

Research Article

Otolith shape analysis as a tool for stock identification of the southern blue whiting, *Micromesistius australis*

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ABSTRACT. The southern blue whiting, *Micromesistius australis* (Norman, 1937), is an important demersal resource associated with the slope and continental shelf of southern Chile, Argentina and the Malvinas/Falkland Islands. Recent studies have reported schools of adult fish from Atlantic waters migrating along the southern Chilean coast in mid-winter, moving northwards to spawn in August (47°-51°S), and then returning to Atlantic waters, presumably to feed. The migratory pattern suggests the presence of one or more stock units associated with the South American coast. In the present study, “otolith morphometry” is used to determine the stock structure of *M. australis* based on applications of basic size descriptors (SDs) (area, perimeter and otolith size), shape indices (SIs) (circularity, squareness, shape factor, roundness, ellipticity), and normalised elliptical Fourier descriptors (NEFDs). Samples were collected during the winter and spring of 2010, during the reproductive period, in the economic zone of southern Chile (36°-57°S), in the Pacific Ocean and around the Falkland Islands economic zone (50°-52°S) in the Atlantic Ocean. Analyses were conducted to include the effects of size, sex and age. A stepwise canonical discriminant analysis showed that fish were successfully discriminated with SDs, SIs and NEFDs. In this analysis, 86.4% and 70.1% of the fish were correctly classified as belonging to the Atlantic and Pacific stocks, respectively. A multivariate analysis of variance showed that the mean values of the NEFDs, SDs, and SIs did not vary significantly between sexes within areas ($P > 0.05$), but varied significantly between the Pacific and Atlantic oceans ($P < 0.05$). These results highlighted that otolith shape analysis can be a useful tool to evaluate the potential level of mixing in feeding areas where both stocks, the Pacific and Atlantic units, are expected to co-occur.

Keywords: otolith morphometry, demersal fish, southern blue whiting, *Micromesistius australis*, stock identification.

Análisis morfométrico de los otolitos como herramienta para la identificación de stock de la merluza de tres aletas, *Micromesistius australis*

RESUMEN. La merluza de tres aletas, *Micromesistius australis* (Norman, 1937), es un importante recurso demersal asociado al talud y plataforma continental en la región sur-austral de Chile, Argentina e islas Malvinas. Estudios recientes han reportado la existencia de cardúmenes de peces adultos procedentes de aguas atlánticas migrando hacia el extremo sur de la costa chilena a mediados de año, desplazándose al norte, en agosto (47°-51°S), para desovar, retornando posteriormente a aguas atlánticas, presumiblemente para alimentarse. El patrón migratorio plantea la presencia de una o más unidades de stock asociadas al cono sur de América. En el presente estudio se utiliza la “morfometría de otolitos” para determinar la estructura del stock, basada en parámetros básicos de tamaño (área, perímetro y tamaño), índices de forma (circularidad, rectangularidad, factor de forma, redondez y elipticidad), y descriptores elípticos de Fourier normalizados (NEFDs). Se analizaron dos zonas geográficas: océanos Pacífico y Atlántico. Las muestras fueron colectadas en primavera e invierno de 2010, durante el período reproductivo en el sur de Chile (36°-57°S), y alrededor de la zona económica exclusiva de las islas Falkland (50°-52°S). El análisis se efectuó considerando el efecto de tamaño, sexo y edad. Se realizó un análisis discriminante canónico tipo inclusión por pasos y se diseñó una función de clasificación, con una discriminación exitosa de 86,4% y 70,1% para los océanos Atlántico y Pacífico, respectivamente. Se realizó un análisis de varianza multivariado (MANOVA), que indicó que los valores medios de NEFDs, SDs y SIs no variaron significativamente entre sexos dentro de las áreas ($P > 0,05$),

pero variaron significativamente entre ambas zonas ($P < 0,05$). Los resultados mostraron que el análisis de la forma de otolitos puede ser utilizado para evaluar niveles potenciales de mezcla en áreas de alimentación donde concurren ambas unidades de stock.

Palabra clave: morfometría de otolitos, pez demersal, merluza de tres aletas, *Micromesistius australis*, identificación de stock.

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INTRODUCTION

The concept of stock in fisheries science has historically been defined in different ways, but the genetic and operational definitions are used most often. The genetic definition states that a stock is a reproductively isolated unit that is genetically different from other stocks (Carvalho & Hauser, 1994). The operational definition was proposed by Begg & Waldman (1999) and states that a stock is a semidiscrete group of fish with definable attributes of interest to managers. In a population with non-detectable genetic differences, it might be possible to find discrete separated units (*i.e.*, stocks), having their own life history characteristics (*i.e.*, growth, mortality, reproduction), and showing a different response to fishery exploitation (Gauldie, 1988; Begg & Waldman, 1999). Hence, it is crucial to identify these units, including their distribution, migration, spawning areas, and temporal and spatial degree of overlap with other stocks. Indeed, the reliability of stock assessments and therefore the effectiveness of fishery management are severely limited for many principal fishery resources because stock structure and delineation are uncertain (Waldman, 2007).

In recent years, otolith shape analyses have been shown to be promising tools for stock identification (Campana & Casselman, 1993; Begg & Brown, 2000; Tuset *et al.*, 2003). To date, the simpler otolith shape analyses are based on the comparison of linear distances among otolith shapes. More recent procedures use geometric outline methods to quantify the boundary shapes of otoliths, so that patterns of shape variation within and among groups are evaluated (Cadrin & Friedland, 1999; Cadrin *et al.*, 2005). At present, the most common outline methods involve Fourier analysis (Campana & Casselman, 1993), and elliptical Fourier series analysis to describe the shape of otoliths (Kuhl & Giardina, 1982; Stransky *et al.*, 2008a, 2008b). If this discrimination is statistically significant, otolith shape analysis proves to be more advantageous as a monitoring tool, particularly to detect mixing levels in common fishery areas, because of its lower cost and easier preparation

procedures. This advantage of otolith shape analysis appears to hold for the southern blue whiting, *Micromesistius australis*, in which mixing is expected to occur.

The southern blue whiting is a commercially important demersal species associated with sub-Antarctic waters (Saavedra *et al.*, 2012). To date, two populations, viewed as two separate subspecies, have been described. *M. australis pallidus* occurs in waters around New Zealand, and *M. australis* is found in the southwest Atlantic and southeast Pacific oceans (Ryan *et al.*, 2002). *M. australis* occurs primarily from 38°S to Burwood Bank and the Scotia Sea in the south. North of 47°S, *M. australis* is confined to the shelf break and slope and is primarily associated with the Subantarctic and Antarctic Intermediate water masses. In the Falkland-Patagonian region, *M. australis* primarily occurs near the bottom. In the southeast Pacific, *M. australis* occurs along the continental shelf between 24°26'S and 57°00'S at depths from 150 to 700 m (Céspedes *et al.*, 1998). In both the southern Atlantic and southern Pacific, the activity of the *M. australis* fishery is linked to the patterns of the reproductive migration to its spawning grounds. In the southwest Atlantic, the principal spawning ground is located south and southwest of the Falkland Islands. In the southeast Pacific, the spawning areas are located primarily around the Penas gulf (Pájaro & Macchi, 2001). On both spawning grounds, the reproductive period appears to extend from August through November (Macchi & Pájaro, 1999).

Despite the known activity of the *M. australis* fishery, in both of these regions of the Pacific and Atlantic oceans, the stock structure of the species is still controversial. Certain authors have hypothesized the existence of only one stock with two separate spawning grounds and with a unique feeding area around the Scotia Sea, where the fish feed primarily on krill during the summer and beginning of autumn. Recent studies have shown the genetic homogeneity of *M. australis* from Chile and the Falkland Islands (Shaw, 2005). Late in the autumn, *M. australis* initiates a migration to its spawning areas in both the southwest Atlantic and southeast Pacific oceans. This

hypothesis requires a high level of homing ability. Recently, Arkhipkin *et al.* (2009) determined, using the otolith microchemistry and the concentrations of four elements (Li, Mg, Sr and Ba), that approximately 80% of the adult fish have a strong affinity with areas of origin, their spawning grounds on the Pacific or Atlantic side. Moreover, that study showed that the Chilean spawning population includes approximately four-fifths of the native fish and approximately one-fifth of migrants from the Atlantic, and vice-versa. Similarly, Niklitschek *et al.* (2010) used parasite assemblages in conjunction with otolith microchemistry to resolve population substructure on this species, demonstrating the existence of at least two ecologically distinct sub-populations of southern blue whiting in South America. Hence, it appears that the stock structure of this species is characterized by a high degree of homing but also reflects a certain amount of mixing in the Scotia Sea. Moreover, the amount of mixing could vary among different years. Under this scenario, it is advisable to evaluate the performance of stock identification methods that can be applied with relative ease to the monitoring process. As otoliths are commonly collected as part of the stock assessment protocols for *M. australis*, a shape analysis of the otoliths would provide a useful tool for monitoring mixing levels in this species. Therefore, the aim of the current study was to determine the stock structure of southern blue whiting, *M. australis*, with otolith shape analysis. For this purpose, sagittal otoliths collected from spawning areas southwest of Chile (36°S to 57°S) and around the Falkland Islands (50°S to 52°S) in the Atlantic Ocean, were compared using basic shape descriptors and shape indices and normalised elliptical Fourier analysis.

MATERIALS AND METHODS

Study area and image-capture procedures

Samples of adult southern blue whiting, *M. australis*, were collected from commercial and research midwater trawls in two geographic areas: (i) in the southwest Atlantic around the Falkland Islands (50°-52°S) and (ii) in the southeast Pacific off the shelf and slope of the Chilean coast (36°-57°S) (Fig. 1). The collections were performed during the reproductive period (June-October) to minimise the mixing effects of fish migration between spawning areas. To reduce the effects of ontogeny, the analysis was performed on a restricted range of fish lengths that included only fish between 50 and 60 cm in total length (Fig. 2). Overall, 239 pairs of sagittal otoliths were available for analysis. In this sample of otoliths, 107 (47 males

and 60 females) and 132 (86 males and 46 females) corresponded to the Pacific and Atlantic areas, respectively. To maintain consistency and avoid asymmetric effects, only the left sagittal otolith was used for otolith shape analysis, whereas the right otolith was stored as a replacement sample. Digital images of otoliths were obtained following the recommendations of Stransky *et al.* (2008a), to minimise the distortion error. Each otolith was placed on a Petri capsule on a dark background and digitized with a high-resolution video camera connected to a PC with an Image Analysis System (Olympus model SZ-61; Fig. 3).

Basic morphometric characterisation of sagittal otoliths

The basic size descriptors (SDs) (area, perimeter, maximum and minimum feret) and shape indexes (SIs) (circularity, shape factor, rectangularity, roundness, and ellipticity) were obtained with Image Pro-plus software based on specified mathematical equations (Table 1). A black mask was used for the otolith, and a white background was used to improve the contour. Image noise was manually removed.

Elliptical Fourier descriptors (EFDs)

EFDs (Kuhl & Giardina, 1982) can outline any type of shape with a closed two-dimensional contour. In the present paper, SHAPE software (Iwata & Ukai, 2002) was used to calculate EFDs. First, the Chain Coder package was used to convert the black-masked image into a binary image to extract its contour. Then, the software was used to generate 11 harmonics for each individual. Each harmonic was composed of four coefficients. This analysis initially produced 44 coefficients per individual. Each otolith was then normalised through the first harmonic for size and orientation. This calculation resulted in the elimination of the first three coefficients (a_0 , b_0 , and c_0). The fourth coefficient (d_0) was also deleted to yield a balanced multivariate statistical analysis of 40 coefficients for use in the shape analysis.

Multivariate analysis of variance (MANOVA)

A MANOVA was used to test for significant differences in normalized elliptical Fourier descriptors (NEFDs), and size descriptors (SDs), between sexes and geographic spawning areas. Only the NEFDs and SDs that met the assumptions of normality and homoscedasticity were included in the MANOVA analysis. The following MANOVA model was used:

$$MO_{ijk} = \mu + area_i + Sex_j + (area * sex)_{ij} + \varepsilon_{ijk}$$

In this model, MO_{ijk} : response vector containing the morphometric and harmonic variables for the

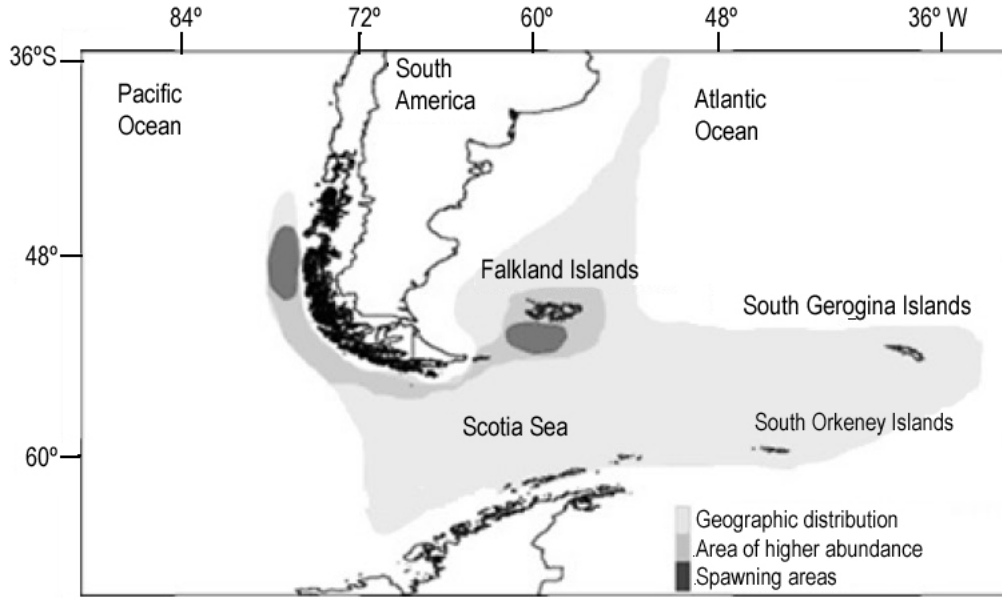


Figure 1. Geographic distribution of the southern blue whiting *Micromesistius australis* in South America, illustrating the location of the Atlantic and Pacific spawning areas.

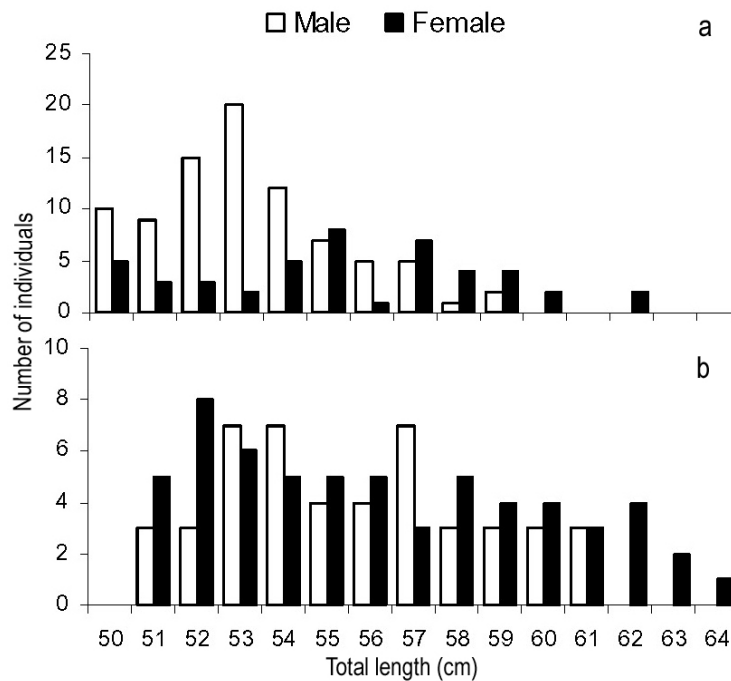


Figure 2. Frequency distribution (number of individuals) of total length (cm) of the southern blue whiting, *Micromesistius australis*, collected from winter through spring 2010 in the Atlantic Ocean (a) and Pacific Ocean (b).

otolith; μ : overall mean; $area_i$: main effect caused by sampling area ($i = 1, 2$); sex_j : main effect caused by sex in each sampling area ($j = 1, 2$); $(area*sexo)_{ij}$: interaction effect between sampling area and sex; ϵ_{ijk} : random error.

The response vector contained 14 dependent morphometric variables Y_{ijk} . Seven of these variables (c2, c5, c6, c8, d3, d6, d7) were NEFDs, and seven were SDs. $Area_i$ and Sex_j were fixed factors. $Area_i$, the sampling area, has two levels (the Atlantic and Pacific

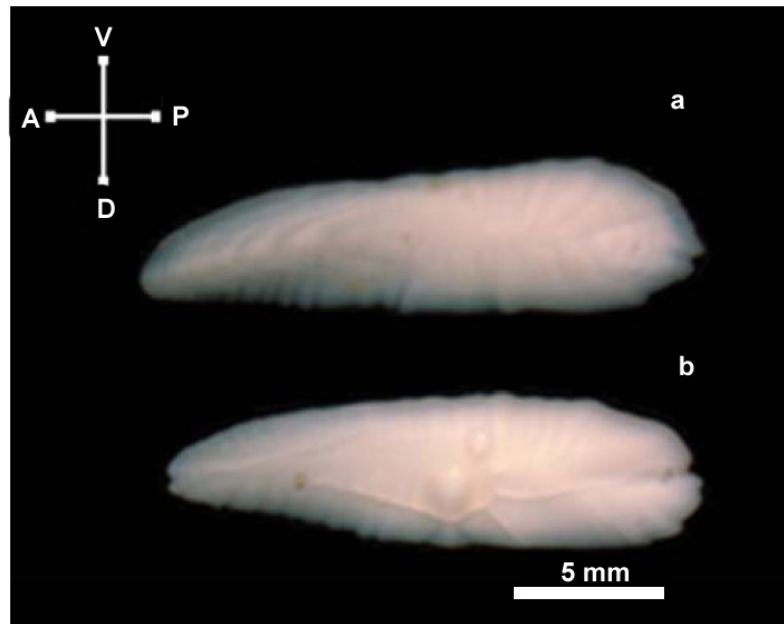


Figure 3. Illustration of left sagittal otoliths taken at 5x magnification. Otoliths from two 52 cm TL females collected in the Atlantic a), and Pacific b), spawning areas. V: ventral, D: dorsal, A: anterior, P: posterior.

Table 1. Nomenclature used to identify basic size descriptor (SD) and shape indexes for stock identification of the southern blue whiting *Micromesistius australis*.

Size descriptor	Shape indexes
Area (A)	Circularity = P^2/A
Perimeter (P)	Squareness = $A / (Fl * Fw)$
Feret weight (Fw)	Form-Factor = $(4\pi * A) / P^2$
Feret length (Fl)	Roundness = $(4A) / (\pi * Fl^2)$
	Ellipticity = $(Fl - Fw) / (Fl + Fw)$

areas). Sex_j also has two levels (male and female). The interaction effect, (Area x Sex)_{ij}, represents the interaction between the two fixed factors. The Kolmogorov-Smirnov (K-S) and Levene tests were used to test for normality and homogeneity of variance, respectively.

Canonical discriminant analysis (CDA)

A CDA was performed to determine a multiple classification function with geographic area as a categorical dependent variable. The independent variables included the NEFDs that met the assumptions of normality and homoscedasticity and the SDs corrected for the effect of otolith length, with the common within-group slope (Cardinale *et al.*, 2004). A forward stepwise CDA was used to detect

differences in otolith morphometrics and harmonics among individuals from the Atlantic and Pacific spawning areas, using the statistical package SPSS 17. Cross-validation procedures were used to calculate an unbiased estimate of classification success. Three trials were conducted to verify the discriminatory power of morphometric variables (Table 3). The first one was performed using the standardized residuals of the size descriptors, also including a shape index. The second trial only included the NEFDs, whereas the third trial (termed global) contained NEFDs, standardized residuals of size descriptors and a morphometric index.

RESULTS

General morphological characterisation of sagittal otoliths

Sagittal otoliths of *M. australis* from the Pacific and Atlantic Oceans have an elliptical shape sharpened to the rostrum and forming a blunt point (Figs. 3a, 3b). Irrespective of location, the dorsal edge is minimally lobed and becomes almost smooth in adult fish, with small irregularities (soft indentations), whereas the ventral margin is curved (Fig. 3a), and almost linear (Fig. 3b) towards the rostrum in sagittae from the Pacific and Atlantic, respectively. Otoliths from the two areas share a heightened outwards concavity in the anterior-posterior axis.

Multivariate analysis of variance

Three trials were used to test for significant differences in otolith shape between the Atlantic and Pacific oceans. In each approach, a multivariate analysis of variance (MANOVA) was used to contrast the null hypothesis associated with the factors (Area x Sex) with the mean values of the SDs, NEFDs, and SIs. In the first approach, seven dependent variables were used. Of these variables, six corresponded to unstandardized residuals of SDs (URSD) and one to a SIs that was significant for the normality K-S and Levene tests. In the second approach, the dependent variables corresponded to the NEFDs that were statistically significant for the normality K-S and Levene tests. In the third approach, all variables from the previous two trials were combined. The MANOVA results (Table 2) show that for all these tests, the mean values of the size and shape variables (NEFDs, SDs and SIs) did not differ significantly between the sexes within the areas (MANOVA, $P > 0.05$) but varied significantly between the Pacific and Atlantic oceans (MANOVA, $P < 0.05$).

Discriminant analysis

A stepwise discriminant analysis was performed using the area as the grouping variable (*i.e.*, the Atlantic and Pacific oceans), and the SDs and NEFDs as explanatory variables, with the same three approaches that had previously been used in the MANOVA (Table 4). The results of the discriminant analysis showed that, based on the SDs and SIs, the sagittal otoliths can be assigned to the Atlantic and Pacific spawning areas with accuracies of 84.8% and 63.6%, respectively. The overall percentage of correct classifications was 75.3% of the total, for the second approach, with the NEFDs as input variables, the discriminant classification matrix correctly classified 75.8% and 61.7% of the sagittal otoliths for the Atlantic and Pacific spawning areas, respectively. The overall percentage of correct classifications was 69.5%. If the input variables were combined, the model correctly classified 86.4% and 70.1% of the sagittal otoliths for the Atlantic and Pacific spawning areas, respectively, and had the best overall classification percentage (79.1%). The final discriminant model included five output variables. The discrimi-

Table 2. Summary statistics of three multivariate analysis of variance (MANOVA) test for significant differences between the Pacific and Atlantic spawning areas in mean values of variables derived from otolith shape analysis of sagittal otoliths of the southern blue whiting *Micromesistius australis*. a) Basic size descriptors (SDs) and circularity index (CI); b) normalised elliptical Fourier descriptors (NEFDs); and c) combined model ((SDs+CI) + (NEFDs)).

Effect /trials	Wilks' Lambda			F-value			Effect DF			Error DF			P-value		
	A	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Intercept	0.00	0.08	0.00	62,690	361	34,213	7	7	14	229	229	222	0.00	0.00	0.00
Area	0.78	0.83	0.69	9.15	6.70	7.04	7	7	14	229	229	222	0.00	0.00	0.00
Sex	0.91	0.98	0.89	3.23	0.50	2.01	7	7	14	229	229	222	0.00	0.84	0.02
Area * Sex	0.96	0.99	0.94	1.45	0.49	0.97	7	7	14	229	229	222	0.19	0.84	0.48

Table 3. Nomenclature used in discriminant analyses. Three trials conducted with different approaches. a) Basic size descriptors (SDs) and circularity index (CI), b) Normalised elliptical Fourier descriptors (NEFDs), and c) combined model ((SDs+CI) + (NEFDs)). N entry and N output denote the number of initial variables and the number of variables after the stepwise process, respectively.

Trials	Descriptions	N entry	N output
A	UR size + Index N&H test	7	2+c
B	NEFDs (N&H test)	7	6+c
C	Global (NEFDs N&H + UR size + I)	14	5+c
Acronyms	description		
N&H test	Normality & homogeneity test		
UR size	Unstandardised residuals of size		
NEFDs	Normalised coefficients of the EFDs		
CI	Circularity Index		
C	Constant		

Table 4. Illustration of analyses with three classification matrices summarising the percentage of correct classifications derived from a linear discriminant analysis (LDA) for stock identification of the southern blue whiting *Micromesistius australis*. LDA based on a) Basic size descriptors (SDs) and circularity index (CI), b) normalized elliptical Fourier descriptors (NEFDs), and c) combined model ((SDs+CI) + (NEFDs)).

Group / trials	Classification matrix								
	Percent correct			N. Atlantic			N. Pacific		
	a	b	c	a	b	c	a	b	c
Atlantic Ocean	84.8	75.8	86.4	112	100	114	20	32	18
Pacific Ocean	63.6	61.7	70.1	39	41	32	68	66	75
Total	75.3	69.5	79.1	151	141	146	88	98	93

Table 5. Classification functions derived from an otolith shape analysis of the southern blue whiting *Micromesistius australis* based on three trials. a) Basic size descriptors (SDs) and Circularity index (CI), b) normalized elliptical Fourier descriptors (NEFDs), and c) combined model ((SDs+CI) + (NEFDs)). C2 to D10 correspond to NEFDs, CI: circularity index, UR_Area: unstandardized residuals of SDs of area. Variables C2 to D10 come from elliptical Fourier analysis.

Group	Classification functions; grouping: area					
	Atlantic Ocean			Pacific Ocean		
Variable/ Trials	a	b	c	a	b	c
C2		226.58	418.48		331.28	524.76
C3						
C5		-104.27	3,376.95		35.74	3,505.30
C6		251.20			335.10	
C7						
C8		133.36	1,836.25		296.42	1,976.27
C10						
D3		-182.52			-246.84	
D4						
D6		1,024.93			934.61	
D10						
CI	7,043.24		7,374.37	6,871.20		7,216.12
UR_Area	-93.74		-95.71	-91.45		-93.45
Constant	-1,024.93	-5.49	-1,075.67	-975.71	-6.80	-1,031.51

minatory power of the combined model (Fig. 4c) is graphically illustrated by the canonical scores for this model compared with the canonical scores from the classification models derived from the SDs and from the NEFDs (Figs. 4a, 4b). A classification function was derived from the discriminant analysis for each trial (Table 5).

DISCUSSION

The morphological and morphometric characterization of otoliths from Southern blue whiting furnished a basis for qualitative and quantitative otolith shape analyses of this species. Otolith shape analysis is based on the principle that the shape of the otolith

varies geographically, even within a species (Campana & Casselman, 1993) and has high morphological specificity (Aurioles, 1988, 1988, 1991; Lowry *et al.*, 1990, 1991; García, 1995, Hernández *et al.*, 2004; Martínez *et al.*, 2007). Due to these properties, otolith shape is a useful tool for identifying intraspecific relationships (biological interactions within a population or stock groups within a population) and interspecific relationships.

Basic size descriptors and the contour morphology of otoliths determined by the analysis of elliptical Fourier descriptors are demonstrated to be useful and practical tools for stock discrimination of *M. australis* between the Atlantic and Pacific oceans. These findings are consistent with the findings of previous

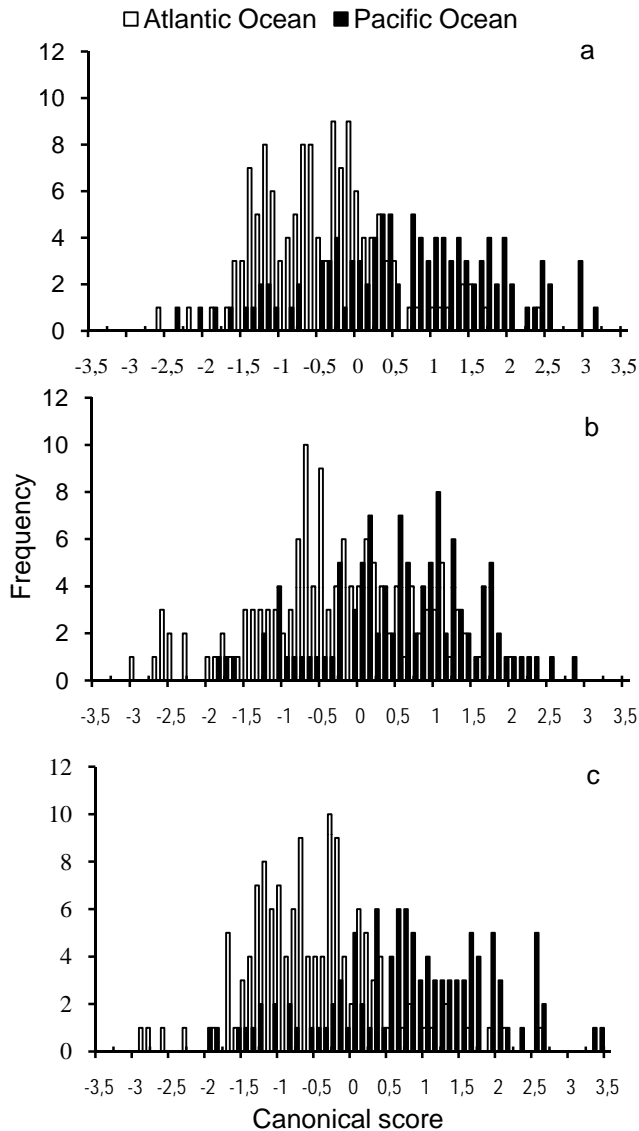


Figure 4. Frequency distribution of canonical scores derived from three discriminant function analyses illustrating separation of adult southern blue whiting, *Micromesistius australis*, collected in the Atlantic and Pacific spawning grounds. a) Basic size descriptors (SDs) and Circularity index (CI), b) normalized elliptical Fourier descriptors (NEFDs), and c) combined model ((SDs+CI) + (NEFDs)).

works that the elliptical method, in conjunction with the basic morphometry and shape indices, reveals small variations that are difficult to assess with linear morphometry if examined in isolation (Bird *et al.*, 1986; Castonguay *et al.*, 1991; Campana & Casselman, 1993; Friedland & Reddin, 1994; Begg *et al.*, 2001; De Vries *et al.*, 2002; Tracey *et al.*, 2006; Turan, 2006; Turan *et al.*, 2006; Burke *et al.*, 2008;

Stransky *et al.*, 2008a, 2008b; Farias *et al.*, 2009). According to Cadrin & Friedland (1999), the Fourier analysis technique is an efficient method for describing all of the variation in the shape contours and the individual differences in the scale of the otolith contour (Campana & Casselman, 1993), although the biological interpretation of the results obtained from this technique is more complex than the biological interpretation of the results obtained from linear morphometry (Stransky & MacLellan, 2005).

Three multivariate trials were performed for stock identification of the southern blue whiting in the current study. In the first two trials, the discriminatory capacity of the basic size descriptors and the discriminatory capacity of the normalised elliptical Fourier descriptors were evaluated separately. The third trial involved an approach in which the two types of descriptors were combined. The third approach showed the best performance. This approach achieved correct classification percentages of 86.4% and 70.1% for the Falkland Islands and southern Chile, respectively. These results agree with the results of previous studies in which contour analyses of otoliths were performed for stock discrimination purposes (Bird *et al.*, 1986; Castonguay *et al.*, 1991; Campana & Casselman, 1993; Friedland & Reddin, 1994; De Vries *et al.*, 2002; Tracey *et al.*, 2006; Turan, 2006; Turan *et al.*, 2006; Tuset *et al.*, 2003; Burke *et al.*, 2008; Stransky *et al.*, 2008a, 2008b; Farias *et al.*, 2009). The incorrect classification of the remaining 20% of the otoliths could reflect a mixture of adults. This mixture would include individuals that did not follow the pattern of migration to their natal spawning grounds. These results support previous works on population structure of *M. australis* (Arkhipkin *et al.*, 2009 & Niklitschek *et al.*, 2010), which state the existence of two ecologically distinct sub-populations maintained by a high degree of fidelity to spawning sites and that a certain level of mixing occurs in the Scotia Sea during the immature stage. During this stage, the fish are in their common feeding area in the Scotia Sea during the Antarctic summer. To corroborate the discriminatory capacity of otolith shape, a multivariate analysis of variance (MANOVA) was performed. The analysis revealed a significant area-sex interaction effect. This significant difference strengthens the high classification rate found in the discriminant model and supports the hypothesis that two distinct groups, representing discrete populations of the southern blue whiting, occur in South American waters. The degree of mixing occurs between these groups and should be addressed in future studies.

The present study underscores the importance of ensuring that certain sampling conditions hold (Farias *et al.*, 2009). These authors emphasised the need to use otoliths from fish in similar ranges of length and age. The present study used otoliths from fish whose total length ranged between 50 and 60 cm to ensure that the specimens used were limited to the adult stage. This approach would avoid the effect of allometric growth (Cardinale *et al.*, 2004). Moreover, Cardinale *et al.* (2004) showed that the normalised Fourier descriptors are independent variables unrelated to otolith size. In addition, the correlation between otolith length and basic size descriptors (perimeter, area, aspect ratio, maximum diameter, medium and minimum width and maximum Feret) was removed from each dependent variable to remove the effects of covariance. Consequently, the conclusions of the current study about the stock structure of the southern blue whiting appeared to be reliable. Furthermore, an attempt to minimise errors resulting from the migratory behaviour of this species was made by collecting adult fish during the spawning season. However, possible additional sources of misclassification in the analysis of otolith shape cannot be excluded. These sources include methodological inaccuracies, individual variability and migration (Campana & Casselman, 1993; Tracey *et al.*, 2006). Cardinale *et al.* (2004) showed that even at the same temperature and growing conditions, different populations have very different otoliths. He also observed differences in the shape of the otoliths, as a result of genetic effects (L'Abée-Lund & Jensen, 1993). Certain authors have suggested that environmental factors are more influential in determining the shape of the otolith because they affect the growth rate of the fish (Smith, 1992; Campana & Casselman, 1993). A study under controlled temperature and food conditions (Hüssy, 2008), showed that for *Gadus morhua*, the size and shape of the otoliths are ontogenetic effects but small-scale differences in shape can result from environmental factors, specifically the availability of food.

The water masses associated with the two spawning grounds of the southern blue whiting in South America are clearly different. The Chilean spawning grounds are occupied by the waters of the West Wind Drift, which meets the Chilean shelf at approximately 42°S and splits into two principal branches. One of these branches, the Humboldt Current System, comes from the north. Another branch, the Cape Horn Current, comes from the south and meets the Antarctic Circumpolar Current (ACC) in the Drake Passage (Orsi *et al.*, 1995). During the austral winter, water temperatures vary between 8° and

9°C at the spawning depths of 200-300 m (Reyes, 2002). In contrast, the Atlantic spawning grounds to the south of the Falkland Islands are occupied by the Falkland Current, a derivative of the ACC, with much colder waters (4°-5°C) at the spawning depth of 200-300 m (Zyrjanov & Severov, 1979). This difference in environmental conditions would perhaps explain the differences in otolith shape between the Atlantic and Pacific stock units. Given this scenario, it is mandatory to evaluate the performance of otolith shape analysis, as applied in the present study, on an interannual basis. As otoliths are commonly collected as part of the stock assessment protocols for *M. australis* in both oceans, otolith shape analysis would provide a useful tool for monitoring mixing levels on the common feeding grounds used by this species. Furthermore, otolith shape analysis can support and/or complement other techniques used for stock identification in this species (Galleguillos *et al.*, 1997; Ferrada *et al.*, 2002; Valdivia *et al.*, 2007; Niklitschek *et al.*, 2010) to contribute to the development of reliable conclusions about the population structure of this species, to ensure the sustainable management of this transoceanic fishery resource.

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