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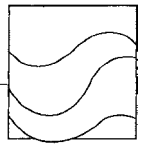

Otoliths imprinting of sole (*Solea solea*) from the Bay of Biscay: a tool to discriminate individuals from nursery origins?

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Abstract – Sole nurseries are located in the main bays and estuaries of the Bay of Biscay, where juveniles from the same stock concentrate and constitute temporarily isolated groups. This context being favourable for elemental analyses of juvenile otoliths, this study has been initiated with the aim of obtaining environmental imprints of the main nurseries of origin of juveniles recruiting to the adult stock, and of evaluating the relative contribution of these nurseries to the stock. The objectives were to compare (i) the otolith elemental imprints obtained by sampling juveniles in the Loire and the Gironde nurseries, and (ii) two multi-elemental analysis techniques: laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) and solution-based ICPMS (SB-ICPMS) adapted to small volumes. Depending on the fish origin, differences in Sr and Rb concentrations were shown using LA-ICPMS. Comparisons between the antero-dorsal and postero-ventral sites from where otolith material was ablated also suggested a spatial heterogeneity in otolith composition at least for some metals. From linear discriminant analyses, 73 % and 79 % of individuals (bootstrap estimations) were correctly classified with respect to their origin from the composition of the antero-dorsal and postero-ventral areas, respectively. The SB-ICPMS analysis was more powerful, which resulted in an 89 % rate of correct classification from a 2-variable model (Mg and Cd), whereas a 5-variable model (Li, Mg, Rb, Cd, Th) resulted in a 91 % rate of correct classification (bootstrap estimations). These results confirm that sole juveniles from the main estuaries of the French Atlantic coast could be discriminated by the elemental fingerprints of their otoliths.

otolith / trace elements / ICPMS / estuarine nursery / juvenile / recruitment

Résumé – Empreintes élémentaires des otolithes de la sole (*Solea solea*) : un outil pour discriminer les nourriceries du golfe de Gascogne ? Les nourriceries de la sole sont localisées dans les principales baies et estuaires du golfe de Gascogne où les juvéniles se concentrent et constituent des groupes du même stock, temporairement autonomes, caractérisés par leurs traits démographiques. L'isolement des juvéniles dans des zones sous diverses influences continentales offre un contexte propice à l'analyse élémentaire de leurs otolithes. Cette méthode permet de rechercher les éléments traces susceptibles de représenter une signature environnementale de la

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nourricerie afin de tenter une estimation des proportions relatives de juvéniles (recrutement) issus de ces nourriceries et rejoignant le stock d'adultes. Pour définir les bases de la méthode, nous avons comparé (i) les signatures élémentaires obtenues pour les otolithes de juvéniles prélevés sur les nourriceries de Loire et Gironde, et (ii) les potentialités de deux techniques de la spectrométrie de masse à plasma inductif (ICPMS) reposant, l'une sur des prélèvements par ablation laser d'échantillons solides (LA-ICPMS) et l'autre, sur le dosage d'échantillons mis en solution (SB-ICPMS), dosage adapté à de très petits volumes. Les concentrations en Sr et Rb mesurées en ablation laser (LA-ICPMS) diffèrent avec l'origine des juvéniles. La comparaison des données acquises sur les aires antéro-dorsale et postéro-ventrale de l'otolithe suggère par ailleurs, pour certains métaux au moins, une hétérogénéité spatiale de composition de l'otolithe. À partir d'analyses linéaires discriminantes, 73 % et 79 % des individus ont été correctement classés (*bootstrap estimations*) selon leur origine à partir de la composition élémentaire des otolithes dans les aires antéro-dorsale et postéro-ventrale respectivement. L'analyse SB-ICPMS, plus sensible du fait de limites de détection très basses, s'avère beaucoup plus performante. Sur la base des concentrations en Mg et Cd, on obtient 89 % d'échantillons biens classés (*bootstrap estimation*). Ce taux s'élève à 91 % en utilisant un modèle discriminant à cinq variables (Li, Mg, Rb, Cd, Th). L'ensemble des résultats confirme que les otolithes des juvéniles de la sole s'imprègnent d'éléments traces authentifiant la nourricerie d'où ils sont issus, ce qui devrait permettre de retracer l'histoire récente des sub-adultes migrant vers les frayères. © 2000 Ifremer/CNRS/IRD/Éditions scientifiques et médicales Elsevier SAS

otolithe / éléments traces / ICPMS / nourricerie estuarienne / juvénile / recrutement

1. INTRODUCTION

In ecology and fisheries research, recruitment is defined as the processes leading juvenile fish to reinforce or replace the adult population and, by extension, this term refers to the estimations of the number of juveniles (recruits) in a population. In practice, for exploited fish populations, these estimations are based on the number of juveniles reaching a fishable size [37, 43]. During the last decade, the programmes dealing with recruitment processes have focused on environmental factors which are likely to contribute to annual recruitment variations and thus could also govern adult stock dynamics [7, 36]. In agreement with Hjort's theory [22], an important part of recruitment variations was shown to be linked to environmental (density-independent) factors acting upon survival of eggs and larvae, whereas the study of flatfish populations has led to evidence that (i) biological (density-dependent) factors can regulate the pre-recruit abundance and (ii), this regulation can be more drastic for species whose juveniles concentrate within changeable coastal nurseries (bays, estuaries and lagoons) (see review in Leggett and Frank [30]). The North Sea plaice (*Pleuronectes platessa*) is a good example of this nursery-stage regulation [3, 51]. The Bay of Biscay sole (*Solea solea*) also offers a valuable model to illustrate some of the concepts relative to the recruitment regulation processes.

Sole distribution ranges from Norwegian to North African coasts, including the Mediterranean [42]. The Bay of Biscay corresponds to the geographical centre of this distribution and the sole stock of the Bay of Biscay ranks among the most important species of fisheries landings from this area [43]. It is identified as a homogeneous genetic entity [16, 24], with limited recruitment fluctuations (1-group juveniles), in contrast to northern stocks [17, 44]. This characteristic suggested that inshore transfers of eggs and larvae to coastal nurseries were mainly driven by 'robust' processes [25, 26]. Supporting this assumption, Amara et al. [1] showed that most of the new settlers (0-group pre-recruits) were survivors of the spawning peak of a given season. Nevertheless, evidence has arisen that hydro-climatic conditions of the spawning season induce some flexibility in the process: differences between mild and cold winters can affect pre-recruit abundance by a 4x factor [Koutsikopoulos unpubl. data].

A first analysis of the nursery functioning was based on the existence of relatively isolated units during the summer concentration periods [14, 26]. Interactions between nurseries could not be excluded, during off-

shore winter migrations made by juveniles of age 0 and 1^+, but they seem limited. However, the extents of these between-nursery exchanges have not yet been evaluated. Do the relatively reduced fluctuations of the recruitment imply that regulation intervenes within the nurseries through mortality-dependent processes, or outside the nurseries during offshore winter migrations? In other words, we are faced with the question of the relative contribution of each nursery to the adult stock, which is of major importance for both fisheries management and nature conservation [44].

In the context of isolated nurseries under continental influences, elemental imprints incorporated in otoliths offer integrative methods to test these hypotheses, with an attempt to identify juveniles from their nursery of origin [49, 50]. The otoliths grow by centrifugal deposition of new materials in more or less concentric patterns, recording the main events of the fish life span. They also incorporate, as they grow, various chemical elements present in the environment, with the advantage of no further resorption or alteration [9, 18]. Due to decreasing values of Sr concentration when continental waters mix with marine waters, the otolith Sr/Ca ratio is a good indicator of estuarine migration [47]. In estuarine areas undergoing anthropogenic inputs, metals incorporated in trace quantities have also been used as tracers of river sources. Among them, zinc and lead, associated with other elements, characterize the sediments exported by the two main estuaries on the French Atlantic coast, the Loire and Gironde estuaries [23]. As far as Cd is concerned, this element is proved to be a major contaminant of waters and substrates at the Gironde mouth [5, 21].

Separated by a distance of about 200 km, these estuaries offer nursery areas in well-delimited locations, facing distinct sole spawning grounds located on the shelf [2] (*figure 1a*). With around 50–70 % of the juvenile fish occurrence, sole is one of the 'key-species' of these nurseries [Guérault, unpubl. data] and thus it was selected as a model to develop methodology before monitoring of the coastal fish communities. This study was focused on the juveniles settled in the Loire and Gironde nurseries, with the dual purpose of (i) the detection of differences in the otolith elemental composition, enabling identification of environmental signatures of the main nurseries and (ii) the comparison of multi-elemental analytical techniques: Laser Ablation Inductively Coupled Plasma-mass spectrometry (LA-ICPMS) and solution-based ICPMS (SB-ICPMS).

2. SAMPLES AND METHODS

2.1. Fish sample locations

Sampling series of juveniles were taken aboard small research vessels or fishing boats in both estuaries. For the Loire estuary, 0-group juveniles were sampled during a single cruise in September 1996 from an area located at the river mouth (*figure 1b*). In contrast, sampling series were taken from the upper and mid-Gironde (*figure 1c*), irrespective of the season. The 1-group fish were sampled from March to May 1996 and the 0-group fish sample was caught in October 1996 (*table I*). The fish were frozen until subsequent measurement of the standard length (SL mm) and otolith extractions in the laboratory.

The main hydrological characteristics having prevailed in each estuary from winter to autumn 1996 are based on the French monitoring program *Réseau National d'Observation de la qualité du milieu marin* (R.N.O.). Seasonal records of temperature and

Table I. Sample characteristics: analyses were performed using left sagittae. (SL: standard length; OW: otolith weight; SE: standard error).

Analyses	Estuarine origins	Age groups	Sample sizes	Dates of capture	SL (mm) mean ± 1SE	OW (mg) mean ± 1SE	mean dilution factor
LA-ICPMS	Loire	0	16	09/96	102.7 ± 2.3	2.46 ± 0.10	–
	Gironde	0	16	10/96	89.8 ± 3.4	2.55 ± 0.17	–
SB-ICPMS	Loire	0	12	09/96	111.8 ± 4.8	2.82 ± 0.21	268
	Gironde	1	15	03–05/96	100.3 ± 4.1	3.23 ± 0.23	338

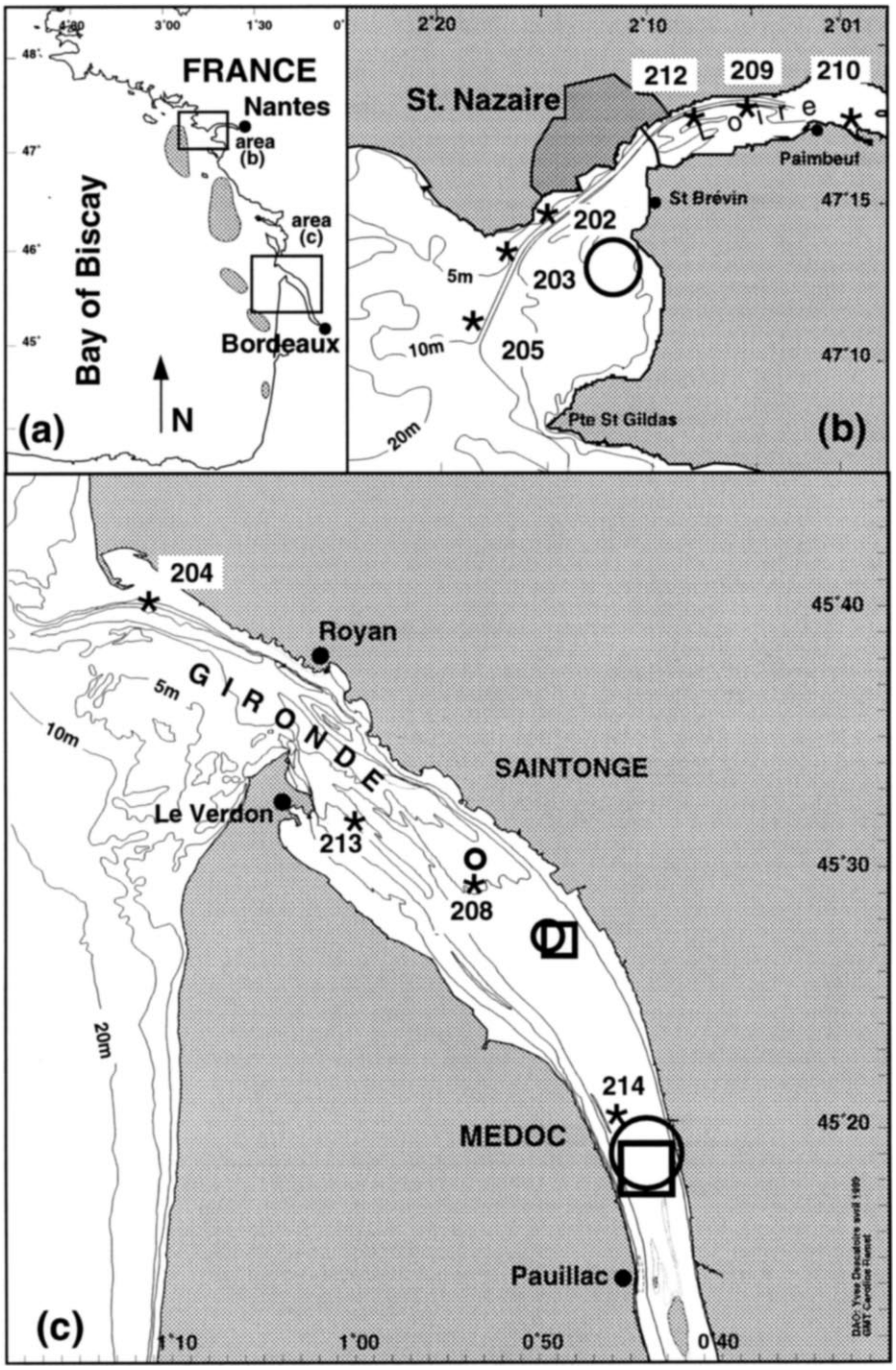

Figure 1. (a) Location of the juvenile sampling areas (b and c), with respect to shelf spawning grounds of sole (dotted areas; from Arbault et al. [2]); (b) samplings taken at the mouth of the Loire river and (c) samplings taken from the Gironde estuary (circles: 0-group sole; squares: 1-group sole; size of symbols represents the relative proportions of individuals; stars: R.N.O. seasonal hydrological stations).

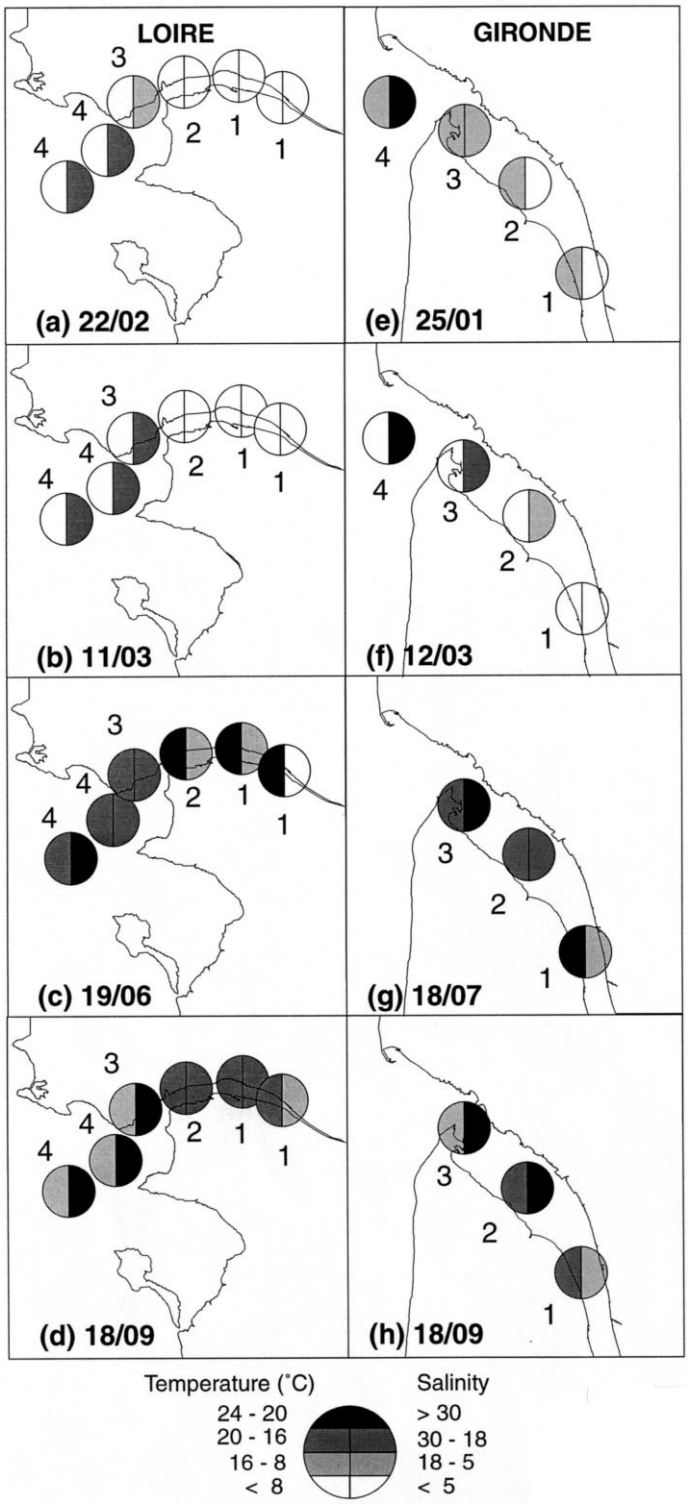

Figure 2. Seasonal gradients of temperature and salinity taken from the Loire (a–d) and Gironde (e–h) estuaries at R.N.O. fixed stations. Areas are defined as: 1. upstream, 2. mid-estuary, 3. downstream, and 4. marine.

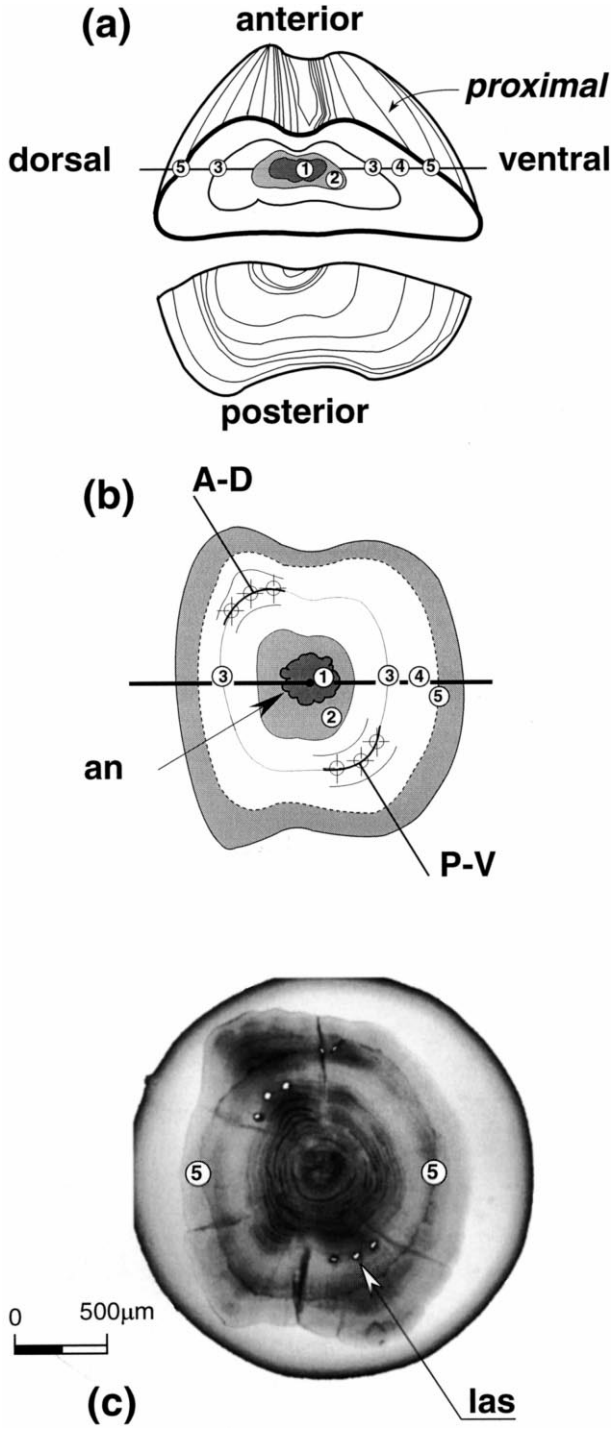

Figure 3.

salinity were taken from the surface at fixed stations during similar tide conditions. They are given in *figure 2* in four areas potentially used by the fish, from upstream (1) to outside of the mouth of the river (4), according to a 4-scale gradient. The hydrological differences between these areas from late spring to autumn were slight: low winter and high summer temperatures occurred in both estuaries, but from mid-estuary in the Loire and upstream in the Gironde (*figure 2a,d* and *2e,h*, respectively). However, salinity records indicated that marine-water entrance was favoured in the Gironde while the Loire estuary was dominated by the river flow.

2.2. Otolith preparations

Sagittae were removed from each fish, cleaned from adhering tissues, then triple-rinsed in Milli-Q water and air-dried for 24 h. Otoliths were weighed to the nearest 0.005 mg (*table I*) and were stored dry in ultra-pure acid-washed polypropylene vials awaiting further preparations.

LA-ICPMS and SB-ICPMS analyses were performed on left otoliths, less asymmetrical in shape and structure than the right ones [27]. For LA-ICPMS assays, hemisections were produced in the sagittal plane according to three successive stages: (1) embedding in epoxy resin on a glass slide in order that the proximal side be exposed (*figure 3a*), (2) grinding with silicium carbide and (3) polishing with diamond pastes of 9 µm, 3 µm, and 1 µm, successively until the core was reached (*figure 3b*). To remove surface contamination, each slide was sonified in Milli-Q water at the end of each polishing step. These standardised sections did not expose the marginal increments because of the otolith thickness, but they guaranteed a spatial homogeneity of otolith sampling sites analysed by LA-ICPMS.

Prior to SB-ICPMS analyses, otoliths were dissolved in a 10 % suprapure nitric acid (Merck S.A. ref.

Figure 3. Preparation and sampling sites for LA-ICPMS analysis on otoliths of 0-group sole. (a and b) Diagrammatic representation of (a) a transverse section showing the main structures crossed by polishing the proximal side until reaching the core and (b) the resulting sagittal hemisection, with indications of the antero-dorsal (A-D) and postero-ventral (P-V) areas selected for laser ablation sites. (c) Photograph of a preparation after LA-ICPMS analyses showing the laser ablated sites (las) ((1) larva otolith, from the core to accessory nuclei (an); (2) opaque central area; (4) growth marks corresponding to the A-D and P-V sites; (4) translucent summer zone and (5) limits of sectioning).

100441) solution containing indium at 10 ppb (indium being used as internal standard). The volume of the acid solution was adjusted to obtain homogeneous factors of dilution (*table I*), as required for the elemental analysis using ICPMS. Each solution sample was weighed and individual dilution factors were calculated to convert subsequently solution concentrations to otolith concentrations.

2.3. ICPMS assays

LA-ICPMS analyses were carried out using a Plasma Quad 3 (VG Elemental) ICPMS coupled to a laser ablation system constituted by a laser Nd/Yag (Spectron SL402) quadrupled to 256 nm. Laser operating conditions (700 V, 7 Hz, acquisition time of 10 s) induced a crater of around 20 μm diameter. Calcium set to 40 % (stoichiometric proportion for $CaCO_3$) was used as an internal standard for the quantification. Instrumental calibration was carried out using a NIST 61O CRM. Limits of detection (LOD) were estimated as 3*standard deviation of ten replicates made on this standard. Two sessions of analyses (Session 1 and 2) were run for two consecutive days. To avoid the possibility of confusing instrumental-drift effects with actual differences in the elemental composition, analyses were run following a systematic order, alternating Loire and Gironde samples during each session. Three ablations were carried out in each of the otolith antero-dorsal and postero-ventral sampling sites (*figure 3b*, *c*). These six measurements were taken on the same growth marks, the initiation of the first translucent summer zone being used as a standardisation criterion. This zone was shown to develop in the Bay of Biscay sole when coastal water temperatures are above 16–18 °C [27] and was deposited, in our samples, at a distance to the nucleus varying from 500 μm for the smallest otoliths (*figure 3c*) to 800 μm for longer fish. The relationship between otolith radius vs. fish standard length was examined on a data set obtained in July 1995. The fitted regression model [$y = 95.887x - 0.2691$; $R^2 = 0.8954$; with y (SL) and x (otolith radius) in mm] inferred that juveniles were from 50 to 80 mm long when the analysed material was deposited.

SB-ICPMS individual analyses were run using the previously mentioned ICPMS equipped with a micronebulizer (CETAC MNC100) allowing us to assay small volumes of solutions. LOD were estimated as $3*SD_{mean}$ of ten replicates on two blanks (10 % suprapure nitric acid solution with 10 ppb indium). Note that these estimations concern dissolved otoliths and thus have to be multiplied by the dilution factor to refer to otolith concentrations. All solution-based samples were processed in a single session (Session 3). ICPMS was run in peak jumping mode, the relevant isotopes having been previously determined from a preliminary study.

2.4. Data and statistical analyses

After blank removal, results of LA-ICPMS and SB-ICPMS analyses were expressed as elemental concentrations, based on percentages of isotopic natural abundance. Solution elemental concentrations were converted to otolith elemental concentrations from the individual dilution factors. In both methods, data below the LOD were set to zero, prior to the subsequent data analyses.

LA-ICPMS results were tested by analysis of variance (ANOVA) using a design for repeated measures. Fish origin and session number were considered as fixed effects, whereas otolith site (antero-dorsal vs. postero-ventral) was considered as a repeated measure factor. Statistics were thus performed using the mean value of the three measurements made on each otolith site. The discriminant power of the elemental composition was checked through linear discriminant analysis which is a special case of multiple regression when only two groups have to be separated. Variables to be included in the models were thus selected by using the 'leaps and bounds' algorithm of Furnival and Wilson available in SPAD N for Unix [29]. It provides the best fittings for $n = 1$ to p dependant variables, p being the maximum number of variables which can be included in the model with respect to the sample size. Correct classification rates were estimated in each model using the 'SPAD.N for Unix' discriminant procedure which includes bootstrap estimations. A similar statistical approach was used for the SB-ICPM data set.

Table II. LA-ICPMS analyses: elemental concentrations in ppm (mean ± 1 SE) and *percentages* of data below the limit of detection (LOD) with respect to session number, sample origins and otolith sites, antero-dorsal (AD) vs. postero-ventral (PV).

	Session 1				**Session 2**			
	Gironde		**Loire**		**Gironde**		**Loire**	
	A-D	P-V	A-D	P-V	A-D	P-V	A-D	P-V
Li^{7}	0.00	0.00	0.00	0.00	0.06	0.06	0.03	0.00
	±0.00	±0.00	±0.00	±0.00	±0.04	±0.04	±0.003	±0.0
	100	*100*	*100*	*100*	*81.8*	*81.8*	*90.9*	*100*
Mg^{24}	52.62	48.00	50.95	46.50	32.33	25.27	35.56	30.84
	±4.67	±8.22	±7.69	±5.43	±3.08	±2.57	±2.10	±3.63
	0	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Fe^{54}	32.61	34.25	29.54	33.48	33.19	34.50	32.80	34.56
	±2.66	±2.21	±2.12	±2.99	±1.89	±1.54	±1.58	±1.06
	0	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Mn^{55}	15.35	17.44	15.64	12.48	10.97	12.55	14.03	14.24
	±3.20	±2.42	±2.39	±2.35	±1.42	±1.10	±1.43	±1.80
	0	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Co^{59}	31.55	32.12	28.57	31.25	25.45	30.19	24.59	29.12
	±4.13	±2.28	±3.09	±5.08	±2.50	±2.52	±1.93	±2.49
	0	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Ni^{62}	25.04	24.74	14.81	21.19	17.94	19.56	20.65	19.09
	±3.02	±3.56	±3.41	±4.57	±3.28	±3.35	±4.41	±2.16
	0	*0*	*0*	*0*	*0*	*9.1*	*9.1*	*0*
Cu^{65}	1.39	1.08	1.73	2.05	0.04	0.24	0.20	0.13
	±0.41	±0.49	±0.49	±0.34	±0.04	±0.18	±0.07	±0.05
	0	*40*	*0*	*0*	*90.9*	*72.7*	*54.5*	*63.6*
Zn^{66}	0.20	0.50	0.32	0.00	0.25	0.24	0.17	0.07
	±0.12	±0.23	±0.20	±0.0	±0.17	±0.09	±0.10	±0.04
	60	*40*	*60*	*100*	*72.7*	*54.5*	*72.7*	*81.8*
As^{75}	2.43	3.69	2.64	3.12	0.56	0.98	0.53	0.59
	±0.23	±0.42	±0.32	±0.34	±0.10	±0.14	±0.15	±0.06
	0	*0*	*0*	*0*	*18.1*	*9.1*	*36.4*	*0*
Rb^{85}	2.22	2.13	2.15	1.43	0.82	0.64	0.52	0.29
	±0.33	±0.41	±0.40	±0.13	±0.30	±0.15	±0.19	±0.08
	0	*0*	*0*	*0*	*36.4*	*18.2*	*45.4*	*45.4*
Sr^{86}	1560	1556	1370	1466	1427	1513	1260	1322
	±124	±79	±109	±51	±46	±64	±44	±73
	0	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Ba^{138}	1.23	0.94	0.51	0.58	5.41	7.02	6.62	8.62
	±0.67	±0.29	±0.39	±0.28	±1.14	±1.94	±1.62	±1.83
	40	*20*	*60*	*40*	*9.1*	*0*	*0*	*0*
Pb^{208}	0.00	0.00	0.00	0.00	0.00	0.00	0.77	0.08
	±0.00	0.00 ±	±0.00	±0.00	±0.00	±0.00	±0.77	±0.08
	100	*100*	*100*	*100*	*100*	*100*	*18.2*	*81.8*

3. RESULTS

Table I gives the characteristics of the different samples. These results indicated a tendency for the Loire samples to have lighter otoliths despite longer SL than the Gironde samples.

3.1. Elemental composition determined from LA-ICPMS analyses (Sessions 1 and 2)

In each session, elemental concentrations were determined from the following isotopes: Li^{7}, Mg^{24}, Fe^{54}, Mn^{55}, Co^{59}, Ni^{62}, Cu^{65}, Zn^{66}, As^{75}, Rb^{85}, Sr^{86}, Ba^{138},

Pb^{208}. *Table II* summarises the corresponding results with respect to origin, otolith site and session of analyses. In both sessions, the percentages of data below the respective LOD were particularly high for Li, Pb and Zn and these elements were thus ignored in the subsequent statistical analyses. Nevertheless, it is worth noting that Pb was detected only in some Loire samples (9 out of the 11 samples of Session 2) and more particularly in the antero-dorsal area of the otolith.

No drift was noticed within sessions. However, we observed a poor consistency between sessions for some elements, although patterns of variations (between origins and within otolith sites) are generally conserved (*figure 4*). This phenomenon was clearly related to the apparatus calibration procedure, which remains a difficult stage for reasons discussed later.

ANOVA for repeated measures showed that concentrations differed significantly with respect to session number for Mg, Cu, As, Rb and Ba (*Table III*). In addition, As concentrations differed significantly between antero-dorsal and postero-ventral otolith sites, whereas elements such as Mg, Fe reached the marginal level of significance for this factor. ANOVA also indicated that concentrations of Sr differed significantly between estuaries, with higher Sr values for the Gironde samples than for the Loire's, Rb reaching the marginal level of significance for this factor. Interactions were found for As, whose concentration was greater in the postero-ventral area, this effect being more marked for the Gironde individuals and for Session 1 data. A 3rd-order interaction was found for Cu. In Session 1 data, this element showed a greater concentration in the Loire samples, this effect being more marked in the postero-ventral area,

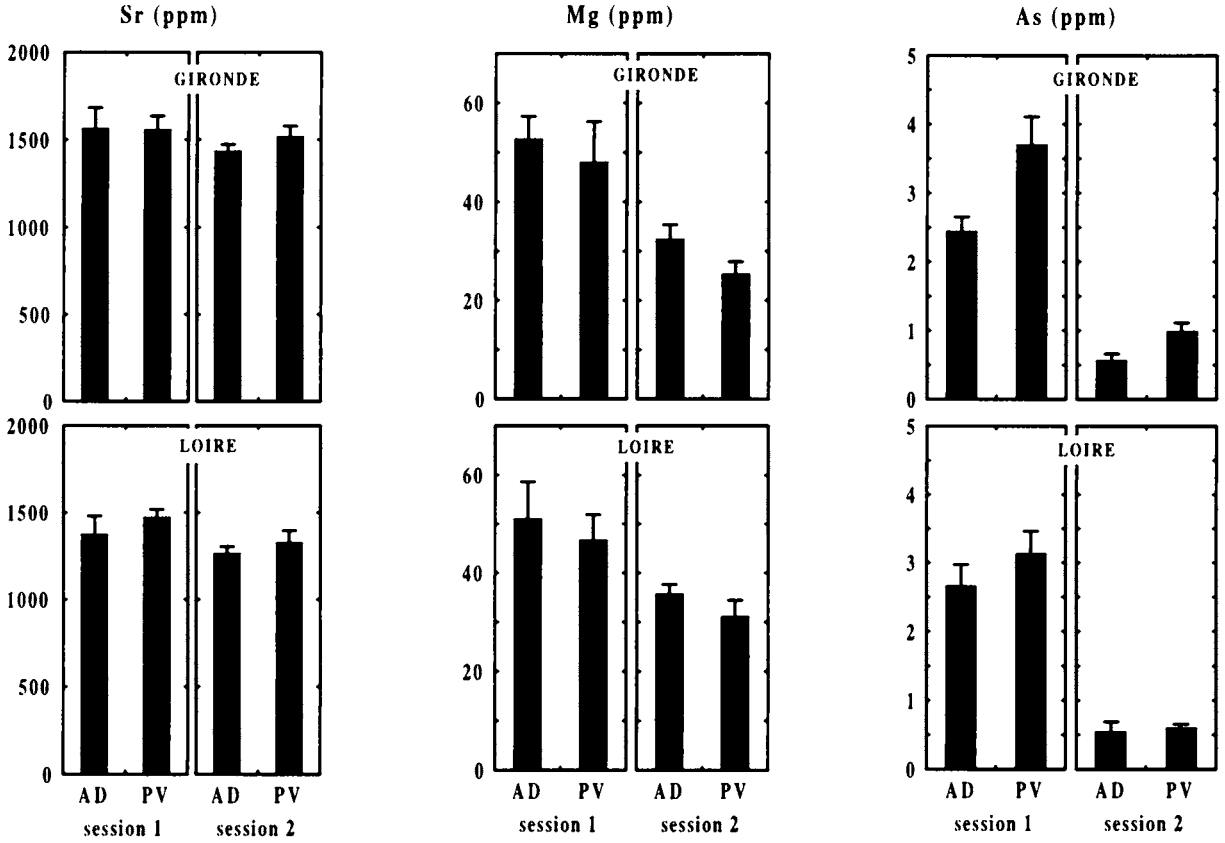

Figure 4. Sr, Mg and As otolith concentrations (mean ± 1SE) from LA-ICPMS analyses, with respect to the origins (Gironde vs. Loire) of sole juveniles, the otolith sites (AD = antero-dorsal vs PV = postero-ventral) and the session number.

Table III. ANOVA on LA-ICPMS results. Session number (1) and origin of individuals (2) are fixed effects. Otolith sites (3) is a repeated measure factor. Only significant interactions are reported.

	factor	MS effect	MS error	$F_{1,28}$	p-level
Mg^{24}	session	4713.444	133.763	35.237	<0.0001
	origin	27.311	133.763	0.2042	0.6549
	site	373.273	125.680	2.9700	0.0958
Fe^{54}	session	23.024	35.377	0.6508	0.4266
	origin	14.799	35.377	0.4183	0.5230
	site	64.257	20.220	3.1779	0.0855
Mn^{55}	session	71.439	31.850	2.2430	0.1454
	origin	0.007	31.850	0.0002	0.9882
	site	0.456	21.130	0.0216	0.8843
Co^{59}	session	171.729	69.713	2.4634	0.1278
	origin	28.650	69.713	0.4110	0.5267
	site	134.966	60.072	2.2467	0.1451
Ni^{62}	session	62.842	92.374	0.6803	0.4165
	origin	114.187	92.374	1.2361	0.2757
	site	32.349	127.439	0.2538	0.6183
Cu^{65}	session	27.322	0.633	43.1583	<0.0001
	origin	1.597	0.633	2.5226	0.1235
	site	0.019	0.074	0.2607	0.6137
	1x2x3	0.691	0.074	9.3613	0.0048
As^{75}	session	73.033	0.316	230.823	<0.0001
	origin	0.497	0.316	1.5722	0.2203
	site	4.215	0.234	18.016	0.0002
	1x3	1.358	0.234	5.804	0.0228
	2x3	1.125	0.234	4.8065	0.0368
Rb^{85}	session	27.61710	0.514100	53.71929	<0.0001
	origin	1.752	0.514	3.4083	0.0755
	site	1.289	0.410	3.1418	0.0872
Sr^{86}	session	158201.000	48170.730	3.2842	0.0807
	origin	349524.3	48170.73	7.2559	0.0118
	site	49924.100	30487.000	1.6376	0.2112
Ba^{138}	session	512.0688	33.59813	15.24100	0.0005
	origin	2.600	33.598	0.0774	0.7829
	site	9.884	10.287	0.9608	0.3354

whereas data from Session 2 were obscured by poor precision.

Since there had been an analytical bias between the two analysis sessions, linear discriminant analyses were only carried out on the data from Session 2, due to the small number of samples processed in Session 1. Owing to significant differences of elemental fingerprints between otolith sites, discriminant functions were computed for each data set obtained by site. Bootstrap estimations gave rates of 73.14 % of total correct classification based on Sr and Mn measured on the antero-dorsal area and 79.18 % of total correct classification based on Sr, As and Fe measured on the postero-ventral area, respectively (*table IV*).

Table IV. Sample classification by linear discriminant analysis on LA-ICPMS data: number of individuals (N), correct classification rate (%), standard deviation (SD) of bootstrap estimation. Variables in model 1: [Sr] and [Mn] in the antero-dorsal area. Variables in model 2: [Sr], [As] and [Fe] in the postero-ventral area.

Estuarine origins		Model 1		Model 2		Total
		A priori prediction	Bootstrap estimation	A priori prediction	Bootstrap estimation	
Gironde	N	9	8.51	10	9.18	11
	%	81.82	77.36	90.91	83.45	
	SD		12.35		9.80	
Loire	N	8	7.58	9	8.24	11
	%	72.73	68.91	81.82	74.91	
	SD		12.31		11.37	
Total	N	17	16.09	19	17.42	22
	%	77.27	73.14	86.36	79.18	
	SD		10.33		8.07	

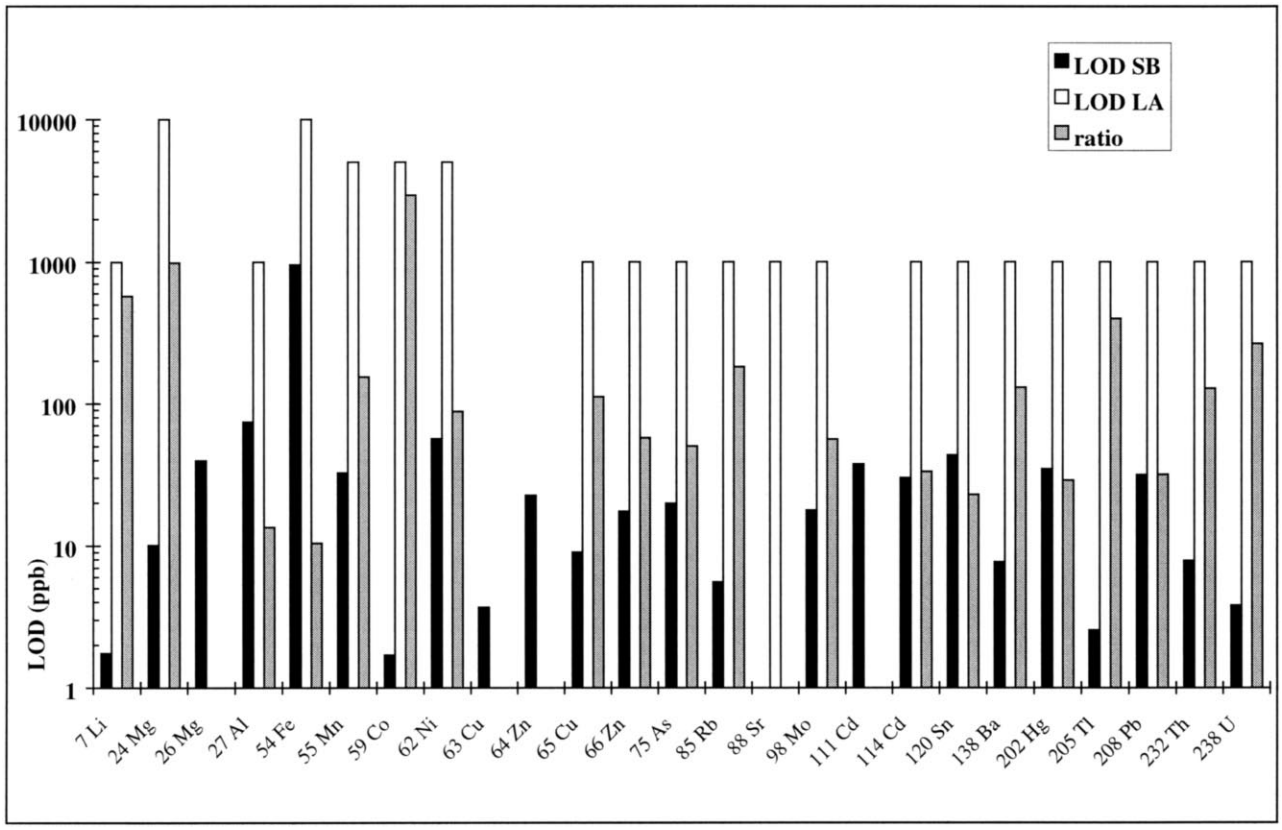

Figure 5. Comparison of limits of detection (in $ng \cdot g^{-1}$ of otolith) obtained using LA-ICPMS (LOD LA) and SB-ICPMS (LOD SB) techniques.

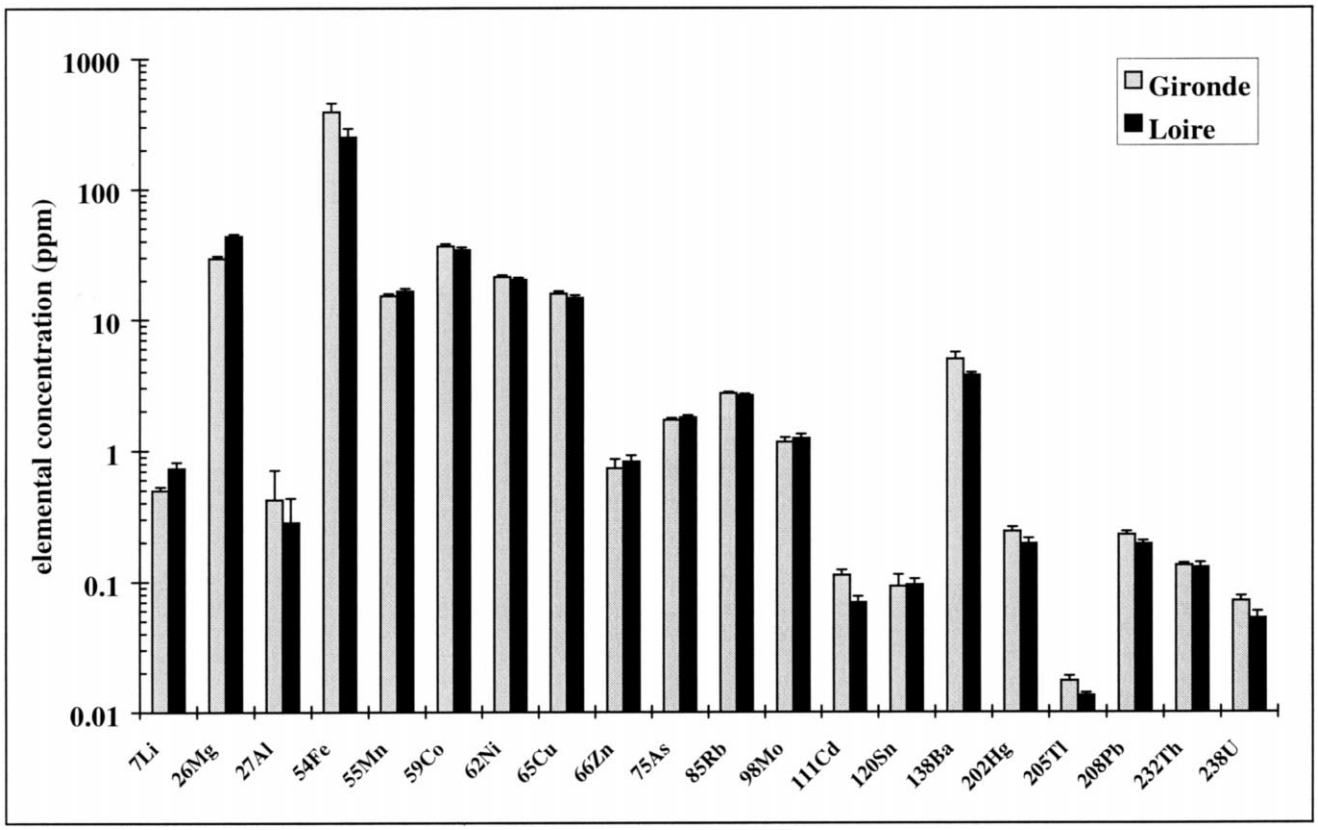

Figure 6. Otolith elemental concentrations measured using SB-ICPMS (mean ± 1SE) with respect to the estuarine origins of sole juveniles (ANOVA showed significant differences for Li, Mg, Cd and Tl).

3.2. Whole otolith elemental composition assessed from SB-ICPMS (Session 3)

By using the SB-ICPMS technique, better LOD were obtained, compared to the LA-ICPMS ones, so that the ratios (LOD LA-ICPMS / LOD SB-ICPMS) varied from 10 to more than 2 900 depending on isotopes (*figure 5*). This allowed us to determine concentration of 20 elements based on the following 24 isotopic concentrations: Li^{7}, Mg^{24}, Mg^{26}, Al^{27}, Fe^{54}, Mn^{55}, Co^{59}, Ni^{62}, Cu^{63}, Zn^{64}, Cu^{65}, Zn^{66}, As^{75}, Rb^{85}, Mo^{98}, Cd^{111}, Cd^{114}, Sn^{120}, Ba^{138}, Hg^{202}, Tl^{205}, Pb^{208}, Th^{232}, U^{238}. When two isotopes were available for the same element and since interferences are always additive, isotopes giving the lowest elemental concentration (Mg^{26}, Cu^{65}, Zn^{66}, Cd^{111}) were selected for further data analyses. *Figure 6* summarises the elemental concentrations obtained with respect to origins of sole juveniles.

ANOVA tests showed that concentrations differed significantly according to fish origins for Li ($F_{1,25} = 6.24$; $p = 0.019$), Mg ($F_{1.25} = 39.62$; $p < 0.0001$), Cd ($F_{1.25} = 8.54$; $p = 0.007$) and Tl ($F_{1.25} = 4.54$; $p = 0.043$). Two models were tested to estimate the discriminant power of the elemental fingerprints assayed with the SB-ICPMS technique (*Table V*). A model based on two variables (Cd and Mg) gave a total correct classification rate of 88.85 % (bootstrap estimation), whereas this rate reached 91.48 % using a 5-variable model (Li, Mg, Rb, Cd and Th). Note that the standard deviations of the bootstrap estimations are much lower for the latter, which indicates a better precision of the estimated values.

4. DISCUSSION

Since its first application to otolith elemental composition analysis [9], ICPMS has proved to be a prime technique, particularly in research concerned with stock discrimination or population characterization purposes, either in the solution-based configuration [18] and its variant isotopic dilution ICPMS [10, 50] or in the laser ablation configuration [19, 49]. As expected, some of the minor and trace elements detected in juvenile otoliths of wild sole obviously accounted for differences in fish origins. However, other variations could be related to intrinsic properties of sole otoliths or could also arise from technical constraints.

4.1. Comparison of ICPMS methods and technical issues

SB-ICPMS proved to be much more sensitive than LA-ICPMS: by detecting a further ten elements than the latter method, SB-ICPMS enhanced the richness of the chemical information obtained (*figure 5*). For the elemental data that could be compared between both methods, a good consistency was observed, except for Fe whose concentrations were obviously biased in the solution-based assays due to high interference with ArN^+. The high sensitivity of SB-ICPMS clearly has to be related to the use, in this experiment, of a micro-nebulizer. The low flow rates of this device strongly minimise the required sample volume, which opens new perspectives for individual analysis of larval and juvenile otoliths. It also offers the possibility to reduce dilution factors with respect to those recommended when using a conventional nebulizer which has a direct effect on LOD levels. However, SB-ICPMS on whole otolith provides time-integrated measurements, i.e. accounting for the entire life span until fish capture. Recent studies have tried to get around this limitation by extracting the portion of otolith to be analysed (acid digestion or cutting) [15].

To investigate short-term migrations of fish, LA-ICPMS remains the preferred tool because it allows samplings of specific otolith loci (*figure 3*). Nevertheless, this technique has a very recent past in biogenic carbonate analysis and still presents some difficulties due to the fact that ICPMS and the laser probe system have to be controlled simultaneously. The lack of certified reference material (CRM) matching calcium carbonate matrix makes it difficult to calibrate the unit correctly and to obtain reliable LOD estimations. This obviously raises several issues pertaining to data quality, especially in the case of multi-sessions analyses. Different alternatives have been proposed, although none of them achieves general agreement. Methods for development of spiked carbonate standards have been proposed [39, 40]. Thorrold et al. [49] used multi-element glass bead standards described in Campana et al. [11], which consist of otolith powder spiked with trace elements and fused with lithium tetraborate. The use of a

Table V. Sample classification by linear discriminant analysis on LA-ICPMS data: number of individuals (N), correct classification rate (%), standard deviation (SD) of bootstrap estimation. Variables in model 1: [Cd] and [Mg]. Variables in model 2: [Li], [Mg], [Rb], [Cd] and [Th].

Estuarine origin		Model 1		Model 2		Total
		A priori prediction	Bootstrap estimation	A priori prediction	Bootstrap estimation	
Gironde	N	13	12.29	15	14.38	15
	%	86.67	81.93	100.00	95.87	
	SD		7.32		2.39	
Loire	N	12	11.70	11	10.32	12
	%	100.00	97.50	91.67	86.00	
	SD		4.03		5.98	
Total	N	25	23.99	26	24.70	27
	%	92.59	88.85	96.30	91.48	
	SD		4.51		2.96	

two-channel sample introduction, allowing calibration with standard aqueous solutions, has also been suggested [12, 13]. Moreover, up to now, another limitation of the technique has been a lack of stability and precision of the laser ablation systems. It induces a non-controlled variability in the amount of ablated material which implies the use of Ca as an internal reference under the hypothesis of a constant concentration of this element in otoliths.

4.2. Otolith composition heterogeneity

The choice of standardised sampling sites on the otoliths could not guarantee the synchronisation of analyses between individual wild fish with variable early life spans. However, by selecting the initiation of the first translucent summer zone as sampling sites, we could assume that these juveniles were in a narrow range of sizes (on average, 50–80 mm long), experiencing water temperatures around 16–18 °C. This thermal threshold induced structural changes in the Bay of Biscay sole otoliths [27], allowing us to refer LA-ICPMS analyses to similar hydroclimatic conditions.

Moreover, it made it possible to test the otolith chemical composition for spatial homogeneity. This issue has been pointed out by several authors [9, 18]: if general mechanisms of elemental incorporation in otoliths are roughly understood, the actual mechanistic process of otolith biomineralisation still remains unknown. Although they require confirmation, our results suggest a spatial variability of some metal concentrations in sole otoliths (*table II*, *figure 4*). Pb was detected in the antero-dorsal area of the Loire otoliths, so systematic contamination would be an unlikely explanation. A differential concentration was also suggested for metals such as As and to a lesser extent, for Mg, Fe, and Rb (*table III*). Payan et al. [38] have recently shown a non-uniform distribution for CO_2 and total proteins in endolymph, two major factors in otolith calcification. According to these authors, endolymph ionic content also presented heterogeneous concentrations for various elements, with a repercussion on otolith concentrations for elements which could be assayed by Wavelength Dispersive Spectrometry. Such phenomena are to be linked to the very specific cellular structure of saccular epithelium, just lately described [35, 41, 48].

4.3. Fish origins

The Loire and Gironde estuaries have relatively synchronous river flows of 900 $m^3\ s^{-1}$ on average, with lowest water levels below 500 $m^3\ s^{-1}$ and spring floods reaching 4000–5000 $m^3\ s^{-1}$ in both rivers [28]. However, the estuaries differ in their topography and geological context. The Gironde estuary covers roughly twice the area of the Loire estuary (*figure 1a*), so that marine waters penetrate further into the former estuary. In the Loire estuary, severe oxygen depletions cause high fish mortality [32, 46], never observed in the Gironde [34], despite elevated temperatures upstream in summer (*figure 2g*). This ecological context seems to explain some discrepancies in the seasonal distribution of juveniles between estuaries. Previous studies had reported that new settlers (from 13–20 mm long) could be sampled in both the Loire [33] and the Gironde [4] estuaries, from April onwards. In the Loire nurseries, a patchy distribution, depending on the river-bed characteristics, partly concealed a size-dependant distribution resulting from the immigration of successive cohorts [31, 32]: sole mainly settled in mid-estuary, a part of them reaching the upper low-salinity areas, not yet hypoxic. Marchand et al. [33] reported that juveniles shifted downstream in autumn, to concentrate out of the river mouth where 0-group fish were sampled in September 1996 (*figure 1b*). In contrast, this segregation by size classes appeared less drastic in the Gironde: 60 % of the October 1996 fish samples were taken in the upper Gironde (*figure 1c*). In this estuary, early sole probably settled from mid-estuary to upstream areas, where juveniles could stay depending on the date of estuarine entrance of the cohorts [4]. Sole could even protract their stay in mid-Gironde, providing river-bed water temperatures were above 8 °C. This was observed during mild winter conditions, in 1995–96 (Mario Lepage, pers. comm.) and permitted samplings of 1-group juveniles over winter and spring.

Wherever fish samplings were located in autumn, LA-ICPMS analyses accounted for water qualities encountered in areas of June–July settlements of juveniles. At this period, juveniles occupied areas of variable salinity in both estuaries, but in a more reduced range in the Loire (salinity 5–18) than in the Gironde (salinity 5–30) (*figure 2c*, *2g*). This can explain that Sr concentrations on LA-ICPMS

sampling sites in the Gironde otoliths were higher than in the Loire's (*figure 4*). In addition, comparisons of autumnal fish sizes and otolith weights indicated that the upper Gironde samples had, on average, heavier otoliths than the samples taken outside the Loire estuary (*table I*). This decoupling of relationships between fish and otolith sizes was shown to indicate differences in growth rates of juvenile fish in an upstream pollution gradient [8]. This suggests that the Gironde fish could belong to late cohorts and/or that they had achieved a slower growth than the Loire juveniles did, lower growth rates being also liable to explain higher Sr incorporation levels [45]. As a result, Sr presented a high discriminant power between estuaries, reinforcing the trace-element based discrimination obtained using the LA-ICPMS technique (*table IV*).

Concerning the results of SB-ICPMS analyses, the use of the Gironde 1-group juveniles compared with the Loire 0-group juveniles could have induced a bias due to (i) differences in relative proportions of estuarine vs. marine residence times and (ii) variations in elemental concentrations according to fish sizes and ages [20]. Our results showed that concentrations did vary for some elements, but were irrespective of age groups, with concentrations significantly higher for Li and Mg in the Loire otoliths and for Cd and Tl in the Gironde otoliths (*figure 6*). That Cd concentrations depended on the origins of sole juveniles can be explained from the R.N.O. data base. Very high concentrations (400 $ng \cdot L^{-1}$) of dissolved Cd have been measured in the Gironde estuarine-mixing zone, due to a zinc mining area upstream in the River Lot [5]. In contrast, Cd concentrations have been shown lower in the Loire estuary and not linked to any intra-estuarine anthropogenic source [6]. This is not the case of Pb distributions in the Loire estuary, largely governed by significant anthropogenic inputs. However, high-dissolved Pb concentrations (up to 1.7 nM) remain confined to the estuarine zone itself, whereas the coastal zone presents markedly lower concentrations [6]. This could support the fact that Pb concentrations measured on the whole otolith (SB-ICPMS) did not differ significantly between estuaries. On the contrary, LA-ICPMS results showed that Pb was only detected in relatively high concentrations (> 1 $g \cdot g^{-1}$, *table II*) in the otoliths of the Loire juveniles, which corroborates our assumption of their location within the estuary at the deposition time of LA-ICPMS sites.

In conclusion to this preliminary study, both techniques identified sole juveniles from environmental imprints characteristic of their nursery of origin. These environmental imprints are based on elemental assemblages whose detection depends on (i) the sensitivity of ICPMS techniques, (ii) the concentrations of elements present in each estuary, liable to vary according to estuarine zonation, and (iii) the actual locations and movements of fish with respect to the investigations carried out on the otoliths. In other words, the elemental imprints obtained from LA-ICPMS could be compared to snapshots of the late spring–early summer locations of new settlers, whereas SB-ICPMS analyses accounted for the whole estuarine residence until capture, and if any, the seaward migration as well. Regarding the integrated juvenile period, the SB-ICPMS technique was proved more powerful, since many trace elements found in otoliths are close to the limits of detection of the LA-ICPMS analysis. The latter technique nevertheless revealed a possible heterogeneous incorporation of some trace elements, which could open up new questions relative to otolith formation. Analytical problems met during this study, due to difficulties in the apparatus calibration, are in the process of being solved and LA-ICPMS remains a realistic goal to track short-term movements of fish [49]. Inshore movements of sole juveniles appear cued by complex interactions, which could act on the within- and between-nursery dynamics through differences in growth and mortality rates. If this hypothesis is confirmed, this could determine the rates of between-nurseries exchanges suggested by Koutsikopoulos et al. [26], leading to variable contributions of these nurseries to the adult stock. Current studies are attempting to obtain further knowledge on these questions.

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