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Interactive comment on "Hyposalinity tolerance inthecoccolithophorid *Emiliania huxleyi* under the influence of ocean acidification involves enhanced photosynthetic performance" *by* Jiekai Xu et al.

Anonymous Referee #1

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General comments:

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

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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'.

Specific comments:

âĂć 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. âĂć 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. âĂć 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. âĂć 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

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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.

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.

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

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. P6, L 124: Were settings and initial cell concentrations comparable in the cited literature? P7, L 135: Gas-tight bottles? 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.

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Could the authors provide cell concentration data including more sampling days? 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. 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. P13, L 260 - 266: Long sentence, difficult to understand. P 14, L 287: The parameter Đd'PSII has not been introduced in material and methods. 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. 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. P16, L 332: smaller than?

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

P18, Line 369: Origin of strain should be mentioned earlier as it is quite substantial for the interpretation of the data P18, L370: Why genetic data? 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. P18, L 377: Which parameter is referred to with 'increased light capturing capability'? Reference to Figure is needed. 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. P18, L 385 – 387: Contradicts the 'no significant change of the carbonate chemistry' (P5, L 122). If the authors really

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think that the increased CO2 concentration in the LC/25‰ salinity treatment is responsible for the observed physiological studies, they should reconsider their experimental because they interpret the data based on an 'outlier' with a drift in carbonate chemistry P 18, L 388. Definition of calcifying capacity missing P 19, L 390 – 395: Rates are not directly comparable, which makes the assumption that the observed culture was 'low-calcifying' difficult to believe. 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. P20, L 406-407: Associated changes in DIC should be mentioned. 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. 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. P20, L 424 – 427: Sentences are difficult to understand and should be rephrased. 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. P20, L 428 – P 21, L 436: This paragraph/argument appears contradictory to the first part of the discussion where all positive 'salinity' effects were associated with CO2. P21, L 443: Beaufort et al. (2011) investigated field samples. The setups can therefore not be 'consistent', but could only show 'similar trends'. P21, L 444 – 447: Calcification rates do not intrinsically correlate with coccolith thickness as up to 80% of coccoliths can be discarded. P21, L 448 – 450.

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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. 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?

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