	Experiment title: Caught in the act (of mineralization): in-vivo study of the shell development in pearl oysters	**Experiment number**: EV 395
Beamline: ID13	**Date of experiment**: from: 03.03.2021 to: 08.03.2021	**Date of report**: 26.08.21
Shifts: 9	**Local contact(s)**: Manfred Burghammer, Michael Sztucki	*Received at ESRF:*

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Report:

Summary:

The aim of this experiment was the in-vivo observation of mineralization in calcifying animals. In our original proposal, we propsed to observe the pearl oyster (Pinctada margaritifera) shell, for which we have developed a strong expertise. However, due to the pandemic situation, it was not possible to get access to these species, only produced in French Polynesia. Thanks to our network, we had access to two complementary species, shells of *Crassostrea gigas* and *Stylophora pistillata coral, in order to establish the whole in vivo* Bragg diffraction experiment. The experimental constraints (closed sample cell for the animal and minimized water gap) necessitated to work in reflection geometry and modify the experimental setup of the beamline considerably by introducing a new microscope and a user-supplied in-vivo cell.

The experimental programm comprised establishing radiation damage thresholds of the two species, evaluating the experiental stability of the setup and finally, collecting datasets at subsequent timepoints to follow up on the growth of the animals.

In summary, we could collect data on the early stages of mineralization in *Stylophora pistillata* as well as following the development of the prismatic units in Crassostrea gigas over a span of 8 hours, evidencing a crystallographic change during development.

Samples and setup:

The experimental setup comprised a in-vivo cell for the animal whose design needs to fulfill requirements in terms of stability, available space, diffraction geometry and biocompatibility. It was chosen to 3D print the cell from a biocompatible resin and manufactur x-ray transparent windows to fit the specimens into the cell. The cell was compatible with the kinematic mounting system of ID13 and a constant stream of sea water was supplied by a peristaltic pump. We acknowledge the help of the PSCM for discussions on flow stabilization and supplying a flow dampener for the setup.

In order to check compatibility of the setup with the beamline, a test fitting sessions were carried out in February. The whole setup was implemented the week before the experiment to check the clearance and facilitate the alignment of the new microscope system.
The in-vivo cell was placed on the ID13 EH3 nanogoniometer and was used in a reflection geometry together with the Eiger 4M detector. The x-ray energy was set to 15.2 keV and a set of Si NFLs produced a spot of 280x300 nm^2 with a flux of $9x10^{10}$ ph/s
Fig 1 shows a schematic rendering of the experimental setup.

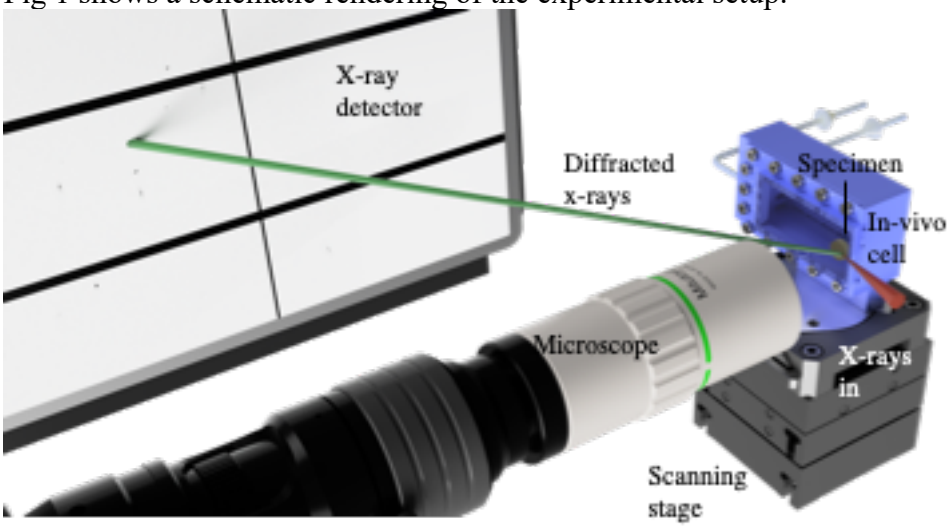

Figure 1 Schematic rendering of the major components of the in-vivo setup

The samples comprised 40 live oysters (Crassostrea gigas) which where kept in four aquariums at the beamline over the course of the experiment. The other sample set comprised 5 corals (Stylophora pistillata) which were grown on Kapton subtrates for 3 month prior to the experiment by collaborators at the Centre Scientifique de Monaco (group of Sylvie Tambutte) and in collaboration with Pupa Gilbert. Due to the sensititiviy of the corals, they were brought directly on-site by the collaboration Monaco group on the third day of the experiment and kept in a dedicated aquarium setup.
Fig 2a) shows the aquarium installation for the corals and Fig 2b) shows a Crassostrea gigas animal in the in-vivo cell during the x-ray experiment

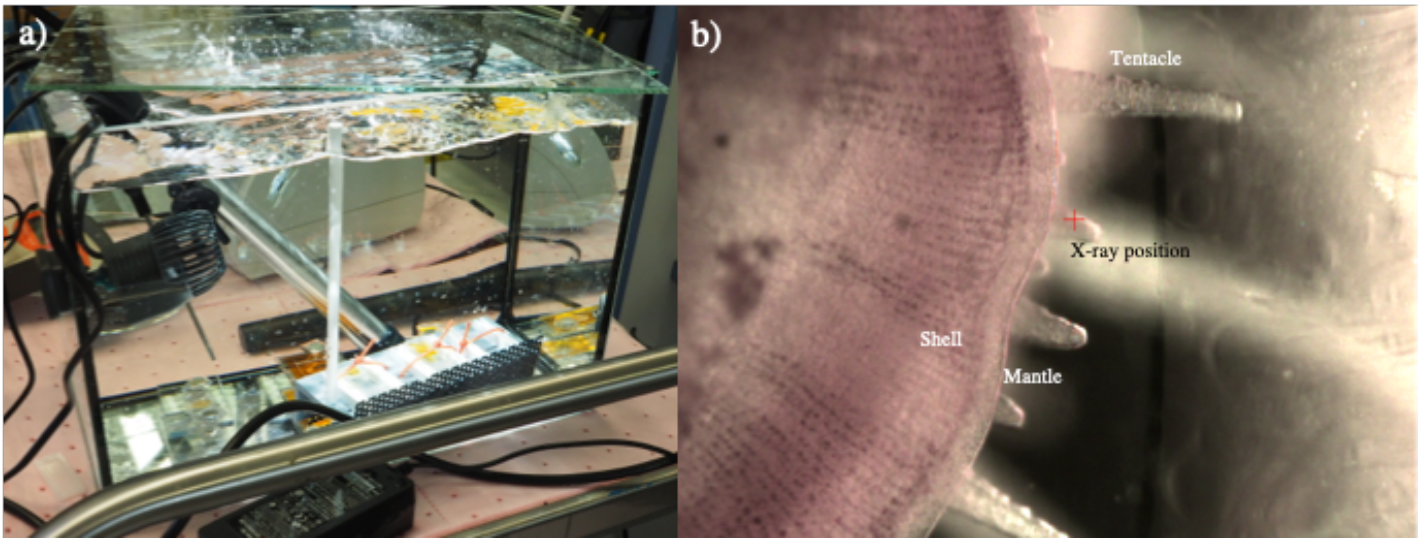

Figure 2 a) Aquarium installation for the corals (on the white sample support) b) A life crassostrea gigas specimen during the x-ray diffraction experiment

Principal outcome:
The principal outcome of this experiment is to establish the feasibility of the experimental nanodiffraction approach and going further to produce insights into a) the early mineralization steps of stylophora pistillata and the involvment of multiple calcium carbonate polymorphs and b) following the shell development of Crassostrea gigas over the course of 8 hours and evidencing the growth crystallograhically . We note that both these

experimental data are unable to obtain with ex-vivo experiments and samples removed from their native environement. Fig 3 shows some preliminary results on Crassostrea gigas.
A careful data analysis has been carried out on the collected data set and we could evidence:
- on stylophora pistillata, the presence of an initial calcite seed crystal before the subsequent growth of the aragonite biomineral.
- on crassostrea gigas, we could observed the slow formation of crystalline domains, way after the initial deposition of the initial bio-mineral precurssor.

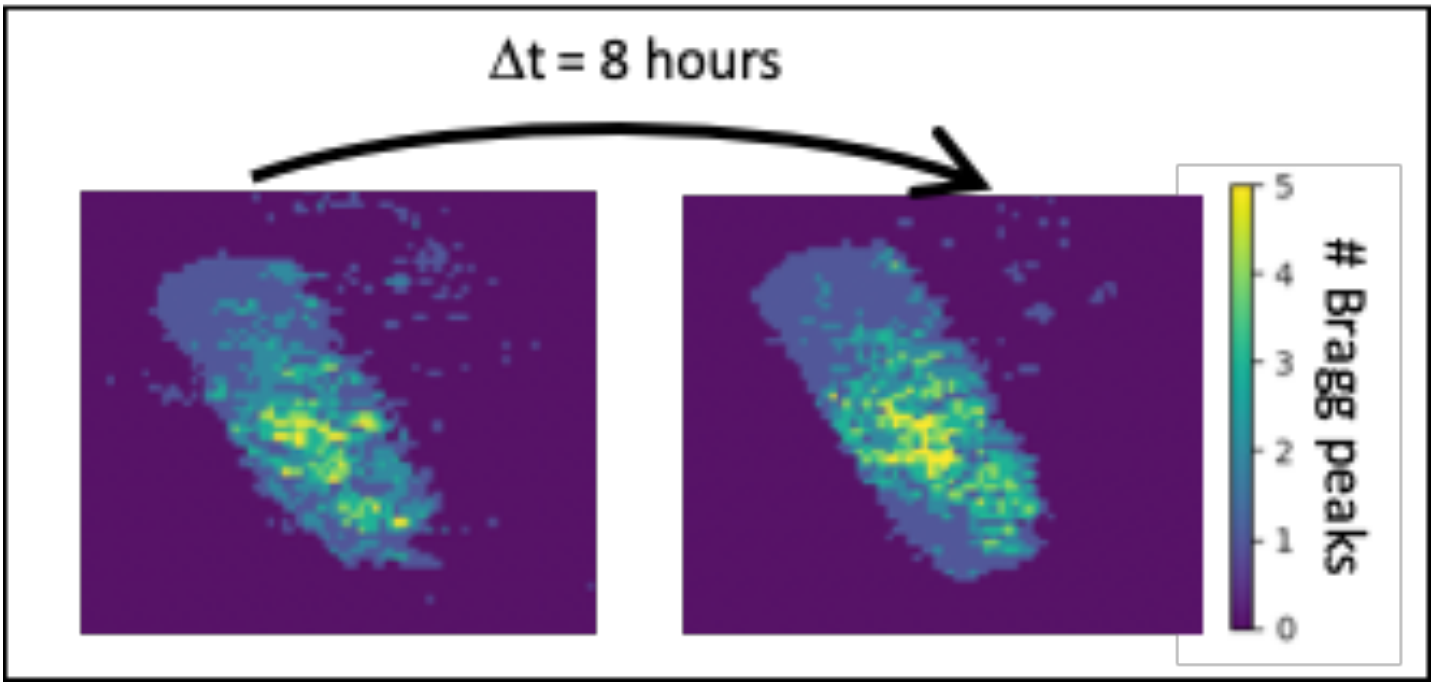

Figure 3 Temportal evolution of the number of Bragg peak components in a Crassostrea gigas prism. A clear increase of Bragg peak components aftr 8 hours is indicative of an ongoing crystallization of the shell.

We conducted first experiments to test the feasibility of coherent diffractive imaging approaches. This experiment aimed assessing the impact of the background (water, window material) on the diffraction pattern as well as establishing the short- and long-term stability of the setup. Our experiments showed that we are able to obtain diffraction patterns with sufficient speckle visibility, but the stability of the current in-vivo cell was not sufficient to carry out extended rocking curves. We identified the pulsation of the water flow as one contributing factor and are confident that a next revision of our cell and pump design can ammeliorate the performance.

Conclusions and further proceedings:
In conclusion, our experiment proofed feasibility of in-vivo diffraction experiments and allowed us to follow up the growth of two different species over time.
A publication reporting these results is in preparation and future experiments that follow-up experiments which exploits the first data we obtained on the two systems are planned alongside with technical improvements to the in-vivo cell and the pumping system. The technical goals here are to be able to combine X-ray fluoresence observation with diffraction experiments as well as increase the experimental stability to conduct coherent diffractive imaging experients on in-vivo systems.
We would also like to point out the great deal of help received from the ESRF staff (user office, safety, PSCM) and in particular the beamline staff of ID13.