

## ***Interactive comment on “Inferring phytoplankton carbon and eco-physiological rates from diel cycles of spectral particulate beam-attenuation coefficient” by G. Dall’Olmo et al.***

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Received and published: 23 August 2011

Reviewer 2

We thank very much the reviewer for his/her comments, which have helped us clarify several parts of our original manuscript.

Below we present the reviewer’s comments in blue and our answers in plain black text. Our answers follow the order of the reviewer’s comments.

Please explain how  $cp$  has been computed from the measurements. It is not clear how the particulate beam attenuation coefficient ( $cp$ ) has been computed. The Acs

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instrument provides total absorption ( $a$ ) and total attenuation ( $c$ ). The contribution of pure water ( $c_w$ ) and colored dissolved organic matter ( $ag$ ) has to be removed from the total beam attenuation coefficient to compute  $cp$ . It is not indicated how the values of  $c_w$  and  $ag$  have been computed. It seems to me that this is a study of  $cpg$ , not of  $cp$

The manuscript presents particulate beam attenuation measurements (i.e.,  $cp$ ), not  $cpg$ . As mentioned in the text (p. 3015 lines 11–12), the methodological details on data processing have been previously published, so it was initially decided not to include these details again in the manuscript. Nevertheless, we agree with the reviewer that it may be best to briefly explain what was done, since the methodology adopted is not well known. In essence, our  $cp$  measurements are derived by subtracting the optical signal generated by 0.2  $\mu m$ -filtered seawater from the bulk raw measurement. These “filtered” measurements are automatically collected by the system every hour for ten minutes, linearly interpolated to match the bulk data, and subtracted to obtain a calibration-independent  $cp$  measurement. We have added a clarification in the methods section “In-situ optical measurements”.

2. Why don’t you examine the diel variability in  $bp$ ? The effects of attenuation and scattering should be separately examined. Currently, the interpretation of the  $cp$  diel cycle does not take into account the contribution of dissolved and particulate absorption ( $apg$ ). This reduces the validity of the assumptions that (i)  $cp$  could be modeled using a power law of wavelength, (ii) the spectral variability of  $cp$  is related to the slope of the particle size distribution (PSD), and (iii) the diel changes in spectral  $cp$  are mostly related to changes in particles size. Accordingly, the following points should be addressed: (i) Is it correct to model  $cp$  using a spectral model when the contribution of absorption might be significant? Indeed, the error analysis simulations conducted by Boss et al. (2001) concludes that “Absorption effects are important [...] when a larger number of small absorbing particles will be present”, which is likely the case here because of the presence of *Synechococcus* and small eukaryotes in the particle community. (ii) The spectral distribution of the scattering coefficient is a power

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function of wavelength if two successive conditions are met (Morel, 1973): 1) The PSD follows a power law function, and 2) Particles are non-absorbing. The limits of these assumptions should be mentioned: 1) The use of a power-law PSD has been challenged by several studies (Jonasz and Fournier, 1996; Reynolds et al., 2010). 2) The second assumption is not valid in the visible range because most marine particles, and in particular phytoplankton, are absorbing. As a result the imaginary part of the refractive index is high enough to impart on the scattering coefficient (Babin et al., 2003, Doxaran et al. 2007). 2. (iii) Changes in  $a_p$  are mostly related to changes in pigments concentration and composition, which can occur independently of changes in Particle Organic Carbon (POC), cell size and/or real refractive index.

We respectfully disagree with the reviewer that the spectral  $cp$  can not be approximated by a power law. Instead, the relationship that would not hold, when the assumptions are violated, is the one that links the slope of  $cp$  with the slope of the particle size distribution. We did not use this relationship in any of our calculations.

In addition and as clarified above, the analysis is based on  $cp$  measurements, not  $cpg$  as the reviewer thought, and we further selected  $cp$  data above 550 nm where absorption becomes negligible with respect to scattering.

We have added a clarification (in the section "Simulation of optical properties") regarding the contribution of absorption to our  $cp$  measurements: above 550 nm, absorption contributed at most 2.5% of  $cp$ . As consequence,  $cp$  at these wavelengths was dominated by scattering and there is no need to distinguish between  $cp$  and  $bp$ .

3. Why don't you provide two additional graphics showing (i) the spectral  $cpg$  and  $bp$  at different times of the diel cycle (e.g. at sunrise, noon, sunset, midnight, following sunrise), and (ii) the temporal variation of their spectral slope for days 195 to 198? It would support (or not) the assumption of using a power-law model for spectral  $cpg$  and  $bp$ . If a power-law model is indeed appropriate, then it would be also useful to show also the diel cycle of the spectral slope for the days 195 to 198.

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(i) Again, we measured  $cp$ , not  $cpg$  and  $bp/cp$  97.5

(ii) Done, see new figure 1.

4. The partition of  $cp$  between a background and a diel component is one of the good points of the paper. Combining flow cytometry and ACs data, it should also be possible to partition the diel component of  $cp$  into the contributions of nano-1, nano-2, syn, euk and bact (e.g. DuRand and Olson, 1996). This would strengthen the conclusions of the paper and consolidate the interpretations of the model results. It would also make it possible to discuss one of the major assumptions of your model, i.e. that only one population of cells is responsible for the observed cycle in  $cp$ .

We agree with the reviewer: the flow-cytometry data could be further exploited to derive phytoplankton beam attenuation coefficients. However, to do so, a laborious empirical calibration of the flow cytometric scattering signals would be required (e.g., [Durand\_Olson\_1996]; [Green\_etal\_2003]). Unfortunately, it was not possible to obtain this calibration for our study and thus we cannot provide this important validation step. Nevertheless, as mentioned in the discussion manuscript, [Durand\_Olson\_1996] did find that the larger cells contributed the majority of the phytoplankton beam attenuation (due to their larger cross sections) and thus supports our hypothesis and results.

5. It would improve the paper to compare the results of your computationally heavy model with more simple models linking particle growth and beam attenuation diel cycle (e.g. Marra 1995, Cullen et al. 1995, Gernez et al. 2011).

We did include such a comparison in the discussion manuscript, but it might have been missed by the reviewer. It was on page 3026 and demonstrated that 1) these simpler models are underestimating growth rates (because they do not account for the background component of  $cp$ ) and 2) the growth rates calculated from those simpler models can vary by a factor 2 depending on which wavelength is selected for the calculations.

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3013:17-27 and 3014:1-3 This paragraph is not accurate enough. Please rewrite it in order to take into account the points mentioned in general comments 2(i) and 2(ii).

We have added the specification that the relationship between the slope of cp and that of the particle size distribution holds if particles are not absorbing.

3014:14-16 What about the variability in the size of heterotrophic picoplankton? From measurements performed during the same cruise, Talarmin et al. (2011) suggest that mixotrophy should be considered in studies of primary and bacterial production.

The relative side scattering of the bacterial population did not show significant diel changes (Fig. 1 in the discussion manuscript). Thus, we believe that it is reasonable to assume that heterotrophic bacteria contribute to the background component of cp and not to the time-varying component. We have added text acknowledging this issue in the "Limitations" section of the discussion.

3015:9-21 This paragraph is not accurate enough. What is the spectral range measured by the ACs?

We have added the requested instrument specification.

More importantly, it is not clear how cp has been computed from ACs measurement because it is not clear how the contribution of pure water and of dissolved substances has been computed (see also general comment 1).

We answered this question at the beginning of this document and added relevant text in the revised manuscript.

3016:18-28 The symbols and subscripts are misleading. (i) The symbol  $r$  is not appropriate for a number of classes. I would rather use  $N$ . Moreover, as the symbol  $r$  is often used to design a growth rate, this is confusing to use it for something else. (ii) I do not like the subscript  $d$  for "division" because it is confusing with "diel" or "daytime". This is all the more confusing as cell division is (incorrectly?) assumed to occur during night-time. (iii) Symbol  $DI$  is not appropriate to design a probability, please use another

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symbol.

The symbols were initially selected to match the notation of the papers that described the population model, to help the reader interested in that model. However, following both reviewers' suggestion we have changed them to match the conventions of the oceanographic literature.

3016:26-27 Is it really inconceivable that cell division can also occur during the day?

No it is not, but data from oligotrophic regions show that most phytoplankton species divide towards the end of the day. That is why, in our initial attempt, we had decided to force division only at night. However, following comments from both reviewers, the model has now been adapted so this feature is no longer forced, although interestingly still emerges from the model. See new Figure 5.

3017:6-9 What is the size range encompasses by the 64 classes: 1 to 30  $\mu\text{m}$ ?

The original text specifies (two sentences after the sentence quoted by the reviewer) that "Although the number of size classes is known, the average diameter of each size class is determined by the model parameters and cannot be specified a priori".

3017:12-13 Is it realistic to assume that the growth rate is uniform among size classes? Chisholm (1992) points out that the growth rate might be size-dependent.

Growth rate could be size-dependent, as the reviewer suggests. However, we do not believe that our model and data could allow to infer a size-varying growth rate. Thus, the decision was made to make growth rate constant across all size-classes.

3019:7-10 Why do you assume that the imaginary part of the refractive index is negligible?

Because we have verified that absorption contributed at most 2.5% of cp for this data set at wavelengths above 550 nm. We did specify this piece of information after eq. (10) in the discussion manuscript, but we have now moved it where the reviewer was

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expecting it (i.e., at the end of the first paragraph of section “Simulation of optical properties”).

Moreover, the real part of the refractive index is not constant during the day. Stramski and Reynolds (1993) report a diel variation between 1.036 and 1.055 at 660 nm.

Other studies (e.g., [DuRand\_etal\_2002]), including a larger number of species than the single species investigated by [Stramski\_Reynolds\_1993], demonstrated that size was the dominant driver of the scattering cross-section and not the refractive index. In addition, and as mentioned in the discussion manuscript, diameter and refractive index are correlated parameters from an optimization point of view, because they are multiplied by each other in the parameter that determines the scattering cross-section. We have added references to justify for our hypothesis.

3019:20 Eqs. 7 and 9. Again, the notation is misleading as subscript “d” in cpd is likely to be confused by the “d” of detritus. I would suggest to replace cpd by cp1 and write  $cp(\lambda, t) = cp0(\lambda) + cp1(\lambda, t)$  in Eq. 9.

Done.

3019:21 How do you reconcile Eq.1 and Eq. 7? Is the spectral slope the same for cp0 and cpd?

Equation 1 is used to introduce the notion of spectral slope of cp. However, as indicated by equations (7-8), the spectral slopes of cp0 and cp1 are calculated independently.

3022:16 and Fig.4. What day is Fig. 4? Why don't you show the temporal variability of cp for the other days (i.e. 195 to 198)? How do you explain the dramatic drop in cp at 4:00? For example, cp(550) drops from 0.078 to 0.075 in less than one hour, which is about 30% of the total diel variation. In order to highlight the spectral difference between cp(550 nm), cp(630 nm) and cp(710 nm), it would be useful to show the full spectrum of cp at key times of the diel cycle for day 196, such as for example: sunrise, noon, sunset, midnight, following sunrise.

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We have specified in the caption that the day for which data are plotted is 196.

The step change observed in the data at 4:00 was also recorded by the C-star transmissometer (data not shown) and thus it was not due an instrument specific problem. In addition, the step change did not occur during a time when the instruments were cleaned (such cleaning was carried out earlier that night and is evident as a gap in the data, but not step change). The in-situ water density instead displayed a slight but sudden variation in correspondence of the step change seen in the cp data (data not shown). Thus, it is likely that the step change in cp was related to crossing a front, even though the ship was following a drifter within the eddy sampled during the long duration station.

The revised figure 1 presents full cp spectra for selected times of the day.

3022:22-26 I may have missed a point, but I do not understand why the fact that parameters are (in)dependent of the refractive index suggests that the estimation of parameters is (in)correct. The explanation for that comes too late (3025:8-16).

We have modified the text in the results section to clarify why the correlation indicates that the parameter estimates are unreliable.

3022:26 How do you determine which pair of refractive index and average size is correct between the three options shown in Table 3?

We do not determine which refractive index or diameter is correct in Table 3, rather we report estimates of the diameter and carbon biomass that take into account of the variability due to the refractive index. This is because, as stated in the text, the refractive index and the inferred diameter are correlated and cannot be independently estimated in this analysis.

3023:10 Why do you select  $n = 1.05$  and not  $n = 1.02$  or  $1.08$ ? Because, this value is what is typically assumed for phytoplankton (e.g., Aas 1996). We have added a reference in the text.

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3025:8-16 I think some parts of this paragraph should be moved in the method section.

We respectfully disagree. This part does not explain why two parameter estimates can not be considered reliable, when they are correlated. Instead, this part is our interpretation of why, in the specific case, the parameters are correlated. However, as specified above, we added text in the result section to clarify why the estimates of correlated parameters are unreliable.

3025:23-24 Why should the average population cell size be independent of losses?

As specified at the end of the sentence 3025:23-24, the statement quoted in the reviewer question holds “provided that these [losses] are not size specific”. This is because the average size of the population depends on the shape of the PSD, not on its magnitude. This was further clarified by equation (11) in the original text.

3026:4 (Eq. 12) Why do you scale by  $f_d$  (i.e. the illuminated fraction of the day)? Doing so the ratio  $f_d / (t_2 - t_1)$  is equal to 1.

Since cells are growing only during the illuminated part of the day, it is necessary to scale their growth rate as determined during the illuminated hours to the entire day. Without such scaling, the calculated growth rate would be significantly overestimated.

The reviewer is correct that the ratio  $f_d / (t_2 - t_1)$  is equal to 1. Nevertheless, we decided to make the scaling explicit, despite its unit value, to ensure the reader followed the reasoning.

3026:13-14 There is no point comparing your volume specific growth rate  $g$  with the diurnal rate of  $cp$  variation: they are two different quantities. Applying Eq. 6 of Gernez et al. (2011) to your data of Fig. 4 gives a diurnal rate of variation around 0.19 d<sup>-1</sup> at 630 nm, which is consistent with their observations at 660 nm (Gernez et al., last line of table 2).

We respectfully disagree with the reviewer, who, on his/her general comment 5, specifically suggested to “compare the results of your computationally heavy model with more

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simple models linking particle growth and beam attenuation diel cycle”.

First, the diel cycles of  $cp$  have been shown to be due to phytoplankton [Durand\_Olson\_1996]. Secondly, many previous studies (e.g., [Siegel\_etal\_1989]; [Cullen\_Lewis\_1995]; [Gernez\_etal\_2011]) have linked “the diurnal rate of  $cp$  variation” and phytoplankton productivity. Therefore, we feel, it is important to show how different the prediction of growth rates are between our model and the previous ones.

3027:16-21 How do you explain that, according to the results of the model, *Synechococcus* does not significantly contribute to beam attenuation diel cycle? Your Fig. 3 displays conspicuous diel variation for the side scatter of *Synechococcus*.

As noted in the original text (3022: 7–12), the more “conspicuous diel variations” for the side scatter (SSC) of *Synechococcus*, as compared to those for the eukaryotic populations (“euk”, “nano-1” and “nano-2”), are not necessarily an indication that the latter population does not show a diel cycle. The larger noise in the eukaryotic populations is likely a result of the larger diversity of eukaryotes than *Synechococcus*. In other words, the SSC of the flow cytometry class “euk” (for example) is more noisy because it includes variations in size and refractive index due to different species. On the other hand, the class “syn” is more likely to be a relatively more homogeneous population (possibly a single species) and thus its SSC varies more regularly.

As stated in the original text (3027: 20–21), our finding that relatively large, but less numerous, cells are responsible for most of the diel cycle of  $cp$  are consistent with the findings of [Durand\_Olson\_1996] and [Claustre\_etal\_1999].

3028:2-3 Oubelkheir et al. (2005) reports a  $cp^*$  of 1.78 m<sup>2</sup> (gC)-1 for the Mediterranean Sea.

We have now added this value to our calculations.

3028:23-26 The partition of  $cp$  into the contribution of nano-1, nano-2, euk, syn and

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bact using flow cytometry data should allow you to discuss the assumption that only population of cells is responsible for the diel cycle in cp (see also DuRand and Olson 1996 and general comment 4).

See answer to general comment 4

Table 1 is not self explanatory. Please define all parameters of the table, including T, DI, rb.

Done, see also Tables 1 and 2 in the revised manuscript.

Table 2. Why do you use the central 68th percentile rather than standard deviation?

To make our estimate more robust to outliers.

Are xrb0 and vrb0 identical?

Yes, corrected.

Fig.4. It would be more appropriate to model bp(lambda, t) rather than cp(lambda, t). It would be useful to show more observations before showing the results of the model. I would suggest to add three figures before the current Fig. 4: (i) temporal variation of bp(t) and/or cp(t) at 440, 550, 630, and 710 nm for days 195 to 198, (ii) spectral variation of bp(lambda) and/or cp(lambda) at various time of the diel cycle (e.g. 4:00, 8:00, 12:00, 15:00, 19:00, and 22:00) for day 196, and (iii) temporal variation of the spectral slope of bp(lambda) and/or cp(lambda) for days 195 to 198.

Done, see new figure 1.

Fig.4. How do you explain the dramatic drop in cp(t) at 4:00?

This question is repeated and has already been answered above.

3019:14 (Eq. 6) Is  $\beta_i$  a function of wavelength?

Yes, corrected.

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3022:12 patterns

Corrected.

3022:24 Are vrd0 and vrb0 identical?

Yes, corrected.

C2648

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Interactive comment on Biogeosciences Discuss., 8, 3009, 2011.