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Comparison of 0- and 2-group otolith elemental signatures to discriminate *Solea solea* nurseries in the bay of biscay

Eric D.H. Durieux [a*], Maylis Labonne [b], Marie-Laure Bégout [c] & Hélène de Pontual [d]

a-Université de Corse Pasquale Paoli, UMR CNRS-UDC 6134 Sciences Pour l'Environnement, BP 52, 20250 Corté, France
b-LEMAR, UMR 6539 IRD/CNRS/UBO, Centre IRD de Bretagne, BP 70, 29280 Plouzané, France
c-Ifremer, Place du Séminaire, BP 5 - 17137 L'Houmeau, France
d-Ifremer, Département Sciences et Technologies Halieutiques, BP 70, 29280 Plouzané, France

***Email contact: durieux@univ-corse.fr**

Introduction

The common sole *Solea solea* (L.) is a commercially important and widely distributed flatfish of the North-East Atlantic. For the Bay of Biscay stock, sole nurseries display differences in terms of quantity and quality. *In fine* the measure of the quality of a habitat for juveniles of a particular species is expressed by the contribution to the recruitment into the adult population. Understanding this connectivity between juvenile and adult habitats, *i.e.* evaluating the contribution of each nursery to a single adult stock appears essential in terms of stock management. However, this critical link is still missing for the Bay of Biscay sole stock.

Determining if nurseries could be discriminated using otolith elemental composition analysis constitutes the first step in evaluating the relative contribution of these nurseries into the adult stock. The aim of this study was to compare otolith elemental signatures of sole juveniles from two different ages (0-group and 2-group) captured at the same time in the six main nurseries of Bay of Biscay along the French Atlantic coast.

Material and methods

Originally these G0 and G2 juveniles samples comes from two different studies : G0 were sampled in order to analyze adults otoliths (G3+) from the same cohort using LA-ICPMS, whereas G2 juveniles were sampled with the objective of analyzing G3 (first reproduction) otoliths of the same cohort using SB-ICP-MS. Here we took advantage of these two separate samples / analysis for a comparison exercise.

Fish collection and otolith preparation

0-group and 2-group sole juveniles were collected in 6 nurseries (embayed and estuarine) as part of the IFREMER sole nursery survey in the Bay of Biscay (Table 1; Fig. 1) during September/October 2003 using a beam trawl at 5-20 m depths. Fish were immediately frozen and stored individually at –20°C. Subsequently fish were measured, weighed after which the sagittal otoliths were extracted, thoroughly cleaned of adhering tissue and then individually stored in plastic tubes. Only left otoliths were selected for multi-elemental analysis. Otoliths were cleaned and decontaminated under a laminar flow positive pressure fume hood by: (1) immersion in ultrapure water, (2) immersion in 0.1% HNO3 for 1 min (induced less than 2 % loss in otolith weight), (3) double immersion in ultrapure water, (4) air dried for 24 hours and (5) stored in decontaminated polypropylene tubes.

Sample analysis

Multi-elemental composition was determined in whole otoliths using solution-based inductively coupled plasma mass spectrometry (SB-ICP-MS, Thermo Element 2). The preparation for analysis was performed in a class 100 clean room.
Otoliths were dissolved in 15N HNO3 and diluted proportionally to otolith mass (x1500) in ultrapure water. The following elements were selected and quantified in Low or Medium resolution as follow: Li, Ga, Rb, Y, Ba, Na, Mg, Mn and Sr. To ensure precision and accuracy the NIES 22 otolith standard was measured at the beginning, the middle and the end of each session.

Data analysis

Data were mean centred. Differences between estuaries were analyzed for each element using ANOVA. Otolith multi-elemental compositions were compared using MANOVA. Linear discriminant function analysis (LDFA) was used to classify individuals to the different sampling sites. The individuals from each site were represented in the first two factors plane of the LDFA for illustrative purposes. The functions generated were then used to classify fish according to nurseries. A cross-validation using jackknife method was applied to determine the classification accuracy.

Table 1. Site code, average fish size (TL), average fish mass (W), mean otolith (left) mass (Wo) and sample size (n) for 0-group and 2-group sole juveniles sampled in the Bay of Biscay

	Site	TL ± SD (mm)	W ± SD (g)	Wo ± SD (mg)	n
G0	1	121.7 ± 13.3	13.2 ± 4.2	2.8 ± 0.4	20
	2	123.2 ± 13.1	14.5 ± 5.3	2.7 ± 0.6	20
	3	126 ± 11.7	14.7 ± 4.6	3.1 ± 0.5	21
	4	121.5 ± 9.4	13.6 ± 3.5	2.8 ± 0.3	10
	5	109.6 ± 7.5	9.3 ± 2.1	2.5 ± 0.3	20
	6	102.9 ± 10.1	8.1 ± 2.6	1.9 ± 0.4	28
G2	1	243.8 ± 18.6	143.2 ± 39.3	12.3 ± 2.9	52
	2	234.1 ± 13.6	124.8 ± 26	11.3 ± 1.8	31
	3	233.7 ± 11.5	112.9 ± 20.2	11.3 ± 1.9	52
	4	224.8 ± 11.7	102.8 ± 19.8	10.7 ± 1.1	49
	5	235.9 ± 11.7	119.9 ± 22	12.1 ± 1.7	46
	6	252.4 ± 19.5	151.7 ± 45.5	14.3 ± 2.7	50

Fig. 1. Locations of the 6 main Solea solea nurseries sampled along the French Atlantic coast off the Bay of Biscay (Sites 1 to 6).

Table 2. Mean ± SD elemental concentrations of otolith in ppm for 0-group and 2-group sole juveniles sampled in the Bay of Biscay

	G0 Concentration (ppm)	G2 Concentration (ppm)
Li	0.37 ± 0.06	0.31 ± 0.03
Ga	0.05 ± 0.02	0.05 ± 0.02
Rb	0.09 ± 0.02	0.04 ± 0.01
Y	0.01 ± 0	0.01 ± 0
Ba	1.16 ± 0.41	1 ± 0.31
Na	2951.82 ± 207.67	2865.03 ± 173.51
Mg	20.25 ± 2.71	14.52 ± 2.85
Mn	10.49 ± 4.44	5.94 ± 2.15
Sr	2057.3 ± 222.06	2030.85 ± 203.53

Table 3. Results of ANOVAs comparison for individual element (mean centered) collected 6 different nurseries.

	G0 F	G0 P	G2 F	G2 P
Li	21.231	***	9.669	***
Ga	14.987	***	10.664	***
Rb	7.855	***	8.956	***
Y	1.46	ns	20.284	***
Ba	11.82	***	9.421	***
Na	10.07	***	1.278	ns
Mg	2.247	ns (=0.05)	29.507	***
Mn	22.047	***	15.089	***
Sr	10.789	***	10.573	***

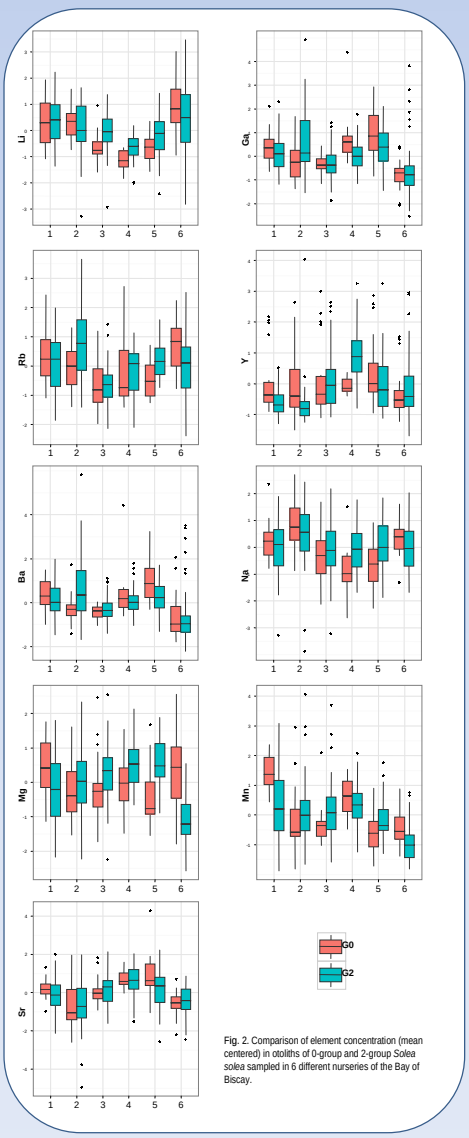

Fig. 2. Comparison of element concentration (mean centered) in otoliths of 0-group and 2-group *Solea solea* sampled in 6 different nurseries of the Bay of Biscay.

Results

Mean otolith element concentrations were similar between G0 and G2 for the measured elements except for Rb, Mg and Mn that was lower in G2 (Table 2). Mean centred element concentration displayed spatial patterns relatively consistent between G0 and G2 with significant difference between sites except Y and Mg (for G0) and Na for G2 (Table 3; Fig. 2). Some sites showed however clear difference in element concentrations between G0 and G2: for instance site 3 (Li), site 2 (Ga and Ba), site 4 (Y), site 5 and 6 (Mg). Multi-elemental composition was significantly different between sampling sites for both G0 (MANOVA, F = 8.496, P < 0.0001) and G2 (MANOVA, F = 14.305, P < 0.0001). The LDFA correctly classified 69 % of G0 and 60 % of G2 individuals to their respective nursery of origin (Fig 3 and 4). Best reclassification scores were obtained for Bay of Bourgneuf (site 3), Pertuis d'Antioche (site 5) and Gironde (site 6) for G0 and Pertuis Breton (site 4) and Gironde (site 6) for G2. Individuals from Loire estuary (site 2) were poorly reclassified especially for G2 individuals.

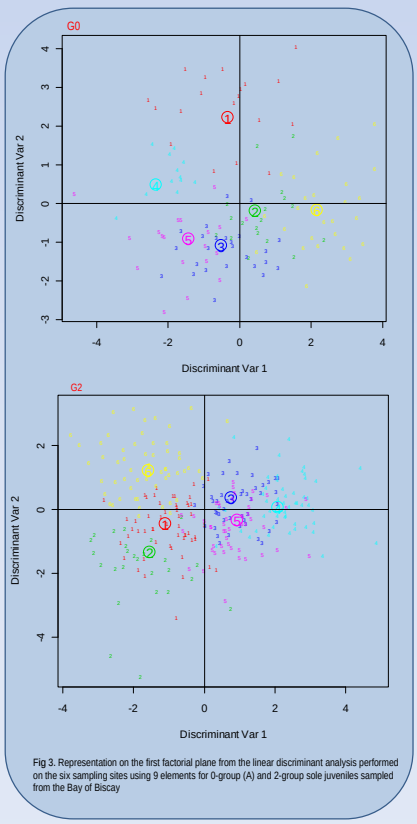

Fig 3. Representation on the first factorial plane from the linear discriminant analysis performed on the six sampling sites using 9 elements for 0-group (A) and 2-group sole juveniles sampled from the Bay of Biscay

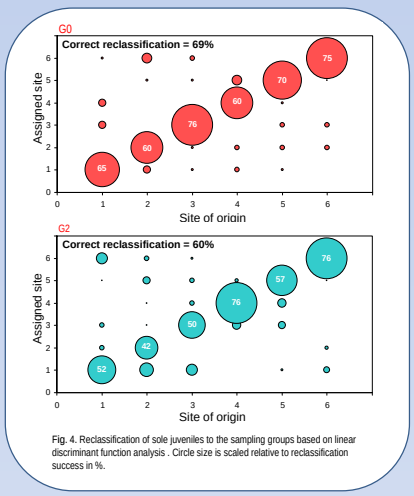

Fig. 4. Reclassification of sole juveniles to the sampling groups based on linear discriminant function analysis . Circle size is scaled relative to reclassification success in %.

Discussion - conclusion

0-group and 2-group sole from the six main nurseries of the Bay of Biscay displayed multi-elemental signatures that allowed to correctly reclassify the individuals to their nursery of origin with 69% and 60% correct reclassification for G0 and G2 respectively. These results confirm previous studies performed on *Solea solea* G0 juveniles: in the Bay of Biscay (De Pontual et al. 2000) on the Portuguese coast (Vasconcelos et al. 2007) and in the North Sea (Cuveliers et al. 2010) using similar approaches.

Although relatively consistent multi-elemental patterns between G0 and G2 could be identified, some sites showed clear difference in element concentrations between G0 and G2. These differences might be due to both interannual variability in otoliths signatures and the potential movements of juveniles between sites. We also need to consider that fish integrate elements into otoliths differentially with age. Finally, analyzing G2 otoliths multi-elemental signatures implies to perform SB-ICP-MS only on G3 for further analysis on adults. These different points are serious limitations for the use of G2 juveniles otoliths for further identification of adults nurseries origin.

In conclusion, this study provides otolith elemental signatures of 0-group and 2-group sole *Solea solea* in the Bay of Biscay with higher discrimination power and less limitations for G0 otoliths. Otolith elemental signatures of 0-group sole can subsequently be used to determine the relative contribution of the different nurseries to the adult stock using Laser Ablation ICP-MS (LA-ICP-MS). In the future, within nurseries interannual and spatial variability of otolith signatures should be identified in order assess more precisely the origin of adult fish.

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