Assessing migration and moulting strategy in closely related taxa based on stable isotope analysis: a population study of Balearic and Yelkouan shearwaters across their breeding range

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ABSTRACT

Animal migrations are unique phenomena involving mass movements of individuals, which pose significant challenges to develop conservation strategies. Migratory seabirds, particularly, face many anthropogenic threats across their distributions, and populations are declining worldwide. We provided a thorough isotopic method to characterise individual migratory patterns and identify main moulting areas of two closely-related *Puffinus* shearwaters breeding in the Mediterranean that produces minimal intrusion on birds. Using tracking data of 64 birds and the stable isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of one wing feather, we built a robust discriminant for rapid geographic assignment of post-breeding grounds, among Atlantic, Mediterranean and Black Sea basins. Afterwards, we applied the method to several wing feathers of non-tracked individuals and populations and assessed their most plausible post-breeding grounds. First, most tracked Balearic shearwaters from Ibiza and 7% from Minorca were moult-ascribed to the Atlantic; the remaining Balearic and all Yelkouan shearwaters displayed what resembled a longitudinal chain-migration, with an easternmost gradual proportion of individuals migrating towards the Black Sea. Second, the isotopic discriminant achieved high accuracy of correct classification (91.0%) with only six non-assigned individuals. Third, >80% of birds moulted all their primary feathers in a single location. Finally, we provided examples assigning moulting areas of non-tracked individuals (e.g., bycaught birds) and entire populations based on a single sampling. This isotopic approach and the examples we provided offer new insights into the study of seabird migration with minimal disturbance, requiring a single capture. The methodology offers valuable tools for studying endangered seabirds and supports conservation efforts by identifying key moulting areas and migration strategies.

Keywords: Balearic shearwater, biologging, discriminant analysis, isotopic assignation, oceanic migration, Yelkouan shearwater.

1. INTRODUCTION

Climate change, habitat alteration, invasive alien species and pollution are modifying landscapes and creating new conservation challenges, especially for migratory species due to their high mobility and reliance on multiple habitats to complete their life history (Lennox et al., 2016). Indeed, the conservation status of many of these species is rapidly deteriorating (Runge et al., 2014), highlighting the urgent need to understand the spatial overlap between potential threats and their distribution throughout the annual cycle. Uncovering migratory patterns and understanding the linkages between areas (i.e., migratory connectivity) of populations or closely-related species is crucial to more directly implement effective conservation measures (Lascelles et al., 2014; Schuster et al., 2019), as there may exist migratory divisions within the species that result in divergent routes and different threats.

Studies of seabird migrations have increased over the last decades with the improvements of tracking methods (López-López, 2016; Davies et al., 2021). Technological advances in tracking devices, such as Global Locators Sensors (GLS) have led to a remarkable expansion in studying the migratory behaviour of many seabird species, since they are durable, lightweight, capable of recording a wealth of data and relatively benign method (Carey, 2011). However, the study of the spatial and migratory ecology of these species through GLS devices has several drawbacks, such as devices must be retrieved from birds to download data and therefore are only useful for easily-recaptured and high site-fidelity species. Alternatively, the analysis of biogeochemical markers, such as the Stable Isotope Analysis (SIA), can complement the insights provided by biologging devices, as only one capture and tissue sampling is needed to assess the geographic location where the animal has generate the tissue (Ramos & González-Solís, 2012). For instance, the isotopic composition of animal tissues typically reflects the isotopic elementary source of every geographic area and the processes that produce every specific tissue. Feathers, for instance, maintain the isotopic signal of resources used during their moult, i.e., where and when they regrow (Pérez et al., 2008). Thus, tracking devices and biogeochemical analysis are often combined in current animal migration studies (e.g., Péron et al., 2013; Austin et al., 2019).

The present study provides a thorough isotopic method, based on $\delta^{13}C$ and $\delta^{15}N$ of feathers, to characterize individual migratory patterns of two *Puffinus* shearwaters endemic as breeders to the Mediterranean basin, the Balearic *(P. mauretanicus)* and the Yelkouan shearwaters *(P. yelkouan)*. In particular, we verified wing moult (i.e., primary feather replacement) of these species, described the isotopic variation of the moulted feathers in their main three post-breeding areas (Atlantic, Mediterranean, and Black Sea; Militão et al., 2013) for seven breeding populations, and developed a robust isotopic assignation method of post-breeding areas for unmonitored and untracked individuals. Finally, we provided four different examples (with data and results) on the applicability of the method and discussed its reliability in ecological and conservation contexts.

2. METHODS

2.1 Study species

In the Mediterranean, there are two recognised endemic species of shearwaters of the genus *Puffinus*: the Balearic shearwater (Lowe, 1921) and the Yelkouan or Mediterranean shearwater (Acerbi, 1827). Recent genetic evidences suggested the possibility of re-considering both taxa as subspecies of a pan-Mediterranean species which might include certain differentiation among populations (Ferrer Obiol et al., 2023), although morphological and behavioural evidence still support the recognition of two species (Altaba, 1993; Curé et al., 2010; Gil-Velasco et al., 2015) with an area of contact (i.e. a population showing signs of hybridization) in Minorca (NE Balearic Islands, Spain) (Genovart et al., 2012; Austin et al., 2019). Balearic shearwater breeds in several islands and islets of the Balearic Archipelago, and most individuals move to the Atlantic Ocean during the post-breeding season (Guilford et al., 2012), with the exception of Minorcan birds, that largely remain in the western Mediterranean (Austin et al., 2019). On the contrary, Yelkouan shearwater occupies areas spread across most of the Mediterranean for breeding, and the central and eastern Mediterranean and the Black Sea for post-breeding (Raine et al., 2013).

The Balearic shearwater is listed as Critically endangered species by the International Union for Conservation of Nature (IUCN; BirdLife International, 2024) while the Yelkouan shearwater is listed as Vulnerable species, in both cases, due to their declining populations and susceptibility to both marine and terrestrial threats. Fishery bycatch is the main threat identified for both species at sea in the Mediterranean, as it poses a major source of mortality with a high influence on their demography due to reductions in adult survival (Arcos, 2011; Oppel et al., 2011; Oliveira et al., 2015; Genovart et al., 2016; Cortés et al., 2017; Ramírez et al., 2024). On land, predation of eggs, chicks and even adults by introduced mammals, such as mice, rats and feral cats, are also relevant demographic constrains in the breeding colonies (Arcos, 2011; Gaudard, 2018). This had already ended with the extinction of, at least, eleven breeding colonies of Yelkouan shearwater over the last decades (Bourgeois & Vidal, 2008) whereas at least one colony of Balearic shearwater has also disappeared (Ruiz & Martí, 2004).

2.2 Moult phenology

As most Procellariiformes do (Ramos et al., 2009), Balearic shearwaters start moulting primary feathers early in the post-breeding period following a simple descending order from the innermost primary (P1) to the outermost primary feather (P10; Meier et al., 2017). Most of the moult of the Balearic shearwater occurs between June and October, while that of the Yelkouan shearwater could start one month later, i.e., in July (Militão et al., 2013; Bourgeois & Dromzée, 2014). Pictures taken at sea during the post-breeding period (collected in Figure S1) further supported these results for both species.

2.3 Sampling strategy

Main sampling sites of the study included ten breeding colonies across the Mediterranean Sea (Figure 1; Table S1): Es Bosc and Sa Conillera (Ibiza, Spain), La Mola de Maó (Minorca, Spain), Port-Cros and Porquerolles (France), Tavolara (Sardinia, Italy), Malta (Malta), Zaklopatica (Croatia), and Lipsi and Gyaros (Greece). At each site and across two study periods (2007-2013 and 2021-2022), breeding birds were captured and deployed with GLS (models MK19, BioTrack© and C65-COOL, Migrate Technology©). GLS weigh of about 2g and were fitted on the tarsus of the animals mounted on a Darvic ring (Pérez-Roda et al., 2017; Grissot et al., 2023). At the time of retrieving the devices, in subsequent breeding seasons, we also sampled one P1 feather of all tracked individuals, or, alternatively, one P3 feather if primary moulting eventually started. As mentioned above, P1-P3 of the breeding adults start moulting when they reach their post-breeding areas primarily, thus, we assumed these areas serve as main moulting areas for both species.

We complemented the main sampling of old feathers from already-tracked birds, with four examples where the isotopic methods could be applied. First, we sampled small pieces of five primary feathers across the moult sequence, i.e., P1, P3, P5, P7, and P9, of 26 untracked individuals of up to four breeding colonies: Minorca (n=8), Sardinia (n=8), and Greece (n=5), and from another colony of the Central Mediterranean without any tracking information (Zembretta Island, Tunisia; n=5). These samplings should allow us clarifying the moulting sequence and areas for each of the populations. Second, we also sampled feathers from untracked individuals from the main study colonies to ascertain the potential winter locations of those individuals and populations. Finally, we collected P1-P3 feathers from bycaught birds off the Catalan coast (NE Spain), both Balearic (n=110) and Yelkouan (n=77) shearwaters, and with unknown breeding origins to infer their moulting and post-breeding grounds, and thus, the potential breeding origins of those birds.

2.4 GLS and tracking data

GLS devices provide light data, among others, that can be converted into geographic locations. The process consists of threshold levels of solar irradiance, arbitrarily chosen to identify the timing of sunrise and sunset (Lisovski et al., 2020) which allows generating a single location based on the estimated times of two successive twilights events. By considering the alternating sequence of sunrise/sunset and sunset/sunrise pairs, two locations can be estimated every 24h. Concurrent deployment of these loggers along with satellite transmitters during breeding has indicated a mean accuracy of 145-185 km in seabirds, which is reasonable for tracking migration ranges of pelagic species (Merkel et al., 2016; Halpin et al., 2021). Given the inherent error of the GLS data and the maximum foraging range of *Puffinus* shearwaters (Péron et al., 2013; Meier et al., 2015), we modelled a Kernel Density Estimation (KDE) with a smoothing factor at 50% for August positions of all tracked individuals and populations to define main moulting areas of the first primary feathers, i.e., P1 to P3 feathers. Individual and population 50% KDE and their respective centroids were calculated using the `kernelUD()` from package `adehabitatHR` in R (Worton, 1989; Calenge & Fortmann-Roe, 2024).

2.5 Laboratory procedures and stable isotope analyses

Feathers and pieces of the feather analysed in Spain were all washed in a 0.25M solution of NaOH, rinsed with distilled water to eliminate any possible residues that may interfere the analysis, and dried at 60 ºC for 48 h in an oven to extract all moisture. Then, the feather was cut into smaller pieces and 0.30 mg of every sample (± 0.05 mg) was enclosed and crimped in tin (Sn) capsules for combustion (Chew et al., 2019). Every sample is combusted by means of elemental analysis-isotope ratio mass spectrometry using a ThermoFisher Scientific elemental analyser Flash IRMS coupled to a Delta-V Advantatge isotope ratio mass spectrometer via a CONFLO IV interface at the *Serveis Científico-Tècnics* of the *Universitat de Barcelona* (SCT-UB; Spain). On the other hand, feathers analysed in France were rinsed in a 2:1 chloroform:methanol solution, rinsed twice in the solution, dried for 48 h at 60°C and homogenized with scissors. Analyses were performed at the Institut Littoral Environnement et Sociétés (LIENSs, La Rochelle, France) on ca. 0,5 mg subsamples of material loaded into tin cups, using an elemental analyser (Thermo Fisher, Flash EA 1112) coupled in continuous flow mode to an isotope ratio mass spectrometer (Thermo Fisher, Delta V Advantage, Bremen, Germany). Stable isotope ratios in both cases were expressed in the standard δ-notation (‰) relative to Vienna Pee Dee Belemnite (VPDB; δ^{13}C), and atmospheric N2 (AIR; δ^{15}N). The isotopic ratio mass spectrometry facility at the SCT-UB applies international inorganic standards (IAEA CH7, IAEA CH6 and USGS-24 for C, and IAEA N1, IAEA N2 and IAEA NO3 for N) inserted every 12 samples to calibrate the system and compensate for any drift over time (Salazar-García, 2011). Replicate assays of standard materials indicated measurement errors of ± 0.2‰, for both C and N, but these are likely underestimates of true measurement error for complex organic compounds like feathers.

2.6 Statistical analyses

First, a total of 64 individuals from 10 colonies were sampled for the creation of the discriminant and the previous analyses (see Table S1). Using stable isotope ratios of feathers from tracked individuals, we evaluated potential differences in δ^{13}C and δ^{15}N between the two study periods (i.e., 2007-2013 and 2021-2022). To do so, we used `glm()` function from the `Stats` package for Generalised Linear Models (GLM) and selected the most parsimonious models based on Akaike's Information Criterion for small sample sizes (AIC_c) with `dredge()` and `model.avg()` functions from the `MuMIn` package in R.

Second, we built a discriminant function based on the stable isotope ratios of feathers of GLS-tracked birds assigned to one specific moulting area (among Atlantic Ocean, Mediterranean basin and Black Sea). As the isotopic data did not comply with normal distribution, we conducted a Quadratic Discriminant Analysis (QDA) that justifies very unequal sizes categories and performs better on non-Gaussian data (Qin, 2018). In brief, the method estimates the probability that an observation, given specific isotopic values as of the predictors, belongs to each of the categories of potential moulting areas (Sohil et al., 2022). QDA was conducted using the `qda()` function from `Caret` package in R. Finally, we determined the correct assignation rate using a cross-validation method and set a threshold of reliable assignment probability to >80% (Rocque et al., 2006).

Finally, we applied the discriminant function to more than 400 feathers from non-tracked individuals, including here primary feathers of moult sequence from four breeding populations (n= 26 individuals), P1 or P3 from individuals of various localities (n=79), and bycaught animals with an unknown origin (n=187). All spatial analyses, exploratory models and QDA were performed in R version 4.2.0.

3. RESULTS

3.1 Moulting and post-breeding areas of Balearic and Yelkouan shearwaters

Dated images we collected (Figure S1) evidenced that individuals of both species retain old primary feathers until mid-June and start the primary feather replacement (i.e., from P1-P3 onwards) primarily somewhen between July and August, when most birds are already in their main post-breeding grounds. Outermost primary feathers, i.e., P7-P10, are likely to be replaced along September, also in the post-breeding grounds of the species (Mouriño et al., 2003; Raine et al., 2013; Meier et al., 2017).

GLS tracking data identified the main moulting areas of the studied populations and defined the most probable non-breeding grounds for every tracked individual (Figure 1 & Table 1). Most individuals from Ibiza wintered in the Atlantic Ocean (90.9%, Table 1), while the four individuals from Minorca remained in the Western Mediterranean, around the breeding area. The individuals from the French population mostly remained in the western-central Mediterranean for post-breeding period, and only 7.7% of them migrated towards the Black Sea. Sardinian and Croatian birds distributed similarly between the Mediterranean Sea (specifically Adriatic and Aegean seas, respectively) and the Black Sea. Most birds from Malta spent the post-breeding period in the Black Sea (83.3%), and finally, all GLS-tracked individuals from Greece spent the post-breeding period in the Black Sea (Table 1).

According to these results and considering the isotopic essence of most marine basins across the distribution of both species (Figure 1), we defined three most regions that are characterized by their isotopic resemblance and consistency: Atlantic waters, Mediterranean basin or Black Sea.

3.2 Effect of the study period and moulting area on stable isotope ratios of feathers

We found larger and more relevant differences among moulting areas than between periods in both δ^{13}C and δ^{15}N modelling (Table S2 & Figure S2). Models only including the moulting area factor always showed the lowest AIC$_c$ values, although they cannot be preferred above others that considered the study period as factors, because ΔAIC$_c$ between models did not go over 2.0 units (Table S2). However, even when carrying out model averaging, the estimates of the study period factor were always much smaller in magnitude and contribution than those of the moulting area factor (Table S3). Thus, we omitted the study period effect while performing the discriminant analysis and only considered the moulting area as a relevant factor influencing the stable isotope ratios of the feathers of both species of shearwaters.

Each isotopic value belonging to specific area allowed us to ascribe the feathers in Atlantic if the isotopic signal is -16.18 $\delta^{13}C$ ± 0.55 and 15.07 $\delta^{15}N$ ± 0.67, Mediterranean whether it is -17.21 $\delta^{13}C$ ± 0.50 and 11.84 $\delta^{15}N$ ± 1.60 or in Black Sea if it is -19.42 $\delta^{13}C$ ± 0.60 and 12.80 $\delta^{15}N$ ± 2.27 (Figure 2). Separating the moulting areas was possible thanks to the isotopic difference between them. The higher $\delta^{13}C$ in the Atlantic and Mediterranean than in the Black Sea expose a gradient. Due to the influence of Atlantic waters entering the Mediterranean the sea has a higher concentration of carbon than Black Sea. And in contrast $\delta^{15}N$ have higher values on Atlantic than the other two moulting areas, highlighting the possible difference in trophic levels.

3.3 Discriminant analysis and assignment method to non-tracked birds

First, and importantly, the cross-validation method of the isotopic discriminant analysis to ascertain potential post-breeding areas showed a 91.0% of correct classifications (Figure 2). Likewise, the correct classification for the test matrix of each group was 75.0% for Atlantic and 100% for both Mediterranean and Black Seas.

Second, the classification of the non-tracked individuals from breeding colonies using their first primary feathers revealed that (1) most of Balearic shearwaters from Ibiza moulted these feathers in the Atlantic (83.4%), (2) some Yelkouan shearwater individuals from the Balearic-Yelkouan hybrid colony of Minorca (6.6%) moulted them in the Atlantic, (3) about 78.4% of the Yelkouan shearwaters from France and the 50.0% from Sardinia moulted the feathers, apparently, in the Mediterranean, while the remaining, i.e., 13.5% and 50.0%, respectively, did that in the Black Sea. Unfortunately, (4) we could not classify 16.7% of the feathers of birds from Minorca and 8.1% from France due to the assignation below the threshold.

Third, the assignation of moulting areas along the sequence of primary feathers, i.e., P1, P3, P5, P7, P9 from some individuals, unravelled that some of them (19.2%) may change areas along the moulting period as feathers were ascribed to different moulting areas (e.g., see two individuals from Minorca in Figure 3). As a whole, 67.5% of these sequenced feathers of birds from Minorca were assigned to the Mediterranean, 27.5% to the Atlantic, and 5.0% non-assigned. For the feathers of individuals from Sardinia, we assigned 75.0% of them to the Mediterranean, 22.5% to the Black Sea, and 2.5% went below the threshold. Similarly, 72.0% of feathers from Greece were assigned to the Black Sea, 16.0% to the Mediterranean, and 12.0% were non-assigned. Lastly, 56.0% of the feathers of birds from Tunisia were assigned to the Mediterranean, 36.0% to the Black Sea, and only 8.0% of them were non-assigned.

Finally, we assigned the moulting areas of the majority of Balearic shearwaters bycaught along the Catalan coast to the Atlantic (70.9%), while the bycaught Yelkouan shearwaters were more prone to use Mediterranean (67.5%), and the Black Sea (29.9%) for moulting. As before, 7.5% of the feathers from bycaught animals were non-assigned to any group due to the threshold settled.

4. DISCUSSION

The present metapopulation study confirms that Balearic and Yelkouan shearwaters moult and spend the post-breeding period in discrete areas in the Atlantic Ocean, the Mediterranean, and the Black Sea. Interestingly, these areas have distinct isotopic natures (Militão et al., 2014) that, with the support of tracking data, have allowed us to build a robust assignment method for determining the post-breeding areas of the birds merely sampling and analysing small pieces of their feathers.

First, the tracking data showed that populations of both taxa display differentiated patterns of migration: most individuals of Balearic shearwater migrated towards the Atlantic Ocean, while those of Yelkouan shearwater spread along the Mediterranean and the Black Sea to spend the post-breeding period (Austin et al., 2019). Therefore, we considered the Atlantic Ocean, the Mediterranean and the Black Sea as the main oceanic basins with discrete biogeochemical natures (Nieblas et al., 2014) where Balearic and Yelkouan shearwaters eventually replace their wing feathers during the post-breeding period. Interestingly, while westernmost populations of Yelkouan shearwater tended to stay in the Mediterranean during the post-breeding period, including the Balearic-Yelkouan hybrid population of Minorca, a gradual proportion of individuals of populations of Yelkouan shearwater seemed to migrate longitudinally towards the Black Sea, in a chain-migration resemblance (e.g., Fort et al., 2012). These migration patterns in Balearic and Yelkouan shearwaters could reflect the differential population sizes across their longitudinal breeding distribution and, possibly, the higher productivity of the Atlantic and Black Sea during the non-breeding season of the species i.e., from July to October (Demidov, 2008; Huguet et al., 2024).

Second, although stable isotopic values of feathers differed between the periods they were sampled (i.e., 2007-13 and 2021-2022), much larger isotopic differences appeared when comparing them among the aforementioned moulting areas (i.e., Atlantic, Mediterranean, and Black Sea). The slight differences between the two considered periods may arise from the small sample size of the present study, the larger variability on the stable isotope ratios associated to specific areas, or, likely, a combination of both factors (Gómez-Díaz & González-Solís, 2007) as well as, the variation in baseline isotopic values (Militão et al., 2014). Therefore, given the magnitude and contribution of the period differences, compared to those provided by the three moulting areas described above, we omitted the potential temporal variability and focussed the study solely on the spatial variability of SIA.

Third, we reported a clear longitudinal gradient in feather values of $\delta^{13}C$ along the three oceanic basins. The highest values were always found in feathers moulted in the Atlantic, while the lowest ones were those from Black Sea, and showing the Mediterranean the mid-term $\delta^{13}C$ values. The relatively small size and close nature of the Black Sea compared to the larger size of the Mediterranean and the vast extension of the Atlantic Ocean could mediate in this gradient, as non-renewal or mixing of water can lead to bicarbonate concentration gradients, which, in turn, leads to differences in carbon availability (Pérez et al., 2008). Additionally, as Black Sea has mainly influence of fluvial discharges, the isolation from the flushing effects of the open ocean, as the Mediterranean Sea has from the Atlantic Ocean (Wells et al., 2021), and long residence time of water masses, have made the Black Sea particularly susceptible to increased production of organic matter with the consequent poor input of inorganic carbon (Bănaru et al., 2007). This phenomenon, combined with factors such as increased primary productivity and degradation processes (Graham et al., 2010), can reflect the local configuration of the Black Sea. Besides, spatial patterns of $\delta^{15}N$ showed a distinct pattern with higher values in the Atlantic than in the Mediterranean and the Black Sea (McMahon et al., 2013). This $\delta^{15}N$ variability in the tissues of shearwaters may be attributed to either differential baseline rates of oceanic basins or differences in food chains and trophic levels (Adams & Sterner, 2000) where shearwaters feed on. N^2 fixation and nitrogen recycling can cause a decrease in the stable isotope composition which occurs in the oligotrophic Mediterranean (Pantoja et al., 2002). Black Sea also has a lower $\delta^{15}N$ when the river flow reduces in autumn (Bănaru et al., 2007). Therefore, the differentiated gradients in isotopic values enable the delineation of ranges for discrete geographic regions. This approach allows for the identification of discernible patterns in these three water masses.

Fourth, the three end-point (Atlantic, Mediterranean, Black Sea basins) discriminant analysis we performed based on $\delta^{13}C$ and $\delta^{15}N$ of feathers moulted in post-breeding areas demonstrated an excellent and reliable accuracy of assignment, as indicated by the validation results. Note that P1 and P3 feathers exhibit highly similar values in most cases, which indicates that the discriminant is not influenced using two different feathers. Consequently, the reliability of the discriminant is not compromised. Consistently with tracking results, our discriminant analysis assessed the moulting region of already-tracked individuals correctly in 91% of the cases, with a margin of error of less than 10%. Such assignment methods based on intrinsic biogeochemical markers become very useful for species that are unable to carry GPS or GLS, for whom key knowledge on their migratory pathways would be beneficial (Flockhart et al., 2013). In addition, other migratory species could be favoured from our method study, particularly those endangered seabirds for those a single capture and a piece of feather sampling would be required. For example, there is poor knowledge on the migration of the European storm petrels (*Hydrobates pelagicus*) that breed along the Mediterranean basin (Metzger et al., 2015; Militão et al., 2022). It is known that western populations of Mediterranean European storm petrels migrated towards the Atlantic (Militão et al., 2022), but there is a gap of knowledge on the rest of populations (Lago et al., 2019), particularly those easternmost. Finally, this approach provides valuable information using only a single capture and isotopic analysis of a feather, being lot less invasive than deploying tracking devices and giving the possibility of applying it to a large number of individuals (Ramos & González-Solís, 2012).

Following up on the reliability of the method, we also found that a few animals of untracked shearwaters sampled opportunistically at colony sites, showed two distinct moulting areas in their primary feather sequence. This could be since a few birds moult their outermost primaries (from P7 to P10), the last to be replaced, in the breeding grounds, after returning from post-breeding areas. Yelkouan shearwaters from Greece showed the highest percentage of non-assigned birds, which unravel that these birds could replace their feathers both in the Mediterranean and the Black Sea, integrating the isotopic signal of both basins in different feathers (e.g., Ramos et al., 2009). In addition, we also unravelled a relevant proportion of shearwaters sampled and breeding in Minorca moulted their primary feathers in the Atlantic Ocean, so, confirming that the animals from this small population exhibit a mixed migratory behaviour between Balearic and Yelkouan shearwaters (Austin et al., 2019). The isotopic assignment method we proposed can also be applied with confidence to other colonies of Balearic and Yelkouan shearwaters for which there is no information on their moulting or post-breeding grounds. We evidenced that Tunisian Yelkouan shearwaters have similar migratory and moulting behaviours than those shearwaters from other central colonies, e.g., Sardinia or Malta. These examples well prove that the isotopic approach based on feather sampling could unravel moulting sites of other Yelkouan shearwater populations, such as those in Corsica, Algeria, Albania or Turkey. Finally, our discriminant method was also useful to infer information from bycaught animals, something that provide useful tools to evaluate the impact of these episodes of high mortality. In our case, as low percentage of Balearic shearwaters were ascribed to the Mediterranean waters, we can tell that Balearic birds prefer to migrate to the Atlantic in the post-breeding period instead of being year-round residents. We assumed that most of the 77 bycaught Yelkouan shearwaters we processed originated from the western-central colonies (e.g., Minorca, France or Sardinia; Courbin et al., 2024) as their moult behaviour matched that of the animals we included in the discriminant analysis.

From the conservation and taxonomic point of view of studied taxa, our study supports the idea that the involved populations show differentiated migratory strategies (cf. Austin et al. 2019). This differentiation in migratory patterns and post-breeding habitats eventually promote local adaptation and could lead to ecological divergence. Combined with high natal philopatry, this often results in a strong genetic structure and evolutionary divergence among populations

(Friesen et al., 2007; Rayner et al., 2011; Friesen, 2015; Ramos et al., 2020; but see Quillfeldt et al., 2017). However, such differentiation in migratory strategies could also merely reflect a behavioural gradient along the breeding range of a given species, previously described in many other seabirds (e.g., Fort et al., 2012). In the case of the Minorcan population, often referred as a Balearic-Yelkouan hybrid population (Austin et al., 2019), it exhibited an intermediate behaviour between the two species, and individuals shared moulting and non-breeding grounds with other populations of Balearic and Yelkouan shearwaters. In addition to this, the low percentage of birds from the French coast moulting in the Black Sea, also supported a longitudinal gradient hypothesis related to the chain migration strategy (Fort et al., 2012). The hypothesis is describe as *"individuals residing at higher latitudes will migrate to even higher latitudes, and vice versa"* and they will orientate uniformly as a metapopulation (Newton, 2008). In our case, the pattern can be considered longitudinally analogous, with individuals breeding in the easternmost longitudes (i.e., populations in Greece), also winters in the even more easterly non-breeding areas, and the reverse for the westernmost populations (i.e., Balearic populations). These findings agree with recent genomic studies that evidenced gene flow between these taxa, suggesting that both could be in an early phase of divergence (Ferrer Obiol et al., 2023; Genovart et al., 2012). Thus, by unravelling a continuous longitudinal trend in the distribution of Balearic and Yelkouan shearwater populations during the moulting period, our results also contributed in defining appropriate limits and robust Conservation Units of these threatened taxa. Defining such Units is an important task for marine conservation aiming at preserving key biodiversity areas in such a rapidly changing and impacted environment.

5. CONCLUSIONS

Our discriminant analysis, based on SIA of specific feathers, has been proved successful in identifying the post-breeding areas of both Balearic and Yelkouan shearwaters. Our study unravelled the primary moult of the Balearic and Yelkouan shearwaters; for both species and for most of the individuals, primary feathers were replaced in a single moulting area, which depended on the breeding population of the individual. This highlights the utility of SIA in assessing moult strategies of species which are hardly accessible during the moulting period, such as seabirds. The discriminant represents a very useful tool for assessing moulting areas of untrack individuals of any of the study species, but also any other seabird inhabiting similar areas (i.e., Northeast Atlantic, Mediterranean and Black Sea). The method can be applied to a large number of individuals, and it is particularly adequate for endangered species as information can be obtained with minimal intrusion and single captures.

Finally, although our results contributed to define Conservation Units for Balearic and Yelkouan shearwaters, there is an urgent need for complimentarily research that help concluding on this complex of species. For instance, SIA studies on non-tracked populations, on sabbaticals or non-breeding birds, on massive sampling of key populations (e.g., Minorca) and on potential prey should reveal key information on the trophic and migratory ecologies of these birds. These new isotopic results will help developing appropriate conservation strategies, for such endangered species, but also for any other migratory species facing similar constraints.

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Authors contribution

Cristina Hernández de Tena: Data curation, Formal analysis, Writing – original draft, Maite Louzao: Data collecting, Revising, Pep Arcos: Data collecting, Writing and Revising, David Garcia: Data collecting, Tim Guilford: Data collecting, Rhiannon Austin: Data collecting, Teresa Militão: Data collecting, Karen Bourgeois: Data collecting, Jacob González-Solís: Data collecting, Clara Perón: Data collecting, Jerome Fort: Data collecting, Writing and Revising, David Grémillet: Data collecting, Marco Zenatello: Data collecting, Nicola Bacetti: Data collecting, Martin Austad: Data collecting, Revising, Nicholas Barbara: Data collecting, Sven Kapelj: Data collecting, George Karris: Data collecting, Stavros Xirouchakis: Data collecting, Danae Portolou: Data collecting, Raül Ramos: Conceptualization, Data collecting, Funding acquisition, Writing – original draft.

REFERENCES

Adams, T. S., & Sterner, R. W. (2000). The effect of dietary nitrogen content on trophic level 15N enrichment. *Limnology and Oceanography*, *45*(3), 601-607. https://doi.org/10.4319/lo.2000.45.3.0601

Altaba, C. R. (1993). La sistemàtica i la conservació de la biodiversitat: El cas de les baldritges (Puffinus). *Anuari Ornitològic de les Balears: revista d'observació estudi i conservació dels aucells*, 3-14.

Arcos, J. M. (2011, enero 1). International species action plan for the Balearic shearwater, Puffinus mauretanicus. *SEO/BirdLife & BirdLife International.* https://www.researchgate.net/publication/235453019_International_species_action_plan_for_the_Balearic_shearwater_Puffinus_mauretanicus

Austin, R. E., Wynn, R. B., Votier, S. C., Trueman, C., McMinn, M., Rodríguez, A., Suberg, L., Maurice, L., Newton, J., Genovart, M., Péron, C., Grémillet, D., & Guilford, T. (2019). Patterns of at-sea behaviour at a hybrid zone between two threatened seabirds. *Scientific Reports*, *9*(1), Article 1. https://doi.org/10.1038/s41598-019-51188-8

Bănaru, D., Harmelin-Vivien, M., Gomoiu, M.-T., & Onciu, T.-M. (2007). Influence of the Danube River inputs on C and N stable isotope ratios of the Romanian coastal waters and sediment (Black Sea). *Marine Pollution Bulletin*, *54*(9), 1385-1394. https://doi.org/10.1016/j.marpolbul.2007.05.022

BirdLife International. (2018). IUCN Red List of Threatened Species: Puffinus yelkouan. *IUCN Red List of Threatened Species*. https://www.iucnredlist.org/en

Bose, S., Pal, A., SahaRay, R., & Nayak, J. (2015). Generalized quadratic discriminant analysis. *Pattern Recognition*, *48*(8), 2676-2684. https://doi.org/10.1016/j.patcog.2015.02.016

Bourgeois, K., & Dromzée, S. (2014). Moulting strategies of the Yelkouan Shearwater Puffinus yelkouan during the breeding season. *Journal of Ornithology*, *155*(1), 265-271. https://doi.org/10.1007/s10336-013-1011-y

Bourgeois, K., & Vidal, E. (2008). The endemic Mediterranean Yelkouan Shearwater Puffinus yelkouan: Distribution, threats and a plea for more data. *Oryx*, *42*, 187-194. https://doi.org/10.1017/S0030605308006467

Calenge, C., & Fortmann-Roe, S. (2024). *adehabitatHR: Home Range Estimation* (Versión 0.4.22) [Software]. https://cran.r-project.org/web/packages/adehabitatHR/index.html

Carey, M. J. (2011). Leg-mounted data-loggers do not affect the reproductive performance of short-tailed shearwaters (Puffinus tenuirostris). *Wildlife Research*, *38*(8), 740. https://doi.org/10.1071/WR11024

Chew, B., Kelly, J., & Contina, A. (2019). *Stable isotopes in avian research: A step by step protocol to feather sample preparation for stable isotope...* https://www.protocols.io/view/stable-isotopes-in-avian-research-a-step-by-step-p-z2uf8ew

Cortés, V., Arcos, J. M., & González-Solís, J. (2017). Seabirds and demersal longliners in the northwestern Mediterranean: Factors driving their interactions and bycatch rates. *Marine Ecology Progress Series*, *565*, 1-16. https://doi.org/10.3354/meps12026

Courbin, N., Besnard, A., & Grémillet, D. (2024). Transnational mortality from Spanish longline fisheries bycatch is shaping the decline of a vulnerable French seabird. *Biological Conservation*, *293*, 110597. https://doi.org/10.1016/j.biocon.2024.110597

Curé, C., Aubin, T., & Mathevon, N. (2010). Intra-sex vocal interactions in two hybridizing seabird species (Puffinus sp.). *Behavioral Ecology and Sociobiology*, *64*(11), 1823-1837.

Davies, T. E., Carneiro, A. P. B., Tarzia, M., Wakefield, E., Hennicke, J. C., Frederiksen, M., Hansen, E. S., Campos, B., Hazin, C., Lascelles, B., Anker-Nilssen, T., Arnardóttir, H., Barrett, R. T., Biscoito, M., Bollache, L., Boulinier, T., Catry, P., Ceia, F. R., Chastel, O., … Dias, M. P. (2021). Multispecies tracking reveals a major seabird hotspot in the North Atlantic. *Conservation Letters*, *14*(5), e12824. https://doi.org/10.1111/conl.12824

Demidov, A. B. (2008). Seasonal dynamics and estimation of the annual primary production of phytoplankton in the Black Sea. *Oceanology*, *48*(5), 664-678. https://doi.org/10.1134/S0001437008050068

Ferrer Obiol, J., Herranz, J. M., Paris, J. R., Whiting, J. R., Rozas, J., Riutort, M., & González-Solís, J. (2023). Species delimitation using genomic data to resolve taxonomic uncertainties in a speciation continuum of pelagic seabirds. *Molecular Phylogenetics and Evolution*, *179*, 107671. https://doi.org/10.1016/j.ympev.2022.107671

Flockhart, D. T. T., Wassenaar, L. I., Martin, T. G., Hobson, K. A., Wunder, M. B., & Norris, D. R. (2013). Tracking multi-generational colonization of the breeding grounds by monarch butterflies in eastern North America. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1768), 20131087. https://doi.org/10.1098/rspb.2013.1087

Fort, J., Pettex, E., Tremblay, Y., Lorentsen, S.-H., Garthe, S., Votier, S., Pons, J. B., Siorat, F., Furness, R. W., Grecian, W. J., Bearhop, S., Montevecchi, W. A., & Grémillet, D. (2012). Meta-population evidence of oriented chain migration in northern gannets (Morus bassanus). *Frontiers in Ecology and the Environment*, *10*(5), 237-242. https://doi.org/10.1890/110194

Friesen, V. L. (2015). Speciation in seabirds: Why are there so many species…and why aren't there more? *Journal of Ornithology*, *156*(1), 27-39. https://doi.org/10.1007/s10336-015-1235-0

Friesen, V. L., Burg, T. M., & McCOY, K. D. (2007). Mechanisms of population differentiation in seabirds. *Molecular Ecology*, *16*(9), 1765-1785. https://doi.org/10.1111/j.1365-294X.2006.03197.x

Gaudard, C. (2018). *International Single Species Action Plan for the Yelkouan Shearwater Puffinus yelkouan. Project LIFE 14 PRE/UK/000002. Coordinated Efforts for International Species Recovery EuroSAP.* (p. 45p). LPO/BirdLife France. Rochefort.

Genovart, M., Arcos, J. M., Álvarez, D., McMinn, M., Meier, R., B. Wynn, R., Guilford, T., & Oro, D. (2016). Demography of the critically endangered Balearic shearwater: The impact of fisheries and time to extinction. *Journal of Applied Ecology*, *53*(4), 1158-1168. https://doi.org/10.1111/1365-2664.12622

Genovart, M., Juste, J., Contreras-Díaz, H., & Oro, D. (2012). Genetic and Phenotypic Differentiation between the Critically Endangered Balearic Shearwater and Neighboring Colonies of Its Sibling Species. *Journal of Heredity*, *103*(3), 330-341. https://doi.org/10.1093/jhered/ess010

Gil-Velasco, M., Rodríguez, G., Menzie, S., & Arcos, J. M. (2015). *Plumage variability and field identification of Manx, Yelkouan and Balearic Shearwaters*. *108*, 514-539.

Gómez-Díaz, E., & González-Solís, J. (2007). Geographic Assignment of Seabirds to Their Origin: Combining Morphologic, Genetic, and Biogeochemical Analyses. *Ecological Applications*, *17*(5), 1484-1498. https://doi.org/10.1890/06-1232.1

Graham, B., Koch, P., Newsome, S., & Mcmahon, K. (2010). Using Isoscapes to Trace the Movements and Foraging Behavior of Top Predators in Oceanic Ecosystems. En *Isoscapes: Understanding Movement, Pattern and Process on Earth Through Isotope Mapping* (pp. 299-318). https://doi.org/10.1007/978-90-481-3354-3_14

Grissot, A., Borrel, C., Devogel, M., Altmeyer, L., Johansen, M. K., Strøm, H., & Wojczulanis-Jakubas, K. (2023). Use of geolocators for investigating breeding ecology of a rock crevice-nesting seabird: Method validation and impact assessment. *Ecology and Evolution*, *13*(3), e9846. https://doi.org/10.1002/ece3.9846

Guilford, T., Wynn, R., McMinn, M., Rodríguez, A., Fayet, A., Maurice, L., Jones, A., & Meier, R. (2012). Geolocators Reveal Migration and Pre-Breeding Behaviour of the Critically Endangered Balearic Shearwater Puffinus mauretanicus. *PLOS ONE*, *7*(3), e33753. https://doi.org/10.1371/journal.pone.0033753

Halpin, L. R., Ross, J. D., Ramos, R., Mott, R., Carlile, N., Golding, N., Reyes-González, J. M., Militão, T., De Felipe, F., Zajková, Z., Cruz-Flores, M., Saldanha, S., Morera-Pujol, V., Navarro-Herrero, L., Zango, L., González-Solís, J., & Clarke, R. H. (2021). Double-tagging scores of seabirds reveals that light-level geolocator accuracy is limited by species idiosyncrasies and equatorial solar profiles. *Methods in Ecology and Evolution*, *12*(11), 2243-2255. https://doi.org/10.1111/2041-210X.13698

Huguet, A., Barillé, L., Soudant, D., Petitgas, P., Gohin, F., & Lefebvre, A. (2024). Identifying the spatial pattern and the drivers of the decline in the eastern English Channel chlorophyll-*a* surface concentration over the last two decades. *Marine Pollution Bulletin*, *199*, 115870. https://doi.org/10.1016/j.marpolbul.2023.115870

Lago, P., Austad, M., & Metzger, B. (2019). *PARTIAL MIGRATION IN THE MEDITERRANEAN STORM PETREL*.

Lascelles, B., Notarbartolo Di Sciara, G., Agardy, T., Cuttelod, A., Eckert, S., Glowka, L., Hoyt, E., Llewellyn, F., Louzao, M., Ridoux, V., & Tetley, M. J. (2014). Migratory marine species: Their status, threats and conservation management needs. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *24*(S2), 111-127. https://doi.org/10.1002/aqc.2512

Lennox, R. J., Chapman, J. M., Souliere, C. M., Tudorache, C., Wikelski, M., Metcalfe, J. D., & Cooke, S. J. (2016). Conservation physiology of animal migration. *Conservation Physiology*, *4*(1), cov072. https://doi.org/10.1093/conphys/cov072

Lisovski, S., Bauer, S., Briedis, M., Davidson, S. C., Dhanjal-Adams, K. L., Hallworth, M. T., Karagicheva, J., Meier, C. M., Merkel, B., Ouwehand, J., Pedersen, L., Rakhimberdiev, E., Roberto-Charron, A., Seavy, N. E., Sumner, M. D., Taylor, C. M., Wotherspoon, S. J., & Bridge, E. S. (2020). Light-level geolocator analyses: A user's guide. *Journal of Animal Ecology*, *89*(1), 221-236. https://doi.org/10.1111/1365-2656.13036

López-López, P. (2016). Individual-Based Tracking Systems in Ornithology: Welcome to the Era of Big Data. *Ardeola*, *63*(1), 103-136. https://doi.org/10.13157/arla.63.1.2016.rp5

McMahon, K. W., Hamady, L. L., & Thorrold, S. R. (2013). A review of ecogeochemistry approaches to estimating movements of marine animals. *Limnology and Oceanography*, *58*(2), 697-714. https://doi.org/10.4319/lo.2013.58.2.0697

Meier, R. E., Votier, S. C., Wynn, R. B., Guilford, T., McMinn Grivé, M., Rodríguez, A., Newton, J., Maurice, L., Chouvelon, T., Dessier, A., & Trueman, C. N. (2017). Tracking, feather moult and stable isotopes reveal foraging behaviour of a critically endangered seabird during the non-breeding season. *Diversity and Distributions*, *23*(2), 130-145. https://doi.org/10.1111/ddi.12509

Meier, R. E., Wynn, R. B., Votier, S. C., McMinn Grivé, M., Rodríguez, A., Maurice, L., van Loon, E. E., Jones, A. R., Suberg, L., Arcos, J. M., Morgan, G., Josey, S. A., & Guilford, T. (2015). Consistent foraging areas and commuting corridors of the critically endangered Balearic shearwater *Puffinus mauretanicus* in the northwestern Mediterranean. *Biological Conservation*, *190*, 87-97. https://doi.org/10.1016/j.biocon.2015.05.012

Merkel, B., Phillips, R. A., Descamps, S., Yoccoz, N. G., Moe, B., & Strøm, H. (2016). A probabilistic algorithm to process geolocation data. *Movement Ecology*, *4*(1), 26. https://doi.org/10.1186/s40462-016-0091-8

Metzger, B., Borg, J., Barbara, N., & Sultana, J. (2015, febrero 20). *Far beyond the horizon—Modern tracking techniques as a tool to identify marine IBAs for Maltese Seabirds. Maltese seabirds*.

Militão, T., Bourgeois, K., Roscales, J., & González-Solís, J. (2013). Individual migratory patterns of two threatened seabirds revealed using stable isotope and geolocation analyses. *Diversity and Distributions*, *19*, 317-329. https://doi.org/10.1111/j.1472-4642.2012.00916.x

Militão, T., Gómez-Díaz, E., Kaliontzopoulou, A., & González-Solís, J. (2014). Comparing Multiple Criteria for Species Identification in Two Recently Diverged Seabirds. *PLOS ONE*, *9*(12), e115650. https://doi.org/10.1371/journal.pone.0115650

Militão, T., Sanz-Aguilar, A., Rotger, A., & Ramos, R. (2022). Non-breeding distribution and at-sea activity patterns of the smallest European seabird, the European Storm Petrel (Hydrobates pelagicus). *Ibis*, *164*(4), 1160-1179. https://doi.org/10.1111/ibi.13068

Mouriño, J., Arcos, F., Salvadores, R., Sandoval, A., & Vidal, C. (2003). Status of the Balearic shearwater (*Puffinus mauretanicus*) on the Galician coast (NW Iberian Peninsula. *Scientia Marina*, *67*(S2), 135-142. https://doi.org/10.3989/scimar.2003.67s2135

Newton, I. (2008). *The Migration Ecology of Birds*. Elsevier.

Nieblas, A.-E., Drushka, K., Reygondeau, G., Rossi, V., Demarcq, H., Dubroca, L., & Bonhommeau, S. (2014). Defining Mediterranean and Black Sea Biogeochemical Subprovinces and Synthetic Ocean Indicators Using Mesoscale Oceanographic Features. *PLOS ONE*, *9*(10), e111251. https://doi.org/10.1371/journal.pone.0111251

Oliveira, N., Henriques, A., Miodonski, J., Pereira, J., Marujo, D., Almeida, A., Barros, N., Andrade, J., Marçalo, A., Santos, J., Oliveira, I. B., Ferreira, M., Araújo, H., Monteiro, S., Vingada, J., & Ramírez, I. (2015). Seabird bycatch in Portuguese mainland coastal fisheries: An assessment through on-board observations and fishermen interviews. *Global Ecology and Conservation*, *3*, 51-61. https://doi.org/10.1016/j.gecco.2014.11.006

Oppel, S., Raine, A. F., Borg, J. J., Raine, H., Bonnaud, E., Bourgeois, K., & Breton, A. R. (2011). Is the Yelkouan shearwater Puffinus yelkouan threatened by low adult survival probabilities? *Biological Conservation*, *144*(9), 2255-2263. https://doi.org/10.1016/j.biocon.2011.05.017

Pantoja, S., Repeta, D. J., Sachs, J. P., & Sigman, D. M. (2002). Stable isotope constraints on the nitrogen cycle of the Mediterranean Sea water column. *Deep Sea Research Part I: Oceanographic Research Papers*, *49*(9), 1609-1621. https://doi.org/10.1016/S0967-0637(02)00066-3

Pérez, G. E., Schondube, J. E., & Martínez del Río, C. (2008). ISÓTOPOS ESTABLES EN ORNITOLOGÍA: UNA INTRODUCCIÓN BREVE. *ORNITOLOGIA NEOTROPICAL*, *19*, 95-112.

Pérez-Roda, A., Delord, K., Boué, A., Arcos, J. M., García, D., Micol, T., Weimerskirch, H., Pinaud, D., & Louzao, M. (2017). Identifying Important Atlantic Areas for the conservation of Balearic shearwaters: Spatial overlap with conservation areas. *Deep Sea Research Part II: Topical Studies in Oceanography*, *141*, 285-293. https://doi.org/10.1016/j.dsr2.2016.11.011

Péron, C., Grémillet, D., Prudor, A., Pettex, E., Saraux, C., Soriano-Redondo, A., Authier, M., & Fort, J. (2013). Importance of coastal Marine Protected Areas for the conservation of pelagic seabirds: The case of *Vulnerable* yelkouan shearwaters in the Mediterranean Sea. *Biological Conservation*, *168*, 210-221. https://doi.org/10.1016/j.biocon.2013.09.006

Qin, Y. (2018). *A review of quadratic discriminant analysis for high-dimensional data*. WIREs Computational Statistics. https://wires.onlinelibrary.wiley.com/doi/epdf/10.1002/wics.1434

Quillfeldt, P., Moodley, Y., Weimerskirch, H., Cherel, Y., Delord, K., Phillips, R. A., Navarro, J., Calderón, L., & Masello, J. F. (2017). Does genetic structure reflect differences in non-breeding movements? A case study in small, highly mobile seabirds. *BMC Evolutionary Biology*, *17*(1), 160. https://doi.org/10.1186/s12862-017-1008-x

Raine, A. F., Borg, J. J., Raine, H., & Phillips, R. A. (2013). Migration strategies of the Yelkouan Shearwater Puffinus yelkouan. *Journal of Ornithology*, *154*(2), 411-422. https://doi.org/10.1007/s10336-012-0905-4

Ramírez, I., Mitchell, D., Vulcano, A., Rouxel, Y., Marchowski, D., Almeida, A., Arcos, J. M., Cortes, V., Lange, G., Morkūnas, J., Oliveira, N., & Paiva, V. H. (2024). Seabird bycatch in European waters. *Animal Conservation*, *27*(6), 737-752. https://doi.org/10.1111/acv.12948

Ramos, R., & González-Solís, J. (2012). Trace me if you can: The use of intrinsic biogeochemical markers in marine top predators. *Frontiers in Ecology and the Environment*, *10*(5), 258-266. https://doi.org/10.1890/110140

Ramos, R., Militao, Teresa, & Gonzalez-Solís, Jacob. (2009). Moulting strategies of a long-distance migratory seabird, the Mediterranean Cory's Shearwater Calonectris diomedea diomedea | Request PDF. *Ibis*, *151(1)*, 151-159. https://doi.org/DOI:10.1111/j.1474-919X.2008.00877.x

Ramos, R., Paiva, V. H., Zajková, Z., Precheur, C., Fagundes, A. I., Jodice, P. G. R., Mackin, W., Zino, F., Bretagnolle, V., & González-Solís, J. (2020). Spatial ecology of closely related taxa: The case of the little shearwater complex in the North Atlantic Ocean. *Zoological Journal of the Linnean Society*, zlaa045. https://doi.org/10.1093/zoolinnean/zlaa045

Rayner, M. J., Hauber, M. E., Steeves, T. E., Lawrence, H. A., Thompson, D. R., Sagar, P. M., Bury, S. J., Landers, T. J., Phillips, R. A., Ranjard, L., & Shaffer, S. A. (2011). Contemporary and historical separation of transequatorial migration between genetically distinct seabird populations. *Nature Communications*, *2*(1), 332. https://doi.org/10.1038/ncomms1330

Rocque, D. A., Ben-David, M., Barry, R. P., & Winker, K. (2006). Assigning birds to wintering and breeding grounds using stable isotopes: Lessons from two feather generations among three intercontinental migrants. *Journal of Ornithology*, *147*(2), 395-404. https://doi.org/10.1007/s10336-006-0068-2

Ruiz, A., & Martí, R. (2004). *La Pardela Balear.* SEO/BirdLife-Conselleria de Medi Ambient del Govern de les Illes Balears.

Runge, C. A., Martin, T. G., Possingham, H. P., Willis, S. G., & Fuller, R. A. (2014). Conserving mobile species. *Frontiers in Ecology and the Environment*, *12*(7), 395-402. https://doi.org/10.1890/130237

Salazar-García, D. (2011). Patrón de dieta en la población púnica de Can Marines (Ibiza) a través del análisis de isótopos estables (C y N) en colágeno óseo. *Saguntum*, *43*, 95-102. https://doi.org/10.7203/SAGVNTVM.43.1213

Schuster, R., Wilson, S., Rodewald, A. D., Arcese, P., Fink, D., Auer, T., & Bennett, J. R. (2019). Optimizing the conservation of migratory species over their full annual cycle. *Nature Communications*, *10*(1), Article 1. https://doi.org/10.1038/s41467-019-09723-8

Sohil, F., Sohali, M. U., & Shabbir, J. (2022). An introduction to statistical learning with applications in R: By Gareth James, Daniela Witten, Trevor Hastie, and Robert Tibshirani, New York, Springer Science and Business Media, 2013, $41.98, eISBN: 978-1-4614-7137-7. *Statistical Theory and Related Fields*, *6*(1), 87-87. https://doi.org/10.1080/24754269.2021.1980261

van Bemmelen, R. S. A., Kolbeinsson, Y., Ramos, R., Gilg, O., Alves, J. A., Smith, M., Schekkerman, H., Lehikoinen, A., Petersen, I. K., Þórisson, B., Sokolov, A. A., Välimäki, K., van der Meer, T., Okill, J. D., Bolton, M., Moe, B., Hanssen, S. A., Bollache, L., Petersen, A., … Tulp, I. (2019). A Migratory Divide Among Red-Necked Phalaropes in the Western Palearctic Reveals Contrasting Migration and Wintering Movement Strategies. *Frontiers in Ecology and Evolution*, *7*. https://www.frontiersin.org/articles/10.3389/fevo.2019.00086

Wells, R. J. D., Rooker, J. R., Addis, P., Arrizabalaga, H., Baptista, M., Bearzi, G., Fraile, I., Lacoue-Labarthe, T., Meese, E. N., Megalofonou, P., Rosa, R., Sobrino, I., Sykes, A. V., & Villanueva, R. (2021). Regional patterns of δ13C and δ15N for European common cuttlefish (Sepia officinalis) throughout the Northeast Atlantic Ocean and Mediterranean Sea. *Royal Society Open Science*, *8*(9), 210345. https://doi.org/10.1098/rsos.210345

Worton, B. J. (1989). Kernel Methods for Estimating the Utilization Distribution in Home-Range Studies. *Ecology*, *70*(1), 164-168. https://doi.org/10.2307/1938423

Table 1: Number of GLS-tracked individuals of Balearic (*Puffinus mauretanicus*) and Yelkouan (*P. yelkouan*) shearwaters sampled in each colony and the percentage (%) of individuals ascribed to each moulting area (among Atlantic, Mediterranean and Black Sea basins).

Species	**Population**	**Colony**	**nº of tracked individuals**	**Individuals in Atlantic (%)**	**Individuals in Mediterranean (%)**	**Individuals in Black Sea (%)**
Balearic	Ibiza	Es Bosc Sa Conillera	7 15	90.9	9.1	0
Balearic-Yelkouan	Minorca	La Mola	4	0	100	0
Yelkouan	France	Porquerolles Port Cros	3 11	0	92.3	7.7
Yelkouan	Sardinia	Tavolara	5	0	0	100
Yelkouan	Malta	Malta	6	0	16.7	83.3
Yelkouan	Croatia	Zaklopatica	9	0	44.4	55.6
Yelkouan	Greece	Gyaros Lipsi	3 1	0	0	100

FIGURE LEGENDS

Figure 1: Moulting areas for the seven GLS-tracked populations of Balearic and Yelkouan shearwaters represented as 50% Kernel Density Estimations (KDE) of the positions of each population for the month of August (when P1-P3 feathers are replaced) and depicted as light blue polygons. Additionally, individual centroids of the 50% KDE of every tracked individual for that month are also depicted in brown dots. Colony sites are depicted as yellow rhomboids,

and sample sizes are: n=22 for Ibiza, Spain (A); n= 4 for Minorca, Spain (B); n= 14 for France (C); n= 5 for Sardinia, Italy (D); n= 6 for Malta (E); n= 9 for Croatia (F); n= 3 for Greece (G).

Figure 2: Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic values in decision boundary plot for P1-P3 feathers of GLS-tracked shearwaters (n= 64). Colours represent the three moulting areas we established: green for NE Atlantic, blue for Mediterranean Sea and in red the Black Sea. Boxplots on the margin indicate the distribution of the data and intervals in isotopic composition of the basins.

Figure 3: *Puffinus* shearwater wing scheme with grey-coloured primary feathers used in the analysis (a), primary moult pattern is shown by the orange arrow (Ramos et al., 2009). $\delta^{13}C$ and $\delta^{15}N$ of the primary feathers analysed from non-tracked individuals sampled along the moult sequence, i.e., 1st, 3rd, 5th, 7th and 9th primary feathers (P1, P3, P5, P7 and P9, respectively). The colours represent the post-breeding area: green (Atlantic), blue (Mediterranean Sea), red (Black Sea) and grey non-assigned. Colony sites are depicted as different symbols: Minorca as dots, Sardinia as rhomboids, Tunisia as triangles and Greece as squares. Symbols belonging to the same individual are connected by a line.

Figure 1.

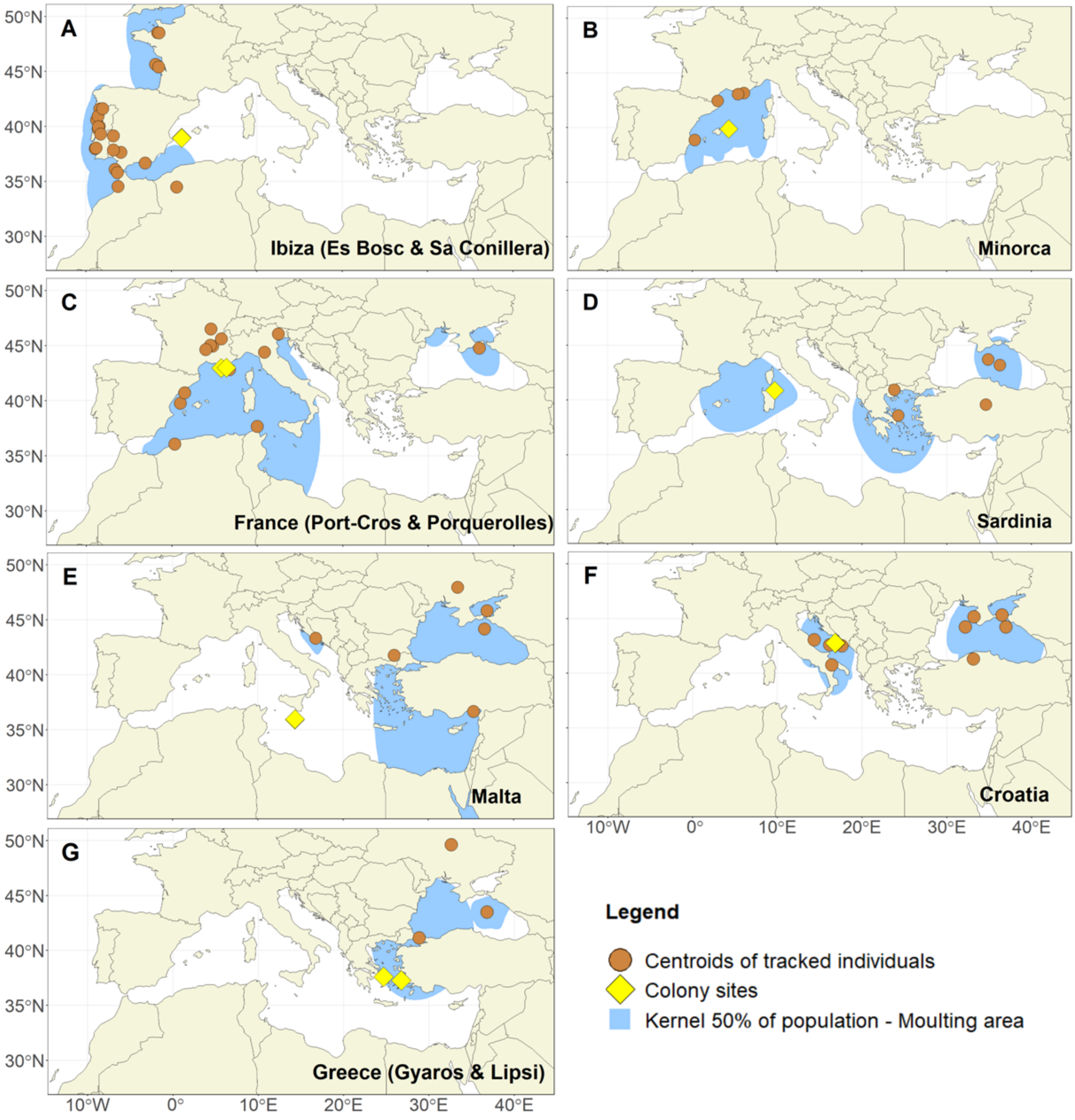

A
Ibiza (Es Bosc & Sa Conillera)
B
Minorca
C
France (Port-Cros & Porquerolles)
D
Sardinia
E
Malta
F
Croatia
G
Greece (Gyaros & Lipsi)
50°N
45°N
40°N
35°N
30°N
10°W
0°
10°E
20°E
30°E
40°E
Legend
Centroids of tracked individuals
Colony sites
Kernel 50% of population - Moulting area

Figure 2.

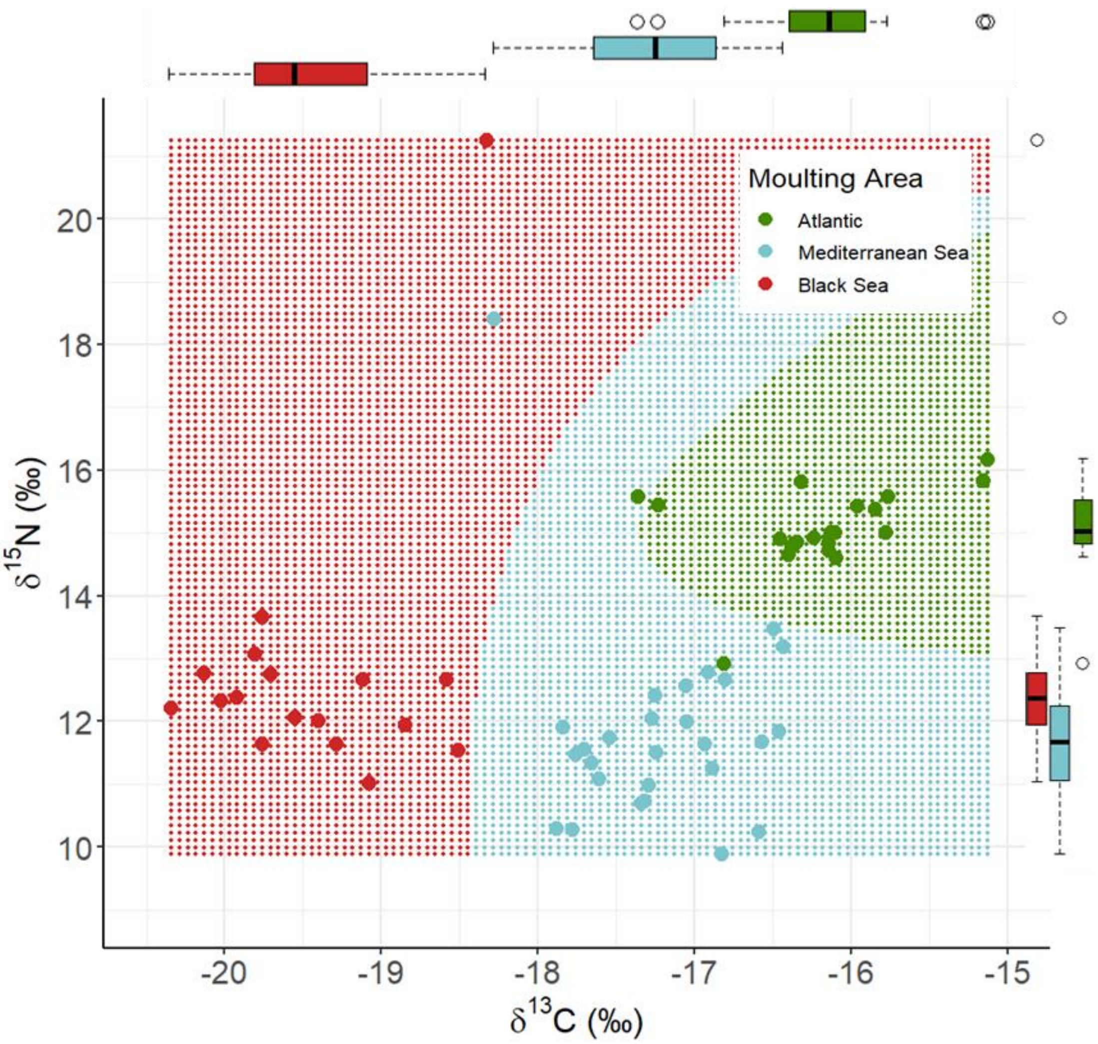
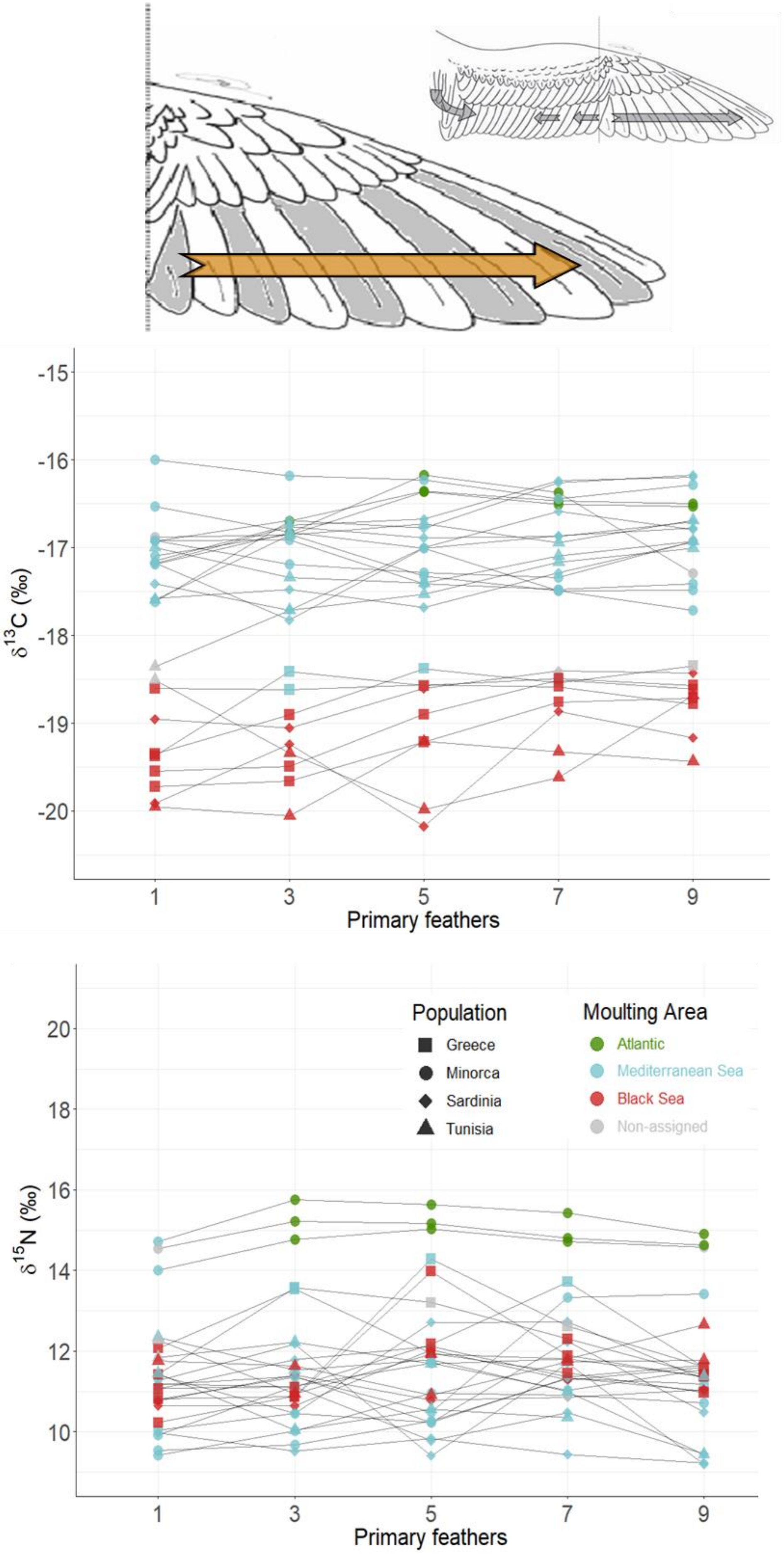


Figure 3.


-15
-16
-17
-18
-19
-20
δ13C (‰)
1
3
5
7
9
Primary feathers
20
18
16
14
12
10
δ15N (‰)
Population
Greece
Minorca
Sardinia
Tunisia
Moulting Area
Atlantic
Mediterranean Sea
Black Sea
Non-assigned
1
3
5
7
9
Primary feathers

SUPLEMENTARY MATERIAL

Assessing migration and moulting strategy in closely related taxa based on stable isotope analysis: a population study of Balearic and Yelkouan shearwaters across their breeding range

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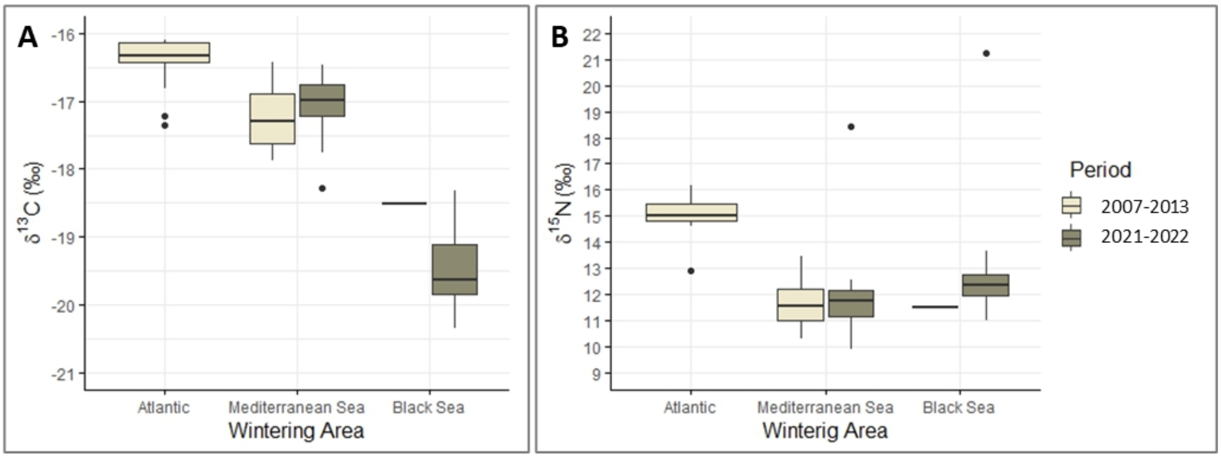
Figure S1: Flight moult pattern of *Puffinus* shearwaters. Compilation of photographs of Balearic and Yelkouan and shearwaters actively moulting feathers. The moulting feathers are marked with arrows. P refers to primary feathers. A-C. Yelkouan shearwater. D-F. Balearic shearwater. A) Actively moulting, P1-P2, 15th August 2014, Topkapı Saray, İstanbul, Turkey, Frank Hawkins. B) Actively moulting P1-P2, France, July 20,12 Porquerolles island, Provence-Cote d'Azur, France, Aurélien Audevard. C) Actively moulting P5-P6, 8th of July 2020, Portland Bill, United Kingdom, Ewan Urquhart. D) Non-active moult yet, 18th of June 2014, Barcelona, Spain, JM Arcos. E) Actively moulting P7-P8, 4th September 2016, Aveiro, Portugal, Pmde Esteves. F) All primary feathers already moulted, 1st of November 2007, Roses, Catalunya, Spain, JM Arcos.


A
B
C
D
E
F

Figure S2: Isotopic values of (A) $\delta^{13}C$ and (B) $\delta^{15}N$ from feathers collected in two study periods and assigned to different moulting areas.



Population	Colony	nº of tracked individuals from 2007-2013	nº of tracked individuals from 2021-2022
Ibiza	Es Bosc	-	7
Ibiza	Sa Conillera	-	15
Minorca	La Mola	-	4
France	Porquerolles	-	3
France	Port Cros	-	11
Sardinia	Tavolara	5	-
Malta	Malta	6	-
Croatia	Zaklopatica	9	-
Greece	Gyaros	3	-
Greece	Lipsi	1	-

Table S1: Summary of GLS-tracking data for each colony that contributed to the study, i.e., number of recovered devices from birds with an associated feather sampling.

Models for $\delta^{13}C$	**df**	**AICc**	**ΔAICc**	**wAICc**
Wintering area	**4**	**109.1**	**0.0**	**0.538**
Period:Wintering area	**6**	**110.3**	**1.2**	**0.295**
Wintering area + Period	5	111.4	2.3	0.167
Period	3	184.9	75.8	0.000
Null	1	550.3	441.2	0.000

Models for $\delta^{15}N$				
Wintering area	**4**	**247.9**	**0.0**	**0.558**
Wintering area + Period	**5**	**248.9**	**1.0**	**0.334**
Period:Wintering area	6	251.1	3.3	0.108
Period	3	280.7	32.8	0.000
Null	1	514.6	266.7	0.000

Table S2: Model-selection for all GLM evaluating the wintering area and the period of sampling on both stable isotope ratios. Degrees of freedom (df), Akaike's Information Criterion corrected for small sample size (AIC_c), difference in the AIC_c from the top-ranking model (ΔAIC_c) and the Akaike weight ($wAIC_c$). The models are sorted from lowest to highest AICc, i.e. from the most parsimonious ones.

Averaged model for $\delta^{13}C$	**Estimate**	**Adjusted SE**	**z value**	**P- value**
WAAtlantic	-10.45	7.74	1.35	0.177
WAMediterraneanSea	-11.49	7.72	1.49	0.137
WABlackSea	-13.75	7.65	1.80	0.072
Period07-13	-5.73	7.74	0.74	0.459
Period21-22	-5.69	7.68	0.74	0.459
Period07-13: WABlackSea	0.39	0.64	0.61	0.542
Period07-13: WAMediterraneanSea	0.00	0.00	NaN	NaN

p averaged $\delta^{15}N$	**Estimate**	**Adjusted SE**	**z value**	**P- value**
WAAtlantic	9.43	7.30	1.29	0.196
WAMediterraneanSea	6.12	7.40	0.83	0.409
WABlackSea	6.91	7.63	0.91	0.365
Period07-13	5.64	7.30	0.77	0.439
Period21-22	5.90	7.65	0.77	0.440

Table S3: Averaged model parameters from the most parsimonious models in Table S2. Wintering Areas (WAAtlantic, WAMediterraneanSea, WABlackSea), and study periods (Period07-13 (2007-2013) and Period21-22 (2021-2022)).