

Reviewer-1

Reviewer comment
<p>The current manuscript describes an analysis primarily focused on evaluating alternative approaches for assessing phytoplankton carbon biomass. This is a challenging and important issue. Unfortunately, none of the measurements conducted during this study were direct analytical measurements of phytoplankton carbon, so the authors are limited to comparisons between different indirect proxies. This limitation is clearly articulated in the manuscript along with a brief discussion of underlying uncertainties with each proxy. The manuscript is written in an almost conversational manner, which I found enjoyable to read. That said, in future versions it would be beneficial if the authors found a colleague whose first language is English to provide a final edit to clean up some of the writing to make it clearer to readers.</p>

Authors response
<p>We appreciate that this reviewer recognises the importance and challenging aspect of the topic, enjoyed reading the manuscript, and acknowledges that we have clearly stated the limitations of the study (no direct analytical measurements of phytoplankton carbon). Several comments provided in this review have helped to significantly improve the manuscript and we thank the reviewer for this.</p> <p>We leave it to the editor to assess whether significant improvements of the writing are still required.</p>

Reviewer comment
<p>I found the manuscript to be fundamentally flawed in ways that compromise the overall conclusions. The central measurements of this study were total chlorophyll (tchl), beam attenuation (cp), particulate backscatter (bbp), POC, and cell counts. Phytoplankton carbon (Cphyto) is assessed from tchl based on the study of Sathyendranath et al (2009) (hereafter S2009), from bbp following Graff et al. (2015) (hereafter G2015), and from cell counts following an approach similar to Martinez-Vincent et al 2013) (hereafter MV2013). It should be noted that in both G2015 and MV2013 a significant relationship is reported between bbp and Cphyto, with the former representing the only assessment where direct measurements of Cphyto were used (as recognized in the current paper). In G2015, it was noted that, while the MV2013 paper reported a linear relationship between bbp and cell volume, the slope was much too large compared to results based on analytical Cphyto data, highlighting a challenge with converting cell number and cell size data into Cphyto that is relevant to the current study. Samples measured during the G2015 and MV2013 studies included phytoplankton populations that were very similar to populations sampled during the current study (e.g., oligotrophic waters</p>

dominated by *Prochlorococcus* and *Synechococcus*). There is no mechanistic reason I am aware of that similar populations measured in different geographical places would be expected to exhibit vastly different relationships with b_{bp} . Thus, one must question why the current study failed to find a significant relationship between b_{bp} and C_{phyto} ?

Authors response

We think that “fundamentally flawed” is somewhat excessive language here. However, we agree with the reviewer that assumptions underlying the three methods we test can be challenged.

We want to clarify here that we do not take for granted any of these approaches. We recognize their inherent limitations and we do not a priori claim that one is better than the others in determining C_{phyto} . The use of the particulate backscattering coefficient to derive C_{phyto} rests on theoretical considerations and on a very limited number of direct analytical measurements of phytoplankton carbon. The approach based on the POC vs. Chl relationship also relies on debatable assumptions (as discussed below by the reviewer). Finally, although the use of cell counts can be seen as a better approach in principle because it is more mechanistic, its application relies on approximations that also generate uncertainty in the derived C_{phyto} values (e.g., average sizes and conversion factors). We think that these differences are precisely why comparing these three methods is useful. That being said, we agree our discussion was maybe not reflecting clearly-enough what was learned here from the comparison.

Another point that needs clarification: we do not claim that we could not “find a significant relationship between b_{bp} and C_{phyto} ”. The only way to find (or not) such a relationship would have required direct C_{phyto} measurements. What we do not find is a relationship between C_{phyto} derived from b_{bp} and C_{phyto} derived through the two other methods (this is Fig. 8).

Of course, this is partly because of the limited range of b_{bp} from the mesotrophic to oligotrophic conditions we sampled. We think this is simply illustrating the limitation of the approach for such waters. It does not mean it cannot be applied generally when a larger range of b_{bp} values is available.

We have rewritten the last two sentences of the abstract in an attempt to avoid misinterpretation. They now read:

“The range of b_{bp} measurements was too narrow to conclusively assess the performance of deriving C_{phyto} from b_{bp} in such an oligotrophic environment. These results highlight the limitations of each method and emphasize the need for careful review before applying them to satellite ocean color observations.”

Reviewer comment

Before investigating this question further, it is important to evaluate the S2009 study. In that study, near surface chlorophyll concentrations (i.e., >95% of samples were from <40m) were compared to POC values. S2009 then assumes that at any given chlorophyll concentration, the lowest observed POC can be assumed to approximately represent C_{phyto} and that POC values above this minimum can be attributed to increasing concentrations of other non-phytoplankton particulate carbon forms (detritus, bacteria, viruses). The fundamental flaw in this approach is that chlorophyll concentration is a reflection of 3 primary determinants; biomass, nutrient limitation of growth, and photoacclimation to light. The fact that physiological factors can be responsible for greater than an order of magnitude variation in chlorophyll concentration invalidates the foundational assumption of the S2009 approach. In addition, since the S2009 data set likely included few if any measurements from below the mixed layer, it is questionable whether the approach can be robustly applied to data in the current study from samples collected below the mixed layer. Given the aforementioned issues, one should be skeptical about the C_{phyto} values presented in the current study that are based on the S2009 approach.

Authors response

As a general remark: we understand that the reviewer is critical of a number of previously published studies, like the S2009 approach here discussed. We do not intend to enter such debates here and we reiterate that we do not take any of the three methods as inherently superior to, or weaker than, the two others. As already stated, it is precisely the goal of our study to assess how these methods perform in the particular case of oligotrophic environments. We think this is relevant precisely because the three methods are based on different assumptions.

We acknowledge the need to be careful with the origin of the data when comparing our results to previously published relationships (e.g., surface vs. below-mixed-layer samples). We actually also generated all results presented in the manuscript by only using samples for depths < 40 m. This is the blue solid line in Fig. 5a, showing a very close relationship to that of S2009 ($79.3 \times \text{Chl}^{0.6}$ vs. $79 \times \text{Chl}^{0.65}$).

Most other relationships we displayed in Figs. 6 and 7 do not hold when the data set is restricted to depths < 25 m (the "remote sensing domain").

Reviewer comment

One of the primary messages in the current study is that bbp provides an unreliable estimate of C_{phyto} (at least for the current study region – but see comment above regarding mechanisms), and indeed this message is the main theme of Section 4.2 and figure 8. However, inspection of figure 2 reveals that the current data set is poorly suited for assessing a bbp:C_{phyto} relationship. Specifically, the dynamic range in observed particle mass for the full cruise transect is driven by differences

between the moderately productive waters sampled at stations 1-4 and oligotrophic sites at stations 5-20. While Tchl, POC, cp and cell count data are available across this gradient, bbp is entirely absent in the more productive waters. This lack of bbp data is a great disadvantage for assessing a bbp:Cphyto relationship, and it also influences the interpretation of histograms presented in the manuscript (Fig. 3, Fig 5). Despite the smaller dynamic range in bbp, the authors nevertheless find good relationships between bbp and cp, bbp and POC, and cp and POC. Where things fall apart is when tchl is compared to the IOPs (fig. 3c,d). So lets give this more consideration.

Authors response

We are sorry that our message was not properly delivered and then interpreted as if the b_{bp} method was not working properly. Again, our study cannot per se assess a b_{bp} : C_{phyto} relationship because we do not have direct C_{phyto} measurements.

As for the range of b_{bp} measurements: it was unfortunate indeed that the backscattering meter could not be deployed at stations 1-4. We have addressed this limitation in the discussion lines 671-673: *"If the b_{bp} vs TChl-a relationship (Fig. 4c) is used to reconstruct b_{bp} values that could have been measured for stations 1 to 4, the associated range in C_{phyto} still remains low."* More precisely here: a Chl of 0.6 mg m^{-3} as measured in stations 1-4 would correspond to a $b_{bp}(470)$ of about 0.0012 m^{-1} if using the relationship in Fig. 4c and other relationships would give similar values, up to 0.002 m^{-1} (e.g., Huot et al., 2008. Biogeosciences, 5, 495–507, www.biogeosciences.net/5/495/2008). Such b_{bp} values translate into a C_{phyto} of about 20/25 mg m^{-3} through the G2015 equation. This is similar (a bit lower) than what is predicted from the S2009 method, both being lower than what is calculated from cell counts (rather averaging 40 mg m^{-3}). Adding points with such values in Fig. 8 would not change much the outcome.

Reviewer comment

In figure 3c we see that there is a curvilinear relationship between bbp and tchl when samples shallower than 150 m are included. Why is this?

In figure 2 we see a clear subsurface chlorophyll maximum in the oligotrophic region (where the bbp data are available). This chlorophyll maximum is primary a consequence of photoacclimation (i.e., not biomass variability). Thus, when bbp and tchl are compared, we get the curvilinear relationship shown in figure 3c, which can be interpreted as a strong indicator that chlorophyll is not a reliable index of C_{phyto} (i.e., in general, the photoacclimation contribution will increase in parallel with increasing chlorophyll concentration along the x axis). If we look at the surface only data in figure 3c (solid symbols), we see little relationship between tchl and bbp. The most straight forward explanation for this is that the dynamic range in C_{phyto} is very limited across stations 5-20, but there still is some variability in tchl

as a result of changing phytoplankton division rates and mixed layer light levels. If we now look at the data in figure 3d, we see essentially the same thing as in 3c, with one minor difference. For the cp data in figure 3d, we now have optical measurements for the mesotrophic stations 1-4, which I believe show up as the cluster of cp values > 0.10. Aside from this data cluster, we again see the curvilinear relationship between cp and tchl for samples shallower than 150 m (open symbols) that is due to photoacclimation impacts in the tchl data, and the constrained range of variability in the surface only samples (which can be interpreted exactly as above for the bbp : tchl data). While I don't have the data to evaluate this, my guess is that the station 1-4 data cluster in figure 3d falls apart from the other open symbol data because these mesotrophic stations actually had higher phytoplankton biomass and that their photoacclimation state was different than the 'below the mixed layer' data from stations 5-20. Since the authors report a strong relationship between cp and bbp, it would seem safe to assume that had bbp data been collected at stations 1-4 they too would have shown a good relationship Cphyto.

Authors response

Note to the editor: the reviewer actually refers here to Fig. 4, not Fig. 3.

About the curvilinear relationship between b_{bp} and Chl: we think that this is what is recurrently observed, with relationships of the form $b_{bp} = A \times [Chl]^B$. These relationships are generally plotted in a log-log space, however. Here we preferred to keep a linear scale for all four panels of Fig. 4. This non-linear behaviour can be indeed caused by photoacclimation, as the reviewer suggests. It can also be generated by a varying contribution of non-algal particles, which have different backscattering properties than living cells.

See our answer above for the point about the missing b_{bp} data for stations 1-4.

Reviewer comment

Given the above, my conclusion from the observations of the current study is that bbp, cp, and POC are all correlated with each other and correlated with Cphyto, while assessments based on tchl are inaccurate. This leaves the question of how to interpret the cell count data. I don't know the answer to this question, but I encourage the authors to rethink their overall interpretations.

Authors response

We agree that b_{bp} , c_p POC and C_{phyto} are correlated with each other, whereas Chl appears less directly linked to these particle-based estimates.

The C_{phyto} we derived using an established approach that converts cell counts to carbon biomass based on literature-reported carbon-per-cell factors provides a biologically grounded measure of C_{phyto} and allows comparison with particle-associated parameters such as b_{bp} , c_p , and POC. This C_{phyto} based on cell counts reflects changes in phytoplankton community composition and size, which likely explains its stronger correlation with particle-based properties.

Other issues:

Reviewer comment

(1) line 421: this conclusion about the PPC data standing out from the PSC data indicating a significant influence of non-algal particles is neither logical nor supported by observations.

Authors response

Sorry, we do not really understand this comment. For a given Chl concentration, a larger presence (proportion) of NAP can indeed lead to a larger b_{bp} . The split between the PSC-dominated and PPC-dominated points in Fig. 4c can also be due to different photo-acclimation states, although both subsets include surface data (the PPC-dominated subset is entirely for depths $< \sim 50$ m, yet the PSC-dominated subset also includes data for such depths at stations 1-6 and 18).

Therefore, we have rephrased as follows:

The PPC-dominated data however stands out when plotting TChl-a as a function of $b_{bp}(470)$ (Fig. 4c), indicating either a significant non-algal contribution to backscattering or different photoacclimation states or both.

Reviewer comment

(2) line 501: this idea that bbp is largely influenced by submicrometer particles has its foundations in Mie theory as applied to homogeneous spheres and is antiquated. It is well recognized now that particles significantly larger than a micron play an important role in bbp variability and that Mie predictions for homogeneous spheres are inadequate for characterizing backscattering properties of natural phytoplankton populations.

Authors response

Yes, some authors have indeed shown that particles somewhat larger than the “sub-micron” category may influence b_{bp} more significantly than previously thought (e.g., reference below). It remains, however, that sub-micron particles still play a

significant role in sizing b_{bp} even if not as predominant as predicted by the theory. It also remains that they do not play a strong role in determining the particulate beam attenuation coefficient, c_p .

We have rewritten as follows:

Particle backscattering is largely influenced by submicrometer particles (Ulloa et al., 1994), although a larger role of phytoplankton-sized particles has also been suggested (Dall'Olmo et al., 2009). The beam attenuation is rather sensitive to the size range ~ 0.5 to $20 \mu\text{m}$ (Boss et al., 2001).

Dall'Olmo, G., Westberry, T. K., Behrenfeld, M. J., Boss, E., and Slade, W. H.: Significant contribution of large particles to optical backscattering in the open ocean, *Biogeosciences*, 6, 947–967, <https://doi.org/10.5194/bg-6-947-2009>, 2009.

Reviewer comment

(3) lines 556-569: these suggestions regarding a role for NAP are simply speculation.

Authors response

We think that the reviewer refers to lines 566–569, where we mention that NAP has a smaller contribution to backscattering compared to PPC in the central part of the transect.

This statement is supported by published results, which we cite in lines 569–573 to substantiate our observation.

Reviewer comment

(4) lines 589-599: comments here about the role of non algal particles also seem weak.

Authors response

Sorry, we are a bit confused here.

The lines referred here do not refer much to the role of NAP.

Reviewer comment

(5) paragraph beginning on line 619: I did not find the discussion in this paragraph regarding phytoplankton physiology mechanistically sound, nor the statements regarding the relative contribution of phytoplankton to POC.

Authors response

This paragraph describes a limitation of the 1% quantile regression of POC:Chl-a due to changes in the photoprotective and photosynthetic pigment ratio impacting the contribution of phytoplankton to POC. We rewrote the section more succinctly and hopefully it clarifies the message.

Reviewer comment

(5) Section 4.4: The conclusions stated in this section regarding the utility of b_{bp} for assessing C_{phyto} are incorrect (as explained above). The proposed role of NAP variability suggested in the two Bellacicco papers is based on a flawed assessment where b_{bp} associated with NAP is assessed through relationships between b_{bp} and chlorophyll even in low chlorophyll waters where chlorophyll variability is predominantly physiological.

Authors response

We remind again that we do not claim that the b_{bp} method is inherently unreliable. Our statement refers specifically to its performance in the mostly oligotrophic conditions of our study region, where we found that C_{phyto} derived from b_{bp} did not compare well with the two other estimates and led to a narrow range of values.

We have rewritten the last paragraph to bring a more nuanced message.

To some extent, the oligotrophic domain represents an “end-member” in the C_{phyto} vs. b_{bp} relationship. When considering only this domain, it is not that surprising not to find a clear relationship.

This is similar to what happens to, e.g., IOPs vs. Chl relationships for open ocean Case I waters. When the full dynamic range observed in the oceans is considered, the relationship is clear and is the basis for interpretation of global satellite ocean colour observations. The relationship vanishes when “zooming” on a limited range of Chl variability.

Reviewer-2

Review of Antoine et al. ‘Potential of optical and ecological proxies to quantify phytoplankton carbon in oligotrophic waters.

Reviewer comment

This article explores the use of various approaches to estimate phytoplankton carbon across a range of waters in the Indian Ocean, with a focus on chlorophyll a, optical backscatter and flow cytometry as predictor variables. Such C_{phyto} estimates are of great value in marine biogeochemical studies, with important

applications to satellite-based measurements. The results presented in this paper show a range of relationships between C_{phyto} and the various input variables, with significant correlations observed for the full data set, and weaker relationships for surface (<25 m depth) data.

Overall, I think that this a valuable and interesting study, with important implications for the field. One significant limitation, however, is the lack of a 'true' (i.e. gold-standard) measurement of C_{phyto}. Without this validation, it is not possible to say which method provides the best approximation for C_{phyto}, as the authors themselves acknowledge. Nonetheless, I think the paper is still useful, as we can (with some modifications to the current text) get a sense of how the different proxies produce different C_{phyto} estimates. Other things I note, is the need for a bit more discussion on the role of light-acclimation in driving some of the observed variability, more discussion of the size biases in the cytometer data, and a more robust application of the pigment data to discuss the role of phytoplankton taxonomy.

Authors response

We are pleased that this reviewer found our work valuable and important for the field and also acknowledged that we have clearly stated the limitations caused by the absence of direct analytical measurements of phytoplankton carbon.

We have tried to improve our discussions to address the points raised here by the reviewer, whom we thank for a constructive and helpful review.

Specific comments are listed below.

Specific comments:

Reviewer comment or question: Line 13: include physiological status as a source of variability

Response: done

Reviewer comment or question: Line 16: 'both are yet each in'?? check grammar here.

Response: the confusion comes from a misplaced comma. The sentence has been rewritten as follows and should be clearer now.

It is accordingly still unclear which of Chl-a or b_{bp} is best suited to quantify C_{phyto} or whether they both are, yet each in specific trophic conditions, especially for low-productivity oligotrophic waters.

Reviewer comment or question: Line 36: 'are' missing before 'accordingly'.

Response: corrected.

Reviewer comment or question: Line 63: reference after 'change significantly'

Response: Two references have been added:

Serra-Pompei, C., Hickman, A., Britten, G. L., & Dutkiewicz, S. (2023). Assessing the potential of backscattering as a proxy for phytoplankton carbon biomass. *Global Biogeochemical Cycles*, 37(6), e2022GB007556.

Xu, Wenlong, et al. "Spatiotemporal variability of surface phytoplankton carbon and carbon-to-chlorophyll a ratio in the South China Sea based on satellite data." *Remote Sensing* 13.1 (2020): 30.

Reviewer comment or question: Line 66. I find the transition to this new paragraph a bit abrupt

Response: we have modified the starting sentence as follows:

Understanding these dynamics is particularly important in regions such as the Eastern Indian Ocean (EIO), which provides food, natural resources and numerous benefits to surrounding countries (Hermes et al., 2019). The EIO encompasses diverse hydrographic regimes that strongly influence phytoplankton productivity and physiology. In the northern EIO, the Bay of Bengal experiences monsoon-driven seasonal circulation changes during summer (southwest monsoon) and winter (northeast monsoon) (Schott and McCreary Jr, 2001), along with large freshwater inputs from the rivers and rainfall that create surface stratification and barrier layer (Vinayachandran, 2009).

Reviewer comment or question: Last line of introduction – do you relate the results to environmental variables also?

Response: No, we have not related the results to any variables. However, in another publication (Parida et al., 2025), we have linked some of the results with environmental variables in the relevant sections.

Reviewer comment or question: Line 93: replace 'going' with 'sent'

Response: done.

Reviewer comment or question: Lines 94/95. Given the significant latitudinal gradient sampled, did these sampling times represent a consistent part of the diel cycle (e.g. xx hours after sunrise or sunset). If not, is it necessary to consider this?

Response: yes, we stayed on the same meridian, and these times always corresponded to dawn and dusk.

Reviewer comment or question: Line 106: was the filtration for pigments conducted under low light?

Response: yes, it was. This is now said.

Reviewer comment or question: Line 111: I think the 'P' in HPLC stands for 'performance'

Response: Yes, indeed. Corrected.

Reviewer comment or question: Line 169: I think it's important to provide more information on the size cutoffs of the instrument (lower and upper). What part of the size spectrum is being missed? This is mentioned very briefly on line 240, but it would be good to see it here, and with an upper cutoff also.

Response: we have added further information at the end of the paragraph here pinpointed.

Reviewer comment or question: Line 200: I would have thought that the value of gamma differed significantly between the different phytoplankton assemblages, based on their size spectra. What was the relative error in the mean gamma value, averaged for all samples?

Response: our data set covers a limited range of variability of phytoplankton assemblages. Therefore, the impact of their variability is not strong enough to generate substantial changes in the spectral slope of the backscattering coefficient (especially when the role of phytoplankton in b_{bp} is rather low).

Reviewer comment or question: Line 208: What was the vertical resolution / sampling frequency of vertical b_{bp} measurements?

Response: The Hydrosat sampling frequency is 1 Hz, and the profiling speed was about 0.25 m s^{-1} . We then had a vertical resolution of about 0.25 m (added in the text).

Reviewer comment or question: Line 213: I don't quite understand this method, based on POC chl regressions. It seems to me that the derived relationship would produce an average C:Chl ration, including a lot of detrital matter. It makes more sense after looking at figure 5, which could be cited here.

Response: here we refer the reviewer to the original paper by Sathyendranath et al (2009) and indeed, Fig. 5 probably makes quite clear what the logic is. We want to add here that we did not consider any of the tested methods, including this one, as necessarily exempt from uncertainty or underlaid by "rock solid" assumptions. It is precisely the goal of the paper to illustrate these uncertainties.

Reviewer comment or question: First paragraph p. 10. At this point, I was wondering about the various error terms. These are addressed below, but it might be good to at least mention this here.

Response: we have added " , and their uncertainties assessed later (section 2.6)." at the end of the first paragraph of section 2.5.

Reviewer comment or question: Line 245: What is the theoretical basis underlying the relationship between the slope of the size distribution and absorption at 676? Does it relate to pigment packaging? Some more information would be helpful for non-specialists.

Response: The rationale is exposed in Roy et al (2013) (their Eq. 10). The connection between the two exists because specific absorption of chlorophyll-a is a function of the cell diameter and the spectral dependence of backscattering has been shown a function of the particle size distribution.

Reviewer comment or question: I gather that there were no size-fractionated chlorophyll data? Those would have been really helpful to validate some of these results.

Response: indeed, we did not perform size-fractionation.

Reviewer comment or question: Line 304: It's true that conditions were more oligotrophic, but it's worth mentioning the significant sub-surface chl maximum, which had chl values higher than observed to the south.

Response: we are unsure what to answer here. We indeed mention this in the text. Or maybe we do not understand the comment.

Reviewer comment or question: Fig. 2. The red stars are not labelled in the legend (only in the main text). I would add dots to show the actual sampling points used for the interpolations. For the bottom panel, rather than repeating chl, why not plot, for example, the PPC/PSP ratio, or POC:Tchl, which is mentioned in the text.

Response: The meaning of the red stars is now added. Note that the dots in panel (e) correspond to the sampling depths for each station so we do not repeat them in the other panels.

Reviewer comment or question: Last paragraph on p. 14. This is very descriptive material, which I think could be removed, as it's apparent from the figures.

Response: it is indeed somewhat descriptive, but this is the results section, so we decided to keep it as it is.

Reviewer comment or question: Fig. 3 could go in a supplement, I think.

Response: we prefer to keep it in the main text because from your comments and those of the other reviewer, it seems important to make clear that the data set covers a rather limited range of values, because mostly from oligotrophic waters.

Reviewer comment or question: Line 370: Cphyto is listed here as having a non-linear relationship, but that variable is not shown in the plots.

Response: yes, indeed, that is unclear. This sentence is not only for C_{phyto} . We have rewritten as follows:

When relationships among the various parameters here assessed were clearly not linear, we assessed them in a log-log space.

Reviewer comment or question: Fig. 4 bottom left panel: the white points look rather non-linearly distributed.

Response: yes, and that is why we used a power law to describe the relationship.

Reviewer comment or question: Table 2: I don't understand the last sentence in the table header. Maybe change 'panel' to 'figure' along the column headers.

Response: what we try to say is that the relationships derived in this work are specific to our data set so great caution should be used if they are applied to predict, e.g., C_{phyto} from IOPs measured in other environments. We have rewritten as:

"None of these relationships are supposed to be applied to data sets collected in environments markedly different from what we encountered during this IIOE voyage." Otherwise, we have indeed changed "panel" to "Figure".

Reviewer comment or question: Line 403 and elsewhere. Overall, it seems that the PPC/PSC ratios provide relatively little explanatory power. Maybe this could be mentioned somewhere and the data not explicitly included, unless they provide additional useful information. In Fig. 7e, for example, all the points fall together.

Response: we think that the fact that these high-PPC points either stand out of the general relationship or, on the contrary and as noted here, fall together with the high-PSC points, show that pigment composition has an impact. These PPC-dominated data correspond to the bulk of surface (<50 m) oligotrophic waters, where the range of variability of IOPs and Chl is small.

Reviewer comment or question: Line 456: It's worth noting that the POC - Tchl data were distributed over a much smaller range. If you took a similarly narrow range of other variables, relationships might also not be statistically significant.

Response: yes, we agree.

Reviewer comment or question: Line 466: worth emphasizing here the significant size bias of the cytometry data

Response: we think this point is addressed in the discussion and not really relevant here in the results section.

Reviewer comment or question: Fig. 6: One on hand, I can understand why the authors used log transformation for visual purposes, but the fact that this is not applied

consistently across the panels makes direct comparison rather difficult. I would use all linear or log scaling.

Response: See our response above and also Fig. S2.

Reviewer comment or question: Line 485. I can see how the black points sit above the line, but I'm not sure if these data 'stand out' considering the magnitude of the error bars.

Response: we have modified the sentence as follows:

"The cluster of PPC-dominated data (black dots) does not overlap the general $b_{bp}(470)$ -derived....."

Reviewer comment or question: Line 501: replace 'when' at the end, by 'while'

Response: the sentence has been further modified following a comment by the other reviewer.

Reviewer comment or question: Line 505: replace 'on' with 'in'

Response: done

Reviewer comment or question: Line 509: I can't really see the green line.

Response: we have made it thicker, so it is hopefully more visible now (same for the dashed blue line)

Reviewer comment or question: Fig. 7: I think it would be useful to plot the various Cphyto estimates against each other.

Response: that is actually what Fig. 8 does.

Reviewer comment or question: Line 520: the PPC-dominated points are distributed over a very narrow range, which could explain the lack of a clear relationship. Are they 'separated' beyond the error bars of measurements?

Response: yes they are.

Reviewer comment or question: Line 573: maybe 'refractive' instead of 'refrangent' (I had to look that up).

Response: we think refringent is the right word here, as a property of cells. Refringent meaning "generating refraction".

Reviewer comment or question: Line 617. In addition to PSC/PPC ratios, there are other approaches to pigment-based taxonomy, including Chemtax, for example. Would different results have been achieved with another method? At the least, I think it would be useful to provide more information on the taxonomic composition of PPC-dominated waters. This comes up again on line 646.

Response: we actually looked at various pigment assemblages and also to the pico-, nano- and micro-phytoplankton relative contributions using the HPLC data and did not find any clear pattern connected to C_{phyto} and IOPs variability. This is now said in that paragraph.

Reviewer comment or question: Line 624: insert reference after 'ratios'

Response:

Gibb, S.W., Barlow, R.G., Cummings, D.G., Rees, N.W., Trees, C.C., Holligan, P., Suggett, D., 2000. Surface phytoplankton pigment distributions in the Atlantic Ocean: an assessment of basin scale variability between 50°N and 50°S. Prog. Oceanogr. 45, 339–368.

Reviewer comment or question: Line 626: insert reference under 'conditions'

Response:

Araujo, Milton Luiz Vieira, et al. "Contrasting patterns of phytoplankton pigments and chemotaxonomic groups along 30 S in the subtropical South Atlantic Ocean." Deep Sea Research Part I: Oceanographic Research Papers 120 (2017): 112-121.

Reviewer comment or question: Line 643: is the higher bbp due to larger surface area / volume ratio?

Response: it might be. Although we do not really have elements to support this we have nevertheless added it in the text.

Reviewer comment or question: Line 690: There is no doubt that the cytometer provides a significantly size-biased view of the community. I wonder how robust the ffc factor is across the different assemblages.

Response: we have rewritten the sentence as follows, because we do not need to question "whether" the cytometry technique misses a "significant" part of the population but how much it misses:

"Therefore, the question arises as to how much of the phytoplankton population the cytometry technique misses"

END OF REVIEW