

Using Otolith Shape Analysis to Identify Different Stocks of *Epinephelus morio* from the Campeche Bank

XIMENA RENÁN^{1*}, ESPERANZA PÉREZ-DÍAZ¹, TERESA COLÁS- MARRUFO¹,
JOAQUÍN GARZA- PÉREZ, JR², and THIERRY BRULÉ¹

¹ CINVESTAV. Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Departamento de Recursos del Mar, Unidad Mérida, México, Antigua Carretera a Progreso Km 6. Cordemex. Mérida Yucatán. México. C.P. 97310. ²UMDI- Sisal Facultad de Ciencias, Universidad Nacional Autónoma de México, Puerto de Abrigo sin número Sisal, Yucatán México. C.P. 97355. *ximenarenan@me.com; ximenarenan@hotmail.com.

ABSTRACT

Otolith shape analysis was used for discriminating stocks of *Epinephelus morio* (red grouper) from the Campeche Bank. Since red grouper is the most commercially exploited specie in Yucatan, to identify different stocks becomes a major issue for fishery management. From a total of 420 red groupers sampled between 1996 and 1999 by commercial and artisanal fleets, 373 individuals were analyzed. Both otoliths (*sagittae*) were removed through the gill arch, alcohol cleaned and stored dry in small paper bags. Each left otolith was measured to obtained otolith length (OL; 0.001cm), otolith width (OWi; 0.001cm) and both otoliths were weighed to obtained the weighted average (OW; 0.0001 g). Each left otolith was consistently oriented at a 45° position (*sulcus* side down, rostrum upwards) and digitally photographed using a stereomicroscope at 0.4× and 0.5× equipped with a videocamera. Otolith shape was determined through Age & Shape software (Infaimon S.L. ®) using wavelets descriptors (WLT), and then compared by principal components (PCA) and Multi-dimensional Scaling analysis (MDS) (Primer 6.1.12, PRIMER Ltd.). Red grouper standard length ranged from 33.0 cm to 83.0 cm and gutted weight 650.0 g to 13200.0 g. Otoliths meristics varied from 0.6679 - 1.1340 cm OWi, 1.2875 - 1.9992 cm OL and 0.0823 - 0.9516 g OW. Through the shape analysis, 512 random measurements for each of 10 WLT scales were calculated. The hierarchical cluster analysis (WPGMA) showed four distinct groups (at 0.005) using only WLT5, WLT6 and WLT7 (established by PCA) and six different groups using all the WLT (at 0.05). Apparently, no differences on the otolith shape could be identified.

KEY WORDS: Red grouper, otolith shape, discriminating stocks

Identificación de diferentes stocks de *Epinephelus morio* del Banco de Campeche mediante la forma del otolito

Se utilizó el análisis de la forma del otolito para discriminar posibles stocks del mero rojo (*Epinephelus morio*) del Banco de Campeche. Debido a la importancia que tiene el mero rojo como la especie más abundante en la pesca de escama del estado de Yucatán, la identificación de la presencia de diferentes stocks cobra relevancia para mejorar el manejo del recurso. De un total de 420 meros rojos capturados entre 1996 y 1999 por las flotas tecnificada y ribereña, 373 individuos fueron analizados. Ambos otolitos (*sagita*) fueron removidos a través del arco branquial, enjuagados con alcohol y empacados secos dentro de bolsas de papel opacas. Cada *sagita* izquierda fue medida para obtener la longitud (LO; 0.001cm) y ancho del otolito (AO; 0.001cm), mientras que ambos otolitos fueron pesados para obtener el peso promedio por otolito (PO; 0.0001 g). Cada *sagita* izquierda fue colocada de manera constante en una posición de 45° (*sulcus* hacia abajo, rostro hacia arriba) y fotografiada de manera digital utilizando un estero-microscopio con videocámara a aumentos de 0.4× y 0.5×. La forma del otolito fue determinada a través del programa Age & Shape (Infaimon S.L. ®) utilizando como descriptores la transformada de Óndula (*wavelets* WLT), y comparadas posteriormente a través de un análisis de componentes principales (ACP) y un análisis de escalamiento multidimensional (EMD) (Primer 6.1.12, PRIMER Ltd.). Las medidas de longitud estándar y el peso del pez eviscerado para los individuos de mero rojo capturados, variaron entre 33.0 cm a 83.0 cm (LE) y entre 650.0 g a 13200.0 g (Pev). Las medidas de los otolitos fluctuaron entre 0.6679 - 1.1340 cm (AO), 1.2875 - 1.9992 cm (LO) y 0.0823 - 0.9516 g (PO). A través del análisis de la forma de los otolitos, 512 medidas aleatorias fueron extraídas por cada una de las 10 transformada de Óndula calculadas. El análisis de agrupamiento jerárquico (WPGMA) identificó cuatro grupos distintos (a 0.005) utilizando únicamente las transformadas WLT5, WLT6 y WLT7 (establecidas previamente por el ACP) y seis grupos diferentes utilizando las 10 transformadas (a 0.05). Aparentemente, no pudieron identificarse diferencias estadísticas significativas en la forma del otolito entre individuos.

PALABRAS CLAVE: Mero rojo, forma del otolito, discriminación de stocks.

Utilisation de l'analyse de la forme de l'otolithe pour l'identification des différents stocks d' *Epinephelus morio* du Banc de Campeche

L'analyse de la forme de l'otolithe a été utilisée pour différencier les stocks d' *Epinephelus morio* (mérrou rouge) du Banc de Campeche. Du au fait que le mérrou rouge est l'espèce commerciale la plus exploitée au Yucatan, l'identification des différents stocks représente un objectif majeur pour l'aménagement des pêches. D'un total de 420 mérrous rouges échantillonnés entre 1996 et 1999 par les flottes commerciale et artisanale, 373 individus ont été analysés. Les otolithes (*sagittae*) ont été extraits au niveau des arcs branchiaux, lavés dans l'alcool et conservés au sec dans de petits sacs de papier. Chaque otolithe droit a été mesuré dans sa longueur (OL; 0,001cm) et sa largeur (OWi; 0,001cm), tandis que les deux otolithes ont été pesés pour calculer un poids moyen (OW; 0,0001 g). Chaque otolithe droit a été positionné à 45° (*sulcus* vers le bas et *rostrum* vers le haut) et photographié à travers d'une loupe binoculaire (0,4x et 0,5x) équipée d'une caméra vidéo. La forme des otolithes a été déterminée à travers de l'utilisation

du programme *Age & Shape* (Infaimon S.L.®), en considérant les descripteurs *ondelette* (wavelets, WLT), lesquels furent comparés par une analyse de composantes principales (PCA) et une analyse multidimensionnelle (MDS) (Primer 6.1.12, PRIMER Ltd.). La longueur standard et le poids du poisson éviscéré des mérours rouges analysés ont varié entre 33 et 83 cm et entre 650 et 13200 g. Les tailles et poids des otolithes ont varié entre 0,6679 et 1,1340 cm OW_i, 1,2875 et 1,9992 cm OL et entre 0,0823 et 0,9516 g OW. Au cours de l'analyse de la forme de l'otolithe, 512 mesures réalisées au hasard pour chaque 10 WLT ont été calculées. L'analyse des conglomerats hiérarchiques (WPGMA) a permis de distinguer quatre groupes (α 0,005) en utilisant seulement WLT5 ; WLT6 et WLT7 (déterminés par le PCA) et six groupes différents en considérant tous les WLT (α 0,05). Apparemment, aucune différence sur la forme des otolithes n'a pu être observée.

MOTS CLÉS: Mérour rouge, forme des otolithes, identification de stocks

INTRODUCTION

It has long been recognized that otolith shape is species-specific, and that could be used to distinguish different stocks of marine fish. This assumption relies on the peculiarities of the otoliths such as constant growth throughout the fish life and metabolic inertness (Campana and Casselman 1993). In addition, otolith morphology can often varied geographically within species because otolith shape changes with fish growth (Wright et al. 2002), diverse environmental conditions (Campana and Neilson 1985) and genetic variations (Galley et al. 2006). Nevertheless, different studies suggested that environmental factors are major determinants of otolith growth, and therefore, on otolith shape (Begg and Brown 2000, Cardinale et al. 2004). This strengthens the validity of shape analysis for stock identification where phenotypic-based characters are just as reliable as genetic-based ones (Begg et al. 2000).

Distinguishing stocks is an integral component of modern fisheries assessment (Tracey et al. 2006). A stock could be defined as a population that is exploited by a fishery and which may be subject to some form of management (Wootton 1999). When a large number of stocks of differing reproductive productivity mix in the fishery and quotas are set at the maximum sustainable yield, this may exterminate the less productive stocks, thereby reducing the productivity of the entire fishery (Watkinson and Gillis 2005).

Estimates based on population dynamics models indicate that red grouper stocks from northern and southern Gulf of Mexico are overfished (Burgos and Defeo 2000, 2004). Furthermore, studies showing declines in biomass (19,886 T to 9,762 T), capture (50% less) and income (Burgos and Pérez-Pérez 2006; SAGARPA 2008), denote that the red grouper fishery of the Campeche Bank, is over-exploited (Giménez-Hurtado et al. 2005, Albañez-Lucero and Arreguín-Sánchez 2009).

However, Mexican fishery regulations are restricted only to license limitation, a closed season from February 15 to March 15 since 2003, and an annual catch quota of 1,200 mt for the Cuban fleet (García-Moliner and Eklund 2004). Although red grouper is the most important commercially exploited species in Yucatan, there is no information about the stocks that compose the fishery. The objective of this study is to identify the red grouper stocks of the Campeche Bank, so that fishery management authorities can determine their contribution to the fishery and monitor accurately the population status.

METHODS

Red grouper were obtained from both inshore (artisanal) and offshore (industrial) Mexican commercial fleet of the northern coast of the Yucatan Peninsula, from April 1996 to December 1999. All fishes were caught during the two last days of each fishing trip, and stored in ice until landing. For each one, standard length (SL), fork length (FL), whole (GW) and gutted weight (gW) were recorded. Gonads were removed for microscopic sexual identification and sexual classes determination and results were previously published in Brulé et al. 1999.

Sagitta used for morphological analysis were removed up through the gills; cleaned in alcohol; stored dry and weighed to the nearest 0.01 mg. Video images of the left otolith were acquired using a software hardware workstation consisting of a stereomicroscope (Zeiss Stemi SV6) with digital camera (SONY CCD-Iris, CXC-107A) connected to a computer. The otolith direction was standardized by positioning each otolith at 45° with the distal side facing up and the anterior side to the right. Each otolith image was taken against a homogeneous black background to achieve good contrast of its contour, using 0.4× and 0.5× objectives with a magnification of × 8. Images were gray scale 720 X 576 pixels size. In each image, otolith length (OL) and width (OW_i) were measured using MicroMED XP (EASY SOFT 2008) software. Afterwards, each pair of otoliths was weighted to estimate the otolith mean weight (OW).

The otolith morphometry was extracted using each otolith digital image exported to *Age & Shape* (INFAIMON S.L.®) software. The *Shape* module automatically determines the contour and calculates Fourier and wavelets transforms. Since wavelet transform allow the irregularities of the contour to be quantified and their precise position to be determined (Parisi-Baradad et al. 2005, Piera et al. 2005), and its suited for describing the frequencies of highly non-stationary signals with sudden peaks or discontinuities (Hubbard 1996, Watkinson and Gills 2005), was chosen over Fourier transform. For each otolith image the scale centroid was determined automatically as the mean *X* and *Y* polar co-ordinates and radius measurements were taken from the centroid to the scale edge. All outlines were interpolated to 512 *radii* measurements and a set of 10 different frequency bands, or scales, obtained.

Once the 512 points for each 10 scales per image, were achieved, the results were tested for normality, an ANOVA and a Principal Components analysis (PCA) were carried

out (Sg- Plus 4.1; STATISTICAL GRAPHICS CORP. 1999). A Hierarchical cluster analysis (Primer 6.1.12, PRIMER Ltd.) was applied using all 10 scales and only the significant variables identified by the PCA, in order to define groups of different otolith shape. This clustering used the Euclidean distance metric and the complete linkage-clustering mode, which takes into account the minimum similarity of the individual modes. To explore the biological variables (SL, age, sex, sexual classes) and its relationship to the otolith shape, a Multi-dimensional Scaling analysis (MDS) (Primer 6.1.12, PRIMER Ltd.) was performed using also Euclidean distances of the complete

Table1. Number of red groupers captured during 1996 to 1999 in the Campeche Bank, indicating fishing zones, min-max SL (cm), GW (g) and number of whole otoliths (sagitta) per year.

	No. Individuals	Fishing Zones	SL (cm)	GW (g)	No. Otoliths
1996	118	I, VI, VIII	33.0-83.0	650-11100	101
1997	290	V,VI, XII	34.7-80.0	900-13200	261
1998	11	V	39.0-67.5	1000-7250	11
TOTAL	419				373

wavelets dataset (10 scales) and to the significant wavelets determined by the PCA, adding biological variables as factors for the graphic visualization in 2-D.

RESULTS

A total of n = 419 red groupers were captured throughout 1996 to 1999. Inshore specimens (n = 28) were collected by hook and line in shallow waters near Ría Lagartos (fishing zone XII), and offshore specimens (n = 391) by long lining in deep waters of the Campeche Bank at depths ranging form 30 to 90 m (fishing zones I, V, VI and VIII) (Figure 1).

Standard length (SL) ranged from 33.0 cm to 83.0 cm and gutted weight (GW) 650.0 g to 13200.0 g. Only 373 whole otoliths were analyzed, otoliths broken were discarded (Table 1).

Of 419 red groupers, assessed histologically, 64.91% were females, 33.17% males, 0.47% transitional and 1.43% were undistinguished. Otoliths meristics varied from 0.6679 - 1.1340 cm OW_i , and 1.2875 - 1.9992 cm OL. The otolith weight ranged from 0.0823 - 0.9516 g (Figure 2)

After automatically extracting the contour, the centroid and the radio of each otolith digital image (n = 373), ten wavelets (WLT) per 512 random points, were calculated. These results do not follow the parameters for normality and so, were analyzed using non- parametric statistics. According to a Kruskal-Wallis test there were no statistical differences between the ten WLT of the 373 otoliths ($p > 0.05$). A hierarchical clustering analysis, with all 10 WLT,

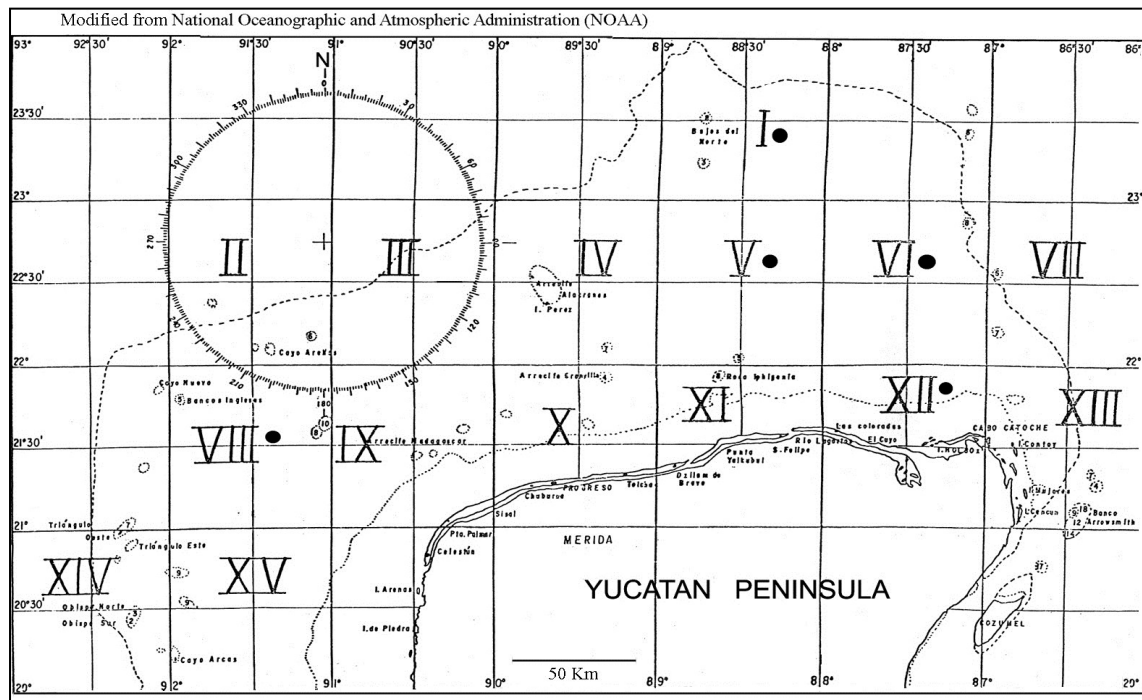


Figure 1. Map of the northern coast of the Yucatan Peninsula and the Campeche Bank divided into XV fishing zones. These zones are established by the INP (National Fisheries Institute) for reference purposes. Black dots represent sampling sites where red grouper individuals were captured by the artisanal and industrial fishing fleet during 1996 to 1999.

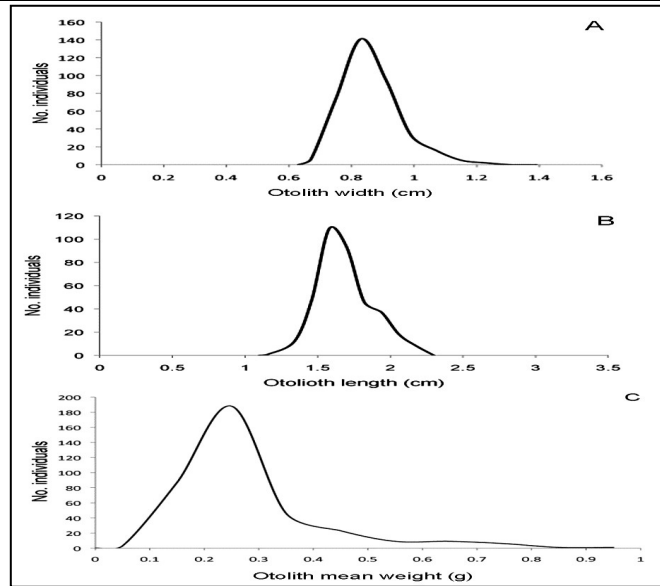


Figure 2. Frequency distributions of otolith meristics of *Epinephelus morio* (N= 373) from the Campeche Bank. (A) Otolith width (cm), (B) otolith length (cm) and (C) otolith mean weight (g).

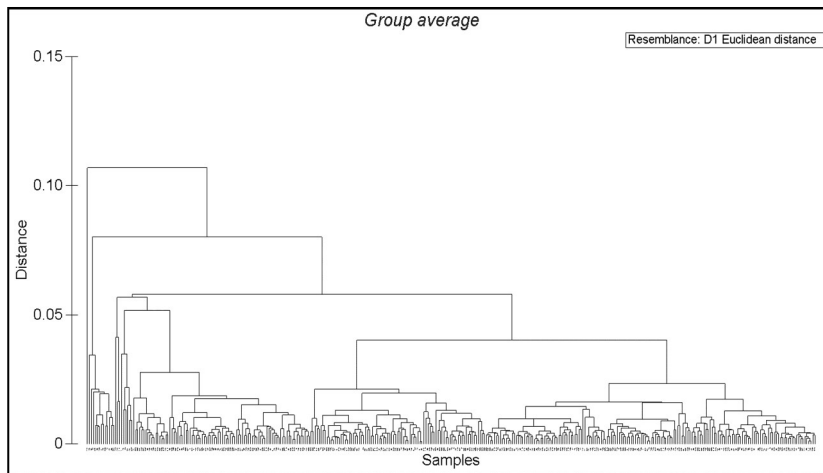


Figure 3. Dendrogram (WPGMA) representing differences between each WLT mean (ten WLT) for n = 373 *Epinephelus morio* respectively. Six groups could be distinguished at a 0.05 Euclidean distance.

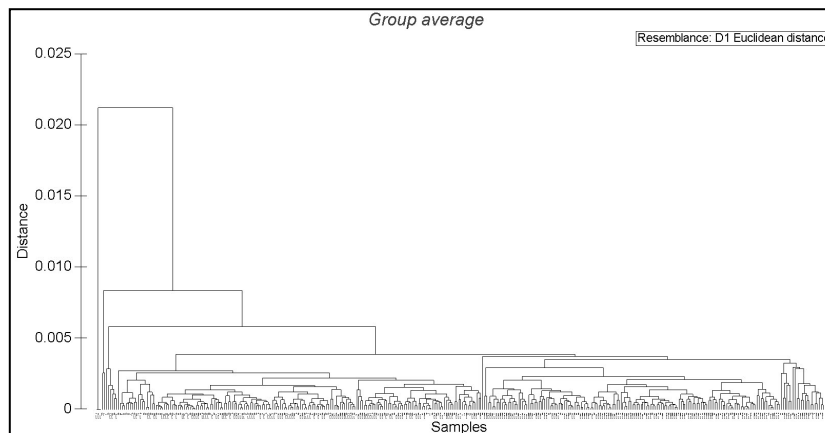


Figure 4. Dendrogram (WPGMA) representing differences between WLT5, WLT6 and WLT7 means for n = 373 *Epinephelus morio*. Four groups could be distinguished at 0.005 Euclidean distance.

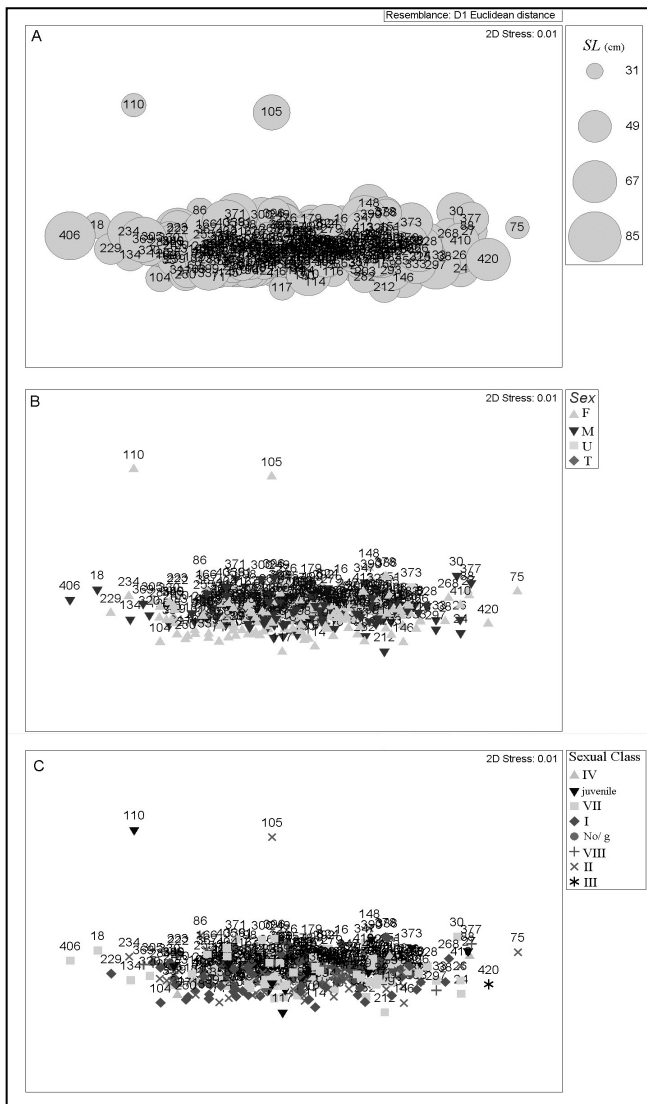


Figure 5. A 2-D visualization of an MDS analysis using the complete wavelets set from otolith shape, showing the Euclidean distance between the individuals and their relationship with some biological variables. The stress values (0.01) in each graphic, established an excellent representation of the data. **A)** Standard length (SL) where the bubble size represents the SL of the fish; **B)** sex per individual (F = females, M = males, U = undetermined and T = transitional) and **C)** sexual classes (I immature/resting female, II ripening female, III ripe-running female, IV spent female, VII ripening males VIII ripe-running male and No/g without gonad sample).

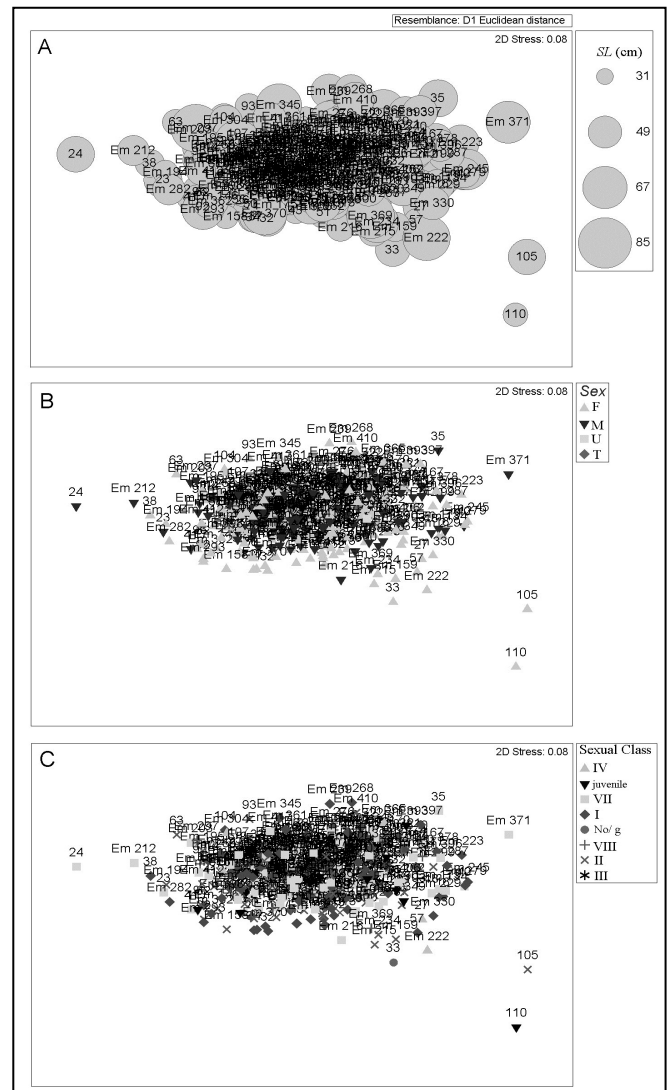


Figure 6. A 2-D visualization of an MDS analysis using the significant wavelets (WLT5, WLT6, WLT 7) from otolith shape, showing the Euclidean distance between the individuals and their relationship with some biological variables. The stress values (0.08) in each graphic, established a good representation of the data. **A)** Standard length (SL) where the bubble size represents the SL of the fish; **B)** sex per individual (F = females, M = males, U = undetermined and T = transitional) and **C)** sexual classes (I immature/resting female, II ripening female, III ripe-running female, IV spent female, VII ripening males VIII ripe-running male and No/g without gonad sample).

produced a cluster where the maximum difference (distance) between groups was 0.12 (out of a possible distance of 1.0), establishing six different groups at 0.05 (Figure 3).

A Principal Components Analysis (PCA) determined WLT 5, WLT 6 and WLT 7 as the best WTL descriptors, which were used to detect potential shape variations

between individuals. Another hierarchical cluster analysis was carried out using only these variables, indicating a distance between groups of 0.025, establishing four different groups at 0.005 (Figure 4).

A MDS analysis was applied to the complete dataset (10 WLT) in order to explore relationships between the otolith shape and the biological variables of the individuals

(Figure 5). The 2-D visualization of this analysis showed very low stress values (0.01), showing that the representation of the input data is excellent.

Similarly a MDS analysis was applied only to the significant WLT (WLT5, WLT6 and WLT7), displaying in a 2-D visualization analysis, a low stress values 0.08 resulting from a good representation of the input data (Figure 6). With these results, there are no apparent relationships between the otolith shapes and the biological variables.

DISCUSSION

Our results showed no statistical differences in otolith shape between individuals. The hierarchical clustering analysis of the otolith shape considering 10 WLT showed that the difference between groups was small; furthermore, when taken into account the 3 WLT determined as the best descriptors by a PCA, the difference between groups was reduced even more and could be considered as negligible.

Wavelets transforms are a very sensitive analysis that could identify small differences in otolith shape. Moreover, wavelet algorithm was preferred, over Fourier transform, because is suited for describing the frequencies of highly non-stationary signals with sudden peaks or discontinuities, such as otolith contour. Likewise, wavelets are considered a multi-resolution analysis where signals are analyzed at different levels of spatial scale, and is designed so that high frequency signal components are resolved better in terms of their location, and low frequencies values are better estimated but deficiently localized (Watkinson and Gillis 2005). This is particularly relevant for indentifying otolith contour, since these imperfections alter Fourier transform globally but only alter the wavelet transform locally (Parisi-Baradad et al. 2010). Another advantage of wavelets is the capacity to determine which points of the contour are more specific regions in the otolith.

Differences in otolith shape may be attributable to growth rate differences, and since growth rate vary more between than within stocks, otolith shape analysis can provide a measure of stock identity, as long as they remain at least partially segregated (Campana and Casselman 1993). Red grouper from the Campeche Bank seems to be distributed widely in different substrates (Albañez-Lucero and Arreguín-Sánchez 2009), presents a bathymetric distribution according to size length, is a generalized opportunistic carnivore (Brulé and Rodríguez-Canché 1993, Brulé and Déniel 1996), a monandric protogynous hermaphrodite, with a single annual spawning peak (January to March), and spawn on various grounds distributed all over the Campeche Bank (Brulé et al. 1999). These characteristics may explain the lack of differences in otolith shape among individuals because there is no evident segregation of different groups, during the red grouper life span.

It is important to address that since all the individuals were taken by the commercial and artisanal fishing fleets

the sampling may be skewed. These results may require additional independent surveys that may evidence geographic differences along de Campeche Bank.

Growth rate, and therefore the otolith shape, may vary by genetic and phenotypic factors being, the later, influence by sex, age, year class and environment (Cardinale et al. 2004). Nevertheless, there were no differences in otolith shape between individuals and their length, or between individuals of different sex and different sexual class (mature vs. immature individuals). The otolith length, width and weight displayed a normal distribution, where the presence of only one mode suggests, that most of the individuals belong to the same year class. To corroborate this, the determination of age per individual through the reading of annuli in otolith thin sections, is currently in process, and the analysis of differences in otolith shape per age and year class will be done. In addition, considering the importance of environmental factors, a micro-chemical analysis on a random number of these otoliths will also be carried out.

For years researchers have considered the red grouper fishery from the Campeche Bank as one stock (Arreguín-Sánchez et al. 1996, Burgos and Defeo 2004, Gimenez-Hurtado et al. 2005) and so far, we cannot state otherwise.

ACKNOWLEDGMENTS

This study was funded by the State of Yucatan Government in alliance with the Science and Technology Council of Mexico (CONACyT) grant FOMIX- Yuc- 2008- C06-108288.

LITERATURE CITED

- Albañez-Lucero M.O. and F. Arreguín-Sánchez. 2009. Modelling the spatial distribution of red grouper (*Epinephelus morio*) at the Campeche Bank, México, with respect substrate. *Ecological Modelling* **220**:2744–2750.
- Arreguín-Sánchez F., M. Contreras V., Moreno, R. Burgos, and R. Valdés. 1996. Population dynamics and stock assessment of Red Grouper (*Epinephelus morio*) fishery on Campeche Bank, Mexico. Pages 202–217 in: F. Arreguín-Sánchez, J.L. Munro, M.C. Balgos and D. Pauly (eds.) *Biology and Culture of Tropical Groupers and Snappers*. ICLARM Conference Proceedings, Manila, Philippines.
- Begg, G. and R. Brown. 2000. Stock identification of haddock *Melanogrammus aeglefinus* on Georges Bank based on otolith shape analysis. *Transactions of the American Fisheries Society* **129**:935–945.
- Begg, G.A., W.J. Overholtz, and N.J. Munroe. 2000. The use of internal otolith morphometrics for identification of haddock (*Melanogrammus aeglefinus*) stocks on Georges Bank. *Fisheries Bulletin* **99**(1):1–14.
- Brulé, T. and L. Rodríguez-Canché. 1993. Food habits of juvenile red groupers, *Epinephelus morio* (Valenciennes, 1828), from Campeche Bank, Yucatan Mexico. *Bulletin of Marine Science* **52** (2):772–779.
- Brulé, T. and C. Déniel. 1996. Biological research on the red grouper (*Epinephelus morio*) from the southern Gulf of Mexico. Pages 28–42 in: F. Arreguín-Sánchez, J.L. Munro, M.C. Balgos and D. Pauly (eds.) *Biology and Culture of Tropical Groupers and Snappers*. ICLARM Conference Proceedings, Manila, Philippines.
- Brulé, T., C. Déniel, T. Colás-Marrufo, and M. Sánchez-Crespo. 1999. Red Grouper Reproduction in the Southern Gulf of Mexico. *Transactions of the American Fisheries Society* **128**:385–402.

- Burgos, R. and O. Defeo. 2000. Un marco precautorio para la pesquería de mero (*Epinephelus morio*) del Banco de Campeche. *Océanides* **15**(2):129- 140.
- Burgos, R. and O. Defeo. 2004. Long- term population structure, mortality and modeling of a tropical multi-fleet fishery: the red grouper *Epinephelus morio* of the Campeche Bank, Gulf of Mexico. *Fisheries Research* **66**:325- 335.
- Burgos, R. and M. Pérez-Pérez. 2006. Mero (*Epinephelus morio*). in: P.J. Cuellar y C.O. Cademna (eds.) *Sustentabilidad y Pesca Responsable en México. Evaluación y Manejo*. SAGARPA- IPN. 544 pp.
- Campana, S.E. and J.D. Neilson. 1985. Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Science* **42**:1014–1032.
- Campana, S.E. and J.M. Casselman. 1993. Stock discrimination using otolith shape analysis. *Canadian Journal of Fisheries and Aquatic Science* **50**:1062- 1083.
- Cardinale, M., P. Doering-Arjes, M. Kastowsky, and M. Mosegaard. 2004. Effects of sex, stock, and environment on the shape of known -age Atlantic cod (*Gadus morhua*) otoliths. *Canadian Journal of Fisheries and Aquatic Science* **61**:158– 167.
- Galley, E.A., P.J. Wright, and F.M. Gibb. 2006. Combined methods of otolith shape analysis improve identification of spawning areas of Atlantic cod. *ICES Journal of Marine Science* **63**:1710-1717.
- García-Moliner G. and A.M. Eklund. 2004. *Epinephelus morio*. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.3. www.iucnredlist.org.
- Giménez-Hurtado, E., R. Coyula-Pérez-Puelles, S.E. Lluch-Cota, A.A. González- Yañez, V. Moreno-García, and R. Burgos-Rosa. 2005. Historical biomass, fishing mortality, and recruitment trends of the Campeche Bank Red Grouper (*Epinephelus morio*). *Fisheries Research* **71**:267–277.
- Hubbard, B.B. 1996. *The World According to Wavelets*. A.K. Peters Ltd., Wellesley, Massachusetts USA.
- Parisi-Baradad, V., A. Lombarte, E. García-Ladona, J. Cabestany, J. Piera, and Ó Chic. 2005. Otolith shape contour analysis using affine transformation invariant wavelet transforms and curvature scale space representation. *Marine and Freshwater Research* **56**:795 –804.
- Parisi-Baradad, V., A. Manjabacas., A. Lombarte., R. Olivella., Ó Chic., J. Piera, and E. García-Ladona. 2010. Automated Taxon Identification of Teleost fishes using an otolith on line database— AFORO. *Fisheries Research* **105**:13–20.
- Piera, J., V. Parisi-Baradad, E. García-Ladona, A. Lombarte, L. Recasens, and J. Cabestany. 2005. Otolith shape feature extraction oriented to automatic classification with open distributed data. *Marine and Freshwater Research* **56**:805–814.
- SAGARPA. 2008. Anuario estadístico de acuicultura y pesca. Comisión nacional de acuicultura y pesca. <http://www.conapesca.sagarpa.gob.mx>.
- Tracey S.R., J.M. Lyle J.M., and S.R. Guy Duhamelb. 2006. Application of elliptical Fourier analysis of otolith form as tool for stock identification. *Fisheries Research* **77**:138–147
- Watkinson D.A. and D.M. Gillis. 2005. Stock discrimination of Lake Winnipeg walleye based on Fourier and wavelet description of scale outline signals. *Fisheries Research* **72**:193–203.
- Wootton, R.J. 1999. *Ecology of Teleost fishes. Second Edition*. Fish and Fisheries Series 24, Kluwer Academic Press, Hingham, Massachusetts USA. 386 pp.
- Wright, P.J., J. Panfili, B. Morales-Nin, and A.J. Geffen. 2002. Types of calcified structures. Pages 31-57 in: J. Panfili, H. de Pontual, H. Troadec, and P.J. Wright (eds.) *Manual of Fish Sclerochronology A. Otoliths*. IFREMER, Plouzane, France.