Journal of Animal Ecology

British Ecological Society

Journal of Animal Ecology 2012, **81**, 1223–1232 doi: 10.1111/j.1365-2656.2012.01998.x

The early bird gets the shrimp: confronting assumptions of isotopic equilibrium and homogeneity in a wild bird population

Michael B. Wunder[1]*, Joseph R. Jehl, Jr[2] and Craig A. Stricker[3]

[1]*Department of Integrative Biology, University of Colorado Denver, PO Box 173364, Denver, CO 80217, USA;* [2]*Division of Birds, US National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, USA; and* [3]*US Geological Survey, Denver Federal Center, Denver, CO 80225, USA*

Summary

1. Because stable isotope distributions in organic material vary systematically across energy gradients that exist in ecosystems, community and population structures, and in individual physiological systems, isotope values in animal tissues have helped address a broad range of questions in animal ecology. It follows that every tissue sample provides an isotopic profile that can be used to study dietary or movement histories of individual animals. Interpretations of these profiles depend on the assumption that metabolic pools are isotopically well mixed and in equilibrium with dietary resources prior to tissue synthesis, and they extend to the population level by assuming isotope profiles are identically distributed for animals using the same proximal dietary resource. As these assumptions are never fully met, studying structure in the variance of tissue isotope values from wild populations is informative.

2. We studied variation in $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$ and $\delta^{18}O$ data for feathers from a population of eared grebes (*Podiceps nigricollis*) that migrate to Great Salt Lake each fall to moult feathers. During this time, they cannot fly and feed almost exclusively on superabundant brine shrimp (*Artemia franciscana*). The ecological simplicity of this situation minimized the usual spatial and trophic complexities often present in natural studies of feather isotope values.

3. Ranges and variances of isotope values for the feathers were larger than those from previously published studies that report feather isotopic variance, but they were bimodally distributed in all isotope dimensions. Isotope values for proximal dietary resources and local surface water show that some of the feathers we assumed to have been grown locally must have been grown before birds reached isotopic equilibrium with local diet or immediately prior to arrival at Great Salt Lake.

4. Our study provides novel insights about resource use strategies in eared grebes during migration. More generally, it demonstrates the utility of studying variance structures and questioning assumptions implicit in the interpretation of stable isotope data from wild animals.

Key-words: capital-income, eared grebe, isotopic niche, migration, trophic diversity

Introduction

At natural abundance levels, stable carbon, hydrogen and oxygen isotopes in plants and water vary with geography along temperature and humidity gradients (West *et al.* 2006). The process of tissue synthesis by animals more or less predictably modifies isotope abundances found in their diets (summarized by Gannes, Martinez del Rio & Koch 1998; Martinez del Rio *et al.* 2009). Therefore, any geographic patterns in isotope abundance are reliably reflected in animal tissues synthesized from local resources at points across these geographic gradients, provided that (i) the animals were in isotopic equilibrium with those resources prior to tissue synthesis, and (ii) that the resultant isotope distributions in tissues are homogeneous within specific locations and distinct among locations across the gradient. Under these conditions, it follows that isotope distributions in feather keratin, for example, reflect diet and environmental conditions where feathers were grown and, because keratin is metabolically inert, those distributions will remain unchanged even after the bird migrates to a new location. In this way, isotope

*Correspondence author. E-mail: michael.wunder@ucdenver.edu

values in feathers can be coupled with knowledge about moult phenology to indirectly link migrants to geographic locations that were occupied at other times of the year. Over the past few decades, this approach has been used to gather new insight about avian migration (Hobson & Wassenaar 2008).

The reliability of estimated geographic links of sampled individuals depends on the degree of understanding for how isotope values vary among and within individuals growing feathers at the same locations (Wunder & Norris 2008a); information about variance distributions leads directly to quantitative statements about the probability that any pre-defined location was the feather origin relative to any other pre-defined candidate location (Wunder & Norris 2008b; Wunder 2010). The spatial resolutions associated with these assignment models are constrained by the isotopic variability of available dietary resources and by variation in allocation of those resources to feathers. Thus, quantifying the minimum expected variance for feathers from a single species grown at a single location is useful for understanding spatial resolution limits for isotope-based models that assign birds to specific locations. As we show in this study, eared grebes stage and moult during autumn in an extremely simple food web at Great Salt Lake, which provides a unique opportunity to study the lower limits for variance in the isotopic composition of feathers, while also evaluating widely held assumptions of isotopic equilibrium with local resources during tissue synthesis.

CASE STUDY

Eared grebes (*Podiceps nigricollis*) breed in fresh-water habitats broadly distributed over western North America. Immediately after nesting, most of the population migrates directly to Great Salt Lake in Utah, or Mono Lake in California to moult flight feathers. These two hypersaline lakes provide grebes with a seasonal abundance of small invertebrates, which at Great Salt Lake comprise brine shrimp (*Artemia franciscana*) and two species of alkali flies (*Ephydra* spp.; Jehl 1988; Cullen, Jehl & Nuechterlein 1999). For most individuals, the migration between the breeding grounds and the lakes involves a 2- to 3-day direct flight, during which they do not feed (Jehl, Henry & Ellis 2003; Jehl & Henry 2010). Migrating grebes arrive at Great Salt Lake from late July through October. The birds are thin on arrival, most weighing 250–300 g. After gaining weight for about 2 weeks and reorganizing body composition from a flying to flightless condition (Jehl 1988, 1997), they begin the pre-basic moult, which for adults is complete; juveniles replace only the body plumage. Flight feathers are moulted simultaneously; they are replaced over a flightless period of 35–40 days (Jehl 1988), and nearly all birds are full-winged by the middle of October.

The grebes fatten slowly over the fall staging period, reaching a peak of 600 g or more. They remain at this weight until food abundance drops below a threshold value (Jehl 2007), after which they begin to reorganize their body for migratory flight to wintering areas in Mexico and southern California. The reorganization involves a decrease in weight to 400–450 g, along with an increase in the size of flight muscles and a decrease in digestive organs (Jehl 1997). In most years, departure from Great Salt Lake occurs between late November and early January, with juveniles departing a week or two before adults (Jehl 1988 and unpublished data).

In early November 2004, thousands of eared grebes died from an outbreak of avian cholera while staging at Great Salt Lake. This event provided an opportunity to investigate isotopic variance within a sample of flight feathers all grown at the same known time and location. Additionally, dietary resources available for feather synthesis are essentially restricted to resident brine shrimp and more marginally alkali flies. These prey items comprise part of a simple food web that consists of phytoplankton (including at least two species of *Dunaliella* algae), shrimp, and flies. We sampled grebe feathers from this comparatively simple food web to quantify what we suspected would be among the narrowest ranges of stable isotope values for feathers from a wild population of birds.

Materials and methods

SAMPLING AND PREPARATION OF FEATHERS, SHRIMP AND FLIES

Grebes in this study were killed by avian cholera at Great Salt Lake in late October or early November 2004. Because avian cholera kills quickly (< 24 h), these birds probably weighed at most 20–30 g less than unaffected birds, the difference because of catabolism of tissue. Over 200 bird carcasses were salvaged from near Promontory Point (40·4975°N, 112·2214°E). We weighed each bird to the nearest gram with a Pesola spring scale (Rebmattli 19; CH-6340 Baar, Switzerland), determined sex by visual inspection and in some cases by dissection (Jehl, Henry & Bond 1998) and evaluated feathers for indication of recent moult. We also attempted to age all birds by plumage and eye colour; however, the latter was not always possible because some specimens were dried out and because of the seasonal change in eye colour from brown in juveniles to orange and red in adults.

Because we were interested in the magnitude of isotopic variability in feathers grown at Great Salt Lake, we restricted the analysis to 75 adult birds that retained varying degrees of partial sheathing material at the base of the flight feathers, which we assumed indicated a locally (recently) grown feather. Not all grebes replace remiges on the staging areas. Jehl & Henry (2010) estimated that 10% of adults replace remiges before leaving the breeding grounds, and Storer & Jehl (1985) found that very few birds moult on the wintering quarters. However, feathers replaced during the previous winter would not likely retain any traces of sheathing upon arrival at the fall staging area.

From each bird, we plucked all primary flight feathers with evidence of basal sheathing. Whole feathers were stored in glass scintillation vials; all feathers from each bird were stored in the same vial. We transported feathers to Colorado, where they were cleaned of surface oils using a 2 : 1 chloroform: methanol solution and allowed to air-dry for 48 h under a fume hood. Approximately 1·7 mg of feather tissue was clipped and loaded into 5 × 9 mm pressed tin capsules for $\delta^{13}C$ and $\delta^{15}N$. For $\delta^{2}H$ and $\delta^{18}O$ analysis, approximately 0·5 mg of feather keratin was loaded into 3 × 5 mm

pressed silver capsules. Only fully developed vanes were used for analysis. We did not use any part of the rachis because it can be contaminated with blood and may yield a different isotope value than the vanes (Wassenaar & Hobson 2006). Grebes have a simultaneous remige moult, which means that all flight feathers were growing at the same time, from the same diet, at roughly the same rate. Therefore, within-population variance related only to timing of moult or arrival schedules. Because we did not know the arrival time of each sampled bird, we clipped vanes from the same regions of the feathers for sampling consistency.

Live brine shrimp (*Artemia franciscana*) were also sampled from the lake a few days after the grebe carcasses were collected. Bulk shrimp samples were stored in glass scintillation vials and transported to Colorado where they were washed in distilled water and dried in an oven at 60 °C for 24 h. Dried shrimp were individually ground to a powder using a mortar and pestle and weighed into tin or silver capsules as described for feathers above. We analysed 11 individual shrimp for all four isotopes and another 46 shrimp for δ^2H and $\delta^{18}O$ only. We sampled live alkali flies (*Ephydra* spp.) from the lake in August 2005. Because individual fly samples were not massive enough for analysis of all four isotopes, we alternately analysed each individual fly either for $\delta^{13}C$ and $\delta^{15}N$ ($n = 13$ flies) or for δ^2H and $\delta^{18}O$ ($n = 13$ flies).

STABLE ISOTOPE ANALYSIS

Samples were analysed for $\delta^{13}C$, $\delta^{15}N$, δ^2H and $\delta^{18}O$ at the U.S. Geological Survey Stable Isotope Laboratory in Denver, Colorado. We report stable isotope ratios as relative values in parts per million (‰) using the standard delta notation: $\delta_{sample} = [(R_{sample}/R_{standard}) - 1] \times 1000$ where R_{sample} and $R_{standard}$ are the ratios of the heavy to light isotopes for the sample and the standard (monitoring gas), respectively.

Samples were analysed for $\delta^{13}C$ and $\delta^{15}N$ by continuous flow methods using an elemental analyzer coupled to either a Micromass Optima or a Finnigan DeltaPlus XP mass spectrometer (Fry *et al.* 1992). C and N isotopic compositions are reported relative to V-PDB and Air, respectively, using internationally distributed primary standards (glutamic acid; USGS 40: −26·24 and −4·52‰, USGS 41: 37·76 and 47·57‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively). Accuracy was approximately ±0·2‰ for $\delta^{13}C$ and $\delta^{15}N$ based on replicate analyses of additional primary standards (NBS 22, USGS 25 and USGS 26); analytical precision and matrix reproducibility (internal keratin standard) were approximately ±0·2‰.

Samples were analysed for δ^2H and $\delta^{18}O$ by continuous flow isotope ratio mass spectrometry using a Finnigan TC/EA interfaced to a Finnigan DeltaPlus XL mass spectrometer (Thermo Scientific, Bremen, Germany) (Farquhar, Henry & Styles 1997; Wassenaar & Hobson 2003). Hydrogen and oxygen isotopic compositions are reported relative to V-SMOW using an internal laboratory standard (benzoic acid, $\delta^2H = -61‰$ and $\delta^{18}O = 25·1‰$) calibrated to IAEA-CH-7 ($\delta^2H = -100‰$) and NBS127 ($\delta^{18}O = 9·0‰$). Non-exchangeable hydrogen isotopic compositions were determined by comparative equilibration techniques described by Wassenaar & Hobson (2003) using internal laboratory keratin standards (AK = −171·5‰, LA = −78·1‰) that have been calibrated to BWB-CFS-CHS (Wassenaar & Hobson 2003). Accuracy and analytical error associated with non-exchangeable hydrogen isotope measurements was ±2‰ based on replicate analyses of a normalization standard treated as an unknown; matrix reproducibility was ±2‰. For organic-$\delta^{18}O$, accuracy was assessed at ±0·1‰ based on replicate analyses of internal standards (AK = 2·0‰, LA = 10·0‰); analytical error and matrix reproducibility were ±0·5‰.

STATISTICAL ANALYSIS

Because we did not have measures of all four isotopes for every individual shrimp and fly, we summarized the data at the population level for each of the four isotopes independently. For consistency, we also independently summarized the distribution for each of the four isotopes in the feather data (Table 1). We found two distinct modes in each of the four isotope dimensions; bird feathers comprising each of the two modes in each of the four isotope dimensions were from the same individuals across all isotopes. A four-dimensional K-means cluster analysis based on the four isotope variables and the associated covariance clearly defined these two distinct groups of individuals; the most parsimonious number of clusters in the data was two. Thus, for subsequent analyses, we considered these two groups of bird feather data as having derived from two independent processes. Group 1 included 23 individual birds with lower feather delta values for all four isotopes; Group 2 included 52 individuals with higher delta values for all isotopes. We tested whether the sex ratio was different from 1 : 1 in each group. For this, we used the binomial distribution to compute the probability of observing a proportion as or more extreme than observed, assuming the true proportion was even among the classes (e.g. $\theta = 0·5$).

A plot of $\delta^{13}C$ vs. $\delta^{15}N$ showed that brine shrimp, but not flies, were likely incorporated into feathers for Group 2 and that feathers for Group 1 were not derived from either prey resource. We therefore used the isotope data from shrimp and feathers from Group 2 to estimate an empirical distribution of trophic discrimination factors for each of the four stable isotopes. We did this by a simple Monte Carlo process whereby we randomly sampled with replacement one feather and one shrimp. We measured the linear distance between

Table 1. Means ± standard deviations, and (ranges) for ^{13}C, ^{15}N, 6^2H and $6^{18}O$ for organic material sampled in November 2004 from Great Salt Lake, Utah. The two groups of grebe feathers were distinguished using a clustering algorithm that assigned individual feather samples to one of two groups based on multivariate distributions of the four stable isotopes

	δ^2H	$\delta^{18}O$	$\delta^{13}C$	$\delta^{15}N$
Brine shrimp	−110 ± 9 (42)	18 ± 1 (6)	−18.1 ± 2.1 (6.5)	10.9 ± 0.7 (2.3)
Brine flies	−165 ± 17 (55)	14 ± 1 (2)	−13.1 ± 1.1 (3.7)	12.1 ± 0.9 (3.4)
Group 1 ($n = 23$)	−134 ± 14 (49)	5 ± 2 (10)	−22.3 ± 3.9 (13.1)	10.3 ± 2.0 (6.4)
Group 2 ($n = 52$)	−57 ± 11 (52)	12 ± 1 (5)	−15.5 ± 1.1 (4.7)	15.9 ± 1.3 (7.8)
Surface water[a]	−113 ± 11 (28.2)	−15 ± 2 (5)		
GSL water[b]	−61	−4		

[a]I. Friedman, unpublished data. [b]Approximate values for September 2006, from Nielson and Bowen, Fig. 4.

feather and shrimp values for each of the four isotope dimensions and summarized the distributions of these distances as estimates of the trophic discrimination factors for each isotope.

Results

K-means cluster analysis based on the joint distribution of all four isotopes in grebe feathers suggested there were two isotopically distinct groups. These two feather groups were consistently different from each other in the marginal distributions for each isotope; Group 1 had systematically lower δ^2H, δ^{18}O, δ^{13}C and δ^{15}N values than did Group 2. For Group 1, feathers were generally characterized by $\delta^2H < -100‰$, $\delta^{18}O < 10‰$, $\delta^{13}C < -20‰$ and $\delta^{15}N < 12‰$. For Group 2, feathers were generally characterized by $\delta^2H > -100‰$, $\delta^{18}O > 9{\cdot}5‰$, $\delta^{13}C > -16{\cdot}5‰$ and $\delta^{15}N > 15‰$ (Table 1). The overall ranges of isotope values for feathers from both Group 1 and Group 2 combined were 130‰ for δD, 15‰ for δ^{18}O, 16‰ for δ^{13}C and 12‰ for δ^{15}N (Fig. 1). The ranges in isotope values for only Group 2 were less than half as narrow as all feathers combined (Table 1), but standard deviations in δ^2H, δ^{13}C and δ^{15}N for feathers from each of the groups were within the range of standard deviations observed for feathers from other bird species sampled outside the laboratory (Fig. 2). All analysed feathers retained basal sheath material (sometimes only traces), indicating recent growth. However, only the isotope values for Group 2 were within the range expected for feathers synthesized from brine shrimp, and values for Group 2 were relatively tightly clustered when compared with those for Group 1 (Fig. 3). Monte Carlo simulations to estimate the discrimination between shrimp and feathers for Group 2 resulted in mean discrimination values of 53 ± 15‰ (SD) for δ^2H, −6 ± 2‰ (SD) for δ^{18}O, 2·5 ± 2·5‰ (SD) for δ^{13}C and 5·0 ± 1·4‰ (SD) for δ^{15}N.

The isotope values for brine shrimp and flies from Great Salt Lake were normally distributed within each isotope dimension (Fig. 4). The overall ranges of values for each isotope were large; brine shrimp δ^2H values ranged 42‰ and δ^{13}C ranged 6·5‰ (Table 1). With the exception of δ^{15}N, the ranges of isotope values for feathers in Group 2 were similar in magnitude to the ranges of values for brine shrimp (Table 1).

The proportion of females was not different from 0·5 for Group 1 ($\theta = 0{\cdot}57$, $P = 0{\cdot}74$) or Group 2 ($\theta = 0{\cdot}46$, $P = 0{\cdot}14$). The group with lower isotope values (Group 1) also had reduced body mass (Fig. 5), averaging 403 ± 58 g (SD), compared with 475 ± 61 g (SD) for Group 2. The 95% confidence interval for difference in mean mass ($\mu_{G1} - \mu_{G2}$) was −101·2 to −41·8.

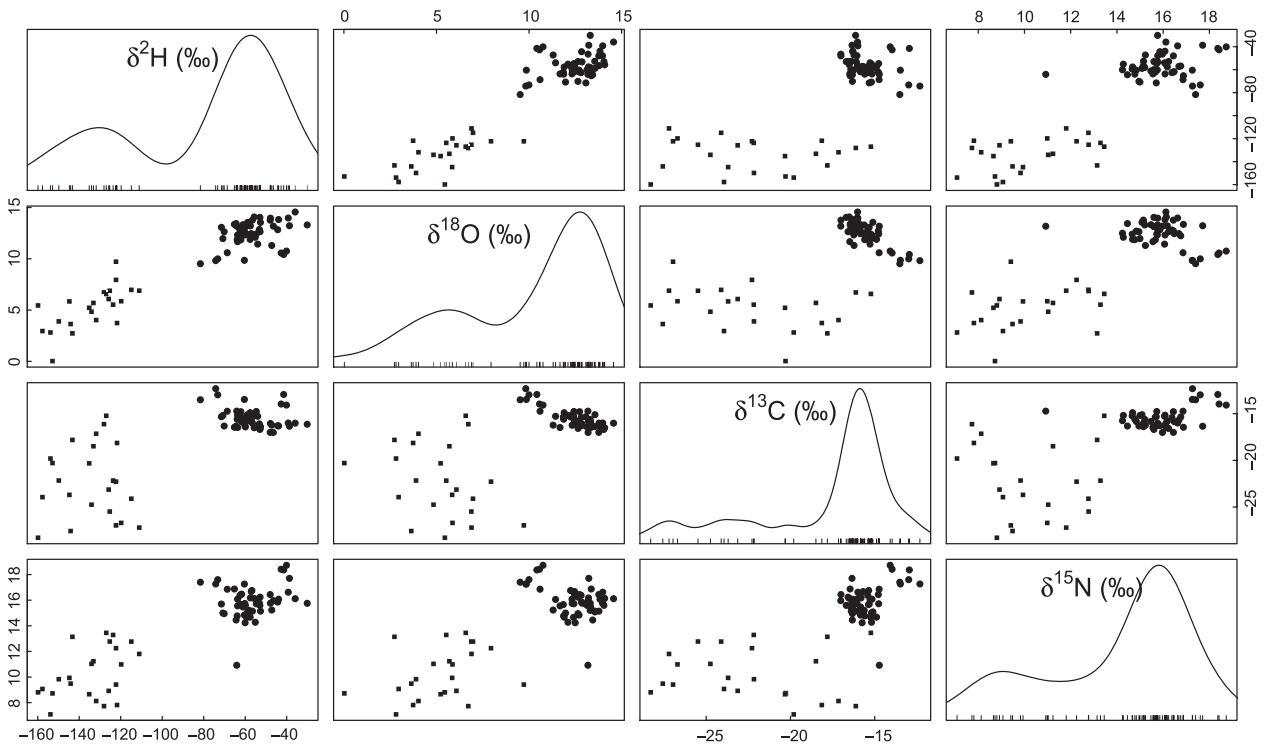

Fig. 1. Distributions of δ^2H, δ^{18}O, δ^{13}C and δ^{15}N for individual eared grebe feathers from Great Salt Lake, Utah, USA. Density histograms for single isotopes appear on the diagonal, where the hash marks indicate locations of the data along the number line that is scaled for each named isotope. To find the specific values, use the numbers that appear on the x-axis for that column. Data distributions in panels on the diagonal are for both groups of feathers combined. Off-diagonal panels show bivariate plots for each pair of isotopes, where Group 1 feathers are plotted as squares, Group 2 as circles (see text for definition of Group 1 and Group 2). Axis labels follow from the named isotopes on the diagonal. For example, the y-axis for all plots in the top row and the x-axis for all plots in the first column are scaled for δ^2H, whereas the y-axis for all plots in the second row and the x-axis for all plots in the second column are scaled for δ^{18}O.

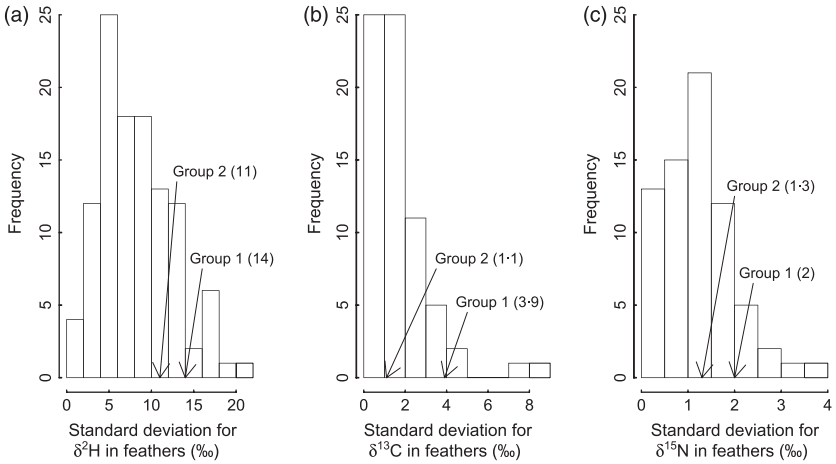

Fig. 2. Distributions of standard deviations in δ^2H (a), δ^{13}C (b) and δ^{15}N (c) for bird feathers summarized from the published literature. For δ^2H, the histogram represents 112 from 36 species (Wunder 2007). For δ^{13}C and δ^{15}N, histograms describe data in Table 2. Standard deviations for the two groups of eared grebe feathers from this study are indicated with arrows and given in parenthesis. See text for definition of Group 1 and Group 2.

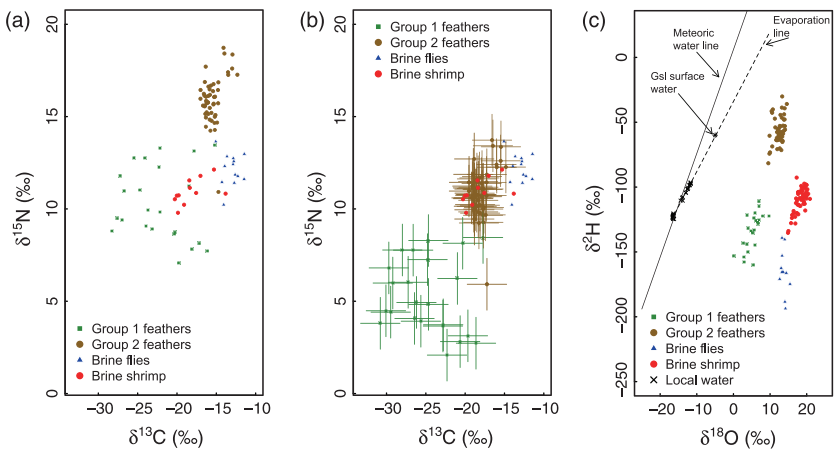

Fig. 3. δ^{13}C, δ^{15}N, δ^2H and δ^{18}O values for eared grebe feathers, dietary items and water sampled from Great Salt Lake, Utah, USA. (a) δ^{13}C and δ^{15}N for Group 1 feathers, Group 2 feathers, brine shrimp and brine flies. (b) δ^{13}C and δ^{15}N for eared grebe feathers projected to locations expected after adjusting for trophic discrimination (see text for details). Error bars reflect the standard deviations in the discrimination values for δ^{13}C and δ^{15}N. (c) δ^2H and δ^{18}O values Group 1 feathers, Group 2 feathers, brine shrimp, brine flies, local ground water and spring water (black crosses; data from USGS collected by I. Friedman). Black star indicates approximate location for Great Salt Lake surface water in September 2006 (from Nielson & Bowen 2010). Solid line shows local meteoric water line ($\delta^2H = 8 * \delta^{18}O + 6$). Broken line is surface evaporation line ($\delta^2H = 5{\cdot}4 * \delta^{18}O - 34$) estimated from Friedman data. See text for definition of Group 1 and Group 2.

Discussion

Because grebes undergo simultaneous moult of remiges, they are flightless during feather synthesis. Therefore, we assumed the presence of basal sheathing material indicated a locally grown feather that was synthesized from two dietary resources. We expected to find relatively small ranges in isotope values of the newly grown feathers. However, the overall range of values was surprisingly large and those data were clearly bimodal not only in the marginal distributions for each isotope, but also in the joint distributions of all four isotopes (Fig. 1).

The δ^2H and δ^{18}O values in feathers from Group 1 were as follows: the mean δ^2H for Group 1 was −134‰, which is comparable to values in meteoric water for locations farther north and east of Great Salt Lake (Bowen, Wassenaar & Hobson 2005), whereas those in Group 2 were closer to what we would expect based on water data from near Great Salt Lake (Nielson & Bowen 2010; Fig. 3c). Lower δ^{13}C and δ^{15}N feather values for Group 1 were also consistent with the use of resources from less saline habitats (Hobson 1990; Bearhop *et al.* 1999; Kelly 2000; Hart & Lovvorn 2005) where grebes would have foraged prior to migrating. The δ^{13}C and δ^{15}N values for brine shrimp were consistent with expectations for a potential dietary resource for the grebes in Group 2, and the values we estimated as discrimination factors between shrimp and feathers were within the ranges reported by other studies (reviewed by Becker *et al.* 2007; Bond & Jones 2009), whereas it would be very unlikely that Group 1 feathers were synthesized from either of the two prey resources available to grebes at Great Salt Lake (Fig. 3a). These observations collectively support the idea that Group 2 was in isotopic equilibrium with local dietary resources (e.g. brine shrimp) prior to feather growth, whereas Group 1 was not, and may even have grown feathers elsewhere.

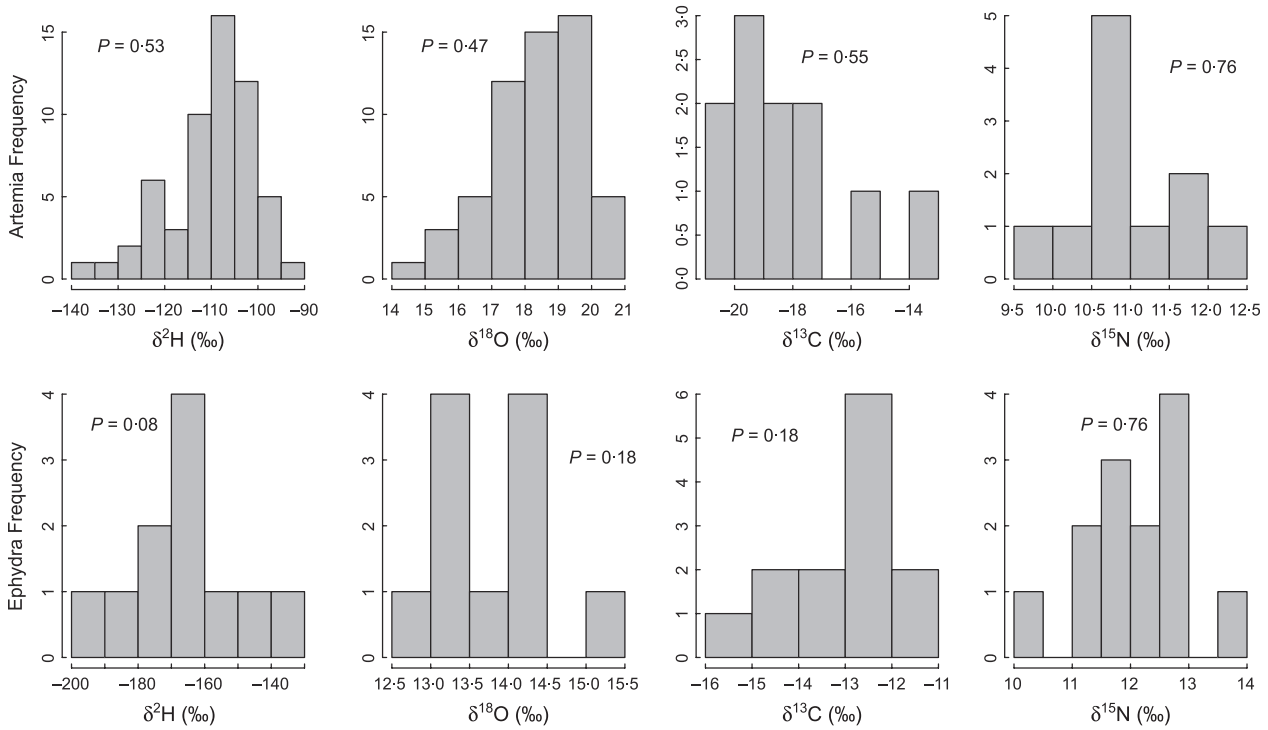

Fig. 4. Distributions of δ^2H, $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$ for brine shrimp (*Artemia franciscana*; top four panels) and brine flies (*Ephydra sp.*; bottom four panels) collected at Great Salt Lake, Utah, USA. *P*-values for each plot are from Anderson-Darling tests for normality.

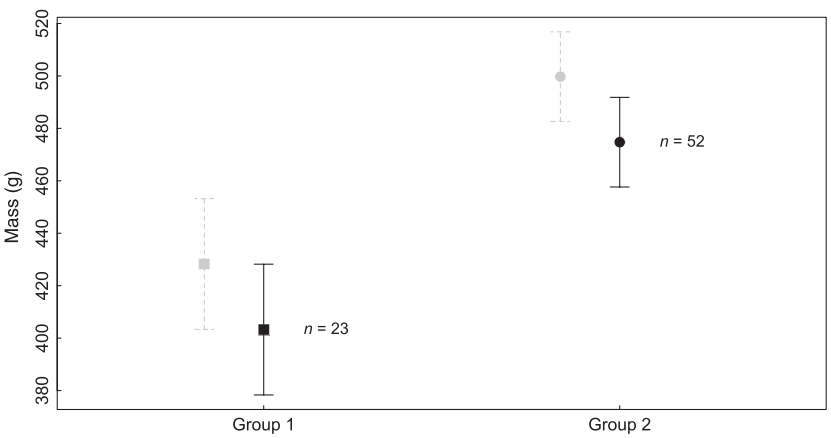

Fig. 5. Body masses (grams) for eared grebes sampled at Great Salt Lake, Utah. Individual birds were separated into two groups based on the joint distribution of δ^2H, $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$ in their feathers (see text for details). Error bars indicate width of 95% confidence intervals. Grey points and dotted bars indicate assumed masses for grebes prior to infection by avian cholera. Sample sizes for each group are indicated. See text for definition of Group 1 and Group 2.

GROUP 2 FEATHERS

Because our original objective was to quantify the variance in isotope values for feathers grown under trophic and geographic constraints, we focus first on the characteristics of the isotope data for Group 2 and for the two available dietary resources. The standard deviation in $\delta^{13}C$ for shrimp was nearly twice that for Group 2 feathers, and the overall range for $\delta^{13}C$ was nearly 40% greater for the shrimp than for the bird feathers (Table 1). This may be because we analysed whole shrimp samples, which contain lipids, whereas we removed all preen wax and other lipids from the feathers prior to analysis.

The difference in variance for $\delta^{15}N$ between feathers and shrimp was opposite that for $\delta^{13}C$; the range of values for feathers was three times as large as that for shrimp, and the standard deviation for $\delta^{15}N$ in feathers was nearly twice that for shrimp (Table 1). However, a single feather assigned to Group 2 was an apparent outlier in terms of $\delta^{15}N$, likely assigned to Group 2 based primarily on the δ^2H value (Fig. 1, lower left corner panel). When this datum is removed, the range in $\delta^{15}N$ for Group 2 reduces to 4·5‰, still about twice as large as that for the shrimp, and the standard deviation reduces to 1·0‰. Although the standard deviation is still approximately 40% larger than that for the shrimp, we point out that it is nearly equivocal when considering the magnitude of analytical error in $\delta^{15}N$ measurements. Analytical error for $\delta^{15}N$ is estimated at 0·2‰, so the difference in standard deviations is about 1·5 times the magnitude of the measurement error. Perhaps more importantly, these

magnitudes of variation in $\delta^{15}N$ in the feathers are not unusual for natural populations (Table 2, Fig. 2). However, neither are they unusually small, as we had expected them to be. Physiological reorganization during staging may influence $\delta^{15}N$ feather values as mass gain, enhancements in digestive efficiency, and moult all result from dietary energy intake.

Wunder (2007) summarized published standard deviations for $\delta^{2}H$ in feathers from 112 different samples for over 35 species, and here, we summarize standard deviations for $\delta^{13}C$ and $\delta^{15}N$ in feathers from 70 different samples for nine different species (Table 2). All individuals within each sample were known or assumed to have grown their feathers at the same site. Thus, the distribution of these estimates (shown in Fig. 2) provides an empirical basis for the range of expected variation in $\delta^{2}H$, $\delta^{13}C$ and $\delta^{15}N$ among populations of feathers grown at the same geographic location. The standard deviations for the two groups of grebe feathers in this study fell within the range of estimates from those previous reports, but neither was near the low end where we expected it for Group 2 (Fig. 2). Group 2 feathers were almost certainly grown from a diet of brine shrimp, and the distributions in $\delta^{2}H$ and $\delta^{18}O$ for brine shrimp were nearly as wide as those for feathers from grebes that foraged on them (Table 1, Fig. 3c).

Nielson & Bowen (2010) report that mean $\delta^{18}O$ values for shrimp chitin collected from Great Salt Lake fluctuate by approximately 5‰ over a single year, and that surface water ranges nearly 6‰ over the same time frame. They also report that sample means for $\delta^{2}H$ in brine shrimp chitin and surface water ranged nearly 16 and 25‰, respectively. Our observed range for $\delta^{2}H$ in whole shrimp was wider, but similar for $\delta^{18}O$ (Table 1). We note that results from the two studies are not directly comparable; our data show among-individual variation for whole animals within a single location at a single time, and their data show variation among mean values (but not variances) of chitin at various locations over time (Nielson & Bowen 2010). The relevant observation, however, is that the ranges of $\delta^{2}H$ and $\delta^{18}O$ in grebe feathers from Group 2 cannot solely be attributed to dietary or physiological differences among individual grebes; the wide ranges were already present at the next lowest trophic level, which was geographically constrained to a single, albeit large, lake.

GROUP 1 FEATHERS

Although all feathers we sampled for birds from both groups retained residual sheathing (which suggested they were grown locally at Great Salt Lake), we observed consistent and marked disparities in all four isotope values for feathers in Group 1 as compared with Group 2. There are at least four potential explanations for these broad deviations in values between the two groups. First, the birds in Group 1 may have been relatively metabolically stressed prior to feather synthesis. Sears, Hatch & O'Brien (2009) found that developing young birds and food-deprived adult birds were depleted in ^{13}C and ^{15}N relative to well-fed adults. Group 1 feather

Table 2 Standard deviations in $\delta^{13}C$ and $\delta^{15}N$ in feathers for distinct populations of various species

Study	Species	$\delta^{13}C$ (SD)	$\delta^{15}N$ (SD)	*n*
Bearhop *et al.* (1999)	Cormorant (*Phalacrocorax carbo*)	2.9	2.0	21
		4.0	2.0	21
		3.0	1.9	21
	Shag (*Phalacrocorax aristoltelis*)	0.5	0.6	12
	Goosander (*Mergus merganser*)	1.8	1.1	8
Bensch, Bengtsson & Akesson (2006)	Willow warbler (*Phylloscopus trochilus*)	3.5	1.9	47
		0.8	1.0	7
		1.1	1.3	15
		4.2	3.7	2
		4.1	1.6	13
		1.9	2.1	8
		3.8	1.5	26
		2.4	1.0	12
		1.2	0.5	5
		1.5	2.2	8
		1.7	2.4	27
		0.7	1.4	4
		1.8	1.9	14
		1.0	2.8	9
Chamberlain *et al.* (2000)	Willow warbler (*Phylloscopus trochilus*)	2.9	2.0	10
		1.8	0.9	10
		2.0	1.7	10
		1.6	1.3	10
		1.4	1.3	10
		1.2	1.5	10
		1.6	2.2	10
		0.7	1.4	10
		0.9	1.7	10
		1.7	2.7	10
		0.6	1.2	10
		0.7	1.5	10
		1.0	2.1	10
		3.7	1.5	8
Hebert & Wassenaar (2001)	Mallard (*Anas platyrhnchos*)	2.9	1.6	6
		1.5	0.8	3
		2.1	0.5	6
		1.7	0.8	5
		0.4	1.2	5
		0.7	0.4	5
		1.9	0.2	2
		0.5	0.5	5
		1.2	3.3	6
		2.9	0.6	6
		0.3	0.2	4
		0.2	0.4	2
		0.1	0.5	6
		0.4	0.3	6
		0.4	0.4	3
		0.4	0.3	6
Kojadinovic *et al.* (2008)	Barau's petrels (*Pterodroma baraui*)	1.3	0.6	5
		0.4	0.4	5
		1.1	0.6	10
Moller *et al.* (2006)	Barn swallow (*Hirundo rustica*)	2.8	0.9	6
		8.6	1.2	39
		1.7	1.1	8
		2.3	1.3	29
		1.0	0.6	3
		7.3	1.4	53
		2.4	1.0	21
		2.5	1.1	25

Table 2 (Continued)

Study	Species	$\delta^{13}C$ (SD)	$\delta^{15}N$ (SD)	*n*
Wunder *et al.* (2005)	Mountain plover (*Charadrius montanus*)	0.9	1.1	17
		1.3	0.7	7
		0.2	0.1	2
		0.9	1.1	103
		2.0	1.4	25
		1.7	1.7	25
		0.7	0.8	25
		0.5	1.0	7
This study	Eared grebe (*Podiceps nigricollis*)	3.9	2.0	23
		1.1	1.3	52

values were depleted relative to those for Group 2 (Table 1). However, all of the grebes we sampled were in the expected weight range for this time of year, and there was no evidence to suggest that any of the birds in either group were food deprived, as shrimp typically remain abundant into early December. Indeed, healthy grebes remained at Great Salt Lake for another month or more; radar data showed that the major departure occurred 11–24 December 2004 (J. R. Jehl, unpublished data). Food shortage seems further unlikely because the differences in mean $\delta^{13}C$ and $\delta^{15}N$ values between the two groups of grebe feathers reported here are 6·7 and 5·6‰, respectively, which is far greater than the approximately 1‰ difference reported by Sears, Hatch & O'Brien (2009).

A second potential explanation is that the grebes in Group 1 arrived late to the staging area and may have used additional food resources that we did not sample, such as might be available at freshwater inlets. However, freshwater sources of food for grebes at Great Salt Lake in the fall are trivial, and grebes that moult on Mono Lake, a similar hypersaline environment, do not congregate at fresh water (Mahoney & Jehl 1985). Moreover, the 95% CI for the difference in mean $\delta^{2}H$ (Group 1 – Group 2) was (−134 and −57‰); an average difference of 77‰ in mean $\delta^{2}H$ between two groups of feathers formed at the same location would be surprisingly large, even if the two different dietary bases were from freshwater and saline sources.

A third and perhaps more plausible explanation is that feathers from Group 1 were replaced on the breeding grounds and develops in large part from the direction and magnitude of the difference in $\delta^{2}H$ between the two groups. In this case, the relatively dispersed distribution of isotope values for Group 1 reflects a mixture of isotopically distinct breeding sites among birds, rather than a mixture of isotopically distinct dietary sources or physiological stresses among birds. If late migrants are time-constrained by late nesting or are otherwise delayed in departure from the breeding grounds, some will moult remiges prior to leaving (Jehl & Henry 2010). If such birds retain traces of sheathing during migration, our assumption that sheathing is an indicator of local (Great Salt Lake) moulting would be invalidated.

Our sample of grebe carcasses from Great Salt Lake is likely representative of the overall population that stages there each fall. We report isotope values only for birds with feathers that retained visible evidence of basal sheathing, because we assumed this indicated a locally grown feather and we were interested in quantifying the range of variability for feathers grown in a simple food web at a single location. Group 1 comprised approximately 30% (23 of 75) of birds with evidence of recent moult, but those individuals comprised only about 10% of the larger original sample of carcasses that we examined. That is, we examined about 230 carcasses in all, only 75 of which showed clear evidence of basal sheathing; about 10% of the birds we examined had recently grown new flight feathers that we now suspect were grown immediately prior to arrival at Great Salt Lake. This would be consistent with findings from Jehl & Henry (2010) who reported that approximately 10% of passage migrants had undergone remige moult prior to stopping over in Wyoming en route to Great Salt Lake, and that grebes that moult on the breeding grounds also migrate several weeks later than those that migrate to moult. The difference in mean body mass for the two groups is also consistent with this hypothesis. Migrating grebes arrive at Great Salt Lake from Wyoming with a body mass on average near 250 g. Grebes add about 40 g prior to initiating moult and then increase in mass to around 500 g just prior to departure for wintering grounds, which in some years may begin by early November (Cullen, Jehl & Nuechterlein 1999). The mean mass of grebes in Group1 was significantly less than that for the grebes in Group 2, which was nearly at average departure mass prior to contracting avian cholera (Fig. 5).

We cannot completely exclude a fourth possibility that if the birds in Group 1 were late migrants, they may have initiated moult almost immediately following arrival at Great Salt Lake. In this case, feather synthesis would have occurred prior to body water pools reaching isotopic equilibrium with local dietary resources, and feather C and N isotope values would therefore reflect a mixture of local brine shrimp and residual resources carried from the breeding grounds. Because grebes arrive thin, lipid reserves and muscle catabolism are less likely to contribute to an isotopic carry-over signal in feathers. The process of growing a complete feather lasts about 30–35 days, marking the minimum integration period over which a mixture of local diet and residual resources in the blood would occur. The isotopic half-life for $\delta^{13}C$ in whole blood is about 11 days (Hobson & Clark 1992), whereas those for the plasma and cellular fractions of blood are about 3 and 30 days, respectively (Hobson & Clark 1993). These rates do not preclude the possibility for residual blood cell contributions to new feather growth. Although this scenario is possible, it seems an unlikely strategy for late migrants to undergo rapid moult upon arrival at the staging areas. Because grebes arrive at the staging area in the 250–300 g mass range (Jehl & Henry 2010) and typically do not initiate moult until after gaining 30–40 g, any endogenous reserves remaining upon arrival would more likely be prioritized for rebuilding the

atrophied digestive system. Mass gains average 3–4 g day^{-1} at the staging area, so a late arrival with a body mass of 300 g would require approximately 2 weeks to attain typical pre-moulting conditions. This would mean that birds that began moulting immediately on arrival would necessarily do so before fully recovering from the metabolic demands of migratory flight.

Conclusion

Grebe feathers grown under isotopic equilibrium conditions with diet (Group 2) were not unusually variable in $\delta^{13}C$ and $\delta^{15}N$ for birds foraging at one trophic level in a simple food web (Kelly 2000). Similarly, the observed variances in $\delta^{2}H$, $\delta^{13}C$ and $\delta^{15}N$ for this group were consistent with other reports for bird feathers from single locations. We expected smaller variances in this system because of restricted dietary resources and geographic constraints. However, variances for $\delta^{13}C$ and $\delta^{15}N$ in both diet and feathers were similar, and those of $\delta^{2}H$ and $\delta^{18}O$ in feathers attained almost half of the range present in water samples at Great Salt Lake. This suggests that local hydrologic processes from freshwater recharge and evaporation were responsible for much of the feather variance in $\delta^{2}H$ and $\delta^{18}O$, leaving biochemical mechanisms responsible for the remainder. Variance in $\delta^{2}H$ and $\delta^{18}O$ for brine shrimp was similar to those in the feathers, suggesting that any additional biochemical sources of variance exist at trophic levels below brine shrimp. These observations imply that limits in isotope variation for wild bird populations are not necessarily associated with food web diversity or differences in individual physiology, a finding that adds complications to the interpretation of isotopic variance for evaluating trophic dynamics and trophic niche width (Bearhop *et al*. 2004).

A growing number of studies illustrate that clear interpretation of patterns in isotope data depends on animals having reached isotopic equilibrium with diet before tissue synthesis (e.g. Hobson & Clark 1992; Cerling *et al*. 2007; Fox, Hobson & Kahlert 2009; Yohannes *et al*. 2010). This study reinforces that point and additionally demonstrates the utility of natural history known from other methods for interpreting patterns in stable isotope data. Only by questioning our assumption that feathers with residual sheathing were grown in equilibrium with locally available resources we were able to explain the unexpected patterns in the isotope data for the birds assigned to Group 1. In so doing, we gained insightful information about resource use in staging areas that enhances the understanding of migration ecology in this species. It appears that late migrants face a potential energetic constraint related to moult; either they forgo the energetic advantages of the super abundant brine shrimp at great Salt Lake and moult on the breeding grounds, or they are forced to initiate moult immediately on arrival at great Salt Lake, prior to fully restoring other tissues that were modified for or damaged from migratory flight. Clearly, this suggests that more experimental work is needed to understand the extent to which isotope variances like those observed here are attributed to ecological strategies or energetic stresses associated with moult.

Acknowledgements

We thank the Utah Division of Wildlife for collecting grebe carcasses and brine shrimp and Fritz Knopf for suggesting this project. We thank Amy Yackel-Adams for assistance with laboratory sample preparation and Cayce Gulbransen for performing the isotope analyses. We thank Stuart Bearhop, Richard Inger, Andrew Jackson, Gabe Bowen, Scott Carleton, Carlos Martinez del Rio and Keith Hobson for discussion that improved this work. We are grateful to Keith Hobson, Jeff Kelly and Peggy Ostrom for providing comments to improve earlier drafts of the manuscript.

References

Bearhop, S., Thompson, D.R., Waldron, S. & Russell, I. (1999) Stable isotopes indicate the extent of freshwater feeding by cormorants *Phalacrocorax carbo* shot at inland fisheries in England. *Journal of Applied Ecology*, **36**, 75–84.

Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A. & Macleod, H. (2004) Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology*, **73**, 1007–1012.

Becker, B.H., Newman, S.H., Inglis, S. & Beissinger, S.R. (2007) Diet-feather stable isotope ($\delta^{15}N$ and $\delta^{13}C$) fractionation in common murres and other seabirds. *Condor*, **109**, 451–456.

Bensch, S., Bengtsson, G. & Akesson, S. (2006) Patterns of stable isotope signatures in willow warbler (*Phylloscopus trochilus*) feathers collected in Africa. *Journal of Avian Biology*, **37**, 323–330.

Bond, A.L. & Jones, I.J. (2009) A practical introduction to stable-isotope analysis for seabird biologists: approaches, cautions, and caveats. *Marine Ornithology*, **37**, 183–188.

Bowen, G.J., Wassenaar, L.I. & Hobson, K.A. (2005) Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia*, **143**, 337–348.

Cerling, T.E., Ayliffe, L.K., Dearing, M.D., Ehleringer, J.R., Passey, B.J., Podlesak, D.W., Torregrossa, A.&. & West, A.G. (2007) Determining biological turnover using stable isotopes: the reaction progress variable. *Oecologia*, **151**, 175–189.

Chamberlain, C.P., Bensch, S., Feng, X., Akesson, S. & Anderson, T. (2000) Stable isotopes examined across a migratory divide in Scandinavian willow warblers (*Phylloscopus trochilus trochilus* and *Phylloscopus trochilus acredula*) reflect their African winter quarters. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 43–48.

Cullen, S.A., Jehl, J.R. Jr & Nuechterlein, G.L. (1999) *Eared Grebe* (*Podiceps nigricollis*)*, the Birds of North America Online* (ed. A. Poole). Cornell Lab of Ornithology, Ithaca. Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/433.

Farquhar, G.D., Henry, B.K. & Styles, J.M. (1997) A rapid on-line technique for determination of oxygen isotope composition of nitrogen-containing organic matter and water. *Rapid Communications in Mass Spectrometry*, **11**, 1554–1560.

Fox, A.D., Hobson, K.A. & Kahlert, J. (2009) Isotopic evidence for endogenous protein contributions to greylag goose (*Anser anser*) flight feathers. *Journal of Avian Biology*, **40**, 108–112.

Fry, B., Brand, W., Mersch, F.J., Tholke, K. & Garritt, R. (1992) Automated analysis system for coupled $\delta^{13}C$ and $\delta^{15}N$ measurements. *Analytical Chemistry*, **64**, 288–291.

Gannes, L.Z., Martinez del Rio, C.M. & Koch, P. (1998) Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comparative Biochemical Physiology, Part A*, **119**, 725–737.

Hart, E.A. & Lovvorn, J.R. (2005) Patterns of macroinvertebrate abundance in inland saline wetlands: a trophic analysis. *Hydrobiologia*, **541**, 45–54.

Hebert, C.E. & Wassenaar, L.I. (2001) Stable nitrogen isotopes in waterfowl feathers reflect agricultural land use in western Canada. *Environmental Science & Technology*, **35**, 3483–3487.

Hobson, K.A. (1990) Stable isotope analysis of marbled murrelets: evidence for freshwater feeding and determination of trophic level. *Condor*, **92**, 897–903.

Hobson, K.A. & Clark, R.G. (1992) Assessing avian diets using stable isotopes I: turnover of 13C in tissues. *Condor*, **94**, 181–188.

Hobson, K.A. & Clark, R.G. (1993) Turnover of 13C in cellular and plasma fractions of blood: implications for nondestructive sampling in avian dietary studies. *Auk*, **110**, 638–641.

Hobson, K.A. & Wassenaar, L.I. (2008) *Tracking Animal Migration with Stable Isotopes*. Elsevier Inc., Amsterdam.
Jehl, J.R. Jr (1988) Biology of the Eared Grebe and Wilson's Phalarope in the nonbreeding season. *Studies in Avian Biology*, **112**, 74.
Jehl, J.R. Jr (1997) Cyclical changes in body composition in the annual cycle and migration of the Eared Grebe *Podiceps nigricollis*. *Journal of Avian Biology*, **28**, 132–142.
Jehl, J.R. Jr (2007) Why do grebes leave hypersaline lakes in autumn. *Waterbirds*, **30**, 112–115.
Jehl, J.R. Jr & Henry, A.E. (2010) Biology of the eared grebe in the postbreeding migration. *Wilson Journal of Ornithology*, **122**, 217–227.
Jehl, J.R. Jr, Henry, A.E. & Bond, S.I. (1998) Sexing eared grebes by bill measurements. *Colonial Waterbirds*, **21**, 98–100.
Jehl, J.R. Jr, Henry, A.E. & Ellis, H.I. (2003) Optimizing migration in a reluctant and inefficient flier. *Avian Migration* (eds P. Berthold, E. Gwinner & E. Sonnenschein). pp. 199–209, Springer-Verlag, Berlin.
Kelly, J.F. (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology*, **78**, 1–27.
Kojadinovic, J., Richard, P., Le Corre, M., Cosson, R.P. & Bustamante, P. (2008) Effects of lipid extraction on $\delta^{13}C$ and $\delta^{15}N$ values in seabird muscle, liver and feathers. *Waterbirds*, **31**, 169–178.
Mahoney, S.A. & Jehl, J.R. Jr (1985) Avoidance of salt-loading by a diving birds at a hypersaline and alkaline lake: Eared Grebe. *Condor*, **87**, 389–397.
Martinez del Rio, C., Wolf, N., Carleton, S.A. & Gannes, L.Z. (2009) Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews*, **84**, 91–111.
Moller, A.P., Hobson, K.A., Mousseau, T.A. & Peklo, A.M. (2006) Chernobyl as a population sink for barn swallows: tracking dispersal using stable-isotope profiles. *Ecological Applications*, **16**, 1696–1705.
Nielson, K.E. & Bowen, G.J. (2010) Hydrogen and oxygen in brine shrimp chitin reflect environmental water and dietary isotopic composition. *Geochimica et Cosmochimica Acta*, **74**, 1812–1822.
Sears, J., Hatch, S.A. & O'Brien, D.M. (2009) Disentangling effects of growth and nutritional status on seabird stable isotope ratios. *Oecologia*, **159**, 41–48.
Storer, R.W. & Jehl, J.R. Jr (1985) Moult patterns and moult migration in the Black-necked Grebe. *Ornis Scandinavica*, **16**, 253–260.
Wassenaar, L.I. & Hobson, K.A. (2003) Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. *Isotopes in Environmental Health Studies*, **39**, 211–217.
Wassenaar, L.I. & Hobson, K.A. (2006) Stable-hydrogen isotope heterogeneity in keratinous materials: mass spectrometry and migratory wildlife tissue subsampling strategies. *Rapid Communications in Mass Spectrometry*, **20**, 2505–2510.
West, J.B., Bowen, G.J., Cerling, T.E. & Ehleringer, J.R. (2006) Stable isotopes as one of nature's ecological recorders. *Trends in Ecology and Evolution*, **21**, 408–414.
Wunder, M.B. (2007) *Geographic structure and dynamics in mountain plover*. PhD Dissertation. Colorado State University, Fort Collins.
Wunder, M.B. (2010) Using isoscapes to model probability surfaces for determining geographic origins. *Isoscapes: Isotope Mapping and its Applications* (eds G. Bowen, J. West, K. Tu & T. Dawson). pp. 251–270, Springer-Verlag, New York.
Wunder, M.B. & Norris, D.R. (2008a) Improved estimates of certainty in stable isotope-based methods for tracking migratory animals. *Ecological Applications*, **18**, 549–559.
Wunder, M.B. & Norris, D.R. (2008b) Analysis and design for isotope-based studies of migratory birds. *Tracking Animal Migration With Stable Isotopes* (eds K.A. Hobson & L.I. Wassenaar). pp. 107–128, Elsevier Inc, Amsterdam.
Wunder, M.B., Kester, C.L., Knopf, F.L. & Rye, R.O. (2005) A test of geographic assignment using isotope tracers in feathers of known origin. *Oecologia*, **144**, 607–617.
Yohannes, E., Valcu, M., Lee, R.W. & Kempenaers, B. (2010) Resource use for reproduction depends on spring arrival time and wintering area in an arctic breeding shorebird. *Journal of Avian Biology*, **41**, 580–590.

Received 12 March 2011; accepted 5 April 2012
Handling Editor: Stuart Bearhop