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Morphological and morphometric changes of *sagittae* otoliths related to fish growth in three Mugilidae species

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Summary

The aim of this study was to analyze morphologic and morphometric changes of *sagittae* otoliths throughout the growth of three mullets: *Mugil liza*, *Mugil cephalus* and *Liza ramada*. Fish were collected seasonally between October 2011 and April 2014, and three methods were used: regression analysis of traditional biometric characters; morphological analysis for group identification; and morphometrical analysis of shape indices among groups (circularity, rectangularity, aspect ratio and percentage occupied by the *sulcus*). In all species, dependence of standard length on otolith length and height were best described as power functions. Two morphological groups were identified for *M. liza* and *M. cephalus*, while three for *L. ramada*. Morphological changes were supported by morphometry differences only for the first two species. Smaller size specimens of *M. liza* had more rectangular otoliths with more percentage covered by the *sulcus* while bigger fish tended to have a more circular shape. For *M. cephalus* smaller size fish had a more elliptical shape. The observed changes may be reflecting life history changes, related to habitat or dietary shifting.

1 | INTRODUCTION

Feeding ecology is relevant for understanding trophic networks, based on the identification of prey and the interactions involved (Gerking, 2014). Different methods are usually employed to identify dietary items such as stable isotopes, or recognition of structures like bones and otoliths (Boecklen, Yarnes, Cook, & James 2011; Jansen et al., 2012; Volpedo & Echeverría, 2000). To analyze feeding habits in marine mammals, birds and fishes the study of stomach contents provides information for recently ingested prey (Bustos, Daneri, Volpedo, Harrington, & Varela 2012, 2014; Pierce & Boyle, 1991).

Otoliths are structures composed mainly of calcium carbonate over an organic matrix (Campana, 1999). Their morphology is species-specific (Avigliano, Jawad, & Volpedo 2016; Callicó Fortunato, Benedito Durà, & Volpedo 2014; Campana, 2004; Hecht, 1987; Tuset, Lombarte, & Assis 2008; Volpedo & Echeverría, 2000, 2001) and particularly, otolith size has a close relationship with fish body size (Martucci, Pietrelli, & Consiglio 1993). These facts show how valuable otolith data are when analyzing feeding relations in different aquatic ecosystems, from the present and the past. These inert structures record life history stage transitions of fishes (Brothers & McFarland, 1981) and may have a substantial importance when working in trophic studies and paleoecology. Identifying otolith morphology and morphometry found in fossils or in diet remains can determine not only species but also specimen size (Barrett et al., 2007; Bustos et al., 2012, 2014; Reichenbacher, Sienknecht, Küchenhoff, & Fenske 2007; Riet-Sapriza et al., 2013; Scartascini & Volpedo, 2013; Tuset, Parisi-Baradad, & Lombarte 2013; Tuset et al., 2015; Veen, Mullié, & Veen 2012). Even though otolith erosion, caused by digestion, is expected to decrease its length, otolith features are still a good approximation for trophic studies on species that are difficult to collect (Harvey, Loughlin, Perez, & Oxman 2000).

Members of the Mugilidae family, generally known as mullets, are coastal marine fishes with a worldwide distribution including all temperate, subtropical and tropical seas. Because of their tolerance to a wide range of salinities, they not only inhabit offshore and coastal waters, but also spend part of their life cycle in coastal lagoons, lakes and/or rivers using these areas for feeding, refuge and development (González-Castro & Ghasemsadeh 2015; González-Castro & Minos, 2015; Heras, Roldán, & González-Castro 2009; Nelson, 2006). Mugilids, after their resting and

TABLE 1 Features of sampling areas and fish captured of the three studied species: *Mugil liza*, *Mugil cephalus* and *Liza ramada*

Species	N	SL (mm)	Sampling location	Sampling latitudes	Latitudinal distribution range
Mugil liza	238	81–488	Coast of Buenos Aires province, Argentina: Samborombon Bay (Estuary), Mar Chiquita coastal lagoon, San Blas Bay (Sea coast).	35°27′–40°37′S	(30°44′N–42°31′S) Menezes et al. (2010) and Froese and Pauly (2016)
Mugil cephalus	164	118–577	Valencia Community coast, Spain: Ebro Delta (Coast and River basin), Albufera of Valencia (Mediterranean lake), Santa Pola (coastal salt marsh).	40°43′–38°09′N	Discontinuous distribution, between 42°N and 42°S Whitfield et al. (2012)
Liza ramada	147	120–584			60°22′–27°49′N Froese and Pauly (2016)

maturing periods in coastal lagoons, estuaries or related environments, perform a reproductive migration towards the sea (González-Castro, Abachian, & Perrotta, 2009; González-Castro, Macchi, & Cousseau 2011; Whitfield, 2015). After spawning, some adults may return to estuaries and others remain in the marine environment (Whitfield, Panfili, & Durand 2012). Mullets are important food items for birds such as cormorants (Martucci et al., 1993; Barquete, Bugoni, & Vooren 2008; Veen et al., 2012; Muñoz-Gil, Marín-Espinoza, Andrade-Vigo, Zavala, & Mata 2013), pinnipeds such as sea lions and grey seals (Alava & Gobas, 2012; Mikkelsen, 2013), and other fishes such as the leerfish (*Lichia amia*), the smoothhound shark (*Mustelus mustelus*) and the giant trevally (*Caranx ignobilis*) (Morte, Redon, & Sanz-Brau 1997; Whitfield & Blaber, 1978). They are also commercially-relevant species in industrial fisheries with the extractions greater than 550,000 t/year all over the world (FAO, 2017). In some regions, like Argentina or Venezuela, mullets are important in artisanal fisheries, supporting local communities as food or bait (Gallardo-Cabello, Espino-Barr, Cabral-Solis, Puente-Gomez, & Garcia-Boa 2012; González-Castro et al., 2009; Marin, Quintero, Bussière, & Dodson 2003).

Given the importance of the Mugilidae family in trophic food webs and in fisheries, the general aim of the present study is to analyze morphologic and morphometric changes of *sagittae* otoliths throughout the development of three selected mullet species: *Mugil liza* Valenciennes, 1836; *Mugil cephalus* Linnaeus, 1758 and *L. ramada* (Risso, 1827); in order to contribute to the general knowledge of Mugilidae species ontogeny and their recognition as prey items. Two specific objectives are set out, (i) general description of the otoliths' growth changes by means of traditional biometric characters related to fish growth; and (ii) morphological analysis of otoliths to characterize the changes in features throughout the growth of the specimens, recognizing groups, that are confirmed by morphometrical analyses to determine if the subjective separation can be sustained by morphometry.

2 | MATERIAL AND METHODS

2.1 | Sample collection

Specimens of three species of Mugilidae family (*M. liza*, *M. cephalus* and *L. ramada*) were collected seasonally between October 2011 and April 2014. Individuals were obtained with gill nets and trammel nets (mesh size ranging from 2.5 to 25 cm between nots) in artisanal catches of local communities in part of their distribution area (Table 1). A wide range of sizes was sampled, for each species, so as to have large number of individuals of the different development stages (Table 1). For species identification, the keys proposed by Trewavas and Ingham (1972); Fischer, Bauchot, and Schneider (1987), Thomson (1997) and Harrison (2002) were employed. The standard length (SL) of each fish was recorded to the nearest millimeter. *Sagittae* otoliths were removed, cleaned and stored in plastic vials for further examination and measurement.

2.2 | Otolith shape measurements

The medial face of each right otolith was photographed with a digital camera attached to a stereomicroscope (Leica® EZ4-HD, Leica Microsystems, Wetzlar, Germany). All images were analyzed and measured using image processing systems (Image-Pro Plus 4.5®, Media Cybernetics Inc., Rockville, MD, USA). Otolith gross morphology descriptions were made according to Tuset et al. (2008). Morphological features analyzed were: otolith shape, anterior/posterior regions, dorsal/ventral margins, *cauda* and *ostium*, presence/absence of culminant point (Mollo, 1981). To characterize otolith growth related to fish size, a regression analysis (Casselman, 1990; Huxley, 1993) of traditional biometric characters was performed. Maximum length (OL) and maximum height (OH) of the otolith, in millimeters, (Figure 1) were measured; the equation $y=ax^b$ was used to fit relations between SL and OL-OH (Huxley, 1993; Lleonart, Salat, & Torres 2000), where *b* represents the "constant of differential growth rate" (Corruccini, 1972).

For the morphometrical analysis of identified groups, a shape indices analysis was used (Avigliano, Martinez, & Volpedo 2014; Rossi-Wongtschowski, 2015; Tuset, Lombarte, González, Pertusa, & Lorente 2003; Volpedo & Echeverría, 2003). Apart from OL and OH, otolith perimeter (OP) and area (OA), and *sulcus* perimeter (SP) and area (SA) were measured in millimeters (linear measurements) or in square millimeters (areas) (Figure 1). Shape indices were then calculated to analyze otolith variations throughout the ontogenetic development of the studied species: Circularity (OP^2/OA), providing information on the complexity of the otolith contour (Tuset et al., 2003, 2008); Rectangularity (OA / [OL × OH]), giving information on the approximation to a rectangular or square shape, being 1 a perfect rectangle or

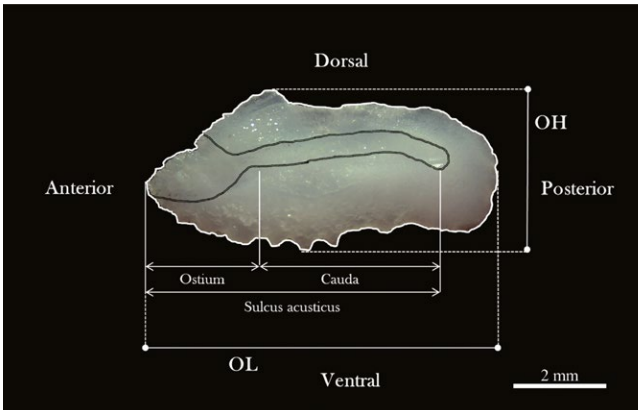

FIGURE 1 Features and measured variables for *sagitta* otolith of mugilids. OL: maximum length; OH: maximum height; continuous white line: otolith perimeter (OP); continuous black line: *sulcus* perimeter (SP)

square (Tuset et al., 2003, 2008); Aspect ratio (OH/OL; %) (Tuset et al., 2008); and Percentage of the otolith area occupied by the *sulcus* (SA/OA; %) (Avigliano et al., 2014).

2.3 | Statistical analysis

Shape indices were corrected to eliminate possible allometry effects in otolith shape related to fish body size, for a proper comparison among groups; the formula proposed by Lleonart et al. (2000): $y' = yi \times (x0/xi)^b$ was used, in which y' is the corrected predictive variable, yi is the original value of the obtained shape index, x0 is a referential SL value (*M. liza*: 360 mm; *M. cephalus*: 300 mm; *L. ramada*: 250 mm), xi is the original SL value, and *b* is the Huxley coefficient of each regressioned variable to SL. For each species, shape indices were compared among previously identified morphological groups. Variables were tested for normality with Shapiro–Wilks test and homogeneity of variances with Levene's test. For normal shape indices, groups were compared with a paired *t* test or with ANOVA when more than two groups were identified. For non-normal variables Mann–Whitney *U* test or a Kruskal–Wallis test were used.

Sex could only be identified for adult individuals, and no significant differences were found for the measured variables between males and females in each studied species (Mann–Whitney *U* test, $p > .05$); thus, all otoliths were pooled to perform the analysis.

Finally, curves of otolith growth relative to fish size were compared among species. For this, SL vs OL and SL vs AO relations were linearized applying a Log transformation, to compare the b parameter (slopes) among the three studied species by means of Statgraphics® software (Statgraphics.Net, Madrid, Spain).

3 | RESULTS

A general Mugilidae *sagitta* pattern can be recognized for adult individuals of the three studied species: *Sagitta* shape is rectangular to

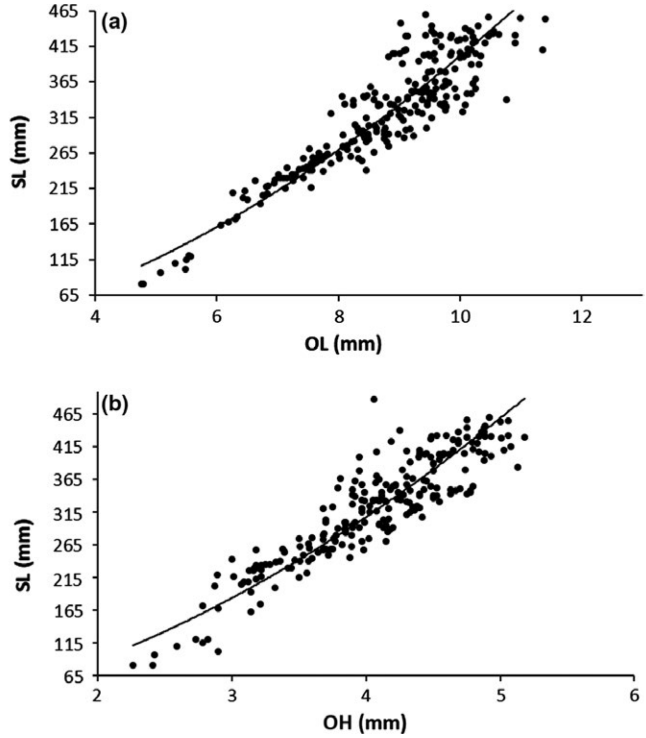

FIGURE 2 Dispersion diagram and regression (power function) of standard length (SL) vs (a) Maximum length of *sagitta* (OL) and (b) Maximum height of *sagitta* (OH), for *Mugil liza*

oblong with irregular margins; the *sulcus acusticus* is heterosulcoid and ostial, formed by a short funnel-like *ostium* open to the anterior margin, and a closed tubular *cauda* at least two times bigger than the *ostium*.

Nevertheless, during their growth, the morphology of the *sagitta* of the studied mullets presents differences. Each species has distinctive otolith morphology patterns as described below.

3.1 | *Mugil liza*

3.1.1 | Otolith biometric characters general description

Dependence of SL on OL and OH is best described as power functions, presenting a significant positive relationship ($p < .01$) for each case. Including all individuals (n = 238), regression equations for both variables are as follows: $SL = 6.53 \times OL^{1.79}$ ($R^2 = .87$); $SL = 25.72 \times OH^{1.79}$ ($R^2 = .84$) (Figure 2a and b).

3.1.2 | Morphological descriptions

Based on the observed morphological characters of the *sagitta*, studied specimens of this species can be separated into two groups: (I) Size range 81–370 mm SL (n = 173): *sagitta* with peaked anterior region and the presence of a dorsal culminant point; (II) Size range >370 mm SL (n = 65): *sagitta* with angled anterior region, and absence of culminant point (Figure 3). All *M. liza* specimens present a rectangular

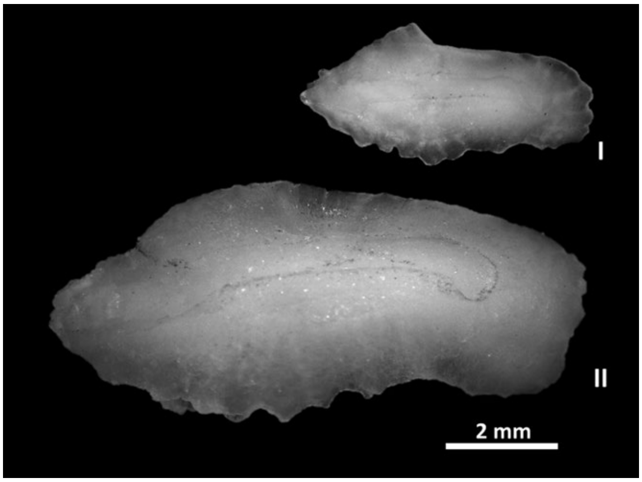

FIGURE 3 General morphology patterns of the identified groups (I and II) for *sagittae* otoliths of *Mugil liza*. Standard lengths of specimens shown in figure: I. 193 mm; II. 460 mm

sagitta with a tubular *cauda* slightly curved, and round to flattened posterior region (Figure 3).

3.1.3 | Shape analysis

All shape indices but circularity, are normal and have homogeneity of variances (Shapiro–Wilk, $p > .05$; Levene, $p > .05$). Identified groups differ significantly on rectangularity, aspect ratio, and percentage of otolith occupied by the *sulcus* (Table 2). Thus, smaller specimens show more rectangular otoliths, longer than wider, with a greater surface of their medial face occupied by the *sulcus*.

3.2 | *Mugil cephalus*

3.2.1 | Otolith biometric characters general description

Dependence of SL on OL and OH is best described as power regressions presenting a significant positive relationship ($p < .01$) for each case. Including all individuals (n = 164), regression equations for both variables are as follows: $SL = 8.53 \times OL^{1.63}$ ($R^2 = .84$); $SL = 34.25 \times OH^{1.56}$ ($R^2 = .89$) (Figure 4a,b).

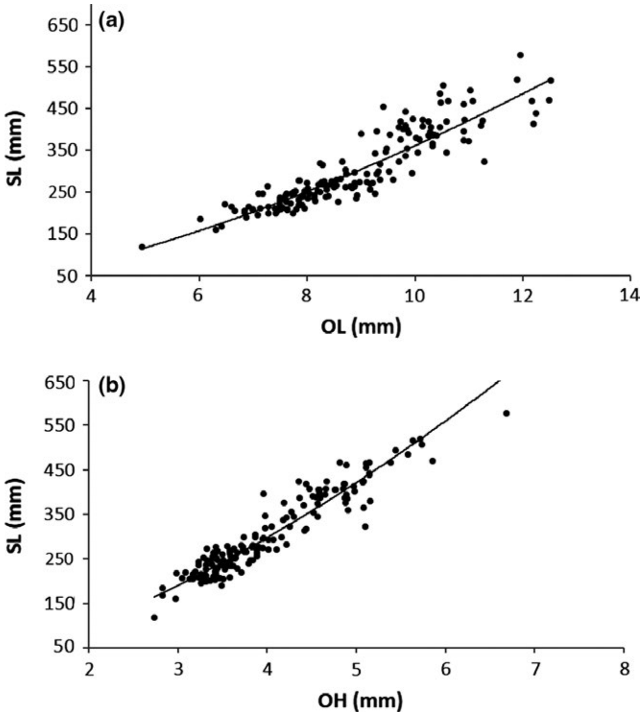

FIGURE 4 Dispersion diagram and regression (power function) of standard length (SL) vs (a) Maximum length of *sagitta* (OL) and (b) Maximum height of *sagitta* (OH), for *Mugil cephalus*

3.2.2 | Morphological descriptions

Based on the observed morphological characters of the *sagitta*, studied specimens can be separated into two groups: (I) Size range 118–465 mm SL (n = 154): *sagitta* with peaked anterior region; (II) Size range >465 mm SL (n = 10): *sagitta* with angled anterior region (Figure 5). All *M. cephalus* specimens present a rectangular *sagitta* shape with a tubular *cauda* straight or slightly curved and flattened to round posterior region (Figure 5).

3.2.3 | Shape analysis

All shape indices but circularity, are normal and have homogeneity of variances (Shapiro–Wilk, $p > .05$; Levene, $p > .05$). Identified groups differ significantly on aspect ratio index (Table 3). Small size individuals present smaller aspect ratios, being their *sagittae* longer than wider.

TABLE 2 Mean and standard deviation of the shape indices analyzed among identified groups for *M. liza*; and their statistical analysis: *W* = Mann–Whitney *U* test and *T* = paired *t* test. In bold, $p < .05$

	Group I (n = 173)	Group II (n = 65)		
	Mean	Mean	Statistic	*p*
Circularity (OP^2/OA)*	24.22 ± 1.91	23.86 ± 2.05	*W* = 6945.00	.082
Rectangularity (OA / [OL × OH])	0.69 ± 0.03[a]	0.68 ± 0.03[b]	*T* = 2.12	**.035**
Aspect ratio (OH / OL)	0.46 ± 0.03[a]	0.47 ± 0.03[b]	*T* = −3.13	**.002**
Percentage occupied by the sulcus (SA/OA)	0.24 ± 0.02[a]	0.23 ± 0.02[b]	*T* = 2.24	**.026**

*Non-normal variable.
Different letters show significant differences ($p < .05$).

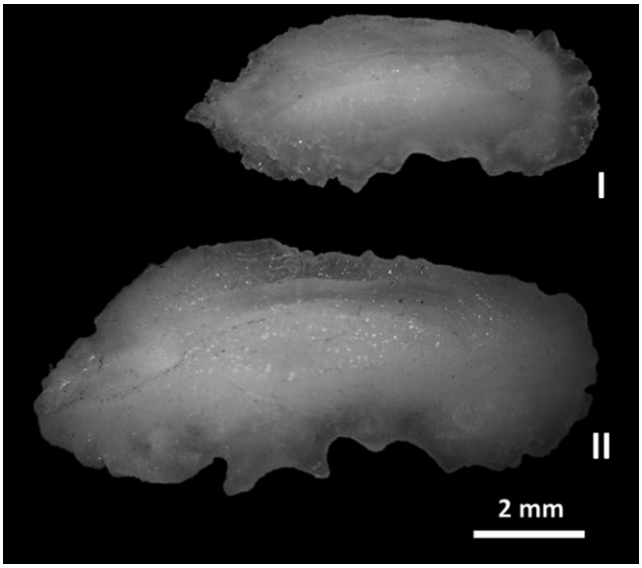

FIGURE 5 General morphology patterns of the identified groups (I and II) for *sagittae* otoliths of *Mugil cephalus*. Standard lengths of specimens shown in figure: I. 238 mm; II. 493 mm

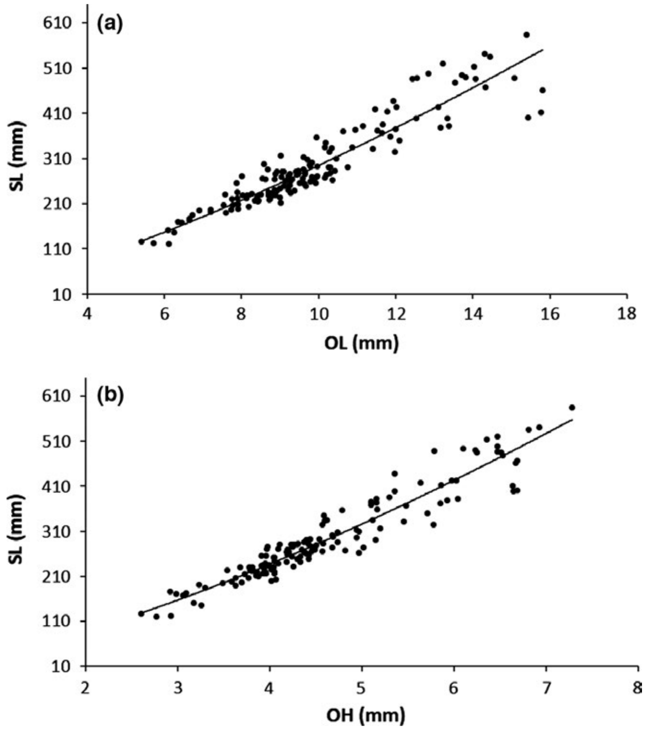

FIGURE 6 Dispersion diagram and regression (power function) of standard length (SL) vs: (a) Maximum length of *sagitta* (OL) and (b) Maximum height of *sagitta* (OH), for *Liza ramada*

3.3 | *Liza ramada*

3.3.1 | Otolith biometric characters general description

Dependence of SL on LO and OH is best described as power regressions showing a significant positive relationship (p < .01) for each case. Including all individuals (n = 147), regression equations for both variables are as follows: SL = 12.72 × $OL^{1.36}$ (R^2 = .90); SL = 32.54 × $OH^{1.43}$ (R^2 = .91) (Figure 6a,b).

3.3.2 | Morphological descriptions

Based on the observed morphological characters of the *sagitta*, the studied specimens can be separated into three groups: (I) Size range <140 mm SL (n = 3): *sagitta* with elliptic shape; (II) Size range 140–275 mm SL (n = 75): *sagitta* with rectangular shape and peaked anterior region; (III) Size range >275 mm SL (n = 69): *sagitta* with rectangular shape and round to irregular anterior region (Figure 7). All *L. ramada* specimens present a sinuous *cauda* markedly bent towards the ventral region and round to angled posterior region (Figure 7).

3.3.3 | Shape analysis

All shape indices but circularity, are normal and have homogeneity of variances (Shapiro–Wilk, p > .05; Levene, p > .05). Identified groups do not differ significantly in any of the obtained shape indices (Table 4).

3.3.4 | Biometric relations among species

When comparing the biometric relations of the otolith related to fish growth among the three studied mullets, significant differences are found (p < .01) for both otolith length and otolith height. *Liza ramada* presents lesser regression slope for the biometric variables (SL vs

TABLE 3 Mean and standard deviation of the shape indices analyzed among identified groups for *M. cephalus*; and their statistical analysis: *W* = Mann–Whitney *U* test and *T* = paired *t* test. In bold, p < .05

	Group 1 (n = 154)	Group 2 (n = 10)		
	Mean	Mean	Statistic	*p*
Circularity (OP^2/OA)*	23.38 ± 2.05	23.36 ± 0.02	*W* = 862.00	.799
Rectangularity (OA / [OL × OH])	0.72 ± 0.02	0.71 ± 0.04	*T* = 1.05	.296
Aspect ratio (OH / OL)	0.45 ± 0.03[a]	0.48 ± 0.01[b]	*T* = −2.29	**.024**
Percentage occupied by the sulcus (SA/OA)	0.23 ± 0.02	0.23 ± 1.82	*T* = 0.04	.969

*Non-normal variable.
Different letters show significant differences (p < .05).

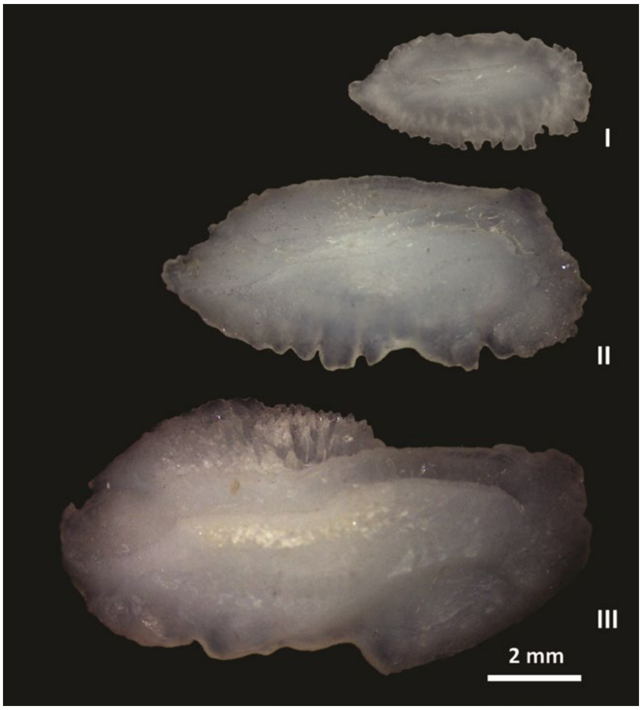

FIGURE 7 General morphology patterns of the identified groups (I, II and III) for *sagittae* otoliths of *Liza ramada*. Standard lengths of specimens shown in figure: I. 117 mm; II. 241 mm; III. 541 mm

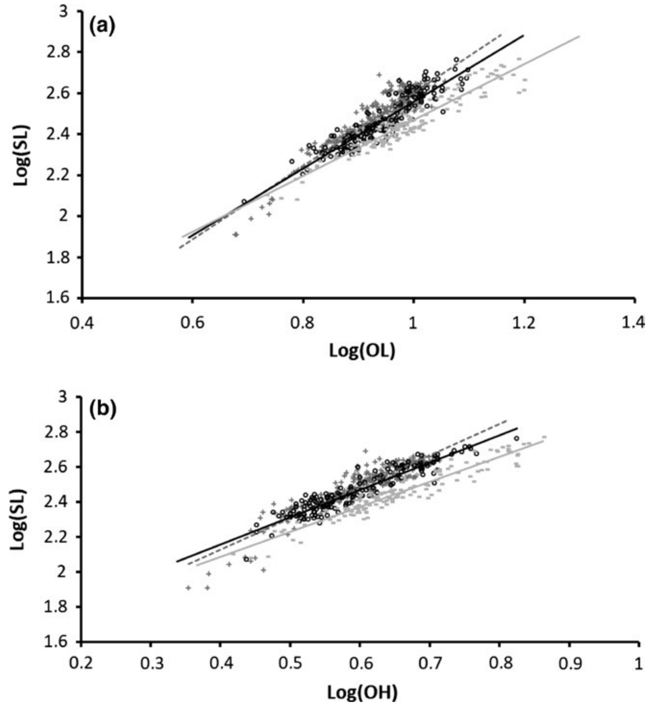

FIGURE 8 Linear regressions of biometric variables for the three analyzed mugilids. (a) Log(SL) vs Log(OL) and (b) Log(SL) vs Log(OH). Black circles and solid trend-line corresponds to *Mugil cephalus* specimens; dark grey crosses and dashed trend-line corresponds to *Mugil liza* specimens; and light grey dashes and solid line corresponds to *Liza ramada* ones. SL: standard length; OL: Maximum length of *sagitta*; and OH: Maximum height of *sagitta*. Data collected between October 2011 and April 2014

OL = 1.36, SL vs OH = 1.43), while *Mugil liza* presents greater slope values (SL vs OL = 1.79, SL vs OH = 1.79) (Figure 8). *Mugil cephalus* shows intermediate values for both slopes (SL vs OL = 1.63, SL vs OH = 1.54) (Figure 8).

4 | DISCUSSION

The results obtained in the present study indicate that the three studied mugilids present ontogenetic changes in the morphology and morphometry of the otolith related to fish growth. Mathematic expressions relating otolith biometric features with fish length were obtained for all species (*M. liza*, *M. cephalus* and *Liza ramada*); these could be useful to estimate size and development stages of ingested mullets by piscivorous predators as done by other authors (Barros & Wells, 1998; Blanco, Salomón, & Raga 2001; Ross, Johnson, & Adams 2005).

Moreover, for the three mullets, different groups were identified by otolith morphology during fishes' development. Common morphologic features that permitted group separation were shape, anterior region, and presence/absence of culminant point. Otolith shape indices showed differences among groups only for *M. cephalus* and *M. liza*. For the latter, a species with an extensive latitudinal distribution, from Florida, USA, throughout the Caribbean Sea to north for the Argentinian Patagonia (30°44′N–42°31′S) (Froese & Pauly, 2016; Menezes, De Oliveira, & Nirchio 2010), morphological groups were

TABLE 4 Mean and standard deviation of the shape indices analyzed among identified groups for *L. ramada*; and their statistical analysis: *F* = ANOVA test and *H* = Kruskal–Wallis test

	Group 1 (n = 3)	Group 2 (n = 75)	Group 3 (n = 69)		
	Mean	Mean	Mean	Statistic	*p*
Circularity (OP^2/OA)*	25.18 ± 2.79	23.49 ± 3.14	23.80 ± 2.85	*H* = 2.02	.364
Rectangularity (OA / [OL × OH])	0.74 ± 0.01	0.74 ± 0.02	0.74 ± 0.02	*F* = 0.02	.983
Aspect ratio (OH / OL)	0.49 ± 0.01	0.47 ± 0.03	0.47 ± 0.03	*F* = 0.82	.441
Percentage occupied by the sulcus (SA/OA)	0.22 ± 0.01	0.20 ± 0.02	0.20 ± 0.02	*F* = 1.10	.337

*Non-normal variable.

differentiated by three shapes indices. Otoliths in group I (smaller sizes) were more rectangular, had more percentage of otolith covered by *sulcus*, and smaller aspect ratio; while otoliths in group II tended to have a more circular shape. Thus, morphometric indices were useful on differentiating changes associated with growth for *M. liza*. *Sagitta* shape analysis of *M. cephalus* also revealed differences among groups. The two identified groups could be differentiated by one shape index. Otoliths of group I were significantly lesser in aspect ratio than group II ones; indicating a more elliptical shape in smaller size fish. This species is the most cosmopolitan Mugilidae with a wide distribution range (mainly between 42°N and 42°S) all along the globe (Whitfield et al., 2012), having great relevance as ichtyofagous prey item (Fury & Harrison, 2011; Liordos & Goutner, 2009; Weyl & Lewis, 2006). For the two mentioned mullets, aspect ratio index was less in smaller size specimens. This variable is useful to differentiate among pelagic fishes (elongated otoliths, lesser aspect ratio) and fishes related to the substrate (greater aspect ratio) (Volpedo & Echeverría, 2003). Therefore, the observed morphometrical variations could be associated to the post-reproductive migrations of larvae from sea to estuarine or freshwater environments (Whitfield et al., 2012). Moreover, mugilid diet changes throughout their development: larvae are planktivorous and juvenile feed firstly in the water column (making vertical migrations) shifting towards a benthic diet when being around 20–30 mm total length (sizes of dietary shifts may vary given they are species-specific) (Cardona, 2015; Whitfield et al., 2012). This feeding behavior could be influencing the changes observed in *M. liza* and *M. cephalus* otoliths. In regards to *Liza ramada*, a species with a more restricted latitudinal distribution (from the coasts of southern Norway to Morocco, including the Mediterranean and the Black Sea (60°22′–27°49′N) (Froese & Pauly, 2016), although three morphologic groups were identified, no differences were found regarding to shape indices.

When comparing the slopes of the regression obtained for the morphometric variables analysed, *L. ramada* presented the least values for both variables (OL and OH), and *M. liza* presented the greatest ones. The observed differences could be related to the dissimilarities in sizes at sexual maturation (L_{50}) of the three studied mullets. While *Liza ramada* has the least sizes reported: L_{50} = a230/255 mm SL males/females (Moura & Gordo, 2000), *Mugil liza* shows notoriously greater sizes than other mugilids: L_{50} = 355/368 mm SL males/females (González-Castro et al., 2011), thus adult individuals reach greater sizes.

Other authors have studied changes in morphology and morphometry of *sagittae* along ontogenetic variations of species not related phylogenetically with mugilids, such as Sciaenidae (Volpedo, 2001; Volpedo & Echeverría, 2001; Waessle, Lasta, & Favero 2003), Atherinidae (Tombari, Volpedo, & Echeverría 2005) and Serranidae (Tuset et al., 2003). They have found that morphological features studied along with shape indices could separate, in most cases, juvenile from adults in species from the Atlantic and Pacific Oceans. Mullets are known to have diverse life-history patterns such as diadromous behavior or permanent open sea residency (Górski, De Gruijter, & Tana 2015; Whitfield et al., 2012). However, it is known that juveniles use coastal areas during their development for refuge and as feeding areas, while adults move to offshore areas for reproductive migrations (Whitfield et al., 2012). The variations observed in the identified groups for the species may as well be reflecting life history changes, related to habitat or dietary shifting.

Our research indicates specific morphologic patterns throughout the growth of three different mugilids. These patterns, along with shape indices analysis, could contribute to the specific identification of prey and prey sizes by the use of otoliths of at least two of the analyzed mullets (*Mugil liza* and *Mugil cephalus*). Moreover, the mathematical expressions of otolith growth associated to fish size presented in the present work could help minimize overestimation of ingested items by a piscivorous predator and improve studies of trophic webs in environments with great variations such as coastal areas. Finally, this simultaneous use of methodologies could be important for fisheries management of this worldwide distributed and poorly studied family.

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REFERENCES

Alava, J. J., & Gobas, F. A. (2012). Assessing biomagnification and trophic transport of persistent organic pollutants in the food chain of the galapagos sea lion (*Zalophus wollebaeki*): Conservation and management implications. In A. Romero & E. O. Keith (Ed.), *New approaches to the study of marine mammals*, (pp. 77-108). INTECH Open Access.

Avigliano, E., Jawad, L. A., & Volpedo, L. A. (2016). Assessment of the morphometry of saccular otoliths as a tool to identify triplefin species (Tripterygiidae). *Journal of the Marine Biological Association of the United Kingdom*, *96*, 1167–1180.

Avigliano, E., Martinez, C. F. R., & Volpedo, A. V. (2014). Combined use of otolith microchemistry and morphometry as indicators of the habitat of the silverside (Odontesthes bonariensis) in a freshwater–estuarine environment. *Fisheries Research*, *149*, 55–60.

Barquete, V., Bugoni, L., & Vooren, C. M. (2008). Diet of Neotropic cormorant (*Phalacrocorax brasilianus*) in an estuarine environment. *Marine Biology*, *153*, 431–443.

Barrett, R. T., Camphuysen, C. J., Anker-Nilssen, T., Chardine, J. W., Furness, R. W., Garthe, S., … Veit, R. R. (2007). Diet studies of seabirds: a review and recommendations. *ICES Journal of Marine Sciences*, *64*(9), 1675–1691.

Barros, N. B., & Wells R. S. (1998). Prey and feeding patterns of resident bottlenose dolphins *(Tursiops truncatus)* in Sarasota Bay, Florida. *Journal of Mammalogy*, *79*(3), 1045–1059.

Blanco, C., Salomón, O., & Raga, J. A. (2001). Diet of the bottlenose dolphin (*Tursiops truncatus*) in the western Mediterranean Sea. *Journal of the Marine Biological Association U.K.*, *81*, 1053–1058.

Boecklen, W. J., Yarnes, C. T., Cook, B. A., & James, A. C. (2011). On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution, and Systematics*, *42*, 411–440.

Brothers, E. B., & McFarland, W. N. (1981). Correlations between otolith microstructure, growth, and life history transitions in newly recruited french grunts [*Haemulon flavolineatum* (Desmarest), Haemulidae]. *Rapports et procès-verbaux des réunions / Conseil permanent international pour l'exploration de la mer*, *178*, 369–374.

Bustos, R. L., Daneri, G. A., Volpedo, A. V., Harrington, A., & Varela, E. A. (2012). The diet of the South American sea lion (*Otaria flavescens*) at Río Negro, Patagonia, Argentina, during the winter-spring period. *Iheringia, Serie Zoologia*, *102*(4), 394–400.

Bustos, R. L., Daneri, G. A., Volpedo, A. V., Harrington, A., & Varela, E. A. (2014). Diet of the South American sea lion *Otaria flavescens* during the summer season at Río Negro, Patagonia, Argentina. *Aquatic Biology*, *20*(3), 235–243.

Callicó Fortunato, R., Benedito Durà, V., & Volpedo, A. V. (2014). The morphology of saccular otoliths as a tool to identify different mugilid species from the Northeastern Atlantic and Mediterranean Sea. *Estuarine, Coastal and Shelf Science*, *146*, 95–101.

Campana, S. (1999). Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series*, *188*, 263–297.

Campana, S. (2004). *Photographic Atlas of Fish Otoliths of the Northwest Atlantic Ocean*. Canadian Special Publication of Fisheries and Aquatic Sciences No. 133. Ottawa, Canada: NRC Research press. 284 p.

Cardona, L. (2015). Food and feeding of Mugilidae. In D. Crosetti& S Blaber (Ed.), *Biology, ecology and culture of mullets (Mugilidae)* (pp. 165–195). Boca Raton, USA: CRC Press.

Casselman, J. M. (1990). Growth and relative size of calcified structures of fish. *Transactions of the American Fisheries Society*, *119*(4), 673–688.

Corruccini, R. S. (1972). Allometry correction in taximetrics. *Systematic Zoology*, *21*(4), 375–383.

FAO. (2017). FishStat – Capture fisheries production 1950–2015. Retrieved from http://www.fao.org/fishery/topic/16140/en

Fischer, W., Bauchot, M. L., & Schneider, M. (1987). Fiches FAO d'identification des espèces pour les besoins de la pêche. (Révision 1). Méditerranée et mer Noire. Zone de pêche 37. Vertébrés. *Rome*, *II*, 761–1530.

R. Froese & D. Pauly (Eds). (2016). FishBase. World Wide Web electronic publication. Retrieved from www.fishbase.org.

Fury, C. A., & Harrison, P. L. (2011). Seasonal variation and tidal influences on estuarine use by bottlenose dolphins (*Tursiops aduncus*). *Estuarine and Coastal Shelf Science*, *93*, 89–395.

Gallardo-Cabello, M., Espino-Barr, E., Cabral-Solis, E. G., Puente-Gomez, M., & Garcia-Boa, A. (2012). Study of the Otoliths of Stripped Mullet *Mugil cephalus* Linnaeus, 1758 in Mexican Central Pacific. *Journal of Fisheries and Aquatic Science*, *7*, 346–363.

Gerking, S. D. (2014). *Feeding ecology of fish*. San Diego, California: Elsevier. 416 p.

González-Castro, M., Abachian, V., & Perrotta, R. G. (2009). Age and growth of the stripped mullet *Mugil platanus* (Actinopterygii, Mugilidae), in a south-western Atlantic coastal lagoon (37°32′S-57°19′W): a proposal for a life- history model. *Journal of Applied Ichthyology*, *25*(2), 61–66.

González-Castro, M., & Ghasemsadeh, J. (2015). Morphology Based Taxonomy. In D. Crosetti & S.. Blaber (Ed.), *Biology, ecology and culture of mullets (Mugilidae)* (pp. 1–21). Boca Raton, USA: CRC Press.

González-Castro, M., Macchi, G. J., & Cousseau, M. B. (2011). Studies on reproduction of the mullet *Mugil platanus* Günther, 1880 (Actinopterygii, Mugilidae) from the Mar Chiquita coastal lagoon, Argentina: similarities and differences with related species. *Italian Journal of Zoology*, *78*(3), 343–353.

González-Castro, M., & Minos, G. (2015). Sexuality and Reproduction. In D. Crosetti & S. Blaber (Ed.), *"Biology, ecology and culture of mullets (Mugilidae)"* (pp. 227–263). Boca Raton, USA: CRC Press.

Górski, K., De Gruijter, C., & Tana, R. (2015). Variation in habitat use along the freshwater–marine continuum by grey mullet *Mugil cephalus* at the southern limits of its distribution. *Journal of Fish Biology*, *87*(4), 1059–1071.

Harrison, I. J. (2002). Mugilidae. In K. Carpenter (Ed.). *The Living Marine Resources of the Western Central Atlantic*. Vol. 2. Bony Fishes part 1 (Acipenseridae to Grammatidae) (pp. 1071–1085). Rome: FAO Species Identification Guide for Fisheries Purposes.

Harvey, J. T., Loughlin, T. R., Perez, M. A., & Oxman, D. S. (2000). *Relationship between fish size and otolith length for 63 species of fish from the eastern North Pacific Ocean*. Seattle, WA: U.S. Dep. Commer., NOAA Tech. Rep. NMFS 15, 36 p.

Hecht, T. (1987). A guide to the otoliths of Southern Ocean fishes. South African. *Journal of Antartic Research*, *17*(1), 2–86.

Heras, S., Roldán, M., & González-Castro, M. (2009). Molecular Phylogeny of Mugilidae fishes revised. *Reviews in Fish Biology and Fisheries*, *19*, 217–231.

Huxley, J. S. (1993). *Problems of relative growth; with a new introduction*. Baltimore: The John Hopkins University Press.31 p.

Jansen, O. E., Aarts, G. M., Das, K., Lepoint, G., Michel, L., & Reijnders, P. J. (2012). Feeding ecology of harbour porpoises: stable isotope analysis of carbon and nitrogen in muscle and bone. *Marine Biology Research*, *8*(9), 829–841.

Liordos, V., & Goutner, V. (2009). Sexual differences in the diet of great cormorants *Phalacrocorax carbo sinensis* wintering in Greece. *European Journal of Wildlife Research*, *55*, 301–308.

Lleonart, J., Salat, J., & Torres, G. J. (2000). Removing allometric effects of body size in morphological analysis. *Journal of Theoretical Biology*, *205*, 85–93.

Marin, B. J., Quintero, A., Bussière, D., & Dodson, J. J. (2003). Reproduction and recruitment of white mullet (*Mugil curema*) to a tropical lagoon (Margarita Island, Venezuela) as revealed by otolith microstructure. *Fishery Bulletin*, *101*(4), 809–821.

Martucci, O., Pietrelli, L., & Consiglio, C. (1993). Fish otoliths as indicators of the cormorant *Phalacrocorax carbo* diet (Aves, Pelecaniformes). *Italian Journal of Zoology*, *60*(4), 393–396.

Menezes, N. A., De Oliveira, C., & Nirchio, M. (2010). An old taxonomic dilemma: the identity of the western south Atlantic lebranche mullet (Teleostei: Perciformes: Mugilidae). *Zootaxa*, *2519*, 59–68.

Mikkelsen, B. (2013). Present knowledge of grey seals (*Halichoerus grypus*) in Faroese waters. *NAMMCO Scientific Publications*, *6*, 79–84.

Mollo, S. M. (1981). Otolitos de peces de la Laguna Chascomús (Provincia de Buenos Aires). Análisis y consideraciones para su identificación en estudios tróficos. *Limnobios*, *2*, 253–263.

Morte, S., Redon, M. J., & Sanz-Brau, A. (1997). Feeding habits of juvenile *Mustelus mustelus* (Carcharhiniformes, Triakidae) in the western Mediterranean. *Cahiers de Biologie Marine*, *38*, 103–107.

Moura, I. M., & Gordo, L. S. (2000). Abundance, age, growth and reproduction of grey mullets in Obidos lagoon, Portugal. *Bulletin of Marine Science*, *67*(2), 677–686.

Muñoz-Gil, J., Marín-Espinoza, G., Andrade-Vigo, J., Zavala, R., & Mata, A. (2013). Trophic position of the Neotropic Cormorant (*Phalacrocorax brasilianus*): integrating diet and stable isotope analysis. *Journal of Ornithology*, *154*(1), 13–18.

Nelson, J. S. (2006). *Fishes of the world*, 4th ed. Hoboken, NJ, USA: John Wiley and Sons. 601 p.

Pierce, G. J., & Boyle, P. R. (1991). A review of methods for diet analysis in piscivorous marine mammals. *Oceanography and Marine Biology*, *29*, 409–486.

Reichenbacher, B., Sienknecht, U., Küchenhoff, H., & Fenske, N. (2007). Combined otolith morphology and morphometry for assessing taxonomy and diversity in fossil and extant killifish (*Aphanius*, †*Prolebias*). *Journal of Morphology*, *268*, 898–915.

Riet-Sapriza, F. G., Costa, D. P., Franco-Trecu, V., Marín, Y., Chocca, J., González, B., … Hückstadt, L. A. (2013). Foraging behavior of lactating South American sea lions, *Otaria flavescens* and spatial-resource overlap with the Uruguayan fisheries. *Deep-Sea Research Part II-Topical Studies in Oceanography*, *88–89*, 106–119.

Ross, M. R., Johnson, J. H., & Adams, C. M. (2005). Use of fish-otolith-length regressions to infer size of double-crested cormorant prey fish from recovered otoliths in Lake Ontario. *Northeastern Naturalist*, *12*(2), 133–140.

Rossi-Wongtschowski, C. L. D. B. (2015). Morfología de otolitos. In A. Volpedo & A. Martins Vaz-dos-Santos, (Eds.), *Métodos de estudos com otólitos: princípios e aplicações* (pp. 30–57), Buenos Aires, Argentina: CAFP-BA-PIESCI.

Scartascini, F. L., & Volpedo, A. V. (2013). White croaker (*Micropogonias furnieri*) paleodistribution in the Southwestern Atlantic Ocean. An archaeological perspective. *Journal of Archaeological Science*, *40*(2), 1059–1066.

Thomson, J. M. (1997). The Mugilidae of the world. *Memories Queensland Museum*, *41*(3), 457–562.

Tombari, A. D., Volpedo, A. V., & Echeverría, D. D. (2005). Desarrollo de la *sagitta* en juveniles y adultos de *Odontesthes argentinensis* (Valenciennes, 1835) y *O. bonariensis* (Valenciennes, 1835) de la provincia de Buenos Aires, Argentina (Teleostei: Atheriniformes). *Revista Chilena de Historia Natural*, *78*, 623–633.

Trewavas, E., & Ingham, S. (1972). A key to the species of Mugilidae (Pisces) in the Northeastern Atlantic and Mediterranean, with explanatory notes. *Journal of Zoology*, *167*, 15–29.

Tuset, V. M., Imondi, R., Aguado, G., Otero-Ferrer, J. L., Santschi, L., Lombarte, A., & Love, M. (2015). Otolith patterns of rockfishes from the northeastern pacific. *Journal of Morphology*, *276*(4), 458–469.

Tuset, V. M., Lombarte, A., & Assis, A. C. (2008). Otolith atlas for the Western Mediterranean, North and central eastern Atlantic. *Scientia Marina*, *72*(1), 7–198.

Tuset, V. M., Lombarte, A., González, J. A., Pertusa, J. F., & Lorente, M. J. (2003). Comparative morphology of the sagittal otolith in *Serranus* spp. *Journal of Fish Biology*, *63*, 1491–1504.

Tuset, V. M., Parisi-Baradad, V., & Lombarte, A. (2013). Application of otolith mass and shape for discriminating scabbardfishes *Aphanopus* spp. in the north-eastern Atlantic Ocean. *Journal of Fish Biology*, *82*(5), 1746–1752.

Veen, J., Mullié, W. C., & Veen, T. (2012). The Diet of the White-Breasted Cormorant *Phalacrocorax carbo lucidus* along the Atlantic Coast of West Africa. *Ardea*, *100*(2), 137–148.

Volpedo, A. V. (2001). Estudio de la morfometría de las *sagittae* en poblaciones de Sciaenidos marinos de aguas calidad del Perú y agua templado-frías de Argentina. PhD Thesis. Buenos Aires: University of Buenos Aires, 234 p.

Volpedo, A. V., & Echeverría, D. D. (2000). *Catálogo y claves de otolitos para la identificación de peces del Mar Argentino*. Peces de importancia comercial. Buenos Aires, Argentina: Editorial Dunken.

Volpedo, A. V., & Echeverría, D. D. (2001). Morfología y morfometría de las *sagittae* de sciaenidos marinos del norte de Perú. *Boletín de la Sociedad de biología de Concepción*. *72*, 147–154.

Volpedo, A. V., & Echeverría, D. D. (2003). Ecomorphological patterns of the *sagitta* in fish on the continental shelf off Argentine. *Fisheries Research*, *60*(2–3), 551–560.

Waessle, J. A., Lasta, C. A., & Favero, M. (2003). Otolith morphology and body size relationships for juvenile Sciaenidae in the Rio de la Plata estuary (35-36°S). *Scientia Marina*, *67*(2), 233–240.

Weyl, O. L. F., & Lewis, H. (2006). First record of predation by the alien invasive freshwater fish *Micropterus salmoides* L. (Centrarchidae) on migrating estuarine fishes in South Africa. *African Zoology*, *41*(2), 294–296.

Whitfield, A. K. (2015). Ecological role of mugilidae in the coastal zone. In D. Crosetti & S. Blaber (Ed.), *Biology, ecology and culture of mullets (Mugilidae)* (pp. 324–348). Boca Raton, USA: CRC Press.

Whitfield, A. K., & Blaber, S. J. M. (1978). Food and feeding ecology of piscivorous fishes at Lake St Lucia, Zululand. *Journal of Fish Biology*, *13*(6), 675–691.

Whitfield, A. K., Panfili, J., & Durand, J.-D. (2012). A global review of the cosmopolitan flathead mullet *Mugil cephalus* Linnaeus1758 (Teleostei: Mugilidae), with emphasis on the biology, genetics, ecology and fisheries aspects of this apparent species complex. *Reviews in Fish Biology and Fisheries*, *22*(3), 641–681.

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