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

Dear referee,

Thank you for the attention that you have given to our work.

We provide below, a feedback about the main points you raised. We are confident that following your remarks and suggestions, as well as the ones from the other anonymous referee, we can improve our manuscript for publication in Biogeosciences. In order to follow our reply, your comments have been copied hereafter the '==>' symbol.

General comments

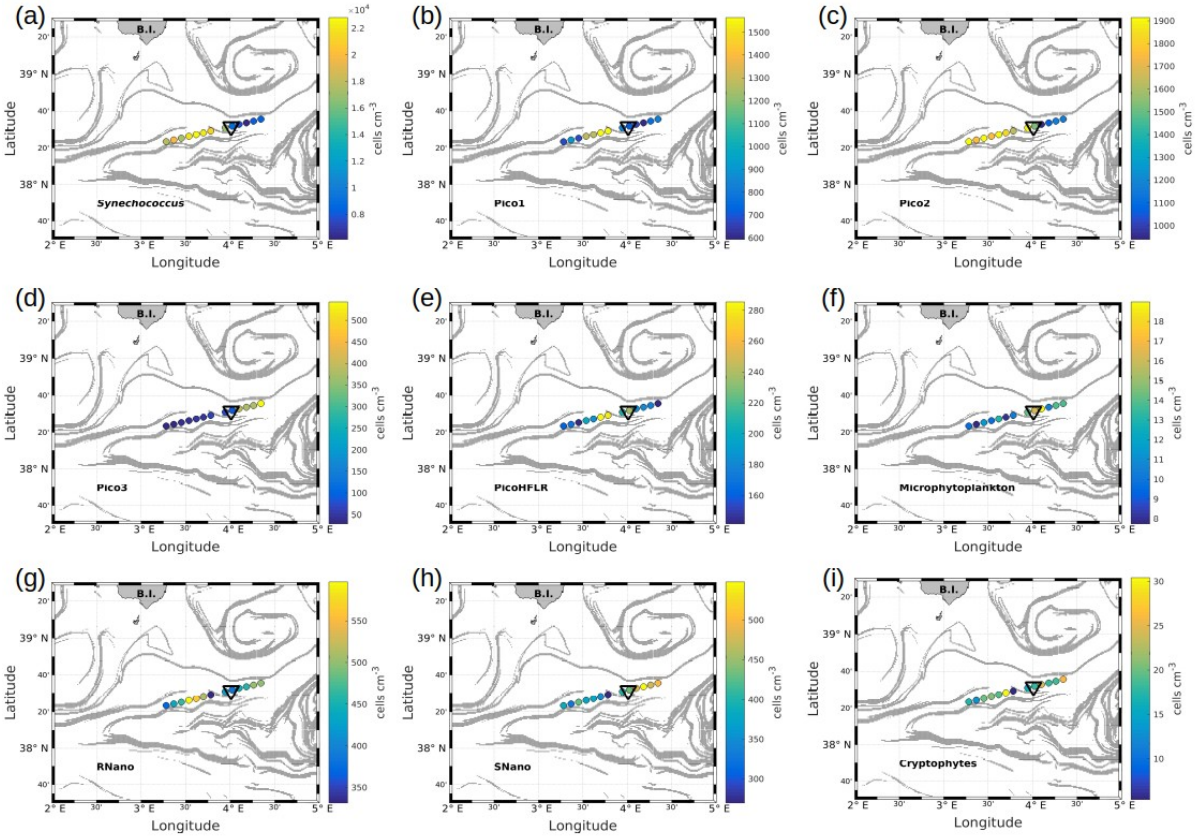
==> "The impact of fine-scale physical processes on plankton community is indeed very important. We have fully realized the significance of this problem, but limited by the observation means (especially biological parameters), the current understanding is very limited. Based on these backgrounds, I think this work is a very good attempt. The author's cruise design is very targeted, various equipment is very effective, the text description is very clear and detailed. However, from the perspective of research papers, I did not see the logical chain driven by scientific hypotheses. Instead, they used various devices to verify some predictable results. If the physical-biological processes and mechanisms in fine-scale are consistent with those in meso-/large- scales, why are they so important and are unique? When the scale becomes smaller, what is the most important scientific question in the process of physical-biological processes? Because this part is not highlighting enough, I have been looking forward to the new results (differences with large-scale and classical observation) and thinking about so what? What is the implication? As a research paper, I would like to see the author's point around a new result, or a logical inference."

First of all, we kindly disagree with the Reviewer on the fact that showing consistency between some biophysical stirring processes in meso- and large- scales is a non-result or a result of poor interest. Such a consistency should not be automatically expected. An equally plausible scenario could have been that, in regions that are much less energetic than boundary currents, like the region we studied, the physico-chemical contrasts induced by the horizontal stirring are not sufficiently strong to spatially reflect into different phytoplanktonic communities. That said, we acknowledge that we did not detail our scientific questioning enough and we were not clear enough about the novelty and the implications of our results. Moreover, we did not sufficiently discuss how the dynamical characteristics of the region specifically structure the phytoplankton community. As mentioned in the Introduction, horizontal fine-scales have been predominantly studied with numerical simulations and satellite observations. The validation of these studies is however difficult due to the lack of in situ observations, especially biological parameters. It is indeed a real challenge to perform in situ measurements of fine-scales processes due to their small physical scales and short lifetimes. In recent years only few in situ samplings targeting the fine scales have been performed, and the important point is that most of these cruises have been led in large and

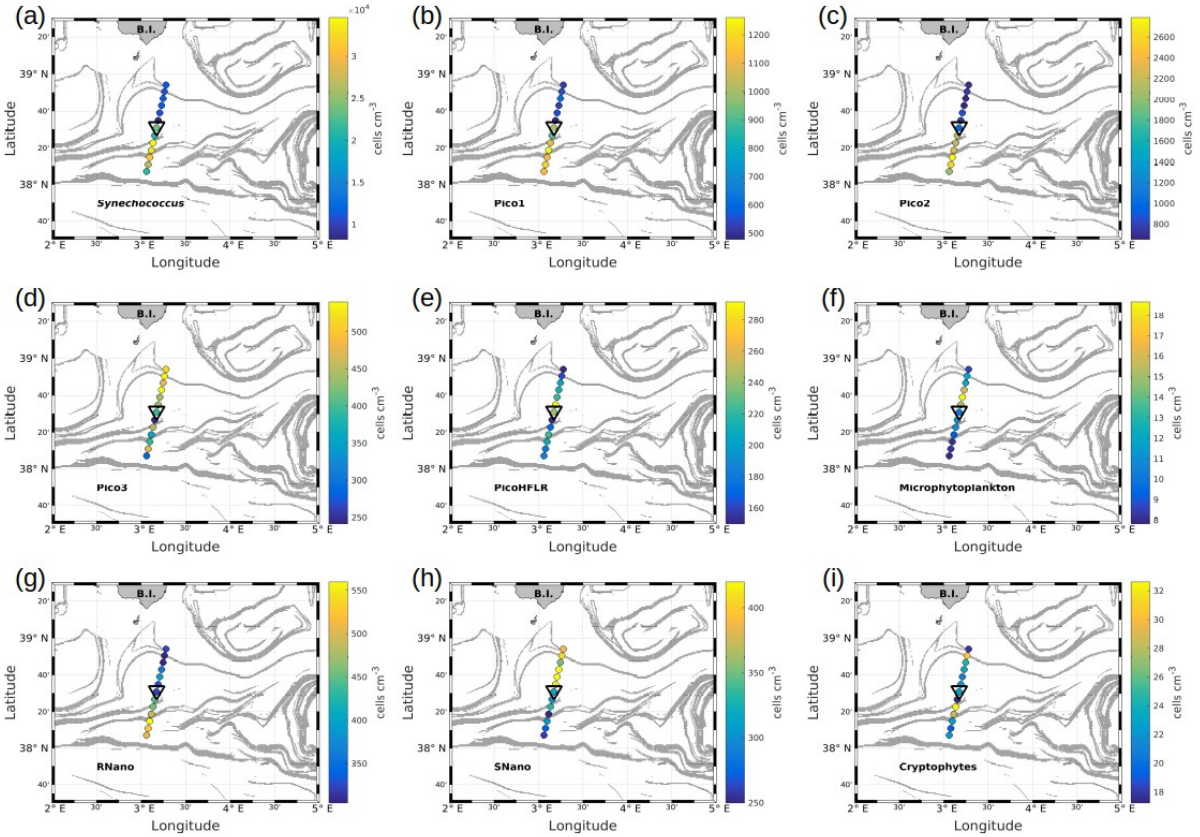
energetic regions such as in coastal upwelling regions (Ribalet et al., 2010) and in boundary currents (Clayton et al., 2014 ; 2017) known to generate persistent fronts and to host biodiversity hotspot areas (Barton et al., 2014 ; Lévy et al., 2015). Contrary to the existing and growing body of literature, this study is one of the only few targeting particularly less energetic and more ephemeral fine-scale processes (e.g., typical of Mediterranean Sea) and their effects on phytoplankton. Our results show a barrier role played by a front on the distribution of phytoplankton abundances. The fact that a moderately energetic fine scale front plays a similar role as large fronts in structuring the phytoplankton community has never been shown before and consists of the key original result of this study. Moreover, contrary to previous studies performed in nutrient-rich areas, our results have been obtained in an oligotrophic region. Since oligotrophic and weaker energetic regions are more representative of the global ocean than highly energetic structures presented in previous works, we are convinced that our results shed light on a better understanding of the functioning of the global ocean, and are therefore of great interest to the readers of Biogeosciences. We plan to stress these findings in the revised version of our manuscript.

==> "I noticed that there are two different water masses, one is old AW and the other is young AW. The later data analysis is almost organized according to this logic. Although the traditional physical ocean observation (such as water mass analysis) can also distinguish these two water masses generally, I found that the biological parameters do not seem to be completely consistent (at least some mathematical analysis is needed to clarify from the seemingly chaotic distribution). If the author can dig in depth according to this logic and see if the underlying mechanism is universal (extrapolation), they may be able to find a clue."

The clustering analysis of flow cytometry data allowed to detect several groups of phytoplankton (cf Fig. 12 in the manuscript), and, in particular, to identify various groups of eukaryotic Nanophytoplankton (RNano and SNano) and eukaryotic Picophytoplankton (Pico1, Pico2, Pico3, and PicoHFLR). Nevertheless, in the first version of the manuscript, we showed figures where the abundances of nanophytoplankton represented the sum of the abundances of RNano and SNano groups. Thanks to your remarks, we have redone Fig. 13 and 14 (cf figures below) taking into account RNano and SNano abundances separately.



Abundances (in cells per cubic centimeter) of the phytoplankton groups along the WE transect, superimposed with the FSLE field. Triangles indicate the front area.



Abundances (in cells per cubic centimeter) of the phytoplankton groups along the NS transect, superimposed with the FSLE field. Triangles indicate the front area.

On these new figures, it is possible to distinguish a clear separation of RNano and SNano abundances by the front. Furthermore, the distribution of RNano abundance is opposite to the SNano abundance. This opposite distribution is very likely the reason for the unclear distribution that you noticed when both groups were merged. For the sake of consistency, the pico-phytoplankton groups (Pico1, Pico2, Pico3 and PicoHFLR) will be separated in the new version of the manuscript, which wasn't the case in the first version. In the new figure above, Pico1, Pico2, and Pico3 abundances appear now clearly separated by the front. The distribution of PicoHFLR, as well as Cryptophytes, remains less clear. These groups do not appear as well correlated with temperature and salinity (used for the characterization of water masses), unlike the other phytoplankton groups. One explanation could be that these cells could be less sensitive to the environmental conditions than the other groups. Another explanation could be their low abundances combined to the fact that these groups are more difficult to define than the other: the limit of the groups is less obvious and maybe some of the events gated as PicoHFLR or Cryptophytes may be in fact some background noise.

The principal component analysis (Fig. 15a) and the K-medoid algorithm (Fig. 15b) already constitute an advanced mathematical analysis. The PCA results (cf Fig. 15a in the manuscript) clearly indicate what is qualitatively observed in the new figures and described above: the opposite distribution of the RNano and SNano abundance, the front separation for

the picophytoplankton abundances.

==> "Although the author defines fine scale (line 23), there are other related descriptions, which are easy to be confused. For example, low energetic front (lines 53, 60) and moderate energetic front (Title. Line 5, 328)."

Thank you. We will harmonize the text in order to use only the term "moderate energetic front".

==> "The size of phytoplankton is also confused. In fact, Synechococcus belongs to picophytoplankton, and I guess the "picophytoplankton" in the article means eukaryotic picophytoplankton. In addition, most cryptophytes are considered to be in nano size. Anyway, there's some confusion."

As mentioned above, we have identified several groups of phytoplankton by flow cytometry and used the conventional names used by flow cytometrists. Phytoplankton groups were resolved on the basis of their light scatter (namely forward scatter FWS and sideward scatter SWS) and fluorescence (red FLR and orange FLO fluorescence ranges) properties (Thyssen et al., 2015 ; Marrec et al., 2018). The name are indeed confusing in the manuscript as some groups are related to the taxonomy (*Synechococcus*, Cryptophytes) while the others are more related to a range of size (picoeukaryotes, nanoeukaryotes). We will correct that in the new version, to help the reader. Indeed, for instance, *Synechococcus* belongs to picophytoplankton, but it is a prokaryote. We have decided to keep it separated in the study because of that and because it was unambiguously put in evidence by flow cytometry thanks to its higher FLO intensity induced by the presence of phycoerythrin pigments. Idem for Cryptophytes which can be pico- or nanoeukaryotes but can also be discriminated from the red-only fluorescing pico- or nanoeukaryotes based on their orange fluorescence.

Minor comments and suggestions

==> "Abstract: It is necessary to present some new results based on fine scale observations."

==> "Line 259, n=≈?"

==> "Line 323-331, It is repeated in the introduction"

==> "Line 365-370: I suggest more analysis, based on your high-resolution results, to give more evidence or new explanations."

We will rework the manuscript to take into account your useful suggestions.

==> "Line 373, The concentration and structure of dissolved organic matter may be controlled by physical processes (aggregation and dilution) on the one hand, and by biological effects (production rate and species composition) on the other. Whether these high-resolution matching data can be further mined."

Thank you for your suggestion concerning the interpretation of the structure of dissolved organic matter.

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 tryptophan- and 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 reveals 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).

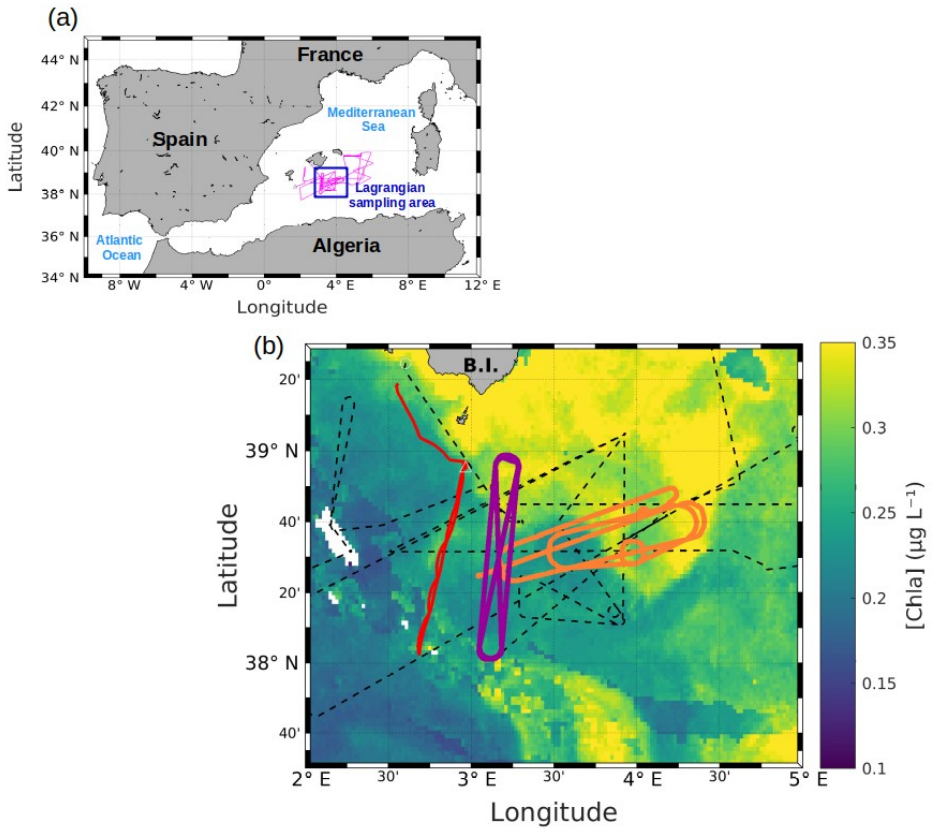
==> "Line 383, "provide an in-situ confirmation of the findings" may not enough. In fact, your data is so good that you don't need to prove other people's opinions at all."

==> "Line 387, change "Kurushio" to "Kuroshio"."

As mentioned above, we will rework the manuscript after the answer of Biogeosciences.

==> "Figure 1, large scale circulation and map may help readers understand better."

Concerning Figure 1, we have done a new figure below with a larger area.



(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.

==> "Some figures can be combined and are more suitable for comparison, as Figures 2 and 4, Figures 3 and 5, and Figures 6-10."

We will also combine figures and, following the suggestion of the other referee, some figures could be moved in supplementary material.

References

Barton, A. D., Ward, B. A., Williams, R. G., and Follows, M. J.: The impact of fine-scale turbulence on phytoplankton community structure, Limnology and oceanography: fluids and environments, 4, 34–49, https://doi.org/10.1215/21573689-2651533, 2014.

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. https://doi.org/10.1016/j.marchem.2006.06.003, 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.

Clayton, S., Nagai, T., and Follows, M. J.: Fine scale phytoplankton community structure across the Kuroshio Front, J. Plankton. Res., 36, 1017–1030, https://doi.org/10.1093/plankt/fbu020, 2014.

Clayton, S., Lin, Y.-C., Follows, M. J., and Worden, A. Z.: Co-existence of distinct Ostreococcus ecotypes at an oceanic front, Limnol. Oceanogr., 62, 75–88, https://doi.org/10.1002/lno.10373, 2017.

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. https://doi.org/10.1016/S0304-4203(98)00026-7, 1998.

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. https://doi.org/10.1007/s10533-009-9336-6, 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. https://doi.org/10.1093/plankt/fbu015, 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.

Lévy, M., Jahn, O., Dutkiewicz, S., Follows, M. J., and d'Ovidio, F.: The dynamical landscape of marine phytoplankton diversity, J. Roy.Soc. Interface, 12, 20150 481, https://doi.org/10.1098/rsif.2015.0481, 2015.

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, https://doi.org/10.5194/bg-15-1579-2018, 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.

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. https://doi.org/10.3390/jmse8110888, 2020.

Ribalet, F., Marchetti, A., Hubbard, K. A., Brown, K., Durkin, C. A., Morales, R., Robert, M., Swalwell, J. E., Tortell, P. D., and Armbrust, E. V.: Unveiling a phytoplankton hotspot at a narrow boundary between coastal and offshore waters, Proc. Nat. Acad. Sci. USA, 107, 16 571–16 576, https://doi.org/10.1073/pnas.1005638107, 2010.

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.

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. https://doi.org/10.1071/EN12081, 2012.

Thyssen, M., Alvain, S., Lefèbvre, A., Dessailly, D., Rijkeboer, M., Guiselin, N., Creach, V., and Artigas, L.-F.: High-resolution analysis of a North Sea phytoplankton community structure based on in situ flow cytometry observations and potential implication for remote sensing, Biogeosciences, 12, 4051–4066, https://doi.org/10.5194/bg-12-4051-2015 , 2015.