

### Response to Anonymous Referee #3

*We thank the reviewer for their very insightful comments and ideas. Following their suggestions we have added substantial additional analysis of our data. This response letter is accompanied by a revised manuscript, which allows to see how we suggest to incorporate these changes into the original narrative. In the revised manuscript, parts which differ from the original discussion paper are written in blue font. All page and line numbers we give in our answers below refer to the revised manuscript.*

#### General Comments

This study examines diel periodicity of photosynthetic electron transport and carbon fixation in iron-limited waters of the subarctic Pacific Ocean. A comparison of active fluorescence light-response curves and  $^{14}\text{C}$ -irradiance curves reveal the stoichiometry between reaction center II (RCII)-specific electron transport rates and carbon fixation rates vary by a factor of 3.5 throughout the day. This diurnal variability confounds the accuracy in which active fluorescence measurements can be scaled into more ecologically relevant carbon fixation rates. The authors provide a robust review of the myriad of non-carbon fixation pathways that consume photosynthetic energy (ATP and reductant), and suggest that endogenous periodicity in these pathways likely cause some of the observed decoupling. The authors also present an empirical relationship demonstrating that non-photochemical quenching (NPQ) explains a significant fraction of the decoupling between RCII-specific electron transport and carbon fixation rates. This study provides a clear demonstration of disparate diurnal variations in RCII specific electron transport and carbon fixation rates. While this lack of co-variation isn't surprising given our understanding of circadian patterns in phytoplankton physiology (e.g. Behrenfeld et al. 2008), this manuscript nevertheless is a useful contribution to the literature. My largest criticism of this manuscript is that the authors cannot address whether this variability is driven by diurnal changes in the electron requirement of carbon fixation ( $\text{Phie,C}$ ) or the number of functional reaction centers normalized to chlorophyll a (nPSII). In fact we know that nPSII decreases in high light (Behrenfeld et al. 2002), and this is generally consistent with the highest  $\text{Phie,C} \times 1/\text{nPSII}$  occurring midday (Fig 2A). Given that a properly calibrated active fluorometer can now estimate nPSII through an instrument specific conversion factor (KR, Oxborough et al. 2012 L&O Methods; Silsbe et al. 2015 L&O Methods), I feel as though the authors have missed an opportunity to more significantly advance the literature.

The authors mention that they did not attempt the new nPSII protocol as it is likely invalid for iron-limited phytoplankton. This is likely true because iron-limited phytoplankton can possess surplus photosynthetic antennae that are decoupled from photosynthetic reaction centers (Schrader et al 2009 PLoSOne). As the new nPSII protocol varies from first principles with  $F_0$ , decoupled antennae increase  $F_0$  independent of nPSII. That said I would be surprised if this overestimation of nPSII has a diel pattern, in other words surplus antennae remain uncoupled from photosynthetic reaction centers over the course of the day so long as iron-limitation remains. If the authors can estimate nPSII from  $F_0$ , then this study could better elucidate the diurnal periodicity of  $\text{Phie,C}$  alone. If the authors do not have access to an oxygen flash yield system that is required to derive KR to estimate nPSII (Oxborough et al. 2012), then I suggest estimating KR using a chlorophyll a standard following Silsbe et al. (2015). Many newer active fluorescence studies implement this approach (e.g. Robinson et al. 2014, J. Mar. Sys), and if the authors can make these changes it would likely increase this manuscript's impact.

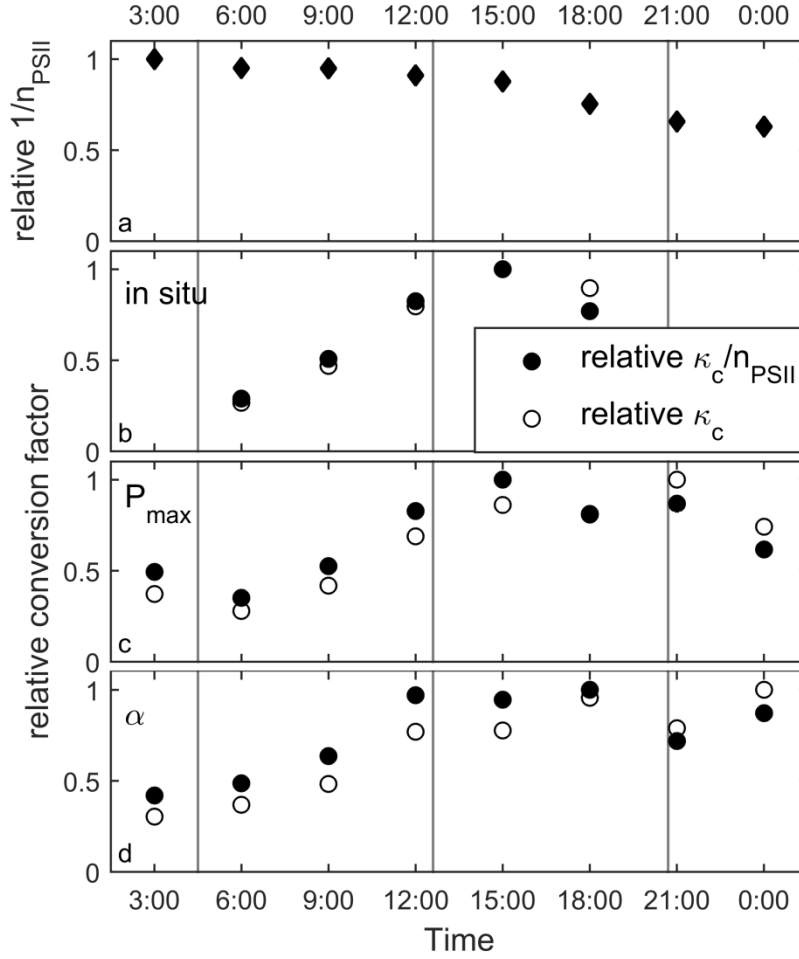
*As the reviewer explains correctly, the Oxborough approach to estimate absolute values of [RCII] was not applied in the original manuscript because the inherent assumption that the ratio of the rate constants of photochemistry ( $k_p$ ) and fluorescence ( $k_f$ ) stay within a narrow range, does not hold under iron limitation, where  $k_p$  decreases while  $k_f$  increases (e.g. Vassiliev et al., 1995) (see also answers to reviewers #1 and #2). As the reviewer points out, the increase in  $k_f$  under iron limitation is likely caused by energetically decoupled light harvesting complexes (DLHCs) (Behrenfeld and Milligan, 2013; Schrader et al., 2011), and we have added this information to the revised manuscript (page 11, line 295-303).*

*As shown clearly in response to reviewer #2, it is not possible to apply the Oxborough approach when comparing data from contrasting levels of iron limitation. However, it can indeed be argued that the phytoplankton assemblage sampled experienced a constant degree of iron limitation during the present study. Therefore, it is likely that the amount of DLHCs remained constant, which could result in a constant (albeit much lower)  $k_p/k_f$ . While this is speculation, and we do not have any experimental data on diurnal behaviour of DLHCs, we do agree with the reviewer's reasoning.*

*The reviewer suggests estimating  $K_R$  using a chlorophyll standard, as has been done in Silsbe et al.(2015), who show that a  $K_R$  value estimated in this way agrees relatively well with a  $K_R$  value derived the 'traditional' way, for six instruments tested. However, the same study also points out that for cultures grown without added Fe (no additional information on the actual degree of Fe limitation is given) the Oxborough approach (applying this  $K_R$  value) predicted higher [RCII] than was measured using oxygen flash yields. Therefore, we do not think it is possible to obtain an instrument specific  $K_R$  value which will result in accurate absolute values of [RCII] using a chlorophyll standard, if the phytoplankton assemblage sampled was iron limited.*

*Lacking the instrument specific calibration factor  $K_R$ , we were not able to derive absolute values for [RCII] (and in turn  $1/n_{PSII}$ ). However, since  $K_R$  can be assumed constant (as long as the degree of iron limitation does not change), changes in of  $F/\sigma_{PSII}$  should represent relative changes in [RCII]. We should thus be able to derive relative changes in  $1/n_{PSII}$ , as suggested by the reviewer.*

*Below, we show how we applied this simplified version of the Oxborough approach to calculate relative diurnal changes in  $1/n_{PSII}$  which, in turn, were used to deduce relative diurnal changes in  $\Phi_{e:C}$  ( $K_c$ ).*



Here, we calculated relative values of  $1/n_{PSII}$  for each TP as  $(F_o/\sigma_{PSII})/[chl\ a]$ , and scaled the values to 1 (panel a). Relative values of  $1/n_{PSII}$  calculated in this way are highest at pre-dawn, stay relatively constant until the afternoon, and decrease by 37% from the first to the last TP. By comparison, the lumped conversion factor  $K_c/n_{PSII}$  changes by 245% for in situ irradiances (panel b), 185% at light saturation ( $P_{max}$ ; panel c) and 138% at light limitation (alpha; panel d).

We used the relative values of  $1/n_{PSII}$  shown in panel (a) to deduce relative changes in  $K_c$  from the lumped conversion factor. The filled symbols in panel (b)-(c) are values of the conversion factor  $K_c/n_{PSII}$ , as shown in Fig. 2 and Fig. 3 of the original manuscript, scaled to 1. The open symbols are relative values of  $K_c$  derived by dividing the relative values of  $K_c/n_{PSII}$  by  $1/n_{PSII}$ . These derived values show that the diurnal change in relative  $K_c/n_{PSII}$  are very similar to diurnal changes in relative  $K_c$ , suggesting that variability in  $K_c$  drives most of the variability in the lumped conversion factor. The above figure and analysis are now included in the manuscript (page 11, lines 292-315; page 14, lines 380-390; page 17, lines 487-488; page 18, lines 494-497)

16805 – 20. Some references for the plasticity in pH<sub>ie</sub>, C and nPSII are needed. As active fluorometers can be calibrated to estimate nPSII, mentioning this technique (Oxborough et al. 2012, Silsbe et al. 2015) is warranted in this paragraph.

*We have added range of variability encountered as  $1.15 - 54.2 \text{ mol } e^- \text{ mol } C^{-1}$  for  $K_c$  (Lawrenz et al., 2013) and approx.  $200 - 950 \text{ mol chl } a \text{ mol } RCII^{-1}$  for  $1/n_{PSII}$  (Suggett et al., 2010) to the revised manuscript (page 11, lines 284-287).*

*Furthermore, we revised the entire manuscript to better acknowledge the Oxborough approach for estimating absolute values of [RCII] directly from FRRF measurements. We also discuss why the approach has limitations under conditions of iron limitation, and how we apply a simplified version of it to our data to obtain relative [RCII] estimates.*

16807 – 5. I would define NPQNSV as the ratio of the total non-photochemical dissipation in the light adapted state to the rate constant of photochemistry (McKew et al. 2013).

*We added a sentence with this definition to the method section of the revised manuscript (page 9, lines 227-230).*

16810 – 22. Are the LED lights at different wavelengths flashed in sequence or at the same time?

*For all data shown in the present study, the four wavelengths were applied simultaneously. This is now clarified in the revised manuscript (page 7, lines 188).*

16810 – Section 2.5. Was background (filtrate) fluorescence measured and subtracted from profile data?

*Background fluorescence was measured and subtracted for each time point. We have added a sentence about this to the method section of the revised manuscript (page 7 lines 181-182).*

16815 – 12. Please verify that daily incident irradiance was  $53\,236 \text{ } \mu\text{mol quanta m}^{-2}$ ? This corresponds to a daily value of  $0.053 \text{ mol quanta m}^{-2} \text{ d}^{-1}$ , which seems a factor of 1000 too small (<http://oceancolor.gsfc.nasa.gov/cgi/13>).

*We thank the reviewer for pointing out this mistake. The daily incident irradiance was  $31.94 \text{ mol quanta m}^{-2} \text{ d}^{-1}$ , which is in agreement with values expected for this oceanic region and time of the year. The value has been corrected in the revised manuscript (page 12, line 326).*

16821 – 3 to 27. This paragraph can be shortened, and you may want to look at Geider et al. (2009 Plant Ecology and Diversity) who tabulate the electron requirement of the dominant non-carbon fixation pathways.

*The section has been shortened.*

Figure 3: Combine with Figure 2 and reduce the range in the Y-axis.

*The two figures have been combined (Fig. 2 in revised manuscript). However, we did not change the range of the Y-axis, as it is our intention to show  $E_k$  in relation to the available irradiance.*

Figure 5 and 6: These figures should probably be combined.

*In response to suggestions from reviewers #1 and #2 we added a panel to both Fig. 5 and Fig. 6 (Fig. 4 and Fig. 5 in revised manuscript). We feel that both figures now contain sufficient information to remain as separate figures, where Fig. 4 deals specifically with pigment ratios and Fig. 5 specifically with FRRF derived parameters only.*