



## Experiment Report Form

**The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.**

Once completed, the report should be submitted electronically to the User Office via the User Portal:

<https://www.esrf.fr/misapps/SMISWebClient/protected/welcome.do>

### ***Reports supporting requests for additional beam time***

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

### ***Reports on experiments relating to long term projects***

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

### ***Published papers***

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

### **Deadlines for submission of Experimental Reports**

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

### **Instructions for preparing your Report**

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.



	<b>Experiment title:</b> Developing new proxy variables to reconstruct past oxygenation in the sea: a synchrotron approach	<b>Experiment number:</b> ES682
<b>Beamline:</b> ID21	<b>Date of experiment:</b> from:21/07/2017, 8am to: 24/07/2017, 8am	<b>Date of report:</b> 04/09/2017
<b>Shifts:</b> 9	<b>Local contact(s):</b> Marine COTTE	<i>Received at ESRF:</i>
<b>Names and affiliations of applicants (* indicates experimentalists):</b> <b>Helena Filipsson, Nadine Quintana Krupinski</b> : Laboratory Lund University Dept. of Geology Sölvegatan 12 SE - 22362 LUND <b>Christine Barras*, Aurélia Mouret*, Sandrine Le Houedec*</b> : Laboratory Angers University LPG-BIAF, UMR CNRS 6112 2, boulevard Lavoisier FR - 49045 ANGERS CEDEX		

## Report:

### Context and objectives:

Our goal is to develop an indicator (proxy variable) for past marine bottom water oxygenation, in particular for low oxygen conditions. Elemental concentration of Manganese (Mn) in biogenic calcite, expressed as Mn/Ca, has the potential to be an indicator of past seawater oxygen levels [1, 2]. Mn is a redox-sensitive element: under oxic conditions it precipitates in solid form Mn oxyhydroxide, whereas Mn is reduced and dissolves under low oxygen (hypoxic) conditions, making dissolved Mn available for incorporation into the shells of bottom-dwelling (benthic) foraminifera (single-celled marine organisms often with a CaCO<sub>3</sub> shell). For this purpose, two benthic foraminiferal species, *Ammonia tepida* and *Bulimina marginata*, were cultured and calcified in controlled conditions under low O<sub>2</sub> conditions with varying seawater [Mn] (2.5 to 600 µmol/L). Laser ablation-ICP-MS analyses show that foraminiferal calcite Mn/Ca values vary by one order of magnitude between different species grown in the same conditions. Such large interspecies differences in Mn/Ca beg the question whether different species calcify differently, and if so, whether the coordination chemistry of Mn differs based on species and calcification strategy. A recent study, using NEXAFS and scanning transmission x-ray microscopy to study Mg, indicates that, for some species (*Orbulina universa* and *Amphistegina lessonii*), the foraminifera's shells have inner distinct layers of high Mg concentration which, is uniformly substituted with Ca within the calcite lattice [3]. The same feature was also observed for other elements such as B, with B-concentration bandings asynchronous with respect to the Mg bandings [4]. The µXRF and µXANES methodologies available at the Beamline ID21 offer high spatial resolution and sensitivity and access to the chemical speciation of the studied element. In our project, we aimed, for two foraminiferal species: (i) to better understand the drivers involved in the incorporation of Mn (and other trace elements) in biogenic calcite, (ii) to detect potential different speciation of Mn in calcite according to changes in the seawater [Mn] and/or species. For this purpose, several specimens of both species (*Ammonia*

*tepida* and *Bulimina marginata*) calcified in different seawater [Mn] were cleaned with NaOCl to removed the cytoplasm, embedded and polished before analyses at the ID21 beamline.

## Experiments performed :

### $\mu$ XANES of reference material

The aim of the XANES analyses was to determine the chemical speciation of the incorporated Mn in the calcite lattice, and if this speciation changed according to species, to the seawater [Mn], and/or to high/low Mn banding within the calcite. Part of our time on the beamline was first dedicated to acquire XANES spectra on reference materials with different oxydation states of Mn and coordination (Mn foil, Mn chloride, Rhodochrosite, Manganite, Manganese oxide...) for further comparison with the spectra acquired on foraminiferal calcite.

### $\mu$ XRF and $\mu$ XANES on *Ammonia tepida*

For  $\mu$ XRF mapping, we first performed analyses with an energy of 6.64 keV on both species, several specimens per species calcified in different [Mn]. Secondly, as preliminary analyses, we performed low energy XRF mapping (2.5 keV+ 3<sup>rd</sup> harmonic at 7.5keV) in order to investigate the distribution of Mn along with the other elements (e.g. Mg) which have shown to be heterogeneously distributed in previously published articles.

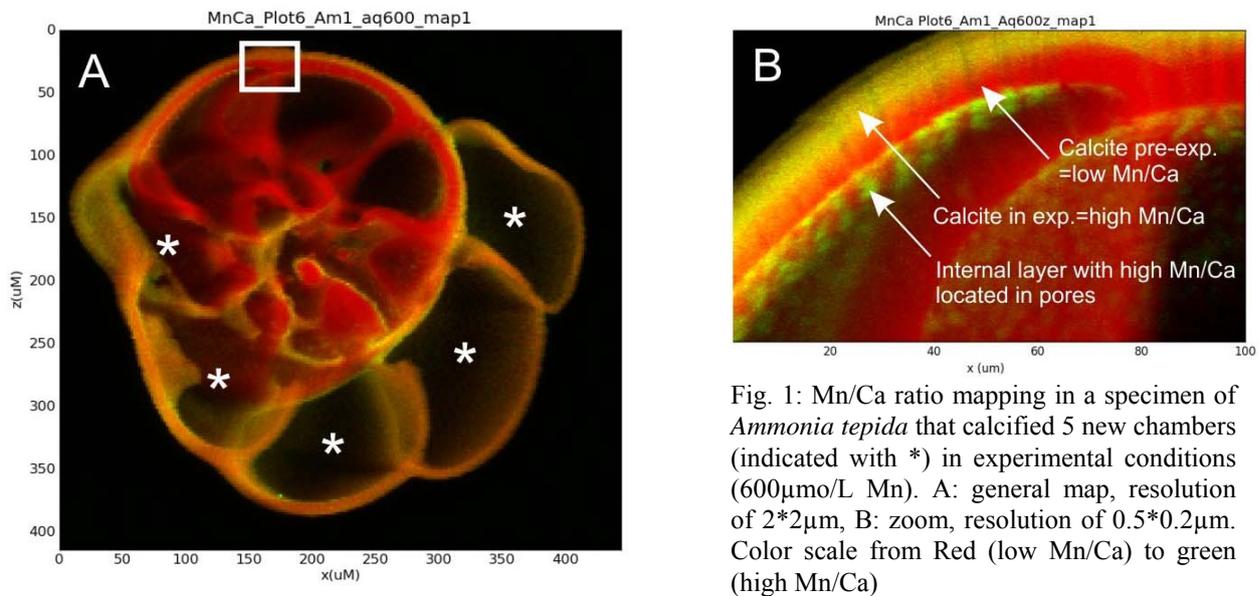


Fig. 1: Mn/Ca ratio mapping in a specimen of *Ammonia tepida* that calcified 5 new chambers (indicated with \*) in experimental conditions (600 $\mu$ mo/L Mn). A: general map, resolution of 2\*2 $\mu$ m, B: zoom, resolution of 0.5\*0.2 $\mu$ m. Color scale from Red (low Mn/Ca) to green (high Mn/Ca)

For *A. tepida*, we could undoubtedly identify the part of the shell calcified under our experimental conditions that were enriched in Mn (high Mn/Ca) compared to the former calcite (low Mn/Ca) (Fig. 1A). Moreover, we could identify a layer of high Mn/Ca ratio covering the internal part of the shell (Fig. 1B) and particularly inside the pores which are known to have a role in gas exchange between the foraminiferal cell and the surrounding environment. This internal high Mn layer was observed in all the seawater [Mn] tested although it seems to present a more patchy distribution at the lowest concentrations.

On the one hand, all the XANES analyses acquired in both low Mn/Ca (calcite pre-experiment) and high Mn/Ca (calcite formed in the experiment) calcite layers, show similar spectra close to the one of the MnCaCO<sub>3</sub> (Fig. 2). On the other hand, the internal enriched layer was characterised by XANES spectra close to the one of MnSO<sub>4</sub> and correlates with Mg, P and S enrichments (identified thanks to low energy  $\mu$ XRF). (Fig. 2). These results suggest that this layer could be related to organic material, or at least that Mn exhibits a different speciation than in calcite.

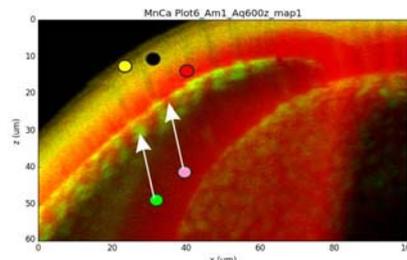
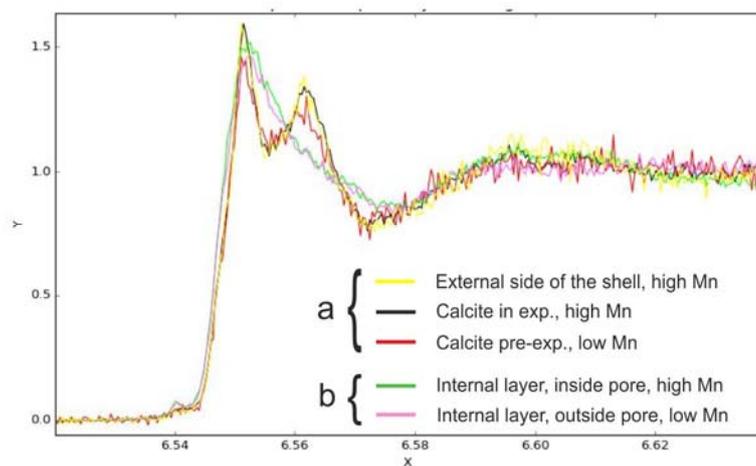


Fig. 2: Average spectra of XANES analyses performed in the same specimen of *Ammonia tepida* than in Fig. 1 showing 2 different speciation for Mn (type a and b). See map for location of the sites of interest.

Further process of XRF maps will allow to investigate the Mn concentration in this internal layer according to the [Mn] of our experimental conditions. In order to define if this layer corresponds to the cytoplasm's remains or come from the cleaning procedure applied to the shell before embedding, we would need to test different type of cleaning solutions (e.g. NaOCl, H<sub>2</sub>O<sub>2</sub>) and for different duration. This question is important since usual elemental analyses are performed on bulk samples or with LA-ICPMS measurements; therefore we need to identify the contribution of this high Mn layer on the global Mn/Ca ratio of calcite.

For *A. tepida*, we also observed a high Mn layer located on the external side of the suture (calcite wall between 2 chambers). This layer is different from the one described above since the XANES spectra are closer to the spectra from the calcite itself (type "a" in Fig. 2) than from the "organic" internal layer (type "b" in Fig. 2).

### **μXRF and μXANES on *Bulimina marginata***

For *B. marginata*, which incorporated more Mn in the experiments than *A. tepida*, the internal enriched layer was also observed but it was more patchy and thinner. The thickness of the secondary calcite layer (i.e. the covering of the previous chambers with calcite formed in the experimental conditions) is also thinner than the one observed in *A. tepida*.

Moreover, it was possible to observe bandings of high and low Mn/Ca within the calcite thickness (Fig. 3). Although this feature was observed in other studies (tropical and planktonic species) for other elements (Mg and B) [e.g. 3, 4], it is the first observation done for Mn and particularly for temperate benthic foraminifera. There is no obvious difference in the μXANES spectra acquired in these different bands suggesting that Mn is incorporated under the same form regardless the Mn concentrations. However, we had not enough time (and specimens) to validate and confirm that this feature is observed systematically and in all conditions.

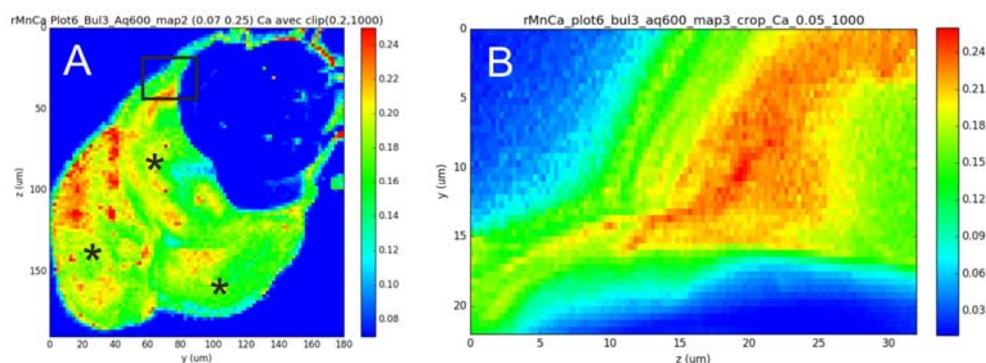


Fig. 3: Mn/Ca ratio mapping in a specimen of *Bulimina marginata* that calcified 3 new chambers (indicated with \*) in experimental conditions (600 μmo/L Mn). A: general map, resolution of 2\*2 μm, B: zoom, resolution of 0.5\*0.3 μm.

### **Conclusions and perspectives**

We acquired a very high quantity of data using μXRF (high and low energies) and μXANES during this 9 shift at the Beamline ID21. Initially we required 15 shifts, but only 9 shifts could be allocated to promote the proposal from the reserve list. The quantitative analyses of the spectra are under progress (using ATHENA) in order to precisely evaluate the similarity between the XANES spectra obtained in the calcite itself and in

the internal high Mn layers. To better constrain the chemical speciation of Mn in these sites of interest, we will work on the comparison with the reference material spectra measured during the session and probably try to obtain further reference spectra. Further treatments and optimisations of the Mn and other elements maps obtained during the session are still needed using PyMCA to finalise the total understanding and interpretation of the recorded data.

Finally, our preliminary results show undoubtedly that synchrotron-based methods are very promising tool to better understand the incorporation of chemical elements in biomineralisation processes for the development of paleoceanographical proxies, although there have only been used very little until now in micropaleontology and paleoceanography compared to other fields.

However, the beamline ID21 show some limitations for the purpose of our study. The spatial resolution of the electron beam ( $0.3 \times 0.7 \mu\text{m}$ ) is probably not small enough to detect thin banding layers in the distribution of Mn in the foraminiferal wall thickness. Moreover, the main limitation comes from the high penetration of the beam inside the samples which is, in addition, different according to the element considered (i.e. 10 to  $50 \mu\text{m}$ ). Therefore, although the spatial resolution is interesting for preliminary results, it is blurred by the depth resolution.

In perspective, we would like to realise thin sections ( $\sim 1 \mu\text{m}$ ) of the samples in order to solve the problem of depth resolution using beamline ID21 and to try to perform the measures by transmission instead of fluorescence. We are although planning to apply at the beamline ID16A to improve the resolution and get nano-XRF maps in order to investigate the Mn/Ca potential bandings in the  $10 \mu\text{m}$  thick shell. Finally, the speciation of the element using the  $\mu\text{XANES}$  available on ID21 is a very powerful tool and we would really like to go further in this direction focalising on elements such as Sulphur which is an innovative proxy for seawater carbonate chemistry.

**References:** [1] Glock et al, 2012. *Biogeosci.*, 9, 341-359. [2] Groeneveld & Filipsson 2013. *Biogeosci.* 10, 5125–5138. [3] Branson et al. 2013. *EPSL* 282 134-141. [4] Branson et al. 2015. *EPSL*, 416, 67-72.