

Is otolith microchemistry (Sr: Ca and Ba:Ca ratios) useful to identify *Mugil curema* populations in the southeastern Caribbean Sea?

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(With 4 figures)

Abstract

The aim of the present study was to evaluate the potential use of otolith microchemistry (Sr:Ca and Ba:Ca ratios) to identify silver mullet, *Mugil curema*, populations in Southeastern Caribbean Sea. Fish samples were collected in 7 areas of Nueva Esparta State (Venezuela). The otolith Sr:Ca and Ba:Ca ratios and water Sr:Ca were determined (by ICP-OES and EDTA volumetric method). Otoliths Sr:Ca and Ba:Ca ratios and Sr:Ca partition coefficient of mullets in Cubagua island (south of the State) were significantly different from ratios in La Guardia (north of the State). A discriminant analysis of otolith Sr:Ca and Ba:Ca ratios separated Cubagua Island from La Guardia values. These results suggest the existence of different mullet groups in the Southeastern Caribbean Sea. For this, the simultaneous use of Sr:Ca and Ba:Ca ratios could be a potential tool to identify populations in the study area.

Keywords: Mugilidae, fish resource, otolith, microchemistry.

A microquímica do otólito é um indicador do habitat de *Mugil curema* no sudeste do Mar Caribenho?

Resumo

O objetivo do presente estudo foi avaliar o potencial uso da microquímica do otólito (razões Sr:Ca e Ba:Ca) para identificar distintas populações de tainha, *Mugil curema*, no sudeste do mar caribenho. Os peixes foram coletados em 7 áreas do estado de Nueva Esparta (Venezuela). As razões Sr:Ca e Ba:Ca do otólito e a razão Sr:Ca da água foram determinadas (pelo ICP-OES e EDTA método volumétrico). As razões de Sr:Ca e Ba:Ca dos otólitos e o coeficiente de partição das tainhas da Ilha Cubagua (sul do estado) foram significativamente diferentes das razões de La Guardia (norte do estado). A análise discriminante das razões de Sr:Ca e Ba:Ca dos otólitos separa os valores da Ilha Cubagua e de La Guardia. Estes resultados sugerem a existência de diferentes grupos de *Mugil curema* no sudeste do mar Caribenho e que o uso simultâneo das razões Sr:Ca e Ba:Ca poderiam ser uma potencial ferramenta para identificar as populações da área de estudo.

Palavras-chave: Mugilidae, recurso pesqueiro, otólito, microquímica.

1. Introduction

In the last decade, the analysis of otolith chemical composition has been increasingly used to study fish movements and identify fish stocks (Zlokovitz et al., 2003; Kraus and Secor, 2004; Schuchert et al., 2010; Tabouret et al., 2010; Avigliano and Volpedo, 2013). In this regard, the determination of strontium (Sr) and barium (Ba) concentrations has proven to be of major importance (Campana, 1999; McCulloch et al., 2003; Elsdon and Gillanders, 2005; Hamer et al., 2006; Tabouret et al., 2010; Avigliano et al., 2013). Sr concentration in otoliths was reported to vary mainly due to the chemical composition of the water where

fish live (Campana, 1999; Wells et al., 2003; Kraus and Secor, 2004). In species that frequent environments with large variations in salinity, the concentration of Sr in the otoliths was found to be positively correlated with salinity (e.g. Kraus and Secor, 2004; Sturrock et al., 2012; Avigliano and Volpedo, 2013). This relationship tends to be weak and ambiguous in marine species due to the relatively constant salinity of this environment (Brown and Severin, 2009; Sturrock et al., 2012). On the other hand, Ba concentrations in otoliths were found to be negatively correlated with water salinity (Miller, 2011; Avigliano et al., 2013). For this, the

simultaneous analysis of the otolith Sr:Ca and Ba:Ca ratios has been applied in previous years to study life history of fish and the differentiation of fish stocks (Tabouret et al., 2010; Ferguson et al., 2011; Avigliano et al., 2013).

The Mugilidae family, with 17 genera and around 72 species (Nelson, 2006; Harrison et al., 2007; González Castro et al., 2008; Heras et al., 2009), is broadly distributed all over the world. It is represented by eight important commercial species in the Caribbean Sea and north of the southwestern Atlantic (Rivera-Reyes, 2009; Mendonça and Bonfante, 2011). In some regions of the southwestern Atlantic, like Brazil, *Mugil curema* has not only aquaculture importance (Mendes, 1983), but also has become one of the most important fisheries exploitation on the region (Mendonça and Bonfante, 2011). Moreover, for the last two decades, Venezuela has become the country with the largest fish catch in the Caribbean Sea (500.000 tons per year); dropping by 35% during 1998-2008 but recovering thereafter (FAO, 2005, 2010). Both artisanal and industrial fishing contribute to the Venezuelan capture production, with the former representing 70% of the total extractive catches (FAO, 2005). Nueva Esparta is the third most important state in terms of capture fisheries production (10.17%), after Zulia (12.13%) and Sucre (57.51%). In Nueva Esparta State there are many local communities undertaking this activity (FAO, 2005), and emergent nutrient-rich waters resulting in high levels of productivity (Muller-Karger et al., 1989).

The silver mullet *M. curema* is the second most abundant fish resource obtained by artisanal extraction, with its capture and culture providing economic support for small local communities (Marin et al., 2003). Ibañez et al. (2012) determined, using a chronological study of the Sr:Ca ratios in otoliths of *M. curema*, that this species remains most of its life in brackish waters of variable salinity and that it spends a short period in marine waters in Mexican coasts. Also, the proportion of Sr:Ca in the otolith core indicated that the individuals can spawn both in freshwater and in marine water. These analyses have special relevance to comprehend the life history of *M. curema*. However, many aspects of the population structure, such as number of stocks or populations connectivity, are yet unknown; especially in Southeastern coasts of the Caribbean Sea where this species is actively exploited. In this sense, the aim of the present study was to evaluate the potential use of otolith microchemistry (Sr:Ca and Ba:Ca ratios) for the identification of *Mugil curema* populations in Southeastern Caribbean Sea. This could provide a tool for further studies on the use of habitat and connectivity of the populations.

2. Material and Methods

2.1. Study area and sample collection

Nueva Esparta State is located in the Caribbean Sea, in northeastern Venezuela, and includes the Coche, Cubagua and Margarita islands. The latter is the largest of the three and has two massifs: the Macanao Peninsula in the west and the Paraguachoa Peninsula in the east, separated by a

lagoon (La Restinga) (Figure 1). The mullets were collected during July 2011, with gill nets and identified using the taxonomic keys of Harrison (2002) and Harrison et al. (2007). A total of 112 adult fish were collected and their total length (TL in cm) recorded. The *sagittae* otoliths were removed from all fish. The right otoliths from individuals of TL between 23 and 35 cm were selected: 20 from Cubagua Island, 6 from Punta de Piedras lagoon, 21 from El Guamache, 13 from La Isleta, 15 from Manzanillo, 19 from La Guardia and 11 from La Restinga lagoon. Collection of water samples was performed simultaneously with fish sampling.

2.2. Elemental otolith chemistry

Otoliths were weighed to the nearest 0.1 mg in an analytical balance and digested with 50% nitric acid during 24 hours. Otolith Sr and Ba concentrations were determined using an inductively coupled plasma-atomic emission spectrometry instrument (ICP-OES Perkin Elmer® Optima 2000 DV, Überlingen, Germany) equipped with cross-flow nebulizer and a quartz ICP torch (method EPA 200.7) (EPA, 1994). Solutions were introduced to the spectrometer using a Perkin-Elmer® AS-90 Plus autosampler. The instrument was cleaned at regular intervals with Milli-Q water (Millipore, São Paulo, Brazil) and 10% nitric acid matrix to prevent sample memory effects. The detection limits of the ICP-OES were 8 and 10 µg/L for Ba and Sr, respectively. All measurements were performed in triplicate (RSD < 4%). External calibration was performed using the atomic spectroscopy standard QCS 21 (Quality Control Standard), Perkin Elmer® Pure (USA). The operating conditions of the ICP-OES instrument are given in Table 1. The concentration of Ca was determined by



Figure 1. Study area: black spots indicate sampling sites.

titration with ethylenediaminetetraacetic acid (EDTA) (APHA, 1993). All measurements were made in triplicate (standard deviation less than 4%). The digestion and analytical procedures were checked by the analysis of Otolith Certified Reference Material for trace elements (FEBS-1, National Research Council, Canada). Replicate analysis of this material showed good accuracy, with the following metal recovery rates: 93% for Sr, 88% for Ba and 110% for Ca. The element:Ca ratios were expressed in mmol mol⁻¹ to standardize the concentrations of trace elements in relation to Ca.

2.3. Elemental water chemistry

Water samples were preserved by the addition of 2 mL of nitric acid per liter of water (APHA, 1993; 3010 B method), maintained at 4 °C until further analysis (Chiba et al., 2011). In the laboratory, samples were filtered through a membrane of 0.45 µm. Water Sr and Ca concentrations were determined as described above. Water Ba concentrations were below the detection limit (8 µg/L). Sr:Ca ratios were expressed in mmol mol⁻¹.

2.4. Relationships between water chemistry and otolith Sr:Ca

In order to examine the relationship between otolith microchemistry and water chemistry, the mean individual elemental ratio (Sr:Ca) and the averages of all water samples within each site were compared using the partition coefficient described as (Tabouret et al., 2010) (Equation 1):

$$D_{Sr} = mSr : mCa_{otolith} / mSr : mCa_{water} \quad (m = \text{molarity}) \quad (1)$$

Table 1. Optima 2000 ICP-OES operating conditions for analysis.

RF Power	1.3 kW
Plasma gas flow rate	15.0 L/min
Nebulizer gas flow rate	0.80 L/min
Auxiliary gas flow rate	0.20 L/min
Pump flow rate	1.2 mL/min
Delay time	20 seg
Flush time	20 seg
Number of replicates	3

Table 2. One-way ANOVA results for the comparison of otolith Sr:Ca and Ba:Ca ratios (mmol/mol) of *Mugil curema* among sampling sites.

	N	Sr:Ca ratio		Ba:Ca ratio	
La Guardia	22	2.65 ± 0.38	A	0.009 ± 0.003	A
Guamache	21	2.97 ± 0.33	A B	0.013 ± 0.007	A B
La Isleta	15	2.86 ± 0.74	A B	0.023 ± 0.013	A B
La Restinga Lagoon	11	3.10 ± 0.29	B	0.013 ± 0.007	A B
Punta de Piedras Lagoon	6	3.12 ± 0.17	B	0.013 ± 0.005	A B
Manzanillo	16	3.12 ± 0.36	B C	0.016 ± 0.008	A B
Cubagua Island	22	3.64 ± 0.49	C	0.024 ± 0.010	B

Significant differences are shown with different letters (p<0.05). N: Sample size.

Partition coefficients allow the evaluation of elemental discrimination in the otolith compared to the environment water chemistry (e.g. Kraus and Secor, 2004; Lin et al., 2007; Tabouret et al., 2010; Santos et al., 2013). Sr was estimated for each locality taking into account mean individual Sr:Ca values in the otolith and mean Sr:Ca ratios in water from the sampling sites where the specimens were caught.

2.5. Statistical analysis

ANCOVA was used to determine the effect of fish length on the magnitude of each studied variable. Sr:Ca ratios were significantly correlated to fish length and were corrected using the common within-group slope (b=0.04) for fish length variable (Begg et al., 2001; Cardinale et al., 2004; Galley et al., 2006; Burke et al., 2008). This adjustment successfully removed this significant correlation. Subsequently, otolith element:Ca ratios means were compared among sampling sites using one-way analysis of variance (ANOVA), followed by Tukey test (Sokal and Rohlf, 1995). Sr:Ca ratios of water samples were compared among study sites with a Kruskal-Wallis test. Finally, partition coefficients were compared among sampling sites using a one-way analysis of variance (ANOVA), followed by Tukey test.

3. Results

3.1. Elemental otolith chemistry

The otolith Sr:Ca ratio ranged from 2.65 to 3.64 mmol/mol, with the lowest value corresponding to mullets from La Guardia and the highest to those collected near Cubagua Island (Table 2). Tukey's test (Table 2) showed three groups: La Guardia, the lagoons (La Restinga and Punta de Piedras) and Cubagua Island. No significant differences were found between Guamache and La Isleta. The mean Sr:Ca ratios of the areas previously mentioned were intermediate values between the ones of the lagoons and the ones of La Guardia. Manzanillo Sr:Ca ratio (3.12±0.49) was higher than the Lagoons and lower than Cubagua Island (Table 2).

The otolith Ba:Ca ratio ranged from 0.009 to 0.024 mmol/mol, with the lowest value corresponding to fish from La Guardia and the highest to fish collected in Cubagua Island (Table 2). Tukey's test (Table 2) showed two distinct groups: La Guardia and Cubagua Island. Ba:Ca ratios for the remaining areas were intermediate between

the two sites previously mentioned, with no significant differences (Table 2). Discriminant analysis showed that 86.5% of the total variation was explained by the first axis. The variable best represented along this axis was Ba:Ca ratio (eigenvalue=0.73), followed by Sr:Ca ratio (eigenvalue=0.45). Values for Cubagua Island were obtained towards the positive values of the first canonical axis, while La Guardia values were obtained towards the negative ones (Figure 2). An overlap of the values corresponding to both lagoons and Guamache localities was observed (Figure 2). Manzanillo site showed widely dispersed values, overlapping with the ones of Cubagua Island.

3.2. Elemental water chemistry and Sr:Ca partition coefficients

The water Sr:Ca ratio ranged between 2.87 and 3.28 mmol/mol, with the highest value corresponding to the localities of La Guardia and Cubagua Island, and the lowest to Manzanillo (Figure 3). The remaining sampling sites presented intermediate values (Figure 3). The analysis of variance for Sr:Ca partition coefficients showed similar values for Cubagua Island and Manzanillo localities (Figure 4). They were significantly different from Guamache, La Isleta and La Guardia sites that presented

the lower partition coefficients (Figure 4). The lagoons Punta de Piedras and La Restinga showed intermediate values (Figure 4).

4. Discussion

The incorporation of Sr:Ca and Ba:Ca in the otolith depends on several factors, particularly, elemental water chemistry, salinity and temperature (Kraus and Secor, 2004; Miller, 2011; Sturrock et al., 2012). Temperature can have a negative, positive or no effect on the incorporation of these elements to the otolith. However, this relationship is dependent on the species and it is necessary to assess it through experimental research like breeding specimens in captivity (Campana, 1999; Brown and Severin, 2009; Sturrock et al., 2012). For mullets these relationships have not yet been studied. In the area selected for this study, temperature (approx. 23.5-24.5 °C) and salinity (35.8-36.6 UPS) of water do not vary significantly throughout the year (Astor and Cárdenas, 1997).

In this work the higher Sr:Ca and Ba:Ca (3.64 and 0.024 mmol/mol, respectively) ratios for *M. curema* otoliths were found in Cubagua Island (Table 2), while the lower ratios correspond to La Guardia locality (2.65 and 0.009 mmol/mol, respectively). The high values

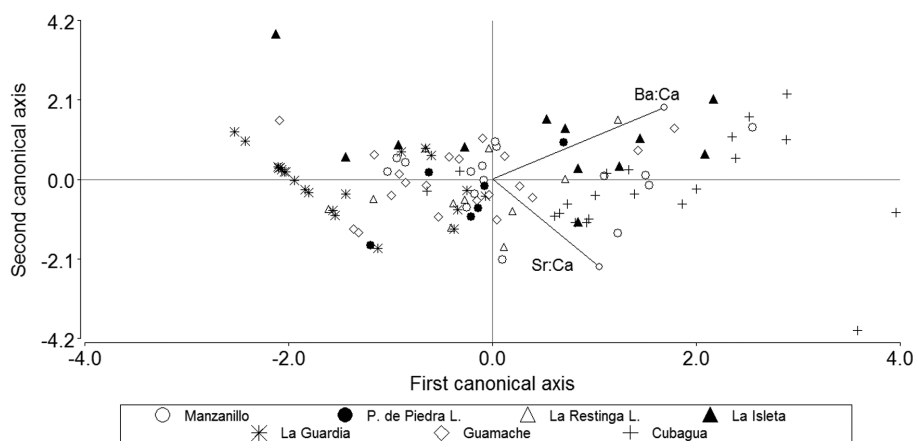


Figure 2. Discriminant analysis for otolith Sr:Ca and Ba:Ca ratios of *Mugil curema* for the sampling sites in Nueva Esparta State.

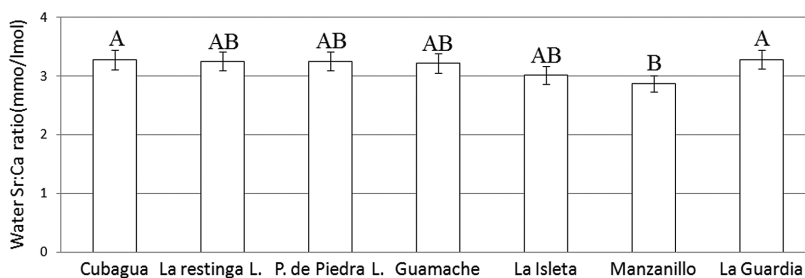


Figure 3. Water Sr:Ca ratios (mmol/mol) for *Mugil curema* sampling sites in Nueva Esparta State. P. de Piedras (Punta de Piedras), L (Lagoon). Error bars represent standard deviation. Significant differences are shown with different letters ($p < 0.05$).

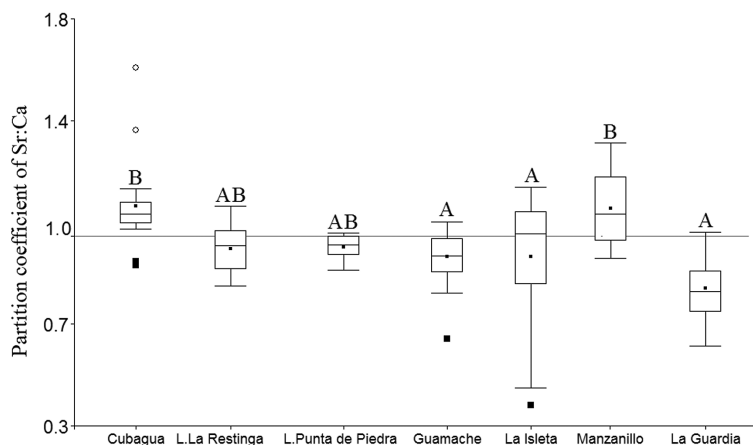


Figure 4. Partition coefficient of Sr:Ca ratios for the sampling sites of *Mugil curema* in Nueva Esparta State. L (Lagoon). Error bars represent standard deviation. Significant differences are shown with different letters ($p < 0.05$).

of the otolith Sr:Ca ratios coincides with water Sr:Ca ratios (Figure 3), as previously reported by other authors (i.e., Kraus and Secor, 2004; Avigliano and Volpedo, 2013). The discriminant analysis among otolith Sr:Ca and Ba:Ca ratios also showed a separation between Cubagua Island and La Guardia values (Figure 2).

In the analysis of partition coefficients, usually used to describe relationships between elemental concentrations in otolith and in water (e.g. Tabouret et al., 2010; Santos et al., 2013), a coefficient of 1 represents elemental deposition into the otolith in equal proportion as the environment concentrations, while values lower than 1 represent elemental discrimination, and values higher than 1 represent active elemental uptake (Elsdon and Gillanders, 2003, 2005). Except for La Guardia ($D_{Sr} = 0.81$), all partition coefficients were close to 1 ($D_{Sr} = 0.92-1.10$), showing that Sr:Ca ratios of otoliths were similar as ratios from surrounding water. Lower values of D_{Sr} were reported by some authors for other marine (0.28) (De Vries et al., 2005) and freshwater species (0.49) (Tabouret et al., 2010), but, the methodology of total otolith digestion used in the present study could be representing a loss in analysis definition, given that it represents an average of the life history of the fish. Additionally, the relatively low partition coefficient of mullets from La Guardia would be partially explained by other factors, such as the migratory behavior of this species. The silver mullet moves from its main spawning area, located near Fosa de Cariaco between La Tortuga and Margarita islands (with a maximum depth above 1500 m), to Margarita Island (Etchevers, 1974). Coastal catches of mullets are uncommon during the spawning period in the open sea (April-July and September-October) (Moore, 1974). Approximately 14 days after hatching, larvae metamorphose and are carried by the currents to depths above 50 m, in the estuarine areas, such as mangles and coastal lagoons, where they live until reaching recruitment age (Marin et al., 2003). Less-intense spawnings have also been reported from March to October in Golfo de Cariaco,

located in the south of Nueva Esparta State, and from July to September in La Restinga lagoon (Marin et al., 2003). Studies carried out in other regions of the Caribbean Sea (Mexican Gulf), demonstrated that *M. curema* spawns both in freshwater and open-sea waters, and that they spend most of their life in brackish waters, migrating annually to the spawning areas (Ibañez et al., 2012).

Baumar et al. (2000, 2003) also reported that *M. curema* uses salt water-lagoons not only as nurseries but also as hatching areas. These environments distinguish for having high salinity concentrations (36-45 g/L) (Rocha et al., 2010). This concurs with high Sr:Ca ratios found in fish otoliths for both lagoons studied. On this basis, the differences found in the otolith microchemistry between fish from the La Restinga and Punta de Piedras Lagoons and south regions of Margarita Island (e.g. Cubagua Island) may be explained by the permanence of fish populations in these different spawning areas.

Significant differences were found in otolith Sr:Ca and Ba:Ca ratios among study areas; particularly between the localities of La Guardia, in the north of the State, and Cubagua Island in the south. This could be showing the possible existence of different groups of mullets in southeastern Caribbean Sea, suggesting that simultaneous use of Sr:Ca and Ba:Ca ratios could be a potential identifier of populations in the study area. However, this identification was not clear enough. This could be due to diverse reasons, but mostly the microchemical analysis of the whole otolith (a methodology reachable in South America and with relatively low-cost) can result in errors or limitations of interpretation because it doesn't take into account the life history of the fish, especially for those species with long distance movements. Further research is needed to confirm and gain a more in-depth insight of the interaction between these fish stocks, with the use of additional methodologies like microchemical analysis of the otolith by age (using the Sr:Ca and Ba:Ca ratios as an indicator of habitat), parasitological studies, etc.

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