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## ***Interactive comment on “Co-limitation by iron, silicate, and light of three Southern Ocean diatom species” by L. J. Hoffmann et al.***

**Anonymous Referee #2**

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The authors aim to unravel the importance of combined iron, silicate and light co-limitation in three Southern Ocean diatom species. An appropriate choice of the high and low iron, silicate, and light levels is central to the relevance of this study for the ‘real’ Southern Ocean. However, the authors do not justify their choice of the specific high and low levels for iron, silicate and light in their bottle incubations, even as they draw conclusions from their findings to controls on Southern Ocean phytoplankton.

Whilst not being an expert on batch incubations of diatoms, I recommend rejection of the article on the grounds that the choice of high and low silicate levels is not appropriate for extrapolation to the Southern Ocean. Information on the iron concentration in the low iron treatments is essential for a comparison of low and high iron treatments. The evolution of the nutrient and iron concentrations during the experiments should be

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given. Furthermore the conclusions are only partly supported by the observations. In addition I recommend review of this article by at least one expert on diatom growth in batch incubations.

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Elaboration on the above comments:

The low silicate level of 20  $\mu\text{M}$  is not necessarily limiting for diatoms north and south of the Polar Front. Sedgwick et al. (2002) determined silicate concentrations of 0.5  $\mu\text{M}$  and 1.2  $\mu\text{M}$  north and south of the Polar Front. These authors used high-silicate treatments of 4.4  $\mu\text{M}$  and 18.3  $\mu\text{M}$  in incubations, respectively, while they had corresponding control (low) silicate treatments of 0.5  $\mu\text{M}$  (north) and 1.2  $\mu\text{M}$  (south) of the front. The length of incubation in their study was 5 (north) and 9 days (south). The 'low-silicate' concentrations in the manuscript are in fact quite high silicate concentrations in the real ocean.

In the high-silicate experiments by Sedgwick and colleagues the silicate concentrations did not decrease by more than a third of the initial concentrations. Thus, there is no justification for a high silicate concentration of 20  $\mu\text{M}$  in the 'low silicate' treatments (in contrast to the statement on p220 lines 26-27).

The high silicate concentration of 200  $\mu\text{M}$  is a very high concentration, which is not found in Southern Ocean surface waters (WOCE Southern Ocean Atlas, 2005).

No justification for the choice of light levels is given. Combining the abstract with Table 1 suggests that the high light level of 90  $\mu\text{mol photons /m}^2\text{/s}$  corresponds to the light in an actively mixed layer of 28 m. However, it is not clear if this is an average light level integrated for the daytime hours and depth range, nor at which latitude and season such a light level is found and whether the light level is for days with clear or overcast skies. The absence of such information makes it difficult to judge how relevant the light level is. Gervais et al. (2002) found depth averaged irradiance of 100-150  $\mu\text{mol}$

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photons  $/m^2/s$  on most days of the EisenEx experiment (in the Polar Frontal Zone, north of the Polar Front) in November 2000, with higher levels of 38–80  $\mu mol$  photons  $/m^2/s$  on two days. From this it appears that the low light level is on the low end of the light levels in parts of the Southern Ocean in spring, while the high light level exceeds that in the springtime EisenEx experiment.

The iron concentration in the low iron treatments is not indicated. No justification is given for the choice of the high iron level. Strzepek and Harrison (2004) use a dissolved inorganic iron concentration of 0.4–0.7 nM  $[Fe']$  in their iron replete treatments. The level in this study (1.55 nM  $[Fe']$ ) is 2–4 times that level.

The absence of information on how the nutrient and iron levels evolve during the incubations makes it difficult to interpret these results. It is questionable whether ‘the low iron and low light conditions represent typical Southern Ocean conditions’ (page 216 line 16).

The duration of some of the experiments (up to 77 days) is long compared to the studies by Sedgwick et al. (2002) (5–9 days).

Abstract:

The conclusions and parts of the abstract are not justified by the data:

The statement that all species are co-limited by iron and silicate is flawed (abstract lines 7–9). The high-iron high-silicate treatments F and H (Figure 4) do not systematically show higher cell numbers for all species than the high-iron low-silicate treatments (E, G). Of course one might argue that the silicate concentration in the low-silicate treatments is non-limiting.

The suggestion that grazing indirectly effects species composition (via different levels of these nutrients, presumably Si and Fe) (abstract lines 14–15) is not justified by these experiments which exclude grazers.

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The authors speculate that the frustule deformation is a useful biological marker in sediments (e.g abstract lines 15-17). However, they do not indicate if such deformation has been observed in sediments.

The observation that the (very) high light conditions have a negative impact on growth does not imply that diatoms do not suffer from low light availability, notably at levels below the low light treatment in these experiments (abstract lines 18-22).

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#### Methods:

Page 214, lines 15-17. Where and when were the diatoms isolated (month, latitude, geographic area)? Where the diatoms isolated in the non-iron enriched waters?

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#### Results:

Several interpretations of the experiments are dubious or possibly not statistically significant. Certainly the reader is easily lost in the large number of treatments and parameters for three different species, especially as the effects on the diatoms are not clear cut. Here some examples:

Page 216. Figure 2. The suggestion that high silicate concentrations lead to cell elongation is not clear for *C. dictyota* in iron replete conditions. The cell length of diatoms is not clearly different in figures E versus F and of G versus H (given the different scale of Figure E).

Page 216 16-21 and Figure 4. It is difficult to judge differences in cell numbers between the treatments as the scales of Figure 4 vary widely, especially for *C. debilis*. Whilst struggling with the scales I would conclude that cell numbers of *C. debilis* are high in the 'low-silicate' treatments A, E and G, moderate in C, and low in the high silicate treatments B, D, F, H. Furthermore *C. dictyota* should be excluded from the analysis

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given the small number of samples.

Page 219-220. In the discussion on chain length it is not clear whether the differences between the treatments are significantly different.

The interpretation of these experiments needs careful thought. There could well be interesting science in them, but this is not obvious from the current manuscript.

References:

Gervais, F., Riebesell, U., Maxim Y. Gorbunov, M.Y., 2002. Changes in primary productivity and chlorophyll a in response to iron fertilization in the Southern Polar Frontal Zone, *Limnol. Oceanogr.*, 47(5), 2002, 1324-1335.

Sedgwick et al (2002). Resource limitation of phytoplankton growth in the Crozet Basin, Subantarctic Southern Ocean. *Deep-Sea Research II*, 49, 3327-3349.

Strzepek, R., Harrison, P.J., 2004. Photosynthetic architecture differs in coastal and oceanic diatoms. *Nature*, 431, 689-692.

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