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SCIENCE FOR A SUSTAINABLE DEVELOPMENT

# CRITICAL EVALUATION OF MARINE CALCAREOUS SKELETONS AS RECORDERS OF GLOBAL CLIMATE CHANGE

## « CALMARS II »

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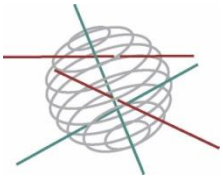

***Climate***

FINAL REPORT (Phase I) Summary

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**SD/CS/02**


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L. André, F. Planchon, F. Dehairs, M. Bauwens, V. Beelaerts, R. Mas, R. Blust, H. Hansen, Ph. Dubois, C. Borremans, Ph. Willenz, P. Gosselin, J. Hermans - ***Critical evaluation of marine calcareous skeletons as recorders of global climate change "CALMARS II"*** Final Report Ph 1. Summary Brussels : Belgian Science Policy 2009 – (Research Programme Science for a Sustainable Development)

During the two first years of the project, the five partners concentrated their efforts in two main directions.

Firstly field work was performed to collect specimens and water samples and to set up continuous monitoring stations.

Sea urchins *Paracentrotus lividus* and water samples were sampled by ULB and RBINSc in different locations around the Mediterranean Sea: Cabo Rasso (Portugal), Marseille (France), Sardinia (Italie), Antiparos (Greece) and stored until analysis.
Starfishes *Asterias rubens* were collected by ULB in April and May 2006 along a gradient of salinity in the Scheldt estuary. Sympatric starfish (*Asterias rubens, Marthasterias glacialis, Echinaster sepositus, Henricia sanguinolenta, Asterina gibbosa, Anseropoda placenta*) were collected in Finistère (Britanny, France) in March and April 2006.

The hypercalcified sponge *Petrobiona massiliana* and seawater samples were collected monthly by RBINSc at La Vesse (Marseille) from June 2006 to January 2008 (20 sampling events). Additional specimen samplings were performed in Sardinia (Italy) and Rhode Island (Greece). Water temperature and salinity probes were installed in all localities for continuous monitoring.

Monitoring of a *Mytilus edulis* colonized site (wave breaker at Knokke) was started in April 2006. For every field survey, the temperature, the salinity, the conductivity, the dissolved $O_2$-concentration and the pH of the seawater were measured. Mussels and seawater were sampled for further analysis. In addition, the analysis of two different datasets was performed: (1) Measurements in *Mytilus edulis* shells collected in Terneuzen, Breskens and Hoofdplaat. (2) Measurements in *Ruditapes philippinarum* shells from the Gulf of Morbihan. Bivalves where dissected after collection of epibionts and hemolymph. Seawater was filtered for analysis of Chlorophyll-a, elemental composition of suspended matter, including major and minor elements and C, N. Filtered seawater was collected for the analysis of Ba, Sr, Mg. The first set of suspended matter and tissue samples has been acid-digested and analysed by ICP-AES and HR-ICP-MS analysis.

Secondly, each partner developed his own experimental techniques, keeping in mind the future utilisation by other participants of the project.

The methodology developments for the analysis of Mg isotopic ratios in biogenic carbonates have been set up in conformity with the initial task program. MRAC focused successively, on state of the art sample preparation protocols and then on the analytical set up of the Multi-Collector Inductively Coupled Plasma Mass Spectrometer (MC-ICP-MS) for Mg isotopes determination. MRAC also set up the analytical procedure on the MC-ICP-MS (Nu instrument) for the reliable determination of Mg isotopes. A measurement routine specific to Mg has been built for the Nu instrument in order to collect and transform the raw data from the different collectors of the spectrometer into isotopic ratios corresponding to $^{26}Mg/^{24}Mg$ and $^{25}Mg/^{24}Mg$.

UA studied the incorporation of key elements in the soft tissues and shells of mussels under different conditions. To do this the transfer of the elements via intercellular and intracellular pathways was studied under controlled conditions. A cocktail of radioactive or stable isotopes of the elements was used as tracers so that the kinetics of the uptake and accumulation processes can be followed and the different pathways can be quantitatively separated.

VUB established a non parametric model (using wood samples as models), enabling to correct for the underestimation of the amplitude variation of a signal linked to the width spanning of a sample as it grows. To resolve the control factors that set the recurrent Ba peaks in bivalve shells, preliminary culturing experiments with different North Sea phytoplankton (diatoms and flagellates) were initiated to identify the factors which control uptake of Ba and other elements.

Sea urchins *Paracentrotus lividus* were grown in aquarium at ULB under controlled conditions of temperature and salinity. Two growth processes were studied: short term regeneration of spines in adults and long term growth of juvenile test. At the end of the experiment, sea urchins were dissected and their skeleton cleaned of associated soft tissues. Newly formed plates of the test were recognized under an epifluorescence microscope using calcein labelling of initial skeleton.

Magnesium partition in the different body compartments of *Asterias rubens* collected on a breakwater in Knokke The uncalcified part of the integument and the skeleton shared similar Mg/Ca ratios. On the contrary, the other uncalcified tissues (gonads, pyloric caeca, coelomic fluid) have a much higher Mg/Ca ratio, closer to that of sea water. This indicates that the discrimination against Mg occurs in the tissues surrounding the endoskeleton. No phylogenetic difference in the skeleton Mg/Ca ratio was detected in sympatric starfishes species living in the same temperature and salinity conditions.

The skeleton of *Petrobiona massiliana* was observed under scanning electronic microscopy (SEM) to understand the morphology of the different structures to be analysed to follow the growth processes. In order to study the biomineralization mechanisms at the cellular level, a protocol of cryofixation of *P. massiliana* in liquid propane (-160°C) was developed, using the newly acquired LEICA EM CPC Cryoplunger and the LEICA EM AFS2 Freeze Substitution Device. The method was first tested on young specimens of the fresh water sponge *Ephydatia fluviatilis* cultivated *in vitro*. Specimens of *Petrobiona massiliana* were also transferred into experimental aquariums to study their skeletal growth under various conditions of temperature and salinity. The growth of *Petrobiona massiliana* specimens was estimated *in situ* by periodic calcein labelling. Sponges marked with calcein before the initiation of this project were collected after one year. An *in situ* regeneration experiment of *P. massiliana* was started in September 2006. At the end of the experiment, concentrations of magnesium (Mg) and calcium (Ca) will be measured in the regenerated parts of the sponges by AL-ICPMS (MRAC) and the $\delta^{18}O$ by mass spectrometry.

The activities of the five partners during the two first years were performed in accordance with the planned program. Activities were coordinated between partners through regular contacts and meetings of the concerned groups.