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Simultaneous use of strontium:calcium and barium:calcium ratios in otoliths as markers of habitat: Application to the European eel (*Anguilla anguilla*) in the Adour basin, South West France

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A B S T R A C T

Sr:Ca and Ba:Ca ratios in water from the Adour estuary show a clear relationship with the salinity of the surrounding water for salinities <20, while ratios are almost constant above this level of salinity. A positive relationship was observed for the Sr:Ca ratio, whereas it was inverse for the Ba:Ca ratio. These two elemental ratios were measured in the otoliths of the European eels (*Anguilla anguilla* L.) using femtosecond laser ablation linked to an ICP-MS (fs-LA–ICP-MS). There was a direct relationship between the elemental ratios recorded in eel otoliths and those found in water from fresh and marine areas, suggesting that Sr:Ca and Ba:Ca ratios in eel otoliths can be used as markers of habitat in this estuary. Continuous profiling allowed the determination of three behaviour patterns in terms of habitat: freshwater, estuary and migratory individuals. Finally, the above results support the simultaneous use of both ratios for a better understanding of the migratory contingents and also as a relevant method to avoid a misidentification of environmental migratory history due to the presence of vaterite crystal in the otolith matrix.

1. Introduction

The European eel *Anguilla anguilla* L. is a high migratory catadromous fish. Leptocephali larvae are transported by the Gulf Stream from the hypothetic spawning ground in the Sargasso Sea (Schmidt, 1922) throughout its distribution area towards the European and North African coasts. Near to the continental shelf, a first metamorphosis takes place giving glass eels which colonize inland and coastal waters. After a growing period of 3–15 years depending on their sex, yellow eels undergo a second metamorphosis, they change into silver eels and emigrate into the Atlantic where the sexual maturation occurs. European eel populations have significantly declined throughout their distribution area since the 1980s (Moriarty and Dekker, 1997). In 2007, the European Council Regulation (CE 1100/2007) for the recovery of eel stocks required the management of eel populations at a local scale, level corresponding to hydrographic basin. At this local level, recent publications have shown that eels are not strict catadromous fish and exhibit a mosaic of habitat use during the growing phase inside a single hydrographic system i.e. strict marine residency, strict freshwater residency, migrations from freshwater habitats to marine habitats and vice versa (Daverat and Tomas, 2006; Tsukamoto and Arai, 2001; Tzeng et al., 2000). In the Adour basin (South West, France), glass eel fisheries in 2000 represented 80% of the figure for the local fishermen's business (Prouzet, 2002) showing the economic importance of this species and its management in this area. In order to better understand the habitat use and the probable interaction between eels and anthropogenic activities (pollution, fisheries, dams…) along the Adour estuary, investigations were conducted using the potential of geochemical indicators in the otoliths to reconstruct life history.

Otoliths are well known calcified structures, metabolically inert and continuously growing throughout life, consisting of successive discrete layers of aragonite crystalline microstructure corresponding to daily growth increments (Campana and Thorrold, 2001) deposited on a protein matrix. These layers called annuli are able to trap minor and trace elements within the matrix during the calcification

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process. In combination with age data, otolith elemental composition has been used to reconstruct migratory environmental history of fish (Elfman et al., 2000; Halden et al., 2000; Daverat et al., 2005; Shiao et al., 2006; Arai et al., 2007). Among elements trapped in the otolith, strontium (Sr), particularly the Sr:Ca ratio, is the most common marker used to describe migratory environmental history of diadromous and catadromous fish (Limburg et al., 2001; Milton et al., 2008), particularly European and Japanese eels (Tzeng et al., 1997; Shiao et al., 2006). On the French coast, Daverat and Tomas (2006) highlighted more than 6 behaviour patterns of European eel in the Gironde–Dordogne–Garonne system based on the analysis of strontium otolith composition. In order to check for the mosaic of habitat use in the Adour estuary and associated watersheds, analysis of Sr composition in yellow eel otoliths was assessed using LA–ICP-MS (laser ablation–inductively coupled plasma mass spectrometry). This method allows the quantitatively determination of trace and minor elements within otolith at a high spatial resolution (Coutant and Chen, 1993; Arai and Hirata, 2006). However, the use of Sr for the reconstruction of the individual history exhibits some limitations. First, variations in ambient chemistry over time, of the water masses in question have to show significant and consistent differences in ambient strontium levels. Chemical signatures inside the water mass also have to be stable over time to ensure that variations incorporated within the otolith matrix reflect or physiological changes or migration of the individual between different water masses. Finally, the accurate reconstitution of habitat histories depends upon the occurrence of vaterite inclusions, one of the three natural polymorphs of $CaCO_3$ (calcite, aragonite and vaterite). Indeed, vaterite was shown to trap less Sr than aragonite (Brown and Severin, 1999; Melancon et al., 2005; Tzeng et al., 2007), resulting in elemental composition not related to the ambient water chemistry leading in a misidentification of habitat use and historical migration (Tzeng et al., 2007; Jessop et al., 2008).

In the following report, we propose the use of Sr as an environmental marker to reconstruct the habitat use of eels in the Adour estuary and associated wetlands. However, in order to overcome the limitations of the presence of vaterite, we propose the association of strontium with barium. Recent findings (McCulloch et al., 2003; Elsdon and Gillanders, 2005a; Hamer et al., 2006) have underlined the potential of the Ba:Ca ratio to track environmental histories of fish, due to the ability of an element such as Sr to be incorporated into otoliths primarily in relation to ambient concentrations (Bath et al., 2000; Milton and Chenery, 2001; Elsdon and Gillanders, 2004; de Vries et al., 2005). Furthermore, McCulloch et al. (2005) highlighted the strong inverse correlation between Sr:Ca and Ba:Ca in barramundi (*Lates calcarifer*) aragonitic otolith whereas both ratios were shown to have similar pattern in vateritic otolith (Tzeng et al., 2007). As a first step, we propose the comparison of ambient water chemistry in the freshwater and marine waters of the Adour estuary to assess if concentration gradients exist for both Sr:Ca and Ba:Ca ratios in this region. Relation between otolith incorporation and water chemistry was then assessed by Comparing the Ba:Ca and Sr:Ca ratios determined from eel (*A. anguilla*) otoliths with water samples. Finally, we tested the reliability of using both elemental ratios recorded from individual otoliths to describe the life history of eels in the Adour estuary and the associated wetlands avoiding possible error caused particularly by the presence of vaterite inclusions.

2. Materials and methods

2.1. Fish sampling

Yellow eel sampling was conducted at four sites in the Adour basin (South West, France) that represent two extremes of water masses: saline and the strictly freshwater environment upstream (Fig. 1). Intermediate water masses were unfortunately not sampled due to the opportunist character of our sampling which was based on two fish population monitoring programs one located in the upstream wet zone of the estuary and the other one in lower part of the estuary. The first site, sampled in July 2005 and July 2007, is situated near the estuary mouth (Redon, $N = 14$) where the influence of marine waters is marked at the bottom with salinity usually between 25 and 35, but around 10–20 during specific tide and flow conditions. Two wetlands corresponding to floodplains and located closed to the upper limit of the saline intrusion were also sampled: one directly connected to the Adour river and subjected to the influx of brackish waters only during spring tides and low flow (St Laurent de Gosse, SLG, $N = 14$) and a second one connected to the Adour river by a tributary, showing brook characteristics (Termi, $N = 49$) and never affected by saline water. Samples were collected five times between October 2005 and July 2007 at these two sites by electrofishing. Finally, a couple of individual fish were collected only once in the Gave de Pau river (Cauneille, $N = 4$), one of the three major tributaries of the Adour estuary, outside the influence of the dynamic tide.

2.2. Water chemistry

Spatial variability in water chemistry (Ca, Sr, Ba) was investigated across the mixing zone of the Adour estuary, within different freshwater tributaries and wetlands where eels were collected (Fig. 1). Water samples were collected twice at the eel freshwater sampling sites (Fig. 1), on 5th September 2007 and 26th June 2008. To assess the spatial variation in water chemistry in the Adour estuary, the entire salinity gradient was also sampled on the same date, September 2007 and June 2008. Additional Ba water chemistry data were also obtained in the saline mixing zone as in the coastal zone using unpublished previous measurements (February and July 1998) and new sampling dates (April and June 2007). In 1998 and 2007, sampling was performed thanks to an ultra trace Go Flo Teflon coated sampler (General Oceanic) on the French oceanographic vessels “Côte d'Aquitaine” and “Côte de la Manche” (CNRS/INSU). Temporal variability in water chemistry during an annual hydrological cycle was checked on the Gave de Pau, a major tributary of the Adour River, based on a weekly sampling strategy from March to October 2007.

Most of the water samples used in this study were collected at subsurface and were processed using ultra-clean protocol as previously published in Point et al. (2007). In the lower estuary where a strong stratification of the water column is observed (Dailloux, 2008), water samples were also taken across the water column during some dates. No significant difference was found between vertical and horizontal concentrations of dissolved trace elements compared to salinity, particularly for Ba as for the other elements. Thereby, water samples collected directly below the surface, in the mixing zone of the estuary, were considered to be representative of the water chemistry variability experienced by eel at the bottom in the downstream estuary.

Sr and Ba concentrations were measured in freshwater and marine water samples using an X7 series CCT ICP-MS (Thermo Electron, Windsford, UK). Marine water samples were diluted to obtain salt content below 2 g L^{-1} prior to the analysis. Internal standard of Indium (2 μg L^{-1}) was used to correct for instrument drift. Ca was determined using ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry – PANORAMA, Horiba Jobin Yvon). Blanks were regularly performed, using the same protocol as for the samples, with 18.2 MΩ MQ water (Millipore). The general performance of the procedure was checked using the certified reference freshwater SLRS-4 (NRCC, Canada) or the CASS-4 (NRCC,