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Interactive comment on "Disparities between *Phaeocystis* in situ and optically-derived carbon biomass and growth rates: potential effect on remote-sensing primary production estimates" *by* L. Peperzak et al.

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Dear Editor, First of all, we like to thank the three anonymous referees for their time they have invested to review our manuscript. We were mildly surprised by their comments, that contained the curious combination of minor criticism on the experiment itself and extremely strong criticism on our interpretation of this experiment. The referees are fully correct that this is only one experiment. However, this experiment has been carried out with extreme caution and state-of-the-art instrumentation. We prove, based on this experiment, that an estimate of the carbon fixation rate for Phaeocystis by re-

mote sensing will likely give erroneous results. As long as no other experiments show that that we did something wrong, we stick with these results and our interpretation and offer this type of experiment to the scientific community in order to provide a more profound base for the coupling between primary production and the optical signature in and above water. In this respect we like to quote anonymous reviewer #2 who wrote "I do not dismiss the value that such experiments provide in explaining general trends or certain ecological scenarios, for example in interpreting behavior observed during the wax and wane of a Phaeocystis bloom in a given locale." Indeed, our ambition at the start of the experiment was that a better characterization of the optical signature would help to understand the wax and wane of Phaeocystis blooms detected by satellites (see also Kurekin, A.A., Miller, P.I, Van der Woerd, H.J., 2014. Discrimination of Karenia mikimotoi and Phaeocystis blooms in European coastal waters: merged classification of MODIS and MERIS ocean color data. Harmful Algae, 31, 163-176.). The point is: we failed in coupling the optical signature to primary production. This article explains why and warns that this can "potentially" be the case for more algal species. We are willing to include references to the (old) literature provided by the referees and a few sentences in the introduction and discussion to put this experiment in more perspective. It is not the purpose of this paper to deny all the experiments on remote sensing estimates of primary production. It is not the purpose of this article to deliberately leave out literature and we certainly have no intention to be "not fair" to any author. But it also cannot be the purpose of the referees to deny new experiments that are well done but produce results that are not in line with the present paradigm. Below we provide a response (A) to the questions and comments (Q) in more detail. Basically, we will provide a more extensive clarification or give extra information in an update of the present manuscript, unless stated explicitly in that answer. Kind regards, Louis Peperzak Hans van der Woerd Klaas Timmermans Texel/Amsterdam, 18 November 2014.

Anonymous reviewer #1 General comments: Q. This reviewer wants to know the question we want to address. A. It simply is: how applicable are potentially suitable proxies

for remote sensing derived primary production estimates (4th paragraph of Introduction)? We do this by comparing such proxies directly with those derived from standard oceanographic techniques (1st paragraph of Introduction). This question is very much alive in the scientific world: see for example the work of Saba et al. (2010) "Challenges of modelling depth integrated marine primary productivity over multiple decades: A case study at BATS and HOT". Glob. Biochem. Cycles, 24: GB3020, doi:10.1029/2009GB003655.

Q. I do not know of any proposed application of φ as a measure of growth rate nor of anyone using it as such (the authors do not cite appropriate literature for their statement). A. We may not have cited all available literature and there may be misunderstanding about the wording of the ms (we do not say φ is equal to Fv/Fm, we say it is equivalent i.e. as a physiological proxy and show this is so with data) but in our view the goal or casting of the paper is clear. We can rewrite this section, including some more literature, see letter to the editor.

Q. Methods are extremely succinct and refer extensively to a previous paper. A. In order to provide a recipe for other scientist to replicate this type of experiment, we have published a detailed description of the set-up and processing of optical data in a separate paper in L&O Methods. More information of the LOM paper can be included to make the BG article more comprehensive.

Q. How was the Fv/Fm measurement carried out? A. The Fv/Fm measurement was made on a dark acclimated sample for >20 min.

Q. In section 3.1 the authors skip over the observation that after day 8, both mesocosms (not only mesocosm 1 where nutrients were added) showed an increase in POC while every other measurement decreased in mesocosm 2. A. Carbon fixation might have taken place because Fv/Fm was still >0.4 Fig 2A), not very low as suggested by the referee. Another explanation might be heterotrophic growth (bacteria) but this is unlikely to be significant (see answer to reviewer 2, specific comment 5).

Specific comments:

Q. Two different symbols for the fluorescence quantum efficiency A. The fluorescence quantum efficiency should always be represented by the symbol ðİIJŚ (See table 1). We will correct this.

Q. To me Figure 5 is probably the most interesting result in this paper showing a relatively constant quantum yield even under starvation (nutrient enrichment not having a strong effect). A. Thanks for this constructive comment. We will address this point in the discussion with reference to some of the suggested literature.

Q. P.6131, line 11. It is not clear at all what the loss of correlation correspond to? There is to my eye a non-linear and fairly nice relationship between ðİIJŚ and growth rate. I note, however, that the quantum efficiency values presented in Figure 6 do not match the values presented on Figure 5. A. Sorry for this: we made a mistake. Figure 6C should be the one as Fig. 1 to this comment(note: y-axis values are to be multiplied by 100% to match description in ms text).

Anonymous reviewer #2

General comments: Q. I have to question if the term "mesocosm" is an accurate description of this approach? A. The definition is different for the various scientific disciplines. Toxicologists name 1 L beakers with monocultures mesocosms; we use a larger volume. The reviewers of the LOM article had no problems with the term and we define the chosen name.

Q. The results are for a single species only. A. Correct, our mesocosm is not representative for a natural system. Instead we aimed to reach a controllable experimental set-up that would allow careful comparison of optical and chemical measurements. Note the word "potential" in the title.

Q. The experiment is a short-term batch culture in which nutrient conditions are constantly changing. A. Likewise, our mesocosm is not a 'real environment' but an excel-

lent controllable experimental set-up. Nutrient conditions were NOT constantly changing. For the growth-limiting nutrient DIN: it was either replete (indeed declining) or depleted (and spiked once).

Q. In my opinion, the authors need to be more realistic about the usefulness of their study for real world applications. A. We simply warn that non-linear changes in optically and chemically derived growth rate proxies take place, and because optically derived proxies must be used for global carbon production estimates such estimates may be less accurate as we would like (again: note the word "potential" in the title).

Q. There is a lack of information provided on cell size and morphological characteristics observed during the experiments A. This isolate did not form colonies. Cell size was checked for every sample by the forward- and side-scattering characteristics, as measured by the flow cytometer.

Specific comments:

Q. Fv/Fm and φ are NOT equivalent. A. Agree. See answer to reviewer 1.

Q. More information on the methods must be included to better understand the results. A. More Information from the L&O Methods paper can be included (see also answers to reviewer 1).

Q. The above inquiry regarding mesocosm depth and clarity of the enclosure sides relates to the calculation of "absorption" by phytoplankton. A. The reviewer is completely correct that great care must be taken to calculate the absorption spectra of Phaeocystis. Water samples were measured with the Integrating Cavity Absorption meter from Hobi Labs. RAMSES irradiance measurements were made at the top and bottom of the mesocosms to monitor the spectral attenuation in the mesocosm. These measurements were combined with a simple spectral attenuation model that incorporates aspects like CDOM absorption and optical thickness. Model and measurements proved to be very robust (see Figures 6 and 8 in the L&O Methods article by Peperzak

et al. (2011). Relevant aspects of the methodology can be added in an annex to the present manuscript.

Q. The calculation of phytoplankton quantum efficiency, φ , here and in the appendix needs further elaboration. A. See previous answer. The method for calculation is described in great detail in the L&O Methods article, including baseline correction, integration of angles, wavelengths and correction for the re-absorption of emission and air-water interface. All relevant aspects of the methodology can be added in an annex to the present manuscript.

Q. All estimates of POC and PON used in growth rate calculations assume that no bacteria or other microorganisms exist in the cultures. Was this verified? A. Flow cytometer diagrams did not suggest the presence of other phytoplankton species. We judged the bacteria concentrations as low. We could make an estimate of an upper limit to the percentage of POC by bacteria. The seawater used originated from the Atlantic Ocean, DOC concentrations (as source for heterotrophic growth) are expected to be low.

Q. I have a difficult time keeping track of which measurements are used in different calculations and why. A. This remark is related to the previous request for more details on the absorption and quantum efficiency calculations. We fully agree that our set-up and calculations should be transparent and reproducible, but hoped to have solved this in the L&O Methods paper. Clearly, from the remarks by 2 out of 3 reviewers we failed and we will take great care in the updated paper to clarify all these quantities.

Q. I do not understand the utility of normalizing POC to Chl a+c. A. As is explained in the manuscript, both forms of chlorophyll were present in the same order of magnitude. Adding them increased the precision of the data.

Q. Fig. 3. Please specify the wavelength range over which these absorption measurements correspond. A. Reflectance was measured from 400 to 750 nm. The absorption algorithm wavelengths are given in Appendix A.

Q. How can the fluorescence emission F be in irradiance units? To be consistent with the described derivation and Table 1, the units should be sr$-$1. A. Yes, sr-1 is correct.

Anonymous reviewer #3

General comments:

Q. The additional change in light environment alone (due to accumulation of chlorophyll biomass during the experiment; Chl goes from $\sim$0 μg/L to $\sim$30 μg/L) would be enough to drive patterns in any of the physiological quantities (e.g., Chl:C). Given this, why is it surprising that none of the physiological parameters correlate with the growth rate? A. Thank you for this constructive comment and suggestions for literature that could shed light on our experiments from a different perspective. We noticed in our experiments that rapid changes occur in absorption when N is depleted or spiked and therefore considered nutrient conditions the main driver. Changes in average light conditions are smaller, but still can be important. We will check calculations and suggested literature and include this aspect.

Q. Why is no attention given to limitation by iron? A. A minimum level of Fe- concentration was established by the addition of Fe (1 μM) to the seawater. Concentrations are given in the L&O Methods article and are not growth limiting. Proof can also derived from the fact that the N-spike gave immediate response in mesocosm-2.

Q. What is new: if you set aside the optics for a moment, then all we are looking at is a short time-course batch culture and its response to nutrient addition. A. Exactly, but we did not "set aside the optics for a moment". Surprisingly, this type of experiments, where simultaneous measurement of optical and chemical proxies are made are few. This is innovative. See also the general comments in the letter to the editor.

Interactive comment on Biogeosciences Discuss., 11, 6119, 2014.