IDENTIFICATION OF GERREID SPECIES (ACTINOPTERYGII: PERCIFORMES: GERREIDAE) FROM THE PACIFIC COAST OF MEXICO BASED ON SAGITTAL OTOLITH MORPHOLOGY ANALYSIS

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Background. Although the species of the family Gerreidae have been subjected to many studies, their taxonomy at specific- and sometimes generic levels is still confusing. To contribute to the clarification of the taxonomy of the Mexican Pacific mojarras, the morphology of sagittal otoliths among six species of mojarras (Diapterus brevirostris, Eugerres lineatus, Eucinostomus dowii, E. entomelas, E. currani, and Gerres cinereus) was compared using size and shape descriptors. Otolith shape has long been known to be species-specific but this has not been tested in species of the family Gerreidae. Therefore, our goal was to explore the effectiveness of otolith descriptors for identifying gerreid fish at species level.

Materials and methods. Gerreid fish were captured between January 2009 and January 2010, off the Pacific coast of Mexico. The right- and left sagittae of 160 individuals were extracted for analysis. Size and shape descriptors considered in the presently reported analysis were: surface area, perimeter, length, width, rectangularity, ellipticity, roundness, circularity, aspect ratio, form-factor, Feret length, Feret width, Feret maximum, diameter maximum, diameter mean, diameter minimum, ratio maximum, and ratio minimum. Other measurements were made in the otolith region of sulcus acusticus, such as: cauda length, ostium length, ostium width, sulcus length, and rostrum width. Canonical discriminant analysis on otoliths morphology was used to identify differences between species.

Results. Rectangularity, roundness, otolith length, and Feret length were the main otolith descriptors that explain the inter-specific variability. Significant differences (Wilks’ lambdas (λ), \( P < 0.001 \)), high canonical correlation coefficients, and also a high classification success (overall mean >90%) allowed the separation of the species by using discriminant functions. Results from both G-test and Cohen’s kappa procedure confirmed the high rates of classification success obtained by the discriminant analysis.

Conclusion. These results suggest the usefulness of otolith morphology for differentiation of Gerreidae species from Mexican Pacific waters, thereby demonstrating that otolith shape is species-specific. Otolith morphology descriptions provided in this study is presented for the first time for the species included.

Keywords: Gerreidae, canonical discriminant analysis, otolith morphology, Mexico
Although gerreid fish have an important value for human consumption in tropical and subtropical regions, separate catch statistics are not reported for the majority of species and the landings are often summarized as mojarras. This lack of species-specific data is partly due to the difficulty in distinguishing many of the species (De La Cruz-Agüero and Galván-Magaña 1993). Traditionally, identification of the Gerreidae taxa has been difficult and time-consuming, because of the subjectivity of characteristics and overlap of meristic and morphometric characters provided in taxonomic keys (Bussing 1995). Other contributions to this taxonomic problem in the family are the inadequate or incomplete descriptions of type materials, their poor current condition, loss of some of them, and lack of published taxonomic reviews (Yáñez-Arancibia 1980).

Otoliths, the calcium carbonate structures located in fish inner ear, have been used as an auxiliary characteristic for identification of fishes in paleoichthyology (Nolf 1995) and stock recognition in fisheries management (Begg and Brown 2000, Tuset et al. 2003a, Neves et al. 2011), as well as a useful morphological character in taxonomy (Torres et al. 2000a, Tuset et al. 2006), systematics (Lombarte et al. 1991, Smale et al. 1995, Volpedo and Echeverría 2000), and food habits analysis (Radhakrishnan et al. 2010). Otolith shape has long been known to be species-specific (L’Abée-Lund 1988) and less variable in growth than body growth, presumably because otoliths are internal organs contributing to audition and balance (Lord et al. 2012), although its morphology can often vary geographically within a species (Lombarte and Lleonart 1993). In the majority of teleost species, sagittal otoliths are the largest pair, so they are the structures most frequently used in comparative studies because of the relative ease with which this structure can be reached (Nolf 1995). In addition, it has been established that from the three otolith pairs, sagitta is the pair with highest interspecific variation (Tuset et al. 2003b).

Comparison of otolith morphology using size parameters and shape indices of six Gerreidae species that inhabit the west Pacific coast of Mexico, including the Gulf of California was performed in the frames of the presently reported study. These species are important in small-scale artisanal fisheries of the country (Aguirre-León and Yáñez-Arancibia 1986): the short-beaked mojarra, Diapterus brevirostris (Sauvage, 1879); streaked mojarra, Eugerres lineatus (Humboldt, 1821); Dow’s mojarra, Eucinostomus dowii (Gill, 1863); dark-spot mojarra, Eucinostomus entomelas Zahuranec in Yáñez-Arancibia, 1980; Pacific flagfin mojarra, E. currani Zahuranec in Yáñez-Arancibia, 1980; and yellow fin mojarra, Gerres cinereus (Walbaum, 1792). Our main objective was to assess otolith morphology variation to test the hypothesis that otolith morphology distinguishes gerreid fish at species level and therefore, to contribute in highlighting mojarras taxonomy in the Pacific coast of Mexico.

MATERIALS AND METHODS

The right and left sagittae of 160 individuals were extracted for analysis, although sometimes it was not possible to obtain the complete pairs (230 sagittae in total). The gerreid fish were captured between January 2009 and January 2010, from the Pacific, off three Mexican states (Fig. 1): Baja California Sur (La Paz Bay, Magdalena Bay, and Espiritu Santo Island), Sinaloa (Mazatlan har-
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bour), and Guerrero (Acapulco harbour). These specimens came from artisanal catches of local fisheries and occasional samples by the authors. Each fish was measured to the nearest mm (standard length, SL). Otoliths were placed in distilled water just after extraction to clean them and stored in labelled glass vials. All fish and sagittae (hereafter called otoliths) were deposited and catalogued in the fish collection of the Centro Interdisciplinario de Ciencias Marinas (CICIMAR-IPN) at La Paz, B.C.S., Mexico.

Considering all sampled fish, body size varied from 55 mm SL in D. brevirostris and 245 mm SL in G. cinereus (Table 1). The sex was not considered separately because gerreid fish do not have sexual dimorphism (De La Cruz Agüero and Galván-Magaña 1993). Digital images of each pair of otoliths by species were taken with an Olympus® stereo-microscope model SZ61 fitted with an adapter for digital camera Olympus® SP-320 with 3× optical zoom and a light source Olympus® SZ2-LGBST to highlight edges of the structures. The otoliths were positioned systematically with the sulcus acusticus up and the rostrum pointing to the left before capture as bright objects on dark background.

Digitized images of each otolith were analyzed using the Image Pro Plus® program (version 6.0) to measure surface area (Ao; units in mm²), perimeter (Po), length (Lo), width (Wo), diameter maximum (Dmx), diameter minimum (Dmn), Diameter mean (Dm), ratio maximum (Rmx) and ratio minimum (Rmn)—all of them taken to the nearest 0.01 mm. Such measurements were used to calculate dimensionless shape indices (Tuset et al. 2003b, Ponton 2006, Pothen et al. 2006) which are independent from differences in otolith size (Tuset et al. 2006, Lord et al. 2012), which made in the otolith region that is associated with the sensory macula: sulcus acusticus (e.g., sulcus length (Sl), cauda length (CL), ostium length (OL), ostium width (OW), and rostrum width (RW)).

We used a Friedman’s Q test (FQT), a non-parametric test used to compare repeated observations on the same subjects belonging to three or more paired groups, to detect differences between sulcus acusticus ratios by species. The FQT makes no assumptions for data distribution (e.g., normality or homoscedasticity of variance) (Zar 2009).

Canonical discriminant analysis (CDA) was run for all fish with both otoliths pooled together as recommended by Morat et al. (2012), and used to assess whether species can be distinguished between each other based on otolith descriptors or not. Percentage of correct assignment of individuals, calculated within classification matrix, can then be used as a morphometric measurement of similarity between taxa and as an evidence for the a-priori established taxonomic structure (e.g., conventional identification produced by taxonomic keys). Quadratic Mahalanobis distances (MD) and their approximations to the F-statistics were used to assess significant differences between species centroids in the multivariate space. Also a dendrogram was constructed by hierarchical cluster analysis (UPGMA), based on the Euclidian distance of MD values to assess the degree of similarity between Gerreidae species by otolith descriptors.

Statistical significance of CDA was assessed by Wilks’ lambda (λ) test. Values close to zero indicate a good model fit and values ≈ 1 indicate lower discrimination power (Klecka 1980, Lord et al. 2012). Another way to judge the relevance of a discriminant function is by examining the canonical correlation coefficient (r²; Klecka 1980). If the groups are no very different on the variables being analyzed, then all of the correlations will be low. Classification efficiency (e.g., estimate error rates in CDA) was cross-validate according to Lachenbruch and Mickey (1968). The bias of the classification was determined with the Cohen’s kappa (κ) coefficient, which estimates the improvement over chance of the percent corrected classification rates (Tuset et al. 2003b).

Values of κ range from 0 to 1, with zero indicating that the discriminant analysis yielded no improvement over chance, while a κ of 1 occurs only with perfect agreement (Titus et al. 1984). The κ values were scaled up to a percentage, and the prior probability of classification was equal for all groups. Finally, a G-test of independence was used to test agreement between observed classification rates and expected classification rates (Tuset et al. 2003a). All statistical analyses were performed in XLStat® 2009, a statistical plug-in for MS Excel® 2007 spreadsheet program. Significance level was set at 0.05 for all statistical tests used.

RESULTS

The gross morphology and morphometry of the otolith are described by species according to Tuset et al. (2003a) and Volpedo et al. (2008), and all ratios are expressed as the average (see also Fig. 2 and Table 2): Diapterus brevirostris. Otolith shape: oblong or semicircular.

Table 1

| Species                  | N  | n  | Standard length [mm] |
|-------------------------|----|----|----------------------|
|                         |    |    | Mean | Range             |
| Diapterus brevirostris  | 22 | 37 | 156.8 | 55.0–170.0      |
| Eugerres lineatus       | 27 | 36 | 166.0 | 79.0–200.0     |
| Eucinostomus dotwii     | 12 | 21 | 162.0 | 120.0–168.0   |
| Eucinostomus entomelas  | 36 | 43 | 178.0 | 83.0–195.0    |
| Eucinostomus currant    | 28 | 45 | 140.3 | 60.0–170.0    |
| Gerres cinereus         | 35 | 48 | 223.0 | 110.0–245.0   |

N = number of individuals, n = number of otoliths (sagittae) examined.
Fig. 2. Morphology of sagittae in six species of the fish family Gerreidae, indicating terminology used in the study; The sulcus acusticus is facing the observer (right sagitae per species; the left sagitta with terminology, modified from Tuset et al. 2003a); Db = Diapterus brevirostris, El = Eugerres lineatus, Gc = Gerres cinereus, Ed = Eucinostomus dowii, Ee = Eucinostomus entomelas, Ec = Eucinostomus currani; Scale bar for otoliths = 1 mm, and 1 cm for fish.
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Otolith shape: semicircular. Sulcus acusticus: ostial to medium. Ostium: rectangular. Cauda: slightly curved. Anterior region: angled. Proximal margin: lobed. Dorsal margin: serrate. Summary of ratios: cauda length / ostium length (CL / OL) = 3.348; ostium length / ostium width (OL / OW) = 1.159; and cauda length / rostrum width (CL / RW) = 3.832.

Eucinostomus dowii. Otolith shape: oval. Sulcus acusticus: ostial to medium. Ostium: funnel-like. Cauda: strongly curved. Anterior region: angled. Proximal margin: lobed. Dorsal margin: dentate. Remarks: Some specimens showed deformations in the posterior region. Summary of ratios: cauda length / ostium length (CL / OL) = 1.413; ostium length / ostium width (OL / OW) = 1.261; and cauda length / rostrum width (CL / RW) = 3.049.

Eucinostomus entomelas. Otolith shape: semicircular. Sulcus acusticus: ostial to medium. Ostium: funnel-like. Cauda: slightly curved. Anterior region: angled. Proximal margin: lobed. Dorsal margin: serrated. Summary of ratios: cauda length / ostium length (CL / OL) = 2.488; ostium length / ostium width (OL / OW) = 1.415; and cauda length / rostrum width (CL / RW) = 1.684.

Eugerres lineatus. Otolith shape: oval. Sulcus acusticus: ostial to medium. Ostium: rectangular. Cauda: slightly curved. Anterior region: angled. Proximal margin: lobed. Dorsal margin: serrations were observed in specimens larger than 40 mm SL. Summary of ratios: cauda length / ostium length (CL / OL) = 2.528; ostium length / ostium width (OL / OW) = 1.251; and cauda length / rostrum width (CL / RW) = 3.049.

Gerres cinereus. Otolith shape: oblong. Sulcus acusticus: ostial to medium. Ostium: funnel-like. Cauda: strongly curved. Anterior region: angled. Proximal margin: sinuous. Remarks: proximal margin presents a projection that is directed toward the external face of the otolith, like a flap. Summary of ratios: cauda length / ostium length (CL / OL) = 2.118; ostium length / ostium width (OL / OW) = 1.261; and cauda length / rostrum width (CL / RW) = 2.664.

FQT was used to compare the data set for each of the twenty three descriptors only ten are not correlated at 5% significance level: diameter maximum (Dmx), ratio maximum (Rmx), rectangularity, otolength length (Lo), area (Ao), perimeter (Po), roundness, Feret length (Fl), Feret maximum (Fmx), and form-factor (Table 3).

Five discriminant functions produced were significant for discriminating the six gerreid species using ten descriptors of otoliths. The first three functions account for 93% of the variation (e.g., F1 (40%): \( \lambda = 0.006, P < 0.001; r^* = 0.905; F2 (33%): \lambda = 0.034, P < 0.001; r^* = 0.885; F3 (20%): \lambda = 0.156, P < 0.001\)) while the discriminant functions F4 and F5 made a negligible contribution and therefore, were not considered (\( r^* = 0.612 \) and \( r^* = 0.443 \), respectively). Interspecific discriminant analysis of otolith descriptors demonstrated that 90% (207) from total of sagittae (230) were correctly classified, ranging from 100% in E. currani to 76% in D. brevirostris (Table 4). Thus, misclassification rates varied between 0% (E. currani) and 24% (D. brevirostris). The Cohen’s kappa indicates that a classification efficiency of 88% better than would have occurred by chance alone (\( \kappa = 0.879; \) standard error = 0.024; 95% confidence interval = 0.832 to 0.926; \( Z = 36.6, P < 0.001\)). Also the G-test indicated that otoliths were assigned to the correct species at rates significantly greater than would be expected by chance (\( G = 669.811; DF = 25, P < 0.000\)).

Score plots for the first three discriminant functions show a separation between the six species although some overlapping can be noticed, all quadratic Mahalanobis distances and species centroids were significant (\( P < 0.001\) (Fig. 3). Among all descriptors, only diameter maximum and ratio maximum were not significant (\( P < 0.57 \) and \( P < 0.31 \), respectively). Otolith descriptors with highest contribution to eigenvalues were (standardized coefficients in parentheses): rectangularity (−24.7), otolength length (−17.9), and roundness (−8.9) for F1; rectangularity (−21.1), Feret length (−13.9), and roundness (5.8) for F2, and rectangularity (−20.6), Feret length (−13.9), and roundness (−8.5) for F3.

DISCUSSION

We found no studies for otoliths of the family Gerreidae, although they were synthetically treated in a study for selected west Atlantic species (Lemos et al. 1993). This family has historically been characterized by the complexity of its

**Table 2**

Otolith shape indices established from morphometric measurements taken on the mesial surface of each sagittae by species (see Tuset et al. 2003b)

| Shape indices | Formulae |
|---------------|----------|
| *Roundness* | \((4 Ao) (\pi FF)^{-1}\) |
| *Circularity* | \(Po^2 Ao^{-1}\) |
| *Ellipticity* | \((FL - Fw)(FL + Fw)^{-1}\) |
| *Rectangularity* | \(Ao(FFw)^{-1}\) |
| *Form factor* | \((4\pi Ao) Po^{-2}\) |

*Not correlated (\( P < 0.05\)).
Fig. 3. Scatter plots of first (F1) and second (F2), and first (F1) and third (F3) discriminant functions; Canonical discriminant analysis using ten otolith shape and size descriptors of the six species of Gerreidae; The first discriminant axis (F1) explains 40%, the second axis (F2) 33%, and the third axis (F3) 20% of the variation; Db = Diapterus brevirostris, El = Eugerres lineatus, Ed = Eucinostomus dowii, Ee = Eucinostomus entomelas, Ec = Eucinostomus currani, Gc = Gerres cinereus
Adaptations of the different sizes of fish to environmental conditions occurring at different depths (Lombarte 1992). This is not the case for Gerreidae species in present research, possibly due to the size range used and shallowness of commercial catches. However, there are certain patterns in proportions CL/OL and CL/RW of taxa that should be investigated: higher values in genus *Eucinostomus*, intermediate values in *G. cinereus*, and lower values in species of *Diapterus* and *Eugerres*. This morphometric pattern corresponds, to some extent, with the taxonomic and phylogenetic arrangements (as discussed below).

CDA results show that this set of morphological variables of otoliths produced a good discrimination among all considered species. Besides, morphological affinity between species corresponds to the current taxonomic arrangement, except for *E. currani*, as shown in the phenogram based on quadratic Mahalanobis distances (Euclidean distance = 75; Fig. 4). This morphological affinity is also close to the two proposed phylogenetic clades based on molecular data for the American gerreid genera: clade 1: *Eucinostomus* / *Gerres* and clade 2: *Diapterus* / *Eugerres* (see Ruiz-Carús and Uribe-Alcocer 2003, Chen et al. 2007), and agreeing with the same two morphological assemblages in taxonomic keys, characterized by the by the shape of preopercle and preorbital bone (Bussing 1995).

Actual separation rate in CDA with otoliths (93% considering the first three discriminant functions) is similar to several CDA’s reports for gerreid species although using variables of body morphology: 97% for *Eucinostomus* spp. in the Pacific coast (De La Cruz Agüero and Galván-Aguero 2000).

### Table 3

Mean values of the main otolith descriptors (not correlated at 5% significance level)

| Descriptor | Species          |
|------------|------------------|
|            | Db               | El     | Ed     | Ee     | Ec     | Gc     |
| Dmx        | 8.11 (0.69)      | 7.27 (0.84) | 6.95 (0.38) | 5.30 (0.35) | 6.81 (0.61) | 6.96 (0.81) |
| Rmx        | 4.14 (0.86)      | 3.76 (0.43) | 3.64 (0.21) | 2.73 (0.19) | 3.48 (0.40) | 3.65 (0.42) |
| Po         | 24.4 (2.41)      | 22.9 (2.95) | 19.7 (1.21) | 16.3 (1.44) | 20.4 (2.10) | 20.2 (2.02) |
| Lo         | 8.06 (0.70)      | 7.29 (0.83) | 7.0 (0.37)  | 5.30 (0.35) | 6.8 (0.60)  | 7.1 (0.82)  |
| Ao         | 28.8 (4.24)      | 21.9 (3.42) | 19.5 (1.82) | 14.4 (1.71) | 20.1 (3.10) | 16.9 (3.82) |
| Fmx        | 8.14 (0.70)      | 7.36 (0.83) | 7.03 (0.38) | 5.32 (0.34) | 6.88 (0.60) | 7.10 (0.82) |
| Fl         | 6.58 (0.51)      | 5.88 (0.51) | 5.59 (0.25) | 4.54 (0.28) | 5.50 (0.40) | 5.34 (0.58) |
| Ff         | 1.99 (0.19)      | 2.16 (0.15) | 2.13 (0.19) | 1.67 (0.19) | 1.87 (0.19) | 2.53 (0.28) |
| Rd         | 1.66 (0.20)      | 1.92 (0.35) | 1.58 (0.10) | 1.47 (0.16) | 1.69 (0.21) | 1.96 (0.24) |
| Rc         | 0.71 (0.02)      | 0.68 (0.03) | 0.72 (0.02) | 0.70 (0.02) | 0.69 (0.02) | 0.70 (0.02) |

Acronyms for species: *Db = Diapterus breviostris*, *El = Eugerres lineatus*, *Ed = Eucinostomus dowii*, *Ec = Eucinostomus entomelas*, *E = Eucinostomus curtani*, *Gc = Gerres cinereus*. Dmx = diameter maximum, Rmx = ratio maximum, Rc = rectangular length, Ao = area, Po = perimeter, Rd = roundness, Fl = Feret length, Fmx = Feret maximum, Ff = form-factor.

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Magaña 1993) and 96% and 93% for Pacific and Atlantic species of *Eugerres*, respectively (González Acosta et al. 2005, 2007). In other studies the classification accuracy obtained using otolith descriptors has varied between 70% and 95% (Tuset et al. 2006), indicating that otolith morphology can be used for identifying and separating Gerreidae species and it is likely that an individual is better characterized by its two otoliths rather than by only one of them (see Morat et al. 2012).

Although it has been established that otolith shape is markedly species-specific (L’Abée-Lund 1988, L’Abée-Lund and Jensen 1993), it is also known that there are external driving forces acting on otoliths shape, whatever the species (Lord et al. 2012) including: age, sex, size, food availability, substrate, water temperature, as well as other biological and environmental factors, linked to the species and geographical sites (Tuset et al. 2003a). In the presently reported study, considering that some of these factors are related to sample locations along the Pacific coast of Mexico, and that our analyses were made regardless of sex and minimizing size effect considering mostly adult specimens, there may be a confounding effect increasing the misclassification rate in some species (e.g., *D. brevirostris*: 24% and *G. cinereus*: 21%; Table 4). Another possibility could have been an inadequate sample size in that species; according to the criterion that relates the number of variables with the number of individuals (Junquera and Peréz-Gándaras 1993).

Nevertheless, we can conclude that otolith morphology can be used for separation of gerreid species through CDA. Otoliths descriptors here studied (e.g., rectangularity, roundness, otolith length, Feret length, and CL/OL and CL/RW ratios) can also be used as additional features to those already used for proper taxonomic identification, with possible implications of phylogenetic value. For further research on otolith morphology of Gerreidae, would be desirable increasing the number of sagittae per species, and the use of geometric morphometry (e.g., contour analysis) because it might increase the discrimination power and precision of species classifications (Chen et al. 2011).

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**Table 4**

| Actual group | Predicted group | SBS | CC [%] |
|--------------|-----------------|-----|--------|
| Db           | El              |     | (37) 75.67 |
| El           | Ed              |     | (36) 97.22 |
| Ed           | Ee              | 20  | (21) 95.232 |
| Ee           | Ec              | 41  | (43) 95.34 |
| Ec           | Gc              | 45  | (45) 100 |
| Gc           |                 | 38  | (48) 79.16 |
| Total        |                 | 35  | (230) 90.43 |

Acronyms for species: Db = *Diapterus brevirostris*, El = *Eugerres lineatus*, Ed = *Eucinostomus dowii*, Ee = *Eucinostomus entomelas*, Ec = *Eucinostomus currani*, Gc = *Gerres cinereus*; SBS = sagittae by species; CC = correct classification percentage by species; Underlined: quantity of correctly classified otoliths for each species; sample sizes in parentheses; Overall classification success of 90.43%; Cohen’s kappa = 0.879, *P* < 0.001; G-test = 669.8, *P* < 0.000.
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