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Nursery habitat colonization by juvenile sole (*Solea solea* L.) in the North Atlantic and Mediterranean – a latitudinal perspective

Ana Catarina Ventura Marques Vaz

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor Miguel Pardal (Universidade de Coimbra) e do Doutor Filipe Martinho (Universidade de Coimbra)

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Resumo

Os ecossistemas estuarinos estão entre os mais produtivos do mundo, conferindo funções importantes como áreas de viveiro para juvenis de peixes cuja desova ocorre em alto mar e as suas larvas migram posteriormente para áreas costeiras e estuarinas. Uma dessas espécies é o linguado comum, *Solea solea*, com elevada importância comercial e vasta distribuição, desde o Senegal até à costa da Noruega. O objetivo do presente trabalho foi analisar a tendência latitudinal nos processos de colonização das áreas de viveiro pelos juvenis de linguado, ao longo da sua área de distribuição geográfica: lagoa de Veneza (Itália), estuário do Mondego (Portugal), estuário de Vilaine (França) e Balgzand (Holanda). A análise da microestrutura de otólitos foi usada para estimar a idade, duração das fases pelágicas, metamorfose e do período de postura, sendo encontrada uma tendência latitudinal para os principais processos do ciclo de vida. A desova iniciou-se em Dezembro nas áreas mais a sul e em Fevereiro na população de Balgzand, assim como a eclosão. Um padrão latitudinal claro também foi encontrado para a forma do otólito, sendo os das regiões mais a norte mais esféricos no entanto, este facto pode estar relacionado com a idade dos indivíduos. Foi encontrada uma clara distinção entre as populações do norte e sul para a relação entre a data eclosão e a data à metamorfose. Finalmente, as populações mais a norte tiveram taxas de crescimento mais elevadas, o que indica a existência de uma compensação do crescimento contragradiente. Concluiu-se assim o importante papel no controlo do ciclo de vida exercido pela temperatura ao longo de um gradiente latitudinal.

Palavras chave: estuário; *Solea solea*; otólitos; padrão latitudinal; temperatura

Abstract

Estuaries are among the most productive ecosystems in the world, providing an important function as nursery grounds for marine fish whose spawning takes place offshore, and larvae migrate towards estuarine and shallow coastal areas. One of these species is *Solea solea*, a commercially important flatfish that is distributed between the Senegalese and Norwegian coasts. The aim of the present work was to analyse the latitudinal trend in nursery habitat colonization processes along its geographical distribution area: Venice lagoon (Italy), Mondego estuary (Portugal), Vilaine estuary (France) and Balgzand (Netherlands). Otolith microstructure was used to estimate age, the duration of the pelagic and metamorphosis stages, and the spawning period. A latitudinal cline was found for the main processes of the life cycle. Spawning started in December in the southernmost areas and in February in the Balgzand population, as well as hatching. A clear latitudinal pattern was also found for otolith shape, being the northern ones more spherical, despite that otolith shape may be related with age. A clear distinction between the northern and southern populations was found for the relationship between the hatch date and the date at metamorphosis. Finally, populations further north had higher growth rates, which indicate a countergradient growth compensation. This work highlighted the control exerted by temperature along the latitudinal gradient on species' life cycle and early life dynamics.

Keywords: estuary; *Solea solea*; otoliths; latitudinal pattern; temperature

Chapter 1 - Introduction

1.1 Estuaries as nursery areas for fish

Estuaries are one of the most productive ecosystems in the world (Costanza et al., 1997; Beck et al., 2001; Elliott & Hemingway, 2002; Kennish, 2002), although they are among the most threatened too (Blaber et al., 2000; Vasconcelos et al., 2007). Over the last years, there has been an increasing of interest in studding these areas, in order to protect, preserve and recuperate them (Blaber et al., 2000; Elliott, 2002). They make the interface between land and ocean, which confers them several important functions, (Costanza et al., 1997; Elliott & Hemingway, 2002), such as the control of river flow, nutrient retention, protection against floods, recreation and navigation areas, nursery areas for fish and invertebrates, migratory routes for several species, preferential feeding areas, wintering areas and low predator pressure (Elliott & Hemingway, 2002; Beck et al., 2001; Elliott et al., 2007). Estuaries are frequently chosen for the implementation of industrial activities, leading to their damage through an increasing in eutrophication (Cardoso et al., 2004), over-exploitation and pollution, affecting the local fish communities (Costanza et al., 1997; Blaber et al., 2000; Elliott & Hemingway, 2002), among others.

Due to the natural mixing of marine and freshwater, the species that live in estuaries have special adaptations (Wolowicz et al., 2007). Once in estuaries, the individuals experiment several unfavourable natural and anthropogenic conditions, such as temperature and salinity fluctuation, chemical contamination and deepening of channels, which lead to an increasing of stress in organisms (Costanza et al., 1997; Kennish, 2002), leading to multiple ecological impacts (Vasconcelos et al., 2012). Since this physic and chemical characteristics vary through estuaries, the densities of juveniles vary among them (Vasconcelos et al., 2007).

One of the most important functions of estuaries is providing nursery areas to several fish species, a large number of them commercially important, including flatfishes (Miller et al., 1984; Blaber et al., 2000; Cabral et al., 2007; Martinho et al., 2009; Vasconcelos et al., 2009). The nursery concept was created for fishes with complex life cycles, where spawning occurs offshore and than the larvae migrate to estuaries and shallow coastal areas. Once in the adult stage, fish leave estuaries and migrate to marine waters and join the adult community (Beck et al., 2001; Vasconcelos et al., 2012). The timing of estuarine colonization depends on the balance between salt and freshwater, due to wind, river flow and tidal cycles (Amara et al., 2000; Vinagre et al., 2009). During the estuarine stage, density-dependent processes influence the success of recruitment to adult habitats, for instance by variations in growth and survival (van der Veer, 1986; Miller et al., 1991), habitat quality and availability (Rijnsdorp et al., 1992). The reasons that make the estuaries such good nurseries for fish are the high food availability, low predation pressure, high turbidity and availability of protection sites, high temperature and multiple habitats that fish can exploit (Dahlgren et al., 2006).

Larval transport depends mainly on passive coastal processes (Rooper et al., 2006), using the currents to reach the estuaries (Churchill et al., 1999). In order for the species enter on estuaries they undergo vertical migrations, by actively moving to the bottom of the water column in order to catch the denser current of marine water that is entering in the estuary, facilitating their movements (Rowe & Epifanio, 1994; Shanks, 1995; Grioche et al., 1997). Changes in the extent of river plumes, as a consequence of different regimes in freshwater discharge, are also an important factor to their successful migration to estuaries, mainly by the presence of chemical cues (salinity, temperature, turbidity and potential higher primary production) that orient the individuals to the estuaries, indicating their proximity (Amara et al., 2000). In highly explored populations by fishing activities, recruitment is very important in order to compensate those losses (Brunel & Bourcher, 2006), since the fluctuation of the adult population size is controlled by the recruitment variability (Amara et al., 2001). The carrying capacity of the nursery and the connexion between estuaries and coastal areas is also very important to recruitment (van der Veer et al., 2000). A rapidly growth in this first months is very important to improve the survival (van der Veer & Bergman, 1987; Sogard, 1992), since fast growth means less time spent in the vulnerable life stages (Sogard, 1992). In addition, the inter-annual variability in juveniles densities and consequently in recruitment is associated to the variation in transport and survival of eggs (Nielsen et al., 1998; van der Veer et al., 2000; Martinho et al., 2009).

1.2 Latitudinal patterns in fish life cycle

Fishes have indeterminate growth patterns influenced by the environment (Campana & Thorrold, 2001) and populations in their limits of distribution seem to be mostly controlled by abiotic factors (Vinagre et al., 2008a; Martinho et al., 2013). The most dramatically variable in marine abiotic gradient is temperature, which decreases towards the poles (Gibson, 2005). The latitudinal variation of temperature leads to differences in the nurseries colonization, occurring earlier in southern areas, where the water temperature is higher (Amara et al., 2000). However, differences in the distance of the spawning areas to the nurseries, topography, transport and retention mechanisms, may also affect the nursery colonization patterns (Bailey et al., 2008).

In fact, a latitudinal cline has been observed in spawning of marine fish, starting earlier at lower latitudes and latter at more northern ones (e.g. Gibson et al., 2005; Vinagre et al., 2008a; Martinho et al., 2013). In southern areas, higher water temperature at the beginning of the spawning season contributes to a faster growth and an earlier colonization of the nursery areas (Martinho et al., 2013). In contrast, northern populations have a tendency to spawn at slightly lower temperatures and at shorter day length (Conover & Present, 1989). In areas with lower water temperature, this is compensated with faster growth during the most favourable period by countergradient compensation growth (Campos et al., 2009). The availability of food and predators pressure are also important factors that can exercise some influence and the food stock seems to be correlated with the latitude; however, a latitudinal gradient in predation is less clear (Miller et al., 1991). Also the recruitment variation exhibits a relationship with latitude (Leggett & Frank, 1997; Philippart et al., 1998) and the time of settlement varies from year to year, depending on the temperature (van der Veer et al., 2001). Some studies suggested that even the photoperiod has an effect on growth and spawning (Gibson, 2005; Laffargue et al., 2007; Vinagre et al., 2009; Martinho et al., 2013).

Over the last few years, climate has been suffering some changes and consequently, the marine ecosystems have been changing too, leading to shifts in geographical distribution of species (Brander et al., 2003; Perry et al., 2005; Grebmeier et al., 2006) and in the seasonal timing of biological events (Sims et al., 2005). The changes introduced by the climate strongly differ throughout the globe, and the increasing of temperature is expected to be more pronounced at poles (MacDonald et al., 2005). Since the southern species are more adapted to deal with the rise of temperatures, it is expected for fish to spread their range (Hermant et al., 2010). As said before, temperature has a main role on the life cycle (Gibson, 2005), regulating directly the metabolic factors (activity and feeding rates), swimming speeds and reproduction (Pörtner et al., 2001). Despite that the temperature has an indirect effect on mortality (Houde et al., 1987), drastical changes can lead to a direct mortality (Colton, 1959) and also the match between prey and predator life cycles can be affected (Drinkwater et al., 2010). For example, considering that spawning depends on temperature, it is expected an earlier occurrence of this stage (Martinho et al., 2013), which will influence the rest of the individual life cycle.

1.3 Fish otoliths

Otoliths are composed by calcium carbonate, and are the first calcified structures formed in the ontogenic process, which can be found in the membranous labyrinth of the inner ear in fishes (Popper et al., 2005). They are metabolic inert, with continuous growth and with the chemical elements of the surrounding ambient impregnated in them (Campana, 1999). Their unique mode of calcification may result from their physical and chemical isolation (Campana & Thorrold, 2001), and their function is related with the postural equilibrium and hearing (Popper et al., 2005). Bony fishes have three pairs of otoliths: sagittae, lapilli and asterisk.

Fish otoliths are a powerful tool in fisheries ecology, due to their use for age estimation (Campana & Thorrold, 2001), since they form daily basis in age and growth (Campana & Thorrold, 2001; Stares et al., 2014). Because they are not digested, they are useful for trophic studies, allowing to identify the prey and their length, permitting also the reconstruction of the environment where the individual was in a specific time (environmental history) by studying their chemical composition (Campana, 1999; Campana & Thorrold, 2001; Brown, 2006). So, they function as “flight–box recorders” for biological and chemical information from the formation of otolith to a death of the individual (Campana & Thorrold, 2001).

Otoliths record important events, allowing the age determination during the early life history – hatching, mouth opening, first feeding (Nash & Geffen, 2004), metamorphosis (Fox et al., 2007; Martinho et al., 2013) and habitat transitions (Tanner et al., 2011). They have a continuously growth through the individual life and during that time there are a formation of growth rings (Campana & Thorrold, 2001). The larval and juveniles stages present daily rings with constant frequency, and in adults they are annual with short periodicity (Campana, 1992). One day after hatching, there is a nuclear area with irregular form (Amara et al., 1998) and the appearance of the increments is more prominent after the mouth opens. Otolith growth is directly proportional to the somatic growth and is used for retro–calculation of growth (e.g. Lea, 1919; Campana & Jones, 1992; Martinho et al., 2013). Even in periods when the somatic growth is non-existent, the otolith growth is maintained (Maillet and Checkley, 1990).

With age, and especially in flatfishes, the complexity of otoliths increase: their shape changes and changes in increments’ appearance at the end of the larval stage are also visible (Neilson, 1992). This is due to the metamorphosis process (Gibson, 2005), changing from spherical to hemispherical form (Nash & Geffen, 2004; Gibson, 2005). On the initial phase of the metamorphosis, occurs the formation of an extra circumference of the otolith and during the first days the number of circumferences increases. These circumferences (i.e. accessory primordia) may appear as a result of the gravity during the transition of the orientation from dorsoventral to lateral (Mondin et al., 1996), and represent the passage from the pelagic to the demersal life - larval to juvenile stage (Lagardére & Troadec, 1997). After the metamorphosis, the increments return to their normal daily formation (Gibson, 2005). Hence, this process leads to marked differences between the increments of larvae and juveniles, since in the larval stages they are thinner and more regular. There also are some differences between the different pairs of otolith: the asterisk otoliths are irregular in shape and produce increments that are not related with daily patterns (Lagardére & Troadec, 1997); the lapilli otoliths have a bigger axis due to their latitudinal asymmetry, because they are relatively thin, with well defined increments and more uniform; the sagittae have the primordial accessory structures, which confers them more information (Vinagre et al., 2008a), being more amplified than the lapilli and easier to calculate the larval age (Lagardére & Troadec, 1997).

Data from otoliths can be used to determine growth rate in early life stages, stages durations and the effects of physical process on larval survival, as well as to reconstruct instantaneously daily growth rates of larvae fish, based on an empirical relationship between otolith size and fish size (Campana & Thorrold, 2001). In some otoliths clear patterns of summer and winter growth has also been found (Nash & Geffen, 2004). By analysing otolith microstructure it is also possible to identify periods of larval stress such as starvation, temperature or salinity stress (Campana, 1983; Eckman & Rey, 1987; Berghahn & Karakiri, 1990). The use of long-term datasets and with a wide spatial coverage will allow determining the influence of changes in climate in population dynamics (Starrs et al., 2014).

1.4 *Solea solea*

Flatfishes are among the marine species with higher commercial value that use estuaries and coastal areas as nursery grounds. Spawning takes place offshore and then larvae and juveniles migrate from the continental coastal to the nursery grounds inshore (Rijnsdorp et al., 1985; Grioche et al., 1997; Cabral et al., 2007), and transport varies seasonally with the meteorological and hydrological conditions (Talbot, 1977; Rijnsdorp et al., 1985) The spawning location is also critical, determining the success of the rest of the life cycle due to the variability in connectivity between habitats (Bailey et al., 2008).

Variations in water temperature, salinity and food availability, lead to a seasonal variation of the pelagic stage duration, since they function as regulators (Champalbert et al., 1992; Leggett & Frank, 1997; Amara et al., 1998). Also hydrodynamics is a key factor (Amara et al., 1993), which may lead to a higher dispersion, genetic variation, but also to higher mortality rates (Chambers & Leggett, 1987; Amara et al., 1993).This stage is responsible for determining the recruitment strength (Leggett & Frank, 1997; Gibson, 2005) and its inter-annual variation result from ambient fluctuations (wind, temperature, currents, tidal transport), fluvial discharges and predation (Leggett & Frank, 1997; van der Veer et al., 2000; Brunel & Bourcher, 2005; Martinho et al., 2012) that vary in geographic scale and along the latitudinal gradient (van der Veer et al., 2000; Vinagre et al., 2008a).

The species in study in this present work is the common sole, *Solea solea*, a marine migrant flatfish species, (Rijnsdorp et al., 1992; Elliott, 2002; Gibson, 2005; Vinagre et al., 2008a). It is characterized for having both eyes on the same side of the head, flattened shape, and ability to match the colour and patterns with their background. This is a species widely distributed in cold, temperate and tropical areas (Gibson, 2005) from the North Atlantic to Senegal (Whitehead et al., 1986). The adults live offshore, reproduce and then produce eggs that will drift in the water column. After hatching, larvae gain some ability to swim and start their migration to estuaries (van der Veer et al., 1990; Rijnsdorp et al., 1992; Bolle et al., 1994) and the year-class strength is determined in the pelagic phase stage (Rijnsdorp et al., 1995). Spawning is mainly controlled by the seasonal photoperiod and water temperatures (Scott, 1979), and sole enter in estuaries after metamorphosis and settlement (Amara et al., 2000; Ramos et al., 2010). The common sole nursery grounds are located in estuaries but also in marine coastal areas, and usually prefers areas with lower salinity; however, depth and sediment type also influence where they are located (Cabral et al., 2007; Ramos et al., 2010).

During the migration towards estuarine and coastal nurseries, they pass through a process of metamorphosis typical of all flatfishes, which include several marked morphological and physiological changes, passing from the pelagic larval stage to a highly specialized benthic life style (Rijnsdorp et al., 1995; Geffen et al., 2007). This period of transition is an important process to the recruitment, and adult success depends on it (Amara et al., 2000). The metamorphosis ends with the gain in length, shape and behaviour of juveniles, occurring morphological, anatomic and physiological transformation without interfering with their continuous growth (Lagardère et al., 1999; Amara et al., 2000). This is a process that depends on energy (Balon, 1986) and include drastic changes in body conformation, food type, in assimilation and digestion, since their internal organs are rearranged, and may become more vulnerable to predators, due to the affected vision and other senses (Geffen et al., 2007). The visible evidence that this process is occurring is the eye migration with a rotation of 90º (Amara et al., 2000; Gibson, 2005).

1.5 Objectives

The aim of this study was to identify the latitudinal patterns in estuarine nursery ground colonization processes for the common sole, *Solea solea*, in the Northeast Atlantic and Mediterranean coasts. Considering a wide geographical area, the specific objectives were: a) to determine the duration of larval and metamorphosis stages; b) estimate the duration of the spawning season; c) determine possible variability in juvenile growth rates across sampled areas; d) and to ascertain the possibility of occurrence of countergradient growth compensation, considering the constraints that latitudinal gradients might impose on specific populations.

Chapter 2 – Materials and Methods

2.1 Study area and sampling process

Juvenile fish for this study were obtained from several nursery areas located in the Northeast Atlantic and Mediterranean, ranging between 40ºN and 53ºN: Balgzand (Wadden Sea) - The Netherlands, Vilaine estuary – France, Mondego estuary – Portugal, and Venice lagoon – Italy (Fig. 1, Table 1). The sampling sites were chosen in order to cover a wide geographical area, which comprises most of the central and northern areas of distribution of *Solea solea* (Whitehead et al., 1986). Fish were collected in the Venice lagoon using a fyke net with 5 chambers and mesh size from 14mm (entrance) and 8mm (cod-end), in the Mondego estuary and Balgzand using a 2m beam trawl and 5mm mesh size in the cod-end and in the Vilaine estuary by using an 8m beam traw with 5mm mesh sizel. After fishing, fish samples were frozen and transported to the laboratory in iceboxes, and kept frozen until further analyses. Since the aim of this project didn't involve analyses of juvenile densities trends, the differences in sampling methods were not considered (as in Martinho et al., 2013).

Sampling was performed in the beginning of summer in all areas, considering the available information of the spawning period in literature, in order to catch individuals once the migration towards the nursery areas was terminated. Sampling in the Balgzand took place on the 7th of Jully 2010, in the Vilaine estuary on the 1th of July, in the Mondego estuary on the 3th of June and the 6th of July 2011, and in the Venice lagoon on the 10th of June 2012.

The average monthly sea surface temperature (SST) data for 2010, 2011 and 2012 for each coastal area near to the chosen estuaries were obtained from the International Comprehensive Ocean Atmosphere Data Set (ICOADS) online database (http://dss.ucar.edu/pub/coads, dataset 540.1, Waley et al., 2005).

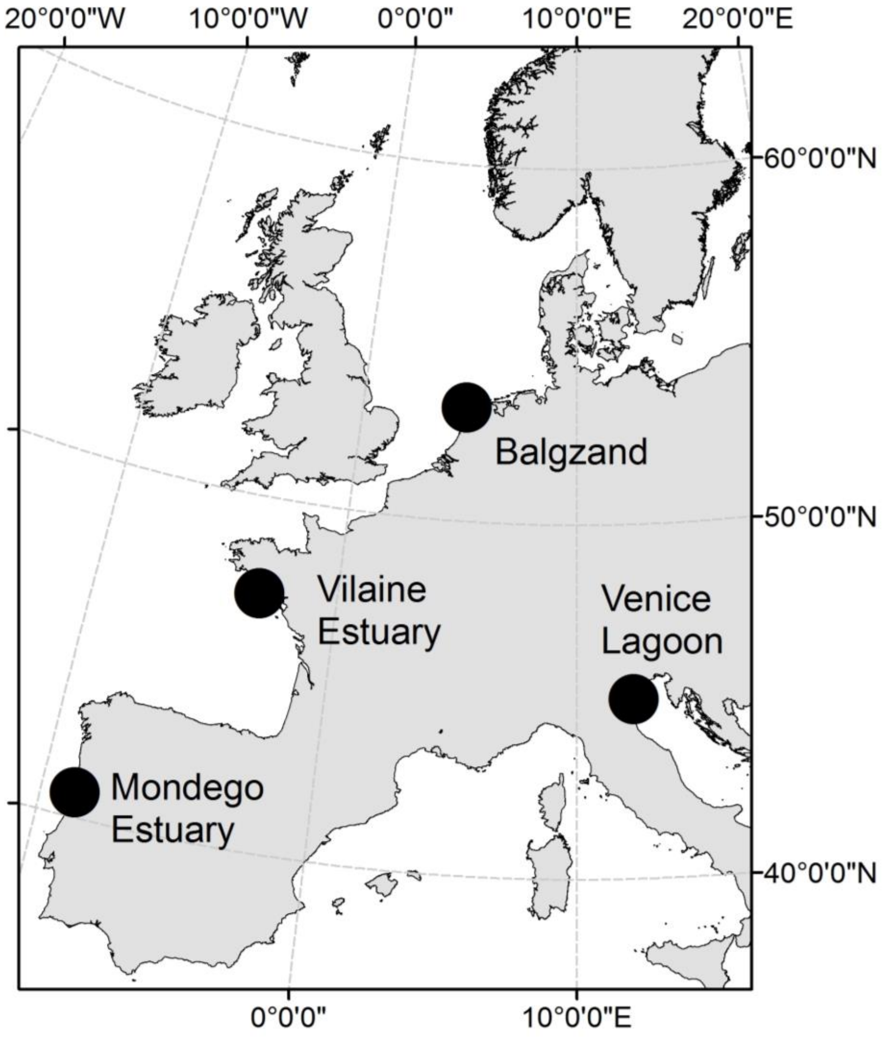


Figure 1 – Geographical location of the sampled nursery areas of 0-group *Solea solea* in the Northeast Atlantic and Mediterranean.

2.2 Otolith microstructure analysis

All fish were measured (total length, TL, mm) and weighted (wet weight, WW, g) (Table 1). Posteriorly, a sub-sample was chosen randomly from each site, representative of range of total lengths. The sagittae otoliths were removed, cleaned and mounted with the help of a resin, Crystalbond 509, on microscope slides. After this process, the otoliths were polished in the sagittal plane using 3µm sandpaper till a clear visibility of the daily rings. Using a light microscope, all microscope slides. Afterthis process, the otoliths were polished in the sagittal plane using 3μm sandpaper till a clear visibility of the daily rings. Using a light microscope, all daily increments were counted at 1000x magnification for the nucleus and 400x magnification for the metamorphosis and peripheral areas. The otoliths measurements were also performed at light microscope at 40x magnification for the otolith greater length and greater width. The otolith microstructure was used to determine the duration of the different stages duration: pelagic larval stage, which correspond to the otolith nucleus; the metamorphosis stage, corresponding to the irregular rings (i.e. accessory primordial); the benthic stage was determined from the first ring after metamorphosis till the edge of the otolith (Fig. 2). The increments were counted three times for each stage and an average was performed.

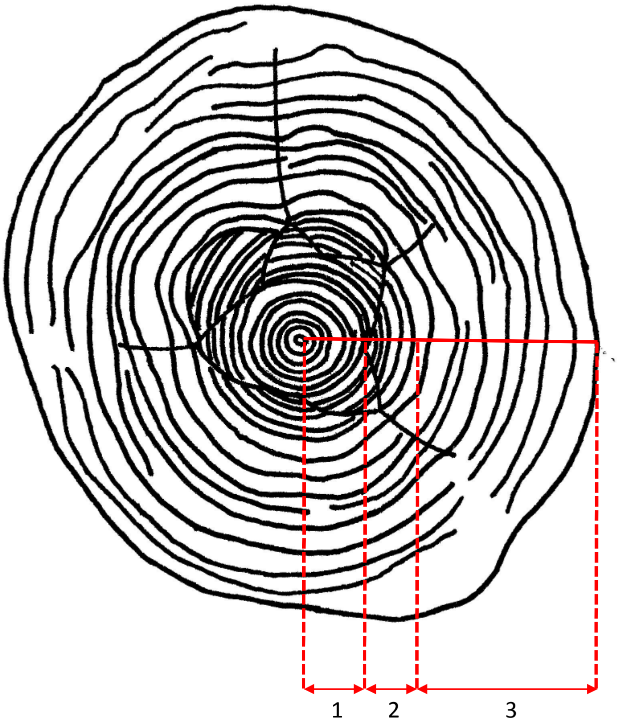


Figure 2 – Main structure of the sagittal otolith of sole juvenile: 1 – pelagic stage; 2 – metamorphosis; 3 – benthic stage. Based on Fig.2 in thePontual et al., 2003.

Whenever there were difficulties in age estimations with the sagittae otoliths due to a more irregular appearance, lapilli otoliths were used to confirm the counts.

Table I – Geographical features of each nursery area sampled for juvenile *Solea solea:* country, latitude (Lat.), longitude (Long.) and area, including also the total number of fish caught, the number of analysed fish for otolith microstructure, and respective mean wet weight (WW) and mean total length (TL).

Nursery area	Country	Lat.	Long.	Area (Km^2)	Total fish (n)	Analysed fish (n)	WW (g)	TL (mm)
Balgzand	Netherlands	N53º06'	E5º06'	52.0	35	34	0.9	40.0
Vilaine estuary	France	N46º30'	W2º30'	11.3	55	45	2.6	75.0
Venice lagoon	Italy	N45º18'	W12º54'	550.0	48	43	6.9	95.0
Mondego estuary	Portugal	N40º09'	W8º53'	8.6	64	56	3.6	80.6

In order to estimate the total age in days, spawning period, beginning of the pelagic stage, age at metamorphosis and hatch date, the following calculations were performed accordingly to Fox et al., (2007) and Martinho et al., (2013):

Estimated age = larval stage + metamorphosis stage + juvenile stage

Spawning period = hatch day of the oldest fish – hatch date of the youngest fish

Beginning of the pelagic stage = sampling date – average of the age

Age at metamorphosis = all increments counted until the onset of metamorphosis

Hatch date = sampling day – duration of the three stages

An estimated timing and duration of the spawning period for *Solea solea* at different latitudes, comprehending the species range, was collected from literature in order to compare with the present work and analyse a possible latitudinal pattern. In order to verify differences in the shape of the sagittae otoliths, the greater length (GL) and the greater width (GW) were measured perpendicularly, and was calculated the ratio between GL and GW; a ratio equal to 1 indicates a rounded shape, whereas a ratio larger than 1 indicated a more oval-shaped otolith.

2.3 Data analyses

The respective total length – otolith length relationship was determined for all populations. After counting all rings, a linear regression was performed between the total length and the estimated age for all populations. Geographical differences in hatch day, duration of the pelagic stage and metamorphosis were assessed using a one way non-parametric Kruskal-Wallis ANOVA, since data did not meet the necessary requirements for an ANOVA, followed by Dunn's pairwise multiple comparison. It was also performed an ANCOVA to compare the slopes of the relationship between total length – otolith length and total length – age relationship amongst all the sampled areas. A significance level of 0.05 was considered in all test procedures.

In order to find a possible latitudinal pattern in the shape of the otoliths it was performed a relationship between the length and the width of the otolith. Finally, a relationship between the hatch day (days) and the day at metamorphosis (days) and between the hatch day and temperature (^{o}C) was also established.

Chapter 3 - Results

3.1 Environmental conditions

Sea surface temperature (SST) for the coastal areas near each nursery followed a typical pattern of temperate regions, with warmer temperatures in the summer months and colder during the winter (Fig. 3). The lowest water temperature was recorded near the Balgzand, with an average of 10.7ºC (±1.0), followed by the Vilaine estuary (13.6ºC ± 1.1). The Mondego estuary was next on the temperature range with an average of 17.2ºC (±0.6), where the water temperature was more constant during the three years, and finally, the Venice lagoon with an average of 18.9ºC (±1.2), being the area with the highest thermal amplitude.

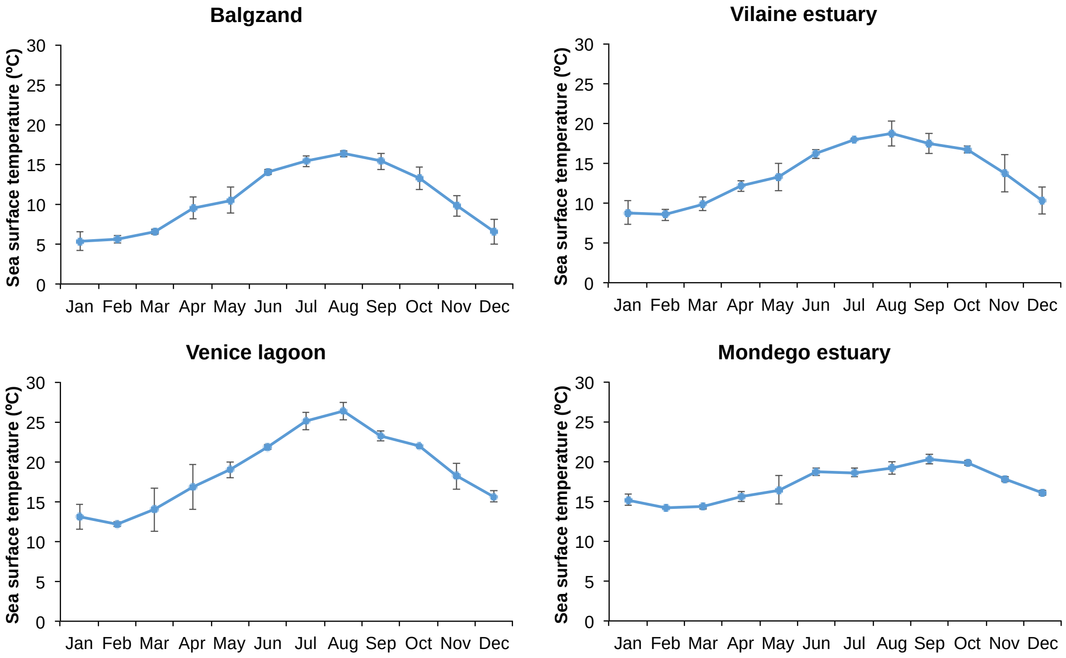
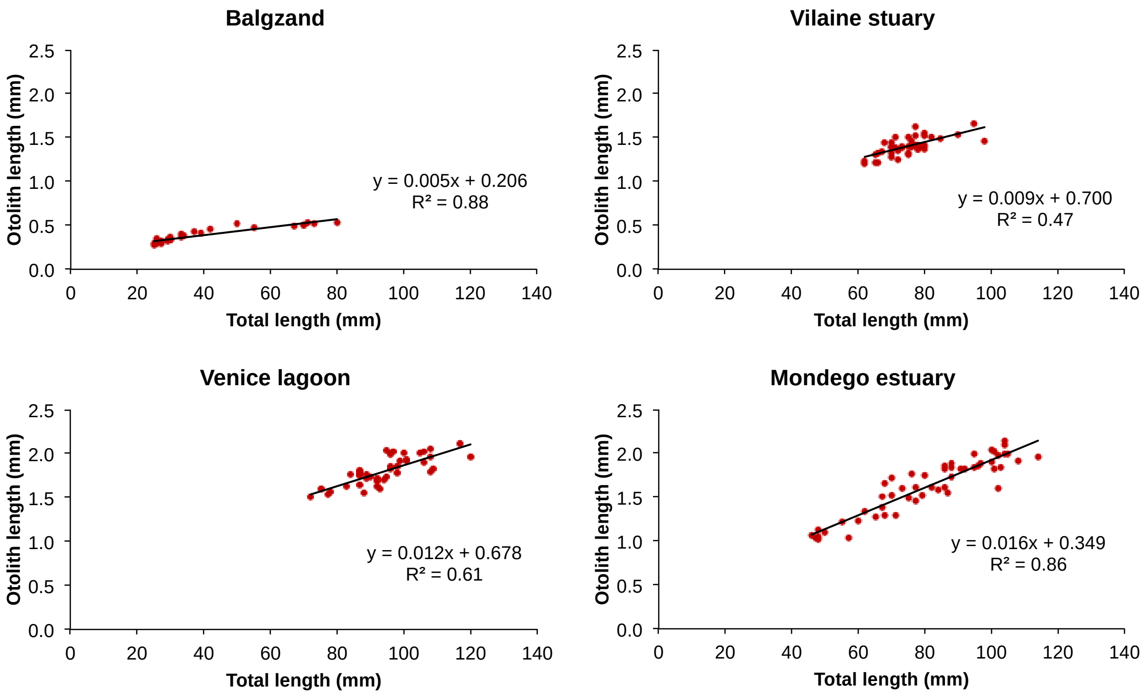


Figure 3 – Average sea surface temperature (SST) values for the period between 2010 and 2012, for the coastal areas near the sampled areas. The vertical bars represent the standard deviation.

3.2 Otolith microstructure analysis

In Figure 4 is represented the relationship between the total length (mm) and otolith length (mm). A significant linear relationship was obtained for all sampling areas, and differences between slopes where observed for the Balgzand and Mondego (F=285.2; $p<0.05$).



Balgzand

Vilaine stuary

Venice lagoon

Mondego estuary

Figure 4 – Linear regression between total length (mm) and the otolith length (mm) for 0-group juvenile *Solea solea* in all sampled areas.

A significant linear regression model was obtained for all the sampled areas between total length (mm) and age (days) (Fig. 5). Differences were observed in the slopes and intercept between the Venice lagoon, Mondego estuary and Balgzand (F=258.2; $p<0.05$).

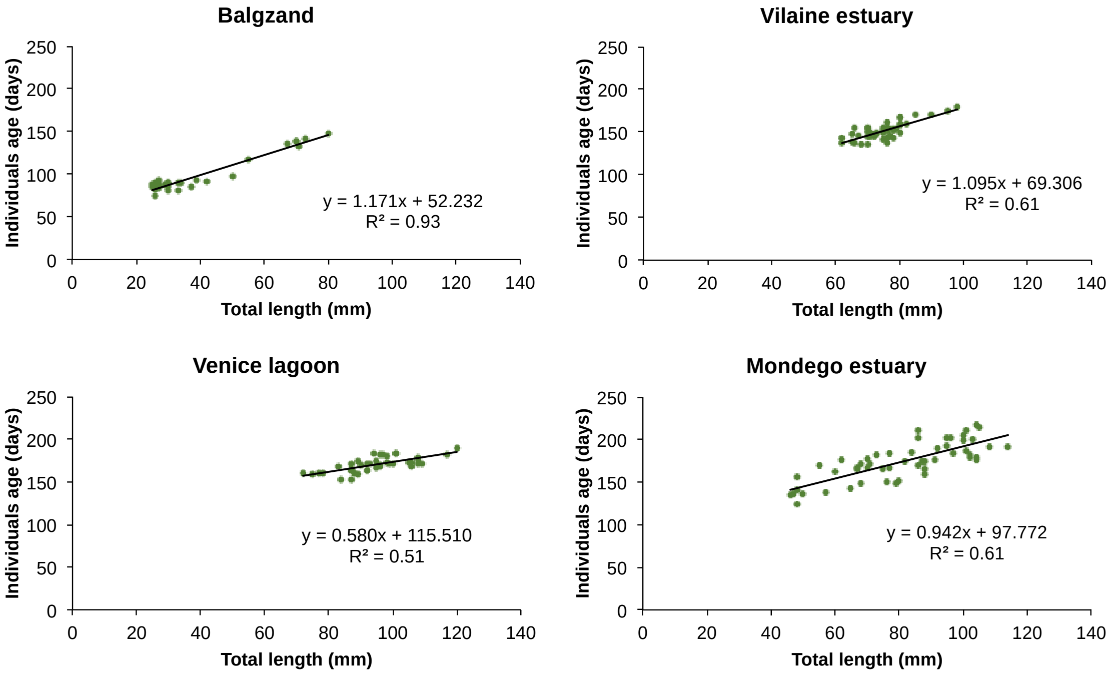


Figure 5 – Linear regression between total length (mm) and estimated age (days) for 0-group juvenile *Solea solea* in all sampled areas.

Age-standardized total length values for fish with 150 days were different across sites, with larger individuals from the Balgzand and the smaller ones from the Mondego estuary (Fig. 6).

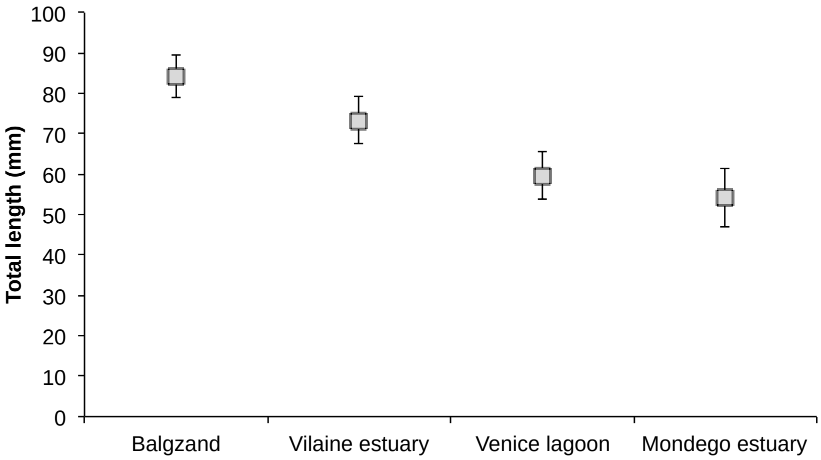


Figure 6 - Age-standardized total length values for fish with 150 days, calculated by means of the significant linear relationships obtained in all sampling sites between total length (mm) and age (days). Error bars represent the 0.95 confidence level.

Otolith shape analysis, given by the ratio between GL and GW (Fig. 7), identified two groups: the north group, composed by the Vilaine estuary and Balgzand, with a more regular and spherical shape, and the south one, composed by the Venice lagoon and Mondego estuary populations, with more oval-shaped otoliths.

Figure 7 – Relationship between greater length (GL) and greater width (GW) for 0-group juvenile *Solea solea* otolith, for all sampled areas.

3.3 Geographical variation in life cycle

In Figure 8 is represented the duration of the pelagic, metamorphosis and benthic stages. A latitudinal cline was observed from the southern to the northern areas, where hatch started earlier at lower latitudes; consequently, the metamorphosis and benthic stages followed this trend. The hatch day was different between sites, particularly between the northernmost and southern areas (H=127.65; p<0.05). Differences in the pelagic stage duration were observed between all sampled areas except between the Mondego estuary and the Venice lagoon (H=128.62; p<0.05). The metamorphosis stage was also different between sites, except between the Vilaine estuary and the Venice lagoon (H=106.03; p<0.05), being longer in theBalgzand population.

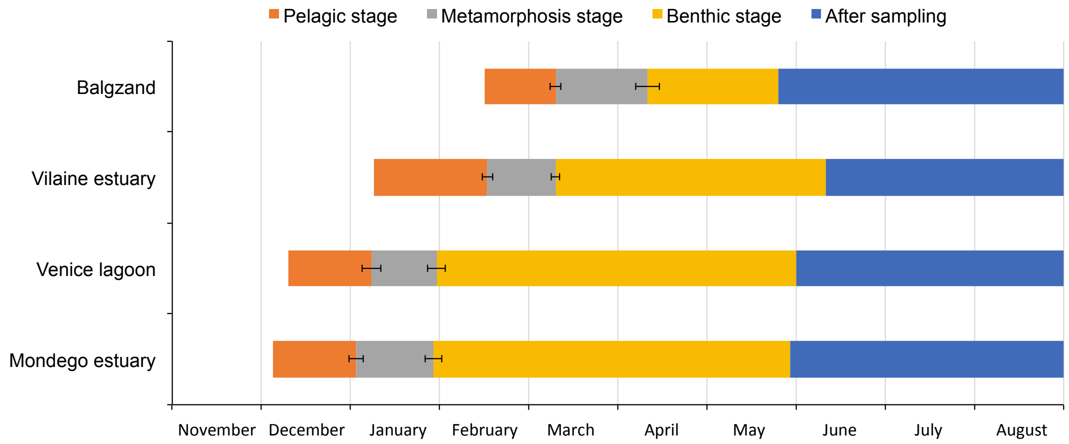


Figure 8 – Life-history stage duration by juvenile *Soleasolea* in all sampled areas.The mean values are presented, and horizontal bars represent the standard deviation.

A latitudinal cline was also observed for the mean hatch day according with sea surface temperature, with the southern populations hatching earlier in the season, at higher seawater temperature (Fig. 9). The more northern populations (Balgzand) hatched nearly 100 days later, at nearly half the seawater temperature when compared to the Venice lagoon populations.

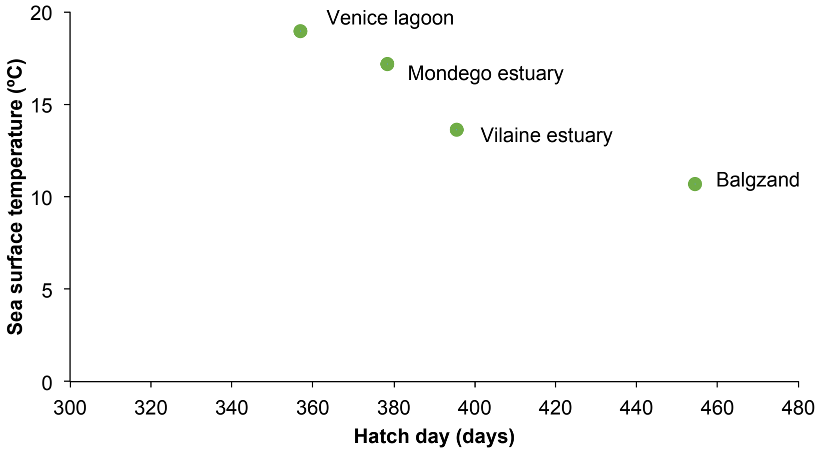


Figure 9 – Average variation of the hatch day with the average of sea surface temperature for 2010, 2011 and 2012 near to all sampled areas.

The duration of spawning according with the present work and published literature is presented on Table II. There was a greater variability in the spawning duration in the central range of the species distribution, being longer in the Vilaine estuary and a smaller in the Venice lagoon.

Table II – Duration of the spawning and metamorphosis for the different sampled areas and literature for 0-group juvenile *Solea solea*.

Site	**Lat.**	**Date**	**Spawning Duration (days)**	**Author**
North Sea	N56°30’	April -May	~60	van der Veer et al., 2001
North Sea	N56°30’	April-August	~120	Cuveliers et al., 2010
Irish Sea	N53°51’	Mid May	peak	Fincham et al., 2013
Wadden Sea	N53°35’	April-June	~90	Fonds, 1979
Balgzand	N53°06’	18th February-29th April	73	Present work
Bristol Channel	N51°22’	End of March	peak	Fincham et al., 2013
Authie estuary	N51°18’	28th January-15th April	78	Vinagre et al., 2008a
Vilaine estuary	N46°30’	3rd December-5th April	123	Vinagre et al., 2008a
Vilaine estuary	N46°30’	4th January-19th February	44	Present work
Venice lagoon	N45°18’	6th December-10th January	36	Present work
Douro estuary	N41°10’	23rd January-3rd March	39	Vinagre et al., 2008a
Mondego estuary	N40°09’	1st December-5th March	94	Present work
Tejo estuary	N38°37’	12th February-2nd April	50	Vinagre et al., 2008a

A relationship between hatch day and the day at metamorphosis was observed for all the sampled areas (Fig. 10), comprising two distinguishable groups: the north group, composed by the Vilaine estuary and Balgzand

Balgzandpopulations, and the south group, composed by the Venice lagoon and the Mondego populations. In more detail, the southern populations presented a positive relationship between hatch day and day at metamorphosis, while a contrasting trend was observed in the northern populations, implying that,for the north group, the pelagic stage duration decreases for the later hatched fish.

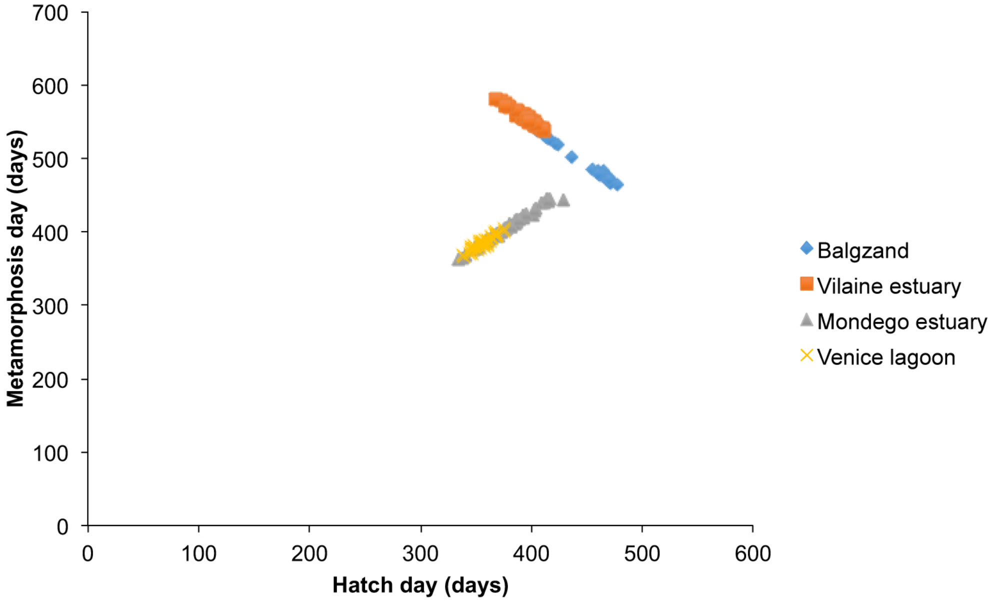


Figure 10 – Relationship between hatch day (days) and the day at metamorphosis (days) for 0-group juvenile *Soleasolea* in all sampled areas.

Larval length at metamorphosis was positively related with hatch day and day at metamorphosis, but only for the Balgzand population (R^2=0.74).The relationship between the larvallength (mm) and hatch day (days) and between the larvallength (mm) and the day at metamorphosis (days) were only significant for the Balgzand population (R^2=0.74 and R^2=0.72, respectively). No relationship between length and age at metamorphosis, and hatch day and age at metamorphosis was observed for any populations.

Chapter 4 - Discussion

4.1 Environmental conditions

This study described the variation of *Solea solea* early life stages along a latitudinal gradient ranging from 40ºN till 53ºN, allowing the establishment of trends along a significant portion of the species' distribution range. In fact, considering large-scale approaches in ecological studies allows determining patterns and processes along species distribution ranges, relating them with the prevailing environmental gradients (Martinho et al., 2013). Site selection was performed taking into consideration the species distribution range in the Northeast Atlantic and Mediterranean, and accessibility to samples: from the Balgzand, Wadden Sea (Netherlands, 53ºN) to the Mondego estuary (Portugal, 40ºN). The two intermediate sites, the Venice lagoon (Italy, 45ºN) and the Vilaine estuary (France, 46ºN), despite being almost at the same latitude, the prevailing environmental conditions were assumedly different, since the Venice lagoon is located in the Mediterranean Sea, where the temperatures and photoperiod are higher, whereas the Vilaine estuary is located in the French Atlantic coast.

During the period considered in this study, sea surface temperature near the sampled nursery grounds showed a typical variation of temperate ecosystems, with colder winter and warmer summer months mean values for 2010-2012: near the Venice lagoon were observed the higher water temperatures (18ºC ± 1.2), especially during summer, while near the Mondego estuary were observed the more constant temperatures during the three years (17ºC ± 0.6). Finally, near the northernmost area (Balgzand, Wadden Sea) were observed the lowest water temperatures (11ºC ± 1.0). Water temperature has a determinant role in the life cycle of fishes (e.g. Brett, 1979), by influencing fecundity (Pauly, 1994), spawning, egg growth and survival (Fonds, 1979), being also highly responsible for variability in recruitment patterns in marine fish (van der Veer et al., 2000). In addition to temperature, a latitudinal gradient can also be observed for photoperiod, which influences the onset of early life stages, their extent and respective growth rates (Devauchelle et al., 1987; Amara et al., 1993; Pörtner et al., 2001; Vinagre et al., 2008b; Dolbeth et al., 2010).

4.2 Otolith microstructure

Otolith growth is a function of somatic fish growth (Lea, 1910; Hickling, 1933), with a recognized function in equilibrium and hearing (e.g. Campana & Neilson, 1985). In agreement, a significant linear relationship between fish length and otolith length was observed for all areas, whose slope was only significantly different for the Balgzand and Mondego populations. Given these results, northern sole populations were characterized by much smaller otoliths than the remaining populations. Considering that otolith shape and growth can be influenced by temperature, growth rates and food consumption (Hussy, 2008; Javor et al., 2011), it seems that the northern populations of the Balgzand face highly contrasting environmental conditions than more southern populations, leading to distinct otolith growth patterns.

Age estimation from calcified structures such as otoliths is based on the assumption that the formation of distinguishable features follows a periodical (i.e. daily) frequency, and that the distance between features is proportional to fish growth (Campana & Neilson, 1985). For *Solea solea*, age reading in otoliths has been performed (e.g. Lagardère and Troadec, 1997; Amara et al., 1998; Vinagre et al., 2008a), providing a powerful tool for estimating age, as well as early life-cycle stage duration (Amara et al., 1998; Vinagre et al., 2008a). A significant linear relationship was found for all sites between the total length and the estimated age of *S. solea* juveniles. Differences between slopes and intercept were also found, in particular between the Mondego and Venice lagoon populations. These results indicate that there were different growth parameters between populations, and that the Mondego populations are older for a given length. Through an age-standardized total length values for fish with 150 days it was evident that individuals from different populations, with the same age, didn't present the same total length, which means that the growth rates were different: the individuals from Balgzand had a higher growth rate when compared with the southernmost populations. This provides evidence for the existence of a countergradient growth compensation mechanism, where individuals further north have a faster growth capability during the time window of more favourable environmental conditions (shorter in the northernmost areas). Similar patterns in growth parameters have been observed in northern populations of both marine fish and crustaceans (e.g. Campos et al., 2009; Martinho et al., 2013).

Otolith shape analysis was also performed, by assessing the relationship between the otolith length and width, which allows determining whether they are more round or oval-shaped. In this case, a value closer to 1 means that they are more spherical. It was evident a separation between northern and southern populations, with the northernmost presenting a more spherical shape, and the ones further south a more hemispherical one. On one hand, and since otolith shape analysis has been used as a tool for stock identification in marine fish (e.g. Aguera & Brophy, 2011; Javor et al., 2011), it might be possible that both groups can be considered as different stocks, given the existence of reproductive barriers at specific areas. Additional documentation has been provided by Symonds and Rogers (1995), who observed that sole adults from different populations in the Irish Sea and Bristol Channel have limited movements, with well-defined and exclusive spawning grounds. On the other hand, the northern populations were also the younger ones, which might explain the more round-shaped otoliths. As fish growth and metamorphosis occurs, otolith suffers morphological changes and tend to become more hemispherical (Nash & Geffen, 2005; Gibson, 2005). Still, more focused investigations on otolith shape at various latitudes will provide further information on the relationship between age, growth and otolith shape for this species.

4.3 Geographical variations in spawning

The most important requirements for a successful spawning are temperature, photoperiod and the presence of suitable hydrographic conditions for the transport of the eggs and larvae for the coastal waters (Symonds and Rogers, 1995; Vinagre et al., 2008a, 2009). Seasonal variations in these abiotic characteristics will lead to changes on the beginning, as well as on duration of this stage (Symonds & Rogers, 1995; Amara et al., 2000). However, spawning is also adapted to the timing of occurrence of both prey and predators (Grioche et al., 1997; Bailey et al., 2005). Populations further north tend to spawn at considerable lower temperatures and shorter day length (Conover & Present, 1990), starting later when compared with the populations further south (Vinagre et al., 2008a; Martinho et al., 2013).

In response to the different temperature regimes, a clear latitudinal cline was found for the day at which the spawning started: first in the Venice lagoon and in the Mondego estuary (6th December and 1st December, respectively), followed by the Vilaine estuary populations (January 4th), and lastly in the Balgzand (February 18th), exhibiting a delay between the southernmost and northern of nearly three months. Similar results were also observed for several other species, such as the European seabass *Dicentrarchus labrax* (Vinagre et al., 2009), European flounder *Platichthys flesus* (Martinho et al., 2013), and even for the studied species *S. solea* (Vinagre et al., 2008a).

The duration of spawning also increased with increasing latitude, with one exception: the Mondego population was out of trend, with an average duration of 94 days, when compared to the other populations: Venice lagoon – 36 days; Vilaine estuary – 44 days; Balgzand – 73 days. At higher latitudes, spawning of *S. solea* starts with increasing temperatures, (e.g. Devauchelle et al., 1987; Van der Land, 1991), with this process being related with gonadal maturation, which needs a minimum temperature to be fully completed (Lam, 1983). Again, with the exception of the Mondego population, the present results seem to agree with the established concept that egg growth and development is related with temperature (Fonds, 1979), and thus, northern populations should present longer spawning periods. Nevertheless, Vinagre et al. (2008a) already reported that major latitudinal gradients might be overrun by local conditions such as photoperiod, water temperature and coastal upwelling, which can provide an explanation for the especially prolonged spawning period in the Portuguese coast.

Based on published literature and on the present results, it was observed that sole spawning season lasts between 36 and 123 days, with an average duration of 73 days. In addition, the duration of spawning seems to follow a latitudinal trend. Comparing the present results with the ones from Vinagre et al. (2008a) also for the Vilaine estuary, it is clear a difference of 79 days in this stage duration, which may be due to the temporal difference on the sampling process (2011 and 1992, respectively), related to the increase in the water temperature over the last two decades, triggering specific responses by early life history dynamics of fish.

Following the onset and respective duration of spawning in all nursery grounds, the first individuals to hatch were the ones from the Mondego estuary and Venice lagoon in early December, followed by the Vilaine juveniles in the beginning of January and the last ones in Balgzand, in mid-February. In result, the onset of hatching exhibited a delay of nearly three months between the south and north populations, a situation similar to other flatfishes in the Northeast Atlantic (e.g. Martinho et al., 2013). These differences can also be explained by the prevailing environmental conditions (water temperature, day length and food availability), since the areas further south have higher water temperatures during all year, especially during winter, when compared with the northern areas. A particularly important negative relationship was observed with water temperature and hatch day. Other factors that might influence hatching are the evolutionary capacity of matching this stage with the ideal environmental conditions, as well as the phenology (Fincham et al., 2013), since the seasonal and interannual variations in climate and habitat influence the life cycle events.

4.4 Pelagic and metamorphosis development stages

The pelagic stage lasted longer in the central area surveyed, with an average of 38 days in the Vilaine estuary populations. In the northernmost area, Balgzand, the pelagic stage duration was the shortest of all sites. In fact, the coastal area exhibited the lowest average water temperature (10ºC ± 1.0), so it would be expected a longer pelagic stage. However, and since the time window of favourable conditions in this area is smaller than in more southern locations, fish larvae tend to grow faster during the time when conditions are more favourable. This mechanism is referred to as countergradient growth compensation as previously mentioned, according to which organisms at higher latitudes have developed microevolutionary adaptation responses for specific climatic patterns, which results in differential physiological performance (Kokita, 2004). Still, the duration of the pelagic stage is similar to what was determined by Rijnsdorp et al. (1992) for the Northeast Atlantic (20-40 days). In addition, shorter stage duration can also be caused by the proximity between the spawning and nursery areas (Martinho et al., 2013), which is the case for this area, as observed by Rijnsdorp et al. (1992).

Flatfishes usually reach the nursery areas in the end of metamorphosis, which sets the end of the pelagic stage; hence, and as stated previously, the longer the distance between the spawning and nursery areas, the longer it is expected for this stage to last. In fact, the spawning area is an initial critical factor for larval transport towards coastal nurseries (Bailey et al., 2008), with specific variability associated to its location, where temperature, winds, hydrographic conditions and retention mechanisms are determinant for a successful transport and dispersion (Fonds, 1979; Amara et al., 2000). The local interannual variability in the transport from the spawning areas to the nursery grounds is large (Tiessen et al., 2014), increasing with the increasing of distance between these two sites (van der Veer et al., 1998). However, different fish populations in different areas can adapt to different strategies (Nash et al., 2000), which may lead to local adjustments to the current or seasonal events (Boehlort & Mundy, 1987). This is what seems to occur in the Vilaine estuary, since the distance between the spawning and nursery areas is supposedly the largest, leading to a larger pelagic stage.

The onset of estuarine colonization can also vary greatly due to the balance between salt and freshwater, regulated by the combination of the wind direction and intensity, currents and tidal cycle (Primo et al., 2013). The extension of river plumes in adjacent coastal areas is also crucial for guiding the individuals to estuaries, given the presence of signals that attract larvae (Arvedlund & Takemura, 2006). In years with higher river flow, these plumes extend over a larger area and increase the probability of being detected by larvae (Vinagre et al., 2007; Martinho et al., 2013).

Metamorphosis corresponds to a transition stage between the pelagic and benthic environments, which in this species co-occurs with settlement (Geffen et al., 2007). Metamorphosis is also considered as a critical period for flatfishes, affecting the population dynamics (Yamashita et al., 2001; Van der Veer et al., 2000). The present results showed a longer duration in the Balgzand populations (31 ± 4.1 days), followed by the Mondego (26 ± 2.8 days). The shorter periods were recorded for the Vilaine and Venice lagoon populations (23 ± 1.4 and 22 ± 3.0, respectively). This lack of pattern may be attributed, once again, to the distance between the spawning and nursery areas, through the currents that allow the transport of the metamorphosing larvae (Rijnsdorp et al., 1985; Grioche et al., 1997; Bailey et al., 2005), combined with adaptations to local environmental conditions. Despite that Amara et al. (2000) associated the time at metamorphosis to the gain of a competent size, no influences of larval length on the onset of the metamorphosis were found in the present study. However, it was observed a clear effect of the hatch day on the onset of metamorphosis, with an evident segregation between the northernmost and southern areas. In the north (Vilaine and Balgzand) the later hatched individuals started earlier the process of metamorphosis, while the contrary was observed for the southern populations (Mondego and Venice lagoon). Such evidence can be explained by the development of adaptation strategies to the time window in which all the environmental conditions are more favourable, which is expectedly higher in more southern latitudes. Hence, northern populations seem to shorten their pelagic stage by growing faster, since their time window with suitable temperature and food availability is shorter.

4.5 Final considerations

The present work elucidated about the importance of otolith microstructure analysis as a powerful tool for estimating age and stage duration of marine fish. It was also clear the main role of temperature as a control mechanism of these processes, leading to a clear latitudinal pattern in spawning, hatch day and consequently in the onset of the remaining stages, which began sooner at lower latitudes. Growth rates also followed this trend, where populations further north presented a slower growth. Considering the present climate change scenarios, with increasing air and water temperatures, it is expected that this species will suffer changes in its distribution range, abundance and life cycle characteristics.

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