Inter- and Intraspecific Differences of *Lutjanus campechanus* and *Lutjanus purpureus* in Otolith Shape

Diferencias Inter- e Intraespecíficas de la Lorma del Otolito en *Lutjanus campechanus* y *Lutjanus purpureus*

Différences Intra- et Interspécifiques de la Forme de L'otolithe chez *Lutjanus campechanus* et *Lutjanus purpureus*

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EXTENDED ABSTRACT

Discrimination of stocks, populations and cohorts are some of the most important issues in fisheries management. The *sagitta* shape analysis is an efficient tool to distinguish between species, populations or stocks components based on phenotypic characteristics, by high morphological variability. The otolith shape is markedly species specific and provides an assessment based on phenotypic characteristics mainly regulated genetically. Nevertheless there is a strong related otolith shape variability due to sex, age, year class and diet, as well as environmental conditions such as depth, temperature and substrate type. Therefore, variations in otolith shapes could be related to population differences and may be used in stock-discrimination and species ID studies. Our study assesses the use of the otolith shape analyses to explore possible inter and intra differences between *L. campechanus* in the Gulf of Mexico and *L. purpureus* in Eastern Venezuela, taking into account factors such as species, fishing areas, sex and group age.

Methods

Samples **—** Individuals were collected in Southwest Gulf of Mexico (*Lutjanus campechanus*) and Eastern Venezuela (*Lutjanus purpureus*), between December 2015 and December 2017 by a multi-species artisanal fishing fleet. 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 stereomicroscope with camera-PC station, previously placed over a black surface (to improve contrast), the *sulcus* always facing down and the *rostrum* to the right.

Otolith morphometrics and shape indexes **—** The software Image-Pro Plus®, was used to measure five morphometric variables (diameter maximum, diameter minimum, area, perimeter and ellipse) and four shape indexes (rectangularity, roundness, aspect and fractal dimension index). Size effects as a consequence of the variation in the growth rate of the fish, were normalized by dividing each otolith shape variable positively correlated, between the within-group slopes (*b*) of the linear regressions of each component on fish length.

Otolith contour **—** wavelet transforms (WLTs) were extracted using Age & Shape program. To obtain the otolith contour, the program automatically determines the otolith centroid as the mean *x* and *y* polar coordinates and traces 512 distances ororthogonal projections from the centroid to the otolith contour (radii) in a clockwise direction. Eleven discrete WLTs 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. As otoliths differentially develop specific characteristics by zones or subsections (dorsal, ventral, posterior and anterior zone), the analyses were performed in the anterior subsection (between 491 and 45 radii), which represents the rostrum size, and the posterior or posterodorsal subsection (between 175 and 374 radii) representing the *postrostrum*.

Statistical analyses **—** To explore the possible inter and intraspecific differences between otolith morphometrics, shape indexes and specific subsections of WLT4 contour among species, fishing areas, sex and group age. Multi-Dimensional Scaling Analyses (MDS), Permutational MANOVA (PERMANOVA) and SIMPER analyses (Primer v.6, PRIMER-E Ltd.) were performed. Otolith morphometrics and shape indexes were transformed before running the MDS in a triangular Euclidean distance matrix.

Results

A total of 132 left sagittae were analyzed: *Lutjanus campechanus* (N = 108) and *Lutjanus purpureus* (n = 24). Morphologically, the otoliths of *Lutjanus campechanus* and *Lutjanus purpureus* are similar in shape, with small specific variations. The otoliths of *L. campechanus* and *L. purpureus* have pentagonal overall shape with a concave-convex profile. For both species, *sulcus acusticus* is heterosucoidal, ostial with middle position and descending orientation. Present developed *rostrum*, *antirostrum* little developed and moderately curved cauda. Between species, otoliths varied markedly with respect

to the anterior and posterior region, the shape of the ostium and the edges. In *L. campechanus*, the anterior region is angular, while the posterior region is oblique, showing sinuous ventral edge with angular dorsal borders, and a funnel -shaped *ostium*. In contrast, otoliths of *L. purpureus* displayed a rounded anterior region with an angular posterior one, ventral and dorsal crenulated rims and a rounded *ostium*.

The MDS + PERMANOVA interspecific analyses discriminate between the two species taking into account morphometric and indexes variables (ED: 2D stress = 0.01; psed-F = 4.38, $p = 0.045$). Even though WLT4 anterior and posterior otolith zone did separate the two species (ED: 2D stress = 0.01) the PERMANOVA analyses did not displayed a statistically significant difference between the two groups (Anterior zone psed-F = 0.600, $p = 0.543$; Posterior zone psed-F = 1.05, $p = 0.375$). With respect to intra-specific comparison, *L. campechanus* morphometric and indexes variables discriminate groups in relation to:
area (ED: 2D stress = 0.01; psed-F = 35.77, $p = 0.001$),
age (ED: 2D stress = 0.01; psed-F = 208.11, $p = 0.001$) and sex (ED: 2D stress = 0.01; psed-F = 192.53, $p = 0.01$).
The three age groups (group age 1, age 2, and age 3), three fishing zones (Campeche, Tabasco and Veracruz) and in two groups of sex (juveniles + females and males). Variables that according to SIMPER analyses discriminate between groups were roundness, fractal index, aspect and area. The contour of the otolith by the anterior and posterior WLT4 descriptors subsections had a higher intrinsic variability for each individual of *L. campechanus* and even though they discriminate groups by the factors, these were not statistically significant. *Lutjanus purpureus* displayed more homogenous otolith morphometrics and indexes within discriminated groups with age as the most robust factor in the discrimination (psed-F = 19.04, $p = 0.001$). SIMPER analyses showed fractal index and roundness as the two variables that discriminate between groups by age, sex and fishing site. MDS analyses of the otolith contour by WLT4 anterior and posterior subsections discriminate by factors: two different areas, Sucre and Nueva Esparta in Venezuela (ED: 2D stress = 0.01); two age groups (ED: 2D stress = 0.01) and two sexes (ED: 2D stress = 0.01). Nevertheless WLT4 PERMANOVA analyses did not found statistically significant differences between the discriminated groups.

Discussion

Results of the otolith shape analysis indicate that otoliths of snappers from the Gulf of Mexico and the Caribbean Sea display very similar features like type of *sulcus acusticus* and *cauda*, but do show morphological differences that are useful to establish a clear differentiation between and within species. Interspecific morphological differences between otoliths of *L. campechanus* and *L. purpureus* were expected since otolith shape is specie-specific. Nevertheless, there has been debate whether these two species could be just one with different adaptations manly due to environmental particularities within the Caribbean and Gulf of Mexico. Our results showed that morphometrics, shape indexes and contour descriptors could discriminate individuals of both species and that otolith shape may be used as natural tags for separating these. As seen in other snappers, the highest interspecific variability was mainly observed in the anterior and posterodorsal otolith contour subsections. A recent study by Sadighzadeh et al. (2014) in snapper's otoliths noted variations in these similar otolith sections, which they associated with diel activity rhythms or ambient light conditions. These species live in distribution areas with different water conditions such as temperature, transparency, primary productivity, with variations in depth and substrate type that may result in otolith growth changes and therefore otolith shape differences. Intraspecific differences observed in the morphometric variables, shape indexes and WLT contour, may be related mainly to ontogenic changes and sex for both species. Particularly for *L. campechanus* differences may be related to the ontogenic stages of the individuals captured: juveniles in Campeche and adult females and males in Tabasco and Veracruz. Vignon (2012), suggest that the development of otolith shape is indeed an ontogenetic process reaching beyond the earliest life stages, that can reshape the overall otolith outline, regardless of individuals and small-scale environmental conditions. Otolith shape discrimination by sex within each species may also be a reflection on the changes in the pattern of otolith growth that may take place near the size at first maturity. At this size, the metabolism of the fish undergoes important changes that provide variations in the growth, affecting the otolith morphology (García-Díaz et al. 1997). Our results showed that morphological, shape indexes and wavelet analysis of otolith could be used as tool to identify and separate species and groups from distinct geographical areas, between sexes and sizes.

KEYWORDS: *Lutjanus*, otolith, morphometric

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