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# Use of stable isotope fingerprints to assign wintering origin and trace shorebird movements along the East Atlantic Flyway

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## Abstract

Migratory connectivity can be defined as the flux of individuals or populations among areas between stages of an animal's life cycle. Many shorebird species perform long-distance migrations and while moving between breeding and wintering grounds, they depend on a network of intermediate wetlands (stopover sites) where populations of different origins extensively overlap. The difficulty to discriminate such populations represents a serious obstacle to the identification of the links between breeding or wintering areas and stopover sites, and also precludes the estimation of demographic parameters for each population. In this study, we test if linear discriminant models based on stable carbon and nitrogen isotope ratios in toenails can be used to identify populations of several shorebird species of different wintering origins overlapping at two stopover sites of the East Atlantic Flyway. In addition, we evaluate the ability of this approach to infer migratory phenological patterns of shorebirds. Linear discriminant analyses performed overall well in distinguishing the isotopic signals of birds from wintering areas (in France, Portugal, Morocco, Mauritania and Guinea-Bissau) in most species, correctly classifying over 80% ($n = 542$) of all wintering

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individuals sampled at these areas. Assignment rates of shorebirds captured during spring migration were also high (96%, $n = 323$) at the Tejo estuary, Portugal, but lower (40%, $n = 185$) at Marennes-Oléron Bay in France, and also differed among species. A large proportion of spring migrants captured in Portugal and France were assigned to Banc d'Arguin in Mauritania, the most important wintering area in the flyway. Phenological patterns derived for dunlins (*Calidris alpina*), common ringed plovers (*Charadrius hiaticula*) and grey plovers (*Pluvialis squatarola*) suggest that the first northward migrants started arriving at the Tejo estuary during the second half of March, with peaking numbers occurring during April or May.

## Zusammenfassung

Migrationskonnektivität kann als die Wechselrate von Individuen oder Populationen in bestimmten Gebieten zwischen zwei Phasen im Lebenszyklus einer Art definiert werden. Viele Watvögel legen weite Strecken zurück, und während des Zuges zwischen Brut- und Überwinterungsgebieten benötigen sie ein Netzwerk von Feuchtgebieten als Rastplätze, wo Populationen unterschiedlicher Herkunft zusammen kommen. Die Schwierigkeit, solche Populationen zu unterscheiden, stellt ein schwerwiegendes Hindernis für die Bestimmung der Verbindungen zwischen Brut- bzw. Überwinterungsgebieten und den Rastplätzen dar und verhindert außerdem die Bestimmung von demographischen Parametern für einzelne Populationen. Wir testeten, ob -basierend auf den Signaturen der stabilen Kohlenstoff- und Stickstoffisotope in Zehennägeln- lineare Diskriminanzmodelle eingesetzt werden können, um die unterschiedlichen Überwinterungsgebiete von Watvögeln zu identifizieren, die an zwei Rastplätzen entlang des Ostatlantischen Zugweges zusammentrafen. Wir bewerteten außerdem die Fähigkeit dieser Methode, Rückschlüsse auf phänologische Muster der ziehenden Watvögel zuzulassen. Die lineare Diskriminanzanalyse bewährte sich für die meisten Arten gut bei der Unterscheidung von Isotopensignaturen der Vögel aus unterschiedlichen Überwinterungsgebieten (Frankreich, Portugal, Marokko, Mauretanien und Guinea-Bissau). Mehr als 80% ($n = 542$) der in diesen Gebieten untersuchten überwinternden Individuen konnten korrekt eingeordnet werden. Die Zuordnungsraten der Watvögel, die während des Frühjahrszuges im Mündungsgebiet des Tejo gefangen wurden, waren ebenfalls hoch (96%, $n = 323$), sie waren aber geringer (40%, $n = 185$) in der Marennes-Oléron-Bucht (Frankreich) und variierten zwischen den Arten. Ein hoher Anteil von Frühjahrsziehern, die in Portugal und Frankreich gefangen wurden, wurde dem Banc d'Arguin-Nationalpark (Mauretanien) zugeordnet, dem wichtigsten Überwinterungsgebiet des Zugweges. Phänologische Muster von Alpenstrandläufer (*Calidris alpina*), Sandregenpfeifer (*Charadrius hiaticula*) und Kiebitzregenpfeifer (*Pluvialis squatarola*) legten nahe, dass die ersten nordwärts ziehenden Vögel während der zweiten Märzhälfte im Mündungsgebiet des Tejo ankamen, wonach die Spitzenwerte im April oder May erreicht wurden.

***Keywords:*** Migratory connectivity; Stable isotopes; Stopover sites; Discriminant analysis; Toenails

## Introduction

The study of migratory connectivity involves not only linking populations across geographic areas between different stages of their annual cycle but also quantifying demographic flows and identifying migratory strategies (Webster & Marra 2005). Many of the ecological processes that determine population dynamics of migrants are triggered by individual behavioural decisions, such as where to go, how long to stay, and when to leave (Bearhop, Hilton, Votier, & Waldron 2004; Schaub, Jenni, & Bairlein 2008; Covino, Holberton, & Morris 2014). Therefore, identifying the origins of individuals sharing migratory routes and stopover sites is crucial to interpret inter-individual behavioural variability and further determine how changes driven by rapid habitat and/or climatic alterations in particular areas will impact populations.

Most shorebird species perform long-distance migrations, the individuals completing, twice a year, lengthy flights linking breeding and wintering grounds (van de Kam, Ens, Piersma, & Zwarts 2004). While breeding areas are mainly confined to the Arctic tundra and other high-latitude grounds, wintering shorebirds disperse over much wider latitudinal ranges, both through the northern and southern hemispheres. Beside their impressive migrations, shorebirds are also notorious for gathering in large numbers and by their dependence on a network of stopover sites along migratory routes (Delany, Scott, Dodman, & Stroud 2009). These areas are critical for birds to rest and to fuel the remaining migratory journey, and may form significant bottlenecks for migration (Buehler & Piersma 2008; Iwamura et al. 2014). At stopover sites, populations of mixed origins often adopting different migratory strategies overlap extensively (e.g. Atkinson et al. 2005; Masero et al. 2009), and the overall inability to discriminate such populations represents a serious obstacle for the estimation of demographic parameters for each one of them.

Tracking technology, which largely replaced ringing approaches that provided low and biased amounts of data, currently faces rapid development and new devices are being tested at high rates (Vandenabeele, Wilson, & Wikelski 2013). Nonetheless, tracking methodologies still present drawbacks, mainly related to the large size of devices (e.g. Hooijmeijer et al. 2014) and the high costs required by studies involving large sample sizes. In this scenario, biochemical markers such as stable isotopes are still the most promising tracers to study migratory connectivity in birds (Hobson 1999; Webster & Marra 2005; Clark, Hobson, & Wassenaar 2006; Rushing, Ryder, Saracco, & Marra 2014). Ratios of isotopes naturally occurring in a particular environment are passed across food webs and integrated in tissues of birds foraging in that environment according to well-understood processes (Hobson & Wassenaar 2008). Hence, birds migrating from one isotopically distinct food web to another will carry the signature of the first in the second, and can thus be assigned to their origin if reference values are known for the areas included in their migratory route. Nevertheless, this requires a careful choice of the tissue to be sampled (Hobson & Wassenaar 2008). Blood might be inappropriate to trace origins and migration patterns of birds due to its fast turnover rate (but see e.g. Dietz et al. 2010). In fact, this metabolic active tissue typically yields an isotopic record of just days to few weeks prior to collection date (Hobson & Wassenaar 2008), requiring tissue sampling within a short period since arrival date at the new grounds. Feathers have successfully been used in migratory studies, including studies with shorebirds, given that isotopic information is integrated during feather growth (in moulting areas), and remains unchanged after synthesis (Atkinson et al. 2005; Mazerolle & Hobson 2005; Clark et al. 2006; Rushing et al. 2014). However, the use of feathers might be problematic if moulting patterns are variable or not understood in detail, that is, when it is unknown which feathers are grown when and where. This applies to several shorebird species, showing variable and complex moulting cycles, with some individuals starting moults at the breeding areas while others replace both body and primary feathers during migration, in moulting areas or at the wintering grounds (Holmgren, Jönsson, & Wennerberg 2001; Meissner, Chylarecki, & Skakuj 2010; Meissner & Cofta 2014; Lourenço & Piersma 2015).

Recently, toenails (or claws) have helped establishing the geographic origins of migrant birds (Clark et al. 2006; Fraser, Kyser, Robertson, & Ratcliffe 2008; Hobson et al. 2006), including shorebirds sampled at their stopover sites (Catry, Martins, & Ganadeiro 2012). The use of toenails, especially with respect to growth and isotopic turnover rates are still under investigation, with several studies calling for accurate data from captive controlled experimental approaches (Bearhop, Furness, Hilton, Votier, & Waldron 2003; Mazerolle & Hobson 2005; Barquete, Strauss, & Ryan 2013; Hopkins, Cutting, & Warren 2013). Recent captivity experiments with a small-sized shorebird, the dunlin (*Calidris alpina*), indicated half-lives of approximately one month for both carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios in toenails, thus further validating the use of this tissue for studies of migratory connectivity (Lourenço, Granadeiro, Guilherme, & Catry 2015).

A recent study used stable isotopes to described the structure and functioning of food webs and shorebird communities in several tidal environments across the East Atlantic Flyway (Catry et al. 2015), a migration route connecting high latitude breeding grounds to temperate and tropical wintering areas located along the western coasts of Europe and Africa (Delany et al. 2009). This research identified considerable differences among study areas in the isotopic signatures of most components of food webs, including shorebirds (Catry et al. 2015). In the present study, we build on such evidence to investigate the potential of $\delta^{13}C$ and $\delta^{15}N$ signatures in toenails of shorebirds to identify their wintering origin and to trace their movements across the East Atlantic Flyway. In particular, we aim to: (1) describe and compare reference $\delta^{13}C$ and $\delta^{15}N$ signatures of shorebirds in five major wintering sites in Europe and Africa (in France, Portugal, Morocco, Mauritania and Guinea-Bissau), (2) identify the wintering origins of shorebirds during spring migration at two stopover areas (in France and Portugal) based on the discriminating power of stable isotope signatures and (3) evaluate the ability of this approach to infer migratory phenological patterns of shorebirds.

## Materials and methods

### Study sites and sample collection

Shorebirds were captured in five wetlands of international importance for birds along the East Atlantic Flyway: Marennes-Oléron Bay—France (45°54′N, 01°07′W), Tejo estuary—Portugal (38°45′N, 09°01′W), Sidi Moussa—Morocco (32°58′N, 8°45′W), Banc d'Arguin—Mauritania (19°52′N, 16°17W) and Bijagós archipelago—Guinea-Bissau (11°12′N, 15°53′W; Fig. 1). The two southernmost sites, in Mauritania and Guinea-Bissau, are the major wintering areas for shorebirds in West Africa, holding more than three million birds in total (Delany et al. 2009). The Tejo estuary and Marennes-Oléron Bay are among the most important wetlands for waders in Europe, holding several tens of thousand wintering birds but also receiving large numbers of migrants that use these areas as stopover sites during migratory periods (Delany et al. 2009; Catry et al. 2011; Bocher et al. 2014). For the sake of simplicity, sites are preferentially designated with the names of the countries. Shorebirds were captured with mist-nets, cannon-nets and crossbow-nets (Martins, Catry, & Granadeiro 2014). Sampling of shorebirds toenails took place between late December and February in two consecutive winters (2012/2013 and 2013/2014) to obtain reference winter isotopic ratios from all study areas. Sampling also took place between March and May (2013 and 2014, with few birds, i.e. 7%, sampled in 2012) in the Portuguese and French study sites, where resident wintering and migrant

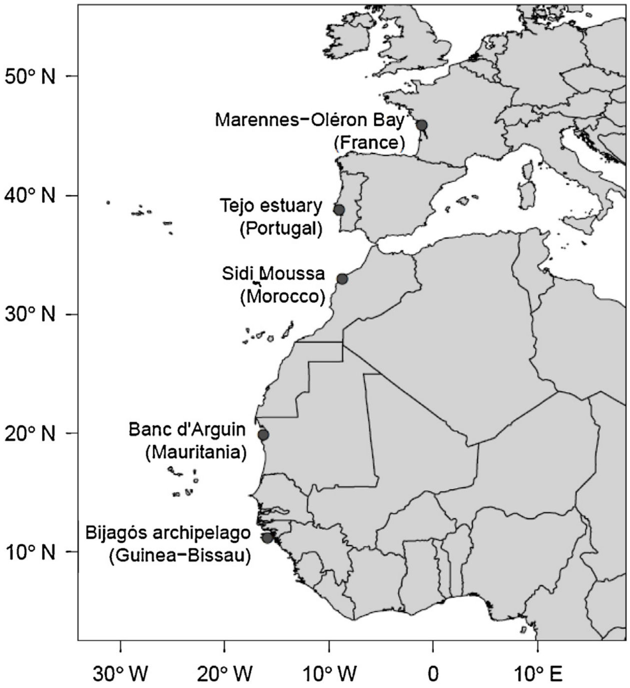

**Fig. 1.** Location of the study areas. Wintering shorebirds were sampled at the five study sites, whereas spring migrants were also sampled at Marennes-Oléron Bay (France) and Tejo estuary (Portugal), where resident wintering and migrant populations from southern areas overlap during spring migration.

populations from southern areas overlap during spring migration (Catry et al. 2011; Bocher et al. 2014). Between 1 and 2 mm of toenail were clipped from three to four toes of each bird using sharp scissors and stored in individual plastic bags.

## Stable isotope analysis

Toenails were washed twice in baths of 0.25 N sodium hydroxide solution for ca. 1 min in a vortex alternated with baths of double distilled water to remove adherent contamination, and then dried at 50 °C for 48 h (Catry et al. 2012). Between 0.6 and 1.0 mg of toenail were stored in tin cups for stable-carbon and nitrogen isotope assays and combusted at 1000 °C in a Euro EA Elemental Analyser, at the Stable Isotopes and Instrumental Analysis Facility of the Faculty of Sciences, University of Lisbon. Isotopic ratios were determined by continuous-flow isotope-ratio mass spectrometer IsoPrime (MicroMass). Results are presented conventionally as $\delta$ values in parts per thousand (‰) relative to the Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}$C, and atmospheric nitrogen ($N_2$) for $\delta^{15}$N. Precision of the isotope ratio analysis, calculated using values from 6 to 9 replicates of laboratory standard material (casein) interspersed among samples in every batch analysis, were 0.11 to 0.25‰ for $\delta^{13}$C and 0.05 to 0.17‰ for $\delta^{15}$N (SD).

## Data analysis

We used a linear discriminant analysis (LDA) to test whether $\delta^{13}$C and $\delta^{15}$N values in toenails can be used to predict the wintering origin of shorebirds. Isotopic signatures of birds captured at the five study areas during the winter season were used as the training data set to derive the discriminant functions for each species. These functions were then used to assess the wintering origin of birds captured during spring in Portugal and France (testing data set). The classification accuracy of discriminant functions was evaluated through the percentage of correctly assigned individuals from the training data set in leave-one-out cross-validations (Zuur, Ieno, & Smith 2007). Individuals of unknown origin were assigned to a specific site only when the probability of group membership was $\geq$95%.

Birds captured in France during the winter were not included in the training set used in the discriminant functions calculated for Portugal. In fact, it is most unlikely that birds captured in spring in Portugal could have come from France, given that they are migrating northwards. Conversely, discriminant functions drawn for France include data of birds from Portugal (except for Bar-tailed Godwit *Limosa lapponica* for which we did not collect samples of wintering birds in Portugal). Very few dunlins winter south of Mauritania (Delany et al. 2009) and none were captured in Guinea-Bissau. Thus, discriminant analysis for this species included all sites except Guinea-Bissau.

We were unable to capture wintering redshanks (*Tringa totanus*) in France. Thus, in order to assign the origin of individuals captured during spring, we used blood signatures, instead of toenails, of 13 birds collected in the same French site during the winter 2006–2007. To validate this option, we used data from dunlins captured at their wintering grounds, which showed coefficients of determination ($R$) and concordance correlation coefficients (CCC) between red blood cells and toenails close to one for both $\delta^{13}$C and $\delta^{15}$N values ($R^2 = 0.93$, $p < 0.001$, $n = 36$ and CCC = 0.93, 95% CI [0.87–0.96] for $\delta^{13}$C; $R^2 = 0.90$, $p < 0.001$, $n = 36$ and CCC = 0.93, 95%CI [0.87–0.96] for $\delta^{15}$N; author's unpublished data). Concordance correlation coefficients evaluate the degree to which pairs of observations fall on the 45° line through the origin (Lin 1989).

Dunlin, common ringed plover (*Charadrius hiaticula*; hereafter ringed plover) and grey plover (*Pluvialis squatarola*) were regularly captured in reasonable numbers across the spring period in Portugal. Therefore, for these species, we were able to estimate the probability of occurrence of passage migrants in relation to calendar date using generalized linear models (GLMs) with a binomial error distribution and a logit link function. For this purpose, linear discriminant functions were drawn using only two groups from the training data set: Portuguese wintering birds and African wintering birds (pooling all individuals from Morocco, Mauritania and Guinea-Bissau). Accordingly, birds captured during spring migration in Portugal were identified as local wintering birds or migrants from Africa.

All analyses were performed with R version 3.0.1 (R Core Team 2013).

## Results

We sampled a total of 542 individuals of nine species during the winter across the five studied areas, plus 508 birds of the same species during spring migration in Portugal and France (see Tables 1 and 2).

### Performance of discriminant functions to segregate shorebirds' wintering grounds

Discriminant analyses did overall well in distinguishing the isotopic signals of birds from wintering areas in most species, correctly classifying over 80% of all individuals in the training set. Lower percentages were found for red knot and redshank in the analyses including France (73 and 75% of correct classifications; Table 1). For dunlin, sanderling, turnstone, ringed plover and curlew sandpiper, the proportion of correctly assigned individuals was ≥90% (Table 1). Overall, the highest corrected assignment rates for the training data set were found for Mauritania and Portugal.

### Origins and phenology of shorebirds captured during spring migration

Overall, of the 321 birds captured during spring migration in Portugal (belonging to eight different species), 96% could be assigned to a wintering origin with a very high (≥95%) probability of group membership (Table 2). Assignment rates were higher than 80% for all species except for redshank and red knot (Table 2). Sample sizes in the testing data set for these two species were, however, very small ($n=4$). With ca. 40% of 185 individuals assigned, in France, assignment rates during spring migration were lower (Table 2).

Dunlins achieved the highest assignment rates in both Portugal and France with most migrating birds coming from Mauritania (Figs. 2 and 3, Table 2). The first West African dunlins started arriving in Portugal by the end of March, and during the first half of April wintering birds became comparatively less abundant (Fig. 4).

**Table 1.** Classification results (% of correctly classified individuals, sample size in parenthesis) of linear discriminant function analysis (LDA) based on carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotope ratios of wintering shorebirds in five study areas within the East Atlantic Flyway, obtained by cross-validation (training data set). Results are presented for two separate sets of discriminant analysis, one excluding and another including birds from France (see the Materials and methods section for details).

| | | Classification results (%) for reference wintering areas (*n*) | | | | | |
|---|---|---|---|---|---|---|---|
| LDA | Species | France | Portugal | Morocco | Mauritania | Guinea-Bissau | Total |
| Without France | Dunlin *Calidris alpina* | – | 100 (56) | 97 (29) | 100 (27) | – | 99 (112) |
| | Sanderling *Calidris alba* | – | 100 (7) | 100 (10) | 95 (22) | 100 (10) | 98 (49) |
| | Curlew sandpiper *Calidris ferruginea* | – | 88 (8) | 0 (3) | 100 (25) | 100 (5) | 90 (41) |
| | Red knot *Calidris canutus* | – | 94 (17) | 0 (5) | 100 (24) | 79 (14) | 85 (60) |
| | Ringed plover *Charadrius hiaticula* | – | 100 (17) | 80 (10) | 100 (11) | 93 (15) | 94 (53) |
| | Turnstone *Arenaria interpres* | – | 91 (11) | 100 (5) | 100 (20) | 100 (8) | 98 (44) |
| | Grey plover *Pluvialis squatarola* | – | 100 (20) | 71 (7) | 67 (6) | 75 (8) | 85 (41) |
| | Redshank *Tringa totanus* | – | 92 (26) | 71 (21) | 90 (10) | 80 (10) | 84 (67) |
| With France | Dunlin *Calidris alpina* | 80 (5) | 100 (56) | 93 (29) | 100 (27) | – | 97 (117) |
| | Red knot *Calidris canutus* | 73 (15) | 59 (17) | 0 (5) | 100 (24) | 71 (14) | 73 (75) |
| | Grey plover *Pluvialis squatarola* | 80 (15) | 100 (20) | 71 (7) | 67 (6) | 62 (8) | 82 (46) |
| | Redshank *Tringa totanus* | 46 (13) | 92 (26) | 62 (21) | 90 (10) | 80 (10) | 75 (80) |
| | Bar-tailed godwit *Limosa lapponica* | 86 (14) | – | 25 (4) | 100 (12) | 71 (7) | 81 (37) |

**Table 2.** Proportion of individuals from different shorebird species captured in spring (testing data set, sample size in parenthesis) assigned to their wintering areas, and identification of winter origins as determined by discriminant analysis based on carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotope ratios. Results are presented separately for birds captured in Portugal and France.

| | | | Winter origin of birds captured during spring | | | | |
|---|---|---|---|---|---|---|---|
| Species | Place of capture | % Assigned | France | Portugal | Morocco | Mauritania | Guinea-Bissau |
| Dunlin | Portugal | 99 (172) | – | 45 | 4 | 121 | – |
| *Calidris alpina* | France | 72 (57) | 2 | 1 | 0 | 38 | – |
| Sanderling *Calidris alba* | Portugal | 100 (18) | – | 16 | 1 | 0 | 1 |
| Curlew sandpiper *Calidris ferruginea* | Portugal | 100 (7) | – | 5 | 1 | 1 | 0 |
| Red knot | Portugal | 0 (4) | 0 | 0 | 0 | 0 | 0 |
| *Calidris canutus* | France | 57 (42) | 0 | 0 | 0 | 24 | 0 |
| Ringed plover *Charadrius hiaticula* | Portugal | 85 (60) | – | 35 | 5 | 9 | 2 |
| Turnstone *Arenaria interpres* | Portugal | 100 (16) | – | 11 | 3 | 2 | 0 |
| Grey plover | Portugal | 86 (42) | – | 28 | 6 | 1 | 1 |
| *Pluvialis squatarola* | France | 50 (30) | 10 | 0 | 2 | 3 | 0 |
| Redshank | Portugal | 75 (4) | – | 2 | 1 | 0 | 0 |
| *Tringa totanus* | France | 47 (38) | 9 | 0 | 0 | 9 | 0 |
| Bar-tailed godwit *Limosa lapponica* | France | 67 (18) | 4 | – | 0 | 8 | 0 |

Grey plovers migrating through Portugal were relatively easy to segregate from the locally wintering birds (assignment proportion = 95%, $n = 42$), but almost half of the migrants could not be accurately assigned to their specific wintering origin (Fig. 2, Table 2). Our results suggest that some birds from southern origins arrive at the Tejo estuary in the second half of March, but most migrants were captured in late April and May (although they were still a minority among all sampled birds; Fig. 4). In France, assignment rates of grey plovers were lower (Fig. 3).

Our results show that the assignment of ringed plovers captured during spring in Portugal to their wintering origin was also accurately performed (85%), with the proportion of assigned birds rising to 98% when considering only Portuguese vs. African winterers. Of the ringed plovers assigned to winter origin, 68% were classified as Portuguese, while among migrants the majority was assigned to Mauritania, followed by Morocco and Guinea-Bissau (Fig. 2, Table 2). The first passing ringed plovers seem to arrive in Portugal by late March, but their number only surpasses that of local wintering birds during the second half of April (Fig. 4).

Discriminant functions presented good classification results for sanderlings, turnstones and curlew sandpipers sampled during spring migration in Portugal. In these species, a high proportion of individuals were classified as local wintering birds, with few assigned to West African wintering areas (Fig. 2, Table 2).

The assignment rate of red knots captured in France was low (57%), which might be due to the similarity in isotopic signatures between birds from France and Guinea-Bissau, precluding an accurate assignment to any of the areas. All knots successfully classified were assigned to Mauritania (Fig. 3, Table 2). Similarly low assignment rates were obtained for redshanks and bar-tailed godwits sampled in France, with most birds assigned either to France or Mauritania. However, in both species a relatively high proportion (ca. 30%) of birds showed isotopic signatures (both $\delta^{13}$C and $\delta^{15}$N depleted) that hardly fit in any of the reference populations (although some of those were classified as local wintering birds by the discriminant analysis; Fig. 3).

## Discussion

Our study demonstrates the potential of stable isotopes in distinguishing shorebirds from distinct origins co-occurring at stopover sites and assigning them to wintering grounds. The use of stable isotopes to trace migration in shorebirds is largely under-explored and to date less than 10 published studies have employed this tool in this particular group. The choice of toenails as sampling tissue proved critical to obtain accurate results within a bird group with poorly understood and highly variable moulting processes (Catry et al. 2012). A recent experimental setup involving a controlled diet switch in captive dunlins during which toenail growth rates and $\delta^{13}$C and $\delta^{15}$N were measured at regular periods, validated the temporal window of this tissue for studies of migratory connectivity (Lourenço et al. 2015). According to this study, toenails have half-lives of 27 and 35 days for carbon and nitrogen isotopes, respectively (Lourenço et al. 2015). Our

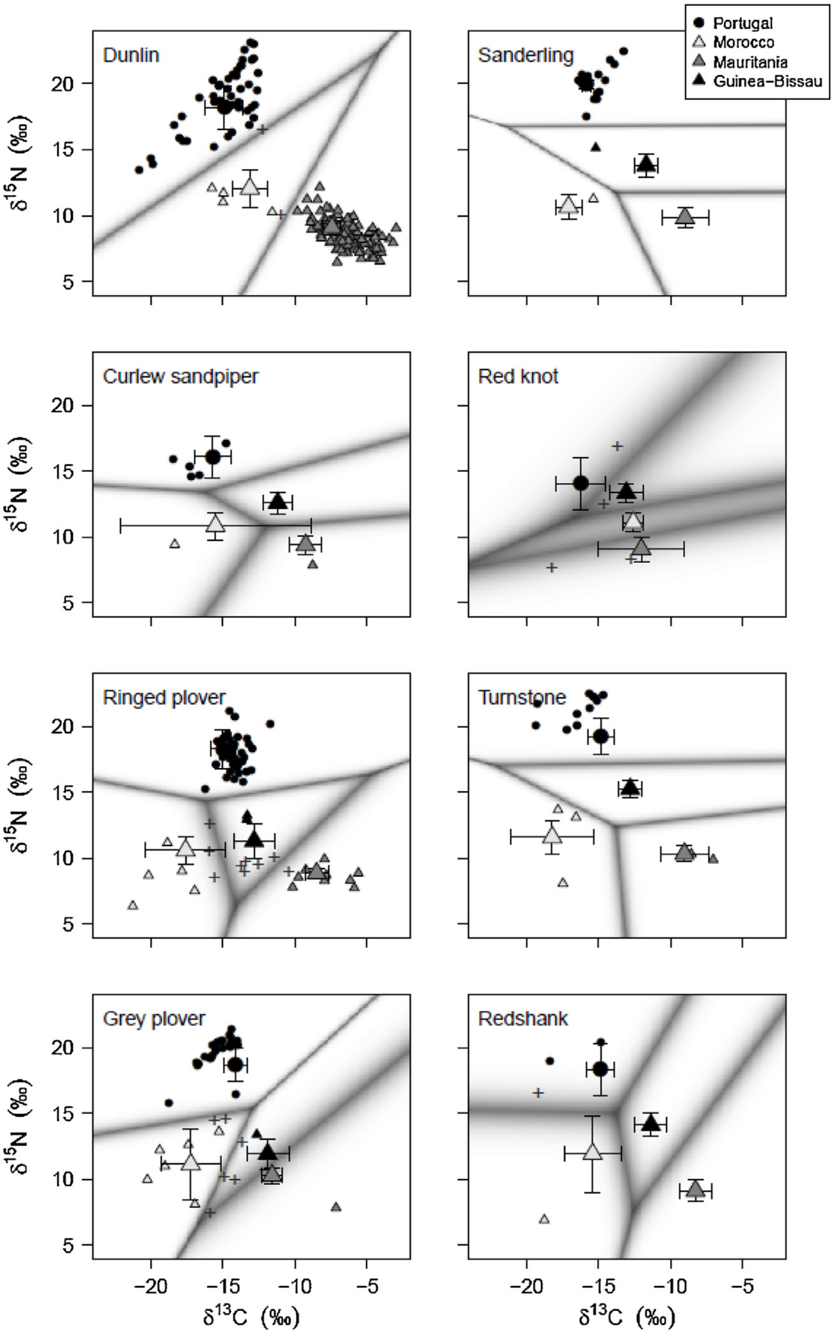

**Fig. 2.** Carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) stable isotope signatures of shorebirds sampled at reference wintering sites in Portugal, Morocco, Mauritania and Guinea-Bissau (mean ± SD; large symbols), and during spring migration (March–May) at the Tejo estuary, Portugal (small symbols). Individuals captured in spring and assigned to a particular wintering area with linear discriminant analysis are represented by the symbol and colour of the corresponding wintering population, whereas all others are represented by a "plus" sign. Lines within each plot represent the discriminant functions obtained from the training data set (see the Materials and methods section) and shades represent the approximate boundaries of group membership (from 50% to 95%).

study further supports these early findings, and represents the first approach conducted at the community-level and one of the few covering a large geographic scale.

Overall, we achieved high assignment rates (>80%) with the training data set, i.e., wintering shorebirds sampled at distinct geographic areas could be segregated from each other and accurately assigned to their origin. Results obtained for the testing data set, i.e., for birds captured during spring migration, showed that the proportion of individuals assigned to wintering areas might vary among species and (stopover) areas. In Portugal, the segregation between local wintering individuals and passage migrants, as well as the classification of migrants from different African origins, was highly successful. In France, however, assignment rates were

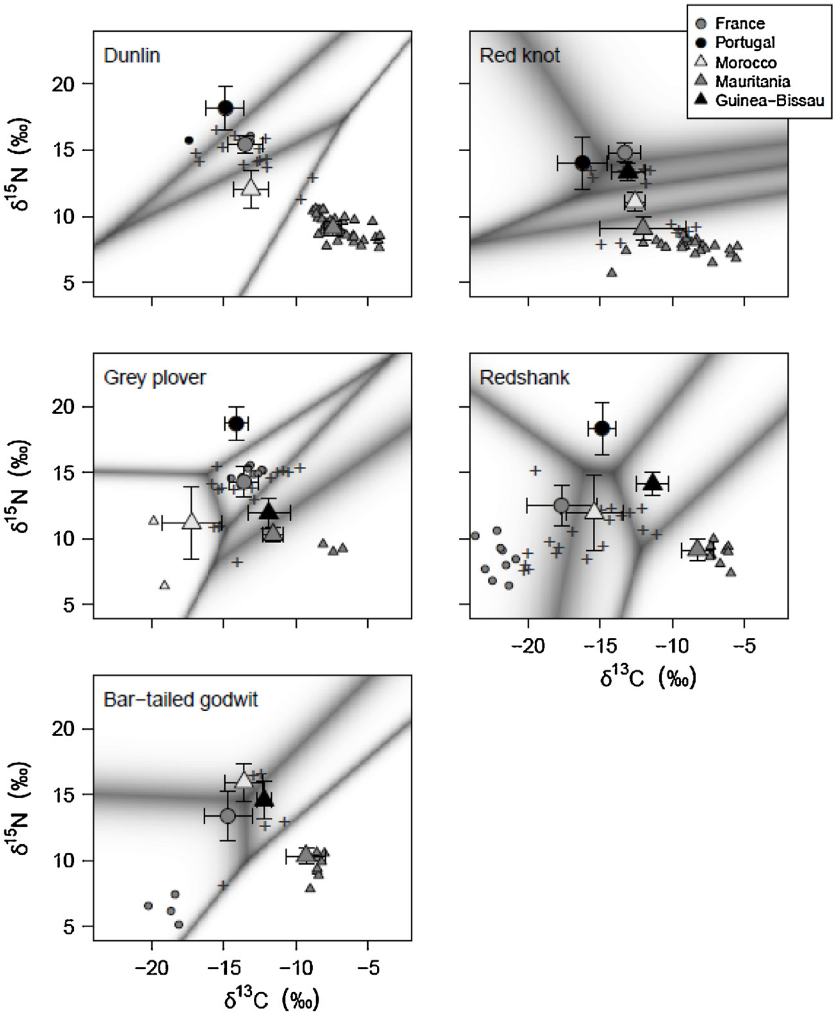

**Fig. 3.** Carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) stable isotope signatures of shorebirds sampled at reference wintering sites in France, Portugal, Morocco, Mauritania and Guinea-Bissau (mean ± SD; large symbols), and during spring migration (March–May) at Moëze, France (small symbols). Individuals captured in spring and assigned to a particular wintering area with linear discriminant analysis are represented by the symbol and colour of the corresponding wintering population, whereas all others are represented by a "plus" sign. Lines within each plot represent the discriminant functions obtained from the training data set (see the Materials and methods section) and shades represent the approximate boundaries of group membership (from 50% to 95%).

generally lower, with shorebirds originating from Mauritania being easily identified, while the more similar signatures between birds from France and Morocco or Guinea-Bissau produced lower membership percentages for these areas.

Adding other chemical tracers could potentially increase discrimination power for some species/localities. Either stable isotopes of other elements (e.g. sulfur, $\delta^{34}$S) or concentrations of several trace elements (e.g. Pb, Cu, Zn, Mn, Mg, Na, Ba, Hg, among others) have been used to track bird migration and/or origins (Parrish, Rogers, & Ward 1983; Hobson 1999). Among shorebird studies, these elements have produced contrasting results, either encouraging (Norris et al. 2007) or dissuading their use (Torres-Dowdall, Farmer, Abril, Bucher, & Ridley 2010). A potential problem for a multi-tracer analyses, however, is the amount of tissue required. In small-sized shorebirds, only ca. 2 mg of toenail can be collected per individual, which might prove insufficient to run analysis for multiple elements (Hobson & Wassenaar 2008).

A large proportion of spring migrants captured during stopover in Portugal and France were assigned to Mauritania. This is not surprising, as Banc d'Arguin, in Mauritania, is the most important wintering area of this flyway, holding more than 2 million shorebirds (Delany et al. 2009). A lower proportion of birds was assigned to Guinea-Bissau, which ranks as the second African wintering ground within the East Atlantic Flyway (with ca. 700 000 birds; Delany et al. 2009). The expected ratios of migrants from the two African areas are difficult to determine accurately given the uncertainty in numbers from wintering counts (Salvig, Asbirk, Kjeldsen, & Rasmussen 1994; Zwarts et al. 1997). Nonetheless, for species such as ringed plover and especially red knot, the low number of birds assigned to Guinea-Bissau might be partly an artefact of the relatively high proportion of birds

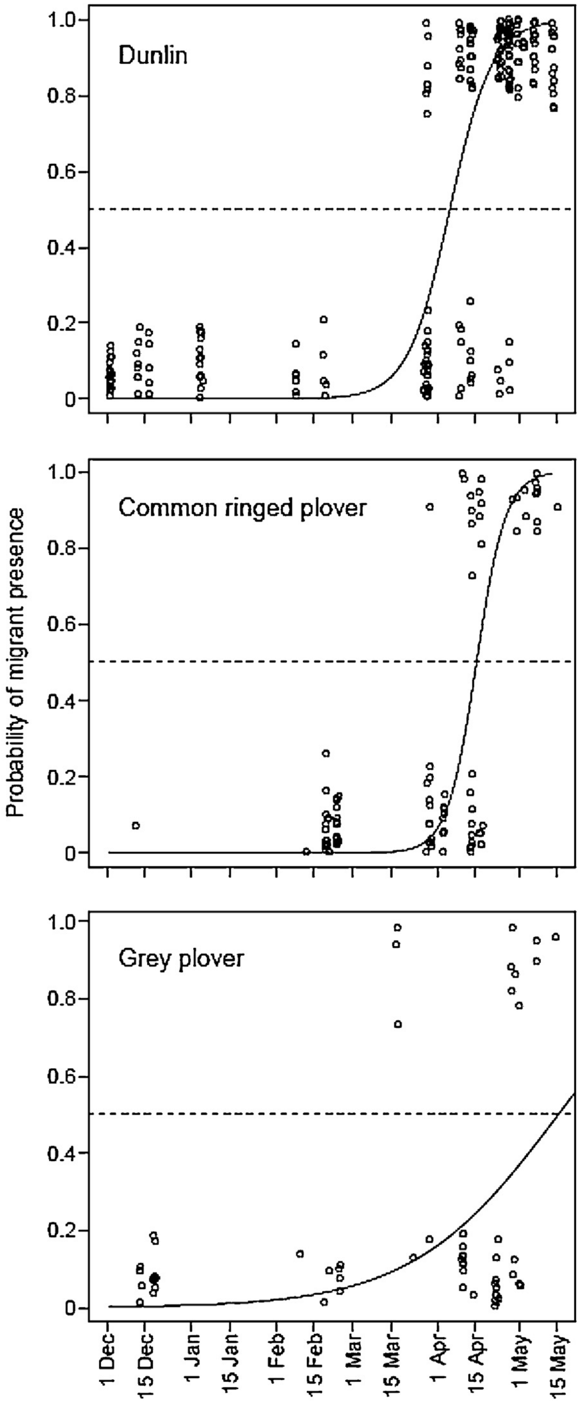

**Fig. 4.** Predicted probability of occurrence of passage migrant dunlins, grey plovers and common ringed plovers at the Tejo estuary, in Portugal, fitted by a logistic regression.

that remained unclassified with our 95% probability membership criteria, but which would fall in the Guinea-Bissau group under a less demanding rule. Also, we cannot exclude the possibility that birds may arrive from other non-sampled wintering areas, namely from Senegal and Guinea-Conakry, although contributions to the pool of migrants should be relatively modest considering the lower importance of these wintering sites (Delany et al. 2009). Nonetheless, further studies focused on particular species, should ideally take into account all wintering areas with relevant numbers of wintering birds from the target species.

The first southern migrant dunlins, ringed plovers and grey plovers, the three species for which we had regular capture events along the spring season in Portugal, all started arriving at the Tejo estuary during the second half of March. Contrasting with the high number of African dunlins captured in Portugal during spring migration, the proportion of migrants vs. local wintering birds in the other two species, and also among sanderlings and turnstones, was biased towards local populations. This observation suggests that spring migratory flows of these species across the Tejo estuary either involve a relatively small number of birds or a high turnover rate of individuals, diluting the number of migrants within the local wintering populations. Different migratory strategies may explain to some extent the inter-specific differences described here. Dunlins, for instance, are known to adopt a “hopper” migratory strategy, with a high number of stopover localities separated by shorter distances (Pienkowski & Dick 1975; Piersma 1987; van de Kam et al. 2004), and are therefore expected to occur in comparatively large numbers in Portugal. For other species, the journey from Northwest Africa to the breeding grounds might be often completed in only two long flights (van de Kam et al. 2004; Delany et al. 2009), with most birds skipping stopover areas in southern Europe. Red knots and bar-tailed godwits caught in France in spring and assigned to Mauritania might be unable to fly directly to the Wadden Sea and use the Atlantic coast of France as an “emergency” stopover area (Delany et al. 2009; Shamoun-Baranes et al. 2010; Bocher, Quaintenne, Robin, Doumeret, & Delaporte 2012).

## Conclusions

Our study shows that stable isotope analysis of toenails can be used to assign winter origins of migrant shorebirds at stopover sites, although the success of this assignment might vary across geographic areas and species. This information will expand current knowledge on migration flyways, but it also offers a unique opportunity to study the different strategies of migrant birds and assess potential carry-over effects of particular strategies. The ability to distinguish the winter origin of individuals allows for a set of comparative ecological studies (e.g. foraging behaviour, habitat and space use) at stopover areas between, for instance, passage migrants and local wintering populations. Moreover, as shown here, this methodology proved to be appropriate to infer migratory phenological patterns of migrant shorebirds, which is often unfeasible given the degree of overlap among different populations in the same area during migratory periods. Long-term datasets of population composition (in terms of winter origin) and phenological patterns of migrating populations at important stopover areas may provide an invaluable contribution to assess changes in population dynamics along the flyway.

In particular, we might be able to monitor the responses of birds to habitat loss and to expected climate-driven changes (e.g. Godet, Jaffré, & Devictor 2011), as well as to identify which populations and areas are in emergent risk.

Future research could evaluate how this approach performs during autumn migration, although it will require getting access to reference isotopic signatures for extremely wide and often inaccessible breeding areas.

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