Ontogenetic and intraspecific variability in otolith shape of anchoveta (*Engraulis ringens*) used to identify demographic units in the Pacific Southeast off Chile

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Abstract. The phenotypical variability in otolith shape of anchoveta (*Engraulis ringens*) was analysed in three zones (I, II and III) from north to south along the Chilean coast, using juvenile and adult fish. Generalised additive models were used to analyse shape indices and canonical discriminant analysis was used to analyse elliptical Fourier harmonics. The form factor and ellipticity indices varied significantly among the three zones, whereas roundness, circularity and rectangularity indices only showed differences between Zones I and III. Fourier reconstructed outlines for five ontogenetic stages suggested important differences among sampling zones, which were larger for sampling Zone III, where, at the same fish length, otoliths were smaller than those sampled in Zones I and II, at least at the pre-recruit stage. Elliptical Fourier descriptors showed significant differences among the three units, with a total percentage of correct classifications for juveniles of 89 and 74% for raw data and cross-validated cases respectively, compared with >85 and ~65% respectively for adult fish. The results support the hypothesis that juveniles and adults of anchoveta have remained segregated throughout their entire, or at least a fraction of, their life cycle, mainly between the extreme northward and southward zones.

Additional keywords: elliptical Fourier analysis, generalised additive model otolith shape indices, stock.

Introduction

A very complex issue in fisheries management is the uncertainty in the number, distribution and connectivity of demographic units stocks (Begg *et al.* 1999; Taylor and Dizon 1999; Luck *et al.* 2003; Kerr *et al.* 2014). In practice, it is not possible to estimate the productive surplus that can be harvested with acceptable precision and accuracy without knowing the effective distribution, abundance, degree of reproductive isolation, self-recruitment potential and dependence on immigration of a management unit (Secor 2013). To date, although control measures have been taken to achieve stock recovery in some overexploited fisheries (Hutchings *et al.* 2010; Murawski 2010; Petitgas *et al.* 2010), their poor success could be linked to a poor match between management units and biological stocks (i.e. demographic units).

Otolith morphology, and shape analysis in particular, have been widely used in fisheries science to determine demographic units (Campana and Casselman 1993; Begg and Brown 2000;

Torres *et al.* 2000; Pothin *et al.* 2006; Canas *et al.* 2012). Otoliths are pairs of calcified structures, located in the inner ear of fishes, whose main functions are balance and hearing (Campana 1999). Although otolith shape is species specific (Hecht and Appelbaum 1982; Gaemers 1984), environmental variability (biotic and abiotic) may lead to intraspecific differences among geographical regions, probably mediated through differences in individual growth rates (Vignon 2012). The use of otolith shape analysis to differentiate demographic units is possible and adequate when enough and prolonged spatial isolation leads to detectable and consistent differences in otolith shape among demographic units (Neilson *et al.* 1985; Bird *et al.* 1986; Campana and Casselman 1993; Begg and Brown 2000; Turan 2000; Turan *et al.* 2006).

Otolith shape analysis includes shape indices (circularity, rectangularity, ellipticity, eccentricity, roundness), shape factors (Pothin *et al.* 2006) and otolith outline analyses, commonly

based on elliptic Fourier and Wavelet transformations (Bird *et al.* 1986; Smith 1992; Campana and Casselman 1993; Begg and Brown 2000; Bergenius *et al.* 2005; Parisi-Baradad *et al.* 2005; Pothin *et al.* 2006; Turan *et al.* 2006). These approaches have benefited from increasing technological ability to produce reliable two-dimensional digital images in most fishery laboratories. Furthermore, otolith shape analysis is far less time consuming and less costly than other techniques used for stock discrimination, such as otolith microchemistry and otolith microstructure analysis (Chittaro *et al.* 2006). Consequently, otolith shape analysis can be used either complementary to or as an effective alternative for other techniques, particularly for long-term monitoring of the degree of separation or mixing of units previously established using multiple approaches.

Anchoveta (*Engraulis ringens*) is distributed from northern Peru (4°30′S) to southern Chile (42°30′S). Two main population units have been identified within this range: one off the northern to central coast of Peru and the other off southern Peru and northern Chile (Pauly and Tsukayama 1987). The northern unit supports important fishery activity throughout its distribution (i.e. from 16°00′S to 24°00′S), representing a substantial fraction of the global anchoveta fisheries (Bakun and Weeks 2008; Schreiber and Halliday 2013). Despite the tremendous ecological role anchoveta plays in the Humboldt Current system (Chavez *et al.* 2003; Espinoza and Bertrand 2008; Karstensen and Ulloa 2009) and its considerable contribution to regional economies from the 1950s to the present, there have been limited efforts to enhance our knowledge regarding the population structure of anchoveta (Galleguillos *et al.* 1996; Ferrada *et al.* 2002; Chávez *et al.* 2007; Valdivia *et al.* 2007; Rojas 2011; George-Nascimento and Moscoso 2013) and the degree of coherence between management and demographic units.

Until now, efforts to identify the number, distribution and connectivity of demographic units within Chilean waters have been limited. Genetic studies have failed to reject the null hypothesis of panmixia and have suggested the existence of a single evolutionary unit along the Chilean (Galleguillos *et al.* 1996; Ferrada *et al.* 2002) and Peruvian (Rojas 2011) coasts. Conversely, parasitological studies (Valdivia *et al.* 2007; George-Nascimento and Moscoso 2013) have indicated the possible existence of at least two separate demographic units, one in the north and another in southern Chile. Therefore, the aim of the present study was to investigate the spatial, temporal and ontogenetic variability in the shape of sagittal otoliths of anchoveta to contribute to efforts to reveal the population structure of this important fisheries resource.

Materials and methods

Study area

The study area was divided in three sampling zones that corresponded to the three main fishing areas off Chile: Zone I, Arica to Antofagasta (18°24′–26°00′S); Zone II, Caldera to Coquimbo (26°01′–32°16′S); and Zone III, Valparaiso to Valdivia (32°17′–41°77′S; Fig. 1).

However, there are also oceanographic and topographic traits to justify this sampling design. First, there is a coastal transition zone (CTZ) between 34 and 39°S (Morales *et al.* 2010), which can act as natural boundary. Although there is no strict
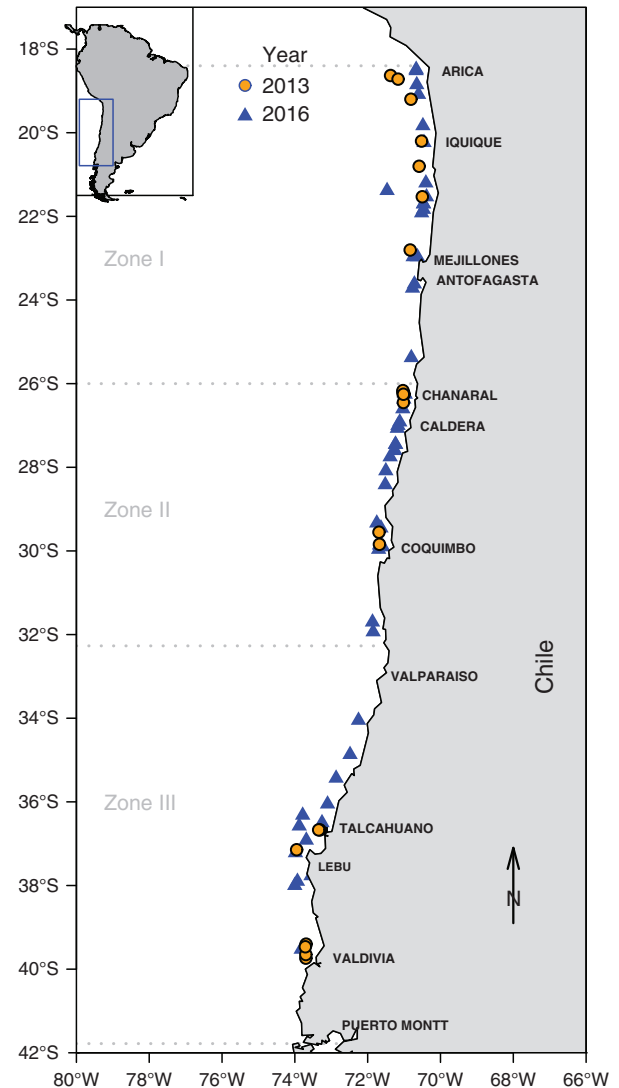

Fig. 1. The anchoveta (*Engraulis ringens*) study area along the Chilean coast. Symbols show the fishing hauls in each year, and the dashed lines demarcate the study zones.

topographic boundary between the northern zones (I and II), there is a critical latitude described around Mejillones and Moreno bays (~22.8°S; Letelier *et al.* 2012). Furthermore, each of these zones has discrete catch and spawning areas, where catches and biological activity (e.g. spawning) are almost non-existent.

The anchoveta fishery in Zone I is concentrated from Arica (18°S) to Antofagasta (26°S; Böhm *et al.* 2018). The spatial distribution of spawning in this area is concentrated in two recurring foci, one from Arica (18°24′S) to Punta Patache (21°S), with another secondary focus located between Copaca (22°20′S) and Mejillones (23°S; Angulo *et al.* 2018). Historically, landings have been lower in Zone II (Caldera–Coquimbo) than Zone I, concentrated primarily in Caldera (26–28°S) and Coquimbo (29–30°S; Böhm *et al.* 2018). In these areas, spawning is distributed from Chañaral (16°10′S) to Tongoy (30°10′S; Reyes *et al.* 2018). In Zone III, anchoveta are distributed from 36

Table 1. Number (*n*) of sagittal otoliths of the anchoveta (*Engraulis ringens*) used in morphological analysis, classified by ontogenetic stages, area and locality of origin off Chile, for the years 2016 and 2013

The range of fish length is included for reference. TL, total length

Ontogenetic stage	Zone			2016			2013	
			n	Length of fish (cm)		*n*	Length of fish (cm TL)	
				Minimum	Maximum		Minimum	Maximum
Juvenile	I	Arica–Iquique	50	6.5	11.5			
		Antofagasta	92	4.0	9.5			
	II	Caldera	103	9.0	11.5			
		Coquimbo	69	6.5	11.5			
	III	Valparaíso	126	2.0	9.5			
		Valdivia	49	2.0	6.0			
Adult	I	Arica–Iquique	51	12.0	14.0	32	14.0	14.5
		Antofagasta	46	12.0	14.0	18	14.0	14.5
	II	Caldera	53	12.0	14.0	20	14.0	14.5
		Coquimbo	55	12.0	14.0	30	14.0	14.5
	III	Valparaíso	45	12.0	14.0	21	14.0	14.5
		Valdivia	50	12.0	14.0	29	14.0	14.5

to 40°S (Aranis *et al.* 2018), and spawning occurs primarily between 35 and 40°S (Cubillos *et al.* 2017).

Sampling and preparation of otoliths

In all, 940 juveniles and adult anchoveta were collected between December 2015 and October 2016 in each of the three sampling zones (Table 1). Juvenile fish corresponded to specimens <12-cm total length (TL), sampled during the main recruitment period (December–February) from annual scientific surveys conducted during this period by the Chilean Instituto de Fomento Pesquero (IFOP). Samples of adult fish corresponded to individuals ≥12 cm TL, sampled during the main spawning period (August–October) from annual scientific surveys conducted during this period by IFOP. A complementary sample of adults was available from the 2013 annual reproductive survey and was included in the present study for an interannual comparison between 2016, a year with a strong El Niño–Southern Oscillation (ENSO), leading to an El Niño event, and a normal year (2013) without an El Niño event. TL to the nearest centimetre and total weight (g) were measured and sagittal otoliths were extracted under a stereomicroscope. Otoliths were cleaned in distilled water just after extraction, with any remaining tissue removed from the macula and vestibule using fine tweezers (Secor *et al.* 1992). Once cleaned, the otolith pairs were dried and kept dry in Eppendorf tubes.

Digital images of each left otolith were acquired using Image-Pro Plus software (Media Cybernetics, Bethesda, MD, USA) which also binarised the images and calculated otolith surface area, perimeter, Feret length (longest calliper length of the otolith) and Feret width (smallest calliper length of otolith). These morphometric variables were corrected to avoid any effects of fish size and the allometry that occurs during otolith growth (Lombarte and Lleonart 1993) using the method proposed by Lleonart *et al.* (2000), whereby the morphometric measures, used as dependent variables, were related to fish TL as an independent variable and then used to calculate five shape indices: form factor, roundness, circularity, rectangularity and ellipticity (Pothin *et al.* 2006). Finally, ~1000 Cartesian

coordinates of each otolith outline were extracted using the 'getaoipoints' macro in Image-Pro Plus and were exported to a text file for further elliptic Fourier analysis.

Analysis of otolith shape indices

Descriptive analyses of shape indices were organised into six length-interval groups linked to ontogenetic stages: two pre-recruit intervals of 4.0–5.5 and 6.0–7.5 cm TL; two recruit intervals of 8.0–9.5 and 10.0–11.0 cm TL; and two adults intervals, one from 2016 (13.5–14.0 cm TL) and another from 2013 (14.0–14.5 cm TL).

The relationship between shape indices and both sampling zone and fish length (as a covariate) was modelled and analysed through a generalised additive model (GAM) using the R package 'mgcv' (see https://cran.r-project.org/web/packages/mgcv; Wood 2006). Both Gaussian and gamma distribution models were compared using the Akaike information criterion (AIC; Akaike 1974) to select the most informative representation of the distribution data. Explanatory variable effects were then evaluated on the selected model through deviance analysis (Venables and Ripley 2002). The general equation of the evaluated models was as follows:

$$SI_i = \varphi\big(\beta_0 + s(TL_i) + Z_{ij}\big)$$

where φ is the link function that links the mean to the model, *SI* corresponds to the shape indices, β_0 is the intercept of model, *TL* is total fish length, *s* is the smoothing function and *Z* (zone) is a dummy variable that represents the origin of the sample with $j = 1,2$ and *i* is the *i*th fish.

Analysis of otolith outline

Elliptic Fourier analysis is a method that allows a closed curve to be described, characterised by equidistant (*x*,*y*) coordinates such as the otolith contour, through the infinite summation of ellipses with different amplitudes and angles. However, the objective of the analysis is to describe the otolith outline using the minimum

number of ellipses. Each ellipse of the Fourier analysis is called an elliptical Fourier descriptor (EFD) or simply a 'harmonic', because of its functional form (sines and cosines). Because the shape described by the EFDs is sensitive to the orientation, size and starting point of the ordered pairs (*x,y*), a normalisation process was performed following Kuhl and Giardina (1982). Although normalisation reduced bias in EFDs due to correlation with other morphological variables, such as fish length, in order to avoid any undesired effect of fish size on otolith outlines, the analyses were conducted according to fish size classes. Hence, separated elliptical Fourier analyses were performed for fish ranging in size from 6.5 to 9.5 cm TL in the case of juveniles and from 13.5 to 14.0 cm in the case of adults collected in 2016; for adults collected in 2013, EFDs were obtained for adult fish ranging in size from 14.0 to 14.5 cm TL. A total of 200 EFDs was initially calculated for each otolith according to the algorithm implemented by Claude (2008) in the R package (R Foundation for Statistical Computing, Vienna, Austria). After a power analysis was performed, only 20 harmonics were sufficient to explain 95% of the variance in otolith outlines. The first EFDs, which corresponded to the ellipse, used to normalise the data were discarded for further analyses.

Stepwise canonical discriminant analysis (CDA) was used to determine whether otoliths collected in different sampling zones could be distinguished based on the 20 selected EFDs. CDA is a standard method where data belonging to known groups (sampling zones) are used to find linear combinations of descriptors that maximise Wilks' lambda (λ; Ramsay and Silverman 2005; Pothin *et al.* 2006). Wilks' λ is the ratio between the intragroup variance and total variance, and provides an objective means of calculating the chance-corrected percentage of agreement between real and predicted group membership. Values of Wilks' λ range from 0 to 1: the closer λ is to 0, the better the discriminating power of the CDA (Lord *et al.* 2012).

Results

Basic otolith shape indices

There were differences in area, perimeter, Ferret length and Ferret width among length groups (ontogenetic state). Overall, shape indices showed a latitudinal gradient for all ontogenetic stages (i.e. the ellipticity and circularity were higher in Zone I and lower in Zones II and III). Conversely, rectangularity, form factor and roundness were higher in Zone III than in the other zones, at least for pre-recruits and recruits. In adults, mean shape indices showed closer values between Zones I and II than between each of these zones and Zone III (Table 2).

Roundness and form factor decreased as fish length increased, whereas the opposite was found for circularity and ellipticity, which tended to increase with fish length. GAM analysis of the relationship between shape indices and fish length (Year 2016) across sampling zones showed an overall trend towards a continuous change in otolith shape with fish length, at least until 14 cm TL. Within this general pattern, more pronounced changes with size were noticeable between 4 and 8 cm TL, and there was slight evidence of stabilisation between 8 and 10 cm TL (Fig. 2).

GAM deviance analysis showed sampling zone and fish length explained over 88% of the deviance in all shape indices

Table 2. Mean (±s.d.) values for basic morphometric variables and shape indices of sagittal otoliths of pre-recruits, recruits and adults of the anchoveta (*Engraulis ringens*) by sampling zone
Zone I, Arica–Antofagasta; Zone II, Caldera–Coquimbo; Zone III, Valparaíso–Valdivia. TL, total length

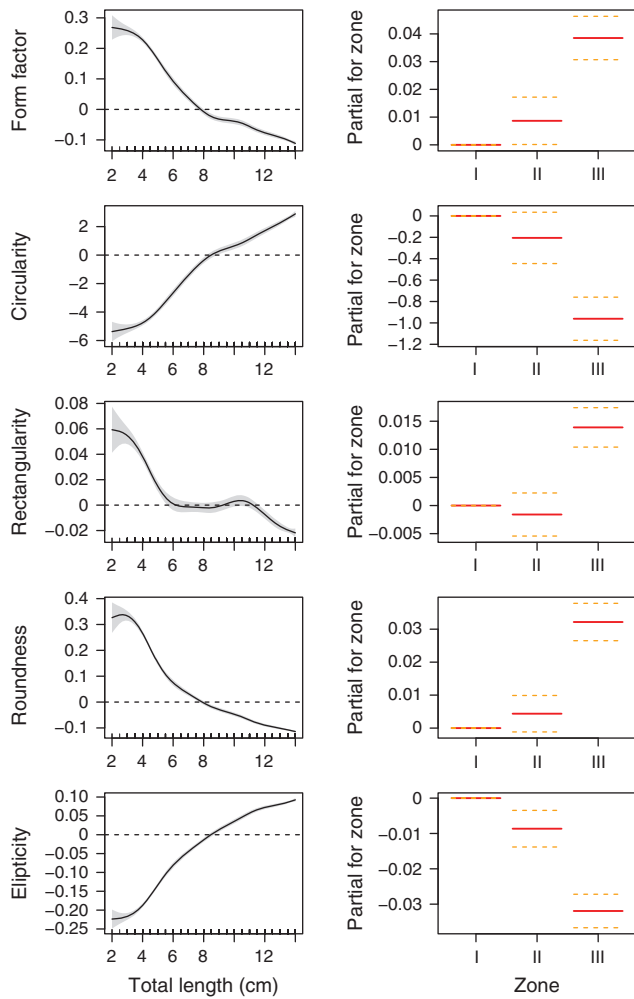| Ontogenetic stage	Length range (cm TL)	Zone	Area	Perimeter	Feret length	Feret width	Ellipticity	Rectangularity	Circularity	Form factor	Roundness
Pre-recruit 1	4.0–5.5	I	2.60 ± 0.25	7.21 ± 0.40	2.93 ± 0.19	1.30 ± 0.05	0.44 ± 0.04	0.68 ± 0.01	20.04 ± 0.67	0.63 ± 0.02	0.39 ± 0.02
		II									
		III	1.49 ± 0.46	5.21 ± 0.78	2.04 ± 0.34	1.01 ± 0.12	0.28 ± 0.06	0.71 ± 0.02	18.51 ± 0.88	0.68 ± 0.03	0.45 ± 0.03
Pre-recruit 2	6.0–7.5	I	2.43 ± 0.21	7.04 ± 0.38	2.80 ± 0.15	1.27 ± 0.06	0.42 ± 0.03	0.68 ± 0.01	20.45 ± 0.92	0.62 ± 0.03	0.39 ± 0.02
		II	2.56 ± 0.47	7.13 ± 0.78	2.84 ± 0.32	1.32 ± 0.11	0.40 ± 0.03	0.68 ± 0.02	19.99 ± 1.01	0.63 ± 0.03	0.40 ± 0.02
		III	2.08 ± 0.48	6.12 ± 0.97	2.44 ± 0.41	1.18 ± 0.15	0.37 ± 0.06	0.71 ± 0.01	18.27 ± 1.04	0.69 ± 0.04	0.44 ± 0.04
Recruit 1	8.0–9.5	I	2.10 ± 0.23	6.56 ± 0.41	2.57 ± 0.18	1.18 ± 0.06	0.40 ± 0.03	0.69 ± 0.01	20.58 ± 0.86	0.61 ± 0.03	0.40 ± 0.02
		II	2.33 ± 0.36	6.81 ± 0.67	2.71 ± 0.27	1.23 ± 0.10	0.39 ± 0.03	0.70 ± 0.02	19.95 ± 1.30	0.63 ± 0.04	0.40 ± 0.03
		III	1.81 ± 0.17	5.83 ± 0.31	2.30 ± 0.14	1.10 ± 0.06	0.37 ± 0.03	0.71 ± 0.01	18.83 ± 0.80	0.67 ± 0.03	0.44 ± 0.03
Recruit 2	10.0–11.5	I	1.95 ± 0.13	6.32 ± 0.19	2.45 ± 0.02	1.14 ± 0.10	0.37 ± 0.04	0.70 ± 0.02	20.43 ± 0.11	0.62 ± 0.00	0.41 ± 0.02
		II									
		III	1.96 ± 0.21	6.2 ± 0.4	2.5 ± 0.2	1.1 ± 0.1	0.37 ± 0.02	0.71 ± 0.02	19.4 ± 1.3	0.65 ± 0.04	0.41 ± 0.02
Adults 2016	13.5–14.0	I	1.91 ± 0.14	5.97 ± 0.29	2.39 ± 0.10	1.13 ± 0.05	0.32 ± 0.02	0.71 ± 0.02	18.70 ± 1.33	0.68 ± 0.05	0.43 ± 0.02
		II	1.76 ± 0.15	5.84 ± 0.27	2.31 ± 0.13	1.10 ± 0.05	0.32 ± 0.02	0.69 ± 0.02	19.41 ± 1.14	0.65 ± 0.04	0.42 ± 0.03
		III	1.76 ± 0.19	5.8 ± 0.3	2.3 ± 0.1	1.10 ± 0.07	0.31 ± 0.02	0.70 ± 0.02	19.47 ± 1.18	0.65 ± 0.04	0.44 ± 0.03
Adults 2013	14.0–14.5	I	1.68 ± 0.12	5.55 ± 0.22	2.23 ± 0.08	1.07 ± 0.04	0.31 ± 0.01	0.70 ± 0.02	18.37 ± 1.25	0.69 ± 0.05	0.43 ± 0.02
		II	1.58 ± 0.13	5.46 ± 0.28	2.16 ± 0.10	1.04 ± 0.05	0.31 ± 0.02	0.70 ± 0.02	18.97 ± 1.48	0.67 ± 0.05	0.43 ± 0.03
		III	1.59 ± 0.11	5.5 ± 0.3	2.1 ± 0.1	1.06 ± 0.05	0.30 ± 0.02	0.71 ± 0.02	19.09 ± 1.93	0.66 ± 0.06	0.45 ± 0.02

Fig. 2. Left-hand panels show generalised additive models for form factor, roundness, ellipticity, circularity and rectangularity as a nonlinear function of the total length of the anchoveta (*Engraulis ringens*) for Zone I (Arica–Antofagasta), Zone II (Caldera–Coquimbo) and Zone III (Valparaíso–Valdivia) off the coast of Chile. In the left-hand panels, the solid black line shows the tendency of partial residual of dependent variable (smooth term) against the fish length as predictor variable, and the grey shaded areas indicate the 95% confidence interval. Panels on the right show the standardised estimated effect, partial residual of dependent variable, for each level of factor zone. In the right-hand panels, the solid line shows the mean value of standardised dependent variable to each level factor zone and the dashed line shows the 95% confidence interval.

except rectangularity, for which these parameters only explained 62.8% of deviance (Table 3). Considering fish length covariance, there were significant differences for all indices between sampling Zones I and III, as well as between Zones II and III. However, significant differences between Zones I and II were limited to form factor and ellipticity.

Otolith outline

A two-function discriminant model fit using otolith EFDs of juvenile fish (6.5–9.5 cm TL) explained 79 and 21% of variance, with canonical correlations of 0.88 and 0.69 respectively. Only

Table 3.   Deviance analysis from generalised additive models for each shape index by sampling zone
Zone I, Arica–Antofagasta; Zone II, Caldera–Coquimbo; Zone III, Valparaíso–Valdivia

Indices factor	Estimate	s.e.	t-value	P-value
Form factor				
Intercept	0.684	0.003	245.29	$<2 \times 10^{-16}$
Zone II	0.009	0.004	2.03	0.043
Zone III	0.039	0.004	9.85	$<2 \times 10^{-16}$
log likelihood	1427.9			
Percentage deviance	91.1			
Circularity				
Intercept	1.892	0.076	248 892.00	$<2 \times 10^{-16}$
Zone II	−0.205	0.120	−1714.00	0.087
Zone III	−0.961	0.101	−9531.00	$<2 \times 10^{-16}$
log likelihood	−1165.2			
Percentage deviance	88.8			
Rectangularity				
Intercept	0.705	0.001	567 309.00	$<2 \times 10^{-16}$
Zone II	−0.002	0.002	−1051.00	0.293
Zone III	0.013	0.002	7558.00	1.2×10^{-13}
log likelihood	2058.3			
Percentage deviance	62.8			
Roundness				
Intercept	0.466	0.002	235 403	$<2 \times 10^{-16}$
Zone II	0.004	0.003	1578	0.115
Zone III	0.032	0.003	11 356	$<2 \times 10^{-16}$
log likelihood	1666.2			
Percentage deviance	94.8			
Ellipticity				
Intercept	0.334	0.002	197.86	$<2 \times 10^{-16}$
Zone II	−0.009	0.003	−3.34	0.001
Zone III	−0.032	0.002	−13.49	$<2 \times 10^{-16}$
log likelihood	1824.933			
Percentage deviance	95.2			

Table 4. Classification matrix of sagittal otoliths of juvenile anchoveta collected in 2016
Bold numbers indicate the numbers and percentage of fish correctly classification to the origin zone. Zone I, Arica–Antofagasta; Zone II, Caldera–Coquimbo; Zone III, Valparaíso–Valdivia

Classification procedure	Output format	Zone	Predicted group			Total
			I	II	III	
Original	Count	I	**74**	10		84
		II	5	**35**	1	41
		III	1	1	**41**	43
	Percentage	I	**88.1**	11.9		100
		II	12.2	**85.4**	2.4	100
		III	2.3	2.3	**95.3**	100
Cross-validation	Count	I	**64**	13	7	84
		II	10	**26**	5	41
		III	3	6	**34**	43
	Percentage	I	**76.2**	15.5	8.3	100
		II	24.4	**63.4**	12.2	100
		III	7.0	14.0	**79.1**	100

the first discriminant function presented a significant value for Wilks' λ of 0.117 ($P < 0.0001$). The lower Wilks' λ of the second function (0.521; $P < 0.2$), suggested overlap between zones regarding this function. These canonical discriminant functions produced an accurate self-classification of 89% of samples, withe accurate classification in 95% of cases for Zone III. The total percentage of correct classifications of cross-validated grouped cases in their original group was 74%, and >76% in Zones I and III (Table 4; Fig. 3a).

The two-function discriminant model fit using otolith EFDs of 2016 adults (13.5–14.0 cm TL) explained 67 and 33% of variance, with canonical correlations of 0.78 and 0.57 respectively. The first discriminant function was significant, with Wilks' λ of 0.222 ($P < 0.000$). The Wilks' λ of the second function was higher, at 0.568, at the edge of significance ($P < 0.053$). Classification reached an overall accuracy of 85%, with all sampling zones exhibiting crude accuracies >81%. The total percentage of correct classifications of cross-validated grouped cases in their original group was 64%, and 68% in Zone III (Table 5; Fig. 3b).

Discriminant functions for 2013 adults explained 63 and 37% of the variance, with canonical correlations of 0.83 and 0.76 respectively. Wilks' λ for the first discriminant function (0.130) was highly significant ($P < 0.000$), whereas the second function showed a much lower Wilks' λ value (0.425; $P = 0.072$), indicating a greater overlap between sampling zones. The accuracy for 2013 adults reached 90% of all samples, with all sampling zones reaching accuracies >87%. The total percentage of correct classifications of cross-validated grouped cases in their original group was 66%, and 70% in Zone III (Table 6; Fig. 3c).

Fourier reconstructed outlines (from the first 20 EFDs) for the six different ontogenetic stages suggested important differences among sampling zones (Fig. 4).

Discussion

For a long time, the anchovy fishery in Chile was managed using a purely administrative criterion without knowing the population structure of the species. In this context, the present study has made a substantive contribution, identifying at least two completely independent demographic units (I and III) of
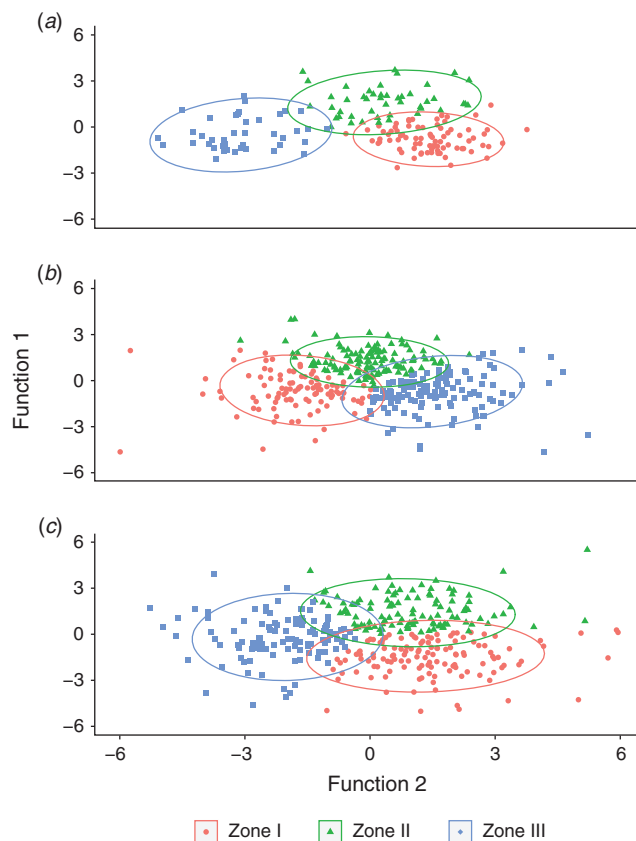

Fig. 3. Scatterplots of the first and second discriminant function scores for Fourier coefficients of sagittal otolith outlines of anchoveta (*Engraulis ringens*) for (a) juveniles in 2016, (b) adults in 2016 and (c) adults in 2013. Zone I, Arica–Antofagasta; Zone II, Caldera–Coquimbo; Zone III, Valparaíso–Valdivia.

anchoveta (*E. ringens*) off the Chilean coast through of otolith shape analysis, where both basic shape indices and EFDs showed consistent results.

Table 5.   Classification matrix of sagittal otoliths of adult anchoveta collected in 2016

Bold numbers indicate the numbers and percentage of fish correctly classification to the origin zone. Zone I, Arica–Antofagasta; Zone II, Caldera–Coquimbo; Zone III, Valparaíso–Valdivia

Classification procedure	Output format	Zone	Predicted Group			Total
			I	II	III	
Original	Count	I	**59**	8	3	70
		II	5	**61**	9	75
		III	4	3	**58**	65
	Percentage	I	**84.3**	11.4	4.3	100
		II	6.7	**81.3**	12.0	100
		III	6.2	4.6	**89.2**	100
Cross-validation	Count	I	**45**	17	8	70
		II	19	**45**	11	75
		III	10	11	**44**	65
	Percentage	I	**64.3**	24.3	11.4	100
		II	25.3	**60.0**	14.7	100
		III	15.4	16.9	**67.7**	100

Table 6.   Classification matrix of sagittal otoliths of adult anchoveta collected in 2013

Bold numbers indicate the numbers and percentage of fish correctly classification to the origin zone. Zone I, Arica–Antofagasta; Zone II, Caldera–Coquimbo; Zone III, Valparaíso–Valdivia

Classification procedure	Output format	Zone	Predicted Group			Total
			I	II	III	
Original	Count	I	**45**	5		50
		II	3	**44**	3	50
		III	1	3	**46**	50
	Percentage	I	**90.0**	10.0	0.0	100
		II	6.0	**88.0**	6.0	100
		III	2.0	6.0	**92.0**	100
Cross-validation	Count	I	**33**	9	8	50
		II	12	**31**	7	50
		III	6	9	**35**	50
	Percentage	I	**66.0**	18.0	16.0	100
		II	24.0	**62.0**	14.0	100
		III	12.0	18.0	**70.0**	100

— Zone I (Arica–Antofagasta)
– – Zone II (Caldera–Coquimbo)
· – · – · Zone III (Valparaíso–Valdivia)

Prerecruit (4.0–5.5 cm TL)   Prerecruit (6.5–7.5 cm TL)   Recruit (8.0–9.5 cm TL)   Recruit (10.0–11.0 cm TL)   Adult 2013 (14.0–14.5 cm TL)   Adult 2016 (13.5–14.0 cm TL)
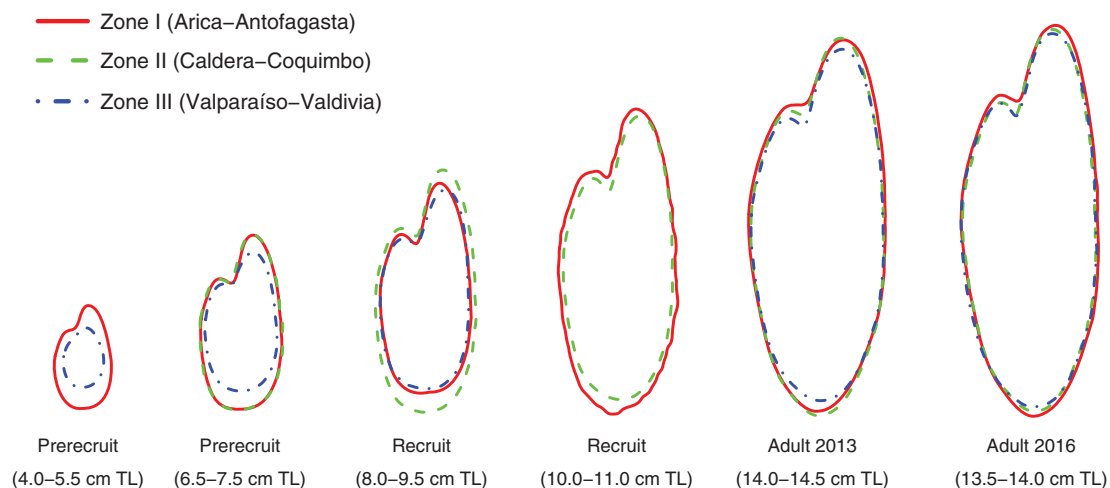
Fig. 4.   Mean otolith shapes from elliptical Fourier coefficients for four length ranges, representing three ontogenetic stages of anchoveta (*Engraulis ringens*). Shape indices were organised into six length interval groups linked to ontogenetic stages: two prerecruit intervals of 4.0–5.5- and 6.0–7.5-cm total length (TL); two recruit intervals of 8.0–9.5 and 10.0–11.0 cm TL; and two adults intervals, one from 2016 (13.5–14.0 cm TL) and another from 2013 (14.0–14.5 cm TL).

These results match with the stocks or management units used today, which support stock assessment of this species based on separated stocks. However, the lower discriminating power between Zones I and II suggests some level of mixing between these zones, which should be elucidated in further studies in order to specify the exact limits between populations to improve the management of this resource. Some of the characteristic features associated with these new findings are discussed below.

Discriminatory capacity of basic otolith shape indices and EFDs

Otolith shape indices (form factor, circularity, rectangularity, roundness, ellipticity) showed high variability and significant differences among sampling zones. Although the magnitude of these morphological differences tended to increase with age and size, we found evidence indicating the effects of fish length on shape indices decreased with size, leading to relative stability in morphological differences among sampling zones after fish reached sexual maturity (~14 cm TL). Such a condition has been reported for the clupeoid *Strangomera bentincki* by Curin-Osorio *et al.* (2012), as well as for other species (e.g. Tuset *et al.* 2003). Consequently, evaluation of otolith shape stability is important to determine when the otolith outline is not affected by fish ontogeny, but rather by genetic and environmental variability. Furthermore, it would be very interesting to evaluate whether the pattern of otolith shape stability found in *E. ringens* in the present study is also applicable to other engraulid species. For example, Zengin *et al.* (2015) and Jemaa *et al.* (2015) identified population units of *Engraulis encrasicolus* in the Black and Mediterranean seas respectively, although in both these studies only immature fish (11–12 cm TL) were used, when the shape or otolith outline has not completely yet. In this sense, it is reasonable to infer that results derived from otolith shape analysis of adult fish in the present study are reliable.

A distinctive finding of the present study was the significant discriminatory capacity of EFDs to distinguish anchoveta from the three localities, even when the analysis was conducted in two different years (i.e. 2013 *v.* 2016). These results support previous findings where a high discriminatory capacity of EFDs has been reported in several species of teleost fishes (i.e. Popper *et al.* 2005; Brophy *et al.* 2016; Afanasyev *et al.* 2017; Duncan *et al.* 2018). Moreover, similar original classification accuracies (>81%) were found for *E. encrasicolus* when this technique was used to differentiate four populations in the Mediterranean Sea (Jemaa *et al.* 2015). These are promising results for fisheries resource monitoring purposes, because otolith shape is a cost-effective technique that would continuously be updated and enhanced because of advances in image-based platforms.

A further finding of the present study was that Fourier reconstructed otolith outlines showed differences between zones that appear to be larger for sampling Zone III (Valparaíso–Valdivia), where at the same fish length otoliths were smaller than those sampled in Zones I and II, at least at the pre-recruit stage (<8 cm TL). The smaller and rounder otoliths of Zone III could be associated with growth differences among juveniles, because a recent study reported much slower growth of pre-recruits in southern (zone III) than northern zones (zone I) (Cerna and Plaza 2015).

Population structure and spatial segregation of anchoveta

The marked differences found in otolith morphology between the two extreme localities in the present study are strong evidence of an environmental spatial heterogeneity of anchoveta along the Chilean coast. This evidence matches well with previous studies, which have proposed that environmental variability can produce important differences in otolith shape (Lombarte and Lleonart 1993; Cardinale *et al.* 2004; Vignon 2012). Indeed, juveniles and adult fish inhabiting the extreme northward and southward zones analysed for anchoveta in this study must cope with important environmental differences in biological productivity (e.g. chlorophyll-*a*), upwelling intensity, temperature and both physical and topographic changes. An important feature here is the CTZ defined between 34 and 39°S (Morales *et al.* 2010). Although the northern zones (Zones I and II) do not have a strict topographic boundary between them, there is a critical latitude (~22.8°S) described around Mejillones and Moreno bays that has higher concentrations of chlorophyll throughout the year and stronger water retention dynamics related to local topography and particular Ekman transport characteristics of adjacent waters (Letelier *et al.* 2012). Hence, it is reasonable to hypothesise that this critical latitudinal zone could enhance retention of early stages of anchoveta and limit the flow of early juveniles between Zones I and II.

Phenotypic differences found among sampling zones were large and consistent between years and through ontogenetic stages, which is indicative of a prolonged and demographically relevant separation among units (Reiss *et al.* 2009). However, these differences do not provide direct evidence of genetic (i.e. nearly complete) isolation between sampling areas. Nonetheless, although classical genetic studies performed for this area and species have failed to reject the conventional hypothesis of panmixia (Galleguillos *et al.* 1996; Ferrada *et al.* 2002; Rojas 2011), new evidence from Ferrada *et al.* (2018) suggests progressive differentiation with distance and may change the current view regarding the population genetics of anchoveta along the Humboldt Current. In this sense, in the present study, otolith-based morphometric differences were larger for sampling Zone III (Valparaiso–Valdivia), than for samples from Zones I and II. The high differences between Zone III and both Zones I and II suggest that genetic or environmental differences are greater between these areas, but lower between Zones I and II, which is consistent with the geographic distribution of the species (Castillo *et al.* 1998; Leiva *et al.* 2016), as well as with previous parasitological studies (Valdivia *et al.* 2007; George-Nascimento *et al.* 2011).

The similar classification accuracies found in 2013 and 2016 provide the first evidence that otolith morphological differences among zones were not sensitive to the El Niño event, which dominated the oceanographic conditions during 2014–16 in the Southern Hemisphere (Vera and Osman 2018). This finding suggests the absence of a generalised movement and mixing of schools in a southward direction, which could be expected as a result of movement of warm water masses from north to south. In fact, latitudinal stability in fish distribution under El Niño events has been observed in recruiting acoustic cruises (Castillo *et al.* 1998; Leiva *et al.* 2016), where a change in bathymetric distribution seems to be the dominant response to warm water

conditions due to the El Niño event, where anchoveta move to deeper waters and are less vulnerable to purse seine fishing gear.

The existence of clear otolith-based morphological differences in both juvenile and adult fish among the two extreme localities supports the hypothesis that individuals have remained segregated throughout the entire (or at least a fraction of) their life cycle, supporting the increasing scientific evidence over the past two decades of homing in marine fishes (e.g. Thorrold *et al.* 2001; Rooker *et al.* 2008; Sólmundsson *et al.* 2015). Hence, further studies focused on extending both the spatial and temporal scale, as well as incorporating additional ecological markers (e.g. otolith microchemistry and microstructure) would provide additional insights to reveal homing and the level of mixing, if any, in this important fishery resource in the Humboldt Current system.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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