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## Preliminary indication of ontogenetic and spatial variations in the whole otolith isotopic and elemental signatures of *Solea solea* in the Gulf of Lions (NW Mediterranean)

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**SUMMARY:** Knowledge of fish life cycles is important for exploited species. In the Mediterranean Sea, catches of common sole (*Solea solea*) have fluctuated widely since the 1970s. *Solea solea* displays an ontogenetic shift in habitat: sole larvae are marine, juveniles inhabit shallow marine waters and coastal lagoons and adults inhabit deeper marine waters. Although the results of this study were obtained using only a small set of individuals, the otolith elemental composition and isotopic ratios were investigated for the three life stages (post-larval, juvenile and adult) in order to acquire a better knowledge of the variability in environmental conditions experienced by the common sole at each life stage in the NW Mediterranean. Moreover, this study provides the first evaluation of the potential of whole otolith microchemistry for investigating habitat connectivity in sole populations from the Gulf of Lions. The elemental and isotopic signatures of otoliths of post-larvae captured in various environments appear to show variations related to the River Rhône inputs (high Ba/Ca and low Sr/Ca ratios). Juveniles appear to show significant variations in otolith elemental and isotopic compositions depending not only on the physico-chemical properties of water in the nurseries they inhabit (Rhône River, Thau or Mauguio lagoons), but also as a result of variations in the chemical composition of their surrounding sediments and in their benthic prey. Adults exhibit otolith differences for isotopic ratios only, which is probably linked to the River Rhône inputs. Finally, several significant relationships were observed between otolith elemental composition and fish size in specimens captured at the River Rhône mouth. However, these variations seem to be an indication of the composition acquired in each environment rather than a sign of ontogeny.

**Keywords:** flatfish, otolith microchemistry, stable isotope, coastal lagoon, River Rhône.

**RESUMEN:** INDICIOS PRELIMINARES DE VARIACIONES ONTOGENÉTICAS Y ESPACIALES EN LAS SEÑALES ISOTÓPICAS Y ELEMENTALES DE OTOLITOS DE *SOLEA SOLEA* DEL GOLFO DE LEÓN (MEDITERRÁNEO NOROCCIDENTAL). – El conocimiento del ciclo de vida es importante para las especies comerciales de peces. En el mar Mediterráneo, las capturas de lenguado común (*Solea solea*) han fluctuado ampliamente desde la década de los 70. *Solea solea* presenta un cambio de hábitat ontogenético, las larvas son marinas, los juveniles viven en aguas marinas y en lagunas costeras, y los adultos viven en aguas marinas más profundas. Aunque los resultados de este estudio fueron obtenidos usando sólo un número pequeño de individuos, la composición elemental los otolitos y las relaciones isotópicas fueron investigados en las tres etapas de vida (post-larval, juveniles y adultos) con el fin de adquirir un mejor conocimiento de la variabilidad en las condiciones ambientales que experimenta el lenguado común en cada etapa de la vida en el mediterráneo noroccidental. Por otra parte, este trabajo permite una primera evaluación del potencial de toda la microquímica de otolitos para la investigación de la conectividad del

hábitat en las poblaciones de lenguado en el Golfo de León. Las señales elementales e isotópicas de los otolitos de las post-larvas capturadas en diferentes entornos muestran variaciones en relación a los materiales aportados por el río Ródano (alta relación Ba/Ca y baja relación Sr/Ca). Los juveniles parecen mostrar variaciones significativas en la composición elemental e isotópica de los otolitos dependiendo no sólo de las propiedades físico-químicas del agua en los viveros que habitan (Thau, Mauguio o el río Ródano), sino también como resultado de las variaciones en la composición química de los sedimentos circundantes y de su presa béntica. Los adultos presentan únicamente diferencias en las relaciones isotópicas de los otolitos, que está probablemente vinculado a por materiales aportados por el río Ródano. Por último, se observaron varias relaciones significativas entre la composición elemental de los otolitos y el tamaño de los peces en las muestras capturadas en la desembocadura del río Ródano. Sin embargo, estas variaciones parecen ser una indicación de la composición adquirida en cada entorno, más que ser debidas a la ontogenia.

*Palabras clave:* peces planos, microquímica de los otolitos, isótopos estables, laguna costera, río Ródano.

## INTRODUCTION

Many marine fish species are subject to intensive fishing and stocks are overexploited (Garcia *et al.* 1999, Myers and Worm 2003). In coastal environments many fish migrate between habitats during larval, juvenile and adult stages. However, these coastal environments are subject to significant pressures in addition to fishing, such as pollution and eutrophication, which lead to habitat loss (Nixon 1995, Kemp *et al.* 2005). A better understanding of fish life cycles and use of these environments is essential for stock management.

The common sole, *Solea solea* (Linnaeus, 1758), is an important commercial flatfish of the eastern Atlantic Ocean and Mediterranean Sea. Catches of this species in the NW Mediterranean Sea from 1990 to 2009 dropped from roughly 520 to 130 tonnes per year, according to FAO figures (<http://www.fao.org>, Demaneche *et al.* 2009). The life cycle of the common sole is characterized by an ontogenetic shift in habitat (Salen-Picard *et al.* 2002), with juveniles inhabiting coastal lagoons and shallow coastal marine waters ( $\leq 10$  m), whereas older individuals occupy the continental shelf from 20 to 150 m depth. Several studies dealing with the distribution of this species in the Gulf of Lions, NW Mediterranean Sea, have shown a higher density of individuals off the River Rhône mouth (Campillo *et al.* 1989, Gaertner *et al.* 1998). Moreover, a positive correlation has been established between the mean annual discharge of the River Rhône and the annual commercial catches of *S. solea*, with a time lag of five years (Salen-Picard *et al.* 2002) related to the fluctuations of soft-bottom communities dominated by polychaetes which are the prey of the common sole (Darnaude *et al.* 2001, Salen-Picard *et al.* 2002). A recent study based on otolith shape analysis (Mérigot *et al.* 2007) showed that several local populations of sole inhabit the Gulf of Lions. However, in addition to the relationship between otolith shape and growth rate, shape is seen to be strongly dependent on changes in environmental conditions and fish size (Reddin *et al.* 1988, Campana and Casselman 1993, Friedland and Reddin 1994, Pothin *et al.* 2006, Tracey *et al.* 2006, Morat *et al.* 2012). However, the exact nature of past environmental changes or migrations is invisible in the studies of otolith shape.

Otoliths, the “flight recorder” of teleost fish (Lecomte-Finiger 1999), have been widely used to

characterize populations and stocks on the basis of chemical elemental composition (Campana *et al.* 1995, 1999, 2000, Gillanders and Kingsford 2000, Rooker *et al.* 2003, Jónsdóttir *et al.* 2006, Vasconcelos *et al.* 2008, Tanner *et al.* 2011). They record environmental changes with great precision using other markers such as the composition of chemical elements and isotopic ratios (see Campana 1999 for an extensive review). Otoliths exhibit three key features: (i) they grow continuously from the birth of fish to their death; (ii) they are metabolically inert, so newly-deposited material is neither resorbed nor reworked after deposition (Campana and Neilson 1985); and (iii) their uptake of trace elements as they grow reflects the physical and chemical environment (Fowler *et al.* 1995, Gallahar and Kingsford 1996), albeit with significant physiological regulation (Kalish 1989, Farrell and Campana 1996). Environmental availability and temperature also affect the isotope ratios of elements. For example,  $\delta^{18}\text{O}$  can be used to estimate the temperatures at which carbonates are formed (Kalish 1991, Thorrold *et al.* 1997, Rooker *et al.* 2008 a,b). The carbon isotopic ratio can provide information on metabolic processes and on the source of the carbon involved during calcification (Kalish 1991, Schwarcz *et al.* 1998). Given the capacity of otoliths to incorporate both chemical elements and isotope ratios (i.e. the “elemental fingerprints”) from the environment and to grow continuously, the otolith can be used as a life-history record reflecting habitat changes during the fish’s lifetime.

This paper investigates differences in the otolith isotopic and elemental signatures between the three life stages (post-larval, juvenile and adult) in order to acquire a better knowledge of the variability in environmental conditions experienced by the common sole at each life stage in the NW Mediterranean. Moreover, this study provides the first evaluation of the potential of whole otolith microchemistry for the investigation of habitat connectivity in sole populations from the Gulf of Lions.

## MATERIALS AND METHODS

### Sampling

In the Mediterranean Sea, the common sole undergoes an ontogenetic shift of habitat during its life cycle. Larvae are marine, juveniles inhabit shallow marine

TABLE 1. – Numbers of left *sagittae* of *Solea solea* analysed for elemental composition (EC) and isotopic fingerprint (I) from different sites, seasons and depths in the NW Mediterranean Sea. TL, total fish length.

Sites	Date	Depth		Size range TL (mm)	Age estimated
		Shallow (5-15 m) EC	I		
Thau	autumn 2003	5	4	194-218	0+
	autumn 2004	5	4	195-222	0+
Mauguio	spring 2004	10	6	95-123	0+
	autumn 2004	10	7	180-229	0+
Rhône River mouth	spring 2000	5		56-91	0+
	autumn 2000	5	3	122-177	0+
Saintes-Maries	spring 2000			207-371	1+,-3+
	spring 2001			310-338	2+

waters, estuaries or coastal lagoons and adults live in deeper marine environments. The sampling of each life stage was conducted according to these characteristics.

**Juvenile habitats.** Juvenile sole inhabit the shallow waters at the River Rhône mouth (5-15 m, Campillo 1992). This river is 812 km long and has a catchment area of 95500 km<sup>2</sup>. Its discharge varies between 500 and 11000 m<sup>3</sup> s<sup>-1</sup>, with a mean value of 1700 m<sup>3</sup> s<sup>-1</sup>. The salinity in very shallow waters off the River Rhône mouth can drop to about 6, and the temperature range is 13°C to 21°C. Juveniles can also inhabit coastal lagoons. Two types of coastal lagoon on the central coast of the Gulf of Lions were sampled: Mauguio Lagoon, a desalted coastal lagoon and Thau Lagoon, a more marine lagoon. Mauguio Lagoon has a surface area of 32 km<sup>2</sup> and a catchment area of 410 km<sup>2</sup>. Its mean depth is 0.8 m (max. 1.30 m) and it has only one permanent communication channel with the open sea. The salinity varies between 18 and 32 and the water temperature ranges from 7°C to 24°C (Ifremer 2008). Thau Lagoon has a surface area of 75 km<sup>2</sup> and a catchment area of 285 km<sup>2</sup>. Its mean depth is 4 m (max. 10 m) and it has two permanent seawater inlets. Salinity usually varies between 32 and 39, and water temperature ranges from 5°C to 25°C (Ifremer 2008).

**Adult habitats.** Adults live on the continental shelf and are abundant off the River Rhône mouth (Campillo 1992, Gaertner *et al.* 1998). The salinity and temperature ranges for this deep water environment are 37-38°C and 12-14°C, respectively. In Saintes-Maries, salinity and water temperature characteristics are similar to those observed in the deep waters off the river mouth.

Fish captured in spring in Mauguio Lagoon and in the shallow waters at the Rhône River mouth were post-larval individuals (Table 1, Fig. 1). Fish captured in lagoons and in shallow waters at the River Rhône mouth in autumn were young of the year (Table 1, Fig. 1). Fish captured in the deep waters at the River Rhône mouth and at Saintes-Maries were older individuals (Table 1, Fig. 1). Their otoliths consist mainly of material deposited during the larval/juvenile or adult life stages, respectively, so they primarily reflect the environmental conditions that prevail in the habitats of larvae, juveniles or adults of the species.

A total of 85 individuals of *Solea solea* were sampled (Table 1). Left otoliths (*sagittae*) were extracted

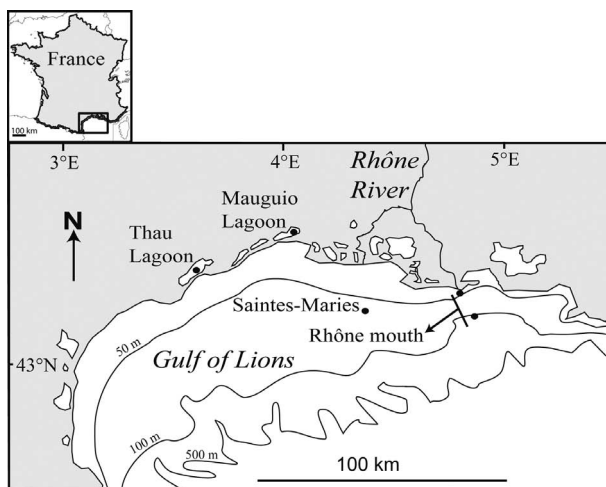


FIG. 1. – Location of study sites in the northwestern Mediterranean Sea. All sites were used for otolith trace elemental analyses and for determination of the stable isotopic signatures of otoliths.

with non-metallic forceps. Immediately following extraction, the otoliths were placed in distilled water for cleaning and for removal of residual tissues from the macula and the vestibule, using fine tweezers. Once cleaned, the otolith pairs were dried and weighed (1.03-42.11 mg) and kept dry in Eppendorf tubes. Fifty-five otoliths were used for the determination of the chemical composition and 30 for  $\delta^{18}\text{O}$ - $\delta^{13}\text{C}$  stable isotope analysis (Table 1). Because the life cycle of the common sole is characterized by an ontogenetic shift of habitat, fish sampled at the four sites showed differences in total fish length (Table 1).

### Elemental analyses

Elemental analyses were carried out to characterize spatial differences at three life stages of the common sole at various sites. The left otoliths were analysed with Inductively Coupled Plasma Mass Spectrometry (ICPMS, mass 700, Varian, Inc. Corporate Headquarters, Palo Alto, CA, USA) for Al, Mn, Co, Cu, Cd, Ba and Pb, and with ICP Atomic Emission Spectroscopy (ICP-AES, Vista-PRO Varian, Inc. Corporate Headquarters, Palo Alto, CA, USA) for Li, Mg, Fe, Ca and Sr. The use of both machines ensured the best resolution for each element. Each otolith was dissolved in

2 mL of Suprapur nitric acid (65%). Solutions were mineralized for 2 hours at 110°C to 120°C and dissolved in 5 mL of Suprapur nitric acid (5%). Indium was used as an internal standard (0.1 mg L<sup>-1</sup>). A standard range was carried out with a multi-element solution (ASTASOL MIX MO101, Analytika, Prague, Czech Republic). The quality control was performed with an international standard (Bone Meal, NIST SRM1486), and two internal standards (TORT 2, lobster hepatopancreas, certified by the National Research Council, Canada, and another obtained from otolith crushing, Campana pers. comm. 2006).

The otolith elemental composition revealed 11 elements above the limit of detection: 1 minor element (<sup>86</sup>Sr) and 10 trace elements (<sup>7</sup>Li, <sup>24</sup>Mg, <sup>26</sup>Fe, <sup>27</sup>Al, <sup>55</sup>Mn, <sup>59</sup>Co, <sup>63</sup>Cu, <sup>111</sup>Cd, <sup>135</sup>Ba and <sup>208</sup>Pb). The elemental classification is in agreement with Campana (1999).

### Stable isotopic composition

Otoliths were cleaned and weighed to ensure a suitable sample mass of carbonate for isotopic analysis. To avoid possible contamination by organic matter (<1 wt %), the samples were baked at 380°C for 45 minutes (Blamart *et al.* 2002). All isotope analyses were carried out on a Finnigan Mat Delta + mass spectrometer coupled with an automated preparation line.

The results are given in the conventional ( $\delta$  ‰) notation expressed in parts per mil against the V-PDB standard (Vienna Pee Dee Belemnite: Coplen *et al.* 1983), where:

$$\delta_{\text{sample}} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 10^3$$

Reproducibility was 0.04‰ and 0.05‰, respectively, for the carbon and the oxygen isotopes. Variation in the isotopic signatures in otoliths was estimated using the coefficient of variation (CV  $\delta^{18}\text{O}$  and CV  $\delta^{13}\text{C}$ ).

$$CV\delta X = \frac{sd_x \times 100}{\bar{m}_x}$$

where  $sd_x$  was the standard deviation of samples and  $\bar{m}_x$  the mean of samples.

### Data analyses

Otolith elemental concentrations and isotopic composition were compared within each life stage by MANOVAs in order to observe differences between sites. All differences between sites were tested with Fisher LSD post-hoc tests. Before statistical testing, elemental ratios were examined for normality and homogeneity among factor levels. Variations in elemental ratios in otoliths according to fish size were analysed with Pearson linear correlation at the River Rhône mouth.

Canonical discriminant analyses (CDA) were performed to investigate the ontogenetic effect on the chemical elemental composition of otoliths. The

performance of the CDA was assessed with Wilk's  $\lambda$ . This statistic is the ratio of intragroup variance to total variance, and provides an objective means of calculating the chance-corrected percentage of agreement between real and predicted group membership (Titus *et al.* 1984). The values of Wilk's  $\lambda$  range from zero to one: the closer Wilk's  $\lambda$  is to zero, the more accurate is the discriminating power of the CDA. The two principal discriminant functions were characterized by linear combinations of variables. To validate the performance of the CDA, a leave-one-out cross-validation statistic was used (Ramsay 2005).

## RESULTS

### Larval signatures

The elemental composition of otoliths revealed significant differences between post-larvae captured in shallow water at the River Rhône mouth and in Mauguio Lagoon in spring 2000 and spring 2004, respectively (Table 2). Post-larvae from Mauguio Lagoon showed significantly lower Sr/Ca and Co/Ca ratios and higher Ba/Ca, Mn/Ca and Mg/Ca ratios than post-larvae from shallow water at the River Rhône mouth (Fig. 2). Moreover, Mauguio fish otoliths had the lowest isotopic ratios (Table 3, Fig. 3) with  $\delta^{13}\text{C} = -10.50$  ( $\pm 0.27$ ) and  $\delta^{18}\text{O} = -1.86$  ( $\pm 0.01$ ).

### Juvenile signatures

No significant difference was observed for either the isotopic or the elemental signatures of otoliths from juveniles captured in Thau Lagoon in autumn 2003 and in autumn 2004 (Table 3). Therefore, these two years were pooled in all other comparisons.

Although sampling year varied according to the nursery site, significant differences were observed for otolith isotopic and elemental signatures of juvenile soles from the River Rhône mouth and from the Thau and Mauguio lagoons (Table 2). Juveniles captured in Thau Lagoon showed higher values of Sr/Ca, Co/Ca, Cu/Ca,  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  than those captured in Mauguio Lagoon. Moreover, they also showed higher values of Sr/Ca, Ba/Ca, Mn/Ca, Co/Ca, Cd/Ca and  $\delta^{13}\text{C}$  than juveniles captured at the River Rhône mouth. The juveniles captured at the River Rhône mouth showed higher values of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  than those captured in Mauguio Lagoon, and no difference for other elements (Tables 2, 3 and Figs 2, 3).

### Adult signatures

The elemental compositions of otoliths of adult fish captured in deep water at the River Rhône mouth and at Saintes-Maries did not reveal significant differences (Table 2, Fig. 2). However, higher  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values were observed for otoliths from adult fish captured at Saintes-Maries (Tables 2, 3, Fig. 3).

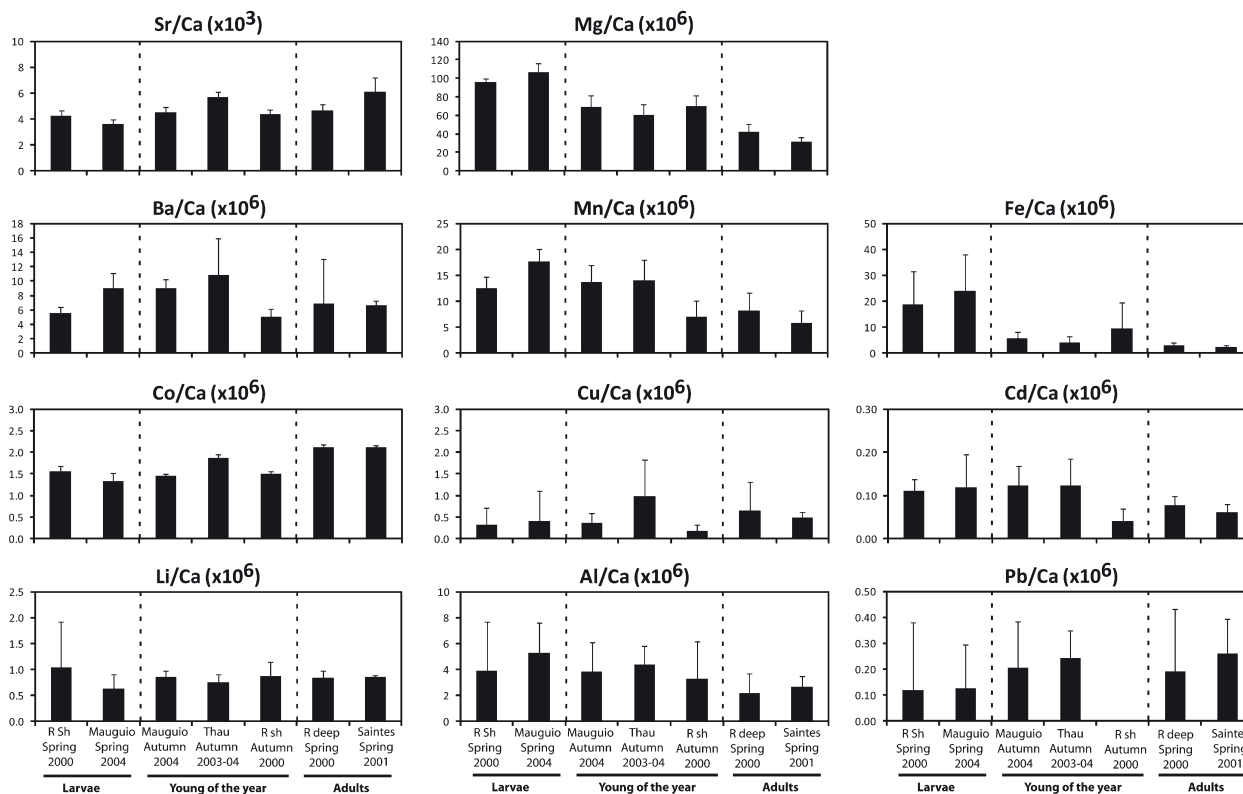


Fig. 2. – Mean ( $\pm$ sd) of elemental ratios by site (R Sh, Rhône shallow; R deep, Rhône deep). Both years in Thau coastal lagoon are grouped. Life stages are mentioned below the graphics.

TABLE 2. – Results of one-way MANOVAs achieved with elemental composition (in ratios to calcium) and isotopic ratios of otoliths for each life stage studied. NS, non-significant; Thau aut. grp, Thau autumn 2003 and 2004 grouped.

One-way MANOVAs comparisons		F	p	LSD Post-hoc elemental differences	
<i>Larval signatures</i>					
Rhône sp. vs Mauguio sp.	EC	33.2	**	Rhône>Mauguio Rhône<Mauguio	Sr, Co Ba, Mn, Mg
<i>Juvenile signatures</i>					
Thau aut. 2003 vs 2004	EC	64.1	Ns		
	I	0.3	Ns		
Thau aut. grp vs Mauguio aut.	EC	25.5	***	Thau>Mauguio	Sr, Co, Cu
	I	26.9	***	Thau>Mauguio	$\delta^{13}\text{C}$ , $\delta^{18}\text{O}$
Thau aut. grp vs Rhône aut.	EC	10.7	*	Thau>Rhône	Sr, Ba, Mn, Co, Cd
	I	6.6	*	Thau>Rhône	$\delta^{13}\text{C}$
Rhône aut. vs Mauguio aut.	EC	2.9	Ns		
	I	9.7	*	Rhône>Mauguio	$\delta^{13}\text{C}$ , $\delta^{18}\text{O}$
<i>Adult signatures</i>					
Rhône sp. vs Saintes sp.	EC	4.6	Ns		
	I	111.0	**	Rhône<Saintes	$\delta^{13}\text{C}$ , $\delta^{18}\text{O}$

TABLE 3. – Mean ( $\pm$ sd) otolith  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  for *Solea solea* caught from four sites in the NW Mediterranean expressed in ‰ versus PDB. Variations in ratios are indicated by the coefficient of variation (CV).

Site and date	N	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	CV $\delta^{18}\text{O}$	CV $\delta^{13}\text{C}$
Mauguio: spring 2004	6	-1.86 ( $\pm$ 0.07)	-10.50 ( $\pm$ 0.27)	3.65	2.38
Mauguio: autumn 2004	7	-0.44 ( $\pm$ 0.59)	-8.78 ( $\pm$ 1.81)	123.70	19.05
Thau: autumn 2003	4	1.11 ( $\pm$ 0.56)	-2.13 ( $\pm$ 0.69)	4.87	15.04
Thau: autumn 2004	4	0.86 ( $\pm$ 0.35)	-2.92 ( $\pm$ 1.86)	3.58	26.10
Rhône River mouth: shallow; autumn	3	1.20 ( $\pm$ 0.07)	-4.59 ( $\pm$ 0.85)	43.54	28.06
Rhône River mouth: deep; spring	3	1.66 ( $\pm$ 0.03)	-3.86 ( $\pm$ 1.23)	35.54	55.27
Saintes-Maries: deep; spring	3	2.35 ( $\pm$ 0.06)	-1.75 ( $\pm$ 0.19)	2.13	9.12

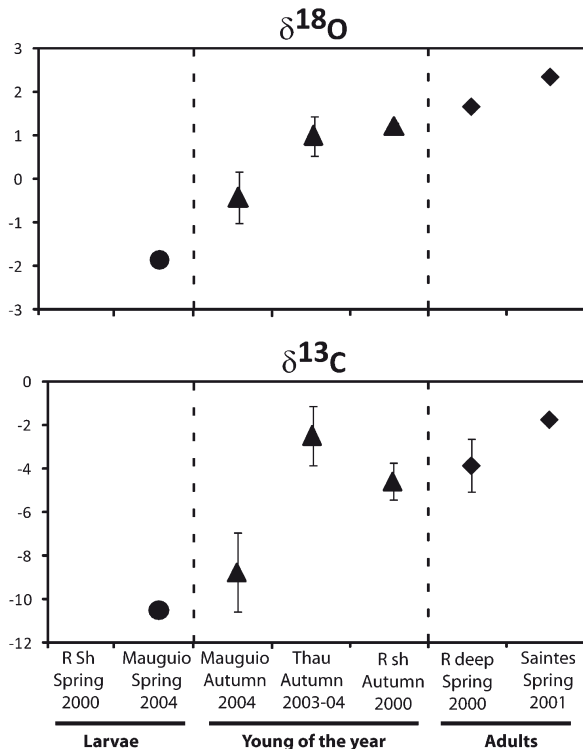


FIG. 3. – Mean (±sd) of isotopic ratios by site (R Sh, Rhône shallow; R deep, Rhône deep). Both years in Thau coastal lagoon are grouped. Life stages are mentioned below the graphics

TABLE 4. – Results of linear regressions between elemental composition and fish size (TL) in the River Rhône mouth.

	Slope	y-intercept	r <sup>2</sup>	p
Sr/Ca	2.0*10 <sup>-3</sup>	4.050	0.178	Ns
Mg/Ca	-24.3*10 <sup>-2</sup>	111.770	0.903	***
Li/Ca	-7.0*10 <sup>-4</sup>	1.043	0.023	Ns
Fe/Ca	-6.9*10 <sup>-2</sup>	22.520	0.429	**
Ba/Ca	4.5*10 <sup>-3</sup>	5.190	0.010	Ns
Mn/Ca	-1.7*10 <sup>-2</sup>	12.402	0.203	*
Al/Ca	-7.6*10 <sup>-3</sup>	4.418	0.082	Ns
Co/Ca	2.8*10 <sup>-3</sup>	1.247	0.744	***
Cu/Ca	1.4*10 <sup>-3</sup>	0.155	0.068	Ns
Cd/Ca	-7.7*10 <sup>-5</sup>	0.093	0.048	Ns

**Ontogenetic effects**

The ontogenetic effect on the elemental composition of otoliths was tested for fish of all three life stages, all captured in 2000 at the River Rhône mouth. Of the 11 elemental ratios, four showed a significant relationships with fish size (Table 4). An increase in the Co/Ca ratio and a decrease in the Mg/Ca, Fe/Ca and Mn/Ca ratios were observed with increasing fish size.

Moreover, the CDA achieved with the elemental composition of otoliths indicated a clear differentiation

TABLE 6. – Results of CDA carried out on elemental ratios. Coefficient-driven functions 1 and/or 2 are presented in bold characters.

	Wilks λ	P value	Variability in %	Function standardized coefficient									
				Sr/Ca	Mg/Ca	Li/Ca	Fe/Ca	Ba/Ca	Mn/Ca	Al/Ca	Co/Ca	Cu/Ca	Cd/Ca
Function 1	0.051	<0.001	88.1	0.328	<b>0.796</b>	<b>0.081</b>	0.117	0.050	<b>0.067</b>	<b>0.223</b>	<b>-0.620</b>	0.096	<b>0.151</b>
Function 2	0.472	<0.001	11.9	<b>-0.589</b>	0.390	-0.152	<b>0.730</b>	<b>0.137</b>	-0.310	-0.534	1.023	<b>-0.376</b>	0.326

TABLE 5. – Percentage success rate reclassifying sole to life stage using a cross-validated CDA and whole otolith elemental ratios. Rows represent actual groups while columns represent predicted groups. Correct classifications are given in bold characters; YOY, young of the year.

	Larvae	YOY	Adults	Total
Larvae	<b>93.3</b>	6.7	0	100
YOY	12.0	<b>88.0</b>	0	100
Adults	0	0	<b>100</b>	100

Function 2 (11.9%), λ=0.472\*\*\*

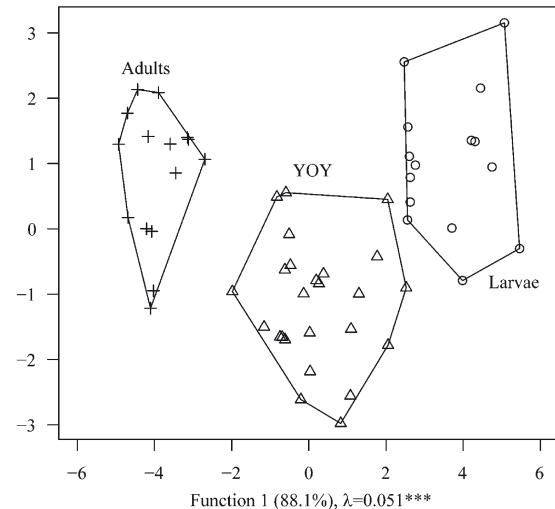


FIG. 4. – Plot of the CDA carried out with multi-element fingerprints of life stages otolith signatures for all sites grouped: (o) larvae, young of the year (YOY, Δ), adults (+) for all sites grouped. Results of cross-validation in percentage are shown in Table 6.

of the three fish life stages: larvae, juveniles (young of the year) and adults, with a correct reclassification of 92.7% of individuals (Fig. 4, Table 5). The first discriminant function represented the majority of the total variability (88.1%) and separated the three life stages. This function was driven by six ratios: Mg/Ca, Mn/Ca, Al/Ca, Co/Ca and Cd/Ca (Table 6). Life stage separation was linked to these ratios and adults were mainly characterized by higher Co/Ca ratios and larvae by Mg/Ca. The second discriminant function represented 11.9% of the total variability and was driven by four ratios: Sr/Ca, Fe/Ca, Ba/Ca and Cu/Ca (Table 6). This function separated out primarily the young of the year, which were linked to higher Sr/Ca and Cu/Ca ratios.

**DISCUSSION**

Many marine applications of multi-element otolith composition analysis have been published (Gillanders and Kingsford 1996, Campana *et al.* 2000, Gillanders

2002, Vasconcelos *et al.* 2008, Cuvelier *et al.* 2010, Tanner *et al.* 2011, Mercier *et al.* 2012), but few studies have investigated concomitantly the use of stable isotopes ratios (carbon and oxygen) and elemental composition (Kerr *et al.* 2007, Tanner *et al.* 2011). Although preliminary, the results from the present work suggest that otolith elemental and isotopic signatures may help to elucidate habitat connectivity of the common sole in the Gulf of Lions.

### Larval life stage

Of the 11 elemental ratios measured in otoliths of the common sole, 5 showed significant differences between fish post-larvae captured in shallow waters at the River Rhône mouth and those caught in Mauguio Lagoon in spring. Although the results were obtained using only a small set of individuals, the differences observed suggest that these fish lived in different environments during the larval stage. Otoliths of post-larvae captured at the River Rhône mouth showed higher values of Sr/Ca and Co/Ca and lower values of Ba/Ca, Mn/Ca, Mg/Ca than those of post-larvae captured in Mauguio Lagoon. Four of the five elements indicated relationships with environmental characteristics of the water. The otolith Sr/Ca ratio has often been used as a proxy of salinity (Limburg 1995, Campana 1999, Secor and Rooker 2000, Zlokovitz *et al.* 2003, Zimmerman 2005). The Co/Ca ratio of otoliths has the same characteristics in higher proportions in marine water. Ba/Ca and Mn/Ca ratios are higher in freshwater than in marine water (Panfili *et al.* 2002, Hamer *et al.* 2006, Bradbury *et al.* 2008, Tabouret *et al.* 2010). All these variations in ratios suggest that the fish post-larvae captured in Mauguio Lagoon in spring had been exposed to more desalted waters than those captured at the River Rhône mouth. However, in the Mediterranean Sea, the common sole reproduces in winter, and this probably takes place in proximity to the River Rhône mouth (Quéroux and Vayne 1997). The fish post-larvae analysed were captured in 2000 at the River Rhône mouth and in 2004 in Mauguio Lagoon. During these years, the winter flow of the River Rhône varied considerably. In winter 1999-2000 the flow was normal, with a maximal water discharge of  $3300 \text{ m}^3 \text{ s}^{-1}$ , whereas in winter 2003-2004 the river experienced a 100-year return flood with a maximal water discharge of more than  $10000 \text{ m}^3 \text{ s}^{-1}$  (Compagnie Nationale du Rhône, Miralles *et al.* 2006). This difference could explain why the otoliths of fish born in winter 2003-2004 exhibit elemental characteristics typical of fish from less salty water than fish born in winter 1999-2000. In otoliths,  $\delta^{13}\text{C}$  reflects the metabolism (diet) and dissolved inorganic carbon (DIC) signatures (Solomon *et al.* 2006). The  $\delta^{13}\text{C}$  of the River Rhône DIC is lower (between  $-10.2$  and  $-9.0\text{‰}$ ) than the  $\delta^{13}\text{C}$  of marine water (between  $0$  and  $2\text{‰}$ ; Aucour *et al.* 2003). The very low  $\delta^{13}\text{C}$  value of otoliths from fish post-larvae captured in Mauguio Lagoon in spring 2004 may be due to the influence of the River Rhône

waters during the larval life stage. These differences in isotopic and elemental compositions of larval stage otoliths seem to indicate inter-annual variations in the environmental conditions encountered by these sole larvae in the periods studied rather than spatial difference in the larval stage habitat of these fish.

### Juvenile life

Otolith growth is greater during the juvenile life stage than in the larval phase and for this reason the signatures observed in otoliths from nurseries seem to reflect principally the juvenile stage. Indeed, the juvenile period makes up ~90% of a juvenile otolith. Although our results were obtained on a small number of individuals for each year, no differences were observed when signatures of otoliths from juveniles captured in Thau Lagoon in autumn 2003 were compared with those from autumn 2004, suggesting that these fish had been exposed to globally homogenous conditions during this period in this lagoon (salinity of  $36.4 \pm 1.1$  and  $35.0 \pm 1.8$ , respectively). Moreover, winter 2003-04 was characterized by a 100-year return flood of the River Rhône, whereas in winter 2002-03 there was no exceptional flood. Otolith signatures from fish born in winter 2003-04 revealed high Ba/Ca, low Sr/Ca and low  $\delta^{13}\text{C}$  in relation to the flood. The absence of difference in juvenile signatures suggests that the larval life stage has not interfered with signatures observed for these two years. Our study thus shows that elemental and isotopic otolith compositions can be used as a tracer in adult populations of fish having used this lagoon as a nursery grounds. However, several studies have shown that group-specific variation in elemental composition is more the rule than the exception (Thorrold *et al.* 1997, Campana *et al.* 2000). Thus, the homogeneity observed in Thau Lagoon could have varied in other years. Use of this elemental composition as a biological marker was only possible in fish that used Thau Lagoon during the two years studied.

Otolith elemental fingerprints separate juveniles from both lagoons and juveniles from Thau Lagoon and the River Rhône mouth. These differences suggest that the environmental characteristics of each lagoon were sufficient to enable the identification of fish origins during this life stage. As noted above, Sr/Ca and Co/Ca ratios are higher in marine water than in freshwater (Limburg 1995, Campana 1999, Secor and Rooker 2000, Panfili *et al.* 2002, Zlokovitz *et al.* 2003, Zimmerman 2005), whereas Ba/Ca and Mn/Ca show higher values in freshwater than in marine water (Panfili *et al.* 2002, Hamer *et al.* 2006, Bradbury *et al.* 2008, Tabouret *et al.* 2010). Higher Sr/Ca, Co/Ca and Cu/Ca ratios were observed in otoliths from juveniles captured in Thau Lagoon than in those captured in Mauguio Lagoon. The differences observed for these ratios may be related to the higher salinity observed in Thau Lagoon (~32-39) than in Mauguio Lagoon (~18-32, Ifremer 2008). The Thau and Mauguio lagoons



have different watersheds, which may explain the differences in Cu/Ca values. Mauguio is very close to the city of Montpellier (400000 inhabitants including the suburbs: Mouillot *et al.* 2005) and Thau is surrounded by several small towns with a total of approximately 90000 inhabitants. The area is the home to considerable agricultural activity such as vineyards, which represent 36% of the watershed (Ifremer 2004). Moreover, tourism activities located around both lagoons explain seasonal swings in population. Through surface runoff, both lagoons accumulate pollution from urban and agricultural activities (such as copper from vineyards).

Although our results were obtained on a small number of individuals, juveniles captured in Thau Lagoon show higher Ba/Ca and Mn/Ca ratios than those captured at the River Rhône mouth. Otoliths from shallow nurseries show high barium concentrations in various species (Hamer *et al.* 2006, Leakey *et al.* 2009, Cuveliers *et al.* 2010). Ambient barium sources include terrestrial runoff, groundwater, pollution and remobilization from sediments (Hamer *et al.* 2006). High manganese values in otoliths have been observed for *Solea solea* in the Wadden Sea (Cuveliers *et al.* 2010) and are explained by an enrichment of Mn linked to increased microbial activity during summer. This is a robust indication that these two ratios (Ba/Ca and Mn/Ca) may be useful to characterize fish from waters having contrasting seasonal parameters. Juveniles captured at the River Rhône mouth show lower Cd/Ca ratio values than those from Thau Lagoon. Cd and Mn concentrations are also higher in lagoonal waters than in sea waters, probably because of the enrichment in both dissolved and particulate elements of brackish lagoonal waters (Cuveliers *et al.* 2010). Cadmium is usually associated with allochthonous inputs, whereas manganese is associated with organic matter inputs (Péna and Picot 1991, Metzger *et al.* 2007). Thau Lagoon is surrounded by several small towns with a total of approximately 90000 inhabitants. Moreover, the human population increases considerably in summer with tourism activities around both lagoons. The lagoon accumulates pollution from urban and agricultural activities through surface runoff. In addition, chemical elements drain into the lagoon from inflow Rivers. Péna and Picot (1991) showed that Thau sediments were principally composed of silts (45%), and found cadmium in the vicinity of the major river flowing into the lagoon. Roussiez *et al.* (2006) showed that metal contamination (cadmium) has accumulated in the silt fraction in the direct vicinity of the River Rhone mouth. Differences in otolith metal concentrations have provided the basis for a tag which can be used to discriminate between coastal and lagoonal marine *S. solea*. Detritivorous “polychaetes” are abundant in lagoons (Duport *et al.* 2007), where they make up the main part of the *S. solea* diet (Darnaude *et al.* 2001). Consequently, these invertebrates have been clearly identified as the major pathway for the transfer of organic matter into those food webs which culminate with the common sole (Darnaude *et*

*al.* 2004). Recently, Amiard *et al.* (2007) illustrated the bioaccumulation of cadmium in a “polychaete”, *Nereis deversicolor*, related to increasing concentrations of labile cadmium in sediment. It is thus likely that metal contamination from sediment to *S. solea* could occur through the trophic network, via detritivorous “polychaetes”. However, incorporation of cadmium through the dissolved inorganic materials from resuspension events may also be another hypothesis. The differences observed in otoliths seem to be due to the differences between both habitats, but a variation of conditions cannot be excluded between both years.

Although the results were obtained using a small number of individuals, nurseries exhibit differences in isotopic ratios, with an increase in  $\delta^{13}\text{C}$  from Mauguio Lagoon to Thau Lagoon, and lower  $\delta^{18}\text{O}$  values in otoliths from juveniles captured in Mauguio Lagoon.  $\delta^{18}\text{O}$  is negatively correlated with temperature and positively correlated with salinity (Panfili *et al.* 2002). Differences in  $\delta^{18}\text{O}$  observed between nurseries may reflect the differences in site salinity, with lower salinity occurring in Mauguio Lagoon (Ifremer 2008). In otoliths,  $\delta^{13}\text{C}$  reflects the metabolism (diet) and DIC signatures (Thorrold *et al.* 1997, Høie *et al.* 2004, Solomon *et al.* 2006). The  $\delta^{13}\text{C}$  variation may thus reflect both a change in diet due to the different prey species eaten in each nursery (Rosenberg *et al.* 2003) and also DIC signatures, which are lower in water influenced by terrestrial inputs (Vizzini *et al.* 2005) than in marine water (Aucour *et al.* 2003). Higher values in otoliths from juveniles captured in Thau Lagoon suggest a stronger marine influence on this lagoon than on the two other nurseries. Moreover,  $\delta^{13}\text{C}$  variations may reflect differences in the portion of  $\delta^{13}\text{C}$  from the diet. Recent studies on common sole muscle tissue have revealed differences in  $\delta^{13}\text{C}$  signatures in relation to differences observed in the  $\delta^{13}\text{C}$  of the particular organic matter (POM) at the base of the trophic network (Darnaude 2003, 2005, Dierking *et al.* 2012). These differences may result from the more terrestrial origin of POM in Mauguio Lagoon than at the River Rhône mouth or in Thau Lagoon.

### Adult life

Although our results were obtained using a small number of individuals, the otoliths of adults show differences for isotopic signatures only. Isotopic signatures are linked to water parameters (such as temperature, salinity and DIC, Thorrold *et al.* 1997, Høie *et al.* 2004, Solomon *et al.* 2006). Adults were captured at the same depth in the eastern part of the Gulf of Lions, where temperature and salinity are similar. The differences observed for  $\delta^{18}\text{O}$  may result from environments previously inhabited by the fish (larval or juvenile life stages). Otolith  $\delta^{13}\text{C}$  was higher in adults captured at Saintes-Maries than in those captured in deep water at the River Rhône mouth. Whereas both sites are located in proximity to the River Rhône mouth, the differences

observed could suggest that the Saintes-Maries area is less influenced by river inputs than the area located near the River Rhône mouth. This signature could also reflect a difference in the signature of the environment previously inhabited by soles during their larval and/or juvenile phases.

### Ontogenetic effect

Of the 11 elemental ratios, four (Fe/Ca, Mn/Ca, Mg/Ca and Co/Ca) showed a significant correspondence with fish size. The life cycle of the common sole in the NW Mediterranean Sea is characterized by an ontogenetic shift in habitat. Juveniles inhabit shallow marine waters, whereas adults inhabit deeper marine environments (Salen-Picard *et al.* 2002). Cobalt is more concentrated in otoliths from marine environments than in those from fish caught in brackish waters, and the opposite is observed for manganese (Panfili *et al.* 2002). Roussiez *et al.* (2006) showed an association between this element and the clay fraction in sediments, which increased with depth. This conformity could explain the increase of the Co/Ca ratio observed for fish size and the decrease observed for the Mn/Ca ratio. Iron and magnesium ratios were seen to decrease with fish size. Relationships between these environmental concentrations and otolith bio-mineralization are not clear (Panfili *et al.* 2002) and variations may be due to ontogenetic effects. These differences could also reflect a migration of individuals between contrasted habitats during their life.

Moreover, otolith chemical composition enabled discrimination between individuals according to their life cycle stage. The differences observed may be due to the ontogenetic shift in habitat use. As we have shown previously, during each life cycle phase the common sole lives in different habitats, each with specific environmental characteristics. Adults showed high Co/Ca ratios associated with the clay sediments present in deep water near the River Rhône mouth. In general, juveniles were a separate group, showing Sr/Ca and Cu/Ca ratios in relation to salinity and allochthonous inputs from the watersheds. The larval stage was associated with higher Mg/Ca values. All these differences seem to suggest that otolith elemental composition of the common sole is more strongly influenced by modifications of environmental characteristics during their life cycle than by ontogeny.

Although preliminary, our results show a differentiation of each life stage by otolith elemental composition, probably related to differences in environmental conditions between sites or years. Moreover, they suggest that the SB-ICPMS of whole otoliths is inappropriate for life-history studies. Indeed, the elemental composition acquired during one life stage is masked by those acquired during the next stages of life. These results, obtained on limited sample numbers, must be confirmed by other analyses involving greater numbers of individuals. However, the differences observed in

various environments for the same stage suggest that otolith microchemistry could provide information on fish migration, if better-adapted techniques such as LA-ICPMS were used.

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