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ScienceDirect
Nuclear Instruments and Methods in Physics Research B 266 (2008) 67–72
NIM B
Beam Interactions with Materials & Atoms
www.elsevier.com/locate/nimb

Quantitative elemental imaging of octopus stylets using PIXE and the nuclear microprobe

Zoë Doubleday [a,*], David Belton [b], Gretta Pecl [a], Jayson Semmens [a]

[a] Marine Research Laboratories, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Private Bag 49, Tasmania 7001, Australia
[b] CSIRO Exploration and Mining, University of Melbourne (School of Physics), Melbourne 3010, Australia

Received 27 July 2007; received in revised form 28 September 2007
Available online 5 November 2007

Abstract

By utilising targeted microprobe technology, the analysis of elements incorporated within the hard bio-mineralised structures of marine organisms has provided unique insights into the population biology of many species. As hard structures grow, elements from surrounding waters are incorporated effectively providing a natural 'tag' that is often unique to the animal's particular location or habitat. The spatial distribution of elements within octopus stylets was investigated, using the nuclear microprobe, to assess their potential for determining dispersal and population structure in octopus populations. Proton Induced X-ray Emission (PIXE) was conducted using the Dynamic Analysis method and GeoPIXE software package, which produced high resolution, quantitative elemental maps of whole stylet cross-sections. Ten elements were detected within the stylets which were heterogeneously distributed throughout the microstructure. Although Ca decreased towards the section edge, this trend was consistent between individuals and remained homogeneous in the inner region of the stylet, and thus appears a suitable internal standard for future microprobe analyses. Additional analyses used to investigate the general composition of the stylet structure suggested that they are amorphous and largely organic, however, there was some evidence of phosphatic mineralisation. In conclusion, this study indicates that stylets are suitable for targeted elemental analysis, although this is currently limited to the inner hatch region of the microstructure.

PACS: 92.20.Jm; 92.20.Wx

Keywords: Nuclear microprobe; Octopus; PIXE; Stylet; Elements; Spatial distributions

1. Introduction

The quantitative and spatial distribution of trace elements within the hard bio-mineralised structures of marine organisms provide a 'story' of an individual's life history and movement patterns, as they commonly reflect changes in behaviour, physiology and the surrounding environment. However, such chronological information can only be accurately measured if the structure contains regular temporally- or age-related growth increments. The targeted microprobe-based analysis of trace elements incorporated within such hard structures, which has included fish otoliths [1], squid and cuttlefish statoliths [2,3], gastropod shells [4], and bivalve shells [5], has provided population-level information on many marine species. However, due to their crumbly conglomerate structure and lack of growth increments [6] octopus statoliths, unlike other cephalopod statoliths or teleost otoliths, are of little use for ageing and therefore time-specific trace element studies. On a global scale, there are many unresolved questions regarding the population biology and dispersal patterns of octopus, and this is largely due to the lack of available methods to study octopus populations. The development of such methods will be crucial for increasing our understanding of octopus populations and enable the sustainable management of increasingly exploited commercial species.

* Corresponding author. Tel.: +61 3 6227 7215; fax: +61 3 6227 8035.
E-mail address: zoeanned@utas.edu.au (Z. Doubleday).

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doi:10.1016/j.nimb.2007.10.027

Stylets (also known as vestigial shells) are a little known structure unique to Octopoda and are thought to represent a remnant shell [7]. Stylets consist of a pair of fine, cartilage-like rods embedded within the dorsal mantle musculature. Their composition has been suggested to be based on a calcium phosphate compound, such as hydroxyapatite [8], or chitin [7]. The stylet microstructure of *Octopus pallidus* has been found to have distinct concentric regions, a visible pre-hatch nucleus, and age-related growth in the form of daily growth increments [9]. Due to this microstructure stylets, like squid statoliths [2,3], are likely to incorporate elements from the environment on a chronological basis, and therefore, may be a useful tool to address ecological questions on the dispersal patterns of both juvenile and adult octopus.

Laser ablation inductively coupled plasma-mass spectrometry (LA ICPMS) is one of the most widely employed microprobe techniques for examining bio-mineralised structures on a temporal level, as it requires relatively little sample preparation and is capable of measuring elements to the trace level [10]. However, LA ICPMS requires compositional information and standard materials to accurately measure trace elemental concentrations. For example, internal standardisation, a calibration method used for the analysis of many biological materials, requires the presence of one element (usually Ca) of known concentration which is homogenously distributed throughout the microstructural region of interest [11].

The nuclear microprobe (NMP) is a powerful microanalytical tool for investigating the distribution of minor and trace elements in biological materials [12]. Proton induced X-ray emission (PIXE) is a reliable and effective NMP-based technique, which quantitatively maps the spatial distributions of elements within a structure [13]. PIXE analysis is also standardless, and therefore does not require compositional information or the use of a standard [14]. Although the use of the NMP for analysing the bio-mineralised structures of marine species are not common, studies have included the analysis of squid statoliths [13], fish otoliths [15], octopus statoliths [16], and octopus stylets [8].

This is the first time octopus stylets have been analysed with the CSIRO-GEMOC NMP (CSIRO Exploration and Mining) using PIXE and the Dynamic Analysis method of analysis, which allows for simultaneous multi-element analysis and quantitative imaging at a high spatial resolution (down to 1.8 μm) and sensitivity (detection limits have been recorded at 0.2 ppm) [14]. Standard comparisons, using accepted reference and secondary standards, have shown that the standardless PIXE method has an accuracy level of 5–10% for major and trace elements [17]. The Dynamic Analysis method produces 'true' quantitative images of the whole section which are resolved of elemental overlaps, background-subtracted, free of artefacts and generated in real-time [18]. This capability will enable the spatial distribution of elements of whole stylet sections to be quantitatively mapped. In comparison, the study by Napoleão et al. [8] examined the elemental distributions by targeting the proton beam at a selected number of single 2–3 μm points across the section of the stylet. Using the Dynamic Analysis method this study assesses the potential of stylets as environmental time-recorders and the suitability of Ca as an internal standard for LA analysis. Furthermore, to broaden our understanding of the stylet structure and stoichiometry, general compositional analyses (including X-ray diffraction and infrared spectrometry) will also be conducted.

2. Materials and methods

All stylets were obtained from *O. pallidus*, a fully benthic shallow-water species found throughout south-east Australia. This species is the target of a small commercial fishery in northwest Tasmania, Australia. Stylets were removed from fresh mantle muscle and air-dried for 48 h.

2.1. General composition analyses

General composition analyses were performed on stylets collected from mature adults sourced from the commercial fishery in October 2005. Powder X-ray diffraction was conducted to determine mineral composition (Mineral Resources Tasmania, Australia). The dried stylet samples were ground to approximately 10–75 μm and pressed into a 25 mm sample holder. The samples were run on an automated Philips X-ray System and analysed with Diffraction Technology software. To determine total inorganic content 'loss-on-ignition' (combustion of organic material) was also conducted (Mineral Resources Tasmania, Australia). Secondly, infrared spectroscopic analysis was conducted on the stylet with most of the outer sheath removed (see [9] for more details on the microstructure). The sheath was removed to help identify, more clearly, the potential discriminating peaks characteristic of the calcified portion of the stylet. A crushed sample was mixed with anhydrous KBr and pressed into a 7 mm diameter pellet. The analysis was performed at 4 cm^{-1} resolution using a Fourier Transform Bruker IFS66 infra-red spectrometer (Central Science Laboratory, University of Tasmania).

2.2. Nuclear microprobe analysis

Five stylets were sourced from adult octopus collected from the commercial fishery in October 2005. The mature adults were of similar size ranging from 550 to 760 g. Two juvenile stylets were also sourced from five-month-old aquaria-reared octopus in January 2006. These juveniles were reared in a natural seawater flow-through system with a simulated natural temperature regime at the Tasmanian Aquaculture and Fisheries Institute, Hobart, Australia. All stylets were removed from fresh mantle muscle and air-dried for 48 h. To remove excess tissue from the juvenile stylets they were soaked in a mixture of 30% H_2O_2 buffered with NaOH for 24 h, and then rinsed thoroughly in ultra-pure water (Milli-Q) prior to drying.