RHINOCEROS AUKLET DEVELOPMENTAL RESPONSES TO MODERATE FOOD RESTRICTION

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RHINOCEROS AUKLET DEVELOPMENTAL RESPONSES TO MODERATE FOOD RESTRICTION

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Abstract

Seabird nestlings are vulnerable to food restriction because their parents may not buffer them from prey shortages. I conducted a captive study to explore how rhinoceros auklet chicks (*Cerorhinca monocerata*) may cope with food restriction and avoid long-term fitness consequences. I predicted auklet nestlings would be adapted to moderate levels of food-stress, and investigated how morphological allocation, glucocorticoid stress response, and fledging behavior change under conditions of a 50% calorie restriction. I also investigated effects of growth and food restriction on carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in auklet tissues. I found that food-restricted auklets allocated resources heavily toward skeletal growth, most notably toward wingchord growth. Restricted auklets exhibited a muted adrenocortical response, increasing glucocorticoid levels only slightly in response to food restriction. Fledging decision was not affected by restriction, with restricted and well-fed chicks fledging at approximately the same age. Both growth and food restriction caused decreases in $\delta^{15}N$ of auklet red blood cells (RBCs), but caused no change in $\delta^{13}C$. Sampling of free-living auklets revealed that natural levels of variability were low for RBC isotope ratios, indicating that the effects of growth and restriction detected in the captive study are of biological significance.

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General Introduction

Avian nestlings routinely experience neonatal nutritional stress. Nest-bound seabird chicks may be particularly prone to food restriction because of long nestling periods and a dependence on an ephemeral and unpredictable food base (Ricklefs 1990, Weimerskirch 2002). Because seabirds are long-lived species with low annual fecundity, a small compromise in adult survival or body condition in one year can substantially reduce breeding success in subsequent years (Williams 1966). Life history theory states that animals should balance the current reproductive event with future survival and breeding attempts in a manner such that lifetime fitness is maximized (Stearns 1992). In long-lived species such as seabirds, parents may favor self-maintenance over the needs of their young and choose not to increase foraging effort when prey is scarce (Cody 1966).

Periods of food shortages may lead to variable or reduced provisioning of nestlings if parents are unable or unwilling to buffer chicks from food shortages. High variation in both the rate of chick meal delivery and the size of chick meals is common in some alcid species (Wilson and Manuwal 1986, Barrett and Rikardsen 1992, Piatt and Kitaysky 2002). Furthermore, numerous studies have documented high annual variability in nestling growth rates indicating nutritional stress during development is routine (Gaston and Dechesne 1996, Litzow et al. 2002, Gjerdrum et al. 2003). Neonatal food deprivation may have important implications for post-fledging survival, recruitment, and future fecundity (Lindstrom 1999, Metcalfe and Monhagen 2001). Thus, a species' ability to cope with food limitation during growth is an important aspect

of its life history (Blount et al. 2003, Searcy et al. 2004, Gasparini et al. 2006). In species where chicks are not buffered from reduced prey availability, chicks should possess some means of mitigating the effects of food restriction to avoid the fitness consequences associated with early food-stress (Kitaysky et al. 2005, Takenaka et al. 2005, Benowitz-Fredericks et al. 2006).

Because nest-bound chicks cannot forage independently, they must either elicit additional food from parents or alter some aspect of their development when faced with food deprivation. In this thesis I investigate responses of captive rhinoceros auklet (*Cerorhinca monocerata*) nestlings to moderate levels of food restriction. Auklet nestlings receive chicks meals that can vary substantially in size (Hatch 1984, Bertram et al. 1991), and as a result may be routinely subjected to moderate levels of food-stress. I predict that auklet nestlings possess some means of coping with energy limitations to avoid substantially compromising their lifetime fitness. Specifically, I examine plasma levels of the stress hormone corticosterone, skeletal allocation, and flexible fledging behavior as potential mechanisms of coping with early food stress. Additionally, to assess a possible long-term consequence of early food restriction, I investigate whether auklets restricted as nestlings experienced permanent skeletal stunting.

Moderate food restriction during growth is fairly common in free-living populations; thus it is critical that researchers consider its biochemical and physiological consequences when employing physiological techniques to make inferences about a species' ecology. This captive study also provided an opportunity to examine effects of food restriction and growth on carbon and nitrogen stable isotope ratios in auklet tissues.

Stable isotope analysis is a commonly used technique in avian field studies and has proven useful in estimating diet composition and foraging location (Hobson et al. 1994, Thompson et al. 1999, Cherel et al. 2006). Based on the observation that isotopes cycle through ecosystems in a predictable manner, isotope analysis provides a valuable tool to examine wildlife foraging ecology and diet composition (Kelly 2000, Bearhop et al. 2006). $\delta^{15}N$ has been shown useful in estimating trophic level, while $\delta^{13}C$ is more suited to examining foraging distribution and food web links (Hobson et al. 1994, Forero et al. 2002).

Many physiological mechanisms underlying isotopic fractionation in animals are poorly understood, and previous research has suggested that physiological factors such as nutritional status and growth may affect an animals' carbon and nitrogen isotope ratios (Hobson et al. 1993, Gaye-Siessegger et al. 2004, Williams et al. 2007). If the effects of these physiological processes are substantial, they may confound ecological interpretation of stable isotope signatures through incorrect estimates of trophic level and foraging location.

Food restriction has been shown to induce depletion in $\delta^{13}C$ of avian whole blood and plasma (Cherel et al. 2005, Williams et al. 2007), attributed to the mobilization of ^{13}C-depleted lipid reserves under conditions of food limitation. In the case of nitrogen, impacts of food restriction on $\delta^{15}N$ are inconsistent and may be driven by differences in individuals' nitrogen balance. Severely restricted animals exhibit increasingly elevated ^{15}N levels (Hobson et al. 1993, Fuller et al. 2005), while more

moderately restricted individuals have shown both no effect of restriction on $\delta^{15}N$, and depletion relative to well-fed individuals (Kempster et al. 2007, Williams et al. 2007). Diet-tissue fractionation of nitrogen is associated with the excretion of ^{15}N-depleted waste products. Severe restriction or fasting may result in elevated $\delta^{15}N$ due to unreplenished loss of ^{14}N to excretion (Cherel et al. 2005). In contrast, more moderately restricted animals may be able to increase their nitrogen-use efficiency (Romano 2000), effectively reducing diet-tissue fractionation by excreting proportionately less nitrogen. A similar effect is predicted to result from growth: growing animals are generally highly efficient in their nitrogen use, assimilating a much greater proportion than they excrete, and presumably fractionating less (Martinez del Rio and Wolf 2005). In the absence of experimental validation, these predictions remain largely speculative.

I set out to clarify effects that growth and moderate, realistic levels of food restriction may have on stable isotope ratios in tissues of seabird nestlings. In this portion of the study, I compared $\delta^{15}N$ and $\delta^{13}C$ of control and food-restricted auklets fed identical diets, as well as those of growing and non-growing auklets to quantify the effects of these physiological factors. In doing so I aimed to identify factors field researchers should account for when interpreting isotopic data to avoid drawing spurious conclusions. To place our results in an ecological context, I also collected blood and feather samples from wild auklets. Through this additional sampling I intended to estimate the level of natural variability present in this species' stable isotope ratios and determine whether the size of physiological effects observed in the captive study were of

biological importance. The following two chapters were prepared as separate manuscripts and include multiple authors.

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Chapter 1: Rhinoceros auklet developmental responses to food limitation: potential coping mechanisms and post-fledging consequences[1]

Abstract

Neonatal nutritional restriction can compromise an individual's lifetime fitness. Seabirds may be particularly vulnerable to such restriction because nestling periods tend to be long and parents may not increase foraging effort during times of prey shortage. Previous studies of alcids have found that parental responses to food scarcity vary; thus chicks exhibit variable growth rates and fledging ages. We performed a captive study of rhinoceros auklet *Cerorhinca monocerata* nestlings to identify adaptations to food shortages, as well as long-term consequences of early restriction on adult morphology. We tested effects of a ~50% caloric restriction on auklet morphological allocation, levels of the stress hormone corticosterone, and fledging behavior. Auklets were reared in captivity and provisioned either ~441 kJ/d or ~227 kJ/d of high-quality forage fish until fledging (n = 13 for both treatment groups). Food-restricted auklets allocated heavily to skeletal growth at the expense of mass reserves, resulting in fledglings that were proportioned very differently—i.e., wing length at fledging was similar between treatments despite a 95 g difference in auklet mass. Restricted nestlings exhibited low plasma concentrations of corticosterone over the course of the experiment, although

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baseline levels were consistently higher than those of non-restricted chicks. We found no effect of treatment on fledging behavior, in contrast to field studies, which generally find well-provisioned alcid chicks fledging at younger ages. At 11 months post-hatching, once-restricted birds were still ~50 g lighter than unrestricted birds. They had smaller tarsi, a marginally smaller culmen, but a similar-sized manus. Our results suggest rhinoceros auklets use both morphological allocation and adrenocortical suppression to cope with energy shortages in the nest. Apparently, they are unable to avoid permanent skeletal stunting.

Introduction

A species' ability to cope with a variable food supply is a crucial aspect of its ecology. Nest-bound seabird chicks are prone to neonatal food restriction because of long nestling periods and a dependence on an ephemeral and unpredictable food base. Parents and nestlings have evolved a variety of reproductive and developmental strategies in response to this variability. A key consideration is the degree to which parents will compromise their own body condition in attempting to buffer chicks from prey shortages. Life history theory predicts that individuals will balance investment between current and future reproductive events to maximize lifetime fitness (Stearns 1992). Because seabirds are generally long-lived with low annual fecundity, a small compromise in adult survival or body condition one year can substantially decrease breeding success in subsequent years and lifetime fitness. Thus, parents may favor self-

maintenance over the needs of their young and choose not to increase provisioning effort during times of prey shortages (Williams 1966).

Prior research has demonstrated potential fitness consequences of neonatal nutritional stress, including compromised immune function (Gasparini et al. 2006, 2007), cognitive ability (Kitaysky et al. 2005a), digestive efficiency (Kitaysky et al. 2001), and skeletal stunting (Searcy et al. 2004). In species in which parents cannot or will not increase provisioning to young during a time of food shortage, young should possess some means of coping that mitigates the long-term effects of food restriction. The avenues available to nutritionally stressed nestlings can be broadly classified as: (1) developmental—preferential allocation of energy to particular skeletal elements and tissues during growth (Ricklefs et al. 1998, Dahdul and Horn 2003), (2) physiological—modulation of thyroid (metabolic) and glucocorticoid (stress) hormones (Kitaysky et al. 2005b), and (3) behavioral—adjustments in begging behavior, siblicide, and timing of fledging (Nunez-de la Mora et al. 1996, Gjerdrum 2004).

Alcids possess a diversity of developmental patterns unparalleled in other avian families (Sealy 1973, Ydenberg 1989). Field studies of parental responses to chick nutritional demands have produced equivocal results, and probably are confounded by variable environmental conditions, parental quality, and inconsistent methodology (Bertram et al. 1996, Takahashi et al. 1999, Harding et al. 2002). Nestling responses to food shortage have been more amenable to both field and captive approaches, revealing in general that nutritionally restricted alcid chicks shunt energy toward structural

elements needed for flight, at the expense of mass reserves (Benowitz-Fredericks et al. 2006, Romano et al. 2006)

Less is known of the physiological response of alcid nestlings to poor nutrition. Food-restricted common murre *Uria aalge* chicks increased secretion of corticosterone (A. Kitaysky, unpubl. data), the primary avian stress hormone, while tufted puffin *Fratercula cirrhata* chicks appeared to suppress or uncouple their hormonal response from nutritional status (Kitaysky et al. 2005b). Corticosterone promotes the breakdown of endogenous protein reserves, an adaptive strategy in the short term. In species subject to intermittent or variable provisioning, however, chronically high corticosterone leads to muscle wasting and reduced cognitive development (Kitaysky et al. 2001, Kitaysky et al. 2003).

Food-restricted chicks may adjust the timing of fledging, departing the nest sooner in an attempt to meet energy demands independently at sea, or remaining in the nest until some critical mass or wing length is attained (Ydenberg 1989). Additionally, synchrony is an important aspect of fledging behavior in colonial species, as chicks that fledge en masse reduce predation risk and have higher survival rates (Daan and Tinbergen 1979, Hatchwell 1991).

We performed a captive feeding experiment to determine how rhinoceros auklet *Cerorhinca monocerata* chicks adjust their developmental, physiological, and behavioral responses to food restriction. Rhinoceros auklets are not true auklets, but rather are medium-sized, colonial-nesting alcids at the base of the puffin tribe (Friesen et al. 1996). Unlike other puffins, which make multiple food deliveries to their chicks in daylight

(Wehle 1983, Harris 1984), rhinoceros auklets are nocturnal on land, and typically an adult makes at most one food delivery during a night (Hatch 1984, Bertram et al. 1991). Not infrequently, one or both parents skip a nightly visit, so fasting is a common experience in the life of the nestling. Because feeding frequency is relatively inflexible, the total quantity of food delivered depends substantially on the mean size of food loads, which can vary from ~19 g to ~35 g depending on foraging conditions (Wilson and Manuwal 1986, Watanuki 1987, Bertram et al. 1991). Not surprisingly, development is characterized by slower growth and a longer and more variable fledging period compared to other puffins (Leschner 1976, Gaston and Dechesne 1996).

We compared auklet morphological allocation, adrenocortical function, and fledging behavior in food-restricted and well-fed chicks to elucidate the mechanisms auklets employ in coping with levels of food restriction similar to what occurs in the wild. We predicted that restricted chicks would possess smaller morphological characters upon fledging, although we also expected nestlings to allocate toward wing and culmen, two highly conserved characters (Oyan and Anker-Nilssen 1996, Takenaka et al. 2005). We examined mass-specific growth trajectories of morphological characters to determine whether growth was affected differentially by energy intake (Benowitz-Fredericks et al. 2006). Given that regular fasting is normal in this species, we considered that corticosterone secretion may not be closely tied to nutritional status, unlike most vertebrate and avian taxa. We examined two components of fledging behavior: age at departure and fledging synchrony. Finally, to identify any lasting effects of neonatal restriction, we kept a subset of birds in captivity following the nestling

period and took measurements of once-restricted and never-restricted birds at 11 months post-hatching, following ~9 months of ad libitum access to food.

Materials and methods

Egg collection and captive conditions

We collected partially incubated auklet eggs from a colony on Middleton Island, Alaska in June 2006. We estimated incubation stage in the field by immersing eggs in water (Hays and LeCroy 1971) and chose eggs estimated to be relatively close to hatching. We kept eggs within their thermoneutral zone (32-37° C) during transport using hot water bottles and placed them immediately into an incubator upon arrival at the University of Alaska-Fairbanks (UAF) animal quarters. The incubator was maintained at constant temperature (37° C) and relative humidity (60%). Chicks were brooded for 24 h after hatching and were then placed in individual nest boxes in a climate-controlled room. The room was maintained at 21° C and 50% humidity.

We weighed chicks approximately 1-2 h after hatching (i.e., once they were dry) and subsequently each morning before feeding. We measured morphological characters once every 7 days, starting on day one. We used digital calipers to measure culmen and tarsus lengths to the nearest 0.1 mm. Wingchord was measured with a metal wingbar. Once the flight feathers emerged, we measured the second primary feather and first secondary feather using a plastic ruler. All wing measurements were taken to the nearest mm.

Beginning at 40 days, the earliest fledging age reported for wild rhinoceros auklets (Gaston and Dechesne 1996), we left nest boxes open at night, providing chicks the opportunity to "fledge". A chick was considered fledged after it jumped out of its nest box on two nights to ensure the departure was intentional. Treatments were terminated upon fledging.

Treatments

We fed nestlings high-quality forage fish purchased from commercial suppliers in Rhode Island, USA. Fish meals were supplemented with a multi-vitamin and offered to chicks on plastic petri dishes placed in the nest boxes. Prior to treatment initiation at 15 days old, we fed chicks silverside *Menidia menidia* ad libitum twice per day. We then began four treatments that achieved two approximately isocaloric diets, each being supplemented with a daily multi-vitamin. High-calorie treatments consisted of 75 g/d of silverside (431 kJ, n = 7) or 100 g/d capelin *Mallotus villosus* (452 kJ, n = 6). Low calorie treatments were 40 g/d silverside (229 kJ, n = 6) or 50g/d capelin (224 kJ, n = 7). Treatments were intended to reflect the range of provisioning commonly seen in the wild (Hatch 1984, Bertram et al. 1991, Wilson and Manuwal 1986).

To minimize any effect of hatch date among treatments, we assigned treatment groups sequentially by hatching order. We attempted to make daily portions equivalent in energy content within treatments (they differed by 5-21 kJ/d) inducing approximately a 50% restriction for all chicks in low-calorie treatments. Although chicks fed high-calorie treatments were not fed ad libitum, their growth rates (~8 g/d) and fledging

masses (~402 g) were in the upper range of those observed in the wild (Gaston and Dechesne 1996).

Post-nestling period

Five birds from the high-calorie treatment and six from the low-calorie group were moved immediately after fledging to an outdoor facility, where they had access to water pools for swimming and ad libitum access to food. The birds were housed at UAF animal quarters until they were ~ 6 months old, when they were moved to similar accommodations at the Alaska SeaLife Center in Seward, Alaska. We collected measurements of mass, culmen length, and tarsus length at 11 months post-hatching. Because auklets experienced some damage to primary feathers, we used manus in lieu of wingchord. We estimated manus length by subtracting the length of the longest primary feather from total wing length. Primary feathers were measured to the nearest mm.

Blood collection

Blood (100-200 μl) was collected weekly from the alar vein using hematocrit capillary tubes, beginning at day seven. We took blood samples within 3 min of removal of chicks from the nestbox to ensure true baseline levels of corticosterone were measured (Romero and Reed 2005).

When chicks were 42 days old, we exposed them to the standardized acute stressor of handling and confinement in a mesh bag to gauge adrenocorticol function and nestling ability to mount a stress response. A baseline blood sample was drawn within 3 min of

handling, and additional stress-series samples were taken at 10, 30, and 50 mins following removal from the nestbox. All blood samples were refrigerated until centrifuged, and plasma was collected and frozen within 8 h.

Laboratory analysis

We determined concentrations of corticosterone in plasma samples via radioimmunoassay according to the methods of Wingfield and Farner (1975). We equilibrated 20-40 μl aliquots of plasma samples overnight with 2000 counts per minute of tritiated corticosterone, extracted in 4 ml of dichloromethane. Steroid concentrations were measured in duplicate. Baseline plasma samples were processed in two assays, and stress-series samples were run in a single third assay. Intra- and inter- assay coefficients of variation were 3 and 6%, respectively. Recovery values of 85-99% were used to adjust final assayed concentrations of corticosterone. We performed molecular sexing of nestlings according to the methods of Griffiths et al. (1998) and determined energy content of fish through proximate analysis (Folch et al. 1957; Parker et al. 2005).

Statistical analysis

Repeated-measures ANOVA revealed that diet type (silverside versus capelin) had no effect on chick growth rate, morphological characters, corticosterone secretion, or fledging behavior within isocaloric treatments. In subsequent analyses, isocaloric diets were pooled and are henceforth referred to as high- and low-calorie treatments. We tested for differences in body mass, baseline corticosterone, and morphometric

measurements between caloric treatment groups prior to treatment (i.e., 14 days old) using multivariate analysis of variance (MANOVA).

We used repeated-measures mixed models to determine the effects of caloric treatment, age, sex, and their interactions with mass, baseline corticosterone, and morphological characters. Nonsignificant terms ($P > 0.10$) were dropped from the model. Simple effects tests were used to examine significant two-way interactions A x B (i.e., treatment by nestling age). This procedure tests for effects of A for each B, which is calculated by extracting the appropriate row from the coefficient matrix for the A x B least-squared means and using it to form an F-test. If there was no interaction but an overall treatment effect was present, we used Tukey's HSD test to determine at what ages significant differences existed between treatments. We tested for effects of treatment on primary and secondary feather lengths at fledging using t-tests. We compared stress response corticosterone levels using a repeated-measures mixed model to determine effects on corticosterone secretion of treatment, bleed time (baseline, 10, 30, or 50 min) and interaction between the two.

We regressed means of log-transformed morphological measures (culmen, tarsus, and wingchord) against means of log-transformed mass for each treatment to determine whether allocation differed between high- and low-calorie treatments (Benowitz-Fredericks et al. 2006). We used linear regression and the resulting slope as the mass-specific growth rate for each character. Regression slopes from high- and low-calorie treatments were compared using t-tests with Bonferonni corrections.

Finally, because previous research (Morbey et al. 1999, Deguchi et al. 2004) suggested that parental provisioning rate and wing length may affect fledging behavior, we constructed a generalized linear model to test the effects on fledging age of treatment and wing length at 42 days. We used 42-day wingchord length in this model to determine whether nestlings with slower growing wings fledged later—i.e., waited until a critical wingchord had been attained. We compared treatment wingchord lengths at fledging using a t-test. We also used an F-test to assess whether sychronization of fledging occurred, as indicated by the variances of hatching and fledging dates.

We used generalized linear models to compare the morphological characters of birds maintained in captivity following fledging. Because adult auklets are known to be slightly sexually dimorphic (Gaston and Dechsne 1996), the models included both caloric treatment and sex as predictor variables and mass, culmen, tarsus, and manus lengths at 11 months post-hatching as dependent variables. All statistical analyses were performed in SAS 8e (SAS Institute 2001). Means are presented ± SE, and we adopted an α-level of 0.05 for all tests. All data met assumptions of normality necessary for parametric tests, although corticosterone data was right-skewed and required log transformation.

Results

Chicks assigned to high- and low-calorie treatments were similar in mass, corticosterone levels, and morphological measures prior to experimental treatments ($P > 0.30$ in all

cases). Sex was not a significant factor in any analysis of nestlings and was subsequently dropped from all models.

Mass and size in the nestling period

Mass was significantly affected by caloric treatment ($F_{1,24} = 130.96$, $P < 0.001$), age, ($F_{6,143} = 1090.29$, $P < 0.001$) and their interaction ($F_{6,143} = 45.20$, $P < 0.001$; Fig. 1.1a). Chicks in the high-calorie treatment averaged 399.4 ± 3.6 g at 42 days and had an average growth rate of 8.2 g/d. One chick was excluded from all analyses of mass at day 42 because it ate poorly starting at ~35 days and analysis revealed it to be an extreme outlier. Chicks in the low-calorie treatment averaged 294.5 ± 3.7 g at 42 days and had a mean daily growth rate of 3.1 g/d. While both culmen and tarsus lengths were smaller (culmen: $F_{1,24} = 5.08$, $P = 0.034$, tarsus: $F_{1,24} = 3.51$, $P = 0.003$; Fig. 1.2) in chicks fed low-calorie (restricted) diets, wings were not ($F_{1,24} = 0.23$, $P = 0.63$). Mass-corrected growth trajectories differed between high- and low-calorie groups for all characters: culmen ($P = 0.002$), tarsus ($P = 0.02$), and wingchord ($P < 0.001$; Fig. 1.3, Table 1.1).

Corticosterone

Baseline corticosterone changed with age ($F_{5,115} = 7.00$, $P < 0.001$), with values appearing to peak at seven days and falling thereafter (Fig. 1.1b). Chicks in the high-calorie treatment exhibited lower baseline levels ($F_{1,24} = 7.52$, $P = 0.01$). Stress-induced maximum corticosterone levels did not differ between treatments ($F_{2,46} = 0.66$, $P =$

0.52); maximum values averaged 8.88 ± 2.44 ng/mL in the high-calorie treatment and 9.37 ± 2.55 ng/mL in the low-calorie treatment (Fig. 1.4).

Fledging Behavior

Fledging age did not differ between treatments ($F_{2,23} = 1.42$, P = 0.24) and was not affected by wing length at 42 days ($F_{2,23} = 1.62$, P = 0.21). Chicks in the high-calorie treatment fledged at 47.8 ± 0.7 days with an average wing length of 150 ± 1.26 mm; restricted chicks fledged at 49.23 ± 0.86 days with a wing length of 152 ± 1.53 mm. Although wingchord at fledging did not differ between treatments ($t_{24} = -0.61$, P = 0.55), restricted chicks fledged lighter (307.0 ± 1.1 g) than those in the high-calorie group (402.1 ± 7.1 g) ($t_{23} = 12.0$, P < 0.0001). There was no evidence of altered fledging synchrony; the variances of hatch dates and fledging dates were nearly identical, approximating a spread of 14-16 days in both treatments ($F_{2,25} = 1.51$, P = 0.31).

Mass and size in the post-nestling period

All auklets continued gaining mass after fledging. Upon final sampling at 11 months, there was no longer a significant difference between birds restricted as nestlings and those from high-calorie treatments ($F_{2,8} = 2.66$, P = 0.14), although the once-restricted birds averaged ~48 g lighter (Fig. 1.1a). Tarsus measurements showed lasting effects of nestling treatment ($F_{2,8} = 5.40$, P = 0.048; Fig. 1.2) and culmen remained marginally affected by treatment ($F_{2,8} = 4.89$, P = 0.058). Manus length at 11 months was not affected by early restriction, with once-restricted birds averaging 71 ± 5 mm and those

never restricted averaging 74 ± 5 mm ($F_{2,8} = 0.28$, P = 0.61). Sex was not a contributing factor in any character except mass, in which it approached significance ($F_{2,8} = 3.23$, P = 0.11).

Discussion

Our results suggest that auklets are adapted to realistic levels of food restriction, using morphological allocation and muted adrenocortical function to cope with energy shortages. This study lends support to the hypothesis that nestlings suppress corticosterone secretion, rather than sustain high circulating levels, because auklet parents are unwilling or unable to buffer nestlings from food stress. The preferential allocation to wing growth implies that rapid and normal growth of this character is important to post-fledging survival. Conservation of wing growth may allow poorly provisioned chicks to attain flight capability and fledge sooner, effectively escaping a food-limited environment. The highly variable fledging behavior seen in wild auklets suggests a behavioral means of coping with nutritional shortages in the nest, although we were unable to replicate this mechanism experimentally. Despite the existence of coping mechanisms, our study revealed apparent long-term morphological consequences of early food-restriction. Birds restricted as nestlings had shorter tarsi and a marginally shorter culmen at 11 months old, having experienced ad libitum food availability for > 9 months.

Morphology and growth

Preferential skeletal growth is documented in other alcid species (reviewed in Gaston 1985). In our study, auklets fledged with similar sized wings, regardless of food intake and body mass, supporting Deguchi et al.'s (2004) conclusion that fledging decision is driven by attainment of a critical wing length. Ultimately, chicks in different treatment groups had remarkably different shapes, with restricted chicks possessing proportionately larger wings and tarsi for a given mass. Similar levels of restriction in murres and Atlantic puffins *Fratercula arctica* caused less pronounced allocation toward skeletal growth (Oyan and Anker-Nilssen 1996, Benowitz-Fredericks et al. 2006). Our results suggest that in auklets, foraging efficiency (diving and flight) achieved through wing development may be of greater importance to post-fledging survival than energy reserves or culmen growth.

By analyzing both absolute measures of skeletal features and mass-specific values, we were better able to assess the degree to which auklet nestlings allocated limited energy. For example, the greatest divergence in absolute values occurred in culmen length, whereas the difference in mass-specific growth rates was less pronounced than in other characters. This result implies that although restriction affected development of the culmen, much of the observed difference was due to allometric scaling rather than true allocation. Chicks of different energy intakes ultimately had culmen sizes consistent with their respective masses, suggesting that post-fledging survival is not highly dependent upon chicks achieving a particular culmen length. Oyan and Anker-Nilssen

(1996) proposed that culmen and head-bill lengths were highly conserved characters in Atlantic puffins because of preferential allocation to brain and central nervous system development. In this study we measured only culmen, not skull, but found it to be the skeletal measure most compromised by food restriction.

Culmens and tarsi of 11 month old auklets exhibited lasting effects of early food-stress, in contrast to mass and manus. Our sample may have been too small to separate the effects of sex and food treatment on mass—similar to Searcy et al. (2004), who reported skeletal stunting in song sparrows *Melospiza melodia* restricted as nestlings. Together these studies imply that birds possess a capacity for remedial growth in mass, but they cannot always recover skeletally from neonatal restriction. Many of the long-term fitness consequences thought to be associated with early nutritional stress remain speculative because it is difficult to follow individuals after fledging. Thus the fitness consequences of shorter tarsi and culmen are unknown. Moreover, studies relating nestling growth rate and fledging mass of seabirds to post-fledging survival have produced contradictory results and may be confounded by variable food availability in the post-fledging period (Williams and Croxall 1991, Gaston 1997).

It is noteworthy that once-restricted birds who were able to compensate their mass but not their skeletal elements would appear to be in better body condition according to most avian body condition indices, because such birds are heavier for their respective skeletal size.

Adrenocortical function

Food-restricted auklets had only slightly higher baseline corticosterone levels over the course of the nestling period (~0.4 - 1.5 ng/ml), variability that may not be picked up in the wild. Therefore, corticosterone may not be a useful indicator of nutritional status in field studies of auklets. Although auklets did not appear to uncouple adrenal function from nutritional status, as tufted puffins do under conditions of moderate food restriction (Kitaysky et al. 2005b), their response (baseline levels of ~4 ng/mL in restricted chicks) was relatively muted compared to similarly restricted chicks of other species. For example, food-restricted kittiwake *Rissa spp.* chicks sustained baseline levels of up to 16 ng/mL, two to four times the levels of non-restricted chicks (Kitaysky 1999, Kitaysky et al. 2001).

Stress-induced corticosterone levels did not differ between treatment groups, either in rate of increase or maximum levels attained. This result may be an artifact of habituation to handling (Collette et al. 2000, Love et al. 2003), or it may indicate that chicks had not attained full hypothalamus-pituitary-adrenal (HPA) axis function. Stress-induced levels under 10 ng/mL were surprisingly low—captive kittiwake chicks exhibited maximum levels of up to 80 ng/mL (Kitaysky et al. 2001). As a burrow-nesting species, auklets may not require a fully functional HPA-axis as chicks because they have few acute stressors (i.e., no predators or nest mates and a constant microclimate). By 42 days however, when stress-induced corticosterone samples were

collected, most nestlings were less than a week from fledging and presumably would need a full stress response to cope with noxious stimuli outside the burrow.

Alternatively, auklet chicks may have modulated their adrenocortical function through corticosterone binding globulins (CBGs), not the stress hormone itself. In binding to corticosterone molecules, CBGs render them biologically unavailable, potentially limiting an individual's overall corticosterone exposure (Mendel 1989). In this study, we measured only total corticosterone (bound and free fractions combined). The possibility exists that in auklets, nestlings modulate their neonatal exposure through CBG levels in addition to, or to a greater extent, than regulation of corticosterone secretion.

Overall, our results support the conclusion that adrenal response to nutritional stress is moderately dampened or even suppressed in auklet nestlings. Their adrenal response appears to fall between that of black-legged kittiwakes, a species in which parents incur high costs of reproduction and respond strongly to begging behavior (Golet et al. 1998, Kitaysky et al. 2001), and tufted puffins (Kitaysky et al. 2005b). Parental response to chick body condition has been difficult to ascertain in puffins, but nestling adrenal function and provisioning rates suggest an inability of parents to adjust feeding rates or unwillingness to compromise their own body condition. We propose that adrenal response of seabird nestlings is directly proportional to parental reproductive strategies.

Fledging behavior

Despite our apparent success in capturing the range of auklet growth rates, we found surprisingly little variation in fledging age. Fledging age appeared to be unaffected by growth rate, mass, or provisioning. All chicks fledged around 40 - 45 days, after growing restless in their boxes. Fledging decision may be genetically or ontogenetically determined to some degree, or our experimental conditions may have lacked the necessary environmental cues to induce normal fledging behavior (e.g., scent, noise, parental prodding). Additionally, fledging is thought to be driven to some extent by a perceived reduction in provisioning, i.e., it may be the rate of provisioning rather than the absolute amount to which chicks respond (Bertram et al. 1991, Morbey et al. 1999). Because we provided a constant amount of food, heavier chicks may not have been inclined to fledge earlier, as Hipfner et al. (2004) found in wild chicks, because they perceived no reduction in provisioning.

Deguchi et al. (2004) proposed that attainment of a critical wing length may dictate the fledging decision. Because wing length was highly conserved by chicks in this study, it is difficult to assess the validity of this hypothesis. The lack of treatment effect on both wing length and fledging behavior provides potential support, however. Previous work on alcids has repeatedly shown an inverse relationship between mass at fledging and age at fledging, with faster growing chicks generally fledging both heavier and at a younger age (Harfenist 1995, Morbey et al. 1999). Although we found no evidence that captive auklets adjusted fledging behavior as a means of coping with food restriction, the wide range of fledging ages seen in wild auklets strongly suggests that such intra-specific

variability is adaptive. We hoped to replicate such variability experimentally; however, our study design did not allow us to address this question adequately.

Our results, in conjunction with those of field studies, suggest that auklets use all three of the proposed avenues—physiological, morphological, and behavioral—available to nest-bound chicks. Our results further suggest that despite these apparent coping mechanisms, auklet nestlings are unable to avoid certain life-long consequences of early food restriction. Future studies should focus on the long-term physiological and behavioral effects of early food limitation and, in particular, the potential fitness consequences of skeletal stunting. Ultimately, the goal is to quantify the effect of a variable prey base during the nestling period on post-fledging survival and recruitment

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Bird Research Fund. Abby Powell and participants in Biology 694 Spring '07 improved this manuscript greatly. All procedures were approved by Institutional Animal Care and Use Committee at the University of Alaska Fairbanks (#06-21) and authorized under state and federal permits.

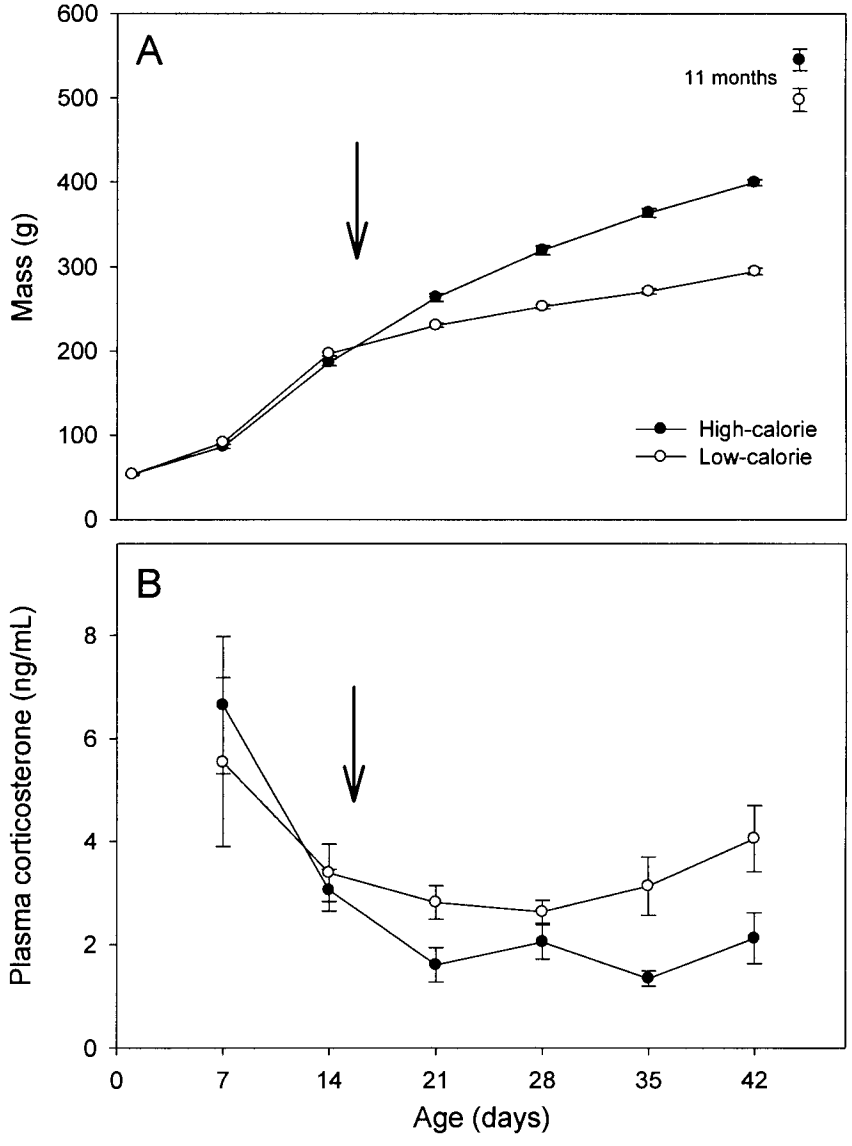

Figure 1.1 Mass and plasma corticosterone concentrations in rhinoceros auklet chicks. Each point is a control or restricted treatment mean ± SE at a given age (n = 13 for both treatments). Arrows represent treatment initiation at 15 days post-hatch. Mass measurements begin at day 1; corticosterone levels were measured at 7 days post hatch and every 7 days thereafter until fledging (~49 days). Mass measurements labeled 11 months include 5 high-calorie and 6 low-calorie birds kept in captivity under ad libitum feeding conditions after fledging. Treatment significantly ($P < 0.05$) affected mass between ages 21-42.

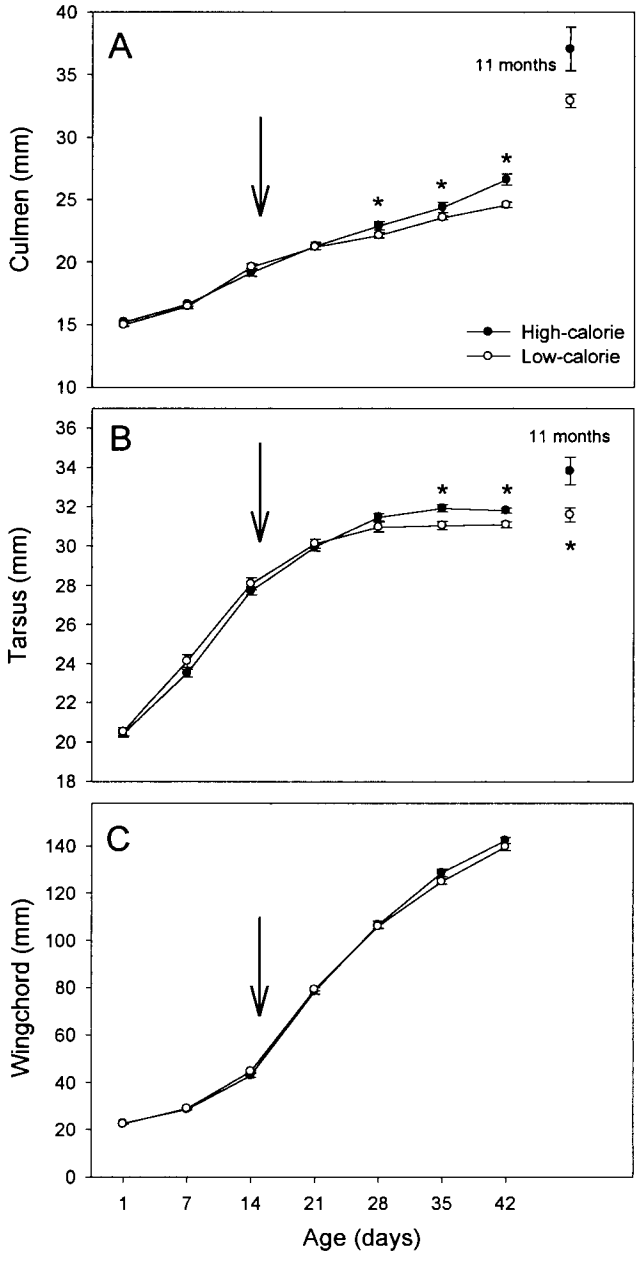

Figure 1.2. Rhinoceros auklets morphological measures. Age dependent growth of (A) culmen, (B) tarsus, and (C) wingchord in chicks on high and low calorie treatments (n = 13 in both). Each point represents a treatment mean ± SE; arrows indicate initiation of treatments at 15 days post-hatch. Asterisks represent points where treatments differed significantly at a particular age. Measures at 11 months include 5 high-calorie and 6 low-calorie birds kept in captivity under ad libitum feeding conditions after fledging.

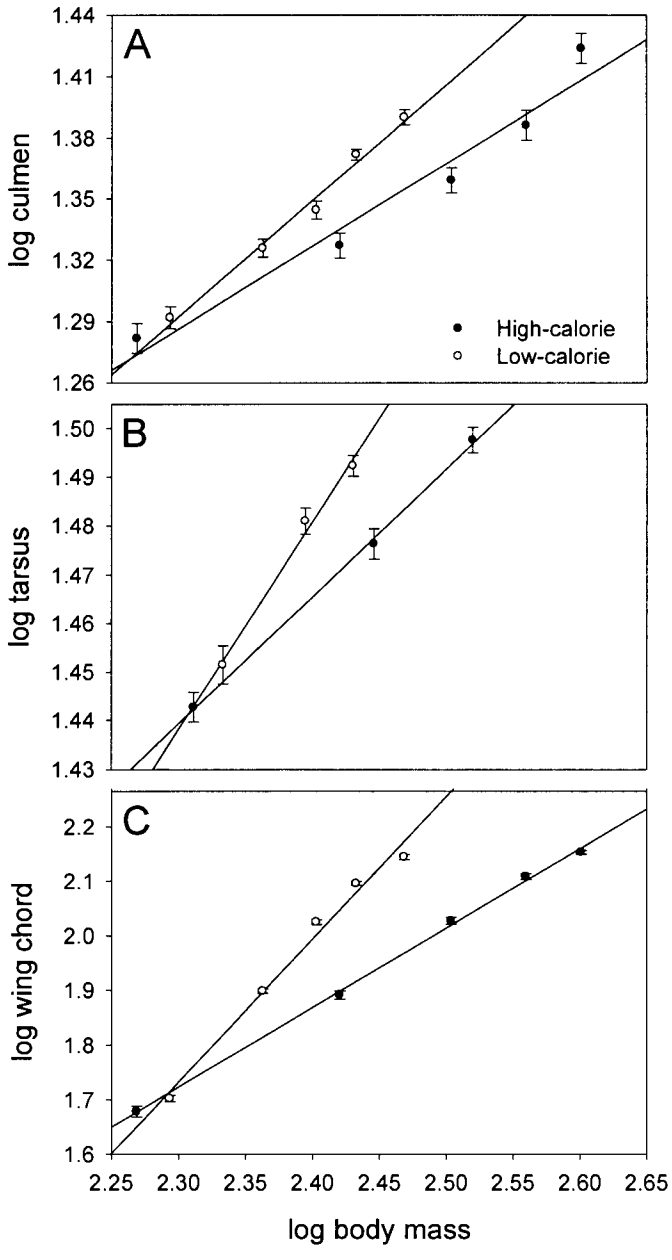

Figure 1.3. Log-transformed growth trajectories of rhinoceros auklet chicks. Relationship between logs of (A) culmen, (B) tarsus and (C) wingchord measurements and log body mass in chicks on high- and low-calorie diets (n = 13 for both). Each point represents an average log mass and log morphological character ± SE for a treatment at a given age. To isolate shifts in nestling energy allocation due to caloric restriction, measurements taken under ad libitum conditions are omitted. Slopes represent relative growth corrected for body mass and differ significantly between treatment groups for all morphological characters ($P < 0.05$ in all cases).

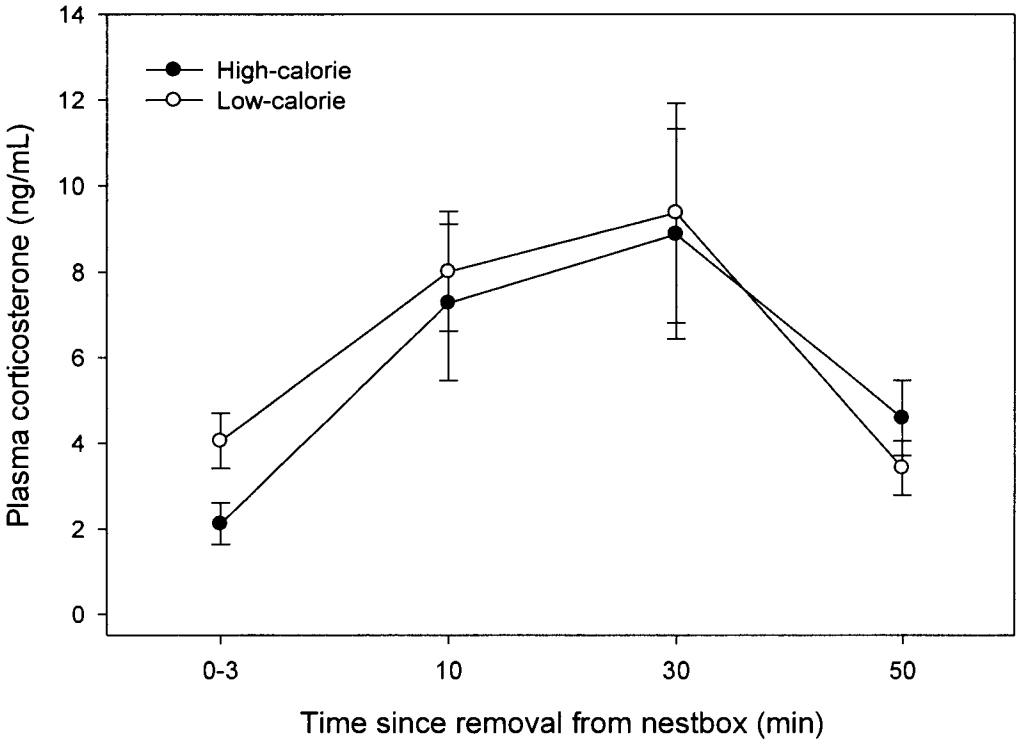

Figure 1.4 Rhinoceros auklet stress-induced plasma corticosterone levels. Chicks were sampled at 42 days old, four weeks after treatments were begun (n = 13 in both groups).

Table 1.1 Slopes of linear regressions of log morphological characters vs. log mass in rhinoceros auklet nestlings.[a]

Morphological Character	Caloric Treatment	Slope
Culmen	High	0.41 ± 0.03
	Low	0.57 ± 0.09
Tarsus	High	0.23 ± 0.01
	Low	0.38 ± 0.04
Wingchord	High	1.45 ± 0.02
	Low	2.62 ± 0.09

[a] Mean log values for high- and low-calorie treatments (441 kJ/day and 227 kJ/day; n = 13 per treatment) at 14, 21, 28 and 35 days post-hatching were used to calculate treatment slopes. Tarsus calculations included days 14, 21, and 28, prior to the levelling off of the tarsus growth curve. $R^2 > 0.95$ and $P < 0.05$ in all cases.

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Chapter 2: Disentangling effects of growth and nutritional status on seabird stable isotope ratios[1]

Abstract

A growing number of studies suggest that an individual's physiology may affect tissue carbon and nitrogen stable isotope signatures, obscuring a signal often assumed to be only a reflection of diet and foraging location. We examined effects of growth and moderate food restriction on red blood cell (RBC) and feather $\delta^{15}N$ and $\delta^{13}C$ in captive rhinoceros auklet chicks (*Cerorhinca monocerata*), a piscivorous seabird. Chicks (n=26) were reared in captivity and fed either control or 50% restricted amounts of high quality forage fish. We maintained a subset of birds (n=11) in an outdoor facility after the nestling period and fed them ad libitum until they were 6 months old to obtain blood samples from non-growing birds on these same diet types. To estimate natural levels of isotopic variation at our study site, we also collected blood from a random sample of free-living rhinoceros auklet adults and chicks (n=15 for each), as well as adult feather samples (n=13). In the captive study, moderate food restriction caused significant depletion in $\delta^{15}N$ values of blood and feathers. Growth also induced depletion in RBC $\delta^{15}N$, with chicks exhibiting lower $\delta^{15}N$ when they were growing the fastest. As growth slowed $\delta^{15}N$ increased, resulting in an overall pattern of enrichment over the course of the nestling period. Combined effects of growth and restriction depleted $\delta^{15}N$ in chick RBCs by 0.69 - 0.92 ‰. We propose that increased nitrogen-use efficiency is

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responsible for ^{15}N depletion in both growing and food restricted chicks. $\delta^{15}N$ values in red blood cells of free-ranging auklets fell within a range of only 1.03‰, while feather $\delta^{15}N$ varied widely. Together our captive and field results suggest that both growth and moderate food restriction can impact RBC stable isotope ratios in an ecologically meaningful way.

Introduction

Analysis of carbon and nitrogen stable isotopes is a common technique for diet estimation in biological research and avian research in particular. Increasingly, studies suggest that individuals' physiology may affect their isotopic signatures, potentially obscuring a signal often assumed to be a reflection only of diet and foraging location. For example, isotopic differences among sexes and age classes within species are generally thought to represent intra-specific niche partitioning (Forero et al. 2005, Bearhop et al. 2006). However, studies by Hobson et al. (1993), Cherel et al. (2005), Gaye-Sessinger et al. (2004a), and others demonstrate that physiological status may present an additional source of isotopic variation. If large enough, factors such as nutritional status and growth could potentially confound dietary interpretation of isotope signatures of wild individuals. The physiological mechanisms underlying patterns of isotopic movement through animals and between trophic levels are still not well understood (Gannes et al. 1997, 1998). This gap in knowledge compromises the ability of researchers to accurately interpret and assign sources of variation present in isotopic data.

Although both carbon and nitrogen isotope ratios may be affected by nutritional status and growth (Gaye-Siessengger et al. 2004b, Barnes et al. 2007), effects on $\delta^{13}C$ typically occur through changes in tissue lipid composition. In tissues composed primarily of protein, growth and nutritional status are more likely to impact $\delta^{15}N$ (Hobson et al. 1993, Fuller et al. 2004, 2005). Enrichment in ^{15}N with increasing trophic level is widely observed, and this diet-tissue fractionation is attributed to the disproportionate loss of ^{14}N during urea production and excretion (Macko et al. 1986). Theoretical models suggest that the magnitude of this fractionation is determined by nitrogen-use efficiency: the amount of ^{15}N-depleted waste products lost through excretion relative to total nitrogen assimilated (Martinez del Rio and Wolf 2005). A decrease in the ratio of nitrogen loss to nitrogen intake is therefore expected to result in decreased $\delta^{15}N$ in an animal's tissues. Growing animals, which excrete relatively small amounts of nitrogen relative to what they are consuming and assimilating for growth, should fractionate less than non-growing individuals. Captive studies of fish support this prediction, reporting an inverse relationship between growth rate and diet-tissue fractionation (Gaye-Siessegger et al. 2004b, Trueman et al. 2005), although data on other taxa are sparse.

Under conditions of food limitations, $\delta^{15}N$ values should be impacted when an individual's nitrogen-use efficiency is altered, or if the restriction is so severe that it results in unreplenished loss of endogenous nitrogen (Ponsard and Averbuch 1999). Romano (2000) found that body composition of seabird nestlings subjected to a 48%

reduction in food (and protein) was only 30% lower in lean mass compared to well-fed controls, suggesting restricted nestlings assimilated protein more efficiently. If dietary restriction does induce increased nitrogen-use efficiency, decreased fractionation and $\delta^{15}N$ values would be expected, whereas if N is being lost without replacement, fractionation and $\delta^{15}N$ values should increase. Experimental research into the interaction of physiological condition and isotopic fractionation has focused largely on severely restricted or fasting animals. Cherel et al. (2005), Hobson et al. (1993) and Fuller et al. (2005) all reported that severe nutritional stress induced increasingly elevated ^{15}N levels in undernourished birds and humans. The isotopic effects of moderate nutritional stress, as would commonly be seen in growing and breeding free-ranging animals, has only recently been explored. Kempster et al. (2007) reported no effect of moderate caloric restriction on nitrogen isotopic fractionation in song sparrow chicks (*Melospiza melodia*), while Williams et al. (2007) showed significant depletion of RBC ^{15}N in food-restricted tufted puffin chicks (*Fratercula cirrhata*).

Clarification of the effects of both food restriction and growth on stable isotope signatures is especially relevant to seabird biology. Prey distribution in the marine environment is known to be patchy and unpredictable, and seabirds may be routinely exposed to bouts of food shortages (Durant et al. 2004). Additionally, although chick meals are relatively easy to collect at colonies (e.g., Gjerdrum 2004, Bertram et al. 1991), adult diets are more difficult to characterize. Stable isotope analysis has great potential to reliably characterize both nestling and adult diets. Differences in isotope values observed between age classes are often attributed to differing diet composition

(Hodum and Hobson 2000, Forero et al. 2002, 2005) and suggest that diet may in fact differ between age classes. To properly interpret such isotopic data however, it must first be confirmed if and how fractionation factors differ between adults and growing juveniles.

We performed a captive feeding experiment to examine the effects of growth and physiological condition on carbon and nitrogen stable isotope signatures in rhinoceros auklet chicks (*Cerorhinca monocerata*), a pelagic species of alcid. Our analysis focused on the two tissue types most commonly used in avian field studies: red blood cells and feathers. Specifically we addressed: 1) effects of moderate nutritional stress on stable isotope ratios in tissues of seabird chicks, and 2) effects of growth on stable isotope fractionation in seabird red blood cells. We also collected blood samples from free-living auklets to assess population-level variability in the field and place our captive results in an ecological context. This additional sampling allowed us to compare the size of the physiological effects detected in our captive study to the amount of natural variability in a free-living population.

Materials and Methods

Captive Experiment

Nestling Period

We collected 29 rhinoceros auklet eggs from Middleton Island, Gulf of Alaska in June 2006. We transferred eggs immediately into an incubator upon arrival at the University of Alaska Fairbanks (UAF). Three eggs failed to hatch, and the remaining 26

chicks were used in the study. Chicks were brooded 24 hrs post-hatch, at which point they were placed in individual nest boxes.

We weighed chicks approximately 1-2 hrs following hatching and then each morning before feeding. Beginning at 40 days, near the earliest fledging age reported for wild rhinoceros auklets (Gaston and Deschesne 1996), we left nest boxes open at night, allowing chicks the opportunity to "fledge." Chicks were considered fledged after jumping from nest boxes two nights, to ensure jumps were intentional.

Dietary Treatments

We fed all chicks ad libitum amounts of the forage fish silverside (*Menidia menidia*) twice per day, starting at hatch. At 15 days old, we assigned chicks to one of four treatment groups sequentially by hatching order. We continued feeding half of the chicks silverside (n = 13), while the other half were switched to capelin (*Mallotus villosus*; n = 13). Within each diet type we created control and restricted diet treatments: silverside: 75g/day (431 kJ; n = 7) and 40g/day (229 kJ; n = 6) or capelin: 100g/day (452 kJ; n = 6) and 50g/day (224 kJ; n = 7). These groups allowed us to explore the effects of food restriction in animals on isotopically uniform diets as well as those undergoing isotopic turnover. Daily portions were similar in energy content within treatments (they differed by 5-21 kJ/day) and implemented a ~50% reduction in caloric intake for chicks in restricted treatments. Portion sizes and chick growth rates were within the range of published values for this species (Gaston & Deschesne 1996). All fish were from a single batch, ordered through Atlantic/Pacific commercial suppliers in Providence,

Rhode Island, USA. Diet restriction was terminated upon fledging (~49 days), although birds were maintained on their experimental diet type to allow continued study of effects of growth on isotopic signatures.

Post-fledging Period

We maintained a subset of birds from the capelin group in captivity for a continued study of the effects of growth on stable isotope signatures. We moved five individuals from the control group and six from the restricted group to an outside facility with access to water pools immediately after fledging, where they also had ad libitum access to food. To track effects of growth and turnover on RBC isotope ratios while on the capelin diet, we collected blood at 72 and 120 days old. We fed birds capelin until ~120 days old to ensure that the turnover to the capelin signature from the nestling period was complete (all birds were switched on the same day but exact ages differed by 13 days maximum due to differing hatch dates). Birds were then switched to a silverside diet and maintained on this regimen for 60 days. This second diet switch allowed us to obtain a non-growing RBC signature for each diet type: capelin at 120 days and silverside at 180 days. By 110 days post-hatch, auklets had stable masses and minimally growing morphological characters (hereafter "subadults"). Subadult $\delta^{15}N$ and $\delta^{13}C$ values were compared to chick values for each diet type to investigate the effects of growth. We collected blood at 170 and 180 days old (50 and 60 days on silverside diet type) to confirm that turnover of capelin carbon and nitrogen was complete.

Blood and Feather Collection

We collected weekly blood samples from captive chicks beginning at day 7. All birds were bled by collecting 100-200 µl of blood from the alar vein using heparin-coated capillary tubes. All blood samples were centrifuged to separate plasma and red blood cell components within 8h. We collected the second primary feather from each captive chick at 56 days old. Primary feathers emerged ~ 10 days post-hatch, and growth continued until ~49 days; thus the vast majority of synthesis occurred while chicks were subject to experimental treatments.

Analysis of Fish

We collected approximately 5 fish of each type at five sampling points over the course of the experiment. Whole fish from each sampling point were pooled together and ground using a mortar and pestle. We then freeze dried these samples and extracted lipids from half of them using 2:1 chloroform: methanol (Folch et al. 1957). Because previous work has suggested that $\delta^{15}N$ signatures may be affected by the extraction process, we performed isotope analysis on both lipid-extracted and non-extracted samples for each sampling point and ran these samples in duplicate (Sweeting et al. 2006). Unless noted, reported results are non-extracted values for $\delta^{15}N$ and extracted values for $\delta^{13}C$.

We estimated protein content for each fish type by elemental analyzer at the time of isotope analysis (% N x 6.5 approximates % protein in lipid free dry matter; Robbins

2001). Lipids were extracted from whole ground fish according to the methods of Folch et al. (1957), and % lipid composition was determined gravimetrically following evaporation under nitrogen. We estimated energy content based on energy equivalents for protein and lipid fractions (39.3 kJ/g lipid; 17.8 kJ/g protein; Schmidt-Nielsen 1997).

Field Methods

We collected blood samples from 15 adult auklets and 15 chicks on Middleton Island in July 2007. Chicks were removed directly from burrows, and adults were captured by net upon their return to the colony. We aged chicks based on wingchord length, and all blood was collected and processed using the same methodology described above. One covert feather was collected from 13 of the 15 adults.

Stable Isotope Analysis

We performed isotope analysis on red blood cells collected from 25 captive chicks (n = 7 in the silverside control group and n = 6 in the remaining treatments), 15 wild chicks and 15 wild adults. Red blood cell samples were freeze-dried for 24-48 hrs prior to analysis. Additional analyses were performed on feather samples collected from the same 25 captive chicks and 13 wild adult auklets (2 adults were not sampled). We washed feathers with ethanol and cut ~1 cm of the most recently grown barbs (those closest to the base of the shaft). A 0.2 - 0.3 mg sample from each bird was analyzed.

We performed analysis with continuous flow isotope ratio mass spectrometry using a Costech Elemental Analyzer (ESC 4010), Finnigan MAT Conflo III interface, and a

Delta+XP Isotope Ratio Mass Spectrometer. Replicate measurements of internal laboratory peptone standards indicated measurement error to be ± 0.20 ‰ for nitrogen, ± 0.15 ‰ for carbon and ± 0.06 ‰ for C:N. We report stable isotope concentrations using 'δ' notation: $\delta X = [(R_{sample}/R_{standard})-1] \times 1000$ where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. $R_{standard}$ values are based on Vienna PeeDee Belmnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. In the captive experiment, we estimated discrimination factors (Δ) by taking the mean difference in $\delta^{13}C$ and $\delta^{15}N$ between RBCs of control chicks and their diet.

Statistical Analysis

We performed statistical analysis in SAS 8e (SAS Institute 2001). Unless noted, means are presented ± SE and we assigned an α-level of 0.05. All data met assumptions of normality necessary for parametric tests. Chicks assigned to control and restricted treatments did not differ in mass prior to experimental treatments ($P > 0.30$). Age at fledging was compared across treatments using a general linear model with diet type and treatment included as factors.

We used separate repeated measures mixed models for each diet type to determine the effects of diet restriction, age, and their interactions on chick mass, and RBC $\delta^{15}N$, $\delta^{13}C$, and C:N. In mass models, we included measurements from day 14, when treatments were initiated, until day 42, when chicks began fledging and treatments were terminated. Models of $\delta^{15}N$ and $\delta^{13}C$ in chicks fed silverside throughout the nestling

period (no diet switch) allow us examine effects of growth on isotopic fractionation in addition to those of restriction. These models include days 28, by which point turnover of yolk was complete (see Results), through day 56. The same range of days in chicks fed capelin allowed us to assess the effects of restriction, but growth is confounded with turnover. We used a repeated measures mixed model to examine effects of growth on $\delta^{15}N$ fractionation. We included control nestlings fed silverside from days 28 to 56 post-hatch, and used $\Delta^{15}N$ (diet-tissue fractionation) as the dependent variable and weekly % mass change as a factor.

Nonsignificant terms ($P > 0.10$) were dropped from models. Simple effects tests were used to examine significant two-way interactions A x B (i.e., treatment x nestling age). Effects of A for each level of B were calculated by extracting the appropriate row from the coefficient matrix for the A x B least-squared means and using it to form an *F*-test. If there was no interaction but an overall treatment effect was present, we used Tukey's HSD test to determine at what ages treatments differed. We estimated average effects sizes of restriction by taking the mean difference in signatures between control and restricted groups.

We analyzed captive feather signatures using t-tests with Bonferonni corrections to compare treatment means within diet types. Effects of lipid extraction on nitrogen signatures were also compared using t-tests, as were samples collected from free-living adults and nestlings. Because we were interested in the natural variability present in signatures of free-living auklets, we report these means ± standard deviation.

Results

Mass and Age at Fledging

Mass was significantly affected by diet restriction ($F_{1,24} = 130.96$, $P < 0.001$), age ($F_{6,143} = 1090.29$, $P < 0.001$) and their interaction ($F_{6,143} = 45.20$, $P < 0.001$), with chicks in control groups averaging 402.1 ± 6.9 g at fledging and chicks in restricted treatments 307.0 ± 4.1 g. Within isocaloric treatments, mass did not differ significantly between diet types over the course of the experiment ($F_{1,22} = 2.07$, $P < 0.16$), although chicks in the control capelin group fledged heavier on average than those in the control silverside group (Fig. 2.1). Chicks in both control groups lost mass following fledging, resulting in all groups exhibiting similar masses at 56 days. Fledging age did not differ significantly between diet type ($F_{1,22} = 1.14$, $P = 0.25$) or treatment ($F_{1,22} = 2.08$, $P = 0.16$), although on average, chicks in the control groups fledged slightly younger: control chicks at 47.7 ± 0.6 days and restricted chicks at 49.2 ± 0.9 days.

All 11 individuals maintained in captivity after the fledging period gained mass, although those birds restricted as nestlings remained consistently ~ 50 g lighter. Masses stabilized by ~ 110 days post-hatching at 415 ± 9 g in previously restricted individuals and 467 ± 16 g in control birds.

Stable Isotope Analysis

Captive Experiment

Red Blood Cells

There was no effect of growth or food restriction on RBC $\delta^{13}C$ in either diet type (Fig. 2.2). Turnover of yolk carbon is apparent between days 7 and 28 in the silverside group. In nestlings switched to capelin, turnover of silverside carbon appeared complete by day 49 post-hatch, and $\delta^{13}C$ remained stable thereafter. Sub-adult $\delta^{13}C$ values remain stable until the second diet switch from capelin to silverside at 120 days. After this second diet switch, $\delta^{13}C$ values plateau at 50-60 days (between 170 and 180 days old), and turnover of capelin carbon appears complete (Fig. 2.3). Using the allometric equation suggested by Carleton and Martinez del Rio (2005) we estimated turnover to be 90% complete 60 days following the diet switch, when birds were 180 days old.

Because neither growth nor restriction affected $\delta^{13}C$ values, we are able to use these values to track turnover and thereby disentangle effects of growth from those of turnover in $\delta^{15}N$. We assumed that rates of turnover were the same for carbon and nitrogen isotopes (Bearhop et al. 2002, Evans-Ogden et al. 2004) and estimated turnover of yolk to be complete by 28 days post-hatch in nestlings fed silverside (Fig. 2.2a). In nestlings switched to capelin, turnover of both yolk and silverside appears complete by 49 days(2.2b). We attribute any changes in $\delta^{15}N$ of control nestlings to growth after 28 days for chicks fed silverside and 49 days for chicks switched to capelin.

RBC $\delta^{15}N$ of nestlings fed silverside increased with age ($F_{4,43} = 3.71$, $P = 0.011$) and showed depletion in response to food restriction ($F_{1,11} = 17.22$, $P = 0.002$; Fig 2.4a). In these nestlings, the mean effect size of restriction between days 14 and 56 was 0.37 ‰. $\delta^{15}N$ values changed most dramatically between days 42 and 56, and showed an overall increase of 0.55 ‰. $\delta^{15}N$ of control nestlings at 56 days was nearly identical to those of subadult birds fed silverside for 60 days (120-180 days old; Fig. 2.4a). Between days 28 and 56, mean $\Delta^{15}N$ between silverside and RBC's increased significantly as growth rate declined ($F_{1,26} = 8.39$, $P = 0.008$; Fig. 2.5), ranging from 2.84 ‰ at 35 days to 3.39 ‰ at 56 days. Subadult RBC $\delta^{15}N$ at 180 days exhibited the highest discrimination factors for this diet type, 3.49 ‰.

Nestlings that were switched to the capelin diet at 14 days post-hatch also exhibited depletion in $\delta^{15}N$ in response to diet restriction by a mean of 0.35 ‰ ($F_{1,10} = 12.98$, $P = 0.005$; Fig. 2.4b). Although RBC nitrogen in these nestlings enriched with age ($F_{7,69} = 3.62$, $P = 0.002$), some of this increase in $\delta^{15}N$ was most likely due to turnover. Capelin and silverside had similar $\delta^{15}N$ values, but discrimination factors were higher in individuals fed capelin. We estimate that turnover from silverside to capelin was complete by day 49. Between 49 and 72 days, RBC $\delta^{15}N$ values of the 11 birds kept in captivity continued to increase, by 0.34 ‰. After 72 days RBC $\delta^{15}N$ remained stable, until the last sampling point on the capelin diet at 120 days. By combining observed effects of growth and food-restriction on $\delta^{15}N$, we estimate that together these factors can cause depletion of 0.69 - 0.92 ‰ relative to well-fed adult or non-growing bird on

the same diet. Nestling C:N did not change with nestling age ($F_{7,164} = 1.09$, $P = 0.37$) and was not affected by caloric treatment ($F_{1,22} = 2.78$, $P = 0.12$). C:N of RBCs did not differ between diet types ($F_{1,22} = 4.20$, $P = 0.07$), and mean C:N in nestling RBC was 3.25.

Feathers

Effects of caloric restriction on the $\delta^{13}C$ and $\delta^{15}N$ of feathers were not consistent across diet types (Fig. 2.6). Feather samples of nestlings fed capelin exhibited no effect of restriction in either isotope (nitrogen: $t_{10} = 0.49$, $P = 0.6$ and carbon: $t_{10} = 1.86$, $P = 0.09$), while restricted chicks fed silverside had feathers significantly depleted in $\delta^{15}N$ ($t_{11} = 5.23$, $P = 0.001$). At 0.6 ‰, the mean effect size of restriction on feather $\delta^{15}N$ values was larger than that in red blood cells.

Diet

Capelin and silverside differed substantially in $\delta^{13}C$ (by 2.48 ‰), but not in $\delta^{15}N$ (by 0.21 ‰). Silverside $\delta^{15}N$ and $\delta^{13}C$ values were 11.10 ± 0.14 ‰ and -16.29 ± 0.32 respectively, and those of capelin were 10.89 ± 0.15 ‰ and -18.77 ± 0.11 ‰. C:N was 5.29 ± 0.12 for silverside and 4.85 ± 0.12 for capelin. Using data from sub-adult birds at 120 days (silverside treatment) and 180 days (capelin treatment), we estimated nitrogen and carbon discrimination between diet and RBCs to be 3.4 ‰ and 0.56 ‰, respectively, for birds on a diet of silverside, and 3.91 ‰ and 0.64 ‰, respectively, for birds on a diet

of capelin. Lipid-extraction significantly enriched nitrogen isotope ratios in the capelin (effect size = 0.68 ‰, $t_{18} = 3.01$. $P = 0.008$) and had no effect on silverside samples. Extraction affected carbon signatures as expected, enriching signatures of both fish by 1.83 - 1.52 ‰.

Silverside had higher lipid content (7.7 ± 0.7 %) than capelin (4.8 ± 0.8 %), but had similar protein content (~15 %). Because nestlings were fed differing amounts of each fish (75 vs. 100 g and 40 vs. 50 g), nestlings in the capelin treatments ultimately received more protein each day than chicks fed an isocaloric silverside diet. Control nestlings fed capelin received 14.9 g protein/day. In comparison, control nestlings fed silverside received 11.5 g/day (23% less), restricted nestlings fed capelin received 7.4 g/day (50% less), and restricted nestlings fed silverside received 6.1 g/day (60% less). Chicks in the control and restricted silverside groups received ~ 1 g/day of additional lipid.

Field Samples

Auklet nestlings ranged in age from 14 to 28 days (mean = 19.6 ± 1.1 days). Adult auklet RBCs exhibited mean values of 13.38 ± 0. 33 ‰ and -19.64 ± 0.24 ‰ for $\delta^{15}N$ and $\delta^{13}C$ respectively. Nestling RBCs exhibited means of 13.14 ± 0.25 ‰ $\delta^{15}N$ and -19.99 ± 0.16 ‰ $\delta^{13}C$. Both nitrogen and carbon isotope ratios differed between age classes (nitrogen: $t_{28} = 2.25$, $P = 0.03$ and carbon: $t_{28} = 4.71$, $P < 0.001$), by 0.24 ‰ for $\delta^{15}N$ and by 0.35‰ for $\delta^{13}C$ (Fig. 2.7). All RBC isotope signatures (nestling and adult

combined) fell within a range of 1.03 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. Adult feather signatures averaged 15.69 ± 1.70 ‰ $\delta^{15}N$ and -20.35 ± 0.63 ‰ $\delta^{13}C$. Feather isotope ratios were much more variable: range = 5.85 ‰ for nitrogen and 2.20 ‰ for carbon.

Discussion

Nitrogen isotope ratios in developing auklet nestlings exhibited consistent depletion in response to food restriction and rapid growth. $\delta^{15}N$ of all nestlings enriched with age, and discrimination factors between fish and RBC were lowest when growth rates were at their highest. $\delta^{15}N$ is generally reported to enrich by approximately 3‰ per trophic level (Minagawa and Wada 1984); therefore, from an ecological perspective, the overall effect sizes in our captive study are small (0.35 – 0.55 ‰). However, the variability present in stable isotope signatures of free-living individuals was surprisingly small as well. RBC $\delta^{15}N$ values of wild birds exhibited standard deviations that were smaller than or comparable to the physiological effects measured in nitrogen isotope values, especially when considering effects of growth and restriction combined. All RBC $\delta^{15}N$ of free-living auklets were contained within a 1 ‰ range, suggesting that physiological factors such as moderate food restriction and growth present a biologically meaningful source of variation in $\delta^{15}N$ values of this and potentially other seabird species.

The depletion of $\delta^{15}N$ values in response to moderate levels of food restriction is consistent with the prediction that restricted auklets increased their nitrogen-use

efficiency and diet-tissue fractionation was reduced. This study is among the first to demonstrate a reduction in $\delta^{15}N$ values in association with moderate nutritional stress. Our results are similar to those of Williams et al.'s (2007) study on tufted puffins, with restricted nestlings exhibiting depleted nitrogen values and tissues of all nestlings enriching in ^{15}N with age. However, these authors were unable to separate effects of growth from those of turnover of yolk nitrogen, due to a lack of sampling points late in the nestling period.

Because we were able to use carbon isotope values to assess when yolk turnover was complete, we could demonstrate that growth affected nitrogen isotope signatures independent of turnover. Discrimination factors were negatively correlated with growth rates, indicating growing birds were more efficient in their nitrogen use. Similar results are reported by Fantle et al. (1999), Gaye-Sessiengger et al. (2004b) and Trueman et al. (2005) who also observed an inverse relationship between nitrogen discrimination and growth rates in crab and fish species. Although we would expect discrimination of all birds with stable masses to be equivalent, discrimination factors for sub-adults were much higher than those of chicks exhibiting a 0% change in body mass (Fig. 2.5). We propose that reduced discrimination in chicks is due to incomplete turnover of RBCs synthesized earlier in the nestling period when growth rates were high and discrimination low.

Carbon isotope values, in contrast to those of nitrogen, showed no effect of growth or restriction, over the course of the captive study, consistent with other studies of food restriction (Hobson et al. 1993, Fuller et al. 2005, Kempster et al. 2007). Cherel et al.

(2005), however, reported reduced $\delta^{13}C$ in plasma of fasting king penguins (*Aptenodytes patagonicus*) and a concurrent increase in plasma C:N. These authors proposed that this reduction in ^{13}C was due to increased mobilization of lipid reserves, which tend to be depleted relative to other tissues and the diet (DeNiro and Epstein 1977). They saw no such effects on RBCs, similar to the results in this experiment. We did not perform isotope analysis on plasma samples, thus are unable to determine whether restricted auklet chicks were also mobilizing lipids.

In free-living auklets, RBC $\delta^{15}N$ of adults was enriched relative to those of chicks, consistent with the results of our captive study. This difference between age classes could easily be explained by differences in N-use efficiency, as observed in the captive experiment where young, rapidly growing chicks had lower $\delta^{15}N$ compared to sub-adult birds that had achieved stable masses. Carbon signatures of samples collected from free-living auklets also differed a small amount between age classes, which may indicate that parents fed chicks slightly different prey than what they fed on themselves. Alternatively, we see from the captive study that turnover of yolk isotopes signatures can take 3-4 wks; thus wild chicks (aged 2-4 wks) may also have differed from adult signatures due to residual effects of the yolk, rather than variation in diet composition.

The combination of laboratory and field data in our study highlights the potential for misinterpretation of field data if physiological effects on $\delta^{15}N$ in free-living birds are not considered. Growth and restriction each affected nitrogen signatures by 0.35 - 0.5 ‰. Combined these effects resulted in restricted chicks exhibiting $\delta^{15}N$ values depleted by

0.69 - 0.92 ‰ relative to a well-fed, non-growing sub-adult bird with the same diet. In light of the small ranges of values present in free-living RBC samples (standard deviations < 0.35 ‰), these physiological effects are biologically meaningful, and we urge researchers to consider them. However, field studies generally report elevated $\delta^{15}N$ in nestling tissues relative to adults (Hodum and Hobson 2000, Forero et al. 2002, 2005). Given our captive findings, these studies may actually be underestimating $\delta^{15}N$ trophic differences between age classes.

In captive chicks, food restriction also induced depletion of $\delta^{15}N$ of feathers, although only in chicks fed the silverside diet. Similar to RBCs, $\delta^{15}N$ depletion may be due to increased efficiency in use of dietary nitrogen. The differing protein content in these diet types may have affected this tissue's isotope values. Because they consumed ~ 1.3 fewer g /d of protein (~ 18% difference between the restricted groups), chicks fed silverside may have been more protein- (and thus nitrogen-) limited than chicks fed capelin, resulting in reduced $\delta^{15}N$. It is not clear why effects of food restriction are inconsistent across feathers and RBCs.

In this study, the nitrogen variability present (ranging 2-5 ‰) in feather samples from wild birds would easily obscure the small effects of growth or moderate diet restriction. Other field studies report similarly high levels of variation in feather $\delta^{15}N$ and $\delta^{13}C$ values (Cherel et al. 2000, Gladbach et al. 2007), and is most likely due to variable timing and location of molt. Tighter geographic constraints on breeding locations may explain the small variation present in RBC isotope ratios. Stable isotope

analysis of feathers is increasing due to the ease of collection of this tissue and the expansive time frame it reflects. Our results indicate that although this tissue may be affected by poor physiological condition, that fact does not invalidate the use of feathers in making ecological inferences.

Due to their pelagic-nature much remains unknown about seabird foraging ecology. As a time-integrated indicator of diet composition, stable isotope analysis has proven to be an invaluable tool in furthering such understanding (Hobson et al. 1994, Thompson et al. 1999, Hedd and Montevecchi 2006). We have shown that growth and moderate diet restriction can alter isotope ratios in a biologically meaningful way in seabird nestlings. We witnessed tissue depletion in ^{15}N in food restricted and rapidly growing chicks and interpret this depletion as evidence of change in nitrogen-use efficiency. Because the magnitude of this depletion is small relative to the magnitude of trophic enrichment in $\delta^{15}N$ (~2-4‰), such depletion does not necessarily reduce the utility of stable isotope analysis. However, it should be considered, particularly when the isotopic variation in field samples is small.

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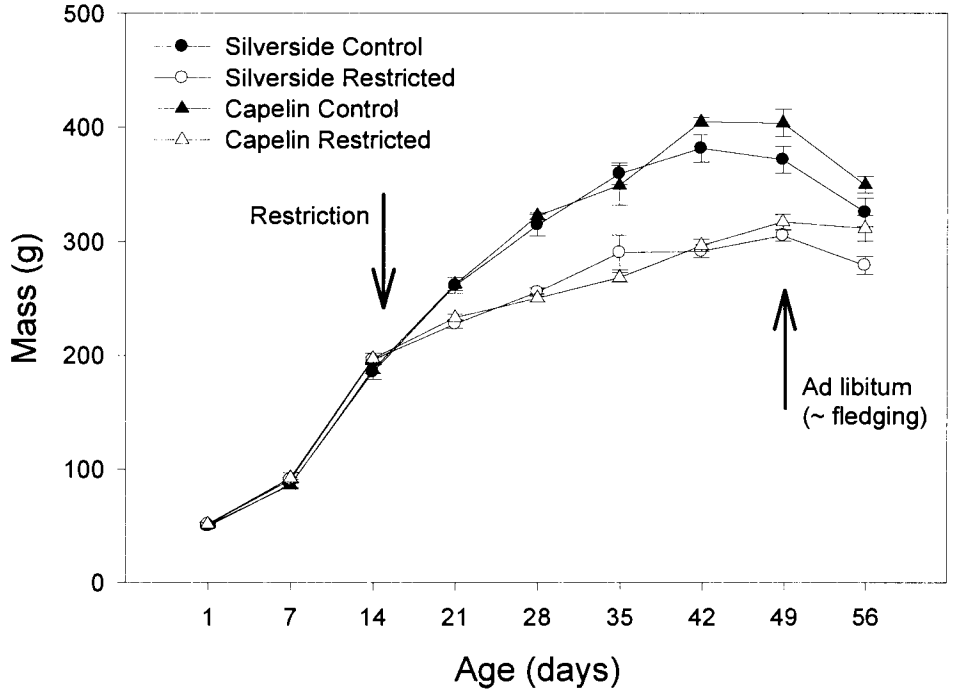

Figure 2.1 Mean body mass of rhinoceros auklet chicks. Each point is a control or restricted treatment mean (± SE). Arrows indicate treatment initiation at 15 days and termination at fledging (mean = 49 days).

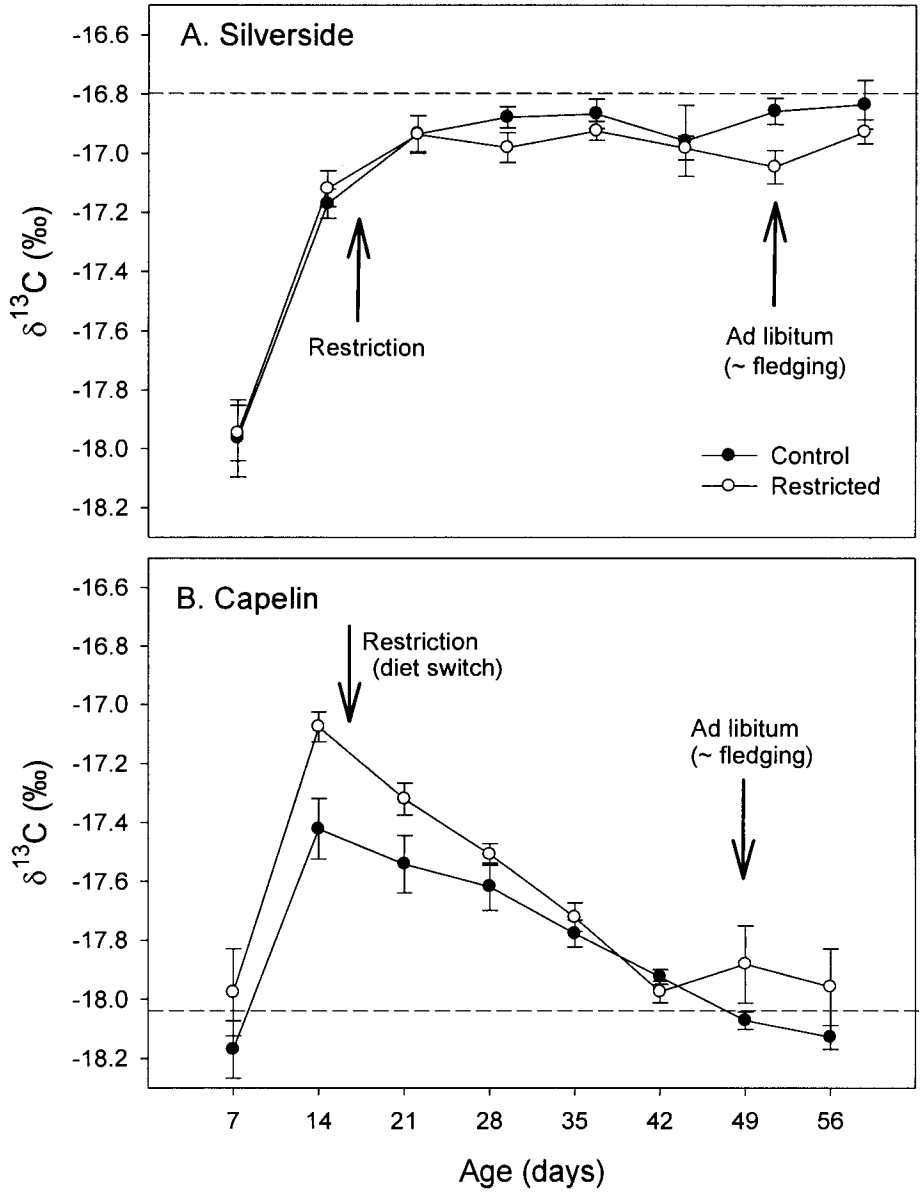

Figure 2.2 Red blood cell $\delta^{13}C$ of rhinoceros auklet chicks. Chicks were fed control and restricted amounts of (A) silverside and (B) capelin fish. Points represents treatment means ± SE. Prior to treatment initiation at 15 days, all chicks were fed silverside ad libitum. Horizontal lines indicate carbon values of capelin chicks maintained in captivity following the nestling period at (A) 180 days and (B) 120 days. These birds were fed capelin (B) ad libitum following fledging until 120 days. They were then switched to silverside for comparison to growing chicks fed the same diet type.

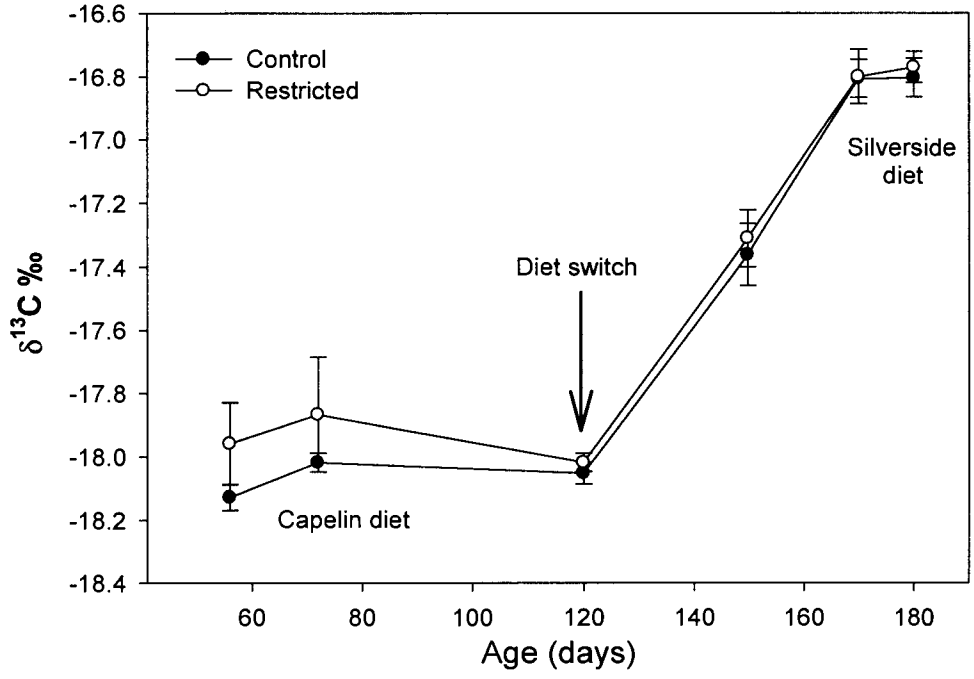

Figure 2.3 Turnover of $\delta^{13}C$ in rhinoceros auklet red blood cells. Auklet diets were switched at 120 days from capelin to silverside. Open symbols represent those birds restricted as nestlings (n = 6) and closed symbols those in the control group as nestlings (n= 5). After fledging at ~49 days all birds were fed ad libitum.

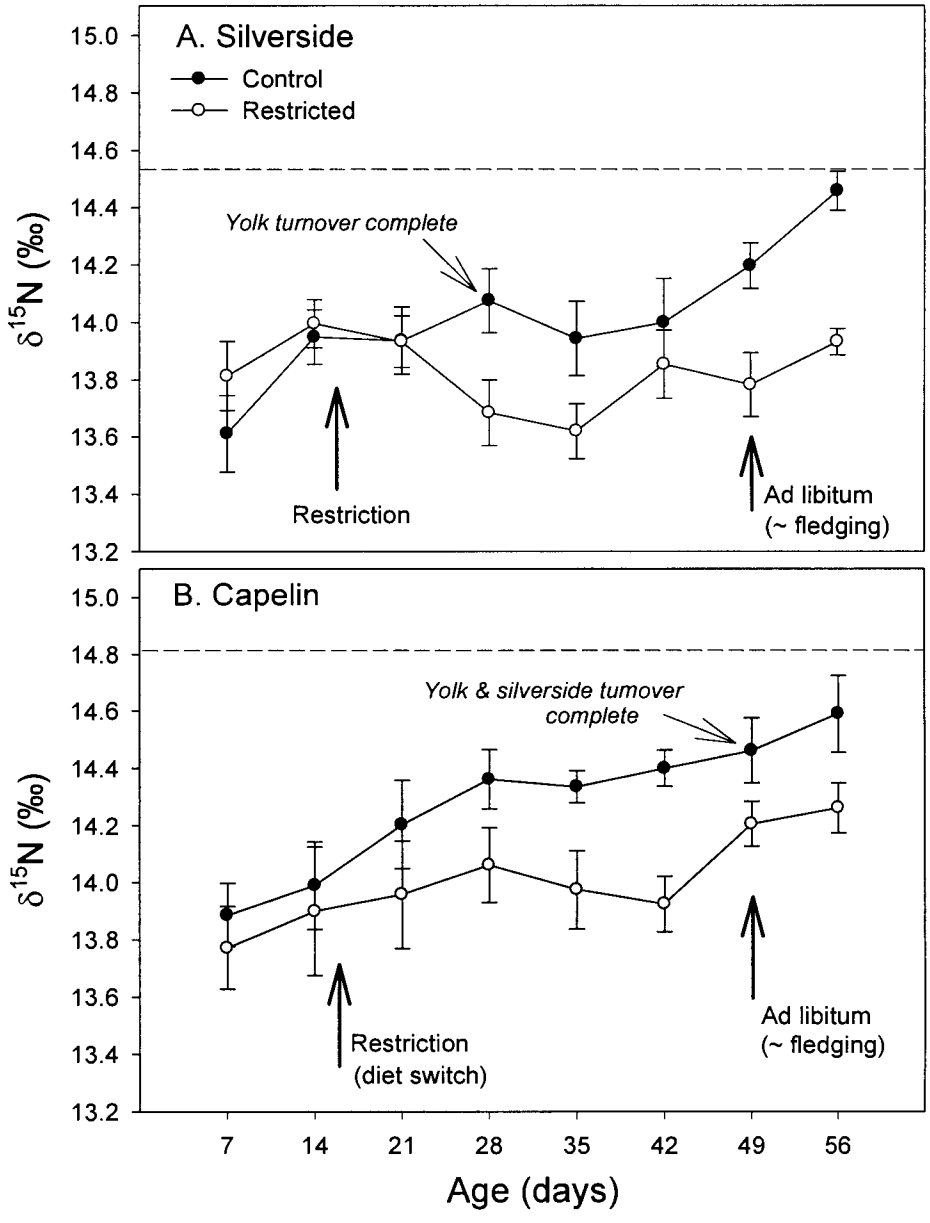

Figure 2.4 Red blood cell $\delta^{15}N$ of rhinoceros auklet chicks. Chicks were fed control and restricted amounts of (A) silverside and (B) capelin fish. Points represent treatment means ± SE. Arrows indicate treatment initiation at 15 days, prior to which all chicks were fed silverside ad libitum, and termination of treatments at fledging. Horizontal lines indicate mean signatures of birds from the capelin treatment groups retained after 56 days and fed capelin until 120 days (B) and then silverside until 180 days (A); silverside signatures at 180 days approximate signatures of non-growing (subadult) individuals. Estimated completion of turnover is derived from carbon signatures (see results).

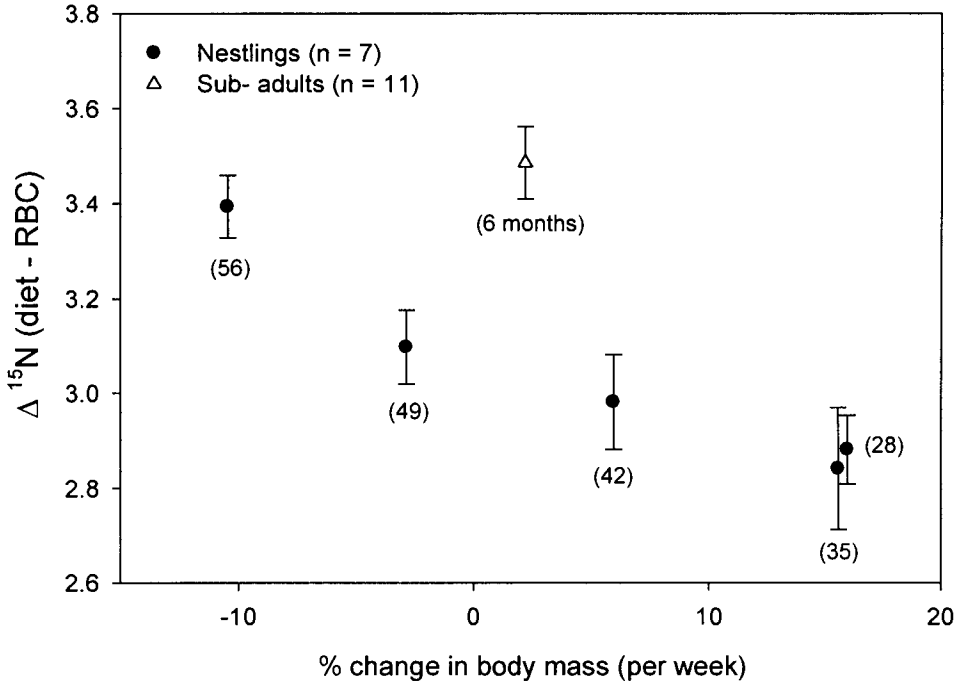

Figure 2.5 Rhinoceros auklet discrimination factor vs. % change in body mass. Auklets were fed control (high calorie) amounts of the fish silverside from hatch. Only ages 28 – 56 days are included to ensure turnover of yolk $\delta^{15}N$ is complete. Each point is a treatment mean (± SE) at a given age. Nestling age (days post-hatch) is indicated in parentheses. Mean fractionation of subadults (6 months post-hatch) also fed silverside is included as a point of reference.

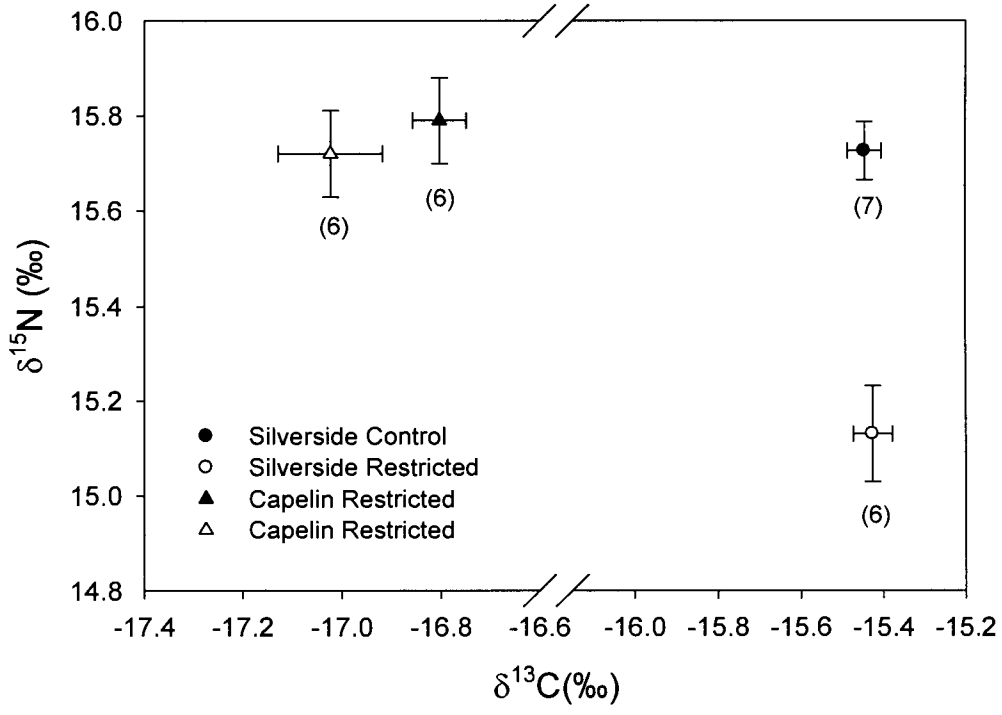

Figure 2.6 $\delta^{13}C$ and $\delta^{15}N$ of captive rhinoceros auklet feathers. Feathers were collected 56 days post-hatch and presented as treatment means ± SE. Sample sizes shown in parentheses.

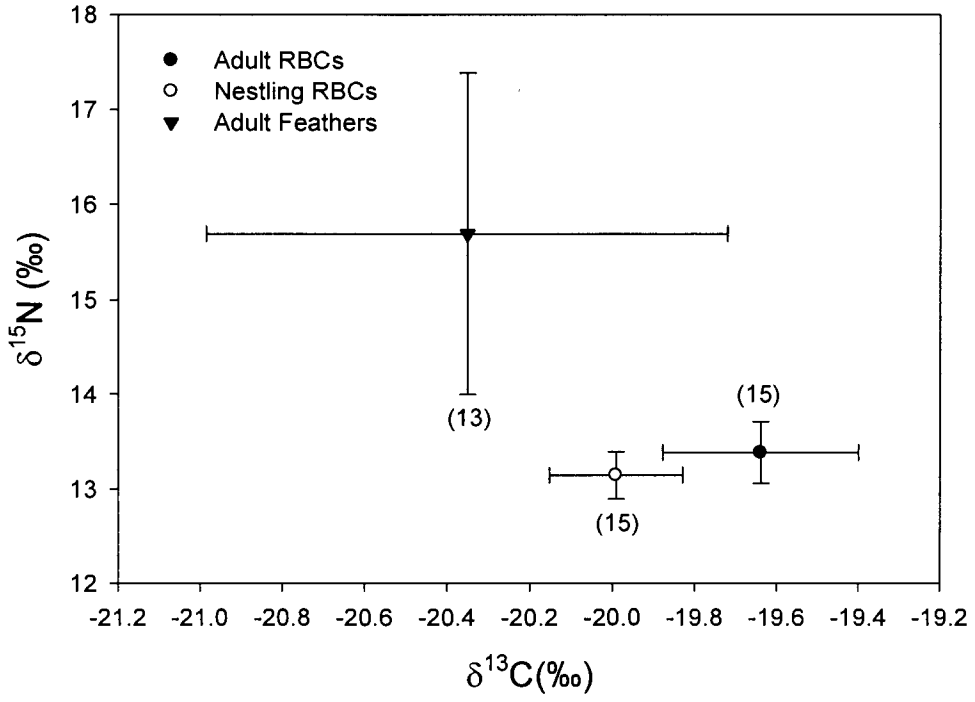

Figure 2.7 $\delta^{13}C$ and $\delta^{15}N$ of free-living rhinoceros auklet feathers and red blood cells. Data are presented as means ± SD and sample sizes are shown in parentheses. Nestlings were 14-28 days old.

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General Conclusion

The first chapter examined developmental responses of rhinoceros auklets to food limitation. I found that food-restricted auklet nestlings exhibited both reduced adrenocortical activity and disproportionate allocation toward skeletal growth as means of coping with moderate levels of food restriction. These results suggest that this species may be adapted to food restriction in the nestling stage. Adrenocortical response (measured through corticosterone levels) was relatively muted in food restricted chicks and displayed a strong ontogenetic effect, peaking just after hatching (days 1-7). Auklet nestlings revealed an adrenocortical response somewhat between that of tufted puffins, which are known to suppress corticosterone secretion in response to food restriction (Kitaysky et al. 2005), and kittiwakes, which are known to increase corticosterone levels dramatically (Kitaysky et al. 2003). This inter-specific variation in nestling adrenocortical function may be directly related to parental willingness or ability to respond to poor chick body condition or begging behavior.

When food-restricted, auklet chicks also allocated heavily toward skeletal growth, with restricted chicks exhibiting skeletal measurements that were very close to those of well-fed chicks, despite their substantially smaller masses. In spite of this allocation toward growth of skeletal characters, residual effects were present in once-restricted auklets at 11 months old, 9 months after the restriction had ended. Those individuals restricted as nestlings remained slightly lighter and had smaller skeletal characters. Although this skeletal stunting cannot be taken as a direct measure of fitness,

it is some indication that auklets could not avoid long-term consequences of early exposure to food limitations.

Surprisingly, I did not find that food restriction affected the timing of fledging, as many wild studies have reported (Morbey et al. 1999, Hipfner et al. 2004). We found no evidence that captive auklets adjusted fledging behavior as a means of coping with food restriction, but the wide range of fledging ages seen in wild auklets strongly suggests that such intra-specific variability is adaptive. Deguchi et al. (2004) proposed that fledging decision may be driven by the attainment of a critical wing length. Because wing length was highly conserved by chicks in this study, it is difficult to assess the validity of this hypothesis. The lack of treatment effect on both wing length and fledging behavior provides potential support, however. My results, in conjunction with other field research, support the notion that auklets are adapted to moderate levels of food-stress, utilizing adrenocortical, morphological and behavioral strategies to protect themselves from long-term consequences of early food-restriction.

I also saw distinct effects of food restriction in auklet stable isotope values. Although no effect was detected in $\delta^{13}C$, $\delta^{15}N$ of both red blood cells (RBCs) and feathers were lower in food-restricted auklets. Growth also induced a reduction in RBC $\delta^{15}N$, with nestlings exhibiting lower $\delta^{15}N$ when they were growing the fastest. As growth slowed $\delta^{15}N$ increased, resulting in an overall pattern of enrichment over the course of the nestling period. An increase in $\delta^{15}N$ is evidence of increased fractionation between the diet and the tissue. Animals that are more efficient in their use of nitrogen

are predicted to fractionate less and thus appear more isotopically similar to their food source (Trueman et al. 2005). I propose that the depletion witnessed in $\delta^{15}N$ values of both rapidly growing and moderately restricted nestlings was due to increased nitrogen-use efficiency in these birds.

To assess whether natural levels of isotopic variability were large in relation to the effects of growth and food-restriction observed in the captive study, I sampled free-living birds in the field. This sampling of free-living auklets revealed that although feather isotope values varied widely, red blood cells exhibited low levels of natural variability. Thus, the physiological effects of both growth and food restriction could cause detectable differences in the nitrogen isotope values measured in wild nestlings and adults. I urge future researchers to consider physiological factors when interpreting small differences in $\delta^{15}N$ among age classes in field studies, especially when natural variability is small.

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