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Application of otolith morphometry for the study of ontogenetic variations of *Odontesthes argentinensis*

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Abstract The study of otolith morphometry is a tool widely used in numerous studies of fish populations (fish stocks, taxonomic, ecological, ontogeny, among others). The aim of this study was to detect ontogenetic variations in the otolith of *Odontesthes argentinensis* through the application of traditional and geometric morphometry and the association of these variations with ecological and trophic habits of the species. Fish (52 to 360 mm TL) were collected seasonally between years 2013–2016 in the southwest coast of the Atlantic Ocean (36°39'30.96"S - 56°40'40.09"W). Otolith shape indices (circularity, rectangularity, aspect ratio and surface occupied by *sulcus*) and Fourier descriptors were measured and compared through ontogenetic stages (I, II and III). The three stages analysed in the present study were differentiated by both traditional and geometric morphometry. The four analysed indices showed significant differences between stages (ANOVA Kruskal Wallis test, $P < 0.001$) and a simultaneous analysis of the morphometric variables also showed significant differences (Hotelling's $T^2 < 0.001$). The quadratic discriminant analysis performed on the Fourier descriptors showed a clear separation for each defined group. Therefore, the use of both methodologies simultaneously could be considered robust to evaluate the ontogenetic variations in this species. The observed changes could be associated to changes in the habitat throughout its development, to the sexual maturity of fish and to dietary shifting of these organisms.

Keywords *Odontesthes argentinensis* · Argentina · Fourier analysis · Morphometric ontogenetic indices

Introduction

Otoliths are calcareous concretions located in the inner ear of fish and are species specific (Campana 1999; Assis 2000; Tuset et al. 2003a). Otolith shape is a tool widely used for taxonomic studies (Volpedo and Echeverría 1999; Tombari et al. 2005; Tombari et al. 2010; Volpedo and Vaz dos Santos 2015; Jawad et al. 2017), fish stock identification (Avigliano et al. 2015a), nursery areas identification (Avigliano et al. 2016, 2017), discrimination among closely related species (Callicó Fortunato et al. 2014; Avigliano et al. 2015b, 2018), ecological studies (Avigliano et al. 2014, 2015c) and ontogenetic studies (de Carvalho et al. 2015; Callicó Fortunato et al. 2017).

Sagittae otoliths permit the analysis of different aspects of fish biology and their environment (Morales-Nin 1987; Radtke and Shafer 1992). General form of

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otolith is associated with ecological characteristics such as depth, habitat, type of locomotion and feeding (Wilson 1985; Gauldie and Crampton 2002; Lombarte et al. 2003; Volpedo and Echeverría 2003; Sadighzadeh et al. 2014; Tuset et al. 2015). In this sense, otoliths' traditional morphometric analysis has allowed identifying different ecological aspects of fish species. The shape, margins and rostrum of otoliths were used by Volpedo and Echeverría (2003) to identified three ecomorphological groups according to the use of the water column (fish associated with soft and hard substrates, and pelagic fish) from the Argentinian shelf. Volpedo et al. (2008) determined that swimmer fish have a common *sagittae* morphological pattern: oblong shape, marked rostrum and a considerable sulcus area (>20% of the otolith surface, approximately), while fish that make short vertical migrations present rostrum, excisure and antirostrum slightly developed. Lombarte et al. (2010) suggested that there is a clear correspondence between relative otolith size and shape of nototheniids (pelagic and benthic species). In addition, the traditional morphometry has been used to evaluate changes during the development of fish (Volpedo and Echeverría 1999; Waessle et al. 2003; De La Cruz-Agüero et al. 2012; Vignon 2012; de Carvalho et al. 2015). These authors observed morphometric changes in otoliths related to sexual maturation, diet change, or habitat use by swimmer fish. These morphometric variations are due to physiological changes or to endogenous factors that make calcium carbonate to precipitate differently in juveniles and adults.

On the other hand, geometric morphometry, allows to recognize the existence of groups through the identification of *landmarks* (anatomical loci that do not alter their topological position) and *outlines* (Fourier contours) in the profile of different organisms and structures (Turan 2000; Ponton 2006; Burke et al. 2008; Vignon and Morat 2010; Avigliano et al. 2016). The comparison of the otolith shape in fish by geometric morphometric methods (commonly known as "otolith shape analysis") is widely used to study the population structure of different fish species (Orlov and Afanasyev 2013; Valentin et al. 2014; Harbitz and Albert 2015; Cresson et al. 2015). Otolith shape analysis has allowed to study the discrimination of spawning groups (*Gadus morhua;* Galley et al. 2006), to separate population stocks (Tracey et al. 2006; Burke et al. 2008; Vasconcelos et al. 2018) and to identify distinct growth phases in fish like *Anchoa tricolor* and different engraulid species (Ponton 2006; de Carvalho et al. 2015). Ontogenetic otolith shape changes during fish growth have been described and used to identify age (Cardinale et al. 2004), sex and maturity stage (Bird et al. 1986; Lombarte and Castellón 1991; Piera et al. 2005). de Carvalho et al. (2015) determined that changes in the otoliths shape of *A. tricolor* were associated with the changes in swimming ability and sexual maturation during growth. Moreover, Vignon (2012) related ontogenetic changes in otolith shape of a coral reef fish with a shift in habitat use.

The association of traditional morphometry and geometric studies makes it possible to expand the use of otoliths as key elements for understanding species and populations (Rohlf and Marcus 1993; Monteiro et al. 2005; Vignon 2012). As mentioned before, both methods have been used to evaluate otolith changes during the development of fish; but studies that applied them together are very scarce.

One of the most ecologically and economically important marine coastal species on the southwest coast of the Atlantic Ocean is *Odontesthes argentinensis.* This species is distributed along the southwest coast of the Atlantic Ocean, between Rio de Janeiro, Brazil (22°S) and Rawson, Argentina (43°S) (Dyer 2000, 2006; Di Dario et al. 2013). It is present in the diet of marine mammals and seabirds (Silva Rodríguez et al. 2005; Tombari et al. 2005) and is considered a significant economic resource for in local fisheries in southern Brazil, Uruguay and Argentina (De Buen 1953; Chao et al. 1985; Sampaio 2006; Llompart et al. 2013, 2017). In Argentina, *O. argentinensis* is exploited by recreational and artisanal fisheries along the coast of Buenos Aires province (Llompart et al. 2012).

Tombari et al. (2005), studied the ontogenetic variability of the otolith of *O. argentinensis* through morphology, however, there are no studies on their morphometry. The objective of this work is the detection of ontogenetic variations in the otolith of *O. argentinensis* through the application of traditional and geometric morphometry, and the association of these variations with the ecological and trophic habits of the species.

Materials and methods

Study area and fish sample collection

Specimens of *Odontesthes argentinensis* were obtained seasonally from artisanal catches with gill nets or rods between years 2013–2016 in the coastal city of La Lucila del Mar (36°39'30.96"S - 56°40'40.09"W), Buenos Aires province - Argentina - South America (Fig. 1). A total of 290 individuals were collected and taken to the laboratory. Total and standard length (TL and SL in mm) were recorded and their *sagittae* otoliths were removed. A wide range of sizes were sampled (52 to 360 mm TL), so as to have a large number of individuals of the different development stages. After extraction and cleaning, the inner face of left *sagittae* otoliths was photographed with a digital camera attached to a stereomicroscope (Leica® EZ4 HD).

Traditional morphometric analysis

For the study of traditional morphometry, images were analysed and morphometric variables were measured using an image processing system (Image-Pro Plus 4.5®). The variables registered were: otolith length (OL), otolith height (OH), otolith perimeter (OP) and *sulcus* perimeter (SP) in mm; and otolith area (OA) and *sulcus* area (SA) in mm^2 (Fig. 2). Afterwards, four indices were calculated using these variables: *i)* circularity (OP^2/OA), providing information on the complexity of the otolith, taking a minimum value of 4π (Tuset et al. 2003b; Lombarte and Tuset 2015); *ii)* rectangularity (OA / [OL*OH]), giving information on the approximation to a rectangular or square shape, being 1 a perfect rectangle or square, taking the value π/4 for circular objects and smaller values for slender, curved objects (Wu et al. 2008; Lombarte and Tuset 2015); *iii)* aspect ratio (OH/OL), used to distinguished long and thin objects from roughly square or circular objects (Tuset et al. 2003b), and *iv)* percentage of the otolith area occupied by the *sulcus* (SA/OA) (Avigliano et al. 2014; Volpedo and Vaz dos Santos 2015). Indices were corrected to eliminate possible allometry effects in otolith shape related to fish body size, for a proper comparison between groups; the formula proposed by Lleonart et al. (2000): $y' = y_i*(x_0/x_i)^b$ was used, in which y' is the corrected predictive variable, y_i is the original value of the obtained index, x_0 is a referential standard length (SL) value ($SL_{minimum} = 44$ mm), x_i is the original SL value, and b is the Huxley coefficient of each regressioned index to SL.

Three development stages were defined according to Tombari et al. (2005), as follow: stage I: individuals $\leq$130 mm TL ($n = 92$); stage II: individuals >130 mm TL and $\leq$ 210 mm TL ($n = 124$); and stage III: individuals >210 mm TL ($n = 74$). Tombari et al. (2005) also identified a stage IV: (TL > 280 mm and ≤ 320 mm). In the present study, due to the low number of individuals >280 mm TL ($n = 20$), stages III and IV (ca. Tombari et al. 2005) were considered one single group (stage III).

The four shape indices were compared among previously identified development stages by one-way analysis of variance (ANOVA). Since parametric assumptions of normality (Shapiro-Wilks test) and homogeneity of variance (Levene's test) could not be met, the Kruskal-Wallis nonparametric ANOVA tests were used. Stage medians were compared by Dunn's multiple comparison test. Finally, a multivariate analysis of variance (MANOVA) with Hotelling's T^2 test was applied to evaluate differences among stages considering all morphometric variables simultaneously. All differences were tested at a probability (α) of 0.05.

Otolith shape analysis

To study the otolith shape differences among stages, a Fourier shape analysis was performed to a subsample of the individuals ($n = 247$) divided in the same way as in the traditional morphometry analysis (stage I: $n = 97$; stage II: $n = 91$ and stage III: $n = 59$). For this analysis, otolith images were digitalized using the Shape program (SHAPE software ver.1.3, Iwata and Ukai 2002). First, using the chain coding algorithm, the numerical contour of each otolith was extracted. Then the elliptical Fourier analysis (EFA) was performed obtaining data for 20 harmonics to explain the original shape of each otolith (Steer and Fowler 2014). Finally, the number of harmonics required to explain otoliths outlines was estimated using the average Fourier spectrum (FP) as described by Steer and Fowler (2014). Of the 20 harmonics analysed, the first 8 represented 99.99% of the mean cumulative power (Fig. 3), so those were the ones used for later analysis.

Each of these eight harmonics was characterized by four coefficients (a, b, c, d), but the first three (a1, b1, c1) were constant for every sample (Steer and Fowler 2014); therefore, the total number of shape descriptors for each otolith was: (4 * number of harmonics – 3) = 29. Each descriptor was treated as an independent variable. A Quadratic discriminant analysis (QDA) was performed to test the differences in shape of the three defined development stages, using the shape descriptors mentioned before, since this analysis does not require

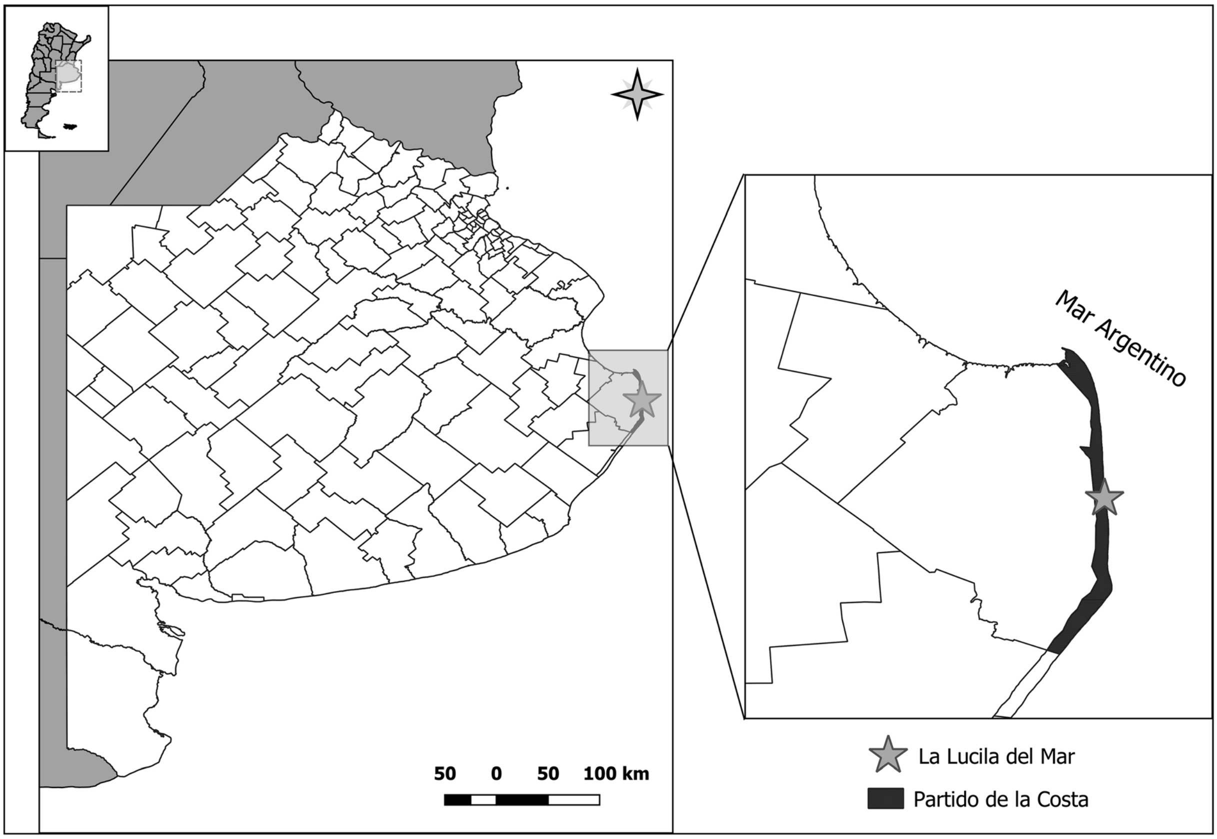

Fig. 1 Location of *Odontesthes argentinensis* sampling site in the coast of Buenos Aires province (La Lucila del Mar: grey star) at Partido de La Costa

the data to meet the assumptions of homogeneity of variance and multivariate normality (Mohan et al. 2012). An error was estimated for each analysed group after obtaining the classification matrix.

Results

Traditional morphometric analysis

The four analysed indices showed significant differences among stages (Table 1). Older specimens (stage III: TL > 210 mm) showed significantly lower circularity and higher percentage occupied by the *sulcus* than younger specimens. Stage II individuals showed significant higher values of rectangularity and aspect ratio indices, than stages I and III, indicating that stage II otoliths were more oval and larger than wider with regard to other stages (Table 1). When analysing morphometric variables simultaneously, the three stages differed significantly (Hotelling's $T^2 < 0.001$).

Otolith shape analysis

The quadratic discriminant analysis showed a clear separation for each defined group (Table 2); mean error of the QDA for all groups was less than 4% (Table 2). When observing the mean shapes (± 2 standard deviation) of each group, smaller individuals (stage I) had a more rounded otolith shape with a long rostrum an almost non-existent antirostrum; medium-size specimens (stage II) had a more round-oval otolith shape with a short rostrum, and stage III individuals had a more oval-elongate shape with moderate rostrum but no antirostrum present (Fig. 4).

Discussion

The results showed the existence of morphometric variations in the otoliths of *O. argentinensis* between 52 and 360 mm total lengths, coinciding with the results

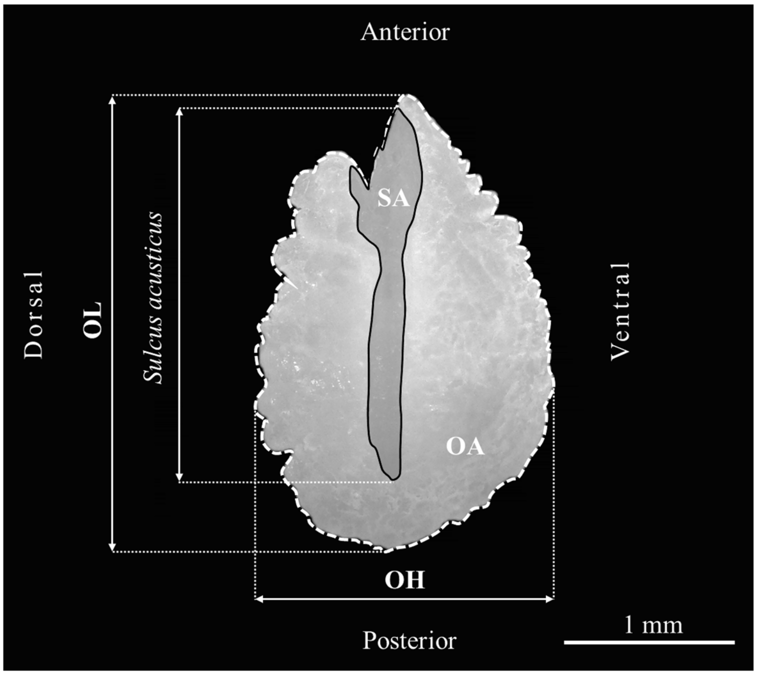

Fig. 2 Left *sagitta* otolith of *Odontesthes argentinensis.* Features and measured variables: OL: otolith length; OH: otolith height; OA: otolith area; SA: sulcus area; otolith perimeter (dash white line); sulcus perimeter (continuous black line)

obtained by Tombari et al. (2005) based on the otoliths morphology of this species.

Differences were determined by traditional morphometry analysing the different morphometric indices. Significantly high circularity values were obtained for stages I and II indicating high edge complexity and circular shape, which is typical of the larval stage (Hüssy 2008; de Carvalho et al. 2015; Joh et al. 2015), while lowest circularity index values were obtained in adult individuals (development stage III). These results agree with those recorded by Callicó Fortunato et al. (2017), for *Mugil liza* and *Liza ramada*, which could be

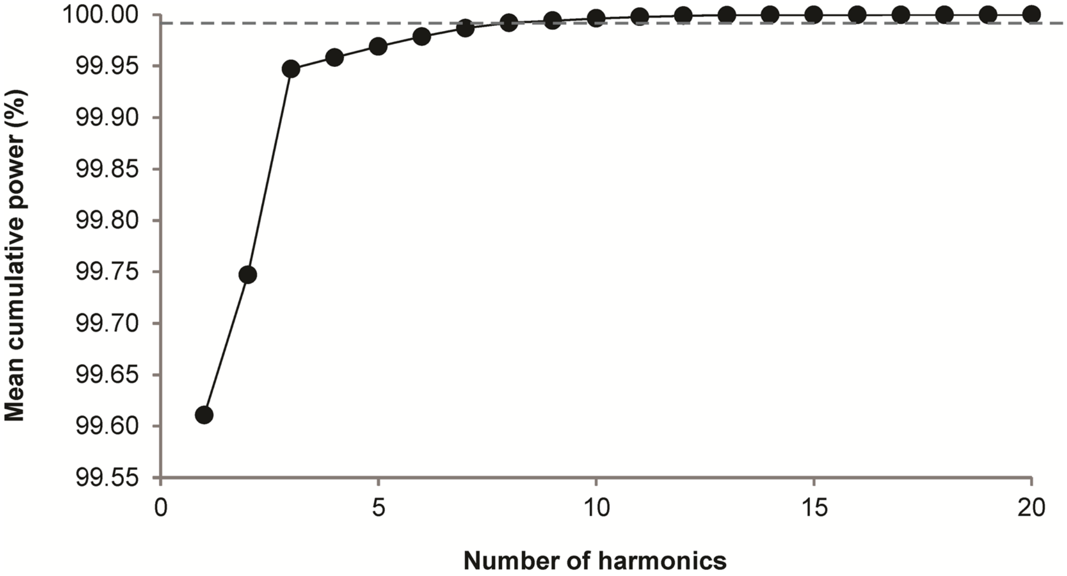

Fig. 3 Fourier mean cumulative power (%) from harmonics analysed to study otolith shape throughout *Odontesthes argentinensis* ontogeny. Dash line shows number of harmonics at 99.99% of cumulative power

Table 1 Statistical analysis performed for the four morphometric variables: H = Kruskal-Wallis test (medians test). Different letters show significant difference in Dunn multiple comparisons. Significant *p* values ($p < 0.05$) are indicated in bold

	Stage I ($n = 92$) Median	Stage II ($n = 124$) Median	Stage III ($n = 74$) Median	Statistic (H)	p
Circularity (OP^2/OA)	18.444^a	18.911^a	17.859^b	8.16	**< 0.001**
Rectangularity (OA / [OL × OH])	0.597^a	0.694^b	0.584^a	89.94	**< 0.001**
Aspect ratio (OH / OL)	0.740^a	0.763^b	0.728^a	7.66	**< 0.001**
Percentage occupied by the *sulcus* (SA/OA)	0.161^a	0.157^a	0.168^b	3.59	**0.032**

associated to movements that smaller fish of these species performed to find their development habitats. Circularity was also a good habitat indicator for species like *Odontesthes bonariensis*, *Prochilodus lineatus, Plagioscion ternetzi, Coryphaenoides rupestris*, *Lophius piscatorius*, *Argyrosomus japonicus* and *Scomberesox saurus* (Longmore et al. 2010; Agüera and Brophy 2011; Ferguson et al. 2011; Cañás et al. 2012; Avigliano et al. 2015a, 2015c, 2017). Rectangularity and aspect ratio index values indicated that the *sagittae* of *O. argentinensis* had a more elongated than wide shape, which could be related to swimmer fishes according to Volpedo and Echeverría (2003). Rectangularity was also considered an efficient habitat indicator for several species like *Coryphaena hippurus* (Duarte-Neto et al. 2008), *Gadus morhua* (Petursdottir et al. 2006) and *Scomberesox saurus* (Agüera and Brophy 2011). The difference recorded in the percentage of otolith surface occupied by the sulcus in stage III, might be due to the fact that this index is higher in pelagic and swimmer fish than in fish with demersal habits (Gauldie 1988; Volpedo and Echeverría 2003) and in migratory species than in those that are not (Tuset et al. 2003a, b; Avigliano et al. 2015c). Moreover, fish with larger sulcus have more contact surface with the nervous macula, thus, capturing greater environmental information compared with fish more frequently related to the bottom that present lower sulcus surface (Volpedo and Echeverría 2003; Popper et al. 2005; Jaramillo et al. 2014). Adults of *O. argentinensis* show a benthic feeding habit (Bemvenuti 1990; Martinetto et al. 2005), inhabiting the coast of the Atlantic Ocean (Dyer 2000).

The shape of the silverside fish otolith presented distinctive patterns during each of the development stages: stage I had a rounded otolith with a marked rostrum, stage II presented a more round-oval otolith with a less marked rostrum and stage III presented an otolith with an elongated shape and moderate rostrum (Fig. 2). Due to the pelagic swimming behaviour of the juveniles of *O. argentinensis*, features of stage I otoliths are in agreement with Volpedo and Echeverría (2003), whom determined that the *sagittae* of pelagic fish possessed a prominent rostrum, a deep V-shaped excisure and ornamented borders. On the other hand, otoliths of the stages II and III show similar characteristics to the *sagittae* of fish associated with soft substrates (circular or polygonal with rounded borders, poorly developed rostrum) (Volpedo and Echeverría 2003) or to the *sagittae* of mesopelagic fish that make short vertical migrations (Volpedo et al. 2008). This suggests that otolith morphometrics can reflect the use of the water

Table 2 Cross-classification table of the quadratic discriminant analysis (QDA) for the three defined development stages of *Odontesthes argentinensis*, based on 29 shape descriptors per otolith obtained by the morphometric Fourier analysis

Development stages	I	II	III	Sampled individuals per stage	Estimated Percentage Error
I	94	2	1	97	3.1%
II	0	91	0	91	0%
III	0	1	58	59	1.7%

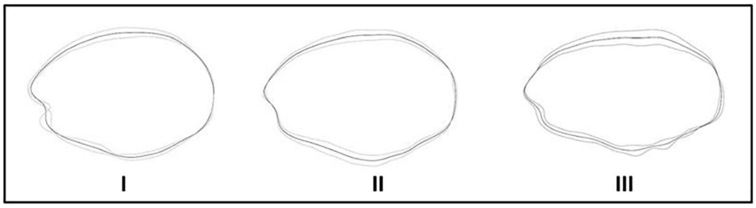

Fig. 4 Mean ± 2 standard deviation of otolith shapes for the three defined development stages (I, II and III). Black line: mean; light-grey lines: ± standard deviations

column by the fish during their life cycle, as seen for *O. argentinensis*.

The variability in otolith shape could be associated with ecological (coastal oceanographic processes, chemical/physical characteristics of water, etc.) and biological (sexual maturity, growth) (Tombari et al. 2005) factors. On the other hand, Hüssy (2008), suggests that the overall shape of the otolith is an ontogenetic process, while the finer details can be modulated by environmental conditions, most particularly related with feeding level, ranging from starvation to ad libitum (Gagliano and McCormick 2004) and food availability (Cardinale et al. 2004). In this sense, the observed changes in stage I, could be due to the fact that the fish have greater swimming habits and feed on plankton (Thompson and Volpedo 2018). In stage II fish broaden their range of prey items by feeding on other organisms associated with the bottom such as Tanaidacea, Polychaeta, Amphipoda and megalopa stage of Crustacea Decapoda larvae (Bemvenuti 1990; Martinetto et al. 2005; Thompson and Volpedo 2018) and these changes in the diet could be reflected in the otoliths shape. On the other hand, stage III includes individuals with the size of first sexual maturation (Bemvenuti 1990; Tombari et al. 2005; Llompart et al. 2013), thus, otolith changes could be associated with displacement habits (de Carvalho et al. 2015; Callicó Fortunato et al. 2017).

The application of morphometric techniques complements the study of morphology to better understand the ontogeny of fish otoliths. Moreover, the simultaneous use of these techniques strengthens the analysis. The morphometric variations observed in the otolith of *O. argentinensis* throughout its ontogeny could be associated to changes in the habitat throughout its development, to the sexual maturity of fish and to the changes in the feeding habits of these organisms.

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