R. E. M. Rickaby[1], ****, J. Henderiks**[2], ***, and J. N. Young**[1]

Perturbing phytoplankton: a tale of isotopic fractionation in two coccolithophore species

Clim. Past Discuss., 6, 257–294, 2010

Overall: Good paper. Please see the final comment regarding the interpretive diagram (Fig 8) especially for Coccolithus braarudii (C.b.). There have been very few attempts to quantitatively model C13 balances, and here you have a great data set for attempting this because you have C13 data for organic and inorganic carbon, plus relative flux rates into organic and inorganic phases. See if you can make a model for C.b. especially, that accommodates all the data. I attempted this with skimpy data in my Coral Reefs 2003 paper, Fig. 6 below (Coral Reefs (2003) 22: 316–327 DOI 10.1007/s00338-003-0325-2 T. A. McConnaughey Sub-equilibrium oxygen-18 and carbon-13 levels in biological carbonates: carbonate and kinetic models)

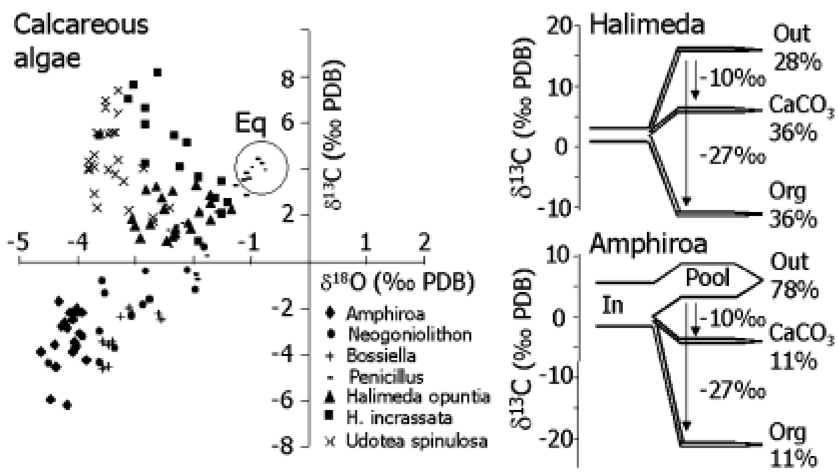

Fig. 6 ^{13}C and ^{18}O in marine algal carbonates analyzed by Lee and Carpenter (2001). ^{13}C budgets for *Halimeda* and *Amphiroa* assume that photosynthesis and calcification withdraw equal amounts of carbon from a common DIC "pool," with ^{13}C fractionations of −27 and −10‰, respectively. Both fractionations involve kinetic effects

Title: Not particularly informative, but cute titles are just fine with me.

Abstract and introduction: well written

p262 L8: 1100, 1600, 2100, 5300 and 7800 μmol kg−1, after which pH was adjusted to 8.13±0.02. Please give estimated CO_2 concentrations after pH adjustment. Was this measured?

P261 L11: nitrate and phosphate concentrations of 100 and 6.25μmol kg−1, respectively Rather lush nutrient soup. Nutrients often seem to suppress calcification, including in coccolithophorids. Likely that there would be more calcification at lower nutrient levels. Were nutrient levels measured? Would nutrients have been substantially depleted under experimental conditions?

P262 L21: the drift in DIC and pH was between 2.35–9% and 0.00–0.08 units for *C. braarudii* and 2.27–9% and 0.00–0.13 units. Was this DIC drift toward NEGATIVE values and pH drift POSITIVE? Also, it is unclear whether DIC drift was -2.35 to -2.39% or -2.35 to -9% (for C.b.).

P263 Eq4: Isotopic fractionation relative to CO_2 implicitly makes assumption that CO_2, not HCO_3^- provides carbon for organic synthesis. Worth stating this. The way Eq 4 is set up, higher positive values of Ep mean more negative $\delta^{13}C$(POC). This reversal is potentially confusing. Why not leave everything in $\delta^{13}C$ units and put $\delta^{13}C$ (CO_2) and $\delta^{13}C$ (HCO_3^-) on the graph for comparison.

P265 L11: Despite seeming adversely affected, calcification rates and photosynthetic carbon Clumsy wording

P265 discussion of uncertainty in cell counts: This is a little distracting. Would it be reasonable to put this in the methods section? Likewise for comparison with Langer et al. (2006) to discussion?

P266 L9: but crucially here, this decrease is only driven by the increasing photosynthetic carbon fixation rate. Good to state this.

P267 L11: The _18OPICtg_18Omedium of *G. oceanic* Find a more intuitive way to represent this. It looks like a comparison to O18 isotopic equilibrium, in which case this is very important. It should be more clear what you are saying here.

P291 Fig 5: This would be clearer if you put O-18 on one graph (with both species) and C-13 on the other graph. Keep shading scheme (open or filled symbols) the same as for previous graphs. The **C. braarudii** data is fascinating.

P268 L5: The distinction between HCO_3^- and $CO_3^=$ based calcification should perhaps go to discussion section. Furthermore, it is not at all clear what this distinction means, or how it might come about. Suggest you drop it. Also drop the suggestion (line 19) of $CO_3^=$ transport. Especially in light of proton transport and pH elevation, $CO_3^=$ transport is unlikely. It just needlessly confuses the situation.

P268 bottom – 269 top: This discussion should be done very differently. It invokes some unlikely physiology, when a much simpler plausible physiology will do. I will try to make suggestions later, particularly if I succeed in figuring out a coherent explanation. (but I am worried that I might not come up with a coherent explanation.)

Fig 6: Possible interpretation:
G. oceanica. Quantitative ppt of DIC into PIC, no C13 isotopic fractionation. If true, then POC comes entirely from a different batch of DIC.
C. braarudii. DIC partitioned between PIC and POC. When PIC gets heavier, POC gets lighter in C13. PIC and POC come from same batch of DIC.
Draw cartoons for both interpretations, showing proton transfer from HCO_3^- at calcification site TO HCO_3^- at photosynthesis site.

Can this relate to O18 in Fig 5?
For C. braarudii, C13 depleted PIC corresponds to O18 depleted PIC. Suggests kinetic effect, most prominent at low PCO2. Maybe the coccolith vesicle is most alkaline under low CO_2 conditions, and absorbs CO_2 from cell to calcify. Seems consistent. At high CO_2, PIC looks close to equilibrium but this needs to be verified.
For G. oceanica, O18 doesn't change much, and always near ?equilibrium? Show equilibrium calculation on graph (maybe based on earlier discussion around P267 L11. If this interpretation is correct, then it seems likely that coccolith vesicle is not particularly alkaline and calcification mainly uses HCO_3^- from environment.

P269 L18: _13CPOCg What's the g?

P270. Agreed that the low Ep values (relative to CO_2) imply CCM or HCO_3^- utilization, which can include protonation of HCO_3^- using protons from calcification.

P271 L7: With the increasing DIC of our experiments, we would expect the leakiness of the cells to decrease since the high DIC creates a gradient able to drive carbon into the cell. Ambiguous. Furthermore it is C uptake mainly by photosynthesis that creates any inward diffusion gradient for CO_2. Calcification is potentially more complicated. If protons from calcification convert HCO_3^- to CO_2 faster than photosynthesis uses CO_2, it might even be possible to create an outward CO_2 diffusion.

P271 L14. Probably right.

Fig 7. Interesting difference in growth response. What's on the X-axis? Looks like specific growth rate (growth rate as from Eq 5, divided by CO_2). The C.b. result seems intuitive, but the G.o. result doesn't. Suggests that G.o. doesn't depend so much on external CO_2.

P271. L26 gn ?
P271 L27. Good. This is a key conclusion regarding C.b.
P276 L25. Good.
P277. L11. Always specify whether you are talking about C13 or O18.
Fig 8. Generally the right idea, but better to re-draw C.b. picture so that vacuoles containing both Ca^{2+} and HCO_3^- are brought into the cell, then split into separate "calcification" and "photosynthesis" vacuoles. The calcification vacuole then exports protons, which are pumped into the photosynthesis vacuole, which exports CO_2. Most of the CO_2 is used in photosynthesis, and some leaks out to the environment, but some also goes into the alkaline calcification vacuole where it contributes to calcification. This is the origin of the isotopic linkage between calcification and photosynthesis, such that heavy carbon in calcification coincides with light carbon for photosynthesis, and vice versa. Alkalinization of the calcification vacuole is also critical to get the CO_2 in, and to account for the O18 depletion in the coccoliths, at low ambient CO_2 levels. (Where did the pH 8.3 number come from? This is quite important.) Note that coccoliths are only O18 depleted at low ambient CO_2.
Also in this figure, you might try drawing it such that Ca^{2+} ATPase simultaneously extracts H+ from the calcifying vesicle while adding Ca^{2+}. Some of the earliest evidence for Ca^{2+} ATPase in calcifying systems came from coccolithophores.