Influence of ontogenetic and environmental changes in the otolith microchemistry of juvenile sole (*Solea solea*)

Hélène de Pontual*a,*, Françoise Lagardèreb, Rachid Amarac, Marcel Bohnad, André Ogora

aLaboratoire de sclérochronologie des animaux aquatiques, IFREMER, DRII/BH/LASAA, BP 70, 29280 Plozané, France
bCresta-L’Houmeau (CNRS - IFREMER), BP 5, 17137 L’Houmeau, France
cUniversité du Littoral - Côte d’Opale, UMR CNRS 8013, Avenue Foch, 62930 Wimereux, France
dLaboratoire de Géochimie, IFREMER, DRO/OM, BP 70, 29280 Plozané, France

Received 18 December 2002; accepted 15 May 2003

Abstract

We analysed otolith composition, specifically Sr/Ca ratios, with the aim of determining whether metamorphosis and the transition to benthic life of Bay of Biscay sole occur in marine or estuarine conditions. Otoliths of wild juveniles (0-group) collected in two estuarine nurseries showed characteristic Sr/Ca chronologies, with a significant decrease of the Sr/Ca ratio from the core to the accessory growth centres. As the otolith Sr/Ca ratio decreases in respect of the ambient salinity, this could suggest a relative synchronism in the timing of estuarine nursery entrance. In order to validate this hypothesis, hatchery-produced larvae were reared in a tidal marsh mesocosm until they completed metamorphosis (around 1 month post-hatching) and settled on the bottom. The otoliths from these juveniles exhibited a similar Sr/Ca pattern, which could not be explained as a result of habitat changes, and especially salinity variations, but rather reflected an ontogenetic signal. Since both wild and reared soles achieved high growth rates during metamorphosis, this suggests a link between a high metabolic rate and the observed Sr/Ca drop. In addition, ontogenetic changes during this transitional phase could involve the setting of functional mechanisms, responsible for the regulation of free Sr\(^{2+}\) in either plasma or endolymph or both. Nevertheless, this common trend appeared to be modulated by environmental cues: Sr/Ca ratios were significantly higher for reared fish compared to wild fish during the larval period. A possible explanation is that experienced temperatures were much higher in the incubator than in the field. Furthermore, Sr/Ca values obtained in the otolith juvenile area, with the lowest ratios for soles from the Loire nursery, suggest that the salinity regime of estuarine nurseries could exert an environmental control on otolith Sr/Ca ratios, thus providing ecological records of up-estuary migration after metamorphosis.

Keywords: Flatfish; Metamorphosing larva; Juvenile; Salinity; Field and experimental studies; Sr/Ca; Na; K; WDS

* Corresponding author.
E-mail address: Helene.De.Pontual@ifremer.fr (H. de Pontual).
1. Introduction

A better understanding of factors acting on recruitment is of major interest in the study of fish populations and fisheries management. Recruitment variability results from the interaction of biological and physical processes such as starvation, predation, temperature and larval transport (Yoklavich and Bailey, 1990). Larval transport can be one of the most important factors affecting recruitment, especially for species which spawn offshore and utilise inshore nurseries (Bailey, 1981; Norcross and Shaw, 1984; Bergman et al., 1989). This is the case of the common sole (Solea solea) in the northern Bay of Biscay (Koutsikopoulos and Lacroix, 1992). The mechanisms of sole larval transport remain unclear, albeit noticeable progress has been obtained (see review in Amara et al., 2000). During the last decade, it has been shown that larvae at the beginning of metamorphosis are found around the spawning area on the continental shelf, 20–90 km offshore (Amara et al., 1993). Nevertheless, very few late transforming larvae and no 0-group juveniles are captured on the shelf around the spawning ground (Amara et al., 1998). Juveniles tend to concentrate in coastal and estuarine nurseries, principally those of the Loire and Vilaine estuaries for this region (Marchand and Masson, 1989; Marchand, 1992; Amara et al., 1994). Koutsikopoulos et al. (1991) hypothesised that pelagic larvae reach nurseries through diffusive transport, which would result in the loss of most of the larvae that settle offshore. However, Amara and Bodin (1995) showed that feeding conditions are suitable offshore for potential settling of sole. In addition, experimental and field studies provided evidence that vertical migrations of larvae (Champalbert et al., 1994; Champalbert and Koutsikopoulos, 1995) and changes in the vertical distribution during metamorphosis (Lagardère et al., 1999) could reinforce the shoreward immigration process from offshore spawning areas to coastal nurseries. In order to determine whether metamorphosis and the shift to benthic life occur in marine or estuarine conditions, we analysed otolith composition and specifically Sr/Ca ratios. We also wished to identify the timing of brackish-water entrance in the life history of young sole.

For most flatfish species, accessory growth centres (AGC) (see Panfili et al., 2002, for terminology used in otolith studies) are easy to locate on sagittae. AGC are known to be synchronous with the transition phase of metamorphosis, which corresponds to the shift from pelagic to bottom-dwelling life of various flatfish such as, for example, the winter flounder Pseudopleuronectes americanus (Sogard, 1991), the plaice Pleuronectes platessa (Modin et al., 1996), the common sole (Lagardère and Troade, 1997), and the windowpane Scophthalmus aequosus (Neuman et al., 2001). In addition, AGC could indicate the date of estuarine nursery entrance for some of those species immigrating inshore, as shown for the starry flounder Platichthys stellatus (Campana, 1984) and the plaice (Al-Hossaini et al., 1989; Karakiri et al., 1989).

The use of Sr/Ca ratios for studying diadromous migrations is probably among the most robust applications of otolith microchemistry (see reviews in Campana, 1999; De Pontual and Gef fen, 2002). A positive relationship between ambient salinity and otolith Sr/Ca ratios has been reported for various species and environments (see review in Secor and Rooker, 2000), although highly variable among taxa. Nevertheless, the incorporation rate of Sr also depends on other environmental (abiotic and biotic) or endogenous (e.g. ontogeny, growth and reproductive status) factors whose relative contributions remain unknown. For instance, Sr/Ca has been reported to correlate negatively with temperature (e.g. Radtke et al., 1990; Townsend et al., 1992, 1995). However, in a study comparing groups of fish (Leiostomus xanthurus) reared at 20° and 25 °C, Bath et al. (2000) showed that Sr/Ca was positively related to temperature. Other studies have indicated that Sr/Ca would depend on metabolic rate (Kalish, 1991; Sadovy and Severin, 1992, 1994; Gallahar and Kingsford, 1996). Consequently, drastic metabolic changes, such as those occurring during metamorphosis, are liable to affect the otolith chemical patterns, irrespective of environmental conditions. This study compares wild and mesocosm-reared sole juveniles to analyse the effects of ontogenetic and habitat changes on otolith composition, specifically K, Na and Sr/Ca ratios.
2. Materials and methods

2.1. Origin of biological samples

Wild sole juveniles were collected in June 1993 in two nurseries of the Bay of Biscay (France) located in the Vilaine and Loire estuaries (Fig. 1, locations 4 and 7, respectively). The 0-group fish were of estimated ages ranging from 110 to 150 d. This implies that they had experienced marine waters of offshore spawning grounds and coastal waters before entering the nurseries for a 1–2 month stay.

Eggs originating from the CEFAS Conwy Hatchery facilities (UK) were transported to the Aqualive Station (Ile de Noirmoutier, location 8) to be reared, after hatching, in an incubator from 28 April to 11 May 1994. They were then transferred to a net cage moored in an earthen pond used as a tidal marsh mesocosm. Metamorphosis took place from 18 to 26 May (20–28 d post-hatching) and the experiment ended on 28 June 1994.

2.2. Field and rearing hydrological conditions

Table 1 provides temperature and salinity conditions, recorded both offshore and in the Vilaine and Loire estuaries during the 1993 field cruises, and those obtained during the 1994 rearing experiment.

The main difference between field and rearing conditions was the wide range of both temperature and salinity experienced by early sole during the transfer to inshore nurseries. This was particularly true for juveniles entering the Loire estuary, whose depth, length and river flow led to a greater variability of hydrological characteristics than in the Vilaine estuary.

2.3. Otolith preparation and analyses

After sampling, wild and reared fish were sacrificed, fixed and stored in 95% ethanol awaiting preparation and analyses.

Fig. 1. Location of hydrological records: northern Bay of Biscay spawning grounds (1), coastal areas (2), estuaries of the River Vilaine (3–4) and the River Loire (5–6–7). Wild juveniles were taken from the estuarine nurseries (locations 4 and 7). Reared samples were produced at the Aqualive Station (location 8, Ile de Noirmoutier).
Hemisections through the core were produced in the sagittal plane following a classical protocol: (1) embedding in epoxy resin on a glass slide in order that the proximal side be exposed, (2) grinding with silicon carbide, and (3) polishing with progressively finer grades of diamond suspensions (9 Å, 3 Å, 1 Å and 0.25 Å grain size). Automatic grinding and polishing machines were used in order to obtain a high quality surface state, as required for electron probe microanalysis. Sections were ultrasonically cleaned with Milli Q water (resistivity \( f 18.2 \text{MΩ}.\text{cm} \)) at the end of each polishing stage. They were stored in a desiccating cabinet and carbon coated under vacuum just before analysis.

Samples were analysed by a wave-length dispersive electron microprobe (WDS) Camebax SX50. Preliminary tests (unpubl. data) helped in the choice of the following beam conditions: 10 kV accelerating voltage, 15 nA beam current, 5 µm spot size, peak acquisition times of 120 s for K, Na, Sr and 40 s for Ca, background acquisition times set to half of peak acquisition times. These settings achieved the best compromise in terms of X-ray emission yields for the four elements. They provided suitable limits of detection (LODs) (Table 2) and permitted analysis of specimens with no or very slight damage under the beam. The setting of voltage and current to 15 kV and 10 nA, respectively, resulted in greater pitting (thus reducing data quality), although the beam power density, as defined by Gunn et al. (1992), remained unchanged.

Two types of analysis were performed on each sample, from the core to the postero-ventral edge of the otolith (Fig. 2):

- OP1, very close to the core (C), between the hatching and mouth-opening checks (see Lagar-
dèr et Troað (1997) for details on otoliths of sole larvae;
- OP2, at ~ 70–80 μm from the core, corresponding to the inner transition zone (TZ) of metamorphosis;
- OP3, at ~ 100–150 μm from the core, within the AGC area, whose deposition indicates the last events of metamorphosis (transformation and shift to benthic life);
- OP4, at ~ 150 μm from the AGC, in an area corresponding to the juvenile period.

To take into account measurement uncertainty, four replicates were made at each position on the otolith.

(2) linear scans (radials) consisting in series of analysis spots from the core to the juvenile area, at ~ 150–200 μm from the AGC. Distances between sampling points on a single radial were set at 12–17 μm depending on the sample.

Both types of analysis were carried out on 11 otoliths for each of the three different origins. We estimated that the beam diameter (5 μm) covered 3 to 8 daily increments during the early larval period and 0.5 to 2 during the metamorphic phase and juvenile period.

2.4. Data analysis

Statistical analyses were performed using SPSS® software. To compare positions within the otolith, we used the averaged value of the four replicate spots. Analysis of variance designed for repeated measures was used in order to eliminate the variability due to differences between otoliths in a same group when variables were measured on various occasions in each otolith (4 positions). Data from individual radial analyses were adjusted to an interval of 17 μm prior to the estimation of mean and standard error values for each origin (reared, Loire and Vilaine). Detailed Sr/Ca chronologies were graphically analysed by comparing the averaged profiles of the 3 juvenile groups.
3. Results

3.1. Otolith position analyses

Sr/Ca ratios, Na and K concentrations, clustered by otolith positions and fish origins, are shown in Fig. 3. Whatever the origin of the fish, the temporal variation of Sr/Ca appeared roughly similar (Fig. 3a): all 3 groups exhibited a decrease of Sr/Ca (due to a decrease of Sr concentration) from the core area (OP1) to the metamorphic transition zone (OP2). In contrast to wild fish, in reared fish this decrease extended to the AGC (OP3) of the last phase of metamorphosis. Wild juveniles originated from the Vilaine and reared juveniles showed similar Sr/Ca ratios at OP4, whereas the Sr/Ca ratio continued to decrease in this part of the otolith for the Loire juveniles.

Na concentrations remained stable over time for all three groups (Fig. 3b), whereas K concentrations exhibited clear temporal variations (Fig. 3c). Whatever the origin of the fish, the concentrations clearly increased from the innermost area (OP1), where most values were below the LOD, to the peripheral juvenile area (OP4).

Statistical treatment indicated that the within-otolith variations in Ca and Na concentrations were generally not significant (Table 3), except for Na concentrations measured in the larval otolith areas (OP1 vs. OP2) of reared and Vilaine samples. Regarding Sr/Ca, significant differences were observed between OP1 and OP2 in all groups, between OP2 and OP3 in reared samples and between OP3 and OP4 in the Loire samples only. In all groups, variations in K concentrations were significant in the otolith areas deposited during the larval period, including meta-
morphosis for the Vilaine and reared individuals (OP1 vs. OP2 and OP2 vs. OP3), whereas these variations were not significant in the juvenile parts (OP3 vs. OP4) of the otoliths.

3.2. Detailed otolith Sr/Ca chronology

The phase coherence of averaged Sr/Ca time series was striking whatever the origin of the sample (Fig. 4). A check of individual data indicated that Ca concentrations were relatively low within the core, explaining the relatively high Sr/Ca level in this structure. The variation pattern of Sr/Ca ratios encompassed 4 phases: (i) a maximum value at ~15–20 μm from the core, i.e. in an area deposited during the first-feeding stage (FF), (ii) a rapid drop during the rest of the pelagic larval stage, including the metamorphic transition zone, (iii) a plateau, concomitant to the completion of metamorphosis, at a similar level whatever the origin of the fish, and (iv) a more variable pattern during the juvenile period, depending on the origin of the fish.

Reared fish had significantly higher Sr/Ca ratios than wild fish in the larval stage (up to ~100 μm from the core). In contrast, the similarity was remarkable between the otolith Sr/Ca of wild larvae, from the core up to ~140 μm toward the edge. However, standard errors indicated relatively high within-group variability, especially during the larval period. During the juvenile period, a divergence between profiles was observed, mostly for the Loire group, whose averaged time series exhibited a gradual decrease, suggesting a progressive ingress in lower-salinity estuarine waters.

4. Discussion

Results of both otolith position analyses and Sr/Ca time series provide clear evidence that drastic changes in otolith Sr/Ca ratios and K concentrations occur during the early-life history of the common sole, especially during the larval period. Similar general patterns were observed in wild and reared fish: increasing K concentration from the core towards the edge, Sr/Ca spike during the earliest stages of development and Sr/Ca plateau coinciding with metamorphosis completion. This makes it impossible to interpret such patterns as a result of habitat changes. Reared and wild fish experienced different habitats (temperature and salinity), which suggests that the common trend corresponds to an ontogenetic signal. Ontogenesis mainly acts through the physiological status of early stages (metabolic stress during the critical stages of first-feeding and metamorphosis), in synchrony with the otolith formation. This could lead to ontogenetic changes in otolith elemental composition. However, Fig. 4 shows a clear discrepancy between Sr/Ca chronologies of reared fish (Welsh brood stock) and fieldsampled fish (French wild stock), which may be explained by at least the strain origin of fish and/or the different environments experienced by reared and wild fish in their early-life histories. In fact, all fish did experience roughly the same salinity during the larval phase (~34, Table 1) but temperatures were much higher for reared fish (ΔT ~ 5 °C, Table 1). The shift in the Sr/Ca chronology of reared fish appears to be consistent with the recent finding that Sr/Ca is positively related to temperature (Bath et al., 2000).

4.1. Are Na and K concentrations informative data?

Atomic characteristics of Sr being close to those of Ca, Sr is incorporated within the otolith crystal by substitution forming SrCO₃. Sr/Ca ratios thus provide
direct measurements of the substitution rates. Conversely, the monovalent ions Na and K are thought to be absorbed onto the crystal in the otolith interstitial space (see e.g. Campana, 1999). Even so, normalisation to Ca is often used to compensate for otolith matrix effects which induce local variations in reaction to the electron beam (Gunn et al., 1992). In the present work, the WDS was set to work in non-destructive conditions and the response patterns for K, Na and normalised data were similar. We thus reported Na and K measurements in weight concentrations (μg g⁻¹). A preliminary question is that of the accuracy of the reported results because the fish were preserved in alcohol prior to otolith extraction and analysis. This storage method is not recommended since it has recently been shown that it can alter otolith composition especially for some elements. Milton and Chenery (1998) found higher Na concentrations in otoliths whose extraction had been delayed after death (fish storage in ethanol or freezing). Proctor and Thresher (1998) also observed depressed Na levels at the otolith margins and elevated K levels at both core and margin in fish stored in ethanol. In both experiments, Sr was reported as being relatively insensitive to the storage method. In our study, Na measurements did not show significant differences in terms of ontogenetic and/or environmental variations. Whatever the origin of the sample, K levels show a clear temporal variation, which may be interpreted as an ontogenetic trend. Similar observations have already been reported and Proctor and Thresher (1998) noted that the ontogenetic trends observed in K concentrations were similar in preserved samples and controls. The reason for this ontogenetic variation is unclear, but has to be associated with the existence of a proximo-distal gradient in the endolymph K concentration (Payan et al., 1999), both facts suggesting that K could be involved in the otolith biomineralisation process.

4.2. Ontogenetic variations in the otolith Sr/Ca ratio

As discussed above, the comparison of Sr/Ca ratios in reared and wild fish provides evidence that both environmental and endogenous factors are involved in the early stages of sole otolith chemistry. However, our results suggest that ontogeny governs the general trend. Ontogenetic changes in structure of early sole otoliths result in the formation of two transition zones at the first-feeding and early-metamorphic stages (FF and TZ areas, respectively). AGC are swollen transformational structures whose deposition, characteristic of late metamorphosis in sole, contributes to moulding the species-specific shape of the juvenile otolith (Lagarðere et al., 1995). These ontogenetic changes appear to correspond to the main variations in the Sr/Ca ratio. Relatively high initial Sr/Ca ratios very close to the core (OP1) are imputed to result from the relatively low Ca concentration in this protein-rich structure (Radtké and Dean, 1982; Brothers, 1984; Dale, 1984). The Sr/Ca peak that is then observed in the FF area results from higher Sr concentrations in increments deposited after the mouth-opening check. Such high Sr levels in the otolith of FF larvae may be due to a low otolith incremental rate (Lagarðere, 1989) and may constitute a stress index associated with this critical stage. In agreement with these findings, Toole et al. (1993) suggested that high Sr incorporation in the otolith area surrounding the core could arise from a combination of low temperature, slow growth rate and high stress. This was experimentally assessed by Mugiya and Satoh (1997). In addition, an increase of Sr uptake by assimilation through the intestinal epithelium could occur, probably mainly from the water (Hoff and Fuiman, 1995; Farrell and Campana, 1996) and from food, which constitutes a second Sr²⁺ source (Limburg, 1995; Gallahar and Kingsford, 1996).

Several underlying causes may explain the subsequent drop of Sr/Ca until the transition zone of metamorphosis (OP2) was reached. In marine species, evidence has been accumulated which closely links Sr/Ca ratios and individual metabolism. Inverse relationships were reported between Sr/Ca and somatic growth rate (Sadovy and Severin, 1994; Friedland et al., 1998), suggesting that less strontium may be incorporated into the otolith during periods of higher otolith protein synthesis and higher accretion rate. In common sole larvae, both the somatic and otolith growth are coupled and exponential (Lagarðere and Troade, 1997), which is consistent with the observed decrease of Sr/Ca. However, the drop in Sr/Ca ratio is particularly abrupt and other processes may be involved, such as the setting of functional mechanisms in the larva’s inner ear (e.g. metal-binding proteins synthesis), responsible for the regulation of the free
metal content in either plasma or endolymph or both systems (Kalish, 1989).

Other species undergo dramatic metamorphosis and it has been shown, for instance, that the onset of metamorphosis from leptocephalus to glass eel induces a rapid drop in otolith Sr/Ca ratios, coinciding with a rapid increase in increment width in several eel species (Arai et al., 1997, 2000). Such drastic changes are believed not to be related to habitat changes.

Otake et al. (1994, 1997) associated the decrease of otolith Sr/Ca ratio with that of the body Sr content, explained by the breakdown of the amount of the gelatinous extracellular matrix composed of sulfated glycoaminoglycans (GAGs), which are known to have a high affinity for Sr. Similar physiological processes could have caused the abrupt changes in sole otolith Sr/Ca.

4.3. Change in habitat: from pelagic to benthic life

Few data are available regarding the relationships between otolith microchemistry and early-life history events in flatfish. Toole et al. (1993) reported Sr/Ca chronologies in Dover sole (Microstomus pacificus) otoliths, which are surprisingly similar in their fluctuation ranges to those measured in this study, although each species strongly differs in their ontogenetic transformations and habitats. In the Bay of Biscay common sole, spawning and early life stages progress from the mid-shelf to inshore areas, whereas the whole cycle of the Pacific Dover sole takes place offshore (Markle et al., 1992). In the latter species a strong Sr/Ca peak also occurred in a ‘clear central area’ surrounding the core and covering the early developmental stage up to the completion of eye migration (Toole et al., 1993). A decline was then observed in a wide peripheral opaque zone and AGC area. This decline took place during a very protracted metamorphosis (around 4 months) and was related to otolith growth rather than somatic growth, both parameters being uncoupled during this developmental stage. The Sr/Ca ratio levelled at the end of metamorphosis and Sr/Ca remained low in the otolith portion corresponding to juvenile settlement in offshore nurseries. Low Sr/Ca levels were here associated with high growth rate and low temperature, suggesting that growth rate overrode temperature as a predictor of Sr/Ca.

In the Bay of Biscay sole, metamorphosis needs about 8 d in reared individuals and at least 10 d in wild individuals. Metamorphosing larvae do not fast and there is no disruption of somatic and otolith growth (Amara et al., 2000). Despite some discrepancies in the early Sr/Ca chronologies in reared and wild samples, one important finding was the occurrence of a plateau during metamorphosis (OP3), at a similar level whatever the origin of the sample. If temperatures had been similar in the mesocosm and the field, the comparison of the experimental and field data could have suggested that wild fish still experienced marine or coastal-water conditions during late metamorphosis (OP3), either offshore or in coastal areas. This was not the case and mesocosm temperatures were much higher than in the field. This may suggest that high metabolic rate during metamorphosis overrides other processes, such as habitat change of transforming larvae, as observed for the Dover sole (Toole et al., 1993). As a consequence, our results do not permit us to state whether larvae shift to benthic life at random, whatever the distance from the coast (hypothesis of Koutsikopoulos et al. (1991)), or whether they concentrate in coastal areas before settlement (hypothesis of Marchand (1991) and Amara et al. (1993)). According to the former authors, the progeny settling offshore is lost for recruitment, whereas the alternative hypothesis is supported by the latest studies (Amara et al., 1998; Lagardère et al., 1999). More work is needed to investigate possible locations of the shift to bottom-dwelling life.

4.4. Environmental effects on otolith: Sr/Ca ratios and estuarine immigration

The field studies described above, confirmed by laboratory validations, have established a clear relationship between ambient salinity and otolith Sr/Ca. However, the relationship between Sr uptake and salinity is not linear (Secor et al., 1995) and otolith Sr/Ca ratios reflect the ambient Sr/Ca ratios rather than salinity per se or absolute Sr water concentration. Studies that manipulated salinity over a narrow range of variations failed to establish a clear relationship between otolith Sr/Ca and ambient salinity (Fowler et al., 1995a,b). Accordingly, only salinity regimes that differ substantially are expected to be
detected by otolith Sr/Ca ratios. The Loire and Vilaine watersheds respond well to this requirement, with very different hydrological characteristics along with specific topology and geology. For instance, river flows averaged from January to June 1993 were 522 m³ s⁻¹ and 67 m³ s⁻¹ for the Loire and Vilaine, respectively (HYDRO database, French Department of the Environment). A dam in the latter system limits upstream migration, whereas in the Loire, sole nurseries can extend to low salinity areas, provided that oxygen requirements are fulfilled (Marchand et al., 1996). Otolith Sr/Ca ratios differed significantly between juveniles collected in each river system for the otolith zone (OP4) deposited after the shift to benthic life in a way that was consistent with the expected scheme. The averaged Loire Sr/Ca profile showed a progressive decrease of the Sr/Ca ratio, which could indicate that, in the Loire system, upstream migration was progressive. This result corroborates Marchand’s (1988) observations in the Loire estuary. In this estuary, recently metamorphosed sole first migrate upstream to the polyhaline waters where there is high food density, and later they migrate progressively to the meso-polyhaline areas. From a metaanalysis, based on the literature data for freshwater and marine species, Campana (1999) deduced that ‘each 1 increase in salinity would be expected to produce a 0.05 10⁻³ increase in the otolith Sr/Ca molar ratio’. Taking the salinity of rearing conditions as reference, we estimated from this equation the salinity of the relative habitats of the wild 0-group juveniles from Sr/Ca ratios measured on otolith position OP4 (Table 4). Although the obtained estimates lie in plausible salinity ranges for both Vilaine and Loire, there is no means to assess their accuracy. Detailed salinity data for both environments are unavailable for the time period considered. Moreover, as mentioned above, ambient Sr/Ca ratios are far from being the unique factor involved in otolith Sr/Ca uptake and, at this time, there is no general model allowing precise inferences on habitat characteristics from otolith Sr/Ca measurements. Nevertheless, our results clearly indicate that nurseries located in areas under marine influence (here the Bay of Vilaine) should be distinguished, at least in the early juvenile period, from those located in areas under continental-water influence (here the Loire estuary). In the Bay of Biscay, this type of nursery area is very rare (mainly the Loire and Gironde estuarine nurseries), and fish using these areas may be distinguished based on otolith elemental fingerprints (De Pontual et al., 2000). These nurseries are believed to make major contributions to the adult stock of the Bay of Biscay. This hypothesis has been recently re-examined by Le Pape et al. (2003) and could be checked using the otolith chemistry of early juvenile soles.

Acknowledgements

We thank Bari Howell (Conwy Laboratory) for providing us with the batch of sole eggs, and Vincent Buchet and Hubert Palvadeau (Aqualive Station) for their expertise during the mesocosm experiment. We also thank two anonymous referees for their helpful comments on this paper.

References


Table 4
Salinity of the wild juvenile habitats estimated from Sr/Ca measured on otolith site OP4, taking rearing conditions as reference

<table>
<thead>
<tr>
<th>Origin</th>
<th>Sr/Ca</th>
<th>Molar Sr/Ca</th>
<th>Estimated salinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reared</td>
<td>0.0037</td>
<td>0.0017</td>
<td>33 (reference)</td>
</tr>
<tr>
<td>Loire</td>
<td>0.0026</td>
<td>0.0012</td>
<td>23</td>
</tr>
<tr>
<td>Vilaine</td>
<td>0.0035</td>
<td>0.0016</td>
<td>31.6</td>
</tr>
</tbody>
</table>

Estimates are derived according to the hypothesis that an increase of 0.05 10⁻³ in the otolith Sr/Ca molar ratio would reflect an increase of 1 in salinity (Campana, 1999).


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