DIFFERENCES IN ELEMENTAL CHEMISTRY AND C-O STABLE ISOPORE COMPOSITION BETWEEN LEFT AND RIGHT OTOLITHS OF A FLATFISH, THE COMMON SOLE SOLEA SOLEA

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ABSTRACT. – To test the hypothesis that both otoliths (left and right sagittae) of a flatfish, Solea solea (Linnaeus, 1758), display the same elemental fingerprint information, we analyzed whole-otolith preparations from coastal lagoons and marine sites in the NW Mediterranean for the presence of 15 elements (⁷Li, ²⁴Mg, ²⁷Al, ⁴⁴Ca ⁵²Cr, ⁵⁵Mn, ⁵⁶Fe, ⁶⁰Ni, ⁶³Cu, ⁶⁸Zn, ⁸⁶Sr, ¹¹¹Cd, ¹³⁷Ba and ²⁰⁸Pb), their ratio to Ca and for carbon and oxygen stable isotope ratios. We found significant concentration differences between the two otoliths for two elements, i.e. ⁷Li (right > left) and ⁸⁶Sr (right > left) all sites pooled together. However, this general trend differed between sites, with coastal lagoons showing significant differences for additional elements between the two otoliths, such as ⁴⁴Ca and ¹³⁷Ba in coastal lagoons for small juveniles, ⁵⁵Mn and ⁶⁸Zn in coastal lagoons for larger juveniles, and for ⁵⁵Mn/⁴⁴Ca for adults in marine sites. Both δ¹⁸O and δ¹³C isotopic ratios were higher in the right than in the left otolith (a difference of ~16% between otoliths in both cases) but these trends were not statistically significant and showed no spatial pattern. The left otolith was significantly heavier than the right otolith, a difference which decreased significantly with increasing fish size. Otolith mass was shown to correlate significantly with the δ¹⁸O and δ¹³C ratios, as well as for concentration in some elements and their ratio to Ca for both otoliths (⁵⁵Mn, ⁶⁸Zn, ⁸⁶Sr and ¹³⁷Ba) and for ²⁷Al on the left otolith only. Our results imply that the two otoliths are not interchangeable for fingerprint analysis. The right vs. left difference for ⁴⁴Ca, ⁸⁶Sr and ¹³⁷Ba decreases with increasing fish size, which suggests that differences in element concentrations may be at least partly driven by fish size. Thus, fish physiology and inner ear functioning may differ between otoliths in intensity and/or type of process as a function of increasing fish size and so possibly explain left vs. right differences in the otoliths of S. solea.

INTRODUCTION

The use of hard structures such as vertebrae, scales and otoliths in various fields of fish biology and ecology has a long history, beginning with the very early pioneering work of Hederström (1759) and Reibisch (1899). However, modern techniques have obviously extended the possibilities of detailed investigations considerably, in particular through the use of otoliths. Indeed, due to their conservative nature (Campana & Neilson 1985), their component material is not subject to resorption or chemical reworking. This has led to otoliths being increasingly considered as recorders of environmental conditions, and has provided the basis for the study of several aspects of fish life history traits (Lecomte-Finiger 1999). A decade ago, the reviews by Campana (1999) and Thresher (1999) were the first to offer a complete synthesis of the advantages of using otoliths for the study of biological and ecological processes in fish. These reviews also proposed important ways to both improve our knowledge of the otolith as a tool and to explore new ecological mechanisms. For instance, the recent and fast-growing development of microchemistry and stable isotope analysis is certainly the most well-known technique for exploiting the potential of the otolith as an ecological recorder (Panfili et al. 2002, Campana 2005). Otolith elemental fingerprints (chemical element and/or stable isotope ratios) have thus been used (1) to reconstruct past movements of individual fish (Campana et al. 1999, Hamer et al. 2006, Rooker et al. 2008a, b) and (2) to discriminate between different local populations and characterize the water mass variables (mostly salinity and temperature) encountered by fish at various spatial and temporal scales (Vasconcelos et al. 2007, Leakey et al. 2009).

In the Mediterranean as well as in the NE Atlantic, Solea solea (Linnaeus, 1758) is important to local fisher-
cies due to its high commercial value. The life cycle of the species can be broadly characterized by an ontogenetic shift in habitat use, i.e. juveniles inhabit coastal lagoons and waters usually shallower than 10 m depth, whereas older individuals inhabit the continental shelf from 20 to 150 m depth. Gaertner et al. (1997) have clearly established the distribution of this species within the NW Mediterranean, especially within the Gulf of Lions, with higher densities off the Rhône River mouth and in deeper waters (to ~150 m) in that area. Salen-Picard et al. (2002) demonstrated that terrestrial material represents an important input into the food webs that end with this species. Due to the ecological and economic importance of *S. solea*, it is of major interest to establish its life history traits. Otoliths are clearly good candidates for such a purpose. Mérigot et al. (2007) distinguished several local populations of *S. solea* within the NW Mediterranean based on otolith morphometrics and shape characteristics. Preliminary examinations of trace elemental composition in otoliths, as well as studies of δ¹³C and δ¹⁸O ratios, reinforce the pattern of population structure previously found by Mérigot et al. (2007) and allow some characterization of an ontogenetic shift in habitat use (Morat et al. 2012). Otolith chemistry is beginning to prove useful for the species, but an important methodological question (the subject of the present paper) has not yet been addressed: this question underlines the pressing need for research into left-right differences, before a much greater wealth of information can be accumulated in the face of this potential pitfall.

*MATERIALS AND METHODS*

**Sampling:** A total of 33 pairs of otoliths (sagittae) of the common sole *Solea solea* were considered for trace metal analyses (Table I). Otoliths were extracted with non-metallic forceps and cleaned with trace metal-free materials (washed for 5 minutes in an ultrasonic bath using deionised water). These fish had sizes ranging from 107 to 334 mm total length (TL) depending on site. Fish were labeled 0+ when < 220 mm (TL), 1+ for 220-260 mm, 2+ for 260-300 mm, and 3+ when > 300 mm (Shétaha 1984, Morat et al. 2012). Ten additional pairs of otoliths (fish size range: 200-329 mm TL) were used to determine and compare isotopic signatures (δ¹⁸O and δ¹³C) (Table I). The different individuals studied were caught in 2004 and came from coastal lagoons and marine sites of the French Mediterranean coast (Fig. 1). More detailed information on environmental conditions, substrate characteristics, etc., can be found in other works on the studied area (Mérigot et al. 2007, Vacquier et al. 2008, Dierking et al. 2009).

**Chemical elemental composition:** The trace metal analysis of each (whole) otolith was performed using an Inductively Coupled Plasma Mass Spectrometer (quadrupole ICP-MS Agilent Technologies, 7500 CE, Santa Clara, CA, USA). Each otolith

![Fig. 1. – Location of the sampled sites in the Gulf of Lions, NW Mediterranean Sea. Lines within the Gulf of Lions indicate depths of 50 m, 100 m and 500 m.](image-url)

**Table I.** – Length (TL) and age (year) of fish analysed in stable isotopes analyses and microchemistry in the different sites studied. YOY = Young of the year. (a) and (b) for coastal lagoons, and (c) and (d) for marine sites distinguished fish size and/or age ranges of individuals caught.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Stable isotope analyses</th>
<th>Microchemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TL (mm)</td>
<td>Age (y)</td>
</tr>
<tr>
<td>Coastal lagoons (a)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Coastal lagoons (b)</td>
<td>200-212</td>
<td>0+ (YOY)</td>
</tr>
<tr>
<td>Marine sites (c)</td>
<td>328-334</td>
<td>3+</td>
</tr>
<tr>
<td>Marine sites (d)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
was dissolved in 2 mL of Suprapur nitric acid (65%) which was obtained by two distillations of commercial acids. Solutions were mineralized for 2 hours at 110-120 °C and solubilised in 5 mL of suprapur nitric acid (5%) which was obtained by the dilution of suprapur acids with MQ element water. 50 µL of internal standard (indium 0.1 mg/L) were added. A standard range was realized with a multi-element solution (AsTAsOL MIX MO101, MO101, Analytika, Prague, Czech Republic).

The quality control was performed on the basis of two certified standards: Bone Meal (NIsT sRM1486), TORT 2 (Lobster hepatopancreas, National Research Council Canada certified) and one internal standard done with otolith crushing (s. Campana, Bedford Institute of Oceanography, Dartmouth, Canada, pers. com.) (Table II). The limits of detection (LOD) of the various elements measured by the ICP-MS are the following, in ppb:

- Li: 5.59*10^-3, Mg: 1.424*10^-1, Al: 7.77*10^-2, Ca: 3.219, Mn: 1.6*10^-1, Fe: 3.8*10^-1, Co: 1.5*10^-1, Ni: 6.27*10^-2, Cu: 1.28*10^-2, Zn: 1.72*10^-1, Sr: 6.13*10^-1.

### Table II. – Elemental certified and measured values for NIST SRM1486 and Campana otolith crushing. C = certified value, NC = non-certified value, NR = non-referenced value.

<table>
<thead>
<tr>
<th>Elements (ppm)</th>
<th>NIST SRM 1486</th>
<th>Campana otolith crushing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>306 507</td>
<td>265 800</td>
</tr>
<tr>
<td>Sr</td>
<td>235</td>
<td>264</td>
</tr>
<tr>
<td>Mg</td>
<td>4.083</td>
<td>4.660</td>
</tr>
<tr>
<td>Mn</td>
<td>2.60</td>
<td>1.00</td>
</tr>
<tr>
<td>Ba</td>
<td>4.37</td>
<td>-</td>
</tr>
<tr>
<td>Zn</td>
<td>150.0</td>
<td>147.0</td>
</tr>
<tr>
<td>Fe</td>
<td>91.0</td>
<td>99.0</td>
</tr>
<tr>
<td>Al</td>
<td>6.05</td>
<td>&lt; 1.00</td>
</tr>
<tr>
<td>In (0.1 mg/L)</td>
<td>0.77</td>
<td>0.80</td>
</tr>
</tbody>
</table>

### Table III. – Mean value (± SD) of concentration in chemical elements (in mg/g) measured in right and left otoliths (N=33 pairs). A. concentration of elements (top); B. ratio of elements to Ca (bottom). Percentage of difference between right (R) and left (L) otoliths are mentioned, with significant results in bold characters. NC not calculated. Presentation of elements by decreasing importance of concentration in the right otolith in A.

#### A

<table>
<thead>
<tr>
<th>Element</th>
<th>Right otolith</th>
<th>Left otolith</th>
<th>% dif. L &gt; R</th>
<th>% dif. R &gt; L</th>
</tr>
</thead>
<tbody>
<tr>
<td>44Ca (x 10^3)</td>
<td>383.1 (36.0)</td>
<td>378.2 (43.8)</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>86Sr (x 10^3)</td>
<td>1.78 (0.41)</td>
<td>1.71 (0.42)</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>24Mg</td>
<td>11.68 (6.18)</td>
<td>12.26 (6.39)</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>55Mn</td>
<td>3.39 (1.36)</td>
<td>3.43 (1.38)</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>137Ba</td>
<td>2.73 (1.02)</td>
<td>2.63 (1.07)</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>68Zn</td>
<td>1.50 (2.60)</td>
<td>1.45 (1.81)</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>27Al</td>
<td>0.59 (1.27)</td>
<td>0.16 (0.40)</td>
<td>269.4</td>
<td></td>
</tr>
<tr>
<td>7Li</td>
<td>0.45 (0.29)</td>
<td>0.38 (0.09)</td>
<td>31.8</td>
<td></td>
</tr>
<tr>
<td>63Cu</td>
<td>0.37 (0.34)</td>
<td>0.49 (1.13)</td>
<td>31.8</td>
<td></td>
</tr>
</tbody>
</table>

#### B

<table>
<thead>
<tr>
<th>Element/Ca</th>
<th>Right otolith</th>
<th>Left otolith</th>
<th>% dif. L &gt; R</th>
<th>% dif. R &gt; L</th>
</tr>
</thead>
<tbody>
<tr>
<td>86Sr (x 10^3)</td>
<td>4.65 (1.11)</td>
<td>4.55 (1.04)</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>24Mg (x 10^-6)</td>
<td>29.97 (15.12)</td>
<td>31.22 (15.56)</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>55Mn (x 10^-6)</td>
<td>8.91 (3.69)</td>
<td>9.06 (3.56)</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>137Ba (x 10^-6)</td>
<td>7.24 (2.09)</td>
<td>7.10 (2.49)</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>68Zn (x 10^-6)</td>
<td>3.45 (5.63)</td>
<td>3.62 (5.32)</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>7Li (x 10^-6)</td>
<td>1.54 (3.39)</td>
<td>0.35 (0.89)</td>
<td>333.5</td>
<td></td>
</tr>
<tr>
<td>63Cu (x 10^-6)</td>
<td>1.17 (0.77)</td>
<td>1.05 (0.31)</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>56Ni (x 10^-6)</td>
<td>1.01 (0.95)</td>
<td>1.46 (3.89)</td>
<td>45.8</td>
<td></td>
</tr>
</tbody>
</table>
Isotopic composition: For stable isotopes analysis, otoliths were cleaned and weighed to ensure an adequate sample mass of carbon (50-70 µg). In order to avoid possible contamination by their organic matter (< 1 wt %), the samples were roasted 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 at the Laboratoire des Sciences du Climat et de l’Environnement (Gif-sur-Yvette, France). The results are given in the conventional (δ ‰) notation expressed in per mil against the VPDB (Vienna Pee Dee Belemnite) standard where:

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

Reproducibility is 0.04 ‰ and 0.05 ‰, respectively, for carbon and oxygen.

Data analysis: The various elements measured were analysed in two ways: the classical ratio of a given element to Ca but also the concentration of each element separately as the combination with another element (i.e. ratio to Ca) may mask a potentially important information. Principal component analyses (PCA) were run on concentration of elements, and their ratio to Ca, to look at possible spatial and/or left vs. right general pat-
### RESULTS

In all, 15 elements (\(^{7}\)Li, \(^{24}\)Mg, \(^{27}\)Al, \(^{44}\)Ca, \(^{52}\)Cr, \(^{55}\)Mn, \(^{56}\)Fe, \(^{58}\)Co, \(^{60}\)Ni, \(^{64}\)Cu, \(^{86}\)Sr, \(^{111}\)Cd, \(^{137}\)Ba and \(^{208}\)Pb) were detected in otoliths. The most abundant elements (except \(^{44}\)Ca), on average and in both otoliths, were the following, in decreasing order: \(^{86}\)Sr, \(^{24}\)Mg, \(^{55}\)Mn, \(^{137}\)Ba and \(^{68}\)Zn (Table III). This trend remains similar when these elements are expressed in ratio to Ca. Four elements were, on average, below the limits of detection (\(^{56}\)Fe, \(^{58}\)Co, \(^{111}\)Cd), meaning that for some fish these elements were not detected and were above these limits for other fish. Two additional elements (\(^{60}\)Ni and \(^{208}\)Pb) were, on average, above the limits of detection, but these means were due to a combination of high values in only a very low number of otoliths and absence of detection in most other otoliths. These six elements were thus not taken into account hereafter.

**Differences between left and right otoliths**

Among the nine elements considered, three showed higher concentrations in the left otolith and six showed higher concentrations in the right otolith (Table III). However, only two of these left vs. right differences were statistically significant: \(^{7}\)Li and \(^{86}\)Sr were significantly more concentrated in the right otolith (Fig. 2). These trends were similar when elements are expressed in ratio to Ca, except for \(^{86}\)Sr (right > left), but surprisingly the significance of differences disappeared. Most often, the previous patterns were also observed according to site and to fish size (Table IV). However, some variations in this general pattern appeared as far as several elements were concerned. For instance, \(^{86}\)Sr showed significant dif-

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**Table IV.** Elemental composition of right and left otolith (Mean ± sd) and percentages of differences in elemental composition between right (R) and left (L) otoliths according to sites. Statistical significance of comparison of means are mentioned by bold characters. A '-' indicates absence of element on both otoliths. TL is the total fish length in mm. A, concentration of elements (top); B, ratio of elements to Ca (bottom). Presentation of elements as in table II and sites according to increasing range of fish size (min-max; TL in mm).

### Table A

<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Right otolith</td>
<td>Left otolith</td>
<td>% dif.</td>
</tr>
<tr>
<td>(^{44})Ca (x10(^3))</td>
<td>3.58 ± 2.60</td>
<td>306.4 ± 23.5</td>
<td>16.9 (L &gt; R)</td>
</tr>
<tr>
<td>(^{60})Sr (x10(^3))</td>
<td>1.11 ± 0.08</td>
<td>0.97 ± 0.09</td>
<td>14.6 (R &gt; L)</td>
</tr>
<tr>
<td>(^{24})Mg</td>
<td>14.83 ± 3.56</td>
<td>15.89 ± 3.13</td>
<td>7.1 (L &gt; R)</td>
</tr>
<tr>
<td>(^{55})Mn</td>
<td>4.12 ± 0.73</td>
<td>3.55 ± 0.84</td>
<td>16.2 (R &gt; L)</td>
</tr>
<tr>
<td>(^{137})Ba</td>
<td>3.27 ± 0.46</td>
<td>2.77 ± 0.60</td>
<td>19.4 (L &gt; R)</td>
</tr>
<tr>
<td>(^{7})Li</td>
<td>4.58 ± 5.30</td>
<td>3.06 ± 3.24</td>
<td>49.8 (R &gt; L)</td>
</tr>
<tr>
<td>(^{27})Al</td>
<td>–</td>
<td>–</td>
<td>1.88 ± 3.92</td>
</tr>
<tr>
<td>(^{68})Zn</td>
<td>0.54 ± 0.28</td>
<td>0.38 ± 0.11</td>
<td>42.5 (R &gt; L)</td>
</tr>
<tr>
<td>(^{63})Cu</td>
<td>0.84 ± 0.44</td>
<td>1.65 ± 2.84</td>
<td>97.6 (L &gt; R)</td>
</tr>
</tbody>
</table>

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**Table B**

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td>Right otolith</td>
<td>Left otolith</td>
<td>% dif.</td>
</tr>
<tr>
<td>(^{60})Sr (x10(^3))</td>
<td>3.09 ± 0.19</td>
<td>3.15 ± 0.17</td>
<td>2.0 (L &gt; R)</td>
</tr>
<tr>
<td>(^{24})Mg</td>
<td>–</td>
<td>39.88 ± 9.00</td>
<td>41.80 ± 6.39</td>
</tr>
<tr>
<td>(^{55})Mn</td>
<td>11.55 ± 2.14</td>
<td>11.53 ± 2.2</td>
<td>0.3 (R &gt; L)</td>
</tr>
<tr>
<td>(^{137})Ba</td>
<td>9.16 ± 1.49</td>
<td>8.90 ± 1.55</td>
<td>3.0 (R &gt; L)</td>
</tr>
<tr>
<td>(^{63})Zn</td>
<td>12.33 ± 14.02</td>
<td>10.02 ± 10.34</td>
<td>23.1 (R &gt; L)</td>
</tr>
<tr>
<td>(^{27})Al</td>
<td>–</td>
<td>–</td>
<td>1.88 ± 3.92</td>
</tr>
<tr>
<td>(^{7})Li</td>
<td>1.48 ± 0.74</td>
<td>1.24 ± 0.38</td>
<td>19.6 (R &gt; L)</td>
</tr>
<tr>
<td>(^{63})Cu</td>
<td>2.32 ± 1.21</td>
<td>5.72 ± 10.10</td>
<td>146.6 (L &gt; R)</td>
</tr>
</tbody>
</table>

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References which always favoured higher concentrations in the right otolith; this difference being the most important for small fish (Table IV). Here also, the ratio to Ca (i.e. $^{86}$Sr/$^{44}$Ca) did not show any significant difference. $^{44}$Ca was significantly more concentrated in the right otoliths of small fish (358.1 vs. 306.4 mg/g for the right and left otoliths, respectively, i.e. a difference of ~15%), but not for larger ones (Table IV). Other elements, such as $^7$Li, $^{63}$Cu, $^{68}$Zn or $^{137}$Ba for instance, displayed marked opposite results in the magnitude of difference between both otoliths, according to site and fish size, even if statistical significance was not always found. The only significant difference between both otoliths for an element expressed in ratio to Ca concerned $^{55}$Mn for large fish from marine sites (Table IV). In general, the magnitude of differences in elemental concentrations between both otoliths was more important for small fish of coastal lagoon than for other individuals, at least for the most common elements, although this trend was less apparent for elements/Ca (Table IV).

Both $^{18}$O and $^{13}$C isotopic mean values were lower in the left otolith (0.73 ± 1.18 ‰ VPDB and -5.29 ± 3.56 ‰ VPDB, respectively) than in the right (0.85 ± 1.05 ‰ VPDB and -4.57 ± 2.72 ‰ VPDB, respectively) (Fig. 3), but these results were not statistically significant (U-test, p > 0.05) and did not show any particular spatial pattern. Overall differences observed were equivalent to average differences of 16.5% for $^{18}$O and 15.8% for $^{13}$C between right and left otoliths.

**Mass of otoliths and relationships with fingerprints**

For all fish, the mass of the left otoliths was significantly greater (p < 0.001) than the right (Fig. 4), with an average mass asymmetry of 8.6% for all fish pooled together. However, the magnitude of this mass asymmetry was significantly influenced by fish size (one-way ANOVA, p < 0.001). There is a significant decreasing relationship ($R^2 = 0.672$, p < 0.01) between fish size and mass asymmetry. The smallest individuals (107-115 mm TL) had a mass asymmetry of 12.7% (1.58 ± 0.03 mg and 1.39 ± 0.04 mg for the left and right otoliths, respectively) whereas the largest individuals (314-334 mm TL) displayed a lower asymmetry of 6.8% (24.54 ± 2.24 mg vs. 22.96 ± 2.45 mg respectively); intermediate fish sizes displayed intermediate percentage differences. The concentration of $^{86}$Sr was significantly and positively correlated with the mass of the two otoliths, whereas significantly negative relationships were found for other chemical elements ($^{55}$Mn, $^{68}$Zn and $^{137}$Ba) vs. mass of the two otoliths (Table V). $^{27}$Al represented a unique case in which a positive correlation was found only for the left otolith. Considering elemental ratio to Ca rather than elemental concentrations did not change this pattern. The $^{18}$O and $^{13}$C signatures were also significantly and positively correlated with the mass of the two otoliths (Table V).

**DISCUSSION**

Our results have highlighted that the left and right otoliths (sagittae) of *Solea solea* were not equivalent in term of mass, thus confirming and reinforcing other similar results for flatfish (Toole *et al.* 1993, Mérigot *et al.* 2007, Loher *et al.* 2008). We also found differences between the two otoliths in concentration, of several chemical ele-
ments, but not in their ratio to Ca. Differences were not seen in carbon and oxygen isotopic values for both otoliths, which, despite a difference of ~16% of the mean values, were not statistically distinguishable. This clearly implies that from a chemical perspective, the otoliths of any individual are not interchangeable, and suggests that studies which assign otoliths to treatment groups at random, or for ‘pragmatic’ reasons (one otolith is broken or lost), may not be valid in terms of elemental fingerprint studies concerning this flatfish species.

**Left versus right otolith**

Although some authors have already reported chemical differences between left and right otoliths (Kalish 1991, Outridge et al. 2002), very few studies have genuinely focused on differences between the two otoliths in terms of chemical concentrations and/or isotopic ratios (Gauldie 1996, Thorrold et al. 1997, Rooker et al. 2001, Høie et al. 2004, Huxham et al. 2007, Loher et al. 2008). Gauldie (1996) described high variations in the concentration of five trace elements (Mg, Mn, Sr, Fe and Zn) between left and right otoliths for the Chinook salmon, but the significance of these differences in concentration was not statistically demonstrated. Rooker et al. (2001) found only one significant difference, in mg concentration, between right and left otoliths among the six trace elements (Na, K, mg, Mn, Sr and Ba) tested, and for only one of the two tuna species that they studied. Høie et al. (2004) did not find any differences in isotopic values between the two otoliths for Atlantic cod, Gadus morhua, whereas Thorrold et al. (1997) and Huxham et al. (2007) found small and insignificant differences in isotopic values, for the Atlantic croaker, Micropogonias undulatus, and for two mangrove species, respectively. These studies focused on ‘round’ (i.e., with a bilateral symmetry) fish species. Even if some studies seem to indicate a relative homogeneity or no statistical difference in the chemical composition between right and left otoliths, more extensive experimentation is needed to test whether this is a general rule or a species effect. As far as flatfish are concerned, very few geochemical and isotopic studies are available. The work of Loher et al. (2008) on a North-Atlantic flatfish, Hippoglossus stenolepis, mentions mass asymmetry, and highlights significant differences in δ¹⁸O and δ¹³C signatures and Sr/Ca ratio between the two otoliths.

Solea solea exhibits mass asymmetry (Mérigot et al. 2007) and our results confirm that left otoliths have greater mass than do right otoliths (8.6% in our case, 12.5% for Mérigot et al. 2007). The development of this asymmetry in the otoliths of flatfish probably occurs after metamorphosis or after settlement on soft bottoms (Graf & Baker 1983, Toole et al. 1993). This process could indicate a differential growth rate between the two otoliths (Sogard 1992, Fischer & Thompson 2004, Helling et al. 2005). Indeed, S. solea shifts from its pelagic larval life to a benthic life after metamorphosis. After lying on its left side, with a concomitant 90° rotation of the whole stato-acoustic system (Graf & Baker 1983), S. solea adopts a typical flat form. The left otolith then begins to become heavier, possibly due to the development of greater sensitivity at the water-bottom interface (Mérigot et al. 2007). It can be suggested that the growth of the otolith increases in mass due to possible feedback caused by the otolith resting on the side of the endolymph chamber, which occurs at settlement. Indeed, Lychakov & Rebane (2000) and Popper et al. (2005) suggested that the sensitivity of the inner ear depends on the otolith mass, and that this sensitivity further increases with the mass of the otolith. The few existing studies illustrate similar observations for other flatfish, such as the Dover sole Microstomus pacificus (Toole et al. 1993) and the Pacific halibut Hippoglossus stenolepis (Loher et al. 2008). More extensive studies are needed to confirm whether mass asymmetry may be considered as general for flatfishes.

The differences in trace element concentrations for Li and Sr (plus Mn, Zn and Ba) for some size-class-

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**Table V. Results of linear correlations between otolith mass and elemental and isotopic fingerprints for the right and the left otoliths**

**A**

<table>
<thead>
<tr>
<th>Element</th>
<th>Right</th>
<th>p-level</th>
<th>Left</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>0.134</td>
<td>0.435</td>
<td>0.367</td>
<td>0.054</td>
</tr>
<tr>
<td>Sr</td>
<td>0.575</td>
<td>&lt; 0.001</td>
<td>0.613</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mg</td>
<td>0.238</td>
<td>0.183</td>
<td>0.206</td>
<td>0.251</td>
</tr>
<tr>
<td>Mn</td>
<td>-0.458</td>
<td>0.007</td>
<td>-0.357</td>
<td>0.036</td>
</tr>
<tr>
<td>Ba</td>
<td>-0.509</td>
<td>0.003</td>
<td>-0.398</td>
<td>0.022</td>
</tr>
<tr>
<td>Zn</td>
<td>-0.347</td>
<td>0.048</td>
<td>-0.439</td>
<td>0.011</td>
</tr>
<tr>
<td>Al</td>
<td>0.308</td>
<td>0.081</td>
<td>0.405</td>
<td>0.019</td>
</tr>
<tr>
<td>Li</td>
<td>-0.073</td>
<td>0.685</td>
<td>0.295</td>
<td>0.095</td>
</tr>
<tr>
<td>Cu</td>
<td>-0.021</td>
<td>0.906</td>
<td>-0.255</td>
<td>0.153</td>
</tr>
<tr>
<td>δ¹⁸O</td>
<td>0.807</td>
<td>&lt; 0.001</td>
<td>0.742</td>
<td>0.007</td>
</tr>
<tr>
<td>δ¹³C</td>
<td>0.719</td>
<td>0.011</td>
<td>0.648</td>
<td>0.016</td>
</tr>
</tbody>
</table>

**B**

<table>
<thead>
<tr>
<th>Element</th>
<th>Right</th>
<th>p-level</th>
<th>Left</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr</td>
<td>0.494</td>
<td>0.004</td>
<td>0.539</td>
<td>0.002</td>
</tr>
<tr>
<td>Mg</td>
<td>0.214</td>
<td>0.231</td>
<td>0.192</td>
<td>0.284</td>
</tr>
<tr>
<td>Mn</td>
<td>-0.519</td>
<td>0.002</td>
<td>-0.499</td>
<td>0.003</td>
</tr>
<tr>
<td>Ba</td>
<td>-0.531</td>
<td>0.002</td>
<td>-0.493</td>
<td>0.004</td>
</tr>
<tr>
<td>Zn</td>
<td>-0.353</td>
<td>0.044</td>
<td>-0.445</td>
<td>0.009</td>
</tr>
<tr>
<td>Al</td>
<td>0.309</td>
<td>0.080</td>
<td>0.426</td>
<td>0.014</td>
</tr>
<tr>
<td>Li</td>
<td>-0.096</td>
<td>0.595</td>
<td>0.107</td>
<td>0.553</td>
</tr>
<tr>
<td>Cu</td>
<td>-0.043</td>
<td>0.811</td>
<td>-0.253</td>
<td>0.155</td>
</tr>
</tbody>
</table>

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*Vie Milieu*, 2013, 63 (3/4)
es and sites, cf. Table IV) may be due to a differential growth rate and/or incorporation rate of chemical elements between right and left otoliths. However, it remains unclear why this is not the case for other trace elements and why such differences are not all systematically in favour of the same otolith. We also highlighted that significant differences between otoliths found for elemental concentrations became insignificant when the considered element is expressed in ratio to Ca. The reason of this remains unclear but underlines that the concentration of Ca, an element usually not taken into account for micro chemical analyses, might be much more important than usually expected. Additionally, an important point is to be careful when normalizing a given element to Ca ratio, as chemical concentrations of otoliths are usually normalized to a constant value of Ca (stoichiometry of CaCO₃, \( \sim 390 \) mg of Ca / g of otolith) which is not always measured. In our case, normalizing concentrations of elements by this constant value of Ca would possibly have generated artificially significant differences between right and left otoliths. This also suggests that the usual manner to consider chemical fingerprints, i.e. the classical element to Ca ratio, appears to be insufficient as it can mask some useful information on elemental concentrations.

Otolith growth through bio-mineralization depends, overall, on endolymp chemistry (Morales-Nin 2000, Borelli et al. 2001, 2003, Hüssy 2008a, b) but also on the transport of the main constitutive elements, e.g. calcium carbonates and proteins, within the sacculum epithelium (Mayer-Gostan et al. 1997, Tohse & Mugiya 2004). The growth regulation processes of otolith daily increment formation are relatively well-known (Morales-Nin 2000), but possible variations in such processes are not documented. Growth conditions probably differ between the larval stage, the metamorphosis period and the subadult stage. It has been suggested that possible changes in inner ear anatomy occurring during the transition between larval and metamorphic stages, and/or between metamorphosis and subadult stages, may provoke changes in mechanisms of otolith growth, generating differences in the width of rings in the otolith at these periods (de Pontual et al. 2003, McCormick & Meekan 2010). It is reasonable to suggest that these processes may also play a role in the rate at which various trace elements are incorporated, although nothing is yet known regarding such physiological and chemical mechanisms. Loher et al. (2008) suggested that the differential carbonate accretion that generates mass asymmetry during flatfish growth may give rise to mass-bias in whole-otolith preparations favoring the heaviest otolith, i.e. the left one for Solea solea. This also suggests that the right sagittae would be the preferred otolith to use (for whole-otolith preparations) for assessing larval and early post-larval stages, because it has not been affected by metamorphosis and by the 90° rotation of the stato-acoustic system (Loher et al. 2008). As these authors suggested for δ¹⁸O in Hippoglossus stenolepis, the systematically higher ⁸⁶Sr concentration in the right otolith in conjunction with a decreasing percentage difference between the two otoliths suggests that larval, early post-larval and possibly even young settlers encountered cooler mean temperature and/or lower salinity than did larger individuals (Campana 1999, Morat et al. 2012).

The role of fish size

One problem we face here is linked to the non-random distribution of individual fish size in the sites studied. Solea solea displayed an ontogenetic shift in its habitat use, with small individuals inhabiting shallow waters, sometimes including coastal lagoons, and larger individuals occurring mainly in deeper waters (Gaertner et al. 1997). In the NW Mediterranean, most coastal lagoons have relatively low salinity, and an important nursery ground is located off the Rhône River mouth in an area thus subjected to high freshwater and terrestrial runoff. We are, therefore, well aware that confounding effects (of site and size) are probably involved here, but we have chosen to focus on possible size effects, because spatial patterns of chemical and isotopic fingerprints have already been documented for the studied area (Morat et al. 2012).

Our findings concerning mass asymmetry between right and left otoliths for Solea solea reinforce those of Mériot et al. (2007) who reported an association between decreasing mass asymmetry and increasing fish size. In our study, both otoliths of small fish showed greater sensitivity to left vs. right percentage difference for ⁸⁶Sr than did those of large individuals. This was also found for other trace elements including ⁴⁴Ca (cf. Table IV). As there is a significant linear relationship between fish size and otolith mass for this species (Morat et al. 2008), our findings indicate that ⁸⁶Sr was mostly incorporated during the early life-span and that incorporation of this element then progressively decreased during later phases of life (de Pontual et al. 2003, Morat et al. 2012). Another and/or complementary explanation could be linked to the significant difference between otoliths for concentration in ⁴⁴Ca. In particular, the concentration of this element in the left otolith of small fish (~304 mg.g⁻¹) is well under the stoichiometry of calcium carbonate (~380 mg.g⁻¹ otolith) (Campana 1999). This suggests that small fish have encountered particular environmental conditions and/or are subjected to unusual metabolic processes compared to larger individuals (see below). Finally, Sr was more concentrated in the right otolith and this is consistent with the finding of Hamer & Jenkins (2007) and suggests higher concentration when otolith growth rate is lower, i.e. the right otolith for S. solea. Conversely, Mn and mg were more concentrated in the left otolith, and this difference became significant in Mn for larger fish, suggesting these elements were more largely incorporated when accretion rates are higher (Hamer & Jenkins 2007).
These ontogenetic changes may be linked to the relatively low Ca concentration in the core region and may constitute a stress index associated with the larval stage (Toole et al. 1993, de Pontual et al. 2003). In contrast with $^{86}$Sr, other trace elements and $^{18}$O and $^{13}$C were apparently poorly incorporated during young phases, but were progressively incorporated in greater concentration with fish growth. This may either be due to environmental conditions, including an ontogenetic shift in habitat use, or to biological processes such as diet, most likely under the control of physiological and perhaps hormonal mechanisms. $^{86}$Sr is often considered to be a good environmental marker reflecting salinity and/or temperature (Campana 1999). The decreasing proportional difference in $^{86}$Sr concentrations between left vs. right otoliths, as well as decreasing mass asymmetry with increasing fish size, indicates that the differential carbonate accretion decreases progressively as the fish grow (Mérigot et al. 2007). This may occur concomitantly to a higher incorporation of $^{86}$Sr per se. Another possibility may be that the strontium incorporation rate simply stabilizes to a constant, such that, as the total carbonate (as reflected by the mass of the otoliths) becomes more equal between otoliths, the differential strontium signal is apparently being established early in life, and is being masked with increasing age and consequent otolith mass. In the case of S. solea, this also suggests that the difference in acoustical sensitivity between the two inner ears, which depends on the otolith mass, also decreases as fish size increases. Mérigot et al. (2007) suggested that higher sensitivity is required for small fish because they need to be able to acoustically detect small prey (e.g., copepods and cumaceans; Darnaude et al. 2001), which can be easily detected visually, perhaps reducing the need for acoustic signals. It is thus also possible that $^{86}$Sr (but also $^{27}$Al, $^{55}$Mn, $^{66}$Zn and $^{137}$Ba; perhaps $^{13}$C) incorporation is partly linked to dietary processes (Buckel et al. 2004), through a shift in prey with increasing fish size. However, it is still not clear how a given elemental concentration could be higher (or lower) in some prey (such as small crustaceans) than in other prey (such as large polychaetes).

The increase of $\delta^{18}$O values with fish size may be linked to the gradually decreasing temperatures which are experienced by individuals, as has been suggested by other authors (Kalish 1991, Campana 1999, Loher et al. 2008). This corresponds roughly to seasonal differences during our sampling, although temperatures were not systematically recorded. The case of $\delta^{13}$C is more complex, because several concomitant processes may be involved, such as general metabolism or dissolved inorganic carbon (Solomon et al. 2006, Loher et al. 2008). Irrespective of the relative importance of the various processes occurring for S. solea, our results clearly imply an ontogenetic change in their relative importance with increasing fish size. As the dissolved inorganic carbon was present in globally close concentrations in the various studied zones, this suggests that metabolism and/or kinetic effects became increasingly important as the fish grew, ultimately affecting $^{13}$C incorporation in otoliths (Kalish 1991, Schwarcz et al. 1998).

ACKNOWLEDGEMENTS. – The first author is supported by the “Fondation Total” and the “Agence de l’Eau Rhône-Méditerranée-Corse”, within a PhD framework funded by the “Conseil Régional Provence-Alpes-Côte d’Azur”. We are grateful to local fishermen for their help in fish catches, to C Bouchyrand for help in chemical preparation, to M Paul and R Mackie for improvements in the English of our manuscript, and to the anonymous reviewers for their valuable suggestions and criticisms which have enabled us to improve the article.

REFERENCES


Vie Milieu, 2013, 63 (3/4)


Toole CL, Markle DF, Harris PM 1993. Relationship between otolith microstructure, microchemistry, and early life history events in Dover sole, Microstomus pacificus. Fish Bull 91: 732-753.


Received on September 19, 2013
Accepted on January 13, 2014
Associate Editor: R Lecomte