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Variation in otolith microchemistry for Lebranche mullet (*Mugil liza*) in southern Brazil and its potential use in identifying their nursery grounds

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Abstract—The Lebranche mullet (*Mugil liza*) is an economically important estuarine species found along the coast of Brazil. The southern population of this species ranges from the coast of the state of São Paulo (23°S) to the coast of Argentina (36°S). It migrates annually among estuaries in Argentina, Uruguay, and southern Brazil to reproduce in their marine spawning grounds (~26°S). We evaluated whether variations in the otolith chemical composition for fish acquired at nursery grounds can be used to make distinctions among 4 nursery areas of the southern population. Analysis of otolith microchemistry included concentrations of 9 elements: lithium (Li), magnesium (Mg), copper, zinc, strontium (Sr), cadmium, barium, lanthanum, and lead. When using random forest classification algorithms, the maximum accuracy of 96% in assignment of nursery habitat for Lebranche mullet between 2 groups (fish caught at nurseries in Brazil and fish migrating from waters of Uruguay and Argentina) was achieved with the combination of Li, Mg, and Sr. Our results indicate that the elemental composition of otoliths can be an important tool for establishing connectivity between nursery areas used by Lebranche mullet. We discuss the implications of this result for the structure of the population and the management of the mullet fishery in southern Brazil relative to the limitations of the methods we employed.

The Lebranche mullet (*Mugil liza*) is one of the most economically important marine species in southern Brazil (Sant'Ana et al., 2017; dos Santos et al., 2018). This species ranges from the Gulf of Mexico to Argentina (Menezes et al., 2015), and the southern population, discriminated on the basis of population genetic analyses, is distributed from the coast of the state of São Paulo in Brazil (23°S) to Buenos Aires Province in Argentina (36°S) (Mai et al., 2014; Lemos et al., 2017). The states of Rio Grande do Sul and Santa Catarina alone account for approximately 90–95% of the total catch in Brazil (MPA and MMA, 2015). In this area in Brazil, the average annual catch of commercial fisheries was 7150 metric tons (t), with a maximum catch of 13,600 t in 2007 (MPA and MMA, 2015; Sant'Ana et al., 2017). To the south, in Uruguay, the average annual catch was 200 t for the period 2000–2007 (Lorenzo, 2010). In Argentina, the Lebranche mullet is a target of a small-scale fishery, mainly off the northern coast of the Buenos Aires Province, where it is a popular food resource and a favorite of sport fishermen (González Castro et al., 2009).

The Lebranche mullet is a marine migrant estuarine species (Mai et al., 2018, 2019). The southern population, to reproduce, migrates annually from estuaries and lagoons along the coasts of Argentina, Uruguay, and southern Brazil to offshore waters off the northern coast of Santa Catarina and the southern coast of the state of Paraná in Brazil (~26°S) (Vieira and Scalabrin, 1991; González Castro et al., 2009; Lemos et al., 2014, 2016). This migration has been reported to start gradually and to follow the temporal movement of the range of sea-surface temperatures of 19–21ºC in a south–north direction from April to July (Lemos et al., 2014, 2016). According to Lemos et al. (2014), the broad geographical range of spawning locations along the coast of Santa

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Catarina indicates that spawning of Lebranche mullet is more closely related to oceanographic conditions than to specific locations. These oceanographic conditions are salinities of 32–35 and sea-surface temperatures of ~20 °C, found at depths of ~50 m in waters on the continental shelf (Lemos et al., 2014, 2016). The larvae and early juveniles of this species are neustonic and are carried, mainly through wind-driven currents, from spawning grounds toward the coast, where they concentrate in surf zones prior to recruitment to estuarine environments (Vieira, 1991; Lemos et al., 2021).

Many estuaries along the southern coast of Brazil and the coasts of Uruguay and Argentina are used by Lebranche mullet as nursery grounds (Ramos and Vieira, 2001; Loebmann and Vieira, 2005; González Castro et al., 2009; del Favero and Dias, 2015; Callicó Fortunato et al., 2017; Mai et al., 2018), and the Patos Lagoon estuary is considered the largest nursery area for the southern population of Lebranche mullet (Lemos et al., 2021). The Lebranche mullet has a flexible life history with a high degree of behavioral plasticity and wide salinity tolerance as juveniles. According to Mai et al. (2019), at least 3 patterns of coastal use and recruitment have been observed for juveniles. The majority (~90%) of juvenile Lebranche mullet migrate toward estuaries before completing their first year of life (64%) or after (25%) their first year of life (Mai et al., 2019). However, approximately 10% of the population does not always use estuaries as nurseries and can complete its entire life cycle in the marine environment (Callicó Fortunato et al., 2017; Mai et al., 2019). Furthermore, some adults return to an estuary after spawning in the ocean, but others never return to an estuary after spawning and remain in the marine environment (Mai et al., 2019).

Schroeder et al. (2023), combining otolith shape analyses and meristic and morphometric analyses of Lebranche mullet captured in waters of Brazil, discriminated 2 subpopulations. From the same study, results of analyses of the deposition of multiple elements in otolith cores from Lebranche mullet collected in Brazil and Argentina indicate the existence of more than one nursery area for this species (Schroeder et al., 2023). The identification of nursery origin of Lebranche mullet is extremely important and may help guide strategies to ensure the efficient management and sustainability of fisheries that target this species (Lotze et al., 2006).

The use of the chemical composition of otoliths has allowed stock identification (Thresher, 1999; Campana et al., 2000), descriptions of migration patterns and habitat utilization (Secor et al., 1995; Kennedy et al., 2002; Elsdon and Gillanders, 2003; Gillanders, 2005), descriptions of the life histories of different commercial fish species (Rooker et al., 2001; Artetxe-Arrate et al., 2019; Moll et al., 2019; Bae and Kim, 2020; Avigliano et al., 2021), and identification of nursery habitats (Gillanders and Kingsford, 2000; Vasconcelos et al., 2008; Reis-Santos et al., 2013; Tournois et al., 2017). Properties of otoliths allow them to form growth increments and retain chemical elements, associated with their metabolically inert capacity, and these signals of growth and chemistry provide a permanent record of the environmental conditions (such as the temperatures and chemical composition of the water) to which a fish was exposed (Campana and Neilson, 1985; Campana, 1999; Panfili et al., 2002). For this reason, researchers have long appreciated the importance of otoliths in studies on the biology of teleost fish (Campana and Neilson, 1985).

In this study, we tested the applicability of the elemental fingerprint in otoliths of Lebranche mullet to identification of nursery grounds utilized by the southern stock. For this purpose, the microchemistry of otoliths from Lebranche mullet was compared between 4 sampling sites, all nursery areas, off the southern coast of Brazil. The concentrations of the following chemical elements in otoliths were examined: lithium (Li), magnesium (Mg), copper (Cu), zinc (Zn), strontium (Sr), cadmium (Cd), barium (Ba), lanthanum (La), and lead (Pb).

Material and methods

Study area and fish collection

Adult Lebranche mullet (n=83) were collected directly from landings of the artisanal and commercial fisheries (gill-net fisheries) that operate in coastal waters and estuarine areas along the coasts of the southern states of Brazil (Fig. 1). Lebranche mullet use as nurseries several estuaries and lagoons that are part of the complex hydrographic system in and along the coastal plain in southern Brazil (Ramos and Vieira, 2001; Lemos et al., 2014). Therefore, we chose to collect specimens caught in 3 estuarine systems used as nursery grounds by juveniles on the southern coast of Brazil: Laguna (LAG, 28°S), Tramandaí (TRA, 29°S), and Patos Lagoon estuary (PLE, 32°S). We compared those specimens with individuals in prespawning migration that were coming from nurseries in Uruguay and Argentina; they were captured at the border between Brazil and Uruguay, near the city of Chuí (CHU, 33°S), by purse seine at the time of northern migration (Table 1). Individuals were collected between May and October 2011. All fish were transported on ice to the laboratory and preserved frozen until dissection. All specimens were measured for total length (TL) in millimeters and total weight (TW) in grams, and the sagittal otoliths were extracted, cleaned, dried, and stored for further analysis.

Otolith preparation and sample analysis

The method we used for obtaining otolith microchemical data follows that of Mai et al. (2018, 2019). Otoliths, one from each of the 83 collected specimens, were embedded in polyester resin, and transverse sections were cut by using an IsoMet Low Speed Saw[1] with a diamond blade (Buehler, Lake Bluff, IL). Prior to analysis through laser

[1] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

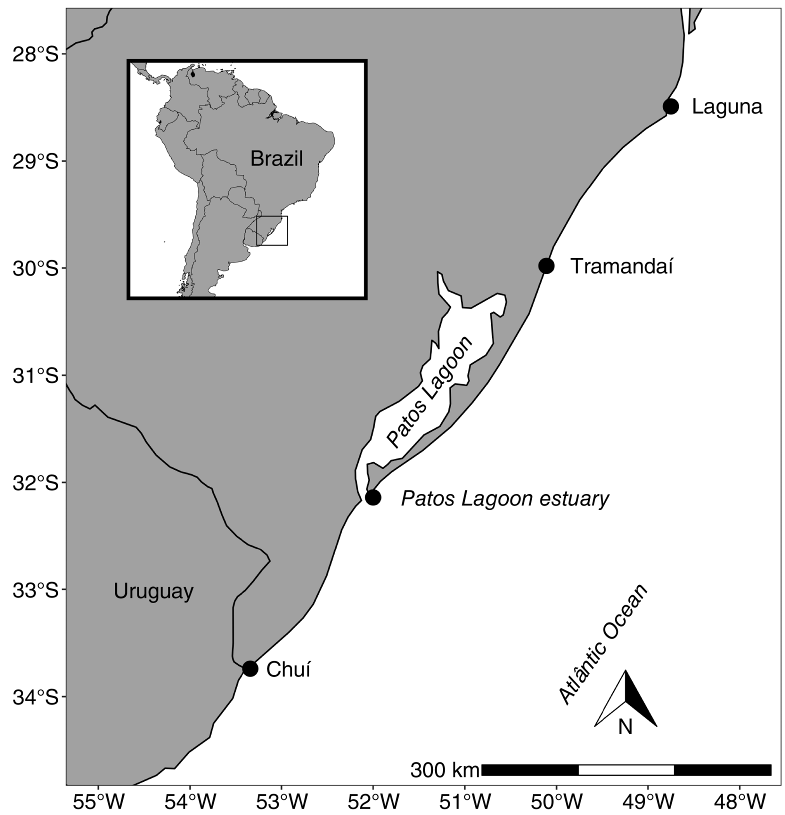

Figure 1

Map of southern Brazil showing the 4 sampling sites where Lebranche mullet (*Mugil liza*) were caught in May–October 2011. Laguna, Tramandaí, and Patos Lagoon estuaries are estuarine systems used by juvenile Lebranche mullet as nursery grounds, and the fourth site is near Chuí, a city at the border between Brazil and Uruguay.

ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), the sections (0.5 mm) were mounted on glass slides (with cyanoacrylate glue), polished (with silicon-carbide sanding paper no. 8000), sonicated for 1 min, rinsed 3 times with ultrapure distilled water (Milli-Q, Merck-Millipore), and dried in a laminar flow cabinet (Mai et al., 2018, 2019).

The LA-ICP-MS analysis was done with a Thermo Scientific iCAP Q ICP-MS (Thermo Fisher Scientific Inc., Waltham, MA) coupled to a 213-nm New Wave neodymium-doped yttrium-aluminium-garnet laser (Electro Scientific Industries Inc., Beaverton, OR) at the Instituto de Geociências da Universidade de São Paulo. Using this analysis, we quantified the concentrations of the following isotopes in the otoliths of Lebranche mullet: ^{7}Li, ^{25}Mg, ^{65}Cu, ^{66}Zn, ^{86}Sr, ^{114}Cd, ^{137}Ba, ^{139}La, and ^{208}Pb. A crater with a width of approximately 30 µm was generated in otoliths with a pulse frequency of the laser of 10 Hz, a scan speed of 30 µm/s, and energy outputs of 10 and 12 J/cm^2 per pulse. The ablated material was placed through a Teflon-coated tube into the ICP-MS by using a combination of helium (^{4}He) and argon (^{40}Ar) as a carrier gas.

Before each otolith ablation, the background signal intensity was measured with the ICP-MS for 60 s. Counts per second for Sr and calcium (Ca) were subtracted from the background level, and the ratios of the concentrations of elements to those of Ca were then calculated for all otoliths analyzed. The maximum analyte intensities and minimum interferences in the performance of the ICP-MS were determined by using oxides and double charged ions. The equipment was calibrated and tuned with the certified reference material MACS-3 (U. S. Geological Survey): 5.7% ^{86}Sr and 3.2% ^{137}Ba. To correct for mass bias and machine drift, after every 10 otoliths, a certified glass reference standard, NIST 612 (National Institute of Standards and Technology), was run.

Otolith transects were ablated from the core to the edge to capture the entire life history of the collected fish. Knowing that Lebranche mullet spawn at sea (Lemos

Table 1

Number and size of Lebranche mullet (*Mugil liza*) caught at each sampling site on the coast of southern Brazil from May through October 2011. n=sample size; SD=standard deviation.

		Total length (mm)		Total weight (g)	
Site (latitude)	n	Range	Mean (SD)	Range	Mean (SD)
Laguna (28°S)	23	353–558	435 (59)	377–1538	749 (300)
Tramandaí (29°S)	22	319–540	403 (49)	271–1260	597 (212)
Patos Lagoon estuary (32°S)	25	360–430	402 (20)	506–919	698 (106)
Chuí (33°S)	13	490–590	545 (34)	1145–2322	1771 (413)

et al., 2014, 2017; Mai et al., 2019), the sampling zone around the otolith core was chosen manually to represent the otolith section formed while the fish was in a nursery area, with the ratios of concentrations of Sr and Ba to concentrations of Ca in otoliths assumed to be characteristic of the water chemistry of an estuarine zone (Mai et al., 2018, 2019). According to Mai et al. (2019), values of 0.02–0.13 µmol/mol for ratios of Ba concentration to Ca concentration after the core zone indicate that a species was living in a nursery area that was an estuarine zone. For characterization of the nursery origins of specimens, at least 20 chemistry readings (by laser ablation spot) in the nursery zone of each otolith were made.

Data reduction, including background corrections, conversion of mass count data to concentrations (in parts per million), and limits of detection, was done for each sample by using Glitter software (Australia Research Council National Key Centre for Geochemical Evolution and Metallogeny of Continents, Macquarie University, Sydney, Australia). In further analysis, we considered the concentrations of 9 elements (Li, Mg, Cu, Zn, Sr, Cd, Ba, La, and Pb) that were all well above the detection limits calculated by using Glitter (0.020, 0.080, 0.025, 0.057, 0.155, 0.004, 0.007, 0.014, and 0.015 ppm, respectively). Concentrations of trace elements (originally in parts per million) were transformed to ratios of concentrations of elements to concentrations of Ca, expressed as units of millimoles per mole or micromoles per mole (Thorrold et al., 1998; Forrester and Swearer, 2002; Swearer et al., 2003; Dorval et al., 2005; Brown, 2006).

Statistical analyses

We used a nonmetric multidimensional scaling plot to visualize the elemental composition of otoliths and potential chemical differences among sampling sites from a dissimilarity matrix of calculating the Euclidean distances. For each nursery area, we analyzed the element concentration distributions by using box plots. We visualized otolith chemical signatures for each nursery area by using radar plots, based on the mean proportion of each element concentration among the 83 otoliths analyzed. Concentrations of all elements were checked for normality and homoscedasticity prior to statistical analyses. Parametric assumptions were not met (according to results of Shapiro–Wilk and Bartlett tests), even after $\log_{10}$ transformation; therefore, we used nonparametric statistics. To detect whether otolith elemental fingerprints differed among nursery areas, we employed the Kruskal–Wallis test with Dunn's post hoc test. Spearman correlation analysis was used to examine the correlation between the ratios of element concentrations in the otoliths.

We used the elemental fingerprinting index (EFI) created by Moll et al. (2019) as a measure of the similarity among individuals when considering numerous chemical signatures in their otoliths. The EFI ranges from 0 to 1, with a value of 0 indicating that the elemental compositions in the otoliths of the 2 compared individuals are most different and a value of 1 indicating the highest similarity in the elemental compositions in the otoliths of the 2 compared individuals. We conducted comparisons of element concentrations in otoliths for all individuals within one sampling area and comparisons for all individuals among the different sampling areas. The EFI values were tested for significant differences within and between the 4 sampling areas by using the Welch test and Games–Howell post hoc test.

To identify the optimal combination of elements for habitat discrimination, we used a machine learning method based on the random forest algorithm developed by Breiman (2001). Machine learning methods, such as random forest, have been reported to have greater classification efficiency for nursery identification based on otolith microchemistry, especially when data sets include multiple specific otolith signatures or several chemical elements (Mercier et al., 2011). Following this method, 5000 classification trees were built, considering all possible combinations of the 9 elements measured. The analysis proceeded in 2 steps. First, a random forest classifier was built by using the chemical signatures in otoliths for 75% of individuals that were randomly selected from specimens of known nursery origin. Second, the otolith signatures from the remaining individuals (25%) were used in the random forest classifier to predict origin and to test the prediction accuracy by measuring the proportion of individuals correctly assigned to their known origin (CHU, LAG, TRA, or PLE). Additionally, the algorithm was also executed with the origin site defined as binary, classified as either the coasts of Uruguay and Argentina (CHU) or estuaries of Brazil (LAG, TRA, and PLE). Details of the method and application are provided in Mercier et al. (2011, 2012).

All statistical analyses were done in statistical software R (vers. 4.2.2; R Core Team, 2022) with the packages dplyr (vers. 1.0.10; Wickham et al., 2022), class (vers. 7.3-22; Venables and Ripley, 2002), FactoMineR (vers. 2.9; Lê et al., 2008), FSA (vers. 0.9.5; Ogle et al., 2023), ggplot2 (vers. 3.4.4; Wickham, 2016), gtools (vers. 3.9.3; Warnes et al., 2022), MASS (vers. 7.3-58.1; Venables and Ripley, 2002), mda (vers. 0.5-3; Hastie et al., 2022), randomForest (vers. 4.7-1.1; Liaw and Wiener, 2002), and stats (vers. 4.2.2; R Core Team, 2022).

Results

Otolith microchemistry

Mean concentrations of elements in examined otoliths varied greatly across the 4 sampling sites, CHU, PLE, TRA, and LAG (Table 2, Fig. 2). The ratios of concentrations of 5 elements, Li, Cu, La, Pb, and Sr, to concentrations of Ca in the otoliths from specimens differed significantly (Kruskal–Wallis pairwise test: $P<0.05$) between 2 or 3 sites. The ratios of the concentrations of Li to Ca in otoliths were significantly higher for fish caught at CHU than for those caught at the other 3 sampling sites. Concentration ratios of La to Ca were lower in otoliths from fish caught at CHU than in those from fish

Table 2

Mean ratios of the chemical concentrations of 9 elements to concentrations of calcium in the nursery zone in otoliths from Lebranche mullet (*Mugil liza*) caught at 4 sampling sites in southern Brazil, Chuí (CHU), Patos Lagoon estuary (PLE), Tramandaí (TRA), and Laguna (LAG), between May and October 2011. The elements are lithium (Li), magnesium (Mg), copper (Cu), zinc (Zn), strontium (Sr), cadmium (Cd), barium (Ba), lanthanum (La), and lead (Pb). Significant differences in chemical signatures in otoliths between sites were examined by using Kruskal–Wallis and Dunn's post hoc tests for each element with the significance level of 0.05. An asterisk (*) indicates that signatures were revealed in Kruskal–Wallis tests to be significantly different between sites. The results of Dunn's test for pairwise comparisons are provided. Standard deviations (SDs) of mean ratios are given in parentheses. NS=not significant.

	Mean ratio (SD)						
Element	CHU	PLE	TRA	LAG	χ^2	*P*	Dunn's test
Li	4.57 (1.62)	1.46 (1.16)	1.91 (1.06)	2.01 (1.11)	33.36	<0.01*	CHU>PLE, TRA, LAG
Mg	3.20 (0.87)	3.47 (1.56)	3.17 (1.20)	3.07 (1.29)	2.87	0.41	All NS
Cu	0.22 (0.20)	0.30 (0.44)	0.22 (0.14)	0.20 (0.24)	7.64	0.05*	PLE>LAG
Zn	0.43 (0.78)	0.60 (0.58)	0.67 (1.35)	0.59 (0.53)	5.24	0.16	All NS
Cd	2.03 (1.75)	2.25 (1.81)	2.57 (1.85)	2.22 (1.82)	1.79	0.62	All NS
Ba	0.07 (0.05)	0.08 (0.07)	0.06 (0.05)	0.07 (0.05)	0.71	0.87	All NS
La	1.22 (1.29)	1.81 (1.62)	1.73 (1.19)	1.44 (0.94)	13.34	<0.01*	CHU<PLE, TRA
Pb	0.21 (0.27)	0.30 (0.21)	0.47 (0.40)	0.34 (0.33)	16.04	<0.01*	TRA>CHU, PLE
Sr	0.43 (0.10)	0.45 (0.12)	0.46 (0.09)	0.48 (0.10)	8.35	0.04*	LAG>CHU, TRA

caught at PLE and TRA. The ratios of concentrations of Pb to Ca were higher in otoliths from specimens caught at TRA than in the otoliths of those caught at CHU and PLE. The concentration ratios of Cu to Ca were significantly higher for otoliths from fish caught at PLE than for otoliths from those caught at LAG. Ratios of the concentrations of Sr to Ca in otoliths was higher for specimens caught in LAG than for those from CHU and TRA, and ratios of the concentrations of Mg, Zn, Cd, and Ba to Ca in otoliths were similar between sites (Table 2).

Results from Spearman correlation analysis (Table 3) indicate that some of the correlations between ratios of element concentrations were significant ($P<0.05$). The correlations between the concentration ratios of Cu, Zn, Cd, La, and Pb to Ca indicate a significantly positive relationship. The only significant negative correlation was between the ratio of the concentration of Li to Ca and the ratio of the concentration of La to Ca.

Chemical signatures in the nursery zone of otoliths

Examination of the data set on the chemical composition of the nursery zone of otoliths included habitat-specific plots of elemental fingerprints with similarities and differences in proportions of element concentrations in otoliths (Fig. 3). The nonmetric multidimensional scaling plot indicates differences in chemical signatures in otoliths for each sampling site. Element concentrations in otoliths of specimens from CHU had lower variability among individuals than those in otoliths of fish from LAG, TRA and PLE, and they are clustered on the left side of the ordination plot. Elemental compositions in the nursery zone of otoliths from specimens caught at PLE, LAG, and TRA were similar and clustered on the right side of the plot, indicating more similarity in otolith compositions among these sites than between these sites and CHU (Fig. 4).

Results from the pairwise comparisons of multiple element concentrations for individuals within a single sampling site, made by using the EFI, indicate that the otolith compositions for individuals collected at CHU are significantly more similar to those of each other (higher EFI value) and are significantly different from those of specimens caught at the other 3 sites ($P<0.001$) (Table 4). Comparisons between sampling sites also identified similarities in element concentrations of otoliths from fish caught at PLE, TRA, and LAG (Table 4). The EFI values for comparisons of chemical signatures in otoliths of fish caught at CHU to those of fish caught at each of the other 3 sites, LAG, PLE, and TRA, indicate that similarities in element concentrations between otoliths within these groups of fish were significantly lower (lower EFI value) than the similarities between otoliths from fish collected at CHU ($P<0.001$). The EFI values from comparisons between 2 sites at a time for all 4 nurseries are smaller than the EFI values from within-site comparisons, indicating that element concentrations in otoliths of specimens are more similar within sites than between all 4 nurseries (Table 4).

Discrimination of nursery grounds

In using the random forest method to compare the 4 nursery grounds sampled, we built classification trees by considering multiple combinations of the 9 chemical elements tested. We observed that the addition of elements generally improved the accuracy of the random forest classifier, increasing the average levels of accuracy in discriminating fish nursery origin of Lebranche mullet (Fig. 5A). The maximum accuracy (55.1%) in assignment of nursery

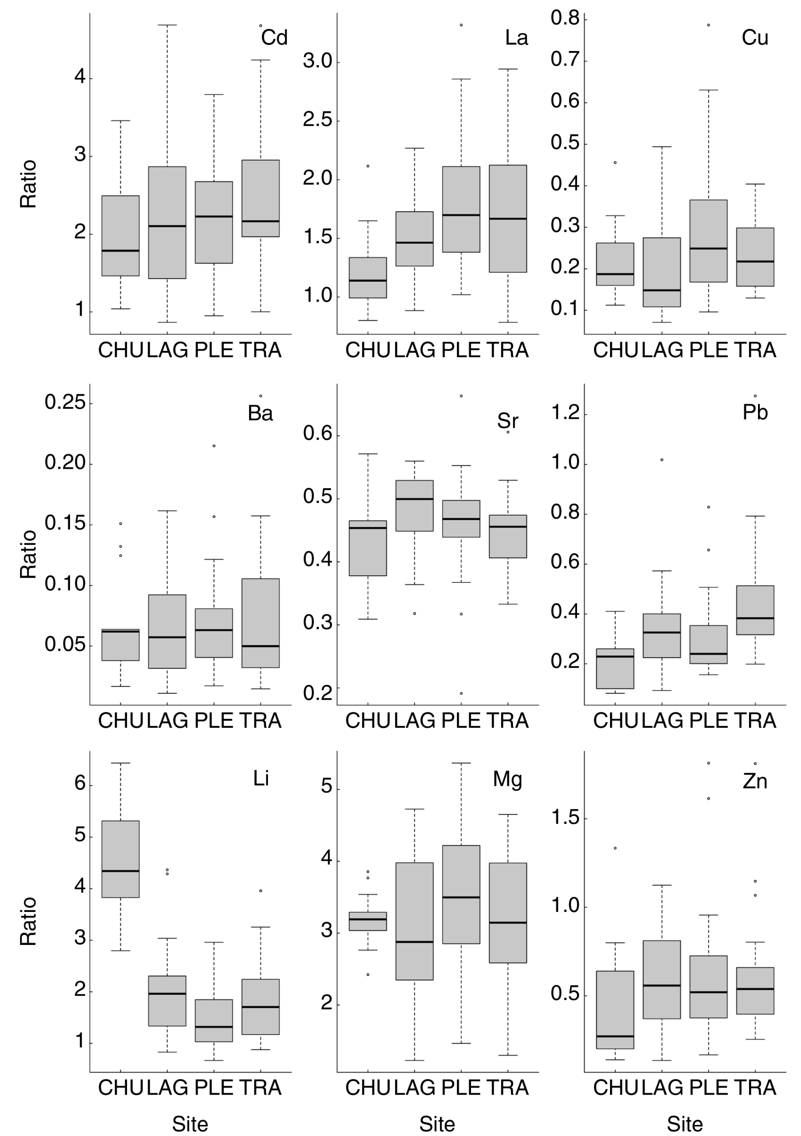

Figure 2

Mean ratios of the chemical concentrations of 9 elements to the concentrations of calcium in the nursery zone of otoliths of Lebranche mullet (*Mugil liza*) collected from May through October 2011 at 4 sampling sites in southern Brazil: Chuí (CHU), Patos Lagoon estuary (PLE), Tramandaí (TRA), and Laguna (LAG). The elements are cadmium (Cd), lanthanum (La), copper (Cu), barium (Ba), strontium (Sr), lead (Pb), lithium (Li), magnesium (Mg), and zinc (Zn). In each box plot, the thick black line is the median. The upper and lower parts of each box represent the first and third quartiles (the 25th and 75th percentiles). The whiskers extend above and below the box no more than 1.5 times the interquartile range. Data points that appear beyond the end of the whiskers are outliers.

origins to the 4 sampling sites was reached with the combination of the 4 elements Cu, Li, Pb, and Sr, specifically the ratios of their concentrations to concentrations of Ca.

On the basis of evidence that element concentrations in otoliths of specimens from CHU were significantly different from those in otoliths of fish from the remaining sites sampled and that the nursery habitats for specimens from PLE, TRA, and LAG could not be discriminated (Figs. 3 and 4, Table 4), we compared data for specimens from CHU to data for fish from the other 3 sampling sites combined (Fig. 5B). The maximum accuracy of 96% in assignment of nursery area was achieved with the combination of values for only 3 elements: the ratios of the concentrations of Li, Mg, and Sr to concentrations of Ca.

Discussion

Otolith chemical composition is not a direct representation of ambient water chemistry, and the relationship between environmental and physiological processes is still poorly understood (Thresher, 1999). Nonetheless, it is widely accepted that the mechanisms controlling the uptake and incorporation of elements into otoliths are mediated by both exogenous (e.g., salinity) and endogenous (e.g., growth) factors (Walther et al., 2010). Not all elements behave similarly in otoliths (Vrdoljak, 2020). Many studies have attempted to determine the causes of variations in otolith microchemistry (Elsdon and Gillanders, 2003; Sturrock et al., 2014, 2015), and knowledge of the factors that influence otolith microchemistry is far from complete. However, several scientists have claimed that it is not necessary to understand all these mechanisms to be able to use otolith microchemistry as a tool in fisheries management (Thorrold et al., 1998; Thresher, 1999; Elsdon et al., 2008).

As expected, results from the analysis in our study of element concentrations in the nursery zone of otoliths from Lebranche mullet indicate that the ratios of concentrations of Li, Sr, La, Pb, and Cu to concentrations of Ca were significantly different among the sampling sites. The concentrations of certain elements varied between nursery grounds in southern Brazil and the southernmost nursery areas in Uruguay and Argentina.

Signatures of Sr and Ba can be used to distinguish residences in waters of different salinities (Walther and Thorrold, 2006; Elsdon et al., 2008) and have been used to successfully reconstruct environmental histories and coast–estuary movement for Lebranche mullet in South America (Callicó Fortunato et al., 2017; Mai et al., 2018, 2019). We found no significant difference between sites in the ratio of concentrations of Ba to Ca in the nursery zone of otoliths from specimens of Lebranche mullet. This finding indicates that all

Table 3

Spearman correlation coefficients for the relationships between ratios of the concentrations of 9 elements, lithium (Li), magnesium (Mg), copper (Cu), zinc (Zn), strontium (Sr), cadmium (Cd), barium (Ba), lanthanum (La), and lead (Pb), to concentrations of calcium in the nursery zone of otoliths from Lebranche mullet (*Mugil liza*) collected from 4 sampling sites in southern Brazil from May through October 2011. An asterisk (*) indicates a significant correlation between concentrations of elements ($P<0.05$).

	Ratio of element to calcium								
Ratio	Li	Mg	Cu	Zn	Cd	Ba	La	Pb	Sr
Li									
Mg	0.17								
Cu	−0.01	0.15							
Zn	−0.13	0.12	0.52*						
Cd	0.02	0.04	0.59*	0.56*					
Ba	−0.23	−0.11	0.14	0.10	−0.04				
La	−0.26*	0.11	0.45*	0.56*	0.47*	0.09			
Pb	−0.08	−0.07	0.33*	0.67*	0.62*	0.07	0.55*		
Sr	−0.06	−0.07	0.02	0.24	0.13	−0.11	0.00	0.17	

sampled individuals developed in estuaries. However, we found significantly higher ratios of concentrations of Sr to Ca in otoliths of Lebranche mullet from LAG, a trend that may be associated with differences in ambient chemistry and physical factors. The other sampled nursery areas are affected by large drainage basins and, therefore, are heavily influenced by freshwater discharges; in comparison, LAG is a saline wedge estuary located at 28°S and is under the influence of higher temperatures than those at other sites (D'Aquino et al., 2011).

Fish absorb heavy metals (e.g., Pb and Cu) from their environments during their lives, and these elements are preserved within otolith structures (Reis-Santos et al., 2013; Sturrock et al., 2014). The accumulation of trace metals in fish otoliths depends on a number of factors, including the concentrations of elements in the water environment (Ranaldi and Gagnon, 2010). We found higher concentration ratios of Pb and Cu to Ca in otoliths from Lebranche mullet caught in the estuary at TRA and in the PLE, respectively. This result may be an indication of anthropogenic effects in estuarine environments. In fact, the average concentrations of metals in estuarine water in Patos Lagoon correspond to the natural base levels described for other estuarine systems, but sporadic increases in Cu and Pb concentrations have been related to the ingress of industrial effluents and activities in the limnic area of Patos Lagoon (Seeliger et al., 1997). Although findings from some other research indicate associations of the highest Pb concentrations in otoliths with port areas (Cuevas et al., 2019), the effects of the time during which fish are exposed to Pb and of physiological processes should be considered (Vrdoljak, 2020). The nurseries sampled at both TRA and PLE are in highly populated areas. Although concentrations of trace metals in the waters at these sites are low, they could be used to identify the contribution of polluted nurseries to the stock of Lebranche mullet in southern Brazil.

We found significantly higher ratios of the concentrations of Li to Ca in the nursery zone of the otoliths of Lebranche mullet sampled from the southern migratory contingent (at CHU) during the time of northern migration than in those of fish caught at other sampling sites, all estuaries in southern Brazil. In fact, Li was the most significant element in the use of otolith microchemistry to discriminate between sampling sites (Table 2, Fig. 2). According to Sturrock et al. (2012), among the various elements found in otoliths, Li has the most positive relationship with the environment and is one of the elements that are reliable geographic markers in marine species. Lithium is highly bioavailable; however, little attention has been given to determining the environmental and physiological factors that affect incorporation of Li into otoliths (Brown, 2006). Furthermore, information about Li in the aquatic environment is limited (Tkatcheva et al., 2015). This element seems to be associated with estuaries in the southern regions of South America, and results from other studies already indicate the power of Li in discriminating habitats for species collected in Brazil, Uruguay, and Argentina: the Brazilian codling (*Urophycis brasiliensis*) (Biolé et al., 2019) and the whitemouth croaker (*Micropogonias furnieri*) (Avigliano et al., 2021).

Through none of our analyses were we able to use the nursery zone of otoliths to differentiate between groups of fish caught in each of the estuaries in southern Brazil (PLE, TRA, and LAG). However, otoliths of fish caught from CHU were different from those of the combined group of fish caught in PLE, TRA, and LAG. The similarity between the chemical signatures in the nursery zone of otoliths of Lebranche mullet from the estuaries of Brazil can be explained by the similar physicochemical conditions of the waters in those estuaries; after all, water chemistry influences the chemical composition of otoliths (Kerr and Campana, 2014). According to Adelir-Alves

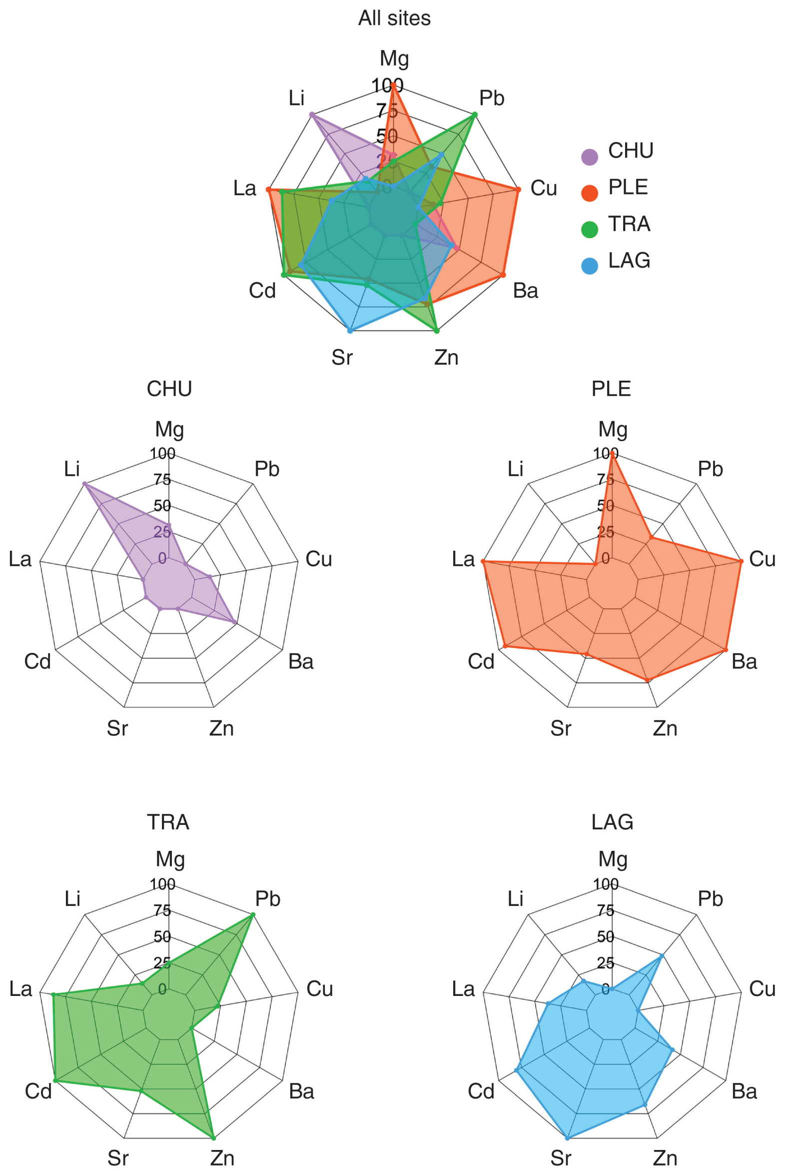

Figure 3

Radar plots of the proportions of element concentrations in the nursery zone of otoliths from Lebranche mullet (*Mugil liza*) collected at 4 sampling sites in southern Brazil in May–October 2011: Chuí (CHU, purple radii), Patos Lagoon estuary (PLE, red radii), Tramandaí (TRA, green radii), and Laguna (LAG, blue radii). The proportions (shown as percentages) are for concentrations of 9 elements, lithium (Li), magnesium (Mg), lead (Pb), copper (Cu), barium (Ba), zinc (Zn), strontium (Sr), cadmium (Cd), and lanthanum (La).

et al. (2019), otolith microchemistry analyses provide information for a given time and on a fine spatial scale, but the usefulness of element concentrations in otoliths as natural biogeochemical markers depends on the existence of measurable differences in otolith microchemistry on a geographic scale relevant to the life history of a species. In fact, the concentration of some elements in otoliths differed significantly between the sampling sites in the estuaries of southern Brazil, but the general signature in otoliths of fish from each habitat had a relatively low level of accuracy (55%) in the analysis done with the random forest method (Fig. 5A). According to Mercier et al. (2011), an acceptable degree of accuracy should be greater than 75%. Only after grouping the specimens from the estuaries in Brazil and comparing the element concentrations in their otoliths with those of specimens from CHU did the accuracy of nursery assignment increase to 96% (Fig. 5B).

Temporal variability in otolith microchemistry must be considered because otolith elemental fingerprints are unlikely to remain consistent over time (Gillanders, 2002; Hamer et al., 2003; Clarke et al., 2009; Walther and Thorrold, 2009). This temporal variability can confuse spatial differences, meaning differences in elemental composition of otoliths among fish from different nurseries. Therefore, it is important to consider interannual differences in otolith microchemistry (Gillanders, 2002; Cuveliers et al., 2010; Reis-Santos et al., 2012), and assignment of origin (to specific nurseries or sampling sites) should be done only for year-classes for which an otolith chemical signature has been identified for juveniles. For this reason, it would be ideal if sampling and monitoring programs systematically collect otoliths over a period of several years, compiling a library of reference elemental markers than can be later used to determine the nursery origin of coastal adult fish (Gillanders, 2002; Reis-Santos et al., 2013).

We assume that individuals collected at the southernmost sampling site, CHU, were from estuaries in Argentina and Uruguay, a notion that is reinforced by the distinction in otolith microchemistry for fish from this site and the low standard deviations of ratios of element concentrations. Several reports on previous scientific research have revealed that Lebranche mullet migrate from Argentina and Uruguay to waters in Brazil in order to reproduce (González Castro et al., 2009; Lemos et al., 2014; Mai et al., 2014; Lemos et al., 2016). This common finding reflects the local ecological knowledge of Brazilian fishermen regarding the existence of various morphotypes of Lebranche mullet with different geographical origins in the southwestern Atlantic Ocean (Herbst and Hanazaki, 2014). Distinct morphological characteristics are acquired in natural conditions of nurseries where

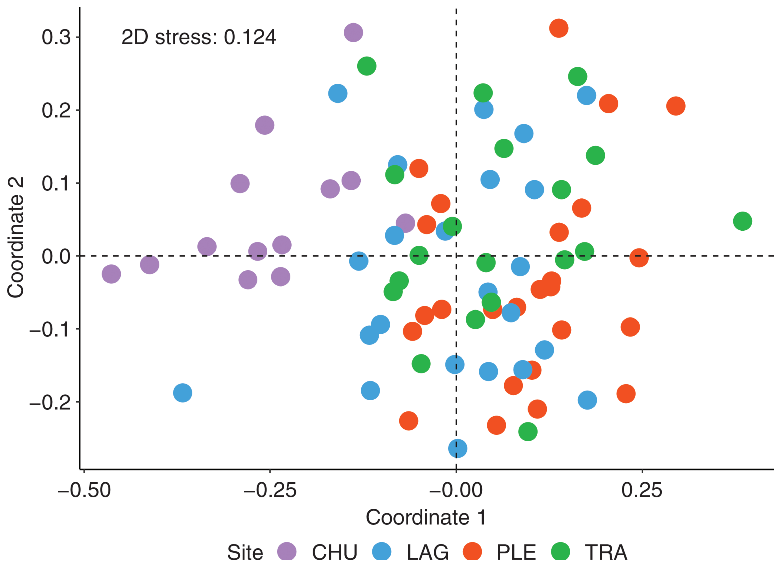

Figure 4

Nonmetric multidimensional scaling plot of element concentrations in the nursery zone of 83 otoliths from Lebranche mullet (*Mugil liza*) caught from May through October 2011 at 4 sampling sites in southern Brazil: Chuí (CHU), Laguna (LAG), Patos Lagoon estuary (PLE), and Tramandaí (TRA). The plot is based on a Euclidean distance matrix.

Table 4

Elemental fingerprinting indices (EFIs) for each sampling site (upper table) and between 2 sites (lower table), based on comparisons of chemical signatures in otoliths from Lebranche mullet (*Mugil liza*) collected in May–October 2011 in southern Brazil. The EFI ranges from 0 to 1, with a value of 0 indicating that the elemental compositions in the otoliths of 2 fish are most different and a value of 1 indicating that the elemental compositions in the otoliths of 2 specimens have the highest similarity (Moll et al., 2019). An asterisk (*) indicates a significant difference between EFI values (P<0.05). Standard deviations (SDs) of mean EFI values are provided in parentheses.

Comparisons within 1 site		
Site	No. of comparisons	Mean EFI (SD)
CHU	78	0.89 (0.04)*
PLE	276	0.86 (0.05)
TRA	231	0.86 (0.04)
LAG	210	0.85 (0.05)

Comparisons between 2 sites				
Site	Site	No. of comparisons	Mean EFI (SD)	P
CHU	PLE	234	0.81 (0.06)*	<0.001
CHU	TRA	208	0.82 (0.06)*	<0.001
CHU	LAG	195	0.83 (0.05)*	<0.001
PLE	TRA	297	0.86 (0.04)	0.960
PLE	LAG	294	0.84 (0.06)	0.203
TRA	LAG	252	0.87 (0.04)	0.074

Lebranche mullet of these morphotypes grow for up to 4–5 years until they migrate to reproduce in coastal zones (~26°S) (Garbin et al., 2014; Lemos et al., 2014, 2016). Schroeder et al. (2023) recognized 2 subpopulations of Lebranche mullet: one subpopulation is associated with the central stock in Brazil, and another subpopulation is associated with the southern stock that comprises a mix of individuals from Brazil and Argentina. Locations or regions where Lebranche mullet are known to spawn are more associated to oceanographic conditions than to specific geographical locations (Lemos et al., 2014, 2016). The geographic breadth of the spawning region for this species varies annually, as does the annual contribution of individuals to the different subpopulations or contingents.

The mullet fishery in southeastern Brazil targets a stock that has been reported to be overfished in recent decades (de Miranda et al., 2011; MPA and MMA, 2015). This exploitation is further aggravated by the fact that Lebranche mullet are fished more intensively during the reproductive period of this species, especially when shoals are formed and gradually thickened as they migrate north from the nursery areas in Argentina, Uruguay, and southern Brazil (MPA and MMA, 2015; Lemos et al., 2016).

Proving that the 2 subpopulations described by Schroeder et al. (2023) mix with each other during the reproductive period and determining in which proportions this mixing occurs were the objectives of our study. Our work did not incorporate analysis of individuals from the same cohort that were born in different locations; therefore, it was not possible to estimate the actual contributions of specific nurseries to the adults of the southern population caught by commercial fisheries during the reproductive period of this species. However, we were able to conclude that the 2 subpopulations do mix during the reproductive period, and we have established that it is possible to estimate the relative contributions of different estuarine nurseries to the adult population of Lebranche mullet by using random forest classifiers generated with fingerprints of a combination of multiple elements in otoliths.

Conclusions

The results of our study indicate that the elemental composition of otoliths from Lebranche mullet differed significantly between groups of nurseries and can potentially be used to estimate the relative contributions of nurseries to adults in the southern population. Using random forest classification algorithms with the chemical signatures of 3 elements (Li, Mg, and Sr) combined, we achieved the

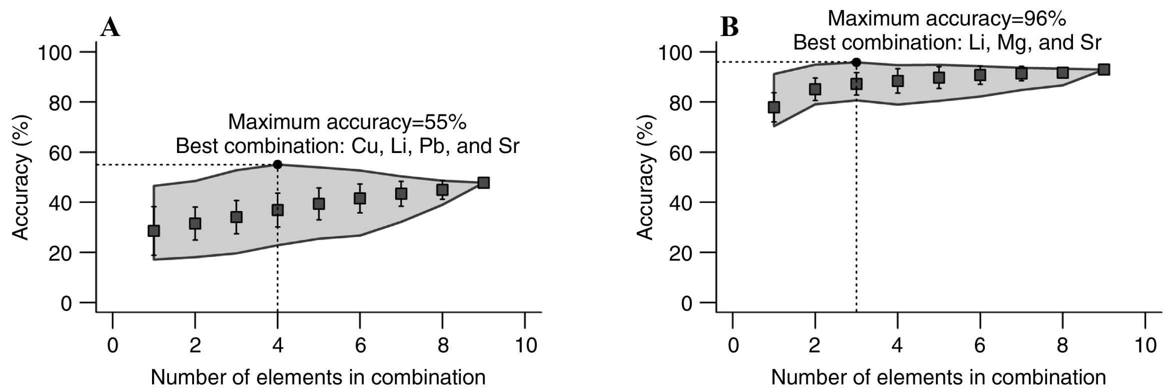

Figure 5

Accuracy in assigning nursery origins by using microchemistry in otoliths from Lebranche mullet (*Mugil liza*) caught between May and October 2011 at 4 sampling sites in southern Brazil, (**A**) when the origin was defined in a random forest algorithm as the site Chuí, Patos Lagoon estuary, Tramandaí, or Laguna and (**B**) when the origin was defined as either Chuí or the 3 nursery sites combined (Patos Lagoon estuary, Tramandaí, and Laguna). The classification trees of the algorithm were based on different combinations of up to 9 elements in otoliths. The polygon in each panel indicates the range of accuracy values. Error bars indicate standard deviations of mean accuracy levels. The dashed lines indicate maximum model accuracy. Cu=copper; Li=lithium; Mg=magnesium; Pb=lead; Sr=strontium.

maximum accuracy of 96% in identification of nursery origin for Lebranche mullet when assigning origin between CHU, where individuals of the southern migratory contingent were caught, and the nurseries in southern Brazil (PLE, TRA, and LAG combined). Given that analyses were done with individuals from the same population, our findings corroborate the existence of 2 subpopulations of Lebranche mullet off the coast of southern Brazil on the basis of their use of different nursery areas. Our results also confirm that signatures based on the concentrations of a combination of multiple elements in otoliths provide information valuable in establishing connectivity between nursery grounds and adult reproductive aggregation. Therefore, such analysis based on otolith microchemistry can be used to aid in the management of mullet fishing.

Resumen

El lisa Lebrancha (*Mugil liza*) es una especie estuarina de importancia económica que se encuentra a lo largo de la costa de Brasil. La población meridional de esta especie se extiende desde la costa del estado de São Paulo (23°S) hasta la costa de Argentina (36°S). Migra anualmente entre estuarios de Argentina, Uruguay y el sur de Brasil para reproducirse en sus zonas de desove marinas (~26°S). Evaluamos si las variaciones en la composición química de los otolitos de los peces colectados en las áreas de crianza pueden utilizarse para distinguir entre las 4 áreas de crianza de la población meridional. El análisis de la microquímica de los otolitos incluyó concentraciones de 9 elementos: litio (Li), magnesio (Mg), cobre, zinc, estroncio (Sr), cadmio, bario, lantano y plomo. Al utilizar algoritmos de clasificación de bosque aleatorio, se alcanzó una máxima precisión del 96% en la asignación del hábitat de críanza de la lisa Lebrancha entre 2 grupos (peces capturados en criaderos de Brasil y peces migrando desde aguas de Uruguay y Argentina) con la combinación de Li, Mg y Sr. Nuestros resultados indican que la composición de elementos de los otolitos puede ser una herramienta importante para establecer la conectividad entre las áreas de crianza utilizadas por la lisa Lebrancha. Discutimos las implicaciones de este resultado para la estructura de la población y el manejo de la pesquería de lisa en el sur de Brasil en relación con las limitaciones de los métodos que empleamos.

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