Reviewer1

Comment

In their paper, Xu et al. address the question how different salinity levels affect Emiliania huxleyi's physiological response to changes in pCO2. This is a novel research question that the authors address with valid experiments. The authors, however, manipulated seawater in a way that also levels of total inorganic carbon varied between the salinity levels, which is neglected in their discussion and only becomes clear on closer inspection of the provided carbonate chemistry data. Combined changes in salinity and inorganic carbon concentrations may be natural and therefore a valid treatment if discussed appropriately. Although the dataset may indeed provide insights on the salinity (and associated DIC) effect on pCO2 responses, the authors focus on changes in CO2 levels between different pCO2 treatments, but not on pCO2 changes between salinity treatments. This makes some of their arguments contradictory and difficult to follow. Other aspects that are associated with changes in salinity, such as osmolality, ion concentrations and electrochemical gradients are not discussed. Overall, the manuscript would benefit from discussing different drivers in a consistent manner. The authors need to better embed their findings into the related findings. As it stands, this study is not suitable for publication in "Biogeosciences".

Response: This is a constructive comment which will allow us to fine tune our discussion. We are aware of the fact that both elevated CO_2 and changed salinities alter the parameters of seawater carbonate chemistry. Therefore, we provided the carbonate chemistry parameters at different CO2 and salinity levels in Table 1. Since co-variation of CO_2 and salinity together alters the carbonate chemistry, we set both low and high CO_2 levels at each salinity treatment. Since this study is scenario-based as suggested for multiple driver studies (Boyd et al. 2018, Global Change Biol), our aim in this study was to examine the physiological responses of *Emiliania huxleyi* to ocean acidification and desalination. While there are a number of published studies regarding OA impacts on this species under the influences of light, temperature, nutrients and UV radiation (see the review by Gao et al. 2019, Frontiers in Mar Sci, and references therein), to the best of our knowledge little has been documented on the combined effects of OA and salinity. Our findings suggest that *Emiliania huxleyi* can tolerate low salinity plus acidification conditions by up-regulating its photosynthetic performance.

We accept that, as pointed out by the reviewer, the discussion is less detailed less about salinity effects, such as possible impacts of osmolality, and we will add relevant analysis and discussion on this point in a revised version of the manuscript.

Specific comments:
Major parts of the discussion and the author's conclusions (for example that the effective photochemical efficiency is significantly different under 'low salinity' (LC, 25‰ refer Table 2)) are based on one treatment that is an 'accident', i.e., on a treatment in which the authors state that the carbonate chemistry substantially drifted by the end of the treatment. The growth rate is determined on two measurements of cell concentrations only, one of which was taken a very low cell concentration. This approach is specifically prone to errors. Rates of photosynthesis and calcification were measured by 14C incorporation experiments. The authors do not mention whether they exchanged medium for the measurements and measured photosynthesis and calcification under standardized conditions, or whether they added 14C to the growth media. Whereas the first approach would measure a 'capacity' for photosynthesis and calcification rather than in situ rates, the second approach delivers a parameter that may indeed reflect in situ conditions. The authors should also provide details about the quantities of added 14C and whether the addition changed the carbonate chemistry significantly.

Response:

The methods to culture *E. hux* and measure its specific growth rate of cells in the present study has been documented in many other studies, including several publications from our group (Gao et al. 2009 L&O; Jin et al. 2003 Plant Physiol.; Jin et al. 2017 MEPS; Tong et al. 2018 Global change Biol.). Initiation of the cultures at very low cell concentration can avoid impacts of biological activities on the chemistry of the culture medium, which indeed led to stable carbonate chemistry at the various combinations of CO_2 and salinity treatments.

In terms of the ^{14}C incorporation experiment, we have described details of our ^{14}C incorporation experiment, which are also reflected in our previous publications (Gao et al. 2009 L&O; Jin et al. 2003 Plant Physiol.; Jin et al. 2017 MEPS). As outlined in the main text at line 216, the quantities of added ^{14}C is 5 μCi in 20 ml culture, which is exactly same as in our previous publications. The method used to measure photosynthesis and calcification rate at the same time with the ^{14}C technique is based on our previous work of Gao et al. (2009; L&O).

Comment: Throughout the manuscript, the authors 'jump' between the effects of salinity (and the associated DIC), of pCO2, and combined effects for many, but not all parameters. This makes it difficult to understand the results and discussion parts, and to subtract true 'salinity' effects. A consistent order in which all different parameters

are discussed, and defining reference treatment, would help to point out the actual effects of salinity and to follow the provided arguments. Also, the authors should be more precise in defining which parameters they refer to when using the terms 'OA, 'seawater acidification, pCO2, low/high carbon', LC/HC'etc., especially as they work in a decoupled system.

Response: We appreciate these constructive comments and will revise the manuscript following the suggestions to analyze and point out effects of each driver and their combinations with greater clarity.

Technical corrections:
Abstract: New structure would improve comprehensibility of the abstract P2 L20: 'Combined effects' only becomes meaningful when the reader knows about the individual effects.
Response: We agree with the reviewer, and will revise our manuscript accordingly.

Introduction: P2, Line 24-28: C:P is not a parameter for calcification, but consist of two parameters.

Response: Thanks for pointing this out and we will rephrase the manuscript to clarify that this is a ratio of 2 parameters rather than a single parameter in its own right.

P4, Line 62: Please provide reference P4, Line 76: Mentioned reference does not quantify morphological changes P4, Line 78: Several more recent papers compiled or discussed literature on ocean acidification effects on E. huxleyi and dis- cussed factors as strain-specific differences and optimal curves in photosynthesis and calcification, all of which show that opposite trends in POC or PIC quotas in different studies are not of a clear 'contradictive' nature. P5, Line 99-100: More literature on growth responses to various salinity levels would be interesting. In terms of morphology changes, also refer to 'Morphological variation of Emiliania huxleyi and sea surface salinity. J Bollmann, JO Herrle - Earth and Planetary Science Letters, 2007 – Elsevier' P6, Line 108-109: Maybe mention experimental setup

Response: We thank the reviewer for the constructive comments, and will revise the manuscript accordingly, including citation of the missed literature.

Material and Methods P6, L 122-123: Reference to carbonate chemistry data table (Table 1 and 2) is missing. The authors later mention that a significant drift in carbonate chemistry occurred in the LC/ 25‰ salinity treatment over time (refer to P 18, L 385) that the reader should be made aware off here.

Response: Thank you for pointing out our omission here. This will be rectified in the revised manuscript.

P6, L 124: Were settings and initial cell concentrations comparable in the cited literature?

Response: The use of similarly low cell concentrations is common in the literature and is considered best practice to avoid biological activity disrupting the carbonate chemistry system. This was verified by taking samples for carbonate chemistry measurements at the beginning and end of the experiment (lines 137-138).

P7, L 135: Gas-tight bottles?
Response: Bottles were always kept sealed in the process of culture. This will be clarified in the revision.

P8, L 161-162: Specific growth rates based on measurements taken at the first day and last day of the cultures are very error-prone when initial cell concentrations are low (here 400 cells mL-1 (cp., P6, L 120)), especially in case cells undergo an initial lag phase. Could the authors provide cell concentration data including more sampling days?

Response: Cells were still in exponential phase at the final measurement, thus avoiding the common mistake of using a final cell density outside exponential phase. Care was taken to obtain accurate cell density measurements for the initial reading where three times measure was made for one parameter.

P11, L229 f.: In case that the authors think it is necessary to analyse linear relationships of their data, statistics for the linear regression procedure should be provided.

Response: A good point and these details will be added in the revised manuscript.

P12, L 251: 'Range' could also be the range of minimal and maximal cell size within one sample. Sentence should be rephrased in order to clarify which range is meant. It stands to question how relevant changes in cell size on such small scales are, or whether these changes may be caused by a measurement or natural treatment-independent variability.

Response: Here we meant to point out that cell size altered with salinity and with OA (Fig 1b), though such changes are small. We will clarify this carefully in the revision.

P13, L 260 - 266: Long sentence, difficult to understand.

Response: This sentence will be rephrased for clarity to " Under HC for growth, cultures at 25‰ cells showed an increased chlorophyll *a* content. Cellular chlorophyll *a* at this salinity was 72% ($p < 0.001$) higher than in cells grown at 30‰ and 1.8 times higher ($p < 0.001$) than in cells at 35‰ (Fig. 2a)"

P 14, L 287: The parameter Đd'PSII has not been introduced in material and methods.

Response: This is in fact mentioned on line 189 in the methods but was not properly defined there. This will be rectified in the revised manuscript.

P 14, L 295: The authors do not mention whether they measured photosynthesis and calcification under standardized conditions, and the quantities of 14C that were added. The according information should be provided.

Response: See our first response above. We will carefully clarify the conditions used for measurements of photosynthesis and calcification (growth conditions) in the revised manuscript.

P15, Line 314-325: How do the C:P values contribute to a deeper understanding of the physiology, given that the individual processes calcification and photosynthesis are already discussed? Neither salinity/the given DIC nor OA has an effect on C:P by itself here. It's therefore difficult to argue that one or the other effect is more pronounced.

Response: This is an interesting point. There did appear to be significant interactions such that HC and high salinity caused the greatest ratio of calcification to photosynthesis, but we take the point that it is hard to argue the importance of one condition over another and will revise the manuscript accordingly.

P16, L 332: smaller than?

Response: This should have read "……smaller decrease in Φ_{PSII} than cells grown at the higher salinities." This will be clarified in the revision.

Discussion: Focus is on carbon uptake and therefore neglects other aspects of salinity such as osmolality, ion concentrations, electrochemical gradients and implications.

Response: As mentioned above, we are grateful to the reviewer to point this out and will rectify the omission in the revised version.

P18, Line 369: Origin of strain should be mentioned earlier as it is quite substantial for the interpretation of the data

Response: Good point - which will be rectified in the revised manuscript.

P18, L370: Why genetic data?
Response: We should have specified that we cannot provide genetic data to prove similarity of our strain to the ecotypes identified by Paashe et al (1996). This will be corrected in the revision.

P18, L 373: Compared to which condition? More precise description of which treatments the authors compare, and a reference to the respective Figures should be provided.

Response: We will clarify these points in the revision.

P18, L 377: Which parameter is referred to with 'increased light capturing capability'? Reference to Figure is needed.

Response: We do refer to increased cellular photosynthetic pigments here, but will specify the relevant Fig (2a) in the revision.

P18, L 376 – 379: Definition of the 'tolerance' that authors refer to would improve the comprehensibility of the sentence. Under which treatments are photosynthetic pigment and light use efficiency [..]' increased? The authors should be more precise in referring to treatments, data and Figures.

Response: We will clarify these points in the revised manuscript

P18, L 385 – 387: Contradicts the 'no significant change of the carbonate chemistry' (P5, L 122).

Response: This is not in fact contradictory. The reference on p 5 line 122 is to changes during the culture process i.e. for a given treatment the carbonate chemistry was stable. Here (p18) we mean that the carbonate chemistry differed between treatments. We will clarify the statement on p 5 in revision.

P 18, L 388. Definition of calcifying capacity missing

Response: We will add this in the revised manuscript

P 19, L 390 – 395: Rates are not directly comparable, which makes the assumption that the observed culture was 'low-calcifying' difficult to believe.

Response: We will revise the manuscript to incorporate the proviso that rates may not be directly comparable due to differences in conditions etc.

P19, L 395 – 400: Discussion is based on the data point with an unintended drift in carbonate chemistry. Besides this, not only CO2 is increased in the LC/25‰ treatment (Table 2), but also pH is decreased. If CO2 is really the driver for the observed increase

in growth, growth should should also be increased under ocean acidification. Instead, growth is impaired under ocean acidification. The argument should be carefully thought through. Instead of focussing on the unintended drift in carbonate chemistry here, the authors could discuss that the increased growth occurs independently of ocean acidification and may be a 'true' salinity effect.

Response: Thank you for this insight. We will incorporate these possibilities in the revision.

P20, L 406-407: Associated changes in DIC should be mentioned.

Response: The differences in DIC will be incorporated

P20, L 408 – 411: Respiration is generally small in *Emiliania huxleyi* and unlikely to affect net photosynthesis by such a large dimension. Respective literature should be provided.

Response: We will provide more details on the magnitude of respiration rates and analyze more carefully if respiration could play a role as suggested.

P20, L 412 - 424: This paragraph does not consider recent studies on changes in carbon uptake of *Emiliania huxleyi* under ocean acidification. It is furthermore based on CO2 as main driver of physiological responses although treatments are ought to have equal CO2 concentrations. It neglects that the major difference in the discussed treatments are salinity and HCO3- concentrations.

Response: This is a good point. We should have taken into consideration the differences in bicarbonate concentrations in relation to salinity and this will be carefully considered in the revision.

P20, L 424 – 427: Sentences are difficult to understand and should be rephrased.

Response: This will be done.

P20, L 432: Changes in the pH of the chloroplast have, according to my knowledge, not been resolved in *Emiliania huxleyi*. Suffrian et al. 2011, for example, measured cytosolic pH.

Response: Good point. We will rewrite this to incorporate the fact that we have no direct measurements of chloroplast pH and be more cautious in our interpretation.

P20, L 428 – P21, L 436: This paragraph/argument appears contradictory to the first part of the discussion where all positive 'salinity' effects were associated with CO2.

Response: We will clarify this to better draw out the balance between positive effects of elevated CO_2 under OA and the negative aspects caused by pH alterations and a need to maintain intracellular pH homeostasis.

P21, L 443: Beaufort et al. (2011) investigated field samples. The setups can therefore not be consistent, but could only show similar trends.

Response: Good point. We will rephrase this to point out the studies showed similar trends.

P21, L 444 – 447: Calcification rates do not intrinsically correlate with coccolith thickness as up to 80% of coccoliths can be discarded.

Response: Thanks for pointing this out. We will modify the text to add this precautionary note.

P21, L 448 – 450.
Discussion is in contradiction with general OA literature that shows that show that OA impairs calcification and also contradicts the finding that calcification here drops under HC.

Response: A good point. We will modify the discussion to take this into account

P21, L 451 – 458: In that case, calcification should have significantly gone up under low salinity under LC and HC. Instead, there are hardly any salinity-driven changes in calcification P 22, L 458 – 464: This argument seems a bit far-fetched. It would, among others, imply that the intracellular pH goes up under osmotic shocks, which had to be experimentally proven P22. : 465- 466: 'reversed' not clear. P22, L 465 – P 23, L 477: Line of reasoning not quite clear to me P23, L 480 – 486: Certainly, increased photosynthesis can, under some circumstances, be reflected in increased cell diameters (especially if the specific growth rate stays constant). However, photosynthetic and calcification rates are not generally correlated to cell size or coccolith thickness. Cell size is instead regulated by an interaction of changes division rates and photosynthetic rates/calcification rates. In general, I do not quite understand how the presented cell sizes improve our knowledge about combined ocean acidification responses. P23, L 491: At the given DIC levels? How were the DIC levels during 14C incubation? P23, L 492- 493: To understand this, it should be mentioned that the changes in HCO3- between LC and HC and more pronounced in the LC/35‰ treatment than in the low-salinity treatments. Redirection of excess carbon to calcification when carbon cannot be used for photosynthesis has been discussed in previous studies (please refer to literature) P23, L 495 – 497: This 'decoupling' of salinity and HCO3- as drivers could be mentioned earlier P24, L 501 – 504: Would be nice to have main findings rephrased here. How do they adjust to the different conditions?

Response: All these are excellent points which we will incorporate into a revised manuscript, in which we will be less speculative about pH effects and pay more consideration to alterations in bicarbonate as well as CO_2 levels in the experimental treatments.

Reviewer 2

Reviewer #1 has already written a detailed review which contains most of my concerns and I have only few comments to add. I was not able to reproduce the calculated carbonate system parameters given in Tables 1 and 2.

Response: We have checked our calculations, which we believe to be correct. We would explain the discrepancies as being due to differences in nutrient levels etc.

p. 6, L108: If the manuscript by Wulff et al. (2016, BGD) is cited at all, it should be clearly stated that it has never been accepted by Biogeosciences.

Response: We will add the statement about the state of the Wulff et al manuscript.

p. 6, L116: ”Sterilized seawater was enriched with Aquil medium (Sunda et al., 2005).” How much Aquil medium did you add to sterilized seawater?

Response: 1 ml Aquil medium was added to 1 L sterilized seawater so the content of nitrogen and phosphorus of the medium was 10 μ mol/L

p. 7, L127-128: ... the salinity of our artificial seawater (Harrison, 2005) ...” Which culture medium (sterilized seawater or artificial seawater) was used for the experiments?

Response: We used artificial seawater for our experiments. This will be clarified in a revised manuscript.

p. 41 Table 1: I have used the MATLAB version of CO2SYS (CO2SYS originally by Lewis and Wallace 1998; Converted to MATLAB by Denis Pierrot at CIMAS, University of Miami, Miami, Florida; Vectorization, internal refinements and speed improvements by Steven van Heuven, University of Groningen, The Netherlands. Uploaded to CDIAC (http://cdiac.ornl.gov/oceans/co2rprt.html) at June 11th, 2009.) and obtained results that are different from the ones given in Table 1 (same for Table 2, data not shown). My input parameters are listed in the MATLAB script given below.

The differences might be caused by deviations in nutrients or salt composition of the culture medium from seawater, however, I could not find any information about such deviations in the manuscript.

Response: As stated above, we have checked our calculations, which we believe to be correct. We would explain the discrepancies as being due to differences in nutrient levels etc.