THE ROLE OF CORTICOSTERONE AND CORTICOSTEROID-BINDING GLOBULIN IN REPRODUCTION OF RED-LEGGED KITTIWAKES (*RISSA BREVIROSTRIS*)

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A

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Abstract

The goal of this study was to determine to what extent, and by what physiological and behavioral mechanisms, avian reproductive performance is related to environmental variability. Specifically, I explored relationships between components of the physiological stress response, reproductive behavior, and reproductive performance in the red-legged kittiwake (*Rissa brevirostris*), a long-lived seabird. I found that individuals respond to food-related stress by altering levels of both corticosteroid-binding globulin (CBG) and the primary avian glucocorticoid, corticosterone (Cort). I also found a negative association between breeding adults' Cort concentrations and population-level reproductive success; although no such association existed between CBG and reproductive success. The relationship between Cort and reproductive performance does not appear to result from Cort concentrations affecting an adult's decision to initiate or forego reproduction in a given year. In conclusion, this study suggests that environmentally-induced physiological stress affects reproductive success of red-legged kittiwakes, although the behavioral mechanism remains unknown.

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GENERAL INTRODUCTION

Seabird reproductive success varies inter-annually; however, the proximate causes of this variation are not well understood. The goal of this study is to determine to what extent, and by what mechanisms, avian reproductive performance is related to physiological signals of stress. Specifically, we explore the relationships between components of the physiological stress response, reproductive behavior, and reproductive performance in red-legged kittiwakes (*Rissa brevirostris*).

Reproduction is central to Darwinian fitness, yet costs associated with breeding attempts often reduce individual survival and likelihood of successful future reproductive attempts. Long-lived, iteroparous species, including seabirds, must balance the potential benefits and costs of initiating breeding in a given year to maximize lifetime fitness. Currently, we do not know if or how seabird species assess the favorability of environmental conditions and adjust reproductive strategy. Here, we explore one potential mechanism by which seabirds might both evaluate the environment and optimize reproductive effort in a given year, the physiological stress response.

The stress response operates to allow individuals to respond to energetic challenges associated with environmental perturbations, decreased foraging success, or increased energy expenditure, resulting from life-history events (e.g. migration, reproduction). An important component of the avian response to stress is the primary glucocorticoid, corticosterone (Cort), which is elevated within minutes of the onset of a perceived stressor. Elevated Cort facilitates energy mobilization and behavioral changes that are thought to be adaptive solutions to overcome short-term challenges. However, chronic exposure to elevated Cort concentrations has negative consequences, including decreased immune efficiency, increased free radical concentrations, and neurological damage.

Recent research suggests that these harmful consequences of prolonged elevation of CORT might be mitigated by increases in a protein, corticosteroid-binding globulin (CBG). This protein binds to Cort with high affinity and is thought to render Cort biologically inactive and, therefore, less harmful. Based on these findings, the Free

Hormone Hypothesis posits that the concentration unbound hormone (free Cort) provides more information regarding an individual's experienced stress than does the concentration of total Cort. The dynamics of CBG and free Cort levels are not well known for red-legged kittiwakes specifically or for seabirds in general.

In the first part of this study, I investigate the relationships between components of the stress response (total Cort, CBG, free Cort) and reproductive performance to determine what portion of the inter-annual variation in kittiwake reproduction can be explained by stress physiology. Next, I explore reproductive behavior as a potential mechanism underlying these relationships. Specifically, I ask whether stress physiology affects reproductive performance by informing the decision to reproduce and/or mediating costs of reproductive behavior. Finally, I investigate whether individuals buffer themselves from the harmful effects of prolonged Cort exposure by increasing CBG levels (thus reducing circulating free Cort). I address this by quantifying changes in total Cort and free Cort levels in captive chicks exposed to prolonged nutritional restrictions.

Admittedly, without *in situ* experimental manipulations, my ability to suggest causal relationships between stress physiology, reproductive behavior, and reproductive success is limited. However, I believe that the current study is valuable in understanding the potential associations between components of the stress response and reproduction in wild seabird populations. This knowledge should improve the application of field endocrinology to seabird monitoring efforts and direct future research.

CHAPTER 1 CORTICOSTERONE LEVELS PREDICT REPRODUCTIVE SUCCESS IN RED-LEGGED KITTIWAKES*

1.1 Abstract: Concentrations of the primary avian glucocorticoid, corticosterone (Cort), have been used to predict reproductive performance in some avian species. However, relationships between Cort and reproduction are not always consistent. In such cases, concentrations of corticosteroid-binding globulin (CBG), a protein which binds Cort with high affinity and potentially reduces its availability to receptors, might more accurately predict reproductive success. The goal of this study was to determine whether Cort and/or CBG reliably predict population-level reproductive success in red-legged kittiwakes (*Rissa brevirostris*). Such analysis first requires examination of the relative effects of ecological factors and endogenous seasonal changes in Cort and CBG concentration. First, we examined factors contributing to inter-annual and intra-seasonal variations in concentrations of Cort (baseline and stress-induced) and CBG at three colonies (St. George Island, St. Paul Island, and Buldir Island, Alaska). We then used an information-theoretic approach to evaluate relationships between Cort (baseline, stress-induced) and CBG concentrations and reproductive performance. We found that concentrations of baseline Cort, but not of stress-induced Cort or CBG, varied significantly between colonies, years, and reproductive stages. Baseline Cort during incubation was the best predictor of productivity, and inclusion of CBG levels did not improve model predictions. We found no evidence to support the hypothesis that CBG dynamics alter relationships between Cort and productivity in this species. We conclude that baseline concentrations of Cort, but not CBG, are reliable predictors of productivity in this species and that further development of field endocrinology techniques might augment traditional monitoring efforts.

Key words: stress response, seabird productivity, corticosteroid-binding globulin

*Dempsey TD, Kitaysky AS. 2006. Corticosterone levels predict reproductive success in red-legged kittiwakes. Prepared for submission to J Comp Physiol B.

1.2 INTRODUCTION

Studies have suggested that circulating concentrations of the primary avian glucocorticoid, corticosterone (Cort), might predict reproductive performance in free-living avian populations. For example, Kitaysky et al. (1999) showed that black-legged kittiwakes (*Rissa tridactyla*) breeding at a colony with poor local foraging conditions had higher total Cort concentrations and lower reproductive success than those breeding at a neighboring colony (<100 km away) with good local foraging conditions. More recently, Lanctot et al. (2003) and Kitaysky et al. (2006 in review) found negative relationships between total Cort levels of adult seabirds and population-level reproductive success. These relationships between total Cort and reproductive success are likely the result of direct effects of elevated Cort on adult breeding behavior (Astheimer et al. 1992; reviewed in Wingfield et al. 1997; Kitaysky et al. 2001) and associations between Cort and other factors (e.g. body condition, parasite load) affecting reproduction (Chastel et al. 2005; Raouf et al. 2006). However, relationships between total Cort and reproduction have not been consistent in all cases and can vary among colonies, years, and reproductive stages (Kitaysky et al. 1999; Lanctot et al. 2003).

One explanation for this inconsistency might be that dynamics of corticosteroid-binding globulin (CBG) influence the relationship between total Cort and productivity (Breuner and Orchinik 2002; Love et al. 2004). CBG is a protein which binds Cort with high affinity (Breuner and Orchinik 2001; Breuner et al. 2003) and renders the hormone less available for some target tissue receptors (Hammond 1995; Breuner and Orchinik 2001) and for metabolic clearance (Bright 1995). According to the Free Hormone Hypothesis, it is the 'free' fraction of hormone (i.e., that portion not bound to CBG or similar proteins) that is most physiologically relevant (Mendel 1989). Supporting this hypothesis, research has shown that CBG levels may vary independently of total Cort on several temporal scales (Breuner and Orchinik 2001) and in response to perceived stressors (Lynn et al. 2003; Love et al. 2004), such that levels of unbound Cort are not necessarily tightly linked with total Cort concentrations. In addition, Love et al. (2004) found lower CBG levels in successful breeders relative to failed breeders in the European starling (*Sturnus vulgaris*), but no difference in total Cort between the groups. These results suggest that analysis of CBG dynamics might be integral to any investigation of relationships between total Cort and reproduction and that, in some cases, CBG levels might more reliably predict reproductive performance than total Cort. However, few studies have simultaneously examined the relationships between total Cort, CBG, and reproductive success, and none have done this for a long-lived avian species.

Before evaluating relationships between Cort or CBG and reproductive success, one must examine the relative effects of ecological factors and endogenous seasonal changes on Cort and CBG levels for a given species. Concentrations of both Cort and CBG have been shown to vary among colonies (or populations), years, and seasons in several avian species (Kitaysky et al. 1999, 2002; Breuner and Orchinik 2001, 2002; Breuner et al. 2003; Holberton and Wingfield 2003; reviewed in Romero 2002). Thus, multi-year and multi-colony studies are required to quantify the potential effects of ecological factors and endogenous seasonal changes and to accurately detect relationships between Cort and CBG and productivity. Yet, relatively few previous studies simultaneously assessed inter-colony, inter-annual, and seasonal differences in Cort and/ or CBG (but see Kitaysky et al. 1999, 2006 in review).

The goal of this study is to determine whether Cort and/ or CBG concentrations are useful predictors of population-level reproduction in red-legged kittiwakes (*Rissa brevirostris*). Our specific objectives are 1) to assess variability in concentrations of baseline and stress-induced Cort concentrations as well as CBG levels among three colonies (St. George Island, St. Paul Island, and Buldir Island), among years (2000, 2003, and 2004), and between reproductive stages (incubation and chick-rearing), 2) to determine which Cort measures (baseline or stress-induced; incubation or rearing) best predicts productivity at these colonies, and 3) to determine whether inclusion of CBG parameters improves the accuracy of these predictions. Because over 95% of the world's red-legged kittiwake population breeds at the three colonies sampled here (Byrd and William 1993), this study offers a unique opportunity to assess variation in Cort and CBG concentrations throughout most of its breeding range.

1.3 MATERIALS AND METHODS

1.3.1 STUDY SPECIES AND STUDY LOCATION

Red-legged kittiwakes are long-lived seabirds endemic to the Bering Sea (Byrd 1978; Byrd et al. 1997) and show no consistent genetic pattern of population differentiation among major colonies (Patirana et al. 2002). Adults typically rear a single chick per successful reproductive attempt, and both parents provision young at the nest (Byrd and Williams 1993). Population-level reproductive success in this species is highly variable among years and colonies (Byrd and Williams 1993; Thompson 2005), suggesting that reproductive success is closely tied to foraging conditions during the reproductive season.

We conducted this study on the three major colonies known for this species: St. George Island and St. Paul Island (Pribilof Island group; southeastern Bering Sea) and on Buldir Island (Rat Island group; western Aleutian chain). These islands are oceanographically different from one another. Specifically, St. George Island is located on the continental shelf but near the shelf-edge (an area of high productivity), St. Paul Island is located on the continental shelf but farther from the shelf-edge, and Buldir Island is located in the ocean basin. These differences in oceanographic characteristics among islands have been linked to variation in biological communities and food webs in this region (Springer et al. 1996a).

1.3.2 FIELD SAMPLING

We measured Cort in blood samples from kittiwakes at St. George (2000, 2003, 2004), St. Paul (2000, 2003, 2004), and Buldir (2003, 2004) islands. We also measured CBG in samples from all islands during 2003 and 2004. We collected blood samples from 151 breeding adults during incubation and chick-rearing. We defined breeders as individuals attending a nest which contained an egg (incubation) or chick (rearing). Actual dates of sampling differed slightly among colonies and years based on chronology; in all cases, we collected samples over 1-3 consecutive days between 12-26 June (incubation) and again between 12-31 July (rearing).

We captured individual adults at nest sites using fiberglass noose-poles and collected blood from the brachial vein using 26-guage needle and 100-µl heparinized hematocrit tubes. We collected a baseline blood sample (~200 µL) within 3 min of initial contact, prior to significant increase in circulating Cort levels associated with handling stress (baseline Cort = 0.14*min post-capture + 0.27; $r^2 = 0.09$). We then collected additional samples (~100 µL) 10, 30 and 50 min after capture, following protocols for quantifying adrenocortical response to standardized acute handling and restraint stress (Wingfield 1994). Birds were kept in cloth bags during the sample protocol. Baseline Cort levels are typically negatively correlated with current foraging conditions, while stress-induced 'peak' levels (the highest concentration of Cort measured among the four successive samples) reflect an individual's adrenal capacity and are negatively associated with foraging conditions ~4-6 weeks prior to sampling (Kitaysky et al. 2001, 2002, 2006 in review).

We transferred collected blood into 0.5-ml vials and stored samples on ice. Within 12 hours, we centrifuged the blood, separated plasma, and kept all samples frozen. We later transported samples on ice to the University of Alaska Fairbanks (Fairbanks, AK) and stored them at -20°C until analysis. Based on results from a separate study of red-legged kittiwakes in which there was no effect of sex (assigned according to morphology) on Cort parameters (Dempsey and Kitaysky 2006), we did not determine the sex of individuals sampled in this study.

1.3.3 CORTICOSTERONE ASSAYS

We determined total Cort concentrations via eight separate radioimmunoassays (one for each colony-year) using 20 µl plasma (for details see Wingfield et al. 1992). Each sample was extracted with 4.0 ml dicholoromethane, dried under nitrogen, and re-suspended in phosphate-buffered saline with 1% gelatin. We analyzed samples in duplicate and corrected final assay values for individual recoveries of 2000 cpm tritiated Cort added prior to extraction (recoveries ranged from 72% to 100 %; average 87%). Inter-assay variability was approximately 12.4% (estimated from repeated analysis of assay standards, there was no consistent pattern in the variability of these standards among years or colonies) and intra-assay variability (estimated from variation between duplicate samples) was 1.8%. Sensitivity of this assay was 7.8 pg/ tube. To quantify 'peak' levels for each individual, we used the highest Cort concentration measured during the stress protocols. All individuals in this study reached peak Cort secretion at either 30 or 50 min following capture.

1.3.4CORTICOSTEROID-BINDING GLOBULIN ASSAY

We measured plasma CBG in three assays according to established protocols (Breuner and Orchinik 2002; Love et al. 2004). Because acute stress handling protocols were shown by Lynn et al. (2003) to affect CBG levels in white-crowned sparrows (*Zonotrichia leucophrys*), we tested for similar effects in red-legged kittiwakes. We found no effect of acute handling stress on CBG levels in plasma collected <3, 10, or 50 min following capture ($F_{2,23} = 1.06$ $P = 0.36$). Therefore, the decision regarding which sub-sample to assay was based upon volume of available plasma.

We determined optimal incubation time (2 hr), final plasma concentration (1:450), and Cort affinity (Kd = 1.585; see Figure 1.1). We stripped endogenous Cort from plasma by incubating each 10 µl sample with 20 µl dextran-coated charcoal solution for 20 min at room temperature prior to centrifuging for 10 min at 4000 rpm, 4°C. We conducted the assay in triplicate and simultaneously determined non-specific binding in duplicate (using unlabeled Cort) for each individual sample. Following incubation, we separated bound and free fractions of labeled Cort using glass fiber filters (Whatman GF/B) pre-soaked for 1 hr in 25 nM Tris with 0.3% PEI. We suspended filters containing bound Cort in scintillation liquid and measured radioactivity after 12 hr. We calculated average intra-assay variation (10.8 %) as well as inter-assay variation (3.3 %) using three repeated point sample determinations (adjusted CBG capacity nM) of a single plasma pool in each assay.

1.3.5 REPRODUCTION ESTIMATES

Estimates of reproductive success were collected by the Alaska National Maritime Refuge (ANMR) as part of their ongoing seabird monitoring effort. Productivity is a cumulative measure calculated from stage-specific reproductive parameters (laying success, hatching success, fledging success) and is equal to the number of nest sites with a chick fledged divided by the total number of nests in ANMR sampling areas (Thomson 2005).

Kittiwake reproductive performance differs greatly among colonies, potentially due to intrinsic differences including oceanographic characteristics (e.g. continental shelf or oceanic basin), extent of winter ice cover, timing of spring ice-retreat, and predation pressure (Hatch et al. 1993; Frederiksen et al. 2005). Specifically, among the three colonies included in this study, red-legged kittiwake productivity was consistently higher at St. George than either St. Paul or Buldir, which might reflect differences in avian predator pressure among the colonies (Barrett et al. 2005; Thomson 2005; Wright et al. 2005). To correct for these “intrinsic” colony-specific differences in reproduction, we calculated 20-year means and standardized reproductive parameters for each colony by using the residuals from these means in our analyses.

1.3.6 STATISTICAL METHODS

Prior to analysis, we tested initial data for assumptions associated with parametric tests (Sokal and Rohlf 1981). In cases where data violated assumptions, we employed $\log_{10}$-transformations and re-examined the data. In all such cases, transformed data met the assumptions for parametric tests. We examined the effects of colony, year, and reproductive stage (and all interactions) on Cort (baseline and peak) and CBG levels for birds sampled during 2003 and 2004 using three-way analysis of variance (ANOVA). We examined orthogonal contrasts for planned *post hoc* comparisons.

We used a model-selection approach based on Akaike Information Criterion (AIC) to determine which of several *a priori* models (Table 1.1) was the most parsimonious (fewest estimable parameters, ‘K’) and accurate predictor of productivity

(Burnham and Anderson 1998). In the first analysis, we were interested in comparing the relative predictive power of baseline and peak Cort concentrations during incubation and rearing (n = 8 colony-years for all models). After converting AIC values to AIC_c values for each model, as suggested when sample size is small relative to the number of estimable parameters (Burnham and Anderson 1998), we identified the 'best' model based on the lowest AIC_c (Analysis 1, Table 1.2). We ranked all models according to AIC_c and calculated the ΔAIC_c (difference between the AIC_c of each approximating model and that of the best model), the AIC_c weights (measure of the likelihood of a model being the best given the data), and the evidence ratios (comparisons between the support for a given model relative to that the support for the best model). We identified a set of models which, given the data, would include the best model with approximately 95% confidence (Burnham and Anderson 1998). Secondly, we used the same modeling procedures to compare the models in this 95% confidence set with models that also included the CBG parameter for that reproductive stage (Analysis 2, Table 1.2). Because we were unable to quantify CBG levels for individuals sampled during 2000, we conducted the second analysis using only 2003 and 2004 Cort and CBG data (n = 6 colony-years for all models).

1.4 RESULTS

1.4.1 COLONY, YEAR AND STAGE EFFECTS

1.4.1.1 BASELINE CORTICOSTERONE

We found effects of colony ($F_{2,\,109} = 8.02$ $P < 0.001$) and year ($F_{1,\,109} = 5.02$ $P = 0.03$), but not reproductive stage ($F_{1,\,109} = 2.91$ $P = 0.09$), on baseline total Cort (Figure 1.2). We found interactions between colony and year ($F_{2,\,109} = 4.81$ $P = 0.01$), year and stage ($F_{1,\,109} = 4.43$ $P = 0.04$), but not colony and stage ($F_{2,\,109} = 2.10$ $P = 0.13$) or colony*year*stage ($F_{2,\,109} = 2.82$ $P = 0.06$). Across years and stages, birds sampled on Buldir and St George had higher baseline Cort than those on St. Paul (Buldir, $P = 0.001$; St. George, $P = 0.02$), but there was no difference between Buldir and St. George ($P = 0.07$).

1.4.1.2 PEAK CORTICOSTERONE

We found no effect of colony ($F_{2, 109} = 1.47$ $P = 0.24$), year ($F_{1, 109} = 0.04$ $P = 0.84$), or stage ($F_{1, 109} = 3.01$ $P = 0.09$) on peak total Cort (Figure 1.2). There was an interaction between colony and year ($F_{2, 109} = 7.59$ $P < 0.001$), but not between colony and stage ($F_{2, 109} = 0.43$ $P = 0.65$), year and stage ($F_{1, 109} = 1.45$ $P = 0.23$), or colony*year*stage ($F_{2, 109} = 2.76$ $P = 0.07$).

1.4.1.3 CORTICOSTEROID-BINDING GLOBULIN

We found no effect of colony ($F_{2, 109} = 2.37$ $P = 0.10$), year ($F_{1, 109} = 1.11$ $P = 0.29$), or stage ($F_{1, 109} = 0.17$ $P = 0.68$) on CBG capacity (Figure 1.2). Nor were there any interactions between colony and year ($F_{2, 109} = 1.14$ $P = 0.33$), colony and stage ($F_{2, 109} = 0.45$ $P = 0.64$), year and stage ($F_{1, 109} = 1.36$ $P = 0.25$), or colony*year*stage ($F_{2, 109} = 0.82$ $P = 0.44$).

1.4.2 PRODUCTIVITY

The model-selection approach indicates that baseline Cort during incubation (model 3, Table 1.1) was the single best Cort parameter for predicting productivity (i.e. model with the lowest AIC_c and highest AIC weights; Table 1.2). Peak Cort during incubation (model 1) and peak Cort during rearing (model 2) were also included in the ~95% confidence set of models. All three Cort parameters included in these models were negatively correlated with productivity (Figure 1.3). Based on evidence ratios, model 3 is only approximately 1.2 times more likely to be the best model than either model 1 or 2; and, therefore, these models are indistinguishable from one another (Burnham and Anderson 1998). There was, however, substantially less support for Models 4, 5, and 6 (Table 1.2), and we excluded these models in subsequent analyses below.

In the second model-selection procedure, based on the truncated dataset (2003 and 2004 only), the 95% confidence set again included models 1, 2, and 3 (Table 1.2). However, the relative support for these models changed, such that peak Cort during incubation (model 1) was the best predictor of productivity (Table 1.2). Based on evidence ratios, this model was 4.2 and 6.4 times more likely the best model than baseline Cort during incubation (model 3) or peak Cort during rearing (model 2), respectively. Including CBG as a parameter did not appear to improve predictions of productivity.

1.5 DISCUSSION

1.5.1 COLONY, YEAR, AND STAGE EFFECTS

This study found colony and year effects on baseline Cort concentrations as well as colony-year and year-stage interactions. The colony-year and year-stage interactions suggest that ecological factors (presumably local foraging conditions), rather than consistent colony-specific differences or endogenous seasonal dynamics, largely determine baseline Cort concentrations in this species. This finding is consistent with Kitaysky et al. (2002, 2006 in review), which found colony-year interactions in baseline Cort levels of red-legged kittiwakes and common murres (*Uria aalge*), and contributes to a growing body of research linking baseline Cort concentrations to environmental conditions (e.g. Chastel et al. 2005; Benowitz-Fredericks et al. in press; reviewed in Wingfield and Kitaysky 2002; Boonstra 2004).

It is important to note that we sampled only active breeders; therefore, we cannot distinguish between the effects of reproductive stage and those of season (independent of reproduction) on baseline Cort. However, a separate study of Cort dynamics in breeding and non-breeding red-legged kittiwakes indicated that baseline Cort values reflect reproductive effort rather than endogenous seasonal dynamics (Dempsey and Kitaysky in prep). In that study, non-breeding adults' baseline Cort concentrations during the rearing period remained low and similar to those experienced during incubation, while breeders' baseline Cort levels significantly increased. In a related study, Golet et al. (2004) found elevated baseline Cort concentrations in breeding black-legged kittiwakes relative to non-breeders. Thus, although we did not directly distinguish between the effects of reproduction and the potential effects of endogenous seasonal patterns in this study, it appears appropriate to infer that ecological factors and reproduction interact to determine baseline Cort levels in this species.

We found no effect of colony, year, or stage on peak Cort, although there was a colony-year interaction, indicating no consistent colony or year differences. The lack of consistent colony effect on stress-induced peak Cort levels and the significant colony-year interaction found in this study agree with earlier findings for this species (Kitaysky et al. 2002). Also, although there was no effect of stage on peak Cort, we did find substantial variation in the intra-seasonal dynamics of peak Cort (Figure 1.3). These results suggest that, similar to baseline Cort, peak Cort levels in this species are not population-specific, but instead determined by ecological factors.

Although the lack of detectable inter-colony, inter-annual, or stage-specific differences in CBG levels does not refute the hypothesis that individuals mediate their Cort response via modulation of CBG levels, it provides no evidence that quantifying CBG levels is necessary for gauging stress status in red-legged kittiwake populations. These findings are in contrast to those of Breuner and Orchinik (2001) who found population-specific differences, and to those of Breuner and Orchinik (2002) and Romero (2002) who found seasonal patterns in CBG levels. The dissimilarity might be due to differences in life-history among the various study species. Breuner and Orchinik (2001) attributed population-specific differences in CBG capacity in white-crowned sparrows (*Zonotrichia leucophrys*) to differences in the length of breeding season. Specifically, the population with the longest breeding season (and subsequently highest probability of re-nesting following failed breeding attempts and highest possible number of successful breeding attempts) had the lowest mean CBG concentration and was most behaviorally sensitive to poor conditions. Since the current study is among the first to report CBG dynamics in a free-living, long-lived species, it seems likely that vast differences in the life history traits of such species relative to passerines might explain the discrepancy in CBG patterns. It is possible that long-lived species simply do not respond to changing conditions via modulation of CBG levels. More data on this and other long-lived species are required to evaluate the generality of our findings.

1.5.2 PRODUCTIVITY

Baseline Cort during incubation, peak Cort during incubation, and peak Cort during rearing are comparable predictors of productivity and are more reliable predictors than other Cort parameters; highlighting the importance of early-season environmental conditions on the eventual success of reproduction. As stated previously, baseline Cort levels tend to reflect current foraging conditions while peak levels are more tightly linked to foraging conditions 4-6 weeks previously (Kitaysky et al. 2001, 2002, 2006 in review). Hence, though we did not directly quantify food availability in this study, peak Cort levels during incubation likely reflect foraging conditions during the pre-laying period while both baseline Cort during incubation and peak Cort during rearing most likely reflect foraging conditions during the incubation stage.

The superiority of models representing early season conditions is consistent with previous findings in both red-legged and black-legged kittiwakes (Hatch et al. 1993; Gill and Hatch 2002; Lanctot et al. 2003; Barrett et al. 2005; Thomson 2005; Wright et al. 2005). Ongoing monitoring efforts at the colonies included in this study indicate that the majority of reproductive failure in both kittiwake species is attributable to laying or hatching failures (Barrett et al. 2005; Thomson 2005; Wright et al. 2005). Similarly, Hatch et al. (1993) and Gill and Hatch (2002) found that the majority of kittiwake breeding failure (~60-70%) at North Pacific colonies occurs prior to hatching, suggesting that chick-rearing is of secondary importance relative to earlier reproductive stages. The relative importance of early reproductive stages does not appear to be explained by consistent patterns in prey availability during the chick-rearing period (given the variation in Cort measures during this stage presented above). Instead, it might be explained by heterogeneous kittiwake quality as found by Cam et al. (1998) in black-legged kittiwakes. If substantial heterogeneity exists in red-legged kittiwakes, individuals representing a wide range of quality and experience might attend a colony to breed. In more difficult years, challenging early season conditions (e.g., low food availability) would force inferior individuals to forego breeding or to abandon breeding efforts; however, in less difficult years, more of these inferior individuals would initiate reproduction. In this way, early season conditions could drive population-level productivity. The degree of heterogeneity in breeder quality in red-legged kittiwakes has not yet been directly studied.

According to our analyses, the inclusion of CBG parameters does not increase the accuracy of productivity predictions based on total Cort concentrations. This is not surprising given the constancy of CBG levels among colonies, years, and stages observed in this study. However these results are in contrast to previous studies in which CBG concentrations varied seasonally (Breuner and Orchinik 2001; Romero 2002), in response to food stress (Lynn et al. 2003), and in association with reproduction (Love et al. 2004). As stated above, this pattern might be explained by differences between the life-history characteristics of passerine species studied previously and the long-lived red-legged kittiwake. As this study is among the first to record CBG levels in free-living seabirds, more research is required to improve our understanding of the role and dynamics of CBG in long-lived avian species.

1.6 CONCLUSIONS

We found significant spatial and temporal variation in baseline and peak Cort, as well as a general and predictive pattern between elevated early season Cort concentrations and lower population-level reproduction. We found no evidence that CBG dynamics alter the relationship between Cort and reproduction at the population level or improve the accuracy of productivity predictions. Therefore, though CBG dynamics are a potentially important component of the avian stress response and warrant further research, our results suggest that measurement of CBG levels does not provide additional insights into the relative stress levels in this species.

We believe that field endocrinology methods similar to those employed here have the potential to augment traditional seabird monitoring methods currently employed in the Bering Sea and elsewhere. Monitoring the relative stress status of seabird populations could provide valuable information on the demography of these important consumer species and, more generally, on the conditions of oceanic ecosystems. This clearly depends, however, on continued research into the generality of these relationships to other seabird species and other regions.

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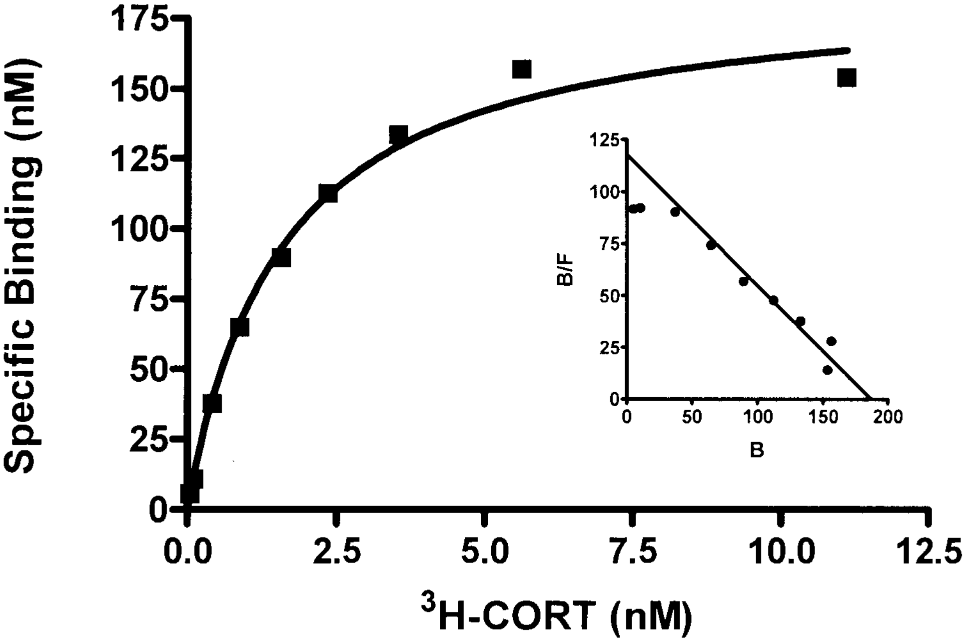
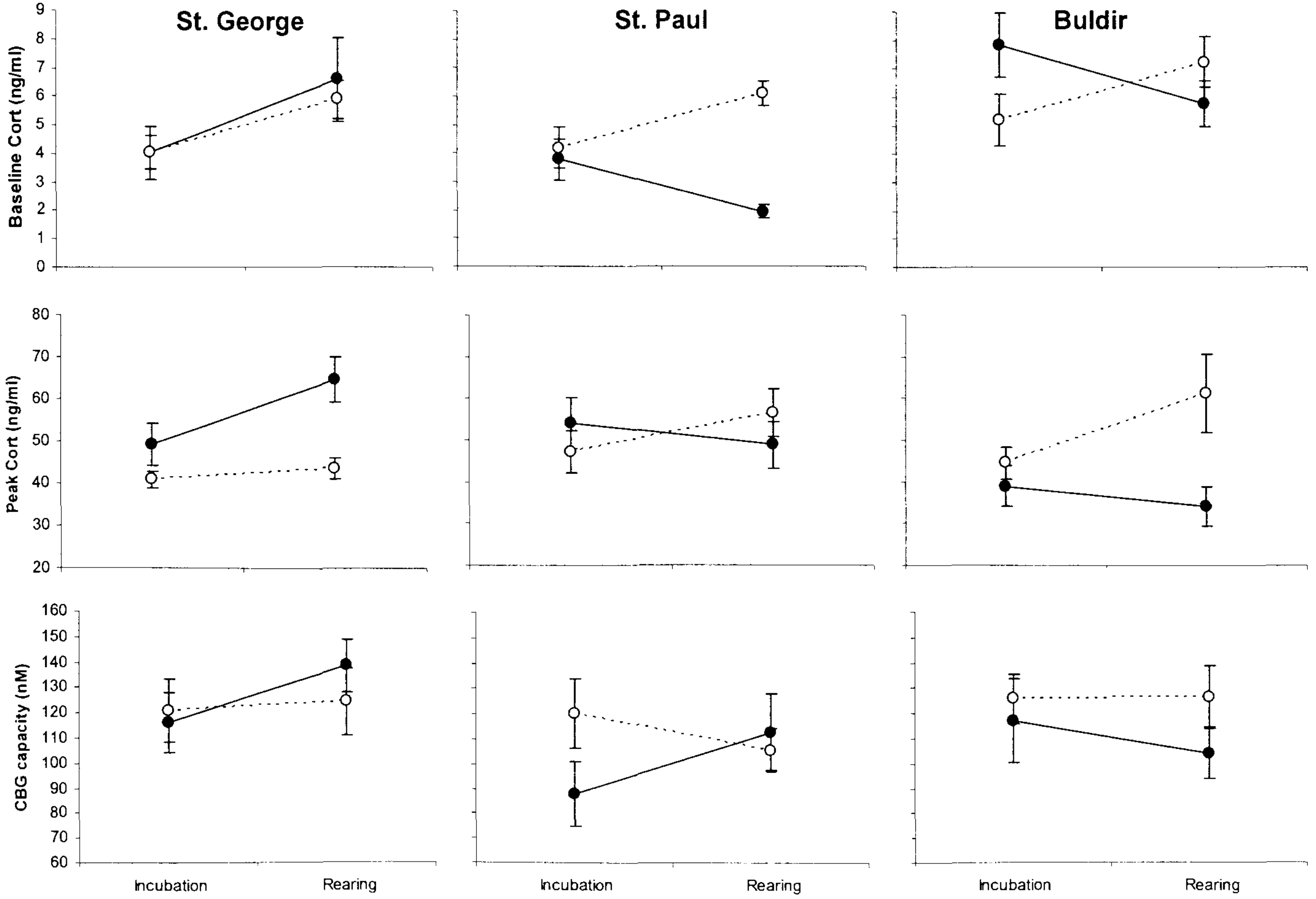


Figure 1.1 Equilibrium saturation binding curve showing specific binding of [^{3}H]Cort to red-legged kittiwake plasma associated with increasing concentrations of [^{3}H]Cort (means ± SE). The inlay is a Scatchard–Rosenthal re-plot of the data.

Figure 1.2 Inter-annual and intra-seasonal dynamics of baseline and stress-induced peak concentrations of corticosterone (Cort) and corticosteroid-binding globulin (CBG) in red-legged kittiwakes breeding at St. George Island, St. Paul Island, and Buldir Island, Alaska (mean ± SE), in 2003 (filled circles, solid lines) and 2004 (open circles, dashed lines).



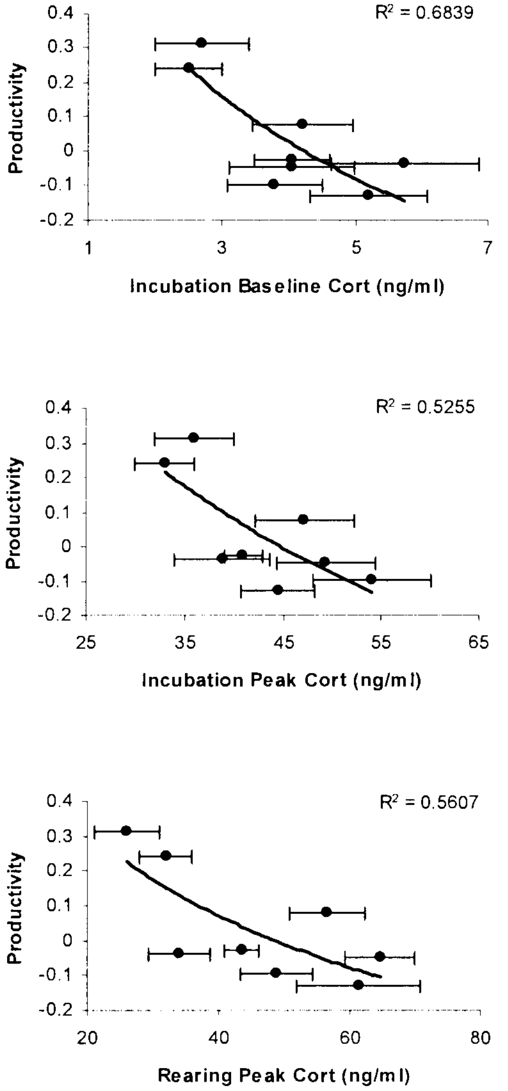


Figure 1.3 Productivity relative to population means (± SE) of baseline corticosterone (Cort) during incubation (upper panel; productivity = -0.47Ln(BL Cort) + 0.67, r^2 =0.68), peak Cort during incubation (middle panel; productivity = -0.70Ln(Peak Cort) + 2.67, r^2 =0.53) and peak Cort during rearing (lower panel; productivity = -0.36Ln(Peak Cort) + 1.41, r^2 =*0.56*) of adult red-legged kittiwakes breeding at St. George Island, St. Paul Island, and Buldir Island, AK

Table 1.1 Parameters included in models assessing the relative power of incubation and rearing corticosterone (Cort) concentrations (baseline and peak) and corticosteroid binding globulin (CBG) levels in predicting productivity in red-legged kittiwake (*Rissa brevirostris*) at three colonies in the Bering Sea 2000-2005.

Model	Parameters included
1	Peak Cort: incubation
2	Peak Cort: rearing
3	Baseline Cort: incubation
4	Baseline Cort: rearing
5	Peak Cort: incubation * Peak Cort: rearing
6	Baseline Cort: incubation * Baseline Cort: rearing
7	Peak Cort: incubation * CBG: incubation
8	Peak Cort: rearing * CBG: rearing
9	Baseline Cort: incubation * CBG: incubation

Table 1.2 Relative power of incubation and rearing corticosterone (Cort) concentrations (baseline and peak) and CBG levels in predicting productivity in red-legged kittiwake (*Rissa brevirostris*) at three colonies (2000, 2003, 2004). Models are ranked from most to least likely given the data.

Model	Parameters	N	K	ΔAIC_c	AIC weight[a]	∑AICc weight	Evidence ratio
Analysis 1							
3	Baseline Cort: incubation [b]	8	3	0.000	0.351	0.351	1.000
2	Peak Cort: rearing [b]	8	3	0.396	0.288	0.640	1.219
1	Peak Cort: incubation [b]	8	3	0.409	0.286	0.926	1.227
4	Baseline Cort: rearing	8	3	3.347	0.066	0.992	5.331
5	Peak Cort: incubation * Peak Cort: rearing	8	4	8.666	0.005	0.997	76.180
6	Baseline Cort: incubation * Baseline Cort: rearing	8	4	9.287	0.003	1.000	103.885
Analysis 2							
1	Peak Cort: incubation [b]	6	3	0.000	0.691	0.691	1.000
3	Baseline Cort: incubation [b]	6	3	2.853	0.166	0.857	4.163
2	Peak Cort: rearing [b]	6	3	3.722	0.108	0.965	6.429
8	Peak Cort: rearing * CBG: rearing	6	4	7.694	0.015	0.980	46.857
9	Baseline Cort: incubation * CBG: incubation	6	4	7.694	0.015	0.995	46.857
7	Peak Cort: incubation * CBG: incubation	6	4	11.061	0.003	0.997	252.311

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[a] Measure of likelihood that specific model is the best model given the data

[b] Denotes models included in the ~95% confidence set based on cumulative AICc weight

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CHAPTER 2 PHYSIOLOGICAL FACTORS ASSOCIATED WITH BREEDING IN RED-LEGGED KITTIWAKES*

2.1 Abstract: Intermittent breeding is widespread among long-lived birds; however, mechanisms underlying initiation or inhibition of breeding in a given year are poorly known. We examined physiological and ecological factors associated with breeding decision and reproductive costs in red-legged kittiwakes (*Rissa brevirostris*). We compared baseline and stress-induced concentrations of Total and Free corticosterone (Cort), corticosteroid-binding globulin (CBG), body condition, ectoparasite load, as well as Carbon and Nitrogen stable isotopic signatures (as a proxy for diet) between breeders and non-breeders attending nests on St George Island, AK. If physiological condition influences breeding decision, we predicted non-breeders would have higher Cort, lower BC, and higher ectoparasite loads than breeders during the incubation period. We expected that demands of chick-rearing would reverse these patterns, indicating consequences of reproduction. We found no difference in baseline Total or Free Cort between groups during the incubation period; however, during the chick-rearing period, breeders' showed elevated baseline Total and Free Cort. Also, though peak Total Cort was similar between stages and groups, breeders had elevated peak Free Cort compared to non-breeders during the rearing period. Ectoparasite load was greater in breeders than non-breeders only during the incubation period. We found no difference in CBG or body condition between groups at either reproductive stage and no differences between breeders' and non-breeders' isotopic signatures. We suggest that breeders incurred reproductive costs during incubation (higher parasite load) and chick-rearing (elevated Total and Free Cort). However, these results provide no evidence that physiological condition or foraging ecology (as indicated in isotopic signatures) determine breeding decision in kittiwakes.

Key words: *breeding decision, corticosterone, corticosteroid-binding globulin*

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2.2 INTRODUCTION

Intermittent breeding, defined as instances of non-breeding in individuals that have previously reproduced, is a common phenomenon among long-lived seabirds (Coulson and Thomas 1985; Jones and Montgomerie 1992; Cam et al. 1998; Bradley et al. 2000). Since reproduction is energetically costly and potentially detrimental to survival and future reproductive prospects (Williams 1966; Aebischer and Wanless 1992; Stearns 1992; Golet et al. 1998), individuals might maximize their lifetime fitness by forgoing reproduction during years in which they experienced poor physiological condition at the start of the reproductive season or during years of suboptimal environmental conditions. However, the mechanism(s) underlying intermittent breeding are largely unknown. One hypothesis is that physiological condition of an individual at the start of the breeding season determines whether that individual would initiate or forego reproduction (hereafter: 'breeding decision').

While much has been written about the costs and consequences of avian reproduction (Stearns 1992; Erikstad et al. 1998; Golet et al. 1998), relatively little is known about the physiological mechanisms associated with breeding decision and reproductive costs. Here, we use the term 'reproductive costs' to mean the measurable deterioration of physiological condition resulting from reproduction. This definition differs considerably from that used by Stearns (1992), who defined 'reproductive costs' as reduced survival and/or future fecundity resulting from a reproductive attempt. Clearly, although the deterioration of physiological condition might lead to reduced survivorship and fecundity, the two are not synonymous.

In this study, we compare physiological condition of breeding and non-breeding red-legged kittiwakes (*Rissa brevirostris*) attending the colony at St. George Island during the 2005 breeding season. My primary goal is to identify physiological mechanisms which might both influence the breeding decision and indicate consequences of reproductive costs. We focus on three candidates.

The first is the physiological stress response, which is primarily controlled by the Hypothalamus-Pituitary-Adrenal axis (HPA-axis) and regulates circulating levels of the primary avian glucocorticoid, corticosterone (Cort). Shortly after the onset of a perceived stressor, Cort concentrations increase and facilitate behavioral and physiological changes that maintain positive energy balance over short periods (Wingfield et al. 1997; reviewed in Wingfield et al.1998; Boonstra 2004). Specifically, elevated Cort results in increased protein catabolism (Le Ninan et al. 1988; Cherel et al. 1992), gluconeogenesis (Remage-Healey and Romero 2001), and lipogenesis (Gray et al. 1990), as well as increased foraging activity (Astheimer et al. 1992; Kitaysky et al. 2001). Despite apparent benefits of the stress response, prolonged elevation of Cort is associated with costs (reviewed in Sapolsky et al. 2000), including reduced immune defense efficiency (Barriga et al. 2001), muscle deterioration (Gray et al. 1990; Harvey et al. 1984), as well as reproductive abandonment and irruptive migration (reviewed in Wingfield et al. 1997; Wingfield and Ramenofsky 1997; Breuner and Hahn 2003).

In free-living avian species, circulating Cort concentrations have been tightly linked to forage availability (e.g. Wingfield 1994; Kitaysky et al. 1999), which, in turn, affects both the relative costs of reproduction (Tinbergen and Dietz 1994; Blondel et al. 1998; Török et al. 2004) and reproductive success (Kitaysky et al. 1999, 2000; Gill et al. 2002; Lanctot et al. 2003). In this study, we use two separate Cort measures: 1) 'baseline' levels, which are measured in an individual prior to the Cort increase associated with handling stress and 2) 'peak' levels, which are the highest concentration of Cort measured over the application of standardized acute handling stress (Wingfield 1994). Baseline Cort is tightly linked with current food availability while peak Cort is a more integrated measure of the forage conditions over the previous 4-6 weeks (Kitaysky et al. 2002). Recently, Chastel et al. (2005) found that breeding black-legged kittiwakes (*Rissa tridactyla*) had higher baseline Cort but similar peak Cort to failed breeders, while Lanctot et al. (2003) found elevated baseline Cort during the pre-laying period to be associated with laying failure in black-legged kittiwakes.

The role and regulation of Cort in responding to the challenges of reproduction are likely associated with the dynamics of corticosteroid-binding globulin (CBG), a protein that binds Cort with high affinity and renders the hormone less biologically available for most target tissue receptors (Hammond 1995). According to the Free Hormone Hypothesis, it is the free 'unbound' fraction of hormone that is most biologically active (Mendel 1989); therefore, Total Cort and CBG dynamics represent two distinct but interrelated means of regulating Free Cort concentrations (Breuner and Orchinik 2002). Supporting this hypothesis, Love et al. (2004) found lower CBG levels, but similar total Cort concentrations, in actively breeding European starlings (*Sturnus vulgaris*) relative to failed breeders. Hence, it appears necessary to simultaneously quantify plasma concentrations of Total Cort, CBG and Free Cort when evaluating the effect of reproductive effort on adrenocortical function. If adrenocortical function influences the breeding decision, we predict that non-breeders (presumably in poorer condition) will have higher circulating Total and Free Cort levels relative to actively breeding birds during the incubation period. However, during the energetically demanding chick-rearing period, we predict that active breeders will have higher Total and Free Cort levels than non-breeders (reflecting reproductive costs as demonstrated by Golet and Irons (1999) and Golet et al (1998)). CBG dynamics in free-living kittiwakes have not been documented; therefore, the ability to make specific predictions regarding CBG levels is limited. However, if CBG dynamics in this species are similar to those of European starlings, we expect lower concentrations in breeders than non-breeders (Love et al. 2004).

A second candidate mechanism is adult body condition (body mass scaled to size). Though approximate, such an index estimates an individual's accumulated energy reserves (primarily lipids). Many studies have reported that individuals in better condition (signaled by larger lipid reserves) are more likely to survive and breed (Pugesek and Diem 1990; Moe et al. 2002) and that breeding individuals often deplete energy reserves over the course of the reproductive season (Drent and Daan 1980; Golet and Irons 1999; Kitaysky et al. 1999; Stjernman et al. 2004). Monitoring the rate and magnitude of decline in body condition in breeding birds can provide important information about the physiological and energetic consequences of a reproductive attempt (Golet and Irons 1999; Vleck and Vleck 2002). If early-season body condition influences breeding decision, we predict that non-breeders will be in poor condition relative to actively breeding birds during the incubation period. Since we sampled birds during the incubation period (not during laying or pre-laying periods), this prediction assumes that an individual's condition at the time of sampling is tightly linked to that at the time of the breeding decision. During the chick-rearing period, we predict that active breeders will decline in condition to the point that they are similar to or in poorer condition than non-breeders (again, indicative of the consequences of reproduction).

A third candidate which may influence breeding decision and mediate the consequences of reproduction is immune function, indicated by resistance to ectoparasitism. Studies have found higher parasite loads among breeding birds than failed breeders (Norris et al. 1994; Richner et al. 1995; Oppliger et al. 1996; Nordling 1998) and a negative relationship between parasite load and post-breeding survival (Nordling et al. 1998; Dawson and Bortolotti 2000; Stjernman et al. 2004). This relationship is reflected both in physiological factors, including suppression of the immune response and reduced parasite resistance, and behavioral factors, including increased time at nest sites (where ectoparasites aggregate) and decreased preening behavior in breeding adults (Nordling 1998). We predict that if early-season immune function influences breeding decision, non-breeders will have higher ectoparasite loads than breeders during the incubation period (due to reduced resistance). Again, this assumes that parasite loads during the incubation period are tightly linked to those during the preceding pre-laying and laying periods when the breeding decision is made. Alternately, if ectoparasite load is largely determined by reproductive behavior, we expect breeders to have higher ectoparasite loads than non-breeders during the incubation period. We predict that the demands of reproduction will reduce the immune efficiency and parasite resistance of breeders, who, therefore, will have higher ectoparasite loads than non-breeders during the rearing period.

Finally, we compared stable isotope ratios of carbon (C) and nitrogen (N) in the red blood cells of breeders and non-breeders during the incubation period. Isotopic analyses of these elements have been used repeatedly to indicate differences in foraging strategy and relative diet composition in free-living avian populations (e.g. Hobson et al. 1994). Such comparisons are valuable since adult diet composition can influence breeding chronology, egg size, clutch size, nestling survival, and overall productivity in seabirds (Bolton et al. 1992; Bolton et al. 1993; Abraham and Sydeman 2004). We predicted that if pre-breeding foraging patterns influenced the breeding decision, breeders and non-breeders would have different isotopic ratios of C and/or N.

2.3 METHODS

2.3.1 STUDY SITE AND SPECIES

The red-legged kittiwake (*Rissa brevirostris*) is a small (290-450 g; Byrd et al. 1997) seabird of the family Laridae, and is known to breed at only four locations worldwide: the Pribilof Islands, Bogoslof Islands, Buldir Island and the Commander Islands (Byrd 1978). Red-legged kittiwakes breed in multi-species colonies on vertical cliffs and typically rear a single semi-precocial chick, provisioned by both parents (Byrd and Williams 1993). Intermittent breeding has not been explicitly documented for this species; however, previously breeding adults that were not sighted at the colony during one season were observed breeding there in subsequent years (Thomson 2005). In addition, in this study, some previously breeding adults were associated with nests without eggs or chicks. Finally, Cam et al. (1998) recorded intermittent breeding in the closely related black-legged kittiwake (*Rissa tridactyla*).

We conducted this study on St. George Island (Pribilof Islands, southeastern Bering Sea; 56°36'20" North, 169°33'35" West), breeding island for ~80% of the global population (Kildaw 1997). Red-legged kittiwakes typically arrive at the colony in late spring and lay eggs in late June to early July, peak hatching occurs in early July to mid-August, and peak fledging in early to mid-September (Byrd and Williams 1993).

2.3.2 SAMPLE COLLECTION AND PREPARATION

During the summer of 2005, we collected baseline and acute stress-induced blood samples from 38 breeding and non-breeding adults during two reproductive stages: incubation (27-29 June) and early chick-rearing (14-18 July). We define 'non-breeders' as adults attending nest sites at the colony but foregoing other stages of reproduction (indicated by the absence of egg or chick). This categorization was verified by the presence of completely denuded brood patches of breeders and the partially re-feathered brood patches in all non-breeders during the incubation period. In this study, we assume that nests without an egg or chick never contained either. This assumption is supported by the near-complete absence of avian predators at this colony, the inaccessibility of sampled nests to Arctic foxes, and observations of abandoned eggs remaining in nests during chick-rearing (Thomson 2005; Dempsey pers obs.).

We captured adults at nest sites using a fiberglass noose-pole and collected blood from the brachial vein using 26-guage needle and 100-µl heparinized hematocrit tubes. We collected a baseline blood sample (~200 µL) within 3 min of initial contact, prior to significant increase in circulating Cort levels associated with handling stress (Romero and Reed 2005; Kitaysky, unpublished data). We collected additional samples (~100 µL) 10, 30 and 50 minutes after capture, following protocols for quantifying adrenocortical response to standardized acute handling and restraint stress. Following each sample collection, we staunched bleeding with cotton, transferred blood into 0.5-ml vials, and stored samples on ice. Within 12 hours, we centrifuged the blood, separated plasma, and stored samples frozen. We later transported samples to the University of Alaska Fairbanks laboratories (Fairbanks, AK) and stored them at -20°C until analysis.

2.3.3 BODY CONDITION AND ECTOPARASITE LOAD

We recorded the mass to the nearest 5g using a Pesola spring balance: we recorded tarsus, culmen, and head plus bill (back of the head to the tip of the bill) to the nearest 0.1 mm using calipers; we measured flattened wing chord with a stopped ruler from the carpal joint to the tip of the longest primary. We also recorded the number of ticks on the head and neck of each individual. Similar to the methods used by Chastel et al. (2005) for black-legged kittiwakes, we employed multiple regression to determine the relationship between morphometric measurements (headbill, tarsus) and body mass ($r^2 = 0.32$, $n = 50$, $P < 0.0001$).

Predicted mass = 1.407(headbill*tarsus*0.1) + 27.945

We then calculated an index of body condition (hereafter: BCI) as the residual between the predicted value and measured mass of an individual. We found no effect of putative sex on BCI ($F_{1,36} = 0.06$, $P = 0.81$).

2.3.4 SEXING

Sex was estimated using headbill length with 82.0 mm as the discriminant value (S. D. Kildaw, UAF, unpublished data). Individuals with headbill lengths below this value were assumed to be females, individuals with headbill lengths equal to or greater than this value were assumed to be males. Including sex as a factor in preliminary analyses did not change the nature or statistical significance of relationships during the incubation period. There were no significant differences between males and females in components of the physiological stress response, parasite load, isotopic ratios, or BCI. Therefore, we did not include sex as a class variable in the analyses presented here in the interests of maximizing statistical power.

2.3.5 CORTICOSTERONE ASSAYS

We determined Total plasma Cort concentrations for all samples via radioimmunoassay using 20 µl plasma (for details see Wingfield and Farner 1975; Wingfield et al. 1992). We conducted two assays: one for incubation samples and one for chick-rearing samples (randomizing individual sample positions). Each sample was extracted with 4.0 ml dicholoromethane, dried under nitrogen, and re-suspended in phosphate-buffered saline with 1% gelatin. We analyzed samples in duplicate and corrected final assay values for individual recoveries following extraction (92% on average). The intra-assay coefficients of variation were 2.5% (incubation) and 2.3% (chick-rearing); inter-assay variation was 7.9%; and sensitivity of each assay was 7.8 pg/ tube.

2.3.6 CORTICOSTEROID-BINDING GLOBULIN ASSAY

We measured plasma CBG in a single assay according to established protocols (Breuner and Orchinik 2002; Love et al. 2004). Because Lynn et al. (2003) found changes in CBG levels during acute stress handling, we tested for similar effects in red-legged kittiwakes. Our results indicated no significant effect of acute handling stress on CBG levels in plasma collected <3, 10, or 50 minutes after capture (Dempsey, unpublished data, appendix). Therefore, we chose which sub-sample to assay based on plasma availability.

We determined optimal incubation time (2 hr), final plasma concentration (1:450), and Cort affinity (Kd = 1.585; Figure 2.1) for adults of this species. We stripped endogenous Cort from plasma by incubating each 10 μl sample with 20 μl dextran-coated charcoal solution for 20 min at room temperature before centrifuging for 10 min at 4000 rpm, 4°C. We assayed each sample in triplicate at 4°C and determined nonspecific binding (in duplicate) simultaneously using unlabeled hormone. Following incubation, we separated bound and free fractions of labeled Cort using pre-soaked glass fiber filters (Whatman GF/B). We suspended filters containing bound Cort in scintillation liquid and measured radioactivity after 12 h. We calculated intra-assay variation (3.2%) using 3 repeated point sample determinations of a single plasma pool comprised of plasma from multiple individuals.

2.3.7 FREE CORTICOSTERONE ESTIMATES

We estimated concentrations of Free Cort based on Total Cort levels and binding capacity using the Barsano and Baumann (1989) equation (below):

$$Hfree = 0.5 \times \left[Htotal - B\max - \frac{1}{Ka} \pm \sqrt{\left(B\max - Htotal + \frac{1}{Ka} \right)^2 + 4\left(\frac{Htotal}{Ka} \right)} \right]$$

2.3.8 STABLE ISOTOPE ANALYSIS

We conducted isotopic analysis on freeze-dried red blood cells from 11 breeders and 9 non-breeders sampled during the incubation period (IRMS, Europa Scientific 20/20

Continuous Flow IRMS, Europa Scientific, Chestershire, UK). Isotope ratios are expressed in delta (δ) notation wherein $\delta^{15}N$ represents the deviation in parts per thousand (‰) of the N isotope ratio of the sample from that of the established standard (air) and $R={}^{15}N/{}^{14}N$, while $\delta^{13}C$ represents the deviation of the C isotope ratio of the sample from that of the established standard (Peedee Belemnite) and $R= {}^{13}C/{}^{12}C$. We conducted all analyses at the Alaska Stable Isotope Facilities at the University of Alaska Fairbanks in Fairbanks, AK.

2.3.9 STATISTICAL

Using two-way ANOVA, we examined the effects of reproductive status and stage on Total Cort (baseline and peak), CBG capacity, Free Cort (baseline and peak), body condition, and ectoparasite load. We analyzed isotopic data using a one-way ANOVA with reproductive status as a factor. We used regression analysis to quantify associations between BCI and Total Cort (baseline and peak), CBG capacity, Free Cort (baseline and peak). We repeated this procedure to analyze relationships between ectoparasite load and these measures of the physiological stress response.

Prior to each analysis, we tested initial data for assumptions associated with parametric tests (Sokal and Rohlf 1981). In cases where data violated assumptions, we employed standard statistical transformations (logarithmic or square root) and reanalyzed the data. We performed all analyses using the Statistica statistical package (StatSoft, Inc. 2006) and set $\alpha = 0.05$.

2.4 RESULTS

2.4.1 TOTAL CORT LEVELS

There were effects of reproductive stage ($F_{1,34} = 25.189$ $P < 0.0001$) and reproductive status (hereafter "status"; $F_{1,34} = 16.379$, $P < 0.001$) on baseline Total Cort and a significant stage-status interaction ($F_{1,34} = 6.826$, $P < 0.01$).

Peak Total Cort varied between stages ($F_{1,33} = 27.442$ $P < 0.0001$) but not by status ($F_{1,33} = 1.703$, $P = 0.20$), and the stage-status interaction was not significant ($F_{1,33} = 0.254$, $P = 0.62$).

2.4.2 CORTICOSTEROID-BINDING GLOBULIN LEVELS

There was no effect of reproductive stage ($F_{1,34} = 0.412$ $P = 0.53$), status ($F_{1,34} = 0.882$, $P = 0.35$), or stage-status interaction ($F_{1,34} = 1.380$, $P = 0.25$) on CBG capacity.

2.4.3 FREE CORT LEVELS

Similar to Total Cort results, there were highly significant effects of reproductive stage ($F_{1,34} = 15.884$ $P < 0.001$), status ($F_{1,34} = 14.946$, $P < 0.001$), and stage-status interaction ($F_{1,34} = 7.589$, $P < 0.01$) on baseline Free Cort.

There were also significant effects of reproductive stage ($F_{1,33} = 17.345$ $P < 0.001$) and status ($F_{1,33} = 4.459$, $P < 0.04$) on peak Free Cort, but no significant stage-status interaction ($F_{1,33} = 3.739$, $P < 0.06$).

2.4.4 BODY CONDITION

There was an effect of reproductive stage ($F_{1,33} = 4.055$, $P < 0.03$) on BCI, but no effect of reproductive status ($F_{1,33} = 3.934$, $P = 0.06$) and no significant stage-status interaction ($F_{1,33} = 0.127$, $P = 0.72$).

Based on regression analysis, we found no significant relationship between BCI and Total Cort (baseline, $F_{1,35} = 1.335$, $P = 0.26$; peak, $F_{1,35} = 2.301$, $P = 0.14$), CBG capacity ($F_{1,35} = 0.505$, $P = 0.48$), or Free Cort (baseline, $F_{1,35} = 0.683$, $P = 0.41$; peak, $F_{1,35} = 0.716$, $P = 0.40$).

2.4.5 ECTOPARASITE LOAD

There was an effect of status ($F_{1,33} = 10.698$, $P < 0.003$) on ectoparasite load with breeders having higher loads than non-breeders during the incubation period, but no effect of stage ($F_{1,33} = 0.052$, $P = 0.82$) and no stage-status interaction ($F_{1,33} = 0.131$, $P = 0.72$).

There was no relationship between ectoparasite load and Total Cort (baseline, $F_{1,35} = 1.447$, $P = 0.24$; peak, $F_{1,35} = 0.826$, $P = 0.37$), CBG capacity ($F_{1,35} = 1.199$, $P = 0.28$), or Free Cort (baseline, $F_{1,35} = 2.038$, $P = 0.16$; peak, $F_{1,35} = 0.047$, $P = 0.83$).

2.4.6 ISOTOPIC ANALYSIS

There was no significant effect of reproductive status on the isotopic ratios of N ($F_{1,18} = 1.241$ $P = 0.28$) or C ($F_{1,18} = 0.127$ $P = 0.73$). There was also no difference between breeders and non-breeders in the ratio of carbon to nitrogen in samples ($F_{1,18} = 0.689$ $P = 0.42$).

2.5 DISCUSSION

In this study, we examined physiological (endocrine, CBG and body condition) and ecological (ticks and SI) factors which potentially influence breeding decision and could reflect consequences of reproduction. We predicted that if early-season physiological condition had influenced breeding decision, breeders would have lower baseline and stress-induced Cort levels, better body condition, and fewer ectoparasites than non-breeders during the incubation period. Also, if foraging ecology during the pre-laying or early-incubation periods influenced breeding decision, we expected to detect differences between the isotopic signature of breeders and non-breeders during the incubation period (reflecting different diets). Finally, we predicted that if these factors reflect physiological consequences of reproduction, breeders would have higher Cort levels, poorer condition, and more ectoparasites than non-breeders during the chick-rearing period.

We found no effect of reproductive status on components of the stress response (Total and Free Cort, CBG), body condition, or isotopic signatures of red-legged kittiwakes during the incubation period (Table 2.1). Contrary to our prediction, breeders had higher ectoparasite loads than non-breeders at this stage. During chick-rearing, breeders had higher baseline Total and Free Cort and higher peak Free Cort than non-breeders; however, differences in peak Total Cort, CBG levels, body condition, and ectoparasite load were not significant (Table 2.1).

2.5.1 STRESS RESPONSE

Although Cort has previously been linked to breeding behavior in black-legged kittiwakes (Golet et al. 1998; Gill et al. 2002; Lanctot et al. 2003; Chastel et al. 2005), none of the stress response components measured in this study (Total or Free; baseline or stress-induced; or CBG) was associated with breeding status during the incubation period in attending red-legged kittiwakes. As Cort levels in seabirds are primarily thought to indicate food availability (Kitaysky et al. 2001, Kitaysky et al. 2006 in review), our findings suggest that neither the foraging conditions during the incubation period (reflected in baseline levels) nor those during the pre-laying period (reflected in peak levels) strongly influenced breeding status in this species. In contrast, Cort concentrations of black-legged kittiwakes during the pre-laying period were negatively associated with subsequent laying success (Lanctot et al. 2003). The discrepancy might be attributable to species-specific differences or to poor conditions at St George in 2005, a year in which productivity of red-legged kittiwake on the island was the lowest recorded in approximately 20 years (Thomson 2005). Such poor conditions could potentially prohibit lower quality adults from attending the colony, thus biasing the distribution of sampled birds and altering apparent relationships between Cort and reproduction.

Despite the lack of relationship between current physiological condition during the incubation period and previous breeding decision, some Cort measures do appear to provide an accurate index of accrued reproductive costs in kittiwakes as indicated by breeders' elevated baseline Total Cort, baseline Free Cort, and stress-induced Free Cort during chick rearing. Furthermore, the elevated hormone concentrations in breeders and the relatively unchanging levels in non-breeders during the chick-rearing period suggest that these factors are more closely related to the demands of reproduction than to endogenous seasonal dynamics or changing foraging conditions.

We found that peak Total Cort did not differ between breeders and non-breeders at either stage, similar to the pattern found in black-legged kittiwakes (Chastel et al. 2005) for black-legged kittiwakes. It is not surprising that peak Total Cort levels during early chick-rearing period were unaffected by reproductive status, given that peak Total Cort concentrations tend to reflect conditions approximately 4-6 weeks prior to sampling (Wingfield et al. 1994; Kitaysky et al. 2001, 2002, 2006 in review). Such a measure would reflect the same time period as baseline Cort levels in birds sampled during the incubation period, which similarly did not differ between breeders and non-breeders. It is possible that breeders sampled later during the rearing period (closer to the time of fledging) would have elevated peak Total Cort concentrations; however such a comparison was not possible during this study.

Due to the similarity of CBG levels in breeders and non-breeders during both reproductive stages, Free Cort estimates closely paralleled Total Cort. The one exception involved peak Total and Free Cort during the rearing period. Despite similar CBG and peak Total Cort between groups (though breeders had a nonsignificant tendency toward lower CBG levels and higher peak Total Cort), peak Free Cort was significantly higher in breeders. Thus, we cannot reject the possibility that individuals modulate Free Cort levels via CBG as suggested by Breuner and Orchinik (2002) and observed in studies of passerines (Lynn et al. 2003; Love et al. 2004). These earlier studies support the 'Free Hormone Hypothesis' which posits that it is the unbound fraction of hormone that is most available for tissue receptors (Hammond 1995; Breuner and Orchinik 2001) and, hence, is the most physiologically relevant factor (Mendel 1989). In our study, it is unclear why baseline Free Cort dynamics mirrored those of baseline Total Cort, while the dynamics of peak Free Cort differed from those of peak Total Cort. More studies of the CBG and Free Cort dynamics of free-living, long-lived avian species are needed to assess the generality of our results.

We found no evidence of adrenocortical suppression during reproduction in this species. This agrees with previous studies of other long-lived birds (Wingfield et al. 1995; Kitaysky et al. 1999, 2001; Chastel et al. 2005) but is in contrast to adrenocortical suppression observed in passerines (Astheimer et al. 1994; Wingfield et al. 1995). The difference is likely related to life-history strategies and linked to the relative costs and benefits of a single reproductive attempt (Stearns 1992). In long-lived species, a single breeding attempt is of lesser relative value; hence, overall fitness is likely maximized by maintaining a robust stress response and by abandoning reproduction if adult survivorship will be severely compromised (reviewed in Wingfield and Sapolsky 2003). In shorter-lived species with relatively lower chances of surviving from one breeding season to the next, this relationship is reversed and the high potential value of each reproductive attempt might support adrenocortical suppression and a strategy which accepts high costs of reproduction (Wingfield et al. 1995; Wingfield and Sapolsky 2003).

2.5.2 BODY CONDITION

The absence of a relationship between body condition during the incubation period and previous breeding decision appears to be in contrast to several studies of birds in which body condition at the beginning of the breeding season is positively correlated to both initiation and success of reproduction (Ankney and Afton 1988; Moe et al. 2002; Bleeker et al. 2005). These results suggest that the relationship between endogenous energy (primarily lipid) stores and initiation of reproductive behavior is species-specific and that, in red-legged kittiwakes, the relative amount of energy stores during the incubation period is not associated with reproductive status. Additionally, though the lack of detectable body condition decline associated with a reproductive attempt is in accord with some seabird studies (e.g. Wernham and Bryant 1998), it is in contrast to several others (Golet and Irons 1998; Kitaysky et al. 1999; Moe et al. 2002; Shultz 2006, in prep). Taken together, our results suggest that the amount of endogenous energy reserves is variable even among individual red-legged kittiwakes of the same breeding status, and, because of this variability, this measure does not reliably reflect reproductive status or accurately indicate physiological consequences of reproduction. Interestingly, Shultz (2006 in prep) found that body condition (along with Cort concentrations) was a reliable predictor of black-legged kittiwake productivity in a study that included the St George colony. The difference between these two closely related species might be related to their different foraging strategies. Specifically, forage trip duration is typically much longer in red-legged kittiwakes (Dempsey and Shultz, unpublished data), leading to prolonged nest attendance bouts by one adult while its partner forages. This pattern could lead to greater fluctuations in body condition, as some birds would likely be sampled shortly after returning to the colony (presumably after accumulating lipid stores) while others would be sampled near the end of extended nest attendance bouts (presumably after depleting their lipid stores). The potential effect of the duration of nest attendance on body condition remains to be tested in this species.

2.5.3 ECTOPARASITE LOAD

The higher ectoparasite load of breeders relative to non-breeders during the incubation period and the similarity between the groups during the rearing period suggest that reproductive behavior (e.g. time spent at nest during incubation bouts and reduced preening) rather than immune system suppression determines ectoparasite load. Alternately, this finding could result from the higher abundance of ectoparasites in breeders' fully-formed nests than non-breeders' often incomplete or deteriorating nests (Dempsey pers. obs.). Had reproduction itself induced a suppression of the immune response and reduced parasite resistance, we would have expected higher ectoparasite loads during both reproductive stages. Thus, we believe that this factor is more likely to be a consequence of reproductive behavior rather than a factor influencing the breeding decision. Similar positive relationships between ectoparasite load and reproduction have been reported previously in other avian species (Norris et al. 1994; Richner et al. 1995; Oppliger et al. 1996; Nordling 1998). The effects of this increased parasitism on the survival and future reproduction of adult red-legged kittiwakes are currently unknown.

2.5.4 ISOTOPIC ANALYSIS

Based upon previous studies linking adult diet composition and various measures of reproductive success in seabirds (Bolton et al. 1992; Bolton et al. 1993; Abraham and

Sydeman 2004), we expected that diet composition during pre-laying or early-incubation periods (as indicated by the isotopic signatures during the incubation period) might influence the breeding decision. Yet, our results for peak Cort provide no evidence that foraging ecology or diet composition of attending kittiwakes prior to or near the time of their arrival at the colony affects breeding decision. As we analyzed isotopic signatures in breeders and non-breeders only during the incubation period, we are unable to compare the diet composition of these groups during the chick-rearing stage, though such differences could exist. Furthermore, the foraging ecology of this species during winter is not well known (Byrd and Williams 1993), and it is possible that differences in winter diet composition affect the decision to attend the colony and initiate reproduction.

2.6 CONCLUSIONS

Our results indicate that breeding red-legged kittiwakes incur physiological costs during both incubation (higher parasite load in male breeders) and chick-rearing (elevated Total and Free Cort). However, these findings do not provide clear evidence of a relationship between physiological condition or foraging ecology and breeding decision in this species. Admittedly, this study does not preclude the possibility that the breeding decision is, in fact, based on the early- season physiological condition of adults. There are several possibilities which might explain our inability to detect a physiological basis, if one indeed exists.

First, we were only able to sample individuals that attended the colony and maintained nest sites: these individuals might represent only one portion of a wider range of condition. It is possible that the breeding decision is, in fact, based on components of the stress response, body condition, immune function, and/or foraging ecology; but that these factors actually influence the earlier decision to attend the colony and establish/defend a territory rather than the subsequent female decision to lay an egg. Also, as we were not able to sample during the pre-laying or laying stages, we based our predictions on the untested assumption that an individual's condition during the incubation period was tightly linked to that individual's condition during earlier reproductive stages. It is, therefore, possible that breeders and non-breeders differed in condition during these prior stages, but that these differences were quickly negated during early incubation.

Second, we assumed that all nests that did not contain an egg or chick during the rearing period had never previously contained an egg or chick. We believe that this assumption is largely supported due to the scarcity of avian predators at this colony, and inaccessibility of sampled nests to fox predation. However, it is possible that a fraction of the birds measured during the rearing period, which we refer to here as 'non-breeders', were actually individuals that had produced and lost eggs that year.

Despite these considerations, our study suggests that the breeding decision might be based on factors other than the physiological condition of adults. One possible explanation is that adult quality drives the breeding decision in kittiwakes. Research on black-legged kittiwakes (*Rissa tridactyla*) indicates that certain individuals are more likely to survive and reproduce in sequential years than others; in other words, better quality birds live longer and reproduce more often compared to inferior individuals (Cam et al. 1998). If similar heterogeneity exists in red-legged kittiwakes, it is possible that superior individuals initiate reproduction regardless of their current physiological condition. Such differences in quality might overwhelm or disguise tradeoffs (e.g. current reproduction vs. survival) that are thought to be central to life-history theory. Finally, these differences in quality might be most obvious during years of challenging conditions (as in 2005 at St George; Thomson 2005) that negatively affect all potential breeders but which only some are able to overcome.

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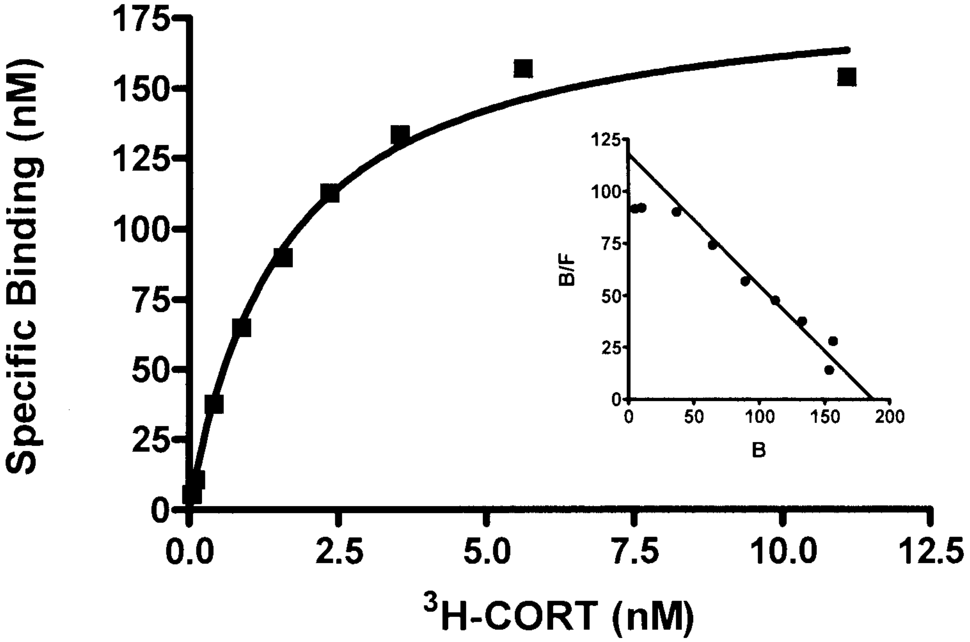

Figure 2.1 Equilibrium saturation binding curve showing specific binding of [^{3}H]CORT to red-legged kittiwake plasma (4°C) associated with increasing concentrations of [^{3}H]CORT (means ± SE). The inlay is a Scatchard–Rosenthal plot of the data.

Table 2.1: Baseline and peak Total and Free Cort concentrations, CBG levels, body condition and ectoparasite load of breeding and non-breeding adult red-legged kittiwakes during the incubation and chick-rearing periods on St George Island (2005).

Breeding Stage	**Factor**	**Reproductive Status**		
		Breeder	P value	Non-breeder
Incubation	Baseline Total Cort (ng/ml)	2.17 ± 0.45	NS	1.55 ± 0.15
	Baseline Free Cort (ng/ml)	0.02 ± 0.01	NS	0.02 ± 0.00
	Peak Total Cort (ng/ml)	27.97 ± 2.95	NS	26.24 ± 1.74
	Peak Free Cort (ng/ml)	0.52 ± 0.10	NS	0.50 ± 0.09
	CBG (nM)	174.31 ± 8.77	NS	174.54 ± 15.48
	Body Condition Index	1.8 ± 0.0	NS	1.7 ± 0.1
	Ectoparasite Load	13.5 ± 5.5	$P < 0.003$	2.0 ± 1.3
Chick-rearing	Baseline Total Cort (ng/ml)	6.01 ± 1.07	$P < 0.0001$	2.35 ± 0.41
	Baseline Free Cort (ng/ml)	0.07 ± 0.02	$P < 0.0001$	0.02 ± 0.00
	Peak Total Cort (ng/ml)	40.39 ± 1.24	NS	36.48 ± 1.46
	Peak Free Cort (ng/ml)	1.74 ± 0.41	$P < 0.010$	0.81 ± 0.21
	CBG (nM)	162.05 ± 9.83	NS	200.07 ± 18.52
	Body Condition Index	1.9 ± 0.1	NS	1.8 ± 0.0
	Ectoparasite Load	3.1 ± 0.6	NS	0.8 ± 0.4

Note: Values shown as means ± SE. NS indicates statistical non-significance

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CHAPTER 3 PROLONGED DIETARY RESTRICTION AFFECTS CORTICOSTEROID-BINDING GLOBULIN LEVELS IN CAPTIVE RED-LEGGED KITTIWAKE CHICKS*

3.1 Abstract: Nest-bound seabird chicks routinely experience nutritional stress if parents are unable or unwilling to provision them adequately. In some species, increased plasma corticosterone (Cort) during short periods of food limitation facilitates behavioral and physiological changes, maintaining positive energy balance. Despite apparent benefits of this response, prolonged exposure to elevated Cort has physiological and fitness costs. One mechanism which might mitigate these deleterious effects is modulation of corticosteroid-binding globulin levels (CBG), a protein that binds Cort with high affinity and renders the bound hormone less biologically available for both tissue receptors and metabolic clearance. We examined effects of long-term nutritional restriction (limited energy intake; lipid and/or protein limitation) on the dynamics of CBG and Free 'unbound' Cort in captive red-legged kittiwake chicks (*Rissa brevirostris*). We found no ontogenetic effects on CBG or Free Cort. Free Cort dynamics paralleled the previously reported Total Cort response to food restriction. We also found that, relative to controls, protein-restricted chicks (but not lipid-restricted chicks) reduced CBG. This suggests that chicks might adjust CBG in response to protein limitation, possibly by reducing synthesis and/ or increasing metabolism of endogenous proteins. Our results indicate kittiwake chicks do not mitigate deleterious effects of chronic Cort exposure via CBG modulation.

Key words: corticosterone, CBG, food stress, seabird chicks

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3.2 INTRODUCTION

Nest-bound seabird chicks routinely experience periods of nutritional stress if parents are either unable or unwilling to provision them adequately due to factors including fluctuations in food resources, inclement weather, or altered nutritional requirements (Takahashi et al. 1999; Kitaysky et al. 2000). Substantial interspecific variation exists in the adrenocortical response of chicks to these periods of nutritional stress during development. Precocial and semi-precocial chicks increase plasma levels of corticosterone (Cort), the primary avian glucocorticoid (Holmes and Phillips 1976), in response to nutritional limitations (Nunez-de la Mora et al. 1996; Kitaysky 1999; Kitaysky et al. 1999, 2001a). Elevated Cort levels in both adults and chicks facilitate behavioral and physiological changes, presumably aimed at maintaining positive energy balance over short periods (Wingfield et al. 1997; reviewed in Wingfield et al. 1998; Boonstra 2004). Specifically, elevated Cort results in increased protein catabolism (Cherel et al. 1992; Le Ninan et al. 1988), gluconeogenesis (DeFronzo et al. 1980; Remage-Healey and Romero 2001) lipogenesis (Gray et al. 1990), and, in some seabirds, increased chick begging behavior (Kitaysky et al. 2001b; Quillfeldt et al. in press) and increased adult and chick feeding rates (Koch et al. 2002; Vallarino et al. 2006).

Despite apparent benefits of the stress response, prolonged exposure to elevated corticosterone in chicks and adults is associated with costs (reviewed in Sapolsky et al. 2000) including reduced growth efficiency (Kitaysky et al. 2003), reduced immune defense efficiency (Barriga et al. 2001), neurological damage (Sapolsky 1987), muscle deterioration (Harvey et al. 1984; Gray et al. 1990). These costs are particularly severe for individuals that experience nutritional stress during development; they can suffer retarded growth, higher degrees of fluctuating asymmetry (Pravosudov and Kitaysky 2006), enduring cognitive deficiency (Kitaysky et al. 2003; Kitaysky et al. 2006; Pravosudov et al. 2005; Pravosudov and Kitaysky 2006), and decreased lifelong fitness (Nowicki et al. 2000; van der Ziel and Visser 2001). Hence, the adrenocortical response must be precisely regulated to allow an individual to cope with a perceived stressor while minimizing the associated costs.

Possibly, seabird chicks could mitigate the deleterious effects of elevated Cort by modulating levels of corticosteroid-binding globulin (CBG), a protein that binds Cort with high affinity and renders it less available for most target tissue receptors (Hammond 1995; Breuner and Orchinik 2001a) and for metabolic clearance (Bright 1995). According to the Free Hormone Hypothesis, it is the unbound 'free' fraction of hormone that is most biologically active and the most physiologically relevant (Mendel 1989). Though this hypothesis does not specify a mechanism for CBG action, CBG decreases receptor binding of Cort as well as increasing plasma storage and localized redistribution of bound hormone (for review of CBG function see Breuner and Orchinik 2002).

Supporting the Free Hormone Hypothesis, recent studies have shown that avian CBG levels vary independently of Total Cort levels on several temporal scales (Breuner and Orchinik 2001a) and in response to a number of perceived stressors (Breuner and Hahn 2003; Lynn et al. 2003; Love et al. 2004) such that Total and Free Cort may not be tightly coupled. Lynn et al. (2003) showed that specific binding of CBG decreased in fasting birds relative to controls after only 22 h, leading to significantly higher Free Cort levels in fasted birds despite Total Cort concentrations similar to controls. CBG levels also might respond differently to specific nutritional limitations. For instance, decreased CBG levels might result from reduced protein synthesis observed in birds fed protein-restricted diets during development (MacDonald and Swick 1981). However, currently the regulation and respective roles of Total Cort, CBG and Free Cort are not well understood for developing chicks. It is unknown whether food stress affects CBG and Free Cort levels of developing semi-precocial chicks and, if so, what nutritional factors (e.g. restriction of energy, protein or lipid) influence this response.

We examined the effects of long-term dietary restriction on CBG and Free Cort concentrations in red-legged kittiwake chicks (*Rissa brevirostris*). Previously, Kitaysky et al. (2001a) found that captive red-legged kittiwake chicks exposed to restrictions in energy content and/or nutritional quality (high vs. low-lipid content) had elevated baseline and stress-induced Total Cort levels relative to chicks fed *ad libitum* rations. Subsequently, within 3 weeks of the onset of restriction, chicks fed low-lipid diets showed a reversal, to reduced baseline and stress-induced Total Cort levels. The authors suggested this reduction in Cort represents a protective mechanism to avoid deleterious effects of chronic Cort exposure. However, these authors proposed no specific mechanism to explain the reduction in Cort concentrations. Here, we investigate 1) the ontogenetic development of two components of the avian response to stress (CBG, Free Cort) and 2) the effect of nutritional limitation on CBG levels. Specifically, we are interested in whether CBG dynamics could account for the Cort reduction observed in chronically food-stressed chicks by Kitaysky et al. (2001a) and whether CBG levels might be sensitive to diet composition (relative protein, lipid content) as well as daily energy intake.

To address these questions, we assigned captive chicks to one of four dietary treatments: 1) controls not limited in lipid, protein, or energy content 2) limited in lipid, protein, and energy content; 3) limited in lipid and energy but not protein content; and 4) limited in protein and energy but not lipid content. Importantly, the latter two treatments were iso-caloric and allow us to compare the effects of lipid and protein restriction on CBG and Free Cort. Such comparisons are necessary, because red-legged kittiwake chicks are sensitive to diet composition as well as overall energetic intake. Specifically, Kitaysky et al. (2001a) showed that diet composition influenced chicks' Total Cort response to food stress, and Kitaysky et al. (2006) showed that the cognition of lipid-restricted chicks was inferior to that of protein-restricted chicks fed iso-caloric diets of high- and low-lipid fish. If CBG capacity mitigates the effects of nutritional stress, we would expect CBG dynamics be sensitive to both energy intake and diet composition.

If chicks adjust CBG levels to maximize Cort clearance (explaining the previously reported Cort reduction in chronically stressed chicks), we expect energy-limited chicks will lower CBG levels compared to controls. Alternatively, if chicks adjust CBG either to maximize Cort binding and avoid harmful effects of chronic Free Cort exposure or to maximize Cort storage, we expect energy-limited chicks might increase CBG compared to controls (though this would not explain the observed Cort reduction in chronically stressed chicks). We also predicted that protein limitation would reduce CBG concentrations. This prediction was based on earlier studies of both avian and mammalian species which showed decreased synthesis of endogenous proteins during periods of dietary protein limitation (Young et al. 1971; MacDonald and Swick 1981; Pacy et al. 1994).

3.3 MATERIALS AND METHODS

3.3.1 ANIMALS AND EXPERIMENTAL DESIGN

We collected twenty partially-incubated red-legged kittiwake eggs from St George Island, Alaska in the southeastern Bering Sea (56°36'20" North, 169°33'35" West). We transported the eggs to animal care facilities at the University of Washington in Seattle, Washington, and continued incubation at 37.2°C and 56% relative humidity. When eggs hatched, we placed chicks in individual nest boxes at 30°C and 80% relative humidity until 10 days post-hatch, then at 25°C and 70% relative humidity from 10-20 days post-hatch, and, finally, at outdoor ambient temperatures and humidity through fledging (42-55 days post-hatch). All chicks were kept in the same room in physical, but not in visual or acoustic, isolation from each other. Prior to the experiment, we fed all chicks a high-quality mixed-species diet of forage fish diet *ad libitum*.

At 15 days post-hatch, we assigned chicks to each of four diet treatments (n = 5 individuals per treatment) maintained over 21 days: 50 g day^{-1} low-lipid (LL), 80 g day^{-1} LL, 50 g day^{-1} high-lipid (HL), 80 g day^{-1} HL. The 'low-lipid diet' (LL) was rainbow smelt, *Osmerus mordax* (lipid to protein ratio, LPR = 0.61), and the 'high-lipid diet' (HL) was silverside, *Menidia menidia* (LPR = 1.47). For full analysis and description of the energy content of each diet treatment see Table 1, Kitaysky et al. (2001a). Briefly, the 80 g day^{-1} LL and the 50 g day^{-1} HL diets were iso-caloric, and the respective treatments were equivalent to ~40%, 65%, and 100% of *ad libitum* rations (Table 3.1). Therefore, chicks fed 50 g day^{-1} LL were limited in both lipid and protein content; chick fed 80 g day^{-1} LL were limited in lipid but not in protein; chicks fed 50 g day^{-1} HL were limited in protein but not in lipid; and control chicks (fed 80 g day^{-1} LL) were not limited in either lipid or protein. To avoid any effects of nutritional restriction unrelated to energy, protein, or lipid content, each chick received a multi-vitamin/mineral supplement daily (one tablet USP quality Kirkland Signature).

After 21 days, caloric restriction ended, and we fed all chicks *ad libitum* rations of their treatment (LL or HL) diets until 47 days post-hatch. At that time, we switched all chicks maintained on LL diet to the HL diet and continued to feed all chicks *ad libitum* rations of HL diet.

3.3.2 PLASMA COLLECTION AND PREPARATION

We collected baseline (<3 min after initial disturbance) blood samples from the alar vein of post-absorptive (following overnight fast) chicks using 100-μl heparinized hematocrit tubes. We sampled chicks prior to dietary restriction (15 days post-hatch), after two and three weeks of restriction (29 and 36 days post-hatch, respectively), and two weeks after the end of restriction (49 days post-hatch).

Following each sample collection, we staunched bleeding with cotton, transferred blood into 0.5-ml vials, and stored samples at 4°C. Within 6 hours, we centrifuged the blood, collected plasma, and stored the plasma frozen at -20 °C until analysis.

3.3.3 CORTICOSTEROID-BINDING GLOBULIN ASSAY

We measured plasma CBG in a single assay according to established protocols (Breuner and Orchinik 2002; Love et al. 2004). Because acute stress handling protocols were shown by Lynn et al. (2003) to affect CBG levels in white-crowned sparrows (*Zonotrichia leucophrys gambelii*), we tested for similar effects in red-legged kittiwakes. We found no effect of acute handling stress on CBG levels in plasma collected <3, 10, or 50 minutes following capture (Dempsey, unpublished data). Therefore, we based the decision of which sub-sample to assay on the volume of available plasma.

Based on data from earlier validation assays, we determined optimal incubation time (2 hr), final plasma concentration (1:450), and Cort affinity (Kd = 1.585; Figure 3.1). We stripped endogenous Cort from plasma by incubating each 10 μl sample with 20 μl dextran-coated charcoal solution for 20 min at room temperature prior to centrifuging for 10 min at 4000 rpm, 4°C. We conducted the assay in triplicate at 4°C and simultaneously determined non-specific binding (in duplicate) using unlabeled Cort. Following incubation, we separated bound and free fractions of labeled Cort using glass fiber filters (Whatman GF/B) pre-soaked for 1 h in 25 nM Tris with 0.3% PEI. We suspended filters containing bound Cort in scintillation liquid and measured radioactivity after 12 h. We calculated intra-assay variation (1.96%) using 4 repeated point sample determinations (adjusted CBG capacity nM) of a single plasma pool.

3.3.4 FREE CORT ESTIMATES

We used Cort concentrations previously reported by Kitaysky et al. (2001a) for determination of Free Cort in this study. In brief, we measured Total plasma Cort concentrations for all samples via a single radioimmunoassay using 20 μl plasma (for details see Wingfield and Farner 1975; Wingfield et al. 1992). Samples were analyzed in duplicate and final assay values were corrected for individual recoveries following extraction (recoveries ranged from 76% to 100 %; mean 89%). The intra-assay coefficient of variation was 5%. Sensitivity of this assay was 7.8 pg/ tube.

Using the Barsano and Baumann (1989) equation (below), we estimated concentrations of Free Cort for each sample based on Total Cort levels and binding capacity:

$$Hfree = 0.5 \times \left[Htotal - B\max - \frac{1}{Ka} \pm \sqrt{\left(B\max - Htotal + \frac{1}{Ka} \right)^2 + 4\left(\frac{Htotal}{Ka} \right)} \right]$$

Total Cort and CBG were measured in the same plasma samples collected at the end of restriction and two weeks after the end of restriction. Samples used for CBG analysis prior to dietary restriction and after 15 days of dietary restriction were collected one day after those used to determine Total Cort levels. We did not expect that Total

Cort levels changed significantly in chicks over this 24 h period; therefore, we do not believe that this biases our estimates of Free Cort levels.

3.3.5 STATISTICAL ANALYSES

We examined the effects of experimental treatment on CBG capacity and Free Cort with repeated measures two-way ANOVA, where dietary restriction treatment and duration of restriction were used as factors. We examined the effect of age on CBG capacity and Free Cort of control chicks with repeated measures two-way ANOVA including age as a covariate. For subsequent planned comparisons, we used LSD statistical tests.

Prior to analysis, we initially tested data for assumptions associated with parametric tests (Sokal and Rohlf 1981). In cases where data violated assumptions, we employed $\log_{10}$-transformations and re-examined the data. In all cases where transformation was necessary, the transformed data met assumptions for parametric tests. We performed all analyses using the STATISTICA statistical package (StatSoft, Inc. Tulsa, USA) and set $\alpha = 0.05$.

3.4 RESULTS

3.4.1 DEVELOPMENTAL EFFECTS

For control chicks fed 80 g day^{-1} HL, there was no significant effect of age on CBG (age $F_{3,16} = 0.290$, $P = 0.83$; Figure 3.2), or Free Cort levels (age $F_{3,16} = 0.920$, $P = 0.45$; Figure 3.2).

3.4.2 RESPONSES TO LONG-TERM DIETARY RESTRICTIONS

3.4.2.1 CORTICOSTEROID-BINDING GLOBULIN LEVELS

There was no significant overall effect of dietary restriction treatment or duration on CBG capacity; however, there was a significant treatment-duration interaction term (treatment $F_{3,12} = 0.600$ $P = 0.62$; duration $F_{2,30} = 2.126$, $P = 0.14$; treatment*duration interaction term $F_{6,30} = 2.615$, $P < 0.04$; Figure 3.2). We found no significant difference between controls and chicks fed 80 g day^{-1} LL after 14 days of restriction ($P = 0.54$) or after 21 days of restriction ($P = 0.07$). However, chicks fed 50 g day^{-1} HL, though similar to controls after 14 days of restriction ($P = 0.90$), had significantly lower CBG levels after 21 days of restriction ($P < .02$). Additionally, relative to controls, chicks fed 50 g day^{-1} LL had marginally lower CBG levels after 14 days of restriction ($P = 0.05$) and significantly lower levels after 21 days of restriction ($P < 0.001$).

3.4.2.2 FREE CORT LEVELS

For calculation of Free Cort levels, we used Total Cort data previously reported in Kitaysky et al. (2001a). Briefly, there were significant effects of dietary restriction treatment and duration on Total Cort levels (Figure 3.2). Prior to restriction, there was no significant difference between chicks later assigned to control group and those assigned to any of the food-limited treatments (50 g day-1 LL, $P = 0.94$; 80 g day-1 LL, $P = 0.71$; 50 g day-1 HL, $P = 0.99$). After 14 days of restriction, there was no significant difference between controls and chicks fed 50 g day^{-1} HL; however chicks fed either 50 g day^{-1} LL or 80 g day^{-1} LL had significantly elevated Total Cort levels. After 21 days of restriction, Total Cort levels were similar to controls for chicks fed 50 g day^{-1} HL or 80 g day^{-1} LL, while chicks fed 50 g day^{-1} LL maintained elevated Total Cort.

There was no significant difference in Free Cort levels between controls and any of the food-limited treatments prior to restriction (50 g day-1 LL, $P = 0.94$; 80 g day-1 LL, $P = 0.70$; 50 g day-1 HL, $P = 0.53$). There were highly significant but complex effects of dietary restriction treatment and duration on Free Cort levels (treatment $F_{3, 15} = 8.900$, $P < 0.001$; duration $F_{2, 30} = 10.860$, $P < 0.001$; treatment*duration interaction term $F_{6, 30} = 4.277$, $P < 0.01$; Figure 3.2). Similar to results for Total Cort after 14 days of restriction, we found no significant difference between controls and chicks fed 50 g day^{-1} HL ($P = 0.341$); however, chicks fed either 50 g day^{-1} LL or 80 g day^{-1} LL had significantly elevated Free Cort levels (50 g day-1 LL, $P < 0.0001$; 80 g day-1 LL, $P < 0.0001$). After 21 days of restriction, Free Cort levels of control chicks were similar to those of chicks fed 50 g day^{-1} HL ($P = 0.18$) or 80 g day^{-1} LL ($P = 0.16$), while chicks fed 50 g day^{-1} LL maintained elevated Free Cort ($P < 0.01$).

There were no significant differences in CBG or Free Cort levels among treatments in samples collected 4 weeks after the restriction period (CBG, P = 0.63; Total Cort, P = 0.94; Free Cort, P = 0.88; Figure 3.2).

3.5 DISCUSSION

We found no ontogenetic effect on Cort or CBG levels and no overall effect of restriction treatment or duration on CBG levels. Though we expected that chronically food stressed chicks might adjust CBG levels either to increase Cort clearance or Cort binding, we found no evidence that chicks buffered themselves from the deleterious effects of exposure to free glucocorticoids in this way. In short, Free Cort dynamics in all treatments paralleled those of Total Cort. However, as we predicted, protein-restricted chicks had lower CBG levels than controls during the period of restriction.

3.5.1 ENDOCRINE DEVELOPMENT IN UNSTRESSED CHICKS

Substantial interspecific variation exists in the adrenocortical response of chicks to nutritional stress. According to the Developmental Hypothesis, these differences in the development of the adrenocortical response are largely explained by the species' life-history strategy (Sims and Holberton 2000; Kitaysky et al. 2003; Blas et al. 2006). Specifically, because of the differing abilities of dependent and independent chicks to respond behaviorally to overcome challenges, the Developmental Hypothesis predicts an inverse relationship between the degree of nestling dependence on parental care and the robustness of their adrenocortical response to stressors. Altricial and semi-altricial chicks, which are entirely reliant on their parents for provisioning while in the nest, should suppress the adrenocortical response during development (with robust response developing close to fledging). In contrast, precocial and semi-precocial chicks, presumably better able to overcome challenges behaviorally (e.g. increased begging, siblicide), should show a robust stress response at or very soon after hatching.

This hypothesis is supported by a number of recent studies demonstrating the suppression and/or delayed development of adrenocortical function in altricial and semi-altricial species (Schwabl 1999; Sims and Holberton 2000; Sockman and Schwabl 2001; Love et al. 2003; but see Pravosudov and Kitaysky 2006) and the robust early-life adrenocortical response of precocial or semi-precocial chicks (Freeman et al. 1981; Holmes et al. 1990; Nunez-de la Mora et al. 1996; Kitaysky et al. 1999, 2001a). Also, several studies have found that baseline Total Cort levels of nest-bound chicks increase with developmental age, possibly to facilitate fledging (Heath and Dufty 1998; Walker et al. 2005; Kitaysky et al. 2005; Blas et al. 2006).

Here, we found no effect of developmental age on the CBG or Free Cort levels in *ad libitum* fed captive red-legged kittiwake chicks. These results are similar to those from a previous study of chicks of the closely related black-legged kittiwake (*Rissa tridactyla*) which also detected no ontogenetic changes in baseline Total Cort (Kitaysky et al. 1999). We also found that, by 4 wks old, food-limited red-legged kittiwake chicks are capable of modulating CBG levels in response to prolonged nutritional stress. This result, along with the previously reported ability to modulate Cort at this age (Kitaysky et al. 2001a), indicates either that kittiwakes hatch with a robust physiological stress response or that its development is well-advanced within a 4 weeks of hatching.

3.5.2 RESPONSES TO LONG-TERM DIETARY RESTRICTIONS

3.5.2.1 CORTICOSTEROID-BINDING GLOBULIN LEVELS

Our results suggest that moderate protein restriction, rather than daily energy intake or lipid restriction, drives changes in CBG levels (Table 3.1). The evidence for this is the difference in CBG levels between chicks in the two iso-caloric treatments, 80 g day^{-1} LL and 50 g day^{-1} HL. Relative to controls, the protein-rich group showed no significant reduction in CBG levels, whereas the protein-limited group showed reduced CBG levels. Previous studies of both birds and mammals exposed to dietary protein restriction have shown that such limitations result in decreased endogenous protein synthesis (Liu et al. 1995; MacDonald and Swick 1981; Pacy et al. 1994; Young et al. 1971) and/or increased protein degradation (Millward et al. 1976). Though the pattern of decreased protein synthesis during dietary protein-limitation is robust, the effect of protein-limitation on degradation of endogenous proteins appears more complex. In some instances, severe protein-limitation results in decreased breakdown and, consequently, decreased protein turnover (Liu et al. 1995; MacDonald and Swick 1981); yet more moderate restrictions result in increased degradation (Millward et al. 1976; Pacy et al. 1994). Given the moderate protein limitations used in this study, both reduced synthesis and increased degradation appear to be plausible mechanisms for CBG reduction. The reduction of CBG might also be mediated by Cort, as elevated Cort levels have been shown to increase protein catabolism (Cherel et al. 1992; Le Ninan et al. 1988). Further research is needed to evaluate the relative contribution of these mechanisms.

3.5.2.2 TOTAL CORT AND FREE CORT LEVELS

Total Cort and Free Cort showed similar patterns as chicks in both low-lipid treatments had significantly elevated levels after 14 days of restriction, though chicks in the 50 g day^{-1} HL group did not differ from controls. By 21 days of restriction, chicks fed either 50 g day^{-1} HL or 80 g day^{-1} LL showed similar Cort levels to controls, while the Cort levels of the most restricted group (50 g day^{-1} LL) remained elevated. This pattern suggests that Free Cort levels in chronically stressed seabird chicks are not independent of Total Cort levels, and that the response of both are driven by nutritional restriction and exacerbated by lipid-poor diet (Table 3.1).

These results provide further evidence of reduced Cort secretion (Total and Free Cort) within 3 weeks in chicks exposed to severe or intermediate levels of dietary restriction (50 g day^{-1} LL, 80 g day^{-1} LL). We found no evidence of a pattern similar to that reported by Lynn et al. (2003) in which Total Cort levels decreased rapidly following the onset of short-term stress while Free Cort levels remained elevated. However, this study was not designed to detect the short-term fluctuations of CBG or Cort, and we can not rule out the existence of such patterns on finer temporal scales.

3.6 CONCLUSIONS

We found no evidence to indicate that changes in CBG levels are the mechanism responsible for previously reported Cort reductions in kittiwake chicks (Kitaysky et al. 2001a). Here, we suggest that observed decreases in CBG levels are likely due to protein limitation rather than modulation of CBG levels for the purpose of Cort clearance or for maximization of Free Cort. This conclusion is based on the increased protein catabolism resulting from elevated Cort (Cherel et al. 1992; Le Ninan et al. 1988); the reduced synthesis of endogenous proteins found in previous studies where chicks were fed protein-limited diets (e.g. MacDonald and Swick 1981); and two key findings of this study: 1) that of the two treatments which showed significant reduction of the Cort response, only one (50 g day^{-1} LL) showed CBG reduction and 2) that the 50 g day^{-1} HL showed a reduction in CBG levels despite no subsequent Cort reduction.

Moreover, if the function of CBG modulation is to protect chicks from the deleterious effects associated with prolonged Cort exposure, we would expect chicks that altered CBG levels to show superior cognitive performance following the temporary dietary restriction. However, when Kitaysky et al. (2006) tested the red-legged chicks used in this study for lasting cognitive impairment, they found that only chicks fed high-lipid diets demonstrated the ability to learn and perform simple associative tasks (Table 3.1). Thus, the simultaneous decrease in CBG and Cort observed in the 50 g day^{-1} LL treatment was unsuccessful in avoiding cognitive impairment.

Other potential mechanisms by which an individual might reduce circulating corticosterone associated with chronic food stress are via reduced sensitivity of the pituitary gland to releasing factors from the hypothalamus and/ or via reduced sensitivity of the adrenal gland to adrenocorticotropic hormone (ACTH) from the pituitary (Romero et al. 1998). If chronic stress decreased pituitary or adrenal sensitivity to releasing factors, this could explain decreased Cort concentrations during prolonged food restriction. Additionally, increased Cort clearance resulting from processes unrelated to CBG dynamics might also explain the reduction in Cort concentrations observed by Kitaysky et al. (2001a). Finally, it is important to note that in this study we did not measure changes to the density or distribution of target receptors. It is possible that elevated levels of Total and Free Cort might be less physiologically relevant when compared to altered receptor properties (Breuner and Orchinik 2001a, 2001b).

In conclusion, our findings do not support the previously suggested hypothesis that exposure to free glucocorticoids is modulated by CBG. However, this study does provide evidence that CBG dynamics are dependent, at least in part, on diet composition and further underscores the sensitivity of developing chicks to changing foraging conditions.

3.7 ACKNOWLEDGEMENTS

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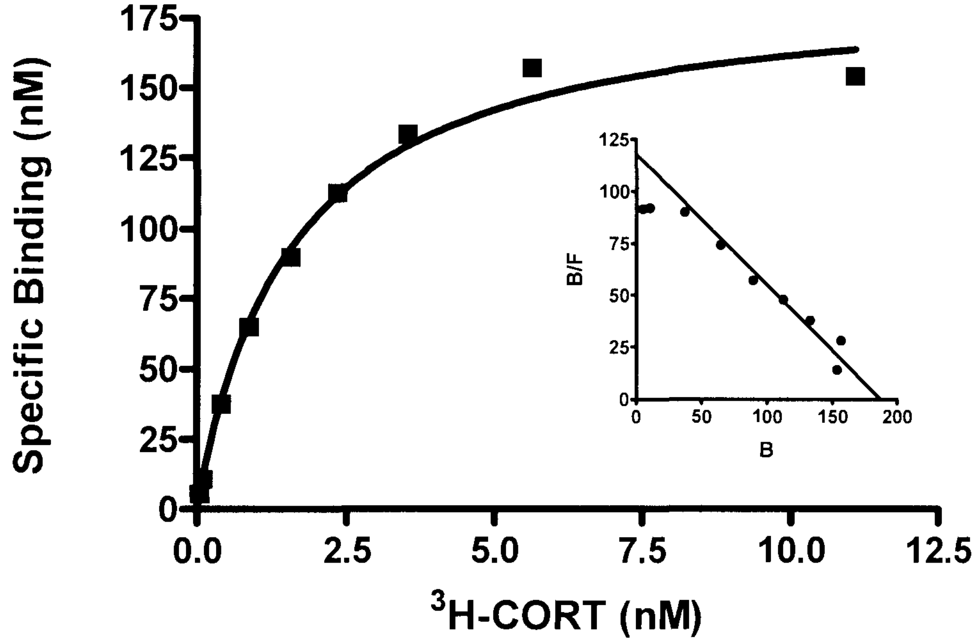

Figure 3.1 Equilibrium saturation binding curve showing specific binding of [^{3}H]Cort to red-legged kittiwake plasma (4°C) associated with increasing concentrations of [^{3}H]Cort (means ± SE). The inlay is a Scatchard–Rosenthal plot of the data.

Figure 3.2 Baseline total corticosterone (*upper panel;* re-drawn from Kitaysky et al. 2001a with permission), corticosteroid binding globulin (*middle panel*) and free corticosterone (*lower* panel) levels (means ± SE) of red-legged kittiwake chicks in relations to their diet treatment (50 or 80 g d^{-1}; LL = low-lipid diet LPR 0.61, HL = high-lipid diet LPR 1.47) and duration of restriction (prior to restriction; 2 wks into restriction; 3 wks into restriction; 2 wks after end of restriction). The dark bar below indicates the period of restriction. Letters over columns indicate significant differences only within a sampling period (P < 0.05).

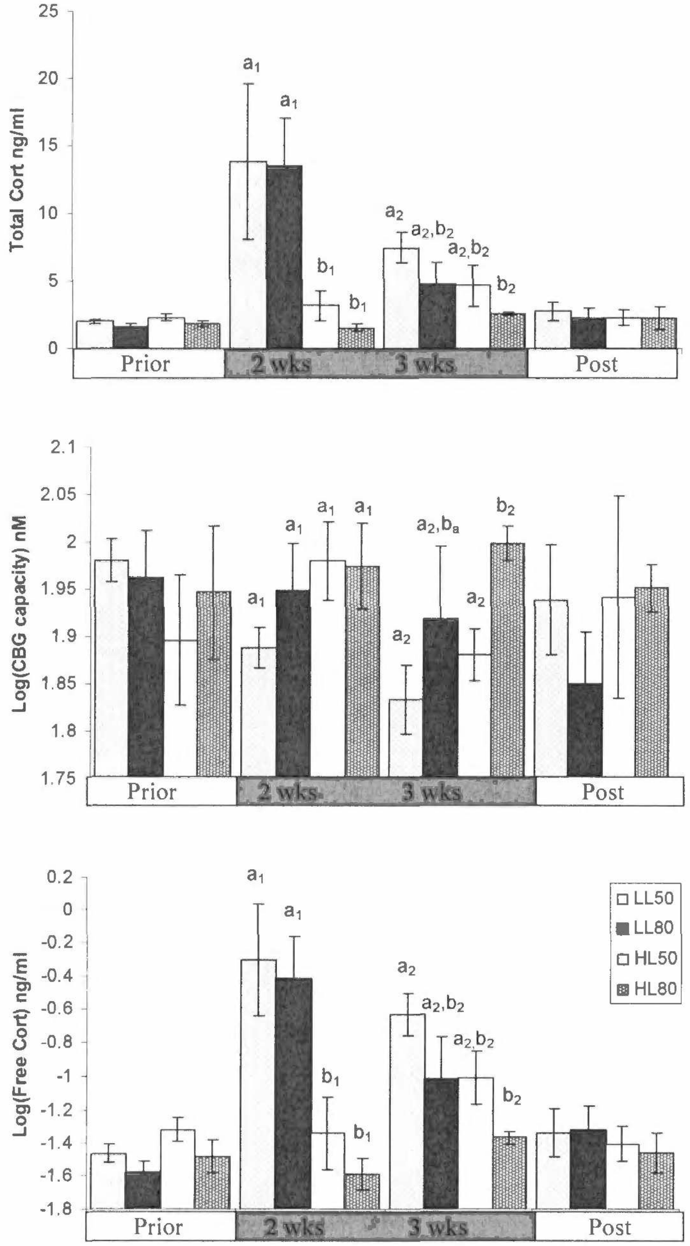

Table 3.1 Daily total energy and protein intake; detectable adrenocortical suppression; CBG dynamics; and enduring cognitive impairment in red-legged kittiwake chicks exposed to four nutritional treatments (50 or 80 g day-1 of low-lipid or high-lipid forage fish diet).

diet treatment	energy intake ($kJ\ d^{-1}$)	protein intake[a] ($g\ d^{-1}$)	adrenocortical[b] suppression	CBG	cognitive[c] impairment
50 g day^{-1} LL	222.7	7.8	yes	↓	severe
80 g day-1 LL	356.3	12.4	yes	no Δ	severe
50 g day-1 HL	353.2	8.0	no	↓	mild
80 g day-1 HL	565.1	12.9	no	no Δ	none

a estimate based on Schmidt-Nielsen (1997) value for uric acid (17.8 kJ/g)
b after three weeks of dietary treatments (Kitaysky et al. 2001a).
c Kitaysky et al. 2006

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GENERAL CONCLUSIONS

The goal of this study was to determine to what extent and by what mechanisms avian reproductive performance is related to stress physiology. Specifically, I asked whether variation in reproductive success or the physiological consequences of reproduction are associated with components of the stress response in red-legged kittiwakes.

The first objective of this study was to investigate the relationships between components of the stress response and reproductive performance in red-legged kittiwakes (*Rissa brevirostris*). Because of the restricted geographic distribution of red-legged kittiwakes, this study system offers a unique opportunity to sample a species throughout almost its entire breeding range. Using an information-theoretic approach, I found that baseline corticosterone (Cort) during incubation and peak Cort during incubation and chick-rearing were the parameters which most accurately predict population-level reproductive performance. Due to the lack of variation in corticosteroid-binding globulin (CBG) levels among colonies, years, and reproductive stages I sampled, CBG levels were not valuable in predicting reproductive performance.

My results confirmed findings of previous studies of the closely related black-legged kittiwake (*Rissa tridactyla*), which highlighted the importance of food availability and stress status during early reproductive stages on reproductive success (Hatch et al. 1993; Gill and Hatch 2002; Lanctot et al 2003). Taken together, these results might indicate a critical period during which, in sub-optimal years, a portion of attending adults forego breeding or abandon breeding efforts and avoid costs associated with rearing a chick to fledging.

These findings also suggest that, although CBG dynamics might be an important component of the avian stress response, measuring CBG levels for this species does not provide information essential in assessing either a population's stress status or the effects of stress on reproductive success. These results do not support the hypothesis that individuals adjust circulating Cort concentrations via modulation of CBG levels (Breuner and Orchinik 2002) and conflict with previous studies in which changes in CBG concentrations were associated with food stress (Lynn et al. 2003) and with reproductive behavior (Love et al. 2004). Differences in life-history traits between previously studied passerines and kittiwakes studied here might explain this incongruity. Breuner and Orchinik (2001) attributed population-specific differences in CBG capacity in white-crowned sparrows (*Zonotrichia leucophrys*) to the length of the breeding season and the possible number of clutches per season. Hence, the role and variation of CBG might fundamentally differ between taxa with dissimilar life histories. Because the current study is among the first to record CBG levels in free-living non-passerines, more research is needed to understand the role and dynamics of CBG levels in long-lived species and to assess the generality of patterns reported here.

My second objective was to explore the role of reproductive behavior in explaining the relationship between stress physiology and reproductive success. Specifically, I asked whether the current physiological condition or foraging ecology of attending adults affects reproductive performance by influencing the breeding decision and/or mediating costs of reproductive behavior. I found no evidence to suggest that the parameters of physiological condition investigated in this study (Cort and CBG concentrations, body condition, and ectoparasite load) or foraging ecology (stable isotope signatures) influenced the breeding decision of adults attending the colony. I did, however, find that Cort concentrations and ectoparasite load, but not body condition or CBG levels, provided indices of physiological deterioration associated with reproduction.

These results suggest that the breeding decision in this species is not predicated upon current physiological condition. However, as I was able to sample only attending adults, the results do not preclude the possibility that an individual's current physiological condition influences the 'decision' to attend the colony and establish/ defend a nest site. Alternately, the observed pattern might be explained by heterogeneity in breeder quality, although such a pattern has not yet been directly investigated in red-legged kittiwakes.

The difference in stress status between breeders and non-breeders during chick-rearing period indicates that reproductive status, rather than reproductive stage, largely determines concentrations of baseline Cort in this species. This also suggests that reproduction in red-legged kittiwakes is associated with deteriorating physiological condition, which could lead to reduced post-breeding survival and lower future fecundity similar to patterns observed in black-legged kittiwakes (Golet et al. 1998; Golet and Irons 1999; Golet et al. 2004). Interestingly, I found no effect of reproductive status on body condition in this species. It is possible that condition differed between breeders and non-breeders prior to sampling, but that the costs of incubation accrued by breeders quickly eliminated this difference. However, this lack of detectable difference in body condition and the conflicting results of previous studies on several seabird species (Golet and Irons 1999; Wernham and Bryant 1998; Kitaysky et al. 1999), suggest that the relationship between adult body condition and reproductive effort is variable and species-specific.

I found no effect of reproductive stage or status on CBG levels in this species. Here again, this study does not support the hypothesis that individuals modulate CBG levels in response to stress associated with environmental conditions or the demands of reproduction (Breuner and Orchinik 2002; Love et al. 2004).

The third objective of this study was to investigate whether individuals buffer themselves from the harmful effects of prolonged Cort exposure by modulating CBG levels (thus altering circulating free Cort concentrations). I addressed this by asking: 1) whether captive red-legged kittiwake chicks respond to prolonged nutritional restrictions by altering CBG levels and 2) whether this response explains reduced total Cort concentrations observed in the same chicks following prolonged food restriction (Kitaysky et al. 2001). I found no evidence suggesting this response explains the previously reported reduction in total Cort concentrations following extended food-limitation, and free Cort concentrations closely paralleled those of total Cort. Rather, it appears that reduced CBG levels are associated with protein limitation and not with mediating concentrations of circulating total and/or free Cort.

Similar to the findings in both of the previous components of this study, these results do not support the hypothesis that individuals buffer themselves from the negative consequences of Cort exposure via modulation of CBG levels, as suggested by Breuner and Orchinik (2002). It is possible that adults (with more endogenous protein reserves) are less sensitive to protein limitation than developing chicks, which have relatively small endogenous protein stores and high protein demands for growth and development. Thus, the lack of variation in CBG levels among colonies, years, and stages might indicate that adult red-legged kittiwakes do not typically experience protein limitation during the breeding season. However, due to the logistical difficulty of conducting controlled experimental food manipulations with adults, the CBG response of adult kittiwakes to food stress remains largely unknown.

In conclusion, this study provides evidence that reproductive performance of red-legged kittiwakes is affected by environmentally-induced stress. Based on other studies in seabirds, it is likely that this environmental stress reflects changes in food availability. Specifically, Cort secretion in incubating individuals appears to reliably predict population-level reproductive performance in this species. However, I was unable to identify the mechanism(s) explaining this relationship, since none of the physiological or ecological parameters I measured differed between birds that succeeded or failed to lay eggs. Whether the decision to reproduce is influenced by a long-term reproductive strategy and/ or by an individual's physiological condition during the pre-breeding stage (e.g., the nest initiation stage of reproduction) remains to be examined. I found no evidence that kittiwakes buffered themselves from the harmful effects of prolonged exposure to high Cort by modulating CBG levels. Thus, it appears that the role and response of CBG levels are not consistent among all species. Finally, though there are costs associated with chronic Cort exposure in kittiwakes, the presence of a robust adrenocortical response in breeding adults suggests that the benefits of such a response (e.g. increased activity, foraging) offset these costs and maximize an individual's fitness.

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