

# REPORT FROM A BOU-FUNDED PROJECT

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## **Birds of a feather flock together: characterising Scopoli's Shearwater year-round foraging ecology by means stable isotope signatures across the central Mediterranean**

**LETIZIA CAMPIONI<sup>1\*</sup>, JACOPO G. CECERE<sup>2</sup>**

<sup>1</sup> MARE – Marine and Environmental Sciences Center, ISPA - Instituto Universitário, Rua Jardim do Tabaco 34, 1149-041 Lisboa, Portugal

<sup>2</sup> ISPRA (Institute for Environmental Protection and Research), Via Ca Fornacetta 9, 40064 Ozzano dell'Emilia, Italy

\* letziacampioni@hotmail.com

### **BACKGROUND**

Tracing the use of trophic resources made by marine top-predators at different spatiotemporal scales is crucial to understand their interactions with marine environment and human activity, offering an important support to investigate mechanisms behind population dynamics across specie's distribution range. Scopoli's Shearwater *Calonectris diomedea* (SS) is a long-distance migrant, breeding in the Mediterranean Sea and spending the non-breeding period mainly along the western coast of Africa, in the Atlantic Ocean (González-Solís et al. 2007, Muller et al. 2014). Albeit detailed information is available for SS populations in the West Mediterranean (Afán et al. 2014), the use of trophic resources during the breeding and non-breeding periods is still unknown for most of the central- and east-Mediterranean populations (Ramos et al. 2009)

In recent years, stable isotope analysis (SIA) has become a useful tool for marine food-web analysis when direct observation of bird is not possible. Stable carbon and nitrogen are the most commonly used isotope ratios employ as natural tracers of animals' diet and as endogenous markers to identify and link breeding and wintering sites of migratory seabird species (Ramos et al. 2009). As feathers grow, the isotopic forms assimilated through the diet are incorporated into the keratin structure. Once formed, feathers become metabolically inert integrating the composition of the local food web where they were grown (Ramos et al. 2009).

According to that, we took advantage of the known moulting pattern of SS (Ramos et al. 2009) to characterize and compare the isotopic niche during both breeding and non-breeding period of three geographically distinct SS populations breeding in the central Mediterranean. During the breeding period, the foraging areas of the three populations are spatially segregated and characterized by different marine environments (Cecere et al 2013). Unfortunately, the Atlantic non-breeding areas are only known for birds from the Pelagie archipelago (Muller et al. 2014).

The SS mainly feed on small pelagic fish (Afán et al. 2014), but evidence suggests it can also exploit fish discards, at least during the breeding period (Cecere et al 2015). Fish discards are generally composed by demersal fishes, which are isotopically distinct from naturally caught small pelagic prey (Navarro et al 2009), here we also aim to understand the potential contribution of fish discards to the diet of SS.

### **DATA COLLECTION AND ANALYSIS**

Fieldwork was carried out in three archipelagos along the cost of Italy in the central Mediterranean (Tremi, Maddalena and Pelagie, Figure 1b) between May and July 2016. During the breeding period we

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sampled primary P1 known to be moulted in mid-chick-rearing period and secondary feathers S8 generally moulted in the non-breeding period (Ramos et al. 2009). Sampled feathers were prepared following the procedure in Ramos et al (2009) and analyzed in the SIAF Laboratory. Four S8 from Maddalena and one from Pelagie were not included in the analyses because showing values typical of the breeding season.

## OUTPUTS AND CONCLUSION

The SIA analysis of 61 SS showed that both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of the P1 and S8 feathers varied among colonies (ANOVA  $\delta^{13}\text{C}$ :  $F_{2,58}=8.93$ ,  $P < 0.0001$ ;  $\delta^{15}\text{N}$ :  $F_{2,58}=90.97$ ,  $P < 0.0001$ ) and between breeding and inter-breeding periods ( $\delta^{13}\text{C}$  ANOVA:  $F_{1,52}=73.5$ ,  $P < 0.0001$ ;  $\delta^{15}\text{N}$ :  $F_{1,52}=456.7$ ,  $P < 0.0001$ ). The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values for P1 of Tremiti birds ( $\delta^{15}\text{N}$ , LMM Estimate $\pm$ SE:  $3.00\pm 0.23$ ,  $df=58$ ,  $t=12.9$ ,  $P < 0.0001$ ) were remarkably different from those of Linosa and Maddalena ( $\delta^{15}\text{N}$ , Estimate $\pm$ SE:  $-0.52\pm 0.22$ ,  $df=58$ ,  $t=-2.33$ ,  $P=0.02$ ; Table1, Figure 1). These differences allowed us to assign any individual to the Tirreanean-Sicily Channel sub-region (Maddalena and Pelagie) or to the Adriatic Sea (Tremiti) breeding area (100% correct classification, Table 2). These results from central Mediterranean complemented the information for SS provided by Ramos et al (2009).

SIA of P1 showed clear isotopic niche segregation among colonies during the breeding period. The enrichment in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  found in birds from Tremiti ( $\delta^{15}\text{N}$  mean differences between colonies: 3.01-3.52‰) suggests those birds might be feeding at a higher trophic level (Table1).

**Table 1.** Mean  $\pm$ SD isotopic signatures ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of primary P1 and secondary S8 feather sampled in bird from three Italian archipelagos and those from a close colony from a Tunisian island.

| Archipelago<br>s           | N  | $\delta^{15}\text{N} \pm \text{SD}$<br>(‰) | Range       | $\delta^{13}\text{C} \pm \text{SD}$<br>(‰) | Range            |
|----------------------------|----|--|-------------|--|------------------|
| <i>Italy</i>               |    |  |             |  |                  |
| <b>Feather P1</b>          |    |  |             |  |                  |
| Maddalena                  | 23 | $8.87 \pm 0.22$                            | 8.45-9.18   | $-16.61 \pm 0.31$                          | -17.64 to -16.15 |
| Pelagie                    | 19 | $9.38 \pm 0.54$                            | 8.75-10.75  | $-17.08 \pm 0.35$                          | -17.69 to -16.43 |
| Tremiti                    | 19 | $12.39 \pm 0.43$                           | 11.74-13.15 | $-16.02 \pm 0.28$                          | -16.65 to -15.70 |
| <b>Feather S8</b>          |    |  |             |  |                  |
| Maddalena*                 | 18 | $12.74 \pm 2.16$                           | 11.34-14.6  | $-15.57 \pm 0.85$                          | -16.54 to -13.05 |
| Pelagie*                   | 19 | $13.06 \pm 1.98$                           | 11.90-14.70 | $-15.47 \pm 1.18$                          | -17.18 to -13.38 |
| Tremiti                    | 19 | $13.14 \pm 0.99$                           | 11.37-15.49 | $-15.02 \pm 0.90$                          | -16.35 to -13.43 |
| <i>Tunisia<sup>1</sup></i> |    |  |             |  |                  |
| Zembra                     | 18 | $9.9 \pm 1.0$                              | 7.6-11.5    | $-17.1 \pm 0.3$                            | -17.5 to -16.0   |

\* We removed five S8 which were likely moulted in the Mediterranean then with anomalous nonbreeding isotopic values.

<sup>1</sup> Gremillét et al. (2014).

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**Table 2.** Linear discriminant classification based on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of P1 sampled in SS from three Italian archipelagos.

| Breeding area           | N  | Stable isotope (%) | P1 |
|-------------------------|----|--------------------|----|
| <i>Original data</i>    |    |                    |    |
| Maddalena               | 23 | 100                |    |
| Pelagie                 | 19 | 100                |    |
| Tremiti                 | 19 | 100                |    |
| <i>Cross-validation</i> |    |                    |    |
| Maddalena               | 22 | 95.7               |    |
| Pelagie                 | 15 | 79                 |    |
| Tremiti                 | 19 | 100                |    |
| <i>Cross-validation</i> |    |                    |    |
| Maddalena + Pelagie     | 42 | 100                |    |
| Tremiti                 | 19 | 100                |    |

The isotopic niche of SS from Pelagie overlapped with that of SS from the neighbor colony of Zembra (Gremill t et al. 2014) suggesting the occupancy of a similar trophic niche. Conversely to Zembra,  $\delta^{15}\text{N}$  values of SS from our three colonies ranged over about 0.75 to 2‰ denoting a higher diet specialization at colony level for the Italian populations. However, similar to Zembra,  $\delta^{15}\text{N}$  values for Pelagie and Maddalena ranged over low values suggesting that birds might be foraging less on small pelagic fish relying more on preys as planktonic crustaceans, fish larvae (Af n et al. 2014, Gremill t et al. 2014). Such a strategy has been proposed also for SS exploiting the French Mediterranean (Gremill t et al. 2014), not far from Maddalena archipelago. Diet depletion in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  would also exclude bird from Maddalena and Pelagie feeding heavily on fishery waste. However, this does not seem to be the case of SS from Tremiti.

Tremiti is located in the Adriatic Sea highly exploited for trawl fishery of demersal species (Cecere et al 2015) along with the fishery of small pelagic species (anchovy and sardines). Here, trawling produce high amount of discards including anchovy and sardine (Tsagarakis 2014). Those trawlers overlap with Tremiti SS main foraging areas during the breeding period (Cecere et al. 2013, 2015). Because such discards represent also naturally caught preys of SS and because demersal fishes are enriched in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  (Navarro et al. 2009), then the higher isotopic levels of Tremiti might be explained by a larger consumption of fishery waste or it might be caused by higher levels of Nitrogen and Carbon at the base of the food web characterizing a semi-closed water body as the Adriatic Sea, further analysis are needed.

The SIA of S8 shows that the isotopic signatures of feathers replaced in the Atlantic region are well differentiated by those of the Mediterranean, being the latter enriched in both C (LMM:  $1.61 \pm 0.24$ ,  $df=53$ ,  $t=6.8$ ,  $P<0.001$ ) and N (LMM:  $3.67 \pm 0.24$ ,  $df=52$ ,  $t=15.6$ ,  $P<0.001$ ) (Figure 1c, d). During the nonbreeding period the isotopic niche of SS from the three colonies overlapped extensively (ANOVA:  $P>$

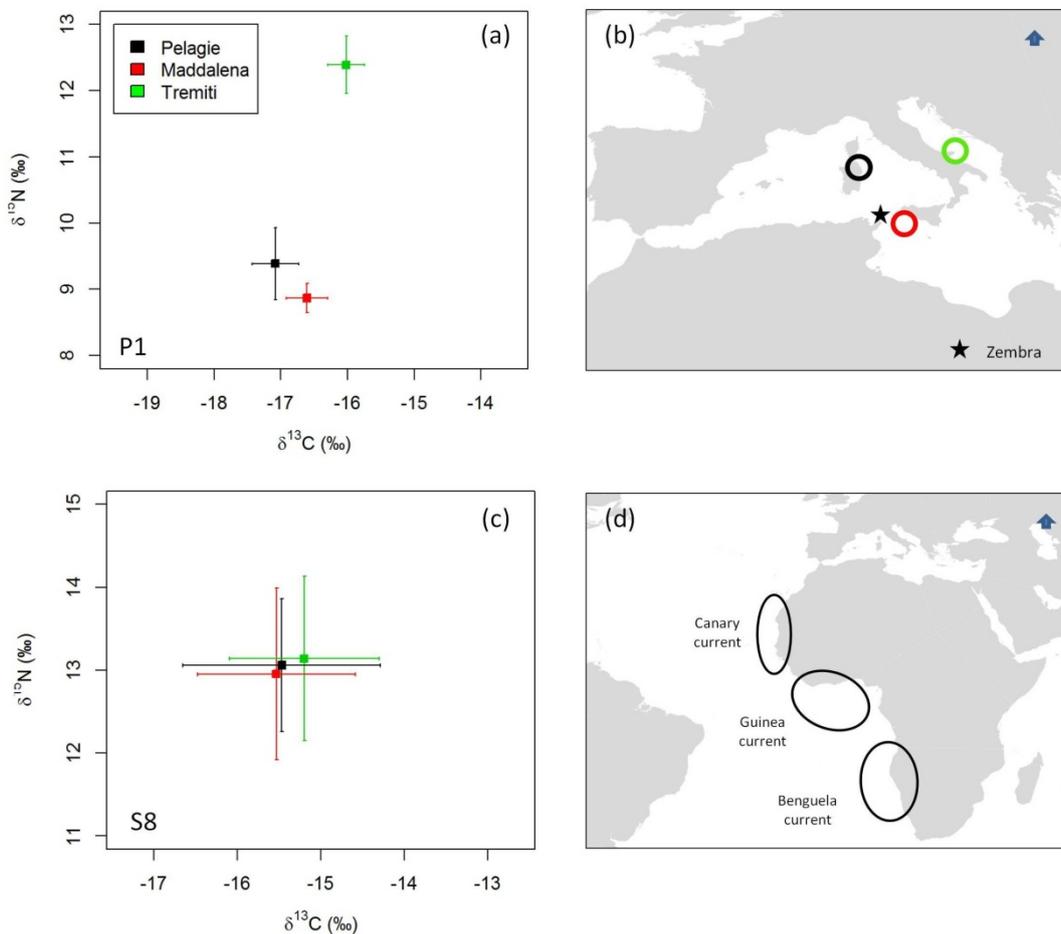
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0.1; Figure 1) suggesting that during the nonbreeding period they are remarkably mixed up (González-Solís et al. 2007). S8  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  range of variation were 4.13 and 4.15‰ respectively, indicating a large variation and then the use of different areas during the nonbreeding period (González-Solís et al. 2007, Muller et al. 2014). By comparing feathers' carbon and nitrogen signatures with those of *C. edwardsii*, *C. borealis* and *Morus capensis* wintering also along the coast of West-Africa (Figure 2) (from Navarro et al. 2009), we can cluster SS in different groups. From Figure 2 we can see that some birds fit well within the range of value characterizing the Canary current; the majority of signatures fall close to those of Mauritania area (i.e., Cape Verde) whereas just a few are similar to those typical of Benguela current (Muller et al. 2014).



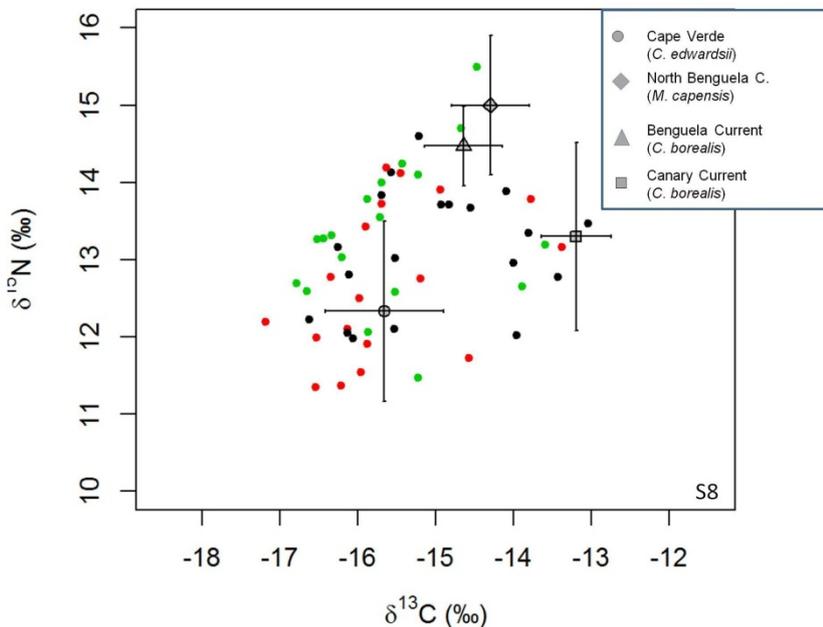
**Figure 1.** Isotopic  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of (a) primary feathers P1 moulted in the breeding colonies (b) and (c) secondary S8 replaced in the nonbreeding period sampled in SS from three archipelagos during summer; (d) known west-coast areas of Africa used by SS from the central Mediterranean during the nonbreeding period (Muller et al 2014).

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**Figure 2.** Isotopic  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of secondary S8 replaced in the nonbreeding period sampled in SS from three Italian archipelagos (Tremiti =green, Pelagie=red, Maddalena=black). Gray symbols are available isotopic signatures of seabirds wintering on the west coast of Africa at different latitudes (Navarro et al. 2009) corresponding to the upwelling regions used by SS (Muller et al 2014).

## ACKNOWLEDGEMENTS

This project was awarded a £1479 BOU - Small Research Grant - in 2016 with an additional contribution from ISPRA. We are very grateful to G. Dell' Omo, D. Rubolini, L. Serra and M. Griggio for helping in data collection. A special thanks to the Parco Nazionale Arcipelago di La Maddalena for their logistic support. L.C. was supported by post-doctoral grant (BPD/89904/2012) from Fundação para a Ciência e a Tecnologia.

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