Cah. Biol. Mar. (2002) 43 : 253-257

Biological versus environmental control on shell chemistry of the vent clam *Calyptogena magnifica*

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Introduction

Dense populations of the vesicomyid clam *Calyptogena magnifica* Boss & Turner, 1986 thrive in active hydrothermal areas of the East Pacific Rise (EPR). *C. magnifica* is strictly dependent on the vent fluid supply through a symbiotic nutritional relationship with chemoautotrophic bacteria (Fiala-Medoni, 1988). Clams live at moderate temperatures where hot vent fluids are mixed with cold ambient seawater. Then, clam shell growth takes place under variable thermal and oxic conditions, mainly depending on the hydrothermal vent activity. Despite the lack of an extensive set of physico-chemical data around the clump of vent bivalves, it was shown that stable isotopic compositions (O and C) and Sr/Ca values of *C. magnifica* shells provide a record of water temperature and seawater chemistry in agreement with the mean values measured in situ (Rio et al., 1992; Hart & Blusztajn, 1998). Such a record would be useful for studying environmental exposure or biomineralization processes.

The aim of our study is: *i*) to investigate spatial and temporal variations of shell chemistry by measuring trace element (Mg, Sr, Mn) and stable isotopic ($\delta^{18}O$ and $\delta^{13}C$) variations along a growth axis, *ii*) to discriminate between physico-chemical and biological causes of these chemical variations.

We investigate the impact of physico-chemical conditions on shell chemistry by comparing trace elements with O and C stable isotopes, which are traditionally used as temperature and metabolic proxies.

Material and methods

Living specimens of *Calyptogena magnifica* were collected in 1982 on the East Pacific Rise (EPR 21°N, 2600 m water depth, 5 specimens) and in 1985 on the Galapagos Spreading Center (GSC) (Rose Garden vent field, 2450 m water depth, 4 specimens). The shells were thoroughly cleaned and rinsed with de-ionized water to remove any external organic material and prevent sample contamination. Two series of 10 mg-samples of quasi-pure carbonate powder (all aragonitic) were collected from each specimen with a 0.5 mm dental drill.

We drilled first consecutive grooves about 1 mm deep in the outer shell layer, avoiding the inner shell, and collected the powder of each groove (Fig. 1a). This procedure provides a time-series of shell carbonate deposited during the life of the organism. Because of the dissolution of the outer layers in the oldest part of the shell, samplings were conducted only up to 2 or 3 cm from the ventral margin, where shell thickness is between 2 and 5 mm.

Then, we sampled large areas on the inner shell surface, paying careful attention to be as superficial as possible (Fig. 1b). By this way, the carbonated layer sampled corresponds to a short period of calcification. It was then possible to examine the modification of the shell chemistry according to the distance to the ventral margin.

Each sample was splitted into two fractions. The first fraction (~10 mg) was digested with 6% ultrapure nitric acid at 90 °C in Teflon reactors to determine Mg, Sr and Mn concentrations. Reactor contents were diluted in 25 ml of ultrapure water before analyses by absorption spectrometry (FAAS and GFAAS). The second fraction (~1 mg) was reacted with 100% orthophosphoric acid at 50 °C to measure stable isotope ratios ($\delta^{18}O$, $\delta^{13}C$). Results of the isotopic analyse of the CO_2 gas are presented in permil notation with respect to the PDB carbonate standard. The standard deviation of replicate carbonate standards was 0,1‰ for $\delta^{13}C$ and 0,2‰ for $\delta^{18}O$. These skeletal $\delta^{18}O$ values and a seawater $\delta^{18}O$ value of 1.3‰ (Fatton et al.,

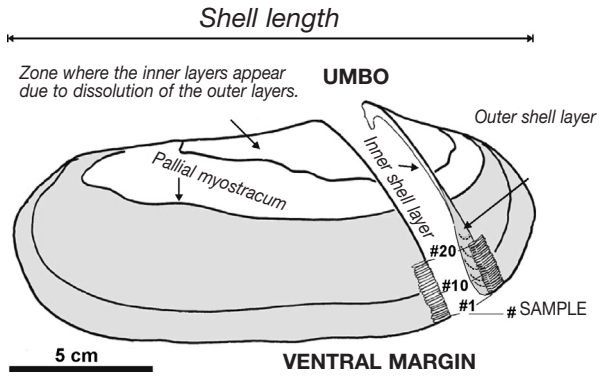

Figure 1a. Example of a *C. magnifica* shell which has been drilled incrementally along the height axis (shell height is perpendicular to length). The shell has been cut to check that powder is drilled only from the outer shell layers. This procedure provides a time series of shell carbonate.

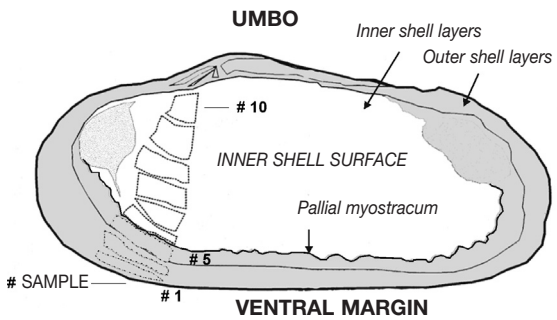

Figure 1b. Consecutive samplings on the inner shell surface. Sampling was as superficial as possible in order to collect a layer formed during a period as short as possible. The mantle located near the ventral margin, which is considered as the main place for length and thickness growth, forms the "outer layers" (shaded area). The mantle located behind the pallial myostracum (muscular fixation of the mantle to the shell) is less active with regard to shell growth. It forms the "inner layers".

1981) were used to predict seawater temperature by solving a biogenic aragonite palaeotemperature equation given by (Grossman & Ku, 1986).

Results

Range of trace metal concentrations

In all cases, the aragonitic shells of *C. magnifica* from active hydrothermal areas are depleted in Mg (30 - 200 ppm) when compared to littoral ones (100 - 800 ppm, Morrison & Brandt, 1986). Sr contents (900 - 2000 ppm) are in the range of aragonitic bivalves (Morrison & Brandt, 1986). The Mn concentrations are very low in outer layers but clearly discriminate the two sites (Fig. 2) (EPR 21°N: average [Mn] = 1,230 ppb, range of [Mn] = 820 ppb; GSC: average [Mn]= 12,260 ppb, range of [Mn] = 7,300 ppb). On the inner surface, Mn concentrations drop below the detection limit (500 ppb, taking into account the dilution effect in acidic solution).

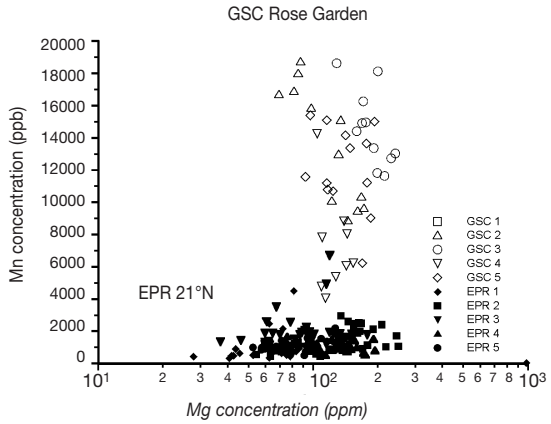

Figure 2. Site effect on trace element composition of the shells (filled symbols: EPR shells; open symbols: GSC shells). Mg concentrations (30 - 200 ppm) are very low when compared to littoral ones (100 - 800 ppm, Morrison & Brandt, 1986). Mn concentrations clearly discriminate the two hydrothermal sites.

Temporal evolution (outer shell layer profile)

Variations in the trace elements content from outer shell layer display sinusoidal patterns depending on specimens and on sampling site. Shells present more important variations for Mg and Sr at EPR 21°N than at GSC. On the contrary, Mn variations are much more important at GSC than at EPR 21°N. The Mg and $\delta^{18}O$ variations covary well but present a relatively short period (i.e., over several millimeters) during which the isotopic evolution does not coincide with Mg variation (Fig. 3). In the shells from both sites, Mg and Sr concentrations increase gradually with the distance from umbo, i.e. with the age of the bivalve (Fig. 4a, 4b). In the shells from the same site, trends are similar.

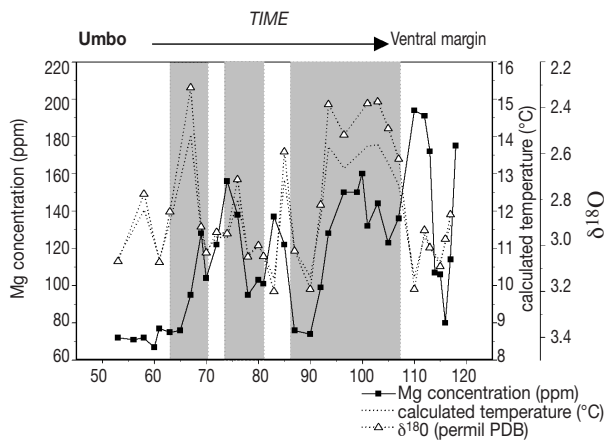

Figure 3. Profiles of Mg (black squares; in ppm) and $\delta^{18}O$ (open triangles; in ‰) in the outer shell layer of a C. magnifica specimen from 21°N (1223-5L). We show also the theoretical temperature profile (doted line), calculated on the basis of the skeletal $\delta^{18}O$ values and the $\delta^{18}O$ temperature relationship given by Grossman & Ku (1986) for biogenic aragonite. The shaded areas show periods when Mg and $\delta^{18}O$ covary. Near the ventral margin (recent part of the shell), the Mg and $\delta^{18}O$ evolutions are opposite.

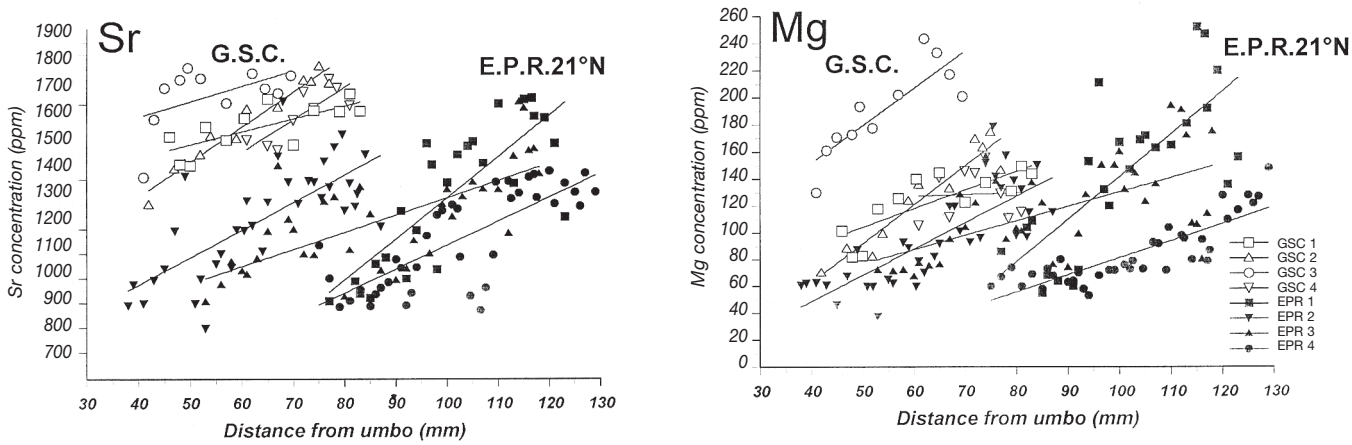

Figure 4. Temporal variations of Sr and Mg concentrations in the outer shell layers of *C. magnifica*. All profiles show a gradual increase with age. Specimens from East Pacific Rise: filled symbols (EPR 21°N N=4), specimen from Galapagos Spreading Center: open symbols (G.S.C. N=4).

Inner shell surface

In all specimens, concentrations in Sr and Mg decrease sharply from the ventral margin to the internal zones of the inner surface. Sr decreases by a factor of 2 and Mg by a factor of 5 (Fig. 5a, 5b). A concomitant depletion in $\delta^{13}C$ is observed (Fig. 5c). Along the same axis, $\delta^{18}O$ values decrease gradually about 1‰ from the ventral margin to the internal zones of the inner surface.

Discussion

The water temperatures deduced from $\delta^{18}O$ in shells from EPR 21°N range from 7 °C to 15 °C in accordance with in situ measurements near the shells and 30 cm into the clump of Bivalves (Hessler et al., 1985; see Table 1). For GSC, calculated temperatures range from 12.4 °C to 17.2 °C and are higher than those measured near the clams siphons (Fisher et al., 1988) but are similar to those measured in end-member fluids (see Table 1). It suggests that the shell chemistry records the temperature of the vent fluids at the ground level, inside the clump. The shell chemistry could then be used to investigate the physico-chemical parameter of vent fluids before the rapid mixing with seawater.

The low Mg contents in *C. magnifica* shells are consistent with the in situ measurements, showing the lack of Mg in the mixing water zone due to the supply of Mg-depleted warm hydrothermal fluid of the bottom seawater. However, the skeletal Mg variations cannot be explained exclusively by the fluctuations in the Mg/Ca ratios in seawater: the covariation between short-term increase of Mg and calculated temperature (reflected by decreasing $\delta^{18}O$) suggests that the incorporation of Mg in the carbonate is related to temperature. The use of growth models (Roux et al., 1985; Lutz et al., 1988) allows us to estimate a periodicity approaching several months for short-term variations.

We show that Mn can be used to discriminate two hydrothermal sites (Fig. 2). This observation is in agreement with the enrichment of dissolved Mn in the fluids at the site of Rose Garden vent field compared with those of EPR 21°N (Klinkhammer & Bender, 1980) (see Table 1). Mn concentrations in *C. magnifica* shell may be mainly environmentally controlled. However, our observations do not preclude that intra-specific variations of metal metabolism may have an effect on skeletal Mn content (a more important incorporation at the site Rose Garden), either through metal assimilation and transport or through mechanism of deposition.

The composition of *C. magnifica* shell is then influenced by the physicochemical properties of the surrounding seawater. However, it is clear that biological factors are involved in the control of the carbonate chemistry. The calcification occurs within the extrapallial fluid secreted by the mantle. The composition of this fluid, which is relatively isolated from the external medium, is partly dependant on the intensity of the transport of metals or of metabolic carbon through the mantle. Carbonates secreted at the ventral margin are expected to incorporate trace elements with about the same proportions as in ambient water. On the inner shell surface, the drop of the skeletal $\delta^{13}C$ and $\delta^{18}O$ just behind the ventral margin (i.e., in the region of shell secretion) may result of local changes in the $\delta^{13}C$ and $\delta^{18}O$ of CO_2 and HCO_3^- in the extrapallial fluid due to biological effects. The decrease of about 1‰ in skeletal $\delta^{18}O$ values could not be explained by a temperature effect (calculated dTp = 4.3 °C). The similar evolution of stable isotopes and trace metals (Mg, Sr) supports the idea that the major control of the inner shell chemistry is mostly the metabolism of mantle epithelium which builds the shell carbonate. Such a metabolic control can naturally be related with the functional characteristics of the ventral margin, considered as the place for length and thickness growth (Wilbur & Saleuddin, 1983). On the one hand, the sharp decrease of the Mg concentrations behind the ventral margin, in connection with the intensity of the calcification processes, is not surprising if one considers that Mg is

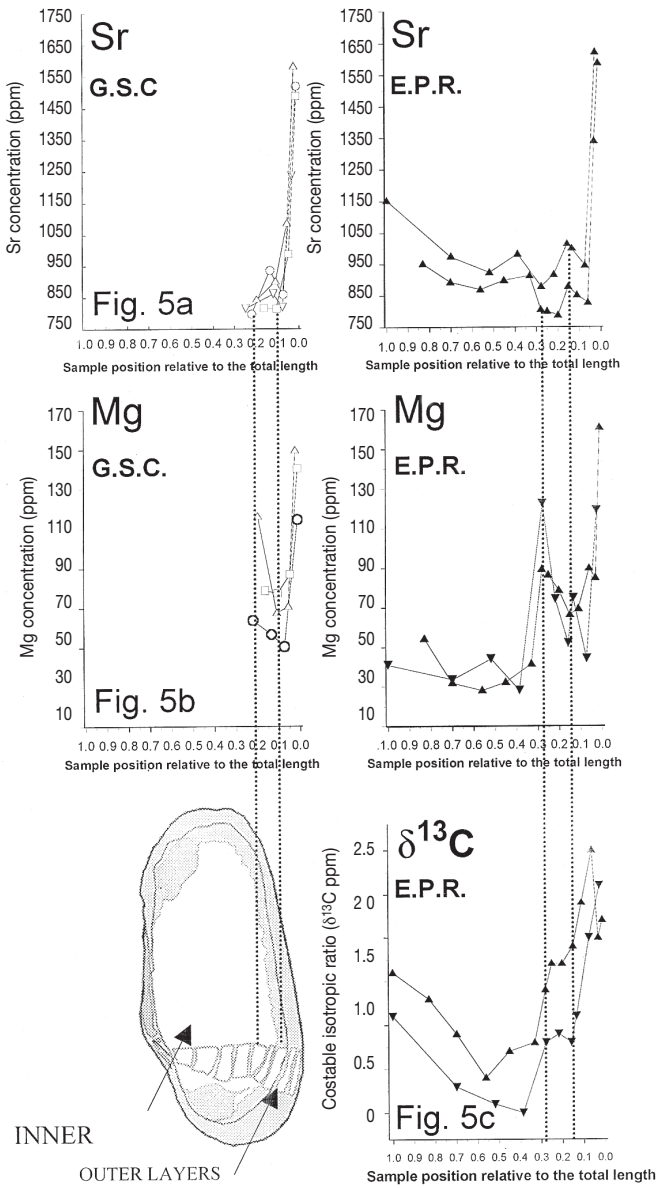

Figure 5a, 5b, 5c. Spatial variability in shell chemistry of the inner surface from the umbo (left) to the ventral margin (right). The x-axis corresponds to the position of the sampling point from the umbo relative to the total length (umbo-ventral margin). This ratio allows us to directly compare chemical profiles obtained on specimen with different sizes. Specimens from East Pacific Rise: filled symbols (EPR 21°N N=2), specimen from Galapagos Spreading Center: open symbols (G.S.C. N=4). The vertical doted lines locate the transition zone (pallial myostracum) between the "outer layers" and the "inner layers".

involved in the energetic cost of the biomineralization (Rosenberg, 1990). On the other hand, the concomitant

Table 1. General characteristics of fluids and bottom sea water.

	GSC bottom seawater	**EPR 21°N**	Abyssal
Dissolved Mn (nmol/kg^{-1})	10 – 15(1)	3.22 – 12.5(1)	0.3(1)
Dissolved Mg (mmol/kg^{-1})	~ 0	~ 0	53
Endmember fluids T_{max}(°C)	20(2)	350(1)	~ 2
Bivalves community T(°C)	2.5<Tp<4.5(2)	2.6<Tp<16.4(3)	~ 2

(1) Klinkhammer & Bender (1980)
(2) Fisher et al. (1988): highest temperature was recorded near the clams shell.
(3) Hessler et al. (1985): highest temperature was recorded about 30 cm inside the clump of clams.

variations of Sr concentrations remain to be clarified. A further characterization of this chemical contrast is expected to reveal a taxon-specific pattern for biomineralization processes. The observed trends on outer shell profile may be reliable to ontogeny. For each shell, regression lines could be determined to propose a theoretical curve corrected for this ontogenetical increase.

Despite hydrothermal fluxes are one of the most important input of Mn in seawater, concentrations of Mn in clam shell are very low. It may indicate the efficiency of the detoxification processes as shown for hydrothermal mussels (Rousse et al., 1998). It may also indicate the removing of large amount of dissolved Mn, which is catalyzed by bacterial oxidation (Mandernack & Tebo, 1993). As a result, Mn would not be under a bioavailable shape for the incorporation in carbonates (only the ionic form Mn^{2+} can substitute the Ca^{2+} in calcium in the crystalline network of carbonates).

Our study suggests that shell chemistry of *C. magnifica* is controlled firstly by metabolic activity of the mantle and secondly by the chemistry of the mixing zone. Although the chemical profiles in the outer shell layers clearly present ontogenetic trend, the part of the shell secreted by the ventral margin is expected to contain valuable records of the environmental conditions over a decade of giant clam life span. A better knowledge of the biological effect on shell chemistry (experimental studies in situ with incubation chambers or in aquarium) would help to decipher the environmental signal and thus allow the reconstruction of the long-term environmental history.

Acknowledgements

This work was supported by a grant from the DORSALE program (CNRS/INSU-CNRS/SDV-IFREMER). We express our gratitude to Pr. A. Mariotti and members of the Laboratoire de Biogéochimie Isotopique (UPMC, Paris 6) for the use of the mass spectrometer and for human and technical support during the analyses.

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