Anonymous Referee #2

General comments: In this paper by Ryan-Keogh et al the authors present data on nutrient (iron) addition bioassay style experiments conducted in the sub-Antarctic zone of the S. Ocean. The papers describes the varying response to iron–addition on the phytoplankton community over the growing season and characterises changes in physiology, nutrient uptake and community composition. The paper then discusses potential causes of the relationship between biological demand for Fe and supply. The paper is well presented and is a useful addition to the important understand of the controls and limitations on primary production in this important oceanographic region. My main concern is that the authors are too strong in their conclusions (especially relating to the seasonal cycle) from a limited dataset and sections of the paper should better reflect these limitations of the study.

Specific comments:

Three incubation experiments have been conducted. Which this is valuable data it is still only three data points throughout the growing season. As such conclusions as to how this data relates to a seasonal cycle should be stated with a bit more consideration. Especially as often the authors claim a development in iron stress over the growing season while the most iron-stressed community seems to be mid-season? While this could be due to the selection of for example cells with reduced iron-requirements as iron-limitation develops it needs more openly discussed.

> The most iron stressed community is mid-season, if examining the photophysiology alone, however experiment 3 displayed the greatest increases in growth rates following iron addition. A statement to this effect has been added to the discussion.
>
> Line 412: “When examining the photophysiology alone, experiment 2 displayed the greatest response to iron addition (Fig. 2c) with significant responses also observed in Chl-a derived net growth rates (Fig. S1) and nitrate drawdown rates (Table 2). Experiment 3 displayed the greatest increases in growth rates following Fe addition (Fig. S1), while significantly higher F_v/F_m was similarly observed (Fig. 2e).”
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> We agree with reviewer 2, who similarly raised concerns like reviewer 1, that more consideration should be taken in regards to the conclusions. As such, the text has been modified to discuss the implications of iron limitation here on the potential maximal growth rates and productivity.
>
> Some examples of how the conclusions have been adjusted are provided below.
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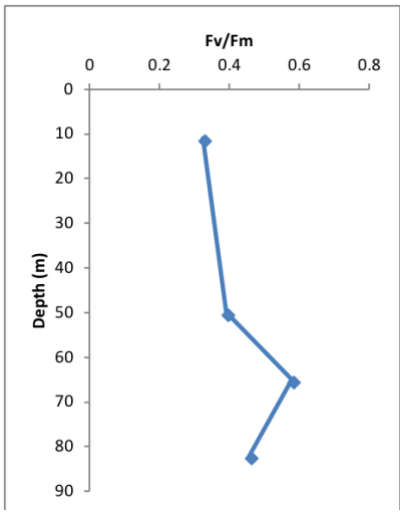
> Line 25: “Here we demonstrate that at the beginning of the growing season, there is sufficient iron to meet the demands of the phytoplankton community, but that as the growing season develops the mean iron concentrations in the mixed layer decrease and are insufficient to meet biological demand.”
>
> Line 434: “Irrespective of the different supply mechanisms; winter-entrainment, storm driven entrainment, diapycnal diffusion, photochemical reduction or microbial regeneration, the iron supply to the mixed layer is not sufficient for phytoplankton to

reach to reach maximum growth potential and completely drawdown all available macronutrients."

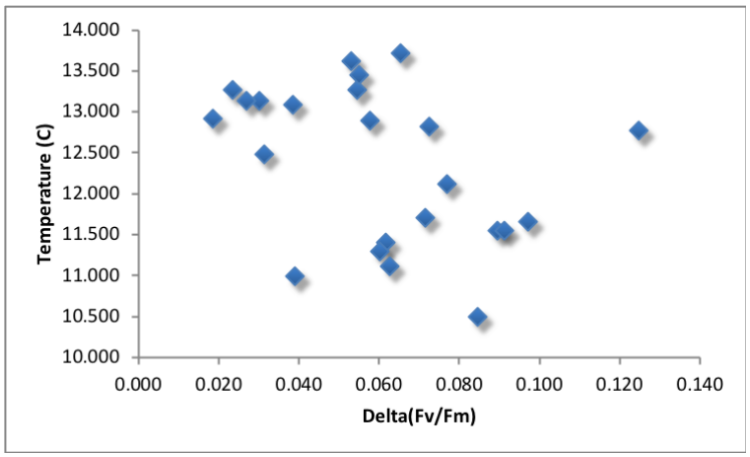
The authors infer accumulation of detached chlorophyll-binding protein as a mechanisms for low Fv/Fm during iron stress. If this is the case why does Fv/Fm not reduce in the set-up conditions (table 1). This is potentially a change in community – although the authors suggest the community is pretty consistent. Can the authors give a reason for this. Possibly plot Fm:Chl or similar to help support the arguments? Is there a water temperature effect on magnitude of the deltaFv/Fm throughout similar studies from the author?

One potential reason for the F_v/F_m not reducing during the set-up conditions is that the phytoplankton species (haptophyte dominated - see Fig. S2) are living under steady-state iron limited conditions during experiments 2 and 3, high values of F_v/F_m have previously been observed under steady state iron limitation in culture (Parkhill et al. 2001; Price 2005). However, with the lack of sufficient community structure data it is hard to determine which signal is dominating the measurements presented here. As the experiments will display a physiological signal (i.e. nutrient stress), superimposed with a taxonomic signal (i.e. different phytoplankton groups have different baseline F_v/F_m). It is also not possible to rule out the effects of light intensity on suppressing the initial F_v/F_m measured, through the downregulation of PSII, when setting up the experiments. During the cruise, a CTD was deployed at 03:00 local time before sunrise, and a depth profile of FRRf was collected. Samples collected at 10m and 50m indicated a much higher F_v/F_m, with 0.32 and 0.39 respectively, in comparison to the initial samples collected 4 hours later after sunrise. Potential reasons for this discrepancy is that the dark acclimation step may have not fully relaxed the initial samples before measurement. Indeed, one of the results of the incubation was a ~62% decrease in the light exposure that the experimental bottles received, which potentially explains why both the controls and Fe increase their F_v/F_m by ~0.15.

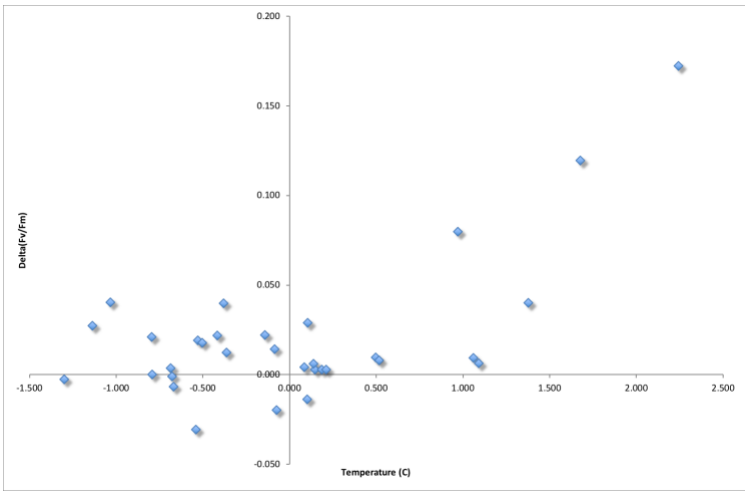


Depth profile of F_v/F_m of the same station for samples that were collected on a CTD cast 4 hours prior to the experimental CTD cast.

Temperature was examined as a potential driver of $\Delta(F_v/F_m)$ during previous studies as part of my PhD, but was not found to be a significant driver in these studies and therefore excluded from the analysis (Ryan-Keogh et al., 2013; Ryan-Keogh et al., 2017). I have attached here 2 figures of data from these studies (unpublished), showing $\Delta(F_v/F_m)$ against temperature. However, given the small temperature range in the experimental set up, 10.44 - 10.8, it is unlikely that there would be any temperature effect in dictating the range of $\Delta(F_v/F_m)$ values measured in the experiments.

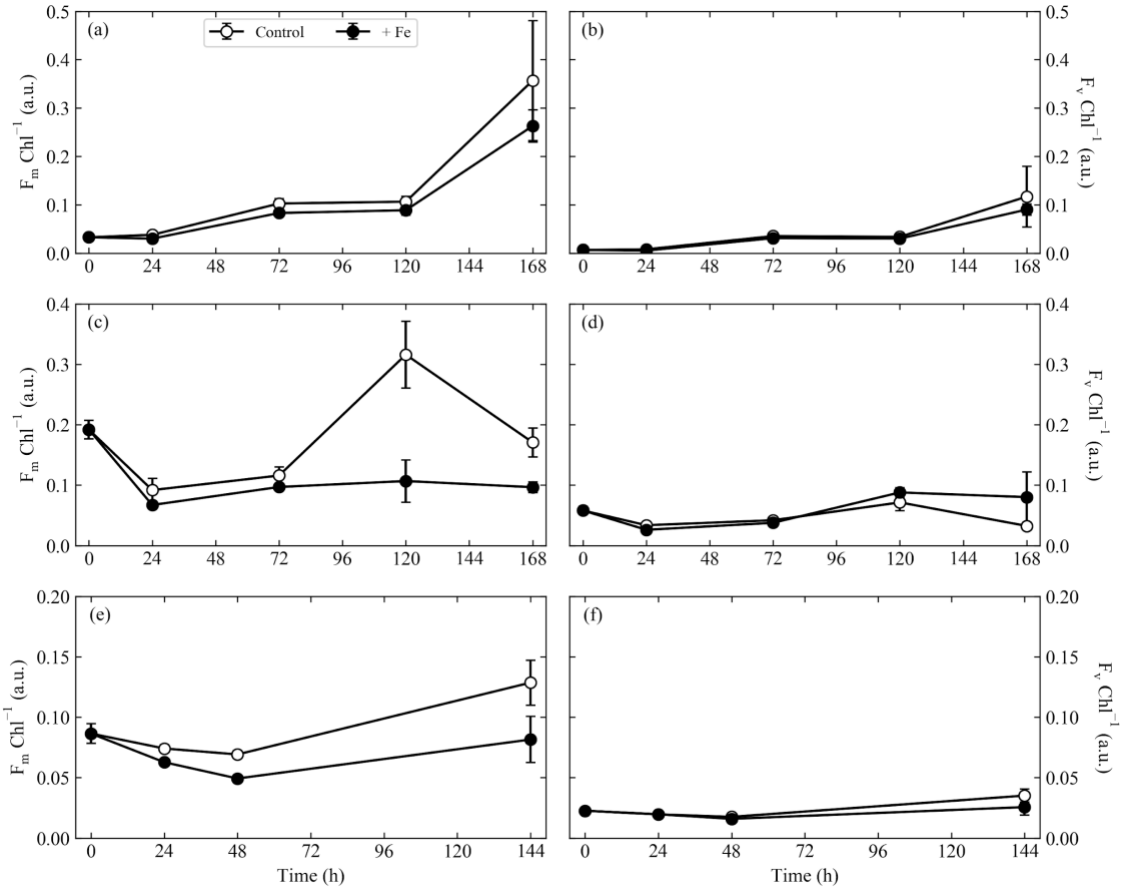


HLNA results from Ryan-Keogh et al. 2013



Ross Sea results from Ryan-Keogh et al. 2017

Displayed here is the full time series of the changes in F_m Chl^{-1} and F_v Chl^{-1} from each experiment, evident in panels c & e is that the iron addition creates a difference in F_m Chl^{-1} between the treatments; there is no evidence of changes in F_v Chl^{-1} (panels d & f). However, I feel that the information in this figure is already displayed in figure 3c and 3d.



Technical corrections:

Line 27 – suggests depend is greater than supply – the supply rate could still be high

This sentence has now been modified to:

Line 31: “suggestive of seasonal iron depletion and an insufficient resupply of iron to meet biological demand.”

Line 74 – include a reference for this statement

The following reference has been added to the text on line 80 and included in the references.

Boyer, T. P., Antonov, J. I., Baranova, O. K., Coleman, C., Garcia, H. E., Grodsky, A., Johnson, D. R., Locarnini, R. A., Mishonov, A. V., and O'Brien, T. D.: World Ocean Database 2013, NOAA Printing Office, Silver Spring, MD, 2013.

Line 87 – define more what eddy-strom interactions mean

This section has now been updated to only discuss the effects of storm on shear mixing, rather than the effects of eddy-storm interactions on 3D mixing. The

complexities of these different mixing mechanisms is beyond the scope of this paper and is discussed in greater detail elsewhere. See line 93 for clarification.

Line 232 – Be clear if there was no sig difference throughout the experiment

There was no significant difference at any timepoint during the experiment, the text has been updated to state this more clearly.

Line 254: “Statistical analysis confirmed that there were no significant differences in F_v/F_m or chlorophyll throughout the experiment.”

Line 235 – I think the sigmaPSII data is in supplementary but please refer to this in the text

References to supplementary figure S1 which shows σ_{PSII} has been added to the text on lines 256, 264 and 272.

Figure 2 – I think the lines are mis-labelled - +Fe and control should be the other way around?

The labelling is correct on Figure 2, open circles which show the lowest values of F_v/F_m and chlorophyll are the controls. The closed circles represent the +Fe treatment which has the higher values of chlorophyll and F_v/F_m.

Line 304 – can you reference a paper that shows or discussed this bottle effect in more detail

Additional references have now been added - see line 327. This section discussing the bottle effects evident here have also been greatly expanded and discussed in detail.

“The rapid increase in F_v/F_m in both treatments from 24 h onwards is likely due to potential bottle effects i.e. a change in the light environment (Martin and Fitzwater, 1988; de Baar et al., 1990; Coale 1991; de Baar et al., 2005). The total daily PAR in the incubators ranged from 6.52 - 6.99 mol photons m^{-2} d^{-1}, which is in good agreement for the in situ light environments of experiments 2 and 3. However, this was a ~62% decrease in the daily PAR that the phytoplankton community in experiment 1 were previously subjected to. Such a decrease in PAR would be expected to lead to a decrease in the downregulation of PSII by photodamage, coincident with an anticipated response in community structure. This could explain the observed increase in F_v/F_m and decrease in σ_{PSII}, as larger cells tend to have a higher F_v/F_m and small σ_{PSII} in comparison to smaller cells (Suggett et al., 2009). Indeed, we did observe a change in the community structure for experiment 1 (Fig. S2), suggestive that a decrease in light pressure resulted in a community response in the control treatment. However, the lack of taxonomic data at 72 h makes it difficult to distinguish whether the primary driver of this response is physiological, taxonomic or a combination of both.”