Ocean and Coastal Research

Original Article
Special Article Collection
Braga, E. S.; Azevedo, J.S.; Harari, J.; Castro, C. G. (Eds.),
Research in a RAMSAR site: The Cananéia-Iguape-Peruibe estuarine-lagoon complex, Brazil.
http://doi.org/10.1590/2675-2824071.23017im
ISSN 2675-2824

Morphometric analysis of lapillus otoliths in two estuarine bioindicator catfish (Siluriformes, Ariidae) from a marine protected area in Brazil

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Abstract

Some fish species can be used as target species and bioindicators of environmental disturbances. In this context, biological indicators such as the length, weight, and height of lapillus otoliths of the ariid species *Cathorops spixii* and *Genidens genidens* were considered bioindicators of contamination of the Cananéia-Iguape estuarine-lagoon complex (CIELC) in this study. The CIELC is an estuary with spatial and temporal differences regarding its hydrobiochemical properties and this characteristic has been related to changes in biological predictors such as the otolith morphometrics of estuarine fish. Therefore, this study aimed to verify the responses of otolith dimensions to sexual and temporal changes, throughout the CIELC. In total, 163 wild catfish *C. spixii* and 55 *G. genidens* were collected during the winter period of 2009, 2017, and 2018 in the CIELC. In general, the weight and length of lapillus otoliths of *C. spixii* and *G. genidens* were good metrics adjusted to fish growth. Females of *C. spixii* were significantly larger than males and subadults and there were no significant differences regarding the sexual approach of *G. genidens*. The temporal approach showed significant differences between the years for both catfish. *C. spixii* sampled in 2009 and 2018 showed greater total length (TL) and total weight (TW) when compared with fish from 2014 and 2017 ($p < 0.05$). *G. genidens* collected in 2018 had greater TL and TW than the specimens sampled in the other years ($p < 0.05$). Otolith dimensions seem to have followed this same pattern, since females of *C. spixii* had higher values for otolith weight, height, and length than males and subadults ($p < 0.05$). The data obtained in this study can be a potential tool for application combined with other ecological analyses and conservation studies in the CIELC.

Descriptors: *Cathorops spixii*, *Genidens genidens*, Otoliths, Growth, Estuary

INTRODUCTION

Otoliths are structures rich in calcium carbonate ($CaCO_3$) found in the inner ear (labyrinth) of all teleost fish, responsible for hearing and balance (Popper et al., 2005; Quist and Isermann, 2017). Bony fish have three types of otolith structures, called sagitta, lapilli, and asteriscus, which differ in their morphology and internal location in the ear capsule (Popper et al., 2005). These structures are formed during embryonic development and continue to grow in incremental layers of $CaCO_3$ in an organic matrix (Campana, 1999). Since fish

Submitted: 18-Mar-2022
Approved: 29-Sep-2023

Associate Editor: Carmen G. Castro

body growth and otolith growth are closely linked (Campana, 1999), the thickness and increment formation, and consequently the shape of otoliths, reflect periods of environmental and physiological stress, despite the fluctuations in fish growth caused by metabolic deceleration (Morales-Nin, 2000). For this reason, as these structures grow, variations in their size and shape can be detected, while maintaining the morphology of the species, since these are species-specific characters (Campana and Casselman, 1993).

The analysis of otoliths, based on traditional measurements related to size, length, and width, as well as weight, has been a particularly significant quantitative tool for studies in the identification of fish species and populations, as well as to elucidate how different individual biological processes, such as ontogenetic development and maturity, and geographic factors affect the shape of otoliths during the life cycle of populations (Campana and Casselman, 1993; Vaz-dos-Santos, 2015). Otolith growth can be used to estimate the size at first maturity. For example, Agostinho (2000) analyzed a method based on the diphasic growth as an alternative method for estimating the size at sexual maturity of females of *Plagioscion squamosissimus* (Perciformes, Sciaenidae). In this context, Maciel et al. (2019) verified that the Huxley and polyphasic allometric models were adequate in revealing differential growth patterns between males and females of the species *G. genidens*. Therefore, understanding the processes involved in sexual dimorphism and maturity, the applications of the morphometrics of lapillus otoliths in *C. spixii* and *G. genidens* is an interesting and accurate tool, combining allometric models and growth assessments. A more accurate assessment of the size at first maturity between male and female growth may help in stock assessment and fisheries management. Different growth patterns are observed throughout the life cycle of fish, detected using growth rates in length and weight and the biometric relationships between different parts of the body, such as otoliths (Casselman, 1990). Therefore, otolith measurements have been used to detect changes in the growth rate of several fish species (Lombarte and Tuset, 2015; Afanasyev et al., 2017; Taştan and Sönmez, 2021). The development, sex, and sexual maturity of fish, in addition to spatio-temporal variations in environmental conditions, may reflect changes in the shapes and growth of otoliths within the same species in different places and times (Duarte Neto, 2005).

Catfish species of the Ariidae family occur in freshwater, marine and estuarine systems in tropical and subtropical regions, and are widely distributed along the Brazilian coast (Figueiredo and Menezes, 1978; Fischer et al., 2011), and are very important for ecological studies regarding ecophysiological responses in environments with saline and temperature gradients, as found in estuaries. In ariids, lapilli otoliths have been commonly used in growth and population biology studies in general, since they are larger than sagittae and asterisci otoliths (Fuchs and Volpedo, 2009; Carvalho et al., 2014; Maldonado-Coyac et al., 2021). In this context, some authors have used lapillus otoliths to determine age and growth in populations of ariids that inhabit the Brazilian coast, such as *Genidens genidens, Genidens barbus, Netuma barba*, and *Sciades proops* (Reis, 1986; Oliveira and Novelli, 2005; Carvalho, et al., 2014; Freire et al., 2017; Maciel et al., 2018, 2019). Some studies on otolith morphometrics in ariids have also been performed to aid in species identification (Chen et al., 2008; Vaz-dos-Santos, 2015), differentiation of sexual dimorphism (Maciel et al., 2019), and as an indication of habit use and movement between regions of the species by chemical evaluation (i.e., Ca/Sr, Ca/Ba) in lapillus otoliths (Avigliano et al., 2015, 2020, 2021; Fortunato et al., 2017; Condini et al., 2019; Maciel et al., 2020). Finally, lapillus otoliths of *C. spixii* have been used to compare with those of the species *G. genidens* (Carvalho et al., 2014), and these structures have been used in assessments of the somatic growth-otolith growth relationship (Azevedo et al., 2019). However, none of these were focused on sexual approach and maturity.

The ariids *G. genidens* (Cuvier, 1829) and *Cathorops spixii* (Agassiz, 1829) are distributed throughout the east coast of South America, from the Guianas to the Rio de la Plata in Argentina, and are one of the most commons ariids on the

Brazilian coast (Marceniuk and Menezes, 2007), especially abundant in coastal lagoons and estuaries (Andrade Tubino et al., 2008; Schmidt et al., 2008; Silva Junior et al., 2013). *C. spixii* has been used to provide response as a bioindicator of contamination in the Brazilian coastal area under different anthropogenic influences (Azevedo et al., 2009; Azevedo and Braga, 2011; Azevedo et al., 2012). In addition, Azevedo et al. (2012) suggested that *G. genidens* has significant potential as a sentinel species for biomonitoring, including the Santos-São Vicente estuarine system, one of the most degraded estuarine systems on the southern Brazilian coast (Meniconi et al., 2012). On the other hand, the Cananéia-Iguape estuarine-lagoon complex (CIELC) is an aquatic ecosystem subject to minimal anthropogenic influence, where *C. spixii* and *G. genidens* have a complete life cycle (Mishima and Tanji, 1981) with current habitat use partitioning models, as expected in the literature (Yànez-Arancibia and Lara-Dominguez, 1988; Carvalho et al., 2014; Torres, 2009).

The development of management criteria and conservation strategies in aquatic environments to improve or protect ecological properties and functions requires the use of biological indicators, such as fish, and target species to assess habitat quality (Chovanec et al., 2003). Thus, studies with bioindicator species, such as the Ariidae representatives *C. spixii* and *G. genidens* (Azevedo et al., 2009; Azevedo et al., 2012; Maciel et al., 2018), are very important, since they can be used to improve knowledge about life history, growth, and habitat use (Avigliano et al., 2014), in association with subcellular biochemical, genetic, and histological responses (Chovanec et al., 2003; Azevedo et al., 2013). Although the CIELC is anthropogenically influenced by continental discharges in the northern area, by the Ribeira de Iguape River inflow via Valo Grande (Mahiques et al., 2009, 2013; Cornaggia et al., 2018), this estuarine system still has characteristics of a healthy ecosystem based on its hydrobiogeochemical characteristics (Chiozzini et al., 2010; Amaral et al., 2021). Therefore, the use of otolith measurements as a predictor of temporal changes in fish from the CIELC is highly valid. In this context, the present study aimed to analyze whether there were sexual and temporal changes in otolith dimensions within the study period based on the responses of otolith dimensions along the Cananéia-Iguape estuarine-lagoon complex (CIELC).

METHODS

Study area and fish collection

The study area is located on the south coast of São Paulo State (SP), in the Cananéia-Iguape estuarine-lagoon complex (CIELC) (25°00’28.27S/47°56’01.89W). This area is part of the Lagamar Mosaic of Conservation Units (Cananéia-Iguape-Peruíbe Environmental Protection Area), recognized as the Atlantic Forest Biosphere Reserve since 1992, and considered a Natural World Heritage site since 1999 (Côrrea, 1995). Since 2017, it has been an international priority area for conservation, as it was recognized as a Ramsar site (https://rsis.ramsar.org/ris/2310). The northern region of the CIELC is mainly influenced by the Ribeira de Iguape fluvial inputs (Prado et al., 2019), and where the salinity is lower, ranging from 10 to 30 (Barcellos et al., 2005; Pecoraro et al., 2018). On the other hand, the southern region is significantly influenced by the ocean and is a region with conserving natural conditions attributed to the major marine influxes (Chiozzini et al., 2010). Wetlands considered Ramsar sites are priority regions and very important for achieving sustainable development via local and national actions and international cooperation, for example. Therefore, all abiotic and biological aspects of the CIELC reinforce the importance of obtaining empirical data on the general biology of resident species, such as the ariids *C. spixii* and *G. genidens*, to promote their conservation and sustainable use.

Due to the differences between the species regarding habitat use and distribution (Mishima and Tanji, 1981; Rios, 2001), *C. spixii* were collected only in the southern region (site 1) and *G. genidens* in the northern region (site 2) of the CIELC (SP, Brazil) (Figure 1). The fish were collected in August 2009, 2014, 2017, and 2018, on board the research vessel Albacora of the Oceanographic Institute of the University of São Paulo (USP), using a bottom otter trawl (1.6” mesh wall, 1.2” mesh cod end, and 11 m long), lasting 10 minutes each, trawling at a mean depth of 6 m at 3 mph. The catfish were immediately separated from the other fish, and the species were identified according to Figueiredo and Menezes (1978) and Marceniuk (2005), considering the main characteristics of the occipital process of the skull and the dentition. All analyzed and collected *C. spixii* and *G. genidens* specimens were authorized by the Chico Mendes Institute for Biodiversity Conservation (ICMBio; SISBIO Processes No. 20398-1; 63087-1) and the recommendations of the Animal Ethics Committee of the Federal University of São Paulo were followed (CEUA-UNIFESP; No. 2597030815; 9841150518). The voucher specimen MZUSP-48529 is deposited in the ichthyological collection of the Museum of Zoology, USP.

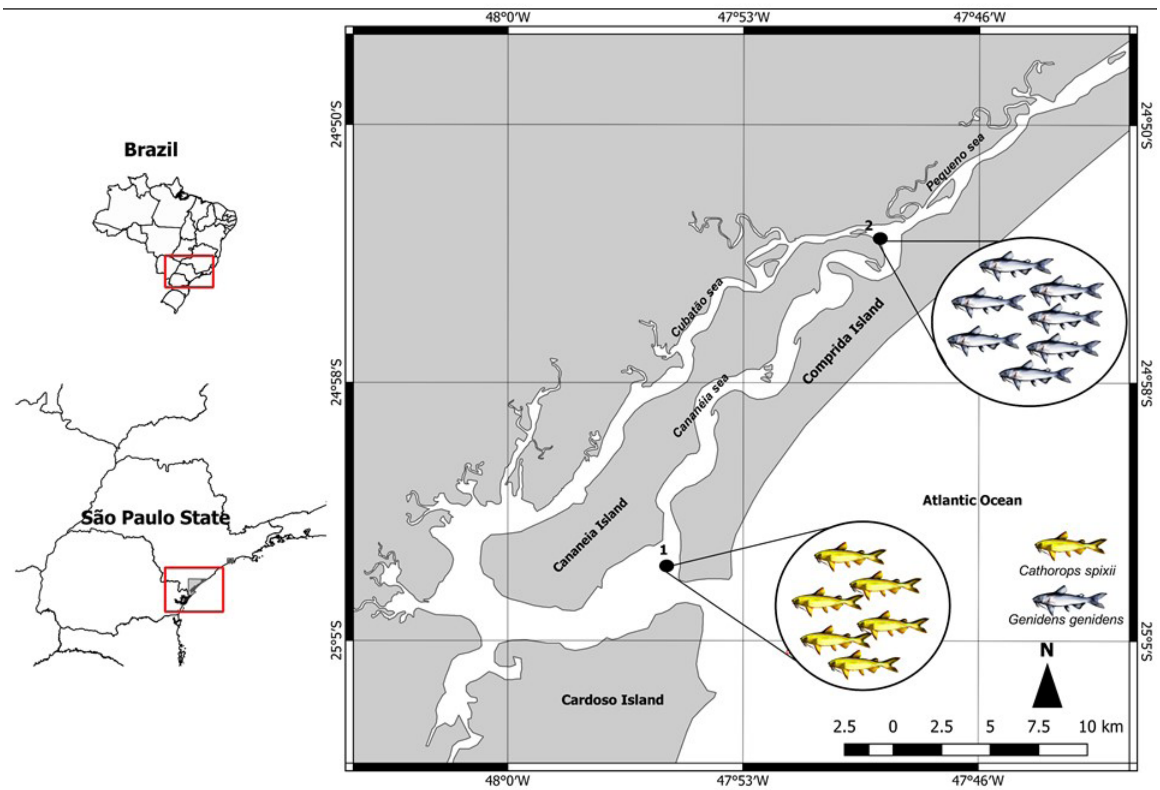

Figure 1. Cananéia-Iguape estuarine-lagoon complex (CIELC), Southeastern Brazil, with sampling sites: 1) South; 2) North.

Laboratory procedure

After collection, the fish were transported to the laboratory of the “Dr. João de Paiva Carvalho” Research Base of the Oceanographic Institute, in Cananéia, using an icebox. Sex identification was performed by identifying the macroscopic, morphological, and color characteristics of the gonads: females – orange gonads, with developing oocytes; males – white-colored tissue in the abdomen. All the fish that did not show a defined gonadal pattern were considered subadults (Fávaro et al., 2005; Gomes et al., 1999), who have passed the free embryo stage, which is the juvenile of the free -swimming juvenile (Lima et al., 2012), as they are below length at first maturation, being 9.66-9.80 cm for *C. Spixii* (Mishima and Tanji, 1983) and 13 cm for *G. Genidens* (Araújo et al., 1998). Therefore, we consider subadult fish to be those that most resemble the morphological characteristics of adult fish (Lima et al., 2012), but which are not yet able to reproduce, as they have not yet reached adult size and reproductive maturity. Despite the low number of subadults of *C. spixii* and females of *G. genidens* collected, these data were recorded and shown, since there is a paucity of morphometric data on these catfish species concerning their sexual approach in marine protected area such as the CIELC.

The total length (TL; cm) and total weight (TW; g) of *C. spixii* and *G. genidens* were obtained, and the catfish were dissected for the removal of the pair of lapilli otoliths from the ear capsule. The lapillus is the most robust pair of otoliths of *C. spixii* and *G. genidens* (Carvalho et al., 2014). Otoliths were extracted using a cross-sectional cut on the inner surface of the fish's skull to expose the inner ear in which otoliths are inserted (Pisam et al., 1998; Popper et al., 2005).

Lapilli otoliths were washed in deionized water (≥ 18.2 MΩ cm) obtained from a Milli-Q water purification system (Millipore), dried, and stored in polypropylene microtubes (2 mL). Otoliths were measured for length (OL: the longest horizontal measurement between the posterior and anterior margins of the otolith), height (OH: measured between the dorsal and ventral margins), and weight (OW) (Figure 2). OL and OH were measured using a manual caliper with an accuracy of ± 0.01 mm. OW was obtained using a digital scale with an accuracy of ± 0.001 mg. The left and right otoliths were included in the collection of the Aquatic Toxicology and Fish Ecophysiology Group (AquaTox) in the Federal University of São Paulo (UNIFESP), Diadema, Brazil.

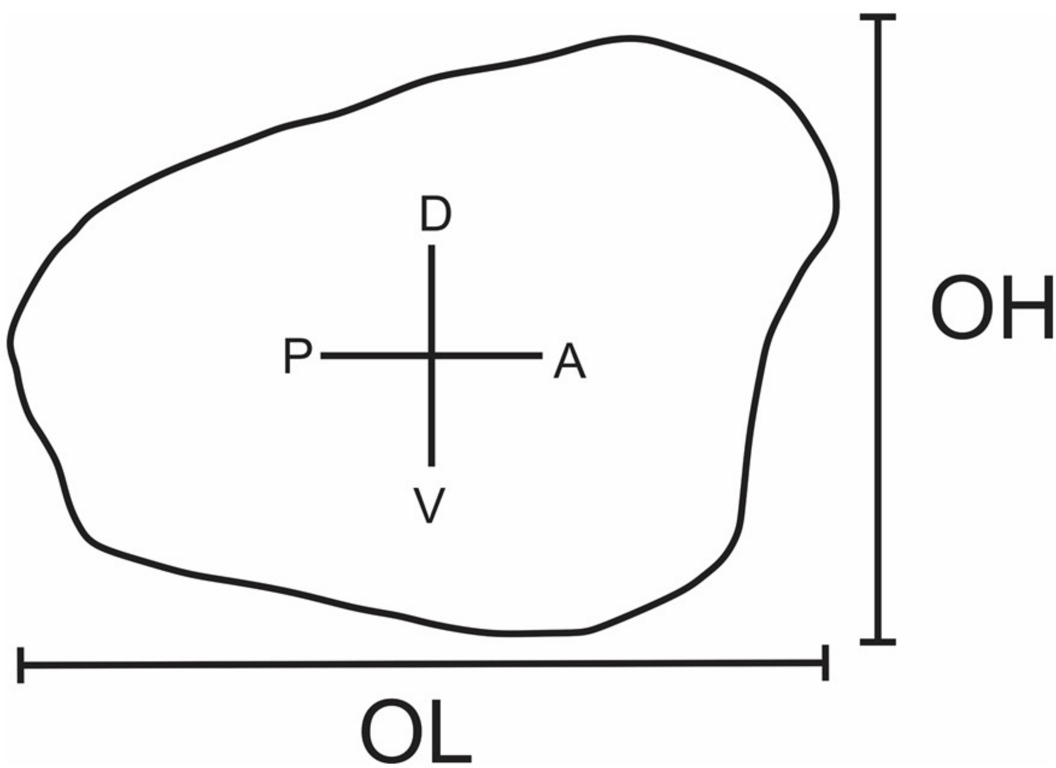

Figure 2. Schematic drawing of the left lapillus otolith of *C. spixii* with identification of measurements and morphological structures. Otolith length (OL) and otolith height (OH). Position relative to the fish: D: Dorsal; P: Posterior; A: Anterior; and V: Ventral.

Data analysis

Non-linear (exponential) regressions were used to determine the relationships between otolith variables (length, height, and weight) and fish weight and length ($Y = a \times X^b$), where a is the intercept of the regression curves and b is the slope of the regression (Harvey et al., 2000; Zar, 2009).

Differences between otolith measurements regarding the sexual and temporal approach of both species were tested using nonparametric analysis of variance (ANOVA) with post-hoc test (Kruskal-Wallis test with Dunn's multiple comparisons). The significance level considered was $p < 0.05$. The dispersion of the data between the response variable and the exploratory variable was verified by R-squared (R^2). Since there was no difference in the measurements of the left and right otoliths (length, weight, and height) ($r_s = 0.95$), in this study, only the left otoliths were processed for morphometric analysis.

The accuracy of the measurements was determined by calculating the mean percentage error. The mean percentage prediction error was calculated as average of the percentage prediction error (%PE) values for all specimens, in accordance with Smith (1980), following the formula:

$$\%PE = \frac{|X_{Predicted} - X_{Observed}|}{X_{Observed}} \times 100$$

RESULTS

In total, 163 wild catfish *C. spixii* and 55 *G. genidens* were collected during the winter period (August) of 2009 (*C. spixii*: n = 17), 2014 (*C. spixii*: n = 27; *G. genidens*: n = 21), 2017 (*C. spixii*: n = 35; *G. genidens*: n = 7), and 2018 (*C. spixii*: n = 33; *G. genidens*: n = 25). Otolith measurements were self-consistent, with %PE ranging from 0.80 (TL x OW) to 20.90 (TW x OL) for *G. genidens* and from 3.35 (TL x OW) to 20.68 (TW x OH) for *C. spixii* (Table 1). Table 2 shows the measurements of the catfish species and the biometric data of lapillus otoliths. In general, females of *C. spixii* were significantly larger than males and subadults ($TL_{females}$ = 29 ± 86 cm; TL_{males} = 20 ± 60 cm; $TL_{subadults}$ = 15 ± 8 cm; $p < 0.05$). On the other hand, no significant differences were observed regarding the sexual approach (females and males) of *G. genidens* ($TL_{females}$ = 23 ± 28 cm; TL_{males} = 21 ± 48 cm; $p > 0.05$). With respect to the temporal approach, *C. spixii* sampled in 2009 and 2018 showed greater total length (TL) and total weight (TW) when compared with fish from 2014 and 2017 ($p < 0.05$). *G. genidens* collected in 2018 had greater TL and TW than specimens sampled in the other years ($p < 0.05$). The same pattern was observed for otolith measurements (Table 2), since females of *C. spixii* had higher values for otolith weight, height, and length than males and subadults ($p < 0.05$); and no statistically significant differences were observed regarding these measurements between males and females of *G. genidens* ($p > 0.05$). In the temporal context, otoliths of *C. spixii* from 2009 and 2018 had greater OW, OH, and OL, while in *G. genidens*, higher values were observed only in otoliths of fish from 2018 (Table 2).

Table 1. Percentage prediction error (%PE) regarding fish size and weight (TL: Total length) and otolith measurements (OW: Otolith weight; OH: Otolith height; OL: Otolith length) for males, females, and subadults and the temporal approach of *Cathorops spixii* and *Genidens genidens* from the CIELC, SP, Brazil.

%PE *Cathorops spixii*							
	2009	2014	2017	2018	Males	Females	Subadults
TL x OL	5.47	9.39	3.59	7.07	7.90	8.13	3.46
TL x OH	12.23	6.06	3.83	6.02	5.04	5.75	2.95
TL x OW	5.03	4.48	3.42	4.74	3.35	4.98	3.57
TW x OL	20.03	19.47	13.84	20.61	20.13	16.91	13.30
TW x OH	19.66	20.68	15.31	19.33	19.66	20.39	14.86
TW x OW	45.41	17.55	13.63	12.12	13.65	19.62	14.63
%PE *Genidens genidens*							
	2009	2014	2017	2018	Males	Females	Subadults
TL x OL	-	8.44	5.92	5.38	9.65	5.63	3.97
TL x OH	-	6.26	2.36	6.17	12.309	5.65	4.45
TL x OW	-	4.51	0.80	5.70	5.54	5.44	4.51
TW x OL	-	20.01	20.90	15.99	17.98	10.56	12.07
TW x OH	-	17.90	11.74	15.33	13.64	12.40	11.46
TW x OW	-	12.81	3.28	16.35	14.46	19.63	10.87

Table 2. Mean and standard deviation of otolith length (OL), weight (OW), and height (OH) —total length (TL) and total weight (TW)— of adults (males and females) and subadults of catfish species.

Group	n	OW (g)	OH (cm)	OL (cm)	TL (cm)	TW (g)
Cathorops spixii						
Females	71	0.54±0.32ᵃ	0.83±0.19ᵃ	1.11±0.22ᵃ	29±16ᵃ	281±200ᵃ
Males	36	0.24±0.20ᵃ	0.65±0.15ᵃ	0.89±0.18ᵇ	20±6ᵇ	92±93ᵃ
Subadults	05	0.12±0.01ᵇ	0.52±0.02ᵃ	0.76±0.04ᶜ	15±8ᶜ	32±7ᵇ
2009	17	0.79±0.17ᵃ	0.99±0.07ᵃ	1.26±0.12ᵃ	358±36ᵃ	447±147ᵃ
2014	27	0.19± 0.11ᵇ	0.63±0.09ᵇ	0.86±0.13ᵇ	190±36ᵇ	71±52ᵇ
2017	35	0.15± 0.05ᵇ	0.57±0.05ᵇ	0.82±0.09ᵇ	168±25ᵇ	52±32ᵇ
2018	33	0.71±0.19ᵃ	0.95±0.07ᵃ	1.25±0.10ᵃ	367±125ᵃ	335±37ᵃ
Genidens genidens						
Females	06	0.32±0.01ᵃ	0.71±0.12ᵃ	0.89±0.07ᵃ	23±28ᵃ	103±36ᵃ
Males	27	0.26±0.16ᵃ	0.73±0.05ᵃ	0.78±0.19ᵃᵇ	21±48ᵃ	84±63ᵃ
Subadults	20	0.13±0.04ᵇ	0.56±0.06ᵇ	0.70±0.08ᵇ	16±22ᵇ	31±12ᵇ
2014	21	0.15±0.04ᵃ	0.64±0.09ᵃ	0.69±0.09ᵃ	170±20ᵃ	38±12ᵃ
2017	07	0.10±0.03ᵃ	0.50±0.05ᵇ	0.62±0.08ᵃ	130±13ᵇ	20±7ᵃ
2018	25	0.31±0.16ᵇ	0.71±0.12ᵃ	0.87±0.14ᵇ	224±44ᶜ	103±60ᵇ

n = number of analyzed fish. For the same variable, different letters indicate statistical differences in the Kruskal-Wallis test at $p < 0.05$.

The relationships between fish and otolith measurements are shown in Figures 3 (fish length and otolith length, height, and weight) and 4 (fish weight and otolith length, height, and weight) for both the sexual and temporal approaches of *C. spixii* specimens. Regarding *G. genidens*, the same relationships are expressed in Figures 5 (fish length and otolith length, height, and weight) and 6 (fish weight and otolith length, height, and weight). Regression analysis shows significant differences ($p < 0.05$) between catfish size and weight (*C. spixii* and *G. genidens*) and otolith measurements (length, height, and weight) (Figures 3, 4, 5, and 6).

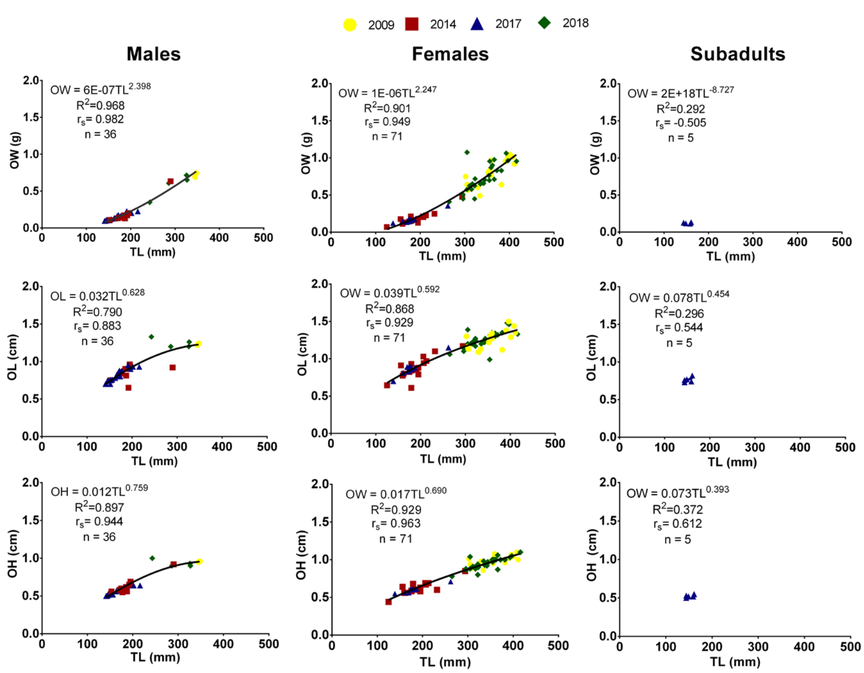

Figure 3. Relationship between fish size (TL: Total length) and otolith measurements (OW: Otolith weight; OH: Otolith height; OL: Otolith length) of males, females, and subadults of *Cathorops spixii* from the CIELC, SP, Brazil.

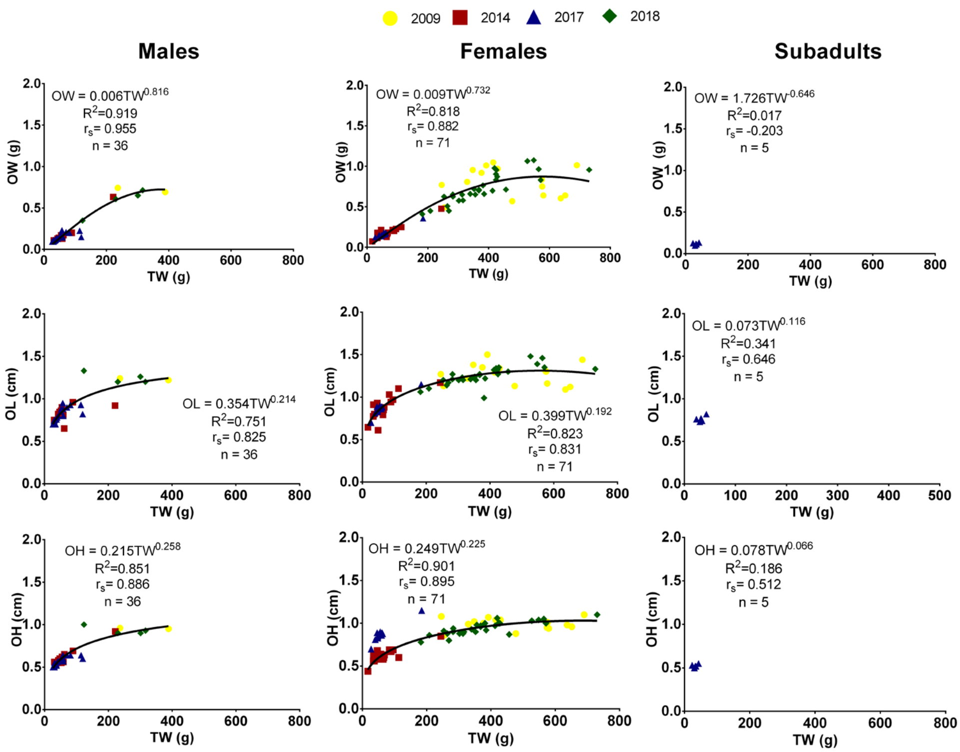

Figure 4. Relationship between fish weight (TW: Total weight) and otolith measurements (OW: Otolith weight; OH: Otolith height; OL: Otolith length) of males, females, and subadults of *Cathorops spixii* from the CIELC, SP, Brazil.

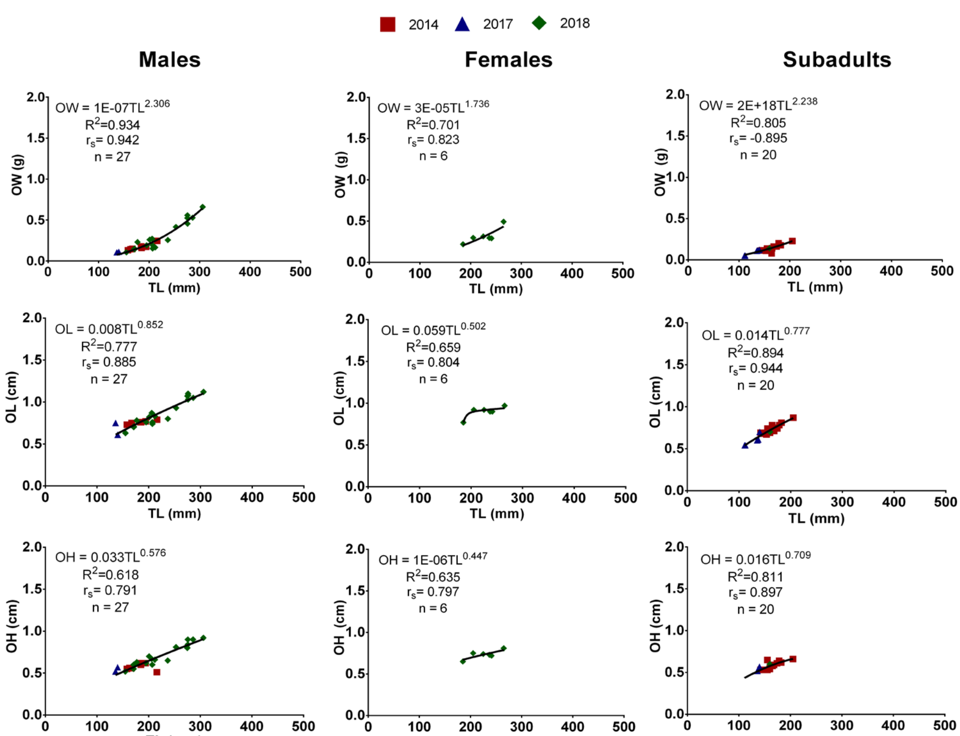

Figure 5. Relationship between fish size (TL: Total length) and otolith measurements (OW: Otolith weight; OH: Otolith height; OL: Otolith length) of males, females, and subadults of *Genidens genidens* from the CIELC, SP, Brazil.

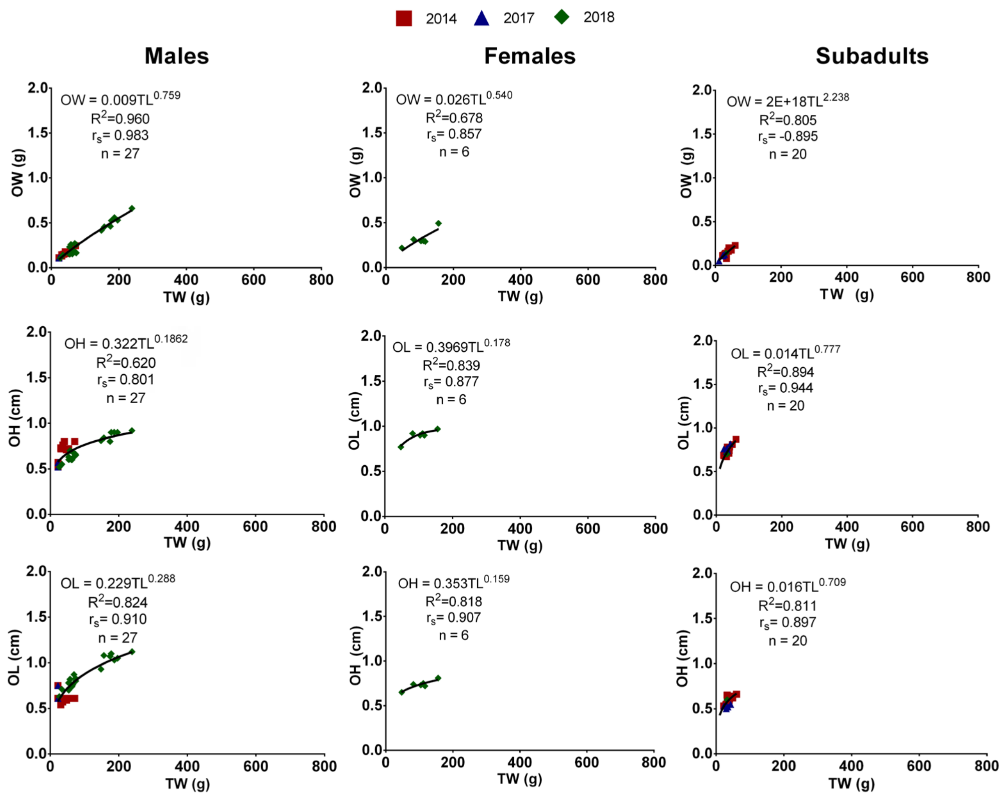

Figure 6. Relationship between fish weight (TW: Total weight) and otolith measurements (OW: Otolith weight; OH: Otolith height; OL: Otolith length) of males, females, and subadults of *Genidens genidens* from the CIELC, SP, Brazil.

Regarding *C. spixii*, there were no significant differences in weight and height between males and females of *C. spixii*, except that otolith length of females was greater than that of males and subadults ($OL_{females}$ = 0.94 ± 0.26; OL_{males} = 0.82 ± 0.20; $OL_{subadults}$ = 0.61 ± 0.17). Subadults were lighter than males and females (OW_{males} = 0.24 ± 0.17; $OW_{females}$ = 0.36 ± 0.29; $OW_{subadults}$ = 0.09 ± 0.03). It was possible to observe that otolith length and weight of females of *C. spixii* were greater compared with males (Figures 3 and 4). Regarding *G. genidens*, the same pattern was observed, with no significant sex differences in otolith weight, length, and height between males and females (Figures 5 and 6). In fact, the number of females was very low, and this aspect may have influenced these results. Subadults of *G. genidens* had a lower weight than adults (OW_{males} = 0.24 ± 0.17; $OW_{females}$ = 0.36 ± 0.29; $OW_{subadults}$ = 0.09 ± 0.03). The temporal approach showed significant differences within the study period for both catfish species *C. spixii* (Figures 3 and 4) and *G. genidens* (Figures 5 and 6).

DISCUSSION

In this study, we could verify the potential of applying regression analysis for testing morphometric relationships between the total length and lapillus otolith dimensions of adults and subadults of *Genidens genidens*. In general, otolith weight, length, and height were good metrics adjusted to fish growth, as has also been reported in other studies with the same species. Although using adjusted linear regression analysis, Carvalho et al. (2014) also found high correlation coefficient values between otolith height, weight, and length versus fish biometrics when analyzing the same species studied from another location. Otolith length of the catfish *C. spixii* and *G. genidens* proved to have a stronger relationship with fish length than otolith weight (Figures 3 and 5). This relationship has been previously demonstrated in the literature (Avigliano and Volpedo, 2016; Winkler et al., 2019; Ozpicak et al., 2021).

In this study, we intended to analyze whether there are sexual and temporal changes in otolith dimensions of the two ariid species *C. spixii* and *G. genidens*. Some studies have shown that, although *G. genidens* does not present evident sexual dimorphism demonstrated by body morphometrics, significant differences are observed, mainly in head and mouth measurements, related to the oropharyngeal incubation of offspring performed by males (Paiva et al., 2015). In addition, studies using otolith measurements of *G. genidens* found differences between the sexes with respect to growth parameters such as length and age rings (Maciel et al., 2018).

An important highlight of this study is the lack of significant differences regarding otolith height in adults (males and females) and subadults of catfish species (Figures 3, 4, 5, and 6). In fact, in some cases, it is possible that otolith weight fits better to fish length and height, since otoliths stop growing in size when the fish reaches its maximum length. Therefore, once the fish reaches its asymptotic length, the otolith mainly increases in weight. Thus, otolith height does not grow proportionally to fish growth and these results also may suggest that, in the case of these catfish species, the variable is not reliable for growth studies.

As dispersion can indicate the degree of variation in otolith biometric data, the dispersion found in the morphometric relationship of *C. spixii* and *G. genidens* may indicate the effect of temporal variability. In this sense, it is possible that there is more than one cohort collected with a difference in otolith growth rate, since the specimens collected in 2009, 2014, 2017, and 2018 were exposed to different environmental conditions (Amaral et al., 2021). Some studies have reported the environmental effect on otolith growth (Lombarte and Lleonart, 1993a; Sadovy and Severin, 1994; Chen et al., 2008). Morais and Azevedo (2021) reported the existence of abnormal age rings in lapillus otoliths of *C. spixii* from the same sampling site of this study, the region of greatest marine influx in the CIELC, indicating the potential effect of environmental conditions of this coastal region on age ring formation.

Estuarine ecosystems are highly dynamic environments, subject to changes in the water regarding salinity, pH, dissolved oxygen levels, and temperature. These abiotic changes promote environmental modifications in the habitat that are reflected in fish growth (Bøeuf and Payan, 2001). Some authors have reported changes in the growth of otoliths of other species collected in different coastal regions (Buttler et al., 1996; Dehghani, 2016). These variations in otolith growth could be due to differences in habitat, food availability, and physiochemical factors of the environment waters (Aydin et al., 2004; Javor et al., 2011). Furthermore, the difference in otolith growth may be related to the changes in life history between juveniles, subadults, and adults.

The majority of subadults of *C. spixii* were collected in the northern region of the CIELC. These specimens had a total length above first maturity (10.6 to 16.1 cm) compared with those reported by Mishima and Tanji (1983) of 9.6 and 9.8 cm. Therefore, this result is interesting to be assessed regarding the presence of subadults in both regions of the CIELC (north and south), as well as the variation in length at first maturity in this species over the years, since it can respond to environmental variables (Lombarte and Lleonart, 1993b; Ozpicak et al., 2021). Moreover, the variation in length at first maturity may be related to the difficulty in separating subadults from those starting a new reproductive cycle. Especially when the assessment of length at first maturity is based on analyses of gonadal maturity, as in the present study and the study by Mishima and Tanji (1983).

Finally, the results presented here are fundamental for the advancement of age and growth studies in catfish such as *C. spixii* and *G. genidens*, since the proportionality between fish and otolith weight and length was verified. Although otolith length of *C. spixii* and *G. genidens* had a strong relationship with fish length, for this information to be effectively used, especially with regard to fishery stock assessments, the relationship has to be linked to fish age. To achieve this, further studies would be needed to assess how otolith growth relates to fish growth. Important perspectives that can be explored by this study refer to the combined use of otolith morphometrics with other analyses, such as otolith microchemistry, to investigate the life history and habitat use of these bioindicator catfish species.

CONCLUSION

The morphometric data of lapillus otoliths of these catfish species, regarding the temporal approach, evidenced the potential of these metrics to predict temporal variations in aquatic ecosystems with differential hydrobiogeochemical characteristics such as those observed in the CIELC.

ACKNOWLEDGMENTS

The authors would like to thank the Oceanographic Institute of the University of São Paulo (IOUSP), Brazil, and the team of the IOUSP research vessel Albacora for their support in fish sampling. This study was funded in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Brazil – Finance Code 001 (Morais, I.S.).

AUTHOR CONTRIBUTIONS

I.S.M.: Methodology; Formal analysis; Investigation; Writing – original draft; Writing – review & editing.

E.S.B.: Writing – review & editing; Resources; Supervision.

J.S.A.: Conceptualization; Formal analysis; Data curation; Writing – review & editing; Supervision.

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