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# Exploring morphometric frontiers: A comprehensive study of otolith growth patterns in brown comber *Serranus hepatus* (Linnaeus, 1758)

Jairo Castro-Gutiérrez[1,2] | Sara Madera-Santana[1] | Carlos Rodríguez-García[1,3] | Ángel Rafael Domínguez-Bustos[1] | Jésica Sarmiento-Carbajal[1,3] | José Belquior Gonçalves-Neto[1,4] | Remedios Cabrera-Castro[1,3]

[1]Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Campus Río San Pedro, Universidad de Cádiz, Puerto Real, Spain

[2]Departamento de Ciencias Agroforestales, Escuela Técnica Superior de Ingeniería, Campus El Carmen, Universidad de Huelva, Huelva, Spain

[3]Instituto Universitario de Investigación Marina (INMAR), Campus de Excelencia Internacional del Mar (CEIMAR), Puerto Real, Spain

[4]Marine Vertebrate Evolution and Conservation Lab-EvolVe, Departamento de Biologia, Centro de Ciências, Universidade Federal do Ceará, Campus do Pici, Bloco 909, Fortaleza, Brazil

**Correspondence**
Jairo Castro-Gutiérrez, Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Campus Río San Pedro, Universidad de Cádiz, 11510 Puerto Real, Spain.
Email: jairo.castro@uca.es

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## Abstract

Otoliths are widely employed in marine sciences to gain insights into fish growth, age, migrations, and population structure. This study investigates the relationships between morphometric measurements, otolith characteristics, and length size patterns in the brown comber (*Serranus hepatus*) from the Gulf of Cádiz, a species discarded in artisanal trawl fisheries. Our findings reveal significant changes in otolith shape indices as fish grow, with symmetry observed between left and right otolith measurements. Otolith size is found to be related to fish size, supporting its use in estimating body length at different life stages. Otolith shape analysis has potential applications in stock identification, detecting catch misreporting, and studying marine predator diets. Combining otolith shape analysis with other data types can clarify relationships among taxa and inform spatial management strategies, contributing to the long-term sustainability of fish populations and the assessment of the impact of management strategies on fish size and growth. This study enhances our understanding of the broader implications of morphometric and otolith analyses in fisheries research and supports the development of more sustainable fisheries management practices.

KEYWORDS
artisanal trawl fisheries, discards, morphometrics, otolith analysis, stock identification

## 1 | INTRODUCTION

Fisheries play a vital role in global food security and socioeconomic stability, but they are also subject to various challenges such as overfishing and discards that have made it essential to develop effective and sustainable fisheries management and strategies (Pauly et al., 2002). Discards in fisheries have become a major concern due to their ecological and economic impacts (Bellido et al., 2011; Despoti et al., 2020; Kelleher, 2005). Discarded species are frequently caught unintentionally during fishing operations targeting more commercially valuable species. These discarded species, which are often returned to the sea either dead or injured, represent a significant portion of the total marine life caught (Kelleher, 2005). Among the discarded species is the brown comber, *Serranus hepatus* (Linnaeus, 1758), a small benthopelagic fish commonly found in various marine habitats, including rocky reefs and sandy bottoms, and is often encountered by artisanal trawl fisheries in the Gulf of Cádiz (Rodríguez-García et al., 2023). Given its status as a discarded species, the brown comber has received limited attention from researchers and stakeholders. Consequently, there is a pressing need for comprehensive studies on the biology and ecology of this species to support informed fisheries management decisions in the region. The Sustainable Fisheries and Fisheries Innovation Act (Ley 5/ , 2023) explicitly advocates for sustainable management, innovation, local development, scientific research, and cooperation; therefore, conducting comprehensive studies on the biology and ecology of the brown comber, a discarded and understudied species, directly aligns with these principles and is essential for ensuring informed and sustainable decision-making in the management of the region's fisheries.

In recent years, research on morphometrics has been increasingly employed to inform sustainable fisheries management practices (Begg et al., 2001; Canty et al., 2018; Hilborn & Walters, 1992). Knowledge of growth patterns and morphometric characteristics is crucial for understanding their population dynamics and informing these management efforts (Cadrin, 2000). Moreover, the study of otolith characteristics can provide valuable insights into fish growth, age, migrations, and population structure (Agüera & Brophy, 2011; Bacha et al., 2014; Francis & Campana, 2004; Morales-Nin, 2000; Sturrock et al., 2012; Tanner et al., 2016). These calcified structures are located in the inner ear of fishes and grow incrementally throughout the life of a fish (Campana & Thorrold, 2001).

Research on otolith shape has proven to be valuable across various research areas (Campana & Thorrold, 2001). Campana and Casselman (1993) employed otolith shape analysis to discriminate between fish stocks, demonstrating the utility of morphometric indices in fisheries management. Besides that, Cardinale et al. (2004) examined the effects of sex, stock, and environment on the otolith shape of Atlantic cod (*Gadus morhua*) of known age, showing that these factors can influence otolith morphology. Lombarte and Lleonart (1993) investigated otolith size changes related to body growth, habitat depth, and temperature, indicating that morphometric indices can be useful in ecological and evolutionary studies. Tuset et al. (2008) presented an otolith atlas for the western Mediterranean, north Atlantic, and central eastern Atlantic, facilitating the identification and classification of different fish species. Lastly, Volpedo and Echeverría (2003) analysed the ecomorphological patterns of the sagitta in fish from the Argentine continental shelf, exploring the relationships between otolith morphology and fish habitat. Within the same genus, otoliths have a similar basic evolutionary design that may differ between species due to the different environmental factors to which individuals are exposed (Arellano et al., 1995; Lombarte, 1992; Tuset et al., 2003). Morphometric and otolith analyses have also been applied to various fish species of the genus *Serranus* such as *Serranus scriba*, *Serranus cabrilla*, and *Serranus atricauda* (Bilge et al., 2014; Bilge et al., 2018; Bilge & Filiz, 2018a; Bilge & Filiz, 2018b; Karakulak et al., 2006; Tuset et al., 2003).

Therefore, our study aims to elucidate the relationships between morphometric measurements, otolith characteristics, and length size patterns in brown comber from the Gulf of Cádiz. Given that otolith shape is highly species-specific (Gaemers, 1984; Nolf, 1985), this study will facilitate comparisons with similar research on other *Serranus* species or related taxa, enhancing our understanding of the broader implications of morphometric and otolith analyses in fisheries research. By providing this information this study can help to inform more sustainable fisheries management practices in the region, benefiting both the ecosystem and the communities that rely on these resources for their livelihoods.

## 2 | MATERIALS AND METHODS

### 2.1 | 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.

### 2.2 | Study design

A total of 1534 individuals of *S. hepatus* were collected through the ECOFISH, ECOFISH2, ECOFISH+, and ECOFISH 4.0 projects, carried out by the University of Cádiz (Spain). Specimens were captured using trawls by the artisanal fleet operating in the Gulf of Cádiz in southwestern Spain (36°51′ N, 06°55′ W; Figure 1). Sampling was conducted over 3 years (from 2019 to 2022) with a total of 90 hauls made at depths ranging from 15 to 550 m.

### 2.3 | Morphometric fish sampling

The total length (TL) was measured to an accuracy of ±0.1 cm and total weight (TW) to an accuracy of ±0.01 g. The length-weight relationship was modeled using the allometry equation $TW = a TL^b$ (Ricker, 1975), where TW is the total weight (in g), TL is the total

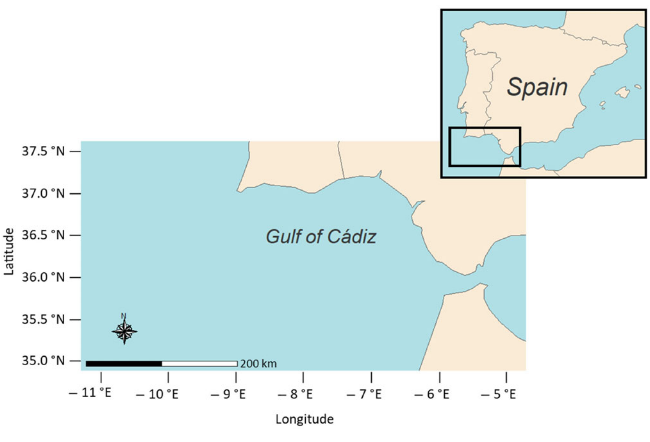


FIGURE 1 Map of the Gulf of Cádiz (southwest Spain).

length (in cm), *a* is the intercept of the regression curve related to body shape, and *b* is the slope related to growth type. Isometric growth is considered when $b = 3$, indicating that weight and length increase in the same proportion. When $b > 3$, individuals show a greater increase in weight versus length, with a tendency to positive allometric growth, but when $b < 3$, the rate of weight increase is less than length, so growth tends to be negative allometric (Froese, 2006).

## 2.4 | Otolith shape analysis

The otoliths were extracted, cleaned, and stored dry for later analysis. Each otolith was weighed using an Explorer Semi-Micro EX125D balance with an accuracy of ±0.01 mg. Otoliths were measured for their length and width (in mm), weight (in g), area (in mm$^2$), and perimeter (in mm). For this purpose, they were photographed using a Moticam 580 camera coupled to a Leica Wild M10 loupe, and the measurements were calculated using the image analysis software ImageJ version 1.53 (Schneider et al., 2012).

To determine the appropriate statistical test for comparing the means of otolith measurements, the normality of each variable in the dataset was first assessed using the Shapiro–Wilk test. Statistical comparison of the means of left and right otolith measurements was performed using the Wilcoxon signed-rank test, as it often exhibits asymmetry between the left and right structures (Vignon & Morat, 2010).

Circularity, rectangularity, form factor, and *E*-value indices were calculated for the otoliths to characterize their shape and structure (Table 1). Circularity is an index of how closely the otolith shape resembles a circle, with higher values indicating more circular shapes. Rectangularity describes the variations in length and width with respect to the area, 1.0 being a perfect square. Form factor is an index that takes into account the otolith area and perimeter, with higher values indicating more compact shapes. *E*-value is the ratio of the otolith width to its length, providing information about the elongation of the otolith (Russ, 1990).

To investigate the relationships between TL and otolith morphometric variables, linear regression models were fitted for otolith weight, otolith area, and otolith perimeter against total fish length.

**TABLE 1** Summary of otolith measurements and otolith shape indices derived from otolith dimensions in brown comber *Serranus hepatus* (Linnaeus, 1758).

| Otolith measurements (mm) | Otolith shape index |
|---|---|
| Length ($L_o$) | Circularity $= \frac{P^2}{A}$ |
| Width ($W_o$) | Rectangularity $= \frac{A}{L_o \cdot W_o}$ |
| Area ($A$) | Form factor $= \frac{4\pi A}{P^2}$ |
| Perimeter ($P$) | *E*-value $= \frac{W_o}{L_o}$ |

*Note*: Length, width, and perimeter were measured in mm, and area in mm$^2$.

To examine how otolith shape changes with fish length size, the relationships between different indices and TL were assessed using correlation analyses. The dataset was then divided into length classes based on TL (Ketchen, 1950), and the Kruskal-Wallis test was performed to determine if there were significant differences in the indices among the length classes. Dunn's test with Bonferroni correction was conducted for pair-wise comparisons between length classes for each index (Dunn, 1964). The significant comparisons were identified. All statistical analysis were performed using R version 4.1.2 (R Core Team, 2021) and a significance level of $\alpha = 0.05$.

# 3 | RESULTS

The mean TL of the individuals was 8.99 cm (ranging from 3.20 to 13.70 cm). The mean TW was 13.36 g (ranging from 1.02 to 43.64 g). Length-weight relationship followed Equation 1:

$$\mathrm{TW} = -4.71988 + \mathrm{TL}^{3.27155}, \quad (1)$$

where TW is the total weight (in g) and TL is the total length (in cm). Brown comber from our study area showed a positive allometric growth ($b > 3$).

Shapiro–Wilk test for normality showed that normality could not be assumed for any of the variables ($p < 0.05$). Therefore, non-parametric tests were employed to compare the means of left and right otolith measurements. Wilcoxon signed-rank tests revealed no significant differences ($p > 0.05$) between left and right otolith measurements in terms of length, width, area, perimeter, and weight. Consequently, further analyses were conducted using only the left otolith measurements (Tuset et al., 2008).

A summary of the otolith measurement characteristics is presented in Table 2.

A linear regression model was fitted to analyse the relationship between fish length and otolith weight, area, and perimeter, resulting in an $R^2$ value of 0.759, 0.822 and 0.821, respectively.

Mean values for circularity, rectangularity, form factor, and *E*-value indices are presented in Table 3.

The relationships between TL and the four different morphometric indices of otoliths in brown comber were analysed.

**TABLE 2** Statistics results summary of the different morphometrics measured in brown comber *Serranus hepatus* (Linnaeus, 1758) otoliths.

| Otolith measure | *n* | Mean (±SD) | Min. | Max. |
|---|---|---|---|---|
| Length (mm) | 1351 | 4.33 (±0.87) | 1.51 | 7.67 |
| Width (mm) | 1351 | 2.19 (±0.47) | 1.12 | 4.04 |
| Weight (g) | 1200 | 8.00 (±4.56) | 0.96 | 28.00 |
| Perimeter (mm) | 1194 | 11.11 (±2.19) | 5.68 | 17.31 |
| Area (mm$^2$) | 1194 | 6.58 (±2.45) | 1.86 | 14.01 |

Abbreviations: Max., maximum value; Min., minimum value; *n*, sample size; S.D., standard deviation.

The Pearson correlation coefficient result between the circularity index and TL was 0.29 ($p < 0.05$). The rectangularity index showed a weak correlation with TL, having a non-significant correlation coefficient of 0.00 ($p > 0.05$). The form factor index exhibited a negative correlation with TL, with a significant correlation coefficient of −0.30 ($p < 0.05$). Lastly, the *E*-value index displayed a weak negative significant correlation with TL, with a correlation coefficient of −0.07.

Scatterplots and linear regression analyses were conducted for each morphometric index, and the results are presented in Figure 2.

The variables were further analysed for the magnitude of their variation as a function of different discrete length ranges of the individual. For this, TL was divided into 10 discrete length ranges, and Kruskal-Wallis tests were performed to analyse the differences between each TL size interval. The *p*-value for the Kruskal-Wallis test was less than 0.05 for all morphometric indices, indicating statistically significant differences among the medians of the groups for each index. Then Dunn's post-hoc tests were performed to analyse specific differences between TL classes, adjusting *p*-values with the Bonferroni method. The results of Dunn's test comparisons for each otolith shape index are presented in Table 4.

As fish length increases, the otolith shape undergoes several significant changes in morphometric indices (Figure 3). The circularity index, which measures the roundness of the otolith by comparing its perimeter and area, shows some fluctuations along the length range of the fish. The values increase from 18.43 in the 3.2–6.3 cm length range to 19.07 in the 6.3–7.5 cm length range, then remain fairly stable around 19.50 from the 7.5–10.3 cm length range, and finally increase to 19.58 in the 11.1–13.5 cm length range. These changes in circularity suggest that the otolith's roundness varies across different fish lengths, with a tendency to become slightly more rounded in the final stages.

As fish length increases, the rectangularity index, which represents the ratio of otolith area to the product of otolith length and width, exhibits a general increasing trend. The values change from 0.68 in the 3.2–6.3 cm length range to 0.73 in the 11.1–13.5 cm length range. This pattern suggests that the otolith shape becomes more rectangular as the fish grows.

The form factor index, which quantifies the compactness of the otolith by comparing its area and perimeter, demonstrates a general decreasing trend as the fish length increases. It starts at 0.68 in the 3.2–6.3 cm length range, decreases to 0.66 in the 6.3–7.5 cm range, and then to 0.64 in the 7.5–8.1 cm range. After this, the values remain relatively stable around 0.64–0.65 across the 8.1–11.1 cm length ranges, and finally decrease slightly again to 0.64 in the 11.1–13.5 cm range. These variations in the form factor indicate that the otolith

**TABLE 3** Summary of the results of the otolith shape indices calculated for brown comber *Serranus hepatus* (Linnaeus, 1758).

| Otolith shape index | *n* | Mean (±S.D.) | Min. | Max. |
|---|---|---|---|---|
| Circularity | 1202 | 19.42 (±0.95) | 16.63 | 23.64 |
| Rectangularity | 1202 | 0.70 (±0.16) | 0.14 | 2.24 |
| Form factor | 1202 | 0.65 (±0.03) | 0.53 | 0.76 |
| *E*-value | 1360 | 0.51 (±0.06) | 0.32 | 1.24 |

Abbreviations: Max., maximum value; Min., minimum value; *n*, sample size; S.D., standard deviation.

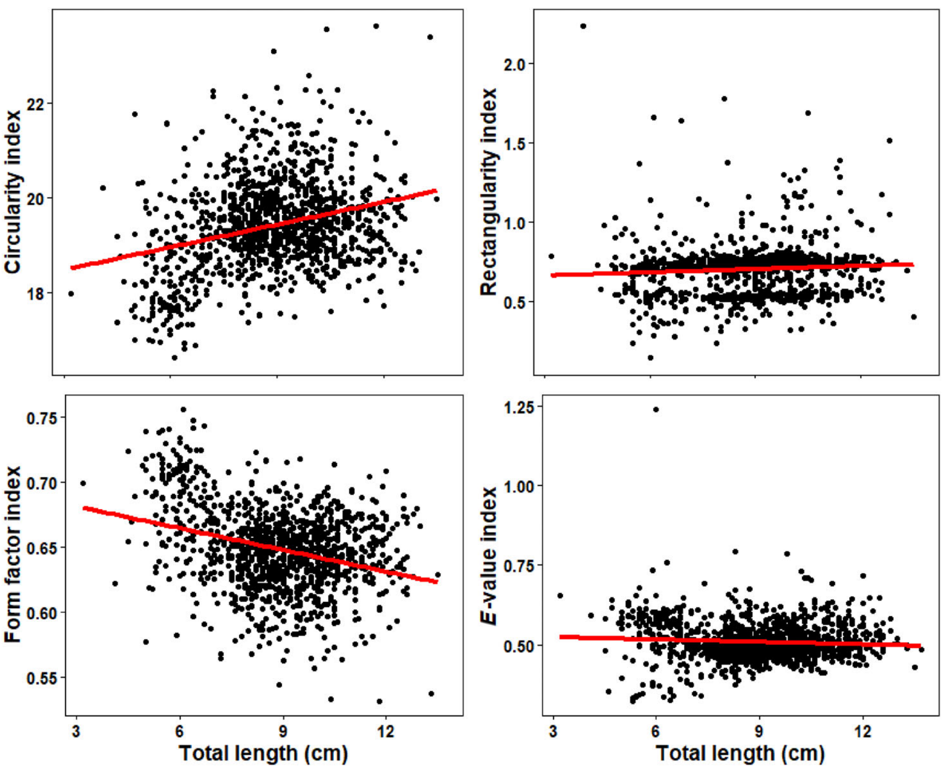


**FIGURE 2** Relationship between morphometric indices and total length of specimens. Each plot shows the relationship between total length (in cm) and a specific morphometric index: circularity index (top-left), rectangularity index (top-right), form factor index (bottom-left), and *E*-value index (bottom-right). The red lines represent linear regressions.

**TABLE 4** Mean otolith shape indices for different length ranges in brown comber *Serranus hepatus* (Linnaeus, 1758).

| | Otolith morphometric index | | | | |
|---|---|---|---|---|---|
| Length range (cm) | *n* | Circularity | Rectangularity | Form factor | *E*-value |
| 3.2–6.3 | 119 | 18.43[a] | 0.68[a] | 0.68[a] | 0.55[a] |
| 6.3–7.5 | 119 | 19.07[b] | 0.69[b] | 0.66[b] | 0.50[b] |
| 7.5–8.1 | 119 | 19.58[a] | 0.69[b] | 0.64[c] | 0.49[c] |
| 8.1–8.5 | 119 | 19.51[a] | 0.69[b] | 0.65[a] | 0.49[c] |
| 8.5–8.9 | 119 | 19.68[a] | 0.70[b] | 0.64[a] | 0.49[c] |
| 8.9–9.4 | 118 | 19.52[a] | 0.68[b] | 0.64[a] | 0.49[c] |
| 9.4–9.9 | 118 | 19.66[a] | 0.71[b] | 0.64[a] | 0.50[b] |
| 9.9–10.3 | 118 | 19.65[a] | 0.71[b] | 0.64[a] | 0.50[a] |
| 10.3–11.1 | 118 | 19.50[a] | 0.72[b] | 0.65[a] | 0.50[b] |
| 11.1–13.5 | 118 | 19.58[c] | 0.73[c] | 0.64[c] | 0.52[a] |

*Note*: For each otolith shape index, means with the same superscript lowercase letter are not significantly different ($p > 0.05$). Sample size is shown as "*n*."

**FIGURE 3** Comparison of otoliths from three individuals of brown comber *Serranus hepatus* (Linnaeus 1758) displaying varying sizes. Left image corresponds to an otolith from an individual with a total length (TL) of 4.10 cm and a total weight (TW) of 1.13 g. The middle image corresponds to an otolith from an individual with a TL of 8.9 cm and a TW of 10.06 g. The right otolith is from an individual with a TL of 13.3 cm and a TW of 35.02 g. Scale shown at 1.5 mm. "TL" refers to the total length of the fish, and "TW" refers to the total weight.

compactness generally decreases with medium lengths and then increases as the fish grows larger.

The *E*-value, which represents the ratio of otolith width to length, shows a decreasing trend in the initial stages up to 8.5 cm, with values decreasing from 0.55 to 0.49. However, in the later stages, the *E*-value starts to increase again, reaching a value of 0.52 in the 11.1–13.5 cm length range. This pattern suggests that the otolith shape becomes less elongated in the initial growth stages, whereas in the later stages, the shape becomes slightly more elongated again.

## 4 | DISCUSSION

In our study, the shape of the otoliths in the brown comber (*S. hepatus*) and the relationship between fish length and growth patterns of the otolith shape indices (circularity, rectangularity, form factor, and *E*-value) were analysed. The results indicate that otolith shape indices change significantly: circularity fluctuates with a tendency to become more rounded at larger sizes; rectangularity increases, indicating a more rectangular shape; form factor decreases, suggesting reduced compactness; and *E*-value shows an initial decrease, followed by a slight increase, reflecting changes in elongation.

Our study found no significant differences between left and right otolith measurements in terms of length, width, area, perimeter, and weight, suggesting symmetry in brown comber otoliths. This symmetry in otolith shape has also been observed in other fish species, such as *Gadus morhua*, *Synechogobius ommaturus*, *Coryphaena hippurus*, *Xiphias gladius*, *Scomber scombrus*, and *Lutjanus kasmira*. In contrast, several roundfish and flatfish species have been reported to exhibit directional asymmetry in otolith shape, such as *Chelon ramada*, *Diplodus annularis*, *Diplodus puntazzo*, *Clupea harengus*, and *Scomberomorus niphonius* (Mahé et al., 2019; and citations therein). Mahé et al. (2019, 2021) indicate that otolith directional asymmetry may be linked to geographical population structuring, potentially due to phenotypic plasticity in response to environmental factors or genetic differentiation between locations, enabling stock differentiation in the Mediterranean Sea, and could stem from differences in biomineralization between left and right inner ears.

The relationship between fish length and otolith weight, area, and perimeter revealed a strong correlation. Those results are in line with previous studies on otolith morphometrics in other fish species (Bolles & Begg, 2000; Boudinar et al., 2015; Burke et al., 2008; Campana & Casselman, 1993; Tuset et al., 2008), supporting the notion that otolith size is related to fish size. This allows the estimation of body length at different life stages using growth marks on otoliths by retrospective analysis (Francis, 1990; Ricker, 1975). This technique is useful for monitoring average growth rates of various age classes in the population, identifying periods of slow or rapid growth and correlating them with abiotic factors (Mahé et al., 2019; Smith, 1983). Furthermore, differences in otolith sizes for the same TL of individuals are not trivial. These differences may be based on information about the different growth rates of the individual (Secor & Dean, 1989). Thus, individuals that have had slower growth rates will have had greater assimilation of calcium carbonate ($CaCO_3$), resulting in larger otoliths.

Our findings on the relationship between otolith indices and fish length in brown comber show similarities and differences when compared to the study conducted by Tuset et al. (2003) in the Canary Islands. Following the results of this author, *S. atricauda*, *S. scriba*, and *S. cabrilla* showed higher circularity values, similar rectangularity, and a lower form factor than brown comber from this study. Libungan et al. (2015) analysed differences in the otolith shape of *C. harengus* in Norwegian waters, demonstrating that semi-enclosed systems, where local populations live and breed, are effective barriers for dispersal.

Otolith shape analysis has potential applications beyond stock identification, such as detecting catch misreporting by area or stock, and studying diets by identifying otoliths collected from stomachs or scats, which could help determine the feeding area or test for size-selective prey mortality (Campana & Casselman, 1993; Canty et al., 2018; de Carvalho et al., 2019). The use of otolith shape in fish stocks identifications has been widely used. Those indices can be used as a validation tool for other techniques, thus providing valuable complementary information. For example, it can be combined with age-based studies in tropical environments, where traditional otolith growth ring analysis can be challenging due to the lack of pronounced seasonal temperature variations (Morales-Nin, 1992). By combining otolith shape analysis with age studies, researchers may be able to validate and refine their findings, overcoming the limitations associated with otolith growth ring analysis in tropical regions (Morales-Nin, 2000). Burke et al. (2008) used the otolith shape analysis for discriminating between seasonal spawning stocks of juvenile herring collected from nursery grounds in the Irish Sea. Cadrin and Friedland (1999) demonstrated that image processing can be very useful in analysis using morphometric techniques to differentiate fish stocks. This is in line with the study of Bolles and Begg (2000) who demonstrated that otolith morphometric indices specific to fish age such as perimeter, circularity, and rectangularity extracted by image processing are useful in identifying silver hake stocks and can be a useful tool in identifying other fish stocks in northwest Atlantic. Castonguay et al. (1991) found that otolith shapes were more distinct between stocks than between contingents in Atlantic mackerel, suggesting greater genetic differentiation and/or greater environmental differences between stocks. Conversely, Morales-Nin et al. (2022) examined otolith shape and chemical composition as markers for European hake stock discrimination, finding that a continuous gradient rather than distinct stocks was more plausible, due to low classification success rates using these otolith markers.

Although the precise factors that influence otolith shape are not yet fully comprehended, recent studies suggest various aspects could play a role understood (Burke et al., 2008; Mahé et al., 2019). Environmental disturbances during the early life stage, fish length, age, year class, sexual maturity, and sexual dimorphism have all been identified as potential contributors to shape differences (Vignon, 2018, Mahé et al., 2019 and cites therein). Otolith shape has been linked to fish growth, which in turn is connected to size. Additionally, ontogenic changes in otolith shape, often measured as size-related variation, may stem from differences in growth rates due to habitat quality and developmental processes (Mahé et al., 2019). Therefore, although regional differences can be described by shape indices, the combination of otolith shape with other types of data may help clarify the relationship among taxa (Tuset et al., 2003).

The information obtained from otolith shape analysis regarding the spatial distribution and connectivity of fish stocks can help to identify local communities that are being pressured and could play a key role in a specific ecosystem, and to establish spatial management strategies, such as marine protected areas (MPAs) and fishery closures (Mahon et al., 2008; Tuset et al., 2008). By protecting critical habitats and ensuring the persistence of discrete stocks, these spatial management measures can contribute to the long-term sustainability of fish populations and reduce the pressure to discard fish from overexploited areas (Gaines et al., 2010; Lubchenco et al., 2003). By analysing the otoliths of fish from both landed catches and discards, researchers can assess the impact of management strategies on fish size and growth, and identify areas where further improvements may be needed (Campana, 2001; Morales-Nin et al., 2022).

## 5 | CONCLUSIONS

Our findings indicate significant changes in otolith shape indices as fish grow, with symmetry observed between left and right otolith measurements. The results support the notion that otolith size is related to fish size, and thus can be used for estimating body length at different life stages.

Otolith shape has potential applications beyond stock identification, such as detecting catch misreporting, and studying marine predator diets. Although the exact factors influencing otolith shape are not fully understood, variables like environmental disturbances, fish size, and sexual maturity may contribute to shape differences. The combination of otolith shape analysis with other data types may help clarify the relationship among taxa. Information obtained from otolith shape analysis can inform spatial management strategies, contributing to the long-term sustainability of fish populations and helping assess the impact of management strategies on fish size and growth.

### AUTHOR CONTRIBUTIONS

Jairo Castro-Gutiérrez: conceptualization, software, formal analysis, data curation, writing—original draft, writing—review and editing. Sara Madera-Santana: methodology, formal analysis, writing—original draft, writing—review and editing. Carlos Rodríguez-García: methodology, data curation, writing—review and editing. Ángel Rafael Domínguez-Bustos: data curation, resources, writing—review and editing. Jésica Sarmiento-Carbajal: resources, writing—review and editing. José Belquior Gonçalves-Neto: resources, writing—review and editing. Remedios Cabrera-Castro: conceptualization, methodology, writing—review and editing, supervision. All authors have read and agreed to the published version of the manuscript.

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## ORCID

*Jairo Castro-Gutiérrez* https://orcid.org/0000-0002-4466-3645
*Sara Madera-Santana* https://orcid.org/0009-0001-2125-2624
*Carlos Rodríguez-García* https://orcid.org/0000-0003-4372-5442
*Ángel Rafael Domínguez-Bustos* https://orcid.org/0000-0003-3129-9765
*José Belquior Gonçalves-Neto* https://orcid.org/0000-0002-9209-9479
*Remedios Cabrera-Castro* https://orcid.org/0000-0002-0388-1937

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