



## Differentiation of *Lesueurigobius sanzi* and *L. suerii* through otolith morphometric analysis

Jaime S. Rubiales-Gómez<sup>a</sup>, Jairo Castro-Gutiérrez<sup>a,b,\*</sup> , Carlos Rodríguez-García<sup>a,c</sup> ,  
 Jesica Sarmiento-Carbajal<sup>a,c</sup>, Remedios Cabrera-Castro<sup>a,c</sup> 

<sup>a</sup> Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Campus Río San Pedro, Universidad de Cádiz, Puerto Real, 11510, Spain

<sup>b</sup> Departamento de Ciencias Agroforestales, Escuela Técnica Superior de Ingeniería, Campus El Carmen, Universidad de Huelva, Huelva, 21007, Spain

<sup>c</sup> Instituto Universitario de Investigación Marina (INMAR), Campus de Excelencia Internacional del Mar (CEIMAR), Puerto Real, 11510, Spain

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### ABSTRACT

Otolith morphology is a valuable tool for species identification, particularly for morphologically similar species. This study evaluates the potential of otolith morphometry to distinguish *Lesueurigobius sanzi* and *L. suerii*, two closely related species commonly found as fishery discards in the Gulf of Cádiz. A total of 1019 individuals (*L. sanzi*: 921, *L. suerii*: 98) were collected and their otoliths were analysed using the R package "shapeR" to obtain morphometric parameters and standardised wavelet coefficients. Statistical analyses revealed significant morphological differences between the species. Principal Component Analysis (PCA) showed that otolith length, perimeter, and shape indices contribute significantly to species differentiation. Furthermore, a Linear Discriminant Analysis (LDA) based on standardised wavelet coefficients demonstrated high classification accuracy (91.6%), confirming the effectiveness of this approach for species identification. These findings highlight the reliability of otolith morphometry as a robust method for distinguishing *L. sanzi* and *L. suerii*, providing valuable insights for fisheries management and biodiversity studies. This research contributes to the development of classification methods, essential for sustainable fishery assessments and ecological research.

### 1. Introduction

Fishing has grown significantly due to technological advancements, leading to higher fishing efforts and intensified overexploitation of fishery resources, alongside a rise in discards (Rousseau et al., 2019; Gilman et al., 2020). According to the Food and Agriculture Organization of the United Nations (FAO, 1996), fishery discards are defined as the portion of the catch that, during a fishing operation, is not retained on board and is returned to the sea alive or dead. These discard dynamics highlight the need to integrate sustainable management measures that balance technological advancements with resource conservation (Aranciaga, 2003; Coronil-Huertas et al., 2024).

The decision to discard catches is influenced by multiple factors, primarily economic reasons and fishing regulations, with four main causes identified: the use of non-selective fishing techniques (Catchpole et al., 2005), biological and environmental factors affecting species distribution (Maynou and Sardá, 2001), social and cultural reasons (Morandau et al., 2014) and economic and regulatory drivers (Feekings

et al., 2012). These causes include specific situations such as the capture of individuals below the legal size, species with low or no commercial value, damaged catches, or exceeding quotas (Coronil-Huertas et al., 2024). High levels of discards pose serious ecological problems, as they can destabilize marine ecosystems, impacting biodiversity and ocean health (Bellido et al., 2011).

Among the discards from the artisanal bottom trawling fleet in the Gulf of Cádiz (located in the northeastern Atlantic Ocean along the southwestern Iberian Peninsula; Fig. 1), are Sanzo's goby, *Lesueurigobius sanzi* (de Buen, 1918) and Lesueur's goby, *L. suerii* (Risso, 1810), two species belonging to the family Gobiidae. Both are small benthic species inhabiting sandy or muddy substrates between 30 and 200 m depth, usually associated with burrows or sediment shelters. Although they have little or no commercial value, these species are a common fishery bycatch that play a key ecological role within the demersal community of the Gulf of Cádiz. They act as essential trophic links, transferring energy from benthic invertebrates to higher trophic levels, including commercially important predators such as hake (*Merluccius merluccius*)

\* Corresponding author. Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Campus Río San Pedro, Universidad de Cádiz, 11510, Puerto Real, Spain.

E-mail address: [jairo.castro@uca.es](mailto:jairo.castro@uca.es) (J. Castro-Gutiérrez).

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(Velasco and Olaso, 1998; Torres et al., 2013; Riccioni et al., 2022). However, their strong visual similarity makes the species identification based on external morphology particularly difficult. Due to this difficulty, species of the genus *Lesueurigobius* have often been misidentified, making it necessary to interpret the available information on their habitat and biology with caution (Lloris, 2015). This persistent misidentification affects the reliability of catch data reporting, obscuring actual abundance patterns and hindering the evaluation of their ecological contribution within the Gulf of Cádiz ecosystem (García-Vázquez et al., 2012).

In recent years, the morphometry of otoliths has emerged as accessible and cost-effective methodology for identifying stocks and analysing the life histories of commercial and non-commercial fish species (Abaunza et al., 2008; Karahan et al., 2014). Otoliths are calcified structures located in the inner ear of teleost fishes, involved in balance and hearing, and characterized by incremental growth throughout the life of the individual, preserving species-specific information on growth and morphology (Campana, 1999). Otoliths are particularly valuable for studies on age, growth, and stock differentiation (Volpedo and Vaz-dos-Santos, 2015). Moreover, their characteristic shape allows the identification and differentiation of species (Lombarte and Castellón, 1991; Delpiani et al., 2012). Otolith morphology has been increasingly applied to support taxonomic and ecological studies, providing reliable discrimination even taxa with similar external body morphology (Campana and Casselman, 1993; Francis and Campana, 2004).

Several analytical approaches have been developed to describe otolith contours, including landmark-based geometric morphometrics, Fourier elliptic descriptors, and wavelet transforms. Each method captures different aspects of shape variation: geometric morphometrics relies on homologous landmarks to analyse shape geometry, Fourier descriptors are efficient for smooth outlines, whereas wavelet analysis allows a more precise detection of local irregularities and complex contours (Libungan and Pálsson, 2015; Tuset et al., 2021). In gobiid otoliths, which often exhibit intricate shapes, wavelet-based morphometry provides an advantageous approach for detailed shape characterization.

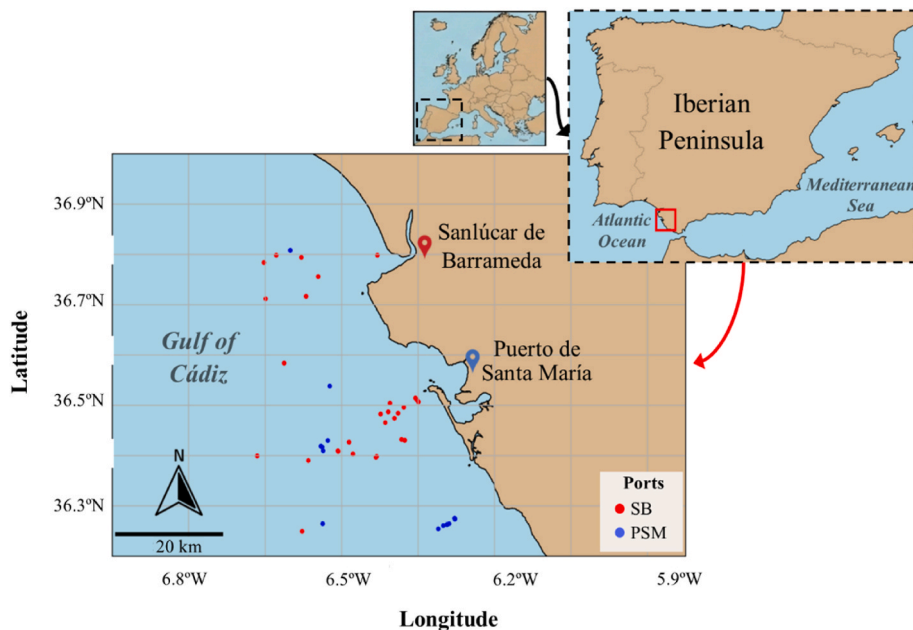
Although otolith extraction and analysis require additional effort

compared to external identification, otolith-based approaches offer clear advantages in contexts where specimens are damaged, partially consumed, or fragmented, as is common in trawl discards, stomach content analyses, and habitat-based biodiversity surveys (Campana, 2004; Tuset et al., 2008). In such cases, otolith morphology represents a persistent and species-specific structure that enables reliable identification when external characters are unavailable (Campana and Casselman, 1993). This is particularly relevant for discard-associated demersal assemblages, where routine monitoring based on external morphology is prone to misidentification, generating uncertainty in discard composition, biodiversity indicators, and subsequent management inferences (Bellido et al., 2011). Moreover, small benthic gobies constitute an important prey resource for commercially valuable predators such as juvenile European hake (*Merluccius merluccius*), which feeds on small benthic fishes during early ontogenetic stages (Carpentieri et al., 2005). Accurate prey identification from stomach contents relies on species-specific hard structures such as otoliths, as soft tissues are rapidly digested.

Considering the challenges in species discrimination described above, this study aims to develop a reliable morphometric approach for distinguishing *L. sanzi* and *L. suerii* in contexts where external identification is constrained. Specifically, this study addresses the following questions: (1) do sagittal otoliths of *L. sanzi* and *L. suerii* exhibit consistent interspecific differences in size and shape? (2) which morphometric descriptors contribute most strongly to species discrimination? and, (3) can wavelet-based contour analysis provide sufficient discriminatory power to support species-level identification in mixed discard samples. By explicitly linking these questions to otolith-based analyses, this study evaluates the applicability of morphometric approaches for improving species identification in discard-associated demersal assemblages.

## 2. Material and methods

The samples were collected during campaigns conducted under the framework of the ECOFISH, ECOFISH2, ECOFISH+, and ECOFISH 4.0 projects whose objective was to characterize and improve fisheries



**Fig. 1.** Map of sampling locations in the Gulf of Cádiz (located in the northeastern Atlantic Ocean along the southwestern Iberian Peninsula), showing the capture sites of *Lesueurigobius sanzi* and *L. suerii*. Red dots represent samples collected by the fleet from the port of Sanlúcar de Barrameda (SB), while blue dots correspond to samples from the fleet of Puerto de Santa María (PSM). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

discards, in collaboration with the Fishermen's Guilds of Sanlúcar de Barrameda and El Puerto de Santa María two fishing ports located in the Gulf of Cádiz, southwestern Spain (Fig. 1).

### 2.1. Study area and sampling

The Gulf of Cádiz is a highly productive region of great fishing interest due to its unique oceanographic and climatic dynamics. Surface temperature follows a seasonal pattern, with peaks in summer and lows in winter, influenced by atmosphere-ocean exchange and local winds (Vargas et al., 2003). Additionally, ocean tides, combined with input from the Guadalquivir River and coastal upwelling processes, enrich the area with nutrients and chlorophyll, promoting the early life stages of fish, including eggs and larvae (García Lafuente and Ruiz, 2007).

Specimens of *Lesueurigobius sanzi* and *L. suerii* were obtained from artisanal bottom trawl fisheries operating in the Gulf of Cádiz, where both species are commonly discarded. These operations involved fleets from two different ports, Sanlúcar de Barrameda and El Puerto de Santa María. The collection period spanned from 2019 to 2022, excluding the annual closure period for bottom trawl fleets in the Gulf of Cádiz, which runs from September 15 to October 31.

Sampling was opportunistic and based on fishery discards provided by the trawl fleet. As such, the collected material reflects the discard fraction generated under routine fishing practices and may be influenced by gear selectivity, rather than representing population-level abundance.

### 2.2. Sampling processing

A total of 1019 individuals were analysed in this study. Fish samples were initially identified based on specific meristic and morphometric characteristics, according to identification guides (Lloris, 2015). For *L. sanzi*, the first dorsal fin ray, which is noticeably longer, was used as a distinguishing feature. When this fin was damaged or not in suitable condition to be used as a diagnostic character, species identification was supported using alternative morphological traits described by Lloris (2015). In particular, *L. sanzi* is also characterised by having 22 pectoral fin rays, which provided an additional criterion for identification. This morphological information, together with the visual assessment performed by experienced technicians specialised in fish identification in the Gulf of Cádiz, allowed reliable species identification even when external diagnostic features were not well preserved.

Data collection included measurements of the fish total length (TL, in cm) and weight (in g). Then, right and left otoliths were extracted and photographed, focusing on the otolith edges (Fig. 2), using a Moticam 580 camera attached to a Leica Wild M10 stereomicroscope, with the assistance of the Motic Images Plus 2.0 software. Filenames were coded to associate each image with its corresponding individual. The pixel-to-millimetre ratio for each image was calibrated in ImageJ using a

reference scale. A fixed ratio of 240.53 px/mm was adopted to maintain measurement consistency, and values were expressed with two decimal places to ensure precision while avoiding excessive rounding noise in morphometric calculations.

### 2.3. Otolith morphometric analysis

Otolith shape analyses were conducted in R (version 4.1.3; RStudio Team, 2022) using the package “shapeR” for image processing and extraction of morphometric measurements (Libungan and Pålsson, 2015). A significance level of  $\alpha = 0.05$  was applied for all analyses.

Once the outline is accurately detected, the “shapeR” software calculates a set of morphometric parameters based on the pixel-to-millimetre ratio. The automatically extracted variables include otolith area (OA, in  $\text{mm}^2$ ), otolith perimeter (OP, in mm), otolith length (OL, in mm), defined as the longest distance along the longitudinal axis, and otolith width (OW, in mm), defined as the maximum perpendicular dorso-ventral distance (Fig. 3). In addition, otolith contour shape was characterized using wavelet coefficients derived from outline analysis. Using the extracted parameters, several morphometric indices were subsequently calculated (Muñoz-Lechuga et al., 2023; Park et al., 2023), as summarized in Table 1. An exploratory analysis of the indices and parameters was conducted following Zuur et al. (2010) to identify potential outliers in the data.

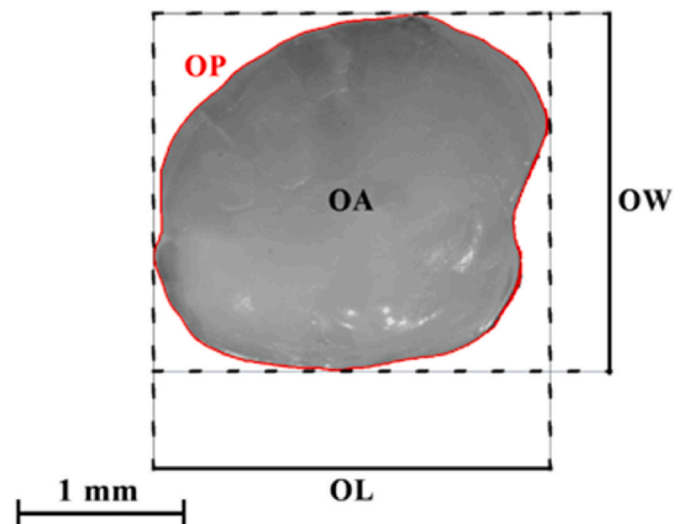


Fig. 3. Representation of a processed otolith photograph with its shape detected. Otolith measurements are represented as follows: OL = Otolith Length (mm), OW = Otolith Width (mm), OA = Otolith Area ( $\text{mm}^2$ ), OP = Otolith Perimeter (mm).

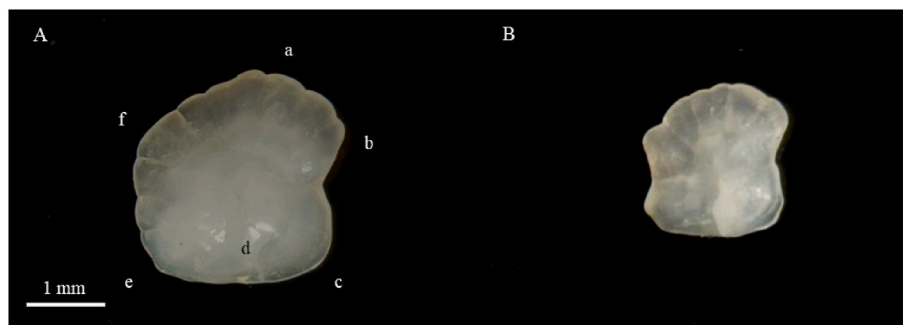


Fig. 2. Sagittal otoliths photographs obtained with a stereomicroscope at  $8\times$  magnification. Shown are the left otoliths of (A) *Lesueurigobius sanzi* and (B) *L. suerii*. In panel A, the main morphological features are indicated: (a) dorsal margin, (b) postrostrum (posterior region), (c) ventral (inferior) margin, (d) sulcus acusticus, (e) rostrum, and (f) antirostrum. Scale bar = 1 mm.

**Table 1**

Calculation of morphometric indices based on morphometric parameters. Key: OP = otolith perimeter; OA = otolith area; OL = otolith length; OW = otolith width.

Morphometric Indices	Formula
Circularity	OP <sup>2</sup> /OA
Rectangularity	OA/(OL*OW)
Form Factor	(4πOA)/OP <sup>2</sup>
Roundness	(4OA)/πOL <sup>2</sup>
Aspect Ratio	OL/OW
Ellipticity	(OL-OW)/(OL + OW)

To verify the allometric relationship of otoliths in the samples, Pearson correlations (Pearson, 1895) were analysed between otolith areas and the total length of the individuals. Once this relationship was confirmed, the morphometric indices and parameters were standardised using Equation (1) (Elliott et al., 1995).

$$M_s = M_o * \left(\frac{\bar{x}}{x}\right)^b \tag{Eq.1}$$

Where “M<sub>s</sub>” represents the standardised measurement, “M<sub>o</sub>” is the original measurement,  $\bar{x}$  is the mean total length of the entire dataset, x is the total length measurement of each fish and b is the slope of the regression between M<sub>o</sub> and log x. Wavelet coefficients were automatically standardised using the “shapeR” package. Morphometric variables were standardised to remove size-related scaling effects and to focus the analyses on shape differences between species, rather than on intra-specific ontogenetic variation.

**2.4. Statistical analysis of morphometric standardised indices and parameters**

The standardised morphometric indices and parameters were subjected to the Shapiro-Wilk normality test (Shapiro and Wilk, 1965) to assess normality, as it is highly sensitive for detecting deviations from normal distribution in moderate sample sizes and performs reliably with continuous morphometric data. For variables that did not meet normality assumptions, the Kruskal-Wallis test (Kruskal and Wallis, 1952) was applied to identify significant differences between species. This non-parametric approach provides robust comparisons when data depart from normality, ensuring consistent analytical treatment across all indices. Homoscedasticity and independence were verified prior to its application to confirm the reliability of the results. To assess otolith bilateral asymmetry, differences between left and right otoliths were analysed using Wilcoxon signed-rank (Wilcoxon, 1945) tests for morphometric variables.

Additionally, a Principal Component Analysis (PCA) was conducted to identify primary patterns and trends, analyse differences between groups, and determine the variables most influential in the observed variation (Avigliano et al., 2015). This multivariate approach efficiently reduces data dimensionality while retaining the variables that best describe the structure and differentiation between species.

**2.5. Statistical analysis of standardised wavelet coefficients**

To evaluate significant differences between groups and assess otolith bilateral asymmetry, we performed a PERMANOVA based on dissimilarity matrices (Good, 2000). This non-parametric method does not assume normality or homogeneity of variances. A total of 1000 permutations were conducted to generate a null distribution of the test statistics. The p-value was calculated as the proportion of permutations in which the test statistics were at least as extreme as the original statistics.

A Linear Discriminant Analysis (LDA) model was performed to classify the samples by species. The model was trained using the

standardised wavelet coefficients. This supervised classification method was selected because it maximises the separation between predefined groups, allowing the evaluation of how effectively otolith contour descriptors differentiate *L. sanzi* and *L. suerii*. Cross-validation was performed to estimate the model's accuracy and confirm the consistency between the LDA classification and the PCA results to differentiate the studied species.

**3. Results**

In total, 921 individuals of *L. sanzi* and 98 *L. suerii* were identified. From both species, a total of 2038 otolith photographs were taken and analysed. The pixel-to-millimetre ratio was calculated and determined to be 240.5327 pixels per millimetre.

The total length of the specimens ranged from 3.9 to 11.5 cm in *L. sanzi* and from 3.8 to 6.1 cm in *L. suerii*. These ranges approach the maximum reported lengths for each species (approximately 12.3 cm for *L. sanzi* and 7.5 cm for *L. suerii*; Froese and Pauly, 2025), indicating that the analysed samples likely include both juvenile and adult individuals. Although *L. sanzi* reached larger maximum total lengths than *L. suerii*, the size ranges of both species partially overlapped, and size differences alone do not account for the observed otolith morphometric differentiation.

**3.1. Analysis of otolith indices and morphometric parameters**

Minimum, maximum, mean, and standard deviation values for each morphometric measurement and index derived from otoliths are summarized in Table 2. Overall, *L. sanzi* exhibited higher values in most of the measured characteristics. Conversely, *L. suerii* showed slightly higher roundness values.

Significant bilateral asymmetry was detected in the otolith morphometric variables and shape indices for both species. Paired Wilcoxon signed-rank tests revealed consistent statistical differences between left and right otoliths in *L. sanzi* (p < 0.05) and *L. suerii* (p < 0.05).

A strong positive allometric relationship was observed between otolith size and total length in both species, with slightly higher correlation values in *L. sanzi* (r = 0.93, p < 0.001) compared to *L. suerii* (r = 0.87, p < 0.001). This pattern indicates that otolith area increases with fish size.

After standardising the indices, the Shapiro-Wilk test revealed that, except for the rectangularity variable (p > 0.05), the other indices did not follow a normal distribution (p < 0.05). Subsequently, the non-parametric Kruskal-Wallis test confirmed the presence of significant differences in all non-normally distributed indices (p < 0.05). Additionally, the ANOVA performed on the rectangularity index indicated

**Table 2**

Descriptive statistics of otolith indices and morphometric parameters for *Lesueurigobius sanzi* and *L. suerii*. Key: Min = Minimum; Max = Maximum; SD = Standard Deviation.

Morphometric Variables	<i>L. sanzi</i>	<i>L. suerii</i>
	Min-Max (Mean ± SD)	Min-Max (Mean ± SD)
Otolith Area (mm <sup>2</sup> )	2.64 – 17.60 (8.42 ± 2.37)	1.05 – 4.92 (3.16 ± 0.73)
Otolith Perimeter (mm)	5.76 – 15.37 (10.64 ± 1.52)	3.90 – 8.32 (6.58 ± 0.84)
Otolith Width (mm)	1.76 – 4.57 (3.15 ± 0.44)	1.08 – 2.44 (1.98 ± 0.24)
Otolith Length (mm)	1.63 – 5.06 (3.34 ± 0.52)	1.20 – 2.53 (1.96 ± 0.26)
Circularity	13.09 – 17.29 (13.75 ± 0.44)	12.86 – 16.43 (13.93 ± 0.44)
Rectangularity	0.73 – 0.84 (0.79 ± 0.02)	0.75 – 0.83 (0.80 ± 0.02)
Form Factor	0.72 – 0.96 (0.91 ± 0.03)	0.76 – 0.97 (0.90 ± 0.03)
Roundness	0.71 – 1.11 (0.95 ± 0.06)	0.81 – 1.18 (1.03 ± 0.06)
Aspect Ratio	0.90 – 1.34 (1.06 ± 0.05)	0.87 – 1.18 (0.99 ± 0.05)
Ellipticity	–0.05 – 0.13 (1.06 ± 0.05)	–0.06 – 0.08 (–0.01 ± 0.02)

significant differences between the otoliths of the species ( $p < 0.05$ ).

The indices that contributed most to the observed separation were circularity, form factor, and aspect ratio, which reflect the contrast between the more elongated otoliths of *L. sanzi* and the more rounded shape of *L. suerii*.

### 3.2. Principal Component Analysis (PCA)

After standardising the indices and parameters, the Principal Component Analysis (PCA) revealed that the first two dimensions accounted for a cumulative variance of 76.60%, capturing most of the variability present in the original dataset. This indicates that the variables with the highest contributions to these two principal components are sufficient to adequately represent the underlying structure of the data, significantly reducing model complexity without substantial information loss. The remaining dimensions accounted for a very small proportion of the variance, suggesting their limited relevance in the overall data representation.

The first two PCA dimensions were mainly structured by different sets of morphometric variables (Table 3). Dimension 1 was primarily driven by otolith length and perimeter, which showed the highest absolute contributions and  $\cos^2$  values, followed by otolith area and aspect ratio. In contrast, circularity and form factor showed negligible contributions to this dimension. Dimension 2 displayed a different contribution pattern, being mainly influenced by circularity, with rectangularity and form factor also contributing substantially.

The PCA revealed a tendency for *L. suerii* samples to cluster towards negative values along the first two dimensions (Fig. 4), indicating species-related differences in otolith morphology. These differences were mainly associated with size-related descriptors along Dimension 1 and shape-related descriptors, such as circularity and form factor, along Dimension 2.

### 3.3. Wavelet coefficient analysis

After standardising the Wavelet coefficients, the PERMANOVA revealed statistically significant differences in the standardised Wavelet coefficients between *L. sanzi* and *L. suerii* ( $p$ -value  $< 0.05$ ).

Regarding classification analysis using the Linear Discriminant Analysis (LDA) method, the model achieved a classification error rate of 0.084, as determined by 10-fold cross-validation. This indicates that, on average, the LDA model misclassifies approximately 8.4% of cases, highlighting its high accuracy in differentiating the two species.

The LDA scores showed a clear separation between groups, with limited overlap in the discriminant space. Classification accuracy reached 92.4% for *L. sanzi* and 88.7% for *L. suerii*.

**Table 3**

Contributions and squared cosine ( $\cos^2$ ) values of each variable in the first two dimensions of the principal component analysis.

Variable	Dimension 1		Dimension 2	
	Absolute contribution (Relative)	$\cos^2$	Absolute contribution (Relative)	$\cos^2$
Otolith Area	0.807 (14.462)	0.651	0.564 (10.058)	0.318
Otolith Perimeter	0.838 (15.591)	0.702	0.418 (5.542)	0.175
Otolith Width	0.665 (9.833)	0.443	0.667 (14.097)	0.445
Otolith Length	0.963 (20.594)	0.927	0.260 (2.145)	0.068
Circularity	0.031 (0.021)	0.001	-0.687 (14.92)	0.471
Rectangularity	-0.553 (6.798)	0.306	0.533 (9.005)	0.284
Form Factor	-0.033 (0.024)	0.001	0.690 (15.057)	0.476
Roundness	-0.725 (11.663)	0.525	0.613 (11.878)	0.375
Aspect Ratio	0.679 (10.241)	0.461	-0.549 (9.551)	0.302
Ellipticity	0.696 (10.774)	0.485	-0.495 (7.744)	0.245

## 4. Discussion

This study addressed whether otolith morphometry, particularly wavelet-based contour descriptors, can reliably discriminate *Lesueurigobius sanzi* and *L. suerii* within the context of discard monitoring in the Gulf of Cádiz. The clear differences observed in otolith indices and contour parameters highlight the robustness of this approach, particularly the use of standardised wavelet coefficients, which allowed a precise characterisation of otolith shape and an accurate classification of both species.

The strong and significant correlation detected between otolith dimensions and fish length confirms that otolith growth is closely linked to somatic growth, a relationship widely recognised in previous research. Gutiérrez and Morales-Nin (1986) demonstrated that otolith size in European seabass (*Dicentrarchus labrax*) reflects daily growth patterns influenced by both physiology and environmental conditions, while Castro-Gutiérrez et al. (2023) observed similar relationships in the brown comber (*Serranus hepatus*). Given this strong relationship, standardising morphometric data (by removing allometric effects) is essential to decouple otolith shape from fish total length. This ensures that the observed differences are driven by species identity rather than by differences in fish size.

After standardisation, significant differences remained between *L. sanzi* and *L. suerii*. Circularity, form factor and aspect ratio indices were particularly informative for distinguishing the two species. Comparable results were reported by Bani et al. (2013), who achieved over 94% correct classification among three gobiids (Caspian goby *Neogobius caspius*, deepwater goby *Ponticola bathybius* and bighead goby *Ponticogoriscus*) species using the same type of morphometric descriptors. This consistency across studies confirms the usefulness of shape indices for resolving taxonomic ambiguities in closely related species.

Principal Component Analysis (PCA) showed that the first two components explained 76.6% of the total variance, confirming their suitability for interpreting the main axes of morphological differentiation. As noted by Ringnér (2008), focusing on the first two components is appropriate when they represent most of the cumulative variance. In this study, the first component was driven mainly by otolith size variables (length and perimeter), whereas the second captured shape-related features such as circularity and rectangularity. These variables together accounted for the main patterns of differentiation between *L. sanzi* and *L. suerii*. Overall, the PCA demonstrated that shape traits associated with elongation and compactness drive most of the morphological separation between species, in agreement with previous otolith-based discrimination studies (Pavlov, 2016; Sadighzadeh et al., 2012).

The differences observed between *L. sanzi* and *L. suerii* likely result from the combined influence of genetic and environmental factors. While genetics exert a dominant effect on the general otolith contour (Radtke and Shafer, 1992; Campana et al., 2001), environmental variables such as depth, temperature, and substrate type are known to modulate finer-scale morphological details (Pavlov, 2016). In the Gulf of Cádiz, *L. sanzi* typically inhabits shallower sandy bottoms (40–120 m) and is exposed to warmer and more hydrodynamically active conditions, whereas *L. suerii* occupies deeper muddy habitats (80–200 m) characterised by lower temperatures and reduced energy (Lloris, 2015; Fanelli et al., 2009). Importantly, samples of both species were obtained by artisanal trawl fleets operating from both ports and across overlapping operational depth ranges. While this suggests that the observed differences are unlikely to be driven exclusively by fleet- or port-specific sampling, spatial and operational effects cannot be fully excluded.

Habitat type and depth can significantly influence otolith morphology (Lombarte, 1992). Specifically, otolith shape complexity has been linked to substrate characteristics (Volpedo and Echeverría, 2003). The rounder and smoother otoliths of *L. suerii* align with descriptions of fish associated with soft, muddy substrates, which tend to

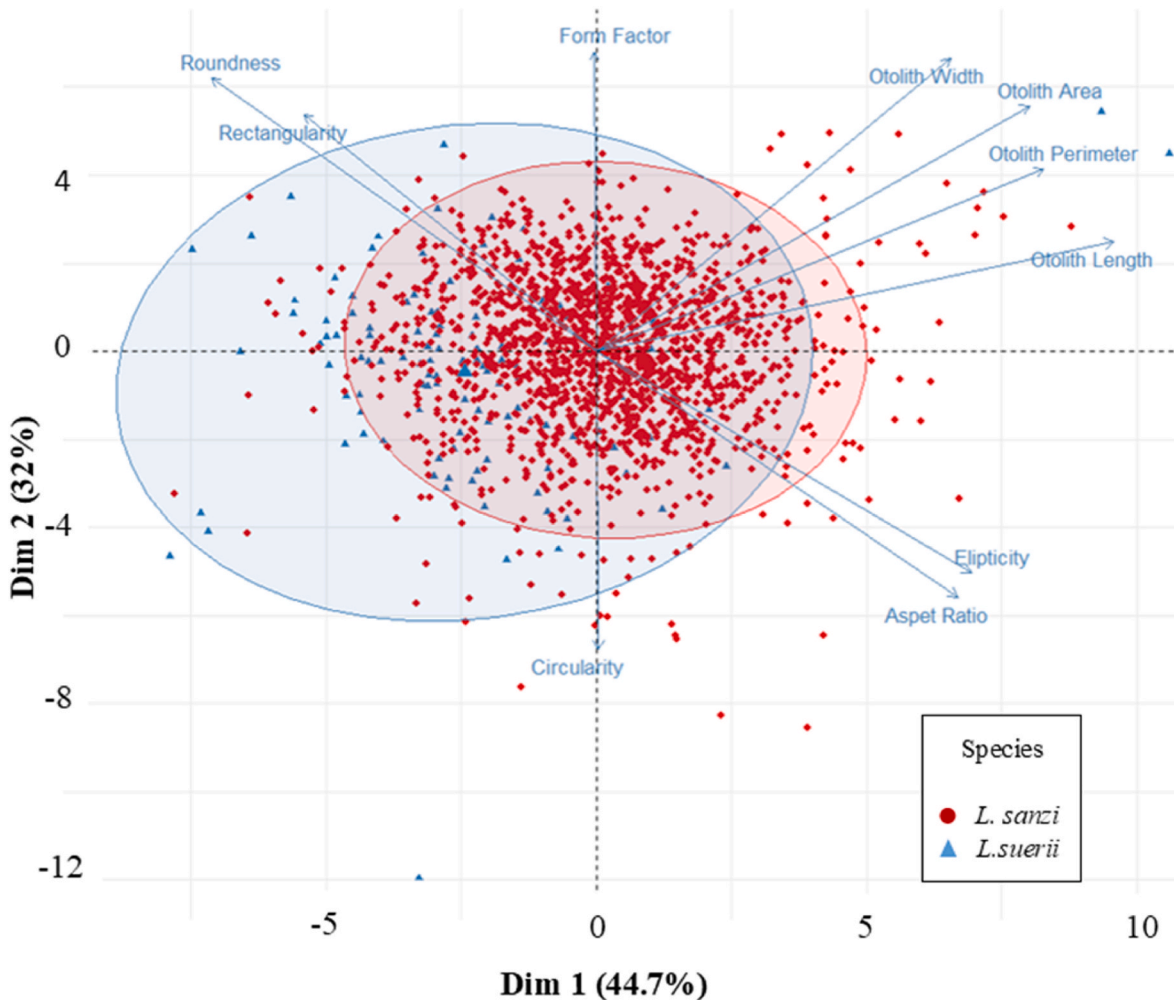


Fig. 4. Representation of the first two dimensions of the PCA. The eigenvectors representing the variables show their direction and contribution relative to the two principal components. The observations of *Lesueurigobius sanzi* are represented as red circles, while the observations of *L. suerii* are shown as blue triangles. Coloured ellipses represent 95% confidence intervals around the group centroids. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

exhibit simpler margins and circular forms (Volpedo and Echeverría, 2003). Conversely, the more elongated and angular otoliths of *L. sanzi* may reflect adaptations to the sensory requirements of high-energy sandy environments. Similar divergence in otolith shape driven by habitat segregation has been documented in other gobiids (Bani et al., 2013), supporting the hypothesis that environmental gradients act synergistically with genetic differentiation to shape the observed morphometric divergence. Rather than merely indicating species identity, these morphological distinctions reflect the specific functional adaptations required by their respective ecological niches. The congruence between the observed environmental segregation and the otolith shape divergence suggests that habitat-specific selective pressures could have played a role in shaping these structures.

The classification accuracy obtained through the Linear Discriminant Analysis (LDA) confirms the strong discriminative power of wavelet coefficients. These results are in line with previous findings demonstrating that wavelet descriptors outperform traditional shape indices in distinguishing closely related species (Tuset et al., 2021; Bostanci et al., 2024). However, as reported for other taxa (Wujdi et al., 2022), the precision of LDA-based discrimination can vary across populations, reflecting the combined influence of both genetic and environmental variability.

From a management perspective, these morphological and environmental distinctions are consistent with the known ecological segregation

between *L. sanzi* and *L. suerii*, even when both species co-occur in trawl discards. Recognising such habitat-linked differentiation is valuable for discard monitoring programs and ecosystem-based fisheries management, as it facilitates more accurate species identification within mixed trawl catches and improves biodiversity and impact assessments in the Gulf of Cádiz.

Although this approach is particularly effective for gobiid species, whose external morphology often hampers reliable identification, it also highlights the broader value of otolith-based methods as practical classification tools. Otolith morphometry offers a cost-effective and objective alternative that does not require specialised genetic analyses or extensive taxonomic expertise, making it especially suitable for routine fisheries monitoring and discard assessment programmes.

Beyond the focal species, the methodological framework developed here has broader ecological implications. Small benthic gobies play a key role as prey in demersal food webs, transferring energy from benthic invertebrates to higher trophic levels, including commercially important predators (Carpentieri et al., 2005). The otolith-based discrimination approach presented here can therefore be extended to other gobiid taxa and applied in stomach content analyses of piscivorous predators, where morphological identification of partially digested prey is often unreliable.

#### 4.1. Limitations of this study

A recognised challenge in studies of these species is the difficulty of distinguishing them based on external morphology (Lloris, 2015), particularly when specimens originate from trawl discards where diagnostic features may be obscured. This limitation was addressed during sampling through visual identification performed by experienced personnel from the ECOFISH project, who have extensive expertise in the identification of demersal species from the study area. To minimise potential bias associated with field sorting, subsequent analyses relied on otolith-derived morphometric measurements, which provide an objective basis for species comparison. Under this approach, any residual misclassification is expected to be low and non-differential with respect to the contrasts examined.

Moreover, the sample size of *L. suerii* was smaller compared to *L. sanzi*, which may have a slight influence on the interpretation of morphological differences between the two species. However, the results remain valuable for identifying trends and providing relevant information on their otolith characteristics. This imbalance reflects the natural abundance patterns encountered during routine fishing operations, as samples were collected directly from the artisanal trawl fleet, making equal representation difficult to achieve in field conditions. Future studies with a larger sample size could further strengthen and expand these findings.

Age information was not available for the analysed individuals, and published age–size relationships for *Lesueurigobius* species are limited, preventing a direct assessment of age-specific structure relative to the population. Consequently, data were pooled across individuals to encompass the full size range available in the catches. While this approach limits the ability to detect specific ontogenetic shape transitions, it ensures that the resulting discriminatory model incorporates the natural morphological variability present across the catchable population structure. To address this limitation and mitigate potential size-related effects associated with ontogenetic variation, all morphometric variables were standardised prior to analysis. While otoliths increase in size as fish grow, this standardisation allows for comparisons that are independent of otolith size, effectively reducing the influence of allometry and scaling effects across the sampled size range.

However, we acknowledge that standardisation does not entirely remove intraspecific shape variation associated with size, and size-related effects may operate within species as well as between species. This represents a common methodological trade-off in otolith morphometry studies focused on species discrimination, where the primary objective is to maximise interspecific separation rather than to characterize intraspecific ontogenetic trajectories.

A critical aspect of otolith-based identification tools is their applicability in routine fishery monitoring. Field applications face constraints such as damaged specimens and limited processing time, so both left and right otoliths were included in model development to ensure reliable performance regardless of which otolith is available. Although significant bilateral asymmetry was detected (PERMANOVA:  $p < 0.001$ ), incorporating this variation enhances classification robustness by producing a more generalized model.

#### 4.2. Conclusions

The results of this study highlight that the morphometric indices of the otoliths of *L. sanzi* and *L. suerii* are significantly different, as demonstrated clearly and reliably through Principal Component Analysis (PCA). This approach effectively distinguishes between the two species and reinforces its utility in taxonomic studies. Furthermore, the Linear Discriminant Analysis (LDA) confirmed that wavelet coefficients are the most accurate tool for otolith classification, owing to their low error rate in the classification process.

The use of otolith morphometry emerges as an effective, cost-efficient, and accessible method for classifying species within the same

genus that are difficult to differentiate using traditional meristic-based techniques. Although otolith extraction and analysis require additional effort, this approach becomes particularly valuable in contexts where external morphological characters are damaged, incomplete, or unreliable, such as trawl discards, stomach content analyses, or habitat-based biodiversity assessments.

This study provides a robust methodology for differentiating between *L. suerii* and *L. sanzi* improving species identification in situations where conventional morphological traits are often deteriorated. Beyond this specific case, the approach presented here can be extended to other demersal species and ecological contexts where accurate taxonomic resolution is limited. Otolith-based morphometry is therefore proposed as a complementary tool that supports species identification when external diagnostic characters are compromised or when taxonomic expertise is not readily available. Accurate species identification is a key step toward effective management, as it underpins reliable ecological assessments, informs population-level analyses, and supports sustainable exploitation and conservation of marine resources.

#### Ethics statement

The specimens used in this work have never been subjected to animal experimentation. These specimens come from catches made by professional fishermen and are subject to European regulations on Fish Discards.

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#### CRediT authorship contribution statement

**Jaime S. Rubiales-Gómez:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft. **Jairo Castro-Gutiérrez:** Conceptualization, Formal analysis, Methodology, Resources, Supervision, Validation, Writing – review & editing. **Carlos Rodríguez-García:** Data curation, Methodology, Resources, Validation, Visualization, Writing – review & editing. **Jesica Sarmiento-Carbajal:** Investigation, Resources, Writing – review & editing. **Remedios Cabrera-Castro:** Conceptualization, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability

Data will be made available on request.

## References

- Abauza, P., Murta, A.G., Campbell, N., Cimmaruta, R., Comesaña, A.S., Dahle, G., García Santamaría, M.T., Gordo, L.S., Iversen, S.A., MacKenzie, K., Magoulas, A., Mattiucci, S., Molloy, J., Nascetti, G., Pinto, A.L., Quinta, R., Ramos, P., Sanjuan, A., Santos, A.T., Stransky, C., Zimmermann, C., 2008. Stock identity of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean Sea: integrating the results from different stock identification approaches. *Fish. Res.* 89 (2), 196–209. <https://doi.org/10.1016/j.fishres.2007.09.022>.
- Aranciaga, I., 2003. Mapa pesquero patagónico austral hacia una demanda de ciencia y tecnología en el desarrollo del sector. *Cadernos de Ciencia & Tecnología*. Retrieved from <https://seer.sct.embrapa.br/index.php/cct/article/view/8737>.
- Avigliano, E., Comte, G., Rosso, J.J., 2015. Identification of fish stocks of river croaker (*Plagioscion ternetzi*) in the Paraná and Paraguay rivers using otolith morphometric analysis. *Latin American Journal of Aquatic Research* 43 (4), 684–692. <https://www.lajar.cl/index.php/rlajar/article/view/vol43-issue4-fulltext-10>.
- Bani, A., Poursaeid, S., Tuset, V.M., 2013. Comparative morphology of the sagittal otolith in three species of south Caspian gobies. *J. Fish. Biol.* 82 (4), 1321–1332. <https://doi.org/10.1111/jfb.12073>.
- Bellido, J.M., Santos, M.B., Pennino, M.G., Valeiras, X., Pierce, G.J., 2011. Fishery discards and bycatch: solutions for an ecosystem approach to fisheries management? *Hydrobiologia* 670 (1), 317–333. <https://doi.org/10.1007/s10750-011-0721-5>.
- Bostanci, D., Yilmaz, S., Kostas, S., Öztürk, T., 2024. Comparative analysis of otolith morphology and wavelet descriptors in *Pagellus* species (Teleostei: sparidae). *Oceanol. Hydrobiol. Stud.* 53 (1), 88–101. <https://doi.org/10.26881/oahs-2024.1.10>.
- Campana, S.E., 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Mar. Ecol. Prog. Ser.* 188, 263–297. <https://doi.org/10.3354/meps188263>.
- Campana, S.E., 2004. *Photographic Atlas of Fish Otoliths of the Northwest Atlantic Ocean*, vol. 133. Canadian Special Publication of Fisheries and Aquatic Sciences, pp. 1–284.
- Campana, S.E., Fowler, A.J., Jones, C.M., 2001. Otolith elemental fingerprinting for stock identification. *ICES (Int. Counc. Explor. Sea) J. Mar. Sci.* 58 (2), 330–338. <https://doi.org/10.1139/f94-196>.
- Campana, S.E., Casselman, J.M., 1993. Stock discrimination using otolith shape analysis. *Can. J. Fish. Aquat. Sci.* 50 (5), 1062–1083. <https://doi.org/10.1139/f93-123>.
- Carpentieri, P., Colloca, F., Cardinale, M., Belluscio, A., Ardizzone, G.D., 2005. Feeding habits of European hake (*Merluccius merluccius*) in the central Mediterranean Sea. *Fish. Bull.* 103 (2), 411–416.
- Castro-Gutiérrez, J., Madera-Santana, S., Rodríguez-García, C., Domínguez-Bustos, Á.R., Sarmiento-Carbajal, J., Gonçalves-Neto, J.B., Cabrera-Castro, R., 2023. Exploring morphometric frontiers: a comprehensive study of otolith growth patterns in brown comber (*Serranus hepatus*). *J. Fish. Biol.* 103 (6), 1374–1381. <https://doi.org/10.1111/jfb.15544>.
- Catchpole, T., Frid, C., Gray, T., 2005. Discards in North Sea fisheries: causes, consequences and solutions. *Mar. Pol.* 29 (6), 421–430. <https://doi.org/10.1016/j.marpol.2004.07.001>.
- Coronil-Huertás, D.J., Rodríguez-García, C., Pavón-Quintana, S., Vidal-Pérez, J.M., Sarmiento-Carbajal, J., Cabrera-Castro, R., 2024. Optimization of the storage of fishing discards: innovations in trawlers in the Gulf of Cadiz (SW Iberian Peninsula). *Reg. Stud. Mar. Sci.* 76, 103593.
- Delpiani, Sergio Matías, González-Castro, Mariano, de Astarloa, Díaz, Martín, Juan, 2012. El uso de otolitos y huesos de la cabeza para la identificación de dos especies del género *Merluccius*, en estudios de predador-presa. *Rev. Biol. Mar. Oceanogr.* 47 (2), 351–357. <https://doi.org/10.4067/S0718-19572012000200018>.
- Elliott, N.G., Haskard, K., Koslow, J.A., 1995. Morphometric analysis of orange roughy (*Hoplostethus atlanticus*) off the continental slope of southern Australia. *J. Fish. Biol.* 46 (2), 202–220. <https://doi.org/10.1111/j.1095-8649.1995.tb05962.x>.
- Fanelli, E., Badalamenti, F., D'Anna, G., Pipitone, C., 2009. Diet and trophic level of scald fish *Arnoglossus laterna* in the southern Tyrrhenian Sea (western Mediterranean): contrasting trawled versus untrawled areas. *J. Mar. Biol. Assoc. U. K.* 89, 817–828. <https://doi.org/10.1017/s0025315409000137>.
- Feelings, J., Bartolino, V., Madsen, N., Catchpole, T., 2012. Fishery discards: factors affecting their variability within a demersal trawl fishery. *PLoS One* 7 (4), e36409. <https://doi.org/10.1371/journal.pone.0036409>.
- Food and Agriculture Organization, 1996. *Technical Guidelines for Responsible Fisheries: No. 5 - Fishing Operations*. FAO. <https://www.fao.org/3/w3591e/w3591e00.htm>.
- Francis, R.C., Campana, S.E., 2004. Inferring age from otolith measurements: a review and a new approach. *Can. J. Fish. Aquat. Sci.* 61 (7), 1269–1284. <https://doi.org/10.1139/f04-063>.
- Froese, R., Pauly, D. (Eds.), 2025. *FishBase*. World Wide Web Electronic Publication. [www.fishbase.org](http://www.fishbase.org).
- García Lafuente, J., Ruiz, J., 2007. The Gulf of Cádiz pelagic ecosystem: a review. *Prog. Oceanogr.* 74 (3–4), 228–251. <https://doi.org/10.1016/j.pocean.2007.04.001>.
- García-Vázquez, E., Machado-Schiaffino, G., Campo, D., Juanes, F., 2012. Species misidentification in mixed hake fisheries may lead to overexploitation and population bottlenecks. *Fish. Res.* 114, 52–55.
- Gilman, E., Perez Roda, A., Huntington, T., Kennelly, S.J., Suuronen, P., Chaloupka, M., Medley, P.A.H., 2020. Benchmarking global fisheries discards. *Sci. Rep.* 10 (1), 14017.
- Good, P.I., 2000. *Permutation Tests: a Practical Guide to Resampling Methods for Testing Hypotheses*, second ed. Springer-Verlag.
- Gutiérrez, E., Morales-Nin, B., 1986. Time series analysis of daily growth in *Dicentrarchus labrax* otoliths. *J. Exp. Mar. Biol. Ecol.* 103 (2–3), 163–179. [https://doi.org/10.1016/0022-0981\(86\)90139-5](https://doi.org/10.1016/0022-0981(86)90139-5).
- Karahan, A., Borsari, P., Gucu, A.C., Kandemir, I., Ozkan, E., Ak Orek, Y., Acan, S.C., Koban, E., Togan, I., 2014. Geometric morphometrics, Fourier analysis of otolith shape, and nuclear-DNA markers distinguish two anchovy species (*Engraulis* spp.) in the Eastern Mediterranean Sea. *Fish. Res.* 159, 45–55. <https://doi.org/10.1016/j.fishres.2014.05.009>.
- Kruskal, W.H., Wallis, W.A., 1952. Use of ranks in one-criterion variance analysis. *J. Am. Stat. Assoc.* 47 (260), 583–621. <https://doi.org/10.1080/01621459.1952.10483441>.
- Libungan, L.A., Pálsson, S., 2015. ShapeR: an R package to study otolith shape variation among fish populations. *PLoS One* 10 (3), e0121102. <https://doi.org/10.1371/journal.pone.0121102>.
- Lloris, D., 2015. *Marine ichthyofauna: identification manual of marine fishes from the Iberian Peninsula and Balearic Islands*. OMEGA Publishing.
- Lombarte, A., 1992. Changes in otolith area: sensory area ratio with body size and depth. *Environ. Biol. Fish.* 33, 405–410.
- Lombarte, A., Castellón, A., 1991. Interspecific and intraspecific otolith variability in the genus *Merluccius* as determined by image analysis. *Can. J. Zool.* 69 (9), 2442–2449. <https://doi.org/10.1139/z91-343>.
- Maynou, F., Sardá, F., 2001. Influence of environmental factors on commercial trawl catches of *Nephtrops norvegicus*. *ICES Journal of Marine Science* 58 (5), 1318–1325.
- Morandeau, G., Macher, C., Sanchez, F., Bru, N., Fauconnet, L., Caill-Milly, N., 2014. Why do fishermen discard? Distribution and quantification of the causes of discards in the Southern Bay of Biscay passive gear fisheries. *Mar. Pol.* 48, 30–38. <https://doi.org/10.1016/j.marpol.2014.02.022>.
- Muñoz-Lechuga, R., Sow, F.N., Constance, D.N., Angueko, D., Macías, D., Massa-Gallucci, A., Lino, P.G., 2023. Differentiation of spatial units of genus *Euthynnus* from the Eastern Atlantic and the Mediterranean using otolith shape analysis. *Fishes* 8 (6), 317. <https://doi.org/10.3390/fishes8060317>.
- Park, J.M., Kang, M.G., Kim, J.H., Jawad, L.A., Majeed, S., 2023. Otolith morphology as a tool for stock discrimination of three rockfish species in the East sea of Korea. *Front. Mar. Sci.* 10, 1301178. <https://doi.org/10.3389/fmars.2023.1301178>.
- Pavlov, D.A., 2016. Differentiation of three species of the genus *Upeneus* (Mullidae) based on otolith shape analysis. *J. Ichthyol.* 56 (1), 37–51.
- Pearson, K., 1895. Notes on regression and inheritance in the case of two parents. *Proc. Roy. Soc. Lond.* 58, 240–242.
- Radtke, R.L., Shafer, D.J., 1992. Environmental sensitivity of fish otolith microchemistry. *Aust. J. Mar. Freshw. Res.* 43, 935–951.
- Riccioni, G., Stagoni, M., Manfredi, C., Tinti, F., Piccinetti, C., Libralato, S., 2022. DNA metabarcoding suggests dietary niche partitioning in the Adriatic European hake. *Sci. Rep.* 12 (1), 1343.
- Ringné, M., 2008. What is principal component analysis? *Nat. Biotechnol.* 26 (3), 303–304. <https://doi.org/10.1038/nbt0308-303>.
- Rousseau, Y., Watson, R.A., Blanchard, J.L., Fulton, E.A., 2019. Evolution of global marine fishing fleets and the response of fished resources. *Proc. Natl. Acad. Sci.* 116 (25), 12238–12243.
- Sadighzadeh, Z., Tuset, V.M., Valinassab, T., Dadpour, M.R., Pazira, A., 2012. Comparison of different otolith shape descriptors and morphometrics for the identification of closely related species of *Lutjanus* spp. from the Persian Gulf. *Mar. Biol. Res.* 8 (9), 802–814. <https://doi.org/10.1080/17451000.2012.692163>.
- Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52 (3–4), 591–611. <https://doi.org/10.1093/biomet/52.3-4.591>.
- Torres, M.Á., Coll, M., Heymans, J.J., Christensen, V., Sobrino, I., 2013. Food-web structure of and fishing impacts on the Gulf of Cadiz ecosystem (South-western Spain). *Ecol. Model.* 265, 26–44.
- Tuset, V.M., Lombarte, A., Assis, C.A., 2008. Otolith atlas for the western Mediterranean, north and central eastern Atlantic. *Sci. Mar.* 72 (S1), 7–198. <https://doi.org/10.3989/scimar.2008.72s17>.
- Tuset, V.M., Otero-Ferrer, J.L., Siliprandi, C., Manjabacas, A., Martí-Puig, P., Lombarte, A., 2021. The paradox of otolith shape indices: routine but overestimated use. *Can. J. Fish. Aquat. Sci.* 78 (6), 681–692. <https://doi.org/10.1139/cjfas-2020-0369>.
- Vargas, J.M., García-Lafuente, J., Delgado, J., Criado, F., 2003. Seasonal and wind-induced variability of sea surface temperature patterns in the Gulf of Cádiz. *J. Mar. Syst.* 38 (3–4), 205–219. [https://doi.org/10.1016/S0924-7963\(02\)00240-3](https://doi.org/10.1016/S0924-7963(02)00240-3).
- Velasco, F., Olaso, I., 1998. European hake *Merluccius merluccius* (L., 1758) feeding in the Cantabrian Sea: seasonal, bathymetric and length variations. *Fish. Res.* 38 (1), 33–44.
- Volpedo, A.V., Echeverría, D.D., 2003. Ecomorphological patterns of the sagitta in fish on the continental shelf off Argentina. *Fish. Res.* 60 (2–3), 551–560.
- Volpedo, A.V., Vaz-dos-Santos, A.M. (Eds.), 2015. *Methods of Studies with Otoliths: Principles and Applications*, first ed., bilingual edition. CAFP-BA-PIESCI.

- Wilcoxon, F., 1945. Individual comparisons by ranking methods. *Biometrics Bull.* 1 (6), 80–83. <https://doi.org/10.2307/3001968>.
- Wujdi, A., Kim, H.J., Oh, C.W., 2022. Population structure of Indian mackerel (*Rastrelligerkanagurta*) in Java and Bali Island, Indonesia inferred from otolith shape. *Sains Malays.* 51 (1), 39–50. <https://doi.org/10.17576/jsm-2022-5101-04>.
- Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol.* 1 (1), 3–14. <https://doi.org/10.1111/j.2041-210X.2009.00001.x>.