Timing of fledging is influenced by glucocorticoid physiology in Laysan Albatross chicks

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ABSTRACT

Fledging is a major life transition for birds, when juveniles move 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 likely a primary factor, other physiological changes, such as elevated plasma corticosterone (CORT), may affect fledging behavior. Laysan Albatross (*Phoebastria immutabilis*) chicks have an extended post−hatching period during which they reach 150% of adult mass. However, approaching fledging, chicks fast for days to weeks and lose mass while still putting energy into feather growth. We evaluated chick morphology and physiology to elucidate proximate triggers of fledging. As in some other species, CORT increased as chicks fasted and lost body mass. At the same time, corticosteroid binding globulin (CBG) declined, thus amplifying free CORT prior to fledging. Once chicks reached a morphological threshold, free CORT levels predicted how long they stayed at the colony: chicks with higher free CORT fledged sooner. To perturb the relationship between body condition, endocrine physiology, and fledging behavior, we supplementally fed chicks for the month before fledging. 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 threshold. Our study suggests that as chicks lose mass, free CORT acts as a signal of energetic or nutritional state to adjust the timing of fledging.

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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 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, 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). Wing development is particularly critical for pelagic species: even when food restricted, Common Murre (*Uria algae*) chicks disproportionately allocate growth to wing development rather

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than body mass (Benowitz-Fredericks et al., 2006). In the single study that has examined the relationship between morphology at fledging and survival, Tufted Puffin (*Fratercula cirrhata*) chicks that fledge with longer wings have better juvenile survivorship (Morrison et al., 2009).

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 2000 mi 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?

Glucocorticoid (GC) hormones rise during development in many species across different taxa and may assist with transitions between life history stages (Wada, 2008). Elevations in GCs are seen prior to parturition or hatching in mammals, birds, lizards, and large fish species (reviewed in Wada, 2008). In mammals, the rapid rise in GCs promotes maturation of many critical organs immediately prior to birth (Liggins, 1994). For amphibians and flatfishes, GCs promote metamorphosis in concert with thyroid hormones (Denver et al., 2002). GCs are also implicated as an important mediator of the transition to independence in birds. 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; Leshner, 1971; 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, 1999, 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 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) and 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.

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 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 *Procellariiforms* 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 (mean lay date = 30 November 2003; mean hatch date = 2 February 2004; mean fledge date = 3 July 2004). 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 chicks 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 min 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 3 h, 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 min 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 captured an additional group of chicks only once at banding with USFWS bands (n = 27, mean = 125 days post hatch), 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 every 4 days beginning 14 June (chicks of this age usually lose 100–300 g every 4 days). Approximately 65% of Laysan Albatross diet is squid (Whittow, 1993) and chicks can gain as much as 1 kg from one parental feeding (R. Sprague, unpublished data). At the time our study was conducted, albatross chicks were undergoing their developmental mass recession, so the 150 g we fed was purely supplemental and not intended to cause chicks to maintain body mass. We collected blood samples and morphological measurements every 4 days from fed chicks in the same way 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 h) 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 μl 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 μl 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 coefficients 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 min at room temperature and then centrifuged at 4500 rpm for 10 min 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 μl buffer, 50 μl [^{3}H]-CORT, and 50 μl diluted plasma. NSB was determined using 50 μl of 1uM unlabeled CORT instead of buffer. Bound and free radioligands 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 h 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):

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

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 ± 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 (LME; 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 π $(1/2\, w)^2$ where w is the wingspan from tip to tip. A lower WDL provides lower induced drag, a lower rate of decent, and generally higher flight efficiency. We did not have wingspan measurements for the chicks in this study so we first examined the correlation between individual hand wing length (wrist and primary feathers) and wingspan from other birds of this species ($F_{1,35}$ = 33.17, $P<0.0001$, r^2 = 0.49, S. Shaffer, unpublished data). We used this correlation and the hand wing length from our birds to calculate an estimate of wingspan for our chicks. 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 minimum wing length, the fledging threshold was defined as the first day a chick fell under the heaviest WDL with which any chick successfully fledged.

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,
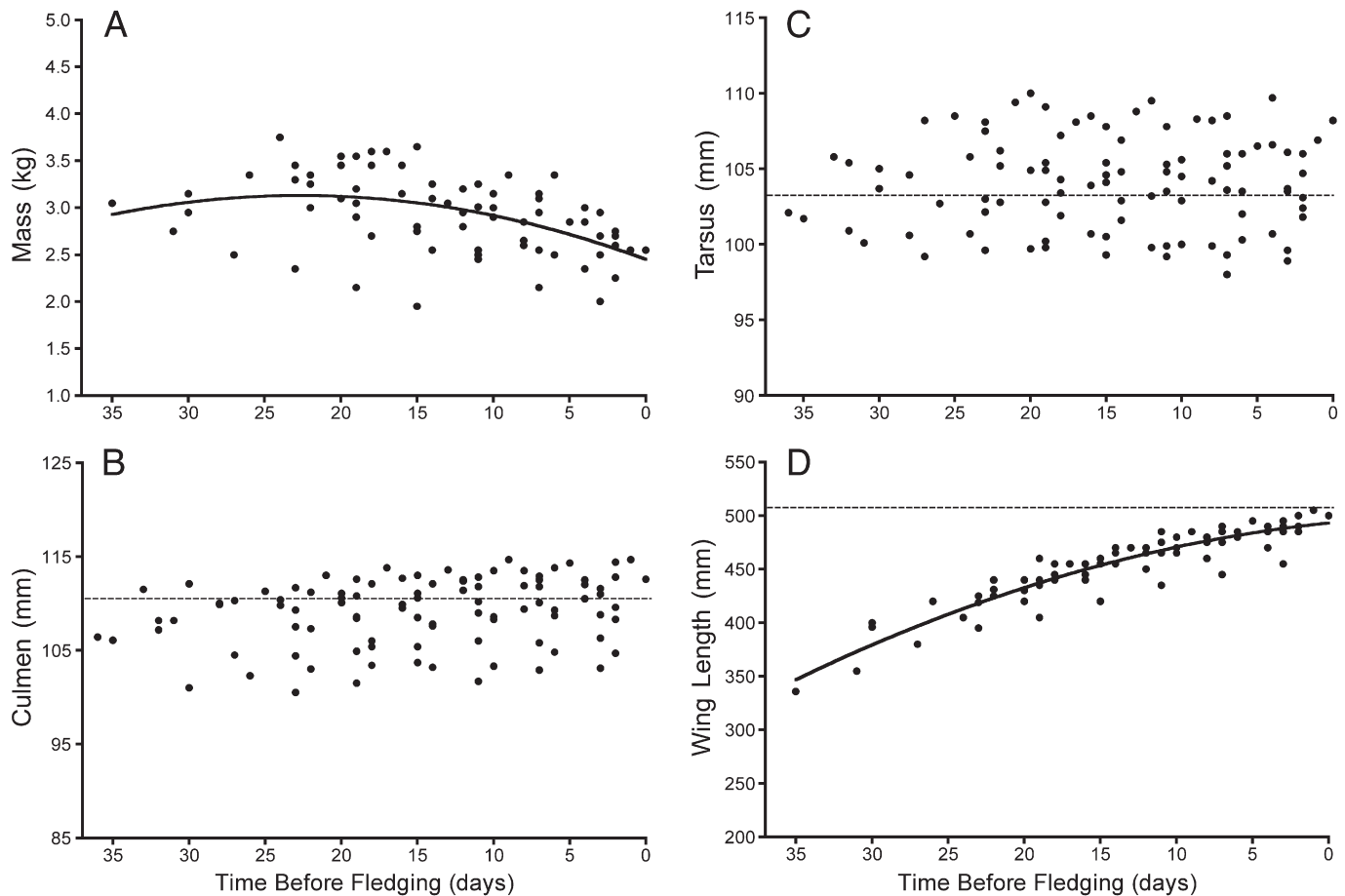
Fig. 1. Change in mass (A), culmen (B), tarsus (C), and wing length (D) approaching fledging in Laysan Albatross chicks ($N = 12$). Solid lines indicate significant relationships. Dashed lines indicate average adult size.

Fig. 1A). However, tarsus and culmen length were near maximum size by the time we started taking measurements (average adult values (mean ± SE): tarsus = 103.9 ± 0.24 mm, culmen = 111.7 ± 0.34 mm, Fig. 1B, C). Note that while tarsus bone length was likely near maximum size, our tarsus measurement declined as the chicks lost weight and fat off their legs. Primary feather growth was extremely fast, up to 1 cm per day (average adult wing length (mean ± SE) = 507.8 ± 0.81 mm, Fig. 1D).

Glucocorticoid physiology

The equilibrium saturation binding experiment demonstrated a single binding site on CBG for CORT in Laysan Albatross chick plasma (Kd = 3.3 ± 0.23 nM, Fig. 2). Total CORT levels significantly increased in unfed chicks as they approached fledging (Table 1, Fig. 3A). CBG capacity significantly declined over the same time period (Table 1, Fig. 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, Fig. 3C).

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 rate of 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, Fig. 3D). CBG capacity in fed chicks declined but at a slightly slower rate than unfed chicks (Table 1, Fig. 3E). 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, Fig. 4F). In fact, free CORT levels measured at the last sample before fledging were significantly lower in fed chicks than in unfed ($df = 16$, $t = 2.76$, $P = 0.01$, Fig. 4A).
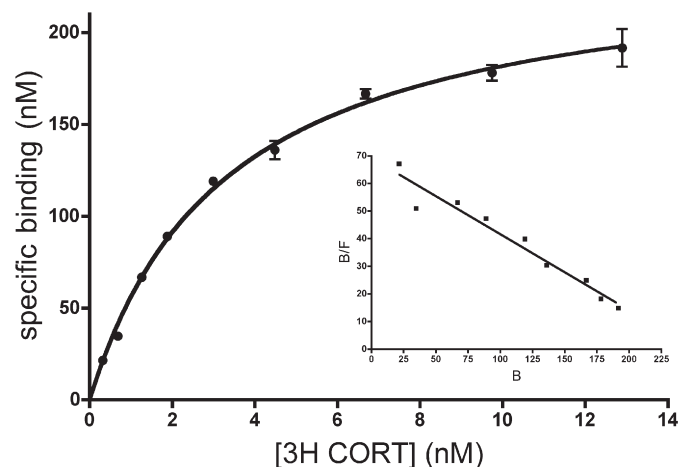

Fig. 2. Equilibrium saturation binding of [³HCORT] to Laysan Albatross chick plasma. Data shown are specific binding (means ± SE at each concentration). Data are best fit by a one-site model with a K_d of 3.3 ± 0.23 nM. Inset: Scatchard–Rosenthal replot of the data.

Table 1
Changes in total CORT, CBG, and free CORT as chicks approach fledging.

Response variable	Unfed			Fed			Difference between groups		
	df	t-value	P-value	df	t-value	P-value	df	t-value	P-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	1.71	0.09	134	−1.83	0.06
Free CORT	73	−6.12	**<0.0001**	61	−2.11	**0.04**	134	4.28	**<0.0001**

The fixed effect in all models was "number of days before fledging."
Individual ID was included as a random effect in every model. Significant variables are indicated in bold.
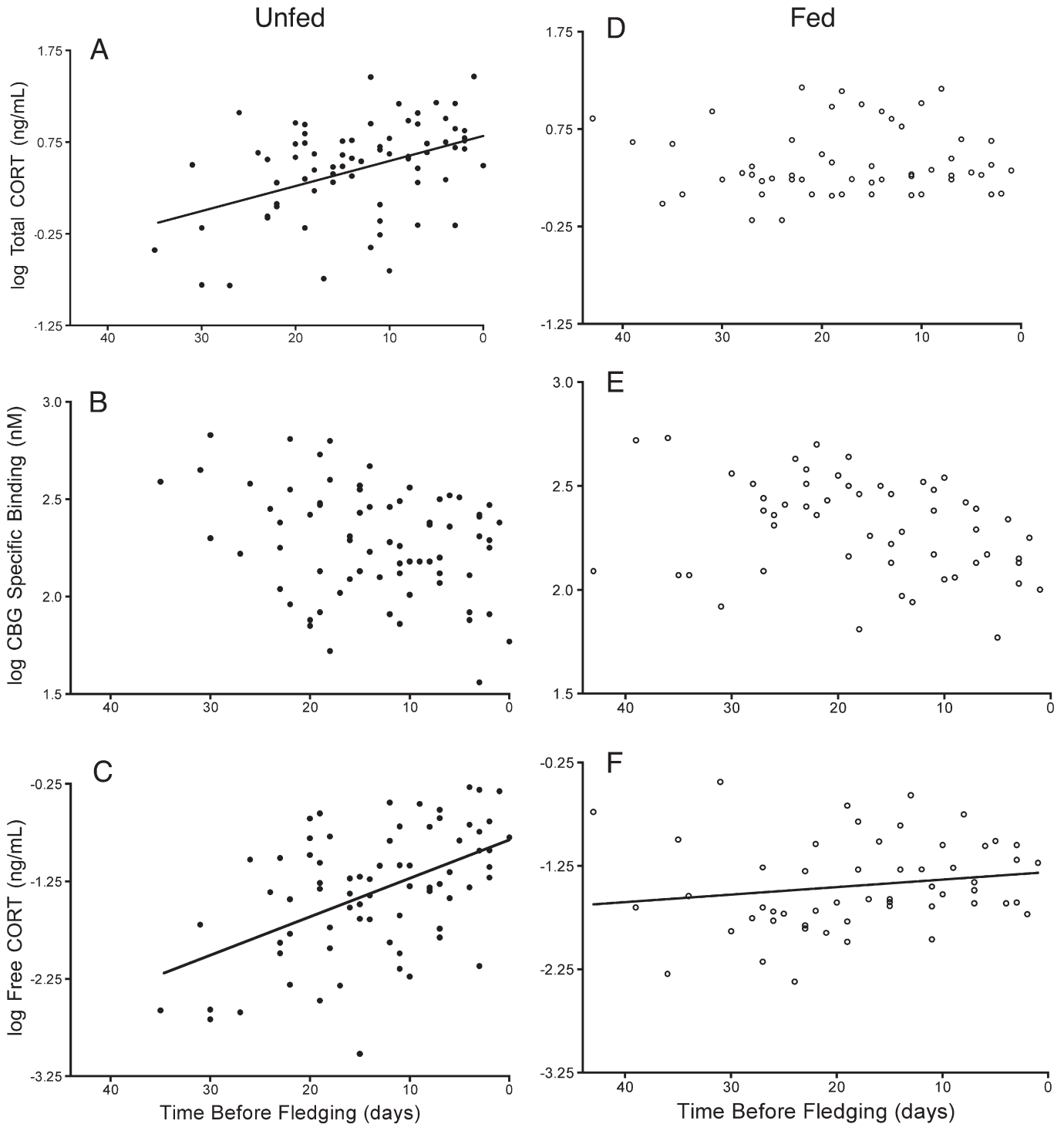

Fig. 3. Changes in GC physiology (total CORT: A, D; CBG: B, E; and free CORT: C, F) as unfed (filled circles) and fed (open circles) chicks approach fledging. Solid lines correspond to significant relationships as determined by LME models ($P<0.05$, Table 1).

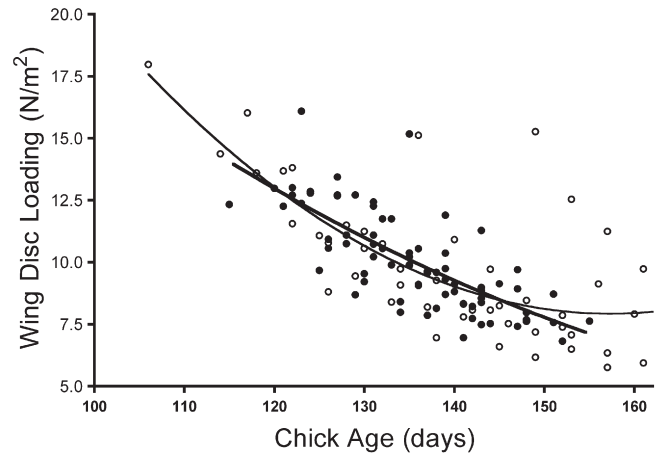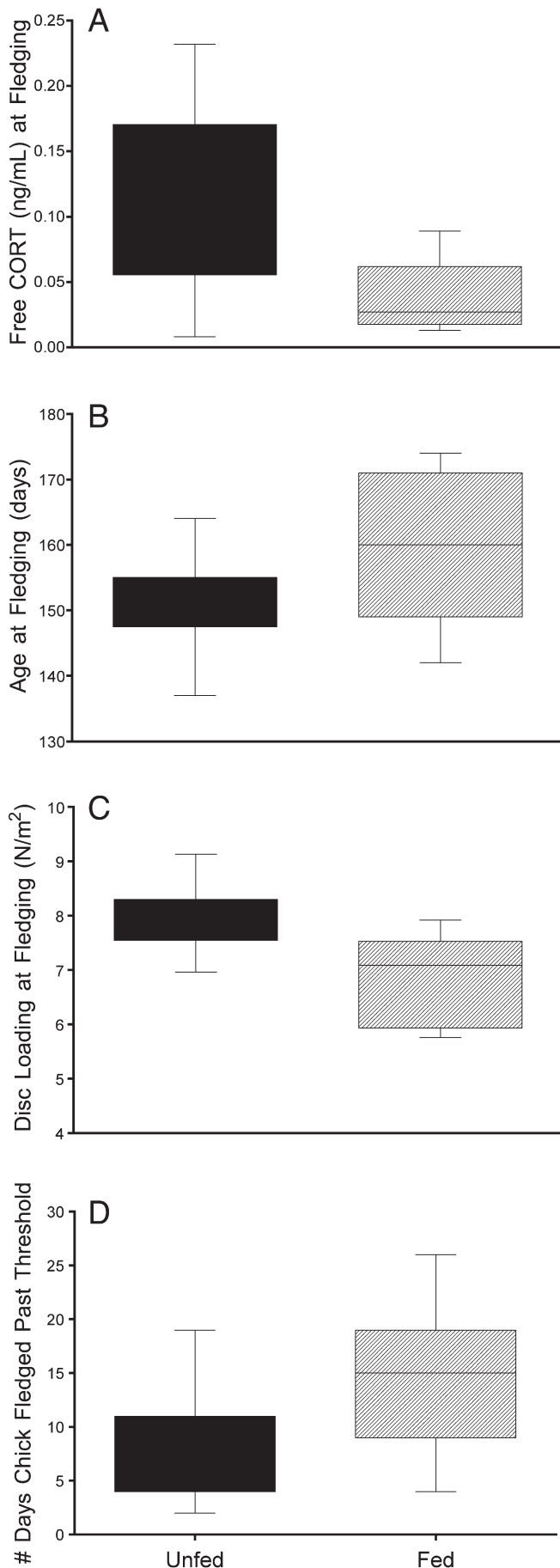**Fig. 5.** Change in wing disc loading as chicks age. Closed circles (and thick trendline) denote unfed chicks while open circles (and thin trendline) denote fed chicks.

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; $df = 43$, $t = 2.78$, $P = 0.008$, Fig. 4B).

Wing disc loading and the fledging threshold

Wing disc loading (WDL) declined as chicks approached fledging ($F_{1,70} = 112.0$, $P < 0.0001$, $r^2 = 0.56$, Fig. 5). Chicks that stayed at the colony longer and fledged older had lower WDL at fledging ($F_{1,17} = 4.65$, $P = 0.045$, $r^2 = 0.21$, Fig. 6). Fed and unfed chicks reached the fledging threshold at the same age ($df = 18$, $t = 1.23$, $P = 0.23$). However, unfed chicks fledged significantly sooner after reaching the fledging threshold of wing length and WDL ($df = 18$, $t = 2.23$, $P = 0.038$, Fig. 5D). Fed chicks fledged with significantly lower WDL ($df = 17$, $t = 2.98$, $P = 0.008$, Fig. 4C). 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: $F_{1,5} = 0.49$, $P = 0.51$, Fig. 7).

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 that fledged at an older age, 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.

Fig. 4. 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 ($P < 0.05$).
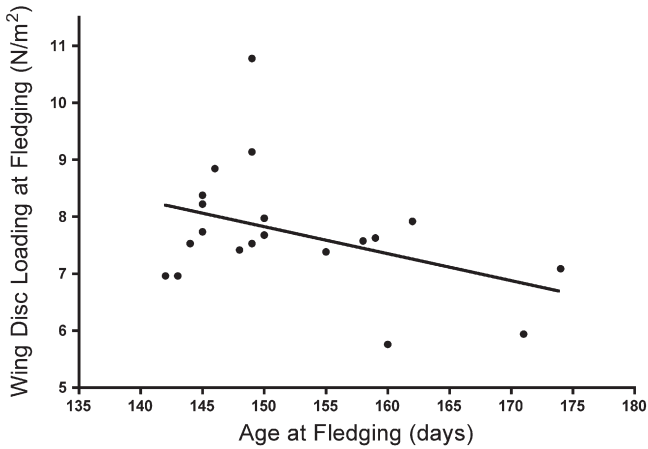
Fig. 6. Relationship between age at fledging and disc loading at fledging for Laysan Albatross chicks.

GC physiology prior to fledging

Across avian species, the range of CORT patterns prior to fledging may depend on life history and the 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 and CBG may have developmentally programmed changes, 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).

To help separate these hypotheses, we supplementally fed chicks to manipulate mass and energy availability. 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.

The linkage of nutritional deprivation and elevated GC physiology may depend strongly on species and life history. A number of species show an increase in CORT and a corresponding increase in begging behavior in response to food deprivation (Kitaysky et al., 2001, 1999; Loiseau et al., 2008). Food restriction induces a faster rise in CORT approaching fledging in White Stork nestlings (Corbel and Groscolas, 2008) and elevated CORT in Steller's sea lions (*Eumetopias jubatus*) (du Dot et al., 2009). On the other hand, food-restricted Tufted Puffin (*F. cirrhata*) chicks mount a behavioral response (increased begging) but do not demonstrate an increase in total or free CORT (Williams et al., 2008). Given the results of this study, energetic or nutritional deprivation is the most likely driver of GC physiology in albatross chicks approaching fledging. As albatross chicks fast and lose weight,
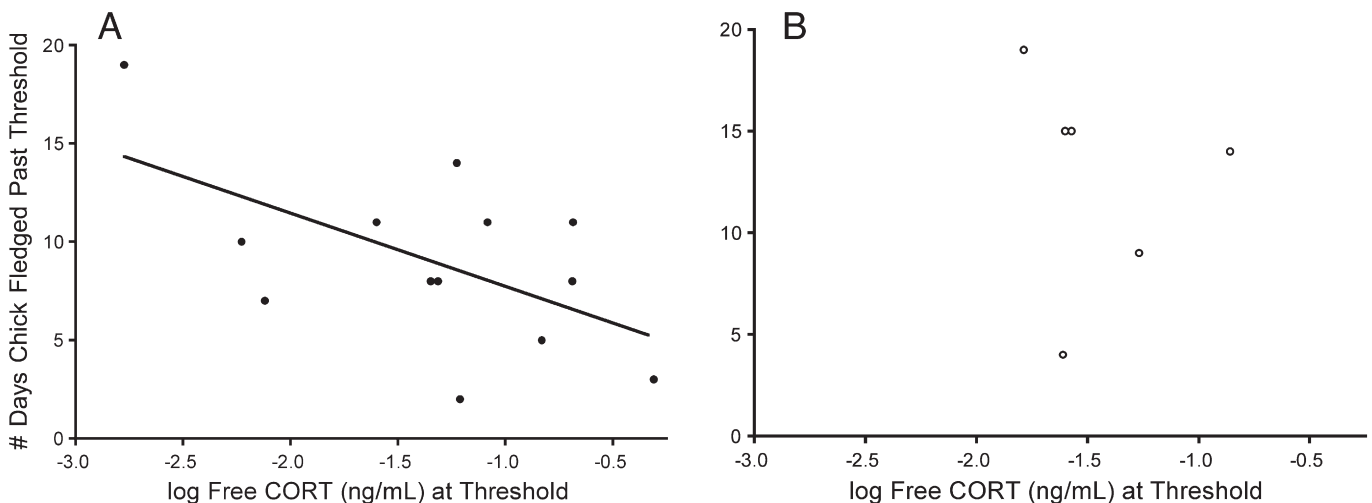

Fig. 7. Relationship between free CORT of unfed (A) and fed (B) chicks at the fledging threshold and the number of days those chicks fledged after reaching the threshold. Solid line corresponds to a significant regression (P<0.05).

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. We found that free CORT levels predicted the amount of time chicks stayed at the colony after they reached a morphological fledging threshold: chicks with higher CORT fledged sooner after reaching the threshold. The significance of this relationship is strongly influenced by the individuals with the highest and lowest free CORT at the fledging threshold that fledged 3 and 19 days later, respectively (Fig. 7A). Removal of either individual makes the relationship non-significant but the direction of the relationship remains.

The relationship between GC physiology and energetic state in fledglings is mirrored in other life history transitions such as dispersal and migration. CORT is often elevated prior to juvenile dispersal in birds (Belthoff and Dufty, 1998; Silverin, 1997), mammals (Mateo, 2006), and reptiles (Hamann et al., 2007), allowing for mobilization of energy and promoting locomotor activity. For migrating animals, CORT may be elevated immediately after arrival at stopover sites (Landys-Ciannelli et al., 2002), and animals that have been re-feeding exhibit lowered plasma CORT titers corresponding to the replenishment of energetic stores (Astheimer et al., 1992; du Dot et al., 2009; Landys-Ciannelli et al., 2002). Thus, in both migrating adults and fledgling juvenile albatross, energetic depletion (low resource availability in migratory birds and fledglings) is associated with elevated CORT, while feeding reduces CORT. Variation between strategies is apparent in the effect of CORT on activity, in that elevated CORT is associated with cessation in migratory birds, whereas elevated CORT induces earlier fledging and extended flight in fledglings.

Timing of fledging

Fledging in birds is an important life-history transition and the timing should be influenced by individual physiological and morphological state. 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 the idea that albatross chicks remain at the colony to grow their wings 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 that 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 (Cherel et al., 1987; 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 that 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.

Acknowledgments

References

Alexander, S.L., Irvine, C.H.G., 1998. The effect of social stress on adrenal axis activity in horses: the importance of monitoring corticosteroid-binding globulin capacity. J. Endocrinol. 157, 425–432.

Astheimer, L.B., Buttemer, W.A., Wingfield, J.C., 1992. Interactions of corticosterone with feeding, activity, and metabolism in *Passerine* birds. Ornis Scandinavica 23, 355–365.

Barsano, C.P., Baumann, G., 1989. Simple algebraic and graphic methods for the apportionment of hormone (and receptor) into bound and free fractions in binding equilibria - or how to calculate bound and free hormone. Endocrinology 124, 1101–1106.

Belthoff, J.R., Dufty, A.M., 1998. Corticosterone, body condition and locomotor activity: a model for dispersal in screech-owls. Anim. Behav. 55, 405–415.

Benowitz-Fredericks, Z.M., Kitaysky, A.S., Thompson, C.W., 2006. Growth and allocation in captive common murre (*Uria aalge*) chicks. Auk 123, 722–734.

Berrow, S.D., Croxall, J.P., 2001. Provisioning rate and attendance patterns of Wandering Albatrosses at Bird Island, South Georgia. Condor 103, 230–239.

Berrow, S.D., Huin, N., Humpidge, R., Murray, A.W.A., Prince, P.A., 1999. Wing and primary growth of the Wandering Albatross. Condor 101, 360–368.

Bojarinova, J.G., Rymkevich, T.A., Smirnov, O.P., 2002. Timing of autumn migration of early and late-hatched Great Tits *Parus major* in NW Russia. Ardea 90, 401–409.

Breuner, C.W., Hahn, T.P., 2003. Integrating stress physiology, environmental change, and behavior in free-living sparrows. Horm. Behav. 43, 115–123.

Breuner, C.W., Orchinik, M., 2002. Plasma binding proteins as mediators of corticosteroid action in vertebrates. J. Endocrinol. 175, 99–112.

Breuner, C.W., Greenberg, A.L., Wingfield, J.C., 1998. Noninvasive corticosterone treatment rapidly increases activity in Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). Gen. Comp. Endocrinol. 111, 386–394.

Breuner, C.W., Wada, H., Shyu, J., Love, O.P., 2003. Corticosteroid binding globulin capacity responds to chronic hormone treatment but not acute stressors. Integr. Comp. Biol. 43, 1013-1013.

Cherel, Y., Stahl, J.-C., Yvon Le, M., 1987. Ecology and physiology of fasting in King Penguin chicks. Auk 104, 254–262.

Cherel, Y., Robin, J.P., Walch, O., Karmann, H., Netchitailo, P., Lemaho, Y., 1988. Fasting in King Penguin: 1. Hormonal and metabolic changes during breeding. Am. J. Physiol. 254, R170–R177.

Cooch, E.G., 2002. Fledging size and survival in snow geese: timing is everything (or is it?). J. Appl. Stat. 29, 143–162.

Corbel, H., Groscolas, R., 2008. A role for corticosterone and food restriction in the fledging of nestling White storks. Horm. Behav. 53, 557–566.

Corbel, H., Morlon, F., Groscolas, R., 2008. Is fledging in king penguin chicks related to changes in metabolic or endocrinal status? Gen. Comp. Endocrinol. 155, 804–813.

Cornelius, J.M., Breuner, C.W., Hahn, T.P., 2004. Effects of food reduction on stress reactivity in an irruptive nomad, the red crossbill. Integr. Comp. Biol. 44, 684–684.

Davies, C.T.M., Few, J.D., 1973. Effects of exercise on adrenocortical function. J. Appl. Physiol. 35, 887–891.

Davis, H., Levine, S., 1982. Predictability, control, and the pituitary-adrenal response in rats. Journal of Comparative and Physiological Psychology 96, 393–404.

Dawson, R.D., Clark, R.G., 2000. Effects of hatching date and egg size on growth, recruitment, and adult size of Lesser Scaup. Condor 102, 930–935.

Deguchi, T., Watanuki, Y., 2005. Prefledging mass recession and timing of fledging in Rhinoceros Auklets Cerorhinca monocerata. Ibis 147, 217–220.

Deguchi, T., Takahashi, A., Watanuki, Y., 2004. Proximate factors determining age and mass at fledging in Rhinoceros Auklets (Cerorhinca monocerata): Intra-and interyear variations. Auk 121, 452–462.

Denver, R.J., Boorse, G.C., Glennemeier, K.A., 2002. Endocrinology of complex life cycles: amphibians. In: Pfaff, D.E.A. (Ed.), Hormones, Brain, and Behavior. Academic Press, Inc., San Diego, pp. 469–513.

du Dot, T.J., Rosen, D.A.S., Richmond, J.P., Kitaysky, A.S., Zinn, S.A., Trites, A.W., 2009. Changes in glucocorticoids, IGF-I and thyroid hormones as indicators of nutritional stress and subsequent refeeding in Steller sea lions (Eumetopias jubatus). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 152, 524–534.

Hamann, M., Jessop, T.S., Schauble, C.S., 2007. Fuel use and corticosterone dynamics in hatchling green sea turtles (Chelonia mydas) during natal dispersal. J. Exp. Mar. Biol. Ecol. 353, 13–21.

Heath, J., 1997. Corticosterone levels during nest departure of juvenile American Kestrels. Condor 99, 806–811.

Hedd, A., Gales, R., Brothers, N., 2002. Provisioning and growth rates of Shy Albatrosses at Albatross Island, Tasmania. Condor 104, 12–29.

Hetmanski, T., 2007. The timing of fledging and annual post-fledging survival of juvenile Feral Pigeons, Columba livia, in a city area (Pomerania, NW Poland). Pol. J. Ecol. 55, 367–375.

Holmes, D.J., Ottinger, M.A., Ricklefs, R.E., Finch, C.E., 2003. SOSA-2: Introduction to the Proceedings of the Second Symposium on Organisms with Slow Aging. Exp. Gerontol. 38, 721–722.

Kern, M., Bacon, W., Long, D., Cowie, R.J., 2001. Possible roles for corticosterone and critical size in the fledging of nestling pied flycatchers. Physiol. Biochem. Zool. 74, 651–659.

Ketterson, E.D., Nolan, V., Wolf, L., Ziegenfus, C., Dufty, A.M., Ball, G.F., Johnsen, T.S., 1991. Testosterone and avian life histories—the effect of experimentally elevated testosterone on corticosterone and body-mass in Dark-eyed Juncos. Horm. Behav. 25, 489–503.

Kitaysky, A.S., Piatt, J.F., Wingfield, J.C., Romano, M., 1999. The adrenocortical stress-response of Black-legged Kittiwake chicks in relation to dietary restrictions. J. Comp. Physiol. [B] 169, 303–310.

Kitaysky, A.S., Kitaiskaia, E.V., Wingfield, J.C., Piatt, J.F., 2001. Dietary restriction causes chronic elevation of corticosterone and enhances stress response in Red-legged Kittiwake chicks. J. Comp. Physiol. [B] 171, 701–709.

Kitaysky, A.S., Romano, M.D., Piatt, J.F., Wingfield, J.C., Kikuchi, M., 2005. The adrenocortical response of Tufted Puffin chicks to nutritional deficits. Horm. Behav. 47, 606–619.

Landys-Ciannelli, M.M., Ramenofsky, M., Piersma, T., Jukema, J., Wingfield, J.C., 2002. Baseline and stress-induced plasma corticosterone during long-distance migration in the bar-tailed godwit, Limosa lapponica. Physiol. Biochem. Zool. 75, 101–110.

Leshner, A.I., 1971. Adrenals and regulatory nature of running wheel activity. Physiology and Behavior 6, 551–558.

Levin, B.E., Richard, D., Michel, C., Servatius, R., 2000. Differential stress responsivity in diet-induced obese and resistant rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 279, R1357–R1364.

Liggins, G.C., 1994. The role of cortisol in preparing the fetus for birth. Reprod. Fertil. Dev. 6, 141–150.

Loiseau, C., Sorci, G., Dano, S., Chastel, O., 2008. Effects of experimental increase of corticosterone levels on begging behavior, immunity and parental provisioning rate in house sparrows. Gen. Comp. Endocrinol. 155, 101–108.

Lynn, S.E., Breuner, C.W., Wingfield, J.C., 2003. Short-term fasting affects locomotor activity, corticosterone, and corticosterone binding globulin in a migratory songbird. Horm. Behav. 43, 150–157.

Martin, T.E., 1993. Nest predation among vegetation layers and habitat types—revising the dogma. Am. Nat. 141, 897–913.

Martin, T.E., 2002. A new view of avian life-history evolution tested on an incubation paradox. Proc. R. Soc. Lond. B Biol. Sci. 269, 309–316.

Martins, T.L.F., 1997. Fledging in the common swift, Apus apus: weight-watching with a difference. Anim. Behav. 54, 99–108.

Mateo, J.M., 2006. Developmental and geographic variation in stress hormones in wild Belding's ground squirrels (Spermophilus beldingi). Horm. Behav. 50, 718–725.

Mauck, R.A., Ricklefs, R.E., 2005. Control of fledging age in Leach's Storm-Petrel, Oceanodroma leucorhoa: chick development and prefledging mass loss. Funct. Ecol. 19, 73–80.

Michaud, T., Leonard, M., 2000. The role of development, parental behavior, and nestmate competition in fledging of nestling Tree Swallows. Auk 117, 996–1002.

Morbey, Y.E., Ydenberg, R.C., Knechtel, H.A., Harfenist, A., 1999. Parental provisioning, nestling departure decisions and prefledging mass recession in Cassin's auklets. Anim. Behav. 57, 873–881.

Morrison, K.W., Hipfner, J.M., Gjerdrum, C., Green, D.J., 2009. Wing length and mass at fledging predict local juvenile survival and age at first return in Tufted Puffins. Condor 111, 433–441.

Naef-Daenzer, B., Widmer, F., Nuber, M., 2001. Differential post-fledging survival of great and coal tits in relation to their condition and fledging date. J. Anim. Ecol. 70, 730–738.

Nilsson, J.A., Svensson, M., 1993. Fledging in altricial birds—parental manipulation or sibling competition. Anim. Behav. 46, 379–386.

Parsons, J., Chabrzyk, G., Duncan, N., 1976. Effects of hatching date on post-fledging survival in Herring Gulls. J. Anim. Ecol. 45, 667–675.

Pennycuick, C.J., 1975. Mechanics of Flight. In: Farner, D.S., et al. (Ed.), Avian Biology. Academic Press, New York, pp. 1–76.

Perrins, C.M., Harris, M.P., Britton, C.K., 1973. Survival of Manx Shearwaters Puffinus puffinus. Ibis 115, 535–548.

Phillips, R.A., Hamer, K.C., 1999. Lipid reserves, fasting capability and the evolution of nestling obesity in procellariiform seabirds. Proc. R. Soc. Lond. B Biol. Sci. 266, 1329–1334.

Remes, V., Martin, T.E., 2002. Environmental influences on the evolution of growth and development in Passerines. Evolution 56, 2502–2518.

Ricklefs, R.E., Schew, W.A., 1994. Foraging stochasticity and lipid-accumulation by nestling petrels. Funct. Ecol. 8, 159–170.

Roff, D.A., Remes, V., Martin, T.E., 2005. The evolution of fledging age in songbirds. J. Evol. Biol. 18, 1425–1433.

Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comp. Biochem. Physiol. A Mol. Integr. Physiol. 140, 73–79.

Romero, L.M., Holt, D.W., Maples, M., Wingfield, J.C., 2006. Corticosterone is not correlated with nest departure in snowy owl chicks (Nyctea scandiaca). Gen. Comp. Endocrinol. 149, 119–123.

Schwabl, H., 1995. Individual variation of the acute adrenocortical response to stress in the White-throated Sparrow. Zool. Anal. Compl. Syst. 99, 113–120.

Schwabl, H., 1999. Developmental changes and among-sibling variation of corticosterone levels in an altricial avian species. Gen. Comp. Endocrinol. 116, 403–408.

Shultz, M., Sydeman, W.J., 1997. Pre-fledging weight recession in Pigeon Guillemots on Southeast Fallalon Island, California. Colon. Waterbirds 20, 436–448.

Silverin, B., 1997. The stress response and autumn dispersal behaviour in willow tits. Anim. Behav. 53, 451–459.

Sims, C.G., Holberton, R.L., 2000. Development of the corticosterone stress response in young Northern Mockingbirds (Mimus polyglottos). Gen. Comp. Endocrinol. 119, 193–201.

Smith, G.T., Wingfield, J.C., Veit, R.R., 1994. Adrenocortical response to stress in the Common Diving Petrel Pelecanoides urinatrix. Physiol. Zool. 67, 526–537.

Spencer, R.L., Miller, A.H., Moday, H., McEwen, B.S., Blanchard, R.J., Blanchard, D.C., Sakai, R.R., 1996. Chronic social stress produces reductions in available splenic type II corticosteroid receptor binding and plasma corticosteroid binding globulin levels. Psychoneuroendocrinology 21, 95–109.

Stempniewicz, L., Iliszko, L., 2002. Body size and timing of fledging of Atlantic Puffins in the Faeroes and Northwest Norway. Waterbirds 25, 164–172.

Tinnikov, A.A., 1993. Corticosteroid-binding globulin levels in the rat serum under conditions of starvation and restriction of motion. Horm. Metab. Res. 25, 88–89.

Tinnikov, A.A., 1999. Responses of serum corticosterone and corticosteroid-binding globulin to acute and prolonged stress in the rat. Endocrine 11, 145–150.

Visser, M.E., Verboven, N., 1999. Long-term fitness effects of fledging date in great tits. Oikos 85, 445–450.

Wada, H., 2008. Glucocorticoids: mediators of vertebrate ontogenetic transitions. Gen. Comp. Endocrinol. 156, 441–453.

Wada, H., Hahn, T.P., Breuner, C.W., 2007. Development of stress reactivity in white-crowned sparrow nestlings: total corticosterone response increases with age, while free corticosterone response remains low. Gen. Comp. Endocrinol. 150, 405–413.

Walker, B.G., Boersma, P.D., Wingfield, J.C., 2005. Physiological and behavioral differences in Magellanic Penguin chicks in undisturbed and tourist-visited locations of a colony. Conserv. Biol. 19, 1571–1577.

Whittow, G.C., 1993. Laysan Albatross. In: Poole, A., Gill, F. (Eds.), The Birds of North America, No. 66. The Birds of North America Inc., Philadelphia, PA.

Williams, C.T., Kitaysky, A.S., Buck, C.L., 2008. Food restricted Tufted Puffin (Fratercula cirrhata) nestlings increase vocal activity during handling without modulating total or free corticosterone. J. Ornithol. 149, 277–283.

Wingfield, J.C., 1994. Modulation of the adrenocortical response to stress in birds. In: Davey, K.G., et al. (Ed.), Perspectives in Comparative Endocrinology. National Research Council Canada, Ottawa, pp. 520–528.

Wingfield, J.C., Farner, D.S., 1975. Determination of 5 steroids in avian plasma by radioimmunoassay and competitive-protein-binding. Steroids 26, 311–327.

Wingfield, J.C., Suydam, R., Hunt, K., 1994. The adrenocortical responses to stress in Snow Buntings (Plectrophenax nivalis) and Lapland Longspurs (Calcarius lapponicus) at Barrow, Alaska. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 108, 299–306.

Wright, J., Markman, S., Denney, S.M., 2006. Facultative adjustment of pre-fledging mass loss by nestling swifts preparing for flight. Proc. R. Soc. B Biol. Sci. 273, 1895–1900.