Dear Editor,

We are grateful for your interest in our study, and for accepting our manuscript in revision for a second time. We are also grateful to the two anonymous reviewers for their helpful and constructive comments.

We thank the referee 1 who has decided to accept “as it” our manuscript after the first revision. We also thank referee 2 for his detailed and constructive suggestions that helped us to further improve the presentation of our work. In addition to reworking the scientific aspect we also hired a native English-speaking proofreader to accurately verify the grammar of our text. We hope that our actual revised version, that precisely takes into account all his comments, will be accepted for publication.

Below you find the point-by-point reply to the referee 2 comments.

Authors's reply to Anonymous Referee #2 comments on bg-2021-38 Tzortzis et al.

Dear referee,

Thank you very much for the attention that you have given to our work for a second time, as well as for your English corrections. We have reworked the manuscript, taking into account your suggestions. We have also hired a native English-speaking proofreader to accurately verify our manuscript. As in our previous reply, your comments have been copied here in blue, after the ⇒ symbol. You can find the revised manuscript and also the marked-up manuscript version showing the changes made on the Biogeosciences website. Note that on the marked-up version the modifications concerning your comments are in red and the English corrections suggested by the English-speaking appear in blue.

General comments:

⇒ This is my second time reviewing the manuscript of Tzortiz et al. Overall, the datasets and plots are convincing and the results constitute a novel and interesting contribution to the study of phytoplankton community dynamics in the Mediterranean Sea. I can see that attempts have been made to address my previous comments and indeed, some parts of the manuscript have been improved. However, unfortunately the manuscript appears hastily written at times, and contains sections that are either confusing or grammatically incorrect. In addition, I still feel the authors need to spend some more time on their discussion to really smooth out their interesting story. For examples, the results are extremely detailed, yet the discussion seems speculative and broad at times. Overall, the manuscript has potential, but in my opinion, is currently not at an adequate level for publication in Biogeosciences. I recommend the manuscript for major revisions.

We acknowledge that we were not clear enough in some parts of the manuscript, in particular in sections 4.2 and 4.3 of our discussion. In this revised version of the manuscript, we have improved these sections, taking into account your comments. Furthermore, we have given particular attention to improve English grammar, following your suggestions.

However, we kindly disagree with you on the fact that our discussion is speculative. Indeed, in our discussion we have highlighted important aspects concerning the physical and biological coupling at fine scale, providing an in situ confirmation to the theories advanced by previous numerical simulations studies. And although some points remain uncertain at the end of this very study due to some missing variables (i.e., nutrients), we will improve that in the future thanks to the new campaigns already planned with an optimal sampling strategy.

Specific comments:

Abstract:

⇒ Line 2. I would re-phrase "samplings" to something like "in situ – based studies". It sounds a bit strange grammatically otherwise. I would also mention that you are referring to fine scale ocean dynamics when mentioning the lack of in situ data.

Following your suggestions, we have modified the abstract (see line 3).

⇒ Line 8. Please move "at high spatial resolution" to come after "both physical and biogeochemical variables".

Following your suggestions, we have modified the abstract (see lines 8).

⇒ Line 9. Particular attention was "given to"?

Following your suggestions, we have modified the abstract (see line 9).

⇒ Line 13. I would remove "With respect to previous studies".

Following your suggestions, we have removed that (see line 13).

Introduction:

⇒ Line 20. Phytoplankton "are"? Please also add "the" before "ocean", and also change ocean to oceans.

Following your suggestions, we have corrected that (see line 20).

⇒ Line 21. "It is"? I presume you mean phytoplankton. Please rephrase to "they are" and check the grammar for this elsewhere (e.g., in line 22).

Following your suggestions, we have corrected that (see lines 21-22).

⇒ Line 23. Can probably remove Line 23 (e.g., from "Ptacnik...)". Not sure if it really adds anything extra to your first paragraph.

Following your suggestions, we have removed this sentence.

⇒ Line 26. Change "but also", to "and also".

Following your suggestions, we have modified that (see line 25).

⇒ Line 57 – 58. Please rephrase this. It sounds a little bit contradictory as you state that the effect of fine scale is non predictable (which in some aspect is what you're trying to do in

this manuscript?)...

We have replaced “non predictable” by “more elusive” (Merriam-Webster dictionary: meaning “hard to comprehend or define”), (see lines 59-60).

⇒ Line 75. “have allowed researchers/the authors to capture”?

Following your suggestions, we have corrected these lines (see line 77).

⇒ Line 82. “dedicated to providing”? Would also make “surface current” plural.

Following your suggestions, we have corrected these lines (see line 83).

Results:

⇒ Lines 205-213. You state the existence of a fine-scale structure (e.g., line 211) and then state that the FLSE/current direction are likely influenced by the presence of a fine scale structure. I would rephrase as this sounds a little bit contradictory.

We have removed this line. Indeed, thanks to your comment, we have realized that this sentence was not necessary for the description of our results and that it anticipates our discussion.

⇒ Line 248. Please correct the grammar in this sentence (e.g., “this can be explained because...”). Also, I’m not sure what you mean by “were realized”. Please check carefully your sentences throughout the MS.

Following your suggestions, we have modified this part (see lines 256-257).

⇒ Line 252 – 260. Is it possible to just re-arrange this paragraph slightly. I think it would be good to start the paragraph by stating that the two different surface water masses can be clearly seen in the glider transects – your most important result from the plot I suppose? Currently, you focus on distinguishing between surface and intermediate waters.

We are a bit confused as in this paragraph, we are not referring to the glider transects but we focus on the vertical sections of the Seasoar (i.e., Fig. 5 in the paper). However, you are right concerning the structure of this paragraph. Thanks to your suggestions, we have modified the text in the revised manuscript, highlighting our most important result, which is indeed that the two different surface water masses can be clearly identified on these vertical sections. Furthermore, in order to clarify this part, we have moved it above the paragraph concerning the description of the water masses with the Θ-S_A diagrams of the glider (see lines 242-252).

⇒ Some of the figures (e.g., figure 5) are a little difficult to read, particularly in terms of the size of axis labels and the tick markers. If possible, I would increase their size for the reader.

We have increased the size of axis labels and tick markers on figure 5 (see Fig. 5 in the revised manuscript), and also on figure A4 (see Fig. A4 in the revised manuscript).

⇒ Section 3.3. This is a nice analysis, and the cytometry results are clear. If possible, please try and improve the grammar and smoothness of how the paragraphs are written, as there are several instances where it is difficult to follow (e.g., "the distribution of this latter", "unambiguously put in evidence", "thanks to their light scatter").

We have corrected that (see lines 262-264, 267, 285).

Discussion:

⇒ Lines 368-374. I can see a deepening of the DCM at around 38 degrees 30', but not sure if you can state that this continues (at least clearly) north of that latitude. The actual maximum Chl-a value (0.8-0.9 µg/l) appears to remain at a fairly constant depth along the transect. That being said, I can see the lower "boundary" of the DCM is generally deeper. I would be careful with the wording here.

In order to follow the evolution of the deepening of the DCM, we have selected the maximum of [Chla] along the outward and the return route of the SeaExplorer glider (see figure below). Indeed, the maximum [Chla] value (0.8-1 µg L^{-1}) is constant in depth (it varies between 50 and 80 m) along the outward route of the glider (Fig. a), except at the beginning of the transect where the maximum is located on the surface. On the return route (Fig. b), some of the values are located on the surface, but the majority of the maximum [Chla] value varies between 50 and 80 m as on the outward route. As you pointed out, this is the lower "boundary" of the layer containing the DCM which is deeper. Thanks to your remark, we have modified the text in the revised manuscript (see lines 370-373).

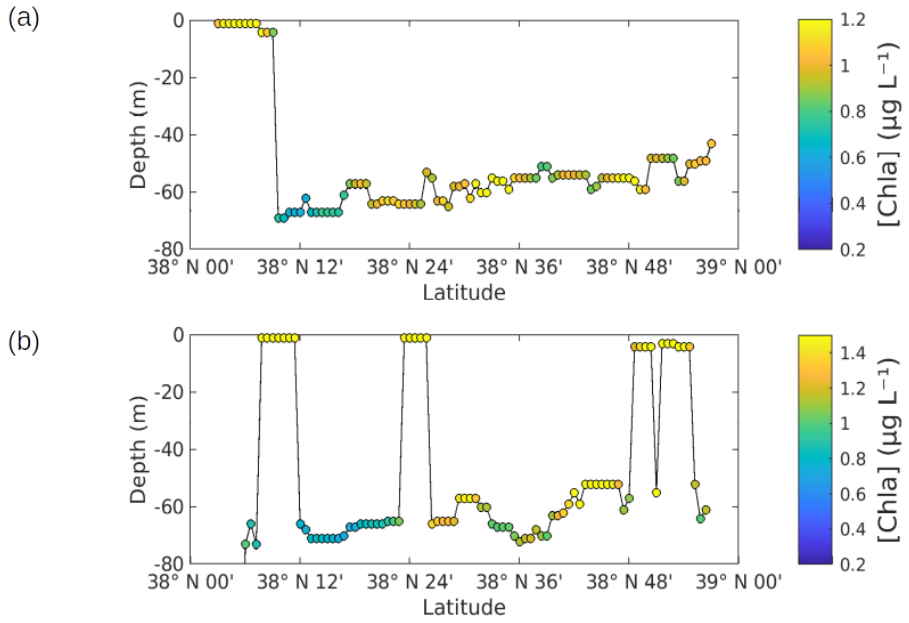

Figure: Vertical profiles of the maximum of [Chla], measured by the SeaExplorer glider, along the outward route (a): 6 May 2018 00:00 - 9 May 2018 21:00 UTC, and along the return route (b): 10 May 2018 00:00 - 13 May 2018 21:00 UTC.

The DCM is clearly identified on the vertical sections of the glider, that is why, we think the figure above is not necessary for the paper, and we have not added this figure in the revised manuscript.

⇒ Line 385. I do not see any correlation analysis presented in Figure A5?

Indeed, this figure represents Box-and-whisker plots and thus does not show any correlation analyses. We apologize for this mistake. However, as mentioned in figure A5 caption, t-test have been performed to compare the mean values of Chla, Peak B and Peak T in "older AW" and in "younger AW". Mean values were significantly higher in "older AW" than in "younger AW" (t-test, $p < 0.0001$). Correlation analyses have been made however on glider data (the two transects from 5 to 200 m depth) to determine correlations between [Chla], [O2], tyrosine-, and tryptophan-like fluorophores. We found a very highly significant linear positive correlation between [Chla] and tyrosine-, and between [O2] and tryptophan-like fluorophores ($r = 0.88$ and 0.84, $n \sim 32595$, $p < 0.0001$).

Therefore, this sentence has been modified. Please see our answer below since the whole paragraph has been changed (see lines 376-396 in the revised manuscript).

⇒ Section 4.2 Although I appreciate the authors considered my previous comment regarding further discussion of this analysis (it was generally skipped over in the last version's discussion), this section is a little bit confusing, especially towards the end. For example, in line 391, the authors state that the high contents of tryptophan and tyrosine must be correlated

with microphytoplankton. In the next sentence, it states that microphytoplankton might produce these fluorophores. General, loose connections are made and further justification of your statements or a re-wording of the text is needed. Overall, I'm not sure of the strength of the author's discussion here. In addition, you are now discussing the results you have already presented, but rather presenting new results/figures not included in the main manuscript. I don't want to discredit the author's hard work in this revision, but some improvement is still needed in the flow/organisation of the discussion section.

We agree with you that this paragraph deserves clarification and modifications. Also, we propose **to replace the part :**

"The vertical sections of tyrosine- and tryptophan-like fluorophores (respectively peak B and peak T) fluorescence intensities revealed distribution patterns very close to that of [Chla] for tyrosine-, and very close to that of [O_2] for tryptophan-like fluorophore (data not shown). These results highlight the strong coupling between hydrology, phytoplankton activity and DOM concentration in this area. Indeed, tryptophan- and tyrosine-like fluorophores are recognized to have an autochthonous origin (Coble et al., 2014), being produced through the activity of autotrophic and heterotrophic plankton organisms, in particular phytoplankton and heterotrophic bacteria (Stedmon and Cory, 2014), and are known to be indicators of bioavailable/labile DOM (C and N) (Hudson et al., 2008; Fellman et al., 2009). Even though phytoplankton activity is considered a source of tryptophan- and tyrosine-like fluorophores (Determann et al., 1998; Stedmon and Markager, 2005; Romera-Castillo et al., 2010), bacterial degradation appears to be a source, but also a sink for these fluorophores, depending on the availability in nutrients (Cammack et al., 2004; Nieto-Cid et al., 2006; Biers et al., 2007). These similarities in the profiles of [Chla] and [O_2] were confirmed by correlation analyses (Fig. A5), which pointed out a very highly significant linear positive correlation between [Chla] and tyrosine-, and between [O_2] and tryptophan-like fluorophores when considering all glider data for the two transects from 5 to 200 m depth ($r = 0.88$ and 0.84, $n \sim 32595$, $p < 0.0001$). The fact that tyrosine-like fluorophore was rather associated with [Chla] and tryptophan-like with [O_2] reveal that these two fluorophores were probably not issued from the same phytoplankton groups. Moreover, it seems that tryptophan would be more susceptible to be released by heterotrophic bacteria (in addition to be released by phytoplankton) than would be tyrosine-like material (Hudson et al., 2008; Tedetti et al., 2012; Stedmon and Cory, 2014). The high contents in tryptophan- and tyrosine-like fluorophores found in the northern part of the transect ("older" AW) must be correlated with the abundances of Microphytoplankton at this place. Indeed, it has been recently shown that various groups of microphytoplankton might produce tryptophan- and tyrosine-like fluorophores (Romera-Castillo et al., 2010; Fukuzaki et al., 2014; Retelletti Brogi et al., 2020)."

By the part:

The vertical sections of tyrosine- and tryptophan-like fluorophores (peak B and peak T respectively) from the glider (not shown) reveal distribution patterns very close to that of [Chla] for tyrosine-, and very close to that of [O_2] for tryptophan-like fluorophore. These similarities in the profiles of [Chla] and [O_2] were confirmed by correlation analyses (not

shown), which indicate a very highly significant linear positive correlation between [Chla] and tyrosine-, and between [O_2] and tryptophan-like fluorophores when considering all glider data for the two transects from 5 to 200 m depth ($r = 0.88$ and 0.84, $n \sim 32595$, $p < 0.0001$). Tryptophan- and tyrosine-like fluorophores are recognized as having an autochthonous origin (Coble et al., 2014), as being produced through the activity of autotrophic and heterotrophic plankton organisms, in particular phytoplankton and heterotrophic bacteria (Stedmon and Cory, 2014), and as being indicators of bioavailable/labile DOM (C and N) (Hudson et al., 2008; Fellman et al., 2009). Even though phytoplankton activity is considered a source of tryptophan- and tyrosine-like fluorophores (Determann et al., 1998; Stedmon and Markager, 2005; Romera-Castillo et al., 2010), bacterial degradation appears to be not only a source, but also a sink for these fluorophores, depending on nutrient availability (Cammack et al., 2004; Nieto-Cid et al., 2006; Biers et al., 2007). The fact that tyrosine-like fluorophore was fairly associated with [Chla] and tryptophan-like with [O_2] reveals that these two fluorophores were probably not produced by the same phytoplankton groups. Moreover, it seems that tryptophan is more susceptible to release by heterotrophic bacteria (in addition to being released by phytoplankton) than is tyrosine-like material (Hudson et al., 2008; Tedetti et al., 2012; Stedmon and Cory, 2014). Figure A5 shows the comparison of distribution of [Chla], and fluorescence intensities of tyrosine- and tryptophan-like fluorophores between the "younger and older AW". It appears that the content of Chla, tyrosine- and tryptophan-like fluorophores was higher in "older AW" than in "younger AW" (the mean values of Chla, Peak B and Peak T in "older AW" being significantly higher than those in "younger AW" ; t-test, $p < 0.0001$). These results are in line with the distribution of microphytoplankton, which exhibited higher abundances in the northern part of the transect ("older AW"), thus highlighting the strong coupling between hydrology, phytoplankton activity and DOM concentration in this area.

See lines 376-396 in the revised manuscript.

⇒ Section 4.3. Some work is needed here. The last paragraph particularly seems hastily written, is grammatically incorrect and diverges from other parts of the manuscript that are generally well-written. E.g., Lines 430 – 436. It is currently not at publication level.

In your general comments, you have highlighted that our discussion seems speculative. As specified in the beginning of this document, we kindly disagree with you on this point. However, we acknowledge that we were not clear enough on this section. Thanks to your comments, we have rearranged this section in order to clarify our development.

We have also given particular attention to improving English grammar.