Population connectivity of *Solea solea* and *Solea senegalensis* over time

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ABSTRACT

A better understanding of connectivity patterns between estuarine and coastal areas is fundamental towards sustainable management of estuarine associated fish and identification of ecologically important habitats. Otolith geochemistry (Li:Ca, Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca, Pb:Ca) determined in juveniles of *Solea solea* and *Solea senegalensis* in two year classes was used to determine the estuaries of origin of age 3 + and age 2 + fish sampled off the Portuguese coast. The contributions of estuarine nursery areas to coastal adult populations obtained using maximum likelihood estimation varied significantly over the two years. However, one estuary (Ria Aveiro) consistently contributed a large proportion (30%–87%) to the adult populations of both sole species while a second estuary (Mira) contributed almost no adults (0%–3%). Comparison of otolith geochemistry between juveniles from characterized estuarine nurseries and adults indicated that unknown nursery areas may have contributed to the coastal adult populations, particularly in one year class. Nevertheless, the estimated relative contributions, based on otolith geochemistry, successfully identified the estuarine systems that accounted for most of the recruitment to adult stocks in the two years. Ultimately, this information should promote the development of effective conservation strategies and integrated fisheries management plans for these two commercially important species.

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1. Introduction

Many marine fish species use estuaries, bays or shallow coastal areas to complete their life cycles (Able, 2005; Beck et al., 2001). Juveniles spend months and up to years in these more sheltered areas before recruiting to adult populations offshore. Connectivity rates between estuarine and coastal environments have important implications for understanding fish population dynamics, colonization patterns of new habitats and populations' resilience to harvest (Thorrold et al., 2001). Information on connectivity among these habitats is considered a fundamental component towards sustainable management of estuarine associated fishes and identification of ecologically important habitats yet, the rate and timing of movement at different spatial scales is still poorly understood for many marine species (e.g. Gillanders, 2002; Rooker et al., 2010; Secor and Rooker, 2005).

Quantifying the movement of fishes is a difficult undertaking that, over the decades, has been tackled by a variety of approaches such as artificial and natural tagging techniques (reviewed in Gillanders et al., 2003). Among the natural markers, otolith geochemistry has demonstrated to be a particularly useful tool to determine connectivity rates and track movement patterns in fish (Elsdon et al., 2008; Thorrold et al., 2002). The metabolic inertness and continuous growth of otoliths allow for these applications (Campana, 1999). Calcium carbonate is

continuously deposited at the otolith surface, and periodic deposition of protein and aragonite forms daily and annual increment patterns that provide, when combined with chemistry measurements of individual increments, a chronological record of environmental conditions (Campana and Thorrold, 2001). Thus, the environmental conditions experienced by adult fish during their juvenile estuarine residency can be assessed by analyzing the portion of the otolith that corresponds to the juvenile life stage.

Understanding connectivity and quantifying the contributions, in terms of recruits, of different estuarine juvenile sources to the coastal adult population is particularly important for species that have commercial value. Since these populations are constantly harvested, the different sources of recruitment need to be identified and their relative importance evaluated. Flatfish fisheries, including the two sole species addressed in the present study, are widely spread along the Portuguese coast and play an important socio-economic role (Teixeira and Cabral, 2009). Although flatfishes account for less than 4% in the total fish biomass landed, these species and particularly soles account for almost 11% of the economic value of all landings (source: DGPA-national directorate of fisheries and aquaculture). Common sole (*Solea solea*) and Senegalese sole (*Solea senegalensis*) occur sympatrically from the Bay of Biscay to Senegal and the western Mediterranean Sea (Quéro et al., 1986). The two species are similar in terms of morphology and ecology; nonetheless they are easily distinguished based on the pigmentation of the right side pectoral fin. Spawning and larval development takes place in shelf waters with metamorphosis, and the resulting shift to a benthic

life form, occurring during transport from spawning to nursery grounds (Marchand, 1991). Juveniles of both species spend up to two years in estuarine nursery grounds before migrating to shelf waters (Cabral, 2003; Dolbeth et al., 2008; Quéro et al., 1986) where they reach sexual maturity at 3–4 years of age (Ramos, 1982). Estuaries along the Portuguese coast are recognized as important nursery areas for *S. solea* and *S. senegalensis* (Cabral et al., 2007; Vasconcelos et al., 2010) while juveniles of the two species do not occur in shallow coastal areas (e.g. Cabral et al., 2003; Prista et al., 2003).

The aim of this study was to determine the relative contributions of several estuarine juvenile sources to the coastal adult populations of two commercially important sole species along the Portuguese coast. Furthermore, by assessing the contributions of two different year classes the inter-annual consistency of the importance of juvenile sources was investigated. A previous study established the geochemical baseline values of juvenile *S. solea* and *S. senegalensis* of the 2006 and 2009 year classes in the major nursery areas along the Portuguese coast (Tanner et al., 2012). The high inter-annual variability of the otolith geochemical signatures in juveniles of both sole species implied that only adults belonging to the 2006 and 2009 year classes could be retrospectively assigned to the potential source estuaries (Tanner et al., 2012).

2. Material and methods

2.1. Geochemistry of estuarine nursery areas

Baseline values for otolith geochemistry of estuarine nursery areas in *S. solea* and *S. senegalensis* were characterized previously from juveniles sampled in the major estuarine systems along the Portuguese coast in 2006 and 2009 (Tanner et al., 2012) (Fig. 1). While otolith geochemical values for juvenile *S. solea* and *S. senegalensis* showed strong variability among sampling years, we generally found good discrimination results among estuaries (average cross-validated accuracy of 74%, Tanner et al., 2012).

2.2. Sampling of adult soles

Length at age relationship in both sole species is well established for the Portuguese coast (Teixeira and Cabral, 2010). We therefore collected age 3 + and age 2 + individuals of *S. solea* and *S. senegalensis* from the 2006 and 2009 year classes, respectively, based on fish size. Samples were obtained directly from professional fishermen working along the Portuguese coast during winter 2009/2010 and 2011/2012 (Fig. 1). Fish were transported on ice to the laboratory and dissected. Total length of each individual was determined and sagittal otoliths were extracted, cleaned of adhering tissue and stored dry.

2.3. Geochemical analyses of adult otoliths

Preparation and analysis of otoliths from adults followed the same methods as those used to determine the baseline values of otolith geochemical composition in juveniles (Tanner et al., 2012). Briefly, after grinding right otoliths to the midplane, possible surface contamination was removed by immersing otoliths in ultrapure water, sonicating for 2 min and triple-rinsing with ultrapure water in a class-100 clean room.

Otolith elemental ratios were quantified by measuring ^{7}Li, ^{25}Mg, ^{48}Ca, ^{55}Mn, ^{63}Cu, ^{88}Sr, ^{138}Ba and ^{208}Pb on a Thermo Finnigan Element2 single collector inductively coupled plasma mass spectrometer (ICP-MS) coupled to a New Wave 193 nm excimer laser ablation system. The laser software was used to trace 100×150 μm rasters (75 μm spacing) along the otolith increments that correspond to the juvenile life stage of the adult individuals (hereafter referred to as juvenile portion of adult otolith). The positions of the rasters ablated were standardized to ensure that approximately the same area of otolith material was ablated as that analyzed in juvenile otoliths (Tanner et
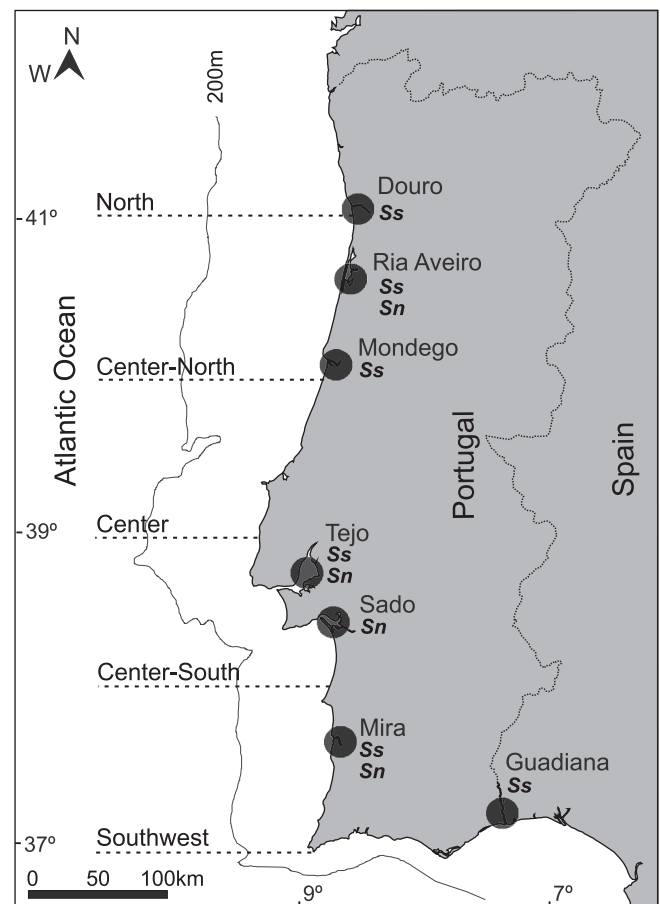

Fig. 1. Sampling of adult *Solea solea* (*Ss*) and *Solea senegalensis* (*Sn*) was carried out in 5 sectors off the Portuguese coast. Also shown, estuaries (black circles) where baseline geochemical values were obtained previously for juveniles of the two species (Tanner et al., 2012).

al., 2012). These areas were determined by measuring the distance from the otolith cores to the ablation lines of 100 randomly selected juvenile otoliths per species and year class that constitute the baseline data (Hamer et al., 2005; Tanner et al., 2012). The ablated material was transported via a He gas stream to the dual-inlet quartz spray chamber where it was mixed with a 2% HNO$_3$ aerosol from a self-aspirating PFA 20 μl min^{-1} nebulizer. The analyte was then transported to the ICP-MS via an Ar carrier gas. Instrumental blanks (2% HNO$_3$) were run at the beginning and end of each set of 10 otoliths. Blank correction of all measured raw values was done by calculating a blank value for each sample by linear interpolation of the measured blanks. A dissolved otolith certified reference material (CRM) (Sturgeon et al., 2005), diluted to a Ca concentration of 40 μg g^{-1}, was used to correct for instrument mass bias. Instrument precision was assessed by running another CRM (Yoshinaga et al., 2000), similarly dissolved and diluted to a Ca concentration of 40 μg g^{-1}. External precision (relative standard deviation) for this CRM ($n = 100$) was as follows: Li:Ca = 4.2%, Mg:Ca = 5.0%, Mn:Ca = 14.6%, Sr:Ca = 0.7%, Ba:Ca = 4.2%, Pb:Ca = 8.7%. Due to low precision of Cu:Ca in the adult sole otoliths, this elemental ratio was excluded from further analyses.

2.4. Data analysis

Accurate estimation of relative contributions of different source nursery areas to a mixed stock is based on the assumption that all possible sources have been included in the baseline data (Campana, 1999). The juvenile baseline datasets of *S. solea* and *S. senegalensis* included the geochemical signatures of all major nursery areas in