

## ***Interactive comment on “Regulation of inorganic carbon acquisition in a red tide alga (*Skeletonema costatum*): the importance of phosphorus availability” by Guang Gao et al.***

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Review of Gao et al. "Regulation of inorganic acquisition in a red tide alga (*Skeletonema costatum*): the importance of phosphorus availability

The authors present a manuscript detailing culture studies of a common algae species under varying CO<sub>2</sub> and phosphorus concentrations. Algal blooms can draw down dissolved CO<sub>2</sub> to very low levels, and some species have developed mechanisms to compensate for decreased CO<sub>2</sub> availability. Some of these mechanisms may be influenced by the presence or absence of bio-available phosphorus, leading to the study design of varying phosphorus levels across test populations of two CO<sub>2</sub> levels. While there

C1

is probably a compelling study underlying this manuscript, in my opinion there are too many flaws as presented to encourage publication. I feel compelled to point out here that my background is in seawater carbonate chemistry, and my knowledge of cellular biochemical processes is limited. However, based solely on the description of inorganic carbon system measurements I would advise rejecting this manuscript until serious revisions have been done. I will present some major comments below, followed by more minor concerns.

**MAJOR COMMENTS** -The Materials and Methods section, in particular the sections pertaining to pH and alkalinity measurements, is totally inadequate. Is the pH system an electrode-type system? What pH scale are measurements presented in? How was the pH system calibrated? How were alkalinity titrations performed? No information is presented. These questions are especially critical in the calculation of DIC from pH and alkalinity (P7L129-131), which is very sensitive to relatively minor changes in pH and alkalinity. With no information about the quality of pH and alkalinity measurements, the results of this analysis are impossible to interpret.

-Besides using the barely-described pH system, how did the authors know the CO<sub>2</sub> levels of their cultures? Also, adding phosphate to the cultures, at concentrations ranging from 0.5-10  $\mu\text{mol/kg}$  adds a potent buffering agent, as monosodium phosphate has a pK<sub>a</sub> around 7. How did the authors alter or maintain the pH in the cultures? Were the cultures open to the ambient atmosphere?

-As previously mentioned, my knowledge of some of the biochemical processes presented here is minimal, and the Introduction did little to help readers like myself. There seem to be connections between plasma membrane redox activity, CAext, rETR, but the manuscript does not explain them. Some terms (i.e. rETR) are presented with no explanation or definition. Thus the reason for some of the analyses presented was unclear to me. What did measuring the chlorophyll fluorescence inform? The cultures were initiated at the same cell density, but surely the cell density varied between cultures after the treatment period- how was this accounted for?

C2

-In the Results section the authors present their statistical findings in the form  $F(1,20)=XX$  or  $F(4,20)=XX$ . I'm assuming these are the results of the two-way ANOVA test mentioned in the 2.8 Statistical Analysis section, but no explanation is given as to what is signified. Are the numbers in parentheses indicating degrees of freedom? What is the threshold for significance?

-The Results section is extremely repetitive. Much of the information presented could be more effectively summarized in a table.

#### MINOR COMMENTS

-The English usage in much of the manuscript could be improved. I will note some points below.

-Define rETR in the Abstract (P2L10)

-P2L3 and throughout: define the abbreviations when first used: CO<sub>2</sub>, DIC, HCO<sub>3</sub><sup>-</sup>, rETR

-P2L16 change "is" to "was"

-P3L26 change to "the marine biological"

-P3L31 need a different word than compelling

-P3L33 and throughout: don't finish sentences with "etc".

-P3L34 change "could" to "can".

-P3L36 misspelled "dominate"

-P3L37 P3L40-41 How is RUBISCO important? What is it, an enzyme?

-P4L45-48: the carbon concentrating mechanisms are named but not explained. A reader like myself has no way to know what "multiple carbon anhydrase, assumed C4-type pathway" represents

#### C3

-P4L54 define CCMs (CO<sub>2</sub> concentrating mechanisms, right?)

-P4L57: keep consistent units between discussions of CO<sub>2</sub> or pCO<sub>2</sub>. Discuss either CO<sub>2</sub> concentration or partial pressure. Reader has no way to compare 5  $\mu\text{mol/L}$  CO<sub>2</sub> to a pCO<sub>2</sub> of 1800  $\mu\text{atm}$ .

-P5L69: cite the references yourself, don't refer to references therein

-P5L70: what is the relationship?

-P5L75-76: remove "the capacity of"

-P5L78-79: all these mechanisms/pathways! How do they interrelate?

-P5L82: as CO<sub>2</sub> is removed by diatom growth, the inorganic carbon equilibria will shift to convert HCO<sub>3</sub><sup>2-</sup> to CO<sub>2</sub>. How do the kinetics of this potentially affect this study?

-P6L105: what does "algae after in light" mean?

-P6L108: change to "photosynthetic and respiration rates"

-P7L114-116: why measure photosynthesis for 5 minutes but respiration for 10?

-P7L126: what is "Ci-saturated maximum rate"?

-P8L149: by "samples" do you mean the diatom cells?

-P9L156: what is the exofacial ferricyanide reduction reaction?

-P9L158: "pH drift" connotes instrument drift to me, not pH changes due to cellular activity

-P9L169: change "on differences"

-P9L174: Need more information on the Bonferroni correction.

-P11L200: is the Bonferroni correction the same as the "Post hoc LSD comparison" mentioned here? I don't think it is? What is this comparison?

#### C4

- P11L213: change "access" to "assess"
- P11L216: what does "interplayed" signify?
- P12L230: is the peak the same as the plateau mentioned earlier?
- P12L231: what do you mean by "assayed"?
- P13L247: how was the pH compensation point identified?
- P13L260-261: not sure what "comparative photosynthetic rates" means
- P15L293: does "inorganic carbon" here mean both carbonate and bicarbonate?
- P15L303: change to "increased the redox"
- P15L304: misspelled extracellular
- P16L315: change to "as the CO<sub>2</sub>"
- P16L317: change to "with a strong"
- P16L319: change to "the red macroalgae"
- P17L340: change to "the potential mechanisms"
- P17L342: change to "are hampered"
- P17L348: change to "growth"

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