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# On the intraspecific variation in morphometry and shape of sagittal otoliths of common sardine, *Strangomera bentincki*, off central-southern Chile

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SUMMARY: Size and shape of fish otoliths are species-specific, but some species also display intraspecific variations. The common sardine, *Strangomera bentincki*, is a small pelagic fish inhabiting a seasonal upwelling ecosystem off central-southern Chile, having two discrete spawning sites along its latitudinal distribution. Otoliths of specimens were collected from commercial catches in Talcahuano and Corral, representing the central and south spawning zones. On the basis of otolith images, size-based shape descriptors were used to detect ontogenetic variation, and morphometric variables (length, breadth, area, perimeter and weight) were used to detect geographical differences in size and shape of otoliths. Outline analysis was studied on the basis of elliptic Fourier descriptors through multivariate statistical procedures. Size-based shape descriptors showed that otolith shape starts to be stable for fish larger than 12 cm total length, which keep an elliptical form. Morphometric variables for fish larger than 12 cm revealed intraspecific variation between central and south zones, which were associated with otolith weight and breadth. Outline analysis did not reveal significant spatial differences, but extreme intraspecific variation was due to the *antirostrum*, *excisure*, and *posterior* part of otoliths. Intraspecific variation in otolith size could be linked to differences in each spawning habitat and related to geographical origin, whose differences are not clearly identified. It is concluded that intraspecific variability in morphometric variables of sardine otoliths revealed geographic differences in size that are not attributable to allometric effects, and that otolith shape was similar between specimens from different geographic origin.

*Keywords*: otolith, outline, shape descriptors, intraspecific, Fourier, sardine, pelagic.

RESUMEN: Sobre la variación intra-específica en la morfometría y forma del otolito sagita de sardina común (*Strangomera bentincki*) en la zona centro-sur de Chile. – El tamaño y la forma de los otolitos de los peces son específicos, pero algunas especies también muestran variaciones intraespecíficas. La sardina común, *Strangomera bentincki*, es un pez pelágico pequeño que habita en un ecosistema de afloramiento estacional en la zona centro-sur de Chile, y tiene dos áreas discretas de desove a lo largo de su distribución latitudinal. Se obtuvieron otolitos a partir de las capturas comerciales en Talcahuano y Corral, que representan las zonas de desove del centro y sur. Sobre la base de imágenes de otolitos, los descriptores de la forma basados en el tamaño fueron usados para detectar variación ontogenética, y las variables morfométricas (longitud, ancho, área, perímetro, y peso) se utilizaron para detectar diferencias geográficas en el tamaño y la forma de los otolitos. El análisis de contorno fue estudiado sobre la base de los descriptores elípticos de Fourier a través de procedimientos estadísticos multivariados. Los descriptores de forma mostraron que la forma del otolito comienza a ser estable en peces mayores de 12 cm de longitud total, manteniendo la forma elíptica. Las variables morfométricas de los peces mayores de12 cm reveló variación intraespecífica significativa entre las zonas centro y sur, las que se asociaron con el peso y amplitud del otolito. El análisis de contorno no reveló diferencias espaciales significativas, pero la variación extrema intraespecífica se debió al *antirostrum*, *excisura*, y la parte *posterior* de los otolitos. La variación intraespecífica en el tamaño del otolito podría estar relacionado con diferencias en cada hábitat de desove y en relación con el origen geográfico, cuyas diferencias no están claramente identificadas. Se concluye que la variabilidad intraespecífica en las variables morfométricas de los otolitos de sardina reveló diferencias geográficas en el tamaño que no son atribuibles a efectos alométricos, y que la forma del otolito fue similar entre especímenes de diferente origen geográfico.

*Palabras clave*: otolito, contorno, descriptores de forma, intraespecífico, Fourier, sardina, pelágico.

## INTRODUCTION

Otoliths are polycrystalline hard structures that form part of the inner acoustic system of teleost fish. They are located in the membranous labyrinths within the otic capsules located at each side of the neurocranium, and their functions are equilibrium and hearing (Harder 1975, Popper and Lu 2000). Otoliths are composed of precipitate calcium carbonate (usually aragonite) and other minerals in small amounts, all of which are immersed in a protein organic matrix (Degens *et al*. 1969). Their shape and form is due to differential accretion of minerals, producing spherical forms in early larvae and the characteristic morphology of otoliths in later stages of a given species (Gauldie 1988, Lagardère *et al*. 1995). The sagittal otoliths are the largest of the three types of otoliths and are the ones most used for fish ageing (Morales-Nin 1985, Secor *et al*. 1992). The morphology and morphometry of sagittal otoliths are species-specific feature traits (Hecht and Appelbaum 1982, Volpedo and Echeverría 2000), and they are very useful for population or stock identification (Campana and Casselman 1993, Bird *et al*. 1986, Smith 1992, Friedland and Reddin 1994, Begg and Brown 2000, Agüera and Brophy 2010). In addition, morphology and morphometry of otoliths have been important for trophic ecology studies (Härkönen 1986, Tuset *et al*. 1996, Tombari *et al*. 2000), for taxonomy (e.g. Lombarte and Castellón 1991, Martínez and Monasterio de Gonzo 1991), and also for palaeontological studies (e.g. Nolf 1985).

Genetic and environmental effects play a role in determining the shape of otoliths (Vignon and Morat 2010). Ontogeny and environmental conditions influence otolith shape in an interactive way, potentially mediated by growth rate (Vignon 2012). Factors that may contribute to intraspecific variations in otolith morphology are depth, temperature, salinity and diet, but otoliths may also vary morphologically within the same species during ontogenic development and among individuals of geographically distant populations (Paxton 2000, Volpedo and Echeverría 2000, Volpedo 2001, Tombari *et al*. 2005, Lombarte and Cruz 2007, Tombari 2008, Reichenbacher *et al*. 2009). In Chile, the small pelagic fish *Strangomera bentincki* (Norman 1936) is known locally as the common sardine and is an important commercially exploited fish in the area off central-southern Chile (33-42°S), with Talcahuano (36°30'S) and Corral (39°49'S) as the main ports for landings (Yáñez *et al*. 1990, Cubillos *et al*. 2002). At present, this fishery is managed by setting an annual global quota, which is allocated to industrial and artisanal fishermen. The quota is established on the basis of a stock assessment model, which assumes that a single, homogeneous fish population is present in the distribution area. However, two main spawning areas have been recognized during the reproductive peak (Cubillos *et al*. 2007, Cubillos *et al*. 2010) and are separated by Punta Lavapié (37°10'S, Fig. 1). Close to this latitude, the narrow (3-10 km) and deep (1 km at 15 m from the coast) submarine canyon of the Biobío river (36°05'S) divides the continental shelf into two sectors, affecting the circulation over the shelf north of Arauco gulf (Sobarzo and Djurfeldt 2004). In addition, south of Punta Lavapié, the submarine Lleulleu valley (38°S) is recognizable to the north of the Isla Mocha (Fig. 1). In the spawning area located in the north of the Arauco Gulf, eggs are less abundant than the spawning area located in the southern sectors of Isla Mocha. The latter spawning area extends to Corral and it is persistent and characterized by higher egg abundance (Castillo-Jordán *et al*. 2007, Cubillos *et al*. 2007). The environmental conditions during the spawning time showed latitudinal gradients in sea surface temperature and salinity, with warmer water in the north and colder and fresher water in the south (Cubillos *et al*. 2010). Otolith shape is species-specific, and the morphology can vary geographically within a species (Lombarte and Lleonart 1993). Also, variation in the shape of sagittal otoliths has been used to distinguish between groups of fish that have been separated or are growing in different environments (Pullianen and Korhonen 1994, Galley *et al*. 2006). Because *S. bentincki* is a population having two separate spawning areas, individuals originating in each spawning area could express intraspecific variability, and this variability could be identified in the morphology and morphometry of their sagittal otoliths. The main reason for investigating intraspecific variability in size and shape of otoliths is to identify groups of fish that have originated in each spawning zone, and to compute their contribution to the catch or population abundance.

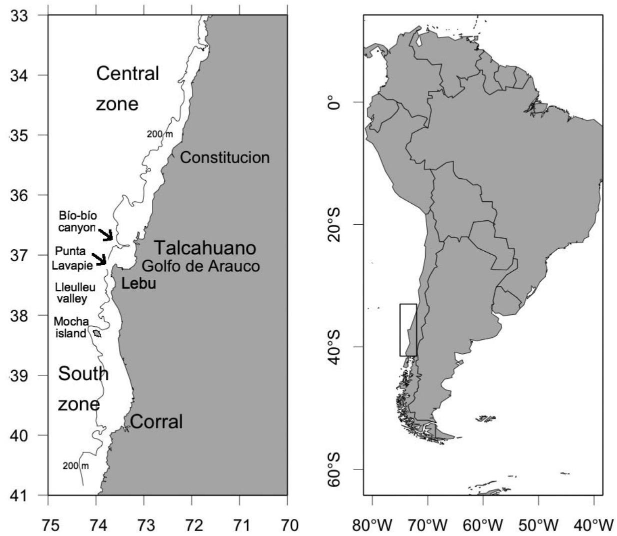


FIG. 1. – Study area off central-southern Chile; specimens of *Strangomera bentincki* were sampled in Talcahuano and Corral.

The objective of this study was to determine the intraspecific variability in the morphometry and shape of sagittal otoliths of the common sardine, *S. bentincki*. The main techniques used to study morphometrics and variability in otoliths have been: i) shape indices such as rectangularity, circularity, ellipticity, and derived characters such as form-factor and roundness (e.g. Pothin *et al*. 2006); ii) traditional multivariate analysis of measurements such as length, weight, width, area and perimeter (e.g. Bolles and Begg 2000, Reichenbacher *et al*. 2007); and iii) otolith outlines, in which the contour of the otoliths can be analysed on the basis of a given number of harmonics which are obtained with elliptic Fourier analysis (e.g. Kuhl and Giardina 1982, Begg and Brown 200, Campana and Casselman 1993, Tracey *et al*. 2006). In the present study, shape indices were used to identify the size at which shape of sagittal otoliths of *S. bentincki* was stable and not influenced by growth. Furthermore, traditional multivariate analysis of otolith measurements and multivariate analysis of elliptic Fourier descriptor were used to investigate intraspecific variability in size and shape.

## MATERIALS AND METHODS

### Sampling and otoliths collection

Specimens of *Strangomera bentincki* were sampled from the fishery catch in waters of Talcahuano (36°43'S, 73°07'W) and Corral (39°49'S, 73°14'W) in 2008 and 2009. The geographic strata were called "central" for specimens obtained off Talcahuano, and "southern" for specimens obtained off Corral, representing the central and south spawning areas. Random samples of fish were obtained, and otoliths of specimens were obtained according with total length classes (Table 1). Although the number of otoliths was not balanced between central and southern sectors for the first and second length classes, there were enough specimens for fish larger than 12 cm (Table 1). Total length (±0.1 cm) and total weight (±0.01 g) were recorded for each specimen, and sagittal otoliths were removed from the neurocranium. The otoliths were then cleaned with water, air-dried and stored in individual labelled Eppendorf tubes. Only entire, undamaged and non-decalcified otoliths were selected.

### Otolith measurements and procedures

The right otolith of each individual was used and their weight was obtained in an analytical balance (±0.0001 g). Measurements were carried out using an image of the otolith, which was obtained under stereomicroscope Zeiss Stemi 2000-C (×5.0 and ×3.2 depending on otolith size) with a Canon PowerShot A640 digital camera (10 megapixels) adapted to the stereomicroscope. The images of otolith were processed with IMAGE J software (http://rsbweb.nih.gov/ij/) and used to measure otolith length (Lo, mm), otolith breadth (Bo, mm), otolith area (Ao, mm$^2$), and otolith perimeter (Po, mm). In addition, we used IMAGE J to compute size-based shape descriptors, i.e. rectangularity index, ellipticity index, circularity index, aspect ratio, form-factor and roundness. The rectangularity, ellipticity and circularity indices were computed as the ratio between the otolith area and the area of a rectangle, the area of an ellipse, and the area of a circumference, each one covering the major axis length (otolith length) and minor axis length (otolith breadth, Fig. 2). In this context, the three indices are dimensionless numbers between 0 and 1. The form-factor, aspect ratio, and roundness were computed following the formulae given by Tuset *et al*. (2003), Pothin *et al*. (2006) and Agüera and Brophy (2010). The size-based shape descriptors were used to analyse the stabilization in the otolith form as a function of total body length. Generalized Additive Model (GAM) techniques, contained in the mgcv package (Wood 2006), were used to describe the relationship between each shape descriptor and total body length. The five morphometric variables were corrected to avoid body size and allometric effects that occur during otolith growth (Lombarte and Lleonart 1993). For each of the five measurements, the allometric relationship to total body length was calculated. The power model, i.e. $Y=aX^b$, was fitted using logarithmic transformation to homogenize the residuals. Each measurement $Y$ was transformed into $Z$, according to $Z_i=Y_i(X_0/X_i)^b$, where $X_i$ is the otolith length of the i-th specimen in the sample, and $X_0$ is the total reference total body length. A total length of 14 cm was selected for standardization. The principal components analysis (PCA) was used to compare the five morphometric variables on the basis of the correlation matrix, which is described as a size and shape analysis. The main reason for the use of the correlation matrix is that it makes the principal components independent of the order of magnitude and the scale of the variable measurements (Torres *et al*. 2000).

Table 1. – Number of otoliths, which were selected per length classes.

| Total Length (TL) strata (cm) | Central area (Talcahuano) | Southern area (Corral) | Total |
|---|---|---|---|
| TL<8 cm | 38 | - | 38 |
| 8≤TL<12 | - | 87 | 87 |
| 12≤T <16 | 48 | 91 | 139 |
| TL≥16 | 8 | 3 | 11 |
| Total | 94 | 181 | 275 |

### Outline analysis

The elliptic Fourier analysis consists of fitting a given number of harmonics to the original otolith outline on the basis of a given number of coordinates, which have been sampled along the otolith contour (Kuhl and Giardina 1982, Tracey *et al*. 2006, Claude 2008). Otolith images were binarized, and 50 coordinates distributed sequentially along the outline were sampled from each image. Subsequently, 25 elliptic

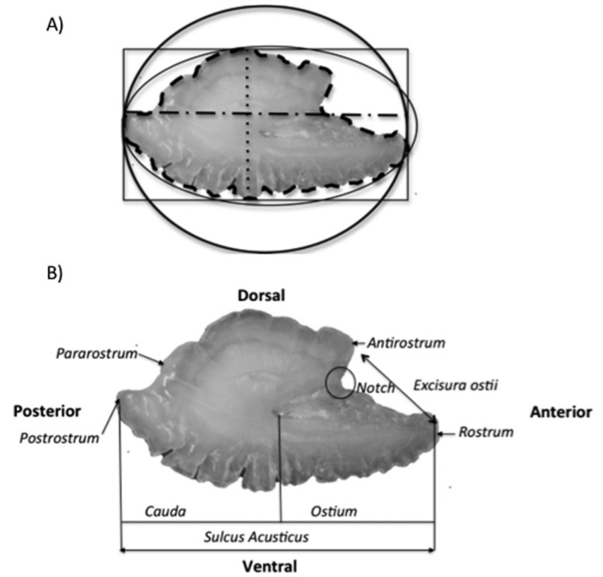


FIG. 2. – Otolith of *Strangomera bentincki*, showing: A) the main measurements, such as length (- × -) and breadth (×××), perimeter (- - -), and areas of circle, ellipse, and rectangle covering the major and minor axis of otolith; and B) morphological description of the sagittal otolith.

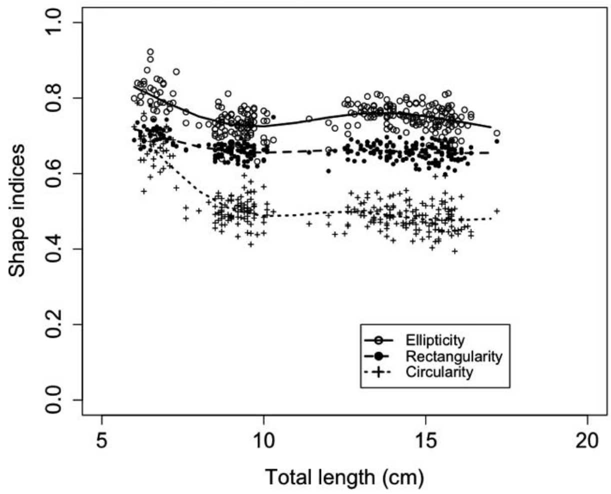


FIG. 3. – Size shape descriptors as a function of total body length of *Strangomera bentincki* off central-southern Chile.

Fourier harmonics were calculated on the basis of the 50 pseudo-landmarks per otolith, according to the algorithms described by Claude (2008) for the software R version 2.11.1 (R Developer Team 2010). Each harmonic is composed of four coefficients, resulting in 100 elliptic Fourier coefficients per otolith. A random subsample of 30 otolith per zone were used, representing fish larger than 12 cm total body length. For each otolith, elliptic Fourier coefficients were normalized for size, location, and starting point. The number coefficients were selected by analysing the power and proportion of variance explained by harmonics (Claude 2008). Once the number of harmonics had been defined, the PCA was used to describe the extreme shape variation of otoliths. Also, MANOVA was applied to detect geographical differences. Finally, to identify intraspecific groups from geographical zones, linear discriminant function (LDF) was used for elliptic Fourier coefficients. LDF is an appropriate analysis when the populations of the two zones have identical variance-covariance matrices.

The allometric analysis, PCA, ANOVA, and MANOVA were carried out using the statistical packages for the software R version 2.11.1 (R Developer Team 2010). For LDF, the MASS package of Venables and Ripley (2002) for the software R was used.

## RESULTS

### Otolith shape descriptors, allometric analysis, and morphometric analysis

Otoliths of sardine are elliptic, with the ventral margin crenate and the posterior region round to irregular. The anterior region is peaked, with the rostrum very broad, short; the antirostrum broad, very short, and round; the excisure wide with a shallow notch (Fig. 2).

According to GAM analysis, the rectangularity (RI), ellipticity (EI), and circularity (CI) indices showed that otolith shape was close to an elliptical form, and that this shape was stable for fish larger than 10 cm total fish length. All the indices showed a decline from fish length of 6 to 10 cm, particularly the circularity index (Fig. 3). The aspect ratio showed a significant increase from 6 to 10 cm, and the contrary occurred for roundness and form-factor (Fig. 4). The results mean that otolith shape starts to be more stable in fish larger than 12 cm. All morphometric measurements were allometric functions of total body length (Table 2). The least allometric was otolith breadth, with the lower 95% confidence interval close to 1, i.e. IC=[1.001, 1.110]. However, all morphometric measurements were corrected to avoid correlation with body size.

The five principal components computed from corrected morphometric measurements for fish larger than 12 cm total length revealed that the two first principal

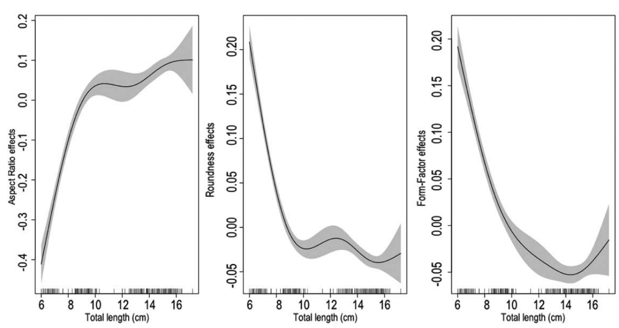


FIG. 4. – GAM modelling for the aspect ratio, roundness and form-factor as a nonlinear function of total body length of *Strangomera bentincki* off central-southern Chile.

Table 2. – Allometric coefficient for each morphometric measurements of *Strangomera bentincki* otoliths as a function of total body length (cm).

| Morphometric measurements and units | Allometric coefficient and standard error | Pr(>\|t\|) | $F_{1,273}$ | $r^2$ |
|---|---|---|---|---|
| Otolith weight (OTW, mg) | 1.23 (0.04) | <0.01 | 925 | 0.77 |
| Length (Lo, mm) | 1.29 (0.02) | <0.01 | 2691 | 0.91 |
| Breadth (Bo, mm) | 1.05 (0.03) | <0.01 | 1444 | 0.84 |
| Area (Ao, mm$^2$) | 2.29 (0.05) | <0.01 | 2005 | 0.88 |
| Perimeter (Po, mm) | 1.29 (0.02) | <0.01 | 2851 | 0.91 |

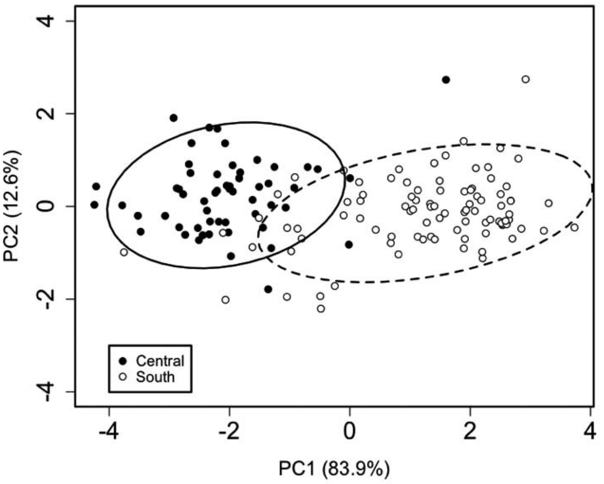


Fig. 5. – Scatterplot of factor scores from principal components analysis for otoliths of *Strangomera bentincki*. The ellipses represent a 95% confidence interval.

components explained 96.5% of the variance. The first PC explained 83.9% and the second 12.6%, showing that otoliths of specimens tended to segregate along the PC1 (Fig. 5). The first PC was negatively correlated with the corrected morphometric measurements, with the lowest correlation with otolith weight (-0.668) and the highest with otolith breadth (-0.987). These results showed differences in the size of the otoliths between central and southern areas.

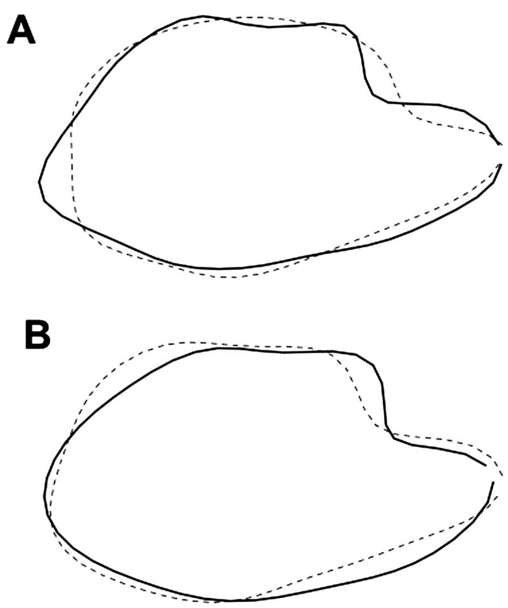


Fig. 6. – Extreme shape variation of otolith outline as explained along maxima and minima scores along the first PC (A) and second PC (B).

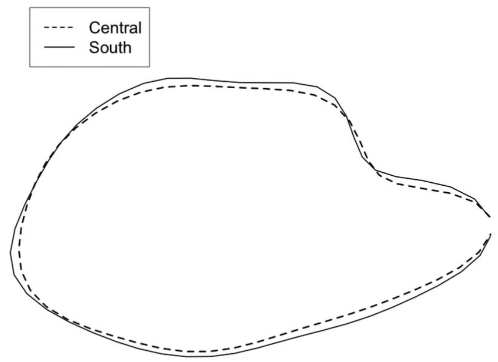


Fig. 7. – Average outline from the average elliptical Fourier coefficients of each locality.

## Outlines analysis

From the elliptical Fourier analysis (EFA), the first 12 harmonics had 99.2% of cumulative power and contributed 96.2% to the total shape variation. Therefore, discarding the first harmonic, only 11 harmonics were selected for multivariate analysis. On this basis, the first and second PC explained 40% and 17% of the variance, respectively. The EFA descriptors did not reveal geographical segregation along the first PC, showing similarity in shape. However, the extreme variation along the first PC (40%) was due to variation in the *antirostrum*, *excisure* and *posterior* part (Fig. 6a). The extreme variation explained by the second PC (17%) was due to some variation in the ventral and dorsal shape of the otoliths (Fig. 6b). The MANOVA did not show significant differences between zones in the 11

Table 3. – Classification matrix (%) of sagittal otoliths for *Strangomera bentincki* from central (n=30) and southern areas (n=30).

| | Predicted classification | | |
|---|---|---|---|
| | Central area | Southern area | Total |
| Central area | 70 | 30 | 100 |
| Southern area | 10 | 90 | 100 |
| Total | 80 | 120 | 200 |

EFA descriptors (Wilks' lambda = 0.426, *F*=0.460, *P*=0.977). The discriminant analysis showed correct classification for 70% of central zone specimens, and 90% in the case of the specimens from south sector (Table 3). The minor differences in average otolith outlines, as reproduced by considering the mean of the first 12 EFA descriptors by zone (Fig. 7), showed the similarity in shape.

## DISCUSSION

Intraspecific variability in the morphometry and shape of sagittal otoliths of the common sardine, *Strangomera bentincki,* was studied by considering geographic variation in the size and shape of otoliths. The size-based shape descriptors showed that shape of sagittal otoliths started to be stable for fish ≥12 cm total body length, which was close to the length at 50% maturity (11 cm) that has been reported (Cubillos *et al*. 1999).

The size-based shape indices showed that otoliths of sardine lost circular and rectangular shapes quickly, and retained elliptic shape for fish ≥12 cm. It must be mentioned that the circularity index used here was computed on the basis of otolith area and the area of a circle whose diameter was the otolith size. In fact, the circularity index has usually been based on squared perimeter divided by otolith area: for example, Tuset *et al*. (2003), Pothin *et al*. (2006) and Agüera and Brophy (2010).

The GAM was a satisfactory technique for revealing growth effects on size-based shape descriptors and detecting the size ranges that are least influential on the otolith shape. Roundness and form-factor describe shape in the same direction while aspect ratio is inversely correlated with the previous shape descriptors. All shape descriptors showed significant ontogenetic changes that occur in the otolith of sardine, allowing us to conclude that otolith shape stabilizes after size of maturity, as has been reported for some species (Tuset *et al*. 2003). In addition, the allometric effects on otolith size were less great for fish larger than 12 cm. However, when allometric effects were removed, otoliths of common sardine revealed geographical differences in size. The morphometric variables were negatively correlated with the first PC, representing the component of relative size (otolith weight and otolith breadth) that was not linked with individual allometric growth. The intraspecific variability in otolith size is probably linked to differences in each spawning habitat and related to geographical origin, whose differences are not clearly identified. It must be mentioned that both genetic and environmental effects play a role in determining the shape of otoliths (Vignon and Morat 2010). Furthermore, ontogeny and environmental conditions influence otolith shape in an interactive way, potentially mediated by growth rate (Vignon 2012). The otolith weight and breadth of *Strangomera bentincki* could be affected by potential environmental differences in the spawning habitat where fish develop and grow.

In terms of shape, the EFA showed that only 12 harmonics were satisfactory in explaining the otolith outline. Variations in otolith shape of a species from different geographical regions are at least partially expressed during its life history, thereby representing a phenotypic measurement of stock identification (Bolles and Begg 2000). MANOVA and discriminant linear analysis showed that there were no significant geographic differences in the sardine otolith shape. However, the extreme variation explained by the first PC revealed that almost 40% of variation occurs in the *antirrostrum*, *excisure*, and *posterior* part. This intraspecific variation, however, was not sufficient to conclude that sardine is divided into more than one subpopulation between Talcahuano and Corral. This result is consistent with Galleguillos *et al*. (1997), who using 5 enzyme polymorphic loci found no genetic differences between three geographical origins. The authors indicate that *Strangomera bentincki* showed a high genetic homogeneity. However, the otoliths of specimens vary in size (breadth and weight) between zones. If these differences in size persist in time and can be monitored in time, then otolith morphometry could help to quantify the mix of fish coming from different geographic origins (spawning habitats) and their contribution to catch and population abundance. In fact, otolith morphometry and shape analysis from specimens originating in each spawning habitat can contribute to population dynamics by quantifying the mix of those fish and/or monitoring the contribution of each spawning site to the recruitment. Further research should include efforts to use otolith chemistry to elucidate population structure (e.g. Newman *et al*. 2010) and natal origins of fish (e.g. Schloesser *et al*. 2010, Barnett-Johnson *et al*. 2010). Meanwhile, it is concluded here that the intraspecific variability in morphometric variables of *S. bentincki* otoliths revealed geographic differences in size that are not attributable to allometric effects, and that otolith shape was similar between specimens from different geographic origins.

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