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Interactive comment on "Growth of the coccolithophore *Emiliania huxleyi* in light- and nutrient-limited reactors: relevance for the BIOSOPE deep ecological niche of coccolithophoresbatch" *by* L. Perrin et al.

Anonymous Referee #2

Received and published: 19 August 2016

GENERAL COMMENTS The manuscript by Perrin et al differs from much of the physiological work in the coccolithophore literature in that it examines co-limitation (nutrients and light) and uses modelling to further parameterise key physiological parameters. It is generally well written, although at times it does become repetitive and parts could be tightened and shortened to avoid this, also there are a few grammar/syntax errors which distract from the work. 16 figures and 5 tables is excessive, especially when the same data appears in both and the authors should consider the best way to convey their results and key points without repeatedly presenting the same data. The authors also need to think over their central messages and conclusions from this work

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(see comments below) which would lead to a better structure of the paper (e.g., what belongs as supplementary material or doesn't need to be emphasised repeatedly). Overall the scientific work is excellent and will provide significant new insights into E. huxleyi physiology which can be taken up by other studies.

1. Emiliania huxleyi, not all coccolithophores. Unavoidably, this is a central bugbear of much of the literature – information on E. huxleyi does not equal information on coccolithophores as a whole. E. huxleyi is just one species of a group which exists in a wide diversity of ecological niches. Although E. huxleyi is particularly cosmopolitan in its distribution and often becomes an exhibitionist when forming mesoscale sub-polar blooms, it is lightly calcified and contains little POC. Though E. huxleyi may dominate calcite fluxes in high-latitude environments, other (often deep-dwelling) species dominate fluxes to sediments below oligotrophic gyres. Please be specific when discussing coccolithophores as a group or E. huxleyi as a single species.

2. Why the deep niche focus? Despite the introduction, it is not clear why the focus on the deep E. huxleyi communities in the South Pacific. Co-limitation and the physiological parameters determined in this study are relevant to E. huxleyi growth in many other environments. Growth in this environment is likely to be taxing (and potentially on the outer envelope of the E. huxleyi global niche), but so is growing in the cold and dark, nutrient poor Arctic or Antarctic (where E. huxleyi also has sizable communities). The strength of the work is outlined in the introduction (Lns 79-80): 'understanding the development of (deep) coccolithophore populations in low nutrient, low irradiance environments does contribute to building a global picture of coccolithophore ecology and biogeography'. At times the authors seem overly fixated on the deep communities in the South Pacific – despite having used a surface water isolate potentially adapted to growth with low inorganic nutrients and readily available organic nutrients.

3. What are the main messages from this work? That batch cultures and modelling can determine good estimates of physiological parameters is a nice result, but is it the main message of the paper (and does it need repeating several times throughout the

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manuscript?) What is the main message? The physiological parameters determined are fascinating and have potentially global implications (and don't appear to differ with other E. huxleyi strains). Did you need to look at a BIOSOPE strain for this work and are the results only relevant to the 'deep ecological niche' of coccolithophores. This issue over the main message of the paper partly results in the 16 figures and 5 tables and repetitive nature of parts of the manuscript (please note that much of the data in tables is in the figures and vice versa and consider deleting figures where the data is better shown in a table – note you don't have to show figures just to highlight statistical relationships). Could some of the modelling plots go in the Supplementary material?

4. Light dose. It should be acknowledged that light dose is important, not just the incidental amount of light. For example, a 12:12 L:D day at 30 umol photons m-2 s-1 may be similar to a 16:8 L:D day at 20 umol photons m-2 s-1. This should be made clear when comparing between in situ and laboratory, and also between various laboratory studies.

8. Alternative sources of N and P. Several papers (e.g., Benner & Passow (2010) Utilization of organic nutrients by coccolithophores. Marine Ecology Progress Series 404, 21-29) have shown that E. huxleyi can utilise organic sources of N and P, whereas the focus in this paper is on inorganic (nitrate, phosphate) sources only. In an oligotrophic environment, alternative sources of nutrition (organic substrates) will be especially important. The existence of these other nutritional strategies should be acknowledged and discussed – how reliant are the conclusions of this paper on the assumption that E. huxleyi is not utilising organic nutrient sources?

SPECIFIC COMMENTS Ln 18: What does 'coccolithophore ecosystem' mean? Revise use of ecosystems. Also, why are they important – production, export?

Ln 18-19: Did you actually 'investigate the conditions that regulate the development of a deep coccolithophore niche' or rather investigate the physiological responses of an E. huxleyi strain to living in simulated conditions of living deep in the oligotrophic

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gyres?

Ln 30: How can an ecosystem be disadvantageous? I am sure the other members of the DCM community don't feel that it is disadvantageous.

Lns 38-39: Consider you use of 'participate' – a better word would be contribute.

Ln 40-41: The ratio of calcification and photosynthesis are not the only factors contributing to ocean-atmosphere CO2 fluxes: what about dissolution, respiration, advection, sinking, or carbonate chemistry? This sentence oversimplifies something far more complex than PIC:POC.

Ln 42-43: Sure all these factors influence cellular PIC:POC (in laboratory cultures) for coccolithophores, but not for the whole phytoplankton community (i.e. the communities POC). Consider being more specific in this opening paragraph to avoid confusion.

Ln 44: biogeography is not just growth rates, what about mortality?

Ln 47: This list of references is not extensive, but more examples of relevant literature (the list would be much longer) – hence start the list with "e.g.". See also the list on Ln 50.

Ln 60: Communities of deep living coccolithophores were not first discovered by BIOSOPE – the existence of deep shade flora has been known since the 1970's (at least). Rather, BIOSOPE observed it in the South Pacific Ocean. See also comments on the deep niche of coccolithophores.

Ln 62: Firstly, nitracline (nitrate) or nutricline (all nutrients) – do all nutrients have a 'cline' at 200 m (nitrate, phosphate, silicate, ammonia?)? Secondly, the nitracline is defined as the depth were nitrate concentrations increase over a threshold, it doesn't not have a fixed depth as is inferred in this line.

Ln 114: What is the rationale for using a surface E. huxleyi isolate to replicate the biology of a deep E. huxleyi community? Are they the same?

Lns 120-123: Surely daily light dose is more relevant to replicating in situ conditions that instantaneous light levels? 12 hours of 30 umol m-2 s-1 is not the same as 16 hours of 20 umol m-2 s-1 (or is it?).

Ln 159: Where nutrients analysed on a CHN Auto-analyzer? Or a Seal Analytical AAIII? I cannot find this equipment on the Seal website.

Ln 168-170: How was POP measured? Fumed or not fumed? It is not clear from this text, and as these are among the few measurements of cellular P for E. huxleyi it is quite important to state the method.

Ln 189: N fixation rate? Or N uptake rate?

Ln 192-194: Why use the cellular carbon quota and Redfield to calculate cellular N content? The N content has been measured and why assume Redfield?

Ln 202-205: Why has two methods used to determine cell volume and surface area? When was one used and not the other in the calculations?

Ln 214: PON and POP are not equivalent to cellular NO3 or PO4, or do you mean the media concentrations? This sentence is confusing.

Ln 216: What other internal cellular quota of N is there? What is nutrient N?

Ln 243: What is KN/P? N to P ratio? Same for QN/P in Ln 246.

Ln 250-251: Do you mean nutrient data points (i.e. media nutrient concentration) or nutrient-quota (i.e. cellular elemental quota)?

Ln 269,279,288,306: Do you need sub-headings for the sub-section of the results?

Ln 293: Please make it clear why you express as P:C and not C:P.

Ln 379-381: How do new insights into E. huxleyi physiology tell you about the other coccolithophores living deep in the water-column? Maybe other Isochrysidales or Noelaerhabdaceae, but not species such as Florisphaera profunda.

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Ln 409: Rephrase ‘however, large error margins do not allow explaining these observations ..’

Ln 442: Decreasing phosphate/nitrate or phosphate/nitrate-limitation? A drop in nutrients or their complete absence?

Ln 447: Please, the review by Zondervan (2007) is almost entirely E. huxleyi (maybe a little Gephyrocapsa), but it certainly isn’t ‘coccolithophores’.

Ln 452-460: Such a comparison between studies needs to account for day length differences between experiments – i.e. where the light doses different?

Ln 463-465: The relationship between coccolith size and coccosphere size is likely to be (very!) species-specific and hence this sentence should be rephrased. Also, PIC quota of what? Coccoliths yes, but not coccospheres – there is considerable intra- and inter-species variability in the number of coccoliths per cell.

Ln 467,476, 483: Having three separate summary sections mid-discussion breaks up the flow of the paper and doesn’t seem necessary if they are combined and written clearly.

Ln 561: What other nitrate is there?

Ln 573: What about comparing these growth rates with other measurements/estimates? Figure 13B. What are the red triangles?

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