

Interactive comment on “Satellite-detected fluorescence reveals global physiology of ocean phytoplankton” by M. J. Behrenfeld et al.

J. Cullen

John.Cullen@Dal.CA

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Abstract

Behrenfeld and colleagues claim that satellite-detected fluorescence measurements can be used as a global physiological indicator of iron-limited growth conditions in marine phytoplankton. Their analysis involves correcting the sun-induced chlorophyll fluorescence signal for phytoplankton pigment, pigment packaging, and the effects of irradiance; results are used to derive estimates of fluorescence quantum yield that are interpreted as a diagnostic of nutrient stress in phytoplankton. The authors' conclusion that they have found a globally applicable diagnostic is unprecedented. However, a brief review of relevant literature shows that their approach is not new and that aspects of the authors' analyses are at odds with published results. Here I discuss research that should be considered and assumptions that must be more thoroughly tested before satellite-detected fluorescence measurements can be used as a robust diagnostic of phytoplankton physiology.

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1 Introduction

Behrenfeld and colleagues claim to have demonstrated the utility of satellite-detected fluorescence measurements as a global physiological indicator of iron-limited growth conditions in marine phytoplankton. This would be a very significant accomplishment, so the claim and the supporting research merit careful examination. In this commentary, I will refer to the manuscript as BFL08, with page number and lines in parentheses (e.g., 4240: 5-7). These comments are guided by the itemized list in BGD Evaluation Criteria (http://www.biogeosciences.net/review/ms_evaluation_criteria.html).

2 Evaluating results in the context of relevant research

The authors describe their principal findings in the following concise statement (4239: 1-10):

“We find that three primary factors regulate global fluorescence distributions: (1) phytoplankton pigment concentrations, (2) a photoprotective response aimed at preventing high-light damage (i.e., ‘non-photochemical quenching’), and (3) ‘pigment packaging’, a self-shading phenomenon influencing light absorption efficiencies (Duysens, 1956; Bricaud et al., 1995, 1998). Additional information on nutrient stressors is resolved by first accounting for these three primary factors and then deriving global distributions of fluorescence quantum yield, the ratio of photons fluoresced per photons absorbed. As described below, iron-stress was anticipated a priori to be a key factor influencing satellite quantum yields (Behrenfeld et al., 2006b, 2008), and this expectation is upheld by a close correspondence between elevated satellite fluorescence yields and low-iron conditions predicted from ecosystem models with active iron cycling.”

The statement can be summarized and evaluated in three parts:

2.1 “We find that three primary factors regulate global fluorescence distributions. . .”

Criticism: The authors represent this as their finding rather than a confirmation of what has already been shown. This statement is not an isolated lapse; throughout the manuscript the authors fail to give proper credit to related work or to clearly indicate their own new/original contributions.

Details: The authors fail to cite the comprehensive study by Babin et al. (1996), which treats the influences of phytoplankton pigments, nonphotochemical quenching and pigment packaging thoroughly and quantitatively, with extensive references to the relevant literature. BFL08 provides references on pigment packaging, but not to compare or contrast their results with related work. For example, the study of Huot et al. (2005) is referred to for a few details of their analysis, but not its principal findings with respect

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to effects of pigment packaging on patterns of fluorescence yield as estimated from satellites (and the uncertainty inherent in parameterizations of absorption coefficients as a function of satellite chlorophyll). The effects of irradiance (directly relevant to remote sensing) have been addressed in several publications (e.g., Babin et al., 1996; Cullen et al., 1997; Ostrowska et al., 1997; Maritorena et al., 2000; Morrison, 2003; Laney et al., 2005; Huot et al., 2007; Schallenberg et al., 2008). BFL08 allude to the fact that Morrison (2003) and Schallenberg et al. (2008) present models of fluorescence yield vs irradiance, but nowhere do the authors provide a comparison of their model of fluorescence yield vs irradiance (shown in Fig. 3) with well described models that have been published previously.

The authors do compare their approach with previous studies when they discuss their use of normalized water leaving radiance, thereby removing the influence of $iPAR$ on F_{sat} . However, the authors' statement, "This property of line height products has been overlooked in earlier treatments, resulting in additional division by $iPAR$ in quantum yield calculations and introduction of errors into derived fields." (4241: 8-10) is incorrect. The product used by Huot et al. (2005) is the FLH calculated as the top of atmosphere radiances corrected by the Rayleigh scattering (see p. 111 in that paper), and thus their analysis is not subject to the error suggested by BFL08.

Sections in the introduction also fall short on appropriate recognition of related work:

(4238: 9-12). Key studies of chlorophyll fluorescence are reviewed in the introduction. Rather than referring to decades of research on the effects of light and nutrient growth conditions on stimulated fluorescence yields (e.g., Kiefer, 1973b; Kiefer, 1973a; Loftus and Seliger, 1975; Vincent, 1979; Cullen, 1982; Cleveland and Perry, 1987), the authors conflate studies of fluorescence induction (Krause and Weis, 1991; Behrenfeld et al., 2006) with those of fluorescence yield (the central topic of this study), potentially leading to confusion. It is important to recognize that fluorescence induction (e.g., F_v/F_m and related measures) is not the same thing as sun-induced fluorescence yield. As shown by Schallenberg et al. (2008, see their Fig. 11), the factors that determine F_v/F_m have very little direct influence on fluorescence yield near the sea surface.

(4238: 20-24). Providing context for their study, the authors state, "To date, application of satellite chlorophyll fluorescence observations has been limited and largely focused on geographically-restricted studies assessing near-shore chlorophyll concentrations or detecting harmful algal species." This ignores the study of Huot et al. (2005) that applied satellite chlorophyll fluorescence observations toward improving global applications of satellite-derived fluorescence, using two examples from the open ocean for illustration.

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2.2 The influence of nutrient stressors can be resolved by accounting for the first three factors, then interpreting the remaining variability.

The authors' analysis depends on three important assumptions: i) corrections for the influences of chlorophyll concentration, irradiance and pigment packaging are acceptably accurate; ii) the effects of particular nutrient stressors (e.g., iron) on fluorescence yield in nature are known (i.e., described and validated) and iii) the influence of other environmental factors on fluorescence yield are insignificant for the global application in BFL08. In my opinion, the authors should examine these assumptions more thoroughly than they do in this manuscript in order to support their conclusion that they have developed a robust, global physiological indicator of iron-limited growth conditions.

The three key assumptions can be examined with corresponding questions:

2.2.1 Are the three corrections justified and accurate?

Chlorophyll. The correction for chlorophyll concentration would seem to be straightforward, but for a global analysis, it is important to verify that the estimates are robust. Consider that estimated Chl_{sat} in large parts of the ocean (including biomes that are very important in the global analysis) is less than 0.1 mg m^{-3} . Quantitative evaluation of the BFL08 analysis would require explicit consideration of limits of detection, and some estimate of propagation of error for estimates of fluorescence yield based on F_{sat} and Chl_{sat} . Based on what is known about retrieval of FLH and Chl_{sat} from MODIS, what are the uncertainties in retrieval of F_{sat}/Chl_{sat} , particularly for oligotrophic waters with very low concentrations of chlorophyll?

Irradiance. The authors present their correction for irradiance ($1/iPAR$) as a fundamental characteristic of sun-induced fluorescence yield, referring inaccurately to the study of Morrison (2003), which does not use a simple $1/iPAR$ function. The authors should support their model of irradiance with comparisons to other descriptions in the literature, of which there are several (see Sect. 2.1 in this commentary). Alternatively, they could present it as an empirical function that does a good job correcting for a major source of variability in the data, and downplay its utility as a general model of fluorescence vs irradiance.

Pigment packaging. The correction for pigment packaging, in which the light absorption coefficient is calculated as the product of Chl_{sat} and the spectrally-averaged phytoplankton absorption coefficient, normalized to chlorophyll, $\langle a_{ph}^* \rangle$, is problematic. Eq. A13 is used to calculate $\langle a_{ph}^* \rangle$ using a function that increases very sharply at low Chl_{sat} . Calculated $\langle a_{ph}^* \rangle$ is $0.09 \text{ m}^2 \text{ mg Chl}^{-1}$ for $Chl_{sat}=0.03 \text{ mg m}^{-3}$, and $0.059 \text{ m}^2 \text{ mg Chl}^{-1}$ for $Chl_{sat}=0.1 \text{ mg m}^{-3}$. This calculated value for $Chl_{sat}=0.1 \text{ mg m}^{-3}$ — and consequently the calculated $\langle a_{ph}^* \rangle$ for all waters with $Chl_{sat} < 0.1 \text{ mg m}^{-3}$ — is higher than all the points in the extensive compilation of spectrally averaged specific absorption coefficients presented by

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Babin et al. (1996) (see also Babin, 2008). It thus appears that a very significant proportion of estimated absorption coefficients in the BFL08 study are unrealistically high, and more so in the most oligotrophic waters. The implication is that estimates of fluorescence yields are not accurate. It is possible that the calculations were inadvertently misrepresented in Eq. A13: BFL08 reports $\langle a_{ph}^* \rangle = 0.027 \text{ m}^2 \text{ mg Chl}^{-1}$ for $\text{Chl}_{sat} = 0.03 \text{ mg m}^{-3}$ (4245: 2), inconsistent with their own equation.

2.2.2 Are the effects of iron known?

In their Sect. 3.2, the authors make a case for iron stress having a strong influence on the quantum yield of fluorescence, focusing in particular on the ratio of PSII:PSI (4246: 20-24), arguing that iron stress leads to increased quantum yield of fluorescence:

“Importantly, iron stress is a key environmental factor influencing PSII:PSI ratios in natural phytoplankton populations. Under low iron conditions, phytoplankton increase PSII:PSI by a factor of 2.5 to 4.0 (Sandmann, 1985; Vassiliev et al., 1995; Ivanov et al., 2000; Strzepek and Harrison, 2004).”

To support this statement about natural populations of marine phytoplankton, the authors cite laboratory studies on: i) the cyanobacterium *Aphanocapsa*, ii) *Dunaliella tertiolecta*, iii) the freshwater cyanobacterium *Synechococcus* sp. PCC 7942, and iv) two cultured marine diatom species, respectively. From this foundation, the authors present in Fig. 3c a model of the influence of changing PSII:PSI on the relationship between fluorescence quantum yield and *iPAR*. This model, which does not include supporting equations, is the foundation of their “global physiological indicator of iron-limited growth conditions.” It shows higher quantum yields of fluorescence with iron stress. The authors do not cite studies by Greene and colleagues (Greene et al., 1991; Greene et al., 1992) who show decreased quantum yields of fluorescence under iron stress.

Clearly, the authors are making some assumptions that should be discussed in some detail. For example:

i) What is the BGFL08 model of fluorescence vs irradiance, and what are its foundations in the published literature? On p. 4243, the model is described with no references to published models except a comment (4243: 20-21) suggesting that the scaling of a graph (log vs linear) alters the relevance of its results when applied to remote sensing. It would be more useful for the authors to consider explicitly the parameters of fluorescence-irradiance models — e.g., fluorescence yields of open and closed reaction centers, saturation irradiance for photosynthesis, threshold irradiance for NPQ, r , the fraction of reaction centers insensitive to NPQ, and q_T , the reduction of fluorescence yield at all irradiances due to “slow quenching” (Morrison, 2003) — then compare their model to what has already been published.

ii) On what basis can it be assumed that the principal effect(s) of iron are those shown in Fig. 3c, to the exclusion of others? In particular, what are the possible influences of nutrient stress (not necessarily

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iron) on susceptibility to NPQ (cf. Babin et al., 1996), and how might this affect the model predictions? Further, how should we interpret the findings of Strzepek and Harrison (2004) that the low-iron adapted *Thalassiosira oceanica* was particularly susceptible to photoinhibition of PSII reaction centers because of its limited ability to carry out energy dependent NPQ? Surely, such responses would influence the shape of predicted fluorescence yield vs irradiance relationships that are based on photosynthesis and the different types of NPQ; these include energy-dependent quenching (as evident in the BFL08 model) and q_I , the quenching highlighted by Morrison (2003) and Schallenberg et al. (2008), but which seems not to be considered by BFL08.

2.2.3 Are the effects of other environmental factors insignificant?

Fig. 3 of BFL08 indicates that the authors have a model that describes the relationship between fluorescence yield and irradiance. The results presented in BFL08 should be discussed in a broader context. In addition to exploring how the model might describe the possible effects of iron nutrition on more than just PSII:PSI as illustrated in Fig. 3c, it is important to examine the potential effects of other environmental factors on the modeled relationship. For example, what are the three curves in Fig. 3d telling us about acclimation as it influences parameters of the photosynthesis- and fluorescence vs irradiance relationships? Part of that discussion should include explicit justification for: i) not including equivalents of the q_I and r terms in Morrison (2003), ii) assuming that fluorescence is constant as a function of irradiance above saturation (Fig. 3a; contrast with Laney et al. (2005) who find differently), and iii) more discussion of the assumptions that go into Fig. 3d, and how they relate quantitatively to predictions of fluorescence quantum yield as observed from satellites. I am very interested in the justification for not invoking q_I as an important factor (if indeed this is the case), since it is so prominent in other analyses of variability of fluorescence yield vs irradiance (Morrison, 2003; Schallenberg et al., 2008).

The above comments on the BFL08 mathematical model are not meant to imply that BFL08 ignores the relationships between NPQ and environmental forcing such as mixed layer irradiance. Indeed, this is discussed on p. 4251, and the authors acknowledge that it would be useful to characterize the relationship between NPQ and photacclimation state. However, they do not attempt to characterize acclimation state (4251: 7-11): “In the current study, we have not implemented such an approach because light-acclimation responses in natural phytoplankton assemblages remain poorly-constrained and large uncertainties still exist in assessing physiologically relevant surface mixing layer depths (required for calculating mixed layer acclimation light levels).” This leads one to wonder what the authors now think about the influential study of Behrenfeld et al. (2005), which is fundamentally based on assessing physiologically relevant surface mixing layer depths to calculate mixed layer acclimation light levels.

Relation of the BFL08 approach to other research. The authors do not mention that the study by

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Schallenberg et al. (2008) is similar to BFL08 in that it includes correction of the fluorescence signal for phytoplankton pigment, pigment packaging, and the effects of irradiance, to derive estimates of fluorescence quantum yield at an irradiance typical for remote sensing, for examination of the variability of quantum yield as a possible indicator of nutrients stress. That is, although the system in which Schallenberg et al. (2008) worked (records from optical drifters) was different, the approach that they developed (based on foundations established by Babin et al., 1996; Letelier et al., 1997; Morrison, 2003; Huot et al., 2005) was the same in its principal elements as that presented by BFL08. Note that the core analysis of Schallenberg et al. was presented as a poster with an extended abstract on CD-ROM for the Ocean Optics XVI meeting in Santa Fe, NM in 2002. Like BFL08, the study by Schallenberg and colleagues concluded that high fluorescence yields were associated with inferred nutrient stress in phytoplankton (reinforcing results presented by Letelier et al., 1997) and that nonphotochemical quenching is a dominant physiological factor. Unlike BFL08, they were cautious in their assessment of the results, concluding that the use of fluorescence yield as a diagnostic would require much more knowledge about “the mechanistic links among environmental forcing, physiological state, and nonphotochemical quenching (q_E , q_I and possibly other processes) as a function of irradiance” (Schallenberg et al., 2008). One can argue that these mechanistic links have yet to be described and verified conclusively with direct observations from the ocean.

2.3 “[Our] expectation is upheld by a close correspondence between elevated satellite fluorescence yields and low-iron conditions predicted from ecosystem models.”

The authors state that “iron-stress was anticipated a priori to be a key factor influencing satellite quantum yields (Behrenfeld et al., 2006b, 2008), and this expectation is upheld by a close correspondence between elevated satellite fluorescence yields and low-iron conditions predicted from ecosystem models with active iron cycling.” This is a start, and it certainly merits the development of a working hypothesis. However, the hypothesis should be tested rigorously under conditions that allow its falsification. As part of this, alternate hypotheses explaining high fluorescence yields should be rejected. Can confounding influences, such as sources of error (including bias) in the estimation of fluorescence, chlorophyll or packaging, potentially explain some of the pattern? What is the role of q_I ? Do regions of natural iron enrichment show the expected patterns of fluorescence yield?

Note that if results of other studies do not seem to support the conclusions of BFL08, it may not be adequate to discount their findings because they may reflect non-steady (transient) responses (4251: 14-16). The corresponding implicit assumption that much of the ocean is in something approaching steady state with respect to phytoplankton physiology may not be justified (as discussed by Parkhill et al., 2001). Regardless, the authors state in their abstract that their method may be useful for appraising phytoplankton responses to natural iron enrichments or purposeful iron fertilizations activities — very much transient responses.

3 Comment on the analysis of correspondence between global distributions of fluorescence yield and other measures

An unfortunate trend followed in this manuscript is the authors' reliance on visual comparisons of patterns in images to determine correspondence between results. For example, on (4246: 5-7), the reader is invited to visually compare Fig. 4a with Figs. 4b and 4c and to agree with the authors' conclusion that there is no apparent correspondence between ϕ_{sat} and the

distributions of two macronutrients. To establish the quantitative foundations of such comparisons, the authors should consider including some readily interpretable plots of relationships between variables, and statistical summaries with estimates of errors. Their Fig. 2 includes plots that are easy to evaluate.

4 Concluding comments

Since the early studies of Lorenzen (1966), Kiefer (1973b; 1973a) and Loftus and Seliger (1975), physiologically based variability in chlorophyll fluorescence yield has intrigued oceanographers, as has the possibility of interpreting the variability of sun-induced fluorescence observed at the sea surface (e.g., Letelier et al., 1997). There is a rich legacy of research on which to build new analyses. Studies describing physiological interpretations of chlorophyll fluorescence detected from satellites should be reported in the context of the relevant literature, so the assumptions underlying new diagnostics can be properly evaluated.

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