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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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Abstract:

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.

Keywords: Otoliths; *Anguilla anguilla*; Estuaries; Water chemistry; Strontium; Barium; Laser ablation; Inductively coupled plasma mass spectrometer

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 to 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 et 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 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, 2003; Arai and Hirata, 2006; Chu *et al*., 2007). 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 (*Anguilla 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, Canada), the latter being doped with Ba and Sr and diluted in the same manner as the samples. Mean Sr:Ca and Ba:Ca ratios (µg g^{-1}) were calculated for each site. However, due to a lack of samples from the two wetland areas, an overall average was established for freshwater and for the marine water (> 20) end-members.

2.3. Otolith preparation and determination of Sr:Ca and Ba:Ca ratio by fs LA-ICP-MS analysis

Sagittal otolith pairs were excised from the fish, cleaned according to Jones and Chen (2003) with H_2O_2 (ultrex), rinsed with 18.2 MΩ MQ water and dried. They were embedded in araldite resin (araldite DBF CH Escil, Chassieu, France), ground on a sagittal plan to expose their cores and polished with diamond paper of decreasing grain diameter (9.0, 3.0 and 0.1 µm) (Escil, Chassieu, France). Finally, the otoliths were rinsed with 18.2 MΩ MQ water and dried prior to the laser ablation-ICP-MS analysis.

Otolith samples were analysed with an IR 1030 nm femtosecond laser (Alfamet-Novalase, France) in conjunction with an Elan DRC II (Perkin Elmer). A linear raster scan ablation (width: 80 µm) was taken along the longest radius of the otolith. The laser beam, applied with a 500 Hz frequency, is rapidly moved by a back and forth displacement along the axis parallel to the annuli (y axis; speed 1 mm s^{-1}) while the otolith was slowly advanced in the perpendicular direction (x axis; speed 5 µm s^{-1}). This leads to a N shaped scan formed by the partly overlapped pulses of 20 µm (87% in the y axis and 94% in the x axis) and results over a whole ablated surface of a desired transect of 80 µm (y axis) whose length depends on the sagittal otolith size (x axis). The raster scan is taken from one edge to the other edge passing through the nucleus (Fig. 2). This allows two independent transects to be examined that would help in the identification of possible perturbations resulting from the crossing of vaterite inclusions.

One (or both) of the two transects was selected in order to determine chronological variation of ^{86}Sr and ^{138}Ba. A pre cleaning ablation, consisting in a very fast (50 µm s^{-1}) ablation and removing the outermost surface (~ 2 µm), was applied in order to avoid contamination of the sample surface (Wyndham *et al.*, 2004). Table 1 gives the characteristics of the ablation and the coupling. Analytical accuracy was achieved with the fish otolith certified reference material n°22 (National Institute for Environmental Studies, Japan) (Yoshinaga *et al.*, 2000). ^{43}Ca was used as an internal standard for each ablation to check for variation in ablation yield. Strontium and barium were standardised to calcium (i.e. Sr:Ca and Ba:Ca) based on the stoichiometry of calcium carbonate (400 000 µg Ca g^{-1} otolith), as these elements can substitute for calcium in the otolith matrix (Campana, 1999). Quantification of ^{86}Sr and ^{138}Ba concentration were allowed with the use of a standard curve based on the analysis of calcite pellets (Barats *et al.*, 2007). The average detection limit based on three standard deviations (SD) of the blank gas was 767 ng g^{-1} for ^{86}Sr and 32 ng g^{-1} for ^{138}Ba.

After the laser ablation, the otoliths were etched with EDTA 5% (Ultra grade, Fluka). A first observation of the otoliths by stereomicroscopy (x50) (Optech, Sodipro, France) under reflected light was carried out in order to check the presence of vaterite inclusions that appear opaque under these conditions (Tzeng *et al.*, 2007). In the second step, the otoliths were stained with Toluidine blue 5% (Standard, Fluka). Finally, annual growth layers, and particularly the annual growth stop mark, revealed by this coloration were observed by stereomicroscopy (x50) and counted to estimate individual age (Berg, 1985; Svedang *et al.*, 1998).

2.4. Relationships between water chemistry and otolith Sr:Ca and Ba:Ca ratios

In order to examine the relationship between otolith microchemistry and water chemistry, the mean individual elemental ratios (Sr:Ca and Ba:Ca ; µg g^{-1}) and the averages of all water samples within each site were compared. Mean otolith Sr:Ca and Ba:Ca values were calculated for each otolith using the portion of individual profile out of the elver mark up to the edge. Previous to this step, erratic peaks on otolith elemental profiles defined as peaks greater than 3 times the standard deviation of the mean were excluded from the calculation. Eel data from freshwater sites were compared to mean freshwater elemental ratios comprising all freshwater sites data (SLG, Termi, GP), whereas data from eels caught in the estuary were compared to data from the estuary water.

Mean elemental ratios from water (Me/Ca_{water}) (µg g^{-1}) and otolith (Me/Ca_{otolith}) (µg g^{-1}) were also used to calculate partition coefficients for Sr (D_{Sr}) and Ba (D_{Ba}) described as:

$D_{Me} = [Me/Ca]_{\text{otolith}} / [Me/Ca]_{\text{water}}$ (Morse and Bender, 1990).

Partition coefficients allow the evaluation of elemental discrimination in the otolith compared to the ambient water chemistry. D_{Me} was estimated for each site taking into account mean individual elemental ratio in the otolith and mean elemental ratio in the water for the site where the individual was caught. The partition coefficient for the whole freshwater environment was calculated as the average of D_{Me} from all freshwater eels (SLG, Termi, Gave de Pau).

All the elemental means were expressed within a 95% confidence interval. Differences in elemental ratio in water and otolith between sites were tested using non–parametric Kruskal Wallis test, Bravais Pearson tests were also performed to confirm the correlation between otolith and water data (Xlstat-Pro 7.5.2 ; Addinsoft, France).

2.5. Qualitative presence of vaterite inclusions as perturbation of continuous elemental profiles

Deficiency in both Sr:Ca and Ba:Ca ratios in vaterite sections compared to aragonite (Brown and Severin, 1999 ; Melancon *et al.*, 2005; Tzeng *et al.*, 2007; Jessop *et al.*, 2008) was used to discriminate possible affected regions in the otolith ablated sections. As in eel otoliths, both Sr:Ca and Ba:Ca ratios have been shown to be 7 times lower in vaterite than in aragonite (Tzeng *et al*., 2007), we visually checked for concomitant and drastic decrease in both Sr:Ca and Ba:Ca ratios from the two transects performed on both sides of the nucleus as indicative of possible vaterite inclusions. Because of the otolith asymmetry giving one transect longer than the other, and thus differential elemental dilutions during the analysis (depending on carbonate increments, analytic sampling and resolution), each side of the otolith nucleus was regarded as an independent profile. Furthermore, as vaterite crystal could be present across the whole ablated sections, mean elemental ratios calculated for individual otoliths were also compared to previously published Sr:Ca and Ba:Ca ratios in vaterite crystal from European eels *Anguilla Anguilla* (Tzeng *et al.*, 2007). Vaterite inclusions appear darker than aragonite under reflected light after 5% EDTA etching (Tzeng *et al.*,

2007) and can be easily differentiated by optical microscopy. Finally, since the otoliths were etched with 5% EDTA for the age estimation, the presence of large vaterite inclusions along the otolith ablated profiles was also checked for.

3. Results

3.1. Sr:Ca and Ba:Ca ratios in water bodies of the Adour estuary (freshwater, marine waters and salinity gradient)

Regarding elemental ratio (µg g^{-1}), the Sr:Ca ratio points to a linearly increasing trend between salinity >0.5‰ and ≤20‰ (Fig. 3a) as Ba:Ca shows a linearly decreasing trend (Fig. 3b) for the same salinity interval (r = -0.806 ; *P*<0.0001). These contrasting and opposite behaviours were found consistently whatever the flow conditions and result in a negative linear correlation (Fig. 3c) between the two elemental ratios along the >0.5‰ and ≤20‰ salinity range (r = -0.884; *P*<0.0001). Beyond 20‰ salinity, both Sr:Ca and Ba:Ca ratios do not seem to vary with salinity. As expected, a very high Sr:Ca ratio ($19.7 \pm 3.2 \times 10^{-3}$) and relatively low Ba:Ca ratio ($21 \pm 19 \times 10^{-6}$) were found at these high salinities (>20‰, Fig. 3a and b). With the freshwater samples, lowest Sr:Ca ratios were encountered over a large range (Fig. 3a), varying from 2.08×10^{-3} to 5.51×10^{-3} and averaging $2.75 \pm 0.96 \times 10^{-3}$. On the contrary, the highest Ba:Ca ratios were always found at the freshwater sites (Fig. 3b), $\geq 223 \times 10^{-6}$ with an average value of $404 \pm 68 \times 10^{-6}$. Whatever the situation no overlap between freshwater data and estuary data was found for the Sr:Ca ratio (Fig. 3a). As regards the Ba:Ca ratio (Fig. 3b), that of freshwater can sometimes be similar to that observed in low salinity (0.5‰ and 5‰) estuary water ($165\text{-}342 \times 10^{-6}$). Nevertheless, the combined use of both Sr:Ca and Ba:Ca ratios seems to resolve the uncertainty of the Ba:Ca ratio at low salinity (Fig. 3c). There were too few water samples among sites to investigate possible difference in elemental ratios within the freshwater tributaries. The variability recorded on the tributary Gave de Pau (Fig. 3d) across almost an entire hydrological cycle (March to October 2007), was found to encompass the overall dispersion of Ba:Ca data including all freshwater sites (Fig. 3a, b). Sr:Ca ratios in water were found to be very stable over time in the Gave de Pau river.

3.2. Sr:Ca and Ba:Ca continuous profiles in otoliths

For all otoliths, high Sr:Ca and Ba:Ca ratios (Sr:Ca = 10×10^{-3} ; Ba:Ca = 3.5×10^{-6}) were observed in the central region corresponding to the leptocephalus stage from the core and 150 µm out. These ratios decrease after the elver mark and is thought to be linked to the metamorphosis and the remobilization of element stock more than to the salinity occurring during this event (Otake *et al.*, 1997) (Fig. 4), and thus cannot be used for habitat reconstitution. The latter is based on the elemental signature of the part out of the elver mark up to the otolith edge. This section represents 4 to 10 years according to age estimation. Femtoseconde laser scanning of eel otoliths revealed three main types of combined Sr and Ba profiles out of the elver mark. The first one was observed for all the individuals caught at the freshwater sites, which showed consistently low Sr:Ca mean ratios ranging from 0.94×10^{-3} to 2.57×10^{-3} with an average of 1.59×10^{-3}, and relatively high Ba:Ca mean ratios comprised between 4.54×10^{-6} and 21.8×10^{-6} with an average of 12.1×10^{-6} maintained throughout their life (Fig. 4a). Contrary to the Sr:Ca ratio which is relatively constant throughout the life of eels caught in freshwater sites, the Ba:Ca ratio exhibited irregular variations, some eels showing a progressive decrease in the ratio with age while others presenting relatively constant or very fluctuating Ba:Ca ratios (Fig.4a). The two other types of profiles were only found in eels caught within the lower part of the estuary. 49% of yellow eels from this area showed a consistently high Sr:Ca mean ratio (from 4.78×10^{-3} to 7.91×10^{-3} with an average of 6.45×10^{-3}) and a low Ba:Ca mean ratio (0.51×10^{-6} and 4.42×10^{-6} with an average of 1.16×10^{-6}) after the elver mark (Fig. 4b). Elemental profiles of 51% of eels sampled in the estuary revealed large variations of both elements that would indicate a third pattern of life-history (Fig. 4c). For almost all eels of the third group, large variations observed for Sr:Ca ratios are characterised by a decrease of the ratio after the elver mark, then ratio remains stable for a period depending on the individual, two to four years according to age estimation, with values similar to those found in freshwater individuals. After this period, an increase of Sr:Ca ratio is observed until a ratio value corresponding to those described in the second profile (from 4.78×10^{-3} to 7.91×10^{-3}) which is maintained until the sampling date. In very few eels, the Sr:Ca ratio exhibited multiple fluctuations between values found for freshwater and lower estuary sites. For all these individuals in the third group, Ba:Ca exhibits a strongly inverse relationship.

3.3. Otolith mean ratios and water chemistry

3.3.1. Otolith vs ambient water

Mean ratios cited above and calculated from elemental continuous profiles from the elver mark to the edge were compared to elemental mean ratios in water (Fig. 5). Otolith ratios from eel caught in freshwater sites were compared to the mean ratio given by all freshwater data of all freshwater sampling sites ($\text{Sr:Ca}_{\text{water}}$: $2.8 \pm 0.5 \times 10^{-3}$; $\text{Ba:Ca}_{\text{water}}$: $408.5 \pm 58.7 \times 10^{-6}$), whereas otolith ratios from eels caught in the estuary were compared to elemental mean ratios based on water data from the estuary (September 2007 and June 2008 ; $\text{Sr:Ca}_{\text{water}}$: $19.7 \pm 1.6 \times 10^{-3}$; $\text{Ba:Ca}_{\text{water}}$: $21.3 \pm 9.7 \times 10^{-6}$). The variability (standard deviation SD) around the average ratio for each individual otolith was lower for Sr:Ca in each end-member (SD averaging 20%) compared to that observed for Ba:Ca (SD generally >30%). The Mann-Whitney *U*-test confirmed a significant difference in Sr:Ca and Ba:Ca levels in otolith between strict estuary (salinity >20) and freshwater sites (*P* value of Sr:Ca < 0.007 and Ba:Ca < 0.005), and reflected the observed water chemistry differences (Fig. 5).

3.3.2. Partition coefficient

Partition coefficients for Sr and Ba, namely D_{Sr} and D_{Ba}, characterised the relationship between otolith and the ambient water chemistry. Mean D_{Sr} and D_{Ba} differed slightly between freshwater and saltwater of the estuary, 0.49 ± 0.24 and 0.33 ± 0.08 for Sr and 0.036 ± 0.024 and 0.043 ± 0.04 for Ba respectively. Greater variability in D_{Ba} occurred as indicated by greater confidence intervals. Regarding the partition coefficient values for each site, the Gave de Pau site showed the lowest D_{Ba} of freshwater sites (0.016±0.04) whereas the Termi site values were the highest (0.040±0.011) contributing to the large variability observed for the overall D_{Ba} average for freshwater sites. Eels from St Laurent de Gosse showed a D_{Sr} (0.314±0.042) similar to the estuary value although eels from Termi and the Gave de Pau river showed the highest values (0.549±0.087 and 0.438±0.089 respectively). Slightly higher D_{Sr} mean in freshwater could indicate a more active uptake of Sr:Ca at low ambient Sr levels, or other sources of Sr than water.

3.4. Sr:Ca and Ba:Ca association

3.4.1. Sr vs Ba in otolith for determination of environmental trend

As Sr and Ba exhibit inverse relationship and otolith composition reflects Sr and Ba behaviours in water, mean Ba:Ca ratios of individual otoliths were plotted versus Sr:Ca in Figure 6 for all sampling sites. This approach indicates the dispersion of individuals according their Sr and Ba ratios reflecting the environmental signature and thus the environmental trend of their entire life. Furthermore, such an illustration aims to determine Sr:Ca and Ba:Ca limits which define a specific habitat characteristic (freshwater or saline or intermediate salinity). Three groups were identified. The first one includes the 67 eels caught in freshwater sites which are constrained within $4.54\text{x}10^{-6}$ for the lowest Ba:Ca ratio and $2.57\text{x}10^{-3}$ for the highest Sr:Ca ratio. The second encompasses eels caught in the estuary and exhibiting a stable elemental otolith profile meaning no habitat change during the growing phase. These individuals show Sr:Ca up to $5.90\text{x}10^{-3}$ and Ba:Ca ratios lower than $1.80\text{x}10^{-6}$. These two first groups do not overlap and clearly distinguish eels which have a growing phase dominated by a freshwater habitat from eels which have a growing phase dominated by a saline environment. However, 51% of the eels caught in the estuary fall between these two distinct groups. These eels correspond to those showing a drastic change in elemental composition in the otolith matrix during the growing phase and constitute the third group.

3.4.2. Check for vaterite disturbance

For most of the ablated transects made from the nucleus to the opposite edge of the individual otolith, elemental ratio profiles do not show strong and simultaneous depletion of both elements suggesting that vaterite inclusions would be rather limited along the ablated sections. In addition, as illustrated on Figure 6, when Sr:Ca mean ratios in the otolith from freshwater eels do not really differ from the values found in the vaterite crystal sections of the same eel species (Tzeng *et al.*, 2007), then the Ba:Ca ratio is far from the ratio encountered in this carbonate polymorph. The reverse is also shown suggesting that otoliths in the present study do not suffer from inclusions such as vaterite.

3.5. Sr:Ca and Ba:Ca to reconstruct historical migrations

Reconstruction of the history of eel habitat-use was conducted using the combination of both ratios in otoliths. Three assumptions were thus made based on observed ranges of Sr:Ca and Ba:Ca ratios in otoliths, that would allow the interpretation of residency in the salt waters (estuary or coastal) or the freshwater environment of the Adour basin or migration between the two habitats for all eels irrespective of sampling location. The three assumptions are as follow:

(1) Background Sr:Ca ratios < $2.23\text{x}10^{-3}$ (mean Sr:Ca background ratios of 67 eels caught in the freshwater catchment plus 2 S.D.) and Ba:Ca ratio > $4.84\text{x}10^{-6}$ (mean Ba:Ca background ratios of 67 eels caught in the freshwater catchment minus 2 S.D) were considered as freshwater residency.

(2) Background Sr:Ca ratios >$4.93\text{x}10^{-3}$ (mean Sr:Ca background ratios of 14 eels caught in the lower estuary minus 2 S.D.) and Ba:Ca ratio <$3.2\text{x}10^{-6}$ (mean Ba:Ca background ratios of 14 eels caught in the lower estuary plus 2 S.D.) were considered as salt water residency with salinity >20, either in the coastal zone as in the lower estuary area.

(3) Otolith with intermediate Sr:Ca and Ba:Ca values, were regarded as having residence in the salinity gradient (2 – 20) or associated to interhabitat shifters (see Fig. 4) moving from one environment to the other. In this latter case, each end-member signature could be recorded successively, if an eel resides long enough in each environment for the elemental ratio to be imprinted in detectable levels.

Based on the three assumptions defined above, life-history scans of the otolith, using simultaneous high resolution Sr:Ca and Ba:Ca ratios out of the elver mark, allowed three types of profiles to be distinguished. The first one was observed for all the individuals caught at the freshwater sites, which showed consistently low Sr:Ca ratios and high Ba:Ca ratios throughout their life (Fig. 4a). This pattern confirms that eels collected in the freshwater area of the Adour estuary did not subsequently move between fresh and salt waters throughout their life, but rather continuously resided in freshwater until the sampling date. The two other types of profiles were only found in eels caught within the lower part of the estuary. 49% of yellow eels from this area showed consistently high Sr:Ca ratio and low Ba:Ca ratio after the elver mark (Fig. 4b). Inversely to the previous type, this simultaneous Sr:Ca and Ba:Ca trend illustrates a permanent residency in the estuary at salinity > 20 without ever entering freshwater. For the second half of the individual eels within the estuary, one drastic and simultaneous change in Ba and Sr ratio occurred. Indeed, a period of low Sr:Ca and high Ba:Ca ratios, corresponding to ratios observed for freshwater eels, appeared before a high Sr:Ca and low Ba:Ca, similar to ratios found for eels from the Adour estuary (Fig. 4c).

4. Discussion

4.1. Water and otolith chemistry

There is a growing literature that explores the otolith microchemistry especially to distinguish populations or stocks, or to reconstruct migratory histories of fish (Campana, 1999). Nevertheless, spatial differences in elemental concentrations in waters have rarely been measured and are often derived from predictable relationships with salinity. Such relationships were used for Sr despite the fact that some freshwater locations may have water with Sr:Ca ratios close to or greater than seawater (Limburg, 1995; Wells *et al.*, 2003; Kraus and Secor, 2004). In addition, limited emphasis has been placed on examining temporal variability (Elsdon *et al.*, 2008) although Ca, Sr and Ba are expected to regularly change in concentration within estuaries or coastal zones with different time-scales (day, week, month, season) (Elsdon and Gillanders, 2006) due to the interplay of several processes (tidal cycling, freshwater flow, flushing time …). Indeed, the distribution of ambient Ba:Ca along estuary gradients would be largely influenced by the release of Ba-enriched fluvial particles at low salinities in the upper estuary zone (Li and Chan, 1979; Coffey *et al.*, 1997), benthic–water column fluxes (Colbert and McManus, 2005), mixing of fresh and salt water (Coffey *et al.*, 1997; Elsdon and Gillanders, 2006) and uptake in association with biological activity (Guay and Falkner, 1998). In a recent review, Elsdon *et al.* (2008) insisted on the necessity of acquiring more knowledge of variability such as on factors influencing otolith chemistry, before elemental information recorded in the otolith can be used to accurately describe life history events and fish movement patterns.

In this study, spatial and temporal variability of water chemistry on Sr:Ca and Ba:Ca ratios in the Adour estuary were investigated. The behaviour of Sr and Ba were clearly related to the salinity in accordance with other studies (Coffey *et al.*, 1997; Guay and Falkner, 1998; Secor and Rooker, 2000; Howland *et al*., 2001; Kraus and Secor, 2004; Colbert and McManus, 2005; Moore and Shaw, 2008), resulting in relationships between Sr:Ca and Ba:Ca ratios and salinity. Nevertheless, such relationships appeared to be valid only for a salinity levels below 20‰. Within this salinity interval, clear close to linear relationships between Sr:Ca (positive) and Ba:Ca (negative) and salinity were defined whatever the flow regime (i.e. spring runoff and low flow periods).

A negative trend between Ba:Ca and salinity was previously observed by Elsdon and Gillanders (2005b) but only for salinity above the Ba peak concentration expected at low to mid salinity and depending on the river flow, the suspended load and how compressed the salinity gradient is along the estuary (Coffey *et al.*, 1997; Guay and Falkner, 1998; McCulloch *et al.*, 2003; Moore and Shaw, 2008).We found significant variations in ambient Ba:Ca concentration (30 to 100 µmol mol^{-1}) at low salinities (0 - 10‰), but of a lesser magnitude to those observed by Elsdon and Gillanders (2005b) in southern Australian estuaries. Taking into account of the low turbidity of the Adour estuary and the Ba load measurements performed in both suspended particles from the fluvial zone, the estuary salinity gradient and the bed sediments within the estuary (Bareille, unpublished results), a limited release of Ba from fluvial particles towards the dissolved pool would be expected, leading to a reduced dissolved Ba peak at low salinity and thus less variability in the Ba:Ca ratio. This is in agreement with the maximum dissolved Ba concentration (100–150 nmol L^{-1}) measured at low salinities (0-10‰) in the Adour that occurred at a magnitude similar to the lower end-member found in the literature (Guay and Falkner, 1998). Concerning the freshwater end-member, relatively large variations were found apart from the average for both ratios. This variability can be related, for the Ba:Ca ratio, to the seasonal flow pattern and to weekly changes for which the cause is as yet unclear, while they appear to be more largely driven by seasonal scale for the Sr:Ca ratio, as previously found by Elsdon and Gillanders (2006). Part of the variability can be associated to underlying geology as well as processes influencing elemental concentrations in water (dilution, active binding to fluvial sediments, benthic-water fluxes…). In addition, lowest ambient Ba:Ca concentrations from the freshwater end-member exhibited values that could overlap with those found in low salinity estuary water (0-5‰), confusing any attempt to distinguish freshwater occupancy from upper estuary occupancy of fish by using the otolith Ba:Ca ratio. Nevertheless, as no water Sr:Ca concentration overlapping was observed between freshwater and low salinity water in this study, one would expect that this limitation can therefore be resolved based on the simultaneous use of both Ba:Ca and Sr:Ca ratios. This is strongly supported by the distribution of Sr:Ca and Ba:Ca ratios in otolith, where these ratios show an interesting complementarity as shown in figure 6 (see below).

Beyond 20-25‰ salinity, both ratios did not vary according to the salinity. They remained almost constant as was observed in other systems (Kraus and Secor, 2004; Elsdon and Gillanders, 2005b), at values similar to normal seawater for the Sr:Ca (18-20 µg mg^{-1}) and to Atlantic surface water for the Ba:Ca (around 5-11 µg mg^{-1}) (Guay and Falkner, 1998). Some variations in the Ba:Ca ratio can be found in the 20-35 salinity interval but they appeared to be very small compared to the observed gradient between the two end-members. They are either due to isolated high peak concentrations of Ba or to higher global levels of dissolved Ba for some dates which may reflect changes in particulate load or difference in the mixtures of river inflow and marine end-member (Fry, 2002). Furthermore, it would imply that discriminating eels living in strict marine habitat (i.e., the coastal zone) from those living in the lower estuary (i.e., mixing zone corresponding to a salinity range between 20 - 30) would be more difficult with these two elemental ratios in the Adour estuary and along the Basque coast.

Our results clearly support the potential use of Sr:Ca and Ba:Ca ratios to trace freshwater and salt water environmental histories of eels and other fish inhabiting this estuary, but the simultaneous use of the two ratios seems to be strongly advised to resolve uncertainties especially regarding the Ba:Ca ratio. Similar trends in water and otolith ratios in our study were consistent with previous studies on other fish species confirming the growth evidence that incorporation of both Sr and Ba into otoliths is primarily driven by ambient concentrations (Bath *et al.*, 2000; Milton and Chenery, 2001; Elsdon and Gillanders, 2004; Lin *et al.*, 2007). Nevertheless, although freshwater displays around seven times less Sr:Ca and twenty times more Ba:Ca than saltwater, we found that freshwater otoliths display around four times less Sr:Ca and twelve times higher Ba:Ca ratio than in the waters with salinity >20. This can be attributed to physiological regulations of elements at several biological interfaces (gills, intestine, inner ear membrane…) before they can be deposited into the crystallizing otolith (Campana, 1999). Several parameters can in fact affect elemental uptake through the gills (salinity, pH, dissolved oxygen concentrations, hardness, temperature …). Furthermore, geochemical processes in carbonate mineral precipitation may further influence element incorporation through competition or facilitation between elements (de Vries *et al.*, 2005). Partition coefficients are subsequently used to describe the relationship between elemental concentrations in otolith and in the ambient water (Bath *et al.*, 2000; Elsdon and Gillanders, 2003; Martin *et al.*, 2004; Elsdon and Gillanders, 2005b). All partition coefficients in our study were below one, meaning that Sr:Ca and Ba:Ca ratios in otoliths were lower than those of the surrounding water. The overall D_{Sr} for freshwater (0.49±0.24) was similar to those found by Wells *et al.* (2003) and De Vries *et al.* (2005). For saltwater, the overall D_{Sr} (0.33±0.08) was also in the range of 0.28 described by De Vries *et al.* (2005) and

Elsdon and Gillanders (2005b), but slightly higher than coefficient found (D_{Sr} = 0.18) by Kalish (1991) and Bath *et al.* (2000) in several marine fish species. We observed D_{Ba} of 0.036±0.024 and 0.043±0.04, respectively in freshwater and saltwater, close to those cited for other species by Bath *et al.* (2000) in marine water and De Vries *et al.* (2005) and Wells *et al.* (2003) (D_{Ba} = 0.04) in freshwater. However these data were largely less than those found by Elsdon and Gillanders (2005b) (D_{Ba} = 0.136±0.005) in marine water for the black bream (*Acanthopagrus butcheri*).

Alternatively, D_{Sr} and D_{Ba} in this study present relatively large standard deviations especially for freshwater. This could be due first to the inter freshwater site heterogeneity in the partition coefficient values, and supported by the wide variability we encountered in Ba:Ca and Sr:Ca ratios of the otolith from the freshwater end-member (see Fig. 4). It is difficult to speculate the cause of this large variability as it can be linked to biological (sex, reproductive status, dietary sources, growth rates…) and kinetic effects. Bath *et al.* (2000), Elsdon and Gillanders (2005a) and De Vries *et al.* (2005), suggested an active incorporation of Sr and Ba at low ambient water ratios. It was proposed that changes in elemental discrimination would be linked to regulation of the amount of Sr and Ba transported in the endolymphatic fluid (Bath *et al.*, 2000; Elsdon and Gillanders, 2003). The role of available binding sites on the calcite crystal surface was also suggested. At low ambient elemental concentrations, adsorption of elements may increase, while binding sites become saturated as elemental concentrations rise and equilibrium is reached in the solution interface and growing crystal surface (Watson, 1996). According Bath *et al.* (2000), Sr and Ba incorporation in fish otoliths were independent from growth rates of individual fish. No significant effect of temperature was found on Ba uptake (Bath *et al.*, 2000; Elsdon and Gillanders, 2005a). Despite large seasonal temperature variations (from 6°C to 23°C), eels in this study came from habitats geographically close to each other and thus underwent similar influence of temperature. As a result, this influence on otolith composition can be considered as negligible. A small proportion of elements probably originated from food sources (Limburg, 1995; Farrell and Campana, 1996; Gallahar and Kingsford, 1996). Walther and Thorrold (2006) recently highlighted the water as the major source of Sr and Ba with more than 83% and 95% respectively, in otoliths from juvenile mummichogs (*Fundulus heteroclitus*). Finally, we cannot exclude that some individual eels caught in the different freshwater areas did not move within several tributaries that could have had significant water Ba:Ca and Sr:Ca ratios. However, this does not affect the ability to recover significant differences in otolith ratios between contrasted fresh and seawater end-members in the Adour estuary.

4.2. Simultaneous use of strontium and barium to avoid vaterite disturbance

Interpretations of chemical profiles along the otolith axis also rely on the presence of vaterite inclusions that could lead to misinterpretation of migratory history (Brown and Severin, 1999; Tzeng *et al.*, 2007; Jessop *et al.*, 2008). The occurrence of such inclusions was clearly demonstrated in several fish such as the European eel (Bowen Li *et al.*, 1999; Brown and Severin, 1999; Tomas and Geffen 2003; Tzeng *et al.*, 2007; Jessop *et al.*, 2008; Morat *et al.*, 2008). Chemical composition of vaterite inclusions in the otoliths were showed to be significantly different from the aragonite matrix as, fewer Sr:Ca and Ba:Ca ratios were measured in vaterite crystal than in aragonite of European and American eel otoliths (Jessop *et al.*, 2008; Tzeng *et al.*, 2007). The elemental differences between the two carbonate polymorphs can be attributed to the different coordination numbers of cations in substitution of Ca^{2+}, limiting the entry of Sr, Ba ions to the vaterite lattice while facilitating the entry of Mg and Mn during crystallisation (Tzeng *et al.,* 2007), and resulting in systematically lower Sr:Ca and Ba:Ca concentration ratios in vaterite than in aragonite. Sr:Ca ratios in otoliths of eels from freshwater sites on the Adour basin ($1.59 \pm 0.32\ 10^{-3}$) could be confused with average Sr:Ca ratio found within vaterite inclusions ($0.56 \times 10^{-3} – 1 \times 10^{-3}$) (Tzeng *et al.*, 2007; Jessop *et al.*, 2008). Moreover, Ba:Ca ratios in vaterite crystal ($0.53 \pm 0.74 \times 10^{-6}$) (Tzeng *et al.*, 2007) did not really differ from ratios we found in otoliths from marine waters ($1.16 \pm 1.02 \times 10^{-6}$ – Fig. 6). Using only the Sr:Ca ratio could lead to confusing vaterite signature with freshwater habitat signature, while using only Ba:Ca could cause misinterpretation of vaterite composition as marine water habitat. However, combining both elemental signatures and profiles leads to the easy discrimination of such polymorph inclusion. In the absence of vaterite when one ratio is low (close to the ratio that can be confused with vaterite) the other may be high and vice versa, allowing the definitive differentiation of aragonite from the vaterite signal. According Tzeng *et al.* (2007), the percentage of otoliths of European eel with vaterite inclusions can be very high. 48% of the 108 eels they analysed showed this type of inclusion and illustrates the necessity to distinguish vateritic profiles from aragonitic ones based on a relevant and easy tool. In the Adour estuary, the screening of the analysed otoliths carried out by optical microscopy confirmed the chemical results, which are the low occurrence of these inclusions in this area.

4.3. Reconstruction of eel habitat-use.

Otolith Sr:Ca ratio has been widely used to determine habitat discrimination and the reconstruction of the movements between freshwater and marine habitats of catadromous fish such as anguillids (Tsukamoto and Arai, 2001; Daverat *et al.*, 2005; Arai and Hirata, 2006; Shiao *et al.*, 2006). Conversely, Ba:Ca ratio has been largely unexplored in any fish species (Arai and Hirata, 2006; Hamer *et al.*, 2006) in spite of its great promise (Bath *et al.*, 2000; de Vries *et al.*, 2005; Elsdon and Gillanders, 2005b; Gillanders, 2005). McCulloch *et al.* (2005) highlighted the sensitivity of the Sr:Ba otolith ratio to environment chemical signature especially for transitional environments such as estuaries. Sr and Ba levels in the otolith of barramundi (*Lates calcarifer*) leads to the determination of a mosaic of behaviours in terms of habitat use in this species. Our investigations on Sr:Ca and Ba:Ca profiles into eel otoliths of the Adour estuary, using laser ablation-ICP-MS, confirm the potential of both ratios to help distinguish the freshwater and seawater habitats of the eel and support the results obtained by Arai and Hirata (2006).

Previous studies (Feunteun *et al.*, 2003), and especially those using Sr:Ca otolith microchemistry described different behaviours or tactics of habitat use within an eel population (Tsukamoto and Arai, 2001; Daverat *et al.*, 2005). Three major contingents were defined for several eel populations coming from different water systems around the world: Europe (Daverat *et al.*, 2005), Lithuanian and Baltic (Tzeng *et al.*, 2000; Shiao *et al.*, 2006), Asia (Tsukamoto and Arai, 2001; Tzeng *et* *al.*, 2002), America (Jessop *et al.*, 2002) and New Zealand (Arai *et al.*, 2004): a river resident group, estuary and marine resident groups, and a last one with eels called “migrants”, “transients” or “nomads” that moved from one habitat to another once or more during their growth phase. These three types of behaviour were also found in our study using only the Sr:Ca profiles provided by fs-LA-ICP-MS. However the same scheme and distribution of individuals were obtained with the Ba:Ca profiles supporting the use of this ratio as a relevant marker for the discrimination of habitat use pattern on the Adour basin. Moreover, mean values of Sr:Ca ratios for each situation were in agreement with those found for European eels from other French watersheds (Daverat *et al.*, 2005) and for *Anguilla japonica* (Tsukamoto and Arai, 2001; Tzeng *et al.*, 2003; Arai and Hirata, 2006). Our conclusion on the Ba:Ca ratio also supports the results of Elsdon and Gillanders (2005b) using Ba to trace the freshwater environmental histories of black bream (*Acanthopagrus butcheri*).

The presence of migrant eels was observed in European eels as in the other eel species like *A. japonica* (Tsukamoto and Arai, 2001; Tzeng *et al.*, 2003), *A. rostrata* (Morrison *et al*., 2003; Jessop *et al*., 2006; Lamson et al., 2006), *A. diffenbachii* and *A. australis* (Arai *et al.*, 2004). In the sampling group from the Adour Basin used to test the methodology, only one pattern of migration was observed, a downstream migration with a single drastic shift of habitat. This shift occurs after 2 to 4 years spent in freshwater. Daverat *et al.* (2005) observed this phenomenon for 91% of the migrants before age class 5. In addition, Daverat *et al.* (2006) underlined the possibility of a size dependent migration for downstream nomads as the migration occurred in a similar range of age for all migrants. There was also evidence of upstream migratory behaviour, always with only one drastic change of habitat during the lifespan in the Gironde watershed (Daverat *et al.*, 2005) as in Lithuanian waters (Shiao *et al.*, 2006). For *A. japonica* (Tsukamoto and Arai, 2001) and *A. australis* in New Zealand (Arai *et al.*, 2004), migratory behaviour with two drastic changes of habitats were also observed. In our study, only one eel from the estuary sampling exhibited a Sr:Ca profile showing regular variations within the range of estuary or marine water signature. This individual suggests that several migratory patterns could also be observed in the Adour estuary. The opportunistic sampling used in this study probably did not encompass all the migratory patterns especially those that are less frequent in the population. The absence of upstream migratory behavior as observed for *A. japonica* or *A. australis* may be due to the limited sampling in coastal zones near the Adour estuary. Moreover, the mobility of a group might be different according the species and the system considered. However, all the studies highlight the evidence of the semi catadromous life history of the eel. Furthermore, a large proportion of the eels caught in the downstream part of the Gironde and Adour estuaries exhibit a resident behaviour, 44% (Daverat and Tomas, 2006) and 50% (this study) respectively. In both studies, 100% of freshwater individuals were freshwater residents, illustrating also in the Adour Basin the decrease of migratory behaviour from the upstream river to marine environments. This was also observed in other systems for *A. rostrata* and *A. japonica* (Tsukamoto and Arai, 2001; Morrison and Secor, 2003).

Conclusions

In summary, a wide range of literature deals with the complexity of the interpretation of the otolith elemental signature, and the care that must be taken on all aspects of the links between environment and elemental composition, non biotic and biotic factors influencing elemental uptake and also variability of matrix structure. In this study, we illustrated the relevant use of Sr:Ca and Ba:Ca ratio chronology in the otolith in order to reconstruct the migratory history of the European eel avoiding misinterpretation due to a change in the otolith structure (vaterite inclusion) as well as reducing the uncertainty at low salinities by using the Ba:Ca ratio alone. Based on rigorous environmental monitoring, this combination can be a powerful tool for a better understanding and management of the habitat of interests for this declining species.

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Figures

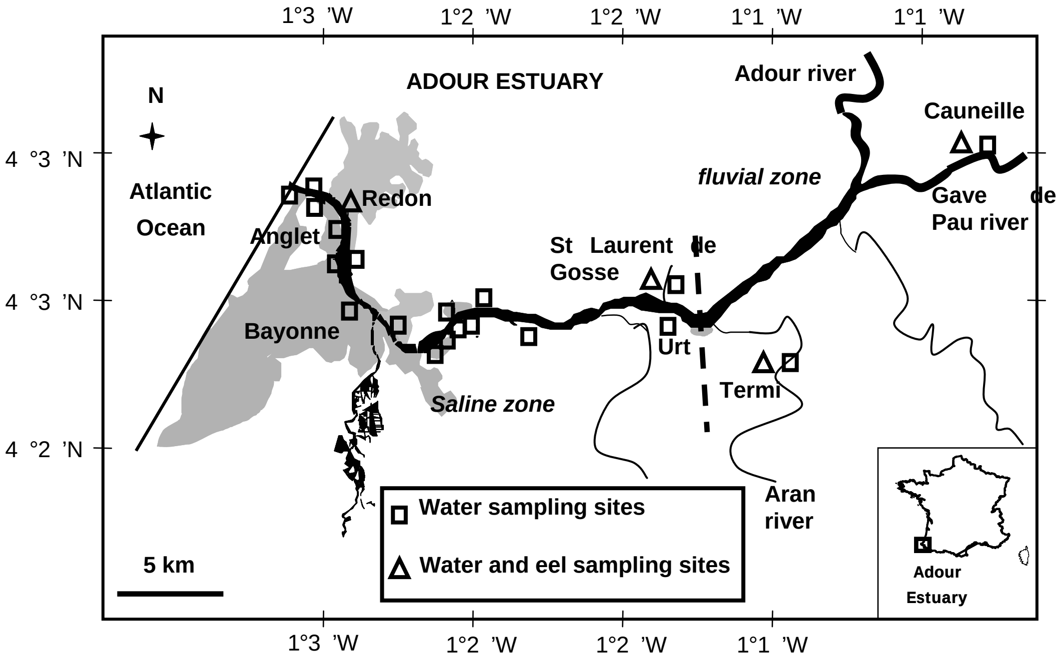

Figure 1: Map of the Adour basin showing the sampling sites for water (square) and yellow eels (triangle). Dotted line: Limit of the saline tide influence.

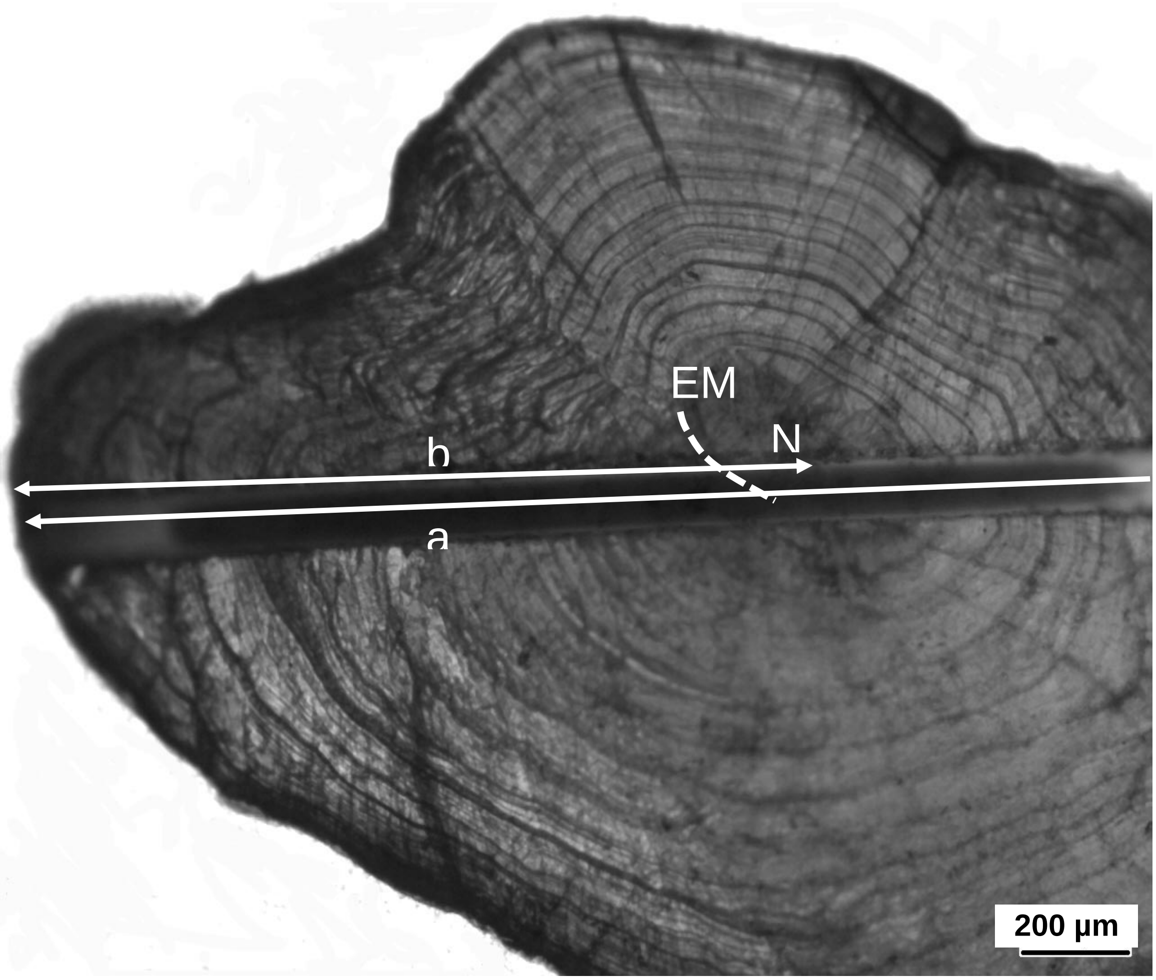

Figure 2: Otolith of yellow European eel (33.6 cm total length, age estimated 8 years) from freshwater site showing the entire continuous transect made by fs-LA-ICP-MS (a) from the edge to the other, and the section used for analysis (double arrows, b). EM: elver mark; N: nucleus.

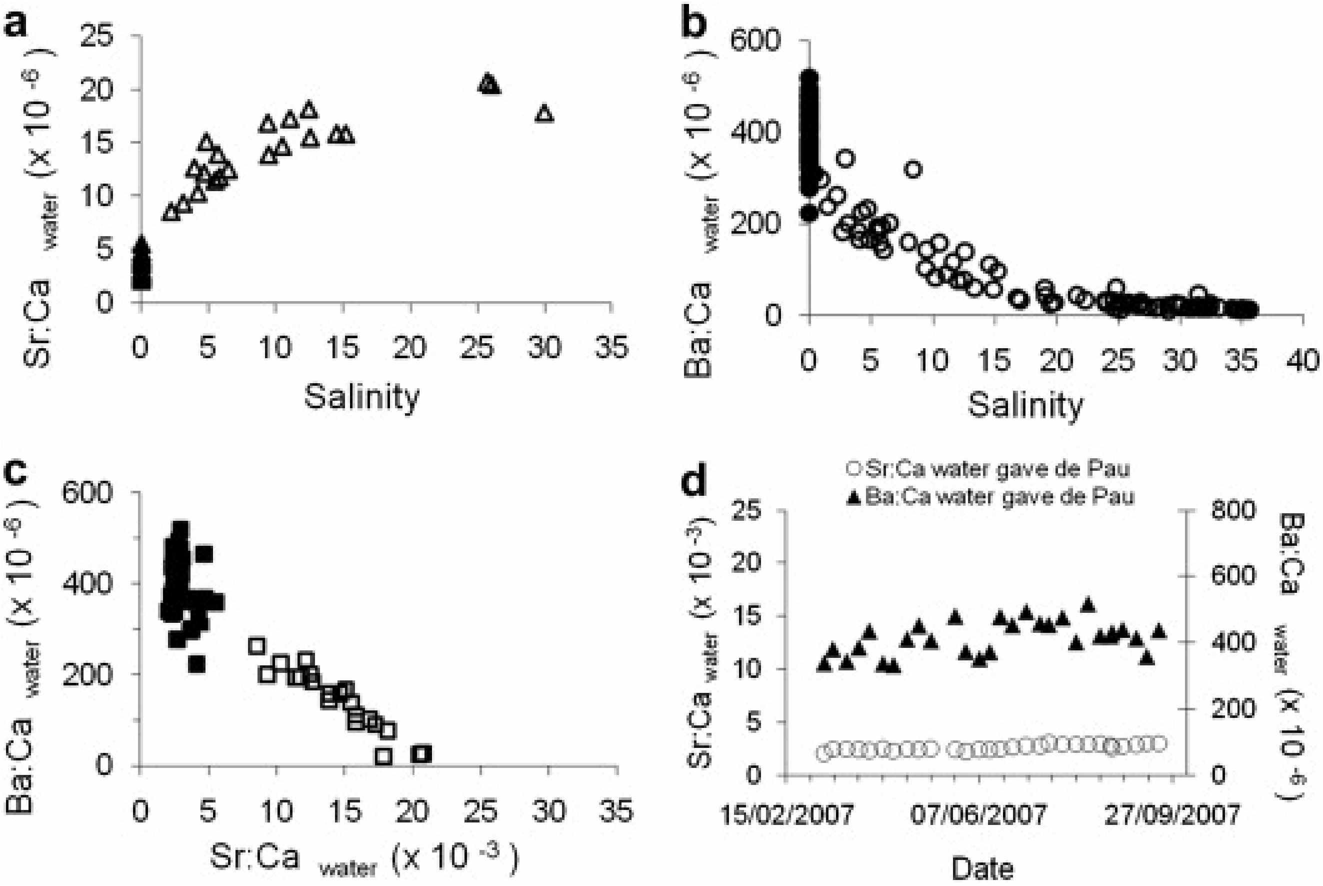

Figure 3: Relationship between element: Ca ratio in water of the Adour estuary and salinity: Sr:Ca ratio (a), Ba:Ca ratio (b), relationship between Ba:Ca and Sr:Ca ratios (c), and temporal evolution of Sr:Ca and Ba:Ca ratios along 2007 in the Gave de Pau river (d). Filled symbols represent samples with salinity <0.5‰, whereas open symbols consist of saline water (>0.5‰) in figure 3a), 3b) and 3c).

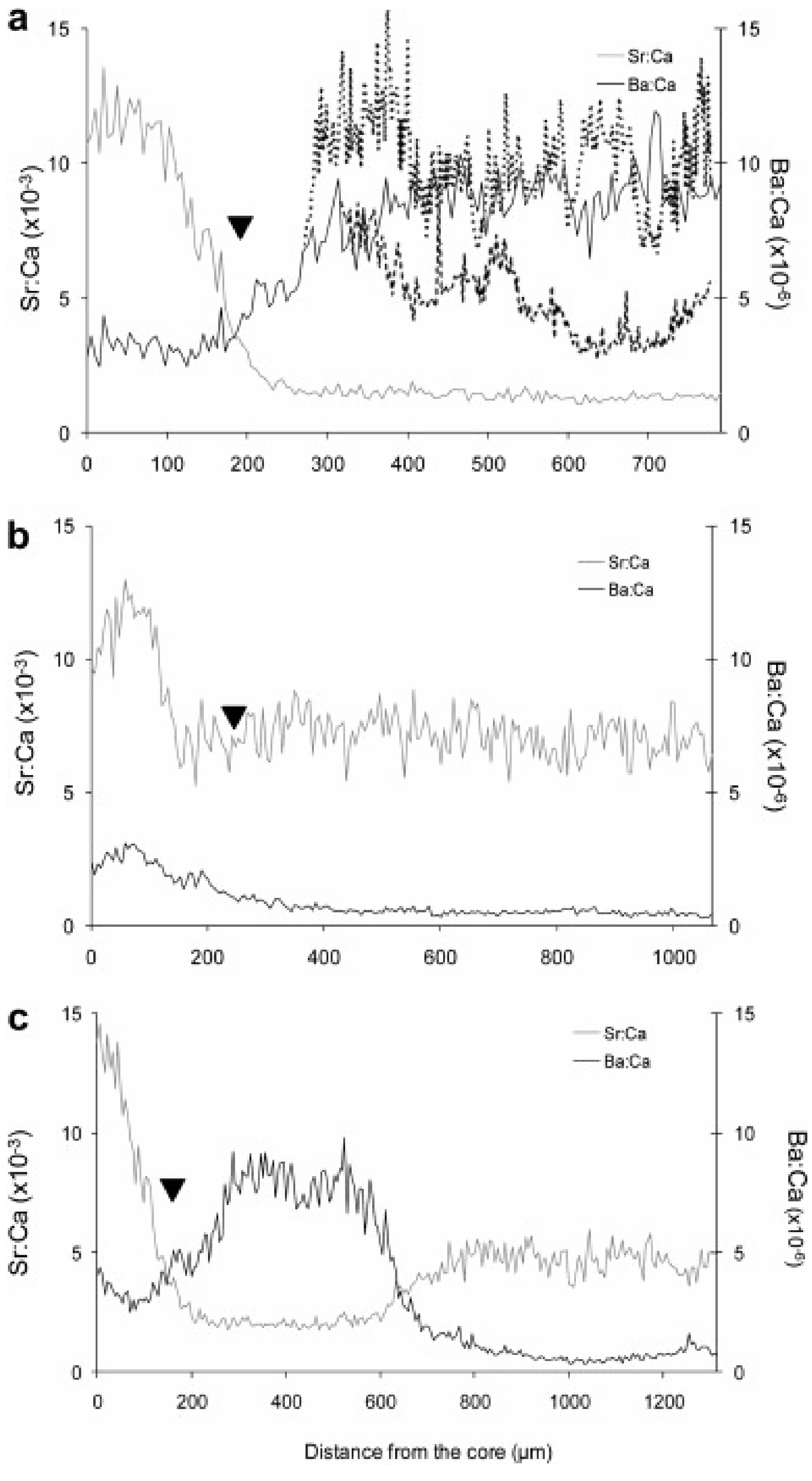

Figure 4: Continuous profiles showing the evolution of Sr:Ca (grey line) and Ba:Ca (black line) ratios (µg.g^{-1}) in otoliths of a) an eel caught in freshwater b) an eel caught in the estuary without drastic change in element ratios c)

an eel caught in the estuary with a drastic change in element ratios ▼ : elver mark. Because the high variability in the Ba:Ca ratio out of the elver mark, the three main types of profiles are shown in figure 4a.

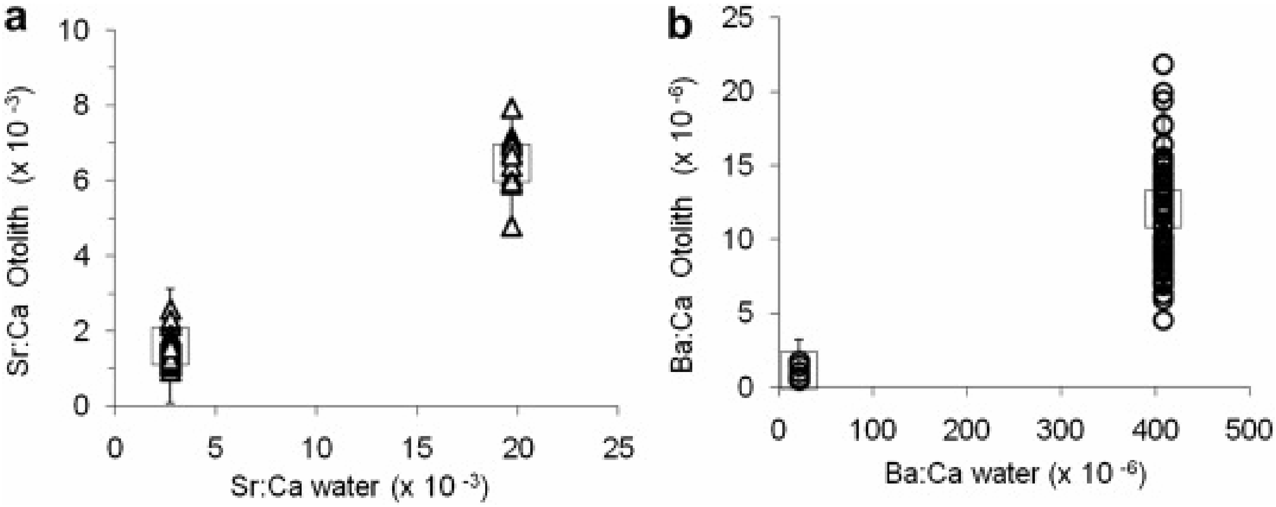

Figure 5: Relationship between Sr:Ca ratio in otolith and Sr:Ca in water (a), Ba:Ca ratio in otolith and Ba:Ca in water (b). Grey dots are individual otolith background data, open square are mean ratios with 95% CI illustrated by a double bar.

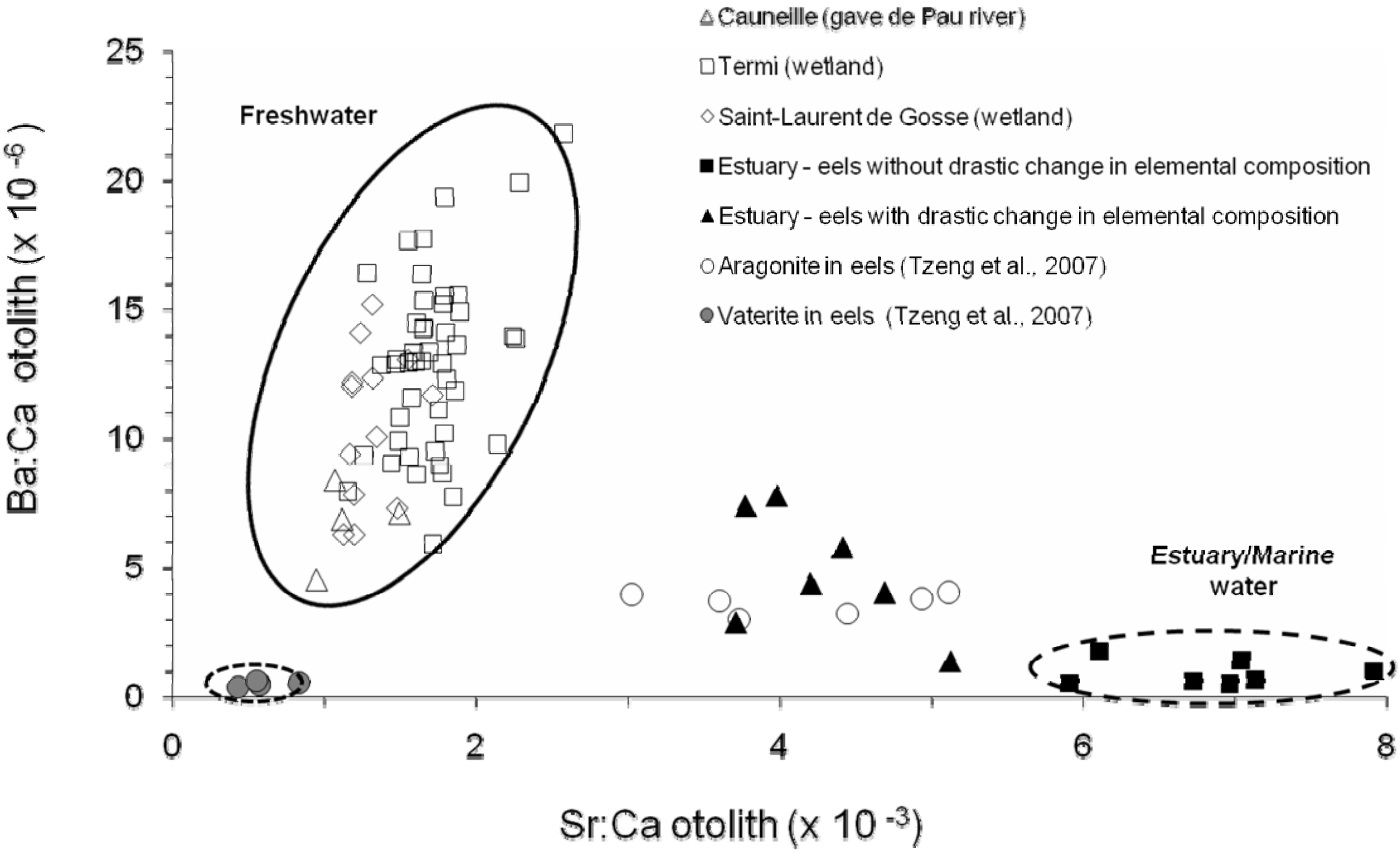

Figure 6: Relationship between Sr:Ca ratio and Ba:Ca in otolith of eel *Anguilla anguilla* from the Adour estuary (filled square and triangle) and associated watersheds (open rhombus, triangle and square). Ratios found in the same species by Tzeng *et al.* (2007) in both aragonite (open circle) and vaterite (filled circle) otolith microstructures are also shown for comparison.

Tables

Table 1: Operating conditions of the fs-LA-ICP-MS

<u>*Laser ablation*</u>	
Instrumentation	Femtolaser (Alfamet – Novalase, France)
Wavelength	1030 nm
Repetition rate	500 Hz
Energy	31 µJ pulse^{-1}
Sampling strategies	Linear raster scan
Platine speed	50 µm s^{-1} (pre-ablation), 5 µm s^{-1} (ablation)
Scan speed	1000 µm s^{-1} (pre-ablation and ablation)
<u>*ICP-MS*</u>	
Instrumentation Dual sample introduction system Torch Nebulizer Spray Chamber	Thermo elemental X7 CCT serie 2 inlets – Internal injector diameter 1.5mm Pneumatic concentric nebulizer 1ml min^{-1} Cooled Impact bead (2°C)
Coolant gas flow (Ar)	15 L.min^{-1}
Auxiliary gas flow (Ar)	0.8 L.min^{-1}
Nebulizer gas flow (He)	0.6 L.min^{-1}
Isotopes and Dwell time (ms)	^{138}Ba, (25 ms, 0.75 amu) ^{86}Sr, ^{43}Ca (5 ms, 0.3 amu)
Internal standard (m/z)	^{43}Ca