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First record of anomalous otoliths of *Menticirrhus americanus* in the South Atlantic

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Abstract

Sagitta otoliths are usually formed of calcium carbonate polymorphs as aragonite. The objective of this study was to verify which carbonate polymorph is predominant in the sagitta otolith of *Menticirrhus americanus* and check whether this pattern remains in otoliths with morphological alterations. Otoliths of *M. americanus* were obtained from five sites on the southeast-south coast of Brazil (São Sebastião (SS) 23°45′S–45°24′O, $n = 29$; Cananéia-Iguape Estuarine Complex (CI) 25°02′S–47°54′O, $n = 30$; Paranaguá Estuarine Complex (PEC) 25°28′S–48°20′O, $n = 35$; Itapoá (IT) 26°07′S–48°36′O, $n = 31$; Laguna (LA) 28°28′S–48°46′O, $n = 13$). The characterization of carbonate polymorphs of otoliths was performed through Raman spectroscopy, a photonic and non-destructive technique that analyzes molecular vibrations induced by laser. We analyzed 138 pairs of *M. americanus* otoliths, of which eight otoliths from different pairs presented morphological alterations (SS $n = 1$, CEP $n = 5$, IT $n = 1$, LA $n = 1$). The Raman spectra show that normal otoliths, that is, without morphological alterations, presented only aragonite in their structure. Among the otoliths that presented morphological alterations, the Raman spectra allowed to identify in six otoliths the deposition of aragonite and in only two otoliths the deposition of vaterite (one specimen of the PEC and one of SS).

1 | INTRODUCTION

Otoliths are metabolically inert carbonate structures, that is, after their formation there are no chemical alterations or reabsorption. They are sensory organs immersed in the endolymph that help in the balance and hearing of the fish (Ladich & Schulz-Mirbach, 2016). Chemically, they are mainly composed of calcium carbonate, precipitated as aragonite, under a high molecular weight protein matrix. This process is regulated by hormones and influenced by environmental factors (Popper & Fay, 2011).

Precipitation of calcium carbonate may occur in the form of vaterite and calcite, this change in carbonate crystallization has been attributed to chemical changes in endolymph and/or environmental stress (David & Grimes, 1994; Gauldie, 1993; Tomás, Geffen, Allen, & Berges, 2004). Experimental studies have pointed out that changes in physicochemical parameters such as pH and temperature of the marine environment can cause alterations in the acidity of endolymph, a liquid in which otoliths are immersed (Martino, Doubleday, Woodcock, & Gillanders, 2017;Munday, Hernaman, Dixson, & Thorrold, 2011). Due to the alkaline property of endolymph, saturation and deposition of aragonite occurs in otoliths (Payan, Pontual, Boeuf, & Mayer-Gostan, 2004). Alterations in the endolymph pH can favor the precipitation of vaterite and calcite, which are less dense than aragonite (Holmberg et al., 2019).

Studies have shown that crystallographic anomalies may be due to a variety of factors, such as physical trauma in the macula (Strong, Neison, & Hunt, 1986), deficient nutrition and high phosphate concentration in water (David & Grimes, 1994), high population density in captivity (Sweeting, Beamish, & Neville, 2004) and salinization of the environment (Avigliano, Tombari, & Volpedo, 2012). For demersal species, it has been reported different frequencies of occurrence of anomalies: *Pollachius virens* 48% of anomalous otoliths (Strong et al., 1986), *Oncorhynchus kisutch* 16,7% (Sweeting et al., 2004), *Platichthys flesus* 43% (Neves, Guedes, Valentim, Campos, & Freitas, 2017); *Pseudorhombus malayanus* 50% (Manizadeh, Teimori, Askari Hesni, & Motamedi, 2018). Species of the family Sciaenidae presented lower frequency of anomalies in relation to the species mentioned above (David & Grimes, 1994; Béarez, Carlier, Lorand, & Parodi, 2005).

The demersal species, *Menticirrhus americanus* (Linnaeus, 1758), object of this study, belongs to the family Sciaenidae and is distributed between latitudes 41°N and 51°S (Chao et al., 2015). *Menticirrhus americanus* is an important resource in the trophic web of ichthyophagous in the southern Brazilian region (Bornatowski, Braga, Abilhôa, & Corrêa, 2014). The species dwells estuarine areas, usually associated with unconsolidated substrate and makes use of the internal continental shelf (Passos et al., 2013). This study aimed to evaluate the occurrence of macroscopic and crystallographic morphological anomalies in *M. americanus* otoliths on the southeast-south coast of Brazil.

2 | MATERIALS AND METHODS

Specimens of *M. americanus* were caught and purchased from the fishing fleet between June 2015 and May 2016 in five sites along the southeast-south coast of Brazil (Figure 1).

Along the sampled region there are densely urbanized locations with the presence of ports and petroleum terminals (SS – 23°S, CEP – 25°S and IT – 26°S) and without terminals (LA - 28°S) (Table 1). Sampling was also performed in a region with less urbanization, without ports and petroleum complexes (CI - 25°S) (Table 1). The lowest water temperatures are recorded for Laguna and the highest for São Sebastião (Table 1).

Menticirrhus americanus specimens were measured for total length (TL) in cm, total weight (TW) in grams. The otoliths were removed, cleaned and stored dry and classified morphologically (Corrêa & Viana, 1992). Areas with alterations were photographed in a scanning electron microscope. From the microphotographs, the crystals were morphologically classified: calcite crystals were classified as cuboidal with larges crystals, botryoidal and fusiforms vaterite and laths and blocks aragonite according to Béarez et al., (2005); Gauldie (1993); Tzeng et al., (2007) and Kern et al., (2017).

The crystallographic characterization of otoliths was performed using a Confocal Raman Witec alpha 300R spectrometer, the spectra were excited by the 532 nm, using an 50× microscope objective, laser penetration was 200 μm and the results were collected 10 times with an exposure time of 3 s. According to Melancon, Fryer, Ludsin, Gagnon, and Yang (2005), aragonite and vaterite in otoliths are characterized by vibrations at different wavelengths, in which aragonite is characterized by a more intense vibration between 1,075 and 1,090 cm^{-1} and two small vibrations between 100 and 400 cm^{-1}. Vaterite differs from aragonite because it presents two more intense vibrations between 1,075, 1,081 and 1,090 cm^{-1} and three small vibrations between 100 e 400 cm^{-1}.

3 | RESULTS

For the morphological classification were observed 676 otoliths of *M. americanus*. Morphological alterations were found in 8 otoliths of 676 observed, representing 1.18% of all otoliths observed. The highest sampling occurred in the PEC with 538 specimens, followed by CI, IT, SS and LA, with 45, 44, 32 and 17, respectively. The frequency of otolith anomalies differs between sites, in the PEC we observed five otoliths with alterations (1% frequency) and one otolith in SS (3% frequency), IT (2% frequency) and LA (6% frequency). No otolith with morphological anomaly was observed in CI.

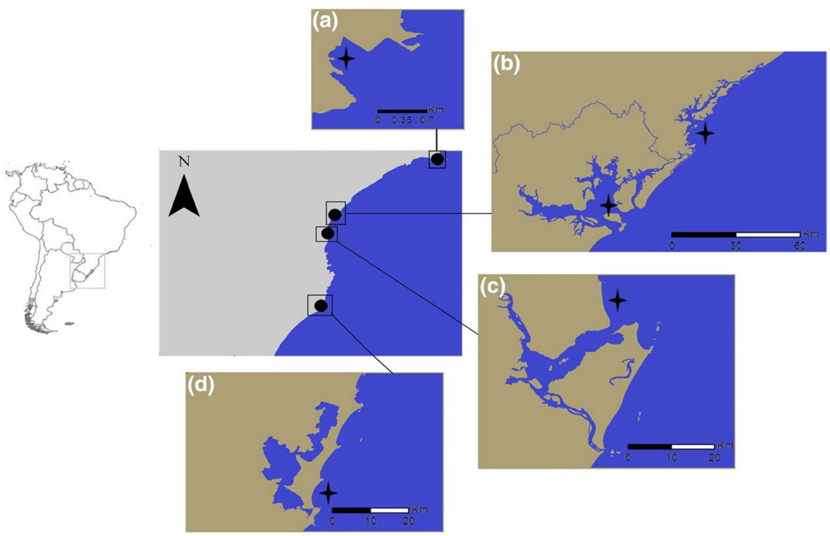

FIGURE 1 Sampling sites of *Menticirrhus americanus* specimens along the South Atlantic. (a) Location of the sampling sites at latitude 23°S - São Sebastião (SS), (b) at latitude 25°S - Cananéia (CI) and Paranaguá Estuarine Complex (PEC), (c) at latitude 26°S - Itapoá (IT) and (d) at latitude 28°S - Laguna (LA). Stars indicate the sampling sites

TABLE 1 Physical-chemical parameters and population of the environments in which sagittae otoliths were sampled *M. americanus* from the South Atlantic

Site	Pop	T°C	*Sal*	Author
São Sebastião (SS)	>85 mil	15–28	32–35	Siegle et al. (2017)
Cananéia-Iguape Estuarine Complex (CI)	<13 mil	20–26	<35	Barcellos et al. (2005)
Paranaguá Estuarine Complex (PEC)	>140 mil	20–26	<33.2	Mizerkowski et al. (2012)
Itapoá (IT)	>14 mil	17.3–26.8	24.7–36	Carvalho et al. (1998)
Laguna (LA)	>51 mil	<18	>35.5	Campos et al. (2013)

Morphological alterations in the anterior region were observed in four otoliths (Figure 2a–c,H), while in the posterior region, in only one otolith (Figure 2f). Three completely deformed otoliths were recorded, with rough features throughout their length (Figure 2d,e,g). Only one otolith showed anomalies on the outer face of the otolith (Figure 2e).

The morphological analysis of the otolith crystals, by means of scanning electron microscopy (Figure 3), allowed to classify the crystals of normal otoliths as laths aragonite. The deformed regions were classified as calcite (Figure 3b,c,h), tabular vaterite crystal (Figure 3f), fibrous vaterite (Figure 3e,g), calcite prism and granular vaterite prism (Figure 3d) and irregular aragonite (Figure 3a).

For crystallographic analysis were selected 122 otoliths of *M. americanus* that showed TL range of 14.9–44 cm and TW range of 30.95–717.5 g. From the Raman spectroscopy, in specimens without morphological alterations, we observed only aragonite at all sites sampled with a peak of greater intensity between 1,075 and 1,090 cm^{-1}. The Raman spectra of otoliths with morphological alterations demonstrated two distinct patterns of crystallization. Two presented precipitated vaterite one collected in the PEC and the other in SS, (Figure 4a) and six presented precipitated aragonite (Figure 4b).

4 | DISCUSSION

This study showed the occurrence of morphological alterations associated with the deposition of vaterite and aragonite in *M. americanus* otoliths. The low frequency of morphologically anomalous otoliths observed for *M. americanus* in the analyzed region indicates that species of the family Sciaenidae present low frequency of alterations as also observed in other studies (David & Grimes, 1994; Béarez, Carlier, Lorand & Parodi, 2005). However, other demersal species presented frequency of anomalies higher than 30%, which can be a consequence of high population density in captivity (Sweeting et al., 2004) and salinization (Neves et al., 2017).

According to the *SEM* images, crystals precipitated in altered areas of the *M. americanus* otoliths were morphologically classified as calcite, vaterite and aragonite. However, Raman microscopy indicates only the precipitation of vaterite and aragonite in the anomalous otoliths analyzed. The morphological alteration associated with vaterite and aragonite has already been reported for other species (Gauldie, 1993; David & Grimes, 1994; Béarez et al., 2005; Neves et al., 2017). But the presence of vaterite in *M. americanus* otoliths is presented here for the first time. The precipitation of vaterite in the otoliths may be, among other factors, a consequence of the reduction in the endolymph pH (Holmberg et al., 2019). According to Feng (2011), vaterite is the most unstable calcium carbonate and has more frequent deposition under extreme conditions of pH, temperature and pressure. As vaterite becomes more frequent under extreme conditions, its presence suggests that modifications occurred in the endolymph pH of two individuals of *M. americanus* that presented anomalous otoliths with carbonate precipitated as vaterite. Due

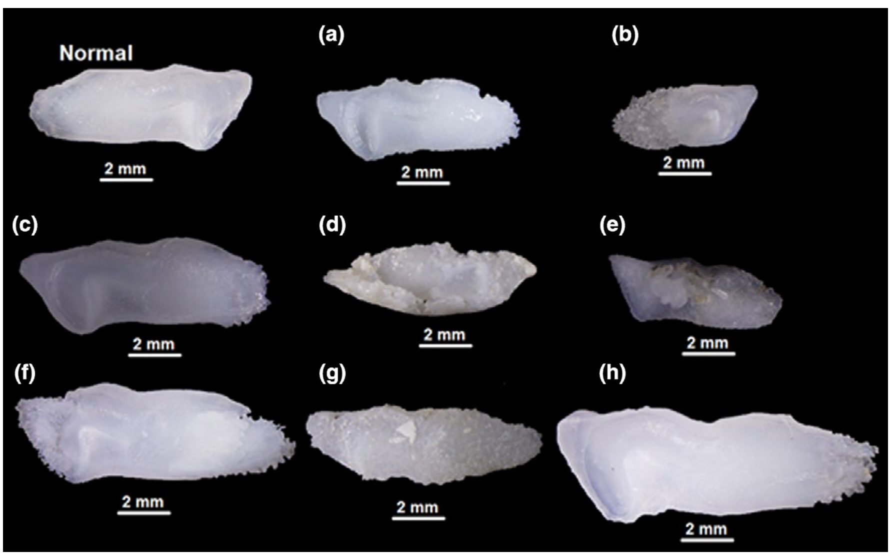

FIGURE 2 Anomalous sagitta otoliths of *Menticirrhus americanus* from the South Atlantic (a-h) and without alterations (normal)

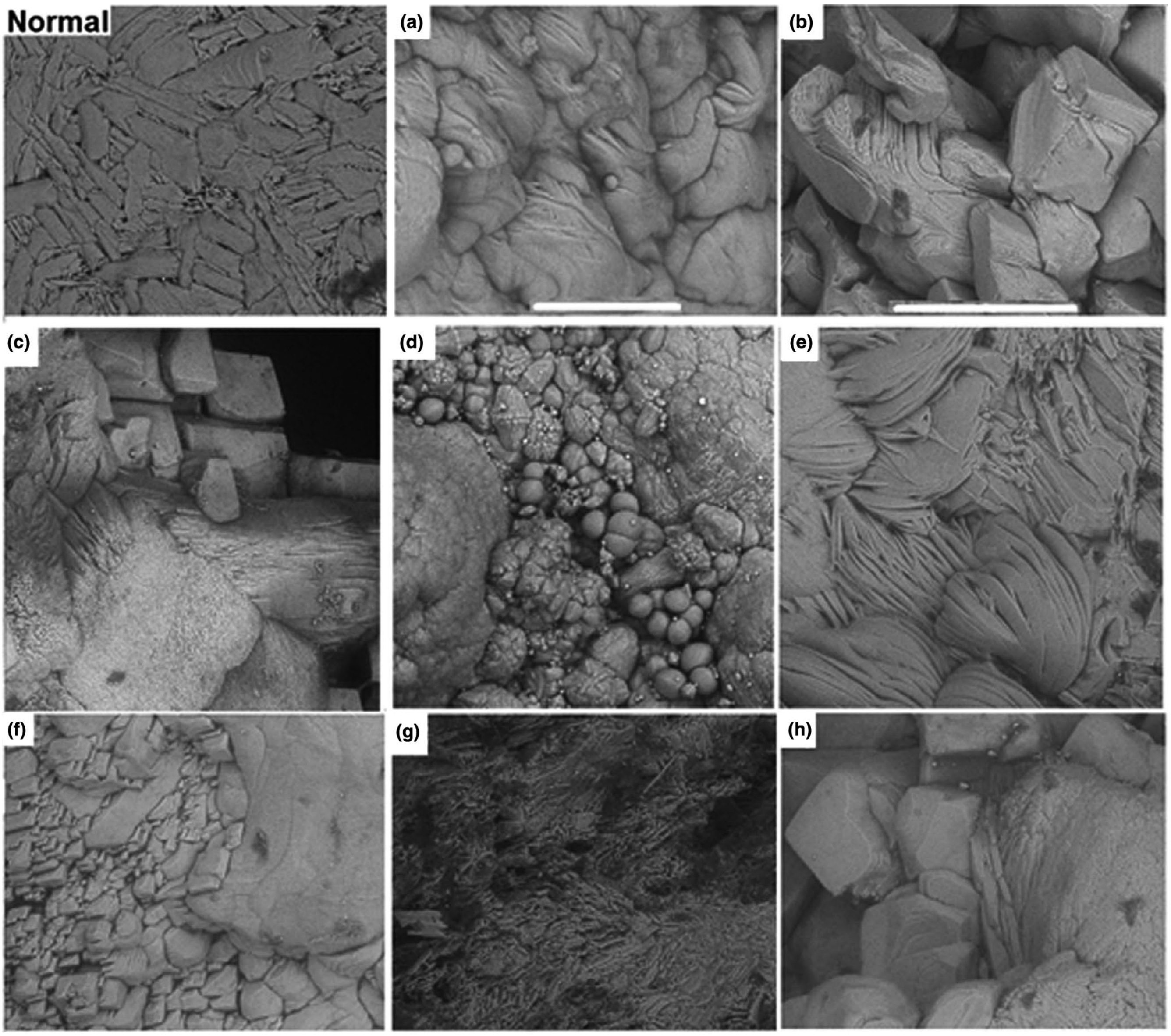

FIGURE 3 Scanning micrograph of the anomalous and normal sagitta otoliths of *M. americanus* from the South Atlantic shelf. Scale a, d, Normal = 20 μm and b, c, e, f, g, h = 50 μm

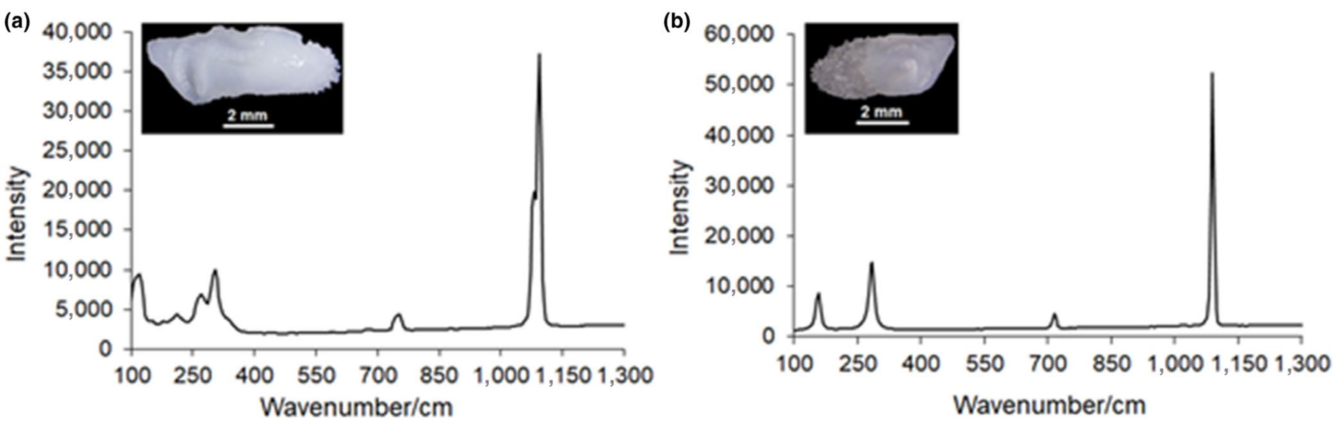

FIGURE 4 Raman spectra of sagitta otoliths with morphological alterations of *Menticirrhus americanus* sampled in five sites of the South Atlantic. (a) vaterite and (b) aragonite

to the low density of vaterite, specimens with this crystallization in the otoliths may present hearing deficits becoming more susceptible to predation and/or capture by fishing (Oxman et al., 2007).

Anomalous otoliths were not observed in IC; this environment houses the smallest population and no port or petroleum terminals, differing from the other environments that presented alterations. According to David & Grimes (1994), the high introduction of phosphate in the aquatic environment would affect the solubility of the endolymph, thus causing an interruption in the deposition of aragonite and resulting in morphological changes in the otoliths. PEC, SS, IT and LA are more populated habitat in comparison to CI and with port and petroleum terminals. These differences could indicate anthropic influence on the presence of anomalous otoliths. However, in this study, data are biased due to differences in sampling effort, hindering to reach a final conclusion on human influence in the presence of anomalies in otoliths.

The morphological alterations observed in sagitta otoliths of *M. americanus* had low frequency, being considered rare events in the analyzed population. Vaterite was present in only two morphologically anomalous otoliths and it is not possible to correlate it with morphological alterations in *M. americanus*.

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