

Interactive comment on “Effects of CO₂ and iron availability on *rbcL* gene expression in Bering Sea diatoms” by H. Endo et al.

Anonymous Referee #3

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General comments:

This manuscript presents pigment composition and *rbcL* transcription data from on-deck incubation experiments manipulating carbonate chemistry and Fe concentration using HNLC waters from the Bering Sea. A follow-up to Sugie et al. 2013 and Yoshimura et al. 2014, it aims at revealing, at molecular level, the responses of natural phytoplankton assemblages, in particular diatoms, to interactive impacts of ocean acidification and Fe availability. The authors concluded that CO₂ enrichment and Fe limitation synergistically caused a negative effect on diatom growth, as demonstrated by fucoxanthin concentration and the expression and diversity of *rbcL*.

Albeit the results presented in this manuscript are somewhat interesting, I found that overall the article is rather difficult to understand, as it doesn't properly provide the

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context of the study, and that the discussion is overly speculative.

Specific comments/questions:

Page 18106, lines 11-15. This is misleading! 1) “At the END of incubation, the relative contributions of diatoms to. . .”. According to Materials and Methods, the incubation last for 7 days and therefore day 7 should be the END; however, Fig. 2A. only shows data collected on days 3 and 5. 2) The contribution of diatom to total Chla biomass actually increased over the course of the experiment regardless of CO₂ or Fe treatments (Fig. 2). It is the extent of this increase that was less at high CO₂.

Page 18106, line 21. No, it is not the “activity” – there is no RubisCO activity measurement in this study – it should be RubisCO transcription.

Page 18109, lines 2-4. Is the effect of CO₂ and/or Fe availability on rbcL transcription in diatoms really COMPLETELY unknown? Here I just give two examples: Granum et al. 2009 J Phycol; Shi et al. 2013 Appl Environ Microb.

Pages 18109-18110, “Experimental setup”. More details on how trace metal clean techniques were applied should be provided. For instance, under what conditions and how was the seawater poured into 50 L carboys? Did the CO₂ gas pass through 0.22 filters before being introduced into the incubation bottles?

The authors discuss the roles of the CCM in the response of diatoms to CO₂ and Fe. They first (page 18121, lines 4-5) suggest that CCM may have been down-regulated at high CO₂, resulting in the decrease in biomass in both Fe-deficient and Fe-added bottles; however, later on (page 18123, line 26 to page 18124, line 1) they suggest that “diatoms can upregulate CCM activity at elevated CO₂. . . , photosynthetic carbon fixation in diatoms could not be limited by CO₂ availability as a consequence of the CCMs”. These two statements are contradictory to each other. Please clarify! Without any direct experimental evidence it would be impossible to evaluate the roles the CCM may play in this paper.

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Page 18124, lines 2-8. Fv/Fm indicates the maximum photochemical quantum yield of PSII. An increase in Fv/Fm doesn't necessarily mean more energy for CCMs.

Figs. 1, 2, and 4. The time points at which the data presented in these figures were collected are inconsistent. Fig. 1 shows pigment data from the first and the last day (day 7, I presume), Fig. 2 shows data from days 3 and 5 for the Fe-deficient and days 4 and 6 for the Fe-added treatments, and Fig. 4 shows data from day 3 for the controls and day 2 for the Fe-added bottles. The authors need to clarify why the samplings/measurements were performed in such a way, which makes it difficult to compare the results among the treatments to arrive at conclusions.

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