Comments by Anonymous Referee #2 and our responses

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.

We are grateful to this reviewer for their time and helpful comments.

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)

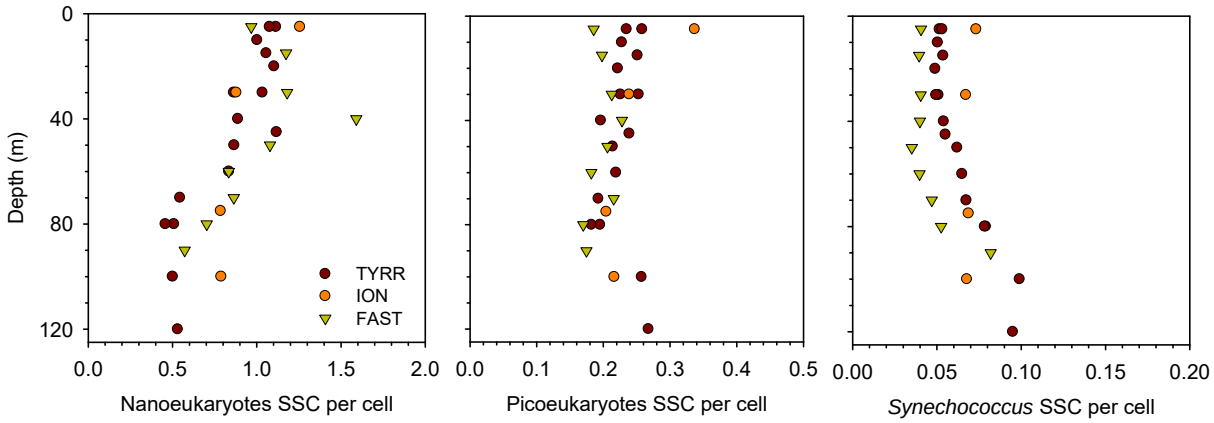
The observation that phytoplankton growth rates were rather invariant across the euphotic layer does seem counterintuitive in the face of strong gradients in irradiance and temperature. However, the same pattern (i.e. similar growth rates in the surface layer and near the base of the euphotic layer) has often been reported by other studies, such as (cited in the ms) Pérez et al. (2006) and Berthelot et al. (2019) and also (not cited in the ms) Cáceres et al. (2013), Landry et al. (2004) and Armengol et al. (2019). Specifically, Cáceres et al. (2013) found virtually the same growth rate throughout the euphotic layer in a station located in the eastern subtropical North Atlantic (their Fig. 7, bottom panels). Landry et al. (2004) measured the same growth rate at the surface and at 60 m in an offshore oligotrophic station off Southern California (their Fig. 2, Cruise P0605 Cycle 5). Armengol et al. 2019 reported (their Table 2, stations 1-7) a mean growth rate of 0.28±0.18 d^{-1} at the surface compared with 0.21±0.07 d^{-1} at the DCM in the central tropical Atlantic. In the revised version of the manuscript, we will add a reference to these additional studies in the Discusion (section 4.3).

The paradox of relatively constant phytoplankton growth throughout the euphotic layer in oligotrophic settings can perhaps be explained by considering that the physiological effect of a given environmental factor tends to decrease when another factor is limiting. Most laboratory experiments are designed to determine the effect of a single environmental driver while keeping other variables under optimal levels. For instance, under nutrient-sufficient conditions the effect of irradiance on growth is strong, and under optimal nutrient and irradiance conditions the effect of temperature is also strong. However, the temperature dependence of phytoplankton growth is greatly reduced under conditions of light (Edwards et al. 2016) or nutrient (Marañón et al. 2018) limitation. Conversely, the effect of increasing nutrient supply on growth is modest when temperatures are strongly limiting (see review by Cross et al. 2015). Thus the lack of irradiance effects on the growth rate of acclimated phytoplankton assemblages may result from the

fact that nutrient limitation prevails throughout the water column. We will add this suggestion to section 4.3 in the revised version of the manuscript.

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).

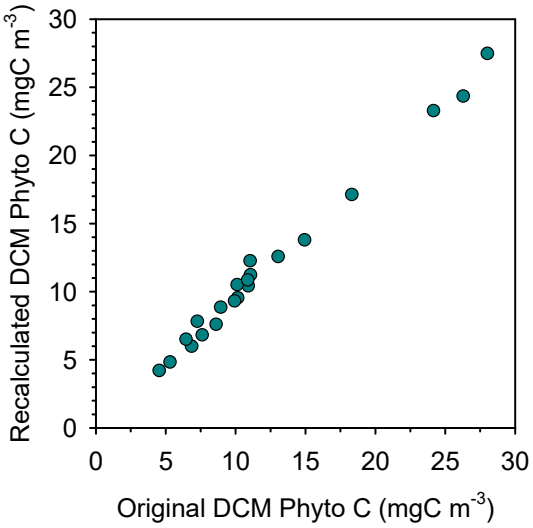
Although estimates of cell biovolume based on the side scattering (SSC) signal were not routinely available for the cruise, we have examined a few profiles of SSC per cell at the long stations to assess depth-related changes in cell biovolume of *Synechococcus*, picoeukaryotes and nanoeukaryotes. As shown in the plots and table below, we found that cell biovolume of nano- and pico-eukaryotes decreased with depth whereas the opposite was true for *Synechococcus*.



Mean (and standard deviation) of the side-scatering signal per cell of different groups in the surface layer (0-40 m) and at the DCM (including also the sample obtained immediately above the DCM) in the three long stations (data from all stations were pooled together).

	SSC per cell		
	Nanoeukaryotes	Picoeukaryotes	*Synechococcus*
Surface (n = 13)	1.06 (0.11)	0.24 (0.04)	0.052 (0.010)
DCM (n = 11)	0.64 (0.15)	0.21 (0.03)	0.074 (0.017)

We have recalculated the total biomass of phytoplanton at the DCM taking into account these depth-related changes in cell volume of pico and small nanophytoplankton. Specifically, the value of C biomass per cell used in the original calculations was multiplied by the observed DCM to surface SSC ratio for each group, which was 0.61 for nanoeukaryotes, 0.87 for picoeukaryotes and 1.42 for *Synechococcus*. The figure below shows that taking into account these changes in cell volume with depth has negligible effects on the estimated total phytoplankton biomass at the DCM:



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.

Samples were run at a flow rate of 145 µL min^{-1} for 5 min so that analysed volume for each sample was 725 µL. This information will be added to section 2.3 in the revised manuscript.

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.

As shown in the response above, when phytoplankton biomass at the DCM is recalculated taking into account depth-related changes in SSC the new biomass data are virtually identical to the original ones. This results from several factors: i) the vertical changes in cell volume of picoeukaryotes were minor, ii) the change of *Synechococcus* and that of nanoeukaryotes were substantial but showed opposite trends, thus counterbalancing each other, and iii) the biomass of all groups measured with flow cytometry represent, on average, ≤40% of total phytoplankton biomass at the DCM.

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.

Following the reviewer's advice, we have calculated the fucoxanthin:(19'hex-fuco+19'but-fuco) ratio and verified that it increases consistently with depth. In fact, the vertical distribution of the fucoxanthin:(19'hex-fuco+19'but-fuco) ratio is nearly identical to that of the fucoxanthin:chlorophyll a ratio, which supports our conclusion of increased diatom contribution at the DCM. The new pigment ratio will be added to the supplementary information in the revised version of the manuscript. We will also add mosaics of all cells imaged by the IFCB in surface and DCM samples from the three long stations. These

mosaics show that diatoms were abundant at the DCM of all three stations and virtually absent in surface samples.

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.

It has to be noted that our cruise took place during late spring whereas the survey reported by Crombet et al. (2011) was conducted in summer, more than 1.5 months later in the year. We estimated the diatom contribution to total chl a by using three different pigment coefficients. The lowest pigment coefficient used (1.41, taken from Uitz et al. 2006), which gives a lower-bound estimate of diatom contribution, is derived from a large database covering a broad range of trophic situations and including the entire water column, not just the surface layer as is typically the case. We therefore consider that the resulting estimate of diatom contribution is robust. Note that station ION, which has the highest estimated contribution of diatoms in the DCM, is also the one that shows the highest abundance of diatoms in the IFCB images.

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.

We agree with the reviewer that the high diatom abundance and productivity observed during our cruise are not necessarily persistent features of the DCM in the Mediterranean sea. In fact, we end the Conclusions section by pointing out that future, high-resolution studies are needed to ascertain if the observed peak in productivity is a persistent feature of the DCM in the Mediterranean Sea. It may well be the case that, as indicated by the reviewer, the significant biomass contribution of diatoms observed at the DCM results from the sedimentation of the earlier spring bloom. In the revised version of the manuscript, we will re-write the relevant passages of sections 4.3 and 4.5 to point out that 1) our results have a limited temporal coverage and therefore cannot be used to ascertain if the deep productivity maxima are persistent during the stratification season and 2) it is possible that the enhanced biomass contribution of diatoms at the DCM results from the sedimentation of the spring bloom in the weeks prior to the cruise.

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.

In two of the TYRR profiles mentioned (sampled on 18 and 19 May) there is indeed a disconnection between the deep PP maximum (located at the DCM depth) and the biomass maximum (located at 40 m) but it has to be noted that the magnitude of the deep PP peaks is minor. The rates of primary production measured on those 2 profiles at the DCM are only slightly higher than those measured at the surface (2 vs 1.7 and 3 vs 2.3 mgC m^{-3} d^{-1}, respectively). On 17 May, PP at the DCM was twice as large as that at the surface, but biomass was also higher by a factor of 2. Large discrepancies between biomass and primary production would have resulted in anomalous values of biomass turnover rates, which were not found. The temperature correction used assumes a strong sensitivity of photosynthesis to temperature (Ea = 0.61 eV, approximately equivalent to Q_{10} = 2.3), which only moderates the magnitude of the deep PP peaks, as explained in the first version of the manuscript (section 4.1).

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).

The response to atmospheric deposition may not be necessarily symmetrical between phytoplankton and heterotrophic bacteria, as the latter tend to respond faster and more intensely to the nutrients injected from the atmosphere (see review of dust addition bioassays in Guieu et al. 2014). In fact, the superior ability of heterotrophic bacteria to compete for inorganic nutrients is also shown by the budget analysis and experimental observations of Van Wambeke et al. (2020), who concluded that dry atmospheric deposition could supply nearly 40% of the hetetrophic bacteria N demand in the upper mixed layer.

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.

Other results from dilution experiments, not cited by Laws (2013), show that slow growth rates prevail in low-productivity waters. For instance, Landry et al. (2008) measured rates around 0.3 d^{-1} in oligotrophic waters off Hawaii not affected by a cyclonic eddy, while finding rates as high as 0.6 d^{-1} in stations inside the eddy. In another study, Landry et al. (2009) found euphotic layer-integrated phytoplankton growth rates of 0.1-0.2 d^{-1} in oceanic, well-stratified stations off southern California, compared with rates of 0.2-0.5 d^{-1} in stations within the coastal upwelling region (their Fig. 3). More recently, Armengol et al. (2019) obtained (also with the dilution method) mean growth rates around 0.3 d^{-1} across the oligotrophic tropical Atlantic (10°N-0°S).

We agree with the reviewer that different taxa have different maximum growth rates. However, even though some strains of *Prochlorococcus* and *Synechococcus* may have relatively low maximum growth rates (<0.5 d^{-1}), picoeukaryotes of wide distribution such as *Ostreococcus* sp. and *Micromonas* sp. can indeed grow at rates ≥ 0.5 d^{-1} (Six et al. 2008, Demory et al. 2019). The results of Berthelot et al. (2018) that we cite are especially relevant because they were based on measurements of isotope uptake by intact, single cells, thus avoiding some of the uncertainties involved in bulk methods. They found that growth rates of picoeukaryotes were 0.15-0.26 d^{-1} in the North Pacific subtropical gyre compared with 0.42-0.50 d^{-1} in stations within the California coastal current. Also, in situ experiments in HNLC waters have shown unequivocal increases in growth rates once Fe limitation was removed (Boyd et al. 2008). Finally, flow cytometry measurements of single-cell fluorescence (a proxy for abundance of photosynthetic units) in subtropical gyres (Davey et al. 2008, Browning et al. 2017) show that investment in photosynthetic machinery increases markedly after nutrient addition, again supporting the view that nutrient limitation in oligotrophic regions causes physiological impairment and thus reduced growth rate.

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

The scale in Fig 3i will be changed accordingly.

References (not cited in the ms)

Armengol et al. (2019) Planktonic food web structure and trophic transfer efficiency along a productivity gradient in the tropical and subtropical Atlantic Ocean. Sci Rep 9, Article No 2044

Boyd et al. (2008) Mesoscale Iron Enrichment Experiments 1993-2005: Synthesis and Future Directions Science 315 :612-617

Browning et al. (2017) Nutrient co-limitation at the boundary of an oceanic gyre. Nature, doi:10.1038/nature24063.

Cáceres et al. (2013) Phytoplankton Growth and Microzooplankton Grazing in the Subtropical Northeast Atlantic. PlosOne 8(7) e69159.

Davey et al. (2008) Nutrient limitation of picophytoplankton photosynthesis and growth in the tropical North Atlantic. Limnol Oceanogr 53:1722-1733.

Demory et al. (2019) Picoeukaryotes of the Micromonas genus: sentinels of a warming ocean. The ISME Journal, 13:132–146

Edwards et al. (2016) Phytoplankton growth and the interaction of light and temperature: A synthesis at the species and community level. Limnol Oceanogr, doi: 10.1002/lno.10282

Landry et al. (2008) Depth-stratified phytoplankton dynamics in Cyclone Opal, a subtropical mesoscale eddy. Deep Sea Res 55:1348-1359

Landry et al. (2009) Lagrangian studies of phytoplankton growth and grazing relationships in a coastal upwelling ecosystem off Southern California. Prog Oceanogr 83:208-216

Six et al. (2008) Contrasting photoacclimation costs in ecotypes of the marine eukaryotic picoplankter Ostreococcus. Limnol Oceanogr 53:255

Van Wambeke et al. (2020) Influence of atmospheric deposition on biogeochemical cycles in an oligotrophic ocean system. Biogeosciences, under revision.