

## ***Interactive comment on “Effects of CO<sub>2</sub> and iron availability on *rbcL* gene expression in Bering Sea diatoms” by H. Endo et al.***

### **Anonymous Referee #2**

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#### General comments:

This paper reports on a subset of results from shipboard incubations of natural phytoplankton communities from the Bering Sea, where CO<sub>2</sub> and Fe concentrations were manipulated. Their conclusions were that under Fe-limitation, elevated CO<sub>2</sub> had a negative effect on diatom growth, as demonstrated by pigment composition and *rbcL* expression. There are clear differences in fucoxanthin content and *rbcL* expression between the CO<sub>2</sub> treatments, which I find interesting, along with a nice relationship between *rbcL* copy number and fucoxanthin concentration, which opens up the possibility of using *rbcL* copy number as an indicator of diatom biomass. However, I feel that this paper is difficult to read without first reading an earlier paper by Sugie et al (2013), which gives a much more detailed picture about what is happening over the course of

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the incubation and provides the much needed context in which the results of this paper can be based. I find the author's results are somewhat interesting but the discussion is overly speculative with little supporting data. In particular, the speculation about the role of the CCM seems very unsupported by the results.

Specific comments to improve the paper:

The abstract states: "At the end of the incubation, the relative contributions of diatoms to chl a biomass decreased significantly with increased CO<sub>2</sub> levels in the controls". This is misleading as the contribution of diatoms to chl a biomass increased over the course of the incubation in all bottles; it is the extent of this increase that is less at high CO<sub>2</sub>.

In addition, the sentence starts with "At the end of the incubation...". This would be after 7 days when the bottles were clearly depleted of nutrients. Table 2 gives insufficient information to know when nutrient limitation occurred and I would also like information on how long it took the bottles to equilibrate with CO<sub>2</sub> (this information is given in Sugie et al, 2013 but is not sufficiently discussed in this manuscript). In addition, it is confusing to know when the data points were collected. Table 2 and Figure 1 show data from the final day (7?) whereas Figure 2 shows data from days 3 – 6. This lack of clarification makes it difficult to draw conclusions to what is happening and raises question to whether the results are purely due to CO<sub>2</sub> manipulation and not due to nutrient limitation.

The abstract further states "These results indicate that under Fe-deficient conditions, the growth of diatoms was negatively affected by the increase in CO<sub>2</sub> availability". I would be careful with this statement. I would say their ability to compete is better at high CO<sub>2</sub>. I am interested in what is happening with the haptophytes. Like diatoms they also increase in abundance over the course of the incubation but this increase is less under high CO<sub>2</sub>. However, in Fig 2 it looks like they increase their contribution to total chl a at high CO<sub>2</sub> in control bottles. Perhaps the story is more about the competition

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between diatoms and haptophytes under different CO<sub>2</sub> rather than just interpreting everything in terms of diatom growth.

A lower expression of diatom *rbcL* normalized to *rbcL* gene number certainly implies the diatoms are less active. This finding supports other studies that show Rubisco is regulated at the expression level in diatoms. However, this has not been absolutely proven yet, and given the tight regulation of Rubisco protein activation in plants, it is hard to accept that *rbcL* expression equals photosynthetic rates in this paper without more study.

How do the authors reconcile that *rbcL* expression is lower in both Fe-added and Fe-limited incubations whereas fucoxanthin concentrations are only lower in Fe-limited cultures?

It is difficult to tell from the rarefaction curves whether they are approaching saturation. As such, it is difficult to say whether the number of OTUs are different between the treatments. I do not have a good understanding on whether the differences found in the Shannon Index and Simpson diversity are significant. More details would be appreciated.

Significant differences were found in the cDNA libraries under different CO<sub>2</sub> within the Fe-treated incubations. Are the authors certain that this is due to a change in diatom *rbcL* sequences rather than a change in the non-diatom *rbcL* sequences that were detected? (in the initial treatment it seems that ~ 17 % of the *rbcL* cDNA library comes from other eukaryotes).

The authors discuss the influence of Fe and CO<sub>2</sub> on the CCM. However, I feel that their link between Fe and the CCM is tenuous. Fe is important for PSII, and Sugie et al (2013) found increased F<sub>v</sub>/F<sub>m</sub> with increased Fe, which is to be expected. However, speculating that the Fe limitation down-regulates the CCM through lack of energy provided by PSII seems tenuous. Without any further measurements it is difficult to draw any conclusions about the role of the CCM in this paper.

**BGD**

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In the discussion about *Chaetoceros*, it should be noted that Trimborn et al, 2013 *Limnol. Oceanogr.*, 58(3), 2013, 997-1007 | DOI: 10.4319/lo.2013.58.3.0997, found that *Chaetoceros debilis* increased growth rates under high CO<sub>2</sub> and that Tortell et al 2008 *GRL*, 35 (4) DOI: 10.1029/2007GL032583 found *Chaetoceros* spp. to dominate shipboard incubations from the Ross Sea under high CO<sub>2</sub>.

Technical Comments:

I presume figure 1 is the concentration of fucoxanthin ( $\mu\text{g/L}$ ) in the final sampling day (day 7?) divided by the fucoxanthin concentration ( $\mu\text{g/L}$ ) in the initial bottle? This should be clearer.

Fucox is a strange abbreviation and I would keep the full word fucoxanthin.

In abstract “To confirm this. . .” (that diatom growth is negatively affected by high CO<sub>2</sub>) seems to be too strong a statement as mRNA of *rbcL* is not a direct measurement of growth. “To further support this finding. . .” would be better.

In the supplementary I would appreciate more information about how the conditions changed in the bottles over time. Perhaps a Table showing the measured values from every day so the readers can see when nutrient limitation occurs, how long it takes CO<sub>2</sub> to equilibrate, the increase of total phytoplankton biomass (POC or Chla) over time and when different samples were collected.

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Interactive comment on *Biogeosciences Discuss.*, 11, 18105, 2014.

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