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NOTE

Element characterization of the vestigial shell of *Octopus vulgaris* Cuvier, 1797

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ABSTRACT

The present study represents the first elemental distribution and composition characterization of the vestigial shell in *Octopus vulgaris* Cuvier, 1797. Ten specimens of *O. vulgaris* were collected monthly between January and July 2002, on the central coast of Portugal. Using nuclear microscopy, Rutherford Backscattering Spectrometry (RBS) and Particle Induced X-ray Emission (PIXE) techniques, it was found that along vestigial shell transversal sections, the elemental concentrations were differential. Most striking were those of P, Ca, S, and Cl. At the core of the vestigial shell, high concentration levels of Ca and P were determined, declining towards the periphery. Also, trace concentrations of elements such as Fe, Cu and Zn were found to be higher in the external rings than the inner regions. Future complementary studies of vestigial shell chemical composition, as well as histology studies, are necessary to gain better understanding of the vestigial shell microstructure.

Keywords: Nuclear microprobe, vestigial shell, trace elements, *Octopus vulgaris.*

RESUMEN

Caracterización elemental de la concha vestigial del pulpo* Octopus vulgaris *Cuvier, 1797

Se presenta la primera caracterización de la distribución elemental y de la composición de la concha vestigial del pulpo Octopus vulgaris *Cuvier, 1797. Diez muestras de* O. vulgaris *fueron recogidas mensualmente, entre enero y julio del 2002, en la costa central de Portugal. Utilizando microscopía electrónica, técnicas de espectrometría Rutherford de refracción (RBS) y emisión de rayos X inducida por partículas (PIXE), se pudieron encontrar, a lo largo de las secciones transversales de la concha vestigial, diferencias en las concentraciones elementales, en particular en las de P, Ca, S, y Cl. En el núcleo de la concha vestigial, se pudieron determinar altas concentraciones de Ca y P, que se iban reduciendo hacia la periferia. Asimismo, se encontraron microelementos, como el Fe, Cu y el Zn, a concentraciones que eran mayores en los anillos externos que en las regiones internas. Para una mejor comprensión de la microestructura de la concha vestigial, es necesario llevar a cabo estudios complementarios a éste, y también estudios histológicos.*

Palabras clave: *Microprobetas nucleares, concha vestigial, microelementos,* Octopus vulgaris.

Interest in the elemental composition of marine organisms' mineral structures has increased recently. For *Octopus vulgaris* Cuvier, 1797, the morphophysiological description of vestigial shells represents a possible tool for age determination; however, such a method has yet to be established and validated, based on this or another structure. The vestigial shell is described by Wells (1978) as a reduced shell restricted to two very fine long structures inserted into the mantle muscle at the gill base. Previously, Young (1960) pointed out the existence of concentric rings on statoliths of *O. vulgaris*, but no other reference to periodic ring deposition on those structures has been noted. The presence of concentric rings in *O. vulgaris* structures has been reported for the beak (Smale *et al.*, 1993), internal vestigial shell and lens (Gonçalves, 1993), but no deposition rate was proposed. Raya and Hernández-González (1998) described a regular pattern of micro-increments in beaks of this species, which suggests a constant deposition (probably daily) during growth. However, these authors presented no age validation results. Sousa Reis and Fernandes (2002) have noted a pattern for the increments in the internal vestigial shell of *O. vulgaris* which seems to be related to its life cycle. The present paper reports on our preliminary data for element distributions in the vestigial shells of *O. vulgaris*, obtained with nuclear microscopy techniques. Ten specimens of *O. vulgaris* were collected monthly between January and July 2002, by commercial fishing vessels at Cascais, on the central coast of Portugal (9-9.30° W, 38.30-38.50° N). For each specimen, the vestigial shell was removed, quickly frozen in liquid nitrogen and stored at –20 °C in the laboratory. The post-rostral zone near the bend of the vestigial shell was selected for transversal sectioning (10 to 15 µm thick) in a cryostat at –20 °C, since this appears to be the best region for identifying concentric bands, according to Sousa Reis and Fernandes (2002). The nuclear microscopy experiments were performed using Portugal's Instituto Tecnológico e Nuclear (ITN) Van de Graaff accelerator, which provides incident proton beams of 2.0 MeV that can be focused to 2-3 µm. Nuclear microscopy comprises various techniques that made it possible to obtain density, morphology, and elemental distribution images of the sample. The concentrations of chemical elements detected could also be determined by combining Rutherford Backscattering Spectrometry (RBS) and Particle Induced X-ray Emission (PIXE) techniques, also available at the ITN. OM_DAQ and Dan32 programs were used to perform the RBS and PIXE spectral data acquisitions, analysis and element quantification (Watt and Grime, 1995).

The element distribution maps obtained using nuclear microscopy showed that along vestigial shell transversal sections, the elemental concentrations were differential. Most striking were those of P, Ca, S, and Cl. At the core of the vestigial shell, high concentration levels of Ca and P were determined, declining towards the periphery. Contrary to the gradients observed for Ca and P, the S and Cl concentrations increase from the core out to the external rings (figure 1). Also, trace concentrations of elements such as Fe, Cu and Zn were found to be higher in the external rings than the inner regions.

The average concentrations obtained for the vestigial shell matrix constituents (C, O and N), major and minor elements (K, Ca, Cl, P and S), and trace elements (Si, Ti, Cr, Mn, Fe, Cu, Zn, Br and Sr) are listed in table I.

In the inner regions of the vestigial shell, the P and Ca contents were found to be much higher than for the external rings (P and Ca were 5 % and 18 % w/w respectively, for the inner regions, versus 3 % and 9 % w/w, for the outer rings), although the P/Ca contents

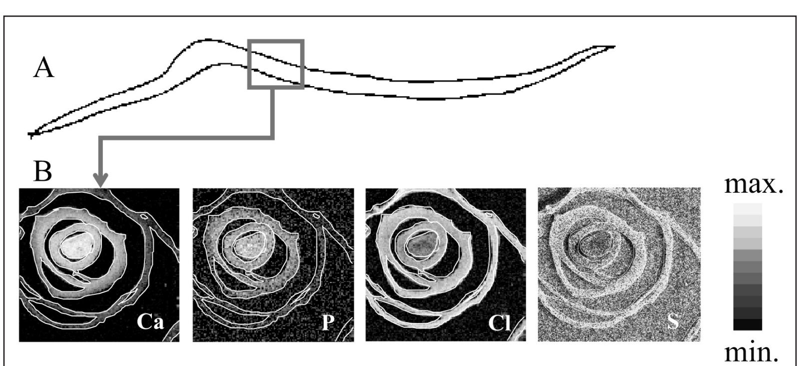

Figure 1. Elemental distributions in *O. vulgaris* vestigial shell, obtained for transversal cryosections. The vestigial shell area studied is indicated in A, and the transversal section P, Ca, Cl and S distribution images are represented in B. The scanned area was 1 060 µm × 1 060 µm. Elemental concentration differences are shown by colour gradient (min.-max.), and white lines represent the concentric rings that partially individualise when cut

Tabla I. Elemental concentrations, in mg/kg unless otherwise indicated in the table, of the *O. vulgaris* vestigial shell obtained from RBS and PIXE analyses. Values are median, minimum and maximum. (n): number of analysed cases where the element was detected

Elements	n	Median	Mimimum	Maximum
C (%)	118	27.4	4.21	59.6
N (%)	119	14.5	2.61	25.3
O (%)	119	33.5	9.39	51.7
P (%)	118	0.595	0.008	19.6
Ca (%)	117	6.57	0.219	49.4
Si (mg/kg)	46	6 087	77.0	39 085
S (mg/kg)	116	5 693	100	31 827
Cl (%)	114	2.41	0.280	16.5
K (mg/kg)	120	2 793	836	16 541
Ti (mg/kg)	73	18.0	2.00	180
Cr (mg/kg)	65	45.0	4.00	1 861
Mn (mg/kg)	76	42.5	2.00	345
Fe (mg/kg)	101	144	6.00	6 519
Cu (mg/kg)	53	58.0	17.0	1 098
Zn (mg/kg)	44	140	18.0	2 863
Br (mg/kg)	60	401	62.0	1 348
Sr (mg/kg)	56	3 766	210	18 274

ratio was quite constant along the vestigial shell. The major elemental contents determined, and their proportions, suggest a basic constitution in Ca and P compounds for the vestigial shell. As to the concentrations of trace element, Sr showed the highest levels, whilst the lowest levels were determined for Ti (table I).

Like other mineral structures of marine organisms, including cephalopods (Ikeda *et al.*, 1998, 1999) and other molluscs (Carriker *et al.*, 1996), Ca is one of the main components of the *O. vulgaris* vestigial shell. Of all the trace elements detected, Sr presented the highest concentrations, and the distribution pattern found was similar to that of other cephalopod statoliths (Ikeda, Arai and Sakamoto, 1996; Ikeda *et al.,* 1998, 1999; Bettencourt and Guerra, 2000) and bivalves (Carriker *et al.*, 1991, 1996). The chemical behaviour of this element is analogous to Ca, competing with Ca sites in the mineral lattices of marine organisms' hard structures (Brown *et al.*, 1995). In statoliths of the giant octopus *O. dolfleini*, Ikeda *et al.* (1999) also detected Cr, Mn, Fe, Cu, Zn and Br, although in levels that were lower than those determined in the present work. The presence of Ti and Si has also been reported for the *Crassostrea* sp. shell (Carriker *at al.*, 1991, Almeida *et al.*, 1998).

In summary, the present study represents the first elemental distribution and composition characterization of the vestigial shell in *O. vulgaris*. However, our results highlight the need for complementary studies of vestigial shell chemical composition, as well as histology studies, to gain better understanding of the vestigial shell microstructure.

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