Response of juvenile sole (*Solea solea* (L.)) to environmental changes investigated by otolith microstructure analysis

Constantin Koutsikopoulos, Maria Karakiri, Yves Desaunay, and Didier Dorel

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Otolith microstructure analysis of sole juveniles, *Solea solea* (L.) (0–2 groups) sampled in the Bay of Vilaine, Bay of Biscay, was carried out using scanning electron microscopy (SEM). Otoliths of sole juveniles used in SEM analyses were randomly sampled from the median length classes of the extensive samples of a seven-year survey from 1981 to 1987. Daily increment analysis showed in two cases (1982 and 1986) growth retardation, recorded as narrow increment deposition during the first weeks after transition to the bottom-dwelling mode of life. It is assumed that the observed growth retardation of 0-group sole in the summer of 1982 might have been caused by the oxygen deficiency recorded in the Bay of Vilaine at that time. The same observation in otolith microstructure during late spring 1986 might be associated with a strong cold freshwater discharge recorded in the sampling area that affected both water salinity and temperature.

Constantin Koutsikopoulos, Yves Desaunay, and Didier Dorel. IFREMER, Centre de Nantes, B.P. 1049, 44037 Nantes Cedex, France. Maria Karakiri: Biologische Anstalt Helgoland, Notkestr. 31, 2000 Hamburg 52, Federal Republic of Germany.

Introduction

Otolith increment analysis has often been used in recent years for age and growth studies of early stages of fish. Daily growth patterns have been studied for various fish species under laboratory conditions or in the field (Brothers *et al.*, 1976; Pannella, 1980; Campana and Neilson, 1982; Geffen, 1982; Victor, 1982; Jenkins, 1987). For sole *Solea solea* (L.), daily patterns of otolith growth have been analysed by means of light microscopy on larval otoliths under laboratory conditions and in the field (Lagardère and Chaumillon, 1988).

In the present study otolith microstructure of sole juveniles was analysed by scanning electron microscopy (SEM). Juveniles were sampled in the Bay of Vilaine to study the ecology of early stage sole (Desaunay *et al.*, 1987; Marchand and Masson, 1989). The main objective of otolith analysis was to demonstrate the suitability of the method in detecting growth events by increment readings and to correlate these with environmental events during the growth period of the individual.

Material and methods

Sole juveniles (0-2 groups), were sampled during a seven-year survey from 1981 to 1987 in the Bay of

Vilaine, Bay of Biscay (Fig. 1). Two surveys were carried out each year (summer/autumn) using a 3 m wide beam trawl (20 mm stretched inside mesh net, mean duration 20 min). The sampling area covered the major part of the nursery ground.

From this material, specimens of the median length class for October 1982, November 1983, June 1984, and May 1987 were randomly taken for otolith analysis. Sixty-eight sagitta otoliths were prepared and examined by SEM. Whenever the preparation quality was poor, otoliths were eliminated from further analysis. Thus a total of 55 sagittae were considered in the final analysis. Since both left and right sagittae proved to be identical regarding their microstructure, only one specimen was analysed further.

Otoliths were attached on SEM stubs and ground to the midplane using a rotating glass plate with silicon carbide as an abrasive (details in Karakiri and von Westernhagen, 1988). After grinding, otoliths were etched using 0.1 M EDTA, pH 7.4 for 4–6 min, according to size, dried in an oven (24 h, 60°C), coated with gold and viewed in SEM.

Increment analysis was carried out from SEM photographs. The mean width and standard deviation of the increments deposited during the first months of the bottom-living stage were estimated.



Figure 1. Research area. The arrows indicate position and direction of trawls of a typical survey in the Bay of Vilaine.

Results

The sagittae of sole juveniles showed the typical flatfish clustered pattern: (P) primordium, the calcification nucleus formed during the late embryonal stages, the relatively narrow larval increments, the accessory primordia, four to six, denoting the transition to the bottom-dwelling mode of life and the later deposition of wide increments (Fig. 2a, b, c). Otolith increment measurements were made along axis A, situated 35° from the posterior radius along the sulcus. During the larval stage increment width ranged from 0.4 to 1.2 μ m.

Width analysis of otolith increments deposited during the bottom-living stage revealed in two cases unusually low values (Fig. 3). Extremely narrow increments ranging from 1.7 to 4.2 μ m were recorded in July 1982 at the bottom-living stage in otoliths of 0-group sole, sampled in October 1982. The daily increments deposited after this period (in August 1982) ranged in width from 3.0 to 9.3 μ m. In contrast to this, no extremely narrow increments were recorded in otoliths of 1-group sole sampled on the same date at the same location or in other groups examined, from samplings in November 1983 and June 1984 (Table 1).

Extremely narrow increments were also recorded during the first three to four weeks after the transition to the bottom-living stage in May 1986, in otoliths of 1group sole sampled in May 1987. No unusual minimal widths were, however, recorded in otoliths of 2-group sole sampled on the same date. The mean increment width and the standard deviation during May 1986, at the bottom-living stage, was estimated and compared with the mean width of the increments deposited during the following four weeks in June 1986 (Table 1).

Discussion

Sagittae of sole juveniles showed the typical flatfish clustered pattern described for other flatfish-species like English sole *Parophrys vetulus* (Rosenberg, 1982),



Figure 2. Otolith microstructure of a sole juvenile in SEM: a: sagitta ground down to the midplane, P = primordium, A = axis along which increment width measurements were carried out; b: higher magnification of daily increments deposited after the transition to the bottom-living stage, DI = daily increment; c: higher magnification of an area (R) showing growth retardation (increment width $<3 \mu$ m).



Figure 3. Mean increment width of extreme narrow increments (summer 1982 and late spring 1986) and normal wide increments deposited after transition to the bottom-dwelling mode of life.

starry flounder *Platichthys stellatus* (Campana, 1984) and plaice *Pleuronectes platessa* (Karakiri *et al.*, 1989). Increment width measurements were carried out along a standard axis from the edge of the otolith (capture date) to the nucleus. Employing SEM analysis no repetitive counts were necessary, since preparation quality allowed clear discrimination of increments while light artifacts or difficulties in distinction among daily and subdaily increments were excluded.

The anticipated daily periodicity of increment deposition in sole otoliths was not validated in laboratory or field experiments. However, as stated in recent review work (Campana and Neilson, 1985), daily increment deposition is considered a common phenomenon for all fish species and thus we are convinced that the increment observed in sole otoliths were of a daily nature.

Width measurements of increments deposited during the larval phase, as well as of those deposited in the months after transition to the bottom-dwelling mode of life, showed a trend of increasing width with age. After the first year of life there was, in contrast, a tendency for relatively thin increment deposition, These observations are consistent with the fact that growth rates are higher in younger stages. Radtke and Dean (1982) described the same effects for mummichog *Fundulus heteroclitus* otoliths.

While the number of otolith increments is strictly agedependent, the increment width is growth-dependent, reflecting closely the somatic growth (Karakiri and von Westernhagen, 1989). Thus, analysis of increment width variations can provide a picture of growth events, especially during the early life of fish. In our study, the presence of extremely narrow increments shortly after transition to the bottom-living stage, in 1982 and 1986, indicates growth retardation, or even growth cessation, during that period. For larval sole *Solea solea* it was

Table 1. Daily otolith increment analysis of sole juveniles from surveys in the Bay of Vilaine. Mean width of 30 increments, A: mean width of the narrower increments deposited in 0- and 1-group otoliths in the period from May to September, and B: mean width of the following 30 increments in each case. * deposition of extremely narrow increments in summer of 1982 and in late spring of 1986.

Sampling date	Age group	N	Mean increment width (µm) (A)	s.d.	Mean increment width (µm) (B)	s.d.
Oct 1982	0	8	2.68*	0.47	7.30	0.62
Oct 1982	1	8	7.92	0.58	8.47	0.45
Nov 1983	Ô	12	7.91	0.62	8.63	0.47
Nov 1983	1	8	7.33	0.45	7.85	0.57
June 1984	î	8	6.96	1.98	8.18	0.43
May 1987	î	7	3.09*	0.56	6.83	0.73
May 1987	2	4	7.55	0.33	8.60	0.47



Figure 4. Physical parameters which probably led to oxygen depletion in July of 1982 in the Bay of Vilaine (from Merceron, 1987).

experimentally shown that hyaline bands consisting of low contrast increments were formed in fish subjected to food deprivation when growth was retarded (Lagardère and Chaumillon, 1988). Other experimental studies on factors influencing fish growth and otolith increments showed short-time starvation effects in otoliths of *Coregonus* spp. (Eckmann and Rey, 1987) as well as feeding frequency, photoperiod and temperature effects in otoliths of chinook salmon *Oncorhynchus tschawytsha* (Neilson and Geen, 1982).

The above-described lifespan of young sole with extremely narrow increments coincided with a pronounced oxygen deficiency in the Bay of Vilaine in July 1982 (Fig. 4). The oxygen deficiency had obviously affected the benthic fauna of the area, leading to limited food supply for newly-settled sole and growth retardation. Growth retardation may have also been caused directly due to a cessation of feeding activity at low oxygen concentrations. Similar growth retardation, reflected in narrow increment deposition during late summer 1981, was detected in otoliths of juvenile dabs Limanda limanda in the Kiel Bight (Karakiri and Temming, 198). In this case oxygen deficiency in the Kiel Bight caused complete disappearance of the benthic organisms (Weigelt, 1987). Unfortunately, the feeding conditions in the Bay of Vilaine during and after the oxygen deficiency are not known. It is therefore assumed that oxygen deficiency influenced the growth rate on the one hand indirectly through food limitation and on the other hand directly through a depression of activity.

In the late spring of 1986 when narrow increments were again deposited in otoliths of sole juveniles shortly



Figure 5. Water density variations and abundance of early stages of sole at the entrance of the Vilaine estuary in spring 1986 (from Marchand and Masson, 1989).

after the transition to the bottom-living stage, a heavy discharge of cold freshwater was recorded in the nursery ground. This modified the circulation of the water masses, led to lower temperatures and negatively affected the abundance of early stage sole in the Bay of Vilaine (Fig. 5 from Marchand and Masson, 1989). Since temperature is accepted as a major factor influencing fish growth and otolith microstructure, it is assumed that it was the low temperature that led to growth retardation of 0-group sole.

It is remarkable that during both events, in 1982 and 1986, narrow increments were only detected in otoliths of 0-group sole. This observation suggests that either the 0-group is more strongly affected by environmental changes than the 1- and 2-group sole or that older groups, being distributed over wide areas, may more easily evade unfavourable conditions by emigration.

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