Effects of pCO_2 on production of $CaCO_3$ by skeletal organic matrix in coral cell cultures

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I. Research Context

We examined the production of new $CaCO_3$ and five skeleton organic matrix (SOM) proteins in primary cell cultures of the stony coral, *Stylophora pistillata*. Cell cultures of *S. pistillata* were incubated in four CO_2 treatments (400, 700, 1000 and 2000 ppm pCO_2). Cultures were evaluated for photosynthetic efficiency, calcification and SOM protein expression rates, and mineralogical, elemental and isotopic composition. The primary cell cultures assembled into organic "proto-polyps" precipitating aragonite crystals, which formed on the external face of the proto-polyps and were identified by their distinctive elongated crystallography and X-ray diffraction pattern. The preliminary data show an apparent link between the protein expression and $CaCO_3$ mass accumulation. $CaCO_3$ mass decreased substantially at pCO_2 levels above 700 ppm, which could be related to the apparently inhibited protein expression at high pCO_2 or to re-dissolution of mineral following initial formation in more acidic media. Boron isotope ratios, a proxy for pH, of culture-precipitated aragonite follow the inorganic precipitation line with a relatively constant positive offset, consistent with observations from nubbin cultures, providing evidence for pH increase at the calcification site occurring at the molecular or cellular level.

Stylolophora pistillata

II. Methods

1. Medium & Culture Preparation

•Artificial seawater was equilibrated in a controlled CO2 growth chamber to the desired pCO2 before addition of growth medium (DMEM to 12.5% final volume). Growth medium + seawater was then equilibrated.
•*S. pistillata* nubbins were treated in Ca-free seawater followed by equilibrated growth medium for 1-2 days.
•Dissociated *S. pistillata* cells were filtered to 20 mm and grown in Primaria culture dishes in equilibrated medium for 9 days.
•All samples were grown as independent triplicates for each analysis type.

2. SOM Protein Expression

•Expression rates of a cadherin (GenBank AGG36361.1) were measured by quantitative western blot.
- Standardized to total protein content. Expression fold change was calculated relative to T_0.

•Expression rates of CARP4 and STPCA2 (GenBank AGG36357.1 andACE95141) were measured by qPCR.
- 18S as housekeeping gene and **ΔΔ**Ct converted to expression fold change from 400 ppm pCO_2 T_0.

3. Sr-based Calcification Rates

- Total Sr content of dissolved $CaCO_3$ measured by ICP-MS.
- The distribution coefficient for Sr incorporation into aragonite is used to calculate total mass of carbonate mineral precipitated per culture well.

4. Boron Isotope ratios

•Each cell culture was bleached of organic matrix, and treated with NaOH, with repeated rinses between each step. $CaCO_3$ was preserved by extended centrifugation. Residual organics were floated off with Tetrachloroethylene.
•Carbonate was dissolved in 2 N HNO_3 and micro-sublimated at 85ºC for 12 hours.
•Boron isotope ratios of the sublimate were measured on a Thermo Neptune multi-collector ICP-MS.

III. Preliminary Results

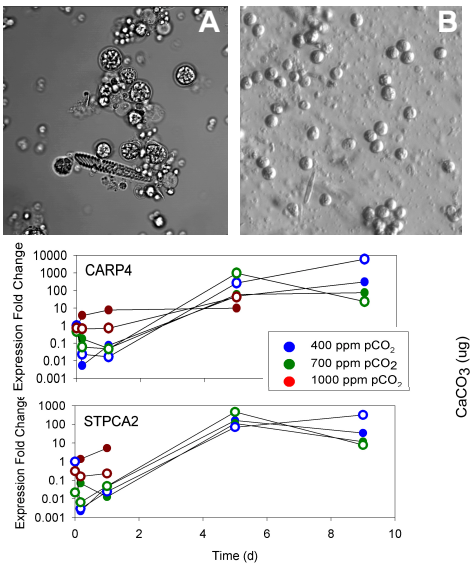

Figure 2. Expression of coral acid rich protein-4 (CARP4) and a carbonic anhydrase previously detected in coral skeleton (STPCA2) in initial, 4hr-, 1d-, 5d-, and 9d-old *S. pistillata* cultures as determined by qPCR. Closed and open circles represent 0.1 mM and 0.2 mM glucose, respectively. Circle coloring follows Figure 3. No CARP4 or STPCA2 expression was detected at 1000 ppm pCO_2 after 5 days.

Figure 1. Nanopolyp formation in 5-day-old *S. pistillata* cultures at 400 ppm (A; 630x mag.) and 1000 ppm (B; ~630x mag.) CO_2. Nanopolyps >10 cells in diameter were frequently observed at 400 ppm, but nanopolyps remained smaller than 5 cells in diameter at 1000 ppm. C. SEM images of columnar aggregates of aragonite crystals formed on the surface of the cell culture (Mass et al., 2012)

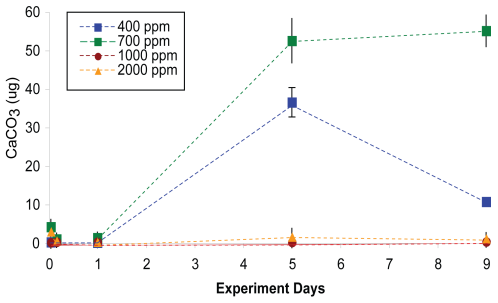

Figure 3. Mass of carbonate per culture well precipitated at each time point based on measurements of Sr content of cleaned samples. Sr is an unequivocal tracer for precipitated aragonite because very little is incorporated into organic material, whereas Ca is biologically active and thus may be biased by small amounts of residual organics.

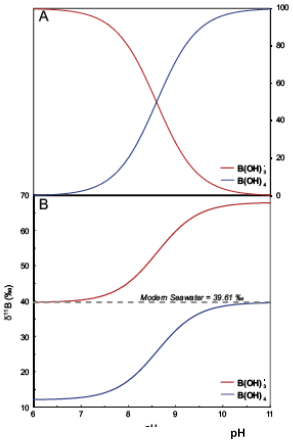

Figure 4. A. Boron speciation between boric acid and borate in seawater with respect to pH at 25°C. B. Isotope value of the boron species in seawater at a range of pH values (After Henehan et al., 2013). At lower pH boric acid is the dominant species. However, borate is the B species understood to be incorporated into precipitating aragonite. At changing pH, the isotope fractionation follows:

$^{10}B(OH)_3 + {}^{11}B(OH)_4^- \leftrightarrow {}^{11}B(OH)_3 + {}^{10}B(OH)_4^-$

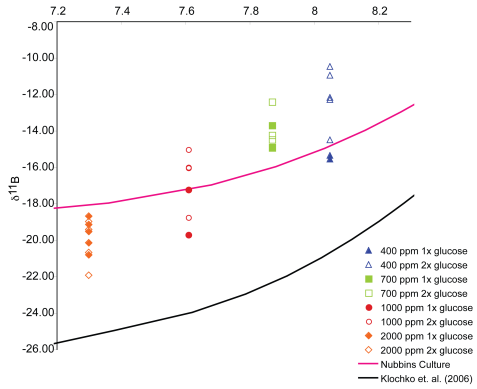

Figure 5. Boron isotope ratio of aragonite precipitated under various pH conditions measured on the in-vitro cultures grown in atmospheres of 400, 700, 1000 and 2000 ppm CO_2. The $\delta^{11}B$ of borate in the artificial seawater is 0 ‰. The $\delta^{11}B$ of the cell culture carbonate is consistent with measurements of skeleton from natural colonial "nubbins" cultures, indicating that chemical modification of the calcification site occurs at a cellular level. The differential offset from the inorganic precipitation line (Klochko et al., 2006) suggests that aragonite precipitation occurs at pH' s (and omega values) significantly higher than that of the ambient seawater. Samples yield precise values at twice the signal of the instrument blank, with an analytical error of ± 0.7 ‰.

IV. Discussion and Future Work

- CARP4 and STPCA2 expression is not significantly different at pCO_2 levels of 400 and 700 ppm. At both levels we see an increased precipitation of aragonite to near-maximum mass in the first 5 days coincident with increasing protein expression. The decrease in $CaCO_3$ between 5 and 9 days at 400 ppm, is coincident with the decrease in protein expression.
- In the 1000 and 2000 ppm conditions significantly less aragonite was measured, which could be related to the apparently inhibited protein expression at high pCO_2 (as manifested by the flat line of CARP4 in the 1000ppm experiment) or to re-dissolution of mineral following initial formation in more acidic media.
- $\delta^{11}B$ is consistent with measurements of nubbins cultures, indicating that chemical modification of the calcification site occurs at a cellular level. The offset from the inorganic precipitation line suggests that precipitation occurs at pH levels significantly higher than in the ambient medium, indicating that CARPs are active in modifying the calcification site.

V. Acknowledgements

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