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**GLUCOCORTICOID PHYSIOLOGY AND BEHAVIOR DURING LIFE HISTORY
TRANSITIONS IN LAYSAN ALBATROSS (*PHOEBASTRIA IMMUTABILIS*)**

By

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Dissertation

presented in partial fulfillment of the requirements
for the degree of

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Glucocorticoid physiology and behavior during life-history transitions in Laysan Albatross (*Phoebastria immutabilis*)

Chairperson: Creagh W. Breuner

Organisms have different life history stages where their energetic priorities differ between the goals of growth, survival, and reproduction. Behavioral decisions within stages and in the transition between stages have significant repercussions for future success so the timing of behaviors is extremely important. In this dissertation, I ask: How do intrinsic and extrinsic factors control the timing of behavioral decisions during tradeoffs within life history stages (the tradeoff between reproduction and self maintenance in breeding adults), and transitions between life history stages (the transition to independence in young animals) in a pelagic seabird, the Laysan Albatross (*Phoebastria immutabilis*).

First, I ask how physiology changes during incubation fasts over the entire 2-month incubation period. I found total corticosterone (CORT) increased both within individual incubation shifts and over the season as body mass declined. Corticosteroid binding globulin (CBG) significantly increased as individual body condition declined. Though CORT secretion steadily increased during long, repeated fasts in this species, CBG may also rise to protect the reproductive attempt from the inhibitory effects of CORT on parental behavior.

Next, I explore individual variation in parental provisioning trips during the chick rearing period. I found that surprisingly, adults expected to invest highly in their current reproductive effort, performed a pattern of foraging trips thought to be the strategy of adults investing in self-maintenance. This challenges previous work from the southern hemisphere and indicates that environmental differences in the northern hemisphere may change the distribution or predictability of ocean resources such that the benefit of different types of trips may not be fixed.

Finally, I investigate morphology and physiology in chicks approaching fledging. I suggest that there are two primary factors contributing to the timing of fledging in Laysan Albatrosses: wing disc loading and plasma free CORT levels. After chicks reach a critical developmental threshold, they may fledge if food delivery is inadequate or stay if it is plentiful. When food delivery is unpredictable and chicks lose energetic stores, free CORT levels may fine-tune the timing of fledging: chicks with high free CORT when they reached the fledging threshold fledged sooner than chicks with low CORT levels.

DEDICATION

This dissertation is dedicated to my family: my mother and Jason, who have always given their unconditional love and support; my father, whose time here was too short but who encouraged his children to follow their passions; and finally, my husband Jon, who has been there every single step along the way and without whose love, this would not have been possible.

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INTRODUCTION

Glucocorticoids and Life-history Transitions

All organisms face challenges as they move through the environment and must respond behaviorally and physiologically to successfully surmount those challenges. Challenges can be both predictable, such as moving between life history stages, or unpredictable, such as social interactions, food shortages, storms, or predation. In response to challenges, most vertebrates mount a neuroendocrine “stress response,” a primary physiological component of which is the release of glucocorticoids (Sapolsky et al. 2000; Wingfield and Sapolsky 2003). This dissertation addresses the question: **What roles do energetic challenges and glucocorticoid hormones play in shaping the timing of optimal behavior during tradeoffs within life history stages and transitions between life history stages?**

Glucocorticoids are normally maintained in the blood at relatively low levels that fluctuate on circadian and circannual cycles to regulate energy availability and use (Dallman et al. 1995). However, when the brain perceives a stressor or challenge, the hypothalamus upregulates a hormone cascade resulting in the further release of glucocorticoids from the adrenal gland into the plasma. Elevated glucocorticoids then act through receptors in target tissues to alter behavior and physiology in a variety of ways, including glucose mobilization through gluconeogenesis (Dallman et al. 1989; Plaschke et al. 1996), increased lipogenesis (Cherrington 1999; Landys et al. 2004) and fat deposition (Dittami et al. 2006; Yuan et al. 2008), impairment of immune function (Morici et al. 1997; Rubolini et al. 2005), abandonment of the reproductive effort (Breuner et al. 1998; Love et al. 2004; Silverin 1986; Wingfield and Silverin 1986), promotion of escape behavior (Wingfield et al. 1998), and increases in locomotor activity, foraging behavior and food intake (Landys et al. 2006). Glucocorticoids are thought to appropriately redirect energy and behavior to move animals between normal life history stages or trigger an emergency life history stage in response to unpredictable events (Orchinik 1998; Wingfield et al. 1998). In this body of work, I investigate the

relationship between glucocorticoids and behavior during: 1) the tradeoff between reproduction and self-maintenance, and 2) the transition to independence (fledging).

1. Survival-Reproduction Tradeoff

Glucocorticoid hormones may act as a proximate mechanism mediating the tradeoff between current reproductive effort and self-maintenance (future reproduction)(Wingfield and Sapolsky 2003). The behavioral and physiological effects of glucocorticoids are classically thought to decrease activities or functions associated with reproduction, and promote those associated with survival or self-maintenance:

H₁: Self Maintenance: Glucocorticoids move energy expenditure from current breeding effort to self-maintenance physiology and behavior.

Hence elevated glucocorticoids: ↓REPRODUCTION ↑SURVIVAL

Behaviorally, glucocorticoids tend to decrease reproductive behaviors such as territory defense, territory size, and parental provisioning rates in favor of increased locomotor activity, foraging behavior, and escape behavior (Wingfield and Sapolsky 2003). Pied Flycatchers (*Ficedula hypoleuca*) implanted with glucocorticoids feed their nestlings less frequently, have nestlings with lower mass, and have a lower % nestlings fledged (Silverin 1986). Glucocorticoid treatment in male Barn Owls (*Tyto alba*) also reduces provisioning rate (Almasi et al. 2008). Pied Flycatcher and Black-legged Kittiwake (*Rissa tridactyla*) parents implanted with glucocorticoids lose less mass (or gain more mass) than control adults also raising nestlings, presumably because they have shifted their investment from reproduction to self-maintenance (Angelier et al. 2007; Silverin 1986). At the nest success level, baseline glucocorticoids are significantly higher in female European Starlings (*Sturnus vulgaris*) that abandon their nests in the day following blood sampling (Love et al. 2004). Physiologically, elevated glucocorticoids have negative feedback on reproductive endocrine systems, shutting down production of testosterone (involved in territorial and aggressive behavior in males) and prolactin

(associated with parental behavior in both sexes)(Boonstra and Singleton 1993; Delehanty et al. 1997; Lee and McDonald 1985; Schoech et al. 1991).

However, as empirical tests of the relationship between glucocorticoids and parental behavior continue, it is becoming clear that the classic hypothesis may not explain all parental behavior seen in response to elevated glucocorticoids. In response, an alternative hypothesis for glucocorticoid action during reproduction has emerged in the field:

H₂: Reproductive Enhancement: Elevated glucocorticoids enhance the current reproductive effort:

- a. Slightly elevated glucocorticoids may induce greater foraging effort in parents
- b. Elevated glucocorticoids in development means fewer males and slower male growth, so remaining offspring get more resources per unit body size.
- c. Offspring physiology is prepared for lean times so offspring from more reactive mothers may have better physiology for lean environments than less reactive mothers.

Hence elevated glucocorticoids: ↑ REPRODUCTION

Glucocorticoid levels may be elevated for a number of reasons, many of them thought to enhance the reproductive effort. First, baseline glucocorticoids change seasonally, often increasing as the breeding season approaches (Romero 2002). This is thought to be preparatory for the increased energetic demand of reproduction, allowing for more flexibility in glucose and protein mobilization during breeding. Second, glucocorticoids may become elevated above baseline levels in response to environmental challenge, such as low food availability, increased predator pressure, or increased social challenge. An elevation of glucocorticoids here does not necessarily lead to lower reproductive effort or abandonment of young. Elevated glucocorticoids increase parental feeding rates in Common Murres (*Uria aalge*), House Sparrows (*Passer domesticus*), and Meerkats (*Suricata suricatta*)(Carlson et al. 2006; Doody et al. 2008; Lendvai and Chastel 2008).

Additionally, elevated maternal glucocorticoids during offspring development may affect offspring phenotype, increasing the likelihood that the parents end the breeding season with measurable reproductive success. Elevated maternal glucocorticoids at laying result in a female-biased offspring sex ratio and smaller males who grow more slowly (Bonier et al. 2007; Hayward et al. 2006; Love et al. 2005; Love and Williams 2008b). In many species, males are the more expensive sex to raise as they are often larger at birth/hatch, and grow at a faster rate (Cameron-MacMillan et al. 2007). By decreasing the overall number of males, and male growth rate, offspring demand can then match maternal ability to deliver food under challenging environmental conditions (Breuner 2008; Love and Williams 2008a; Love and Williams 2008b).

2. Transition to Independence

The consequences of elevated glucocorticoids are not consistent between life history and behavioral stages. Rather, the costs and benefits of elevated glucocorticoids change throughout development and are highly context-dependent (Orchinik 1998; Wada 2008; Wada et al. 2007). Excess glucocorticoids have negative effects on growth (Glennemeier and Denver 2002; Hayward and Wingfield 2004; Janczak et al. 2006; Meylan and Clobert 2005; Morici et al. 1997; Saino et al. 2005; Spencer et al. 2003; Wada and Breuner 2008), immune function (Morici et al. 1997; Rubolini et al. 2005), cognition (Kitaysky et al. 2003), and neuronal survival (Howard and Benjamins 1975). So in early development, high glucocorticoids have extremely high costs and should potentially be suppressed (Blas et al. 2006; Kitaysky et al. 2003; Schwabl 1999; Sims and Holberton 2000; Wada et al. 2007). However, elevated glucocorticoids can also stimulate behaviors necessary for the transition to independence: in young animals, exogenous glucocorticoids increase begging behavior (Rubolini et al. 2005; Wada and Breuner 2008), food intake (Kitaysky et al. 2003), and locomotor activity (Crespi and Denver 2004). Hence, in animals with an abrupt departure from their natal site, elevated glucocorticoids may facilitate the successful transition from a sedentary neonate to a mobile, independent adult.

Study System

The class Aves (birds) has over 9,600 species with incredible diversity of life history strategies. Several bird orders have adopted a life history that makes them better described as marine vertebrates. Birds such as penguins (*Sphenisciformes*), alcids (i.e. puffins, auklets, murrelets: *Alcidae*), and the tube-nosed seabirds (i.e. albatrosses, shearwaters, fulmars, petrels, storm-petrels: *Procellariiformes*) require land solely because eggs do not float and cannot be incubated on water. These seabirds tend to inhabit the extreme end of the life-history spectrum: they are long-lived, do not reach maturity for 3-12 years, usually lay only one egg per year, and often skip years between breeding attempts. Even for their size, seabirds are long-lived: Leach's Storm-petrels (*Oceanodroma leucorhoa*) regularly live >25 years as opposed to the comparably sized American Robin (*Turdus migratorius*) at 5-6 years. One of the longest-lived birds in North America is the Laysan Albatross (*Phoebastria immutabilis*), which currently holds the age record at 65 years old (Whittow 1993). They nest in the Hawaiian Islands Archipelago, the most isolated island chain in the world, Laysan Albatrosses travel over 2,000 km to forage in the cold, productive sub-Arctic waters. Albatross parents alternate incubation bouts, and they repeatedly fast for 2-3 weeks on the nest while taking turns foraging up to 4,000 km away in the North Pacific. After the egg hatches, parents brood the chick until it develops endothermy (14-21 days) and then depart again for the North Pacific, leaving the chick alone. As a result, the chicks also fast repeatedly approaching fledging. Fledging in albatross chicks involves the first flight, departure from the nesting colony, and the cessation of parental care. With the repeated long fasts in both adults and chicks and an extremely abrupt transition to independence in chicks, Laysan Albatrosses are an ideal system in which to study glucocorticoid hormones and life history transitions.

Dissertation Summary

This dissertation addresses the role of glucocorticoid hormones in shaping behavior during reproduction and approaching fledging in a long-lived, pelagic seabird, the Laysan Albatross. First, I ask how glucocorticoid physiology changes within adult birds during long incubation bouts and over the two-month incubation period. Second, I examine how parental body condition, glucocorticoid physiology, and age affect individual variation in

parental provisioning patterns during chick rearing. These two chapters test the self-maintenance and reproductive enhancement hypotheses regarding the role of glucocorticoids in modulating parental effort. Finally, I examine morphological and physiological changes in chicks as they approach fledging and whether glucocorticoids fine-tune the chick's decision to depart the colony. These studies represent a comprehensive analysis of physiology and behavior during two energetically demanding stages in an extremely long-lived seabird. Understanding the glucocorticoid response to energetic challenges, and the correlated behaviors, in an animal near the extreme of the life history spectrum will inform our overall understanding of the physiology underlying life history transitions.

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Chapter 1: GLUCOCORTICOID PHYSIOLOGY DURING INCUBATION FASTS IN LAYSAN ALBATROSS

Abstract

Laysan Albatross undergo repeated 2-3 week fasts throughout incubation, during which they can drop 25% of their body mass. In some species, during short, unpredictable fasts, glucocorticoid stress hormones (corticosterone in birds: CORT) rise as body mass declines. However, it is not clear whether the relationship between CORT and body mass holds during long-term, predictable fasting. Given that elevated CORT levels are associated with nest abandonment, species with repeated, long fasts during incubation may delay or depress the secretion of CORT to preserve their reproductive effort. We monitored body mass, CORT, and, for the first time, corticosteroid binding globulin (CBG), during incubation fasts over the entire incubation period in Laysan Albatross (*Phoebastria immutabilis*). As expected, body mass declined both within fasts and over the season. At the same time, total CORT increased both within individual incubation shifts and over the season. As supported by many other studies, baseline CORT was negatively correlated with body mass. CBG significantly *increased* as individual body condition declined. We also found that birds captured at a higher frequency (every 3 days) had a greater increase in CORT and smaller increase in CBG over the 11-14 days than did birds captured only twice. In summary, we found that CORT secretion does steadily increase during long, repeated fasts in this species, though CBG may also rise to protect the reproductive attempt from the inhibitory effects of CORT on parental behavior.

Introduction

Glucocorticoids (GCs) act as transitional hormones, moving animals between physiological, behavioral or life history states (Orchinik 1998; Wada 2008; Wingfield et al. 1998). These transitions between states (e.g. breeding abandonment) are often triggered by declines in food availability, and/or internal energetic resources (i.e. body condition). Animals experiencing poor body condition or low environmental food availability often elevate glucocorticoid (GC) hormone release in the plasma (Breuner and Hahn 2003; Buck et al. 2007; Doody et al. 2008; Kitaysky et al. 2001; Kitaysky et al.

1999; Mateo 2006; Shankar et al. 2007). This GC elevation may be useful in fine-tuning the shift away from non-critical activities toward self-maintenance through a wide range of physiological and behavioral effects including: glucose mobilization through gluconeogenesis (Dallman et al. 1989; Plaschke et al. 1996), increased lipogenesis (Cherrington 1999; Landys et al. 2004), fat deposition (Dittami et al. 2006; Yuan et al. 2008), abandonment of the reproductive effort (Breuner et al. 1998; Love et al. 2004; Silverin 1986; Wingfield and Silverin 1986), and increases in locomotor activity, foraging behavior and food intake (Landys et al. 2006).

Given the often negative effects of elevated GCs on reproductive behavior, one might expect fasting during reproduction to promptly induce abandonment or decrease reproductive effort. This is generally the case in income breeders, or species that directly convert resources from the environment into breeding effort rather than storing that energy. Because of their low capacity for energy storage, these animals must have the ability to transition between strategies or states quickly in the face of declining conditions. Low tolerance for food deprivation is reflected in their GC response to challenges: short-term fasting rapidly induces secretion of GCs in many income breeders (Breuner and Hahn 2003; Dempsey et al. 2005; Lynn et al. 2003). Elevation of GCs at this exhaustion stage should assist the bird in reacting to lowered food availability by shifting it into a temporary state of energy mobilization and food acquisition, and then move the animal towards cessation of reproductive activity in favor of self-maintenance if conditions do not change.

By contrast, capital breeders are defined as species that store large reserves of energy to fuel reproduction. Many use this energy while predictably and repeatedly fasting during breeding (Bernard et al. 2003; Bourgeon et al. 2007; Criscuolo et al. 2005; Groscolas and Robin 2001; Vleck and Vleck 2002). These species would not benefit from rapidly switching to self-maintenance physiology or behavior immediately upon the onset of fasting. The GC response in capital breeders should thus be buffered if elevated GCs shut down reproductive activities (Love et al. 2004; Silverin 1986; Wingfield and Sapolsky 2003). Rather, they may delay the GC response to fasting until they reach

critically low levels of energy reserves and require a transition away from breeding. The GC physiology of capital breeders is expected to reflect their internal energy stores and remain low until animals are close to exhausting fat stores (Vleck and Vleck 2002). Elevation of GCs at this stage may mediate a shift from lipid metabolism to mobilization of protein stores and signal a life-history stage transition: birds are in critical body condition and should abandon reproduction (Groscolas and Robin 2001; Hood et al. 1998; Vleck and Vleck 2002). However, GCs do still rise steadily during incubation fasts in some capital breeders (Hector and Harvey 1986). The variation among capital breeders in the magnitude of CORT increase during fasting may reflect differences in the length of time different species fast. Species that fast for months (e.g. penguins: *Sphenisciformes*) may suppress GC increase more than a species that fasts for days to weeks (e.g. albatrosses: *Procellariiformes*), but studies of such possible differences are lacking.

Physiological and behavioral effects of GCs depend on activation of receptors in target tissue. In the plasma, corticosterone (CORT: the primary glucocorticoid in birds) interacts with corticosteroid binding globulin (CBG), which may restrict the ability of CORT to cross capillary beds and activate receptors (Breuner and Orchinik 2002). As a result, CBG binding capacity in the blood may significantly affect the ability of CORT to have biological effects. In fact, evidence supports a role for CBG in modulating GC physiology in fasting animals with CBG declining over 22 hours of fasting in a passerine and 2 days in rats (Lynn et al. 2003; Tinnikov 1999). No study thus far has measured CBG during long fasts in any species.

Does GC physiology (both CORT and CBG) reflect condition indices of fasting seabirds in a manner consistent with the fine-tuning of life history transitions? Previous studies have assessed CORT during single fasts or through the breeding season using cross-sections of individuals. This is the first detailed, longitudinal evaluation of CORT or CBG within individuals through the entire incubation period in a capital breeder. Laysan Albatrosses (*Phoebastria immutabilis*) exemplify the extreme of long-lived capital breeders: they fast repeatedly during incubation bouts as adults take turns incubating and

foraging. Moreover, they hold the age record for North American birds with a maximum recorded age of 65 years old (Whittow 1993). Laysan Albatrosses may in fact have the longest incubation fasts of any bird outside of penguins because their nesting colonies in Hawaii are thousands of miles away from their foraging grounds near the Aleutian Islands. In this comprehensive study, we used a repeated measures approach to assess changes in CORT and CBG within individual Laysan Albatross adults both during individual incubation shifts (fasts) and across the entire two-month incubation period.

Materials and Methods

All procedures were approved by the University of Montana Institutional Animal Care and Use Committee (042-06CBDBS-083106) and University of Texas Institutional Animal Care and Use Committee (04040503).

Study Site and Species

Laysan Albatrosses are large, pelagic seabirds that nest through the Hawaiian Islands Archipelago. Animals for this study were captured at Midway Atoll National Wildlife Refuge (28°12' N, 177°21' W), where approximately 75% of the world's population (> 400,000 pairs) of Laysan Albatrosses nest on 623 hectares of emergent islets. Laysan Albatrosses nest in shallow depressions on the ground and can be easily picked up off their nests for blood sampling. When adults are not at the colony, they forage in the North Pacific along the sub-arctic frontal zone south of the Aleutians, 2,000 miles north of the Hawaiian Islands. Both parents provide extensive parental care (Whittow 1993) so both sexes were included in this study. All birds were banded with USFWS bands and engraved plastic bands.

Nest Monitoring

We monitored 57 nests every day from 15 November 2004 to 27 January 2005 (eggs were laid between 19 November and 7 December). The nests were spread throughout the colony so as not to disturb our other study birds when sampling nests. One egg was abandoned within a week of laying and two other nests were found unattended with a broken egg in late January. All other eggs hatched in late January or early February.

Adults from 35 nests were captured every 3 days during their incubation shifts starting 5 December 2004 (repeated measures group). To control for the possible chronic elevation of CORT resulting from repeated handling, we captured adults from 12 nests on only the 1st and once between the 11th and 14th day of a single incubation shift (control group). In all, we collected blood from 76 individuals during 97 incubation shifts for a total of 366 samples.

Blood Sample Collection and Morphological Measurements

All blood samples were collected within 3 minutes of initial disturbance (approach to within sight of the bird). There was no relationship between CORT level and the amount of time it took to get the blood sample. Therefore, any sample collected within 3 minutes of initial disturbance represents baseline levels (Romero and Reed 2005). Blood (400-500 μ l) was collected from the alar vein using an uncoated 1 ml tuberculin syringe with a 26 G needle. Blood was immediately expelled into a BD Microtainer® tube coated with Potassium-EDTA and placed on ice. All samples were centrifuged within 2h, plasma was removed with a Hamilton Syringe and stored at -20°C.

Following blood sampling, we measured flattened hand-wing length (wrist to end of longest primary) using a 60 cm wing ruler. We measured tarsus and culmen length to the nearest 0.1 mm using calipers, and mass to the nearest 50 g using a 5 kg Pesola spring scale. All sampling lasted less than 6 minutes from initial disturbance to when birds were returned to their nest. Occasionally, birds did not immediately settle on the egg but all birds returned to incubating within 10 minutes.

Corticosterone Assay

Plasma CORT levels were measured using direct radioimmunoassay (after Ketterson et al. 1991; Wingfield and Farner 1975). Briefly, 20-50 μ l of plasma from each individual was equilibrated overnight with distilled water and a tracer of 2000 cpm [³H]-CORT to calculate recoveries. Samples were extracted with 4 ml diethyl ether, evaporated in a waterbath, and resuspended in 550 μ l phosphate buffer. Samples were then assayed in duplicate using CORT antibody (#20-CR45, Fitzgerald Industries) and individual values

were later corrected for plasma volumes and recovery values after extraction. Recoveries averaged 91% (range = 72 - 99%). Each assay included 3-4 'blanks' (samples consisting of distilled water only). Detectability was determined by calculating the mean minus two standard deviations of the % bound values for the blanks. This established the lowest level of hormone that was significantly different from zero. The average detection limit was 6.73 pg/tube (0.35 ng/ml). Any sample results below the detection limit were corrected to that value for the assay. Samples were run in 4 assays; average inter- and intra-assay coefficients of variation 14.72% and 3.78% respectively.

Corticosteroid Binding Globulin Assay

Plasma CBG levels were measured using a radio-ligand-binding assay with tritiated corticosterone (after Breuner et al. 2003). Samples were stripped of endogenous hormone by incubating in a 1:3 dilution of dextran-coated charcoal for 20 minutes at room temperature and then centrifuged at 4500 rpm for 10 minutes at 4°C.

We first characterized the binding protein for Laysan Albatross. We optimized plasma dilution (1:500), incubation time (2h 15min), and incubation temperature (4°C). Affinity (K_d) of CORT for CBG was determined in an equilibrium saturation binding experiment where pooled plasma was incubated with [3 H]-CORT between 0.23 and 12 nM with or without unlabeled CORT to determine non-specific binding.

For the point samples assay (identification of individual CBG levels), total binding (TB) was determined using 50 μ l buffer, 50 μ l [3 H]-CORT, and 50 μ l diluted plasma. Non-specific binding (NSB) was determined using 50 μ l of 1 μ M unlabeled CORT instead of buffer. Bound and free radioligand were separated using rapid vacuum filtration (Brandel Harvester) over glass fiber filters (Whatman GF/B) soaked in 25 mM Tris with 0.3% PEI for 1 hour prior to filtering. After filtration, radioactivity bound to the filters was measured by liquid scintillation spectroscopy. All samples were run in duplicate in one assay; intra-assay variation was 15.79%. The inter-filter coefficient of variation was 18.76% determined from a single plasma pool run 30 times in duplicate (in order for one TB and one NSB of the pool to be on each filter).

Estimation of Free Corticosterone

Free CORT levels were estimated using the mass action-based equation from Barsano and Baumann (1989):

$$H_{free} = 0.5 \times [H_{total} - B_{max} - 1/K_a \pm \sqrt{(B_{max} - H_{total} + 1/K_a)^2 - 4(H_{total}/K_a)}]$$

In the equation, H_{free} = free hormone, H_{total} = total hormone, B_{max} = total binding capacity of CBG, and $K_a = 1/K_d$ (nM). The affinity of CORT for CBG was determined using equilibrium saturation binding analysis on pooled plasma samples. Individual CBG capacity estimates represent approximately 81% of B_{max} , so capacity values were increased to 100% for free CORT calculations. Results are given as means \pm standard errors for all individuals.

Statistics

We used a morphological body condition index of the residuals from the first principle component (using SPSS 11.5) of wing, culmen, and tarsus regressed against mass to obtain a relative measure of mass per skeletal size. We refer to this throughout as body condition index or BCI. Because the study design involved taking repeated samples from individuals, we used Linear Mixed Effects Models (Holmes et al. 2003) that include individual ID as a random effect in all models. LME models (R version 2.6.2 for Mac OS X) were first used to test the effects of number of days fasting and time since egg lay on body condition index. We then used LMEs to test the fixed effects of BCI, number of days fasting, and time since egg lay (incubation day) on the response variables of CBG, total CORT, and free CORT. Total CORT and free CORT were log-transformed to achieve normality. To determine the effects of repeated handling, we used two-tailed t -tests (when needed, we used Welch's correction for unequal variances) to compare the change in CBG, total CORT, free CORT, and BCI between birds caught every 3 days and birds caught only on the first and between the 11-14th day of their incubation shift. Residuals for BCI, linear regressions, and t -tests were run in GraphPad Prism version 4.0c for Mac.

Results

Nest Attendance

Each parent performed up to three fasting shifts during the 65-day incubation period: usually two long shifts and one short shift (as hatching approached). Long incubation shifts ranged from 6 to 34 days with an average length of 15 days (Figure 1).

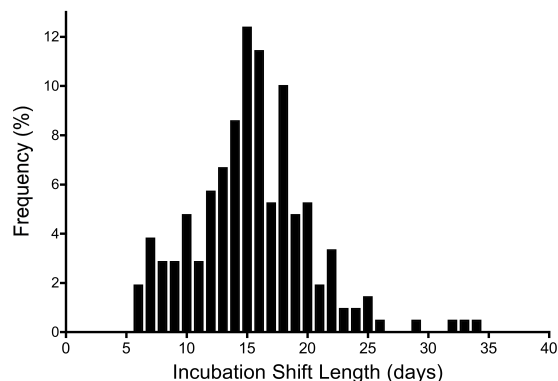


Figure 1. Frequency distribution of incubation shift lengths in Laysan Albatross (mean \pm SE = 15 \pm 0.33 days).

Mass Loss and Body Condition Index

Adults steadily lost mass through each incubation shift, though the slope became more shallow in extremely long-fasting birds (Figure 2). During the longest fasts (> 30 days), adults lost close to 25% of their initial body mass. Within individuals, condition also declined over the 2-month incubation period (Table 1), with an interaction between the number of days fasting and time since egg lay (Table 1).

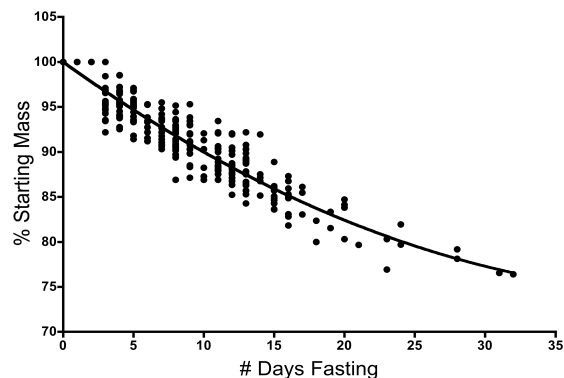


Figure 2. Condition loss (% of starting) as birds fast during individual incubation shifts.

Total Corticosterone

Total CORT increased both within individual incubation shifts and over the entire incubation period (Table 1, Figure 3a, 3b). Total CORT was negatively correlated with condition index (BCI) and there was an interaction effect between BCI and the number of days fasting (Table 1, Figure 4a). As birds fasted and BCI declined, their total CORT levels rose.

Corticosteroid Binding Globulin

The equilibrium saturation binding experiment demonstrated a single binding site for CORT in Laysan Albatross plasma ($K_d = 5.9 \pm 0.49$; Figure 5). CBG capacity had a

significant negative correlation with BCI (Table 1, Figure 4b): as BCI declined, CBG capacity increased. However, CBG capacity did not significantly change within individual birds either within incubation shifts or over the two-month incubation period (Table 1, Figure 3c, Figure 3d).

Table 1. Linear Mixed Effects Models (LME) to test changes in body condition with # days fasting and time since egg lay (incubation day), and effects of body condition index, # days fasting and time since egg lay (incubation day) on CBG, total CORT, and free CORT. Individual ID is included as a random effect in all models; $N = 366$ samples from 76 individuals.

Response Variable	Fixed Effects	df	t-value	P-value
Body Condition Index	# Days Fasting	287	-14.17	<0.0001
	Incubation Day	287	-5.16	<0.0001
	# Days Fasting x Incubation Day	287	3.13	0.001
Total CORT	# Days Fasting	286	5.36	<0.0001
	Incubation Day	286	6.97	<0.0001
	BCI	286	-4.10	0.0001
	BCI x # Days Fasting	286	3.21	0.002
CBG	BCI	289	-3.37	0.001
Free CORT	# Days Fasting	286	4.72	<0.0001
	Incubation Day	286	6.51	<0.0001
	BCI	286	-3.71	0.0002
	BCI x # Days Fasting	286	2.89	0.004

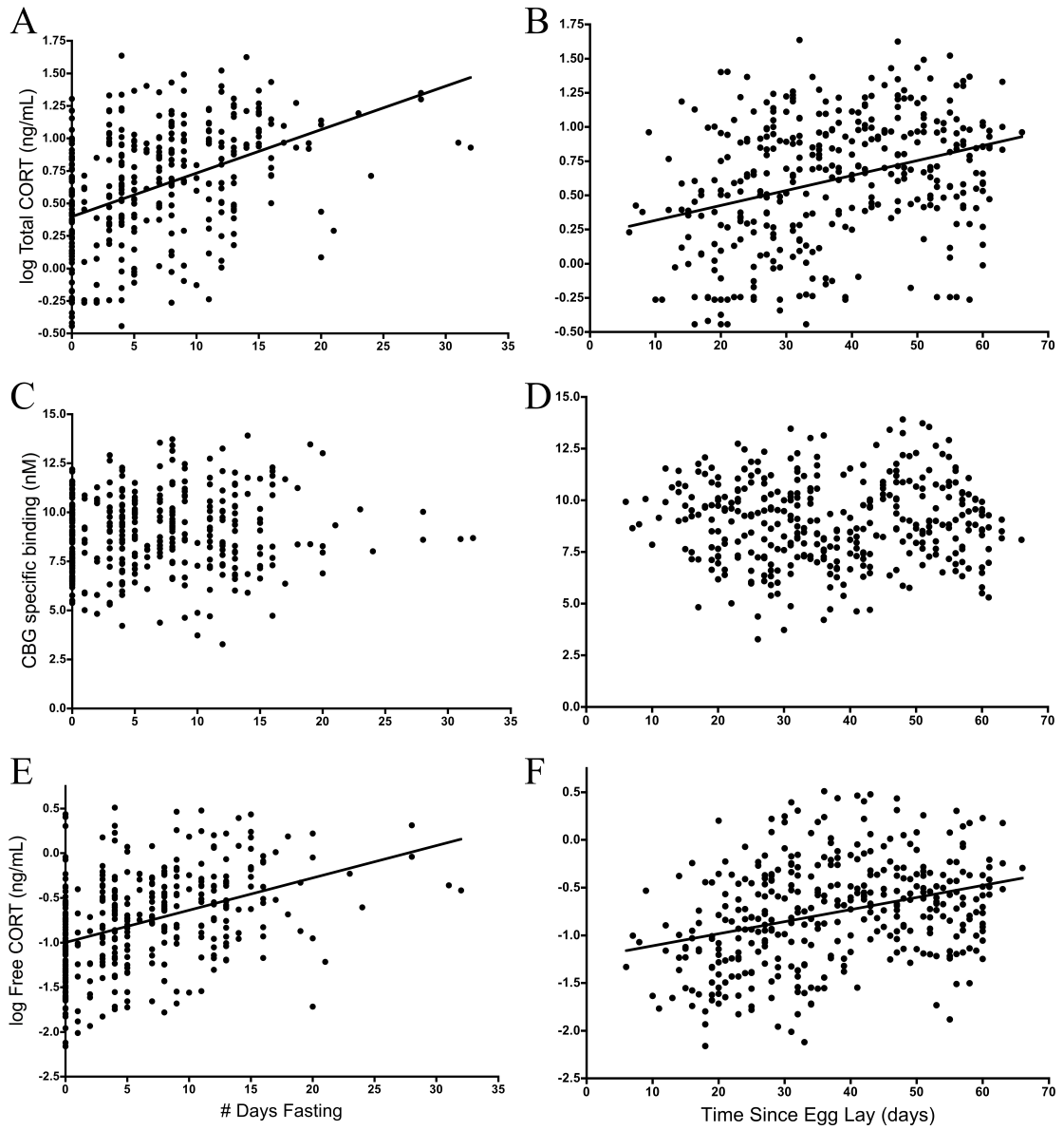


Figure 3. Changes in total CORT, CBG, and free CORT over the season (A,B,C), within individual incubation shifts (D,E,F). (N = 366 samples from 76 individuals.) Trendlines indicate significant relationships as determined from LME models (Table 1).

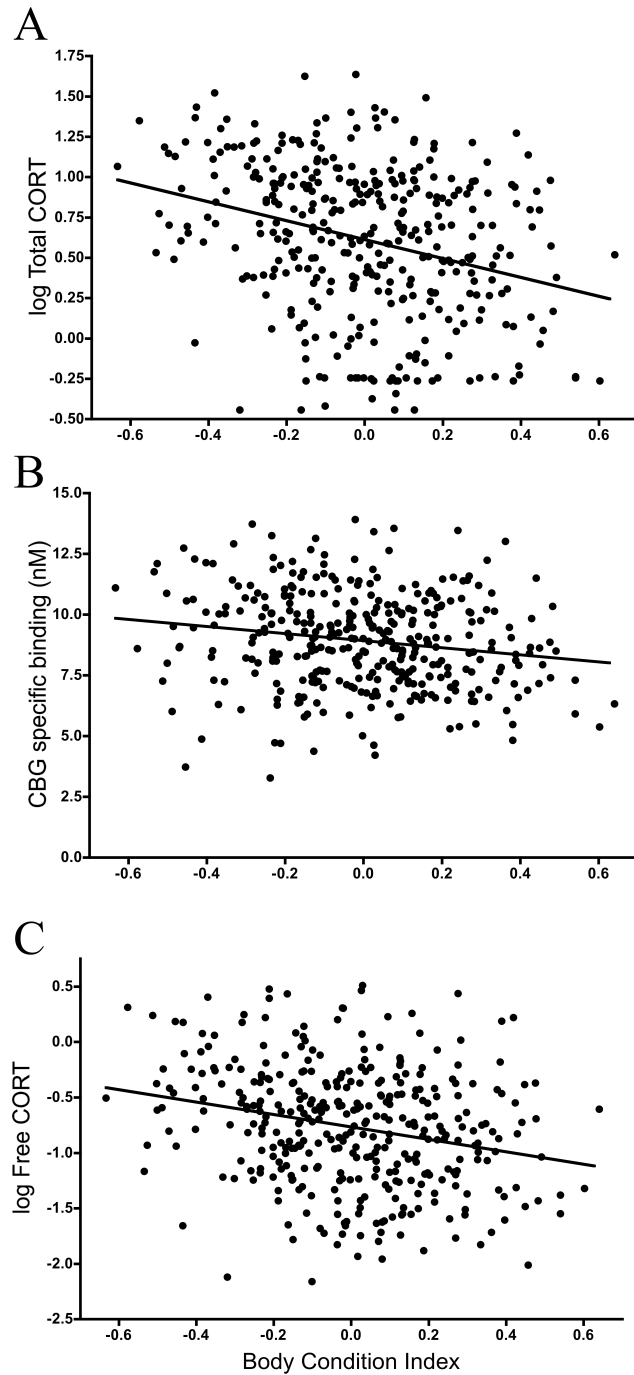


Figure 4. Relationship between adult body condition index and total CORT (A), CBG capacity (B), and free CORT (C). Trendlines indicate significant relationships as determined from LME models (Table 1).

Free Corticosterone

Free CORT displayed the same patterns of change as total CORT: increasing within individual incubation shifts and over the entire incubation period (Table 1, Figure 3e, 3f), and negatively correlated with BCI (Table 1, Figure 4c).

Effects of Capture Frequency

We compared the change in CBG, total CORT, free CORT, and body condition on day 1, and day 11-14 of fasting between birds only caught at the beginning and end of their fast (control) and birds caught every 3 days (repeated measures). All birds had a significant increase in both CBG and CORT during the individual incubation shifts. However, control birds had a significantly greater increase in CBG during the incubation shift than repeated measures birds (t -test, $df = 45$, $t = 2.150$, $P = 0.037$, Figure 6a). Control birds also had a smaller increase in total and free CORT than repeated measures birds during incubation shifts (total CORT: t -test with Welch's correction, $df = 31$, $t = 2.361$, $P = 0.025$, Figure 6b; free CORT: t -test with Welch's correction, $df = 41$, $t = 2.584$, $P = 0.0134$, Figure 6c). There was no difference in % body mass loss between groups during fasting (t -test, $df = 45$, $t = 0.4451$, $P = 0.658$, Figure 6d).

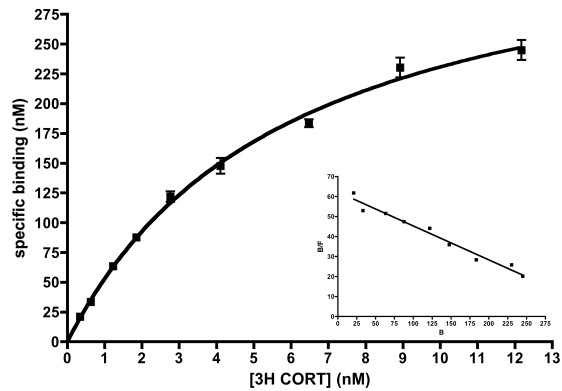


Figure 5. Equilibrium saturation binding of [³H]CORT to Laysan Albatross plasma. Data shown are specific binding (means \pm SE at each concentration). Data are best fit by a one-site model with a K_d of 5.9 ± 0.49 nM. Inset: Scatchard-Rosenthal replot of the data (*included to visualize whether or not the receptor (CBG) has one binding site or two. In this case, the linear relationship indicates that there is a single binding site.*)

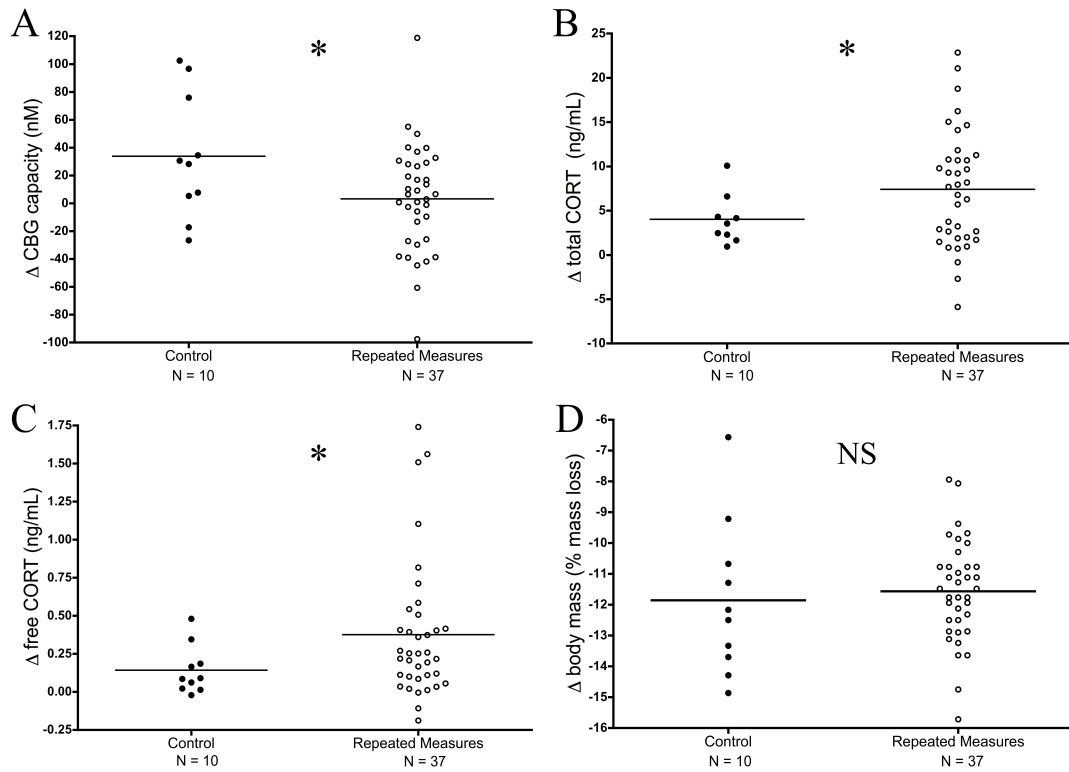


Figure 6. Change from day 1 to day 11-14 in CBG capacity (A), total CORT levels (B), free CORT levels (C), and body mass (D). Control birds were only caught on day 1 and day 11-14. Repeated measures birds were caught every three days between d1 and d11-14. Asterisks denote $P < 0.03$ as determined by a t -test with Welch's correction for total and free CORT.

Discussion

For the first time, we used a repeated-measures approach to examine changes in body condition, CORT, and CBG during long incubation shifts and during the entire incubation period in a long-lived, long-fasting capital breeder. We found that total CORT and free CORT increased within individual incubation bouts and over the entire incubation period as body condition declined. While CBG did not change significantly over the two-month incubation period, it was negatively correlated with body condition index so rose overall as birds fasted.

Incubation bouts and body mass

Many seabird species nest on isolated islands but forage on patchy and widely distributed resources in the ocean. Depending on the location of the breeding islands, birds may need to travel a variety of distances to food and thus must fast during incubation for several hours to a month or more. Laysan Albatrosses have some of the longest average incubation shifts (~ 15 days) of *Procellariiforms*. In fact, along with the much larger Wandering Albatross, they appear to have the longest incubation fasts of any bird outside of the multi-month fasts of penguins (*Sphenisciformes*), which lose $\leq 50\%$ of their body mass over ≤ 100 days (Groscolas 1988; Robin et al. 1988). During incubation bouts, Laysan Albatrosses lost up to 25% of their starting mass at a rate of about 1% per day, though the rate slowed down in the longest-fasting birds. Of *Procellariiforms*, this is again similar to Wandering Albatrosses (~1% per day; Ackman 1989), but somewhat slower than the much smaller-bodied Antarctic Fulmars (~3.91-4.46% per day; Weimerskirch 1990) and Wilson's Storm Petrels (~6% per day; Ackman 1989).

We found that body condition index was negatively correlated with total CORT levels. Though there was considerable individual variation, the relationship was highly significant. Recent studies of seabirds have found no relationship between body condition and total CORT (Angelier et al. 2008; Angelier et al. 2007b). In fact, a study using single time samples from individual Laysan Albatrosses during incubation in a different year found no relationship between body condition and total CORT (R. Sprague, unpublished data). The primary difference may be cross-sectional studies as opposed to

repeated measures studies. At any given point, there may not be a visible relationship between body condition and CORT because of large variation between individuals. However, when tracked within individuals over time, the relationship is apparent.

GC response to fasting - Corticosterone

Elevated CORT is classically thought to have negative effects on reproduction (Silverin 1986; Wingfield et al. 1998; Wingfield and Sapolsky 2003; Wingfield and Silverin 1986). Though fasting and food deprivation usually cause increased CORT, some long-fasting species delay CORT elevation until individuals have exhausted fat stores (Hood et al. 1998; Vleck and Vleck 2002). Laysan Albatross undergo long and repeated fasts during incubation so could be expected to suppress CORT secretion until the depletion of fat stores. However, we found that total CORT increased steadily during individual incubation shifts and increased overall during the incubation period. This is consistent with other studies where elevated plasma CORT in response to fasting is pervasive and seen across taxa (Angelier et al. 2007c; Astheimer et al. 1992; de la Mora et al. 1996; Guinet et al. 2004; Lynn et al. 2003; Nieminen et al. 2007; Tinnikov 1999; but also see: Cherel et al. 1988; Hood et al. 1998; Vleck and Vleck 2002). CORT elevation during fasting may serve a transitional role by mobilizing energy and redirecting investment away from non-critical behaviors (such as breeding), to self-maintenance activities (such as increased foraging effort)(Orchinik 1998; Wingfield et al. 1998). However, Laysan Albatrosses experience repeated multi-week fasts during each breeding season, such that shutting down reproduction in response to fasting would be detrimental. Even so, exhibit a steady elevation in CORT secretion as their mass drops and do not abandon their nests. There are at least two possibilities as to why CORT rises in this long-fasting species when birds have not yet reached stage III fasting (exhaustion of fat stores) and do not show the expected behavioral output of elevated CORT (nest abandonment).

First, it is possible that this species has decoupled the stress response associated with fasting from reproductive endocrine systems. CORT tends to shut down reproductive hormones to redirect energy away from breeding toward self-maintenance. This occurs through negative feedback on release of testosterone or prolactin, hormones involved in

territory defense and parental behavior (Boonstra and Singleton 1993; Delehanty et al. 1997; Schoech et al. 1991). Recent work in petrels and albatrosses indicates that stress is tied to decreased levels of prolactin (Angelier et al. 2007a; Angelier et al. 2006; Chastel et al. 2005). Occasionally however, it may be advantageous for species to decouple stress and reproduction. Some examples of this may occur when there are limited opportunities for reproduction: i.e. aged individuals with minimal future reproductive success, seasonal breeders reproducing in severe environmental conditions where time for reproduction is extremely short, or semelparous species (Wingfield and Sapolsky 2003). Pied Flycatchers and House Sparrows who have lost a mate exhibit both elevated CORT levels and increased chick feeding rates (Lendvai and Chastel 2008; Silverin and Wingfield 1998). Garter snakes in Canada with only a few days to mate show no change in CORT or testosterone with handling stress during the breeding season (Moore et al. 2001). Mechanistically, animals may block secretion of GCs, downregulate GC receptors, or alter binding protein levels (among other strategies) during breeding to protect against the inhibitory effects of CORT on reproduction (Jennings et al. 2000; Stein-Behrens and Sapolsky 1992).

Alternatively, long-term fasting may require energy mobilization and slowly elevated CORT could be a metabolic necessity to deal with lack of food and water for 2-3 weeks at time. It should be noted that the highest levels of total CORT measured during the incubation shifts were still well below capture and handling induced levels (mean ~ 8 ng/mL as opposed to 30-40 ng/mL after 30-60 minutes of handling (R. Sprague, unpublished data). Interestingly, out of 58 nests, only 3 nests failed during incubation (broken eggs) and an additional 5 did not hatch, or failed prior to chick fledging. This indicates that very few birds may have reached CORT levels high enough to induce nest abandonment. Baseline CORT titers elevated to intermediate levels during reproduction may be necessary for the energetic demands of reproduction in birds and other taxa (Love et al. 2004; Moore and Jessop 2003; Romero 2002). Energy regulation tends to be mediated through the high-affinity mineralocorticoid receptors (MR) at low CORT levels whereas the emergency life history stage (here, nest abandonment) may be triggered by high CORT levels via the lower-affinity glucocorticoid receptors (GR) (Wingfield 2005;

Wingfield et al. 1998). As a result, CORT levels may have stayed in an energy regulation state as opposed to triggering an emergency life history stage, potentially facilitated by the CBG response to fasting.

GC response to fasting - Corticosteroid Binding Globulin

Regulation of plasma CBG levels may allow for elevation or reduction of tissue-levels of CORT, independently of CORT secretion (Breuner and Orchinik 2002; Hammond 1995; Rosner 1990). If CBG restricts hormone access to tissues (the free hormone hypothesis), then declining CBG (e.g. in response to fasting) would increase the amount of CORT entering tissues. There is disagreement over whether the total, bound, or free plasma CORT is the biologically relevant fraction. Because of this, incorporation of CBG measurements is important to evaluate each fraction and gain a more complete evaluation of the organismal response.

Overall, CBG tends to decline under conditions of chronic stress (Alexander and Irvine 1998; Spencer et al. 1996; Tinnikov 1993; Tinnikov 1999). In response to fasting specifically, CBG has been studied only in income breeders (Cornelius et al. 2004; Lynn et al. 2001; Lynn et al. 2003). In all of these cases, fasting caused a decline in CBG, presumably increasing the amount of free CORT available to tissues without further secretion of CORT. We found that CBG *increased* as body condition declined but did not change significantly over the 2-month incubation period. This is the first time that CBG has been demonstrated to increase during fasting in any bird species. Free CORT still increased (driven by the robust increase in total CORT) but it is possible that CBG rose to help buffer the reproductive attempt from negative effects of high CORT (Breuner and Orchinik 2001).

Capture Frequency

As part of our control for repeated sampling from individuals, we captured one group at a lower frequency (only twice during their incubation shift instead of every three days). We found that birds captured at the higher frequency had a greater increase in CORT and smaller increase in CBG over the 11-14 days than did birds captured only twice. As a

result, higher sampling frequency birds had a significantly greater increase in free CORT during their incubation shift than did the low sampling frequency birds. Interestingly, birds captured at a higher frequency were only caught twice more than the low-frequency birds and all captures were baseline samples (birds were returned to their nest within 6 minutes of capture, so extended elevation of CORT was avoided).

The higher capture frequency could have also been perceived as a chronic stressor by the incubating birds, who upregulated their baseline CORT levels in response (Creel et al. 2002; Homan et al. 2003; Hopkins et al. 1997; Wasser et al. 1997). Chronic stress has been shown to decrease CBG levels (Alexander and Irvine 1998; Spencer et al. 1996; Tinnikov 1993; Tinnikov 1999). While CBG did not decrease in the more frequently handled birds, it did increase at a slower rate. Our “high capture frequency” may have been considered a chronic stressor by the incubating birds even though it occurred only once every 4 days for a short period of time. Even short-term stressors may have delayed effects on CBG: recent work shows that quail subjected to a single acute capture and handling protocol had significantly lowered CBG 24-hours later (Malisch et al. unpublished data).

Summary

Traditionally, elevated CORT (along with decreased prolactin and exhaustion of body reserves) is thought to initiate nest abandonment in critically depleted birds, especially long-fasting species (Groscolas et al. 2008; Silverin 1986; Silverin 1998; Vleck and Vleck 2002). In at least one case in songbirds, nest abandonment is not driven solely by an elevation in total CORT but because of lower CBG, resulting in higher plasma free CORT (Love et al. 2004). We observed a steady increase in total CORT during fasting in albatrosses but interestingly, accompanied by a corresponding increase in CBG even as birds' body condition decreased. While the CBG increase could have a buffering effect on free CORT reaching receptors in tissue, the increase in total CORT was still robust enough to see significant increase in free CORT as well. Though again, free CORT levels in most individuals never reached stress-induced levels seen in this species that could behaviorally result in nest abandonment (R. Sprague, unpublished data). During

incubation in this species, CORT may be playing an energy regulation role during long fasts or may be seasonally upregulated through the breeding season.

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Chapter 2: INDIVIDUAL VARIATION IN PARENTAL INVESTMENT STRATEGIES IN ALBATROSS

Abstract

Many *Procellariiform* seabirds alternate short and long foraging trips while feeding their chicks. In Weimerskirch's (1994) paradigm based on the southern hemisphere, short trips into warm, nearshore waters provide frequent and consistent food for the chick at a net energetic loss to the parent. On the other hand, long trips into the productive waters off the continental shelf augment adult condition while the condition of the chick suffers by less frequent feedings. To examine this parental tradeoff between self-maintenance and investment in offspring, we characterized individual variation in the pattern of provisioning trips made by 26 adult Laysan Albatross (*Phoebastria immutabilis*) on Kauai, Hawaii, from hatching to fledging (Jan-July). We then evaluated adult body condition, glucocorticoid physiology, age, and environmental variation as potential mechanisms underlying behavioral variation. Glucocorticoid physiology is often negatively associated with body condition and could be a signal redirecting parental effort away from reproductive activities toward self-maintenance. Body condition index strongly predicted free corticosterone (CORT) levels at the end of incubation. Surprisingly, adults with lower free CORT at the end of incubation performed fewer short trips for every long trip (thought to be the strategy of adults in lower condition with high CORT) during chick rearing. Older birds, expected to invest highly in their current reproductive effort, also performed fewer short trips for every long trip and began performing long trips when their chicks were significantly younger. Age was not correlated with body condition or free CORT. Interestingly, adults who made more long trips and had a lower ratio of short to long trips, had chicks that fledged older; i.e. more long trips (thought to be less beneficial for chicks) correlated with higher than average chick condition indices. In general, our results do not match the expectations provided by the southern hemisphere *Procellariiformes*. However, North Pacific species forage north of a mobile transition zone chlorophyll front (TZCF) instead of a continental shelf. In early 2007, the TZCF was farther north than the 4-year average, potentially indicating that the benefit of short and long trips in the northern hemisphere may vary based on the location of the TZCF.

Introduction

A key life history decision for organisms is whether to invest in current reproduction or self-maintenance for future reproduction. Because investing in one process reduces the materials and energy available for the other, this allocation decision constitutes a tradeoff (Stearns 1992). Intrinsic and extrinsic factors such as body condition, age, or environmental food availability can influence the optimal decision for any individual. In the extreme, the tradeoff may be binary – in any reproductive season, individuals either reproduce or maintain themselves, but not both (Stearns 1992). However, more subtle modulation of the tradeoff is also possible. For example, even within a single clutch, parents may feed some offspring more than others (Clutton-Brock 1991). More dramatically, in response to changing conditions, animals may decide to abandon clutches entirely. Between individuals, there is wide variation in the extent to which intrinsic or extrinsic factors shift their investment between current and future reproduction.

Offspring provisioning is a major component of parental investment. Many seabirds have an extreme and unusual chick provisioning pattern that begs for investigation into individual parental investment strategies. During chick provisioning, some *Procellariiformes* (albatrosses and petrels) and *Sphenisciformes* (penguins) alternate long and short foraging trips (Chaurand and Weimerskirch 1994; Clarke 2001; Tremblay and Chérel 2005; Weimerskirch 1998; Weimerskirch et al. 1994; Weimerskirch et al. 1997). In the southern hemisphere, short trips to warm, coastal waters allow frequent delivery of food to their chick. However, these short trips result in a net energetic loss to the parent because warm waters have lower productivity and short trips do not yield enough food for both parent and chick (Weimerskirch et al. 1994). Conversely, long trips to cold water off the continental shelf produce larger meals for the chick and a net energetic gain for the parent. Yet, the chick loses mass during long trips because of the greater time between feedings (Weimerskirch et al. 1994). In this paradigm, the pattern of alternating short and long foraging trips exemplifies the tradeoff between current and future reproduction: increased frequency of short trips represents parental investment in the

current attempt, whereas increased frequency of long trips represents increased self-maintenance.

Given that the benefits of short and long trips accrue differently to chicks and parents, we expect to see individual variation in the trip pattern that reflects the level of parental investment. While no study has characterized individual variation in species that make short and long feeding trips, factors expected to affect parental investment include 1) age or experience (Angelier et al. 2007c; Ardia and Clotfelter 2007; Hatch and Westneat 2007; Pugesek 1983), and 2) parental body condition or food availability (Chastel et al. 1995a; Chastel et al. 1995b; Dearborn 2001; Lormee et al. 1999; Robinson et al. 2005). Additionally, 3) stress hormones (i.e. glucocorticoids such as corticosterone) are often cited as likely candidates translating differences in age or body condition into shifts from reproduction to self-maintenance, probably through their inhibitory effects on hormones that promote reproductive behavior (i.e. testosterone, prolactin).

1. Age/Experience

Empirical studies across taxa have demonstrated an increase in reproductive effort and success with age (Angelier et al. 2007b; Hatch and Westneat 2007; Mauck et al. 2004; Pugesek 1981; Pugesek 1983; Pyle et al. 1991; Reed et al. 2008). This pattern may be attributed to increased foraging efficiency with age and experience (Curio 1983), increased effort because of declining residual reproductive value (Stearns 1992), or a disappearance of poor-quality phenotype individuals, leaving the older, higher-quality individuals (Cam and Monnat 2000).

2. Body Condition/Food Availability

In long-lived species, adults in poor body condition tend to invest relatively less in current reproductive effort and more in future reproduction (Chastel et al. 1995a; Weimerskirch 1992). For example, body condition predicts both the decision to reproduce and within-season reproductive success in several species (Chastel et al. 1995b; Fowler et al. 1994; Vleck and Vleck 2002). Within colony, female Adeline Penguins (*Pygoscelis adeliae*) that return but do not breed are also 10-12% lighter than

females that both return and breed (Vleck and Vleck 2002). This positive relationship between body condition and reproductive behavior within colonies generally holds between colonies as well: adult body condition and reproductive success are higher in colonies with higher local food availability and marine productivity (Cairns 1987; Cairns 1992; Monaghan et al. 1989; Montevecchi et al. 1992).

3. Role of Glucocorticoid Hormones

Elevated glucocorticoids (GCs) are often implicated as proximate mediators of some life-history tradeoffs because of their pervasive effects on physiology and behavior. In particular, GCs are thought to enhance self-maintenance (e.g. energy mobilization, foraging behavior) while inhibiting reproductive behavior and parental care (Silverin and Wingfield 1982; Sinervo and Svensson 1998; Wingfield and Sapolsky 2003). GC response to a stressor can be modulated by environmental and organismal characteristics and thus, should act to translate physiological state into an appropriate behavioral response. For instance, the GC response to an acute stressor can decline with age, potentially explaining increased reproductive effort and success in older individuals (Heidinger et al. 2006; Heidinger et al. 2008). GCs also link body condition and food availability at both the individual and population levels. Within individuals, GC levels and body condition are negatively correlated across taxa (Astheimer et al. 1992; Breuner and Hahn 2003; Cabezas et al. 2007; Hanna et al. 2008; MacDonald et al. 2007; Mateo 2006; Shankar et al. 2007). At the population level, average GC levels are higher in years or in colonies with lower environmental food availability and lower reproductive performance (Barbraud and Weimerskirch 2005; Buck et al. 2007; Doody et al. 2008; Kitaysky et al. 2007; Kitaysky et al. 1999; O'Reilly and Wingfield 2001; Romero and Wikelski 2002; Shultz and Kitaysky 2008).

Here we used a long-lived northern hemisphere seabird to examine individual variation in parental investment during chick provisioning. We assessed individual variation in the number of short and long foraging trips made during chick rearing and considered adult age, body condition, corticosterone (CORT: the primary glucocorticoid in birds), and corticosteroid binding globulins (CBG: a secondary regulator of CORT action) as

potential factors regulating parental investment. To further evaluate the output of parental investment, we measured chick growth rates and body condition at fledging. We predicted that individual variation in parental feeding strategies would reflect differences in age/experience and body condition among individuals. If the paradigm from the southern hemisphere holds true in the north Pacific as well, birds investing in current reproduction (old birds and birds in good body condition) should take more short trips, and birds investing in self (young birds and birds in poor body condition) should take more long trips.

Materials and Methods

All procedures were approved by the University of Montana Institutional Animal Care and Use Committee (042-06CBDBS-083106).

Study Site and Species

Laysan Albatrosses (*Phoebastria immutabilis*) are large, pelagic seabirds that nest throughout the Hawaiian Islands Archipelago. Animals for this study were captured on the Albatross Hill area at Kilauea Point National Wildlife Refuge (22°13' N, 159°24' W), where 43 pairs of Laysan albatrosses nested in 2006-2007. Laysan Albatrosses nest in shallow depressions on the ground and can be easily picked up from their nests for blood sampling. When adults are not at the colony, they forage anywhere from the Musician Seamounts several hundred kilometers north of the Hawaiian Islands to the sub-arctic frontal zone south of the Aleutians, 2,000 – 3,000 km north. Both parents provide extensive parental care (Whittow 1993) so both sexes were included in this study. All birds on Kauai are banded with USFWS bands and engraved plastic bands.

Nest Monitoring

We monitored 19 study nests every day from 15 November 2006 to 28 January 2007. The nests were spread throughout the colony so as not to disturb our other study birds when sampling nests. Five of the 19 eggs were abandoned or did not hatch. The failure rate of the experimental nests was not significantly different from the rest of the birds nesting on Albatross Hill (5 failed of 24 nests: Fisher's Exact Test, $P = 0.73$).

Blood Sample Collection and Morphological Measurements

Both adults from each nest were captured on the day they arrived for their first incubation shift after the egg hatched (arrival) and again on the day they arrived for their last shift before the egg hatched (hatching). One nest was not added until partway through incubation so we have only hatching samples. All blood samples were collected within 3 minutes of initial disturbance (approach to within sight of the bird). There was no increase in CORT levels with collection time so our samples represent baseline (Romero and Reed 2005). Blood (400-500 μ l) was collected from the alar vein using an uncoated 1 ml tuberculin syringe with a 26 G needle. Blood was immediately expelled into a BD Microtainer® tube coated with Potassium-EDTA and placed on ice. All samples were centrifuged within 2h, and plasma was removed with a Hamilton Syringe and stored at -20°C.

Following blood sampling, we measured flattened wing length (wrist to end of longest primary) using a 60 cm wing ruler. We measured tarsus and culmen length to the nearest 0.1 mm using calipers, and mass to the nearest 50 g using a 5 kg Pesola spring scale. Sampling lasted less than 6 minutes from initial disturbance to when birds were returned to their nest. Occasionally, birds did not immediately settle on the egg but all birds returned to incubating within 10 minutes.

Radio Transmitters

At the end of incubation (the second sampling event), we attached radio transmitters to both parents from the 14 successful nests (for one nest, we were able to capture only one adult so there were a total of 27 adults with transmitters). We attached transmitters (Advanced Telemetry Systems Inc., model A2930) to scapular feathers with acrylic-coated cloth tape (tesa #4651). The transmitters weigh 23 g (< 1% of adult body mass) and have a 98% retention success over 8 months (P. Sievert and S. Shaffer, pers. comm.). Radio transmitters are also smaller than satellite transmitter and that do not affect the aerodynamics or energetics of the adult birds (Anderson et al. 1998; Fernandez et al. 2001; Jouventin and Weimerskirch 1990; Weimerskirch and Robertson 1994). We used

a stationary receiver at the colony to monitor presence or absence of all 27 frequencies every 6 minutes from late-January until after the chicks fledged (August).

Chick Fledging

To monitor how parental provisioning strategy affected chick growth, we measured chick morphology (mass, culmen, tarsus, and wing length) every 4 days as chicks approached fledging and determined chick age at fledging.

Corticosterone Assay

Plasma CORT levels were measured using a direct radioimmunoassay (after Ketterson et al. 1991; Wingfield and Farner 1975). Briefly, 50 μ l of plasma from each individual was equilibrated overnight with distilled water and a tracer of 2000 cpm [3 H]-CORT (to calculate recoveries). Samples were extracted with 4 ml diethyl ether, evaporated in a water bath, and resuspended in 550 μ l phosphate buffer. Recoveries averaged 93% (range = 77-98%). Samples were then assayed in duplicate using an antibody to CORT (#20-CR45, Fitzgerald Industries), and individual values were later corrected for plasma volumes and recovery values after extraction. Each assay included 3-4 blanks (samples consisting of distilled water only). Detectability was determined by calculating the mean minus two standard deviations of the % bound values for the blanks. This established the lowest level of hormone that was significantly different from zero. The average detection limit was 16.98 pg/tube (0.93 ng/ml). Any sample results below the detection limit were corrected to that value. All samples were run in one assay; the intra-assay coefficient of variation was 6.40%.

Corticosteroid Binding Globulin Assay

Plasma CBG levels were measured using a radio-ligand-binding assay with tritiated corticosterone (after Breuner et al. 2003). We previously characterized the binding protein for Laysan Albatross (Chapter 1). Samples were stripped of endogenous hormone by incubating them in a 1:3 dilution of dextran-coated charcoal for 20 minutes at room temperature. They were then centrifuged at 4500 rpm for 10 minutes at 4°C. For the point samples assay (identification of individual CBG levels), total binding (TB) was

determined using 50 µl buffer, 50 µl [³H]-CORT, and 50 µl diluted plasma. Non-specific binding (NSB) was determined using 50 µl of 1 µM unlabeled CORT instead of buffer. Bound and free radioligand were separated using rapid vacuum filtration (Brandel Harvester) over glass fiber filters (Whatman GF/B) that had been soaked in 25 mM Tris with 0.3% PEI for 1 hour prior to filtering. After filtration, radioactivity bound to the filters was measured by liquid scintillation spectroscopy. All samples were run in duplicate in one assay. Intra-assay variation was 17.42%. The inter-filter coefficient of variation was 16.94% determined from a single plasma pool run 8 times in duplicate (in order for one TB and one NSB of the pool to be on each filter).

Estimation of Free Corticosterone

Free CORT levels were estimated using the mass action-based equation from Barsano and Baumann (1989):

$$H_{free} = 0.5 \times [H_{total} - B_{max} - 1/K_a \pm \sqrt{(B_{max} - H_{total} + 1/K_a)^2 - 4(H_{total}/K_a)}]$$

In the equation, H_{free} = free hormone, H_{total} = total hormone, B_{max} = total binding capacity of CBG, and $K_a = 1/K_d$ (nM). The affinity of CORT for CBG was determined using equilibrium saturation binding analysis on pooled plasma samples. Individual CBG capacity estimates represent approximately 87% of B_{max} , so capacity values were increased to 100% for free CORT calculations. Results are given as means ± standard errors for all individuals.

Data Analysis

Sexes were determined by microsatellite analysis at the University of Hawaii (by Lindsay Young). We used a morphological body condition index of the residuals from the first principle component (using SPSS 11.5) of wing, culmen, and tarsus regressed against mass to obtain a relative measure of mass per skeletal size. We refer to this throughout as body condition index (BCI). Linear regressions, residual analysis, and *t*-tests were performed in GraphPad Prism (version 4.0c for Mac; GraphPad Software Inc). Multiple regressions were performed in R (version 2.6.2 for Mac OS X). Total and free CORT levels were square-root transformed to achieve normality. We used multiple regression

and linear regression analysis to test the relationships between BCI, GC physiology, age, the ratio of short:long trips, and chick condition. For significant regressions, we report the r^2 . Preliminary analysis indicated no relationship between total CORT or CBG and any test parameter, supporting the hypothesis that free CORT may be the biologically relevant plasma fraction (Breuner and Orchinik 2002). We therefore present only the free CORT results graphically, with total CORT and CBG appearing in table form. We used t -tests to determine if there were differences between males and females in BCI, GC physiology (total CORT, CBG, or free CORT), and the total number or ratio of short:long trips made. We also used t -tests to compare trip statistics (total and ratio of short:long trips) between male-female pairs and female-female pairs.

Receiver Data

Laysan Albatross parents brood the chick for approximately 18 days after hatching until chicks develop endothermy and during this time, they only perform short trips. Given that parental attendance during the brooding stage is dependent on endothermy and not food requirements, we restricted our analysis to trips after chicks were at least 18 days old when parents begin making both long and short trips. Short and long trips in *Procellariiform* seabirds are generally determined by trip duration and foraging location. We categorized short and long foraging trips based on trip lengths from satellite tracking data from other studies in the Hawaiian

Archipelago (Fernandez et al. 2001). We used a cutoff of <6 days for short trips and >6 days for long trips. This 6-day cutoff was validated by assessing the frequency distribution of trip lengths for each nest; in each case, there was a distinct bimodal distribution with a gap at 6 days that represented a cutoff between short and long trips (Figure 1).

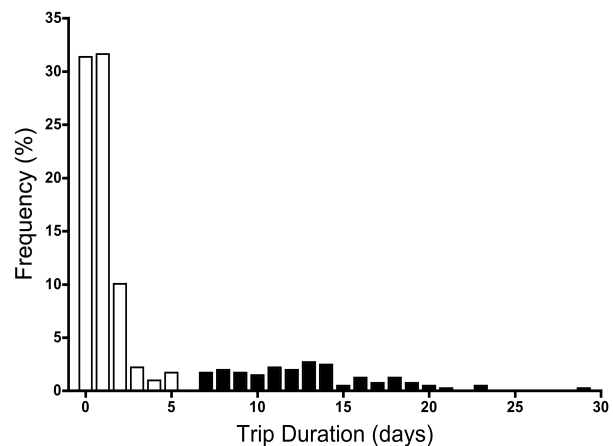


Figure 1. Frequency distribution of foraging trip durations made by Laysan Albatross parents during the chick provisioning period (N = 514 trips).

Age Determination

All albatrosses on Kauai are banded either as chicks or the first year they arrive to breed. Albatrosses have extremely high breeding site philopatry, so the first time a bird is observed breeding is likely to be the first time that bird breeds anywhere. Laysan albatross begin breeding between 6 and 11 years old (Vanryzin and Fisher 1976). Six of the 27 monitored birds on Kauai were banded as chicks, so their exact ages were known (between 8 and 16 years old). For the remaining 21 birds, we assigned a minimum age of 6 years old to the first year they were observed breeding on Kauai. These estimates gave us conservative minimum ages (between >6 and 24 years old) to use in our analysis.

Results

Sex Differences

There was no difference between males and females in arrival BCI, % mass loss during incubation, free CORT measures, or trip statistics (total # trips and the ratio of short:long trips) (Table 1). Because we found no difference between sexes, we pooled males and females for the remainder of analyses in this study. Five of the 14 nests with transmitters were unrelated female-female pairs in long-term pair bonds (Young et al. 2008). There was no difference in trip statistics between male-female and female-female pairs (Table 1).

Table 1

BCI, baseline free CORT, and trip statistics of males v. females and male-female v. female-female pairs.

Males v. Females	Males	Females	df	<i>t</i>	<i>P</i>
arrival BCI	0.14 ± 0.05 (9)	0.13 ± 0.06 (17)	24	0.14	0.89
% mass loss during incubation	-3.12 ± 2.12 (9)	-8.32 ± 2.64 (17)	24	1.32	0.20
[sqrt] baseline free CORT (ng/ml)	0.29 ± 0.02 (9)	0.30 ± 0.04 (17)	19	0.15	0.88
total # trips	32.67 ± 3.55 (9)	32.67 ± 2.45 (18)	25	0.00	1.00
ratio of short:long trips	3.41 ± 0.54 (9)	3.88 ± 0.39 (18)	25	0.70	0.49
Male-Female v. Female-Female Pairs	Male-Female	Female-Female	df	<i>t</i>	<i>P</i>
total # trips	62.00 ± 5.73 (9)	68.60 ± 6.42 (5)	12	0.73	0.48
ratio of short:long trips	3.72 ± 0.35 (9)	3.66 ± 0.59 (5)	12	0.10	0.92

t-tests were used to test for differences between sexes. All data are presented as means ± SE with sample size in parentheses.

Arrival

We found no relationship between BCI and any measure of GC physiology when birds arrived at the beginning of the breeding season (total CORT: $F_{1,24} = 2.16$, $P = 0.16$; CBG: $F_{1,24} = 1.39$, $P = 0.25$; free CORT: $F_{1,24} = 0.70$, $P = 0.41$). No BCI or GC physiology parameters were significant using backwards elimination in multiple regression models with the response variable of the total # trips or the ratio of short:long trips.

Hatching

At the end of incubation (right before chick hatching), there was a strong negative relationship between BCI and both total and free CORT levels (total CORT: $F_{1,19} = 7.12$, $P = 0.02$, $r^2 = 0.27$; CBG: $F_{1,24} = 0.16$, $P = 0.69$; free CORT: $F_{1,19} = 14.55$, $P = 0.001$, $r^2 = 0.43$); birds with a higher BCI had lower free CORT (Figure 2a). Free CORT levels were similarly correlated with the % mass loss during incubation (Figure 2b, $F_{1,19} = 7.55$, $P = 0.013$, $r^2 = 0.28$); birds that lost less weight during incubation had lower free CORT levels. Mean values (\pm SE) for total CORT, CBG, and free CORT at hatching are found in Table 2.

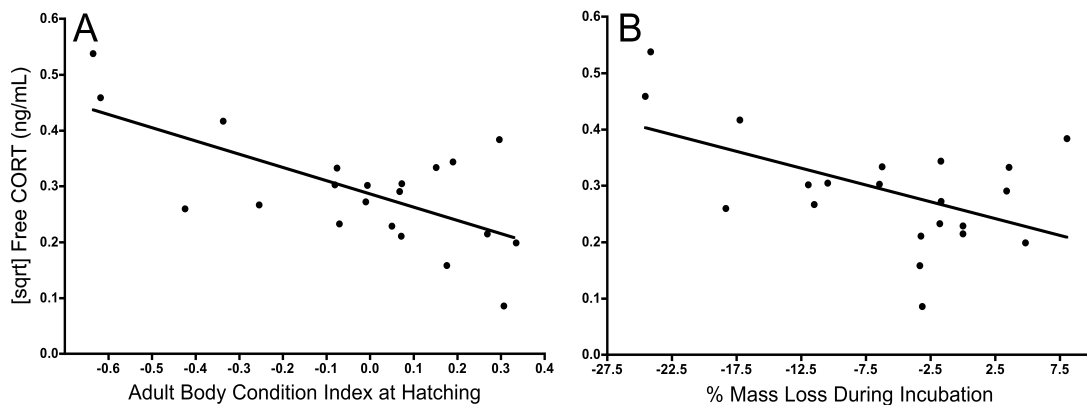


Figure 2. Relationship of free CORT levels to (A) adult body condition index at chick hatching (the end of incubation) and (B) % mass loss during incubation. Adult BCI and % mass loss strongly predicts free CORT levels at hatching: birds in good condition (heavy for their body size) and birds that lost less mass have significantly lower free CORT than do birds in lower condition or birds that lost more mass.

Table 2

Average values of total CORT, CBG, and free CORT at arrival and hatching for breeding Laysan Albatross. All data are presented as means \pm SE.

	Arrival	N	Hatching	N
Total CORT (ng/ml)	3.62 \pm 0.65	26	5.19 \pm 0.72	21
CBG specific binding (nM)	198.02 \pm 12.68	26	187.96 \pm 11.51	21
Free CORT (ng/ml)	0.08 \pm 0.02	26	0.10 \pm 0.01	21

Free CORT level at hatching was the only GC parameter that significantly predicted the ratio of short:long trips made during the 5-month chick provisioning period: birds with low free CORT (in good condition) performed relatively fewer short trips, while birds with high free CORT (in poor body condition) performed more short trips (multiple regression, adjusted $r^2 = 0.15$, $F_{1,17} = 4.28$, $P = 0.05$, Figure 3, Table 3).

Both CBG and free CORT levels predicted the total number of trips made by adults (multiple regression, adjusted $r^2 = 0.31$, $F_{4,14} = 3.05$, $P = 0.05$, Table 3). Though BCI was correlated with free CORT, it was not a significant parameter in either of the multiple regression models predicting adult trip patterns (Table 3).

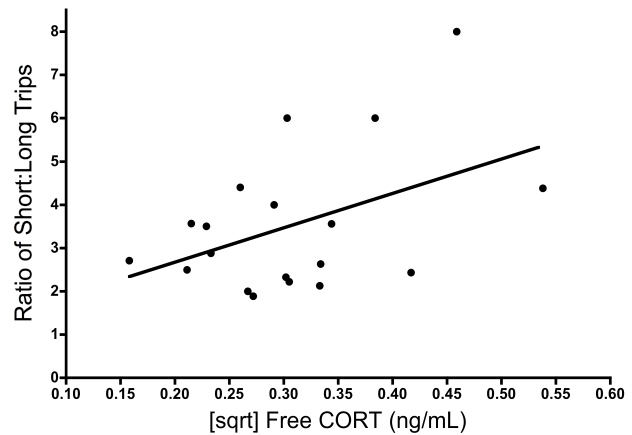


Figure 3. Relationship between adult free CORT at the end of incubation and the ratio of short:long trips performed by adults during chick provisioning.

Table 3

Multiple regression models predicting total # trips or the ratio of short:long trips made by albatross adults.

Parameter	β	r^2	Std. Error	P
<i>Total # Trips</i>				
BCI	0.45	0.11	8.71	0.09
Total CORT	-0.76	-0.06	5.36	0.06
CBG	0.51	0.15	0.05	0.05
Free CORT	1.21	0.27	40.52	0.009
<i>Ratio of short:long trips</i>				
Free CORT	0.45	0.2	3.84	0.05

Age

Age did not predict arrival BCI ($F_{1,24} = 1.28, P = 0.27$), % mass loss during incubation ($F_{1,24} = 0.29, P = 0.60$), total CORT levels at hatching ($F_{1,17} = 0.43, P = 0.51$), or free CORT levels at hatching ($F_{1,17} = 0.56, P = 0.46$). We found a significant relationship between age and CBG capacity at hatching: older birds had higher CBG at the end of incubation (Figure 4, $F_{1,17} = 5.19, P = 0.04, r^2 = 0.23$). Female age did not affect the date of egg lay ($F_{1,12} = 0.09, P = 0.77$).

Adult age significantly predicted the ratio of short:long trips performed: older adults made fewer short trips for every long trip while younger adults made more short trips for every long trip (Figure 5a, ratio of short:long trips: $F_{1,25} = 5.49, P = 0.027, r^2 = 0.18$). This relationship appears to be driven by older adults making more long trips (though not significant). Moreover, older adults started making long trips when their chicks were significantly younger (Figure 5b, $F_{1,25} = 21.57, P < 0.001, r^2 = 0.46$). Adult age did not affect the total number of trips made (total # trips: $F_{1,25} = 0.207, P = 0.653$).

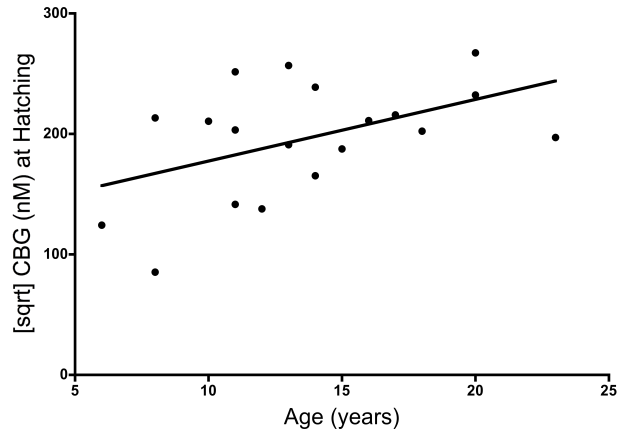


Figure 4. Relationship between age and CBG specific binding (nM) at hatching.

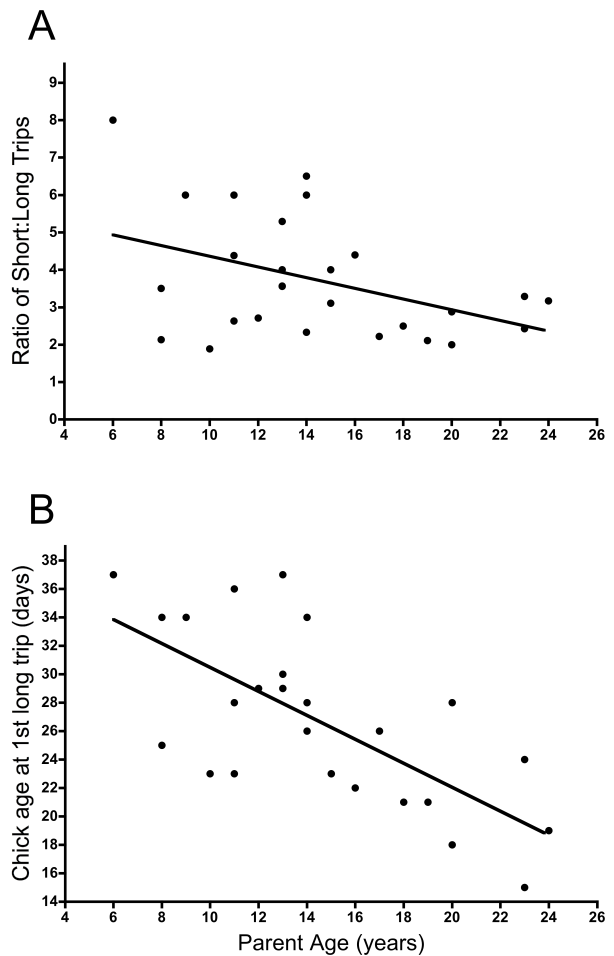


Figure 5. Effect of parent age (years) on the ratio of short:long trips performed (A) and the chick age when parents performed the first long trip (B).

Chick Physiology: Chicks who fledged older tend to fledge with better flight efficiency (R. Sprague, unpublished data). Chicks whose parents performed more short trips fledged at a younger age (Figure 6, $F_{1,10} = 8.501$, $P = 0.015$, $r^2 = 0.46$).

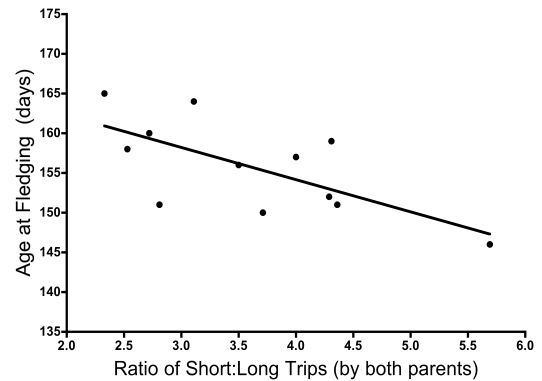


Figure 6. Relationship between the ratio of short:long trips performed on chick age at fledging. Chicks whose parents performed more short trips for every long trip fledged at a younger age.

Discussion

This study examined sources of individual variation in parental behavior during the entire chick rearing period in a long-lived seabird. We found that free CORT and age predicted the pattern of foraging trips adults made. Adults with low free CORT levels (in better condition) made more long trips than did birds with high CORT (in lower condition). Though age did not influence CORT or body condition, older birds also made relatively more long trips than did younger birds. Based on the southern hemisphere paradigm, we had expected to see a higher proportion of short trips from individuals who should be investing highly in reproduction: adults in better condition, adults with lower CORT, and older adults. Instead, we found that birds expected to invest in current effort made more *long* trips and overall, had chicks in better condition that fledged an older age.

Taken together, our results do not support our *a priori* predictions based on results previously reported in species with the same patterns of long and short trips during chick provisioning. Why this apparent disparity between southern hemisphere *Procellariiformes* and Laysan albatross? There are several assumptions inherent in our original predictions. First, based on empirical work in other seabird species, we assumed that for chicks, fledging heavier and older is reflective of greater parental investment and

an indicator of better condition and higher survivorship (Michaud and Leonard 2000; Perrins et al. 1973; Reid et al. 2000; Sagar and Horning 1998). Second, we assumed that the Southern Hemisphere paradigm of short and long trip benefits holds true in all systems in which the same phenomenon of long and short chick provisioning trips is found. Third, based on much previous theoretical and empirical work, we assumed that older birds and birds in good condition with low CORT should invest highly in their offspring (Angelier et al. 2006; Angelier et al. 2007c; Chastel et al. 1995a; Chastel et al. 1995b; Hatch and Westneat 2007; Love et al. 2005; Pugesek 1981; Silverin 1986). Violation of any of these assumptions would clearly change our predictions and interpretation of results. The optimality of chick fledging time and condition is important and under-studied, but here, we focus on alternatives related to parental investment through environmental variation or intrinsic characteristics.

1. Parental Provisioning and Oceanographic Variation

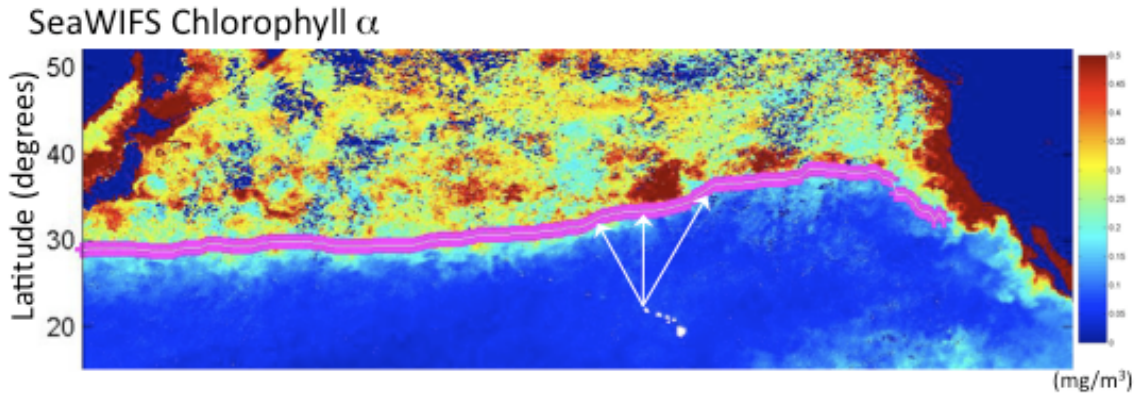
It is possible that, in the northern hemisphere, short trips benefit the adult and long trips benefit chicks. Or, long trips may be beneficial for both adults and chicks but some short trips are still required, particularly for very young chicks that may have the ability to fast for long periods of time. However, based on the similar productivity in cold and warm water in both hemispheres, this is unlikely. Rather, there may be greater variation in the value of short trips between years in the northern hemisphere. The northern and southern hemispheres experience different large-scale oceanographic conditions that could lead to different chick provisioning strategies.

Many southern hemisphere seabirds forage along a continental shelf (Chaurand and Weimerskirch 1994; Weimerskirch et al. 1997). While the food resources along this shelf certainly fluctuate, the location and distance from land generally remains the same (Inchausti et al. 2003). In contrast, in the northern hemisphere, Laysan albatross forage along the mobile Transition Zone Chlorophyll Front (TZCF). The TZCF is the boundary between the warm, nutrient-poor North Pacific Subtropical Gyre and the cold, highly productive Subarctic Gyre. The boundary zone moves both seasonally and interannually,

and affects apex marine vertebrates that forage near Hawaii (Baker et al. 2007; Bograd et al. 2004; Chai et al. 2003; Polovina et al. 2001).

In El Nino years when there is overall warming of the waters near Hawaii and the TZCF moves farther away, species such as the Hawaiian monk seal experience a depleted prey base and lower survival (Baker et al. 2007). The primary foraging location of Laysan albatrosses is north of the TZCF, so their reproductive productivity is also likely to be affected by the distance to the TZCF (Fernandez et al. 2001; Hyrenbach et al. 2002). There are often significant differences in avian reproductive success based on variation in ocean temperatures because of the El Nino Southern Oscillation (ENSO) or other large-scale oceanic variability (Anderson et al. 2007; Buck et al. 2007; Croxall et al. 1999; Jodice et al. 2006a; Jodice et al. 2006b; Kitaysky et al. 2007; Suryan et al. 2006).

In fact, recent evidence indicates an interannual correlation between Laysan albatross reproductive success and distance to the TZCF. In years when the TZCF is farther away, albatross in the Hawaiian Islands make longer trips during chick provisioning and have lower reproductive success (Kappes et al., unpublished manuscript). During the time our study was conducted, in early 2007, the TZCF was farther north than usual (the end of the most recent ENSO event), requiring the Hawaiian seabirds to travel ~10% farther than in average years to reach high food concentrations (Figure 7). It is possible that during 2007 when we were monitoring trips, short trips were particularly depauperate and highly investing adults tried to compensate for this by doing more long trips that would provide more food for the chicks.



Average distance from Kauai to the TZCF in kilometers:

	January	February	March	April	May	June
4yr mean	1384.08	1338.57	1339.12	1506.73	1668.61	1977.37
2007	1481.85	1470.38	1461.50	1457.06	1564.73	1846.30
anomaly	97.77	131.81	122.38	-49.67	-103.88	-131.07

Figure 7. Location of the Transition Zone Chlorophyll Front (TZCF) based on data from the SeaWIFS satellite sea surface chlorophyll α measurements. The location of the TZCF extracted by Michelle Kappes, UCSC, using a protocol developed by Dave Foley, NOAA-SWFSC. Distance to the TZCF was measured at due North, 30 degrees East, and 30 degrees West from Kauai (depicted by the white arrows) for January, February, March, April, May, and June from 2003-2007. The distance (in kilometers) to the TZCF in each month is shown in the accompanying table as the 4 year means (2003-2006), the values for 2007, and the amount that 2007 varied from the mean (anomaly).

2. Age or Quality and Parental Investment

In many long-lived species, reproductive performance and investment increase with age (Angelier et al. 2007c; Mauck et al. 2004; Pyle et al. 1991). This can be ascribed to several explanations, including increased foraging efficiency with age and experience (Curio 1983), increased effort because of declining residual reproductive value (Stearns 1992), or a disappearance of poor-quality individuals, leaving only older, higher-quality individuals (Curio 1983). We found that older birds performed relatively more long trips and also fledged significantly heavier chicks. Recent evidence from Laysan albatrosses also indicates that older parents raise chicks that are more likely to survive and grow rapidly (Dearborn et al. 2008). Older birds may be more efficient at foraging and provisioning as a result of either age-related experience or intrinsic quality (Weimerskirch et al. 2005). Because we did not know the exact amount of food delivered from each foraging trip, we used the number of foraging trips of each type as our measurement of investment. It is possible that older birds deliver more food per trip and thus do not need to make more short trips, a subtlety missed by our measure of the number of trips (Weimerskirch et al. 2005).

We also found that older adults started making long trips when their chicks were significantly younger. Our results could reflect an age or experience-related response to changing environmental conditions if the optimal pattern of short and long trips changes from year to year as the TZCF moves: old birds may have responded faster to particularly bad conditions near Hawaii by starting long trips when their chicks were younger and did more long trips overall. As a result of this strategy, chicks from higher quality or more experienced parents who did more long trips were in better condition at fledging. So older birds may not be more efficient at obtaining food, but may be more experienced with the resource fluctuations in the North Pacific, resulting in greater net efficiency.

Classic life-history theory assumes that all individuals face a survival-reproduction tradeoff where it is not possible to invest maximally in both simultaneously. However, our results could be explained by the existence of high-quality birds with sufficient resources to mask this tradeoff. Rather than age conferring increased experience or efficiency, only high quality birds may survive to old ages. Tradeoffs are thought of as allocations of energy from a resource pool, but some individuals may have a larger pool. In that case, the traits of age and condition in albatrosses may be indicators of intrinsic quality where those individuals have overall more resources, or a greater ability to obtain them (Van Noordwijk and de Jong 1986). High-quality parents may be able to simultaneously make more long trips for themselves and fledge high-quality chicks, while poor-quality parents may be energetically taxed by chick provisioning and cannot provide enough resources to fledge a high-quality chick.

Role of Glucocorticoid Hormones

Glucocorticoid hormones are released in response to stressors (e.g. environmental, social, or physiological), and are transitional hormones, moving animals between life history stages (Orchinik 1998; Wingfield et al. 1998). CORT is classically viewed as a down-regulator of reproductive behavior (Wingfield and Sapolsky 2003). Under this idea, and the southern hemisphere paradigm that short trips benefit chicks at a cost to the parent,

we expected birds with lower CORT to perform more short trips. Instead, we found that birds with higher CORT made more short trips. And surprisingly, their chicks were significantly lighter at fledging. The assumption that high CORT causes birds to transition energy expenditure away from reproduction is not entirely accurate. In some cases, elevated CORT is correlated with *increased* parental effort. In most studies, the CORT elevation is reflective of that effort, not predictive: CORT may be elevated as a result of increased energetic demand in raising offspring (Romero 2002). So birds that have recently invested large amounts of energy into raising offspring may have higher CORT. In this context, high CORT should not be thought of as reducing parental effort. Samples for our study were taken prior to the provisioning trips so CORT levels in them are not reflective of parental effort but could be preparatory for increased energy expenditure during chick provisioning.

Even when thought of in a predictive sense, intermediate levels of CORT could enhance parental effort (Carlson et al. 2006; Doody et al. 2008; Lendvai and Chastel 2008). Elevated CORT below a nest desertion threshold could actually increase parental foraging effort though CORT's facilitation of locomotor activity and foraging behavior in other contexts (Astheimer et al. 1992; Breuner et al. 1998). Some species of seabirds have shown this increased foraging in response to elevated baseline CORT but it is not clear whether foraging time or effort should be equated to parental provisioning (Angelier et al. 2008; Angelier et al. 2007a). Elevated baseline CORT could also be an indicator of poor individual quality. Poor-quality birds may be inefficient foragers (during both incubation and chick provisioning) and thus, may display elevated CORT levels at the end of incubation as a result of increased energy expenditure as they try to breed.

Suggestions for Future Research Directions

More studies are required to elucidate the potential differences in provisioning strategies between the Northern and Southern Hemispheres. First, multi-year studies of North Pacific albatrosses would let us know whether the short:long trip patterns we saw with free CORT and age were plastic or fixed strategies. Do more long trips always produce heavier chicks? The most powerful studies would track individuals to see if provisioning

strategies change with age or are an individual quality characteristic. Secondly, detailed, intra-annual studies would give us the first look at the amount of food brought back from short and long trips and tell us whether quantities changes throughout the season or are constant as assumed from previous work in the Southern Hemisphere. Short trips may be most beneficial early in the season when the chicks are young and the TZCF is close to Hawaii. But as the TZCF moves farther away, more long trips may be best for chick growth. Finally, there is already some work in progress (at University of California at Santa Cruz) using satellite transmitters to examine age-specific foraging locations of Laysan albatross. In the future, more detailed work examining the amounts of food brought back from short and long trips by old and young parents would give us valuable insight into whether some adults are simply higher quality, or whether age imparts experience and higher foraging efficiency that affects how many chick feeding trips parents need to make.

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Chapter 3: TIMING OF FLEDGING IS INFLUENCED BY GLUCOCORTICOID PHYSIOLOGY IN LAYSAN ALBATROSS CHICKS

Abstract

Fledging is a major life transition for birds, moving from the safety of a nest into an environment where they must find food and avoid predators. The timing of fledging within a season can have significant effects on future survival and breeding success. Proximate triggers of fledging are unknown, though wing development is clearly important and several studies have documented elevated plasma corticosterone (CORT) in the days before fledging. Laysan Albatross (*Phoebastria immutabilis*) chicks have an extended post-hatching period of 165 days where they peak at a mass of 1 ½ times the weight of an adult. However, as they approach fledging, chicks fast for days to weeks and lose significant mass while still putting energy into feather growth. We evaluated morphological and physiological measures, and manipulated body mass to identify proximate triggers of fledging. As expected, we found that CORT increased as chicks approached fledging. Interestingly, corticosteroid binding globulin (CBG) declined, thus amplifying free CORT prior to fledging. CORT levels were negatively correlated with body mass while CBG was positively correlated – as chicks fasted longer and lost weight, their CORT levels rose and CBG fell. Finally, free CORT levels of chicks who had reached a morphological fledging threshold predicted how long those chicks would stay at the colony: chicks with higher free CORT fledged sooner. To test for a causal relationship between body condition and endocrine physiology, we manipulated body condition by supplementally feeding chicks for an average of four weeks before fledging (after the majority of skeletal development). Fed birds had a slower decrease in body mass, slower decrease in CBG, slower increase in free CORT, and stayed at the colony longer after reaching a morphological fledging threshold. Our study suggests that free CORT acts as a signal of energetic or nutritional state as the chicks lose mass to adjust the timing of fledging.

Introduction

Across taxa, the transition to independence is a critical life history event. The onset of independence is generally associated with high mortality rates. As a result, an

individual's condition and timing of leaving parental care can have substantive effects on that individual's probability of survival to adulthood (Cooch 2002; Naef-Daenzer et al. 2001; Stempniewicz and Iliszko 2002; Visser and Verboven 1999). For birds, the transition to independence ends parental care and in some cases, requires both an abrupt transition from immobility to sustained flight, and learning to forage independently.

Depending on life history, optimal timing of fledging may be subject to different selective pressures (Roff et al. 2005). Many situations may lead chicks to develop as quickly as possible – often going from hatching to fledging in less than one or two weeks (Remes and Martin 2002). In small songbirds, predation is a huge risk and generally the primary cause of nest failures (Martin 1993; Martin 2002). As a result, there is strong pressure for the chicks to develop quickly and gain mobility to aid in escaping predators. In temperate breeding species, chicks are also under pressure to fledge quickly due to dwindling resources as the season progresses and the need to gain body condition before the change of seasons necessitates migration (Bojarinova et al. 2002; Dawson and Clark 2000). Finally, in multiple-chick nests, leaving the nest sooner allows chicks to more aggressively solicit parental care (Michaud and Leonard 2000; Nilsson and Svensson 1993). For many of these species, the timing of fledging (either date or individual age) has marked effects on survivorship: chicks that fledge earlier in the season have higher survivorship and greater reproductive success as adults (Hetmanski 2007; Naef-Daenzer et al. 2001; Parsons et al. 1976).

In a few cases, these pressures to develop quickly are absent. Some species have a single chick, lack predation, and have access to year-round resources. Seabirds, many with little to no predation, tend to have prolonged incubation and nestling periods. For most of these species, fledging (departure from the nest) and the transition to independence (cessation of parental care) are synonymous. In several cases, chicks fledging later in the season have higher survivorship because the lack of predation allows them to stay, continue to be fed by parents, and thus, fledge in a more developed state (Morbey et al. 1999). Fledging represents an action by the chick but may be influenced by parental behavior (Nilsson and Svensson 1993). Chicks should leave when parental feeding

becomes so infrequent and unpredictable that chicks could obtain more food through independent foraging.

Laysan Albatrosses (*Phoebastria immutabilis*) are pelagic seabirds that have one of the most extreme life history strategies among birds. They lack terrestrial predators, live close to six decades, and raise only one chick in years when they breed (Whittow 1993). The chicks have an extremely long developmental period compared to other birds (65 day incubation, 165 day nestling period), and because parents forage over 2,000 miles away from the colony, chicks must fast for up to several weeks at a time during development. Unlike the majority of bird species (primarily *Passerines*), when albatross chicks leave their natal island on their first flight, they become completely independent of parental care and must learn to forage on their own as they spend the entirety of their first 3-5 years at sea. This represents an abrupt transition to independence and there can be lethal consequences to fledging at the wrong time or in the wrong condition. What proximate triggers do fledglings use to time this important life-history transition?

Glucocorticoids

Glucocorticoid (GC) hormones rise during development in many species and may assist with the transition to independence (reviewed in Wada 2008). Recent studies have demonstrated correlations of GC hormone levels with fledging (Corbel and Groscolas 2008; Heath 1997; Kern et al. 2001). Many factors are correlated with elevated GC secretion in nestlings: sibling competition or social stress (Nilsson and Svensson 1993), reduction in nutritional condition (Kitaysky et al. 1999), an increase in activity (Davies and Few 1973; Luger et al. 1988; Tinnikov 1999), or unpredictability of food delivery (Davis and Levine 1982). The observed increase in GCs may be adaptive during the transition to independence: elevated plasma levels of GCs are associated with an increase in locomotor activity and foraging behavior (Astheimer et al. 1992; Breuner et al. 1998). In some seabird chicks, GCs reflect nutritional condition and quality of food (Kitaysky et al. 2001; Kitaysky et al. 1999; Kitaysky et al. 2005). Thus, elevated GCs observed prior to fledging may help mobilize energy for chicks preparing to fledge, signal declining nutritional condition, and act as a proximate trigger initiating fledging behaviors.

Physiological and behavioral effects of GCs, however, may not depend simply on total plasma concentration. In the plasma, CORT interacts with corticosteroid binding globulin (Breuner and Orchinik 2002). Regulation of plasma CBG levels may allow for elevation or reduction of tissue-levels of GCs, independently of GC secretion. CBG levels may increase slightly with age in White-crowned Sparrow (*Zonotrichia leucophrys nuttalli*) nestlings (Wada et al. 2007). On the other hand, CBG levels decline under conditions of chronic stress (Alexander and Irvine 1998; Lynn et al. 2003; Spencer et al. 1996; Tinnikov 1993) so may decline in fasting seabird chicks approaching fledging. This divergence reinforces the importance of understanding CBG dynamics during development in birds with different life history strategies.

Morphology

Developing chicks also face morphological constraints on the transition to independence. As such, feather growth and wing length are clearly primary predictors of fledging in most species (Berrow et al. 1999; Deguchi et al. 2004; Deguchi and Watanuki 2005; Kern et al. 2001). In addition, mass recession should also be important in avian species where chicks reach a mass much heavier than that of adults prior to fledging: *Procellariiformes*, *Sulidae*, *Phaethontidae*, *Falconiformes*, *Apodidae*, *Hirundinidae*, some *Alcidae*, and Oilbirds (*Steatornis caripensis*). Depending on the species, this mass recession may be chick driven (anorexia or increased nutritional demand) or parent driven (by reduction in feeding rate or meal size) (Berrow and Croxall 2001; Hedd et al. 2002; Mauck and Ricklefs 2005). In both cases, chicks must first reach a certain level of wing development, and then drop below a critical ratio of mass to wing area or wing length in order to physically fly.

In this study, we comprehensively integrated analysis of structural development, mass recession, wing morphology, and GC physiology into understanding the timing of fledging; additionally, we took an experimental approach, by supplementally feeding chicks, to elucidate the triggers for fledging in a species with an abrupt transition to independence. Given the repeated fasting and mass decline in *Procellariiformes*

approaching fledging, we expected that corticosterone (CORT: the primary glucocorticoid in birds) and CBG levels might act as a signal of nutritional condition in Laysan Albatross chicks to finely tune the timing of fledging once chicks have reached an aerodynamic threshold where flight is possible. By supplementing food to late-stage chicks, we predict that CORT levels should drop relative to control birds and this drop should be associated with delayed fledging after reaching a minimum level of morphological development.

Methods

All procedures were approved by the University of Texas Institutional Animal Care and Use Committee (04040503).

Study Site

Fieldwork was conducted at the Kilauea Point National Wildlife Refuge, Kauai, Hawaii (22°13'49.65"N, 159°24'10.08"W) from 23 May to 27 July 2004. In 2004, 136 pairs of Laysan Albatrosses nested on the refuge and surrounding area with 65 chicks fledged. All chicks on Kauai are banded with USFWS bands and engraved plastic bands.

Blood Sample Collection and Morphological Measurements

Laysan Albatrosses nest in shallow depressions on the ground and the chicks do not move far so they can easily be picked up from their nests for blood sampling. We captured 19 chicks every 4 days starting on 23 May (resulting in 175 samples). All blood samples were collected within 3 minutes of initial disturbance (when the chick saw us or began to react, i.e. running away, snapping bill). There was no relationship between blood sample time and CORT levels so our samples represent baseline hormone levels (Romero and Reed 2005). Albatross nest in close groups; to reduce the possibility of disturbance before our capture, we staggered birds so only one bird in a group was caught in any given day. Blood (300-400 µl) was collected from the alar vein using an uncoated 1 ml tuberculin syringe with a 26G needle. Blood was immediately expelled into a BD Microtainer® tube coated with Potassium-EDTA and placed on ice. All samples were centrifuged within 3h, plasma was removed with a Hamilton Syringe and stored at -20°C.

Following blood sampling, we measured flattened wing length (wrist to end of longest primary) using a 60 cm wing ruler. We measured tarsus and culmen length to the nearest 0.1 mm using calipers, and mass to the nearest 50 g using a 5 kg Pesola spring scale. All sampling lasted less than 7 minutes from initial disturbance to when birds were returned to the vicinity of their nest site.

There was a possibility that repeated handling could influence the timing of fledging. To control for this, we had an additional group of chicks that were either captured only once at banding with USFWS bands ($n = 27$) or not at all ($n = 14$) (control groups).

Food Supplementation

In order to experimentally manipulate body condition, we supplemented food to 7 chicks (fed group; a subset of the 19 multi-capture chicks) by feeding them ~150 g fresh squid beginning 14 June (chicks of this age usually lose 100-300 g every 4 days). These chicks were captured every 4 days with the same blood samples and morphological measurements as the other 13 chicks (unfed group).

Determination of Fledging Age

The refuge biologist (B.J. Zaun) recorded hatch date for each chick, so age (within 24 hours) was known for all individuals. We monitored all chicks daily in June and July to obtain exact age at fledging and compare between unfed, fed, and control groups. All chicks fledged between 23 June and 26 July. One multi-capture chick died and was excluded from the analysis. A veterinarian determined the cause of death as blockage from over 200 g of plastic debris removed from the bird's proventriculus and gizzard.

Corticosterone Assay

Plasma CORT levels were measured using a direct radioimmunoassay (after Ketterson et al. 1991; Wingfield and Farner 1975). Briefly, 40-50 μ l of plasma from each individual was equilibrated overnight with distilled water and a tracer of 2000 cpm [3 H]-CORT to calculate recoveries. Samples were extracted with 4 ml diethyl ether, evaporated in a

water bath, and resuspended in 550 μ l phosphate buffer. Recoveries averaged 84% (range = 61-98%). Samples were then assayed in duplicate using CORT antibody (#20-CR45, Fitzgerald Industries) and individual values were later corrected for plasma volumes and recovery values after extraction. Each assay included 3-4 “blanks” (samples consisting of distilled water only). Detectability was determined by calculating the mean minus two standard deviations of the % bound values for the blanks. This established the lowest level of hormone that was significantly different from zero. The average detection limit was 6.33 pg/tube (0.63 ng/ml). Any samples measuring below the detection limit were corrected to that value. Samples were run in two assays; the inter- and intra-assay coefficient of variation were respectively 15.88% and 4.16%.

Corticosteroid Binding Globulin Assay

Plasma CBG levels were measured using a radio-ligand-binding assay with tritiated corticosterone (after Breuner et al. 2003). Samples were stripped of endogenous hormone by incubating in a 1:3 dilution of dextran-coated charcoal for 20 minutes at room temperature and then centrifuged at 4500 rpm for 10 minutes at 4°C.

We first characterized the binding protein for Laysan Albatross chicks. We optimized plasma dilution (1:500), incubation time (2 h), and incubation temperature (4°C). Affinity (K_d) of CORT for CBG was determined in an equilibrium saturation binding experiment where pooled plasma was incubated with [3H]-CORT between 0.23 and 12 nM with or without unlabeled CORT to determine non-specific binding (NSB).

For the point samples assay (identification of individual CBG levels), total binding (TB) was determined using 50 μ l buffer, 50 μ l [³H]-CORT, and 50 μ l diluted plasma. NSB was determined using 50 μ l of 1 μ M unlabeled CORT instead of buffer. Bound and free radioligand were separated using rapid vacuum filtration (Brandel Harvester) over glass fiber filters (Whatman GF/B) soaked in 25 mM Tris with 0.3% PEI for 1 hour prior to filtering. After filtration, radioactivity bound to the filters was measured by liquid scintillation spectroscopy. All samples were run in triplicate in one assay. Intra-assay variation was 11.82%. The inter-filter coefficient of variation was 19.38% determined

from a single plasma pool run 12 times in triplicate (in order for one TB and one NSB of the pool to be on each filter).

Estimation of Free Corticosterone

Free CORT levels were estimated using the mass action-based equation from Barsano and Baumann (1989):

$$H_{free} = 0.5 \times [H_{total} - B_{max} - 1/K_a \pm \sqrt{(B_{max} - H_{total} + 1/K_a)^2 - 4(H_{total}/K_a)}]$$

In the equation, H_{free} = free hormone, H_{total} = total hormone, B_{max} = total binding capacity of CBG, and $K_a = 1/K_d$ (nM). The affinity of CORT for CBG was determined using equilibrium saturation binding analysis on pooled plasma samples. Individual CBG capacity estimates represent approximately 85% of B_{max} , so capacity values were increased to 100% for free CORT calculations. Results are given as means \pm standard errors for all individuals.

Data Analysis

Our study design involved taking repeated samples from individuals. To statistically control for individual differences and avoid pseudoreplication, we used Linear Mixed Effects Models (Holmes et al. 2003) that include individual ID as a random effect in all analyses. Total CORT, CBG, and free CORT data were log-transformed to reach normality. LME models (R version 2.6.2 for Mac OS X) were used to test for changes in morphology and GC physiology within individual chicks as they approached fledging. In most cases, we report the LME results in tables and show scatterplots with trendlines to graphically describe the relationships. We used ANOVA and t -tests (GraphPad Prism version 4.0c for Mac; GraphPad Software Inc.) to compare fledging age between experimental, fed, and control chicks. We also used t -tests to compare morphological, physiological, and behavioral parameters between unfed and fed chicks.

Wing loading (Newtonian mass per unit wing surface area) is often cited as a critical threshold for flight in birds with developmental mass recession approaching fledging (Martins 1997; Wright et al. 2006). However, Pennycuick (1975) suggested that wing

disc loading (WDL: Newtonian mass divided by the area of a disc of which the radius is half the wingspan) may be more aerodynamically important for flight efficiency in gliding birds such as albatrosses. We calculated WDL as $\pi (\frac{1}{2} w)^2$ where w is the wingspan from tip to tip. A lower WDL provides lower induced drag, a lower rate of descent, and generally higher flight efficiency. We did not have wingspan measurements for these chicks so we estimated wingspan based on the correlation between hand wing length (the wrist and primary feather length measure we did have) and wingspan from other birds of this species ($F_{1,35} = 33.17$, $P < 0.0001$, $r^2 = 0.49$, Figure 1., S. Shaffer, unpublished data). We then used the estimated wingspans to calculate WDL and used WDL and wing feather development to conservatively define a fledging threshold. The fledging threshold was defined by two criteria: 1) the chick must have reached the minimum wing length with which any chick successfully fledged, and 2) after reaching that wing length, the fledging threshold was defined as the first day a chick fell under the heaviest WDL with which any chick successfully fledged.

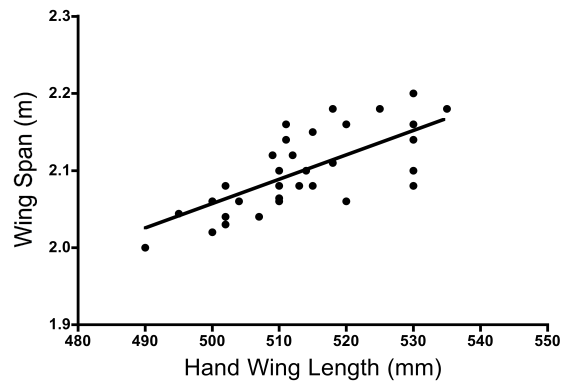


Figure 1. Correlation between hand wing length (wrist to end of longest primary) and wing span in Laysan Albatrosses (N = 37).

Results

Morphology

We confirmed that mass in Laysan Albatross chicks decreases significantly approaching fledging ($F_{1,70} = 13.23$, $P = 0.0005$, $r^2 = 0.22$, Figure 2A). However, tarsus and culmen length were near maximum size by the time we started taking measurements (average adult values (mean \pm SE): tarsus = 103.9 ± 0.24 mm, culmen = 111.7 ± 0.34 mm, Figure 2B, 2C). Primary feather growth was extremely fast, up to 1 cm per day (average adult wing length (mean \pm SE) = 507.8 ± 0.81 mm, Figure 2D).

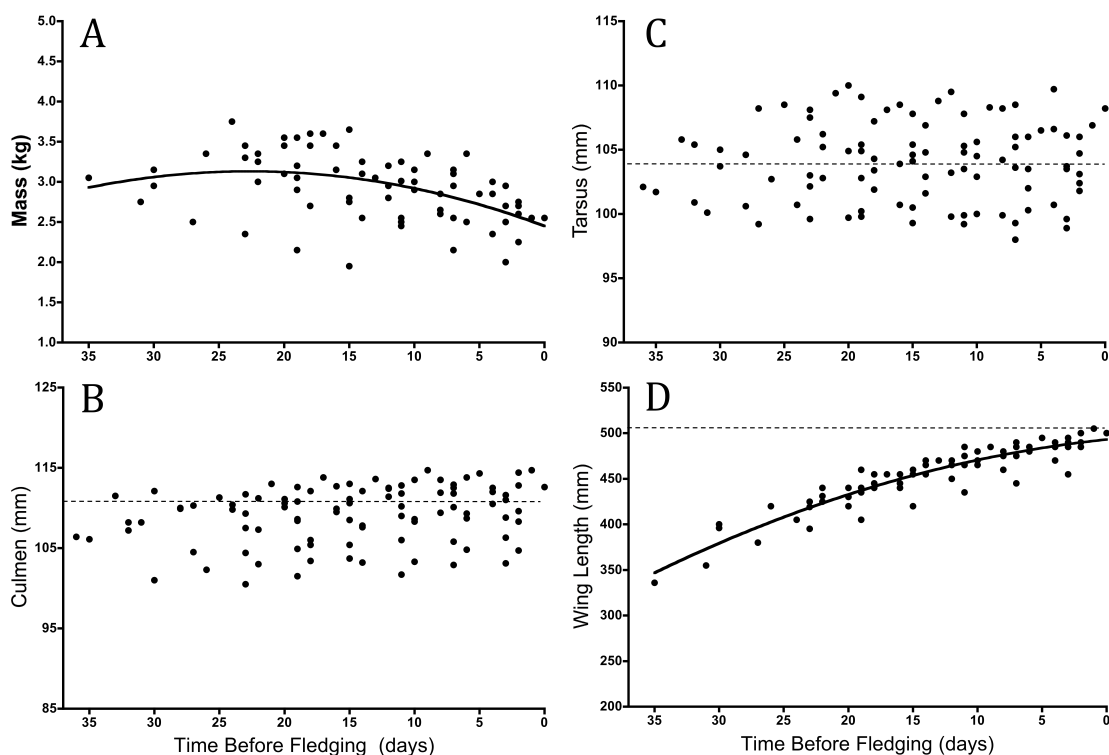


Figure 2. Change in mass (A), culmen (B), tarsus (C), and wing length (D) approaching fledging in Laysan Albatross chicks (N = 12). Solid lines indicate significant relationships. Dashed lines indicate average adult size.

Glucocorticoid Physiology

The equilibrium saturation binding experiment demonstrated a single binding site on CBG for CORT in Laysan Albatross chick plasma ($K_d = 3.3 \pm 0.23$ nM, Figure 3). Total CORT levels significantly increased in unfed chicks as they approached fledging (Table 1, Figure 4A). CBG capacity significantly declined over the same time period (Table 1, Figure 3B). As a result of the concomitant increase in total CORT and decline in CBG capacity, free CORT increased dramatically in unfed chicks nearing fledging (Table 1, Figure 4C).

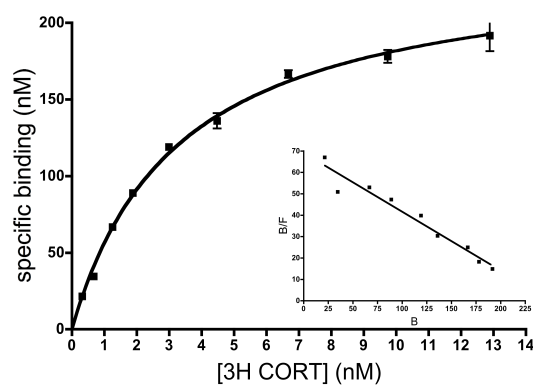


Figure 3. Equilibrium saturation binding of $[^3\text{H}]\text{CORT}$ to Laysan Albatross chick plasma. Data shown are specific binding (means \pm SE at each concentration). Data are best fit by a one-site model with a K_d of 3.3 ± 0.23 nM. Inset: Scatchard-Rosenthal replot of the data.

Table 1. Changes in total CORT, CBG, and free CORT as chicks approach fledging. The fixed effect in all models was “number of days before fledging.” Individual ID was included as a random effect in all models.

Response Variable	Unfed			Fed			Difference Between Groups		
	df	<i>t</i> -value	<i>P</i> -value	df	<i>t</i> -value	<i>P</i> -value	df	<i>t</i> -value	<i>P</i> -value
Total CORT	73	-4.54	<0.0001	61	-1.46	0.15	134	3.39	0.0009
CBG	73	3.53	0.0007	61	1.712	0.09	134	-1.83	0.06
Free CORT	73	-6.12	<0.0001	61	-2.11	0.04	134	4.28	<0.0001

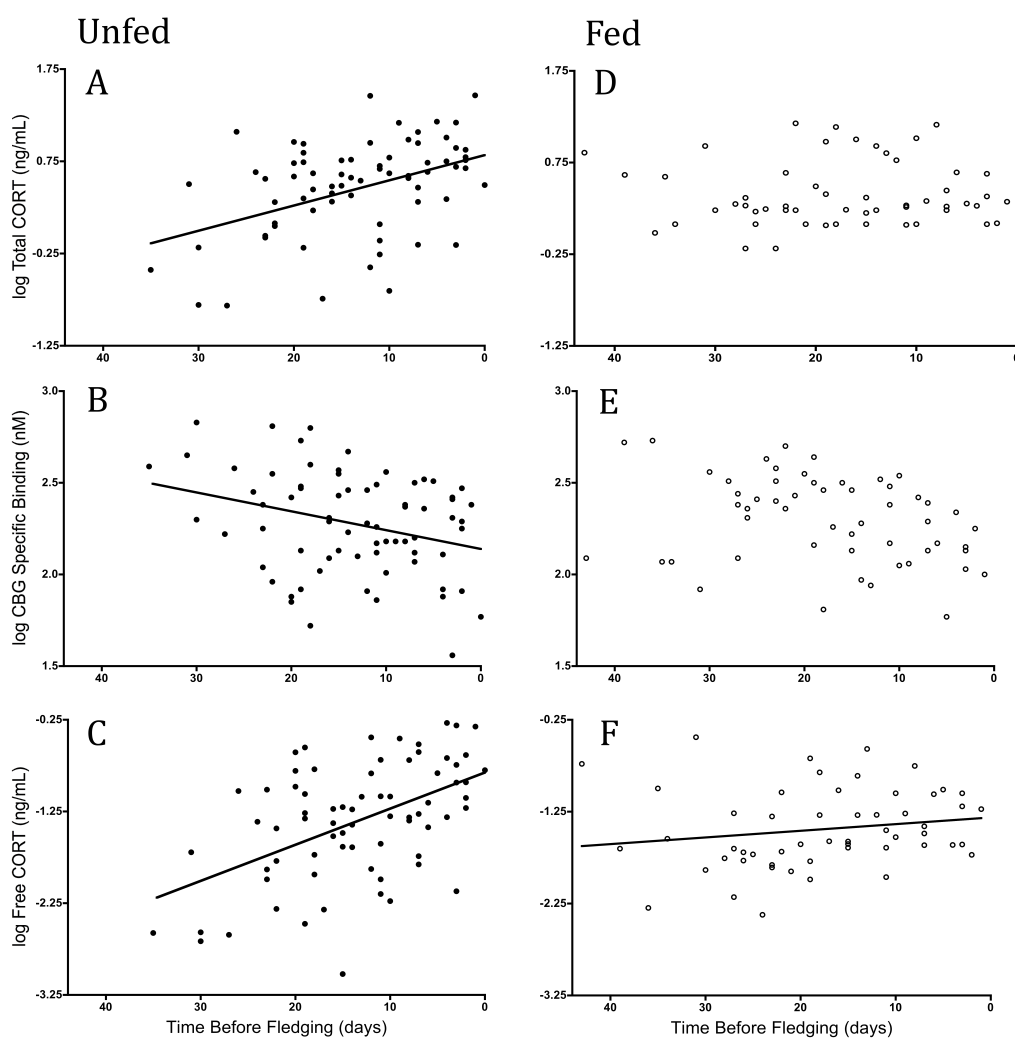


Figure 4. Changes in GC physiology (total CORT: A,D; CBG: B,E; and free CORT: C,F) as unfed (filled circles) and fed (open circles) chicks approach fledging. Solid lines correspond to significant relationships as determined by LME models ($P < 0.05$, Table 1).

Effect of Supplemental Feeding on Morphology and GC Physiology

There was no difference in culmen length between unfed and fed chicks ($F_{1,159} = 0.14$, $P = 0.71$). Supplementally fed chicks had a slightly slower decline in tarsus length than unfed chicks ($F_{1,159} = 2.83$, $P = 0.09$). Wing growth rate also did not differ between unfed and fed groups ($F_{3,120} = 1.64$, $P = 0.18$). Fed chicks declined in mass, although the decline was significantly less rapid than that of unfed chicks ($F_{3,120} = 2.98$, $P = 0.03$).

Total CORT did not significantly increase approaching fledging in the fed chicks (Table 1, Figure 4D). CBG capacity in fed chicks declined but at a slightly slower rate than unfed chicks (Table 1, Figure 4E). Because supplemental feeding eliminated or slowed both the increase in total CORT and decline in CBG, there was only a very slight increase in free CORT approaching fledging and this increase was significantly slower than that of unfed chicks (Table 1, Figure 4F). In fact, free CORT levels measured at the last sample before fledging were significantly lower in fed chicks than in unfed ($df = 16$, $t = 2.76$, $P = 0.01$, Figure 5A).

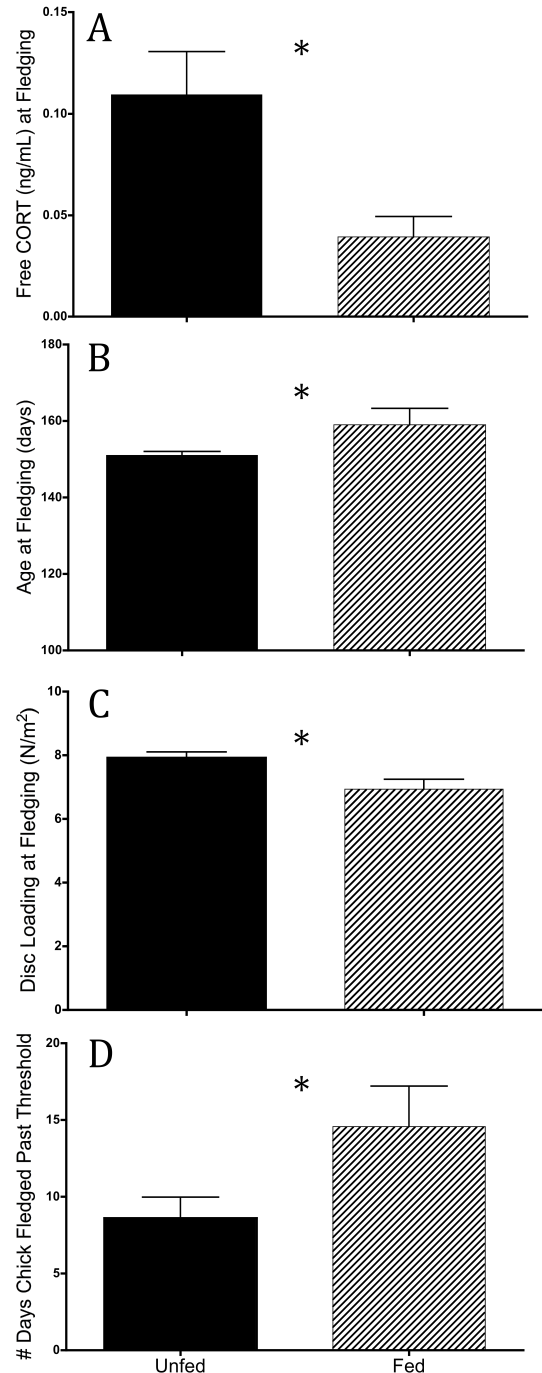


Figure 5. Difference between unfed (solid bars) and fed (dashed bars) chicks in free CORT at fledging (A), age at fledging (B), disc loading at fledging (C), and number of days chicks fledged past threshold (D) (mean \pm SE). Asterisks indicate significant relationships determined from t -tests ($P < 0.05$).

Age at Fledging

The age at fledging was not significantly different between the multiple-capture unfed chicks (mean \pm SE = 148.9 \pm 5.02 days old at fledging) and chicks that were only captured once or not at all (mean \pm SE = 152.1 \pm 6.04 days old at fledging, N = 26; df = 36, $t = 1.59$, $P = 0.12$). Supplementally fed chicks fledged from the colony significantly older than both unfed and control chicks by an average of 9 days (mean \pm SE = 160.5 \pm 11.40 days old at fledging; df = 43, $t = 2.78$, $P = 0.008$, Figure 5B).

Wing Disc Loading and the Fledging Threshold

Wing disc loading (WDL) declined as chicks approached fledging ($F_{1,70} = 112.0$, $P < 0.0001$, $r^2 = 0.56$, Figure 6). Chicks that stayed at the colony longer and fledged older had lower WDL at fledging ($F_{1,17} = 4.65$, $P = 0.045$, $r^2 = 0.21$, Figure 7). Fed and unfed chicks reached the fledging threshold at the same age (df = 18, $t = 1.23$, $P = 0.23$). However, fed chicks fledged with significantly lower WDL (df = 17, $t = 2.98$, $P = 0.008$, Figure 5C). Unfed chicks fledged significantly sooner after reaching the fledging threshold of wing length and WDL (df = 18, $t = 2.23$, $P = 0.038$, Figure 5D). For unfed chicks, free CORT levels at the time of reaching the fledging threshold predicted how many more days the chick would stay before fledging: chicks with higher CORT levels at the threshold fledged sooner (unfed chicks: $F_{1,11} = 5.36$, $P = 0.04$, $r^2 = 0.34$, fed chicks: $F_{1,5} = 0.49$, $P = 0.51$, Figure 8).

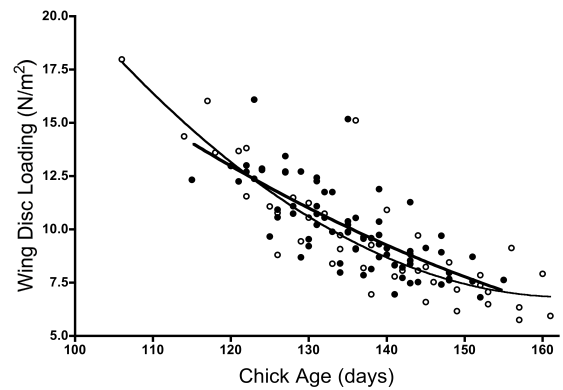


Figure 6. Change in wing disc loading as chicks age. Closed circles (and thick trendline) denote unfed chicks while open circles (and thin trendline) denote fed chicks.

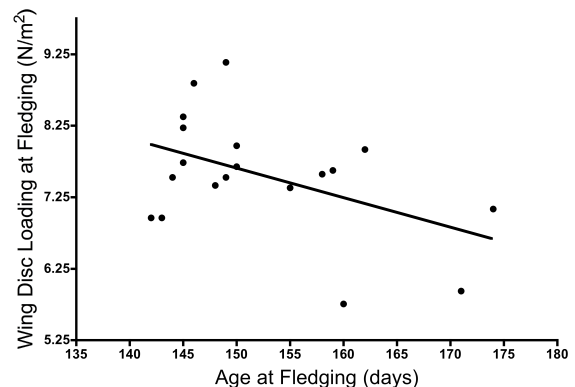


Figure 7. Relationship between age at fledging and disc loading at fledging for Laysan Albatross chicks.

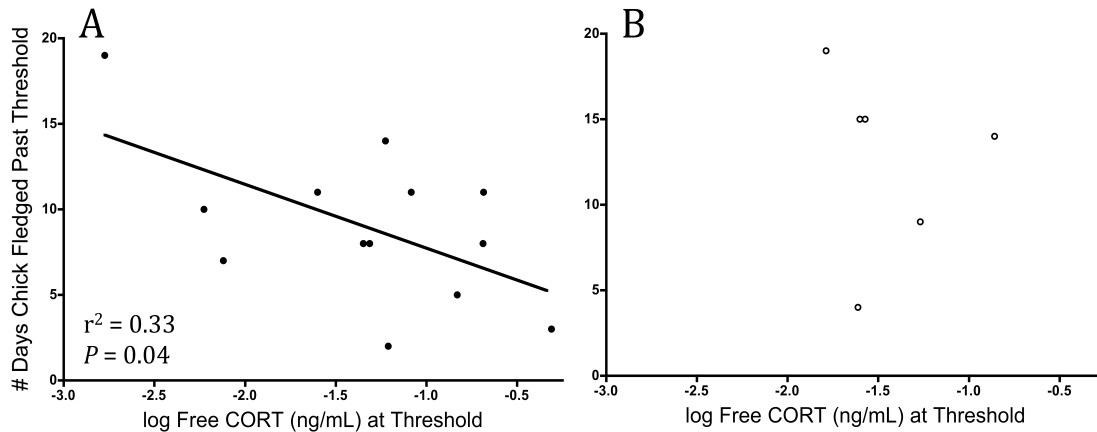


Figure 8. Relationship between free CORT of unfed (A) and fed (B) chicks at the fledging threshold and the number of days those chicks fledged after reaching the threshold. Solid line corresponds to a significant regression ($P < 0.05$).

Discussion

We assessed morphological and physiological changes approaching fledging in a seabird species with an abrupt transition to independence. The most dramatic changes were in wing length, mass, and GC physiology. Wing length increased at close to 1 cm per day while mass significantly declined in the last month before fledging. At the same time, free CORT increased dramatically, driven by a significant increase in total CORT and decrease in CBG capacity. Chicks who fledged older, fledged with lower wing disc loading (WDL), indicating better flight efficiency. Finally, free CORT levels of chicks who had reached a morphological fledging threshold predicted how long those chicks would stay at the colony: chicks with higher CORT fledged sooner.

GC physiology prior to fledging

Across avian species, the range of CORT patterns prior to fledging may depend on the system and abruptness in which the chick departs and becomes independent. A number of species elevate CORT prior to fledging (Belthoff and Dufty 1998; Corbel and Groscolas 2008; Heath 1997; Schwabl 1999; Sims and Holberton 2000). However several species of penguins and owls have no change in CORT before fledging (Corbel et al. 2008; Romero et al. 2006; Walker et al. 2005). In these few cases, chicks are raised on the ground and walk (or swim) off the nest to fledge, which may not be as

physiologically or energetically demanding as flying. For Laysan Albatross chicks, fledging is synonymous with the first flight, cessation of parental care, and departure from the natal colony to spend 3-5 years at sea. Here, elevated CORT levels could be adaptive as Laysan Albatross chicks rapidly shift from a sedentary lifestyle to flying and foraging independently.

Changes in the capacity of CBG in the plasma may further restrict or allow access of CORT to receptors in target tissues. This study is the first to measure both CORT and CBG levels in chicks with the intent of understanding how CBG changes prior to fledging. CBG in chicks has only been measured in one other study where it increased slightly with age in White-crowned Sparrow nestlings (Wada et al. 2007). We found that CBG significantly declined during the mass-recession phase in the last month before fledging. If CBG restricts hormone access to tissues (the free hormone hypothesis), then declining CBG would increase the amount of CORT entering tissues as chicks neared fledging (Breuner and Orchinik 2002).

There are three main hypotheses that explain the rise in CORT (and possibly the decline in CBG) prior to fledging. First, CORT may have a developmentally programmed rise and CBG, a programmed decline prior to fledging, as chicks prepare for the important life history transition to independence. Second, shifts in the behavior and activities of the chicks may change CORT levels. For instance, physical activity causes elevation of plasma CORT so wing exercise as chicks approach fledging could have caused the elevation in CORT (Corbel and Groscolas 2008). Finally, numerous studies tie elevated CORT secretion to poor body condition in both adults and chicks, so declining body mass due to the reduction in parental provisioning prior to fledging may cause the CORT elevation seen in albatross chicks (Breuner and Hahn 2003; Cherel et al. 1988; Kitaysky et al. 1999; Levin et al. 2000; Schwabl 1995; Smith et al. 1994; Wingfield 1994; Wingfield et al. 1994).

The feeding manipulation of chicks attenuated the mass decline, total CORT increase and CBG decline such that at fledging, fed chicks had significantly lower free CORT than

unfed chicks. Because manipulation of mass and energetics affected changes in GC physiology, the full increase in total CORT and decline in CBG is unlikely to be endogenously programmed approaching fledging (Corbel and Groscolas 2008). Increased food intake or more predictable food delivery may cause differences in activity rates that could have explained the differences in CORT secretion between fed and unfed chicks. All chicks did fledge so they must have exercised their wings, but it is not clear whether there was a distinct reduction of wing exercise in fed chicks.

Energetic or nutritional deprivation is the most likely driver of GC physiology in albatross chicks approaching fledging. Kittiwake chicks fed a nutritionally stressful diet (low lipid to protein ratio) have higher CORT levels than chicks fed a high-quality, isocaloric diet (Kitaysky et al. 2001; Kitaysky et al. 1999). In the wild, food restriction induces a faster rise in CORT approaching fledging in White Stork nestlings (Corbel and Groscolas 2008). As albatross chicks fast and lose weight, CORT may rise to assist with energy mobilization and could prompt fledging if energetic stores drop too low. CBG also responds to food restriction, declining under continued fasting, potentially causing a further elevation of free CORT (Cornelius et al. 2004; Lynn et al. 2003). Overall, GC physiology should signal energetic or nutritional state to adjust the timing of fledging after the appropriate morphological development is achieved.

Timing of Fledging

Our supplemental feeding experiment altered the timing of fledging, with fed chicks fledging significantly later than unfed chicks. This delay in fledging time could have been observed for two reasons: 1) feeding may have slowed down pre-fledging mass recession to the point where chicks remained too heavy to fly, so stayed later in order to lose the necessary mass; or 2) chicks may have remained at the colony longer because there was a predictable source of food and fledging later (i.e. more developed) confers a survival benefit.

To discriminate between these alternate explanations, we examined wing disc loading, both as a measure of flight efficiency, and as a way to determine the minimum

aerodynamic requirements for fledging. In order to successfully fledge, chicks must first reach a minimum level of wing development and then lose mass to fall below a maximum amount of disc loading. Lower WDL is equated with lower induced drag, a lower rate of descent, and overall higher flight efficiency. There should be an optimal window for fledging during mass recession: chicks must fall below a critical WDL in order to fly, but should fledge before jeopardizing the energetic reserves they rely on while learning to forage post-fledging. Fed chicks reached a minimum fledging threshold at the same age as unfed chicks. However, fed chicks stayed longer after reaching that threshold and fledged with significantly better flight efficiency. This supports that idea that albatross chicks remain at the colony if food delivery is relatively predictable. Because lower WDL confers improved flight efficiency, it should be beneficial to stay at the colony longer if the chick has enough energetic stores to weather longer fasting.

As chicks remain at the colony growing their wings, they steadily lose mass unless a parent returns to feed them. The origin of pre-fledging mass recession has been attributed to several different sources in the body. Most studies assume that mass recession is the loss of fat reserves (Ricklefs and Schew 1994; Shultz and Sydeman 1997). Since chicks often need to learn to fly and forage independently after fledging, the fat loss hypothesis implies they should leave when their energetic stores become low, but not entirely depleted, so they have some energetic buffer after fledging (Perrins et al. 1973). However, one of the few studies that actually measured body composition during mass recession found that the primary cause of the recession was water loss, not lipid metabolism (Phillips and Hamer 1999). Most mortality for *Procellariiform* chicks appears to be dehydration rather than starvation (R. Sprague, pers. obs.). Because the only external source of both nutrients and water is parental food deliveries, albatross chicks face lethal consequences if they stay at the colony too long in the summer sun regardless of their fat stores.

Summary

Our study indicates there are two primary factors contributing to the timing of fledging in Laysan Albatrosses: wing disc loading and plasma free CORT levels. After Laysan

Albatross chicks reach a critical developmental threshold, chicks may fledge if food delivery is inadequate or stay if it is predictable and plentiful. When food delivery is unpredictable and chicks are losing energetic stores and water, free CORT levels may fine-tune the timing of fledging. Chicks with high free CORT when they reached the fledging threshold left the colony sooner than chicks with low CORT levels. This study suggests that free CORT acts as a signal of energetic or nutritional state as the chicks lose mass to adjust the timing of fledging.

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