PIC methods for quantifying calcification

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Indirect calculation of PIC

• Aliquot cultures are filtered onto pre-combusted glass fiber filters (GF/C)

• One of two replicate filter sets are treated with a solution of HCl [e.g., 230 µL of an HCl solution (5 mol/L) added on top of the POC filters] to dissolve the coccoliths and both filters are then analyzed for particulate carbon on a CHN analyzer.

• The concentration of PIC is determined from the difference between the total particulate carbon and the particulate organic carbon concentration.
Direct calculation of particulate inorganic carbon

• Aliquots of cultures are filtered through 0.22 \(\mu m\) polycarbonate filters

• Filters are washed (before and after filtration) with dilute ammonium hydroxide solution (pH ~9) to remove seawater. NaOH is avoided as sodium is used as a proxy of seawater contamination.

• \(\text{CaCO}_3\) is dissolved using 0.4 M \(\text{HNO}_3\) (Romil UpA grade) and keeping the tubes in a rotating platform overnight
Particulate inorganic carbon analysis

• The resulting solution is filtered through 0.45 µm hydrophilic PTFE membranes and analyzed using a Perkin Elmer Optima 4300 DV inductively coupled plasma - optical emission spectrometer (ICP-OES)

• Calibrations are conducted using standard solutions bracketing the range of concentrations measured.

• Sodium concentration was used as a proxy for seawater contamination.
Semicontinuous cultures

Cell density

Time of population development

\[ t_0 \rightarrow \text{Lag} \rightarrow \text{Exponential} \rightarrow \text{Exponential} \rightarrow \text{Exponential} \rightarrow t_n \]
Continuous cultures

Cultures are kept at ~constant conditions

\[ D = \frac{\text{vol. medium supplied/hr}}{\text{vol. the culture}} \]
Normalization of PIC

• Typically, rates are expressed per cell basis (cellular calcification, e.g. pmol C cell\(^{-1}\) d\(^{-1}\)) (biologically relevant)

• Growth rates of the culture under nutrient-saturated conditions are based on cell counts made at the same time of day each day

\[ \mu = \ln\left(\frac{C_{t+1}}{C_t}\right) + D \]

where \(\mu\) and \(D\) are the growth rate and dilution rate (d\(^{-1}\)), respectively, and \(C_{t+1}/C_t\) is the ratio of cell counts on successive days. Adjustments must be made to the dilution rate.

• Normalization to organic carbon should accompany cellular measurements of PIC (biogeochemically relevant)
Effect of CO$_2$ partial pressure on *E. huxleyi* physiology

Short-term CO$_2$ incubations with coccolithophore species

*Batch cultures*

*Riebesell et al. (2000)*
Short-term CO₂ incubations with coccolithophore species

Langer et al. (2006)
Limitations in inferring CaCO$_3$ from Ca$^{2+}$ measurements

• Magnesium tends to substitute Ca in the calcite lattice, forming “low-Mg calcite” when %MgCO$_3$ <4, and “high-Mg calcite” when it is >4.

• Calcifiers incorporate substantial amounts of Mg, which is often produced as MgCO$_3$ (Weber, 1969; Vinogradov, 1953; Chave, 1954; Lowenstam, 1954, 1964; Clarke, 1917).

• The degree of Mg incorporation varies widely amongst different organisms, as well as amongst different skeletal components within a single organism (Ries, 2004).

• Mg incorporation is also known to be largely influenced by seawater Mg/Ca (Ries, 2004) and temperature (Chave, 1954).
Merely calculating the saturation state as:

$$\Omega = \frac{[Ca^{2+}][CO_3^{2-}]}{K_{sp}}$$

of the biomineral (calcite or aragonite) is not adequate for estimating the organism’s susceptibility to elevated $pCO_2$.

CaCO$_3$ calculations from Ca$^{2+}$ measurements could underestimate calcification.
Implications on dissolution of biomineral - susceptibility of organisms to high CO$_2$

- The saturation state of seawater with respect to Mg calcite:

$$\Omega = [\text{Mg}^{2+}]^x [\text{Ca}^{2+}]^{(1-x)} [\text{CO}_3^{2-}] / K_x$$

(Plummer and Mackenzie, 1974).

$x =$ mol fraction of Mg ions, and $K_x$ is the equilibrium constant with respect to Mg calcite (ion activity product at equilibrium since stoichiometric solubility products have not been determined).
\[ \Omega = [\text{Mg}^{2+}]^x [\text{Ca}^{2+}]^{(1-x)} [\text{CO}_3^{2-}]/K_x \]

Mg is five times more abundant than Ca in seawater:

- substituting Mg for Ca in the above equation will effectively increase the ion concentration product for high Mg calcite
- However, this increase is offset by a proportionally greater increase in the solubility product for high Mg calcite.

\[ \Downarrow \]

The solubility of Mg calcite in seawater will exceed that of aragonite when MgCO\textsubscript{3} in calcite exceeds about 11 mole %

[modelling work suggests that Mg calcite with greater than 17 mole % MgCO\textsubscript{3} will be undersaturated in surface seawater by the year 2230 (Morse et al, 2006)].
- Need to report the pH scale to avoid confounding tipping points
- Saturation states need to be more clearly defined and developed within the concept of a species depending on the Mg content
- Avoid possible confounding tipping points at Omega=1
- We need to re-assess if this is relevant for model outputs
Technical recommendation

• Determine co-variation of calcium and magnesium incorporation into biomineral in response to environmental conditions (effect of nutrient availability, diurnal cycles (sampling time), temperature, Mg:Ca ratios).

Future needs

• Characterization of Mg contribution to the biomineral in calcifiers

• Develop new technologies to ‘clean’ chlorophyll fraction and potential cellular contributors to Mg other than the biomineral fraction
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