

Review of Gao et al., “Regulation of inorganic carbon acquisition in a red tide alga (*Skeletonema costatum*): the importance of phosphate availability.

This is a review of a resubmitted, revised manuscript, which I originally reviewed. The authors should be commended for taking the original reviews from myself and another reviewer and making substantial changes and additions to their manuscript. The revised manuscript is improved, and I believe is suitable for publications following some minor revisions I list below.

Response: We appreciate these comments very much.

-One overarching question still remains to me: if P concentrations are important to these carbon concentrating mechanisms, which in turn promote bloom formation, what is the likelihood that these higher P concentrations will be available during a bloom, as P is drawn down along with CO₂ during a bloom? It seems that the P would become as unavailable as CO₂ during bloom formation, and thus not able to be utilized by in these CCMs, unless nutrient conditions are quite eutrophic. Perhaps the authors can speculate on this question in the discussion?

Response: We appreciate this constructive suggestion. Although P is replete in eutrophic waters at the early stage of algal blooms, P limitation may occur at the late stage of algal bloom, which leads to shift of dominating algae. We have added this point to the text “The CCMs of *S. costatum* are hampered under P limiting conditions and only function when P is replete. This finding may explain why diatoms could overcome carbon limitation and dominate red tides when P is replete and as well as the shift from diatoms to dinoflagellates when P is limiting (Mackey et al., 2012)” at P20L388-392.

Minor Comments:

-P5L51: these common and unusual family notations (alpha, beta, sigma etc.) are not really explained, and unfamiliar to me

Response: It has been clarified to “multiple carbonic anhydrase (including both common (α , β , γ , found in all algae) and unusual (δ , ζ , found only in diatoms) families that carries out the fast interconversion of CO₂ and HCO₃⁻)” at P5L51-15.

P5L54: remove “.” after “fixation”

Response: Corrected.

-P5L67 remove “in” from “that in S.”

Response: Corrected.

-P6L71 define ATP

Response: It has been revised to “adenosine-triphosphate (ATP)”.

-P6L90- change to “Our study provides helpful...”

Response: Corrected.

-P7L98- change to “set as 200...”

Response: Corrected.

-P7L109- change to “blooms”

Response: Corrected.

-P8L114 change “till” to “until”

Response: Corrected.

-P8L116 changes to “units”

Response: Corrected.

-P8L122-134- these lines are all indented and do not need to be

Response: Corrected.

-P8L130-132- this is a direct copy of the previous statement in P8L118-120

Response: We apologize for the repetition. These lines have been removed.

-P9L135- what is “photosystem II”?

Response: It has been clarified to “photosystem II (the first protein complex in the light-dependent reactions of photosynthesis)”.

-P9L139 change to e-1

Response: We think it would be better to keep it as here e⁻ means electron with negative charge and this is the usual expression for electron transport rate in photosystems (Alderkamp et al, 2012; Perkins et al., 2018).

Alderkamp, A. C., Kulk, G., Buma, A. G., Visser, R. J., Van Dijken, G. L., Mills, M. M., & Arrigo, K. R. (2012). The effect of iron limitation on the photophysiology of *Phaeocystis antarctica* (Prymnesiophyceae) and *Fragilariopsis cylindrus* (Bacillariophyceae) under dynamic irradiance. *Journal of Phycology*, 48(1), 45-59.

Perkins, R., Williamson, C., Lavaud, J., Mouget, J. L., & Campbell, D. A. (2018).

Time-dependent upregulation of electron transport with concomitant induction of regulated excitation dissipation in *Haslea* diatoms. *Photosynthesis Research*, 1-12.

-P12L203- change to “no further”

Response: Corrected.

-P18L337- change to “exactly the same”

Response: Corrected.

-P18L348- change to “based on experiments”

Response: Corrected.

-P18L352- change to “directly, and whose photosynthesis...”

Response: Corrected.

-P19L366- change to “In the development of red tides, the pH in seawater can be...”

Response: Corrected.

-P19L376- change to “*S. costatum* in order to overcome ...”

Response: Based on the previous comment, it has been revised to “This finding may explain why diatoms could overcome carbon limitation and dominate red tides when P is replete and as well as the shift from diatoms to dinoflagellates when P is

limiting (Mackey et al., 2012).” at P20L389-392.

-P20L382- change to “overcome CO2 limitation...”

Response: Corrected.

-P20L385- change to “mechanisms that help *S. costatum* dominate algal blooms.”

Response: Corrected.

The authors grew the model diatom *Skeletonema costatum* in seawater media at pH 8.2 at 5 [PO₄⁻]. They shifted samples to pH 8.2 (no change) or to pH 8.7 and measured the photosynthetic responses to a matrix of imposed [PO₄⁻] and [CO₂]. They do not state (although they have the data) the [DIC] under their treatments, and I question their protocol for lowering [CO₂] which depends on blocking equilibration between the culture suspension and the headspace, if any. I offer suggestions for major (although easy) redesign of the figures that might help convey the findings.

best regards, Doug Campbell

Response: We appreciate the reviewer’s comments and the manuscript has been revised by responding to these comments.

Figure 1:

This figure should be an X-Y plot of photosynthesis (A) or respiration (B) vs. $\mu\text{mol Phosphate L}^{-1}$. Each graph should have 2 series, a low carbon dioxide and a high carbon dioxide.

Do not erect unnecessary levels of coding; 'AC'? 'LC'?? Why? Just give the CO₂ concentrations.

Response: Corrected.

Fig. 2 should be panel C of Figure 1, same comments as above.

Response: Corrected.

rETR $\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$ is not a meaningful unit for a suspension of cells. Think about what is being measured and how.

Same comment regarding XY plot as Figure 1

Response: We thank the reviewer for this constructive comment. By following the method of Perkins et al (2018), the absolute photosynthetic electron transport rate through PSII (ETR, $\mu\text{mol e}^- \text{PSII}^{-1} \text{s}^{-1}$) can be estimated by the following equation: $\text{ETR} = E \times \sigma_{\text{PSII}} \times \Phi_{\text{PSII}} / ((F_M - F_0) / F_M)$. However, we do not have a fluorometer that can measure σ_{PSII} . To estimate the absolute ETR, we would like to use the following equation: $\text{ETR} (\mu\text{mol e}^- (\text{mg Chl } a)^{-1} \text{s}^{-1}) = 0.5 \times E \times \Phi_{\text{PSII}} \times \bar{a}^*$ (Dimier et al., 2009; Alderkamp et al., 2012), where Φ_{PSII} (dimensionless) is the PSII photochemical efficiency, E ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) is the ambient light density and \bar{a}^* is Chl *a*-specific absorption coefficient ($\text{m}^{-2} (\text{mg Chl } a)^{-1}$). Since \bar{a}^* is light-dependent, we used the value of 0.0138 based on Lefebvre et al’s (2007) study in which the light density is very close to ours.

- Alderkamp, A. C., Kulk, G., Buma, A. G., Visser, R. J., Van Dijken, G. L., Mills, M. M., & Arrigo, K. R. (2012). The effect of iron limitation on the photophysiology of *Phaeocystis antarctica* (Prymnesiophyceae) and *Fragilariopsis cylindrus* (Bacillariophyceae) under dynamic irradiance. *Journal of Phycology*, 48(1), 45-59.
- Dimier, C., Brunet, C., Geider, R., & Raven, J. (2009). Growth and photoregulation dynamics of the picoeukaryote *Pelagomonas calceolata* in fluctuating light. *Limnology and Oceanography*, 54(3), 823-836.
- Lefebvre, S., Mouget, J. L., Loret, P., Rosa, P., & Tremblin, G. (2007). Comparison between fluorimetry and oximetry techniques to measure photosynthesis in the diatom *Skeletonema costatum* cultivated under simulated seasonal conditions. *Journal of Photochemistry and Photobiology B: Biology*, 86(2), 131-139.
- Perkins, R., Williamson, C., Lavaud, J., Mouget, J. L., & Campbell, D. A. (2018). Time-dependent upregulation of electron transport with concomitant induction of regulated excitation dissipation in *Haslea* diatoms. *Photosynthesis Research*, 1-12.

Fig. 4 same comment

Response: Corrected.

Fig. 5, OK, now we switch the rules and plot photosynthesis vs. DIC, with series for [PO₄-].

Response: Yes. This is what we did.

Fig. 6, same comment as Fig. 1. Do not put a quantitative data series as a categorical axes. 0.05 to 10 $\mu\text{mol PO}_4^-$ are not different categories, they are arbitrary measurement points along a (potentially) continuous axes of PO_4^- .

Use a log scale if necessary.

Response: Corrected.

Fig. 7, same comment.

Response: Corrected.

Materials & Methods:

Lines 101-103

This is an odd, incomplete way of expressing the applied DIC treatment. What was the [DIC] under the two treatments? A shift from bubbling with ambient air (outdoor? indoor?) pH 8.2 to pH 8.2 (no change) or pH 8.7 (increase) would lead to increased dissolution of head space CO₂ into water at pH 8.7, with a concomitant increase in [DIC] if the cell suspension is allowed to equilibrate with a headspace. If not headspace is provided the suspension will be in [CO₂] deficit as pH equilibration drives [DIC] to [HCO₃⁻], lowering [CO₂]. Then, any exposure to gas with ambient CO₂ will lead to uptake.

Response: The concentrations of DIC were 2109 ± 36 and $1802 \pm 38 \mu\text{mol (kg seawater)}^{-1}$, respectively. As mentioned in the text, the cultures were open to the ambient atmosphere but this did not lead to the increase of DIC or CO₂. Instead, there

was a slight decrease in CO₂ because of the algal photosynthesis. The text has been clarified to “Afterwards, cells were resuspended in fresh media with two levels of pH (8.20 and 8.70, respectively corresponding to ambient CO₂ (12.6 μmol L⁻¹, AC) and low CO₂ (2.8 μmol L⁻¹, LC) under corresponding phosphate levels for two hours before the following measurements, with a cell density of 1.0 × 10⁶ mL⁻¹. The concentrations of DIC were 2109 ± 36 and 1802 ± 38 μmol (kg seawater)⁻¹, respectively.” at P37L102-107 and “The cultures were open to the ambient atmosphere and the rise of culture pH due to algal photosynthesis was below 0.02 units (corresponding to the decrease of CO₂ less than 0.7 and 0.2 μmol L⁻¹ for pH 8.20 and 8.70 treatments respectively) during the two hours of pH treatment.” at P8L118-121.

Line 140: the rETR units are algebraically correct, but are not meaningful in the sense of a cell suspension.

Response: This has been corrected and please see the response above.