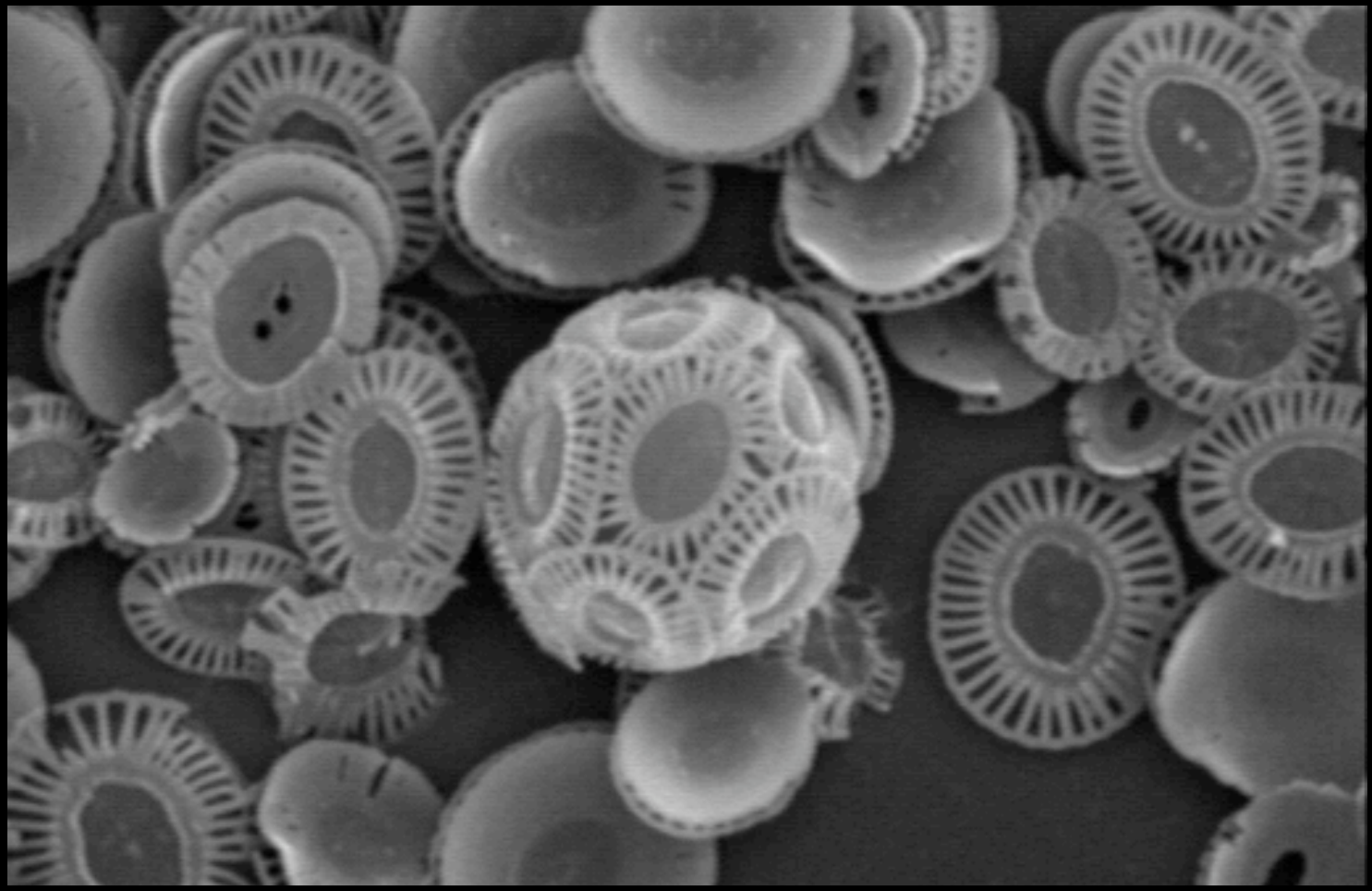
PIC methods for quantifying calcification



Débora Iglesias-Rodríguez
National Oceanography Centre, Southampton.

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 µm 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.

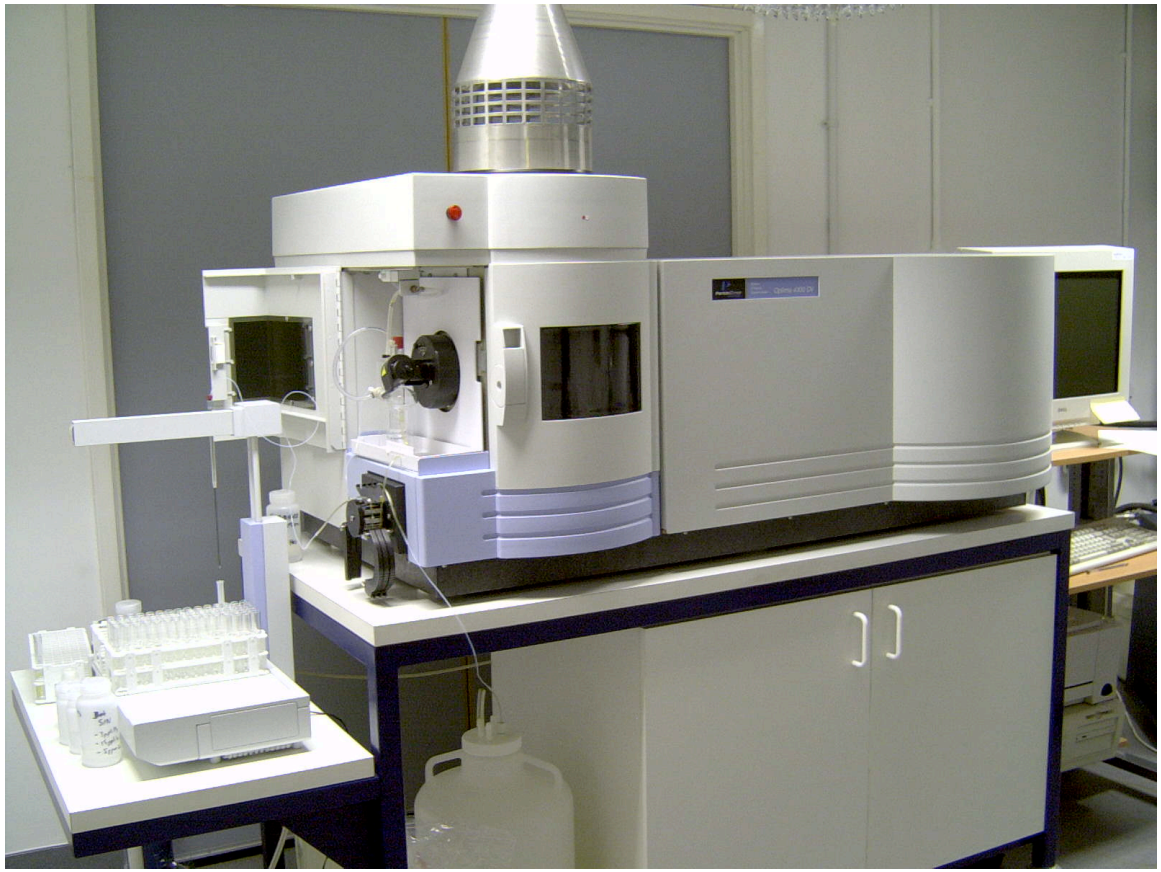
- $CaCO_3$ is dissolved using 0.4 M HNO_3 (Romil UpA grade) and keeping the tubes in a rotating platform overnight

Particulate inorganic carbon analysis

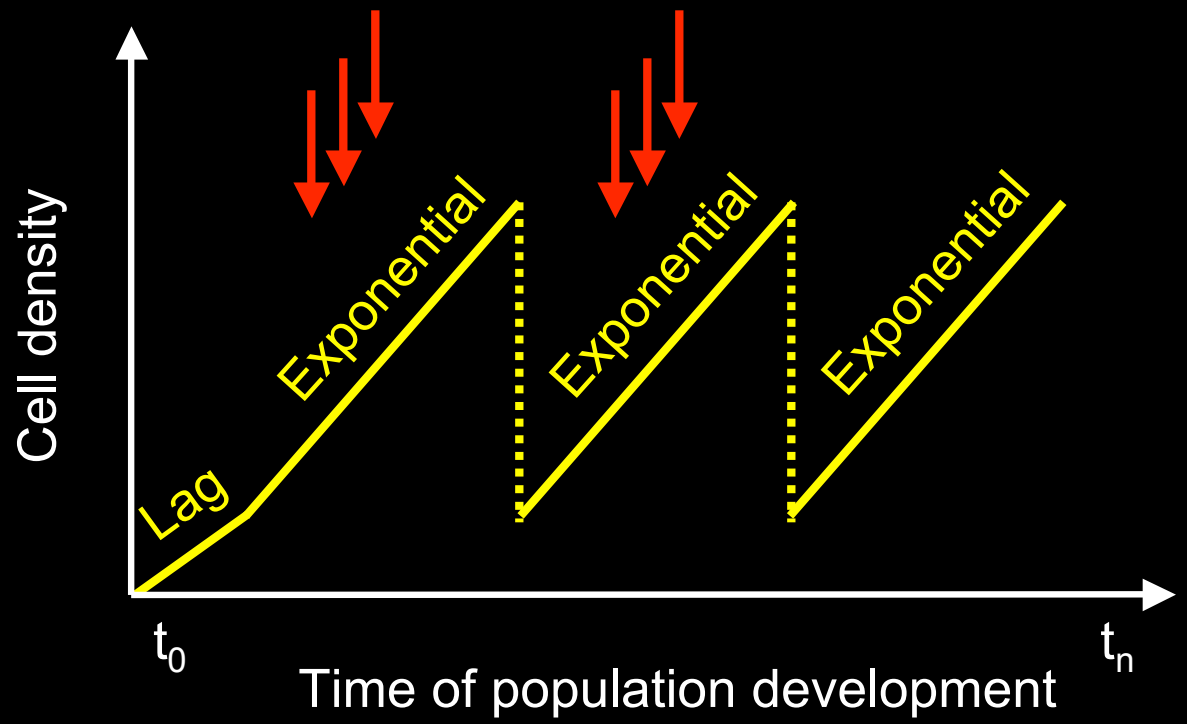
• The resulting solution is filtered through 0.45 µm hydrophilic PTFE membranes and analyzed using a Perkin Elmer Optima 4300 DV inductively coupled plasma - optical emission spectrometer (ICP-OES)

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

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

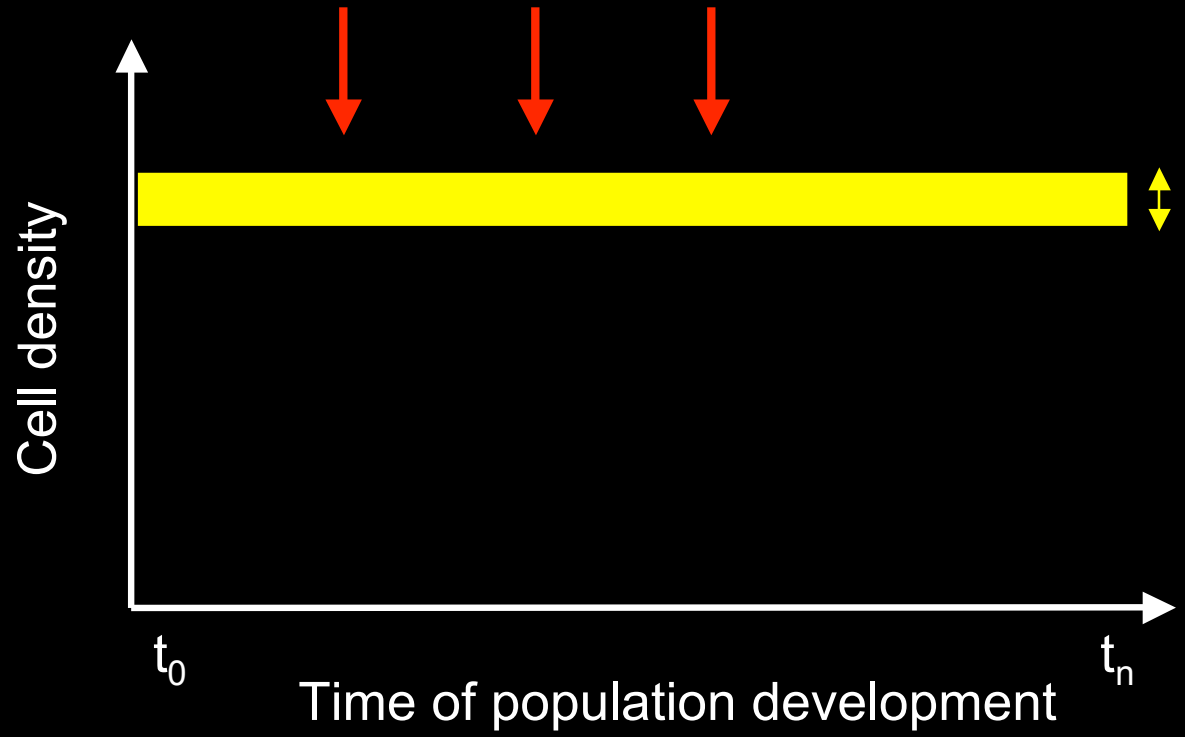


Semicontinuous cultures

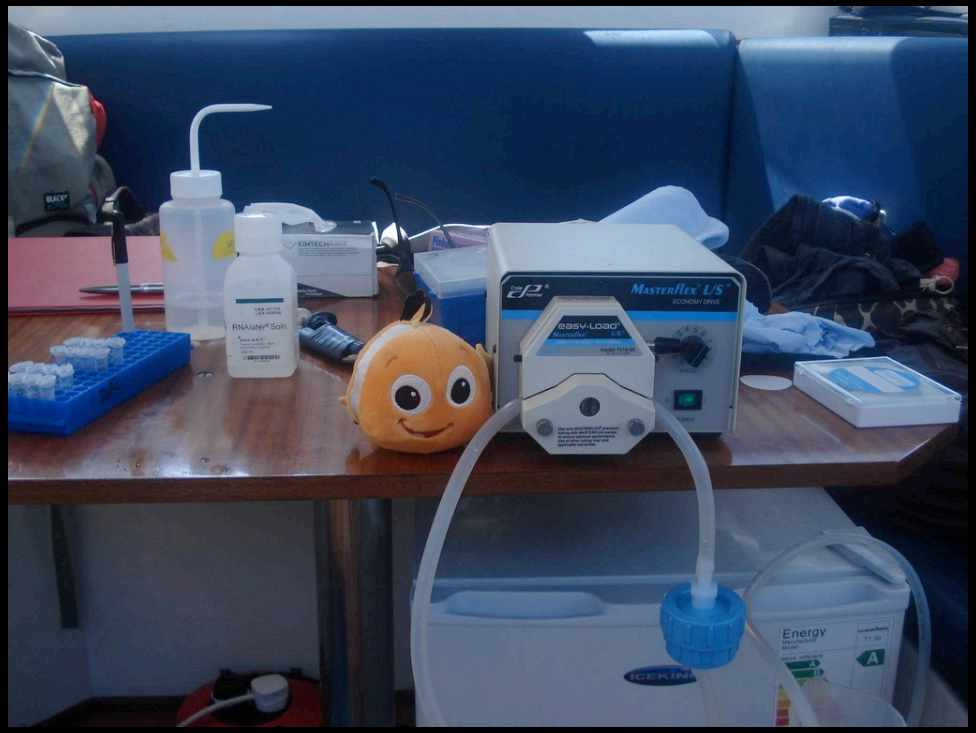


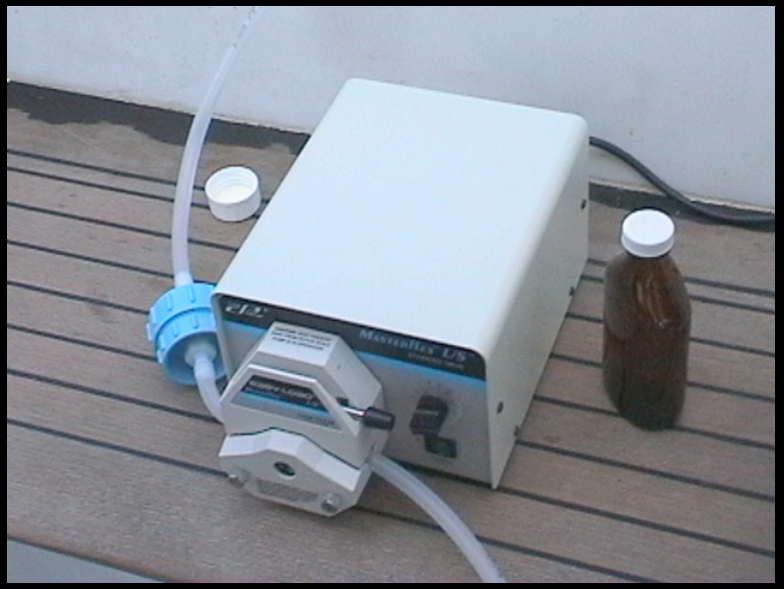
Continuous cultures

Cultures are kept at ~constant conditions



D = (vol. medium supplied/hr)/ vol. the culture


Masterflex L/S
ECONOMY DRIVE
easy-load
RNAlater Soln
Energy


Masterflex L/S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Normalization of PIC

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

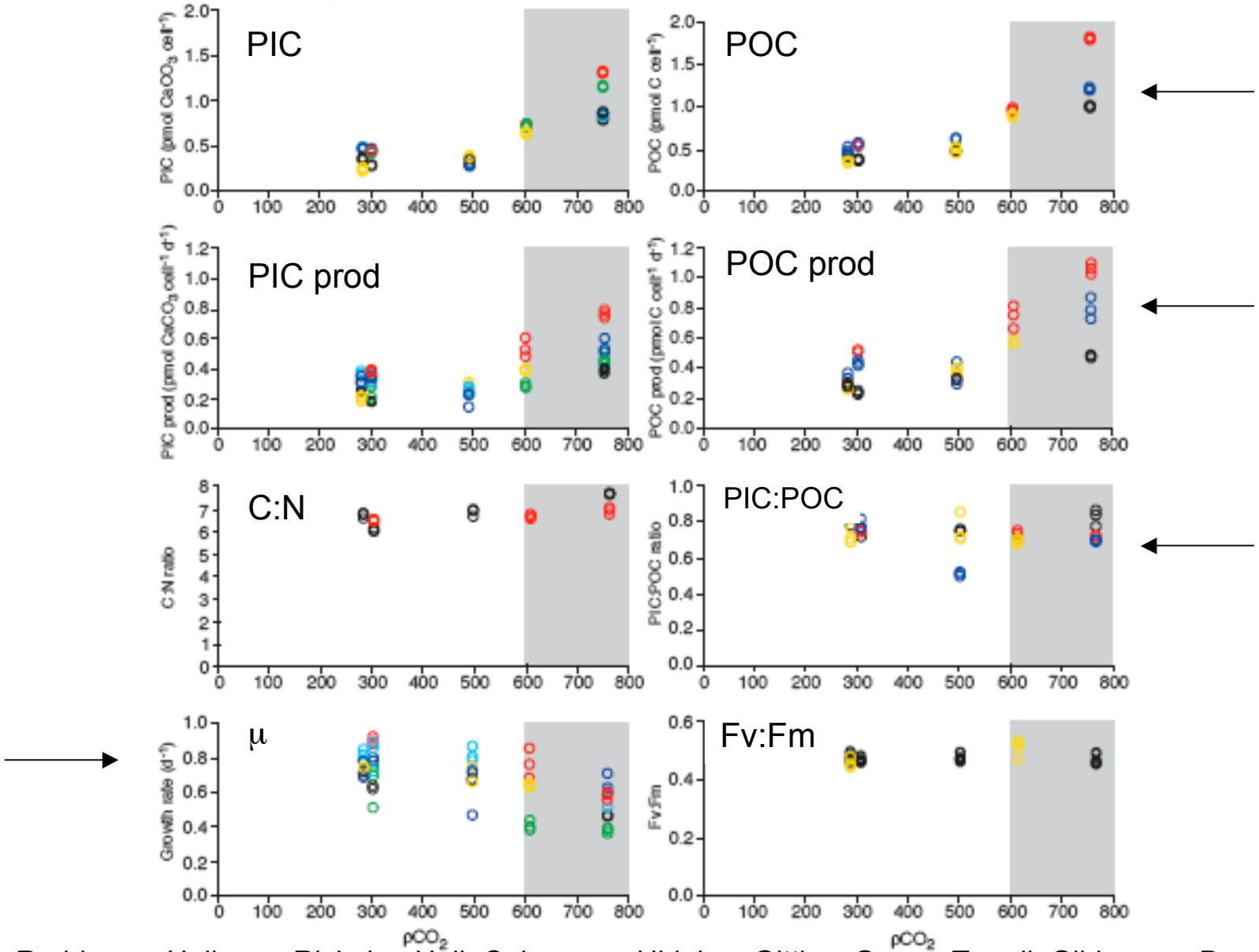
- Growth rates of the culture under nutrient-saturated conditions are based on cell counts made at the same time of day each day

$$\mu = \ln\left(C_{t+1}/C_t\right) + D$$

where μ 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)

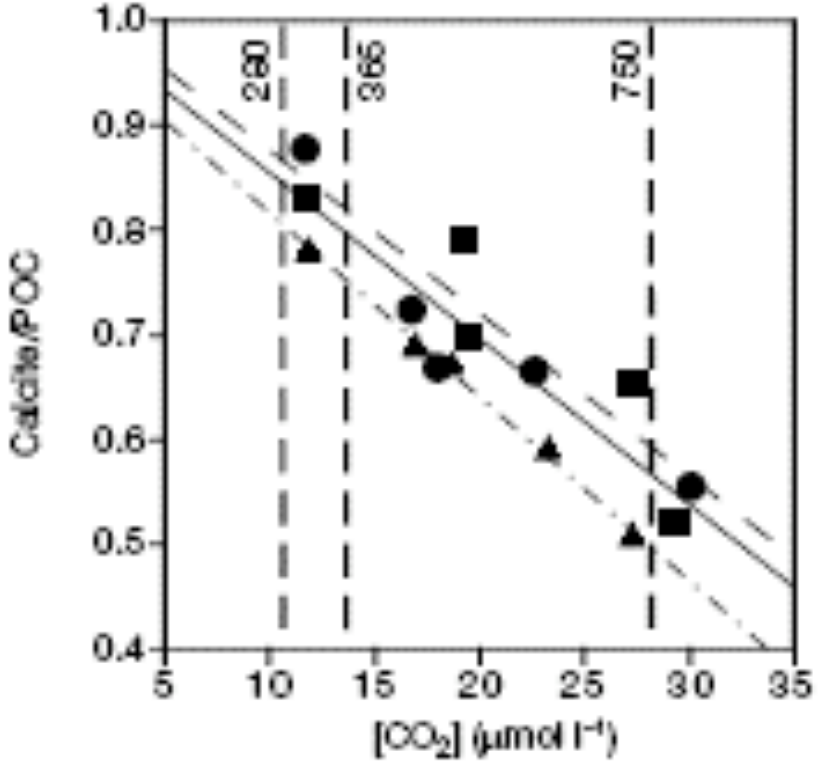
Effect of CO_2 partial pressure on *E. huxleyi* physiology



Iglesias-Rodriguez, Halloran, Rickaby, Hall, Colmenero-Hidalgo, Gittins, Green, Tyrrell, Gibbs, von Dassow, Rehm, Armbrust and Boessenkool, 2008.

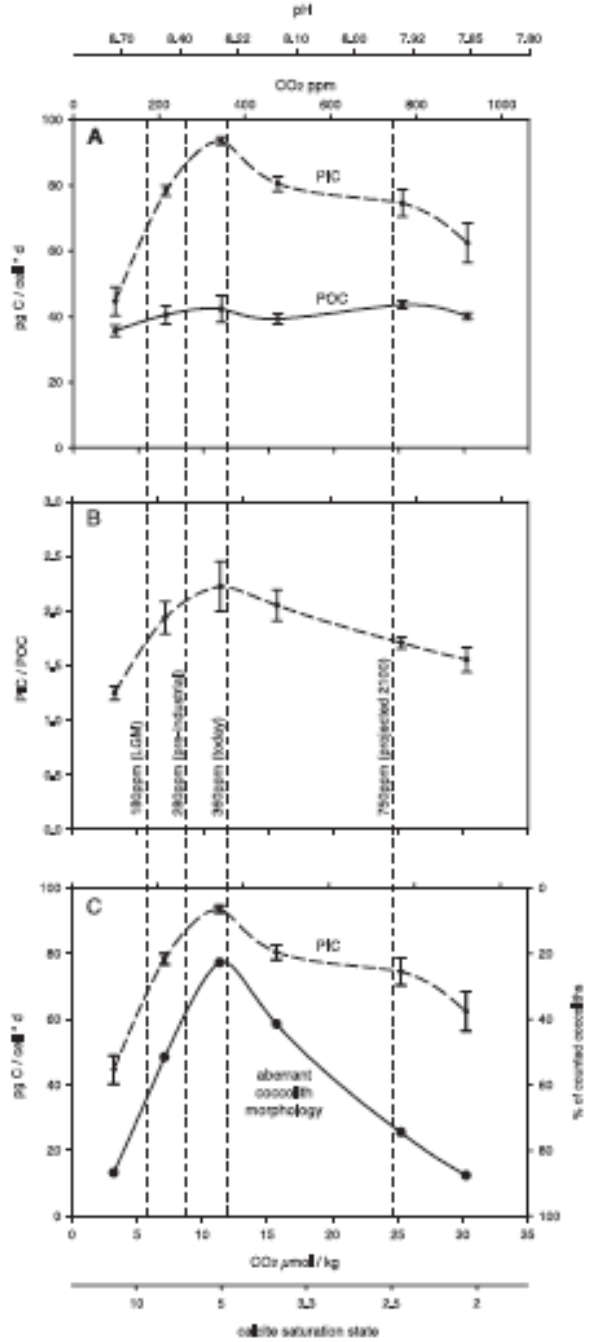
Short-term CO_2 incubations with coccolithophore species

Batch cultures

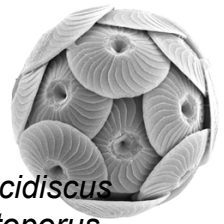


Riebesell et al. (2000)

Short-term CO_2 incubations with coccolithophore species



Langer et al. (2006)



Calcidiscus leptoporus

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:

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

of the biomineral (calcite or aragonite) **<u>is not adequate</u>** 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:

$\Omega = [Mg^{2+}]^{x} [Ca^{2+}]^{(1-x)} [CO_3^{2-}]/K_x$
(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).

$$\Omega = [Mg^{2+}]^{x} [Ca^{2+}]^{(1-x)} [CO_3^{2-}]/K_x$$

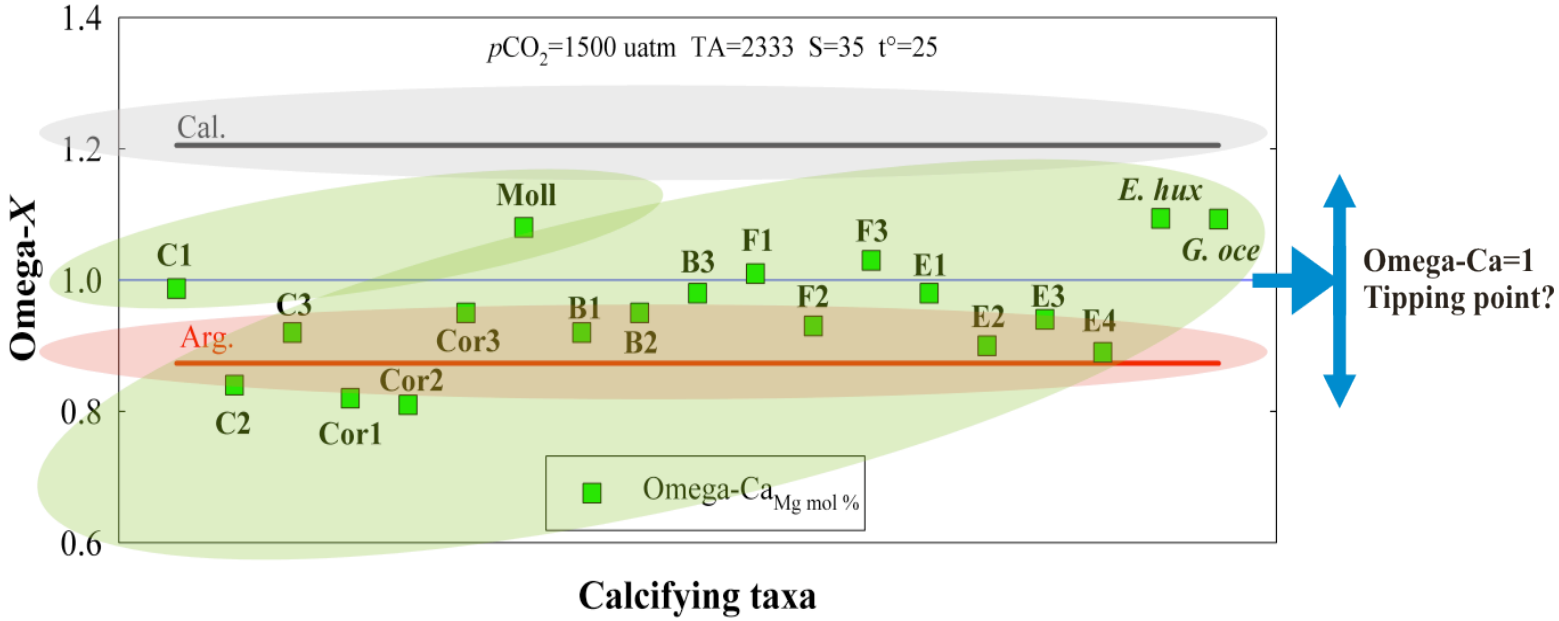
Mg is five times more abundant than Ca in seawater:

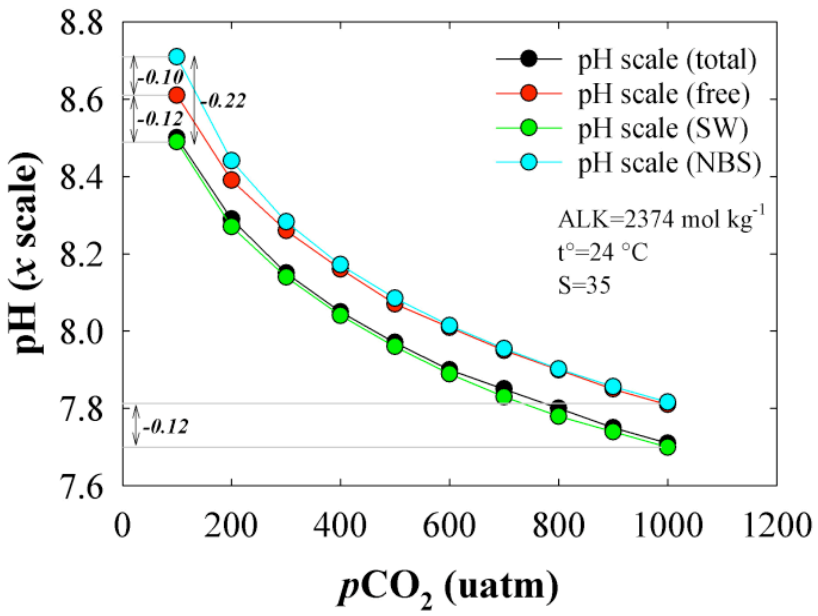
- substituting Mg for Ca in the above equation will effectively increase the ion concentration product for high Mg calcite
- However, this increase is offset by a proportionally greater increase in the solubility product for high Mg calcite.

The solubility of Mg calcite in seawater will exceed that of aragonite when $MgCO_3$ in calcite exceeds about 11 mole %

[modelling work suggests that Mg calcite with greater than 17 mole % $MgCO_3$ will be undersaturated in surface seawater by the year 2230 (Morse et al, 2006)].

Tipping points - WP9 re-analysis (Lebrato et al. pers. Com)





- 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

Thanks to:

- Darryl Green (SOES, NOC, Univ. Southampton, U.K.)
- John Gittins (SOES, NOC, Univ. Southampton, U.K.)