

# Disentangling effects of growth and nutritional status on seabird stable isotope ratios

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**Abstract** A growing number of studies suggest that an individual's physiology affects its 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  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in rhinoceros auklet chicks (*Cerorhinca monocerata*), a piscivorous seabird. Chicks were reared in captivity and fed either control (75 g/day;  $n = 7$ ) or  $\sim 40\%$  restricted (40 g/day;  $n = 6$ ) amounts of high quality forage fish. We quantified effects of growth on isotopic fractionation by comparing  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in control chicks to those of captive, non-growing sub-adult auklets ( $n = 11$ ) fed the same diet. To estimate natural levels of isotopic variation, we also collected blood from a random sample of free-living rhinoceros auklet adults and chicks in the Gulf of Alaska ( $n = 15$  for each), as well as adult feather samples ( $n = 13$ ). In the captive experiment, moderate food restriction caused significant depletion in  $\delta^{15}\text{N}$  of both RBCs and feathers in treatment chicks compared to control chicks. Growth also induced depletion in RBC  $\delta^{15}\text{N}$ , with chicks exhibiting lower  $\delta^{15}\text{N}$  when they were growing the fastest. As growth slowed,  $\delta^{15}\text{N}$  increased, resulting in an overall pattern of enrichment over the course of the nestling period. Combined effects of growth and restriction depleted  $\delta^{15}\text{N}$  in chick RBCs by

0.92‰. We propose that increased nitrogen-use efficiency is responsible for  $^{15}\text{N}$  depletion in both growing and food-restricted chicks.  $\delta^{15}\text{N}$  values in RBCs of free-ranging auklets fell within a range of only 1.03‰, while feather  $\delta^{15}\text{N}$  varied widely. Together, our captive and field results suggest that both growth and moderate food restriction can affect stable isotope ratios in an ecologically meaningful way in RBCs although not feathers due to greater natural variability in this tissue.

**Keywords** Diet-tissue fractionation · Food restriction ·  $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$  · Nitrogen-use efficiency

## 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 an individual's physiology may affect its isotopic signature, potentially obscuring a signal often assumed to be only a reflection 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-Siessegger 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) and this gap in knowledge compromises the ability of

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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-Siessegger et al. 2004b; Barnes et al. 2007), effects on  $^{12}\text{C}/^{13}\text{C}$  typically occur through changes in tissue lipid composition. In tissues comprised primarily of protein, growth and nutritional status are more likely to impact  $\delta^{15}\text{N}$  (Hobson et al. 1993; Fuller et al. 2004, 2005). Enrichment in  $^{15}\text{N}$  with increasing trophic level is widely observed, and this diet-tissue fractionation is attributed to the disproportionate loss of  $^{14}\text{N}$  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  $^{15}\text{N}$ -depleted waste products lost through excretion relative to total nitrogen assimilated (Martinez del Rio and Wolf 2005; Koch 2007). A decrease in the ratio of nitrogen loss to nitrogen intake is therefore expected to result in decreased  $\delta^{15}\text{N}$  in an animal's tissues. Growing animals, which excrete relatively small amounts of nitrogen relative to what they consume and assimilate 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 limitation,  $\delta^{15}\text{N}$  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 that restricted nestlings assimilated protein more efficiently. If dietary restriction does induce increased nitrogen-use efficiency, decreased fractionation and  $\delta^{15}\text{N}$  values (i.e., depletion in  $^{15}\text{N}$ ) would be expected, whereas if N is being lost without replacement, we would expect increased fractionation and  $\delta^{15}\text{N}$  values (i.e., enrichment in  $^{15}\text{N}$ ). Experimental research into the interaction of physiological condition and isotopic fractionation has focused largely on severely restricted or fasting animals. Chérel et al. (2005), Hobson et al. (1993) and Fuller et al. (2005) all reported that severe nutritional stress induced increasingly elevated  $^{15}\text{N}$  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 red blood cell

(RBC)  $^{15}\text{N}$  in food-restricted tufted puffin chicks (*Fratercula cirrhata*).

Clarification of the effects of both food restriction and growth on stable isotope signatures is needed for future applications in 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 characterize reliably 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 first must be confirmed if and how fractionation differs between adults and growing juveniles.

We performed a captive feeding experiment to examine the effects of growth and nutritional status on carbon and nitrogen stable isotope signatures in avian tissues. Our study focused on rhinoceros auklet chicks (*Cerorhinca monocerata*), a species of seabird, and analysis was limited to the two tissue types most commonly used in avian field studies: RBCs 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 present in a free-living population.

## Materials and methods

### Captive experiment

#### Nestling period

We collected 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). Chicks ( $n = 13$ ) were brooded for 24-h post-hatch, at which point they were placed in individual nest boxes.

We weighed chicks approximately 1–2 h following hatching and then each morning before feeding. Beginning at 40 days, near the earliest fledging age reported for wild rhinoceros auklets (Gaston and Dechesne 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 with the forage fish silverside (*Menidia menidia*) twice per day, starting at hatch. At 15 days old, we assigned chicks to either control (75 g/day;  $n = 7$ ) or restricted (40 g/day;  $n = 6$ ) treatment groups. Portion sizes and chick growth rates were within the range of published values for this species (Gaston and Dechesne 1996). All fish were from a single batch, ordered from a commercial supplier in Providence, Rhode Island. Diet restriction was terminated upon fledging (~49 days), and we fed birds silverside ad libitum until the experiment was ended at 56 days.

To examine effects of growth on isotopic fractionation, we compared RBC  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of chicks with those of a separate group of non-growing, subadult auklets (~6 months old;  $n = 11$ ) also raised in captivity. We collected blood from subadults after 50 and 60 days on the silverside diet.

#### *Blood and feather collection*

We collected weekly blood samples from captive chicks beginning at day 7. All birds were bled by collecting 100–200  $\mu\text{l}$  blood from the alar vein using heparin-coated capillary tubes. All blood samples were centrifuged within 6 h to separate plasma and red blood cell components. 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 five fish 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 the samples and extracted lipids from half of them using 2:1 chloroform:methanol (Folch et al. 1957). Because previous work has suggested that  $\delta^{15}\text{N}$  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 the samples in duplicate (Sweeting et al. 2006). Unless otherwise noted, reported results are non-extracted values for  $\delta^{15}\text{N}$  and extracted values for  $\delta^{13}\text{C}$ .

#### 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

Red blood cell samples were freeze-dried for 24–48 h prior to analysis. Feathers were washed with ethanol after drying, and a 0.2–0.3 mg sample was cut from the most recently grown barbs (those closest to the base of the shaft). 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. We report stable isotope concentrations using ‘ $\delta$ ’ notation:  $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1,000$ , where  $X$  is  $^{13}\text{C}$  or  $^{15}\text{N}$  and  $R$  is the corresponding ratio  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ .  $R_{\text{standard}}$  values are based on Vienna PeeDee Belemnite for  $\delta^{13}\text{C}$  and atmospheric nitrogen for  $\delta^{15}\text{N}$ . Replicate measurements of internal laboratory peptone standards indicated measurement error to be  $\pm 0.20\%$  for nitrogen,  $\pm 0.15\%$  for carbon and  $\pm 0.06$  for C:N. In the captive experiment, we estimated discrimination factors ( $\Delta$ ) by calculating the mean difference in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between RBCs of chicks and their diet.

#### Statistical analysis

We performed statistical analysis in SAS 8e (SAS 2001). Unless otherwise noted, means are presented  $\pm$ SE, and we assigned an  $\alpha$ -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 between treatments using a  $t$ -test. We used separate repeated measures mixed models to determine the effects of food restriction, age, and their interaction on chick mass, RBC  $\delta^{15}\text{N}$ , RBC  $\delta^{13}\text{C}$ , and C:N. In mass models, we included measurements from day 14, just prior to treatment initiation, until day 42, when chicks began fledging and treatments were terminated. Models of  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ , and C:N included days 28–56. We excluded earlier measurements due to incomplete turnover of nutrients from yolk (see results). We also used a repeated measures mixed model to examine effects of growth on  $\delta^{15}\text{N}$  fractionation. We included control nestlings from days 28 to 56 post-hatch, and used  $\Delta^{15}\text{N}$  (diet-tissue fractionation)

as the dependent variable and weekly % mass change as a factor.

Nonsignificant terms with  $P > 0.10$  were dropped from models. Significant terms retained in each model are reported in the “Results” section. Simple effects tests were used to examine significant two-way interactions (i.e., treatment  $\times$  nestling age). Effects of age for each level of treatment were calculated by extracting the appropriate row from the coefficient matrix for the age  $\times$  treatment 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 effect sizes of restriction by taking the mean difference in signatures between control and restricted groups at each sampling point during experimental treatments.

We compared treatment means of captive feather isotope ratios using  $t$ -tests with Bonferroni corrections. 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 mean  $\pm$  SD.

## Results

### Mass and age at fledging

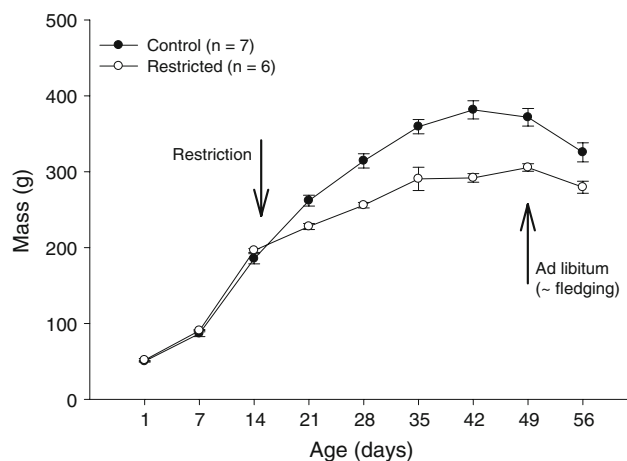
Mass was affected by diet restriction ( $F_{1,11} = 49.68$ ,  $P < 0.001$ ), age ( $F_{6,65} = 613.2$ ,  $P < 0.001$ ) and their interaction ( $F_{6,65} = 27.15$ ,  $P < 0.001$ ), with chicks in control groups averaging  $397.3 \pm 7.7$  g at fledging and chicks in restricted treatments  $301.6 \pm 5.8$  g (Fig. 1). Fledging age did not differ between treatments ( $t_{11} = 2.08$ ,  $P = 0.41$ ), and mean fledging age was  $49.1 \pm 0.63$  days for all chicks.

### Stable isotope analysis

#### Captive experiment

**Diet** In silverside,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were  $11.10 \pm 0.14\text{‰}$  and  $-16.29 \pm 0.32$ , respectively, and C:N was  $5.29 \pm 0.12$ . Lipid-extraction had no effect on  $\delta^{15}\text{N}$  ( $t_{18} = 0.21$ ,  $P = 0.81$ ) but enriched  $\delta^{13}\text{C}$  by  $1.83\text{‰}$  ( $t_{18} = 5.82$ ,  $P < 0.001$ ).

**Red blood cells and feathers** There was no effect of food restriction on RBC  $\delta^{13}\text{C}$  ( $F_{1,11} = 3.20$ ,  $P = 0.10$ ). Apparent turnover of yolk carbon occurs between days 7 and 28 and  $\delta^{13}\text{C}$  values did not change with age thereafter ( $F_{4,43} = 0.94$ ,  $P = 0.44$ ; Fig. 2a). There did not appear to be any effect of growth on  $\delta^{13}\text{C}$  values: once yolk turnover

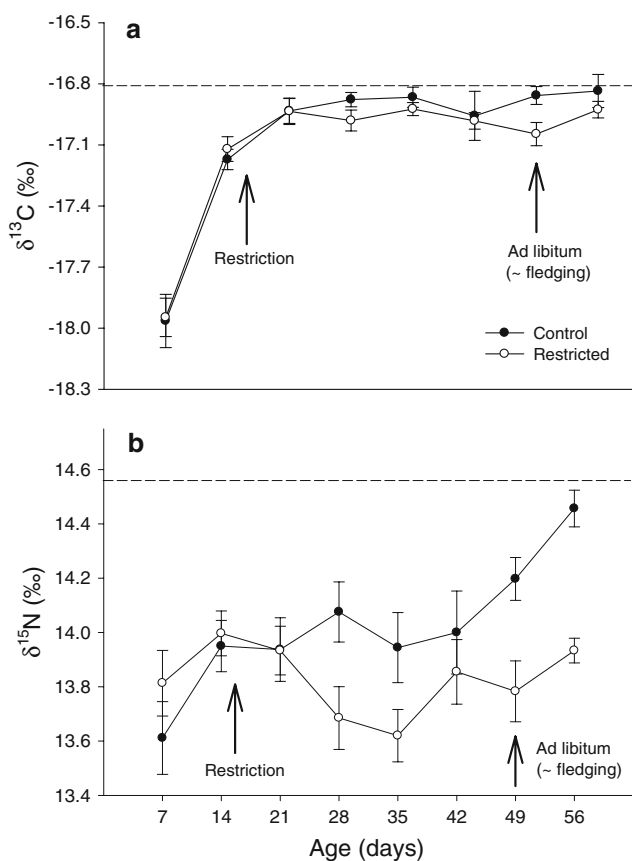


**Fig. 1** Mean body mass ( $\pm$ SE) of rhinoceros auklets on control and 40% food restricted treatments. Arrows Treatment initiation at 15 days and termination at fledging (mean = 49 days)

was complete, there was no difference in RBC  $\delta^{13}\text{C}$  between growing chicks and non-growing subadults on the same diet (Fig. 2a). In subadults,  $\delta^{13}\text{C}$  values were stable between 50 and 60 days on the silverside diet. Using the allometric equation suggested by Carleton and Martinez del Rio (2005) we estimated turnover to be 90% complete 60 days following the switch to silverside.

Because neither growth nor restriction affected RBC  $\delta^{13}\text{C}$  values, we are able to use these values to track turnover and thereby disentangle effects of growth from those of turnover in  $\delta^{15}\text{N}$ . We assumed that rates of turnover were the same for carbon and nitrogen isotopes, as found by previous research in birds on high-protein diets (Bearhop et al. 2002; Evans-Ogden et al. 2004), and estimated turnover of yolk to be complete by 28 days post-hatch (Fig. 2a). After 28 days, we attribute any changes in  $\delta^{15}\text{N}$  of control nestlings to growth.

RBC  $\delta^{15}\text{N}$  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. 2b). Between days 14 and 56, restricted chicks exhibited  $\delta^{15}\text{N}$  values an average of  $0.37\text{‰}$  lower than those of control chicks.  $\delta^{15}\text{N}$  values of control chicks changed most dramatically between days 42 and 56, and showed an overall increase of  $0.55\text{‰}$ .  $\delta^{15}\text{N}$  of control nestlings at 56 days was similar to those of subadult birds fed silverside for 60 days (Fig. 2b). Between days 28 and 56, mean  $\Delta^{15}\text{N}$  between silverside and RBCs increased significantly as growth rate declined ( $F_{1,26} = 8.39$ ,  $P = 0.008$ ; Fig. 3), ranging from  $2.84\text{‰}$  at 35 days to  $3.39\text{‰}$  at 56 days. Subadult RBC  $\delta^{15}\text{N}$  at 180 days exhibited the highest discrimination factors for this diet type,  $3.49\text{‰}$ . Chick C:N did not change with chick age ( $F_{4,43} = 1.09$ ,  $P = 0.37$ ) and was not affected by caloric treatment ( $F_{1,11} = 2.78$ ,  $P = 0.12$ ).

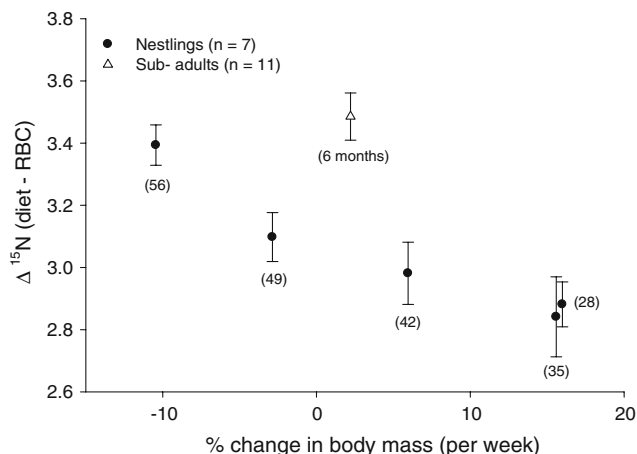


**Fig. 2**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of red blood cells (RBCs) of rhinoceros auklet chicks fed control ( $n = 7$ ) and restricted ( $n = 6$ ) amounts of silverside fish. *Data points* Treatment means  $\pm$  SE; *arrows* treatment initiation at 15 days, prior to which all chicks were fed silverside ad libitum, and termination of treatments at fledging, *horizontal lines* carbon and nitrogen values of non-growing, subadult birds (6 months post-hatch) also fed silverside

Feathers of restricted chicks were significantly depleted in  $\delta^{15}\text{N}$  relative to control chicks ( $t_{11} = 5.23$ ,  $P = 0.001$ ). Control chicks exhibited a mean  $\delta^{15}\text{N}$  of  $15.72 \pm 0.63\text{‰}$  and restricted chicks a mean of  $15.13 \pm 0.10\text{‰}$ . At  $0.59\text{‰}$ , the mean effect size of restriction on feather  $\delta^{15}\text{N}$  values was larger than that seen in red blood cells.  $\delta^{13}\text{C}$  values were not affected by food restriction ( $t_{11} = 0.28$ ,  $P = 0.78$ ) and all chicks exhibited a mean  $\delta^{13}\text{C}$  of  $-15.54 \pm 0.04$ .

*Field samples*

Auklet nestlings ranged in age from 14 to 28 days (mean =  $19.6 \pm 1.1$  days). Adult auklet RBCs exhibited mean values of  $13.38 \pm 0.33\text{‰}$  and  $-19.64 \pm 0.24\text{‰}$  for  $\delta^{15}\text{N}$  and for  $\delta^{13}\text{C}$ , respectively. Nestling RBCs exhibited means of  $13.14 \pm 0.25\text{‰}$   $\delta^{15}\text{N}$  and  $-19.99 \pm 0.16\text{‰}$   $\delta^{13}\text{C}$ . 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  $0.24\text{‰}$  for  $\delta^{15}\text{N}$  and by  $0.35\text{‰}$  for



**Fig. 3** Mean discrimination factor vs mean percentage change in body mass for RBCs of rhinoceros auklets fed control (high calorie) amounts of the fish silverside from hatch. Ages 28–56 days only are included to ensure turnover of yolk  $\delta^{15}\text{N}$  is complete. Each *data point* is a treatment mean ( $\pm$ 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

$\delta^{13}\text{C}$ . All RBC isotope signatures (nestling and adult combined) fell within a range of  $1.03\text{‰}$  for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Adult feather signatures averaged  $15.69 \pm 1.70\text{‰}$   $\delta^{15}\text{N}$  (range =  $5.85\text{‰}$ ) and  $-20.35 \pm 0.63\text{‰}$   $\delta^{13}\text{C}$  (range =  $2.20\text{‰}$ ).

**Discussion**

Nitrogen isotope ratios in developing auklet nestlings exhibited consistent depletion in response to food restriction and rapid growth. RBC  $\delta^{15}\text{N}$  of all nestlings enriched with age, and discrimination factors between fish and RBC were lowest when growth rates were at their highest.  $\delta^{15}\text{N}$  is generally reported to enrich by approximately  $3\text{‰}$  per trophic level (Minigawa and Wada 1984); therefore, from an ecological perspective, the overall effect sizes in our captive study are small ( $0.37\text{--}0.55\text{‰}$ ). However, the variability present in stable isotope signatures of free-living individuals was also surprisingly small. RBC  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  of free-living auklets were contained within a  $1\text{‰}$  range, suggesting that physiological factors such as moderate diet restriction and growth present a biologically meaningful source of variation in  $\delta^{15}\text{N}$  values of this, and potentially other, seabird species. It is important to note that, in this study, all wild samples were collected at a single colony, where individuals were most likely consuming the same

prey species and chicks were in similar nutritional states. Samples collected across different years or locations would exhibit greater variability due to differences in prey species and availability.

The depletion in  $\delta^{15}\text{N}$  values that we witnessed in both feathers and RBCs of restricted auklets is consistent with the prediction that moderate food restriction increases nitrogen-use efficiency and thus diet-tissue fractionation was reduced. This study is among the first to demonstrate a reduction in  $\delta^{15}\text{N}$  values in association with moderate nutritional stress. Our results are similar to those of Williams et al.'s (2007) study on tufted puffins, in which restricted nestlings exhibited reduced  $\delta^{15}\text{N}$  values and blood of all nestlings enriched in  $^{15}\text{N}$  with age. The puffin study, however, was 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 in RBCs, we could demonstrate that growth affected nitrogen isotope signatures independent of turnover. RBC 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-Siessegger 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, we see that discrimination factors for sub-adults is much higher than those of chicks exhibiting no change in body mass (Fig. 3). 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). Chérel et al. (2005), however, reported reduced  $\delta^{13}\text{C}$  in plasma of fasting king penguins (*Aptenodytes patagonicus*), and a concurrent increase in plasma C:N. The authors proposed that such reduction in  $^{13}\text{C}$  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 we are unable to determine whether food restricted auklet chicks were also mobilizing lipids.

In free-living auklets, RBC  $\delta^{15}\text{N}$  of adults was enriched relative to 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  $\delta^{15}\text{N}$  compared to sub-adult birds that had achieved stable masses. Carbon signatures of samples collected from free-living auklets also differed by 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 isotope signatures can take 3–4 weeks, thus wild chicks (aged 2–4 weeks) 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  $\delta^{15}\text{N}$  in free-living birds are not considered. Growth and restriction each affected nitrogen signatures by 0.37–0.55‰. Combined, these effects resulted in restricted chicks exhibiting  $\delta^{15}\text{N}$  values depleted by 0.92‰ relative to a well-fed, non-growing sub-adult bird on the same diet. In light of the small ranges of values present in free-living RBC samples (standard deviations <0.35‰), these physiological effects should be considered in dietary studies. Field studies generally report elevated  $\delta^{15}\text{N}$  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 trophic differences between age classes.

In this study, the nitrogen variability (ranging 2–5‰) present 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  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (Chérel et al. 2000; Clark et al. 2006; Gladbach et al. 2007) and this is most likely due to variable timing and location of molt and associated differences in diet composition. 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 feather isotope ratios may be affected by poor nutritional status, the effect is likely to be small relative to the levels of isotope variation observed in the field. Thus, the effects of nutritional status on feathers does not invalidate the use of this tissue in making ecological inferences.

Due to the pelagic nature of many seabirds, much remains unknown about their 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; Chérel et al. 2007). 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  $^{15}\text{N}$  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  $\delta^{15}\text{N}$  (~2–4‰), such depletion does not necessarily reduce the utility of stable isotope analysis to future trophic studies. However, it should be considered, particularly when the isotopic variation in field samples is small.

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