International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 10, Issue 5, pp: 58-66, 2025
https://doi.org/10.55126/ijzab.2025.v10.i05.008 **http://www.ijzab.com**

Research Article

DIRECTIONAL ASYMMETRY OF OTOLITHS OF *CHRYSICHTHYS NIGRODIGITATUS* (LACEPEDE 1803) INDICATOR OF ENVIRONMENTAL STRESS IN THE COMOE RIVER BASIN (IVORY COAST)

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Article History: Received 29th July 2025; Accepted 30th August 2025; Published 30th September 2025

ABSTRACT

The impact of pollution on the otoliths of Chrysichthys nigrodigitatus organisms from the middle course of the Comoé River was assessed through the study of asymmetry. Quarterly sampling of 201 specimens of Chrysichthys nigrodigitatus was carried out over one year, from February 2021 to March 2022 at five sampling stations (M'Basso, Manzan, YèrèYèrè, Abradinou and Bettié). The otoliths were extracted and then measured. The Shapiro-Wilk test was performed to statistically verify the normality of the variables (weight, area, width, length and perimeter). Asymmetry was assessed through the AF1 and AF2 indices. After this assessment, analysis of variance (ANOVA) and the Tukey-Kramer post-hoc test were used to test for differences in directional asymmetry between sites. Directional asymmetry values varied significantly between sampling sites. Otolith analysis revealed marked directional asymmetry, predominantly oriented toward the right side. The Manzan, Bettié, Abradinou, and YèrèYèrè stations displayed the highest average directional asymmetry, while M'Basso recorded the lowest values.

Keywords: *Chrysichthys nigrodigitatus*, Echantillonnage, Erreur de mesure, Points aberrants, Pollution.

INTRODUCTION

Directional asymmetry (DA) is defined as a mean of d = r - l that is significantly different from zero (Baamrane *et al.,* 2018). It is a difference between sides, but where the more developed side is always the same for the entire population (Baamrane *et al.,* 2018). It represents small directional deviations from perfect symmetry in bilateral structures expressed by a genome (Møller, 1990). These deviations may arise due to variations in the ability of individuals to develop both sides of the body identically following possible exposure to environmental stress (Baamrane *et al.,* 2018). The use of otoliths, which are paired structures, could therefore be an appropriate tool for studying directional asymmetry. Otoliths are complex polycrystalline structures composed of calcium carbonate (approximately 96%) and trace elements embedded in a protein matrix (Wright *et al.,* 2002). These structures are in the inner ear of fish and play a role in hearing and maintaining balance (Popper & Lu, 2000). They are enclosed within three terminal organs of the inner ear of the teleost fish (Popper & Lu, 2000). The saccular otolith (sagitta) is the largest pair in most teleost families and is therefore used in most studies (Mahé, 2019). Otolith morphometry and morphology have been widely used to identify fish species. Fortunato *et al.* (2014) used otolith morphometry to identify mullet species (Mugilidae) from the Northeast Atlantic and the Mediterranean Sea, while Tuset *et al.* (2008) characterized 348 fish species using otolith morphometry. Otolith morphometry has also been used for fish stock identification (Avigliano *et al.,* 2014). However, studies related to otolith directional asymmetry of *Chrysichthys nigrodigitatus* populations are lacking in the middle reaches of the Comoé River. In this work, we describe for the first time the directional asymmetry of otoliths in *Chrysichthys nigrodigitatus* populations caused by environmental stress in the middle reaches of the Comoé River. Otolith asymmetry is considered a potentially

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important tool for assessing the level of environmental stress.

MATERIALS AND METHODS

Study environment

Figure 1 shows the map of sampling stations. The study area is located in the south-east of Ivory Coast. It is bordered by the middle course of the Comoé River. Five stations from upstream to downstream were selected on the Comoé River. These are : M'Basso, Manzan, YèrèYèrè, Abradinou and Bettié.

Sampling ichthyological

Figure 2 represents a specimen of *Chrysichthys nigrodigitatus.* The quarterly sampling method was carried out over one year, from March 2021 to March 2022. The catches were made using nets of different mesh sizes (7, 10, 12, 13, 18, 20, 25, 28, 30, 35, 50, 60 and 70 mm) in order to capture the maximum number of specimens of *Chrysichthys nigrodigitatus* The initial biological material consisted of 201 specimens of *Chrysichthys nigrodigitatus*. However, due to losses during handling and laboratory extraction, 40 otoliths corresponding to 20 specimens could not be included in the final analysis. Therefore, the final sample of *C. nigrodigitatus* was 181 individuals, or 362 otoliths

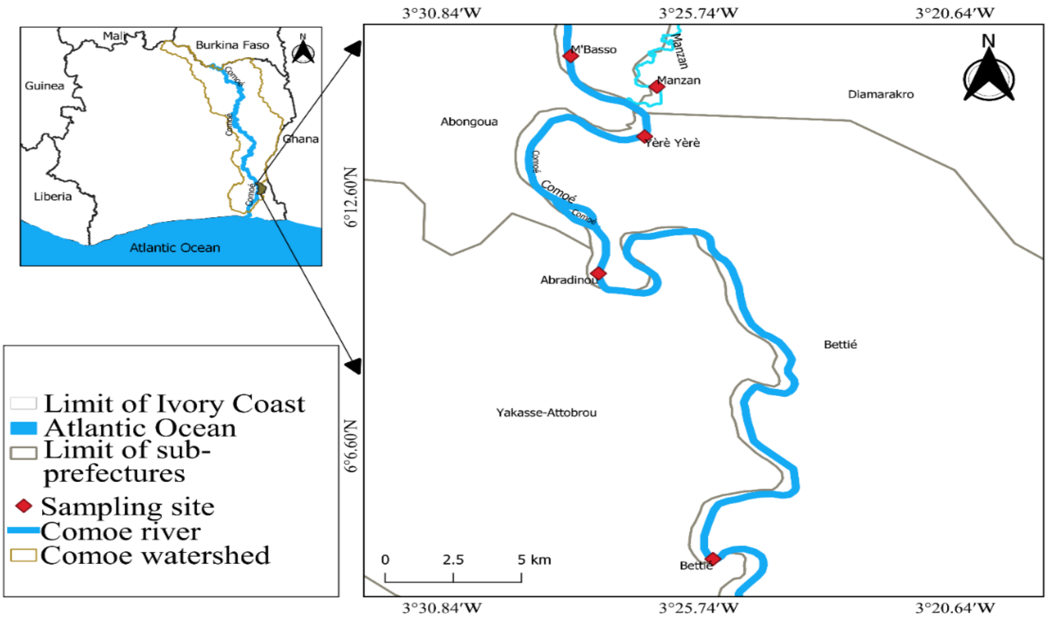

Figure 1. Sampling map of the study stations.

Figure 2. Specimen of *Chrysichthys nigrodigitatus.*

Extraction and photography of otoliths

Figure 3 shows the otolith extraction steps. The saccular otoliths were extracted by turning the fish's ventral side upward to remove the gills and hypobranchial apparatus, exposing the base of the skull. Fine forceps were used for extraction. To remove the thin membrane covering the otoliths, they were carefully cleaned with distilled water and 70% ethanol (Panfili & Ximenes, 1992). They were air-dried and stored in numbered plastic tubes. The two (2) sagittal otoliths from each fish were photographed and then coded (Figure 4). According to the terminology used by Avigliano *et al.* (2014), morphometric variables such as, otolith length (Lo, mm), otolith width (lo, mm), otolith perimeter (Po, mm), otolith area (Ao, mm2) were measured using the software (ImageJ version 1.52a).

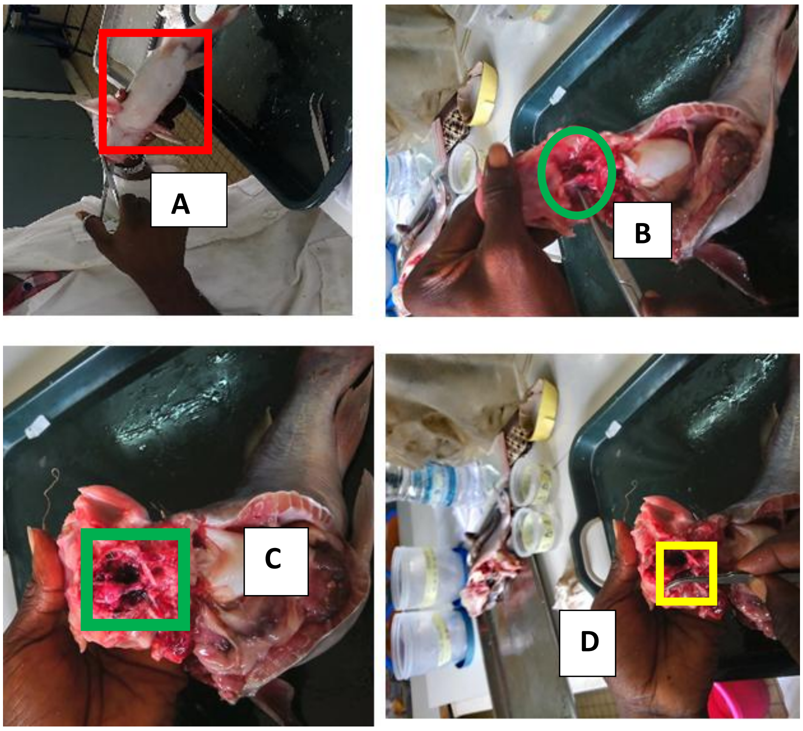

Figure 3. Otolith extraction steps (A: ventral side of the fish and cutting of the lower jaw, B: exposure of the otolithic chamber, C: opening of the otolithic chamber, D: highlighting the otolith).

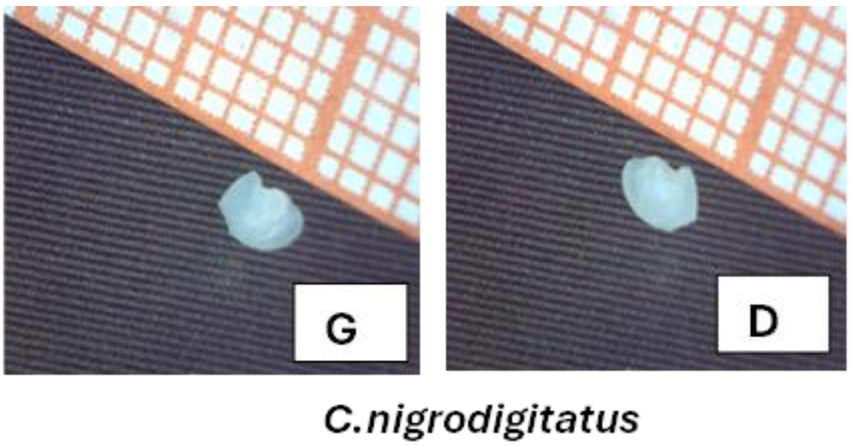

Figure 4. Pair of sagittal otoliths of *Chrysichthys nigrodigitatus* after photography (L: left, R: right).

Statistical processing

First, for each site studied and for each parameter analyzed, the otolith pairs (sagittae) were subjected to an outlier elimination process. Potentially outlier individuals were then statistically evaluated using the Grubbs test (Rohlf & Sokal, 1995; Palmer & Strobeck, 2003). This test allows us to objectively determine whether the exclusion of these points is statistically justified. Furthermore, measurement errors were considered in this study on a subset of 30 individuals, in accordance with Pither & Taylor, (2000). To assess whether the variation between sides significantly exceeded the measurement error, a mixed-model ANOVA (side x individual) was performed for each trait studied, considering two factors: sides and individuals. In addition, the normality of the variables was verified using a Shapiro-Wilk statistical test using R Studio software (Bertrand *et al.,* 2019). Finally, to detect directional asymmetry, a t-test was used to test whether the means (d = r - l) differed from zero. A mean distribution of traits significantly different from zero would then indicate directional asymmetry (Palmer & Strobeck, 1986). Meanwhile, in the present study, antisymmetry was examined using the Kolmogorov-Smirnov test applied to the frequency distribution of deviations between right and left sides (Palmer &Strobeck, 1986). We used analysis of variance (ANOVA) to compare asymmetry between sides and sampling sites. To precisely determine significant differences between sites, we then applied the Tukey-Kramer post-hoc (HSD) test.

Determination of asymmetry

Among the different indices for measuring asymmetry proposed by the literature (Palmer, 1994) , we opted for the AF1, AF2 indices. The AF1 index is frequently used for its simplicity of calculation. It quantifies the average difference between the right and left sides for a specific character and treatment (Palmer, 1994) . This index makes it possible to obtain a numerical measure of asymmetry. It is obtained by the following relationship:

$$AF1 = |average\ right\ otolith - average\ left\ otolith|$$

To account for the potential influence of otolith size on asymmetry measurements, we applied a correction to our calculations. Instead of simply using the raw difference between the right and left otolith (AF1), we employed the following formula (AF2) for the meaning of each character

$$AF2 = average\ [|\text{right otolith} - left\ otolith|/((\text{right otolith} + left\ otolith)/2)]$$

This approach allows us to normalize the difference in total otolith size, including biases related to size variations between individuals (Palmer, 1994) .

Organic pollution index versus asymmetry

Table 1 illustrates the class boundaries of the organic pollution index. Here, we used the organic pollution index (Leclercq & Vandevenne, 1987) calculated from ammonium, nitrite, and orthophosphate measurements. The organic pollution level according to Buhungu *et al.* (2018) is:

IPO = 5.0 - 4.6: no organic pollution.

IPO = 4.5 - 4.0: low organic pollution.

IPO = 3.9 - 3.0: moderate organic pollution.

IPO = 2.9 - 2.0: high organic pollution.

IPO = 1.9 - 1.0: very high organic pollution.

To analyze the relationship between the OPI and the asymmetry of each sampling site, we used Pearson correlation.

RESULTS AND DISCUSSION

Figure 5 illustrates the distribution of normal and outlier values within *C. nigrodigitatus* populations. Of 362 otoliths analyzed, 11 (3.04%) were identified as outliers ($p < 0.05$). Grubb 's test confirmed the statistical significance of the outliers ($p < 0.05$). This outlier data was excluded from further analyses to ensure the reliability of the results. Table 2 presents the results of the mixed analysis of variance (ANOVA), carried out on a subsample of 30 specimens of *Chrysichthys nigrodigitatus*.

Table 1. Limits of the classes of the organic pollution index (Buhungu *et al.,* 2018).

Classes	NH4+ (mg/l)	NO $^{2-}$ (ug /l)	PO $^{3-4}$ (ug / l)
5	<0.1	<5	<15
4	0.1-0.9	6-10	16-75
3	1-2.4	11-50	76-250
2	2.5-6	51-150	251-900
1	>6	>150	>900

The precision of the measurements was found to be remarkable, with a measurement error between otolith replicates of less than 1%. Figure 6 presents the distributions of each parameter of the *Chrysichthys nigrodigitatus* populations. The histograms show a shape that appears to be that of a normal distribution. The Shapiro-Wilk test revealed a normal distribution ($P > 0.05$) in all stations and all parameters. Figure 7 represents the intra-population variation of the directional asymmetry of otoliths *Chrysichthys nigrodigitatus* according to the side. Graphically we observe that the directional asymmetry is more pronounced on the right side (R) of the otoliths in the studied population. The ANOVA shows a very significant difference between the right and left sides ($p < 0.001$) for all characters. Figure 8 illustrates the spatial differences in the average directional asymmetry of otoliths. Statistical analyses (ANOVA and Tukey HSD test) revealed significant effects of stations on otolith directional asymmetry (DA) ($p < 0.05$). Station Manzan has the highest DA (14.92%), indicating more asymmetric otoliths. Then, stations Bettié, YèrèYèrè and Abradinou show intermediate values. M' Basso stands out for the lowest DA values, about five times lower than the observed maximums.

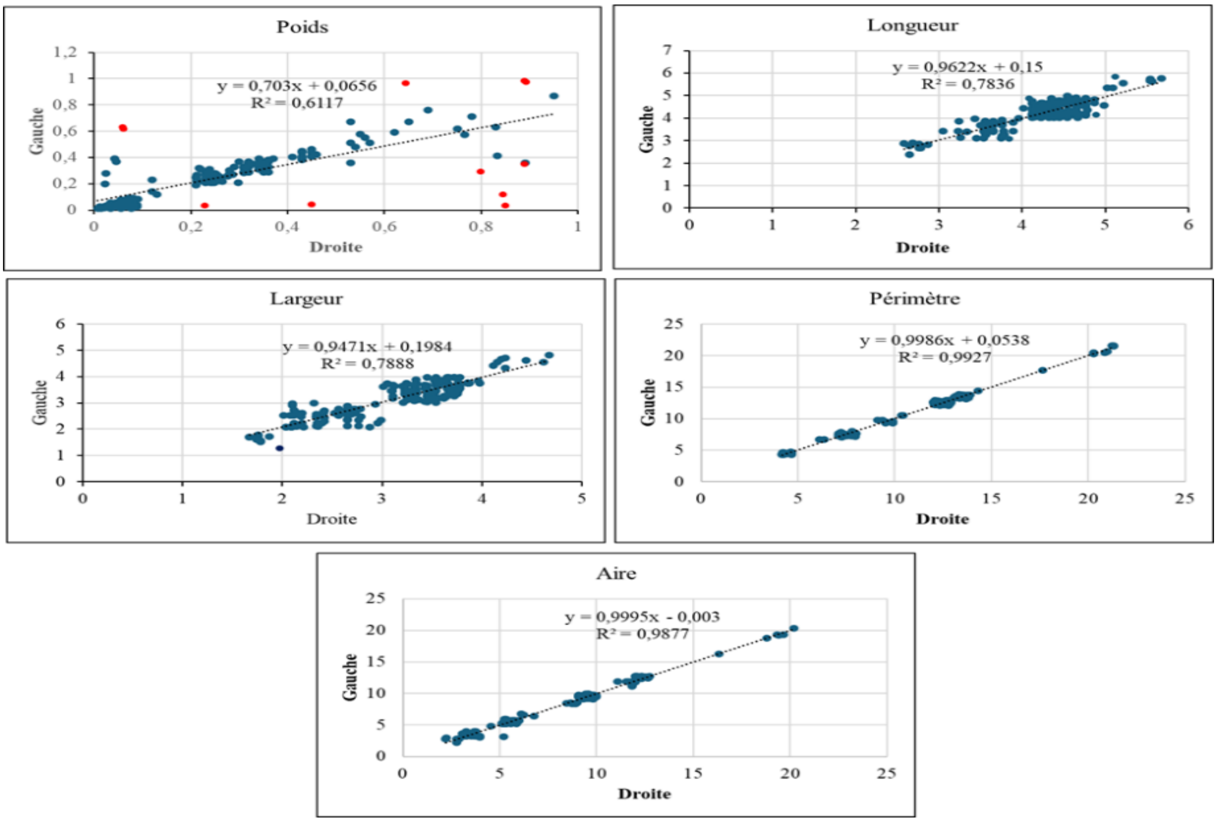

Figure 5. Results of outliers (red dots) of *Chrysichthys nigrodigitatus*.

The values of the organic pollution index (IPO), and the average directional asymmetry are represented in table 3. Overall, the values of the index oscillate between 1 (very high organic pollution) and 2.8 (high organic pollution). It is noted that the stations (Manzan, Betié, Abradinou and YèrèYèrè) which are polluted with very high values of pollution have a high average directional asymmetry. The result of the Spearman correlation test between the pollution index (IPO) and the directional asymmetry (AD) indicates a strong negative correlation (-0.73) between IPO and AD. This relationship is statistically significant ($P=0.0009$) for the populations.

We analyzed otolith pairs of *Chrysichthys nigrodigitatus* from five sites in the Comoé River, measuring various morphological parameters (weight, area, width, length, and perimeter). The Shapiro-Wilk test confirmed the normality of the data, allowing for skewness analysis. The t-test and ANOVA revealed significant directional skewness, consistently oriented toward the right side ($p < 0.001$) for all the characters studied. The directional skewness of *Chrysichthys nigrodigitatus* otoliths appears to result from ecological adaptation and varies among sampling stations. ANOVA and the Tukey HSD test revealed that the Manzan station exhibited significantly higher skewness than the others, while M'Basso displayed the lowest values.

Table 2. Mixed model ANOVA result performed on a subsample of 30 individuals of *Chrysichthys nigrodigitatus.*

		Area			
Source of variation	Df	Sum	Medium square	F	P
Side	29	0.7	0.69	0.012	0.913
Individual	29	57.06	57,056	0.085	0.0002

Individual side	29	0.84	0.841	0.0001	0.992
Measurement error	116	23.16	0.0023		
			Width		
Source of variation	Df	Sum	Medium square	F	P
Side	29	0.009	0.009	0.018	0.892
Individual	29	6,261	6,260	12,467	0.0005
Individual side	29	0.92	0.916	0.1824	0.6701
Measurement error	116	58,254	0.0022		
			Length		
Source of variation	Df	Sum	Medium square	F	P
Side	29	0.065	0.0651	0.140	0.708
Individual	29	5,343	5,342	11,524	0.0009
Individual side	29	0.6	0.58	0.0124	0.911
Measurement error	116	53,777	0.0036		
			Perimeter		
Source of variation	Df	Sum	Medium square	F	P
Side	29	0.002	0.0021	0.281	0.992
Individual	29	129.96	129,962	9,081	0.0003
Individual side	29	0.61	0.612	0.0004	0.984
Measurement error	116	16.15	0.009		

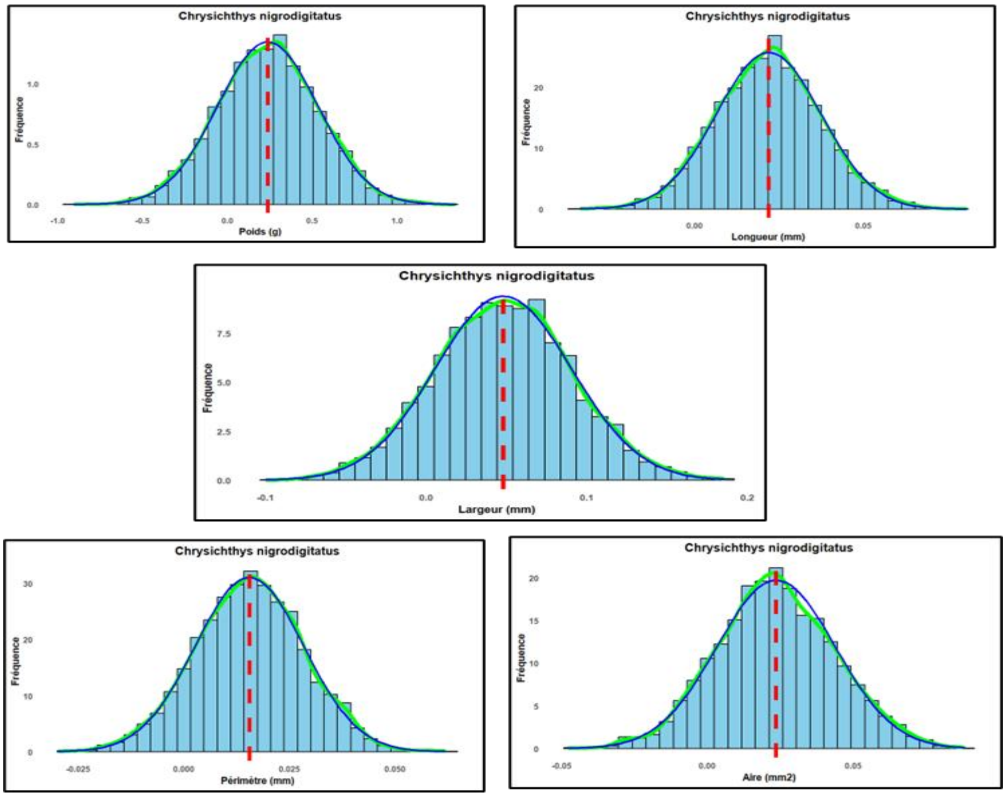

Figure 6. Normality graphs of populations *Chrysichthys nigrodigitatus*.

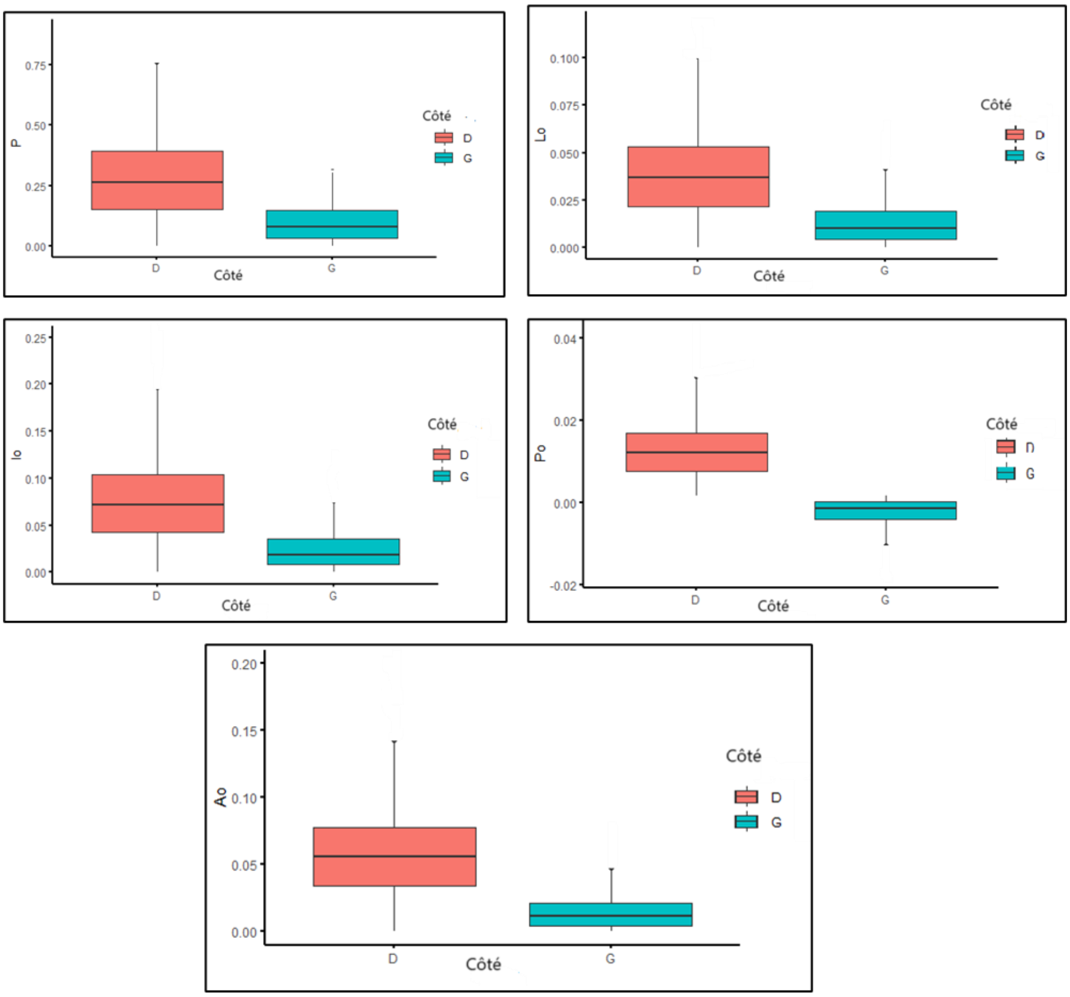

Figure 7. Variations in directional asymmetry of otoliths *Chyrsichthys nigrodigitatus* depending on the side (P: weight, Lo: length, lo: width, Po: perimeter, Ao: area, D: right and G: left).

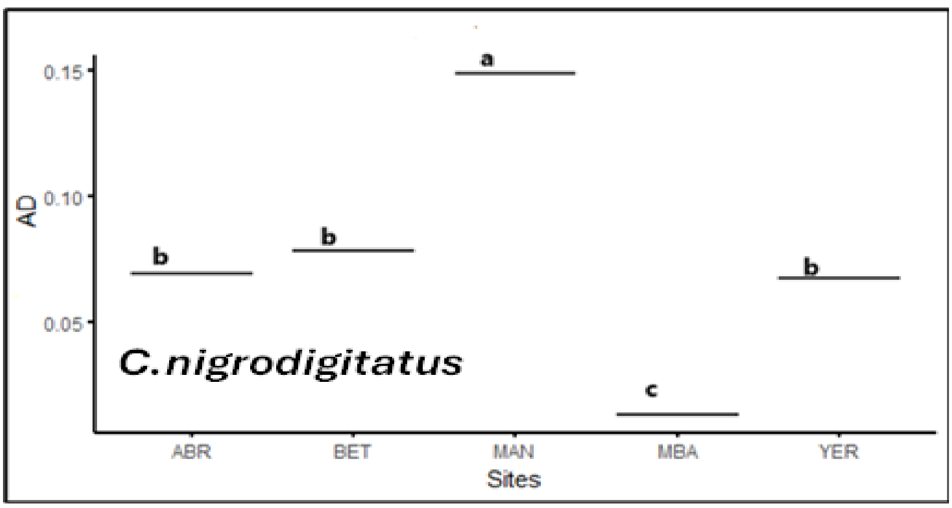

Figure 8. Spatial variations in mean directional asymmetry (AD) of otoliths from the *population Chrysichthys nigrodigitatus* (stations with common letters do not differ, ABR: Abradinou, BET: Bettié, MAN: Manzan, MBA: M'Basso, YER: YèrèYèrè).

Table 3. Relationship between organic pollution index (OPI) and average directional asymmetry (DA) of the studied fish populations *(Cn: Chrysichthys nigrodigitatus*).

Site	Esp	Class NH4+	Class NO2-	Class PO4-	IPO	Pollution	AD	Horn	*P*
My	C,n	3	1	1	1	Very strong	0.149	-0.73	0.00
Be	C,n	4	3	1	1.6	Very strong	0.078	-0.73	0.00
Ab	C,n	4	3	2	1.8	Very strong	0.069	-0.73	0.00
Ye	C,n	4	2	1	1.4	Very strong	0.068	-0.73	0.00
Mb	C,n	5	5	4	2.8	Strong	0.013	-0.73	0.00

These results can be explained by several environmental and biological factors (Mille *et al.,* 2015). First, environmental conditions, such as temperature, pH, and substrate nature, play a crucial role in otolith formation and morphology. Nasreddine (2010) and Mahé (2019) demonstrated that temperature accelerates otolith morphogenesis and changes their growth trajectory, which can lead to more pronounced directional asymmetry in certain geographic areas. According to Tissot & Souchon, (2010), water temperature can also influence fish embryonic development, including otolith development. According to these authors, abnormally high temperatures can lead to abnormalities in otolith development, which can have consequences on the fish's sensory function. Mille (2015) demonstrated that diet composition contributes more significantly to otolith morphological variability than ingested quantity. Thus, differences in food availability and type between the Manzan, Bettié, and M'Basso stations could explain the variations related to otolith asymmetry. Another hypothesis is that phosphate exerts indirect chemical stress effects on otolith pairs (Perennou & Aufray, 2007). The presence of high phosphate concentrations in a freshwater environment can lead to excessive algal blooms, which can degrade water quality and lead to eutrophication. This eutrophication phenomenon can generate significant stress on living organisms, particularly fish, and potentially increase their asymmetry rate. The observation of the presence of algae at the Bettié and Manzan sites, as well as the poor water quality observed during the sampling periods, support this hypothesis. Such an indirect effect of phosphate could thus contribute to the increase in the level of otolith asymmetry. Our hypotheses corroborate the findings of Østbye *et al.* (1997). Their research revealed that perch from acidic lakes rich in phosphate and aluminum showed a more pronounced asymmetry than those from non-acidified lakes. Similarly, it is possible that the polluted environment and the stress experienced by *C. nigrodigitatus* populations in the waters of Bettié and the Manzan River are sufficient to generate a strong asymmetry of the otoliths. This idea is supported by the organic pollution index (OPI) calculated in our study, which indicates very strong organic pollution in Manzan, YèrèYèrè, Abradinou and Bettié.

CONCLUSION

This study highlighted the significant influence of human activities on the morphology of *Chrysichthys nigrodigitatus* fish in the middle reaches of the Comoé River. Otolith analysis revealed significant directional asymmetry oriented towards the right side. The Manzan, Bettié, Abradinou and YèrèYèrè stations showed the highest average directional asymmetry, while M'Basso had the lowest values. The results of this study highlight the urgency of implementing conservation and sustainable management measures to preserve aquatic biodiversity.

ACKNOWLEDGMENT

The authors would like to thank the Sustainable Management of Fisheries Resources Support Program (PAGDRH) of the Directorate of Aquaculture and Fisheries (DAP) for the funding provided to carry out this work. Indeed, this study was carried out within the framework of the DAP/PAGDRH-UNA project on the assessment of the impact of agricultural and gold mining on the water quality and fishery resources of the Comoé River. The authors also thank the researchers and others who participated in the data collection and writing of this article.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received DAP/PAGDRH-UNA project on the assessment of the impact of agricultural and gold mining on the water quality and fishery resources of the Comoé River.

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

REFERENCES

Avigliano, E., Martinez, C, F, R., & Volpedo, A, V, (2014). Combined use of otolith microchemistry and morphometry as indicators of the habitat of the silverside (*Odontesthes bonariensis*) in a freshwater-estuarine environment, *Fisheries Research*, *149*, 55-60,

Baamrane , M, A., Znari , M., & Abáigar , T, (2018). Comparative study of fluctuating cranial asymmetry in two subspecies of the dorcas gazelle (Gazella dorcas massaesyla vs. G, d, Neglecta), *Bulletin of the Scientific Institute, Rabat, 40,* 01-09,

Bertrand, F, Claeys, E, & Maumy -Bertrand, M, (2019). Statistical modeling through practice with R: Course and corrected exercises, Dunod,

Buhungu , S., Montchowui , E., Barankanira , E., Sibomana, C., Ntakimazi , G., & Bonou , C, (2018). Spatio-temporal characterization of the water quality of the Kinyankonge River, a tributary of Lake Tanganyika, Burundi, *International Journal of Biological and Chemical Sciences, 12* (1), 576-595.

Fortunato, R, C., Dura, V, B., & Volpedo, A, (2014). The morphology of saccular otoliths as a tool to identify different mugilid species from the Northeastern Atlantic and Mediterranean Sea, *Estuarine, Coastal and Shelf Science*, *146*, 95-101.

Leclercq, L., & Vandevenne , L, (1987). Impact of a discharge of salt-laden water and organic pollution on diatom populations in the Gander (Grand Duchy of Luxembourg), *Cahiers de Biologie Marine, 28* (2), 311-318.

Mahé, K. (2019). Sources of variation in otolith shape: Implications for fish stock discrimination. University of the Littoral Côte d'Opale.

Mille, T. (2015). Sources of intra- population variation in otolith morphology: directional asymmetry and diet (PhD thesis, University of Lille 1-Sciences and Technologies).

Mille, T., Mahe, K., Villanueva, M., De Pontual , H., & Ernande , B, (2015). Sagittal otolith morphogenesis asymmetry in marine fishes, *Journal of Fish Biology, 87* (3), 646-663.

Møller, A. (1990). Fluctuating asymmetry in male sexual ornaments may reveal male quality, *Mamm Rev, 5,* 121-172.

Nasreddine, K. (2010). Signal registration and pattern recognition. Application to the analysis of fish otoliths (Doctoral dissertation, University of Western Brittany-Brest).

Østbye, K., Øxnevad, S, A., & Vøllestad, L, A, (1997). Developmental stability in perch (Perca fluviatilis) in acidic aluminium-rich lakes, *Canadian Journal of Zoology*, *75*(6), 919-928.

Palmer, A. R. (1994). Fluctuating asymmetry analyses: a primer. In Developmental instability: its origins and evolutionary implications: proceedings of the international conference on developmental instability: its origins and evolutionary implications, Tempe, Arizona, 14–15 June 1993 (pp. 335-364). Dordrecht: Springer Netherlands.

Palmer, A, R, & Strobeck, C, (1986). Fluctuating asymmetry: Measurement, analysis, patterns, *Annual review of Ecology and Systematics*, 391-421.

Palmer, A, R., & Strobeck, C, (2003), CH 17. Fluctuating asymmetry analyses revisited, *Developmental Instability: Causes and Consequences, Oxford University Press, Oxford*, 279-319.

Panfili, J., & Ximenes, M, (1992). Measurements on ground or sectioned otoliths: Possibilities of bias, *Journal of Fish Biology, 41* (2), 201-207.

Perennou , C., & Aufray , R, (2007). Observatoire Camargue, *Evolution de la Camargue: chasse, pêche, protection* .

Pither, J., & Taylor, P, D. (2000). Directional and fluctuating asymmetry in the black-winged damselfly Calopteryx maculata (Beauvois) (Odonata: Calopterygidae), *Canadian Journal of Zoology*, *78*(10), 1740-1748.

Popper, A, N., & Lu, Z. (2000). Structure–function relationships in fish otolith organs, *Fisheries research*, *46*(1-3), 15-25.

Rohlf, F, J., & Sokal, R, R. (1995). Statistical tables, Macmillan.

Tissot, L., & Souchon, Y. (2010). Summary of thermal tolerances of the main fish species in the rivers and streams of the lowlands of western Europe, *Applied Hydroecology* , *17,* 17-76.

Tuset, V, M., Lombarte , A., & Assis, C, A, (2008). Otolith atlas for the western Mediterranean, north and central eastern Atlantic, *Scientia Marina, 72* (S1), 7-198.

Wright, P., Panfili, J., Morales-Nin, B., & Geffen, A, (2002). Different types of calcified piece, A/the otoliths, *Handbook of fish sclerochronology (eds J, Panfili , Hd Pontual , H, Troadec & PJ Wright),* 31-57.