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Biogeosciences Discussions

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

M. J. Behrenfeld et al.

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Preamble for Biogeosciences Discussion Readers: This document provides responses to an *Interactive Comment* by John Cullen (hereafter referred to as JC08). The format of the JC08 comment followed that of a normal manuscript submission (Abstract, Introduction, Main Text with subheadings, Conclusion Statement, Acknowledgment of funding, and References). Unfortunately, and likely due to the manuscript-style format, this *Interactive Comment* was mistaken as a new manuscript submission to *Biogeosciences Discussion* and thus not posted on the Discussion website prior to the closing date. Consequently, the two Referees of the paper and the Authors were not aware of this additional comment until after the open period ended, responses to reviewer comments were completed, and a final revised manuscript submitted. It is for this reason that the following responses were prepared independently of the Responses to Reviewer Comments and not posted on-line prior to the end of the discussion period.

As with the earlier Reviewer responses, I have italicized the comments of JC08 below and provided responses in normal text font. As the JC08 Abstract is a reiteration of points made in the main text and the Introduction contains no specific comments, the following responses begin with the Main Text section of JC08.

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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., 'nonphotochemical 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 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.

RESPONSE #1: We appreciate this comment by JC08 and have included the suggested additional ref-

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erences in the manuscript, along with additional new references (increasing the total reference list by ∼50%). In addition, we have changed the cited sentence in paragraph 3 to recognize the earlier work:

In agreement with earlier studies, 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', NPQ), and (3) 'pigment packaging', a self-shading phenomenon influencing light absorption efficiencies (Duysens 1956, Bricaud et al. 1995, 1998)...."

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 Fsat. 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.

RESPONSE #2: The cited statement was not intended to mean that all previous studies were in error. In the case of the Huot et al. study, for example, FLH values were calculated by the authors using MODIS radiances at 667, 678, and 748 nm, as per MODIS ATBD's. To ensure that there is no confusion on this point, we have removed the first sentence of the cited paragraph and now begin with:

"A critical distinction between equations (1) and (2) in that the latter does not include *iPAR*. This difference results ...."

However, other users have attempted to work with the MODIS FLH data products. The important issue is that, at the time when we began our work, MODIS fluorescence quantum yield fields were based on FLH values calculated from normalized water leaving radiances and divided again by iPAR. Thus, the scientific community was trying to work with flawed data fields. In the revised manuscript, we changed the cited statement to:

"Consequently, the direct dependence of F_{sat} on *iPAR* is already removed (Appendix A). This property of line height products was earlier overlooked in the processing of MODIS data, resulting in additional division by *iPAR* in quantum yield calculations and introduction of errors into derived fields.

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,

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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., Fv/Fm 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 Fv/Fm have very little direct influence on fluorescence yield near the sea surface.

RESPONSE #3: We did not suggest that stimulated fluorescence induction was equivalent to natural fluorescence. We only used stimulated fluorescence induction as one example of how fluorescence is used to study phytoplankton physiology before moving directly to the issue of natural fluorescence. However, we greatly appreciate the additional references provided by JC08 and have added them in the revised sentence:

"Accordingly, detailed measurements of stimulated fluorescence have been widely used to study light- and nutrient-stress effects on PSII functioning and fluorescence yields (Kiefer 1973b, Kiefer 1973a, Loftus & Seliger 1975, Vincent 1979, Cullen 1982, Cleveland & Perry 1987, Krause & Weis 1991, Falkowski & Kolber 1995, Behrenfeld et al. 2006b)."

(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.

RESPONSE #4: Our original statement was correct. Fluorescence data have been highly un-utilized compared to other satellite products (e.g., chlorophyll) and the studies that have been conducted were *largely* limited to near-shore studies. However, we have added to the references the following:

"(e.g., Gower et al. 2004, Hu et al. 2005, Ahn & Shanmugam 2007, Gilerson et al. 2007, Gower & King 2007, Huot et al. 2007, Gilerson et al. 2008 - but see also the broader analysis of Huot et al. 2005)"

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

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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 Chlsat 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 Fsat and Chlsat. Based on what is known about retrieval of FLH and Chlsat from MODIS, what are the uncertainties in retrieval of Fsat/Chlsat, particularly for oligotrophic waters with very low concentrations of chlorophyll?*

RESPONSE #5: One approach to evaluating the performance of satellite chlorophyll products is through comparison with field match-up data. Such comparisons have been made extensively by the NASA Goddard team and can be viewed on their website and in related MODIS and algorithm publications. For the current study, we were particularly concerned with chlorophyll and fluorescence products degrading in very clear ocean waters, especially since the fluorescence products were earlier expected to be valid only at chlorophyll concentrations greater than 1 mg m^{-3} (Abbott and Letelier 1999). However, our analysis yields a relationship between NPQ- and package-corrected fluorescence and chlorophyll that is linear, has an intercept of zero, and exhibits a relatively uniform distribution of scatter across the full range of open ocean chlorophyll concentrations (Fig. 2E,F). This result therefore indicates that there is no apparent limit of detection for the fluorescence and chlorophyll products, or at least that the uncertainty in the two products is highly correlated, and thus not a serious issue in the calculation of fluorescence quantum yields (ϕ_{sat}). The significance of the zero intercept result is it implies that a bias is not introduced as chlorophyll decreases. In other words, if the intercept was > 0, then calculated ϕ_{sat} would increase with decreasing chlorophyll. Finally, the relatively conserved distribution of scatter suggests that there is no systematic decrease in product quality as chlorophyll decreases. We use in the main text chlorophyll products from the NASA standard algorithm for MODIS (OC-3), but also provide a comparison in Appendix A Figure 2 between NPQ-corrected F_{sat} data and chlorophyll estimates from 3 different algorithms. This comparison yields similar correlation coefficients for all 3 algorithms, with intercepts of zero and relatively uniform distributions of scatter for the full range of chlorophyll concentrations. Nevertheless, there are some differences between data distributions for the three algorithms, and these differences do introduce some uncertainty in derived ϕ_{sat} distributions.

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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.*

RESPONSE #6: The inverse-light-function introduced in our manuscript for NPQ correction is nowhere proposed as a general model of fluorescence changes with incident light, but only an approximation for satellite applications under supersaturating conditions. In addition, we clearly show in figure 3A,B that the relationship does not hold at light levels near or below the saturation point. Further still, we show in figure 3D that a single inverse-light-function does not capture variability in NPQ for different photoacclimation states. We also call this issue out in the final section of our paper and describe how a characterization of photoacclimation effects will be necessary to further advance the analysis of satellite ϕ_{sat} data as a physiological tool. So we believe that the limited application of our NPQ correction has been clearly stated throughout. Nevertheless, we have added additional text in the revised manuscript and the legend for figure 3 to more fully explain factors not considered in any one given ϕ-*iPAR* function, how qI will influence these curves, and the basic assumptions of our ϕ-*iPAR* calculations.

With respect to the model of Morrison (2003), our full light model describing fluorescence changes with iPAR (gray lines in Fig. 3) is consistent with this earlier model for a given photoacclimation state and value of qI. If there was any issue here, J. Morrison, one of the solicited reviewers for this manuscript, would certainly have raised it in his review. As stated above and in the manuscript, the inverse-light-function is only an appropriate approximation at supersaturating light levels. At these light intensities, the model of Morrison for any given qI also approximates a 1/iPAR form. Regarding the Schallenberg et al. (2008) manuscript, their model is a modification of the Morrison (2003) model and again exhibits a similar decrease in fluorescence with increasing light at supersaturating levels for a given qI.

Pigment packaging. *The correction for pigment packaging, in which the light absorption coefficient is calculated as the product of Chlsat and the spectrally-averaged phytoplankton absorption coefficient, normalized to chlorophyll, $<$ a*ph $>$, is problematic. Eq. A13 is used to calculate $<$ a*ph $>$ using a function that increases very sharply at low Chlsat. Calculated $<$ a*ph $>$ is 0.09 m2 mg Chl-1 for Chlsat=0.03 mg m-3, and 0.059 m2 mg Chl-1 for Chlsat=0.1 mg m-3. This calculated value for Chlsat=0.1 mg m-3 — and consequently the calculated $<$ a*ph $>$ for all waters with Chlsat $<$0.1 mg m-3 — is higher than all the points in the extensive compilation of spectrally averaged specific absorption coefficients presented by 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*

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*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 < a*ph >=0.027 m2 mg Chl-1 for Chlsat=0.03 mg m-3 (4245: 2), inconsistent with their own equation.*

RESPONSE #7: For open ocean conditions, light absorption by photosynthetically active pigments is dominated by wavelengths between 400 to 530 nm (e.g., see Bricaud et al. 1995, 1998). Package effects on fluorescence are therefore best described by this waveband. Babin et al. (1996) calculated a spectrally-averaged chlorophyll-specific absorption coefficient for the waveband 400 to 700 nm. While this value may improve the characterization of packaging when phytoplankton absorption spectra are broad, it will tend to underestimate packaging effects on fluorescence when light absorption for photosynthesis is dominated by shorter wavebands.

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.

RESPONSE #8: In the revised manuscript, we have expanded the section describing iron stress effects on phytoplankton physiology and fluorescence yields, including a description of consequences on qI. In Figure 3C, we illustrate how a change in the PSII:PSI ratio can influence fluorescence quantum yields, but now also include a statement on how qI changes result in similar 'whole curve' shifts. We have also included in the figure legend additional information on our calculations and underlying assumptions. The legend now reads:

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"Relationships between fluorescence quantum yield (ϕ) and incident light (*iPAR*). **(a)** Changes in ϕ with *iPAR* for a phytoplankton population acclimated to a single light level. Blue line = absorbed light energy (relative). Green line = fluorescence (relative). Gray line = ϕ. Red line = scaled $^1/_{iPAR}$ function. **(b)** Same ϕ data as in panel (a) but plotted against a log-transformed *iPAR* axis, making this plot directly comparable to ϕ-*iPAR* relationships shown in figure 9 of Morrison (2003) and figure 11 of Schallenberg (2008). Red line = scaled $^1/_{iPAR}$ function. **(c)** Change in the ϕ- *iPAR* relationship corresponding to a PSII:PSI ratio shift from 0.5 to 2.0, assuming that pigment distribution between the two photosystems varies in proportion to their relative abundances. A decrease in PSII reaction center quenching (qI) results in a similar 'whole curve' upward shift in ϕ. Iron stress influences both PSII:PSI and qI (see text). **(d)** Changes in the ϕ-*iPAR* relationship resulting from photoacclimation to different light levels (the three curves correspond to light-saturation levels (E_k) of 50, 150, and 250 μmol photon m^{-2} d^{-1}). Phytoplankton grown at low-light become light saturated at low *iPAR* levels and then exhibit strong NPQ upon exposure to higher *iPAR* levels (lower curve). Higher-light acclimated phytoplankton require higher light levels to saturate photosynthesis and engage NPQ (middle and upper curves). For any one of these three curves, the reduction in ϕ from NPQ at supersaturating light levels follows an inverse function of *iPAR*. However, the NPQ effect *at any particular saturating iPAR level* is stronger in the lower-light acclimated phytoplankton than in the higher-light acclimated cells. This dependence of NPQ on photoacclimation state implies that a single, globally-applied inverse-light function will tend to under-correct for NPQ at high *iPAR* and overcompensate for NPQ at low *iPAR* if photoacclimation is positively correlated with *iPAR* (as occurs with changes in latitude). **(a-d)** Gray bar on x-axis indicates *iPAR* range for MODIS fluorescence measurements. For each panel, we assume that qI is constant, light absorption increases linearly with *iPAR*, and light energy delivered to the core of PSII saturates in an exponential fashion with *iPAR*. The fraction of this PSII core energy lost as fluorescence increases from an initial value (F_o) to a maximum value (F_m) at saturating light levels."

Let us now consider the two citations to Greene et al. Inspection of the Greene et al (1991) manuscript shows that no determinations of fluorescence per unit chlorophyll or fluorescence per unit of light absorption were made. This work therefore is not relevant to the current investigation. What they reported was (see their figure 3) iron-replete and iron-limited fluorescence yields for pump and probe measurements (panel a) and the same fluorescence yields normalized to the maximum yield (panel b). In their Discussion section, however, they state that "fluorescence per unit chlorophyll is higher in Fe-deficient than in Fe-replete cells" (citing earlier investigations).

The problem with the Greene et al. (1992) citation is the methodological approach. In that study, *D. tertiolecta* and *P. tricornutum* were severely iron limited, such that specific growth rates were 0.1 d^{-1} over the growing period. In addition, analyses were not conducted during the growth period, but rather after the cells had entered early *stationary* phase. Such conditions have little relevance to fluorescence per

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chlorophyll or fluorescence per light absorption in natural populations growing under chronic low-iron conditions and may also be responsible for the curious fluorescence/chlorophyll and fluorescence/absorption response curves reported by Greene et al. (1992).

Let us now look at two other studies. In the work of Sakshaug and Holm-Hansen (1977), *Skeletonema costatum* and *Pavlova lutheri* were grown under modest iron stress (83% and 63% of maximum growth rates, respectively) and then fluorescence per unit chlorophyll observed over a subsequent starvation period. What these investigators found is that fluorescence per chlorophyll increased rapidly with increasing iron stress and then decreased smoothly upon iron addition, without the transient features reported by Greene et al. (1992). If we then look at a later study by Rueter and Ades (1987), we find fluorescence per chlorophyll similarly increases with increasing iron stress. In this later study, the investigators took the precaution to maintain the cultures at relatively steady-state conditions through semi-continuous culturing techniques and monitored cell properties to ensure steady state. We have added these references in the revised manuscript

As a final investigation, we also have data on light-saturated fluorescence values (Fm) (measured as in Greene et al. 1992) and chlorophyll data for natural phytoplankton populations from the tropical Pacific (Behrenfeld et al. 2006). The plot on the right shows all of the Fm/chlorophyll data for the 25 experimental cites described in our 2006 paper. Here, samples from iron-stress populations (as determined from nutrient enrichment experiments) are shown in red, while samples from iron-sufficient populations are shown in gray. On average, Fm/chlorophyll is a factor of 2.1 higher under iron stressed conditions.

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 qI , the reduction of fluorescence yield at all irradiances due to "slow quenching" (Morrison, 2003) — then compare their model to what has already been published.

RESPONSE #9: As stated above, we have now added further information of the fluorescence model to Figure 3. We have also provided the light saturation values assumed for the three curves in Figure 3D. We do not state that "*the scaling of a graph (log vs linear) alters the relevance of its results when applied to remote sensing*". What we have tried to do is to illustrate the fluorescence versus light relationship on both a linear and log scale. This is to help the reader have a clearer view of changes in fluorescence

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with increasing supersaturating light level (Figure 3A) and also to provide the same data on a log scale so that it is easier to compare with figures in previous manuscripts (Fig. 3B) (e.g., Morrison 2003). For our satellite application, fluorescence changes at subsaturating light levels (e.g., yields for open and close reaction centers) are irrelevant. However, we have revised the manuscript text to carefully distinguish between qE and qI effects on fluorescence yields and state how the later influences ϕ-iPAR relationships. It is important to recognize that Figure 3 is intended to illustrate general fluorescence properties and we show all curves on relative scales. The similarity between our fluorescence curves and those previously described for any given qI implies that the inverse light function will reasonably approximate the other models at *supersaturating light levels*. Thus, the same degree of variability in satellite package-corrected F_{sat}/Chl_{sat} (Figure 2D) is accounted for by our simple inverse light function and the model of Morrison (2003). We also explicitly call-out in the final section of our manuscript that improvements in the NPQ correction are needed:

"One factor that is particularly influential on satellite-derived fluorescence yields is NPQ. In the current treatment, a simple inverse-light function has been applied for the qE-based NPQ correction. While such a relationship ...., the actual reduction in ϕ_{sat} from NPQ expressed at any given saturating *iPAR* differs between phytoplankton acclimated to different light levels (Fig. 3d). .... Applying a single inverse-light function at all latitudes, therefore, will tend to over-correct for NPQ at high latitudes, yielding dampened ϕ_{sat} values. .... Addressing this issue represents an important challenge for future satellite fluorescence analyses and will require the description of NPQ as a function of both *iPAR* and photoacclimation state, where the latter term should be considered from the perspective of the mixed layer light environment and its fluctuations on physiologically-relevant time scales."

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 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 qI , the quenching highlighted by Morrison (2003) and Schallenberg et al. (2008), but which seems not to be considered by BFL08.

RESPONSE #10: Iron stress has a wide range of effects on phytoplankton physiology and not all of them have been considered here. However, we have expanded our description of these physiological effects in the revised text, including the influence on photoinhibition. Particularly noteworthy is that the laboratory

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results of Strzepek and Harrison (2004) are not consistent with other findings in the laboratory or, more importantly, in the field. More typically it is found that iron stress reduces susceptibility to photoinhibition (as described and referenced in the revised manuscript). Again, it is important to recognize that Figure 3 is intended to illustrate some basic concepts. Our link between satellite quantum yields and iron stress is not based on Figure 3, but rather empirical comparisons with distributions of iron deposition and model predictions of iron stress. When we compare elevated fluorescence yields with low macronutrient distributions, we do not find a similar correspondence. We also do not state that iron stress is the only factor influencing satellite fluorescence yield distributions, although it does appear to be an important one. In the final section of our manuscript, we discuss some of the additional issues that need to be considered and have expanded the final paragraph to:

"In addition to photoacclimation-dependent variability in the ϕ-*iPAR* relationship, other factors can also be considered in future interpretations of global ϕ_{sat} distributions. For example, it may be fruitful to consider how non-steady-state growth conditions and additional nutrient stressors influence qI-type NPQ (e.g., Schallenberg et al. 2008). Taxonomic factors may also be important, particularly with respect to their influence on ϕ_{sat} through changes in accessory pigments or PSII:PSI responses to iron-stress. As these remaining issues are resolved, we can anticipate new insights on phytoplankton physiology and ecology to emerge from the satellite chlorophyll fluorescence record, as well as an independent global data set for regionally- and globally evaluating satellite chlorophyll retrieval algorithms (e.g., *Fig. A2*)."

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 of 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 qI 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 qI 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).

RESPONSE #11: See Responses #6, #8, #9, and #10 above.

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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.

RESPONSE #12: This sentence has been changed to now read:

"Addressing this issue represents an important challenge for future satellite fluorescence analyses and will require the description of NPQ as a function of both *iPAR* and photoacclimation state, where the latter term should be considered from the perspective of the mixed layer light environment and its fluctuations on physiologically-relevant time scales."

***Relation of the BFL08 approach to other research**. The authors do not mention that the study by 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 iradiance 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 (qE, qI 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.*

RESPONSE #13: We have added the reference to Schallenberg et al. (2008) in our description of NPQ

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normalization to average *iPAR,* clearly indicated when we are referring to NQP in general or qE or qI specifically, and have expanded our description of qI effects and their link to iron.

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 qI? Do regions of natural iron enrichment show the expected patterns of fluorescence yield?

RESPONSE #14: Issues of fluorescence and chlorophyll retrievals and pigment packaging uncertainties are addressed in Responses #5 and #6 above. The revised manuscript now specifically treats issues regarding qI (*see above*). As we mention in our text, it will certainly be exciting to see if the fluorescence signal changes in a consistent manner during both artificial iron enrichments and natural enrichments, but this is far beyond the scope of the current contribution.

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.

RESPONSE #15: We do not discount the results of Schallenberg et al. (2008), but instead cite it as evidence of additional factors influencing quantum yields. This has been clarified in the revised mansucript. With respect to iron fertilization, see Response #14 above. Also note that in the revised manuscript, we have removed the term "purposeful iron enrichments".

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

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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 ϕsat 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.

RESPONSE #16: With respect to Figure 4, we state that there is no apparent correspondence between ***elevated*** ϕ_{sat} and ***low*** macronutrient levels, not simply ϕ_{sat} and macronutrient levels. We then proceed to discuss the apparent correspondence with iron deposition data. At the end of this section, we raise the issue that iron deposition may not accurately represent nutrient stress levels because of additional physical and ecosystem phenomena. The same is true for macronutrient stress, so we have revised this paragraph to now state:

"The physiological phenomena thus described provide a mechanistic link between iron stress and fluorescence yields, but realization of this relationship may not be fully captured in comparisons of high ϕ_{sat} and low iron deposition because aeolian deposition alone is not the only factor controlling iron stress in the open ocean. Additional important factors include upwelling of iron from depth, ecosystem recycling, and biomass-dependent competition for available iron. Likewise, macronutrient stress is not fully represented by surface concentrations because of similar ecosystem processes. To further investigate the link between nutrient stress and ϕ_{sat}, we therefore employed coupled ocean circulation-ecosystem models with active iron cycling."

In the subsequent section (3.3), we compare our ϕ_{sat} distributions with model predictions of iron stress and macronutrient/light stress. At this point, we are now in the position to make a quantitative assessment of the match-ups (as per JC08's suggestion), which has been added in the revised manuscript:

"Likewise, model predicted macronutrient- or light-limited waters are typically associated with low ϕ_{sat} values (average = 82% of global area), such as across the Atlantic where the GCI indicates a general absence of iron-stress. Recognizing the spatial (satellite = 9 km at equator; model = 3.6^o longitudinal, $>1^o$ latitudinal) and temporal (satellite = single season; model = climatology) inconsistencies between these data sources, this agreement of satellite observations and model predictions is rather remarkable (over the annual cycle, 66% of the global ocean area exhibits high ϕ_{sat} values in predicted iron-stress regions and low ϕ_{sat} in predicted iron-sufficient regions)."

FINAL RESPONSE TO JC08

All of the authors wish to thank Dr. Cullen for his evaluation of our manuscript and the effort put forward. We believe it has resulted in a more thorough treatment.

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ADDITIONAL REFERENCES NOT PROVIDED IN JC08

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