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Influence of Lipid and Uric Acid on $\delta^{13}C$ and $\delta^{15}N$ Values of Avian Blood: Implications for Trophic Studies

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The use of nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) stable isotopes to infer trophic relationships in food webs has become a common and widely accepted technique (see Michener and Schell 1994). Stable-isotope ratios in the tissues of consumers reflect those in their diets in a predictable fashion, being isotopically enriched in ^{15}N (up to 4‰) and ^{13}C (usually ca. 1‰; DeNiro and Epstein 1981, Peterson and Fry 1987). Thus, unlike conventional dietary studies, stable-isotope analyses reflect assimilated (vs. merely ingested) foodstuffs. In addition, depending on the tissue chosen, dietary information spanning different temporal scales can be obtained (Hobson and Clark 1992a). Typical proteins used in such analyses are bone collagen and muscle (e.g. Hobson 1987, 1990; Hobson et al. 1994), which usually require the sacrifice of live animals or the opportunistic sampling of carcasses.

Isotopic analyses can also be performed on tissues that can be sampled nondestructively, such as blood, feathers, and hair (Hobson and Clark 1993, Thompson and Furness 1995, Bearhop et al. 1999). Nondestructive sampling is desirable when dealing with rare species, when individuals are part of ongoing studies, and when investigating variation within individuals over time. However, there are consistent differences in isotopic signatures among tissue types (e.g. Tieszen et al. 1983). Some of this variation can be linked to differences in tissue metabolic rates, but some reflects differences among tissues in biochemical composition (e.g. Hobson and Clark 1992a). Lipid presents a particular problem. The lipid component of a given tissue type can be quite variable among individuals and generally is depleted in ^{13}C compared with whole tissues (e.g. Tieszen et al. 1983). For these reasons, it is common practice to remove lipids from tissue samples where they may be present (Hobson 1987, 1990; Hobson and Clark 1992b; Thompson and Furness 1995).

One of the most obvious ways to take nondestructive samples from animals is to obtain blood. The lipid component of blood generally is very low and is carried mostly in the plasma (Deuel 1955). Researchers have either analyzed whole blood (Hobson and Clark 1993, Hobson et al. 1997) or removed the serum fraction and analyzed the cells (Ben-David et al. 1997). However, the concentration of uric acid and urea (the end products of protein catabolism) reach substantial levels in blood plasma, particularly in times of high protein turnover such as during growth (Skadhauge 1983, Wolf et al. 1985, Alonso et al. 1991). Indeed, the concentration of uric acid in blood plasma is close to its solubility limit, and in avian urine, it grossly exceeds this limit and is present in colloid suspension (Skadhauge 1983). The mechanism by which ^{15}N enrichment occurs is largely due to the excretion of isotopically light nitrogen (^{14}N) in nitrogenous waste (Peterson and Fry 1987). Thus, if blood levels of urea or uric acid are elevated, then whole blood measurements will appear to be depleted in ^{15}N. To date, no workers have assessed the influence that the lipid component and the uric acid / urea content may have on the isotopic signature of whole blood.

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In this paper, we compare the isotope signatures measured in whole blood of Great Skua (*Catharacta skua*) adults and chicks (more than 28 days old) with those of the same samples after they have been subjected to an aggressive lipid and uric acid/urea extraction technique. As top predators in many marine ecosystems, seabirds frequently have been used in isotopic studies (Hobson 1987, 1990; Hobson et al. 1994; Thompson and Furness 1995). Seabirds tend to share a number of life-history characteristics, so the Great Skua should be a good model for seabirds in general. Moreover, because elevated levels of uric acid have been reported in the blood of chicks from a wide range of taxa (including pelecaniforms [Wolf et al. 1985], ciconiiforms [Alfonso et al. 1991], and galliforms [Featherston 1969]), it might be expected that this phenomenon would apply to a great many species.

Methods.—Great Skuas were sampled on Foula, Shetland (60°08′N, 2°05′W), during summer 1996 and on St. Kilda, Western Isles (57°49′N, 8°35′W), during summer 1997. Blood was collected from the tarsal vein using a 23-gauge needle and transferred to a glass vial, where it was stored frozen until analysis. Prior to analysis, samples of whole blood were freeze dried and powdered. Part of each dried sample was subjected to a standard technique for the extraction of lipids using a soxhlet apparatus with refluxing chloroform for 4 to 6 h. Unfortunately, the samples were not weighed.

All $\delta^{13}C$ and $\delta^{15}N$ analyses were carried out by continuous-flow isotope ratio mass spectrometry (CF-IRMS) using a Europa Anca 20–20 C/N/S apparatus. Samples were loaded into miniature tin cups (4 × 6 mm) for combustion. A typical run consisted of a reference sample, duplicate analyses of two standards (internal laboratory carbon standard and IAEA-N-2), and then 8 to 10 samples. This sequence was repeated throughout the run, allowing a correction to be made for drift, if necessary. Isotope ratios are expressed in parts per thousand (‰) according to the following equation:

$$\delta X = \left[\left(\frac{r_{\text{sample}}}{r_{\text{standard}}}\right) - 1\right] \times 1{,}000, \qquad (1)$$

where X is ^{15}N or ^{13}C and r is the corresponding ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. The r_{standard} for ^{13}C is Pee Dee belemnite and for ^{15}N is atmospheric nitrogen. Measurements of $\delta^{13}C$ and $\delta^{15}N$ are precise and accurate to ±0.2‰ and ±0.4‰, respectively. To adjust for test-wise error, we applied a sequential Bonferroni adjustment for α ($\alpha = 0.006$ for 8 statistical tests).

Results.—The $\delta^{13}C$ signatures of blood from adult Great Skuas did not change significantly after washing in hot chloroform (before, $\bar{x} = -17.1 \pm$ SD of 0.6‰, $n = 23$; after, $\bar{x} = -17.2 \pm 0.8$‰, $n = 23$; paired $t = 1.61$, $P = 0.122$). The same was true of $\delta^{15}N$ values in adult blood (before, $\bar{x} = 12.6 \pm 0.9$‰, $n = 21$; after, $\bar{x} = 12.6 \pm 0.9$‰, $n = 21$; paired $t = 0.76$, $P = 0.94$). The $\delta^{13}C$ values of chick blood also remained unaffected by the treatment (before, $\bar{x} = -17.6 \pm 0.6$‰, $n = 30$; after, $\bar{x} = -17.7 \pm 0.9$‰, $n = 30$; paired $t = 0.43$, $P = 0.43$). However, the mean $\delta^{15}N$ value of chick blood increased significantly after treatment (before, $\bar{x} = 11.6 \pm 0.5$‰, $n = 30$; after, $\bar{x} = 12.8 \pm 0.7$‰, $n = 30$; paired $t = 11.5$, $P < 0.001$). Before extraction with hot chloroform, the mean $\delta^{13}C$ value of adult whole blood was lower than that of chicks ($t = 2.9$, df = 51, $P = 0.005$), as was the mean $\delta^{15}N$ value ($t = 4.3$, df = 29, $P < 0.001$). However, after treatment with hot chloroform, we detected no significant differences between blood from adults and chicks ($\delta^{13}C$, $t = 1.9$, df = 51, $P = 0.052$; $\delta^{15}N$, $t = 1.1$, df = 49, $P = 0.27$).

Discussion.—Given that other researchers have measured the ^{13}C content of lipid as being depleted relative to whole tissues (e.g. Tieszen et al. 1983), carbon-isotope ratios suggest that the lipid component of adult blood is so small that it does not significantly affect the whole-tissue isotope signatures. These findings are supported by studies that determined that the lipid component of avian blood is less than 1% of the total wet mass (e.g. Wolf et al. 1985, Rosa et al. 1993, Sartori et al. 1995); hence, it is unlikely to be of sufficient magnitude to influence the overall $\delta^{13}C$ value. The pattern for skua chicks appears to be similar, which is somewhat surprising because young birds tend to have larger stores of lipid than adults (see Blem 1990), and one might have expected to detect a similar pattern in blood. However, even if lipid levels in chick blood were much higher than those in adult blood, they would still comprise only a small proportion of the whole tissue and as such would probably not be substantial enough to affect the $\delta^{13}C$ ratio significantly.

The results for ^{15}N were different than those for ^{13}C. After washing in hot chloroform, $\delta^{15}N$ values in chick blood were significantly higher than those of whole tissue, whereas no such difference was detected in adult blood. Moreover, after the aggressive extraction procedure, the mean $\delta^{15}N$ signature of chick blood did not differ significantly from that of adult blood. These observations suggest that washing whole blood with hot chloroform results in the removal of isotopically light nitrogenous compounds, which are present in high concentrations in chick blood but in low amounts in adult blood.

Excreted nitrogenous compounds, such as urea and uric acid, are isotopically depleted in ^{15}N (Petersen and Fry 1987). Assessment of plasma concentrations of these components in adults and chicks of the White Stork (*Ciconia ciconia*) yielded higher overall concentrations in chicks (Alfonso et al. 1991). Furthermore, in Brown Pelicans (*Pelecanus occidentalis*) and domestic fowl (*Gallus gallus*), large chicks tend to have elevated levels of uric acid in blood due to their large daily food intake (Featherston 1969, Wolf

et al. 1985). Uric acid is the end product of protein catabolism and is produced mostly in the kidney and liver, and the serum concentration of uric acid is employed as an indicator of rates of protein catabolism (Stevens 1996). Rates of protein turnover are substantially higher during growth periods, with muscle protein turnover of 30% per day in chicks versus 5% per day in adults (Swick 1982).

We suggest that the aggressive extraction technique using refluxing hot chloroform removes uric acid from whole blood. The solubility of uric acid in chloroform increases substantially with temperature from ca. 0.11 mM at 25°C to more than 0.25 at 50°C (M. Teece unpubl. data). Furthermore, in a study of mono- and dimethyl uric acids in incubation media, a mixture of chloroform and 2-propanol (85:15 v/v) was used to extract the compounds of interest (Benchekroun et al. 1990). Because uric acid is assumed to be isotopically light (Petersen and Fry 1987) and is present in substantially higher concentrations in chicks than in adults (Alfonso et al. 1991), removal of this compound during the extraction procedure would account for the observed results.

Our results indicate that there is no need to extract lipids prior to isotopic analysis of avian blood. However, if protein turnover in the individuals being studied is thought to be high, then the possibility of elevated uric acid levels should be considered, particularly when comparing the diets of adults and young. It is worth noting that adult birds also can experience periods of high protein turnover, for example during rapid molt or egg formation (Blem 1990), at which time they also may have high levels of uric acid in their blood. Therefore, the influence of elevated concentrations of nitrogenous waste on the $\delta^{15}N$ of blood must be considered when assessing trophic status based solely on the measurement of bulk tissue.

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Dive Depth and Diet of the Black-vented Shearwater (*Puffinus opisthomelas*)

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In general, procellariiforms have been considered to be surface and shallow-dive foragers (Brooke 1990, Warham 1990). This view has persisted despite Kuroda's (1954) demonstration that Short-tailed Shearwaters (*Puffinus tenuirostris*) and Sooty Shearwaters (*P. griseus*) have streamlined tarsi and a narrow pelvis that should make them more proficient divers than some other procellariiforms. Weimerskirch and Sagar (1996) deployed maximum depth gauges and found that Sooty Shearwaters dive to 67 m. These depths are surprising for such a proficient flier. Weimerskirch and Sagar's data corroborate Kuroda's predictions from his morphological data and challenge previous assumptions about shearwater foraging ecology. However, it is difficult to determine whether diving is widespread in shearwaters because no studies have been published on the diving capabilities of species other than the Sooty Shearwater. Here, we examine maximum dive depths and diet of the Black-vented Shearwater (*Puffinus opisthomelas*).

The Black-vented Shearwater is endemic to islands off the Pacific coast of Mexico and is the only shearwater that breeds on islands in the California Current (Everett 1988). Anecdotal observations of this species began at the turn of the century (Anthony 1896), but only recently have detailed studies been conducted on the biology of this species (Keitt 1998). Natividad Island, Baja California, Mexico, with a breeding population of about 150,000 pairs, supports most of the world population. The Black-vented Shearwater is a summer breeder with a four-month reproductive cycle that is very similar to that of the well-studied Manx Shearwater (*Puffinus puffinus*; Harris 1965, Perrins et al. 1973, Brooke 1990). At-sea observations indicate that the Black-vented Shearwater, unlike many other procellariiforms, is a near-shore forager that does not regularly make extended pelagic foraging trips (Ainley 1976).

Methods.—Maximum depth gauges (MDG) were deployed at Natividad Island between 13 May and 30 June 1998. The MDGs were constructed similar to those described in Croll et al. (1992). Briefly, lengths of Tygon tubing (15 to 18 cm) were lined with a soluble indicator (confectioner's sugar), and one end was securely tied to allow water entry only at the

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