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Interactive comment on "Interactive effects of seawater carbonate chemistry, light intensity and nutrient availability on physiology and calcification of the coccolithophore *Emiliania huxleyi*" *by* Yong Zhang et al.

Anonymous Referee #1

Received and published: 18 February 2018

Review on 'Interactive effects of seawater carbonate chemistry, light intensity and nutrient availability on physiology and calcification of the coccolithophore Emiliania huxleyi' by Zhang et al.

General comments:

The present manuscript presents a comprehensive dataset investigating the interactive effects of carbonate chemistry, light intensities and nutrient availabilities on the coccolithophore E. huxleyi. The dataset consists of an impressively high number of treat-

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ments, replicates and measured parameters therein. Given the fact that interaction of multiple drivers are often impossible to predict from simpler experiments, datasets as this one are indispensable to understand the expected natural complexity in climate change effects.

This vast amount of data is, however, somewhat overwhelming and it seems to me that the authors also got lost in it a bit. In its current form, the manuscript is poorly written (also with respect to language) and lacks a story line. While I do acknowledge the difficulty to find overarching patters in such a complicated dataset, the current manuscript does not make it easy for the reader to take home any conclusions. In the discussion, individual paragraphs are often not connected to each other (and sometimes even not between sentences therein). I suggest the authors to focus on the main aspects they want to interpret and discuss these in more than a few sentences, and to omit some of the other (side-)aspects. Likewise, parameters that do not get discussed in detail also do not need to be described in great detail in the (currently quite long) results section. In my opinion, quite some of this information could be sufficiently described in tables and the supplement.

With respect to the general interpretation of the data, I disagree with the way the nutrient treatments are regarded. Despite the fact that cells divided 1-2 times per day (μ >1 in almost all cases) and were clearly exhibiting non-limited exponential growth, the data is discussed as if the cells were nutrient limited and compared to previous studies that investigated strong nutrient limitation. Regarding nitrogen limitation, for example, residual DIN was 1.0 0.4 μmol L-1 in LN treatments, which is known to not limit growth, and the molar drawdown in HN and LN treatments is actually similarly high. The same is true for the molar drawdown of DIP. Thus, the discussion needs to be refocussed by considering different but not strongly limiting nutrient concentrations rather that limiting vs. non-limiting conditions. This is particularly the case as growth rates are integrated over the whole duration of the experiment (i.e. mixing phases of non-limited growth with potential limitation towards the end of the experiment), while photophysiological

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measurements are only taken at the final (potentially more limited) stage.

Specific comments:

L33-34: The interaction between CO2 and N is actually the least significant term, why do you focus on this interaction and not the others?

L36-37: The authors do not provide any data that would allow to conclude on the competitive abilities of this species. If they want to, they would need to either conduct competition experiments, or compare nutrient uptake kinetics with those of competing species.

L56: Why only from media, not generally from seawater?

L60-65: I do not understand why the authors mention two opposing interpretations of multiple stressor effects (i.e. linearly increasing/decreasing/non-affected vs. optimum curve response) without clarifying why they use the linear trends even though they are aware of the fact the responses follow more complex optimum curves.

L65-67: Really? These is also plenty of evidence for the opposite effect, also published by some of the authors.

L67-70: Intraspecific differences are another well-established reason for differing responses (e.g. Langer et al. 2009).

L75: Photo-acclimation to HL or LL? Both are photo-acclimative processes.

L86-92: The same information is presented in the discussion. Is it really necessary to present it twice with the same level of detail?

L180: How did you measure the pressure inside the syringe filter?

L193: How similar were the PAM light values to those during the incubation? Please provide a quantitative comparison.

L198-203: How was the “cellular absorption value” determined? This parameter most

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likely changes strongly with light-acclimation, so I do not think that one constant value can be used to convert relative ETR to absolute ones for all treatments. If the authors did not determine this values for each treatment, they should rather report the ETR in their relative unit.

L214: Is it really true that the authors did not even measure the initial cell count but just assumed inoculation to be perfectly equal among all bottles? I do not trust the growth rate estimates at all if this is the case, especially as small differences in the low abundance range will have huge effects on the final counts.

L234-235: Why were the two nutrient treatments analysed separately?

L266 ff.: It is no clear to me to which of the two tests (i.e. ANOVA vs. post hoc tests) the statements regarding the p values refer to. These are two different things. Please clearly state if you base a statement of "significance" on the ANOVA itself or a post-hoc test in the whole results section. If you describe an optimum-curve behaviour, for example, the ANOVA cannot capture both increasing and decreasing phases of it, but would indicate that one of the two is more dominant.

L412 ff: Quite often, single sentences are not clearly connected. The discussion thus seems like a long list of ideas, but without any structure or line of thought.

L414-416: Why "synergistic negative effects"? This a priory expectation is not stated (nor argued for) in the intro.

L423-430: What does the content of this paragraph mean for the interpretation of the results with respect to nutrient limitation?

L435-439: This could be explained by an excess of PSII reaction centres (Behrenfeld et al. 1998).

L445-447: See my comment regarding competition above.

L466-467: looking at the fit on figure 5, I am not convinced by this, as the fit does not

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run close to the data in the relevant part of the curve (i.e. the slope).

L480-482: In the natural environment, 10 μM NO3 is definitely not “low nutrients”.

L483-485: What is “alkaline phosphate activity”? There seems to be a word missing. Also, please explain why this is relevant.

L487-492: I do not understand how this is related to LP conditions. Wouldn’t one expect that P limitation would increase energy demand due to upregulated P uptake machinery?

L498-499: This can be solely explained by increasing levels of energy saturation of C acquisition and fixation with increasing light.

L506-509: Seems completely unrelated to the presented and discussed data.

L509-511: Seems completely unrelated to previous discussion.

L515-527: Here, results from really nutrient-limited cultures are compared to the data from this study without discussing the lack of considerable nutrient-limitation of growth. Please rewrite this section by taking this into consideration. Also, take into account that under intermediate light levels, growth rates under P limitation and LC are as high as in the full media.

L535-536: ETRmax were measured at high light, so it cannot be limited by low energy input. Instead, previous acclimation to low light may have hampered usage of the provided energy.

L541: Please clarify that you have no data on CCM down-regulation but that this is speculation based on previous publications.

L547-550: Of course these processes are correlated. Can you provide something new that further elucidates this fact?

L555-558: I do not understand this line of thought. Please explain in more detail.

L563-566: The authors correctly state that highly labour-intensive experiments like the current one are necessary because interactions between multiple stressors cannot be inferred from isolated effects. I therefore do not understand why they speculate on an interaction they did not investigate.

Figure 5 legend: The method description should move into the method section and be more detailed, e.g. were growth rates integrated over 4 days? Were the cultures pre-acclimated to the conditions? If not, which conditions were they acclimate to before?

Technical corrections:

Generally, there are a lot of instances where grammar and wording need to be improved. I strongly suggest the native speakers in the author list to thoroughly correct the final revised version of this manuscript. Below a few examples:

L34-35: Please correct/rephrase this sentence.

L48-49: Please correct/rephrase this sentence.

L55: Replace “in the UML” by “therein”

L57-60: Please correct/rephrase this sentence. Why “counteract”?

L84: Consider replacing “decreased” by “suboptimal”

L86-92: Combine first two sentences into one.

L93-101: Indicate at which pCO2 levels these studies were conducted.

L119: Why “Even”?

L398-403: This is not discussion later on. Is it needed then?

L142-145: Please correct/rephrase this sentence.

L158: For clarity, please add “For each nutrient treatment, [. . .]”

L158-159: Add standard errors for light levels.

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L165-167: Please correct/rephrase this sentence.

L178: “CO2 System” should read “CO2SYS”

L182: “Dickson et al. 2003” should read “Dickson et al (2003)”.

L185: “equimolal” should read “equimolar”.

L186-187: I assume you did not calculate K1 and K2, but used these constants from Roy et al. for your calculations. . . If so, please correct accordingly.

L194-196: Please correct/rephrase this sentence.

L226: Replace “their” by “cellular”.

L256-264: Estimates of uncertainty are missing.

L274-279: Units of the treatments are missing.

L346-349: Replace “At each nutrient condition, at both LC and at HC“ by "At all nutrient and CO2 levels“.

L356: Why "both“?

L439-441: This sentence sounds as if the authors would have observed the first statement, and the reference refers to the latter, while the opposite is true. Please rephrase.

L465: “saturation condition, relationship” should read “saturated conditions, the relationship”.

L467-471: Please correct/rephrase this sentence.

L480-482: Please correct/rephrase this sentence.

L496-502: Please correct/rephrase this sentence.

L503: omit first “-“

L516: Insert “could have” between “which” and “led”.

L553-555: I do not understand this sentence. Please rephrase.

Figures: Consider using a dashed line for one of the fits to distinguish between the twoCO2 levels.

L869: Based on which test?

L902: Explain letters to abbreviate pCO2 and light intensity.

L920-921: Please rephrase to make it a sentence.

Figure 2: Indicate if the PIC:POC is molar- or weight-based.

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