Photophysiological response of autumn phytoplankton in the Antarctic Sea-Ice Zone

"Absence of photophysiological response to iron addition in autumn phytoplankton in the Antarctic Sea-Ice Zone"

Singh et al.

RC2: 'Comment on bg-2022-245', Anonymous Referee #2, 06 Mar 2023

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Singh et al examine the photophysiological response of phytoplankton communities during autumn in the Southern Ocean via iron addition incubations. No significant differences were observed in Fv/Fm and σ PSII and the authors conclude that there was not iron-limitation at these times and locations. I commend the authors for presenting what some would consider "negative results." The data are clearly presented and methods are described in detail. Please see my minor comments below.

Note from Authors: We thank the reviewer for their appreciation and encouragement for presenting our data, as well as the constructive feedback and suggestions that will assist in refining our manuscript. Please find below our specific responses as well as indicated changes made to the manuscript.

Please note that, as per the suggestion of Reviewer #1, we have considered changing the manuscript title as follows:
"Absence of photophysiological response to iron addition in autumn phytoplankton in the Antarctic Sea-Ice Zone"

1. **Abstract (Lines 24-26) and Line 399** - The authors state that this study confirms that the phytoplankton communities "were not iron limited and…ambient iron concentrations were sufficient." I suggest that the authors rephrase these sentences to reduce their high confidence in their assessment that there was no iron limitation although I agree with the authors that these results suggest that Fe was not the sole limiting nutrient.

Authors' response:
Original:
"The photophysiological response of phytoplankton to iron addition, measured through the photosynthetic efficiency and the absorption cross-section for photosystem II, showed no significant responses. This confirms that phytoplankton were not iron-limited at the time and that ambient iron concentrations were sufficient to fulfill the cellular requirements."

This has been changed to read as follows, in addition to a change in the title, as suggested by reviewer 1:

Modified:
"Contrary to expectation, the photophysiological response of phytoplankton to iron addition,

measured through the photosynthetic efficiency and the absorption cross-section for photosystem II, showed no significant responses. It is thus proposed that the autumn phytoplankton in the SIZ exhibited a lack of an iron limitation at the time of sampling, and that ambient iron concentrations may have been sufficient to fulfill their cellular requirements."

2. An alternative explanation is that there is Fe-Mn colimitation. Ratios of dMn to dFe relatively close to the study region suggest that Mn limitation or Fe-Mn limitation is possible (Browning et al. Nature Communications 2021 Supplementary Fig 5). As Mn is critical for PSII, the photophysiology results presented here could be influenced by Mn-Fe colimitation. The authors very briefly hint at this at the end of the conclusion (line 430).

Authors' response:
We appreciate the reviewer's suggestion to look into the Fe-Mn co-limitation. We agree that Mn is critical for PSII, and there could be a potential Mn-Fe co-limitation in the Sea-Ice Zone close to Dronning Maud Land, based on the study by Browning et al. (2021) who found low coastal dMn concentrations towards the west of our study region.

However, our intention was not to explicitly claim or suggest the limitation of any specific trace metals but encourage an interest among the community to consider this as an option when planning future campaigns in the Southern Ocean, as more seasonal studies other than in summer is needed, particularly close to the sea-ice edge of the Antarctic coast. Our concluding remarks thus strive to highlight future objectives and aims which can be achieved in this study region. Nevertheless, we have removed the specific part mentioning manganese as an example in the conclusion, so as to avoid confusion:

"It is recommended that future studies in this region help to bridge the knowledge gaps by studying the varying impacts of light in tandem with iron and other trace metals which may instead be limiting during this time of the year, with an emphasis on short-term studies to understand the photophysiological response of phytoplankton in the absence of community induced responses."

Consequently, we have included a sentence in the final paragraph of the discussion suggesting the possibility of other limitations in the region, such as Mn when listing the possible contributions to high iron concentrations (also included in the comment response #4 on high dFe:nitrate ratios below and suggestions from Reviewer #3):

"And finally, considering factors that determine the bloom end, it may not be confined to a bottom-up limitation or the possibilities of light and/or other micronutrients such as manganese instead being limiting in this sea-ice region which is close to the coast of Antarctica (Browning et al., 2021)."

Reference:
Browning, T.J., Achterberg, E.P., Engel, A., and Mawji, E.: Manganese co-limitation of phytoplankton growth and major nutrient drawdown in the Southern Ocean. Nat. Commun. 12, 1–9. https://doi.org/10.1038/s41467-021-21122-6, 2021.

3. Also, much of the dFe is < 1 nM (lines 279-287), and only a fraction of dFe is bioavailable, which should also be mentioned in the results/discussion.

Thank you, we have made the changes as follows:
Original:
"Silicate concentrations showed a higher mean (48±1 µM) and less variability around Astrid Ridge with concentrations ranging from 46 to 52 µM, compared to a lower mean (46±2 µM) and larger range (41 to 49 µM) observed in the 6°E SIZ (Fig. 1g). Despite the limited number of dFe measurements, a wide range of surface concentrations (Fig. 1h) were evident around Astrid Ridge with concentrations as low as 0.27 nM and as high as 1.39 nM (mean 0.64±0.49 nM). Mean dFe concentrations in the 6°E SIZ were slightly lower (0.59±0.05 nM) compared to Astrid Ridge and varied over a narrow range between 0.56 to 0.63 nM. Furthermore, the mean PAR in the mixed layer for the 6°E SIZ was lower (29.71 µmol photons m^{-2} s^{-1}) in comparison to the Astrid Ridge (59.37 µmol photons m^{-2} s^{-1})."

Modified:
"Silicate concentrations showed a higher mean (48±1 µM) and less variability around Astrid Ridge with concentrations ranging from 46 to 52 µM, compared to a lower mean (46±2 µM) and larger range (41 to 49 µM) observed in the 6°E SIZ (Fig. 1g). Despite the limited number of dFe measurements, a wide range of surface concentrations (Fig. 1h) were evident around Astrid Ridge with concentrations as low as 0.27 nM and as high as 1.39 nM (mean 0.64±0.49 nM). Mean dFe concentrations in the 6°E SIZ were slightly lower (0.59±0.05 nM) compared to Astrid Ridge and varied over a narrow range between 0.56 to 0.63 nM. However, it is noted that only a fraction of the dFe is bioavailable to the phytoplankton, where this fraction can vary regionally and thus influence the variability in iron stress which may not mirror the ambient concentrations (Lis et al., 2015). Furthermore, the mean PAR in the mixed layer for the 6°E SIZ was lower (29.71 µmol photons m^{-2} s^{-1}) in comparison to the Astrid Ridge (59.37 µmol photons m^{-2} s^{-1})."

Reference:
Lis, H., Shaked, Y., Kranzler, C., Keren, N., and Morel, F.M.: Iron bioavailability to phytoplankton: an empirical approach. The ISME journal, 9(4), 1003-1013. https://doi.org/10.1038/ismej.2014.199, 2015.
https://www.nature.com/articles/ismej2014199

4. It may also be useful to report the range in dFe:NO3 (nmol:umol) which appear to be quite high so it is also surprising that Fe did not have an effect. Again, I largely agree with the authors' conclusions; however, I believe some altered wording and added discussion of potential Fe-Mn colimitation is warranted.

Authors' response:
We appreciated the advice of the reviewer. Indeed, we are aware that the iron ratio to both macronutrients nitrate and phosphate render high values for each experiment (please see below).

Exp	dFe:nitrate (nmol:μmol)	dFe:phosphate (nmol:μmol)
Exp01	n.d	n.d
Exp02	0.86 : 26.2 = 0.033	0.86 : 1.71 = 0.50
Exp03	1.39 : 25.5 = 0.055	1.39 : 1.69 = 0.82
Exp04	0.56 : 25.8 = 0.022	0.56 : 1.72 = 0.33
Exp05	0.63 : 25.7 = 0.025	0.63 : 1.75 = 0.36

In the publication of Ellwood et al. (2008), during winter-time in the South Tasman Sea of the Southern Ocean, it is reported that the dFe:nitrate ratios (as Fe:NO3) in Figure 3 exhibited a decrease in surface concentrations southward (~0.005 nmol:μmol) from 52 - 53°S. The experiments reported in our manuscript were conducted further south, between 68.56° - 69.07°S. Furthermore, Ellwood et al. (2008) report that low Fe:NO3 ratios in the south (0.005–0.018 nmol:μmol) corresponded with other HNLC regions which reported iron limiting conditions under low Fe:NO3 ratios (~0.01 nmol:μmol). Thus, we agree that the dFe:nitrate ratios in our study do indeed appear to be high; however, we disagree that it is surprising that iron addition did not have any effect on the phytoplankton, as they were not iron-limited from our results.

Moreover, we had intended to establish a proxy for potential iron limitation by assessing the uptake ratio between iron and PO4 as the tracer Fe* (Parekh et al., 2005; Rijkenberg et al., 2018). The Fe* is defined as the difference between the dFe concentration and the PO4 concentration, multiplied by a dFe:PO4 ratio (Twining et al., 2014), and is used to quantify the extent of iron limitation in the water mass. Negative Fe* values for the surface waters would thus suggest the potential for iron limitation (Parekh et al., 2005; Rijkenberg et al., 2018).

Using the ratio estimated for iron-limited Southern Ocean species (0.18 $mol.mol^{-1}$; Strzepek et al., 2011), no negative Fe* values were obtained for any of the 4 experiments (Exp01 did not have a dFe value), which suggests that there was no iron deficiency in respect to PO4 as could be expected a priori on those values. Yet, this uptake ratio does have several assumptions (most of these ratios represent laboratory conditions for single species cultures (Strzepek et al., 2011)), and by including other ratios (0.47 (Parekh et al., 2005) and 0.56 (Twining et al., 2014)), some of the experimental stations would have negative Fe* values and thus suggest an iron limitation. Given this ambiguity, we have refrained from including the Fe* values in the discussion.

However, we have included the above table depicting the dFe:nitrate and dFe:phosphate ratios into Table 1 in our manuscript and we include the following sentence in the discussion (rewritten in the paragraph from line 402 onward, taking into consideration the suggestions made by Reviewer #3) which compares these values and the interpretation thereof as described above:

“Furthermore, upon evaluating the initial dFe:nitrate (nmol:µmol) and dFe:phosphate (nmol:µmol) ratios (Table 1) for the experimental stations, it is worthy to note that the dFe:nitrate ratios appear to be higher than reported values, where for example, the winter-time assessment of dFe and nitrate distributions of Ellwood et al. (2008) in the South Tasman Sea of the Southern Ocean. Ellwood et al. (2008) reported a low range of dFe:nitrate ratios (0.005–0.018 nmol:µmol) further south from ~52°S, which corresponded with other HNLC regions which reported iron limiting conditions under low dFe:nitrate ratios (~0.01 nmol:µmol) (Ellwood et al., 2008 and references therein). Based on this evidence, the high dFe:nitrate ratios from our study indicate very little probability for an iron limitation, but rather a limitation on light and/or other trace metals such as manganese instead (Browning et al., 2021).”

References:

Ellwood, M.J., Boyd, P.W., and Sutton, P.: Winter-time dissolved iron and nutrient distributions in the Subantarctic Zone from 40–52S; 155–160E. Geophysical Research Letters, 35(11). https://doi.org/10.1029/2008GL033699, 2008.

Parekh, P., Follows, M.J., and Boyle, E.A.: Decoupling of iron and phosphate in the global ocean. Global Biogeochem. Cycles 19, 1–16. https://doi.org/10.1029/2004GB002280, 2005.

Rijkenberg, M.J., Slagter, H.A., Rutgers van der Loeff, M., Van Ooijen, J., and Gerringa, L.J.: Dissolved Fe in the deep and upper Arctic Ocean with a focus on Fe limitation in the Nansen Basin. Frontiers in Marine Science, 5, 88. https://doi.org/10.3389/fmars.2018.00088, 2018.

Twining, B.S., Nodder, S.D., King, A.L., Hutchins, D.A., LeCleir, G.R., DeBruyn, J.M., Maas, E.W., Vogt, S., Wilhelm, S.W., and Boyd, P.W.: Differential remineralization of major and trace elements in sinking diatoms. Limnol. Oceanogr. 59, 689–704. http://dx.doi.org/10.4319/lo.2014.59.3.0689, 2014.

Strzepek, R.F., Maldonado, M.T., Hunter, K.A., Frew, R.D., and Boyd, P.W.: Adaptive strategies by Southern Ocean phytoplankton to lessen iron limitation: Uptake of organically complexed iron and reduced cellular iron requirements. Limnology and Oceanography, 56(6), 1983-2002. https://doi.org/10.4319/lo.2011.56.6.1983, 2011.

5. Line 424 – The authors state that they observed “high Fv/Fm” although I would consider many values to be relatively low (< 0.3). I suggest changing this sentence.

Authors' response:
Thank you, we have made the changes as follows:
Original:
“The results from this study show that although in theory it is expected that parts of the Southern Ocean are iron-limited during autumn, it is not necessarily true for the Sea-Ice Zone region surrounding Astrid Ridge and along the 6°E transect, where high Fv/Fm and σPSII, i.e. efficient photophysiology was observed in situ, and where iron addition did not lead to more efficient photophysiology.”

Modified:
“The results from this study show that although in theory it is expected that parts of the

Southern Ocean are iron-limited during autumn, it is not necessarily true for the Sea-Ice Zone region surrounding Astrid Ridge and along the 6°E transect. The observed *in situ* Fv/Fm and σPSII is suggestive of efficient photophysiology, and where the iron addition did not lead to increased efficiency in phytoplankton photophysiology.

6. The recent paper in Science by the corresponding author here (Ryan-Keogh et al 2023) seems relevant to include in the Discussion. Specifically, its stated that irradiance normalized NPQ is higher in Spring/Summer compared to Fall/Winter which aligns with the results in these studies.

Authors' response:
We thank the reviewer for suggesting the addition of the recent publication, where the conclusions of Ryan-Keogh et al. (2023) do indeed align with our conclusion that the phytoplankton studied in our manuscript was not iron-limited. The iron limitations seen in spring and summer are much greater than that seen in autumn and winter (Ryan-Keogh et al., 2023).

Original:
"As such, iron was not considered limiting to photosynthesis at any of the autumn stations in the DML SIZ. This was unexpected and implies that despite the timing of the cruise occupation relative to the seasonal bloom termination, iron was unlikely the primary driver of the bloom's ending."

Modified:
"As such, iron was not considered limiting to photosynthesis at any of the autumn stations in the DML SIZ. This unexpected finding implies that despite the timing of the cruise occupation relative to the seasonal bloom termination, iron was unlikely the primary driver of the bloom's termination (Kauko et al., 2021). Coincidently, a recent study by Ryan-Keogh et al. (2023) proposed a greater probability of iron limitation in spring and summer in comparison to autumn and winter, which aligns with the results of our study."

Reference:
Ryan-Keogh, T.J., Thomalla, S.J., Monteiro, P.M., and Tagliabue, A.: Multidecadal trend of increasing iron stress in Southern Ocean phytoplankton. Science, 379(6634), 834-840. https://doi.org/10.1126/science.abl5237, 2023.