# Different migration patterns of European anchovy and sardine around Iberian Peninsula revealed by eye lens isotopes

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**Keywords**

Eye lens, stable isotope, migration, population structure, anchovy, sardine, fishery management

**Highlights**

- The first movement analysis using eye lens isotopes for European anchovy and sardine
- Anchovy populations were separated between west and south Iberian coasts
- In contrast, sardine was mixed considerably between west and south coasts
- Trans-boundary connectivity between west and north coasts may exist in anchovy
- Inferences from eye lens isotopes will help fisheries management under climate change

**Abstract**

Small pelagic fish are key components of productive coastal ecosystems, yet their migration ecology remains poorly understood, causing challenges for management. We applied stable carbon and nitrogen isotope ($\delta^{13}$C and $\delta^{15}$N) analyses of eye lenses to investigate movements of European anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) around the Iberian Peninsula. Muscle isotopes showed strong spatial heterogeneity, largely consistent between species and reflecting differences in baseline values. Eye lens centres of small anchovy, and to a lesser extent sardine, also showed clear geographic variation: higher $\delta^{15}$N off the Atlantic south coast, lowest $\delta^{15}$N in the

Alboran Sea, and lower $\delta^{13}C$ off the west coast. These patterns persisted across years and fish sizes in anchovy, with only minor outliers, suggesting limited cross regional migration. An exception was the overlap between west coast and Cantabrian Sea values, consistent with connectivity supported by cohort tracking. In contrast, sardine isotopes from the west and south coasts converged into a unimodal distribution with growth, indicating frequent exchange between these regions. These findings support recent revision of stock limits that separate south and west coast anchovy stocks and maintain a single Iberian sardine stock, but they question the current assumption of separation between western and northern anchovy stocks. Eye lens isotopes provide a powerful complementary tool to resolve nursery origin and connectivity, offering new opportunities for fisheries management in shorter time scales than molecular techniques, which is paramount to be able to cope with rapid changes of fish distribution due to climate change, and for spatially explicit management.

## Introduction

Marine fish often change their habitats with ontogeny and seasons to avoid predation, meet physiological requirements and maximise fitness, resulting in diverse migration patterns among and within species or populations (Bauer and Hoye, 2014). Resolving each unique migration pattern is critical for defining management boundaries and assessing connectivity (Frisk et al., 2013), which is key for developing effective management practices. Stock assessment models, that provide the framework for setting exploitation limits, often rely on the assumption of well-mixed, self-recruiting population within boundaries (Cadrin et al., 2023). Connectivity determines whether local populations persist through self-recruitment or depend on external sources, with direct consequences for resilience to environmental change and fishing pressure (Sakamoto et al., 2024). Misspecifications of boundaries or inaccurate estimation of connectivity can therefore lead to biased assessments (Berger et al., 2021), overexploitation and stock collapses (Petitgas et al., 2010). Mass migrations of fish can also have substantial ecological impacts on ecosystems through alterations of energy flow, food-web topology and stability, and trophic cascades (Bauer and Hoye, 2014). Understanding migration patterns of abundant species and its ecological function should significantly contribute to ecosystem-based managements (Link et al., 2020).

European anchovy *Engraulis encrasicolus* and European sardine *Sardina pilchardus* are key components in the Northeast Atlantic and Mediterranean shelf ecosystems. These small pelagic fishes are short-lived and abundant plankton feeders (Garrido et al., 2015) and, being fed by many predators including fish (Cardona et al., 2015), mammals (Santos et al., 2014) and seabirds (Martínez-Abraín et al., 2019), they play a critical role in energy transfer from low to high trophic levels (Veiga-Malta et al., 2019). They are also important targets of pelagic fisheries, particularly off Western Moroccan waters and around the Bay of Biscay and Iberian Peninsula, providing several hundred thousand tonnes of landing annually (FAO 2025). However, the sustainability of the fishery is challenged by biological changes in recent years, such as a reduction of body sizes of both species in the Bay of Biscay (Véron et al., 2020; Taboada et al., 2024), declined sardine abundance off the Iberian west coast where anchovy bloomed for the first time since it started being recorded (Ferreira et al., 2023; 2024). Their migrations can cause significant changes in trophic structures, predator distribution and abundance, and increase challenges for fishery management. Despite their importance, the migration ecology of both species remains poorly studied, primarily due to the lack of appropriate techniques to track individual movements.

Population structure of the two species have been studied mainly by using genomics and morphological traits (e.g., Zarraonaindia et al., 2012), although many issues remain regarding the connectivity of Iberian Peninsula populations (Caballero-Huertas et al., 2022a). Currently, different anchovy stocks are assumed in the Bay of Biscay, western Iberian coast (hereafter West coast), and south Iberian coast (hereafter, the South coast), Alboran Sea and Atlantic Africa (three different stocks assessed by FAO) for management (Fig. 1). The border between the anchovy populations inhabiting the West and South coasts has been added only recently (ICES, 2024a), which was supported by a compilation of published and unpublished evidences from genomics, larval dispersal, fish and fisheries distribution, morphometrc and genomic studies (Garrido et al., 2024) but would benefit from further analysis. In particular, potential connectivity between the populations of the West coast and the Bay of Biscay has been suggested recently (Teles-Machado et al., 2024; Pujolar et al., 2025), against the current assumption of two different stocks in these areas. Adding another layer of complexity, two genetically and morphologically distinct ecotypes, namely marine and coastal ecotypes, have been identified in anchovies in the Mediterranean, Bay of Biscay and the North Sea (Le Moan et al., 2016; Huret et al., 2020). The former thrives preferentially in pelagic systems and the latter in estuaries and lagoons, whose distributions and proportions in other regions are unknown.

Sardine in the Cantabrian Sea, West and South coasts is managed as one stock (Fig. 1). This is consistent with the genomic analyses that assigned those surrounding the Iberian

Peninsula into a single cluster (da Fonseca et al., 2024; Sabatino et al., 2025), and cohort tracking analysis that suggests that the connectivity across these regions is mediated by migrating adults (Silva et al., 2019). Meanwhile, morphometric analysis suggests the existence of several clusters between the northern and southern Iberia (Neves et al., 2023), and the connectivity between west and east of the Strait of Gibraltar have been indicated, with unknown extent (Caballero-Huertas et al., 2022b; Hidalgo et al., 2024).

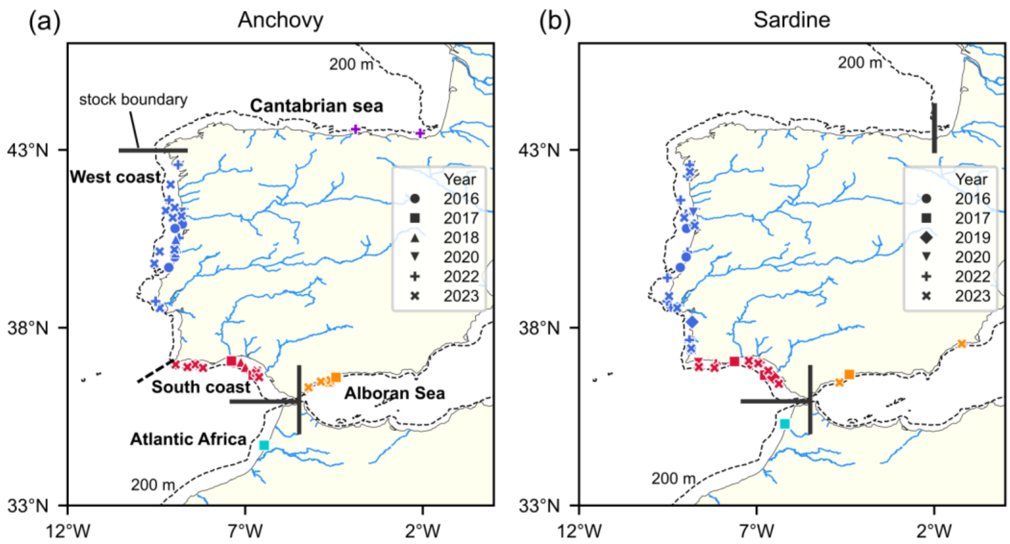


**Figure 1. Sampling locations of anchovy (a) and sardine (b) used for isotope analyses, with representation of stock boundaries (black lines), 200 m bathymetric contour and major rivers.** The broken line shows the new stock boundary added recently between west and south coast populations (a). River positions were taken from Global River Width from Landsat dataset (Allen and Pavelsky, 2018).

Stable isotopes in eye lenses may provide a complementary measure of fish movements, which can be a particularly helpful tool to understand the migrations of abundant small pelagic fish. Eye lenses are incrementally growing protein structure that lack turnover (Wallace et al., 2014), and therefore the history of isotopes of diet are recorded in their layers i.e. laminae with some isotopic offsets (Yoshikawa et al., 2025). The carbon and nitrogen stable isotopes of marine organisms often show significant gradients across oceanographic conditions and from inshore to offshore (Minagawa and Wada 1986; Rau et al., 1989; Montoya and McCarthy, 1995), reflecting differing primary producer isotopes and dietary plasticity (Bode et al., 2007; Chouvelon et al., 2015; Sakamoto et al., 2023b). Thus, the isotopes recorded in eye lenses can be used as a marker of organisms' locations (Vecchio and Peebles, 2022; Sakamoto et al., 2023a; 2025), making them one of the rare natural tags applicable to full-marine systems where water chemistry is largely homogenous. Sequential analysis from the inner to outer laminae to infer ontogenetic habitat and trophic changes has been the major usage, although analysing only the central part as a marker of nursery origin can be beneficial to analysis broad scale mixing from larger datasets (Vecchio and Peebles, 2022; Sakamoto et al., 2025). As eye lenses can be delaminated manually, the analysis is less time-consuming and equipment-dependent when compared to conventional otolith chemical analyses that requires substantial sample preparation efforts. This analysis can also detect movements on an ecological timescale that is directly relevant to management, which distinguishes it from genetic markers that indicate connectivity on an evolutionary timescale. However, overlapping isotope values do not necessarily lead to the same nursery, as organisms from distinct locations can show similar isotope values from different dynamics. This limitation should be considered, being preferable to complement this method with other approaches, to accurately understand mixing processes.

In this study, we investigated migration patterns of European anchovy and sardine around the Iberian Peninsula. To understand the geographical variations of $\delta^{13}$C and $\delta^{15}$N of their diets, muscles isotopes were first analysed. The isotopes of eye lens centre, which reflect the diet isotopes during early life stages, were then analysed for the two species across different size classes, to infer their nursery origin and detect ontogenetic migrations between areas. To aid the inferences obtained from eye lens analysis, we also investigated the connectivity by cohort tracking, comparing the abundance at ages of anchovy from the Cantabrian Sea, West coast and South coast, estimated during acoustic surveys in the last 10 years and interpreting the results in light of previously published results of sardine cohort tracking (Silva et al. 2019).

The water surrounding the Iberian Peninsula includes regions of distinct oceanographic conditions. The West coast of the Peninsula is a highly productive region, as a consequence of strong and frequent upwelling events, particularly during spring and summer, and due to freshwater discharge by several rivers and rías, particularly during fall and winter months (Ferreira et al. 2021). The Cantabrian Sea, in the north, is the transition zone between the western upwelling region and strong freshwater input in the eastern end, and has lower and more variable productivity compared to western waters.

The southern Atlantic coast including the Gulf of Cadiz, hereafter the South coast, is generally warm and oligotrophic except for some local upwelling and freshwater-input spots (Lafuente and Ruiz, 2007). The Alboran Sea in the east of the Strait of Gibraltar is even warmer in summer to which a jet current persistently flows (Hidalgo et al., 2024). Located further south is the northwest African coast, whose Atlantic side is a coastal area with permanent upwelling, and is more productive and warmer than the Iberian west coast. This environmental heterogeneity offers a unique opportunity to study how European anchovy and sardine move across different oceanographic conditions.

## Material and Methods

### *Sample collection*

European anchovy and sardine samples for stable isotope analysis were collected during acoustic and trawl surveys during 2016 to 2023 conducted by multiple institutes, which includes the spring acoustic survey PELAGO off Atlantic west and south Iberian coasts (2016, 2018, 2020, 2022, 2023), the autumn acoustic recruitment survey IBERAS off the west coast (2018, 2022, 2023), the autumn demersal survey IBTS off the west coast, summer MEDIAS survey in the Alboran Sea (2023), SARLINK 1117 winter survey covering the Alboran Sea, Atlantic African coast and south Iberian coast (2017), and PELACUS spring survey in the Cantabrian Sea (2022). A total of 691 anchovy and 590 sardine specimens were available. After measuring the total length (TL), wet weight and gonad weight and recording sex, white muscle tissues were extracted from dorsal side and stored in 1.5 ml plastic tubes. Eye lenses were also extracted from both eyes and stored in 96-well plates. Muscle and eye lenses were frozen at −20°C until later use. Fish were then classified into three size ranges, small (< 120 mm TL for anchovy and < 140 mm TL for sardine), medium (120–140 mm and 140–180 mm, respectively) and large (>140 mm and >180 mm, respectively), which roughly correspond to typical sizes of age 0, 1 and ≥ 2 of each species around Iberian Peninsula with 1−2 errors (Uriarte et al., 2016; Silva et al., 2008).

### *Analysis of muscles*

To understand the geographical variations of stable isotopes of the diet, the $\delta^{15}N$ and $\delta^{13}C$ of muscles were analysed for a subset of the samples. As fish of similar sizes in the same haul often have similar values (Sakamoto et al., 2023b), up to six individuals per size range were selected. In total, 106 anchovy from 18 hauls and 112 sardine from 19 hauls collected in 2016, 2017, 2022 and 2023 were selected for the isotope analysis to cover the geographical and temporal range of the target. Muscle tissues were freeze-dried and ground into powder. Lipids were extracted using a 2:1 chloroform:methanol solution, freeze-dried again and 800 µg of a subsample was extracted. The $\delta^{15}N$ and $\delta^{13}C$ values of the samples were analysed at GeoScience Laboratory (Nagoya, Japan) using a continuous-flow stable isotope ratio mass spectrometer (IsoPrime100, Elementar, Stockton, UK) coupled to an elemental analyser (vario MICRO cube, Elementar; FLASH2000, Thermo Fisher Scientific, Yokohama Japan). The $\delta^{15}N$ and $\delta^{13}C$ values were reported in $\delta$-notation against the atmospheric $N_2$ standard and the VPDB reference standard (Vienna Pee Dee Belemnite), respectively, and given as a ‰ value. Analytical precisions assessed by repeated measurements of laboratory standards were ± 0.2‰ for $\delta^{15}N$ and $\delta^{13}C$.

*Analysis of eye lenses*

Thawed eye lenses were placed on a slide glass under a stereo microscope with a micrometre scale for delamination. Using forceps, gelatinous cortex was removed and outer laminae were peeled off until the diameter of the remaining lens became smaller than 1 mm, typically 0.7 to 0.9 mm, under 10−20X magnification. The eye lens centre diameter corresponds to a fish size of 3 to 4 cm SL, for Japanese sardine (Sakamoto et al., 2025), which has similar morphology with European sardine and anchovy. The remaining centre part was rinsed with distilled water to remove potentially tangled fibres from outer laminae, then attached to the inner wall of 1.5 ml tube. After drying the lens centres for more than a day at a room temperature, tube lids were sealed, then the samples were sent to Stable Isotopes Analysis Facility, Sciences Faculty at the University of Lisboa, Portugal. Samples were weighted, then $\delta^{15}N$ and $\delta^{13}C$ were determined by continuous flow isotope mass spectrometry, on a Sercon Hydra 20-22 (Sercon, UK) stable isotope ratio mass spectrometer, coupled to a EuroEA (EuroVector, Italy) elemental analyser for online sample preparation by Dumas-combustion. The $\delta^{15}N$ and $\delta^{13}C$ values were reported in $\delta$-notation against the atmospheric $N_2$ standard and the VPDB reference standard (Vienna Pee Dee Belemnite), respectively, and given as a ‰ value. Analytical precisions calculated using repeated measurements of laboratory standard in every batch of analysis were ≤ 0.2‰ for $\delta^{15}N$ and $\delta^{13}C$.

*Correction of eye lens isotope values*

As larger fish tend to have higher $\delta^{13}C$ and $\delta^{15}N$ values due to the increase of trophic position, higher eye lens isotopes are expected in larger eye lens centres. To account for size variations in the peeled eye lens centre, effects of sample dry weight on isotope values were assessed using linear models including fish sampling region as a factor for each species ($\delta^{13}C$ or $\delta^{15}N$ ~ *Region* + *Sample weight*). When a significant effect of sample weight was detected, which was the case for $\delta^{13}$C and $\delta^{15}$N, the isotope values were corrected to the value at 0.3 mg of sample weight using the estimated slope.

*Data analysis*

Consistent analysis flow was used for the two species to reveal their different migration ecologies. To confirm that the isotopes values of eye lens centre are linked to nursery area, the geographical differences in the value distributions were visualised using scatter plots and kernel density estimations for each fish size class (small, medium, large). If many fishes migrated to different regions with growth, this would result in significant changes of data distributions between size classes. The temporal stability as a marker of nursery origin was assessed by calculating the mean isotope values of small-sized anchovy and sardine for each sampling year. Geographical variations of eye lens isotopes of small−medium-sized fish were compared to those of muscles of small−medium sized fish to test whether the eye lens isotopes reflect prey isotopes in each region. The inclusion of medium sized fish here was to cover regions with limited number of small fishes available.

To investigate the temporal variations in the extent of fish mixing/separation, overlaps of eye lens isotope value distributions were quantified between each group of sampling region/sampling year/fish size. For each pair of groups, two-dimensional histogram with 0.5 and 1.0‰ bins for $\delta^{13}$C and $\delta^{15}$N respectively, reflecting the greater variations in $\delta^{15}$N, were calculated for each group. For each bin, the smaller proportion between the two groups was retained, and the sum of these across all bins provided a metric of distributional overlap. While this allows the visualisation of major mixing/separations in a group level, minor differences between individuals of different origins can be of significant importance in terms of gene flow cannot be detected. As a complement, data points outside the 95% or 99% confident ellipses, calculated based on Mahalanobis distance, for fish across all size classes in each region were considered outliers. When outliers were just one in a given station, they were considered potential analytical errors and discarded. When multiple outliers were detected for a given station, they were considered valid and used for further analysis. The nursery origins of the valid outliers were inferred based on main data distributions of other sampling regions, defined as the 50% probability mass area of kernel density estimation, and their muscle isotope values when available.

*Cohort tracking analysis*

To contrast our results with observations of potential source and sink areas based on cohort analysis, we used published literature for the sardine (Silva et al. 2019) and conducted a cohort tracking analysis for the anchovy. Particularly, we compared the abundance at age of anchovy between contiguous areas using estimates of two complementary spring acoustic surveys used for stock assessment: PELACUS, conducted in the Cantabrian Sea and Western Galician waters and PELAGO, conducted in the Western Portuguese coast and South Iberia, including the Spanish Gulf of Cadiz.

Natural logarithms of abundance indices (ln(N+k)) per age were compared between the contiguous areas by adjusting a linear regression model to the data, where N is number of individuals at age and K is half the minimum but non-zero N observed for scaling. The abundance-at-age was compared either for individuals of same age in a given year for different areas (corresponding to matching dynamics of cohorts) or comparing the abundance of individuals at age $x$ and year $y$ in a given area, with the abundance of individuals of age $x+1$ in year $y+1$ in the contiguous area (testing for migration from one area to the other during the time between surveys). Correlations were tested between the Cantabrian Sea and the West coast and between the West coast and the South coast.

## Results

*Muscle isotope distribution*

The $\delta^{13}$C and $\delta^{15}$N values of the muscles of anchovy (70–184 mm TL) and sardine (104–220 mm TL) showed significant variations among and within regions (Fig. 2a–j). The $\delta^{13}$C value ranged from –20.1‰ to –15.6‰ in anchovy and from –19.6‰ to –16.2‰ in sardine, with the general tendency of higher values in sardine (Fig. 2a, c). The $\delta^{15}$N values ranged from +8.1‰ to +14.1‰ and from +8.1‰ to +12.9‰ for anchovy and sardine, respectively (Fig. 2c, d). Exceptionally high $\delta^{15}$N anchovy were found for large individuals (151–173 mm) caught during October 2022 in the Ría de Arousa estuary (+13.4 ± 0.8‰, Fig. 2a, c), and from medium individuals (124–134 mm) caught close to the Tagus River estuary during March 2022, both in the West coast (Fig. 2c). As such, only in the West coast, significant correlations were detected between anchovy $\delta^{13}$C and $\delta^{15}$N and logarithm of distance from the coast ($\delta^{13}$C: Peason's $r = –0.50$, $p = 7.7*10^{-3}$; $\delta^{15}$N: Peason's $r = –0.50$, $p = 1.5*10^{-6}$, $n = 27$), revealing the marked inshore-offshore gradients there. The geographical variation of $\delta^{13}$C was less clear, except for low values of anchovy caught off the South coast and Cantabrian Sea, which were not observed for sardine (Fig. 2i). On the other hand, $\delta^{15}$N values were significantly different between regions for both species, with higher values (10–12‰) mainly observed in the Cantabrian Sea, inshore (< 10 km) the West coast and in the South coast. Moderate values (9–10‰) were found offshore (> 10 km) the West coast and Atlantic Africa, and the lowest (< 9‰) in the Alboran Sea (Fig. 2j), likely reflecting different isotope baselines across regions.

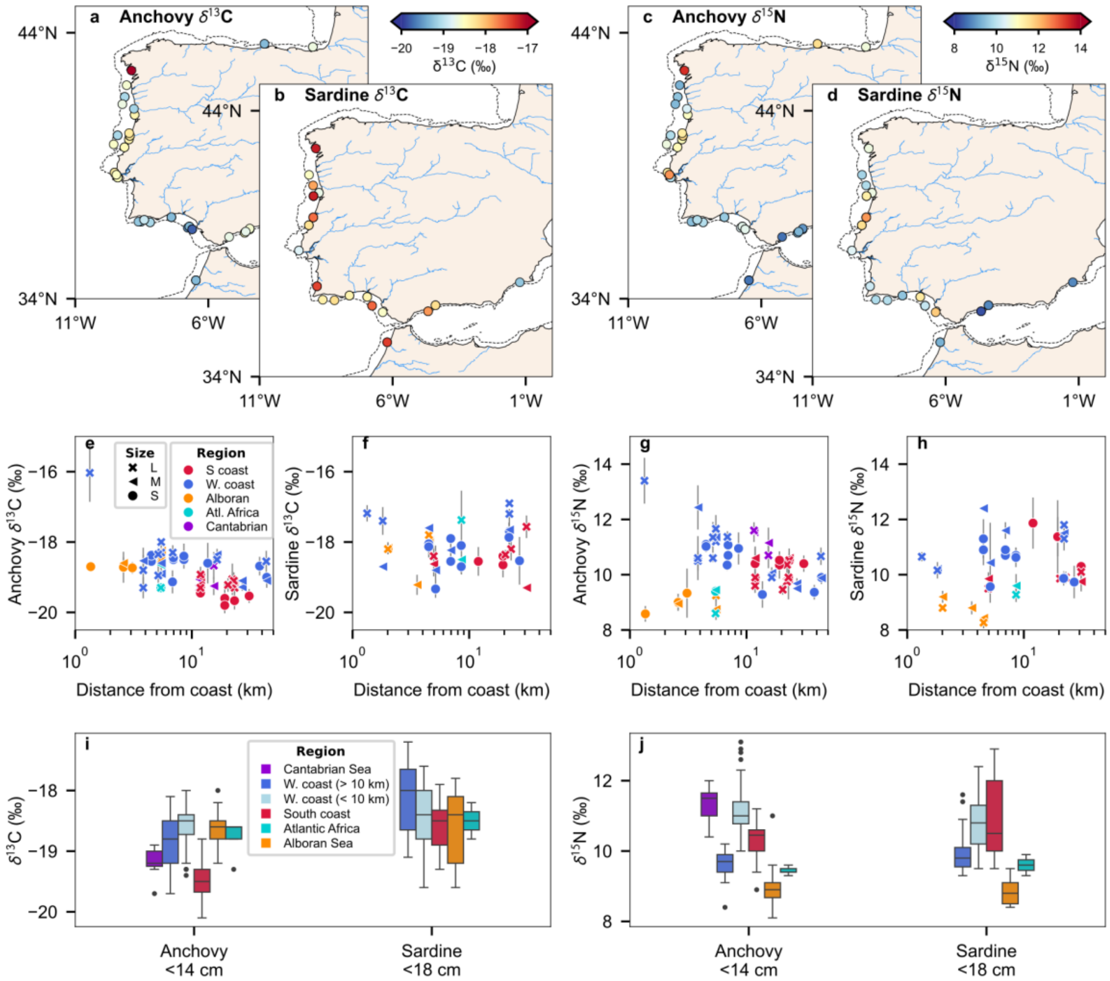


**Figure 2. Variations of muscle isotopes.** Station-mean $\delta^{13}$C (a, b) and $\delta^{15}$N (c, d) of anchovy (a, c) and sardine white muscles (b, d). Relationships between distance from coast and station/size class-mean $\delta^{13}$C (e, f) and $\delta^{15}$N (g, h) of anchovy (e, g) and sardine (f, h). Regional difference of $\delta^{13}$C (i) and $\delta^{15}$N (j) of small to medium anchovy and sardine, shown as boxplots. The west coast data is split into close or far from the coast at 10 km (i, j), given the significant gradient there (g).

*Eye lens isotopes*

The dry weight of delaminated eye lens centres ranged between 0.10 and 0.53 mg, whose effect on isotope values were assessed by linear model (Supplementary Table 1; Supplementary Fig. 1). For anchovy, the estimated slope of the effect was 1.14‰/mg for $\delta^{13}$C and 6.71‰/mg for $\delta^{15}$N, likely reflecting the change of trophic position with growth. For sardine, the effect on $\delta^{13}$C was not significant, while the effect on $\delta^{15}$N was 4.78‰/mg.

Hereafter, the eye lens $\delta^{13}C$ and $\delta^{15}N$ values corrected to those at 0.3 mg of sample weight using the slopes were used in downstream analyses to account for this effect.

The $\delta^{13}C$ and $\delta^{15}N$ of eye lens centre values varied significantly by species, sampling area and ontogeny (Fig. 3a–f). Anchovy showed distinct values between region, largely consistent across size ranges (Fig. 3a–c): high $\delta^{15}N$ (+9.6 ± 1.3‰ (1SD)) in the South coast, lower $\delta^{15}N$ (+6.8 ± 1.8‰) and $\delta^{13}C$ (–20.7 ± 0.8‰) in the West coast, low $\delta^{15}N$ (+5.9 ± 0.8‰) and higher $\delta^{13}C$ (–19.2 ± 0.6‰) in the Alboran Sea, and moderate $\delta^{15}N$ (+7.7 ± 0.6‰) and higher $\delta^{13}C$ (–19.1 ± 0.9‰) in the Atlantic Africa. The Cantabrian Sea showed values similar to those of the West coast, but also included higher ranges (Fig. 3b, c). In sardine, $\delta^{13}C$ values showed less geographical variations and mostly fell in the range between –21‰ and –17‰, though tended to be lower in the Alboran Sea (Fig. 3d–f). In small sardine (< 140 mm), $\delta^{15}N$ values were higher in the South coast (+10.1 ± 1.3‰) and lower in the West coast (+8.7 ± 0.8‰). This difference between the West and South coasts decreased in medium and large sizes (Fig. 3d–f). In medium and large sardines, $\delta^{15}N$ were lower in the Alboran Sea (+6.5 ± 1.6‰) and moderate in the Atlantic Africa (+8.1 ± 1.2‰), and were distinct from those in the West and South coasts. Among sampling years, mean eye lens isotope values of small anchovy and sardine in each region varied by < 1‰ for $\delta^{13}C$ and < 1.5‰ $\delta^{15}N$, showing the robustness against inter-annual variations. Compared to muscle isotopes, eye lens isotopes showed greater extent of geographical variation (Fig. 4a, b). The difference of mean $\delta^{13}C$ in eye lens and muscle for each region was less than 1‰ in most cases, except for the West coast and Cantabrian Sea anchovy that differed by 1–2‰. The $\delta^{15}N$ were generally lower in eye lens centre by 1–3‰, although the ranks were mostly consistent between muscles and eye lens centres if we use offshore (>10 km) mean for the West coast for the muscle (Fig. 4b). The exception here was the Cantabrian Sea, where muscles showed highest $\delta^{15}N$ values but lower in eye lenses.

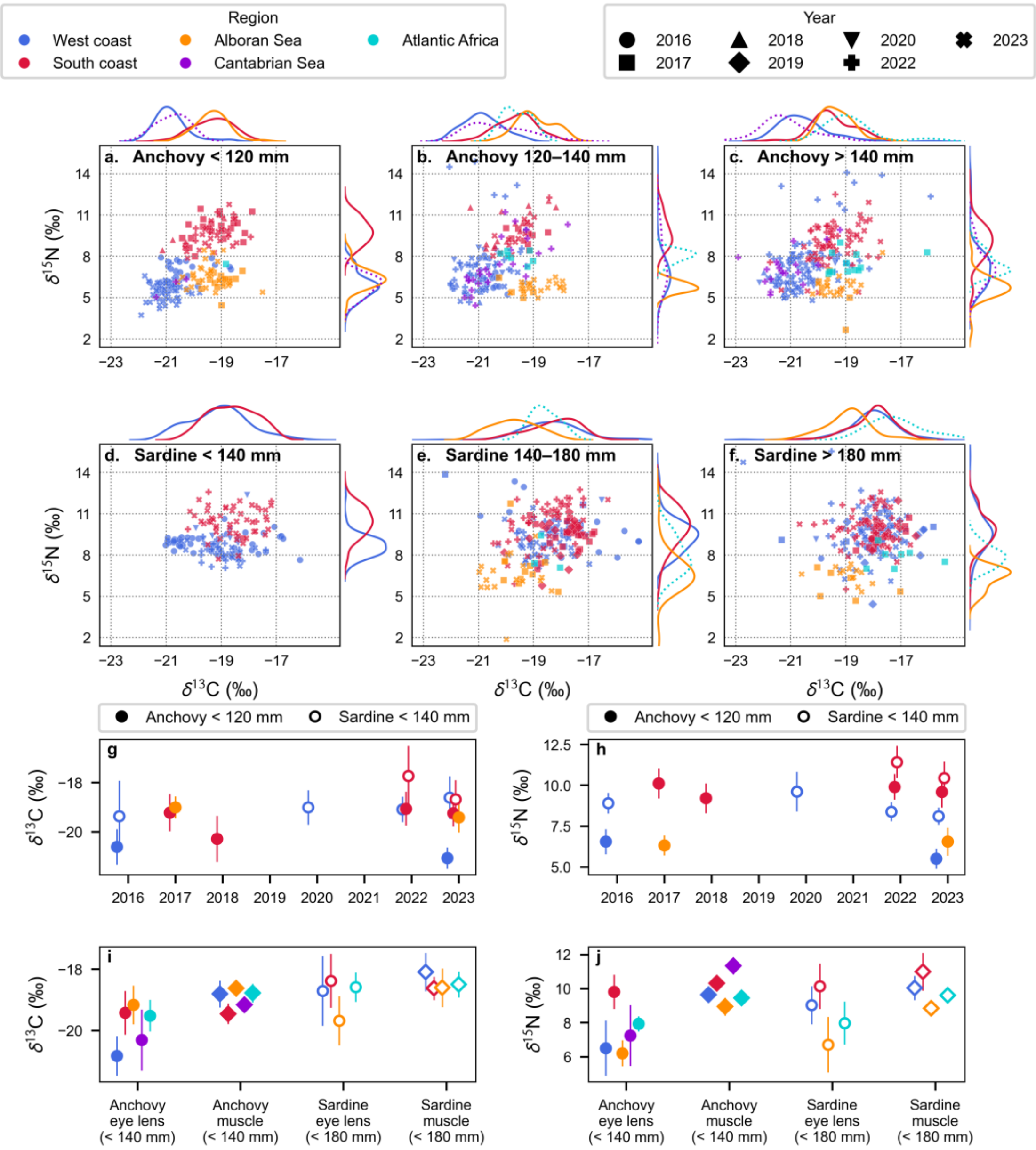


**Figure 3. Variations of eye lens centre isotopes.** Eye lens centre $\delta^{13}$C and $\delta^{15}$N of anchovy (a, b, c) and sardine (d, e, f) of small (a, d), medium (b, e) and large size (c, f), where colours and symbols represent sampling regions and years, respectively. Inter-annual variations of eye lens $\delta^{13}$C (g) and $\delta^{15}$N (h) of small anchovy (filled) and sardine (open). Comparison of geographical differences in $\delta^{13}$C (i) and $\delta^{15}$N (j) between eye lens centre and muscle. The nearshore muscle data (< 10 km) in the West coast fishes are excluded given the strong inshore-offshore gradient there (i, j). The plots and error bars show mean and 1 SD, respectively (g–j).

The similarity of eye lens centre isotopes between regions, size classes and sampling year were assessed as the overlaps of 2-D histograms (Fig. 4). For anchovy, higher overlaps (> 0.5) were mostly observed within each sampling region and not between the regions across size classes and years, except for the West coast and Cantabrian Sea that showed high overlaps (Fig. 4a). These results are indicative of limited movements of anchovies between the Atlantic Africa, Alboran Sea, South coast and West coast, and potential mixing between the West coast and the Cantabrian Sea. Despite this general trend, there were outliers in each region that suggest individuals of different origins (Fig. 4b). In the West coast, high eye lens $\delta^{15}$N outliers were found in hauls in the Ría de Arousa and close to the Tagus River mouth (Fig. 4b, c), which mostly matched the exceptionally high muscle $\delta^{15}$N individuals (Fig. 4d). This suggests the individuals' strong preference for coastal environment potentially throughout life stages. At around the western edge of South coast (stations "14", "15" and "17", Fig. 4c), six large anchovies showed similar isotope values with the Alboran Sea or West coast fishes (Fig. 4b), which are likely migrants from the adjacent regions. Similarly, the three small individuals found in the westmost station ("67") in the Alboran Sea with higher $\delta^{15}$N may have originated from the South coast.

For sardine, higher histogram overlaps were shown within the Alboran Sea stations, but also between medium to large individuals in the West and South coasts (Fig. 4e). These are indicative of general separation between the Alboran Sea and the South coast at the Strait of Gibraltar, and the mixing between the West and South coasts with age. The highest overlap between the 2022 small and 2023 medium sardines stood up as an exception, showing the strength of West coast recruits in 2022. Many of the outliers in the West coast were collected in March 2016 ("W12", "22") in the low biomass period. Outliers found in the South coast in 2022 ("S12") and 2019 ("1") with low $\delta^{15}$N likely originated from the Alboran Sea, showing the possibility of minor migration across the Strait.

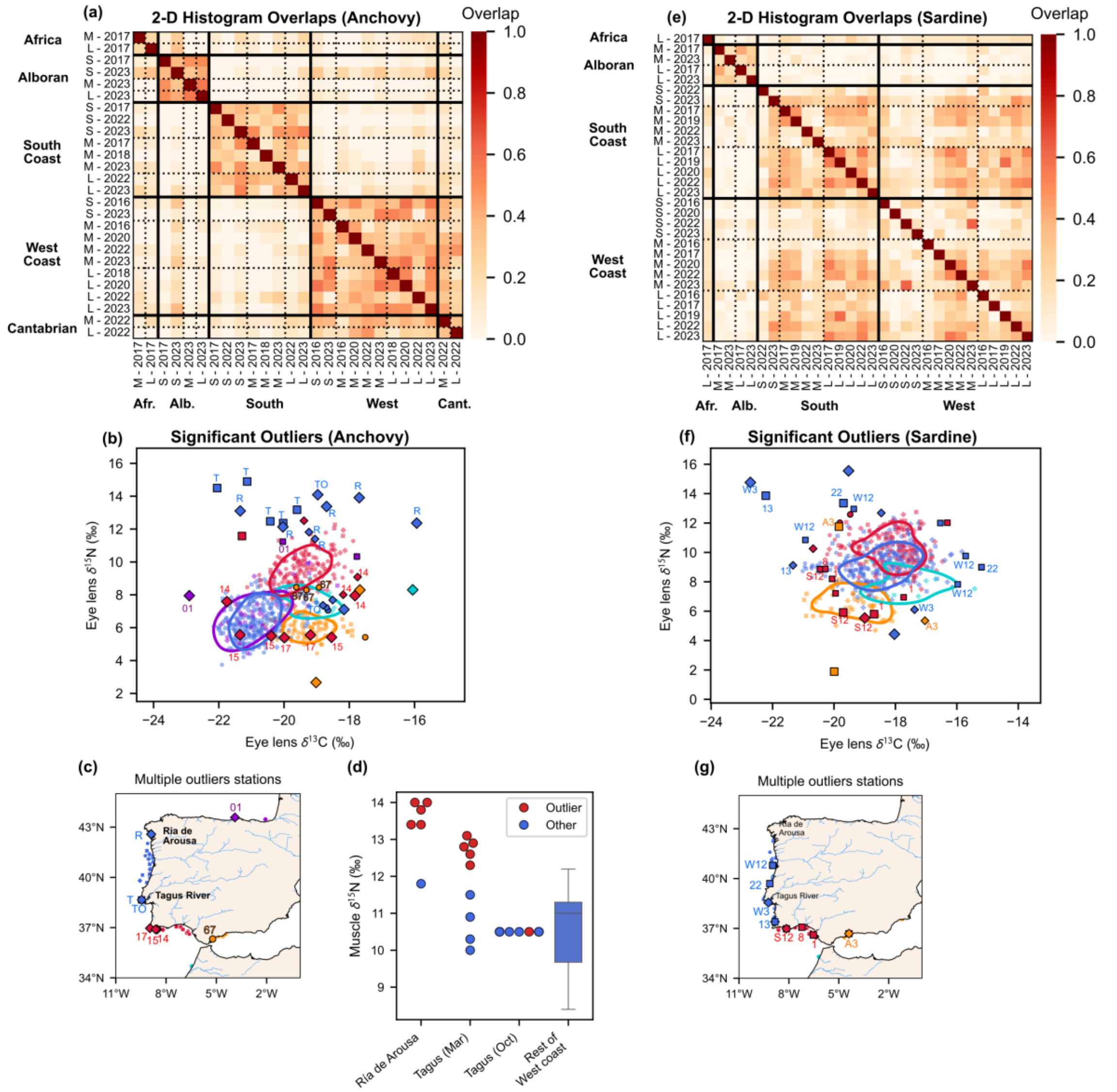


**Figure 4. General trends and outliers of eye lens isotopes.** Histogram overlaps between different sampling year/size class (Small, Medium, Large)/region groups for anchovy (a) and sardine (e). The outliers out of 95% (larger plots) and 99% (largest plots) confident ellipse based on Mahalanobis distance for each region, with small plots showing non-outliers and lines showing main data distributions based on kernel density estimation (50% probability mass area) for anchovy (b) and sardine (f). The annotations in (b, f) are station IDs from which multiple outliers were detected (c, g). The muscle $\delta^{15}$N of West coast anchovy, with the detected high eye lens $\delta^{15}$N outliers shown in red, others in blue plots or a boxplot.

*Cohort tracking of anchovy*

Potential connectivity of anchovy populations from the North, West and South Iberia was investigated by cohort tracking, comparing the abundance-at-age of fish from synoptical spring acoustic surveys. The correlation of the abundance of fish from the West and South Iberia was not significant for fish of the same age (p-values > 0.35, Fig. 5a, b, c). Moreover, no significant correlation was found between age 1 individuals from the South and age 2 individuals from the West in the following year (Fig. 5d), discarding the hypothesis of a significant migration of recruits from the Gulf of Cadiz recruitment hotspot to the West coast.

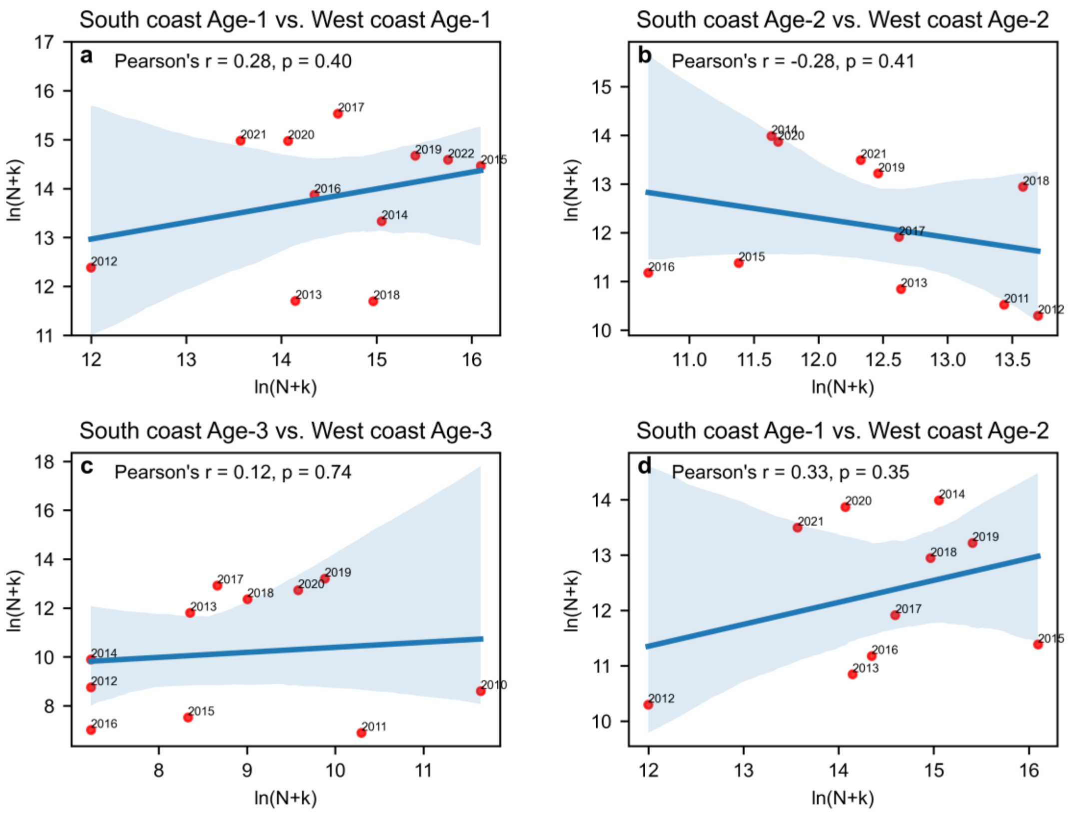


**Figure 5. Cohort tracking of the West coast and South coast anchovies.** Relationship between the abundance of Age 1, 2 and 3 individuals estimated in the PELAGO+PELACUS survey series in the West and the South Atlantic Iberian coast (a, b, c), and with Age 1 in the South and Age 2 in the West coast (d). Units for both axes are Log the number of individuals (N) + K, being K half the minimum non-zero N observed, method described in ICES, 2004; Payne et al., 2009). The labels represent year-classes.

The potential connectivity between the West coast and the North (Cantabrian Sea, corresponding to Division 8c which is part of the Bay of Biscay stock) was tested using the same approach. A significant correlation was found between the abundance of fish of the same age between both areas, for the three ages groups tested (p-values < 0.012, Fig. 6a, b, c). Moreover, a significant correlation was found between age 1 individuals in the North with age 2 individuals found in the West Iberia in the following year, suggesting a potential southern migration during the juvenile stage (Fig.6d).

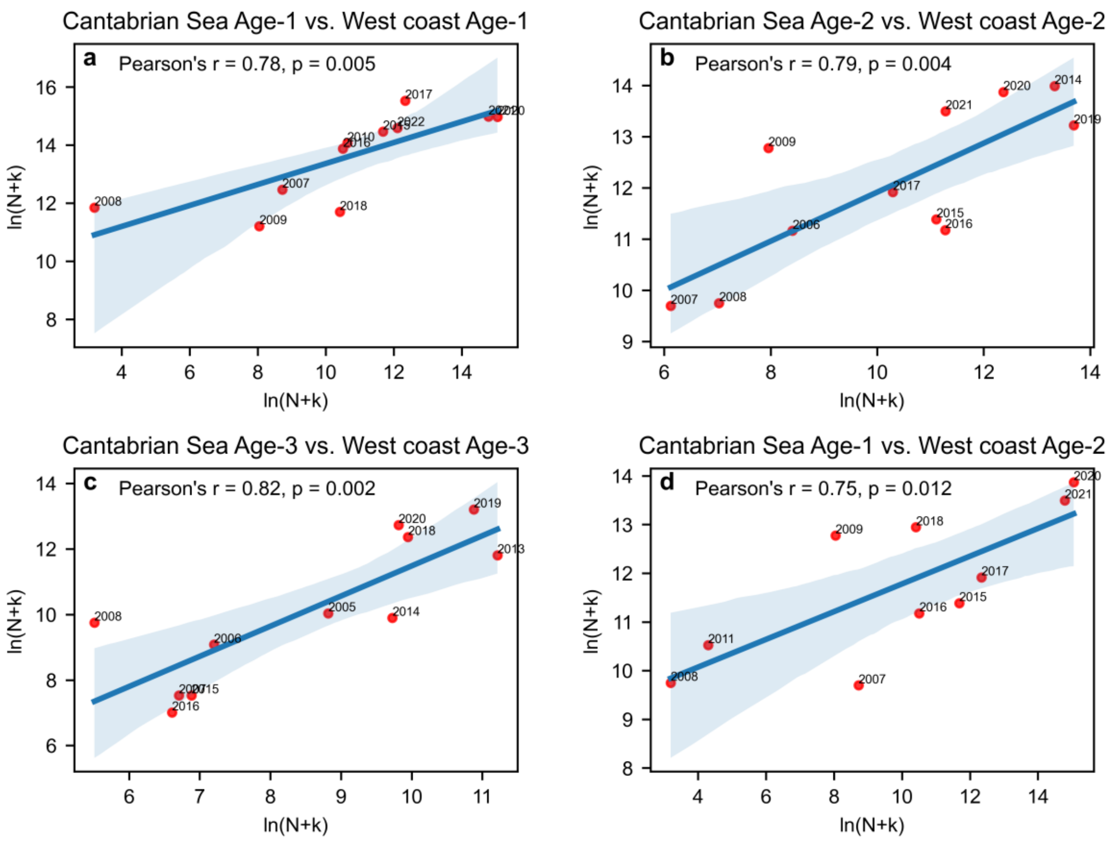


**Figure 6. Cohort tracking of the West coast and Cantabrian Sea anchovies.** Relationship between the abundance of Age 1, 2 and 3 individuals estimated in the PELAGO+PELACUS survey series and in the Cantabrian Sea (division 8c) (top panels and right bottom panel), and with Age 1 in the Cantabric and Age 2 in the West Iberian coast (left bottom panel). Units for both axes are Log the number of individuals + K, being K half the minimum N observed, method described in ICES, 2004; Payne et al., 2009). The labels represent year-classes.

## Discussion

In this study, we analysed the migration patterns of European anchovy and sardine around the Iberian Peninsula, by introducing stable isotopes of eye lenses for the first time for the species. The eye lens centre isotopes, particularly $\delta^{15}$N, were significantly different between recruitment areas around the Iberian Peninsula, thereby showing its utility as a marker of individual nursery origin in a shelf ecosystem of a scale of several-hundred kilometres. The analysis allowed to us to effectively visualise the overall mixing and separation patterns across regions that changed with ontogeny, and detected the outliers representing rare migrants or different ecotypes. With the support from cohort tracking analysis, our results revealed the difference in migration ecology between the two small pelagic species of similar niches, which have important implications for management.

The geographical variations of eye lens isotopes are mainly driven by difference in primary producer isotopes. The eye lens centre record body isotope values during larval stage with several permill offset in $\delta^{15}$N (Sakamoto et al., 2025), which sensitively reflects local prey isotopes in a short time period (Sakamoto et al., 2023a). This explains the lower $\delta^{15}$N, and similar but more exaggerated geographical isotopic differences in eye lenses than in muscles of juveniles and adults (Fig. 3). The important factors affecting phytoplankton isotopes are nutrient source and availability for $\delta^{15}$N (Minagawa and Wada, 1986; Montoya and McCarthy, 1995), and temperature-dependent phytoplankton growth rates for $\delta^{13}$C (Rau et al., 1989). The lower $\delta^{15}$N in the Alboran Sea likely reflect the contribution of nitrogen originated from $N_2$ fixation that are active in the Mediterranean (Béthoux and Copin-Montégut, 1986). In contrast, the higher $\delta^{15}$N in the West coast nearshore waters likely reflects inputs from anthropogenic origin nitrogen that has high $\delta^{15}$N (Vinagre et al., 2011; Bode et al., 2025). On the other hand, the West coast and Atlantic Africa are both part of Canary Current upwelling system, where abundant nutrients supplied from deep waters. Preferential uptake of lighter nitrogen by phytoplankton from a rich pool likely led to the lower baseline $\delta^{15}$N there, which may have worked otherwise in the nutrient-limited South coast. For $\delta^{13}$C, lower values in anchovy eye lens in the Cantabrian Sea and the West coast likely reflect lower nursery temperatures than in southern areas. Trends in $\delta^{13}$C were inconsistent across species and tissues, suggesting that the spatial differences in baselines can be outweighed by species or life-stage dependent factors such as spawning seasonality or local-scale habitat selection particularly near river mouth. The results for the Cantabrian Sea anchovy were unexpected, having high $\delta^{15}$N and moderate $\delta^{13}$C in muscle but both lower in eye lens centres (Fig. 3j). This is attributable to the unique movement pattern of anchovy there, where some individuals spend their early life stage off the shelf before migrating back closer to the coast (Irigoien et al., 2008), where zooplankton $\delta^{15}$N and $\delta^{13}$C are likely lower.

A self-recruiting anchovy population likely exists in the South Atlantic coast of Iberian Peninsula. The separations of eye lens centre isotopes between the Atlantic Africa, Alboran Sea, South coast and West coast–Cantabrian Sea regions, consistently evident across different size classes and sampling years, suggest limited migrations across these regions. The separation between the West and South coasts is also corroborated with the lack of correlations of abundance at ages between the two areas (Fig. 5). While our analyses cannot reject the mixing during larval stage via ocean transport (Casaucao et al., 2021), the genomic differentiation between fish from the West and South coasts also suggest the separation there (Pujolar et al., 2025). The Strait of Gibraltar, between the South coast and the Alboran Sea, have not been identified as a significant barrier of gene flow (Zarraonaindia et al., 2012; Alexandridis et al., 2025), although distinct otolith morphometrics have been detected (Bacha et al., 2014), consistently with our inference from eye lens isotope. Importantly, the existence of some large-sized migrants in the western part of the South coast, likely from the West coast or the Alboran Sea, suggest that the separation is not complete. As large anchovies are often collected as a minor portion of a mixture with other species such as sardine and chub mackerels in trawl surveys in the area (0.03–12% in 2023 PELAGO survey, ICES, 2024b), they may have been trapped in the schools of larger fishes (Bakun and Cury, 1999) and taken to the area. However, given the lack of such migrants in the Gulf of Cadiz, the eastern part South coast where the major spawning activities in the South coast occur, the impact of the migrants on abundance are likely limited. This line of evidence supports the recent decision to split the South coast from the West coast stocks (ICES, 2024a) and different managing units.

The anchovy populations in the West coast likely have a more complex structure. Despite the current management assumption of considering the West populations as a stock unit, the existence of a self-recruiting population here is disputable, as shown in the recent genomic analysis that have suggested connectivity between anchovies from the West coast and Ireland (Pujolar et al., 2025). Larval dispersal simulations also suggest that the rapid population increase in the West coast since 2015 may be a result of colonisation from Bay of Biscay populations, driven by anomalously strong and persistent westward currents that occurred in the Cantabrian Sea in 2014 and 2015 (Teles-Machado et al., 2024). Eye lens $\delta^{13}$C and $\delta^{15}$N were overlapping between anchovies in the West coast and the Cantabrian Sea even in small sizes, but there was a discrepancy between $\delta^{13}$C of eye lens centre and muscle in the West coast (Fig. 3i). This can be reasonably explained if most of the West coast anchovy spent their early life stage in the Cantabrian Sea and then moved to the West coast. Moreover, the significant correlation of abundance at age between the west and north Iberian coasts (Fig. 6) support the mixing. The western and northern populations are currently managed as two independent stock units, but it is advised to review stock limits, which is critical as underlying basis of stock assessment and fisheries management. The West coast is characterised by frequent and strong coastal upwelling events during spring and summer months, which provides a cooler and productive environment for the mid-latitude area, with potential to host a population adapted to higher latitudes.

Furthermore, the high $\delta^{15}$N individuals in the Ría de Arousa and Tagus River estuaries may be coastal ecotypes such as the ones identified in the Bay of Biscay, Mediterranean Sea and North Sea (Huret et al., 2020; Le Moan et al., 2016) and have not been recognised yet in the West coast. The impact of the estuarine ecotypes on stock assessment is likely limited, as they were only found in low proportions. As the scattered estuarine ecotype populations are known to be genetically differentiated while having a shared ancestry, however, they deserve attention for conservation of genetic diversity. These are the remaining issues for West coast anchovy managements that need to be solved by future investigations, which should include whole-genome sequencies (e.g., Pujolar et al., 2025) with spatially dense sampling, and inferences about individual movement histories based on otolith oxygen isotopes (Sakamoto et al., 2024).

Sardine likely have broader migration ranges than anchovy. The previous cohort tracking analysis across the Bay of Biscay shelf and the Iberian coasts predicted significant migrations of sardines of age 1 to 3 among adjacent areas (Silva et al., 2019). The converged eye lens isotope distributions of larger sardines in the West and South coasts, despite the difference in small sardines, suggest the gradual ontogenetic mixing with age, thereby providing empirical support for the prediction. The stock biomass of Iberian sardine is under recovery from the historical low-levels during the 2010s. The suggested mixing of the medium and large sardines in 2020s indicates that the recruitment hotspots off the West and South coasts that respond differently to environmental variabilities (Ferreira et al., 2023) both contributed significantly to the recovery, showing the importance of the both for population fluctuations. Migrations though the Strait of Gibraltar may occur occasionally from the East to the West against the Atlantic Jet, as a minor number of outlier individuals with low eye lens $\delta^{15}$N similar to the those in Alboran Sea can be found in the South coast. The migrants may mediate gene flows suggested by genomic studies (da Fonseca et al., 2024; Sabatino et al., 2025), although their low proportions suggest limited impact on abundance. For practical management and stock assessment, therefore, we present results support the assumption of a single metapopulation extending from the north, west and south Iberian coasts, and splitting the Alboran Sea and Africa.

The distinct migration patterns of sardines and anchovies off the Iberian Peninsula are likely influenced by a combination of ecological and physiological factors. Anchovy experience peak spawning in the spring and summer months, when upwelling events enhance productivity, especially along the West coast where they are frequent and intense. On the South coast, upwelling events occur less often and with lower intensity. Anchovy larvae require higher energy intake to meet their physiological demands by developing in warmer temperatures. Therefore spawning in the proximity of river runoff might be essential, and this requirement to find such spawning locations for adults can limit their migration far away from nursery areas. In contrast, sardines typically spawn between October and March in colder waters, a period when downwelling conditions dominate off western Iberia. The reduced metabolic costs of larvae in lower temperatures may relax selections for spawning location of spawners, which may allow the adults to migrate more freely to maximise energy acquisition. This is consistent with the view that sardine in the European Atlantic do not show natal homing for spawning (Silva et al., 2019). Leaving the nursery area and reproducing in other area has also been observed in *Sardinops* species off South Africa coast and in the western North Pacific (Teske et al., 2021; Sakamoto et al., 2025), which could be a general feature of sardines.

The different migration ecology of anchovy and sardine indicates that significant changes in their abundance, which have been observed elsewhere (Chavez et al., 2003), can have ecosystem-level impacts. Anchovy and sardine are both predominant zooplankton feeders that prey on secondary productions and transfer the energy to higher trophic levels. Their migrations from a nursery to different regions is therefore an energy export, suggesting that the primary production in migration source areas can enrich higher level production in the sink neighbouring areas (Hutchings et al., 2010). The excretion and death of fish in the sink can also enhance nutrient recycling (Bauer and Hoye, 2014). The greater ability of sardine to migrate suggests that the lateral energy transfer process would be pronounced under sardine dominant condition and less in anchovy flourishing environment. This leads to the hypothesis that the distribution and abundances of high trophic level predators can be geographically more homogenised under sardine-dominance but patchier and more concentrated under anchovy-dominance. Future quantifications of the biomass of migrators, which could be predicted by cohort tracking (Silva et al., 2019) and calibrated by eye lens isotopes analyses as in this study, may allow assessments of the significance of the processes when combined with ecosystem models (Veiga-Malta et al., 2019). This quantification should also be of great help to develop spatial explicit management, which is crucial in the Iberian shelf system with significant environmental heterogeneity.

Overall, we demonstrated that the stable isotopes in eye lenses are useful to understand movement dynamics of marine fish. Given the similar isotopic trends between species, the method would also be applicable for other species around Iberian Peninsula, and beyond. Using only two isotope values for broader geographical range, however, increases the risk of confusion of different nursery areas accidently showing similar values through different dynamics. The combination with other natural tags is therefore the key for geographical expansions. Nevertheless, eye lens isotopes would remain an important option due to the feasibility to generate large dataset, which allows to resolve spatiotemporally varying mixing processes (Sakamoto et al., 2025). Such analysis allows to remove spatial components from the temporal variation of population dynamics, which would be of significant help to understand and predict marine fish population fluctuations. in shorter time scales than molecular techniques, which is paramount to be able to cope with rapid changes of fish distribution due to climate change and for spatially explicit management.

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**CRediT authorship contribution statement**

**Tatsuya Sakamoto:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Susana Garrido:** Writing – review & editing, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation.

**Data availability**

Data will be made available on request.

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**Supplementary Information for: Different migration patterns of European anchovy and sardine around Iberian Peninsula revealed by eye lens isotopes**

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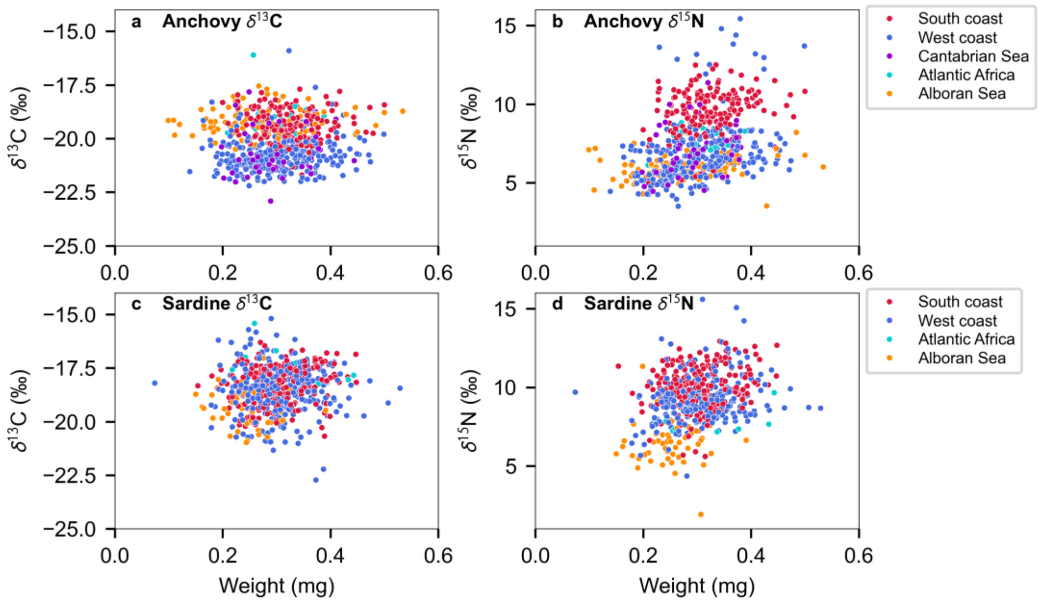


**Supplementary Figure 1. The effect of sample weight on eye lens centre $\delta^{13}C$ (a, c) and $\delta^{15}N$ (b, d) in anchovy (a, b) and sardine (c, d).** Colours indicate sampling region.

**Supplementary Table 1.**

Summary results of linear model ($\delta^{13}C$ *and* $\delta^{15}N$ *~ Sample weight + Region*).

***Anchovy*** $\boldsymbol{\delta^{15}N}$ ***~ Sample weight + Region***

| | Estimate | Std. Error | t-value | p-value | |
|---|---|---|---|---|---|
| (Intercept) | 4.07 | 0.28 | 14.34 | < 2E-16 | *** |
| Sample weight | 6.71 | 0.88 | 7.59 | 1.2.E-13 | *** |
| Region:Atlantic Africa | 1.58 | 0.36 | 4.40 | 1.3.E-05 | *** |
| Region:Cantabrian Sea | 1.14 | 0.26 | 4.45 | 1.0.E-05 | *** |
| Region:South coast | 3.36 | 0.19 | 17.80 | < 2E-16 | *** |
| Region:West coast | 0.76 | 0.17 | 4.39 | 1.3.E-05 | *** |

Residual standard error: 1.46 on 621 degrees of freedom

Multiple R-squared: 0.483, Adjusted R-squared: 0.4788

F-statistic: 116 on 5 and 621 DF, p-value: < 2.2e-16

***Anchovy*** $\boldsymbol{\delta^{13}C}$ ***~ Sample weight + Region***

| | Estimate | Std. Error | t-value | p-value | |
|---|---|---|---|---|---|
| (Intercept) | -19.55 | 0.15 | -129.50 | <2e-16 | *** |
| Sample weight | 1.14 | 0.47 | 2.43 | 0.016 | * |
| Region:Atlantic Africa | 0.12 | 0.19 | 0.61 | 0.54 | |
| Region:Cantabrian Sea | -1.44 | 0.14 | -10.51 | <2e-16 | *** |
| Region:South coast | -0.23 | 0.10 | -2.29 | 0.022 | * |
| Region:West coast | -1.50 | 0.09 | -16.28 | <2e-16 | *** |

Residual standard error: 0.7764 on 621 degrees of freedom

Multiple R-squared: 0.4403, Adjusted R-squared: 0.4358

F-statistic: 97.69 on 5 and 621 DF, p-value: < 2.2e-16

**Supplementary Table 1 (continued).**

***Sardine $\delta^{15}N$ ~ Sample weight + Region***

| | Estimate | Std. Error | t-value | p-value | |
|---|---|---|---|---|---|
| (Intercept) | 5.30 | 0.31 | 17.16 | < 2e-16 | *** |
| Sample weight | 4.16 | 0.97 | 4.28 | 2.23E-05 | *** |
| Region: Atlantic Africa | 1.33 | 0.47 | 2.89 | 0.0040 | ** |
| Region: South coast | 3.50 | 0.22 | 16.09 | < 2e-16 | *** |
| Region: West coast | 2.70 | 0.21 | 12.66 | < 2e-16 | *** |

Residual standard error: 1.337 on 589 degrees of freedom

Multiple R-squared: 0.3551, Adjusted R-squared: 0.3508

F-statistic: 81.1 on 4 and 589 DF, p-value: < 2.2e-16

***Sardine $\delta^{13}C$ ~ Sample weight + Region***

| | Estimate | Std. Error | t-value | p-value | |
|---|---|---|---|---|---|
| (Intercept) | -19.43 | 0.24 | -81.99 | < 2e-16 | *** |
| Sample weight | 0.18 | 0.75 | 0.25 | 0.81 | |
| Region: Atlantic Africa | 1.79 | 0.37 | 5.06 | 5.66E-07 | *** |
| Region: South coast | 1.12 | 0.17 | 6.74 | 3.88E-11 | *** |
| Region: West coast | 0.90 | 0.17 | 5.49 | 6.16E-08 | *** |

Residual standard error: 1.061 on 589 degrees of freedom

Multiple R-squared: 0.07797, Adjusted R-squared: 0.07171

F-statistic: 12.45 on 4 and 589 DF, p-value: 9.932e-10