Anonymous Referee #2

Dear reviewer,

First, thank you foryour careful review of our manuscript and your remarks. They have been really helpful to improve the overall structure and content of the manuscript and we have addressed them into the new version. Now we have re-written the introduction and discussion section to improve their readibility. We hope that thanks to your suggestions we have managed to improve the manuscript, and that it suits now the standards of Ocean Science. Best regards

Best regards,

Xabier Davila

AR = Author's response
AC = Author's changes in the manuscript

General responses:

The manuscript describes mesoscale processes in the shelf of the Southern Bay of Biscay and tries to relate that physical environment with the occurrence and distribution of phytoplankton in the area. The approach presented is very interesting and the manuscript provides a detailed description of a snapshot of the circulation in the SE BoB in August.

I have to acknowledge that I am not an expert in ocean circulation, so although I found this part well described and thoughtful, I am not fully capable of reviewing the method- ological details of the description of the mesoscale ocean processes.

Since my expertise includes the phytoplankton community of the BoB, my main con- cerns are related to the fact that the aim of the manuscript is to relate the physical environment to the phytoplankton community structure, and I found this connection poorly supported by the data presented.

AR: We thank the reviewer for this valuable comment. We agree that we need to be less assertive when relating the physical environment to the phytoplankton community structure and rather focus on phytoplankton spectral groups distribution / dynamics based on the information that can actually by extracted by the data we have.

AC: We have changed the title of the paper and reviewed the discussion accordingly.

First, phytoplankton distribution is presented though accessory pigments fluorescence data, which is variable depending on the proportion of accessory pigments with respect to chlorophyll and depending on the proportion of chlorophyll to phytoplankton carbon. I think these fluorescence data do not represent phytoplankton distribution as straight-forward as the authors claim.

AR: The data presented correspond to an automated in situ approach of the contribution of different pigmentary groups to total clorophyll-a concentration, estimated by multispectral fluorometry (MacIntyre et al., 2010).

Also, not all phytoplankton groups are presented in the results, only "green" and "brown" algae, which leaves out all the cyanobacteria, very relevant in the phytoplankton community of the BoB in summer.

Regarding writing and composition, the manuscript is a bit difficult to follow, the physi-cal part is better explained (although there are some typos and acronyms not defined, listed below), but the biology part is very confusing, with many concepts not fully explained.

AR: The data presented as total and spectral group fluorescence are in fact Chl-a Equivalents units concentration (µg ChlaEq L-1) after manufacturer's calibration with microalgal cultures. Therefore, they are not technically raw fluorescence data (the units label was corrected in the MS).

We agree on the fact that relationships between fluorescence and chlorophyll-a estimations from one side, chlorophyll to C (biomass) as well as the accurate discrimination of the different phytoplankton groups, depend on phytoplankton community composition, physiology and light history of cells (Lawrenz et al., 2010; MacIntyre et al., 2010; Catherine et al., 2012; Escoffier et al. 2015; Garrido et al., 2019). Moreover, one of the caveats of this technique is that obtained fingerprints are not stable, but vary between species and physiological conditions. Nevertheless, the signal found is strong and correspond to what other studies has identified as the chlorophyll deep maximum with in vivo total chlorophyll a fluorescence.
We can reasonably hypothesize that during the short period sampled, the changes observed might have corresponded to changes in phytoplankton pigmentary composition as no important changes were recorded in meteorological conditions (which could have influenced water column irradiance and, consequently, physiological state of phytoplankton cells which were always measured during day time). Phytoplankton communities in surface waters might have been affected by hourly changes in irradiance and might have been submitted to Non Photochemical Quenching (NPQ) of the fluorescence signal. The comparison between some surface chlorophyll-a concentrations and some chl-a concentrations around the SCM measured on filters (data not shown) confirmed the difference encountered between surface waters and 30-40-50m-depth.

Besides, cyanobacteria were abundant in surface waters (continuous FCM recording, counts not shown) but not very important in terms of total red fluorescence (chl-a indication) what was confirmed by the very low amount of chlorophyll a attributed to this group (as well as to Cryptophytes) by Fluoroprobe "blue-green" and "red" signal (compared to that of "green" and "brown" algae). Therefore we decided not analyzing their variability as the majority of the total chl-a signal was attributed, , to "Green" and "Brown" algae, according to the Fluoroprobe and manufacturer algorithms (Beutler et al., 2002).

AC: The manuscript was improved to make it easier to follow and concepts are now better explained. Several of these statements are now explicitly included in the main text of the manuscript to clearly show what are the limitations and potential of the data used in this study.

Specific responses:

Introduction

25 That's an unclear sentence, it is not clear which is the subject (it?) of the first part.

AR: The authors agree that the sentece is unclear.

AC: This sentece was removed since part of the introduction was rewritten.

27 "this cross-self transport" does refer to the complex ocean dynamics mentioned before (26)?

AR: Yes.

AC: This sentence was also removed due to the restructuring of the introduction.

64 Some word is missing here: "different phytoplankton groups" or "different groups of phytoplankton".

AC: We changed the wording to "different phytoplankton groups".

74 MFSD not defined.

AC: This sentence was also removed due to the restructuring of the introduction.

Material and Methods

90 Is the FluoroProbe deployed together with the CTD casts?

AR: Not simultaneously but it was deployed at the same stations right before the CTD casts.

95 Typo: "Cryoyptophytes" should read Cryptophytes.

AC: Corrected.

138 I think there is a typo here: "enough resolution for resolving".

AC: Corrected.

149 Another typo, a parenthesis or a preposition is missing: "of the analysed field (Gamis et al. 2001)".

AC: Corrected.

150 The treatment of fluorescence data is not very well explained. Only the method to interpolate the values to a regular grid is explained. But regarding the FluoroProbe data themselves, if FluoroProbe provides Chla values (95) why are they not showed and it is instead fluorescence? Are the fluorescence values calibrated with filtered samples in any way? Even though chlorophyll is not the same as phytoplankton biomass (given the variability in the chlorophyll to carbon ratios), it is more interpretable and comparable among groups than fluorescence. Fluorescence is also variable depending on the content of accessory pigments which is also subjected to photoacclimation and hence variable with phytoplankton physiological state. That's for me the weakest point of the manuscript, that the fluorescence values presented hardly represent the actual biomass or abundance of the phytoplankton community.

AR: The FluoroProbe data characteristics and limitations is now more clearly explained and results are now presented in chl-a Equivalent values according to manufacturer's calibration. Some filtered samples were taken to measure chlorophyll-a concentration, mainly in surface waters and at one deep sample near the DCM. Even though the relationship was significant, we decided to use the manufacturer's calibration to express our results in terms of chl-a equivalents concentration. Presenting the values in chl-a also allow us to make comparable the results among groups. Besides, no significant meteorological changes occurred during the survey, therefore we assume that during the short-term study described in our manuscript not big physiological changes have occurred from one profile to another at the same depth.

We agree that the relation between the actual phyoplankton biomass and the total chl a fluorescence is not straightforward. However, many studies dealing with the DCM and physical constrains deal with total chlorophyll fluorescence as the method allowing to record changes at a fine scale. In our preliminary study, we used a multispectral fluorometer in order to have a first idea of the different pigmentary/spectral groups or signal that contributed the most to total chlorophyll-a fluorescence at

different depths. Unfortunately, we could not get a detailed information of the distribution of phytoplankton taxa and cell abundance. We are conscious of the need, for further studies, to make as much sampling as possible, with horizontal hydrological bottles (as Lunven et al., 2005) to be able to catch the thin layers of accumulation of the different phytoplankton taxa by different complementary methods as microscopy, pigment analysis and flow cytometry (as Latasa et al., 2017) .

AC: We provided a more detailed information: During the cruise, chlorophyll-a (chl-a) was estimated by a FluoroProbe (Bbe Moldakenke) multi-spectral fluorometer, which measures chl-a and accessory pigments using LEDs with different wavebands. Therefore, it is possible to distinguish between four algal pigmentary groups: "Blue algae" (e.g. Cyanobacteria), "Green algae" (e.g.Chrolorophytes, Chrysophytes), "Brown algae" (e.g. Diatoms, Dinoflagellates) and "mixed red group" (e.g. Cyanobacteria, Cryptophytes). It estimates chl-a equivalent concentrations for these four groups and total chl-a following the algorithms of (Beutler et al., 2002) as explained in (MacIntyre, 2010) and a manufacturer's calibration, and also provides an estimation of the concentration of chromophoric dissolved organic matter (CDOM or yellow substances).

160 This methodology is not very clear, "smaller subsets in relation to the fluorescence" do refer to the spectral groups retrieved by the FluoroProbe?

AR: This section referred to the filtering technique we applied in the old version of the manuscript.

AC: This sentence has been removed in the new version of the manuscript.

Results

182 Correct punctuation: "the distribution of the SST, as well as the position of the river plumes".

AR: Agree.

AC: Changed

Figure 5 (186) It seems the names of the eddies are duplicated in panels.

AR: This was fixed in the revised MS.

Figure 7 (226) Please, indicate which are the units for fluorescence, even if they are arbitrary units.

AR: The units are in fact Chlorophyll a equivalents (µg chla Eq L-1)

AC: The units were changed.

Figure 8 (230) Why values of total and groups of phytoplankton are not given in chlorophyll if the output of FluoroProbe is equivalent chlorophyll (95)? Maybe explaining the FluoroProbe technique with more detail would help with the interpretation of the data, or at least including some references about the technique.

AC: This was corrected in the revised version.

246 Figure 8 shows depth profile not surface fluorescence, maybe the text should read: "From satellite imagery and continuously recorded surface salinity and fluorescence data (Figure 4 and 7)".

AR: Figure 8 was a typo.

AC: Changed.

Discussion

325 I don't think this sentence is correct. Which varies depending on the position in the water column could be which physical driver affects most the occurrence or distribution of phytoplankton, but not the interplay between physics and phytoplankton in general.

AC: We agree on that, the sentence is modified.

333 The authors seem to insist throughout the manuscript on the role of salin- ity/ freshwater as one of the main drivers of the distribution of phytoplankton above the picnocline, which is more likely an effect of nutrient-availability (river discharge re- lated). I would suggest the authors to take care of these kind of sentences that relate so directly salinity and phyto distribution.

AR: We agree that the nutrient availability related to river discharge is the most likely explanation for the relation between phytoplankton and salinity above the pycnocline.

339-348 This paragraph seems methods to me, not results. Maybe could be useful to have this paragraph in the methods section where the filtering technique is introduced to help explain its relevance (160), which is not very clear (see below).

AR: The authors agree that this part of the methodology is confusing and therefore it was removed from the manuscript.

AC: This paragraph was removed from the manuscript since the filtering technique was substituted by an additional GAM which focuses on the DCM.

350 I don't quite understand the point of this filtering technique. If I understood correctly with each iteration only the larger values are selected, and regarding chlorophyll this eventually considers only the large values in the DCM. But, with larger values cor- relation coefficients are also larger, not necessary meaning a higher correlation among data, so I am not sure that correlation coefficients between iterations are comparable. Also, with each iteration sample size, range and probably also variability are smaller which influences the comparability of correlation coefficients among iterations. I would suggest the authors to clarify the relevance of this statistical analysis.

AR: The goal of this technique was to remove those areas where the phytoplankton concentration is low that add noise to the relations between chl-a and the environmental variables. Due to this low values of chl-a, the GAM that comprehends the whole section below the pycnocline eclipses the relations in the DCM, which are ultimately the most relevant ones.

AC: Now we have substituted this technique by an additional GAM which comprehends the DCM (> 1.5 chl-a eq µg.L-1). This subset still comprehends the 20% of the data below the pycnocline and the relations are significative. This highlights the difference between in modulating mechanisms for the whole section and the DCM.

353 "The strong negative correlation points suggest that in general brown algae are highly conditioned by the salinity range". Conditioned by the salinity range in which sense?

AR: The new GAM for the DCM was performed

AC: This new GAM shows that vorticity is the factor that explains most of the deviance for Total chl-a and Brown algae chl-a, whereas salinity explains most of the deviance for Green algae chl-a and the B:G ratio.

372 Data presented are not of phytoplankton concentration.

AR: This was corrected in the revised version : estimation of chl-a due to two spectral groups.

403 The variable fluorescence to chlorophyll ratios could amplify or decrease the signal depending on if the fluorescence comes from accessory photosynthetic pigments (that increase relative to chlorophyll with depth) or from accessory photoprotective pigments (that decrease relative to chlorophyll with depth).

AR: In the present study, we assume that the sharp deep equivalent chlorophyll maximum addressed by fluorescence is of high magnitude and that even though affected also by physiological changes, it may reflect a peak in chlorophyll-a concentration and, most probably, a peak in phytoplankton

biomass as one can assume that environmental conditions are not very different from those one meters above or below even though we did not measure them.

409 "The latter (dinoflagellates) can easily regulate their optimum depth by altering their swimming behaviour." Not sure about that, dinoflagellates can swim but not at the spatial scale necessary to change their position in the water column, working against turbulence, mixing and so on. If I am wrong, the authors should include some reference for this statement.

AR: Some studies address that issue, that dinoflagellates might be more eager to change that much their position but at low temporal rates (see Wirtz & Smith, 2020). However, we agree with the reviewer that these would not explain big amplitude changes in the water column and definitely not working against turbulence/mixing. That's why even this group would be submitted to hydrological and hydrodynamic forcing, as the other phytoplankton groups.

AC: The sentence was removed from the manuscript due to the restructuring of the section.

427 Possible references for fluorescence to chlorophyll ratios and for fluorescence fingerprint variable within groups and within populations: Estrada, Marrasé and Salat. In vivo fluorescence/chlorophyll a ratio as an ecological indicator in oceanography. Sci. Mar. (1996) 60(1) : 317-325. Kruskopf and Flynn. Chlorophyll content and fluorescence responses cannot be used to gauge reliably phytoplankton biomass, nutrient status or growth rate. New Phytologist (2006) 169: 525–536.

AR: We thank the reviewer for these references. Some of them were added to the revised version. Indeed, as no biomass estimations were made, we cannot be sure that there was a deep phytoplankton biomass maximum. Nevertheless, the signal found is very strong and correspond to what other studies has identified as the chlorophyll deep maximum (from total in vivo chl-a fluorescence measurements). However, we acknowledge that pigmentary supposed changes recorded could be due to a strict change in phytoplankton composition or to physiological acclimation, nor to changes in biomass.

429 "In any case, vorticity creates a dynamical niche that plays a major role shaping the phytoplankton community". I find this is a too ambitious sentence, the "shape" of the phytoplankton community is not fully addressed in the manuscript and hence the major role of these vorticity-created niches has not been really evaluated.

AR: We agree on this observation. Our study was a first attempt on understanding how physical forcing played a role in chlorophyll a total distribution and by spectral groups, as a proxy of pigmentary groups composition).

AC: The link with vorticity is now explained as it follows: Vorticity is the factor that explains most of the deviance in Total chl-a and Brown algae chl-a concentrations. The more negative (positive) the vorticity, the more anticyclonic (cyclonic) is the circulation and the more positive (negative) is the effect on Brown algae chl-a concentrations. Due to Ekman transport, anticyclones have a small component of the velocity that is directed to the core that is able to gather phytoplankton at its core (Mahadevan et al., 2008).

435 This last paragraph is a mix of many concepts, phytoplankton functional types, biogeochemical models, harmful algae, fisheries. . . I would suggest to reorganize it and focus more clearly on the aims and findings of the manuscript.

AR: We agree that too many concepts were included.

AC: In the revised version, we point to the specifics aims and findings of the study: We believe that the observed submesoscale processes during the Etoile cruise would have perturbed an already existing horizontal layer of DCM, not necessarily enhancing primary production (not measured during our study) by themselves, but rather isolating, advecting and gathering the phytoplankton in the region of anticyclonic circulation.

Conclusions

447 ". . . joint analysis of remote and operational together with discrete data. . ." is confusing. Maybe repeat data after remote and operational.

AC: Done