

Fluctuating Asymmetry of *Elops lacerta* (Valenciennes, 1847) Otoliths in the Western African Waters

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Abstract

The importance of this study lies in the in-depth exploration of the ecological diversity of otoliths in *Elops lacerta*, based on the analysis of data from 260 individuals collected from various sites, including the Porto Novo lagoon, the Cotonou lagoon, Lake Nokoué, and the Atlantic coast in southern Benin. The results highlight significant variations in otolith morphology, establishing relevant links with the biological parameters of the fish at each site. Exploration of the asymmetry between the right and left sides reveals notable distinctions between these two aspects. Analysis of otolith shape thus emerges as a valuable tool for discriminating between stocks and providing a better understanding of ecological variations. The local diversity observed highlights the crucial importance of implementing adaptive management strategies to ensure effective conservation of the species and its habitat.

Keywords

Analysis, Porto-Novo Lagoon, Cotonou Lagoon, Lake Nokoué, Coast Atlantic

1. Introduction

West Africa's aquatic ecosystems are the habitat of remarkable biodiversity and

play an essential role in the subsistence of local communities [1]. These systems, which include lakes, lagoons and coastal areas, are habitat to many species of fish and other marine organisms, contributing to the region's food and economic security. However, these ecosystems are under increasing pressure from urbanization, pollution and environmental change [2]. It is essential to understand the complex dynamics of the species to ensure its long-term conservation and management. This is also a study of an economically crucial fish species in Beninese waters.

It is in this perspective that this study was initiated and seeks to develop our understanding of the ecological relationships associated with the West African ladybug, *Elops lacerta*, based on otolith analysis. Member of the Elopidae family, this species comprises one genus and six distinct species.

Otoliths are a pair of calcified white pieces that develop during the fish's lifetime. This continuous development makes it possible to determine age by reading the growth bands. Otoliths are also sound transducers, playing an important role in fish hearing. The shape of the otoliths is directly related to the spatial development of the lobes. Otoliths provide basic data and are used as a tool in taxonomy and phylogeny [3], evolution and ontogeny [4], spatial and temporal stock discrimination [5] [6] [7], population management [8] [9] [10]. Otolith shape and size are used because they reflect the environment in which the species inhabits [11] [12]. They depend on endogenous and exogenous factors such as environmental temperature and salinity [13] [14], luminosity [15] and depth [16] [17]. Otolith shape reveals both interspecific [18] and intraspecific differences [4]-[11]. Otolith shape within a population can be influenced by several confounding factors, including sex, age, fish size [19] and sexual maturity [7] [8] [9]. These factors, which describe the fish's life history, reflect the ontogenetic component [20]. However, we must also take into account the possibility of intra-individual variability linked to a possible asymmetry between the otoliths of the right and left ears. Of the three types of asymmetry that exist (fluctuating asymmetry, bilateral asymmetry, antisymmetry), fluctuating asymmetry is a characteristic that can potentially reflect stressful situations or environmental heterogeneity [21] [22] [23] [24]. Directional asymmetry, on the other hand, is not the result of chance, but of genetic components and/or phenotypic plasticity. Thus, the degree of directional asymmetry could also vary between different geographical areas for the same species.

The study of otoliths is therefore a means of stock discrimination for better management of fishery resources [8]-[26]. The study of otoliths is therefore a means of stock discrimination for better management of fishery resources [6]-[25]. In this context, the objectives of the study were to explore the fluctuating asymmetry of otoliths within *E. lacerta* and to explore any spatial variation within the study areas, which are: the Atlantic coasts in southern Benin, Lake Nokoué, Cotonou lagoon and Porto-Novo lagoon. These sites are distinctive for their unique characteristics in terms of geography, biodiversity and environmen-

tal constraints.

Elops lacerta is very common in tropical waters and can sometimes be observed in temperate zones [27]. This fish reproduces in the marine environment and their larvae disperse in estuarine habitats which, according to [27], are in decline and degradation. Most *E. lacerta* populations are faced with significant challenges related to habitat degradation and alteration, due to human activity, making them vulnerable to habitat degradation and overfishing [26]. It is also a fish species that has received little attention internationally due to its low economic status [28]. *E. lacerta* coexists with *E. senegalensis* in tropical and subtropical marine and estuarine areas, and is often confused in the West African estuaries of Benin and Nigeria. It has 17 to 19 branchiospines on the lower part of the first gill arch and 72 to 83 scales on the lateral line, adorned with unbranched tubules. Its flanks are silvery and shiny; the back is grey-black and the fins are all pale, tinged with yellow and more or less broadly edged with dark grey [27] [29] [30]. *Elopes* species reproduce at sea [31] [32] [33]. According to Beckley (1984) and McBride *et al.* (2001), juvenile fish ecophases take place in the low-salinity areas of estuaries without entering freshwater, but juveniles are capable of surviving hypersaline conditions. *E. lacerta* can withstand salinities ranging from 0 to 110 [34]. *E. lacerta* is a carnivorous species with piscivorous tendencies [35]. It is a marine-spawning species [36]. It generally develops rapidly in lagoons, where it is represented by its immature phasing. There is a positive correlation between age and length of fork in *E. lacerta* [35].

This study is based on an analysis including otolith shape and morphometric parameters (length, width, area, perimeter) of the otolith, as well as the morphological data of the fish. The aim of this study was to assess whether spatial variation in fluctuating asymmetry and differences in fish otolith shape could be exploited as a potential tool for differentiating fish stocks. This approach was intended to improve our understanding of the species' adaptability to various habitats, and to contribute to efforts to manage and conserve aquatic resources.

2. Material and Methods

2.1. Sampling Site

This comparative study examined the waters of the Porto-Novo lagoon, the Cotonou lagoon and Lake Nokoué (Figure 1). The Porto-Novo lagoon, covering an area of around 35 km², is located in southeastern Benin between parallels 6°25' and 6°30' North and meridians 2°30' and 2°38' East. Lake Nokoué lies between parallels 6°20' and 6°30' N and meridians 2°20' and 2°35'E. The Porto-Novo lagoon is located in the Ouémé department, between parallels 6°25' - 6°30' N and meridians 2°30' - 2°38'E. Both the Nokoué and Porto-Novo lagoons function as catchment basins for the waters of the Ouémé and Sô rivers. These two environments are interconnected and receive water inflows during low-water periods, when the Atlantic tides are strong. However, they become highly brackish for an extended period of 4 to 5 months each year. The Cotonou lagoon has a northern latitude of 6°22' and a longitude of 2°25'60" E. Benin's coastline, link-

ing the marine and coastal environment to the mainland, represents a transition zone where interactions between the Atlantic Ocean and the mainland are intensive. This part of southern Benin is home to several bodies of water in communication with the sea, geographically located between 1°20' and 3°00' E longitude and 3°00' and 6°40' N latitude.

2.2. Sampling

The sample totaled 260 individuals of *Elops lacerta*, distributed as follows: 64 from the Porto-Novo Lagoon, 62 from the Cotonou Lagoon, 74 from Lake Nokoué, and 60 from the Atlantic Sea. For each fish, the total length (TL \pm 0.1 cm) and the total weight (W \pm 0.1 g) were measured. (Table 1)

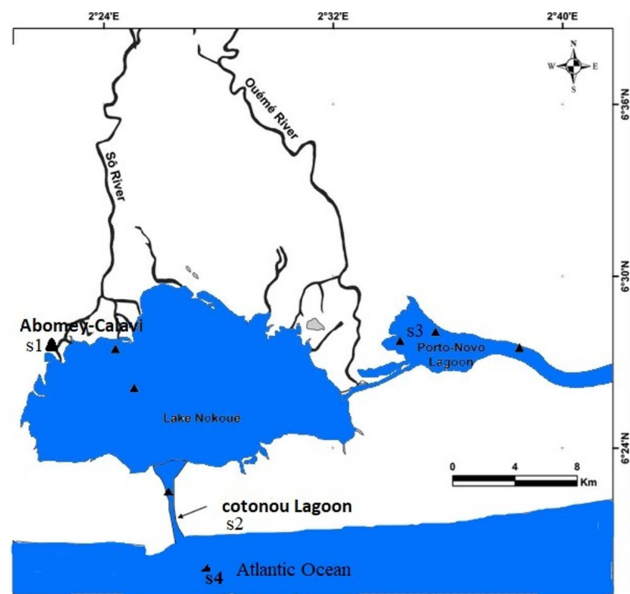


Figure 1. Map of *Elops lacerta* sampling sites (S1: Lake Nokoué; S2: Cotonou Lagoon, S3 Porto-Novo Lagoon, S4 Atlantic coast in southern Benin).

Table 1. Sampling information (total length and total weight) of *E. lacerta* in the Benin waters according to the sampling sites.

		Lagoon of Porto Novo	Lagoon of Cotonou	Lake Nokoué	Atlantic coast south of Benin
Standard Length (cm)	Mean \pm CV	23.0 \pm 2.87	25.0 \pm 3.13	22.65 \pm 1.91	35.36 \pm 1.83
	max	31.0	33.4	28.2	39.2
	min	16.6	19.2	18.0	30.0
Total Weight (g)	Mean \pm CV	60.5 \pm 24.8	74.2 \pm 31.3	53.7 \pm 15.2	256.7 \pm 128.7
	max	140	192	190	1178
	min	20	28	24	134

2.3. Of the Otolith and Image Processing

The present study was based on the *sagittae* (left and right inner sides). Extraction of this pair of otoliths (The left and right *sagittae*) was performed by cutting off the head and then removing the gills. The otoliths were collected with forceps, cleaned with distilled water, dried and preserved in Eppendorf tubes. The species name, area, and date of sampling were noted on a label adhered to the tube. Otoliths image acquisition was realized from a Canon powershot digital camera (12.1 Mega pixels) through a binocular microscope. Image processing of the whole otoliths was performed using Photoshop CS6 image processing software. In order to compare the shapes of the left and right otoliths, a mirror image of the left otolith was used.

2.4. Otoliths Shape Analysis

The otolith outline was described using Fourier elliptic analysis [37] on each otolith. The contour was delimited and extracted after image pre-processing leading to a transformation of the original image into a binary image. The OpenCV library was then used to detect the otolith contours in the pre-processed image. This enabled the shape of the otolith to be represented as accurately as possible. The coordinates (x, y) of the main contour describing the shape of the contour were extracted [38]. Elliptical Fourier analysis [37] was done on each otolith contour delimited and extracted after binarization of the image. For each otolith, the first 100 elliptical Fourier harmonics (H) were extracted and normalized with respect to the first harmonic and were therefore invariant to size, rotation and the starting point of the otolith contour description [39]. To determine the number of harmonics required to reconstruct the otolith contour, the cumulative Fourier power (F) was calculated for each individual otolith as a measure of the accuracy of the contour reconstruction obtained with n harmonics): nk harmonics (*i.e.* the proportion of variance in the contour coordinates explained by the k

$$F(nk) = \sum_{k=0}^{nk} \frac{Ai^2 + Bi^2 + Ci^2 + Di^2}{2}$$

where Ai , Bi , Ci and Di are the harmonic coefficients and nk is the total number of harmonics included.

The value of nk was chosen so that $F(nk)$ explains 99.99% of the variance in contour coordinates, *i.e.* it reconstructs the shape with 99.99% accuracy [37].

In the second part of the study, ImageJ software was used (using a predefined scale of 1 millimeter) to determine otolith biometric parameters (length (Lo), width (Wo), area (Ao) and perimeter (Po)).

Size parameters are measures directly related to otolith size, unlike shape indices, which are dimensionless measures and therefore independent of otolith size. The shape of the otolith relative to a geometric reference shape such as an ellipse for ellipticity (E), and a square for aspect ratio, was determined. They are simple to obtain, and the biological interpretation of the associated results is less complex than that of results obtained from multivariate data [18] [40].

3. Statistical Analysis

Length-weight relations in fish are considered to be allometric growth models of the type:

$$W = a \times TL^b$$

Three types of descriptors were analyzed, including otolith size parameters, shape indices (Ellipticity and aspect ratio), and EFDs.

The analysis focused on the asymmetry between left and right otolith shapes, examining the impact of the side on their morphology. To evaluate fluctuating asymmetry, the absolute value of the difference between the right and left sides for length, width, area and perimeter measurements was calculated. Then, the mean of the absolute value of the difference was calculated for each measurement. A Shapiro-Wilk normality test for each measurement was performed to assess the distribution of the data. Finally, a Student's t-test to determine whether the mean differs significantly from zero was performed. The percentage of asymmetry using the mean of the absolute difference of otolith size parameters and the mean of the right side for each species was calculated. Finally, whisker box plots for each measure (length, width, area and perimeter) as a function of species and sampling site selected to visualize data distributions were produced. Principal component analysis (PCA) was applied to an otolith size matrix and the elliptical Fourier descriptor (EFD) matrix [41]. It enabled us to reduce the data size of the elliptical Fourier descriptor (EFD) matrix while retaining as much information as possible, and to obtain a subset of the principal components. The selected principal components (PCs) can be used as shape descriptors of the otolith in our analysis [42]. Each principal component represents a specific shape feature. Then a matrix of the selected EFDs was created by organizing the selected elliptical Fourier descriptors into columns and the individual otoliths into rows [42]. Each cell of the matrix represents the value of the descriptor for a given otolith. For each pair of otoliths, the Euclidean distance was calculated.

A mixed-effects model was used to test the effects of inner ear side, sampling site, sex, fish size and fish weight on otolith shape, but also the effect of sampling site, sex and side on size parameters. Their interactions were also taken into account. Analysis of variance (ANOVA) was performed. Using the mixed-effects model. This statistic measures the difference between the estimated variance of the model's random effects and the residual variance (or error).

For a better estimate of the divergences between samples, we performed multivariate analyses treating all traits simultaneously. Linear discriminant analysis (LDA) is a statistical analysis commonly used for classification and dimension reduction [43]. It is used to extract discriminant information from multivariate data for classification.

Applied LDA is a classification algorithm that seeks to maximize the separation between classes using a linear combination of features.

LDA using geographical positions to define the groups to be tested revealed

principal components that significantly explained the variation in otolith shape. Canonical discriminant analysis (CDA) and mixed factorial discriminant analysis (MDFA) were then performed to assess the effect of gender, side and sampling site.

All statistical tests were performed using the following packages in a Python environment (Numpy, matplotlib, pyplot, Scikit-learn, Pandas, mapply, Plotnine, Plydata, statsmodels, seaborn, scipy) [44].

4. Results

4.1. Relations between Length and Weight of Fish

The relationship between fish length and fish weight (**Figure 2**) varied considerably from site to site. Lagoon Porto-Novo and Lake Nokoué show a strong linear relationship between these two variables, with a large proportion of the variability explained. The Atlantic Sea, on the other hand, shows a very weak and insignificant relationship.

4.2. Relations between Fish Size and Otolith Morphometrics

The correlation between total fish length and otolith size (Supplementary Appendix **Table S1**), examined at several fishing sites, reveals significant variations in the strength of this relation from one site to another. In particular, at Lake Nokoué, the correlation is remarkably robust, indicating a significant influence of otolith length on total fish length (**Figure 3**). In the South Atlantic coast of Benin, on the other hand, the correlation is lower, indicating a less significant contribution of otolith size to variation in total fish length. Data analysis reveals a significant relation between total fish length and otolith length, with substantial

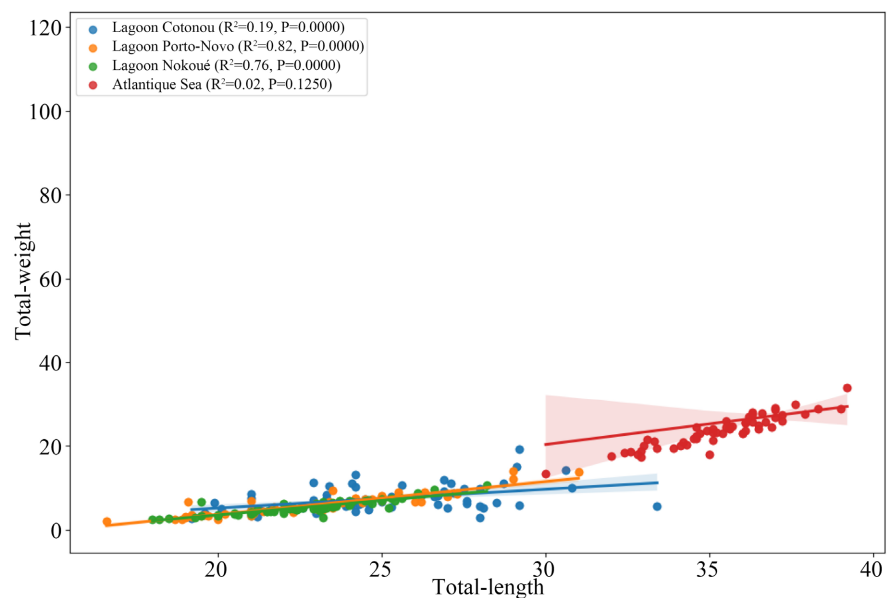


Figure 2. Regression showing the correlation between a fish total length (TL) and weight (W).

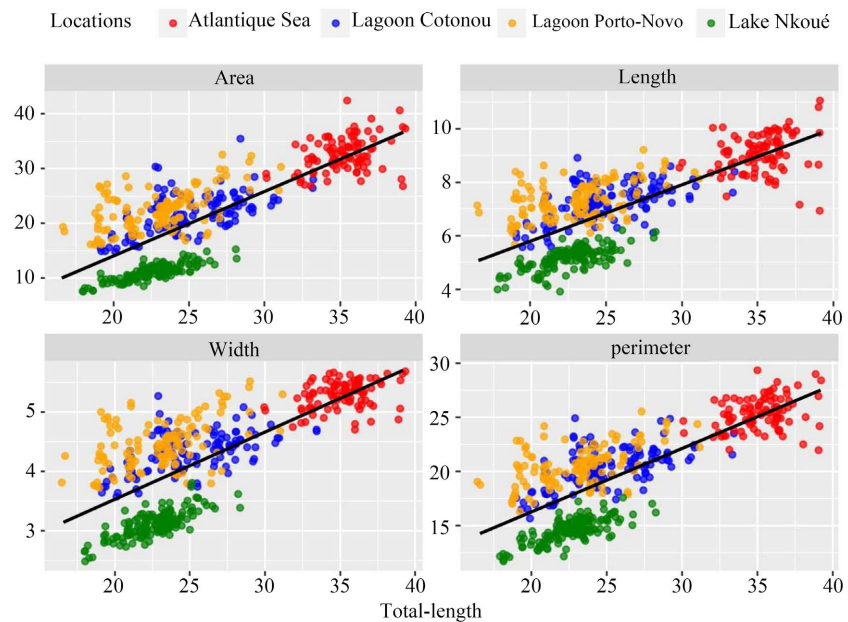


Figure 3. Regression showing the correlation between fish size and otolith morphometrics (A: Area; B: Length, C: Width, D: Perimeter).

variation in this relation according to the fishing site. The other otolith variables (width, area, perimeter) also have significant relationships with total fish length, but their impact also varies from site to site.

a) Analysis of Euclidean Distances

The analysis of Euclidean distances between the right and left sides at the different study sites offers interesting insights into ecological symmetry or asymmetry in the ecosystems examined. Average distances vary from site to site, reflecting different levels of similarity between the right and left sides.

b) Mixed-Effect Linear Analysis

At Lagoon Cotonou and Lake Nokoué, mean distances are relatively low (4.49 and 4.17 respectively), indicating some regularity or similarity between the sides. These results suggest ecological consistency, although local variations may also be present.

In contrast, the Lagoon Porto-Novo and southern Atlantic coast sites show higher mean distances (6.17 and 6.26 respectively), suggesting greater disparity between the right and left sides. These results could indicate greater ecological variation or significant differences between habitats on these two sides.

The results of analysis of variance (ANOVA) tests for different morphological variables (Length, Width, Area, Perimeter) as a function of the Sites:Side and Sites:Sex factors (Table 2) reveal that for Length, the Sites:Side factor (P-value > 0.05), while the Sites:Sex factor shows a tendency towards significance (P-value approaching 0.06). As for width, the Sites:Side factor shows no significant difference, but the Sites:Sex factor is significant (P-value < 0.05). Finally For Area and Perimeter, neither the Sites:Side nor Sites:Sex factors show significant differences.

4.3. Analysis of Otolith Fluctuating Asymmetry

Wilcoxon analysis was used to estimate asymmetries in otolith size (length, width, area, perimeter) at various study sites (Supplementary Appendix **Table S2**). The results show that there are disparities between right and left (**Figure 4**) otolith lengths in the Lake Nokoué and Cotonou lagoons ($p < 0.05$), while the Porto-Novo (0.071) and Atlantic coast (0.480) lagoons show no significant difference. Lake Nokoué and the Atlantic coast reveal a significant fluctuating asymmetry in width ($p < 0.05$), suggesting significant variations between these sites. On the other hand, no significant difference was found in the Porto-Novo (0.899) and Cotonou lagoons (0.422). For otolith surface area, Lake Nokoué,

Table 2. Linear mixed model on biometric data.

Otolith parameters		sum_sq	df	F	P-value
Length	Sites:Side	1.844	3.0	1.493	2.153e-01
	Sites:Sex	2.657	3.0	2.485	0.060
Width	Sites:Side	0.676	3.0	1.783	1.492e-01
	Sites:Sex	8.726e-01	3.0	2.761	0.041
Area	Sites:Side	35.607	3.0	1.181	3.161e-01
	Sites:Sex	6.667e+01	3.0	2.488	6.003e-02
Perimeter	Sites:Side	35.607	3.0	1.181	3.161e-01
	Sites:Sex	1.583e+01	3.0	2.045	0.107

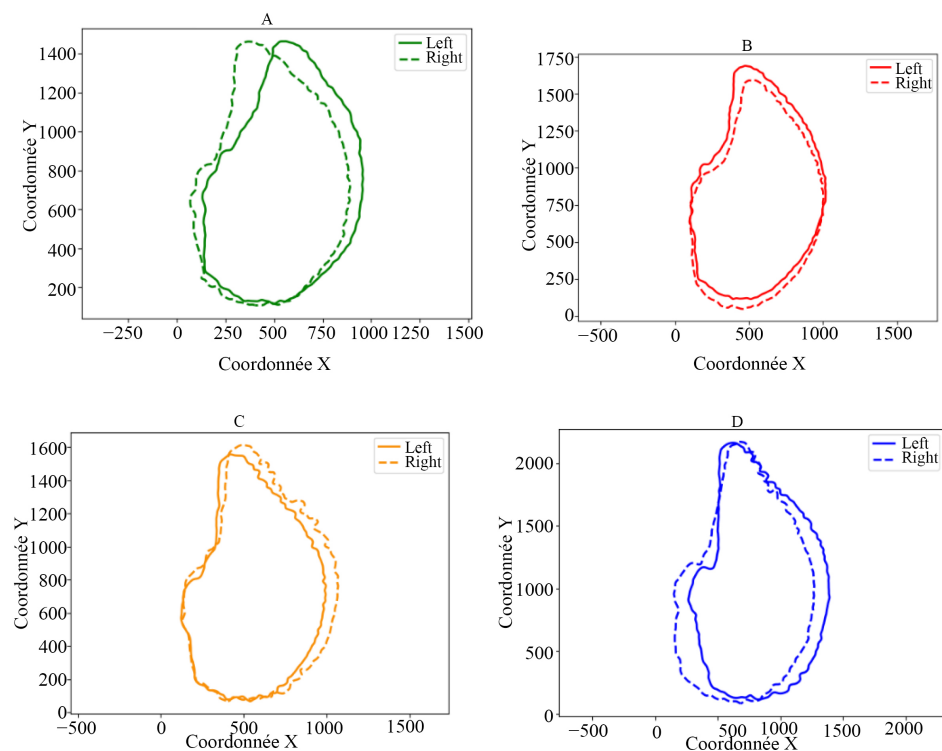


Figure 4. Difference between the mean shapes of the reconstructed left and right otoliths at each station (A: Porto Novo Lagoon, B: Cotonou Lagoon, C: Lake Nokoué, D: Atlantic coast).

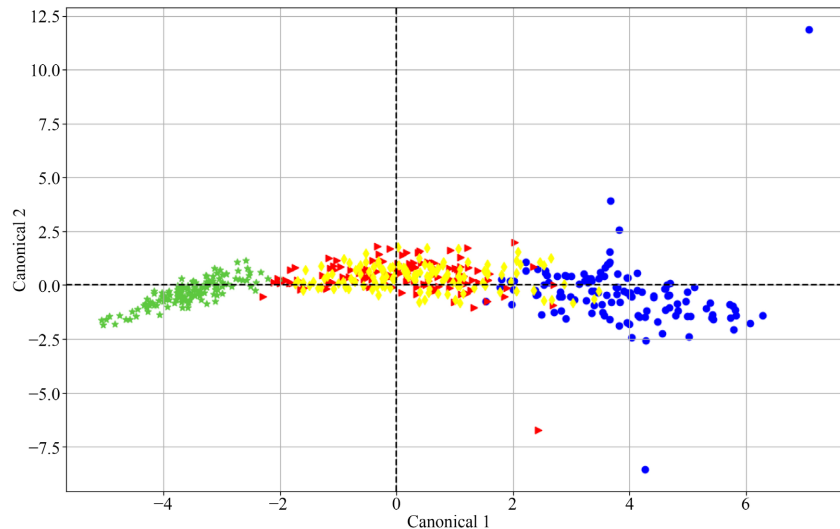


Figure 5. Canonical Discriminant Analysis with the first two canonical variables of the biometric parameters for 4 classes (Lake Nokoué (green), Porto-Novo Lagoon (yellow), Cotonou Lagoon (red), Atlantic Coast (blue)).

Cotonou Lagoon and the Atlantic coast showed significant fluctuating asymmetry ($p < 0.05$), highlighting differences between these sites.

Analysis of Otolith Characteristic Variations

ANOVA results indicate that otolith length, width and area are variables that significantly differ between groups ($p < 0.05$). Width and area appear to be particularly important in discriminating between groups as a function of the independent variable. Perimeter also shows a significant but less pronounced difference (Supplementary Appendix **Table S3**).

The graphical representation of the first two canonical variables of the biometric parameters (**Figure 5**) offers a significant visualization of the complex relationships between these variables. The scattering of points in the graph indicates a grouping of the two lagoon environments.

The absence of a statistically significant Mantel correlation (-0.20) with a p -value greater than 0.05 suggests that no clear association was identified between the Mahalanobis distances of otolith biometric parameters and geographical differences. In other words, spatial variation in otolith biometric characteristics does not appear to be closely related to the geographical differences examined in this analysis.

4.4. Otolith Shape Analysis

The results of the analysis of variance (ANOVA) reveal that height, weight and side have significant effects ($p < 0.05$) on otolith shape variation, while gender shows no significant difference ($p = 0.31$). The various interactions between these variables are presented in the table, along with F-statistics, explained variability and corresponding p -values.

The interaction between gender and sites shows no significant difference ($p =$

0.233). For the interaction between side and sites, although the p-value is slightly above the traditional threshold ($p = 0.084$), it indicates a trend towards significance. Finally, the interaction between length and sites shows ($p > 0.05$), also suggesting a trend towards significance, albeit just above the standard threshold.

These results suggest subtle relationships between the biological characteristics studied and otolith shape (**Table 3**).

The MANOVA analysis (Supplementary Appendix **Table S4**) suggests that there are significant differences between the different sites ($p < 0.0001$). The graphical representation of the first two canonical variables of the morphological parameters (**Figure 6**) offers a significant visualization of the complex relationships in otolith shape at each site. The dispersion of points in the graph clearly indicates a distinct grouping of the two lagoon environments, the lake environment and the marine environment.

The Mantel correlation between the mahalanobis distance of morphological parameters and geographic distance is -0.34 , with a $p > 0.05$. This correlation is not statistically significant, meaning that there is no clear association detected between the two data sets. In other words, there is no significant linear relationship between the observations of the two datasets.

Table 3. Mixed linear shape model.

	sum_sq	df	F	P-value
Sex:Sites	35.732	3.0	1.427	0.233
Side:Sites	54.591	3.0	2.225	0.084
Length:Sites	61.469	3.0	2.470	0.060

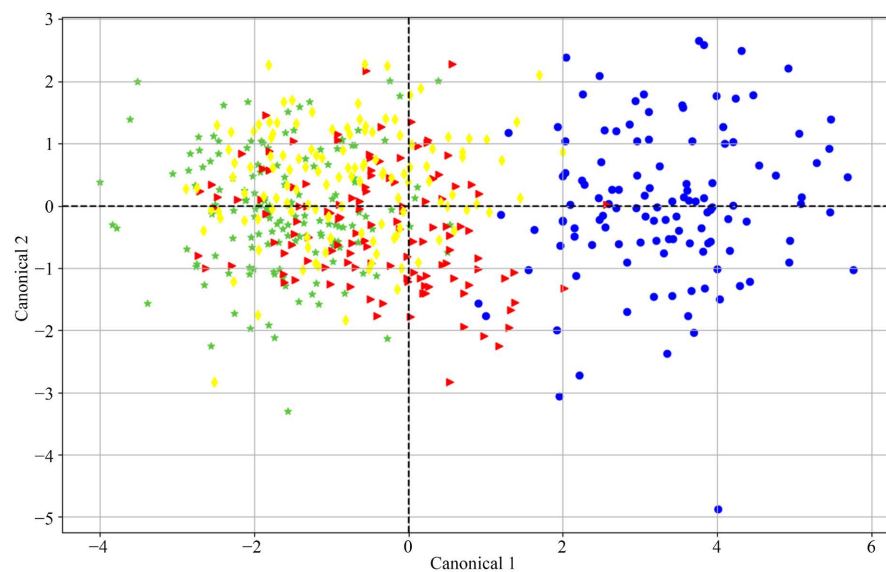


Figure 6. Canonical Discriminant Analysis with the first of two canonical otolith shape variables for 4 classes (Lake Nokoué (green), Porto-Novo Lagoon (yellow), Cotonou Lagoon (red), Atlantic Coast (blue)).

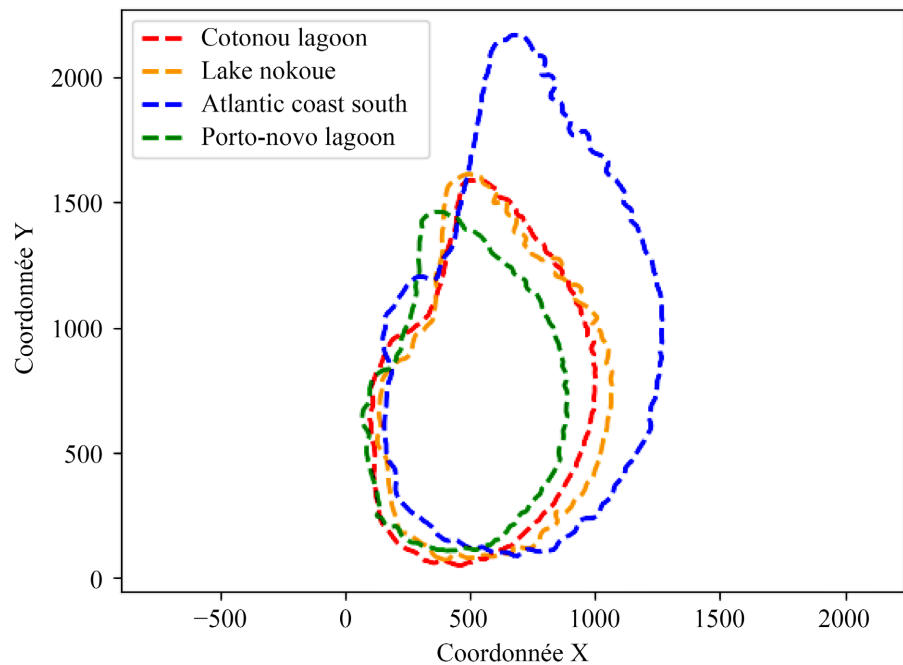


Figure 7. Difference in mean shapes of reconstructed right otoliths between the four sites used for *Elops lacerta* identification.

Dendrogram analysis based on Euclidean distance reveals (Supplementary Appendix **Figure S1**) distinct groupings between the sites studied. More specifically, the Porto lagoon and Lake Nokoué are grouped on the same branch, indicating a higher morphometric similarity between these two sites. The Cotonou lagoon follows this grouping, showing an affinity with the Porto lagoon and Lake Nokoué. In contrast, the sea is separated from these three sites, forming a distinct branch, highlighting significant differences in otolith morphology between marine and lagoon environments. This organization of the dendrogram suggests structured relationships between sites in terms of otolith morphometric variability.

The lowest Width/Length ratio is associated with the “Atlantic Sea” class, with a value of 0.59. Otoliths from the Porto Novo lagoon were closer to those from Lake Nokoué, which in turn were closer to those from the Cotonou lagoon (**Figure 7**). The amplitudes of directional asymmetry at all sites, measured as the percentage of non-overlapping area between right and left otolith shape, averaged 6.793%, 6.921, 5.627% and 5.181% respectively for *E. lacerta* individuals sampled on the southern Atlantic coast, in Lake Nokoué and in the Cotonou and Porto-Novo lagoons). These mean values show a significant directional asymmetry for *E. lacerta*.

5. Discussion

The present study has shown that the *Elops lacerta* population differs by geographical area. Overall, the results are coherent with those of previous studies on pelagic fish species [45] [46]. The results of our analysis reveal substantial varia-

tion in the relationship between otolith morphology and fish biological parameters at different study sites, demonstrating the importance of taking local variability into account in these aquatic ecosystems.

The linear relation observed between fish length and weight in the Porto-Novo lagoon and Lake Nokoué suggests a significant correlation between these two parameters at these sites. In contrast, the southern Atlantic coast shows a weak, non-significant relation. These differences could be attributed to variations in environmental conditions, such as the availability of food resources [47]. The significant variation in the influence of otolith biometric parameters on the total fish length between sites suggests a differentiated morphological response of the *Elops lacerta* species to the local environment.

This first study of the morphology of *Elops lacerta* otoliths at different sites in Benin provides crucial information on ecological relationships in these aquatic ecosystems. Analysis of the asymmetry between the right and left sides revealed that the differences observed could be linked to specific environmental conditions, such as the availability of food resources, water quality or other ecological factors [6]-[52]. This type of asymmetry is very common in biology, and represents developmental instability, *i.e.* the inability of an organism to produce a regular phenotype under several stress conditions [50] [51] [52] [53]. Fluctuating asymmetry, defined as random deviations from perfect symmetry between the left and right otoliths, has been reported for some species of roundfish and flatfish [22]-[56]. The significant variations in fluctuating otolith asymmetry between sites underline the morphological plasticity of the *Elops lacerta* species. These differences could be influenced by local selective pressures, such as predation, competition or site-specific environmental conditions [19]. However, the fluctuating asymmetry detected within each site for any biometric parameter (length, width, area, perimeter) could be explained by the vulnerability of this trait to immediate changes in environmental factors, such as pollution, extreme physical conditions and habitat quality [57] [58] [59]. In the same context, some works have published the presence of a fluctuating asymmetry between the two sides of the otoliths, right and left, and have suggested this variability to numerous biotic and abiotic factors and explained that these factors can be a tool to measure developmental instability [60] [61].

In biology, symmetry in bilaterally symmetrical organisms such as vertebrates is so consistent that it is generally regarded as the norm, supported by homeostatic processes [62]. Symmetry between the left and right otoliths, showing the two strictly similar parts of the vestibular system, has been observed in *Gadus morhua* [7]-[63], *Coryphaena hippurus* [64], *Xiphias gladius* [65], *Scomber scombrus* [8], *Melanogrammus aeglefinus* [12], *Mullus barbatus* [12], *Clupea harengus* [61] and *Lutjanus kasmira* [65]. Directional asymmetry has been measured for several fish species. This directional asymmetry of otoliths, showing lateralisation, was observed for the round fish *Liza ramada* [66], *Diplodus annularis* [67], *Scomberomorus niphonius* [68] and *Merlangius merlangus* [61] *Solea solea* [9], *Pleuronectes platessa* [12], *Limanda limanda* [61] and *Lepi-*

dorhombus whiffiagonis [60]. This study identified a directional asymmetry in the shape of the otoliths of *Elops lacerta*. Sex, weight, size and their interaction were analyzed to assess sources of uncertainty and reveal actual differences in otolith shape between the four sampling sites. The results obtained suggest its utility as a stock differentiation tool. The results of the otolith shape analysis suggest its potential use as a stock differentiation tool. Asymmetry in otolith shape was observed both within and between populations. These results showed the significant impact of size, weight and side on variation in otolith shape, suggesting that factors intrinsic to the fish, such as its size or weight, could play a crucial role in determining otolith morphology [6]. Volpedo and Echeverria (2003), have also confirmed this hypothesis. There is no difference in otolith shape between the sexes. This result has already been proven in Atlantic mackerel *Scomber scombrus* [8], Atlantic cod (*Gadus morhua* [49]) and blue whiting (*Micromesistius poutassou* [42]). Differences in environmental conditions can have a considerable influence on otolith growth and, consequently, on the shape of the otoliths formed [69]. The shape of sagittas is conservative enough and reflects many life histories to be considered specific [70] [71]. Its capacity for morphological variability is considerable and strongly influenced by biotic and abiotic parameters [72] [73]. In addition, the composition of the diet in terms of essential polyunsaturated fatty acid concentration affects endolymph composition and thus generates shape variability. The composition of the alimentary bolus explains otolith shape variation better than the amount of food ingested by the animal [61]. The importance of otolith shape analysis goes beyond differentiation, as it also enables us to understand the events that influence the life history of each individual [73] [74]. Mentel's test indicates that spatial variability in otolith characteristics does not necessarily follow geographical differences. This could be due to active fish migration or to local adaptations independent of geography.

In conclusion, this study contributes significantly to the discrimination of *Elops lacerta* sampled at different sites. The asymmetry found between the right and left otoliths validates previous research findings on stock distinction, using otolith morphology and biometric parameters. Understanding the variability of morpho-biological relationships is crucial for aquatic resource management.

6. Conclusion

This study makes a significant contribution to the discrimination of *Elops lacerta* populations at different sites. The asymmetries observed between otoliths validate the potential use of morphology and biometric parameters to differentiate stocks. Understanding the variability of morpho-biological relationships is essential for effective management of aquatic resources.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Villanueva, M.C. (2004) Biodiversity and Trophic Relationships in Some Estuarine and Lagoon Environments of West Africa: Adaptations to Environmental Pressures. Ph.D. Thesis, Institut national polytechnique de Toulouse, Toulouse.
- [2] Ben Bouih, H., Nassali, H., Leblans, M. and Srhiri, A. (2004) Contamination en métaux traces des sédiments du lac Fouarat. *Afrique Science: Revue Internationale des Sciences et Technologie*, **1**, 109-125. <https://doi.org/10.4314/afsci.v1i1.35403>
- [3] Chao, L.N. (1978) A Basis for Classifying Western Atlantic Sciaenidae (Teleostei: Perciformes). NOAA Technical Report NMFS Circular.
- [4] Mahé, K., Ider, D., Massaro, A., Hamed, O., Jurado-Ruzafa, A., Goncalves, P., *et al.* (2019) Directional Bilateral Asymmetry in Otolith Morphology May Affect Fish Stock Discrimination Based on Otolith Shape Analysis. *ICES Journal of Marine Science*, **76**, 232-243. <https://doi.org/10.1093/icesjms/fsy163>
- [5] Bolles, K.L. and Begg, G.A. (2000) Distinctions between Silver Hake (*Merluccius bilinearis*) Stocks in U.S. Waters of the Northwest Atlantic Based on Whole Otolith Morphometric. *Fishery Bulletin*, **98**, 451-462.
- [6] Campana, S.E. and Casselman, J.M. (1993) Stock Discrimination Using Otolith Shape Analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, **50**, 1062-1108. <https://doi.org/10.1139/f93-123>
- [7] Cardinale, M., Doering-Arjes, P., Kastowsky, M. and Mosegaard, H. (2004) Effects of Sex, Stock, and Environment on the Shape of Known-Age Atlantic Cod (*Gadus morhua*) Otoliths. *Canadian Journal of Fisheries and Aquatic Sciences*, **61**, 158-167. <https://doi.org/10.1139/f03-151>
- [8] Castonguay, M., Simard, P. and Gagnon, P. (1991) Usefulness of Fourier Analysis of Otolith Shape for Atlantic Mackerel (*Scomber scobrus*) Stock Discrimination. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 296-302. <https://doi.org/10.1139/f91-041>
- [9] Merigot, B., Letourneur, Y. and Lecomte-Finiger, R. (2007) Characterization of Local Populations of the Common Sole *Solea solea* (Pisces, Soleidae) in the NW Mediterranean through Otolith Morphometrics and Shape Analysis. *Marine Biology*, **151**, 997-1008. <https://doi.org/10.1007/s00227-006-0549-0>
- [10] Mejri, M., Trojette, M., Allaya, H., Ben Faleh, A., Jmil, I. and Chalh, A. (2018) Use of Otolith Shape to Differentiate Two Lagoon Populations of *Pagellus erythrinus* (Actinopterygii: Perciformes: Sparidae) in Tunisian Waters. *Acta Ichthyologica et Piscatoria*, **48**, 153-161. <https://doi.org/10.3750/AIEP/02376>
- [11] Begg, G.A. and Brown, R.W. (2000) Stock Identification of Haddock *Melanogram-*

- mus aeglefinus* on the Georges Bank Based on Otolith Shape Analysis. *Transactions of the American Fisheries Society*, **129**, 935-945. [https://doi.org/10.1577/1548-8659\(2000\)129<0935:SIOHMA>2.3.CO;2](https://doi.org/10.1577/1548-8659(2000)129<0935:SIOHMA>2.3.CO;2)
- [12] Mille, T., Mahe, K., Villanueva, M.C., De Pontual, H. and Ernande, B. (2015) Sagittal Otolith Morphogenesis Asymmetry in Marine Fishes. *Journal of Fish Biology*, **87**, 646-663. <https://doi.org/10.1111/jfb.12746>
- [13] Lombarte, A. and Lieonart, J. (1993) Otolith Size Changes Related with Body Growth, Habitat Depth and Temperature. *Environmental Biology of Fishes*, **37**, 297-306. <https://doi.org/10.1007/BF00004637>
- [14] Ben Labidi, M., Mejri, M., Shahin, A., Quignard, J.P., Trabelsi, M. and Ben Faleh, A. (2020) Asymetrie fluctuante des otolithes dans les *Boops boops* (actinopterygii, sparidae) de deux stations marines (bizerte et kelibia) dans les eaux tunisiennes. *Journal of the Marine Biological Association of the United Kingdom*, **100**, 2-16. <https://doi.org/10.1017/S0025315420001022>
- [15] Paxton, J.R. (2000) Fish Otoliths: Do Sizes Correlate with Taxonomic Group, Habitat and/or Luminescence? *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, **355**, 1299-1303. <https://doi.org/10.1098/rstb.2000.0688>
- [16] Tuset, V.M., Lombarte, A., Gonzalez, J.A., Pertusa, J.F. and Lorente, M.J. (2003) Comparative Morphology of the Sagittal Otolith in *Serranus* spp. *Journal of Fish Biology*, **63**, 1491-1504. <https://doi.org/10.1111/j.1095-8649.2003.00262.x>
- [17] Lombarte, A. and Cruz, A. (2007) Otolith Size Trends in Marine Fish Communities from Different Depth Strata. *Journal of Fish Biology*, **71**, 53-76. <https://doi.org/10.1111/j.1095-8649.2007.01465.x>
- [18] Stransky, C. and MacLellan, S.E. (2005) Species Separation and Zoogeography of Redfish and Rockfish (Genus *Sebastes*) by Otolith Shape Analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 2265-2276. <https://doi.org/10.1139/f05-143>
- [19] Hüssy, K. (2008) Otolith Shape in Juvenile Cod (*Gadus morhua*): Ontogenetic and Environmental Effects. *Journal of Experimental Marine Biology and Ecology*, **364**, 35-41. <https://doi.org/10.1016/j.jembe.2008.06.026>
- [20] Lleonart, J., Salat, J. and Torres, G.J. (2000) Removing Allometric Effects of Body Size in Morphological Analysis. *Journal of Theoretical Biology*, **205**, 85-93. <https://doi.org/10.1006/jtbi.2000.2043>
- [21] Downhower, J.F., Blumer, L.S., Lejeune, P., Gaudin, P., Marconato, A. and Bisazza, A. (1990) Otolith Asymmetry in Cottus Bairdi and Cottus Gobio. *Polskie Archiwum Hydrobiologii*, **37**, 209-220.
- [22] Lemberget, T. and McCormick, M.I. (2009) Replenishment Success Linked to Fluctuating Asymmetry in Larval Fish. *Oecologia*, **159**, 83-93. <https://doi.org/10.1007/s00442-008-1212-x>
- [23] Díaz-Gil, C., Palmer, M., Catalan, I.A., Alos, J., Fuiman, L.A., Garcia, E., Del Mar Gil, M., *et al.* (2015) Otolith Fluctuating Asymmetry: A Misconception of Its Biological Relevance? *ICES Journal of Marine Science*, **72**, 2079-2089. <https://doi.org/10.1093/icesjms/fsv067>
- [24] Green, A.A., Mosaliganti, K.R., Swinburne, I.A., Obholzer, N.D. and Megason, S.G. (2017) Recovery of Shape and Size in a Developing Organ Pair. *Developmental Dynamics*, **246**, 451-465. <https://doi.org/10.1002/dvdy.24498>
- [25] Bird, J.L., Eppler, D.T. and Checkley Jr., D.M. (1986) Comparison of Herring Otoliths Using Fourier Series Shape Analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, **43**, 1228-1234. <https://doi.org/10.1139/f86-152>

- [26] Begg, G.A. and Brown, R.W. (2000) Stock Identification of Haddock *Melanogrammus aeglefinus* on the Georges Bank Based on Otolith Shape Analysis. *Transactions of the American Fisheries Society*, **129**, 935-945.
[https://doi.org/10.1577/1548-8659\(2000\)129<0935:SIOHMA>2.3.CO;2](https://doi.org/10.1577/1548-8659(2000)129<0935:SIOHMA>2.3.CO;2)
- [27] Adams, A.J., Horodysky, A.Z., McBride, R.S., Guindon, K., Shenker, J., MacDonald, T.C., *et al.* (2013) Global Conservation Status and Research Needs for Tarpons (Megalopidae), Ladyfishes (Elopidae) and Bonefishes (Albulidae). *Fish and Fisheries*, **15**, 280-311.
- [28] Levesque, J. (2010) Habitat Use Patterns of Juvenile Ladyfish (*Elopes saurus*) in Florida (USA) Estuaries. *Wildlife Biology in Practice*, **6**, 39-56.
<https://doi.org/10.2461/wbp.2010.6.4>
- [29] Agboola, J.I. and Anetekhai, M.A. (2008) Length-Weight Relationships of Some Fresh and Brackish Water Fishes in Badagry Creek, Nigeria. *Journal of Applied Ichthyology*, **24**, 623-625. <https://doi.org/10.1111/j.1439-0426.2008.01079.x>
- [30] Abowei, J.F.N. (2010) The Condition Factor, Lengthweight Relationship and Abundance of *Elops senegansensis* (Regan, 1909) from Nkoro River, Niger Delta, Nigeria. *Advance Journal of Food Science and Technology*, **2**, 16-21.
- [31] Ugwumba, O.A. (1989) Distribution and Growth Pattern of the Ten-Pounder (Val.) in the Freshwater, Estuarine and Marine Environments of Lagos, Nigeria. *Archiv für Hydrobiologie*, **115**, 451-462.
<https://doi.org/10.1127/archiv-hydrobiol/115/1989/451>
- [32] Aceves-Medina, G., Jim Enez-Rosenberg, S.P.A. and Hinojosa-Medina, A. (2003) Fish Larvae from the Gulf of California. *Scientia Marina*, **67**, 1-11.
<https://doi.org/10.3989/scimar.2003.67n11>
- [33] Tzeng, W.N., Chen, H.L. and Teng, H.Y. (2005) The Recruitment Dynamic of *Elops hawaiiensis* Leptocephalus in Gongshyuan Creek Estuary of Northern Taiwan. *Journal of the Fisheries Society of Taiwan*, **32**, 129-130.
- [34] Pandare, D. and Tamoikine, M.Y. (1997) Etude de l'ichtyoplancton dans les eaux cotieres et estuariennes bordees de mangroves en afrique de l'ouest, guineeesen egal. In: Kjerfve, B., Drude De Lacerda, L. and El Hadji Salif, D., Eds., *Mangrove Ecosystem Studies in Latin America and Africa*, UNESCO, Paris, 241-263.
- [35] Hie-Dare, J.P. (1982) Croissance de la phase lagunaire de *Elops lacerta*. Documents Scientifiques, Centre de Recherches Océanologiques, Abidjan (Côte d'Ivoire).
- [36] Albaret, J.J. (1987) Les peuplements de poissons de la casamance (senegal) en periode de secheresse. *Revue d'hydrobiologie tropicale*, **20**, 291-310.
- [37] Lestrel, P.E. (1997) Fourier Descriptors and Their Applications in Biology. Cambridge University Press, Cambridge.
<https://doi.org/10.1017/CBO9780511529870>
- [38] Andrialovanirina, N., Caillault, É.P., Couette, S., Laffont, R., Poloni, L., Lutet-Toti, C. and Mahé, K. (2023) Asymmetry of Sagittal Otolith Shape Based on Inner Ear Side Tested on Mediterranean Red Mullet (*Mullus barbatus* Linnaeus, 1758): Comparative Analysis of 2D and 3D Otolith Shape Data. *Symmetry*, **15**, Article 1067.
<https://doi.org/10.3390/sym15051067>
- [39] Kuhl, F. and Giardina, C. (1982) Elliptic Fourier Features of a Closed Contour. *Computer Graphics and Image Processing*, **18**, 236-258.
[https://doi.org/10.1016/0146-664X\(82\)90034-X](https://doi.org/10.1016/0146-664X(82)90034-X)
- [40] Cadrin, S.X. and Friedland, K.D. (1999) The Utility of Image Processing Techniques for Morphometric Analysis and Stock Identification. *Fisheries Research*, **43**, 129-139. [https://doi.org/10.1016/S0165-7836\(99\)00070-3](https://doi.org/10.1016/S0165-7836(99)00070-3)

- [41] Rohlf, F.J. and Archie, J.W. (1984) A Comparison of Fourier Methods for the Description of Wing Shape in Mosquitoes (Diptera: Culicidae). *Systematic Biology*, **33**, 302-317. <https://doi.org/10.2307/2413076>
- [42] MahE, K., Evano, H., Mille, T., Muths, D. and Bourjea, J. (2016) Otolith Shape as a Valuable Tool to Evaluate the Stock Structure of Swordfish *Xiphias gladius* in the Indian Ocean. *African Journal of Marine Science*, **38**, 457-464. <https://doi.org/10.2989/1814232X.2016.1224205>
- [43] Allam, N. (2018) Application de l'analyse discriminante lineaire (lda) dans la reconnaissance du visage. Memoire de fin D'etude.
- [44] Boschetti, A. and Massaron L. (2016) Python Data Science Essentials. 2nd Edition, Springer, Dordrecht.
- [45] Moreira, C., Froufe, E., Vaz-Pires, P. and Correia, A.T. (2019) Otolith Shape Analysis as a Tool to Infer the Population Structure of the Blue Jack Mackerel, *Trachurus picturatus*, in the NE Atlantic. *Fisheries Research*, **209**, 40-48. <https://doi.org/10.1016/j.fishres.2018.09.010>
- [46] Muniz, A.A., Moura, A., Triay-Portella, R., Moreira, C., Santos, P.T. and Correia, A.T. (2020) Population Structure of the Chub Mackerel (*Scomber colias*) in the North-East Atlantic Inferred from Otolith Shape and Body Morphometrics. *Marine and Freshwater Research*, **72**, 341-352. <https://doi.org/10.1071/MF19389>
- [47] Ndour, F., Le Loc'H, O.T., Thiaw, J.M. and Laë, R. (2011) Étude du régime alimentaire de deux espèces de Cichlidae en situation contrastée dans un estuaire tropical inverse d'Afrique de l'Ouest (Casamance, Sénégal) [Study of the Diet of Two Species of Cichlidae in Contrasting Situation in a West African (Casamance, Senegal) Inverse Tropical Estuary]. *Journal des Sciences Halieutiques et Aquatiques*, **4**, 120-133.
- [48] Torres, G.J., Lombarte, A. and Morales-Nin, B. (2000) Sagittal Otolith Size and Shape Variability to Identify Geographical Intraspecific Differences in Three Species of Genus *Merluccius*. *Journal of the Marine Biological Association of the United Kingdom*, **80**, 333-342. <https://doi.org/10.1017/S0025315499001915>
- [49] Cardinale, M., Doerin-Arjes, P., Kastowsky, M. and Mosegaard, H. (2004) Effects of Sex, Stock, and Environment on the Shape of Known-Age Atlantic Cod (*Gadus morhua*) Otoliths. *Canadian Journal of Fisheries and Aquatic Sciences*, **61**, 158-167. <https://doi.org/10.1139/f03-151>
- [50] Gagliano, M. and McCormick, M.I. (2004) Feeding History Influences Otolith Shape in Tropical Fish. *Marine Ecology Progress Series*, **278**, 291-296. <https://doi.org/10.3354/meps278291>
- [51] Swan, S.C., Geffen, A.J., Morales-Nin, B., Gordon, J.D.M., Shimmield, T., Sawyer, T. and Massuti, E. (2006) Otolith Chemistry: An Aid to Stock Separation of *Helicolenus dactylopterus* (Bluemouth) and *Merluccius merluccius* (European Hake) in the Northeast Atlantic and Mediterranean. *ICES Journal of Marine Science*, **63**, 504-513. <https://doi.org/10.1016/j.icesjms.2005.08.012>
- [52] Vignon, M. and Morat, F. (2010) Environmental and Genetic Determinant of Otolith Shape Revealed by a Non-Indigenous Tropical Fish. *Marine Ecology Progress Series*, **411**, 231-241. <https://doi.org/10.3354/meps08651>
- [53] Grønkaer, P. and M.K. (2003) Fluctuating Asymmetry and Nutritional Condition of Baltic Cod (*Gadus morhua*) Larvae. *Marine Biology*, **143**, 191-197. <https://doi.org/10.1007/s00227-003-1064-1>
- [54] Lychakov, D.V. and Rebane, Y.T. (2005) Fish Otolith Mass Asymmetry: Morphometry and Influence on Acoustic Functionality. *Hearing Research*, **201**, 55-69.

- <https://doi.org/10.1016/j.heares.2004.08.017>
- [55] Lychakov, D.V., Rebane, Y.T., Lombarte, A., Demestre, M. and Fuiman, L.A. (2008) Saccular Otolith Mass Asymmetry in Adult Flatfishes. *Journal of Fish Biology*, **72**, 2579-2594. <https://doi.org/10.1111/j.1095-8649.2008.01869.x>
- [56] Lychakov, D. (2013) Behavioral Lateralization and Otolith Asymmetry. *Journal of Evolutionary Biochemistry and Physiology*, **49**, 441-456. <https://doi.org/10.1134/S0022093013040099>
- [57] Møller, A.P. and Shykoff, J.A. (1999) Morphological Developmental Stability in Plants: Patterns and Causes. *International Journal of Plant Sciences*, **160**, S135-S146. <https://doi.org/10.1086/314219>
- [58] Cañas, L., Stransky, C., Schlickeisen, J., Sampedro, M.P. and Fariña A.C. (2012) Use of the Otolith Shape Analysis in Stock Identification of Anglerfish (*Lophius piscatorius*) in the Northeast Atlantic. *ICES Journal of Marine Science*, **69**, 250-256. <https://doi.org/10.1093/icesjms/fss006>
- [59] Al-Rasady, I.H., Jawad, L.A., Al-Mamry, J.M., Al-Mamari, H.M., Al-Yarubi, M. and Mamary, D.S. (2010) Fluctuating Asymmetry in the Otolith Length and Width of Rhynchorhamphus Georgi (Valenciennes, 1846) (Family: Hemiramphidae) Collected from the Sea of Oman. *Museo Civico di Storia Naturale—Ferrara*, **13**, 85-89.
- [60] Jawad, L.A. and Al-Mamry, J.M. (2012) Relationship between Fish Length and Otolith Dimensions in the Carangid Fish (*Carangoides Coeruleopinnatus* (Rüppell, 1830)) Collected from the Sea of Oman. *Journal of FisheriesSciences.com*, **6**, 203-208.
- [61] Mille, T. (2015) Sources de variation intra-populationnelle de la morphologie des otolithes: Asymétrie directionnelle et régime alimentaire. Master's Thesis, Université de Lille, Villeneuve-d'Ascq.
- [62] Palmer, A.R. (2009) Animal Asymmetry. *Current Biology*, **19**, 473-477. <https://doi.org/10.1016/j.cub.2009.04.006>
- [63] Petursdottir, G., Begg, G.A. and Marteinsdottir, G. (2006) Discrimination between Icelandic Cod (*Gadus morhua* L.) Populations from Adjacent Spawning Areas Based on Otolith Growth and Shape. *Fisheries Research*, **80**, 182-189. <https://doi.org/10.1016/j.fishres.2006.05.002>
- [64] Duarte-Neto, P., Lessa, R., Stosic, B. and Morize, E. (2008) L'utilisation d'otolithes sagittaux dans la discrimination des stocks de coryphènes communes (*Coryphaena hippurus*) au large du nord-est du Brésil à l'aide de descripteurs multiformes *ICES Journal of Marine Science*, **65**, 1144-1152. <https://doi.org/10.1093/icesjms/fsn090>
- [65] Mahé, K., Oudard, C., Mille, T., Keating, J., Goncalves, P., Worsoe Clausen, L., *et al.* (2016) Identifying Blue Whiting (*Micromesistius poutassou*) Stock Structure in the Northeast Atlantic by Otolith Shape Analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, **73**, 1-10. <https://doi.org/10.1139/cjfas-2015-0332>
- [66] Rebaya, M., Ben Faleh, A.R., Allaya, H., Khedher, M., Trojette, M., Marsaoui, B., Fatnassi, M., Chalh, A., Quignard, J.P. and Trabelsi, M. (2017) Otolith Shape Discrimination of *Liza ramada* (Actinopterygii: Mugiliformes: Mugilidae) from Marine and Estuarine Populations in Tunisia. *Acta Ichthyologica et Piscatoria*, **47**, 13-21. <https://doi.org/10.3750/AIEP/02006>
- [67] Trojette, M., Ben Faleh, A., Fatnassi, M., Marsaoui, B., Mahouachi, N.H., Chalh, A., Quignard, J.P. and Trabelsi, M. (2015) Stock Discrimination of Two Insular Populations of *Diplodus annularis* (Actinopterygii: Perciformes: Sparidae) along the Coast of Tunisia by Analysis of Otolith Shape. *Acta Ichthyologica et Piscatoria*, **45**,

- 363-372. <https://doi.org/10.3750/AIP2015.45.4.04>
- [68] Zhang, C., Ye, Z., Li, Z., Wan, R., Ren, Y. and Dou, S. (2016) Population Structure of Japanese Spanish Mackerel *Scomberomorus niphonius* in the Bohai Sea, the Yellow Sea and the East China Sea: Evidence from Random Forests Based on Otolith Features. *Fisheries Science*, **82**, 251-256. <https://doi.org/10.1007/s12562-016-0968-x>
- [69] Campana, S.E. and Neilson, J.D. (1985) Microstructure of Fish Otoliths. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**, 1014-1032. <https://doi.org/10.1139/f85-127>
- [70] Abée-Lund, J.H. (1988) Otolith Shape Discriminates between Juvenile Atlantic Salmon, *Salmo salar* L., and Brown Trout, *Salmo trutta* L. *Journal of Fish Biology*, **33**, 899-903. <https://doi.org/10.1111/j.1095-8649.1988.tb05538.x>
- [71] Tuset, V.M., Rosin, P.L. and Lombarte, A. (2006) Sagittal Otolith Shape Used in the Identification of Fishes of the Genus *Serranus*. *Fisheries Research*, **81**, 316-325. <https://doi.org/10.1016/j.fishres.2006.06.020>
- [72] Monteiro, L.R., Di Benedetto, A.P.M., Guillermo, L.H. and Rivera, L.A. (2005) Allometric Changes and Shape Differentiation of Sagittal Otoliths in Sciaenid Fishes. *Fisheries Research*, **74**, 288-299. <https://doi.org/10.1016/j.fishres.2005.03.002>
- [73] Reichenbacher, B., Feulner, G.R. and Schulz-Mirbach, T. (2009) Geographic Variation in Otolith Morphology among Freshwater Populations of *Aphanius dispar* (Teleostei, Cyprinodontiformes) from the Southeastern Arabian Peninsula. *Journal of Morphology*, **270**, 469-484. <https://doi.org/10.1002/jmor.10702>
- [74] Campana, S.E. and Thorrold, S.R. (2001) Otoliths, Increments, and Elements: Keys to a Comprehensive Understanding of Fish Populations. *Canadian Journal of Fisheries and Aquatic Sciences*, **58**, 30-38. <https://doi.org/10.1139/f00-177>

Appendix

Table S1. Regression results for biometric data.

Species	Variable	R-Squared	P-Value
1) Lagoon Cotonou	Length	0.312310	1.528003e-11
2) Lagoon Cotonou	Width	0.288971	1.214285e-10
3) Lagoon Cotonou	Area	0.336841	1.607686e-12
4) Lagoon Cotonou	Perimeter	0.348560	5.330115e-13
5) Lagoon Porto-Novo	Length	0.212284	4.435396e-08
6) Lagoon Porto-Novo	Width	0.225754	1.453738e-08
7) Lagoon Porto-Novo	Area	0.292954	4.215640e-11
8) Lagoon Porto-Novo	Perimeter	0.271582	2.852656e-10
9) Lake Nokoué	Length	0.477551	2.474792e-22
10) Lake Nokoué	Width	0.579334	3.039971e-29
11) Lake Nokoué	Area	0.686779	1.248478e-38
12) Lake Nokoué	Perimeter	0.557913	1.162805e-27
13) Atlantique Sea	Length	0.054956	2.295220e-02
14) Atlantique Sea	Width	0.092842	2.826567e-03
15) Atlantique Sea	Area	0.130784	3.418303e-04
16) Atlantique Sea	Perimeter	0.086244	4.068379e-03

Table S2. P-value of asymmetry fluctuating between right and left.

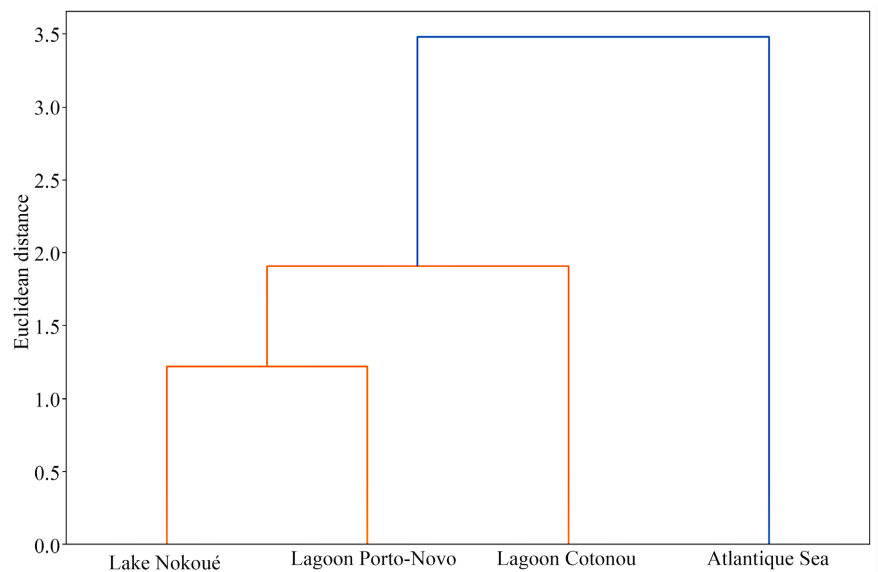
SITES	P VALUE			
	LN	LP	LC	AS
Length	0.0009 ***	0.071	0.007***	0.480
Width	3.66e-08***	0.899	0.422	7.57e-05***
Area	1.79e-12***	0.859	0.018**	0.0002***
Perimeter	4.21e-08***	0.373	0.0003***	0.005***

Table S3. ANOVA results for otolith length, width and surface area between groups.

Parameter	Wilks L.	Partial L.	F	P-Value
Length	0.106	0.977	3.95	<0.0001
Width	0.119	0.865	26.57	<0.0001
Area	0.119	0.869	25.757	<0.0001
Perimeter	0.105	0.978	3.75	<0.0001

Table S4. MANOVA of principal components.

	Value	DF	F Value	P Value
Wilks' Lambda	0.19	39.00	27.77	<0.0001
Pillai's Trace	0.87	39.00	16.12	<0.0001
Hotelling-Lawley Trace	3.64	39.00	47.01	<0.0001
Roy's Greatest Root	3.54	13.00	137.87	<0.0001

**Figure S1.** Dendrogram of studied sites.