Connectivity between the marine coast and estuary for white mullet (Mugil curema) in northeastern Brazil revealed by otolith Sr:Ca ratio

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ABSTRACT

Microchemical analyses were carried out in order to estimate the Strontium:Calcium (Sr:Ca) ratio in the otolith of the white mullet, Mugil curema, in the Pernambuco (at the Santa Cruz channel, Brazil) in order to determine its connectivity between the estuary and ocean. Variation in the otolith Sr:Ca ratio was directly related to salinity, with greater salinity denoting a higher otolith Sr:Ca value. Data on the otolith Sr:Ca ratio demonstrates that the individuals analyzed are born in areas of salinity that are characteristic of the estuary, where they develop until approximately one year of age, at which point they migrate to areas of greater salinity until reaching sexual maturity (3 years of age) in the sea. Spawning occurs in the ocean, after which M. curema individuals may either remain or return to the estuary until the next spawning. Differences in estuarine salinity were found for young-of-year individuals and may be related to the season when spawning took place, since M. curema females are found spawning throughout the year. The hypothesis is that higher salinity in the dry season leads to a greater otolith Sr:Ca signature among individuals spawned in this season from birth until one year of life. On the other hand, the lower salinity in the rainy season leads to a lower otolith Sr:Ca signature among individuals spawned in this season. These information are important for the adequate management of the white mullet stock in northeastern Brazil.

1. Introduction

Fish from the family Mugilidae are distributed throughout the tropical and subtropical regions of the world, mainly in estuarine and coastal waters, where they are commercially exploited by artisanal fisheries, displaying economic and social importance to human populations (Menezes et al., 2015). According to FAO statistics, Brazil accounted for an annual mean of 10.9% in weight of the global productions of mullets between 1950 and 2004, ranking the eighth largest accounting for 96% of mullet catches (Santana da Silva, 2007). M. curema has euryhaline habits and can be found throughout Brazil’s coast. The species uses estuarine regions as nurseries, while spawning occurs at sea (Ibáñez Aguirre, 1993; Ditty and Shaw, 1996; Marin et al., 2003; Ibáñez and Gutiérrez Benítez, 2004; Ibáñez et al., 2012).

A number of studies confirm the correlation between the otolith Sr:Ca ratio and salinity (Secor et al., 2001; Zimmerman, 2005; Labonne et al., 2009), whereas others have not found this correlation (Elsdon and Gillanders, 2002; Rooker et al., 2004). Thus, other factors likely interact with salinity, such as the water chemical composition, temperature or ontogenetic and/or physiological changes (Kawakami et al., 1998; Elsdon and Gillanders, 2002, 2003, 2004, 2005; Pontual et al., 2003; Martin et al., 2004). As the incorporation of elements and the

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salinity effects on otoliths differs from species to species, thereby limiting the determination of general validation models, studies for specific validation are needed to confirm the relation between salinity and the Sr:Ca ratio in otoliths (Secor et al., 1995; Tzeng, 1996). In tropical regions, there is little variation in temperature (2–4 °C) during the year (Santos et al., 2000). However there is seasonality regarding rainfall, which leads to differences in salinity between rainy and dry seasons. In the Santa Cruz channel, the largest estuary in Pernambuco, salinity ranges from 7 to 10 in the rainy season (March to August) and from 34 to 37 in the dry season (September to February) due to the rainfall patterns (Medeiros and Kjerfve, 1993). In this region, *M. curema* is under fishing pressure, especially in the estuary, where there is a high frequency of juveniles (Santana da Silva, 2007). Hence, studies investigating the use of estuarine and marine environments by this species are necessary to better manage its stock.

To date, only one study was conducted on *M. curema*'s migratory pattern using otolith Sr:Ca ratios (Ibáñez et al., 2012), and none in Brazil so far. The present study aimed at identifying the migratory pattern, connection, and environmental history between the estuary and ocean for *M. curema*. A comparison of the transects of Sr:Ca ratio from the nucleus to the edge of the otolith enables an assessment of which environments should be protected in order to assist in the species management. Variations in the otolith Sr:Ca ratio and salinity are also studied in order to validate the relation between these variables for the species.

2. Materials and methods

2.1. Sample collection

Specimens between two and four years old were collected at Santa Cruz channel (08°43′00″S - 08°43′00″S/034°51′00″W - 034°54′00″W) on the northern coast of Pernambuco. Sampling took place in January 2006 (dry season, *n* = 12) and May and June 2006 (rainy season, *n* = 11), from the artisanal fisheries landings that used gillnets with 40 and 50 mm mesh sizes. For each specimen, fork length (FL) was recorded (dry season between 25.2 and 32.6 cm FL and rainy season between 21.3 and 25.7 cm FL) and the sagittal otoliths were removed using ceramic pincers washed in nitric acid (HNO₃) and stored in Eppendorf tubes.

2.2. Validation of the relationship between otolith Sr:Ca ratio and salinity

Five live individuals that were collected at Santa Cruz channel in salinities of 18.1 (*n* = 4) and 31.3 (*n* = 1) (Table 1) were maintained in captivity to validate the influence of salinity on the otolith chemical composition in relation to Strontium. One of these specimens was collected in November 2005 and the others in March 2006. Fish were kept in a 150-L tank equipped with a biological filter and aerator. Specimens were acclimated (salinity 25.3) for one week following capture and were fed with a microalgae-based ration. Fishes were stained with alizarin complexone in a bath at a concentration of 250 mg/l for one day, and were kept in a 150-L tank at a constant salinity of 25.3, where they remained from 19 to 190 days (Table 1). To determine the relation between salinity and otolith Sr:Ca ratio, spots were made near the alizarin complexone labels. Labels corresponded to salinities at capture (18.1 and 31.3) with spots after labels corresponding to tank salinities (25.3) (Fig. 1).

![Fig. 1. Spots (electron beam focalisation) of 10 μm in the otolith transversal cut before (a) and after (b) of alizarine dyes used for the validation of *M. curema* from Pernambuco state (Brazil).](image)

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>SC</th>
<th>FL (cm)</th>
<th>Before Mark</th>
<th>After Mark (Salinity = 25.3)</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sr:Ca (×10⁻³)</td>
<td>Sr:Ca (×10⁻³)</td>
<td>NS</td>
</tr>
<tr>
<td>V1</td>
<td>18.1</td>
<td>15.9</td>
<td>7.4 ± 0.8</td>
<td>8.0 ± 0.8</td>
<td>3</td>
</tr>
<tr>
<td>V2</td>
<td>18.1</td>
<td>9.2</td>
<td>6.0 ± 0.1</td>
<td>7.8 ± 0.9</td>
<td>3</td>
</tr>
<tr>
<td>V3</td>
<td>18.1</td>
<td>16.3</td>
<td>6.4 ± 0.3</td>
<td>7.5 ± 0.9</td>
<td>3</td>
</tr>
<tr>
<td>V4</td>
<td>18.1</td>
<td>15.8</td>
<td>6.4 ± 1.1</td>
<td>7.9 ± 1.3</td>
<td>4</td>
</tr>
<tr>
<td>V5</td>
<td>31.3</td>
<td>17.5</td>
<td>9.7 ± 0.9</td>
<td>7.8 ± 1.0</td>
<td>5</td>
</tr>
</tbody>
</table>

2.3. Sr:Ca ratio analyses in otolith

In all wild specimens, the left sagittal otolith was used to determine age based on annual rings on whole otoliths and longitudinal sections (Santana et al., 2009) (Fig. 2), thus enabling the identification of distinct zones of otolith microstructures. The first two points of the transect between the core and the posterior edge of otolith corresponded to the egg (E) and larval (L) phases of *M. curema*, respectively. The first two rings correspond to checks related to the initial phase of life. The first check (*C₁*) corresponds to 62 days after hatching when the individual reaches approximately 3.5 cm. The second check (*C₂*) emerges 160 days after *C₁*, in fishes of 9.8 cm in length, which leave protected areas start being caught by fisheries. The remaining rings are formed annually (Fig. 2).

The right otolith was embedded in epoxy resin and a longitudinal section including the nucleus was cut using an Accutom-50 micro-cutting instrument (Struers). Each section was sanded up to the nucleus, using a TegraPol 35 (Struers) with abrasive discs of 15 μm, 10 μm and 5 μm calibers, then polished with a diamond solution (0.25 μm) (Gunn et al., 1992). The preparations were cleaned by ultrasound using milliQ water and stocked in a desiccator cabinet.

Samples were prepared prior to electron microprobe analysis ( Cameca-SX50, Ifremer, Brest, France) by applying a fine layer of...
carbon to avoid charge accumulation. Transects from the nucleus to the edge of the otoliths were made in 50-μm steps. Spots (electron beam focalisation) were 10 μm in diameter, the acceleration voltage was 12 kV, and the intensity was 12 nA. The wavelength dispersive spectrum at the Sr Lα peak position was measured for 120 s and 40 s on the Kα calcium radius. Ca, Sr, and C (Carbon) concentrations were measured, and those for O (Oxygen) were calculated. A ZAF matrix correction was then used to obtain Sr and Ca concentrations (% in weight). Calcite (CaCO3) and strontianite (SrCO3) standards from the CNRS-UMR N° 6538 laboratory (Ifremer, Brest, France) were used to calibrate the microprobe. Limits of detection were 360 ppm and 270 ppm for Ca and Sr, respectively. Measurement precision was 0.3% for Ca and 1.8% for Sr.

The Sr:Ca data were separated by age and each 50-μm step and statistically analyzed using non-parametric methods (Mann-Whitney U test and ANOVA Kruskal-Wallis test). The level of significance was set at α = 0.05. The level of significance for all tests was set at α = 0.05 and estimated using the Statistica program.

3. Results

Analyzing the 5 specimens of Table 2, otolith Sr:Ca ratios showed significant differences (Mann-Whitney U test, P < 0.05) for all regarding spots before and after Alizarin labels. Concerning the specimen V1, three spots corresponded to salinity 18.1, resulting in an average value of 7.4 × 10^{-3} (standard deviation – SD ± 0.8 × 10^{-3}). Furthermore, in the area corresponding to the salinity of 25.3, nine spots led to an average of 8.0 × 10^{-3} (SD ± 0.8 × 10^{-3}). In specimens V2 and V3, the same number of spots as V1 were done for both the anterior and posterior regions to the Alizarin label, thus leading to an otolith Sr:Ca ratio of 6.0 × 10^{-3} (SD ± 0.1 × 10^{-3}), 6.4 × 10^{-3} (SD ± 0.3 × 10^{-3}) for salinity of 18.1 respectively, and 7.8 × 10^{-3} (SD ± 0.9 × 10^{-3}) and 7.5 × 10^{-3} (SD ± 0.9 × 10^{-3}) for V2 and 3 in salinity of 25.3. For V4, the four spots of salinity 18.1 led to 6.4 × 10^{-3} (SD ± 1.1 × 10^{-3}), and 7 spots of 25.3, 7.9 × 10^{-3} (SD ± 1.3 × 10^{-3}). Finally, in V5, salinity spots corresponding to 25.3 (n = 7) led to an average value similar to other individuals for the same salinity (7.8 × 10^{-3}, SD ± 1.0 × 10^{-3}), whereas the 5 salinity spots of 31.3 corresponded to the highest salinity of 9.7 × 10^{-3} (SD ± 0.9 × 10^{-3}).

Overall, the otolith Sr:Ca ratio exhibited a direct relation with water salinity in all specimens, with significant differences between different salinity degrees (Kruskal-Wallis, P < 0.05). Mean otolith Sr:Ca ratio for salinity of 18.1 in four specimens (number of spots - NS = 13) was 6.5 × 10^{-3}, progressively increasing to 7.8 × 10^{-3} in the salinity of 25.3 (n = 5, NS = 25) and reaching 9.7 × 10^{-3} in the salinity of 31.3 (n = 1, NS = 5) (Fig. 3). Therefore, the otolith Sr:Ca ratio increased with salinity.

There was a considerable variability in the otolith Sr:Ca ratio between phases/ages in all specimens (2.2 × 10^{-3} in C1 and 11.9 × 10^{-3} at 3 years). In the initial phase of life, there was a slight drop in the otolith Sr:Ca ratio between the E and L phases, which was more accentuated through to C1, when the lowest Sr:Ca values were found (Table 2). Beginning at C1, the Sr:Ca increased progressively until reaching maximum values at the age of maturity (3 years) (Table 2). These variations were significant between phases/ages (Mann-Whitney U test, P < 0.05), caused mainly by the C1 and C2 values (lowest values) and those at 2, 3 and 4 years of age (highest values) (Table 3).

Mullet specimens formed checks 1 and 2 when in the estuary. Therefore, the Sr:Ca ratio in the otoliths was estimated for these two checks in order to establish the variation in this ratio corresponding to estuarine life, thereby allowing comparisons to other phases/ages. Mean otolith Sr:Ca ratio for the 23 specimens analyzed between checks 1 and 2 was 6.2 × 10^{-3}, with a standard deviation of 1.2 × 10^{-3}. Therefore, the variation in otolith Sr:Ca corresponding to estuarine life is between 5.0 × 10^{-3} and 7.4 × 10^{-3}.

Analyzing the data on each 50 μm of the transects (Fig. 4), the egg and larval phases were spent in the estuary (mean Sr:Ca = 6.4 × 10^{-3}) and, beginning at C2, the Sr:Ca increased gradually until 2 and 3 three years of age (mean Sr:Ca = 7.4 × 10^{-3}), when the values were near the upper bound of the Sr:Ca corresponding to the estuary. This indicates migration within the estuary toward areas with greater salinity. After reaching the age at first maturity (3 years), individuals tended to remain in the marine environment (Fig. 4).

Analyzing the otolith Sr:Ca ratio in each phase of life according to season, different values were found for each season, mainly in early phases. In the dry season, all individuals (except the number 1) presented otolith Sr:Ca values in E, L, C1 and C2 near the upper bound of the estuary (7.0 × 10^{-3} ± 0.3 × 10^{-3}) and remained relatively constant until one year of age, when fish started to migrate toward areas of greater salinity (Fig. 5). The egg and larval phases of some individuals occurred in the ocean, thereby diminishing the Sr:Ca to estuarine levels (Fig. 5). This pattern could indicate that these individuals were born in a period of high salinity in the estuary.

In individuals from the rainy season, otolith Sr:Ca values were near the lower bound of the estuary in the four initial phases (6.0 × 10^{-3} ± 0.4 × 10^{-3}), increasing to a similar pattern of the dry season, beginning at one year of age (Fig. 5). In this season, all individuals (except the number 37) spent the E and L phases in the estuary with C1 and C2 values lower than those in the dry season (Fig. 6). Such results show that individuals of this group were born and developed in a period of very low salinity.

Table 2

<table>
<thead>
<tr>
<th>Phase</th>
<th>FL (cm)</th>
<th>Age (years)</th>
<th>n</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>0.19</td>
<td>0</td>
<td>23</td>
<td>4.72</td>
<td>9.7</td>
<td>6.58</td>
<td>1.45</td>
</tr>
<tr>
<td>Larvae</td>
<td>0.68</td>
<td>0.04</td>
<td>23</td>
<td>4.81</td>
<td>8.93</td>
<td>6.45</td>
<td>1.36</td>
</tr>
<tr>
<td>C1</td>
<td>3.51</td>
<td>0.17</td>
<td>23</td>
<td>2.21</td>
<td>9.78</td>
<td>6.15</td>
<td>1.10</td>
</tr>
<tr>
<td>C2</td>
<td>9.75</td>
<td>0.61</td>
<td>23</td>
<td>3.39</td>
<td>9.03</td>
<td>6.37</td>
<td>1.00</td>
</tr>
<tr>
<td>1</td>
<td>16.46</td>
<td>1</td>
<td>23</td>
<td>4.07</td>
<td>10.06</td>
<td>6.64</td>
<td>0.96</td>
</tr>
<tr>
<td>2</td>
<td>21.34</td>
<td>2</td>
<td>23</td>
<td>5.13</td>
<td>11.24</td>
<td>7.05</td>
<td>0.97</td>
</tr>
<tr>
<td>3</td>
<td>25.00</td>
<td>3</td>
<td>15</td>
<td>4.94</td>
<td>11.91</td>
<td>7.53</td>
<td>1.16</td>
</tr>
<tr>
<td>4</td>
<td>27.73</td>
<td>4</td>
<td>2</td>
<td>4.83</td>
<td>11.19</td>
<td>7.66</td>
<td>1.07</td>
</tr>
</tbody>
</table>
4. Discussion

The Sr:Ca ratio analysis in otoliths of *M. curema* was effective to identify migrations between areas of considerable salinity variation, as observed by Ibáñez et al. (2012). This direct relation between salinity and otolith Sr:Ca ratio has also been found for other fish species that either migrate to or use different degrees of salinity in some phase of life, such as *Anguilla japonica* (Tzeng et al., 2002), *M. cephalus* (Chang et al., 2004a; Callicó Fortunato et al., 2017a), *M. liza* (Callicó Fortunato et al., 2017b), *Leiostomus xanthurus* (Martin et al., 2004), salmonids...
Fig. 5. Sr:Ca transect in otoliths of *M. curema* collected in Pernambuco (Brazil) on dry season; life phases and age in larger points, indicated by arrows; lines represent lower ($5.0 \times 10^{-3}$) and upper ($7.4 \times 10^{-3}$) bounds of Sr:Ca in the estuary.
Fig. 6. Sr:Ca transect in otoliths of *M. curema* collected in Pernambuco (Brazil) on rainy season; life phases and age in larger points, indicated by arrows; lines represent lower ($5.0 \times 10^{-3}$) and upper ($7.4 \times 10^{-3}$) bounds of Sr:Ca in the estuary.
The data obtained demonstrate that the bounds of the estuarine zone defined by the Sr:Ca values in the otoliths between checks C1 and C2 correspond to those obtained in the validation experiment. The lowest otolith Sr:Ca value between C1 and C2 is attributed to the low salinity (< 5, close to fresh water) and the highest value (upper bound of the estuary – 7.4 × 10⁻³) corresponds to the typical salinity of the marine environment (> 25). Individuals caught in salinity of 18.1 had an otolith Sr:Ca ratio within the estuary bounds, whereas the specimen caught in salinity of 31.3 had an otolith Sr:Ca ratio above the upper bound of the estuary, which is characteristic of the marine environment (Fig. 3).

Variation in otolith Sr:Ca values among the transects also demonstrate the relation between the otolith Sr:Ca ratio and salinity. Medeiros and Kjerfve (1993) found salinity in the Santa Cruz channel to range from 7 to 37, with minimal and maximal Sr:Ca values in otoliths from M. curema in this same site of 2.2 × 10⁻³ and 11.9 × 10⁻³. These values are similar to those found by a number of authors who described salinity values ranging from 0 to 38.7 and Sr:Ca values in otoliths ranging from 0 to 16 × 10⁻³ (Pontual et al., 2003; Chang et al., 2004b; Daverat et al., 2005; Crook et al., 2006).

Based on current results, M. curema spawns in coastal zones and eggs and larvae are transported into estuaries by coastal currents. Such a general pattern agrees with descriptions by Marín et al. (2003) off Venezuela, by Alvarez-Lajonchere (1976) off Cuba, and by Collins and Stender (1989) and Dittvy and Shaw (1996) who describe the spawning taking place in the ocean. Mean otolith Sr:Ca value estimated for the egg and larval phases (6.5 × 10⁻³) is close to the mean Sr:Ca value in the otolith, which is characteristic of the estuarine zone (6.2 × 10⁻³). This implies that the two phases are spent in the estuary, thus agreeing with Alvarez-Lajonchere (1980) who mentioned the presence of white mullet larvae and post-larvae in estuarine zones. Ibáñez et al. (2012) in Mexico found the same migratory pattern, spawning in nearshore waters with fertilized eggs carried by currents to the lower estuarine salinities.

Upon entering the juvenile phase, check C3 is formed with the lowest mean otolith Sr:Ca values (6.0 × 10⁻³). Accordingly, specimens under one year of age (young-of-year) are only found in estuarine zones, as explained by Santana da Silva (2007) for the Santa Cruz channel, where they are caught by gillnets. Juvenile mullets have similar behavior as reported by Vieira (1991), Monteño (1994) and Carvalho et al. (2007) using estuaries as nurseries. After the formation of C3, the otolith Sr:Ca increases gradually until reaching the age of first sexual maturity (3 years).

When maturity is acquired, M. curema is distributed in salinities that are characteristic of the marine environment (mean otolith Sr:Ca at three years of age = 7.6 × 10⁻³), where spawning occurs. Ibáñez Aguirre (1993) and Ibáñez and Gutiérrez Benítez (2004) also found adult mullets in the ocean, thus conforming to Santana da Silva (2007) for the Santa Cruz channel. After spawning, the majority of individuals remain in ocean areas, but some returned to the estuary at the study area (Santana da Silva, 2007).

The connectivity and migratory behavior between estuary and ocean demonstrated by the otolith Sr:Ca ratio has been analyzed by Ibáñez et al. (2012) for M. curema, M. cephalus (Chang et al., 2004a, 2004b; Callicó Fortunato et al., 2017a), M. liza (Callicó Fortunato et al., 2017b), and other Mugilidae species (Chang and Iizuka, 2012), as well as on other catadromous species such as eels (Tsubamoto and Arai, 2001; Jessop et al., 2002; Tzeng et al., 2002; Daverat et al., 2005). These species exhibited an identical behavior and similar habitat use, remaining in estuaries the entire juvenile phase and spending the adulthood (mainly reproductive) in the ocean.

The two season dependent patterns for the early life history seem to be possible prior to one year of age. Individuals born and develop in the estuary in high salinity near the marine zone (estuarine upper limit) characterize the first. The second is characterized by birth and development in waters with low salinity near the lower edge of the estuary. In both, otolith Sr:Ca values are similar after one year of age and all individuals have the same pattern in pre-adult and adult life. This behavior may indicate different recruitment periods depending on salinity. Studying the migration and Sr:Ca ratio in otoliths from M. cephalus in Taiwan, Chang et al. (2004b) report a similar pattern of two types of migration, with some juveniles developing between the estuary and sea, rarely reaching fresh water, whereas others migrate from fresh to salt water only passing through the estuarine region. Similar results are described for M. liza in the Southwestern Atlantic Ocean (Callicó Fortunato et al., 2017b).

Although similar, patterns of the early phases of life for M. curema appear to be related to the species recruitment in the estuary rather than migration, as occurs in M. cephalus. The difference between the two types appears to be linked to the season in which fish are born. Thus, the white mullet has two spawning peaks – one occurring between November and February (dry season) and another in June (rainy season) (Santana da Silva, 2007). Individuals born in the dry season may use the estuary when salinity is at its highest due to the lack of rainfall, whereas in the rainy season a salinity reduction occurs in the estuary and the individuals born in this season have lower otolith Sr:Ca values, which are near the lower limit of the estuary.

Together with reproduction and catch data, juvenile recruitment patterns may assist in decision making for which specific periods and locations for the adequate management of the white mullet stock in the studied region can be developed.

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