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Using the Shape of *Sagitta* Otoliths in the Discrimination of Phenotypic Stocks in *Scomberomorus sierra* (Jordan and Starks, 1895)

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Abstract: Geometric morphometric analysis was used to evaluate the shape of complete and broken otoliths in stock discrimination of *Scomberomorus sierra*. Data come from three different fishing locations in the mouth of the Gulf of California. Of the 70 otoliths pairs from location, 35 were broken. On the Complete Otoliths (CO) image 41 marks were located and 31 on Broken Otoliths (BO), the latter lacking rostrum. To evaluate the potential loss of BO resolution, the last 10 marks were eliminated from the CO and a third set of information was integrated as Virtually Broken Otoliths (VBO). The effect of the size, position and orientation of the spatial configurations of the otoliths were eliminated through the procedure of Procrustes superimposition. A canonical variable analysis detected significant differences ($p < 0.05$) in the three data sets and the distribution of the scores indicated a clear separation of the three groups, suggesting a morphologic difference of the otoliths from the three fishing locations. Also, the pairwise differences based on the Procrustes distances were statistically significant in all cases ($p < 0.05$). In agreement with Mahalanobis distances, the average percentage of correct assignation varied from 89% in CO and VBO to 80% in BO. It is ruled out that the morphologic differences are attributed to an allometric effect, since, in none of the cases the size of the centroid showed a significant correlation with the canonical axes. The present study constitutes an important referent to maximize the use of the information about otoliths, which due to their fragility break during extraction and manipulation.

Key words: Otoliths, pacific sierra, *Scomberomorus sierra*, geometric morphometrics, Gulf of California

INTRODUCTION

The body shape analysis of the organisms plays an important role in many biological studies focused on identifying the processes responsible for these morphological variations. The corporal changes can be a consequence of disease, ontogeny development, phenotypical plasticity, or evolutionary adaptation related to particular geographic factors (Zelditch *et al.*, 2004).

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The analysis of the morphological variations has made it possible to distinguish individuals from different locations. On fishes, these analyses commonly based on morphological quantification of the external features have been useful in the evaluation and support of the existence of phenotypic stocks (Cadrin and Friedland, 1999; Cadrin, 2000; De la Cruz-Aguero and García-Rodríguez, 2004; Ibañez-Aguirre *et al.*, 2006). Alternatively, the delimitation of phenotypical groups has been based upon morphometric analysis of specific structures such as otoliths (DeVries *et al.*, 2002; Felix-Uraga *et al.*, 2005) or scales (Ibañez *et al.*, 2007).

Otoliths are hard elements found in the internal ear of fishes and have been useful in diverse studies. Otoliths are structures of great relevance because they store information that helps to understand many aspects of the life history of fishes (Campana and Thorrold, 2001; Elsdon *et al.*, 2008). For example, the analysis of the chemical composition of otoliths can be used to identify stocks because otoliths contain trace elements from the water where fishes live. The trace elements can function as a geochemical signature and serve as an additional natural tag (Rooker *et al.*, 2003; Petterson *et al.*, 2004). On the other hand, the contour of the otoliths, the characteristics of their sulcus and in general the type of the relief allow otoliths to be a useful tool for the identification of prey species in studies of their feeding (Labbé and Arana, 2001; Markaida and Sosa-Nishizaki, 2003). At an intra-specific level, otoliths have been relevant to distinguishing different groups of fishes from different locations (Campana and Casselman, 1993; Torres *et al.*, 1996; Smith *et al.*, 2002; Monteiro *et al.*, 2005). One of the main inconveniences of using otoliths for morphometric analysis is that they may break during the extraction (Yaremko, 1996; Granadeiro and Silva, 2000; Furlanni *et al.*, 2008) and as a result of processing for age determination and searching for trace elements (Polat *et al.*, 2005; Limburg *et al.*, 2007) potentially relevant information regarding their shape might get lost. Therefore, the sample size might decrease and the results could lack statistical accuracy if the sample size is limited.

The Pacific sierra *Scomberomorus sierra* is distributed from the Southern part of California to Peru, including the Galapagos Islands. It is a coastal migratory fish that concentrates in the Southern Gulf of California during the period from November to July and disperses in a spawning migration during the summer (Collette and Nauen, 1983). Its seasonal availability in the Mexican Pacific makes this species an opportunity fishing resource with an important contribution to coastal fishing catches. The historical trend of catches (1964-2004) of Pacific sierra in the Eastern tropical Pacific Ocean indicates an average production of 5,575 annual tons including catches from Colombia, Nicaragua, Panama, Peru and Mexico. Mexico's catch contributes 73% (4, 024 tons) of the annual total production (<http://www.fishbase.org/report/FAO/FAOCatchList.cfm?scientific=Scomberomorus+sierra>).

Despite the importance of the Pacific sierra as a fishing resource, knowledge needed to evaluate the status of its population is limited. The few scientific reports on this species provide information regarding size ranges, weight, gonad maturity stages, size at first maturity and age and growth of the local catches (Arámburo-Paéz *et al.*, 1984; Pérez-Ramos, 1994; Cervantes-Escobar, 2004; Aguirre-Villaseñor *et al.*, 2006; Medina-Gómez, 2006). The results found by Medina-Gómez (2006) indicate that the growth curves of the Pacific Sierra are significantly different among the fishing locations in the Southern Gulf of California. At the same age, the specimens from the La Paz fishing area were larger and had a bigger otolith than the ones from the Mazatlan and Guaymas areas (Fig. 1). The work of Medina-Gomez (2006) constitutes the first suggestion of the existence of different stocks in this species.

Pacific sierra constitutes an important economic resource for Mexico. For this reason the National Fish Institute (INP, 2001) recommends increasing knowledge of this species in the Mexican Pacific. Such knowledge is relevant because the identification of stocks should be considered a basic requirement for proper fishery management (Cadrin and Friedland, 1999).

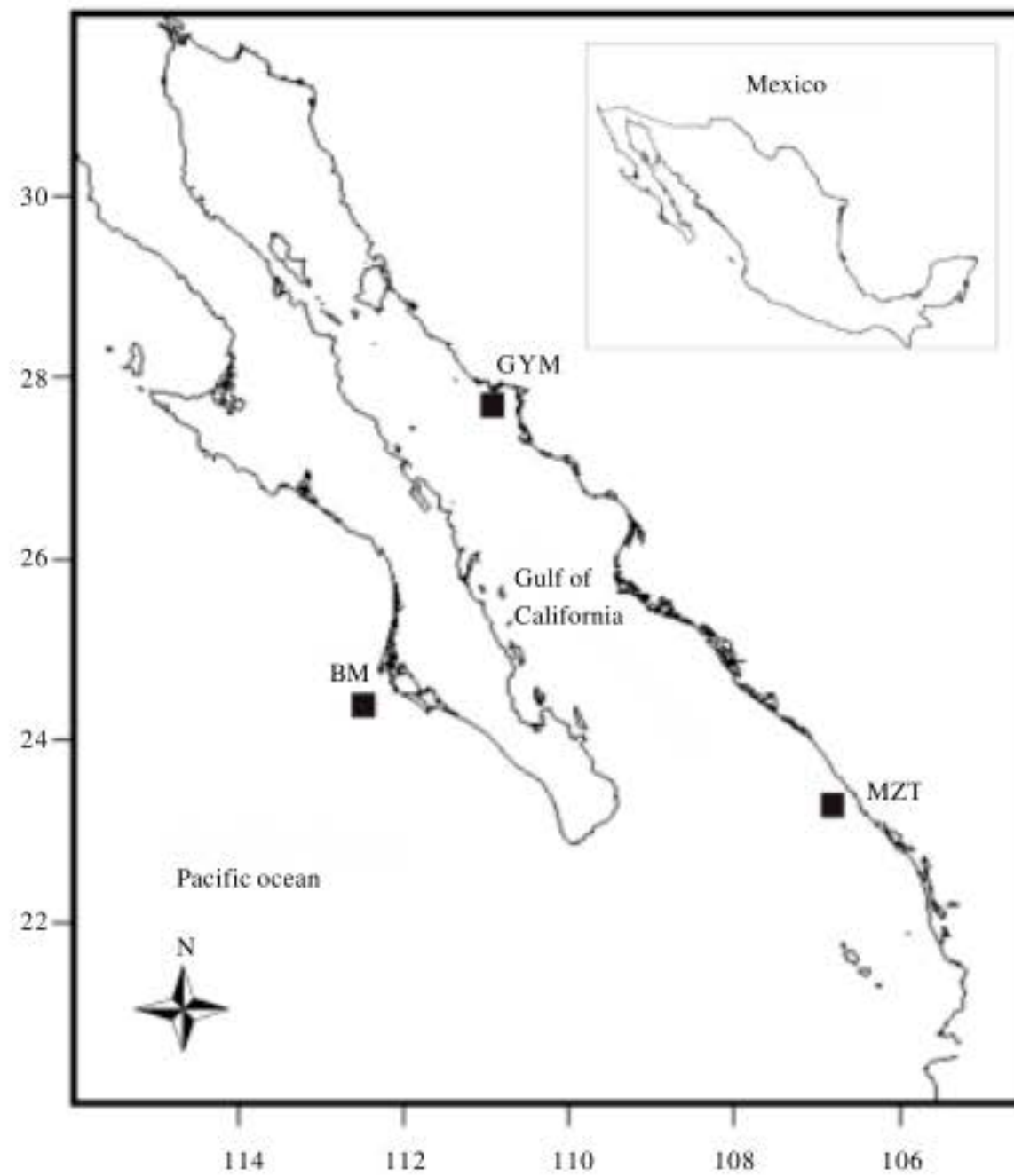


Fig. 1: Study area. Location of collection sites: Bahía Magdalena (BM), Guaymas (GYM) and Mazatlán (MZT)

Morphometric methods are useful when it is necessary to describe and compare the shape of the organisms or their structures (e.g., otoliths, vertebrae, bones, etc.) This information can indicate the geographic location, stage of development and genetic or environmental effects, which have a direct impact on the phenotype (Rohlf and Marcus, 1993). In the present study, samples from three locations from Northwest of Mexico were analyzed applying geometric morphometric analysis with the intention of (1) evaluating the importance of the otolith shape in identifying the phenotype stocks of the Pacific Sierra and (2) determining the level of discrimination afforded by damaged otoliths.

MATERIAL AND METHODS

Data Collection

Pacific sierra were sampled monthly from artisanal fisheries at Guaymas, Sonora, (GYM), Bahía Magdalena, Baja California Sur, (BM) and Mazatlán, Sinaloa, (MZT), during November 2006 to May 2007 (Fig. 1). The sampling consisted on selecting at random up to 100 organisms, from the catch of three fishing boats and to measure the Fork Length (FL). Length measurements were grouped into 5 cm FL intervals; three Pacific sierra chosen at random from each interval were sexed and their otolith sagittae were extracted to determine age and for morphometric analysis. Otolith were cleansed of adhering tissue and stored dry in vials until they could be examined further.

Two hundred and ten Pacific sierra otoliths were random selected, 70 from each fishing site. Thirty-five otoliths from each location were broken and 35 were complete. In the present study just the left otoliths were used. The otoliths were photographed using a digital Sony camera connected to an Olympus stereoscopic microscope. All images were digitalized to 10x augmentation including a scale of measurement.

A template on the digital image was constructed using the MakeFan program (H.D. Sheets, available as part of the Integrated Morphometrics Package (IMP) at: <http://www2.canisius.edu/~sheets/morphsoft.html>) to assist in the placement of points. Forty-one points were located and digitized on the Complete Otoliths (CO), one placed on the core and the rest over the contour. To evaluate the consistency of the results and potential resolution loss, ten marks were eliminated from the ventral area to the rostrum of the otoliths, where they normally break. We called these Virtually Broken Otoliths (VBO). Additionally, to complement the above stated analysis, we used 35 Broken Otoliths (BO) from each sampling location on which thirty-one marks were digitalized (similar to the VBO). Digitalization of points was done using the TPsDig program (F.J. Rohlf, available at <http://life.bio.sunysb.edu/morph/>) (Fig. 2A and B). These three sets of data were used to perform similar geometrical and statistical analysis.

Methods

Otoliths were submitted to three geometric processes (translation, scaling and rotating) that do not affect the variations of the shape and allow standardization of the comparisons. This is possible because all configurations are located over the same position, scaling to the same unit of the centroid size and rotating to minimize the differences between homologous points through least squares (Rohlf and Bookstein, 1990; Rohlf and Slice; 1990). The rotation

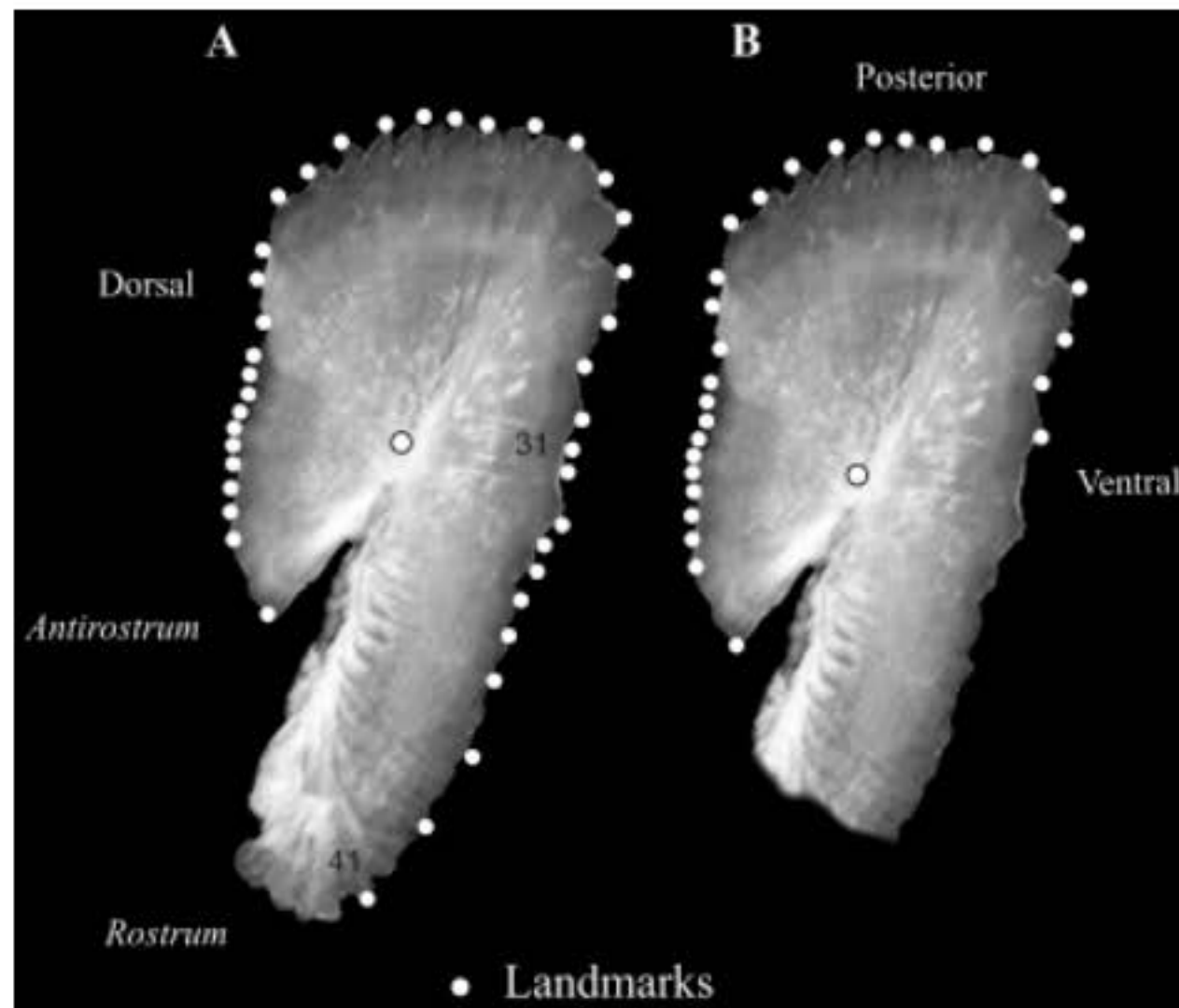


Fig. 2: (A) *Scomberomorus sierra* CO (41 points) and (B) BO (31 points)

of the configurations was done through Procrustes superimposition method. These analyses were performed using CoorGen6 (IMP). To evaluate the variation of the otolith shapes among sampling locations, we did an analysis of Canonic Variables (CV) using the CVAGen6M (IMP) program. The VC makes possible an evaluation of the differences of the groups through the Wilk's Lambda value of significance ($\tilde{\epsilon}$). The values closer to zero mean a perfect discrimination while values closer to one represent a lack of discrimination. To test the differences among the locations, the Procrustes distance was compared between each pair set of the average configurations of the three groups. The significance of the test was based on the comparison of the values observed with those obtained through 900 permutations. These analysis were carried out using TwoGroup6A (IMP). An assignation matrix was obtained using Mahalanobis distances on the scores produced by CV. The assignation is based on the existing distance between each individual and the average of the closest group. To see the trend of the variation of the shapes, an average shape of the otolith and its variation in each location were obtained through thin plate-spline.

The geometric morphometric method was used to remove the differences related to the size but not the differences in the shapes associated with the changes related to the size of the individuals (allometry). To avoid the possible effects of allometry, Pacific sierra with a smaller size than the size of first maturity (443 mm of fork length FL) (Aguirre-Villaseñor *et al.*, 2006) were excluded in this analysis. An ANOVA was performed to compare the significance of the size of the centroid among the locations. Furthermore, the Centroid Size (CS) was correlated with each axis of CV. A high correlation indicates the existence of allometric growth in the individuals.

RESULTS

Complete Otoliths

The canonical variable analysis of CO data detected significant differences between groups ($\tilde{\epsilon} = 0.11$, $p < 0.05$ for CV1; $\tilde{\epsilon} = 0.43$, $p < 0.05$ for CV2). The distribution of the scores of CV1 and CV2 indicated a clear separation of the three groups, suggesting a morphologic differentiation of the otoliths from the three locations (Fig. 3). The pairwise differences based on the Procrustes distances were statistically significant in all cases ($F = 9.52$, $p < 0.05$ for BM vs GYM; $F = 5.09$, $p < 0.05$ for BM vs MZT; $F = 13.03$, $p < 0.05$ for GYM vs MZT). According to the Mahalanobis distances, the average percentage of correct assignation was 88.6%, while the lowest value was 86% (GYM and MZT) (Table 1). The most important variations observed through thin plate-spline indicated differences between locations. The variation of the otoliths in GYM occurred primarily towards the dorsal side, while in BM and MZT they were on the back side of the otolith (Fig. 3). MZT otoliths were significantly larger (CS = 129.11, ± 12.6) than BM (CS = 128.9 ± 8.2) and GYM (CS = 119.9 ± 12.3) (Test t $p < 0.05$). However, the differences attributed to the allometric effect were not statistically significant according to the correlation of Pearson calculated by the centroid size and the first two canonical axes (for CV1, CS-BM, $r = -0.312$, $p = 0.073$; CS-GYM, $r = -0.149$, $p = 0.39$; CS-MZT, $r = 0.057$, $p = 0.74$; for CV2, CS-BM, $r = -0.279$, $p = 0.11$; CS-GYM, $r = -0.228$, $p = 0.195$; CS-MZT, $r = 0.018$, $p = 0.92$).

The canonical variable analysis of the data obtained from VBO (by reducing the number of marks from 41 to 31 on CO) showed similar results to those obtained by using all the marks. Two canonical variables were statistically different ($\tilde{\epsilon} = 0.13$, $p < 0.05$ for CV1; $\tilde{\epsilon} = 0.48$, $p < 0.05$ for CV2) and the distribution of the CV1 and CV2 scores also indicated a clear separation of the three groups (Fig. 4). Significant differences were found in all pairwise comparisons ($F = 11.36$, $p < 0.05$ for BM vs. GYM; $F = 5.58$, $p < 0.05$ for BM vs. MZT; $F = 13.47$,

Table 1: Classification results (percentages of otoliths)

Types of otoliths	BM	GYM	MZT	Mean
Complete otoliths (CO)				
BM	94	0	6	88.6
GYM	3	86	11	
MZT	11	3	86	
Virtually broken otoliths (VBO)				
BM	91	3	6	89.3
GYM	3	86	11	
MZT	3	6	91	
Broken otoliths (BO)				
BM	89	0	11	80.3
GYM	0	80	20	
MZT	14	14	72	

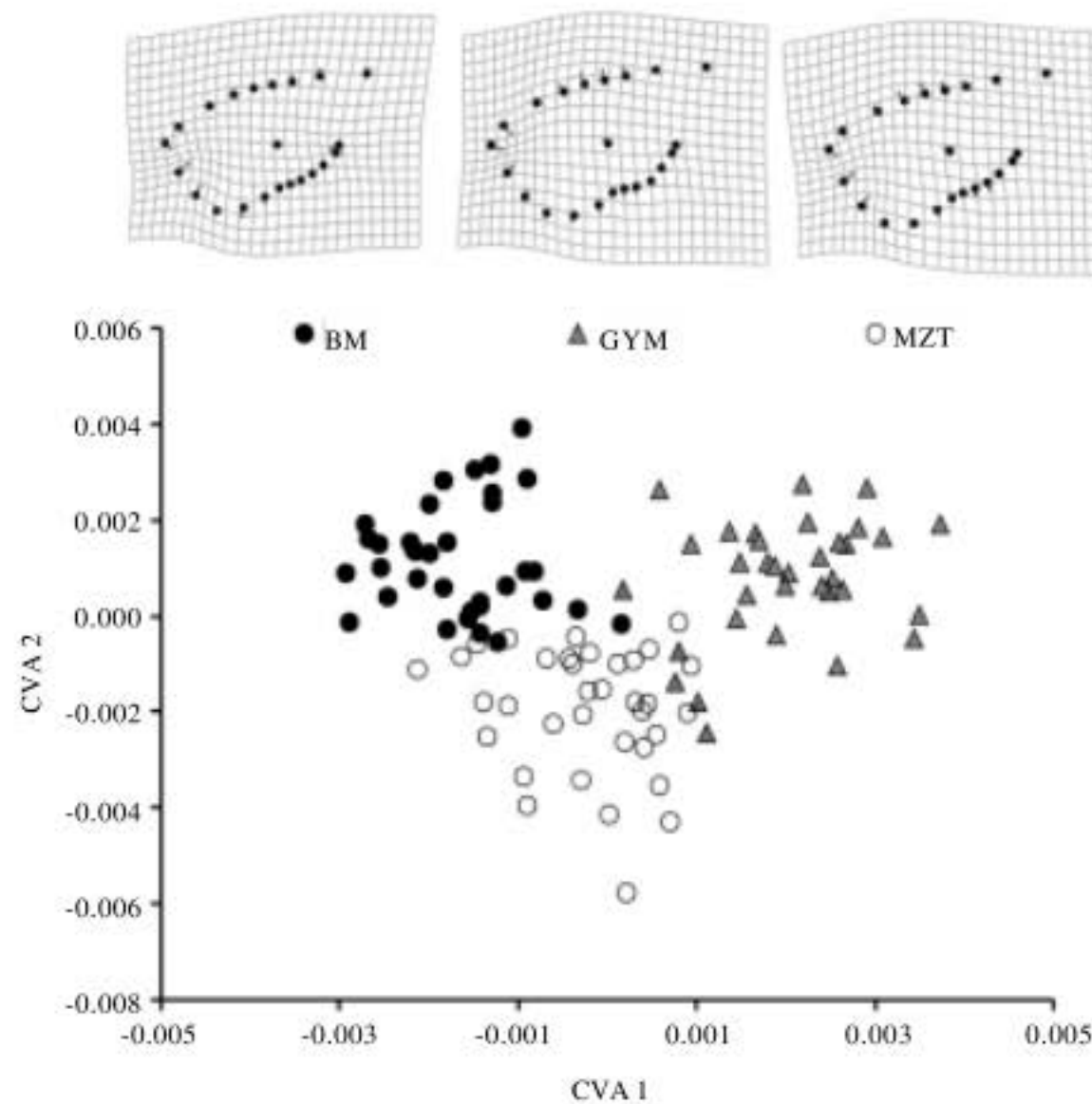


Fig: 3: Scores of canonical variables generated from CO data. Above, deformation graphics of CV1 for each location

$p < 0.05$ for GYM vs. MZT) and the average percentage of the correctly assigned organisms was 89.3%. The lowest value of assignment (86%) was found in GYM (Table 1). The main variation observed in each group through thin plate-spline showed differences among locations (Fig. 4).

Validation of Broken Otoliths

The canonical variable analysis performed on the samples of BO detected significant differences ($\bar{E} = 0.17$, $p < 0.05$ for CV1). The distribution of the scores estimated from CV1 and

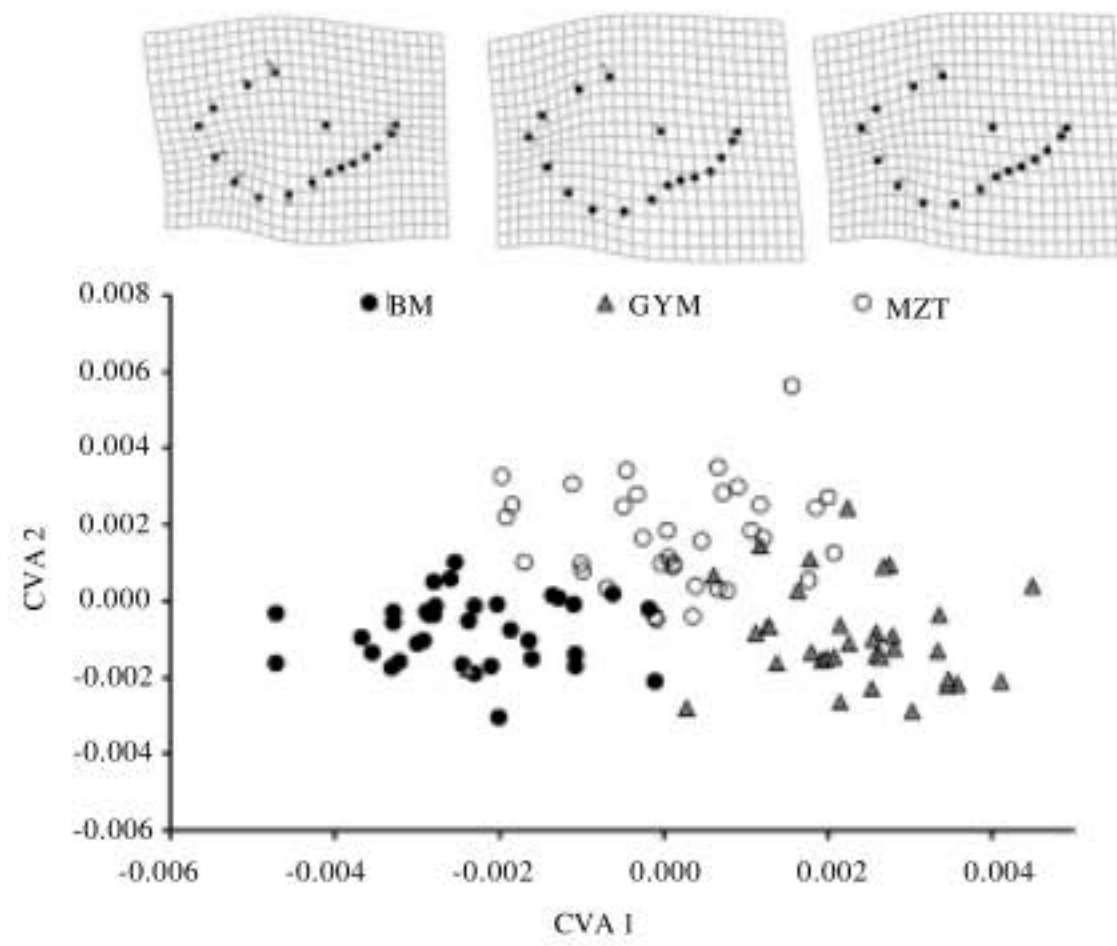


Fig. 4: Scores of canonical variables generated from VBO data. Above, deformation graphics of CV1 for each location

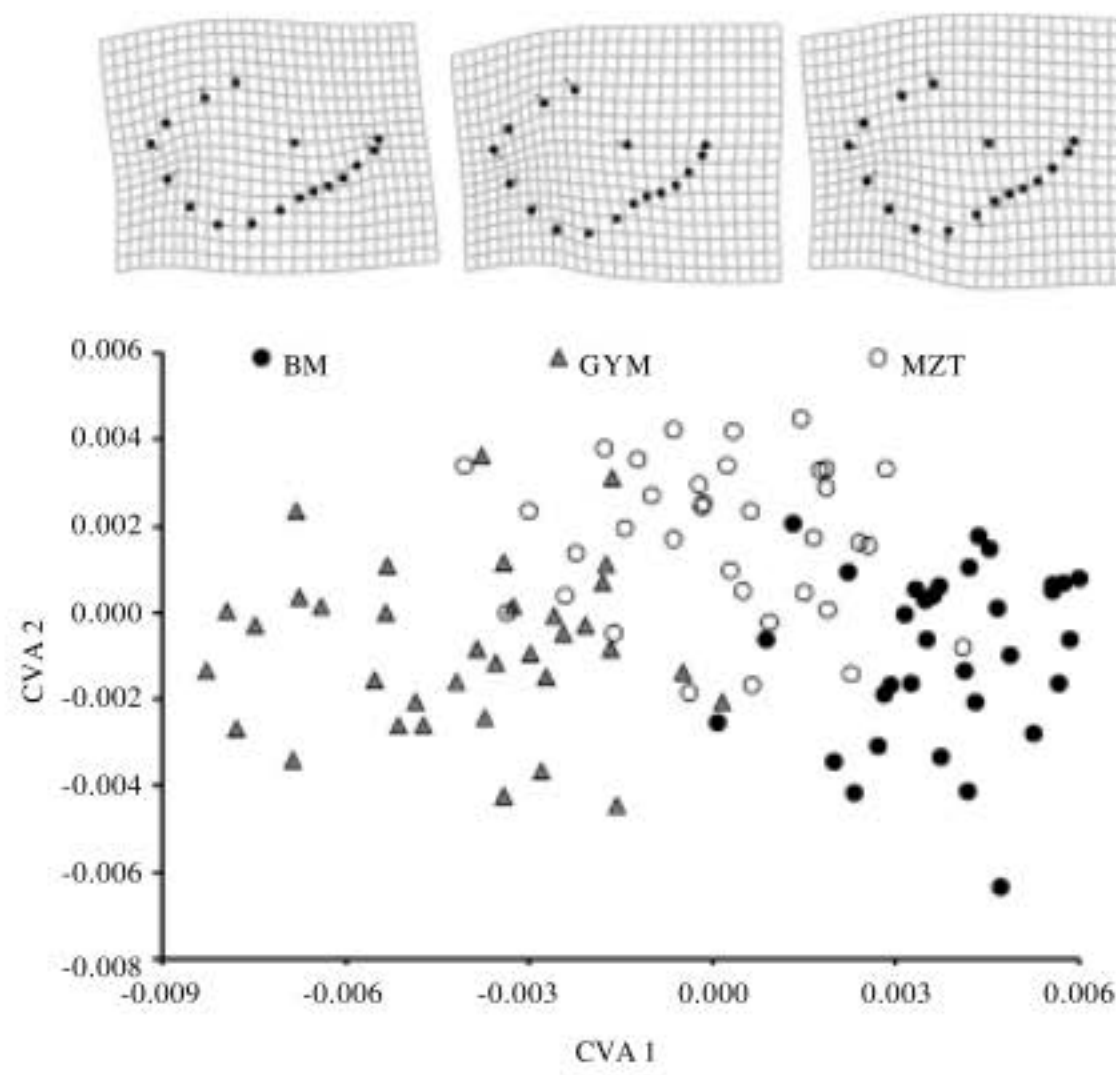


Fig. 5: Scores of canonical variables generated from BO data. Above, deformation graphics of CV1 for each location

CV2 indicated a separation in the shape of the otoliths from the three collection areas (Fig. 5). Significant differences were found in all pairwise comparisons ($F = 8.31, p < 0.05$ for

BM vs. GYM; $F = 3.89$, $p < 0.05$ for BM vs. MZT). The average percentage of organisms correctly assigned was 80.3% and the lowest value was found in MZT (72%) (Table 1).

Upon analyzing the thin plate-spline, it was noticed that there was a higher intraspecific variation in the shape of the otoliths. As with the complete otoliths, the broken otoliths from MZT were significantly larger ($CS = 99.92 \pm 9.25$) than BM ($CS = 97.45 \pm 6.63$) and GYM ($CS = 93.76 \pm 11.47$) (test t , $p < 0.05$). It is ruled out that for these data sets the morphologic differences are attributed to an allometric effect, because in none of the cases the centroid size showed a significant correlation with the canonical axes (for CV1, CS-BM, $r = -0.033$, $p = 0.85$; CS-GYM, $r = -0.034$, $p = 0.85$; CS-MZT, $r = 0.245$, $p = 0.162$; for CV2, CS-BM, $r = 0.166$, $p = 0.34$; CS-GYM, $r = -0.142$, $p = 0.42$; CS-MZT, $r = 0.169$, $p = 0.33$).

DISCUSSION

Present findings support that the use of geometric morphometric on the otoliths of Pacific sierra has considerable potential for the analysis of its shape and consequently for the delimitation of phenotypical groups. This study also constitutes an important reference for those works based on otoliths, which due to their fragility, break during their extraction and manipulation. Previous works have used broken or manipulated (burnt) otoliths. However, their application has been focused on recognizing their efficiency for the determination of age and for comparing with other structures such as scales (Blood, 2003; Edwards *et al.*, 2005; Polat *et al.*, 2005).

The analysis of the otolith shape was commonly addressed through traditional morphology and external otolith morphology. The latter was based upon Fourier analysis (Campana and Casselman, 1993; Tuset *et al.*, 2006) due to the apparent difficulty in defining homologous points to characterize the otolith shape and in applying geometric morphometric. Recently, Ponton (2006) evaluated the variations of the shape of otoliths of different species through geometric morphometric. His study showed the potential of geometric morphometric to find differences in the shape and to analyze the direction and magnitude of both intra and inter specific variations.

The analysis of the otolith shape from a reduced number of homologous points can be complemented with information about curves found along the contour of the otolith. These curves can be represented by points (called semi-marks) which can be located by taking into account natural points as references on the otoliths. The alignment of these curves through thin plate-spline allows the later use of the semi-marks as landmarks to give a good representation of the shape of the structure (Bookstein, 1991). Regardless of the methods used to distinguish differences in the shape (e.g., traditional morphometrics, outline analysis, geometric analysis), Tuset *et al.* (2006) mentioned that the percentage of the classification has varied between 70 and 95%, indicating and separating species. In this study, we used geometric analysis and found a correct average classification between 80 and 89%, with broken and unbroken otoliths, respectively, separating Pacific sierra stocks.

This information indicates that the shape of the otolith of Pacific sierra is statistically sufficient to discriminate individuals that come from different locations. In either case, it was found that the shape of the otoliths was associated with the allometry. This effect could have been reduced by selecting adult individuals for our analysis.

The results of VBO (by reducing the number of marks) indicated no loss of resolution in the discrimination of the three groups. The average percentage of correct classification was 89.3%. One explanation for these findings may be that the ventral side of the otolith (area on which marks were eliminated to generate VBO) presents no significant changes in

the otolith. This is supported by thin plate-spline, which indicate that the dorsal and posterior regions were most important in distinguishing variations in the shape of otoliths from the three locations. The information obtained from BO allowed discrimination between the three groups, which positively validates the objective of this study. Nevertheless, the percentage of correct classification (80.3%) was lower than that obtained with complete otoliths. However, the decrease in the resolution of discrimination of BO should not be attributed to a lesser number of analyzed marks. The main causes could have been due to the use of different samples since no decrease in the resolution of the discrimination between CO and VBO was detected.

The thin plate-spline in otoliths of Pacific sierra from the three locations allowed recognizing both location and direction of the changes of variation. Such differences may be the reflection of a phenotypical plasticity or as genetic consequence (Borges, 2005). In fish, the phenotypical variations may be the result of a variety of environmental factors such as temperature, salinity, oxygen and depth (Winberger, 1992). Thus, changes in the behavior, physiology, mortality, growth, life cycle, or demography may be expressed in both individual life cycles or between generations (Miner *et al.*, 2005). A deeper explanation of causes and possible implications of these differences requires more specific studies.

A similar case study to Pacific sierra is *Scomberomorus cavalla* from the Atlantic ocean and the Gulf of Mexico. Prior to 1985 it was considered as only one stock along its distribution, but mark and re-capture studies allowed recognizing the existence of migrating groups. In 1990 the existence of these groups was confirmed through allozyme electrophoresis (Johnson *et al.*, 1994). Later, DeVries and Grimes (1997) recognized through variation of age and growth analysis that fish (mainly females) from the Eastern side of the Gulf of Mexico grew faster and reached larger sizes than the ones from the Atlantic coast. The management measures for the species were set considering only the mark-recapture study, allowing fishing off Northeast Florida from November to March (Winter) and for the Gulf group catch quotas were set. Such management measures have been criticized since, the percentage of the true mix of groups is unknown and the above referred mark-recapture study suggests that more than half of the fish along the East coast of Florida belong to the Gulf stock (Sutter *et al.*, 1991). DeVries *et al.* (2002) used the otolith shape of the *S. cavalla* as a robust analysis to distinguish the differences between both groups and provide more information about the real percentage of mix of both groups. They estimated that 99.8% of the individuals captured within the management zone belonged to the Atlantic stock and just 0.2% belonged to the Gulf group. In this way, they mentioned that the management of this species is inadequate and may cause over-exploitation of the Atlantic stock.

The existing information about Pacific sierra biology is still insufficient and research efforts carried out to date have been fewer than those related to *Scomberomorus cavalla*. Pacific sierra is a commercially important species and it is convenient to define managing units along the area of its distribution. Collette and Nauen (1983) mentioned that the species is highly migratory, considering the relationship that it keeps with the rest of the Scombridae family. However, to date, there are no mark-recapture studies that would give a better picture of the migratory behavior of Pacific sierra, nor is there any information regarding the reproduction zones. Klawe (1966) did an analysis of ictioplankton trawls collected along the Eastern Pacific and found that the spawning of the Pacific sierra occurs off Mexico from July to September. This conclusion was based on a small number of larvae found between Bahia Tortuga and Bahia Magdalena, B. C. S. and Guerrero. Therefore, it is important to conduct research about the dynamic of the population. This could explain the differences in growth found by Medina-Gomez (2006), as well as the present study, in recognizing the integrity of

the stocks of Pacific sierra. Paraphrasing Ponton (2006), as comparisons of the otolith shape of adult fishes proved difficult, the next step will be examination of the processes producing these shapes during ontogeny.

The present study constitutes part of a research program of Pacific sierra. Future analysis should be aimed at evaluating the existence of phenotypic groups to estimate the population parameters of these groups and to recognize the genetic structure of the sierra along the Mexican Pacific. All the above-mentioned will allow a better understanding of the biology of the species and to reinforce management of the fishery.

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