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Is *Chelidonichthys lucerna* (Linnaeus, 1758) a Marine Estuarine-Dependent Fish? Insights from Saccular Otolith Microchemistry

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Abstract: Tub gurnard, *Chelidonichthys lucerna* (Linnaeus, 1758), is a Mediterranean-Atlantic benthic species usually captured as by-catch by Portuguese traditional fisheries and considered the most important commercial fish species of the family Triglidae. However, to date, little is known about its habitat residency and whether the species can be considered a marine estuarine-dependent fish. Otolith microchemistry has proven effective in providing information about fish movement patterns throughout different water salinities. In this study, core-to-edge transects of Sr:Ca in the fish saccular otoliths of 35 juveniles of *C. lucerna* captured in March 2007 by a scientific survey along the Portuguese coast were used to assess the species movement between brackish and marine waters. Data suggest that most individuals (97%) have an estuarine-dependent profile, with 63% showing a clear presence in marine waters during the early life history periods. Evidence of an estuarine residence throughout the fish life cycle was found in only 3% of individuals. Ba:Ca profiles did not reflect an inverse relationship with Sr:Ca salinity results but corroborated findings from other authors about the influence of upwelling processes and freshwater runoffs on Ba incorporation into the otoliths of coastal fish. Furthermore, the data also showed that *C. lucerna* can occupy and migrate among habitats with diverse salinity degrees, showing high environmental plasticity and adaptation.

Keywords: Triglidae; sagitta; LA-ICP-MS; Element:Ca; habitat residency; migratory behavior

Key Contribution: This is the first study to investigate *C. lucerna* migratory movements between salinity gradients along the Portuguese coast through saccular otolith elemental chemistry.

1. Introduction

Nearshore environments, including estuaries, have been widely recognized among the scientific community as important nursery areas for a variety of fish species, providing food and shelter from predators to a wide number of marine fish and invertebrate species, with

many ending up moving to different habitats in their adult stage [1–3]. Estuaries are also highly productive environments with abundant sources of food, providing nutrient-rich habitats and important feeding grounds that are ideal for juvenile and adult fish, thus playing a crucial role in maintaining fish stocks in the marine environment [4–6].

Some fish species inhabit estuaries throughout their entire life, while others enter these productive environments for variable periods during a particular stage of their life, and others just migrate through estuaries from their spawning grounds at sea to feeding areas in freshwater or vice-versa [5–7]. Marine estuarine-dependent fish, for example, spend part of their life cycle in low-saline estuaries for growth, feeding, reproduction, and sexual maturation [5,8,9]. Understanding fish movements is crucial for the management and conservation efforts of a species, and the migratory routes can be tracked by using physical, electronic, or natural tags [9–11].

Otoliths are calcium carbonate structures, mainly in the mineral form of aragonite, located in the inner ear cavity of teleost fish that belongs to the vestibular organ [12]. These structures grow continuously over a fish's life, are metabolically inert, and incorporate several minor and trace elements derived from the fish environment, allowing several ichthyological applications [12–14]. Otoliths can provide a wide range of information about fish species such as age estimation, environmental history, taxonomic issues, and prey identification, among others [15–17]. Their chemical composition has also shown to be a powerful tool to study fish population structure [18–20], movement patterns [9,21,22], and habitat connectivity [23–25].

Chemical otolith analysis provides information about the environmental conditions and life history traits of fish throughout their whole life [18,26,27] or for a particular life history stage [9,28,29]. The mechanism behind the incorporation of minor and trace elements into the otolith aragonite matrix is still poorly understood. However, strontium (Sr) and barium (Ba) have shown to be related to water salinity, with Ba concentrations being generally higher in fresh and brackish environments, and, conversely, Sr concentrations are assumed to be lower in these environments [9,30,31]. In particular, Sr:Ca profiles from the core to the otolith edge have been widely used as a powerful tracer to infer fish movement among fresh, brackish, and marine waters [9,21,22].

The assessment of the Sr:Ca threshold that represents the transition between habitats of distinct salinity (fresh, estuarine and marine environments) has been carried out through a variety of methods within the scientific community, such as controlled field data, laboratory experiments, or mathematical approaches [21,32,33]. Most of these studies have been applied to species that spend most of their adult lives at sea and return to freshwater to spawn (i.e., anadromous) and for fish that, oppositely, live most of their adult lives in freshwater but migrate to saltwater to spawn (i.e., catadromous) [34–36]. The Sr:Ca threshold from field studies is estimated by assessing the Sr:Ca ratios from a fish species captured from different salinity environments [21]. In laboratory experiments, these ratios are analyzed from fish reared in a controlled environment with varying salinity levels [32]. Mathematical approaches for estimating otoliths Sr:Ca thresholds are based on the average Sr:Ca ratio for fish otoliths collected from different salinity environments and the associated variability around the average ratio (mean ± standard deviation) [9,30,33].

Chelidonichthys lucerna (Linnaeus, 1758) is a benthic species that usually lives in the bottom of the continental shelf [37–39], being found in the NE Atlantic, Mediterranean and Black Sea [39]. In Portuguese fish markets, *C. lucerna* is the Triglidae species that achieves the largest size and greatest commercial interest. It is sold under a commercial category ("Ruivos") that also comprises other gurnard species [40] and, according to the Portuguese fishing statistics, a total of 285 tons of "Ruivos" were landed in 2022 at an average annual price of 1.93 EUR; this means a 5-ton increase in landings and €0.03 EUR increase in price compared to the previous year [41,42].

Nursery areas along coastal waters and a spatial separation between *C. lucerna* juveniles and adults have been reported, with younger individuals being more frequently found in shallow coastal waters and adjacent estuaries, considered feeding areas, while

adults are more dispersed towards offshore grounds, where spawning takes place [38,43,44]. However, although a few studies on the species have investigated population structure, diet composition, and age, growth, and reproductive biology [23,45,46], specific research on the species movement pattern, particularly during its early life history, is inexistent, with a single recent study suggesting that this marine species is apparently estuarine-dependent [47].

The purpose of this study was to assess, for the first time, if *C. lucerna* is a strict marine estuarine-dependent species, or presents some environmental plasticity, using mainly otolith Sr:Ca ratios from core to edge as a water salinity tracer. The gathered knowledge will be important for the rational management and sustainable conservation of this halieutic resource.

2. Materials and Methods

2.1. Fish Sampling

Fish were collected during a scientific survey that took place in the west and south Portuguese continental shelves in March 2007 by bottom trawl at depths ranging between 38 m and 145 m. Thirty-five individuals (five individuals per site), ranging from 21.0 to 45.3 cm (total length, TL), were selected from different sampling points along the Portuguese coast, namely Caminha, Matosinhos, Aveiro, Berlengas, Sines, Milfontes-Arrábida, and Sagres-Portimão. In addition, a total of five individuals (TL: 18.0 to 21.6 cm) were collected from the Douro lower estuary, at 0.5 to 2.5 km from the river mouth, in December 2016 with bottom trawl fishing (Figure 1, Table 1). The Douro estuary has an extent of about 21 km and is a narrow mesotidal, semi-diurnal, and vertically stratified estuary, with an average depth of 8 m [48]. In its lowerection, water temperature and salinity range between 10–15 °C and 10–30 ppt, respectively [49].
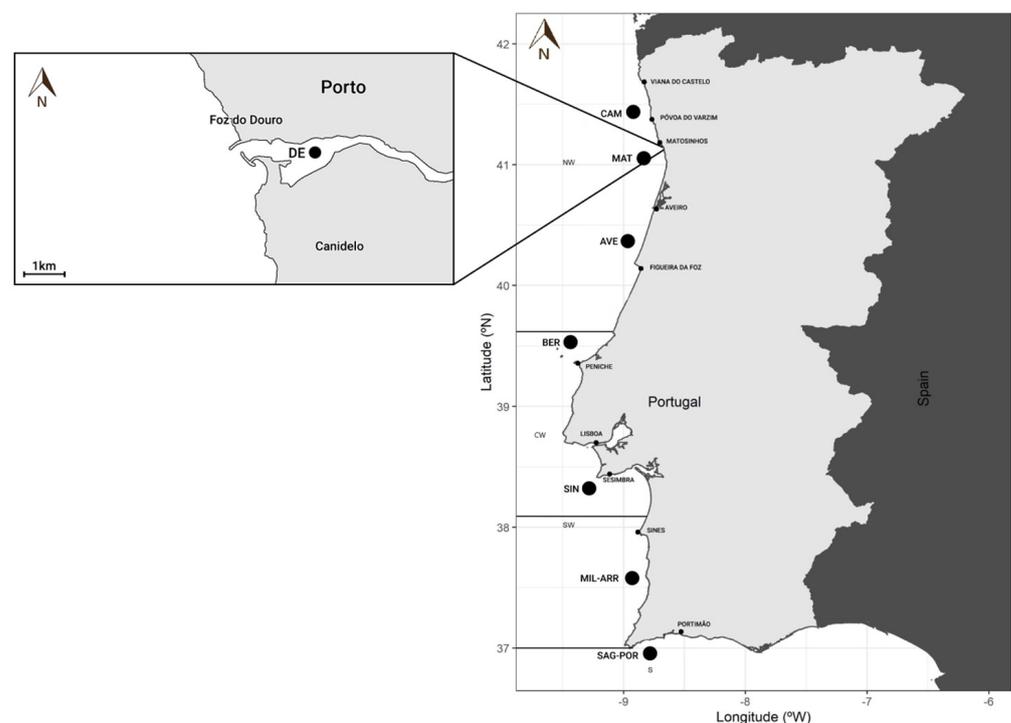

Figure 1. Map of the Portuguese coast indicating the sampling sites (•) of *Chelidonichthys lucerna*, Caminha (CAM), Matosinhos (MAT), Aveiro (AVE), Berlengas (BER), Sines (SIN), Milfontes-Arrábida (MIL-ARR), Sagres-Portimão (SAG-POR), including the Douro estuary (DE) location.

Table 1. Collection site, capture date, sample size (n), total length (TL) and estimated Sr:Ca thresholds for *Chelidonichthys lucerna* individuals used in this study. Values are presented as mean, range and standard deviation (SD).

Location	Date	N	TL (cm)		Sr:Ca (mmol.mol^{-1})
			Mean $\pm$ SD	Range	
Sagres-Portimão	13–14 March 2007	5	27.16 $\pm$ 4.75	21.60–32.90	Mean = 6.63 SD = 1.59 Mean $-$ 1 $\times$ SD = 5.04 Mean $-$ 2 $\times$ SD = 3.45
Arrábida-Milfontes	10–12 March 2007	5	31.68 $\pm$ 8.10	26.10–45.30	
Sines	17–18 March 2007	5	24.54 $\pm$ 3.01	22.80–29.80	
Berlengas	30 March 2007	5	22.56 $\pm$ 1.00	21.20–23.90	
Aveiro	29 March 2007	5	23.04 $\pm$ 0.93	21.80–23.90	
Matosinhos	26 March 2007	5	22.42 $\pm$ 0.93	21.00–23.30	
Caminha	25 March 2007	5	22.50 $\pm$ 1.02	21.20–23.80	
Douro estuary	12 December 2016	5	20.16 $\pm$ 8.38	18.00–21.60	Mean = 4.89
					Maximum = 5.41
					Minimum = 4.28

The salinity and temperature in the upper 100 m of the Portuguese continental shelf usually varies between 35.8 and 36.0 and between 14 °C and 19 °C, respectively, depending on whether upwelling (cooler waters on summer) or downwelling (warmer waters on winter) dominates [50].

2.2. Otolith Preparation

Upon collection or immediately after landing, fish were preserved on ice and processed in the laboratory. The individuals were measured for total length (TL, cm) and saccular otoliths (sagittae) were removed from the inner ear, rinsed with distilled water, air-dried, and stored in plastic vials until further analysis.

Left sagittal otoliths were embedded in transparent epoxy resin (Buehler, Epothin), and a transverse cross-section (0.5 mm) was taken out, preserving the core region, using a precision diamond saw (Buehler, Isomet Low-speed Saw). Slices were ground with abrasive grinding papers of 800, 1200, and 2400 grit (Buehler, Ø 200 mm SiC Paper) to expose the primordium and further polished with 6, 3, and 1 µm diamond pastes (Buehler, Metadi II). Thereafter, the transverse otolith sections were attached to a glass slide with epoxy resin (Buehler, Epothin), cleaned in an ultrasonic bath with ultrapure water (Milli-Q-Water) for 5 min, and dried in a laminar flow cabinet [51,52].

Otolith microchemistry was used to examine *C. lucerna* movement behaviors through different salinity water masses by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). Elemental concentrations in transverse otolith sections were measured using a 193 nm ArF*Excimer Laser Ablation System (Photon Machines Analyte G2) coupled to an ICP-MS (7700× from Agilent Technologies). Concentrations of isotopes ^{7}Li, ^{25}Mg, ^{43}Ca, ^{55}Mn, ^{57}Fe, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{88}Sr, and ^{138}Ba were determined from the core to the ventral-proximal otolith edge using a continuous transect along the radius of the otoliths (Figure 2).

The laser ablation settings used were as follows: spot diameter 50 µm, nominal fluence 5 J cm^{-2}, repetition rate 10 Hz, and scan speed 10 µm s^{-1}. Helium was used as a carrier gas in the ablation cell (at a flow of 800 mL/min) and Argon was added before entering the ICP, operated at 1600W in Ar plasma gas. Analysis conditions of the LA-ICP-MS system were optimized in NIST612 (trace elements in glass, NIST, USA) to minimize fractionation effects that might induce quantification uncertainties. ^{238}U/^{232}Th ratio (below 120%) was used to control plasma robustness and ^{232}Th^{16}O/^{232}Th (less than 0.5%) to control the oxide production rates. To compensate for any variation in ablation yield along the laser transect and improve the reliability of the measurements, ^{43}Ca was used as the internal standard [53]. External calibration was performed using SRM NIST612, SRM NIST610 silicate glass (www.nist.gov, accessed on 2 March 2020), and USGS MACS-3

(https://www.usgs.gov/, accessed on 2 March 2020) that were measured in triplicate at the beginning and at the end of the entire sequence, and after every twelve otoliths during the sequence analysis [54]. During the analysis, all the operating conditions (e.g., spot size, repetition rate, scan speed, laser energy/fluence, and gas flow) were kept constant in both the reference material and otoliths. In order to avoid contamination of the sample surface, a pre-cleaning ablation using a spot diameter of 65 µm, a repetition rate of 10 Hz, and a scan speed of 50 µm s^{-1} was run prior to the main transects. Before each ablation, 20 s were employed to measure the background chemical signals for each isotope in the ICP-MS with the laser switched off. The background average value of each isotope was used as a blank correction [54]. The average relative standard deviation for 20 NIST612 transects of two millimeters each was less than 5%, regardless of the element. All isotope data were given as a concentration relative to ^{43}Ca (element:Calcium).

Figure 2. Transverse section from the left sagittal otolith of a *Chelidonichthys lucerna* individual (total length, TL = 22 cm) collected along the Portuguese coast showing the entire continuous laser ablation transect made by LA-ICP-MS from core (C) to edge (E).

After the laser ablation, ablated otolith cross-sections were photographed using a microscope with transmitted light (Olympus CX41) coupled to a 3 megapixels USB camera (Olympus, SC30) at 40× magnification. Laser transects were measured using the software ImageJ (1.51j version) and ablation time was converted to distance from core to edge (initial and final laser positions, respectively) of otoliths. Elemental ratios were converted to mmol mol^{-1} for Sr:Ca and to µmol mol^{-1} for Ba:Ca using elementR (v 4.0.3) [54] in the R environment [55].

2.3. Data Analysis

The assumption of a positive correlation between otolith Sr:Ca and habitat salinity [9,21,56] was used to discriminate the different water environments and assess *C. lucerna* movement patterns between marine and estuarine waters.

To facilitate the interpretation of core-to-edge transects, transition salinity otolith thresholds were estimated for the 35 *C. lucerna* individuals captured along the Portuguese coast. Two different transition thresholds between marine and estuarine waters were estimated following a well-accepted mathematical approach: the otolith edge Sr:Ca ratios average minus one standard deviation [Mean − 1 × SD] [33] and the otolith edge Sr:Ca

ratios average minus two standard deviations [Mean $- 2 \times$ SD] [9,30]. For each threshold, the mean calculation was carried out considering the three last laser spots on each otolith's edge, which were assumed to represent the capture environment. For a more comprehensive understanding, five additional individuals collected in the Douro estuary were sampled with the goal of validating the relevancy of these methodological approaches to assess fish movement between different salinity environments. In this context, the minimum and maximum means of the otolith edge Sr:Ca ratios from these individuals collected in the Douro estuary were also used as boundaries for the estuarine zone to assess which of the two mathematical approaches better represents the fish transition from marine coastal waters to estuarine brackish waters [21].

The mathematical threshold ratios between marine and brackish waters obtained from the 35 individuals collected on the continental shelf were 5.04 mmol mol^{-1} [Mean $- 1 \times$ SD] and 3.45 mmol mol^{-1} [Mean $- 2 \times$ SD] (Table 1). The minimum and maximum mean otolith edge Sr:Ca ratios for the five individuals collected in the estuarine waters were 4.28 and 5.41 mmol mol^{-1}, respectively. It suggested that the Mean $- 1 \times$ SD threshold value provided a more accurate estimate of estuarine residency. Sr:Ca ratios below the maximum estuarine threshold determined by the individuals collected in the Douro estuary (5.41 mmol mol^{-1}) were used to identify fish movements within estuarine environments.

After a preliminary visual analysis of the different transects for *C. lucerna* samples caught off the Portuguese coast, individuals were separated based on the oscillation of the Sr:Ca ratio between the marine and estuarine thresholds calculated for the Portuguese coast individuals using the above-mentioned approaches. Thereafter, the algorithm "Identifying Changes in Mean" (ICM) in the changepoint package [50] in the R environment [55] was used to interpret the fish movement patterns and to infer the number of habitat changes that took place during the fish life history based on each individual Sr:Ca ratio profile. Finally, the recorded individual Sr:Ca ratio profiles were grouped to minimize the existing number of patterns without losing relevant information.

Assuming that Ba:Ca ratios could vary in opposite directions compared to Sr:Ca ratios in relation to water salinity, with increased Ba:Ca ratios usually associated with low-salinity habitats [31,32,57], any specific pattern for Ba:Ca or potential relationship between these two ratios (Sr:Ca and Ba:Ca) was also assessed.

Unfortunately, the most accepted protocol for *C. lucerna* age estimation requires otolith burning followed by its reading in a clearing agent, which is not compatible with the microchemical methodology stated here [58]. Therefore, the otolith ratio from core-to-edge was considered a proxy for fish age.

3. Results

Sr:Ca ratios in *C. lucerna* otoliths varied significantly along the core-to-edge otolith transects, suggesting distinct ambient water salinities residencies during the fish's lifetime history. Otolith Sr:Ca ratios along the entire ablation process (core-to-edge transect) ranged from 2.5 to 12.4 mmol mol^{-1}.

In total, six main patterns of fish movement were identified: Marine-Estuarine-Marine (MEM), Estuarine-Marine (EM), Estuarine-Marine-Estuarine-Marine (EMEM), Marine-Estuarine-Marine-Estuarine (MEME), Marine-Estuarine (ME), and Estuarine (E). Individuals characterized as MEM, EMEM, and MEME showed several Sr:Ca oscillations between the marine and estuarine environments; EM and ME individuals showed otolith Sr:Ca ratios switching once between the two environments; individuals were characterized as E when otolith Sr:Ca ratios remained at estuarine levels during all life histories.

Sr:Ca ratios from core-to-edge allowed us to identify several types of residency patterns among *C. lucerna*. Most of the individuals (63%) showed a clear presence in marine waters during early life stage periods, suggesting these were spawned in open waters, with one (90%) or two (10%) incursions into estuarine waters throughout their lifetime, reaching values below the lower limit recorded for the estuarine zone (4.28 mmol mol^{-1}). The remaining individuals (37%), however, apparently seem to have spawned in estuar-

ine waters, with 92% of them migrating to saltier environments until the time of capture. Of these, a smaller fraction of individuals (38%) showed one additional incursion into estuarine brackish waters before returning to marine waters throughout their lifetime. Of the total *C. lucerna* individuals, a large majority (97%) showed migratory movements between marine and brackish waters, with only a minority (3%) showing an apparent estuarine residency profile until the moment of capture. Based on Sr:Ca ratios, a total of six different movement profiles were identified based on the number of migratory movements of the species between marine and brackish waters (Figure 3). All individuals' core-to-edge profiles are included in the supplementary material (Supplementary Figure S1).
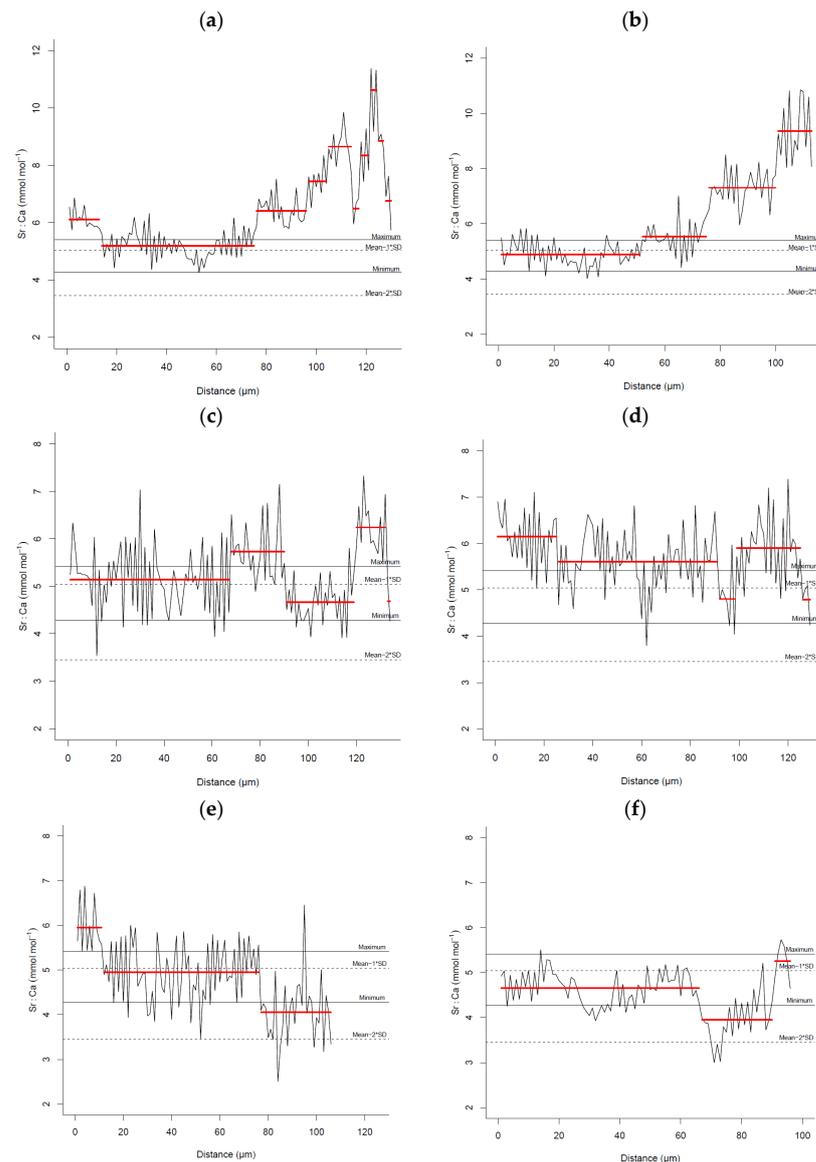

Figure 3. Otolith microchemical (Sr:Ca) individual profiles representing the six different patterns of *C. lucerna* classified as (**a**) Marine-Estuarine-Marine (MEM); (**b**) Estuarine-Marine (EM); (**c**) Estuarine-Marine-Estuarine-Marine (EMEM); (**d**) Marine-Estuarine-Marine-Estuarine (MEME); (**e**) Marine-Estuarine (ME); (**f**) Estuarine (E). The solid line lines represent the minimum and maximum values recorded for the individuals collected in the Douro estuarine zone in 2016. The dashed lines represent the estimated value for [Mean − 1 × SD] and [Mean − 2 × SD] regarding the 35 individuals collected in the Portuguese coastal area during the research vessel in 2007. The red line corresponds to the Sr:Ca associated change points.

When compared to Sr:Ca profiles, individual Ba:Ca profiles showed very different patterns and variations, with the majority (97%) exhibiting pronounced Ba:Ca peaks at the onset of their life history. For 40% of the individuals, these peaks occurred at both Sr:Ca marine and estuarine levels, for 12% only at marine levels, and for 8% only at estuarine levels (Figure 4).
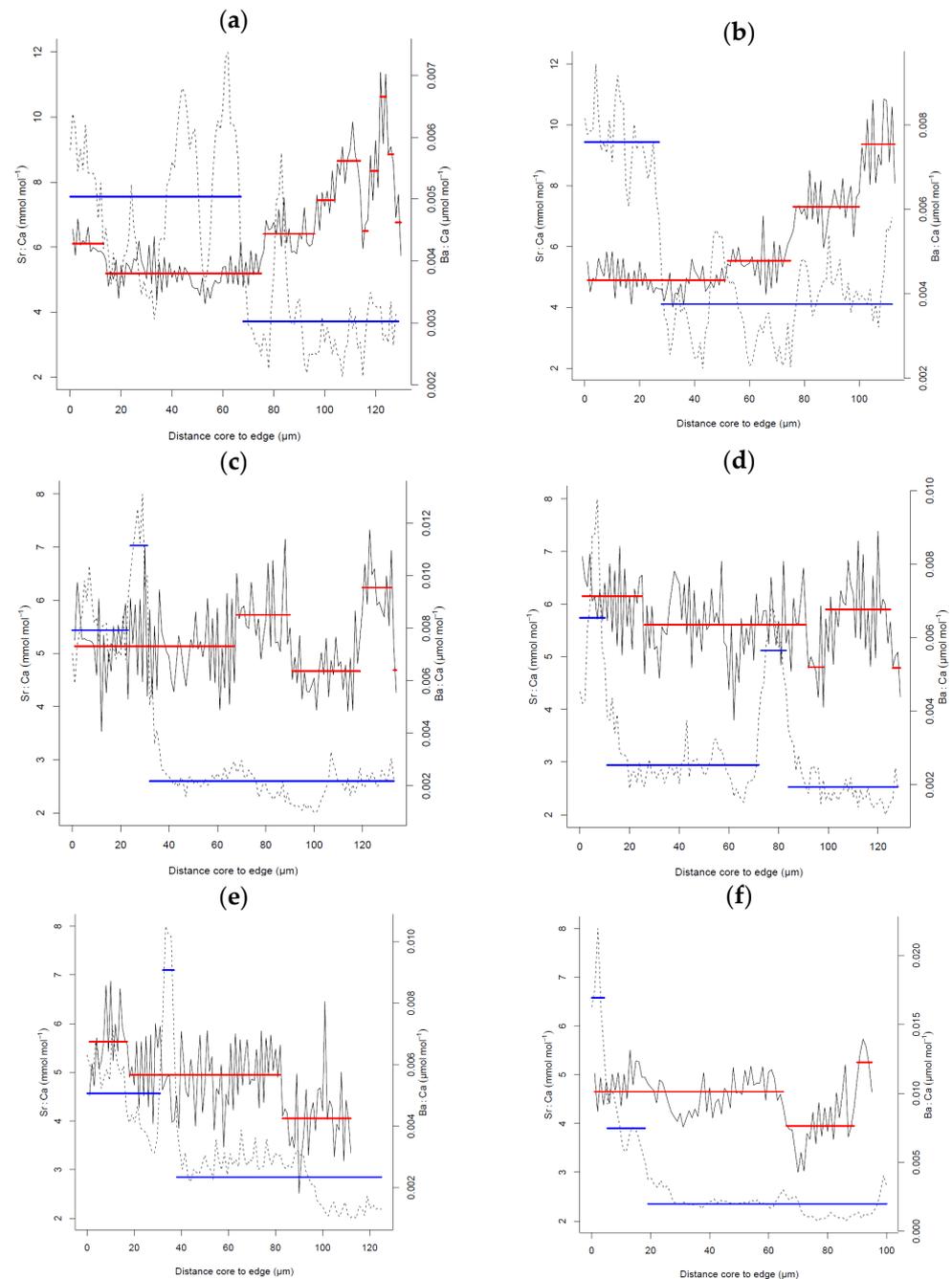

Figure 4. Otolith microchemical (Ba:Ca) profiles of the six different patterns of *C. lucerna* classified as (**a**) Marine-Estuarine-Marine (MEM); (**b**) Estuarine-Marine (EM); (**c**) Estuarine-Marine-Estuarine-Marine (EMEM); (**d**) Marine-Estuarine-Marine-Estuarine (MEME); (**e**) Marine-Estuarine (ME); (**f**) Estuarine (E). The solid and dahs lines corresponds respectively to Sr:Ca and Ba:Ca values and associated change points (red and blue, respectively).

4. Discussion

This study aimed at inferring *C. lucerna* movement patterns within Portuguese waters through different salinity environments and assessing the species' eventual dependency on estuarine areas during its early life through otolith microchemical analysis by LA-ICP-MS.

Previous studies on *C. lucerna* have reported a relationship between fish size and depth, with younger fish more frequently found in shallower coastal waters such as estuaries where food is abundant and larger and older fish more dispersed seaward [43,45,58]. In addition, a few authors have also suggested that the species exhibits seasonal migratory movements, from younger individuals in shallower coastal nursery areas during the spring and summer months to greater depths in oceanic waters during the winter period [37,47,59].

The deposition of Sr and Ba in fish otoliths is positively correlated with their occurrence in the water environment [31,32,60]. Variations in Sr and Ba within otoliths, particularly otolith Sr:Ca and Ba:Ca ratios, are positively related to salinity, with high otolith Sr:Ca ratios indicating marine environments and increased Ba:Ca ratios suggesting freshwater and brackish environments [30,32,61]. Therefore, several authors have already explored the potential of combining both Sr:Ca and Ba:Ca ratios to assess the movement patterns of diadromous species [9,32,62].

In the present study, two different Sr:Ca ratio thresholds between estuarine and marine environments were mathematically estimated (i.e., Mean $- 1 \times$ SD and Mean $- 2 \times$ SD) following a standard procedure used by different authors to delimit habitat residency according to their water salinity [9,21,33]. Simultaneously, the otolith's Sr:Ca ratios of individuals collected in a northern Portuguese estuary (Douro) were recorded (minimum and maximum values recorded in the otolith peripheral zones) in an attempt to assess if the mathematical approach makes sense compared to the real values and which of the two threshold limit estimates was the more accurate. Overall, the threshold estimated through the Mean $- 1 \times$ SD approach resulted closer to the estuarine values (4.28 mmol mol^{-1} and 5.41 mmol mol^{-1}, respectively, minimum and maximum values) determined by Sr:Ca ratios obtained from the Douro estuary individuals, suggesting that these values seem to be a more conservative approach to use for the species. Using the estuarine maximum value from the Douro individuals as the threshold between brackish and salt waters, Sr:Ca ratios in *C. lucerna* otoliths showed that the vast majority of individuals have migrated between marine and estuarine environments throughout their lifetime, apparently corroborating the findings from other authors that have identified a seasonal pattern of migratory movement of the species within its overall depth range during its life cycle [37,47,58]. In addition, the high levels of Sr:Ca ratios at the beginning of the core-to-edge transect (i.e., early life history otolith section) of most *C. lucerna* profiles indicate seawater signatures that quickly decline to estuarine levels, suggesting coastal spawning and a quick larval/juvenile ingress into brackish waters, which is consistent with a nursery role from estuaries, also described in other studies [5,47,63]. Furthermore, a few individuals also showed a progressive evolution of Sr:Ca estuarine levels at the otolith core to marine levels at the otolith edge, indicating an apparent estuarine spawning followed by a seaward migration from a food-rich shallow estuarine environment to deeper waters. Furthermore, while the migration of larger fish to deeper waters aligns with findings from other authors on the species [43,45,58], there has not been clear evidence of *C. lucerna* spawning in brackish environments. Existing studies on the species found that spawning occurs mostly around the winter months [45,59,64], which is also when fish show a more pronounced presence in deeper waters [37,47,65]. In addition, it is also important to note that besides habitat changes and migrations in distinct salinity environments, the otolith's Sr:Ca ratios can also record other critical events in a fish's life. Indeed, other studies focused on several anguilliform species [66–68] have found that a drop in Sr:Ca levels at the core-to-edge otolith transect can also be related to physiological events, such as the onset of the leptocephalus larval metamorphosis. Therefore, some atypical otolith Sr:Ca profiles recorded here should be regarded with caution and further investigated.

The overall behavior of all Ba:Ca profiles did not reflect an inverse relationship with Sr:Ca ratios and salinity, as described by other authors [30,32,69]. However, several studies have also shown that Ba incorporation into otoliths can be affected by factors such as upwelling phenomena [70–72] and terrestrial freshwater contributions (river runoff, groundwater inputs) [73–75]. The vast majority (97%) of *C. lucerna* individuals exhibited variations and peaks in Ba:Ca ratios in the natal region of otoliths that seem to corroborate these findings. A few individuals have shown Ba:Ca peaks only when Sr:Ca ratios were at marine levels, which is consistent with the upwelling of Ba enriched by cold, deep waters (e.g., the southern flow of the Portuguese summer current). Others have shown Ba:Ca peaks only when Sr:Ca ratios are at estuarine levels, indicating potential freshwater runoff from surrounding rivers (e.g., Douro River and Leça River). However, the majority of individuals displayed Ba:Ca peaks at both Sr:Ca marine and estuarine levels, consistent with the influence of both factors on Ba absorption into otoliths (Figure 4). These results have shown that, although individual Ba:Ca profiles would not be sufficient to infer *C. lucerna* movements between different habitat salinities, the combination of their analysis with the Sr:Ca profiles showed to be consistent with the results obtained by other authors on the influence of other factors than salinity in Ba incorporation into otoliths, in particular upwelling processes and freshwater discharges [71,72,75].

5. Conclusions

This is the first study to investigate *C. lucerna* migratory movements between salinity gradients along the Portuguese coast through saccular otolith elemental chemistry.

The results of this study have shown that *C. lucerna* seems to occupy and migrate between different salinity habitats throughout its life cycle, showing a high ability to respond and adapt to different environments. In this context, Sr:Ca ratios allowed us to infer that along the mainland Portuguese coast, the species can be classified as marine-estuarine-dependent, which aligns with findings from other studies that have identified other Portuguese rivers (Arade, Tagus and Mondego) as nurseries for the species [47,76,77]. However, further investigations using wild contemporary animals collected in the same temporal window in inshore areas and estuaries, including water sample analyses, would be useful to make more accurate inferences.

Overall, the information provided by this study is essential to improving the conservation of *C. lucerna*, the most important commercial species in the Triglidae family in Portugal, as it confirmed the fundamental role of estuarine habitats as nursery areas and feeding grounds for the species and the reliability of the Sr:Ca ratio as a tracer to investigate the migration patterns of teleost species between habitats with different salinities.

Finally, given the apparently critical support that estuaries provide for *C. lucerna* survival along the Portuguese coast, it is also noteworthy that the management efforts of fisheries through promoting the conservation, protection, and restoration of these environments can help ensure the long-term sustainability of the species.

Author Contributions: I.F.: formal analysis; investigation; methodology; validation; visualization; writing—original draft; and writing—review and editing. F.A.D.: data curation and writing—review and editing. C.M.: methodology and writing—review and editing. D.F.: data curation and writing—review and editing. A.R.: data curation and writing—review and editing. A.M.-V.: formal analysis; investigation; visualization; writing—review and editing. J.P.: writing—review and editing. A.T.C.: conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; resources; supervision; validation; visualization; writing—original draft; and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: For vertebrates, fishes in our case, the national (Portuguese) legislation regarding the animal welfare was only published in August 2013 (Decreto-Lei 113, 2013)—our research survey was from 2007 (research vessel samples, n = 35), and does not include the use of fish obtained from fisheries (bottom trawl samples, n = 5). It means that we do not have any legal constraint in the submitted MS.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Dahlgren, C.; Kellison, G.; Adams, A.; Gillanders, B.; Kendall; Layman, C.; Ley, J.; Nagelkerken, I.; Serafy, J. Marine nurseries and effective juvenile habitats: Concepts and applications. *Mar. Ecol. Prog. Ser.* **2006**, *312*, 291–295. [CrossRef]
2. Sharpe, C.; Carr-Harris, C.; Arbeider, M.; Wilson, S.M.; Moore, J.W. Estuary habitat associations for juvenile Pacific salmon and pelagic fish: Implications for coastal planning processes. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2019**, *29*, 1636–1656. [CrossRef]
3. Arevalo, E.; Cabral, H.N.; Villeneuve, B.; Possémé, C.; Lepage, M. Fish larvae dynamics in temperate estuaries: A review on processes, patterns and factors that determine recruitment. *Fish Fish.* **2023**, *24*, 466–487. [CrossRef]
4. Wilson, J. Productivity, Fisheries and Aquaculture in Temperate Estuaries. Estuarine, Coast. *Shelf Sci.* **2002**, *55*, 953–967. [CrossRef]
5. Potter, I.C.; Warwick, R.M.; Hall, N.G.; Tweedley, J.R. The physico-chemical characteristics, biota and fisheries of estuaries. In *Freshwater Fisheries Ecology*; Wiley Blackwell: Chichester, UK, 2015; pp. 48–79. [CrossRef]
6. Potter, I.C.; Tweedley, J.R.; Elliott, M.; Whitfield, A.K. The ways in which fish use estuaries: A refinement and expansion of the guild approach. *Fish Fish.* **2013**, *16*, 230–239. [CrossRef]
7. Dando, P.R. Reproduction in Estuarine Fish. In *Fish Reproduction: Strategies and Tactics*; Academic Press: London, UK, 1984; pp. 155–170.
8. James, N.C.; Leslie, T.D.; Potts, W.M.; Whitfield, A.K.; Rajkaran, A. The importance of different juvenile habitats as nursery areas for a ubiquitous estuarine-dependent marine fish species. *Estuar. Coast. Shelf Sci.* **2019**, *226*, 106270. [CrossRef]
9. Soeth, M.; Spach, H.L.; Daros, F.A.; Castro, J.P.; Correia, A.T. Use of otolith elemental signatures to unravel lifetime movement patterns of Atlantic spadefish, *Chaetodipterus faber*, in the Southwest Atlantic Ocean. *J. Sea Res.* **2020**, *158*, 101873. [CrossRef]
10. Bartes, S.; Simpfendorfer, C.; Walker, T.I.; King, C.; Loneragan, N.; Braccini, M. Conventional tagging of sharks in Western Australia: The main commercial species exhibit contrasting movement patterns. *Mar. Freshw. Res.* **2021**, *72*, 1643–1656. [CrossRef]
11. Braun, C.D.; Gaube, P.; Afonso, P.; Fontes, J.; Skomal, G.B.; Thorrold, S.R. Assimilating electronic tagging, oceanographic modelling, and fisheries data to estimate movements and connectivity of swordfish in the North Atlantic. *ICES J. Mar. Sci.* **2019**, *76*, 2305–2317. [CrossRef]
12. Popper, A.N.; Ramcharitar, J.; Campana, S.E. Why otoliths? Insights from inner ear physiology and fisheries biology. *Mar. Freshw. Res.* **2005**, *56*, 497–504. [CrossRef]
13. Rodriguez-Mendoza, R. Otoliths and their Applications in Fishery Science. *Croat. J. Fish.* **2006**, *64*, 89–102.
14. Volpedo, A.V.; Vaz-dos-Santos, A.M. *Métodos de Estudios con Otolitos: Principios y Aplicaciones/Métodos de Estudos com Otólitos: Princípios e Aplicações*, 1st ed.; CAFP-BA-PIESCI: Ciudad Autónoma de Buenos Aires, Argentina, 2015.
15. Ibáñez, C.M.; Riera, R.; Leite, T.; Díaz-Santana-Iturrios, M.; Rosa, R.; Pardo-Gandarillas, M.C. Stomach content analysis in cephalopods: Past research, current challenges, and future directions. *Rev. Fish Biol. Fish.* **2021**, *31*, 505–522. [CrossRef]
16. D'iglio, C.; Natale, S.; Albano, M.; Savoca, S.; Famulari, S.; Gervasi, C.; Lanteri, G.; Panarello, G.; Spanò, N.; Capillo, G. Otolith Analyses Highlight Morpho-Functional Differences of Three Species of Mullet (Mugilidae) from Transitional Water. *Sustainability* **2021**, *14*, 398. [CrossRef]
17. Korostelev, N.; Frey, P.; Orlov, A. Using different hard structures to estimate the age of deep-sea fishes: A case study of the Pacific flatnose, *Antimora microlepis* (Moridae, Gadiformes, Teleostei). *Fish. Res.* **2020**, *232*, 105731. [CrossRef]
18. Soeth, M.; Spach, H.L.; Daros, F.A.; Adelir-Alves, J.; de Almeida, A.C.O.; Correia, A.T. Stock structure of Atlantic spadefish *Chaetodipterus faber* from Southwest Atlantic Ocean inferred from otolith elemental and shape signatures. *Fish. Res.* **2018**, *211*, 81–90. [CrossRef]
19. Schroeder, R.; Schwingel, P.R.; Correia, A.T. Population structure of the Brazilian sardine (*Sardinella brasiliensis*) in the Southwest Atlantic inferred from body morphology and otolith shape signatures. *Hydrobiologia* **2021**, *849*, 1367–1381. [CrossRef]
20. Franco, A.; Franzoi, P.; Malavasi, S.; Riccato, F.; Torricelli, P.; Mainardi, D. Use of shallow water habitats by fish assemblages in a Mediterranean coastal lagoon. *Estuar. Coast. Shelf Sci.* **2006**, *66*, 67–83. [CrossRef]

21. Daros, F.A.; Spach, H.L.; Correia, A.T. Habitat residency and movement patterns of *Centropomus parallelus* juveniles in a subtropical estuarine complex. *J. Fish Biol.* **2016**, *88*, 1796–1810. [CrossRef]

22. Avigliano, E.; Leisen, M.; Romero, R.; Carvalho, B.; Velasco, G.; Vianna, M.; Barra, F.; Volpedo, A.V. Fluvio-marine travelers from South America: Cyclic amphidromy and freshwater residency, typical behaviors in *Genidens barbus* inferred by otolith chemistry. *Fish. Res.* **2017**, *193*, 184–194. [CrossRef]

23. Ferreira, I.; Santos, D.; Moreira, C.; Feijó, D.; Rocha, A.; Correia, A.T. Population structure of *Chelidonichthys lucerna* in Portugal mainland using otolith shape and elemental signatures. *Mar. Biol. Res.* **2019**, *15*, 500–512. [CrossRef]

24. Moura, A.; Muniz, A.; Mullis, E.; Wilson, J.; Vieira, R.; Almeida, A.; Pinto, E.; Brummer, G.; Gaever, P.; Gonçalves, J.; et al. Population structure and dynamics of the Atlantic mackerel (*Scomber scombrus*) in the North Atlantic inferred from otolith chemical and shape signatures. *Fish. Res.* **2020**, *230*, 105621. [CrossRef]

25. Daros, F.A.; Spach, H.L.; Sial, A.N.; Correia, A.T. Otolith fingerprints of the coral reef fish Stegastes fuscus in southeast Brazil: A useful tool for population and connectivity studies. *Reg. Stud. Mar. Sci.* **2016**, *3*, 262–272. [CrossRef]

26. Moreira, C.; Froufe, E.; Sial, A.; Caeiro, A.; Vaz-Pires, P.; Correia, A. Population structure of the blue jack mackerel (*Trachurus picturatus*) in the NE Atlantic inferred from otolith microchemistry. *Fish. Res.* **2018**, *197*, 113–122. [CrossRef]

27. Correia, A.; Moura, A.; Triay-Portella, R.; Santos, P.; Pinto, E.; Almeida, A.; Sial, A.; Muniz, A. Population structure of the chub mackerel (*Scomber colias*) in the NE Atlantic inferred from otolith elemental and isotopic signatures. *Fish. Res.* **2020**, *234*, 105785. [CrossRef]

28. Correia, A.; Pipa, T.; Gonçalves, J.; Erzini, K.; Hamer, P. Insights into population structure of *Diplodus vulgaris* along the SW Portuguese coast from otolith elemental signatures. *Fish. Res.* **2011**, *111*, 82–91. [CrossRef]

29. Moreira, C.; Froufe, E.; Vaz-Pires, P.; Triay-Portella, R.; Méndez, A.; Castro, J.P.; Correia, A.T. Unravelling the spatial-temporal population structure of *Trachurus picturatus* across the North-East Atlantic using otolith fingerprinting. *Estuar. Coast. Shelf Sci.* **2022**, *272*, 107860. [CrossRef]

30. Tabouret, H.; Bareille, G.; Claverie, F.; Pécheyran, C.; Prouzet, P.; Donard, O. Simultaneous use of strontium:calcium and barium:calcium ratios in otoliths as markers of habitat: Application to the European eel (*Anguilla anguilla*) in the Adour basin, South West France. *Mar. Environ. Res.* **2010**, *70*, 35–45. [CrossRef]

31. Stanley, R.R.E.; Bradbury, I.R.; DiBacco, C.; Snelgrove, P.V.R.; Thorrold, S.R.; Killen, S.S. Environmentally mediated trends in otolith composition of juvenile Atlantic cod (*Gadus morhua*). *ICES J. Mar. Sci.* **2015**, *72*, 2350–2363. [CrossRef]

32. Macdonald, J.; Crook, D. Variability in Sr:Ca and Ba:Ca ratios in water and fish otoliths across an estuarine salinity gradient. *Mar. Ecol. Prog. Ser.* **2010**, *413*, 147–161. [CrossRef]

33. Menezes, R.; Moura, P.E.; Santos, A.C.; Moraes, L.E.; Condini, M.V.; Rosa, R.S.; Albuquerque, C.Q. Habitat use plasticity by the dog snapper (*Lutjanus jocu*) across the Abrolhos Bank shelf, eastern Brazil, inferred from otolith chemistry. *Estuar. Coast. Shelf Sci.* **2021**, *263*, 107637. [CrossRef]

34. Gillanders, B.M. Otolith chemistry to determine movements of diadromous and freshwater fish. *Aquat. Living Resour.* **2005**, *18*, 291–300. [CrossRef]

35. Walther, B.D.; Limburg, K.E. The use of otolith chemistry to characterize diadromous migrations. *J. Fish Biol.* **2012**, *81*, 796–825. [CrossRef]

36. Brown, R.J.; Severin, K.P.; Martin, J.; Rougemont, Q.; Drouineau, H.; Launey, S.; Jatteau, P.; Bareille, G.; Berail, S.; Pécheyran, C.; et al. Otolith chemistry analyses indicate that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. *Can. J. Fish. Aquat. Sci.* **2009**, *66*, 1790–1808. [CrossRef]

37. Işmen, A.; Işmen, P.; Başusta, N. Age, Growth and Reproduction of Tub Gurnard (*Chelidonichthys lucerna* L. 1758) in the Bay of İskenderun in the Eastern Mediterranean. *Turk. J. Vet. Anim. Sci.* **2004**, *28*, 289–295.

38. Quigley, D. Gurnards (Triglidae) in Irish and European Atlantic Seas. *Sherkin Comment*. 2005, p. 21. Available online: https://www.researchgate.net/publication/277305043 (accessed on 4 May 2023).

39. FAO. Fisheries and Aquaculture Department, Species Fact Sheets, *Chelidonichthys lucerna* (Linnaeus, 1758). Rome. 2023. Available online: https://www.fao.org/figis/pdf/fishery/species/2530/en?title=FAO%20Fisheries%20%26%20Aquaculture%20-%20Species%20Fact%20Sheets%20-%20Chelidonichthys%20lucerna%20(Linnaeus%2C%201758) (accessed on 20 May 2023).

40. Feijó, D.; Rocha, A.; Santos, P.; Saborido-Rey, F. Statistical Species characterization of Gurnard Landings in North of Portugal. Conference handbook (ICES CM 2008/K:15). In Proceedings of the ICES Annual Science Conference, Halifax, NS, Canada, 22–26 September 2008.

41. Instituto Nacional de Estatística. Estatísticas da Pesca: 2021. *Lisboa*. 2022. Available online: https://www.ine.pt/xurl/pub/36828280 (accessed on 1 July 2023).

42. Instituto Nacional de Estatística. Estatísticas da Pesca: 2022. *Lisboa*. 2023. Available online: https://www.ine.pt/xurl/pub/66322600 (accessed on 1 July 2023).

43. Colloca, F.; Ardizzone, G.D.; Gravina, M.F. Trophic ecology of gurnards (Pisces: Triglidae) in the Central Mediterranean Sea. *Mar. Life* **1994**, *4*, 45–57. Available online: https://www.researchgate.net/publication/284081952 (accessed on 4 May 2023).

44. Montanini, S.; Stagioni, M.; Benni, E.; Vallisneri, M. Feeding strategy and ontogenetic changes in diet of gurnards (Teleostea: Scorpaeniformes: Triglidae) from the Adriatic Sea. *Eur. Zool. J.* **2017**, *84*, 356–367. [CrossRef]

45. El-Serafy, S.S.; El-Gammal, F.I.; Mehanna, S.F.; Abdel-Hamid, N.-A.H.; Farrag, E.-S.F. Age, Growth and Reproduction of the Tub Gurnard, *Chelidonichthys lucerna* (Linnaeus, 1758) from the Egyptian Mediterranean waters off, Alexandria. *Int. J. Fish. Aquat. Sci.* **2015**, *4*, 13–20. [CrossRef]
46. McCarthy, I.D.; Marriott, A.L. Age, growth and maturity of tub gurnard (*Chelidonichthys lucerna* Linnaeus 1758; Triglidae) in the inshore coastal waters of Northwest Wales, UK. *J. Appl. Ichthyol.* **2018**, *34*, 581–589. [CrossRef]
47. Campos, J.; Dias, S.C.; Bio, A.; Santos, P.T.; Jorge, I. Age and Growth of Tub Gurnard *Chelidonichthys lucerna* (Linnaeus, 1758) during Estuarine Occupation of a Temperate Atlantic Nursery. *Int. J. Environ. Sci. Nat. Resour.* **2022**, *31*, 1–14. [CrossRef]
48. Azevedo, I.C.; Bordalo, A.A.; Duarte, P. Influence of freshwater inflow variability on the Douro estuary primary productivity: A modelling study. *Ecol. Model.* **2014**, *272*, 1–15. [CrossRef]
49. Rodrigues, S.M.; Silva, D.; Cunha, J.; Pereira, R.; Freitas, V.; Ramos, S. Environmental influences, particularly river flow alteration, on larval fish assemblages in the Douro Estuary, Portugal. *Reg. Stud. Mar. Sci.* **2022**, *56*, 102617. [CrossRef]
50. Ambar, I.; Serra, N.; Brogueira, M.; Cabeçadas, G.; Abrantes, F.; Freitas, P.; Gonçalves, C.; Gonzalez, N. Physical, chemical and sedimentological aspects of the Mediterranean outflow off Iberia. *Deep. Sea Res. Part II Top. Stud. Oceanogr.* **2002**, *49*, 4163–4177. [CrossRef]
51. Correia, A.T.; Ramos, A.A.; Barros, F.; Silva, G.; Hamer, P.; Morais, P.; Cunha, R.L.; Castilho, R. Population structure and connectivity of the European conger eel (*Conger conger*) across the north-eastern Atlantic and western Mediterranean: Integrating molecular and otolith elemental approaches. *Mar. Biol.* **2012**, *159*, 1509–1525. [CrossRef]
52. Correia, A.; Hamer, P.; Carocinho, B.; Silva, A. Evidence for meta-population structure of Sardina pilchardus in the Atlantic Iberian waters from otolith elemental signatures of a strong cohort. *Fish. Res.* **2013**, *149*, 76–85. [CrossRef]
53. Campana, S.E. Chemistry and composition of fish otoliths:pathways, mechanisms and applications. *Mar. Ecol. Prog. Ser.* **1999**, *188*, 263–297. [CrossRef]
54. Sirot, C.; Ferraton, F.; Panfili, J.; Childs, A.; Guilhaumon, F.; Darnaude, A. elementr: An R package for reducing elemental data from LA-ICPMS analysis of biological calcified structures. *Methods Ecol. Evol.* **2017**, *8*, 1659–1667. [CrossRef]
55. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria. 2023. Available online: https://www.r-project.org/ (accessed on 6 June 2023).
56. Secor, D.H.; Rooker, J.R. Is otolith strontium a useful scalar of life cycles in estuarine fishes? *Fish. Res.* **2000**, *46*, 359–371. [CrossRef]
57. Martinho, F.; Pina, B.; Nunes, M.; Vasconcelos, R.P.; Fonseca, V.; Crespo, D.; Primo, A.; Vaz, A.; Pardal, M.A.; Gillanders, B.M.; et al. Water and Otolith Chemistry: Implications for Discerning Estuarine Nursery Habitat Use of a Juvenile Flatfish. *Front. Mar. Sci.* **2020**, *7*, 347. [CrossRef]
58. Papaconstantinou, C. Age and growth of the yellow gurnard (*Trigla lucerna* L. 1758) from the Thermaikos Gulf (Greece) with some comments on its biology. *Fish. Res.* **1984**, *2*, 243–255. [CrossRef]
59. Rodrigues, J.; Feijó, D.; Rocha, A.; Erzini, K.; Correia, A.T. Age, growth and reproductive biology of the tub gurnard (*Chelidonichthys lucerna*) in North-East Portugal. *Front. Mar. Sci.* **2019**, *6*, 158. [CrossRef]
60. Walther, B.D.; Thorrold, S.R. Water, not food, contributes the majority of strontium and barium deposited in the otoliths of a marine fish. *Mar. Ecol. Prog. Ser.* **2006**, *311*, 125–130. [CrossRef]
61. Nelson, T.R.; Powers, S.P. Validation of species specific otolith chemistry and salinity relationships. *Environ. Biol. Fishes* **2019**, *102*, 801–815. [CrossRef]
62. Elsdon, T.S.; Wells, B.K.; Campana, S.E.; Gillanders, B.M.; Jones, C.M.; Limburg, K.E.; Secor, D.H.; Thorrold, S.R.; Walther, B.D. Otolith chemistry to describe movements and life-history parameters of fishes: Hypotheses, assumptions, limitations and inferences. *Oceanogr. Mar. Biol. Annu. Rev.* **2008**, *46*, 297–330. Available online: https://www.researchgate.net/publication/231180600 (accessed on 10 May 2023).
63. Reis, P.A.; Feijó, D.; Seixas, F.; Pimenta, J.; Rocha, A.; Santos, P. Fishery of *Chelidonichthys lucerna* (Linnaeus, 1758) in portuguese northwest atlantic coast: Exploratory baseline study. *Int. J. Fish. Aquat. Stud.* **2020**, *8*, 5. [CrossRef]
64. İlhan, D.; Toğulga, M. Age, growth and reproduction of tub gurnard *Chelidonichthys lucernus* Linnaeus, 1758 (Osteichthtyes: Triglidae) from İzmir Bay, Aegean Sea, Eastern Mediterranean. *Acta Adriat.* **2007**, *48*, 173–184.
65. Vallisneri, M.; Stagioni, M.; Montanini, S.; Tommasini, S. Body size, sexual maturity and diet in *Chelidonichthys lucerna* (Osteichyes: Triglidae) from the Adriatic Sea, north eastern Mediterranean. *Acta Adriat.* **2011**, *52*, 141–148.
66. Correia, A.T.; Antunes, C.; Isidro, E.J.; Coimbra, J. Changes in otolith microstructure and microchemistry during larval development of the European conger eel (*Conger conger*). *Mar. Biol.* **2003**, *142*, 777–789. [CrossRef]
67. Correia, A.T.; Able, K.W.; Antunes, C.; Coimbra, J. Early life history of the American conger eel (*Conger oceanicus*) as revealed by otolith microstructure and microchemistry of metamorphosing leptocephali. *Mar. Biol.* **2004**, *145*, 477–488. [CrossRef]
68. Ling, Y.; Iizuka, Y.; Tzeng, W. Decreased Sr/Ca ratios in the otoliths of two marine eels, *Gymnothorax reticularis* and *Muraenesox cinereus*, during metamorphosis. *Mar. Ecol. Prog. Ser.* **2005**, *304*, 201–206. [CrossRef]
69. Bath, G.E.; Thorrold, S.R.; Jones, C.M.; Campana, S.E.; McLaren, J.W.; Lam, J.W.H. Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochim. Cosmochim. Acta* **2000**, *64*, 1705–1714. [CrossRef]
70. Lin, Y.T.; Wang, C.H.; You, C.F.; Tzeng, W.N. BA/CA ratios in otoliths of southern bluefin tuna (*Thunnus maccoyii*) as a biological tracer of upwelling in the great Australian bight. *J. Mar. Sci. Technol.* **2013**, *21*, 733–741. [CrossRef]
71. Woodson, L.; Wells, B.; Grimes, C.; Franks, R.; Santora, J.; Carr, M. Water and otolith chemistry identify exposure of juvenile rockfish to upwelled waters in an open coastal system. *Mar. Ecol. Prog. Ser.* **2013**, *473*, 261–273. [CrossRef]

72. Wheeler, S.; Russell, A.; Fehrenbacher, J.; Morgan, S. Evaluating chemical signatures in a coastal upwelling region to reconstruct water mass associations of settlement-stage rockfishes. *Mar. Ecol. Prog. Ser.* **2016**, *550*, 191–206. [CrossRef]

73. Ferguson, G.J.; Ward, T.M.; Gillanders, B.M. Otolith shape and elemental composition: Complementary tools for stock discrimination of mulloway (*Argyrosomus japonicus*) in southern Australia. *Fish. Res.* **2011**, *110*, 75–83. [CrossRef]

74. Morat, F.; Letourneur, Y.; Dierking, J.; Pécheyran, C.; Bareille, G.; Blamart, D.; Harmelin-Vivien, M. The Great Melting Pot. Common Sole Population Connectivity Assessed by Otolith and Water Fingerprints. *PLoS ONE* **2014**, *9*, e86585. [CrossRef] [PubMed]

75. De Carvalho, B.M.; Volpedo, A.; Dos Santos, A.M.V.; Spach, H.L. Use of otolith microchemistry as habitat indicator of *Anchoa tricolor* (Spix and Agassiz, 1829) in a subtropical estuary. *Lat. Am. J. Aquat. Res.* **2017**, *45*, 457–465. [CrossRef]

76. Costa, M.; Cabral, H. Changes in the Tagus nursery function for commercial fish species: Some perspectives for management. *Aquat. Ecol.* **1999**, *33*, 287–292. [CrossRef]

77. Veiga, P.; Machado, D.; Almeida, C.; Bentes, L.; Monteiro, P.; Oliveira, F.; Ruano, M.; Erzini, K.; Gonçalves, J.M.S. Weight-length relationships for 54 species of the Arade estuary, southern Portugal. *J. Appl. Ichthyol.* **2009**, *25*, 493–496. [CrossRef]