# Overwintering habitat links to summer reproductive success: intercontinental carry-over effects in a declining migratory bird revealed using stable isotope analysis

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Running heading: Winter habitat links to summer breeding success

Summary

**Capsule**: Breeding success in female Pied Flycatchers *Ficedula hypoleuca* is related to isotopic signature of feathers grown in Africa, suggesting wintering habitat links to breeding performance 5000km away.

**Aims**: Better understanding of interseasonal carry-over effects is a research priority, especially for declining migrants. We use stable isotope analysis to relate Pied Flycatcher winter habitat to summer reproductive success.

**Methods**: Flycatchers were captured in three UK woodlands in 2013-2015. An Africa-grown tertial was trimmed and analysed using Isotope Ratio Mass Spectrometry to quantify Nitrogen-15 ($\delta^{15}$N) and Carbon-13 ($\delta^{13}$C). In total, 135 samples were taken from 80 individuals.

**Results**: Wintering $\delta^{15}$N and $\delta^{13}$C differed significantly between years. $\delta^{13}$C correlated with lay date, such that birds with lower carbon levels (indicative of more mesic habitat) bred earlier. There was a significant correlation between wintering $\delta^{13}$C and productivity after allowing for year, site, and lay date; birds with low $\delta^{13}$C were more successful. This suggests $\delta^{13}$C links productivity directly as well as indirectly through phenological effects. $\delta^{15}$N did not relate to phenology or productivity.

**Conclusion**: This is the first evidence of carry-over effects between geographical regions for a European passerine. Conservation measures should focus on all aspects of seasonal cycles, not just breeding grounds.

**Keywords**: Breeding success, Carbon-13, *Ficedula hypoleuca*, Migratory ecology, Nitrogen-15, Pied Flycatcher, Stable isotope analysis.

Introduction

Migratory species have complex life-histories, which are influenced by abiotic and biotic factors at breeding grounds, over-wintering sites and during migration. This geographical complexity means that migrants are often more sensitive to environmental and climatic change than resident species (Newton 2008). Indeed, Afro-Palaearctic migrants (i.e. birds that breed in Europe and winter in sub-Saharan Africa) are currently undergoing population decline more regularly, more severely, and more rapidly, than resident or short-distance migrant species (Sanderson et al. 2006). The proximate reasons for such declines are often not fully understood. This is symptomatic of the general lack of knowledge regarding the ecology of migrant species when they are not at their breeding grounds (Gregory et al. 2005; Morrison et al. 2013). Improving ecological knowledge of long-distance migrants throughout their entire annual cycle is important in developing appropriate conservation strategies and has thus been highlighted as a research priority, especially in the face of rapid climatic change (Holmes 2007; Goodenough et al. 2009a; Kelly and Horton 2016).

Although ecologists have known for many years that the different phases of a migrant's annual cycle are co-dependent (Fretwell 1972), studies in the last decade or so have provided increasing evidence that conditions experienced on wintering grounds are a key determinant of population dynamics through their effect on survival, condition, and future reproductive success (e.g. Saino et al. 2004; Eraud et al. 2009; Evans et al. 2012; Leyrer et al. 2013). These carry-over effects can be hard to study because of limitations in tracking individual birds. For many species, there are very poor ringing recovery data and using satellite trackers is currently impossible on many small passerines due to tracker weight. One alternative approach is to use stable isotope analysis to provide valuable insights into the ecology of species that are poorly-studied in parts of their migratory range (Norris and Marra 2007). Unlike living tissue, feathers are keratinised and are thus metabolically inert once grown. This means feather stable isotope composition reflects the bird's location, habitat and diet at the time of growth (Kelly 2000; Inger and Bearhop 2008; Eduardo et al. 2010). Depending on moult strategy, it can be possible to quantify the stable isotope profile of winter-grown feathers when birds are captured on breeding grounds, thus enabling insight into wintering conditions while studying breeding ecology and allowing direct linkages to be made. This approach has been used to enhance knowledge about migration in several Neotropical-Nearctic migrants such as Wilson's Warbler *Wilsonia pusilla* and Red Knot *Calidris canutus* (Kelly et al. 2002; Atkinson et al. 2005), as well as Afro-Palearctic migrants including Aquatic Warblers *Acrocephalus paludicola* (Oppel et al. 2011), Marsh Warblers *A. palustris* and Whitethroats *Sylvia communis* (Yohannes et al. 2005), and Willow Warblers *Phylloscopus trochilus* (Morrison et al. 2013).

To date, the majority of studies have used feather-derived isotopic profiles have been used in a biogeographical context to identify likely wintering grounds or migratory stop-over areas (and, ergo, flyways). This is often undertaken through analysis of Hydrogen-2 stable isotopes (amount of $^{2}H$ relative to $^{1}H$, referred to as deuterium or $\delta D$), which can be used to infer latitude, continentality and altitude where spatial patterns of precipitation are known (e.g. Kelly et al. 2002).

However, isotope profiles can also provide information on carry-over effects between wintering and breeding ground or vice versa (Norris and Marra 2007; Inger and Bearhop 2008; Oppel et al. 2011). There are two elements where stable isotope ratios can provide particularly-used insights for migratory birds:

**Carbon**: analysis of Carbon-13 stable isotopes (amount of $^{13}C$ relative to $^{12}C$; referred to as $\delta^{13}C$) can be useful in establishing habitat type, especially habitat moisture (Kelly 2000). This is because the dominant control of $\delta^{13}C$ within animal tissue is the relative proportion of plants using water-inefficient C3 or water-efficient C4 biochemical photosynthetic pathways in the locale, upon which a food chain is founded (Inger and Bearhop 2008; Paxton and Moore 2015). C3-dominated habitats tend to be more mesic, whereas C4-dominated habitats tend to be more xeric. Feather-derived $\delta^{13}C$ values have been used previously in avian research as a proxy for whether wintering habitat is mesic or xeric (e.g. Paxton and Moore (2015) for Black-and-White Warblers *Mniotilta varia*).

**Nitrogen**: analysis of Nitrogen-15 stable isotopes (amount of $^{15}N$ relative to $^{14}N$; referred to as $\delta^{15}N$) can be useful for assessing diet, particularly in relation to tropic level (Kelly 2000; Inger and Bearhop 2008). In insectivorous birds, this can indicate whether birds are primarily feeding on herbivorous, predatory or detritivorous invertebrates. In terrestrial environments, $\delta^{15}N$ can also indicate water stress, at least in mammals, where $\delta^{15}N$ level increases (Kelly 2000).

Carbon isotope signatures from wintering sites have been found previously to correlate significantly with condition of birds immediately prior to departure for breeding grounds (American Redstarts *Setophaga ruticilla* – Marra et al. 1998) or upon arrival at breeding grounds (Black-throated Blue Warblers *Dendrocia caerulescens* – Bearhop et al. 2004). Significant correlations have been found between $\delta^{13}C$ and $\delta^{15}N$ isotope signatures and: (1) lay date (American Redstarts – Norris et al. 2004; Cassin's Auklets *Ptychoramphus aleuticus* – Sorensen et al. 2009); (2) egg volume (Cassin's Auklets - Sorensen et al. 2009); (3) likelihood of raising chicks (Black-tailed Godwit - Gunnarsson et al. 2005); (4) number of young (American Redstarts – Norris et al. 2004); and (5) condition of offspring (Barn Swallows *Hirundo rustica* - Møller and Hobson 2004).

The Pied Flycatcher *Ficedula hypoleuca* is a migratory passerine that breeds in Europe and winters in sub-Saharan Africa. Comparatively little is known about wintering location and ecology. The core range thought to be Cameroon to Sierra Leone (Dowsett 2010) but information is sketchy. Despite >1 million rings being put on Pied Flycatchers between 1971 and 2008 in the UK and Sweden alone (EURING data), there were just 11 ringing recoveries in Africa of individuals on their wintering grounds during this same period (Ouwehand et al. 2016). Analysis of these recoveries, supplemented by data on 14 male Pied Flycatchers carrying light-level geolocators in 2011/12 suggests that bird occur in Ghana, Ivory Coast, Liberia, Sierra Leone, Guinea and southern Mali (Ouwehand et al. 2016). If data on location of wintering grounds are sparse, data on habitat use within breeding grounds is almost non-existent.

Salewski et al. (2002) found that Pied Flycatchers in the Ivory Coast tended to use isolated forest (66%), followed by savanna and gallery forest (wet woodland) (17% each) but there has been very little other research. This is concerning given recent declines in breeding populations in the UK and in many other European countries (Amar et al. 2006, Baillie et al. 2010; Pan-European Common Bird Monitoring Scheme 2010). Reasons for decline are poorly understood. One study in the UK found that a substantial element of the 73% population decline was due to factors extrinsic to the breeding site, including climatic variation (winter North Atlantic Oscillation and resultant precipitation in the Sahel) (Goodenough et al. 2009a), a pan-European study in the same year found that vegetation growth in the Sahal, itself linked to precipitation and NAO, was a significant predictor of lay date for European-breeding Pied Flycatchers (Both et al. 2006). Finally, a Finnish study showed that winter rainfall in the Sahel correlated with clutch size skewness on European breeding grounds, possibly due to increased survival of low-quality birds in years with high rainfall (Laaksonen et al. 2006). Together, these studies suggest that there could be important overwintering carry-over effects on survival and reproductive success.

In this study, we quantify $\delta^{13}$C and $\delta^{15}$N profiles for wintering grounds in female Pied Flycatchers breeding in the UK. Adult Pied Flycatchers undertake a complete moult on the breeding grounds, but birds of all age groups undertake a partial moult on wintering grounds; this includes all tertial (inner secondary) feathers, but not tail feathers (Lundberg and Alatalo 1992). This means that $\delta^{13}$C and $\delta^{15}$N profiles derived from the tertial feathers taken from adult birds on their breeding grounds should be related to where they undertook their partial moult the previous winter some 5000km away. After first testing to establish if there is a statistical difference in isotopic signature of feathers grown in the UK and Africa, we relate isotopic signatures both to female condition and reproductive success to test the hypothesis that there could be important carry-over effects of winter habitat use or diet on summer reproductive success. We also assess inter-annual variation in wintering (African) isotopic profiles, both at population-level and, where the same birds were captured in multiple years, at individual-level.

## Materials and methods

### Site descriptions and breeding data

Nests of Pied Flycatchers were monitored throughout the 2013 2014 and 2015 breeding seasons in three woodlands in Herefordshire and Powys on the English/Welsh border centred on 2°92’18’’W/52°13’33’’N. Three individual woodlands, about 20km apart, were used: (1) Crow Wood, Vowchurch, Herefordshire 2°55’14’’W/52°01’21’’N); (2) Mansel Lacy, Herefordshire 2°50’26’’W/52°6’18’’N); and (3) Paradise Farm, Presteigne, Powys, 3°01’49’’W/52°16’12’’N). Monitoring was undertaken weekly (by DGC) using standard British Trust for Ornithology Nest Record Scheme protocols. Clutch size, number of young to hatch, and number of young to fledge, were recorded. The date on which the first egg of each clutch

was laid was calculated by counting the eggs in an incomplete clutch and counting back the same number of days using the assumption that one egg was laid per day (Perrins and McCleery 1989). All nests were located in wooden nextboxes.

**Feather samples**

Female Pied Flycatchers were lifted from eggs during incubation for ring details to be obtained (ringed individuals) or to allow a ring to be attached (non-ringed individuals). This procedure was completed as part of normal ringing for the Re-trapping Adults for Survival (RAS) scheme and was undertaken under BTO licence. At the same time, feather samples were taken under an endorsement to the aforementioned licence, issued by the Special Methods Technical Panel of the BTO Ringing Committee in May 2013 (reissued 2014 2015).

Sampling involved cutting 1-2 cm of the outer end of the innermost tertial feather on the left wing using scissors. These feathers would have been grown on African wintering grounds following partial moult (Lundberg and Alatalo 1992) and removal of part of these feathers was considered unlikely to affect flight or the tertials' protective function in the limited time remaining prior to the complete post-breeding moult. The innermost tertial feather has been used previously for stable isotope analysis (Bearhop et al. 2004; Reichlin et al. 2010; Ouwehand et al. 2016), including Pied Flycatchers and other species with a similar partial moult strategy such as Collared Flycatchers *Ficedula albicollis* (Veen et al. 2007; Hjernquist et al. 2009). Over the three years 105 samples were taken from a total of 80 individual birds. Of these, 58 samples were from birds that were only sampled once (2013 = 14 2014 = 17 2015 = 27). In total 22 birds were sampled more than once, with 19 birds being sampled in two years (2013/14 = 8 birds (16 samples) 2014/15 = 10 birds (20 samples) 2013/15 = 1 bird (2 samples)); three birds were sampled in all three years giving 9 samples in total.

In addition to the tertial clipping, a small piece (1-2 cm) of the outer end of one tail feather was removed, again using scissors, for a sub-sample of birds. As tail feathers are not subject to the partial winter moult, these feathers would have grown during the last full moult whilst birds were on their UK breeding grounds the preceding year (Lundberg and Alatalo 1992) and so provided a UK isotopic signature for comparison purposes. Over the three years, 30 samples were taken from a total of 19 individual birds. Of these 10 samples were from birds that were only sampled once (2013 = 4 2014 = 2, and 2015 = 4), seven birds were sampled in two years (2013/14 = 5 birds (10 samples) 2014/15 = 2 birds (4 samples)); two birds were sampled in all three years giving six samples in total.

**Quantifying body condition**

During the ringing and feather-clipping process, female maximum-chord wing length was taken using a stopped ruler (± 1 mm), while weight was taken using a Pesola 0-50 g spring balance (± 0.1 g). Body condition was calculated using a Q-value index, whereby wing length (the single best measure of body size: Gosler et al. 1998) was divided by weight on the basis that birds in better condition would be heavier relative to their size than birds in poorer condition (Gosler 2004). This

metric of body condition is widely used, including in stable isotope research (Marra et al. 1998), and is less prone to intra-observer variability than other, more complex, measures of condition (Goodenough et al. 2010 and references therein). It should be noted that ideally, from a scientific perspective, body condition should be immediately after the birds returned to the breeding ground (as per Marra et al. 1998). However, as this would have involved mist netting during the crucial settlement period, when it could have disturbed female nest site choice and mate selection, we instead opted to take biometric measurements when females were lifted from eggs during incubation as part of standard ringing processes.

**Stable Isotope Analysis**

Stable isotope analysis was undertaken, in total, for 135 samples (105 Africa-grown tertial feathers for wintering signatures; 30 tail UK-grown feathers for breeding signatures). Two chemical elements were considered: Nitrogen-15 and Carbon-13 as per previous studies on migratory passerines both in Europe (e.g. Oppel et al. 2011; Evans et al. 2012; Morrison et al. 2013) and North America (Bearhop et al. 2004; Norris et al. 2004). Hydrogen/Deuterium was not analysed as it has traditionally been regarded as of limited use in isotopic studies of Afro-Palearctic migrants (Møller and Hobson 2004; Oppel et al. 2011; but see recent study by Veen et al. 2014). Analysis was conducted though ISO-Analytical (Crewe, UK) using Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS).

Before processing, feather samples were washed once in 0.25M sodium hydroxide solution and twice in purified water before being oven-dried for 12 hrs at 50 °C. Dried feathers were cut into fine sections using surgical scissors. Feather samples (and reference samples – see below) were placed into tin capsules, sealed, and loaded into a Europa Scientific elemental analyzer, from where they were dropped, in sequence, into a furnace held at 1000 °C. Each capsule flash-combusted, exposing the sample contained therein to ~1700 °C. The resultant combusted gases ($N_2$, $NO_x$, $SO_2$, $H_2O$, $O_2$, and $CO_2$) were swept via a helium stream through several processing chambers. Firstly, to remove sulphur and halides, gases were passed over combustion catalyst ($Cr_2O_3$), copper oxide wires (to oxidize hydrocarbons), and silver wool. Secondly, to remove any $O_2$, and convert $NO_x$ to $N_2$, gases were passed over pure copper wires held at 600 °C. Finally, to desiccate the combusted gases by removing residual water vapour, a magnesium perchlorate chemical trap was used. After this, the combusted gases (then comprising only $N_2$ and $CO_2$) were separated using a packed-column gas chromatograph held at a constant 65 °C. The resultant $N_2$ peak entered the ion source of the Europa Scientific 20-20 IRMS first, where it was ionized and accelerated. Gases of different masses were separated in a magnetic field before being quantified using a Faraday triple cup collector array to simultaneously measure the isotopomers of $N_2$ at *m/z* 28 29, and 30. After a delay, the $CO_2$ peak entered the ion source and was, in turn, ionized and accelerated. $CO_2$ gases were separated and measured in the same way as $N_2$, but using isotopomers of $CO_2$ at *m/z* 44, 45, and 46.

Reference samples were loaded into the analyser such that they were processed before the first feather sample, after the last feather sample, and at regular intervals between feather samples (every third sample). The reference

materials used were: (1) IA-R042 (NBS-1577B, powdered bovine liver, $\delta^{13}C_{V\text{-}PDB}$ = -21.60 ‰, $\delta^{15}N_{AIR}$ = 7.65 ‰); (2) a mixture of IA-R005 (beet sugar, $\delta^{13}C_{V\text{-}PDB}$ = -26.03 ‰) and IA-R045 (ammonium sulphate, $\delta^{15}N_{AIR}$ = -4.71 ‰); and (3) a mixture of IA-R006 (cane sugar, $\delta^{13}C_{V\text{-}PDB}$ = -11.64 ‰) and IA-R046 (ammonium sulphate, $\delta^{15}N_{AIR}$ = 22.04 ‰) as per previous analysis on feather samples from migratory passerines (Oppel et al. 2011). All reference samples were calibrated to International Atomic Energy Agency (IAEA) standards IAEA-CH-6 and IAEA-N-1. As is typical, stable isotope ratios were reported in delta $\delta$ notation as parts per million using the equation: $\delta_{sample} = [(R_{sample} / R_{standard}) -1] * 1000$

where: $\delta_{sample}$ was the isotope ratio of a sample relative to a standard, with $R_{sample}$ and $R_{standard}$ signifying the ratio of heavier to lighter isotopes in the sample and the standard, respectively. In the case of Nitrogen-15 (hereafter $\delta^{15}N$), the relevant atoms were $^{15}N$ / $^{14}N$, while in the case of Carbon-13 (hereafter $\delta^{13}C$), the relevant atoms were $^{13}C$ / $^{12}C$. $\delta^{15}N$ and $\delta^{13}C$ were standardised relative to the respective international constants: atmospheric nitrogen (AIR) for N and Vienna Pee Dee Belemnite (V-PDB) for C.

**Statistical analysis**

All statistical analysis was undertaken using SPSS version 21. Firstly, to establish whether there were differences in the wintering $\delta^{15}N$ profile (from tertial feathers) and the breeding $\delta^{15}N$ profile (from tail feathers) we used a paired samples t-test to compare feather types for the 30 birds from which both samples had been collected. This approach has been used previously for comparing isotope signatures from different feather regions of the same birds (e.g. Evans et al. 2012). The same test was used to quantify wintering/breeding differences in $\delta^{13}C$.

To examine overall inter-year variation in isotope profiles, a one-way ANOVA was used. This tested whether the mean isotopic profile for the overall population differed significantly between years. Two analyses were done for each isotope, one for African wintering signatures from tertial feathers and one for UK breeding signatures from tail feathers. Then, to test whether the wintering isotopic profile of specific individuals changed significantly over time, a paired samples t-test was undertaken for the 22 birds sampled more than once. This allowed for the possibility that any change in mean signatures (analysed using the ANOVA approach) could have been confounded by different individuals being tested in different years, or that high variability between individuals could mask annual change. In the paired analyses, the isotopic signature for the first year the bird was caught was standardised to zero and the difference to the isotopic signature in the second year was calculated. In the case of the three birds caught in three years, the first and last profiles were used. This standardisation was as per Hjernquist et al. (2009) and allowed the absolute difference to be calculated such that there was no underlying assumption of whether differences were positive or negative. This analytical approach also allowed repeat records of all 22 birds to be considered in one analysis since it de-coupled the link between an individual's profile in one year and the annual mean. Finally, we used Pearson's correlation to test for

a relationship in the isotopic profiles of the same birds in successive years. This was used in tandem with the t-test as the former tested whether the values were the same (or, more correctly, whether they differed significantly) while the second tested whether they were systematically related, either positively or negatively.

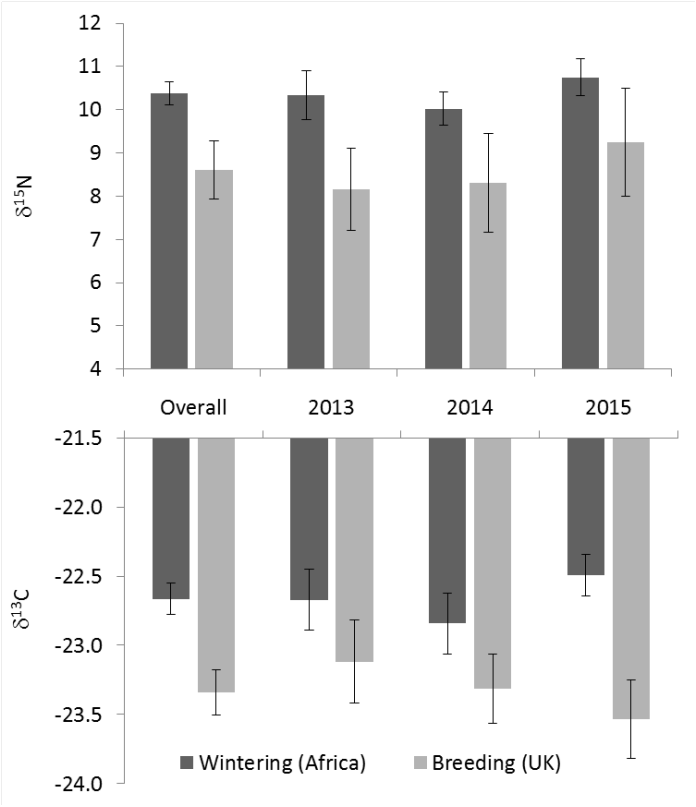
To establish whether wintering isotope signatures for $\delta^{15}$N and/or $\delta^{13}$C correlated with reproductive success the following year (i.e. whether there were carry-over effects), we ran a series of hierarchical multiple linear regression models. Several different models were constructed, each with a different breeding parameter (clutch size, number of young to hatch, number of young to fledge) entered as the dependent variable. In all cases, variables likely to affect productivity, and that thus needed to be allowed for in the analysis, were entered before isotope data using a hierarchical framework. These baseline variables were: (1) year, (2) site, (3) lay date. Isotope data for $\delta^{15}$N and $\delta^{13}$C were then added to establish if this significantly improved the model through carry-over effects. A separate model was also constructed to analyse lay date as the dependent variable with just two baseline variables: year and site. This was done because it was important to understand any link between isotopic signature and the ability of a bird to breed early (which could then indirectly affect breeding success given that early clutches are usually larger in many species, including Pied Flycatchers: Goodenough et al. 2009b) as well as then allowing for the potential effect of lay date when quantifying any direct link between isotope signatures and breeding success. In this way, lay date was regarded as a dependent variable in the one model that specifically focussed on lay date and was then included as an independent model in all analyses of productivity. Overall, this approach allowed for the temporal and spatial variation in both isotopic signatures and breeding success. This allowed us to disentangle whether any productivity-isotope relationships were due to underlying correlations between isotope profile and other independent variables (such as lay date) or were additional to any such patterns. This goes some way to de-coupling the effects of the intrinsic quality of individual birds and the extrinsic effects of wintering habitat (as quantified by stable isotope), which is a vital step in understanding what is driving observed patterns (Inger and Bearhop 2008).

## Results

### Wintering *versus* breeding profiles

There was a substantial significant difference between isotope values for both $\delta^{15}$N and $\delta^{13}$C between tertial feathers grown while wintering in Africa and tail feathers grown while breeding in the UK (paired samples t-test: t = -3.092, d.f. = 29, P = 0.004 and t = -3.681, d.f. = 29, P = 0.001, respectively). Mean wintering $\delta^{15}$N values were higher than breeding $\delta^{15}$N values (10.068‰ ± 0.239 SEM versus 8.612‰ ± 0.343 SEM), while $\delta^{13}$C showed the same pattern (-22.780‰ ± 0.100 SEM versus -23.342‰ ± 0.084 SEM) (Fig. 1). This supports the hypothesis that feathers grown by the same bird in different environments differ significantly in isotopic signature and thus suggests that using stable isotopes to study migration ecology in this species is appropriate.

**Figure 1** – Difference in isotopic profiles for tail feathers grown on breeding grounds and tertial feathers grown on wintering grounds for Nitrogen-15 and Carbon-13 in relation to International standards. Error bars show 95% CI.



**Inter-year variation**

There was no significant difference in $\delta^{15}$N and $\delta^{13}$C values between years for all birds combined, either for UK-grown or African-grown feathers ($P \geq 0.061$ in all cases). This was probably partly due to there being such a wide range of isotopic signatures between different birds in the same year (Fig. 2), giving rise to large standard errors in each individual year (Fig. 1). However, when inter-year variation was compared for birds sampled in two different years on a per-bird basis using a paired samples design, there was a significant difference for wintering and breeding $\delta^{15}$N values (paired samples t-test: $t = -4.050$, d.f. = 21, $P = 0.001$ and $t = -2.792$, d.f. = 8, $P = 0.023$, respectively) and wintering and breeding $\delta^{13}$C values (paired samples t-test: $t = -4.422$, d.f. = 21, $P < 0.001$ and $t = -3.782$, d.f. = 8, $P = 0.005$, respectively).

Despite the significant differences in $\delta^{15}$N isotopic signatures for individual birds in different years, most individual birds sampled in multiple years usually stayed fairly similarly ranked relative to the annual mean (Fig. 2a). In other words, individuals that were around the mean in one year tended to remain around the mean the following year, while those that were substantially above/below the mean tended to remain in that position. This suggested that although the isotopic profile of individual birds was significantly variable between years, individual birds exhibited a tendency to hold the same general position in relation to the overall population. This contrasted to the situation for wintering $\delta^{13}$C values (Fig. 2b) when the gradient of the line joining measurements in different years for the same bird was often steep and birds frequently moved from being substantially one side of the mean in one year to substantially the other side of the mean in subsequent year(s).

To further understand this, we correlated the winter isotope profiles of individual birds as per Hjernquist et al. (2009) using raw data. We extended this by calculating the deviation of each annual measurement from the annual mean, such that the sign of the difference denoted the direction of any change and the value denoted the magnitude of the change (further from zero = bigger change). For $\delta^{15}$N, both individual signatures in successive years using raw data and birds' deviation from the mean in successive years were significantly positively correlated (Pearson correlation $r = 0.879$, $n = 22$, $P < 0.001$ and $r = 0.804$, $n = 22$, $P < 0.001$; Fig. 3.a-b). Thus a bird with a low $\delta^{15}$N value in one year was likely to have a low $\delta^{15}$N value in another year, despite the small (but significant) changes in the values themselves and the fact that between-year changes could be in the same direction as changes in mean values, or counter to them: Fig 2a. This did not occur for $\delta^{13}$C, for which there was no significant correlation between individual signatures in successive years and birds' deviation from the mean in successive years (Pearson correlation $r = -0.95$, $n = 22$, $P = 0.673$ and $r = 0.167$, $n = 22$, $P = 0.458$; Fig. 3c-d). These last two correlations did not change when the single extreme outlier was removed and the analyses were re-run. This suggested that isotopic values themselves, and the way in which the isotopic signature of an individual bird relates to the rest of the population, were both annually variable for this element.

**Breeding success and lay date**

Wintering $\delta^{13}$C profile was a significant predictor of breeding *phenology*, as quantified by lay date, after effects of both year and site had been taken into account. The significance of the lay date model improved after wintering $\delta^{13}$C was

**Figure 2** – Comparison of isotopic signature for feathers grown in Africa on wintering grounds in different years (2013 2014 2015) for: (a) Nitrogen-15 and (b) Carbon-13. Each datapoint is an individual bird, and birds caught in multiple years are linked either with a dotted line (sampled in two years) or a solid line (sampled in three years); dots that are unlinked were sampled in one year only. Annual means are shown by black dashes.

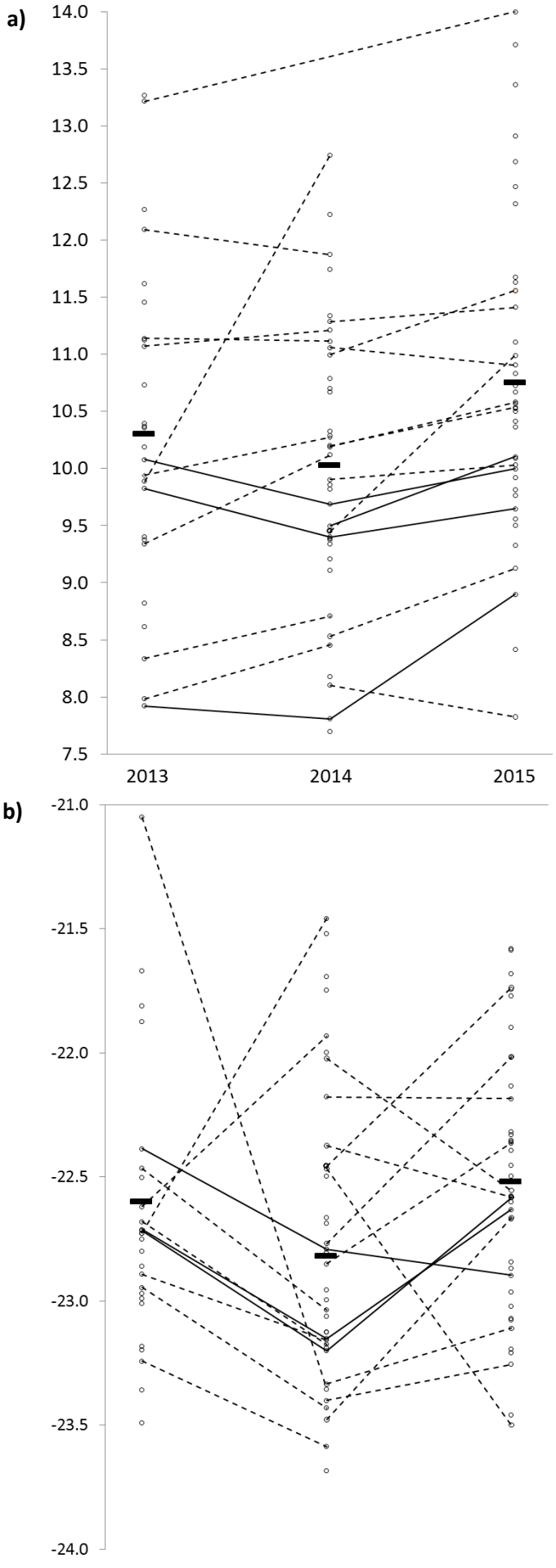
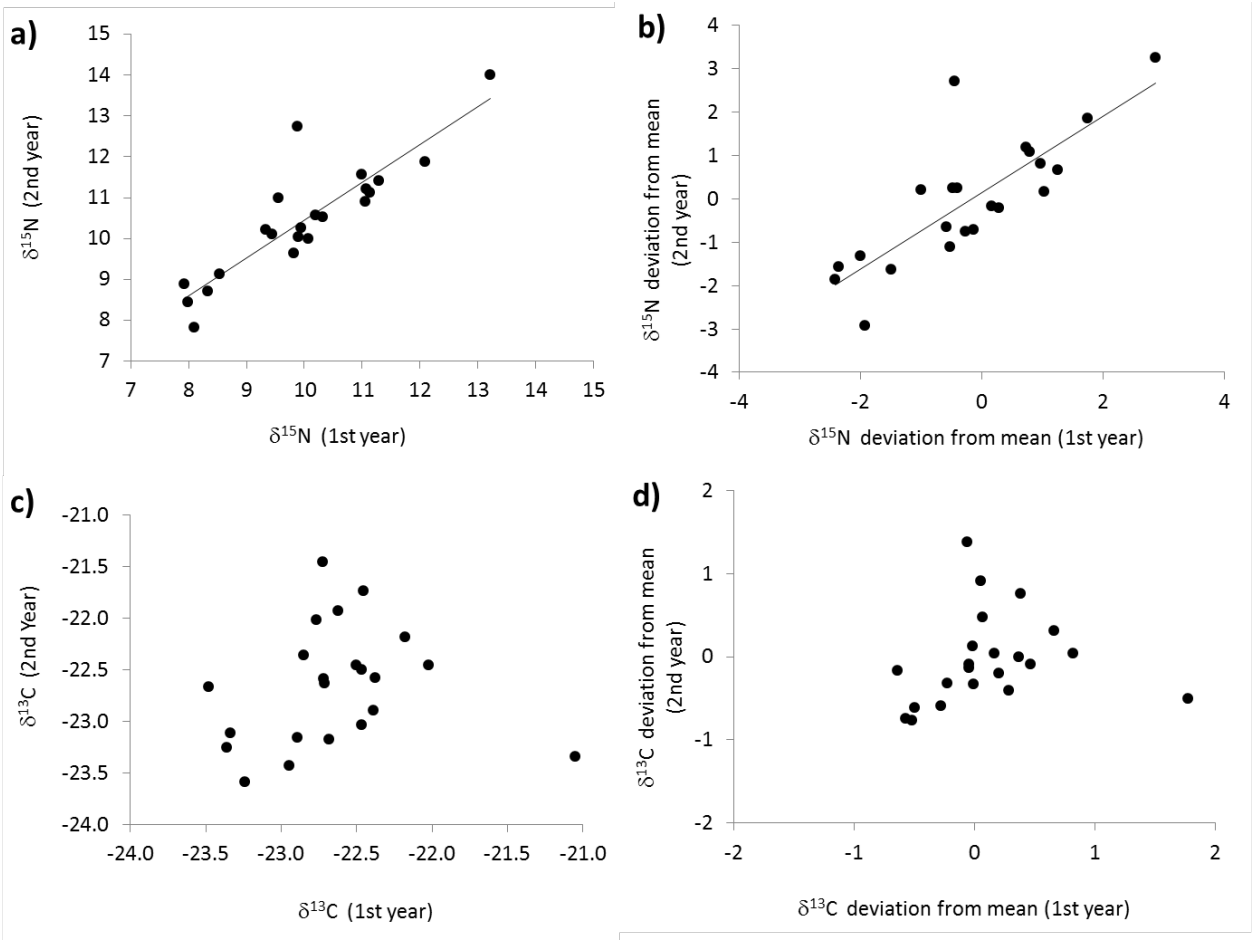


**Figure 3** – Correlation between Nitrogen-15 and Carbon-13 values for feathers grown in Africa on wintering grounds in successive years on the same bird within the period 2013-2015. In most cases birds were caught twice; either 2013/2014 or 2014/15; for the three birds caught in all three years, only the first and last are shown. Relationships are shown for both elements for: (a, c) actual values for $\delta^{15}N$ and $\delta^{13}C$, respectively; and (b, d) deviation from annual mean for $\delta^{15}N$ and $\delta^{13}C$, respectively. Trend lines are shown for significant relationships (see main text)



added and predictive ability more than doubled compared to the model with only year and site variables ($R^2$ = 0.238 versus 0.123; Table 1). This was a positive relationship such that birds with lower $\delta^{13}$C values bred relatively early, which is generally more advantageous (Fig. 4a).

Wintering $\delta^{13}$C profile was significantly related to all stages of breeding *success* (clutch size, number of young to hatch, number of young to fledge), after allowing for the effect of year, site and lay date (Table 1). In all cases, the significance of the final models, which included $\delta^{13}$C values, was improved relative to the models including baseline variables only. All relationships were positive, such that birds with lower $\delta^{13}$C values had higher breeding success (Fig. 4b). The improvement to models after wintering $\delta^{13}$C was added was substantial, especially in the early stages of the breeding process: the predictive power of the model for clutch size more than doubled after $\delta^{13}$C was added (106% improvement), while the predictive power of the hatching and fledging models increased by 68% and 13%, respectively. It should also be noted that these figures are based on direct contribution to the model. The true values would be somewhat higher given the underlying positive correlation between $\delta^{13}$C and lay date (see above) and the negative correlation between lay date and clutch size (P = 0.002), which together suggest that $\delta^{13}$C has an indirect effect on breeding success as well as a direct one. There was no relationship between wintering $\delta^{15}$N and either lay date or any of the breeding success variables. Female body condition was unrelated to $\delta^{13}$C or $\delta^{15}$N isotopic data, lay date, and breeding success.

## Discussion

### Wintering *versus* breeding profiles

Significant differences in $\delta^{15}$N and $\delta^{13}$C profiles from UK-grown tail feathers and Africa-grown tertial feathers suggest stable isotope analysis is appropriate for studying migratory ecology of Pied Flycatchers. The variability within the samples was lower for winter-grown feathers, but this might just reflect the lower sample size. Both isotopes occurred at higher levels in winter-grown feathers compared to ones grown on breeding grounds. In the case of $\delta^{13}$C, this matches the situation for Wrynecks *Jynx torquilla* (Reichlin et al. 2010). The wintering isotope profiles found here (mean $\delta^{15}$N = 10.4‰; mean $\delta^{13}$C = -22.7‰) differ substantially from Pied Flycatchers breeding on Gotland (mean $\delta^{15}$N = 6.9‰; mean $\delta^{13}$C = -20.1‰) (Veen et al. 2014). The $\delta^{13}$C values are lower than for other African-wintering birds, such as Collared Flycatchers (mean $\delta^{13}$C ca -19‰: Hjernquist et al. 2009) and Willow Warblers (mean $\delta^{13}$C = ca -22‰). $\delta^{13}$C values are also similar to American Redstarts and Black-and-White Warblers, which winter at similar latitudes in the Americas (Marra et al. 1998; Norris et al. 2004; Paxton and Moore 2015).

### Inter-year variation

This study has demonstrated a significant year effect for individual birds' wintering isotopic signatures for both $\delta^{15}$N and $\delta^{13}$C. This differs from the situation at population-level where there was no significant between-year difference,

**Figure 4** - The relationship between Carbon-13 profile of winter-grown feathers, indicative of different overwintering habitat, and: (a) lay date and (b) breeding success. Error bars (where possible) show standard error. Note that data are rounded here for display purposes only; unrounded (non-integer) values were used in all analyses,

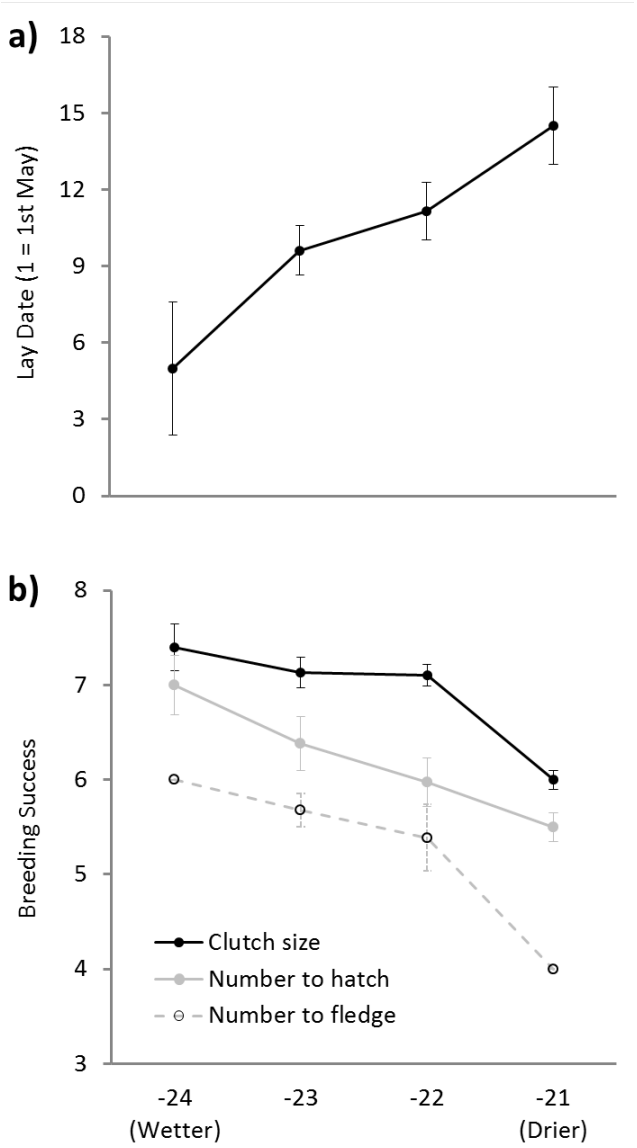


**Table 1:** Hierarchical regression models for breeding success building in baseline variables first followed by wintering (African) Carbon-13 isotope profile.

| Model | | Lay Date | | | Clutch Size | | | Number of Young to Hatch | | | Number of Young to Fledge | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | F | $R^2$ | P | F | $R^2$ | P | F | $R^2$ | P | F | $R^2$ | P |
| 1a: | Baseline variables: Year, Site | 7.009 | 0.123 | 0.001 | - | - | - | - | - | - | - | - | - |
| 1b: | Baseline variables: Year, Site, Lay Date | - | - | - | 3.608 | 0.067 | 0.031 | 4.640 | 0.083 | 0.012 | 11.509 | 0.197 | <0.001 |
| 2: | Baseline variables + Wintering $\delta^{13}C$ | 8.411 | 0.248 | <0.001 | 3.917 | 0.138 | 0.005 | 5.426 | 0.139 | 0.002 | 9.883 | 0.223 | <0.001 |

* Female condition did not improve any model either before or after isotope data were entered and so was not retained as a baseline variable

possibly because of the substantial within-year variation across the overall population. In the case of $\delta^{15}$N, there is an underlying tendency for individuals to have similar profiles in different years in relation to other birds in the same population. This differed for $\delta^{13}$C, where birds displayed much more inter-annual variability in isotopic signature and both exact values and deviation from the annual mean were not correlated between years.

Although temporal variation in stable isotope profiles of the same birds has been used previously to determine prey-switching over the course of a year or less (e.g. Michalik et al. 2013), comparatively little work has been done on inter-year variation. One of the few studies to have examined this for birds was on the closely-related Collared Flycatcher breeding in Gotland (Hjernquist et al. 2009). The findings of Hjernquist's study and the current one are comparable. In both cases, there were inter-year differences in actual values for both isotopes but $\delta^{15}$N profiles of birds caught in multiple years correlated with one another over time (both with $R^2$ values ~0.65). The main difference is that whereas Hjernquist's study found a strongly significant inter-year correlation between $\delta^{13}$C, we found no such correlation.

**Breeding success and lay date**

There is a significant correlation between wintering $\delta^{13}$C and all stages breeding success (clutch size, number of young to hatch and number of young to fledge). Although the $R^2$ values remain fairly low in all models, the relative effect of winter habitat as measured by $\delta^{13}$C is substantial, especially in initial stages of breeding, with $\delta^{13}$C values doubling the amount of variation in clutch size that could be explained by the combination of year, site, and lay date. All relationships are positive, such that birds with lower $\delta^{13}$C values had higher breeding success.

The dominant control of $\delta^{13}$C within avian tissue is the relative proportion of C3 and C4 plants within the locale (Inger and Bearhop 2008; Evans et al. 2012l Paxton and Moore 2015). Different plants use different biochemical photosynthetic pathways: C4 plants use water-efficient C4 carbon fixation whereas C3 plants use water-inefficient C3 carbon fixation. As a result, C3-dominated habitats tend to be mesic (plant $\delta^{13}$C values = -35‰ to -20‰), while C4-dominated habitats tend to be xeric ($\delta^{13}$C values = -18‰ to -7‰). Accordingly, it would appear that Pied Flycatchers that overwinter in mesic habitat have higher productivity the following summer. Based on the only detailed survey of Pied Flycatcher habitat use on wintering grounds by Salewski et al. (2002) in the Ivory Coast, this would suggest that those birds using gallery forest (wet woodland) over the winter tend to have higher success than those using dry forest. This mirrors previous research for American Redstarts, where carbon-13-deficient birds (which had overwintered in higher-quality mesic habitat) fledged more offspring (Marra et al. 1998; Norris et al. 2004). The fact that there was no link between $\delta^{15}$N and breeding success suggests that even though birds might be using wintering habitats that differed in water availability (as suggested by $\delta^{13}$C) birds were either not water-stressed or any effects of water-stress did not carry over to affect breeding success (Kelly 2000).

The main questions that need considering are: (1) whether the relationship between $\delta^{13}C$ and breeding success is causal and (2) how much of this relationship direct and how much is indirect as a result of underlying correlations between isotope profile and other independent variables such as female condition and breeding phenology.

The most intuitive possibility is that wintering habitat correlated with female body condition. This could either be non-causal (i.e. birds in better condition outcompete others for wetter winter habitats and $\delta^{13}C$ is simply a proxy for condition, such that the relationship between $\delta^{13}C$ and breeding is simply indicative of the expected relationship between condition and breeding success), or a causal individual-level seasonal interaction (i.e. birds that select wetter winter habitats improve in condition relative to those in drier habitats and this effect carries over to breeding success the following year: Norris and Marra 2007). However, hierarchical modelling demonstrated that female condition was not significantly related to breeding success at any stage. This, coupled with the lack of any relationship between $\delta^{13}C$ and body condition, suggests that the isotope-productivity relationship is not driven by a female condition interaction. This contrasts with some previous studies, such as American Redstarts breeding at Hubbard Brook (USA) where $\delta^{13}C$ profile was inversely correlated with condition measured in the same way as in this study (Marra et al. 1998), and Black-throated Blue Warblers where again $\delta^{13}C$ profile was inversely correlated with condition as measured by fat reserves (Bearhop et al. 2004). Such patterns, however, are not universal; there was no relationship between winter $\delta^{13}C$ profile and condition in female Cassin's Auklets (Sorensen et al. 2009).

Alternatively phenology could be important, with birds that had wintered in better habitat potentially migrating earlier (Marra et al. 1998) or faster (Bearhop et al. 2004), thereby arriving at the breeding grounds earlier and having a higher probability of securing a good-quality territory. In this case, clutch sizes might be higher because of a plastic response to territory quality or simply the result of the general decline in clutch size and overall breeding success as the season progresses (Perrins and McCleery 1989). There is good evidence for this as $\delta^{13}C$ profile was a strong predictor of lay date, with the addition of the isotope data more than doubling the predictive power of the model relative to year and site alone. Effects of winter habitat, as measured by stable isotopes, on phenology has been previously considered to be the causal mechanism for higher breeding success in American Redstarts breeding in Canada (Norris et al. 2004), Black-tailed Godwit (Gill et al 2001; Gunnarsson et al. 2005), and potentially Black-and-White Warblers (Paxton and Moore 2015). In the case of the Pied Flycatchers studied here, though, there were also a significant relationships between isotopic signature and lay date (which influences success), and significant relationships between $\delta^{13}C$ and breeding success after lay date had been allowed for. It would appear that the effect of winter habitat, as measured by isotope signature, is both indirect, through its effect on ability to breed early, and direct. This in turn suggests that there is another mechanism through which $\delta^{13}C$ links to productivity. Possible mechanisms include:

1. **Seasonal matching in habitat quality**: This might occur if birds that winter in good habitat are at some competitive advantage in securing better breeding territories for reasons other than having better body condition. In this case, seasonal matching of habitat quality could allow upwards adjustment of clutch size. This is feasible in clutch-size adjusting birds, such as the Pied Flycatcher (Järvinen 1989), and has been seen previously in Black-tailed Godwits using stable isotope profiling (Gunnarsson et al. 2005), where winter settlement decisions were considered critical in determining future success.

2. **Related to age/experience**: Older or more experienced birds might preferentially choose wetter habitat and would also be likely to have higher clutch sizes. This effect is often independent of any effect of habitat condition, instead often being linked to experience and the capacity to breed earlier (e.g. in the case of song sparrows *Melospiza melodia*: Nol and Smith (1987)). Although precise age and experience of the birds in the study was not known for the majority of the birds studied, two lines of evidence potentially support this possibility: firstly clutch size is known to relate to age in this species (Järvinen 1991) and secondly winter isotope profiles are known to differ with age (Reichlin et al. 2010), implying that different sub-sections of the population use different wintering grounds.

3. **Driven by residual resources**: Birds are either capital breeders (breeding using resources stored from previous phases of their annual cycle) or income breeders (breeding using resources acquired locally and, ergo, recently) (Inger and Bearhop 2008). If Pied Flycatchers are capital breeders, it is possible that they access better resources in wetter winter habitats (more protein-rich or greater abundance of diet-derived essential amino acids such as lysine and methionine, which are all important for egg-laying) and this affects clutch size.

**Conclusions**

We have shown stable isotope analysis to be a powerful tool for assessing ecology of migratory birds. Our study indicates that African wintering habitat links strongly to UK breeding performance several months later. This is the first evidence of breeding success carry-over effects between geographical regions for a European passerine. Although the mechanisms for this link remain speculative, this study suggests wintering habitat may not simply drive reproductive success through an influence on female condition or ability to breed early. These strong inter-seasonal interactions on population demography indicate that conservation measures for declining migrant species should include all aspects of the seasonal cycle, and not merely focus on conditions on breeding grounds.

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