Unmasking the otolith using synchrotron-based scanning X-ray fluorescence

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Context

The otolith, a paired caclified structure ($CaCO_3$) found in the inner (**Fig.1**), relaye stato-acoustic functions in teleosts. It is result from biomineralization processus leading to a continous growth which retains the biogeochemical signature of the environement (by elemental substitution) and biological marks of ingrowth. Otoliths provide key information on **life history characteristics**, such as age, behaviour life cycle and information about the environment an individual may have inhabited. Unfortunately, for many species, such as Syngnathidae (seahorses, pipefish), otoliths are highly challenging: small size (less than 400µm), fragile and without discernible growth increments. **Synchrontron X-ray fluorescence (XRF)** has seldom been used in otolith chemistry sudies altough it shows real promise in this fields (Limburg & Elfman, 2017). Synchrotron SOLEIL and the Nanoscopium beamline allow to obtain complete images of the dynamics of elemental incorporation in the otolith by producing two-dimensional scanning of a sample. This new tool allows the characterisation of the elemental spatial heterogeneity more finely.

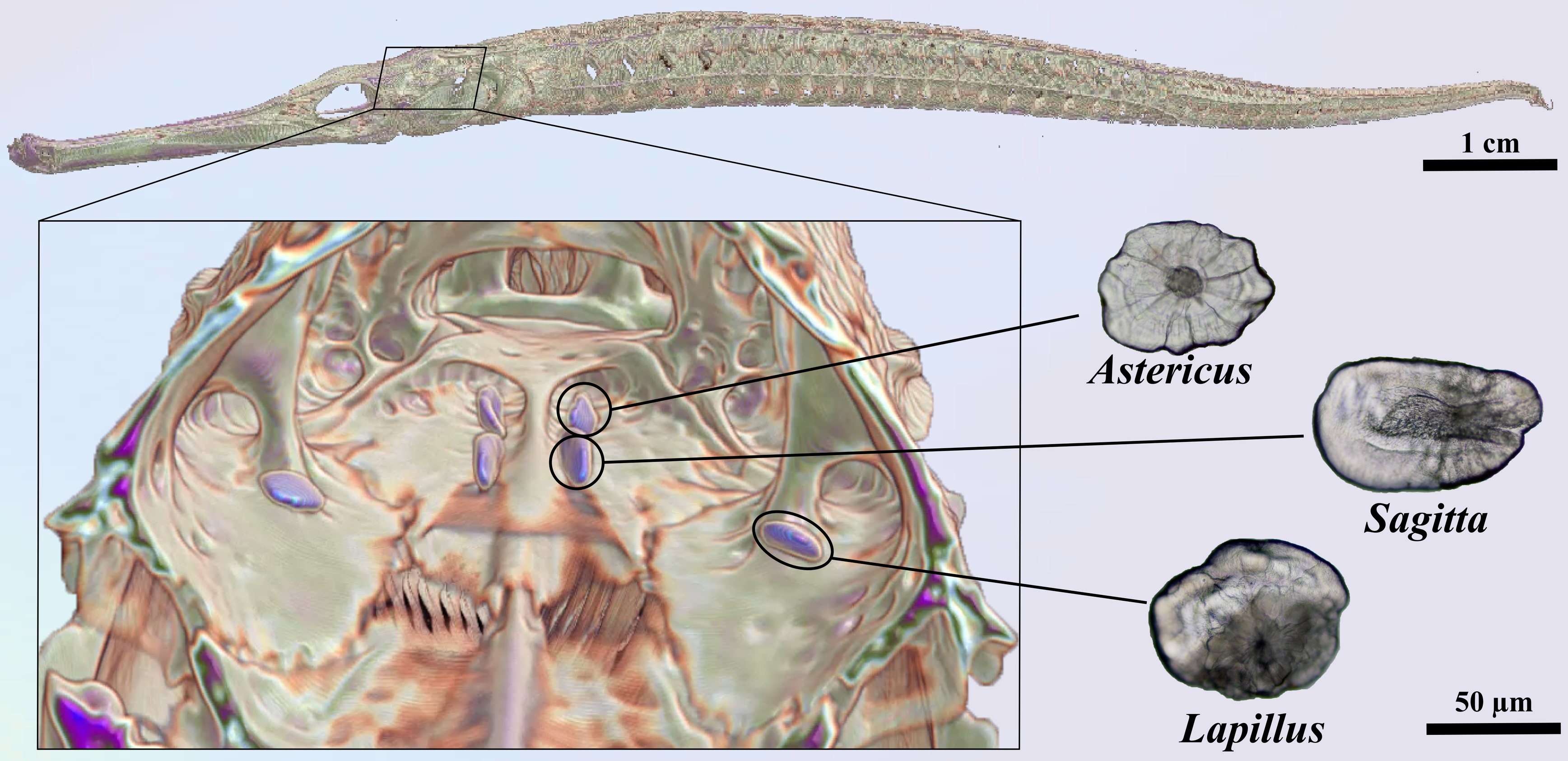

Figure 1: Tomography CT of a specimen of Microphis brachyurus *and zoom on the inner ear and the positionning of the otoliths.*

Aims

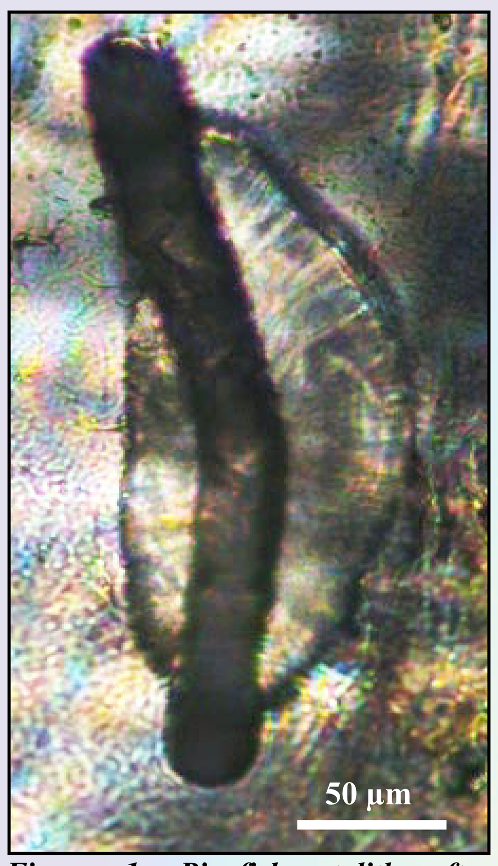

Figure 1: Pipefish otolith after LA-ICP-MS analysis.

Spectrometric methods by laser ablation (LA-ICP-MS), classically used for microchemical analysis, porvide fragmentary information. In addition, destruction of the sample prevents any subsequent analysis (Limburg *et al.,* 2007) (**Fig. 2**). New non-destructive methods such as synchrotron XRF can solve this issue (**Fig. 3**). The present study focuses on **two tropical Indo-Pacific freshwater pipefish species**: ***Microphis brachyurus*** (Bleeker, 1854) and ***Microphis nicoleae*** Haÿ *et al.,* 2023 whose face crucial lack of knowledge about thier ecology and life history traits.

⟶ **Analyse for the first time** with an innovative method, pipefish's otoliths whose size and lack of marks are limiting factors to their analysis.

⟶ **Obtain information on thier life cycle** : Are these species diadromous (transition freshwater/marine/freshwter) or strictly in freshwater ?

⟶ **Define the field of perspective** opened by the use of **XRF methods** for otoliths science.

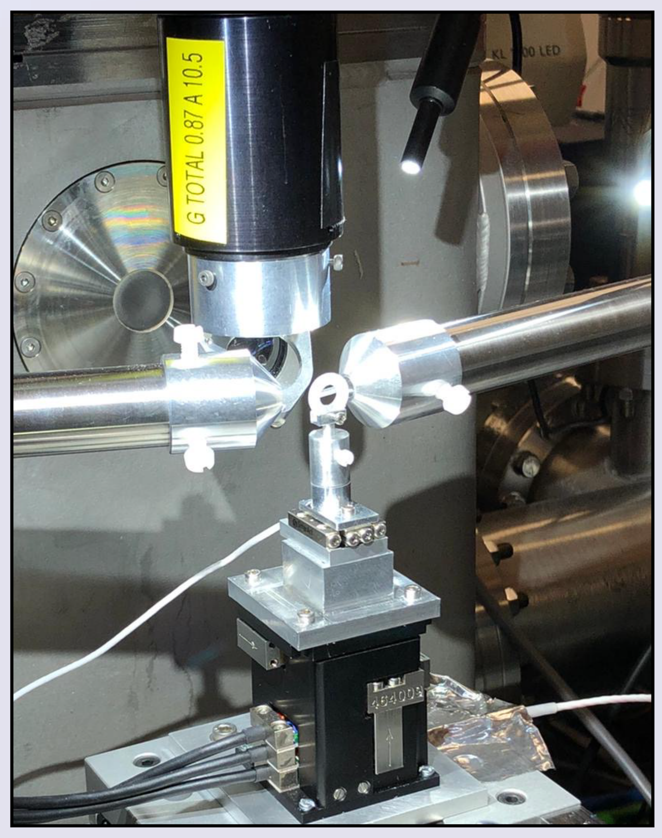

Figure 2: assembly of otolith for XRF analysis on the SOLEIL Nanoscopium beamline.

Results

⟶ **3 zones define according to strontium : calcium ratio intensities (Fig. 4), correlated with water salinity.**

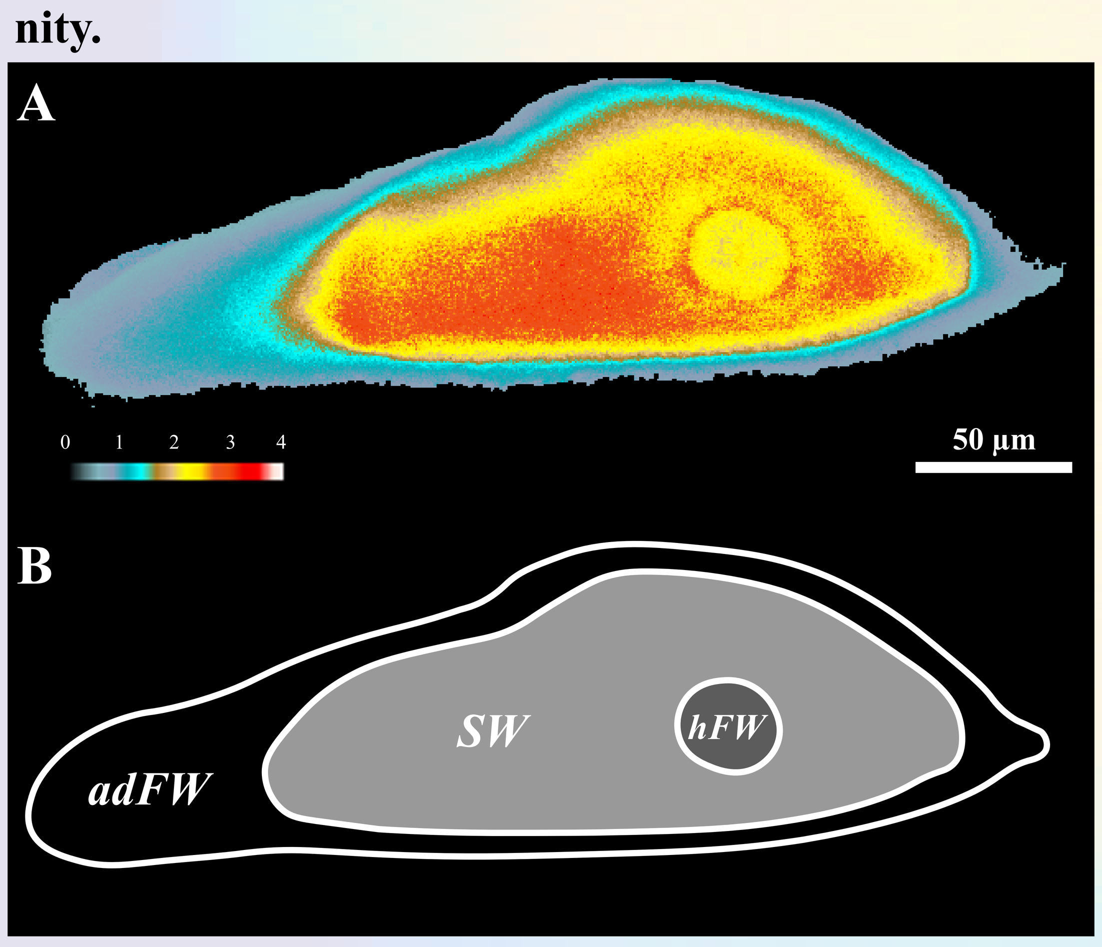

Figure 4: (A) Elemental map of strontium : calcium ratio (Sr:Ca) (X 1000). (B) edge detection unmasking zonation in the otolith depending Sr:Ca values and therefore .

- ***hFW, hatching freshwater,* « birth in river »**
- ***SW, seawater,* « marine dispersal »**
- ***adFW, adult freshwater,* « adult life in river »**

⟶ **Diadromous life cycle , and more precisely amphidromous (for the two species studied) (Fig. 5).**

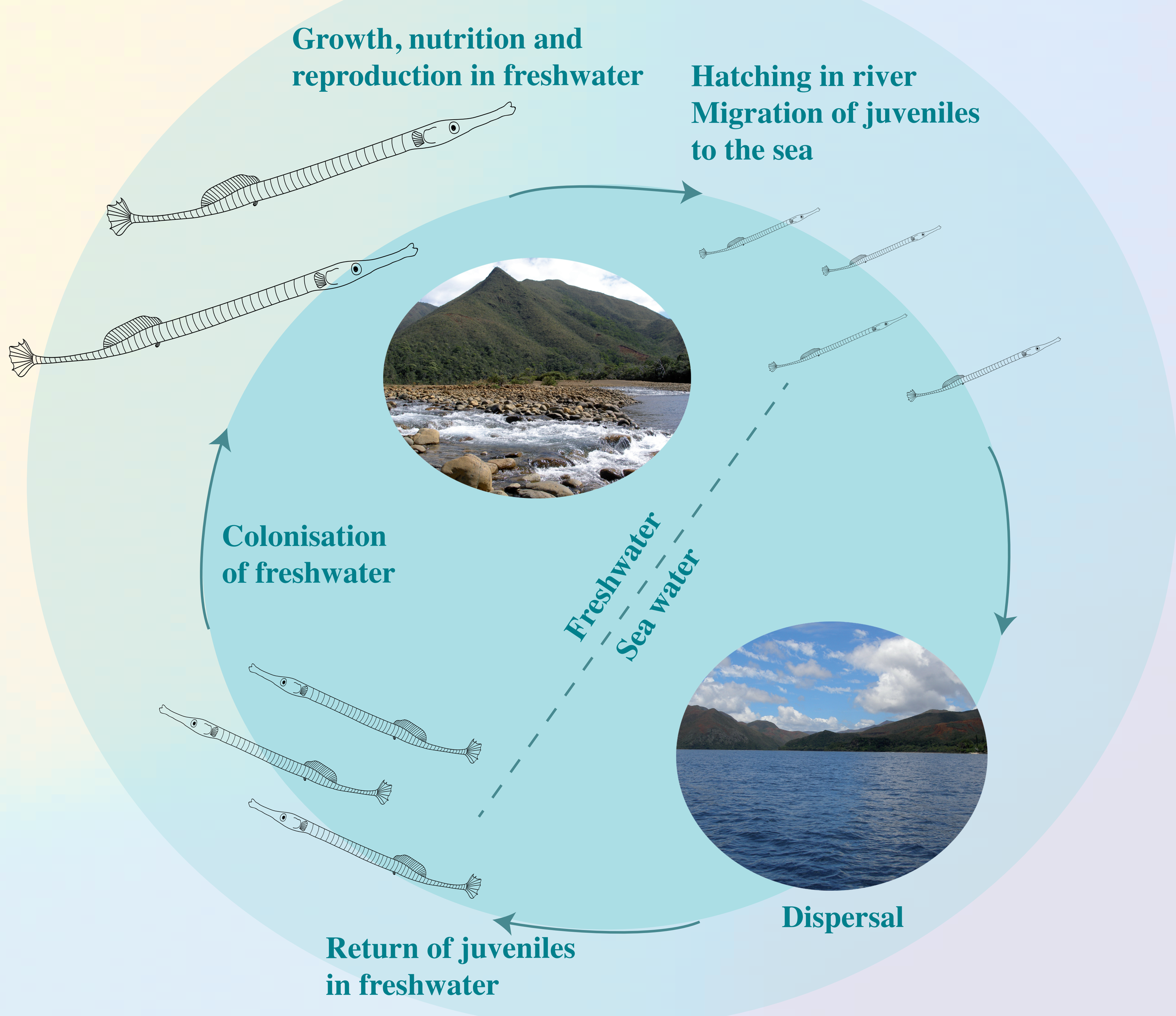

Figure 5: amphidromous life cycle of freshwater pipefish (modified from Lord, 2009).

⟶ **Retrace migration patterns and individual histories through the study of trace elements (Fig. 6).**

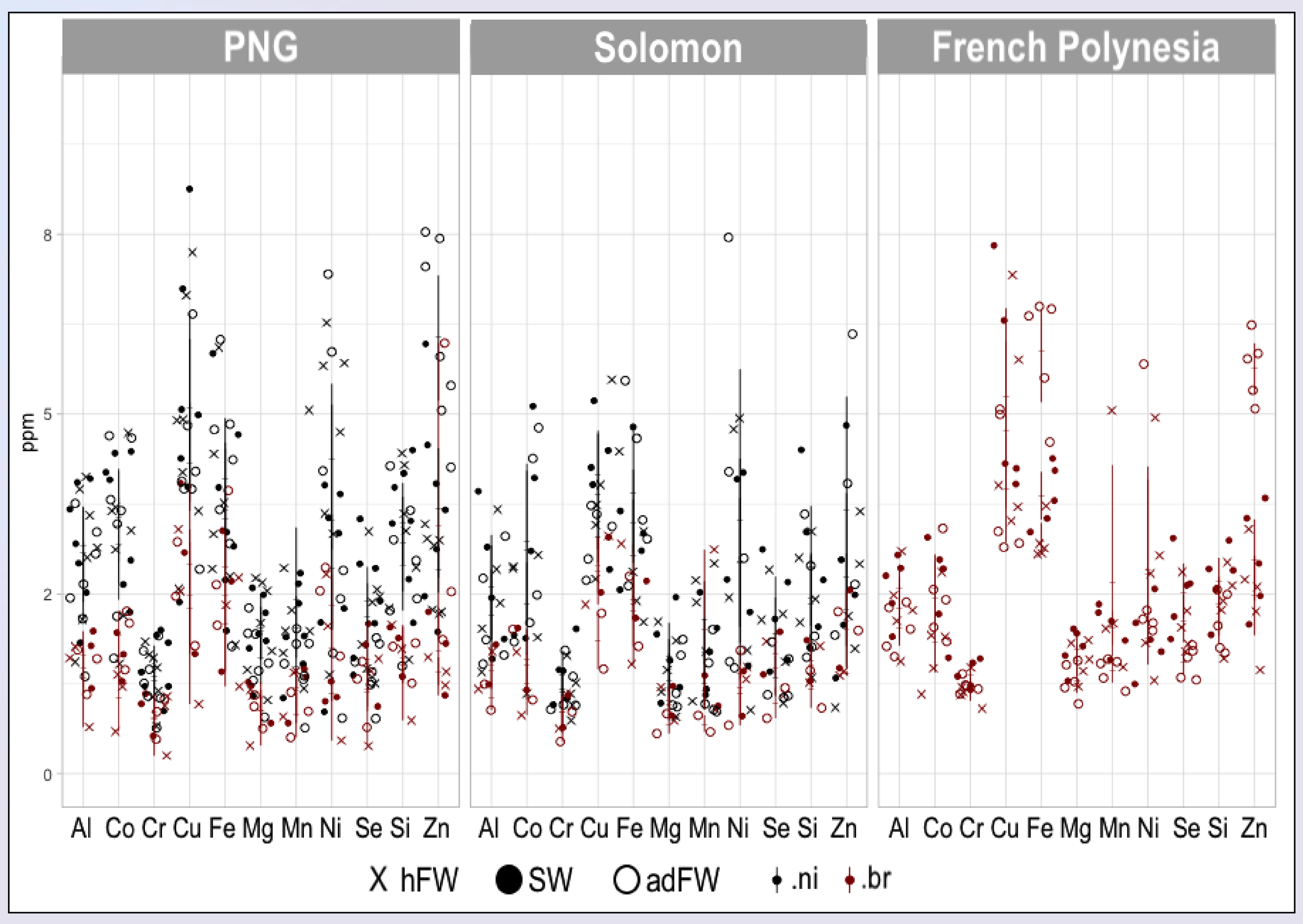

Figure 6: trace element (ppm) variations in the otolith zones (hFW, SW et adFW) according to the geographic region. ni : Microphis nicoleae *; br :* Microphis brachyurus*; PNG: Papua New Guinae*

- **Differences between localities studied (Papua New Guinae, Solomon islands and French Polynesia), inside otolith between different areas (*hFW*, *SW* and *adFW*) and between species (*M. brachyurus* and *M. nicoleae*).**

⟶ **Access to growth dynamic**

Growth increments in the otolith : alternation of **L-Zones (rich in $CaCO_3$)** and **D-Zones (rich in organic material), can be tricky to unmask in otoliths.**

Counting method relies on the higher sulphur concentration in the D-Zones (Mc Fadden *et al.,* 2015).

- **«Chemical» counting of growth increments by sulfur and SEM validation (Fig. 7 and 8). Estimation of the durations spent in the different environments.**

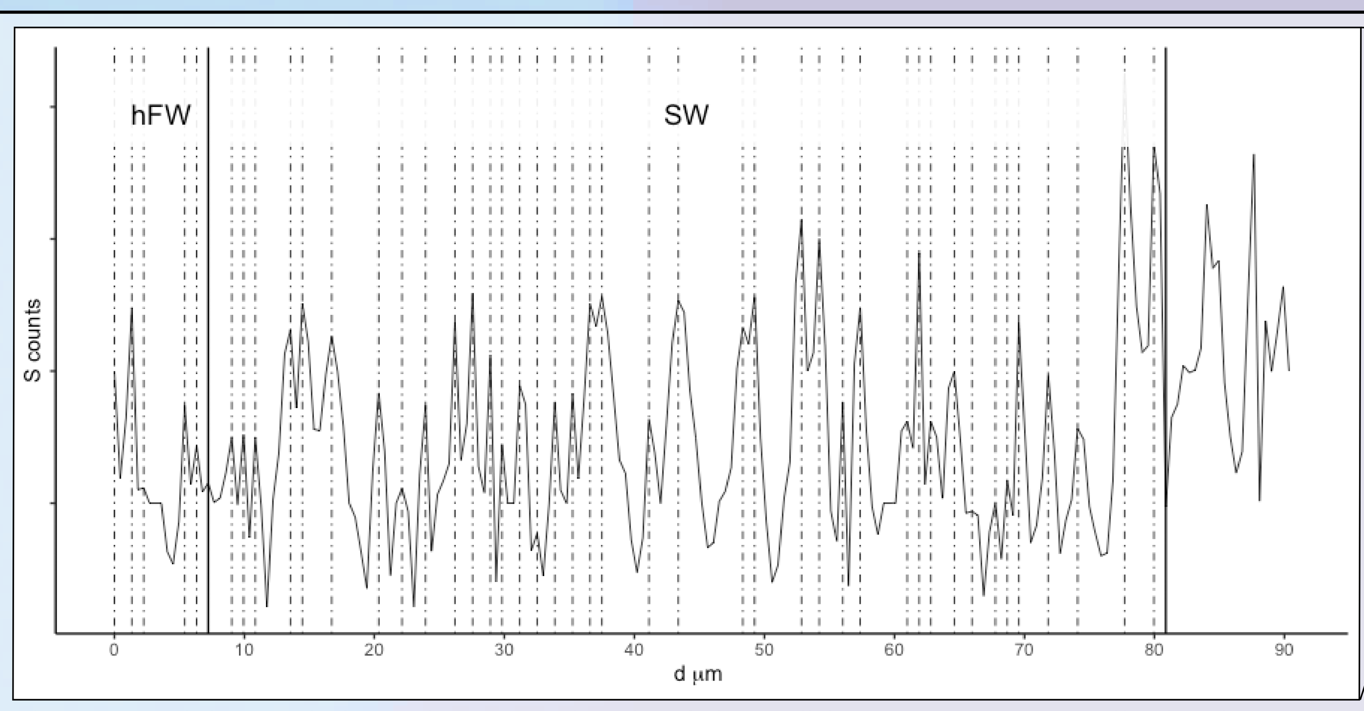

*Figure 7: sulphur variations along growth axis of the otolith (*M. brachyurus*) allowing increments count. Dotted lines : increment equivalent ; solid lines : edges deduced from Sr:Ca values relaying the environmental transition (insert Fig.8).*

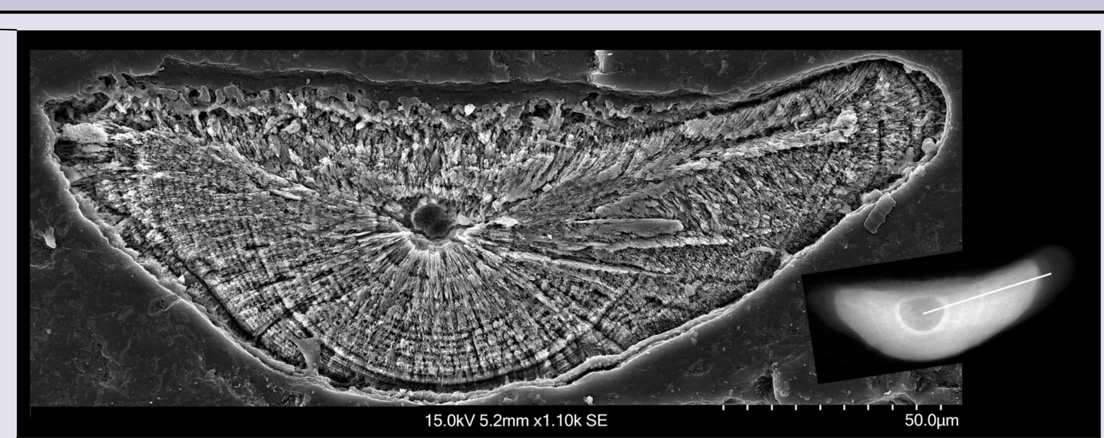

Figure 8 : SEM image of the otolith use to compare «chemical» and «manual» counts.

Automated counting

More accurate estimation

Circumvents reader bais issues

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