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## ***Interactive comment on “From the shape of the vertical profile of in vivo fluorescence to Chlorophyll-*a* concentration” by A. Mignot et al.***

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We would like to thank both reviewers for giving valuable and constructive comments. Most of the time, their suggestions have been carefully addressed. If not, the reasons are explained in our response.

Details answers to the review (Referee #1):

SPECIFIC COMMENTS:

Referee#1: P5, 3-5: Please provide reference for Zpd.

Authors: The definition of Zpd is found p.3702 l.11: “The first penetration depth, Zpd (m) is defined as  $Z_e/4.6$ . Note that this quantity is also used for ocean color remote

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sensing studies, where it delineates the surface layer actually “seen” by the satellite. ”

Referee#1: P6, 21: the sentence “All fluorescence and . . . calibrated and validated.” This sentence is too ambiguous.

Authors: The sentence was indeed unclear because we spoke of “calibration”. The fluorescence data used here were only validated meaning that spurious data (e.g. spikes in the fluorescence profile) had been corrected or removed. The fluorescence data are not calibrated in units of Chla. The sentence has been corrected.

Referee#1: P9, 13-15: Why were there the Gaussian profiles under eutrophic conditions?

Authors: We agree with this remark that the vertical shape of Chla is not well defined for the eutrophic conditions. Nevertheless, in our dataset, we still notice a very shallow DCM, in both fluorescence and HPLC profiles, under eutrophic conditions.

Referee#1: P10, 14-17: the sentence “Several studies (Herland and Voituriez, 1979; Varela et al., 1992; Estrada et al., 1993; Ediger and Yilmaz, 1996 and Mantyla et al., 2008) found that the depth of the DCM,  $Z_{max}$ , was tightly coupled with the upward nutrient flux (high upward nutrient flux, corresponds to a shallow DCM), and consequently to the Chl-a concentration in the upper layer,  $Chl_{surf}$ .” This sentence is difficult to read. Rewrite.

Authors: The new sentence is: “Several studies (Herland and Voituriez, 1979; Varela et al., 1992; Estrada et al., 1993; Ediger and Yilmaz, 1996 and Mantyla et al., 2008) have demonstrated that the depth of the DCM,  $Z_{max}$ , was tightly coupled with the upward nutrient flux (a high upward nutrient flux corresponds to a shallow DCM and reciprocally). Since the Chla concentration ( $Chl_{surf}$  and  $Chl_{ze}$ ) and  $Z_{max}$  are inversely related (Fig. 4a), we state that the upward nutrient flux also controls Chla concentration.”

Referee#1: P10, 17-20: If the increase in water transparency was due to decrease in Chl concentrations, [ $Chl_{ze}$ ] would not increase with deepening of  $Z_e$ .

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Authors: You're right, [Chlze] decreases with a deepening of Ze.

Referee#1: P11, 13-24: I would like to have seen some attempt to examine the direct relationships between Zm, Chlsurf and Chlzm (column integrated content with Zm). It would have been informative to present some attempt so that the reader can assess the differences with the previous studies.

Authors: We agree with this statement. Unfortunately, we do not have the ancillary data necessary to propose some attempt about the relationship between Zm and [Chlzm]. Note that the paper aims at developing a method which a priori does not require such ancillary information. (Furthermore, as an extension of this study, we have begun to download fluorescence profiles from large databases and the ancillary data are generally missing).

Referee#1: P11, 26-28: You should properly explain about the daytime-fluorescence quenching because this process is important for the relationship between in vivo fluorescence and Chl a concentration.

Authors: This was not the aim of the section to detail the mechanism of quenching which has been clearly detailed in the references given in the text (e.g. Cullen and Lewis, 1995; Holm- Hansen et al., 2000; Sackmann et al., 2008). We nevertheless propose a new sentence which briefly describes the issue of quenching. "The fluorescence signal is depressed in the upper layer of the water column, when the phytoplankton is exposed to high irradiance."

Referee#1: P12, 13-21: the sentence "For gaussian profiles, a group of stations mainly representative of spring bloom conditions in temperate areas (e.g. POMME2 and some BOUSSOLE cruises) have, for the same Chlsurf value, a much deeper Z1/2 and larger dz than for the global trend. For the same stations, Zmax (shallow) remains poorly scattered with respect to the global trend. These stations correspond to the typical situation described in Fig. 9a. Another distinct group of stations also suffers from an overestimation of dz due to the daytime-fluorescence quenching (Fig. 7c) while Z1/2

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and  $Z_{\max}$  remain weakly scattered with respect to the global trend. This is another typical case (Fig. 9b) where fluorescence quenching is superimposed onto a DCM (many stations of the subequatorial and the sub-tropical South Pacific: BIOSOPE and OLIPAC cruises).” Unclear. This sentence needs to be rewritten.

Authors: The new sentence is: “For gaussian profiles, we identified two typical situations of quenching that change the shape of the fluorescence profile (Figs. 9a and b). In the first situation (Fig. 9a), a fake DCM appears near the surface, due to the depression of fluorescence at the surface. As a consequence, a group of stations mainly representative of spring bloom conditions in temperate areas (e.g. POMME2 and some BOUSSOLE cruises) have, for the same  $Chl_{\text{surf}}$  value, a much deeper  $Z_{1/2}$  and larger  $dz$  than for the global trend (Figs. 7b and c). At the same time  $Z_{\max}$ , which is very shallow, remains poorly scattered with respect to the global trend (black line on Fig. 7a). In the second situation (Fig. 9b), the fluorescence quenching enlarges the width of the DCM. The depth of the DCM remains unchanged. As a consequence, a group of stations (representative of the subequatorial and the sub-tropical South Pacific: BIOSOPE and OLIPAC cruises), reveal an overestimation of  $dz$  (Fig. 7c), while  $Z_{1/2}$  and  $Z_{\max}$  remain weakly scattered with respect to the global trend (black line on Figs. 7a and b).”

Referee#1: Also, explain why  $Z_{\max}$  remains poorly scattered.

Authors:  $Z_{\max}$  remains poorly scattered because, in the two typical cases of quenching identified (Figs. 9a and b), the surface depression of fluorescence weakly affects the depth of the DCM.

Referee#1: P14, 15-16: How did you derive the equations 10 and 11?

Authors: Equations 10 and 11 are derived from the equation 2. We resolve this equation for  $z=0$  and  $z=Z_{\max}$ , which give  $F(z)=Chl_{\text{surf}}$  equation (10) and  $F(z)=Chl_{\text{max}}$  equation (11)

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Referee#1: P14, 22-25: Please show the error in the regression slope between Fc and F. Also, was there a difference in slopes among the oceanic regions?

Authors: We are not sure to catch the meaning of the question.

Referee#1: P15, 15-27: You should describe more carefully about the results of statistical analysis. For example, the  $r^2$  alone is no statistical meaning.

Authors: We use the coefficient of determination  $r^2$  to assess the quality of the linear regression fitted within the log-10 transformed data. We are then able to compare the quality of the 3 linear regression (gaussian, sigmoid and both).

Referee#1: What are the meanings of RMSE and APD in this study? Why are the Chla-calibrated fluorescence values underestimated for gaussian profiles and overestimated for sigmoid profiles?

Authors: The meanings of RMSE and APD are described in the paragraph “3.4 Statistical indicators”. The RMSE indicate the scatter of the data between fc and Chla. The APD is the absolute percent deviation between fc and Chla. The under or over estimation of Chla-calibrated fluorescence values fc are estimated with the median RPD . A negative median RPD implies that fc are in average lower than the HPLC Chla value, and inversely for a positive median RPD.

Referee#1: P16, 24-28: the sentences “For given trophic conditions, a certain “natural” noise characterizes the relationship between shape and concentration. It was not the purpose of this study to analyze the sources of this noise (except for the specific and well-identified case of fluorescence quenching). A consequence of this noise is that the parameterization proposed here, like any global parameterization (Uitz et al., 2006), is of global relevance. ” Unclear. Please rewrite to clarify.

Authors: The new sentence is: “For given trophic conditions, a certain “natural” variability characterizes the relationship between the depth-dependent shape parameters and the Chla concentration. It was not the purpose of this study to analyze the sources of

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this variability (except for the specific and well-identified case of fluorescence quenching). As a consequence, the parameterization proposed here, is only of global relevance, smilarity to the Uitz et al. (2006) parameterization.”

TECHNICAL CORRECTIONS: P2, Equation 1: Typographical errors of units (E, not d-1 but s-1 or time-1; a\*, not m-2 but m2). P9, 13: Miswriting “r2= 0, 59”

All the technical corrections have been done.

Details answers to the review (Referee #2 R.Forster):

Referee#2: Initial thoughts are that this is a very useful meta-analysis, which will be of wide use to the oceanographic community. The authors correctly (in my view) foresee a rapid increase in the number of vertical F profiles available, primarily from long-term glider and profiling mooring deployments. As none of these remote deployments will be accompanied by calibration data (except for co-location of surface measurements with ocean colour), there is a real need to understand the variability in the fluorescence-to-chlorophyll ratio. As a side note, a similar analysis is urgently needed for understanding the F-chl ratio from continuous measurements of surface fluorescence on ships-of-opportunity (FerryBox systems), as the number of routes for these is also expanding rapidly. The paper and its rationale are clearly laid out and, the equations can be easily used and tested on other data tests, which I'm sure many colleagues will do. My only general point is that the authors do not thoroughly explore the variability between their derived chlorophyll and measured chlorophyll. I would like to see more detailed analysis of the results in Figure 11, particularly with respect to depth.

Authors: See later, this point is discussed in the part Method Validation.

Additional comments:

Referee#2: Introduction p.3699 “. . .most measured biological property (together with O2).. “ Along- side oxygen, underwater light is also relevant here as a proxy for biological status of the water. I would argue that, as with fluorescence, irradiance profiles are

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also under-utilised at present.

Authors: We thank the reviewer for his suggestion , we have incorporated it into the introduction as follows: "Subsequently, the development of in-situ fluorometers that are coupled to CTD sensors has resulted in fluorescence measurements being likely the most measured biological property (together with O<sub>2</sub> and irradiance profiles) in the open Ocean."

Referee#2: p.3699 l.18 Fluorescence equation. I think the spectral dependence of E and  $a^*$  should be noted here. See my comments later on the choice of excitation wavelength for the fluorometer.

Authors: Although the referee is correct with the spectral dependence of E and  $a^*$ , given that the method presented here is "bulk" and that the two fluorometers used in this study have quite similar excitation / emission characteristics, we prefer to leave the equation in its simpler form.

Referee#2: There should also be a term (+F<sub>b</sub>) for the background fluorescence response of the instrument in the absence of algal fluorescence, this is not negligible and typically increases with depth due to bleaching of the "cDOM or fDOM" pool at the surface.

Authors: We agree with the referee but this is accounted for in the way the raw fluorescence profile is treated. This was not explicit in the previous version and is now described in p. 3704 3.1 Dataset and quality control: "l.22: ...in D'Ortenzio et al. (2010). To account that the deep fluorescence values are still non-null in the absence of algal fluorescence, the fluorescence profile is set to 0 at the bottom by subtracting the mean of the 10 deepest points from the profile."

Referee#2: p.3699 l. 19 Units of E normally expressed per s<sup>-1</sup> rather than d<sup>-1</sup>. Three lines later, f has units of mol photon m<sup>-3</sup> s<sup>-2</sup>

Authors: Ok, we corrected in the manuscript.

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Referee#2: Please change “Chl-a” to use a subscript or superscript rather than ‘-’, it is confusing in the equations later on.

Authors: Ok, we changed it throughout the manuscript

Referee#2: P3704 I. 11 Please state the types of fluorometers used on the CTDs on these cruises. Were only blue-excitation light sources used, or a range of lamp or LED types?

Authors: Only two fluorometers were used for the cruises, which were conducted over a 20-year period. A Seatech fluorometer (excitation:  $425 \pm 200$ ; emission:  $685 \pm 30$ ), used for early cruises, and then a Chelsea fluorometer (excitation:  $430 \pm 105$ ; emission:  $685 \pm 30$ ) used afterwards for the more recent cruises. Unfortunately, this information is not available precisely for each cruise. In any case, no LED fluorometer was used.

Referee#2: p.3705-06 Sorting the Data. A world map showing the distribution of the three profile types would be useful.

Authors: The specificity of the dataset is that it covers all the trophic conditions (Chl<sub>surf</sub> range from 0.02 mg.m<sup>-3</sup> to 32 mg.m<sup>-3</sup>) including the most extreme conditions (very deep mixed layer in North Atlantic to the center of a very stratified ocean gyre). We therefore do not believe that a world map showing the geographical location of the data would be relevant for the study.

Referee#2: Results and Discussion p.3709 I. 4 Replace “[chl ze] increases” with “decreases”

Authors: ok, done

Referee#2: p.3711. I.1-5. There are other reasons for the change in F/chl with depth in addition to quenching. A slope  $> 0.3$  with depth can also be seen in nighttime CTD profiles. I think this is due to spectral acclimation at depth, where the excitation cross-section of photosystem-II in the blue-green is enhanced at the DFM relative to the

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surface. This leads to more effective capture of the blue excitation light used by most fluorometers, per unit chlorophyll.

Authors: This aspect is now mentioned in the paragraph dealing with quenching: p. 3711 l.4: " . . . . respectively. A change in fluorescence/Chla ratio with depth could be also seen in nighttime fluorescence profiles due to spectral acclimation. However, when plotting Figs. 7 and 8 regarding day.vs.night, the slope>0.3 occurs predominantly during the day."

Referee#2: p.3714-3715 Method Validation This section could be expanded in a number of ways, most obviously by looking at different regions. However, in order not to expand the paper too much, what I would most like to see is a grouping of the validation results according to optical depth bins. A 'day' versus 'night' comparison of predictions would be also be useful.

Authors: Before the initial submission, we had investigated the potential effect of phytoplankton light acclimation on fluorescence response through an optical depth binning of the data ( see below in Fig.1). Since we did not see any significant results, we decided not to include this figure (and associated presentation / discussion). This figure is now included for the referee only, but we still believe it does not deserve to be included in the revision.

Referee#2: Conclusions p. 3716. Some mention should also be made of the 'blank' problem and how best to remove non-algal signal from the profiles, as this can be a serious contamination in some low-biomass waters.

Authors: The 'blank' problem has been treated in the Section 3. Materials and Methods.

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Interactive comment on Biogeosciences Discuss., 8, 3697, 2011.

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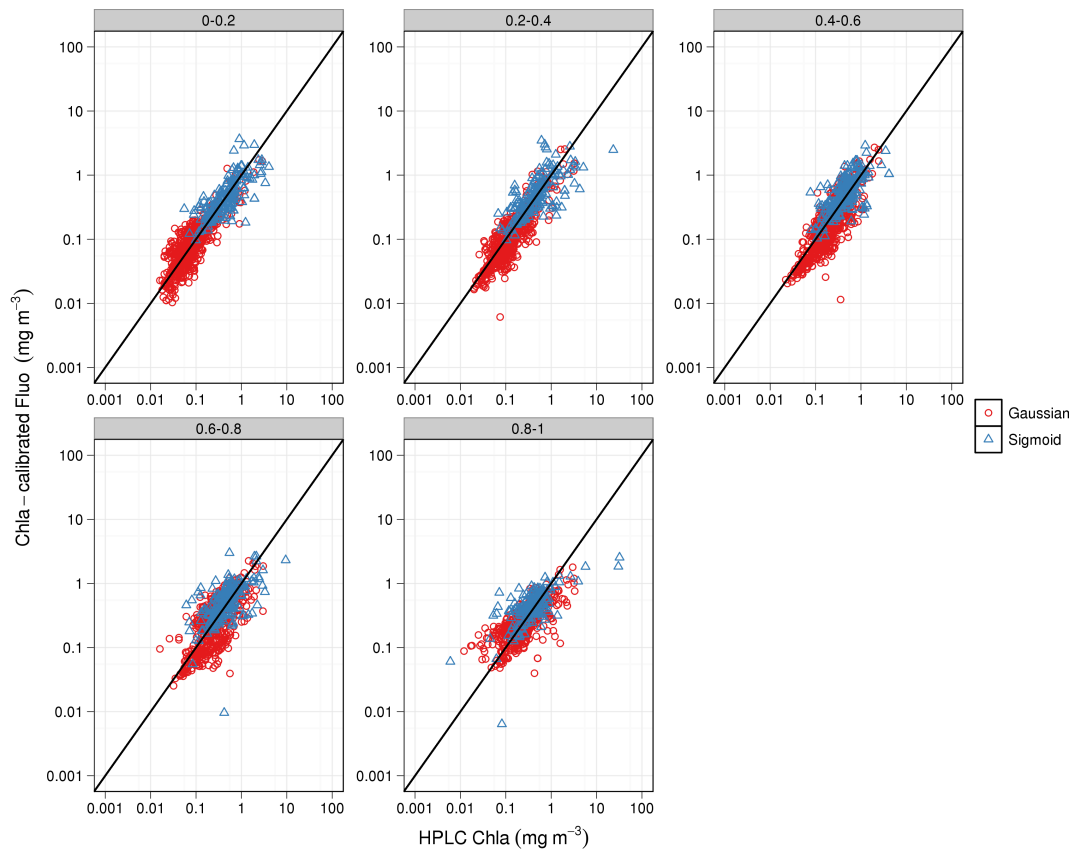
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**Fig. 1.** Optical depth binning (between the surface and Ze; 0 and 1 ) of the scatterplot presented in Fig. 11 (discussion paper)

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