

## Dietary isotopic discrimination in gentoo penguin (*Pygoscelis papua*) feathers

Michael J. Polito · Stephanie Abel ·  
Craig R. Tobias · Steven D. Emslie

Received: 17 November 2010 / Revised: 13 January 2011 / Accepted: 28 January 2011 / Published online: 15 February 2011  
© Springer-Verlag 2011

**Abstract** Feathers are used commonly for stable isotope analysis to assess the foraging ecology and migration patterns of birds. However, these studies often require knowledge of species-specific feather isotopic discrimination factors (the differences in isotopic ratios between a species' diet and feathers), which can be influenced by a species' physiological state during molt. In this study, we determined the isotopic discrimination factors ( $\Delta^{13}\text{C}_{\text{diet-feather}}$  and  $\Delta^{15}\text{N}_{\text{diet-feather}}$ ) between adult gentoo penguin (*Pygoscelis papua*) diet and feathers in a controlled study. In addition, we tested whether molt duration or the magnitude of voluntary dietary reduction during molt influenced isotopic discrimination, as previous studies have found that nutritional stress can exaggerate  $^{15}\text{N}$  enrichment and in some cases lead to  $^{13}\text{C}$  depletion in feathers. Contrary to this hypothesis, we found no effect of molt duration or dietary reduction on discrimination factors, suggesting that isotopic discrimination is not linearly related to these measures of fasting intensity in penguins. Furthermore, we found that the range of  $\Delta^{15}\text{N}_{\text{diet-feather}}$  found in several species of penguins, which fast while they molt, was similar to discrimination factors in fish-eating birds, which do not fast during molt. It is likely that species-specific metabolic adaptations that limit nutritional

stress while fasting and variation in their relative reliance on endogenous vs. dietary pools during feather growth may confound the use of  $\Delta^{15}\text{N}_{\text{diet-feather}}$  as a general measure of nutritional stress when comparing among species.

**Keywords** Discrimination factors · Gentoo penguin · Molt · Nutritional stress · *Pygoscelis papua* · Stable isotopes

### Introduction

Feathers are used commonly by ecologists for stable isotope analysis to assess foraging ecology and migration patterns of birds (Rubenstein and Hobson 2004). As feathers are metabolically inert after synthesis, they encapsulate information on a bird's diet and foraging habitat during molt (Hobson 1999; Cherel et al. 2000; Phillips et al. 2007). However, to quantitatively assess avian diets and accurately track migratory patterns using stable isotope values requires knowledge of tissue-specific isotopic discrimination factors (the differences in isotopic ratios between diet and consumer tissues). This information is necessary because the isotopic values of feathers and other tissues often exhibit a general enrichment in the heavier isotopes relative to their diet (Minagawa and Wada 1984). However, the amount of enrichment in feathers depends on the isotopes, species, tissue type, and the physiological state of the bird at the time of molt (Hobson and Clark 1992; Hobson et al. 1993; Vanderklift and Ponsard 2003; Cherel et al. 2005a, b).

Carbon ( $\Delta^{13}\text{C}_{\text{diet-feather}}$ ) and nitrogen ( $\Delta^{15}\text{N}_{\text{diet-feather}}$ ) isotopic discrimination factors of feathers have been determined for representatives of several major avian families using controlled laboratory or zoo experiments

M. J. Polito (✉) · S. D. Emslie  
Department of Biology and Marine Biology,  
University of North Carolina, Wilmington, NC, USA  
e-mail: mjp7454@uncw.edu

S. Abel  
Henry Doorly Zoo, Omaha, NE, USA

C. R. Tobias  
Department of Geography and Geology,  
University of North Carolina, Wilmington, NC, USA

(see review by Cherel et al. 2005b). Feather isotopic discrimination has been quantified in three species of penguins: the king penguin (*Aptenodytes patagonicus*), Humboldt penguin (*Spheniscus humboldti*), and rockhopper penguin (*Eudyptes chrysocome*; Cherel et al. 2005b). Unlike many other species of birds, adult penguins undergo a catastrophic molt in which all of their feathers are replaced over a 2- to 3-week period while fasting (Stonehouse 1967). There is evidence that nutritional stress such as fasting can exaggerate  $^{15}\text{N}$  enrichment and in some cases lead to  $^{13}\text{C}$  depletion in feathers (Hobson et al. 1993; Cherel et al. 2005a; although see Kempster et al. 2007). This effect may be due to the mobilization of endogenous protein and lipid stores during fasting, which tend to have higher  $^{15}\text{N}/^{14}\text{N}$  ratios and lower  $^{12}\text{C}/^{13}\text{C}$  than dietary protein sources (Cherel et al. 2005a). However, in zoos, penguins are generally offered food during the molt period (Crissey et al. 2005), and previous studies of captive penguins have not quantified the extent of fasting and/or reduction in dietary intake experienced by adults during molt or examined its effect on dietary isotopic discrimination.

In this study, we determined isotopic discrimination factors between adult gentoo penguin (*Pygoscelis papua*) diet and feathers in a controlled study at the Henry Doorly Zoo, Omaha, Nebraska. Furthermore, we tested whether the length of the molt period and the magnitude of voluntary dietary reduction during molt influence dietary isotopic discrimination factors in this species. Based on the findings of Hobson et al. (1993) and Cherel et al. (2005a), we hypothesize that penguins that have longer molt durations or reduce their food intake by a higher magnitude will have higher  $\Delta^{15}\text{N}_{\text{diet-feather}}$  and lower  $\Delta^{13}\text{C}_{\text{diet-feather}}$  due to the increased use of endogenous protein and lipid relative to recent dietary protein sources. We compare our results with diet to feather isotopic discrimination factors in other penguin and fish-eating bird species from both captive and wild studies.

## Materials and methods

We maintained a captive population of 20 gentoo penguins (8 males and 12 females) on a consistent diet of Atlantic Herring (*Clupea harengus*) for 10 months prior to the start of molt. To confirm the isotopic consistency of penguin diets, we randomly sampled five individual herring per month over the 3 months leading up to and during the molt period (January to March 2008). We measured the weight (g) and standard length (mm) of these 15 fish and stored them frozen until later isotopic analysis. During the study, we hand-fed penguins ad libitum allowing us to record the approximate mass of herring each individual consumed per

day during the 30 days prior to molt and throughout the molt period. We calculated the length of the molt period as the number of days between when flippers swell in size and old feathers begin to lift and stand out from the body to the end of molt when new body feathers are fully grown. Prior to molt in January 2008, we measured the mass of penguins to the nearest 10 g. However, due to logistical constraints, we could not measure the mass of penguins at the end of the molt period. Lastly, we collected three newly grown breast feathers from each adult gentoo penguin following their annual molt in late March 2008.

We homogenized whole-fish and fish muscle samples and dried them for 48 h in an oven at 60°C. We extracted lipids, which are depleted in  $^{13}\text{C}$ , from dried fish tissues using a Soxhlet apparatus with a 1:1 petroleum ether/ethyl ether solvent mixture for 8 h (Seminoff et al. 2007). Feathers were cleaned of surface contaminants using a 2:1 chloroform/methanol rinse and cut into small fragments with stainless steel scissors. Approximately 0.5 mg of each of the above materials was loaded into tin cups for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis. Samples were flash-combusted (Costech ECS4010 elemental analyzer) and analyzed for carbon and nitrogen isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) through an interfaced Thermo Delta V Plus continuous flow stable isotope ratio mass spectrometer (CFIRMS). Raw  $\delta$  values were normalized on a two-point scale using depleted and enriched glutamic acid standard reference materials USGS-40 ( $\delta^{13}\text{C}$ :  $-26.389 \pm 0.042$ ;  $\delta^{15}\text{N}$ :  $-4.5 \pm 0.1$ ) and USGS-41 ( $\delta^{13}\text{C}$ :  $37.626 \pm 0.049$ ;  $\delta^{15}\text{N}$ :  $47.6 \pm 0.2$ ). Sample precision based on internal repeats and duplicate standard reference materials was 0.1 and 0.2‰, for  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$ , respectively.

Stable isotope ratios are expressed in  $\delta$  notation in permil units (‰), according to the following equation:

$$\delta X = \left[ \left( R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right] 1000$$

where  $X$  is  $^{13}\text{C}$  or  $^{15}\text{N}$  and  $R$  is the corresponding ratio  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ . The  $R_{\text{standard}}$  values were based on the Peedee belemnite (VPDB) for  $^{13}\text{C}$  and atmospheric  $\text{N}_2$  for  $^{15}\text{N}$ .

We calculated  $\Delta^{13}\text{C}_{\text{diet-feather}}$  and  $\Delta^{15}\text{N}_{\text{diet-feather}}$  by subtracting the isotope values of each individual's feathers from the mean isotopic values of their diet (herring). Statistical calculations were performed using SAS (version 9.1, SAS Institute 1999) and Number Cruncher Statistical Systems (NCSS; Hintze 2004). Prior to analysis, we examined data using an omnibus normality test and modified Levene equal-variance test and used non-parametric methods when appropriate. We used Kruskal–Wallis tests and paired  $t$  tests to test for consistency in the length, mass, and isotopic values of the herring fed to penguins during our study. We also used two-sample  $t$  tests to test for differences in the molt characteristics and isotopic values of

feathers between sexes. We tested for a relationship between molt duration and the extent of voluntary food reduction using simple linear regression. Lastly, we used general linear models (GLM) and simple linear regressions when testing for an influence of molt duration, reduction in dietary intake, or their interaction on isotopic discrimination factors. All tests were two-tailed, significance was assumed at the 0.05 level, and means are presented  $\pm$ SD.

## Results

The size and mass of individual herring fed to penguins did not differ over the course of our study ( $H_{3,15} = 1.94$  and  $1.46$ ;  $P = 0.379$  and  $0.482$ , for herring length and mass). In addition, the isotopic value of herring did not differ over the course of our study ( $H_{4,25} = 2.78, 0.50, 2.22$ , and  $0.18$ ;  $P = 0.249, 0.779, 0.330$ , and  $0.914$ , for whole-fish  $\delta^{13}\text{C}$ , fish muscle  $\delta^{13}\text{C}$ , whole-fish  $\delta^{15}\text{N}$ , and fish muscle  $\delta^{15}\text{N}$ ), and we found no differences in the isotopic signatures of herring muscle relative to whole fish (Table 1). Therefore, we grouped these 15 samples together and all subsequent analyses are based solely on whole-fish values. In addition, we found no differences in the isotopic values of breast feathers between male and female gentoo penguins (Table 1).

The molt period ranged from 11 to 22 days and averaged  $15.5 \pm 2.5$  days in length but did not differ between males and females (males:  $16.1 \pm 2.7$  and females  $14.5 \pm 1.9$ ;  $t = 1.41$ ,  $P = 0.175$ ). Penguins in our study voluntarily reduced their dietary intake from  $6.7 \pm 1.7\%$  of their body mass in herring  $\text{day}^{-1}$  prior to molt to  $1.8 \pm 1.2\%$  of body mass in herring  $\text{day}^{-1}$  during the molt period. This represents an average reduction in food consumption of  $71.7 \pm 22.1\%$  (median:  $78.9\%$ ; range:  $13.1$ – $94.6\%$ ). Voluntary reduction in dietary intake did not differ between males and females (males:  $75.0 \pm 19.0$  and females:  $66.8 \pm 26.7$ ;  $t = 0.81$ ,  $P = 0.429$ ). We found no

relationship between molt duration and the extent of voluntary food reduction ( $R^2 = 0.01$ ,  $P = 0.804$ ).

Due to the lack of differences in the isotopic values of breast feathers, duration of molt, and reduction in dietary intake among sexes, they were pooled to conserve sample size when calculating isotopic discrimination factors and testing for an influence of molt duration and reduction in dietary intake. Breast feathers were significantly enriched in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values relative to their diets (Table 1;  $t = 5.23$  and  $24.9$ ;  $P < 0.001$  and  $0.001$ , for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and calculated  $\Delta^{13}\text{C}_{\text{diet-feather}}$  and  $\Delta^{15}\text{N}_{\text{diet-feather}}$  averaged  $1.3 \pm 0.5\%$  and  $3.5 \pm 0.4\%$ , respectively (Table 2).  $\Delta^{13}\text{C}_{\text{diet-feather}}$  and  $\Delta^{15}\text{N}_{\text{diet-feather}}$  did not appear to be significantly influenced by molt duration ( $F_{1,20} = 3.51$  and  $0.01$ ;  $P = 0.079$  and  $0.933$ , for  $\Delta^{13}\text{C}_{\text{diet-feather}}$  and  $\Delta^{15}\text{N}_{\text{diet-feather}}$ ), reduction in dietary intake ( $F_{1,20} = 0.40$  and  $0.35$ ;  $P = 0.534$  and  $0.560$ , for  $\Delta^{13}\text{C}_{\text{diet-feather}}$  and  $\Delta^{15}\text{N}_{\text{diet-feather}}$ ), or their interaction terms ( $F_{1,20} = 0.00$  and  $0.20$ ;  $P = 0.350$  and  $0.663$ , for  $\Delta^{13}\text{C}_{\text{diet-feather}}$  and  $\Delta^{15}\text{N}_{\text{diet-feather}}$ ). The lack of significant relationships between isotopic discrimination factors and molt characteristics was also apparent when testing each relationship separately using simple linear regressions (Fig. 1). We also noted a single outlier in our study with both low  $\Delta^{13}\text{C}_{\text{diet-feather}}$  and  $\Delta^{15}\text{N}_{\text{diet-feather}}$  (Fig. 1); however, the results of both our GLM and simple linear regression analyses did not vary when excluding this individual.

## Discussion

While isotopic discrimination factors have been described in other penguin genera, our data represent the first such values for *Pygoscelis* penguins. The gentoo penguin  $\Delta^{15}\text{N}_{\text{diet-feather}}$  value calculated in our study is similar with those determined in other penguin species (Table 2) and consistent with the generalized  $3.0$ – $5.0\%$  enrichment in  $\delta^{15}\text{N}$  predicted per trophic level (DeNiro and Epstein 1981;

**Table 1** The stable nitrogen and carbon isotope ratios (mean  $\pm$  SD) in food and breast feathers of captive gentoo penguins

Sample	<i>n</i>	C/N	$\delta^{15}\text{N}$ (‰)	<i>t</i>	<i>P</i>	$\delta^{13}\text{C}$ (‰)	<i>t</i>	<i>P</i>
Herring								
Whole fish	15	$3.2 \pm 0.1$	$13.7 \pm 0.4$	1.81 <sup>a</sup>	0.090	$-17.2 \pm 1.0$	0.73 <sup>a</sup>	0.472
Fish muscle	15	$3.2 \pm 0.1$	$13.8 \pm 0.3$			$-17.1 \pm 1.0$		
Breast feathers								
Male	8	$3.1 \pm 0.1$	$17.2 \pm 0.6$	0.11 <sup>b</sup>	0.911	$-16.0 \pm 0.6$	0.61 <sup>b</sup>	0.548
Female	12	$3.1 \pm 0.1$	$17.2 \pm 0.2$			$-15.8 \pm 0.3$		
All	20	$3.1 \pm 0.1$	$17.2 \pm 0.4$			$-15.9 \pm 0.5$		

<sup>a</sup> Paired *t* test between whole fish and fish muscle

<sup>b</sup> Two sample *t* test between male and female breast feathers

**Table 2** Estimates of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  discrimination factors between food and penguin breast feathers

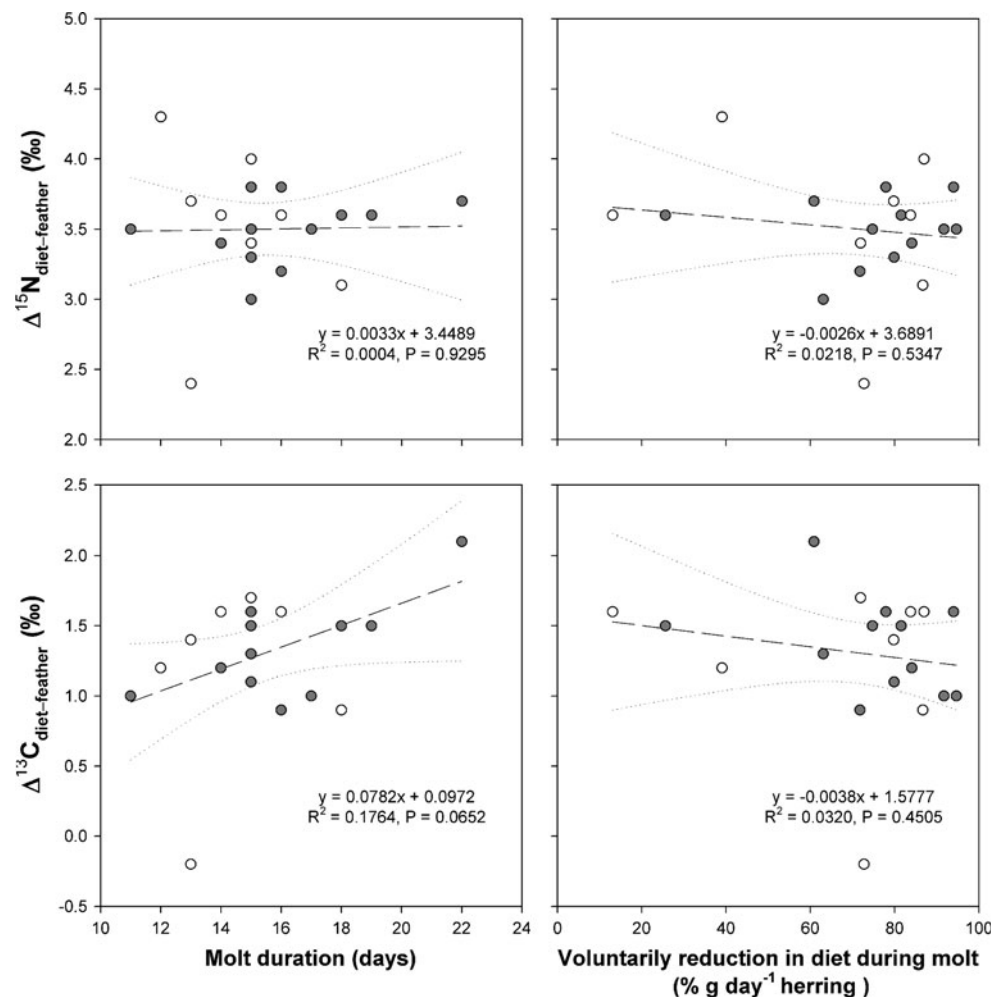
Species, age, tissue	Diet, molt condition	Discrimination Factor (‰)		
		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Reference <sup>a</sup>
<b>King penguin <i>Aptenodytes patagonicus</i></b>				
Adult, body feather	Herring <sup>b</sup> , captive (reduced feeding)	0.1	3.5	1
Adult, body feather (distal region removed)	Wild diet, fasting on land	1.6	3.6	2
Adult, body feather (distal region only)	Wild diet, feeding at sea	0.4	2.1	2
Chick, down	Wild diet, fed by parents	2.4	3.6	2
Chick, body feather	Wild diet, fed by parents (under fed)	1.5	3.4	2
<b>Gentoo penguin <i>Pygoscelis papua</i></b>				
Adult, body feather	Herring <sup>b</sup> , captive (reduced feeding)	1.3 ± 0.5	3.5 ± 0.4	3
<b>Humboldt penguin <i>Spheniscus humboldti</i></b>				
Adult, body feather	Anchovy <sup>b</sup> , captive (unknown if feeding)	–	4.8	4
<b>Rockhopper penguin <i>Eudyptes chrysocome</i></b>				
Adult, body feather	Capelin <sup>b</sup> , captive (reduced feeding)	0.1	4.4	1

Diet and molt conditions are taken from references listed. Gentoo penguin discriminations factors from this study are provided as mean ± SD

<sup>a</sup> (1) Cherel et al. 2005a, (2) Cherel et al. 2005b, (3) This study, (4) Mizutani et al. 1992

<sup>b</sup> Herring (*Clupea harengus*), anchovy (*Engraulis japonica*), capelin (*Mallotus villosus*)

**Fig. 1** The relationships between adult gentoo penguin feather dietary discrimination factors ( $\Delta^{13}\text{C}_{\text{diet-feather}}$  and  $\Delta^{15}\text{N}_{\text{diet-feather}}$ ), the length of the molt period, and the magnitude of voluntary dietary reduction during molt. *Open symbols* represent males, and *closed symbols* represent females. Linear regression equations, regression lines (*dashed*), and 95% confidence lines (*dotted*) are presented for each relationship



Minagawa and Wada 1984). Similarly, our gentoo penguin  $\Delta^{13}\text{C}_{\text{diet-feather}}$  value was within the range of discrimination factors determined for other penguin species (Table 2). The gentoo penguin  $\Delta^{15}\text{N}_{\text{diet-feather}}$  and  $\Delta^{13}\text{C}_{\text{diet-feather}}$  values derived from our study will allow for greater application of the use of feathers to quantify *Pygoscelis* penguin diets using stable isotopes (Tierney et al. 2008). However, it is important to note that gentoo penguins naturally feed on variable amounts of crustaceans and fishes and not herring as in our captive study (Miller et al. 2009). We therefore recommend the inclusion of a sensitivity analysis of the potential error involved with quantifying wild diets using isotopic discrimination factors derived from captive populations (Bond and Diamond 2010; Polito et al. 2011).

Contrary to our hypotheses, we found no significant relationships between molt duration and the magnitude of reduction in dietary intake with the isotopic discrimination factors calculated in our study. Our results differ with findings from previous studies on captive and wild birds, including penguins. Hobson et al. (1993) found that tissue  $\delta^{15}\text{N}$  values of food-restricted Japanese Quail (*Coturnix japonica*) chicks were significantly higher than in chicks fed ad libitum. In the wild, fasting Ross' Geese (*Chen rossii*) have tissues enriched in  $^{15}\text{N}$  (Hobson et al. 1993), and the proximal ends of adult king penguin body feathers grown while fasting are enriched in  $^{15}\text{N}$  and depleted in  $^{13}\text{C}$  relative to the distal tips and sheathes of body feathers that are grown at sea (Cherel et al. 2005a). However, in our study, gentoo penguin  $\Delta^{15}\text{N}_{\text{diet-feather}}$  was not significantly affected by the duration of molt or intensity of fasting. Furthermore, while we did observe an apparent slight increase in  $\Delta^{13}\text{C}_{\text{diet-feather}}$  values with molt duration, this trend was not significant and likely influenced by a single outlier with a low  $\Delta^{13}\text{C}_{\text{diet-feather}}$  (Fig. 1).

Unlike wild penguins, adults in our study had access to food while molting. It may be that while captive adults were molting, they did not attain a similar physiological state to wild penguins and as such any fasting-induced variation in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values was not apparent in their feathers. In addition, not all studies have found evidence of  $^{15}\text{N}$  enrichment and/or  $^{13}\text{C}$  depletion in tissues produced while under nutritional stress (Kempster et al. 2007; Williams et al. 2007; Sears et al. 2009). A recent review and case study using song sparrows suggest isotopic values may not be linearly related to nutritional stress and there may be a threshold level of stress below which such isotopic changes are likely to be negligible (Kempster et al. 2007). Unfortunately, due to logistical constraints, we could not quantify individuals' mass loss over the molt period. It might be possible that if we had used this metric of fasting intensity, any moderate effects of fasting on feather isotopic discrimination would have become apparent.

The feather discrimination factors we calculated in this study were similar to those previously estimated in both captive and fasting wild king penguins (Table 2). Similar to our study, in a captive study of king penguins, individuals were offered food during the molt period but voluntarily reduced their food consumption dramatically (Cherel et al. 2005b, Y. Cherel, pers. comm.). Even so, the  $\Delta^{15}\text{N}_{\text{diet-feather}}$  derived from Cherel et al. (2005b) captive study was nearly identical to the  $\Delta^{15}\text{N}_{\text{diet-feather}}$  estimated in fasting wild king penguins (Table 2; Cherel et al. 2005a). This result suggests that any food provided to adults in Cherel et al.'s (2005b) captive study had little effect on feather  $\delta^{15}\text{N}$  discrimination relative to naturally fasting wild king penguins. Most penguins gain a significant amount of weight prior to molt, including the preferential buildup and storage of proteins in skeletal muscles (Stonehouse 1967; Cherel et al. 1993; Cherel 1995). Furthermore, pectoral muscles and integument protein metabolism provide approximately 77% of the total protein needed for new feather synthesis in wild penguins (Cherel et al. 1994). These findings suggest that adult penguin feathers are preferentially synthesized from a pool of body proteins and that any dietary proteins ingested during molt may contribute little to feather synthesis. However, it is important to note that unlike  $\Delta^{15}\text{N}_{\text{diet-feather}}$ , estimates of  $\Delta^{13}\text{C}_{\text{diet-feather}}$  differed between captive and wild king penguins by approximately 1.5‰ (Cherel et al. 2005a, b). Seasonal variation in the diets of wild king penguins and thus isotopic differences in the  $\delta^{13}\text{C}$  signatures of their endogenous vs. dietary carbon pools may account for differences in  $\delta^{13}\text{C}$  discrimination factors in comparison with captive penguins kept on a constant diet. In addition, carbon and nitrogen used in feather synthesis may be sourced independently from each other (Klasing 1998).  $\Delta^{13}\text{C}_{\text{diet-feather}}$  values may be affected not only by variation in the contribution of different carbon pools (endogenous vs. dietary) as in  $\Delta^{15}\text{N}_{\text{diet-feather}}$  values but possibly also by carbon sources within each pool (protein vs. lipid; Bearhop et al. 2002). These results highlight the inherent complexity of the metabolic pathways involved in the synthesis of feather keratin.

When examining captive studies in general, the range of  $\Delta^{15}\text{N}_{\text{diet-feather}}$  in adult penguins, which fast while they molt, and other fish-eating birds, which do not, is similar (2.1–4.8‰ vs. 3.0–5.3‰; see review by Cherel et al. 2005a). As penguins are suggested to “feed on themselves” during molt, one might expect penguins to have higher  $\Delta^{15}\text{N}_{\text{diet-feather}}$  values than other seabirds on average. We suggest two possible explanations for this observation: (1) penguins in captive studies may not attain a similar fasting state such that their physiology is more similar to non-fasting fish-eating birds and/or (2) species-specific metabolic adaptations to limit nutritional stress

while fasting and their relative reliance on endogenous vs. dietary pools during feather growth may confound comparisons across species. These two explanations are not exclusive and both may contribute to the lack of differences between captive studies of penguin and non-fasting, fish-eating birds. While captive penguins may not be under the same metabolic constraints during molt as wild penguins, small amounts of food provided to adults in captive studies appear to have little to no effect on  $\Delta^{15}\text{N}_{\text{diet-feather}}$  (Cherel et al. 2005a, b). Thus, species-specific and even age-specific differences in the relative importance of income and capital investment and variation in the biochemical pathways involved in feather synthesis are likely to confound such comparative examinations. For example, growing chicks have metabolic constraints that differ from adults and can often lead to differences in isotopic discrimination factors with age (Williams et al. 2007; Sears et al. 2009). While this may be true in some species, there is little evidence to suggest such age effects in penguins as the  $\Delta^{15}\text{N}_{\text{diet-feather}}$  values of king penguin chicks, which are fed during feather synthesis, are similar to those calculated for fasting adult king penguins (Table 2; Cherel et al. 2005a). It may be that king penguin chicks and some other fish-eating birds that do not fast during molt also utilize variable amounts of amino acids from endogenous pools for feather synthesis (Murphy 1996; Bearhop et al. 2002). A recent study suggests up to 60% contribution of endogenous proteins to greylag geese (*Anser anser*) flight feather synthesis (Fox et al. 2009). In many avian species, the utilization of endogenous pools in addition to dietary pools during feather growth could be an adaptation to molting during metabolically stressful periods such as migration or reproduction (Merilä 1997; Yuri and Rohwer 1997; Bridge 2006; Ramos et al. 2009).

In summary, the diet to feather isotopic discrimination factors calculated in our study are similar to those estimated in wild penguins and as such will allow for a greater application of the use of feathers to quantify *Pygoscelis* penguin diets using stable isotopes. However, further work is needed to elucidate the biochemical pathways involved in feather synthesis, the relative importance of income and capital investments, and how these factors interact and affect isotopic discrimination in captive and wild seabirds.

**Acknowledgments** This research was funded by NSF OPP grants ANT-0125098 and ANT-0739575, with additional support from a Ralph W. Brauer Fellowship to M. Polito. We thank K. Vires, J. Beck, K. McGrath, T. Solberg and D. Rivard, and the staff of the Scott Kingdoms of the Seas Aquarium at Omaha's Henry Doorly Zoo for their invaluable assistance with this study. T. Lankford and K. Durenberger provided assistance with lipid extractions and stable isotope analysis. We also thank Y. Cherel and K. Hobson for helpful insights during the preparation of this manuscript. This manuscript was improved through the comments of three reviewers: M. Beaulie,

A. Bond, and C. Williams. This work complies with, and was completed in accordance to, IACUC permit number HDZ#07-800.

## References

- Bearhop S, Waldron S, Voiter SC, Furness RW (2002) Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Phys Biochem Zool* 75:451–458. doi:10.1086/342800
- Bond AL, Diamond AW (2010) Recent Bayesian stable isotope mixing models are highly sensitive to variation in discrimination factors. *Ecol Appl* (In press). doi: 10.1890/09-2409.1
- Bridge EL (2006) Influences of morphology and behaviour on wing-molt strategies in seabirds. *Mar Ornithol* 34:7–19
- Cherel Y (1995) Nutrient reserve storage, energetics, and food consumption during the prebreeding and premoulted foraging periods of King Penguins. *Polar Biol* 15:209–214. doi:10.1007/BF00239060
- Cherel Y, Charrassin JB, Handrich Y (1993) Comparison of body reserve buildup in prefasting chicks and adults of King Penguins (*Aptenodytes patagonicus*). *Physiol Zool* 66:750–770
- Cherel Y, Charrassin JB, Challet E (1994) Energy and protein requirements for molt in the King Penguin *Aptenodytes patagonicus*. *Am J Physiol* 266:1182–1188
- Cherel Y, Hobson KA, Weimerskirch H (2000) Using stable-isotope analysis of feathers to distinguish moulting and breeding origins of seabirds. *Oecologia* 122:155–162. doi:10.1007/PL00008843
- Cherel Y, Hobson KA, Bailleul F, Groscolas R (2005a) Nutrition, physiology, and stable isotopes: new information from fasting and molting penguins. *Ecology* 86(11):2881–2888. doi:10.1890/05-0562
- Cherel Y, Hobson KA, Hassani S (2005b) Isotopic discrimination factors between food and blood and feathers of captive penguins: Implications for dietary studies in the wild. *Physiol Biochem Zool* 78(1):106–115. doi:10.1086/425202
- Crissey S, Rice DF, Rice AL, McGill P, Slifka KS (2005) Diet and Nutrition. In: Penguin Husbandry Manual, 3rd edn. Penguin taxon advisory group of the American zoo and aquarium Association, pp 86–111 <http://www.aviansag.org/standards.html>
- DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta* 45:341–351
- Fox AD, Hobson KA, Kahlert J (2009) Isotopic evidence for endogenous protein contributions to greylag goose *Anser anser* flight feathers. *J Avian Biol* 40:108–112. doi:10.1111/j.1600-048X.2009.04720.x
- Hintze J (2004) NCSS and PASS. Number cruncher statistical systems, Kaysville <http://www.ncss.com>
- Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120:314–326. doi:10.1007/s004420050865
- Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes II. Factors influencing diet-tissue fractionation. *Condor* 94:189–197
- Hobson KA, Alsaukas RT, Clark RG (1993) Stable nitrogen enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analysis of diet. *Condor* 95:388–394
- Kempster B, Zanette L, LongstaVe FJ, MacDougall-Shackleton SA, Wingfield JC, Clinchy M (2007) Do stable isotopes reflect nutritional stress? Results from a laboratory experiment on song sparrows. *Oecologia* 151:365–371. doi:10.1007/s00442-006-0597-7
- Klasing KC (1998) Comparative avian nutrition. CAB International, New York

- Merilä J (1997) Fat reserves and moult-migration overlap in goldcrests, *Regulus regulus*—a trade-off? *Ann Zool Fenn* 34: 229–234
- Miller AK, Karnovsky NJ, Trivelpiece WZ (2009) Flexible foraging strategies of gentoo penguins *Pygoscelis papua* over 5 years in the South Shetland Islands, Antarctica. *Mar Biol* 156(12): 2527–2537. doi: [10.1007/s00227-009-1277-z](https://doi.org/10.1007/s00227-009-1277-z)
- Minagawa M, Wada E (1984) Stepwise enrichment of  $^{15}\text{N}$  along food chains: further evidence and the relation between  $\delta^{15}\text{N}$  and animal age. *Geochim Cosmochim Acta* 48:1135–1140
- Mizutani H, Fukuda M, Kabaya Y, Wada E (1992)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  enrichment factors of feathers of 11 species of adult birds. *Ecology* 73(4):1391–1395. doi: [10.2307/1940684](https://doi.org/10.2307/1940684)
- Murphy ME (1996) Energetics and nutrition of molt. In: Carey C (ed) *Avian energetics and nutritional ecology*. Chapman & Hall, New York, pp 158–198
- Phillips RA, Catry P, Silk JRD, Bearhop S, McGill R, Afanasyev V, Strange IJ (2007) Movements, winter distribution and activity patterns of Falkland and brown skuas: insights from loggers and isotopes. *Mar Ecol Prog Ser* 345:281–291. doi: [10.3354/meps06991](https://doi.org/10.3354/meps06991)
- Polito MJ, Lynch HJ, Naveen R, Emslie SD (2011) Stable isotopes reveal regional heterogeneity in the pre-breeding distribution and diets of sympatrically breeding *Pygoscelis* penguins. *Mar Ecol Prog Ser*. In press. doi: [10.3354/meps08863](https://doi.org/10.3354/meps08863)
- Ramos R, Militã T, González-Solís J, Ruiz X (2009) Moulting strategies of a long-distance migratory seabird: the Mediterranean Cory's shearwater. *Ibis* 151:151–159. doi: [10.1111/j.1474-919X.2008.00877.x](https://doi.org/10.1111/j.1474-919X.2008.00877.x)
- Rubenstein DR, Hobson KA (2004) From birds to butterflies: animal movement patterns and stable isotopes. *Trends Ecol Evol* 19:256–263. doi: [10.1016/j.tree.2004.03.017](https://doi.org/10.1016/j.tree.2004.03.017)
- Sears J, Hatch SA, O'Brien DM (2009) Disentangling effects of growth and nutritional stress on seabird stable isotope ratios. *Oecologia* 159:41–48
- Seminoff JA, Bjorndal KA, Bolten AB (2007) Stable carbon and nitrogen isotope discrimination and turnover in pond sliders *Trachemys scripta*: Insights for trophic study of freshwater turtles. *Copeia* 2007(3):534–542. doi: [10.1643/0045-8511\(2007\)2007\[534:SCANID\]2.0.CO;2](https://doi.org/10.1643/0045-8511(2007)2007[534:SCANID]2.0.CO;2)
- Stonehouse B (1967) The general biology and thermal balance of penguins. *Adv Ecol Res* 4:131–196
- Tierney M, Southwell C, Emmerson LM, Hindell MA (2008) Evaluating and using stable-isotope analysis to infer diet composition and foraging ecology of Adélie penguins *Pygoscelis adeliae*. *Mar Ecol Prog Ser* 355:297–307. doi: [10.3354/meps07235](https://doi.org/10.3354/meps07235)
- Vanderklift MA, Ponsard S (2003) Source of variation in consumer-diet  $\delta^{15}\text{N}$  enrichment: a meta-analysis. *Oecologia* 136:169–182. doi: [10.1007/s00442-003-1270-z](https://doi.org/10.1007/s00442-003-1270-z)
- Williams CT, Buck CL, Sears J, Kitaysky AS (2007) Effects of nutritional restriction on nitrogen and carbon stable isotopes in growing seabirds. *Oecologia* 153:11–18
- Yuri T, Rohwer S (1997) Moulting and migration in the northern rough-winged swallow. *Auk* 114:249–262