doi:10.1017/S1431927614013695
Microsc. Microanal. 21 (Suppl 6), 2015

Micro-scale elemental partition in brain structures of the fish *Liza aurata*

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Studies of metal contamination of fish mainly focused the issues of environmental quality and risk assessment for human populations. However, the influence of metal pollutants in fish physiology has not been extensively addressed. Alterations in brain function due to metal imbalances may endanger not only the individual but poses risks to the population. This issue is relevant considering population dynamics of fisheries.

The aim of our study was to initiate an elemental imaging inventory of fish brain structures and to quantitatively evaluate their elemental distributions. The study used will provide the first elemental mapping of neuronal tissue of fish.

Liza aurata was chosen as model because is a widespread marine species with edible value and often used as bioindicator [1]. Brains of *Liza aurata* captured at Tagus estuary (Portugal) were dissected and deep-frozen in liquid nitrogen. Transversal sections of 20 µm were obtained from the frozen material in a cryo-microtome. The elemental distributions were assessed quantitatively using nuclear microscopy techniques based on accelerated beam of protons with 2 MeV and focused to micrometer dimensions. Mass density images were obtained with STIM technique (scanning transmission ion microscopy) and elemental quantification was carried out by combining PIXE (particle induced X-ray emission) and RBS (Rutherford backscattering spectrometry) [2].

Major brain regions were identified using both mass density variations and elemental distributions, having optical micrographs of stained sections as reference. The cellular layers of supra ventricular regions were distinguished from intercalary white and grey layers. In Figure 1 a section of mesencephalon illustrates the stratification of the *Liza aurata* brain. The layers of grey and white matter of tectum opticum displayed below the subarachnoid space, are well evidenced in density maps (high density zone Fig. 1, scan 1) and are characterised by high Cl, K, Ca contents (see Fig. 2). Details of vessels penetrating the surface of the brain were also visualized (Fig. 2). The peripheral grey zone showed higher concentrations of Ca, Fe, Cu and Zn when compared to central white zones (see Fig. 2). Cell clustering in peripheral tectum opticum zone and inner regions seem to correlate with increases in Zn concentration.

The importance to reference elemental mapping is the possibility to associate these distributions with the tissue function. For instance, superficial layers of tectum opticum have primarily been associated with vision and deeper layers with goal-directed locomotion [3]. Moreover, by identifying elemental changes in brain structures, neuronal health effects can be predicted. Therefore, it is useful to catalogue fish brain images and elemental compositions, which can be further used in environmental biomonitoring assessments and neurotoxicity studies.

First three authors acknowledge financial support received from the Portuguese Science and Technology foundation.

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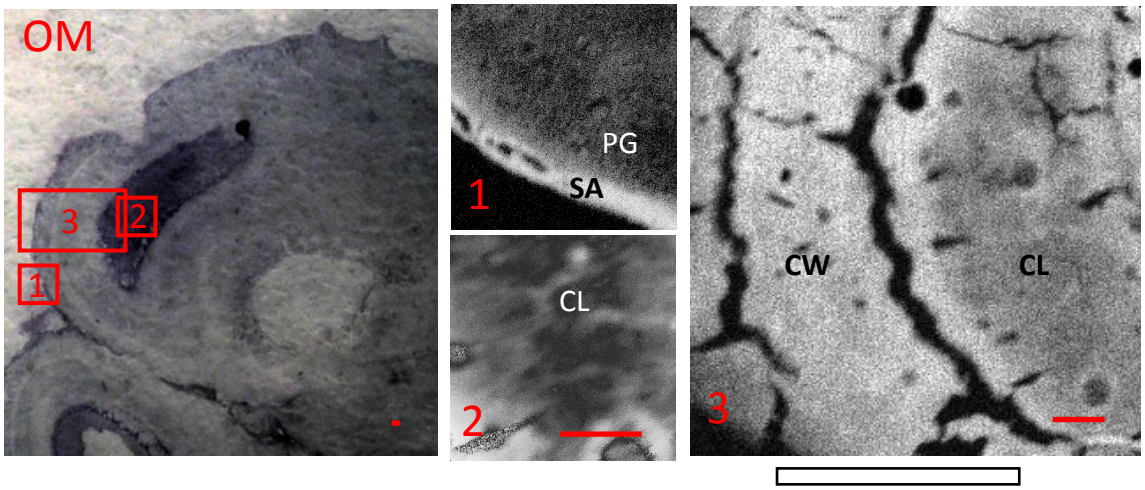

Figure 1. Mass density (STIM) in a cross section of the mesencephalon of the brain *of Liza aurata* showing different structures of tectum opticum region: SA-subarachnoid space; CL – inner cellular layers, CW – central white zones; PG – peripheral grey zone. The scanned regions (1, 2 and 3) are identified in the optical microscopy image (OM). Density gradient represented by a dynamic colour scale: high density - white, to low density – black. Horizontal line denotes 100 µm.

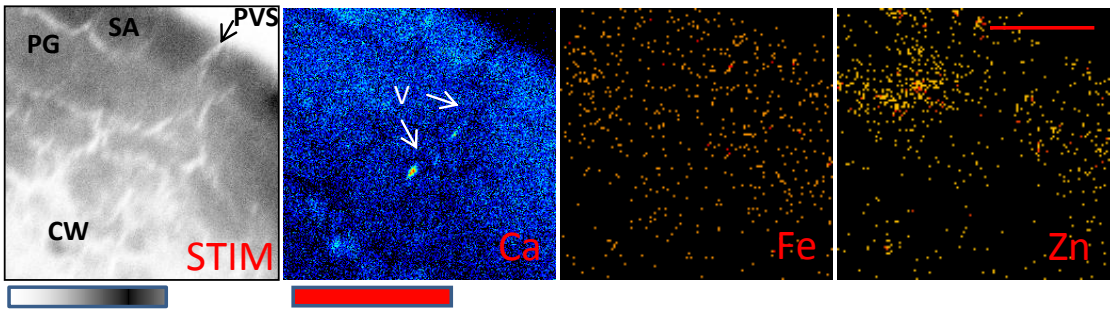

Figure 2. Detail of the tectum opticum of the brain of *Liza aurata*. Mass density (STIM) and elemental distribution maps: perivascular space (PVS), subarachnoid space (SA) and vessels (V) penetrating the surface of the brain through peripheral grey zone (PG) can be depicted. CW – central white zone. Density gradient represented by a dynamic colour scale: high density – black/red, to low density – white/dark blue. Horizontal line denotes 100 µm.

https://doi.org/10.1017/S1431927614013695 Published online by Cambridge University Press