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ARTICLE

Bilateral asymmetry in the mass and size of otolith of two cichlid species collected from Lake Ahémé and Porto-Novo Lagoon (Bénin, West Africa)

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Resumen

Asimetría bilateral en la masa y el tamaño de otolitos de dos especies de cíclidos capturados en el lago Ahémé y la laguna de Porto Novo (Benín, África Occidental)

Se estudia la asimetría de la masa del otolito sacular en dos especies de peces cíclidos *Sarotherodon melanotheron* y *Coptodon guineensis* recolectadas del lago Ahémé y la laguna Porto-Novo, Benín, África occidental. La longitud y el ancho de los otolitos de especímenes adultos de ambas especies se usaron para calcular la asimetría fluctuante en estos dos caracteres. Los resultados muestran que el nivel de asimetría del ancho del otolito es el más importante. La posible causa de la asimetría en estas especies se ha discutido en relación con diferentes contaminantes en el área. Nuestros datos no muestran diferencias significativas, aunque varios estudios demostraron una asimetría más alta en individuos más grandes (de más edad).

Palabras clave: Factores ecológicos; Contaminación acuática; Cichlidae; *Sarotherodon melanotheron*; *Coptodon guineensis*.

Abstract

Saccular otolith mass asymmetry is studied in two cichlid fish species *Sarotherodon melanotheron* and *Coptodon guineensis* collected from Lake Ahémé and Porto-Novo Lagoon, Bénin, West Africa. The length and width of otoliths from adult specimens of both species were used to calculate the fluctuating asymmetry in these two characters. The results show that the level of asymmetry of the otolith width is the most important. The possible cause of the asymmetry in these species has been discussed in relation to different pollutants in the area. Our data show no significant difference, although several studies proved a higher asymmetry in larger (older) individuals.

Key words: Ecological factors; Water pollution; Cichlidae; *Sarotherodon melanotheron*; *Coptodon guineensis*.

Introduction

Among the interesting character of the vertebrate inner ear is the presence of statolithic organs that are linked to hearing and gravity. In fishes, the statoliths are compacted and can thus be easily measured (Anken *et al.* 1998). Otoliths have played a vital role in fisheries science for many years (Kingsmill 1993), and reliable methods to determine the size of a given otolith as a parameter of its mass are readily available (Alemany & Alvarez 1994).

It has been well documented that variation in the shape of fish otoliths is affected by external factors, e.g. environmental conditions, or individual characteristics, e.g. the individual genotype or state, but the possible intra-individual cause of variation, specifically the difference in otolith shape between the right and left inner ears (referred to as otolith location side), are poorly studied (Mille *et al.* 2015). Under normal conditions, the three orthogonal semicircular otoliths at both sides of the head are morphologically similar in roundfishes (Panfili *et al.* 2002), although there are some inter-specific modifications in the size and shape (Popper & Lu 2000). A typical bilateral asymmetry of the otoliths is clearly seen in flatfish species, which endure profound asymmetric morphological changes throughout their lives, including a cranial distortion and the relocation of one eye to the other side of the head (Bao *et al.* 2011).

The studies were few on the otolith asymmetry of the freshwater fish (e.g., Downhower *et al.* 1990, Østbye *et al.* 1997, Anken *et al.* 1998, Øxnevad *et al.* 2002, Novak *et al.* 2013) in general and the otolith mass asymmetry in particular (e.g., Scherer *et al.* 2001, Takabayashi & Ohmura-Iwasaki 2003, Lychakov *et al.* 2006, Yedier *et al.* 2018). The researchers on the otolith mass asymmetry of the freshwater fishes have reached to a conclusion that the differences in weight of the otolith found on both sides of the fish head is either large (Scherer *et al.* 2001) or small as suggested by the work of Takabayashi & Ohmura-Iwasaki (2003). The latter work was based on members of the family Cyprinidae, where the sgitta otolith is very small and the asteriscus otolith is the largest among the three otoliths found in the inner ear of the fish.

The asymmetry in the otolith mass can have a negative impact on the life of a fish, particularly on the hearing and the balance (Lychakov *et al.* 2008). It has been employed as a bio-indicator to check the state between different aquatic habitats (Grønkjær & Sand 2003) and it was also used to test different environmental effects in fish populations. The most obvious effect of a bilateral asymmetry in fish otolith mass is an abnormal swimming activity (Helling *et al.* 2003) and intrusion with precise sound localization resulting in failure of individuals to mix with the habitat they are living in (Lychakov & Rebane 2005). Gagliano *et al.* (2008) investigated whether otolith asymmetry influenced the capability of returning larvae to detect and effectively recruit to favorable reef habitats and they found that larvae with asymmetrical otoliths not only faced greater troubles in identifying appropriate settlement niches, but may also risk considerably higher rates of mortality. They also concluded that otolith asymmetries occurring early in the embryonic stage were not modified by any compensational growth mechanism during the larval stage as these changes are permanent and that phenotypic selection is disturbed by asymmetrical individuals. Hence, asymmetry is likely to play a vital role in the dynamics of wild fish populations.

The natural dispersal of *Sarotherodon melanotheron* Rüppell, 1852 and *Coptodon guineensis* (Günther, 1862) is limited to West Africa, Benin (Froese & Pauly 2018). The presence of *S. melanotheron* in Mauritania is doubtful and it has not been introduced to any other country in the world (Froese & Pauly 2018). *C. guineensis*, however, was introduced in several countries, e.g. Cambodia (Kottelat 1985), the United States of America (Welcomme 1988), Hawaii (Yamamoto 1992) and Surinam (Kullander & Nijssen 1989), where it has become an established species. The latter species is not considered established in other countries such as Japan and Russia (Welcomme 1988).

An earlier study on bilateral asymmetry in *S. melanotheron* and *C. guineensis* from Lake Ahémé and Porto-Novo Lagoon is that of Jawad *et al.* (2016), which did not consider the otoliths. The present study aims at examining the level of bilateral asymmetry in the mass and size of the otoliths in these freshwater species and, consequently, delivers the first data on this subject.

Materials and methods

Study Area and Sampling Sites

Two sites were sampled: Lake Ahémé and Porto-

Novo Lagoon. Lake Ahémé is situated in southern Benin between 6.20º-6.40ºN and between 1.55º-2.00ºE (Fig. 1). The lake mainly obtains its freshwater input from the Couffo River, its surface area is about ~78 km^2 in the dry season and ~100 km^2 in the wet season (Dissou 1986). It is 24 km long, the northern part is deeper than the southern and it is connected to the sea by the 10 km long Aho channel. Throughout the dry season, the sea water runs into this channel, producing an increase in water salinity in the southern part of the lake (Niyonkuru & Lalèyè, 2012). Porto-Novo Lagoon (6º25 N, 2º38 E) is found south of the political capital Porto-Novo (Fig. 1). It is triangular in shape with an area of ~30 km^2 in the wet season and ~20 km^2 in other seasons. Its length is 6 km and its width varies between 2-4 km. It is linked to the Lagos Lagoon (Nigeria) in the east and to Nokoué Lake in the west. The salinity of the water in the lagoon differs from oceanic via tidal influence to freshwater via several tributaries (Adandédjan *et al.* 2011).

Fish collection

Fish specimens were attained from each locality, collected by fishermen. For the squared coefficient asymmetry, 33 individuals of each species were used from Lake Ahémé and 43 individuals of each species from Porto-Novo Lagoon. Multimesh gillnets (200 m x 1.30 m, 25, 40 and 50 mm mesh) and cast nets (6 diameter, 20 mm mesh) were used to catch the fish. Lake Ahémé was sampled in August 2014 and Porto-Novo Lagoon in February 2015. The depth at all sampling sites ranged from 0.5 to 2.4 m (See Jawad *et al.* 2016). Juvenile fish, which were identified by the gonad stage, were not used in this study due to the incomplete development of their otoliths.

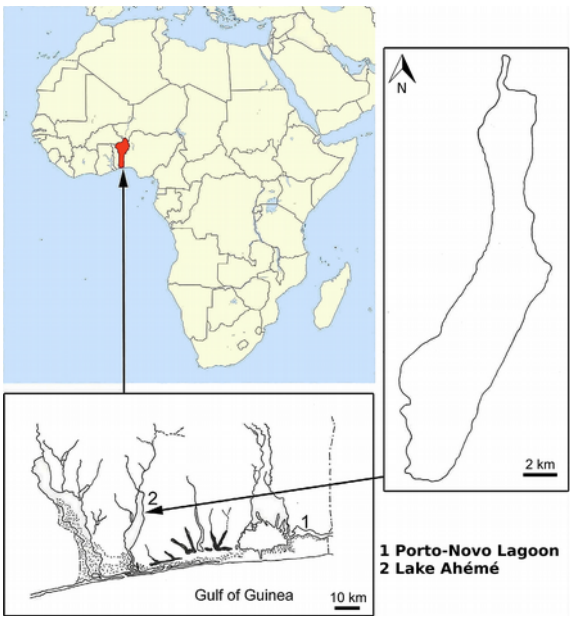

Figura 1. Mapa mostrando los puntos de captura de *Sarotherodon melanotheron* and *Coptodon guineensis* en el lago Ahémé y la laguna de Porto-Novo, Benín.

Figure 1. Map showing capture points of *Sarotherodon melanotheron* and *Coptodon guineensis* in Lake Ahémé and Porto-Novo Lagoon, Bénin.

Otolith mass asymmetry analysis

Standard and total lengths were measured following the method of Jawad *et al.* (2016) preceding to removal of the otoliths. After the dissection of the auditory capsules, the otoliths were detached from each side, washed in distilled water, air-dried at room temperature for a few days, and then weighed on a Sartorius TE 313S analytical balance to accuracy of 0.0001g. Differences between left and right otolith mass values for each species was tested using t-test. Also, differences of otolith weight between males and females was tested using t-test.

The otolith mass asymmetry (X) was computed from $X=(mr-ml)m^{-1}$, where mr and ml are the otolith masses of the right and left paired otoliths and m is the mean mass of the right and left paired otoliths. In theory, the X value can vary between -2 and 2, X=0 represents the absence of mass asymmetry (mr-ml), and X=-2 or X=2 represents the maximal asymmetry (absence of one otolith). A positive value of X means a larger mass for the right otolith mass than the left whereas a negative value signifies the opposite. The relation between the absolute value of X and the otolith growth rate was studied. The relationship between otolith mass and fish total length, $m=al+b$, was calculated in order to assess the otolith growth rate, where m stands for the mass of the otolith, l for the total length of the fish, a for the coefficient characterizing the growth rate of the otolith, and b for a constant for the species in question.

Otolith size analysis

Otolith length and width were measured to the nearest millimetre under a dissecting microscope. The differences between left and right otolith measurements (length and width) were tested using t-test. Also, differences of otolith sizes between males and females was tested using t-test for both species.

The statistical analysis was based on the squared coefficient of the asymmetry variation (CV^2_a) for the two otolith dimensions according to Valentine *et al.* (1973):

$$CV^2_a = (S_{r-l} * 100/X_{r+l})^2$$

where S_{r-l} is the standard deviation of the signed differences and X_{r+l} is the mean of the character, which is calculated by adding the absolute scores for both sides and divided by the sample size. Coefficients of asymmetry were compared between the populations of the two species using ANOVA tests.

Results

Mass asymmetry of otolith

Lake Ahémé

For *S. melanotheron*, the mean value of X is 0.0066±0.0223, n=33 (Fig. 2A) and the mean value of |X| is 0.0167±0.0133, n=33 (Fig. 2B). The difference of the mean otolith mass of *S. melanotheron* between both sides of the fish head in this lake was significant (t-test, p<0.05). There were no differences in otolith weight between males and females (t-test, p>0.05).

According to the regression analysis there was no relationship between fish total length and both X (y=0.05x+0.0037) (p>0.005, R^2=0.0022) and |X| (y=0.0001x-0.0082) (p>0.05, R^2=0.0784). The relation between the otolith mass difference (mr-ml), and the fish length is more complex than the relation between x and fish length (n=33, total length=130-240 mm, p>0.05, y=0.05x-0.0014, R^2=0.0090) (Fig. 2C). For *C. guineensis*, the mean value of x is 0.0086±0.0133, n=33 (Fig. 3A) and the value of |X| is 0.0188±0.0143, n=33 (Fig. 3B). According to the regression analysis, there was no relationship between fish total length and both X (y=0.05X–0.0009) (p>0.005, R^2=0.0009) and |X| (y=0.0001X-0.0083) (p>0.05, R^2=0.0613). The relation between the otolith mass difference (mr-ml), and the fish length is more complex than the relation between x and the fish length (n=33, total length=130-240 mm, p>0.05, y=0.05x-0.0001, R^2=0.0025) (Fig. 3C). In both species, the saccular otolith mass difference increases with the fish length.

Porto-Novo Lagoon

For *S. melanotheron*, the mean value of X is 0.0281±0.0123, n=43 (Fig. 4A) and the mean value of |X| is 0.0127±0.0153, n=43 (Fig. 4B). The difference of the mean otolith mass of *S. melanotheron* between both sides of the fish head in this lagoon was significant (t-test, p<0.05). There were no differences in otolith weight between males and females (t-test, p<0. 05) for both species from this lagoon.

According to the regression analysis, there was no relationship between the fish length, and both X (y=0.05X–0.0079) (p>0.005, R^2=0.0203) and |X| (y=0.05x–0.0033) (p>0.05, R^2=0.0404). The relation between the otolith mass difference (mr - ml), and the fish length is more complex than the relation between X and the fish length (n=43, total length=120-340 mm, p>0.05, y=0.05X-0.0028, R^2=0.0560) (Fig. 4C). For *C. guineensis*, the mean value of X is 0.0355+0.0233, n=33 (Fig. 5A) and the value of |X| is 0.0343±0.0152, n=33 (Fig. 5B2). According to the regression analysis, there was no relationship between the fish length, and both X (y=-0.0003x+0.0103) (p>0.005, R^2= 0.0001) and IXI (y=0.0014x-0.0184) (p>0.05, R^2= 0.0117). The relation between otolith mass difference (mr-ml) and fish length is more complex than the relation between x and fish length (n=33, total length=120-340 mm, p>0.05, y=0.05x-0.0007, R^2=0.0016) (Fig. 5C). In both species, the saccular otolith mass difference increases with the fish length.

Otolith length and width asymmetry

Lake Ahémé

The results of the asymmetry data analysis of the otolith length and width of *S. melanotheron* and *C. guineensis* are shown in the table 1. For both species, the results show that the level of asymmetry of the otolith width was the higher of the two values. The difference of the mean otolith length and width of both species in both localities *S. melanotheron* between both sides of the fish head in this lake were significant (t-test, p<0.05). There were no differences in otolith sizes between males and females (t-test, p<0.05) for this locality. For the two otolith characters studied in both species, the lowest and highest values of asymmetry are found in fish between 130-160 and 211-250 mm total length respectively, whereas the highest percentage of asymmetry was found in the otolith width for both species (Table 1). Individuals of *S. melanotheron* and *C. guineensis* were grouped in length classes (Table 2). The asymmetry values for the otolith length and width tend to increase as

	Character	CV^2_a	N	Character mean ± SD	% of individuals with asymmetry
Lake Ahémé	*Sarotherodon melanotheron*				
	Otolith length	78.5	33	2.7234±0.6	42%
	Otolith width	74.3	33	2.1102±0.3	48%
	Coptodon guineensis				
	Otolith length	80.2	33	2.6561±0.5	58%
	Otolith width	82.3	33	2.2002±0.2	62%
Porto-Novo lagoon	*Sarotherodon melanotheron*				
	Otolith length	95.4	43	3.66700±0.4	76%
	Otolith width	96.3	43	2.1001±0.6	79%
	Coptodon guineensis				
	Otolith length	99.7	43	3.1223±0.3	89%
	Otolith width	101.3	43	2.1334±0.7	90%

Tabla 1. Valor del coeficiente cuadrado de asimetría (CV^2_a) y media del carácter (X_{r+l}) de *Sarotherodon melanotheron* y *Coptodon guineensis* capturados en el Lago Ahémé y la laguna Porto-Novo (Benin)

Table 1. Squared coefficient of asymmetry (CV^2_a) value and character means (X_{r+l}) of *Sarotherodon melanotheron* and *Coptodon guineensis* collected from Lake Ahémé and Porto-Novo lagoon (Benin).

		Character	CV^2_a	N	Character mean	% of individuals with asymmetry
Lake Ahémé	*Sarotherodon melanotheron*	Otolith length				
		130-160	74.8	9	2.5±0.3	0.9%
		161-190	75.2	11	2.5±0.6	2.75%
		191-220	77.8	10	2.6±0.8	2.0%
		221-250	78.1	3	2.8±0.2	3.%
		Otolith width				
		130-160	70.2	9	2.0±0.3	9.0%
		161-190	73.1	11	2.1±0.8	8.8%
		191-220	74.3	10	2.0±0.6	10.0%
		221-250	75.2	3	2.2±0.2	3.0%
	Coptodon guineensis	Otolith length				
		130-160	79.9	8	2.7±0.9	8.0%
		161-190	80.1	10	2.6±0.8	9.5%
		191-220	80.3	10	2.5±0.2	10.0%
		221-250	80.4	5	2.6±0.3	5.0%
		Otolith width				
		130-160	80.3	8	2.3±0.3	8.0%
		161-190	81.7	10	2.2±0.8	9.5%
		191-220	82.3	10	2.1±0.6	9.5%
		221-250	84.2	5	2.4±0.2	5.0%
Porto-Novo lagoon	*Sarotherodon melanotheron*	Otolith length				
		120-160	94.5	5	3.7±0.3	5.0%
		161-200	95.2	7	3.6±0.2	6.65%
		201-240	97.7	9	3.4±0.8	9.0%
		241-280	97.9	10	3.6±0.3	9.5%
		281-320	98.2	9	3.5±0.2	9.0%
		321-360	99.2	3	3.5±0.6	3.0%
		Otolith width				
		120-160	94.1	5	2.1±0.8	5.0%
		161-200	95.3	7	2.0±0.2	7.0%
		201-240	95.6	9	2.1±0.3	9.0%
		241-280	96.2	10	2.1±0.8	9.5%
		281-320	96.5	9	2.1±0.9	9.0%
		321-360	96.9	3	2.1±0.6	3.0%
	Coptodon guineensis	Otolith length				
		120-160	97.2	5	3.1±0.3	5.0%
		161-200	97.6	7	3.0±0.8	6.65%
		201-240	98.4	9	3.3±0.2	8.55%
		241-280	98.5	10	3.2±0.3	9.5%
		281-320	99.2	9	3.3±0.8	9.0%
		321-360	99.6	3	3.2±0.6	3.0%
		Otolith width				
		120-160	98.2	5	2.0±0.2	5.0%
		161-200	98.6	7	2.0±0.3	7.0%
		201-240	99.5	9	2.1±0.2	8.55%
		241-280	99.7	10	2.1±0.6	9.5%
		281-320	99.9	9	2.1±0.8	9.0%
		321-360	100.5	3	2.2±0.3	3.0%

Tabla 2. Valor del coeficiente cuadrado de asimetría (CV^2_a) y media del carácter (X_{r+l}) por clase de edad de *Sarotherodon melanotheron* y *Coptodon guineensis* capturados en el Lago Ahémé y la laguna Porto-Novo (Benin)

Table 2. Squared coefficient of asymmetry (CV^2_a) value and character means (X_{r+l}) by size class of of Sarotherodon melanotheron and *Coptodon guineensis* collected from Lake Ahémé and Porto-Novo lagoon (Benin).

the fish grows and this for both species.

Porto-Novo Lagoon

The results of the asymmetry data analysis of the otolith length and width of *S. melanotheron* and *C. guineensis* in Porto-Novo Lagoon are shown in the table 1, they are similar to those obtained for Lake Ahémé. For the two otolith characters studied in both species, the lowest and highest values of asymmetry are found in fish between 120-60 and 321-360 mm total length respectively, whereas the highest percentage of asymmetry was found in the otolith width for both species (Table 1). Individuals of *S. melanotheron* and *C. guineensis* were grouped in length classes (Table 2). The asymmetry values for the otolith length and width tend to increase as the fish grows and this for both species. There were no differences in otolith weight and sizes between males and females for this locality (t-test, p>0.05).

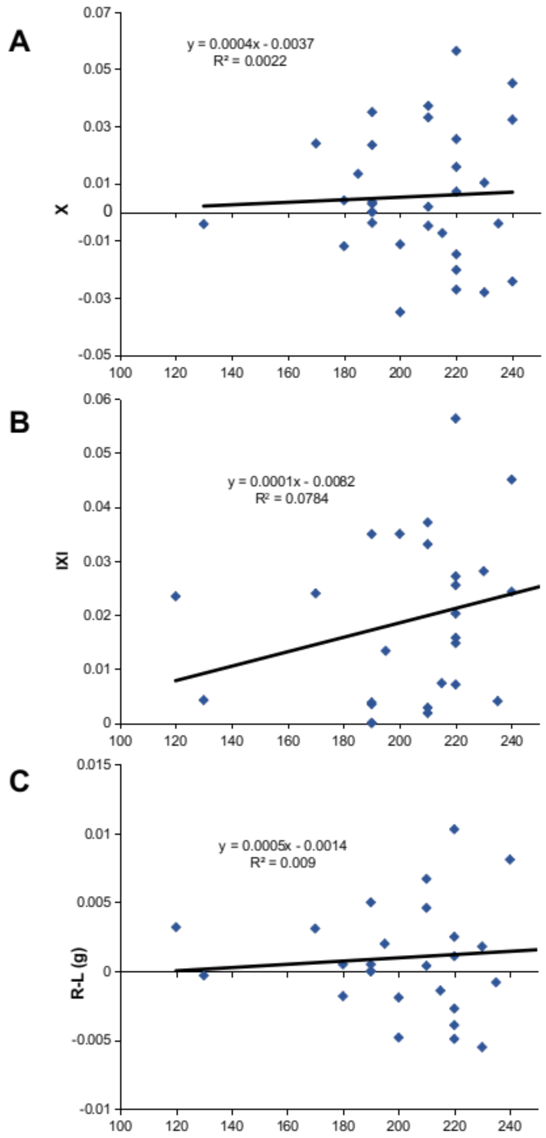

Figura 2. Masa del otolito sacular de *Sarotherodon melanotheron* capturados en el lago Ahémé en función de la longitud total del pez. **A:** Asimetría de la masa (X); **B:** Asimetría absoluta de la masa (|X|); **C:** Diferencia de la masa.

Figure 2. Saccular otolith mass of *Sarotherodon melanotheron* collected from Lake Ahémé as a function of the fish total length. **A:** Mass asymmetry (X); **B:** Absolute asymmetry (|X|); **C:** Mass difference. R & L= right and left side otolith mass.

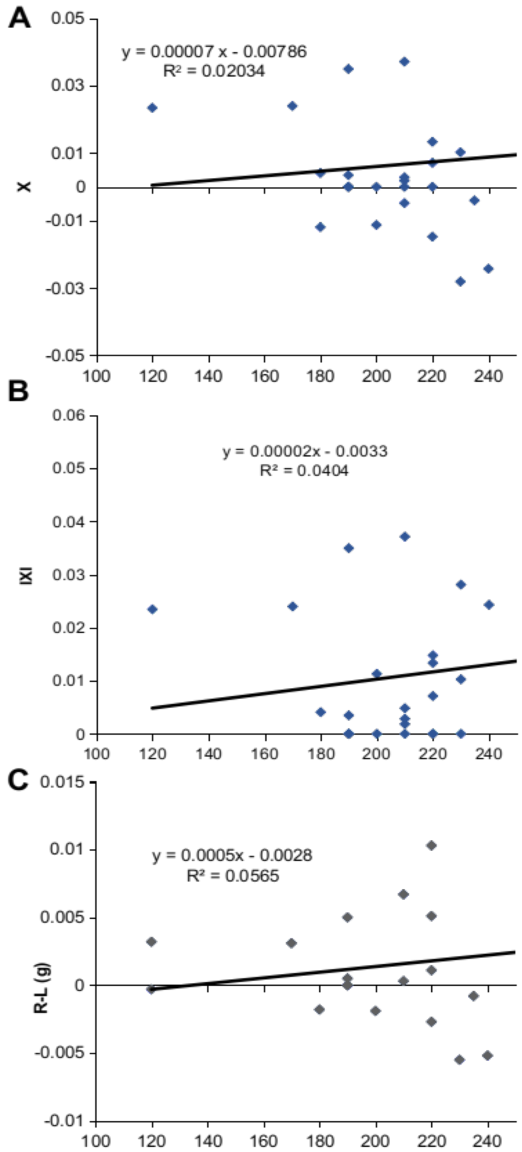

Figura 3. Masa del otolito sacular de *Coptodon guineensis* capturados en el lago Ahémé en función de la longitud total del pez. **A:** Asimetría de la masa (X); **B:** Asimetría absoluta de la masa (|X|); **C:** Diferencia de la masa.

Figure 3. Saccular otolith mass of *Coptodon guineensis* collected from Lake Ahémé as a function of the fish total length. **A:** Mass asymmetry (X); **B:** Absolute asymmetry (|X|); **C:** Mass difference. R & L= right and left side otolith mass.

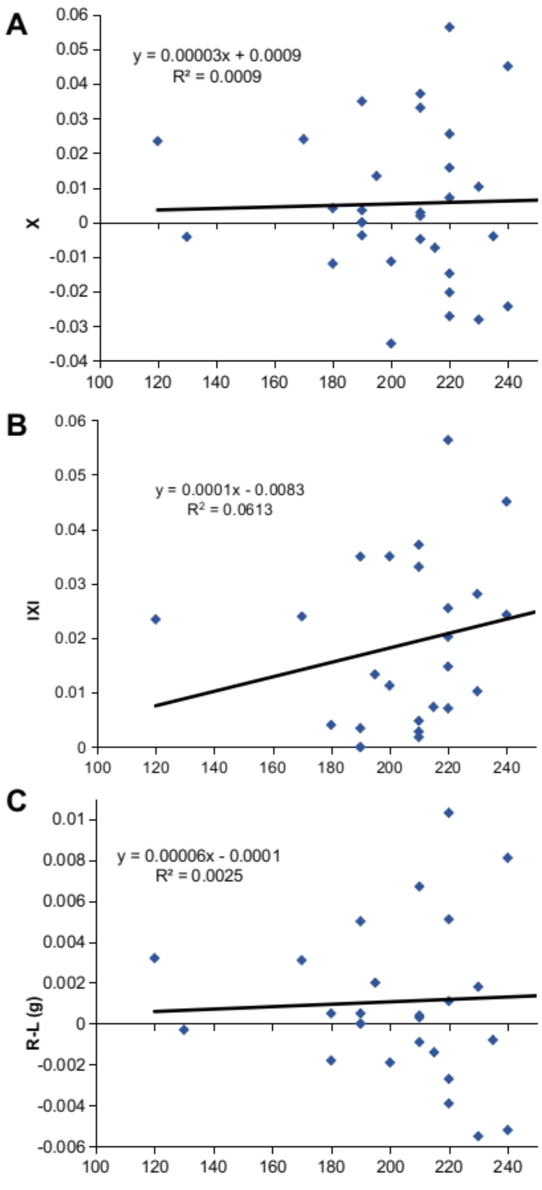

Figura 4. Masa del otolito sacular de *Sarotherodon melanotheron* capturados en laguna de Porto Novo en función de la longitud total del pez. **A:** Asimetría de la masa (X); **B:** Asimetría absoluta de la masa (|X|); **C:** Diferencia de la masa.

Figure 4. Saccular otolith mass of *Sarotherodon melanotheron* collected from Porto-Novo Lagoon as a function of the fish total length. **A:** Mass asymmetry (X); **B:** Absolute asymmetry (|X|); **C:** Mass difference. R & L= right and left side otolith mass.

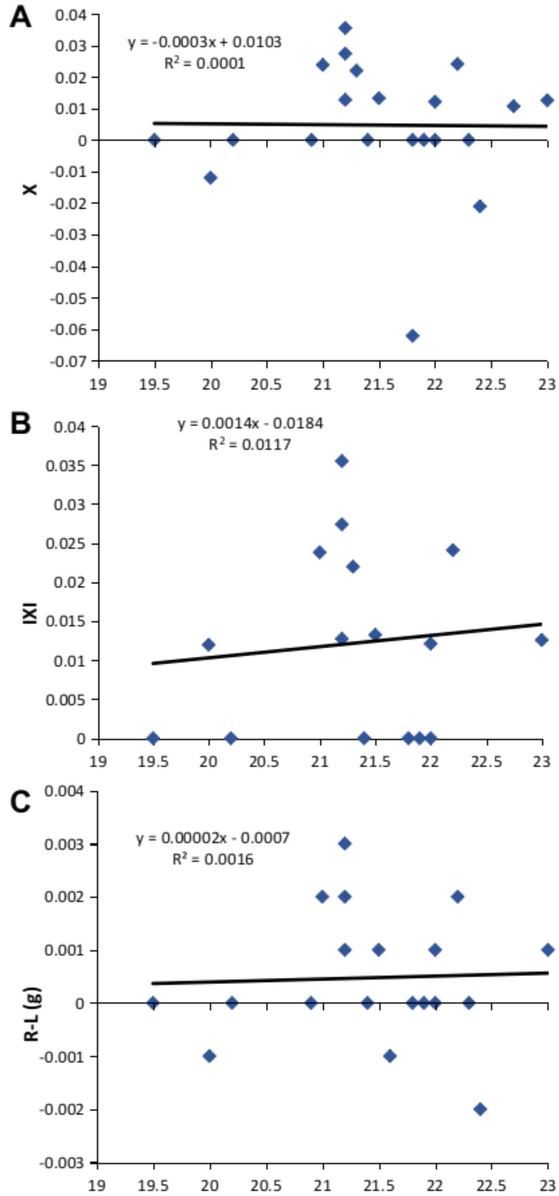

Figura 5. Masa del otolito sacular de *Coptodon guineensis* capturados en laguna de Porto Novo en función de la longitud total del pez. **A:** Asimetría de la masa (X); **B:** Asimetría absoluta de la masa (|X|); **C:** Diferencia de la masa.

Figure 5. Saccular otolith mass of Coptodon guineensis collected from Porto-Novo Lagoon as a function of the fish total length. **A:** Mass asymmetry (X); **B:** Absolute asymmetry (|X|); **C:** Mass difference. R & L= right and left side otolith mass.

Discussion

The otolith mass asymmetry in freshwater fish species has not been investigated well as in the marine species. In certain studies (Scherer *et al.* 2001, Takabayashi & Ohmura-Iwasaki 2003), otolith mass asymmetry was covered as a secondary aim of the investigation by the scientist. The main result obtained by these studies that there are either small differences in mass of the left and right otoliths of the fish (Scherer *et al.* 2001) or small differences as it has been observed by Takabayashi & Ohmura-Iwasaki (2003). The results obtained in the present study showed that there is a large difference between the mass of the otolith found on the left and right sides of the fish head of the species in question. This backs the finding of Scherer *et al.* (2001) on trout, salmon and *Xiphophorus helleri* Heckel, 1848. They suggested that the fish individuals with high otolith mass asymmetry showed an abnormal swimming compared to those with normal otolith. It is not possible to compare our results with those of Takabayashi & Ohmura-Iwasaki (2003) as the later have used asteriscus and lapillus otolithes in their study rather than sagittae. This is because the fish species they investigated are belong to the family Cyprinidae, where the sagitta is smaller than both the asteriscus and lapillus. The results of the work at hand is complete agreement with that of Lychakov *et al.* (2008) on different fish species both freshwater and marine. They obtained a mathematical model showed that relatively large values of otolith mass asymmetry in fishes, which they suggested can affect acoustic performance and may be accountable for irregular fish swimming behavior. They also found that the value of X did not be influenced by fish size (length or mass), systematic or ecological position of the fish. Their results showed that in the bulk they studied, the saccular otolith X was small $|X|<0.05$ (or <5%). Mathematical modeling indicates that values of X vary among individual fish, but that the value is probably stable during a fish's lifetime.

Since there are no similar studies on freshwater fish species like those obtained in the investigation at hand, it preferable to compare them with those results obtained from marine fish species. This will aid the readers to compare otolith mass asymmetry of freshwater and marine species.

Lychakov *et al.* (2006) suggested that otolith mass asymmetry shows similarities and differences among the marine roundfishes and flatfishes (Lychakov *et al.* 2008). They concluded that the saccular otolith mass asymmetry is not influenced by the fish length or the otolith growth rate.

As in other marine fish (Lychakov *et al.* 2008), the value of X attained in the present study falls between -0.2 and +0.2. The saccular otolith mass asymmetry, however, was less than 0.05, a value that concurs with that obtained for a large number of marine species (Lychakov *et al.* 2006). The saccular otolith mass difference increases with the fish length as is the case for other fish species (Lychakov & Rebane 2004). Lychakov & Rebane (2004, 2005) showed in their mathematical model that the acoustic and vestibular functionality of a fish ear can decrease due to otolith mass asymmetry. Nevertheless, in the majority of fish species (Lychakov *et al.* 2006), including those studied, the saccular otolith mass asymmetry is very low ($|X|<0.5$), regardless of the fish length. This low level of otolith asymmetry is characteristic of the utricular and lagenar otoliths. By contrast, Lychakov & Rebane (2005) showed that only fish with the largest otoliths and $|X| > 0.2$ could, in theory, have problems with sound processing due to the unsuitability and incongruity of the movement of the two otoliths on both sides of the head. Therefore, most teleost species can evade a functional disability as they have an otolith mass asymmetry below the critical value.

The effect of otolith mass asymmetry on the vestibular function was studied and explained by Lychakov & Rebane (2000, 2004) in their mathematical model that showed the response of the ellipsoid-shaped otolith to the action of the force of gravity. They calculated the difference between the static displacement of the right and left ellipsoid-shaped otoliths and found that it depends on the otolith mass asymmetry. Such a displacement difference, they concluded, can be the chief cause of the different discharge features of the paired otolithic organs and hence the functional otolith asymmetry.

The results acquired in the present study of the saccular otolith mass asymmetry in *S. melanotheron* and *C. guineensis* show that it does not depend on the fish size. This agrees with the results gained by other researchers on several marine and freshwater fish species (Lychakov & Rebane 2004, 2005, Lychakov *et al.* 2006, Jawad 2013, Jawad & Sadighzadeh 2013, Jawad *et al.* 2011, 2012a, 2012b). However, the relationship between otolith mass difference and fish length is more complex. In the present work, no association between fish length and otolith mass has been found. This is in support with the results attained by Lychakov & Rebane (2004, 2005) on several teleost species. Lychakov *et al.* (2006) suggested that an unproved connection could be due to the small sample used in the study or when specimens do not visibly diverge in size. Both suggestions can be applied to our dataset as only 33 (*S. melanotheron*) and 43 specimens (*C. guineensis*) with total lengths between 130-250 and 120-360 mm LT were used.

Otolith mass asymmetry can negatively impact the life of a fish, so the study of asymmetry is very vital. In the present study, the average otolith mass asymmetry of *S. melanotheron* and *C. guineensis* from the two localities was lower than that obtained on an individual basis. These results show that both populations of the two species are under environmental stress. It has been found that the otolith mass asymmetry of *S. melanotheron* and *C. guineensis* in Lake Ahémé is higher than that in Porto-Novo Lagoon. Therefore, it may be extrapolated that populations of both species in Lake Ahémé are more subjected to pollution or other factors originating from the stress, and this is reflected by the otolith mass asymmetry of the fish. Additional investigations with larger numbers of specimens and a wider range of body size are essential to explore the association between the otolith mass difference and the fish length, especially in view of the absence of studies on otolith mass asymmetry in Benin. This study is offers a starting point for future studies in marine and freshwater fish species in Benin and will allow researchers to make associations between species in Benin and species living in the adjacent areas.

We found that the level of asymmetry is the highest in the otolith width for both *S. melanotheron* and *C. guineensis* ranging in length between 130-250 and 120-360 mm LT and this at both sampling sites. Moreover, the asymmetry values of the length and width of the otolith were higher in Porto-Novo Lagoon than in Lake Ahémé for both species.

A large bilateral asymmetry value for otolith width has earlier been recorded in several fish species (Jawad *et al.* 2012c), which could have to do with the susceptibility of the otolith width to immediate fluctuations in the environment. Therefore, this value could be used as an actual biomarker of stress in the environment. By contrast, the otolith length showed lower bilateral asymmetry values in both species examined, which suggests that this character may be less subjected to environmental stress factors, including pollution. Alternatively, this lower bilateral asymmetry value may be explained by the fact that the developmental period of otolith length may not concur with the presence of adverse environmental events (Jawad 2003).

The obvious effect of bilateral asymmetry in fish otoliths is the abnormal swimming activity (Helling *et al.* 2003) and interference with correct sound localization resulting in the inability of individuals to integrate with the habitat they are living in (Lychakov & Rebane 2005). The capability of young individuals of *S. melanotheron* and *C. guineensis* to find and settle down in a suitable environment can be hindered by the variations in the dimensions of the otolith (Gagliano & McCormick 2004, Gagliano *et al.* 2008). Thus, the settlement of the larvae of the species studied in the present work might be affected by the asymmetry in the morphological characters of their otoliths.

The low bilateral asymmetry value in otolith size at Lake Ahémé can be regarded as usual since biological systems cannot have a perfect bilateral symmetry even in a perfect environment. Minor inconsistencies during development can occur in normal developmental processes (Palmer & Strobeck 1992). These anomalies may be due to the quality and quantity of food, extreme temperatures, parasites, disease and behavioral stress forced by relations with conspecifics (Markov 1995).

Due to the lack of data regarding the natural asymmetry in Benin, it is impossible to evaluate the level of asymmetry of the two morphological characters of the otolith of *S. melanotheron* and *C. guineensis* and to determine whether they are higher or lower than average.

The analysis of variance reveals that bilateral asymmetry values for the otolith mass, length and width, differs significantly between *S. melanotheron* and *C. guineensis* from Porto-Novo Lagoon and Lake Ahémé ($p<0.001$). There is a noteworthy level of pollution by different types of contaminants such as organic pollutants and heavy metals in the areas where fish were collected at both localities (Yèhouénou *et al.* 2013). It is likely that pollution may be responsible for the high bilateral asymmetry values in these areas as frequent studies have confirmed (Elie & Girard 2014). Overall, the levels of toxicity by several trace metals and chemicals is known to upsurge with the increase in both temperature and salinity (Kwok & Leung 2005, Elie & Girard 2014). The average water temperature of both water bodies is similar (28 °C), does not help in understanding the role of the water temperature in an environment of growing toxicity. By contrast, the salinity as a factor enhancing the toxicity by pollutants can be taken into consideration in this case, because the salinity level of Porto Novo Lagoon (>28 g/l) (Gnohossou 2006) is higher than that of Lake Ahémé (<5 g/l) (Niyonkuru 2007).

Developmental disarrays from chemical and organic pollution can lead to severe morphological deformities (Bengtsson *et al.* 1988). In the two localities studied, fish abnormalities were reported, related to heavy metal and organic pollution, in addition to a bilateral asymmetry detected in some morphological characters of both species (Jawad *et al.* 2016).

As shown in preceding investigations (Al-Mamry *et al.* 2011a, 2011b, Jawad *et al.* 2012a, 2012b, 2012c, Mabrouk *et al.* 2014), the ANOVA test analysis in the current study reveals that large specimens of *S. melanotheron* and *C. guineensis* at the two localities have higher bilateral asymmetry values than smaller, younger specimens ($p< 0.001$). It was obvious that the values of fluctuating bilateral asymmetry of the two otolith dimensions, length and width, increased with fish size in both species (Table II). This tendency of increase in the otolith dimensions is probably due to due to the result of imperfect development (Valentine *et al.* 1973). Similar results were attained by Valentine *et al.* (1973), who advocated two possible hypotheses to support such a trend: ontogenetic changes linked to an increase in bilateral asymmetry with size (age) or possible historical processes which result in a secular increase in bilateral asymmetry. However, Thiam (2004) proposed that an increasing tendency of bilateral asymmetry values with fish size may be due to the fact that large size individuals had longer periods of contact with adverse environmental situations and therefore lost their steadiness in such environments. Further studies are needed to support either the suggestion of Valentine *et al.* (1973) or Thiam (2004).

An administration plan is immediately essential in order to reinstate a healthy environment in both Porto-Novo Lagoon and Lake Ahémé. Numerous aquatic plant and animal species within these water bodies have already been harmfully affected (Gnohossou 2006).

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