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Eric D.H. Durieux[1], Maylis Labonne[2], Marie-Laure Bégout[3], Claire Bassoulet[4] & Hélène de Pontual[2]

[1] University of California Davis, One Shields Avenue, Davis, CA 95616, USA; [2] Laboratoire de Sclérochronologie des Animaux Aquatiques, IFREMER - IRD, BP 70, 29280 Plouzané, France; [3] IFREMER, Place du Séminaire, BP 5 - 17137 L'Houmeau, France; [4] Institut Universitaire Européen de la Mer, Place Nicolas Copernic, 29280 Plouzané, France

Introduction

The common sole *Solea solea* (L.) is a commercially important and widely distributed flatfish of the North-East Atlantic. Like most demersal marine fishes around the world, sole stocks suffer from overexploitation. For the Bay of Biscay stock, sole nurseries display differences in terms of quantity (Le Pape *et al.* 2003) and quality (Gilliers et *al.* 2003). *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 (Beck et *al.* 2001). 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.

Otoliths are calcium carbonate structures located in the inner ear of fish. Throughout the life of fish, otoliths grow continuously through accretion forming easily identifiable daily, seasonal and annual marks. During this process, they incorporate chemical elements that indirectly reflect the ambient conditions (e.g. temperature, chemical composition of the water) experienced by the fish. Due to these unique properties, otolith elemental composition analysis has become a powerful tool to determine the nursery origin in adult fish, to discriminate between stocks and sub-populations, and to reconstruct lifetime migration patterns (Campana & Thorrold 2001).

The aim of the present study was to determine if the main sole nurseries along the French Atlantic coast could be discriminated using otolith elemental composition analysis of age 0-group juveniles. This study constitutes the first step in evaluating the relative contribution of the different nurseries into the adult stock of the Bay of Biscay.

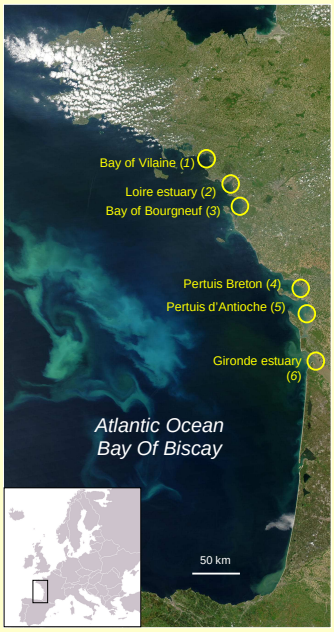


Fig. 1. *Locations of the 6 main* Solea solea *nurseries sampled along the French Atlantic coast off the Bay of Biscay (Sites 1 to 6).*

Material and methods

Fish collection and otolith preparation

0-group sole juveniles were sampled in 6 nurseries (embayed and estuarine) as part of the IFREMER sole nursery survey in the Bay of Biscay (***Fig. 1***) during September/October 2003 using a beam trawl (2.9 m wide and 0.5 m high, mounted with a 20 mm stretched mesh net at the codend) at 5-20 m depths. Fish were immediately frozen and stored individually at –20°C. Subsequently fish were measured (nearest mm), weighed (nearest 0.01 g) (***Table 1***) 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 (n=119) 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.

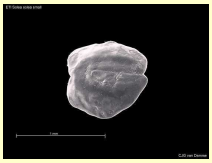
Sample analysis

Multi-elemental composition was determined in whole otoliths using solution-based inductively coupled plasma mass spectrometry (ICP-MS, Thermo Element 2). The preparation for analysis was performed in a class 100 (ISO class 5) clean room.

Decontaminated otoliths were weighed on a precision scale to the nearest 0.1 µg (***Table 1***). Otoliths were dissolved in 15N HNO3 and diluted proportionally to otolith mass (x1500) in ultrapure water overnight. The following elements were quantified: ^{7}Li, ^{23}Na, ^{25}Mg, ^{43}Ca, ^{55}Mn, ^{88}Sr, ^{63}Cu, ^{66}Ca, ^{85}Rb and ^{138}Ba. To ensure precision and accuracy between sessions, Thulium was used as an internal standard to correct for instrumental drift during analytical sessions. Otolith samples were read sequentially in random sets of 3; a multi-element laboratory standard was analyzed between each sample sets; the NIES 22 otolith standard was measured at the beginning, the middle and the end of each session; blanks were measured at the start and at the end of each session. The limit of detection (mean of 10 measurements of the procedural blank + 3 x SD in ppb) were: Li = 0.03, Na = 23.63, Mg = 0.91, Ca = 145.58, Mn = 0.04, Sr = 0.06, Cu = 0.14, Ga = 0.07, Rb = 0.01, Ba = 0.02. No samples were below these limits of detection. Differences between our analyses and the NIES standard certified concentrations were 4%, 2%, 1%, 7% and 7% for, Na, Mg, Cu, Sr and Ba respectively.

Data analysis

Data were transformed to ratio elements to Ca (element:Ca) and then Ln transformed in order to reach normality and equality of variances. Differences between estuaries were analyzed for each element:Ca ratio using ANOVA. In order to remove any otolith effect, the relationship between otolith mass and each element:Ca ratio was analyzed using linear regression. When the regression was significant the residuals were used instead of the original transformed element:Ca ratios in the subsequent analysis. 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 and then each individual was assigned *a posteriori* to a site of origin which was compared with the real site.



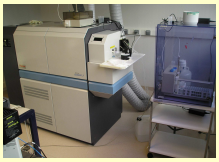


Table 1. *Site code, river flow, average fish size (L_T), average fish mass (M_W), mean otolith (left) mass (M_O) and sample size (n)*

Site code	River flow (m^3 s^{-1})	L_T ± SE (mm)	M_W ± SE (g)	M_O ± SE (mg)	*n*
1	72	121.7 ± 3.0	13.2 ± 0.9	2.8 ± 0.1	20
2	855	123.9 ± 3.0	14.8 ± 1.2	2.7 ± 0.1	19
3	5	126.0 ± 2.5	14.7 ± 1.0	3.1 ± 0.1	21
4	10	121.1 ± 3.9	13.7 ± 1.5	2.7 ± 0.1	10
5	58	110.5 ± 1.6	9.5 ± 0.5	2.6 ± 0.1	22
6	1000	102.7 ± 2.0	8.0 ± 0.5	1.9 ± 0.1	27

Results

Otolith element concentrations showed different patterns (***Fig. 2***) and were significantly different between the different sites (nurseries) in terms of element:Ca ratio (***Table 2***). Element:Ca ratios were significantly correlated to otolith weight (***Fig. 3***). Multi-elemental composition was significantly different between sampling sites (MANOVA, F = 5.853, P < 0.0001). The LDFA correctly classified 68.1 % of the individuals to their respective nursery of origin (***Table 3***). Best reclassification scores were obtained for embayed nurseries compared to estuarine nurseries. Individuals from Gironde estuary (site 6) were relatively poorly reclassified.

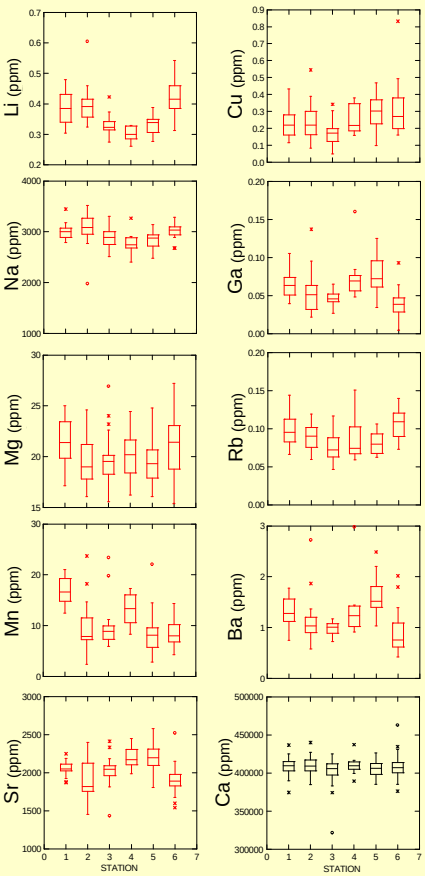


Fig. 2. *Average element concentration (in ppm) in otoliths of 0-group* Solea solea *sampled in 6 different nurseries of the Bay of Biscay.*

Table 2. Results of ANOVAs comparison individual element:Ca ratios in collected 6 different nurseries.

Element	Fisher's F	*P*
Li:Ca	14.767	< 0.0001***
Na:Ca	6.963	< 0.0001***
Mg:Ca	2.045	0.078
Mn:Ca	15.664	< 0.0001***
Sr:Ca	13.770	< 0.0001***
Cu:Ca	5.439	< 0.0002***
Ga:Ca	10.259	< 0.0001***
Rb:Ca	7.126	< 0.0001***
Ba:Ca	10.284	< 0.0001***

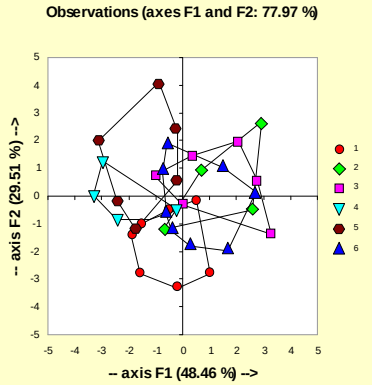


Fig. 4. *Convex hulls representation on the first factorial plane from the linear discriminant analysis performed on the six sampling sites using 9 elements (element:Ca used, corrected for otolith mass).*

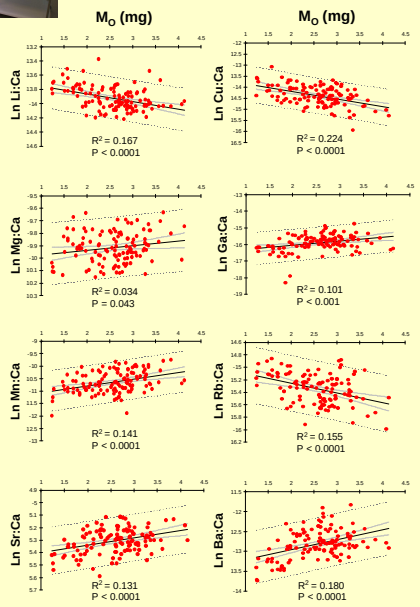


Fig. 3. *Significant linear regression between Ln transformed element:Ca ratios and otolith mass (M_O).*

Table 3. *Cross-validated classification results (%) of linear* discriminant *function analysis of juveniles to the different nurseries sampled. Bold % represent % of individuals correctly classified to their nursery of origin.*

Nursery	Predicted nursery 1	2	3	4	5	6
1	**65.0%**	0	0	10.0%	0	25.0%
2	10.5%	**63.2%**	10.5%	0	0	15.8%
3	0	4.8%	**71.4%**	4.8%	4.8%	14.3%
4	0	0	0	**80.0%**	10.0%	10.0%
5	4.5%	4.5%	0	4.5%	**81.8%**	4.5%
6	14.8%	11.1%	14.8%	0	3.7%	**55.6%**

68.1% of all individuals correctly classified to their nursery of origin

Summary of findings

Otolith elemental signatures can be used to distinguish the six main nurseries of *Solea solea* in the Bay of Biscay.

Discussion-Conclusion

0-group sole from the six main nurseries of the Bay of Biscay displayed different multi-elemental signatures that allowed to correctly reclassify 68.1% of the individuals. These results confirm previous studies performed on *Solea solea*: in the Bay of Biscay (De Pontual *et al.* 2000) and on the Portuguese coast (Vasconcelos *et al.* 2007) using a similar approach.

In conclusion, this study provides otolith elemental signatures of 0-group sole *Solea solea* in the Bay of Biscay. This information can now be used to determine the relative contribution of the different nurseries to the adult stock (of the same cohort) using Laser Ablation ICP-MS (LA-ICP-MS). This overall approach might provide useful information in terms of fisheries and coastal habitat management.

In the future, improvement in otolith elemental signatures of this flatfish species might be obtained by:
- Measuring additional trace elements;
- Analyzing the isotopic composition of specific elements such as Sr, S or O;
- Taking into account the within habitat small-spatial scale variation.

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References
Beck et al. 2001. Bioscience 51: 633-64; ***Campana & Thorrold 2001.*** Canandian Journal of Fisheries and Aquatic Sciences 58: 30-38; ***De Pontual et al. 2000.*** Oceanologica Acta 23: 497-513; ***Gilliers et al. 2006.*** Scientia marina 70: 183-192; ***Le Pape et al. 2003.*** Journal of Sea Research 50: 139-149; ***Vasconcelos et al. 2007.*** Marine ecology progress series 350: 117-126.

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