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Discrimination of *Solea solea* nurseries along the French Atlantic coast using otolith elemental signatures

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**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 sole, stock distributions differ in terms of quantity (Le Pape et al. 2003) and quality (Gilliers et al. 2000). Among the measures of the quality of a habitat for juveniles of a particular species is exposed 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 distinguishable 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 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.

**Material and methods**

**Fish collection and otolith preparation**

A total of 857 sole were sampled as part of a representative and exhaustive part of the IFREMER sole nursery survey in the Bay of Biscay (Fig. 1) during September October 2010 using a beach seine (39° 40’ N, 4° 40’ W). Juveniles of 0-group were retained for this study. Otoliths were removed from each fish by drilling a hole in the otolith core in order to collect otoliths for elemental analysis. No otoliths were excluded from the study for this reason. Otoliths were collected from 4 different nursery areas, 3 in France (Bay of Villaine, Bay of Bourgneuf, Port la Forêt) and 1 in Portugal (Gronde estuary). Only otoliths with a maximum diameter greater than 1.2 mm were retained. All otoliths were scanned using energy dispersive X-ray fluorescence (ED-XRF) at the University of California Davis, ON (USA). 0-group juveniles from each nursery were analyzed in ED-XRF at the University of California Davis, ON (USA). Otoliths were treated and dried as described in Gilliers et al. (2000). Otoliths were measured under a binocular fluorescence microscope (Leica, Wetzlar, Germany). Using the QBOAT software the external radius (a) and thickness (t) of each otolith were measured for each individual. An example of an otolith with its analysis parameters is shown in Table 1.

**Sample analysis**

Multi-element composition was determined in whole otoliths using solution-based inductively coupled plasma mass spectrometry (ICP-MS, Thermo Scientific). The preparation for analysis was performed in a Serafino 2800 ultra-clean laboratory.

Demineralized otoliths were weighed on a precision scale to the nearest 0.1 mg. Table 2. Otoliths were demineralized in orthophosphoric acid (H3PO4) water or in a mixture of oxalic acid water (HCOOH) and orthophosphoric acid (H3PO4) water. The following elements were quantified: Li, Na, K, Ca, Mg, Mn, Sr, Ba, Cu, Co, Zn, Fe and Ti. To reduce interferences and sensitivity problems, the following elements were analyzed after addition of 1% HNO3 to the sample solutions. The 0.13% of the total variance of the 14 elements was explained by their inter-elemental correlation analysis. The data was not used for further analysis. The concentration of each element was determined with the use of a multielement standard solution having a concentration similar to the average concentration of each element in the samples. The concentration of each element in the sample was determined through the use of an external standardization of the instrument for the concentration of each element of the samples. The multielement standard composition was assumed to be that of the standard solution.

**Data analysis**

Data were transferred to ratio elements to Ca (element Ca which the Le Pape transformed in order to reach normality of variance and of the variance of the variance) and to each element Ca was using ANOVA. In order to resolve any overlap effects, the relationship between otolith mass and each element Ca was analyzed using ANOVA. When the homogeneity of variance is not satisfied, comparing two groups we used the following tests: Student’s t-test, ANOVA. When the homogeneity of variance was satisfied, comparing two groups we used Student’s t-test. For all tests, differences were considered significant when the following were not satisfied: P < 0.05.

**Results**

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

**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 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.

**Summary of findings**

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