TECHNICAL COMMENT

# Isotopic Discrimination between Food and Blood and Feathers of Captive Penguins: Implications for Dietary Studies in the Wild

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## ABSTRACT

Using measurements of naturally occurring stable isotopes to reconstruct diets or source of feeding requires quantifying isotopic discrimination factors or the relationships between isotope ratios in food and in consumer tissues. Diet-tissue discrimination factors of carbon ($^{13}C/^{12}C$, or $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, or $\delta^{15}N$) isotopes in whole blood and feathers, representing noninvasive sampling techniques, were examined using three species of captive penguins (king *Aptenodytes patagonicus*, gentoo *Pygoscelis papua*, and rockhopper *Eudyptes chrysocome* penguins) fed known diets. King and rockhopper penguins raised on a constant diet of herring and capelin, respectively, had tissues enriched in $^{15}N$ compared to fish, with discrimination factors being higher in feathers than in blood. These data, together with previous works, allowed us to calculate average discrimination factors for $^{15}N$ between whole lipid-free prey and blood and feathers of piscivorous birds; they amount to +2.7‰ and +4.2‰, respectively. Both fish species were segregated by their $\delta^{13}C$ and $\delta^{15}N$ values, and importantly, lipid-free fish muscle tissue was consistently depleted in $^{13}C$ and enriched in $^{15}N$ compared to whole lipid-free fish. This finding has important implications because previous studies usually base dietary reconstructions on muscle of prey rather than on whole prey items consumed by the predator. We tested the effect of these differences using mass balance calculations to the quantification of food sources of gentoo penguins that had a mixed diet. Modeling indicated correct estimates when using the isotopic signature of whole fish (muscle) and the discrimination factors between whole fish (muscle) and penguin blood. Conversely, the use of isotopic signatures of muscle together with discrimination factors between whole fish and blood (or the reverse) leads to spurious estimates in food proportions. Consequently, great care must be taken in the choice of isotopic discrimination factors to apply to wild species for which no controlled experiments on captive individuals have been done. Finally, our results also indicate that there is no need to remove lipids before isotopic analysis of avian blood.

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## Introduction

The stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in consumer and prey tissues have a broad array of applications in ecology. For example, they provide powerful tools for estimating the trophic position of and mass flow to consumers in food webs. Trophic delineation is based on the fact that the $\delta^{15}N$ value of a consumer is enriched in a predictive way relative to its diet, the difference being the so-called discrimination, or enrichment, factor (Minagawa 1984). Mass balance equations, applied to cases where distinct isotopic signatures exist for various dietary sources, can be used to determine the relative contributions of different diets to a mixed signature in consumers (Phillips and Gregg 2001). During recent years, a number of mathematical methods have developed to improve mixing models (Phillips and Gregg 2003) and to estimate trophic position (Vander Zinden and Rasmussen 1999). However, the weakest link in these applications relates to the estimation of appropriate discrimination factors (Phillips and Koch 2002; Post 2002). Both estimation and choice of appropriate factors require a better knowledge and understanding of their sources of variation.

Values for discrimination factors of $\delta^{13}C$ and $\delta^{15}N$ are generally in the ranges 0‰–2‰ and 2‰–5‰, respectively (Peterson and Fry 1987; Kelly 2000). Experimental controlled feeding experiments demonstrate multiple potential sources of variation. For example, a recent bibliographical synthesis highlighted changes in discrimination factors related to consumer

tissues, nature and quality of the diet, the main biochemical form of nitrogen excretion, and nutritional status (Vander Zinden and Rasmussen 2001; McCutchan et al. 2003; Vanderklift and Ponsard 2003). Avian field works have assumed that the results of a few controlled laboratory studies on captive species (Hobson and Clark 1992*b*, 1993; Mizutani et al. 1992) are representative of a wide taxonomic range of birds. However, two recent studies on great skuas *Catharacta skua* and yellow-rumped warblers *Dendroica coronata* indicate that variation of discrimination factors is probably more complex (Bearhop et al. 2002; Pearson et al. 2003), thus emphasizing the need for more methodological information on different avian species.

The purpose of this article was to determine discrimination factors and their variation in captive penguins feeding on controlled diets. Three species were investigated, the king *Aptenodytes patagonicus*, gentoo *Pygoscelis papua*, and rockhopper *Eudyptes chrysocome* penguins. They belong to three different genera that include most of the living species of penguins and cover a wide range in size, from the genus (*Aptenodytes*) with the largest species to a genus with small species (*Eudyptes*), pygoscelid penguins being of medium size (Williams 1995). Before our study, no data for diet-tissue discrimination factors were available for penguins, except one work dealing with feathers of a *Spheniscus* species, the Humboldt penguin *Spheniscus humboldti* (Mizutani et al. 1992). Taken together, the Mizutani et al. study and this one allow a reasonably good coverage of penguins, an ecologically important group of diving seabirds from the Southern Hemisphere, where they are the major seabird consumers (Woehler 1993; Guinet et al. 1996; Van Franeker et al. 1997). This work is the first part of a larger investigation on the food and feeding ecology and resource partitioning within the penguin community living in subantarctic and Antarctic waters.

We focused on two avian tissues, whole blood and feathers, because they can be sampled easily and nondestructively in the field. Moreover, they provide different time-integrated information about diet. Whole blood provides short- to medium-term information (about 1–5 wk), while feathers reflect the diet at the time they were grown (Hobson and Clark 1992*a*; Haramis et al. 2001; Bearhop et al. 2002; Hobson and Bairlein 2003; Pearson et al. 2003). Methodological investigations dealt first with the effect of lipid extraction on the blood isotopic signature. Lipids are depleted in $^{13}C$ relative to protein (DeNiro and Epstein 1977; Tieszen et al. 1983), and they have thus the potential to lower $\delta^{13}C$ values in blood if not removed, but quantitative estimates of this effect are poorly known (Bearhop et al. 2000). Second, we looked at the effect of using isotopic signatures of whole prey versus prey muscle to calculate discrimination factors. In many cases, it is more practical to measure isotope ratios of a part of an animal rather than its whole body for dietary analysis (DeNiro and Epstein 1978*b*, 1981). In general, stable isotope measurements are determined on skeletal muscle, since $\delta^{15}N$ values of muscle more closely reflect those of the whole body than do those of other tissues (Kelly 2000). Surprisingly, however, the influence of differences in isotopic signatures of prey muscle versus whole prey on discrimination factors was not, to our knowledge, previously investigated.

## Methods

### *General*

Penguins were studied in captivity at Océanopolis, Brest (France). All individuals were held on a constant diet of thawed Atlantic herring *Clupea harengus* and male Icelandic capelin *Mallotus villosus* for at least 6 mo before blood sampling. King and rockhopper penguins were fed exclusively with herring and capelin, respectively, while gentoo penguins had ad lib. access to both herring and capelin. According to the Océanopolis staff, individual gentoos had differential habits, with some birds feeding on both fish species while others preferred either herring or capelin (see below). Consistency of dietary isotopic composition was investigated by measuring isotopic signatures of five individuals of each fish species randomly taken each month during the 3 mo before sampling.

Penguins were not fed the day before sampling. Blood was collected in January 2002 from 10 king, 11 gentoo, and nine rockhopper penguins via venipuncture of one flipper vein. Feathers were collected in the subsequent spring from the backs of newly molted king and rockhopper penguins. Seventy-percent ethanol was then added to whole blood, which was kept at −20°C until analysis. Blood samples were stored in ethanol because that procedure does not significantly alter the isotopic composition of tissue (Hobson et al. 1997) and, in many cases, freezing is not possible in the field.

### *Sample Preparation and Isotopic Analysis*

Before isotopic analysis, whole fish, fish muscle, and blood were dried in an oven at +60°C. Following drying, tissues were ground to a fine powder in an analytical mill. Because whole fish were extremely oily, they were subjected to coarse lipid removal by solvent rinsing before grinding in the analytical mill. Lipids were then removed from prey items and from a blood subsample using a Soxhlet apparatus with chloroform solvent for 4–6 hr. Consequently, all subsequent mentions of prey (either whole fish or muscle sample) refer to their lipid-free component. Feathers were cleaned of surface contaminants using a 2 : 1 chloroform : methanol rinse, air dried, and then cut with stainless steel scissors into small fragments.

Stable carbon and nitrogen isotope assays were performed on 1-mg subsamples of homogenized materials by loading into tin cups and combusting at 1,800°C in a Robo-Prep elemental analyzer. Resultant $CO_2$ and $N_2$ gases were then analyzed using an interfaced Europa 20:20 continuous-flow isotope ratio mass spectrometer (CFIRMS) with every five unknowns separated

by two laboratory standards. Stable isotope abundances were expressed in δ notation as the deviation from standards in parts per thousand (‰), according to the following equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1{,}000,$$

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 (PDB) for $^{13}C$ and atmospheric $N_2$ (AIR) for $^{15}N$. Replicate measurements of internal laboratory standards (albumen) indicate measurement errors of ±0.1‰ and ±0.3‰ for stable carbon and nitrogen isotope measurements, respectively.

### *Model and Statistics*

We quantified the contribution of capelin and herring to the diet of gentoo penguins by using a linear mixing model (Phillips and Gregg 2001). This single-isotope, two-source mixing model provided 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 $\delta^{15}N$ rather than $\delta^{13}C$ values because the proportional standard errors varied inversely with the signature difference between sources (Phillips and Gregg 2001) and the difference in isotopic signatures of the two fish species was higher for $\delta^{15}N$ than for $\delta^{13}C$ (Table 1).

Data were statistically analyzed using SYSTAT 9 for Windows (Wilkinson 1999). Values are mean ± SD, unless otherwise stated.

## Results

### *Fish*

There were no significant differences in the main characteristics of fish collected over three different months before blood sampling of penguins (Kruskall-Wallis tests; capelin: $H_{2,15} = 0.07$, 0.14, 0.10, 3.14, 2.94, and 4.87, and $P = 0.968$, 0.932, 0.954, 0.208, 0.230, and 0.087 for body mass, standard length, muscle $\delta^{13}C$, whole-fish $\delta^{13}C$, muscle $\delta^{15}N$, and whole-fish $\delta^{15}N$, respectively; herring: $H_{2,15} = 0.38$, 1.41, 1.22, 2.21, 0.08, and 0.86, and $P = 0.827$, 0.493, 0.543, 0.331, 0.961, and 0.651, respectively). Results from the 15 individuals, either capelin or herring, were therefore pooled for subsequent analysis.

Specimens of the two fish species differed. Capelin were smaller (length = 148 ± 8 and 181 ± 8 mm, respectively; two-sample $t$-test, $t = 11.57$, $P < 0.0001$) and lighter than herring (27.8 ± 5.7 and 80.2 ± 15.8 g, respectively; $t = 12.07$, $P < 0.0001$). Both fish species were segregated by their stable isotope values when considering either the whole fish (MANOVA, Wilks's λ, $F_{2,27} = 107.02$, $P < 0.0001$) or muscle ($F_{2,27} = 118.30$, $P < 0.0001$). The $\delta^{13}C$ and $\delta^{15}N$ values were lower in whole specimens and in muscle of capelin than in those of

Table 1: Stable-carbon and nitrogen isotope concentrations (mean ± SD) in food, blood, and feathers of captive penguins

| | | $\delta^{13}C$ | | | $\delta^{15}N$ | | |
|---|---|---|---|---|---|---|---|
| | | | Statistics | | | Statistics | |
| Sampling Group | Samples ($n$) | Concentration (‰) | $t$ | $P$ | Concentration (‰) | $t$ | $P$ |
| Herring: | | | | | | | |
| Whole fish | 15 | −18.2 ± .3 | 2.76[a] | .015 | 12.6 ± .8 | 13.50[a] | <.0001 |
| Muscle | 15 | −18.4 ± .3 | | | 13.4 ± .8 | | |
| Capelin: | | | | | | | |
| Whole fish | 15 | −19.5 ± .4 | 5.83[a] | <.0001 | 10.5 ± .5 | 15.68[a] | <.0001 |
| Muscle | 15 | −19.9 ± .3 | | | 11.4 ± .5 | | |
| King penguins: | | | | | | | |
| Blood | 10 | −19.0 ± .4 | 2.88[b] | .018 | 14.6 ± .2 | 6.68[b] | <.0001 |
| Delipidated blood | 10 | −18.5 ± .3 | | | 14.4 ± .2 | | |
| Feathers | 10 | −18.1 ± .3 | | | 16.1 ± .2 | | |
| Rockhopper penguins: | | | | | | | |
| Blood | 9 | −19.5 ± .3 | 3.93[b] | .004 | 13.2 ± .1 | 6.39[b] | <.0001 |
| Delipidated blood | 9 | −19.0 ± .2 | | | 13.0 ± .2 | | |
| Feathers | 9 | −19.4 ± .3 | | | 14.9 ± .3 | | |
| Gentoo penguins: | | | | | | | |
| Blood | 11 | −19.2 ± .2 | 4.34[b] | .001 | 13.8 ± .5 | 2.74[b] | .021 |
| Delipidated blood | 11 | −18.8 ± .3 | | | 13.6 ± .6 | | |

[a] Paired $t$-test between whole fish and muscle.

[b] Paired $t$-test between whole blood and lipid-extracted blood.

herring ($t = 11.43$ and 8.68, both $P < 0.0001$ for $\delta^{13}C$ and $\delta^{15}N$ in whole specimens, respectively, and $t = 14.02$ and 9.20, both $P < 0.0001$ in muscle; Table 1). Elemental compositions of lipid-extracted whole capelin and herring were also different. Whole capelin contained less carbon (35.8% ± 4.2% and 42.6% ± 3.6%, respectively; $t = 4.66$, $P < 0.0001$) and nitrogen (10.6% ± 1.2% and 12.5% ± 1.1%, respectively; $t = 4.68$, $P < 0.0001$) than herring, but the C/N ratio was identical in the two species (3.38% ± 0.09% and 3.39% ± 0.07%; $t = 0.50$, $P = 0.619$).

Whole fish and muscle samples had different isotopic signatures (Table 1). Whole fish were significantly enriched in $^{13}C$ and depleted in $^{15}N$, compared to muscle tissue, and this was found for both capelin and herring. The difference between whole specimens and muscle was species dependent for $\delta^{13}C$ (0.44‰ ± 0.29‰ and 0.20‰ ± 0.28‰ for capelin and herring, respectively; $t = 2.35$, $P = 0.026$) but not for $\delta^{15}N$ (−0.87‰ ± 0.22‰ and −0.84‰ ± 0.24‰, respectively; $P = 0.33$ and $P = 0.745$).

### *Intraspecific Differences in Stable Isotope Ratios of Penguins*

Blood and feathers of king penguins had different isotopic signatures (Table 1). Blood was significantly depleted in $^{13}C$ and $^{15}N$ compared to feathers (paired *t*-tests, $t = 4.49$, $P = 0.002$ and $t = 16.85$, $P < 0.0001$, respectively). In rockhopper penguins, blood and feathers had identical $\delta^{13}C$ values ($t = 0.76$, $P = 0.471$), but, again, blood was depleted in $^{15}N$ ($t = 23.22$, $P < 0.0001$). The difference between blood and feathers in $\delta^{15}N$ value was species dependent (−1.42‰ ± 0.27‰ and −1.67‰ ± 0.22‰ for king and rockhopper penguins, respectively; $t = 2.23$, $P = 0.040$).

In all penguin species, whole blood had significantly lower $\delta^{13}C$ values and higher $\delta^{15}N$ values than delipidated blood (Table 1). Isotopic differences between blood and lipid-extracted blood were consistent among species, with no significant variations in $\delta^{13}C$ (−0.52‰ ± 0.13‰, −0.45‰ ± 0.14‰, and −0.35‰ ± 0.12‰ for king, rockhopper, and gentoo penguins, respectively; ANOVA, $F_{2,27} = 0.45$, $P = 0.644$) and in $\delta^{15}N$ values (0.21‰ ± 0.04‰, 0.22‰ ± 0.04‰, and 0.11‰ ± 0.03‰, respectively; $F_{2,27} = 3.10$, $P = 0.062$).

### *Isotope Ratios between Fish and Penguin Tissues and Discrimination Factors*

Overall, penguin blood (three species) and whole fish (two species) were segregated by their stable isotope values (MANOVA, Wilks's λ, $F_{8,108} = 67.55$, $P < 0.0001$), as were penguin blood and fish muscle ($F_{8,108} = 48.52$, $P < 0.0001$). In the same way, penguin feathers (two species) and either whole fish ($F_{6,88} = 122.86$, $P < 0.0001$) or fish muscle ($F_{6,88} = 102.37$, $P < 0.0001$) were segregated by their $\delta^{13}C$ and $\delta^{15}N$ values (Table 1).

Since king and rockhopper penguins were fed exclusively

Table 2: Statistics (two-sample *t*-tests) and discrimination factors between penguin tissues and penguin food

| Penguin Tissue | Food | Isotope Ratio | *t* | *P* | Discrimination Factor (‰) |
|---|---|---|---|---|---|
| King penguin: | | | | | |
| Blood | Whole fish | $\delta^{13}C$ | 5.70 | <.0001 | **−.81** |
| | | $\delta^{15}N$ | 8.49 | <.0001 | **2.07** |
| | Muscle | $\delta^{13}C$ | 4.01 | .001 | **−.61** |
| | | $\delta^{15}N$ | 5.20 | <.0001 | **1.23** |
| Feathers | Whole fish | $\delta^{13}C$ | .55 | .589 | .07 |
| | | $\delta^{15}N$ | 14.11 | <.0001 | **3.49** |
| | Muscle | $\delta^{13}C$ | 1.95 | .064 | .26 |
| | | $\delta^{15}N$ | 11.04 | <.0001 | **2.65** |
| Rockhopper penguin: | | | | | |
| Blood | Whole fish | $\delta^{13}C$ | .18 | .859 | .02 |
| | | $\delta^{15}N$ | 14.58 | <.0001 | **2.72** |
| | Muscle | $\delta^{13}C$ | 4.02 | .001 | **.46** |
| | | $\delta^{15}N$ | 11.51 | <.0001 | **1.86** |
| Feathers | Whole fish | $\delta^{13}C$ | .80 | .430 | .11 |
| | | $\delta^{15}N$ | 22.51 | <.0001 | **4.40** |
| | Muscle | $\delta^{13}C$ | 4.70 | <.0001 | **.55** |
| | | $\delta^{15}N$ | 20.64 | <.0001 | **3.53** |

Note. Food is herring for king penguins and capelin for rockhopper penguins. Values in boldface are significantly different from 0.

with herring and capelin, respectively, we compared the stable isotopic composition of tissues of a given penguin species with that of its food (Table 2). Surprisingly, king penguin blood, but not feathers, was significantly depleted in $^{13}C$ compared with whole herring and herring muscle, resulting in negative discrimination factors (−0.8‰ and −0.6‰, respectively). In contrast, $\delta^{13}C$ values in blood and feathers of rockhopper penguins were not different from those in whole capelin, but they were enriched in $^{13}C$ compared to those in capelin muscle (discrimination factors of 0.5‰ and 0.6‰, respectively). All penguin tissues were significantly enriched in $^{15}N$ compared to fish. However, the magnitude of the enrichment depended on penguin species, penguin tissue, and whether whole fish or fish muscle was tested (Table 2). Discrimination factors were lower in tissues of king penguins (1.2‰–3.5‰) than in those of rockhoppers (1.9‰–4.4‰). They were lower in blood (1.2‰–2.7‰) than in feathers (2.7‰–4.4‰), and they were higher when calculated with isotopic ratios from whole fish (2.1‰–4.4‰) than they were when calculated with ratios from fish muscle (1.2‰–3.5‰; Table 2).

*Gentoo Penguins*

Among gentoo penguins, one individual fed exclusively on herring, three fed exclusively on capelin, and the remaining seven birds had a mixed diet. The mixed diet was dominated by capelin in five individuals; one penguin fed almost equally on the two fish species, and the last bird fed more on herring (visual observations). No significant differences were found in the stable isotopic composition of blood between the capelin feeders and the penguins feeding on a mixed diet ($\delta^{13}C$: −19.3‰ ± 0.1‰ and −19.2‰ ± 0.2‰, respectively; Mann-Whitney, $U = 6.5$, $P = 0.360$; $\delta^{15}N = 13.7‰ \pm 0.3‰$ and 13.6‰ ± 0.3‰, respectively, $U = 11.0$, $P = 0.909$). However, the gentoo penguin feeding on a mixed diet dominated by herring was the only individual in the mixed group to have a $\delta^{15}N$ value higher than 14‰, and only the herring feeder had isotopic ratios quite different from those of the other gentoo penguins ($\delta^{13}C = -18.6‰$, $\delta^{15}N = 14.9‰$; Fig. 1).

Using isotopic signatures of blood and whole fish with discrimination factors between blood and whole fish, the isotopic model with $\delta^{15}N$ values estimated that gentoos ($n = 11$) fed overall more on capelin than on herring (62.5% vs. 37.5%; Table 3). Birds from the mixed group ($n = 7$) fed on a higher proportion of capelin (68.8% vs. 31.2%). The use of isotopic values of fish muscle with discrimination factors between blood and fish muscle gave identical estimates (62.9% vs. 37.1% and 69.2% vs. 30.8% for all the birds and the mixed group, respectively). The model indicated that only the penguin from the mixed group known to feed more on herring fed on 33.3%–33.6% capelin. Removing that single individual, it estimated that the six remaining birds ingested 75.0% capelin and 25.0% herring (Table 3).

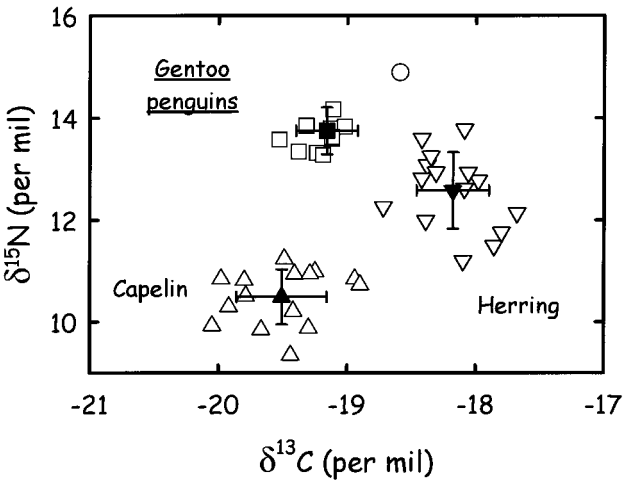


Figure 1. Stable carbon and nitrogen isotope values for whole blood of gentoo penguins and for their food, whole capelin and herring. Mean values (*filled symbols*) are mean ± SD. Open triangles represent fish individual values. Open squares represent individual penguins feeding either on capelin or on a mixed diet, and the circle represents the only bird feeding on herring.

## Discussion

Accurate stable isotope discrimination factors are critical for calculating trophic levels of animals and estimating the relative contribution of several potential food sources using mixing models, since both applications are very sensitive to these values (Minagawa 1992; Phillips and Koch 2002; Post 2002). Our results indicate that discrimination factors depend on several parameters, including predator species and, more important, whether one models prey muscle tissue versus whole prey. Consequently, great care must be taken in the choice of discrimination factors to apply to species for which no control experiments on captive individuals have been done or can be done.

*Whole Blood and Lipid-Extracted Blood*

The protocols put forth in most previous works using stable isotope measurements of seabirds and their prey included no lipid extraction from whole blood of the consumers, but lipids were extracted from the food before isotopic analysis. We investigated possible effects of lipid removal in blood on its isotope signature, since this issue was generally overlooked (Bearhop et al. 2000). Lipid-extracted blood was consistently enriched in $^{13}C$ and depleted in $^{15}N$, compared to whole blood, in the three species of penguins. Differences were small but significant. Small increases in $\delta^{13}C$ values were expected, since avian blood, including penguin blood, usually has a low fat content (Groscolas 1982; Ghebremeskel et al. 1991; Rosa et al. 1993) and lipids are depleted in $^{13}C$ compared to proteins, carbohydrates, and the total organism (DeNiro and Epstein

Table 3: Estimates of the proportion of capelin in the diet of gentoo penguins

| | | | | | Standard | 95% CI | |
|---|---|---|---|---|---|---|---|
| | *n* | $\delta^{15}$N Signature of the Food | $\delta^{15}$N Discrimination Factor | % Capelin | Error | Lower | Upper |
| All birds | 11 | Whole fish | Whole fish/blood | 62.5 | 12.4 | 36.8 | 88.2 |
| | | Muscle | Muscle/blood | 62.9 | 12.2 | 37.7 | 88.1 |
| | | Whole fish | Muscle/blood | 4.1 | 15.9 | .0 | 37.0 |
| | | Muscle | Whole fish/blood | 123.4 | 14.8 | 92.9 | 100.0 |
| Mixed diet | 7 | Whole fish | Whole fish/blood | 68.8 | 11.3 | 45.0 | 92.5 |
| | | Muscle | Muscle/blood | 69.2 | 10.9 | 46.1 | 92.4 |
| Mixed diet, more capelin | 6 | Whole fish | Whole fish/blood | 75.0 | 10.3 | 53.4 | 96.6 |
| | | Muscle | Muscle/blood | 75.5 | 9.8 | 55.0 | 96.0 |
| Mixed diet, more herring | 1 | Whole fish | Whole fish/blood | 33.3 | 9.5 | | |
| | | Muscle | Muscle/blood | 33.6 | 9.4 | | |

Note. Estimates use the single isotope ($\delta^{15}$N), two-source (capelin and herring) mixing model described by Phillips and Gregg (2001). CI = confidence interval.

1977; Tieszen et al. 1983). On the other hand, the difference in $\delta^{15}$N value was unexpected, since lipid removal generally does not affect tissue $\delta^{15}$N values or increases them only slightly (Pinnegar and Polunin 1999; Bearhop et al. 2000). The most likely explanation is that lipid extraction in penguin plasma removed not only lipids but also some isotopically heavy nitrogenous compounds, perhaps some lipoproteins, thus inducing a decrease in blood $\delta^{15}$N value.

Our results for penguin blood are not in agreement with those previously obtained on the blood of great skuas (Bearhop et al. 2000). Bearhop et al. (2000) found no change in $\delta^{13}$C value and either no change or an increase in $\delta^{15}$N value after blood delipidation. We have no explanation other than potential species-dependent differences for these discrepancies, because both studies apparently used the same lipid extraction procedure. However, since that procedure induced the removal of some nitrogenous compounds and resulted in only relatively small changes in $\delta^{13}$C and $\delta^{15}$N values, we concur with Bearhop et al. (2000) that there is no need to extract lipids before isotopic analysis in avian blood. It is worth noting that birds' plasma, including penguin plasma, has a milky appearance in well-fed birds with a high digestion rate (Griminger 1986; Le Ninan et al. 1988) and in females during egg formation (Groscolas 1982; Griminger 1986; Cherel et al. 1994). Therefore, the influence of elevated lipid concentrations on the isotopic signatures of plasma and of whole blood must be considered in these two physiological situations.

*Blood, Feathers, and Avian Discrimination Factors*

Blood and feathers are standard target tissues in ecological studies involving birds because they can be sampled easily and nondestructively in the field. Feathers, moreover, offer the opportunity to study poorly known aspects of avian biology. First, keratin is metabolically inert following synthesis, allowing investigation of feeding ecology during the molting period per se, that is, the nonbreeding period, and/or in connection with migration routes (Hobson 1999; Cherel et al. 2000). Second, comparisons of isotopic signatures of feathers from museum specimens with those from living birds permit the investigation of dietary changes of a given species over decades and even centuries (Thompson et al. 1995). Within that context, it is of primary importance to have good estimates of discrimination factors between food and both blood and feathers.

Results from king and rockhopper penguins showed higher $\delta^{15}$N discrimination factors for feathers than for blood. This is in agreement with previous observations that tissue type is a source of variation in $\delta^{15}$N enrichment (Vanderklift and Ponsard 2003) and is consistent with previous avian studies in which blood and feathers have been investigated (Bearhop et al. 2002; Pearson et al. 2003; Hobson and Bairlein 2003), with the exception of that of Hobson and Clark (1992*b*). A review of the literature on birds reinforces the idea that $\delta^{15}$N discrimination factors for feathers are higher than those in blood, regardless of diet (Table 4). However, another potential source of variation is the diet itself (McCutchan et al. 2003; Pearson et al. 2003; Vanderklift and Ponsard 2003). Ideally, discrimination factors should be applied on a case-by-case basis, and attempts should be made to calculate them for the species and diet in question. In many cases, however, experimental trials of wild animals feeding on their natural diet are not feasible. Penguins, for example, do not naturally prey upon capelin and herring; instead, king penguins are myctophid fish eaters (Cherel et al. 2002), rockhopper penguins mainly feed on swarming crustaceans and myctophids (Cooper et al. 1990), and gentoo penguins have large spatiotemporal variations in their diet (Ridoux 1994). We therefore propose to use the following mean $\delta^{15}$N discrimination factors for wild fish-eating

Table 4: Estimates of $\delta^{13}C$ and $\delta^{15}N$ discrimination factors between food and birds' blood and feathers

| Tissue, Species | Food Items | Discrimination Factors (‰) $\delta^{13}C$ | Discrimination Factors (‰) $\delta^{15}N$ | References |
|---|---|---|---|---|
| Whole blood: | | | | |
| King penguin *Aptenodytes patagonicus* | Herring | −.8 | 2.1 | This study |
| Rockhopper penguin *Eudyptes chrysocome* | Capelin | .0 | 2.7 | This study |
| Ring-billed gull *Larus delawarensis* | Perch | −.3 | 3.1 | Hobson and Clark 1992*b* |
| Great skua *Catharacta skua* | Sprat | 1.1 | 2.8 | Bearhop et al. 2002 |
| | Beef | 2.3 | 4.2 | Bearhop et al. 2002 |
| Peregrine falcon *Falco peregrinus* | Quail | .2 | 3.3 | Hobson and Clark 1992*b* |
| Canvasback *Aythia valisineria* | Commercial diet | 1.5 | 3.0 | Haramis et al. 2001 |
| Japanese quail *Coturnix japonica* | Commercial diet | 1.2 | 2.2 | Hobson and Clark 1992*b* |
| Dunlin *Calidris alpina pacifica* | Mixed diet | 1.3 | 2.9 | Evans Ogden et al. 2004 |
| Garden warbler *Sylvia borin* | Control diet | 1.7 | 2.4 | Hobson and Bairlein 2003 |
| Feathers: | | | | |
| Humboldt's penguin *Spheniscus humboldti* | Anchovy | | 4.8 | Mizutani et al. 1992 |
| King penguin *A. patagonicus* | Herring | .1 | 3.5 | This study |
| Rockhopper penguin *E. chrysocome* | Capelin | .1 | 4.4 | This study |
| Common cormorant *Phalacrocorax carbo* | Mackerel | | 3.7 | Mizutani et al. 1992 |
| | Sprat | 2.6 | 4.9 | Bearhop et al. 1999 |
| European shag *Phalacrocorax aristotelis* | Sprat | 2.0 | 3.6 | Bearhop et al. 1999 |
| Ring-billed gull *L. delawarensis* | Perch | .2 | 3.0 | Hobson and Clark 1992*b* |
| Black-tailed gull *Larus crassirostris* | Saurel | | 5.3 | Mizutani et al. 1992 |
| Great skua *C. skua* | Sprat | 2.1 | 4.6 | Bearhop et al. 2002 |
| | Beef | 2.2 | 5.0 | Bearhop et al. 2002 |
| Nankeen night heron *Nycticorax caledonicus* | Saurel | | 4.2 | Mizutani et al. 1992 |
| Great white egret *Egretta alba* | Saurel | | 3.9 | Mizutani et al. 1992 |
| Grey heron *Ardea cinerea* | Saurel | | 4.3 | Mizutani et al. 1992 |
| Scarlet ibis *Eudocimus ruber* | Mixed diet | | 4.5 | Mizutani et al. 1992 |
| White ibis *Eudocimus albus* | Mixed diet | | 4.3 | Mizutani et al. 1992 |
| Flamingo *Phoenicopterus* spp. | Mixed diet | | 5.6 | Mizutani et al. 1992 |
| Peregrine falcon *F. peregrinus* | Quail | 2.1 | 2.7 | Hobson and Clark 1992*b* |
| Chicken *Gallus gallus* | Commercial diet | −.4 | 1.1 | Hobson and Clark 1992*b* |
| Japanese quail *C. japonica* | Commercial diet | 1.4 | 1.6 | Hobson and Clark 1992*b* |
| Garden warbler *S. borin* | Control diet | 2.7 | 4.0 | Hobson and Bairlein 2003 |

Note. Data from published studies in which diet was known or controlled. Food, but not blood, was lipid-extracted for isotope measurements, which were performed on the whole food items, not on a given prey tissue. Since Mizutani et al. (1992) did not remove lipids from birds' food, discrimination factors for $^{13}C$ were not included in the table.

birds in which no direct investigations on captive individuals have been done: +2.7‰ (2.7‰ ± 0.4‰, $n = 4$) and +4.2‰ (4.2‰ ± 0.7‰, $n = 12$) between lipid-extracted whole prey and whole blood and feathers, respectively (Table 4). Moreover, these average values should be used with a sensitivity analysis in order to estimate how much error there might be.

Unlike the case for $\delta^{15}N$, there is generally a minor or no trophic enrichment in $\delta^{13}C$ value documented among consumers (Kelly 2000; McCutchan et al. 2003). Previous experiments on captive seabirds feeding on fish showed either a slight depletion or an enrichment in whole blood and either no change or a significant enrichment in feathers (Table 4). Our results are in general agreement with these results, that is, almost no changes between the $\delta^{13}C$ signature of prey and either penguin feathers or the blood of rockhopper penguins. Surprisingly, however, the blood of king penguins was significantly depleted in $^{13}C$ compared to diet. Removing lipids decreased the difference between the $\delta^{13}C$ value of herring and that of blood, which nevertheless remained significantly depleted (two-sample *t*-test, $t = 2.49$, $P = 0.020$). The most likely explanation is that since herring is a fatty fish (Lawson et al. 1998), carbon relatively depleted in $^{13}C$ derived from dietary lipids was incorporated

into blood proteins of king penguins. This is in agreement with the hypothesis of lipid ingestion as a potential source of $^{13}C$-depleted carbon in oceanic seabirds (Thompson et al. 2000). The data also suggest differences in metabolic pathways among penguins, because the blood of rockhopper penguins was not depleted in $^{13}C$ when they were fed with capelin that also had a high lipid content (Lawson et al. 1998).

*Whole Fish, Fish Muscle, and Discrimination Factors*

In previous studies investigating isotopic relationships between predators and their prey, the isotopic signature of the food was determined either on the whole item (Bearhop et al. 2002; Kurle 2002) or on the muscle tissue of the prey (Hobson et al. 1996; Lesage et al. 2002). It is well known, however, that $\delta^{13}C$ and $\delta^{15}N$ values can differ between tissues and the whole body (DeNiro and Epstein 1978*b*, 1981; Tieszen et al. 1983). Accordingly, we found differences between the isotopic signatures of lipid-free whole specimens and lipid-free muscle tissue in the two species of fish investigated, muscle tissue being depleted in $^{13}C$ and enriched in $^{15}N$, compared to whole herring and capelin. Such differences may result from differential protein turnover in the whole body and fish skeletal muscle and from differential metabolic routing of elements from diet to various tissues characterized by different macromolecules, including proteins.

Our findings have important implications because we find significant changes in discrimination factors for both $^{13}C$ and $^{15}N$ depending on whether they are calculated using values obtained from whole prey specimens or from prey muscle samples. From a biological point of view, it is better to use data from whole specimens, since penguins swallow their prey whole (but see discussion below). Alternatively, for carnivorous species feeding on flesh only, it would be better to use isotopic signature of the skeletal muscle. Thus, the feeding ecology of the predators has to be taken into account when using the stable isotope technique to reconstruct diets. In the same way, it was recently found that an identical methodological problem arises when using lipids as trophic markers, another fruitful indirect method of investigating animal food and feeding ecology. Indeed, the lipid content and lipid signature (fatty acid pattern) of cephalopods differed depending on whether tissues or the whole individual was examined (Phillips et al. 2002).

*Gentoo Penguins, Models, and Discrimination Factors*

Gentoo penguins fed on both herring and capelin, and they were segregated isotopically from both king and rockhopper penguins that fed only on herring and capelin, respectively. This emphasizes the usefulness of the stable isotope method to investigate resource partitioning among closely related species when the isotopic signatures of the various food items are different (DeNiro and Epstein 1978*a*; Bocher et al. 2000; Lesage et al. 2001; Sabat and del Rio 2002).

Mixing models must account for differences in elemental concentrations of food sources (Phillips and Koch 2002). Their effects are, however, minimized if all sources are animal matter. In this study, the C/N ratios of lipid-extracted capelin and herring were identical. Accordingly, the single-isotope, two-source mixing model correctly calculated an increasing proportion of capelin in the food of gentoo penguins in the following order: the single bird with a mixed diet dominated by herring (33%), all 11 gentoo penguins (63%), the seven birds with a mixed diet (69%), and the six birds with a mixed diet dominated by capelin (75%). Importantly, similar estimates (within 1%) were found when using either the isotopic signature of whole fish with the discrimination factors between whole fish and penguin blood or the isotopic signature of prey muscle with the discrimination factors between muscle and penguin blood. Conversely, the use of isotopic signatures of muscle with discrimination factors between whole fish and blood (or the reverse) leads to spurious estimates in food proportions (Table 3). Modeling either estimated obviously erroneous consumption of capelin (>100%) or induced a misleading estimate (4%) that has no biological value, since gentoo penguins mainly fed on capelin.

These results have two important implications. First, from a practical point of view, the isotopic signature from muscle tissue of prey can be used to estimate food proportion even for animals consuming their prey whole. This has the advantage of greatly reducing the handling time of the samples. Second, when estimating food source and trophic level, a crucial issue is to use the isotopic signatures of whole body or a given tissue together with the associated discrimination factors. This, together with the nature of the consumer tissue (see above), has to be carefully taken into account in the choice of the appropriate factors for wild species. Indeed, experimental feeding trials either used prey muscle tissue or whole items, and factors were calculated using isotopic ratio from various consumer tissues including blood, feathers, and skeletal muscle (Hobson and Clark 1992*b*; Hobson et al. 1996; Bearhop et al. 1999, 2002; Kurle 2002; Lesage et al. 2002; this study). In conclusion, our study broadens the sources of variation of discrimination factors, and it expands the range of avian species for which these factors are known, providing the first data from subantarctic seabirds.

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