Authors's reply to Anonymous Referee #1 comments on bg-2021-38 Tzortzis et al.

Dear referee,

Thank you very much for your constructive comments and suggestions, as well as your English corrections.

General comments

You have highlighted that the major problem in the manuscript is that it contains superfluous information, in particular in the Results and in the Discussion. We will modify these parts taking into account your suggestions. The second referee has also sent his comments. Following the Biogeosciences guidelines, we have first to provide you a feedback about the main points you raised, and then, only after the Editor's green light, we will modify the manuscript. In order to follow our reply, your comments have been copied here after the '==>' symbol.

Specific comments

Abstract and Introduction

Thank you for your English corrections, as mentioned above, we will rework the manuscript after the answer of Biogeosciences.

Materiels and Methods

==> "If I am not mistaken, you selected the two sampling trajectories based on two regions of Chl-a concentration using the satellite-based SPASSO tool. Based on Figure 1, I can see a region of high Chl-a corresponding to the WE transect, but have trouble distinguishing the second region of unique surface Chl-a that justifies the position of the NS transect. Perhaps it is the colour scale/colorbar limits. Are the regions also selected based on SST and currents? In any case, I would rephrase or try and be more specific of why these two sampling transects were selected, and which areas you are referring to. I believe the colour scale can be improved to highlight this."

SPASSO was used to follow both the temporal and spatial variability of the horizontal finescale features of interest. SPASSO combines satellite-derived currents, SST and [Chla], to provide maps of dynamical and biogeochemical structures in both Near Real Time (NRT) and Delayed Time (DT). For simplicity, we have chosen to show only a map of [Chla] in Fig. 1. During the cruise, the analysis of maps provided by SPASSO suggested the presence of two different regions characterized by their different surface [Chla]. The complete archive of figures is available at https://spasso.mio.osupytheas.fr/PREBIOSWOT/Figures_web/. A new figure is proposed below with modified color ranges in the hope that the gradients of [Chla] are better put in evidence.



(a) Route of the RV Beautemps-Beaupré during PROTEVSMED-SWOT (pink line). The blue box corresponds to the area sampled with Lagrangian strategy. (b) Map of satellite-derived [Chla] provided by CLS for 3 May 2018, selected in the Lagrangian area and superimposed on the route of the ship (black dotted line). The orange and purple lines delimit the two areas called "hippodromes": West-East (orange) and North-South (purple). The red line represents the route of the SeaExplorer glider.

The high [Chla] region of interest, i.e., with a concentration greater than 0.3 μ g L⁻¹, is located between 3° E and 4° E 30', with its southern latitude varying from 38° N 20' for longitudes between 4° E and 4° E 30' to 38° N 40' for longitudes around 3° E. The Lagrangian strategy consisted in sampling longitudinally and latitudinally the high and low regions of [Chla]. The dedicated route of the ship across these two regions is represented in purple and in orange on Fig. 1. A special attention was paid to adapt the temporal sampling in these different water masses to the biological time scales, i.e. trying to catch the diurnal cycle. Because of the shape of the ship track, in the following we refer to these areas as "West-East (WE) hippodrome" (in orange on Fig. 1) performed from 8 May 15:30 to 10 May 17:30 UTC, and "North-South (NS) hippodrome" (in purple on Fig. 1) performed between 11 May 02:00 and 13 May 08:30 UTC.

==> "I am not really familiar with FSLEs or Lagrangian techniques. Thus, out of curiosity, is the FSLE a commonly used index for detecting fronts/fine scale features? Can you provide citations supporting this?"

The first study that showed the interest of using FSLE-derived fronts for biogeochemical studies was probably Lehahn et al., 2007 (see in particular their Fig. 8 and 9). Before, Abraham and Bowen (2002) have been the first to apply the Lyapunov exponent technique (although finite-time, not finite-size) to the ocean, in turn borrowing some ideas from dynamical system theory (see in particular Boffetta et al., 2001). For campaign studies, the FSLE analysis permits to identify biogeochemical regions of potential interest. This strategy has already been tested, either in post-cruise or real-time analysis, during many campaigns such as LOHAFEX (Smetacek et al., 2012), Latex10 2010 (Petrenko 2010), KEOPS2 (d'Ovidio et al., 2015), STRASSE 2012, OUTPACE 2015 (Rousselet et al., 2018; de Verneil et al., 2019), OSCAHR 2015 (Marrec et al., 2018; Rousselet et al., 2019), PEACETIME 2017, SARGASSES 2017, FUMSECK 2019 (Barrillon 2019; Comby et al., 2021), TONGA 2019 (Benavides et al., 2021) and SWINGS 2021 to identify structures of interest. A review on the FSLE and other satellite-based Lagrangian techniques can be found in Lehahn et al., 2018).

==> "Do maps of altimetry/SST also show the existence of the front between the two water masses?"

In our work altimetry is the input data for the FLSE (see above). At 38° N 20', the altimetryderived surface current directions change drastically along the NS transect, suggesting a front. However, this is not clearly the case for the WE transect. We don't think altimetric maps are essential for the paper because the front is clearly visible with the VM-ADCP current and the FSLE (see figures above, or eventually in the future supplementary material). Maps of SST can provide another view of fine-scale dynamics. However, the front is not clearly visible on the map of SST. Indeed, gradients of temperature are not enough contrasted in spring, in the Mediterranean Sea. It is easier to locate the front with the map of [Chla] (cf Fig. 1) than the SST, that is why we think these maps aren't necessary for the paper.



Horizontal velocity measured by VMADCP at 25 m, along the WE (a) and the NS (b) transects, superimposed on altimetry-derived surface velocity provided by AVISO. Temperature measured by TSG along the WE (c) and the NS (d) transects, and superimposed on SST. The dates of AVISO and SST correspond to the dates of each transect, i.e., 9 and 11 May.

==> "Is Figure 2 absolutely necessary to include in your results? It relates mainly to your methodology and I suppose isn't overly important for the story you are trying to tell. I would consider moving this to supplementary material."

Following your suggestion, we will move figure 2 in supplementary material.

==> "I think it would be helpful to modify your figure 1 to show broader study region/familiar landmarks, so readers not familiar with the Mediterranean Sea can get more of an idea of the region you are working in."

Cf figure above.

<u>Results</u>

==> "It would help to try and highlight the specific zonal feature being discussed in Figure 3. I can see several features based on the FSLE map, corresponding to the latitude 38° N 20'."

In the paper, figure 3 shows several features of FSLE. On the NS transect, two FSLE features cut the transect at around 38°N 20' exactly where the horizontal current directions change drastically. The orientation of the WE transect makes it harder to distinguish a clear separation of the current direction, due to its alignment with the fine scale structure. However, a FSLE feature cuts this transect just above 38°N 20' and, at this point, the current begins to change and turns to the North-East. We will clarify this in the revised manuscript.

==> "Out of curiosity, do the other transects (not presented) show the same results?"



We obtained similar results for the other transects (see figure below).

Horizontal velocities measured by VMADCP, along transects of the WE hippodrome (a), (b), (c) and the NS hippodrome (d), (e), (f), (g), (h), (i).

(a) 8 May 12:50 - 9 May 00:30
(b) 9 May 09:00 - 9 May 15:30
(c) 9 May 16:50 - 9 May 23:45
(d) 11 May 02:00 - 11 May 08:40
(e) 11 May 10:00 - 11 May 16:45
(f) 11 May 17:55 - 12 May 00:50
(g) 12 May 01:50 - 12 May 08:20
(h) 12 May 09:30 - 12 May 16:40
(i) 12 May 17:30 - 13 May 00:20

The lines in bold correspond to the WE and the NS transects presented in the paper. In our study, we have chosen to select the transect (c) for the WE hippodrome, because we deplore a lack of temperature and salinity data for the other transects of the WE hippodrome, due to technical problems with the Seasoar.

==> "Line 218. I would help the reader and refer to your figures here (Figs. 5b and d). I suppose the triangles indicate the position of this?"

Triangles in Fig. 5b and 5d indicate the geographical positions of the best separation between the two types of AW, as also described in Table 1.

==> "Line 225 onwards. This is your results section and thus I would avoid trying to discuss your observations using citations. Perhaps this information can be moved to the discussion."

==> "Lines 237-239. Again, this seems more like discussion material. Furthermore, although it is nice that you have shown similar results in temperature and salinity using an independent glider dataset, is the addition of a figure necessary here? You can probably briefly mention that the glider dataset showed similar results. I only mention this as the manuscript text is relatively short, and yet you have 16 figures. I would think about condensing your analysis slightly and think about where figures may be more appropriate as supplementary material."

==> "Following my previous comment, Figures 7 and 8 are not described in much detail. For example, Lines 241 – 244 are fairly broad, considering you are talking about three separate transects, for each hippodrome in Figures 7 and 8. I would try and be more clear and descriptive with your results here. Indeed, the data show a clear, interesting separation between the two different water masses (although note that this is less apparent in your density plots...)."

We will rephrase this part following your suggestions. Some aspects could be included in the Discussion section. We will also combine figures (see below) and maybe move some glider figures in supplementary material.



Vertical sections of conservative temperature Θ (a, d), absolute salinity S_A (b, e), and density ρ (c, f), sampled by the Seasoar along the WE (a, b, c) and the NS (d, e, f) transect. The Seasoar trajectory is represented by the black lines. Triangle indicates the localisation of the front area between the two types of AW represented in light and dark blue (see Fig. 5b and 5c in the manuscript).



Vertical profiles of [Chla] (a, c) and dissolved oxygen concentration (b, d), measured by the SeaExplorer glider, along the outward route: 6 May 2018 00:00–9 May 2018 21:00 UTC (a, b) and the return route: 10 May 2018 00:00–13 May 2018 21:00 UTC (c, d). The SeaExplorer glider trajectory is represented by the black lines. The data have been selected between the surface and 250 m for a better visualization of the surface layer.

==> "Line 247. For your DO and Chl-a plots, please re-clarify what hippodrome you are referring to (the NS one)."

The vertical sections of [Chla] and O_2 have been obtained with the SeaExplorer glider. The glider has performed an outward and a return route, parallel to the NS hippodrome (cf Fig. 1).

==> "Lines 247 – 249. What does "richness of structures" mean? Please be more descriptive with your results, or otherwise, remove superfluous material."

==> "Line 251. Chla is higher where exactly? Please expand and provide detail to your analysis."

==> "Lines 251 – 254. What plots are you referring to here? Also avoid general explanations in your results, especially without providing evidence or context e.g. "probably associate with vertical dynamics of the front". Provide more details (in the discussion) or remove. Please go through the whole manuscript and avoid such general statements."

As mentioned above, we will rework and clarify these parts of the manuscript after the answer of Biogeosciences.

==> "Line 255 onwards. I am struggling to understand the connection you are trying to make between Chl-a/DO and your "peak T/peak B". In the methodology, it is not fully clear to me what the motivation was for measuring these parameters. Please clarify. Furthermore, you do not really discuss these parameters in your discussion. Please consider what information is directly relevant to your analysis and justify the inclusion of each of your figures with corresponding text."

Tryptophan- and tyrosine-like FDOM fluorophores (peaks T and B, respectively) are recognized to have an autochthonous origin in the marine environment, being produced through the activity of autotrophic and heterotrophic plankton organisms, in particular phytoplankton and heterotrophic bacteria (Stemond 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; Romero-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).

In the present work, higher contents in tryptophan- and tyrosine-like fluorophores were found in the northern part of the transect ("older" AW) relative to the southern part ("young" AW). The same distribution pattern was observed for total Chla and O2 concentrations, as well as microphytoplankton abundance. These results highlight the strong coupling between hydrology, phytoplankton activity and DOM concentration in this area. In addition, it has been recently shown that various groups of microphytoplankton might produce tryptophanand tyrosine-like fluorophores (Romero-Castillo et al., 2010; Fukuzaki et al., 2014; Retelletti Brogi et al., 2020), which is in agreement with our observations. The fact that tyrosine-like fluorophore was rather associated with Chla concentration and tryptophan-like with O2 concentration 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; Stemond and Cory, 2014).



Vertical profiles (5-200 m depth) of fluorescence intensities of tyrosine-like fluorophore (peak B in RU) (a, b)

and tryptophan-like fluorophore (peak T in RU) (c, d) measured by the SeaExplorer glider (Mini-Fluo sensors), along the outward route: 6 May 00:00 - 9 May 21:00 (a, c) and the return route: 10 May 00:00 - 13 May 21:00 (b, d). Slight spatial interpolation was made using Data-Interpolating Variational Analysis (DIVA) method from Ocean Data View (ODV) software version 4.6.5, Schlitzer, R., http://odv.awi.de, 2014.

Discussion and Conclusion

==> "Line 361. I would avoid using informal text like "thanks to the flow cytometry measurements"."

==> "Lines 349 -359. Why don't you mention the consequences of these dynamics in terms of upwelling/downwelling here? This is what is driving your phytoplankton variability after all?"

==> "379 -380. Please expand on this! How does all of your statistical analysis support your results? And why is there no reference to the figures highlighting this analysis?"

==> "Lines 337 – 348. This is quite confusing as written."

==> "Lines 362 onwards. What about the other phytoplankton groups identified by flow cytometry? You simplify here that there is two main groups, and yet quite an in depth analysis is presented for the other groups in figures 12 -15."

==> "What contributes to the variability in phytoplankton community structure along the WE hippodrome? It is quite clear you have two distinct northern and southern water masses, but I wonder if there are other physical mechanisms that may be driving the variability you see longitudinally? What about the horizontal movement of water masses?"

==> "Overall, the discussion needs to fully encapsulate the results that you present. As it stands currently, it appears at times to only take bits and pieces of your story and I feel much of your previous analysis is ignored."

We will clarify the aspects that you have mentioned with your suggestions and according to the modifications of the Results.

References

Abraham, E.R., Bowen, M.M.: Chaotic stirring by a mesoscale surface-ocean flow. Chaos, 12(2):373-381. https://doi.org/10.1063/1.1481615, 2002.

Barrillon S.: FUMSECK cruise, RV Téthys II, https://doi.org/10.17600/18001155, 2019.

Benavides, M., Conradt, L., Bonnet, S. *et al*. Fine-scale sampling unveils diazotroph patchiness in the South Pacific Ocean. *ISME COMMUN*. 1, 3. <u>https://doi.org/10.1038/s43705-021-00006-2</u>, 2021.

Biers, E.J., Zepp, R.G., Moran, M.A.: The role of nitrogen in chromophoric and fluorescent dissolved organic matter formation. Marine Chemistry, 103, 46–60. <u>https://doi.org/10.1016/j.marchem.2006.06.003</u>, 2007.

Cammack, W.K., Kalff, J., Prairie, Y.T., Smith, E.M.: Fluorescent dissolved organic matter in lakes: Relationships with heterotrophic metabolism. Limnology and Oceanography, 49, 2034–2045. https://doi.org/10.4319/lo.2004.49.6.2034, 2004.

Boffetta G., Lacorata G., Redaelli G., Vulpiani A.: Detecting barriers to transport: a review of different techniques. Physica D: Nonlinear Phenomena, 159, 58-70, <u>https://doi.org/10.1016/S0167-2789(01)00330-X</u>, 2001.

Comby C., Barrillon S., Fuda J.-L., Doglioli A.M., Tzortzis R., Gregori G., Thyssen M., and Petrenko A.A.: Implementation of a new methodology for in situ measurement of vertical velocities. J. Atmos. Ocean. Technol, [in revision], 2021.

Determann, S., Lobbes, J.M., Reuter, R., Rullköter, J.: Ultraviolet fluorescence excitation and emission spectroscopy of marine algae and bacteria. Marine Chemistry, 62, 137–156. <u>https://doi.org/10.1016/S0304-4203(98)00026-7</u>, 1998.

de Verneil, A., Franks, P., and Ohman, M.: Frontogenesis and the creation of fine-scale vertical phytoplankton structure. Journal of Geophysical Research: Oceans, 124(3):1509–1523. <u>https://doi.org/10.1029/2018JC014645</u>, 2019.

d'Ovidio, F., Fernández, V., Hernández-García, E., and López, C.: Mixing structures in the Mediterranean Sea from finite-size Lyapunov exponents, Geophys. Res. Lett., 31, <u>https://doi.org/10.1029/2004GL020328</u>, 2004.

d'Ovidio, F., Della Penna, A., Trull, T. W., Nencioli, F., Pujol, M.-I., Rio, M.-H., Park, Y.-H., Cotté, C., Zhou, M., and Blain, S.: The biogeochemical structuring role of horizontal stirring: Lagrangian perspectives on iron delivery downstream of the Kerguelen Plateau, Biogeosciences, 12, 5567–5581, <u>https://doi.org/10.5194/bg-12-5567-2015</u>, 2015.

Fellman, J.B., Hood, E. D'Amore, D.V., Edwards, R.T., White, D.: Seasonal changes in the chemical quality and biodegradability of dissolved organic matter exported from soils to streams in coastal temperate rainforest watersheds. Biogeochemistry, 95, 277–293. <u>https://doi.org/10.1007/s10533-009-9336-6</u>, 2009.

Fukuzaki, K., Imai, I., Fukushima, K., Ishii, K.-I., Sawayama, S., Yoshioka, T.: Fluorescent characteristics of dissolved organic matterproduced by bloom-forming coastal phytoplankton. Journal of Plankton Research, 36, 685-694. <u>https://doi.org/10.1093/plankt/fbu015</u>, 2014.

Hudson, N., Baker, A., Ward, D., Reynolds, D.M., Brunsdon, C., Carliell- Marquet, C., Browning, S.: Can fluorescence spectrometry be used as a surrogate for the Biochemical Oxygen Demand (BOD) test in water quality assessment? An example from South West England. Science of the Total Environment, 391, 149–158. https://doi.org/10.1016/j.scitotenv.2007.10.054, 2008.

Lehahn, Y., d'Ovidio, F., Lévy, M., and Heifetz, E.: Stirring of the northeast Atlantic spring bloom: A Lagrangian analysis based on multisatellite data, J. Geophys. Res., 112, C08005. https://doi.org/10.1029/2006JC003927, 2007. Lehahn, Y., d'Ovidio, F., and Koren, I.: A Satellite-Based Lagrangian View on Phytoplankton Dynamics, Annual Review of Marine Science. 10:1, 99-119, <u>https://doi.org/10.1146/annurev-marine-121916-063204</u>, 2018.

Marrec, P., Grégori, G., Doglioli, A. M., Dugenne, M., Della Penna, A., Bhairy, N., Cariou, T., Hélias Nunige, S., Lahbib, S., Rougier, G., Wagener, T., and Thyssen, M.: Coupling physics and biogeochemistry thanks to high-resolution observations of the phytoplankton community structure in the northwestern Mediterranean Sea, Biogeosciences, 15, 1579–1606, <u>https://doi.org/10.5194/bg-15-1579-2018</u>, 2018.

Nieto-Cid, M., Alvarez-Salgado, X.A., Perez, F.F.: Microbial and photochemical reactivity of fluorescent dissolved organic matter in a coastal upwelling system. Limnology and Oceanography, 51, 1391–1400. https://doi.org/10.4319/lo.2006.51.3.1391, 2006.

Petrenko, A.A.: LATEX10 cruise, RV Téthys II, https://doi.org/10.17600/10450150, 2010.

Retelletti Brogi, S., Charrière, B., Gonnelli, M., Vaultier, F., Sempéré, R., Vestri, S., Santinelli, C.: Effect of UV and Visible Radiation on Optical Properties of Chromophoric Dissolved Organic Matter Released by Emiliania huxleyi. Journal of Marine Science and Engineering. 28, 888. <u>https://doi.org/10.3390/jmse8110888</u>, 2020.

Romera-Castillo, C., Sarmento, H., Álvarez-Salgado, X.A., Gasol, J.M., Marrase C.: Production of chromophoric dissolved organic matter by marine phytoplankton. Limnology and Oceanography, 55, 446–454. https://doi.org/10.4319/lo.2010.55.1.0446, 2010.

Rousselet, L., de Verneil, A., Doglioli, A. M., Petrenko, A. A., Duhamel, S., Maes, C., and Blanke, B.: Large-to submesoscale surface circulation and its implications on biogeochemical/biological horizontal distributions during the outpace cruise (southwest pacific). Biogeosciences, 15(8):2411–2431. <u>https://doi.org/10.5194/bg-15-2411-2018</u>, 2018.

Rousselet, L., Doglioli, A., de Verneil, A., Pietri, A., Della Penna, A., Berline, L., Marrec, P., Grégori, G., Thyssen, M., Carlotti, F., et al.: Vertical motions and their effects on a biogeochemical tracer in a cyclonic structure finely observed in the Ligurian Sea, J. Geophys. Res.-Oceans, 124, 3561–3574, https://doi.org/10.1029/2018JC014392, 2019.

Smetacek, V., Klaas, C., Strass, V. *et al.* Deep carbon export from a Southern Ocean iron-fertilized diatom bloom. Nature 487, 313–319 (2012). <u>https://doi.org/10.1038/nature11229</u>.

Stedmon, C.A., Markager, S.: Tracing the production and degradation of autochthonous fractions of dissolved organic matter using fluorescence analysis. Limnology and Oceanography, 50, 1415–1426. https://doi.org/10.4319/lo.2005.50.5.1415, 2005.

Stedmon, C.A., Cory, R.M., 2014. Biological origins and fate of fluorescent dissolved organic matter in aquatic environments. In: Aquatic organic matter fluorescence. Edited by P.G. Coble, J. Lead, A. Baker, D.M. Reynolds and R.G.M. Spencer. Cambridge Environmental Chemistry Series, Cambridge University Press, New York, USA, pp. 278–299. ISBN: 9780521764612.

Tedetti, M., Longhitano, R., Garcia, N., Guigue, C., Ferretto, N., Goutx, M.: Fluorescence properties of dissolved organic matter in coastal Mediterranean waters influenced by a municipal sewage effluent (Bay of Marseilles, France). Environmental Chemistry, 9, 438–449. <u>https://doi.org/10.1071/EN12081</u>, 2012.