Journal of the Marine Biological Association of the United Kingdom

cambridge.org/mbi

Original Article

Cite this article: de Carvalho BM, Spach HL, Vaz-Dos-Santos AM, Volpedo AV (2019). Otolith shape index: is it a tool for trophic ecology studies? *Journal of the Marine Biological Association of the United Kingdom* **99**, 1675–1682. https://doi.org/10.1017/S0025315419000729

Received: 13 September 2018
Revised: 18 July 2019
Accepted: 30 July 2019
First published online: 9 September 2019

Key words:
In vitro digestion; Sciaenidae; shape; trophic ecology

Author for correspondence:
Barbara Maichak de Carvalho, E-mail: bmaicarvalho@gmail.com

Otolith shape index: is it a tool for trophic ecology studies?

Barbara Maichak de Carvalho[1], Henry Louis Spach[2], André Martins Vaz-Dos-Santos[3,4] and Alejandra Vanina Volpedo[5]

[1]Programa de Pós-Graduação em Engenharia Ambiental, Departamento de Engenharia – UFPR, Centro Politécnico, CEP 81531-970, Bairro Jardim das Américas, Curitiba, Paraná, Brazil; [2]Programa de Pós-Graduação de Sistema Costeiro e Oceânicos, UFPR, Av. Beira-Mar, s/n, CEP 83255-976, caixa postal 61, Bal. Pontal do Sul, Pontal do Paraná, PR, Brazil; [3]Departamento de Biodiversidade, Laboratório de Esclerocronologia, UFPR, Rua Pioneiro, 2153, CEP 85950-000, Palotina PR, Brazil; [4]Programa de Pós-Graduação em Aquicultura e Desenvolvimento Sustentável – UFPR/Bolsista, CNPq 305403/2015-0, Palotino PR, Brazil and [5]Instituto de Investigaciones en Producción Animal (INPA-UBA-CONICET)/Centro de Estudios Transdisciplinarios del Agua (CETA- Universidad de Buenos Aires), Av. Chorroarin 280 Buenos Aires CP (1427), Argentina

Abstract

The aim of this study was to test the effective separation of shape indices of otoliths of three species belonging to the family Sciaenidae before and after *in vitro* digestion. We measured 328 sagittal otoliths and applied six shape indices. Before the experiment, the aspect ratio (otolith height/otolith length%), circularity, ellipticity and relative surface of the sulcus acusticus were suitable for differentiating the species of genera Paralonchurus and Stellifer. Among the species of Stellifer, the aspect ratio and rectangularity were suitable. Otoliths exposed to *in vitro* digestion showed no significant differences in their morphometry before and after the experiment. After *in vitro* digestion, the aspect ratio and circularity were effective in separating Paralonchurus and Stellifer. However, none of the indices used in the present study were efficient to separate otoliths of congeneric species after *in vitro* digestion.

Introduction

The understanding of trophic webs in marine environments depends on the identification of the trophic levels occupied by organisms (Young *et al.*, 2015), which in turn is based on analysis of the contents of the digestive tract (Baker *et al.*, 2014). The identification of prey, number and size of prey ingested are limiting factors in trophic ecology studies (Bowen & Iverson, 2013). The identification of prey is possible using sophisticated methods such as DNA analysis (Bowles *et al.*, 2011) and isotopic analysis (Silva-Costa & Bugoni, 2013; Nielsen *et al.*, 2019), and more traditional methods such as analysis of rigid structures (for example, otoliths and bones) (Sekiguchi & Best, 1997; Tarkan *et al.*, 2007; Rupil *et al.*, 2019).

Otoliths are complex structures of calcium polycarbonate, precipitated mainly as aragonite (Popper & Fay, 2011). Phylogenetic proximity influences the similarity of otolith morphology between species (Avigliano *et al.*, 2015; Tuset *et al.*, 2016). Although otoliths have intraspecific patterns, some morphological changes may occur under the influence of physiological or external factors (Volpedo & Echeverría, 1999). The onset of the reproductive process is one of the physiological factors that influence otolith shape (Gonzalez-Naya *et al.*, 2012; Carvalho & Correia, 2014; Carvalho *et al.*, 2015), as is sexual dimorphism (Maciel *et al.*, 2019). In addition, food availability can cause morphological changes (Gagliano & McCormick, 2004). Environmental factors, including depth (Torres *et al.*, 2000), temperature (Lombarte & Lleonart, 1993), pH (Schulz-Mirbach *et al.*, 2011) and salinity (Capoccioni *et al.*, 2011; Avigliano *et al.*, 2012) may also influence the shape of otoliths.

Several studies have used morphometric relationships between otoliths and fish size to estimate the length and weight of prey ingested by ichthyophagous organisms (Di Beneditto & Lima, 2003; Cremer *et al.*, 2012; Silva-Costa & Bugoni, 2013; Miotto *et al.*, 2017*a*). However, the digestion process causes erosion and breaks in the otoliths, modifying their measurements and proportions (Sekiguchi & Best, 1997; Tollit *et al.*, 2004; Christiansen *et al.*, 2005; Bowen & Iverson, 2013). *In vitro* digestion demonstrated that estimates of prey length using otoliths overestimate prey size by 35% (Tollit *et al.*, 1997, 2004; Bowen & Iverson, 2013).

Otoliths of species belonging to the family Sciaenidae are considered robust and have been described in several studies (Sasaki, 1989; Volpedo & Echeverría, 2000; Oliveira *et al.*, 2009; Siliprandi *et al.*, 2014; Volpedo *et al.*, 2017). Species of the family Sciaenidae have been identified as prey of ichthyophagous organisms (Giberto *et al.*, 2007; Bornatowski *et al.*, 2014; Rupil *et al.*, 2019) and, in some studies, the length of prey were estimated (Santos *et al.*, 2002; Cremer *et al.*, 2012; Miotto *et al.*, 2017*b*).

Traditionally, the morphological characteristics of otoliths have been used to identify prey, but shape indices could also be an efficient tool in identifying prey in stomach contents of ichthyophagous organisms. Shape indices of otoliths are widely used for differentiation of fish stocks (Avigliano *et al.*, 2015; Zischke *et al.*, 2016; Vaz-dos-Santos *et al.*, 2017) and

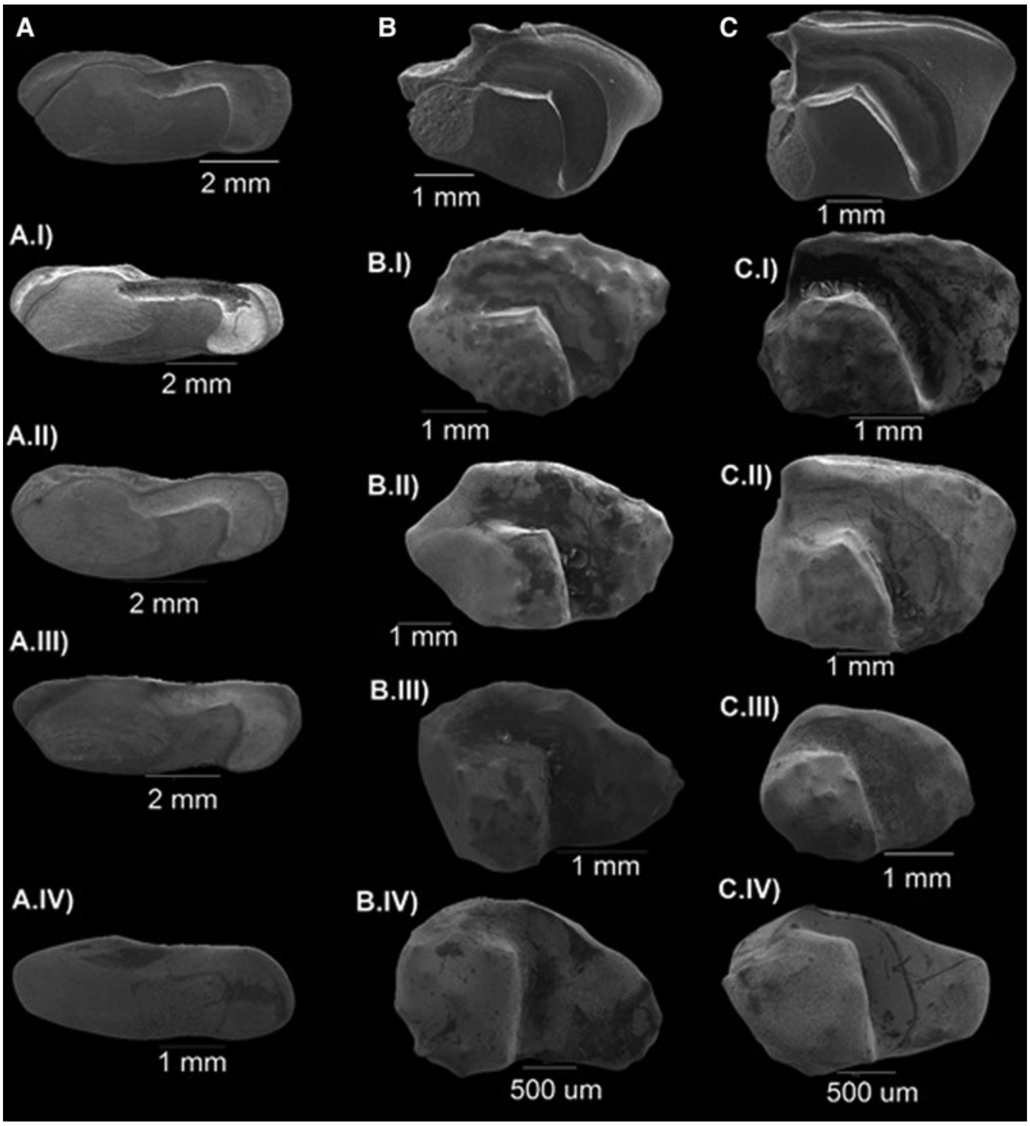

Fig. 1. Micrographs of the three Sciaenidae species: (A) *Paralonchurus brasiliensis*, (B) *Stellifer brasiliensis* and (C) *Stellifer rastrifer*. Micrographs A.I, B.I and C.I correspond to the respective species after 6 h; A.II, B.II and C.II, after 12 h; A.III, B.III and C.III, after 18 h; and A.IV, B.IV and C.IV, after 24 h of *in vitro* digestion.

identification of ontogenetic variations (Cañas *et al*., 2012; Carvalho *et al*., 2015). However, otolith shape indices have not yet been used as a tool to aid in the identification of species in stomach contents of ichthyophagous organisms, although these indices are easy to apply and provide excellent results for description and differentiation of species (Tuset *et al*., 2003; Volpedo & Echeverría, 2003; Rondon *et al*., 2014). Therefore, the present study was conducted to test the efficiency of separation of otolith shape indices of three species of the family Sciaenidae before and after *in vitro* digestion.

Materials and methods

Sampling was performed monthly between August 2000 and July 2001 on the continental shelf of the State of Paraná (25°30′–25°45′S 48°07′–48°30′W), Brazil. In the laboratory, the specimens *Paralonchurus brasiliensis*, *Stellifer rastrifer* and *Stellifer brasiliensis* were identified using specific literature (Menezes & Figueiredo, 1980), measured for total length (TL, in mm) and sagittal otoliths were extracted from the palate region (Rossi-Wongtschowski, 2015). After extraction, otoliths were photographed with the aid of ImageJ, and measured for otolith length (OL, greater longitudinal distance), otolith height (OH, greater perpendicular distance), otolith area and perimeter (AO and PER, respectively), and the area and perimeter of the sulcus acusticus (AS and PERS, respectively). The otolith shape, type, position and opening of sulcus acusticus, and presence or absence of rostrum and antirostrum were classified according to Tuset *et al.* (2008).

To calculate the shape indices, it was initially necessary to remove the effect of the individual size of each specimen. For this, an allometric model of estimation with the normalization of Lombarte & Lleonart (1993) was applied using the equation $y' = y\ (x_0/x)^b$, where: y' is the normalized variable, y is the raw value of the data, x_0 is the reference value, in the case of the lowest length of each species analysed (*P. brasiliensis* < TL = 68 mm, *S. brasiliensis* < TL = 56 mm and *S. rastrifer* < TL = 54 mm), and b is the allometric coefficient between TL and OL as the dependent variable.

After normalization, six indices of shape were applied to verify the ontogenetic variation in three species: OL/TL and OH/OL × 100 (aspect ratio), which indicated to what extent the otolith is circular or elongated (Volpedo & Echeverria, 2003); rectangularity [Rc = AO/(OL × OH)], ellipticity [E = (OL – OH/OL + OH)] (Tuset *et al*., 2003); relative surface of the sulcus acusticus [SRS = AS/AO] (Lombarte, 1992); and circularity (PER^2/AO) (Tuset *et al*., 2003; Volpedo & Vaz-dos-Santos, 2015).

https://doi.org/10.1017/S0025315419000729 Published online by Cambridge University Press

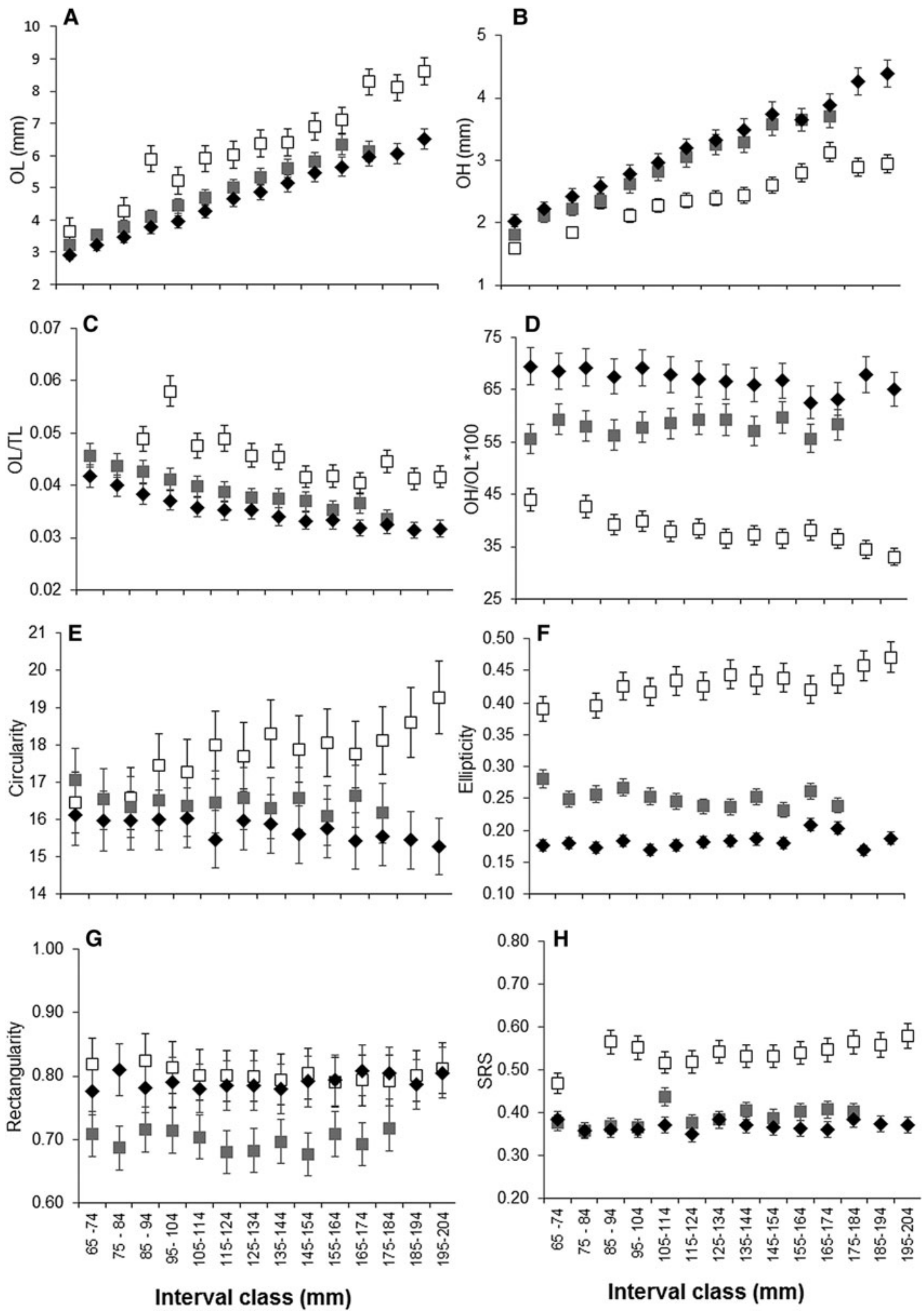

Fig. 2. Mean and standard error of the shape indices of the otoliths of *Paralonchurus brasiliensis* (square), *Stellifer rastrifer* (diamond) and *Stellifer brasiliensis* (grey square): (A) length and (B) height of otoliths, (C) OL/TL shape aspect ratio, (D) OH/OL × 100 (shape aspect ratio), (E) Circularity, (F) Ellipticity, (G) Rectangularity, and (H) SRS (relative surface of the sulcus acusticus).

The data were analysed in *R* with non-parametric tests. To check for morphometric differences between the three species analysed, the Kruskal–Wallis test and the Nemenyi post-hoc test (Pohlert, 2016) were used. To evaluate which of the shape indices is most correlated with a particular species, a Canonical Analysis of Principal Coordinates (CAP) was applied using Pearson's correlation ($r = 0.8$) (Anderson *et al.*, 2008). Significant values were considered at $P < 0.05$.

To simulate the digestion of an aquatic mammal, the otoliths were randomized and maintained in 2% hydrochloric acid at a constant temperature of 37 °C for different durations (T1 = 6, T2 = 12, T3 = 18 and T4 = 24 h). After the completion of each experimental batch (T1, T2, T3 and T4), the otoliths were washed with distilled water, photographed and re-measured, and shape indices and statistical analyses were used as described above. A *t*-test was also performed between the length and height of the

https://doi.org/10.1017/S0025315419000729 Published online by Cambridge University Press

otolith before and after digestion for each duration to measure changes in otolith dimensions after verification of the normality and homogeneity of variances assumptions.

After digestion, we calculated percentage mean size reduction (MSR): MSR = 100 × (1 − (mean size after digestion/mean size before digestion)) to measure the reduction of otoliths in length and height at different periods of the experiment (Tollit *et al.*, 1997).

Results

A total of 328 otoliths of *P. brasiliensis* (TL range = 68 to 220 mm; N = 121), *S. rastrifer* (TL range = 54 to 201 mm; N = 114) and *S. brasiliensis* (TL range = 56 to 177 mm, N = 93) were measured and classified morphologically.

According to the morphological classification, the three species presented otoliths of distinct shapes: *P. brasiliensis* bullet-shaped, *S. rastrifer* hexagonal and *S. brasiliensis* rhomboidal. The sulcus acusticus was heterosulcoid for the three species. The type of opening of the sulcus acusticus was pseudo-ostial for *S. brasiliensis* and *S. rastrifer*. In the three species, the sulcus acusticus is located in the median region of the otoliths, with absence of rostrum and anti-rostrum in the three species (Figure 1A–C). However, in *P. brasiliensis*, there were two types of openings of the sulcus acusticus – predominant para-ostial (86.77%) and pseudo-ostial (12.39%).

Before the experiment

Both the OL and OH tend to increase with fish growth (Figure 2A, B). For the three species studied, the OL/TL and aspect ratio showed a tendency of reduction along with growth; probably owing to a reduction in the growth rate of the antero-posterior axis of the otolith, a reduction that should not occur in the body growth of the fish (Figure 2C). In Figure 2D, it is possible to observe higher values of the aspect ratio for *S. rastrifer* and *S. brasiliensis* in relation to that of *P. brasiliensis*, indicating the first two have more rounded otoliths throughout their ontogeny in comparison to *P. brasiliensis*.

The circularity index resulted in similar values between *S. rastrifer* and *S. brasiliensis*; *P. brasiliensis* with the range 165–174 mm TL presented an upward trend in values of this index increasing the complexity of its contour (Figure 2E). In contrast, the ellipticity index for *P. brasiliensis* presented a growth tendency as the fish grew, thus having greater growth in the antero-posterior axis of the otolith. Minor variation in the values of this index was found for *S. brasiliensis* and *S. rastrifer* (Figure 2F).

The rectangularity showed almost constant values along the ontogeny of the three species, indicating it is not an adequate index to distinguish these species (Figure 2G). The SRS was higher for *P. brasiliensis* than that for the two species of the genus *Stellifer* (Figure 2H).

The Kruskal–Wallis test evidenced significant differences for OL ($k = 130$; df = 2; $P < 0.001$) and OH ($k = 285$; df = 2; $P < 0.001$) between the three species. The Nemenyi post-hoc test indicated significance in the interaction of OL and of OH for the three species. For shape indices, significant probability values were also found between species in the Kruskal–Wallis test ($P < 0.001$). The results of the Nemenyi post-hoc test are presented in Table 1 and only the interaction in SRS between *P. brasiliensis* and *S. rastrifer* was not significant.

In Figure 3, the graphed result of CAP illustrates the indices most correlated with the three different species. The vector of the aspect ratio and the rectangularity influenced the separation of the two species of the genus *Stellifer*; *S. rastrifer* otoliths were smaller in length and more rounded and *S. brasiliensis* otoliths were longer and more elongated. The ellipticity, circularity and relative surface indices of the sulcus acusticus separate *P. brasiliensis* from the other two species.

Table 1. Probability values (*P*) obtained from the Nemenyi post-hoc test of the shape index related to *Paralonchurus brasiliensis*, *Stellifer rastrifer* and *Stellifer brasiliensis* in the State of Paraná, Brazil (significant differences, $P < 0.05$)

Index/Species		*P. brasiliensis*	*S. brasiliensis*
OL/TL	*S. brasiliensis*	<0.001	
	S. rastrifer	<0.001	<0.001
(OH/OL)*100	*S. brasiliensis*	<0.001	
	S. rastrifer	<0.001	<0.001
Circularity	*S. brasiliensis*	<0.001	
	S. rastrifer	<0.001	<0.001
Rectangularity	*S. brasiliensis*	<0.001	
	S. rastrifer	0.55	<0.001
Ellipticity	*S. brasiliensis*	<0.001	
	S. rastrifer	<0.001	<0.001
SRS	*S. brasiliensis*	<0.001	
	S. rastrifer	<0.001	0.18

OL, otolith length; OH, otolith height; TL, total fish length.

After the experiment

At 12 h of the experiment, the otoliths of *S. brasiliensis* did not resist digestion and only a few specimens were retrieved (Table 2). For *S. rastrifer*, few otoliths resisted digestion after 18 h. The otoliths subjected to *in vitro* digestion were measured before the experiment and after the different treatments (Table 2). The three species presented a reduction in the OL and OH when comparing before and after treatment measurements. However, no significant differences were detected ($P > 0.05$).

Owing to the high erosion of the otoliths of *S. rastrifer* and *S. brasiliensis* after 24 h of *in vitro* digestion, these otoliths were not measured again. It was not possible to apply the index of SRS, since the ostium of the species of the *Stellifer* genus was very worn out, making it impossible to define the area of sulcus acusticus in any of the treatments (Figure 1).

After *in vitro* digestion, the otoliths belonging to the genus *Stellifer* continued to be more rounded irrespective of the duration they were digested for compared with those of *P. brasiliensis* (Figure 4A). The pattern observed in Figure 2 for the circularity and the ellipticity indices with higher values for *P. brasiliensis* was maintained even after *in vitro* digestion (Figure 4B, C). The rectangularity index also presented a pattern similar to that observed before the experiment with higher values for *P. brasiliensis* (Figure 4D); however, a separation of *Stellifer* species was observed with higher values for *S. rastrifer* in relation to that for *S. brasiliensis*.

The graphed result of CAP shows after *in vitro* digestion, the indices of circularity and aspect ratio are suitable for the separation of the genera *Paralonchurus* and *Stellifer*. However, the indices were not efficient in the separation of species of the genus *Stellifer* (Figure 5).

In Figure 6, MSR of the OL demonstrated the otolith of *P. brasiliensis* reduced by 13% at T1 and 26% at T4, *S. rastrifer* presented a greater reduction in otolith length at T1 and *S. brasiliensis* a greater reduction at T3. The MSR of the OH demonstrated patterns similar to the length, greater reductions for *P.*

https://doi.org/10.1017/S0025315419000729 Published online by Cambridge University Press

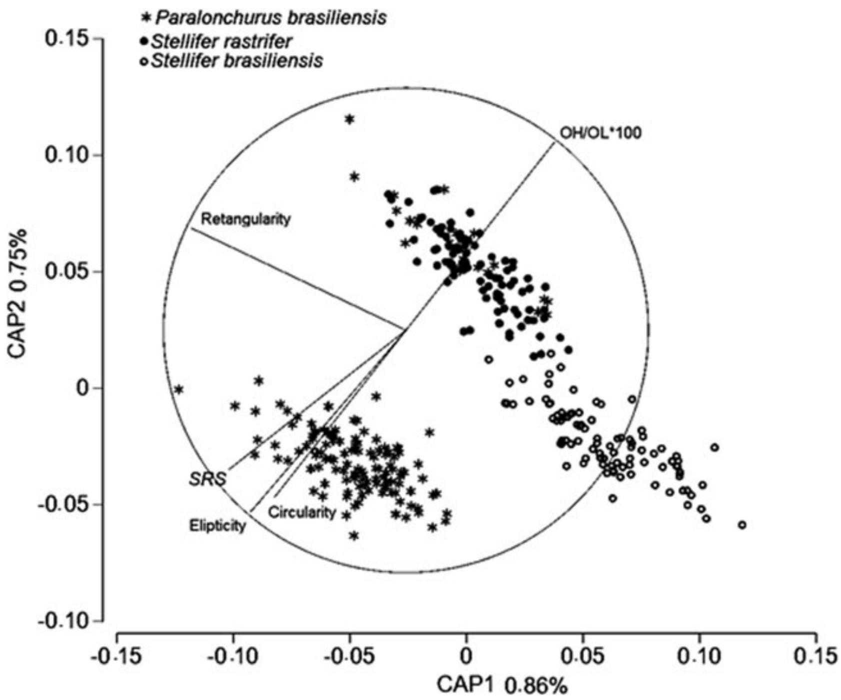

Fig. 3. Results of the Canonical Analysis of Principal Coordinates (CAP) with shape index for different species. Vectors of the index based on Spearman correlation of 0.8. OL, otolith length; OH, otolith height; SRS, relative surface of the sulcus acusticus.

Table 2. Mean and standard deviation of the length and height of the otoliths of *Paralonchurus brasiliensis*, *Stellifer rastrifer* and *Stellifer brasiliensis* used in the experiment

Species	OL (mm)	OH (mm)		OL (mm)	OH (mm)
P. brasiliensis	N = 29, 7.21 ± 1.32	2.71 ± 0.46	T1	N = 29, 6.26 ± 1.52	2.42 ± 0.51
S. rastrifer	N = 29, 4.04 ± 0.84	2.78 ± 0.55		N = 26, 3.43 ± 0.75	2.42 ± 0.62
S. brasiliensis	N = 22, 4.48 ± 0.76	2.69 ± 0.51		N = 16, 3.83 ± 0.47	2.55 ± 0.25
P. brasiliensis	N = 23, 6.63 ± 1.26	2.51 ± 0.44	T2	N = 21, 5.35 ± 1.41	2.06 ± 0.46
S. rastrifer	N = 26, 3.63 ± 0.90	2.51 ± 0.59		N = 20, 3.61 ± 0.68	2.47 ± 0.47
S. brasiliensis	N = 21, 4.43 ± 0.96	2.60 ± 0.57		N = 7, 3.92 ± 0.94	2.47 ± 0.33
P. brasiliensis	N = 29, 6.96 ± 1.13	2.62 ± 0.38	T3	N = 26, 5.47 ± 1.06	2.02 ± 0.38
S. rastrifer	N = 27, 4.24 ± 1.02	2.92 ± 0.68		N = 25, 4.17 ± 0.90	2.87 ± 0.55
S. brasiliensis	N = 27, 4.25 ± 0.90	2.52 ± 0.51		N = 4, 3.37 ± 0.86	2.32 ± 0.45
P. brasiliensis	N = 25, 7.09 ± 1.65	2.65 ± 0.47	T4	N = 22, 5.24 ± 1.50	2.01 ± 0.42
S. rastrifer	N = 28, 4.96 ± 0.97	3.38 ± 0.60		0	0
S. brasiliensis	N = 23, 4.40 ± 1.09	2.57 ± 0.69		0	0

N, number of otoliths measured; OL, otolith length; OH, otolith height; T1, otoliths exposed for 6 h; T2, otoliths exposed for 12 h; T3, otoliths exposed for 18 h; T4, for 24 h of *in vitro* digestion.

brasiliensis at T4, for *S. rastrifer* at T1 and for *S. brasiliensis* at T3 (Figure 6B).

Discussion

Some morphological characteristics were constant for the species analysed in this study; rostrum and excisura were absent, and heterosulcoid sulcus acusticus was located in the medial portion of the otolith, representing the diagnostic features of otoliths of species belonging to the family Sciaenidae from the South-west Atlantic coast (Sasaki, 1989; Volpedo & Echeverría, 2000; Siliprandi *et al.*, 2014).

Otoliths of the three species tend to increase in length and height according to the somatic growth of fish. Consequently, shape indices showed a variation along the ontogenetic development of the otoliths of *S. rastrifer*, *S. brasiliensis* and *P. brasiliensis*. These changes in patterns between the otolith growth and the growth of the body in the fish are often associated with reproduction or sexual dimorphism (Carvalho & Correia, 2014; Maciel *et al.*, 2019) with a reduction in somatic growth owing to energy expenditure with gonadal development (Quince *et al.*, 2008). The existence of these shape variations of the otoliths along ontogeny is a pattern also described for other species (Curin-Osorio *et al.*, 2012; Rondon *et al.*, 2014; Carvalho *et al.*, 2015).

To distinguish the species of the genera *Paralonchurus* and *Stellifer*, the aspect ratio, circularity, ellipticity and SRS were efficient. The separation is because of distinct morphometric and morphological variations among the otoliths of these two genera; for example, the otoliths of *Paralonchurus* are larger and more elongated resulting in larger area and length. They also have a well-developed sulcus acusticus, mainly due to the direct influence of the ostium on the SRS. In the genus *Stellifer*, the otoliths are wider and shorter in length, being more rounded. Diagnostic characteristics of these genera allow their identification in

https://doi.org/10.1017/S0025315419000729 Published online by Cambridge University Press

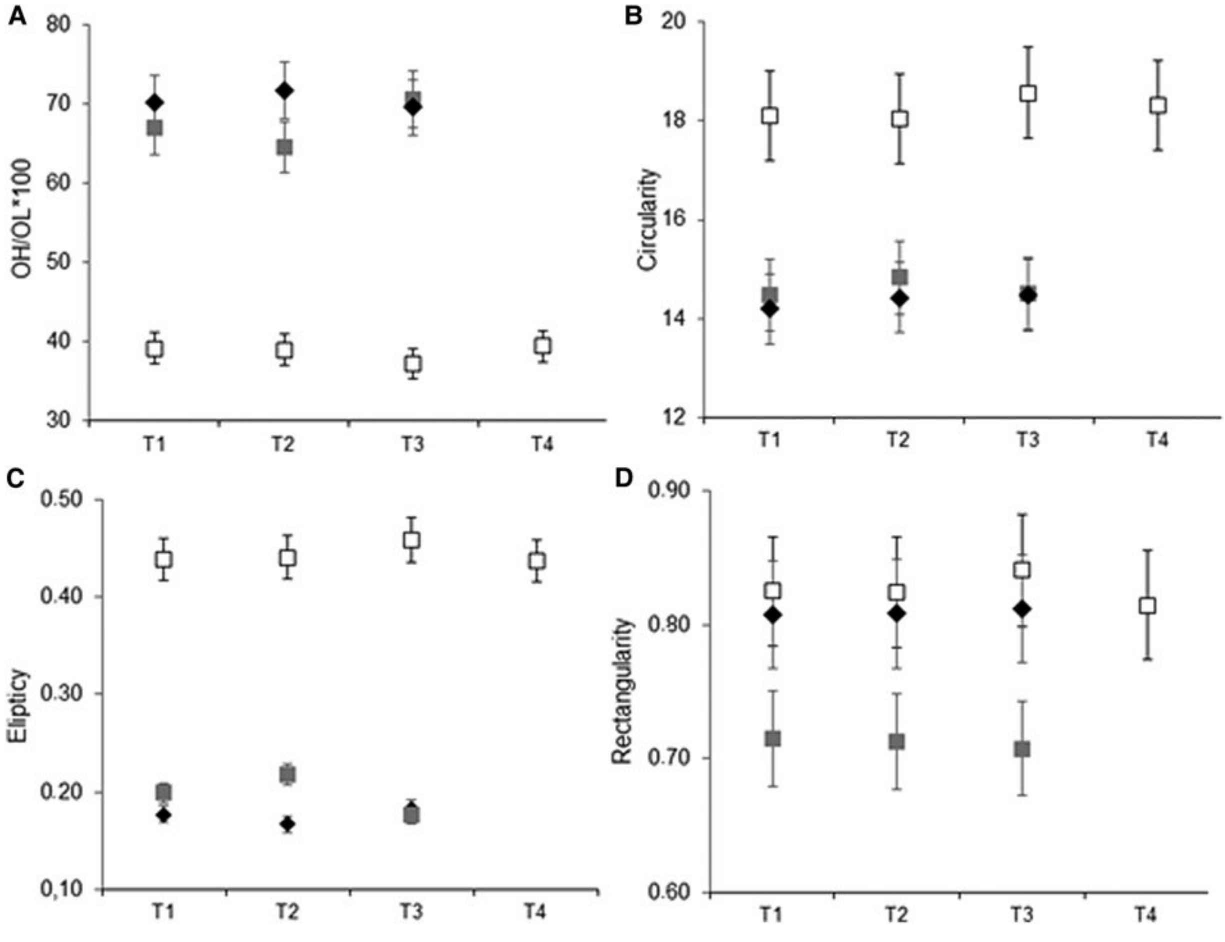

Fig. 4. Mean and standard error of the shape indices of otoliths of *Paralonchurus brasiliensis* (square), *Stellifer rastrifer* (diamond), *Stellifer brasiliensis* (grey square): (A) Shape aspect ratio (OH/OL × 100), (B) Circularity, (C) Ellipticity, (D) Rectangularity in the different treatments (T1: 6 h, T2: 12 h, T3: 18 h and T4: 24 h of *in vitro* digestion).

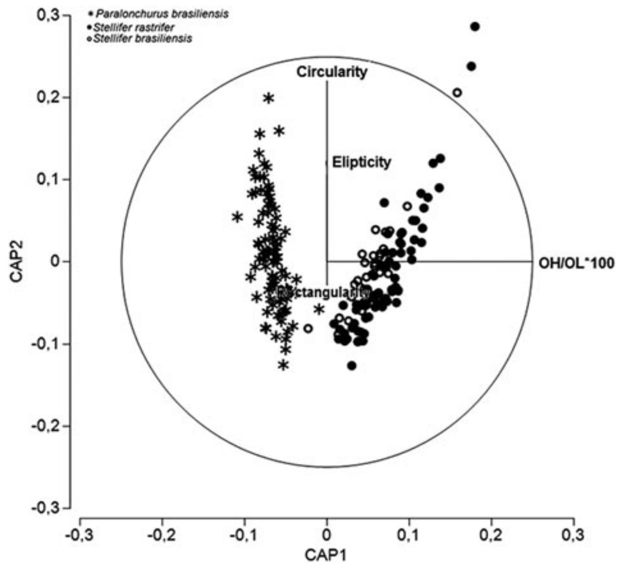

Fig. 5. Results of the Canonical Analysis of Principal Coordinates (CAP) with shape index for differences species. Vectors of the index based on Spearman correlation of 0.8. OL, otolith length; OH, otolith height.

stomach contents when they are not highly digested, while the indices were efficient up to 18 h of digestion (T3).

In the present study, it was possible to verify morphological and morphometric differences between the species of the genus *Stellifer* as reported in other studies (Corrêa & Viana, 1992; Siliprandi *et al*., 2014). The shape aspect ratio and rectangularity were efficient in separating the congeneric species *S. brasiliensis* and *S. rastrifer*.

Otoliths exposed to *in vitro* digestion reduced both in length and height in the different treatments of the present study. The reduction in the dimensions of otoliths exposed to *in vitro* digestion was also observed in other studies and in some cases the otoliths broke after 12 h of experimentation (Sekiguchi & Best, 1997; Wijnsma *et al*., 1999; Christiansen *et al*., 2005). The exposure of *S. brasiliensis* and *S. rastrifer* otoliths for long periods (12 and 18 h) to gastric acid may lead to an underestimate of how much these species are preyed upon by aquatic mammals, and the few otoliths that resist no longer have morphological characteristics that allow their identification. Therefore, previous knowledge of the otolith morphology is necessary for the separation of the otoliths of these species.

Even after digestion, the otoliths maintained their standards of shape independent of the duration of exposure in the experiment: otoliths of the *Stellifer* species with higher values in the shape aspect ratio and otoliths of *P. brasiliensis* retained greater circularity, rectangularity and ellipticity (Figure 4). This demonstrates digestion does not alter the shape of the otolith, but rather only its linear dimensions. Linear reductions can cause errors in prey length estimates when based only on morphometric ratios between otolith length and fish length.

The percentage mean size reduction of both the length and the height of the otoliths of the three species analysed in this study presented a reduction of less than 30% as also reported by

https://doi.org/10.1017/S0025315419000729 Published online by Cambridge University Press

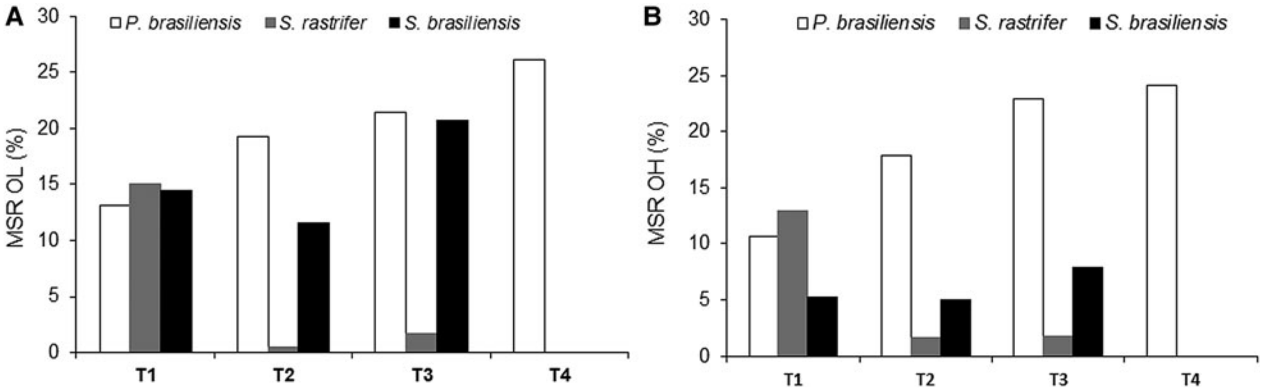

Fig. 6. Frequency histogram of the percentage reduction: (A) in length (OL) and (B) the height (OH) of the otolith before and after the different treatments (T1: 6, T2: 12 h, T3: 18 h, and T4: 24 h of *in vitro* digestion) of three species of the Sciaenidae.

Tollit *et al.* (1997). This indicates the experiment efficiently simulated the stomach of an aquatic mammal.

In conclusion, the shape indices circularity and aspect ratio were efficient in separating the worn otoliths from the genera *Stellifer* and *Paralonchurus* but no index distinguished the two species of the genus *Stellifer* before the experiment. After digestion, the shape indices circularity and the aspect ratio remained efficient in separating the genera *Stellifer* and *Paralonchurus*. However, the application of these indices should be further investigated with artificial digestion of otoliths of other species of the family Sciaenidae and in other fish taxa.

Acknowledgements. The authors are grateful for funding from the project CAFP-BA/SPU and 292/14 UFPR/Fundação Araucária. They also thank CONICET and UBACYT, CNPQ for the PhD scholarship to the first author (141267/2015-1), LAFMA (CEM-UFPR) for the use of the stereomicroscope and the Olympus DP71 camera, and the Centro de Microscopia Eletrônica (CME-UFPR) for the micrographs. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001.

References

Anderson MJ, Gorley RN and Clarke KR (2008) *PERMANOVA. PRIMER: Guide to Software and Statistical Methods.* Plymouth: PRIMER-E.

Avigliano E, Tombari A and Volpedo AV (2012) ¿EL otolito de pejerrey (Odontesthes bonariensis), refleja el estrés ambiental? *Biología Acuática* **27**, 1–7.

Avigliano E, Comte G, Rosso JJ, Mabragaña E, Paola DR, Sanchez S, Volpedo AV, Rosso FD and Schenone NF (2015) Identificación de stocks pesqueros de la corvina de río (*Plagioscion ternetzi*) de los ríos Paraguay y Paraná, mediante el análisis morfométrico de sus otólitos. *Latin American Journal Aquatic Research* **43**, 718–725.

Baker R, Buckland A and Sheaves M (2014) Fish gut content analysis: robust measures of diet composition. *Fish and Fisheries* **15**, 170–177.

Bornatowski H, Braga RR, Abilhôa V and Corrêa MFM (2014) Feeding ecology and trophic comparisons of six shark species in a coastal ecosystem off southern Brazil. *Journal of Fish Biology* **85**, 246–263.

Bowen WD and Iverson SJ (2013) Methods of estimating marine mammal diets: a review of validation experiments and sources of bias and uncertainty. *Marine Mammal Science* **29**, 1–36.

Bowles E, Schulte PM, Tollit DJ, Deagle BE and Trites AW (2011) Proportion of prey consumed can be determined from faecal DNA using real-time PCR. *Molecular Ecology Resources* **11**, 530–540.

Cañas L, Stransky C, Schlickeisen J, Sampedro MP and Fariña AC (2012) Use of the otolith shape analysis in stock identification of anglerfish (*Lophius piscatorius*) in the Northeast Atlantic. *ICES Journal of Marine Science* **69**, 250–256.

Capoccioni F, Costa C, Aguzzi J, Menesatti P, Lombarte A and Ciccotti E (2011) Ontogenetic and environmental effects on otolith shape variability in three Mediterranean European eel (*Anguilla anguilla*, L.) local stocks. *Journal of Experimental Marine Biology and Ecology* **397**, 1–7.

Carvalho BM and Corrêa MFM (2014) Morphometry of the sagittal otolith from *Atherinella brasiliensis* (Quoy & Gaimard, 1824) (Actinopterygii – Atherinopsidae), at the coast of Paraná. *Tropical Oceanography* **42**, 1–6.

Carvalho BM, Vaz-dos-Santos AM, Spach HL and Volpedo AV (2015) Ontogenetic development of the sagittal otolith of the anchovy, *Anchoa tricolor*, in a subtropical estuary. *Scientia Marina* **79**, 409–418.

Christiansen JS, Moen AG, Hansen TH and Nilssen KT (2005) Digestion of capelin, *Mallotus villosus* (Muller), herring, *Clupea harengus* L., and polar cod, *Boreogadus saida* (Lepechin), otoliths in a simulated seal stomach. *ICES Journal of Marine Science* **62**, 86–92.

Corrêa MFM and Viana MS (1992) Catálogo dos otólitos *sagitta* de Sciaenidae (Ostheichthys – Perciformes) do litoral do estado do Paraná, Brasil. *Nerítica* **7**, 13–41.

Cremer MJ, Pinheiro PC and Simões-Lopes PC (2012) Prey consumed by Guiana dolphin *Sotalia guianensis* (Cetacea, Delphinidae) and franciscana dolphin *Pontoporia blainvillei* (Cetacea, Pontoporiidae) in an estuarine environment in southern Brazil. *Iheringia* **102**, 131–137.

Curin-Osorio S, Cubillos LA and Chong J (2012) On the intraspecific variation in morphometry and shape of sagittal otoliths of common sardine, *Strangomera bentincki*, off central-southern Chile. *Scientia Marina* **76**, 659–666.

Di Beneditto APM and Lima NRW (2003) Biometria de teleósteos da costa norte do estado do Rio de Janeiro para estudos sobre piscivoria. *Biotemas* **16**, 135–144.

Gagliano M and McCormick MI (2004) Feeding history influences otolith shape in tropical fish. *Marine Ecology Progress Series* **278**, 291–296.

Giberto DA, Bremec CS, Acha EM and Mianzan HW (2007) Feeding of the Whitemouth croaker *Micropogonias furnieri* (Sciaenidae; Pisces) in the estuary of the Rio de La Plata and adjacent Uruguayan coastal waters. *Atlantica* **29**, 75–84.

Gonzalez–Naya MJG, Tombari A, Volpedo AV and Gomez SE (2012) Size related changes in sagitta otoliths of *Australoheros facetus* (Pisces; Cichlidae) from South America. *Journal of Applied Ichthyology* **28**, 1–4.

Lombarte A (1992) Changes in otolith area: sensory area ratio with body size and depth. *Environmental Biology of Fish* **33**, 405–410.

Lombarte A and Lleonart J (1993) Otolith size changes related with body growth, habitat depth and temperature. *Environmental Biology of Fishes* **37**, 297–306.

Maciel TR, Vaz-dos-Santos AM, Barradas JRS and Vianna M (2019) Sexual dimorphism in the catfish *Genidens genidens* (Siluriformes: Ariidae) based on otolith morphometry and relative growth. *Neotropical Ichthyology* **17**, 1–9.

Menezes NA and Figueiredo JL (1980) Manual de peixe marinhos do sudeste do Brasil Teleostei I. *São Paulo: Museu de Zoologia, Universidade de São Paulo* **3**, 42–59.

Miotto M, Carvalho BM and Spach HL (2017*a*) Does the closed fishing season influence the ichthyofauna consumed by *Larus dominicanus*? *Brazilian Journal of Oceanography* **65**, 9–18.

Miotto M, Carvalho BM, Spach HL and Barbieri E (2017*b*) Ictiofauna demersal na alimentação do gaivotão (*Larus dominicanus*) em um ambiente subtropical. *Ornitología Neotropical* **28**, 27–36.

Nielsen J, Christiansen JS, Grønkjær P, Bushnell P, Steffensen JF, Kiilerich HO, Præbel K and Hedeholm R (2019) Greenland shark

(*Somniosus microcephalus*) stomach contents and stable isotope values reveal an ontogenetic dietary shift. *Frontiers in Marine Science* **125**, 1–11.

Oliveira MdA, Di Beneditto APM and Monteiro LR (2009) Variação geográfica na forma e nas relações alométricas dos otólitos sagitta da maria-luiza *Paralonchurus brasiliensis* (Steindachner, 1875) (Teleostei, Sciaenidae) no litoral norte do Rio de Janeiro (21°S–23°S), Brasil. *Boletim do Instituto de Pesca* **35**, 475–485.

Pohlert T (2016) The Pairwise Multiple Comparison of Mean RanksPackage (PMCMR). R package. Available at http://CRAN.R-project.org/package=PMCMR.

Popper AN and Fay RR (2011) Rethinking sound detection by fishes. *Hearing Research* **273**, 25–36.

Quince C, Shuter BJ, Abrams PA and Lester NP (2008) Biphasic growth in fish II: empirical assessment. *Journal of Theoretical Biology* **254**, 207–214.

Rossi-Wongtschowski CDB (2015) Morfologia de otólitos. In Volpedo AV and Vaz-dos-Santos AM (eds), *Métodos de estudos com otólitos: princípios e aplicações*. Buenos Aires: CAFP-BA-PIESCI, pp. 29–46.

Rondon AS, Vaz-dos-Santos AM and Rossi-Wongtschowski CLDB (2014) Morfologia e biometria dos otólitos de *Beryx splendens* e *Hoplostethus occidentalis* (Beryciformes) no Atlântico Sudoeste. *Boletim do Instituto de Pesca* **40**, 195–206.

Rupil G, Barbosa L, Marcondes MC, Carvalho BM and Farro APC (2019) Franciscana dolphin (*Pontoporia blainvillei*) diet from Northern Espírito Santo State coast, Brazil. *Biotemas* **32**, 87–96.

Santos MCO, Rosso S, Santos RA, Lucato SHB and Bassoi M (2002) Insights on small cetacean feeding habits in southeastern Brazilian. *Aquatic Mammalian* **28**, 38–45.

Sasaki K (1989) Phylogeny of the family Sciaenidae, with notes on its zoogeography (Teleostei, Perciformes). *Memoirs of the Faculty of Fisheries Hokkaido University* **36**, 1–137.

Schulz-Mirbach T, Riesch R, León FJG and Plath M (2011) Effects of extreme habitat conditions on otolith morphology – a case study on extremophile livebearing fishes (*Poecilia mexicana*, *P. sulphuraria*). *Zoology* **114**, 321–334.

Sekiguchi K. and Best P.B. (1997) In vitro digestibility of some prey species of dolphins. *Fishery Bulletin – National Oceanic and Atmospheric Administration* **95**, 386–393.

Siliprandi CC, Rossi-Wongtschowski CLDB, Brenha MR, Gonsales SA, Santificetur C and Vaz-Dos-Santos AM (2014) Atlas of marine bony fish otoliths (sagittae) of southeastern – southern Brazil. Part II: Perciformes (Carangidae, Sciaenidae, Scombridae and Serranidae). *Brazilian Journal of Oceanography* **62**, 28–101.

Silva-Costa A and Bugoni L (2013) Feeding ecology of kelp gulls (*Larus dominicanus*) in marine and limnetic environments. *Aquatic Ecology* **47**, 211–224.

Tarkan AS, Gaygusuz CG, Gaygusuz O and Acipinar H (2007) Use of bone and otolith measures for size-estimation of fish in predator-prey studies. *Folia Zoologica* **56**, 328–336.

Tollit DJ, Steward MJ, Thompson PM, Pierce GJ, Santos MB and Hughes S (1997) Species and size differences in the digestion of otoliths and beaks: implications for estimates of pinniped diet composition. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 105–119.

Tollit DJ, Heaslip SG, Zeppelin TK, Joy R, Call KA and Trites AW (2004) A method to improve size estimates of walleye pollock (*Theragra chalcogramma*) and Atka mackerel (*Pleurogrammus monopterygius*) consumed by pinnipeds: digestion correction factors applied to bones and otoliths recovered in scats. *Fishery Bulletin – National Oceanic and Atmospheric Administration* **102**, 498–508.

Torres GJ, Lombarte A and Morales-Nin B (2000) Variability of the sulcus acusticus in the sagittal otolith of the genus *Merluccius* (Merlucciidae). *Fisheries Research* **46**, 5–13.

Tuset VM, Lozano IJ, Gonzalez JA, Pertusa JF and Garcia-Diaz MM (2003) Shape indices to identify regional differences in otolith morphology of comber, *Serranus cabrilla* (L., 1758). *Journal of Applied Ichthyology* **19**, 88–93.

Tuset VM, Lombarte A and Assis CA (2008) Otolith atlas for the western Mediterranean, north and central eastern Atlantic. *Scientia Marina* **72**, 7–198.

Tuset VM, Otero-Ferrer JL, Omez-Zurita JG, Venerus LA, Stransky C, Imondi R, Orlov AM, Ye Z, Santschi L, Afanasie PK, Zhuang L, Farré M, Love MS and Lombarte A (2016) Otolith shape lends support to the sensory drive hypothesis in rockfishes. *Journal of Evolutionary Biology* **29**, 2083–2097.

Vaz-dos-Santos AM, Santos-Cruz NN, Souza D, Giombelli-da-Silva A, Gris B and Rossi-Wongtschiwski CLDB (2017) Otoliths sagittae of *Merluccius hubbsi*: an efficient tool for the differentiation of stocks in the Southwestern Atlantic. *Brazilian Journal of Oceanography* **65**, 520–525.

Volpedo AV and Echeverría DD (1999) Morfología de los otolitos sagittae de juveniles y adultos de *Micropogonias furnieri* (Desmarest, 1823) (Sciaenidae). *Revista de Ciencias del Mar Thalassas* **15**, 19–24.

Volpedo AV and Echeverría DD (2000) Catálogo y claves de otolitos para la identificación de peces del Mar Argentino. Buenos Aires: Editorial Dunken, 90 [Peces de importância económica].

Volpedo AV and Echeverría DD (2003) Ecomorphological patterns of the sagitta in fish on the continental shelf off Argentine. *Fisheries Research* **60**, 551–560.

Volpedo AV and Vaz-dos-Santos AM (2015) Métodos de estudios con otolitos: principios y aplicaciones/Métodos de estudos com otólitos: princípios e aplicações – Ciudad Autónoma de Buenos Aires. CAFP-BA-PIESCI.

Volpedo AV, Biolé FG, Callicó Fortunato RG, Tombari AD and Thompson GA (2017) Otolitos de peces de la costa bonaerense. In Volpedo AV, Thompson GA, Avigliano E (eds), *Atlas de otólitos de Peces de Argentina*. Buenos Aires: CAFP-BA-PIESCI, pp. 181–248.

Wijnsma G, Pierce GJ and Santos MB (1999) Assessment of errors in cetacean diet analysis: *in vitro* digestion of otoliths. *Journal of the Marine Biological Association of the United Kingdom* **79**, 573–575.

Young JW, Hunt BPV, Cook TR, Llopiz JK, Hazen E, Pethybridge HR, CeccarellI D, Lorrain A, Olson RJ, Allain V, Menkes C, Patterson T, Nicol S, Lehodeym P, Kloser RJ, Arrizabalaga H and Choy AC (2015) The trophodynamics of marine top predators: current knowledge, recent advances and challenges. *Deep-Sea Research II* **113**, 170–187.

Zischke MT, Litherland L, Tilyard BR, Strarford NJ, Jones EL and Wang YG (2016) Otolith morphology of four mackerel species (*Scomberomorus* spp.) in Australia: species differentiation and prediction for fisheries monitoring and assessment. *Fisheries Research* **176**, 39–47.