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Animal Ecology through Stable Isotope Analysis

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ANIMAL ECOLOGY THROUGH STABLE ISOTOPE ANALYSIS

Stable Isotope Ratios techniques raised in the last thirty years as a novel approach very useful for researchers and ecologists that intend to deepen into manifold aspects of animal ecology. In particular, isotopic ratios in animal tissues ultimately reflect diet, and the isotopic composition of diet reflects biogeochemical attributes of environments that may, in turn, show spatial structure and pattern, both at local and continental scale.

Here, SIRs techniques were applied to birds, and, in particular, the stable isotope ratios of hydrogen, carbon, nitrogen, oxygen and sulfur were determined in bird feathers.

The study was articulated in three different case studies.

In the first two studies, SIRs technique was applied to migratory birds, sampled during post-breeding migration on Italian Alps. In particular, feathers were sampled on juvenile Passerines captured in some ringing stations of ‘Progetto Alpi’ long-term monitoring project. The focus was put both on commonly and less commonly observed species (more than 800 individuals of 48 species). The first study aimed to determine the geographical breeding origin of migrants using the stable isotope ratios of hydrogen and oxygen. The study allowed 1) to perform an analyses on the relationship between hydrogen and oxygen isotope ratios of several species, 2) to verify whether migratory populations have distinct geographical origins, different timing of passage and a comparison between species. To achieve the aims, a recovery data set was also used.

The second study focused on the application of stable isotope ratios of carbon, nitrogen and sulfur to infer the trophic attitudes of different passerine species during the breeding season. In this study more species were analyzed, aiming to understand if isotopic variability of carbon, nitrogen and sulfur were able to distinguish trophic relationships between species which have similar diets. It was found that species are isotopically different grouping them for migratory phenology.

These first two studies want to be a start point to better understand migrant trends across the Alps and Europe, and to improve knowledge in using stable isotopes in European continent.

The third case study focused on lesser kestrel breeding ecology in the Gela Plain, in Sicily. In particular the stable isotope ratios of hydrogen, carbon, nitrogen, oxygen and sulfur were measured in feathers of nestlings. The aim of this study was to delineate the isotopic fingerprint of the hunting areas of their parents, making possible to distinguish different spatial uses of adults within and between colonies, and verifying if different habitat selected may be translated into distinct isotopic composition in keratinous tissues. Findings underlined the potential that a multi-isotope approach has in studying animal ecology, especially in detecting trophic partitioning and habitat selection at local and regional scale.

Index

Introduction

Studying animal ecology

In the last few decades, the study of animal ecology has developed in an astonishing manner thanks to the introduction of new and innovative technologies and methods, allowing increasing knowledge about more and more aspects of wildlife. Researchers and scientists are interested in understanding the ecological patterns of animals, including population and ecosystem dynamics and interaction between natural environments and human activities, aiming to guarantee the conservation of terrestrial biodiversity. Thus zoologists are always looking for optimal approaches, making it possible to gather as much information as possible from each observation and encounter. Generally, studying animal ecology means examining in depth the habits of one or more species, understanding their use of space in relation to environmental characteristics and community dynamics. It is evident how important it is to understand population density, movement habits and habitat preferences as well as possible. Hence, counts of individuals and estimates of the number of animals, together with morphometric and habit parameters, are the bread and butter of an animal ecologist. One of the main interests regards understanding how animals use space, from local to continental level. One practical method is to mark an animal in order to follow its movements, understanding its habitat selection and observing its food preferences, during different life stages or meteoric seasons. Animals have been tracked with several kinds of methods and devices, from morphometric detection and coloured tags (rings, collars, ear-tags), radio telemetry and radar to modern geo-locators and satellite-tags, which have allowed impressive movement reconstruction for many taxa (Leech et al. 2001, Balkiz et al. 2007, De La Hera et al. 2007, Heithaus et al. 2007, Nebuloni et al. 2008, Bächler et al. 2010, Mellone et al. 2011, Morganti et al. 2011, Panuccio et al. 2013, Brambilla et al. 2013, Bridge et al. 2013). Moreover, in addition to extrinsic marker approaches, animal ecology also involves the use of intrinsic markers. This group of markers has the advantage that initial marking of individuals is not necessary and that every capture becomes a recapture, since it provides information about the location of origin (Hobson and Norris 2008). Typical intrinsic markers fixed in animal tissues are contaminants, such as mercury (Frederick et al. 2002, Cristol et al. 2008), parasites and pathogens (Reed et al. 2003, Liu et al. 2005, Fallon et al. 2006), genetics (Vignal et al. 2002, Webster et al. 2002), trace elements (Szép et al. 2003, 2009, Schwägele 2005, Norris et al. 2007), and last but not least stable isotopes (Rundel et al. 1988, Fry 2006, Michener and Lajtha 2007, Hobson and Wassenaar 2008, Hobson et al. 2010). As regards the use of stable isotopes of light elements (H, O, C, N and S) in particular (Meier-Augenstein et al. 2013), it is worth noting their constantly increasing employment in studies and research examining various aspects of terrestrial animal ecology in depth: connectivity and migration (Hobson and Wassenaar 1997, Marra et al. 1998, Pain et al. 2004, Rubenstein and Hobson 2004, Bowen et al. 2005, 2009, Hobson 2005a, Wassenaar 2008), trophic position and partitioning in animal diets (Hobson and Clark 1992a, b, Post 2002, McCutchan et al. 2003, Martínez Del Rio et al. 2009a, Wolf et al. 2009, Boecklen et al. 2011), and the study of habitat selection and preferences through the definition of trophic isotopic niches (Koch et al. 1995, Herrera et al. 2003, Bearhop et al. 2004, Cherel et al. 2007, Jackson et al. 2011, Yohannes et al. 2013). Stable isotopes are powerful and efficient recorders of the isotopic fingerprints of dietary sources, which can be spatially linked to the isotopic patterns of landscapes and the hydrosphere at very different levels (Wassenaar 2008, Bowen 2010, Wunder 2010). They are fixed in animal tissues, and depending on biochemical status, the isotopic signature may be fixed, as in keratinous tissues (hairs, feathers), or dynamic, as in blood or muscles (Hobson 2008, Wassenaar 2008). Hence, depending on different tissue growing rates, stable isotope ratios (hereafter referred to as SIRs in this thesis) represent the isotopic variability of the animal’s diet, carrying isotopic evidence about the location where it fed, providing information on sources of nutrients in local food webs and trophic level (Hobson 2008).

In the next few paragraphs, the main aspects regarding the use of stable isotopes in animal ecology will be briefly illustrated, providing a short but complete description of light elemental isotopology.

Stable isotope ratios

The main elemental constituents of bio-organic material are hydrogen, carbon, nitrogen, oxygen and sulphur (H, C, N, O, S) (Wassenaar 2008). These elements have different stable isotopes, which are present in nature with forms having different atomic numbers (^{2}H, ^{1}H; ^{13}C, ^{12}C; ^{15}N, ^{14}N; ^{18}O, ^{17}O, ^{16}O; ^{36}S, ^{34}S, ^{33}S, ^{32}S). Generally, the lighter isotopes are the most abundant (Table 1). They are called stable because the time of decay for this kind of isotope is very long, in the order of the millions of years, in contrast to radioactive isotopes which have a time of decay in the order of thousands of years. The isotopic composition of organic compounds can be precisely and accurately measured using dedicated analytical techniques such as Isotope Ratio Mass Spectrometry (IRMS).

Table 1: Mean natural abundance of some stable isotopes and relative international reference standards.

Element	**Stable isotope**	**Mean natural abundance (%)**	**International reference standard**
Hydrogen	^{1}H	99.99	V-SMOW (Vienna –Standard Mean Ocean Water)
	^{2}H (D)	0.01	
Carbon	^{12}C	98.89	V-PDB (Vienna-Pee Dee Belemnite)
	^{13}C	1.11	
Nitrogen	^{14}N	99.63	AIR (Molecular air nitrogen)
	^{15}N	0.37	
Oxygen	^{16}O	99.76	V-SMOW (Vienna –Standard Mean Ocean Water)
	^{17}O	0.04	
	^{18}O	0.20	
Sulphur	^{32}S	95.00	V-CDT (Vienna – Canyon Diablo Troilite)
	^{33}S	0.76	
	^{34}S	4.22	
	^{36}S	0.02	

Measurements are obtained by comparing organic sampled material to international reference materials (Table 1, VPDB: Vienna - Pee Dee Belemnite for δ^{13}C, Air-N_2 for δ^{15}N, VCDT: Vienna - Canyon Diablo Troilite for δ^{34}S, VSMOW -Vienna Standard Mean Ocean Water- for δ^{18}O and δ^{2}H). Results are denoted in delta (δ) notation (Coplen 2011) and are calculated according to the following general equation (Brand and Coplen 2012):

$$\delta^{i} E = \frac{{}^{i}R_{SAM} - {}^{i}R_{REF}}{{}^{i}R_{REF}}$$

where i is the mass number of the heavier isotope of element E, R_{SAM} is the respective isotope ratio of a sample and R_{REF} is the relevant internationally recognised reference material.

Natural variation in isotopic composition is due to the different chemical-physical characteristics of the different isotopes of a certain element, resulting from different atomic weight and nuclear spin (Rundel et al. 1988). In a chemical/biochemical reaction or phase transition, these differences can both interfere with the speed of reaction (kinetic effect) and affect the energetic state of the system (thermodynamic effect). Greater mobility and smaller bond strength are traits of lighter isotopes, due to their lower weight, consequently they have lower activation energies (kinetic effect). The different free energy of isotopically different molecular species causes the thermodynamic effect: heavier molecules have lower free energy, so they have greater inertia in reaction and tend to concentrate in the condensed phase (Rundel et al. 1988, Fry 2006, Michener and Lajtha 2007). Isotopic fractionation can also be due to situations with an altered reaction equilibrium, such as an instantaneous change in temperature, or removal of a reactant or reaction product. This kind of fractionation (of non-equilibrium, such as enzymatic reactions) determines the enrichment of a particular isotopic species, but without pre-established rules (Rundel et al. 1988).

Hence there are two main types of isotopic fractionation: equilibrium fractionation, leading to isotopic separation of a substance to chemical equilibrium, and non-equilibrium or kinetic fractionation, which separates isotopes from each other during unidirectional processes, typical of biological reactions. Subsequently, there are biological and abiotic factors that can affect the variability of the isotopic ratios of $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$ observed in animal tissues (Fry 2006). However, the same biological or abiotic factors do not necessarily have the same effects on the ratios of different elements. Some of these factors are related to geochemical phenomena, and others to physiological and biochemical processes that occur during the assimilation of nutrients and the fixation of tissues performed by organisms.

Subsequently, factors affecting the variability of the isotopic ratios investigated in this thesis ($^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$) were examined in depth, in order to clarify their applications. In order to understand the isotopic variability of animal tissues, it would be good practice to understand how the stable isotopes of light elements enter food webs, starting from comprehension of the isotopic variability underlying the trophic chain.

Carbon

The carbon of plants is depleted in ^{13}C compared with external CO_2, which is their carbon source (the so-called biological isotopic effect), due to the process of CO_2 fixation (Galimov 1985). Furthermore, fractionation varies considerably in photosynthetic mechanisms, clearly differentiating the carbon isotopic ratio in plants that adopt different carboxylation systems. The result is that plants with a low photosynthetic rate and/or high stomatal conductance (C_3 plants, i.e. using the Calvin cycle for carboxylation) have much more depleted isotopic ratios (-32 to -22 ‰) compared to plants that use the C_4 photosynthetic cycle (Hatch-Slack cycle, -18 to -10 ‰) (Farquhar 1983, Farquhar et al. 1989). The strong dichotomy of values between the two types of photosynthesis creates the conditions for observing strong spatial differences at continental level, on the basis of distribution of the two types of plants over the globe (Still et al. 2003). Specifically, C_3 plants include all types of arboreal plants and several herbaceous plants of temperate, Mediterranean and

Arctic climates, whereas C_4 plants are typically herbaceous species of tropical and equatorial zones (savannah). This strong dichotomy has allowed researchers to reconstruct amazingly precise African food webs (Ambrose and DeNiro 1986, Koch et al. 1995) or migratory connectivity for Afro-Palearctic and Neotropical birds (Reichlin et al. 2009, Hobson et al. 2014a). Moreover, while the variability of isotopic ratios is fairly limited for C_4 plants, given their limited spatial distribution, a certain discrimination in values, measured as about 10 ‰, has been observed in C_3 plants (Körner et al. 1988, 1991). This is due to physiological factors related to gas-exchange during breathing adjustment that are influenced by environmental conditions such as climate, temperature and humidity (Suits et al. 2005, Männel et al. 2007). In general, the transition from mesic to xeric environments determines a decrease in $\delta^{13}C$ values, while an increase in altitude or the transition from forest environments to open lands and agricultural landscapes causes enrichment in ^{13}C (Hobson 2005a). It has been noted that these differences have clear spatial trends at continental scale, based on plant distribution and climatic effects (Still et al. 2003, Suits et al. 2005, Bowen 2010, Still and Powell 2010).

Animals assimilate carbon through their diet from three main dietary macromolecules: proteins, fats and carbohydrates (Hobson 2008). When an animal feeds, the 'isotopic fingerprint' is inherited from its diet, but due to the fractionation occurring during assimilation, it undergoes low isotopic discrimination (Hobson 2008). Hence, the $\delta^{13}C$ in animal tissues will be enriched in heavy isotopes, generally by about 1 ‰ (McCutchan et al. 2003), although $\delta^{13}C$ increases in animal body tissues with nutritional stress and low energy intake (Kempster et al. 2007, Thomas and Crowther 2015).

Nitrogen

Nitrogen isotope ratios in animal tissues are primarily a measure of the trophic position occupied within the food web (Kelly 2000, McCutchan et al. 2003, Boecklen et al. 2011). Unlike carbon, nitrogen represents a means to specifically measure and trace protein pathways deriving from the diet (Hobson 2008). It has been observed that for each step up the trophic chain, there is an enrichment in heavy isotopes in consumer tissues (Hobson and Clark 1992a, Bearhop et al. 2002, Robbins et al. 2005). At all events, global δ^{15}N patterns are determined by nitrogen cycles across ecosystems (Pardo and Nadelhoffer 2010). The most important natural source of nitrogen is air, which contains about 0.4 % ^{15}N. Through several biogeochemical processes also linked to microbial activity, air nitrogen is transformed into inorganic forms (nitrate, ammonia) and organic forms (amino acids, proteins) present and available in the soils (Rundel et al. 1988). The natural cycle of nitrogen in the environment is complex, and according to the extent of each of the processes that occurs within it, δ^{15}N can vary considerably (e.g. the δ^{15}N values of soils can range between -10 and +15 ‰) (Martinelli et al. 1999, Amundson et al. 2003, Craine et al. 2009). The gradients observable at regional and continental scale can be attributed to rapid changes in pedo-climatic characteristics, such as the transition from marine and coastal environments to continental terrestrial environments or mesic to xeric environments (due to water stress), or canopied environments to open or agricultural environments (Högberg 1997, Hobson 2005a). Specifically, marine/coastal, xeric, open and agricultural environments are generally characterised by higher δ^{15}N compared respectively to freshwater/terrestrial, mesic, forest and natural environments (Hobson 1999, 2003, Martínez Del Rio et al. 2009b). Furthermore, in agro-ecosystems managed by man, the use of fertilisers can change the normal δ^{15}N pattern of soils in a decisive manner (Michener and Lajtha 2007, Craine et al. 2015). For example, synthetic fertilisers, i.e. those produced industrially using atmospheric nitrogen with the Haber process, have δ^{15}N values between -4 and +4 ‰ (Bateman and Kelly 2007). On the other hand, organic fertilisers generally have much higher values than synthetic ones, ranging between +0.6 and +36 ‰ (e.g. animal manure has values between +10 and +25 ‰, green manure has values around 0 ‰) (Bateman et al. 2007). The nitrogen content in the soil enters the food web assimilated by plants (Rundel et al. 1988, Yoneyama 1995, Amundson et al. 2003). Spatial variations due to natural and anthropogenic input in terms of the δ^{15}N values of the soil then also pass into animal tissues through the diet. Then, as previously anticipated, each step along the food chain leads to an enrichment in $\delta^{15}N$, which implies progressive enrichment through the trophic levels (Minagawa and Wada 1984, McCutchan et al. 2003, Bearhop et al. 2004, Robbins et al. 2005, Hobson 2008). Depending on several factors, including species-specific physiology and nutritional stress, as well as variability related to environmental source, $\delta^{15}N$ increases of between 1 and 5 ‰ for each step were noted moving from the trophic position of the primary producer to the consumer gradually higher and higher up the scale (primary, secondary and tertiary) (Minagawa and Wada 1984, Hobson et al. 1993, McCutchan et al. 2003, Vanderklift and Ponsard 2003, Robbins et al. 2005).

Sulphur

There are several inorganic compounds containing sulphur, the most important of which are sulphates (SO_4^{2-}), elemental sulphur (S_0) and hydrogen sulphide gas (H_2S) (Rundel et al. 1988, Fry 2006). The average value of the isotope ratio for sulphur on Earth reflects that of the solar system, which has a value of around +22.22 ‰ (Thode et al. 1961). However, the sulphur cycle is conditioned by biological activities through the bacterial reduction and oxidation of sulphates (Mook 2000). These processes have a strong influence on the distribution of the different sulphur isotopes throughout global ecosystems, determining a natural $\delta^{34}S$ range of about 150 ‰ units (Wassenaar 2008). Seawater sulphates make up most of redox processes occurring in the sea (mean value of around +21 ‰) (Rees et al. 1978). In contrast, terrestrial systems tend to have negative $\delta^{34}S$ values (Wassenaar 2008). Sea spray is one of the most important sources of atmospheric sulphur and causes an enrichment in $\delta^{34}S$ values around coastal regions (Lott et al. 2003, Hobson 2005a, Zazzo et al. 2011), while water precipitation can be a vector of sulphur variability crosswise continental gradients (Pichlmayer et al. 1998). Moreover, the sulphur cycle may be also conditioned by anthropogenic input (Wynn et al. 2014). The combustion of fossil fuels containing sulphur and industrial processes involving sulphur compounds are the major anthropogenic sources of atmospheric primary SO_2, with δ^{34}S values ranging from very depleted to very enriched values (from -35 to + 30 ‰) (Krouse and Grineko 1971). The most common natural processes that can cause sulphur input into the atmosphere with typically depleted δ^{34}S values are volcanic activities and forest fires (Camin et al. 2007). Biogenic sulphur is another important source of atmospheric sulphur, but when it is released from soils, especially on wetlands, stable sulphur variability will reflect that of the geology and chemistry of the sites sampled (Hebert and Wassenaar 2005, Hobson 2005a). Within a food web, similarly to δ^{13}C and unlike δ^{15}N, δ^{34}S does not usually seem to suffer high fractionation (McCutchan et al. 2003). In any case, it is closely linked to protein pathways, as well as nitrogen isotopes (Hobson 2008). It is a particularly suitable marker for distinguishing between marine and terrestrial habitats, as well as habitats affected by human activities, contributing both to animal movement research and trophic ecology (Connolly et al. 2004, Hebert and Wassenaar 2005, Tanz and Schmidt 2010, Resano-Mayor et al. 2014). Finally, it has also been noted that δ^{34}S can vary due to the action of atmospheric agents, such as photodegradation of keratinous tissue (Schnyder et al. 2006).

Hydrogen and oxygen

Hydrogen and oxygen isotopes (water isotopes) will be dealt with together because they are strongly related to the water cycle, and the isotopic landscapes global patterns of reference are similar (isoscapes, Bowen and West 2008, Bowen et al. 2009, West et al. 2010). Dansgaard (1964) first described global spatial variability in the isotopic composition of meteoric water with the Global Meteoric Water Line (GMWL), defined by the equation:

$$\delta^{2}\mathrm{H}\ ‰ = 8 \times \delta^{18}\mathrm{O} + 10$$

Variations in stable hydrogen and oxygen isotopes are mainly due to the progressive drying of air masses as they lose moisture in the form of precipitation (Bowen and West 2008). This strong variation gradient is due to chemical reactions that lead to an equilibrium isotope effect, which favours the incorporation of 'heavy' isotopic species in the liquid or solid phase of rainfall during condensation (Clark and Fritz 1997, Bowen and West 2008, West et al. 2010). Then, whilst clouds lose water as rainfall, the isotopic composition of water vapour becomes incrementally depleted in ^{2}H and ^{18}O, according to the Rayleigh equation (given in terms of ratios):

$$R = R_0 f^{(\alpha - 1)}$$

where R is the isotope ratio of cloud vapour at any point in time, R_0 is the initial isotope ratio of the air mass, f is the fraction of vapour remaining, α the residual vapour produced through the condensation process (lower than 1), leading to a progressive decrease in vapour isotope ratio values as an air mass dries (Bowen and West 2008). However, re-evaporation processes for water of different origin and consequent hydrological mixing alter the simple equilibrium model, and the distribution of water isotope gradients is better described with the "Craig-Gordon model" (Craig and Gordon 1965). Other factors shape water isotope fractionation through continental gradients, in addition to the Rayleigh distillation process (depletion in the heavy fraction of water vapour due to rainout): δ^{2}H and δ^{18}O values are influenced by 1) temperature (cooler ambient temperatures result in a greater loss of heavy isotopes), 2) altitude (following the temperature gradient, water vapour decreases with altitude), 3) seasonal alternation (cool seasons have lower precipitation than warmer seasons), 4) the continental effect (precipitation becomes lower further from the source), 5) the source effect (isotopically distinct water bodies), and 6) the amount effect (typical of tropical regions) (reviewed by Hobson 2011). A couple of decades ago, researchers discovered that these isotopic gradients are transferred to food webs and are fixed and expressed in animal tissues (Chamberlain et al. 1997, Hobson and Wassenaar 1997), providing a powerful technique capable of tracing animal movement between different isotopic environments. In effect, hydrogen isotopes are particularly suitable for tracking animal movement, while oxygen is less reliable in geographical assignment (Hobson 2008). Spatial δ^2H patterns are characterised by a wide range of values, modelled on the basis of the observed data (for example sampled at meteorological centres) and predicted on the Earth's surface. Global terrestrial isotopic landscapes (hereafter isoscapes) for hydrogen isotopes cover a huge range (a shift of over 300 ‰ units in the isotopic range), while δ^{18}O shows a small (~ 15 ‰) variation along the global geographical gradient (Bowen and Revenaugh 2003, Wassenaar 2008, Bowen 2010). Moreover, hydrogen isotopes are assimilated exclusively through diet (~ 70 % in bird tissues) and as drinking water (~ 30 %), while oxygen isotopes, although having diet and drinking water as their main source, are also assimilated through respiration (Hobson et al. 1999, Wolf et al. 2011, Vander Zanden et al. 2016). Many authors have described the relationships between δ^2H and δ^{18}O measured in meteoric water and those measured in animal tissues (Hobson et al. 1999, 2004, 2012a, b, Bowen et al. 2005, Ehleringer et al. 2008, Popa-Lisseanu et al. 2012, Hobson and Koehler 2015). Generally, the δ^2H of precipitation has a more correlated relationship with that of animal tissues as compared to δ^{18}O. Furthermore, the total contribution of environmental water to consumer tissue δ^2H depends on its trophic level, and every step up the trophic levels causes an increase in δ^2H values (Birchall et al. 2005, Fraser et al. 2011, Vander Zanden et al. 2016). As regards oxygen isotopes, it is worth remembering that plants, which may be considered as the basis of trophic webs, have an additional source of oxygen through respiration, involving CO_2 and O_2 (Schmidt et al. 2001, Barbour 2007). These passages through trophic levels thus cause isotopic fractionation, and final consumer tissues may have a more enriched isotopic signature compared to that of precipitation water (Hobson 2008). For example, plants experiencing water stress (drought) undergo evapotranspiration, which causes enrichment in heavy portion foliar water isotopes (Flanagan et al. 1999, Yakir and Sternberg 2000). At all events, in the case of bird feathers, particularly of small songbirds, the relationship between the δ^2H measured in feathers and the predicted δ^2H of rainwater is well established and robustly documented (Hobson et al. 2004, 2012a, Bowen et al. 2005, Hobson 2008, 2011).

Stable isotopes in ornithology

The *Aves* class (Linneus 1758) has frequently been studied by ecologists, since in addition to the undeniable charm that birds evoke, many *taxa* are relatively easy to monitor and can be considered good ecological indicators and umbrella species (Carignan and Villard 2002, Roberge and Angelstam 2004, 2006, Padoa-Schioppa et al. 2006, Siddig et al. 2016). Consequently, the use of the SIR approach has grown enormously in the last thirty years, also in the field of ornithological science (Hobson 2005b, 2011, Inger and Bearhop 2008). Stable isotopes have been applied to study the most varied facets of avian ecology: 1) to delineate diets and trophic positions within various food webs (Hobson et al. 1994, Birchall et al. 2005), 2) to understand spatial use and sources of nutrients (Hobson et al. 1997, McKechnie et al. 2004, Hobson 2006, Gagnon and Hobson 2009), and 3) to track bird movements and migrations (Chamberlain et al. 1997, Hobson and Wassenaar 1997, 2008, Marra et al. 1998, Wassenaar and Hobson 2001, Hobson et al. 2003). As for other *taxa*, SIRs can be measured in a broad variety of avian tissues. However, feathers have the advantage of carrying the isotopic imprint of the growth location, because keratinous tissues are metabolically inert (Hobson and Wassenaar 1997, 2008). Therefore, if plumage moulting strategies are known, it is possible to delineate the isotopic patterns related to the growing time of feathers. In the case of European birds, moulting strategies are very well known (Svensson 1992, Baker 1993, Jenni and Winkler 1994, Shirihai et al. 2001), and ornithologists are able to date the growth of feathers during the year and consequently to potentially link each sampled specimen to the most probable locations and trophic webs. For this reason, stable isotopes may offer a useful means for extending knowledge about avian ecology, thanks to their relative flexibility of use and the ease with which they can be measured and obtained. Tying such an approach to the reality of already established scientific research would decisively promote the pursuit of new insight and research lines. In the case of long-term, constant-effort ringing projects, which aim to carry out in-depth research into bird migratory connectivity or breeding and wintering, stable isotope analysis can facilitate the achievement of these goals, by defining geographical origin or outlining trophic niches and habitat use.

In Europe, isotopic ornithology has developed significantly since the early 2000s (Hobson et al. 2004). Most of the research has focused on migratory connectivity between African and Western Palearctic wintering and breeding areas. The goals were to understand the wintering ranges of different populations and species (Pain et al. 2004, Yohannes et al. 2011, Hobson et al. 2012b, 2014b, Reichlin et al. 2013, Procházka et al. 2014), and to delineate their breeding ranges and migratory divides along the main trans-Saharan and trans-Mediterranean flyways (Bearhop et al. 2005, Hobson et al. 2009, 2013, Marquiss et al. 2012, Procházka et al. 2013, Arizaga et al. 2014, Tonra et al. 2014). Moreover, SIRs have also been applied to studies examining trophic ecology in depth, both in wet and terrestrial ecosystems (Yohannes et al. 2013, Resano-Mayor et al. 2014, Catry et al. 2016), and to trace site-specific or regional isotopic signatures, related to short altitudinal movements or habitat selection (Evans et al. 2012, Charmantier et al. 2014, Dehnhard et al. 2016, Morganti et al. 2016). Most of the species involved in such studies are small to medium-sized birds. The δ^2H gradient of precipitation water, which runs south-west to north-east across the Western Palearctic, makes it possible to distinguish populations with different geographical breeding origins well (Hobson et al. 2004, Bowen et al. 2005). The possibility of exploring food web trophic relationships between individuals and species, using environmental isotopic variability for high numbers of individuals, would improve knowledge about bird habits, underlying trophic niches and habitat preferences. For example, as regard bird connectivity, Procházka and collegues (2013) found impressive matches in terms of assigning European Passerines with a pronounced migratory divide to their natal origin. Feathers made it possible to reconstruct the origins of an irruptive species using museum collection material, and to date invasions back to the 19th century (Marquiss et al. 2012). Moreover, important information regarding the management of wildlife can also be obtained by tracing the origin of hunted species (Hobson et al. 2009, 2013a). Lately, the use of stable hydrogen isotopes to trace the natal origins of European migratory birds has increased considerably (Andueza et al. 2013, Arizaga et al. 2014a, b, 2015, 2016, Tonra et al. 2014). Likewise, studies adopting stable isotope approaches and aiming to study trophic ecology in depth and deduce information about trophic webs are constantly increasing (Yohannes et al. 2013, Charmantier et al. 2014, Cross et al. 2014, Resano-Mayor et al. 2014, Catry et al. 2016, Morganti et al. 2016). The stable isotope approach would definitely appear to be an indispensable tool for developing appropriate and aware policy and strategies for avian conservation and management.

Aims of the study

The aim of this thesis is to focus specifically on the application of SIRs to animal ecology, especially birds. The main uses of stable isotope analysis in avian ecology will be tested in three case studies:

1- the geographical breeding origin of 21 species of European Passerines captured in the Italian Alps during post-breeding migration will be determined. For this purpose, stable isotopes in oxygen and hydrogen will be compared. Then δ^2H will be used to delineate assignment to a rainwater isoscape map, and finally both stable isotope ratios will be combined with a EURING recovery data set;

2- trophic ecology related to the nestling diets of several migratory songbirds will be examined in depth, aiming to understand whether different migratory strategies may be related to distinct feeding habits;

3- feeding partitioning and habitat selection will be considered in a colonial raptor, the Lesser Kestrel (*Falco naumanni)*, aiming to understand within and between-colony spatial use of hunting grounds in a Sicilian breeding population.

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Materials and Methods

Sampling in the field

When the stable isotope approach is taken into consideration in order to answer ecological questions about wildlife, it is important to think about the most appropriate tissues to sample. Depending on the kind of sampled tissue and different growing rates, period and timing of growth, the isotopic information which can be gathered may vary considerably in the same specimen (Hobson 2011). Samples that can be collected can be divided into two categories, those from *fixed* tissues and those from *dynamic* tissues (Wassenaar 2008). Metabolically active tissues (*dynamic*) 'turn over' in an isotopically continuous manner. Depending on metabolic rates, the timing of isotopic turnover is very variable, ranging from days (in liver and blood) and weeks (in muscle), to a lifetime (in bone collagen). Moreover, the metabolic rates of tissues can also vary within and between species, and specific laboratory and field experiments have been carried out aimed at understanding the timing of metabolic rates in different species (Hobson and Clark 1992, Hobson and Bairlein 2003, Carleton and Martínez Del Rio 2005, Bauchinger et al. 2010). It is therefore advisable to investigate animal physiology in depth before using *dynamic* tissues. On the other hand, *fixed* tissues are defined as the kind of animal parts that are isotopically inert once they have grown. Hence, "dead" materials such as keratinous hairs, nails, claws and feathers are included in this group. Fixed tissues have the advantage of carrying isotopic information about the location in which they grew, reflecting local diet (Hobson 1999, Wassenaar 2008). In the case of isotopic ornithology involving the use of SIRs measured in feathers, there are some precautions that should be taken into consideration. The isotopic variability which can be observed in a feather refers to the diet which the sampled individual has assimilated during the period of feather growth. Hence, depending on how long the feather takes to complete its growth, together with the movement habits of the species, its SIRs may refer to one or more locations or diets (when the species changes feeding habits), which could be isotopically distinct, providing a “mean” in terms of isotopic information as a result. Generally speaking, body feathers grow faster than wing and tail feathers, especially in the case of large birds such as raptors and herons. However, small songbirds have flight feathers that grow in a relatively short time. Typically, European Passerines have moulting strategies which provide for the replacement of body and flight feathers at least once a year (Svensson 1992). Depending on species strategy, feather moult takes place in breeding or wintering distribution ranges, allowing the ‘isotopic’ ornithologist to speculate on diets and locations which the birds have visited while moulting. Moreover, moulting strategies for most European birds, especially Passerines, have been well described (Baker 1993, Jenni and Winkler 1994, Shirihai et al. 2001), and ornithologists are therefore able to choose between different feather generations, which may be linked to different diets, geographical areas and obviously distinct isoscapes.

This work focuses on three different aspects related to breeding areas. Firstly, the natal origin of several migratory Passerines was investigated. Then the nestling diets of species with different migratory and feeding habits were compared. Finally, the hunting grounds used by adults of a colonial raptor to catch prey for chicks were investigated, along with the isotopic variability measured in keratinous tissues. All three studies involved juvenile individuals, juveniles being intended as individuals born during the breeding season preceding the post-breeding migration when sampling took place. This choice was linked to the fact that the diet of these individuals grown in the nest is the expression of the isotopic variability in the diet brought to the nestlings by their parents. For kestrels, sampling the correct feathers was not a problem, since nestlings are ringed in their nests at the age of about 20 days, and body feathers on the back and flanks are sufficiently developed to be sampled. No discrepancies with local isotopic variability in terms of the isotopic composition of chick feathers which could be related to *capital breeding* (Drent and Daan 1980, Meijer and Drent 1999, Gauthier et al. 2003, Hobson 2006, Langin et al. 2006) should be observed, since egg-laying occurs more than a month after the arrival of the adults in the colonies (Sarà et al. 2012).

Once sampled, the kestrel feathers of each individual were stored separately in paper envelopes until laboratory processing. On the other hand, small songbirds were sampled during migration, at a time when post-breeding moulting had already occurred. However, juveniles of many species experience partial post-breeding moulting, with some of the feathers grown in the nest being retained, usually tail and wing feathers, while the body feathers are renewed (Svensson 1992, Jenni and Winkler 1994). The post-breeding generation feathers are not certainly related to natal location, since after fledging dispersal can occur, and the diet may vary considerably. In contrast, juvenile feathers grown in the nest are undoubtedly related to natal (and breeding) isoscapes, which are assimilated and fixed in keratins through the parental diet. Experienced ornithologists working in ringing stations can easily recognise feather generations on sampled individuals and collect those they need for their research. According to the main moulting and ageing guides available for European Passerines (Svensson 1992, Jenni and Winkler 1994, Shirihai et al. 2001), juvenile feathers were sampled from the tail and wings. The sampling criteria were designed to collect enough keratinous material to measure the SIRs of hydrogen, carbon, nitrogen, oxygen and sulphur. 1.5 milligrams of cleaned keratinous material for each individual was calculated to be enough to perform the analysis. Therefore, two feathers were sampled for small birds (from *Regulids* to *Fringillids*), generally one internal rectrices and an internal secondary remiges, while for medium-sized birds (large *Thrushes*) one feather was enough for analysis. The feathers were pulled out and not cut off, in order to induce immediate renewal of the lost feather. Personal observations of local birds showed complete substitution of the feather within one month of sampling. Subsequently, the feathers were catalogued and stored in plastic binder envelopes, being stored at room temperature until cleaning and analytical processing in the laboratory.

Stable isotope analysis

In order to measure the SIRs of H, O, C, N and S, feathers must be cleaned to remove dirt and natural oils which cover the keratin (Wassenaar 2008). Thus the feathers were washed in a solvent mixture (diethyl ether-methanol 2:1, Bontempo et al. 2014), washing each one three times in a test tube immersed in an ultrasonic tank for one minute. The feathers were then stored in glass vials and oven-dried at 60°C overnight. Pairs of feathers from the same individual were kept together. After cleaning, the feathers needed to be prepared in order to obtain a homogenised powder, avoiding the rachis but using only the vane. Therefore, wearing antistatic gloves and working on an antistatic pad, each feather vane was separated from its rachis manually, simply by tearing it off with the hands. The vanes were subsequently powdered inside the glass vials using stainless steel surgical scissors. The rachis were preserved and stored together with corresponding powdered feathers in the vials, plugged with a rubber stopper. The feathers were then ready to be analysed in order to obtain SIRs. The samples were therefore weighed on a microbalance (CP2P, Sartorius AG, Goettingen, Germany). For δ^2H and δ^{18}O analysis, about 0.2 mg for each sample was placed in silver capsules. Once weighed, the samples and reference materials were left in laboratory air moisture for at least 96 h, then placed in a desiccator with P_2O_5 under vacuum for a further 96 h. Samples were then loaded onto the autosampler tray, put on the carousel, sealed with a cover and purged with argon.

For δ^{13}C, δ^{15}N and δ^{34}S determination, about 0.3 mg of the samples were weighed into tin capsules. Each sample was weighed and analysed twice for δ^2H and δ^{18}O, and three times for δ^{13}C, δ^{15}N and δ^{34}S. Determination of δ^{13}C, δ^{15}N and δ^{34}S in one run was carried out using an isotope ratio mass spectrometer (Vario Isotope Cube, Elementar Analysensysteme GmbH, Germany). δ^2H and δ^{18}O were determined using an isotope ratio mass spectrometer equipped with a TC/EA (thermo combustion pyrolyser - elemental analyser; Delta Plus XP -ThermoFinnigan, Bremen, Germany), with a 1.2 m long GC column (Packed Column Molecular Sieve 5A, 1.2 m, 5.4 mm ID, 1/4 in OD Restek U.S.A). The use of a longer than usual GC column (0.6 m) made it possible to avoid the problem of overlapping of N_2 and CO peaks during δ^{18}O measurement (RIF). The isotope ratios were expressed in δ against V-PDB (Vienna - Pee Dee Belemnite) for δ^{13}C, Air for δ^{15}N, V-SMOW (Vienna - Standard Mean Ocean Water) for δ^{2}H and δ^{18}O, and V-CDT (Vienna – Canyon Diablo Triolite) for δ^{34}S according to the following formula:

$$\delta = \frac{R_{sample} - R_{standard}}{R_{standard}}$$

where R_{sample} is the isotope ratio measured for the sample, and $R_{standard}$ is the isotope ratio of the international standard.

Accurate determination of the relative stable isotope ratios of hydrogen (δ^{2}H), carbon (δ^{13}C), oxygen (δ^{18}O) and nitrogen (δ^{15}N) requires two-point normalization, using at least two isotopic reference materials with different isotopic compositions to (i) anchor the isotopic scale and (ii) compensate for differences in instrumental responses, which commonly compress isotope-δ scales (Schimmelmann et al. 2016). The isotopic values of δ^{18}O and δ^{2}H were calculated as specified in Bontempo et al. (2014). In particular, a regression line was built using the two reference materials Caribou Hoof Standard (CHS, USGS - United States Geological Survey, Reston Stable Isotope Laboratory, Virginia, USA) and Kudu Horn Standard (KHS, USGS). The reference values of the reference materials considered were δ^{2}H = -197.0 ‰ and δ^{18}O = +3.8 ‰ for CBS and δ^{2}H = -54.1 ‰ and δ^{18}O = +20.3 ‰ for KHS. Therefore, the δ^{2}H and δ^{18}O values of the samples were expressed in comparison to V-SMOW on scales normalized in such a way that the δ^{2}H and δ^{18}O values of SLAP (Standard Light Antarctic Precipitation) were –428 ‰ and –55.5 ‰ respectively, as recommended by IUPAC (Brand et al. 2014). In each analytical sequence, analysis of an internal quality control material (cheratin, Camida Ltd., Tipperary, Ireland) was included to check analytical system performance.

One of the problems for meaningful determination of δ^{2}H is taking into account the presence of exchangeable hydrogen atoms. Indeed, in matrices that contain hydrogen atoms in exchangeable groups such as amino, hydroxyl or carboxyl groups (e.g. cheratin and protein in general), these atoms can exchange with atmospheric water vapour and this can potentially strongly affect the δ^{2}H values determined in the samples (Meier-Augenstein et al. 2013). The treatment of samples carried out in this study, in particular the elimination of any residual moisture, as well as simultaneous equilibration with lab air moisture, together with the use of matrix-matched reference materials, overcame the problem of exchangeable hydrogen atoms.

δ^{13}C, δ^{15}N and δ^{34}S isotopic values were calculated against working in-house standards (casein and wheat), which were themselves calibrated against international reference materials: fuel oil NBS-22 (IAEA International Atomic Energy Agency, Vienna, Austria; -30.031 ‰) and sugar IAEA-CH-6 (-10.449 ‰) for δ^{13}C, L-glutamic acid USGS 40 (-26.389 ‰ and -4.5 ‰ for δ^{13}C and δ^{15}N), hair USGS 42 (δ^{15}N = +8.05 ‰ and δ^{13}C = -21.09 ‰) and USGS 43 (δ^{15}N = +8.44 ‰ and δ^{13}C = -21.28 ‰) for ^{13}C/^{12}C and ^{15}N/^{14}N; USGS 42 (+7.84 ‰) and USGS 43 (+10.46 ‰) for ^{34}S/^{32}S. δ^{13}C values were expressed versus V-PDB on a scale normalized using the two reference materials NBS 19 and LSVEC, with consensus δ^{13}C values of +1.95 and -46.6 ‰ (Brand et al., 2014). δ^{15}N values were expressed versus Air-N2 on a scale normalized using the two reference materials IAEA-N-1 and USGS32, with consensus values of +0.4 ‰ and +180 ‰ (Brand et al., 2014). δ^{34}S measurements of all sulphur-bearing materials were expressed relative to VCDT on a scale defined by assigning a δ^{34}S value of exactly -0.3 ‰ to the reference material IAEA-S-1.

Method uncertainty (calculated as one standard deviation) was 0.1 ‰ for δ^{13}C, 0.2 ‰ for δ^{15}N, 0.3 ‰ for δ^{18}O and δ^{34}S and 2 ‰ for δ^{2}H.

Stable isotope ratios

Stable isotope ratios were measured in 807 individuals of 48 Passerines and 45 individuals of Lesser Kestrel. In Appendix 1, together with information about the species, date of sampling in the field and ecological characteristics. In Chapter 5 the isotopic composition of the Kestrels.

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IDENTIFYING THE NATAL ORIGIN OF MIGRATORY PASSERINES USING HYDROGEN AND OXYGEN ISOTOPES (δ^2H AND δ^{18}O) AND RECOVERY DATA

Introduction

The stable isotope ratios of hydrogen and oxygen (δ^2H and δ^{18}O) fixed in animal tissues through diet reflect the spatial continental gradients of the isotopic composition of precipitation water isoscapes (see Chapter 1). They are therefore excellent intrinsic tracers of isotopically distinct geographical locations to adopt in studies of animal movement ecology. However, of δ^2H and δ^{18}O, deuterium is by far the most widely used to detect geographical origin (Hobson and Wassenaar 2008, Inger and Bearhop 2008, Hobson 2011). Indeed, while stable hydrogen composition in animal tissues depends only on diet and drinking water, the oxygen isotope is also found in molecular oxygen breathed in (Wassenaar 2008, Hobson and Koehler 2015). At all events, researchers studying bird migration can benefit extensively from using stable isotope approaches to determine connectivity in population movements. In the case of long-term ringing projects monitoring migratory birds trends annually, the use of stable isotopes can provide a high level of certainty in terms of distinguishing populations by their geographical origin (Dunn et al. 2006), expanding the information gained from recovery data sets (Van Wilgenburg and Hobson 2011) or that can be inferred from morphometrics and genetics (Kelly et al. 2005, Morganti et al. 2014, Bazzi et al. 2016).

This chapter reports on testing of the reliability and efficiency of δ^2H and δ^{18}O measured in bird feathers for the purpose of geographical detectability. Feathers were sampled from 21 Passerine species at two ringing stations in the Italian long-term monitoring project on post-breeding migration through the Italian Alps called ‘Progetto Alpi’ (Pedrini et al. 2003, Spina and Volponi 2008). The scope was to evaluate the relationship between δ^2H and δ^{18}O measured in feathers, to determine whether δ^2H and δ^{18}O measurements are able to delineate intra-seasonal population trends within sampled migratory species, and to reconstruct breeding geographical origin using the relationship between δ^2H measured in feathers and the δ^2H precipitation isoscape, improved by known breeding-range maps and predicted areas of provenance provided by a recovery data set.

Materials and methods

Sampling in the field

The feathers of 715 young belonging to 21 species born that year were sampled (only juvenile feathers), in order to relate isotopic composition to natal origin (see Chapter 2). The 21 sampled species represented those most commonly observed at the alpine ringing stations in the *Progetto Alpi* (Pedrini et al. 2008), namely a mixture of trans-Saharan, regular and irruptive intra-Palaearctic, nocturnal and diurnal migrants. Sampling took place in 2010, 2011, 2012 and 2013. The species were expected to originate from a wide area north-east of Alps, between Central and Eastern Europe and Fennoscandia (Jenni and Kéry 2003), and the migratory flux through Alps should follow a route running mainly north-east to south-west (Bruderer and Jenni 1990, Micheli and Pedrini 2000, Pedrini unpublished data). Specifically, the 21 species were the following: House Martin (*Delichon urbicum*), Tree Pipit (*Anthus trivialis*), Meadow Pipit (*Anthus pratensis*), Yellow Wagtail (*Motacilla flava*), Dunnock (*Prunella modularis*), European Robin (*Erithacus rubecula*), Common Redstart (*Phoenicurus phoenicurus*), Black Redstart (*Phoenicurus ochruros*), Song Thrush (*Turdus philomelos*), Blackcap (*Sylvia atricapilla*), Garden Warbler (*Sylvia borin*), Common Chiffchaff

(*Phylloscopus collybita*), Willow Warbler (*Phylloscopus trochilus*), Goldcrest (*Regulus regulus*), Pied Flycatcher (*Ficedula hypoleuca*), Coal Tit (*Periparus ater*), Common Chaffinch (*Fringilla coelebs*), Brambling (*Fringilla montifringilla*), Eurasian Siskin (*Carduelis spinus*), Common Crossbill (*Loxia curvirostra*), Hawfinch (*Coccothraustes coccothraustes*). The number of specimens and year of capture are shown in Appendix 1. The sampling sites were two ringing stations in the *Progetto Alpi*, set in the central Italian Alps, under the scientific supervision of the Vertebrate Zoology Section of the Museum of Science (MUSE) in Trento, and ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale, Ozzano dell'Emilia, Bologna, Italy): 'Bocca di Caset' (Tremalzo, Ledro, in the province of Trento, 45°51'N, 10°41'E) and 'Passo Broccon' (Passo Broccon, Castello Tesino, in the province of Trento, 46°06'N, 11°41'E). It should be noted that the birds were sampled almost entirely at Bocca di Caset, and 23 Meadow Pipit of 27 are sampled at Passo Broccon ringing station; anyway, the two ringing stations are relatively close to each other (~ 90 Km as the crow flies). The sampling period was between August and October, according to the field activity of the ringing stations. Therefore, the predicted timing of the migratory passage of the sampled species through the Alps was monitored (Pedrini et al. 2012). Finally, morpho-biometrics are available for each sampled individual (Progetto Alpi database).

The feathers were sampled, stored, cleaned and analysed to obtain SIRs as already described in Chapter 2. The isotopic values of different years were pooled together, since the feathers of different species were sampled in different years and the numbers were not large.

Relationship between δ^2H and δ^{18}O

The degree of relationship between δ^2H and δ^{18}O measured in the feathers of each species was calculated. It was expected to find a low statistical correlation (r^2 ~ 0.35, Hobson and Koehler 2015). Therefore, robust fitting of linear models was performed in an R environment (R Core Team

2013), using the *MASS* and *foreign* R packages (Venables and Ripley 2002), for which coefficients of determination were obtained using the *sfsmisc* package (Maechler 2016).

Intra-seasonal trends of δ2H and δ18O measured in feathers and isotopic populations

Pedrini and collegues (2008, 2012) described the intra-seasonal patterns and timing of migration of the most common migratory Passerines passing through the Italian Alps. Aiming to verify whether individuals belonged to one or more geographical (and isotopic) populations, and whether individuals with distinct isotopic composition passed through together or were staggered, the relationship between δ^2H and δ^{18}O measurements and the date of capture was tested for each species with robust fitting linear models. In addition, in order to compare species with different migration timing (typically, trans-Saharans are late-summer migrants, while intra-Palaearctics are autumnal migrants), the date of capture was normalized for each species, obtaining values between 0 and 1. Thus normalization of the date of capture was calculated as follows:

$$DAY_{normalized} = \frac{DAY^{X}_{sampling} - DAY^{X}_{min}}{DAY^{X}_{max} - DAY^{X}_{min}}$$

where DAY_{max} is the latest expected day for observing species X migrating through the Italian Alps, DAY_{min} the earliest expected day for observing species X migrating through the Italian Alps, and $DAY_{sampling}$ is the day on which each individual of species X was sampled (Pedrini et al. 2008, see Table 1). Therefore, individuals with $DAY_{normalized}$ below 0 were considered to be local or to originate from a location close to the ringing stations, while individuals with $DAY_{normalized}$ greater than 1 were considered to be migrants experiencing delays in their journeys, and thus outside the normal specific trends. Hence, neither group was considered when processing the linear models. Moreover, differences between species in terms of δ^2H and δ^{18}O composition were verified, performing analysis of variance between species and *post-hoc* tests (Tukey's Honestly Significant Test -HSD- Test, Abdi and Williams 2010, *stats* package R).

Table 2: List of the 21 sampled species and their post-breeding migratory season as predicted by Pedrini and colleagues (2008).

Species	**Migratory season**	**Pentads**
House Martin (*Delichon urbicum*)	14 Aug - 7 Oct	46-56
Meadow Pipit (*Anthus pratensis*)	3 Oct - 27 Oct	56-60
Tree Pipit (*Anthus trivialis*)	14 Aug - 27 Sep	46-54
Yellow Wagtail (*Motacilla flava*)	24 Aug - 22 Sep	48-53
Dunnock (*Prunella modularis*)	13 Sep - 1 Nov	52-61
European Robin (*Erithacus rubecula*)	3 Sep - 1 Nov	50-61
Common Redstart (*Phoen. phoenicurus*)	14 Aug - 17 Oct	46-58
Black Redstart (*Phoenicurus ochruros*)	23 Sep - 1 Nov	54-61
Song Thrush (*Turdus philomelos*)	18 Sep - 1 Nov	53-61
Garden Warbler (*Sylvia borin*)	14 Aug - 12 Oct	46-57
Blackcap (*Sylvia atricapilla*)	14 Aug - 1 Nov	46-61
Willow Warbler (*Phylloscopus trochilus*)	14 Aug - 12 Oct	46-57
Common Chiffchaff (*Phyllos. collybita*)	8 Sep - 1 Nov	51-61
Goldcrest (*Regulus regulus*)	8 Sep - 1 Nov	51-61
Pied Flycatcher (*Ficedula hypoleuca*)	14 Aug - 2 Oct	46-55
Coal Tit (*Periparus ater*)	8 Sep - 1 Nov	51-61
Common Chaffinch (*Fringilla coelebs*)	23 Sep - 1 Nov	54-61
Brambling (*Fring. montifringilla)*	3 Oct - 1 Nov	56-61
Eurasian Siskin (*Carduelis spinus*)	23 Sep - 1 Nov	54-61
Hawfinch (*Coccot. coccothraustes)*	23 Sep - 1 Nov	54-61
Common Crossbill (*Loxia curvirostra*)	14 Aug - 1 Nov	46-61

Geographic origins of birds

The geographic assignment to the natal origin was calculated for each species basing on a likelihood-based approach, within a Bayesian framework. This type of assignment allows to take into account all kinds of errors (analytical errors, within-population variance, errors associated with the isoscapes) which can influence the probability of correctly assigning of birds to their origins (Wunder and Norris 2008, Wunder 2010, Van Wilgenburg and Hobson 2011). One crucial step of producing an assignment is the choice of the correct calibration equation, which is a regression between δ^2H measured in feathers of sure location of growth and the hydrogen isoscape, and permits to translate the δ^2H measured in feathers (hereafter δ^2H_f) into the δ^2H of precipitation (hereafter δ^2H_p). This equation should be preferably species-specific, in order to reduce the variance due to p.e. different feeding habits of the different species (Hobson et al. 2012), but nevertheless there are not equations for each of the 21 species. Therefore, the mean annual δ^2H_p isoscape (Bowen and Revenaugh 2003) was calibrated following the equation which Bowen and colleagues (2005) modelled using a wide number of different species. The gridded map of δ^2H_p can be found and downloaded online at www.waterisotopes.org (Bowen 2016). The assignment was calculated via selection of likely (1) versus unlikely (0) origins based upon odds ratios (Hobson et al. 2009, Van Wilgenburg and Hobson 2011). The global δ^2H_p isoscape was limited within the extensions of the breeding ranges of each of the 21 species (BirdLife 2015). In addition, the breeding ranges were limited to an area obtained with a recovery data set of regular migrants with first capture occurred during breeding season and consequent re-capture in the same year during post-breeding migration on Italian Alps and foothills. This step allowed to reduce the calculation of the assignment to a restricted area of origin given by the direction of origin of recoveries. However, it was not produced a probability surface built on the basis of directions and distances (Van Wilgenburg and Hobson 2011). Otherwise, it has been produced a simple polygon (QGIS Geographic Information System 2016) which has been used to limit the isoscape together with the breeding range. The assignment has been calculated for each individual, obtaining a probability surface of being (1) or not (0) assigned to each unit cell of the isoscape; afterwards, these surfaces have been summed for each species, obtaining the population assignments to the natal origins, given by the number of individuals of the sampled population which would likely originate from each unit cell (Van Wilgenburg et al. in prep.). Recovery data were obtained from EURING (European Union for Bird Ringing, du Feu et al. 2016), and included almost 100 years of ringing data (1910-2008). All statistical operations were developed within R software, using *raster*, *maps* and *maptools* packages (Becker et al. 2016, Bivand and Lewin-Koh 2016, Hijmans 2016).

Results

Relationship between δ^2H and δ^{18}O measured in feathers

Trend lines, p-values and coefficients of determination (r^2) of relationships between δ^2H and δ^{18}O measured in the feathers of the 21 species are reported and represented in Figures 1 to 21. The coefficients showed a certain variability among species (r^2 max = 0.61, min = 0.004). This could be due in many cases to the small number of specimens per species. However, for species with statistically significant numbers (e.g. European Robins and Pied Flycatchers), the coefficients were quite low. It is worth noting the case of Meadow Pipits, for which oxygen isotopes seem to distinguish two distinct groups characterised by very different relationships between δ^2H and δ^{18}O values.

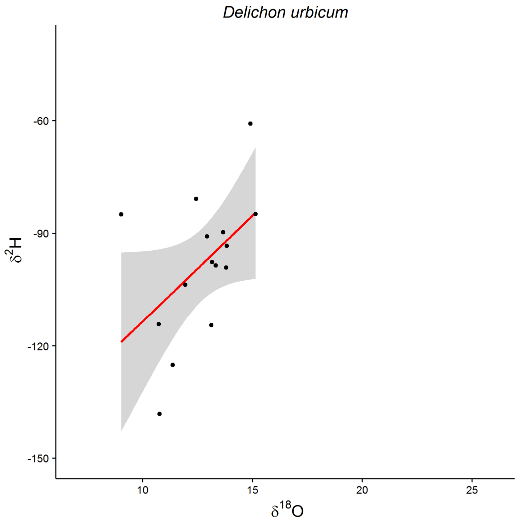

Figure 1: Relationships between $\delta^{2}H_{f}$ and $\delta^{18}O_{f}$ values for the House Martin *Delichon urbicum* (n=16). Trend line y=7.730x-195.644, p value < 0.001, r^2 0.56.

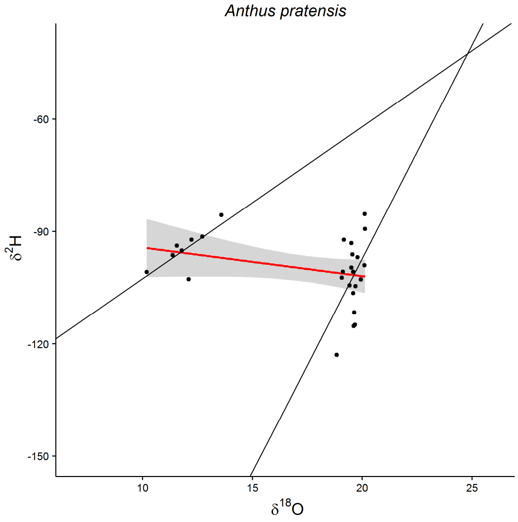

Figure 2: Relationships between $\delta^{2}H_{f}$ and $\delta^{18}O_{f}$ values for the Meadow Pipit *Anthus pratensis* (n=27). Trend line 1 y=4.0575x-143.17, r^2 0.56 (n=8); trend line 2 y=11.401-325.21, r^2 0.19 (n=19).

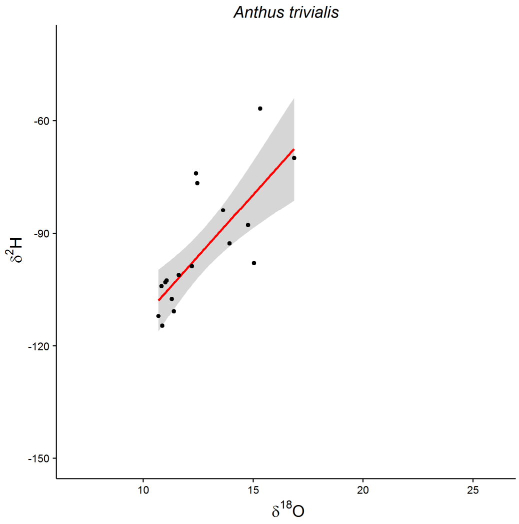

Figure 3: Relationships between $\delta^{2}H_{f}$ and $\delta^{18}O_{f}$ values for the Tree Pipit *Anthus trivialis* (n=18). Trend line y=6.537x-177.707, p value < 0.001, r^2 0.61.

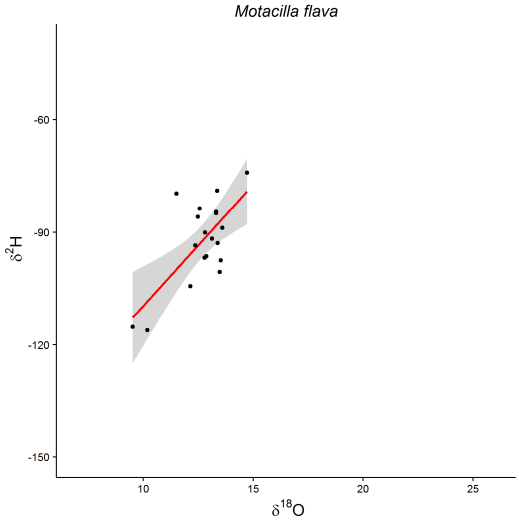

Figure 4: Relationships between $\delta^{2}H_{f}$ and $\delta^{18}O_{f}$ values for the Yellow Wagtail *Motacilla flava* (n=19). Trend line y=6.467x-174.33, p value < 0.01, r^2 0.47.

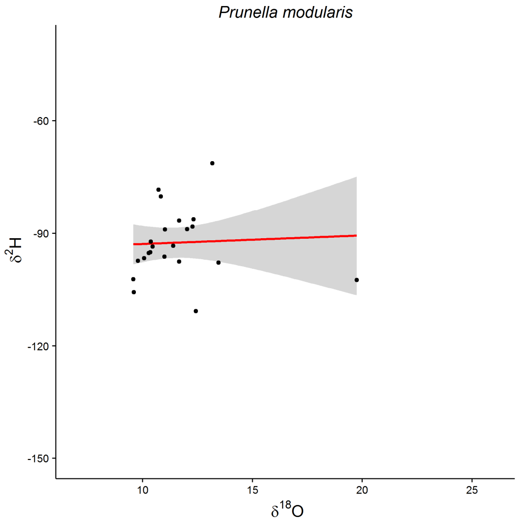

Figure 5: Relationships between δ^2H_f and $\delta^{18}O_f$ values for the Dunnock *Prunella modularis* (n=20). Trend line y=0.2271x-95.096, p value = 0.80, r^2 0.004.

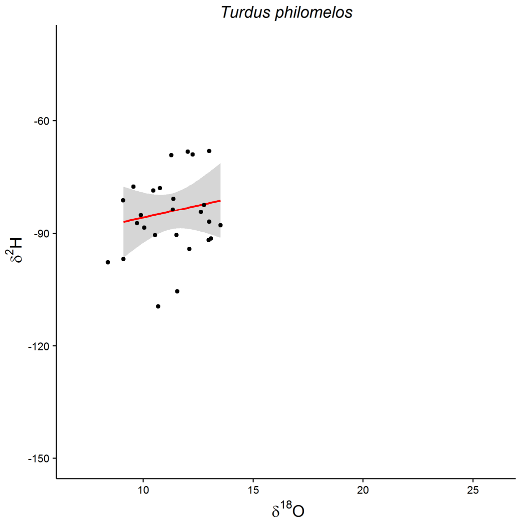

Figure 7: Relationships between δ^2H_f and $\delta^{18}O_f$ values for the Song Thrush *Turdus philomelos* (n=26). Trend line y=1.304x-98.816, p value = 0.48, r^2 0.03.

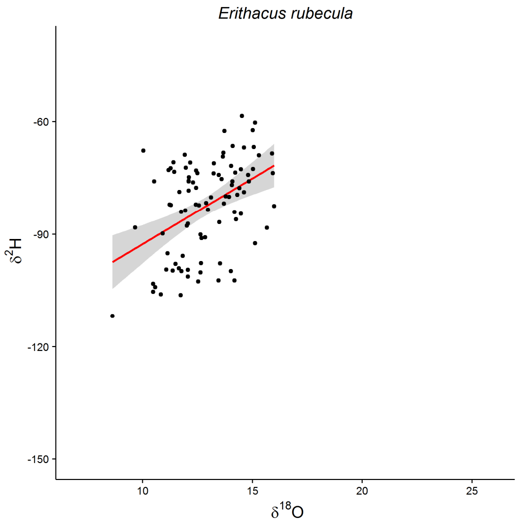

Figure 6: Relationships between δ^2H_f and $\delta^{18}O_f$ values for the European Robin *Erithacus rubecula* (n=96). Trend line y=3.4959x-127.5652, p value < 0.001, r^2 0.18.

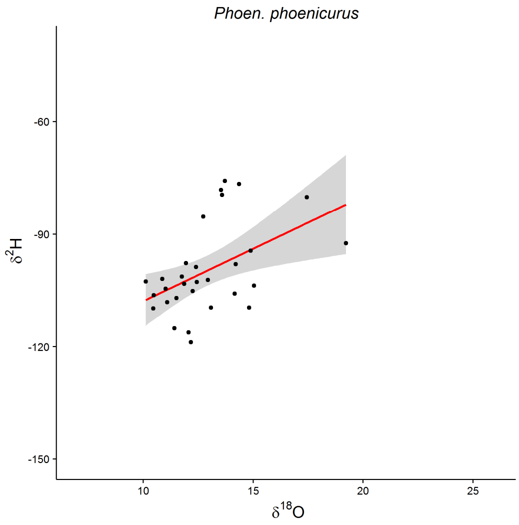

Figure 8: Relationships between δ^2H_f and $\delta^{18}O_f$ values for the Common Redstart *Phoenicurus phoenicurus* (n=30). Trend line y=2.792x-135.7274, p value < 0.01, r^2 0.23.

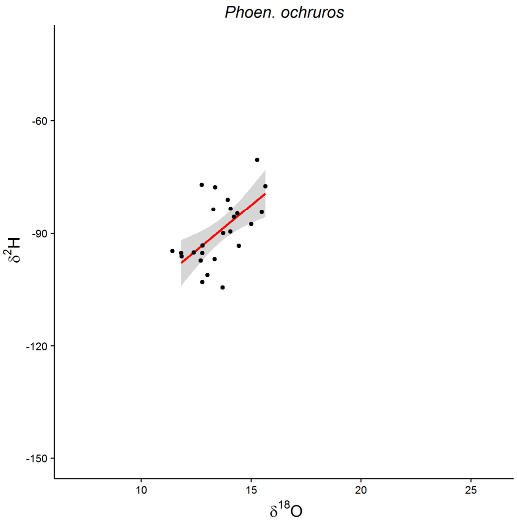

Figure 9: Relationships between δ^2H_f and $\delta^{18}O_f$ values for the Black Redstart *Phoenicurus ochruros* (n=25). Trend line y=4.83x-154.897, p value < 0.01, r^2 0.41.

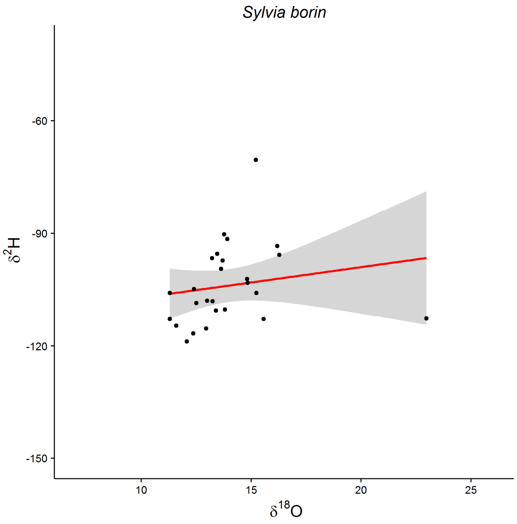

Figure 11: Relationships between δ^2H_f and $\delta^{18}O_f$ values for the Garden Warbler *Sylvia borin* (n=26). Trend line y=0.8127x-115.1986, p value = 0.39, r^2 0.03.

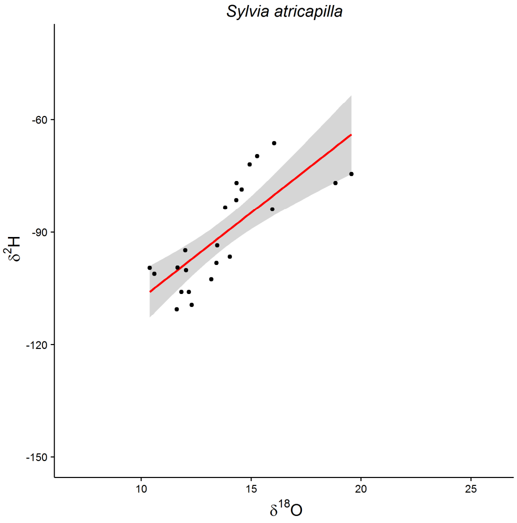

Figure 10: Relationships between δ^2H_f and $\delta^{18}O_f$ values for the Blackcap *Sylvia atricapilla* (n=23). Trend line y=4.5681x-153.2843, p value < 0.001, r^2 0.60.

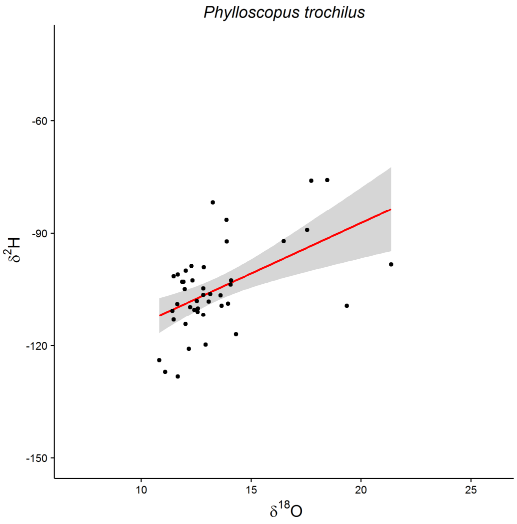

Figure 12: Relationships between δ^2H_f and $\delta^{18}O_f$ values for the Willow Warbler *Phylloscopus trochilus* (n=43). Trend line y=2.6985x-141.178, p value < 0.001, r^2 0.28.

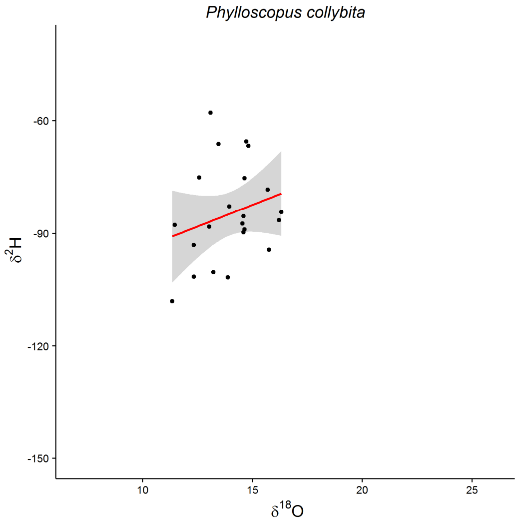

Figure 13: Relationships between $\delta^{2}H_{f}$ and $\delta^{18}O_{f}$ values for the Common Chiffchaff *Phylloscopus collybita* (n=23). Trend line y=2.303x-116932, p value p = 0.26, r^{2} 0.06.

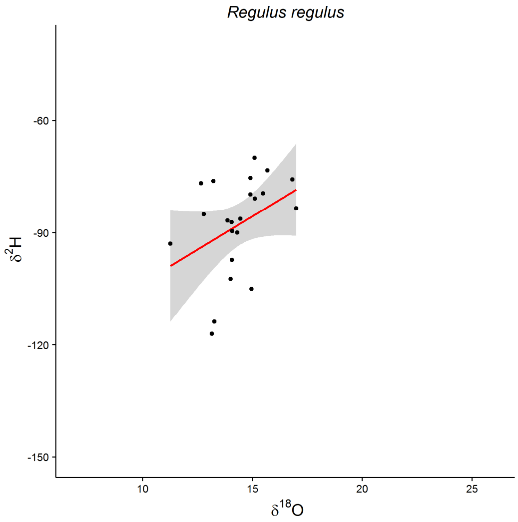

Figure 14: Relationships between $\delta^{2}H_{f}$ and $\delta^{18}O_{f}$ values for the Goldcrest *Regulus regulus* (n=22). Trend line y=3.565x-139.018, p value =0.099, r^{2} 0.16.

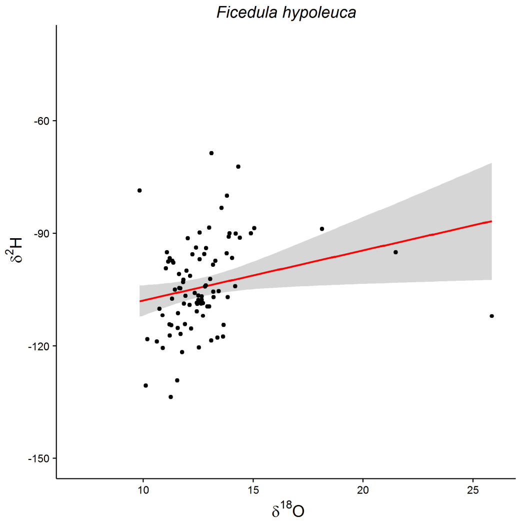

Figure 15: Relationships between $\delta^{2}H_{f}$ and $\delta^{18}O_{f}$ values for the Pied Flycatcher *Ficedula hypoleuca* (n=88). Trend line y=1.326x-121.032, p value < 0.05, r^{2} 0.06.

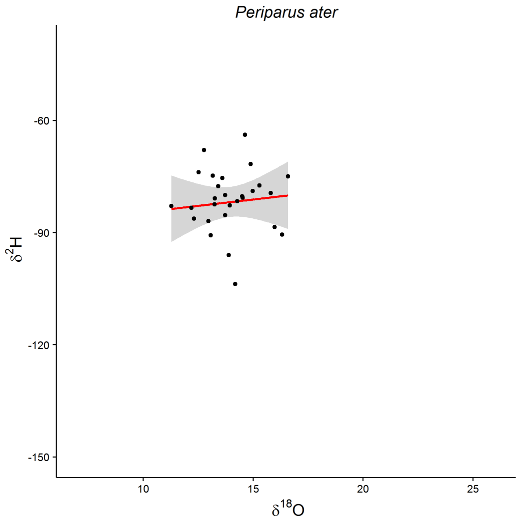

Figure 16: Relationships between $\delta^{2}H_{f}$ and $\delta^{18}O_{f}$ values for the Coal Tit *Periparus ater* (n=28). Trend line y=0.678x-91.164, p value = 0.64, r^{2} 0.01.

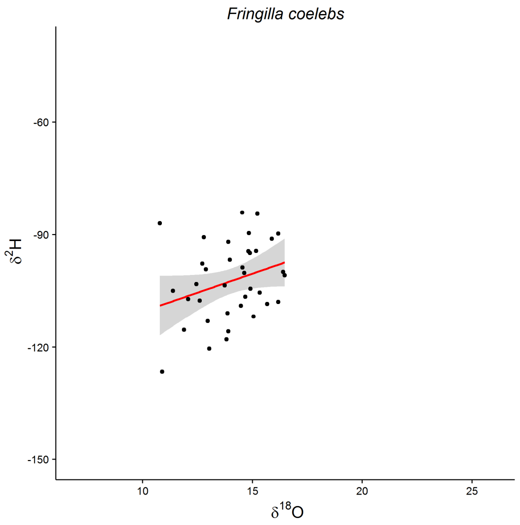

Figure 17: Relationships between δ^2H_f and $\delta^{18}O_f$ values for the Common Chaffinch *Fringilla coelebs* (n=37). Trend line y=2.019x-130.634, p value = 0.07, r^2 0.09.

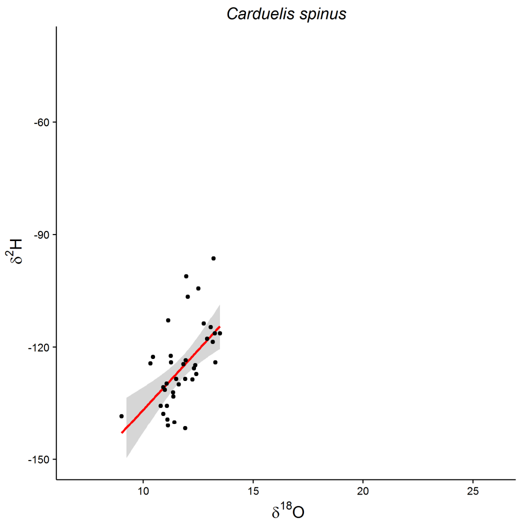

Figure 19: Relationships between δ^2H_f and $\delta^{18}O_f$ values for the Eurasian Siskin *Carduelis spinus* (n=38). Trend line y=6.357x-200.217, p value < 0.001, r^2 0.35.

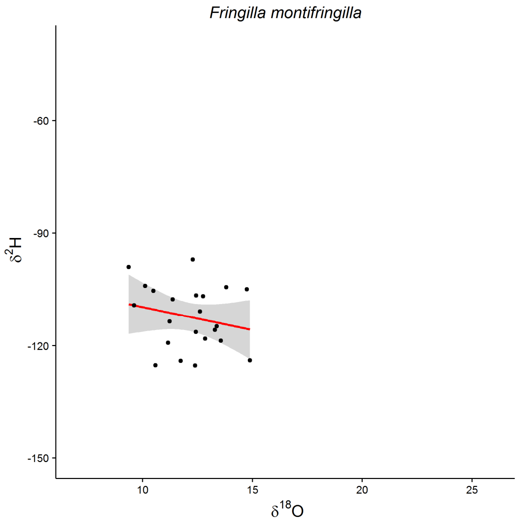

Figure 18: Relationships between δ^2H_f and $\delta^{18}O_f$ values for the Brambling *Fringilla montifringilla* (n=22). Trend line y=-1.223x-97.449, p value = 0.32, r^2 0.05.

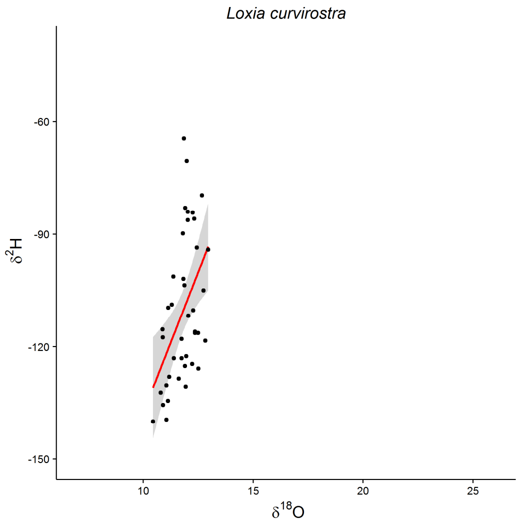

Figure 20: Relationships between δ^2H_f and $\delta^{18}O_f$ values for the Common Crossbill *Loxia curvirostra* (n=41). Trend line y=14.979x-287.298, p value < 0.01, r^2 0.23.

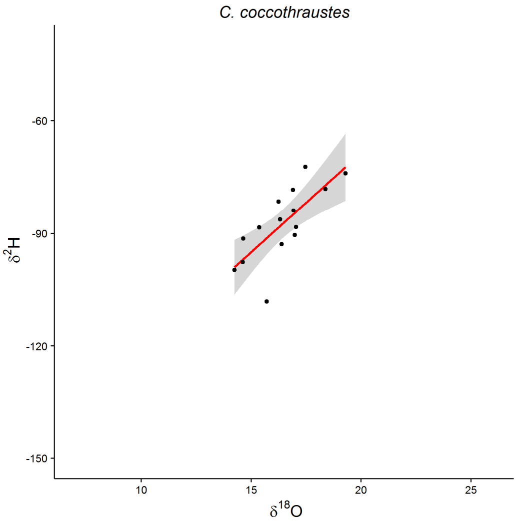

Figure 21: Relationships between δ^2H_f and $\delta^{18}O_f$ values for the Hawfinch *Coccothraustes coccothraustes* (n=15). Trend line y=5.272x-174.049, p value < 0.01, r^2 0.55.

Intra-seasonal trends of δ^2H and δ^{18}O measured in feathers and isotopic populations

An overview of the variability of δ^2H and δ^{18}O isotopic composition in the species is given in Figures 22 and 23 respectively. Statistical differences (*post-hocs*) between species are shown in Table 2. The δ^2H values varied considerably among species. In particular, there were some species, such as the Coal Tit and Goldcrest, with enriched values as compared to others, while species such as the Eurasian Siskin had clearly depleted values compared to others.

On the other hand, δ^{18}O values seemed less dispersed among species, showing two species with considerably enriched values (Meadow Pipit and Hawfinch), while the most depleted values were seen in the Dunnock, Song Thrush, Eurasian Siskin and Common Crossbill.

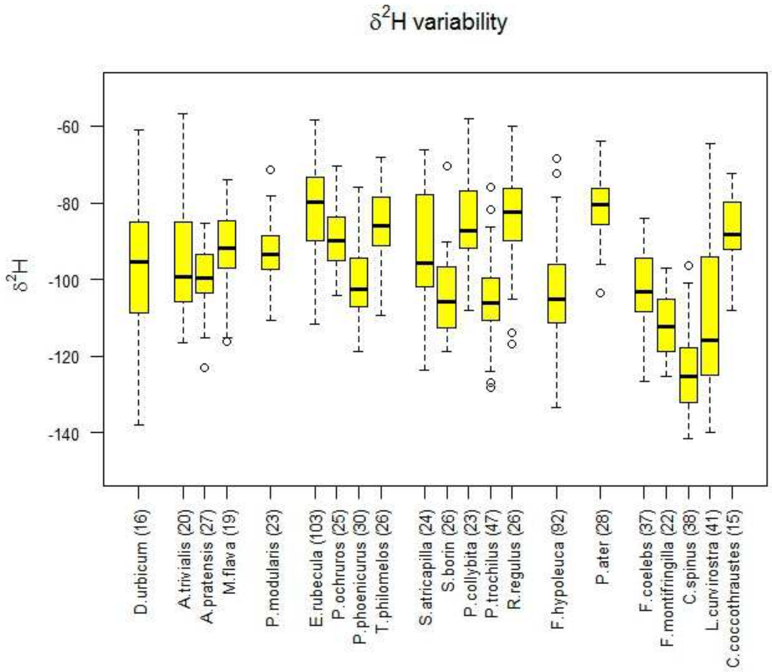

Figure 22: Box-and-whisker plots of δ^2H_f variability in the 21 species. Medians, 25% quartiles, maximum, minimum and outliers (empty dots) are reported. The number of individuals is shown in brackets.

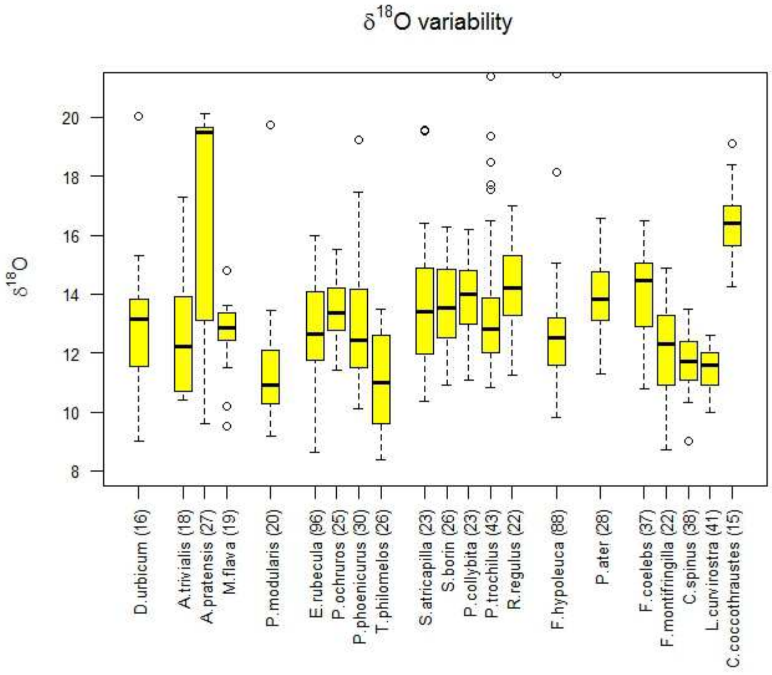

Figure 23: Box-and-whisker plots of $\delta^{18}O_f$ variability in the 21 species. Medians, 25% quartiles, maximum, minimum and outliers (empty dots) are shown. The number of specimens is shown in brackets.

Table 3: Tukey's Honest Significance Test p-values of analysis of variance for δ^{2}H and δ^{18}O (///-+++ = p<0.001; //-++ = p<0.01, /-+ = p<0.05). The table should be read from left to right up to the black boxes for δ^{2}H difference, and downwards up to the black boxes for δ^{18}O differences. + means more enriched values compared to the opposite species, / more depleted.

		Tukey's Honest Significance Distance p-values for δ^{18}O																				
	SPECIES	H.Martin	Meadow Pipit	Tree Pipit	Y.Wagtail	Dunnock	E.Robin	Song Thrush	C.Redstart	B.Redstart	Blackcap	Garden Warbler	Willow Warbler	C.Chiffchaff	Goldcrest	Pied Flycatcher	Coal Tit	C.Chaffinch	Brambling	E.Siskin	C.Crossbill	Hawfinch
Tukey's Honest Significance Distance p-values for δ^{2}H	H.Martin	■	+++																			+++
	Meadow Pipit		■	///	///	///	///	///	///	///	///	///	///	///	///	///	///	///	///	///	///	
	Tree Pipit			■																		+++
	Y.Wagtail				■																	+++
	Dunnock					■					+	++		+			+	++				+++
	E.Robin						■	/													/	+++
	Song Thrush		++					■		+++	+++	+++	+++	+++			+++	+++				+++
	C.Redstart						///	//	■													+++
	B.Redstart								+++	■										/	//	++
	Blackcap										■										///	+
	Garden Warbler						///	///		//	///	■								//	///	+
	Willow Warbler					/	///	///		///	/		■							/	///	+++
	C.Chiffchaff		++						++			+++	+++	■						/	//	+
	Goldcrest											+	++		■	/			/	///		
	Pied Flycatcher				/	/	///	///		///	//			///	///	■						+++
	Coal Tit		+++						+++			+++	+++			+++	■			//	///	+
	C.Chaffinch						///	///						/			///	■	/	///	///	+
	Brambling	//		//	///	///	///	///		///	///			///	///		///		■			+++
	E.Siskin	///	///	///	///	///	///	///	///	///	///	///	///	///	///	///	///	///		■		+++
	C.Crossbill	/		//	///	///	///	///		///	///			///	///		///			+++	■	+++
	Hawfinch												++			++			+++	+++	+++	■

Graphs of the trends, trend lines and statistical significance observed for $\delta^{2}H_{f}$ and $\delta^{18}O_{f}$ against the normalised day of capture are shown in Figures from 24 to 44 for $\delta^{2}H_{f}$ and from 45 to 65 for $\delta^{18}O_{f}$. $\delta^{2}H_{f}$ varied for most of the species, with a negative slope on the date of capture, which means more enriched values at the beginning of migratory timing and lower values at the end (eight species with statistically significant p). However, the relationship was not statistically significant for all species. Moreover, there were some species for which there was an almost flat relationship, probably because one isotopic population is observed. Furthermore, Common Crossbills had a statistically significant positive slope, meaning that individuals with more depleted values were observed at the beginning of migratory timing, while individuals with more enriched values were observed at the end.

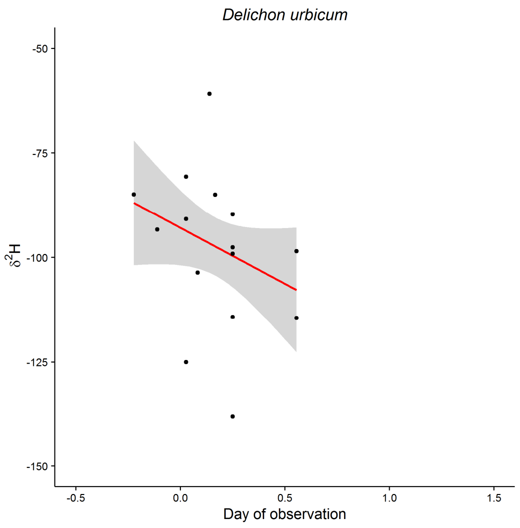

Figure 24: Relationships between $\delta^2\mathrm{H}_\mathrm{f}$ and the normalised day of capture for the House Martin *Delichon urbicum* (n=16). Trend line y=-27.0106x-91.2985, p value = 0.2097.

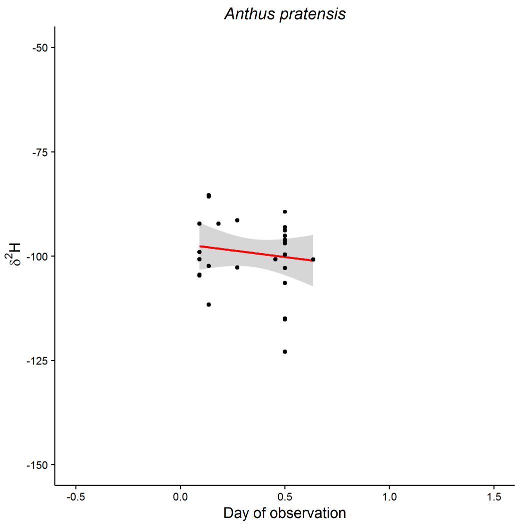

Figure 25: Relationships between $\delta^2\mathrm{H}_\mathrm{f}$ and the normalised day of capture for the Meadow Pipit *Anthus pratensis* (n=27). Trend line y=-6.3487x—97.0064, p value = 0.5394.

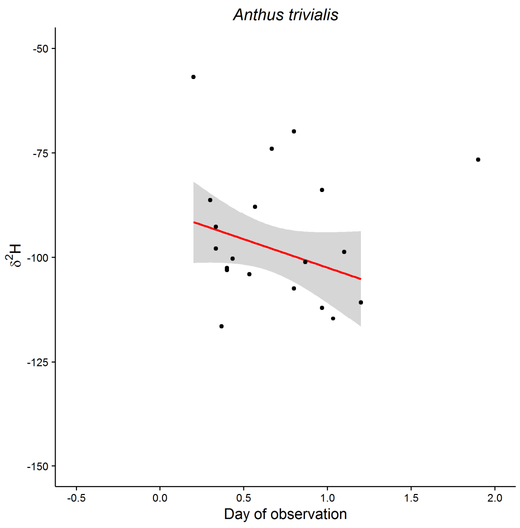

Figure 26: Relationships between $\delta^2\mathrm{H}_\mathrm{f}$ and the normalised day of capture for the Tree Pipit *Anthus trivialis* (n=20). Trend line y=-13.5676x-88.8606, p value =2659.

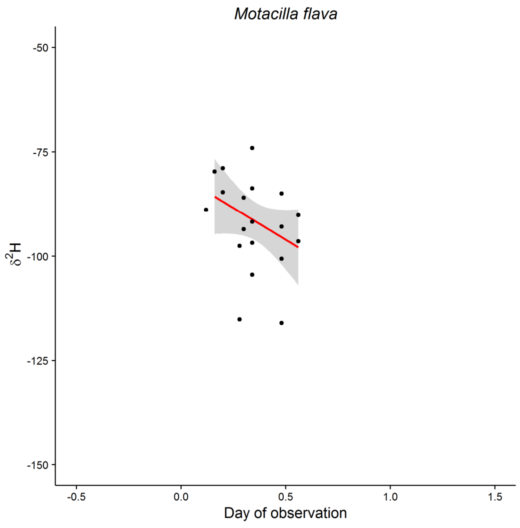

Figure 27: Relationships between $\delta^2\mathrm{H}_\mathrm{f}$ and the normalised day of capture for the Yellow Wagtail *Motacilla flava* (n=19). Trend line y=-30.4154x-80.7955, p value = 0.1668.

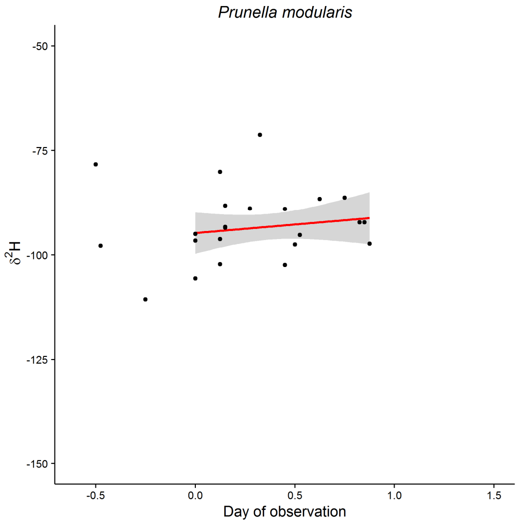

Figure 28: Relationships between $\delta^{2}\mathrm{H}_{\mathrm{f}}$ and the normalised day of capture for the Dunnock *Prunella modularis* (n=23). Trend line y=4.0774x-94.7611, p value = 0.4573.

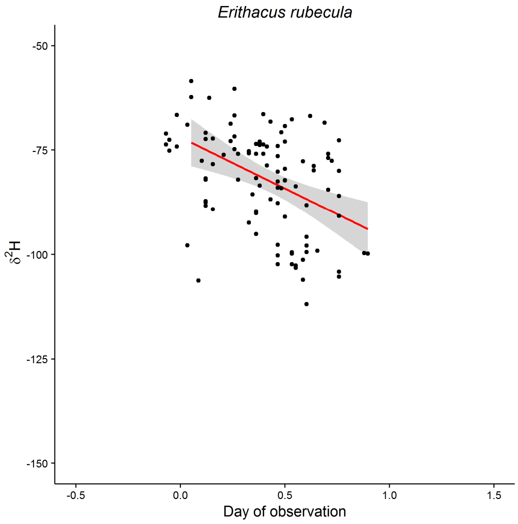

Figure 29: Relationships between $\delta^{2}\mathrm{H}_{\mathrm{f}}$ and the normalised day of capture for the European Robin *Erithacus rubecula* (n=103). Trend line y=-24.4733x-71.9813, p value < 0.001.

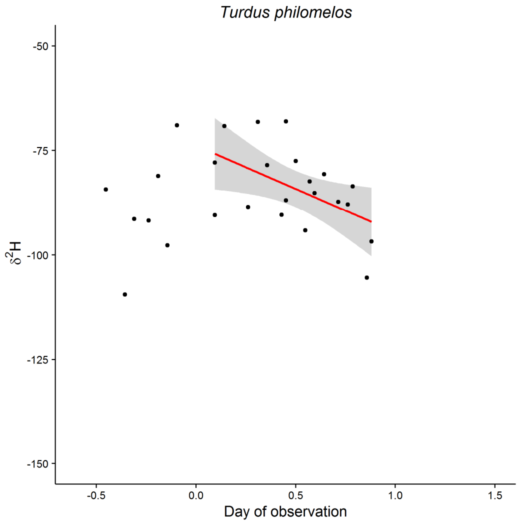

Figure 30: Relationships between $\delta^{2}\mathrm{H}_{\mathrm{f}}$ and the normalised day of capture for the Song Thrush *Turdus philomelos* (n=26). Trend line y=-20.7183x-73.8348, p value < 0.05.

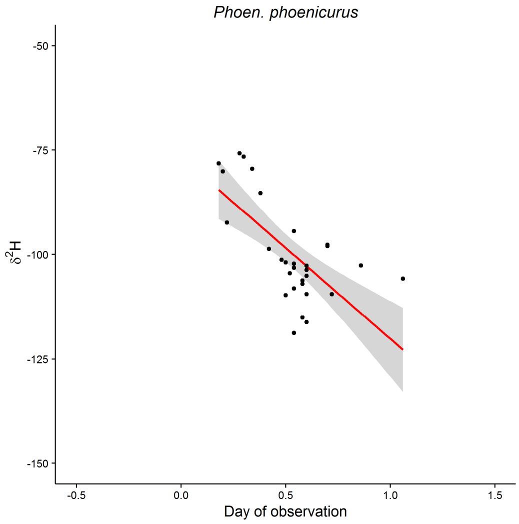

Figure 31: Relationships between $\delta^{2}\mathrm{H}_{\mathrm{f}}$ and the normalised day of capture for the Common Redstart *Phoenicurus phoenicurus* (n=30). Trend line y=-43.4621x-76.7013, p value < 0.001.

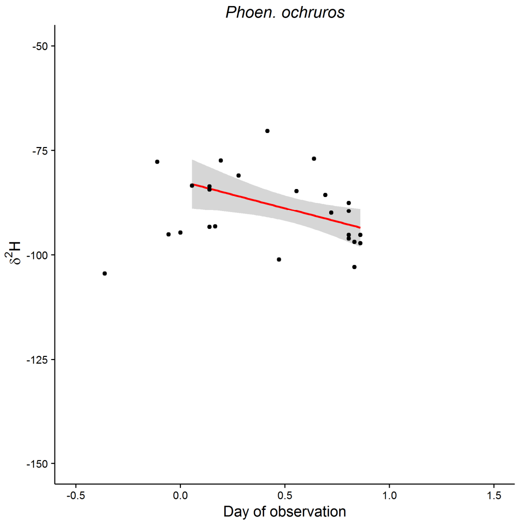

Figure 32: Relationships between δ^2H_f and the normalised day of capture for the Black Redstart *Phoenicurus ochruros* (n=25). Trend line y=-13.0313x-82.2648, p value < 0.05.

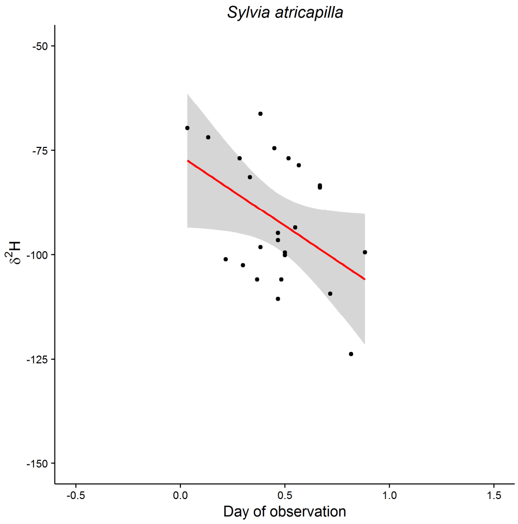

Figure 33: Relationships between δ^2H_f and the normalised day of capture for the Blackcap *Sylvia atricapilla* (n=24). Trend line y=-33.4941x-76.3099, p value < 0.05.

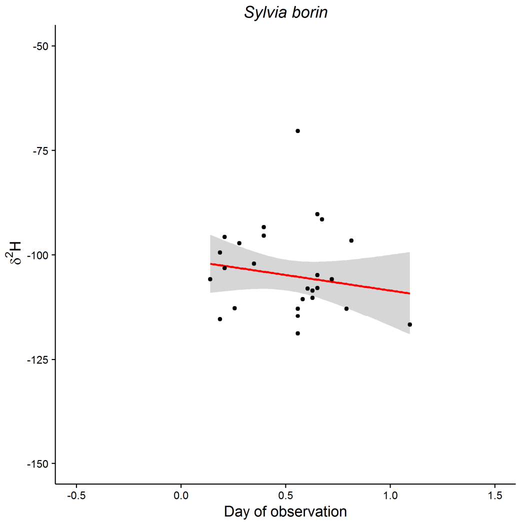

Figure 34: Relationships between δ^2H_f and the normalised day of capture for the Garden Warbler *Sylvia borin* (n=26). Trend line y=-7.4073x-101.0472, p value = 0.3999.

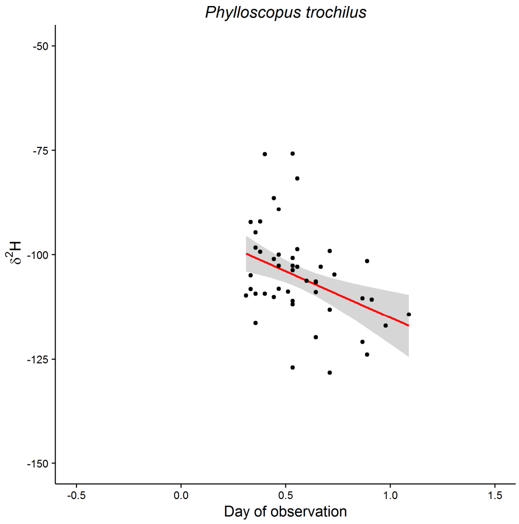

Figure 35: Relationships between δ^2H_f and the normalised day of capture for the Willow Warbler *Phylloscopus trochilus* (n=47). Trend line y=-22.2194x-92.8122, p value < 0.01.

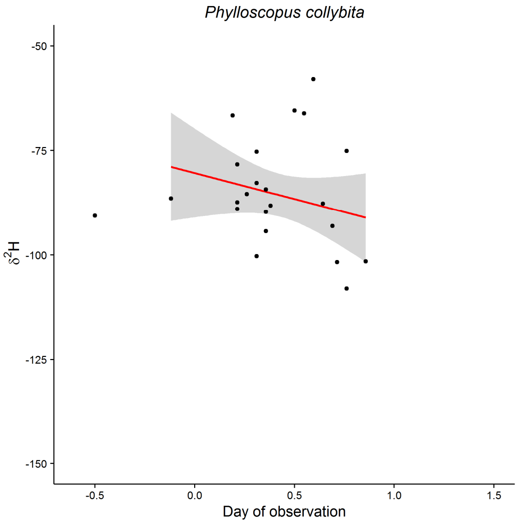

Figure 36: Relationships between δ^2H_f and the normalised day of capture for the Common Chiffchaff *Phylloscopus collybita* (n=23). Trend line y=-12.4599x-80.3779, p value p = 0.3232.

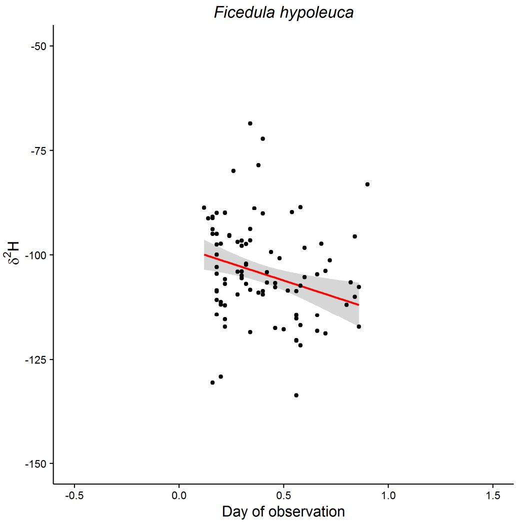

Figure 38: Relationships between δ^2H_f and the normalised day of capture for the Pied Flycatcher *Ficedula hypoleuca* (n=92). Trend line y=-16.1851x-97.9718, p value < 0.01.

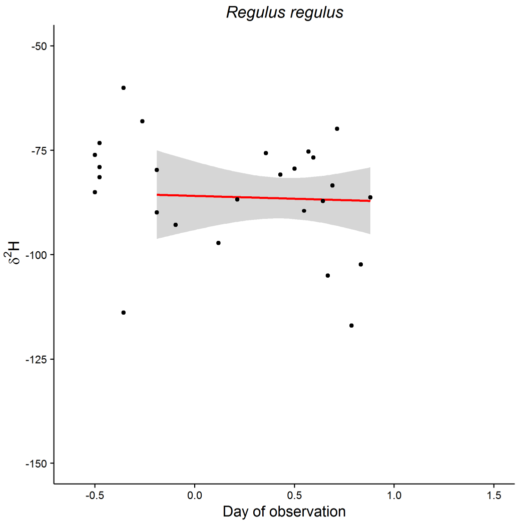

Figure 37: Relationships between δ^2H_f and the normalised day of capture for the Goldcrest *Regulus regulus* (n=26). Trend line y=-1.3511x-85.8698, p value = 0.8832.

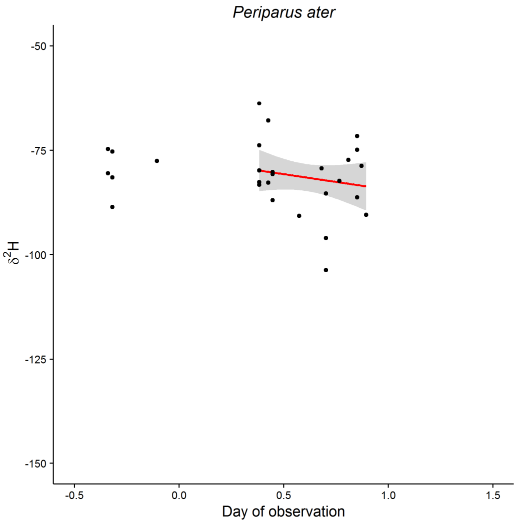

Figure 39: Relationships between δ^2H_f and the normalised day of capture for the Coal Tit *Periparus ater* (n=28). Trend line y=-7.4137x-76.9639, p value = 0.4625.

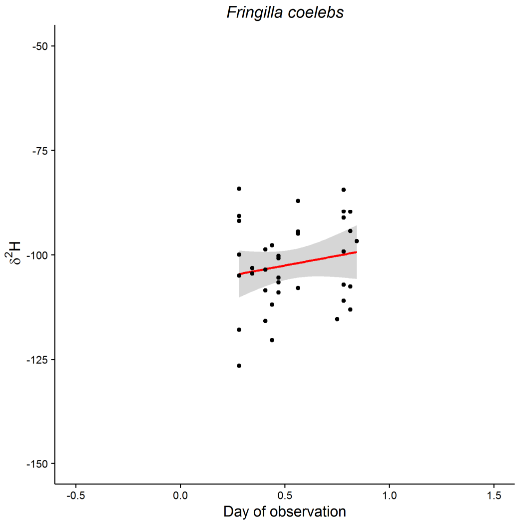

Figure 40: Relationships between $\delta^{2}H_{f}$ and the normalised day of capture for the Common Chaffinch *Fringilla coelebs* (n=37). Trend line y=-9.277x-107.1309, p value = 0.3281.

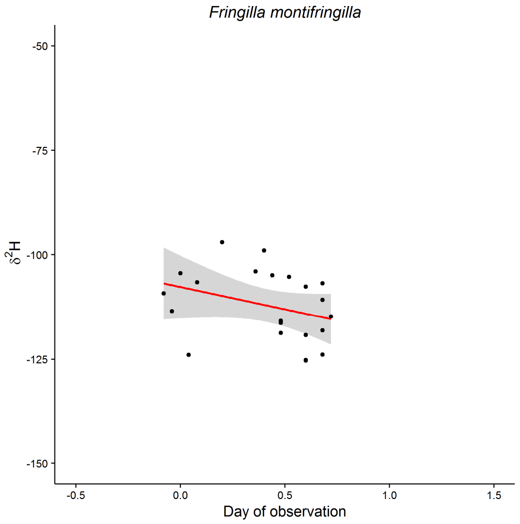

Figure 41: Relationships between $\delta^{2}H_{f}$ and the normalised day of capture for the Brambling *Fringilla montifringilla* (n=22). Trend line y=-10.6742x-107.702, p value = 0.1847.

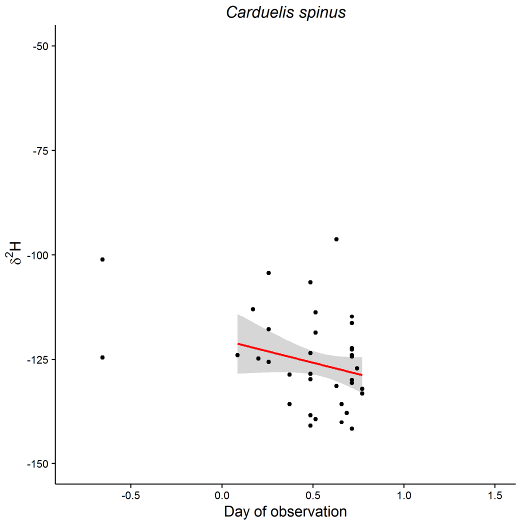

Figure 42: Relationships between $\delta^{2}H_{f}$ and the normalised day of capture for the Eurasian Siskin *Carduelis spinus* (n=38). Trend line y=-10.9127x-120.2989, p value = 0.3394.

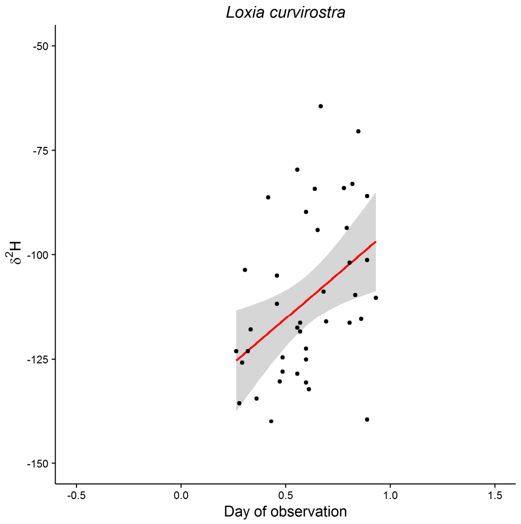

Figure 43: Relationships between $\delta^{2}H_{f}$ and the normalised day of capture for the Common Crossbill *Loxia curvirostra* (n=41). Trend line y=42.7664x-136.626, p value < 0.01.

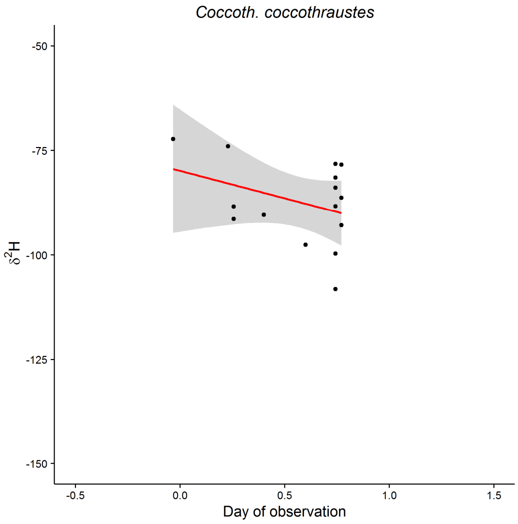

Figure 44: Relationships between δ^2H_f and the normalised day of capture for the Hawfinch *Coccothraustes coccothraustes* (n=15). Trend line y=-13.1351x-79.8444, p value = 0.2071.

The relationships between δ^{18}O and the date of capture are less evident compared to those observed for δ^2H. However, negative slopes were also generally observed for the oxygen isotope, in six cases with statistically significant slopes, although the species were not exactly the same as for δ^2H. Some species which did not show a trend for δ^2H in contrast showed statistically significant negative relationships (e.g. the Garden Warbler).

Finally, normalization of the date of capture highlighted 37 individuals from eight species, common alpine breeders as well as post-breeding migrants, that can be used as detectors of local isotopic variability compared to other species (δ^2H -83.76 ± 13.05 ‰, transformed δ^2H -65.8 ± 14.66 ‰, δ^{18}O 13.17 ± 1.81 ‰).

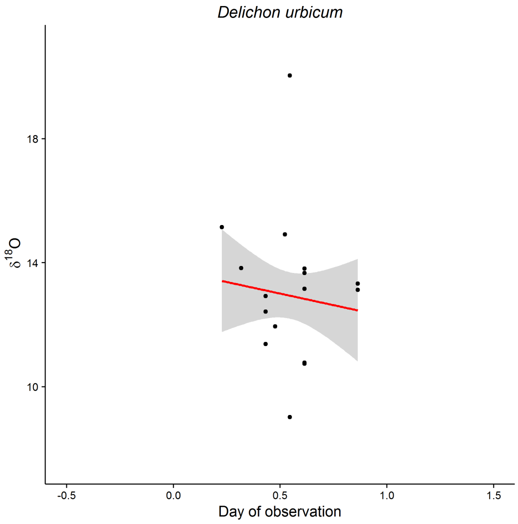

Figure 45: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the House Martin *Delichon urbicum* (n=16). Trend line y=-1.4859x+13.7535, p value = 0.6392.

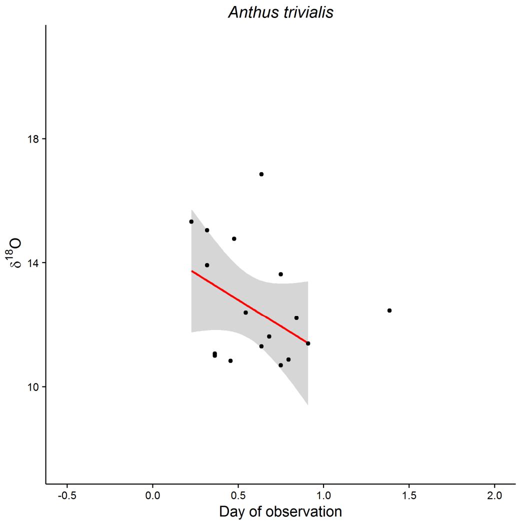

Figure 47: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Tree Pipit *Anthus trivialis* (n=18). Trend line y=-3.4258x+14.5169, p value = 0.133.

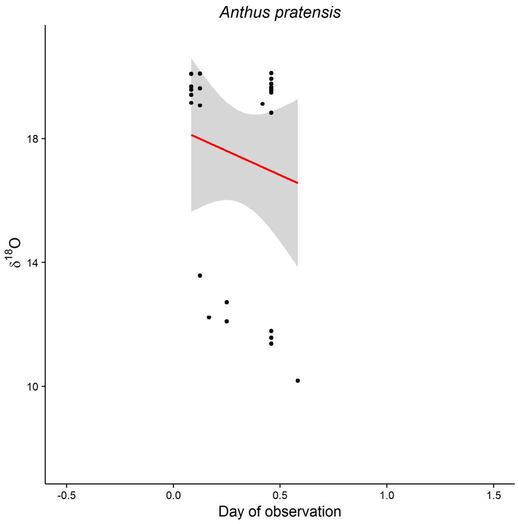

Figure 46: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Meadow Pipit *Anthus pratensis* (n=27). Trend line 1 y=-3.1027x+18.3802, p value = 0.5323.

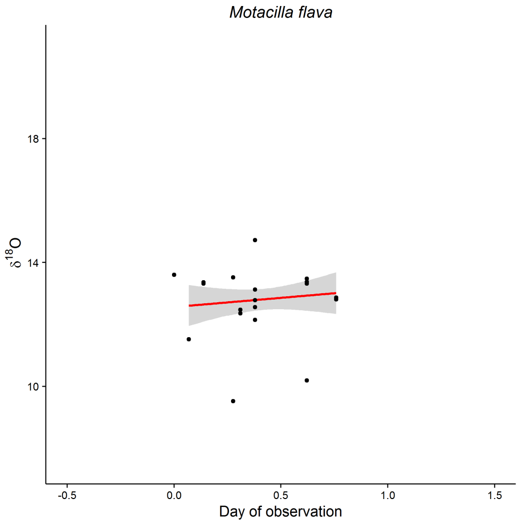

Figure 48: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Yellow Wagtail *Motacilla flava* (n=19). Trend line y=0.5885x+12.5696, p value = 0.5635.

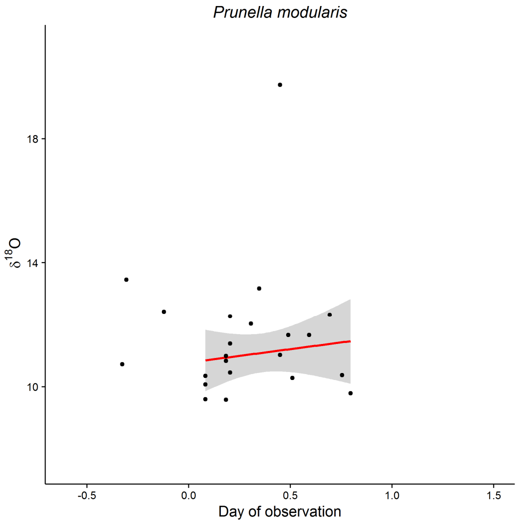

Figure 49: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Dunnock *Prunella modularis* (n=20). Trend line y=0.8646x+10.7779, p value = 0.4944.

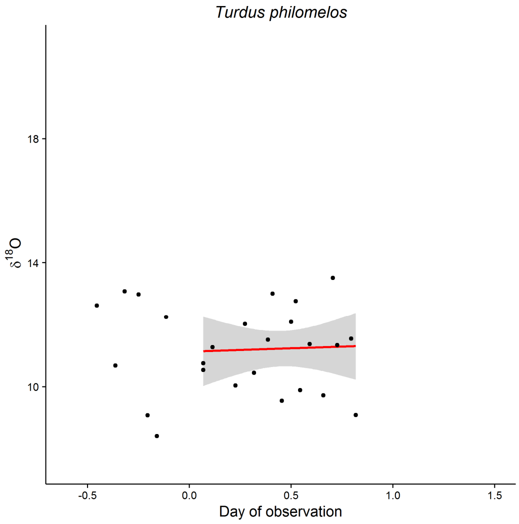

Figure 51: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Song Thrush *Turdus philomelos* (n=26). Trend line y=0.2164x+11.1276, p value = 0.8943.

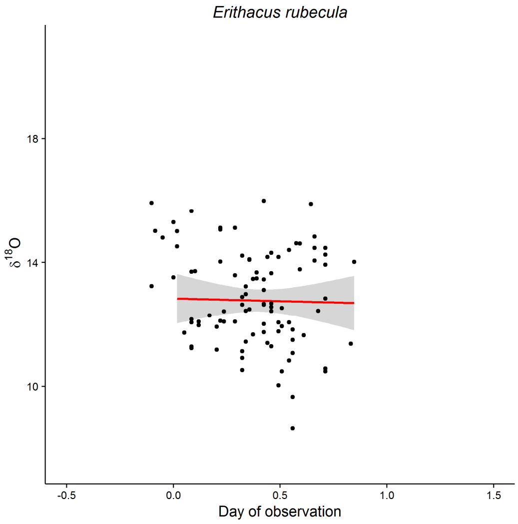

Figure 50: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the European Robin *Erithacus rubecula* (n=96). Trend line y=-0.1708x+12.8372, p value = 0.8376.

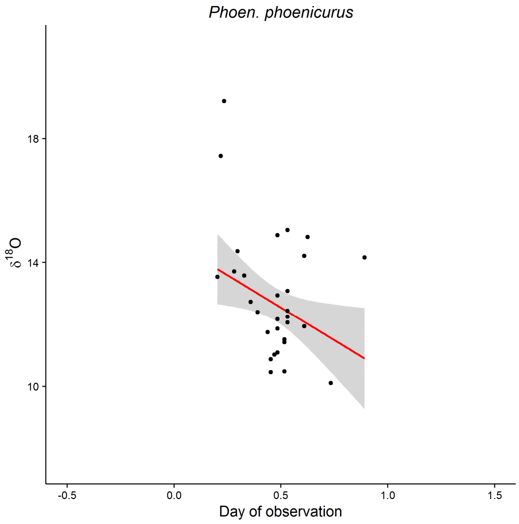

Figure 52: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Common Redstart *Phoenicurus phoenicurus* (n=30). Trend line y=-4.1934x+14.638, p value = 0.1247.

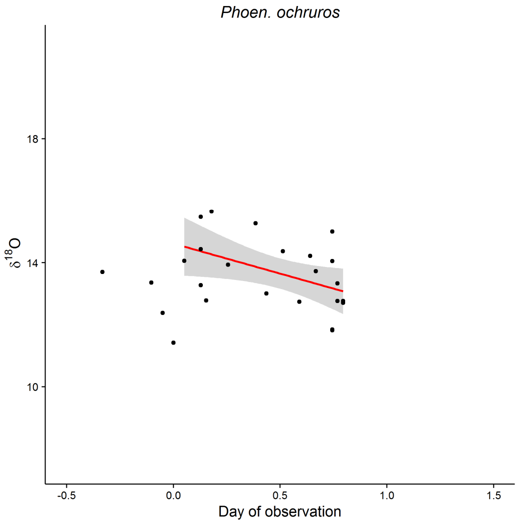

Figure 53: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Black Redstart *Phoenicurus ochruros* (n=25). Trend line $y=-1.9292x+14.6181$, p value < 0.05.

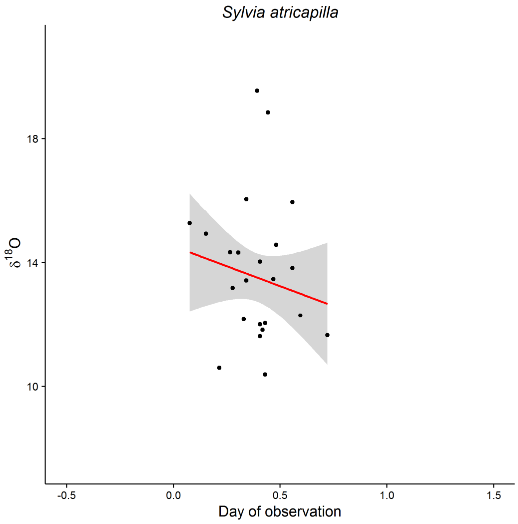

Figure 54: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Blackcap *Sylvia atricapilla* (n=23). Trend line $y=-2.5719x+14.5248$, p value = 0.4203.

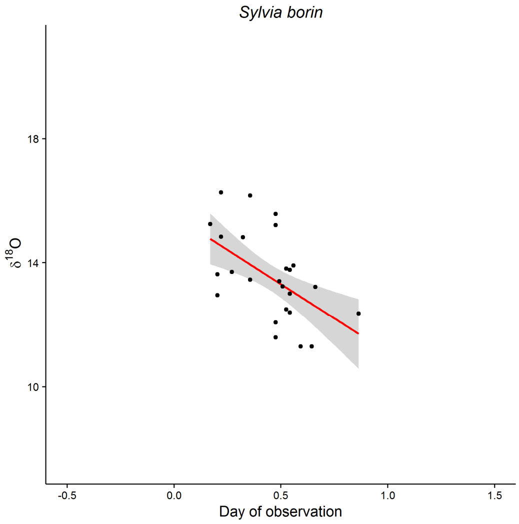

Figure 55: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Garden Warbler *Sylvia borin* (n=26). Trend line $y=-5.0473x+15.9057$, p value < 0.01.

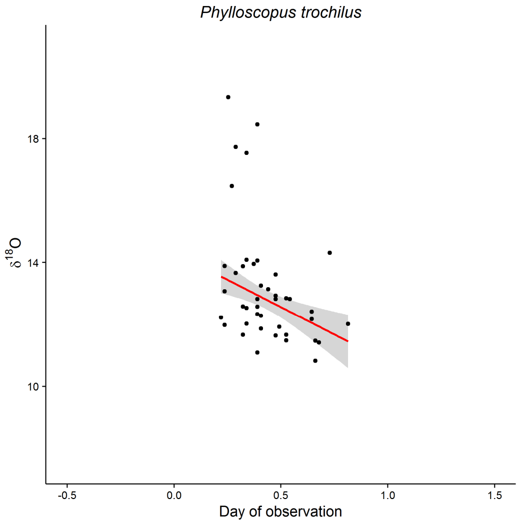

Figure 56: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Willow Warbler *Phylloscopus trochilus* (n=43). Trend line $y=-3.9914x+14.5841$, p value < 0.01.

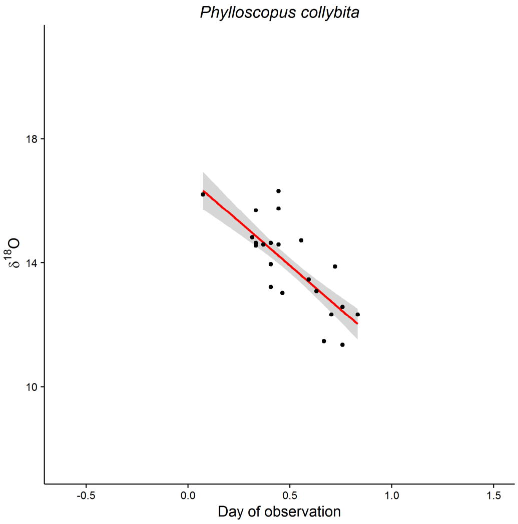

Figure 57: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Common Chiffchaff *Phylloscopus collybita* (n=23). Trend line y=-5.6816x+16.7537, p value $p < 0.001$.

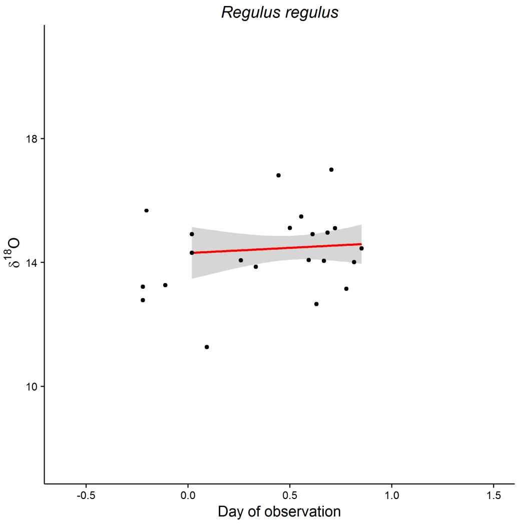

Figure 58: Relationships between and $\delta^{18}O_f$ and the normalised day of capture for the Goldcrest *Regulus regulus* (n=22). Trend line y=0.3369x+14.3035, p value =0.4785.

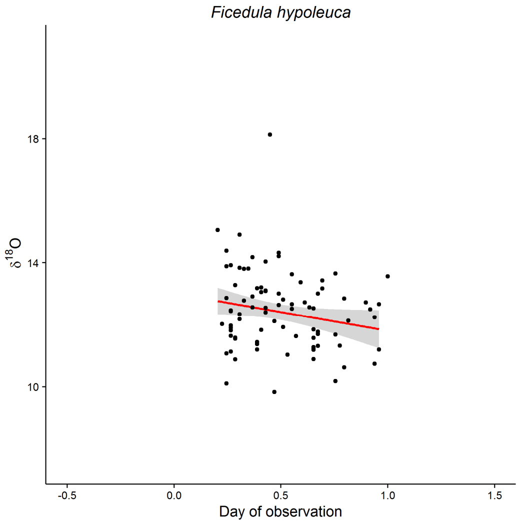

Figure 59: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Pied Flycatcher *Ficedula hypoleuca* (n=88). Trend line y=-1.4435x+13.1785, p value < 0.05.

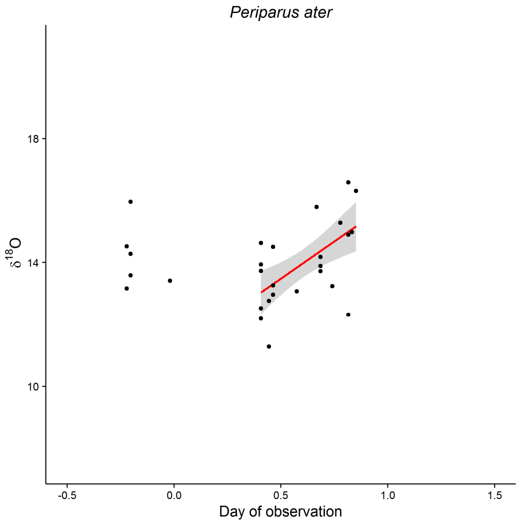

Figure 60: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Coal Tit *Periparus ater* (n=28). Trend line y=4.7881x+11.0872, p value < 0.01.

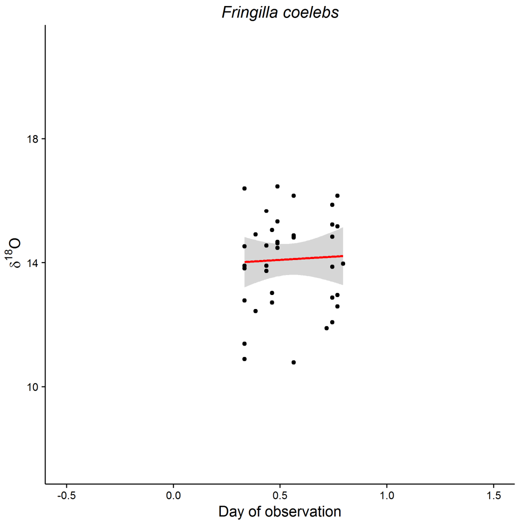

Figure 61: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Common Chaffinch *Fringilla coelebs* (n=37). Trend line y=0.4138x+13.8844, p value = 0.8112.

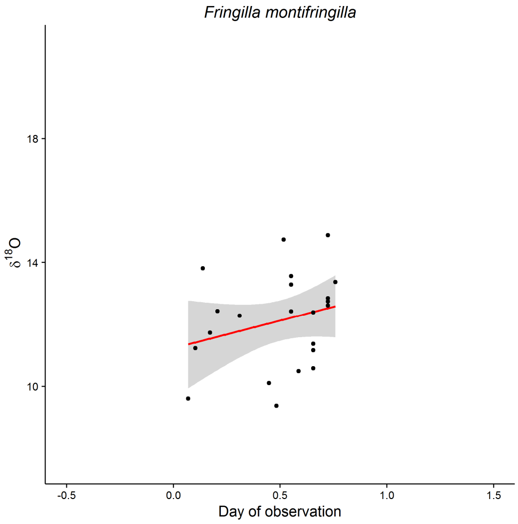

Figure 62: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Brambling *Fringilla montifringilla* (n=22). Trend line y=1.785x+11.2321, p value = 0.291.

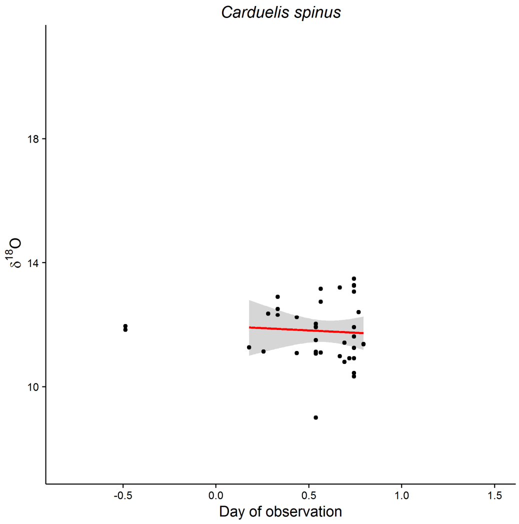

Figure 63: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Eurasian Siskin *Carduelis spinus* (n=38). Trend line y=-0.2966x+11.9568, p value = 0.7745.

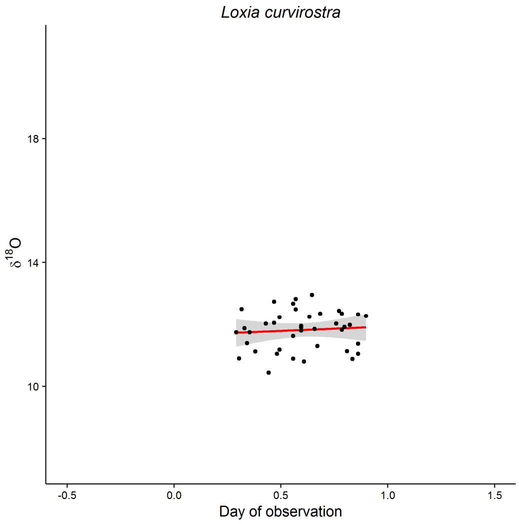

Figure 64: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Common Crossbill *Loxia curvirostra* (n=41). Trend line y=0.2886x+11.64, p value = 0.6468.

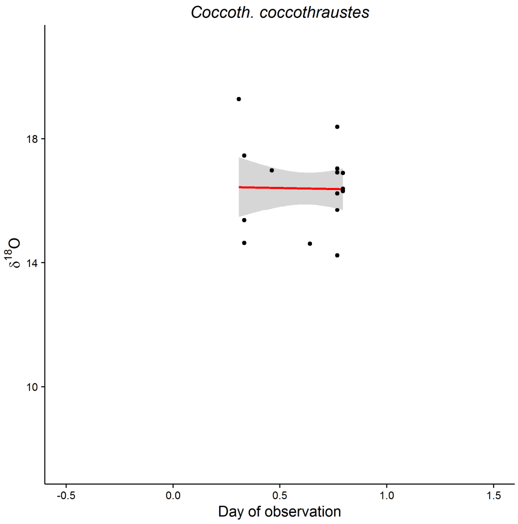

Figure 65: Relationships between $\delta^{18}O_f$ and the normalised day of capture for the Hawfinch *Coccothraustes coccothraustes* (n=15). Trend line y=-0.1262x+16.4792, p value = 0.9541.

Detection of geographical origin

In the recovery data set, 332 individuals corresponded with the requirements (marking and recapture in the same year in the Italian Alps). Moreover, two distinct polygons of probable area of origin were defined. The first one was built with only Pied Flycatcher data (n=174), which as expected showed breeding capture sites in Central and Eastern Europe up to the north in Fennoscandia (Figure 66). The second was delineated using the other thirteen species' breeding sites of first capture (n=158, Figure 67), but included an area between Alps and Fennoscandia, without Eastern Europe. Recovery data were used to build the polygons, which in turn were used to cut out the breeding ranges of each of the 21 species. Cutting of the polygon was done manually, because the intention was to provide general, but effective information about origin. Once the breeding and recovery 'stencils' had been prepared, the isoscape of $\delta^{2}H$ of precipitation (Figure 68) was cut out for each of the 21 species.

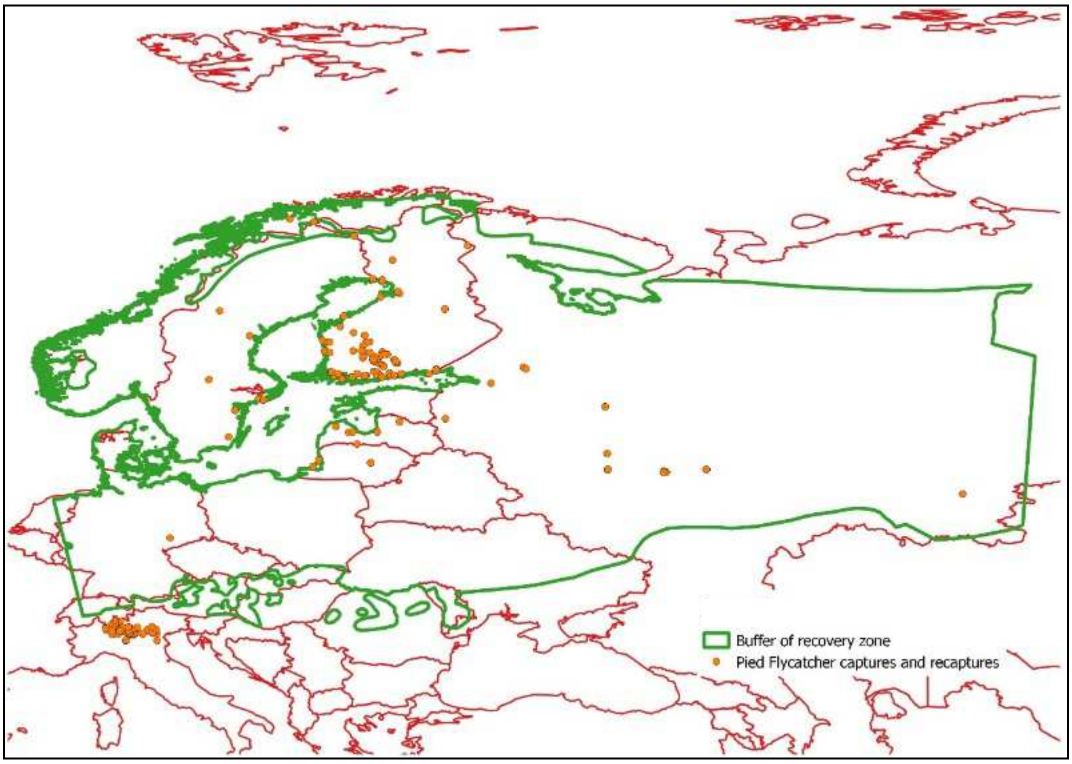

Figura 66: Capture sites for breeding Pied Flycatchers (n=174) and recapture sites in the Italian Alps and foothills.

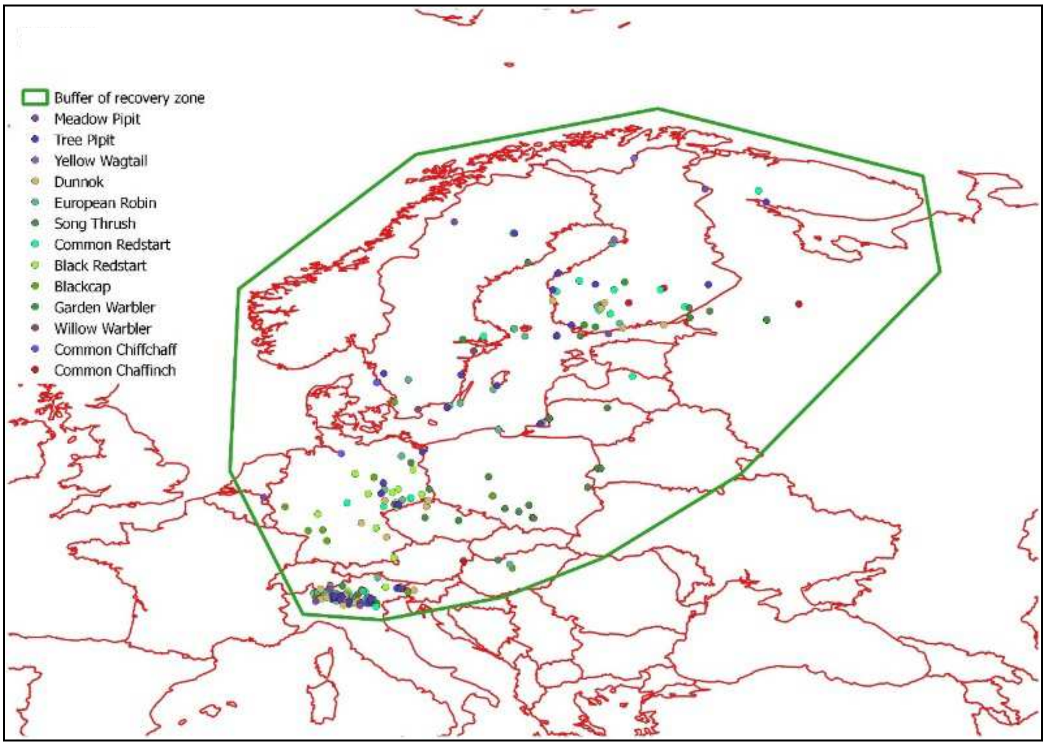

Figure 67: Capture sites for 13 breeding species (n=158) and recapture sites in the Italian Alps and foothills.

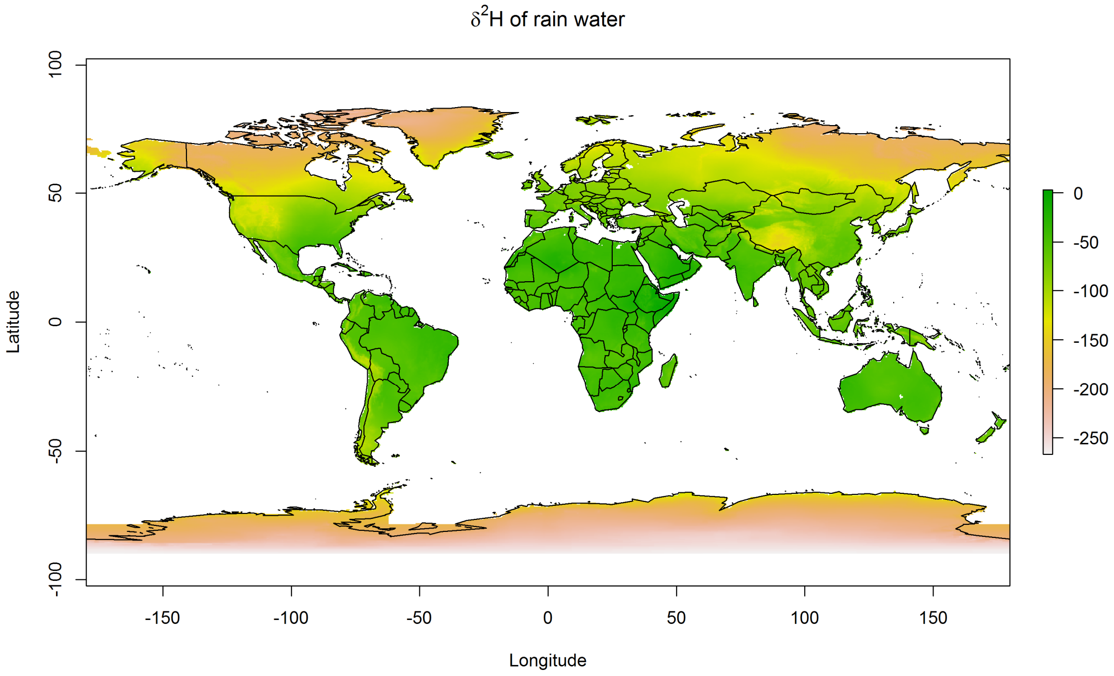

Figure 68: Isoscape of the world δ^2H in rain water (Bowen 2016).

In the next pages, the maps of geographical assignment are shown for each of the 21 species (Figures 69 to 89).

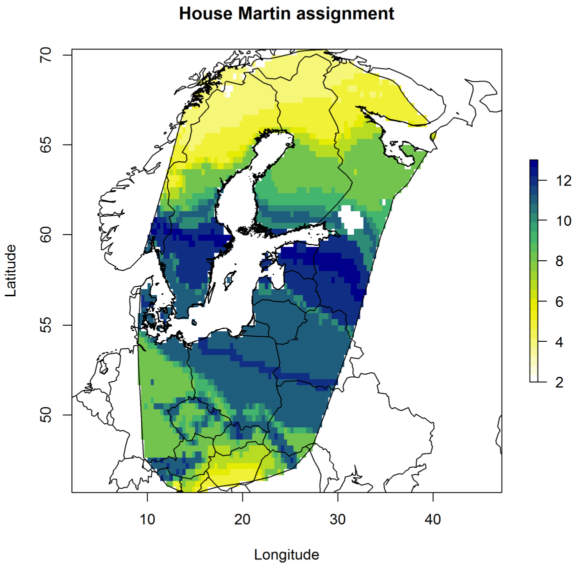

Figure 69: Assignment to the most likely geographic origin for 16 House Martins sampled at Bocca di Caset. At least 12 individuals of 16 are associated with the blue colored areas (isotopically).

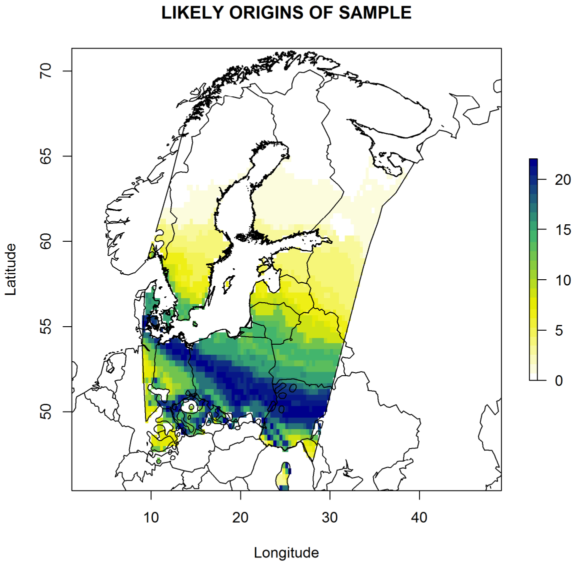

Figure 70: Assignment to the most likely geographic origin for 27 Meadow Pipits sampled at Passo Broccon (n=23) and Bocca di Caset (n=4).

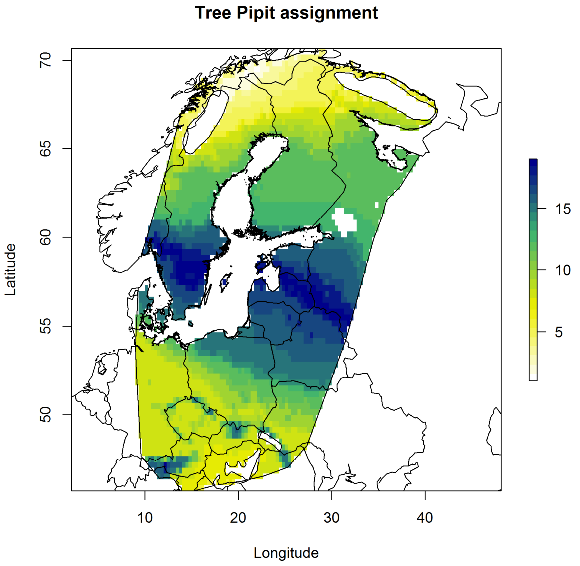

Figure 71: Assignment to the most likely geographic origin for 20 Tree Pipits sampled at Bocca di Caset.

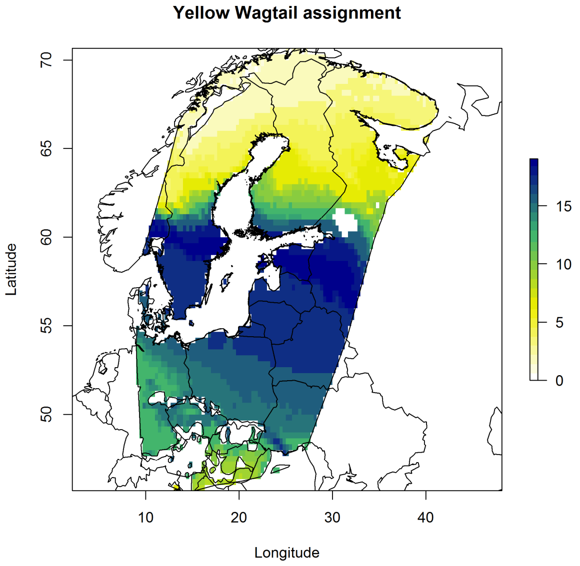

Figure 72: Assignment to the most likely geographic origin for 19 Yellow Wagtails sampled at Bocca di Caset.

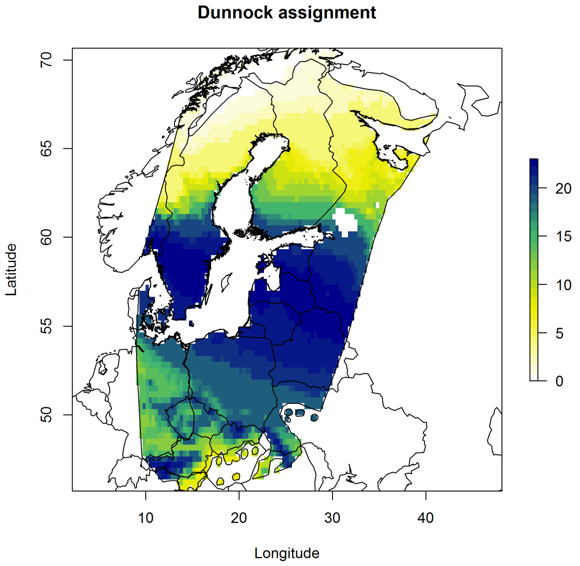

Figure 73: Assignment to the most likely geographic origin for 23 Dunnock sampled at Bocca di Caset.

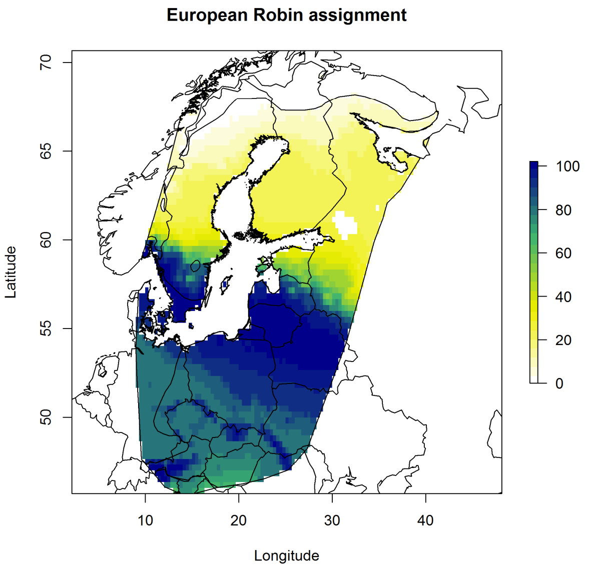

Figure 74: Assignment to the most likely geographic origin for 103 European Robins sampled at Bocca di Caset.

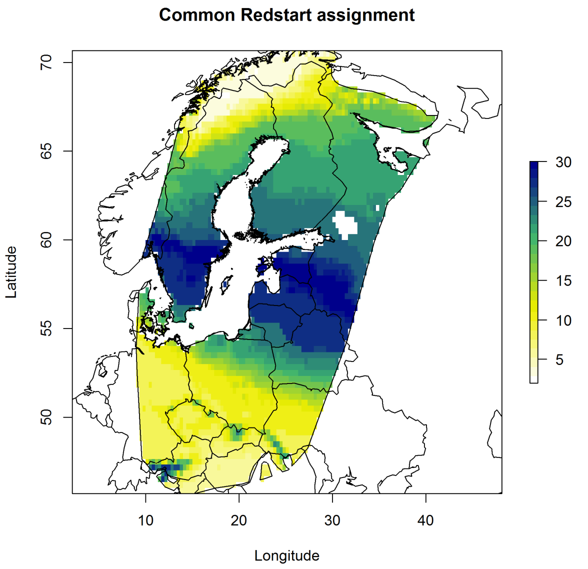

Figura 76: Assignment to the most likely geographic origin for 30 Common Redstarts sampled at Bocca di Caset.

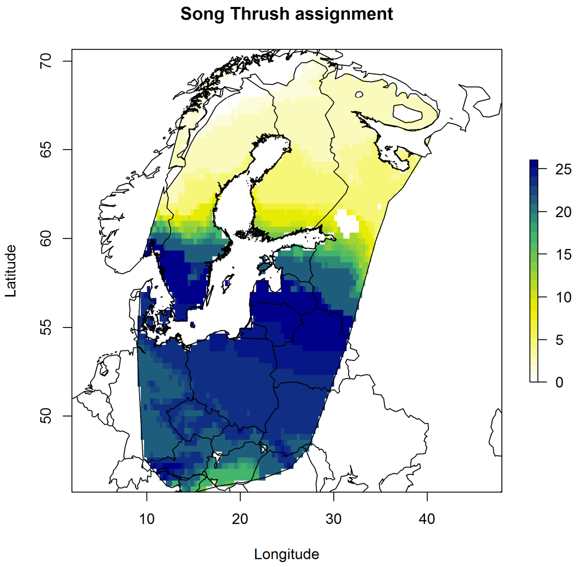

Figure 75: Assignment to the most likely geographic origin for 26 Song Thrushes sampled at Bocca di Caset.

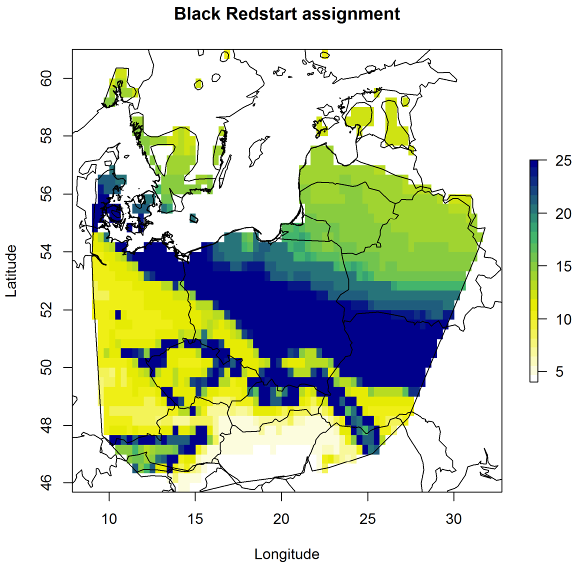

Figure 77: Assignment to the most likely geographic origin for 25 Black Redstarts sampled at Bocca di Caset.

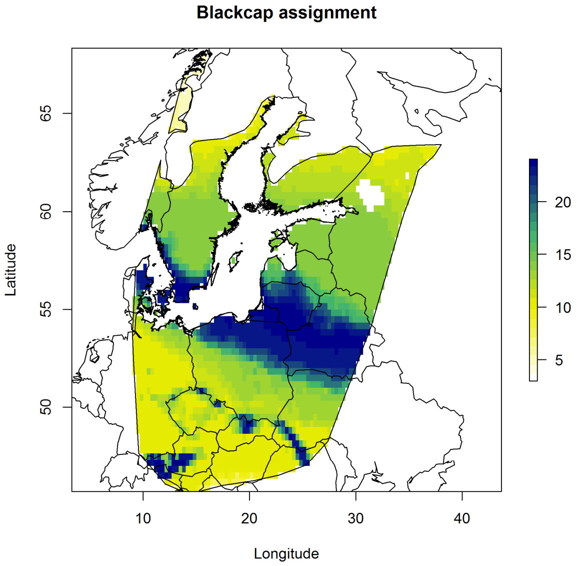

Figure 78: Assignment to most likely geographic origin for 24 Blackcaps sampled at Bocca di Caset.

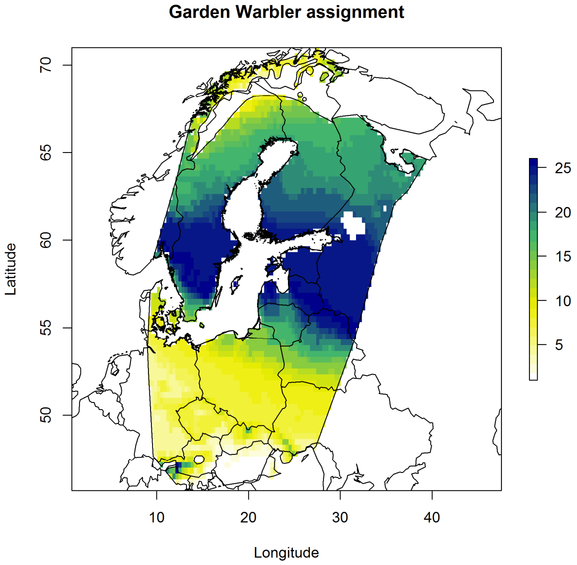

Figure 79: Assignment to the most likely geographic origin for 26 Garden Warblers sampled at Bocca di Caset.

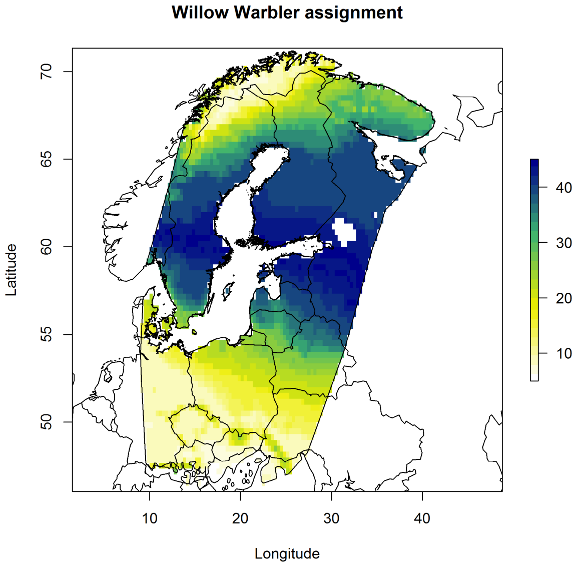

Figure 80: Assignment to the most likely geographic origin for 47 Willow Warblers sampled at Bocca di Caset.

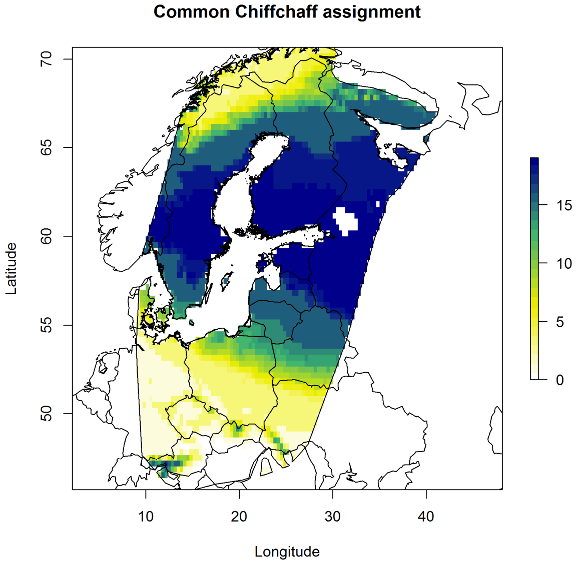

Figure 81: Assignment to most likely geographic origin for 23 Common Chiffchaffs sampled at Bocca di Caset.

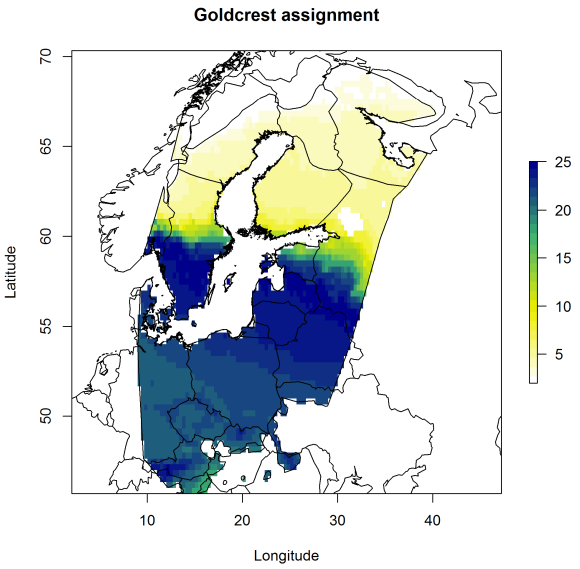

Figure 82: Assignment to the most likely geographic origin for 26 Goldcrests sampled at Bocca di Caset.

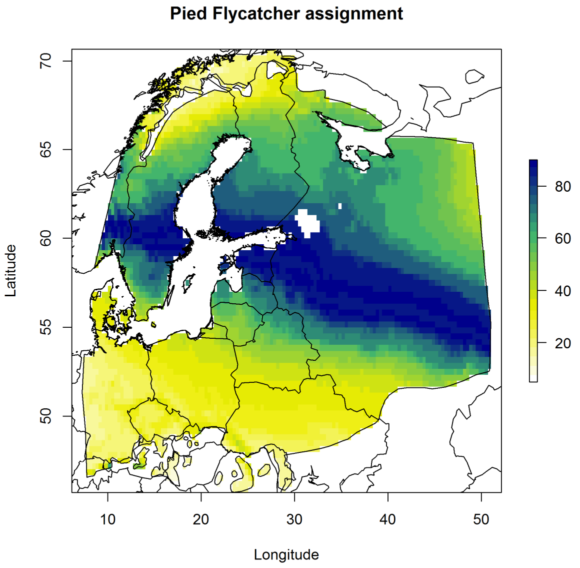

Figure 83: Assignment to the most likely geographic origin for 92 Pied Flycatchers sampled at Bocca di Caset.

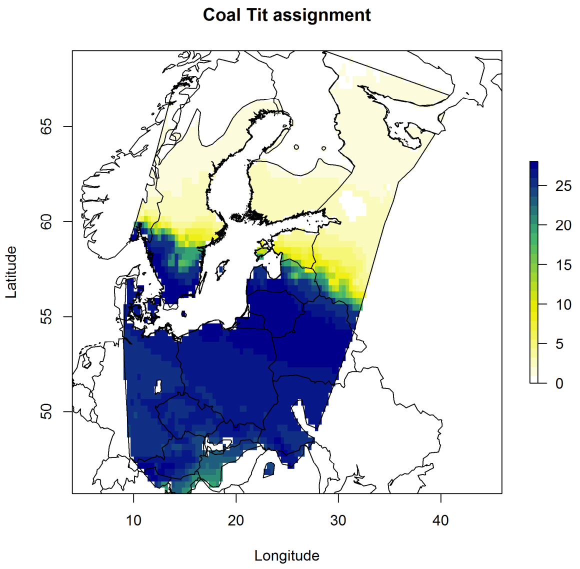

Figure 84: Assignment to the most likely geographic origin for 28 Coal Tits sampled at Bocca di Caset.

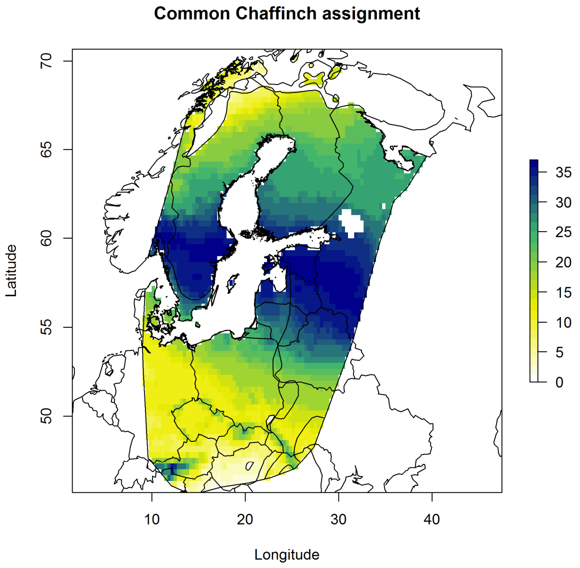

Figure 85: Assignment to the most likely geographic origin for 37 Common Chaffinches sampled at Bocca di Caset.

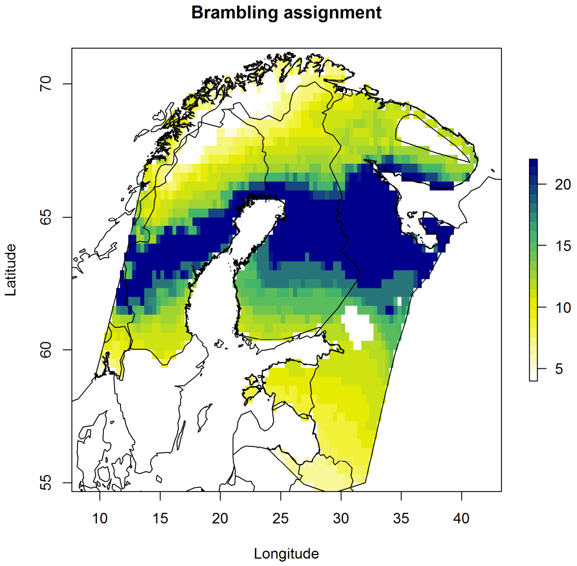

Figure 86: Assignment to the most likely geographic origin for 22 Bramblings sampled at Bocca di Caset.

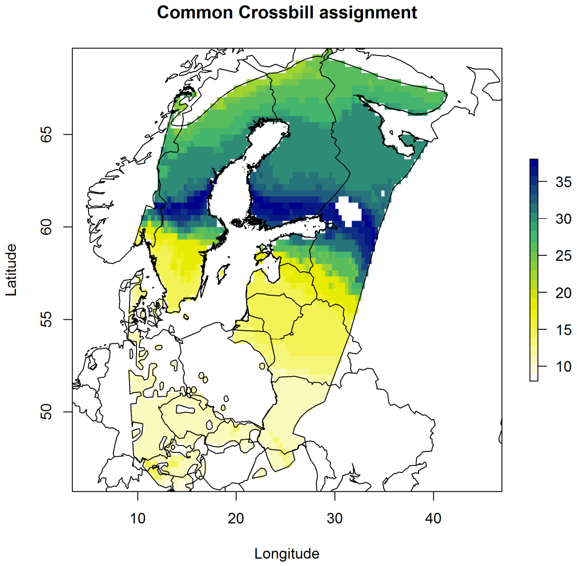

Figure 88: Assignment to the most likely geographic origin for 41 Common Crossbills sampled at Bocca di Caset.

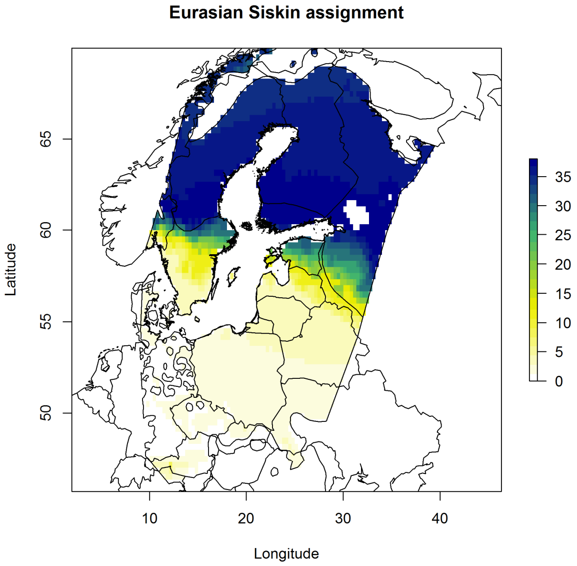

Figure 87: Assignment to the most likely geographic origin for 38 Eurasian Siskins sampled at Bocca di Caset.

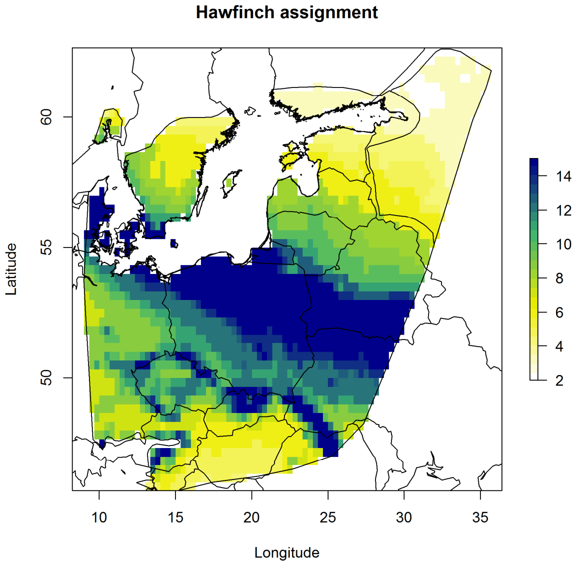

Figure 89: Assignment to the most likely geographic origin for 15 Hawfinches sampled at Bocca di Caset.

Discussion

Relationship between δ^2H and δ^{18}O measured in feathers

As could be expected, the relationship between the two water isotope values depends on a number of factors and dynamics which determine a certain variability in the fixation of δ^{18}O in animal tissues, different and in addition to the spatial patterns related to the stable oxygen precipitation isoscape (Hobson and Koehler 2015). The first evidence of this variability can probably be found in Meadow Pipit values: δ^{18}O probably distinguishes two distinct geographic populations, which hydrogen assigns to a single geographical origin (see subsequent information). These pronounced differences could be due to dynamics related to the peculiarities of the habitat. Meadow Pipits breed in grasslands: open habitats are subject to evaporation, and the moisture gradient in the different types of grassland (e.g. from peat bogs to alpine meadows) could be behind the isotopic niches observed. Additional evidence regards the extremely wide variability that can be observed in the δ^2H-δ^{18}O relationships for the 21 species. The reason for this could be similar to that used to explain the variability observed in Meadow Pipits. All species have their own habitat preferences, but within their breeding ranges the same habitat can change depending on local weather and climatic conditions. Moreover, water stress in birds causes increased breathing, which can affect final isotopic composition locally (Hobson et al. 2009). Finally, these results generally agree with the relationships between H and O isotopes found in other works (Hobson et al. 2004, Bowen et al. 2005, Hobson and Koehler 2015).

Intra-seasonal trends for δ^2H and δ^{18}O and geographical origin

In general, the trends have a negative slope, with starting values close to those observed for local alpine birds. This means that species closer to Alps generally pass through before species from regions with lower H isotope values in the isoscape, typically northern European areas. Distinctions between species in terms of statistical significance and the steepness of slopes could be due to several factors. Firstly, ringing activities cover most post-breeding migration through the Alps, but migration tails could be missed for some species. Trans-Saharan species in particular are early post-breeding migrants, mainly passing in the period between August and September, while intra-Palaearctic species are later migrants, mainly passing between September and early November. For logistical reasons, ringing stations are active between 20 August and the end of October. Hence inevitably some species may lack sampled populations. For example, Pied Flycatchers and Willow Warblers could be affected by this problem, since migratory individuals are also seen resting in the Italian Alps at the end of July in some years, and the hydrogen isotope assigns these two species to northern (and eastern) parts of Europe as a mean. On the other hand, information on species such as the European Robin and the Song Thrush may be lacking for the terminal part of their migration. Finally, data of four different years of migration were pooled together, assuming that no differences are occurring in timing of passing of the individuals among the different years, and that hydrogen isoscapes of distinct years would not change and the isotopic composition in feathers would be constant. Indeed, this facts could affect the isotopic composition observed in the samples (Tonra et al. 2014). However, the number of specimens per year was not consistent for most of the species, and seasonal trends were not equally monitored for the most sampled species, and for some species this may have added variability to the results (Dunn et al. 2006).

Species without statistically significant trends can be separated into two groups. The first is made up of birds that can be mainly assigned to a geographical origin inlcuded between the Alps and the southern shore of the Baltic Sea. This group includes Meadow Pipits, Goldcrests, Coal Tits and

Hawfinches, for which no trends are observable and the values are similar to those of local birds. The second group is composed of species which have a northern origin, located in the Baltic area including Polish, Finnish and Swedish seashores. The species in this group are theYellow Wagtail, the Dunnock, the Garden Warbler, the Common Chiffchaff and the Common Chaffinch, for which no trends were observable. There may be different reasons for sampling only one isotopic (and geographical) population. In the case of Coal Tits, this species makes irruptive movements through the Alps periodically, alternating years of almost total absence with its presence as a captured and observed migratory species in the Progetto Alpi. The origin of this irruptive migrant may vary over the years, and those sampled in this study may mainly be related to populations of Central European and alpine origin. In the case of the Meadow Pipits and Goldcrests, migration can also continue in November, hence individuals from northern Europe may not be sampled. On the other hand, there could be several reasons about why some species seem to come mainly from northern Europe, although they use to breed also on Alps and in Central Europe. The Common Chaffinch is an abundant alpine breeder, but the assignment set the majority of the individuals in northern Europe; this fact could be related to a migratory peculiarity of the species, such as only northern populations are fully migratory whereas southern should be more sedentary (BirdLife 2015). Conversely, the Garden Warbler is not an abundant breeder on Alps, while in northern Europe is more common, hence it could be associated with an abundance effect on the sampling which could be integrated in the prosecution of the analysis (Royle and Rubenstein 2004). Another reason could lay in the different flyways that different populations may follow due to the alpine barrier. Therefore, individuals with longer wings which are generally related to northern populations could be favoured in passing over the southern corridor Alps more than those with shorter wings (Bruderer and Jenni 1990, Berthold 2001). Typically, longer wings are a prerogative of long-distance migrants, and in an intra-Palaearctic species, as well trans-Saharan species, and this could be also related to a leapfrog migration. It would be interesting to measure stable hydrogen ratios in migratory birds captured along the northern alpine corridor passing through southern Germany, Switzerland and finally France.

The case of the Common Crossbill is also rather interesting. This species saw a positive trend in the H isotope during its migratory season. Specifically, in August and September the sampled individuals had significantly lower values compared to those sampled in October, although the variability remained quite wide. In Great Britain Marquiss and collegues (2008, 2012) observed that during years of irruption, Common Crossbills from northern Europe arrived in summer. It seems that the individuals sampled in this study during the summer, as in the British case, came from a northern area of Europe, probably located between Fennoscandia and western Russia, while those sampled in October have a probable origin closer to Alps. Eurasian Siskins and Bramblings were assigned to a geographical area very far away, in the north of Fennoscandia. If for Bramblings it was expected, contrariwise the Siskin is also an alpine breeder but almost all the individuals are assigned more likely in the very north of Europe. Since the species periodically makes irruptive movements from northern Europe to coincide with an abundance of spruce cone seeds (Clement 2015), it seems that 2010 and 2011 (the sampled years for Siskin) where characterised by abundant invasion of northern populations. However, Marquiss and colleagues (2012) suggested an increase in the δ^2H of juvenile Crossbill feathers to be compared with those of the summer-grown of the adults because they usually hatch (and grow feathers) during winter. Hence, in general Crossbills' and Siskins' δ^2H_f could be lower compared to other species because they often breed during winter, and it may be appropriate to do something similar. In any case, more research is needed to understand Siskin isotopology.

For the remaining species, the individuals were assigned to almost the whole geographic range and the abundances of geographic assignments per individuals were changing probably in relationship with the distinct sampling effort along the migratory season. Trends were generally negative, highlighting the passage through Italian Alps of more and more northern populations during the post-breeding migration season (Figures 90 and 91).

Finally, the simultaneous use of recovery data and stable isotopes is worth noting. Together, the information gained from the two separate techniques helped to improve precision and accuracy in terms of detecting the geographical origin of several migrants. Distinction of the two areas of origin was only possible with reencountered ringing data, while quantification and studies on population trends are well described with isotopic information. The development of this approach will be the transformation of the polygons build with the recovery data in a probabilistic prior, which will be used to refine the geographic assignment (Van Wilgenburg and Hobson 2011). Furthermore, the inclusion of priors such as species abundances across their breeding ranges, and the use of isoscapes built on the species-specific breeding period would surely improve precision and accuracy of the assignments.

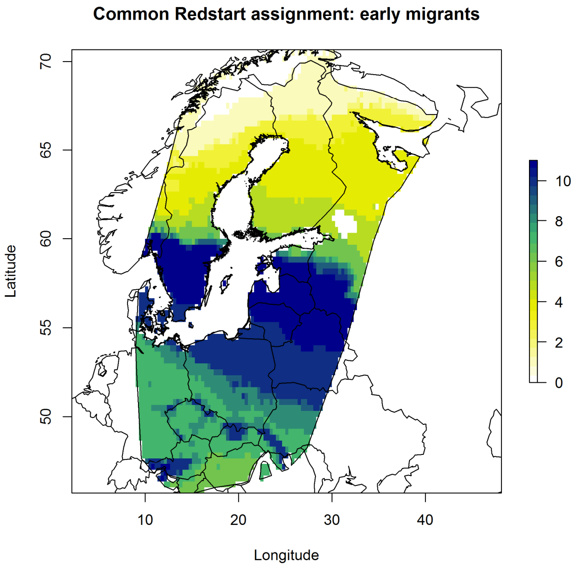

Figure 90: Assignment to the most likely geographic origin for 12 Common Redstarts, captured before predicted migratory peak (September 7th).

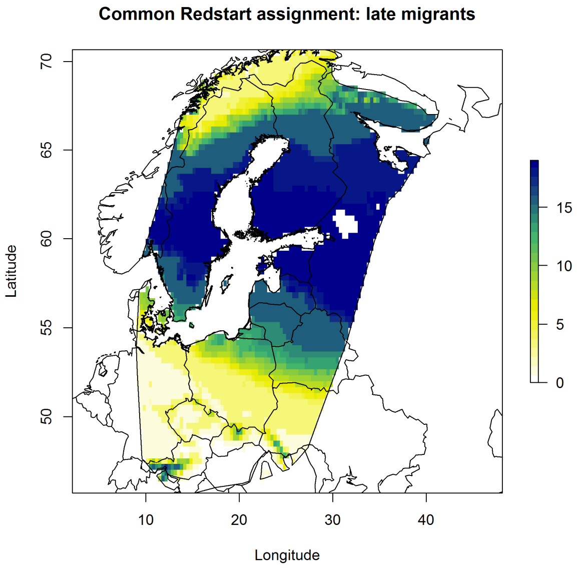

Figure 91: Assignment to the most likely geographic origin for 18 Common Redstarts, captured after predicted migratory peak (September 7th).

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THE TROPHIC ECOLOGY OF EUROPEAN PASSERINES

Introduction

This chapter investigates use of the stable isotope ratios of feathers to reconstruct trophic niches in different bird species. It is by now well-known that stable isotope ratios measured in organism tissues reflect the diet (Boecklen et al. 2011), which in turn reflects the isotopic variability of environmental, biogeochemical and climatic gradients (Rundel et al. 1988, Fry 2006, Hobson and Wassenaar 2008, West et al. 2010). Studies focusing on trophic dynamics using stable isotope ratios usually refer to well-defined and known community structures, in order to reconstruct the mechanisms of local trophic chains (Tieszen et al. 1983, Ambrose and DeNiro 1986), or try to assess spatial movement patterns in two distinct isotopic environments, such as wintering and breeding ranges or fresh versus marine-water feeding grounds (Hobson 1987, Caccamise et al. 2000, Kelly 2000, Gauthier et al. 2003). Furthermore, it has been noted that when ‘climbing’ the steps in the trophic chain, stable isotope ratios undergo fractionation, due to physiological and biogeochemical processes, with depletion or enrichment of the heavy fraction of stable isotopes with every passage in terms of trophic position (Minagawa and Wada 1984, Post 2002, McCutchan et al. 2003). Generally speaking, the isotope ratios of carbon, nitrogen and sulphur are used to study trophic relationships, although water isotopes are also increasingly taken into consideration in studies regarding trophic ecology (Vander Zanden et al. 2016). Moreover, it is necessary to bear in mind that isotopic landscapes, which could be considered as the basic mass of global isotopic variability, change spatially due to several biogeochemical factors, led by natural and anthropogenic processes that cause variations in isotopic signatures in ecosystems, both at regional and continental level (Bowen 2010, West et al. 2010).

The previous chapter focused on the geographical traceability of European birds, studying the post-breeding migration of several species of Passerines over the Italian Alps and trying to assign a geographical origin to them. For each of these species, all the measurements were obtained from juvenile individuals, so the isotopic information is related to the parental diet received in the nest. Small songbirds generally feed their broods a high energy diet, in order to support faster and healthier growth of the nestlings. Typically, depending on the species, Passerines take a huge variety of invertebrates to their brood, such as insects, molluscs, arachnids and crustaceans, preyed on at different stages of life (eggs, larvae or adults; many authors, del Hoyo et al. 2015). However, there is diversification of the diet introduced by the parents for the 21 species previously dealt with: indeed, most of the species are purely insectivorous (Pied Flycatcher, Common Chaffinch; Clement 2015a, Taylor and Christie 2015), but some of them may integrate their diets with vegetal food such as fruits, seeds or buds (Coal Tit, Eurasian Siskin; Clement 2015b, Gosler and Clement 2015), or have an almost completely granivorous diet (Common Crossbill; Clement and Christie 2015). This feeding structure could be linked to the following ecological aspects concerning the species:

- trans-Saharan migrants come to the Western Palaearctic to take advantage of the huge abundance of prey (mainly insects) available during the summer;
- intra-Palaearctic migrants have a longer breeding period and can vary their diets more extensively, including plant food more frequently;
- Red Crossbills are an exception and typically feed their brood on cone seeds;
- species such as the Eurasian Siskin, usually insectivorous, can exceptionally breed concurrently with cone maturation, leading to post-breeding invasive movements (Chambert et al. 2015).

Furthermore, their use of habitat is quite different, since the species dealt usually live in woodlands and grasslands, and can be affected by different levels of anthropogenic input, depending on their adaptation to agro-ecosystems, for example.

All these ecological traits suggest that the isotopic variability of carbon, nitrogen and sulphur may be extensive in these species, and that a species-specific trophic position could be found. To investigate this aspect, δ^{13}C, δ^{15}N and δ^{34}S were measured in the feathers of 48 species, in order to establish whether their ecological characteristics can be explained by isotopic variability.

Methods

Sample preparation and analysis

Feather sampling, cleaning, preparation and stable isotope measurements (δ^{13}C, δ^{15}N and δ^{34}S) were carried out as already described in Chapter 2.

Data handling

In order to classify the ecological differences between species, two different approaches were adopted:

1) The species were grouped according to their migratory habits (hereafter *migration* group), distinguishing trans-Saharan (see table in Appendix 1) from intra-Palaearctic migrants, in turn divided into regular and invasive species. This categorisation can suitably reflect trophic dynamics, as trans-Saharans are mainly insectivorous and intra-Palaearctics are potentially more omnivorous, while invasive species are potentially more granivorous.
2) In order to assess the degree of relationship of isotopic variability with environmental gradients, the migratory categories were then further split on the basis of three habitat classes (hereafter *habitat* group), defining generic habitat use by the species: a) open lands

(grasslands and wetlands), b) open woodlands and c) forests. Using this grouping seven categories were obtained: trans-Saharan and intra-Palaearctic migrants represented all three habitat classes, while invasive species were represented only by forest species.

For each stable isotope ratio, the degree of similarity of isotopic values (δ^{13}C, δ^{15}N and δ^{34}S) in the groups (*migration* and *habitat*) was measured, carrying out ANOVA for an unequal number of samples with relative *post hoc* tests (Tukey's Honestly Significant Test -HSD- Test, Abdi and Williams 2010, *stats* package R).

It was then verified whether the isotopic variability of δ^{13}C, δ^{15}N and δ^{34}S measured at *migration*, *habitat* and species level could be assigned to the correct *migration* category. In order to test this, a multinomial logistic regression model was estimated, with *migration* as the outcome variable and δ^{13}C, δ^{15}N and δ^{34}S as the predictor variables. The *multinom* function of the *nnet* package (Venables and Ripley 2002) in R (R Core Team 2016) was used. This function does not require reshaping of the data. Before running the model, it is necessary to choose a reference class, and the trans-Saharan category was chosen. Each function gives the AIC (Akaike's Information Criterion, Akaike 1973) of the model, while p-values were calculated using Wald tests (here z-tests, Wald 1945). Finally, using the *fitting* function of the *stats* package in R, the predicted probability of choosing one outcome category over the probability of choosing the baseline category were calculated (IDRE 2016) for each of the *migration* levels (trans-Saharan, regular intra-Palaearctic and irruptive species).

Considering that isotopic variability can also be affected by geographical and climatic factors, and that the previous chapter highlighted that the species have various geographical origins, the possibility that part of the observed variability was due to the isotopology of the areas of provenance and not to the trophic habits of the birds needs to be taken into consideration. To avoid the geographical effect, δ^{13}C, δ^{15}N and δ^{34}S values were transformed by calculating residuals of the regressions between stable isotope ratios and normalised dates of capture (see Chapter 3). This calculation was only possible for the same 21 species stated in the previous chapter, because there were sufficient individuals to perform the regression. Then, the means for the stable isotope ratio of each of the 21 species were calculated and added to the residuals, obtaining new species isotopic variability excluding geographical information. After this, ANOVA regression and the multinomial logistic estimation model for the *migration* group were estimated again. Finally, the probability of assignment to each class was calculated again, and as above, the probability was reported as the probability mean for individuals assigned to the same class and species.

Results

Comparison between the mean isotopic signatures of carbon, nitrogen and sulphur for samples grouped according to migratory categorisation showed statistically significant differences between the three classes ($p < 0.001$). The number of specimens varied depending on the reliability of the measurement.

In particular, trans-Saharan species showed higher δ^{15}N values compared to regular and invasive intra-Palaearctic migrants ($p < 0.001$, Figure 2a). Invasive species had higher δ^{13}C compared to regular intra-Palaearctics and trans-Saharans ($p < 0.001$, Figure 1a), and showed lower δ^{15}N compared to regular intra-Palaearctic species ($p < 0.001$). As regards δ^{34}S, regular intra-Palaearctic migrants showed different values compared to trans-Saharan species ($p < 0.05$). The second categorisation method, which included habitat preferences and subdivided migratory groups into seven sub-groups, highlighted diversity in the isotopic fingerprint in different habitat groupings belonging to the same migratory category. In particular, species from open lands showed more enriched δ^{15}N compared to those from woodlands and forests, both in trans-Saharan and regular intra-Palaearctic migrants ($p < 0.001$, Figure 2b). Comparison of δ^{13}C (Figure 1b) revealed that trans-Saharan migrants from open lands had more enriched values compared to all other classes. No differences with open land intra-Palaearctic migrants were identified. For δ^{34}S (Figure 3b), it is evident that regular intra-Palaearctic migrants from open lands showed higher values compared to all other categories ($p < 0.001$). Trans-Saharan migrants from forests had more enriched $\delta^{34}S$ compared to those from trans-Saharan open lands and to regular and irruptive intra-Palaearctic migrants from wooded environments ($p < 0.001$ to 0.05). Finally, regular intra-Palaearctic migrants from forest habitats had lower values compared to trans-Saharan migrants from wooded environments ($p < 0.001$ to 0.05).

Normalization of carbon, nitrogen and sulphur isotopic signatures according to the date of capture did not seem to improve the observations previously made (Figures 4 a and b), especially as regards $\delta^{15}N$ and $\delta^{34}S$. The differences within and between groups remained almost equivalent with or without normalization, although the carbon isotope underlined its statistical significance in differences between trans-Saharan open land migrants and other classes, except irruptive species ($p < 0.001$, Figure 5).

The multinomial logistic regressions which were run were based on these two models:

1) $\mathit{Migration} = \delta^{13}C + \delta^{15}N + \delta^{34}S$
2) $\mathit{Migration} = \delta^{13}C_n + \delta^{15}N_n + \delta^{34}S_n$

where *migration* is a three-level categorical outcome variable (trans-Saharan, regular intra-Palaearctic, irruptive), and raw ($\delta^{nn}X$) and normalized ($\delta^{nn}X_n$) stable isotopes are the predictor variables. The regressions obtained are described in Tables 1 and 2 and characterised by the coefficients, standard errors and p-values shown (Wald tests):

Table 1: Coefficients, standard errors and p-values (Wald tests) of model 1.

Model 1 AIC 931.1129	**Intercept**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{34}S$**
Coefficients				
Intra-Palaearctic	-0.2072	-0.0918	-0.2325	-0.0968
Irruptive	21.7158	0.8951	-0.8271	-0.0277
St. errors				
Intra-Palaearctic	1.859	0.0778	0.0332	0.0387
Irruptive	2.9339	0.1234	0.0977	0.1199
p-values				
Intra-Palaearctic	9.1127e-01	2.3801e-01	2.5531e-12	0.0124
Irruptive	1.3456e-13	4.1144e-13	0	0.8176

Table 2: Coefficients, standard errors and p-values (Wald tests) of model 2.

Model 2 AIC 751.4667	**Intercept**	**$\delta^{13}C_n$**	**$\delta^{15}N_n$**	**$\delta^{34}S_n$**
Coefficients				
Intra-Palaearctic	-2.7755	-0.2115	-0.2405	-0.1242
Irruptive	30.0843	1.2518	-0.7782	-0.1008
St. errors				
Intra-Palaearctic	2.4718	0.1039	0.0378	0.0432
Irruptive	4.5454	0.1929	0.1103	0.149
p-values				
Intra-Palaearctic	2.6149e-01	4.1687e-02	1.933e-10	0.0041
Irruptive	3.6267e-11	8.589e-11	1.7546e-12	0.4988

Each row in the six blocks of values (coefficients, standard errors and p-values) corresponds to a calculated model equation. Values in the rows must be compared to the *migration* = *trans-Saharan* baseline. If we consider the coefficients in the first row (e.g. in model 1) to be q_1 and the coefficients in the second row to be q_2, the model equations can be written as follows:

$$ln\left[\frac{P_{migration=intra-Palearctic}}{P_{migration=trans-Saharan}}\right] = q_1^0 + q_1^1\delta^{13}C + q_1^2\delta^{15}N + q_1^3\delta^{34}S$$

and

$$ln\left[\frac{P_{migration=irruptive}}{P_{migration=trans-Saharan}}\right] = q_2^0 + q_2^1\delta^{13}C + q_2^2\delta^{15}N + q_2^3\delta^{34}S$$

For example, an increase of 1 delta for δ^{34}S is associated with a statistically significant decrease ($p < 0.05$) in the log odds of being an intra-Palaearctic migrant vs a trans-Saharan, amounting to 0.0968 with a standard error of 0.0387. These probabilities were calculated for all the outcome levels of the two models, or rather for each individual. In Tables 1 and 2 the probabilities are reported as the means for *migration* and *habitat* groups and species. The trends for coefficients and their relative p-values appeared to coincide for models 1 (with raw data) and 2 (with normalized values). On observing averaged probability, this seems to be confirmed, establishing that eliminating geographical variability does not improve the model's significance, and that differences between classes are therefore due to trophic position, while geographical origin is not a determinant in the expression of isotopic composition (see Tables 3 and 4). Model 2 does however have a lower AIC, but this could be determined by the higher number of parameters considered in model 1. AIC is also calculated using the number of parameters and the likelihood of the model, hence model 2, which includes fewer individuals, should have a lower AIC value.

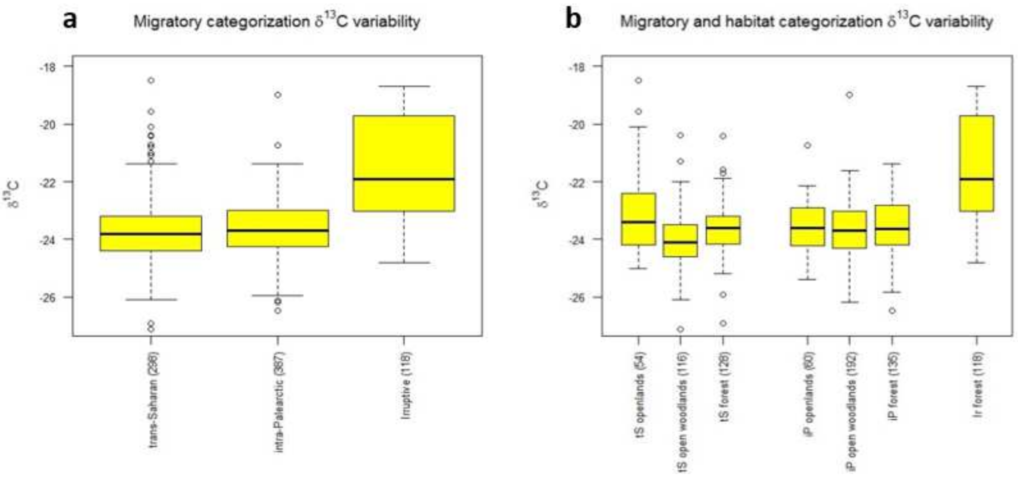

Figure 1: δ^{13}C variability by migratory categorisation (a) and migratory and habitat categorisation (b).

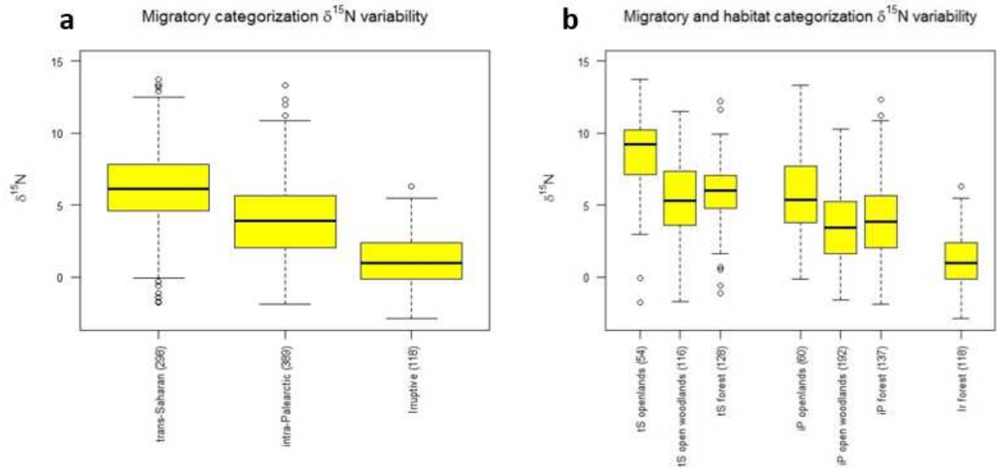

Figure 2: δ^{15}N variability by migratory categorisation (a) and migratory and habitat categorisation (b).

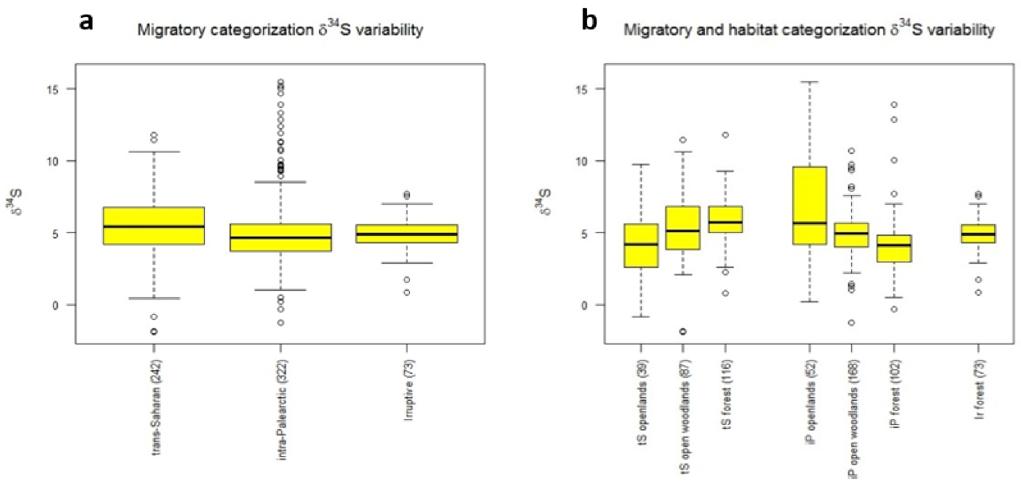

Figure 3: δ^{34}S variability by migratory categorisation (a) and migratory and habitat categorisation (b).

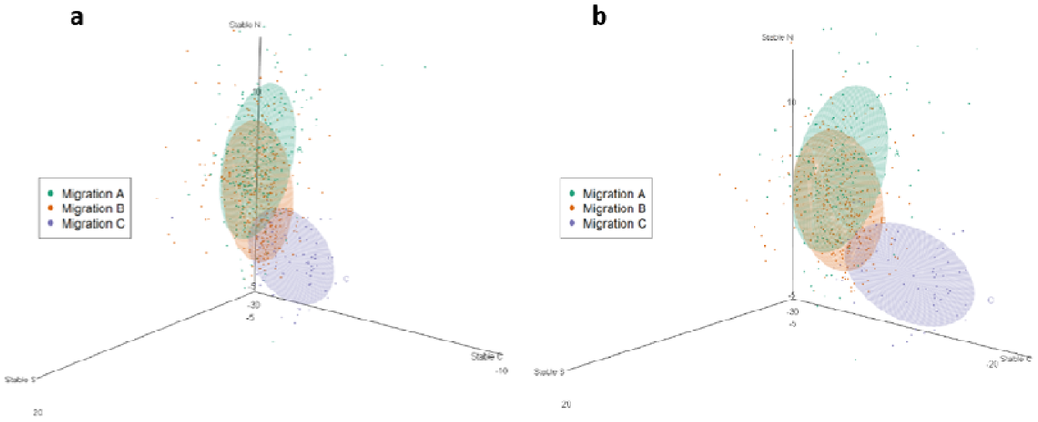

Figure 4: Three-dimensional plot of δ^{13}C, δ^{15}N and δ^{34}S values (a) and normalized δ^{13}C, δ^{15}N and δ^{34}S values (b), by migratory categories, with concentration ellipsoids.

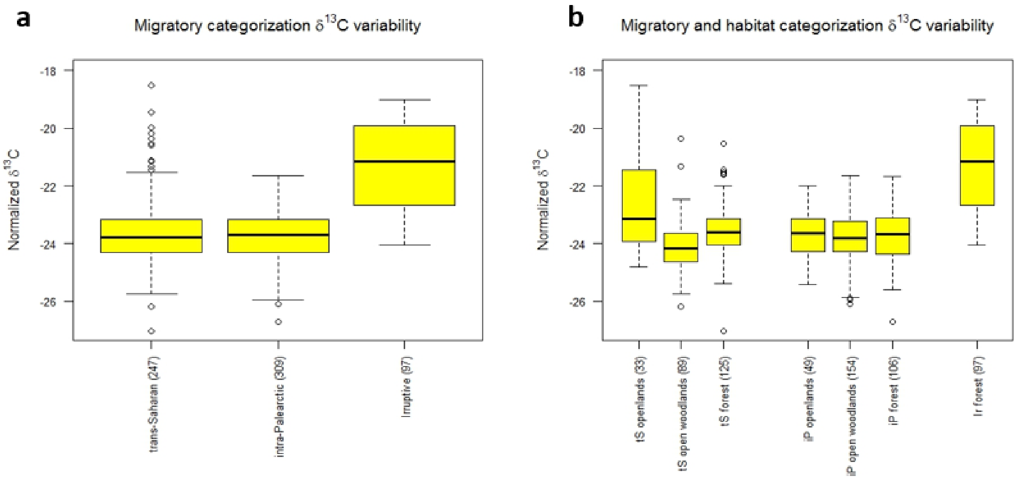

Figure 5: Normalized δ^{13}C variability by migratory categorisation (a) and migratory and habitat categorisation (b).

Table 3: Probability of assignment to the correct migration category for model 1, subdivided by migration and habitat categories and species.

Classes	***P of being in t-S***	***P of being regular i-P***	***P of being irruptive i-P***	***Number of specimens***
trans-Saharan	**0.484**	**0.483**	**0.033**	**242**
from open lands	**0.629**	**0.334**	**0.037**	**39**
Acrocephalus scirpaceus	0.683	0.317	0.000	2
Delicon urbicum	0.560	0.351	0.089	16
Motacilla Flava	0.687	0.312	0.001	19
Saxicola rubetra	0.584	0.415	0.001	2
from open woodlands	**0.414**	**0.547**	**0.039**	**87**
Anthus trivialis	0.327	0.583	0.090	20
Emberiza Hortulana	0.614	0.385	0.001	4
Hyppolais icterina	0.606	0.392	0.002	1
Lanius collurio	0.616	0.360	0.024	1
Muscicapa striata	0.294	0.605	0.101	1
Phylloscopus trochilus	0.412	0.552	0.036	35
Sylvia borin	0.433	0.560	0.007	23
Sylvia communis	0.682	0.317	0.000	1
Sylvia curruca	0.450	0.517	0.033	1
from forests	**0.488**	**0.485**	**0.027**	**116**
Ficedula hypoleuca	0.507	0.474	0.019	86
Phoenic.phoenicurus	0.434	0.517	0.049	30
regular intra-Palaearctic	**0.367**	**0.567**	**0.066**	**319**
from open lands	**0.497**	**0.463**	**0.040**	**52**
Anthus pratensis	0.585	0.403	0.012	26
Anthus spinoletta	0.256	0.690	0.055	1
Carduelis cannabina	0.395	0.579	0.026	1
Phoenicurus ochruros	0.417	0.515	0.069	24
from open woodlands	**0.331**	**0.595**	**0.074**	**166**
Erithacus rubecula	0.322	0.591	0.087	97
Fringilla montifringilla	0.363	0.620	0.017	20
Prunella modularis	0.293	0.589	0.118	22
Regulus ignicapillus	0.267	0.651	0.082	1
Sylvia atricapilla	0.376	0.590	0.035	24
Troglodytes troglodytes	0.353	0.621	0.026	2
from forests	**0.359**	**0.574**	**0.067**	**101**
Coccoth. coccothraustes	0.243	0.637	0.120	15
Fringilla coelebs	0.297	0.627	0.076	26
Phylloscopus collybita	0.505	0.453	0.042	19
Regulus regulus	0.278	0.643	0.080	12
Turdus philomelos	0.411	0.550	0.039	26
Turdus torquatus	0.432	0.507	0.061	3
irruptive intra-Palaearctic	**0.106**	**0.291**	**0.602**	**73**
from forests	**0.106**	**0.291**	**0.602**	**73**
Carduelis spinus	0.088	0.280	0.631	5
Certhia familiaris	0.090	0.395	0.516	1
Loxia curvirostra	0.043	0.097	0.860	40
Parus major	0.195	0.748	0.058	1
Periparus ater	0.196	0.571	0.233	25
Pyrrhula pyrrhula	0.420	0.576	0.004	1
Overall total	**0.382**	**0.503**	**0.115**	**634**

Table 4: Probability of assignment to the correct migration category for model 2, subdivided by migration and habitat categories and species.

Row Labels	P of being in t-S	P of being regular i-P	P of being irruptive i-P	Number of specimens
trans-Saharan	**0.475**	**0.496**	**0.029**	**197**
from open lands	**0.630**	**0.334**	**0.035**	**33**
Delicon urbicum	0.572	0.348	0.081	14
Motacilla Flava	0.674	0.325	0.002	19
from open woodlands	**0.367**	**0.595**	**0.038**	**50**
Anthus trivialis	0.306	0.614	0.079	16
Phylloscopus trochilus	0.396	0.586	0.018	34
from forests	**0.478**	**0.499**	**0.023**	**114**
Ficedula hypoleuca	0.495	0.485	0.020	85
Phoenic.phoenicurus	0.427	0.540	0.033	29
regular intra-Palaearctic	**0.347**	**0.599**	**0.054**	**282**
from open lands	**0.504**	**0.468**	**0.028**	**47**
Anthus pratensis	0.587	0.405	0.008	26
Phoenicurus ochruros	0.401	0.546	0.053	21
from open woodlands	**0.307**	**0.631**	**0.062**	**146**
Erithacus rubecula	0.297	0.625	0.078	89
Fringilla montifringilla	0.326	0.662	0.012	17
Prunella modularis	0.280	0.645	0.075	16
Sylvia atricapilla	0.349	0.624	0.027	24
from forests	**0.329**	**0.615**	**0.056**	**89**
Coccoth. coccothraustes	0.225	0.662	0.113	14
Fringilla coelebs	0.257	0.683	0.060	26
Phylloscopus collybita	0.505	0.474	0.021	19
Regulus regulus	0.220	0.698	0.081	11
Turdus philomelos	0.390	0.583	0.027	19
irruptive intra-Palaearctic	**0.089**	**0.244**	**0.667**	**63**
from forests	**0.089**	**0.244**	**0.667**	**63**
Carduelis spinus	0.158	0.422	0.420	5
Loxia curvirostra	0.022	0.031	0.947	38
Periparus ater	0.198	0.605	0.197	20
Overall total	**0.363**	**0.520**	**0.116**	**542**

Discussion

The aim of this chapter was to verify whether it is possible to distinguish trophic guilds among several Passerine species using carbon, nitrogen and sulphur isotopes. The isotopic variability measured in keratin samples in this study was undoubtedly related to the dietary composition used by adults to feed their chicks, because only feathers from nest-grown juveniles were sampled.

Generally, δ^{15}N appeared to be higher for trans-Saharan than for regular intra-Palaearctic migrants, which in turn had more enriched signatures compared to irruptive species. This is in agreement with the fact that birds usually migrating over long distances should have a higher trophic position compared to short-distance migrants. Their adaptation during long journeys could be related to their trophic needs. In any case, most songbirds, including long and short-distance migrants, have a high protein diet, consisting almost entirely of insects during breeding (Bairlein 2002). The reason for the marked distinction between the three classes should not be ascribed to different habitat selection, considering that comparison of the habitat values used by different migratory classes confirms this trend. Rather, habitat classification underlines the differences in the isotopic signatures of species within the same migratory guild from open habitats, more enriched, and those from woodlands, more depleted, as was expected. Thus it is necessary to look elsewhere for the reason behind the differences in the stable nitrogen signature of the three migratory classes. First of all, the hypothesis of the “insectivorous to herbivorous” gradient mentioned in the introduction could be confirmed. Trans-Saharan species are strictly insectivorous, while intra-Palaearctic migrants may be more omnivorous, and others are completely granivorous, as in the case of the invasive Common Crossbill. Crossbills effectively had the lowest δ^{15}N values of the species measured in this work, and their diet should consist exclusively of cone seeds. Moreover, regular intra-Palaearctic migrants have longer breeding seasons compared to species wintering in sub-Saharan Africa, which could allow them to expand their nestling diet to include vegetal ingredients such as fruits, seeds and buds with a higher frequency as compared to trans-Saharan migrants (del

Hoyo et al. 2011). Another possibility could be the different prey choice made by trans-Saharan and regular intra-Palaearctic migrants. It could be argued that short-distance migrants have greater availability of prey at the larval stage to feed their brood, because hatching occurs earlier as compared to long-distance migrants. On the other hand these have greater availability of prey at adult growth stages. Tibbets and collegues (2008) found that insect metamorphism causes enrichment in heavy nitrogen isotopes from the larvae to adult stages, comparable to a trophic shift. Could the stable nitrogen ratio in juvenile avian tissues be related to the different prey preferences of the two groups? It is difficult to demonstrate this hypothesis here, but it could be verified with a specific study. The easiest way would be to measure nitrogen isotopic variability in trans-Saharan and intra-Palaearctic juvenile birds sampled at the same location for which the diet and date of birth is known.

While nitrogen stable isotopes mainly characterise trophic levels in migratory guilds, δ^{13}C definitely separates irruptive species from the other two classes. Indeed, this class showed highly enriched values, completely different from intra-Palearctic and trans-Saharan species. It was expected to find more enriched values in classes more closely related to the agricultural environment, but on looking at *habitat* classification, the relationship found here was weak. Moreover, the irruptive species dealt with here are typical of forest environments and are expected to have more depleted stable carbon isotope values. Furthermore, the carbon isotope ratio should become more enriched following the trophic positions, similarly to the nitrogen isotope, albeit to a lesser extent. It is worth noting that irruptive species differed totally from this trend. Although these species are potentially totally herbivorous (mainly granivorous), occupying low trophic positions and living in a forest habitat, they showed highly enriched carbon isotope ratios. There may be two explanations for this:

- The irruptive species in this study were represented mainly by the Common Crossbill, Eurasian Siskin and Coal Tit. All these species, and particularly crossbills, may breed during the cold season, between January and May depending on the species, in order to take advantage of the ripening of coniferous cones. Crossbills are extremely well-adapted to this life and they showed the majority of the most enriched carbon values. It may be that breeding in the cold season involves a physiological condition increasing the carbon turnover rate, which translates into more enriched stable carbon ratios in tissues, as observed by Bauchinger and collegues (2010) in a laboratory experiment on Zebra Finches. Similar observations have also been described by Carleton and Martínez Del Rio (2005).

- The second possibility concerns the use of grit by granivorous species during the initial digestive phase (Best and Gionfriddo 1991). The grit used by birds is largely made up of carbonates, which typically have extremely enriched δ^{13}C values (Salomons 1975). It is possible that part of the elemental carbon making up grit minerals was gobbled up by the birds, digested and consequently fixed in their tissues. In any case, this hypothesis remains relatively unlikely, since birds should assimilate calcium from grit, while carbon should be dispersed as a gas (Hobson, pers.com.). The temperature-related hypothesis therefore becomes the most credible. In effect, on looking at the carbon isotope values of irruptive species, there are several individuals with a similar signature to trans-Saharan and regular intra-Palaearctic species. This suggests that δ^{13}C values could distinguish differences in the timing of nesting by the same irruptive species: those breeding during the cold season would show more enriched values, while those breeding during the growing season should show stable carbon ratios similar to summer breeders. Indeed, crossbills, typically winter breeders, had the highest carbon isotope values, while siskins, which only occasionally anticipate reproduction, and even fewer Coal Tits, had a carbon isotope signature closer to other intra-Palaearctic species. Moreover, siskins showed a trend linking carbon isotope values and the date of capture: more enriched values were found in samples at the beginning of the migratory season, confirming this assumption.

Finally, δ^{34}S variability among classes appears to be quite limited, although in the regular intra-Palaearctic open land migrants subgroup several individuals showed much more enriched values compared to all others. Specifically, these individuals were Meadow Pipits. If the variability of the sulphur isotope is so limited, this could mean that it is not affected by the different trophic positions of species, but that the discontinuity could be related to strong differences in habitat or geographical origin. In the previous chapter Meadow Pipits were assigned to northern origin. Effectively, it seems strange that only this species showed such enriched sulphur isotope ratio values, so it is probably related to the habitats used by Meadow Pipits. Hebert and Wassenaar (2005) observed that the sulphur isotope can be considerably enriched in wetlands, due to biochemical processes, but proximity to the sea may also cause an enrichment in heavy sulphur in animal tissues (Caccamise et al. 2000, Lott et al. 2003, Zazzo et al. 2011). So Meadow Pipits originating in Scandinavia probably come from grasslands close to the sea or habitats such as peat bogs and marshes. This aspect could be further developed, because although the variation observed in the sulphur isotope signature within groups is not extensive, it could follow regional gradients related to sea proximity or the deposition of precipitation sulphur, at least for some species (Pichlmayer et al. 1998).

The calculation of probability assigning each individual to a migratory guild provided a sort of estimate of correct affiliation, computable as the mean of *migration*, *habitat* and species category. Although nitrogen separated migratory guilds well, the multinomial models found a wide overlap. Even eliminating the bias provided by different geographical origin did not improve this estimate. Hence, well-defined habitat use and species-specific habits should separate species better, characterising the isotopic variability found more precisely. Using these models, around 50 % of trans-Saharan species were correctly assigned to their class, while the other 50 % were assigned to the regular intra-Palaearctic migrants category. More than 60 % of this latter class was correctly assigned, while 35% fell into the long-distance migrants group. For irruptive species, more than 60% of individuals were correctly assigned. At species level, it was possible to distinguish which species were correctly assigned to their predicted migratory category. For example, around 90% of crossbills were correctly assigned to the right migratory (and feeding) class, while around 60% of Coal Tits (irruptive) were wrongly assigned to the intra-Palaearctic group.

However, it is worth noting that by using the combination of δ^{13}C, δ^{15}N and δ^{34}S, it was possible to gain further knowledge of isotopic trophic partitioning and specialisation in relatively ecologically similar species, highlighting trophic specialisation, for example, and the extent to which the species is generalist.

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THE SPATIAL ECOLOGY OF THE LESSER KESTREL IN SICILY

Introduction

The ecology of the Lesser Kestrel (*Falco naumanni*) has been increasingly studied in the last twenty years, especially throughout the Mediterranean (e.g., Donàzar et al. 1993, Bustamante 1997, Tella et al. 1998, Tella and Forero 2000, Vlachos et al. 2003, 2014, Catry et al. 2009, Di Maggio et al. 2013, Sarà et al. 2014). The reason for this interest is its critical conservation status, which lasted until the beginning of the new millennium (BirdLife 2004), when the status of the species was downgraded from Vulnerable to Least Concern (IUCN categories). It was found that its breeding abundance and success are related to habitat quality and food availability, and are also connected to climate change (Rodríguez and Bustamante 2003, Rodrìguez et al. 2006, Sarà 2010, Catry et al. 2012). Lesser Kestrels need a significant amount of prey, consisting mainly of *Orthoptera* and *Coleoptera*, to grow their brood (Pérez-Granados 2010, Rodríguez et al. 2010). Insect abundance and quality are dependent on the degree of deterioration of the environments making up the hunting grounds surrounding breeding colonies, typically pseudo-steppes such as cereal and arable crops (Di Maggio et al. 2016). Recently, the dietary habits of the Lesser Kestrel, including diet composition and habitat use, have been studied using the stable isotope approach (Catry et al. 2016, Morganti et al. 2016). In particular, there has been investigation of the composition of the parental diet given to chicks, whether individuals from the same colony use the same hunting grounds, and what isotopic information can be gained by measuring stable isotope ratios in feathers from chicks growing in the nest. This chapter is the continuation of a recent study (Morganti et al. 2016), in which δ^{2}H, δ^{18}O, δ^{13}C, δ^{15}N and δ^{34}S were measured in the feathers and claws of 13 Lesser Kestrel chicks. It was found that the isotopic fingerprint could be related to the environmental diversity around the different colonies, reflecting surrounding habitat types, and that it was congruent with the social organisation of Lesser Kestrel breeding in the Gela plain in southern Sicily. The Lesser Kestrel's home range varies considerably during nesting, depending on habitat quality and prey availability (Donàzar et al. 1993, Tella et al. 1998, Vlachos et al. 2014). Nevertheless, recently Bondì (2016) reported that the Lesser Kestrel breeding population in this work mainly use an area with a 1 Km radius around their home colony as hunting grounds. This study is in accordance with a study by Bonal and Aparicio (2008), whilst others have reported more extensive hunting grounds (Catry et al. 2012, Vlachos et al. 2014).

As previously mentioned, a thorough knowledge of the relationships between colonies and their hunting areas is important for the conservation of Lesser Kestrels, in order to understand spatial use of the area surrounding the colonies. As already noted by Morganti and colleagues (2016), stable isotope ratios measured in the keratin of nestlings delineate the isotopic fingerprint of the parents' hunting areas, making it possible to distinguish different spatial use by adults within and between colonies. This study is a continuation of the previous study, and the SIRs of hydrogen, oxygen, carbon, nitrogen and sulphur were determined in the feathers of 45 chicks from seven colonies and seventeen nests from the same population in the Gela plain. It was then verified whether the 45 chicks could be isotopically discriminated by looking at the isotopic composition of the individuals, grouped by colony and selected habitat.

Methods

Study area and sample collection

The feathers of 45 Lesser Kestrel chicks from the Gela plain breeding area (474 Km^2, 37°15’N, 14°35’E, south-eastern Sicily, Figure 1) were sampled. The period of sampling was restricted to 17-18 June 2015, so that juvenile feathers would reflect the diet received during the nesting phase after hatching. Feathers were sampled from posterior and pectoral growth areas. Sampling took place in 7 colonies on the Gela plain. At least 4 individuals were sampled for each colony. Specifically, two nests and six individuals were sampled (4 and 2 individuals per nest) at the colony named ‘Casa Sotto Canalotto’ (ID 1), three nests and eight individuals (5, 1 and 2) at ‘Giaurone’ (ID 2), one nest and four individuals at ‘Magazzinazzo’ (ID 3), three nests and seven individuals (3, 1 and 3) at ‘Monteleone’ (ID 4), three nests and five individuals (1, 2 and 2) at ‘San Gregorio’ (ID 5), two nests and six individuals (2 and 4) at ‘Satellite Canalotto’ (ID 6) and three nests and nine individuals (4, 3 and 2) at ‘Torre Vecchia’ (ID 7). The hunting grounds surrounding the colonies are characterised by a mosaic of pseudo-steppes dominated by artichoke (*Cyanara* spp.), wheat and legume cultivation (Triolo et al. 2011). The habitat variability within 1 km of each colony was verified in the field, and the habitat types were defined and reduced to three main functional groups (see Di Maggio et al. 2016; Morganti et al. 2016 for further details):

1) semi-permanent irrigated arable land (mainly made up of artichoke fields, hereafter referred to as *Artichoke*);

2) non-irrigated arable land (hereafter *Arable*);

3) dry-grasslands and other semi-natural vegetation (hereafter *Grassland*).

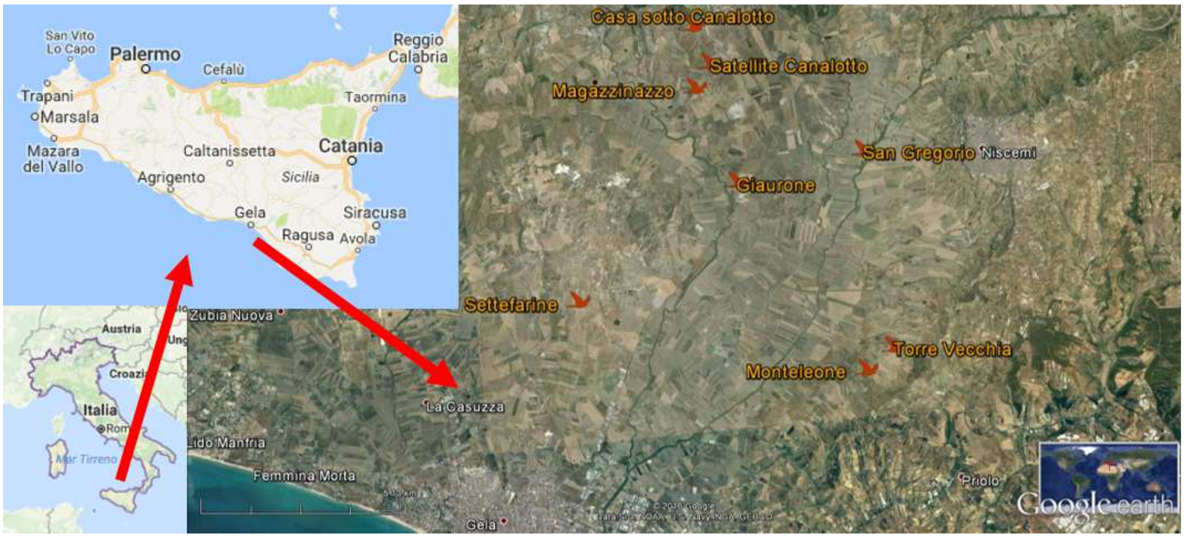

Figure 92: The Gela plain and the seven Lesser Kestrel colonies considered in this chapter. The Settefarine colony was monitored by Morganti and colleagues (2016) but was not sampled in this work. It was associated with *grassland*.

On the basis of this classification, the habitats surrounding the Torre Vecchia and Monteleone colonies (ID 4 and 7) were classified as ‘arable’, those surrounding Giaurone and San Gregorio (colonies ID 2 and 5) as ‘artichoke’ and those surrounding Casa Sotto Canalotto, Satellite Canalotto and Magazzinazzo (colonies 1, 3 and 6) as ‘grassland’.

The sampled feathers were cleaned and prepared as described in Chapter 2, at the Traceability Unit of the Fondazione Edmund Mach, in order to measure the stable isotope ratios of hydrogen, oxygen, carbon, nitrogen and sulphur.

Statistical analysis

First of all, the similarity of isotopic values (δ^{2}H, δ^{18}O, δ^{13}C, δ^{15}N and δ^{34}S) in habitat categories and colonies was measured for each stable isotope ratio, carrying out ANOVA with relative *post hoc* tests (Tukey’s Honestly Significant Difference – HSD - Test, Abdi and Williams 2010, *stats* package R) for an unequal number of samples. Then, Discriminant Function Analysis (DFA) was performed, in order to assess whether colonies and their hunting grounds were isotopically distinguishable (Pérez-Tris et al. 1999, De La Hera et al. 2007, Morganti et al. 2014), based on the δ^{2}H, δ^{18}O, δ^{13}C, δ^{15}N and δ^{34}S values measured in feathers, using SPSS for Windows (IBM 2013), in order to obtain solid assignment of each sampled individual to its habitat (hereafter, DFA-hab) and colony (DFA-col). The isotopic ratios of the five elements were used as predictors in the two separate DFA procedures, assuming the prior probabilities of assignment to a given habitat or colony to be equal between groups, because the sample size was almost equally distributed between colonies and habitat respectively. The number of habitats and colonies was known, respectively three and seven, making it possible to allocate each individual to a habitat type or colony *a priori*, and then check the posterior assignment probability to a given group (a score calculated by the DFA procedure from 0 to 1), based on the structure matrix of DFA. By testing the correlation between the scores of discriminant functions and predictor values (stable isotopes), the structure matrix of the analysis returns functions significantly correlated to predictors.

Results

Table 1 shows the means and standard deviations of the δ^{2}H, δ^{18}O, δ^{13}C, δ^{15}N and δ^{34}S values measured for the whole Gela plain population, and then according to habitat types and colonies. The analysis of variance performed showed statistically significant differences between habitat categories for all isotope ratios ($p < 0.001$, Figure 2), and *post-hoc* tests confirmed the distinction of the three categories. On the other hand, analysis of variance performed on colonies was statistically significant only for δ^{2}H, δ^{18}O and δ^{34}S ($p < 0.05$, Figure 3), and *post-hoc tests* revealed that the detected differences between groups of colonies were related to the habitat category.

Table 4: Mean values ± s.d. of δ^{2}H, δ^{18}O, δ^{13}C, δ^{15}N and δ^{34}S measured in feathers of 45 Lesser Kestrel chicks. Values are pooled on a different spatial scale: whole population, habitat or colony. Numbers in brackets indicate sample size.

Spatial scale	**Name**		**δ^{2}H**	**δ^{18}O**	**δ^{13}C**	**δ^{15}N**	**δ^{34}S**
Whole population	Gela plain (45)		-48.01 ± 12.42 ‰	16.56 ± 1.37 ‰	23.47 ± 0.35 ‰	8.23 ± 1.51 ‰	-2.58 ± 2.13 ‰
Habitat type	Arable (16)		-50.37 ± 10.60 ‰	16.26 ± 0.91 ‰	-23.46 ± 0.31 ‰	8.31 ± 0.43 ‰	-2.88 ± 0.82 ‰
	Artichoke (13)		-59.97 ± 5.48 ‰	15.22 ± 0.60 ‰	-23.84 ± 0.14 ‰	10.12 ± 0.77 ‰	-0.12 ± 0.62 ‰
	Grassland (16)		-35.94 ± 5.54 ‰	17.94 ± 0.83 ‰	-23.17 ± 0.18 ‰	6.62 ± 0.38 ‰	-4.29 ± 1.97 ‰
Colony	*Arable*	Torre Vecchia (9)	-58.64 ± 4.03 ‰	15.53 ± 0.35 ‰	-23.71 ± 0.12 ‰	8.36 ± 0.36 ‰	-3.37 ± 0.50 ‰
		Monteleone (7)	-39.73 ± 4.97 ‰	17.20 ± 0.31 ‰	-23.14 ± 0.09 ‰	8.64 ± 0.27 ‰	-2.25 ± 0.74 ‰
	Artichoke	San Gregorio (5)	-62.12 ± 6.76 ‰	15.12 ± 0.53 ‰	-23.92 ± 0.10 ‰	10.28 ± 0.53 ‰	-0.54 ± 0.21 ‰
		Giaurone (8)	-58.43 ± 4.47 ‰	15.28 ± 0.67 ‰	-23.79 ± 0.13 ‰	10.02 ± 0.91 ‰	0.14 ± 0.66 ‰
	Grassland	Magazzinazzo (4)	-42.85 ± 5.85 ‰	17.10 ± 0.36 ‰	-23.36 ± 0.23 ‰	7.08 ± 0.06 ‰	-4.11 ± 0.31 ‰
		Satellite Canalotto (6)	-36.02 ± 1.56 ‰	17.58 ± 0.47 ‰	-23.04 ± 0.08 ‰	6.43 ± 0.12 ‰	-5.25 ± 0.74 ‰
		Casa sotto Canalotto (6)	-31.60 ± 3.71 ‰	18.85 ± 0.23 ‰	-23.18 ± 0.10 ‰	6.51 ± 0.43 ‰	-3.44 ± 3.01 ‰

DFA-hab correctly classified all the chicks (100 % of cases, see Table 2) to the habitat type surrounding the corresponding colony. In Figure 4 the plot of samples collected, grouped according to the three habitats, is shown according to the two 'new' discriminant functions. The first function has a high eigenvalue (14.138) with a high explanatory power (% of Variance explained= 98.7) and is mainly positively loaded by δ^{15}N (loading of δ^{15}N in the first functions: r = 0.887, see Table 2) and to a lesser extent negatively loaded by δ^{18}O (r = -0.501). The second discriminant function has an eigenvalue of 0.185 and explains the remaining 1.3% of model variability. Furthermore, it is positively correlated to δ^{2}H and negatively to δ^{13}C (respectively, r = 1.195, r = -1.214; see Table 2).

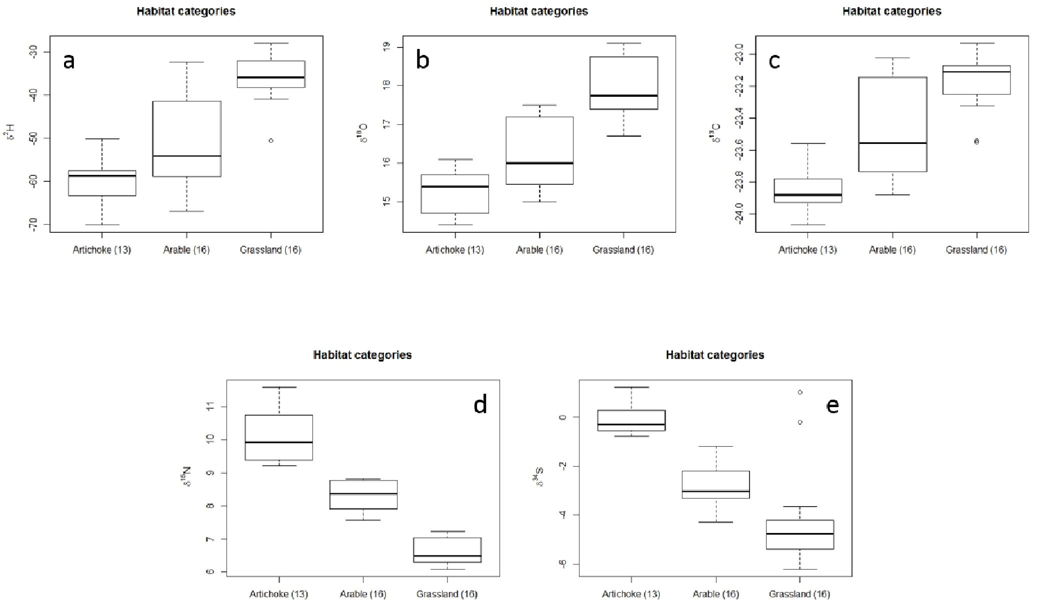

Figure 93: Box-and-Whisker plots of variability of δ^2H (a), δ^{18}O (b), δ^{13}C (c), δ^{15}N (d) and δ^{34}S (e) measured in Lesser Kestrels feathers of chicks, grouped by habitat categories. Anova and *post-hoc* tests all resulted significant (p-values between < 0.01 and < 0.001).

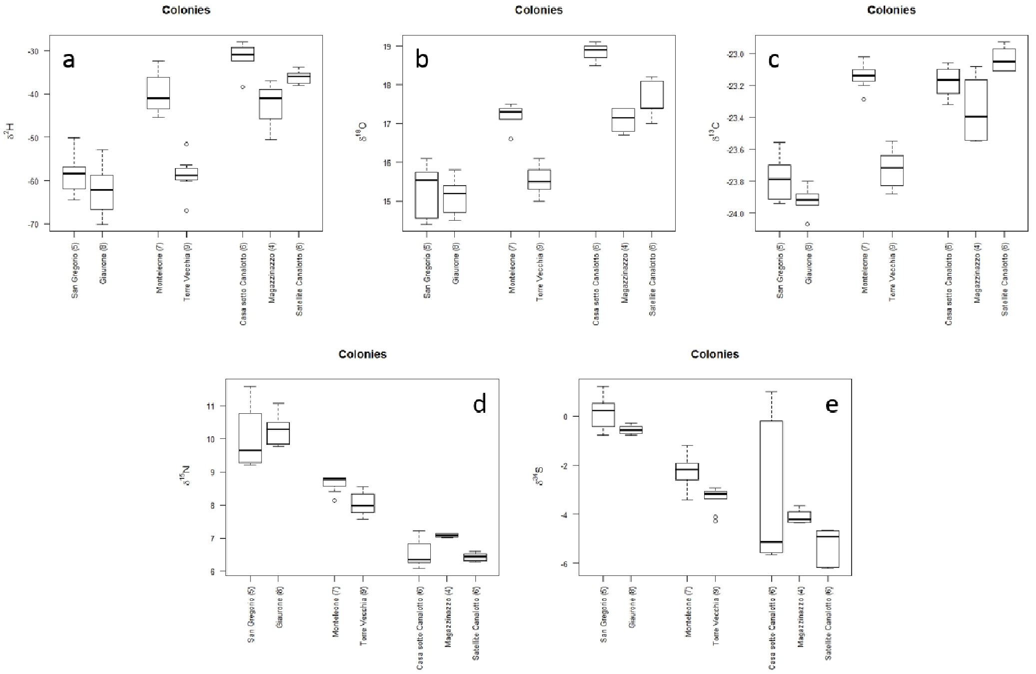

Figure 94: Box-and-Whisker plots of variability of δ^2H (a), δ^{18}O (b), δ^{13}C (c), δ^{15}N (d) and δ^{34}S (e) measured in Lesser Kestrels feathers of chicks, grouped by colony categories. Anova tests resulted statistically significant for δ^2H (a), δ^{18}O (b) and δ^{34}S (e) ($p < 0.05$); the *post-hoc* tests revealed differences in the *habitat* gradient for all colonies.

DFA-col classified each individual with 95.6% correct classification to its colony (Table 3). In Figure 5 the plot of samples grouped within each colony is displayed according to the first three discriminant functions. The first function has an eigenvalue of 40.677 and explained 89.9 % of total variance. It is negatively correlated with δ^{15}N (r = -0.847) and positively with δ^{18}O (r = 0.880). The second function is mainly positively correlated to δ^{13}C (r = 0.643), δ^{34}S (r = 0.616) and δ^{15}N (r = 0.613), whereas the third function was negatively correlated to δ^{13}C (r = -0.783) (Table 3). Together they explained 99.8 % of variance.

Table 5: Eigenvalues, standardised canonical function coefficients and predicted group memberships of DFA-hab.

Eigenvalues

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	14.138	98.7	98.7	0.966
2	0.185	1.3	100.0	0.395

Standardised Canonical Discriminant Function Coefficients

Function	δ^{2}H	δ^{18}O	δ^{13}C	δ^{15}N	δ^{34}S
1	-0.330	-0.501	0.117	0.887	0.287
2	1.195	0.083	-1.214	-0.178	0.511

Classification results – Predicted Group Membership

100% of original grouped cases correctly classified	**Type of habitat**	**Grassland**	**Arable**	**Artichoke**	**Total**
	Grassland (n=16)	100%			**100%**
	Arable (n=16)		100%		**100%**
	Artichoke (n=13)			100%	**100%**

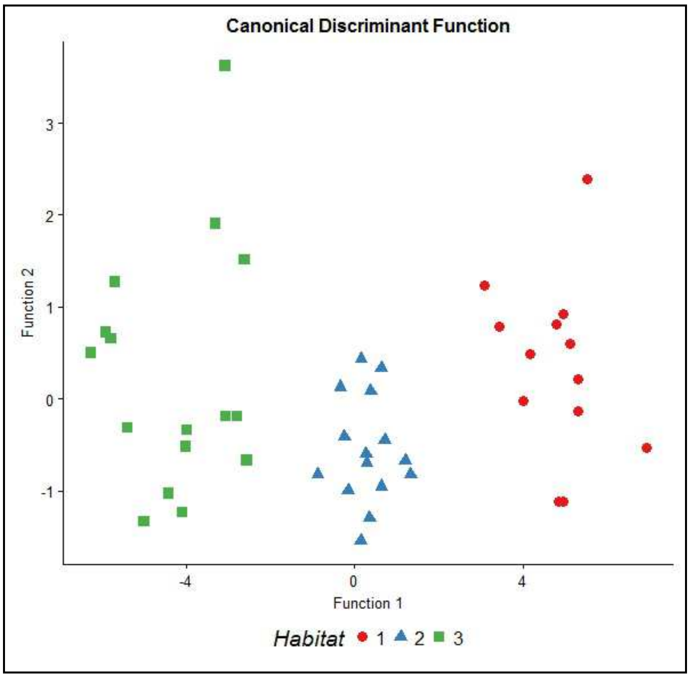

Figure 95: Plot of individuals and habitats in the bi-dimensional discriminant space, as identified by discriminant functions 1 and 2 of DFA1. Habitat 1 ~ *artichoke*, 2 ~ *arable*, 3 ~ *grassland*.

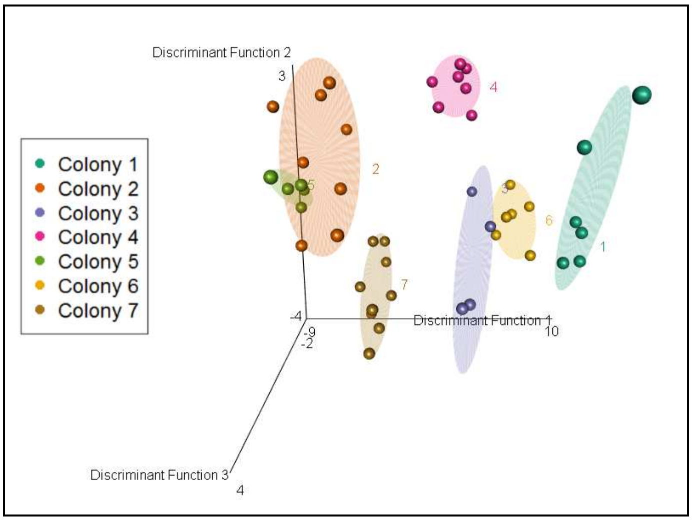

Figure 96: Plot of individuals and colonies in the three-dimensional discriminant space, as identified by discriminant functions 1, 2 and 3 of DFA-col. Colony 1 ~ Casa sotto Canalotto, 2 ~ Giaurone, 3 ~ Magazzinazzo, 4 ~ Monteleone, 5 ~ San Gregorio, 6 ~ Satellite Canalotto, 7 ~ Torre Vecchia.

Table 6: Eigenvalues, standardised canonical function coefficients and predicted group membership of DFA-col.

Eigenvalues

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	40.677	89.9	89.9	0.988
2	3.360	7.4	97.4	0.878
3	1.091	2.4	99.8	0.722

Standardised Canonical Discriminant Function Coefficients

Function	$\delta^{2}H$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
1	0.225	0.880	0.396	-0.847	0.336
2	-0.021	0.252	0.643	0.613	0.616
3	0.265	0.444	-0.240	-0.783	0.503

Classification results – Predicted Group Membership

	Colony	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Total**
95.6% of original grouped cases correctly classified	**1 (n=6)**	100%							**100%**
	2 (n=8)		75% (n=6)			25% (n=2)			**100%**
	3 (n=4)			100%					**100%**
	4 (n=7)				100%				**100%**
	5 (n=5)					100%			**100%**
	6 (n=6)						100%		**100%**
	7 (n=9)							100%	**100%**

Discussion

The utility of measuring stable isotope ratio variability in feathers of a colonial species was investigated in order to verify whether it is possible to distinguish spatial use within a relatively small breeding area. As previously emerged in the preliminary work of Morganti and colleagues (2016), the five stable isotopes together delineated a clear pattern related to the habitat types surrounding the colonies. DFA-hab supported this thesis, and seemed to definitely discriminate habitat types. It is worth noting that this distinction was also sustainable for colonies, which surprisingly resulted almost completely isotopically distinct, despite the fact that some of them depend on the same habitats. In effect, on observing mean values, it would seem that the strongest distinction occurs between grassland and artichoke habitats, while the arable type appears to lie in the middle, between these two categories. Indeed, the two colonies in the arable group were markedly different from each other for δ^{2}H, δ^{18}O and δ^{13}C. In particular, the Torre Vecchia colony was similar to the artichoke group colonies, whereas the Monteleone colony was similar to the grassland group colonies. More specifically, the grassland group, together with Monteleone colony, showed the most enriched values for δ^{2}H, δ^{18}O and δ^{13}C, while the artichoke group, together with the Torre Vecchia colony, had the most depleted values. The variability of δ^{2}H, δ^{18}O and δ^{13}C in colonies and habitats may be associated with different water sources and the different levels of evaporation occurring in different areas. It is interesting to note that more enriched δ^{2}H and δ^{18}O values are typical of semi-natural environments. In contrast, lower values are the prerogative of artichoke fields, which are heavily irrigated. In addition, colonies in the arable category, which can be differently managed depending on the type of crop sown, especially in terms of irrigation, showed a marked dichotomy that surprisingly followed the distinction between artichoke and grassland habitat types. It is known that drier seasons may cause an enrichment in water isotopes, due to evaporation that can be transferred locally into the trophic chain (Langin et al. 2007, Hobson et al. 2012, Bortolotti et al. 2013). Here we are probably observing how human action, specifically the irrigation of fields with water from elsewhere, can cause a variation in the water isotope signature in a location which should not show such variability (~ 25 ‰ for δ^{2}H), according to its restricted spatial (474 Km^{2}) and altitudinal (mean altitude 160.3 ± 14.27 m a.s.l.) gradients. δ^{13}C measurements seem to support this hypothesis. Indeed, it is known that water stress can induce plants to close stomata in order not to disperse water, also causing an enrichment in the heavy fraction of carbon isotopes (Männel et al. 2007). It can be supposed that non-irrigated habitats such as grasslands and cereal arable land are drier compared to those irrigated (artichoke fields), especially in a warm region such as Sicily, and locally this can lead to higher δ^{13}C values.

As regards δ^{15}N and δ^{34}S values, these changed according to habitat categorisation, with more depleted values for grassland individuals, intermediate values for the arable type and more enriched values for artichoke samples. It can be supposed that this diversity between colonies and habitat types is related to the fertilisers applied in agriculture, which may alter the δ^{15}N of soils and enter local trophic chains (Amundson et al. 2003, Bateman and Kelly 2007, Pardo and Nadelhoffer 2010). In this study, the δ^{15}N values increased when passing from semi-natural (grassland) to heavily human-managed (artichoke) environments, with intermediate values in less managed crops (arable). Indeed, the artichoke is a plant needing high nitrogen fertilisation to improve crop growth, early ripening and yield (Lo Giudice et al. 2014). It can be deduced from the δ^{15}N values found in artichoke and arable samples that the fertilisers applied are organic (such as animal manure) and not synthetic (Bateman et al., 2007). Finally, δ^{34}S behaved in a relatively similar manner to δ^{15}N, with a similar, albeit narrower, habitat gradient according to agricultural management. The predominantlynegative δ^{34}S values are in agreement with the expected values for terrestrial soils (Wassenaar 2008), although they could be related to volcanic soils, which have already been observed in Sicily (Camin et al. 2007), despite the fact that the expected values could be higher due to the proximity of the region to the sea (Manca et al. 2006, Zazzo et al. 2011). With reference to colonies, δ^{15}N and δ^{34}S values were grouped according to habitat categorisation, with the exception of two siblings from the ‘Casa sotto Canalotto’ grassland habitat colony, which had more enriched

δ^{34}S values, similar to those in the artichoke group. This may suggest that adults from the same colony are hunting in the same area, but selecting different patches and fields inside this area, here expressed by different δ^{34}S values.

The discriminant functions for colony and habitat worked extremely well in distinguishing the individuals. The only incorrect assignment was for two Giaurone chicks which were assigned to San Gregorio, but the two colonies both belonged to the artichoke habitat. It is evident that a multi-isotope approach has the potential to make accurate predictions of trophic partitioning and niche related to habitat selection. In the case of DFA-hab, the nitrogen isotope explains the majority of variance, meaning that agricultural management of the Gela plain is the main factor affecting the isotopic composition of Lesser Kestrel feathers. This would make it possible to have an indication of the effect of human activities on Gela plain population trends, for example by studying the relationship between breeding success and the habitats used. DFA-col confirms this hypothesis, since the function was able to distinguish the colonies almost perfectly, even when the habitat category was the same. Hence, the multi-isotopic approach can be extremely precise in studies wishing to investigate trophic ecology.

This study was a preliminary investigation, but these encouraging results demonstrate that the multi-isotope approach could work well. Future perspectives for this study will involve larger numbers of sampled individuals, including more colonies, and if possible more habitat types, as well as samples from different years, in order to compare inter-annual variability.

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Conclusions

The aim of this PhD thesis was to carry out an in-depth study of bird ecology using a stable isotope ratio approach. Three main ecological cases were investigated: migration, trophic ecology and habitat selection. Furthermore, the PhD project also included the possibility of integrating past experience and performing laboratory analysis and activities in the field.

As expected, the use of hydrogen and oxygen stable isotopes to detect the geographical origin of trans-Alpine migratory birds was both challenging and promising. This case study focused on 21 species, with a total of 715 samples collected at two Italian ringing stations. Isotopic data were combined with ringing data in order to improve the precision of detection for these two techniques taken individually. The study made it possible to improve knowledge of the geographical origin of post-breeding migrants passing through the Italian Alps and foothills. Recovery data underlined that in all probability most of the species that can be observed come from a geographical region between the Alps and Fennoscandia. Only Pied Flycatchers were shown to also have an eastern origin, including territories further east of the Urals. This is a crucial point for small migratory songbird conservation. In Italy, particularly in central and eastern alpine regions, traditional hunting of Passerines during the post-breeding season is still very popular. To date, some species of thrushes have been legally hunted, but unfortunately there is still poaching of protected species, including practically all songbirds, although this is decreasing. Hence, the hope is to further improve the detection and traceability of these migrants. Future perspectives for this study also include morphological and biometric measurement data. In particular, the relationship between the isotopic composition of feathers and the anatomical characteristics of birds (e.g. wing length, wing pointedness) should be further studied. Understanding these relationships could make the ringing databases for previous years (e.g. Progetto Alpi databases) useful in terms of reconstructing inter-seasonal trends for migratory populations.

The second case study dealt with the trophic ecology of 48 species (807 samples) collected during post breeding migration through the Alps. In particular, the stable isotope ratios of carbon, nitrogen and sulphur were investigated, to determine whether it was possible to trace species with different migratory habits (trans-Saharan, regular and irruptive intra-Palaearctic migrants) and habitat use (open lands, open woodlands and forest). Using this approach it was possible to distinguish species which are apparently quite similar in terms of feeding. As regards bird conservation and global change, this research has implications in terms of the possibility of defining species-specific trophic niches, also in comparison to other species. This could make it possible to verify which factors affect bird decline, in relation to their diets.

The last chapter focuses on the potential use of a multi-isotope approach to define the use of habitat by Lesser Kestrels from different colonies with hunting grounds in different habitats. Specifically, the stable isotope ratios of hydrogen, carbon, nitrogen, oxygen and sulphur were determined in samples from 45 individuals belonging to 7 colonies, collected in the Gela plain in Sicily. In a relatively small area spatial use was defined almost individually and it was possible to define differences in the isotopic composition of different habitats, confirming that individuals from the same colony depend on the same hunting grounds, but suggesting a certain intra-colony competition. In the future this research line could be improved by studying the isotopic composition of prey and defining fractionation factors occurring, in order to better define local trophic dynamics, also increasing the number of specimens per colony.

The analytical approach applied throughout this thesis proved to be highly reliable, as it was successfully applied to three different case studies. Currently, isotope ratio analysis definitely offers one of the most promising hypothesis-driven approaches for establishing the provenance and the ecological habits of birds.

Despite the high potential of stable isotope ratios, it is important to mention some common limitations and reservations highlighted during the drawing up of this thesis. Firstly, classic ‘parameters’ (e.g. ringing data, morphological data) should be not overlooked, as they often provide invaluable additional information assisting with interpretation of isotopic data, or enhancing the potential of this approach. This analytical approach is therefore based on a database, in which the number of samples must be sufficient in order to truly reflect reality.

Appendix 1

Table of average values and standard deviations of isotopic composition of hydrogen, oxygen, carbon, nitrogen and sulphur measured in juvenile feathers of 48 Passerine species reported for year of sampling, with the average day of sampling during the migratory season.

	n	δ^{2}H	δ^{18}O	δ^{13}C	δ^{15}N	δ^{34}S	Day
Common Swallow ***Hirundo rustica***	**1**	**-108.41**	**11.8**	**-23.15**	**9.09**		**258**
2011	1	-108.41	11.8	-23.15	9.09		258
House Martin ***Delichon urbicum***	**16**	**-94.09 ± 25.15**	**13.07 ± 2.52**	**-21.3 ± 2.77**	**7.45 ± 2.99**	**5.43 ± 1.94**	**250**
2011	10	-88.42 ± 27.14	13.5 ± 2.81	-20.65 ± 3.18	7.23 ± 3.62	5.13 ± 1.77	251
2012	5	-106.08 ± 21.26	12.24 ± 2.18	-22.38 ± 1.81	7.93 ± 1.9	6.2 ± 2.43	248
2013	1	-90.8	12.8	-22.42	7.19	4.58	245
Tawny Pipit ***Anthus campestris***	**1**	**-90.78**	**13.34**	**-23.3**	**6.3**		**260**
2011	1	-90.78	13.34	-23.3	6.3		260
Rock Pipit ***Anthus spinoletta***	**2**	**-84.01 ± 3.24**		**-22.97 ± 1.1**	**3.46 ± 1.69**	**4.69**	**279**
2011	2	-84.01 ± 3.24		-22.97 ± 1.1	3.46 ± 1.69	4.69	279
Meadow Pipit ***Anthus pratensis***	**27**	**-99.86 ± 8.99**	**17.27 ± 3.68**	**-24.06 ± 0.74**	**6.22 ± 2.59**	**9.75 ± 3.51**	**284**
2011	23	-100.39 ± 9.43	18.27 ± 2.95	-24 ± 0.76	5.88 ± 2.28	10.06 ± 3.71	284
2012	4	-96.78 ± 5.81	11.5 ± 1.31	-24.4 ± 0.57	8.18 ± 3.76	8.05 ± 1.4	284
Tree Pipit ***Anthus trivialis***	**20**	**-94.82 ± 16.14**	**12.58 ± 2.09**	**-23.91 ± 1.08**	**3.34 ± 3.06**	**4.99 ± 1.69**	**251**
2010	1	-87.79	14.77	-23.4	2.7	2.36	247
2011	11	-97.31 ± 12.57	12.6 ± 1.48	-23.47 ± 0.68	3.03 ± 3.76	5.44 ± 1.64	249
2012	5	-85.72 ± 25.62	13.31 ± 2.95	-24.79 ± 1.6	2.88 ± 2.25	5.29 ± 1.51	261
2013	3	-103.17 ± 0.76	10.57 ± 0.15	-24.21 ± 0.58	5.45 ± 0.2	3.73 ± 1.41	243
Yellow Wagtail ***Motacilla flava***	**19**	**-92.41 ± 11.28**	**12.67 ± 1.21**	**-23.26 ± 1.26**	**10.55 ± 2.19**	**3.22 ± 2.99**	**247**
2010	4	-88.42 ± 10.98	12.17 ± 0.48	-23.23 ± 1.4	12.65 ± 0.86	3.55 ± 3.06	244
2011	9	-98.58 ± 10.68	12.38 ± 1.49	-23.4 ± 1.58	10.03 ± 2.54	3.53 ± 3.71	252
2012	5	-85.22 ± 9.22	13.4 ± 0.83	-23.07 ± 0.73	10.35 ± 1.08	2.73 ± 2.11	244
2013	1	-88.8	13.6	-23	7.83	1.66	236

	n	δ^{2}H	δ^{18}O	δ^{13}C	δ^{15}N	δ^{34}S	Day
Dunnock ***Prunella modularis***	**23**	**-92.88 ± 8.87**	**11.38 ± 2.16**	**-23.51 ± 0.83**	**2.8 ± 2.27**	**5.17 ± 1.44**	**271**
2011	15	-95.14 ± 8.59	11.58 ± 2.6	-23.22 ± 0.55	2.07 ± 2.18	4.86 ± 0.7	264
2012	8	-88.64 ± 8.25	11.04 ± 1.11	-24.05 ± 1.03	4.37 ± 1.64	5.75 ± 2.22	283
European Robin ***Erithacus rubecula***	**103**	**-82.26 ± 12.21**	**12.88 ± 1.55**	**-23.66 ± 1**	**3.47 ± 2.44**	**4.64 ± 1.75**	**267**
2010	45	-82.43 ± 12.95	13.11 ± 1.42	-24.15 ± 0.84	4.28 ± 2.45	4.36 ± 1.82	274
2011	58	-82.12 ± 11.71	12.67 ± 1.64	-23.27 ± 0.94	2.84 ± 2.24	4.83 ± 1.69	262
Common Redstart ***Phoen. phoenicurus***	**31**	**-99.66 ± 11.95**	**12.92 ± 2.03**	**-23.66 ± 0.79**	**4.81 ± 2.42**	**6.35 ± 1.75**	**256**
2010	5	-82.53 ± 11.3	13.06 ± 1.68	-23.22 ± 0.76	6.02 ± 1.35	4.54 ± 0.97	250
2011	26	-103.08 ± 8.83	12.89 ± 2.13	-23.74 ± 0.78	4.58 ± 2.53	6.63 ± 1.69	257
Black Redstart ***Phoenicurus ochruros***	**25**	**-89.48 ± 8.81**	**13.51 ± 1.14**	**-23.3 ± 0.79**	**5.54 ± 3.13**	**4.06 ± 1.33**	**281**
2010	4	-89.94 ± 7.09	13.15 ± 1.59	-23.55 ± 0.64	8.78 ± 1.31	3.09 ± 0.44	290
2011	13	-93.08 ± 8.09	13.33 ± 0.99	-23.34 ± 0.86	4.41 ± 2.92	3.94 ± 1.48	279
2012	8	-83.41 ± 8.13	13.97 ± 1.15	-23.1 ± 0.78	5.76 ± 3.14	4.61 ± 1.11	281
Northern Wheatear ***Oenanthe oenanthe***	**5**	**-109.7 ± 13.86**	**11.85 ± 1.19**	**-23.55 ± 0.86**	**7.07 ± 3.66**		**259**
2011	5	-109.7 ± 13.86	11.85 ± 1.19	-23.55 ± 0.86	7.07 ± 3.66		259
Whinchat ***Saxicola rubetra***	**9**	**-95.5 ± 13.02**	**12.82 ± 1.3**	**-24.08 ± 0.59**	**7.26 ± 3.61**	**5.08 ± 1.91**	**247**
2010	4	-84.54 ± 4.17	13.42 ± 0.19	-24.28 ± 0.15	8.35 ± 1.59	3.73	242
2011	4	-103.67 ± 11.97	12.28 ± 1.89	-23.83 ± 0.87	5.66 ± 5.08		254
2012	1	-106.7	12.6	-24.3	9.27	6.43	236
Song Thrush ***Turdus philomelos***	**26**	**-85.52 ± 10.63**	**11 ± 1.55**	**-23.46 ± 0.6**	**5.35 ± 2.72**	**3.77 ± 1.6**	**272**
2011	11	-89.75 ± 8.45	11.33 ± 1.78	-23.19 ± 0.66	5.35 ± 3.46	3.11 ± 1.61	260
2012	15	-82.43 ± 11.25	10.76 ± 1.37	-23.65 ± 0.48	5.34 ± 2.15	4.25 ± 1.46	282
Redwing ***Turdus iliacus***	**3**	**-100.65 ± 6.14**	**10.64 ± 1.34**	**-23.19 ± 0.6**	**5.77 ± 1.81**		**296**
2011	3	-100.65 ± 6.14	10.64 ± 1.34	-23.19 ± 0.6	5.77 ± 1.81		296
Mistle Thrush ***Turdus viscivorus***	**4**	**-93.52 ± 11.08**	**11.1 ± 1.37**	**-22.99 ± 0.91**	**5.68 ± 2.64**		**284**
2011	4	-93.52 ± 11.08	11.1 ± 1.37	-22.99 ± 0.91	5.68 ± 2.64		284
Blackbird ***Turdus merula***	**4**	**-96.55 ± 9.68**	**10.52 ± 1.47**	**-23.69 ± 0.62**	**3.73 ± 1.61**		**287**
2011	4	-96.55 ± 9.68	10.52 ± 1.47	-23.69 ± 0.62	3.73 ± 1.61		287
Ring Ouzel ***Turdus torquatus***	**4**	**-92.74 ± 5.38**	**8.83 ± 0.7**	**-21.93 ± 0.43**	**5.16 ± 0.79**	**4.88 ± 1.71**	**262**
2011	4	-92.74 ± 5.38	8.83 ± 0.7	-21.93 ± 0.43	5.16 ± 0.79	4.88 ± 1.71	262

	n	δ^{2}H	δ^{18}O	δ^{13}C	δ^{15}N	δ^{34}S	Day
Garden Warbler ***Sylvia borin***	**26**	**-103.86 ± 10.68**	**13.89 ± 2.38**	**-24.56 ± 0.81**	**6.09 ± 1.92**	**4 ± 2.21**	**252**
2010	5	-97.93 ± 4.37	15.11 ± 1.16	-24.7 ± 0.69	5.68 ± 2.48	4.48 ± 2.05	243
2011	13	-104.7 ± 13.19	14.11 ± 2.9	-24.53 ± 0.98	6.25 ± 2.01	3.63 ± 2.85	255
2012	5	-106.77 ± 9.11	12.41 ± 1.11	-24.76 ± 0.54	5.72 ± 1.94	4.18 ± 1.13	256
2013	3	-105.27 ± 7.81	13.33 ± 2.27	-24.11 ± 0.65	6.67 ± 0.73	4.68 ± 0.57	247
Blackcap ***Sylvia atricapilla***	**24**	**-91.85 ± 15.09**	**13.77 ± 2.45**	**-23.79 ± 0.78**	**4.67 ± 2.71**	**3.89 ± 1.63**	**258**
2010	2	-105.22 ± 5.8	11.45 ± 1.2	-23.95 ± 0.21	5.45 ± 4.6	0.93 ± 3.1	258
2011	8	-100.7 ± 14.13	13.06 ± 3.06	-23.93 ± 0.72	4.85 ± 2.9	3.23 ± 1.13	261
2012	8	-87.45 ± 13.81	14.5 ± 2.66	-23.76 ± 1.17	4.94 ± 2.28	4.93 ± 1.05	261
2013	6	-81.47 ± 11.69	14.42 ± 0.87	-23.59 ± 0.19	3.8 ± 3.03	4.37 ± 0.91	250
Whitethroat ***Sylvia communis***	**4**	**-98.68 ± 3.91**	**13.78 ± 0.61**	**-24.45 ± 0.59**	**7.78 ± 2.03**	**3.87**	**249**
2010	1	-99.12	13.72	-24.6	10.5	3.87	237
2011	3	-98.54 ± 4.78	13.79 ± 0.74	-24.4 ± 0.72	6.87 ± 1.1		253
Lesser Whitethroat ***Sylvia curruca***	**4**	**-99.41 ± 12.58**	**12.96 ± 2.48**	**-22.88 ± 1.23**	**5.68 ± 1.7**	**5.31**	**247**
2010	3	-94.76 ± 10.36	13.42 ± 2.83	-22.27 ± 0.25	5.4 ± 1.97	5.31	245
2011	1	-113.37	11.58	-24.7	6.5		251
Reed Warbler ***Acroceph. scirpaceus***	**3**	**-108.64 ± 24.58**	**13.01 ± 1.05**	**-24.57 ± 0.4**	**10.63 ± 1.18**	**5.22 ± 0.08**	**242**
2010	1	-85.35	13.75	-25	10	5.16	242
2011	2	-120.29 ± 19.86	12.26 ±	-24.35 ± 0.21	10.95 ± 1.48	5.28	242
Melodious Warbler ***Hippolais icterina***	**1**	**-88.28**	**13.03**	**-23.6**	**7.4**	**6.97**	**246**
2010	1	-88.28	13.03	-23.6	7.4	6.97	246
Willow Warbler ***Phylloscopus trochilus***	**49**	**-104.83 ± 11.5**	**13.4 ± 2.32**	**-23.97 ± 0.87**	**4.56**	**6 ± 1.89**	**250**
2010	14	-101.01 ± 14.9	14.2 ± 2.35	-23.71 ± 1.1	4.96 ± 1.96		247
2011	35	-106.44 ± 9.53	13.01 ± 2.24	-24.08 ± 0.76	4.4 ± 2.93	6.08 ± 1.86	252
Bonelli's Warbler ***Phylloscopus bonelli***	**1**	**-116.55**	**11.91**	**-23.4**	**8.1**		**244**
2011	1	-116.55	11.91	-23.4	8.1		244
Common Chiffchaff ***Phylloscopus collybita***	**23**	**-85.02 ± 12.8**	**13.86 ± 1.45**	**-23.05 ± 0.78**	**6.24 ± 3.05**	**5.08 ± 4.03**	**276**
2010	2	-91.64 ± 23.14	13 ± 2.33	-22.7 ± 0.99	5.45 ± 2.62	5.68	283
2011	11	-88.4 ± 9.65	14.48 ± 1.27	-22.98 ± 0.84	4.72 ± 3.09	3.14 ± 3.35	269
2012	10	-79.97 ± 13.62	13.41 ± 1.38	-23.2 ± 0.72	8.08 ± 2.15	6.95 ± 4.13	283

	n	δ^{2}H	δ^{18}O	δ^{13}C	δ^{15}N	δ^{34}S	Day
Goldcrest ***Regulus regulus***	**26**	**-85.06 ± 13.49**	**14.34 ± 1.35**	**-23.65 ± 0.63**	**1.79 ± 2.27**	**3.97 ± 0.89**	**268**
2010	2	-94.19 ± 15.3	15.98 ± 1.44	-24.75 ± 0.92	4.45 ± 3.46	3.24	289
2011	14	-83.77 ± 13.22	13.75 ± 1.21	-23.34 ± 0.55	0.55 ± 1.76	3.76 ± 1.34	251
2012	10	-85.04 ± 14.36	14.6 ± 1.23	-23.76 ± 0.38	2.86 ± 1.81	4.08 ± 0.88	287
Firecrest ***Regulus ignicapilla***	**6**	**-85.68 ± 10.52**	**14.36 ± 1.55**	**-20.97 ± 4.55**	**1.03 ± 1.63**	**3.3**	**243**
2011	6	-85.68 ± 10.52	14.36 ± 1.55	-20.97 ± 4.55	1.03 ± 1.63	3.3	243
Wren ***Troglod. troglodytes***	**8**	**-77.41 ± 9.81**	**12.56 ± 1.79**	**-23.51 ± 0.81**	**3.53 ± 2.12**	**4.58 ± 0.94**	**280**
2010	5	-76.73 ± 10.21	13.07 ± 1.68	-23.62 ± 0.43	3.1 ± 2.05	4.58 ± 0.94	283
2011	3	-79.11 ± 12.36	11.28 ± 1.85	-23.31 ± 1.36	4.25 ± 2.46		273
Spotted Flycatcher ***Muscicapa striata***	**2**	**-88.52 ± 21.18**	**13.52 ± 1.25**	**-23.09 ± 1.11**	**6.96 ± 5.18**	**3.63**	**247**
2010	1	-73.54	14.4	-22.3	3.3	3.63	237
2011	1	-103.5	12.63	-23.87	10.62		256
Pied Flycatcher ***Ficedula hypoleuca***	**96**	**-103.85 ± 11.97**	**12.72 ± 2.12**	**-23.55 ± 1.24**	**6.19 ± 2.05**	**5.79 ± 1.45**	**250**
2010	27	-100.58 ± 13.18	12.77 ± 1.78	-23.95 ± 0.93	6.79 ± 1.52	5.99 ± 1.41	252
2011	69	-105.21 ± 11.26	12.7 ± 2.27	-23.39 ± 1.31	5.96 ± 2.18	5.7 ± 1.46	249
Great Tit ***Parus major***	**1**	**-68.55**	**19.14**	**-24.3**	**1.3**	**3.88**	**288**
2010	1	-68.55	19.14	-24.3	1.3	3.88	288
Coal Tit ***Periparus ater***	**28**	**-81.28 ± 8.21**	**13.96 ± 1.3**	**-23.03 ± 0.62**	**1.21 ± 1.7**	**4.63 ± 0.73**	**275**
2010	20	-81.26 ± 9.14	13.82 ± 1.32	-23.01 ± 0.58	1.76 ± 1.53	4.54 ± 0.79	283
2011	8	-81.35 ± 5.77	14.31 ± 1.23	-23.11 ± 0.76	-0.16 ± 1.36	4.93 ± 0.46	255
Crested Tit ***Lophophanes cristatus***	**2**	**-91.97 ± 4.99**	**19.08 ± 0.19**	**-22.9 ± 0.22**	**1.77 ± 1.16**		**285**
2011	2	-91.97 ± 4.99	19.08 ± 0.19	-22.9 ± 0.22	1.77 ± 1.16		285
Eurasian Nuthatch ***Sitta europaea***	**1**	**-83.52**	**17.63**	**-22.61**	**1.73**		**256**
2011	1	-83.52	17.63	-22.61	1.73		256
Common Tree-kreeper ***Certhia familiaris***	**3**	**-77.2 ± 2.91**	**13.4 ± 0.22**	**-22.58 ± 0.12**	**1.75 ± 2.16**	**5.35**	**268**
2011	3	-77.2 ± 2.91	13.4 ± 0.22	-22.58 ± 0.12	1.75 ± 2.16	5.35	268
Red-Backed Shrike ***Lanius collurio***	**4**	**-77.13 ± 2.95**	**14.32 ± 1.79**	**-22.96 ± 1.01**	**7.16 ± 2.03**	**11.46**	**246**
2011	4	-77.13 ± 2.95	14.32 ± 1.79	-22.96 ± 1.01	7.16 ± 2.03	11.46	246

	n	δ^{2}H	δ^{18}O	δ^{13}C	δ^{15}N	δ^{34}S	Day
Common Chaffinch ***Fringilla coelebs***	**37**	**-102.29 ± 10.32**	**14.04 ± 1.53**	**-24.43 ± 0.95**	**3.26 ± 2.43**	**4.11 ± 1.32**	**287**
2010	17	-99.64 ± 9.74	13.97 ± 1.66	-24.37 ± 0.9	3.57 ± 2.36	5.09 ± 1.23	292
2011	20	-104.54 ± 10.51	14.1 ± 1.45	-24.48 ± 1.02	2.99 ± 2.51	3.75 ± 1.19	283
Brambling ***Fring. montifringilla***	**22**	**-112.29 ± 8.47**	**11.97 ± 1.72**	**-24.66 ± 0.85**	**3.95 ± 1.37**	**6.29 ± 2.52**	**290**
2010	5	-120.37 ± 4.63	11.97 ± 1.09	-24.78 ± 0.54	4.56 ± 0.61	6.57 ± 2.5	294
2011	4	-114.88 ± 7.59	13.27 ± 1.08	-25.05 ± 0.3	4.17 ± 1.77	6.56 ± 0.44	297
2012	13	-108.38 ± 7.62	11.56 ± 1.93	-24.49 ± 1.04	3.64 ± 1.45	6.13 ± 2.98	287
Linnet ***Carduelis flammea***	**6**	**-113.27 ± 7.76**	**18.2 ± 1.3**	**-23.03 ± 1.52**	**4.57 ± 3.38**	**2.66**	**284**
2010	1	-107.23	15.55	-22.4	5.2	2.66	288
2011	5	-114.47 ± 8.02	18.73 ± 0.11	-23.16 ± 1.67	4.44 ± 3.77		283
Eurasian Siskin ***Carduelis spinus***	**38**	**-124.79 ± 11.15**	**11.77 ± 0.98**	**-21.84 ± 1.35**	**1.93 ± 1.84**	**4.6 ± 0.74**	**287**
2010	32	-124.22 ± 11.64	11.8 ± 1.04	-21.91 ± 1.44	2.1 ± 1.77		287
2011	6	-127.84 ± 8.21	11.59 ± 0.56	-21.46 ± 0.7	0.99 ± 2.06	4.6 ± 0.74	286
Serin ***Serinus serinus***	**2**	**-118.06 ± 5.4**	**16 ± 3.94**	**-23.34 ± 0.75**	**6.73 ± 1.49**		**299**
2011	2	-118.06 ± 5.4	16 ± 3.94	-23.34 ± 0.75	6.73 ± 1.49		299
Bullfinch ***Pyrrhula pyrrhula***	**4**	**-102.72 ± 11.21**	**13.51 ± 1.3**	**-23.78 ± 0.41**	**0.13 ± 3.63**	**4.42**	**271**
2011	4	-102.72 ± 11.21	13.51 ± 1.3	-23.78 ± 0.41	0.13 ± 3.63	4.42	271
Hawfinch ***Coccot. coccothraustes***	**15**	**-87.42 ± 9.87**	**16.48 ± 1.38**	**-23.42 ± 0.85**	**2.4 ± 1.91**	**3.43 ± 1.28**	**290**
2011	11	-89.01 ± 9.07	16.34 ± 1.16	-23.46 ± 0.67	2.37 ± 2.23	3.36 ± 1.26	294
2012	4	-83.08 ± 12.08	16.85 ± 2.05	-23.31 ± 1.38	2.5 ± 0.68	3.6 ± 1.51	282
Common Crossbill ***Loxia curvirostra***	**41**	**-110.44 ± 19.64**	**11.48 ± 0.7**	**-19.81 ± 1.7**	**0.41 ± 1.68**	**5.25 ± 1.5**	**273**
2010	9	-108.41 ± 18.26	11.89 ± 0.46	-19.2 ± 0.35	0.72 ± 1.79	5.99 ± 1.52	285
2011	2	-118.02 ± 10.96	12.39 ± 0.16	-20.49 ± 0.41	-2.71 ± 0.27	6.25 ± 0.49	274
2012	30	-110.55 ± 20.76	11.3 ± 0.69	-19.95 ± 1.94	0.52 ± 1.51	4.99 ± 1.47	270
Hortulan Bunting ***Emberiza hortulana***	**6**	**-94.53 ± 11.09**	**13.75 ± 1.3**	**-24.12 ± 0.67**	**8.99 ± 0.8**	**4.62 ± 1.54**	**245**
2010	2	-88.13 ± 0.11	14.5 ± 0.04	-24.45 ± 0.21	9.45 ± 0.21	2.49	246
2011	4	-107.34	12.25	-23.96 ± 0.8	8.76 ± 0.92	5.33 ± 0.71	245
Rock Bunting ***Emberiza cia***	**1**		**13.06**	**-22.92**	**2.27**		**286**
2011	1		13.06	-22.92	2.27		286