Research Article

Otoliths morphology and age-record in *Bagre panamensis* (Siluriformes: Ariidae) inhabiting at the southeast of Gulf of California

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ABSTRACT. Among *Bagre* genera, there is a high variation in the estimation of age, a concern due to overexploitation risk in fisheries because of age underestimation. *Bagre panamensis* is an important fishery resource of the Mexican Pacific and the Gulf of California. Its age is known from otoliths, but its accuracy needs to be confirmed, and the periodicity of the otoliths record validated. The external morphology, some microstructure attributes, and age record of *B. panamensis* otoliths were described from 371 specimens collected southeast of the Gulf of California. The *lapilli* otoliths were larger than the *sagitta* and *asteriscus* otoliths. The *lapilli* otoliths present aragonite crystals with a prismatic shape, and their growth is radial, from the core to the otolith edge. The *lapilli* otoliths form an annual growth ring, defined by the slowdown in the growth that occurs during April to July, during the breeding season. The ages of the individuals ranged from 1 to 15 years, and the applied method is considered adequate and accurate for its estimation (otolith cross-sectioning and red-neutral staining).

Keywords: *Bagre panamensis*; sea catfish; *lapillus* otolith; growth rings; aragonite crystals; accurate age method; otolith staining

INTRODUCTION

Otoliths are structures used for balance and hearing in all teleost fish (Campana 1999). They are composed of inorganic material (>90% calcium carbonate; Campana 1999) and ≤10% of organic material (glycoproteins, proteoglycans, and collagens; Lundberg et al. 2015), which grows throughout the life of fish (Schulz-Mirbach et al. 2018). Fish have three different pairs of otoliths (*sagitta, lapillus, and asteriscus*) that can be developed by any combination of the three most common forms (calcite, aragonite, or valerite) in which calcium carbonate crystallizes (Gauldie 1993, Oliveira et al. 1996, Pracheil et al. 2019, Thomas & Swearer 2019). These can be located in membranous chambers (saccule, utricle, and lagena) connected by semicircular canals in the inner ear of fish (Campana 1999, Schulz-Mirbach et al. 2018). In most fish species, *sagitta* otolith is the largest and it is the most frequently used in estimating age (Panfili et al. 2002). However, in the superorder Ostariophysi including the sea catfishes (Siluriformes: Ariidae), the *lapilli* otoliths tend to be larger and more robust (Assis 2005, Diogo 2005) and they are the most suitable structures for fish age studies (Maciel et al. 2018), by the method of cutting, polishing and defining the periodicity deposition of material (Reis...
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1986, Cheragh-Shevi et al. 2015, Maciel et al. 2018, Flinn et al. 2019; Table 1).

The knowledge of fishes age is necessary for growth rate, mortality rate, and productivity estimates (Caillet et al. 2001, Campana 2001, Piddocke et al. 2015), particularly for biomass yield estimation and monitoring fisheries and aquaculture for sustainable management purposes. In Mexico, 25 sea catfishes species have been registered to inhabit the Pacific Ocean and the Gulf of California and 30 species in the Atlantic Ocean and the Caribbean waters (Marceniuk & Ferraris 2003, Robertson & Allen 2015). Some of them are currently fished mainly by Mexican small-scale fisheries (DOF 2012, 2018). *Bagre panamensis* (Gill, 1863) (Siluriformes: Ariidae) has been one of the most intensively exploited species (Arreóin-Sánchez & Arcos-Huitrón 2011) and also it is part of bycatch of the industrial shrimp fishery (Amezcua et al. 2006, López-Martínez et al. 2010, Muro-Torres & Amezcua 2011). It is worth mentioning that nowadays, there is a growing interest in their conservation and responsible use in fisheries and aquaculture (Maldonado-Coyac et al. 2018), but their management is still incipient.

The sea catfish *B. panamensis* is a bentopelagic fish widely distributed from southern California, USA, the Gulf of California to northern Peru (Robertson & Allen 2015). Previous studies of *B. panamensis* from the southeastern Gulf of California have defined age by counting growth rings in whole *lapilli* otoliths without any treatment (Table 1). The age definition from whole otoliths could be easy and accurate in young individuals (Khan et al. 2016). But it is impossible to visualize all growth rings in older individuals (i.e. age >10; VanderKooy 2009). Since the growth rings are more overlapping near the edge of older otoliths, and usually, the age could be underestimated (Panfili et al. 2002, Easley & Millner 2008, Volpode & Vaz-dos-Santos 2015, Khan et al. 2016). Therefore, there is a greater interpretation error (Campana 2001). While, in the youngest individuals could be difficult to identify the first annual growth ring (i.e. *Brachyplatystoma rousseauxii*) due to the presence of false rings (Hauser et al. 2018). Unfortunately, the process of estimating fish age based on any bony structure incorporates error due to subjectivity which originates with the preparation and interpretation of the periodic features in the calcified structures, and even in the same structure as the otolith, results in age estimates can differ among researchers (Campana 2001). In fisheries, particularly, this is a concern due to an aging error that can contribute to overexploitation of a population or species, often because age underestimation (rather than overestimation) results in overly optimistic estimates growth and mortality rate (review by Campana 2001).

There is a high variation in age estimation among sea catfishes in the Ariidae family and even in *Bagre* genera, with maximum ages from 3.5 to 36 years. Even different studies have gotten maximum ages differing by more than 50% in the same species. In *Bagre marinus*, the maximum ages of 10, 24, and 25 years have been defined, considering very close maximum sizes (55.7 cm in total length, 57.7 cm in furcal length, and 55 cm in total length, respectively) (Table 1).

In this context, it is not always appropriate to use a unique method to estimate the age (Beamish & McFarlane 1983, Maciel et al. 2018). It is recommended to re-estimate the age using improved or better methods. The cross-sectioning and neutral-red staining methods have been widely used to improve the contrast of growth rings and to estimate the age in *sagittae* otoliths of several marine fish species (Richter & Mcdermott 1990, Arneri et al. 1998, 2001, Franks et al. 2001, Peltonen et al. 2002, Easley & Millner 2008), and therefore to reduce the interpretation error (Easley & Millner 2008). While scanning electron microscopy (SEM) is a powerful tool that provides useful information about the structure and composition of fish otoliths, even if daily resolution is required (Panfili et al. 2009). In this sense and using these mentioned techniques, the age-record in *lapillus* otolith of *B. panamensis* and some morphological attributes of the external shape and microstructure of inorganic crystals of otoliths were described.

**MATERIALS AND METHODS**

A monthly sample of 10 to 49 specimens (371 total sample) of *Bagre panamensis* was collected from Mazatlán, Sinaloa, from September 2014 to November 2015 and January 2015, with the support of small-scale fishery. The total length (TL), eviscerated body weight (We), and sex of each organism were recorded, and *lapilli* otoliths were extracted, washed with water, marked, and stored to estimate age. Also, gonads weights were registered (Gw).

The three pairs of otoliths (*lapillus*, *sagitta*, and *asteriscus*) from 10 individuals were extracted, photographed, and identified in order to describe their external morphology according to Martínez & Monasterio de Gonzo (1991), Volpode & Echeverría (2000), Acero & Betancur (2007), Chen et al. (2011), Aguilera et al. (2013), Santificetur et al. (2017) and Volpode et al. (2017). Also, these *lapilli* otoliths were prepared to observe their microstructure using a Tescan MIRA 3 LMU scanning electron microscope; this procedure consisted of embedding the *lapillus* otolith in resin and cutting them transversely across the core with a circular diamond-tipped blade. Right away, the cut
Table 1. Some age studies realized using lapilli otoliths of sea catfishes of Ariidae family, Siluriformes. NS: not specified, TL: total length, FL: fork length.

<table>
<thead>
<tr>
<th>Species and aging method</th>
<th>Sex</th>
<th>Maximum Age</th>
<th>Maximum Length</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole otolith and whole operculum</td>
<td>Both</td>
<td>3.5</td>
<td>41 TL</td>
<td>India</td>
<td>Dan (1981)</td>
</tr>
<tr>
<td>Bagre bivit (Linnaeus, 1766)</td>
<td>Both</td>
<td>6</td>
<td>43.4 FL</td>
<td>Mexico</td>
<td>Warburton (1978)</td>
</tr>
<tr>
<td>Ariopsis guatemalensis (Günther, 1864)</td>
<td>Both</td>
<td>6</td>
<td>52.5 TL</td>
<td>Mexico</td>
<td>Muro-Torres (2011)</td>
</tr>
<tr>
<td>Occidentarius platypogon (Günther, 1864)</td>
<td>Both</td>
<td>8</td>
<td>50.5 TL</td>
<td>Mexico</td>
<td>Muro-Torres (2011)</td>
</tr>
<tr>
<td>Bagre panamensis</td>
<td>Both</td>
<td>8</td>
<td>50.5 TL</td>
<td>Mexico</td>
<td>Muro-Torres (2011)</td>
</tr>
<tr>
<td>Sectioned otolith</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Netuma thalassina (Rüppell, 1837)</td>
<td>Both</td>
<td>11</td>
<td>NS</td>
<td>India</td>
<td>Dmitrenko (1975)</td>
</tr>
<tr>
<td>Netuma thalassina</td>
<td>Both</td>
<td>19</td>
<td>NS</td>
<td>Kuwait</td>
<td>Bawazeer (1987)</td>
</tr>
<tr>
<td>Picofollis dussumieri (Valenciennes, 1840)</td>
<td>Both</td>
<td>12</td>
<td>76.7 FL</td>
<td>Iran</td>
<td>Cheraghi-Shevi et al. (2015)</td>
</tr>
<tr>
<td>Galeichthys feliceps (Valenciennes, 1840)</td>
<td>Male</td>
<td>16</td>
<td>37.9 FL</td>
<td>South Africa</td>
<td>Tilney (1990)</td>
</tr>
<tr>
<td>Galeichthys feliceps</td>
<td>Female</td>
<td>18</td>
<td>37.9 FL</td>
<td>South Africa</td>
<td>Tilney (1990)</td>
</tr>
<tr>
<td>Galeichthys ater (Castelnau, 1861)</td>
<td>Male</td>
<td>15</td>
<td>31.9 FL</td>
<td>South Africa</td>
<td>Tilney (1990)</td>
</tr>
<tr>
<td>Galeichthys ater</td>
<td>Female</td>
<td>15</td>
<td>31.9 FL</td>
<td>South Africa</td>
<td>Tilney (1990)</td>
</tr>
<tr>
<td>Genidens barbus (Lacépède, 1803)</td>
<td>Female</td>
<td>36</td>
<td>98 TL</td>
<td>Brazil</td>
<td>Reis (1986b)</td>
</tr>
<tr>
<td>Genidens genidens (Cuvier, 1829)</td>
<td>Male</td>
<td>11.5</td>
<td>38 TL</td>
<td>Brazil</td>
<td>Maciel et al. (2018)</td>
</tr>
<tr>
<td>Genidens genidens</td>
<td>Female</td>
<td>11.5</td>
<td>47 TL</td>
<td>Brazil</td>
<td>Maciel et al. (2018)</td>
</tr>
<tr>
<td>Ariopsis felis (Linnaeus, 1766)</td>
<td>Male</td>
<td>20</td>
<td>34 TL</td>
<td>USA</td>
<td>Armstrong et al. (1996)</td>
</tr>
<tr>
<td>Ariopsis felis</td>
<td>Female</td>
<td>23</td>
<td>43.9 FL</td>
<td>USA</td>
<td>Armstrong et al. (1996)</td>
</tr>
<tr>
<td>Ariopsis felis</td>
<td>Both</td>
<td>24</td>
<td>49.2 TL</td>
<td>USA</td>
<td>Flinn et al. (2019)</td>
</tr>
<tr>
<td>Bagre marinus (Mitchill, 1815)</td>
<td>Male</td>
<td>18</td>
<td>49.5 FL</td>
<td>USA</td>
<td>Armstrong et al. (1996)</td>
</tr>
<tr>
<td>Bagre marinus</td>
<td>Female</td>
<td>24</td>
<td>57.7 FL</td>
<td>USA</td>
<td>Armstrong et al. (1996)</td>
</tr>
<tr>
<td>Bagre marinus</td>
<td>Both</td>
<td>10</td>
<td>55.7 TL</td>
<td>USA</td>
<td>Flinn et al. (2019)</td>
</tr>
<tr>
<td>Bagre marinus</td>
<td>Male</td>
<td>14</td>
<td>50 TL</td>
<td>USA</td>
<td>Miguez (2019)</td>
</tr>
<tr>
<td>Bagre marinus</td>
<td>Female</td>
<td>25</td>
<td>55 TL</td>
<td>USA</td>
<td>Miguez (2019)</td>
</tr>
</tbody>
</table>

Faces were polished using 1000, 300, and 50 nm alumina powders in order to eliminate possible scratches on the surface of the structure. Subsequently, the otolith sections were immersed in 1% HCl solution for 25 s, washed with double distilled water, and dried to reveal the microstructure. Then, the otoliths were mounted on a holder-sample by conductive carbon film, and the surface was coated with a thin layer of gold (Au) of about 2 nm to avoid the electron charge effect. The images were recorded by utilizing SEM. Then, some morphologies and attributes of lapilli otolith microstructure were described according to Tomás & Geffen (2003), Cermeño et al. (2006), Panfili et al. (2009), and Green et al. (2009). The microstructures were measured from the digital images using SigmaScan Pro, version 5.0 (Systat Software Inc.) software.

One lapillus otolith (left) from all collected otoliths pairs was first photographed whole beside a scale (0.01 mm precision). It was then embedded in epoxy resin and cut transversely across the core using a circular diamond-tipped blade. One cut face of each otolith was stained for 30 min in a neutral-red solution prepared with 100 mL of distilled water, 0.2 g of neutral red, 0.5 mL of glacial acetic acid, and 1 g of sodium chloride (Easey & Millner 2008). It was washed with distilled water to remove solution excess and photographed. The neutral-red staining decalcified a thin layer of the otoliths' cut-face surface to expose the protein matrix to be stained by the neutral-red (Arneri et al. 1998, Easey & Millner 2008). The staining is less intense where there is a lower concentration of proteins and higher concentration of inorganic material (mainly calcium carbonate crystals), and vice versa (Richter & McDermott 1990, Arneri et al. 1998, Easey & Millner 2008).

The photographs of whole and cut-stained otoliths were obtained through a stereomicroscope with reflected light. Two independent readers counted the otoliths growth rings on the digital images of cut-stain otolith; one growth ring was composed of red-light (RL) and red-dark (RD) adjacent bands (Fig. 1). It was also recor-
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Figure 2. Morphological characteristics of otoliths of the sea catfish *Bagre panamensis*. Position of otoliths: D: dorsal, V: ventral, A: anterior, and P: posterior. a) *Lapillus*: upper dorsal face and lower ventral face. 1) Core, 2) antirostrum, 3) rostrum, 4) growth rings, 5) semipronounced anterodistal ditch, 6) superficial mesial depression, 7) internal mesial curve, 8) indistinct acoustic sulcus, 9) gibbus maculae. Red dots indicate the extremes of the otolith’s diameter; b) *Sagitta*: upper dorsal face and lower ventral face. 1) Ventral wing, 2) dorsal wing, 3) dorsal fissure, 4) ventral fissure, 5) growth rings; c) *Asteriscus*: upper dorsal face and lower ventral face. 1) Face, 2) antirostrum, 3) core, 4) growth ring, 5) lobus major, 6) sulcus "acoustic fossa", 7) canalliculum, 8) excisura "opening".

The diameters of otolith were measured from the digital images of the whole otolith using SigmaScan Pro, version 5.0 (Systat Software Inc.) software (Fig. 2a).

The ring counting accuracy was evaluated with the average error rate (APE, Beamish & Fournier 1981) and the coefficient of variation (CV, Chang 1982). The monthly relative frequency (%) of the percentage of RL and RD bands at the otolith edge by sex was explored throughout the year to define the deposition periodicity of the growth rings (Reis 1986, Fowler 2009, Maciel et al. 2018). Moreover, the frequencies were correlated with the gonadosomatic index (GSI = Gw / We × 100; Pinheiro et al. 2006) to explore the interaction between reproductive and growth processes, using the Spearman’s rank correlation coefficient \( r_s \) and a *t*-test with *n*-2 degree of freedom and a significance level \( P = 0.05 \) (Zar 2010). Furthermore, the statistical relations of otoliths diameter with TL and the number of growth rings were defined using linear and power models, respectively, fitted by the least-squares method (Zar 2010). Finally, the age structures of *B. panamensis* of...
each sex were presented and described. The Kolmogorov-Smirnov two-sample test was performed to compare age structure between females and males, with a significance level $P = 0.05$ (Zar 2010).

**RESULTS**

**Otoliths**

The lapilli of the sea catfish Bagre panamensis are large (>12 mm), globose or compressed, thick circle, with the rostrum more developed than the anterostrum; it has growth marks on the dorsal surface, difficult to identify in old fish because they overlap near the edge (Fig. 2a). While the sagitta is elongated (<10 mm), the anterior margin is more pointed than the posterior margin. It has a pair of dorsal and ventral wings that twist from the middle to the posterior margin of the otolith; it is a fragile structure. Some growth rings in the middle part up to the anterior margin of the otolith are difficult to identify (Fig. 2b). On the other hand, the asteriscus is a tiny structure (4.5 mm), smaller than the lapillus and sagitta. It is very fragile, translucent, and rounded, with slightly wavy edges, angular face, while the anterostrum is more or less pointed and little developed, existing between both a wide excisura; its acoustic fosse has granulations (Fig. 2c).

Under the SEM, the low-magnification microphotography of lapillus otolith's cut face showed dark and whitish bands (Figs. 3a-b). Darker areas appeared from electron absorption associated with the presence of insoluble organic material. Dark tonalities are seen throughout the otolith (Fig. 3a), including in whitish bands, indicating that the organic matrix is distributed throughout the otolith but with greater concentration in the darker areas. The lapilli otoliths present aragonite crystals with prismatic shape and long grouped (Figs. 3e-f), and the prismatic growth is radial, from the core to the otolith edge (Figs. 3c-d). The surface of aragonite crystals is smooth, and the prismatic form is variable (square, rectangular, pentagonal, hexagonal, or irregular) (Figs. 3g-h). The width of aragonite crystals is approximately 3.7 ± 1.3 μm.

**Growth rings periodicity**

A total of 371 lapilli otoliths from 176 males and 195 females of B. panamensis were suitably cut, stained, and examined. Lapilli otoliths form growth rings with an RL band wider and with less fixation of red-neutral dye. An indication of greater calcium crystals accumulation into the protein matrix due to growth. While the RD band of growth rings was the narrower and with greater fixation of red-neutral dye, indicating a minor accumulation of calcium crystals into the protein matrix due to growth slowdown (Fig. 1). The monthly RL and RD bands frequencies at the edge of the otolith show that one growth ring is formed annually because their behavior presented one periodical oscillation per year. The RD bands at the edge of the otolith (growth slowdown) were less frequent from October to February. Their frequencies began to increase during March, reaching a maximum frequency during April to July (growth slowdown season) decreased in August and September. The behavior of RL bands frequencies was reversed and presented the higher frequencies during August to March (fast-growth season) (Fig. 4). The growth slowdown season coincides with the highest values of the GSI in both sexes of B. panamensis, supported by a positive correlation between RD bands frequencies at the edges and the GSI (Fig. 4, Table 2).

**Particularities of growth rings**

Some growth rings of lapilli otoliths in B. panamensis presented different RL and RD bands composition in 35% of individuals concerning the previously described. Whereby, three different types of growth rings (GR) were identified, characterized by single (S, normal), double (D), and triple (T) RD bands in their composition (Fig. 5a). The additional RD bands in D and T growth rings are discontinuous. That is, the bands do not show continuity around the entire otolith (Fig. 5a). The T growth rings were observed in the first to fifth rings in males and the first to fourth in the females, but the highest frequency (42%) was observed in the second ring in both sexes (Fig. 5b). The D growth rings were observed from the first to the ninth rings (decreased in the latter) (Fig. 5b), and they were more frequent (31%) than grow rings T (4%).

Additionally, width alteration of one RL band was detected between the third and sixth growth rings of lapillus otolith in 8.6% of individuals. The width alteration is seen as a widening of the RL band from the right posterior margin to the middle part of the otolithic structure (Fig. 6).

**Rings counting and otolith size relationships**

The processing and ring counting of 371 lapilli otoliths was performed without difficulty. These otoliths have 4.6 to 12.1 mm in diameter. The accuracy of the ring counting between both readers was high (APE = 5.8% and CV = 8.1%). The individuals presented 13 to 49.1 cm in total length (TL) and one to 15 years old (age, AG). The relationships of lapillus otolith diameter (OD) as a function of TL and AG showed high coefficients of determination, demonstrating a clear positive proportionality between the increase in OD concerning the TL and AG (Fig. 7). The relationship between OD and TL was linear in both sexes ($r^2 = 0.7825$)
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Figure 3. Scanning electron micrographs of the sea catfish Bagre panamensis' lapilli otoliths. a-b) Dark (arrows) and whitish bands on the otolith's cut face, c-d) stretch marks from the core (cr) indicating the radial growth (dotted arrows) of the aragonite crystals (arrows with solid line), e-f) the long grouped aragonite crystals (arrow and dotted marks), g-h) view of aragonite crystals that reveal the variable prismatic form (arrows).

Figure 4. Monthly red-light (RL) and red-dark (RD) bands frequencies at the otoliths edge and monthly average of gonadosomatic index (GSI) of males (a) and females (b) of Bagre panamensis from the southeastern Gulf of California. Standard error (bars).

males and $r^2 = 0.8752$ females) (Fig. 7a). Meanwhile, the relationship between OD and AG presented a suitable fit to the power model in both sexes ($r^2 = 0.7788$ males and $r^2 = 0.7712$ females) (Fig. 7b).
Table 2. Correlations between the monthly red-light (RL) and red-dark (RD) bands frequencies at the otoliths edge and monthly average of gonadosomatic index (GSI) of males and females of *Bagre panamensis* of southeastern Gulf of California. n: data number, \( r_s \): Spearman’s rank correlation coefficient, \( t \): \( t \)-test, \( P \): \( P \)-value, asterisk (*) indicates a significant correlation \((P < 0.05)\).

<table>
<thead>
<tr>
<th>Correlations</th>
<th>n</th>
<th>( r_s )</th>
<th>( t_{(n-2)} )</th>
<th>( P )</th>
</tr>
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<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RD band vs. GSI</td>
<td>14</td>
<td>0.6703</td>
<td>3.1292</td>
<td>0.008704*</td>
</tr>
<tr>
<td>RL band vs. GSI</td>
<td>14</td>
<td>-0.6703</td>
<td>-3.1292</td>
<td>0.008704*</td>
</tr>
<tr>
<td><strong>Females</strong></td>
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<td></td>
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<tr>
<td>RD band vs. GSI</td>
<td>14</td>
<td>0.6828</td>
<td>3.2376</td>
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<tr>
<td>RL band vs. GSI</td>
<td>14</td>
<td>-0.6828</td>
<td>-3.2376</td>
<td>0.007118*</td>
</tr>
</tbody>
</table>

Figure 5. a) Cross-sections of *lapillus* of the *Bagre panamensis* with different types of growth rings, S: single, T: triple, D: double, b) relative frequency of the different type growth rings (single, double, and triple) regarding the total number of growth rings.

**Age structure**

The age structure of the *B. panamensis* sample from the southeastern Gulf of California was composed of organisms from one to 14 years in females and one to 15 years in males. The five to nine years old individuals were the most frequent (Fig. 8). The age structures were similar between females and males \((D_{max} = 0.021329; \ P = 0.10)\).

**DISCUSSION**

**Otoliths**

The descriptions made on *Bagre panamensis* otoliths correspond to that reported for freshwater catfishes (Martínez & Monasterio de Gonzoz, 1988, 1991) and sea catfishes (Volpedo & Echeverría, 2000, Acero & Betancur 2007, Chen et al. 2011, Aguilera et al. 2013, Santificetur et al. 2017, Volpedo et al. 2017), in which the *lapilli* exceed in size the *sagitta* and *asteriscus*. The *lapilli* of the Ariidae is larger than the *lapilli* found in the Plotosidae, *Horabagrus*, and Archariidae catfishes (Oliveira et al. 2001, Diogo 2005). The large size of the *lapillus* is attributed to the fact that this structure occupies the area corresponding to several bones of the cranial otic region (Oliveira et al. 2001, Diogo 2005), which allows it to expand within the cavity and increase its size. Hence, the *lapillus* otolith is one of the most used structures to estimate the age and growth of the catfishes.
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**Figure 6.** Cross-sections *lapillus* otolith of *Bagre panamensis* with width alteration of one red-light (RL) band (black arrows).

**Figure 7.** a) Relationship between the otolith diameter-total length, b) otolith diameter-age of males and females of *Bagre panamensis* in the southeast of the Gulf of California.

The *lapilli* otoliths presented aragonite micro-crystals with a prismatic form, the typical shape of these structures (Tomás & Geffen 2003, Panfili et al. 2009). In some acid-treated otoliths, the crystals were degraded partially or almost totally due to exposure with HCl (Cermeño et al. 2006, Green et al. 2009). The electron absorption was different along the otolith, revealing dark and whitish bands similar in width and shape to the RD and RL bands showed by the neutral-red stain, respectively. Therefore, they are representing the same areas in the otoliths. The darker zones (dark bands) due to electron absorption are associated with insoluble organic material (Cermeño et al. 2006) in higher concentrations. While a more reddish stain (RD bands) due to neutral-red stain is attributed to a higher concentration of proteins (Richter & McDermott 1990, Arneri et al. 1998, Easey & Millner 2008). This coherence between the two methods supports that the narrower bands of rings otoliths (dark bands and RD bands) of *B. panamensis* present a higher concentration of proteins and a minor accumulation of aragonite crystals concerning the wider bands (whitish bands and RL bands).

**Growth rings periodicity**

*B. panamensis* forms one growth ring annually in *lapilli* otoliths. Similar to other species of sea catfishes (Reis 1986, Tilney 1990, Mehanna et al. 2012, Cheraghi-Shevi...
et al. 2015, Maciel et al. 2018, Flinn et al. 2019). The annual growth pattern of rings presented similar periodicity to the annual reproductive pattern, so the fast-growing season coincided with the time of reproductive rest. In contrast, the slowdown growth season was overlapped with the time of reproduction and oral incubation (eggs and offspring) of B. panamensis in the southeast of the Gulf of California (Muro-Torres 2011, Muro-Torres & Amezcua 2011, Zavala-Leal et al. 2019). Similar results have been reported in the sea catfish Galeichthys feliceps and G. ater of South Africa coasts (Tilney 1990), Genidens barbus (Reis 1986), and G. genidens (Maciel et al. 2018) of Brazil coasts. They ensure that reproductive activity and oral incubation (eggs and offspring) entails a high energy expenditure (Rimmer & Merrick 1983, Tilney 1990), and this is a critical factor in reducing the sea catfish growth rate (Reis 1986, Velasco & Reis 2004, Velasco et al. 2007, Maciel et al. 2018).

**Particularities of growth rings**

The S growth rings were considered normal because they are the type of growth ring commonly found and counted to assess the age in fishes (Campana 2001, Green et al. 2009). The additional discontinuous RD bands in D and T growth rings were considered false bands since they were not continuously registered around the otolith. Hauser et al. (2018) recorded similar growth patterns and types of rings in lapillus otolith of the freshwater catfish Brachyplatystoma rousseauxii (Castelnau, 1855) from the Orinoco River, as in B. panamensis. The authors documented that it was difficult to define age due to D and T growth rings presence. They reported that the stress caused by the increased salinity in the Orinoco River during August and September and competition for food, and long periods of starvation were critical factors that led to the formation of these otoliths anomalies.

Similarly, D and T growth rings were found in the sagittae otoliths of adult fish of the American eel Anguilla rostrata (Lesueur, 1817) and the Swedish eel A. anguilla (Linnaeus, 1758), attributed to the stress caused by high-temperature levels and low oxygen concentrations during the summer, that cause slow-or-stop growth and the deposition of false bands (ICES 2009). Likewise, D growth rings were found in daily growth rings of sagittae otoliths in juveniles of European anchovy Engraulis encrasicolus (Linnaeus, 1758) (Cermeño et al. 2006, 2008) and Peruvian anchovy E. ringens (Jenyns, 1842) (Cerna & Plaza 2016). They contained a large amount of protein matrix (Cerdeño et al. 2006), deposited as evidence of metamorphosis stages and habitat changes (Morales-Nin & Aldebert 1997, Tomás & Panfili 2000).

The false bands’ formation (discontinuous RD bands) and the width alteration of one RL band in lapillus otolith could be related to various biological process such as habitat change during the juvenile stage (from the ocean to estuarine systems and vice versa; Amezcua et al. 2006, Madrid-Vera et al. 2007), sexual maturity (35.5 cm LT in Muro-Torres & Amezcua 2011; 3.8 years in Muro-Torres 2011), reproduction and oral incubation of offspring (Muro-Torres & Amezcua 2011), and also with changes in environmental conditions (Beamish & McFarlane 1983, Reis 1986, Velasco & Reis 2004). Those otoliths structure alterations are occurring at the time in which such processes could also be occurring in B. panamensis. Those "alterations" are an abnormal change in the deposition of protein matrix (false bands) and the inorganic material (width alteration of RL band), and this alteration may occur during a short time since it is not fully developed around the otolith structure. Perhaps, those "alterations" are caused by a circumstantial and abnormal limitation (false bands) or availability (width alteration of RL band) of food or energy reserves during the fast growth and slowdown growth seasons, respectively.

**Rings counting and otolith size relationships**

The accuracy of ring counting was high, according to Campana (2001), and the growth rings were distinguishable, even in older individuals. However, the rings were closer together but the neutral-red staining provided adequate contrast. It is worth mentioning that neutral-red staining has been widely used to improve the contrast of growth rings and for the age study in cross-sections of sagittae otoliths of several marine fish species (Richter & Mcdermott 1990, Arneri et al. 1998, 2001, Franks et al. 2001, Peltonen et al. 2002, Easey & Millner 2008).

![Figure 8. The age structure of males and females of the Bagre panamensis sample from the southeastern Gulf of California.](image-url)
The proper contrast of the growth rings and their clear temporal formation pattern in *lapilli* otoliths, in addition to the clear positive proportionality in the relationships of otolith size (OD) as a function of TL and AG, confirm that *lapilli* otoliths are adequate structures to estimate the age and growth of *B. panamensis* as having been reported for other species of sea catfishes (Reis 1986, Tilney 1990, Mehanna et al. 2012, Cheraghi-Shevi et al. 2015) and freshwater catfishes (Hauser et al. 2018).

### Age structure

The age structure of *B. panamensis* was composed of organisms between one to 15 years old. The most frequent ages in the sampled were five to nine years old, mainly adult organisms since *B. panamensis* reaches size and age of sexual maturity at 35 cm of TL and 3.8 years (Muro-Torres 2011, Muro-Torres & Amezcua 2011). The maximum observed age was 15 years, almost twice the maximum age recorded (8 years) by Muro-Torres & Amezcua (2011). Also, for *B. panamensis* in the same sampling area, despite that analyzed individuals in both studies had similar sizes (13 to 49.1 cm TL in the present study; 14 to 52 cm TL in Muro-Torres & Amezcua, 2011). This difference could be due to Muro-Torres & Amezcua (2011) counted growth rings using the whole *lapilli* otolith. Because the growth rings can be masked and poorly distinguished toward the edge of the whole otoliths, fewer growth rings are counted. Whence, the age can be underestimated from whole otoliths, compared to the sectioned otoliths (Panfili et al. 2002, Easey & Millner 2008, Volpedo & Vaz-dos-Santos 2015). For the freshwater catfish *Sperata aor* (Hamilton, 1822), growth rings of whole *lapilli* otoliths are unclear in the otolith edge due to the curvature in older individuals causing underestimating age (Khan et al. 2016), and its cross-sectioned otoliths had more distinguishable growth rings than whole otoliths (Nazir & Khan 2020). Also, for the sparid fish *Argyrozoa argyrozoa* (Valenciennes, 1830), the number of growth rings number is underestimated when using whole *sagitta* otoliths of fish older than ten years old concerning counts made from cross-sectioned otoliths (Brouwer & Griffiths 2004).

Although growth ring counting from whole otoliths is a valid technique and still applied in several fish species (Volpedo & Vaz-dos-Santos 2015), given the present study results and the notable differences with the previous studies, it is not recommended for age estimation in the sea catfish *B. panamensis*. Instead of that, the cross-sectioning and red-neutral staining of otoliths are more suitable for age estimation in *B. panamensis*, like in other fish species, to reduce the interpretation error and age underestimation (Campana 2001, Easey & Millner 2008). It is worth noting that *B. panamensis* is a fishery resource in Mexico and is considered a species with aquaculture potential. The age data are important for fisheries and aquaculture management, such as estimating growth and mortality rate in productivity assessment. Moreover, the age underestimation (rather than overestimation) could cause overly optimistic productivity estimates that may contribute to overexploitation of a population in a fishery (review by Campana 2001) and inaccurate yield estimates in aquaculture.

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