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Connectivity within estuaries: An otolith chemistry and muscle stable isotope approach

Patrick Reis-Santos [a, b, *], Susanne E. Tanner [a], Susana França [a], Rita P. Vasconcelos [a], Bronwyn M. Gillanders [b, c], Henrique N. Cabral [a]

[a] MARE – Marine and Environmental Sciences Centre, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
[b] Southern Seas Ecology Laboratories, DX650 418, School of Biological Sciences, The University of Adelaide, SA 5005, Australia
[c] Environment Institute, School of Biological Sciences, The University of Adelaide, SA 5005, Australia

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ABSTRACT

Understanding whether fish move among estuarine areas and habitats or show high site fidelity has major implications for habitat conservation and the safeguard of estuarine ecological integrity. Muscle stable isotopes ($\delta^{13}C$, $\delta^{15}N$) and otolith elemental composition (Li:Ca, Mg:Ca, Mn:Ca, Cu:Ca, Sr:Ca, Ba:Ca and Pb:Ca) were used to evaluate connectivity between two separate estuarine nursery areas in summer and autumn for juvenile age-0 *Dicentrarchus labrax* and *Pomatoschistus microps*. Distinct isotopic ratios and otolith elemental signatures were found between areas for *D. labrax* and *P. microps*, and in both sampling times. High classification accuracies to collection sites were achieved via otolith elemental signatures (80–94%), and a combined analysis using both muscle stable isotopes and otolith chemistry resulted in increased accuracy with no classification errors. Overall, low site connectivity was found for both species. The use of two distinct natural tags provided corroborative and complementary information on fish movement and intra-estuarine habitat use at different temporal scales, whilst elucidating distinct ecological and environmental linkages. Ultimately, the combined use of distinct natural tags showed great promise to unravel intra-estuarine connectivity patterns.

1. Introduction

Estuaries have long been regarded as highly productive and valuable ecosystems. They provide critical habitats and nursery areas to many marine fish species, where age-0 juveniles settle and individuals remain for protracted periods ranging from months to years (Able, 2005; Beck et al., 2001). Likewise, resident fish species, those which complete their entire life cycle within an estuary, rely on a range of habitats and play a fundamental role in the overall dynamics and functioning of estuarine systems (Dolbeth et al., 2007; Elliott and Dewailly, 1995; França et al., 2009). Yet, detailed knowledge on the extent over which individual fish occupy particular areas is lacking. Moreover, fish movement and temporal habitat use patterns among adjacent or segregated habitats within estuaries are still poorly understood (Able et al., 2012; Green et al., 2012).

A better knowledge of whether fish move among estuarine areas and habitats or show high site fidelity has major implications for habitat conservation and the safeguard of estuarine ecological integrity, particularly in areas that play a major role as nurseries for multiple fish species. Ecological (or demographic) connectivity determines the spatial scales over which fish populations function, and over which management and conservation strategies should be designed and implemented. This is particularly critical considering that estuaries are threatened by extensive habitat loss and degradation due to widespread anthropogenic pressures (Courrat et al., 2009; Kennish, 2002; Vasconcelos et al., 2007).

Fish movement can be quantified by a variety of approaches from abundance and size-frequencies distributions to artificial and natural tagging methods (reviewed by Gillanders, 2009). However, conventional tagging methods are logistically difficult to use on small fishes, namely juveniles and early life history stages. Hence, natural tags have become increasingly applied. Among these, tissue stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) (Herzka, 2005; Trueman et al., 2012) and otolith chemical composition (Elsdon et al., 2008; Sturrock et al., 2012) are prevailing tools in

* Corresponding author. MARE – Marine and Environmental Sciences Centre, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.
E-mail address: pnsantos@fc.ul.pt (P. Reis-Santos).

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assessing fish movement and habitat use.

Stable isotope analysis has been used to examine movement to, from and within estuaries (Herzka, 2005; Hobson, 1999; Trueman et al., 2012). The premise for its use is based on primary producer groups exhibiting distinctive isotopic ratios that are propagated through local food webs; and if food sources are distinct regarding their isotopic ratios, fish feeding in these habitats will also have distinct isotopic ratios. Thus, non-migratory individuals are expected to exhibit stable isotopic ratios in equilibrium with local food webs, while transient individuals moving between habitats should display intermediate or greater isotopic variation (Fry et al., 2003; Herzka, 2005; Rubenstein and Hobson, 2004); with the identification of migrants dependent on the speed at which individuals reach equilibrium following shifts to isotopically distinct prey (turnover rate). The latter can vary from a few days or weeks in larvae and juveniles, to months in larger fish (Herzka, 2005). Overall, stable isotope ratios in soft tissues reflect an organism's diet, spatial variation of food webs and local biogeochemistry, and highlight connectivity and movement patterns (e.g. Suzuki et al., 2008; Verweij et al., 2008), geographic origin, habitat use and site fidelity (e.g. Green et al., 2012; Vinagre et al., 2008).

The chemical composition of otoliths has been widely used to assess population structure (e.g. Tanner et al., 2012; Thresher and Proctor, 2007), reconstruct migration patterns (e.g. Hamer et al., 2006; Morales-Nin et al., 2012), identify estuarine nurseries (e.g. Gillanders and Kingsford, 2000; Reis-Santos et al., 2012), and assess connectivity between juvenile and adult populations (e.g. Chittaro et al., 2009; Reis-Santos et al., 2013b). The use of otoliths as a natural tag is possible due to their continuous growth, metabolic inertness and the fact that trace element incorporation is influenced by physical and chemical properties of the surrounding water (Campana, 1999). Hence, over time, fish occupying different estuarine sites or habitats can be expected to have distinct otolith elemental compositions. Overall, successful application of otolith elemental signatures depends on their variation at relevant scales; and several studies have shown that areas within estuaries can be successfully discriminated over time (e.g. Chittaro et al., 2009; Gillanders and Kingsford, 2000; Miller, 2007; Tournois et al., 2013).

Despite the increased application of natural markers, studies using both tissue stable isotopes and otolith chemistry to assess connectivity or population structure are scarce (but see Dierking et al., 2012; Fodrie and Herzka, 2013; Lawton et al., 2010; Verweij et al., 2008) with none combining tissue isotope and otolith chemistry in an integrated manner. Movement and habitat use patterns of fish within estuaries may vary as a function of life history strategies, size- or age-specific habitat preferences and are also influenced by a combination of behavioural, ecological and environmental factors (e.g. salinity, temperature, food availability) (Stoner et al., 2001; Vasconcelos et al., 2010). Individuals respond to these processes at distinct spatio-temporal scales and therefore the study of movement and connectivity in estuarine fish may benefit from combined approaches of muscle stable isotope ratios and otolith chemistry. The use of multiple distinct natural markers is expected to enhance connectivity assessments as they respond at different spatio-temporal scales (e.g. Abaunza et al., 2008; Fodrie and Herzka, 2013; Lawton et al., 2010; Perrier et al., 2011; Thorrold et al., 2002; Verweij et al., 2008). Specifically, tissue isotopes are expected to integrate information over days to months (Herzka, 2005; Rubenstein and Hobson, 2004), whereas otoliths incorporate temporally resolved information throughout a fish's life history (Campana, 1999; Elsdon et al., 2008).

The aim of the present study was to use muscle stable isotopes and otolith elemental composition to evaluate the connectivity/fidelity between estuarine areas for juvenile age-0 European sea bass *Dicentrarchus labrax* (a species renown to use estuaries as nurseries) and common goby *Pomatoschistus microps* (an estuarine resident) and if connectivity patterns varied over time. Specifically, we examined if tissue stable isotopes ($\delta^{13}C$, $\delta^{15}N$) and otolith elemental composition varied between estuarine sites over time and if local or transient individuals from each site could be distinguished. We also examined whether the integrated use of these techniques enhanced our ability to unravel intra-estuarine connectivity, and evaluated the complementarity between these two natural tags. We were interested in both ecological and methodological issues, and when evaluating if habitat utilization patterns varied over time or as a function of life-history strategies we hypothesised that for *P. microps* site fidelity and low connectivity between estuarine areas would occur, due to low foraging and habitat range of these species (Cattrijse and Hampel, 2006; Salgado et al., 2004), whilst for *D. labrax* connectivity among sites would potentially increase over time, in line with juvenile growth and increase in size during their permanence within nursery areas (Kopp et al., 2013; Vinagre et al., 2008). Ultimately, knowledge on connectivity among segregated estuarine areas and habitats, or by contrast high site fidelity, will be essential for the development of effective estuarine management and habitat conservation strategies.

2. Materials and methods

2.1. Study area

The Tejo estuary is a large partially mixed estuary with c. 320 km^2 and tidal amplitude of c. 3 m. In the present study, two segregated sites were sampled: Vila Franca de Xira (VFX) in the upper estuary and Alcochete (ALC) in the middle reaches of the estuary (Fig. 1). These locations comprise the two existing nursery areas for juveniles of many fish species in the Tejo estuary, including *D. labrax* (e.g. Cabral and Costa, 2001; Vasconcelos et al., 2010). Both are fringed by extensive salt marshes, and are the main feeding and shelter habitats for the estuarine resident *P. microps* (e.g. Salgado et al., 2004). The latter are abundant, key benthic predators and an important species in food webs of estuarine salt marsh systems (Cattrijse and Hampel, 2006). In addition, previous studies have highlighted that organic matter sources and local food webs differ in isotopic composition between these two estuarine areas (e.g. França et al., 2011), and so does water chemistry (e.g. Tanner et al., 2013), further underpinning the use of soft tissue stable isotope ratio analyses and otolith chemistry to assess connectivity in the present study.

2.2. Sample collection and preparation

Beam trawls were conducted at both sites in July (early summer) and October (early autumn) 2009 to capture age-0 *D. labrax* and young adult *P. microps*. Once collected, fish were transported on ice and frozen at the laboratory (−20 °C). All fish were measured (total length to the nearest mm) (Table 1). For stable isotope analysis dorsal white muscle samples were extracted as $\delta^{13}C$ and $\delta^{15}N$ tend to be less variable in this tissue (Pinnegar and Polunin, 1999). For each species five replicates were run per site and sampling time. All instruments and tissue used in sample preparation for stable isotope analyses were cleaned with deionised water. For otolith chemistry, sagittal otoliths were extracted (c. 15 fish per site and season for each species, including those used for stable isotope analysis), washed and cleaned of adhering tissue with ultra-pure water and allowed to air dry in a positive pressure laminar flow hood.