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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: 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 de 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.
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, Demanche 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 (≤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 (Durnau de 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. 1998, 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, δ18O 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...
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 km². Its discharge varies between 500 and 11000 m³ s⁻¹, with a mean value of 1700 m³ s⁻¹. 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 km² and a catchment area of 410 km². 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 km² and a catchment area of 325 km². 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 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 for each element. Each otolith was dissolved in metallic forceps. Immediately following extraction, the otoliths were placed in distilled water for 10 minutes 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 for each element. Each otolith was dissolved in 100 ml of a 2:1 mixture of nitric and perchloric acids for 24 hours. The solution was evaporated to dryness and the otolith was re-dissolved in distilled water. The solution was filtered through a 0.45 µm filter and divided into two aliquots for analysis by the X-ray fluorescence spectrometer (XRFS) and the ICP-MS. The XRFS analysis was performed using a S8-βEND (Bruker AXS) spectrometer equipped with a Rigaku Xeocube X-ray tube with an integrated antiscatter foil. The ICP-MS analysis was performed using a Nicolet 380 (Thermo Finnigan) spectrometer equipped with a nebulizer and a cyclonic spray chamber. The software used for the data acquisition and processing was the spectral and atomic software (SAS) and the spectral diagnostic software (SDS). The data were analyzed using the Statistica software (Statsoft). The differences in the elemental composition of the otoliths were analyzed using the non-parametric Kruskal-Wallis test (p < 0.05). The differences in the stable isotopic signatures of the otoliths were analyzed using the Mann-Whitney U test (p < 0.05).

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.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Date</th>
<th>Depth</th>
<th>Size range</th>
<th>Age estimated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Date</td>
<td>Shallow (5-15 m)</td>
<td>Deep (30-70 m)</td>
<td>TL (mm)</td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>I</td>
<td>EC</td>
<td>I</td>
</tr>
<tr>
<td>Thau</td>
<td>autumn 2003</td>
<td>5</td>
<td>4</td>
<td>194-218</td>
</tr>
<tr>
<td></td>
<td>autumn 2004</td>
<td>5</td>
<td>4</td>
<td>195-222</td>
</tr>
<tr>
<td>Mauguio</td>
<td>spring 2004</td>
<td>10</td>
<td>6</td>
<td>95-123</td>
</tr>
<tr>
<td></td>
<td>autumn 2004</td>
<td>10</td>
<td>7</td>
<td>180-229</td>
</tr>
<tr>
<td>Rhône River</td>
<td>spring 2000</td>
<td>5</td>
<td>3</td>
<td>56-91</td>
</tr>
<tr>
<td></td>
<td>autumn 2000</td>
<td>5</td>
<td>3</td>
<td>122-177</td>
</tr>
<tr>
<td></td>
<td>spring 2000</td>
<td>10</td>
<td>3</td>
<td>207-371</td>
</tr>
<tr>
<td>Saintes-Maries</td>
<td>spring 2001</td>
<td>5</td>
<td>3</td>
<td>310-338</td>
</tr>
</tbody>
</table>
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⁻¹). 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 (86Sr) and 10 trace elements (7Li, 24Mg, 26Fe, 27Al, 55Mn, 59Co, 63Cu, 111Cd, 135Ba and 208Pb). 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 (δ ‰) 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) for δ18O and CV δ13C.

\[
CV\delta X = \frac{sd_X}{\bar{m}_X} \times 100
\]

where sdₓ was the standard deviation of samples and mₓ 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 analyzed 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 δ13C=-10.50 (±0.27) and δ18O=-1.86 (±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, δ13C and δ18O than those captured in Mauguio Lagoon. Moreover, they also showed higher values of Sr/Ca, Ba/Ca, Mn/Ca, Co/Ca, Cd/Ca and δ13C than juveniles captured at the River Rhône mouth. The juveniles captured at the River Rhône mouth showed higher values of δ13C and δ18O 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 δ13C and δ18O values were observed for otoliths from adult fish captured at Saintes-Maries (Tables 2, 3, Fig. 3).
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.

<table>
<thead>
<tr>
<th>One-way MANOVAs comparisons</th>
<th>F</th>
<th>p</th>
<th>LSD Post-hoc elemental differences</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Larval signatures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhône sp. vs Mauguio sp.</td>
<td>EC</td>
<td>33.2</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rhône&gt;Mauguio Sr, Co</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rhône&lt;Mauguio Ba, Mn, Mg</td>
</tr>
<tr>
<td><strong>Juvenile signatures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thau aut. 2003 vs 2004</td>
<td>EC</td>
<td>64.1</td>
<td>Ns</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>0.3</td>
<td>Ns</td>
</tr>
<tr>
<td>Thau aut. grp vs Mauguio aut.</td>
<td>EC</td>
<td>25.5</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>26.9</td>
<td>***</td>
</tr>
<tr>
<td>Thau aut. grp vs Rhône aut.</td>
<td>EC</td>
<td>10.7</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>6.6</td>
<td>*</td>
</tr>
<tr>
<td>Rhône aut. vs Mauguio aut.</td>
<td>EC</td>
<td>2.9</td>
<td>Ns</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>9.7</td>
<td>*</td>
</tr>
<tr>
<td><strong>Adult signatures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhône sp. vs Saintes sp.</td>
<td>EC</td>
<td>4.6</td>
<td>Ns</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>111.0</td>
<td>**</td>
</tr>
</tbody>
</table>

Table 3. – Mean (±sd) otolith $\delta^{13}$C and $\delta^{18}$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).

<table>
<thead>
<tr>
<th>Site and date</th>
<th>N</th>
<th>$\delta^{18}$O (‰)</th>
<th>$\delta^{13}$C (‰)</th>
<th>CV $\delta^{18}$O</th>
<th>CV $\delta^{13}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mauguio: spring 2004</td>
<td>6</td>
<td>-1.86 (±0.07)</td>
<td>-10.50 (±0.27)</td>
<td>3.65</td>
<td>2.38</td>
</tr>
<tr>
<td>Mauguio: autumn 2004</td>
<td>7</td>
<td>-0.44 (±0.59)</td>
<td>-8.78 (±1.81)</td>
<td>123.70</td>
<td>19.05</td>
</tr>
<tr>
<td>Thau: autumn 2003</td>
<td>4</td>
<td>1.11 (±0.56)</td>
<td>-2.13 (±0.69)</td>
<td>4.87</td>
<td>15.04</td>
</tr>
<tr>
<td>Thau: autumn 2004</td>
<td>4</td>
<td>0.36 (±0.35)</td>
<td>-2.92 (±1.86)</td>
<td>3.58</td>
<td>26.10</td>
</tr>
<tr>
<td>Rhône River mouth: shallow; autumn</td>
<td>3</td>
<td>1.20 (±0.07)</td>
<td>-4.59 (±0.85)</td>
<td>43.54</td>
<td>28.06</td>
</tr>
<tr>
<td>Rhône River mouth: deep; spring</td>
<td>3</td>
<td>1.66 (±0.03)</td>
<td>-3.86 (±1.23)</td>
<td>35.54</td>
<td>55.27</td>
</tr>
<tr>
<td>Saintes-Maries: deep; spring</td>
<td>3</td>
<td>2.35 (±0.06)</td>
<td>-1.75 (±0.19)</td>
<td>2.13</td>
<td>9.12</td>
</tr>
</tbody>
</table>
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 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, Campagna 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 (Quéro 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 m³ s⁻¹, whereas in winter 2002-2003 there was no exceptional flood. Otolith signatures from fish born in winter 2003-04 revealed high Ba/Ca, low Sr/Ca and low δ¹³C 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 Ba/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

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±1.1 and 35.0±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 δ¹³C 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 Ba/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 \( \text{Cu/Ca} \) 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 \( \text{Ba/Ca} \) and \( \text{Mn/Ca} \) 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 manganeuse values in otoliths have been observed for \( S. \ solea \) 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 (\( \text{Ba/Ca} \) and \( \text{Mn/Ca} \)) may be useful to characterize fish from waters having contrasting seasonal parameters. Juveniles captured at the River Rhône mouth show lower \( \text{Cd/Ca} \) ratio values than those from Thau Lagoon. \( \text{Cd} \) and \( \text{Mn} \) 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 accumulating 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 Rhône 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 (Dupot 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, Amiad 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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