

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

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General comments: The authors conducted a 2009 Bering Sea 6 day shipboard bottle incubation experiment with Fe enrichment across a range of pCO₂ levels. They tracked the cell numbers (and identities), pigment-based biomass allocations, *rbcL* transcript and gene copy numbers, fucoxanthin and haptophyte indicator pigments and the nutrient levels in the bottles. The Bering Sea water was HNLC and addition of Fe provoked an accumulation of cells and a drawdown of nutrients. Under elevated pCO₂ diatoms did not increase as much as they did at ambient pCO₂. This fits some other literature. Under elevated pCO₂ and elevated Fe, diatoms appeared to escape the pCO₂ limitation on their increase.

rbcL transcripts decreased under elevated pCO₂, whether or not Fe was added. The

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authors speculate this might relate to limitation on RuBP regeneration under elevated pCO₂.

There is interesting calibration data here showing that *rbcL* gene copy correlates well with fucoxanthin, and could thus be used as a proxy for diatom biomass, since large diatoms carry more *rbcL* gene copies than small diatoms.

The data is OK, but the discussion is preliminary; some points are overly speculative, with others are insufficiently developed.

I offer some comments below that may help the authors. best regards, Doug Campbell
Scientific questions/issues

Materials & Methods: Given the importance to the findings, I think the authors should include a diagrammatic figure of the standards, the amplification primers, and the amplicons used for the DNA and cDNA quantitations. From the text, I infer that the standard is only 113 bp long, for the DNA quantitations, but that a different standard was used for cDNA (length?). The primers 5'- GATGATGARAAYATTAATC-3', reverse primer: 5'-TAWGAACCTTTWACTTCWCC-3'.

Discussion: “Our study indicates that the decrease in diatom biomass given elevated CO₂ levels was unique to the Bering Sea basin.” No. Unique would mean that this response is only present in the Bering Sea, and we do not know that yet. In fact a preceding sentence mentions similar responses in the Okhotsk Sea.

are 19-20 bases long, leaving an amplified region of only 60 bp between the primers. It appears (but I am not sure) that the same primers are used for both DNA and cDNA quantitation. If so, why would you use two different quantitation standards?

“However, we speculate that CCMs in the diatoms might not be active in the control treatments because Fe deficiency could reduce the functionality of algal CCMs due to a reduction in their light energy-harvesting ability (Giordano et al., 2005).” This needs to be better explained. It is unlikely that Fe deficiency would limit CCM simply through

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a limitation on light energy harvesting.

“However, because carbon fixation in diatoms is controlled not only by RubisCO activity but also by CCMs (Rost 20 et al., 2003),” Actually, in the discussion you raised the issue of RuBP regeneration as a limiting factor under elevated CO₂ as well.

Technical corrections: Table 1: “Macronutrients and Fe parameters are the values at the initial or final sampling days.” Is the final sampling day 4? or day 6? Or either depending upon the particular treatment? I think this needs to be defined.

Given the large drops in NO₃⁻, PO₄³⁻ and silicic acid, what is the time course? By the final sampling points the cells were likely limited by macronutrients.

Figure 1 legend: define the basis of the normalization (g pigment/g chlorophyll a, I think).

Figure 3 & Results: Fucox This is not a standard abbreviation. Why use it? Why not just write Fucoxanthin? Fucox also has an unfortunate pronunciation in English. Discussing the ‘Fucox’ graph is going to make people think of rude behaviour with neutered male cattle ;)

Fig. 4: Nice.

Dinoflagellates, not dinoflagillates

“and diatoms that were neither centrics nor pennates” Do you mean diatom sequences that could be assigned to centrics or pennates? Or diatoms that are actually something other than centric or pennate? I did not know about any.

“A significant correlation between rbcL copy number in diatoms and Fucox concentration was found in this study (Fig. 3), suggesting the usefulness of the rbcL gene fragment as a proxy for diatoms as well as Fucox.” I think, rather:

“A significant correlation between diatom rbcL copies per litre and Fucox concentration was found in this study (Fig. 3), suggesting the usefulness of the rbcL gene fragment

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as a proxy for diatom biomass.”

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