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Population structure, habitat connectivity and fish movement of *Chelidonichthys lucerna* in the northeast Atlantic revealed by natural tags

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Tese apresentada à Universidade Fernando Pessoa como parte dos requisitos para obtenção do grau de Doutor em Ecologia e Saúde Ambiental, sob a orientação científica do Prof. Doutor Alberto Teodorico Correia da Universidade de Trás-os-Montes e Alto Douro.

Previous Note: This thesis integrates several articles published, accepted or submitted to international peer-reviewed scientific journals. Some of the results have also been partially presented at international congresses. The candidate also states that she conceived the ideas, compiled the data, analyzed and discussed the results, and led the writing of the different chapters.

(i) Published in international peer-reviewed indexed scientific journals

Ferreira I., Santos D., Moreira C., Feijó D., Rocha A., Correia A.T., 2019. Population structure of *Chelidonichthys lucerna* in Portugal mainland using otolith shape and elemental signatures. Marine Biology Research. 15 (8–9): 500–512. https://doi: 10.1080/17451000.2019.1673897

Ferreira I., Daros F.A., Moreira C., Feijó D., Rocha A., Mendez-Vicente A., Pisonero J., Correia A.T., 2023. Is *Chelidonichthys lucerna* (Linnaeus, 1758) a marine estuarine-dependent fish? Insights from saccular otolith microchemistry. Fishes. 8(7):383. https://doi.org/10.3390/fishes8070383

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(ii) International congresses

Ferreira I., Santos D., Moreira C., Feijó D., Rocha A., Correia A.T., 2019. Population structure of *Chelidonichthys lucerna* in Portugal mainland using otolith shape and elemental signatures. XIX Iberian Symposium on Marine Biology Studies, Porto, Portugal. Expanded summary (Poster). http://dx.doi.org/10.3389/conf.FMARS.2016.05.00200

Ferreira I., Daros F.A., Moreira C., Feijó D., Rocha A., Mendez-Vicente A., Pisonero J., Correia A.T., 2023. Is *Chelidonichthys lucerna* (Linnaeus, 1758) a Marine Estuarine-Dependent Fish? Insights from Saccular Otolith Microchemistry. XVII European Congress of Ichthyology, Prague, Czechia. Expanded summary (Poster). http://eci23.agrobiologie.cz/wp-content/uploads/2023/08/ECI-Abstract-Book_29AUG2023B.pdf

Ferreira I., Carrola J.S., Correia A.T., 2023. Otoliths as a powerful tool for studying fishes biology: uses and abuses. Jornada Técnica e Científica de Peixes de Rio, Castelo Branco, Portugal. Expanded summary (Poster). https://jtcpr2023.weebly.com/uploads/1/1/9/2/119259848/livro_resumos_abstracts.pdf

RESUMO

Estrutura populacional, conetividade de habitat e movimento de *Chelidonichthys lucerna* no nordeste Atlântico revelada por marcadores naturais

(Sob orientação do Prof Alberto Teodorico Correia)

Compreender a estrutura da população de peixes, os padrões de movimentos e a conetividade dos habitats são aspectos críticos para uma gestão sustentável e racional das pescas. O cabra-cabaço, *Chelidonichthys lucerna*, é uma espécie de peixe demersal amplamente distribuída no Atlântico nordeste, no Mediterrâneo e no mar Negro, sendo geralmente capturada pela pesca artesanal. Existe um interesse comercial crescente nesta espécie, o que se reflecte na tendência crescente de desembarques declarados pela pesca, a maioria dos quais ocorre na região do Atlântico Nordeste. No entanto, a compreensão da sua dinâmica populacional, movimentos migratórios e conetividade entre habitats, particularmente na região do Atlântico Nordeste, permanece limitada. Para colmatar esta lacuna de conhecimento, o presente trabalho utilizou uma abordagem holística para estudar a estrutura populacional desta espécie ao longo do Atlântico Nordeste através da utilização de diferentes técnicas, nomeadamente da análise da forma dos otólitos, das assinaturas químicas elementares dos otólitos e da geometria morfométrica do corpo. A combinação da análise da forma dos otólitos, obtidas por índices de forma e descritores elípticos de Fourier, e da microquímica elementar dos otólitos, aplicada a indivíduos de *C. lucerna* capturados em diferentes pesqueiros ao longo da costa portuguesa, sugere a existência de alguma conetividade entre estas regiões e que estas agregações de peixes, embora não totalmente homogéneas, constituem uma única unidade populacional que se mistura parcialmente durante a sua história de vida. A relação de Sr:Ca do core para a periferia dos otólitos também se revelou valiosa na avaliação dos padrões de migração da espécie entre diferentes ambientes de salinidade, mostrando que a espécie é marinha estuarino-dependente, como sugerido anteriormente. Finalmente, a análise da geometria morfométrica do corpo dos indivíduos de *C. lucerna* capturados ao longo do Atlântico nordeste (Mar da Irlanda, Mar da Cantábria e águas do noroeste de Portugal) revelou a existência de diferenças regionais significativas, sugerindo que estamos a lidar com stocks diferentes. Em conclusão, este trabalho recomenda que as estratégias de gestão da espécie no Atlântico nordeste considerem os requisitos da estrutura dinâmica da espécie para garantir a sua sustentabilidade a longo prazo. Nas águas portuguesas deve ser tratada e gerida como um stock único, embora não homogéneo. No entanto, as pescarias ao longo do Atlântico Nordeste devem ser geridas regionalmente como unidades populacionais diferentes. Continua a ser necessária mais investigação sobre a espécie, utilizando ferramentas alternativas e complementares, para uma compreensão mais assertiva da sua estrutura populacional.

Palavras-chave: Triglidae, saggitta, Marcadores naturais, Stocks de peixes, Estrutura populacional, Gestão de pescas, LA-ICP-MS, Elemento:Ca, Residência no habitat, Comportamento migratório, Morfometria geométrica, Rede de treliças.

ABSTRACT

Population structure, habitat connectivity and fish movement of *Chelidonichthys lucerna* in the northeast Atlantic revealed by natural tags

(Under the supervision of Professor Alberto Teodorico Correia)

Understanding the fish population structure, movements patterns and habitat connectivity are critical aspects for a sustainable and rational fisheries management. The tub gurnard, *Chelidonichthys lucerna*, is a demersal fish species widely distributed across the northeast Atlantic, Mediterranean and Black Sea and usually captured as by-catch by the artisanal fisheries. There is a commercial growing interest in the species, which is reflected by the increased trend of declared fisheries landings, most of which occur in the northeast Atlantic region. However, the understanding of its population dynamics, migratory movements, and habitat connectivity, particularly in the northeast Atlantic region, remains limited. To address this knowledge gap, the present work employed a holistic approach to study the population structure of this species along the northeast Atlantic through the use of different techniques, namely otolith shape analysis, otolith elemental fingerprints, and body morphometric geometrics. The combination of otolith shape analysis, obtained by shape indices and elliptic Fourier descriptors, and whole otolith elemental fingerprints applied to *C. lucerna* individuals captured in different fishing grounds along the Portuguese coast suggests the existence of some connectivity between these regions and that these fish aggregations, albeit not entirely homogenous, are a single population unit that partially mixes during their lifetime history. Sr:Ca core-to-edge transects also proved valuable in assessing the species migration patterns between different salinity environments, showing that the species is a marine estuarine-dependent, as previously suggested. Finally, the analysis of the body morphometric geometrics of *C. lucerna* individuals captured along the northeast Atlantic (Irish Sea, Cantabria Sea and northwest Portuguese waters) revealed the existence of significant regional differences, suggesting that we are dealing with different stocks. In conclusion, this work recommends that management strategies for the species in the northeast Atlantic consider the requirements of the dynamic structure of the species to ensure its long-term sustainability. In Portuguese waters it should be treated and managed as a unique, although not homogeneous, stock. However, fisheries along the northeast Atlantic should be managed regionally as different stocks. Further research on the species using alternative and complementary tools, remains necessary for a more reliable understanding of its population structure.

Keywords: Triglidae, saggitta, Natural tags, Fish stocks, Population structure, Fisheries management, LA-ICP-MS, Element:Ca, Habitat residency, Migratory behavior, Geometric morphometrics, Truss network.

RÉSUMÉ

Structure de la population, connectivité de l'habitat et mouvements des poissons de *Chelidonichthys lucerna* dans l'Atlantique Nord-Est révélés par les marques naturelles

(Sous l´orientation du Prof Alberto Teodorico Correia)

La compréhension de la structure de la population de poissons, des mouvements et de la connectivité de l'habitat sont des aspects critiques pour une gestion durable et rationnelle de la pêche. Le grondin perlon, *Chelidonichthys lucerna*, est une espèce de poisson démersale largement répandue dans l'Atlantique Nord-Est, la Méditerranée et la mer Noire et généralement capturée en tant que prise accessoire par les pêcheries artisanales. Cette espèce fait l'objet d'un intérêt commercial croissant, comme en témoigne la tendance à la hausse des débarquements déclarés, dont la plupart ont lieu dans la région de l'Atlantique Nord-Est. Cependant, la compréhension de la dynamique de sa population, de ses mouvements migratoires et de la connectivité de son habitat, en particulier dans la région de l'Atlantique Nord-Est, reste limitée. Pour combler cette lacune, le présent travail a utilisé une approche holistique pour étudier la structure de la population de cette espèce le long de l'Atlantique Nord-Est en utilisant différentes techniques, à savoir l'analyse de la forme de l'otolithe, les empreintes chimiques de l'otolithe et la géométrie morphométrique du corps. La combinaison des empreintes de forme des otolithes obtenues par les indices de forme et les descripteurs elliptiques de Fourier, et la microchimie élémentaire des otolithes appliquée aux individus de *C. lucerna* capturés dans différentes zones de pêche le long de la côte portugaise suggèrent l'existence d'une certaine connectivité entre ces régions et que ces agrégations de poissons, bien qu'elles ne soient pas entièrement homogènes, constituent une unité de population unique qui se mélange partiellement au cours de leur histoire de vie. Les raisons Sr:Ca du centre vers la périphérie de l´otolite sont également avérée précieuses pour évaluer les schémas de migration de l'espèce entre différents environnements de salinité, montrant que l'espèce est tributaire des estuaires marins, comme cela avait été suggéré précédemment. Enfin, l'analyse de la morphométrie corporelle des individus de *C. lucerna* capturés le long de l'Atlantique Nord-Est (mer d'Irlande, mer de Cantabrie et eaux portugaises du Nord-Ouest) a révélé l'existence de différences régionales significatives, suggérant que nous avons affaire à des stocks différents. En conclusion, ce travail recommande que les stratégies de gestion de l'espèce dans l'Atlantique Nord-Est prennent en compte les exigences de la structure dynamique de l'espèce afin d'assurer sa durabilité à long terme. Dans les eaux portugaises, l'espèce devrait être traitée et gérée comme un stock unique, bien que non homogène. Toutefois, les pêcheries de l'Atlantique Nord-Est devraient être gérées au niveau régional comme des stocks différents. Des recherches supplémentaires sur l'espèce, utilisant des outils alternatifs et complémentaires, restent nécessaires pour une compréhension plus fiable de la structure de sa population.

Mots-clés : Triglidae, saggitta, Marques naturelles, Stocks de poissons, Structure de la population, Gestion des pêches, LA-ICP-MS, Elément:Ca, Résidence dans l'habitat, Comportement migratoire, Morphométrie géométrique, Réseau en treillis.

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INDEX

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

A

ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance

B

BRTA	Basque Research and Technology Alliance

C

CAP	Canonical Analysis of Principle Components
CI	Circularity
CIIMAR	Centro Interdisciplinar de Investigação Marinha e Ambiental

D

DNA	Deoxyribonucleic Acid
DPA	Departamento de Produção Aquática
DWd	Discrete Wavelet descriptors
DT	Transformed Distances

E

ECVA	Escola de Ciências da Vida e do Ambiente
EFD	Elliptic Fourier Descriptors
EL	Ellipticity
ERDF	European Regional Development Fund
EUR	Euro

F

FAO	Food and Agriculture Organization
FCS-UFP	Faculdade de Ciência da Saúde da Universidade Fernando Pessoa
FCT	Fundação para a Ciência e Tecnologia
FCT-UFP	Faculdade de Ciência e Tecnologia da Universidade Fernando Pessoa
FCUP	Faculdade de Ciências da Universidade do Porto
FDA	Flexible Discriminant Analysis
FF	Form Factor

FP	Fourier Power
FP-ENAS	Unidade de Investigação da Universidade Fernando Pessoa em Energia, Ambiente e Saúde

I

ICBAS	Instituto de Ciências Biomédicas Abel Salazar
ICES	International Council for the Exploration of the Sea
ICM	Identifying Changes in Mean
ICP	Inductively Coupled Plasma
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
INE	Instituto Nacional de Estatística
IPMA	Instituto Português do Mar e da Atmosfera
IR-MS	Isotope Ratio Mass Spectrometry
IUCN	International Union for Conservation of Nature

L

LA-ICP-MS	Laser Ablation Inductively Coupled Plasma Mass Spectrometry
LDFA	Linear Discriminant Function Analyses
LOD	Limit of Detection

M

MANOVA	Multivariate Analysis of Variance
MC-ICP-MS	Multicollector Inductively Coupled Plasma Mass Spectrometer

N

NE	Northeast
NIST	National Institute of Standards and Technology
NW	Northwest

P

PERMANOVA	Permutational Multivariate Analysis of Variance

R

RE	Rectangularity
RNA	Ribonucleic Acid
RO	Roundness
RSD	Relative Standard Deviation

S

SAG-POR	Sagres-Portimão
SB-ICP	Solution-Based Inductively Coupled Plasma
SB-ICP-MS	Solution-Based Inductively Coupled Plasma Mass Spectrometry

SL	Standard Length
SD	Standard Deviation
SI	Shape Indices
SL	Standard Length
SNPs	Single Nucleotide Polymorphisms
SRM	Standard Reference Materials

T

TL	Total Length

U

UK	United Kingdom
UNESP	Universidade Estadual Paulista
UNIOVI	Universidad de Oviedo
UNIVALI	Universidade do Vale do Itajaí
UP	Universidade do Porto
USA	United States of America
USB	Universal Serial Bus
USGS	United States Geological Survey
UTAD	Universidade de Trás-os-Montes e Alto Douro

CHAPTER I. General Introduction

1.1 Ecology and biology of *Chelidonichthys lucerna*

1.1.1. Habitat and geographic distribution

Gurnards are medium-sized demersal fishes belonging to the Triglidae family (order: Scorpaeniformes) that can be found in different types of bottom substrates, such as sand, muddy sand or gravel (FAO, 2016; FishBase, 2023). The designation "gurnard" is thought to derive from the French word for "grunt", "grogner", as these animals can produce audible grunting noises with their swim bladder (Quigley, 2005; Green & Lart, 2014).

There are over one-hundred gurnard species worldwide occurring in brackish and marine environments of tropical and temperate sea (Colloca et al., 1994; Nelson, 2006; FishBase, 2023) and although most of the species are not the object of any particular type of fisheries, they are often caught as bycatch in bottom-trawl and in artisanal gears like beam trawl and trammel nets. Despite the majority of the species are not considered desirable as seafood, some of the larger ones are used for human consumption (Feijó et al., 2008; Green & Lart, 2014; FAO, 2016).

There are eight species that can be found across the northeast Atlantic (Olim & Borges, 2006; Martins & Carneiro, 2018; Rocha et al., 2018): *Chelidonichthys cuculus* (Linnaeus, 1758), *Chelidonichthys obscurus* (Walbaum, 1792), *Eutrigla gurnardus* (Linnaeus, 1758), *Chelidonichthys lucerna* (Linnaeus, 1758), *Chelidonichthys lastoviza* (Bonnaterre, 1788), *Lepidotrigla cavillone* (Lacepède, 1801), *Lepidotrigla dieuzeidei* (Blanc & Hureau, 1973) and *Trigla lyra* (Linnaeus, 1758).

C. lucerna is a Mediterranean-Atlantic species, distributed along the northeast Atlantic coast, from Norway to the northwest coast of Africa, being also found around the British Isles, in the Mediterranean and Black Seas (Richards & Saksena, 1990; Vallisneri et al., 2011; McCarthy & Marriott, 2018) at a water temperature ranging from 8.0°C to 24.0°C (Işmen et al., 2004; Vallisneri et al., 2011; El-Serafy et al., 2015). However, there are no records of its occurrence in the Madeira or Azores archipelagos (Nunoo et al., 2015). The species usually lives in small shoals and can be found in depths ranging from 20 m to 318 m, but it is more abundant in inshore waters up to 150 m (Mytilineou et al., 2005; ICES, 2010; El-Serafy et al., 2015).

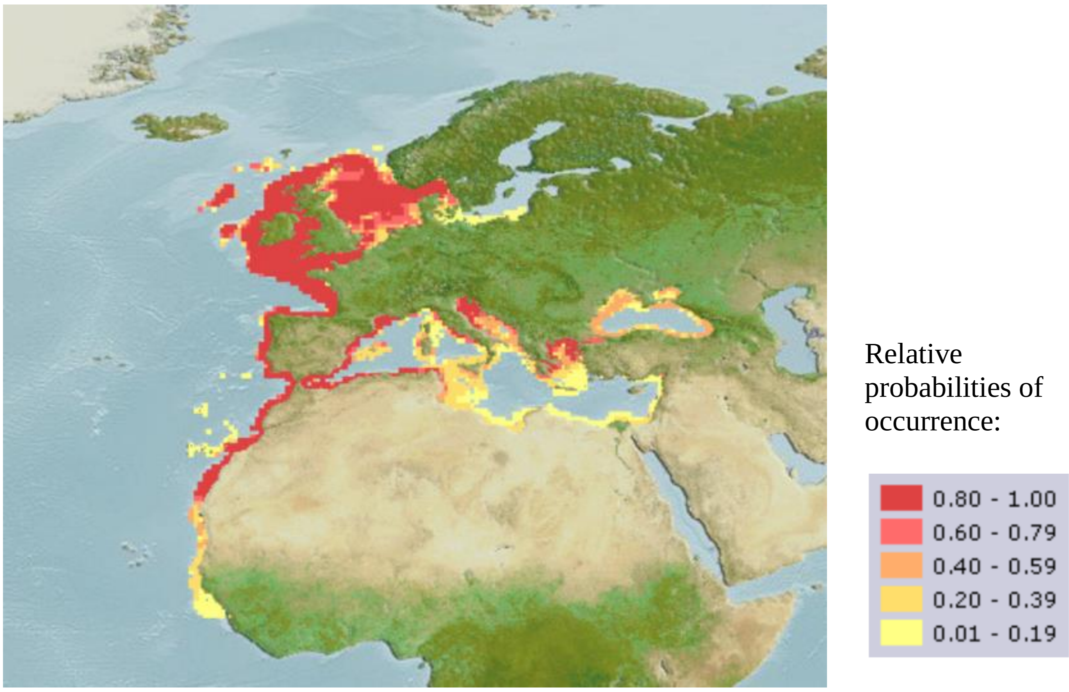

Figure 1-1. Distribution map for *Chelidonichthys lucerna*
Source: AquaMaps, 2019.
Note: Distribution range colours indicate degree of suitability of habitat which can be interpreted as probabilities of occurrence.

1.1.2. Morphology and feeding regime

Similarly to other gurnards species, *C. lucerna* is a distinctive looking fish, with a number of unique features, including elongated bodies covered in spines and large armored bony heads with large eyes located on the top. Its body is covered with small cycloid scales and the lateral line is smooth, without spiny scales. The species has three free pectoral-fin rays that are used for propulsion and as tactile organs for foraging. During the fish's larval and post-larval stages these three digitiform rays are still connected to the fin membrane (Muñoz et al., 2002; Jamon et al., 2007; Mazza, 2023).

Its body color varies from red or reddish brown on the back of the head and across the upper body, and yellowish on the rest of the body with a pale belly. This species has coloured pectoral fins with brilliant peacock blue spots and outer rim which distinguishes it from other gurnards. The pectoral fins sometimes also have a large black circular patch near the base (Allué et al., 1981; FAO, 2016; Martins & Carneiro, 2018) (Figure 1-2).

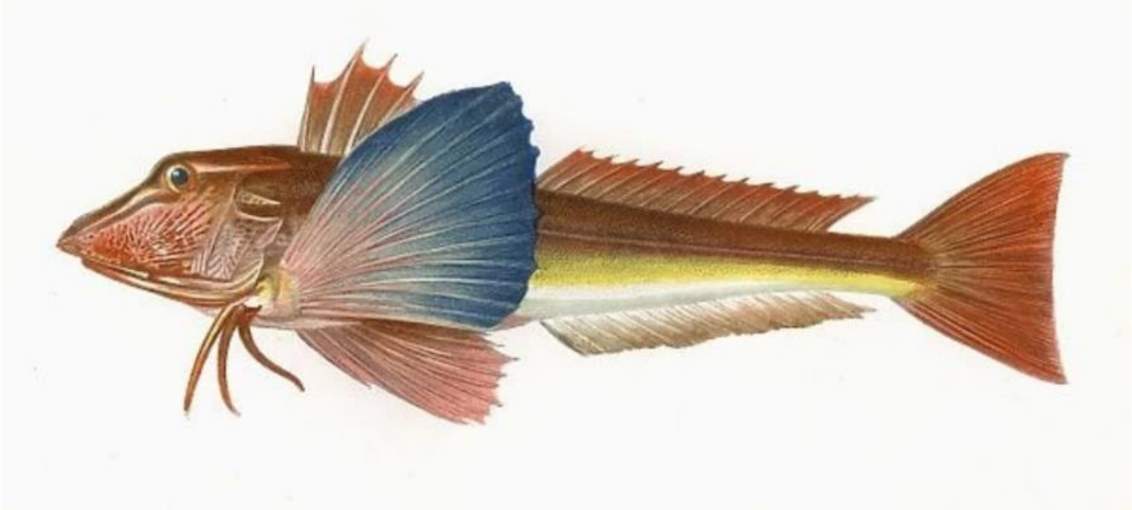

Figure 1-2. *Chelidonichthys lucerna* (Linnaeus, 1758).
Source: The Sharp Illustration Collection / Mary Evans Picture Library.

C. lucerna shows an opportunistic foraging behaviour. The species mainly feeds on crustaceans and small teleost fish, occasionally also feeding on molluscs and polychaetes. Depending on the geographic location, its feeding regime varies according to the food availability (Colloca et al., 1994; Stagioni et al., 2012; McCarthy & Marriott, 2018). A few authors have found that the species changes their diet with size, with smaller individuals feeding upon mysids and larger individuals preying on smaller fishes and decapodes (Colloca et al., 1994; Boudaya et al., 2007; Montanini et al., 2017), and with the season, feeding more on fish during the winter period, and crustaceans during the summer months (Stagioni et al., 2012). A rapid change in the feeding strategy coincidental with the onset of the sexual maturity, when there is a higher energy requirement for gonadal development and reproduction, was also observed by some authors (Colloca et al., 1994; Vallisneri et al., 2011; Montanini et al., 2017). However, no differences between the feeding regimes of males and females were found (Stagioni et al., 2012).

1.1.3. Life cycle and population structure

C. lucerna lives occasionally in solitarily, but more often forms small schools (FAO, 2016) and exhibits a particular pattern of seasonal migratory movement within its depth ranges throughout the year showing a more pronounced concentration in shallower depths during spring and summer, moving progressively to deeper waters in the winter period (Montanini et al., 2017; Carbonara & Follesa, 2019; Campos et al., 2022).

Nursery areas along the coastal and estuarine 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, where food is abundant, while larger and older individuals are more dispersed towards offshore grounds (Colloca et al., 1994; Quigley, 2005; Montanini et al., 2017). A recent study investigated the species growth within an estuarine environment and confirmed an estuarine occupation during the fish early life, and the nursery role provided by these types of environments (Campos et al., 2022).

However, at the time of starting the present work, information about *C. lucerna* population dynamics, movement patterns and habitat connectivity was limited to a single research on the eastern Mediterranean population (Uyan & Turan, 2017).

1.1.4. Age, growth and reproductive biology

C. lucerna is a moderately long-living species with a maximum reported estimated age of 14 years (Baron, 1985a). Age estimations of the species are usually determined using fish otoliths through a burning technique followed by reading the sagittal otoliths under a stereomicroscope (Holden & Raitt, 1974; Papaconstantinou, 1984) (Figure 1-3).

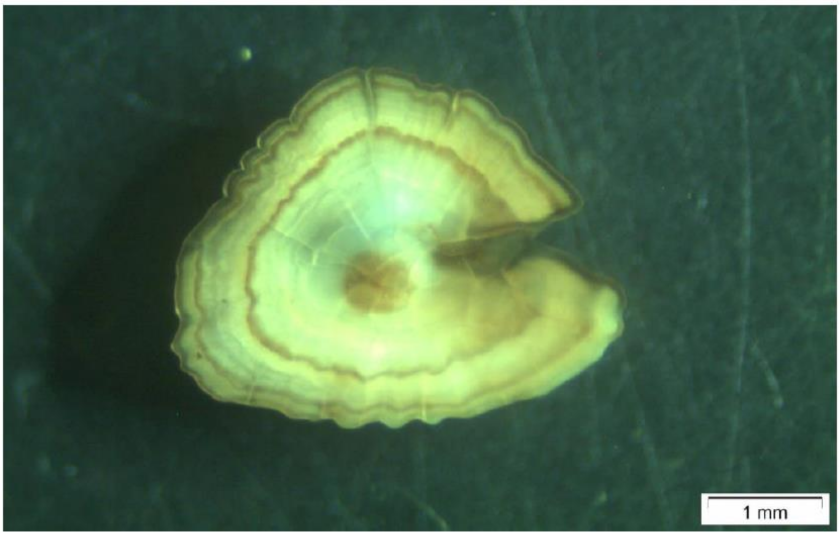

Figure 1-3. Photograph of a *Chelidonichthys lucerna* burned otolith with an opaque margin. **Source:** Rodrigues, 2020.

A few authors have reported that the species presents a faster growth rate during the first year of life, with a rapid drop thereafter (Işmen et al., 2004; İlhan & Toğulga, 2007; El-Serafy et al., 2015). Females seem to grow slower than males (Eryilmaz & Meriç, 2005; El-Serafy et al., 2015; McCarthy & Marriott, 2018). Differences regarding overall length and lifespan between males and females have also been found across different geographic locations, with females growing faster and living longer than males (Table 1-1). In addition, according to McCarthy & Marriott (2018), *C. lucerna* individuals in the Mediterranean region have smaller lengths and mature at smaller sizes, when compared to the Atlantic populations, probably due to differences in seawater temperature. The maximum length recorded for the species was 82.8 cm at the Saros Bay, Northern Aegean Sea (Hasimoğlu et al., 2016).

Table 1-1. Maximum lengths (Lmax.) and estimated *Chelidonichthys lucerna* ages in different locations.

Region	Sex	Lmax.	Estimated age	Reference
NW Wales, UK	F	57.5	7	McCarthy & Marriott, 2018
	M	41.0	5	
NE Atlantic, Portugal	F	46.2	8	Rodrigues, 2020
	M	30.3	4	
Gulf of Gabès	F	36.0	9	Boudaya et al., 2007
	M	26.0	7	
Bay of Iskenderun	F	30.3	4	Işmen et al., 2004
	M	24	3	

Egyptian Mediterranean	F	28.2	5	El-Serafy et al., 2015
	M	23.3	4	
Izmir Bay, Aegean Sea	F	34.4	5	İlhan & Toğulga, 2007
	M	29.9	3	
Sea of Marmara	F	41.5	6	Eryilmaz & Meriç, 2005
	M	36.5	4	

NW – northwest; NE – northeast; F – female; M – male.

C. lucerna is a demersal species with a pelagic phase during its early life period (Dulčić et al., 2001; Vallisneri et al., 2012). Apart from a MSc thesis on the Portuguese coastal waters (Rodrigues, 2020), since Baron (1985b) there has been no detailed study on *C. lucerna*'s reproduction and spawning periods in the northeast Atlantic region, with most of the research being focused on populations from the Mediterranean (including the Sea of Marmara) (Eryilmaz & Meriç, 2005; İlhan & Toğulga, 2007; El-Serafy et al., 2015). Although *C. lucerna* reproduction seems to occur throughout the year, studies have found greater incidences during certain periods in different regions, particularly during the winter months (Table 1-2). The shifts in the observed reproductive periods can be a reflection of different spatial abiotic variables across these geographic areas, since several authors have found a relationship between the seawater temperature and the fish spawning and hatching periods (Kashiwagi et al., 1987; Sheaves, 2006; Xia et al., 2021).

Table 1-2. Spawning periods of *Chelidonichthys lucerna* in different locations.

Location	**Spawning period**												**Reference**
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Ago**	**Set**	**Oct**	**Nov**	**Dec**	
Portugal	▨	▨	▨								▨	▨	Rodrigues, 2020
Duarnenez Bay (France)				▨	▨	▨	▨	▨	▨				Baron, 1985b
Gulf of Gabès (Tunisia)	▨	▨	▨	▨								▨	Boudaya et al., 2007
Bay of Iskenderun (Turkey)	▨	▨	▨	▨	▨							▨	Işmen et al., 2004
Mersin Bay (Turkey)		▨	▨	▨	▨								Cicek et al., 2008
Egyptian Mediterranean (Alexandria)	▨	▨									▨	▨	El-Serafy et al., 2015
Izmir Bay, Aegean Sea	▨	▨	▨									▨	İlhan & Toğulga, 2007
Sea of Marmara			▨	▨			▨	▨		▨	▨		Eryilmaz & Meriç, 2005

Similarly to other fish species, some authors have found that *C. lucerna* males attain their sexual maturity at younger ages and at smaller sizes compared to females (İlhan & Toğulga, 2007; Vallisneri et al., 2012; El-Serafy et al., 2015). This is attributed to the fact that body size is an important factor for females, as larger sizes at maturity probably imply less fitness costs as are often linked to higher fecundity, higher offspring fitness, and lower mortality due to lower predation risk following the larger body size (Boudaya et al., 2008; Pauly, 2019; Niu et al., 2023). Overall, studies (Eryilmaz & Meriç, 2005; İlhan & Toğulga, 2007; El-Serafy et al., 2015) in the Mediterranean region have found females predominate over males (Eryilmaz & Meriç, 2005; İlhan & Toğulga, 2007; El-Serafy et al., 2015), a situation that may attributed to the shorter lifespan of males, associated with their early onset of sexual maturity and migration elsewhere for spawning (Hashem, 1981). However, a few authors (Papaconstantinou, 1984; Işmen et al., 2004; Ahamed, 2012) have also found seasonal variations, with males dominating the females during some periods of the year, that may be associated with an early departure of the females from the spawning and nursery grounds to deeper waters (Eryilmaz & Meriç, 2005; İlhan & Toğulga, 2007; El-Serafy et al., 2015).

1.1.5. Fisheries

C. lucerna is either landed for human consumption or used for baiting traps used to harvest large crustaceans (ICES, 2010). The interest in the species has increased when ICES classified it, along with other gurnard species, as a potential species for commercial exploitation (ICES, 2006). In this context, to derive information on biological parameters for a better assessment of stocks, ICES has made recommendations to monitor landings and discards. However, to date, the data available about the stocks is currently limited and information on the species landings estimates is not accurate as gurnards are usually landed without species discrimination (Rocha et al., 2008; Feijó et al., 2008; Feijó & Rocha, 2018). For example, the various gurnard species are classified, in the official Portuguese statistics, only under the designation "Ruivo" (Instituto Nacional de Estatística, 2023) and information on each species' captures must be estimated based on model projections (Rocha et al., 2018). In this context, information on minimum landing sizes, allowed quotas, fishing closure seasons, or other fishery regulations needed to ensure a sustainable management of the species is still lacking.

Like most gurnards worldwide, in Portugal *C. lucerna* is not targeted by fishing fleets and is often caught as "by-catch" in trawler (bottom trawl and beam trawl) and artisanal fleets (Rocha et al., 2018). Given it reaches bigger sizes and has high commercial values, *C. lucerna* is considered the most important species of the Triglidae family captured by the Portuguese traditional fisheries (Feijó et al., 2008). According to the Portuguese fishing statistics, a total of 285 tonnes of "Ruivos" were landed in 2022 at an average annual price of 1.93 EUR, a five-ton increase in landings and 0.03 EUR increase in price compared to the previous year.

Worldwide, although with slight decreases between 2006-2010 and 2016-2018, *C. lucerna* landings have shown an increased trend since 2000, peaking in 2016 at 8023 tonnes (Figure 1-4), most likely a result of improved landings data rather than increased fishing activity (McCarthy & Marriott, 2018). During this period, the northeast Atlantic region has been responsible for providing the majority of fish (Figure 1-5). In 2021, the Netherlands (1894 t), France (972,27 t), Italy (584,52 t), and Belgium (638 t) landed 95% of the declared tub gurnard catch (FAO, 2023).

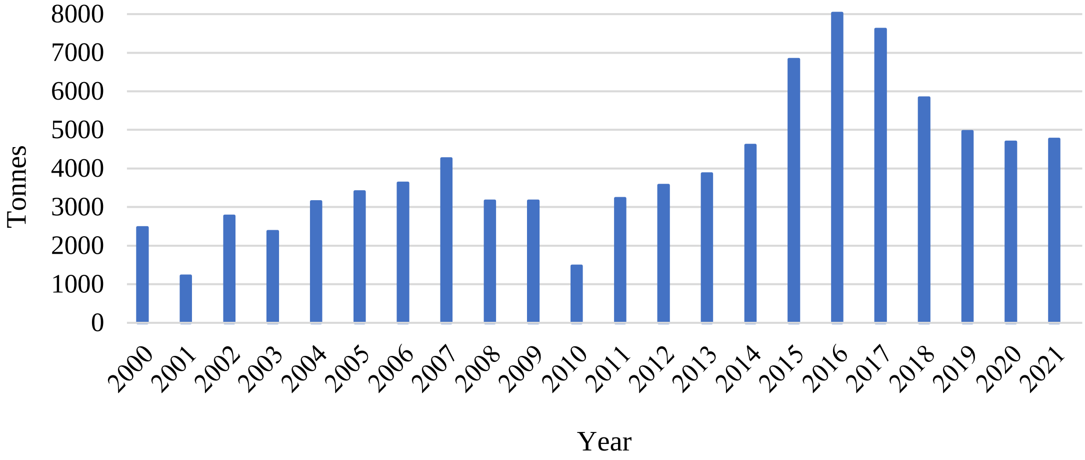

Figure 1-4. Global capture production for *Chelidonichthys lucerna* (tonnes).
Source: Adapted from FAO, 2023.

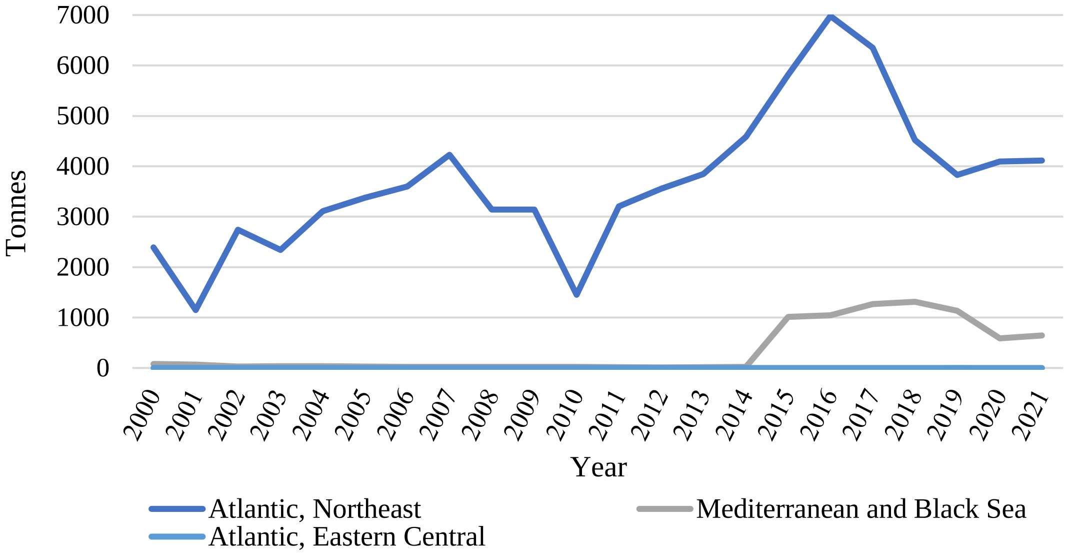

Figure 1-5. *Chelidonichthys lucerna* capture production by FAO region between 2000-2021 (tonnes).
Source: Adapted from FAO, 2023.

In addition, information on the species biology, population dynamics, movement patterns and habitat connectivity is scarce, but of utmost importance for a successful and sustainable management and conservation of the fishery resource.

1.2 Natural tags as tools to study the fish life cycles

1.1.6. Otolith shape and geochemical signatures

Teleost fish's inner ears contain three semicircular canals and three chambers. In each chamber, there is a pair of otoliths (sagittae – the largest pair, asterisci and lapilli), acellular structures that have an important role in fish water balance and hearing functions (Popper et al., 2005; Volpedo & Vaz-dos-Santos, 2015). Otoliths grow throughout fish's life by the successive addition of calcium carbonate, mainly in the mineral form of aragonite, and incorporating some chemical elements and isotopes present in the water surrounding the animal; otoliths do not suffer reabsorption, allowing a complete environmental record throughout the entire fish life history (Campana, 1999). These unique biogeochemical characteristics makes them excellent natural tags to study fish population structure, movement patterns and habitat use at different spatial and time scales (Correia et al., 2014; Daros et al., 2016; Moreira et al., 2018). Moreover, approaches based on features of otoliths such as chemical and shape signatures have been regarded as natural proxies to discriminate fish stocks (Ferreira et al., 2019; Soeth et al., 2020; Correia et al., 2021).

The study of otoliths morphological characteristics has proven successful in the assessment of fish population structure as the shape of the otoliths appears to be mainly influenced by environmental, rather than by genetic factors (Campana & Casselman, 1993; Ferguson et al., 2011; Rodriguez-Mendoza, 2006). Otolith shape is species-specific, less variable in growth than somatic growth, and while it can differ among stocks of a species, it can also differ among ages, sexes, and year classes within the same stock (Campana & Casselman, 1993; Begg et al., 2001; Cardinale et al., 2004). Otolith morphology has shown to be influenced by an interplay of environmental, ontogenic and genetic factors (Cardinale et al., 2004; Vignon & Morat, 2010; Biolé et al., 2019) and may vary according to fish ontogeny, geographic location, water depth, feeding regime, as well the water environmental proprieties (Campana & Neilson, 1985; Hüssy, 2008; Schwarzhans & Gerringer, 2023). Moreover, genetically driven changes can locally affect otolith shape (Berg et al. 2018).

Otolith shape analysis has been successfully applied in fish stock identification through different analytical processes such as Shape Indices (SIs), Elliptic Fourier descriptors (EFd) and, more recently, Discrete Wavelet descriptors (DWd) (Tuset et al., 2003; Soeth et al., 2019; Schroeder et al., 2022). While SIs are calculated based on otolith morphometric size parameters that represent the growth of otoliths such as length, width, area and perimeter (Tuset et al., 2003), Elliptic Fourier Analysis represents the outline of the otolith as a closed contour, capturing its unique shape in a coordinate system, decomposing it into a set of Fourier descriptors that capture its shape information in terms of harmonic functions, representing different scales and orientations of its shape (Kuhl & Giardina, 1982; Campana & Casselman, 1993; Volpedo & Vaz-dos-Santos, 2015). DWd on the other hand, operates approximating functions on finite domains, making it suitable for sharp edges being, therefore theoretically, more sensible to local differences along the otolith contour (Graps, 1995; Parisi-Baradad et al., 2005; Volpedo & Vaz-dos-Santos, 2015).

Otolith elemental and isotopic composition has also proved to be a useful technique in determining population structure and connectivity of fish stocks as it allows to create a life history profile of a fish, from their embryonic stages until they die, providing information about the environmental conditions and characteristics of the fish habitat and movement patterns throughout their whole life (Correia et al., 2012; Higgins et al., 2013; Moreira et al., 2018). Otolith elemental analysis allows, for instance, the identification of the natal origin of fish and the contribution of nurseries to the adult population (Correia et al., 2014; Silva et al., 2011; Vasconcelos et al., 2007), the assessment of fish migratory paths (D'Avignon & Rose, 2013; Daros et al., 2016; Soeth et al., 2020), and fish exposure to environmental contaminants (Ranaldi & Gagnon, 2010; Nimesh & Jain, 2018; Vrdoljak et al., 2020).

The factors that control elemental deposition in otoliths are only partially understood and although the mechanisms and pathways by which elements are deposited in otoliths are still poorly understood, a few studies have already been able to directly link the incorporation of some of these elements (e.g. Sr and Ba) with specific exogenous (e.g., salinity, temperature) (Moreira et al., 2018; Martinho et al., 2020; Soeth et al., 2020) and endogenous (e.g. metamorphosis, diet) factors (Busbridge et al., 2020; Doubleday et al., 2013). Similarly, to trace and minor elements, stable isotopes have also successfully been used as natural tags for fish population structure studies (Campana, 1999; Correia et al., 2021; Reis-Santos et al., 2015). Oxygen and carbon stable isotope composition (δ^{18}O and δ^{13}C), for instance, can provide valuable information on the environmental conditions experienced by fish, being successfully used in fish stock assessment studies (Campana, 1999; Silva et al., 2011; Ferreira et al., 2019). The oxygen isotopic composition has been found to be deposited in fish otoliths in equilibrium with surrounding water and highly influenced by salinity and temperature (Kerr et al., 2007; Correia et al., 2021; von Leesen et al., 2021). However, the incorporation of δ^{13}C into the otoliths' aragonite seems to be mainly influenced by endogenous (i.e., fish diet, growth and metabolism (Tohse & Mugiya, 2007; Correia, Barros, et al., 2011; J. Nelson et al., 2011) and exogenous (i.e., the dissolved inorganic carbon in the water) (Thorrold et al., 1997; Solomon et al., 2011; Daros et al., 2016) sources. Additionally, and although not suited for marine species, since seawater shows a uniform stable Sr signal, stable strontium isotopes (δ^{87}Sr/δ^{86}Sr) have been identified as valuable otolith markers in freshwater fishes (Campana, 2005; Avigliano et al., 2020; Zitek et al., 2023).

One of the most used analytical techniques to determine otolith elemental fingerprints in fishes is the inductively coupled plasma mass spectrometry (ICP-MS) and there are currently two main analytical methods when applying ICP-MS to otoliths: (i) solution-based (SB-ICP-MS) that aims at analyzing the chemical content of the whole otoliths (i.e. all the minor and trace elements incorporated in the otoliths from birth to death, or capture) and (ii) laser ablation (LA-ICP-MS) that allows to analyze a specific loci of the otolith (e.g. growth bands, core or the otolith edge), that represents a particular stage of the fish life, instead of its entire life (Fowler et al., 1995; Correia et al., 2011; Hoover & Jones, 2013). Stable isotopes are usually measured by isotope ratio mass spectrometry (IR-MS) which often involves a milling process of the whole otolith to obtain a relatively large amount of otolith powder for analysis (Shiao et al., 2014; Moreira et al., 2018; Hane et al., 2020). Isotopic signatures measured through IR-MS are determined based on the quotient between the heavy (e.g., δ^{18}O) and the light (e.g., δ^{16}O) isotope (Fischer et al., 2016). ICP-MS has been also used to measure natural isotopes of the elements, and modern equipment such as the multicollector-inductively coupled plasma mass spectrometer (MC-ICP-MS) allows the determination of isotopic ratios of heavier elements such as $\delta^{87}Sr/\delta^{86}Sr$ (Hauser et al., 2019; Avigliano et al., 2020; Hermann et al., 2021).

1.1.7. Body morphometry

Fish morphological studies have been widely used to describe fish spatial distributions as fish body morphological differences (e.g., length, width and depth) can be associated with genetic (Robinson & Wilson, 1996; Turan et al., 2006; Crispo, 2008) or with processes of phenotypic plasticity as a response to different environmental conditions (Hammami & Bahri-Sfar, 2013; Hoff et al., 2020; Moreira, Presa,et al., 2020). Factors such as temperature, salinity and food availability, can lead to different behaviors (e.g., isolation, swimming) and adaptation strategies, which could be reflected on fish morphometric features and contribute to the definition of different phenotypic stocks (O'Dea et al., 2019; Pulkkinen et al., 2022; Quadroni et al., 2023).

There are two main categories of morphometric analysis: outline methods that deal with perimeter shapes and landmark methods that analyze data derived from discrete morphometric points, either being linear distances between two points, or geometric relationships among several points (Stransky, 2014). The process of identifying fish stocks based on body shape has shifted from traditional morphometrics, that merely measures basic linear dimensions, to calculating more complex geometric functions parameters, which was facilitated by the recent development of image processing and computing tools (Begg et al., 1999; Cadrin & Friedland, 1999; Cadrin et al., 2014).

One of the landmark-based approaches that has been increasingly employed for fish stock discrimination purposes is the Truss network system, a geometric morphometrics method that represents the complete shape of the fish by connecting measurements between morphometric landmarks, forming a polygon network across the animal's body (Figure 1-6), and imposes no restrictions on the direction of variation or localization of shape changes (Cadrin & Friedland, 2005; Mojekwu & Anumudu, 2015; Mallik et al., 2020). Truss network measurements are then a series of distances calculated between landmarks and have shown effective in capturing information on an individual's body shape and detecting variations among fish populations (Kumar & Pandey, 2017; Hoff et al., 2020; Moreira, Froufe,et al., 2020) as they can systematically be used to detect shape differences in oblique, horizontal, and vertical directions (Strauss & Bookstein, 1982).

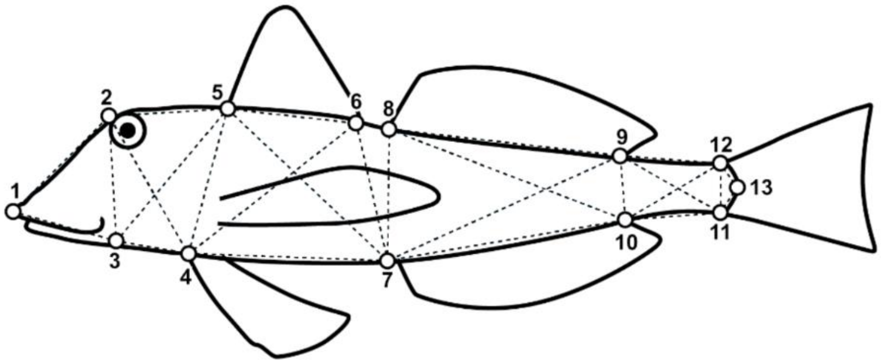

Figure 1-6. Truss network used for morphometric analysis (personal diagram).

1.1.8. Other methods

Molecular genetic techniques have proven to be reliable and effective tools in the identification of geographic ranges of species and populations, supporting the definition of fish stocks management units and the evaluation of conservation priorities (Carvalho & Pitcher, 1995; Begg et al., 1999; Antoniou & Magoulas, 2014). These techniques employ inherited, discrete, and stable genetic markers, which are fragments of DNA and/or RNA, to identify genotypes that characterize individuals, populations or species. These markers are considered ideal stock discriminators as they remain unaffected by environmental changes over a fish's lifetime and are composed of discrete units of information making it easy to quantify population differences (Antoniou & Magoulas, 2014). For fisheries purposes, mitochondrial (mtDNA), nuclear genetic markers and SNPs are the most commonly used (Antoniou & Magoulas, 2014; Moreira, Presa, et al., 2020; Franco et al., 2023).

Parasites fauna have also been widely used as indicators of various aspects of fish biology with the first publication reporting the use of a naturally occurring parasite as a biological tag in a marine fish population study dating back to 1939 (Herrington et al., 1939). Many different taxonomic groups of parasites have been used as tags for commercial marine species (e.g., protozoans, myxosporidians, larval and adult helminths and parasitic crustaceans), but larval nematodes are the most common and widespread parasites in teleost fish (Mosquera & M. Gómez-Gesteira, 2003). When fish are within the endemic area of a certain parasite and all the transmission conditions (e.g., temperature, salinity) are suitable, they can become infected. When this parasite is detected in fish found outside this endemic area, we can infer the fish, at some point in its life, passed within that area. Additional information on the parasite ecology and biology such as the maximum lifespan within the host is valuable to assess and estimate the time span since the fish left the endemic area (Sindermann, 1983; Mosquera & M. Gómez-Gesteira, 2003). In this context, an analysis of geographical variation in the distribution and abundance of parasites can provide an excellent source of information on fish movement.

Despite the existence of several natural tags that have been successfully applied in stock discrimination purposes, a holistic approach involving a broad spectrum of complementary techniques may maximize the likelihood of correctly delineating stocks (Begg & Waldman, 1999; Cadrin et al., 2014).

1.3 Objectives

The main goal of this work was to provide additional research on the northeast Atlantic population of *Chelidonichthys lucerna* given the increasing interest in the species for commercial exploitation. To do so, indispensable information about the population structure and dynamics, stock(s) identification and delineation, habitat connectivity and migration patterns of the species in Portugal mainland and across the northeast Atlantic was gathered.

Specifically, the objective was to answer the following questions:

i. Is the Portugal mainland population a single population-unit?
ii. Is there habitat connectivity among different fishing grounds along the Portuguese coast?
iii. What are *C. lucerna* habitat residency patterns?
iv. Can the species be considered marine estuarine-dependent?

v. Do populations along the northeast Atlantic belong to a single and homogeneous population-unit?
vi. What are the implications of this work for the species stock assessment and fisheries management?

1.4 Thesis Organization

The present and first chapter of this thesis (Chapter 1: General Introduction) is focused on providing context and background information on the species and on the different available methods (natural tags) for fish stock assessment. It also intends to provide an overview of the main objectives of the work, and the thesis structure.

The main body of this thesis (Chapters 2, 3 and 4) are results already published in peer-reviewed international journals and are described below.

Chapter 2: “Population structure of *Chelidonichthys lucerna* in Portugal mainland using otolith shape and elemental signatures”. This Chapter focuses on analyzing the population structure of the species along the Portuguese coast, by analyzing the shape and elemental composition of otoliths of 90 *C. lucerna* individuals collected from three different fishing grounds (north, center and south Portugal). Otolith shape was analyzed using shape indices and elliptic Fourier descriptors and elemental signatures (Ba:Ca, Fe:Ca, Mg:Ca, Mn:Ca, Sr:Ca and Zn:Ca) were determined by solution-based inductively-coupled plasma mass spectrometry (SB-ICP-MS).

Chapter 3: “Is *Chelidonichthys lucerna* a marine estuarine-dependent fish? Insights from otolith microchemistry”. This Chapter focuses on analysing *C. lucerna* movement patterns through different salinity environments in northern Portugal using otolith microchemistry laser-ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Specifically, Sr:Ca and Ba:Ca ratios from core-to-edge transects of thirty-five individuals were used as a water salinity tracer.

Chapter 4: “*Chelidonichthys lucerna* population structure in the northeast Atlantic from landmark-based body morphometry”. This Chapter looks into discriminating *C. lucerna* populations caught in three different fishery grounds areas along the northeast Atlantic (United Kingdom, Spain and Portugal) through body geometric morphometrics using landmark-based truss network on 129 individuals.

Finally, Chapter 5 (Final Discussion and Conclusions) is the final chapter of this thesis and integrates and provides a summary discussion and conclusion of the major findings of the work and further suggestions for rational management and sustainable conservation of the species.

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CHAPTER II. Population structure of *Chelidonichthys lucerna* in Portugal mainland using otolith shape and elemental signatures

Population structure of *Chelidonichthys lucerna* in Portugal mainland using otolith shape and elemental signatures

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Abstract

The tub gurnard, *Chelidonichthys lucerna*, is considered the most important commercial fish species of the family Triglidae captured by the Portuguese traditional fisheries. However, the data available about its population structure, movement patterns and habitat connectivity is, at present, scarce. In this study, otolith chemistry and shape analyses of 90 individuals captured between March and June 2016, in the three main fishing grounds of the Portuguese mainland coast (Matosinhos, Aveiro and Peniche) were investigated. Otolith morphological (shape indices and elliptic Fourier descriptors) and elemental (Ba:Ca, Fe:Ca, Mg:Ca, Mn:Ca, Sr:Ca, and Zn:Ca) signatures of individuals with similar length range were assessed through uni and multivariate statistical tests. The overall combination of otolith elemental chemistry and morphology techniques revealed the highest re-classification success (74%) of samples to their original location and the existence of significant differences among sampling regions. However, linear discrimination function analyses and canonical analyses of principal coordinates did not fully discriminate *C. lucerna* individuals from the three sampling regions of Portugal. Moreover, the hereby data revealed a partial overlap among individuals from the different sampling regions. The obtained shape and chemical signatures suggest that *C. lucerna* is apparently a unique, although not necessarily homogenous, single population-unit in Portugal mainland, and that these fish populations should not be managed separately for fisheries purposes.

Keywords: Triglidae, Sagitta, Natural tags, Population structure, Fisheries Management.

2.1.Introduction

Gurnards are fishes belonging to the Triglidae family (order: Scorpaeniformes) with over 100 species worldwide (Nelson, 2006), from which eight can be found in the eastern Atlantic (Olim & Borges, 2006). *Chelidonichthys lucerna* (Linnaeus, 1758) commonly known as tub gurnard, is a Mediterranean-Atlantic species, distributed along the eastern Atlantic coast, from Norway to the northwest coast of Africa, being also found around the British Isles, in the Mediterranean and Black Sea (FAO Fisheries and Aquaculture Department, 2017). It is a benthic species that usually inhabits sand, muddy sand or gravel bottoms of the continental shelf (from about 20 to 300 m depth) at a water temperature ranging from 8.0°C to 24.0°C; it feeds on fish, crustaceans and molluscs (FAO Fisheries and Aquaculture Department, 2017; Fischer W. et al., 1981; Richards & Saksena, 1990). It is a marine estuarine dependent fish and considered the most important species of the family Triglidae captured by the Portuguese traditional fisheries (trawl and artisanal gears), as it reaches bigger sizes in Portuguese waters and has higher commercial values (Rocha et al., 2008). The various gurnard species are classified, in the official Portuguese statistics, only under the designation “Ruivos” (Instituto Nacional de Estatística, 2017) and information on each species’ captures has to be estimated based on model projections (Rocha et al., 2018b). In 2016, 364 ton of “Ruivos” were captured in Portuguese waters (Instituto Nacional de Estatística, 2017). Despite the abundance of gurnards, the data available about the stocks (Olim & Borges, 2006) and in Portugal (Rocha et al., 2008) is currently limited. Therefore, proper identification of this species population dynamics, stock structure and habitat connectivity is of utmost importance for a successful and sustainable management and conservation of this fishery resource (Rocha et al., 2008).

Otoliths are calcium carbonate structures that are located within the fish’s inner ears and have an important role in the balance and hearing functions of the fish (Popper et al., 2005). Otoliths grow throughout the life of the fish by the addition of calcium carbonate, are constantly up-taking chemical elements from the water that surrounds the fish, and do not suffer reabsorption, allowing for a complete environmental record throughout the entire fish life history (Campana, 1999). These unique biogeochemical characteristics makes them excellent natural tags to study the population structure, movement patterns and habitat use by fish at different spatial and time scales (Correia et al., 2014; Daros et al., 2016; Moreira et al., 2018). The chemical otolith analysis allows to create a life history profile of a fish, from their embryonic stages until they die, that gives us information about the environmental conditions and characteristics of the fish habitat and movement patterns throughout their whole life (Campana & Thorrold, 2001; Silva et al., 2011; Volpedo & Vaz-dos-Santos, 2015). This could allow us, for instance, to identify the natal origin of fish and the contribution of nurseries to the adult population (Vasconcelos et al., 2008; Silva et al., 2011; Correia et al., 2014) as well as the migratory paths (Campana et al., 2007; Secor & Piccoli, 2007; Silva et al., 2011). Although the mechanism of incorporation of the elements into the otolith is still unknown, their chemical composition is affected by endogenous (e.g., metabolic rate, feeding and growth rate) and exogenous (e.g., water salinity and temperature) factors (Elsdon & Gillanders, 2002; Sturrock et al., 2012; Volpedo & Vaz-dos-Santos, 2015). Additionally, otolith shape is species-specific, shows inter-regional variation and appears to be an ideal marker for fish population studies (Campana & Casselman, 1993; A. R. Vieira et al., 2014; Jemaa et al., 2015). This method has been successfully applied in fish stock identification through the use of shape indices (SI) and elliptic Fourier descriptors (EFD) (Mérigot et al., 2007; Stransky et al., 2008; Agüera & Brophy, 2011). The shape of the otoliths appears to be mainly influenced by environmental factors, meaning this is also a useful method to use for fish population structure assessment (Campana & Casselman, 1993; Rodriguez-Mendoza, 2006; Ferguson et al., 2011). Furthermore, the combination of chemical and shape analyses of otoliths proved to be a good complementary tool to discriminate fish stocks (Longmore et al., 2010; Ferguson et al., 2011; Soeth et al., 2019).

The main goal of the present study was to evaluate the use of the elemental composition and shape analyses of the otoliths from *C. lucerna* individuals captured from three different regions along the Portuguese coast as a tool to: (i) determine if the otolith's shape and elemental signatures are site-specific and therefore can be used to infer about the degree of separation between stocks; (ii) infer about the spatial fish population structure; and (iii) assess the habitat connectivity and intermixing between these NW Atlantic Portuguese fishing grounds.

2.2.Materials and Methods

2.2.1. Sample Collection

Chelidonichthys lucerna individuals were acquired directly from fishermen and caught by the artisanal fleets in three major fisheries regions in the NW Portuguese coast (Matosinhos 41°10'N, 08°41'W, Aveiro 40°38'N, 08°38'W and Peniche 39°21′N, 09°23’W) between March and June 2016 (Figure 2-1). After collection, fish were transported on ice to the laboratory and all individuals were measured (Total Length, TL, 0.1 cm) and weighted (W, g). A total of 90 adults (30 per location) with similar TL (range between 27 cm and 30 cm) (One-Way ANOVA: F2,87=0.008, p>0.05), as a proxy of age, were selected to meet the microchemistry and shape otolith analyses criteria (Adelir-Alves et al., 2018; Moreira et al., 2018, 2019). Sagittal otoliths were carefully extracted from the fish head using plastic forceps to avoid metallic contamination, cleaned with ultrapure water (Milli-Q Water), weighted (0.0001 g) and stored in dry acid-washed plastic vials (Eppendorf’s) for further analysis.

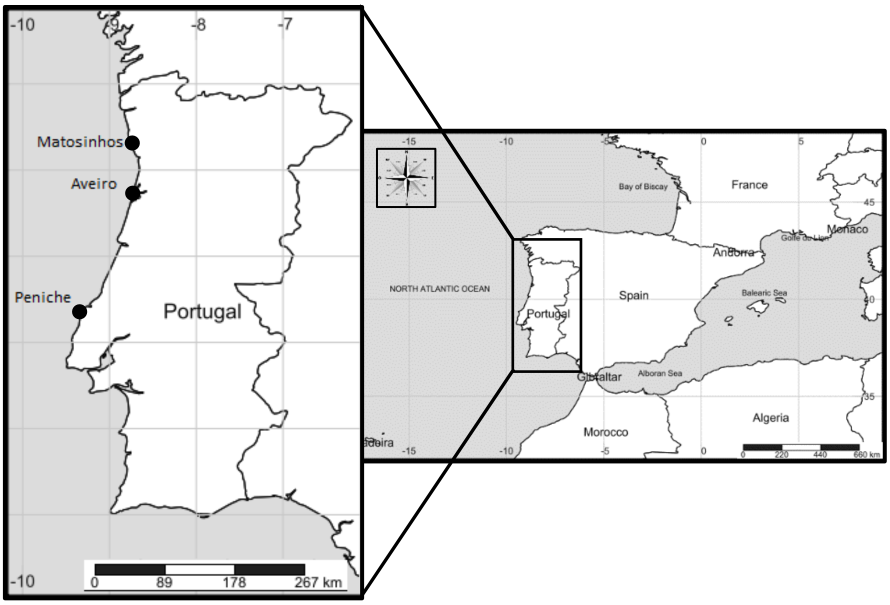

Figure 2-1. Maps representing Portugal’s geographic location with the indication of the three major sampling regions located in the Portuguese western coast (Matosinhos, Aveiro and Peniche) where *C. lucerna* individuals were caught by the artisanal fisheries in 2016.

2.2.2. Otolith Shape Analysis

i. Image Acquisition

Left otoliths were placed convex side up, showing the *sulcus acusticus*, and with the *rostrum* pointing to the left (Figure 2-2A). Orthogonal two-dimensional digital images of otoliths were recorded using a high-resolution USB camera (Olympus, SC 30) mounted on a binocular stereomicroscope (Meiji, EMZ-13TRX) at 15X magnification under reflected light and dark field. Final images were obtained using a computer program (Olympus, AnalySIS getIT SC30).

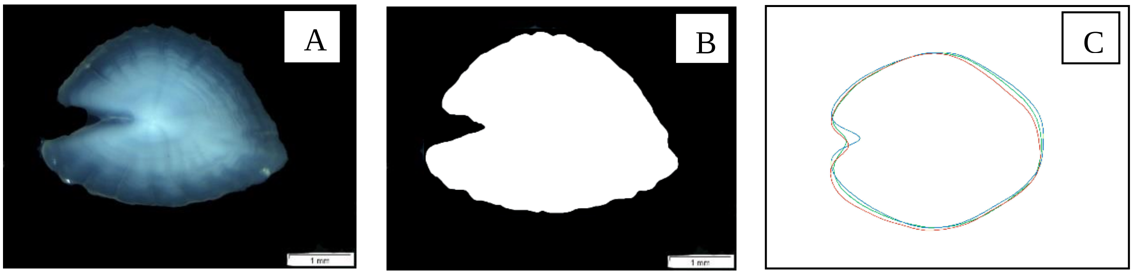

Figure 2-2. *Chelidonichthys lucerna* inner face of the left sagitta showing the original photograph (A), the binary image (B) and the averaged outline contour for each location (C). Regions: Matosinhos (green), Aveiro (red) and Peniche (blue).

ii. Shape Indices

Morphometric measurements were made from each binary B&W otolith image (Figure 2-2B) using the program ImageJ 1.51j8 (Rasband, 2009). Measurements of perimeter (P), area (A), width (W), and length (L) were used to calculate five otolith shape indices (SI): form factor (FF), roundness (RO), circularity (CI), rectangularity (RE) and ellipticity (EL) (Tuset et al., 2003) (Table 2-1).

Table 2-1. Otolith shape indices used in this study.

Otolith Shape Indices	**Formulae**
Form factor (FF)	$(4\pi A)/P^2$
Roundness (RO)	$(4A)/(\pi L^2)$

Circularity (CI)	P^2/A
Rectangularity (RE)	$A/(L \times W)$
Ellipticity (EL)	$(L-W)/(L+W)$

Note: Variables: area (A, mm^2), perimeter (P, mm), length (L, mm), and width (W, mm) of otoliths (from Tuset et al., 2003).

Because otolith shape can change throughout ontogenetic development of fish, biasing comparisons among populations with different age-length structures (Campana & Casselman, 1993), the effect of fish size (i.e., otolith length) on the magnitude of each morphometric index was tested using an ANCOVA (factor: location; co-variate: otolith length). For SI significantly correlated with otolith length (RO, RE and EL), the original values (V_o) were corrected (V_c) by removing the common between group slope (b) using the following formula: $V_c = V_o - b \times L$ (Campana et al., 2000).

iii. Elliptic Fourier Descriptors

Elliptical Fourier Descriptors (EFD) for each otolith were estimated using the program SHAPE V. 1.3 (Iwata & Ukai, 2002). The Fourier power (FP) spectrum was used to determine the number of Fourier descriptors required to adequately describe the otolith outline (Figure 2-2C) (Crampton, 1995; Pothin et al., 2006; Ferguson et al., 2011). The first three harmonics reached >95% of the cumulative power indicating that the otolith shape could be adequately summarised by 12 (i.e., 3 harmonics x 4 coefficients) EFDs. The elliptical Fourier harmonics for each otolith were normalized to the first harmonic in the SHAPE program and were thus invariant to otolith size (Kuhl & Giardina, 1982; Pothin et al., 2006; Mérigot et al., 2007). As a consequence of the normalization, the first three coefficients (a1, b1, and c1) were constant for all outlines, reducing the number of EFDs to 9 (i.e., 9=12-3) (Adelir-Alves et al., 2018).

2.2.3. Otolith Elemental Analysis

Multi-elemental (ME) composition of whole left otoliths (i.e., elemental signatures recorded from the fish birth until capture) was determined using solution-based inductively coupled plasma mass spectrometry (SB-ICP-MS). Prior to the analyses, otoliths were cleaned in an ultrasonic bath for 5 min in ultrapure water (Milli-Q-Water) followed by a 15 min immersion in 3% hydrogen peroxide (H_2O_2, Fluka TraceSelect) to remove any existing adherent biological tissues. Otoliths were decontaminated by immersion in 1% nitric acid (HNO_3, Fluka TraceSelect) for 10 s followed by a double-immersion in ultrapure water (Milli-Q-Water) for 5 min to remove the acid (Rooker et al., 2001). Otoliths were afterwards stored in new, previously decontaminated, plastic vials (Falcon TM tubes), where they were allowed to air dry in a laminar-flow fume hood (Patterson et al., 1999). Decontaminated otoliths were weighed (0.0001 g) and dissolved for 15 min in 10% ultrapure HNO_3 (HNO_3, Fluka TraceSelect) to a final volume of 10 mL (Silva et al., 2011).

Otolith SB-ICP analyses were conducted using a sequential ICP-OES (Activa M, Horiba Jobin Yvon) and a quadrupole ICP-MS (Thermo ICP-MS x series, Thermo Electron Corporation), for major (Ca and Sr) and minor (others) elements, respectively. Both instruments were equipped with a peristaltic pump and a Burgener Mira Mist nebulizer (sample uptake rate 1 l min^{-1}). Ni signal was corrected for the CaO interference. Quantification of trace elements was based on the external calibration method, preparing multi-element standards that contained the elements of interest in the expected concentration range (Merck KGaA). To minimize the effect of any plasma fluctuations or different nebulizer aspiration rates between the samples, ^{115}In of a known concentration was added to all samples and standards as an additional internal standard to adjust for instrument drift (except for ICP-OES). Concentrations were calculated by linear interpolation (sum of least squares) based on normalization with the internal standard, and on calibration curves made from single element standards covering the individual expected concentration ranges. The entire set of calibration standards was made at the beginning of each session. The matrix of both the blank and the standard solutions was 1% HNO_3 (HNO_3, Fluka TraceSelect). Otolith samples were analysed in random order to avoid possible sequence effects.

A preliminary analysis was made to determine the most abundant and likely informative elements present in whole otoliths of *C. lucerna*, taking into consideration the trace elements usually recorded in marine fish otoliths (e.g., Correia et al., 2014; Daros et al., 2016; Silva et al., 2011), but excluding elements under strictly physiological regulation (Campana 2005). Seven elements (^{137}Ba, ^{44}Ca, ^{54}Fe, ^{26}Mg, ^{55}Mn, ^{88}Sr and ^{66}Zn) were detectable and displayed variation in the whole otoliths and were used for further SB-ICP. Three additional trace elements were also analysed (^{63}Cu, ^{7}Li and ^{208}Pb) but their concentrations were consistently below the limit of detection (LOD). For quality control, accuracy was assessed by measurements of one fish otolith reference material: FEBS-01 (National Research Council Canada, Institute for National Measurement Standards, Ottawa, Ontario, Canada). Recovery rate varied between 95% and 110%. The precision of replicate analyses of both standard materials ranged between 2.0% and 5.0% relative standard deviation (RSD). LODs were calculated from the individual calibration curves using the three sigma criteria and were (in µg l^{-1}): ^{137}Ba (0.3), ^{44}Ca (10 000), ^{63}Cu (3), ^{54}Fe (5), ^{7}Li (6), ^{26}Mg (12), ^{55}Mn (0.3), ^{208}Pb (0.3), ^{86}Sr (300) and ^{66}Zn (0.3). Concentrations of trace elements, originally in µg element l^{-1} solution were transformed to µg element g^{-1} otolith, and then converted to µg element g^{-1} calcium (element:Ca) for further statistical analyses (Higgins et al., 2013).

Although there were no significant differences in the TL among regions, relationships between elemental concentration and fish size (otolith weight) were tested with analysis of covariance (ANCOVA, otolith weight as co-variate). All element:Ca ratios varied significantly with otolith mass: Ba and Sr recorded a positive relationship, while Fe, Mg, Mn and Zn showed a negative relationship (ANCOVAs, $p<0.05$). 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).

2.2.4. Statistical analyses

Raw data was checked for normality (Shapiro-Wilk’s test), variance homogeneity (Brown-Forsythe’s test) and equal within-group covariance matrices (inspection of discriminant function scores) according to Quinn & Keough, 2002. These assumptions were met after $\log_{10}$ transformation (e.g. Mg:Ca, Sr:Ca and Zn:Ca). One-way analysis of variance (ANOVA) was used to explore individual elemental fingerprints, SI and EFD differences between regions. If significant differences were found ($p<0.05$), this was followed by a Tukey *post hoc* test. Multivariate analysis of variance (MANOVA) was used to test for spatial differences in otolith multi-elemental (element:Ca) and shape signatures (SI, EFD) and both combined (element:Ca, SI and EFD). For the MANOVA, we reported the approximate F-ratio statistic for the most robust test of multivariate statistics (Pillai's trace). Post-hoc multivariate pairwise comparisons between regions were performed using the Hotelling's T-square test. A Linear Discriminant Function Analysis (LDFA), in complete mode, was also used to visualize spatial differences and to examine the re-classification accuracy success of fish to their original location using a jackknifed (''leave one out'') procedure. A correlation matrix for the elemental and shape data sets was analysed by a Canonical Analysis of Principle Components (CAP) based on Euclidian distances (Spearman correlation of 55%) and the results are presented in a two-dimensional biplot (Moreira et al., 2018). All these statistical analyses were performed using Systat (version 13.0) and PRIMER 6 + PERMANOVA softwares. The statistical level of significance (α) was 0.05. Data are presented as mean values ± standard errors.

2.3.Results

2.3.1. Otolith Shape Analysis

Univariate tests showed no regional statistical differences for a few SI (e.g., RO, EL) and EFD (e.g., D1, B2, C2, B3 and C3) (One-Way ANOVAs: $p>0.05$) (Table 2-2). There was spatial discrimination among regions using multi-shape indices (SI) (MANOVA, Pillai's Trace $F_{10,168}=2.421$, $p<0.05$), with Peniche being different from the other regions (Hotelling's T-square tests $p<0,05$). The circularity was the main variable responsible for this difference (Tukey test, $p<0.05$) (Table 2-2).

Table 2-2. Otolith shape signatures (detrended values for RO, RE and EL) differences among the three sampling regions along the western coast of Portugal for *Chelidonichthys lucerna* individuals sampled in 2016.

	Location		
	Matosinhos	**Aveiro**	**Peniche**
SI			

FF	A	AB	B
RO	A	A	A
CI	A	A	B
RE	A	AB	B
EL	A	A	A
EFD			
D1	A	A	A
A2	A	AB	B
B2	A	A	A
C2	A	A	A
D2	A	A	B
A3	A	AB	B
B3	A	A	A
C3	A	A	A
D3	A	AB	B

Note: SI and EFD mean shape indices and elliptical Fourier descriptors, respectively. Different letters indicate statistically different results (Tukey tests, $p<0.05$).

Regarding the EFD, there were, however, no significant multi-variate differences among regions (MANOVA, Pillai's Trace $F_{18,160}$ =1.642, p=0.056). Overall, when SIs and EFDs were combined, LDFA based on morphometric variables provided a low re-allocation success of individuals to the original regions (an overall re-classification of 51%) (Table 2-3) and the respective plots did not clearly discriminate individuals from the three sampling regions with Aveiro being the least different as it highly overlapped the other two (Figure 2-3A). On the other hand, the CAP was not able to identify individual groups (Figure 2-4A).

Table 2-3. Jackknifed cross validation re-classification matrix following a linear discriminant function analysis based on otolith multi-trace elements (ME), otolith shape signatures (SI and EFD) and all otolith natural tags (ME, SI and EFD) of *C. lucerna* individuals sampled in 2016 from the three regions (Aveiro, Matosinhos and Peniche) along the Portuguese western coast.

SI and EFD	**Predicted Location**			
Original location	**Matosinhos**	**Aveiro**	**Peniche**	**% Correct**
Matosinhos	15	10	5	50

Aveiro	8	15	7	50
Peniche	6	8	16	53
Total	29	33	28	51

ME	**Predicted Location**			
Original location	**Matosinhos**	**Aveiro**	**Peniche**	**% Correct**
Matosinhos	22	4	4	73
Aveiro	8	22	0	73
Peniche	8	0	22	73
Total	38	26	26	73

ME, SI and EFD	**Predicted Location**			
Original location	**Matosinhos**	**Aveiro**	**Peniche**	**% Correct**
Matosinhos	20	7	3	67
Aveiro	8	22	0	73
Peniche	5	0	25	83
Total	33	29	28	74

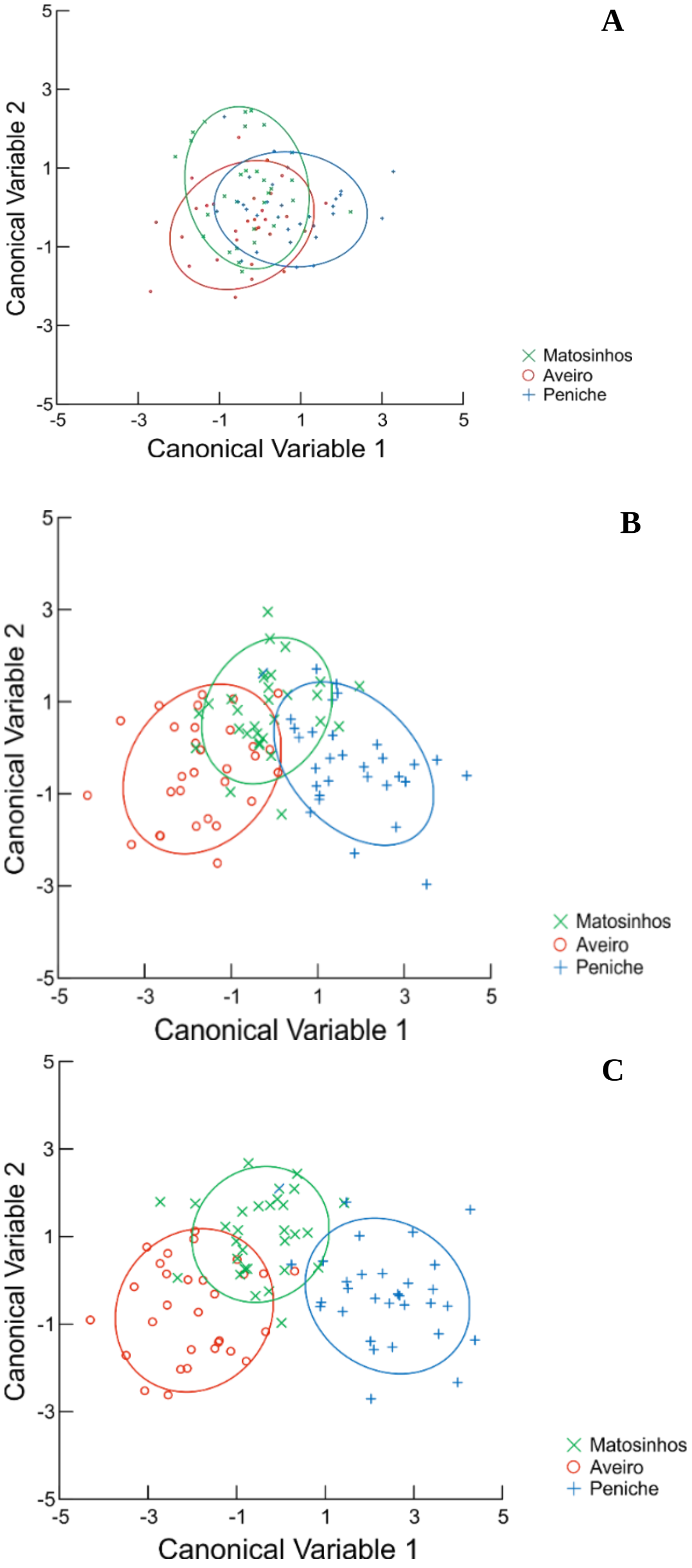

Figure 2-3. Linear discriminant function analyses (LDFA) plots displaying spatial differences for (A) otolith shape signatures (shape indices and elliptic Fourier coefficients), (B) multi-trace elements in whole otoliths and (C) all natural tags combined from *Chelidonichthys lucerna* individuals collected in the three coastal regions of the Portuguese coast in 2016. Ellipses represent 95% confidence intervals around the data, and each data points represent individual fish. Regions: Matosinhos (green x), Aveiro (red o) and Peniche (blue +).

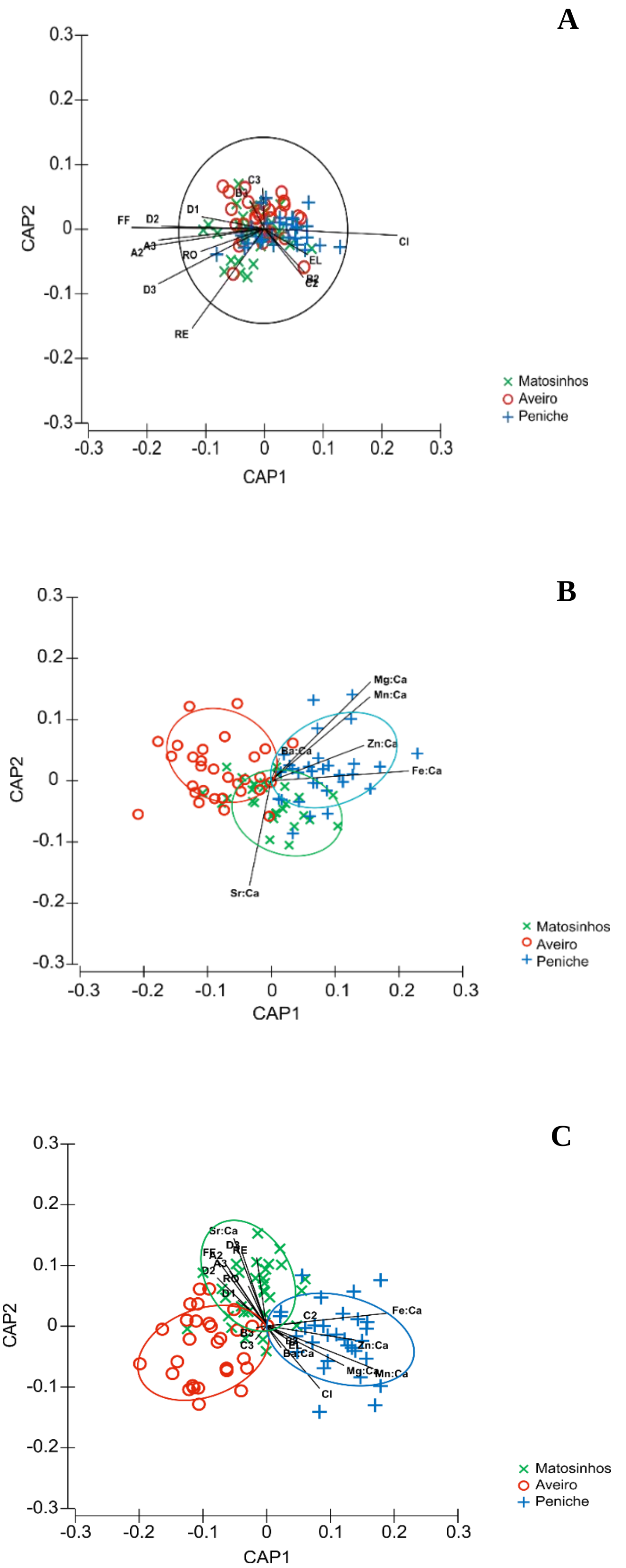

Figure 2-4. Canonical analysis of principal coordinates (CAP) plots for (A) otolith shape signatures (shape indices and elliptic Fourier coefficients), (B) multi-trace elements in whole otoliths and (C) all natural tags combined from *Chelidonichthys lucerna* individuals collected in the three coastal regions of the Portuguese coast in 2016. Regions: Matosinhos (green x), Aveiro (red o) and Peniche (blue +).

2.3.2. Otolith Elemental Analysis

Sr:Ca content differed among regions (One-Way ANOVA: $F_{2,87}$=7.930, $p<0.05$), being highest in otoliths from Matosinhos comparatively to the other regions (Tukey test, $p<0.05$) (Figure 2-5A). Ba:Ca, however, showed similar concentrations among all regions (One-Way ANOVA: $F_{2,87}$=0.575, $p>0.05$) (Figure 2-5B). Mg:Ca, Mn:Ca, Zn:Ca, and Fe:Ca differed among regions (Mg:Ca: One-Way ANOVA, $F_{2,87}$=12.014, $p<0.05$; Mn:Ca: One-Way ANOVA, $F_{2,87}$=26.689, Zn:Ca: One-Way ANOVA, $F_{2,87}$=14.661, $p<0.05$; Fe:Ca: One-Way ANOVA, $F_{2,87}$=238.368, $p<0.05$ (Figure 2-5C-F), being the highest ratios recorded in the otoliths from Peniche region (Tukey tests, $p<0.05$).

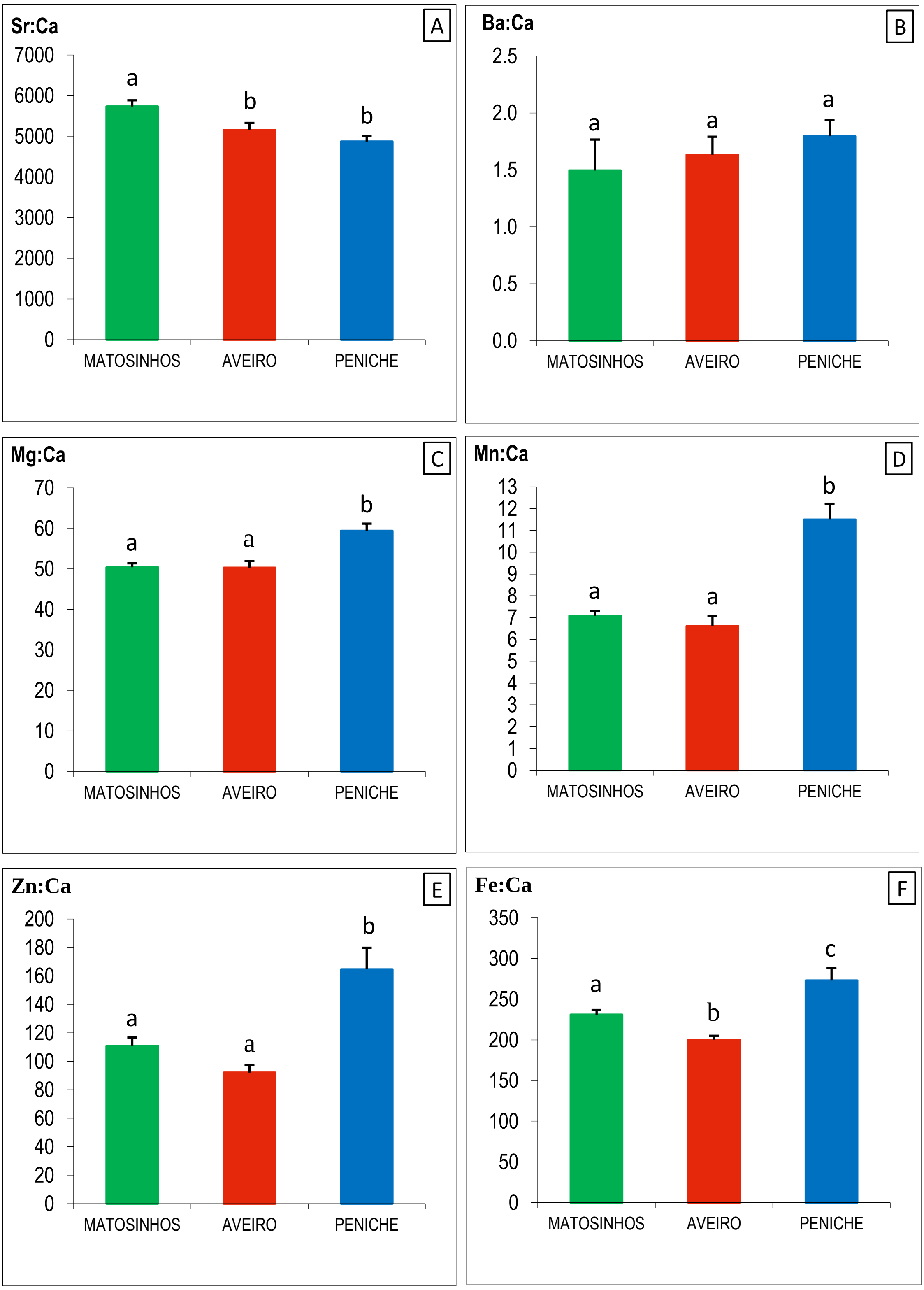

Figure 2-5. Elemental concentrations (detrended concentrations: mean ± SE) recorded in the whole otoliths of *Chelidonichthys lucerna* sampled in 2016 from the three regions along the Portuguese western coast. Concentrations are given in µg element g^{-1} calcium. Different letters in the error bars indicate statistically different results (Tukey Test, $p < 0.05$).

Multi-elemental concentrations of otoliths also differed among regions (MANOVA, Pillai's Trace: $F_{12,166}$=12.094, p<0.05). All pairwise multi-elemental comparisons also gave significant results (Hotelling's T-square tests, p<0.05).

Fish from the three regions could be re-classified to their original location with a relatively high degree of accuracy (73% of correct overall re-classification), although a partial overlap was observed in LDFA between regions (Table 2-3, Figure 2-3B). The CAP identified three groups and the vector for Sr:Ca was aligned with Matosinhos group while the vectors for the remaining element:Ca ratios were aligned with Peniche (Figure 2-4B).

2.3.3. Otolith Elemental and Shape Analyses

All natural otolith tags combined (ME, SI and EFD) gave statistical differences among regions (MANOVA, Pillai's Trace $F_{40,138}$=4.654, p<0.05) and all pair-wise comparisons resulted in significant differences (Hotelling's T-square tests p<0,05). Fish from the three regions could be classified to their collection location with a reasonably high degree of accuracy (74% of correct overall re-classification) (Tale 2-2). The LDFA plot (Figure 2-3C) did not fully discriminate regions as they revealed a partial overlap among them. The CAP correlation matrix identified three groups with most of the variable vectors aligned with Matosinhos and Peniche (Figure 2-4C).

2.4.Discussion

Otolith shape analyses can provide a valuable tool for discriminating between fish populations (Stransky et al., 2008; Agüera & Brophy, 2011; Moreira et al., 2019). Otolith shape has shown to be influenced by a series of environmental (Tuset et al., 2003; B. M. de Carvalho et al., 2015; Vignon, 2015) and endogenous factors (Castonguay et al., 1991; Simoneau et al., 2000; Cardinale et al., 2004), and while it can differ among stocks of a species, it can also differ among ages, sexes, and year classes within the same stock (Campana & Casselman, 1993; Cardinale et al., 2004; Ladroit et al., 2017). Otolith morphology may vary according to fish ontogeny, geographic location, water depth, feeding regime, as well the water environmental properties (Capoccioni et al., 2011; Bacha et al., 2016; Moreira et al., 2019). Moreover, genetically driven changes can locally affect otolith shape (Berg et al., 2018).

Morphometric analysis of *C. lucerna* otoliths revealed significant differences among all sampling regions, but mostly between Matosinhos and Peniche, as some statistics for SI and EFDs clearly discriminated fish from these two sampling regions. Considering that EFDs results do not provide an intuitive understanding of the reasons that may cause outline differences, the SIs, namely FF, RE and CI, are more valuable for explaining which otolith characteristics varied between regions. The LDFA combining both morphometric descriptors (SI+EFD), however, revealed a low re-allocation success of individuals to the original regions (overall of 51%) and the corresponding plot presented a high overlap between all sampling regions. This observation is also corroborated by CAP visualization. A previous study also based on elliptic Fourier analysis of otoliths of Triglidae in the north-middle Adriatic Sea revealed a high intraspecific variability on adult specimens of *C. lucerna* linked both to growth and to environmental conditions (Montanini et al., 2010). Thus, the observed overall *C. lucerna* otolith shape variation among regions most probably resulted from the influences of both genetic and environmental factors, but not great enough to consider it as different spatial populations.

Minor and trace elements deposited into fish otoliths during their life history, when site specific, could provide natural tags of their inhabited areas (Correia et al., 2012, 2014; Moreira et al., 2018). Furthermore, otolith elemental composition has proved to be a useful technique in determining population structure and connectivity of fish stocks (Silva et al., 2011; Higgins et al., 2013; Moreira et al., 2018). Exogenous (e.g., water temperature and salinity) and endogenous (e.g., age, diet and metabolism) factors can influence the complex process of incorporation of these elements in the otoliths (Ranaldi & Gagnon, 2008; Stanley et al., 2015; Turner & Limburg, 2015). Just a few studies have been able to directly link the incorporation of some of these elements (e.g., Sr and Ba) with specific exogenous environmental factors (Bath et al., 2000; Elsdon & Gillanders, 2002; Elsdon Travis S. et al., 2008). However, the mechanisms and pathways by which elements are deposited in otoliths is still poorly understood for many trace elements (Halden & Friedrich, 2008; D'Avignon & Rose, 2013b; Limburg et al., 2015).

The chemical analysis from *C. lucerna* otoliths detected the presence of six trace elements (Sr, Ba, Mn, Mg, Fe and Zn) at informative levels (>LOD) which were within the general ranges of concentration found in other coastal marine species (Silva et al., 2011; Correia et al., 2012; Moreira et al., 2018). Almost all element:Ca ratios were higher in otoliths of fish collected from Peniche. Strontium, as expected, was the most abundant element in *C. lucerna* otoliths; Sr has a positive correlation with salinity thus it is a trace element that is generally associated with higher salinity environments (Silva et al., 2011; Reis-Santos et al., 2013; Moreira et al., 2018). The hereby data, however, revealed a somewhat unexpected higher Sr:Ca concentration in otoliths of fish from Matosinhos. Matosinhos is adjacent to Douro River which is a major source of freshwater along the Portuguese coast (M. E. C. Vieira & Bordalo, 2000). Furthermore, this northern Portuguese coastal area is known to have a low salinity environment as result of the river discharge and continental run-off extending along the shelf off Northwest Iberia (Otero et al., 2008). For Ba, statistics results for all three regions showed no differences among them. Ba concentration is often related to terrestrial freshwater sources and coastal upwelling processes (Bath et al., 2000; Hamer et al., 2006). Thus, the joined effect of the freshwater input from the nearby surrounding rivers (i.e., Douro River for Matosinhos, Vouga River into the Ria de Aveiro, and São Domingos River for Peniche) and the Portuguese coastal upwelling could explain this similarity. Mg, Mn, Zn and Fe concentration ratios were higher in Peniche comparatively to the other two sampling regions. The higher concentration for most of the elements found in fish otoliths for Peniche may then be due to its particular geological and environmental characteristics, including the complex oceanographic patterns: this area is influenced by two different climatic conditions, the Atlantic in the northern cliffs, and the Mediterranean climate in the southern cliffs (Pardal & Azeiteiro, 2001). However, some physiological processes (e.g., diet ontogeny and growth) could also influence the incorporation of Mg, Mn, Zn and Fe into fish otoliths (Thomas et al., 2017). Finally, higher levels of heavy metals in otoliths could be consistent with an environmental exposure history of fish to aquatic contamination (e.g., Arslan & Secor, 2005; Geffen et al., 2003; Ranaldi & Gagnon, 2010). Some studies have already shown substantial anthropogenic metal contamination in the study area, namely in the Douro estuary (Magalhães et al., 2007), Ria de Aveiro (Martins et al., 2013) and Óbidos lagoon (Pereira et al., 2009). These factors contribute to the input of a large quantity of contaminants flushed downstream towards the estuaries and coastal lagoons. These contaminants could be later on transported to the adjacent coastal areas through the

Portuguese southward superficial current, namely in summer (Fiúza, 1984). High concentrations of Mn and Fe have been reported, for instance, in Óbidos, a northern coastal lagoon, nearby Peniche (Pereira et al., 2009), partially explaining the greater abundance of some trace elements found in otoliths of the individuals collected in this area. Furthermore, concentrations of contaminants are higher in sediment than in the water column, which may result in higher exposure levels for epibenthic fish, such as *C. lucerna*, than for pelagic organisms (Ruus et al., 2005). Although the mechanism behind the incorporation of trace elements into fish otoliths is complex and poorly understood, the use of otoliths as natural biogeochemical tags depends on the existence of some measurable differences in otolith chemistry on a geographic scale relevant to the life history of the species (Adelir-Alves et al., 2018). Overall for the multi-elemental elemental analysis, LDFA and CAP plots suggest that these fish aggregations are not a single population unit and partially mix during their life-time history.

Finally, when multivariate analysis of otolith chemistry and morphology were combined, the overall re-classification of the LDFA analysis improved significantly (74%) showing moderate discrimination among samples from the different regions, similar to the results obtained for the elemental composition. LDFA was also unable to isolate different population units and CAP revealed that the chemical elements are what most differentiate Peniche location from the other two sampling regions, corroborating the results obtained with the separate analysis of the different techniques tags (ME and SI + EFD), but not being great enough, however, to assume that it is an isolated population unit.

In short, neither separately or combined, otolith elemental chemistry and morphology techniques did not clearly discriminate *C. lucerna* individuals from the three sampling regions of Portugal, suggesting that there is some habitat connectivity between these NW Portuguese fishing grounds and that the species is unique, but not necessarily a homogenous, single population and cannot be assumed to be separate for fisheries management purposes. Further studied, with the use of other natural tags (e.g., genetics and parasitic fauna), are however needed to unravel *C. lucerna* population structure considering its broad geographic range.

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CHAPTER III. Is *Chelidonichthys lucerna* a marine estuarine-dependent fish? Insights from otolith microchemistry

Is *Chelidonichthys lucerna* a marine estuarine-dependent fish? Insights from otolith microchemistry.

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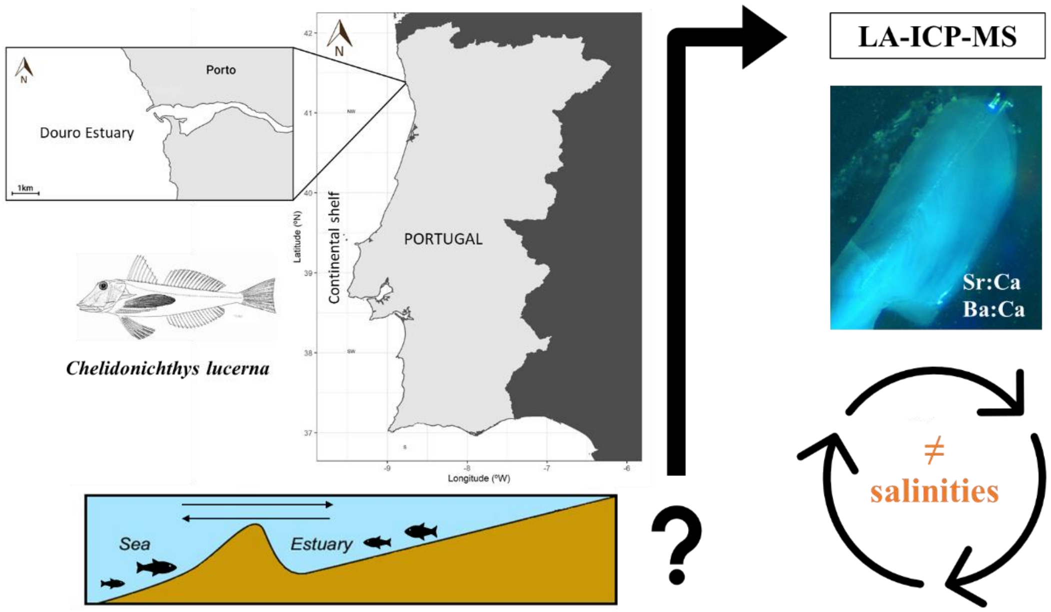
Graphical Abstract



Abstract

Tub gurnard, *Chelidonichthys lucerna* (Linnaeus, 1978), is a Mediterranean-Atlantic benthic species usually captured as by-catch by the 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 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.

3.1.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 (Dahlgren et al., 2006; Sharpe et al., 2019; Arevalo et al., 2023). 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 (Wilson, 2002; Potter et al., 2013, 2015).

Some fish species inhabit estuaries throughout their entire life, 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 (Dando, 1984; Potter et al., 2015; James et al., 2019). Marine estuarine-dependent fish, for example, spend part of their life cycle in low-saline estuaries for growth, feeding, reproduction, and sexual maturation (Potter et al., 2015; James et al., 2019; Soeth et al., 2020). 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 (Braun et al., 2019; Soeth et al., 2020; Bartes et al., 2021).

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 (Popper et al., 2005). 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 (Popper et al., 2005; Rodriguez-Mendoza, 2006; Volpedo & Vaz-dos-Santos, 2015). Otoliths can provide a wide range of information about fish species such as age estimation, environmental history, taxonomic issues, and prey identification, among others (Korostelev et al., 2020; Ibáñez et al., 2021; D'iglio et al., 2022). Their chemical composition has also shown to be a powerful tool to study fish population structure (Franco et al., 2006; Soeth et al., 2019; Schroeder et al., 2022), movement patterns (Daros, Spach, & Correia, 2016; Avigliano et al., 2017; Soeth et al., 2020), and habitat connectivity (Daros, Spach, Sial, et al., 2016; Ferreira et al., 2019; Moura et al., 2020).

Chemical otolith analysis provides information about the environmental conditions and life history traits of fish throughout their whole life (Moreira et al., 2018; Soeth et al., 2019; Correia et al., 2021) or for a particular life history stage (Correia et al., 2011; Soeth et al., 2020; Moreira et al., 2022). 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 (Tabouret et al., 2010; Stanley et al., 2015; Soeth et al., 2020). 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 (Daros, Spach, & Correia, 2016; Avigliano et al., 2017; Soeth et al., 2020).

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 (Macdonald & Crook, 2010; Daros, Spach, & Correia, 2016; Menezes et al., 2021). 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) (Gillanders, 2005; Brown & Severin, 2009; Walther & Limburg, 2012). The Sr:Ca threshold from field studies is estimated by assessing the Sr:Ca ratios from a fish species captured from different salinity environments (Daros, Spach, & Correia, 2016). In laboratory experiments, these ratios are analyzed from fish reared in a controlled environment with varying salinity levels (Macdonald & Crook, 2010). 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) (Tabouret et al., 2010; Soeth et al., 2020; Menezes et al., 2021).

Chelidonichthys lucerna (Linnaeus, 1758), is a benthic species that usually lives in the bottom of the continental shelf (Işmen et al., 2004; Quigley, 2005; FAO Fisheries and Aquaculture Department, 2023), being found in the northeast Atlantic, Mediterranean and Black Sea (FAO Fisheries and Aquaculture Department, 2023). 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 (Feijó et al., 2008) and, according to the Portuguese fishing statistics, a total of 285 ton 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 (Instituto Nacional de Estatística, 2022, 2023).

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 (Colloca et al., 1994; Quigley, 2005; Montanini et al., 2017). However, although a few studies on the species have investigated population structure, diet composition, and age, growth and reproductive biology (El-Serafy et al., 2015; McCarthy & Marriott, 2018; Ferreira et al., 2019), 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 (Campos et al., 2022).

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.

3.2.Materials and Methods

3.2.1 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 3-1, Table 3-1). 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 (Azevedo et al., 2014). In its lowerection, water temperature and salinity range between 10–15 °C and 10–30 ppt, respectively (S. M. Rodrigues et al., 2022).

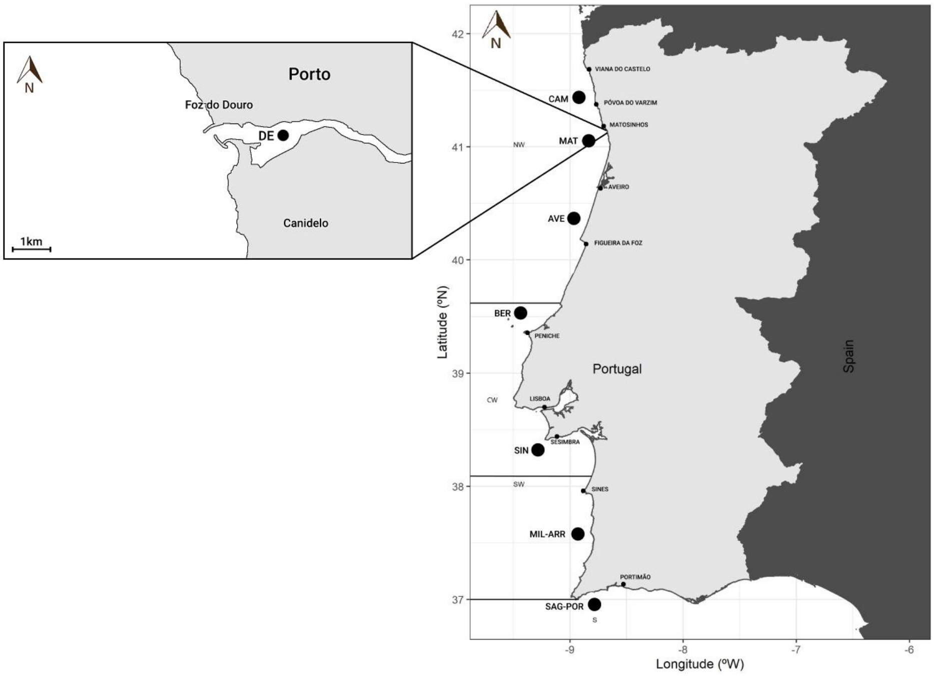


Figure 3-1. Map of the Portuguese coast indicating the sampling sites (•) of *Chelidonichthys lucerna*, Caminha (CAM), Matosinhos (MAT), Aveiro (AVE), Berlengas (BER), Sines (SIN), Milfontes-Arrábida (MIL-ARR), Sagres-Portimão (SAG-POR), including the Douro estuary (DE) location.

Table 3-1. 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 ± SD	**Range**	
Sagres-Portimão	13–14 March 2007	5	27.16 ± 4.75	21.60–32.90	Mean = 6.63 SD = 1.59 Mean -1 x SD = 5.04 Mean - 2 x SD = 3.45
Arrábida-Milfontes	10–12 March 2007	5	31.68 ± 8.10	26.10–45.30	
Sines	17–18 March 2007	5	24.54 ± 3.01	22.80–29.80	
Berlengas	30 March 2007	5	22.56 ± 1.00	21.20–23.90	
Aveiro	29 March 2007	5	23.04 ± 0.93	21.80–23.90	

Matosinhos	26 March 2007	5	22.42 ± 0.93	21.00–23.30	
Caminha	25 March 2007	5	22.50 ± 1.02	21.20–23.80	
Douro estuary	12 December 2016	5	20.16 ± 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 (Ambar et al., 2002).

3.2.2 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 (saggitae) 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 (Correia et al., 2012, 2014).

Otolith microchemistry was used to examine *C. lucerna* movement behaviors through different salinities 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 (7700x 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 ventral-proximal otolith edge using a continuous transect along the radius of the otoliths (Figure 3-2).

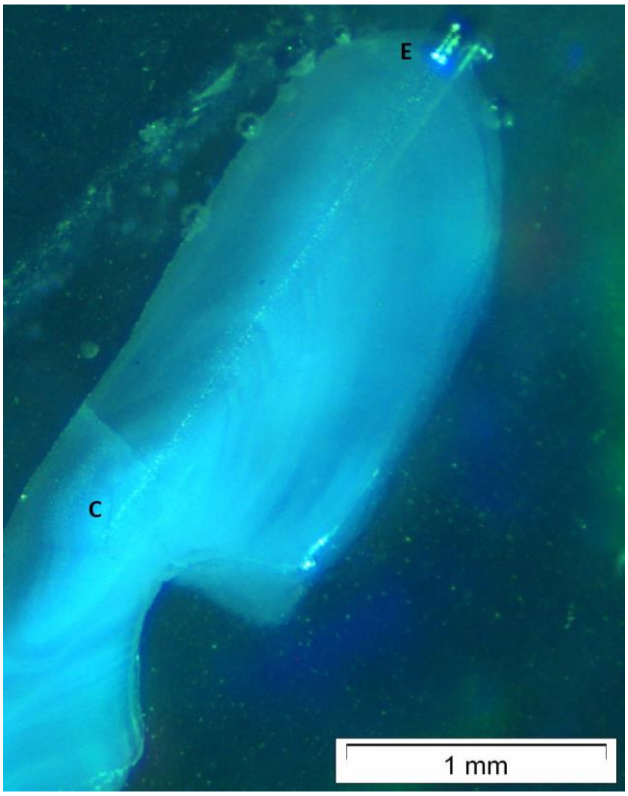


Figure 3-2. Transverse section from the left sagittal otolith of a *Chelidonichthys lucerna* individual (total length, TL = 22cm) collected along the Portuguese coast showing the entire continuous laser ablation transect made by LA-ICP-MS from core (C) to edge (E).

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 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 (Campana, 1999). 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 (Sirot et al., 2017). 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 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 (Sirot et al., 2017). 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 concentration relative to ^{43}Ca (element:Calcium).

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) (Sirot et al., 2017) in the R environment (R Core Team, 2023).

3.2.3 Data Analysis

The assumption of a positive correlation between otolith Sr:Ca and habitat salinity (Secor & Rooker, 2000; Daros, Spach, & Correia, 2016; Soeth et al., 2020) 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 x SD] (Menezes et al., 2021) and the otolith edge Sr:Ca ratios average minus two standard deviations [Mean – 2 x SD] (Tabouret et al., 2010; Soeth et al., 2020). 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 wo mathematical approaches better represents the fish transition from marine coastal waters to estuarine brackish waters (Daros, Spach, & Correia, 2016).

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 x SD] and 3.45 mmol mol^{-1} [Mean – 2 x SD] (Table 3-1). 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 x 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 (Ambar et al., 2002) in the R environment (R Core Team, 2023) 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 (Macdonald & Crook, 2010; Stanley et al., 2015; Martinho et al., 2020), any specific pattern for Ba:Ca or potential relation 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 (Papaconstantinou, 1984). Therefore, the otolith ratio from core-to-edge was considered a proxy for fish age.

3.3.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 estuarine 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 3-3). All individuals' core-to-edge profiles are included in the supplementary material (Supplementary Figure S1).

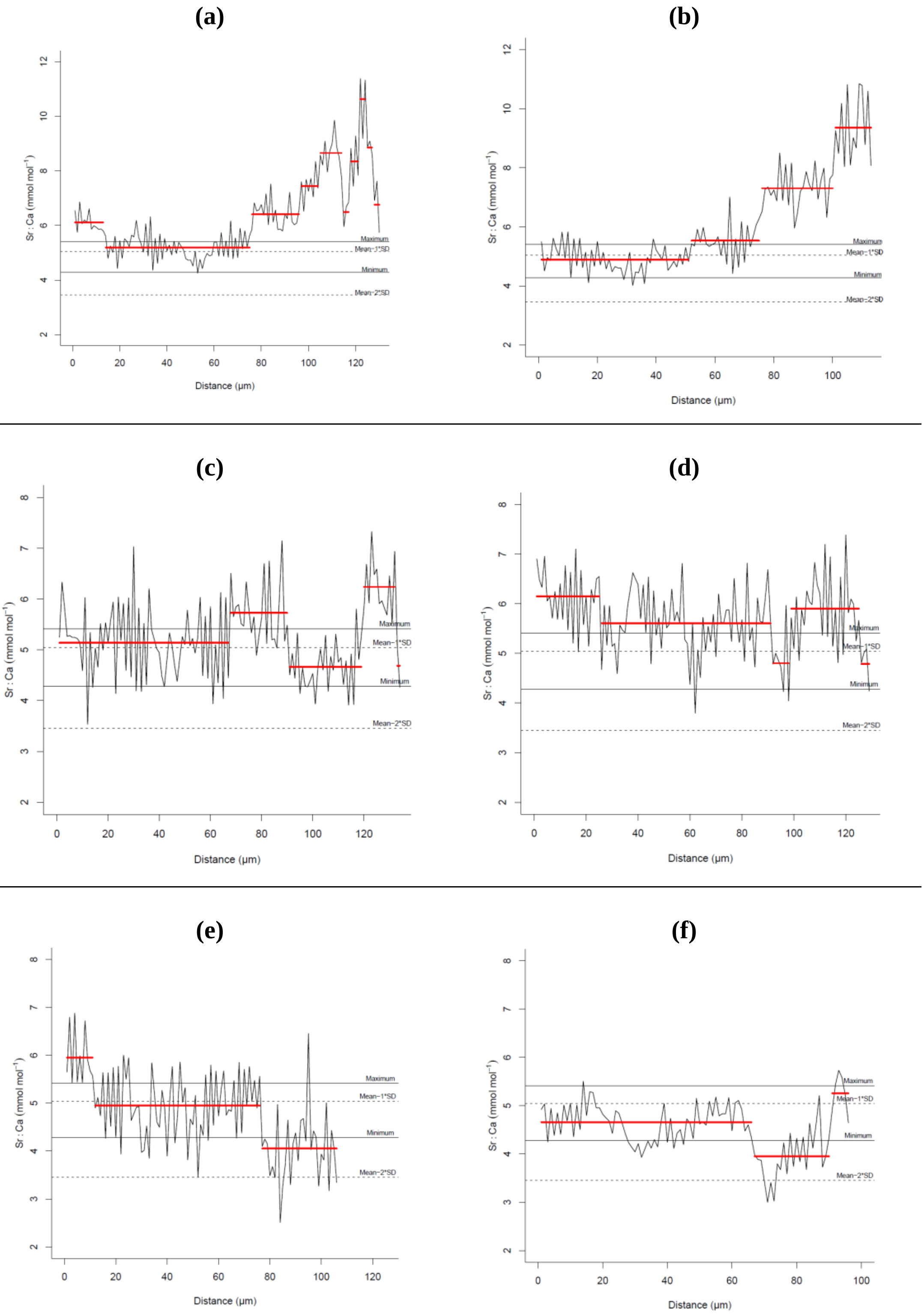


Figure 3-3. Otolith microchemical (Sr:Ca) individual profiles representing the six different patterns of *C. lucerna* classified as (a) Marine-Estuarine-Marine (MEM); (b) Estuarine-Marine (EM); (c) Estuarine-Marine-Estuarine-Marine (EMEM); (d) Marine-Estuarine-Marine-Estuarine (MEME); (e) Marine-Estuarine (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 x SD] and [Mean – 2 x 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 its life history. For 40% of the individuals, these peaks occurred at both Sr:Ca marine and estuarine levels, 12% only at marine levels; and for 8% only at estuarine levels (Figure 3-4).

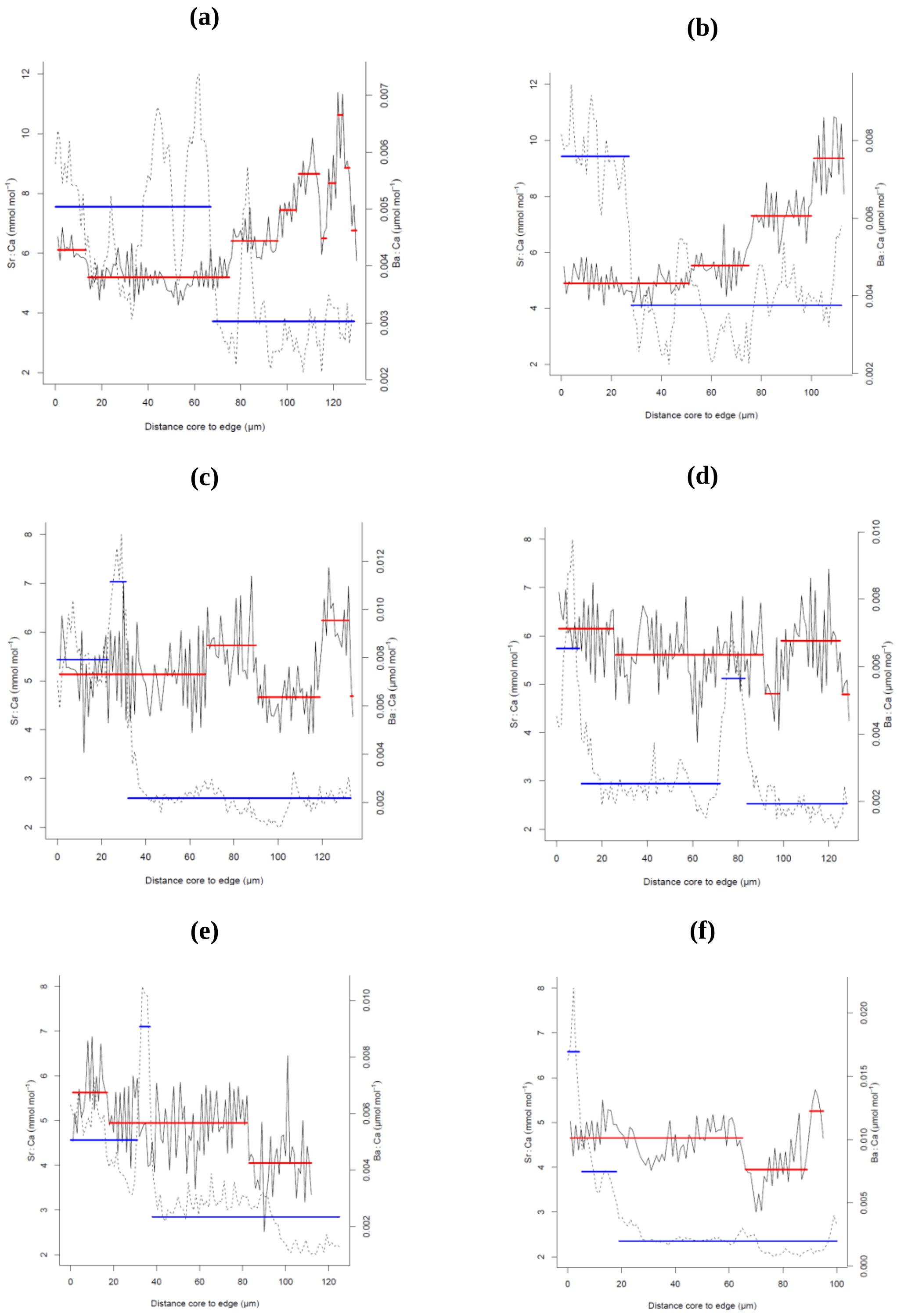


Figure 3-4. Otolith microchemical (Ba:Ca) profiles of the six different patterns of *C. lucerna* classified as (a) Marine-Estuarine-Marine (MEM); (b) Estuarine-Marine (EM); (c) Estuarine-Marine-Estuarine-Marine (EMEM); (d) Marine-Estuarine-Marine-Estuarine (MEME); (e) Marine-Estuarine (ME); (f) Estuarine (E). The solid and dahs lines corresponds respectively to Sr:Ca and Ba:Ca values and associated change points (red and blue, respectively).

3.4. 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 (Papaconstantinou, 1984; Colloca et al., 1994; El-Serafy et al., 2015). 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 (Işmen et al., 2004; J. Rodrigues, 2020; Campos et al., 2022).

The deposition of Sr and Ba in fish otoliths is positively correlated with their occurrence in the water environment (Walther & Thorrold, 2006; Macdonald & Crook, 2010; Stanley et al., 2015). 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 (Macdonald & Crook, 2010; Tabouret et al., 2010; Nelson & Powers, 2019). 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 (Elsdon Travis S. et al., 2008; Macdonald & Crook, 2010; Soeth et al., 2020).

In the present study, two different Sr:Ca ratio thresholds between estuarine and marine environments were mathematically estimated (i.e., Mean – 1 x SD and Mean – 2 x SD) following a standard procedure used by different authors to delimit habitat residency according to their water salinity (Daros, Spach, & Correia, 2016; Soeth et al., 2020; Menezes et al., 2021). 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 limits estimates was the more accurate. Overall, the threshold estimated through the Mean – 1 x 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 (Papaconstantinou, 1984; Işmen et al., 2004; Campos et al., 2022). 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 a 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 (Potter et al., 2015; Reis et al., 2020; Campos et al., 2022). 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 (Papaconstantinou, 1984; Colloca et al., 1994; El-Serafy et al., 2015), there has not been clear evidence of *C. lucerna* spawning in brackish environments. Existing studies on the species found spawning occurs mostly around the winter months (İlhan & Toğulga, 2007; El-Serafy et al., 2015; J. Rodrigues, 2020), which is also when fish show a more pronounced presence in deeper waters (Işmen et al., 2004; Vallisneri et al., 2011; Campos et al., 2022). In addition, it is also important to note that besides habitat changes and migrations in distinct salinity environments, otolith's Sr:Ca ratios can also record other critical events in a fish's life. Indeed, other studies focused on several anguilliform species (Correia et al., 2003, 2004; Ling et al., 2005) have found that a drop in Sr:Ca levels at the core-to-edge otolith transect can also be related with physiological events, such as the onset of the leptocephalus larval metamorphosis. Therefore, some atypical otolith's 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 (Bath et al., 2000; Macdonald & Crook, 2010; Tabouret et al., 2010). However, several studies have also shown that Ba incorporation into otoliths can be affected by factors such as upwelling phenomena (Lin et al., 2013; Woodson et al., 2013; Wheeler et al., 2016), and terrestrial freshwater contributions (river runoff, groundwater inputs) (Ferguson et al., 2011; Morat et al., 2014; de Carvalho et al., 2017). 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 3-4). 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 (Woodson et al., 2013; Wheeler et al., 2016;de Carvalho et al., 2017).

3.5.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 (Costa & Cabral, 1999; Veiga et al., 2009; Campos et al., 2022). However, further investigations using wild contemporary animals collected in the same temporal window in inshore areas and estuaries, including water samples 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.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8070383/s1, Figure S1: Individual *C. lucerna* Sr:Ca core-to-edge profiles.

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.

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3.6.References

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CHAPTER IV. *Chelidonichthys lucerna* (Linnaeus, 1758) population structure in the northeast Atlantic inferred from landmark-based body morphometry

Chelidonichthys lucerna (Linnaeus, 1758) population structure in the northeast Atlantic inferred from landmark-based body morphometry

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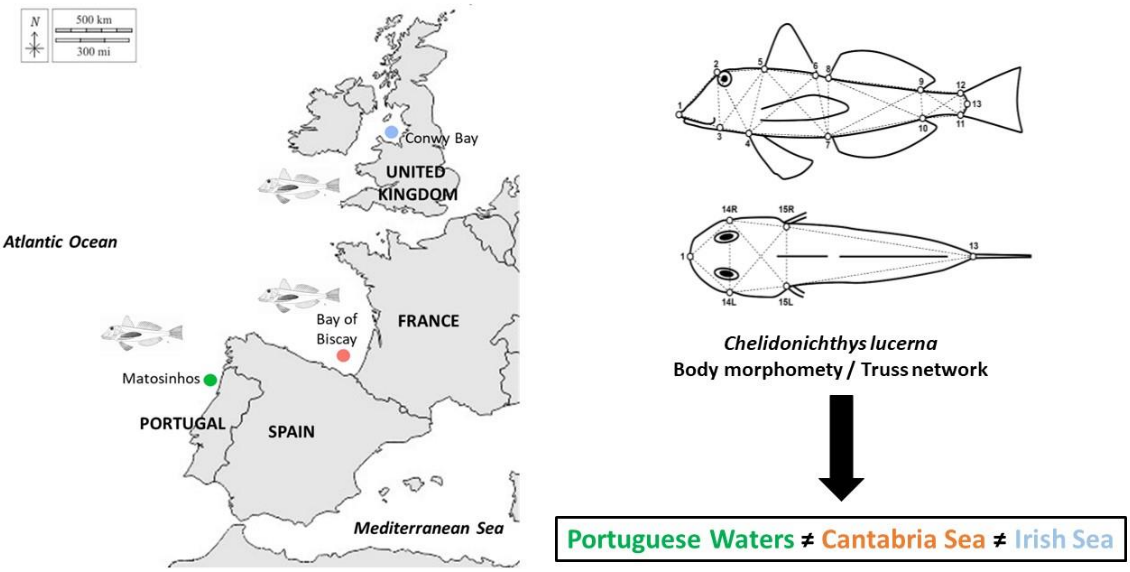
Graphical Abstract



Abstract

The study of geometric morphometrics among stocks has proven to be a valuable tool in delineating fish spatial distributions and discriminating distinct population units. Variations in fish body morphology can be linked to genetic factors or to phenotypic adaptability in response to environmental variables. The tub gurnard (*Chelidonichthys lucerna*) is a demersal species that usually lives in the bottom of the continental shelf, being widely distributed along the northeast Atlantic, Mediterranean and Black seas. Worldwide interest in the species has increased since 2006, when ICES recognized its potential for commercial exploitation. However, despite its broad geographic occurrence, to date, research on *C. lucerna* population structure at large spatial scales is still lacking. In this paper, body geometric morphometrics, using a landmark-based truss network, was applied in order to discriminate *C. lucerna* populations caught in three different fishery grounds areas along the northeast Atlantic: Conwy Bay (United Kingdom), Biscay Bay (Spain) and Matosinhos (Portugal). The results obtained in this study revealed a high overall relocation success (95%) of samples to their original locations, thus demonstrating the existence of significant regional differences and indicating that we are dealing with different fish population units. Moreover, the data revealed a partial overlap between individuals from Spain and United Kingdom, suggesting that in geographically distant areas these populations may inhabit similar environments. However, to corroborate these findings, future works using a holistic approach with alternative and complimentary stock assessment tools (e.g., genetic and phenotypic natural tags) are highly recommended.

Keywords: Triglidae, Fish stocks, Natural tags, Geometric morphometrics, Truss network.

4.1. Introduction

Fish populations can be distributed over extensive geographical areas characterized by distinctive environmental characteristics (e.g., temperature, salinity, depth, habitat, currents), which together with the challenges related to food availability and predators can exert influence over significant demographic aspects, thereby affecting the dynamics of the populations including reproductive patterns, fecundity and lifespan (Harvey et al., 2013; Brooker et al., 2020; Costa et al., 2022). In addition, other factors, such as fishing pressure, anthropic pollution, habitat destruction and climate change, can also affect the abundance and distribution of fish species and consequently fish stocks dynamics (Franssen et al., 2012; Wright et al., 2020; Santi et al., 2021). Understanding population structure is, therefore, an important component of fisheries management as it allows to effectively estimate stock-wise population abundance, determine how each stock responds to fisheries exploitation or environmental changes and the potential impacts on related and dependent species, thereby ensuring species sustainability (Begg et al., 1999; Cadrin et al., 2014; Rawat et al., 2017).

Fish morphometric variation among stocks has been shown to be a useful tool to describe fish spatial distributions and to identify different population units, as fish body morphological differences (e.g., length, width and depth) can be associated with genetic background (Robinson & Wilson, 1996; Turan et al., 2006; Crispo, 2008) or processes of phenotypic plasticity as a response to different environmental conditions (Hoff et al., 2020; Moreira et al., 2020; Muniz et al., 2021). Exposure to variations in factors, such as temperature, salinity, and food availability, can result in different behavioral patterns (e.g., aggregation, migration and others) and the adoption of different adaptation strategies, which could be reflected in fish morphometric features and contribute to the definition of different phenotypic stocks (Pulkkinen et al., 2022; Schroeder et al., 2022; Quadroni et al., 2023). The truss network system is a geometric morphometrics method commonly used for stock discrimination purposes that provides information on phenotypic traits (Rawat et al., 2017; Mallik et al., 2020; Chakraborty, 2022). This approach is a powerful tool for the analysis of the fish contour shape and consists of covering all or most of the animal’s body with a landmark-based uniform network that allows the measurement of a series of distances across the body form (Strauss & Bookstein, 1982).

Chelidonichthys lucerna (Linnaeus, 1758), commonly named tub gurnard, can be found in the northeast Atlantic, from Norway and the southern North Sea extending along the Atlantic shoreline of Europe around to the British Isles, but also in the Mediterranean and Black Seas and the northwest coast of Africa (FAO Fisheries and Aquaculture Department, 2023). The species is demersal and usually inhabits sand, muddy or gravel substrates of the continental shelf in depths ranging from 20 m to 318 m, but it is more abundant in inshore waters up to 150 m (Mytilineou et al., 2005; ICES, 2010; El-Serafy et al., 2015). Following a larval pelagic phase (Dulčić et al., 2001; Vallisneri et al., 2012), *C. lucerna* exhibits a particular pattern of seasonal migratory movements during the juvenile and adult stages within its depth ranges throughout the year, showing a more pronounced concentration of individuals in shallower depths during spring and summer, moving progressively into deeper waters in the winter period (Montanini et al., 2017; Carbonara & Follesa, 2019; Campos et al., 2022). Recently, it has been shown that *C. lucerna,* although mainly a marine fish, can occupy and migrate among habitats with diverse salinity degrees, thereby showing high environmental plasticity and adaptation (Ferreira et al., 2023). In 2006, ICES classified *C. lucerna* as a potential species for commercial exploitation and has recommended that monitoring programs should be conducted to acquire information on biological parameters for stock assessment purposes (ICES, 2006). Since 2010, although with a slight decrease between 2017 and 2020, worldwide fisheries landings have shown an increasing trend, reaching 4759 tons in 2021 (FAO, 2023). At present, there is no minimum landing size, allowed quotas, fishing closure seasons, or other fishery regulations. According to the International Union for Conservation of Nature (IUCN), *C. lucerna* is listed as Least Concern, but it would be helpful to quantify the population trend of this species throughout the Atlantic Ocean and Mediterranean Sea (Nunoo et al., 2015).

Due to its broad spatial distribution and the presence of several physical and oceanographic barriers within its wide distribution range, the species could potentially consist of various distinct population units. However, to date, information about the stock structure of the species is scarce. A study, conducted using genetic and morphological analyses of fish caught in the Black Sea, Marmara, Aegean and northeastern Mediterranean coasts of Turkey, showed that only the Black Sea population is differentiated from the other populations (Uyan & Turan, 2017). More recently, a study conducted in the Portuguese Atlantic waters using otolith shape and microchemistry fingerprints suggested that along the mainland coast the species is, although not homogeneous, apparently a single-population unit (Ferreira et al., 2019). However, its population structure at the larger northeast Atlantic spatial scale is unknown.

Therefore, the present study aimed at investigating the spatial morphological variability of *C. lucerna* among three fishery grounds (British, Cantabrian and Portuguese waters) in the northeast Atlantic, using a truss network approach.

4.2.Materials and Methods

4.2.1 Sampling

A total of 129 fish were collected between October 2020 and December 2021 in three different fishery grounds in the northeast Atlantic: Conwy Bay, United Kingdom (Irish Sea), Bay of Biscay, Spain (Cantabrian Sea), and Matosinhos, Portugal (northwest Portuguese waters) (Figure 4-1, Table 4-1). All individuals were captured using bottom trawl fishing. Upon collection, or immediately after landing, fish were transported to the laboratory in isothermal containers preserved with ice for biological processing.

Figure 4-1. Sampling locations of *Chelidonichthys lucerna* individuals collected between October 2020 and December 2021 in the northeast Atlantic (the blue, red and green solid circles represent the eastern Irish Sea, the Cantabria Sea and the northwest Portuguese waters, respectively).

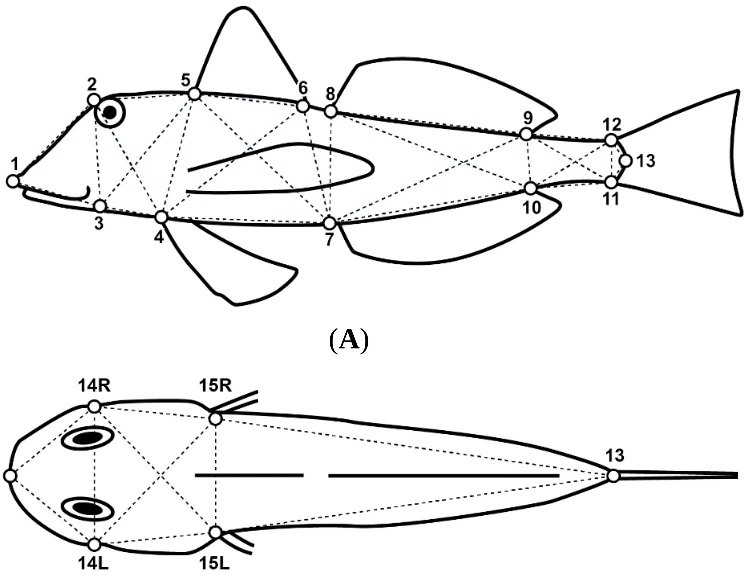
Table 4-1. Sampling locations, date, sample size (N) and standard length (SL: mean ± standard deviation) of *Chelidonichthys lucerna* used in this study.

Location	Date	N	SL (cm)
Irish Sea	October 2020	29	19.4 ± 2.9
Cantabria Sea	April 2021	50	28.3 ± 2.0
Portuguese Waters	December 2021	50	22.1 ± 1.7

4.2.2 Body morphometric analysis

The body morphometrics of individual *C. lucerna* were analyzed using a truss network system standard protocol (Strauss & Bookstein, 1982). All individuals were measured for standard length (SL, 1 mm) (Table 4-1), and their left (lateral) and dorsal (upper) sides were photographed from a fixed distance using a precise scale (10 mm), with a high-quality digital camera for the body morphometric analysis, following recommendations, to minimize the effects of distortion (Muir et al., 2012). Instructions of landmark criteria and a reference image of where to place each landmark were shared among researchers to avoid image-based bias (O´Malley et al., 2021).

A total of 13 and 6 landmarks were defined along the body contour on the fish's lateral and dorsal views, respectively (Figure 4-2). Location coordinates of homologous landmarks were processed and digitized using tpsUtil Version 1.83 (Rohlf, 2023) and tpsDig Version 2.32 (Rohlf, 2021) software and used to determine 37 linear distances (D) of the box-truss network (Table 4-2).



(**A**)

(**B**)

Figure 4-2. Illustration of a *Chelidonichthys lucerna* specimen showing the selected landmarks for the lateral (**A**) and dorsal (**B**) body views. See Table 4-2 for further details.

Table 4-2. Body landmarks defined along the body contour of *Chelidonichthys lucerna* and morphometric distances used for the body shape analysis. For more details, please see Figure 4-2.

Body landmarks	
Number	**Location**
1	Anterior tip of the mouth
2	Anterior margin of the eye
3	Posterior tip of the mouth
4	Anterior insertion of the pelvic fin
5	Anterior insertion of the 1st dorsal fin
6	Posterior insertion of the 1st dorsal fin
7	Anterior insertion of the caudal fin
8	Anterior insertion of the 2nd dorsal fin
9	Posterior insertion of the 2nd dorsal fin
10	Posterior insertion of the caudal fin
11	Ventral insertion of the caudal fin
12	Dorsal insertion of the caudal fin

13		Posterior margin of the caudal peduncle
14R		Right dorsal margin of the head (centre of the eye)
14L		Left dorsal margin of the head (centre of the eye)
15R		Insertion of the right pectoral fin
15L		Insertion of the left pectoral fin
Morphometric Distances		
Distances	**Landmarks**	**Description**
D1	1 to 2	Head length
D2	1 to 3	Maxilla length
D3	2 to 3	Anterior height of head
D4	2 to 4	Posterior height of head
D5	2 to 5	Distance from most posterior aspect of neurocranian to 1st dorsal fin
D6	3 to 4	Distance from maxilla to pelvic fin
D7	3 to 5	Distance from the posterior tip of the mouth to the anterior insertion of the 1st dorsal fin
D8	4 to 5	Anterior body height
D9	4 to 6	Distance from the anterior insertion of the pelvic fin to the posterior insertion of the 1st dorsal fin
D10	4 to 7	Distance between pelvic fin and anal fin
D11	5 to 6	Length of 1st dorsal din
D12	5 to 7	Distance between the origin of 1st dorsal fin and origin of anal fin
D13	6 to 7	Posterior body height
D14	6 to 8	Distance between 1st and 2nd dorsal fins
D15	7 to 8	Distance from the anterior insertion of the caudal fin to the anterior insertion of the 2nd dorsal fin
D16	7 to 9	Distance from the anterior insertion of the caudal fin to the posterior insertion of the 2nd dorsal fin
D17	7 to 10	Length of anal fin
D18	8 to 9	Length of 2nd dorsal fin
D19	8 to 10	Anterior diagonal height of posterior body
D20	9 to 10	Anterior caudal peduncle height
D21	9 to 11	Anterior diagonal of caudal peduncle
D22	9 to 12	Distance between 2nd dorsal fin and caudal fin
D23	10 to 11	Distance between anal fin and caudal fin
D24	10 to 12	Posterior diagonal of caudal peduncle
D25	11 to 12	Posterior caudal peduncle height
D26	11 to 13	Distance between ventral insertion of caudal fin and posterior end of vertebrate column
D27	12 to 13	Distance between dorsal insertion of caudal fin and posterior end of vertebrate column
D28	1 to 14R	Distance from the anterior tip of the mouth to the right dorsal margin of the head
D29	1 to 14L	Distance from the anterior tip of the mouth to the left dorsal margin of the head
D30	14R to 14L	Distance between the right and left dorsal margins of the head

D31	14R to 15R	Distance from the right dorsal margin of the head to the insertion of the right pectoral fin
D32	14L to 15L	Distance from the left dorsal margin of the head to the insertion of the left pectoral fin
D33	14R to 15L	Distance from the right dorsal margin of the head to the insertion of the left pectoral fin
D34	14L to 15R	Distance from the left dorsal margin of the head to the insertion of the right pectoral fin
D35	15R to 15L	Distance between the right and left insertions of the pectoral fins
D36	15R to 13	Distance from the insertion of the right pectoral fin to the posterior margin of the caudal peduncle
D37	15L to 13	Distance from the insertion of the left pectoral fin to the posterior margin of the caudal peduncle

4.2.3 Statistical analysis

The relationship between the thirty-seven morphometric distances (D1 to D37) and fish standard length (SL) was verified using One-Way Analysis of Covariance (ANCOVA). All of them presented a significant positive correlation ($p<0.05$). Each distance was corrected to remove the size effect, and the positive allometric relationship between variables was corrected using the following transformation (Reist, 1986): $DT = 10^\wedge[\log(D) - \beta [\log(SL) – \log(SL_{mean})]$, where DT is the transformed distance, D is the original distance, β is the slope of the regression of log(D) on log(SL), SL is the standard length of the individual, and SL_{mean} is the overall mean of standard length for all locations.

For the univariate statistics, the DT dataset was checked for normality (Shapiro-Wilk test) and homogeneity of variances (Levene test), but only eight DTs (DT2, DT6, DT8, DT9, DT10, DT11, DT32 and DT35), fulfilled the parametric prerequisites. For this case, One-Way Analysis of Variance (ANOVA) was used to explore statistical differences of each morphometric distance among the three sampling locations, followed by a Tukey post-hoc test if significant differences exist ($p<0.05$). However, the majority of the DTs did not fulfil the above-mentioned prerequisites, even after being log (x+1) transformed. For them, non-parametric statistics were then used. So, One-Way ANOVA On Ranks, followed by a Dunn’s test, if needed ($p<0.05$), was performed.

Regarding the multivariate statistics, non-parametric tests were also performed. A permutational multivariate analysis of variance (main PERMANOVA) was used to compare DTs among locations, and when statistically significant ($p<0.05$), it was followed by a permutational pairwise comparisons (pseudo t-statistic).

Finally, a flexible discriminant analysis (FDA) followed by a Jackknifed re-classification matrix (leave-one-out cross-validation), was used to calculate the percentage of correctly re-classified individuals into the original location. FDA first randomly split the data into training (80%) and test (20%) sets. Predictors' parameters were estimated by subtracting the mean of the predictor and scaling by its standard deviation. Estimated parameters were used to transform the train and test sets. The correct re-classification percentage of the discriminant functions was calculated using a Jackknifed matrix for the transformed training and test sets (Kassambara, 2017).

The univariate statistical analyses were performed using SigmaPlot 11.0. Multivariate tests were performed using R 4.3.0 (R Core Team, 2023). A statistical level of significance (α) of 0.05 was considered. Morphometric data is presented as mean ± standard error deviation.

4.3.Results

Univariate tests showed significant differences in body morphology among the locations for 36 out of 37 DTs (Table 4-3). Nearly one-quarter of all measurements (DT15, DT23, DT24, DT26, DT27, DT31, DT33, DT34) presented significant differences between all three sampling locations, 46% (DT1, DT3, DT7, DT8, DT9, DT12, DT13, DT16, DT17, DT18, DT19, DT21, DT28, DT29, DT30, DT35, DT37) differentiated Spain from the other locations, 19% (DT4, DT5, DT6, DT11, DT20, DT22, DT32) differentiated Portugal from the other locations, and only one measurement (DT10) differentiated the United Kingdom from other locations. Only 6% of the measurements showed no significant differences between Spain and the other two sampling locations (DT14) and between Portugal and the other two locations (DT25). DT2 was the only measurement that revealed no significant differences among the three locations.

Table 4-3. Body morphometric transformed distances (DT: mean ± standard error) calculated for *Chelidonichthys lucerna* individuals. DTs, showing different letters, means that significant regional differences exist. For most DTs, One-Way ANOVA On Ranks, followed by a Dunn test ($p<0.05$), if needed, was carried out. However, for DT2, DT6, DT8, DT9, DT10, DT11, DT32 and DT35 a One-Way ANOVA, and a post hoc pairwise Tukey test were used. For more details, see M&M.

Distance	Irish Sea	Cantabria Sea	Portuguese Waters
DT1	3.820 ± 0.068 ^{a}	3.573 ± 0.028 ^{b}	3.910 ± 0.041 ^{a}
DT2	2.545 ± 0.068 ^{a}	2.547 ± 0.035 ^{a}	2.633 ± 0.035 ^{a}
DT3	3.820 ± 0.068 ^{a}	3.573 ± 0.028 ^{b}	3.910 ± 0.041 ^{a}
DT4	4.265 ± 0.079 ^{a}	4.148 ± 0.038 ^{a}	4.645 ± 0.059 ^{b}
DT5	4.294 ± 0.081 ^{a}	4.128 ± 0.024 ^{a}	4.575 ± 0.058 ^{b}
DT6	2.668 ± 0.111 ^{a}	2.522 ± 0.068 ^{a}	2.991 ± 0.058 ^{b}
DT7	6.228 ± 0.126 ^{a}	5.843 ± 0.037 ^{b}	6.496 ± 0.077 ^{a}
DT8	4.799 ± 0.075 ^{a}	4.544 ± 0.043 ^{b}	4.944 ± 0.062 ^{a}
DT9	7.175 ± 0.119 ^{a}	6.830 ± 0.084 ^{b}	7.360 ± 0.084 ^{a}
DT10	8.036 ± 0.143 ^{a}	7.311 ± 0.085 ^{b}	7.429 ± 0.111 ^{b}
DT11	3.776 ± 0.069 ^{a}	3.738 ± 0.059 ^{a}	4.088 ± 0.051 ^{b}
DT12	7.081 ± 0.110 ^{a}	6.628 ± 0.050 ^{b}	7.142 ± 0.087 ^{a}
DT13	4.291 ± 0.090 ^{a}	3.963 ± 0.041 ^{b}	4.492 ± 0.057 ^{a}
DT14	1.401 ± 0.093 ^{a}	1.194 ± 0.046 ^{a,b}	1.139 ± 0.048 ^{b}
DT15	3.721 ± 0.072 ^{a}	3.528 ± 0.033 ^{b}	4.147 ± 0.049 ^{c}
DT16	7.632 ± 0.197 ^{a}	7.226 ± 0.053 ^{b}	8.020 ± 0.110 ^{a}
DT17	7.402 ± 0.210 ^{a}	6.818 ± 0.057 ^{b}	7.627 ± 0.111 ^{a}
DT18	7.970 ± 0.162 ^{a}	7.399 ± 0.059 ^{b}	7.957 ± 0.115 ^{a}
DT19	8.372 ± 0.178 ^{a}	7.669 ± 0.057 ^{b}	8.415 ± 0.110 ^{a}
DT20	1.433 ± 0.041 ^{a}	1.433 ± 0.014 ^{a}	1.658 ± 0.020 ^{b}
DT21	1.854 ± 0.077 ^{a}	1.895 ± 0.023 ^{b}	2.197 ± 0.040 ^{a}
DT22	1.368 ± 0.106 ^{a}	1.416 ± 0.028 ^{a}	1.739 ± 0.048 ^{b}
DT23	1.114 ± 0.090 ^{a}	1.441 ± 0.035 ^{b}	1.730 ± 0.050 ^{c}
DT24	1.759 ± 0.060 ^{a}	1.966 ± 0.028 ^{b}	2.212 ± 0.043 ^{c}
DT25	1.198 ± 0.023 ^{a}	1.115 ± 0.009 ^{b}	1.159 ± 0.015 ^{a,b}
DT26	1.484 ± 0.033 ^{a}	1.286 ± 0.017 ^{b}	1.133 ± 0.026 ^{c}
DT27	1.365 ± 0.033 ^{a}	1.209 ± 0.017 ^{b}	1.095 ± 0.025 ^{c}
DT28	3.437 ± 0.084 ^{a}	3.227 ± 0.025 ^{b}	3.426 ± 0.042 ^{a}

DT29	3.482 ± 0.086	a	3.297 ± 0.026	b	3.506 ± 0.037	a
DT30	3.687 ± 0.084	a	3.424 ± 0.028	b	3.771 ± 0.047	a
DT31	2.920 ± 0.093	a	2.690 ± 0.042	b	3.206 ± 0.046	c
DT32	2.786 ± 0.089	a	2.600 ± 0.039	a	3.247 ± 0.055	b
DT33	4.845 ± 0.109	a	4.552 ± 0.041	b	5.248 ± 0.063	c
DT34	4.955 ± 0.122	a	4.560 ± 0.042	b	5.207 ± 0.054	c
DT35	4.313 ± 0.085	a	4.008 ± 0.035	b	4.473 ± 0.053	a
DT36	16.872 ± 0.218	a	15.893 ± 0.076	b	16.592 ± 0.184	a
DT37	17.015 ± 0.215	a	15.952 ± 0.081	b	16.603 ± 0.194	a

When analyzed together, the morphometric distances also presented significant differences among the three locations (PERMANOVA, p<0.05, Table 4-3), and all pairwise tests also revealed significant differences between the locations (Pseudo-t test, p < 0.05, Table 4-4).

Table 4-4. Mean and pairwise PERMANOVA comparisons for the 37 body morphometric transformed distances among the three *Chelidonichthys lucerna* sampling locations.

PERMANOVA	**Df**	**SSq**	**R2**	**F**	**Pr(>F)**
Region	2	0.057	0.228	18.48	0.0001
Residual	125	0.192	0.772		
Total	127	0.249	1		

Pairwise PERMANOVA	**Df**	**SSq**	**F.Model**	**R2**	**p.value**	**p.adjusted**
Cantabria Sea vs. Irish Sea	1	0.021	13.989	0.154	0.001	0.003
Cantabria Sea vs. Portuguese Waters	1	0.046	38.707	0.285	0.001	0.003
Irish Sea vs. Portuguese Waters	1	0.014	6.805	0.082	0.002	0.006

Abbreviations: Df - degrees of freedom, SSq - sums of squares, R2 - partial R2, F -pseudo-F statistic, Pr(>F) – P value for each term.

The FDA conducted showed a clear discrimination for all locations (Table 4-5, Figure 4-3) with an overall high classification accuracy reported for all three sampling locations (95%). Individuals from Portugal were reclassified correctly in 98% of the cases; it was 96% for the United Kingdom and 93% for Spain. The high discrimination pattern for

Portugal was mainly driven by distances DT19 and DT16, which are related with fish posterior body height. DT7 and DT18, related with fish posterior body length, were mainly responsible for the discrimination of the United Kingdom individuals, while DT12 and DT9, related with fish anterior height, were responsible for the differences in the Spanish individuals.

Table 4-5. Summary of the percentage of correct reclassification using the training base set following a flexible discriminant analysis (FDA) for the body morphometric transformed distances calculated for *Chelidonichthys lucerna* individuals.

Original location	**Predicted location**			**% of correct re-allocation**	**% of overall re-allocation**
	Irish Sea	**Cantabria Sea**	**Portuguese Waters**		
Irish Sea	23	1	0	96	95
Cantabria Sea	3	37	0	93	
Portuguese Waters	0	1	39	98	

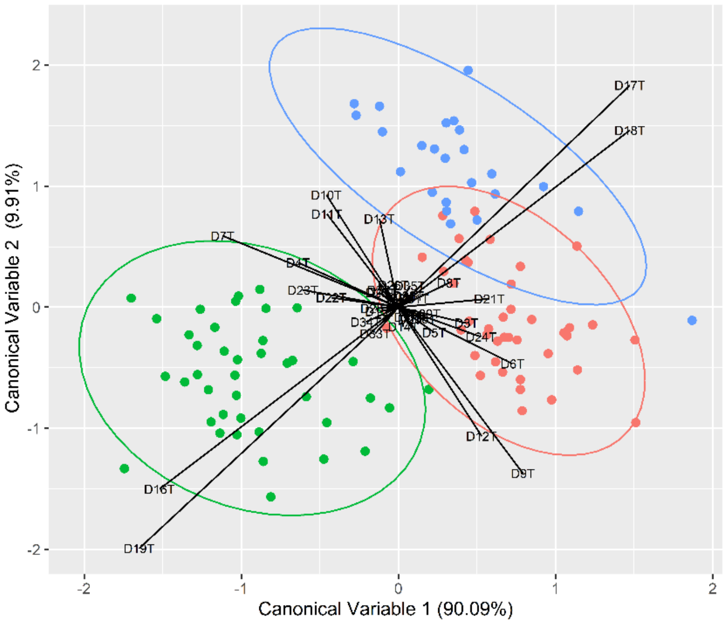


Figure 4-3. Flexible discriminant function analysis plot obtained from body morphometric transformed distances (the blue, red and green solid circles represent the fish from the eastern Irish Sea, the Cantabria Sea and the northwest Portuguese waters, respectively).

4.4.Discussion

The present study aimed at investigating the differences in the body shape of *C. lucerna* along the northeast Atlantic using landmark-based truss network morphometrics.

Morphometric studies on *C. lucerna* are limited and, to date, only one study has been carried out in the Black Sea, Marmara, Aegean and eastern Mediterranean Sea (Uyan & Turan, 2017) that combined genetics with body geometric morphometrics to analyze the existent population structure within Turkish marine waters. As in the former study, the sex of the animals was not considered in this study, since it was demonstrated that there are no morphometric differences between sexes of a related species (*C. obscurus*) (Boudaya et al., 2020), although the potential effect of the reproductive season on the sexual dimorphism should not be excluded.

Phenotypic variation of fish morphometric characteristics provides valuable information about population units and has long been used for stock identification as morphological differences are commonly explained as a response to dissimilar environmental conditions (Begg et al., 1999; Cadrin & Friedland, 2005; Turan et al., 2006).

Advances in digital imaging systems and in analytical methods in the past decades have facilitated progress and diversification of morphometric techniques, expanding the potential for using morphometric analysis as a stock identification tool (Cadrin & Friedland, 2005; Rawat et al., 2017; Mallik et al., 2020). In this context, landmark-based truss analysis has been successfully used alone by several authors for the discrimination of fish stocks (Hoff et al., 2020; Moreira et al., 2020; Rasheeq et al., 2023), or combined with other discrimination methods such as genetics (Kaouèche et al., 2013; Hammami et al., 2016; Zhang et al., 2023), otolith shape (Hari et al., 2020; Muniz et al., 2020; Schroeder et al., 2022) and otolith elemental analyses (Khan et al., 2012; Miyan et al., 2016; Schroeder et al., 2023).

Regarding body shape, the Spanish individuals, although bigger in SL (Table 4-1), after size allometric correction were found to have the smallest body measurements (75% of the total recorded distances), namely in terms of head length, heights and widths, the majority of fins lengths and distances and the anterior and posterior fish heights (DT1, DT3-DT13, DT15-DT19, DT25 and DT28 and DT37). The hydrology of the Spanish sampling location (Bay of Biscay) is particularly complex due to interactions between the general oceanic circulation, topography, highly energetic tidal currents, wind-induced currents and river inputs of freshwater, mainly located on the French coast (Druon et al., 2005; Karagiorgos et al., 2020). Other authors have reported that body, head and fins in fish are highly affected by water velocity and fish with a streamlined morphology exhibit enhanced capability to counter hydrodynamic resistance within fast-flowing water (Páez et al., 2008; Akin & Geheber, 2020; Sánchez-González et al., 2022). In this context, the particular oceanographic characteristics of the Bay of Biscay could have induced some morphological adaptive variations in *C. lucerna*'s body shape and explain the smaller distances within fish shape, despite the larger size of the sampled individuals.

The United Kingdom individuals, that recorded a smaller SL (Table 4-1), have also recorded the smaller mouth sizes and caudal peduncle areas (DT2 and DT20-DT24). Conwy Bay is an inlet of the Irish Sea, which is generally characterized by large tidal energy input from the Atlantic (Hadziabdic & Rickards, 1999). The Bay is recognized for its unusual and varied coastal and intertidal habitats and their associated reef communities (Natural Resources Wales, 2015), which are factors that can influence *C. lucerna*'s feeding regimes and fish habitats and explain the recorded phenotypic regional differences.

The larger overall measurements (70% of total recorded distances) were found on the Portuguese individuals, namely in terms of head length, mouth and anterior body size and peduncle area (DT1-DT9, DT11-DT13, DT15-DT17, DT20-DT24 and DT30-DT35). Fish head and mouth sizes may reflect differential habitat use, variations in feeding behaviors or the capacity to explore different ecological niches with different types of prey (Park et al., 2001; Kaouèche et al., 2017; Baldasso et al., 2019), while the lengthening of the caudal peduncle is usually associated with fish swimming ability in strong hydrodynamic environments (e.g., water currents) (Hammami et al., 2013; de Barros et al., 2019; Larouche et al., 2020). The Portuguese coast presents different hydrographical features influenced by the Canary and the Portuguese currents, both connected to the North Atlantic Subtropical Gyre (Barton, 2001) which could induce morphological variances in body shape, namely in the peduncle area. The largest distances between fins, larger 2nd dorsal fins, higher posterior body height, larger caudal fin areas and posterior body length (DT10, DT14, DT18-DT19, DT25-DT29 and DT36-DT37) were also recorded for the United Kingdom individuals. Fish body form, fin length and location are adaptations for movement that indicate differences in habitat exploitation (Webb, 1984), which is aligned with the distinctive and diverse environments found in eastern Irish Sea (Hadziabdic & Rickards, 1999; Natural Resources Wales, 2015).

Finally, the effect of the sampling period in the truss networking results cannot be disregarded in this study. At each site, samples were collected at different times of the year, which, in conjunction with the life cycle (e.g., spawning period), may have a significant impact on body shape. It is well known that for the Mediterranean Sea this species has a protracted spawning season but with peaks occurring at different sites (Boudaya et al., 2008; El-Serafy et al., 2015). However, data for the Atlantic Ocean is limited. Anyway, a previous study that was conducted in the NE Atlantic reported that females attained maturity at smaller sizes (27.7 cm vs. 29.1 cm) and younger ages (2.7 years vs. 2.8 years) compared to males (McCarthy & Marriot, 2018). This shows that basic data about the reproductive biology of *C. lucerna* are still needed in the Atlantic waters.

Another factor that can play a role in the differences observed among the three sampling locations could be attributed to distinct regional anthropogenic influences (e.g., pollution, habitat alteration) (Kruitwagen et al., 2006; Franssen et al., 2012; Santi et al., 2021). But further investigation is needed.

4.5.Conclusions

Regardless of the reason behind the regional morphological differences, our results concerning the geometric morphometrics analyses have shown significant differences among the three sampling locations, with a high overall reallocation success (95%) of individuals to the original locations. These data indicate that *C. lucerna* individuals caught in the three fishery grounds along the northeast Atlantic do not belong to a single and homogeneous population unit, despite a slight visual overlap in the FDA between the English and Spanish individuals, which suggests that fish from these locations may somewhat inhabit similar environments. Finally, the results suggest that these fisheries should be managed regionally as different population units.

However, since the regional differences found in this study regarding the sample number, size range, temporal collection window, sex ratio and age structure of the caught individuals could somewhat confound the ontogenic effects on phenotypic body variation (Cadrin, 2000), it is recommended to conduct future studies with a holistic approach using other natural tags, such as genetics, parasites fauna and otolith chemistry. In addition, studying individuals from a broader number of sampling locations would also allow for a better understanding of the northeast Atlantic population structure.

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Informed Consent Statement: Not applicable.

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

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CHAPTER V. Final Discussion and Conclusions

Stock identification is an essential component of the modern fisheries stock assessment models, and the understanding of a species population structure is vital to design rational management measures and appropriate conservation strategies (Begg & Waldman, 1999; Begg et al., 1999; Cadrin et al., 2014). Although different approaches have been used to infer stock structures, based mostly on genetic or phenotypic variations, there is currently not a single methodology that can discriminate between all fish stocks (Begg & Waldman, 1999).

Hydrodynamic processes in marine ecosystems, such as water temperature, primary productivity and ocean circulation interact with biological processes affecting species distribution and population dynamics (Cowen, 2002). The population structure of most marine organisms, including most demersal fishes, is influenced by a biphasic lifecycle determined by the free-swimming pelagic larval stage (high dispersive) and by the settlement-recruitment stage of the juvenile and adult individuals (low dispersive) (Leis, 1991; Schunter et al., 2019; Morgan, 2022). Since marine fish populations are regulated by both the larval and juvenile-adult phases, understanding their stock dynamics is especially challenging (Morgan, 2022).

Connectivity within marine species plays a fundamental role in population replenishment, genetic diversity, spread of diseases and invasive species, and resilience to human exploitation (Cowen et al., 2006; Robins et al., 2013). It depends on early life-history traits (e.g., pelagic larval duration, growth rates, larval condition and mortality rates) (Galarza et al., 2009; Shima & Swearer, 2010; Selkoe & Toonen, 2011), larval behavior (e.g., vertical migration, swimming and orientation capabilities) (Leis et al., 2007; Martínez-Quintana et al., 2015; Gary et al., 2020), habitat characteristics (e.g., bottom topography, water depth and distance from shore) (Largier, 2003; Roughan et al., 2005; Pineda et al., 2007), reproductive ecology (e.g., migration movements, spawning patterns and reproductive strategies) (Riginos & Victor, 2001; Bradbury et al., 2008; Treml et al., 2012), and oceanographic processes (e.g., circulation patterns, upwelling/downwelling phenomena, tides, currents and eddies) (White et al., 2010; Munguia-Vega et al., 2018; Torrado et al., 2021). Nonetheless, fish population structure, connectivity and behavior patterns have been inferred using diverse approaches, namely through otolith shape, otolith microchemistry and fish morphology (Begg & Waldman, 1999; Begg et al., 1999; Cadrin et al., 2014).

The tub gurnard, *Chelidonichthys lucerna*, is a benthic fish found in depths from 20 m to 381 m, although it seems to be more abundant in shallower waters up to 150 m, being widely distributed along the northeast Atlantic and the Mediterranean Sea. Due to its broad spatial distribution and the presence of several geographic and oceanographic barriers within its wide distribution range, the species could potentially consist of various distinct population units.

C. lucerna exhibits a pelagic phase during its larval stage (Dulčić et al., 2001; Vallisneri et al., 2012) and younger individuals are frequently found in shallower waters, along coastal and estuarine areas (Quigley, 2005; Montanini et al., 2017; Campos et al., 2022). The abundance of food and shelter provision in these environments make these areas ideal nurseries for juvenile fish (Potter et al., 2015; James et al., 2019; Whitfield, 2020). Older and larger individuals, on the contrary, are usually found more dispersed towards offshore waters (Papaconstantinou, 1984; Colloca et al., 1994; Eryilmaz & Meriç, 2005). Fish migration between shallower and deeper waters seems to happen not only as fish grow but also throughout the year: during spring and summer individuals show a more pronounced concentration in shallower waters, and during the winter months in offshore waters (Montanini et al., 2017; Carbonara & Follesa, 2019; Campos et al., 2022). The species feeds mostly on epibenthic and nectobenthic organisms (Vallisneri et al., 2011; Stagioni et al., 2012; Montanini et al., 2017) and has shown to have an important nutritional value as seafood (Roncarati et al., 2014; Duyar & Özdemir, 2022).

The oldest *C. lucerna* on record is estimated to be 14 years old (Baron, 1985). Although during its first year of life *C. lucerna* overall growth has been recorded to be faster than during the following years (Işmen et al., 2004; İlhan & Toğulga, 2007; El-Serafy et al., 2015), females seem to grow at a slower pace (Eryilmaz & Meriç, 2005; El-Serafy et al., 2015; McCarthy & Marriott, 2018). Similarly to other fish species, *C. lucerna* males mature earlier than females (İlhan & Toğulga, 2007; Vallisneri et al., 2012; El-Serafy et al., 2015). *C. lucerna* females have recorded larger sizes and lifespans (Işmen et al., 2004; McCarthy & Marriott, 2018; Rodrigues, 2020) which have been associated to their increased fecundity, higher offspring fitness and reduced mortality consequence of a diminished predation risk due to their greater body size (Boudaya et al., 2008; Pauly, 2019; Niu et al., 2023) . Although *C. lucerna* reproduction occurs all year round, the spawning peak appears to occur in different regions during the winter months (İlhan & Toğulga, 2007; El-Serafy et al., 2015; J. Rodrigues, 2020).

Although usually captured as by-catch in demersal fisheries (ICES, 2007, 2010), the species has important economic value in the eastern Mediterranean fishery markets (İlhan & Toğulga, 2007; Cicek et al., 2008; Duyar & Özdemir, 2022). Moreover, it is the most important gurnard species for the Italian fisheries (Vallisneri et al., 2011), and the most important Triglidae species captured by the Portuguese traditional fisheries (Feijó et al., 2008). In 2006 *C. lucerna* was classified as a potential species for commercial exploitation and, although like other gurnards the species discard rates are tough to be very high (ICES, 2013, 2019, 2022), since 2010 worldwide fisheries landings have shown a trending increase with the Atlantic region providing most of the declared fish landings (FAO Fisheries and Aquaculture Department, 2023). To maintain high yields while avoiding putting stocks under pressure and potentially at risk of depletion, it is critical to better understand the species ecology and population biology. In this context, ICES has recommended monitoring programs to assess retained and discarded catches information on population biology (ICES, 2006, 2007, 2010).

Although at the onset of the present doctoral work, a few studies have investigated the population structure, diet composition, age, growth and reproductive biology of the species in the Mediterranean region (Stagioni et al., 2012; El-Serafy et al., 2015; Uyan & Turan, 2017), and in Northwest Wales (UK) (McCarthy & Marriott, 2018), specific research on the species' population dynamics, stock structure, movement patterns and habitat connectivity in the northeast Atlantic was almost inexistent.

To improve this gap, this thesis focused directly on the study of the species' population structure, habitat residency and connectivity. To accomplish this aim, a variety of natural tags-based approaches have been applied, namely otolith shape analysis, otolith chemical fingerprints, and body morphometric geometrics of individuals captured in the northeast Atlantic region.

Regarding Chapter 3, otolith shape fingerprints obtained by shape indices and elliptic Fourier descriptors did not clearly discriminate individuals from three northwest Portugal fishing grounds, with fish sampled revealing a low overall allocation rate to their original regions (51%). However, otolith microchemistry fingerprints showed different elemental signatures among the three fishing locations, with a relatively high overall re-classification rate of 73%. When combined, both otolith shape and elemental analyses corroborated the results obtained by the separate analysis, showing a moderate discrimination of fish to their original sampling location (74% re-classification rate). The hereby data suggest some connectivity between these fishing grounds and that these fish aggregations, albeit not entirely homogenous, are a single population unit and partially mix during their lifetime history.

Concerning Chapter 4, otolith core-to-edge transects of Sr:Ca from individuals collected from seven locations along the Portuguese continental shelf revealed a variety of migration patterns between brackish and marine environments throughout the fish lifecycle, with the large majority of sampled individuals (97%) showing a marine estuarine-dependent profile, as previously suggested. In addition, over half (63%) of the individuals showed a clear presence in marine waters during early life periods with quick ingresses into brackish waters, suggesting a coastal spawning and corroborating the nursery role provided by estuarine areas to the species. Moreover, the hereby results showed *C. lucerna* exhibits high environmental plasticity and adaptation.

Finally, in Chapter 5, morphometric geometrics of fish body through landmark-based truss network revealed the existence of significant regional differences resulting from the phenotypic variation among *C. lucerna* individuals collected along the northeast Atlantic, namely in the Irish Sea, Cantabrian Sea, and northwest Portuguese waters, with a high relocation success (95%) of fish to their original locations. This suggests the existence of different fish population units among these locations. Results also revealed a partial overlap between the body shape of individuals collected in the Cantabria Sea and in the Irish Sea, indicating these fish populations may inhabit similar environments.

Overall, the hereby results suggest that *C. lucerna* presents a dynamic migratory behavior throughout its lifecycle between adult´s marine and juvenile’s estuarine habitats, showing high environmental plasticity and adaptation, confirming that the species is clearly a marine estuarine-dependent fish species. Moreover, in the northwest Portuguese mainland coast, the species should be treated as a unique, but not necessarily homogenous, stock. However, in the larger northeast Atlantic region, the existence of distinct population units should be considered, suggesting that these stocks should be managed regionally. In short, the results provided valuable insights into the habitat residency, movement patterns, and population structure of *C. lucerna* in Portugal and in the northeast Atlantic region that underscore the urgent need for appropriate national and regional fisheries management strategies that consider the unique characteristics of each population unit and prioritize the preservation of critical habitats to ensure the conservation and sustainability of the species.

According to the last data reviewed by ICES, the exploitation of *C. lucerna* follows the general regulations in the areas where it is harvested, but no minimum landing size nor technical measures specifically dedicated to the species are yet considered (ICES, 2010). Given the increased trend of declared landings and the large contribution of the northeast Atlantic region to global production (FAO, 2023), the acquired findings of the present work are valuable to advise an adequate management framework of *C. lucerna* fisheries in Portugal and northwest Europe that would ensure maintaining healthy fish stocks in the long-term. Regulations that would contribute to ensuring the sustainability of the species stocks in the northeast Atlantic could consider, for example, the establishment of minimum landing sizes, so fish are not captured before reaching their first reproductive length, and the implementation of strategies for the protection and conservation of key habitats for the species life cycles, namely closed fishing grounds in the estuarine nursery areas.

Nevertheless, further research, encompassing the use of holistic approaches that consider a combination of a variety of techniques or the use of additional natural tags such as genetics and parasitic fauna, is recommended to make more accurate inferences and attain a more comprehensive understanding of the species' population structure across its expansive geographic range. In addition, further research to assess the fishing pressure and to collect more detailed information on the discard rates of the species would also be recommended. In the meantime, and similar to ICES management advice to other gurnard species (ICES, 2019, 2022), a precautionary approach that uses conservative reference points for the implementation of conservation and management measures to help ensure the fish stocks are maintained within safe biological limits would be recommended for *C. lucerna*.

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