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Using stable isotopes to investigate migratory connectivity of the globally threatened aquatic warbler *Acrocephalus paludicola*

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Abstract Understanding the links between breeding and wintering areas of migratory species has important ecological and conservation implications. Recently, stable isotope technology has been used to further our understanding. Stable isotope ratios vary geographically with a range of biogeochemical factors and isotope profiles in organisms reflect those in their food and environment. For inert tissues like feathers, isotope profiles reflect the environment in which they were formed. Following large-scale habitat destruction, the globally threatened aquatic

warbler *Acrocephalus paludicola* has a fragmented breeding population across central Europe, largely in Belarus, Poland and Ukraine. The species' sub-Saharan African wintering grounds have not yet been discovered, and this significantly hampers conservation efforts. Aquatic warblers grow their flight feathers on their wintering grounds, and we analysed stable isotope ratios ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$, δD) in rectrices of adults from six main breeding sites (subpopulations) across Europe to determine whether different breeding subpopulations formed a single mixed population on the wintering grounds. $\delta^{15}\text{N}$ varies considerably with dietary trophic level and environmental factors, and δD with the δD in rainfall; neither varied between aquatic warbler subpopulations. Uniform feather $\delta^{15}\text{N}$ signatures suggest no major variation in dietary trophic level during feather formation. High variance and inter-annual differences in mean δD values hinder interpretation of these data. Significant differences in mean $\delta^{13}\text{C}$ ratios existed between subpopulations. We discuss possible interpretations of this result, and consider differences in moulting latitude of different subpopulations to be the most parsimonious. $\delta^{13}\text{C}$ in plants and animals decreases with latitude, along a steep gradient in sub-Saharan Africa. Birds from the most north-westerly breeding subpopulation (Karsibor, Poland) had significantly lower variance in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than birds from all other sites, suggesting either that birds from Karsibor are less geographically dispersed during moult, or moult in an area with less isotopic heterogeneity. Mean $\delta^{13}\text{C}$ signatures from winter-grown feathers of different subpopulations were positively correlated with the latitude and longitude of breeding sites, suggesting a strong relationship between European breeding and African winter moulting latitudes. The use of stable isotopes provides novel insights into migratory connectivity and migration patterns in this little-known threatened species.

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Introduction

The ecological and conservation importance of understanding the links between breeding and non-breeding areas, i.e. migratory connectivity, is well understood (Esler 2000; Rubenstein et al. 2002; Webster et al. 2002). For example, the geographical extent, and degree of overlap, of breeding subpopulations' wintering areas have ecological and conservation implications. Populations with restricted ranges and strong site fidelity at any stage of their life cycle are particularly vulnerable to environmental changes.

Until recently, migration studies have relied largely upon recoveries of birds ringed in large scale ringing programmes. However, this approach is insufficient for rare species and those that have low recovery rates because they spend time in areas with sparse human populations. Satellite telemetry has now become a valuable technique for large birds such as albatrosses and some raptors, but many species of interest are too small to carry satellite-tags. Recent advances in the analysis of molecular genetic markers, and in stable isotopes, are for the first time helping to answer questions of migratory connectivity for small and widely dispersed species (for a recent review see Webster et al. 2002).

Stable isotope ratios in plants and animals vary according to a range of biogeographical factors such as habitat type, trophic level of diet and geographical location. Isotope ratios in consumers reflect those of their prey; thus animals carry in their tissues an isotopic record of what they have eaten, and local environmental conditions. The residence time of dietary isotopic signatures varies from days to year according to the tissue analysed (Hobson and Clarke 1992a, 1992b; Hobson 1999a). For metabolically inert tissues, such as feathers and hair, an animal will have a signature reflecting diet at the time of tissue formation. For many bird species, the sequence and timing of moult is well known, and over the last decade feather stable isotope ratios have been used to investigate dietary factors, habitat use, and more recently migratory connectivity in passerines, primarily in the Americas (Hobson 1999a; Rubenstein et al. 2002). The main isotopes used to date have been $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and δD . Nitrogen isotopes vary with a range of factors, but undergo a marked trophic level fractionation and are often used as dietary markers (Thompson and Furness 1995; Romanek et al. 2000); carbon isotopes vary predictably and systematically with photosynthetic pathway (C3, C4 or CAM) and are used as habitat indicators (Hobson 1999a); they also vary over larger geographical scales with latitude, largely as a result of varying proportions of C3/C4 plants at different latitudes (Still et al. 2003), and have been used in migration studies (e.g. Chamberlain et al. 1997, 2001; Kelly 2000). Deuterium isotope ratios in the feathers of insectivorous birds vary across continents in relation to the δD in precipitation, and are used to trace origins and migration of wildlife (Chamberlain et al. 1997; Hobson and Wassenaar 1997; Cherel et al. 2000; Wassenaar and Hobson 2000, 2001; Rubenstein et al.

2002). In this paper, we use all three isotopes to investigate migratory connectivity in different breeding subpopulations of the aquatic warbler *Acrocephalus paludicola*.

The aquatic warbler is a globally threatened species (BirdLife International 2000). Its breeding range is restricted to the western Palearctic from 47° to 59°N. The species formerly bred in western Europe [France, Belgium, the Netherlands, former West Germany, Latvia, former Czechoslovakia and Yugoslavia, Austria and Italy (de By 1990; Cramp 1992)] across to Russia and Western Siberia. It became extinct in Western Europe in the 20th century, largely due to loss of its fen-mire habitat from factors such as drainage for agriculture and peat extraction (AWCT 1999). Today the breeding population is fragmented and still declining; over 90% of the world population (estimated at 12,500–20,000 singing males, Aquatic Warbler Conservation Team, personal communication) occurs in Belarus (58%), Ukraine (16%) and Poland (18%), where habitat loss and alteration is a continuing threat.

Following breeding, aquatic warblers migrate through the Netherlands, Belgium, Luxembourg, France and Spain across the Mediterranean to Africa (AWCT 1999; Atienza et al. 2001). Wintering areas are not known with any confidence, but aquatic warblers are believed to moult and winter in sub-Saharan West Africa, possibly in wetlands and floodplains in Mauritania, Mali, and Senegal. However, this information is based on very few records (Curry-Lindahl 1981; Hendenström et al. 1990). Only one ringing record of a wintering aquatic warbler exists in sub-Saharan Africa outside the migration period. This bird was caught along with more than 200 individuals of other *Acrocephalus* spp. in Tono, northern Ghana (ca. 11°N, 2°W) on 15 November 1987 (Hendenström et al. 1990). The bird was a juvenile in fresh plumage, indicating a rapid moult just after arrival in the wintering quarters (Bensch et al. 1991). Similarly, one historical record exists for an aquatic warbler in winter in the Niger Inundation Zone in West Africa, although this is a vague record as country of location was not given and there was no indication of whether this bird was trapped or observed (Moreau 1972).

In this paper, we use isotope ratios in feathers from adult aquatic warblers from six breeding populations (subpopulations) across Europe to investigate whether these form a single mixed population on the wintering grounds, or winter in discrete areas.

Conservation of the species requires that the wintering grounds are located. $\delta^{13}\text{C}$ signatures in feathers, which should reflect geographical variation of $\delta^{13}\text{C}$, may help to locate aquatic warbler wintering grounds at a broad geographical scale. We compare $\delta^{13}\text{C}$ signatures from aquatic warbler feathers with those from simulated environmental variables in an attempt to narrow down potential wintering latitudes of aquatic warblers.

Materials and methods

Sampling aquatic warblers

Adult aquatic warblers were caught between 10 May and 23 June 1999 at four sites across the breeding range in Europe: Karsibor in Poland, Zvanets and Dikoe in Belarus and Uday in Ukraine. Between 27 May and 11 July 2000, samples were collected from Uday and two additional sites, Supoy in Ukraine and Yaselda in Belarus (Table 1, Fig. 1). Adults of 1 and more than 1 year cannot be distinguished, so the sample includes both. One of the fifth rectrices was taken from each bird and stored in a self-sealing, polythene bag prior to analysis. Feathers were handled as little as possible. Birds do not moult flight feathers in Europe (Svensson 1984) or north of the Sahara, despite considerable ringing effort and the capture of a large number of passage birds in Europe and North Africa. For example, in La Nava (Spain), none of the hundreds of aquatic warblers ringed every year have been found to have moulted flight feathers, although partial body moults of body and head feathers have been recorded (Fernando Jubete, personal communication). We can therefore be confident that birds moult on their wintering grounds, probably in the areas that they first occupy after the trans-Saharan migration, as happens with the closely related congeneric sedge warbler *A. schoenobaenus* (Bensch et al. 1991). Consequently, feathers taken from aquatic warblers in their European breeding grounds will reflect the isotope ratios of areas where their feathers were grown in Africa. Moulting areas may not be totally representative of wintering areas, as birds may not necessarily remain in the same part of their wintering area after moult. Bensch et al. (1991) found that in Ghana, sedge warblers ($n=9$) grew approximately 5.8% of a primary feather's length per day, and great reed warblers *A. arundinaceus* 5.3% ($n=7$) per day. Complete primary moult took place in 5–6 weeks, although longer moult periods have been observed in other countries. If aquatic warbler rectrices are similar, our feathers will give an integrated signal reflecting diet, and location, during the 3 or more weeks of feather growth.

Analytical methods

Stable isotope ratios are reported as deviations from a standard in per mil (‰) using the δ notation:

$$\delta_{\text{sample}} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

Table 1 Mean isotope ratios in adult aquatic warbler rectrices from six breeding sites across Europe, 1999 and 2000. ^{13}C ratios of winter grown feathers differed between breeding sites [general linear models (GLM), site $F_{5,127}=2.29, P=0.034$, Fig. 2]. Tukey pairwise

Site and year	Latitude	Longitude	n	$\delta^{15}\text{N}(\text{‰})$		$\delta^{13}\text{C}(\text{‰})$		$\delta\text{D}(\text{‰})$	
				Mean	SE	Mean	SE	Mean	SE
Karsibor 1999	53.85°N	14.32°E	28	10.78	0.19	-21.97 ^{a,b}	0.38	-71.76	1.60
Dikoe 1999	52.75°N	24.21°E	12	10.40	0.36	-20.62	1.08	-69.03	2.68
Yaselda 2000	52.45°N	25.02°E	27	10.89	0.31	-21.02	0.61	-75.99	1.68
Zvanets 1999	52.03°N	24.81°E	28	11.00	0.35	-19.55 ^a	0.73	-70.36	1.65
Supoy 2000	50.40°N	31.75°E	17	10.84	0.38	-19.73	0.83	-75.34	2.47
Uday 1999	50.87°N	32 12°E	8	11.23	0.34	-19.48	1.27	-69.71	2.00
Uday 2000			13	11.41	0.38	-19.25	0.94	-79.62	1.93
Uday combined			21	11.34	0.27	-19.34 ^b	0.74	-75.85	1.75

^a ($T=2.791; P=0.065$)

^b ($T=2.807; P=0.063$)



Fig. 1 Sampling sites locations of aquatic warblers in Europe; Karsibor (Poland), Dikoe, Yasaelda and Zvanets (Belarus), and Supoy and Uday (Ukraine)

where δ_{sample} is the isotope ratio of the sample relative to a standard, R_{sample} and R_{standard} are the fractions of heavy to light isotopes in the sample to standard respectively.

Feathers were washed in a 0.25 M sodium hydroxide solution followed by two separate washes in distilled water. Samples were dried at 50°C for 12 h. Whilst most isotopes, including C and N, are stable after feather formation, a proportion of the hydrogen is exchangeable with water/water vapour in the environment. Chamberlain et al. (1997) measured this experimentally and found it to be about 13%. Consequently, all feathers were equilibrated with laboratory water vapour for 2 weeks prior to analysis, and control samples were analysed along with each batch to control for any temporal variation in δD in laboratory water vapour.

Feathers were clipped lengthwise into fine sections in the sample vials using surgical scissors. The clipped feather contained sections of up to 2 mm in length. This was done to ensure an integrated isotopic signal representing diet during the whole period of feather growth, rather than during the growth period of one section of the feather.

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were analysed using EA-C-IRMS (elemental analyser combustion isotope ratio mass spectrometry). NBS-1577a bovine liver [National Bureau of Standards (NBS), United States] was used as a reference material, as it has similar isotopic and elemental composition to the feathers. NBS-1577a has ~50% C and ~10% N with an isotope signature of -21.68‰ $\delta^{13}\text{C}$ versus PDB (Protein Data Bank) and +7.25‰ $\delta^{15}\text{N}$ versus air. NBS-1577a was

comparisons suggested that the major differences lie between Karsibor and Zvanets and between Karsibor and Uday (see table notes)

calibrated against IAEA-CH6 sucrose ($\delta^{13}\text{C}_{\text{V-PDB}}-10.4\text{‰}$) for $\delta^{13}\text{C}$ and IAEA-N1 ammonium sulphate ($\delta^{15}\text{N}_{\text{AIR}}+0.4\text{‰}$) for $\delta^{15}\text{N}$, [reference standards, International Atomic Energy Agency (IAEA), Vienna]. Each feather sample (0.5 mg) was analysed once. Duplicate subsamples were analysed for each of four feathers on separate occasions, with a mean standard deviation of 0.20‰ for $\delta^{15}\text{N}$ and 0.20‰ for $\delta^{13}\text{C}$. Test samples of NBS-1577a were run along with the feather samples to confirm that the system was performing to specifications. All test data was collated from the 3 days of analysis. Test sample mean signatures were $+7.15\text{‰}\pm 0.27\text{‰}$ ($n=24$) for $\delta^{15}\text{N}$ and $-21.69\text{‰}\pm 0.04\text{‰}$ ($n=24$) for $\delta^{13}\text{C}$.

Deuterium analyses were conducted using EA-Pyr-IRMS (elemental analyser pyrolysis isotope ratio mass spectrometry). NBS-22 mineral oil (reference standards, IAEA) was used as a reference material. NBS-22 has a signature of $-118.5\text{‰}\delta^{-2}\text{H}$ versus SMOW (standard mean ocean water). Each feather sample (1.0 mg) was analysed once. Test samples of NBS-22 and PEF-1 (polyethylene, IAEA +100.3‰) were run along with the feather samples to confirm that the system was performing to specifications. All test data was collated from 3 days of analysis. Test sample mean signatures were $-115.2\text{‰}\pm 3.0\text{‰}$ ($n=12$) for NBS-22 and $-100.5\text{‰}\pm 2.9\text{‰}$ ($n=12$) for PEF-1.

All isotope analyses were performed on an ANCA-GSL sample preparation module and GEO 20–20 IRMS, Europa Scientific, Crewe England. All analysis was performed at Iso-Analytical, Sandbach, Cheshire.

Results

We tested the null hypothesis that birds from different breeding subpopulations form a single mixed population on the wintering grounds. This implies that there will be no difference in the isotope space (means and variances) occupied by each breeding subpopulation. Data were analysed using general linear models (GLM), with ‘site’, and ‘sex’ as factors with six and two levels respectively. Minimum adequate models were obtained by sequential removal of non-significant ($P>0.05$) factors.

$\delta^{13}\text{C}$ data from Zvanets (Anderson-Darling $\text{Asq.}=1.19$, $P=0.003$) and δD for Dikoe ($\text{Asq.}=0.73$, $P=0.04$) showed a significant departure from normality whereas data for all other isotopes and all sites were normally distributed.

Our data were gathered during 1999 and 2000. There may be between-year variation in isotope ratios, however, we have values for both years for only one site: Uday. At Uday, there was a significant year effect on δD ratios (sex $F_{1,18}=0.23$, $P=0.64$; year $F_{1,19}=11.5$, $P=0.003$), but not on $\delta^{15}\text{N}$ (sex $F_{1,19}=1.17$, $P=0.29$; year $F_{1,18}=0.38$, $P=0.55$) and $\delta^{13}\text{C}$ (sex $F_{1,19}=0.62$, $P=0.44$; year $F_{1,18}=0.27$, $P=0.73$;) ratios. For $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, we therefore assume that there are no year effects, and we pool data from 1999 and 2000 in subsequent analyses. For δD , it is impossible to estimate year-effects for the other five sites, since we have no way of assessing whether the observed year-effect applies across sites. Hence, for δD , we analyse data from 1999 and 2000 separately.

Between-site differences in mean isotope signatures

There was no between breeding site variation in $\delta^{15}\text{N}$ of winter-grown feathers (GLM, sex $F_{1,129}=2.18$, $P=0.14$;

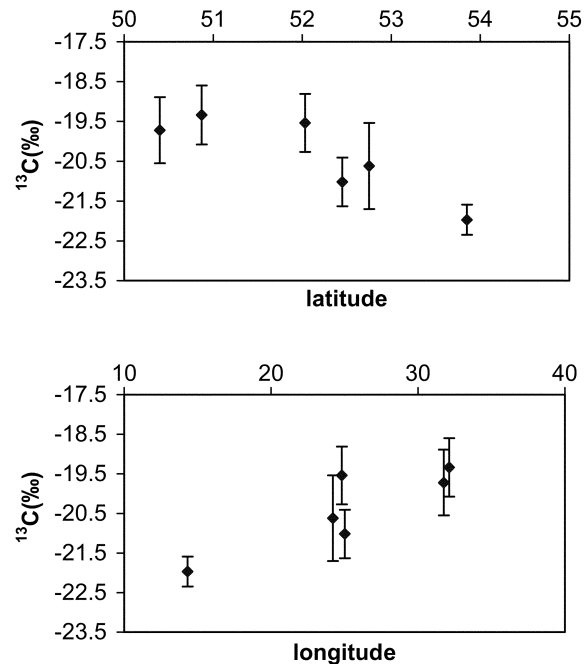


Fig. 2 Mean $\delta^{13}\text{C}$ of the rectrices of adult aquatic warblers in relation to the latitude ($^{\circ}\text{N}$) and longitude ($^{\circ}\text{E}$) of their European breeding sites. General linear models (GLM) weighted by $1/\text{SEM}$, latitude $F_{1,4}=18.2$, $P=0.013$; GLM weighted by $1/\text{SEM}$, longitude $F_{1,4}=19.0$, $P=0.012$

site $F_{5,124}=0.73$, $P=0.60$). Neither was there between breeding-site variation in δD of winter-grown feathers (GLM, year=1999; sex $F_{1,69}=0.01$, $P=0.92$; site $F_{3,72}=0.35$, $P=0.79$. year=2000; sex $F_{1,53}=0.22$; site $F_{2,54}=1.00$, $P=0.38$).

^{13}C ratios of winter grown feathers did differ between breeding sites (GLM, sex $F_{1,124}=0.02$, $P=0.88$; site $F_{5,127}=2.29$, $P=0.034$) (Fig. 2). Tukey pairwise comparisons suggested that the major differences lie between Karsibor and Zvanets ($T=2.791$; $P=0.065$) and between Karsibor and Uday ($T=2.807$; $P=0.063$).

Between-site differences in variances of isotope signatures

ANOVA test for equality of variance using all sites found significant site differences for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Bartlett's test statistic=11.49, $P=0.04$ for $\delta^{13}\text{C}$; 11.54, $P=0.04$ for $\delta^{15}\text{N}$). No significant differences in variance existed between sites for δD in 1999 or 2000 (Bartlett's test statistic=1.96, $P=0.58$ in 1999; 1.85, $P=0.40$ in 2000).

To examine where site differences in variance lay, an F -Test was used to compare all sites except $\delta^{13}\text{C}$ at Zvanets and δD at Dikoe, where Levene's test was used.

Birds from Karsibor had significantly lower variance in $\delta^{13}\text{C}$ than birds from any other site (Levene's test statistic=6.368, $P=0.015$ for Zvanets; $F=0.35$ –2.96, $P=0.008$ –0.02 for all other sites). Birds from Karsibor also had significantly lower variance in $\delta^{15}\text{N}$ than birds from Yaselda, Supoy and Zvanets ($F=0.28$ –2.58,

$P=0.002-0.05$). No other significant between-site differences in variance were found

Relationship between winter $\delta^{13}\text{C}$ signatures and breeding location

Figure 2 shows a relationship between the geographical location of breeding subpopulations and their winter $\delta^{13}\text{C}$ signatures. As latitude and longitude of the sampling sites were highly correlated ($r=-0.959$, $P=0.003$), it was impossible to determine the true geographical axis along which the observed variation lies. Mean winter $\delta^{13}\text{C}$ signatures for each site were highly significantly related to both latitude (GLM weighted by $1/\text{SEM}$, latitude $F_{1,4}=18.2$, $P=0.013$), and longitude (GLM weighted by $1/\text{SEM}$, longitude $F_{1,4}=19.0$, $P=0.012$).

Discussion

Between-site differences in mean isotopic signatures

No between site differences in $\delta^{15}\text{N}$ were found. The isotopic composition of an animal is enriched in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ relative to its diet; more so with $\delta^{15}\text{N}$ (de Niro and Epstein 1978; Mizutani et al., 1992); within biomes, there is a broadly stepwise enrichment in $\delta^{15}\text{N}$ with trophic level (Hobson 1999b). The similar $\delta^{15}\text{N}$ values for different breeding subpopulations of aquatic warblers suggests that they are all feeding at a similar trophic level during moult in Africa.

High variance and inter-annual differences in δD make values difficult to interpret. Mean δD did not differ between subpopulations. Whilst significant intra-annual differences in δD between populations may indicate different feather growth locations, birds with similar δD are not necessarily growing their feathers in the same place. Unlike most of Europe and the Americas, where distinct and predictable geographical variation in δD occurs (e.g. latitudinal trends in the United States, Rubenstein et al. 2002), similar δD signatures can be found at many different latitudes in Africa (IAEA 2001). Significant annual variation in δD for birds from Uday, which had near identical $\delta^{13}\text{C}$ and δN signatures between years, suggest that δD may be of limited use for identifying wintering locations or separating subpopulations of aquatic warblers in Africa.

The most interesting result is the difference in $\delta^{13}\text{C}$ of winter-grown feathers between different breeding subpopulations (Table 1). There are several possible interpretations of this result; different subpopulations could: (1) moult in areas of different habitat type, (2) moult at similar locations but with a temporal separation (3) moult at different latitudes.

Plants with different photosynthetic pathways (C3, C4 and CAM) have different $\delta^{13}\text{C}$ signatures (Smith and Epstein 1971); within an area, the proportions of C3/C4 plants in a habitat type will confer a characteristic $\delta^{13}\text{C}$

signature to the habitat and the animals using it (Hobson 1999a). We consider it unlikely that differences in mean aquatic warbler $\delta^{13}\text{C}$ signatures results from the use of different habitat types during moult, as they are habitat specialists (de By 1990; AWCT 1999), using fen mires and stands of reed and sedge on their breeding grounds, and similar habitats on migration. Studies of other *Acrocephalus* warblers have found species-specific patterns of habitat selection during post-breeding migration to be very similar to those during the breeding season (Honza and Literák 1997).

We do not know the chronology of moult of different aquatic warbler subpopulations. Consequently, a second potential explanation is that some subpopulations arrive south of the Sahara and moult earlier than others, and that differences in $\delta^{13}\text{C}$ reflect temporal changes in environmental $\delta^{13}\text{C}$ (possibly from a similar moulting location). $\delta^{13}\text{C}$ values in plants could change in response to changing environmental factors, such as temperature and light intensity (Smith et al. 1976), water stress (Bowman et al. 1989) or relative humidity (Madhavan et al. 1991; Lipp et al. 1998). For this to explain our $\delta^{13}\text{C}$ differences would require that environmental factors change rapidly and fairly consistently, and that this is translated into significant changes in the $\delta^{13}\text{C}$ of plants and their invertebrate consumers within the potential moulting period of aquatic warblers. Whilst this possibility cannot be discounted, it appears unlikely; we have no evidence to support or refute this hypothesis.

A final interpretation of the variation in $\delta^{13}\text{C}$ is that different subpopulations moult at latitudes with different proportions of C3/C4 plants. A recent modeling exercise has produced a global carbon isotope map showing strong latitudinal trends in $\delta^{13}\text{C}$; simulated annual mean $\delta^{13}\text{C}$ in terrestrial plants decreases with latitude from around 10°N (Suits et al., unpublished data. Simulated annual mean $\delta^{13}\text{C}$ in terrestrial plants can be accessed on http://biocycle.atmos.colostate.edu/html/stable_isotope_biogeochemistry.html; personal communication). This trend broadly reflects the global distributions of C3 and C4 plants with a band of predominantly C4 plants at around 10°N (Still et al. 2003). C4 plants have more positive $\delta^{13}\text{C}$ signatures (mean of -13) than C3 (mean of -27 ; Smith and Epstein 1971). Juvenile aquatic warblers breeding in Europe ($52^\circ27'\text{N}$) had mean $\delta^{13}\text{C}$ signatures of -24.4‰ ($n=12$, authors' unpublished data), supporting inverse latitudinal trends in $\delta^{13}\text{C}$ when compared with wintering $\delta^{13}\text{C}$ signatures (Table 1). In a recent review paper, Kelly (2000) found that $\delta^{13}\text{C}$ signatures in carnivores decreased with latitude, probably reflecting an inverse relationship between the proportion of carbon in the food chain fixed by C4 plants and latitude. Similarly, Chamberlain et al. (1997) found that $\delta^{13}\text{C}$ decreased systematically with latitude in black-throated blue warblers *Dendroica caerulescens* in North America. We are only aware of one paper documenting isotope ratios in the feathers of passerines grown in sub-Saharan Africa (Chamberlain et al. 2001). These authors found that sub-species of willow warblers *Phylloscopus trochilus* breeding at different

latitudes in Europe, and known from ringing studies to winter at different latitudes in Africa, had isotopically distinct $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures.

The wide range of $\delta^{13}\text{C}$ signatures found in aquatic warbler feathers (95% confidence intervals of -13.74 to -27.13) suggest that these subpopulations may be moulting across a rapidly changing gradient of $\delta^{13}\text{C}$ signatures. Predicted C3/C4 plant distributions for sub-Saharan Africa suggest that a rapid gradient is likely to occur at between approximately 5 – 15°N (Still et al. 2003; Suits et al., unpublished data; personal communication). Birds from Karsibor, with a minimum $\delta^{13}\text{C}$ of -16.92 and the lowest mean $\delta^{13}\text{C}$ value (-21.97 , Table 1), are likely to moult to the north of other subpopulations. Independent evidence supports the idea that Polish birds are isolated in some way from other populations. A recent study using microsatellite analyses showed only a limited amount of gene flow between the northwest Polish and the more easterly populations (B. Gießing, author's unpublished data). The most parsimonious interpretation of our results appears to be that different aquatic warbler subpopulations are moulting at different latitudes.

For the six subpopulations studied, there was a strong relationship between $\delta^{13}\text{C}$ signatures of feathers grown on the wintering grounds and the location (latitude and longitude) of breeding grounds (Fig. 2). This provides compelling evidence for a link between the location of breeding and wintering grounds. Along with data on latitudinal trends in $\delta^{13}\text{C}$, this also suggests a possible leapfrog migration pattern. Only one easterly migration route in autumn is known for this species, and our results suggest that birds from the east (Ukraine) travel further south in Africa than birds from the northwest (Karsibor, Poland). Kelly et al. (2002) have similarly used isotopic signals (δD) in feathers to imply leapfrog migration in Wilson's warbler *Wilsonia pusilla*.

Between-site differences in variances of isotopic signatures

Aquatic warblers from Karsibor had lower variance in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than birds from most other sites. These differences could reflect lower isotopic heterogeneity at the moulting site of Karsibor birds. Isotope signatures can vary over small spatial scales due to processes like microbial degradation and methanotrophy, or localised eutrophication, particularly in freshwater wetlands (Hornibrook et al. 2000; Rice 2000; Lake et al. 2001).

Alternatively, Karsibor birds may be less geographically dispersed during moult. Some *Acrocephalus* spp. appear to exhibit site-fidelity in Africa between and within winters (King and Hutchinson 2001). Whilst we do not know the extent of wintering site-fidelity in aquatic warblers, a subpopulation wintering in a small, discrete area would be particularly vulnerable to changes in the wintering area.

Conclusions

We conclude that aquatic warbler subpopulations across Europe do not form a single mixed wintering population. There is a strong relationship between the latitude/longitude of breeding sites and the $\delta^{13}\text{C}$ of wintering sites; birds breeding further north/west in Europe have the most negative $\delta^{13}\text{C}$ signatures. We believe that the most likely explanation for this is that birds breeding further north/west in Europe moult, and possibly winter, further north in sub-Saharan Africa.

The relatively isolated northwesterly breeding population in Poland not only has the most negative $\delta^{13}\text{C}$ signatures, but also has the least variance in $\delta^{13}\text{C}$, thus may moult in a more geographically restricted area than its eastern (Belarussian and Ukrainian) counterparts, or in an area with less isotopic heterogeneity. This could have conservation implications as populations that show strong migratory connectivity are likely to have little genetic variation for migratory behaviour, and as such they may be less able to respond to changing selection pressures, such as those that could result from climate change (Both and Visser 2001; Webster et al. 2002). This is a particular threat for palearctic migrants that winter south of the Sahara, as drought in sub-Saharan west Africa may restrict the availability or quality of wintering habitat (Cave 1983; Kanyamibwa et al. 1990), or increase the size of the Sahara–Saudia Arabian desert belt that they have to cross. Birds wintering in more geographically restricted areas are also more vulnerable to habitat destruction or alteration. Identifying the moulting/wintering grounds of this subpopulation is of high priority for the conservation of the species and its genetic diversity. It is notable that the Pomeranian subpopulation has declined more rapidly than other subpopulations since 1990, from approximately 400 to 80 singing males (Aquatic Warbler Conservation Team data, personal communication).

Further work using common, sedentary, insectivorous African wetland passerines (e.g. winding cisticola, *Cisticola galactotes*) as aquatic warbler surrogates is underway to help 'fingerprint' wetland areas at different latitudes in sub-Saharan Africa. This, and the use of multiple isotopes, may help identify aquatic warbler wintering areas.

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References

- Atienza JC, Pinilla J, Justríbó JH (2001) Migration and conservation of the aquatic warbler *Acrocephalus paludicola* in Spain. *Ardeola* 48:197–208
- AWCT–Aquatic Warbler Conservation Team (1999) World population, trends and conservation status of the aquatic warbler *Acrocephalus paludicola*. *Vogelwelt* 120:65–85

- Bensch S, Hasselquist D, Hendenström A, Ottosson U (1991) Rapid moult among palearctic passerines in West Africa—an adaptation to the oncoming dry season? *Ibis* 133:47–52
- BirdLife International (2000) Threatened birds of the world. Lynx Ediciones/BirdLife International, Barcelona and Cambridge
- Both C, Visser ME (2001) Adjustment to climate change is constrained by arrival date in a long-distance migrant bird. *Nature* 411:296–298
- Bowman WD, Hubick KT, von Caemmerer S, Farquhar GD (1989) Short term changes in leaf carbon isotope discrimination in salt and water-stressed C₄ grasses. *Plant Physiol* 90:162–166
- By RA de (1990) Migration of aquatic warbler in western Europe. *Dutch Bird* 12:165–181
- Cave AJ (1983) Purple heron survival and drought in tropical West Africa. *Ardea* 71:217–224
- Chamberlain CP, Blum JD, Holmes RT, Feng X, Sherry TW, Graves GR (1997) The use of isotope tracers for identifying populations of migratory birds. *Oecologia* 109:132–141
- Chamberlain CP, Bensch S, Feng X, Akesson S, Anderso T (2001) 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
- Cherel Y, Hobson KA, Weimerskirch H (2000) Using stable isotope analysis of feathers to distinguish moulting and breeding origins of seabirds. *Oecologia* 122:155–162
- Cramp S (ed) (1992) The birds of the western Palearctic, vol 6. Oxford University Press, Oxford
- Curry-Lindahl K (1981) Bird migration in Africa, vol. I. Academic, London
- DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 42:495–506
- Esler D (2000) Applying metapopulation theory to conservation of migratory birds. *Conserv Biol* 14:366–372
- Hendenström A, Bensch S, Hasselquist D, Ottosson U. (1990) Observations of palearctic migrants rare to Ghana. *Bull Br Ornithol Club* 110:194–197
- Hobson KA (1999a) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120:314–326
- Hobson KA (1999b) Stable-carbon and nitrogen isotope ratios of songbird feathers grown in two terrestrial biomes: implications for evaluating trophic relationships and breeding origins. *Condor* 101:799–805
- Hobson KA, Clarke RW (1992a) Assessing avian diets using stable isotopes. I. Turnover of carbon-13. *Condor* 94:181–188
- Hobson KA, Clarke RW (1992b) Assessing avian diets using stable isotopes. II. Factors influencing diet-tissue fractionation. *Condor* 94:189–197
- Hobson KA, Wassenaar LI (1997) Linking breeding and wintering grounds of neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. *Oecologia* 109:142–148
- Honza M, Literák I (1997) Spatial distribution of four *Acrocephalus* warblers in reedbeds during the post-breeding migration. *Ring Migr* 18:79–83
- Hornibrook ERC, Longstaffe FJ, Fyfe WS (2000) Factors influencing stable isotope ratios in CH₄ and CO₂ within subenvironments of freshwater wetlands: implications for delta-signatures of emissions. *Isot Environ Health Stud* 36:151–176
- IAEA (2001) GNIP maps and animations. International Atomic Energy Agency, Vienna. <http://isohis.iaea.org>
- Kanyambwa S, Schreider A, Pradel R, Lebreton JD (1990) Changes in adult annual survival rates in a western European population of the white stork *Ciconia ciconia*. *Ibis* 132:27–35
- Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Can J Zool* 78:1–27
- Kelly JF, Atudorei V, Sharp ZD, Finch DM (2002) Insights into Wilson's warbler migration from analyses of hydrogen stable-isotope ratios. *Oecologia* 130:216–221
- King JMB, Hutchinson JMC (2001) Site fidelity and recurrence of some migrant bird species in the Gambia. *Ring Migr* 20:292–302
- Lake JL, McKinney RA, Osterman FA, Pruell RJ, Kiddon J, Ryba SA, Libby AD (2001) Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems. *Can J Fish Aquat Sci* 58:870–878
- Lipp J, Trimborn P, Payer H-D (1998) Influence of relative humidity on the ¹³C content in bean plants (*Vicia faber*): a model experiment in exposure chambers. In: International Atomic Energy Agency proceedings series; isotope techniques in the study of environmental change. IAEA 14–18 April 1997, Vienna, pp 878–881
- Madhavan S, Treichel I, O'Leary MH (1991) Effects of relative humidity on carbon isotope fractionation in plants. *Botan Acta* 104:292–294
- Mizutani H, Fukuda, M, Kabaya, Y (1992) ¹³C and ¹⁵N enrichment factors of feathers of 11 species of adult birds. *Ecology* 73:1391–1395
- Moreau RE (1972) The Palearctic–African bird migration systems. Academic, London
- Rice SK (2000) Variation in carbon isotope discrimination within and among sphagnum species in a temperate wetland. *Oecologia* 123:1–8
- Romanek CS, Gaines KF, Bryan Jr AL, Brisbin Jr IL (2000) Foraging ecology of the endangered wood stork recorded in the stable isotope signature of feathers. *Oecologia* 125:584–594
- Rubenstein DR, Chamberlain CP, Holmes R, Ayres MP, Waldbauer JR, Graves GR, Tuross NC (2002) Linking breeding and wintering ranges of a migratory songbird using stable isotopes. *Science* 295:1062–1065
- Smith BN, Epstein S (1971) Two categories of ¹³C/¹²C ratios for higher plants. *Plant Physiol* 47:380–384
- Smith BN, Oliver J, McMillan C (1976) Influence of carbon source, oxygen concentration, light intensity and temperature on ¹²C/¹³C ratios for higher plants. *Bot Gaz* 137:99–104
- Still CJ, Berry JA, Collatz GJ, DeFries RS (2003) Global distribution of C3 and C4 vegetation: carbon cycle implications. *Global Biogeochem Cycle* 17:1006–1029
- Svensson L (1984) Identification guide to European passerines, 3rd edn. Stockholm, British Trust for Ornithology, Thetford
- Thompson DR, Furness RW (1995) Stable isotopes ratios of carbon and nitrogen in feathers indicate seasonal dietary shifts in northern fulmars. *Auk* 112:493–498
- Wassenaar LI, Hobson KA (2000) Stable carbon and hydrogen isotope ratios reveal breeding origins of red-winged blackbirds. *Ecol Appl* 10:911–916
- Wassenaar LI, Hobson KA (2001) A stable isotope approach to delineate geographical catchment areas of avian migration monitoring stations in North America. *Environ Sci Technol* 35:1845–1850
- Webster MS, Marra PP, Haig SM, Bensch S, Holmes RT (2002) Links between worlds: unraveling migratory connectivity. *Trends Ecol Evol* 17:76–83