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Analysis of the 90º and 150º angles for increment counting in otoliths of estuarine catfish

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Abstract

Fish age can be estimated with the use of age rings in calcified structures, such as otoliths. However, it is important to select an appropriate axis for counting the number of increments and to confirm that the age rings are visible in the otolith and interpreted correctly. In this study, the distance between consecutive age rings in the *lapillus* otoliths of *Cathorops spixii* and *Genidens genidens* catfish from the Cananéia-Iguape Estuarine–Lagoon Complex was measured along otoliths at predetermined 90° and 150° axes. In general, the number of increments observed per otolith ranged from 1 to 14 for both species. The positive linear relationship between the total length of the fish and the radius of the age rings suggests that otolith size is a reliable somatic proxy of *C. spixii* (=0.872) and *G. genidens* (=0.896). The axes chosen to measure the distances of each increment were proven accurate, especially the straight axis. Our study indicates that the 90º and 150º angles can be used for increment counting, but caution is required for otoliths with the same growth morphology: potentially confusing increments are more prevalent along the 150º axis, and this may hinder increment analysis of *G. genidens* and *C. spixii*. Therefore, the 90º axis provides better visualization of the closest opaque and translucent zones and should be prioritized for increment counting in the *C. spixii* and *G. genidens* ariids.

Descriptors: *Cathorops spixii*, *Genidens genidens*, Age Rings, Otolith Radius, Biomonitoring

The catfish belonging to the Ariidae family are widely distributed near the coast, in estuaries, inland and marine tropical and temperate waters (Marceniuk and Menezes, 2007). Ariids such as *Cathorops spixii* (Agassiz, 1829) and *Genidens genidens* (Cuvier, 1829) are distributed throughout the eastern coast of South America, where they are especially abundant in coastal lagoons and estuaries (Andrade Tubino et al., 2008; Schmidt et al., 2008; Silva Junior et al., 2013). In general, ariids have a benthic feeding habit, thus maintaining an intimate association with the sediment (Figueiredo and Menezes, 1978). Thereby, these species have been considered to be sentinel species, responding to contamination issues in the Brazilian coast, which is under various anthropogenic influences (Azevedo et al., 2011, 2013), present in environments such as the Santos-São Vicente estuarine system (Azevedo et al., 2009), one of the most degraded on the southern Brazilian coast, and the Cananéia-Iguape Estuarine Lagoon–Complex - CIELC (Azevedo et al., 2012a;

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Amaral et al., 2021), an aquatic ecosystem subject to minimal anthropogenic influence (Meniconi, 2012), but under the influence of human-made materials coming from the Ribeira de Iguape region, located mainly in the northern region of the CIELC (Schmidt et al., 2008; Souza et al., 2012; Mahiques et al., 2013; Amaral et al., 2021).

Despite the scarcity of more current data, Mishima and Tanji (1981) considered that *C. spixii* and *G. genidens* have a complete life cycle in the CIELC (25°00'28.27S/47°56'01.89W). *G. genidens* are found in greater number in further northern areas of this system, where there are major continental and anthropic influences, mainly by the Ribeira de Iguape fluvial inputs (Prado et al., 2019), and where salinity ranges from 0 to 16 (Barcellos et al., 2005; Pecoraro et al., 2018; Amaral et al., 2021). Conversely, *C. spixii* is distributed throughout the estuary, but more abundantly in its southern region (Mishima and Tanji, 1981), where marine influxes are more accentuated (Chiozzini et al., 2010; Aguiar et al., 2013). The CIELC is part of the Mosaic of Conservation Units of Lagamar (Cananéia-Iguape-Peruibe Environmental Protection Area), which is recognized as the Biosphere Reserve of the Atlantic Forest since 1992 and considered a Natural Heritage of Humanity since 1999 (UNESCO, 2011). This mosaic was designated as a Ramsar site in 2017, and has been an international priority area for conservation ever since (RAMSAR, 2017). Understanding the ecological and biological aspects, such as age and growth, of sentinel ariids, is very important, since these animals' responses to damages and injuries, used as contamination biomarkers (ei. Micronuclei, enzymatic changes, pathological responses and metal input and biotransformation - Azevedo et al., 2012a, 2012b, 2013; Amaral et al., 2021), can be altered by factors such as the animals' age (van der Oost et al., 2003). However, there are few studies on these species in this system (Denadai et al., 2013; Maciel et al., 2018, 2019). Bioecological data collected in the CIELC, such as increments in ariids' calcified structures, are important, as they will enrich the biological database for future and integrated analyses in this area, which has been biomonitored using *Cathorops spixii* as a contaminator bioindicator since 2004 (Azevedo et al., 2009, 2012a, 2012b, 2013, 2019; Pecoraro et al., 2018; Amaral et al., 2021; Morais and Azevedo, 2021). This database will be useful to assist decision-makers in the environmental assessment and conservation of local biodiversity (Amaral and Jablonski, 2005).

Age estimation is the basis for calculating growth rate, mortality rate, and productivity (Campana and Thorrold, 2001; Mendoza, 2006). Fish age can be estimated by counting and interpreting structural patterns that occur in calcified structures such as scales, spines, and otoliths. Otoliths are calcareous structures rich in calcium carbonate ($CaCO_3$) found in the inner ear (labyrinth) of all teleost fish and responsible for their hearing and balance. These structures grow throughout their lifetimes and act as a permanent life history record. There are three types of otolith structures: *sagitta, lapilli*, and *asteriscus*. They differ in their morphology and location in the internal ear of fish (Popper et al., 2005).

In fish from the Ostariophysi superorder, such as ariids, the *Lapilli* otoliths are the most used in age-estimation studies. These structures are larger and more robust than the sagitta otoliths and, therefore, are preferential for growth and age estimation in these taxa (Assis, 2005; Santificetur et al., 2017). It has already been observed that, in the *lapillu*s otolith, the narrow opaque zones represent slow growth, while the wide hyaline or translucent zones represent rapid growth. For instance, some researchers have used the *lapilli* otolith to estimate the age and growth of ariids from different regions, such as *Bagre panamensis,* from the United States, California (Maldonado-Coyac et al., 2021), *Arius maculatus,* from Thailand (Phaeviset et al., 2021), and *Plicofollis tenuispinis*, *Netuma bilineata*, *Netuma thalassina*, and *Plicofollis dussumieri,* from Kuwaiti waters (Al-Husaini et al., 2021). In Brazil, some researchers have studied *G. genidens* and *G. barbus* (Reis, 1986; Oliveira and Novelli, 2005; Gibbs et al., 2013; Maciel et al., 2018). For the *Cathorops spixii* species, there is still a scarcity of data on the analysis of the age rings in *lapillus* otoliths (Azevedo et al., 2019).

This report is, therefore, fundamental: it provides descriptions of increments or age rings present in the *lapillus* otoliths of the *Cathorops spixii* species, which helps to estimate the age of these animals. For age validation, the first essential step is the selection of an appropriate counting path or axis to assess the number of increments, which is an important component of any age and growth study, and to confirm that the age rings are visible and being interpreted correctly (Campana and Thorrold, 2001).

In total, 100 wild *C. spixii* catfish and 33 *G. genidens* were collected during the winter periods (August) of 2017 (*C. spixii* – n=41; *G. genidens* – n=07) and 2018 (*C. spixii* – n=59; *G. genidens* – n=26). *C. spixii* (TL: 13.5-41.6 cm) and *G. genidens* (TL: 11.1-30.6 cm) were dissected to allow the removal of the *lapilli* otolith pair from the auditory capsule. The *Lapillus* is the most robust otolith pair of *C. spixii* and *G. genidens* (Santificetur et al., 2017). Only the left otoliths were processed for rings analysis (observation of opaque and translucent increments) and sectioned transversally, to a cross-section thickness of approximately 3 mm, using a low-speed metallographic saw (Buehler Isomet) (Morais and Azevedo, 2017, 2021). The left otoliths were deposited in the collection of the Group of Aquatic Toxicology and Fish Ecophysiology (AquaTox), in the Universidade Federal de São Paulo (UNIFESP), Diadema, Brazil. The sections were then embedded in water and observed under transmitted light using a Zeiss Discovery V20 stereo microscope under 40X magnification and photographed using the AxioVision 4.8 program. There were no significant differences between the otolith measurements of male and female C. *spixii* from the two regions (northern and southern) of the CIELC (t-test, $p>0.05$). For this reason, otolith measurements of both sexes were pooled. The distance (mm) between consecutive rings was measured along the otolith at pre-determined 90° and 150° axes (Figure 1), in order to be compared with the literature (Cheraghi et al., 2015; Hauser et al., 2018; Maciel et al., 2018; Flinn et al., 2019; Maldonado-Coyac et al., 2021). The validation of the periodicity of formation of the age rings has not been conducted for these catfish species from the CIELC. Therefore, the ring groups were only treated as $CaCO_3$ increments.

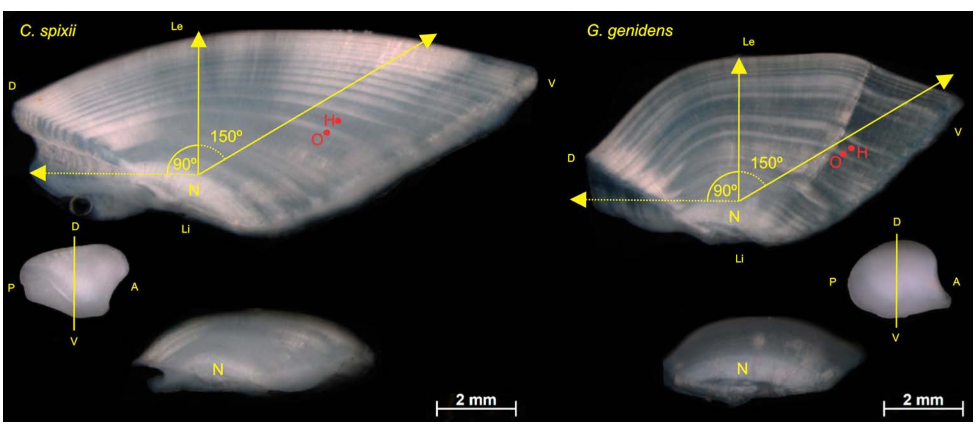

Figure 1. Cross-section of the left *lapillus* of *Cathorops spixii* and *Genidens genidens* under reflected light. Structures: nucleus (N), translucent zone (H), opaque zone (O), dorsal (D), ventral (V), posterior (P), anterior (A), internal lateral face (Li), and external lateral face (Le) (positions relative to the fish). The dashed line indicates the orientation of the otolith section. The red dots indicate the growth rings and the measured axis.

In total, 100 *C. spixii* otoliths were analyzed, of which 20 (20%) were discarded because they could not be interpreted. For the same reason, 6 (20%) *G. genidens* otoliths were discarded, while 27 were analyzed. The maximum number of increments observed per otolith ranged from 1 to 13 (0.17–4.4 mm from the core) for *C. spixii* and from 1 to 14 (0.30–3.31 mm from the core) for *G. genidens* (Table 1). The positive linear relationship between the total length of the fish and the radius of the age rings (Figure 2) suggests that otolith size is a reliable somatic proxy of C. *spixii* (R^2=0.872) and *G. genidens* (R^2=0.896). These results confirm that growth rings are visible in the *lapilli* otolith of *C. spixii* and *G. genidens* from the CIELC, which makes it possible to determine this species' ages, a fact corroborated by Carvalho et al. (2014), Santificetur et al. (2017), Azevedo et al. (2019), Morais and Azevedo (2021). The use of *lapilli* otoliths for age estimation in other sea catfish species has also been made by Reis (1986), Cheraghi-Shevi et al. (2015), Al-Husaini et al. (2021) and Phaeviset et al. (2021), and Hauser et al. (2018) applied this method to freshwater catfishes.

Table 1. Summary of descriptive statistics of the average radius (mm) of otolith increments in *Cathorops spixii* and *Genidens genidens* from the CIELC, Brazil. Rn: number of rings (Mean ± SD, standard deviation) of otolith increments for 90º and 150º axes.

	Cathorops spixii		*Genidens genidens*	
Rings	**90º**	**150º**	**90º**	**150º**
R1	0.68±0.19	1.02±0.29	0.66±0.22	0.96±0.33
R2	1.18±0.30	1.96±0.54	1.22±0.32	1.85±0.48
R3	1.55±0.37	2.76±0.74	1.65±0.37	2.50±0.52
R4	1.79±0.45	3.31±0.80	2.00±0.43	3.06±0.66
R5	2.14±0.43	3.91±0.87	2.45±0.47	3.69±0.70
R6	2.38±0.37	4.50±0.73	2.70±0.49	4.09±0.82
R7	2.67±0.25	4.99±0.49	2.98±0.37	4.49±0.47
R8	2.79±0.26	5.29±0.59	3.38±0.09	5.10±0.19
R9	2.98±0.33	5.47±0.64	3.59±0.05	5.32±0.25
R10	3.39±0.39	5.87±0.81	3.73±0.06	5.33
R11	3.54±0.60	6.55±0.69	3.85	5.58
R12	3.66±0.62	6.75±0.96	3.99	5.70
R13	3.31	6.38	4.07	5.84
R14	-	-	4.44	5.95

As observed in other studies, the axes chosen to carry out the measurements of the distances between increments proved to be accurate (especially the straight axis) (Cheraghi et al., 2015; Hauser et al., 2018; Maldonado-Coyac et al., 2021) and were, therefore, used in this study. Hauser et al. (2018) and Maldonado-Coyac et al. (2021) reported similar types of rings in *lapillus* otoliths of the *Brachplatystoma rousseuxii*, a freshwater catfish from the Orinoco River, and the *Bagre panamensis*, a freshwater catfish from the southeast of the Gulf of California. Hauser et al. (2018) reported that the stress caused by the increased salinity in the Orinoco River during August and September, competition for food and long periods of starvation were critical factors in the formation of ring anomalies. Regarding *C. spixii* collected in the same area of our study (the CIELC), Morais and Azevedo (2021) have reported anomalies in the age rings of *lapillus* otoliths of *C. spixii*, indicating a possible relationship between these anomalies and environmental stressors, such as increased salinity, in the Cananéia-Iguape estuarine region. Although this has not been tested on the ariids from the CIELC, previous evidence does link salinity stress to anomalies on age rings (Hauser et al., 2018). Given the particular results of our study and its notable differences from previous studies, it is important to recommend cross-sectioning for age estimation, which helps reduce errors, since our study and its methods are more suitable for age estimation in *C. spixii*. Maciel et al. (2018) suggest the same for the *G. genidens* species.

Increment counting in otoliths does not have to be performed along a straight line —it can be useful to change the count axis (Campana and Thorrold, 2001). Our study suggests that the 90º and 150º angles can be used for increment counting, but caution is necessary for otoliths with the same growth morphology, since potentially confusing increments are more prevalent along the

150º axis, which may hinder increment analysis in *G. genidens* and *C. spixii*. Moreover, the same pattern of distance between otoliths in *C. spixii* and *G. genidens* that contain more than eight increments or age rings increases the difficulty in visualizing them, due to the approximation of the opaque and translucent zones. This can lead to underestimation in the age ring count. Therefore, we suggest using the 90-degree axis when evaluating these ariid species.

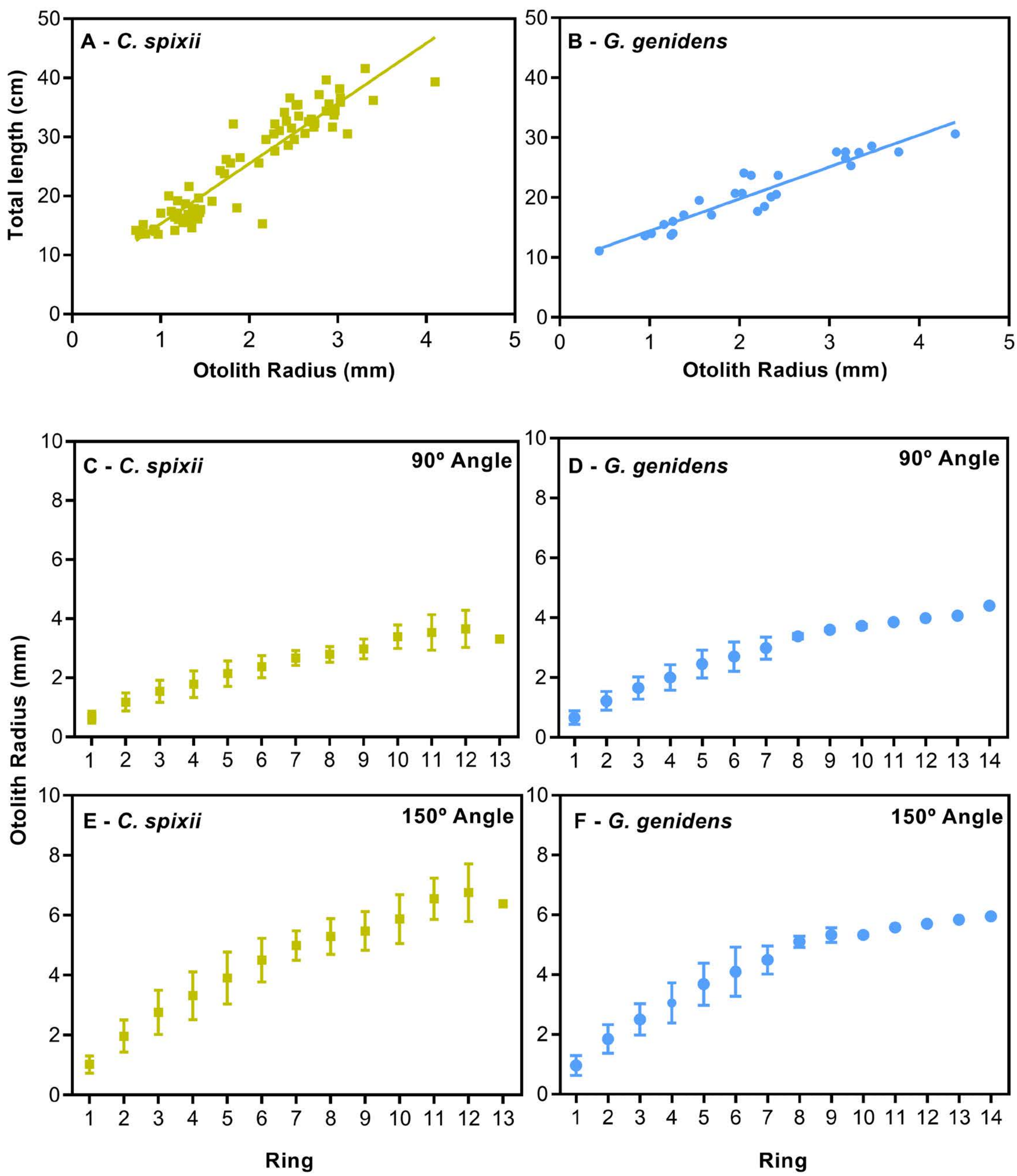

Figure 2. Scatterplots correlating otolith variables. A - B: correlation between the total length of the fish and the radius of the rings. C – F: correlation between ring radius (mean with SD) of *lapillus* of *Cathorops spixii* (yellow color) and *Genidens genidens* (blue color) from the Cananéia-Iguape Estuarine-Lagoon Complex.

Despite not being validated in this study, the periodicity of age ring formation in *C. spixii* and *G. genidens* from the CIELC, along with previous studies on age and growth in ariid catfish, indicate that one age ring is formed per year following the reproductive cycle (Mishima and Tanji, 1981; Gomes and Araújo, 2004; Oliveira and Novelli, 2005). Conversely, Maciel et al. (2018) reported the formation of two rings per year in the *lapillus* otoliths of *G. genidens* from the Guanabara Bay (Rio de Janeiro, Brazil), which is related to the species' reproductive cycle and period of lower metabolism due to cooler water temperatures. This reinforces the importance of carrying an analysis of the periodicity of the rings in the two species from the CIELC, since this area has fluctuating environmental conditions due to continental input in the northern portion and marine flow in the southern portion (Chiozzini et al., 2010; Azevedo and Braga, 2011; Azevedo et al., 2013; Amaral et al., 2021), which can lead to the formation of abnormal age rings.

During the interpretation of the otolith increments in *C. spixii* and *G. genidens*, some difficulties were encountered: 1- the lesser thickness of the increments in the 7-8 ring made measurements difficult and even impossible in some otoliths, which may increase the variance of measurements in these rings; 2- the reading of the first ring was particularly difficult due to the opacity in the initial phase of otolith growth (Maciel et al. (2018) also state that accurately verifying the approximation of the rings may be difficult in otoliths with more than 10 increments); 3- a 90º angle is better for visualization and counting in species such as *G. genidens*, due to the lower variation and opacity of their increments.

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AUTHOR CONTRIBUTIONS

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

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

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