

1 **White mullet *Mugil curema* population structure from Mexico and Brazil revealed by otolith**  
2 **chemistry**

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26

27 **Abstract**

28 The white mullet *Mugil curema* supports several fisheries in the neotropic, nevertheless, the  
29 population structure is still elusive. The goal of this study was to assess the presence of adult  
30 management units and nursery areas from five sampling sites throughout the Gulf of Mexico and  
31 northern Brazil using otolith microchemistry. The Li/Ca, Na/Ca, Mn/Ca, Sr/Ca, Ba/Ca, and Pb/Ca  
32 ratios were measured in otolith core (juvenile stage) and edge (adult stage) (N=131) by LA-ICPMS.  
33 Several ratios were significantly different between sampling sites for core and edge ( $p<0.05$ ). For  
34 otolith edge, PERMANOVA showed significant differences ( $p<0.05$ ) between all sampling sites  
35 from Mexico (except between Mecoacán and Tamiahua,  $p>0.05$ ) and between Mexico (pooled  
36 samples) and Brazil. Quadratic discriminant analyses showed jackknifed classification higher in the  
37 edge (66.6% and 99.5% for Mexico and Brazil plus Mexico, respectively) than in the core (46.3%  
38 and 76.5% Mexico and Brazil plus Mexico, respectively). The two cluster analyzes based on the core  
39 microchemistry (Mexico and Brazil plus Mexico) produced three main clusters, which did not  
40 coincide with catchment areas. These results support the segregation of the *M. curema* adult life  
41 stages among several sampling sites from Mexico and Brazil, moreover, core analysis suggested that  
42 the nursery areas did not correspond to the capture sites or adults stocks.

43 **Keywords:** catadromous; mugilidae; fish stock; otolith microchemistry; laser ablation; LA-ICP-MS

## 44 **1. Introduction**

45 *Mugil curema* (Valenciennes 1836), commonly known as white mullet, is a widely distributed  
46 species from the Mugilidae family that inhabits the Atlantic Ocean (from Nova Scotia to Argentina  
47 in the east, from Gambia to the Congo in the west coast of Africa), and the eastern Pacific Ocean  
48 (from the Gulf of California to Northern Chile) (Crosetti *et al.*, 2016a; Froese & Pauly, 2019). Even  
49 though *M. curema* was considered a species well differentiated from its congeners by its meristic  
50 counts and morphological characters, in the last decade, genetic studies have shown that the *M.*  
51 *curema* found in the Americas was a complex of cryptic species (Nirchio *et al.*, 2005; Heras *et al.*,  
52 2006, 2009; Fraga *et al.*, 2007; Durand *et al.*, 2012; Crosetti *et al.*, 2016a, 2016b); while all African  
53 *M. curema* belonged to a unique evolutionary lineage, endemic to Africa (Crosetti *et al.*, 2016b). In  
54 addition, mugilids include a complex of estuarine species morpho-ecologically similar, but with a  
55 long and differentiated evolutionary history. *Mugil curema* and *M. rubrioculos* are among the species  
56 that have diverged in more time; however, they are morphologically more similar than *M. liza*, which  
57 is most recent species (Neves *et al.*, 2020).

58 The white mullet conforms to a bi-partite life history in which different life stages utilize diverse  
59 aquatic environments (Barletta & Dantas, 2016). Adult fish spawn in coastal waters, the eggs and  
60 larvae are dispersed by physical oceanographic processes into estuaries, where late-stage larvae settle  
61 and develop as juveniles (Moore, 1974). This mullet is a commercially important resource from  
62 Brazil all through the Caribbean, in aquaculture as well as in fisheries exploitation, industrial and  
63 artisanal (Avigliano *et al.*, 2015a; Pacheco-Almanzar *et al.*, 2017). In Brazil, *M. curema* is one of the  
64 most consumed fishing resources (>18,000 tons/year estimated catches) (Santana *et al.*, 2018; Lima  
65 *et al.*, 2019), as well as in Mexico, where it is the most abundant and consumed mullet species  
66 (Avigliano *et al.*, 2015a; Pacheco-Almanzar *et al.*, 2017).

67 The information available on the population structure and migration of *M. curema* in the Atlantic is  
68 scarce. Based on microsatellite markers (Pacheco-Almanzar *et al.*, 2017) and scale shape (Pacheco-

69 Almanzar *et al.*, 2020) the presence of at least three groups of *M. curema* was suggested for the Gulf  
70 of Mexico. Nevertheless, in spite of the commercial and social importance of this mullet all  
71 throughout the western Atlantic coast, the population structure of young and adult stages is not well  
72 known along this region. The knowledge about the structuring of the population in different nursery  
73 groups and adults is a necessary input to design adequate management strategies and guarantee the  
74 sustainability of the resource (Cadrin *et al.*, 2013).

75 Different methods have been used to identify fish stocks and nursery areas such as mark-recapture,  
76 parasites and the analysis of otolith features, such as morphometry and chemical composition  
77 (MacKenzie *et al.*, 2008; Sturrock *et al.*, 2012; Avigliano *et al.*, 2014; Clément *et al.*, 2014). Otolith  
78 microchemistry has been a good alternative for the study of fisheries because it reflects both  
79 exogenous (e.g. environment) or endogenous (e.g. genetics) factors, which can contribute to  
80 detecting differences in chemical signatures between different stocks (Tzadik *et al.*, 2017; Hüseyin *et al.*,  
81 *et al.*, 2020). Otoliths are calcium carbonate structures located in the inner ear of fish. Calcium  
82 carbonate, precipitated mainly in the form of aragonite together with different trace elements, is  
83 continually deposited throughout the life of the fish (Campana, 1999; Benson *et al.*, 2019). Since the  
84 structure of otoliths is acellular and metabolically inert, once elements are incorporated from the  
85 endolymphatic fluid, their concentrations remain fixed over the life history of the fish (Thomas &  
86 Swearer, 2019). Elemental deposition in the otolith is influenced by physiological and environmental  
87 factors, then spatial and temporal differences in otolith elemental composition can be interpreted to  
88 distinguish between groups of fish that experienced different endogenous and exogenous conditions  
89 (Wang *et al.*, 2010; Campana, 2014; Avigliano, *et al.*, 2019). In other words, major and trace  
90 inorganic elements (e.g. Li, Na, Mn, Sr, Ba, and Pb) deposited represent a permanent record of the  
91 environmental conditions where the fish was at a particular time and they are a useful tool to study  
92 the population structure when there are physiological or environmental differences between study  
93 sites (Campana, *et al.*, 2000; Ruttenberg *et al.*, 2005; Thomas & Swearer, 2019).

94 There are many precedents on the use of otolith microchemistry for the study of population structure  
95 of fish (Moreira *et al.*, 2018; Soeth *et al.*, 2019; Macdonald *et al.*, 2020), even in mugilids species  
96 such as *M. liza* (Callicó Fortunato *et al.*, 2017b; Lemos *et al.*, 2017) and *M. cephalus* (Callicó  
97 Fortunato *et al.*, 2017c). Particularly for *M. curema*, otoliths have been used to study their habitat use  
98 in Brazil (Mai *et al.*, 2018), the discrimination of phenotypic stocks, age determination and migratory  
99 history in Mexico (Ibañez-Aguirre & Gallardo-Cabello, 1996; Ibañez *et al.*, 2012, 2017), and  
100 population identification and recruitment in Venezuela (Marin *et al.*, 2003; Avigliano *et al.*, 2015a);  
101 applying different techniques like trace elemental ratio analyses and morphometry and  
102 microstructure analyses. Avigliano *et al.* (2015) have suggested the existence of potential segregation  
103 of this mullet in the Caribbean Sea (Venezuela) based on Sr/Ca and Ba/Ca ratios in whole otolith.  
104 Ibañez *et al.* (2012) performed core-to-edge transects of Sr/Ca to study the migratory pattern of *M.*  
105 *curema* from Mexican coastal waters. They reported a high plasticity of euryhaline patterns, as  
106 described in other species such as *M. liza* and *M. cephalus* (Whitfield *et al.*, 2012; Callicó Fortunato  
107 *et al.*, 2017b); nevertheless, these authors observed that the expected catadromous behavior is less  
108 frequent in *M. curema*. On the other hand, based on core-to-edge transects of Sr/Ca and Ba/Ca, Mai  
109 *et al.* (2018), have classified *M. curema* from a small estuary in southeastern Brazil as a marine  
110 migrant species.

111 The aim of this study was to assess the presence of potential adult management units and analyze if  
112 there are different nursery areas used by *Mugil curema* from the Gulf of Mexico to northern Brazil  
113 by means of otolith microchemistry.

114

## 115 **2. Materials and methods**

### 116 **2.1. Sample collection and preparation**

117 Adult fish (N=131) of commercial species *M. curema* (Table 1), were collected between November  
118 2009 to March 2010 using trammel nets from the Gulf of Mexico, and gill nets in Southwestern

119 Atlantic (Figure 1). In the Gulf of Mexico, fish were caught from Mecoacán (N=17, Total length,  
120 TL=29.7±2.4 cm), Alvarado (N=19, TL=29.4±1.6 cm), Tamiahua (N=19, TL=29.9±2.1 cm) and  
121 Laguna Madre (N=20, TL=30.2±1.5 cm) (Figure 1). In the Southwestern Atlantic, fish were caught  
122 from the north-eastern coast of Brazil, in Alagoas state (N=56, TL=29.1±2.0 cm) (Figure 1). The  
123 samples from México used in this study have previously been analyzed to evaluate stock structure  
124 using other methods (Ibáñez *et al.*, 2017; Pacheco-Almanzar *et al.*, 2017).

125

## 126 **2.2. Ethical Statement**

127 The capture complied with animal welfare laws, guidelines and policies, approved by each  
128 competent national authority. In Mexico the white mullet were collected under the Official  
129 Mexican Norm (NOM-016 PESC-1994) for commercial species. In Brazil, fishing license was  
130 granted by the Instituto Chico Mendes de Conservacao de Biodiveridade of Ministerio do Meio  
131 Ambiente (license number 64835-2). For all sites, fish were euthanized humanely by being placed  
132 directly into an ice water bath upon capture.

133

## 134 **2.3. Sample processing**

135 After recording total length (mm), fish were sexed and the sagittal otoliths were extracted using  
136 surgical steel instruments previously washed with distilled water. Otoliths were weighted,  
137 decontaminated with 2% ultrapure HNO<sub>3</sub> (Merck KGaA, Germany) for 10 seconds, rinsed three  
138 times with ultrapure water at resistivity of 18.2 MΩ/cm (Milli-Q, Brazil), and embedded in crystal  
139 epoxy resin (Biolé *et al.*, 2019). They were sectioned transversely through the core by using a  
140 Buehler Isomet low speed saw (Hong Kong, China) (Avigliano, *et al.*, 2019). Otolith sections were  
141 fixed to glass slides with resin, manually polished using a decreasing gradient of sandpaper (from 9  
142 to 3 µm-grit), rinsed with Milli-Q water, and sonicated for 10 min before chemical analysis.

143

#### 144        **2.4. Chemical analysis**

145        One of the main assumptions of otolith chemistry as a method for studying population structure is  
146        that fish of similar age must be compared (or the same life stage between sites) (Campana, 2014). Due  
147        to the difficulty presented in age reading of some specimens, mainly in those caught in tropical areas  
148        (Ibáñez, 2016), the age of the fish was not determined. This issue was solved by using a narrow  
149        size range and collecting the samples in a reduced time window, as it was previously done by other  
150        authors (e.g. Edmonds *et al.*, 1999; Gillanders *et al.*, 2001; Chittaro *et al.*, 2006; Turan, 2006; Callicó  
151        Fortunato *et al.*, 2017a, 2017b; Lemos *et al.*, 2017; Döring *et al.*, 2019). Moreover, several authors  
152        have indicated that the use of total length is a useful estimator of age for *M. curema*, especially in  
153        environments where there is low seasonal heterogeneity (mainly temperature) (Ibáñez & Gallardo,  
154        1996; Ibáñez Aguirre *et al.*, 1999; Ibáñez Aguirre & Gallardo-Cabello, 2004; Ibáñez, 2016). Based  
155        on these studies, we have used specimens between 4 and 5 years old.

156        Because the otolith material is continuously deposited and not reabsorbed (Campana, 1999), the core  
157        area chemistry corresponds to the early stage of life and is a useful nursery area natural marker  
158        (Avigliano *et al.*, 2016, 2018a; Biolé *et al.*, 2019; Lattuca *et al.*, 2020). Otherwise, the outer area  
159        composition, which represents the last time of life, is often used as a stock indicator (Avigliano *et al.*,  
160        2017b; Biolé *et al.*, 2019; Campana, 2014). Here, the last year of life was considered instead of the  
161        outermost spot of the edge because this last one represents the site and moment of capture. In this  
162        sense, the outermost spot of the edge represents a useful habitat marker (environmental picture) but  
163        not population tag, which requires a type of analysis that allows connectivity between the different  
164        sampling sites (Avigliano, *et al.*, 2019).

165        Chemical analyses were based on the core and edge areas. Core was defined as the area within the  
166        first annual mark (first year of life), representing the nursery period (Figure 2). The otolith edge was  
167        defined as the outer portion of the otolith, representing the last year of life.

168 No specimens were used where those rings were not easily identifiable. Thus, the same period of  
169 time was used in all fish (the first and last year of life) (Figure 2).

170 The isotopes  $^7\text{Li}$ ,  $^{23}\text{Na}$ ,  $^{43}\text{Ca}$ ,  $^{55}\text{Mn}$ ,  $^{88}\text{Sr}$ ,  $^{138}\text{Ba}$ , and  $^{208}\text{Pb}$  were quantified in the core and edge areas  
171 of the otoliths by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS)  
172 using a 193 nm ArF Excimer laser (Photon Machines Analyte G2, USA) coupled to an ICP-QMS  
173 Agilent 7700 (Japan). Measurements were performed in scan mode, where the two transects were  
174 oriented radially and moved from otolith interior to exterior at 5  $\mu\text{m/s}$ .

175 Samples were measured during three analytical seasons using a laser-induced spot size of 40  $\mu\text{m}$ , a  
176 laser fluence of 3.75  $\text{J/cm}^2$ , and repetition rate of 10 Hz. He was used as carrier gas in the ablation  
177 cell, and Ar was also added before entering the ICP that was operated at 1600 W in an Ar plasma  
178 gas. To control the oxide production rates and plasma robustness the  $^{238}\text{U}/^{232}\text{Th}$  ( $\sim 1.2$ ) and  
179  $^{232}\text{Th}^{16}\text{O}/^{232}\text{Th}$  ( $< 0.004$ ) ratios were monitored on the reference material NIST 612 (trace elements  
180 in glass, National Institute of Standards and Technology, USA). Three standard reference materials  
181 (SRM), NIST612, NIST610 (trace elements in silicate glass) and MACS-3 (trace elements in  
182 synthetic calcium carbonate), were measured in triplicate at the beginning and at the end of the each  
183 session. These three SRMs were also analyzed every 10 transects to monitor drift. NIST612 and  
184 38.3% weight Ca (Yoshinaga *et al.*, 2000) were used as calibration and internal standards,  
185 respectively, to convert elemental intensities (counts per seconds) to concentrations; while NIST610  
186 and MACS-3 USGS were used as secondary standards (Pearce *et al.*, 1997; Jochum *et al.*, 2011).  
187 Recoveries rates based on NIST610 and MACS-3 USGS were within 0-15 % (Li=105, Mn=97,  
188 Sr=105, Ba=101, Pb=115) and 0-11 % (Li=89, Na=91, Mn=95, Sr=102, Ba=100, Pb=110) of  
189 GeoREM (<http://georem.mpch-mainz.gwdg.de>) preferred values for NIST 610 and MACS-3,  
190 respectively. Precision was estimated by the relative standard deviation percentage (RSD, %) of  
191 quadruplicate secondary standards (Biolé *et al.*, 2019). RSDs were below 4%.

192 Elemental concentrations were expressed in relation to Ca (in mmol/mol and  $\mu\text{mol/mol}$ ).

193

## 194 **2.5. Statistical approach**

195 This study compares four sites in the Gulf of Mexico, that are separated by  $\sim 300$  km, with a site in  
196 Brazil, which is over 10,000 km away (Figure 1). Then, to operate on similar spatial scales and  
197 sample sizes, a statistical design based on two levels was performed. First, the elemental signatures  
198 between the four sites within the Gulf of Mexico were compared. This also allowed for direct  
199 comparison of results with previous studies based on *M. curema* stock structure in this region (Ibáñez  
200 *et al.*, 2017; Pacheco-Almanzar *et al.*, 2017). Second, the elemental signatures between the Gulf of  
201 Mexico and Brazil samples were compared. For that, the Gulf of Mexico sites were pooled to  
202 produce a single signature for the region. All analyzes reported below were performed considering  
203 this statistical design.

204 Elemental ratios were tested for assumptions of normality (Shapiro-Wilk test) and homogeneity  
205 (Levene test) of variance considering the two levels of analysis separately. For the Mexico data set,  
206 Li/Ca (edge) and Mn/Ca, Sr/Ca, Ba/Ca and Pb/Ca (core) met both normality and homogeneity  
207 (Shapiro-Wilk and Levene's,  $p > 0.05$ ) after log transformation. For the Mexico plus Brazil data set,  
208 only Mn/Ca and Li/Ca (edge) and Pb/Ca (core) met both normality and homogeneity after log  
209 transformation (Shapiro-Wilk and Levene's,  $p > 0.05$ ). The rest of the ratios did not meet the  
210 parametric assumptions even after transformation. Given that differences in fish size or age could  
211 affect the incorporation rate of some trace elements into the otolith, it was necessary to remove the  
212 potential growth effect from the statistical analysis (Campana, *et al.*, 2000). Then, to insure that  
213 potential variations in fish growth between samples did not confound any stock-specific differences  
214 in elemental fingerprint, the effect of distance measured for each portion of the otolith (the first  
215 annual increment for the core, and the outer portion representing the last year of life for the edge),  
216 total length and otolith weight on the elemental ratios were tested using analysis of covariance

217 (ANCOVA) and correlation test (Pearson or Spearman depending on the fulfillment of the  
218 parametric assumptions) (Avigliano *et al.*, 2015b, 2018b). No elemental ratios showed a significant  
219 correlation with the analyzed distance of the otolith, total length or otolith weight (ANCOVA and  
220 correlation tests,  $p > 0.05$ ), therefore, no variable was corrected. The sex effect on otolith chemical  
221 composition was tested between sampling sites by using permutational multivariate analysis of  
222 variance (PERMANOVA) with 9999 permutations and based on Mahalanobis distance (Anderson,  
223 2006). No significant differences were found between sexes for any level of analysis, then,  
224 specimens of both sexes were pooled for analysis ( $0.2 < F < 0.7$ ,  $p > 0.05$ ).

225 According to the meeting of the assumptions, parametric (ANOVA) and non-parametric (Kruskal-  
226 Wallis) univariate analysis of variance, followed by pairwise multiple comparison procedures (Tukey  
227 test and Dunn's test for ANOVA and Kruskal-Wallis, respectively), were performed to compare the  
228 elemental ratios between sampling sites from Mexico for otolith edge and core separately. In  
229 addition, pairwise multiple comparisons among Mexico and Mexico plus Brazil were performed by  
230 using T-Student's and Mann Whitney U tests, depending on the fulfillment of the parametric  
231 assumptions. Pairwise tests via PERMANOVA and Hotelling's T-squared were also performed to  
232 test differences in the multi-elemental otolith fingerprints (otolith edge and core separately) between  
233 sampling sites for Mexico and Mexico plus Brazil, respectively. Quadratic discriminant analyzes  
234 (QDA) based on sample sizes and group numbers were performed to assess the ability of the data set  
235 to sort fish into specific catch area. QDA was used instead of linear discriminant analysis because the  
236 assumption of homogeneity of variance co-variances of the matrix was not met (Box test,  $p < 0.05$ ).  
237 The classification accuracy was evaluated by leave-one-out cross-validation and multicollinearity  
238 was tested by obtaining the tolerance value (Hair *et al.*, 2010).

239 Because the chemical composition of the core corresponds to the early stage of life and it may not be  
240 associated with the capture area, a Ward's hierarchical clustering analysis without prior classification  
241 was conducted to explore the similarity between the samples according to (Tanner *et al.*, 2012). The

242 number of clusters was assessed using the visual aspect of the dendrograms. The match between the  
243 dendrograms and the proximity matrix was evaluated by the cophenetic correlation coefficient which  
244 is considered optimal when it exceeds 0.8 (Rohlf, 1970). Finally, nonmetric multidimensional scaling  
245 (nMDS) analyzes, based on Euclidean distances, were also performed to corroborate the obtained  
246 grouping in the multielemental fingerprints between clusters (Tanner *et al.*, 2012). Goodness-of-fit  
247 was evaluated with the Stress value, which was considered fair below 0.1 (Kruskal, 1964).

248 Statistical tests were performed using the Systat 13 and PAST software.

### 249 **3. Results**

#### 250 **3.1. Otolith edge**

##### 251 **3.1.1. Mexico**

252 The Li/Ca and Pb/Ca ratios were significantly lower in Alvarado compared to the other sites (Table 1  
253 and Figure 3). The Mn/Ca ratio was significantly higher in Tamiahua, while no significant  
254 differences were found between the rest of the sites (Table 1 and Figure 3). Laguna Madre showed  
255 the highest values of Sr/Ca, while Alvarado had the lowest values and no significant differences were  
256 found between the other sites (Table 1 and Figure 3). The Ba/Ca ratio was significantly higher in  
257 Alvarado and lower in Mecoacán and Tamiahua (Table 1 and Figure 3). No significant differences  
258 were found between sites for Na/Ca edge ratios (Table 1, Figure 3).

259 Multivariate analysis showed significant multivariate differences between all sampling sites  
260 (PERMANOVA,  $3.10 < F < 5.9$ ,  $0.0001 < p < 0.0008$ ), except between Mecoacán and Tamiahua ( $F=0.8$ ,  
261  $p=0.5$ ). The QDA identified two main discriminant functions (Wilks' Lambda=0.17,  $p < 0.0001$ ),  
262 which accounted for 75% (first function) and 21% (second function) of the variance, respectively.  
263 Based on mean discriminant coefficients, Sr/Ca ( $b=0.73$ ) and Li/Ca ( $b=0.34$ ) were the most  
264 important variables for the first function, and Mn/Ca ( $b=0.95$ ) and Ba/Ca ( $b=-0.43$ ) for the second  
265 one. The jackknifed classifications of the QDA (mean=66.3%) were high/moderate for Alvarado

266 (84.2%) and Laguna Madre (70.0%), but these were low for Tamiahua (52.9%) and Mecoacán  
267 (57.9%) (Table 2, Figure 4a).

### 268 **3.1.2. Mexico plus Brazil**

269 The Ba/Ca, Sr/Ca and Pb/Ca ratios were significantly higher in Brazil than in Mexico, while Li/Ca,  
270 Mn/Ca, and Na/Ca were significantly higher in Mexico (Table 1, Figure 3).

271 The Hotelling's T-squared test showed significant differences between Mexico (pooled sites) and  
272 Brazil samples (Table 1). Based on mean discriminant coefficients of QDA (Wilks' Lambda=0.08,  
273  $p < 0.0001$ ), Na/Ca ( $b = 0.77$ ) and Mn/Ca ( $b = 0.42$ ) were the most important variables for the  
274 discrimination. The jackknifed classifications were 99% for Mexico and 100% for Brazil (Table 4c,  
275 Figure 5).

276

## 277 **3.2. Otolith core**

### 278 **3.2.1. Mexico**

279 The Pb/Ca ratio was significantly lower in Albarado than in the rest of the catch areas (Table 1,  
280 Figure 3). On the contrary, Alvarado showed significantly highest Na/Ca levels, while the lowest  
281 ratios were found in Mecoacán and Tamiahua (Table 1, Figure 3). No significant differences were  
282 found between sampling sites for Ba/Ca, Li/Ca, Mn/Ca, and Sr/Ca core ratios (Table 1, Figure 3).

283 Multivariate analyses indicated significant differences between all pairs of comparisons  
284 (PERMANOVA,  $2.7 < F < 4.9$ ,  $0.0001 < p < 0.004$ ), except Mecoacán-Tamiahua ( $F = 1.0$ ,  $p = 0.4$ ),  
285 Mecoacán-Laguna Madre ( $F = 1.4$ ,  $p = 0.2$ ), and Tamiahua-Laguna Madre ( $F = 1.8$ ,  $p = 0.06$ ). The two  
286 main discriminant functions of QDA (Wilks' Lambda=0.40,  $p < 0.0001$ ) accounted 82% and 12% of  
287 the variance, respectively. The most important variables for the discrimination were Li/Ca ( $b = 1.1$ )  
288 and Na/Ca ( $b = -0.94$ ) for the first function, and Na/Ca ( $b = 0.86$ ) and Ba/Ca ( $b = -0.49$ ) for the second  
289 one. The jackknifed classification rates were relatively low ( $< 47\%$ , mean=46.3%) (Table 2, Figure

290 4b). The nMDS analysis based on the samples from Mexico produced three main clusters with 24,  
291 13, 38 samples, respectively (Figure 5), represented by fish from the four sampling sites. The CCC  
292 obtained was relatively low (0.59), suggesting that the dendrogram did not adequately summarize the  
293 data. The clusters 1 and 2 were characterized by containing 75% of the Albarado samples. With a  
294 stress of 0.17, the nMDS showed low clustering ability, supporting the cluster analysis result.

### 295 **3.2.2. Mexico plus Brazil**

296 The Sr/Ca ratio was significantly higher in Brazil than in Mexico, while Li/Ca, Mn/Ca, and Pb/Ca  
297 were significantly higher in Mexico (Table 1, Figure 3). No significant differences were found  
298 between Mexico sites and Brazil for Ba/Ca and Na/Ca ratios (Table 1, Figure 3).

299 Significant differences between Brazil and the pooled sites of Mexico were found (Hotelling's T-  
300 squared, Table 1). The mean discriminant coefficients of QDA (Wilks' Lambda=0.76,  $p < 0.0001$ ),  
301 revealed that Sr/Ca ( $b = -0.80$ ) and Li/Ca ( $b = -0.70$ ) were the most important ratios for the  
302 discrimination. The jackknifed classifications were 77% for Mexico and 76% for Brazil  
303 (mean=76.5%, Figure 4d). When the samples from Brazil were included in the cluster analysis  
304 (CCC=0.46), three main clusters were produced, which did not coincide with catchment areas,  
305 represented by samples from all sampling sites (Figure 5). The three clusters integrated samples from  
306 Brazil and all sites of Mexico. The Cluster 1 grouped 56 individuals, 22 from Brazil and 34 from  
307 Mexico, where only one corresponded to the Laguna Madre. With 26 individuals, the cluster 2 was  
308 the smallest and included 16 samples from Brazil and 10 from Mexico, with only one from Laguna  
309 Madre and Mecoacán. Moreover, with 60 fish the cluster 3 was the largest and included 18 samples  
310 from Mexico and 42 from Brazil. The 90% of the samples from Laguna Madre were included in this  
311 group. The high stress (0.25) in the nMDS (Figure 5) suggested that the data were poorly displayed  
312 when reduced to two-dimensions, indicating that there were no discrete groupings in the data cloud,  
313 which is supported by the cluster analysis.

314

#### 315 **4. Discussion**

316 Otolith edge results have supported the segregation of the *M. curema* adult stage among several  
317 sampling sites from Mexico and Brazil, while core analysis suggested that the nursery areas did not  
318 correspond to the capture sites or adult stocks.

319 The methodology used in this study, based on the chemical composition of the first and last year of  
320 life, allowed to discriminate between potential nursery areas and stocks, respectively. However, this  
321 approach may have limitations, particularly if the migration patterns are variable among fish or  
322 sampling sites, which could affect the classification rates. However, due to the high variability of the  
323 studied environments, the use of otolith spots or specific areas (for example the outermost spot of the  
324 edge) would only reflect the local environmental variability and not the population structure. Other  
325 techniques have been used to delimit *M. curema* stocks in the Gulf of Mexico, including otolith/scale  
326 shape and genetic analyzes (Ibáñez *et al.*, 2017; Pacheco-Almanzar *et al.*, 2017). The otoliths of the  
327 fish caught in Mexico analyzed in the present study belonged to the specimens on which the  
328 otolith/scale shape and genetic analyses were run (Ibáñez *et al.*, 2017; Pacheco-Almanzar *et al.*,  
329 2017), making the results thoroughly comparable. Thus, the overall QDA classifications performed  
330 better for otolith edge chemistry (mean=66.6%) followed by scale shape (mean=47.4%, Ibáñez *et al.*,  
331 2017), and otolith shape (mean=40.2%, Ibáñez *et al.*, 2017), the latter with remarkably low values of  
332 overall discrimination. Otolith edge chemistry was thus, the most useful in the identification of the  
333 Mexican population, which could be explained by the existence of environmental or genetic  
334 variability, or a combination of both, between the collection sites (see below).

335 The stock structure obtained by microchemistry analysis didn't exactly match with the genetic groups  
336 reported by Pacheco-Almanzar *et al.* (2017) for the Gulf of Mexico, where the lagoons of Alvarado  
337 and Mecoacán form one group and those of Tamiahua and Laguna Madre another. The resemblance  
338 in otolith chemistry recorded for Mecoacán-Tamiahua could be associated with a similarity in  
339 hydrological conditions in these two lagoons. According to Contreras-Espinosa and Castañeda

340 (2004), salinity in Tamiahua and Mecoacán varies from 14.4 to 37.5 and 22.3 to 34 respectively,  
341 while Madre and Alvarado are metahaline (36 to 40 PSU) and brackish (0.5 to 29 PSU),  
342 respectively.

343 The genetic groups of *M. curema* differentiated via microsatellite markers (Pacheco-Almanzar *et al.*,  
344 2017) were positively identified through the shape of their scales, indicating that both genetic and  
345 environmental factors can determine scale shape (Pacheco-Almanzar *et al.* unpublished data). Thus,  
346 it seems that fish scale shape is molded by the interaction between genetic and environmental factors  
347 (Poulet *et al.*, 2004; Salini *et al.*, 2004; Pinheiro *et al.*, 2005).

348 On the other hand, the mean jackknifed classification rate (99.5%) increased significantly when  
349 comparing the grouped sites of Mexico and Brazil, which could be favored by the great distance  
350 between both sites reducing the possibilities of connectivity.

351 The incorporation of trace elements into otoliths can be influenced by environment (depth, salinity,  
352 temperature) (Lin *et al.*, 2007; Reis-Santos *et al.*, 2013), surrounding water chemistry (Elsdon, &  
353 Gillanders, 2003; Walther, & Thorrold, 2006; Avigliano, *et al.*, 2019), food (Ranaldi & Gagnon,  
354 2008), genetics (Clarke *et al.*, 2011), physiological events such as hatching, transition to free-embryo  
355 or juvenile life, among others (Tabouret *et al.*, 2011; Rogers *et al.*, 2019), and even a differential  
356 fishing pressure among catchment areas could alter the otolith composition (Catalán *et al.*, 2018).  
357 Also, in species with prolonged yolk-feeding, the recent life history of mothers (maternal effect)  
358 could have an influence on the incorporation of trace elements into the larval otolith (Kalish, 1990;  
359 Liberoff *et al.*, 2014). In addition, the incorporation of different trace elements is affected differently  
360 by these factors, and there are also inter-specific differences.

361 In this study, Ba/Ca, Li/Ca, Mn/Ca, and Na/Ca for the edge, and Sr/Ca, Na/Ca, Ba/Ca and Li/Ca for  
362 the core, were the most influential elements to differentiate between groups. In some diadromous  
363 species, a positive relationship between the otolith Sr/Ca and salinity has been observed (Brown, &  
364 Severin, 2009; Avigliano & Volpedo, 2013). In mugilids specifically, otolith Sr/Ca ratio showed an

365 increase in relation to salinity, as reported by Santana *et al.* (2018) for *M. curema* from northeast  
366 Brazil, and by several studies based on *M. cephalus* from Taiwan (Chang *et al.*, 2004b, 2004a; Wang  
367 *et al.*, 2010) and Australia (Fowler *et al.*, 2016), and for *M. liza* from Argentina and Brazil (Callicó  
368 Fortunato *et al.*, 2017b). Here, the hypersaline lagoon Laguna Madre showed the highest Sr/Ca  
369 values, so it is possible that salinity is playing an important role in the Sr/Ca differences found. On  
370 the other hand, because a negative relationship between otolith Ba/Ca and salinity has been reported  
371 for many diadromous species, this ratio turned out to be a good salinity proxy (Elsdon & Gillanders,  
372 2005). Wang (2014) and Wang *et al.* (2010) have reported high otolith Ba/Ca levels for freshwater  
373 *M. cephalus* from Taiwan (e.g. Tanshui River), suggesting that this ratio may be useful as a  
374 freshwater marker. Nevertheless, a direct relationship between Ba/Ca and environment is not fully  
375 understood. According to the results obtained in this study, the Laguna Madre hypersaline lagoon  
376 was not associated with the lowest Ba/Ca values, which does not support a strong negative  
377 relationship between otolith and salinity for this element. The Mn/Ca incorporation ways into the  
378 otolith seem to be very complex and variable between species (Sturrock *et al.*, 2014, 2015).  
379 Manganese in the otolith has been linked to the diet (Pentreath, 1973), low levels of dissolved  
380 oxygen (Limburg, *et al.*, 2011; Limburg *et al.*, 2015), environmental concentration (Mohan *et al.*,  
381 2012), and endogenous factors (Sturrock *et al.*, 2014) for different species. For some species,  
382 including mugilids such as *M. cephalus*, it has been suggested that the incorporation of Na/Ca into  
383 otolith is controlled physiologically rather than by an abiotic factor (Wang, 2014; Loewen *et al.*,  
384 2016). The incorporation of Li/Ca into the otolith seems to be complex and depends on endogenous  
385 (growth rate, sex) and exogenous (temperature, salinity, environmental availability) factors  
386 (Bouchard *et al.*, 2015; Sturrock *et al.*, 2015; Thomas *et al.*, 2017). It is not easy to directly infer why  
387 some ratios such as Mn/Ca, Na/Ca, and Li/Ca differed between groups; however, because in this  
388 study the fish size and sex was controlled, different physiological and environmental pressures are  
389 possibly involved.

390 In summary, it was evident that there were strong enough factors (environmental, physiological, or  
391 both) to generate detectable chemical signatures between some catchment areas. Except between  
392 Mecoacán and Tamiahua, the multivariate tests suggested a high/moderate segregation in the adult  
393 stage of life (otolith edge analyzes) among the capture areas. This means that the fish are kept  
394 separated by long enough periods to detect differences in the otolith chemical composition of the last  
395 year of life, suggesting for the first time the presence of potential fishing management units for *M.*  
396 *curema* in the study area. On the other hand, the relatively high misclassification rates obtained for  
397 Tamiahua and Mecoacán could be due to the fact that there is a high level of connectivity between  
398 these sites, or that the environmental or physiological conditions to which the fish were exposed are  
399 not different enough to print unique chemical signatures on the otolith.

400 In relation to the young stage, the nMDS and cluster-based core analyzes indicated that there were no  
401 discrete groupings in the data cloud. High overlap was observed in the core chemistry even when  
402 samples from Brazil, collected far from Mexico, were included (Figure 4c and d and Figure 5),  
403 suggesting two hypotheses: (1) that multiple nursery areas replenish all sampled adult populations; or  
404 (2) that elemental signatures are not capable of delineating between these groups. Given that the  
405 samples from Brazil were collected over 10,000 km away from the Gulf of Mexico sites, hypothesis  
406 2 seems much more probable. However, both hypotheses could be plausible between the sites in  
407 Mexico. In any case, more evidence is needed to understand the composition of the population in the  
408 juvenile stage.

409 Recently, Santana et al. (2018) identified two microchemical patterns in one-year-old *M. curema* and  
410 suggested a multi-batch spawner strategy (Solomon & Ramnarine, 2007). High otolith core Sr/Ca of  
411 juvenile fish from Brazil was found in the dry season (high salinity inside the estuary), while a low  
412 Sr:Ca signature was reported for the rainy season. In this sense, high variability in the otolith core  
413 composition could be expected in relation to local environmental variation such as the influence of  
414 estuaries and river, rainy seasons, etc.

415 *Mugil curema* generally spawns in the open sea, and the eggs and larvae are carried by the currents  
416 to coastal environments (Moore, 1974). Nevertheless, studies developed in the Mexican Gulf and  
417 Venezuela have reported that the white mullet spawns both coastal (mangles and coastal lagoons)  
418 and open sea, and that they spend most of their life in brackish environments (Marin *et al.*, 2000,  
419 2003; Ibáñez *et al.*, 2012; Santana *et al.*, 2018). According to Ibáñez *et al.* (2012) some groups of  
420 adult *M. curema* form schools and migrate to the open sea for spawning. Oceanic waters are more  
421 homogeneous than that in coastal lagoons where rivers with different inputs converge and water  
422 inlets/outlets modify the characteristics of the water (Brown, & Severin, 2009). The results obtained  
423 record a wide variation in otolith chemistry in the different locations which led us to think of the  
424 extensive mobility of the species. Juvenile *M. curema* have been found 700 km upstream in rivers  
425 from the Gulf of Mexico such as Lacantún River (Chiapas, Mexico) (Rodiles-Hernández *et al.*,  
426 2005). This species has also been found in hypersaline estuaries, indicating its wide resistance to  
427 salinity. For example, it has been frequently recorded in salinities of 38 to 55 in the Saloum Delta  
428 (Senegal) (Le Loc'h *et al.*, 2015). In addition to its seasonal spawning migration from estuarine  
429 ecosystems to the sea, *M. curema* also carries out tidal migrations from the shoreline (where it feeds)  
430 to farther offshore (Ibáñez & Gutiérrez Benítez, 2004; Le Loc'h *et al.*, 2015).

431

432 In conclusion, genetics, and scales and otolith shape suggested latitudinal geographic segregation  
433 between the two sites located to the north (Laguna Madre and Tamiahua) and the other to the south  
434 (Alvarado and Mecoacán). The otolith microchemistry not only supported the presence of latitudinal  
435 segregation, but also provided evidence of segregation within these two large groups previously  
436 identified, especially between Laguna Madre and Tamiahua, and Alvarado and Mecoacán. In  
437 addition, there remains to be considerable uncertainty about the structure of nursery areas,  
438 highlighting the importance of studying different microhabitats such as mangles and coastal lagoons  
439 as potential sources of recruitment. With the information available so far, it is recommended to

440 integrate otolith microchemistry to design management strategies that consider the complex  
441 population structure of *M. curema*. Specifically, stock-specific management could contribute to the  
442 sustainable use of the resource in the study area.

443

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448

449

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