SMALL GRANT SG330 REPORT

The small project grant SG330 awarded by the Marine Alliance for Science and Technology for Scotland (MASTS) was used to analyse the crystal structure of fish otoliths subjected to high pCO_2 conditions using Raman spectrometry and scanning electron microscopy (SEM). Originally, the study was planned to be focused on otoliths from Baltic adult cod (*Gadus morhua*) exposed to low (400µatm) and high (1100µatm) pCO_2 for a period of 5 months; however, we were also able to obtain gilthead sea bream larvae (*Sparus aurata*) exposed to different pCO_2 levels (287µatm; 1159µatm; 2650µatm) for a period of 15 days after fertilization. Therefore, the grant was used to make the analyses in both groups of animals.

Raman spectrometry was successfully used to determine the main calcium carbonate crystal polymorph present in otoliths, but unfortunately, the SEM analysis was not doable due to the difficulty to obtain a clear image of the crystals constituting the last-formed ring (edge of the otolith) in the cod otoliths and the impossibility to prepare and mount the tiny otoliths from sea bream larvae.

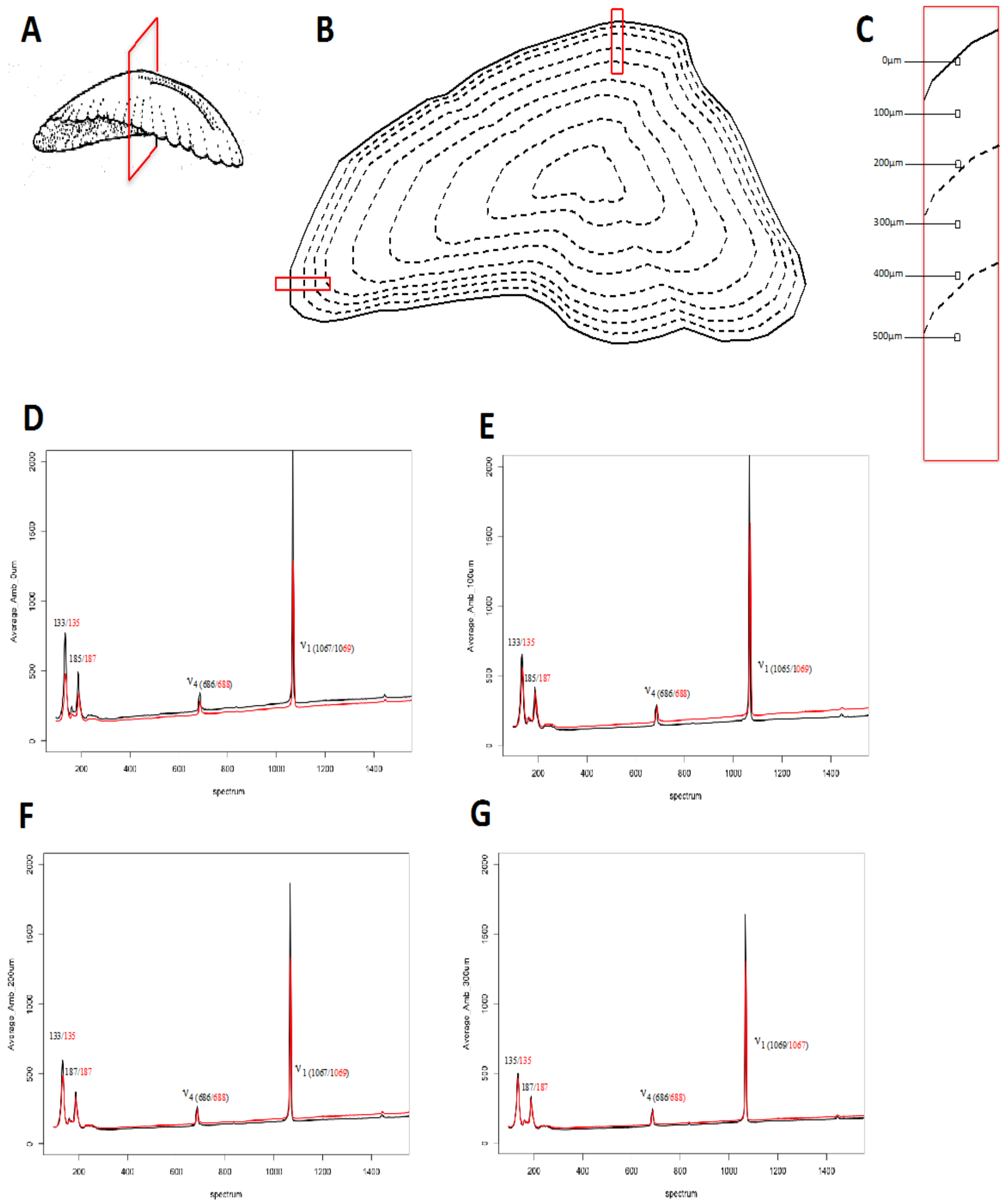
Effect of high pCO2 on adult cod otoliths

Baltic adult cod were exposed to 400µatm and 1100µatm of pCO2 for a period of 5 months. Otolith dimensions (such as *length*, *height*, *area* and *perimeter*) were not significantly affected by treatment. However, we found that some otolith shape parameters were slightly reduced in those animals exposed to 1100 µatm.

Calcium carbonate polymorphs were determined using Raman spectrometry at different distances from the edge of cross-sectioned otoliths (Figure 1). We considered the edge of the otolith (0µm) as the last deposition of calcium carbonate under the experimental conditions, while more internal areas (from 200 to 500µm) were formed during the period the fish lived in the wild (natural pCO_2 levels), which were considered as an internal control (Figure 1). We found that in all areas analysed the main calcium carbonate polymorph in both animal groups was aragonite, the morph normally found in otoliths. Therefore, we concluded that when adult cods were exposed to pCO_2 levels of 1100µatm otolith crystallization is not affected (Figure 1 D-G).

This research was developed in collaboration with Felix Metenmayer and Catriona Clemmensen from Geomar.

Figure 1. Raman spectrometry analysis of adult cod otoliths



A: Adult cod otoliths were cross-sectioned as shown. B: Transversal view of a cross-sectioned otolith. Red rectangles indicate the regions analysed by Raman spectrometry. C: Distances at which Raman spectrometry measurements were taken. D: Raman spectra from otoliths exposed to low (black) and high (red) pCO2 levels measured at 0µm (D), 100µm (E), 200µm (F) and 300µm (G) from the edge of the otolith.

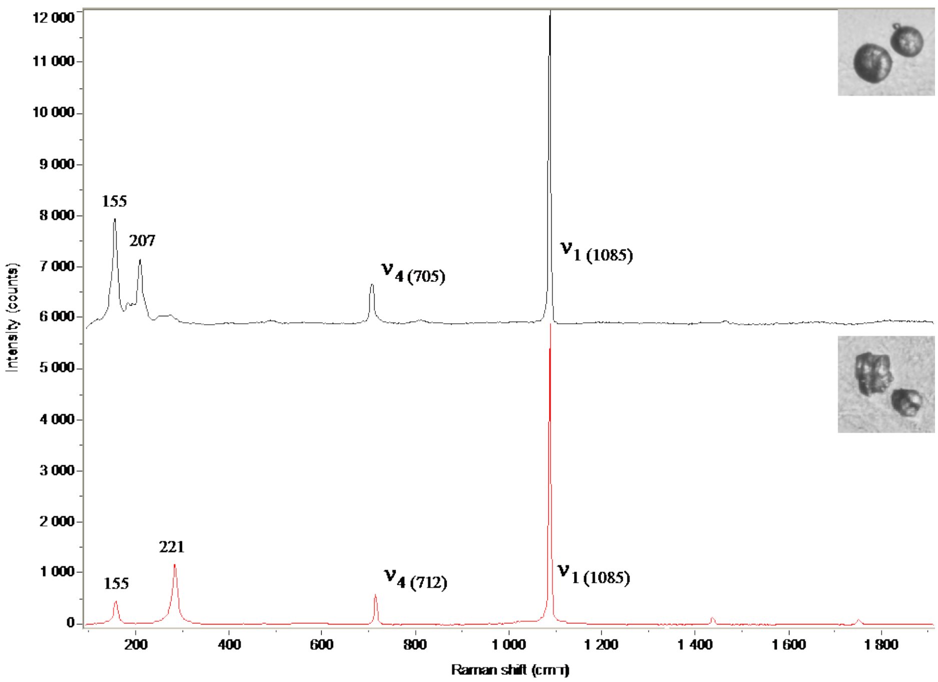
Effect of high pCO2 on gilthead sea bream larval otoliths

Gilthead sea bream larvae were exposed to three different pCO_2 levels (287µatm, 1159µatm and 2650µatm) for 15 days post-fertilization.

We found that some larvae exposed to 2650µatm showed abnormally shaped otoliths (Figure 2), a result never described in larvae in previous studies. In order to determine the main calcium carbonate polymorph, otoliths with normal and abnormal shapes were analysed using Raman spectrometry (Figure 2).

Our results clearly show that abnormal otoliths were formed by calcium carbonate in the form of calcite. This is a very surprising result, not only because it is the first time that it has been shown that high pCO_2 can alter the crystallization of larval otolith, but also because in most of the cases aragonite is replaced by vaterite, and very rarely by calcite. The substitution of aragonite by calcite might have significant implications to the fish physiology affecting their hearing, balance and orientation capacity. This research was developed in collaboration with Jan Giebichenstein and Christopher Bridges from University of Dusseldorf.

Figure 2. Raman spectra of normal and abnormal otoliths



Raman spectra from a normal (black line) and an abnormal (red line) otolith of gilthead sea bream larvae, showing the characteristic peaks of aragonite and calcite, respectively. Typical calcium carbonate v_1 and v_4 peaks are indicated and the shift position of each peak is indicated in numbers. Representative normal and abnormal otoliths are displayed for each spectra.

Small grant main outputs and future directions

The results obtained from our work will be published as two different manuscripts that will be submitted in the following months. The results from the gilthead sea bream larval otoliths will be published as open access to maximize the dissemination of the results. There is a strong possibility that the abnormal mineralization found in larvae might have a strong genetic component and it will be investigated in future research if funding is available.