

## ***Interactive comment on “Diel quenching of Southern Ocean phytoplankton fluorescence is related to iron limitation” by Christina Schallenberg et al.***

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Received and published: 13 November 2019

We thank referee #1 for their thoughtful comments and reply to each in turn below:

Potential changes in community structure during 50-55 h incubations: In the attached Figure (which we plan to add to the Supplementary Material) we show data from Incubation 1, where subsampling took place at 29 h and 52 h (these data were not previously shown). It is evident that the changes observed at 52 h were already underway at 29 h, and in some cases had already taken place, e.g. the majority of data points for NPQSV(1000). We only subsampled this one experiment at 29 h and chose the 50-55 h incubation times for all other experiments due to the low sea water temperature (as

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low as  $\sim 10^\circ\text{C}$ ), which would increase physiological response times compared to temperate waters. If we assume a growth rate no higher than  $0.28\text{ d}^{-1}$  for the subantarctic zone (e.g., see Ryan-Keogh et al. 2018, Table 2), then the phytoplankton community would not have doubled over 50 hours. We thus conclude that 50-55 h is not enough for a significant change in the community composition, and also point to the attached figure as evidence that the overall trends found after 52 hours were generally consistent with those found at 29 h but are more pronounced. Conducting the longer incubations is thus unlikely to have biased the conclusions.

**CHEMTAX vs Diagnostic Pigment Index:** We chose the Diagnostic Pigment Index over CHEMTAX because it assumes less a priori knowledge specific to an oceanic region. CHEMTAX is only as good as the comparison matrix that is specified for it, and as far as we are aware, there is no available matrix specific for the Subantarctic Zone. CHEMTAX would produce a result regardless, but possibly not a very accurate one – without microscopy to match, it would be difficult to know whether the result is valid. In order to avoid overstating what our data can do, we thus decided to show the pigment composition (Figures S2 and S3), which is the raw, unbiased data, and to provide a metric for the community composition with the Diagnostic Pigment Index, while also discussing its suitability in the region and comparing our results to those found previously at the SOTS site.

**Average mixed layer irradiance in the two water masses and its influence on NPQ:** We disagree with the notion that the average light field is the main determinant of NPQ. When realistic vertical mixing is simulated, it appears that low-light (deep ML)-acclimated cells display a larger capacity for NPQ than high-light (shallow ML)-acclimated cells (e.g. Milligan et al. 2012). This phenomenon was likely overlooked in earlier laboratory studies because realistic mixing regimes were rarely simulated. Regardless, we are happy to include estimates of daily integrated median underwater PAR for the respective mixed layers for reference. They are calculated for the nominal date of March 30, 2018, with  $K_d\text{ PAR}$  estimated as a function of Chl following Morel

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et al. (2007). The results are as follows: For the warm regime (average Chl=0.62 mg m<sup>-3</sup> and MLD = 100 m), the median daily mixed layer PAR is 0.13 mol m<sup>-2</sup> d<sup>-1</sup>. For average Chl = 0.38 mg m<sup>-3</sup> and MLD=20 m and MLD=90 m as the two extremes, the median daily mixed layer PAR is 15 and 0.5 mol m<sup>-2</sup> d<sup>-1</sup>, respectively. We propose to include these numbers in section 3.2.2 with an expanded subtitle: Mixed layer depths and average light field.

Paragraph starting line 455, Rates of water column integrated primary productivity (PP) and their dependence on phytoplankton biomass, temperature, and light availability: A comparison between the conditions reported by Westwood et al. (2007) and found in our study shows the following: Sea surface temperatures were similar (12C vs 11C, respectively), mixed layer depths were also similar (mean=38 +/- 11 m and mean=35 +/- 1 m, respectively), but the column-integrated Chl was different: mean=46 +/- 11 mg m<sup>-2</sup> vs mean=13 mg m<sup>-2</sup>. However, the fact that biomass was lower in our study could also be the result of lower iron concentrations (and less primary productivity). Moreover, normalizing the mean column-integrated PP for each study by the mean column-integrated Chl concentration yields 85 mg C (mg Chl)<sup>-1</sup> d<sup>-1</sup> for Westwood et al. and 31 mg C (mg Chl)<sup>-1</sup> d<sup>-1</sup> for our study, indicating that the Chl-normalized PP was lower during our study (in Austral fall) than in the Westwood et al. study in Austral summer. The conclusion that PP was lower in our study thus holds, with iron limitation a probable cause, as discussed in Section 3.2.4. We plan to add the information provided above to the SI of the manuscript.

Line 70, reference for 90% depression of fluorescence by NPQ: Falkowski et al. 2017. We will include this reference in the text at line 70.

Section 2.2, only one replicate 250 mL sample per treatment for the incubations: There was indeed only one 250 mL sample per treatment in each incubation because measurement of all 4 treatments took ~2 h, so measurement of triplicates would have taken ~6 h. Given the sensitivity of fluorescence parameters to the time of day (i.e. to the light history experienced by the phytoplankton), we thought it prudent to stay in a 2-h

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time window in order to be able to compare findings between treatments. We would like to point out that we grouped the three experiments, thus having produced true triplicates. We will clarify this in Section 2.2 in the updated manuscript.

Line 174, 'optimization' of FLCs for estimates of NPQ capacity: This comment is discussed in detail in the response to Referee #2.

Section 2.4.3, fluorometric Chl method: We followed the JGOFS 1994 recommendations for the 'Measurement of Chlorophyll a and Phaeopigments by Fluorometric Analysis', employing the acid ratio method described by Holm-Hansen et al. (1965). We will add this detail to the section indicated.

Line 508, add reference to Figure 8: we will add this figure reference as suggested.

References: Falkowski, P., Lin, H., and Gorbunov, M.: What limits photosynthetic energy conversion efficiency in nature? Lessons from the oceans. *Philos. T. Roy. Soc. B*, 372, 2-8, 2017.

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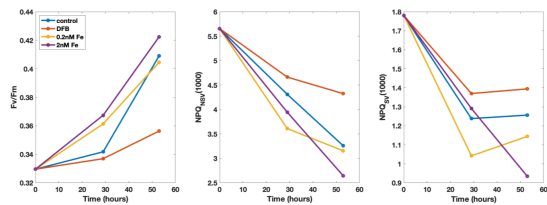
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Supplementary Figure for response to Referee #1



Time course of Fv/Fm and the two NPQ parameters for incubation 1, showing the results at the end point (after 52 hours) as well as at 29 hours. Colours represent different treatments as indicated in the figure legend.

Fig. 1. Figure 1