

# SPATIO–TEMPORAL AND INTRASPECIFIC VARIATIONS IN THE MORPHOLOGY AND MORPHOMETRY OF OTOLITHS IN *Odontesthes bonariensis* (ATHERINIFORMES, ATHERINOPSIDAE)

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## ABSTRACT

The morphological and morphometric features of fish otoliths are species–specific. Moreover, some species are known to *show intraspecific variations*. These could be due to different environmental conditions, but this possibility has scarcely been explored. For the first time, spatio–temporal and intraspecific variations in the shape and size of the otolith of the South American silverside *Odontesthes bonariensis* (Valenciennes, 1835) are reported. For commercial purposes, this species was introduced from the Chascomús Shallow Lake (Argentina) into other environments with different characteristics. The otoliths used in this study belong to silversides sampled in 2006 from the Chascomús Shallow Lake (Argentina), and in 2006 from the Titicaca Lake (Bolivia) and the Kasumigaura Lake (Japan). Otoliths collected in 1980 from the Chascomús Shallow Lake were also included in the analysis. The species identity of some specimens was subsequently confirmed by molecular techniques. Results of multivariate analyses indicated that there were significant differences in otolith morphology and morphometry among the silverside populations studied, probably related to the environmental conditions where silversides developed.

### Key words:

Environmental conditions, sagitta, silversides.

# VARIACIONES ESPACIO-TEMPORALES E INTRAESPECÍFICAS EN LA MORFOLOGÍA Y MORFOMETRÍA DE OTOLITOS DE *Odontesthes bonariensis* (ATHERINIFORMES, ATHERINOPSIDAE)

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## RESUMEN

Las características morfológicas y morfométricas de los otolitos de los peces son especies–específicas. Por otra parte, algunas especies son conocidas por presentar variaciones intraespecíficas. Esto podría ser debido a diferentes condiciones ambientales, pero esta posibilidad ha sido poco explorada. Por primera vez, se reportan variaciones espacio–temporales e intraespecíficas en la forma y el tamaño del otolito del pejerrey *Odontesthes bonariensis* (Valenciennes, 1835) de América del Sur. Para fines comerciales, esta especie fue introducida desde la laguna de Chascomús (Argentina) en otros ambientes con características diferentes. Los otolitos utilizados en este estudio provienen de pejerreyes muestreados en 1999 en la laguna de Chascomús (Argentina), y en 2006 en el lago Titicaca (Bolivia) y el Lago Kasumigaura (Japón). Otolitos colectados en 1980 en la laguna de Chascomús también se incluyeron en el análisis. La identidad de las especies de algunos ejemplares fue confirmada posteriormente por técnicas moleculares. Los resultados de los análisis multivariados indicaron que existen diferencias estadísticamente significativas en la morfología y la morfometría del otolito entre las poblaciones de pejerrey estudiadas, probablemente relacionada con las condiciones ambientales donde pejerreyes se desarrollaron.

### Palabras clave:

Condiciones ambientales, sagitta, pejerreyes.

## INTRODUCTION

Otolith morphology and morphometry are species-specific (Koken, 1884; Hecht, 1987; Volpedo & Echeverría, 2000; Hecht, 1987; Volpedo & Echeverría, 2000; Campana, 2004; Tuset *et al.*, 2008). They are mainly used in trophic ecology studies to identify the dietary items of ichthyophagous fish (Bordino *et al.*, 1999; Koen Alonso *et al.*, 1999; Xavier *et al.*, 2004). Otoliths are also used as tools in studies covering various aspects such as palaeoecology (Nolf, 1995), palaeobiogeography (Elder *et al.*, 1996), phylogeny (Nolf, 1985; Sasaki, 1989; Assis, 2003, 2005), age and growth (Volpedo & Thompson, 1998; Francis & Campana, 2004), determination of fish stocks (Campana, 2005; Volpedo & Fernández Cirelli, 2006; Volpedo *et al.*, 2007), environmental monitoring (Ranaldi & Gagnon, 2010), vertical and horizontal migrations of fish (Campana *et al.*, 2007), trophic ecology (Nonogaki *et al.*, 2007) and eco-morphology (Torres *et al.*, 2000; Lombarte *et al.*, 2003; Volpedo & Echeverría, 2003; Volpedo *et al.*, 2008). The otoliths of teleosts are complex structures (Carlström, 1963; Morales-Nin, 1991; Gauldie, 1993) mainly composed of precipitated calcium carbonate in the form of aragonite and other minerals in small amounts, all of which are immersed in an organic matrix (Degens *et al.*, 1969; Dove, 1998). Calcium deposition is an extracellular process likely to be regulated by hormones and influenced by variations in environmental pH and temperature (Romanek & Gauldie, 1996; Morales-Nin, 1998). These factors directly affect the availability of dissolved calcium in the medium, and therefore the formation of the otolith. Although numerous environmental factors may modify otolith shape and size, the pattern described for the species remains unchanged (Gaemers, 1984; Nolf, 1985; Nolf & Sterbaut, 1989; Tombari *et al.*, 2005). The main factors cited as contributing to variations in otolith morphology are: depth, type of substrate (Lombarte & Fortunato, 1992; Aguirre & Lombarte, 1999; Paxton, 2000; Torres *et al.*, 2000; Gauldie & Crampton, 2002; Volpedo & Echeverría, 2003; Volpedo *et al.*, 2008), resonance frequency relative to the sensory epithelium (Popper *et al.*, 2005), temperature, salinity and diet (Kalish, 1989; Smith, 1992; Lombarte *et al.*, 2003; Cardinale *et al.*, 2004). Otoliths may also vary morphologically within the same species during ontogenetic development and among individuals of geographically distant populations (Volpedo & Echeverría, 1999; Paxton, 2000; Volpedo, 2001; Lombarte & Cruz, 2007; Tombari, 2008; Reichenbacher *et al.*, 2009). More significantly, there is substantial diversity in the shape (principally of the sulcus) and size of sagittae otoliths (Popper *et al.*, 2005). However, spatio-temporal and intraspecific variations have been less studied than interspecific ones.

The silverside *Odontesthes bonariensis* (Valenciennes, 1835), is a freshwater teleostean of great economic importance and it has been studied in the fields of ecology, systematics, morphology, physiology and genetics (Campos, 1984; García, 1987; Piacentino, 1987; Beherengaray, 2000; Grosman *et al.*, 2001; Dyer, 2004; Gómez *et al.*, 2007). The

silverside was introduced for commercial purposes from Argentina into many countries such as Bolivia and Japan, where it adapted to natural and artificial water bodies (Huet, 1978). Silversides from the Chascomús Shallow Lake in Argentina that were introduced into the Poopó Lake in Bolivia migrated upstream along the Desaguadero River and reached the Titicaca Lake in 1955 (Bustamante & Treviño, 1977). In Japan, silversides—also from the Chascomús Shallow Lake— were introduced into the Kasumigaura Lake in 1966 (Berasain *et al.*, 1999).

In the present study, the spatio-temporal and intraspecific variations in the morphology and morphometry of the sagitta otoliths and its relationship with some environmental factors were investigated in the silverside *Odontesthes bonariensis*.

## MATERIALS AND METHODS

### STUDY AREA

Silversides were collected from lentic environments in Argentina, Bolivia and Japan. The physico-chemical features of the water bodies, i.e. altitude, depth, transparency and salinity are summarised in Table 1.

The Chascomús Shallow Lake (35°36'S, 58°00'W; Buenos Aires, Argentina) belongs to the Shallow Lake system "Encadenadas del Este" which lies in the geomorphological unit "Pampa Deprimida" (Frengüelli, 1950). Lakes in this system are subjected to fluctuations in water volume because of their shallowness, as their maximum depth is 1.9 m. This system feeds the Salado River and the direction of the flow is inverted after floods. Water is transparent (10 to 15 cm depth), oligohaline, alkaline, pH range 8.6 – 9.1 and average temperature 15.9 °C (Laprida, 2006) and contains abundant suspended material (Miretzky *et al.*, 2000; Miretzky & Fernández Cirelli, 2004).

The Titicaca Lake (15°49'S, 69°19'W; Bolivia-Perú) is the second largest in South America, with a maximum depth of about 360 m. It is transparent (15 to 65 m depth), alkaline, with a mean pH of 8.4, mesohaline (< 1g/l), and average temperature 13 °C (Iltis, 1987, PNUMA, 2011).

The Kasumigaura Lake (36°0'N, 140°26'E; Ibaraki, Japan) is the second largest in Japan, with a maximum depth of about 7 m, transparency of 2 to 3 m depth, mean pH of 9.2 and average temperature 25.9 °C. It is hyperhaline. The Kasumigaura Lake is currently the main water source of irrigation for domestic, and industrial purposes (Aizaki *et al.*, 1988).

To ensure that all the individuals belong to the same species, their identity was confirmed by comparing the nucleotide sequence of mitochondrial DNA cytochrome oxidase I (COI mtDNA) among them. Samples of muscle tissue of some individuals from each water body (Kasumigaura, Titicaca and Chascomús Shallow Lake 1999) were ob-

tained following the protocol of Aljanabi & Martínez (1997). The amplifications of COI mtDNA were performed by PCR according to Folmer et al. (1994). PCR products were purified using QIAquick (QIAGEN, Mississauga, Ontario, Canada) and sequencing was performed at MacroGen Inc. (www.macrogen.com). Sequences were aligned using MultAlin (Corpet, 1988), available online. The neighbour-joining-distance-based method (NJ) was used to determine the nucleotide relationship among aligned sequences. The reliability of the branching was assessed by bootstrap re-sampling method using 10000 replicates. This analysis was conducted using Mega 4.0 software (Tamura *et al.*, 2007). Sequences of *Odontesthes regia* (Humboldt, 1821) from Iquique, Chile, and of *O. incisa* (Jenyns, 1841) from Mar del Plata, Argentina, were used as outgroups. All haplotypes were deposited in GenBank (accession numbers FJ810251, FJ810254 and FJ810257

	Chascomús Shallow Lake (1980)	Chascomús Shallow Lake (1999)	Kasumigaura Lake (2006)	Titicaca Lake (2006)
Altitude (m.s.l.)	7	7	0.16	3812
Area (km <sup>2</sup> )	30.1	28.7	220	8.372
Media depth (m)	1.53	1.5	4	107
Salinity (g/L)	1–2	1.572	30–50	5.2 – 5.5

**Table 1.** Physical and chemical parameters analyzed from the different locations studied.

#### OTOLITH SAMPLES

A total of 89 adults *Odontesthes bonariensis* were caught by angling from the Chascomús Shallow Lake (in 1980 and 1999; n=30 and n=24, respectively), the Titicaca Lake (n= 19) and the Kasumigaura Lake (n=16) in 2006. Adult individuals were used to perform the morphometric analysis of comparable size in organisms (Tombari *et al.*, 2005, Tombari, 2008). Sample data are presented in Table 2. The specimens were fixed in 70% ethanol and identified using the taxonomic keys of García (1987) and Dyer (1997).

Standard length (SL) was measured for each fish and the sagittae were removed from their otic capsules for examination and measurement. The inner, outer and dorsal faces of each sagitta were photographed. Left sagittae were described using the nomenclature of Volpedo & Echeverría (2000) and Tombari *et al.* (2005). The morphological and morphometric features of the left sagittae from the three silverside populations studied

(Chascomús Shallow Lake 1999, Titicaca and Kasumigaura Lakes) were analysed and compared among each other. Data were then compared with those obtained from the Chascomús Shallow Lake (1980). The sagittae used in this study were deposited in the collection of the Laboratory of Vertebrates under the nomenclature COLV- FCEN-UBA.

The morphological features considered were: presence of rostrum and excisura, shape of the dorsal rim, shape of the ostium (cup-like or funnel-like), width of cauda, shape of the posterior end (blunt, rounded or confluent) and type of depression in the dorsal area (Figure 1). The morphometric characters considered were otolith maximum length straight (OL), otolith maximum width straight (OW), ostium length straight (Osl), otolith surface (OS), sulcus surface (SS) and otolith perimeter (OP). Also, tail width (CW) was calculated as a function of the otolith width (OW) and the length of the face (RL) based on otolith length (OL), and then compared among environments (Figure 2).

Data were first tested for independence with a Contingency Table (CT) (Agresti, 1990). Then, the relationship between otolith morphology and sample sites of silversides was examined with the Correspondence Factorial Analysis (CFA) using Infostat software (2007).

The relationship between silverside growth and otolith growth was determined by linear regressions regarding the morphometric variables SL vs. OL, SL vs. OW and OL vs. OW. The analysis was performed using Statistica 5.0 software (StatSoft 1996).

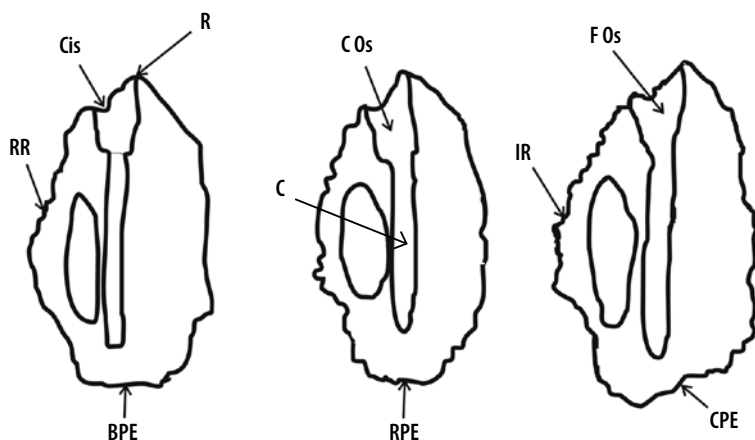
In order to test differences in OL, SL and OW among sites, three different ANCOVA analyses were performed as follows: a) OL as dependent variable and SL as covariate; b) OL as dependent variable and OW as covariate and, c) SL as dependent variable and OW as covariate. In all cases, the site was the independent variable. Tukey HSD test was used to test significant differences for paired measurements.

A Principal Component Analysis (PCA) was carried out to investigate the association between morphometric rates of otoliths (SS/OS, OP/OL, OL/SL, OW/SL, OW/OL and Ost.L/OL) and the origin of sample sites of silversides, to identify the most important standardised morphometric characters. A threshold  $\lambda > 1$  was used to determine the major factors explaining most of the variances observed. A Conglomerate Analysis based on Euclidean distances was used to group otoliths from the three sampling sites according to morphometric characters (SS/OS, OP/OL, OL/SL, OW/SL, OW/OL and Ost.L/OL). This analysis allowed determining the extent of the relationship among the samples.

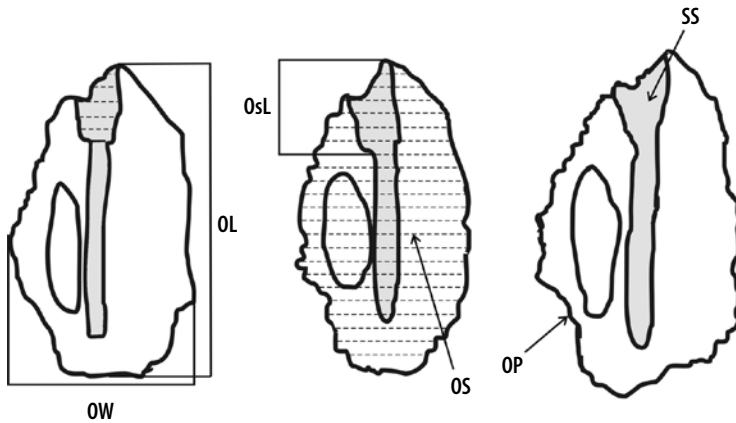
A Discriminant Analysis was carried out to separate the groups analysed and to classify new cases into groups established *a priori* using a classification rule based on the independent variables (SS/OS, OP/OL, OL/SL, OW/SL, OW/OL and Ost.L/OL). The significance association test among dependent variables, a MANOVA analysis, was performed using a Wilks Lambda test. The Principal Component Analysis, the Conglomerate Analysis and the Discriminant Analysis were performed by using the Infostat software (2007).

	Chascomús Shallow Lake	Chascomús Shallow Lake	Titicaca Lake	Kasumigaura Lake
Collected year	1980	1999	2006	2006
N	30	24	19	16
OsL	1.30±0.17	1.21± 0.40	0.94± 0.21	1.16 ±0.27
OS	11.11± 2.03	8.91± 4.80	4.27 ±3.18	4.71 ±4.23
OP	15.43± 1.51	12.83 ±3.73	8.56± 3.59	9.21 ±4.78
SS	1.84±0.35	1.58± 0.76	0.64± 0.48	0.70± 0.64
SL	253.37±22.07	204.58±67.45	185.47±53.34	195.38±54.14
OL	5.38±0.47	4.49± 1.44	3.66 ±1.04	4.41± 1.18
OW	2.92± 0.26	2.65±0.67	1.94 ±0.35	2.44 ±0.53

**Table 2.** Data sampling ( $\bar{X}$  mean  $\pm$  SD standard deviation). OL: otolith maximum length, OP: otolith perimeter; OS: otolith surface; OsL: ostium length; SS: sulcus surface, OW: otolith maximum width.



**Figure 1.** Morphological characters of inner sagittae. BPE: blunt posterior end; C: cauda, Cis: excisura; COs: cup-shaped ostium; CPE: confluent posterior end; FOs: funnel-shaped ostium; IR: irregular dorsal rim; R: rostrum; RPE: rounded posterior end; RR: regular dorsal rim.



**Figure 2.** Morphometric characters of inner sagittae. OL: otolith maximum length, OP: otolith perimeter; OS: otolith surface; OsL: ostium length; SS: sulcus surface, OW: otolith maximum width.

## RESULTS

Nucleotide sequencing (690 bp) of the COI gene revealed that the samples from the lakes studied correspond to a single specific unit (Figure 3). They are separated by at least 4 mutational steps from each other (0.58%) and by at least 20 mutational steps from *O. regia* (2.90%). This information confirms that all the specimens belong to *O. bonariensis*, native of Argentina.

Otolith morphology and morphometry differed among populations (Chascomús, Titicaca, Kasumigaura) and times (Chascomús 1980 and 1999).

The topography of the inner face, i.e., shape of the ostium, width of cauda, and type of depression in the dorsal area, was the most variable feature. Variations were also found in the dorsal and ventral rims, and in the rostrum. However, the general pattern of the species remained unchanged i.e., flat to slightly convex inner face, sulcus divided into ostium and cauda, and concave outer face with grooves and radial folds (Tombari *et al.*, 2005). The sagittae of all the specimens are oblong in shape, but somewhat longer in silversides from the Titicaca and Kasumigaura lakes than in those from the Chascomús Shallow Lake (Figure 4).

The sagittae from the Chascomús Shallow Lake show irregular dorsal and ventral rims, with the former sometimes having the highest point, while those from the Titicaca and Kasumigaura Lakes have slightly irregular rims. A cup-shaped ostium is observed



in sagittae from the three lakes, but the samples from Chascomús 1999 had similar percentages of cup-shaped and funnel-shaped ostium. The cauda of all sagittae is straight and extends along the entire length of the otolith, but it is slightly more than 30% wider in samples from the Chascomús Shallow Lake.

Although the rostrum and excisura were found in most of the otoliths, the rostrum was 40% larger in sagittae from the Kasumigaura Lake than in sagittae from the remaining lakes. The sagittae from Chascomús Shallow Lake is blunt, rounded or confluent, while that of the sagittae from the Titicaca and Kasumigaura Lakes is confluent.

The depression in the dorsal area is joined, parallel to the cauda in sagittae in specimens from the Chascomús Shallow Lake, extends along the entire length of the cauda in sagittae in specimens from the historical collection, and is shallow in those specimens from the Titicaca and Kasumigaura Lakes.

The contingency table (CT) shows a significant relationship of sagitta morphometry with the water body from which the sample was taken ( $\chi^2$  Pearson<sub>36</sub> = 160.08,  $p < 0.0001$ ). The results of the correspondence factorial analysis (CFA) are shown in Figure 5. The first two components explain 97.33% of the variance. The first component explains 72.6% of the variance and is mainly defined by the “absence of excisura”, with an eigenvector of 1.14, also contributing to this first component variables FOS and CPE and less intensely IR. The first component separates the sagittae sampled in Titicaca and Chascomús (1980) from those sampled in the remaining water bodies. The second component explains 24.73% of the variance and is defined mainly by the “absence of excisura”, with an eigenvector of 0.83, but RR and Rp, RPE, CPE and Cisp contributing to this first component. The second component separates the sagittae of the specimen samples from the Chascomús Shallow Lake (1980) and the Kasumigaura Lake from those obtained in the remaining water bodies.

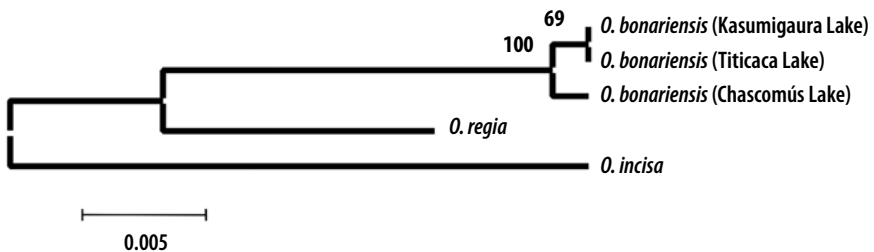
Linear regressions indicate that the sagittae in specimens sampled at the different water bodies grow at the same rate as the standard length of silversides because no differences in the slopes were observed between the otoliths in specimens sampled at the shallow lakes studied. However, the initial otolith size estimated (obtained from the ordinate intercept of the regression line), depends on the water body. The regression coefficients are shown in Table 3. In all cases, the use of the covariate revealed a positive correlation, and significant differences among sites were detected ( $p < 0.001$ ; Table 4). These differences were found mainly in Chascomús (1980) considering all the other sites compared.

The PCA results are shown in Figure 6. The first two components explain 93% of the variance. The first component explains 60% of the variance and is mainly defined by OL/SL; OW/SL; OW/OL and SS/OS, with eigenvectors of 0.45, 0.52, 0.49 and 0.44, respectively. The second component explains 32% of the variance and is mainly defined by OsL/OL and OP/OL, with eigenvectors of -0.58 and 0.69, respectively. The first component se-

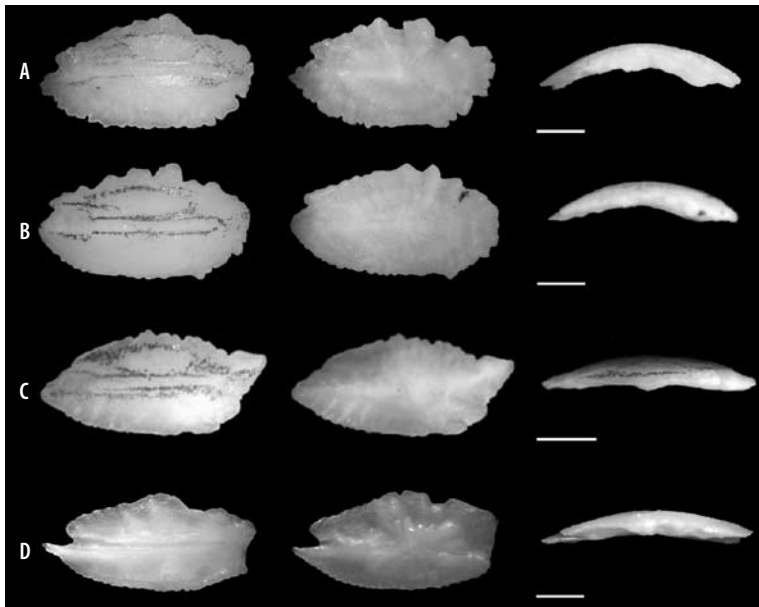
parates the sagittae of specimens from the Kasumigaura and Chascomús Shallow Lake (1999) from those obtained in the Titicaca Lake and the Chascomús Shallow Lake (1980). The second component separates the sagittae of specimens from the Kasumigaura Lake and Titicaca Lakes from those sampled in the Chascomús Shallow Lake (1980 and 1999).

The conglomerate analysis (Figure 7) divided the samples into two groups: one group comprising the sagittae in specimens from the Titicaca Lake and Chascomús Shallow Lake (1980) and the other the sagittae from the Chascomús Shallow Lake (1999) and Kasumigaura Lake.

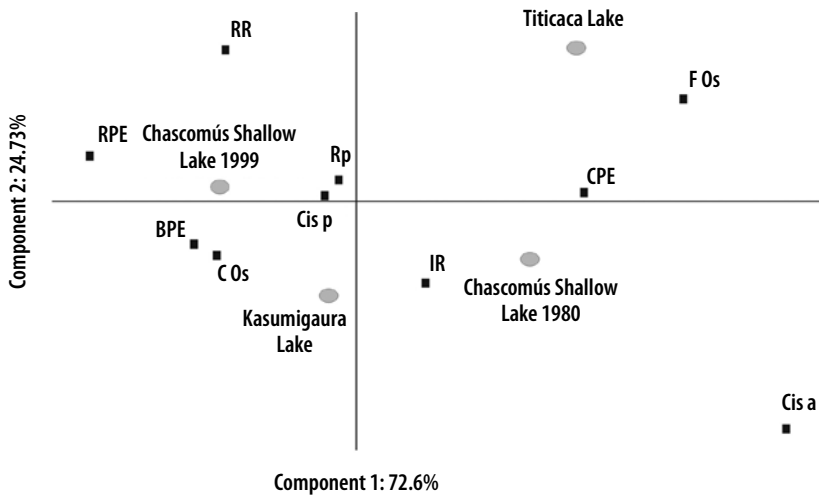
Discriminant Analysis showed statistical significant differences among samples regarding independent variables (Wilks= 0.25,  $p < 0.001$ ). Classification errors are summarised in a cross-classification table (Table 5). The minimum and maximum errors were obtained from the samples of the Chascomús Shallow Lake (1980) (0%) and the Kasumigaura Lake (43.75%), respectively.



**Figure 3.** Neighbour-joining tree analysis of the COI sequences for the *Odontesthes* species. The numbers at the tree nodes indicate bootstrap values from 10,000 replicates.



**Figure 4.** Sagittae of *Odontesthes bonariensis*. A: Chascomús Shallow Lake (1999); B: Chascomús Shallow Lake (1980); C: Titicaca Lake; D: Kasumigaura Lake. From left to right: inner and outer faces of left sagitta and dorsal face of right sagitta. Scale bar: 1 mm



**Figure 5.** Correspondence Factorial Analysis. BPE: blunt posterior end, Cis a: excisura absent; Cis p: excisura present; CPE: confluent posterior end, C Os: cup-shaped ostium, F Os: funnel-shaped ostium; IR: irregular dorsal rim; RPE: rounded posterior end; R p: rostrum present, RR: regular dorsal rim.

Group	SL vs. OL				SL vs. OW				OL vs. OW			
	a	b	r <sup>2</sup>	p	a	b	r <sup>2</sup>	p	a	b	r <sup>2</sup>	p
Chascomús 1999	0.35	0.02	0.90	<0.001	0.72	0.01	0.94	<0.001	0.57	0.46	0.98	<0.001
Chascomús 1980	0.64	0.02	0.75	<0.001	0.43	0.01	0.70	<0.001	0.74	0.41	0.54	<0.001
Titicaca	0.16	0.02	0.94	<0.001	0.78	0.01	0.88	<0.001	0.74	0.33	0.92	<0.001
Kasumigaura	0.22	0.02	0.97	<0.001	0.82	0.01	0.69	<0.001	0.75	0.38	0.71	<0.001

**Table 3.** Coefficients Simple linear regression to *O. bonariensis*. a: y intercept, b: slope of the line, r<sup>2</sup>: coefficient of determination. OL: otolith maximum length, OW: otolith maximum width, SL: Standard length.

	Source of variation	Degree of freedom	Sum of Squares	F-value	P
Dependant Variable	OL				
Covariate	SL	1	86.360	925.697	<0.001
Independent Variable	Sites	3	3.127	11.175	<0.001
	Residuals	84	7.837		
Dependant Variable	OW				
Covariate	SL	1	15.377	381.093	<0.001
Independent Variable	Sites	3	3.264	26.959	<0.001
	Residuals	84	3.390		
Dependant Variable	SL				
Covariate	OW	1	175807	381.093	<0.001
Independent Variable	Sites	3	23639	17.081	<0.001
	Residuals	84	38751		

**Table 4.** Summary of the three ANCOVA analyses performed for the OL, OW and SL variables.

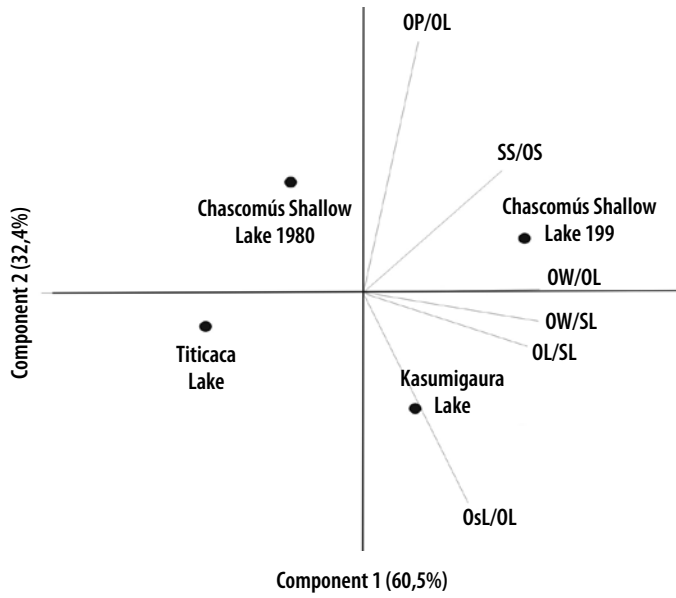


Figure 6. PCA plot. OW: otolith maximum width; OL: otolith maximum length; SL: standard length, OP: otolith perimeter; OsL: ostium length; OS: otolith surface; SS: sulcus surface.

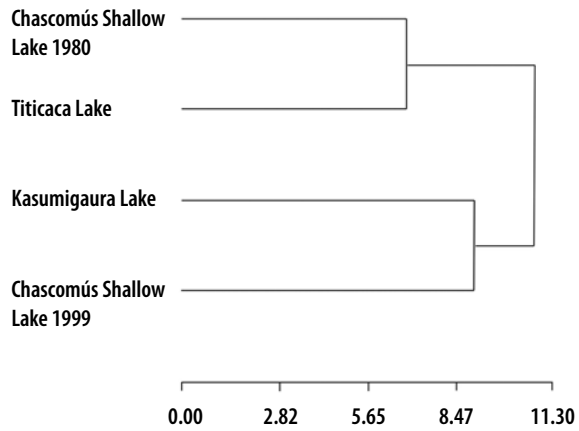


Figure 7. Conglomerate analysis dendrogram for the Chascomús Shallow Lake (1980) and (1999); Titicaca Lake and Kasumigaura Lake.

Group	Chascomús	Chascomús	Titicaca Lake	Kasumigaura Lake	Total	Error (%)
	Shallow Lake	Shallow Lake				
	1999	1980				
Chascomús 1999	18	6	0	0	24	25
Chascomús 1980	0	30	0	0	30	0
Titicaca	2	3	13	1	19	31.58
Kasumigaura	5	0	2	9	16	43.75
<b>Total</b>	25	39	15	10	89	21.35

Table 5. Classification table of Discriminant Analysis.

## DISCUSSION AND CONCLUSIONS

In general terms, asymmetric fish exhibit a high degree of intraspecific variability, particularly those whose asymmetry derives from changes in the neurocranium during larval metamorphosis (Volpedo & Echeverría, 1997; 2000; Díaz de Astarloa, 2005). In fish with bilateral symmetry, intraspecific variation is common in species that have a remarkable ability to adapt to different environmental conditions (Gómez *et al.*, 2007).

It is the first time that spatio-temporal and intraspecific variations *in shape and size of the otolith* of the South American silverside *Odontesthes bonariensis* is reported. The topography of the inner face, i.e., shape of ostium, width of cauda, and type of depression in the dorsal area, was the most variable feature, as exemplified in sciaenid fishes (Chao, 1978; Ramcharitar *et al.*, 2004).

The results of the molecular analysis confirmed that all the fish studied belonged to *O. bonariensis* and the morphological and morphometric analyses of their sagittae revealed space-temporal intraspecific variations among the populations from the Chascomús Shallow Lake (1980 and 1999), the Titicaca Lake and the Kasumigaura Lake.

While the sagittae of fish from the Titicaca Lake differed morphologically and orphometrically from those sampled at the Chascomús Shallow Lake (1999), an explanation could be given on this differentiation. The Titicaca population originated from a stock introduced in 1946. First, the silversides in the Titicaca Lake reproduced successfully, without competing with autochthonous species (Calsina Cota & Salas Piludo, 2004). Even though no massive re-introduction of silversides has been reported for the Titicaca Lake, it is reasonable to assume that this population shares the same genetic pool. In this context, the low number of individuals founding this new population in the Titicaca

Lake probably imported a very narrow and special variation on the morphological attributes that propagated into the present population. In contrast, the Chascomús population interacts with silversides from other water bodies connected by irrigation canals and during flooding events. Furthermore, the Chascomús Shallow Lake is occasionally stocked by silversides from nearby lakes and from Japan (Berasain *et al.*, 1999). Also, the limnological and geomorphological characteristics of the Titicaca Lake and the Chascomús Shallow Lake are different. They show comparable levels of salinity but different altitudes and temperatures, which may affect the amount of precipitated calcium carbonate needed for the development of the sagittae. In contrast to the silversides in the Chascomús Shallow Lake, which are found in surface waters, the silversides in the Titicaca Lake are found at a depth of 50 m (C. Calsina, *com. pers.*) and undergo substantial vertical migrations. This is likely to be reflected in their sagittae, with the longitudinal axis longer than the transverse axis. This type of morphology is in agreement with the results obtained by Volpedo & Echeverría (2003) for active swimming fish and hearing capabilities (Popper *et al.*, 2005). Overall, a common garden experience could improve the more probable scenario for changes in otolith morphology of translocated populations.

The Correspondence Factorial Analysis, Principal Component Analysis, and Conglomerate Analysis revealed similar morphometric characteristics for the sagittae in specimens from the Kasumigaura Lake and the Chascomús (1999) Shallow Lake. This result may be due to gene interchange between populations after the introduction of silversides from the former into the latter (Berasain *et al.*, 1999). Here again, differences in sagitta morphology between these populations emphasise the influence of environmental factors on species, like *O. bonariensis*, that can adapt to different habitats.

The regression lines linking the maximum width of the sagitta with the standard length of the fish showed more variability than other growth lines studied. This could indicate that, unlike otolith length, width is significantly susceptible to environmental variations (Avigliano *et al.*, 2011). As it was previously mentioned, such variations cannot be explained solely by the limnological characteristics of water bodies.

The differences in sagitta morphology and morphometry between the specimens sampled at Chascomús Shallow Lake in 1999 and at the same place in 1980 may result from changes in water quality, because calcium deposition is a process influenced by variations in environmental pH and temperature (Romanek & Gauldie, 1996; Morales–Nin, 1998).

In the sixties, the silversides in the Chascomús Shallow Lake reached a high population density which dropped dramatically in the seventies leading to the closure of commercial fishery and a reduction in the quality of sport fishing (Sendra, 2003).

To summarize, the spatio–temporal and intraspecific variations in the morphology and morphometry of the otolith of silversides could be mainly due to different environmental conditions under which they grow. Reichenbacher *et al.* (2008) have also suggested

that genetics may play a role in the differences in shape and size of sagittae observed among specimens of *Aphanius dispar* (Cyprinodontidae). This study provides evidence supporting the influence of both life history and environment on the shape and size of the otolith of *O. bonariensis*. Consequently, intraspecific variations in this species depend on the intrinsic features of each system. Because of otolith variability, change in otolith morphology and morphometry indicate the need for a greater number of samples and extreme caution when investigating the relationship between otolith morphology and morphometry, and the environment.

### ACKNOWLEDGEMENTS

We are grateful to Dr Pablo Penchaszadeh and collaborators for providing the photography equipment and to Laura López and Silvia Pietrokovsky for their valuable comments on this manuscript. Financial support was provided by grants from Universidad de Buenos Aires, Argentina (UBACYT X504, CC05), ANPCyT (PICT 2010–1372), CONICET, Comisión Nacional de Investigación Científica y Tecnológica (CONICYT), Chile (Basal Grant PFB 023) and Iniciativa Científica Milenio (ICM P05–002).

**Recibido | Received:** 13 de junio de 2011

**Aceptado | Accepted:** 23 de abril de 2012

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