scientific reports

OPEN
Multidimensional natal isotopic niches reflect migratory patterns in birds

A. Franzoi[1,2,3,7], S. Larsen[2,7 ✉], P. Franceschi[2], K. A. Hobson[4], P. Pedrini[1], F. Camin[2,5,6] & L. Bontempo[2]

Naturally occurring stable isotope ratios in animal tissues allow estimation of species trophic position and ecological niche. Measuring multiple isotopes of migratory species along flyway bottlenecks offers the opportunity to sample multiple populations and species whose tissues carry information at continental scales. We measured δ^2H, $\delta^{18}O$, $\delta^{13}C$, $\delta^{15}N$ in juvenile feathers of 21 bird species captured at a migratory bottleneck in the Italian Alps. We examined if trends in individual isotopes reflected known migratory strategies and whether dietary ($\delta^{13}C–\delta^{15}N$) and spatially-explicit breeding origin ($\delta^2H–\delta^{18}O$) niche breadth (NB) differed among long-distance trans-Saharan (TS), short-distance (IP) and irruptive (IR) intra-Palearctic migrants, and whether they correlated with reported populations long-term trends. In both TS and IP groups, species δ^2H declined with capture date, indicating that northern populations reached the stopover site later in the season, following a Type-I migration strategy. Values of δ^2H indicated that breeding range of TS migrants extended farther north than IP and IR migrants. The breeding season was longer for IP migrants whose $\delta^{13}C$ and $\delta^{15}N$ values declined and increased, respectively, with time of capture. Average species dietary NB did not differ among migratory groups, but TS migrants displayed wider breeding origin niches, suggesting that long-distant migration is linked to broader ecological niches. Isotope origin NB well reflected species geographic range extent, while dietary NB did not correlate with literature accounts of species' diet. We found no relationship between species breeding NB and population trends in Europe, suggesting that conditions in the breeding grounds, as inferred by stable isotopes, are not the only determinant of species' long-term persistence. We demonstrate that ringing activities and isotopic measurements of passerines migrating through a bottleneck represents a unique opportunity to investigate large-scale life-history phenomena relevant to conservation.

The concept of species' niche is a fundamental pillar of ecological science[1–3], but remains challenging to quantify empirically. The challenge is particularly acute in the case of long-distance migratory birds due to the shifts in habitat use and distribution throughout their life cycles. Yet, habitat conditions in the breeding, stop-over and wintering grounds profoundly influence individual fitness and demography of migratory populations. Long-distance migrants are showing rapid population declines and appear at greater risk of extinction compared to residents[4–6]. Therefore, assessing niche parameters and differences among migratory groups can help in understanding the drivers shaping migration strategies and setting conservation actions.

Migratory pathways of multiple species often overlap at stopover sites, which can represent bottlenecks, or passage points, causing high spatio-temporal concentration of many individuals and species from different migratory systems[7–9]. Therefore, estimating niche parameters of individuals intercepted along migratory flyways may be a valuable and cost-effective approach to include multiple species potentially conveying information from large, continental scale, breeding areas[9–11].

[1]Vertebrate Zoology Department, MUSE – Science Museum, Corso del Lavoro e della Scienza 3, 38122 Trento, TN, Italy. [2]Research and Innovation Centre, Fondazione Edmund Mach, via E. Mach 1, 38010 San Michele all'Adige, Italy. [3]Department of Earth and Environmental Sciences, Università degli Studi di Pavia, Via Ferrata 1, 27100 Pavia, Italy. [4]Environment and Climate Change Canada, Innovation Blvd., Saskatoon, SK S7N 3H5, Canada. [5]Center Agriculture Food Environment (C3A), University of Trento, Via Mach 1, 38010 San Michele all'Adige, TN, Italy. [6]Present address: Vienna International Centre, International Atomic Energy Agency, PO Box 100, 1400 Vienna, Austria. [7]These authors contributed equally: A. Franzoi and S. Larsen. ✉email: stefano.larsen@fmach.it

Ecological niches of species can be approximated using measurements of naturally occurring stable isotope ratios in their tissues. Such isotopic niches can be used to empirically quantify the realised niche dimensions of species[12,13], since stable isotope measurements in animal tissues provides a time-integrated average of many feeding events[14]. In particular, stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) have been used to represent the "dietary niche" of an organism because they reflect reliance on different primary producers and trophic level, respectively. Although, the dietary niche breadth represented by δ^{13}C and δ^{15}N reflects the range in isotope values of dietary inputs in any given area[15,16], the isotopic niche space produced is a potentially powerful means of deriving information on diets of migratory birds from broad catchment areas[13,17].

Similarly, isotopic values of hydrogen (deuterium; δ^{2}H) and oxygen (δ^{18}O) are linked to isotopic values in environmental waters that can show predictable spatial patterns or isoscapes at continental scales[18]. Therefore, tissue δ^{2}H and δ^{18}O values can be used to represent a spatially-explicit "origin niche" of bird species[19]. Although the meteoric relationship predicts a strong correlation between water δ^{2}H and δ^{18}O, this strong relationship tends to break down in consumer tissues within food webs due to the fact that oxygen in tissues, unlike hydrogen, can be derived from air as well as diet and drinking water. Moreover, metabolic pathways leading to oxygen incorporation in proteins differ from those involving hydrogen[20,21]. The net result is that the two isotopes tend to be poorly correlated in feathers[11]. As such, values of δ^{2}H and δ^{18}O could be considered independent in the analyses of feathers and their combined isotopic space would be highly associated with the geographic origins where feathers were grown[22].

The use of multiple isotopes in animal tissues has allowed ecologists to estimate trophic niche breadths of different species or individuals in populations[12,23], and the geographic origin of migratory species[24,25]. These studies have provided insight into trophic relationships in communities[26–28] as well dietary specialisation[12] and migratory pathways of declining bird species[5,29]. Due to logistic and economic constraints, most studies measuring both dietary and origin isotope niches have focused on single species or local populations and communities but see[10,30,31]. However, examining multiple isotope niches at migratory bottlenecks offers the unique opportunity to quantify and compare niche parameters among several bird species that evolved different migratory behaviours.

In this study, we measured multiple isotopes in the juvenile feathers (remiges and/or retrices) of approximately 800 individuals from 48 species of European passerines captured during the post-breeding migration (August–October) at an Alpine migratory bottleneck over the Italian Alps. Metabolically inert tissues such as feathers show an isotopic composition that reflects the environment during their formation. As a consequence, bird feathers grown in the nest represent "remote carriers" of isotopic information from the breeding grounds[32], since many species retain juvenile plumage during subsequent migration and overwintering. In particular, we examined whether dietary (δ^{13}C–δ^{15}N) and origin (δ^{2}H–δ^{18}O) niche breadths (NB) differed among species belonging to the *trans-Saharan* (TSM), *intra-Palearctic* (IPM), and *irruptive intra-Palearctic* (IRM) migratory systems, and the extent to which feather stable isotope values reflected known migratory patterns.

For both TSM and IPM groups, we expected northern populations to reach the autumn Alpine passage later in the season than populations breeding at lower latitudes. According to the deuterium precipitation isoscape in the Palearctic[18,19], this would result in a negative relation between arrival date and δ^{2}H for each species, as also observed in Neotropical species adopting a Type-I migration strategy whereby southern populations precede northern ones or as "chain migrants"[9].

Since IPM species display a longer breeding season (2–3 clutches) compared to TSM (1–2 clutches), we also compared temporal variation in δ^{13}C and δ^{15}N for each species to examine changes in the availability of main food sources (e.g. larvae to adult insects) during the year of sampling.

Long-distance migration is thought to have evolved primarily in species able to utilise multiple habitat types[33–35], which are encountered *en route* and in wintering areas. More generally, if migratory connectivity occurs at large scales, it could favour the selection for generalist traits. As such, species migration distance is expected to be directly related to their niche breadth. Although intuitively appealing, this hypothesis has received limited empirical support so far[34,36,37]. Here, we specifically examine how migratory distance was related to the origin niche breadth (δ^{2}H–δ^{18}O). We test the hypothesis that long-distance migrants such as TSM species, display broader niches than IPM and IRM species.

Because the relation between isotope niche measures and other estimates of species niche are still poorly understood[23], we also examined whether our measures of dietary and origin isotope niches matched diet and climatic niche breadth derived from the literature and species range maps[35]. While we expected estimates of spatially-explicit isotope origin niche to accord with species range maps, isotope dietary niche breadth is unlikely to match literature data. This is because diet isotopic dimensions largely depend on the inherent isotopic variance of the resources[23], and thus may not adequately reflect the diversity of individual food items in the diet (as reported in the literature), but rather the isotopic diversity of used resources.

Lastly, we assessed whether species isotope niche breadths in the breeding range were related to their long-term population trends as reported in the literature. This is of particular importance for long-distance migrants that show rapid population declines[4,5]. If species with narrow isotope niches systematically displayed negative population trends, it would indicate that conditions at the breeding grounds are key for the long-term persistence of species.

Results

Over the four years of sampling at the two ringing stations, feathers were collected for isotopic analysis from more than 800 individuals of 48 passerine species. After excluding species with less than 15 individuals (see "Methods" section), we included isotope data from 715 individuals of 21 species (Table S1). These belonged to the trans-Saharan (7 species; 241 individuals), intra-Palearctic (11 species; 335 individuals) and irruptive (3 species; 91 individuals) migratory systems. Overall distribution of species over isotope space is shown in Figs. 1
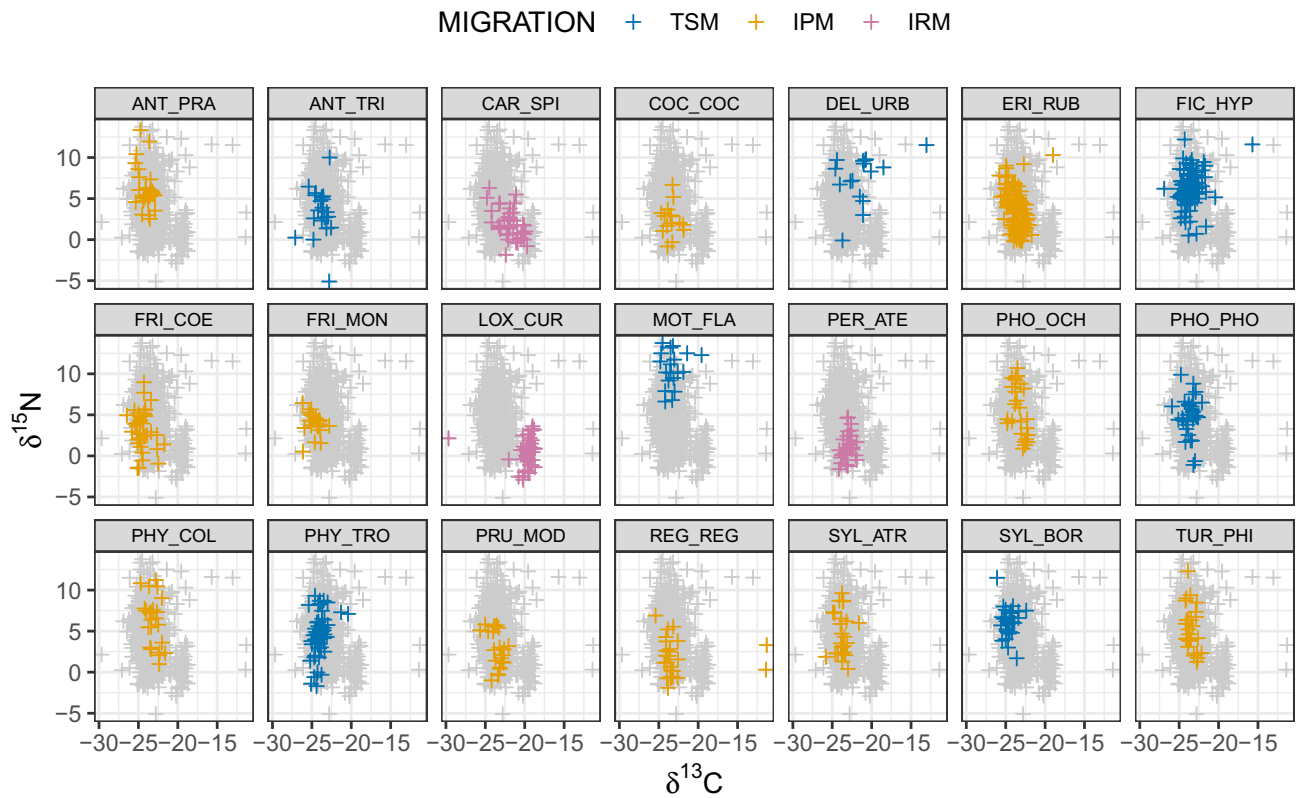
Figure 1. Distribution of each species and individuals over the $\delta^{13}C$-$\delta^{15}N$ isotope space defining the dietary niche. Grey crosses in the background show the overall space occupied by the species (acronyms provided in Table S1). *TSM* Trans-Saharan migrants, *IPM* Intra-Palearctic migrants, *IRM* Irruptive migrants.
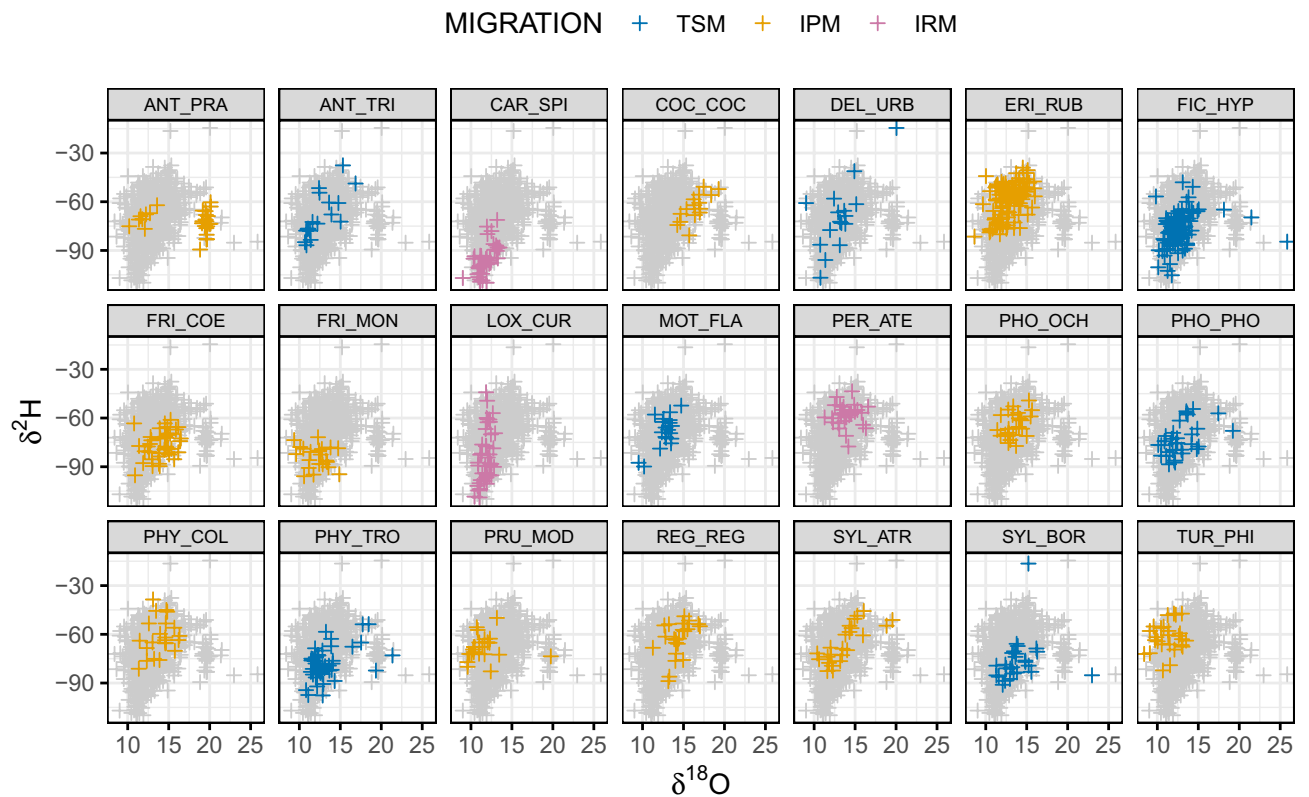

Figure 2. Distribution of each species and individuals over the $\delta^{2}H$-$\delta^{18}O$ isotope space defining the spatial origin niche. Acronyms as in Fig. 1.

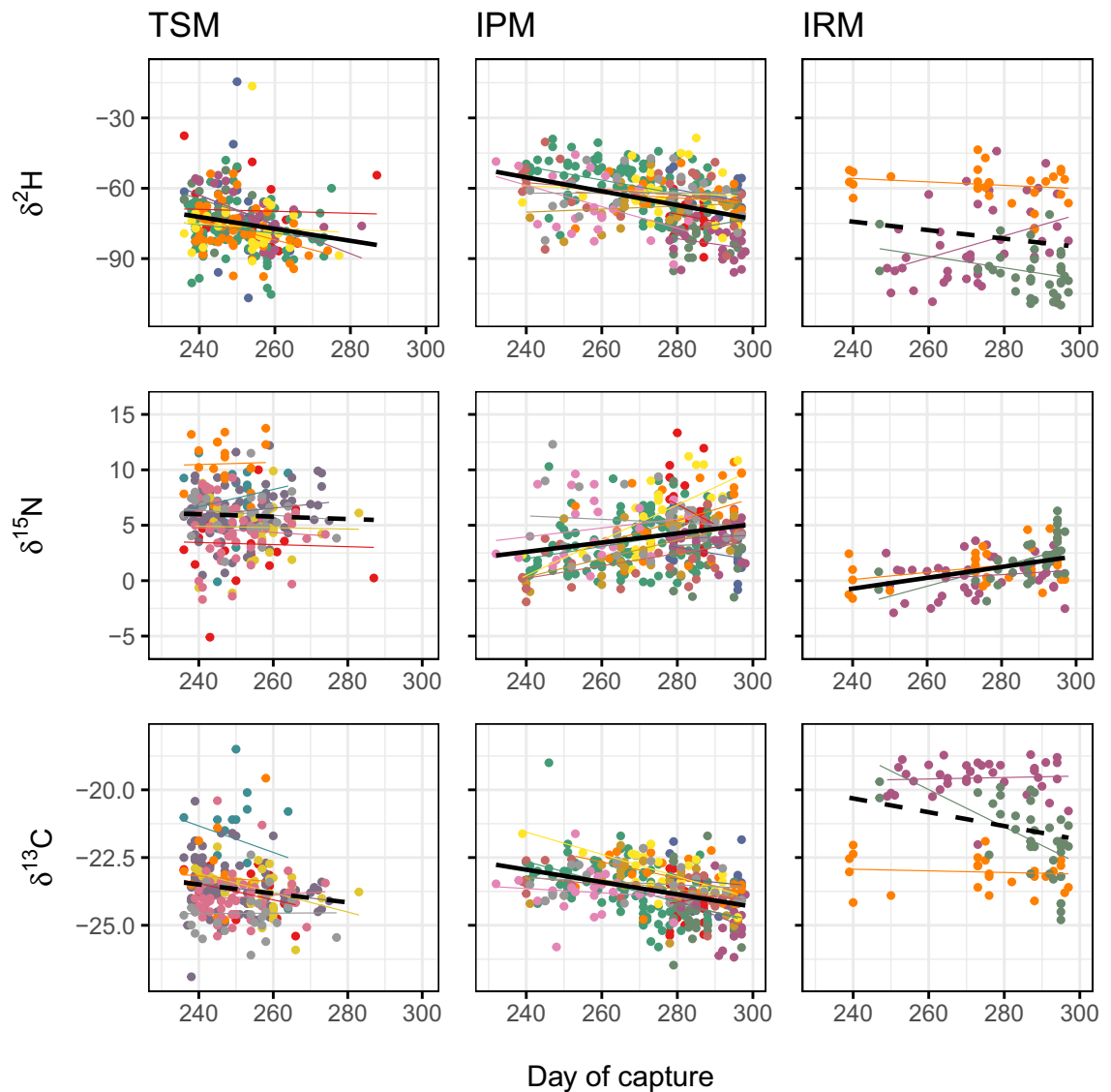**Figure 3.** Temporal trends in δ^2H, δ^{15}N and δ^{13}C values for the TSM, IPM and IRM groups. Black fit line indicates overall trend (dashed line = *NS*), while individual species trends are shown in different colours. Day of capture is expressed as Julian day (1 = 1st Jan). *TSM* Trans-Saharan migrants, *IPM* Intra-Palearctic migrants, *IRM* Irruptive migrants.

and 2. Migratory phenology of each species during the post-breeding passage is shown in Fig. S1 (Supplementary Material), where the temporal patterns in the number of species captured is also shown.

Temporal changes in δ^2H (reflecting species breeding latitude) supported the hypothesis that both TSM and IPM groups display a Type-I (chain) migration, whereby northern populations (with lower δ^2H values) reached the Alpine passage later in the season (Fig. 3). This pattern appeared consistent among species in each group, with a significant effect of capture day in the *lme* model (p < 0.01 for both groups). Mean δ^2H values were evidently lower for TSM, indicating that species in this migratory group tend to breed at higher latitudes than IPM. No temporal trend in δ^2H was observed in IRM species.

A trend in δ^{15}N was evident for IPM and IRM species with a positive effect of capture day (*lme* p < 0.01). This trend was not observed in TSM, which displayed slightly higher mean δ^{15}N values (Fig. 3). Similarly, a significant negative trend in δ^{13}C was observed in IPM (p < 0.01), which was not significant in either TSM and IRM species. Figure 3 also shows that most TSM species reached the capture site by day 280, whereas the season appeared longer for IPM and IRM. The difference in phenology is also clear from Fig. S1.

Average species dietary isotopic NB did not differ significantly among migratory groups (Fig. 4). Conversely, the average isotopic origin niche was significantly broader for TSM, about 30% broader than IPM (GLS model; p = 0.03). However, there was relatively large variation in origin NB within TSM. IRM displayed a much narrower origin NB (Fig. 4). Excluding the apparent outliers from the analysis of dietary NB (δ^{13}C–δ^{15}N; *Regulus regulus* and *Delichon urbicum*), provided qualitatively similar results.
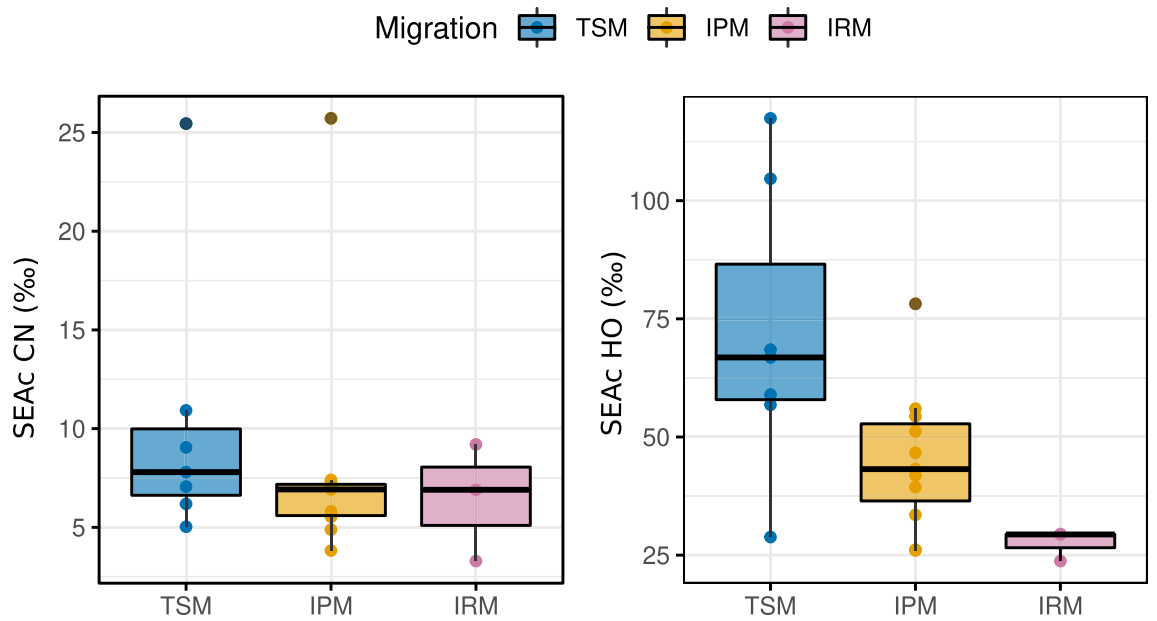
Figure 4. Comparison of the average species dietary and origin isotopic niche breadth among migratory groups. *TSM* Trans-Saharan migrants, *IPM* Intra-Palearctic migrants, *IRM* Irruptive migrants.
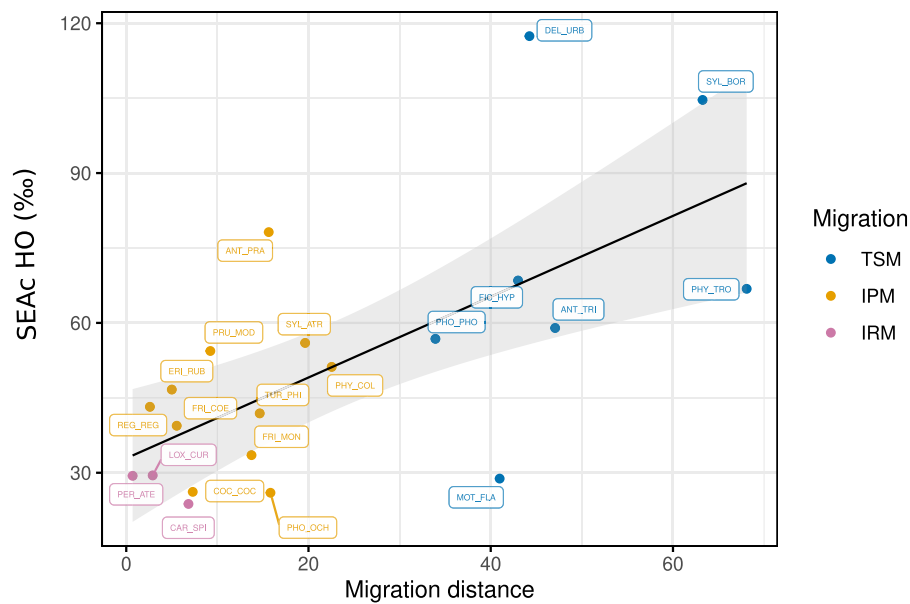

Figure 5. Relationship between species origin niche breadth and migration distance, defined as the latitudinal distance between the centroid of the breeding and wintering ranges. Species identity is labelled. Grey band shows 95% CI of regression fit.

In addition, migration distance was related to the breadth of species origin niche, although there was considerable variation at larger distances (Fig. 5; GLS; p = 0.01). No relationship was observed between dietary isotopic NB and migration distance (Fig. S2).

We compared our estimates of species dietary and origin isotopic niche with those recently derived from species distributional data. We found that the origin NB well matched measures from range maps (Fig. 6), with a positive linear correlation (p = 0.02). In addition, climate niche breadth derived from range maps well reflected the upper distribution of the empirical isotope origin niche. This is evident from the significant quantile regression at q = 0.8 (p < 0.01). Conversely, the dietary isotopic NB was not related to diet specialisation, as derived from the literature.

Finally, we appraised whether long-term population trends were related to the empirical isotope niches. Neither dietary nor origin isotopic niche breadth were related to species population trends as reported in the PECBMS portal (Fig. S3).
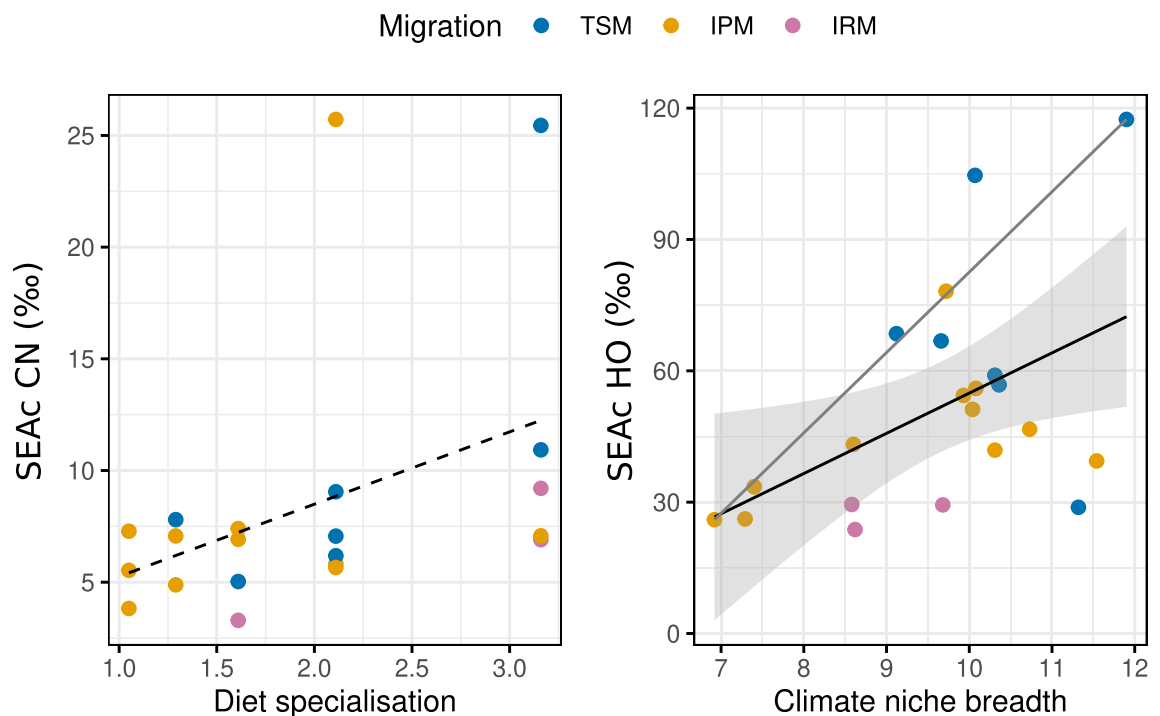
Figure 6. Relation between empirical dietary and origin isotopic niche breadth with literature-derived niche parameters (from Reif et al.[35]). Dashed line = *NS* relation; tick black line = significant regression fit; thin grey line = significant quantile regression fit at q = 0.8.

Discussion

We used juvenile feathers of multiple bird species intercepted along an autumn migratory flyway as 'remote carriers' of isotope niche information from Western Palearctic breeding areas. This allowed quantifying niche parameters from species that evolved different migratory strategies. In addition, despite potential confounding factors related to variance in local isotopic baselines, including individuals from different populations and communities allowed the quantification of species niches that were less influenced by local biotic interactions (e.g. competition), and likely more representative of the realised niche dimensions of the species.

Our results generally supported our initial hypotheses, but also revealed a poor association between isotope niche dimensions and literature-derived niche parameters and long-term population trends.

Temporal trends in feather δ^2H indicated that individuals breeding at higher latitudes (displaying lower δ^2H values) reached the sampling locations later in the season, with patterns that appeared consistent among species and across the two major migratory groups (TSM, IPM). This type of migration, often called Type I (as opposed to Type-II, whereby northern populations leap-frog southern populations during migration), has also been observed across a range of Nearctic-Neotropical migrants[9,38], and appears common in Afro-Palearctic migrants as well[39,40]. Different migration timing of conspecific populations contributes to limit competition among individuals and likely occurs at the species level too. Assessing temporal segregation in the use of migratory stopover helps identify the species most likely to co-occur and compete locally for resources. In our study site, a few species showed complete segregation (Fig. S1), but high overlap was otherwise evident with up to nine migratory species captured in a single day. Bottlenecks can indeed concentrate large numbers of individuals and species during a short period, which not only heightens intra- and inter-specific competition, but also increases vulnerability to localised events and disturbance. The identification and conservation of migratory stopover and bottlenecks are therefore key for the persistence of many declining populations[7–9].

Temporal trends observed in both $\delta^{15}N$ and $\delta^{13}C$ in IPM could be explained by different non-mutually exclusive mechanisms. For instance, the lower latitude breeding range of IPM, as well as IRM (compared to TSM), implies that early captures were dominated by individuals from the Alpine/central-Europe/Carpathian areas at high elevations. This could be associated with higher $\delta^{13}C$[19] also due to lower precipitation compared to northern Europe[41]. Similarly, it is also possible that the extent of anthropogenic nitrogen input from agriculture is lower in mountainous regions, which could contribute to lower $\delta^{15}N$ in early captures. It is known, in fact, that anthropogenic sources such as fertilisers can alter and mostly increase nitrogen isotopic baseline values[23].

Alternatively, seasonally rising values of $\delta^{15}N$ could be associated with the increased availability of adult insects (as opposed to larvae or other arthropods with lower $\delta^{15}N$ values) as the longer breeding season of IPM progresses. Conversely, the shorter season of TSM and their strictly insectivorous diet would result in more seasonally stable $\delta^{15}N$ values.

We found that species breeding origin niche was, on average, broader in long-distance trans-Saharan compared to intra-Palearctic migrants. Long-distance migration is generally thought to have evolved from ancestor species with broad habitat niches: individuals able to exploit a wider range of conditions during migration or

at wintering grounds should be selectively advantaged over more specialised ones[33–35]. Empirical support for this hypothesis is limited[36,42,43], and our data thus provide some additional evidence. However, the link between niche breadth and migration patterns remains unclear, with some evidence suggesting the opposite view that narrower habitat or diet niche may have favoured long-distance migrations[44], because specialists are forced to leave an area when conditions become unfavourable or resources scarce. Yet, our data do not show any correlation between migration distance and dietary niche breadth, providing no support for this alternative hypothesis. It has to be kept in mind, however, that our estimates of ecological niches reflect the breeding diets of species (i.e. isotopes from juvenile feathers grown at nest), and the inclusion of information from wintering grounds would have provided a more complete and likely different picture. This would require measuring isotope ratios in body tissues with different turnover or growth rates[45], or collecting feathers grown in winter[29]. However, for most passerine species, information on wintering locations is still limited, especially for the Afro-Palearctic migration system[46,47]. Similarly, the extent to which migratory species track their climate and habitat niche between breeding and wintering grounds is poorly understood and likely to vary among species[37,47]. Most assessments of seasonal niche shifts during migration relied on large-scale climatic and land-use data[37,48], while those employing tissue isotopes are rare and mostly focussed on a few key species to appraise migratory connectivity[10,45,49]. Therefore, it is difficult to evaluate whether and to what extent our estimates of isotope niche breadth would have differed had we included wintering isotope signals.

We used recently published data from Reif et al.[35] to examine the extent to which isotope niches from our sampled populations reflected diet specialisation and climate niche breadth reported at the species level. As expected, dietary isotopic niche did not match the breadth or diversity of consumed food items reported in the literature. This further supports the notion that the breadth of dietary isotope niche reflects the isotopic variance of the resources and should not be considered synonymous with species diet[23]. Conversely, estimates of spatially-explicit breeding origin niche correlated significantly, albeit weakly, with literature data. In particular, measures of climatic niche from range maps appeared to better predict the upper distribution (quantile: 0.8) of our isotope niche, rather than its central response. A possible interpretation is that niche estimates derived from range maps necessarily reflect the maximum expected value for a given population. In other words, if we assume that a correlation exists between spatially-explicit origin niche inferred from isotope and range maps, any sampled populations should display isotope niches that are either equal or narrower than those derived from species full range maps. This provides additional evidence that species bi-dimensional δ^2H–$\delta^{18}O$ isotope space is a good proxy of their geographic and climatic niche dimensions[30].

Among the key motivations for appraising niche requirements of migratory species, is that their populations appear to be declining more rapidly than residents[4,5,50,51]. The mechanisms underpinning these trends are still unclear and we examined whether narrow isotope breeding niches were associated with stronger population declines. We found no correlation between either dietary or origin breeding niche and long-term population trends as reported in the Pan European Common Bird Monitoring Scheme. This is not surprising as there is increasing evidence that environmental conditions and dynamics in non-breeding areas are key for the long-term persistence of populations[50,52,53]. In particular, habitat change and degradation in tropical wintering areas appear to be a major cause of decline in migrant species. As the conditions in wintering grounds carry-over to influence breeding success[54,55], quantifying both breeding and wintering niche parameters would provide insight not only on basic ecological questions such as the degree of niche tracking, but also on conservation requirements of long-distance migrants[45].

We used multiple stable isotopes to quantify and compare ecological niche parameters between short- and long-distance migrants. We showed that post-breeding migratory bottlenecks, such "Bocca di Caset" and "Passo del Brocon" in the Italian Alps, not only represent important stopovers for many migrants of the Western flyway[40], but also strategic natural laboratories to examine migratory patterns across multiple species. However, not every species or populations are equally likely to use these locations as stopover. For instance, some central-European populations could fly over the Alps during the first migratory flight without stopping. The extent to which the potential selectivity of our study area influenced the results depends on the heterogeneity in isotope profiles within populations, which, however, can be large in some cases[55,56].

Our results illustrate that long-distance trans-Saharan migrants, mostly breeding at higher latitudes than intra-Palearctic migrants, reach the migratory stop-over sooner. This is in line with the notion that early departure allows trans-Saharan migrants to reach the Sahel zone at the peak of vegetation greenness, when feeding conditions are optimal[57,58]. Moreover, in both migratory groups, the onset of breeding for northern populations appeared delayed, likely due to climatic constraints, so that the timing of migration was proportionally shifted later in the season. The delayed timing of northern populations can apparently carry over to the entire annual cycle[39], and likely contributes to limit intra-specific competition. This type of migration pattern appears fairly common in many Nearctic-Neotropical migrants too[9,38].

Our data also provide some support to the hypothesis that broad ecological niches are linked to long-distance migration, although this was only evident for the breeding origin niche dimensions, and requires further testing.

The use of multiple isotope ratios in animal tissues has allowed the quantification of both dietary and spatially-explicit niche aspects. However, the relation between the isotopic ecological niche and other descriptors of species niche (e.g. based on feeding habits, distribution patterns), or conservation status remains vague[23,30]. Here, we found that origin isotopic niche was consistent with the climatic range extent of the species, illustrating how δ^2H and $\delta^{18}O$ measurements can provide insight into the bio-climatic and geographic niche dimensions. However, the breadth of isotope niches was not related to the long-term population trends of the species at the European scale. Appraisal of the complete multi-seasonal niche requirements of long-distance migrants is therefore needed to link ecological information to conservation actions for these declining species.

Methods

Feather sampling. From 2010 to 2013, juvenile wing and tail feathers grown in the nest[59] from 807 migratory birds belonging to 48 passerine species were sampled at the 'Bocca di Caset' and 'Passo del Brocon' ringing stations of MUSE (Province of Trento, Italy, 10° 41′ E, 45° 51′ N, 1605 m a.s.l. and 11° 68′ E, 46° 11′ N, 1720 m a.s.l., respectively) in the central Italian Alps during post-breeding migration (August to the end of October). The study is part of the long-term post-breeding monitoring project 'Progetto Alpi'[60]. We only included species with at least 15 sampled individuals (median individuals per species = 26) to provide a representative measure of species isotopic niches. The remaining species were represented by a median of 3 individuals and were excluded (Fig. S4). Migratory species pass through the Italian Alps at different times during autumn migration[60]. Three migratory groups were considered: long-distance trans-Saharan migrants (TSM), which are fully migratory and leave their breeding ranges to winter in sub-Saharan Africa; short-distance intra-Palaearctic migrants (IPM), which can be fully or partially migratory and which have their breeding and wintering ranges within the Western Palaearctic; irruptive intra-Palaearctic migrants (IRM), which are partial migrants and residents, but show marked invasive and nomadic movements during non-breeding seasons. Date of capture was also recorded for each individual.

Stable isotope analysis. Values of δ^2H, $\delta^{18}O$, $\delta^{13}C$, $\delta^{15}N$ were measured for each individual feather. It was possible to determine all four isotope ratios in the majority of individuals. Feathers were first washed in a solvent mixture (diethyl ether-methanol 2:1) prior to analysis[61]. Simultaneous determination of $\delta^{13}C$ and $\delta^{15}N$ was accomplished using a Vario Isotope Cube isotope ratio mass spectrometer (Elementar, Germany). The δ^2H and $\delta^{18}O$ values were determined through pyrolysis combustion using a TC/EA (Thermo Finnigan, Bremen, Germany) interfaced with a Delta Plus XP (Thermo Finnigan, Bremen, Germany) continuous-flow isotope-ratio mass spectrometer. Pyrolysis was carried out at 1450 °C in a glassy carbon column. The helium carrier flow was 110 ml/min, the GC column was a 1.2 m long molecular sieve 5A, at 110 °C. Isotope ratios were expressed in δ-notation against V-PDB (Vienna-Pee Dee Belemnite) for $\delta^{13}C$, Air for $\delta^{15}N$, V-SMOW (Vienna-Standard Mean Ocean Water) for δ^2H and $\delta^{18}O$. Isotopic values of δ^2H were calculated using the comparative equilibration approach[62,63]. Based on the keratin standards CBS (δ^2H − 197 ‰, $\delta^{18}O$ = + 3.8 ‰) and KHS (− 54.1 ‰, $\delta^{18}O$ = + 20.3 ‰). Values of $\delta^{13}C$ and $\delta^{15}N$ were calculated against working in-house standards (casein and wheat), which were themselves calibrated against international reference materials using multi-point normalisation: fuel oil NBS-22 (IAEA International Atomic Energy Agency, Vienna, Austria; − 30.03 ‰) and sugar IAEA-CH-6 (− 10.45 ‰) for $\delta^{13}C$, L-glutamic acid USGS 40 (− 26.39 ‰ and − 4.5 ‰ for $\delta^{13}C$ and $\delta^{15}N$), hair USGS 42 ($\delta^{15}N$ = + 8.05 ‰ and $\delta^{13}C$ = − 21.09 ‰) and USGS 43 ($\delta^{15}N$ = + 8.44 ‰ and $\delta^{13}C$ = − 21.28 ‰) for $^{13}C/^{12}C$ and $^{15}N/^{14}N$. Values of $\delta^{13}C$ were expressed versus V-PDB on a scale normalised against the two reference materials LSVEC (− 46.6 ‰) and NBS 19 (+ 1.95 ‰)[64]. Values of $\delta^{15}N$ were expressed versus Air-N2 on a scale normalised using the two reference materials IAEA-N-1 and USGS32, with consensus values of + 0.4 ‰ and + 180 ‰[64]. Method uncertainty (calculated as one standard deviation) was 0.1 ‰ for $\delta^{13}C$, 0.2 ‰ for $\delta^{15}N$, 0.3 ‰ for $\delta^{18}O$ and 2 ‰ for δ^2H.

Data analysis. We gathered information on migratory behaviour and distance for each bird species from the literature[65]. Migration distance was estimated as the distance between the breeding and wintering areas. Specifically, distance was estimated as the mean of the two latitudes during breeding minus the mean of the two latitudes during winter[66].

To compare empirical estimates of dietary and origin isotopic niche breadth with literature data, we used recently published measures of diet specialisation and climate niche breadth that were derived from literature data and breeding range maps of European birds[35]. Diet specialisation was expressed as the coefficient of variation in the presence/absence of eight food types in the diet of each species (foliage, fruit, grain, insects, other invertebrates, terrestrial vertebrates, water vertebrates, carrion). Climate niche breadth was measured by overlying the breeding range map of each species to climatic data and calculating the covered temperature range[35].

Information on long-term population trends for each species at the European scale was gathered from the Pan European Common Bird Monitoring Scheme (PECBMS) (https://pecbms.info/trends-and-indicators/species-trends/).

Dietary isotopic niche breadth (NB) was expressed as the Bayesian Standard Ellipse Area corrected for small sample size (SEAc) using the isotopic space defined by $\delta^{13}C$–$\delta^{15}N$. Similarly, spatially-explicit origin NB was expressed as SEAc from the δ^2H–$\delta^{18}O$ space. The SIBER R-package was used for these calculations[30,67].

Differences in NB among migratory groups were examined using generalised least squares (GLS) allowing the variance to differ among groups (with varIdent function in R). Seasonal trends in δ^2H, $\delta^{13}C$ and $\delta^{15}N$ within each migratory group were assessed using linear mixed-effect models including species as random factor to account for different isotope values and trends across species. We used the lme function in R allowing both intercept and slope to differ among species. To examine if origin NB was related to migration distance, we used GLS to regress the mean NB values for each species (across all measured individuals) against the estimated migration distance, allowing the variance to differ among species (with varFixed).

Although migration timing can vary within species depending on age and sex, our analyses only included juveniles born in the year of sampling, which were likely fed a similar diet regardless of sex. In fact, no particular differences in isotope patterns were observed between male and female for the species that we were able to sex (Figs. S5, S6).

Ethics approval. All experimental procedures including the permission to trap and hold birds in temporary captivity were authorized by the Autonomous Province of Trento (Autorizzazione del Comitato faunistico pro-

vinciale n. 549, 17th February 2009; Autorizzazione del Comitato faunistico provinciale n. 608, 21st November 2011) and were carried out in compliance with the current Italian laws regulating scientific research on animals.

All experimental methods, statistical analyses and results were carried out and reported in accordance with the ARRIVE guidelines on animal research.

Data availability

References

1. Hutchinson, G. E. Concluding remarks. *Cold Spring Harb. Symp. Quant. Biol.* **22**, 415–427 (1957).
2. Chase, J. M. & Leibold, M. A. *Ecological Niches. Linking Classical and Contemporary Approaches* (University of Chicago Press, 2003).
3. Holt, R. D. Bringing the Hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. *Proc. Natl. Acad. Sci.* **106**, 19659–19665 (2009).
4. Heldbjerg, H. & Fox, T. Long-term population declines in Danish trans-Saharan migrant birds. *Bird Study* **55**, 267–279 (2008).
5. Evans, K. L., Newton, J., Mallord, J. W. & Markman, S. Stable isotope analysis provides new information on winter habitat use of declining avian migrants that is relevant to their conservation. *PLoS ONE* **7**, e34542 (2012).
6. Vickery, J. A. *et al.* The decline of Afro-Palaearctic migrants and an assessment of potential causes. *Ibis* **156**, 1–22 (2014).
7. Heiss, M. The importance of Besh Barmag bottleneck (Azerbaijan) for Eurasian migrant birds. *Acta Ornithol.* **48**, 151–164 (2013).
8. Buechley, E. R. *et al.* Identifying critical migratory bottlenecks and high-use areas for an endangered migratory soaring bird across three continents. *J. Avian Biol.* **49**, e01629 (2018).
9. Cardenas-Ortiz, L., Bayly, N. J., Kardynal, K. J. & Hobson, K. A. Defining catchment origins of a geographical bottleneck: Implications of population mixing and phenological overlap for the conservation of Neotropical migratory birds. *The Condor* **122**, 004 (2020).
10. Yohannes, E., Hobson, K. A. & Pearson, D. J. Feather stable-isotope profiles reveal stopover habitat selection and site fidelity in nine migratory species moving through sub-Saharan Africa: Feather stable-isotope profiles reveal stopover habitat selection. *J. Avian Biol.* **38**, 347–355 (2007).
11. Hobson, K. A. & Koehler, G. On the use of stable oxygen isotope (δ18O) measurements for tracking avian movements in North America. *Ecol. Evol.* **5**, 799–806 (2015).
12. Bearhop, S., Adams, C. E., Waldron, S., Fuller, R. A. & Macleod, H. Determining trophic niche width: A novel approach using stable isotope analysis: Stable isotopes as measures of niche width. *J. Anim. Ecol.* **73**, 1007–1012 (2004).
13. Newsome, S. D., Martinez del Rio, C., Bearhop, S. & Phillips, D. L. A niche for isotopic ecology. *Front. Ecol. Environ.* **5**, 429–436 (2007).
14. Hobson, K. Isotopic ornithology: A perspective. *J. Ornithol.* https://doi.org/10.1007/s10336-011-0653-x (2011).
15. Hoenighaus, D. J., Winemiller, K. O. & Agostinho, A. A. Landscape-scale hydrologic characteristics differentiate patterns of carbon flow in large-river food webs. *Ecosystems* **10**, 1019–1033 (2007).
16. Hette-Tronquart, N. Isotopic niche is not equal to trophic niche. *Ecol. Lett.* **22**, 1987–1989 (2019).
17. Inger, R. & Bearhop, S. Applications of stable isotope analyses to avian ecology: Avian stable isotope analysis. *Ibis* **150**, 447–461 (2008).
18. Bowen, G. J. Isoscapes: Spatial pattern in isotopic biogeochemistry. *Annu. Rev. Earth Planet. Sci.* **38**, 161–187 (2010).
19. Hobson, K. A., Bowen, G. J., Wassenaar, L. I., Ferrand, Y. & Lormee, H. Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. *Oecologia* **141**, 477–488 (2004).
20. Magozzi, S., Vander Zanden, H. B., Wunder, M. B. & Bowen, G. J. Mechanistic model predicts tissue-environment relationships and trophic shifts in animal hydrogen and oxygen isotope ratios. *Oecologia* **191**, 777–789 (2019).
21. Vander Zanden, H. B., Soto, D. X., Bowen, G. J. & Hobson, K. A. Expanding the isotopic toolbox: Applications of hydrogen and oxygen stable isotope ratios to food web studies. *Front. Ecol. Evol.* https://doi.org/10.3389/fevo.2016 (2016).
22. Pekarsky, S. *et al.* Enriching the isotopic toolbox for migratory connectivity analysis: A new approach for migratory species breeding in remote or unexplored areas. *Divers. Distrib.* **21**, 416–427 (2015).
23. Shipley, O. N. & Matich, P. Studying animal niches using bulk stable isotope ratios: An updated synthesis. *Oecologia* **193**, 27–51 (2020).
24. Hobson, K. A. Tracing origins and migration of wildlife using stable isotopes: A review. *Oecologia* **120**, 314–326 (1999).
25. Hobson, K. A. & Wassenaar, L. I. *Tracking Animal Migration with Stable Isotopes* (Academic Press, 2018).
26. Post, D. M. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology* **83**, 16 (2002).
27. Abrantes, K. G., Barnett, A. & Bouillon, S. Stable isotope-based community metrics as a tool to identify patterns in food web structure in east African estuaries. *Funct. Ecol.* **28**, 270–282 (2014).
28. Wang, J., Chapman, D., Xu, J., Wang, Y. & Gu, B. Isotope niche dimension and trophic overlap between bigheaded carps and native filter-feeding fish in the lower Missouri River, USA. *PLoS ONE* **13**, e0197584 (2018).
29. Steenweg, R. J. *et al.* Stable isotopes can be used to infer the overwintering locations of prebreeding marine birds in the Canadian Arctic. *Ecol. Evol.* **7**, 8742–8752 (2017).
30. Rader, J. A. *et al.* Isotopic niches support the resource breadth hypothesis. *J. Anim. Ecol.* **86**, 405–413 (2017).
31. Ma, C., Shen, Y., Bearup, D., Fagan, W. F. & Liao, J. Spatial variation in branch size promotes metapopulation persistence in dendritic river networks. *Freshw. Biol.* **65**, 426–434 (2020).
32. Langin, K. M. *et al.* Hydrogen isotopic variation in migratory bird tissues of known origin: Implications for geographic assignment. *Oecologia* **152**, 449–457 (2007).
33. Levey, D. J. & Stiles, F. G. Evolutionary precursors of long-distance migration: Resource availability and movement patterns in neotropical landbirds. *Am. Nat.* **140**, 447–476 (1992).
34. Cresswell, W. Migratory connectivity of Palaearctic-African migratory birds and their responses to environmental change: The serial residency hypothesis. *Ibis* **156**, 493–510 (2014).
35. Reif, J., Hořák, D., Krištín, A., Kopsová, L. & Devictor, V. Linking habitat specialization with species' traits in European birds. *Oikos* **125**, 405–413 (2016).
36. Laube, I., Graham, C. H. & Böhning-Gaese, K. Niche availability in space and time: Migration in Sylvia warblers. *J. Biogeogr.* **42**, 1896–1906 (2015).
37. Ponti, R., Arcones, A., Ferrer, X. & Vieites, D. R. Seasonal climatic niches diverge in migratory birds. *Ibis* **162**, 318–330 (2020).

38. Dunn, E., Hobson, K., Wassenaar, L., Hussell, D. & Allen, M. Identification of summer origins of songbirds migrating through southern Canada in Autumn. *Avian Conserv. Ecol.* https://doi.org/10.5751/ACE-00048-010204 (2006).
39. Briedis, M. *et al.* Breeding latitude leads to different temporal but not spatial organization of the annual cycle in a long-distance migrant. *J. Avian Biol.* **47**, 743–748 (2016).
40. Briedis, M. *et al.* Broad-scale patterns of the Afro-Palaearctic landbird migration. *Glob. Ecol. Biogeogr.* **29**, 722–735 (2020).
41. Cortesi, N., Gonzalez-Hidalgo, J. C., Brunetti, M. & Martin-Vide, J. Daily precipitation concentration across Europe 1971–2010. *Nat. Hazards Earth Syst. Sci.* **12**, 2799–2810 (2012).
42. Salewski, V., Bairlein, F. & Leisler, B. Niche partitioning of two Palearctic passerine migrants with Afrotropical residents in their West African winter quarters. *Behav. Ecol.* **14**, 493–502 (2003).
43. Jones, P., Salewski, V., Vickery, J. & Mapaure, I. Habitat use and densities of co-existing migrant Willow Warblers *Phylloscopus trochilus* and resident eremomelas *Eremomela* spp. in Zimbabwe. *Bird Study* **57**, 44–55 (2010).
44. Brändle, M., Prinzing, A., Pfeifer, R. & Brandl, R. Dietary niche breadth for Central European birds: Correlations with species-specific traits. *Evol. Ecol. Res.* **4**(5), 643–657 (2002).
45. Hahn, S., Amrhein, V., Zehtindijev, P. & Liechti, F. Strong migratory connectivity and seasonally shifting isotopic niches in geographically separated populations of a long-distance migrating songbird. *Oecologia* **173**, 1217–1225 (2013).
46. Finch, T., Butler, S. J., Franco, A. M. A. & Cresswell, W. Low migratory connectivity is common in long-distance migrant birds. *J. Anim. Ecol.* **86**, 662–673 (2017).
47. Somveille, M., Manica, A. & Rodrigues, A. S. L. Where the wild birds go: Explaining the differences in migratory destinations across terrestrial bird species. *Ecography* **42**, 225–236 (2019).
48. Zurell, D., Gallien, L., Graham, C. H. & Zimmermann, N. E. Do long-distance migratory birds track their niche through seasons? *J. Biogeogr.* **45**, 1459–1468 (2018).
49. Rubenstein, D. R. Linking breeding and wintering ranges of a migratory songbird using stable isotopes. *Science* **295**, 1062–1065 (2002).
50. Sanderson, F. J., Donald, P. F., Pain, D. J., Burfield, I. J. & van Bommel, F. P. J. Long-term population declines in Afro-Palearctic migrant birds. *Biol. Conserv.* **131**, 93–105 (2006).
51. Ockendon, N., Hewson, C. M., Johnston, A. & Atkinson, P. W. Declines in British-breeding populations of Afro-Palaearctic migrant birds are linked to bioclimatic wintering zone in Africa, possibly via constraints on arrival time advancement. *Bird Study* **59**, 111–125 (2012).
52. Keller, G. S. & Yahner, R. H. Declines of migratory songbirds: Evidence for Wintering-ground causes. *Northeast. Nat.* **13**, 83–92 (2006).
53. Morrison, C. A., Robinson, R. A., Clark, J. A., Risely, K. & Gill, J. A. Recent population declines in Afro-Palaearctic migratory birds: The influence of breeding and non-breeding seasons. *Divers. Distrib.* **19**, 1051–1058 (2013).
54. López-Calderón, C. *et al.* Environmental conditions during winter predict age- and sex-specific differences in reproductive success of a trans-Saharan migratory bird. *Sci. Rep.* **7**, 18082 (2017).
55. Møller, A. P. & Hobson, K. A. Heterogeneity in stable isotope profiles predicts coexistence of populations of barn swallows *Hirundo rustica* differing in morphology and reproductive performance. *Proc. R. Soc. Lond. B Biol. Sci.* **271**, 1355–1362 (2004).
56. Hobson, K., Møller, A. & Wilgenburg, S. L. V. A multi-isotope (δ13C, δ15N, δ2H) approach to connecting European breeding and African wintering populations of barn swallow (Hirundo rustica). *Anim. Migr.* https://doi.org/10.2478/ami-2012-0002 (2012).
57. Newton, I. *The Migration Ecology of Birds* (Academic Press, 2007).
58. Thorup, K. *et al.* Resource tracking within and across continents in long-distance bird migrants. *Sci. Adv.* **3**, e1601360 (2017).
59. Jenni, L. & Winkler, R. *Moult and Ageing of European Passerines* (Academic Press, 2011).
60. Pedrini, P., Rossi, F. & Rizzoli, F. Le Alpi italiane quale barriera ecologica nel corso della migrazione post-riproduttiva attraverso l'Europa. Risultati generali del della prima fase del Progetto Alpi (1997–2002). *Biol. Conserv. Fauna* **116**, 1–336 (2008).
61. Bontempo, L. *et al.* Comparison of methods for stable isotope ratio (δ13C, δ15N, δ2H, δ18O) measurements of feathers. *Methods Ecol. Evol.* **5**, 363–371 (2014).
62. Wassenaar, L. I. & Hobson, K. A. Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. *Isotopes Environ. Health Stud.* **39**, 211–217 (2003).
63. Wassenaar, L. I. & Hobson, K. A. Stable-hydrogen isotope heterogeneity in keratinous materials: Mass spectrometry and migratory wildlife tissue subsampling strategies. *Rapid Commun. Mass Spectrom.* **20**, 2505–2510 (2006).
64. Brand, W. A., Coplen, T. B., Vogl, J., Rosner, M. & Prohaska, T. Assessment of international reference materials for isotope-ratio analysis (IUPAC technical report). *Pure Appl. Chem.* **86**, 425–467 (2014).
65. Del Hoyo, J., Elliott, A., Sargatal, J. & Christie, D. *Handbook of the Birds of the World* (Lynx Edicions, 2013).
66. Møller, A. P., Rubolini, D. & Lehikoinen, E. Populations of migratory bird species that did not show a phenological response to climate change are declining. *Proc. Natl. Acad. Sci.* **105**, 16195–16200 (2008).
67. Jackson, A. L., Inger, R., Parnell, A. C. & Bearhop, S. Comparing isotopic niche widths among and within communities: SIBER—Stable Isotope Bayesian ellipses in R: Bayesian isotopic niche metrics. *J. Anim. Ecol.* **80**, 595–602 (2011).

Acknowledgements

Author contributions

A.F., P.P. and F.C. conceived and designed the study. P.P. led fieldwork organization. A.F and P.P. carried out most fieldwork. S.L., P.F. and A.F. analysed the data. S.L., A.F., L.B. and P.F. wrote the main manuscript. All authors reviewed the manuscript and contributed critically to the final draft.

Funding

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-00373-9.

Correspondence and requests for materials should be addressed to S.L.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.