Comments on the revised manuscript of "The contrasted phytoplankton dynamics across a frontal system in the southwestern Mediterranean Sea", by Roxane Tzortzis, Andrea M. Doglioli, Monique Messié, Stéphanie Barrillon, Anne A. Petrenko, Lloyd Izard, Yuan Zhao, Francesco d'Ovidio, Franck Dumas, and Gérald Gregori.

Dear referee,

We are grateful for your interest in our study and for the attention that you have given to our work for a second time. Your suggestions and constructive comments helped us to further improve our work. We hope that our actual revised version will be accepted for publication. Below you find the point-by-point reply (in blue, with line numbers of the latest version) to your comments (in black).

General Comments

I found that in the revised version of "The contrasted phytoplankton dynamics across a frontal system in the southwestern Mediterranean Sea", what I pointed out has been largely incorporated. I feel a little bit disappointed that there was no additional discussion about the validity of the methodology applied, but I am largely satisfied that the logical consistency of the manuscript has been improved. In particular, the revisions made in Introduction and Conclusion have successfully clarified the location of the present study in a series of past and future expeditions conducted in the Mediterranean Sea.
Hopefully it is plausible that the authors will expand Discussion by adding more about the validation, possibility, and limitation of this methodology. This is just an option, but I believe that it will improve the current manuscript.

Thank you very much for these generally positive comments about our work.
Following your suggestions, we have reworked the Discussion (see new section 4.3 "Limitations of the study and recommendations" (L341-L368)) and the Conclusion, adding comments about the implications and the limitations of our study.

4.3 Limitations of the study and recommendations

Growth and loss rates were estimated using the size-structured population model originally developed by Sosik et al. (2003), which was fitted to a measured diel cycle of cell size distributions. More precisely, the rates were calculated based on the fitted size distribution predicted by the model, and its comparison with the observed size distribution. Because of this, results are sensitive to noise in the measured size distributions. We could not obtain reliable results for the picophytoplankton groups due to noisy distributions, because they probably contained several taxa with differing dynamics (Siokou-Frangou et al., 2010; Le Moal et al., 2011). To take into account this constraint, in future experiments, sorting by flow cytometry and identification with a microscope and/or genetics analysis should be planned to identify taxa in the various phytoplanktonic groups defined by flow cytometry. Nevertheless, these techniques are not easily applicable to large numbers of samples contrary

to automated flow cytometry, which means that a careful selection of samples will be necessary.

For the taxonomic groups where reasonable size distributions could be estimated over a diel cycle, the model fit was evaluated using two metrics: the optimization loss rate $\sum(\theta)$ and the correlation between the observed and modeled mean biovolumes over the diel cycle $corr(\bar{v}_{obs}\ ,\ \bar{v}_{mod}\ )$ (Table 2). These metrics, as well as visual comparisons of the modeled and observed size distributions (Figures 4, 5, 6), indicate differing degrees of confidence in our estimated growth and loss rates, with the highest confidence obtained for *Synechococcus*. In future experiments, these rate estimates could be improved by more accurately measuring the phytoplankton diel cycle (i.e., by continuously sampling the same water mass over 24 h rather than by compiling several days to reconstruct a diel cycle). Furthermore, coupling flow cytometry with NanoSIMS analysis, as in the works of Bonnet et al. (2016) and Berthelot et al. (2019), could be also useful to get independent estimates of the growth rates, although the cost and the successive incubations required by this methodology are not adapted to high-frequency sampling of finescale ocean structures.

Overall, while estimating growth and loss rates by fitting a model to automated flow cytometry data remains limited by our capacity to accurately resolve the size distribution of independent phytoplankton groups over a full diel cycle, the method used here has several advantages. Other methods involve incubations that are dependent on the accurate reproduction of the marine environment in incubators. By contrast, automated flow cytometry as applied here measures the temporal evolution of phytoplankton in situ. The automated CytoSense flow cytometer, deployed underway, requires little maintenance or manipulation during the cruise contrary to time-consuming sampling and incubations. As such, while growth and loss rates obtained from automated flow cytometry would benefit from independent validation, they have the potential to provide in situ estimates of biological rates that are traditionally difficult to measure.

Specific Comments

L34 “Phytoplankton is also responsible for half of the primary production of the planet (Field et al., 1998), while its biomass is only ≤ 1 % of the global biomass” Mentioning primary production again sounds unnatural here, since primary production is essentially the same biogeographical process as CO2 fixation.

We rewrote this paragraph (L14-L22) as follows:

“Phytoplankton forms the basis of the marine food web (Sterner and Hessen, 1994) and it is responsible for half of the primary production of the planet (Field et al., 1998), while its biomass is only ≤ 1 % of the global biomass (Winder and Cloern, 2010). Thanks to photosynthesis, phytoplankton fuels the ocean and the atmosphere in free O_2 and it fixes and exports the CO_2 into the ocean depth (Field et al., 1998; De La Rocha and Passow, 2007). This process called biological carbon pump is critical for global ocean sequestration of carbon and therefore for the modulation of atmospheric CO_2 . The biological carbon pump is

modulated by the size structure of the phytoplankton community. Small or large phytoplankton species are associated with different efficiencies for particle export, remineralization, and transfer to the deep ocean (Boyd and Newton, 1999; Guidi et al., 2009; Hilligsøe et al., 2011; Mouw et al., 2016, etc). That is why, it is primordial to understand the factors that rule phytoplankton abundance and diversity."

L299 "cells...grow more than one size class" This description is should be more specified.
We rewrote it as: (L161-L162) "cells of a specific phytoplankton group are unlikely to grow more than one size class over such a small time duration."

L316 "Normal" should be written in lower case.
It has been done.

L371 If the authors move the detailed description of identification of the phytoplankton functional groups by flow cytometry to appendices, they should refer to it somewhere in the main text, possibly in materials and methods.
It has been done (L128-L129).

L385 Renumber the sections.
It has been done.

L474 Section numbers should be changed.
It has been done.

L625 Although I admit that this is an important point of this study, it is not a point that has been clarified from the present results. I think that the authors had better mention it in any precedent section. It is very important to emphasize the novelty of the study in concluding remarks, but repeated mentioning on the lack of previous studies sounds redundant.
We have reworked the Conclusion, we have also removed the sentence "To our knowledge this had never been done before".