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Interactive comment on "Deep maxima of phytoplankton biomass, primary production and bacterial production in the Mediterranean Sea during late spring" *by* Emilio Marañón et al.

Anonymous Referee #2

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This work reports that the deep chlorophyll maximum (DCM) is a maximum of biomass and primary production in the oligotrophic Mediterranean Sea during late spring. These deep maxima are accompanied by a sub-maximum of bacterial production. The ms is relevant, it reveals that primary production is very significant at the DCM, a component of production undetectable by remote sensing techniques. It is worth mentioning that the biomass data presented are quite new, since the biomass of picoplankton and especially of nanoplankton, the latter seldom directly quantified, were analyzed with specific and appropriate techniques. The ms is well organized and well written and is very easy to read. The figures and tables are clear and explanatory. The results may represent a challenge for some current paradigms of phytoplankton ecophysiology. The main factors that regulate phytoplankton growth rates are light, nutrients and temperature. The study concludes that growth rates remain more or less constant along the water column. Between the surface layers and the DCM, irradiance decreases from saturating to limiting conditions and temperature decreased about 5 C in this study. These two factors alone should have significantly decreased phytoplankton growth rates at the DCM, which could have been compensated somehow by an increase of diffusive nutrient supply to the DCM from the nutricline. However, the measured nutrient supply was low. The authors explain their findings by the presence of a diatom community in the DCM layer that was very efficient at low irradiances (I would add temperature). The implications would be important since these results show that composition conditions the phytoplankton response, which should question general ecophysiological assumptions that are often extrapolated to natural conditions by some models. The following are some issues that I suggest be examined further to reinforce the important findings of the study (sentences copied from the ms are signaled between quotation marks)

Carbon estimates Estimates of C biomass are paramount in this work. More accurate biovolume estimates can be obtained using the scattering properties (forward or side scattering) of single cells than by assuming mean volumes for picoplankton and nanoplankton. In addition, this procedure would take into account the important changes of cell size with depth, often ignored (Binder et al. 1996. Dynamics of picophytoplankton, ultraphytoplankton and bacteria in the central equatorial Pacific. Deep. Res. II 43: 907-931, Mena et al 2019, cited by the authors). Please, specify the volume analyzed for detecting a significant number of cells from the small nanophytoplankton fraction, it is an interesting information that can help other researches and future studies. L. 138. "Thus the increase, from the surface to the base of the euphotic layer, in phytoplankton biomass was ca. 2-fold, compared with ca. 8-fold for TChl a." Please, consider recalculating the biomass taking into account changes of biovolume with depth.

Diatoms at the DCM L. 264. "The fucoxanthin to total chlorophyll a ratio (Fuco: TChl a) consistently increased below the upper 40-50 m in all long stations." From the changes in this ratio it is deduced that diatom contribution increases with depth. Fucoxanthin is also present in haptophytes and pelagophytes, two main components of phytoplankton with 19'hex-fuco and 19'but-fuco as their main diagnostic pigments, respectively. To make sure the increase in fucoxanthin is due to diatoms I would recommend calculating the vertical distribution of fucoxanthin: (19'hex-fuco + 19'but-fuco). The increase of this ratio with depth would be a more convincing evidence of a differential increase in diatoms. The images obtained with the Imaging Flow CytoBot should help to confirm that diatoms dominated or were very abundant in the DCM layer.

L. 375. "...this trend was associated with a significant increase in the contribution of diatoms to total phytoplankton biomass, which reached at least 30 % in the DCM of all stations, and was particularly high (nearly 50 %) in the most stratified station, located in the Ionian Sea." Please, re-check your estimates of diatom contribution at the DCM. Although I agree that diatoms can increase at the DCM, these values appear very high. In addition, the data of Crombet et al 2011 (cited in ms) show a patchy distribution of the Deep Silica Maximum and diatoms in the DCM of the Mediterranean.

Primary production (PP) at the DCM L. 309. "In contrast, during PEACETIME the mean depths of the primary production maximum and the DCM coincided and only on 3 profiles was the primary production peak located above the DCM." The subsequent discussion does not present potential mechanisms to explain the discrepancy in PP estimates at the DCM between this and previous studies cited in the ms, which show a PP maximum above the DCM most of the time. It does argue that the high primary production at the DCM during PEACETIME was due not only to enhanced levels of phytoplankton biomass but also to the presence of a diatom-rich community characterized by high photosynthetic efficiency. It is a bit surprising that the same response has not been found in previous studies in the area. Could it be possible that the presence of diatoms with high photosynthetic efficiency at the DCM discussed by the authors is a consequence of the previous spring bloom at the surface and not a regular feature of the DCM in the Mediterranean? Estrada et al (1993. Variability of deep chlorophyll maximum characteristics in the Northwestern Mediterranean. Mar. Ecol. Prog. Ser. 92: 289–300) reported the occasional presence of diatoms from a decaying bloom that contributed significantly to the DCM biomass but with a very low photosynthetic efficiency, which seems typical of sinking cells. It seems that a large contribution of diatoms in the DCM layer is not a general feature of the Mediterranean Sea, and perhaps could explain the discrepancies in PP estimates at this depth with other studies.

L. 340. "In contrast, during our survey the contribution of increased phytoplankton biomass was similar in all stations, including the one located in the Ionian Sea." An important conclusion is that DCM is a maximum of biomass and production in the Mediterranean, at least during the period of the study. However, in 3 of the 4 profiles obtained in the Tyrrhenian Sea the biomass maximum is well above the DCM. This result is mentioned (line 235) but ignored throughout the ms. Moreover, it is difficult to explain how the PP maximum can be found at 70-80m, at the DCM and below the biomass maxima in these stations without a significant increase of nutrient supply. The correction that has been applied to short-term temperature variations to estimate PP at in-situ temperature from incubations at higher temperatures (about 5 C) could be discussed further to see if they may have distorted the results of the deep layers.

L. 444. "Thus the surface BP (bacterial production) peak observed under in situ conditions was not due to dependence of organic carbon substrates but may have resulted in part from new N and P availability through dry atmospheric deposition." This explanation can be applied to phytoplankton as well. If atmospheric input of inorganic nutrients and recycling are the main reasons for vertical patterns of bacterial production, the same pattern should have been found for primary production (which is the pattern usually found by other studies in the Mediterranean cited in the ms).

L. 335. "Therefore low nutrient availability, which is widespread in the global ocean (Moore et al., 2013), results not only in low phytoplankton biomass but also in slow growth rates." This conclusion is controversial in the scientific community. Another line of research with direct estimates of growth rates using mainly dilution experiments argue that, even with low nutrient concentrations, fast supply of nutrients from recycling results in the predominance of phytoplankton, usually of small size, with relatively high growth rates (Laws, E. A., 2013. Evaluation of in situ phytoplankton growth rates: A synthesis of data from varied approaches. Ann. Rev. Mar. Sci. 5: 247–268, and ref therein), although lower than those of taxa typical for bloom situations. Different optimal growth rates can be a function of taxonomical affiliation or size, among other reasons.

Keep the same y-scale for fig 3g, h and i.

END OF REVIEW

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