Microsc. Microanal. 19 (Suppl 4), 2013

doi:10.1017/S1431927613001050

The Gastropod Statolith Elemental Composition by EMPA

S. Galante-Oliveira*, R. Marçal*, F. Guimarães**, J. Machado*** and C.M. Barroso*

*CESAM & Biology Department, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
**Unit of Science and Mineral Technology, National Laboratory of Energy and Geology (LNEG), R. da Amieira, Apartado 1089, 4466-901 S. Mamede de Infesta, Portugal
***Laboratory of Applied Physiology, Institute of Biomedical Sciences Abel Salazar (ICBAS), R. de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

Gastropods statoliths are biogenic structures of $CaCO_3$, analogous to fish otoliths [1]. Their application in sclerochronology is recent and may be advantageous comparing to the most traditional organisms used hitherto. Moreover, since elements bind within calcified structures [2], statoliths theoretically incorporate chemical information from the surrounding environment over time. So, besides being used for age estimation and growth studies [1], statoliths elemental fingerprinting is recognized as natural tag to track larval dispersal [2] and may also be considered as a potential marker of environmental change and/or pollution. However there is a lack of information on the elemental composition of adult gastropod statoliths. Here we characterize *Nassariusreticulatus* (Gastropoda: Caenogastropoda) statoliths elemental composition by electron microprobe analysis (EMPA).

One statolith from each of 3 adults was prepared for EMPA (Figure 1) and showed the same structural pattern: a nucleus circled by a metamorphic ring and, in addition to this core, 4 other rings delineating 5 increments (Figure 1F). General composition was assessed by Energy and Wavelength Dispersive Spectroscopy revealing C, Ca, O, Sr, Na, S and Mg as the most representative elements. These were then quantified in 3 points per increment. Carbon was not determined since surfaces were C-coated and the amount of oxides of Ca, Sr, Na, S and Mg was estimated from the valence. In addition, Ba – trace element in statoliths of some gastropods larvae [2] – and Al, Cu, Hg, Sn – known as environmental pollutants – were also sought in one of the statoliths. For such, mean values for Ca, Sr, Na, S, Mg and O were set as the sample matrix. Standards and mean detection limits by element and statolith are indicated in Table 1.

Concentrations of Ca, O, Na, Sr and S varied between $3.7x10^5$-$4.2x10^5$, $1.5x10^5$-$1.7x10^5$, $2.5x10^3$-$6.1x10^3$, $1.3x10^3$-$8.9x10^3$ and $4.7x10^2$-$1.3x10^3$ ppm, respectively; Mg, which was not always detected, ranged from $2.0x10^2$-$8.6x10^2$ ppm. No significant differences in these elements mean concentrations were found between increments, except Na in one statolith between increments 1 and 5 (higher at the edge; Dunn's test s=2.014, $p<0.05$). Thus the general elemental composition is nearly constant throughout lifespan (i.e. along statoliths radiuses). Regarding trace elements: Sn was never detected; Al and Hg were at 22.2% of the punctual analyses conducted while Cu was at 66.7% and Ba at 88.9%. Concentrations of Al, Hg, Cu and Ba varied between 55-71, 176-180, 183-302 and 647-969 ppm, respectively.

EMPA proved to be a useful technique in sclerochronology, allowing the characterization of the overall composition of gastropods statoliths and the quantification of elements in specific locations with high resolution.

References

1. Barroso C.M. *et al*., Mar. Biol., 146:1139, 2005
2. Lloyd D.C. *et al*., Mar Ecol. Prog. Ser., 353:115, 2008

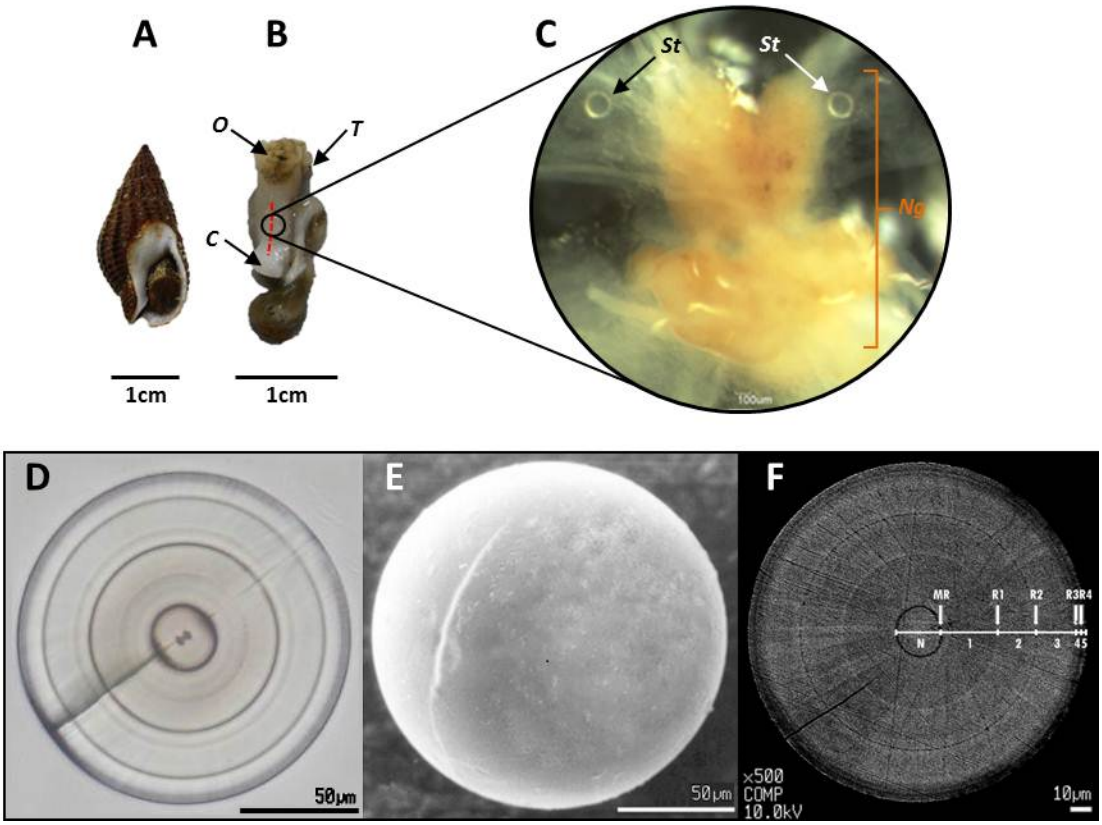

Figure 1.*Nassarius reticulatus*. (A) Ventral surface. (B) Specimen in ventral position after shell removal, indicating the cutting zone (dashed line) for statoliths extraction. (C) Nervous ganglia magnification and statoliths location. (D) Internal microstructure of a statolith grinded to its centerby OM. (E) External surface of a whole statolith by SEM. (F) COMP image of a statolith grinded to its center for EMPA analysis. *C*: Columellar muscle; *MR*: Metamorphic ring; *N*: Nucleus; *Ng*: Nervous ganglia; *O*: operculum; *R1*: First ring; *R2*: Second ring; *R3*: Third ring; *R4*: Fourth ring; *St*: statolith; *T*: Ocular tentacle.

Table 1. Standards, detection limits mean values (D.L.) and respective coefficient of variation (CV, i.e. Standard deviation/Mean) per element and statolith analysed (1, 2 and 3). *n.a.*: not analysed.

	Standard		Statolith.1		Statolith.2		Statolith.3	
Element	Name	Formula	D.L.	CV	D.L.	CV	D.L.	CV
Ca	Calcite	$CaCO_3$	410	0.06	380	0.08	400	0.08
Sr	Tausonite	$SrTiO_3$	222	0.02	221	0.02	211	0.04
Na	Albite	$NaAlSi_3O_8$	192	0.08	193	0.06	159	0.07
S	Pyrite	FeS_2	308	0.07	308	0.10	281	0.09
Mg	Periclase	MgO	170	0.09	168	0.07	121	0.08
Ba	Barite	$BaSO_4$	*n.a.*	*n.a.*	*n.a.*	*n.a.*	53	0.07
Al	Orthoclase	$KAlSi_3O_8$	*n.a.*	*n.a.*	*n.a.*	*n.a.*	26	0.05
Cu	Cuprite	Cu_2O	*n.a.*	*n.a.*	*n.a.*	*n.a.*	94	0.03
Hg	Cinabrio	HgS	*n.a.*	*n.a.*	*n.a.*	*n.a.*	106	0.03
Sn	Cassiterite	SnO_2	*n.a.*	*n.a.*	*n.a.*	*n.a.*	48	0.04

This work was supported by the Portuguese Foundation for Science and Technology (FCT) through the grant SFRH/BPD/70368/2010.

https://doi.org/10.1017/S1431927613001050 Published online by Cambridge University Press