


# Using stable isotopes to assess population connectivity in the declining European Turtle Dove (*Streptopelia turtur*)

Melanie Marx<sup>1</sup>  | Gregorio Rocha<sup>2</sup> | Pavel Zehtindjiev<sup>3</sup> | Strahil Peev<sup>3</sup> |  
Dimitris Bakaloudis<sup>4</sup> | Benjamin Metzger<sup>5</sup> | Jacopo G. Cecere<sup>6</sup> |  
Fernando Spina<sup>6</sup> | Marco Cianchetti-Benedetti<sup>1</sup> | Sylke Frahnert<sup>7</sup> |  
Anita Gamauf<sup>8†</sup> | Christian C. Voigt<sup>9</sup> | Petra Quillfeldt<sup>1</sup>

<sup>1</sup>Department of Animal Ecology & Systematics, Justus-Liebig University Giessen, Giessen, Germany

<sup>2</sup>Department of Zoology, Veterinary School, University of Extremadura, Cáceres, Spain

<sup>3</sup>Bulgarian Academy of Sciences, Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria

<sup>4</sup>Laboratory of Wildlife & Freshwater Fish, School of Forestry and Natural Environment, Aristotle University of Thessaloniki, Thessaloniki, Greece

<sup>5</sup>Rua da Esperança, Lisbon, Portugal

<sup>6</sup>Area Avifauna Migratrice, Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA), Bologna, Italy

<sup>7</sup>Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany

<sup>8</sup>Museum of National History, Vienna, Austria

<sup>9</sup>Leibniz Institute for Zoo and Wildlife Research (IZW), Alfred-Kowalke-Straße 17, Berlin, Germany

## Correspondence

Melanie Marx, Department of Animal Ecology & Systematics, Justus-Liebig University Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany.  
Email: melanie.marx@bio.uni-giessen.de

## Abstract

European Turtle Doves (*Streptopelia turtur*) are long-distance migrants and have experienced a population decline of more than 78% since 1980. Their conservation depends on refined knowledge of breeding origins and population connectivity. Feathers collected at stopover sites, but molted at breeding grounds, provide an opportunity to assign birds to potential regions of origin using tissue stable hydrogen isotope values and relate those to a European feather hydrogen isoscape. Here, 101 feather samples from 13 different breeding countries were analyzed to calibrate the European hydrogen isoscape and 101 feather samples from Spanish, Italian, Maltese, Greek, and Bulgarian stopovers were assigned to potential regions of origin. The assigned range of origin for all 101 individuals grouped together agreed with known distribution patterns. Bulgarian samples were mostly assigned to Russian areas. Possible origins of Greek, Italian, Maltese, and Spanish samples ranged from central to southern Europe. Individual assignments highlighted four broad regions of origin, corresponding to a cool/humid to hot/dry temperature gradient. Proportions of birds assigned to these regions varied among birds sampled at different stopover sites. Therefore, our results provide important information about population connectivity and may be useful to evaluate possible influences of hunting on Turtle Dove populations.

## KEYWORDS

breeding origins, feather samples, IsoriX,  $\delta^2\text{H}$

<sup>†</sup>Deceased.

**Article impact statement:** Using stable isotopes, we infer breeding origins of European Turtle Doves sampled during migration, providing useful information for species protection.

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## 1 | INTRODUCTION

European Turtle Doves (*Streptopelia turtur*, hereafter called Turtle Doves) are the only long-distance migrating columbids in Europe (Glutz von Blotzheim & Bauer, 1994). However, despite a severe population decline of more than 78% since 1980 (European Bird Census Council, 2017), little is known about their population connectivity and important life-cycle stages. Such information about breeding and wintering areas of migrants can be obtained with different methods, such as mark-recapture or tracking studies. Furthermore, details about inhabited breeding and wintering regions are crucial to design and implement effective management plans (Martin et al., 2007; Ocampo-Peñula & Pimm, 2014; Williams & Araújo, 2000). Additionally, such data can be a basis for meta-analyses to estimate potential impacts of threats on distant populations, such as hunting.

The known breeding distribution of Turtle Doves reaches from Europe (except most of northern Europe, e.g., Norway, Sweden, most of Finland) to North Africa and central Asia. The wintering areas are in sub-Saharan Africa (BirdLife International and Handbook of the Birds of the World, 2016; Glutz von Blotzheim & Bauer, 1994). Ring re-encounter and tracking studies highlighted a western and eastern flyway leading to and from breeding and wintering destinations (French birds: Eraud et al., 2013; <http://turtledoverresearch.com/fr/>; British birds: <https://www.rspb.org.uk/our-work/conservation/satellite-tracking-birds/tracking-turtle-doves>; Italian birds: <https://blogs.nabu.de/zugvoegel/category/turtauben/>; Marx et al., 2016). Both methods indicated a split of the eastern flyway into a central (across Italy) and eastern route (across Greece) (Italian birds: <https://blogs.nabu.de/zugvoegel/category/turtauben/>; Marx et al., 2016). Data from birds from the United Kingdom, Spain, and France using the western flyway suggested a strong population connectivity, and data from those following the eastern flyway (e.g., from Czech Republic and Greece) indicated a weak connectivity due to flyway permeability (Marx et al., 2016). This was in line with population genetic analyses showing no genetic structure and indicating gene flow among eastern and western European populations (Calderón, 2016).

However, besides the determination of flyways, identification of important breeding and wintering areas is still pending and probably needs different methodological approaches, due to some disadvantages of previously applied methods. Regarding ring re-encounter analyses, many ring data remain unreported, unprocessed or unavailable due to data loss and because the method can have a delay of several years from initial marking to re-encounter. Specifically in Turtle Dove studies, most populations are hunted during migration. Despite high ringing activity in some countries, only a low number of re-

encounters were recorded at potential winter quarters. Moreover, ringing records mostly represent places with high ringing activity, but not necessarily breeding or wintering sites (Marx et al., 2016). Nevertheless, tracking studies were able to reveal individual breeding sites and movements to and from winter sites, but are limited until now to a low number of individuals because of high costs and the potentially high failure rate of tagged birds due to the aerodynamic effects of tracking devices (Bowlin et al., 2010).

Recently, stable isotope analyses have become an important tool in studies of population connectivity and origin assignment of breeding and wintering areas (Cardador, Navarro, Forero, Hobson, & Mañosa, 2015; Hobson et al. 2012; Hobson, Lormée, Van Wilgenburg, Wassenaar, & Boutin, 2009; Hobson & Wassenaar, 2019). The main advantage of this method is that a very small feather sample (0.3 mg) can be taken from hunted, dead or living birds. Thus, the method is non-invasive, widely applicable and suitable to obtain information quickly from a large number of birds.

Here, feathers known to have grown in breeding regions were used to run origin assignments (Bearhop et al., 2005; Hobson, 2005; Hobson et al., 2006). This is possible due to the metabolically inactive state of feathers and therefore locked-in geographic isotopic information (Hobson, 2005; Hobson et al., 2009; Hobson & Wassenaar, 2019; Wunder, Kester, Knopf, & Rye, 2005). Stable isotopes of the elements carbon, nitrogen and hydrogen are most frequently used in assignment studies because they show predictable patterns over geographic and ecological gradients (Wunder, 2012).

Amount-weighted, long-term average precipitation stable hydrogen isotope values ( $\delta^2\text{H}_p$ ) vary with elevation and latitude (Rozanski, Araguás-Araguás, & Gonfiantini, 1993), particularly in the Northern Hemisphere (Bowen, Wassenaar, & Hobson, 2005; Hobson, 1999, 2005; Hobson & Wassenaar, 2019). Accordingly,  $\delta^2\text{H}$  values in plants and water vary geographically with temperature and humidity gradients (West, Bowen, Cerling, & Ehleringer, 2006). Generally, stable hydrogen isotope values increase towards southern and southwestern directions (Hobson, Bowen, Wassenaar, Ferrand, & Lormée, 2004; Rozanski et al., 1993; West, Sobek, & Ehleringer, 2008) and stable hydrogen ratios in feathers ( $\delta^2\text{H}_f$ ) can be used to assign origins of European migratory birds (Hobson et al., 2009). The Global Network of Isotopes in Precipitation (GNIP; <https://nucleus.iaea.org/Pages/GNIPR.aspx>) database provides an archive on monthly stable hydrogen isotope values in precipitation across Europe. Therefore, a Pan-European breeding assignment using stable hydrogen isotope values is feasible (Bearhop et al., 2005; Cardador et al., 2015; Hobson et al., 2009).

The main emphasis of this study was to assign potential breeding areas (hereafter, regions of origin or origin

**TABLE 1** Overview of European Turtle Dove samples included in this study to calibrate and assign origins of migrating birds. Study sites, sample origins and total sample numbers per place as well as the numbers of calibration and assignment samples per place are given

Country	Place	Longitude	Latitude	Origin	Subspecies	Collection date	N total	N calibration data	Mean $\delta^2H_f$ calibration data	N assignment data
Algeria	Batna	6.166667	35.55	National History Museum Berlin	<i>S. t. arenicola</i>	June 15, 1903	1	1	−24	
Austria	Lower Austria	16.136389	48.618611	Museum of National History Vienna	<i>S. t. turtur</i>	July 20, 1996	1	1	−56	
Austria	Vienna (Prater)	16.398029	48.216727	Museum of National History Vienna	<i>S. t. turtur</i>	June 23, 1980	1	1	−69	
Austria	Vienna (Lobau)	16.516667	48.183333	Museum of National History Vienna	<i>S. t. turtur</i>	June 31, 1925; July 10, 1925; July 10, 1937	3	3	−67	
Bulgaria	North of Kavarna			Hunt	<i>S. t. turtur</i>	August 23, 2014	9			9
France	Auvergne	3.3	45.7	Captured alive	<i>S. t. turtur</i>	June/July 2014	19	19	−54	
France	Chize	−0.3464	46.1164	Captured alive	<i>S. t. turtur</i>	June/July 2014	18	18	−53	
France	Marne	4.358333	48.958333	Captured alive	<i>S. t. turtur</i>	June/July 2014	20	20	−53	
France	Oleron	−1.290278	45.938889	Captured alive	<i>S. t. turtur</i>	June/July 2014	20	20	−46	
Germany	Dahme	11.087778	54.223056	National History Museum Berlin	<i>S. t. turtur</i>	June 26, 1981; July 6, 1981	2	2	−56	
Germany	Undefined	10.451526	51.165691	National History Museum Berlin	<i>S. t. turtur</i>	June 1, 1890	1	1	−55	
Germany	Villingen	8.90742	50.50395	Captured alive	<i>S. t. turtur</i>	June 17, 2014	1	1	−66	
Greece	Levittia	26.459722	37.011111	Museum of National History Vienna	<i>S. t. turtur</i>	June 1, 1935	1	1	−51	
Greece	Dadia Soufli National Park	26.299167	41.191667	Captured alive at nest (nestlings)	<i>S. t. turtur</i>	July 15, 2014; July 17–18, 2014	3	3	−57	
Greece	Soufli			Hunt	<i>S. t. turtur</i>	August 20–25, 2013	20			20
Iran	Abasabad	46.833333	38.9166669	National History Museum Berlin	<i>S. t. arenicola</i>	June 9, 1927	1	1	−63	
Italy	Ferrara	11.619790	44.8381200	Wildlife recovery center	<i>S. t. turtur</i>	July 29, 2014	1	1	−80	
Italy	Rieti	12.856730	42.4045100	Wildlife recovery center	<i>S. t. turtur</i>	June 20, 2014	1	1	−46	

(Continues)

TABLE 1 (Continued)

Country	Place	Longitude	Latitude	Origin	Subspecies	Collection date	N total	N calibration data	Mean $\delta^2\text{H}_f$ calibration data	N assignment data
Italy	Ventotene Island			Captured alive	<i>S. t. turtur</i>	April 28–30, 2014; May 12, May 14, May 15, May 21, May 24, 2014	16			16
Israel	Haifa	34.989571	32.7940463	Museum of National History Vienna	<i>S. t. arenicola</i>	July 14, 1972	1	1	–55	
Israel	Neve Yam	34.93188	32.679769	Museum of National History Vienna	<i>S. t. arenicola</i>	July 19, 1968	1	1	–40	
Kazakhstan	Ilisk	66.923684	48.019573	Museum of National History Vienna	<i>S. t. arenicola</i>	June 7, 1900	1	1	–11	
Latvia	Ziemupe	21.0752181	56.7470561	National History Museum Berlin	<i>S. t. turtur</i>	July 18, 1890	1	1	–58	
Malta	Comino			Captured alive	<i>S. t. turtur</i>	April 21, 2014; May 6, 2014	5			5
Malta	Undefined			Hunt	<i>S. t. turtur</i>	April 2013; October 27, 2014	10			10
Poland	Borowke	22.1389946	49.9509415	National History Museum Berlin	<i>S. t. turtur</i>	June 1, 1944	1	1	–71	
Russia	Kasar	36.845235	52.8980995	Museum of National History Vienna	<i>S. t. turtur</i>	June 1, 1943	1	1	–36	
Spain	Monfrague			Hunt	<i>S. t. turtur</i>	August 29, 2013; August 31, 2013	41			41
Turkey	Ankara	32.85411	39.92077	National History Museum Berlin	<i>S. t. turtur</i>	June 27, 1933	1	1	–34	
N total							202	101		101



regions) of Turtle Doves migrating along the western Palearctic flyways. The study was based on  $\delta^2\text{H}_f$  values of Turtle Doves sampled at different migration stopover sites to a calibrated Pan-European  $\delta^2\text{H}_f$  isoscape. Determined regions of origin can be used to provide information about the geographic range of hunting effects at stopover sites on Turtle Dove populations. Furthermore, the hunting impact on remote populations can be evaluated at different stopover sites and compared between those sites. In particular, we worked on three aims:

1. Estimate the geographic accuracy with which migrating populations and individuals can be assigned to potential breeding origins.
2. Estimate the proportions of birds with different breeding origins for the individual stopover sites.
3. Estimate the proportions of birds with national versus foreign breeding origin for the individual stopover sites.

## 2 | METHODS

### 2.1 | Study species

Turtle Doves prefer warm, temperate climates and usually breed below 350 m elevation in Europe (Bakaloudis, Vlachos, Chatzinikos, Botzorlos, & Papakosta, 2009; Glutz von Blotzheim & Bauer, 1994). Nonetheless, further south in Europe and closer to Mediterranean climates, they breed in areas with elevations over 500 m in temperate zones and up to 1,300 m in southern Europe (del Hoyo, Elliott, & Sargatal, 1997; Glutz von Blotzheim & Bauer, 1994).

While in the breeding areas, the majority of adult and juvenile Turtle Doves start molt in July. They renew the first to occasionally fourth primary feathers, then suspend molt before autumn migration begins in August. They reach the wintering grounds in September where they renew their remaining feathers (Blasco-Zumeta & Heinze, 2011; Cramp, 1985; Glutz von Blotzheim & Bauer, 1994; Marx et al., 2016).

### 2.2 | Feather sampling

Feather samples were collected from migrating individuals, rescued birds from wildlife recovery centers, nestlings or breeding birds. Individuals were either hunted, or intentionally captured alive by hand (nestlings from Greece, rescued birds from Italy), with special bird trapping equipment (e.g., whoosh or mist net), or they originated from museum collections (Table 1). A small subsample of approximately 1 cm<sup>2</sup> was cut from the first

(P1;  $N = 199$ ) or second (P2;  $N = 3$ ) primary feather. In total, we collected 202 feather samples, which originated from 16 different countries (Table 1). Phenological analyses (Marx et al., 2016) and tracking studies (French birds: Eraud et al., 2013; Office National de la Chasse et de la Faune Sauvage, 2017; British birds: Royal Society for the Protection of Birds (RSPB), 2016; Italian birds: Naturschutzbund Deutschland e.V., 2019) showed that birds are at their breeding grounds in June and July. Thus, feather samples obtained in June and July were defined as samples from local breeders and hence had known origins ( $N = 101$ ). The remaining 101 samples were classified as unknown origin and were sampled during spring (April and May) and autumn (August to September) migrations at stopover sites in Spain, Malta, Italy, Greece, and Bulgaria (Table 1; Marx et al., 2016).

### 2.3 | Stable isotope analyses

Stable isotope analyses were run at the Stable Isotope Laboratory of the Leibniz Institute for Zoo and Wildlife Research (IZW). Feather samples were washed with a 2:1 chloroform/methanol solution for 24 hr and then dried for 48 hr under a fume hood. For stable hydrogen isotope analyses,  $0.27 \pm 0.1$  mg were loaded into silver capsules (IVA Analysetechnik e.K., Meerbusch, Germany) and then the capsules were folded to small balls. Loaded samples were stored in 96-well microtiter plates loosely covered with the lid in order to allow exchange with ambient air moisture. Then, trays were placed in a compartment drier at 50°C for at least 24 hr to speed up equilibration and remove extra moisture. Afterwards, samples and standards were loaded into the carousel of a Zero Blank autosampler (Costech Analytical Technologies Inc., Italy) and flushed with chemically pure helium for a minimum of 1 hr. Samples were then dropped one by one into the elemental analyzer (EA; HT Elementanalysator HEKAtech, GmbH, Wegberg, Germany), which operated at 1,350°C and contained a silicon carbide tube filled halfway with glassy carbon chips and including a carbon/water trap. The gas chromatograph separated H<sub>2</sub>, N<sub>2</sub>, and CO. The obtained H<sub>2</sub> sample pulse was then introduced into the stable isotope-ratio mass spectrometer (Delta V advantage, ThermoFisher Scientific, Bremen, Germany) via an interface (Finnigan Conflo III, ThermoFisher Scientific). Samples were analyzed together with three in-house keratin standards, which had previously been calibrated to the USGS42 standard (Soto, Koehler, Wassenaar, & Hobson, 2017; Voigt & Lehnert, 2018). Measured stable isotope ratios are reported in the delta notation ( $\delta^2\text{H}_f$ ) as parts per mil (‰) deviation from V-SMOW. Precision of measurements was

always better than 2‰. We calculated the stable isotope ratio of the non-exchangeable portion of hydrogen in samples based on laboratory keratin standards with known stable isotope ratios of the non-exchangeable portion of hydrogen: sheep wool from Sweden SWE-SHE (−111.65‰), sheep wool from Spain ESP-SHE (−61.54‰), and goat wool from Tanzania AFR-GOA (−26.44‰). Laboratory keratin standards were established by referencing to USGS42, CBS, and KHS keratin standards (Soto et al., 2017).

Isotope data of feather samples with known and unknown breeding origins were compared by country with R 3.3.3 (R Core Team, 2016). For that, analyses of variance (ANOVA) were run for both sample groups (birds with known and unknown breeding origins) followed by a country-wise Tukey Honest Significant Difference (TukeyHSD) test. Subsequently, boxplots were generated for visual data inspection.

## 2.4 | Assignment of breeding origins

The R package IsoriX vs. 0.4-1 (Courtiol et al., 2016a) was used to assign likely regions of origin to the Turtle Doves. The package and the according workflow descriptions (Courtiol et al., 2016a, 2016b, 2019) were explicitly designed to offer a user interface for isoscape modeling and geographic assignments of migrating individuals. Updates appear regularly.

For the determination of regions of origin, isoscapes based on mixed models were built to compute geographic origins of organisms depending on their isotopic values. To assign regions of origin, we followed the instructions specified in the package descriptions and example workflow (Courtiol et al., 2016a, 2016b, 2019). The assignment process consisted of five steps (see R Script in Appendix S1): (1) fitting the environmental isoscape (function Isofit), (2) preparation of the elevation raster (function RElevate), (3) prediction of the isoscape across the area covered by the elevation raster (function Isoscape), (4) fitting the calibration model (function Calibfit), and (5) assignment of spatial origins of samples with unknown origins (function Isorix, Courtiol et al., 2016a). IsoriX depends on several functions provided by other packages to handle the parameters required for the environmental isoscape, relate calibration data to this isoscape, and generate the geographic assignment (for details, see Courtiol et al., 2016a, 2016b, 2019).

For the first processing step, we followed the instructions specified in the package description and workflow (Courtiol et al., 2016a, 2016b). GNIP data from April to August (i.e., the time when Turtle Doves likely stay in Europe) provided continental precipitation  $\delta^2\text{H}_p$  data to

build a first basic isoscape (Courtiol et al., 2016a, 2019). Based on this, a mean model (linear-mixed effects model) and a residual dispersion model (gamma generalized linear-mixed effects model) were generated. The models were used to predict mean isotopic values at different locations and their residual dispersion variance (see for details Courtiol et al., 2016a). The spatial structure of the environmental isoscape was modeled with the Matern autocorrelation function. Hence, spatially close measurements were considered to be more similar compared to those that were spatially distant (Courtiol et al., 2019).

In Step 2, European elevation information was downloaded as a raster file using the function GetElev(). To reduce raster resolution and improve computer performance, every 10 adjacent horizontal and vertical raster cells were aggregated. The raster was restricted to Europe with a latitudinal extent of 31°–69°N and longitudinal range from −27°W to 57°E, which covered the whole known distribution range of Turtle Doves in the western Palearctic (BirdLife International and Handbook of the Birds of the World, 2016; Marx et al., 2016).

In the third processing step, the function Isoscape was used to generate the predicted  $\delta^2\text{H}_p$  isoscape based on the precipitation data and the aggregated elevation raster (for details, see Courtiol et al., 2019).

In the fourth step, we loaded the calibration data (=  $\delta^2\text{H}_f$  values of feathers with known origin) and related the data to the  $\delta^2\text{H}_p$  isoscape from Step 3) by keeping the standard settings (function Calibfit), which use a linear-mixed effects model. The model considers both, the spatially varying prediction variance, but also the prediction covariances between predicted isotope values across the isoscape (see for details Courtiol et al., 2019).

In the final step, assignment data (i.e.,  $\delta^2\text{H}_f$  values of feathers with unknown origin) was implemented into the system and possible origin areas were computed following the workflow (Courtiol et al., 2016b). A group assignment for all assignment samples, group assignments by country of collection and individual assignments for every sample with unknown origin were conducted. Group assignments followed the Fisher's method (Fisher, 1925), which combines all individual probability maps into a single group map in which every raster cell has a deposited probability value which is given as a *p*-value. The *p*-value describes the similarity of the isotopic composition of the assignment data to the isotopic composition of the assigned region and is measured at a 95% confidence level (Courtiol et al., 2019).

The resulting probability maps were exported as rasters and clipped according to the Turtle Doves' known distribution (BirdLife International and Handbook of the Birds of the World, 2016) using ArcGIS 10.2.2. Final maps show most likely origin areas classified by three

probability groups ( $p$ -values  $\geq 0.7$ ,  $\geq 0.8$ , and  $\geq 0.9$ ) and were created for all assignment samples as one group, grouped by country of collection, and individually. In

**TABLE 2** Results of pairwise TukeyHSD tests comparing isotopic values of countries of calibration

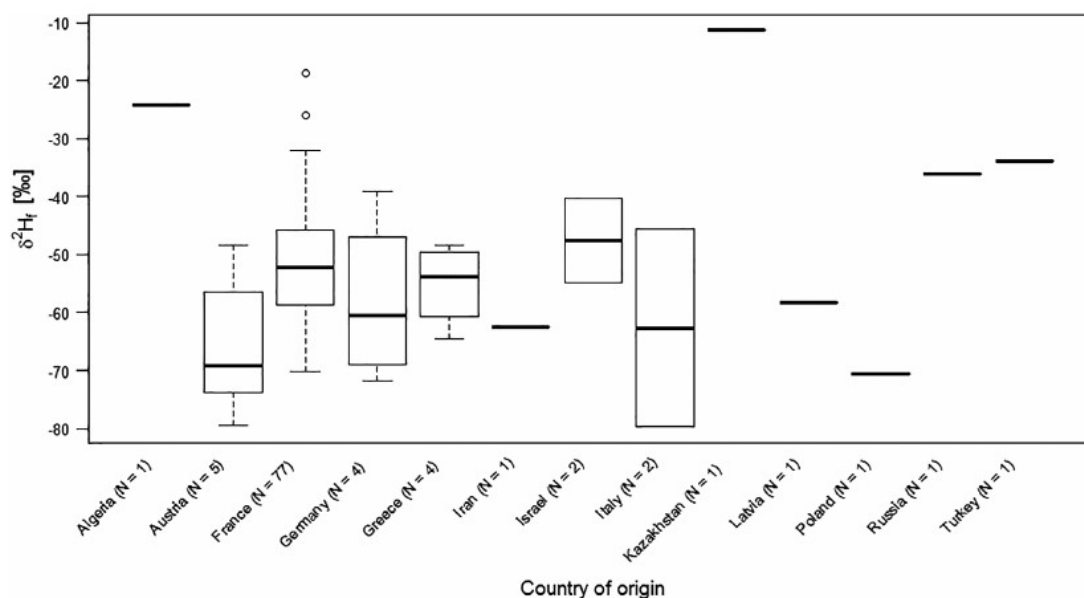
Country	Compared country	$p$ -Value
France	Austria	.06
Germany	Austria	.90
Germany	France	.84
Greece	Austria	.70
Greece	France	.98
Greece	Germany	1.00
Israel	Austria	.33
Israel	France	1.00
Israel	Germany	.86
Israel	Greece	.96
Italy	Austria	1.00
Italy	France	.68
Italy	Germany	1.00
Italy	Greece	.97
Italy	Israel	.70

Note: No significant differences were detected, but a tendency between France and Austria with a  $p$ -value = .06.

every map, the western, central, and eastern flyways are depicted as kernel densities as described by Marx et al. (2016). Furthermore, we analyzed the spatial range with individual results to classify specific regions of origin. Subsequently, the minimal proportion of birds certainly originating from countries other than the country of collection (certain migrants or “foreign” birds) was evaluated.

### 3 | RESULTS

According to statistical analyses, there was a marginally significant effect of country on the  $\delta^2\text{H}_f$  values from calibration samples (ANOVA,  $F_{5,88} = 2.35$ ,  $p \leq .05$ ), which was mainly based on the difference between French and Austrian samples (TukeyHSD,  $p = .06$ , Table 2). Also, boxplots for calibration samples indicated similarities of  $\delta^2\text{H}_f$  values between most of the countries (Figure 1). This weak test result largely originates from the small sample sizes obtained in several countries for the calibration data. In the assignment data, there was a highly significant effect of country on  $\delta^2\text{H}_f$  values (ANOVA,  $F_{4,96} = 9.74$ ,  $p < .001$ ), which was based on the Bulgarian samples having lower values than all other countries (TukeyHSD,  $p < .05$ , Table 3, Figure 2).



**FIGURE 1** Boxplot graphs for  $\delta^2\text{H}_f$  values from feather samples from European Turtle Doves collected from breeding sites (country of origin) and used as calibration data for the assignment of origins of birds captured during migration. The numbers of samples per sampling site are given. The boxes for every country of collection with more than one sample represent the range in which 50% of the data occur (interquartile distance from 25% quartile to 75% quartile). Whiskers indicate extreme values and the median is highlighted as black line within the boxes. The isotopic values for sampling sites with only one sample are represented by a black line only. Circles highlight outliers and extend the interquartile distance for more than 1.5 times

Generally, all assignment maps showed broad possible distribution ranges. Therefore, no spatially accurate localities were assigned, but possible regions of origin, which were distributed along latitudinal ranges. The assignment map for all assigned Turtle Doves combined showed likely regions of origin within southern and central Europe up to 55°N latitude, but no possible origins were in high mountainous areas (Alps, Pyrenees, Pindus, Dinaric Alps or Anatolia; Figure 3).

Group assignments per country showed the following results:

Bulgarian samples were assigned to Russian and Baltic areas primarily. Samples from Spain were mainly assigned to southern, coastal regions. Individuals that migrated through Greece, Italy and Malta mostly had projected possible regions of origin in central and eastern Europe and along a latitudinal band from France to northern Italy, Romania and Bulgaria (Figure 4). Therefore, samples with the most negative  $\delta^2\text{H}_f$  values collected in eastern countries were assigned to more northern regions of origin, and those with more positive  $\delta^2\text{H}_f$  values and more southern/southwestern sampling locations were assigned to more southern and southwestern regions of origin.

With individual assignment data, four large regions of origin could be distinguished (Figures S1–S5, Table 4), corresponding to a hot/dry to cool/humid temperature/humidity gradient, from north to south/southwest. The region covering Russia and the Baltic States (Estonia, Latvia, Lithuania) was the most northern one. To the south, it was followed by a band of origin regions covering central and eastern Europe, which was then followed by a region ranging along a latitudinal band from France to northern Italy, Romania and Bulgaria. The southernmost regions of origin covered coastal areas along the

Mediterranean. Again, birds with the most negative  $\delta^2\text{H}_f$  values were assigned to northern regions and those with more positive  $\delta^2\text{H}_f$  values had regions of origin in southern and coastal areas (Table 4).

Individual assignments were used to estimate the proportions of birds sampled at stopover sites originating from the aforementioned regions. Individuals with  $\delta^2\text{H}_f$  values between  $-77$  and  $-65\text{‰}$  were assigned to Russian and Baltic territories. Predominantly, birds migrating through Bulgaria (89%) were projected to this region, but some individuals sampled at the other migratory sites also came from this region: Malta (27%), Greece (10%), Spain (10%), and Italy (6%; Table 4).

The second region of origin across central and eastern Europe was projected for birds with  $\delta^2\text{H}_f$  values between  $-64$  and  $-59\text{‰}$  and was most common in birds sampled in Greece (45%), Italy (25%), and Malta (20%). This region of origin was absent in migrating individuals collected in Bulgaria and uncommon in Spain (10%; Table 4).

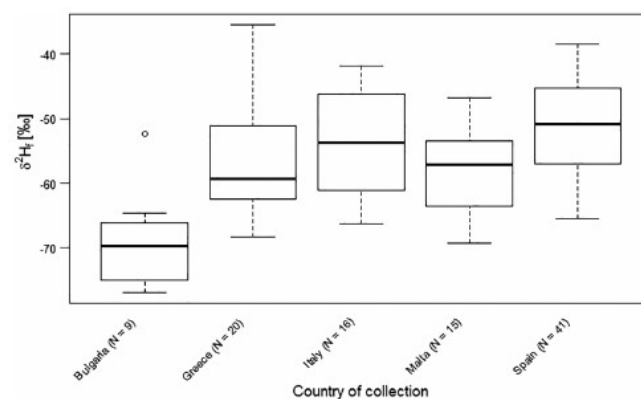
A band of origin regions covering France, northern Italy, south-west England, north-west Spain, Romania, and Bulgaria was modeled for birds with  $\delta^2\text{H}_f$  values between  $-58$  and  $-50\text{‰}$ . The highest proportion of samples originating in these regions was found in Malta (47%), intermediate proportions came from Spain (39%), Italy (38%), and Greece (30%) and low proportions from Bulgaria (11%; Table 4).

Coastal regions of origin were assigned for birds with  $\delta^2\text{H}_f$  values between  $-49$  and  $-36\text{‰}$ . A high proportion

**TABLE 3** Results of pairwise TukeyHSD test to compare isotopic values of countries of assignment

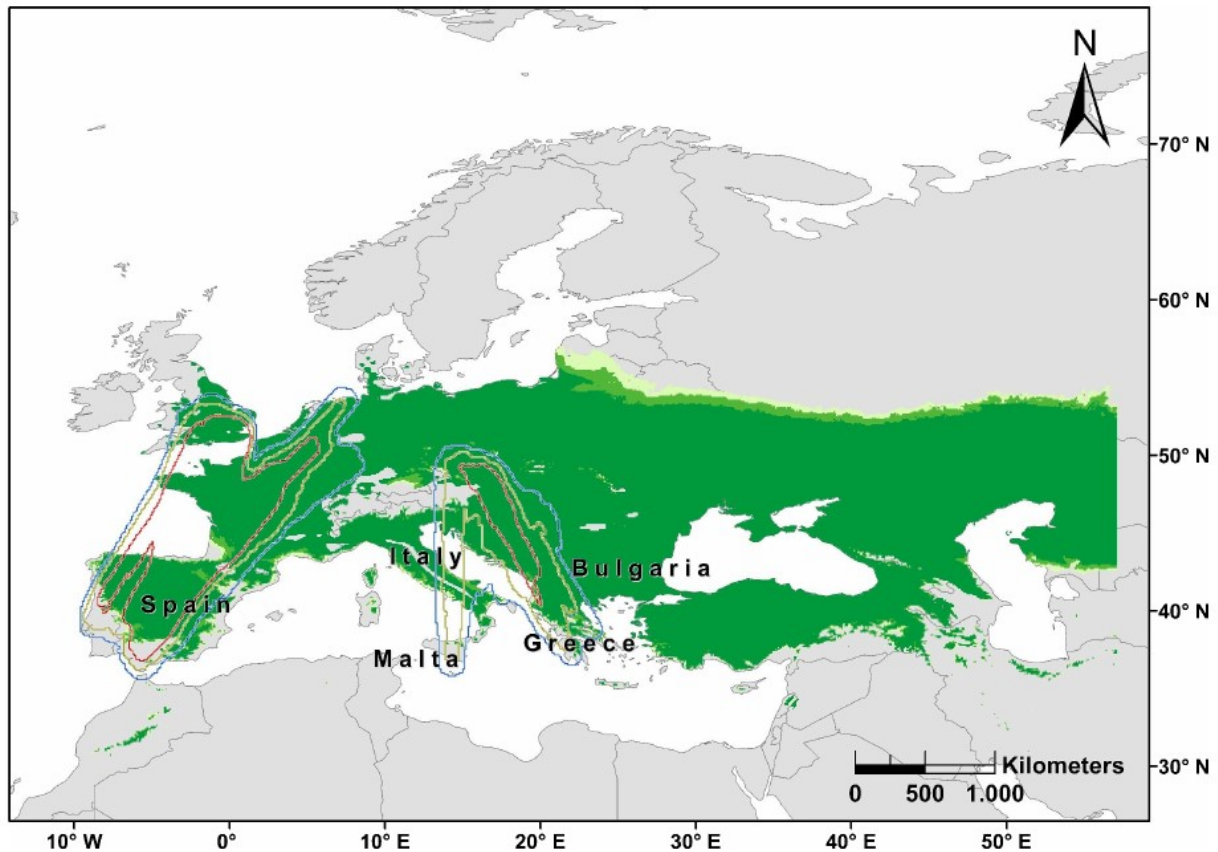
Country	Compared country	p-Value
Greece	Bulgaria	<.05
Italy	Bulgaria	<.05
Malta	Bulgaria	<.05
Spain	Bulgaria	<.05
Italy	Greece	.86
Malta	Greece	.93
Spain	Greece	.26
Malta	Italy	.46
Spain	Italy	.94
Spain	Malta	.06

Note: Values are significantly different when the p-value is <.05.



**FIGURE 2** Boxplot graphs for  $\delta^2\text{H}_f$  values of European Turtle Dove feather samples that were used as assignment data and therefore, had unknown regions of origin. Those samples originated from stopover sites in Europe (country of collection). The numbers of samples per sampling site are given. The boxes for sampling sites with more than one sample represent the range in which 50% of the data occur (interquartile distance from 25% quartile to 75% quartile). Whiskers show extreme values and the median is highlighted as black line within the boxes. Circles highlight outliers and extend the inter-quartile distance for more than 1.5 times





**FIGURE 3** Assignment map showing possible regions of origin grouped for all migrating European Turtle Doves that had unknown breeding origins. Drawn are three classified probability ranges in green shades, highlighting areas with origin probabilities of  $\geq 0.7$ ,  $\geq 0.8$ , and  $\geq 0.9$ . Countries of collection are written in the map. Also, analyzed western, central, and eastern flyways are depicted as kernel densities for 70% (red contour), 80% (yellow contour), and 90% (blue contour) as described by Marx et al. (2016)

of birds assigned to these regions was sampled in Spain (41%), followed by Italy (31%) and Greece (15%), while only one of the samples from Malta and none from Bulgaria fell into this category (Table 4).

After determination of origin regions, minimal proportions of “foreign” birds were determined by evaluating individual results. This proportion was highest in Malta (93%), and Bulgaria (89%), also high in Greece (55%) and low in Spain (20%), while only one of the 16 birds sampled in Italy could be determined as definite “foreign” bird (Table 4).

## 4 | DISCUSSION

We assigned potential breeding regions (i.e., regions of origin) of European Turtle Doves with  $\delta^2\text{H}_f$  values across a validated European hydrogen isoscape. Four different regions of origin were determined corresponding to a hot/dry to cool/humid temperature/humidity gradient. Birds with more positive  $\delta^2\text{H}_f$  values were assigned to more southern/southwestern and coastal regions with a

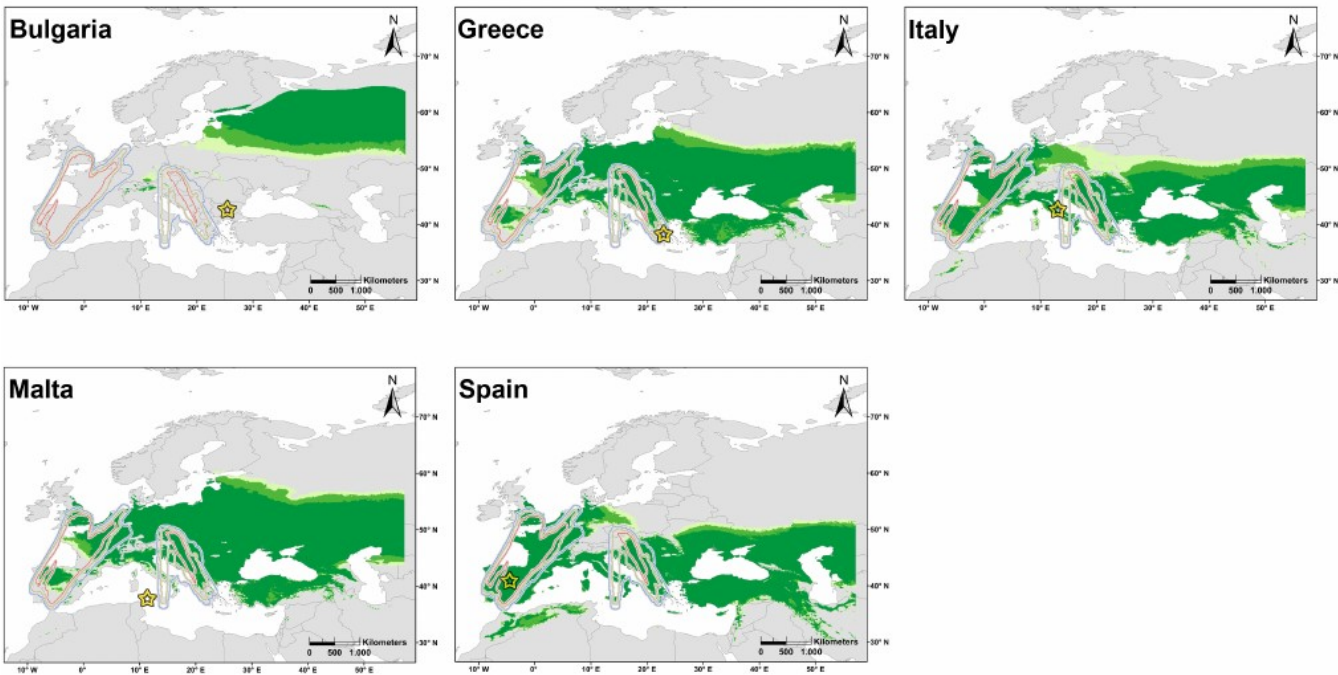
hot and dry climate, and those with more negative  $\delta^2\text{H}_f$  values came from breeding regions in northern areas and towards the interior Eurasian continent with a cool and humid climate (Hobson, 2005; Rozanski et al., 1993; West et al., 2006). Samples collected in eastern countries (Greece and Bulgaria) originated from northern breeding regions and samples collected in south/southwestern countries were assigned to more southern and coastal areas. Thus, stable isotope assignments proved useful in studying regions of origin of Turtle Doves, which is important information in studies about population connectivity. To augment knowledge about population connectivity, a similar approach should be applied using feathers of birds that were molted in the wintering areas on the African continent. For that, a triple-stable isotope approach using stable carbon, nitrogen and hydrogen isotopes could be applied to assign regions of origin as shown in other studies (Hobson et al. 2012).

In line with previous literature, in the resulting maps Turtle Doves were largely absent from mountainous areas as breeding regions (Figures 3 and 4; Browne & Aebischer, 2005; del Hoyo et al., 1997; Glutz



Likely breeding origin measured at p-value  $\geq 0.7$

$\geq 0.7$   $\geq 0.8$   $\geq 0.9$



**FIGURE 4** Assignment maps highlighting estimated regions of origin grouped for  $\delta^2\text{H}_f$  values of European Turtle Doves from Bulgaria, Greece, Italy, Malta and Spain sampled during migration. In every map, three classified probability ranges in green shades are drawn, highlighting areas with origin probabilities of  $\geq 0.7$ ,  $\geq 0.8$ , and  $\geq 0.9$ . Also, western, central, and eastern flyways are depicted as kernel densities for 70% (red contour), 80% (yellow contour), and 90% (blue contour) as described by Marx et al. (2016), and countries of collection are marked with a yellow star

**TABLE 4** Number of individuals and percentage of assigned breeding birds per area for birds sampled during migration in Bulgaria, Malta, Spain, Greece and Italy

Place	Russia and N Baltic States <sup>a</sup>	Central eastern Europe (Germany, Poland, Hungary, Czech Republic and Slovakia)	France, N Italy, SW England, NW Spain, Romania and Bulgaria	Mediterranean coasts <sup>b</sup>	Possible national breeders	Certain migrants
Bulgaria	9 8 (88.9%)	0	1 (11.1%)	0	1	8 (88.9%)
Malta	15 4 (26.7%)	3 (20%)	7 (46.7%)	1 (6.6%)	1	14 (93.3%)
Spain	41 4 (9.8%)	4 (9.8%)	16 (39%)	17 (41.4%)	33	8 (19.5%)
Greece	20 2 (10%)	9 (45%)	6 (30%)	3 (15%)	9	11 (55%)
Italy	16 1 (6.3%)	4 (25%)	6 (37.5%)	5 (31.3%)	11	1 (6.3%)
$\delta^2\text{H}_f$	<b>−77 to −65‰</b>	<b>−64 to −59‰</b>	<b>−58 to −50‰</b>	<b>−49 to −36‰</b>		

Note: Furthermore, numbers of possible national breeders as well as numbers and percentages of sampled birds determined as certain migrants are given per stopover site. Ranges of  $\delta^2\text{H}_f$  values per distribution area are highlighted in bold.

<sup>a</sup>In several birds, the projected area also included the low-Alpine area.

<sup>b</sup>In several birds, the projected area also included the Atlantic coasts of France and Portugal, and south-western Portugal.

von Blotzheim & Bauer, 1994; Sáenz de Buruaga et al., 2012). Yet, some individuals in the present study might inhabit elevated areas, especially when assigned to southern Europe, because the projected areas included mountain foothills. In future studies, calibration samples from those breeding areas might improve assignments.

However, maps generally showed broad ranges of highly likely regions of origin ( $p$ -value  $\geq .9$ ) across the entirety of Europe except areas above approximately 55°N. The results might be affected by small sample sizes but also major isotopic similarity of calibration samples per country (Table 2). Isotopic similarity of samples from different countries can appear when those countries lie

within the same latitudinal band of stable hydrogen isotope values (Hobson, 2005). Thus, assignment of origin regions can differentiate potential breeding ranges along a latitudinal stable hydrogen isotope band, but it is difficult to discriminate populations in more western or eastern directions. On the one hand, those large assigned regions of origin, especially of Turtle Doves from Spain, Italy, Malta, and Greece, support the hypothesis of permeability between flyways (e.g., Guillemain, Sadoul, & Simon, 2005; Marx et al., 2016) and a panmictic population genetic structure as suggested for Turtle Doves (Calderón, 2016). On the other hand, birds sampled in Spain probably follow a western flyway (Marx et al., 2016) and thus the most likely regions of origin may be, for example in Spain, France, United Kingdom, Netherlands or Germany. Also, Turtle Dove feather samples collected in Italy or Malta likely follow the central flyway (Marx et al., 2016) and therefore, probable regions of origin may be, for example, in Italy, Austria, Germany, the Czech Republic or Poland. Greece lies on the eastern flyway, like Bulgaria (Marx et al., 2016). Therefore, likely regions of origin may be in Bulgaria, Romania, the Czech Republic, Austria, Hungary, Poland, the Baltic States or Russia. However, only individuals sampled in Bulgaria were predominantly assigned to an eastern region of origin (Russia and Baltic) and thus confirm the use of an eastern flyway to move between the African winter and European breeding locations (see Figures S1–S5; Marx et al., 2016).

The evaluation of assigned individuals per sampling site to one of the four described regions of origin also highlighted Malta as the main country for “foreign” birds, which coincides with the very low numbers of 0–14 breeding pairs (Fisher, Ashpole, Scallan, Proud, & Carboneras, 2018). Also, individuals sampled in Bulgaria originated from distant regions, and hence were accounted as “foreign” birds. Furthermore, both stopover sites, Bulgaria and Malta, had the highest ratio of birds originating from Russian and Baltic breeding ranges. This is of particular interest, because it suggests a frequent use of eastern breeding areas for birds passing through Malta, but also because recent studies from these Russian and Baltic breeding ranges are scarce despite former high ringing activity (Moscow:  $N > 1,600$ , Marx et al., 2016).

On the opposite side, most birds sampled in Italy during spring migration (Table 1) were possible local breeders, which is in line with similar findings from ring re-encounters (Marx et al., 2016). However, in light of recoveries in different European countries indicated in the Italian migration atlas (Spina & Volponi, 2008) and suggested panmictic behavior (Calderón, 2016), possible regions of origin could also be outside of Italy as the assignments modeled birds along latitudinal bands of

stable hydrogen isotope values. In the future, evaluation of tracking data of Turtle Doves could help to define proportions of local breeders and “foreign” birds along one latitudinal band more precisely.

The gathered information about “foreign” birds is crucial, because it provides data that can be applied in the actions proposed by the International Single Species Action Plan (Fisher et al., 2018). Results about origin regions and minimal proportions of certain migrants came from individuals hunted in five out of ten European Union member states with legal hunting practices. Therefore, these data allow rough assessments of potential effects of hunting on Turtle Dove populations. Because hunting represents an anthropogenic threat, it can have large negative impacts on the population level. Turtle Doves are suggested to be especially vulnerable to those threats due to their natural demographic fluctuations and currently decreasing population sizes (Calderón, 2016). Regarding our findings, legal but unsustainable hunting during Turtle Dove migration (Fisher et al., 2018) could largely affect “foreign” breeding populations (Malta, Greece, Bulgaria), but also possible local breeders, as indicated for Spain and Italy (Table 4, Figures S1 and S2). However, an impact on “foreign” birds cannot be excluded for both sites due to assignments of large regions of origin along latitudinal bands. Regarding Italian results, the data obtained were mainly based on feather samples collected during spring migration, but the official hunting season is open during autumn migration. Therefore, a similar investigation for birds sampled during autumn migration might refine the assumption of large hunting effects on local breeders. However, Marx et al. (2016) already demonstrated high hunting numbers of possible Italian breeding Turtle Doves during autumn migration. Here, our results highlighted the potential geographic reach of hunting on Turtle Dove populations and it appears necessary to implement restrictions for hunting at stopover sites and protect resting, foraging and drinking sites. This could also have positive effects on other migrating bird species likely using similar stopover sites.

To sum up, we were able to assign potential regions of origin to a large number of birds using a stable isotope approach. The use of  $\delta^2\text{H}$  alone results in relatively coarse and large possible origin ranges. Tracking data could identify spatially accurate locations. However, we were able to highlight important regions of origin and discuss possible impacts of hunting at the studied stopover sites on migrating individuals. Thus, together with the previous mark re-encounter analyses (Marx et al., 2016) and data from tracking studies (Eraud et al., 2013; Naturschutzbund Deutschland e.V., 2019; Office National de la Chasse et de la Faune Sauvage, 2017; RSPB, 2016),

stable isotope analyses may be an important tool to implement an adaptive harvest modeling framework for Turtle Doves in order to improve the species conservation status in Europe.

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## CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

## AUTHOR CONTRIBUTIONS

Conceived and designed the study: M.M. and P.Q. Collected the data: M.M., G.R., P.Z., S.P., D.B., B.M., J.G.C., F.S., M.C.B., S.F., A.G., C.C.V. and P.Q. Analyzed the data: M.M., C.C.V. and P.Q. Drafted the manuscript: M.M. and P.Q. Critically revised the manuscript: G.R., P.Z., S.P., D.B., B.M., J.G.C., F.S., M.C.B., S.F., A.G. and C.C.V. All authors read and approved the final manuscript.

## DATA ACCESSIBILITY STATEMENT

All data can be obtained from the contributing author by request. The applied R script is available within the supplementary materials.

## ORCID

Melanie Marx  <https://orcid.org/0000-0001-5939-2369>

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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