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# *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 #2

Received and published: 11 November 2015

General comments The present paper examines the empirical relationships between carbon fixation and electron transfer measured by fast repetition rate fluorometery (FRRF) and their dependency on diel changes in solar irradiance under low iron availability. To largest drawback of the FRRF techniques remaining to this day is the conversion of electron transfer to carbon fixation. This has been the focus of multiple recent studies. New algorithms for the direct derivation of reaction centre 2 concentrations from chlorophyll fluorescence measurements (Oxborough et al. 2012) enabled researchers, for the first time since the introduction of the technique, to measure electron transfer in absolute terms. Any conversion of electron transfer to carbon fixation, however, requires that these new algorithms and subsequent conversion factors hold

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under varying light conditions and nutrient availability. Whether this is actually the case has never been rigorously tested, and thus, the new RCII algorithm should probably used with a certain degree of caution. A RCII-independent approach may, hence, be an alternative to the present approach that should be included in future work and warrants further in-depth studying. I, therefore, consider the present publication a valuable contribution for the field of fluorescence-based primary productivity measurements.

Specific comments 16805 L7/8 – "more recently" – Kolber & Falkowski 1993 is not exactly recent. Furthermore, Kolber et al. (1998) should probably be mentioned here as well, perhaps in favour of Schreiber's work because the latter deals with multiple rather than single turnover techniques. 16805 L 20-23 – Using a combination of Đďe,C and 1/nPSII as a conversion factor is rather new to most people working with FRRF. Frankly, I still have somewhat of a hard time getting my head around this conversion factor with regards to its units and absolute values. To ease the reader into this, dould the authors perhaps mention the previous approach of using Đďe,C alone, emphasize why the new conversion factor is chosen over Đďe,C and what one would expect as theoretical values, similar to the theoretical minimum of Đďe,C of 4-5 mol e- (mol C)-1. 16806 L5-8 References mentioned here did not study the mechanistic underpinning of the uncoupling between ETR and C fixation, but rather the empirical relationships between ETR and Carbon fixation. Either include references that focus on the underlying mechanisms or rephrase the scentence. 16808 L6 – Unfortunate gap in the irradiance data! Could you perhaps just specify when the malfunction occurred and over which times you had to fill the data gap? 16810 L 22: Are the four wavelengths of the Soliense FRRF exciting fluorescence one after another or are they used simultaneously? Please clarify because other (mostly multiple turnover) fluorometers do not allow the user to combine multiple excitation wavelengths at once (instead one has to use them one after another). 16811 – Equation 3 is missing the Đď'PSII term. Đď'PSII = 1 mol electrons (mol quanta)-1, and is needed to end up with units of electrons and cancel out the mol quanta. It is often omitted in the literature because it takes a constant value of 1, however, it should be included. Also, this whole paragraph on

FRR fluorometry makes no mention of a blank measurement. However, the blanks may be very important, especially in waters with low phytoplankton biomass. Please clarify whether blank measurements were carried out and how data were treated for blank correction. 16812 L 4 – The SI unit for radioactivity is Bequerel (Bq), not Curie. Please convert accordingly. 16812 L17 – 10 mL instead of "Ten". 16813 L 18-25. The authors may not yet be aware of an improved technique to fit ETR vs. E curves, which was introduced by Silsbe & Kromkamp (2012). One of the assumptions of a regression analysis is that the y-values are independent of the x-values. With irradiance (E) being a factor in the ETR equation (e.g. Eq. 3), this assumption does not hold. Silsbe and Kromkamp addressed this issue in their 2012 paper in L&O methods (Modeling the irradiance dependency of the quantum efficiency of photosynthesis. Limnol. Oceanogr. Methods 10, 645–652). Their approach also reduces the error of the fit at high irradiances (where quantum efficiency values become highly variable due to low variable fluorescence at high light). It may not make much of a difference in the derived P vs. E parameters, but this is certainly something to keep in mind for future work.

16814 L 10-14 – The authors expect the new RCII algorithm of Oxborough et al. 2012 to not hold under Fe limiting conditions. Could you perhaps elaborate why? As far as I know, the algorithm has never been put to test under low Fe (or any other form of nutrient stress). This needs clarification. 16815 L14 – Could the authors please specify how they define the photic zone, i.e. as the 1% or 0.1% light level because different groups of researchers define it differently, and photosynthesis may take place well below the 1% light level (Kirk 1994) 16816 L 6-27 and conversion factors presented in Fig. 1 and 2 – Why do the conversion factors differ so much between the two figures (2000-8000 e- RCII-1/C Chla-1 in Fig. 1, but only 2-10 e- RCII-1 C Chla-1 in Fig. 2)? If one divides the ETRmax by Pmax for carbon fixation (or the corresponding alpha values by one another), one should end up with values of e few thousand e- RCII-1/C Chla-1. Please clarify/fix accordingly. 16817 – Fig. 4 shows conversion factors of 2-12 x104. This is what the axes in Fig. 3 should probably read as well? 16817 – The in-text reference to Table 2 is misleading. Table 2 defines PPC, PSC etc. but does not actually

present pigment ratios as suggested in the text. I would suggest mentioning this table in the methods and explain how the PPC and PSC were defined there. 16817 L18-29 – The calculated DES ratios account only for taxa containing a xanthophyll cycle based on diadinoxanthin and diatoxanthin but not for taxa containing a violaxanthin-zeaxanthin-based xanthophyll cycle (chlorophytes and prasinophytes). According to the Chemtax results, diadinoxanthin-diatoxanthin containing taxa account for 35 % of the total chlorophyll in the phytoplankton community, chlorophytes and prasinophytes for 28%. Could the authors perhaps also calculate the DES ratios for the "green" group or otherwise explain why they have been left out? Also note that accurate DES calculation requires quick sampling due to rapid epoxidation of the diatoxanthin back to diadinoxanthin (and zeaxanthin to violaxanthin). For such purposes, samples are usually flash-frozen in liquid N2 within 1-2 minutes after their removal from the light source. Given that sampling with Niskin bottles on a rosette and subsequent filtration probably takes on the order of 30 min (?), the ratios presented here may be off. Perhaps the authors could just acknowledge that with one line. Also, please note the typo on line 28: de-epoxidation, not de-epoxilation. 16818 L15 – "Fv/Fm (...) half of the values expected from nutrient DEplete phytoplankton." Is this correct or should this read half the values expected from nutrient REplete phytoplankton? 16820 L22, 23 Superscript '-1' is missing in mol e- mol C-1. 168221 L28- 16822 L14 – It is intriguing to conclude that the observed effects on the conversion factor and optical properties are the result of iron limitation. This, to me, seems rather speculative because we do not have a comparison with iron replete conditions, which would need further field or culture work. I understand that this would be beyond the scope of this paper, but perhaps the authors could acknowledge that and insert a "disclaimer" highlighting possible future work to resolve this issue. 16823 L17-29 – Please note that Fv/Fm also shows considerable taxonomic variability/dependency (Suggett et al. 2009 – MEPS Vol. 376:1-19). Based on the Chemtax results, community composition did not changed throughout the day and, hence, taxonomic dependency of Fv/Fm is probably negligible. Perhaps the authors could acknowledge that with a brief statement. 16824 L22 and Fig. 7.

Please note that correlation and regression are not the same methods and the two terms should not be used interchangeably (e.g. Field 2006 – Discovering Statistics using SPSS, 2nd edition, Sage Publishing. If the authors aim to establish mathematical relationships between NPQ and the conversion factor (and calculate slopes), then they should use a regression. An appropriate description should also be included in the methods. I am also not convinced that one may not miss some essential information by lumping all the data together into one regression. I looks like the slopes of the regression lines may vary with time of day if the data set was broken up according to the different sampling times. Furthermore, for some sampling time points, the relationship between NPQ and the conversion factor seems to have somewhat of a curvature (e.g. 3:00, 6:00).

Interactive comment on Biogeosciences Discuss., 12, 16803, 2015.

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