Articles

High variation reduces the value of feather stable isotope ratios in identifying new wintering areas for aquatic warblers *Acrocephalus paludicola* in West Africa

Steffen Oppel, Deborah J. Pain, Jeremy A. Lindsell, Lars Lachmann, Ibrahima Diop, Cosima Tegetmeyer, Paul F. Donald, Guy Anderson, Christopher G. R. Bowden, Franziska Tanneberger and Martin Flade

S. Oppel (steffen.oppel@rspb.org.uk), D. J. Pain, J. A. Lindsell, L. Lachman, P. F. Donald, G. Anderson and C. G. R. Bowden, Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire, SG19 2DL, UK. DJP also at: Wildfowl and Wetlands Trust, Slimbridge, Gloucestershire, GL2 7BT, UK. LL also at: Polish Society for the Protection of Birds (OTOP), Ul. Odrowaza 24, PL 05-270 Marki, Poland. – I. Diop, Parc National des Oiseaux du Djoudj, SN BP80, St Louis, Senegal. – C. Tegetmeyer, Inst. of Botany and Landscape Ecology, Univ. of Greifswald, Grimmer Str. 88, DE-17487 Greifswald, Germany. – M. Flade, Brandenburg State Office for Environment, Dept Large Protected Areas and Regional Development, Tramper Chaussee 2, DE-16225 Eberswalde, Germany.

Stable isotope analysis of feathers can be useful in the study of seasonal interactions and migratory connectivity in birds. For the Palaearctic–African migration system, however, the lack of isotope data from feathers of known origin in Africa renders the geographic assignment of birds captured on European breeding grounds to potential wintering areas problematic. Rectrices of the threatened aquatic warbler *Acrocephalus paludicola* grown in Africa were sampled across six European countries to assess whether birds in different breeding populations shared similar isotopic signatures and so were likely to have wintered in the same region in Africa. Freshly grown feathers of aquatic warblers collected at the only known wintering site in Senegal showed high variation in carbon, nitrogen, and hydrogen isotope ratios. Due to similarly high variation in isotope ratios of African-grown feathers within all breeding populations, it was not possible to determine whether different populations wintered in different regions. However, isotope signatures of 20% of birds captured on European breeding grounds fell outside the range of those captured in Senegal, suggesting a wider wintering distribution than is currently known. We therefore assessed whether the origin of these feathers could be estimated by trying to establish isotopic gradients across sub-Saharan West Africa. Feathers of three ecologically similar surrogate species were sampled from wetlands across a 3000 km east-west and a 2000 km north–south transect. Within-site variation in feather isotope ratios was frequently larger than the difference predicted by gradients across West Africa. Thus, predicting the origin of individual feathers using single-isotope gradients was not reliable. The large within-site variability of feather isotope ratios of a habitat specialist species like the aquatic warbler indicates that using feather isotope ratios will require large sample sizes from many locations, and may thus not be an efficient tool in identifying wintering areas of Palaearctic–African migrants.

Carry-over effects between seasons and the potential for non-breeding mortality to act as a limiting factor for bird populations render the identification of non-breeding areas very important for the conservation of migratory birds (Webster et al. 2002, Newton 2004, 2006, Norris and Marra 2007). Over the last two decades, the analysis of stable isotope ratios of bird tissues has been developed into a widely used tool for tracking migratory birds across continents (Marra et al. 1998, Kelly et al. 2002, Norris et al. 2005). Stable isotope ratios of terrestrial food-webs, and ultimately bird tissues, vary across spatial scales due to a number of biogeochemical processes, and several isotope gradients exist that can be utilised for the geographic assignment of birds (Hobson 1999, 2005, Inger and Bearhop 2008).

Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have been used to infer migration patterns of several Palaearctic–African migrants (Chamberlain et al. 2000, Evans et al. 2003, Yohannes et al. 2005, 2007, Bensch et al. 2006, Procházka et al. 2008). The use of hydrogen isotopes (δD) may also be valuable in understanding Palaearctic migration systems (Hobson et al. 2004b, 2009a, Bowen et al. 2005b, 2009), but attempts to identify origins of feathers grown in Africa with hydrogen isotopes have so far not been successful (Møller and Hobson 2004, Pain et al. 2004).

While δD in precipitation varies systematically across continents due to atmospheric processes (Craig 1961, Bowen et al. 2005b), the ratios of $\delta^{13}C$ and $\delta^{15}N$ vary among ecosystems as a result of different biochemical

processes during carbon and nitrogen fixation (Peterson and Fry 1987). Some of these differences are large enough to form a predictable gradient for geographic assignment (e.g. freshwater vs marine nutrients, C_3 vs C_4 derived nutrients). However, the assignment of birds to geographic regions based on $\delta^{13}C$ and $\delta^{15}N$ of bird tissues generally requires tissues of known origin to assign those of unknown origin reliably (Wunder et al. 2005, Rocque et al. 2006, Kelly et al. 2008, Oppel and Powell 2008). Such isotopic base-maps or 'isoscapes' are currently lacking for sub-Saharan Africa (Pain et al. 2004, Yohannes et al. 2007). In addition, the scarcity of precipitation sampling stations in western Africa (Bowen et al. 2005b) renders it unclear whether reliable hydrogen isotope gradients exist in this area, and comprehensive feather sampling schemes across Africa are therefore needed to establish whether stable isotopes can be useful to identify the winter distribution of Palaearctic migrants.

Many Palaearctic migrants have suffered population declines in recent decades (Berthold et al. 1998, Sanderson et al. 2006). One of these migrants is the aquatic warbler *Acrocephalus paludicola*, which has been classified as globally threatened ('Vulnerable') due to its limited area of occupancy and recent population declines (Flade and Lachmann 2008, Tanneberger et al. 2008, BirdLife International 2009). The species breeds at a number of widely separated wetland sites from western Poland to southwest Siberia, and is considered a habitat specialist preferring wet open sedge marshes both during and outside the breeding season (Flade and Lachmann 2008). Its winter distribution is very poorly known, and concerns over possible threats on wintering areas triggered research to identify wintering areas using predictive habitat modelling (Walther et al. 2007, Buchanan et al. 2011) and stable isotopes (Pain et al. 2004). The stable isotope study concluded that breeding populations in western Poland, Belarus and the Ukraine might winter at different sites in sub-Saharan Africa, but due to the lack of isotopic baselines from Africa it was not possible to identify potential wintering regions (Pain et al. 2004).

Several approaches contributed to the discovery of the only currently known wintering population in western Africa (in and around Djoudj National Park, Senegal) in 2007 (Flade et al. 2011), which subsequently provided the opportunity to collect freshly grown feathers on the wintering grounds. Using these feathers as an isotopic baseline against which feather isotope ratios from breeding birds could be compared might yield an assessment of 1) whether birds breeding in the same population all wintered at the same site, 2) whether wintering sites outside the known area in Senegal were likely to exist.

Our goal in this study was to extend the work conducted by Pain et al. (2004) to sample aquatic warblers more comprehensively across the breeding range, and to estimate the proportion of European breeding birds that showed feather isotope signatures consistent with those of birds captured at the only known wintering site in Senegal. We also collected feathers from three 'surrogate' species with similar habitat requirements as aquatic warblers at 15 wetland sites along a 3000 km east-west and a 2000 km north-south transect in sub-Saharan West Africa to identify isotopic gradients in bird feathers for an area encompassing the total range of historic aquatic warbler winter sightings

(Schäffer et al. 2006). We assessed whether the stable isotope ratios in feathers of these species carried an identifiable geographic signature that could be used to identify alternative wintering areas of the aquatic warbler. The results also provide an assessment of the likely application of stable isotope analysis in future work of a wide range of Palaearctic migrants.

Methods

Choice of tissue

Palaearctic migrants generally undergo post-breeding moult after having crossed the Sahara, and *Acrocephalus* warblers in particular moult remiges or rectrices in October–December in non-breeding areas south of the Sahara (Aidley and Wilkinson 1987, Bensch et al. 1991, Hedenström et al. 1993). Like related *Acrocephalus* warblers, most aquatic warblers in Africa may have completed moult before mid-December (Bensch et al. 1991, C. Tegetmeyer unpubl.). Rectrices are moulted from the centre outwards (Svensson 1992), and outer rectrices have been sampled from related *Acrocephalus* warblers for isotope work (Yohannes et al. 2008a, b). Outer rectrices were sampled from aquatic warblers both in Africa and on their European breeding grounds, because they reflect the isotope ratios of areas where they were grown in sub-Saharan Africa during the non-breeding season, and because removal of a tail feathers affects flight performance less than the removal of a primary. We ascertained that feathers collected on breeding grounds showed significant wear indicative of completed long-distance migration. By comparing feathers sampled in Europe with freshly moulted feathers that had been accidentally lost and replaced on or near the breeding grounds, we estimated that fewer than 4% of sampled feathers may had been renewed after birds had left the wintering areas.

Sampling aquatic warblers on breeding grounds

Adult aquatic warblers were caught using mist-nets and tape-lures of male song between early May and late July from 1999 to 2009 at 23 sites across the entire known breeding range of the species in central and eastern Europe and southwest Siberia by members of the BirdLife International Aquatic Warbler Conservation Team (Pain et al. 2004, Flade et al. 2011). Sampling locations were assigned to five discrete 'breeding populations' which were separated by > 200 km without any suitable breeding habitat in between (Flade and Lachmann 2008). The 'Pomeranian population' was sampled at three sites in western Poland (Rozwarowo, Karsiborska Kepa, Krajnik); the 'central population' was sampled at three sites in eastern Poland (Biebrza, Bagno Wizna, Chelm), four sites in Belarus (Servech, Sporovo, Zvanets, Dikoe), three sites in Lithuania (Dreverna, Kliosiai, Sausalviai), and one site in the western Ukraine (upper Pripyat); the 'Ukrainian population' was sampled at two sites in the central Ukraine (Supoy, Uday); the 'southern population' was sampled at one site in Hungary (Hortobagy); and the 'west-Siberian population' was sampled at two sites in Russia, northern Omsk Oblast (Lake Busli, Yarovskoe). Sample sizes are presented in Table 1.

One of the outer rectrices was taken from each bird and stored in a self-sealing, polythene bag prior to analysis. Because aquatic warblers cannot be reliably aged by plumage characteristics after their first complete moult, samples from breeding areas included both one-year old and older birds. Sexing of aquatic warblers in the field is only reliable at certain stages of the breeding season, and we therefore do not have sex information for most individuals. However, the majority of the captured birds were singing males attracted by tape lures, and females comprised probably <25% of our dataset.

Sampling aquatic warblers on wintering grounds

After the discovery in 2007 of a wintering site in and around the Djoudj National Park, Senegal (16.4°N, 16.3°W), aquatic warblers were captured between mid-December and mid-March each winter from 2006/2007 to 2008/2009 using mist-nets erected in various habitat types. We collected freshly grown rectrices from birds that had recently undergone complete moult, and assumed that the feathers had been grown near the capture location. It is possible that aquatic warblers may have moulted in nearby wetland areas along the Senegal River or in southern Mauritania, and moved to Djoudj as the progressing dry season rendered other wetlands unsuitable (Aidley and Wilkinson 1987, Bensch et al. 1991, Hedenström et al. 1993).

Aquatic warblers were captured at five sub-sites within the Djoudj area that were approximately 5–10 km apart: 'Grand Lac' was a site with saline influence and vegetation dominated by *Scirpus littoralis*; 'Tiguet' was brackish with vegetation dominated by *Oryza longistaminata*; 'Debi' and 'Croco' were also brackish and dominated by *Eleocharis mutata*; and 'Diadième' was brackish with *Eleocharis mutata*, *Scirpus* sp. and *Sporobolus robustus* contributing fairly equally to the vegetation. *Sporobolus robustus* was present at each sub-site. Aquatic warblers were only captured in water-logged open grassy marshes with very little or no emergent vegetation such as bushes, trees, or *Typha australis* (Flade et al. 2011).

At each sub-site, mist net arrays with a total length between 50 and 335 m were erected and birds driven from the surrounding 1–6 ha into the nets using a line of 2–12 people dragging ropes across the vegetation (Flade et al. 2011). Sexing of aquatic warblers in winter is not possible based on plumage characteristics, and genetic sexing of a sub-sample of 59 birds was performed (Vogel 2009). Males (54%) and females (46%) contributed fairly equally to the sample that was genetically sexed (Vogel 2009), and because there was no evidence for geographical or habitat segregation between sexes we pooled male and female feathers for analysis.

Feather collection of surrogate species

During the winter months, aquatic warblers have historically been recorded in very small numbers between southwest Mauritania, Mali, and northern Ghana (Schäffer et al. 2006). In 2010, no regular wintering areas of aquatic warblers were known outside the Djoudj area, and it was therefore not possible to sample aquatic warbler feathers from other wintering areas. Instead, we collected rectrices from three resident species in sub-Saharan West Africa to establish isotopic gradients across the potential wintering range of aquatic warblers. Three widespread passerine species preferring wetland habitat similar to that of aquatic warblers were selected to serve as surrogate species. Two of those species (greater swamp warbler *Acrocephalus rufescens*, lesser swamp warbler *A. gracilirostris*) are considered as swamp specialists inhabiting reed (*Phragmites* sp.) and cattail (*Typha* sp.) stands (Urban et al. 1997, MacLean et al. 2003), whereas winding cisticola (*Cisticola galactotes*) is considered a swamp opportunist that may also occur outside of wetlands in intermediate grasslands (Urban et al. 1997, MacLean et al. 2003, Gottschalk et al. 2007). These species are known to be resident (Urban et al. 1997, Borrow and Demey 2001) and we therefore assumed that their feathers were grown in the vicinity of their capture location. They tend to breed during the annual rains from April to October (Aidley and Wilkinson 1987, Urban et al. 1997), and perform a complete moult after the breeding season (Dowsett-Lemaire 1997). Thus, their feathers would have grown within two months of the arrival and moult of aquatic warblers in the region (Aidley and Wilkinson 1987).

We collected feathers at 15 sites along an east west transect spanning from 16°W to 13°E, and along a north-south transect spanning from 5°N to 16°N (Appendix 1) during January and February 2004. Birds were sampled with mist-nets in habitats that appeared to be potentially suitable for aquatic warblers, and hence we assumed that surrogate species could reflect the isotopic variation that would be expected in aquatic warbler feathers grown at each site.

Feather isotope analysis

Each feather was removed from its sample bag and washed in 0.25M sodium hydroxide solution followed by two separate washes in purified water. The washed feathers were placed in clean screw top vials and placed in a drying oven at 50°C overnight. After drying, feathers were clipped into fine (up to 2 mm in length) sections using surgical scissors. Results of isotope analyses are reported in delta (δ) notation relative to international standards (Vienna PeeDee Belemnite for C, atmospheric nitrogen for N, Vienna standard mean ocean water for D) according to the following equation: $\delta X = ([R_{sample}/R_{standard}]-1) \times 1000$, with X denoting either ^{13}C, ^{15}N, or ^{2}D, and R representing the ratio of ^{13}C/^{12}C, ^{15}N/^{14}N, or ^{2}H/^{1}H, respectively.

All isotope analyses were conducted at Iso-Analytical, Cheshire, UK. Carbon and nitrogen isotope analysis was undertaken by Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS) using a Europa Scientific 20-20 IRMS. Reference material was NBS-1577B (powdered bovine liver, $\delta^{13}C_{V-PDB} = -21.6‰$, $\delta^{15}N_{Air} = 7.7‰$), and NBS-1577B, IA-R045 (ammonium sulphate, $\delta^{15}N_{Air} = -4.7‰$), IA-R046 (ammonium sulphate, $\delta^{15}N_{Air} = 22.0‰$), IA-R005 (beet sugar, $\delta^{13}C_{V-PDB} = -26.0‰$) and IA-R006 (cane sugar, $\delta^{13}C_{V-PDB} = -11.6‰$) were run as quality control check samples during sample analysis. The standard deviation of replicate measurements of these materials run concurrently with samples was 0.09‰ for δ^{13}C and 0.11‰ for δ^{15}N.

For hydrogen isotope analysis, 1 mg amounts of sample material were weighed into silver capsules (5×8 mm). The filled capsules were left open for a period of 4–10 d to allow the exchangeable hydrogen in the feather keratin to equilibrate fully with the moisture in the laboratory air, and were sealed just prior to analysis. Hydrogen isotope analysis was conducted using EA-Pyr-IRMS (elemental analyser pyrolysis isotope ratio mass spectrometry). The reference material used for δD analysis was IA-R002 (mineral oil, $\delta D_{V\text{-SMOW}} = -111.2‰$). Feather samples were analysed with 20% duplication, and check samples of IA-R002 and IAEA-CH-7 (polyethylene foil, $\delta D_{V\text{-SMOW}} = +100.3‰$) were analysed along with the feather samples as quality control checks. The standard deviation of replicate measurements of these reference materials was 2.0‰ for δD.

Some of the hydrogen in feather keratin can exchange with atmospheric hydrogen (Chamberlain et al. 1997), potentially leading to complications when feather isotope measurements are compared across different laboratories (Smith et al. 2009, Wunder et al. 2009). Because standard methods to estimate δD values of the non-exchangeable fraction of feather keratin had not been published by the time our feather samples from 1999–2002 were analysed (Wassenaar and Hobson 2003, Bowen et al. 2005a, Sauer et al. 2009), we did not have measurements of non-exchangeable δD for those samples. Therefore, we used δD values of total feather hydrogen rather than just the non-exchangeable fraction in this study. All samples were analysed at the same laboratory where they received equal treatment and air equilibration for similar times. The reproducibility of feather hydrogen analysis was very high, with a total feather δD standard deviation of 2.5‰ for six aquatic warbler feathers that were analysed on three separate occasions between April 2000 and July 2002.

Feather samples analysed after 2003 (n $= 128$) were corrected for exchangeable hydrogen by using the comparative equilibration method (Wassenaar and Hobson 2003). Calibrated keratin isotope reference materials (BWB-II, whale baleen, non-exchangeable $\delta D_{V\text{-SMOW}} = -108 \pm 4‰$; and eggshell membrane, non-exchangeable $\delta D_{V\text{-SMOW}}z = -93.8 \pm 2.3‰$) were analysed within each batch of feather samples. These reference materials received identical times of exposure to laboratory air as feather samples. Because samples analysed with the comparative equilibration method indicated an almost perfect correlation ($R^2 = 0.99$, b $= 1.06 \pm 0.01$) between total feather and non-exchangeable fraction δD values for four different analytical runs over a time period of three years, we are confident that the use of total feather hydrogen δD allowed us to make valid comparisons among our samples. Researchers aiming to use our δD values for comparison with samples analysed elsewhere are cautioned that direct comparisons of total feather δD may not be valid. We only report total feather δD.

Statistical analysis

We first assessed whether there was significant temporal variation in isotope ratios of feathers collected in different years. For each population in which >5 aquatic warbler feathers were collected in each of at least three years (Pomeranian, central, and Ukrainian breeding populations;

Senegal winter population), we assessed whether between-year variation in $\delta^{13}C$, $\delta^{15}N$, and δD was greater than within-year variation using analyses of variance (ANOVA).

Because sampling feathers from different years may have confounded our site comparisons, we included year as a factor in all analyses comparing the isotopic variation among locations. ANOVA was also used to assess whether within-site variation in feather $\delta^{13}C$, $\delta^{15}N$, and δD was smaller than between-site variation for aquatic warblers captured at five different sub-sites within the Djoudj area in Senegal. Similarly, we assessed whether there was any evidence that birds captured in different European breeding populations differed from each other or from the known wintering population in their mean $\delta^{13}C$, $\delta^{15}N$ and δD feather signatures. Because the winter location was only discovered in 2007, but many of our feathers in Europe were collected prior to 2005, the analysis comparing European- and African-collected feathers was repeated for a subset of data that reflected the same moult cycle (winter 2008/2009).

Cluster analysis of feather isotopes
To quantify the proportion of adult birds captured on breeding grounds in Europe that had feather isotope ratios consistent with those collected at the single known wintering area in Senegal, the entire adult winter-grown rectrix dataset (including feathers collected in Europe and Africa) was divided into several clusters based on $\delta^{13}C$, $\delta^{15}N$, and δD. The number of clusters will influence the proportion of correct assignments to a cluster (Kelly et al. 2008), and because no a priori hypothesis was available about how many isotopic clusters would exist, the number of clusters was arbitrary. We therefore optimized the number of clusters to achieve best possible classification by using an algorithmic Random Forest model (Breiman 2001, Cutler et al. 2007) with 1200 trees and all three isotope ratios as variables tried at each split. The number of clusters that yielded the lowest out-of-bag error rate in classification was then selected, and the respective Random Forest model was used to assign each feather to a cluster based on its isotopic signature. We identified those clusters that did not include any feathers from the wintering area in Senegal, and calculated the proportion of feathers from each breeding population that fell into those clusters to determine the proportion of birds in each population that showed a feather isotope signature inconsistent with those from the known wintering site in Senegal.

Isotope gradients in surrogate species
Because no wintering sites of aquatic warbler were known outside Senegal, it was not possible to apply robust geographic assignment techniques (Wunder and Norris 2008) to the feathers of unknown origin sampled from birds captured on European breeding grounds. We assessed whether there may be scope for such assignments in the future by determining whether predictable isotope gradients existed among feathers of four passerines (aquatic warbler and three surrogate species) we sampled across sub-Saharan West Africa. Different generalised linear mixed models were fitted to each geographical gradient (latitude and longitude) and each isotope ($\delta^{13}C$, $\delta^{15}N$, δD), and in each model

'species' was included as a random effect to account for error associated with sampling different species (Gillies et al. 2006, Bolker et al. 2009). For each isotopic gradient we constructed one model including the geographic predictor (latitude or longitude), and one model without that predictor (null model, including the same random effect), and estimated slope parameters and AIC weights to evaluate the amount of evidence for the specific isotope gradient (Burnham and Anderson 2002, Bolker et al. 2009). The deviance of the model including the geographic predictor was divided by the deviance of the null model to estimate the proportion of variation explained by the inclusion of the geographic predictor and thus obtain an estimate of model performance (Gillies et al. 2006).

Predicting the wintering area of European aquatic warblers

The simultaneous use of multiple isotopes has improved geographic assignment of several bird species (Chamberlain et al. 1997, Wunder et al. 2005, Oppel and Powell 2008). We therefore assessed whether a predictive model based on carbon, nitrogen, and hydrogen isotope ratios simultaneously could delineate the wintering area of aquatic warblers in West Africa. Boosted regression tree models were used, because these models are widely known for their high predictive ability (Leathwick et al. 2006, Elith et al. 2008, Elith and Graham 2009). The models fitted latitude and longitude as dependent variables in two separate analyses, and were trained with feather isotope ratios of aquatic warblers and the three surrogate species caught in West Africa. Besides the three isotopes, 'species' was also used as predictor to account for species-specific variation. We used a learning rate of 0.005 for latitude and 0.001 for longitude, a tree complexity of 3 (allowing 3-way interactions) and a bag fraction of 0.64 in the R-package 'gbm' as described by Elith et al. (2008), and present the proportional contribution of predictors to evaluate which isotope was the most useful for spatial prediction. After evaluating model performance by the correlation between observed and predicted values in cross-validation of the training data set, the latitude and longitude of aquatic warbler feathers obtained on breeding grounds in Europe was predicted. We then plotted the predicted origin of those feathers on a map of Africa and visually assessed whether the results were broadly consistent with the conclusions provided by the cluster analysis above and other predictions of potential wintering areas of the species (Walther et al. 2007, Buchanan et al. 2011).

Results

Temporal variation in feather signatures

There was no significant variation in $\delta^{13}C$ and δD among feathers collected during three winters from birds wintering in Djoudj National Park, Senegal (ANOVA $\delta^{13}C$: $F_{2,88} = 0.50$, $p = 0.61$; δD: $F_{1,59} = 0.23$, $p = 0.63$). Average feather $\delta^{15}N$ in 2008 was 1.1 and 0.7‰ higher than in 2007 and 2009, respectively ($F_{2,89} = 3.43$, $p = 0.04$).

There was no significant annual variation in $\delta^{13}C$ for three breeding populations in Europe (Pomeranian, central, Ukrainian) where we had collected >5 aquatic warbler

feathers in each of at least three years ($F_{5,255} = 1.83$, $p = 0.11$). In 2003, feather $\delta^{15}N$ was on average 2.4‰ lower than in the other five years from which we had samples ($F_{5,255} = 8.71$, $p < 0.001$). Average δD varied from -76.6‰ in 2000 to -67.7‰ in 2002 ($F_{4,189} = 8.71$, $p < 0.001$), with ranges exceeding 25‰ in every year.

Feathers collected on breeding grounds

Variation in aquatic warbler rectrix $\delta^{13}C$ and $\delta^{15}N$ was not greater among breeding populations than the variation within each population ($\delta^{13}C$: $F_{4,265} = 1.38$, $p = 0.24$; $\delta^{15}N$: $F_{4,265} = 1.26$, $p = 0.29$, Fig. 1). For δD, birds captured in the Ukraine and Russia had hydrogen isotope ratios on average ~ 10‰ depleted compared to birds captured in the central population (Table 1, $F_{3,189} = 4.30$, $p = 0.04$). There was little evidence that different breeding populations grew rectrices in isotopically distinct regions in Africa (Table 1).

Feathers collected on wintering grounds

Total variation in isotope ratios from aquatic warbler feathers that had been recently grown in or near the Djoudj area, Senegal, was >5‰ for $\delta^{15}N$ and >10‰ for $\delta^{13}C$ (Table 2). Birds captured at different sub-sites of the Djoudj area differed statistically in their average $\delta^{15}N$ signatures ($F_{4,89} = 4.89$, $p = 0.001$), but the magnitude of the between-sub-site differences was small (1.2‰) compared to the variation within each sub-site (4‰). Variation in $\delta^{13}C$ and δD was larger within sub-sites than among sub-sites ($\delta^{13}C$: $p = 0.08$; δD: $p = 0.59$, Table 2). Both males and females were captured at each sub-site, and there was no difference between male and female feather isotope ratios ($\delta^{13}C$: $F_{1,46} = 2.50$, $p = 0.12$; $\delta^{15}N$: $F_{1,46} = 0.02$, $p = 0.88$, δD: $F_{1,45} = 1.94$, $p = 0.17$)

The variation in feather isotope ratios of birds captured in Senegal was significantly lower than the variation in feathers from all European breeding populations taken
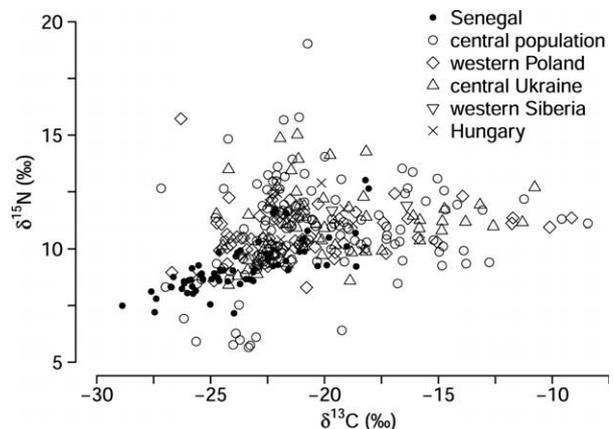
Figure 1. Aquatic warbler tail feather $\delta^{13}C$ and $\delta^{15}N$, reflecting isotope ratios of moulting areas in sub-Saharan West Africa. Filled symbols represent samples collected at the known wintering site in Senegal, unfilled symbols represent samples collected from birds on breeding grounds in Europe and western Siberia. See text for spatial delineation of sub-populations.

Table 1. Carbon, nitrogen, and hydrogen isotope ratios (in ‰) of adult aquatic warbler rectrices collected May–July 1999–2009 from birds at 15 sites representing five discrete breeding populations (see text for spatial definition of breeding populations). Feather isotope signatures reflect isotope ratios of moulting and/or wintering grounds in Africa. δD was not analysed in feathers from the southern population.

Isotope	Population	n	Mean	SD	Min	Max
$\delta^{13}C$	Pomeranian	66	−21.0	3.7	−26.7	−9.2
	Central	137	−20.5	3.5	−27.2	−8.4
	Ukrainian	59	−19.8	3.4	−24.7	−10.8
	Southern	11	−21.4	2.2	−25.6	−18.3
	Siberian	2	−18.0	2.3	−19.7	−16.4
$\delta^{15}N$	Pomeranian	66	10.7	1.1	8.3	15.7
	Central	137	10.7	2.0	5.7	19.0
	Ukrainian	59	11.1	1.5	8.4	15.0
	Southern	11	11.2	1.3	8.9	12.9
	Siberian	2	11.8	0.2	11.7	11.9
δD	Pomeranian	59	−70.3	9.2	−97.0	−50.9
	Central	98	−71.9	9.7	−93.9	−45.9
	Ukrainian	38	−75.6	8.9	−86.3	−48.7
	Siberian	2	−75.6	0.9	−76.3	−75.0

together ($\delta^{13}C$: $F_{1,377} = 78.74$, $p < 0.001$; $\delta^{15}N$: $F_{1,378} = 71.47$, $p < 0.001$; δD: $F_{1,257} = 34.04$, $p < 0.001$; Table 1 and 3, Fig. 1). For $\delta^{13}C$ and $\delta^{15}N$, this pattern was also apparent when we used only feathers from a single moult cycle with equal sample sizes from breeding and wintering areas ($\delta^{13}C$: $F_{1,67} = 16.95$, $p < 0.001$; $\delta^{15}N$: $F_{1,67} = 25.81$, $p < 0.001$); no data are available for δD variation in a single moult season.

Classification of feathers into clusters

All aquatic warbler rectrices reflecting the isotopic signature of African moult sites were classified into four groups, which resulted in a highly accurate assignment using a Random Forest algorithm. With $\delta^{13}C$, $\delta^{15}N$, and δD as classifying variables, and by forcing the algorithm to utilise all three variables, the out-of-bag error rate for classification was 0.4% (1 out of 259 feathers misclassified). Using a larger number of clusters increased the out-of-bag error rate from 3% (6 clusters) to 7.7% (8 or 9 clusters), and we therefore chose four clusters. These four isotope clusters represent statistically optimized units that do not necessarily

Table 2. Isotopic variation in aquatic warbler rectrices captured at five different sub-sites within the wintering area of Djoudj National Park, Senegal, between 2007 and 2009. Feather isotope signatures reflect isotope ratios (in ‰) at a moulting location in sub-Saharan Africa, presumably close to the capture location in Senegal. Sub-sites were <50 ha in size, separated by 2–10 km from each other, and within an area <1000 km².

Isotope	Sub-site	n	Mean	SD	Min	Max
$\delta^{13}C$	Diadième	17	−24.6	1.5	−26.6	−21.6
	Croco	19	−23.5	2.2	−26.7	−18.5
	Debi	7	−24.0	1.7	−26.6	−22.0
	Tiguet	37	−24.4	2.2	−28.9	−19.8
	Grand Lac	15	−22.7	2.7	−26.0	−18.2
$\delta^{15}N$	Diadième	17	8.9	0.6	7.5	9.7
	Croco	19	9.6	0.5	8.7	10.4
	Debi	7	9.6	0.8	8.8	11.4
	Tiguet	38	8.9	0.8	7.2	10.8
	Grand Lac	15	9.7	1.4	8.1	13.0
δD	Diadième	6	−72.6	11.2	−86.4	−56.0
	Tiguet	32	−69.4	5.1	−78.6	−58.4
	Grand Lac	15	−68.9	5.2	−81.6	−60.3

correspond to geographic areas where the respective feathers had grown.

All the feathers collected on the wintering area in Senegal fell into three of the four isotope clusters, leaving one isotope cluster that was inconsistent with any feathers collected in Senegal. In the Ukrainian population, 40% of analysed feathers (n = 38) were assigned to the isotope cluster that was not recorded in Senegal, in the central population 17% (n = 99), and in the Pomeranian population 14% (n = 59). Thus, the proportion of feathers falling outside the Senegal clusters increased from west to east across the European breeding populations. Overall, 20% of 197 aquatic warbler feathers sampled on European breeding grounds therefore had isotope signatures that were inconsistent with any of the 61 birds sampled at the only known wintering area in Senegal. The isotopic cluster that contained 20% of European but none of the Senegalese birds was depleted by about 10‰ in δD compared to other clusters, but overlapped widely with other clusters in $\delta^{13}C$ and $\delta^{15}N$. The feathers in this cluster were collected and analysed in different years, precluding the possibility of an aberrant laboratory run producing these results.

Feathers of surrogate species collected in sub-Saharan West Africa

We collected feathers from three surrogate species in habitats potentially suitable for aquatic warblers, and found high variation in $\delta^{13}C$, $\delta^{15}N$, and δD even within species and locations (Fig. 2).

When including species as random intercepts in generalized linear mixed models, there were statistically well-supported geographic gradients for all three isotopes, except for a longitudinal gradient for δD (Table 3). However, despite their statistical support the geographic gradients explained only 0.8–13.5% of the total isotopic variation, and the magnitude of mean isotopic change projected over our geographic sampling area was often smaller than the isotopic variation recorded within every site (Fig. 2, Table 3). These single-isotope gradients could therefore not be used reliably to assign feathers of unknown origin to a location anywhere in sub-Saharan West Africa.

Table 3. Geographic gradients in feather isotope ratios of four passerine species (aquatic warbler and three surrogate species) across sub-Saharan West Africa (Appendix 1) estimated from generalised linear mixed models accounting for random variation among species. ωAIC indicates the weight of evidence for the model incorporating a linear isotopic gradient compared to a null model assuming no geographic gradient, variation explained indicates the proportional reduction in deviance attributable to the geographic gradient parameter, and predicted change indicates the magnitude of isotopic difference to be expected at the opposite ends of our latitudinal (11°) and longitudinal (30°) transects.

Isotope	Gradient	δ	ωAIC	Variation explained	Isotopic change across range (‰)
$\delta^{13}C$	latitude	−0.732	1.00	0.105	−8.0
	longitude	0.128	1.00	0.025	3.8
$\delta^{15}N$	latitude	−0.180	0.88	0.013	−2.0
	longitude	0.171	1.00	0.135	5.1
δD	latitude	−1.328	1.00	0.037	−14.4
	longitude	−0.134	0.29	0.003	−4.0

Including feathers from five additional species using different habitat types (Appendix 2) increased within-site variation in isotope ratios and weakened the geographic gradients (results not shown).

Predicting the wintering area of European aquatic warblers

The boosted regression tree models predicting the latitude and longitude of feathers from four species based on their isotope ratios and 'species' as explanatory variables performed reasonably well, with cross-validated correlations between observed and predicted values of r = 0.90 for latitude and r = 0.89 for longitude. Of the 61 aquatic warbler feathers in the training data set, 55 (90%) were predicted correctly to have originated from western Senegal, the remaining 6 (10%) were predicted to locations in southern Mauritania (n = 5) and southern Mali (n = 1), up to 850 km east of the capture area.

The most important predictor for latitude was $\delta^{13}C$, with δD and $\delta^{15}N$ having comparatively lower contributions (Table 4). For longitude, $\delta^{15}N$ proved to be the most important predictor, followed by $\delta^{13}C$, and both δD and 'species' of low importance. Hence, despite the large within-site variability in isotope ratios, the multivariate models performed reasonably well using a latitudinal $\delta^{13}C$ and a longitudinal $\delta^{15}N$ gradient.

The predicted origins of aquatic warbler feathers obtained from birds on breeding grounds in Europe were spread throughout sub-Saharan West Africa (Fig. 3). Only 51 out of 197 feathers (26%) were predicted to come from an area within 100 km of the Djoudj area in Senegal, which is substantially less than the 80% inferred from the cluster analysis above. When predicted location was examined separately for those feathers that had been assigned to the cluster inconsistent with feathers from Djoudj (n = 40), all of them were predicted to locations outside of Senegal, from southern Mauritania through Mali and east to Niger.

Discussion

The variation in feather isotope ratios of aquatic warblers and surrogate species spanned > 10‰ in $\delta^{15}N$ and > 20‰ in $\delta^{13}C$, which is similar to the pattern found in related *Acrocephalus* warblers moulting in sub-Saharan West Africa

(Yohannes et al. 2008b). Previous attempts to elucidate the geographic origin of African migrants using feather isotope ratios also found similar levels of variation (Evans et al. 2003, Møller and Hobson 2004, Yohannes et al. 2005, Bensch et al. 2006, Procházka et al. 2008). In many cases, the authors interpreted variation in feather isotope ratios as geographic or habitat differences in moult location. In contrast, our study demonstrates that individuals of a habitat specialist like the aquatic warbler that were captured in a relatively small area in western Senegal represent a high proportion of the isotopic spectrum of several other species
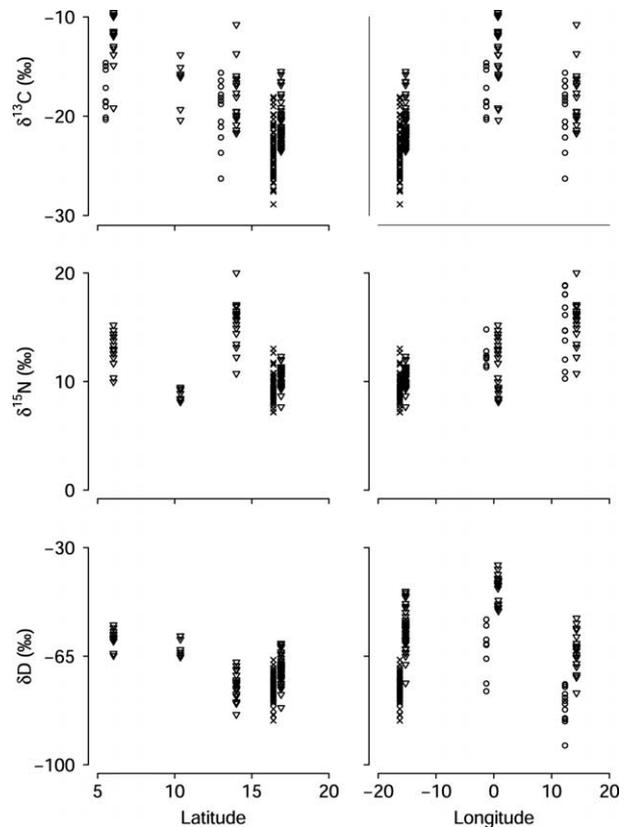
Figure 2. Latitudinal and longitudinal variation in feather $\delta^{13}C$, $\delta^{15}N$, and δD ratios of four species of passerines (winding cisticola = ∇, greater and lesser swamp warblers = ○, aquatic warbler = ×) sampled from wetland habitats across sub-Saharan West Africa. To avoid overlapping symbols winding cisticola data were adjusted by +1°, and swamp warbler data by −1° for both latitude and longitude.

Table 4. Proportional contribution of different predictor variables in boosted regression tree models predicting the latitude and longitude of feather origin. Contribution is assessed via a permutation procedure, whereby the decrease in model accuracy is tested when a given variable is randomly permuted over the data set (Elith et al. 2008).

	Latitude	Longitude
Species	15.5	6.7
$\delta^{13}C$	46.2	13.2
$\delta^{15}N$	17.2	71.1
δD	21.1	9.0

sampled across sub-Saharan West Africa. This large within-site and within-species variation is most likely a result of isotopic habitat heterogeneity, and a clear indication that habitats with a fairly uniform vegetation structure need not necessarily be isotopically uniform, thus reducing the usefulness of large-scale isotopic gradients for geographical assignment (Wunder et al. 2005, Kelly et al. 2008, Rocque et al. 2009).

The range of $\delta^{13}C$ in aquatic warbler rectrices suggests that aquatic warbler diet is derived from both C_3 and C_4 plants. Indeed, the only known wintering area in Senegal is characterised by cattail and bulrush wetlands typical of sub-Saharan floodplains, and includes species with C_3 (e.g. *Scirpus maritimus, S. littoralis, Oryza longistaminata*), C_4 (e.g. *Sporobolus robustus*), and intermediate (e.g. *Eleocharis mutata*) photosynthetic pathways (Flade and Lachmann 2008, Liu et al. 2008). Hence, the actual isotope ratio of a bird's feather may be largely determined by very small-scale foraging behaviour at a specific location, rather than by large-scale isotopic gradients. Similar local variation has recently been found for δD (Hobson et al. 2009b),

indicating that large differences in feather isotope ratios need not always be associated with geographic differences in moult location. While the wetland habitats where we sampled birds may harbour more C_4 species than closed forest habitats, most Palaearctic migrants prefer non-forest habitats in Africa (Moreau 1972, Jones 1985), and thus are likely to consume food with highly variable isotopic composition even if they winter in more arid habitats (Koch et al. 1995, Yohannes et al. 2005).

Plant photosynthetic pathways are not the only factor leading to isotopic variation. Plants can vary in isotope composition due to variation in soil moisture, ambient light and temperature (Dawson et al. 2002), and nutrient inputs from animal activity can additionally affect plant $\delta^{15}N$ (Crait and Ben-David 2007). Although aquatic warblers do not feed on plant material directly, isotopic variation in primary producers is passed on to higher trophic levels (Post 2002, Grey et al. 2004) and could therefore affect bird feather isotope ratios. Anthropogenic alteration of ecosystems can introduce further variation in hydrogen (e.g. through the use of ground water in irrigation), nitrogen (e.g. through the input of sewage, fertilizer, or animal waste products (Lindau et al. 1989, Hebert and Wassenaar 2001)), and carbon isotopes (e.g. through genetic modifications of photosynthetic pathways (Zhang et al. 2010)). Saltwater influence in coastal areas may increase feather isotope ratios (Greenberg et al. 2007), as we found for $\delta^{13}C$ in the saltwater-influenced sub-site 'Grand Lac' (Table 2). All of these factors can confound isotope signatures at very small spatial scales across rural Africa, and render the geographic interpretation of individual feather isotopes extremely challenging. Additionally, individual physiology, nutritional status, diet preferences, and age are all known to
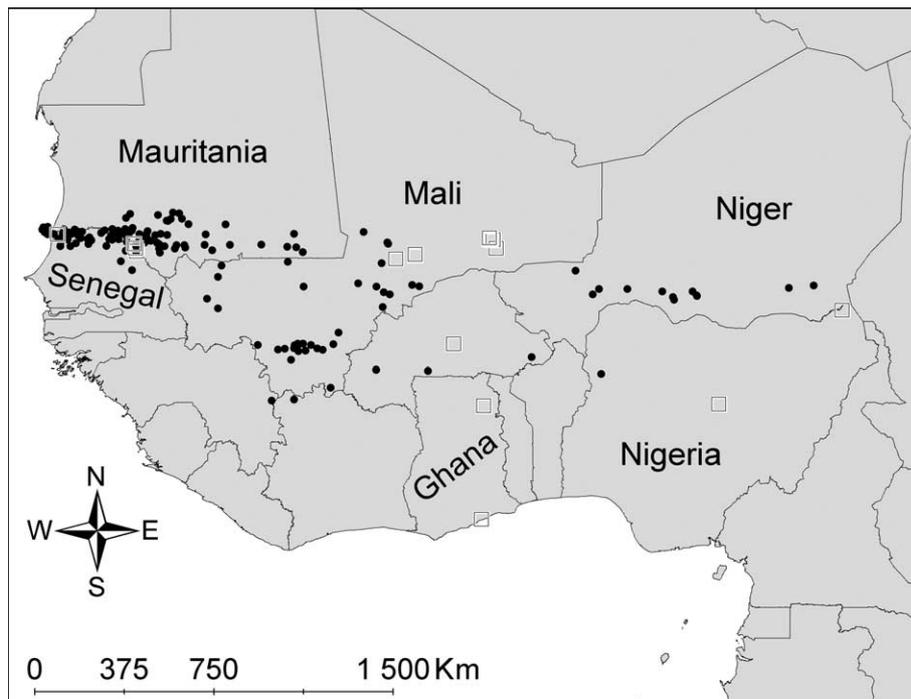
Figure 3. Predicted origin of aquatic warbler rectrices (black dots) sampled from birds on breeding grounds in Europe. Prediction was based on a boosted regression tree model trained on feather isotope data from four species collected at various areas in sub-Saharan West Africa (shaded squares). The known wintering site in the Djoudj National Park is the westernmost square on the map.

add to the isotopic variation among individual birds (McKechnie et al. 2004, Carleton and Martinez del Rio 2005, Kempster et al. 2007, Langin et al. 2007). Differences in migration and moult timing might introduce further isotopic variation among individuals that use the exact same stopover and wintering sites, albeit at different times. In the absence of very strong isotopic gradients and better knowledge about the timing of moult and migration of most species, all the above sources of variation can easily confound or obscure any potential isotopic differences of feather moult locations.

Our extensive sampling scheme across latitudinal and longitudinal transects in sub-Saharan West Africa, encompassing four different species, revealed statistically significant latitudinal and longitudinal isotope gradients. However, the detection of a statistical trend does not necessarily facilitate reliable prediction (Wunder et al. 2005, Rocque et al. 2009, Smith et al. 2009). We do not believe that these isotope gradients by themselves are suitable for geographic inference, because the isotopic variation of feathers collected from particular species within a given site was often larger than the gradient across sub-Saharan West Africa. For example, the frequently cited latitudinal gradient in $\delta^{13}C$, which is based on the composition of the vegetation at a continental scale (with a higher proportion of C_3 plants farther away from the equator), explained only 10.5% of the isotopic variation in the feathers we sampled. In Africa, geographic gradients of $\delta^{13}C$ and $\delta^{15}N$ have been very useful to predict spatial origins of African elephant *Loxodonta africana* tissues (van der Merwe et al. 1990, Vogel et al. 1990). Elephants consume large quantities of vegetation, and isotope ratios in slow-growing tissues such as tusks or bones thus adequately resemble the average vegetation composition of the landscape (van der Merwe et al. 1988). However, even elephant tusks or bones can show high variability among individuals within a small geographic area (Koch et al. 1995). Because insectivorous birds rarely integrate isotope ratios evenly across the landscape, gradients that have been useful for elephants cannot be expected to yield accurate predictions for single feathers from migratory bird species (Graves et al. 2002, Wunder et al. 2005).

Several studies of European migrants have used stable isotopes to reveal patterns among birds captured on breeding grounds. We recommend careful interpretation of literature that reports statistical differences in isotope ratios without considering the range of values, the effect size, and the ecological mechanism needed to cause a biologically meaningful difference in isotope ratios (Pain et al. 2004, Bensch et al. 2006, Procházka et al. 2008). The difference between pattern recognition and prediction has been recognized by some researchers (Wunder et al. 2005, Rocque et al. 2009, Smith et al. 2009), but is frequently disregarded when results of stable isotope projects are reported. An earlier contribution (Pain et al. 2004), which was based on a much smaller dataset than the present analysis, concluded from a statistical relationship between the latitude/longitude of breeding sites and the $\delta^{13}C$ of feathers grown on wintering sites that aquatic warbler subpopulations across Europe do not form a single mixed wintering population. The authors concluded, from broadscale averages of global carbon isotope maps, that birds breeding further north/west in Europe moulted further north in sub-Saharan Africa. Our present analysis shows that with an enlarged dataset, which better represents the isotopic variation within breeding sites, these conclusions cannot be upheld. The feather isotope ratios of birds sampled in all European breeding populations were so variable that they were not distinct at the population level. Due to large variation at the only known African wintering site, any attempt to assign birds from breeding areas to a specific wintering area in Africa could therefore be prone to large and uncontrollable errors.

A further complicating factor for geographic assignment is the imprecise knowledge about the timing of moult (Larson and Hobson 2009). In Africa, the distribution of hydrogen isotope ratios in rainfall changes dramatically within a year, so that even local resident birds could obtain a wide range of feather δD signatures depending on when they moult their feathers and over what time frame rainfall δD is integrated in food sources. For example, at some of our sampling sites δD in precipitation varies between $+20‰$ in November to $-30‰$ in July (Bowen et al. 2005; <http://wateriso.eas.purdue.edu/waterisotopes/pages/data_access/da_main.html>, accessed 27 March 2010). Given that the timing of moult is poorly known and potentially poorly constrained for some resident species (Aidley and Wilkinson 1987, McGregor et al. 2007), isotope gradients derived from multiple species that may moult at different times of the year may not be very meaningful (Farmer et al. 2008, Kelly et al. 2008).

Coarse estimations of aquatic warbler density in the Djoudj area indicate that a part of the world population may winter elsewhere (Flade et al. 2011). Our data also suggest that other wintering areas of aquatic warblers may exist in sub-Saharan West Africa, because not all aquatic warblers sampled on European breeding grounds had feather isotope ratios that fell within the isotopic variation recorded among aquatic warblers at the only known wintering site in Senegal. Three explanations exist for why feathers from aquatic warblers captured in Europe can be isotopically different from birds we captured in Senegal: 1) inaccessible areas of the Djoudj National Park were not represented in our sample, but may hold birds with enriched $\delta^{13}C$ in feathers due to higher concentration of C_4 vegetation, 2) birds may have moulted and wintered elsewhere, or 3) birds may have moulted en route, but still wintered in Senegal, a pattern that is known from other *Acrocephalus* warblers in West Africa (Aidley and Wilkinson 1987, Bensch et al. 1991, Hedenström et al. 1993).

Our two different analyses indicated that 20–74% of aquatic warblers sampled in Europe may have moulted in locations isotopically different from the sub-sites we sampled in the Djoudj area. The predicted origins of aquatic warbler feathers (Fig. 3) were broadly consistent with predictions of suitable aquatic warbler wintering habitat based on remote sensing data (Walther et al. 2007, Buchanan et al. 2011), and confirm earlier suspicions that the Niger river valley in southern Mali may be a potentially important area used by aquatic warblers in winter (Schäffer et al. 2006, Zwarts et al. 2010). However, intensive searches in the inner Niger delta had until 2010 failed to detect any aquatic warblers, and the heavy use by grazing cattle may not only render habitat unsuitable

(Zwarts et al. 2010), but should also increase $\delta^{15}N$ signatures of feathers grown in that region. In that respect, it is important to consider that our predictive model is driven solely by isotope data, and does not recognize any underlying ecological mechanism for isotopic variation. Because the models were constructed with a limited number of geographic sampling points, extrapolation to locations away from sampling localities must be treated with caution and should be viewed with a wide margin of error (Elith and Graham 2009). Additional field efforts are now required to verify whether our model predictions can be substantiated.

Although we are not able to support the earlier hypothesis (Pain et al. 2004) that aquatic warblers from different breeding areas migrate to different wintering areas, the isotope data suggest that aquatic warblers breeding in Europe may winter in areas other than western Senegal in sub-Saharan West Africa. We encourage further searches on the ground for alternative wintering areas of aquatic warblers, focusing on areas where our feather predictions overlap with predicted suitable habitat (Walther et al. 2007, Buchanan et al. 2011).

Conclusions

Stable isotopes have been successfully applied to find previously unknown breeding or wintering regions in eastern North America where favourable isotopic gradients exist (Hobson et al. 2004a, Greenberg et al. 2007). However, failure to consider the underlying mechanisms of isotopic variation across landscapes, insufficient sample sizes that do not adequately represent the isotopic variation within groups, and reliance on coarse gradients that are described in general terms in the literature may lead to erroneous conclusions when using feather isotope ratios for geographic inference (Wunder et al. 2005, Farmer et al. 2008, Rocque et al. 2009). We urge researchers to investigate the nature of isotopic variation in feathers of target species more carefully under controlled conditions and to attempt geographic assignments only under highly constrained scenarios (Larson and Hobson 2009, Rocque et al. 2009). Unfortunately, open wetland habitats in sub-Saharan West Africa do not appear to offer such constrained scenarios. In contrast, latitudinal deuterium gradients in combination with longitudinal marine-freshwater gradients create highly constrained scenarios in eastern North America where stable isotopes have been applied successfully to discover an unknown wintering population of the coastal plain swamp sparrow *Melospiza georgiana nigrescens* (Greenberg et al. 2007).

Our study has shown that isotopic variation in feathers grown by different species of wetland habitat specialists in sub-Saharan West Africa is very large, and that this variation cannot necessarily be interpreted as geographic difference for individual feathers of unknown origin. While it may be possible to predict the origin of feather growth for some species, our results indicate that sampling of ecologically similar species at many potential moulting areas would be required to characterise geographic variation and potentially assign birds to these areas. This limitation undermines one of the main advantages frequently listed for stable isotope analysis – namely that regions could be identified where no sampling has occurred (Hobson 2005, Coiffait et al. 2009). We caution against relying on generalized isotopic gradients in sub-Saharan West Africa to predict a species wintering area when no samples are available from that area, as statistical trends may not be accurate at the desired spatial scale of inference. Resources required for establishing isotopic baselines at sufficient spatial resolution might be better spent on alternative methods to identify the winter distribution of Palaearctic migrants. Alternative methods could include the use of tracking devices (Fiedler 2009, Stutchbury et al. 2009, Bächler et al. 2010) and novel modelling approaches to predict the spatial distribution of suitable habitat (Buchanan et al. 2011).

References

Aidley, D. J. and Wilkinson, R. 1987. The annual cycle of six *Acrocephalus* warblers in a Nigerian reed-bed. – Bird Study 34: 226–234.

Bächler, E., Hahn, S., Schaub, M., Arlettaz, R., Jenni, L., Fox, J. W., Afanasyev, V. and Liechti, F. 2010. Year-round tracking of small trans-Saharan migrants using light-level geolocators. – PLoS One 5: e9566.

Bensch, S., Hasselquist, D., Hedenström, A. and Ottosson, U. L. F. 1991. Rapid moult among palaearctic passerines in West Africa – an adaptation to the oncoming dry season? – Ibis 133: 47–52.

Bensch, S., Bengtsson, G. and Åkesson, S. 2006. Patterns of stable isotope signatures in willow warbler *Phylloscopus trochilus* feathers collected in Africa. – J. Avian Biol. 37: 323–330.

Berthold, P., Fiedler, W., Schlenker, R. and Querner, U. 1998. 25-year study of the population development of central European songbirds: a general decline, most evident in long-distance migrants. – Naturwiss enschaffen. 85: 350–353.

BirdLife International. 2009. Species factsheet: *Acrocephalus paludicola*. – <http://www.birdlife.org>, accessed 16 Feb. 2010.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H. and White, J.-S. S. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. – Trends Ecol. Evol. 24: 127–135.

Borrow, N. and Demey, R. 2001. Birds of western Africa. – Christopher Helm.

Bowen, G. J., Chesson, L., Nielson, K., Cerling, T. E. and Ehleringer, J. R. 2005a. Treatment methods for the determination of delta H-2 and delta O-18 of hair keratin by continuous-flow isotope-ratio mass spectrometry. – Rapid Comm. Mass Spec. 19: 2371–2378.

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

Bowen, G. J., West, J. B. and Hoogewerff, J. 2009. Isoscapes: isotope mapping and its applications. – J. Geochem. Explor. 102: v–vii.

Breiman, L. 2001. Random forests. – Mach. Learn. 45: 5–32.

Buchanan, G. M., Lachmann, L., Tegetmeyer, C., Oppel, S., Nelson, A. and Flade, M. 2011. Identifying the potential wintering sites of the globally threatened aquatic warbler Acrocephalus paludicola using remote sensing. – Ostrich 82: 81–85.

Burnham, K. P. and Anderson, D. R. 2002. Model selection and multimodel inference. A practical information-theoretic approach. – Springer.

Carleton, S. and Martinez del Rio, C. 2005. The effect of cold-induced increased metabolic rate on the rate of 13C and 15N incorporation in house sparrows (Passer domesticus). – Oecologia 144: 226–232.

Chamberlain, C. P., Blum, J. D., Holmes, R. T., Feng, X., Sherry, T. W. and Graves, G. R. 1997. The use of isotope tracers for identifying populations of migratory birds. – Oecologia 109: 132–141.

Chamberlain, C. P., Bensch, S., Feng, X., Akesson, S. and Andersson, 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. – Proc. R. Soc. B 267: 43–48.

Coiffait, L., Redfern, C. P. F., Bevan, R. M., Newton, J. and Wolff, K. 2009. The use of intrinsic markers to study bird migration. – Ringing Migr. 24: 169–174.

Craig, H. 1961. Isotopic variations in meteoric waters. – Science 133: 1702–1703.

Crait, J. R. and Ben-David, M. 2007. Effects of river otter activity on terrestrial plants in trophically altered Yellowstone Lake. – Ecology 88: 1040–1052.

Cutler, D. R., Edwards, T. C., Beard, K. H., Cutler, A., Hess, K. T., Gibson, J. and Lawler, J. J. 2007. Random Forests for classification in ecology. – Ecology 88: 2783–2792.

Dawson, T., Mambelli, S., Plamboeck, A., Templer, P. and Tu, K. 2002. Stable isotopes in plant ecology. – Annu. Rev. Ecol. Syst. 33: 507–559.

Dowsett-Lemaire, F. 1997. Seasonality of breeding and moult in forest and savanna birds in northern Congo. – Rev. Ecol. (Terre et la Vie) 52: 153–171.

Elith, J. and Graham, C. H. 2009. Do they? How do they? WHY do they differ? On finding reasons for differing performances of species distribution models. – Ecography 32: 66–77.

Elith, J., Leathwick, J. R. and Hastie, T. 2008. A working guide to boosted regression trees. – J. Anim. Ecol. 77: 802–813.

Evans, K. L., Waldron, S. and Bradbury, R. B. 2003. Segregation in the African wintering ranges of English and Swiss swallow Hirundo rustica populations: a stable isotope study. – Bird Study 50: 294–299.

Farmer, A., Cade, B. and Torres-Dowdall, J. 2008. Fundamental limits to the accuracy of deuterium isotopes for identifying the spatial origin of migratory animals. – Oecologia 158: 183–192.

Fiedler, W. 2009. New technologies for monitoring bird migration and behaviour. – Ringing Migr. 24: 175–179.

Flade, M. and Lachmann, L. 2008. International species action plan for the aquatic warbler Acrocephalus paludicola. – BirdLife International.

Flade, M., Diop, I., Haase, M., Le Nevé, A., Oppel, S., Tegetmeyer, C., Vogel, A. and Salewski, V. 2011. Distribution, ecology and threat status of the aquatic warblers Acrocephalus paludicola in West Africa. – J. Ornithol. doi: 10.1007/510336-011-0666-5.

Gillies, C. S., Hebblewhite, M., Nielsen, S. E., Krawchuk, M. A., Aldridge, C. L., Frair, J. L., Saher, D. J., Stevens, C. E. and Jerde, C. L. 2006. Application of random effects to the study of resource selection by animals. – J. Anim. Ecol. 75: 887–898.

Gottschalk, T. K., Ekschmitt, K. and Bairlein, F. 2007. Relationships between vegetation and bird community composition in grasslands of the Serengeti. – Afr. J. Ecol. 45: 557–565.

Graves, G. R., Romanek, C. S. and Rodriguez Navarro, A. 2002. Stable isotope signature of philopatry and dispersal in a migratory songbird. – Proc. Natl Acad. Sci. USA 99: 8096–8100.

Greenberg, R., Marra, P. P. and Wooller, M. J. 2007. Stable-isotope (C, N, H) analyses help locate the winter range of the coastal plain swamp sparrow (Melospiza georgiana nigrescens). – Auk 124: 1137–1148.

Grey, J., Kelly, A. and Jones, R. I. 2004. High intraspecific variability in carbon and nitrogen stable isotope ratios of lake chironomid larvae. – Limnol. Oceanogr. 49: 239–244.

Hebert, C. E. and Wassenaar, L. I. 2001. Stable nitrogen isotopes in waterfowl feathers reflect agricultural land use in western Canada. – Environ. Sci. Tech. 35: 3482–3487.

Hedenström, A., Bensch, S., Hasselquist, D., Lockwood, M. and Ottosson, U. 1993. Migration, stopover and moult of the great reed warbler Acrocephalus arundinaceus in Ghana, West Africa. – Ibis 135: 177–180.

Hobson, K. A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. – Oecologia 120: 314–326.

Hobson, K. A. 2005. Stable isotopes and the determination of avian migratory connectivity and seasonal interactions. – Auk 122: 1037–1048.

Hobson, K., Aubry, Y. and Wassenaar, L. 2004a. Migratory connectivity in Bicknell's thrush: locating missing populations with hydrogen isotopes. – Condor 106: 905–909.

Hobson, K. A., Bowen, G. J., Wassenaar, L. I., Ferrand, Y. and Lormee, H. 2004b. Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. – Oecologia 141: 477–488.

Hobson, K. A., Lormée, H., Van Wilgenburg, S. L., Wassenaar, L. I. and Boutin, J. M. Stable isotopes (δD) delineate the origins and migratory connectivity of harvested animals: the case of European woodpigeons. – J. Appl. Ecol. 46: 572–581.

Hobson, K. A., Van Wilgenburg, S. L., Larson, K. and Wassenaar, L. I. 2009b. A feather hydrogen isoscape for Mexico. – J. Geochem. Explor. 102: 167–174.

Inger, R. and Bearhop, S. 2008. Applications of stable isotope analyses to avian ecology. – Ibis 150: 447–461.

Jones, P. J. 1985. The migration strategies of Palaearctic passerines in Africa. – In: MacDonald, A. and Goriup, P. (eds), Migratory birds: problems and prospects in Africa. ICBP, pp. 9–21.

Kelly, J. F., Atudorei, V., Sharp, Z. D. and Finch, D. M. 2002. Insights into Wilson's warbler migration from analyses of hydrogen stable-isotope ratios. – Oecologia 130: 216–221.

Kelly, J. F., Johnson, M. J., Langridge, S. and Whitfield, M. 2008. Efficacy of stable isotope ratios in assigning endangered migrants to breeding and wintering sites. – Ecol. Appl. 18: 568–576.

Kempster, B., Zanette, L., Longstaffe, F. J., MacDougall-Shackleton, S. A., Wingfield, J. C. and Clinchy, M. 2007. Do stable isotopes reflect nutritional stress? Results from a laboratory experiment on song sparrows. – Oecologia 151: 365–371.

Koch, P. L., Heisinger, J., Moss, C., Carlson, R. W., Fogel, M. L. and Behrensmeyer, A. K. 1995. Isotopic tracking of change in

diet and habitat use in African elephants. – Science 267: 1340–1343.

Langin, K., Reudink, M., Marra, P., Norris, D., Kyser, T. and Ratcliffe, L. 2007. Hydrogen isotopic variation in migratory bird tissues of known origin: implications for geographic assignment. – Oecologia 152: 449–457.

Larson, K. and Hobson, K. 2009. Assignment to breeding and wintering grounds using stable isotopes: a comment on lessons learned by Rocque et al. – J. Ornithol. 150: 709–712.

Leathwick, J. R., Elith, J., Francis, M. P., Hastie, T. and Taylor, P. 2006. Variation in demersal fish species richness in the oceans surrounding New Zealand: an analysis using boosted regression trees. – Mar. Ecol. Prog. Ser. 321: 267–281.

Lindau, C. W., Delaune, R. D., Patrick, W. H. and Lambremont, E. N. 1989. Assessment of stable nitrogen isotopes in fingerprinting surface water inorganic nitrogen sources. – Water, Air. Soil Pollut. 48: 489–496.

Liu, K., Eastwood, R. J., Flynn, S., Turner, R. M. and Stuppy, W. H. 2008. Seed information database (release 7.1). – <www.kew.org/data/sid>, accessed 16 April 2010.

MacLean, I. M. D., Hassall, M., Boar, R. and Nasirwa, O. 2003. Effects of habitat degradation on avian guilds in East African papyrus Cyperus papyrus swamps. – Bird Conserv. Int. 13: 283–297.

Marra, P. P., Hobson, K. A. and Holmes, R. T. 1998. Linking winter and summer events in a migratory bird by using stable-carbon isotopes. – Science 282: 1884–1886.

McGregor, R. M., Ottosson, U. and Cresswell, W. R. L. 2007. Moult of Guinea savanna passerines in West Africa. – Ostrich 78: 287–290.

McKechnie, A. E., Wolf, B. O. and Martinez del Rio, C. 2004. Deuterium stable isotope ratios as tracers of water resource use: an experimental test with rock doves. – Oecologia 140: 191–200.

Møller, A. P. and Hobson, K. A. 2004. Heterogeneity in stable isotope profiles predicts coexistence of populations of barn swallows Hirundo rustica differing in morphology and reproductive performance. – Proc. R. Soc. B 271: 1355–1362.

Moreau, R. E. 1972. The Palaearctic-African bird migration systems. – Academic Press.

Newton, I. 2004. Population limitation in migrants. – Ibis 146: 197–226.

Newton, I. 2006. Can conditions experienced during migration limit the population levels of birds? – J. Ornithol. 147: 146–166.

Norris, D. R. and Marra, P. P. 2007. Seasonal interactions, habitat quality, and population dynamics in migratory birds. – Condor 109: 535–547.

Norris, D. R., Marra, P. P., Kyser, T. K. and Ratcliffe, L. M. 2005. Tracking habitat use of a long-distance migratory bird, the American redstart Setophaga ruticilla, using stable-carbon isotopes in cellular blood. – J. Avian Biol. 36: 164–170.

Oppel, S. and Powell, A. N. 2008. Assigning king eiders to wintering regions in the Bering Sea using stable isotopes of feathers and claws. – Mar. Ecol. Prog. Ser. 373: 149–156.

Pain, D. J., Green, R. E., Giessing, B., Kozulin, A., Poluda, A., Ottosson, U., Flade, M. and Hilton, G. M. 2004. Using stable isotopes to investigate migratory connectivity of the globally threatened aquatic warbler Acrocephalus paludicola. – Oecologia 138: 168–174.

Peterson, B. J. and Fry, B. 1987. Stable isotopes in ecosystem studies. – Annu. Rev. Ecol. Syst. 18: 293–320.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. – Ecology 83: 703–718.

Procházka, P., Hobson, K., Karcza, Z. and Kralj, J. 2008. Birds of a feather winter together: migratory connectivity in the reed warbler Acrocephalus scirpaceus. – J. Ornithol. 149: 141–150.

Rocque, D. A., Ben-David, M., Barry, R. P. and Winker, K. 2006. Assigning birds to wintering and breeding grounds using stable isotopes: lessons from two feather generations among three intercontinental migrants. – J. Ornithol. 147: 395–404.

Rocque, D. A., Ben-David, M., Barry, R. P. and Winker, K. 2009. Wheatear molt and assignment tests: ongoing lessons in using stable isotopes to infer origins. – J. Ornithol. 150: 931–934.

Sanderson, F. J., Donald, P. F., Pain, D. J., Burfield, I. J. and van Bommel, F. P. J. 2006. Long-term population declines in Afro-Palaearctic migrant birds. – Biol. Conserv. 131: 93–105.

Sauer, P., Schimmelmann, A., Sessions, A. and Topalov, K. 2009. Simplified batch equilibration for D/H determination of non-exchangeable hydrogen in solid organic material. – Rapid Comm. Mass. Spec. 23: 949–956.

Schäffer, N., Walther, B. A., Gutteridge, K. and Rahbek, C. 2006. The African migration and wintering grounds of the aquatic warbler Acrocephalus paludicola. – Bird Conserv. Int. 16: 33–56.

Smith, A. D., Lott, C. A., Smith, J. P., Donohue, K. C., Wittenberg, S., Smith, K. G., Goodrich, L. and Jones, J. 2009. Poor reproducibility and inference in hydrogen-stable-isotope studies of avian movement: a reply to Wunder et al. (2009). – Auk 126: 926–931.

Stutchbury, B. J. M., Tarof, S. A., Done, T., Gow, E., Kramer, P. M., Tautin, J., Fox, J. W. and Afanasyev, V. 2009. Tracking long-distance songbird migration by using geolocators. – Science 323: 896.

Svensson, L. 1992. Identification guide to European passerines. – Stockholm, publ. by the author.

Tanneberger, F., Bellebaum, J., Fartmann, T., Haferland, H.-J., Helmecke, A., Jehle, P., Just, P. and Sadlik, J. 2008. Rapid deterioration of aquatic warbler Acrocephalus paludicola habitats at the western margin of the breeding range. – J. Ornithol. 149: 105–115.

Urban, E. K., Fry, C. H. and Keith, S. 1997. The birds of Africa. – Princeton Univ. Press.

van der Merwe, N. J., Thorp, J. A. L. and Bell, R. H. V. 1988. Carbon isotopes as indicators of elephant diets and African environments. – Afr. J. Ecol. 26: 163–172.

van der Merwe, N. J., Lee-Thorp, J. A., Thackeray, J. F., Hall-Martin, A., Kruger, F. J., Coetzee, H., Bell, R. H. V. and Lindeque, M. 1990. Source-area determination of elephant ivory by isotopic analysis. – Nature 346: 744–746.

Vogel, A.-L. 2009. Genetische Bestimmung der Herkunft von im Senegal überwinternden Seggenrohrsängern. — MSc thesis, Inst. für Zoologie, Ernst-Moritz-Arndt-Univ., Greifswald, Germany.

Vogel, J. C., Eglington, B. and Auret, J. M. 1990. Isotope fingerprints in elephant bone and ivory. – Nature 346: 747–749.

Walther, B., Schäffer, N., van Niekirk, A., Thuiller, W., Rahbek, C. and Chown, S. 2007. Modelling the winter distribution of a rare and endangered migrant, the aquatic warbler Acrocephalus paludicola. – Ibis 149: 701–714.

Wassenaar, L. and Hobson, K. 2003. Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. – Isot. Environ. Health Stud. 39: 211–217.

Webster, M. S., Marra, P. P., Haig, S. M., Bensch, S. and Holmes, R. T. 2002. Links between worlds: unravelling migratory connectivity. – Trends Ecol. Evol. 17: 76–83.

Wunder, M. B. and Norris, D. R. 2008. Improved estimates of certainty in stable-isotope-based methods for tracking migratory animals. – Ecol. Appl. 18: 549–559.

Wunder, M. B., Kester, C. L., Knopf, F. L. and Rye, R. O. 2005. A test of geographic assignment using isotope tracers in feathers of known origin. – Oecologia 144: 607–617.

Wunder, M. B., Hobson, K. A., Kelly, J., Marra, P. P., Wassenaar, L. I., Stricker, C. A., Doucett, R. R. and Jones, J. 2009. Does a lack of design and repeatability compromise scientific criticism? A response to Smith et al. (2009). – Auk 126: 922–926.

Yohannes, E., Hobson, K. A., Pearson, D. J. and Wassenaar, L. I. 2005. Stable isotope analyses of feathers help identify autumn stopover sites of three long-distance migrants in northeastern Africa. – J. Avian Biol. 36: 235–241.

Yohannes, E., Hobson, K. A. and Pearson, D. J. 2007. Feather stable-isotope profiles reveal stopover habitat selection and site fidelity in nine migratory species moving through sub-Saharan Africa. – J. Avian Biol. 38: 347–355.

Yohannes, E., Bensch, S. and Lee, R. 2008a. Philopatry of winter moult area in migratory great reed warblers *Acrocephalus arundinaceus* demonstrated by stable isotope profiles. – J. Ornithol. 149: 261–265.

Yohannes, E., Hansson, B., Lee, R. W., Waldenström, J., Westerdahl, H., Åkesson, M., Hasselquist, D. and Bensch, S. 2008b. Isotope signatures in winter moulted feathers predict malaria prevalence in a breeding avian host. – Oecologia 158: 299–306.

Zhang, J., Bandyopadhyay, A., Sellappan, K., Wang, G., Xie, H. A., Datta, K. and Datta, S. K. 2010. Characterization of a C_4 maize pyruvate orthophosphate dikinase expressed in C_3 transgenic rice plants. – Afri. J. Biotechnol. 9: 234–242.

Zwarts, L., Bijlsma, R. G., van der Kamp, J. and Wymenga, E. 2010. Living on the edge: wetlands and birds in a changing Sahel. – Zeist, the Netherlands, KNNV Publ.

Appendix 1. Latitude and longitude of sites in sub-Saharan Africa where we captured surrogate bird species for the collection of feather samples.

Country	Site	Latitude	Longitude
Burkina Faso	Koubri Monastery	12.21	−1.34
Ghana	Panbros	5.53	−0.29
Ghana	White Volta River	9.87	−0.21
Ghana	Oti River	9.87	0.21
Mali	Gao 1	15.86	0.27
Mali	Gao 2	16.21	0.04
Mali	Gao 3	16.26	0.01
Mali	Gao 4	16.19	0.15
Mali	Timboktu 1	15.46	−3.52
Mali	Timboktu 2	15.61	−2.79
Nigeria	Jos	9.93	8.66
Nigeria	Lake Chad	13.50	13.30
Senegal	Djoudj National Park	16.40	−16.26
Senegal	Matam 1	16.04	−13.38
Senegal	Matam 2	15.87	−13.32
Senegal	Matam 3	15.75	−13.28

Appendix 2. List of species from which feather isotope ratios were analysed to establish feather isotope gradients across sub-Saharan West Africa. Habitat type indicates main habitat of the species, n indicates number of feather samples with $\delta^{13}C$, $\delta^{15}N$ and δD measurements. Only species marked with an asterisk were used in models predicting aquatic warbler feather origin. Isotope data are available from the authors upon request.

Common name	Scientific name	Habitat type	n
Beautiful sunbird	*Nectarinia pulchella*	dry	14
Cricket warbler	*Spiloptila clamans*	dry	31
Greater swamp warbler*	*Acrocephalus rufescens*	wet	9
Grey-backed camaroptera	*Camaroptera brachyura*	dry	40
Heuglin's masked weaver	*Ploceus heuglini*	dry	13
Lesser swamp warbler*	*Acrocephalus gracilirostris*	wet	12
Tawny-flanked prinia	*Prinia subflava*	dry	8
Winding cisticola*	*Cisticola galactotes*	wet	85