Otolith Shape Analysis in Groupers from Different Southern Gulf of Mexico Habitats

Análisis de la Forma del Otolito en Meros de Diferentes Hábitats del Sur del Golfo de México

Analyse de la Forme de L'otolithe de Mérous du Sud Golfe du Mexique en Relation con su Habitat

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EXTENDED ABSTRACT

Introduction

Otolith morphology has been used in trophic studies and in the identification of population and species. Otolith shape is species–specific with significant interspecific variability mainly due to otolith growth. Otolith growth and therefore otolith shape is affected by genetic and environmental factors, such as depth, water temperature and substrate type, and phylogenetic relationships. But, it is also linked to food, swimming, spatial niche, and producing sounds during courtship or defending the territory. It is known for some fish species that otolith shape may be a reflection of their adaptability to water depth and to different strategies of capturing prey. Groupers are top predators distributed in warm temperate and tropical ecosystems and associated with deep water and hard bottom reefs. Most of them compete with other groupers species for food in the same habitat, therefore developing specialized strategies. The objective of this study was to explore possible differences in groupers otolith shape according to the type of habitat and depth they live in. Otolith shape variation was analyzed in sagittae of sixteen grouper species belonging to four genera (*Cephalopholis* spp. (1)*; Epinephelus* spp. (5); *Hyporthodus* spp. (4); and *Mycteroperca* spp. (6) from southern Gulf of Mexico. Taking into account their distinctive habitats and depths of occurrence, species were classified in:

i) Reef-associated species in shallow depths (10 - 60 m): *C. fulva, E. adscensionis, E. guttatus, E. striatus, M. bonaci, M. interstitialis, M. phenax, M. tigris* and *M. venenosa,*

ii) Soft bottoms and rocky ledges with 60 - 120 m depth: *E. morio* and *M. microlepis,* and

iii) Deep water species (< 120- 600 m) with hard bottoms: *E. drummondhayi*, *H. flavolimbatus, H. mystacinus, H. nigritus* and *H. niveatus*.

Methods

All groupers were captured in the southern Gulf of Mexico on Campeche Bank (19 - 24°N, 93 - 86°W) between April 1996 and April 1999 by the industrial fishing fleet. Total and standard length (nearest 0.1 cm), whole-body weight (nearest 1 g), sex (female, male, and transitional), and reproductive condition, were registered for each individual. The pair of sagittae were extracted through the gill arch, cleaned in alcohol, weighted and stored dry in paper bags. For each left sagitta a greyscale digital image (720 × 576 pixels) was obtained through a digital camera attached to a stereomicroscope under reflected light with the *sulcus acusticus* facing down and the *rostrum* to the right. Since otolith shape can be influenced by many factors, only sexually mature females were selected prior to the analyses.

Otolith morphometrics and shape indices — area, ellipse, rectangularity and roundness were measured using Image Pro Plus version 7.0 (Media Cybernetics Software). Size effects were removed by dividing each otolith shape variable between their respective otolith length.

Otolith contour — was measured by calculating wavelet transforms (WLTs) using the "Shape" module of Age & Shape software (version 1.0; Infaimon). The program automatically determines the otolith centroid as the mean *x* and *y* polar coordinates and then generates the otolith contour by tracing 512 equidistant radii from the centroid to the otolith contour in a clockwise direction. To ensure consistent interpretation of otolith shape across all otolith samples, radii were traced starting from the tip of the rostrum as the common point. Eleven WLT scale signals were generated from the finest (WLT0) to the coarsest (WLT10) but only WLT4 was used as it is considered to be the best contour descriptor. Radii were standardized prior to statistical analysis by dividing each one by the mean radial length.

Statistical analyses — To explore the possible differences between otolith morphometrics and indices per species with their type of habitat and depth Multi-Dimensional Scaling Analyses (MDS; Euclidean distance) with a superimposed Cluster (Hierarchical cluster; Euclidean normalized distance) (Primer v.6, PRIMER-E Ltd.) were performed. A 'Standard' Principal Components Analysis (PCA; CANOCO v4.5, Biometris- Plant Research International) of the species was passively projected to the ordination space to visualize the degree of separation among species taking into account type of bottom and depth.

Following Tuset et al. (2008, 2015) subsections of WLT4 decomposition of the contour (dorsal, ventral, posterior and anterior zone of the otolith) were selected to perform repeated analyses in each zone to find local patterns. The subsections were defined from the graphical representation of mean of the wavelet: anterior zone (*rostrum*; between 491 and 45 radii), ventral zone (from 46 to 174 radii), postero-dorsal zone (*post- rostrum*; between 175 and 374 radii) and antero-dorsal zone (*anti-rostrum*; from 375 to 490 radii). Monte Carlo permutation tests with 499 random permutations were performed for each otolith contour subsection to select the 20 radii with most variability (landmarks). Then as done for otolith morpho-metrics- indices, a MDS with Cluster analyses and a standard PCA were performed with the 20 selected WLT4 landmarks per subsection and related with the type of bottom and depth.

Finally to measure the interaction between type of bottom and depth with morphometrics, indices, and selected-radii of WLT4 a Permutational MANOVA test (PERMANOVA with 999 permutations of residuals; Primer v.6, PRIMER-E Ltd.) was carried out.

Results and Discussion

Of the sixteen grouper species a total of 375 sagittae were analyzed. Specimens' total length ranged from 27.7 cm (TL; *Cephalopholis fulva*) to 160 cm (TL; *Mycteroperca* bonaci). The MDS+ Cluster for otolith morphometrics and indices found three discernable groups at a 150 Euclidean distance (ED: stress = 0.01):

i) *E. striatus* and *C. Fulva*,
ii) *M. bonaci*, *M. venenosa*, *M. interstitialis*, *M. phenax*, *M. microlepis*, *E. morio*, *E. guttatus*, *H. mystacinus* and *H. Nigritus*, and
iii) *E. adscensionis*, *E. drummondhayi*, *H. niveatus*, *H. flavolimbatus* and *M. tigris*.

Nevertheless these groups separation do not relate to depth or type of bottom. The PCA ordination displayed the same individuals belonging to groups 1 and 3 of the MDS opposite in the diagram with the highest degree of separation but without relation to depth or type of bottom.

Through the analyses of the otolith contour by MDS+ Cluster and PCA of the four subsections, there were no ordination of the species related to type of bottom and depth. Nevertheless, through these analyses local patterns of otolith shape characteristics could be accounted. The MDS+ Cluster and the PCA of the anterior zone (*rostrum*) data showed a distinction between species with long peak *rostrum* (*E. drummondhayi* and *H. flavolimbatus*), long rounded *rostrum* (*H. nigritus* and *H. niveatus*), a short peaked *rostrum* (*H. mystacinus*), and a narrow short rounded *rostrum* (all the other species). The analyses of the antero-dorsal zone showed species with a concave thick *antirostrum* (*E. drummondhayi*), concave less developed *antirostrum* (*E. morio*), a *straight* angled *antirostrum* (*M. phenax*), a concave narrow and short *antirostrum* (*E. striatus*), and a narrow straight short *antirostrum* (all the other species). For the postero-dorsal otoliths displayed a high variability in this section. Species with rounded post-dorsal irregularities (*H. nigritus* and *E. drummondhayi*), posterior lanceolated with post- ventral irregularities (*M.*

phenax, M. interstitialis, E. adscensionis, E. guttatus, E. striatus and *E. morio*), posterior lanceolated ventrally irregular (*M. bonaci, M. microlepis, M. venenosa* and *H. mystacinus*), and the other three species (*M. tigris, C. fulva* and *H. flavolimbatus*) each with a particular *post rostrum*. Species with a straight irregular and developed ventral zone (*H. mystacinus, E. drummondhayi*), a concave very irregular ventral zone (*H. nigritus*), straight smooth ventral zone (*H. flavolimbatus*), and a straight less irregular ventral zone (all the other species). The PERMANOVA analysis displayed a P (perm) of 0.966 for depth and 0.642 for type of bottom that shows there is no interaction between otolith morphometrics, shape and selected WLT4 radii.

Even though the otolith shape descriptors used in this analysis could not be related to depth and type of bottom, the *ostium* area (anterior zone) of reef-associated species with shallow depths are wider and more conspicuous than species form other types of bottom or depths. Also, four (*E. drummondhayi, H. mystacinus, H. niveatus* and *H. nigritus*) of the deep-water species with hard bottoms displayed a more developed (thicker) and concave ventral zone than the other species. It is the ventral zone of the otolith that may be related to depth whereas the development of the *rostrum* that may be related to type of bottom (reef associated species). The authors found in other fish species that a development on the anterior and antero-dorsal zone may be related to an increase in the hearing capacities when they have a limited visual field due to habitat characteristics such as high substratum, which could be the case in our reef associated species. Moreover, these species may reduce interspecific competition by segregation their timing of higher behavioral activity to different windows of day-night cycle. Through the otolith shape analyses we could observe a high interspecific variability that may be a reflection of the adaptability of the species to different habitats, type of bottoms and depths. In groupers the ventral, anterior and antero-dorsal otolith contour zones may be important to analyze in order to established shape patterns related to ecological effects.

KEYWORDS: Otolith shape, groupers, southern Gulf of Mexico

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