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

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Running head: *Sagitta* otolith ontogeny of 3 mugilid species

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.

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 (Volpedo & Echeverria, 2000; Boecklen et al., 2011; Jansen et al., 2012). To analyze feeding habits in marine mammals, birds and fishes the study of stomach contents provides information for recently ingested prey (Pierce & Boyle, 1991; Bustos et al., 2012, 2014).

Otoliths are structures composed mainly of calcium carbonate over an organic matrix (Campana, 1999). Their morphology is species-specific (Hecht, 1987; Volpedo & Echeverría, 2000, 2001; Campana, 2004; Tuset et al., 2008; Callicó Fortunato et al., 2014; Avigliano et al., 2015) and particularly, otolith size has a close relationship with fish body size (Martucci et al., 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 (Barret et al., 2007; Reichenbacher et al., 2007; Bustos et al., 2012, 2014; Veen et al., 2012; Riet-Sapriza et al., 2013; Scartascini & Volpedo, 2013; Tuset et al., 2013, 2015). 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 et al., 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 (Nelson, 2006; Heras et al., 2009; González-Castro & Ghasemzadeh, 2015; González-Castro & Minos, 2015). Mugilids, after their resting and maturing periods in coastal lagoons, estuaries or related environments, perform a reproductive migration towards the sea (González-Castro et al., 2009, 2011; Whitfield, 2015). After spawning, some adults may return to estuaries and others remain in the marine environment (Whitfield et al., 2012). Mullets are important food items for birds such as cormorants (Martucci et al., 1993; Barquete et al., 2008; Veen et al., 2012; Muñoz-Gil et al., 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*) (Whitfield & Blaber, 1978; Morte et al., 1997). They are also commercially-relevant species in industrial fisheries with the extractions greater than 550000 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 (Marin et al., 2003; González-Castro et al., 2009; Gallardo-Cabello et al., 2012).

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 *Liza 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, (1) general description of the otoliths' growth changes by means of traditional biometric characters related to fish growth; and (2) 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.

Material and Methods

Sample collection

Specimens of three species of Mugilidae family (*Mugil liza*, *Mugil cephalus* and *Liza ramada*) were collected seasonally between October 2011 and April 2014. Individuals were obtained with gill nets and trammel nets (mesh size ranging from 2.5cm 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 & Ingham (1972); Fischer et al. (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.

Table 1

Otolith shape measurements

The medial face of each right otolith was photographed with a digital camera attached to a stereomicroscope (Leica® EZ4-HD). All images were analyzed and measured using image processing systems (Image-Pro Plus 4.5®). 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, (Fig. 1) were measured; the equation $y=ax^b$ was used to fit relations between SL and OL-OH (Huxley, 1993; Lleonart et al., 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 (Tuset et al., 2003; Volpedo & Echeverría, 2003; Avigliano et al., 2014; Rossi-Wongtschowski, 2015). 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) (Fig. 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 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).

Figure 1

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 U-Mann Whitney 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, $p > 0.05$); 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.

Results

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

Mugil liza

Otolith biometric characters general description

Dependence of SL on OL and OH is best described as power functions, presenting a significant positive relationship ($p < 0.01$) 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 = 0.87); $SL = 25.72 \times OH^{1.79}$ (R^2 = 0.84) (Figs. 2a-b).

Figure 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 (Fig. 3). All *Mugil liza* specimens present a rectangular *sagitta* with a tubular *cauda* slightly curved, and round to flattened posterior region (Fig. 3).

Figure 3

Shape analysis

All shape indices but circularity, are normal and have homogeneity of variances (Shapiro–Wilk, $p > 0.05$; Levene, $p > 0.05$). 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*.

Table 2

Mugil cephalus

Otolith biometric characters general description

Dependence of SL on OL and OH is best described as power regressions presenting a significant positive relationship ($p < 0.01$) 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 = 0.84); $SL = 34.25 \times OH^{1.56}$ (R^2 = 0.89) (Figs. 4a-b).

Figure 4

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 (Fig. 5). All *Mugil cephalus* specimens present a rectangular *sagitta* shape with a tubular *cauda* straight or slightly curved and flattened to round posterior region (Fig. 5).

Figure 5

Shape analysis

All shape indices but circularity, are normal and have homogeneity of variances (Shapiro–Wilk, $p > 0.05$; Levene, $p > 0.05$). Identified groups differ significantly on aspect ratio index (Table 3). Small size individuals present smaller aspect ratios, being their *sagittae* longer than wider.

Table 3

Liza ramada

Otolith biometric characters general description

Dependence of SL on LO and OH is best described as power regressions showing a significant positive relationship ($p < 0.01$) for each case. Including all individuals (n = 147), regression equations for both variables are as follows: $SL = 12.72 \times OL^{1.36}$ ($R^2 = 0.90$); $SL = 32.54 \times OH^{1.43}$ ($R^2 = 0.91$) (Figs. 6a-b).

Fig. 6

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 (Fig. 7). All *Liza ramada* specimens present a sinuous *cauda* markedly bent towards the ventral region and round to angled posterior region (Fig. 7).

Figure 7

Shape analysis

All shape indices but circularity, are normal and have homogeneity of variances (Shapiro–Wilk, $p > 0.05$; Levene, $p > 0.05$). Identified groups do not differ significantly in any of the obtained shape indices (Table 4).

Table 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 ($p < 0.01$) for both otolith length and otolith height. *Liza ramada* presents lesser regression slope for the biometric variables (SL *vs* 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) (Fig. 8). *Mugil cephalus* shows intermediate values for both slopes (SL *vs* OL = 1.63, SL *vs* OH = 1.54) (Fig. 8).

Figure 8

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 (*Mugil liza, Mugil 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 et al., 2001; Ross et al., 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) (Menezes et al., 2010; Froese & Pauly, 2016), morphological groups were 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 (Weyl & Lewis, 2006; Liordos & Goutner, 2009; Fury & Harrison, 2011). 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) (Whitfield et al., 2012; Cardona, 2015). 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} = 230/255 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 & Echeverria, 2001; Waessle et al., 2003), Atherinidae (Tombari et al., 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 (Whitfield et al., 2012; Górski et al., 2015). 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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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; 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)

Table 2. Mean and standard deviation of the shape indices analyzed among identified groups for *M. liza*; and their statistical analysis: W = U-Mann Whitney test and T = paired t-test. * Non-normal variable. Different letters show significant differences.

	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	0.082
Rectangularity (OA / [OL × OH])	0.69 ± 0.03^{a}	0.68 ± 0.03^{b}	T = 2.12	**0.035**
Aspect ratio (OH / OL)	0.46 ± 0.03^{a}	0.47 ± 0.03^{b}	T = -3.13	**0.002**
Percentage occupied by the sulcus (SA/OA)	0.24 ± 0.02^{a}	0.23 ± 0.02^{b}	T = 2.24	**0.026**

Table 3. Mean and standard deviation of the shape indices analyzed among identified groups for *M. cephalus*; and their statistical analysis: W = U-Mann Whitney test and T = paired t-test. * Non-normal variable. Different letters show significant differences.

	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	0.799
Rectangularity (OA / [OL × OH])	0.72±0.02	0.71±0.04	T = 1.05	0.296
Aspect ratio (OH / OL)	0.45±0.03[a]	0.48±0.01[b]	T = -2.29	**0.024**
Percentage occupied by the sulcus (SA/OA)	0.23±0.02	0.23±1.82	T = 0.04	0.969

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. * Non-normal variable.

	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	0.364
Rectangularity (OA / [OL × OH])	0.74±0.01	0.74±0.02	0.74±0.02	F = 0.02	0.983
Aspect ratio (OH / OL)	0.49±0.01	0.47±0.03	0.47±0.03	F = 0.82	0.441
Percentage occupied by the sulcus (SA/OA)	0.22±0.01	0.20±0.02	0.20±0.02	F = 1.10	0.337