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Tissue-specific isotopic discrimination factors in gentoo penguin (*Pygoscelis papua*) egg components: Implications for dietary reconstruction using stable isotopes

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ABSTRACT

Stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) is a useful tool when examining animal diets due to a general enrichment in heavier isotopes from prey tissues to the tissues of the predators consuming them. However, the amount of this enrichment, or discrimination, can vary among taxa and tissue type, limiting the use of stable isotope analysis when estimating diet composition. In this study we calculate the dietary isotopic discrimination factors of specific *Pygoscelis* penguin egg tissues, including eggshell, shell membrane, albumen and yolk, using a captive population of gentoo penguins fed known diets. We found that discrimination factors varied by isotope, tissue, and whether factors were calculated from whole fish or fish muscle. The observed variation in discrimination factors across tissues likely reflects differences in biochemical and metabolic processes during tissue synthesis. We validated the use of tissue discrimination factors derived in this study by independently reconstructing the diet composition of wild gentoo penguins at Cape Sherriff, Livingston Island, Antarctica using the $\delta^{15}N$ values of eggshell organics and shell membrane. While eggshell organics and shell membrane from the same egg differed in raw $\delta^{15}N$ values, modeling confirmed that these differences were due to tissue-specific isotopic discrimination. Furthermore, our results suggest that in 2006, female gentoo penguins at Cape Sherriff consumed a higher percentage of krill than fish during the egg-laying period.

1. Introduction

Stable isotope abundances of carbon and nitrogen in animal biomass are largely determined by isotopic abundances in the animal's food, with specific tissues reflecting the diet at the time of synthesis (Mizutani et al., 1990). Therefore by sampling tissues produced at different locations, or times of the year, it is possible to examine variations in an animal's diet over both time and space (Schell et al., 1989; Hobson, 1999; Cherel et al., 2000; Rubenstein and Hobson, 2004; Quillfeldt et al., 2005). For example, the isotopic analyses of specific avian tissues have the potential to provide information on the diets and foraging habitats of penguins throughout much of their annual cycle. Egg tissues can provide information on female diets during a brief period prior to breeding (Astheimer and Grau, 1985; Emslie and Patterson, 2007; Strickland et al., 2008). Chick feathers reflect parental diets during the chick-rearing period, while adult feathers provide information on diets and foraging habitats after the breeding season when adults undergo molt (Penny, 1967; Cherel et al., 2000, 2005; Ainley et al., 2003; Quillfeldt et al., 2005).

The isotopic values of animal tissues are useful for examining diets due to a general enrichment in the heavier isotopes from prey tissues to the tissues of the predators consuming them (DeNiro and Epstein, 1978, 1981; Minagawa and Wada, 1984). However, the amount of enrichment depends on the tissues and the physiological pathways that produce them (Hobson and Clark, 1992). This can result in predator tissues of differing composition having unique isotope values even though they are synthesized under the same diet. These differing discrimination factors (the differences in isotopic ratios between prey items and consumer tissues) in animal tissues make it difficult to both initially identify prey items and directly compare the isotopic values of different tissue types.

Researchers have been able to determine the discrimination factors of specific animal tissues using controlled laboratory or zoo experiments (Mizutani et al., 1992; Hobson and Clark, 1992; Hobson, 1995; Cherel et al., 2005; Seminoff et al., 2007; Reich et al., 2008). These experiments generally involve keeping animals on an isotopically consistent diet for a period of time. Samples of the animal tissues are then compared to the isotopic values of their food. Most avian studies, including those on penguins, have focused on discrimination factors in blood and feathers and little is known about the magnitude of enrichment in other tissues such as egg components (Vanderklift and Ponsard, 2003).

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Table 1
The carbon to nitrogen ratio (C/N) and stable nitrogen and carbon isotope concentrations (mean ± SD) in food and egg components of captive gentoo penguins.

Captive penguin food and tissue	*n*	C/N ratio	‰	
			δ^{15}N	δ^{13}C
Herring				
Whole fish	10	3.2 ± 0.1	12.3 ± 0.1	−19.4 ± 0.3
Fish muscle	10	3.2 ± 0.1	12.5 ± 0.3	−19.5 ± 0.3
Eggshell organics				
Egg 1	9	3.5 ± 0.1	13.9 ± 0.3	−18.0 ± 0.4
Egg 2	9	3.5 ± 0.1	14.4 ± 0.5	−18.0 ± 0.3
Egg 3	2	3.5	14.1	−17.8
All eggs	20	3.5 ± 0.1	14.1 ± 0.5	−18.0 ± 0.4
Eggshell carbonates				
Egg 1	9	–	–	−12.2 ± 0.4
Egg 2	9	–	–	−12.1 ± 0.8
Egg 3	2	–	–	−12.0
All eggs	20	–	–	−12.1 ± 0.7
Shell membrane				
Egg 1	9	3.2 ± 0.1	16.7 ± 0.4	−16.6 ± 0.5
Egg 2	9	3.2 ± 0.1	16.6 ± 0.5	−16.5 ± 0.4
Egg 3	2	3.2	17.0	−16.6
All eggs	20	3.2 ± 0.1	16.7 ± 0.5	−16.6 ± 0.9
Albumen	5	4.0 ± 0.1	17.0 ± 0.5	−18.6 ± 0.7
Yolk	5	4.2 ± 0.2	16.0 ± 0.5	−19.4 ± 0.5

Using a captive collection of gentoo penguins (*Pygoscelis papua*) at the Henry Doorly Zoo, Omaha, Nebraska, we determined the tissue-specific isotopic discrimination factors between penguin diet and specific egg tissues. When examined in light of their specific discrimination factors it is likely that, similar to blood and feathers, egg tissues can be used to provide information on the diets and foraging habitats of penguins. We tested this possibility by using a single-isotope, two-source linear mixing model to reconstruct the diet composition of a wild population of gentoo penguins at Cape Sherriff, Livingston Island, Antarctica. We compared independent estimates of penguin diets based on isotopic values of eggshell organics and shell membrane, two tissues that are produced during the egg-laying period, to validate the use of the tissue-specific discrimination factors derived in our captive study.

2. Materials and methods

2.1. Captive penguin diet and tissue collection

We studied gentoo penguins from a captive breeding population maintained at the Henry Doorly Zoo in Omaha, Nebraska. Penguins were kept on a consistent diet of Atlantic herring (*Clupea harengus*) for eight months prior to the start of tissue collection. To confirm the isotopic consistency of penguin diets, five individual herring were randomly sampled per month during the two months prior to the mean date of egg-laying (11 November 2007). We measured the weight (g) and standard length (mm) of these 10 fish, which were then stored frozen prior to isotopic analysis.

Eggs were marked as they were laid using a black permanent marker to track laying order. Approximately seven days after laying, eggs were candled to determine if they were fertile. A sub-sample of five infertile eggs was collected and frozen for later isotopic analysis of egg albumen and yolk. Following Hobson (1995), we assume that infertile eggs would be similar to fertilized eggs in isotopic value, as fertilization is independent of egg formation. The remaining eggs were allowed to be incubated by their parents until they either hatched or were determined to be addled, at which time eggshell and membrane samples were collected. This provided us with eggshell and shell membrane samples from nine two-egg clutches and two three-egg clutches for a total of 20 eggs.

2.2. Wild penguin diet and tissue collection

During the austral summer of 2006–07, we collected penguin egg tissues from a colony of approximately 800 breeding pairs of gentoo penguins at Cape Sherriff, Livingston Island, Antarctica (62°28′S, 60°47′W). In November and December 2006, 20 eggshells (including attached shell membranes) were opportunistically collected from hatched, depredated, addled or infertile eggs. Penguin prey items were collected during trawls conducted in the vicinity of the South Shetland Islands by the U.S. Antarctic Marine Living Resource Program (US AMLR) during the austral summers of 2000–01 to 2006–07. Sampled prey species were representative of the two major components of gentoo penguin diets in this region: krill (*Euphausia superba*; $n = 10$) and fish (*Lepidonotothen squamifrons*; $n = 7$; Volkman et al., 1980; Karnovsky, 1997). While whole krill were frozen prior to analysis, fish muscle was stored in 70% ethanol as freezing of these samples was not possible in the field. We assume no effects of the different storage methods as storage in ethanol does not significantly alter the isotopic composition of tissues (Hobson et al., 1997).

2.3. Sample preparation and isotopic analysis

Whole fish, fish muscle, and whole krill samples were homogenized and then dried for 48 h in an oven at 60 °C. Eggshell, shell membrane, albumen and yolk were separated by hand and sub-samples of yolk and albumen were collected and freeze-dried. Dried fish, krill, and penguin albumen and yolk were ground to a powder using an analytical mill. Lipids were then extracted from whole fish and krill, fish muscle, and penguin yolk samples using a Soxhlet apparatus with a 1:1 Petroleum–Ether: Ethyl–Ether solvent mixture for 6–8 h (Seminoff et al., 2007). The mean C/N ratio of lipid-free tissues ranged from 3.1 ± 0.1 to 4.2 ± 0.2; comparable to values found in previous studies (Tables 1–3; Sweeting et al., 2006; Cherel et al., 2007). Lipid extracted herring and wild penguin prey items were not acidified prior to isotopic analysis. Approximately 0.5 mg of each of the above materials was loaded into tin cups for δ^{13}C and δ^{15}N analysis.

Table 2
Discrimination factors and two sample t-tests between captive penguin food and penguin tissues.

Tissue	Food	δ^{15}N		δ^{13}C	
		Discrimination factor (‰)	*t* (P)	Discrimination factor (‰)	*t* (P)
Eggshell organics	Whole fish	1.8	11.73 (<0.001)	1.4	10.82 (<0.001)
	Fish muscle	1.6	10.17 (<0.001)	1.5	11.40 (<0.001)
Eggshell carbonates	Whole fish	–	–	7.2	32.25 (<0.001)
	Fish muscle	–	–	7.4	32.43 (<0.001)
Shell membrane	Whole fish	4.4	27.54 (<0.001)	2.8	17.19 (<0.001)
	Fish muscle	4.2	25.38 (<0.001)	2.9	17.58 (<0.001)
Albumen	Whole fish	4.7	27.10 (<0.001)	0.8	3.00 (0.010)
	Fish muscle	4.5	22.83 (<0.001)	0.9	3.38 (0.005)
Yolk	Whole fish	3.7	23.50 (<0.001)	0.0	0.19 (0.855)
	Fish muscle	3.5	19.07 (<0.001)	0.1	0.42 (0.690)

Table 3
The carbon to nitrogen ratio (C/N) and stable nitrogen and carbon isotope concentrations (mean ± SD) in food and egg components of wild gentoo penguins at Cape Sherriff, Livingston Island, Antarctica, 2006.

Wild penguin food and tissue	*n*	C/N ratio	‰	
			δ^{15}N	δ^{13}C
Krill *E. superba*	10	3.7 ± 0.2	3.3 ± 0.7	−25.3 ± 1.0
Fish *L. squamifrons*	7	3.1 ± 0.1	12.1 ± 0.5	−23.6 ± 0.6
Eggshell organics	20	3.7 ± 0.3	8.2 ± 0.6	−23.4 ± 0.3
Shell membrane	20	3.2 ± 0.1	10.5 ± 0.4	−22.4 ± 1.0

Isotope values of the organic matrix of penguin eggshells (hereafter called eggshell organics) were obtained after the removal of carbonate by dissolving ~10 mg of cleaned eggshell in a silver capsule through titration with five 20 μL aliquots of 6 N HCL. Acidified samples were stored at room temperature under a fume hood for 24 h, and then dried for at least 48 h in an oven at 60 °C. Acidified samples were not rinsed prior to drying so as to avoid biasing δ^{15}N values (Jacob et al., 2005). The above tissues were flash-combusted (Costech ECS4010 elemental analyzer) and analyzed for carbon and nitrogen isotopes (δ^{13}C and δ^{15}N) through an interfaced Thermo Delta V Plus continuous flow stable isotope ratio mass spectrometer (CFIRMS). Raw δ values were normalized on a two-point scale using depleted and enriched glutamic acid reference materials USGS-40 and USGS-41. Sample precision was 0.1‰ and 0.2‰, for δ^{13}C, and δ^{15}N, respectively.

Isotopic values of eggshell carbonates (δ^{13}C) were determined by reacting 0.2 mg of powdered eggshell with 0.1 ml of ultra-pure phosphoric acid at 70 °C. The resulting CO_2 was introduced to a Thermo Delta V Plus CFIRMS through a Thermo Gas Bench II interface. Raw δ^{13}C values were normalized to NBS-19 (calcite) and L-SVEC (lithium carbonate) referenced materials, with a sample precision of 0.05‰.

Stable isotope abundances are expressed in δ notation in per mill units (‰), according to the following equation:

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

Where X is ^{13}C or ^{15}N and R is the corresponding ratio ^{13}C / ^{12}C or ^{15}N / ^{14}N. The R_{standard} values were based on the PeeDee Belemnite (VPDB) for ^{13}C and atmospheric N_2 for ^{15}N.

2.4. Model and statistical analysis

We quantified the contribution of krill (*E. superba*) and fish (*L. squamifrons*) to the diet of gentoo penguins at Livingston Island by using a single-isotope, two-source linear mixing model (Phillips and Gregg, 2001). Model results provide standard errors and confidence intervals for source proportion estimates that account for the observed variability in the isotopic signatures for the sources as well as the mixture. We used δ^{15}N values of the two common prey items in this model because the difference in prey isotopic signatures was larger for δ^{15}N than for δ^{13}C which showed some source/end-member overlap (Fig. 1). A previous study found that using isotopic signatures of whole prey items with discrimination factors of prey muscle (or the reverse) can lead to incorrect estimates of diet composition (Cherel et al., 2005). As our two prey items differ in this regard (whole krill and fish muscle), we corrected whole krill and fish muscle values independently (by adding whole prey and prey muscle discrimination factors, respectively) prior to incorporating them into the model.

Data were examined for normality and equal variance and non-parametric methods were employed when necessary. All tests were two-tailed and significance was assumed at the 0.05 level. Statistical calculations were performed with Number Cruncher Statistical Systems (NCSS; Hintze, 2004).

3. Results

3.1. Captive penguin diet

We found no significant differences in the length and mass of herring collected during the two months prior to egg-laying (Mann–Whitney U; $Z = 0.31$, 0.32 and $P = 0.754$, 0.750, for body mass and standard length respectively). Furthermore, herring fed to penguins during these months did not differ significantly in isotopic values (Table 1; $Z = 1.35$, 1.88, 1.36, 0.94 and $P = 0.175$, 0.060, 0.175, 0.347 for whole fish δ^{15}N, fish muscle δ^{15}N, whole fish δ^{13}C and fish muscle δ^{13}C, respectively). We therefore pooled these 10 herring for subsequent analysis.

The δ^{15}N values of herring muscle were slightly enriched (0.2‰ ± 0.3‰), on average, relative to whole fish. While this trend was statistically significant (Table 1; paired t-test: $t = 2.29$, $P = 0.048$) the

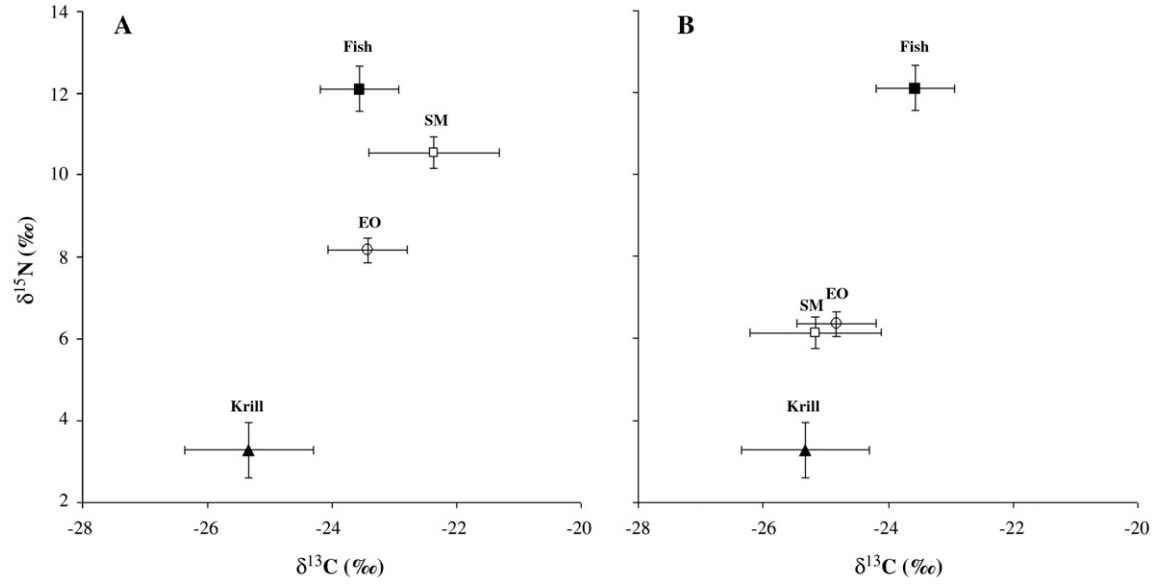

Fig. 1. Raw (A) and corrected (B) stable nitrogen and carbon isotope values for eggshell organics (EO; $n = 20$) and shell membrane (SM; $n = 20$) from gentoo penguin eggshells collected at Cape Sheriff, Livingston Island, Antarctica, 2006, relative to two common prey items, krill (*Euphausia superba; n* = 10) and fish (*Lepidonotothen squamifrons; n* = 7). Penguin tissue values were corrected by subtracting the tissue-specific whole prey discrimination factors derived in this study. Error bars represent standard deviation.

difference between whole fish and fish muscle was within the magnitude of our δ^{15}N sample precision. In contrast, herring δ^{13}C did not differ significantly between whole fish and fish muscle (paired t-test: $t=0.55$, $P=0.595$).

3.2. Isotopic values of captive penguin tissues

The isotopic values of penguin eggs varied significantly among tissue types in both δ^{15}N and δ^{13}C (Table 1). While shell membrane and albumen were isotopically similar in δ^{15}N values, eggshell organics and yolk were significantly segregated and depleted compared to the δ^{15}N values of all other egg tissues (ANOVA: $F_{3,50}=109.52$, $P<0.001$). Egg tissues were also significantly segregated by their δ^{13}C values ($F_{4,70}=420.28$, $P<0.001$), with eggshell carbonates, shell membrane, eggshell organics, albumen, and yolk increasingly depleted in ^{13}C, respectively. Eggshell carbonates were highly enriched in ^{13}C relative to eggshell organics, by 5.8‰ ± 0.5 on average (paired t-test: $t=47.26$, $P<0.001$). Laying order affected the δ^{15}N values of eggshell organics, with second eggs enriched in ^{15}N by 0.5‰ ± 0.4, on average, relative to the first egg of a clutch (Table 1; paired t-test: $t=3.79$, $P=0.005$). In contrast, we found no effect of egg order on the δ^{15}N value of shell membrane (paired t-test: $t=0.43$, $P=0.680$) or the δ^{13}C values of eggshell organics, eggshell carbonates or shell membrane (paired t-test: $t=0.50$, 0.34, 1.11 and $P=0.631$, 0.744, 0.288 for eggshell organics, eggshell carbonates and shell membrane, respectively).

3.3. Diet-tissue discrimination factors

All penguin egg tissues examined were significantly enriched in ^{15}N relative to both whole fish and fish muscle (Table 2). Furthermore, δ^{15}N discrimination factors varied by tissue type and whether values were based off of whole fish or fish muscle. δ^{15}N discrimination factors were lowest in eggshell organics, highest in albumen and were slightly higher when calculated from whole fish (1.8–4.7‰) than from fish muscle (1.6–4.5‰).

Tissues were also variably enriched in ^{13}C relative to diet with yolk as the only tissue not enriched relative to both whole fish and fish muscle (Table 2). δ^{13}C discrimination factors were higher in inorganic (carbonate) egg tissues (7.2–7.4‰) relative to organic egg components (0.0–2.9‰). There were no trends in δ^{13}C discrimination factors with age or when calculated from whole fish compared to when calculated from fish muscle (Table 2).

3.4. Stable isotope values and diet composition of wild penguins

We found significant differences among the raw isotopic values of wild penguin tissues and their common prey species (Table 3; ANOVA: $F_{3,57}=492.88$, 30.24 and $P<0.001$, 0.001, for δ^{15}N and δ^{13}C, respectively). Post-hoc analysis determined that whole krill (*E. superba*), eggshell organics, shell membrane and fish muscle (*L. squamifrons*) all differed significantly in δ^{15}N values from lowest to highest, respectively (Fig. 1A). The δ^{13}C values of eggshell organics and fish muscle were isotopically similar, while these two groups were significantly enriched in δ^{13}C relative to whole krill and depleted in δ^{13}C relative to shell membrane.

Pair-wise comparisons of wild gentoo penguin egg tissues also found that shell membrane was significantly enriched in both δ^{15}N ($+2.4\pm0.8$) and δ^{13}C ($+1.1\pm1.1$) values compared to eggshell organics (Fig. 1A; paired t-test: $t=13.4$, 4.33 and $P<0.001$, 0.001 for δ^{15}N and δ^{13}C respectively). The isotopic differences found between wild penguin shell membrane and eggshell organics were similar to those found in our captive penguins tissues for both δ^{15}N ($+2.5\pm0.5$) and δ^{13}C ($+1.4\pm0.4$) values (t-test: $t=0.73$, 1.30 and $P=0.468$, 0.203 for δ^{15}N and δ^{13}C respectively). Furthermore, after correction with the tissue-specific discrimination factors derived in this study wild eggshell organics and shell membrane no longer differed in isotopic values (Fig. 1B; paired t-test: $t=1.20$, 1.35 and $P=0.243$, 0.193 for δ^{15}N and δ^{13}C respectively).

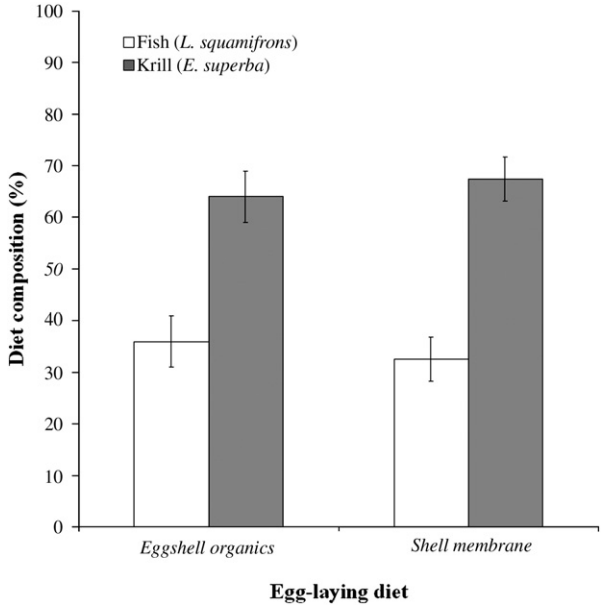

Fig. 2. Predicted diet compositions of gentoo penguins at Cape Sherriff, Livingston Island, Antarctica, 2006–07, during the egg-laying period based on stable isotope analysis of eggshell organics and shell membrane. Estimates use the single-isotope (δ^{15}N), two-source (krill and fish) mixing model described by Phillips and Gregg (2001). Error bars represent 95% confidence intervals.

Using corrected δ^{15}N values, our isotopic model calculated similar diet compositions, within 3.5%, during the egg-laying period whether based on eggshell organics or shell membrane (Fig. 2). Our results suggest that female gentoo penguins at Cape Sherriff fed more heavily on krill than fish (64.0–67.4% vs. 32.6–36.0%) during the egg-laying period of 2006.

4. Discussion

Our study highlights the importance of using tissue-specific isotopic discrimination factors when reconstructing avian diets using isotopic mixing models. Similar to other studies, we found that discrimination factors can differ substantially among specific tissues, likely reflecting differences in the biochemical and metabolic processes of tissue synthesis (Hobson and Clark, 1992; Hobson, 1995). Therefore evaluating the raw isotopic values of tissues, without incorporating tissue-specific discrimination factors, can lead to incorrect or conflicting assumptions about animal diets. Furthermore, these findings reaffirm the idea that in many cases generalized discrimination factors might not adequately represent isotopic discrimination in specific avian tissues and their use could lead to error in dietary reconstruction using isotopic mixing models.

4.1. Discrimination factors in avian egg components

Our study provides the first available data on isotopic discrimination factors in the egg components of penguins and, to our knowledge, the only data available from a captive seabird. In general, the patterns of dietary isotopic fractionation we found in this study agree with data on egg components of other avian species (Von Schirnding et al., 1982; Schaffner and Swart, 1991; Hobson, 1995; Johnson, 1995; Johnson et al., 1998). The δ^{13}C discrimination factors for gentoo penguin eggshell organics, shell membrane, albumen and yolk were comparable to values found in other groups of birds (Table 4). Gentoo penguin eggshell carbonates, which are derived from metabolic carbon

Table 4
Estimates of $\delta^{15}N$ and $\delta^{13}C$ discrimination factors between food and avian egg components.

Egg tissue, species	Food items	Discrimination factor (‰)		Reference
		$\delta^{15}N$	$\delta^{13}C$	
Eggshell organics				
Gentoo penguin *Pygoscelis papua*	Herring	1.8	1.4	This study
Ostrich *Struthio camelus*	C3 plants		2.1	Von Schirnding et al. (1982)
	Commercial diet	3.0	1.5	Johnson et al. (1998)
Quail *Coturnix japonica*	Commercial diet	1.0	2.0	Johnson (1995)
Eggshell carbonate				
Gentoo penguin *Pygoscelis papua*	Herring		7.2	This study
White-tailed tropicbird *Phaethon lepturus*	Fish and squid[a]		13.5	Schaffner and Swart (1991)
Elegant tern *Sterna elegans*	Fish[a]		12.2	Schaffner and Swart (1991)
Ostrich *Struthio camelus*	C3 plants		16.2	Von Schirnding et al. (1982)
	Commercial diet		16.2	Johnson et al. (1998)
Peregrine falcon *Falco peregrinus*	Quail		11.1	Hobson (1995)
Prairie falcon *Falco mexicanus*	Quail		11.6	Hobson (1995)
Gyrfalcon *Falco rusticolis*	Quail		11.2	Hobson (1995)
Mallard *Anas platyrhynchos*	Commercial diet		14.3	Hobson (1995)
Quail *Coturnix japonica*	Commercial diet		15.3	Johnson (1995)
	Commercial diet		15.6	Hobson (1995)
Shell membrane				
Gentoo penguin *Pygoscelis papua*	Herring	4.4	2.9	This study
Peregrine falcon *Falco peregrinus*	Quail	3.5	2.6	Hobson (1995)
Prairie falcon *Falco mexicanus*	Quail	3.2	3.0	Hobson (1995)
Mallard *Anas platyrhynchos*	Commercial diet	4.4	3.7	Hobson (1995)
Quail *Coturnix japonica*	Commercial diet	4.1	3.5	Hobson (1995)
Albumen				
Gentoo penguin *Pygoscelis papua*	Herring	4.7	0.8	This study
Peregrine falcon *Falco peregrinus*	Quail	3.1	0.9	Hobson (1995)
Prairie falcon *Falco mexicanus*	Quail	3.1	0.9	Hobson (1995)
Gyrfalcon *Falco rusticolis*	Quail	3.3	0.8	Hobson (1995)
Mallard *Anas platyrhynchos*	Commercial diet	3.0	1.4	Hobson (1995)
Quail *Coturnix japonica*	Commercial diet	2.4	1.6	Hobson (1995)
Yolk (lipid-free)				
Gentoo penguin *Pygoscelis papua*	Herring	3.5	0.0	This study
Peregrine falcon *Falco peregrinus*	Quail	3.5	0.0	Hobson (1995)
Prairie falcon *Falco mexicanus*	Quail	3.5	0.1	Hobson (1995)
Gyrfalcon *Falco rusticolis*	Quail	3.6	0.1	Hobson (1995)
Mallard *Anas platyrhynchos*	Commercial diet	3.1	−0.1	Hobson (1995)
Quail *Coturnix japonica*	Commercial diet	3.4	0.1	Hobson (1995)

[a] Food items collected in the wild from adult regurgitates or from fish found near nesting sites.

(Schaffner and Swart, 1991), are highly enriched in ^{13}C relative to their diets, similar to other species. However, the magnitude of this enrichment is lowest in gentoo penguins relative to other species with available data. The $\delta^{13}C$ discrimination factor for gentoo penguin eggshell carbonates is 3.9–6.3‰ lower than carnivorous and other piscivorous species and 7.1–9.0‰ lower than herbivorous species (Table 4). However, data for two other seabird species, the white-tailed tropicbird (*Phaethon lepturus*) and the elegant tern (*Sterna elegans*), come from a study in the wild where diets could not be controlled or completely qualified (Schaffner and Swart, 1991). Thus our data may provide a more robust estimate of eggshell carbonate $\delta^{13}C$ discrimination factors in a piscivorous bird.

Our results agree with previous findings that the magnitude of ^{13}C enrichment in eggshell carbonate is higher in herbivores than in carnivores (Table 4). Comparable differences in the $\delta^{13}C$ discrimination factors of herbivores and carnivores have been observed in the inorganic fraction of bone (Krueger and Sullivan, 1984), which suggests a similar mechanism might be responsible for patterns of enrichment in these two tissues. Hobson (1995) proposed that the greater proportion of lipids, which are depleted in ^{13}C, consumed by carnivores could lower the $\delta^{13}C$ values of their eggshell relative to the eggshell of herbivores. This trend also could be due to the export of isotopically light CO_2 via digestive gasification of carbohydrates, leading to more positive $\delta^{13}C$ values in herbivorous birds' eggshell carbonates (Schaffner and Swart, 1991).

In contrast, the $\delta^{15}N$ discrimination factors of egg components do not appear to differ between herbivores and carnivores. Gentoo penguins eggshell organics, shell membrane and yolk $\delta^{15}N$ discrimination factors were similar to values that have been determined in other species, while albumen was slightly more enriched (Table 4). The principle source of nitrogen in egg tissues are the amino and R-groups of amino acids. The nitrogen isotope ratios of individual amino acids exhibit consistent offsets relative to diet, with most amino acids enriched by 3.0‰ on average (Hare et al., 1991). While many of the tissue-specific $\delta^{15}N$ discrimination factors derived in this study significantly differed, the majority exhibited an offset from diet to tissue within the 3.0–5.0‰ enrichment predicted per trophic level (DeNiro and Epstein, 1981; Minagawa and Wada, 1984).

4.2. Mixing models, discrimination factors, and dietary reconstruction

Isotopic mixing models are commonly used to determine the relative contribution of multiple food sources to an animal's diet (Hobson, 1999; Post, 2002). These models are based on geometric procedures that reconstruct animal diets based on the mean $\delta^{15}N$ and/or $\delta^{13}C$ values of each food source after correcting for the discrimination factor of the consumer tissue (Kline et al., 1993; Phillips and Gregg, 2001, 2003; Phillips and Koch, 2002). Accurate discrimination factors are important as models can be very sensitive to variations in these values (Phillips and Koch, 2002; Post, 2002; Caut et al., 2008a). Even so, many studies have

used generalized discrimination factors without taking into account species, age, tissue, or diet (Inger et al., 2006; Major et al., 2006; Reich and Worthy, 2006, Tierney et al., 2008). The use of generalized discrimination factors across the tissues examined in our study would be inappropriate as discrimination factors varied with tissue type and one tissue, eggshell organics, deviated from the generalized discrimination factors of 3.0–5.0‰ for δ^{15}N (DeNiro and Epstein, 1981; Minagawa and Wada, 1984). For example, using a generalized δ^{15}N discrimination factor of 3.0‰ when reconstructing penguin diets based on shell membrane and eggshell organics would lead to conflicting over (+15.1%) and under estimates (−14.5%) of the actual abundance of krill in egg-laying diets, respectively. These results suggest that variation in discrimination factors among avian tissues can lead to erroneous estimates of diet composition and that caution is needed when using generalized discrimination factors.

In many cases the identification of the exact discrimination factors (for a target species, tissue, and natural diet) is not possible in a controlled experiment. Many species are not available in captivity, some tissues must be sampled in an invasive or destructive manner, and, often, natural diets are impractical or impossible to reproduce in a laboratory setting. There is some evidence to suggest that differences in the elemental composition (C/N ratio) or isotopic ratio can affect discrimination factors (see Caut et al., 2008b). Gentoo penguins naturally feed on variable amounts of crustaceans and fishes (Volkman et al., 1980; Karnovsky, 1997), but not on herring, the fish used in our captive study. While the lipid extracted C/N ratio of wild penguin food (krill and fish) and captive penguin food (herring) examined in our study were comparable (Tables 1 and 3), these species do differ in their isotopic composition. It is possible that isotopic differences between natural and captive diets, and their effects on discrimination factors, could affect the accuracy of dietary reconstructions using isotopic mixing models. While further laboratory experiments are needed to verify the effects of dietary isotopic ratios, a proposed linear relationship between dietary isotopic values and discrimination factors could help correct any confounding effects of differing dietary isotopic ratios (Caut et al., 2008b). Alternatively, mixing models that propagate uncertainty in discrimination factors can allow researchers to include these sources of error when calculating diet compositions (Moore and Semmens, 2008).

Cherel et al. (2005) found that using the δ^{15}N values of whole prey items with discrimination factors of prey muscle (or the reverse) led to incorrect estimates of diet composition. This finding was primarily due to an observed ~0.8‰ difference in the δ^{15}N values of fish muscle and whole fish. Disparity between the isotopic values of whole prey and prey muscle may be due to differences in protein turnover, metabolic routing and the macromolecule composition of whole fish and fish muscle (Cherel et al., 2005). When examining one of the same fish species (Atlantic herring) used in Cherel et al.'s study, we observe a much smaller difference between prey muscle and whole prey δ^{15}N values (0.2 vs. 0.8‰). These results suggest that any isotopic differences between prey muscle and whole prey are not constant and that variation from an assumed constant will directly impact the magnitude of isotopic discrimination factors based on prey muscle. However, as the difference between whole individuals and muscle tissue is unknown for gentoo penguin prey species, it is impossible for us to assess how this may have affected our model results. Due to these unknown effects, we suggest the preferential use of whole prey items when estimating the diets of wild penguins. In addition, isotopic discrimination factors based on prey muscle should only be used when the isotopic disparity between specific whole prey items and prey muscle tissue is well known.

In conclusion, the isotopic analysis of penguin tissues, including egg components, has great potential to provide information on the diets and foraging habitats of penguins throughout much of their annual cycles. Additionally, many tissues can be sampled non-invasively, with limited handling of live animals or through the collection of carcasses and depredated, infertile or addled eggs. However, as isotopic discrimination can be affected by species, tissue, and diet-specific variations, we recommend caution when comparing the isotopic values across tissues when specific isotopic discrimination factors are not available. Future work, especially controlled laboratory experiments, is needed to further explore sources of variation in isotopic discrimination factors and the precision with which they need to be estimated in order to yield accurate estimates of diet compositions.

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