BOLETIM DO INSTITUTO DE PESCA

ISSN 1678-2305 online version

Short Communication

THE FIRST REPORT OF ABNORMAL AGE RINGS IN OTOLITHS *LAPILLUS* OF ARIIDS CATFISH

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Received: September 25, 2020
Approved: February 23, 2021

ABSTRACT

The present work aimed to record the first presence of abnormal age rings in *Cathorops spixii lapillus* otoliths from Cananeia-Iguape Estuarine-Lagoon Complex (CIELC), Southern region of Brazilian coast. In August 2018, 59 specimens of *C. spixii* (Siluriformes, Ariidae) were collected during one station sampling in the northern (n = 25) and another in the southern sector (n = 33) of CIELC. In general, among the otoliths that presented age ring alterations, this divergent zone was observed in opaque and translucent layers, on the right side, between the fifth and seventh age rings.

Keywords: *Cathorops spixii*; age rings; Cananeia; estuaries

PRIMEIRO REGISTRO DE ANEIS ETÁRIOS ANORMAIS EM OTÓLITOS *LAPILLUS* DE BAGRES ARÍDEOS

RESUMO

O presente trabalho teve por objetivo apresentar o primeiro relato de anéis etários anormais nos otólitos *lapillus* de *Cathorops spixii* do Complexo estuarino-lagunar de Cananéia-Iguape (CELCI), região sul do litoral brasileiro. Em agosto de 2018, 59 espécimes de *C. spixii* (Siluriformes, Ariidae) foram coletados durante uma estação de amostragem no setor norte (n = 25) e outra no setor sul (n = 33) do (CELCI). Em geral, entre os otólitos que apresentavam alterações nos anéis de idade, essa zona divergente foi observada em camadas opacas e translúcidas, do lado direito, entre o quinto e o sétimo anéis de idade.

Palavras-chave: *Cathorops spixii*; anéis etários; Cananéia; estuários

INTRODUCTION

Otoliths are calcified structures, formed by alternate layers of aragonite and protein, which are continually deposited throughout the lifespan of the fish (Campana and Thorrold, 2001). Three structures (*sagitta*, *lapillus* and *asteriscus*) are found in the internal ear of all teleosts and they are immersed in a liquid called endolymph (Pisam et al., 1998). These calcified structures were known exclusively for the fact that they indicate aspects of the life cycles of these organisms (Campana and Thorrold, 2001; Mendoza, 2006).

Studies about the life cycle of fish show the importance of otoliths as tools of analysis in fisheries science and ichthyofauna characterization (Pracheil et al., 2019; Winkler et al., 2019; Santana et al., 2020). In this context, some characteristics of otoliths have been commonly used and applied to understand the life history of different fish populations, such as morphometry (Morat et al., 2018), morphology (Bostanci et al., 2015; Sanchez and Martinez, 2017) and chemical composition (Avigliano et al., 2017; Rogers et al., 2019). In addition to these, the counting of translucent and opaque zones is emphasized to estimate the age and growth of most teleosts, supporting the understanding of the population dynamics of a given species (Sparre and Venema, 1998; Santana et al., 2020).

The translucent (hyaline) and opaque zones are formed as a consequence of the differential removal and deposition of calcium carbonate ($CaCO_3$) in the protein matrix and are called age rings (Wright et al., 2002; Mendoza, 2006; Thomas and Swearer, 2019). Numerous studies have shown that physiology, age, food energy content and environment affect the composition otoliths growth (Mendoza, 2006; Mille et al., 2016). It can be seen that opaque layers are formed during periods of slow growth and translucent layers are formed during periods of fast growth (Wright et al., 2002).

These growth rings provide a long-term record of the life history of fish by incorporating elemental differences in water chemistry (Campana and Thorrold, 2001). Therefore, otoliths provide a permanent record of environmental changes because they are physiological static accumulations of calcareous material and are, at no point, reabsorbed (Campana and Thorrold, 2001; Panfili et al., 2002).

This alternating formation of the hyaline and opaque bands in otoliths is regulated by the fish physiology, reproduction period, spawning and migration (Grønkjær, 2016; Morrison et al., 2019). In addition, the formation of the age rings can be influenced by abiotic parameters of the environmental, for instance food availability, diet composition, temperature and hydrochemical stress (Barnes and Gillanders, 2013; Mille et al., 2016; Thomas and Swearer, 2019). Therefore, the influence of these factors in the growth process of otoliths at certain times in the species' life cycle can create different types of growth profiles in opaque bands (Katayama, 2018).

Catfish are individuals of the Ariidae family with occurrence in freshwater, marine and estuarine systems in tropical and subtropical regions (Figueiredo and Menezes, 1978). These fish have a benthic feeding-habit, therefore, maintain an intimate association with the sediment (Figueiredo and Menezes, 1978). Concerning catfish from the Brazilian Coast, some studies showed the capacity of the catfish *Cathorops spixii* (Agassiz, 1829) to accumulate some trace metals in different tissues for instance muscle, liver and gills (Azevedo et al., 2009, 2012a), and to supply response as a bioindicator species in the coastal environment under different anthropogenic influences (Azevedo et al., 2012b).

C. spixii have been used since 2004 as a bioindicator species for the purpose of monitoring and the environmental diagnosis of estuarine environments on the São Paulo coast, such as Cananeia-Iguape Estuarine-Lagoon Complex (CIELC) and the Santos-São Vicente estuary and (Azevedo et al., 2012a, 2012b; Pecoraro et al., 2018). However, concerning the biological aspects of *C. spixii*, such as age and growth, there are few studies in this species from the coastal area of São Paulo State (Brazil), especially in CIELC (Denadai et al., 2013; Azevedo et al., 2019).

Cananeia is part of the Mosaic of Conservation Units of Lagamar (Cananeia-Iguape-Peruibe Environmental Protection Area), recognized as the Biosphere Reserve of the Atlantic Forest since 1992, considered a Natural Heritage of Humanity since 1999 (UNESCO, 2011); since 2017, it has been an international priority area for conservation as it was included as a Ramsar site (RAMSAR, 2017). *C. spixii* has a complete life cycle in this estuarine system (Mishima and Tanji, 1981;) and is considered abundant in an area that still has characteristics of preserved regions, that is, the southern region of the area, significantly influenced by the ocean and, therefore, conserving natural conditions attributed to the major marine influxes (Chiozzini et al., 2010). However, individuals of *C. spixii* are also found in the northern area of this estuary (Mishima and Tanji, 1981). In this region, the salinity is smaller, ranging between 10 and 30 (Barcellos et al., 2005; Pecoraro et al., 2018) and is notorious for its major continental and anthropic influences, mainly by the Ribeira de Iguape fluvial inputs (Prado et al., 2019). The aim of this work was to examine the existence of abnormal age rings in *Cathorops spixii* otoliths from the Cananeia-Iguape Estuarine-Lagoon Complex (CIELC) and report it.

MATERIAL AND METHODS

In August 2018, 59 specimens of *C. spixii* (Madamango sea catfish) were collected during one station sampling in the northern (24°53.902'S; 47°48.609'W) (n = 25) and in another in the southern sector (25°01.958'S; 47°54.873'W) (n = 33) of the CIELC (São Paulo State, Brazil) (Figure 1). Catfish were collected on board the *Albacora* research vessel by the Oceanographic Institute of São Paulo University. To catch the fish, a bottom otter trawl net (1.6" mesh wall and 1.2" mesh cod end) 11 m length was used. After collection, the fish were identified following Figueiredo and Menezes (1978), considering the main characteristics of the occipital process of the cranium and dentition. The total length (TL - cm) of *C. spixii* were obtained, and, finally, the catfish were dissected for the removal of the pair of *lapilli* otoliths from the auditory capsule. *Lapillus* is the most robust pair of otoliths of *C. spixii*, since *sagitta* and *asteriscus* are smaller and more fragile. The otoliths were extracted using a cross-section cut on the internal surface of the fish's cranium to expose the inner ear in which the otoliths are inserted (Pisam et al., 1998; Popper et al., 2005).

The otoliths were washed in Mili-Q water, dried and stored in polypropylene microtubes (2 mL) for later laboratory processing. *Lapilli* otoliths were measured concerning the length (OL), height (OH) and weight (OW). OL and OH were performed using a manual caliper with ± 0.01 mm of precision. However, their weight was obtained using a digital balance with ± 0.001 mg of precision. Comparison of the biometric data (height, length and weight) between left-right sides of otoliths was done using the F test (F). Since an absence of statistical differences were observed ($OH_{left\ versus\ right}$: $R^2 = 0.9881$; F = 4586, $p < 0.0001$ / $OL_{left\ versus\ right}$: $R^2 = 0.9775$; F = 2389; $p < 0.0001$ / $OW_{left\ versus\ right}$: $R^2 = 0.9977$; F = 38716, $p < 0.0001$), only the left otoliths were processed for rings analysis, that is the opaque and translucent zone observations). The nucleus of the otoliths was then marked with graphite, embedded in polyester resin for 48 hours, and sectioned transversally to a cross-section thickness of approximately 3 mm using a low-speed metallographic saw (Buehler Isomet). The sections were then embedded in water and observed under transmitted light using a stereo microscope Zeiss Discovery V20 under 40X magnification and photographed using the AxioVision 4.8 program. The left otoliths were deposited in the collection of the Aquatic Toxicology and Ecophysiology of Fish Group (AquaTox) in the Universidade Federal de São Paulo (Unifesp), Diadema, Brazil. All analyzed and collected *C. spixii* specimens were authorized by ICMBio (Instituto Chico

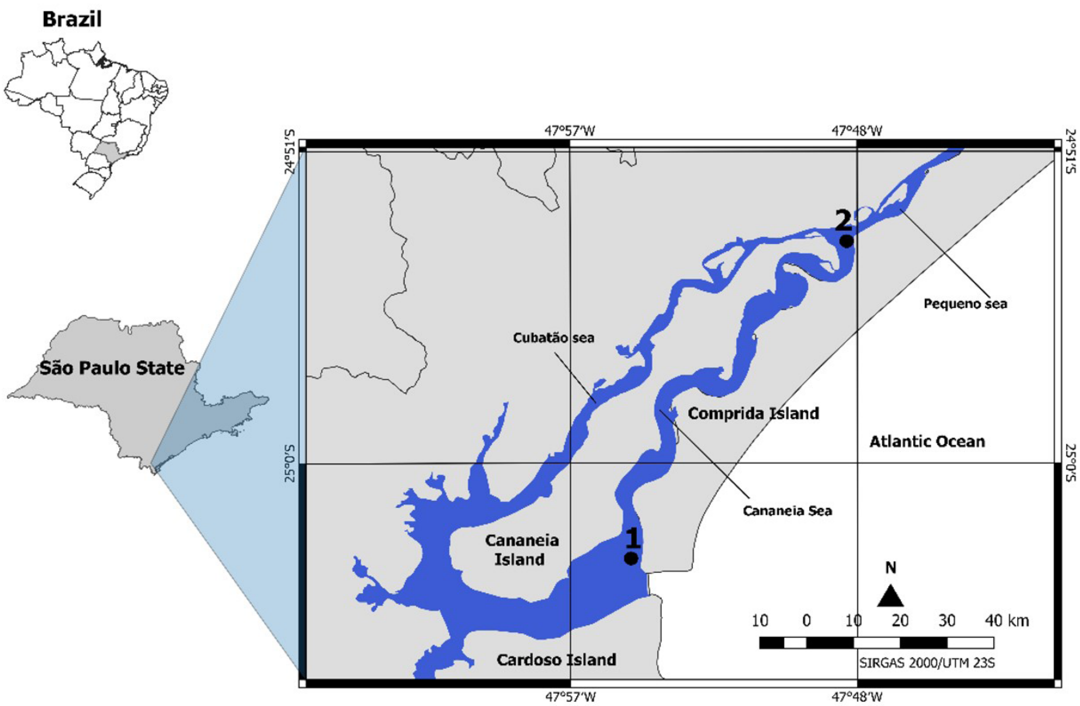

Figure 1. Studied area with sampling fish collection in two sites (1 - southern and 2 - northern) of CIELC, São Paulo State, Brazil.

Mendes de Conservação da Biodiversidade (Sisbio process nº 63087-1). The voucher species MZUSP-48529 is deposited in the ichthyological collection of the Museu de Zoologia da USP.

RESULTS AND DISCUSSION

The *lapillus* otoliths of *C. spixii* has a dorsal body and posterior wedge-shaped region, narrowing from the lateral to the medial side (Figure 2). These otoliths have more length than height (Table 1).

There were no significant differences between males and female's otoliths measurements of *C. spixii* from the two regions (northern and southern) of the CIELC (t-test, $p > 0.05$) (Table 1). For this reason, otolith measurements of both sexes were pooled.

Considering otoliths measurements of *C. spixii* from CIELC, in general, the OL ranged from 0.55 to 1.48 cm, the OH ranged from 0.50 to 1.10 cm, and the OW from 0.088 to 1.075 g. A Mann-Whitney test revealed statistically significant differences ($p < 0.0001$) between the morphometric data of the catfish otoliths between the individuals from the northern and southern areas of the CIELC (Table 2). The exponential regression applied to the relationship between the length of the fish and the morphometric *lapillus* otoliths measurements showed a relative growth profile (Table 2). A positive and significant correlation was found between the measures of the fish and otoliths ($TL_{versus}OH$: $r_s = 0.9238$, $p < 0.0001$ / $TL_{versus}OL$: $r_s = 0.9056$, $p < 0.0001$ / $TL_{versus}OW$: $r_s = 0.9624$, $p < 0.0001$), indicating that the otolith has uniform in length and weight dimensions.

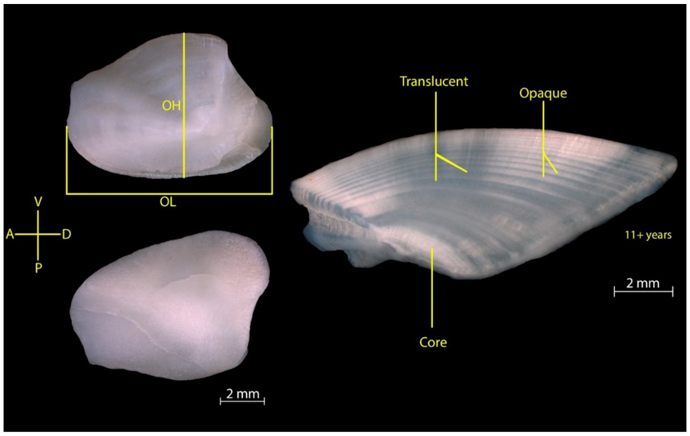

Figure 2. *Lapillus* entire and transverse section (without abnormal age rings) otolith of *C. spixii* from the Cananeia-Iguape Estuarine-Lagoon Complex (CIELC). Image: 40x magnification. OL: otolith length; OH: otolith height; D: Dorsal; V: Ventral; P: Posterior; A: Anterior. Yellow arrow: identification of translucent, opaque rings and the core.

In this study, age rings in otoliths were considered as $CaCO_3$ increments since the periodicity of age rings formation has not been validated. However, previous studies on age and growth in catfish Ariidae indicate the formation of 1 age ring per year in accordance with the reproductive cycle (Mishima and Tanji, 1981; Gomes et al., 1999; Oliveira and Novelli, 2005; Dantas et al., 2010; Maciel et al., 2018) and, therefore, this standard was used as reference in this study. Otoliths having abnormal layers are

Table 1. Statistical results (mean ± standard deviation and *p*-values) of males and females *lapillus* otoliths of catfish from the two sites (northern and southern) of Cananeia-Iguape Estuarine-Lagoon Complex.

	Northern		
	Males (n = 9)	**Females (n = 14)**	***p*-value**
OH	0.62±0.02	0.59±0.02	0.5214
OL	0.80±0.02	0.81±0.03	0.9523
OW	0.188±0.021	0.167±0.028	0.5943
	Southern		
	Males (n = 4)	**Females (n = 29)**	***p*-value**
OH	0.93±0.02	0.95±0.01	0.6891
OL	1.25±0.03	1.25±0.02	0.9105
OW	0.580±0.081	0.731±0.034	0.1246

OH: otolith height, OL: otolith length, OW: otolith weight. Statistical differences were tested by t-test at $p < 0.05$. Juveniles were not included in the table due the sample size (Northern: n = 2; Southern: n = 0).

shown in Figure 3. This data is the first record of this type of abnormal age rings in *lapillus* otoliths *C. spixii*. Concerning the frequency of alterations in the age rings, catfish from the northern site showed no changes. However, 48% of the *C. spixii* from the southern area showed changes in the age rings. In general, this divergent zone was observed to be isolated between opaque and translucent layers, on the right side, between the fifth and seventh age rings.

With respect to the external morphology of *C. spixii lapillus* otoliths, the uniform growth in length and weight dimensions was also observed by Carvalho et al. (2014) in the same species from the estuarine and coastal areas of the Parana State (Brazil). The presence of abnormal age rings was observed mainly in the sixth ring of the *lapillus* otoliths of *C. spixii* from individuals collected in the southern area of CIELC. The presence of the abnormal rings may prevalence in older fish, because it was found in the largest otoliths from *C. spixii* captured in the southern portion.

Table 2. Biometric descriptors (mean ± standard deviation and minimum and maximum values) in the *lapillus* otoliths and their relationship with the length of *C. spixii* from the southern and northern sites of Cananeia-Iguape Estuarine-Lagoon Complex.

		OL (cm)	OH (cm)	OW (g)
Southern		1.25±0.10^{a} (0.99 – 1.48)	0.95±0.73^{a} (0.78 – 1.10)	0.713±0.182^{a} (0.347 – 1.075)
Northern		0.84±0.13^{b} (0.55 – 1.12)	0.66±0.12^{b} (0.50 – 0.92)	0.242±0.151^{b} (0.088 – 0.661)
	TL (cm)	**OL = aTL^{b}**	**OH = aTL^{b}**	**OW = aTL^{b}**
Southern	33.50±3.75	$OL = 0.362\ TL^{0.353}$	$OH = 0.227\ TL^{0.407}$	$OW = 0.001\ TL^{1.936}$
Northern	19.59±5.06	$OL = 0.187\ TL^{0.513}$	$OH = 0.103\ TL^{0.621}$	$OW = 0.001\ TL^{2.053}$

TL: total length of catfish, OL: otolith length, OH: otolith height, OW: otolith weight. Different letters indicate statistical differences Mann-Whitney test at $p < 0.0001$.

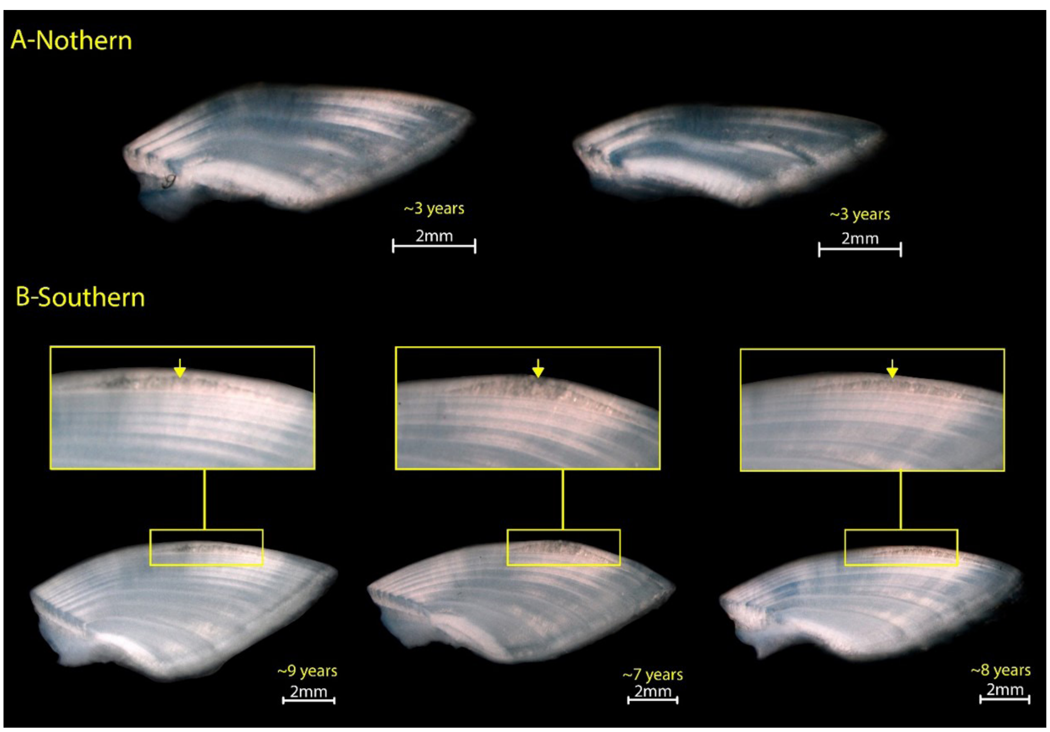

Figure 3. Transverse section of *C. spixii* otoliths from the southern area of CIELC. Yellow arrow: abnormal age rings.

In general, the formation of the age rings can be influenced with several factors, both endogenous and exogenous, which can promote a modification in the development of the individual fish, creating changes in the density and thickness of the calcium carbonate ($CaCO_3$) layers (Morales-Nin, 1987). It is possible that endogenous factors such as reproduction and spawning, and besides abiotic signals for instance water temperature, food availability and diet composition, or another abiotic stress, may induce or disrupt the increment and formation of age rings (Morales-Nin, 1987; Mille et al., 2016).

Furthermore, these factors can promote a modification in the development of different polymorphs of calcium carbonate crystals ($CaCO_3$) in the protein matrix (Vinagre et al., 2014). While aragonite is the norm for *sagittae* and *lapilli*, most *asteriscii* are made of vaterite, thus accounting for their glassy appearance (Fallini et al., 2005). However, studies have reported there is more than one crystallization of calcium carbonate in the same structure otolithic, or also simultaneous deposition, named aberrant or anomalous otoliths, due to the presence of crystallographic anomalies, and it may be indicating natural and/or anthropic influence (Carvalho et al., 2019; Yedier and Bostanci, 2019, 2020).

These different calcium carbonate crystals are attributed to changes in chemical-physical parameters (pH and temperature), physiological disorders in the endolymph, or environmental stress (Morales-Nin, 1987; Payan et al., 2004). Some studies have shown the presence of crystallographic anomalies as vaterite and calcite may be due to a variety of factors, such as physical trauma in the macula, deficient food nutrition, high phosphate concentration in water, a possible stress indicator in hatcheries or native habitats, and the salinization of the environment (David et al., 1994; Ma et al., 2008; Mille et al., 2016).

About the environmental stress, is reported in the literature a major level of otolith asymmetry in more stressed specimens such as in fish from polluted coastal areas under influence of agricultural and mine waster and heavy metals in the aquatic ecosystem (Kontas et al., 2018; Yedier et al., 2018). In fact, the northern area of the CIELC is under influence of the freshwater inflow from an artificial channel that links the Ribeira de Iguape River directly to the estuary, representing an important input of different materials of continental and anthropogenic origin such as waste of an ancient mine and agricultural residues (Mahiques et al., 2009, 2013; Tramonte et al., 2018). This influence is not clearly verified in the southern area of CIELC, that has a more preserved characteristic and marine influence (Azevedo and Braga, 2011; Azevedo et al., 2011). Although otoliths with abnormal age rings have not been found in *C. spixii* from the northern site of CIELC and considering that these catfish are smaller than specimens from southern area, it is possible that *C. spixii* use the estuary differently throughout their life cycle, remaining in the northern sector in the juvenile phase and migrating to the southern region as an adult.

CONCLUSIONS

This study is a first record of abnormal age rings in *lapillus* otoliths of *Cathorops spixii* from Cananeia-Iguape Estuarine-Lagoon Complex (CIELC). It is noteworthy the presence of this abnormality between the 5th and 7th age rings, that is in older fish. In addition, larger fish were found in the southern region of CIELC, where all otoliths with abnormal age rings were recorded. This profile raises the possibility of a relationship between these anomalies and environmental stressors in the Cananeia-Iguape estuarine region.

ACKNOWLEDGMENTS

The authors would like to thank the Oceanographic Institute of São Paulo University (IOUSP), Brazil, and the team from Albacora research vessel of the IOUSP for supporting the sampling fish.

REFERENCES

Avigliano, E.; Carvalho, B.; Velasco, G.; Tripodi, P.; Volpedo, A. 2017. Inter-annual variability in otolith chemistry of catfish *Genidens barbus* from South-western Atlantic estuaries. Journal of the Marine Biological Association of the United Kingdom, 98(4): 855-865. https://doi.org/**10.1017/S0025315417000212**.

Azevedo, J.S.; Fernandez, W.S.; Farias, L.A.; Fávaro, D.T.I.; Braga, E.S. 2009. Use of *Cathorops spixii* as bioindicator of pollution of trace metals in the Santos Bay, Brazil. Ecotoxicology, 18: 577-586. https://doi.org/**10.1007/s10646-009-0315-4**.

Azevedo, J.S.; Braga, E.S. 2011. Caracterização hidroquímica para qualificação ambiental dos estuários de Santos-São Vicente e Cananéia. Arquivos de Ciências do Mar, 44(2): 52-61.

Azevedo, J.S.; Braga, E.S.; Favaro, D.I.T.; Perretti, A.R.; Rezende, C.E.; Souza, C.M.M. 2011. Total mercury in sediments and in Brazilian Ariidae catfish from two estuaries under different anthropogenic influence. Marine Pollution Bulletin, 62(12): 2724-2731.

Azevedo, J.S.; Sarkis, J.E.S.; Hortellani, M.A.; Ladle, R.J. 2012a. Are Catfish (Ariidae) effective bioindicators for Pb, Cd, Hg, Cu and Zn? Water, Air, and Soil Pollution, 223: 3911-3922. https://doi.org/**10.1007/s11270-012-1160-2**.

Azevedo, J.S.; Braga, E.S.; Ribeiro, C.A. 2012b. Nuclear Abnormalities in Erythrocytes and Morphometric Indexes in the Catfish *Cathorops spixii* (Ariidae) from different sites on the Southeastern Brazilian Coast. Brazilian Journal of Oceanography, 60(3): 323-330. http://dx.doi.org/**10.1590/S1679-87592012000300005**.

Azevedo, J.S.; Vaz-dos-Santos, A.M.; Perin, S.; Braga, E.S.; Rossi-Wongtschowski, C.L.D.B. 2019. *Cathorops spixii* (Agassiz 1829) at the Cananéia-Iguape Estuarine system. In: Vaz-Dos-Santos, A.M.; Rossi-Wongtschowski, C.L.D.B. (eds.). Growth in fisheries resources from the Southwestern Atlantic, São Paulo: Instituto Oceanográfico – USP. 127p.

Barcellos, R.L.; Berbel, G.B.B.; Braga, E.S.; Furtado, V.V. 2005. Distribuição e características do fósforo sedimentar no sistema estuarino lagunar de Cananéia-Iguape, estado de São Paulo, Brasil. Geochimica Brasiliensis, 19: 22-36.

Barnes, T.C.; Gillanders, B.M. 2013. Combined effect of extrinsic and intrinsic factors on otolith chemistry: implications for environmental reconstructions. Canadian Journal of Fisheries and Aquatic Sciences, 70(8): 1159-1166. https://doi.org/**10.1139/cjfas-2012-0442**.

Bostanci, D.; Polat, N.; Kurucu, G.; Yedler, S.; Kontas, S.; Darçin, M. 2015. Using otolith shape and morphometry to identify four *Alburnus* species (*A. chalcoides, A. escherichii, A. mossulensis* and *A. tarichi*) in Turkish inland waters. Journal of Applied Ichthyology, 31(6): 1013-1022. https://doi.org/**10.1111/jai.12860**.

Campana, S.; Thorrold, S.R. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Canadian Journal of Fisheries and Aquatic Sciences, 58(1): 30-38. https://doi.org/**10.1139/f00-177**.

Carvalho, B.; Volpedo, A.; Albuquerque, C.Q.; Fávaro, L.F. 2019. First record of anomalous otoliths of *Menticirrhus americanus* in the South Atlantic. Journal of Applied Ichthyology, 35(6): 1286-1291. https://doi.org/**10.1111/jai.13979**.

Carvalho, B.M.; Corrêa, M.F.M.; Volpedo, A. 2014. Lapillus otoliths of the *Cathorops spixii* (Agassiz, 1829) and *Genidens genidens* (Cuvier, 1829) (Actinopterygii, Ariidae). Acta Scientiarum. Biological Sciences, 36(3): 343-347. https://doi.org/**10.4025/actascibiolsci.v36i3.21117**.

Chiozzini, V.G.; Agostinho, K.L.; Delfim, R.; Braga, E. 2010. Tide influence on hydrochemical parameters in two coastal regions of São Paulo (Brazil) under different environmental occupations. In: Safety, Health and Environment World Congress, 1, São Paulo, 2010. Proceedings... São Paulo: COPEC – Science and Education Research Council. p. 25-28.

Dantas, D.V.; Barletta, M.; Costa, M.F.; Barbosa, S.C.T.; Possatto, F.E.; Ramos, J.A.A.; Lima, A.R.A.; Saint-Paul, U. 2010. Movement patterns of catfishes (Ariidae) in a tropical semi-arid estuary. Journal of Fish Biology, 76(10): 2540-2557. https://doi.org/**10.1111/j.1095-8649.2010.02646.x**.

David, A.; Grimes, C.B.; Isely, J.J. 1994. Vaterite sagittal otoliths in hatchery-reared juvenile red drums. Progressive Fish-Culturist, 56(4): 301-303. reared juvenile red drums. Progressive Fish-Culturist, 56(4): 301-303. https://doi.org/**10.1577/1548-8640(1994)056<0301:VSOIHR>2.3.CO;2**.

Denadai, M.; Pombo, M.; Santos, F.B.; Bessa, E.; Ferreira, A.; Turra, A. 2013. Population dynamics and diet of the Madamango Sea Catfish *Cathorops spixii* (Agassiz, 1829) (Siluriformes: Ariidae) in a tropical bight in Southeastern Brazil. PLoS One, 8(11): e81257. https://doi.org/**10.1371/journal.pone.0081257**.

Fallini, G.; Fermani, S.; Vanzo, S.; Miletic, M.; Zaffino, G. 2005. Influence on the formation of aragonite or vaterite by otolith macromolecules. Europen Journal of Inorganic Chemistry, 2005(1): 162-167. https://doi.org/**10.1002/ejic.200400419**.

Figueiredo, J.L.; Menezes, N.A. 1978. Manual de Peixes Marinhos do Sudeste do Brasil, Teleostei (1). São Paulo: Museu de Zoologia da Universidade de São Paulo. 110p.

Gomes, I.D.; Araújo, F.G.; Azevêdo, M.C.C.; Pessanha, A.L.M. 1999. Biologia reprodutiva dos bagres marinhos *Genidens genidens* (Valenciennes) e *Cathorops spixii* (Agassiz) (Siluriformes, Ariidae), na baía de Sepetiba, Rio de Janeiro, Brasil. Revista Brasileira de Zoologia, 16(suppl 2): 171-180. https://doi.org/**10.1590/S0101-81751999000600017**.

Grønkjær, P. 2016. Otoliths as individual indicators: a reappraisal of the link between fish physiology and otolith characteristics. Marine and Freshwater Research, 67(7): 881-888. https://doi.org/**10.1071/MF15155**.

Katayama, S. 2018. A description of four types of otolith opaque zone. Fisheries Science, 84: 735-745. https://doi.org/**10.1007/s12562-018-1228-z**.

Kontas, S.; Bostanci, D.; Yedier, S.; Kurucu, G.; Polat, N. 2018. Investigation of fluctuating asymmetry in the four otolith characters of *Merlangius merlangus* collected from Middle Black Sea. Turkish Journal of Maritime and Marine Sciences, 4(2): 128-138.

Ma, T.; Kuroki, M.; Miller, M.J.; Ishida, R.; Tsukamoto, K. 2008. Morphology and microchemistry of abnormal otoliths in the ayu, *Plecoglossus altivelis*. Environmental Biology of Fishes, 83: 155-167. https://doi.org/**10.1007/s10641-007-9308-4**.

Maciel, T.R.; Vaz-dos-Santos, A.M.; Vianna, M. 2018. Can otoliths of *Genidens genidens* (Cuvier 1829) (Siluriformes: Ariidae) reveal differences in life strategies of males and females? Environmental Biology of Fishes, 101(11): 1589-1598. https://doi.org/**10.1007/s10641-018-0804-5**.

Mahiques, M.M.; Burone, J.; Figueira, R.C.L.; Lavenére-Wanderley, A.A.O.; Capellari, B.; Rogacheski, C.E.; Barroso, C.P.; Samaritano, L.A.; Cordero, L.A.; Cussioli, M.C. 2009. Anthropogenic influences in a lagoonal environment: a multiproxy approach at the Valo Grande mouth, Cananéia-Iguape System (SE Brazil). Brazilian Journal of Oceanography, 57(4): 325-337. http://dx.doi.org/**10.1590/S1679-87592009000400007**.

Mahiques, M.M.; Figueira, R.C.L.; Salaroli, A.B.; Alves, D.P.V.; Gonçalves, C. 2013. 150 years of anthropogenic metal input in a biosphere reserve: the case study of the Cananéia–Iguape coastal system, southeastern Brazil. Environmental Earth Sciences, 68: 1073-1087. https://doi.org/**10.1007/s12665-012-1809-6**.

Mendoza, R.P.R. 2006. Otolith and their applications in fishery science. Ribarstvo, 64: 89-102.

Mille, T.M.; Mahé, K.; Cachera, M.; Villanueva, M.C., de Pontual, H., Ernande, B. 2016. Diet is correlated with otolith shape in marine fish. Marine Ecology Progress Series, 555: 167-184. https://doi.org/**10.3354/meps11784**.

Mishima, M.; Tanji, S. 1981. Distribuição geográfica dos bagres marinhos (Osteichthyes, Ariidae) no Complexo Estuarino-Lagunar de Cananéia (25°S, 48°W). Boletim do Instituto de Pesca, 8: 157-172.

Morales-Nin, B.Y.O. 1987. The influence of environmental factors on microstructure of otoliths of three demersal fish species caught off Namibia. South African Journal of Marine Science, 5(1): 255-262. https://doi.org/**10.2989/025776187784522207**.

Morat, F.; Gibert, P.; Reynauld, N.; Testi, N.; Fayriou, P.; Raymond, V.; Carrei, G.; Maire, A. 2018. Spatial distribution, total length frequencies and otolith morphometry as tools to analyse the effects of a flash flood on populations of roach (*Rutilus rutilus*). Ecology Freshwater Fish, 27(1): 421-432. https://doi.org/**10.1111/eff.12357**.

Morrison, C.M.; Kunegel-Lion, M.; Gallagher, C.P.; Wastle, R.J.; Lea, E.V.; Loewen, T.M.; Reist, J.D.; Howland, K.L.; Tierney, K.B. 2019. Decoupling of otolith and somatic growth during anadromous migration in a northern salmonid. Canadian Journal of Fisheries and Aquatic Sciences, 76(11): 1940-1953. https://doi.org/**10.1139/cjfas-2018-0306**.

Oliveira, M.A.; Novelli, R. 2005. Idade e crescimento do bagre *Genidens genidens* na barra do Açu, Norte do Estado do Rio de Janeiro. Tropical Oceanography, 33(1): 57-66. https://doi.org/**10.5914/tropocean.v33i1.5070**.

Panfili, J.; Meunier, F.J.; Mosegaard, H.; Troadec, H.; Wright, P.J.; Geffen, A.J. 2002. Otoliths. In: Panfili, J.; De Pontual, H.; Troadec, H.; Wright, P.J. (eds.). Manual of fish sclerochronology. Brest, France: Ifremer-IRD coedition. 464p.

Payan, P.; Pontual, H.; Boeuf, G.; Mayer-Gostan, N. 2004. Endolymph chemistry and otolith growth in fish. Comptes Rendus. Palevol, 3(6-7): 535-547. https://doi.org/**10.1016/j.crpv.2004.07.013**.

Pecoraro, G.D.; Hortellani, M.A.; Hagiwara, Y.S.; Braga, E.S.; Sarkis, J.E.; Azevedo, J.S. 2018. Bioaccumulation of total mercury (THg) in catfish (Siluriformes, Ariidae) with different sexual maturity from Cananéia-Iguape Estuary, SP, Brazil. Bulletin of Environmental Contamination and Toxicology, 102: 175-179. https://doi.org/**10.1007/s00128-018-2485-3**.

Pisam, M.; Payan, P.; LeMoal, C.; Edeyer, A.; Boeuf, G.; Mayer-Gostan, N. 1998. Ultrastructural study of the saccular epithelium of the inner ear of two teleosts, *Oncorhynchus mykiss* and *Psetta maxima*. Cell and Tissue Research, 294: 261-270. https://doi.org/**10.1007/s004410051176**.

Popper, A.N.; Ramcharitar, J.; Campana, S.E. 2005. Why otolith? Insights from inner ear physiology and fisheries biology. Marine and Freshwater Research, 56(5): 497-504. https://doi.org/**10.1071/MF04267**.

Pracheil, B.M.; George, R.; Chakoumakos, B.C. 2019. Significance of otolith calcium carbonate crystal structure diversity to microchemistry studies. Reviews in Fish Biology and Fisheries, 29: 569-588. https://doi.org/**10.1007/s11160-019-09561-3**.

Prado, H.M.; Scilndwein, M.N.; Murrieta, R.S.S.; Junior, D.R.N.; Souza, E.P.; Cunha-Lignon, M.; Mahiques, M.M.; Giannini, P.C.F.; Contente, R.F. 2019. O Canal do Valo Grande no Complexo Estuarino Cananéia-Iguape (SP, Brasil): história ambiental, ecologia e perspectivas futuras. Ambiente & Sociedade, 22: e01822. https://doi.org/**10.1590/1809-4422asoc0182r2vu19l4td**.

RAMSAR. 2017. Brazil: environmental protection Area of Cananéia-Iguape-Peruíbe. Gland: Ramsar Information Sheet. 28p.

Rogers, T.A.; Fowler, A.J.; Steer, M.A.; Gillanders, B.M. 2019. Resolving the early life history of King George whiting (*Sillaginodes punctatus*: Perciformes) using otolith microstructure and trace element chemistry. Marine and Freshwater Research, 70(12): 1659-1674. https://doi.org/**10.1071/MF18280**.

Sánchez, R.O.; Martinez, V.H. 2017. Morphological variations of the three otoliths of some species of the family Loricariidae (Ostariophysi: Siluriformes). Neotropical Ichthyology, 15(1): e160058. https://doi.org/**10.1590/1982-0224-20160058**.

Santana, H.S.; Tos, C.D.; Minte-Vera, C.V. 2020. A review on the age and growth studies of freshwater fish in South America. Fisheries Research, 222: e105410. https://doi.org/**10.1016/j.fishres.2019.105410**.

Sparre, P.; Venema, S.C. 1998. Introduction to tropical fish stock assessment. Part 1. Manual. Rome: FAO. FAO Fisheries Technical Paper, vol. 306, 407p.

Thomas, O.R.B.; Swearer, S.E. 2019. Otolith biochemistry: a review. Reviews in Fisheries Science & Aquaculture, 27(4): 458-489. https://doi.org/**10.1080/23308249.2019.1627285**.

Tramonte, K.M.; Figueira, R.C.L.; Majer, A.P.; Ferreira, P.A.L.; Batista, M.F.; Ribeiro, A.P.; Mahiques, M.M. 2018. Geochemical behavior, environmental availability, and reconstruction of historical trends of Cu, Pb, and Zn in sediment cores of the Cananéia-Iguape coastal system, Southeastern Brazil. Marine Pollution Bulletin, 127: 1-9. https://doi.org/**10.1016/j.marpolbul.2017.11.016**.

UNESCO – United Nations Education Scientific and Cultural Organization. 2011. MAB Biosphere Reserves Directory: biosphere reserve information. Available at: <http://www.unesco.org/new/en/natural-sciences/environment>. Accessed: Jan. 25, 2020.

Vinagre, C.; Maia, A.; Amara, R.; Cabral, H.N. 2014. Anomalous otoliths in juveniles of common sole, *Solea solea*, and *Senegal sole, Solea senegalensis*. Marine Biology Research, 10(5): 523-529. https://doi.org/**10.1080/17451000.2013.831178**.

Winkler, A.C.; Duncan, M.I.; Farthing, M.W.; Potts, W.M. 2019. Sectioned or whole otoliths? A global review of hard structure preparation techniques used in ageing sparid fishes. Reviews in Fisheries Science & Aquaculture, 29: 605-611. https://doi.org/**10.1007/s11160-019-09571-1**.

Wright, P.J.; Panfili, J.; Morales-Nin, B.; Geffen, A.J. 2002. Otoliths. In: Panfili, J.; de Pontual, H.; Troadec H.; Wright, P.J. (eds.). Manual of fish sclerochronology. 1st ed. Brest, France: Ifremer-Ird coedition. 464p.

Yedier, S.; Bostanci, D.; Kontas, S.; Kurucu, G.; Polat, N. 2018. Fluctuating Asymmetry in otolith dimensions of *Trachurus mediterraneus* collected from the Middle Black Sea. Acta Biologica Turcica, 31(4): 152-159.

Yedier, S.; Bostanci, D. 2019. Aberrant crystallization of Blackbellied angler *Lophius budegassa* Spinola, 1807 otoliths. Cahiers de Biologie Marine, 60: 527-533. https://doi.org/**10.21411/CBM.A.2389AF48**.

Yedier, S.; Bostanci, D. 2020. Aberrant otoliths in four marine fishes from the Aegean Sea, Black Sea, and Sea of Marmara (Turkey). Regional Studies in Marine Science, 34: e101011. https://doi.org/**10.1016/j.rsma.2019.101011**.