

# EOU Conference, Lund 2023 - poster session: Stable isotope analysis is a reliable method for identifying migrants and residents in a partial migratory European hoopoe (*Upupa epops*) population in southern Spain

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## **Brief author comment**

Here you will find supplementary material of the poster presented (not all poster contents are present but new figures and tables to better understanding of my topic and research).

Data treatment and all statistical analysis were performed in R statistics (R Core Team, 2022) by Mercè Palacios and supervised by David Martín-Gálvez, Elena Arriero and Juan J. Soler.

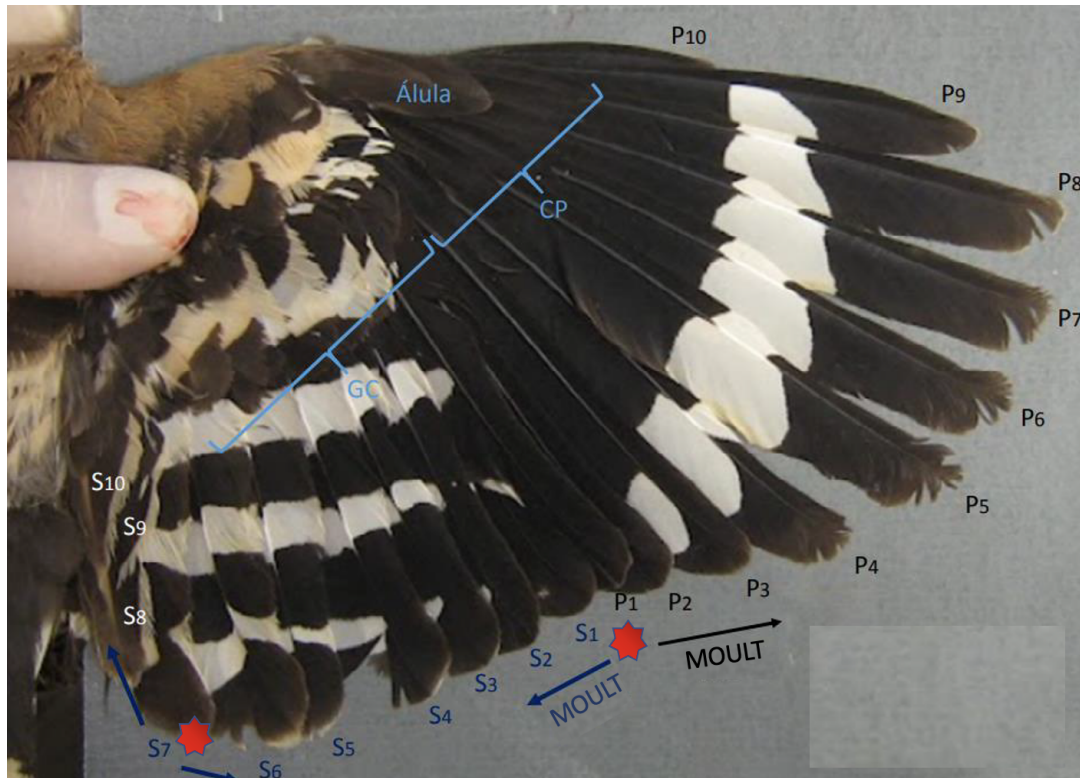
Lab work was conducted in the Stable Isotopes Lab of the Biological Station of Doñana (EBD - CSIC) and by María Herrero during her final degree assignment in the University of Granada supervised by Manuel Martín-Vivaldi.

Principal author, Mercè Palacios, is granted by the Spanish government with a predoctoral fellowship and develop her PhD in the MIGHOOGE project, based in the Complutense University of Madrid (UCM) with reference number PID2020- 116934GB-100.

## **Part 1: Isotope analysis**

### **Feather samples**

We collected 5 different types of feathers: the fourth primary (P4), its covert, the seventh secondary (S7), its covert and feathers from breast. This feathers were selected because of hoopoes moult pattern (Fig 1).



**Fig 1.** Hoopoe wing feathers. Red stars are the moult centers and arrows indicates the direction of moult. Picture was taken by Manuel Martín-Vivaldi and figure is modified from María Herrero final degree assignment (2022).

## Sample size

Migrant	Adult	Male	8
		Female	11
	Juvenile	Male	2
		Female	17
Resident	Adult	Male	7
		Female	4
	Juvenile	Male	2
		Female	4

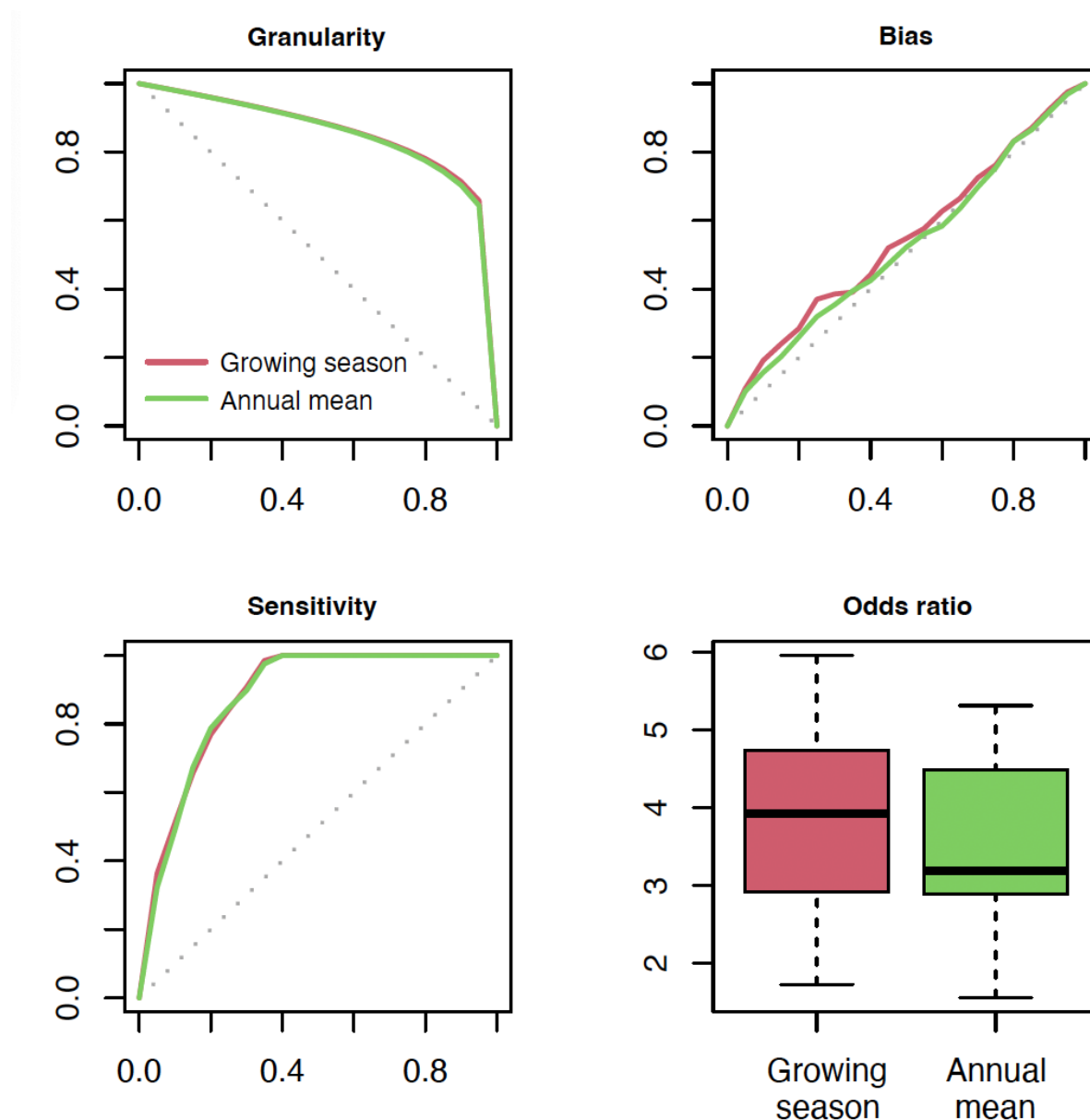
## Assignment of deuterium values to GeoSpace

Different spatial layers were downloaded from [waterisotopes.org](http://waterisotopes.org) containing (1) global annual mean deuterium values and (2) deuterium values during growing season.

I adjusted a correlation model between those environmental deuterium values and the deuterium values from known origin feathers samples. Birds species I selected occur in Europe and north of Africa and the lab protocol for obtaining deuterium values of these samples was the same than mine (Wasenaar & Hobson, 2003).

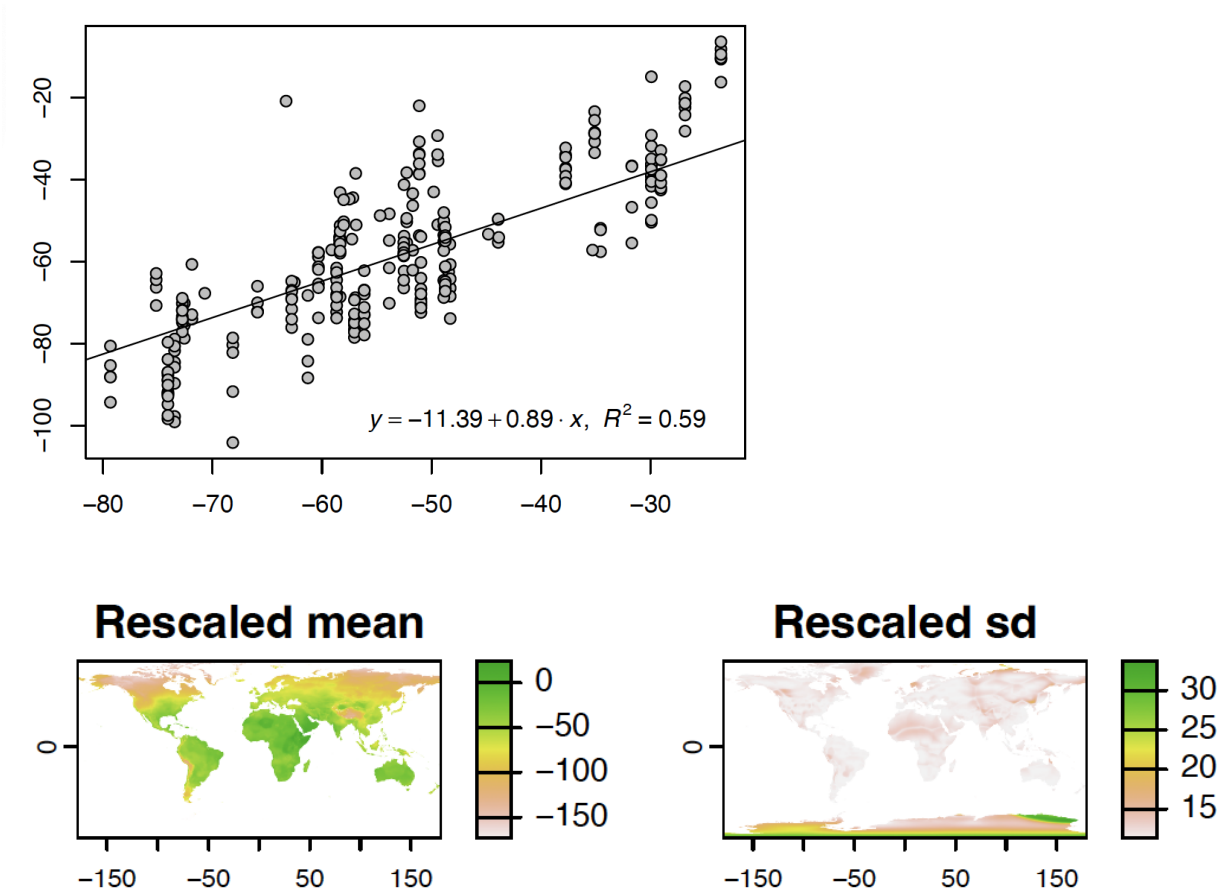
Before calculating the probabilities of my own samples to become from one part or another of the calibrated isoscape, I performed some quality analysis (Fig 2) on the models I had adjusted and trained. It allowed me

to confirm their accuracy and to choose between the isoscape generated with annual mean deuterium values and the other one that used growing season deuterium values.



**Fig 2.** Quality analysis. Isoscape generated using environmental deuterium values from growing season resulted better since it showed similar granularity, bias and sensitivity but higher probabilities of known origin data relative to random ones.

With that best isoscape (Fig 3) I obtained the probabilities of the hoopoe samples following the Bayesian inversion method (Wunder, 2010) and they were represented as density maps one by one. I inspected all of them to decide if the sample was assigned to Europe or Africa. generated with



**Fig 3.** Correlation model used to rescale the deuterium values and resulting isoscape with its standard deviation map.

This part were performed in R using *assignR* package (Ma et al., 2020).

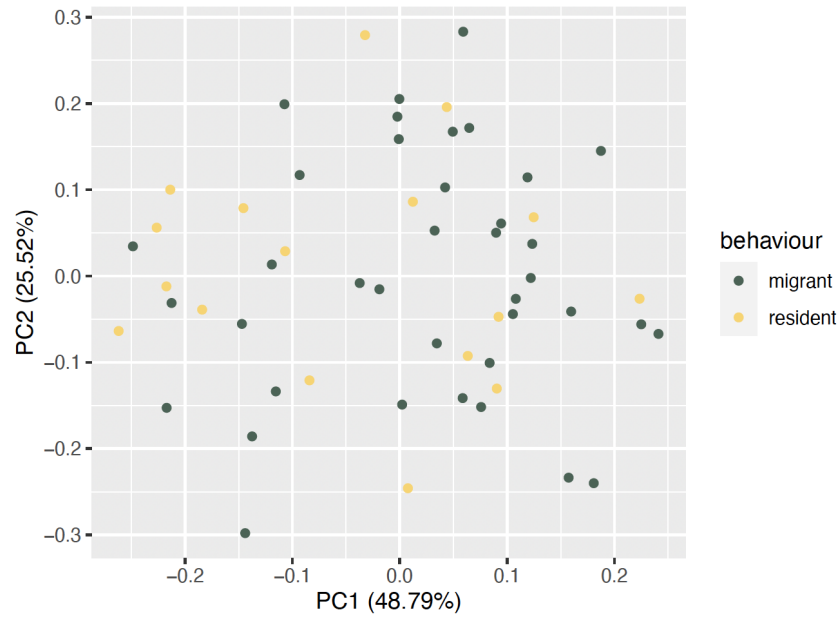
## Linking isotopic profiles with migratory strategies

We identified an individual as migrant when at least one of their feathers showed african isotopic signal. On the other side, we identified an individual as resident when all of their feathers showed european isotopic signal.

## Part 2: Morphological analysis

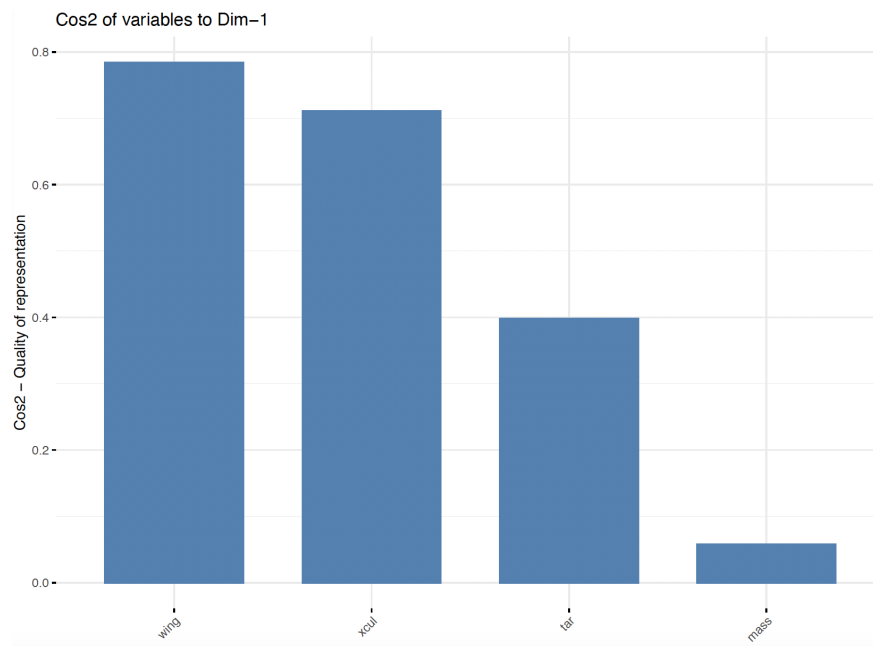
### Variables reduction

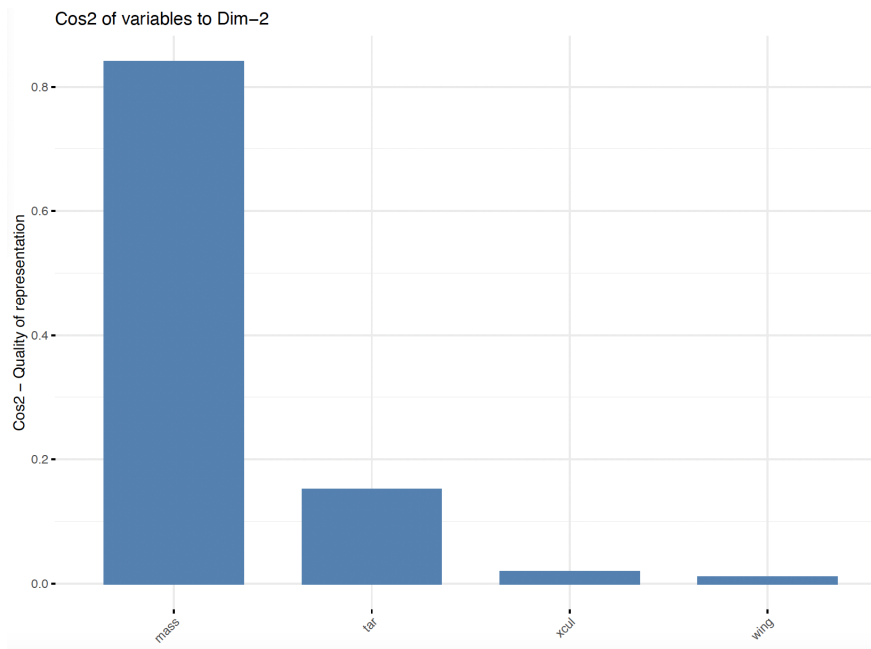
In order to explore phenotypical variation in an integrative and more accurate way I reduced the morphological traits measured in the field to three variables: size, shape and body condition. I performed a Principal Component Analysis (PCA) to see how individuals were distributed (Fig 4).



**Fig 4.** PCA of morphological data. Variables introduced were mass, wing length, bill length and tarsus length. Neither distribution pattern nor clustering was found.

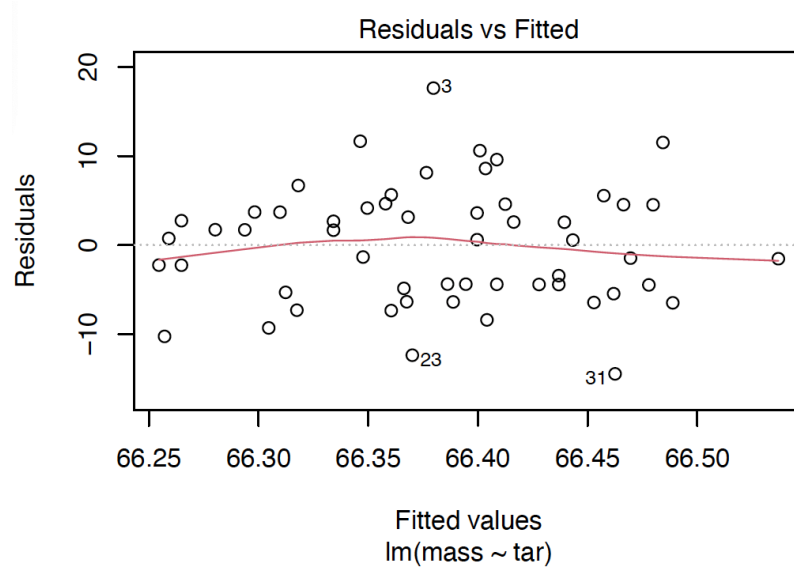
I investigated the variables contribution to each axis (Fig 5) and I decided to use the PC1 values as a proxy of shape and PC2 values as proxy of size.





**Fig 5.** Variation in PC1 (upper) was explained by wing, bill and tarsus length. Variation in PC2 (down) was explained mainly by mass and then tarsus length.

Body condition variable was generated as the regression of mass and tarsus length (Fig 6).



**Fig 6.** Residuals of the loglinear regression of mass and tarsus length.

## Models

- |     |                             |
|-----|-----------------------------|
| 1 : | morph ~ sex                 |
| 2 : | morph ~ age                 |
| 3 : | morph ~ sex*age             |
| 4 : | morph ~ sex + behaviour     |
| 5 : | morph ~ age + behaviour     |
| 6 : | morph ~ sex*behaviour       |
| 7 : | morph ~ age*behaviour       |
| 8 : | morph ~ sex*age + behaviour |
| 9 : | morph ~ sex*age*behaviour   |

**Table 1.** Linear models I adjusted to explore whether morphological variation is influenced by behaviour. In script, “morph” is replaced by each one of the variables. Statistical differences were tested through type II ANOVAs.

## References

- Ma, C., Vander Zanden, HB., Wunder, MB., Bowen, GJ. (2020). assignR: An R package for isotope-based geographic assignment. *Methods in Ecology and Evolution*, 11 pp. 996-1001.
- Wasenaar, L. & Hobson, K. (2003). Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. *Isotopes in Environmental and Health Studies*, 39 pp. 211-217.
- Wunder, M.B. (2010). Using isoscapes to model probability surfaces for determining geographic origins. J.B. West et al. (eds.) *Isoscapes: Understanding Movement, Pattern, and Process on Earth Through Isotope Mapping*. Springer Science.