

# ***Interactive comment on “Diurnal variation in the coupling of photosynthetic electron transport and carbon fixation in iron-limited phytoplankton in the NE subarctic Pacific” by N. Schuback et al.***

## **Anonymous Referee #3**

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### 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  $\sim 3.5$  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

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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 ( $\Phi_{II}$ , 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  $\Phi_{II}$ , 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 PLoS One). 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  $\Phi_{II}$ , 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 sug-

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gest 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.

### Specific Comments

16805 – 20. Some references for the plasticity in pH<sub>le,C</sub> 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.

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).

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

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

16815 – 12. Please verify that daily incident irradiance was 53 236 Umol quanta m<sup>-2</sup>? This corresponds to a daily value of 0.053 mol quanta m<sup>-2</sup> d<sup>-1</sup>, which seems a factor of 1000 to small (<http://oceancolor.gsfc.nasa.gov/cgi/l3>).

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.

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

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

END OF REVIEW.

**BGD**

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