



## Short communication

Spawning period of Senegal sole, *Solea senegalensis*, based on juvenile otolith microstructureCatarina Vinagre <sup>a,\*</sup>, Anabela Maia <sup>b</sup>, Rachid Amara <sup>c</sup>, Henrique N. Cabral <sup>a</sup><sup>a</sup> Centro de Oceanografia, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal<sup>b</sup> Tufts University, Department of Biology, 200 Boston Ave, Medford, MA 02144, USA<sup>c</sup> Université du Littoral côte d'opale, FRE 2816 ELICO, avenue Foch, 62930 Wimereux, France

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## ABSTRACT

The Senegal sole, *Solea senegalensis*, is a flatfish species distributed from the Bay of Biscay to the coast of South Africa. Although it is a species of high commercial value its spawning period remains poorly known. In the Tagus estuary, Portugal, two juvenile cohorts of this species colonize two independent nursery areas within the estuarine system. Left lapilli otoliths of these 0-group juveniles were used to estimate age and back-calculate hatch dates, thus allowing the estimation of *S. senegalensis* spawning period in the Portuguese coast. The two cohorts of *S. senegalensis* completed the colonization of the two nurseries in July. The ages of juveniles were estimated to range between 46 and 111 days in nursery A, and between 33 and 61 days in nursery B. Hatch dates ranged from April to early June for juveniles colonizing nursery A, and from May to early June for juveniles colonizing nursery B. The mean hatch date in nursery A was the 7th of May, while in nursery B it was the 25th of May. It was concluded that the spawning period for this species off the Portuguese coast was from April to June.

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## 1. Introduction

The Senegal sole, *Solea senegalensis*, is a highly valued flatfish that extends its distribution from the Bay of Biscay to the coasts of South Africa (Quéro et al., 1986). Despite being highly valued and having a wide distribution, the spawning period of this species remains poorly known.

There are various difficulties in the study of spawning in this species. One of them is that the location of its spawning grounds is unknown over its entire distribution range. Evidence from multi-cohorts of juveniles colonizing estuarine nursery grounds indicates that there is one main spawning period in spring and a secondary period in summer–autumn (Cabral, 2003). Gonad maturation in the wild and emission of eggs in captivity corroborate this evidence (Anguis and Cañavate, 2005; Dinis, 1986; García-López et al., 2006; Teixeira and Cabral, 2010).

Juveniles concentrate in estuarine nurseries where they benefit from high prey availability, suitable temperatures for rapid growth and refuge from predators (Haedrich, 1983). This species' ecology has been extensively studied in the Tagus estuary, in Portugal (Cabral, 2003; Cabral and Costa, 1999; Vinagre and Cabral, 2008; Vinagre et al., 2006a, 2006b, 2007, 2008a, 2008b, 2009a, 2011). The first cohort arrives in the estuarine nurseries in late spring and its highest densities are observed in summer, another cohort arrives in summer and in some years a late cohort arrives in the middle of autumn (Cabral, 2003).

This last cohort is not present every year, its timing is highly variable and it is generally weaker in abundance (Cabral, 2003).

The Tagus estuary has two distinct nursery areas (Fig. 1). Isotopic studies have shown that the juvenile cohorts of *S. senegalensis* that colonize these areas are independent, have different timing, present high site fidelity and do not migrate between nurseries (Vinagre et al., 2008a, 2011).

The estimation of the spawning period is crucial for species management since early life-stages constitute bottle-necks in the life cycles of most coastal fish species (Van der Veer et al., 2000). This means that recruitment to the adult stock is highly dependent on what happens during and after spawning. Environmental factors acting upon this life history stage strongly influence the spatial and temporal coexistence of larvae, prey availability, predator abundance, thus regulating year-class strength (Houde, 1987). Thus, it is very important to gather knowledge on the period of occurrence of spawning for the effective management of important coastal species, such as *S. senegalensis*.

The otoliths of this juvenile fish can be used to estimate their individual age and back-calculate the spawning period of each cohort. The daily increments that occur in the otoliths of marine fish (Pannella, 1971) are a powerful tool to study the early life history of fish and have been successfully used in the estimation of age and spawning period in other coastal species (Amara et al., 1994; Vinagre et al., 2008c, 2009b).

The aim of the present study is to 1) determine age and hatch-dates of the juveniles that colonize the Tagus estuary nurseries in order to 2) estimate the spawning period of *S. senegalensis* in the Portuguese coast, using otolith daily increments analysis.

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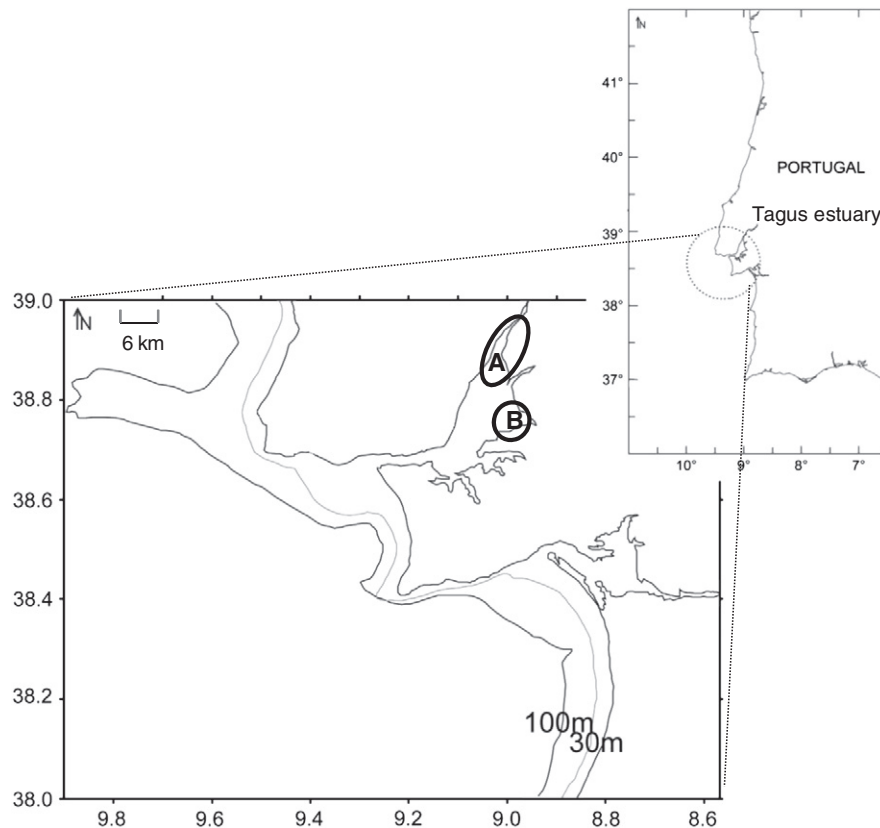


Fig. 1. Map of the Portuguese coast. Inset represents the Tagus estuary and location of its nursery areas (A and B).

## 2. Materials and methods

### 2.1. Study area

The Tagus estuary is located on the Portuguese West coast (Fig. 1). This estuary was chosen for this study because it is the most important nursery area for this species at its subtropical range (Cabral et al., 2007) and also because the ecology of *S. senegalensis* is well studied in this area (Cabral, 2003; Cabral and Costa, 1999; Vinagre and Cabral, 2008; Vinagre et al., 2006a, 2006b, 2007, 2008a, 2008b, 2009a, 2011).

The Tagus is one of the largest estuaries in Europe (320 km<sup>2</sup>) and much of its upper area is composed of extensive intertidal mudflats. Substrate in this area consists of mud or sandy-mud. Climate in this area is Mediterranean with mild winters and warm, dry summers (Aschmann, 1973).

Water temperatures in the adjacent coast of the study areas were compiled during a broad period that encompasses the spawning period (in the area between the 50 m and the 100 m bathymetric) at the World Data Centre for Remote Sensing of the Atmosphere (WDC-RSAT) and consist of Sea Surface Temperature derived from NOAA-AVHRR data. The range of SST values in this database is scaled between 0.125 °C and 31.750 °C (maximum temperature). The radiometric resolution is 0.125 °C. Data from all six of the passes that the satellite makes over Europe in each 24 h period were used. The SST maps were composed according to the maximum temperature value given at every pixel's position to minimise cloud coverage. Weekly values were derived from the daily maximum images using the average at every pixel's position.

### 2.2. Juvenile collections

Both nurseries were surveyed monthly from March to October 2005 in order to determine the beginning and the end of estuarine

colonization by 0-group *S. senegalensis*. From late June and during July surveys were intensified, taking place at approximately two-week intervals, in order to better determine the end of the estuarine immigration process of the first cohort colonizing each nursery area (Fig. 1).

Sampling was carried out in 2005, at eight stations in nursery A and at six stations in nursery B (Fig. 1). Trawls were conducted with a 2.5 m beam trawl with 10 mm mesh size (stretched mesh) and a 5 mm cod end. All samples were immediately frozen. In the laboratory all sole were counted and total length (TL) measured to the nearest 1 mm. Length frequency distributions were analysed in order to determine the end of estuarine colonization (which is detected when no new recruits are arriving). The data were checked for normality with a Shapiro–Wilk test.

### 2.3. Spawning date estimates

Otoliths of each length category (5 mm) were examined. Lapilli were used because they are relatively thin and have well-defined increments spatially more uniform than in sagittae otoliths, which have accessory primordia (Vinagre, personal observation). The left lapillus (Fig. 2), which has the longest axis due to the bilateral asymmetry between the right and left lapillus, was used for all age estimates. The lapilli were removed from the cranium, cleaned and mounted with cyanoacrylate glue on microscope slides. They were polished in the sagittal plane to the central primordia, with a polishing bar of aluminium oxide. All increment counts were made using a light microscope at  $\times 400$  or  $\times 1000$  magnification. Otolith increments were counted three times, and the age was regarded as the mean of the three counts. Precision was estimated by computing the coefficient of variation. Otoliths were eliminated whenever the reading variation was above 5%. Age was determined as described by Amara et al. (1994), for 59 *S. senegalensis* from nursery A and 52 *S. senegalensis* from nursery B.

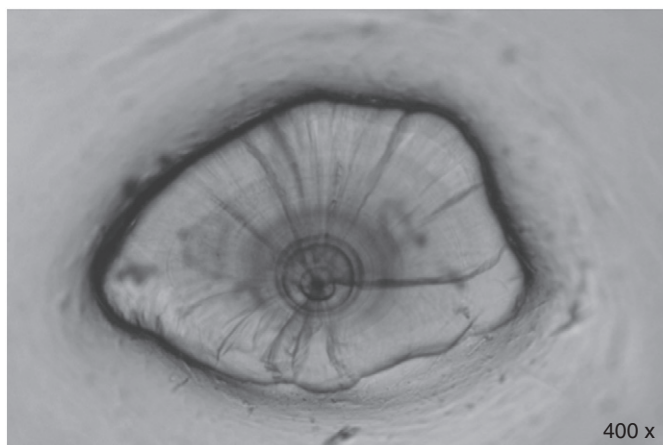


Fig. 2. Image of a left lapillus of *S. senegalensis* juvenile.

Daily formation of increments was also based on *Solea solea*, since it has not been validated for *S. senegalensis* (Lagardère and Troadec, 1997).

Spawning dates were estimated from age and date of capture. Duration of the embryonic period was calculated, according to the water temperature in the coast, based on Fonds (1979) equation for *S. solea*, a very similar species, due to lack of information for *S. senegalensis*. The equation used was:  $D = 34.4 T^{-1.290}$ , where D stands for days and T for temperature in °C.

### 3. Results

The first cohorts of *S. senegalensis* colonized both nurseries in June–July. Length–frequency distribution of 0-group *S. senegalensis* at the end of the colonization period (July) showed a normal distribution fit in both nurseries (Fig. 3). Juveniles' length was 36–99 mm in nursery A (number of individuals=59), while in nursery B it was 19–52 mm (number of individuals=52). The general aspect of the otoliths from nursery A and nursery B was different. More transparent in nursery B, more opaque and with a considerable incidence of deformations in nursery A.

Juveniles' ages were estimated to range between 46 and 111 days in nursery A and between 33 and 61 days in nursery B. Hatch dates from the juveniles that colonized nursery A ranged from 5th of April to June 9th of June, while in nursery B hatch dates ranged from 11th of May to early June 8th of June. The mean hatch date in nursery A was the 7th of May, while in nursery B it was the 25th of May. The distribution of hatch dates reveals that the spawning period was considerably more prolonged in nursery A than in nursery B (Fig. 5).

The first individuals to be spawned in April were exposed to mean coastal temperatures of 15.0 °C, those spawned in May were exposed to mean coastal temperatures of 16.5 °C and those spawned in June were exposed to mean coastal temperatures of 17.6 °C. During the spawning period of the juveniles that colonized nursery A, the mean SST in the adjacent coast was 16.4 °C, during the spawning period of the juveniles that colonized nursery B, yet also in summer (Fig. 4). These values were used to estimate the embryonic period of each cohort.

### 4. Discussion

Unlike what was described by Cabral (2003) in 2005, the two *S. senegalensis* cohorts arrived at the estuarine nurseries at different times but in the same month. In previous years, there was a cohort that arrived at nursery A in late spring, and peaked in summer, and a later one that colonized nursery B, yet also in summer (Cabral, 2003). In 2005, both cohorts peaked in July, which shows the variability

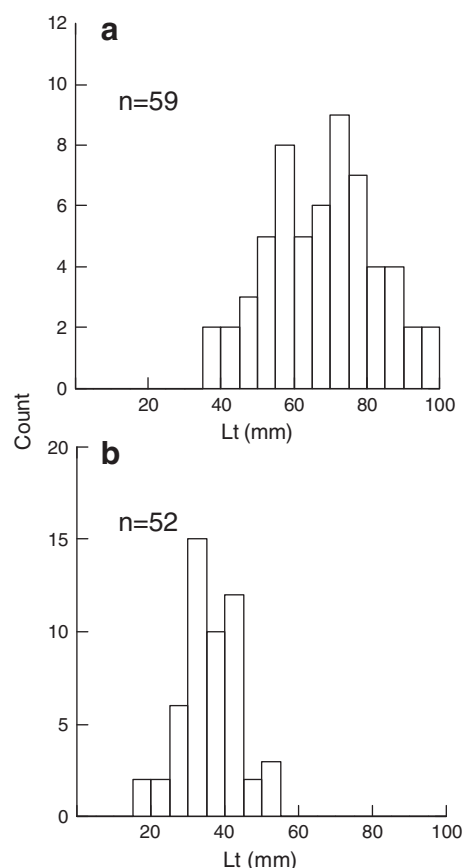


Fig. 3. Length frequency of 0-group juvenile *Solea senegalensis* in nursery A (a) and B (b).

inherent to spawning and recruitment in this species. There is evidence that the two cohorts were independent and that individuals did not mix since their length distributions were very different, as was the general aspect of their otoliths (more transparent in nursery B, more opaque and with a considerable incidence of deformations in nursery A). Isotopic studies done in previous years have already shown that the cohorts colonizing these two nurseries at similar times do not mix and remain spatially segregated for most of the period spent within the estuary (Vinagre et al., 2008a).

The spawning period estimated in this study, between April and June, is in accordance with previous evidence from gonad maturation studies and emission of eggs in captivity (Anguis and Cañavate, 2005;

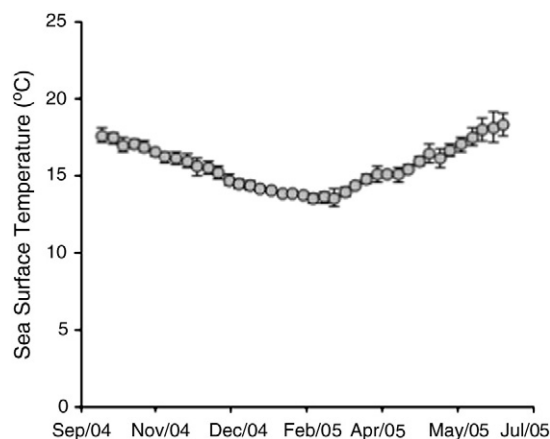


Fig. 4. Sea Surface Temperature in the coastal waters adjacent to the Tagus estuary, in 2005 (bars stand for standard deviation).

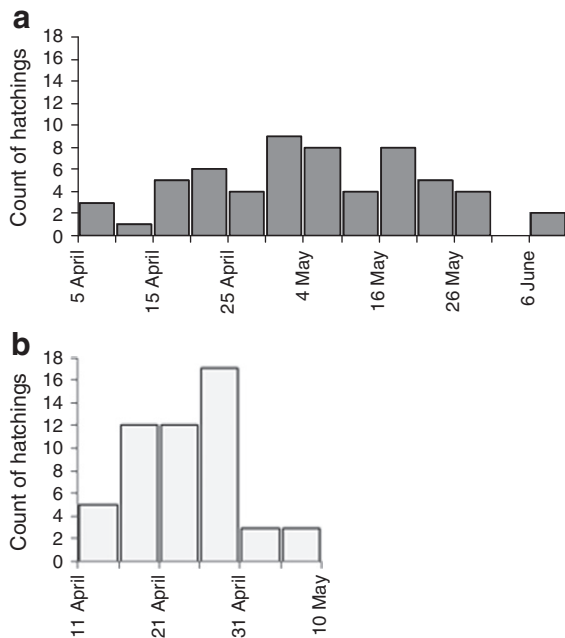


Fig. 5. Distribution of hatch dates for *S. senegalensis* from nursery A (a) and nursery B (b).

Dinis, 1986; García-López et al., 2006), as well as studies with juveniles of this species in the Tagus estuary, which examined length distributions (Cabral, 2003). These previous studies all pointed to a prolonged spawning period in spring, which was confirmed by the analysis of daily increments in lapilli otoliths in the present study.

Spring is a period when SST increases in the adjacent coast (Fig. 4) where adults live, which along with the increasing photoperiod, probably acts as a trigger for the onset of reproduction (Amara et al., 1994, 2000; Devauchelle et al., 1987). A prolonged period of spawning was expected and is typical of fish that inhabit warmer waters, where temperatures are generally favourable to gonad maturation and prey availability is more stable throughout the year (Pauly, 1994; Van der Veer et al., 2000). This contrasts with the spawning patterns of fish inhabiting colder environments, where prey availability and temperatures that allow gonad maturation are only suitable for a very restricted period during the year (Pauly, 1994; Van der Veer et al., 2000).

The high inter-annual variability in recruitment and probably spawning patterns observed for this species, may be related to the particular conditions found along the Portuguese coast. In this area upwelling phenomena occur in summer, with offshore Ekman transport of surface water (Peliz et al., 2002). Although upwelling is more frequent in summer, it is generally considered that winds that favour this phenomena are a recurrent feature of the Portuguese coast (Huthnance et al., 2002). Offshore Ekman transport of surface water will likely direct the eggs and larvae of flatfish away from the coastal nurseries, resulting in high mortality rates that will confound analysis and spawning season estimation based on otolith readings from the survivors. This has been previously noted by Vinagre et al (2008c) for *S. solea*, a flatfish species with a very similar life-cycle.

The reproductive strategy of this species, consisting of several spawning pulses, may be favourable for the survival of juveniles in an area of highly variable oceanographic conditions, such as the Portuguese coast. Survival from the egg stage to a juvenile that can colonize a nursery area depends not only on the timing of the spawning event but also on the conditions that affect the planktonic drift of eggs and larvae and on a successful estuarine colonization, which is affected by conditions in the estuary such as abiotic factors and prey and predators abundance. Thus, having several spawning pulses may be favourable for recruitment since it means that if one cohort is lost, other cohorts spawned at different times may still be successful. This

results in high variability in the number of cohorts colonizing the estuary, as well as in their timing of arrival.

For a more accurate estimation of the spawning period in this species, the methodology used in the present work should be applied to more years and other locations. This will allow the examination of how the spawning period varies inter-annually and will bring new insights into the timing of the different spawning pulses that originate different cohorts. Future studies should also investigate the environmental variables driving spawning in this species and controlling recruitment at early life-stages. The identification of the spawning grounds will also be crucial for stock management, since it would allow the regulation of fishing defence periods.

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