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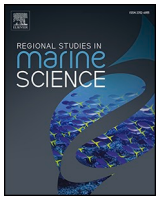

Stock discrimination of the axillary seabream Pagellus acarne (Teleostei: Sparidae) along the mainland Portuguese coast using a complementary otolith geochemistry approach

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ABSTRACT

The identification of population units is essential for proper fishery management to ensure the availability of fish populations. The axillary seabream, *Pagellus acarne* (Teleostei: Sparidae), represents a fishery resource in Portuguese waters; however, its stock structure remains unknown. The objective of this work was to use otolith geochemical signatures to identify the origin, habitat use, and ecological connectivity of P. acarne along the Portuguese coast, and determine whether the species constitutes a single connected demographic unit or multiple isolated population units requiring distinct fishery management strategies. Adult fish were sampled from three fishery grounds: two along the western coast (Matosinhos and Peniche) and one along the southern coast (Portimão). At each site, 25 individuals of the same age (4 years) were selected. Element:Ca ratios of Sr, Ba, Mn, Mg, Zn and Li were measured in otolith cores and edges using laser-ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to characterize early-life and recent environmental histories, respectively. Whole otoliths were also analysed using solution-based ICP-MS (SB-ICP-MS) to obtain life-integrated chemical signatures. Spatial patterns were evaluated using univariate and multivariate analyses, and reclassification success was assessed by jackknife cross-validation. The results showed that whole-otolith geochemistry provided greater spatial discrimination than either core or edge signatures. Core and edge signatures showed limited discriminatory ability, with 56% and 44% reclassification success, respectively. Whole-otolith analysis provided the clearest spatial signal, revealing significant differences between Matosinhos and the other sampling sites and 63% overall reclassification success. This pattern was driven by lower Sr:Ca, Mn:Ca and Zn:Ca values in the northern region, possibly reflecting localized river input and/or adaptive responses. Overall, the results support the hypothesis of a single demographic unit characterized by high connectivity, with limited localized differentiation, particularly in the north. Future research should integrate complementary approaches and broader geographic sampling to detect large-scale population breaks.

1. Introduction

Portugal has an extensive coastline and a strong historical connection with the ocean (de Almeida, 2022). Therefore, seafood consumption has always been an integral part of Portuguese culture, with the country currently ranking first in per capita seafood intake in the European Union, averaging approximately 60 kg per person annually (Guillen et al., 2018; Murthy et al., 2023). This high seafood demand, together

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with the socioeconomic relevance of marine resources, has raised concerns regarding the sustainable management of the fisheries (Almeida et al., 2015; Costa et al., 2022; Murthy et al., 2023). The long-term profitability and persistence of local fisheries depend on effective management measures supported by reliable and up-to-date scientific advice (FAO, 2020; Costa et al., 2022; Petza and Katsanevakis, 2024). A foundational component of this process is the identification and delineation of the different stocks that structure the geographic distribution of an exploited fish species, which differ in life-history traits, habitat use, and population dynamics (FAO, 2020; Cadrin et al., 2023). Different stocks may be exposed and respond differently to fishing pressure, environmental variability, and conservation measures, and therefore require specific management strategies to avoid overexploitation which can lead to ecological and economic consequences (Hilborn et al., 2020; Cadrin et al., 2023; Rumolo, 2023).

Several approaches have been used to investigate stock structure in fish. Genetic analyses can effectively detect long-term reproductive isolation among populations; however, in marine fish species with high dispersal and fecundity, neutral genetic markers often show little spatial differentiation, limiting the ability to resolve fine-scale spatial structure and recent connectivity (Reis-Santos et al., 2018; Moreira et al., 2019a; Cadrin et al., 2023). Consequently, stock assessment studies have increasingly relied on non-genetic natural tags, defined as intrinsic signatures that reflect environmental history, habitat use, or the degree of connectivity across a species' distribution (Hawkins et al., 2016; Thomas and Swearer, 2019; Cadrin et al., 2023). These signatures include phenotypic traits such as body or otolith morphometrics (Ferreira et al., 2019; Moreira et al., 2019b; Kulzer et al., 2025), parasite assemblages (Santos et al., 2009; Braicovich et al., 2025), or the chemical composition of otoliths (Correia et al., 2021; Schroeder et al., 2022; de Almeida et al., 2026).

Otoliths are calcium carbonate structures located in the inner ear of fish that play an important role in balance and hearing (Evans et al., 2013; Thomas and Swearer, 2019). These aragonitic structures grow continuously throughout the fish's life via the incremental deposition of calcium carbonate derived from ambient water (Thomas and Swearer, 2019; Hüssy et al., 2021). Trace and minor elements are also incorporated into the otolith matrix, either through substitution for calcium or incorporation within the organic matrix (Thomas and Swearer, 2019; Hüssy et al., 2021). The incorporation of these elements is influenced by environmental factors, such as temperature and salinity, as well as by diet and physiological processes (Sturrock et al., 2015; Hodkin et al., 2018; Tian et al., 2021). Because otoliths are metabolically inert and not subject to resorption, they serve as permanent chronological archives of an individual's life history (Thomas and Swearer, 2019; Hüssy et al., 2021). Insights into distinct life stages can be drawn from analysing different otolith regions, including the location of spawning (otolith core), the location of capture (otolith edge), and life-averaged signatures (whole otolith) (Elsdon et al., 2008; Thomas and Swearer, 2019). When used together, these otolith chemistry-based techniques provide a more comprehensive understanding of fish connectivity and therefore stock structure (Moreira et al., 2022; Schroeder et al., 2022; de Almeida et al., 2024).

The axillary seabream, *Pagellus acarne* (Risso, 1827), is a demersal sparid species, widely distributed across the eastern Atlantic Ocean, ranging from Denmark to Senegal, and in the Mediterranean Sea (Bauchot and Hureau, 1986; Russell et al., 2014). The species inhabits the continental shelf, preferring sandy and seagrass bottoms and individuals are commonly recorded at depths ranging from 40 to 100 m, although occurrences have been documented at depths of up to 500 m. In Portuguese waters, the spawning season occurs from April to November with individuals gradually moving to deeper habitats as they mature (Bauchot and Hureau, 1986) and similar to other sparids, the axillary seabream exhibits protandric hermaphroditism, first maturing as males and later undergoing sex reversal to females (Coelho et al., 2005; Velasco et al., 2011; Moreira et al., 2025). Its higher abundance occurs south of Lisbon in the west and south coasts (Cardador et al., 2003; Chaves, 2017). The Portuguese coast functions as a biogeographic transition zone, with the northwest coast strongly influenced by coastal upwelling, maintaining colder nearshore waters, and the southwest and southern coasts generally warmer and more influenced by Mediterranean conditions (Relvas and Barton, 2002; Cordeiro et al., 2018; Ferreira et al., 2022).

Pagellus acarne is valued in both commercial and recreational fisheries, being frequently targeted by small-scale commercial fisheries especially using longlines, gillnets, and in bottom trawls (DGRM, 2025). In Portugal, the only management measure currently applied to this species is the official minimum landing size of 18 cm total length (TL), which is below the size at which individuals transition from males to fertile females and complete their natural reproductive cycle (Coelho et al., 2005; Velasco et al., 2011; Moreira et al., 2025). Consequently, harvesting individuals before this sexual transition may disrupt population structure by reducing the number of breeding females (Pavlowich et al., 2018). Hermaphroditic species may be particularly vulnerable to fishing pressure because size-selective harvesting can disrupt natural sex ratios and reproductive dynamics, particularly in species exhibiting size-dependent sex change (Provost and Jensen, 2015). Although *P. acarne* is currently classified as Least Concern by the IUCN (Russell et al., 2014), it has long been recommended that the minimum landing size should be increased to prevent population decline (Coelho et al., 2005). Fisheries data for the species in Portugal indicate that annual catches are variable, with national landings averaging 820 tonnes (t) per year between 1995 and 2023. However, landings have declined markedly since 2012, with recent data showing that total catches decreased to 337 t in 2024, raising concerns about the status of the fishery (DGRM, 2025).

To provide baseline information for further fisheries research and management of *Pagellus acarne* in the mainland Portuguese coastal waters, the population structure of the species was assessed using two complementary otolith geochemical approaches: (1) laser-ablation (core and edge) and (2) solution-based (bulk) analyses. The main objective of this work was to identify whether there is a single demographic unit, with high ecological connectivity and dispersal, or multiple isolated population units, which require a distinct fishery management strategy along the coast of Portugal.

2. Materials and methods

2.1. Study area

The study was conducted at three major fishing grounds in the northeastern Atlantic, along the Portuguese mainland coast: Matosinhos (north), Peniche (central), and Portimão (south) (Fig. 1; Table 1). The Portuguese coast is characterized by marked latitudinal gradients in hydrographic and oceanographic conditions and is commonly divided into three major oceanographic regions (northwestern, southwestern, and southern), which differ in their hydrographic conditions and seasonal upwelling dynamics (Leitão et al., 2019). Such contrasting environmental settings may influence the chemical composition of fish otoliths (Campana et al., 2000; Elsdon et al., 2008) and therefore provide a basis for investigating geographic variation in otolith geochemistry and spatial patterns of population connectivity).

2.2. Fish sampling

The fish were captured by commercial trawling fleets between June and September 2023 and transported to the laboratory in isothermal containers with ice for preservation. Upon arrival, the fork length (FL) of each fish was measured to the nearest 0.1 cm and sagittal otoliths were extracted using plastic forceps to avoid metal contamination (Moreira et al., 2022). The otoliths were cleaned of organic tissues, air-dried, and stored in plastic vials for subsequent age estimation. Both left and

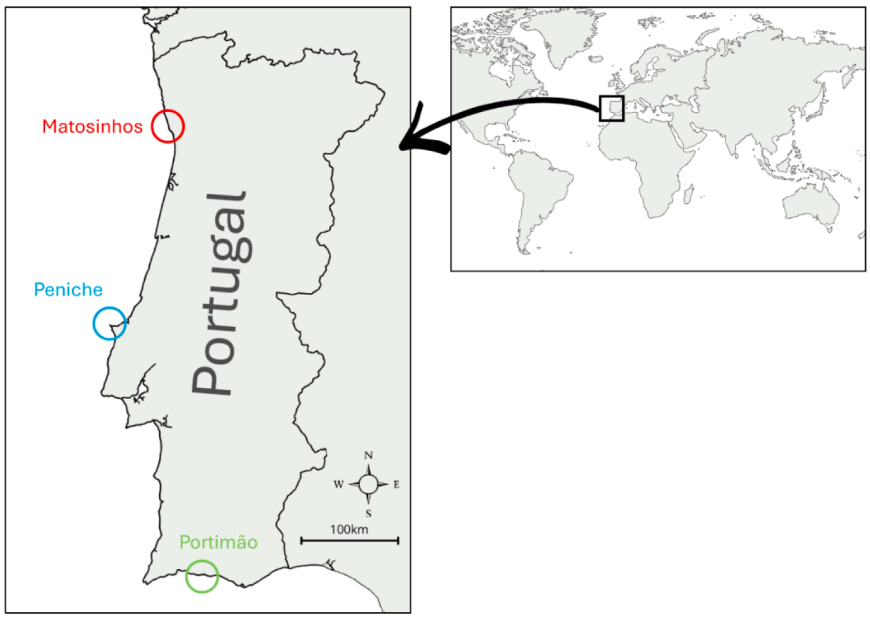

Fig. 1. Location of sampling sites on the coast of Portugal (Matosinhos, Peniche, and Portimão), where *Pagellus acarne* was collected.

Table 1
Sampling site, sample size (N), date of capture and fork length (FL) of *Pagellus acarne* individuals used in this otolith's microchemical study. Values are presented as the mean ± standard error.

Site	N	Date	FL (cm)
Matosinhos	25	25/07/2023	25.4 ± 0.1
Peniche	25	20/06/2023	25.5 ± 0.2
Portimão	25	18/09/2023	24.4 ± 0.2

right sagittal otoliths were distinguished based on the *rostrum* and the *sulcus acusticus* (Tuset et al., 2003). Twenty-five individuals, each aged four years, were selected from each site for further elemental analyses (Table 1). Restricting the analyses to a single age group minimized ontogenetic effects on otolith elemental signatures. Consequently, the present study focused on stock discrimination among adult fish from different sampling locations rather than on characterizing ontogenetic habitat shifts throughout the species' life cycle.

2.3. Age estimates

Otoliths were placed in a Petri dish with the *sulcus acusticus* oriented downward. To enhance the contrast between opaque and translucent bands of each annulus, they were immersed in a 1:1 ethanol–glycerol clearing solution and positioned against a dark, homogeneous background. The otoliths were examined under reflected light using a stereo microscope (Meiji Techno EMZ−13TR) at magnifications ranging from 10 × to 70 × . Age was estimated by counting the translucent bands, which have a dark appearance under reflected light (Fig. 2A) (Coelho et al., 2005). All readings were performed independently and blindly by two experienced readers, and only otoliths with 100% agreement were considered (Moura et al., 2020).

2.4. Laser-ablation analysis

Following age estimation, right sagittal otoliths were embedded in epoxy resin (Struers, epofix) and thereafter sectioned, preserving the core region, using a low-speed diamond saw (Buehler, IsoMet) at 600 rpm, lubricated with ultrapure water (18.2MΩ·cm at 25 ∘C). The otoliths were ground with 1200, 2400 and 4000 grade silicon carbide papers (Buehler Ø 200 mm SiC Paper) until the cores became visible at

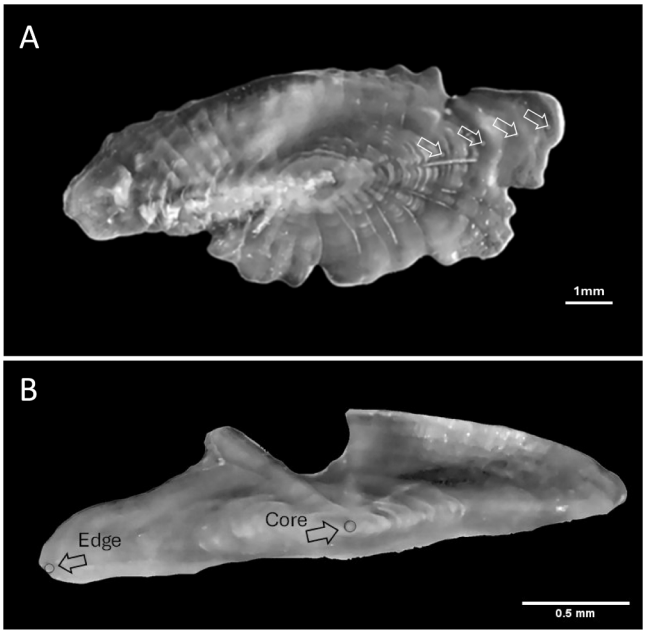

Fig. 2. A) Right whole sagittal otolith from a four-year-old *Pagellus acarne*, sampled in Peniche in September 2023 with a fork length of 21.9 cm showing the translucent bands of each *annulus* (white arrows). B) A transverse section of the same otolith showing the core and edge laser ablation spots (black arrows).

the exposed surface with the help of a metallographic microscope (Meiji, ML7100). Finally, the transverse sections were sequentially polished with 6, 3, 1 μm diamond pastes (Buehler, Metadi II) and mounted on glass slides using a drop of resin. Prior to laser analysis, the slides with the otolith sections were sonicated for 3 min in ultrapure water (18.2 MΩ·cm at 25 °C) and then allowed to dry in a laminar flow cabinet (Correia, et al., 2012b). The otolith core and edge were analysed by LA-ICP-MS. Elemental concentrations of the prepared otolith sections were measured using a femtosecond laser ablation system (NWRFemto, 257 nm; Elemental Scientific Lasers, USA) coupled to an ICP-MS Agilent 7700 (Agilent Technologies). Prior to analysis, each otolith was pre-ablated to remove surface contamination and ensure accurate

detection of endogenous elemental signatures. Helium was used as the carrier gas (flow: 600 mL/ min) in the laser ablation cell and argon was added before entering the ICP, which was operated at a power of 1600 W. NIST 610 and 612 (National Institute of Standards and Technology, USA) and MACS−3 (Geology, Geophysics, and Geochemistry Science Center, USA) reference materials, were measured in triplicate at the beginning and at the end of each batch. The 238U/232Th (<1.2%) and 232Th16O/232Th (<0.40%) ratios in NIST 612 were used for monitoring the plasma robustness. A single ablation with a 65 μm diameter with a repetition rate of 50 Hz and an output energy of 3 J/cm^2 (nominal) was conducted at both the core and the edge of each otolith (Fig. 2B), with three analytical replicates per ablation to ensure statistical robustness. The following isotopes were measured: ^{88}Sr, ^{138}Ba, ^{24}Mg, ^{55}Mn, ^{66}Zn, ^{7}Li as well as ^{43}Ca which was used as an internal standard, based on an assumed calcium concentration of 38.8 wt% in otolith material (Yoshinaga et al., 2000). Ion signals for ^{111}Cd, ^{60}Ni and ^{208}Pb were also measured but these were consistently below the instrument's detection limits and were therefore excluded from downstream statistical analysis. Background ion signals across the different mass-to-charge ratios were recorded at the start of each analytical session. Precision, expressed as relative standard deviation (RSD), ranged from 0% to 16%, while analytical accuracy, expressed as recovery rate (RR), varied between 82% and 111%. Both metrics fall within the accepted analytical limits (RSD < 20% and RR: 75–125%; Dove et al., 1996). The average detection limits (LOD, ppm) were estimated as per Pettke et al. (2012) and are shown between parentheses for: ^{88}Sr (0.0129), ^{137}Ba (0.0032), ^{25}Mg (0.0029), ^{55}Mn (0.0121), ^{7}Li (0.0038), ^{66}Zn (0.0127), ^{111}Cd (0.0378), ^{60}Ni (0.0069), ^{208}Pb (0.0025) and ^{43}Ca (9.18). Raw data (counts/s) were converted to elemental concentrations using the 3D Trace Elements data reduction system on Iolite v4, and presented as molar ratios (μmol element/mol Ca).

2.5. Solution-based analysis

For the bulk elemental analysis, left otoliths from the pre-selected four-year-old fish were used. Otoliths were cleaned with ultrapure water (H_2O, Milli-QR System, Milli-QWater) in an ultrasonic bath for 5 min, immersed in 3% (v/v) ultrapure hydrogen peroxide (H_2O_2, Fluka, TraceSelect) for 15 min to remove adherent organic tissues, following pre-existent protocols (Rooker et al., 2001; Correia, et al., 2012b). Thereafter, the otoliths were decontaminated in 1% (v/v) ultrapure nitric acid (HNO3, Honeywell Fluka, TraceSELECT™, ≥69.0%) for 10 s to remove surface contamination, followed by a triple immersion in ultrapure water (Rooker et al., 2001). Otoliths were allowed to dry overnight in a laminar flow hood and stored in decontaminated plastic tubes. Finally, the otoliths were weighed on an analytical balance (0.01 mg), dissolved for 15 min in 500 μL of 65% ultrapure HNO_3 (v/v), and diluted with ultrapure water (> 18.2 MΩ.cm at 25 ∘C) to a final volume of 15 mL (i.e., 2% HNO_3 v/v and 0.2% TDS m/v). Some pre-selected minor and trace elements (^{88}Sr, ^{137}Ba, ^{25}Mg, ^{55}Mn, ^{7}Li, and ^{66}Zn including calcium (^{44}Ca) were assessed by SB-ICP-MS. Similarly to LA-ICP-MS, data for ^{111}Cd, ^{60}Ni and ^{208}Pb were also measured but were excluded from downstream statistical analysis due to inconsistencies in the LODs. An iCAP™ Q (Thermo Fisher Scientific, Bremen, Germany) equipped with a concentric glass nebulizer, a Peltier-cooled deflector baffled cyclonic spray chamber, a standard quartz torch, and a two-cone interface design (sampler and skimmer cones) was used. A nebulizer and plasma gas, high purity argon (99.9997%) (Gasin II, Leça da Palmeira, Portugal) was used. Equipment control and data acquisition were performed using Qtegra™ software (Thermo Fisher Scientific, Bremen, Germany). The conditions of instrument operation were: RF power, 1550 W; argon flow rate, 14 L/min; auxiliary argon flow rate, 0.8 L/min; nebulizer flow rate, 0.98 L/min. To minimize the effect of plasma fluctuations or different nebulizer aspiration rates among the samples, Indium (^{115}In), Scandium (^{45}Sc), Terbium (^{159}Tb) and Yttrium (^{89}Y) were monitored as internal standards (Moura et al., 2020). To avoid possible sequence influences, the otolith samples were analysed randomized in an analytical triplicate. A certified multi-element reference material for otoliths (FEBS−1) was used to assess analytical precision and accuracy. RSD ranged from 2.5% to 16.1%, while RR varied between 88% and 118%. Both metrics fall within the accepted analytical limits (Dove et al., 1996). The LOD (in ppm) were calculated from the individual calibration of the curves using the three sigma criteria and were: ^{88}Sr (0.00098), ^{137}Ba (0.00005), ^{24}Mg (0.00132), ^{55}Mn (0.00002), ^{66}Zn (0.00065), ^{7} Li (0.00002), ^{111}Cd (0.000012), ^{60}Ni (0.000081), ^{208}Pb (0.000033) ^{43}Ca (0.462). Concentrations of trace elements, originally in μg element/L solution were transformed to μg element/g otolith, and then to molar ratios (μmol element/mol Ca).

2.6. Statistical analysis

Raw data for each element were checked for normality (Shapiro-Wilk) and homogeneity of variances (Levene's test) prior to statistical analysis. A few outliers detected using Grubbs' test were transformed (truncated). For LA-ICP-MS analysis, otolith mass was not considered a relevant covariate for the otolith core or edge comparisons (Hamer et al., 2003). For SB-ICP-MS relationships between elemental concentrations (element: Ca) and otolith mass were tested using Analysis of Covariance (ANCOVA), with otolith mass as a covariate. All elements were significantly correlated except Li ($p > 0.05$). Mn, Mg, Zn, and Ba were negatively correlated to otolith mass ($p < 0.05$), whereas Sr showed a positive relationship ($p < 0.05$). To ensure that differences in fish size among samples did not confound any site-specific differences in otolith chemistry, concentrations of elements were weight-detrended by subtraction of the product of the common within-group linear slope multiplied by the otolith weight from the observed concentration (Campana et al., 2000). Two-way Analysis of Variance (ANOVA) was used to examine differences in single-element signatures measured by LA-ICP-MS among sampling sites (factors: Site × Spot; Table 2). One-way ANOVA was applied to test spatial differences in SB-ICP-MS (factor: site). If significant differences were found ($p < 0.05$), this was followed by a Tukey post hoc pairwise test. Multivariate Analysis of Variance (MANOVA) was used to test for spatial differences in otolith multi-element signatures (core, edge and bulk). For the MANOVA, the approximate F-ratio was reported for the most robust test of multivariate statistics, Pillai's trace. Post-hoc multivariate pairwise comparisons among sites were performed using the Hotelling's T^2 test. Quadratic Discriminant Function Analysis (QDFA) was used to visualize spatial differences and to examine the re-classification accuracy success of individuals to their original site. Cross-validation was performed using a jackknifed leave-one-out cross-validation test (Moreira et al., 2022). All these statistical analyses were performed using Systat (ver. 13.0), Sigmaplot (ver. 16.0) and Past (ver. 4.03). The statistical level of significance (α) was 0.05.

3. Results

3.1. Laser-ablation analysis

The two-way ANOVA revealed different effects of sampling site, otolith region (core vs. edge), and their interaction on the elemental composition of *P. acarne* otoliths (Table 2). Significant Site × Spot interactions were detected only for Zn:Ca and Li:Ca ($P < 0.05$). Furthermore, significant differences between core and edge concentrations were found in all elements (Two-way ANOVAs, followed by a Tukey's post-hoc test $p < 0.05$, Fig. 3). For Sr core elemental concentrations were significantly lower than in the edge in all sites, while for Mg, Mn and Li, the inverse was observed (Fig. 3). Core concentrations of Ba in Portimão, and Zn in Peniche, were higher compared with edges (Fig. 3).

Single-element ratios in the otolith core exhibited spatial variation along the Portuguese coast for Mn:Ca, Zn:Ca and Li:Ca (Two-way ANOVAs, followed by a Tukey test $p < 0.05$, Fig. 3). The remaining

Table 2
Statistics and *p*-values of the Two-Way ANOVA tests used to evaluate the single elemental fingerprint of *Pagellus acarrne* taking into consideration the sampling site (Matosinhos, Peniche or Portimão), the laser spot region (core or edge) and the interaction. DF =degrees of freedom, SS =sums of squares, MS = mean squares. Bold values mean significant statistical differences.

Element:Ca ratio	Source of variation	DF	SS	MS	F	*p*
Sr:Ca	Site	2	958980.790	479490.395	0.503	0.606
	Spot	1	570272729.807	570272729.807	597.649	**< 0.001**
	Site × Spot	2	918287.845	459143.922	0.481	0.619
	Residual	144	137403833.718	954193.290		
	Total	149	709553832.160	4762106.256		
Ba:Ca	Site	2	2.048	1.024	1.024	0.362
	Spot	1	10.783	10.783	10.781	**0.001**
	Site × Spot	2	1.459	0.730	0.730	0.484
	Residual	144	144.024	1.000		
	Total	149	158.314	1.063		
Mg:Ca	Site	2	2318.756	1159.378	1.853	0.160
	Spot	1	18096.489	18096.489	28.930	**< 0.001**
	Site × Spot	2	3012.435	1506.218	2.408	0.094
	Residual	144	90074.667	625.519		
	Total	149	113502.347	761.761		
Mn:Ca	Site	2	36.599	18.299	4.498	**0.013**
	Spot	1	272.461	272.461	66.978	**< 0.001**
	Site × Spot	2	4.419	2.210	0.543	0.582
	Residual	144	585.780	4.068		
	Total	149	899.259	6.035		
Zn:Ca	Site	2	580.596	290.298	9.007	**< 0.001**
	Spot	1	94.381	94.381	2.928	0.089
	Site × Spot	2	211.650	105.825	3.283	**0.040**
	Residual	144	4641.079	32.230		
	Total	149	5527.705	37.099		
Li:Ca	Site	2	7.082	3.541	1.864	0.159
	Spot	1	238.443	238.443	125.551	**< 0.001**
	Site × Spot	2	11.752	5.876	3.094	**0.048**
	Residual	144	273.480	1.899		
	Total	149	530.756	3.562		

elemental ratios showed no significant spatial variation (Two-way ANOVAs, followed by a Tukey's post-hoc test $p < 0.05$, Fig. 3). The Tukey post-hoc tests indicated that Mn:Ca showed lower concentrations in Matosinhos than in Matosinhos and Portimão, Zn:Ca was higher in Peniche than in both Matosinhos and Portimão, and Li:Ca was higher in Portimão than in Peniche. Matosinhos showed intermediate Li:Ca concentrations and did not differ significantly from either site (Fig. 3). MANOVA indicated significant spatial differences in the multi-element ratios of the otolith cores (Pillai's Trace: F ratio = 2.110, $p < 0.05$), separating Peniche from Matosinhos (Hotelling's T^2 tests, $p < 0.05$). The canonical variate plot derived from the QDFA showed substantial visual overlap among the three sites (Fig. 4A). Jackknife cross-validation re-assigned individual fish to their capture sites with a moderate to low degree of accuracy (72%, 44% and 52% for Matosinhos, Peniche and Portimão respectively; 56% of correct overall reclassification) (Table 3).

Single-element ratios in the otolith edge revealed significant variation between sites only in Mg:Ca (Two-way ANOVAs, $p < 0.05$, Fig. 3). Mg:Ca concentrations were significantly lower in Portimão than in Peniche, whereas Matosinhos showed intermediate values and did not differ significantly from either site (Tukey tests $p < 0.05$, Fig. 3). The remaining elemental ratios showed no significant spatial variation (Two-way ANOVAs, followed by a Tukey's post-hoc test $p > 0.05$, Fig. 3). MANOVA performed on multi-elemental signatures of the otolith edges did not reveal significant differences among sites (Pillai's Trace: F ratio = 1.812, $p > 0.05$). The canonical variate plot derived from the QDFA showed substantial visual overlap among the three sampling sites (Fig. 4B). Jackknife cross-validation re-assigned individual fish to their capture sites with a low degree of accuracy (64%, 36% and 32% for Matosinhos, Peniche and Portimão respectively; 44% of correct overall reclassification) (Table 3).

3.2. Solution-based analysis

The single elemental composition of whole otoliths revealed significant spatial differences for Sr:Ca, Mn:Ca, and Zn:Ca (ANOVAs, $p < 0.05$), whereas the remaining elements did not vary significantly among sampling sites (Fig. 5). Tukey post-hoc tests indicated significantly lower Sr:Ca and Zn:Ca concentrations in Matosinhos compared to the other sites, while Mn:Ca concentrations were also lower in Matosinhos but only relative to Portimão (Fig. 5). MANOVA indicated significant spatial differences in the multi-elemental signatures of whole otoliths (Pillai trace, F = 4.312, $p < 0.05$) between Matosinhos and the other two sites (Hotelling's T-square, $p < 0.05$). The canonical variate plot derived from the QDFA demonstrated significant overlap among the three sites with Matosinhos diverging slightly from Peniche and Portimão (Fig. 4C). The jackknife cross-validation procedure re-assigned individual fish to their capture site with a moderate degree of accuracy (76%, 56% and 56% for Matosinhos, Peniche and Portimão, respectively; 63% of correct overall reclassification).

4. Discussion

The present study presents the first assessment of the population structure of *Pagellus acarne* along the Portuguese coast based on otolith geochemical signatures. Overall, the results are consistent with the existence of a single demographic unit characterized by high connectivity, although whole-otolith geochemistry revealed moderate spatial differentiation involving the northern sampling site. There is extensive literature describing the biology of *P. acarne*, particularly in the Mediterranean Sea (İlhan, 2018; Bentata-Keddar et al., 2020; Di Maio et al., 2020), but also in the Atlantic Ocean (Pajuelo and Lorenzo, 2000; Velasco et al., 2011), including Portuguese waters (Coelho et al., 2005; Moreira et al., 2025). In contrast, the stock structure of the species remains largely unassessed, with the only peer-reviewed evidence to date originating from a study in the eastern Mediterranean that suggested regional differentiation along the Turkish coast based on otolith shape analysis (Yedier et al., 2023). By comparison, the population structure of several other sparid species has been investigated using different stock

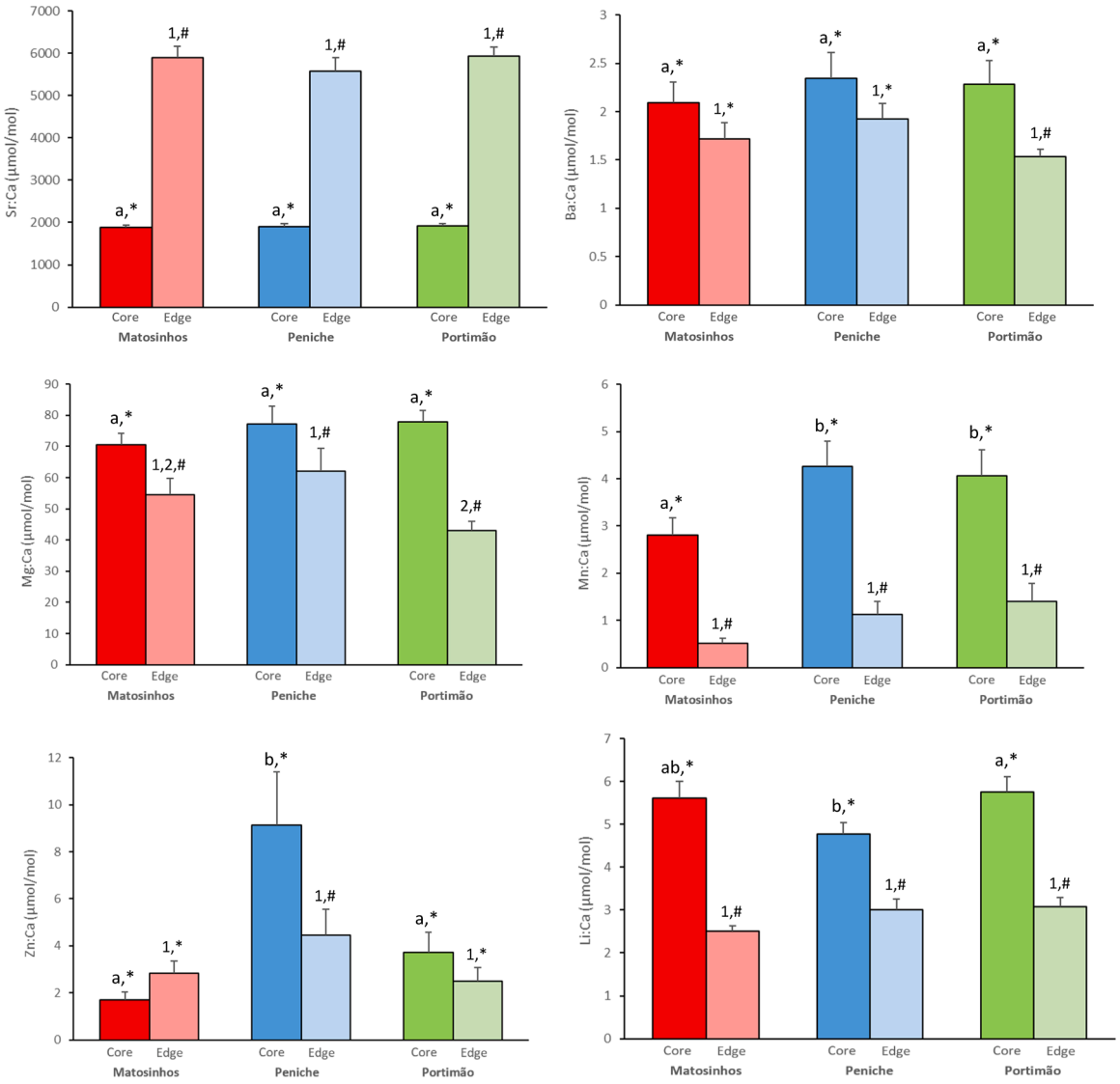

Fig. 3. Element:Ca ratios (mean ± standard errors) of spatially resolved analysis (LA-ICP-MS) of *Pagellus acarne* otolith sections from each sampling site. Different lowercase letters and numbers indicate significant differences among sites for core and edge concentrations, respectively, while symbols indicate significant differences between core and edge concentrations within each site [Two-way ANOVAs followed, if need ($p < 0.05$) by Tukey's post-hoc tests, See also Table 2].

discrimination approaches, including studies conducted along the Portuguese coast. Studies based on nuclear and mitochondrial DNA markers, such as those in the Atlantic for *Sarpa salpa* (Paiva, 2016) and in the Atlantic and Mediterranean for *Diplodus vulgaris* (Stefanni et al., 2015), *Spondyliosoma cantharus* (Neves et al., 2020) and *Boops boops* (Cunha et al., 2022), generally report population structuring at broad geographic scales. Higher-resolution approaches have revealed finer-scale patterns of differentiation. For instance, genotyping-by-sequencing (GBS) in *P. bogaraveo* (Cunha et al., 2024) identified stock structure within the southwestern Iberian Peninsula, and otolith microchemistry studies in *D. vulgaris* (Correia et al., 2011) and *S. cantharus* (Correia et al., 2012a) likewise provided evidence of small-scale spatial structuring.

4.1. Spatially resolved analyses

Geochemical signatures of the otolith cores showed low to moderate discrimination among sites, with an average reclassification success of 56%, indicating limited ability to distinguish spawning provenance of *P. acarne* along the Portuguese coast. Although three of the six analysed elements exhibited spatial differences (e.g., Mn, Zn and Li), these patterns were not consistent and did not allow clear separation of sampling sites. Zinc concentrations were notably higher in the otolith cores of individuals from Peniche, consistent with patterns reported for *Diplodus vulgaris*, where elevated Zn levels were also observed in central Portugal (Correia et al., 2011). The incorporation of Zn, as well as Mn into fish otoliths has previously been linked to physiological processes, particularly diet (Ranaldi and Gagnon, 2008; Hüssy et al., 2021), therefore possibly reflecting differences in early-life feeding ecology and/or local environmental availability of these elements among sampling sites. The mechanisms underlying Li incorporation into otoliths remain relatively poorly understood (Sturrock et al., 2015; Grammer et al., 2017). Moreover, the Li pattern observed in the natal signatures did not align with those of the other elements, further emphasizing the difficulty of discriminating natal origins among the sampled individuals. The absence of pronounced spatial differences in otolith core chemistry is also consistent with extensive larval dispersal during the pelagic early-life stage. Given the relatively short distances among the sampled locations and the potential for coastal circulation to transport larvae over several weeks, recruits originating from spawning areas beyond the Portuguese coast, such as the Gulf of Cádiz or the Gulf of Biscay, may contribute to the observed connectivity. This highlights the need for

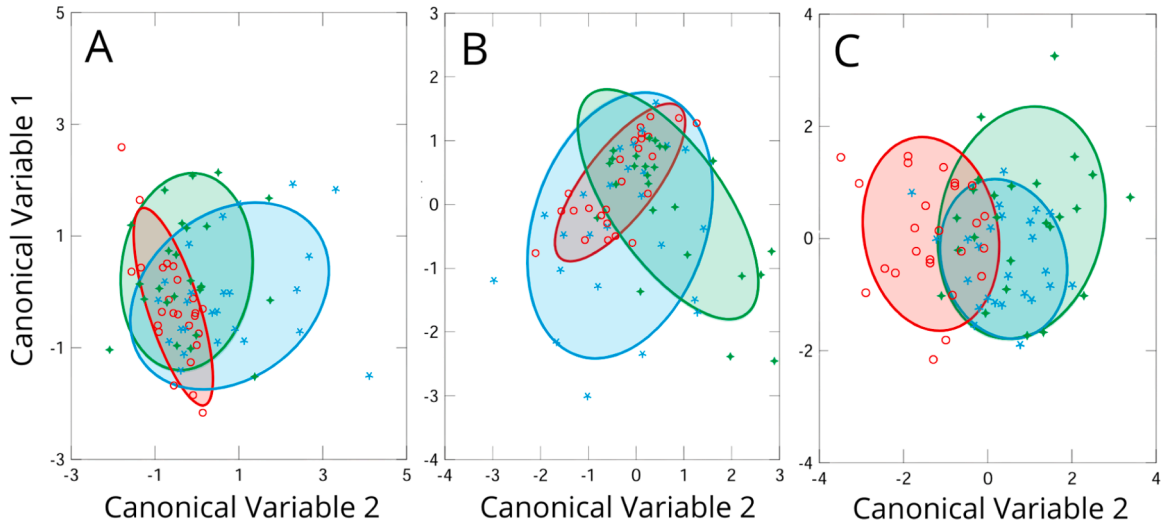

Fig. 4. Quadratic Discriminant Function Analysis (QDFA) plots displaying spatial differences for (A) core (natal signatures), (B) edge (fishing ground signatures) and (C) bulk (life averaged signatures) otolith elemental analyses of *Pagellus acarne*. Ellipses represent 95% confidence intervals around the data, and each data point represents a single individual. Sampling sites: Matosinhos (red circles), Peniche (blue asterisks) and Portimão (green plus signs).

Table 3
Jack-knifed cross validation re-classification matrix based on core, edge and bulk elemental fingerprints of *Pagellus acarne* used in quadratic discriminant function analysis.

Original Site	**Predicted site**			
Core	Matosinhos	Peniche	Portimão	%Correct
Matosinhos	18	1	6	72
Peniche	11	11	3	44
Portimão	10	2	13	52
Total	39	14	22	56
Edge				
Matosinhos	16	6	3	64
Peniche	13	9	3	36
Portimão	14	3	8	32
Total	43	18	14	44
Bulk				
Matosinhos	19	5	1	76
Peniche	2	14	9	56
Portimão	2	9	14	56
Total	23	28	24	63

future studies encompassing a broader geographic range to better resolve large-scale population structure and transboundary connectivity (Ramesh et al., 2019).

Edge signatures showed limited discrimination among sampling sites, with an overall reclassification accuracy of only 44%. The chemistry of recent growth in adult fish was unexpectedly similar across individuals sampled from geographically distant regions, where stronger discrimination would be expected (Correia et al., 2012b; Moreira et al., 2022; de Almeida et al., 2024). While Mg concentrations were significantly lower in fish from Portimão than in those from Peniche, all other elements remained uniform across sites. This isolated difference in Mg is noteworthy, as the element is believed to be influenced by both environmental fluctuations and physiological growth rates (Thomas et al., 2017; Hüssy et al., 2021). However, this variation in Mg was insufficient to provide spatial differentiation, which is particularly notable given the contrasting oceanographic conditions between the west coast (Matosinhos and Peniche) and the south coast (Portimão). The western coast is influenced by strong upwelling and great riverine input, resulting in colder and lower salinity waters, whereas the south coast is generally warmer and more saline (Mendes et al., 2016; Baptista et al., 2018; Ferreira et al., 2022). Thus, the fishing ground signatures suggest a high degree of connectivity among adult individuals across sites and the subsequent integration of environmental variability, resulting in a homogenized chemical signal. However, the limited spatial discrimination achieved by otolith edge chemistry may also have been influenced by the timing of sampling. Fish were collected during the summer season (June–September) to minimize temporal variability among sites, an important assumption in otolith chemistry studies (Elsdon et al., 2008). Nevertheless, the reduced freshwater discharge during this period may also have weakened spatial gradients in coastal water chemistry, reducing the environmental contrast recorded at the otolith edge. Future studies incorporating multiple sampling seasons and contrasting hydrographic conditions would help evaluate the influence of seasonal environmental variability on otolith elemental signatures and their discriminatory power.

Comparing core and edge chemistry provides insight into different ontogenetic phases of *P. acarne*, with discrimination decreasing from core to edge signatures. This pattern contrasts with the classical expectation for many coastal fish species, in which a shared larval origin is followed by increased spatial structuring in adults. In such cases, otolith core chemistry typically reflects a relatively homogeneous early-life environment, whereas edge or whole-otolith signatures show higher discrimination, indicating that individuals occupy distinct areas after settlement (Correia et al., 2011; Moreira et al., 2022; de Almeida et al., 2024). These patterns could be related to the ontogenetic changes in habitat use and movement of *P. acarne* since as individuals grow, they shift towards deeper and more offshore habitats, where they might be less exposed to the strong short-term variability that characterizes shallow coastal environments (Otero et al., 2008; Oliveira et al., 2023). At the spatial scale of the Portuguese mainland coast, this could reduce contrast in otolith edge chemistry among capture sites, although regional hydrographic differences persist. Nevertheless, Matosinhos exhibited the highest reclassification success for both core and edge analyses (72% and 64%, respectively), which may indicate some degree of local retention that should not be ignored.

The concentrations of Sr, Mg, Mn and Li varied consistently between core and edge across all sampling sites. These ontogenetic variations in concentrations of elements from larval to adult signatures have been reported for other marine fish species (Correia et al., 2012b; Moreira et al., 2022; Schroeder et al., 2023). Higher Mn and Mg concentrations in natal signatures are usually attributed to endogenous processes linked to early metabolism in the fish. High metabolic rates during the larval phase enhance Mg incorporation, which subsequently declines as growth and metabolism slow during later life stages (Thomas et al.,

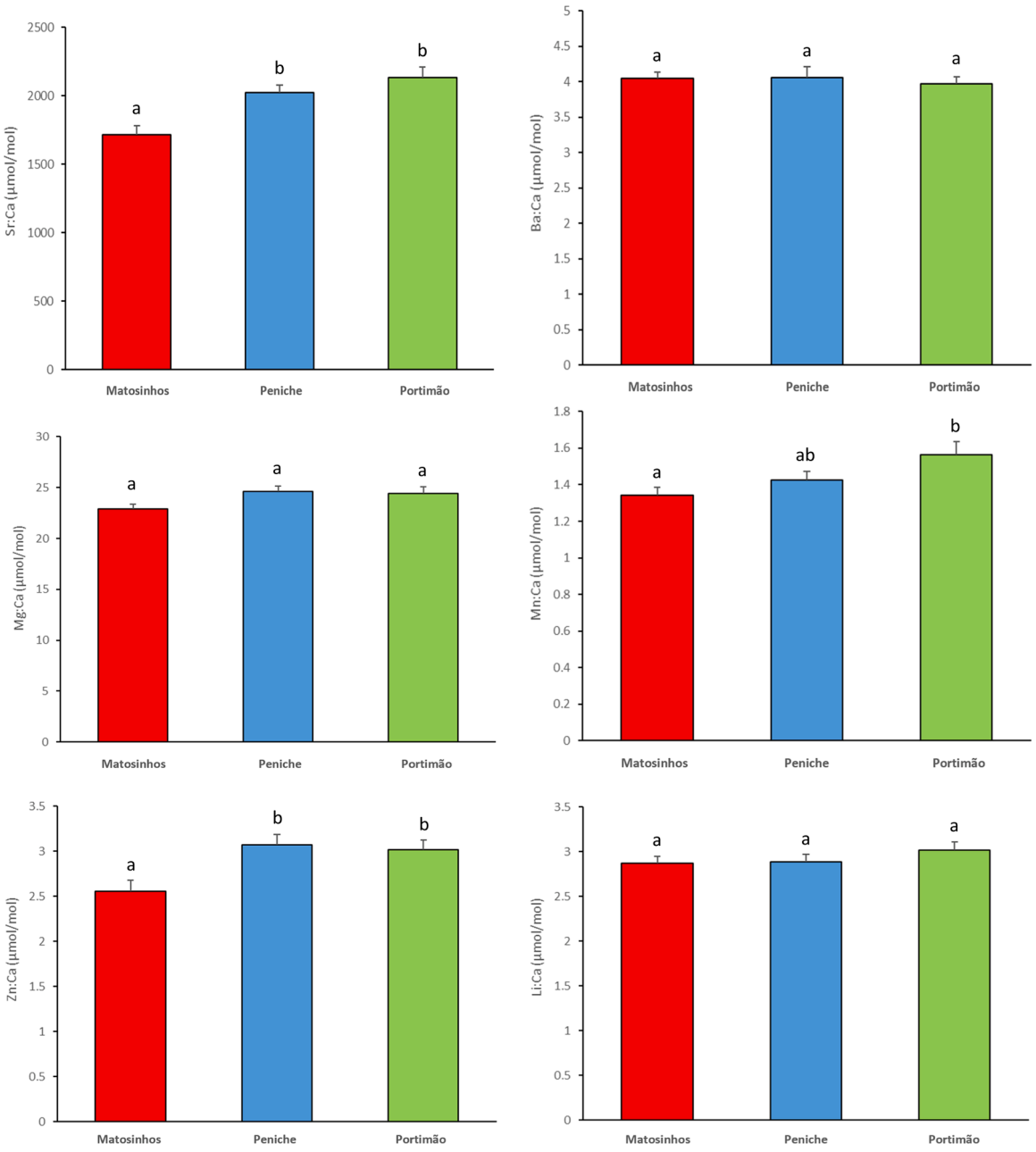

Fig. 5. Element:Ca ratios (mean ± standard error) of bulk otolith elemental analysis (SB-ICP-MS) of *Pagellus acarne* from each sampling site. Sites marked with different letters are significantly different [One-Way ANOVA followed, if need ($p < 0.05$) by Tukey's post-hoc tests].

2017, 2020). Similarly, Mn acts as a cofactor for an otolith protein essential for biomineralization (Thomas et al., 2017, 2020), mainly found in the early larval stages, explaining the higher Mn:Ca ratios observed in otolith cores.

4.2. Life-time integrated analysis

Life-averaged elemental signatures showed the highest discrimination among the three sites, with an overall reclassification success of 63%. Signatures from Matosinhos differed significantly from those of the other sites, mainly due to Sr, Mn and Zn, which were consistently lower in whole otoliths from this site. Sr and Ba are among the elements most strongly influenced by environmental conditions, particularly through their relationships with salinity and, to a lesser extent, temperature (Izzo et al., 2018; Hüssy et al., 2021; Tian et al., 2021). Freshwater input along the western Portuguese coast would therefore be expected to decrease Sr incorporation and increase Ba levels at sites such as Matosinhos and Peniche. However, this pattern was only partially observed, as lower Sr concentrations were restricted to Matosinhos, while Ba remained relatively constant across sites. This localized Sr signal may reflect the influence of freshwater input from the Douro River (Mendes et al., 2016), given its proximity to the sampling site, whereas no comparable signal was detected in Peniche. The absence of a corresponding Ba response further suggests that additional factors may influence elemental incorporation. The lower concentrations of both Zn and Mn observed in whole otoliths from Matosinhos compared to the other sites may indicate some degree of spatial differences in habitat use and diet of this species a localized adaptive response or prey preference in northern individuals, sufficiently pronounced to be reflected in their life-integrated chemical signatures. Li showed no spatial differences in bulk signatures despite significant differences at the otolith edge. Conversely, Sr exhibited no spatial variation in spatially resolved

analyses but differed in life-averaged signatures. The higher discrimination achieved by bulk otolith chemistry likely reflects cumulative environmental exposure experienced throughout the lifetime of individuals, rather than natal origin alone. The higher discrimination observed for the northern group may reflect prolonged residency in environmentally distinct habitats, rather than natal site specificity. Consequently, these differences should be interpreted as evidence of variation in life-integrated environmental histories and habitat use, rather than direct evidence of population differentiation. These contrasting patterns highlight the importance of analysing both bulk and spatially resolved otolith compositions, as each approach provides complementary insights into environmental and physiological histories.

From a fisheries management perspective, the present findings support the managing of *P. acarne* along the Portuguese coast as a single demographic unit. Nevertheless, the localized differentiation detected in the northern region warrants caution and should be further investigated before excluding the possibility of finer-scale stock structuring. Future studies should expand the spatial coverage to encompass the entire Bay of Biscay and Iberian Coast Ecoregion, extending northwards to the Bay of Biscay and southwards to the Moroccan coast, in order to provide a broader understanding of population connectivity. Additionally, multidisciplinary approaches integrating complementary stock discrimination tools such as geometric morphometrics, parasite fauna and molecular analyses will be essential to more robustly assess fish movements and population boundaries.

5. Conclusions

Otolith geochemistry provided limited evidence of population structuring in *P. acarne* along the Portuguese coast. Core and edge elemental signatures showed low discriminatory power, whereas whole-otolith chemistry provided a stronger, although still moderate, spatial signal, particularly distinguishing the northern sampling site (Matosinhos) from the remaining locations. Compared with other sparid species, such as *D. vulgaris* and *S. cantharus*, which exhibited clear spatial differentiation at relatively small geographic scales when investigated using similar approaches, *P. acarne* appears to display greater connectivity within Portuguese waters. Overall, the results support the hypothesis of a single demographic unit characterized by high connectivity, while suggesting limited localized differentiation in the northern region.

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CRediT authorship contribution statement

A.T. Correia: Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **R.P. Vasconcelos:** Writing – review & editing. **C. Schäfer:** Data curation. **A. Almeida:** Resources. **R. Azevedo:** Validation, Methodology. **J. Pisonero:** Resources. **A. Méndez:** Validation, Methodology. **R.M. Silva:** Data curation. **A. Casaca:** Writing – original draft, Methodology, Investigation, Formal analysis. **R.G. Kulzer:** Writing – original draft, Methodology, Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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