Using stable isotopes to investigate the provenance of an Eagle Owl found in Norfolk

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Abstract The stable isotopes of hydrogen, oxygen, carbon and nitrogen were analysed in two generations of feather growth in a second- or third-calendar-year female Eagle Owl *Bubo bubo* found in Norfolk in November 2006. We found that the juvenile primaries and secondaries had a consistently low δ^2H signature, while second-generation remiges, and body feathers, revealed higher values. The pattern in δ^2H between the two generations of feathers from the Norfolk bird corresponds with the known moult patterns of Eagle Owls and suggests that the two generations of feathers were grown in different geographical regions. Although there are a number of alternative explanations for the findings, it seems most likely that the owl was reared somewhere with low local δ^2H precipitation values. An origin in Scandinavia, north-continental Europe or mid-continental Russia is consistent with our findings, but we cannot rule out the possibility that the bird was reared in northern Britain, either in the wild or in captivity.

The recent establishment of breeding pairs of Eagle Owls *Bubo bubo* in Yorkshire and Lancashire has generated a great deal of publicity in recent years and has sparked a debate about whether the species should be considered a native part of the British avifauna (see Melling *et al.* 2008 for a review). The presence of jesses (leather straps used in falconry) indicated that one member of a pair nesting in Lancashire in 2006 was indeed an escaped captive bird and many conservation organisations, including the RSPB, maintain that Eagle Owls should probably not be considered a native element of the British avifauna. However, there is speculation that there could be as many as 40 pairs nesting in the UK (Dennis 2005) and an alternative view among the birding community is that the Eagle Owl should be considered a native British species. This is not a pedantic point. Its status has profound implications for the Eagle Owl's conservation and welfare. Calls have been made for breeding Eagle Owls to be culled, to protect native species that may be predated by the owls. However, if included as part of the native British avifauna, the Eagle Owl would no doubt be subject to a conservation programme to encourage its re-establishment.

Historically, the fossil and archaeological record suggests that the Eagle Owl (or a closely related ancestor) had been present in Britain for up to 700,000 years until the end of the last ice age (Stewart 2007). However, in 1996 the Eagle Owl was removed from the British List by BOURC (BOU 1997), based on a lack of evidence that Eagle Owls have lived in Britain in a wild state for over 200 years. Even though some pairs have bred in Britain, there is no evidence to suggest that these have involved wild birds, and some are known to have been escapes (the species has

been held in captivity in Britain since the seventeenth century). The Eagle Owl is currently regarded as a non-native species in Britain (Dudley *et al.* 2006).

An alternative view is that Eagle Owls are capable of flying across the North Sea and may have colonised Britain from northern Europe or Scandinavia (e.g. Dennis 2005). Although the species is considered to be relatively sedentary, recent evidence has suggested that young birds may disperse widely. The breeding pair in Yorkshire produced 23 young between 1997 and 2005 (Holling *et al.* 2007) before the female was shot. One young bird was recovered dead (having hit power lines) over 200 km away in Shropshire the year after fledging and another was found dead near Peebles, Borders, more than 150 km from the nesting site. Ringing records from Norway show movements of between eight and 220 km (mean 95 km, n = 12), with a tendency for birds to move towards the coast (Cramp 1985). In Switzerland, satellite telemetry and radio-tracking studies showed that young Eagle Owls left the natal site between August and November, covering between four and 35 km per night, and travelling up to 320 km in total before settling up to 100 km from the natal territory (Aebischer *et al.* 2005). More recently, another young Swiss Eagle Owl moved a total of 480 km after leaving its parents, finally settling 120 km from its natal territory (Aebischer *et al.* 2010). One ringed in Finland was recovered more than 400 km from its natal area (A. Aebischer pers. comm.) and another from Schleswig-Holstein, in Germany, where the species is expanding its range, was recovered on the French Atlantic coast, having travelled at least 1,179 km (Hamann 2002). Clearly, individual birds are capable of large movements over land at least.

For the Eagle Owl to be admitted to the British List on the basis of natural occurrence, there would need to be strong supporting evidence (Melling *et al.* 2008). To date there have been no ring-recoveries of continental Eagle Owls in Britain. This is perhaps not surprising given the small number of individuals marked (see Melling *et al.* 2008), and the lack of recoveries is not evidence that the species is incapable of crossing the North Sea. Stable-isotope ratios (e.g. $^{2}H/^{1}H$, expressed as $\delta^{2}H$) have been used extensively in the past decade as a forensic method of determining the origins and movements of migratory animals (e.g. Hobson *et al.* 2004, Rubenstein *et al.* 2004, Bearhop *et al.* 2005, Bowen *et al.* 2005, Neto *et al.* 2006, Fox & Bearhop 2008). For example, Bearhop *et al.* (2005) used hydrogen-isotope ratios in the claws of Blackcaps *Sylvia atricapilla* to infer dichotomous wintering areas of birds returning to their breeding grounds in south-central Europe. Neto *et al.* (2006) used hydrogen, carbon and nitrogen isotope ratios in conjunction with the known moult pattern of Savi's Warblers *Locustella luscinioides* to show that the isotope ratios of feathers grown in Europe differed significantly from those grown in the birds' wintering grounds in sub-Saharan Africa. Newton *et al.* (2006) found a wide range of hydrogen isotope ratios in the feathers of 'Northern Bullfinches' *Pyrrhula p. pyrrhula* sampled from Scotland, Denmark, Sweden and the Amur region of Siberia, with those from Sweden and Amur being significantly more depleted in deuterium (^{2}H). Fox *et al.* (2007) used both hydrogen and oxygen isotope ratios in an analysis of the feathers of a Baikal Teal *Anas formosa* shot in Denmark in 2005, and distinguished juvenile feathers with a strongly continental signature from post-juvenile ones with a signature more typical of a moist, coastal European environment. Stable isotopes of hydrogen have also been used to infer the origins of Goldfinches *Carduelis carduelis* trapped in northeastern Europe and imported into the UK illegally (Kelly *et al.* 2008).

Here, we used stable-isotope analysis to investigate the provenance of a second- or third-calendar-year female Eagle Owl found in Norfolk in November 2006. The bird had no rings and there was no evidence (such as feather wear on the primaries) to suggest that it had been held in captivity. We compared the isotope ratios in the primaries, secondaries and body feathers of the Norfolk bird with those collected from the carcases of five wild-bred Eagle Owls from Norway, two from the Netherlands, and a known captive bird found dead near Bristol.

We tested the hypothesis that the Norfolk bird had crossed the North Sea and predicted

that the stable-isotope signatures of juvenile feathers would be similar to those found in continental Eagle Owls. We compared the hydrogen isotope ratios of the feathers with those shown in maps ('isoscapes') depicting the predicted isotope ratios of precipitation across north and central Europe (www.waterisotopes.org). We are well aware of the limitations of this approach and recognise that assigning geographical origin based on isotope maps can be misleading unless the tissue samples are compared with those of known origin (Wunder *et al.* 2005). However, our intention is to contribute to the body of evidence concerning the status of the Eagle Owl in Britain, rather than to determine conclusively the origin of the Norfolk bird.

On the basis of the known moult patterns of Eagle Owls (Niiranen & Haapala 1987), we looked for dichotomous origins of feathers and predicted that, if the bird had originated in continental Europe or Scandinavia, we should be able to differentiate between juvenile feathers grown at the natal site and post-juvenile feathers grown in Britain. As a control for hydrogen isotope ratios, we also compared the feather samples with those from two Tawny Owls *Strix aluco* from Norfolk (a sedentary and resident species).

In addition, we also measured the carbon and nitrogen stable-isotope ratios of the feathers. This was partly in an attempt to detect any dietary differences among the birds in this investigation; carbon isotope ratios are a broad indicator of carbon source for the diet, for example C3 or C4 primary production, whereas nitrogen isotope ratios generally indicate the trophic level at which these birds are feeding. Secondly, despite the caveats just mentioned, in conjunction with the hydrogen isotope measurements, $\delta^{13}C$ may confirm or support any geographical inferences made from the $\delta^{2}H$ results (Neto *et al.* 2006).

Methods

Circumstances, identification and assessment of moult status

On 20th November 2006, a large owl was found in an aircraft hangar at the former RAF base at Watton, Norfolk, by a member of the public. The bird was subsequently collected by an RSPCA Animal Collection Officer and taken to RSPCA East Winch

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113. Eagle Owl *Bubo bubo*, Helsinki, Finland, December 2006.

Wildlife Centre, where it was identified by KL (a BTO A-permit ringer) as an Eagle Owl, based on its appearance and measurements. The bird was examined by a veterinary surgeon and was found to be emaciated and to have an injured eye. Owing to its poor prognosis, it was subsequently euthanised to prevent further suffering. A post-mortem examination revealed that it was a female. Its wing length (max. chord) was 483 mm, placing it in the mid/upper range for the nominate race *bubo* (Snow & Perrins 1998).

The moult of Eagle Owls is poorly known and the following account is based on Niiranen & Haapala (1987). Juveniles have a dark band near the tip of the primaries, secondaries and tail feathers, but in the case of post-juvenile feathers this dark bar tends to be farther from the feather tip. There is large variation in the moult pattern of Eagle Owls and individuals typically show distinctly asymmetric patterns of wing moult. Primary moult usually starts from two focus points. The first series starts at P6/P7 (primaries numbered descendantly, in other words P10 is the outermost), proceeding inwards and towards the tip, but before the outermost primaries are dropped a second series starts from P1. In most cases P3 or P10 is the last to be moulted. Secondary moult proceeds from three focus points, starting with S16 (the innermost) and moving towards the wingtip. The second series starts at S5 and the third series at S2; in most cases, the outermost (S1) is the last to be moulted. Niiranen & Haapala (1987) stated that Eagle Owls undergo a partial moult between May and October during which the body feathers and some wing-coverts are replaced. The innermost secondaries and the tertials are also replaced. Typically, all the tail feathers are replaced between the second and third calendar-year. However, primary moult does not start until the third calendar-year, with P6 or P7 being replaced first.

The moult details of the Norfolk Eagle Owl were as follows (with feathers classified as adult- or juvenile-type according to pattern and wear):

Left wing P1–P6 juvenile (-type), P7 adult (type), P8–P10 juvenile, S1–S6 juvenile, S7–S14 adult, tertials adult (S15–S16 missing)

Right wing P1–P7 juvenile, P8 adult, P9–P10 juvenile, S1–S3, S6–S7 juvenile, S4–S5, S8–S15 adult, tertials adult (S16 missing)

Tail T1–T4 adult, T5–T6 juvenile (where T1 is the innermost)

Based on this information, the Norfolk specimen was considered to be a second-/third-calendar-year bird.

Sampling

P1–P10 and S1–S14 were taken from the left wing of the Norfolk specimen and placed in polythene sample bags. Tail feathers T1–T6 (left side) and a small number of body feathers removed from the breast were treated similarly. Primary, secondary and breast feathers were taken from two adult Eagle Owls from the Netherlands and secondaries were taken from five adult Eagle Owls from Norway. All the birds from the Netherlands and Norway were recently archived specimens. Primary, secondary and breast feathers were also taken from two adult Tawny Owls from Norfolk and a dead adult Eagle Owl found in Bristol, which was known to be an escaped captive-bred bird. In addition, the outermost primary, outermost secondary and breast feathers of two adult Eagle Owls captive-bred in the UK were collected. Table 1 lists the feathers sampled from each bird. Primaries, secondaries and body feathers were requested for the Norwegian and Dutch birds, but only secondaries were available from the Norwegian birds.

SI preparation and analysis

The methodology is given in Appendix 1. For a full explanation of the stable-isotope technique and its application to ornithology, see Fox & Bearhop (2008).

Results

Hydrogen and oxygen isotope measurements

For the Norfolk Eagle Owl, the $\delta^{18}O$ values overlapped considerably: for juvenile-type feathers 11.9–19.0‰ (mean 16.6, SE ±0.4‰) and for adult-type feathers 12.8–17.4‰ (15.8, ±0.4‰).

Hydrogen isotope ratios of feathers from the Norfolk bird showed a clear pattern (see fig. 1). P7, S7–S14, T1–T4 and breast feathers of the Norfolk Eagle Owl were enriched in

^{2}H, relative to P1–P6, P8–P10 and S1–S6. The δ^{2}H values for juvenile-type feathers ranged from -86.9‰ to -96.5‰ (mean -90.1, SE ±0.5‰) and for adult-type feathers from -24.0‰ to -61.7‰ (-43.8, ±2.7‰). There was no overlap in the δ^{2}H values for juvenile- and adult-type feathers.

The δ^{2}H of the Norwegian Eagle Owls also showed a wide variation, from -1.7‰ to -80.3‰ (-50.6, ±15.3‰) (see fig. 2). This may be related to the variation in latitude from which the birds were obtained (58–69°N) or, alternatively, to the proximity of the ocean of sites from which the samples were obtained. The Norwegian owls consisted of three females and two males. Interestingly, the three birds closest in δ^{2}H values to the Norfolk owl were all females, while the two most enriched birds were males. However, the reasons for this are not clear and the small sample size makes interpretation difficult.

Dual hydrogen and carbon isotope ratios of feathers from the birds from the Netherlands and Norway, two wild-bred Tawny Owls and known captive-bred Eagle Owls are shown in fig. 2.

Carbon and nitrogen isotope measurements

The δ^{13}C values of feathers from the Norfolk Eagle Owl formed a narrow range, from -23.9‰ to -22.3‰. Most of the other UK samples, including those from the Tawny

Table 1. Feathers sampled from the Norfolk Eagle Owl *Bubo bubo*, from wild-bred Eagle Owls from the Netherlands and Norway, and from captive-bred Eagle Owls from Bristol and Wales. Samples from wild Tawny Owls *Strix aluco* bred in Norfolk are included for comparison.

Species	Status	Source	Feather type	Description
Eagle Owl	Unknown	Norfolk	Primary Secondary Body Tail	P1–P10 S1–S14 Breast T1–T6
Eagle Owl	Wild	Netherlands	Primary Secondary Body	P10 S5 Breast
Eagle Owl	Wild	Netherlands	Primary Secondary Body	P10 S5 Breast
Eagle Owl	Wild	Norway	Secondary	S5
Eagle Owl	Wild	Norway	Secondary	S5
Eagle Owl	Wild	Norway	Secondary	S5
Eagle Owl	Wild	Norway	Secondary	S5
Eagle Owl	Wild	Norway	Secondary	S5
Eagle Owl	Captive	Bristol	Primary Secondary Body	P4 S1 Breast
Eagle Owl	Captive	Wales	Primary Secondary Body	P10 S1 Breast
Eagle Owl	Captive	Wales	Primary Secondary Body	P10 S1 Breast
Tawny Owl	Wild	Norfolk	Primary Secondary Body	P10 S1 Breast
Tawny Owl	Wild	Norfolk	Primary Secondary Body	P10 S1 Breast

Owls, were similar to the Norfolk Eagle Owl values, or slightly more ^{13}C-rich. The Dutch and (in particular) the Norwegian feather samples appear to show a positive relationship between δ^{13}C and δ^{2}H, which could be indicative of a marine influence in the signature of the feathers.

The δ^{15}N values of feathers from the Norfolk Eagle Owl ranged from +8.3 to +11.5‰. Most other groupings lie in the same δ^{15}N range, with the exception of one of the Tawny Owl samples, which has feather δ^{15}N values ranging from +2.6 to +5.3‰. It is most likely that this Tawny Owl was feeding at least one trophic level below the other individuals in this study. The adult feathers from the Norfolk Eagle Owl were significantly enriched in ^{15}N and slightly (but not significantly) enriched in ^{13}C compared with the juvenile feathers. This is probably due to a change in the trophic level between the juvenile and adult stages. An alternative explanation is that a marine influence in the diet has resulted in enrichment of ^{15}N and ^{13}C. However, this is unlikely since the natural prey of Eagle Owls is terrestrial.

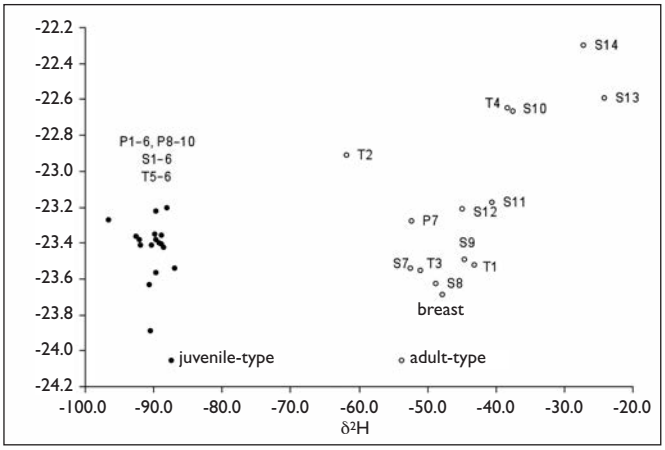

Fig. 1. Dual isotope plot for feathers from the Norfolk Eagle Owl *Bubo bubo*. The closed circles (^{2}H depleted) represent the juvenile-type feathers and the open circles (^{2}H enriched) represent the adult-type feathers.

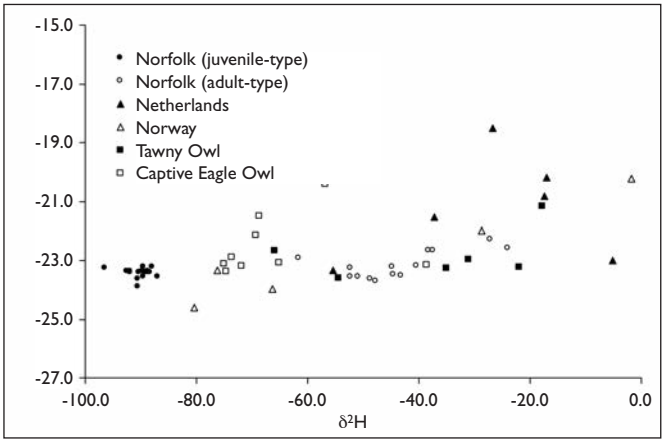

Fig. 2. Dual carbon and hydrogen isotope plot showing the wide range of δ^{2}H values for the sampled feathers from the Norfolk Eagle Owl *Bubo bubo* (juvenile- and adult-type feathers), Eagle Owls from the Netherlands and Norway, Tawny Owls *Strix aluco* from Norfolk and captive Eagle Owls from the UK.

Discussion

The results from our analyses suggest that the two generations of feathers of the Norfolk Eagle Owl were grown in different climatic regions. P7 and S7–S14 (adult feathers) were clearly enriched in deuterium relative to the juvenile primaries and secondaries. The δ^{2}H of the juvenile feathers lay outside the range of values found for the other Eagle Owls, of known origin, measured here and would perhaps be consistent with an origin farther east in continental Europe where the environmental δ^{2}H is lower. The δ^{2}H values of the adult-type feathers from the Norfolk Eagle Owl were similar to those of the Norwegian Eagle Owls and UK Tawny Owls and showed a marked difference from those of the captive-bred (UK) Eagle Owls. In terms of carbon and nitrogen isotopes, only the nitrogen isotope ratio differed between the juvenile- and adult-type feathers, indicating a marginal increase in trophic level.

Owing to the limitations of stable-isotope techniques to pinpoint origins precisely, we cannot rule out the following alternative explanations for the very low δ^{2}H

values of the first-generation feathers:

1. The Norfolk bird was captive-bred and had been fed on ^{2}H-depleted food before escaping and subsequently living for a substantial period in the wild, with post-juvenile feathers reflecting its environment.
2. The bird was captive-bred and had been fed ^{2}H-depleted food for the first year before switching to a ^{2}H-enriched diet in its second calendar-year, prior to escaping.
3. The bird was captive-bred or wild-caught in an area where the δ^2H value of precipitation is low and was then transported to East Anglia before escaping or being released.
4. The bird was wild but had hatched in a region of the UK with depleted local δ^2H values.
5. Physiological differences between the juvenile and adult stage result in differences in the way δ^2H is incorporated into feathers (a growth effect).

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114. Eagle Owl *Bubo bubo*, Helsinki, Finland, November 2006.

There is no evidence to suggest that captive-bred birds in the UK are fed on ^{2}H-depleted food, although the δ^2H values recorded in the feathers of captive birds were considerably lower than those of the other specimens. Most captive-bred Eagle Owls in the UK are fed on day-old chicks, sourced in the UK (Gary Dickenson pers. comm.) and so are most likely to reflect δ^2H values of UK precipitation. Explanations 1 and 2 require that the food fed to a captive-bred bird was sourced from raw materials derived from areas with low δ^2H precipitation values. In terms of explanation 3, Eagle Owls are listed on Annex A of CITES, with trade strictly controlled, and birds legitimately involved in trade are required to be permanently marked; consequently, explanation 3 is unlikely (although illegal importation remains a possibility).

Explanation 5 is not compelling. We can think of no reason why juvenile and adult stages may differ in the way δ^2H is incorporated into feathers, and it is extremely unlikely that annual changes in δ^2H would be as marked as the differences we observed between the juvenile- and adult-type feathers of the Norfolk bird.

We cannot rule out (and cannot test for) explanation 4, that the bird hatched in the wild in the UK in a region with low local δ^2H values before moving to Norfolk. Certainly, published data suggest that δ^2H values in northern Britain and Scandinavia are similar (Bearhop *et al.* 2005), while Newton *et al.* (2006) showed that resident Scottish

Bullfinches (subspecies *pileata*) had δ^2H values similar to those of the juvenile-type feathers from the Norfolk Eagle Owl. The possibility exists, therefore, that this bird may have hatched in an area of Scotland with low local δ^2H values (in the wild or, indeed, in captivity) before moving (or being moved) south to Norfolk.

The juvenile-type feathers suggest that the Norfolk bird's natal area was characterised by precipitation with very low δ^2H values. Such conditions occur in Scandinavia, north-continental Europe and mid-continental Russia – where Eagle Owls are known to occur – but also in northern parts of the UK (Hobson *et al.* 2004; Bowen *et al.* 2005). For example, the δ^2H values of the first-generation feathers from the Norfolk Eagle Owl were similar to those of Grey Partridges *Perdix perdix* and Eurasian Curlews *Numenius arquata* from Russia and of Fieldfares *Turdus pilaris* and Redwings *Turdus iliacus* from Finland (Hobson *et al.* 2004); but also similar to those of resident Scottish Bullfinches (Newton *et al.* 2006). Unfortunately, as discussed above, stable-isotope analysis is not sufficiently precise to identify the natal area of this bird and, in fact, the alternative explanations for the observed isotopic differences between the juvenile and adult-type feathers listed above illustrate well the limitations of the technique.

The strongest objection to Eagle Owls appearing in the UK as natural vagrants appears to be their relatively sedentary behaviour and reluctance to cross large expanses of water (Melling *et al.* 2008). However, although ringing recoveries confirm that Eagle Owls are largely sedentary, they are clearly capable of moving long distances, at least over land, as the examples given at the beginning of this paper show. Indeed, the fact that young birds in Switzerland have been shown to move up to 480 km before settling prompted Aebischer *et al.* (2010) to call for transnational conservation efforts. Moreover, the Eagle Owl's range has been spreading in western Europe, despite declines in many European countries (Hagemeijer & Blair 1997). Although there is no conclusive evidence of the species undertaking a major sea crossing, natural vagrancy may not be as unlikely as suggested by Melling *et al.* (2008).

In future, it may be possible to identify the origin of Eagle Owls genetically using microsatellite markers developed in a captive population used to reinforce the Eagle Owl in Sweden (Isaksson & Tegelstrom 2002). Until then, stable isotopes may provide further information on possible vagrants. We were unable to obtain samples from any of the Yorkshire Eagle Owls and it would be interesting to compare the δ^2H of Eagle Owls known to have been raised in the UK with those from continental Europe and captive-bred birds. We recommend that isotopic values of feathers of Eagle Owls of unknown provenance discovered in Britain in future should be measured and compared with those of specimens of known provenance.

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Appendix 1.

SI preparation and analysis

Hydrogen/oxygen isotope measurements

Feathers were washed in 2:1 chloroform:methanol and dried at room temperature under a fume hood. A small section of mass 0.6–0.8 mg was clipped from the end of all feathers. In the case of the large primaries, small sections were cut from the tip, middle and base. In each case, the sample was weighed into individual silver capsules for hydrogen and oxygen isotope analysis. Isotope ratios were measured via continuous-flow stable-isotope mass spectrometry (CF-IRMS), using a Costech ECS 4010 elemental analyser in oxygen mode with high-temperature induction furnace (HTG-02) interfaced with a ThermoFisher Scientific Delta V Plus isotope ratio mass spectrometer, at the East Kilbride node of the NERC Life Sciences Mass Spectrometry Facility (Scottish Universities Environmental Research Centre).

Repeat analyses of keratin standards incorporated into each hydrogen sample run showed that

$\delta^{2}H$ is measured with an accuracy and precision of ±2‰. Keratin standards CFS (chicken feather), BWB-II (Bowhead Whale *Balaena mysticetus* baleen) (Wassenaar 2008) in conjunction with an internal standard ISB (Icelandic Kittiwake *Rissa tridactyla* feather) were used to correct samples for isotopic exchange between labile hydrogen in feathers and ambient water vapour in the laboratory using a comparative equilibration method (Wassenaar & Hobson 2003). The $\delta^{18}O$ values of samples were compared to those of the reference materials IAEA CH6 (sucrose, Farquhar *et al.* 1997, Kornexl *et al.* 1999) and IAEA 601 and 602 (benzoic acid), though we recognise that the samples here are ^{18}O-poor with respect to the standards used.

Carbon/nitrogen measurements

Sections of the tips of all cleaned feathers were cut and transferred into 3 × 5-mm tin capsules, which were then measured by CF-IRMS, using a Costech ECS 4010 elemental analyser in CHNS mode interfaced with a ThermoFisher Scientific Delta V Plus isotope ratio mass spectrometer, at the East Kilbride node of the NERC LSMSF.

Samples were measured against internal standards gelatin and two alanines with disparate carbon and nitrogen stable-isotope ratios. Long-term reproducibility is around 0.2‰ for $d^{15}N$ and 0.1‰ for $\delta^{13}C$.

Stable-isotope ratios are expressed as the normalised ratio of the sample to a primary international standard, in parts per thousand (per mil, ‰):

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

where R_{sample} and $R_{standard}$ are the ratios of heavy to light isotopes for the sample and the standard