Connectivity between estuarine and coastal fish populations: contributions of estuaries are not consistent over time

Patrick Reis-Santos[1,2,*], Susanne E. Tanner[1], Rita P. Vasconcelos[1], Travis S. Elsdon[2], Henrique N. Cabral[1,3], Bronwyn M. Gillanders[2]

[1]Centro de Oceanografia, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

[2]Southern Seas Ecology Laboratories, DX650 418, School of Earth and Environmental Sciences, and Environment Institute, The University of Adelaide, South Australia 5005, Australia

[3]Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

ABSTRACT: Otolith elemental signatures (Li:Ca, Mg:Ca, Mn:Ca, Cu:Ca, Sr:Ca, Ba:Ca and Pb:Ca) of Age-0 juveniles of flounder *Platichthys flesus* and sea bass *Dicentrarchus labrax*, collected from estuarine systems along the Portuguese coast in 2006 and 2009, were used as baseline signatures to identify the estuarine nursery of Age-3^{+} and 2[+] coastal fish matching the juvenile year classes. Otolith elemental signatures were determined via Laser Ablation Inductively Coupled Plasma Mass Spectrometry, and successfully identified the estuarine origin of the majority of coastal fish to characterized baseline estuaries. Relative contributions of individual estuaries to coastal areas, quantified using maximum likelihood estimation, varied over time for the 2 year classes analysed. However, some general patterns were discernible in terms of important estuaries versus those with minor or negligible contributions. Assigned nursery origins varied among species and suggested large scale movements along the coast. For *P. flesus* the main source for recruits changed between year classes from Ria de Aveiro (69%) to the Douro estuary (59%). The Mondego estuary, located near the latitudinal limit of *P. flesus*, contributed moderate numbers to coastal populations. The Tejo estuary was the most important estuary for *D. labrax* in both years sampled (55 and 50%, respectively), whilst the Ria de Aveiro hardly contributed to sea bass coastal populations (<2%). Ultimately, knowledge of which estuaries replenish coastal adult populations, and both local and distant fisheries, is essential for effective management and conservation of these species and their estuarine juvenile habitats.

KEY WORDS: Connectivity · Juvenile fish · Nursery · Otolith chemistry · Elemental signatures · Portugal

INTRODUCTION

The ecological and economic importance of estuarine nursery function to the replenishment of coastal fish populations has been widely recognized. Many fishes use estuaries to complete their life cycles, with larvae or early juveniles often spending months to years in these environments before recruiting to coastal adult populations (see reviews by Beck et al. 2001, Able 2005). Thus, juveniles and adults of these species are spatially segregated, and it is fundamental to determine the exchange of individuals among these geographically separated groups, i.e. connectivity, which is a key factor in the regulation of population dynamics, colonization patterns and resilience to harvest (Thorrold et al. 2001, Cowen et al. 2007). Assessing fish movement patterns between estuarine and coastal habitats (particularly determining the

source(s) of juveniles to adult populations) is increasingly recognized as a vital link to the identification of ecologically important habitats and the development of effective management strategies for coastal fish populations, many of which are commercially exploited (Gillanders 2005, Vasconcelos et al. 2011). However, the spatial scale and movement patterns between estuarine juvenile and coastal adult populations is still poorly understood for many species, and estimates of the effective contributions of juvenile habitats to coastal adult populations is generally lacking (Gillanders 2002, Hamer et al. 2005, Rooker et al. 2010).

Unravelling fish movement and quantifying connectivity is a complex task. Despite considerable progress, most artificial tagging techniques are not viable for small juvenile fish. However, recent advances enable the interpretation of distinct biological features as natural tags, and among them the chemical composition of otoliths has developed into a prominent technique to quantify individual fish movement and connectivity patterns (Elsdon et al. 2008, Gillanders 2009). Otolith chemical signatures have been successful in discriminating natal or nursery origins (e.g. Rooker et al. 2001, Vasconcelos et al. 2007, Clarke et al. 2009) and assessing the differential contributions of juvenile source areas to adult populations (e.g. Thorrold et al. 2001, Gillanders 2005, Vasconcelos et al. 2008, Wells et al. 2012). The use of otolith chemical composition as a natural tag is possible because otoliths are metabolically inert, grow continuously and incorporate trace elements as they accrete (reviewed in Campana 1999, Elsdon et al. 2008). Hence, as otoliths form, an elemental signature is incorporated, and due to the intrinsic relationship between otolith growth and fish age, the environmental record experienced by adult fish during their juvenile estuarine life period can be determined by analysing the portion of the otolith that corresponds to that specific life period.

Along the Portuguese coast there are several estuaries acknowledged to be important nursery areas for flounder *Platichthys flesus* (Linnaeus 1758) and sea bass *Dicentrarchus labrax* (Linnaeus 1758). These species are valuable resources in Portuguese fisheries, and their use and dependence on estuarine areas as juveniles in this coast is well documented (e.g. Cabral et al. 2007, Martinho et al. 2008, Vasconcelos et al. 2010); juveniles of the 2 species are absent in shallow coastal areas (Prista et al. 2003, Vasconcelos et al. 2008). In view of the commercial importance of these species, a quantitative understanding of the connectivity patterns between estuaries and coastal

populations will provide a valuable framework for the development of appropriate habitat conservation strategies and fisheries sustainable management (Hamer et al. 2005, Hamer et al. 2011). If recruitment is found to be dependent on only one or a few source estuaries, ecologically important habitats with higher degrees of connectivity could be prioritized for management and conservation purposes (Vasconcelos et al. 2011). However, estuarine nursery function and connectivity patterns may be influenced both by species ecological features (e.g. inter-annual fluctuations in larval recruitment to estuarine nursery grounds) or environmental conditions (e.g. freshwater flow), with the value of nursery areas potentially changing with year class (Kraus & Secor 2005). Most studies have only focused on a single year (but see Hamer et al. 2011); however, if estuarine nurseries are to be effectively protected, knowledge on the generality of patterns across multiple species and year classes is required. Therefore, additional research is paramount to ascertain the estuaries that contribute to replenish coastal populations over time and/or year classes.

The aim of the present study was to determine the estuarine nursery origin of *Platichthys flesus* and *Dicentrarchus labrax* along the Portuguese coast and quantify the relative contributions of several estuarine juvenile sources to the coastal populations of these commercially important species. Furthermore, this study assessed connectivity in 2 distinct year classes to evaluate the generality of nursery contributions over time and determine whether the most important source estuaries (i.e. those with higher connectivity estimates) were similar between years. A previous study established the otolith elemental signatures for Age-0 juveniles in 2006 and 2009 in the major estuarine nursery areas where these species occur along the Portuguese coast (Reis-Santos et al. 2012). These estuarine elemental fingerprints accurately characterized the time period spent by juvenile Age-0 fish in estuaries and were used as baseline for the current connectivity assessment.

MATERIALS AND METHODS

Juvenile elemental signatures in estuaries

The baseline multi-elemental otolith signatures used to identify the estuarine nursery of coastal young adult *Platichthys flesus* and *Dicentrarchus labrax* were previously characterized in otoliths of Age-0 juveniles sampled throughout 2006 and 2009

in the major estuarine systems along the Portuguese coast acknowledged as nursery areas for these species (Reis-Santos et al. 2012) (Fig. 1). The estuaries in which juveniles were sampled comprise the most likely source habitats for *P. flesus* and *D. labrax* populations along the Portuguese coast (see also Vasconcelos et al. 2011). Species-specific estuarine otolith elemental signatures were previously established for each year with high discrimination among estuaries (overall cross-validated classification accuracy ranged between 77 and 96%) (Reis-Santos et al. 2012). Estuarine otolith elemental tags were year-specific, which implied that with these natural tags only adults belonging to the 2006 and 2009 year classes could be retrospectively assigned to their estuarine sources (Gillanders 2005, Reis-Santos et al. 2012).

Sampling of coastal adult fish

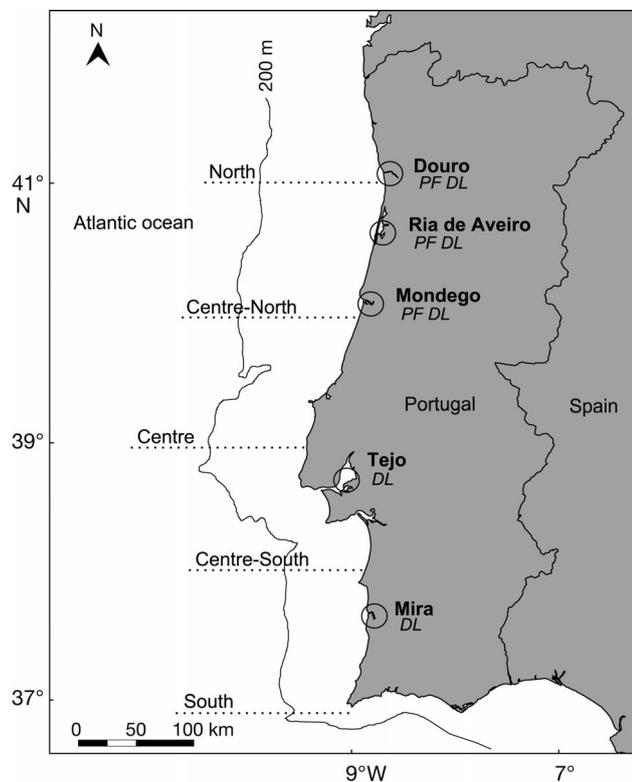Collection of young adults of *Platichthys flesus* and *Dicentrarchus labrax* was carried out along the

Fig. 1. Sampling of adult *Dicentrarchus labrax* (*DL*) and *Platichthys flesus* (*PF*) from 5 sectors along the Portuguese coast. Open circles indicate the estuarine nurseries of the 2 species where estuarine otolith elemental signatures of Age-0 juveniles were previously characterized (Reis-Santos et al. 2012)

Portuguese coast. Specimens were obtained directly from professional fishermen operating locally in 5 different sectors of the Portuguese coast (Fig. 1). These divisions follow previous studies and are based on key hydromorphological and biogeographical criteria (e.g. IPIMAR 1980, Tanner et al. 2013). Upon collection, fish were transported on ice and dissected at the laboratory. We collected Age 3[+] and Age 2[+] individuals of both species belonging to the 2006 and 2009 year classes, respectively, based on fish size, in the winters of 2009–2010 and 2011–2012. Sampling focused on fish that approximated the expected fish lengths for the respective age classes, based on established length at age relationships for the Portuguese coast (e.g. Gordo 1989, Teixeira et al. 2010). Ages were confirmed by counts of annual increments on the otolith sections used for chemical analysis.

Otolith preparation and analyses

Otolith sample preparation and analysis followed the same methodology applied for analyses of juvenile Age-0 fish and the characterization of the baseline estuarine otolith elemental signatures (see Reis-Santos et al. 2012).

Briefly, sagittal otoliths were extracted using plastic forceps, washed, and cleaned of adhering tissue with ultrapure water and allowed to air dry in microcentrifuge tubes in a positive pressure laminar flow hood. The right otolith of each fish was embedded in EpoFix resin (Struers) spiked with indium (^{115}In) at 30 ppm to allow discrimination between otolith material and resin during analysis. Otoliths were sectioned transversely through the nucleus using a low-speed saw (Buehler Isomet). Resulting otolith sections were polished to ca. 250 μm with lapping film (30 μm, 9 μm and 3 μm grit size successively, using ultrapure water to remove surface contamination) and mounted on glass slides with indium-spiked thermoplastic glue (CrystalBond 509). Slides were then cleaned, sonicated and triple-rinsed with ultrapure water, dried under a laminar flow hood and stored individually in sealed plastic bags.

A New Wave 213 nm UV high performance (Nd:YAG) laser microprobe coupled to an Agilent 7500cs inductively coupled plasma mass spectrometer (ICP-MS) was used to quantify ^{7}Li, ^{24}Mg, ^{43}Ca, ^{55}Mn, ^{65}Cu, ^{88}Sr, ^{138}Ba and ^{208}Pb elemental concentrations in otoliths. Laser ablations occurred inside a sealed chamber with resultant material and sample gas transported to the ICP-MS via a smoothing manifold

in an argon and helium stream. We used the same instrument and identical operating parameters as those used by Reis-Santos et al. (2012) to analyse the Age-0 fish otoliths. Ca was used as an internal standard to correct for variation in ablation yield between samples (Yoshinaga et al. 2000). ^{115}In was also measured but solely as a marker to discriminate between spiked resin or CrystalBond and otolith matrices.

Otoliths were analysed using 300 µm rasters along the increments that corresponded to the juvenile life stage. This corresponded to the otolith section formed while Age-0 fish were within estuarine areas and matched the life stage and time period encompassed in the elemental signatures characterized in Reis-Santos et al. (2012). Otoliths were analysed in random order. A certified glass reference standard NIST 612 (National Institute of Standards and Technology) was run at the start and end of each session and after every 10 otoliths to correct for mass bias and machine drift. External precision (% relative standard deviation) was assessed by measurements of a calcium carbonate certified reference material MACS-3 (United States Geological Survey) and were as follows: 4.9% (Li), 6.1% (Mg), 3.3% (Mn), 5.5% (Cu), 2.3% (Sr), 4.2% (Ba), and 7.8% (Pb). These were analogous to those obtained in Reis-Santos et al. (2012). Data reduction, including background corrections, mass count data conversion to concentrations (ppm) and limits of detection were all performed for each individual sample via Glitter software (GEMOC, Macquarie University).

Data analyses

Otolith elemental concentration data were converted to molar concentrations and standardised to calcium. All further data analyses were carried out on log (x+1) transformed element:Ca data. Each species was analysed independently.

Accurate estimations of the relative contributions of estuaries to a mixed coastal stock relies on the assumption that all possible sources that could contribute to that mixed stock have been included in the baseline data set (Campana 1999). Whilst our Age-0 juvenile baseline data incorporated a thorough characterization of the otolith elemental signatures of the major estuarine nursery areas along the Portuguese coast for these species, it is possible that other source areas may have contributed to the analysed adult population. These include other estuaries or inlets along the Portuguese coast where these species may occur, as well as estuarine sites beyond the Por-

tuguese coast. In these cases, the otolith elemental signatures should not match those of the Age-0 baseline data set provided the sites differ in elemental chemistry. Hence, to reduce potential bias of uncharacterized source areas we compared the otolith elemental signatures of adult fish with those of the Age-0 juveniles using principal component analysis (PCA) (Hamer et al. 2005, Chittaro et al. 2009, Tanner et al. 2013). Adult individuals that fell outside a 95% confidence ellipse around the juvenile baseline data (estuarine elemental signatures) were assumed to have originated from an estuarine area not previously characterized in Reis-Santos et al. (2012), and were excluded from further analyses.

We used a maximum likelihood estimation approach (MLE) (Millar 1987, 1990a) to determine the relative contributions of the different baseline nursery areas to populations of *Platichthys flesus* and *Dicentrarchus labrax* along the Portuguese coast for each year class (2006 and 2009). Proportions of adult origins were also evaluated for each of the 5 sectors along the coast. Analyses were performed with HISEA (Millar 1990b), generating maximum likelihood estimates and standard deviations of the proportion of the mixed stock (coastal adult populations of each species) originating from the baseline groups. Bootstrapping with a 1000 resampled baseline and adult datasets was used. For each species and year class analysed, baseline data were the multi-elemental otolith compositions of Age-0 juveniles characterized in Reis-Santos et al. (2012).

RESULTS

Comparison of adult and juvenile otolith elemental ratios

The elemental ratios from the juvenile portion of adult otoliths were mostly distributed within the 95% confidence ellipses of the juvenile baseline data (Figs. 2 & 3). However, for both years and species there were some adult elemental tags that fell outside the confidence ellipses of the juvenile data. We excluded 33% of adult *Platichthys flesus* (32 individuals; Fig. 2a) and 18% of *Dicentrarchus labrax* (23 individuals; Fig. 3a) from the 2006 year class. There was a greater overlap between the otolith chemical composition of adult and juvenile fish belonging to the 2009 year class, with only 23% of *P. flesus* (21 individuals; Fig. 2b) and 6% (7 individuals; Fig. 3b) of *D. labrax* excluded because their elemental signatures fell outside the 95% confidence ellipses of the juveniles.
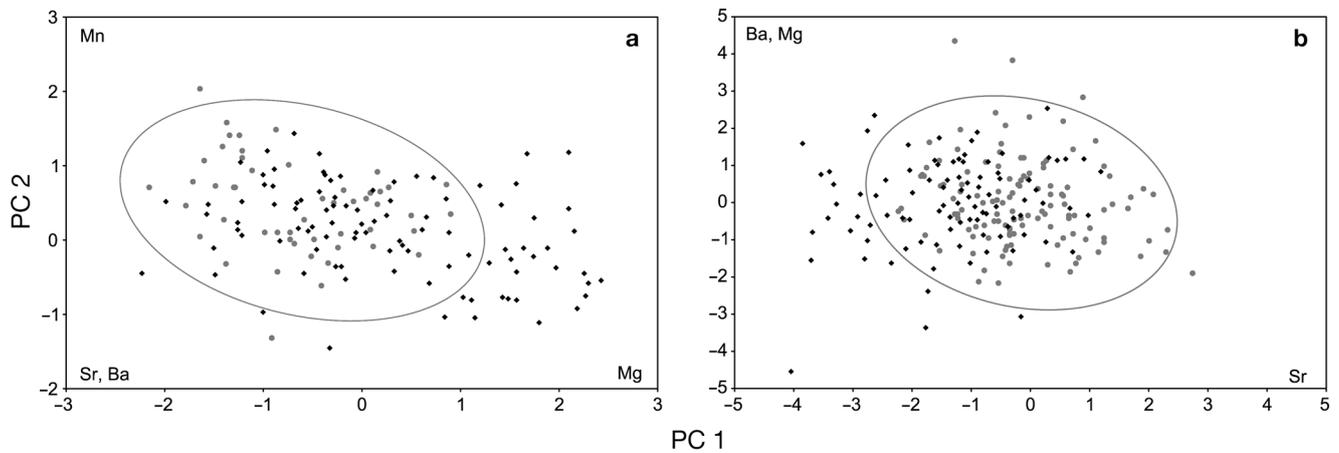
Fig. 2. *Platichthys flesus.* Ordination plot of principal component analysis (PCA) comparing multi-element otolith signatures of adult fish (mixed-stock) (black) and Age-0 juveniles of known estuarine origin (baseline group) (grey) for (a) the 2006 and (b) the 2009 year class. Ellipses are 95% confidence ellipses around the baseline group data. The elements that contributed the most variation to the data are indicated adjacent to the respective axis
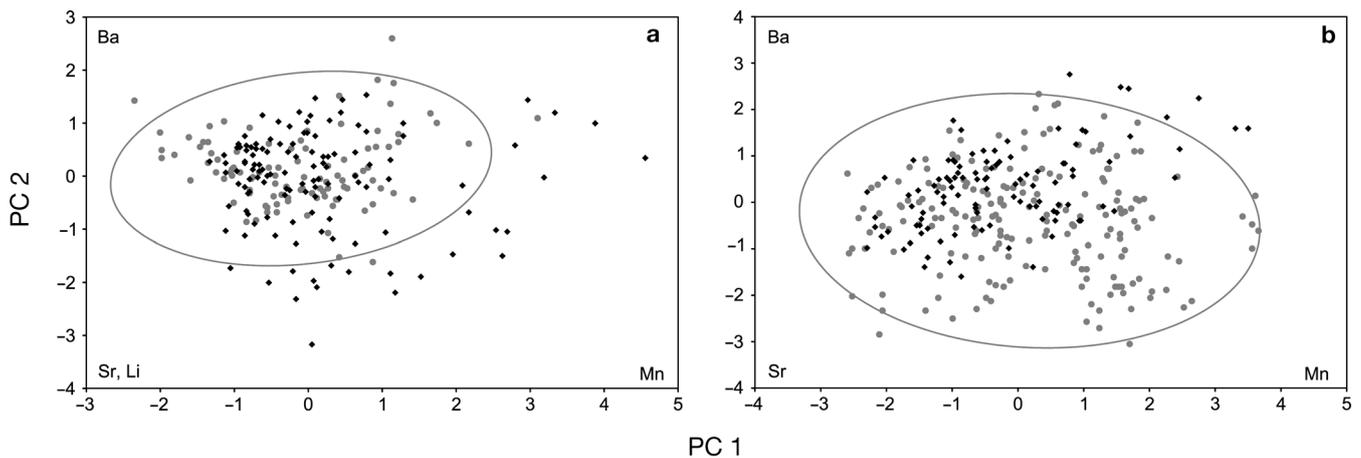

Fig. 3. *Dicentrachus labrax.* Ordination plot of principal component analysis (PCA) comparing multi-element otolith signatures of adult fish (mixed-stock) (black) and Age-0 juveniles of known estuarine origin (baseline group) (grey) for (a) the 2006 and (b) the 2009 year class. Ellipses are 95% confidence ellipses around the baseline group data. The elements that contributed the most variation to the data are indicated adjacent to the respective axis

Variability in contributions of estuarine nurseries

The MLE quantification of the relative contributions of the different estuarine nursery areas to coastal populations along the Portuguese coast varied by species and over time between year classes (Table 1). The Ria de Aveiro estuary contributed the most (69%) to the mixed population of *Platichthys flesus* for the 2006 year class, whilst the Douro contributed the least (3%). In contrast, for the 2009 year class the largest proportion of adult flounder along the Portuguese coast was from the Douro estuary (59%), with the Ria de Aveiro contributing only 26%.

The contributions of the Mondego estuary to the mixed stock ranged from 15 to 28% in the 2 year classes.

For the 2006 year class, when the different sections of the coast were individually analysed, the Ria de Aveiro estuary contributed the highest proportion of *Platichthys flesus* to the northern and northern-central sectors: 63 and 65%, respectively (Fig 4a). For the central sector of the coast, where this species' latitudinal limit of distribution occurs, the majority of fish were identified as having used the Mondego estuary as a nursery (55%). The contributions of the Douro estuary were negligible. For the 2009 year

Table 1. *Platichthys flesus* and *Dicentrachus labrax*. Maximum likelihood estimates of the relative contributions (%) of the Douro, Ria de Aveiro, Mondego, Tejo and Mira estuaries to coastal populations along the Portuguese coast for 2 distinct year classes. Otolith elemental signatures of Age-0 juveniles collected in estuarine nurseries along the Portuguese coast in 2006 and 2009 (previously characterized in Reis-Santos et al. 2012) were used as baseline data to identify the source estuary of coastal adult and sub-adult fish (mixed-stock) of Age 3^+ and 2^+ that matched the 2006 and 2009 baseline year classes, respectively. Mean ± SD and total number of adults (n) are shown

Species	Relative contributions (%) of predicted estuarine nurseries of origin					n
Platichthys flesus	**Douro**	**Ria de Aveiro**	**Mondego**			
2006 Year Class	3 ± 2	69 ± 8	28 ± 8			65
2009 Year Class	59 ± 7	26 ± 4	15 ± 5			72
Dicentrarchus labrax	**Douro**	**Ria de Aveiro**	**Mondego**	**Tejo**	**Mira**	
2006 Year Class	8 ± 3	1 ± 1	39 ± 6	50 ± 7	2 ± 1	99
2009 Year Class	19 ± 4	2 ± 2	16 ± 4	55 ± 5	7 ± 3	100

class, 29% of the flounder from the central sector were also assigned to the Mondego, with contributions to the remaining sectors decreasing to ca. 15% (Fig. 4b). Estimates for the Ria de Aveiro estuary ranged from 1% (Centre) up to 23% (Centre-North), whilst the Douro estuary contributed the majority of *P. flesus* to each sector.

The Tejo estuary made the highest nursery contribution to the adult population of *Dicentrarchus labrax*, with contributions over 50% for both year classes (Table 1). The contribution of the Mondego estuary varied by year class (39% for the 2006 year compared to 16% for the 2009 year class). Contribution from the Douro estuary increased from 8% for the 2006 year class to 19% for the 2009 year class. The Mira, the southernmost estuary, made only small contributions to coastal *D. labrax*, with a maximum of 7% for the 2009 year class. The Ria de Aveiro estuary was of minor significance for this species with contributions under 2% for both year classes.

Observing the contributions to each sector of the coast individually for the 2006 year class, the Tejo estuary contributed between 23% (North) and 89% (Centre-North) of *Dicentrarchus labrax* individuals (Fig 5a). The Tejo and Mondego were the only estuaries to contribute individuals to all the sectors along the coast. Contributions from the Douro and the Mira estuaries were higher in the vicinity of these estuaries. For example, the Douro estuary contributed 41% of coastal fish in the northern section compared to 0 to 21% for the other sections along the coastline; the Mira estuary contributed 7% of coastal fish to the southern sector but zero to < 1% to the remaining

sectors (Centre-North). The same general patterns were found for the 2009 year class (Fig 5b). The Douro, Mondego and Tejo estuaries were the source estuaries for over 80% of adult *D. labrax* from each section of the coastline. Only the Tejo and Mondego estuaries contributed to all 5 sections of the coast, with Tejo contributions dominating (50 to 60%), whilst those of the Mondego ranged between 8% (Centre) and 33% (Centre-South). Once again, the highest contributions for the Mira estuary were to the local southern sector (18%), although in the 2009 year class the Mira estuary contributed to all sections of the coast except to the central-northern sector.
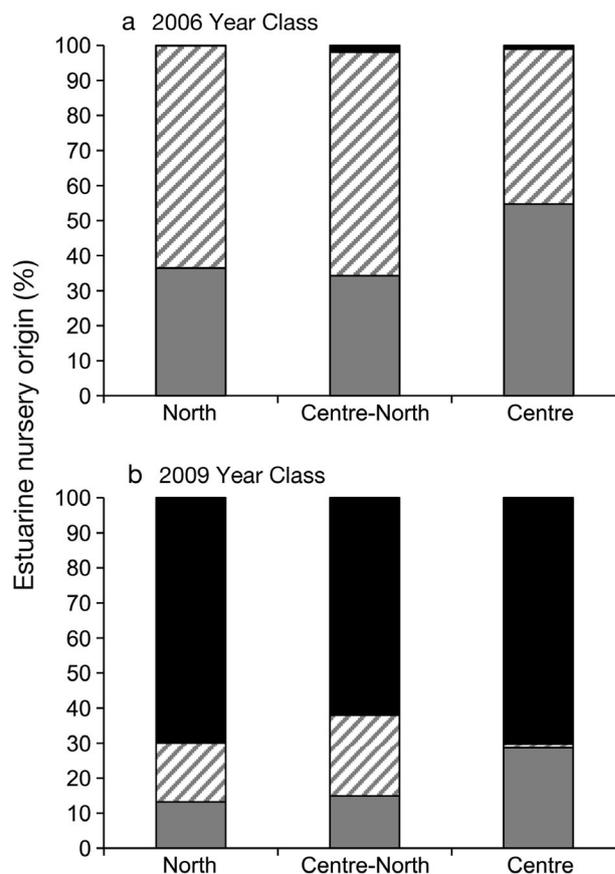

Fig. 4. *Platichthys flesus.* Maximum likelihood estimates of the relative contributions (%) of estuaries to adult fish caught in each of 5 sectors along the Portuguese coast for (a) the 2006 and (b) the 2009 year classes. From top to bottom, represented estuaries are: Douro (black), Ria de Aveiro (grey diagonal lines), Mondego (grey)
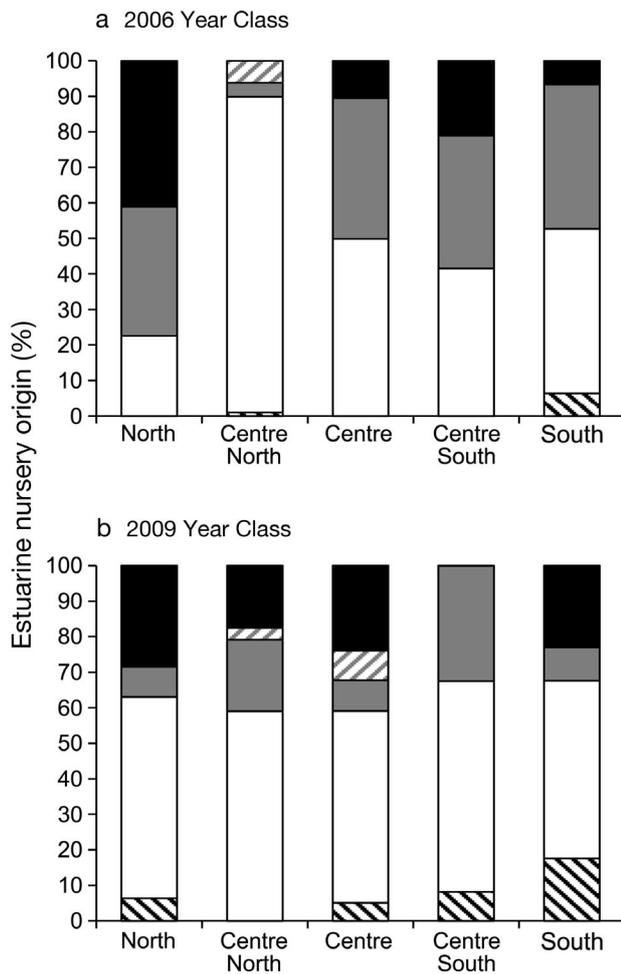
Fig. 5. *Dicentrachus labrax*. Maximum likelihood estimates of the relative contributions (%) of estuaries to adult fish caught in each of 5 sectors along the Portuguese coast for (a) the 2006 and (b) the 2009 year classes. From top to bottom, represented estuaries are Douro (black), Ria de Aveiro (grey diagonal lines), Mondego (grey), Tejo (white) and Mira (black diagonal lines)

DISCUSSION

Understanding patterns of connectivity between juvenile estuarine nurseries and coastal adult populations has significant implications for fisheries management and habitat conservation. In the present study, otolith elemental signatures were a valuable tool for quantifying the contributions of individual estuaries to coastal populations of *Platichthys flesus* and *Dicentrarchus labrax*. Results highlighted that contributions varied over time for the 2 year classes analysed. However, some general patterns were discernible in terms of important estuaries compared to those making minor or negligible contributions.

To avoid confounding effects of inter-annual variations in otolith elemental signatures and consequent bias in the quantification of the relative contributions among source estuaries, we ensured that we matched adult fish to their corresponding Age-0 year class (Gillanders 2005, Reis-Santos et al. 2012). Still, a number of coastal fish were removed from the analyses because their otolith elemental signatures (measured in the otolith area matching the juvenile life period spent within estuaries) did not overlap those of the baseline data (measured in Age-0 juveniles). Thus, alternative source sites along or beyond the Portuguese coast that were not sampled in Reis-Santos et al. (2012) are likely. We are confident that these differences in signatures were not due to variations in analytical procedures, as methodological and analytical procedures were consistent between juvenile and adult otolith analysis. However, movement and dispersal of fish may increase with fish age (e.g. Hamer et al. 2011). Thus, Age 3[+] individuals (year class 2006) potentially disperse further away upon leaving an estuary in comparison to Age 2[+] fish (year class 2009). This suggests that the higher number of adults from uncharacterized sources in the 2006 year class were likely due to increased numbers of stray individuals from estuaries outside the study area, for example, from sites along the neighbouring Spanish coast (Tanner et al. 2013). In fact, tagging studies have highlighted complex dispersal and large scale movement patterns for adult sea bass (Pickett et al. 2004, Pita & Freire 2011) and plaice (Dunn & Pawson 2002), a flatfish phylogenetically close to flounder. Moreover, population structure of *Dicentrarchus labrax* and *Platichthys flesus* based on molecular genetic approaches has been shown to be lacking in the North-Eastern Atlantic (Borsa et al. 1997, Naciri et al. 1999, Coscia & Mariani 2011). Nonetheless, the majority of collected Age 2[+] and 3[+] adult *D. labrax* and *P. flesus* spent their juvenile nursery period within one of the characterized estuaries.

Nursery origins varied among species, and even if many coastal fish were assigned to estuaries in the vicinity of where they were caught (e.g. higher proportions of *Dicentrarchus labrax* from the Mira estuary in the southern sector, or *Platichthys flesus* from the Mondego estuary in the central sector), juvenile contributions suggested wide ranging movements of up to 200 km (*P. flesus*) or 600 km (*D. labrax*). Such movements are in agreement with described movement patterns and homogeneity of coastal population structure for both species (e.g. Borsa et al. 1997, Pickett et al. 2004). As older fish can potentially move further from their recruitment estuaries, it would be of

great interest to run tagging/telemetry studies to link with data acquired via otolith natural tags. Moreover, as these fish are long lived, following year classes through time, analysing a wide range of adult year classes, and increasing the sampling area to investigate contributions to distant coastal locations (Hamer et al. 2005, Rooker et al. 2010, Hamer et al. 2011) could also aid in resolving the unidentified coastal spawning grounds for these species along this coast.

Relative contributions of individual estuarine nurseries to coastal populations varied over the years for analysed age classes. The striking increase in contributions from the Douro estuary for *Platichthys flesus*, from least to most important, was noteworthy and was accompanied by a decrease in contributions from the Ria de Aveiro estuary, even though the latter maintained moderate contributions in the 2009 year class. Overall, particular estuarine nurseries showed consistently higher contributions, whilst others were negligible (e.g. Tejo and Ria de Aveiro for *Dicentrarchus labrax*, respectively). Very few analogous studies have addressed connectivity contributions of source areas over multiple years, with variations over time reported (see Hamer et al. 2005, Kraus & Secor 2005, Chittaro et al. 2009, Tanner et al. 2013). Comparison of the present data to a preliminary single year assessment for these species (Vasconcelos et al. 2008) further outlined annual differences in estuarine relative contributions. However, the use of distinct analytical procedures to determine baseline and adult signatures in that study may be in part responsible for the observed differences between contribution estimates.

Major contributions determined for the Tejo and Mondego estuaries for the 2006 *Dicentrarchus labrax* year class are in agreement with the assumption that higher estuarine juvenile densities account for increased recruitment to coastal populations (e.g. Martinho et al. 2008, Vasconcelos et al. 2010). However, this may not represent a general trend, as there were inconsistent observations of the importance of estuaries to coastal stocks and reported juvenile densities within the estuaries. For instance, while juvenile densities of *Platichthys flesus* were reported to be highest in the Douro estuary in 2006 (Vasconcelos et al. 2010), this was not reflected in its contributions to coastal populations. Overall, assessments combining potential nursery value (e.g. measuring density, growth, available habitat) and effective contributions (e.g. via otolith elemental signatures) have had mixed results depending on species (Vasconcelos et al. 2011), strong matches (Fodrie & Levin 2008) and contributions not explained by variability in juvenile

densities (Chittaro et al. 2009). Still, these assessments do not specifically elucidate why differential connectivity rates are observed. Further studies are needed to examine the link between connectivity and underlying ecological mechanisms, as environmental and biotic factors controlling juvenile survival rates and migration influence recruitment to coastal populations (Levin & Stunz 2005). Density-dependent growth (e.g. through intraspecific competition) and size selective mortality (e.g. due to reduced size-at-age of juveniles at time of emigration or overwintering) can contribute significantly to regulation of year class strength (Craig et al. 2007, Laurel et al. 2007, Searcy et al. 2007, Martino & Houde 2012) and have potential negative feedbacks on estuarine contributions to coastal populations. Furthermore, increased predation pressures may also explain the lack of relation between densities and connectivity (Temming et al. 2007, Dorenbosch et al. 2009).

Assessing the connectivity patterns of *Platichthys flesus* is of particular interest as this species' limit of distribution occurs along the Portuguese coast (central sector), with the Mondego the southernmost estuary where juveniles can now be found (Vasconcelos et al. 2010). The southern limit of *P. flesus* has been receding northward: only 2 decades ago, juvenile *P. flesus* were abundant in the Tejo estuary (Cabral et al. 2001). Populations living at the border of the distribution range are expected to live near the limits of their physiological tolerance, and so are likely vulnerable to changes in abiotic conditions, with adaptive responses to thermal and anthropogenic stress already suggested for the southernmost populations of *P. flesus* (Calvès et al. 2013). In this scenario, changes in population trends may occur at small spatial and temporal scales, and although estuaries further north attained higher contributions, the Mondego estuary still played a role in the replenishment of coastal areas for both year classes.

Overall, little information is available on the dispersal of early life stages (eggs and larvae) of *Platichthys flesus* and *Dicentrarchus labrax*, as well as on the rate and spatial extent of adult movements. Connectivity may vary by life stage, particularly for fish with segregated life histories (e.g. Tobin et al. 2010). Hence, future research should focus on investigating adult movement, spawning areas and larval dispersal for these species, in an attempt to ascertain in which life stage connectivity is maximized.

Complex life cycles and segregated habitats have key implications in the development of management strategies, including protected areas, as both conser-

vation of juvenile habitats as well as sustainable management of coastal adult populations have to be addressed (Kraus & Secor 2005, Di Franco et al. 2012). Regarding estuarine juvenile habitats, management should be prioritised towards those with consistently higher connectivity and contributions, in particular if they act as source sites for multiple species simultaneously (Gillanders 2002, Vasconcelos et al. 2011). Recommended actions may include safeguarding and/or increasing estuarine nursery habitat via conservation and restoration of shallow habitats such as intertidal mudflats, seagrass beds or saltmarshes used by juvenile fish. Further investigations on density-dependent regulation and its underlying effects on recruitment may also contribute to more effective management strategies for estuarine nurseries (Martino & Houde 2012). Together with an increase in available habitat, managers may be able to take better advantage of enhanced larval year class strength and settlement to increase yields of fish growth and survival, and consequently improve recruitment success. In the meantime, considering the variability in connectivity patterns over time, safeguarding estuaries where moderate contributions occur irregularly may act as a buffer to dampen variation in year class strength to coastal populations (Rooper et al. 2003, Chittaro et al. 2009). Ultimately, understanding which estuaries replenish coastal adult population, and fisheries both local and distant, is essential for effective management and conservation of these species and their nursery habitats.

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