

Interactive comment on “Physiological responses of *Skeletonema costatum* to the interactions of seawater acidification and combination of photoperiod and temperature” by Hangxiao Li et al.

Anonymous Referee #2

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The manuscript titled “Physiological responses of *Skeletonema costatum* to the interactions of seawater acidification and combination of photoperiod and temperature” described a research attempting to explore the impact of high pCO₂ (or ocean acidification, OA) under different seasons (combination of photoperiod and temperature) on the diatom *S. costatum*. The experiments are well conceived, and methods are clearly presented. The most interesting observation is that high pCO₂ (OA) does not uniformly impact *S. costatum* under different seasons: with somewhat negative impact on winter conditions. The authors showed the interesting observation, but the experimental

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design and data quality can be improved.

Here are some questions and suggestions for the authors.

On the experimental setup, the authors stated that the cell culture pH did not change over 0.05 units in the 3d of one generation (section 2.3), so one very basic question is: what are the pH values and ranges for the six different conditions (i.e. three seasons and two pCO₂)? Since the manuscript is about how OA impacts *S. costatum* differently during different seasons, the acidification information, which can be presented easily as pH, is very critical to this whole article, however, this information is missing.

Following the cell culture pH question, the authors measured the photosynthesis (P) vs DIC curve at pH 8.12 (section 2.5) and very likely they did the same with P-I curve. It would be better if the authors measure the responses under lower pH for high pCO₂ treated cells, according to the high pCO₂ (OA) conditions. It should be expected that the pH is lower under OA conditions, and *S. costatum* acclimated to OA conditions may not photosynthesize better under the experimental condition with higher pH (8.12). As a result, the presented P-I and P-DIC curves for *S. costatum* cultured in HC (OA conditions) may not reflect their real physiological status in terms of photosynthesis under OA conditions. Also please note that Tris buffer is known to change pH significantly with different temperature, so it is important to measure or calculate the pH at certain temperature.

In the results session, the authors only mentioned and cited Fig. 1, while Figure 2, 3, 4 are listed at the end of the manuscript, none of those was referred in the text. In the tables presented, Table 2 and Table 3 do not show any units.

In section 3.5 where the authors present the “PSII protein concentrations”, RbcL was included as key PSII proteins. Such claim should be red-flagged, likewise the statement of “RbcL is related to the function of QA”. Inclusion of RbcL in PSII proteins is also found in the Abstract and Discussion. The authors should make sure what RbcL really does with creditable citations before writing assumptions or conclusions about RbcL.

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The description of methodology “Values of Actin were divided by other densitometric scanning values of protein to calculate Gray-scale values” should be modified to indicate the supposing meaning of normalizing density to Actin. With the data presented in Figure 4, panel (a) and panel (b) do not seem to agree with each other. The western blot data does not look like a representative of the statistical data. For example, in W-HC (winter high pCO₂) condition, the D1 density is much higher than Actin (Fig 4a), so such value is greater than 2 if analyzed using ImageJ, however, the data presented in statistics showed a value very tightly close to 0.9 (Fig 4b). It would be nice if all raw data (immunoblots) are presented to support the statistics in Fig 4b.

Other minor concerns: Section 2.7, “1000 and 34.5 are constants”, what are the units? Or at least provide the unit of “where C represents total chlorophyll concentration”. The use of letter “C” is ambiguous in the text. C was also used as carbon in line 219: “C fixation”. For the measurement of specific growth rate, more details on how data were collected would be helpful. It would be better to have the raw data, cell concentration vs time (days), presented. Line 229, “initial slop” should be “initial slope”.

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