

Interactive comment on “Perturbing phytoplankton: a tale of isotopic fractionation in two coccolithophore species” by R. E. M. Rickaby et al.

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We are very grateful to the two reviewers for their insightful comments. Below we shall detail how we have dealt with all of the comments and the changes made to the manuscript.

1- At the end of the introduction I have been frustrated to not have seen the reason why they studied coccolith calcification in changing DIC at constant pH. I understand this is important “to navigate through the matrices of factors that can affect cell physiology to understand which aspect of carbon chemistry appears to be so detrimental to the coccolithophores”, but it would be interesting to know exactly why. In the abstract we

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see this sentence “to understand different species response to changing pH and DIC” but the pH is not changing.

The pH is not changing in these experiments, but from our conceptual model, we are able to derive implications for the reported literature responses of the two different species to pH as we do in e.g. Figure 8.

We have now added a sentence to the end of the introduction which makes it clear that our experimental conditions of constant pH but changing saturation state and DIC are more akin to geologically buffered carbonate chemistry than the out of steady state changes to pH associated with anthropogenic change, although our more extreme high DIC conditions in the geological record would have been accompanied by a lower pH:

“Such conditions are analogous to the changes in the carbonate system which evolve on geological timescales in response to relative changes in CO₂ from volcanic out-gassing versus weathering, but which are buffered by the carbonate compensation system leading to minimal changes in ocean saturation state (Ridgwell and Zeebe, 2005). Nonetheless, our high experimental DIC conditions with a saturation state of up to 12, are beyond the realm of reconstructed history, where such high DIC conditions are accompanied by a lower pH”

2- Also it would be informative here to tell if the ocean chemistry used in this work can be found in a real ocean (in the past and/or in the future?). If not in the discussion it would be important to mention that the strains survived in a truly artificial conditions.

See response to the above comment

3- They found the calcification differences between two coccolith strains. They extrapolate those differences at a high taxonomic level saying that Noelarhabdaceae are different from coccolithaceae. Strain-specific differences have been found for the Noelarhabdaceae (e.g. Langer, et al., Strain-specific responses of *Emiliania huxleyi* to changing seawater carbonate chemistry. Biogeosciences Discussions, 2009. 6: p.

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4361-4383.). They should add a caution note in their discussion, stating that they indeed studied only two strains.

We have added the following sentence to our discussion:

*“We add the caveat that we extrapolate our culture results to the higher taxonomic level only tentatively since strain-specific responses are well documented within *E. huxleyi* (Langer et al., 2009).”*

4- I found this section 3.4 very difficult to read by a non specialist. Many parts of the demonstration should be explain in length. For example why is it important to plot epsilon versus μ/CO_2 ? The reason of using the growth rate divided by CO_2 instead of only growth rate should be explain. μ should be redefine there (it is only at the beginning of the paper).

This section now includes the following:

“net carbon isotopic fractionation should show a negative relationship between ϵ_p and $\mu/\text{CO}_2(\text{aq})$ (where μ is growth rate: for details of this theoretical approach see Laws et al., 2002; Keller and Morel, 1999; Cassar et al., 2006) but needs to account for such variables as growth rate, cell geometry, and other factors limiting growth. In this model, $\mu/\text{CO}_2(\text{aq})$ represents the degree of utilization by growth rate, relative to supply rate which occurs in proportion to $\text{CO}_2(\text{aq})$ such that this ratio reflects the degree of utilization of an internal pool hence the Rayleigh distillation factor.”

5- It is particularly intriguing that *C. braarudii*, being the coccolithophore that should be the most adapted to high DIC as other coccolithaceae of large size since they were thriving in an high DIC world (e.g. Paleogene), are showing malformation at high DIC.

Is it not the contrary that was expected ? Why the size of *C. braarudii* diminishes with increasing pCO_2 when larger *Coccolithus* were existing during higher pCO_2 times ?

We agree with the reviewer that this result was not anticipated, and that it poses a paradox to the observation that the Coccolithaceae indeed flourished during the high

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*DIC Paleogene – that is, this family seemed best adapted in the long-term high DIC and pCO_2 world. Modern descendants of *Coccolithus* are only found in cool, nutrient-rich subpolar and upwelling waters, which hold lower carbonate saturation (and likely pH – hence the apparent insensitivity to lowered pH [Langer et al., 2006]) than we imposed in the laboratory.*

*The cell size of our *C. braarudii* strain was indeed smaller under high pCO_2 (high DIC, high saturation state), but we interpret this change in size as a primary result of sub-optimal, if not disrupted cell growth and calcification (malformation) – not as an acclimation or an adaptive response to elevated pCO_2 . It obviously did not perform well under the imposed conditions – and we infer that the strain was affected through the mechanism proposed in our schematic model. As we state above these conditions are rather more saturated than may have been found in the natural environment. Nonetheless, *G. oceanica* was unaffected even by these extreme conditions suggestive that it is well buffered against such change in the carbon system, unlike *C. braarudii*. Again these observations point to a distinct difference in the physiologies of these two strains.*

The authors say that for this taxa does not respond to changing pH and CO_2 (e.g. p275 line 12 or in the conclusion) which is apparently not demonstrated by their experiment here. Could they clarify that point ?

*Since our experiments are all performed at constant pH, we have no further information over and above Langer et al., 2006 to suggest *C. braarudii*'s sensitivity to pH. We therefore clarify in the conclusion:*

*“The larger species (*Coccolithus*), generally insensitive to pH (Langer et al., 2006). . . ”*

Also some very minor things : Title : I do not feel that the title is informative, and although funny it is abstruse and Macconnaughey: Title: Not particularly informative, but cute titles are just fine with me.

We have altered the title to read:

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“Perturbing phytoplankton: Response and isotopic fractionation with changing carbon-ate chemistry in two coccolithophore species”

P.257 Line 1 – “No two...” strange wording.

We have altered the sentence to now read:

“All species of coccolithophore appear to respond to perturbations of carbonate chemistry in a different way.”

P.257 Line 8 - G. should be Gephyrocapsa because it is not correct to start a sentence with an abbreviation. This is made several time (P.262 Line 2, P.265 Line 5....).

We have corrected all G. at the start of sentences to read Gephyrocapsa

P.268 Line 1-3 : *But the trend in isotopic values (Fig. 5) under stable growth rates at the lower DIC levels implies that the isotopic shift cannot only be due to decreased growth rate at high DIC. I do not understand the “only”. This is not the proof that this “shift cannot” be strictly due to decrease growth rate.*

We have removed the “only” so the sentence now reads:

“But the trend in isotopic values (Fig. 5) under stable growth rates at the lower DIC levels implies that the isotopic shift cannot be due to decreased growth rate at high DIC.”

P.269 Line 18 $\delta^{13}C_{POC}$: delete the g and Macconnaughey: P.269 L18: $\delta^{13}C_{POC}$
What's the g?

This has been deleted.

P.270 Line 7 : Before Pagani et al 2005, at least there is Jasper, J.P., et al., Photosynthetic fractionation of ^{13}C and concentrations of dissolved CO_2 in the central equatorial Pacific during the last 255,000 years. *Paleoceanogr.*, 1994. 9(6): p. 781-798.

We have added this reference

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Reviewer Ted Macconnaughey

There have been very few attempts to quantitatively model C^{13} balances, and here you have a great data set for attempting this because you have C^{13} 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. MacConnaughey Sub-equilibrium oxygen-18 and carbon-13 levels in biological carbonates: carbonate and kinetic models)

We have tried to follow the model that MacConnaughey presented in 2003 for macroalgae but it is challenging to apply to the coccolithophore, because there are still so many unknowns: the size and isotopic composition of the internal pool, and the contribution of respiration CO_2 to calcification or photosynthesis. Nonetheless, we have considered the data in the context of a closed or open system model, and it is clear that, in order to account for the divergence between the values in $\delta^{13}C_{POC}$ and $\delta^{13}C_{PIC}$ as carbon availability increases, the closed system must evolve towards a more open system i.e. with the addition of an external source of unfractionated carbon, presumed here to be an increased diffusive supply of CO_2 from the media. We have modified the paragraph in section 3.4 to now read:

*“For *C. braarudii*, there is the additional observation that the shift towards lighter values in $\delta^{13}C_{POC}$ is linearly related to a shift towards heavier values in the $\delta^{13}C_{PIC}$. These potentially related changes in isotopic fractionation imply that the carbon for both calcification and photosynthesis in *C. braarudii* is derived from a common internal pool of HCO_3^- , under low DIC conditions. However, a closed system internal pool must be overprinted by an external source of carbon under the high DIC conditions. Under a closed system, a decreased degree of utilization of this common pool by calcification or photosynthesis under the high carbon conditions, would result in less internal Rayleigh*

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distillation in that common pool and both $d^{13}C_{POC}$ and $d^{13}C_{PIC}$ should tend to lighter values. Instead the divergence between these isotopic values implies that a new, unfractionated source presumably a diffusive supply of CO_2 from the exterior becomes a significant source, as the DIC increases in the external media.

Under normal conditions therefore, we infer that *C. braarudii* contains a common internal pool of carbon. Calcification appears unlikely to provide a direct supply of CO_2 for photosynthesis – but through pH regulation of the coccolith vesicle, provides protons that aid conversion of HCO_3^- into CO_2 near the chloroplast. We propose that *C. braarudii* actively transports HCO_3^- into the cell which then provides CO_3^{2-} for calcification and CO_2 for photosynthesis, perhaps catalysed by a chloroplast carbonic anhydrase and proton transfer between the two equilibria. Such a scenario is consistent with the observation that the addition of HCO_3^- to carbon-starved cells resulted in an increase in pH, while its removal resulted in the cytosolic acidification, suggesting a role of HCO_3^- in buffering cytosolic pH (Brownlee and Taylor, 2004) and the internal proton transfer from calcification to photosynthesis. HCO_3^- influx (Sikes & Wilbur 1982, Nimer & Merrett 1992) was shown previously to be very rapid (Nimer & Merrett 1992). The conversion of HCO_3^- to CO_2 by the enzyme carbonic anhydrase discriminates against ^{13}C by the same sort of value as the inorganic equilibration of $CO_2(aq)$ with HCO_3^- of $\sim 9\%$ (Paneth and O'Leary, 1985). This means that a chloroplast envelope carbonic anhydrase inside the cell enables organic matter to become isotopically heavy because the cellular HCO_3^- would completely convert to CO_2 . There is strong evidence to support a chloroplast carbonic anhydrase within the coccolithophores (Nimer et al., 1994; Quiroga and Gonzalez, 1993)."

p262 L8: 1100, 1600, 2100, 5300 and 7800 $\mu\text{mol kg}^{-1}$, after which pH was adjusted to 8.13 ± 0.02 . Please give estimated CO_2 concentrations after pH adjustment. Was this measured?

CO₂ concentrations were not measured but calculated using CO2.sys from DIC and pH.

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...target concentrations of about 1100, 1600, 2100, 5300 and 7800 $\mu\text{mol kg}^{-1}$, after which pH was adjusted to 8.13 ± 0.02 (see Supplementary datafile for complete initial and final carbonate chemistry of each experiment) .

P261 L11: nitrate and phosphate concentrations of 100 and 6.25 $\mu\text{mol kg}^{-1}$, 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?

*Nutrient levels were not measured throughout the experiment but "after more than 6 (*C. braarudii*) and 7 (*G. oceanica*) generations, when the drift in DIC and pH was between a decrease of 2.35-9.0% and an increase of 0.00-0.08 units for *C. braarudii* and minus 2.27-9.0% and plus 0.00-0.13 units for *G. oceanica*, respectively, samples from the dilute cultures ($< 18500 \text{ cells ml}^{-1}$ for *G. oceanica*; $< 2300 \text{ cells ml}^{-1}$ for *C. braarudii*) were taken" so given the dilute nature of the culture, it would be impossible for any of the nutrients to have become limiting. The experiment was designed to avoid limitation and significant change in carbonate chemistry through time.*

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.).

We have clarified this statement:

*At the end of the experiment, after more than 6 (*C. braarudii*) and 7 (*G. oceanica*) generations, when the drift in DIC and pH was between minus 2.35-9.0% and plus 0.00-0.08 units for *C. braarudii* and minus 2.27-9.0% and plus 0.00-0.13 units for *G. oceanica*, respectively, samples from the dilute cultures ($< 18500 \text{ cells ml}^{-1}$ for *G.**

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oceanica; <2300 cells ml⁻¹ for *C. braarudii*) were taken.

P263 Eq4: Isotopic fractionation relative to CO₂ implicitly makes assumption that CO₂, not HCO₃⁻ provides carbon for organic synthesis. Worth stating this. The way Eq 4 is set up, higher positive values of ϵ_p mean more negative $\delta^{13}\text{C}(\text{POC})$. This reversal is potentially confusing. Why not leave everything in $\delta^{13}\text{C}$ units and put $\delta^{13}\text{C}(\text{CO}_2)$ and $\delta^{13}\text{C}(\text{HCO}_3^-)$ on the graph for comparison.

The ϵ_p calculated for both *G. oceanica* and *C. braarudii* relative to CO₂(aq), assumed to be the substrate for photosynthesis, and plotted versus μ/CO_2 shows that ϵ_p is small (Fig. 7), varying between 4 ‰ and a maximum value of 10 ‰. Using this formulation higher positive values of ϵ_p indicate more negative values of $\delta^{13}\text{C}_{\text{POC}}$.

We have shown the raw data as $\delta^{13}\text{C}$ units on Figure 6 and include the table of the $\delta^{13}\text{C}$ of the different parameters of the carbonate system. By including ϵ_p , we make our data comparable to those that use this parameter as a proxy for past CO₂ so we feel it is useful to leave the data in this format for this particular figure 7.

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

Clumsy wording

We have rewritten the sentence:

*“Calcification rates and photosynthetic carbon fixation rates increased very slightly with increasing DIC in *C. braarudii*, despite the poor visual nature of the cells, but since these were parallel in trend and magnitude, there was no change in the PIC/POC ratio (Fig. 3).”*

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?

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We agree with the reviewer to some extent but consider that both these points are necessary at this stage in the discussion. The PIC/POC response to differing carbonate chemistry appears to be a fairly clear physiological marker. It is useful for us to compare our results on these strains in these experiments with Langer et al. (2006) and also to acknowledge the uncertainty in the calculation of PIC/POC based on cell numbers in the same place. We feel that discussion would lose its relevance if tucked in the methods away from where it is directly useful.

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

We do!

P267 L11: The $\delta^{18}\text{O}_{\text{PIC}}$ of *G. oceanica*. 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.

We have clarified the following sentence to read:

*“The $\delta^{18}\text{O}_{\text{PIC}}$ of *G. oceanica* is within error of 0 ‰ offset from the medium i.e. at equilibrium with the medium; but this value appears to be ~1 ‰ lighter than expected from Ziveri et al., (2003) who obtain a value of between 1-2 ‰ at 18°C.”*

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.

We have revised Figure 5 according to the reviewer's suggestion.

P268 L5: The distinction between HCO₃⁻ and CO₃⁼ 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 CO₃⁼ transport. Especially in light of proton transport and pH elevation, CO₃⁼ transport is unlikely. It just needlessly confuses the situation.

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We have removed the two sentences where we suggest the active transport of CO_3^{2-} . However we would prefer to keep this discussion where it is since it is most relevant to explaining the isotopic composition of the calcite which we discuss in full detail in this section. We refer back to this section when trying to integrate all observations to develop our schematic model.

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.)

We are unclear about the reviewer's comment here because later, the reviewer actually seems to confirm our schematic model (see next comment). As a result we have left this section as it is.

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?

Essentially, the reviewer has encapsulated our exact model that we propose here (see our Figure 8)!

For C. braarudii, C13 depleted PIC corresponds to O18 depleted PIC. Suggests kinetic effect, most prominent at low PCO_2 . Maybe the coccolith vesicle is most alkaline under low CO_2 conditions, and absorbs CO_2 from cell to calcify. Seems consistent. At high

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CO_2 , PIC looks close to equilibrium but this needs to be verified.

We have considered the implications of kinetics on our results, as the reviewer suggests, but given the evidence for both growth rate and calcification rate change under the different conditions, it seems unlikely that kinetics can be the dominant control on these isotopic trends. We state:

“The $\delta^{18}\text{O}_{\text{PIC}}$ and $\delta^{13}\text{C}_{\text{PIC}}$ of C. braarudii, at low DIC and normal growth rates, are 1‰ and 2‰ lighter than the media respectively, and consistent with previous interspecific variations and offsets (Ziveri et al., 2003). But there is a large change in the isotopic composition under the high DIC conditions as $\delta^{18}\text{O}_{\text{PIC}}$ and $\delta^{13}\text{C}_{\text{PIC}}$ record more positive values, within error of calcite equilibrium with the composition of the DIC in the medium.

This isotopic shift towards heavier isotopes under high DIC could reflect a decreasing kinetic fractionation as the growth rates also decrease at these apparently unfavourable conditions. But the trend in isotopic values (Fig. 5) under stable growth rates at the lower DIC levels implies that the isotopic shift cannot be due to decreased growth rate at high DIC. Furthermore, the rate of calcification which should have the greatest influence on the kinetic isotopic fractionation, is unchanged by the DIC conditions.”

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.

We already state in the text (and show on Figure 8):

“The $\delta^{18}\text{O}_{\text{PIC}}$ and $\delta^{18}\text{O}_{\text{medium}}$ of G. oceanica is within error of 0‰, i.e. at equilibrium with the medium but ~1‰ lighter than expected from Ziveri et al., (2003) who obtain a value of between 1-2‰ at 18°C. The $\delta^{13}\text{C}_{\text{PIC}}$ for G. oceanica are within error the same as the $\delta^{13}\text{C}_{\text{DIC}}$ in all experiments, in agreement with Ziveri et al., (2003). This indicates either

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that the pH of the calcification vesicle is very similar to that of the media, or that the pH in the vesicle is in the range where HCO_3^- is the dominant ion, and the primary source of the carbon for calcification as determined elsewhere (Sikes and Wilbur, 1982; Rost et al., 2002).”

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.

We agree with the reviewer that the CO_2 gradient will depend on the relative rate of calcification to photosynthesis, since CO_2 is a sink of carbon drawing CO_2 into the cell and calcification may ultimately lead to CO_2 leaking out of the cell. But when these two processes are taking place in a roughly 1:1 ratio, as in our experiments (the PIC/POC ratio ~ 0.5) it is not clear there will be a net effect driving CO_2 into or out of the cell. During our experiments we increase external DIC to such an extent that the exterior concentration must be greater than the internal one such that net diffusion inwards is induced.

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 .

As we show in the figure, we have plotted the specific growth rate divided by the CO_2 concentration as is the convention when considering variance of ϵ_p .

We do not follow the reviewers next comment. As we state in the text:

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“G. oceanica certainly increases both its growth rate and its rate of photosynthetic carbon fixation as DIC increases in the environment suggestive that it is partly limited by the diffusive supply of CO_2 at low carbon conditions. Extra energy may be allocated to the expression of carbon concentrating mechanisms under low carbon conditions which is then available for growth as DIC becomes more plentiful.”

P271. L26 gn ?

This has been fixed back to “in”

P277. L11. Always specify whether you are talking about C13 or O18.

We have corrected the sentence to read:

The $\delta^{18}\text{O}$ isotopic composition of coccoliths displays a $\sim 5\text{‰}$ array of disequilibrium or “vital effects” across eight different species

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 .

Whilst we appreciate this comment, there is no evidence to date to suggest that vacuolisation of seawater plays any role in the calcification or photosynthesis of coccolithophores, unlike the macroalgae, the foraminifera, and maybe the corals, Indeed the

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very opposite. Jonathan Erez (pers. Comm.) has said that of all organisms that he has investigated for calcification everything he has studied takes up and fixes the fluorescent Ca dye (calcein) into their biominerals EXCEPT for the coccolithophores because they rely solely on diffusion, channels or pumps for the transport of all ions and not vacuolization, as is used by all other calcifying organisms.

We also state clearly in the text that the internal pH values of both 8.3 for the calcifying vesicle and the 7.0 for the cytosol, are taken from Anning et al., 1996. (Pg 268, Line 11)

Also in this figure, you might try drawing it such that Ca²⁺ ATPase simultaneously extracts H⁺ from the calcifying vesicle while adding Ca²⁺. Some of the earliest evidence for Ca²⁺ ATPase in calcifying systems came from coccolithophores.

We have made suggested changes and have drawn ATPase activity at the calcifying vesicle in both species (see revised Figure 8).

Interactive comment on Clim. Past Discuss., 6, 257, 2010.