Scientific Research Publishing

Open Journal of Marine Science, 2024, 14, 41-62
https://www.scirp.org/journal/ojms
ISSN Online: 2161-7392
ISSN Print: 2161-7384

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

Madel Floriane Adjibayo Houeto[1*], Marwa Mejri[1], Malek Tazarki[1], Marius Sounouvou[2], Nicolas Andrialovanirina[3], Meriem Ben Ghorbel[1], Pierre Dossou-Yovo[4], Abdellah Chalh[5], Pierre Quignard[6], Monia Trabelsi[1], Kélig Mahé[3]

[1]Laboratory of Ecology, Biology and Physiology of Aquatic Organisms, Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia
[2]Institute of Mathematics and Physical Sciences, University of Abomey-Calavi (UAC), Abomey-Calavi, Benin
[3]IFREMER, Unit HMMN, Halieutic Resources Laboratory, Boulogne-sur-Mer, France
[4]Laboratory for Research into the Processing and Conservation of Fishery Products (LAREPROH), University of Abomey-Calavi (UAC), Abomey-Calavi, Benin
[5]Unit of Population Genetics and Biological Resources, Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia
[6]Laboratoire d'Ichtyologie, Université Montpellier II, Montpellier, France
Email: *houetofloi@gmail.com

How to cite this paper: Houeto, M.F.A., Mejri, M., Tazarki, M., Sounouvou, M., Andrialovanirina, N., Ben Ghorbel, M., Dossou-Yovo, P., Chalh, A., Quignard, P., Trabelsi, M. and Mahé, K. (2024) Fluctuating Asymmetry of Elopes lacerta (Valenciennes, 1847) Otoliths in the Western African Waters. *Open Journal of Marine Science*, **14**, 41-62.
https://doi.org/10.4236/ojms.2024.143003

Received: February 22, 2024
Accepted: June 7, 2024
Published: June 11, 2024

Abstract

The importance of this study lies in the in-depth exploration of the ecological diversity of otoliths in *Elopes 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, *Elopes 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 environmental constraints.

Elopes 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 km^2, 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, linking 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 *Elopes 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 ± 0.1 cm) and the total weight (W ± 0.1 g) were measured. (**Table 1**)

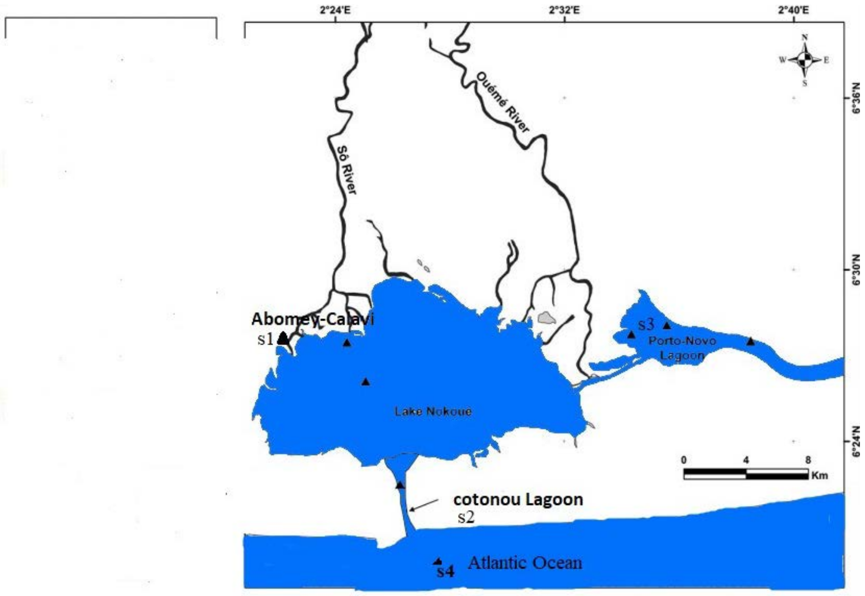

Figure 1. Map of *Elopes lacerta* sampling sites (S1: Lake Nokoué; S2: Cotonou Lagoon, S3 Porto-Novo Lagoon, S4 Atlantic coast in southern Benin).

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 ± CV	23.0 ± 2.87	25.0 ± 3.13	22.65 ± 1.91	35.36 ± 1.83
	max	31.0	33.4	28.2	39.2
	min	16.6	19.2	18.0	30.0
Total Weight (g)	Mean ± CV	60.5 ± 24.8	74.2 ± 31.3	53.7 ± 15.2	256.7 ± 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

$$F\left(nk\right)=\sum\nolimits_{k=0}^{nk}\frac{Ai^{2}i+Bi^{2}+Ci^{2}+Di^{2}}{2}$$

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 $F\left(nk\right)$ 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

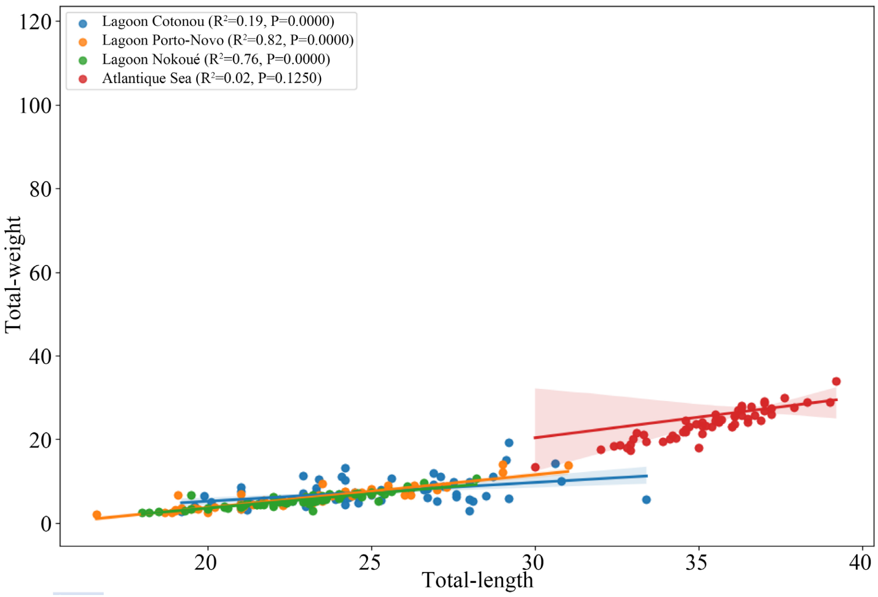

Figure 2. Regression showing the correlation between a fish total length (TL) and weight (W).

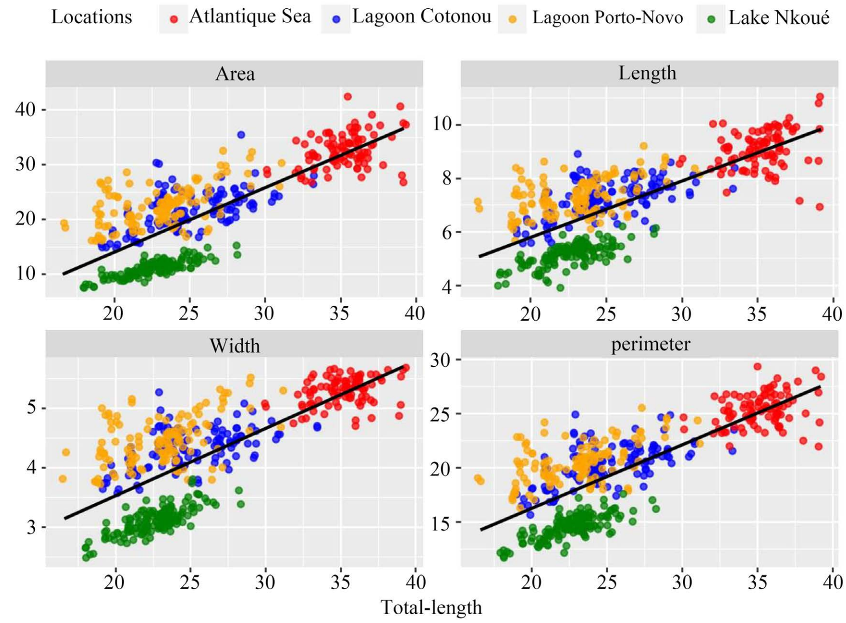

Figure 3. Regression showing the correlation between fish size and otolith morphometrics (A: Area; B: Length, C: Width, D: Perimeter).

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

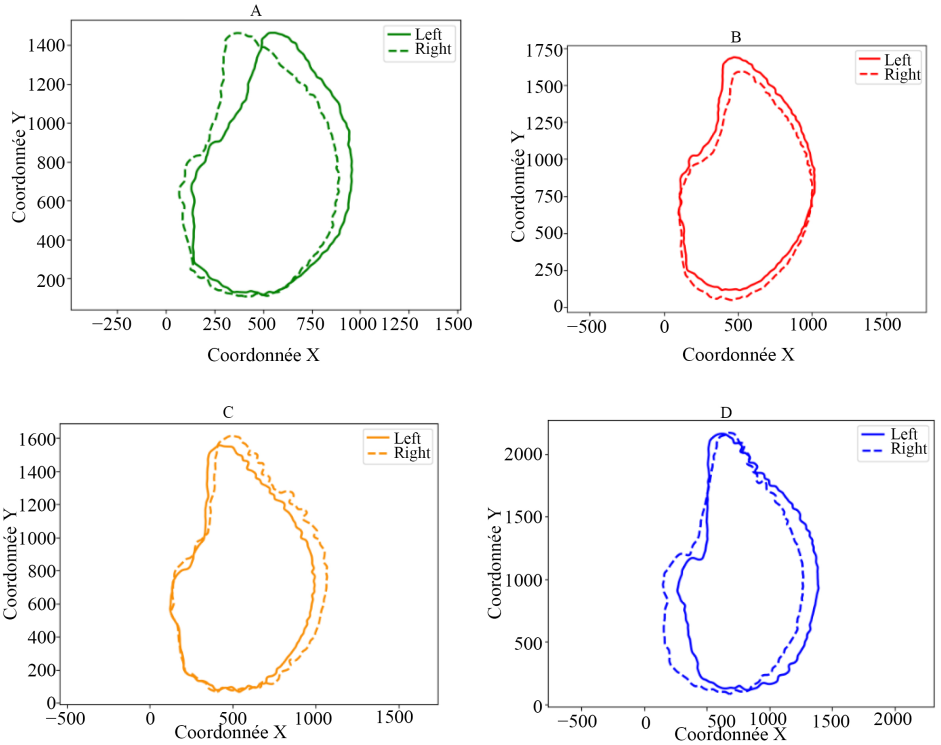

Figure 4. Difference between the mean shapes of the reconstructed left and right otoliths at each station (A: Porto Novo Lagoon, B: Cotonou Lagoon, C: Lake Nokoué, D: Atlantic coast).

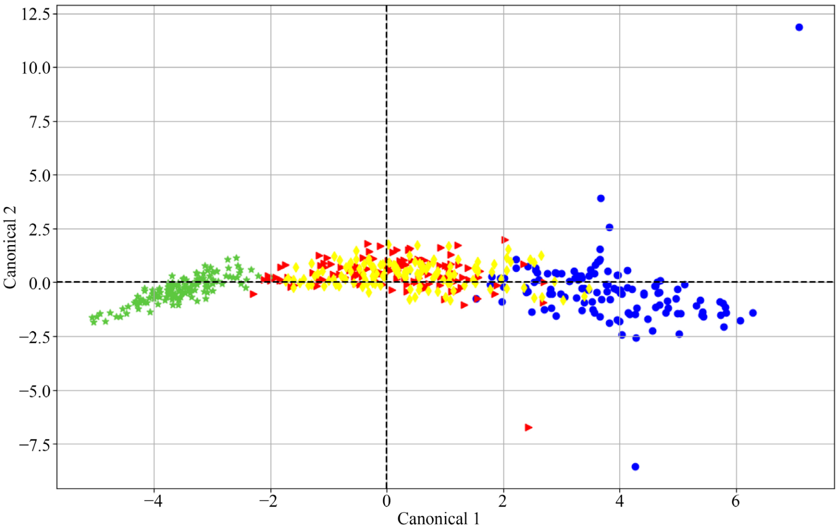

Figure 5. Canonical Discriminant Analysis with the first two canonical variables of the biometric parameters for 4 classes (Lake Nokoué (green), Porto-Novo Lagoon (yellow), Cotonou Lagoon (red), Atlantic Coast (blue)).

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

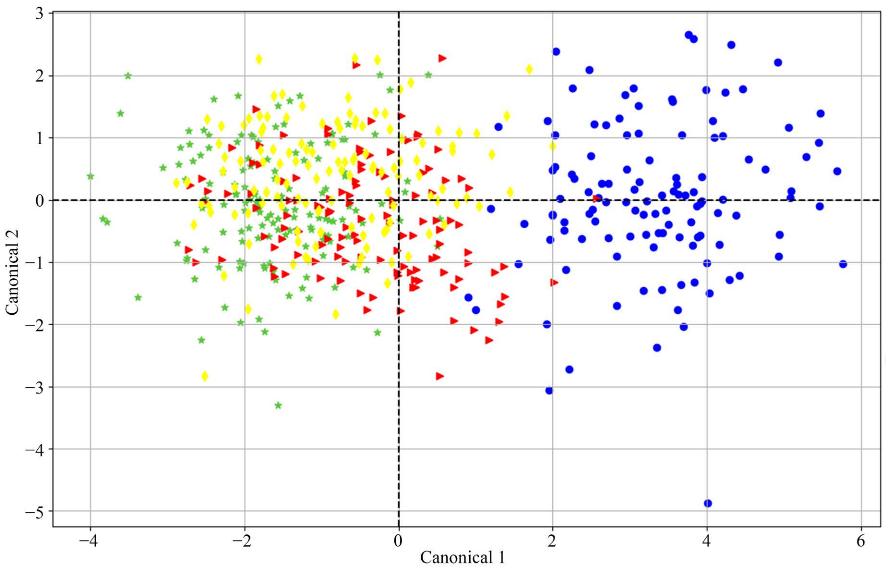

Figure 6. Canonical Discriminant Analysis with the first of two canonical otolith shape variables for 4 classes (Lake Nokoué (green), Porto-Novo Lagoon (yellow), Cotonou Lagoon (red), Atlantic Coast (blue)).

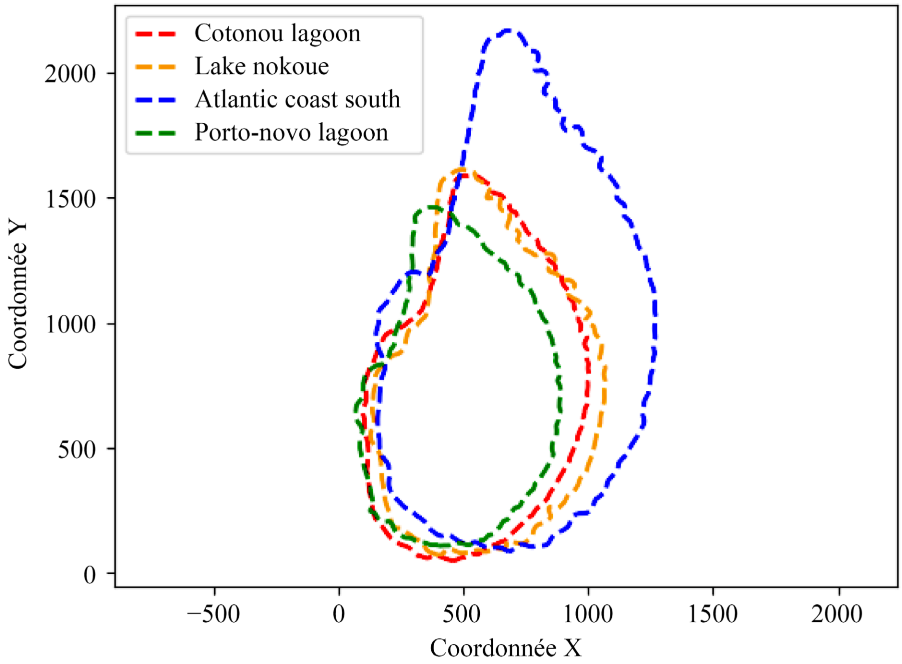

Figure 7. Difference in mean shapes of reconstructed right otoliths between the four sites used for *Elopes lacerta* identification.

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 *Elopes 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 variation 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 *Elopes lacerta* species to the local environment.

This first study of the morphology of *Elopes 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 *Elopes 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 *Elopes 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 *Elopes 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 *Elopes 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.

Acknowledgements

I would like to express my sincere gratitude to Dr. Kakpo Césaire, CEO of K-

POLYGONE multinational, for the financial support you provided for my research work. Your interest and encouragement were essential elements that enriched this experience.

This work has benefited also from the grant "ANR-21-EXES-0011" as part of the IFSEA graduate school (which originates from the National Research Agency under the Investments for the Future program).

Conflicts of Interest

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

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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.

	P VALUE			
SITES	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

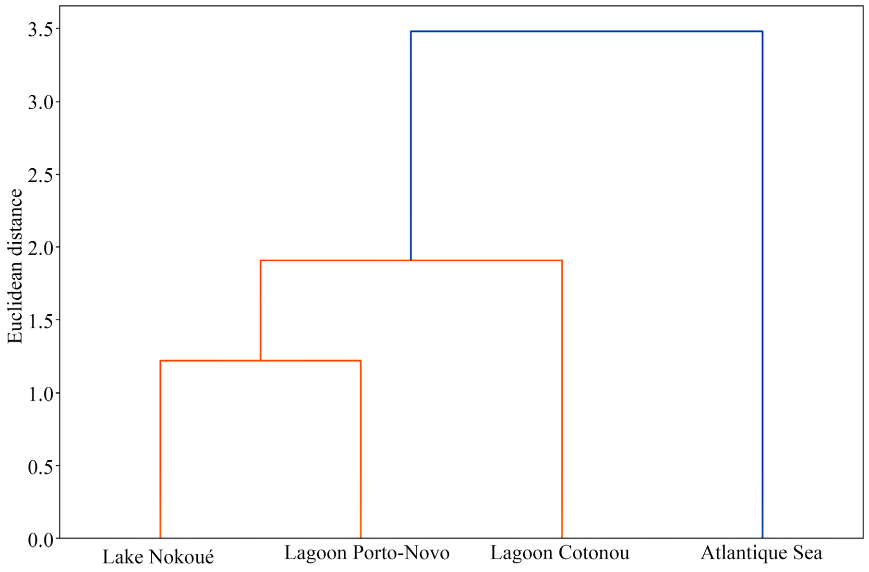

Figure S1. Dendrogram of studied sites.