A forensic approach to understanding diet and habitat use from stable isotope analysis of (avian) claw material

S. BEARHOP*†, R. W. FURNESS*, G. M. HILTON‡, S. C. VOTIER* and S. WALDRON§

*Ornithology Group, Graham Kerr Building, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK, ‡Royal Society for the Protection of Birds, The Lodge, Sandy SG 19 2DL, UK, and §Life Sciences Community Stable Isotope Facility, Scottish Universities Environmental Research Centre, East Kilbride, Glasgow G75 0QF, UK

Summary

1. The potential of using stable isotope signatures of avian claws in order to infer diet and habitat use was investigated.
2. Highly significant relationships observed between stable carbon and nitrogen isotope ratios (\(\delta^{13}C\), \(\delta^{15}N\)) in the claws and body feathers of resident birds were expected since it was predicted that they were synthesized in the same habitat and approximately the same time of year.
3. Likewise the non-significant relationships observed between \(\delta^{13}C\) and \(\delta^{15}N\) in the claws and tertial feathers of neotropical migrant birds were also predicted since the claws were synthesized in the wintering area and the tertials in the breeding area.
4. The growth rates measured in the claws of five species of palearctic passerines provide evidence that this tissue should integrate dietary and habitat information over a medium temporal scale (probably weeks to months).
5. It is suggested that claws may offer a unique combination of attributes to the isotope ecologist: they are non-invasively sampled; metabolically inert but grow continuously, and are therefore a more flexible tool than feathers.
6. It is also suggested that that the stable isotope signatures in the claws of mammals and reptiles may provide similar information.

Key-words: Claw growth rate, habitat marker, neotropical migrant, toenail, trophic marker

Introduction

Variations in the natural abundances of stable isotopes are of considerable importance to ecologists. In particular, they have been used to elucidate avian migration patterns and the processes underlying them (Marra, Hobson & Holmes 1998; Bearhop et al. 1999; Hobson 1999; Kelly et al. 2002; Rubenstein et al. 2002). Such studies utilize the predictable variation in the stable isotopic composition of a number of elements over both broad and narrow geographical scales. These isotopic ratios are in turn stored in the tissues of animals, in varying degrees, as they move from one area to another (Hobson 1999). Thus, it has been possible to identify the natal origins of migratory birds on their wintering grounds based on the hydrogen isotope ratios of their feathers (Hobson & Wassenaar 1997; Chamberlain et al. 1997), investigate the influence of winter habitat quality on breeding season fitness using carbon isotope ratios of muscle tissue (Marra et al. 1998), and study foraging habitat use of birds in winter (Bearhop et al. 1999).

Currently the most commonly used tissues in avian migration studies are feathers, muscle and blood. These types of tissue (and others sampled for isotopic analyses in ecological studies) differ in three aspects. The first difference is in metabolic activity. In general the stable isotope signatures of tissues integrate information over the period during which the tissue was synthesized (although see Bearhop et al. 2002). In the case of metabolically inert tissues such as feathers, this signature remains unchanged over the time following synthesis, recording a discrete period in the past (Hobson & Clark 1992; Hobson & Wassenaar 1997; Chamberlain et al. 1997; Bearhop et al. 2002). An exception to this is provided by the stable hydrogen
isotope ratios of feathers which can alter after synthesis owing to a proportion of the hydrogen bound to feather keratins exchanging with ambient water vapour (e.g. Hobson & Wassenaar 1997). By contrast metabolically active tissues such as blood or muscle continuously turn-over as cells die and are replaced, and hence the isotope signature will change over time to the present, according to switches among isotopically distinct diets or movement between isotopically distinct habitats (Hobson & Clark 1992; Marra et al. 1998; Bearhop et al. 2002). The extent of this dilution will depend on the turnover rate of the tissue in question and the amount of time that has passed since the animal left the original habitat, factors that are particularly difficult to quantify in migrating birds.

During autumn migration it is clear that a number of species make regular stopovers on their way to the wintering areas (Leu & Thompson 2002) and in some cases outward migration may take several months (Berthold 2001). Less is known about spring stopovers, but it seems that these are less frequent and so inward migration flights are much more rapid (Berthold 2001). Lack of knowledge about the precise length of time that it may take individual migrants to move between wintering and breeding areas introduces uncertainty into inferences made from the isotope signatures in metabolically active tissues. Moreover it is clear that a few species can catabolize muscle protein during migratory flights (e.g. Bauchinger & Biebach 2001), which indicates that protein turnover may be more rapid during this period. This, and the elevated metabolic rates exhibited by migrating birds, means that for some species at least the original signature of metabolically active tissues may be diluted even more rapidly than might be predicted from captive studies (Hobson & Clark 1992; Bearhop et al. 2002).

The second difference is in destructiveness: while sampling of tissues that require sacrifice of animals can provide very useful information, non-destructive sampling offers several distinct advantages. When dealing with a species of conservation importance, destructive sampling is usually not an option. Moreover, non-destructive sampling allows serial collection of material from the same individuals, an approach that has provided insights into the role of diet in pollutant accumulation in seabirds (Bearhop et al. 2000), and in migration studies could give valuable information on individual traits, such as site fidelity.

The third difference is in variation in time-integrated signature: since different tissues turnover at different rates they integrate dietary and habitat information over different temporal scales. Thus, blood plasma may yield information that covers 1 or 2 days prior to sampling, whereas the isotopic information in bone may represent integration over several years (Hobson & Clark 1992). Feathers can give information over temporal scales ranging from a few weeks (the growth of a single feather) to several months (the entire moult period).

The main drawbacks with feathers are that detailed knowledge of moult pattern is often required and that moult occurs at the time of interest to the biologist. For example although many migratory species initiate moult on the breeding grounds (Svensson 1992; Pyle 1997), it is often completed elsewhere. Approximately 46% of neotropical migrants and 50% of trans-Saharan migrants continue their flight-feather moult away from the breeding areas (Leu & Thompson 2002; Jenni & Winkler 1994). In fact 50% of trans-Saharan migrants moult completely in the non-breeding areas (Jenni & Winkler 1994). Thus, feathers have to be selected with care and for the most part will yield information covering only one part of a bird’s annual cycle. A few species, such as Willow Warblers *Phylloscopus trochilus*, have biannual moults and isotopic information on both breeding and wintering areas can be accessed by collecting feathers at different times of year (Chamberlain et al. 2000). In addition, some birds have a limited pre-breeding moult, some of which will occur on wintering areas, but as with the postbreeding moult it is often completed away from the region in which it was initiated (Jenni & Winkler 1994; Pyle 1997). In such circumstances is difficult to be certain of the geographical region in which these feathers were grown and distinguishing those grown on the wintering grounds from feathers grown elsewhere is complex.

In this paper we present results of a study into the use of claws in isotopic research. The utility of claws in isotopic studies has rarely been investigated (Hobson et al. 1996; Hobson, Atwell & Wassenaar 1999), yet this tissue potentially contains information complementary to that contained within feathers, blood and muscle. Claws can be sampled non-invasively; their isotope signatures are integrated over an unknown time scale, but we hypothesized it would be of the order of weeks to months. Claw (like whiskers and hair) also has the advantage that, as it is both inert once formed and growing continuously, the stable isotope signatures of this tissue represent a time series that will not dilute over time.

**Methods**

**GROWTH RATES**

Over 200 individuals of five species of palearctic passerines (Great Tit *Parus major* L., Blue Tit *Parus caeruleus* L., Coal Tit *Parus ater* L., Chaffinch *Fringilla coelebs* L. and Robin *Erithacus rubecula* L.) were captured in mist nets at Loch Lomond, Argyll, UK, during autumn and winter 2001. Claws were marked by scoring the keratin sheath with a scalpel, where it erupts from the claw bed. The outer right claw on the right leg and the central claw on the left leg were marked to assess within-individual variability in rates of growth. During four recapture sessions 46 individuals were re-trapped (Great Tit *n* = 5, Blue Tit *n* = 10, Coal Tit *n* = 15, Chaffinch *n* = 11, and Robin *n* = 5) and the
distance between the scalpel mark and the claw bed was measured using Vernier callipers (±0·1 mm). From this a linear daily growth rates was calculated. We acknowledge that growth of claws may not be constant and that they probably do not grow in a simple linear fashion akin to humans (see Discussion). However we believe that our measurement does provide a close approximation of the rate at which claws grow.

STABLE ISOTOPE SIGNATURES

Both resident and wintering neotropical migrant species were captured in mist nets over a 2-week period on North Andros, Bahamas. Resident birds were represented by 13 Thick-Billed Vireos *Vireo crassirostris* (Bryant, H) and 12 Bananaquits *Coereba flaveola* (L.). Migrant birds were represented by 25 Black-Throated Blue Warblers *Dendroica caerulescens* (Gmelin) and 26 Cape May Warblers *Dendroica tigrina* (Gmelin). Between 1 and 2 mm of claw was clipped from two toes of each bird using sharp dissecting scissors. A random sample of 8–12 body feathers was collected from each of the resident birds. In most passerines body feathers are replaced throughout the moult cycle (likely to be during autumn and winter in Thick-Billed Vireos and Bananaquits) with some replacement throughout the year (Svensson 1992; Pyle 1997). Thus body feathers should integrate dietary and habitat information over several months. In the case of migrants 4–6 mm was clipped from the third tertial of one wing. In both Black-Throated Blue Warblers and Cape May Warblers the tertials are replaced in the postbreeding moult, which occurs on or near the breeding areas (Pyle 1997).

If claws do provide a record of dietary and habitat selection over a period of several months prior to sampling, we predict that the following patterns should emerge.

First, for resident birds, claw isotope signatures should be positively correlated with those of body feathers, since they were both synthesized in the same habitat. Although there may be some temporal mismatching between the signatures integrated by the two tissues, we do not expect seasonal effects to introduce disparities. This is because in contrast to aquatic systems (with seasonally variable allochthonous and autochthonous sources) and high-latitude terrestrial systems, there is a year-round growing season in the Bahamas with little variation in N or C input, thus seasonal fluctuations in δ13C and δ15N are likely to be small.

Second, since there is heterogeneity in δ13C at both large and local spatial scales and heterogeneity in δ15N at a mostly local scale, we predict that it is unlikely that migrant birds will spend the winter in areas that are isotopically similar to their breeding grounds. Therefore in the case of migrant birds, whose tertial feathers were grown on the breeding grounds around 7 months prior to sampling, there should be no correlation between the isotope signatures of tertial feathers and claws.

STABLE ISOTOPE ANALYSES

Between 0·6 and 0·8 mg of whole claw and single feather samples were weighed into tin cups for measurement of stable carbon and nitrogen isotope ratios. Stable isotope analysis was carried out at the Life Sciences Community Stable Isotope Facility, East Kilbride. The samples were combusted in a Carlo Erba C/N/S analyser interfaced with a Finnigan Trace Matt continuous flow isotope ratio mass spectrometer (Thermoquest, Hemel Hempstead). All stable isotope ratios are reported in per mil (‰) using the δ notation according to the following equation:

\[
\delta X = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \times 1000
\]

where \(X\) is \(^{13}\)C or \(^{15}\)N, and \(R\) is the corresponding ratio \(^{13}\)C/\(^{12}\)C or \(^{15}\)N/\(^{14}\)N. \(R_{\text{standard}}\) for \(^{13}\)C and \(^{15}\)N is the Pee Dee Belemnite and atmospheric nitrogen (AIR). Repeat analysis of an internal standard showed that \(\delta^{13}\)C and \(\delta^{15}\)N can be measured with accuracy and precision of ±0·2‰.

Results

GROWTH RATES

Mean growth rate (±1 SD) of the central claw (all species combined) was 0·04 ± 0·01 mm day⁻¹ (\(n = 43\) individuals). The mean growth rate (±1 SD) of the right outer claw (all species combined) was also 0·04 ± 0·01 mm day⁻¹ (\(n = 43\) individuals; range = 0·02–0·06 mm day⁻¹). Daily growth rates of the claws of palearctic passerines were converted to μm (to avoid negative numbers in the log-transformation) and then log₁₀-transformed to normalize their distributions in preparation for parametric statistical analyses. There were no significant differences between growth rates of different claws within the same individual (paired \(t\)-test, \(t_39 = 1·478, P = 0·147\)). Moreover growth rates of different claws were significantly correlated with one another (\(R = 0·6, P < 0·01\)) indicating that within-individual variation in approximate growth rates was small, relative to among-individual variation. One way ANOVAs demonstrated that there were no significant differences in claw growth rates among species (Fig. 1; central claw: \(F_{4,38} = 1·42, P = 0·245\). right outer claw: \(F_{4,38} = 1·637, P = 0·185\)). There were no significant relationships between individual body mass and approximate growth rates of either claw (linear regressions using claw growth rate as the dependent variable and body mass as the independent variable, outer: \(F_{1,42} = 0·84, P = 0·37\); central: \(F_{1,42} = 2·08, P = 0·17\)). Lengths of claws in these species ranged from 3·8 mm to 5·9 mm.

STABLE ISOTOPE SIGNATURES

Data for the two species of migrants were analysed...
Stable isotopes in avian claws


separately, since a General Linear Model (General Factorial Design) indicated differences between species. There were no significant linear relationships between claw and feather isotope signatures for either species (Black-Throated Blue Warblers: $\delta^{13}C - F_{1,24} = 0.23$, $P = 0.881$, $\delta^{15}N - F_{1,23} = 0.17$, $P = 0.685$. Cape May Warblers: $\delta^{13}C - F_{1,24} = 0.003$, $P = 0.959$, $\delta^{15}N - F_{1,23} = 0.651$, $P = 0.428$). In the case of resident birds the factor accounting for species had no significant effects on stable isotope signatures and thus isotope data for the two species were combined. Figures 2 and 3 show the significant relationships between claw and feather isotope signatures for resident birds ($\delta^{13}C - F_{1,23} = 66.53$, $P < 0.001$, $\delta^{15}N - F_{1,23} = 58.82$, $P < 0.001$). $R^2$ values were high for both $\delta^{13}C$ ($R^2 = 0.75$) and $\delta^{15}N$ ($R^2 = 0.73$).

Discussion

The significant positive relationships between stable isotope signatures in the claws and feathers of resident birds in the Bahamas (Figs 2 and 3) support our hypothesis that claws reliably integrate dietary/habitat isotope signatures. Based on the results from feathers (Hobson & Clark 1992; Hobson & Wassenaar 1997; Chamberlain et al. 1997; Bearhop et al. 2002) which are also keratin structures, we predict that similar patterns may exist for other elements such as hydrogen. However, the types of keratin constituting feathers and claws differ, as does the surface area to volume ratio, so the amount of exchangeable hydrogen may vary. These results, combined with the lack of any significant relationship between isotope signatures in claw and tertial feathers (representing the breeding areas; Pyle 1997) in migrant birds, indicates that the claws of returning migrants may yield information reflecting the premigration habitat (either wintering or breeding areas).

Consideration of growth rates in palearctic passerines supports the previous interpretation, since (based on claw lengths and linear growth rates) these suggest that keratin erupting from the nail base would take between 95 and 148 days to reach the claw tip. From this it might be inferred that 1–2 mm clipped from the tip of a claw would reflect diet/habitat, not in the month directly preceding sampling, but 2–5 months prior to this. However, we would suggest caution here since avian claw growth is not a simple linear process. There is little or no literature available on this subject, but the presence of a thin finger of pulp running down the centre of the nail indicates that growth occurs within the nail as well as from the nail bed at the end of the toe. This means that the tip of the nail is probably not composed solely of keratin laid down several months prior to sampling, as would be the case if the nail was formed entirely at the nail bed and then grew out along the nail, as in humans. In addition the mark we made for estimating growth rates became more difficult to see as time progressed, indicating that some keratin was being sloughed off. Thus, it is very difficult to estimate exactly what temporal period is represented by the claw tip: most probably it is a combination of old and new keratin and thus provides a short- to medium-term integration. This also means that, for the moment at least, the idea of using claw isotope signatures in the form of a time series should be avoided.
Furthermore, although we found no significant among-species variation in the growth rates of claws of five species palearctic passerines (Fig. 1), our small sample sizes \( n = 5 \) for two of the species mean that the tests were likely to be lacking statistical power. In addition body size had no apparent effect on growth rates, possibly because the ranges of body masses in the birds sampled was relatively small (9–27 g). Based on differences in both size and ecology it would be expected that some bird groups should have faster claw growth rates than others and in some species rates of wear will vary between seasons and thus it would be expected that growth rates would alter correspondingly.

A further point of caution is that since no experimental studies have been conducted, the assumption that most of the protein used in the synthesis of claws comes from immediate diet and not long-term stores has yet to be investigated. However, the 95% confidence intervals for the intercepts and slopes of the relationships between stable isotope signatures in feathers and claws (Figs 1 and 2) overlap those for the line \( y = x \) (slope = 1, intercept = 0). This suggests that isotopic processes are similar during the formation of these two tissues, probably because they are composed of very similar proteins. Therefore, claws and feathers may be very similar in terms of the sourcing of protein for their synthesis.

Nevertheless, variable growth rates are something that warrants further investigation through lab experiments similar to the tracer studies conducted by Hirons, Schell & St. Aubin (2001) to estimate the growth of seal vibrissae. This should be taken into consideration when using the stable isotope signatures of claws to infer diet, habitat use or geographical origins.

Despite uncertainties about the precise temporal period represented by the claw tip, and potential interspecific variability in growth rates, from the results of this study it is clear that claws represent a valuable addition to the stable isotope ecologist’s armoury. With respect to avian migration we believe that claws would continue to provide good information on the premigration habitat for some time after arrival (3–4 weeks at the very least). The slow rates of growth exhibited by claws mean that on inward migration flights (with fewer stopovers than on outward migration), dilution of the original habitat isotope signature should be minimal. This sampling method may be less suitable for species with extremely long outward migration, such as European Marsh Warblers *Acrocephalus palustris* (Berthold 2001). In addition the isotopic record within claws should be preserved indefinitely (as is the case with feathers; Bearhop et al. 1999) and thus will not be influenced by metabolic and energetic demands placed on birds during their migration.

The results also indicate that claws may be a suitable addition in for isotopic investigation of mammalian (as suggested by Hobson et al. 1996) and reptilian ecology. Many mammal and reptile species undergo periodic molts and thus hair or scales may not provide dietary or habitat information that covers the whole of the annual cycle. The isotopic signatures of claw tissue would probably provide longer-term integration than blood, which at the moment can only be accessed via serial sampling of blood or through the use of bone collagen. Drawbacks to the use of bone collagen include a lengthy extraction procedure and animal sacrifice. We also predict that claws could be very useful in paleoecological studies of mammals and reptiles through the use of museum specimens, much in the same way as has already been shown with feathers (Thompson, Furness & Lewis 1995).

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