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Analysis of Otolith Shape as a Tool for Discriminating Stocks of Cassava Croaker (*Pseudotolithus senegalensis*) in Beninese Waters

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Abstract

This study examined the right and left otoliths of 174 individuals of *Pseudotolithus senegalensis* from the Porto-Novo lagoon, Lake Nokoué and the Atlantic coast. The results show a significant variation in the population according to geographic location. Mixed-effects linear analysis showed no significant variation by site, side or sex ($p > 0.05$). Analysis of otolith shape using ANOVA showed significant differences for length, width and area ($p < 0.05$), but not for perimeter ($p > 0.05$). Canonical discriminant analysis revealed significant differences between sites ($p < 0.05$). Finally, shape analyses showed significant differences between sites ($p = 0.0001$) and between right and left sides ($p = 0.007$), but no difference by sex ($p = 0.395$). The study of otolith morphology is therefore proving to be a valuable tool for differentiating stocks and understanding ecological variations.

Keywords

Otoliths, *Pseudotolithus senegalensis*, Factorial Analysis, Porto-Novo Lagoon, Atlantic Coast and Lake Nokoué

1. Introduction

Total local production from marine, river, lagoon and lake fisheries is insufficient to meet the demand of the Beninese population. Artisanal fisheries mainly catch species with a high market value, such as Sciaenidae (21.75%), Polynemidae (8.77%), Lutjanidae (5.52%) and Sparidae (4.07%) [1]. The Sciaenidae are a diverse and abundant family of fish closely related to snappers, but they are distinguished by a short spiny dorsal fin and much longer adipose fin than anal fin, which has only one or two spines [2]. This family includes a large diversity of fishes, such as curvins, drum fishes, meagre fishes and weak fishes. It comprises around 70 genera and 270 species, 14 of which are sampled along the Gulf of Guinea, on the coast of West Africa [2]. The genus *Pseudotolithus senegalensis* (Valenciennes, 1833), belonging to the family Sciaenidae, represents an abundant and economically important group of fish in the coastal waters of Benin [3]-[5], and along the Atlantic coast of West Africa [6].

In tropical waters, it is particularly difficult to determine the age of fish from otoliths because of the similarity of the seasons throughout the year. The otolith is a mineral formation found in the vestibular organ of the inner ear. The sensory structures of the inner ear include the utricle, saccule and *lagena*, with individual otoliths: the *lapillus*, *sagitta* and *astericus* [7]. These otoliths are metabolically inert, meaning that they cannot be modified or generally resorbed [8]. Several studies have exploited otoliths as tools in the field of fisheries science [9]. These formations are considered to be natural archives, permanently preserving information about the environment, past climate and the life history of the individual. Their form has proved useful in fish age studies [10]-[12], ontogenetic processes [13] and the spatial and temporal migrations of fish [14].

Sagittal otoliths show relatively higher inter- and intra-specific variability in terms of shape and size. This makes them effective for identifying fish species [15]-[17], populations [18] and stocks [12]-[20]. In addition, genetic and exogenous factors such as water temperature, salinity, depth and food supply have some influence on otolith morphological variability [21]-[23]. Our aim here is to assess the morphometric and morphological variability of *P. senegalensis* otoliths in lagoon and marine environments.

2. Materials and Methods

2.1. Sampling

This comparative study was carried out at three different locations: the Porto-Novo lagoon, Lake Nokoué and the coast of Benin on the Atlantic Ocean (**Figure 1**). The Porto-Novo lagoon, covering an area of approximately 35 km^2, is located in south-east Benin between latitudes 6˚25' and 6˚30' North and longitudes 2˚30' and 2˚38' East. Lake Nokoué lies between latitudes 6˚20' and 6˚30' North and longitudes 2˚20' and 2˚35' East. These two environments are receptacles for the waters of the Ouémé and Sô rivers, and they communicate with each other, especially during the low-water period when Atlantic tides are strong. The coast of the Atlantic, south of Benin, comprises various bodies of water in communication with the sea, located between 1˚20' and 3˚00' East longitude and 3˚00' and 6˚40' North latitude. The sample totaled 174 individuals of *Pseudotolithus senegalensis*, distributed as follows: 50 from the Porto-Novo Lagoon, 62 from Lake Nokoué, 62 from the Atlantic coast (**Table S1**). Each individual was sampled with measures of total length (TL ± 0.1 cm) and total weight (W ± 0.1 g).

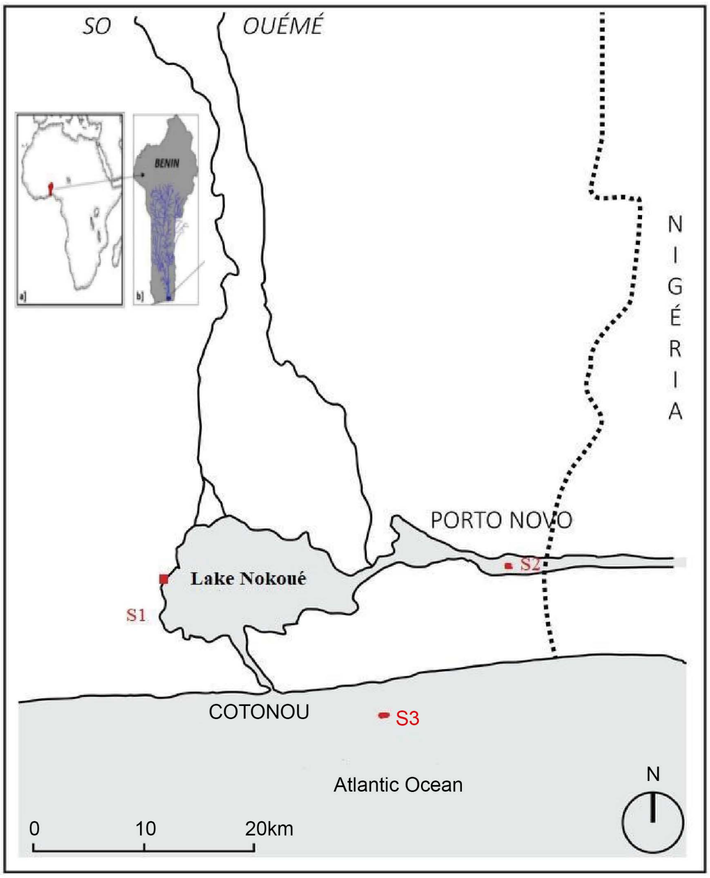

Figure 1. Map of *Pseudotolithus senegalensis* sampling locations (S1: Lake Nokoué; S2: Porto-Novo Lagoon, S3: Atlantic coast).

2.2. Extraction of the Otolith and Image Processing

Extraction of each pair of otoliths (the left and right sagittal otoliths) was performed by cutting off the head and then removing the gills. The otoliths were collected with forceps, cleaned with distilled water, then dried and preserved in Eppendorf tubes. The species name, area, and date of sampling were noted on a label adhered to the tube. Otolith images were acquired using 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.

2.3. Otoliths Shape Analysis

The otolith outline was described using Fourier elliptic analysis [24] 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 [25]. Elliptical Fourier analysis [24] was carried out on each otolith outline 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 [26]. 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): n_k harmonics (*i.e.*, the proportion of variance in the contour coordinates explained by the k

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

where Ai, Bi, Ci and Di are the harmonic coefficients and n_k is the total number of harmonics included. The value of n_k was chosen so that $F(n_k)$ explains 99.99% of the variance in contour coordinates, *i.e.* it reconstructs the shape with 99.99% accuracy [24]. 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 [27] [28].

2.4. Statistical Analysis

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

$$\text{Total Weight} = \text{K} \times \text{Total Length}^{\text{b}}$$

The parameters of such a model are estimated by linear regression on data that have undergone a log-log transformation:

$$\text{Log (Total Weight)} = \text{Log(K)} + \text{b} \times \text{Log(Total Length)}.$$

Three types of descriptors were analyzed, including otolith size parameters (Length: Lo; Width: Wo; Perimeter: Po; Area: Ao), shape indices (Ellipticity and Aspect Ratio), and EFDs. The analysis focused on the asymmetry between left and right otolith shapes, examining the impact of 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 were produced to visualize data distributions. Principal component analysis (PCA) was applied to an otolith size matrix and the Elliptical Fourier Descriptor (EFD) matrix [29], enabling reduction of the data size of EFD matrix while retaining as much information as possible, and acquisition of a subset of the principal components. The selected principal components (PCs) can be used as shape descriptors of the otolith in the analysis [30], where 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 [30]. Each cell of the matrix represents the value of the descriptor for a given otolith. For each pair of otoliths, the Euclidean distance was then 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 (Length; Width; Perimeter; Area). 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. For a better estimate of the divergences between samples, multivariate analyses were performed treating all traits simultaneously. Linear discriminant analysis (LDA) is a statistical analysis commonly used for classification and dimension reduction [31]. 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 sex, side and sampling site. All statistical tests were performed using the following packages in a python environment Numpy [32], matplotlib [32], pyplot, Scikit-learn [32], Pandas, mapply, Plotnine, Plydata, statsmodels [32], seaborn, scipy [32].

3. Results

3.1. W-TL Relationship

The relationship between total weight and total length of fish was examined at different locations, including the Porto-Novo lagoon, Lake Nokoué and the coast of the Atlantic Ocean (Figure 2). The results showed significant differences in the length-weight relationship between the three sites studied. Lake Nokoué showed the most robust correlation, followed by the coast of the Atlantic Ocean, while the Porto-Novo lagoon showed the weakest correlation.

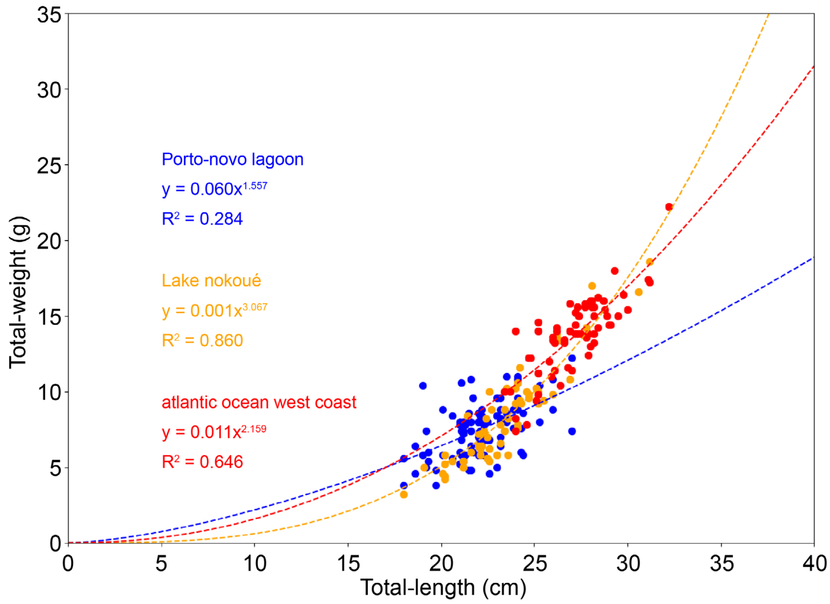

Figure 2. The length-weight relationship of *Pseudotolithus senegalensis* according to the sampling area, each point represents an individual fish.

3.2. TL-Otolith Measurements Relationship

The relationship between total fish length and the different otolith measurements varied considerably between locations (Table S2). Figure 3 shows various morphometric measurements (length, width, area, perimeter, aspect ratio and ellipticity) as a function of total length for different aquatic sites: Porto-Novo lagoon, Lake Nokoué and the coast of the Atlantic Ocean. The Porto-Novo lagoon shows the highest dispersion with weak or moderate exponential trends. Differences between right and left are minimal. As for Lake Nokoué, there are well-defined exponential trends in all the morphometric measurements, with clear differences between right and left. This suggests a stronger correlation between measurements and total length at this site. Finally, for the coast of the Atlantic Ocean, the clusters of points are more scattered and showed few clear trends, while the differences between right and left are also minimal.

3.3. Effect of Various Factors on the Biometric Parameters of Otoliths

The results of the linear mixed-effects analysis (Table 1) examining the effect of site, side and sex on different otolith measurements, such as length, width, area and perimeter, indicate that these otolith characteristics did not vary significantly according to location, side and sex ($p > 0.05$).

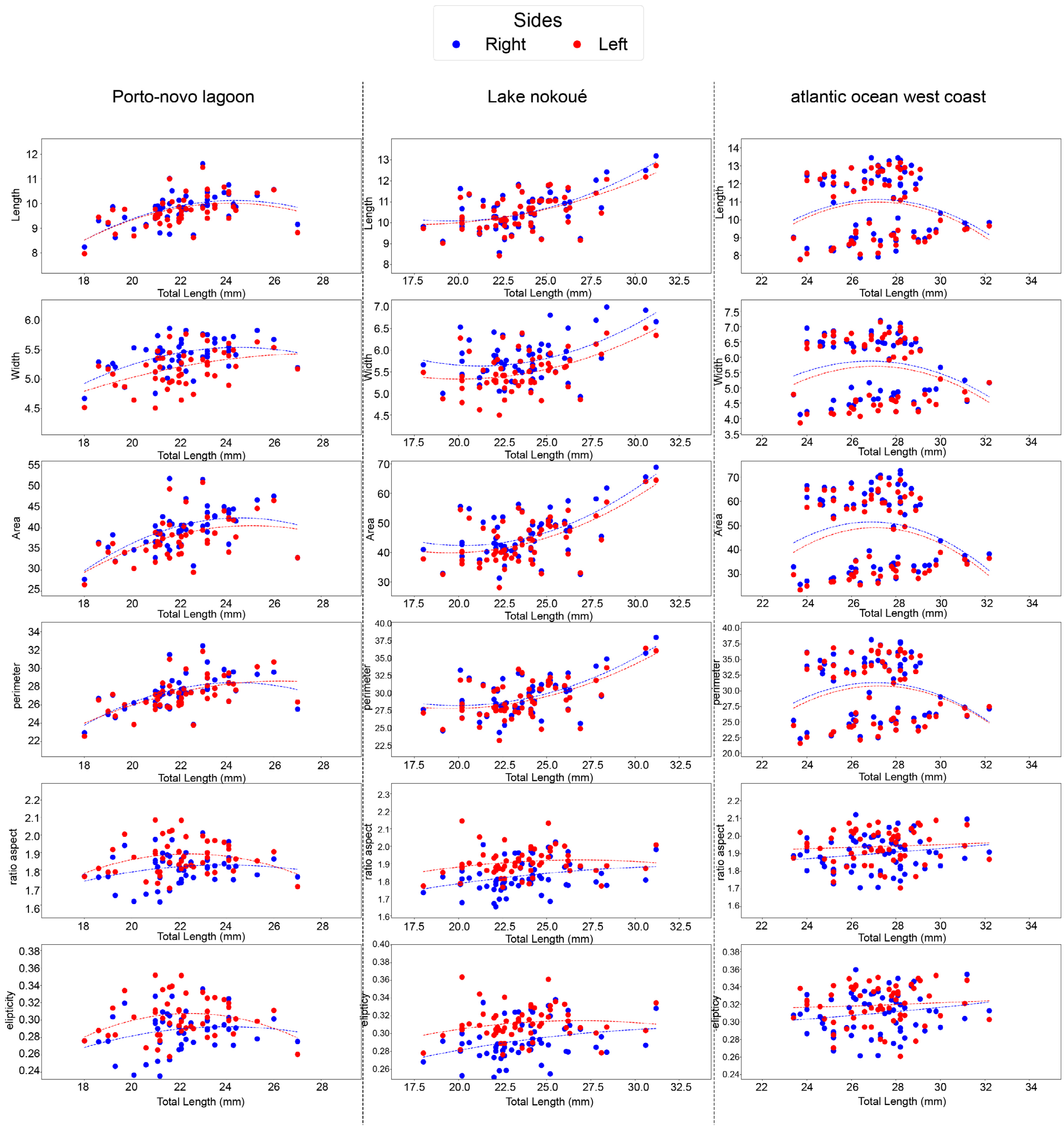

Figure 3. Regression showing the correlation between fish size and otolith morphometrics for right (blue) and left (red) otoliths.

Table 1. Results of linear mixed model by each otolith morphological descriptor with the interaction between location and inner ear side and sex of *Pseudotolithus senegalensis.*

Otolith Parameters		sum_sq	Df	F	P-value
Length	Location:Side	0.124	2	0.040	0.960
	Location:Sex	0.393	2	0.127	0.880
Width	Location:Side	0.147	2	0.153	0.857
	Location:Sex	0.189	2	0.193	0.823

Continued

Area	Location:Side	6.170	2	0.02	0.973
	Location:Sex	52.353	2	0.224	0.799
Perimeter	Location:Side	0.941	2	0.036	0.963
	Location:Sex	2.186	2	0.085	0.918

3.4. Analysis of Fluctuating Otolith Asymmetry

Analysis of the asymmetry percentages (Supplementary Appendix Table S3) by parameter and by site revealed interesting variations in otolith symmetry between the different localities studied (Figure 4). For otolith width, the Porto-Novo lagoon showed an average asymmetry percentage of 4.12%, while the coast of the Atlantic Ocean and Lake Nokoué showed slightly different values, with 3.60% and 4.85% respectively. There are also differences between locations in terms of otolith surface area. The Porto-Novo lagoon has an average asymmetry percentage of 4.66%, while the coast of the Atlantic Ocean has the highest level of asymmetry at 5.78%, and Lake Nokoué has an intermediate value of 4.58%. Similar trends were observed for otolith perimeter and length. The coast of the Atlantic Ocean has the largest percentage of asymmetry, followed closely by the Porto-Novo lagoon, while Lake Nokoué has the lowest values.

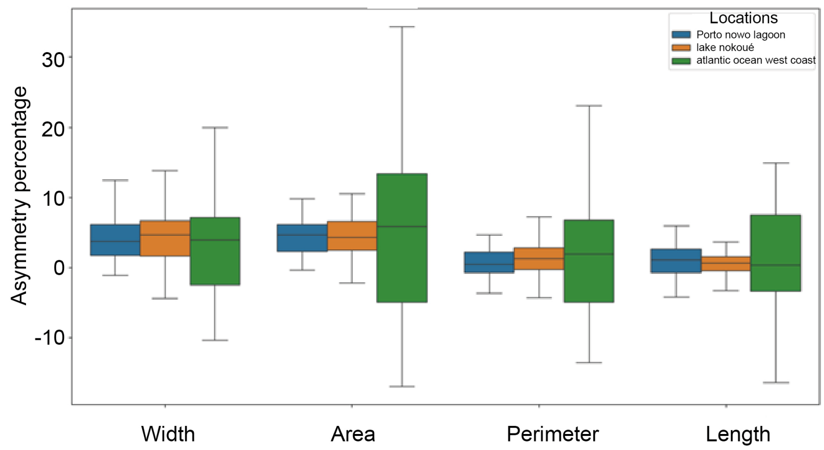

Figure 4. boxplot percentage asymmetry of otolith biometric parameters by each sampling location.

Wilcoxon analysis was used to estimate otolith size asymmetries (length, width, area, perimeter) in the different study locations (Table S4). The results showed that at the Atlantic Ocean coast, there were significant differences between the areas and widths of otoliths on the right and left sides ($p < 0.05$), but no significant differences were observed for length and perimeter ($p > 0.05$).

As for Porto-Novo Lagoon, the results showed significant differences in all otolith characteristics between the right and left sides ($p < 0.05$), suggesting a marked asymmetry in the otoliths of fish from this site. Finally, at Lake Nokoué, analyses indicated significant differences between the length, perimeter, width and area of otoliths on the right and left sides ($p < 0.05$), suggesting a pronounced asymmetry in the otoliths *Pseudotolithus senegalensis* of Lake Nokoué (**Figure 5**).

The results of the ANOVA indicate that otolith length, width and surface area are variables that differ significantly between groups ($p < 0.05$). The perimeter showed a p-value of 0.45, suggesting a non-significant relationship (Supplementary Appendix **Table S5**, Supplementary Appendix **Figure S1**).

3.5. Analysis of Otolith Shape

The results of the analysis of variance (ANOVA) reveal that the interaction of size, weight and side with site have significant effects ($p < 0.05$) on variation in otolith shape. The interaction between sex and site did not show a significant difference ($p > 0.05$). The different interactions between sex, fish length and fish weight as a function of site are presented in **Table 2**, with the corresponding F statistics, variability explained and p-values.

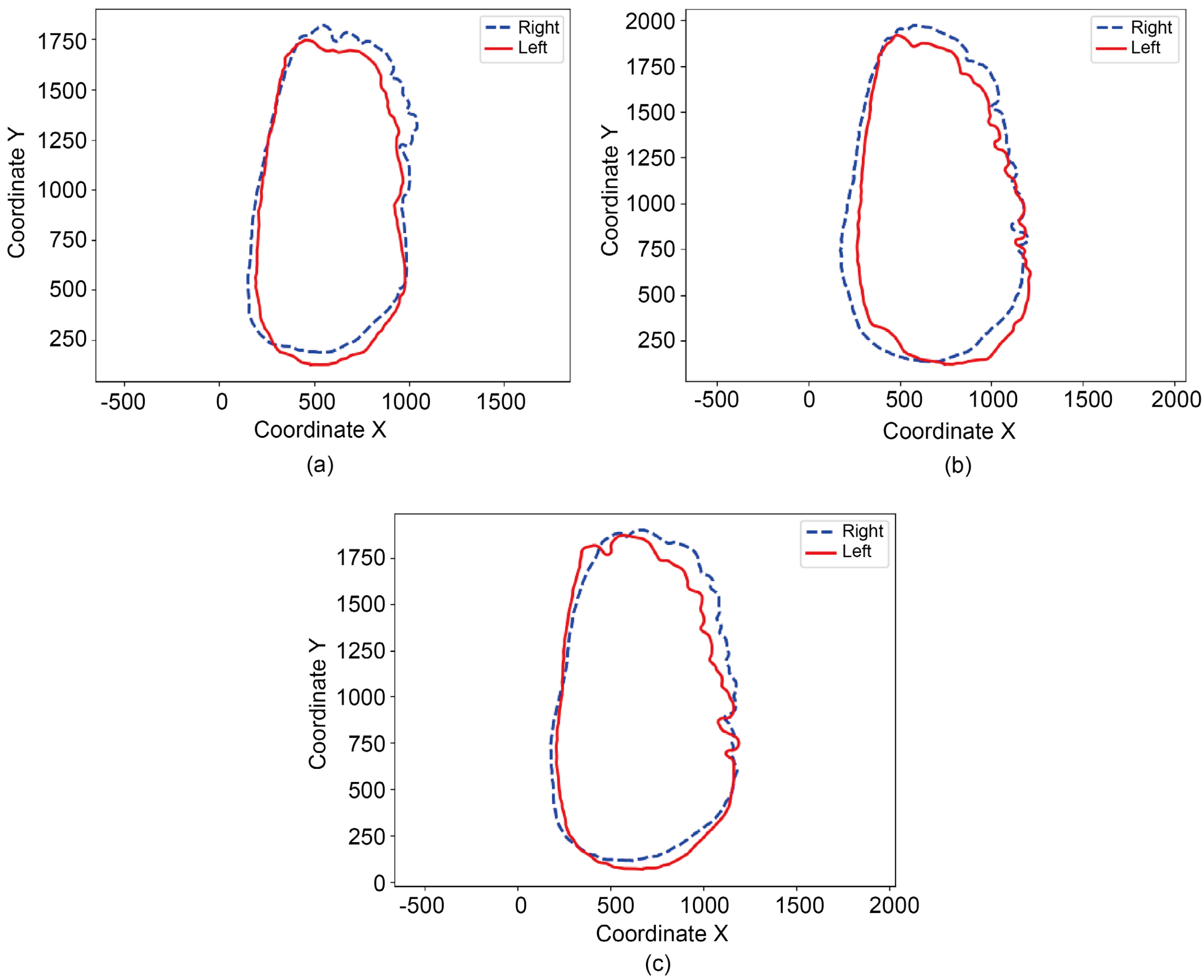

Figure 5. Difference between the mean shapes of the reconstructed left and right otoliths at each station ((a): Lake Nokoue, (b): Atlantic coast, (c): Porto-Novo Lagoon).

Table 2. Mixed linear shape model on otolith morphological parameters.

	sum_sq	df	F	P-value
Sex: locations	4.169e+04	3	1.257	0.285
Side: locations	1.420e+05	3	4.447	0.012
Length: locations	1.016e+05	3	3.859	0.046
Weight: locations	1.263e+05	3	3.859	0.022

The effect of sampling location on otolith shape was significant in the multivariate mixed-effects model (**Table S6**, **Table S7**), suggesting variation in otolith shape that could be used to discriminate between individuals from different stations. The multivariate mixed-effects model on the *S* shape matrix showed that there was a significant difference between left and right otoliths ($p < 0.05$) when considering sampling locations, but no difference between individuals according to sex ($p > 0.05$). After PCA on the Fourier elliptical descriptors (FED), the first two PCs performed (**Figure S2**) explained 82% of the total variance of the FED.

The lowest width/length ratio is associated with the "Atlantic coast" class, with a value of 0.52. Otoliths from the Porto Novo lagoon are closer to those from Lake Nokoué, which are themselves closer to those from the Porto Novo lagoon (**Figure 6**). The magnitudes of directional asymmetry at all locations, measured as the percentage of the surface not overlapping between the shape of the right otolith and that of the left otolith, were on average 10.78%, 4.56% and 4.53% respectively for *Pseudotolithus senegalensis* individuals sampled on the Atlantic coast, in Lake Nokoué and in the Porto-Novo lagoons). These mean values showed a significant directional asymmetry for *Pseudotolithus senegalensis*.

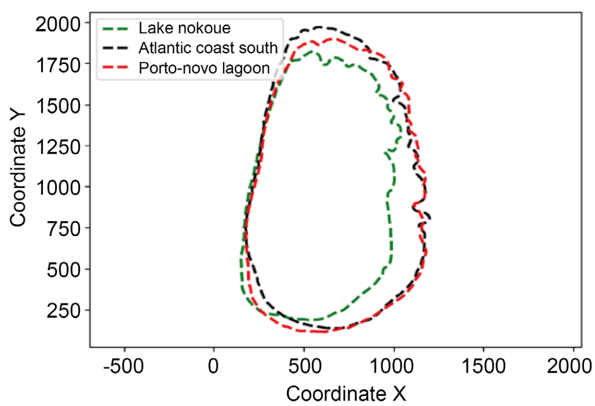

Figure 6. Difference in mean shapes of reconstructed right otoliths between the three locations used for *Pseudotolithus senegalensis* identification.

4. Discussion

4.1. Impact of the Weight-Length Relationship on the Population of *Pseudotolithus senegalensis* According to Sampling Location

The results of this study indicated that the population of *Pseudotolithus* *senegalensis* varies according to geographical area. The Porto-Novo lagoon and the Atlantic coast show cases of positive allometry, with different degrees of growth between the weight and length of the fish. Troadec (1971) and Sun (1975) drew similar conclusions concerning the length-weight relationships of *P. senegalensis* in Congo and Senegal respectively, showing positive allometric growth. This observation contrasts with the results of Sossoukpe *et al*. (2013) who found isometric growth for *P. senegalensis* in Beninese water bodies, but is similar to those of Adjigbe *et al*. (2023) also in Beninese water bodies. In fact, length-weight relationships do not remain uniform throughout the year, but show variations influenced by factors such as food availability, feeding rate, gonad development and the physiological state of the animal, as indicated by Santos *et al*. (2002).

4.2. Influence of Environmental Factors on Otolith Growth

The fluctuation in the relationship between total fish length and various otolith measurements across different geographical locations reveals complex dynamics, suggesting a strong influence on the specific geographical location. The results indicate that the relationship is most robust and significant in the Porto-Novo lagoon, with significant correlations for measures such as otolith length and width, as well as otolith area. This finding suggests that otolith growth and development are closely related to total fish length in this specific environment. However, this relationship loses its strength and significance in other locations such as Lake Nokoué and the coast of the Atlantic Ocean. In these regions, the correlation between total fish length and otolith characteristics is less significant or even weak. For example, although the relationship is significant for the otolith perimeter in the Porto-Novo lagoon, it is less marked in the other locations studied. According to Taylor 1985, given that calcium absorption and serum calcium levels are associated, there is a biological justification for associating changes in fish size with otolith growth. Conceptually, daily variations in fish size are associated with the amplitude of a period of $CaCO_3$ deposition (*i.e.* the area under a peak). Thus, otolith growth is determined by the period of deposition, and each period amplitude can be linked to the age and size of the fish. In all cases, the allometry is positive. This indicates that otolith characteristics increase in proportion to the total length of the fish. Such results have been published by several authors who have provided evidence of the allometric growth of otoliths [13]-[38]. To begin with, the differences observed in the width of the otoliths between the locations suggest variations in the symmetry of this anatomical feature. The Porto-Novo lagoon has a slightly higher average percentage of asymmetry than the coast of the Atlantic Ocean and Lake Nokoué. This could reflect differences in selective pressures or environmental conditions specific to each site, potentially influencing the growth and development of otoliths. The origin and consequences of fluctuating otolith asymmetry in fish remain largely unknown and debated, although it has been documented in several fish species and is regularly associated with stress and/or environmental heterogeneity, and therefore considered an indicator of developmental instability. These results reveal the importance of interactions between several factors, such as size, weight, side and site, on variation in otolith shape in *Pseudotolithus senegalensis*, whereas no influence was observed for the morphometric parameter. They also show the potential of otolith shape as a marker to discriminate between individuals from different stations and suggest promising avenues for future research into the biology and ecology of this species. In our case study, no effect of sex was observed. It has previously been shown that sexual dimorphism has no effect on otolith shape in swordfish, Atlantic mackerel (*Scomber scombrus* [35]), haddock (*Melanogrammus aeglefinus* [39]), Atlantic cod (*Gudus morhua* [40]) or blue whiting (*Micromesistius poutassou* [30]).

4.3. Multifactorial Interactions on Otolith Shape

The shape of otoliths in fish from different geographical origins is influenced by a combination of abiotic environmental factors, such as temperature and salinity, and biotic factors, such as the availability of prey. Moreover, this form also depends on the individual genotype of the fish [41]-[45]. In addition, ontogenic changes in otolith configuration, often assessed through variations associated with size, have been observed to result from the diversity of growth rates as a function of habitat quality and development processes [46] [47].

4.4. Directional Asymmetry between Sites

Analysis of the amplitudes of directional asymmetry reveals significant differences between locations. Individuals sampled on the Atlantic coast show significantly higher directional asymmetry than those sampled in Lake Nokoué and the Porto-Novo lagoons. This observation may indicate differences in environmental constraints or growth patterns that influence otolith symmetry in *Pseudotolithus senegalensis* in these different habitats. Our results show that DA can be observed in otolith shape in round fish such as *Pseudotolithus senegalensis.* DA between right and left otolith shapes has already been described for other roundfish species such as *Liza ramada* [48], *Diplodus annularis* [49], *Diplodus puntazzo* [50], *Clupea harengus* [51] and *Scomberomorus niphonius*. Otoliths from the Porto-Novo lagoon appear to be closer to those from Lake Nokoué, and vice versa, than those collected on the Atlantic coast. This similarity between otolith shapes in lagoons and lakes may reflect similar environmental conditions or comparable growth patterns in these coastal and freshwater habitats, as opposed to the more contrasting marine environment of the southern Atlantic coast.

5. Conclusion

This study revealed variations in the shape of otoliths of *Pseudotolithus senegalensis* across different locations, notably the Porto-Novo lagoon, Lake Nokoué and the Atlantic coast. Analyses revealed that the relationship between otolith morphometric measurements and total fish length was most robust and significant in the Porto-Novo lagoon, moderate on the Atlantic coast, and weak in Lake Nokoué.

Site, side or sex factors had no effect on the biometric parameters. Canonical discriminant analysis showed significant differences between sites, indicating the influence of the environment on otolith shape.

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Conflicts of Interest

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

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Supplementary

Table S1. Sampling information (total length and total weight) of *Pseudotolithus senegalensis* in Benin waters according to the sampling locations.

Sampling locations		Lagoon of Porto Novo	Lake Nokoué	Atlantic coast
Total length (cm)	Mean ± SD	22.1 ± 1.8	23.6 ± 2.5	27.2 ± 1.9
	max	27.0	31.2	32.2
	min	18.0	18.0	23.4
Total weight (g)	Mean ± SD	3.8 ± 1.8	8.70 ± 3.3	13.7 ± 2.5
	max	12.2	18.6	22.2
	min	3.8	3.2	7.4

Table S2. Regression results.

Location	Variable	R-squared	P-value
Lake Nokoué	Length	0.016	1.632e−01
	Width	0.012	2.259e−01
	Area	0.023	9.025e−02
	Perimeter	0.022	9.920e−02
Atlantic coast	Length	2.02e−04	8.838e−01
	Width	0.002	6.106e−01
	Area	0.003	5.566e−01
	Perimeter	3.33e−04	8.512e−01
Porto-Novo Lagoon	Length	0.209	1.664e−06
	Width	0.165	2.638e−05
	Area	0.228	5.018e−07
	perimeter	0.269	3.108e−08

Table S3. Percentage asymmetry of biometric parameters per location.

Otolith parameters	Porto-Novo Lagoon	Lake Nokoué	Atlantic coast
Length	0.774	0.596	1.501
Width	4.118	4.849	3.602
Area	4.656	4.578	5.779
Perimeter	0.906	1.410	1.891

Table S4. P-value of asymmetry fluctuating between right and left.

Otolith parameters	Locations		
	LN	LP	AC
Length	0.015**	0.0282**	0.314
Width	3.352e−10***	4.380e−11***	0.003***
Area	3.282e−11***	5.329e−15***	0.01**
Perimeter	2.367e−05***	0.023**	0.164

: Significant at $p < 0.01$; *: Significant at $p < 0.001$.

Table S5. ANOVA results for otolith length, width and surface area between groups.

Attribute	Wilks L.	Partial L.	F	p-value
Length	0.724	0.981	3.198	P < 0.05
Width	0.862	0.824	36.418	P < 0.05
Area	0.815	0.872	25.071	P < 0.05
Perimeter	0.714	0.995	0.806	P > 0.05

Table S6. MANOVA of principal components: location side.

locations: side	Value	DF	F Value	P value
Wilks' lambda	0.792	44.00	1.796	<0.0001
Pillai's trace	0.215	44.00	1.76	<0.0001
Hotelling-Lawley trace	0.251	44.00	0.72	<0.0001
Roy's greatest root	0.202	22.00	2.96	<0.0001

Table S7. MANOVA of principal components: location sex.

locations: sex	Value	DF	F Value	P value
Wilks' lambda	0.91	44.00	0.72	0.908
Pillai's trace	0.09	44.00	0.73	0.906
Hotelling-Lawley trace	0.10	44.00	1.82	0.909
Roy's greatest root	0.05	22.00	0.84	0.669

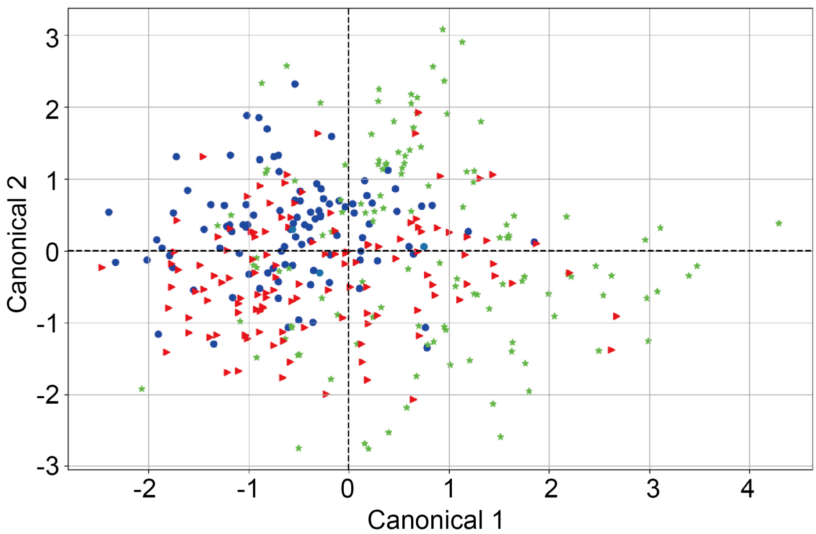

Figure S1. Canonical Discriminant Analysis with the first two canonical variables of the biometric parameters for 4 classes (Lake Nokoué (red), Porto-Novo lagoon (blue), Atlantic coast (green)).

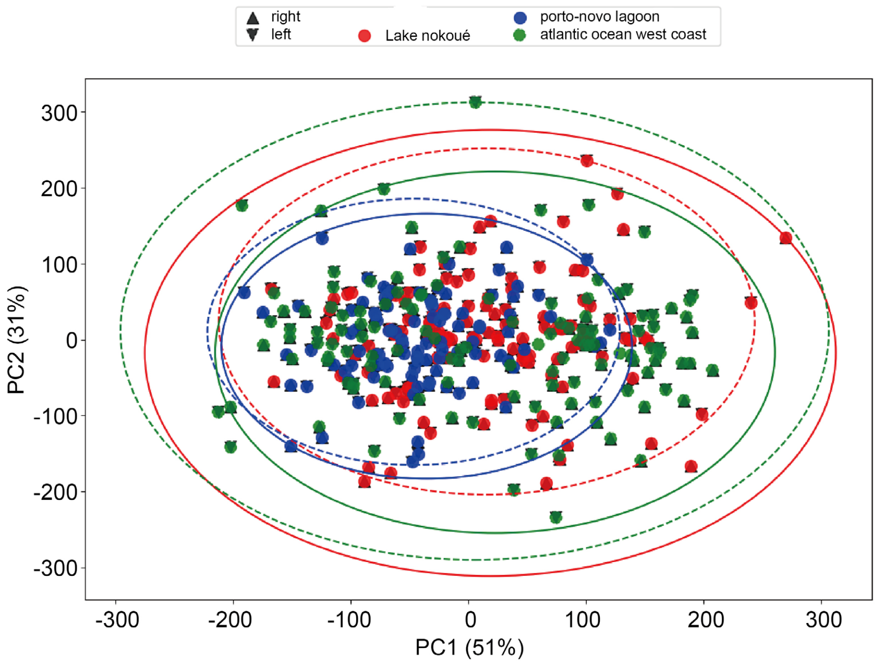

Figure S2. Difference in mean shapes of reconstructed otoliths between identified stock units: Lake Nokoué (red; Left: solid line, Right: dotted line), Atlantic coast (green; Left: solid line, Right: dotted line), Porto-Novo Lagoon (blue; Left: solid line, Right: dotted line).