Comments by Anonymous Referee #3 and our responses

This MS addresses the ubiquitous subsurface feature, the deep chlorophyll maximum, phytoplankton biomass and production, and heterotrophic prokaryotic production in the Mediterranean sea's stratified water column during the later spring season (May 10, 2017- June 11, 2017). This subsurface feature in the world ocean is known for long, more prominent in waters of lower latitude, are often found at nutracline depth well below the remote sensing reach, thus supports the importance of seaboard measurements to capture this feature. Chlorophyll a, an indicator of the phytoplankton biomass, is regulated by light, nutrient, etc. Here, the authors mainly aim to quantify photoacclimation's relative role and enhanced growth as an essential DCM mechanism. Secondly, the trophic coupling between phytoplankton and heterotrophic prokaryotic production is also addressed. Based on shipboard measurements in the Mediterranean sea, authors conclude that the DCM located at subsurface depth coincides with both biomass and primary production but not in growth rate and explains that the photoacclimation process leads to the increased chlorophyll a at the DCM. This study contributes vital insight into likely future ocean changes under the ocean warming scenario, thus merits publication of this work. However, I do not recommend a journal publishing this work in the present form. A few concerns about the methodology and the data interpretation need to be taken care of before considering this work for publication (see below).

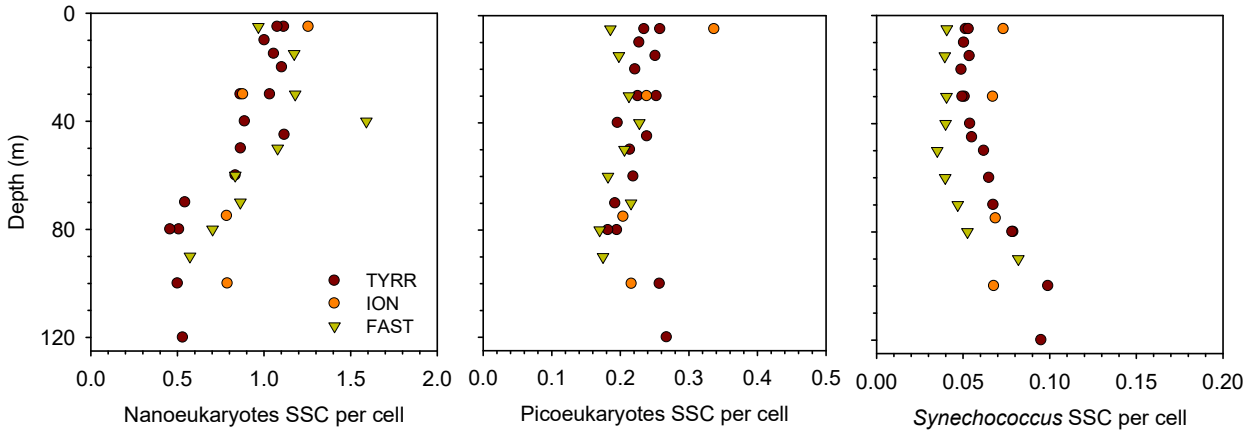
We are grateful to this reviewer for their time and helpful comments.

Flow cytometry tool followed to obtain estimates of the carbon biomass in different size categories does not seem to have taken account of the autotrophic cells >150 microns in size neither their contribution is quantified, if minimal. The authors could have easily viewed these samples (>150micron) under the microscope to support the finding. If this were a significant observed, increased carbon biomass from the surface to the euphotic layer base would have been different and could lead to a different conclusion. The authors need to take care of this part in the section result and subsequently draw a conclusion at the end of the discussion section.

Although a 150-µm mesh is used to pre-filter the samples, particles with a length >150 µm can still be imaged by the IFCB. These include elongated, single cells (such as *Rhizosolenia* sp.) and diatom chains, both of which can be seen in the mosaics that will be included in the revised version of the manuscript. Size-abundance spectra obtained with microscopy image analysis in oligotrophic waters indicate that cells with a volume of ≥10,000 μm^3 (assuming a cylindrical, elongated shape such as that of *Rhizosolenia*, this volume corresponds roughly to cells with a length of 150 µm and a diameter of 10 µm) contribute on average approximately 1% of total biovolume (Huete-Ortega et al. 2011, Marañón 2015). It is thus unlikely that the IFCB has significantly underestimated the total community biovolume. This point will be clarified in the revised version of the manuscript.

Also, it is unclear whether definite size beads were run on flow cytometry to conclude the mean cell diameter used for carbon calculation. It is essential to show the reader the error introduced by assuming the mean cell diameter (2um or 4 um or 6um).

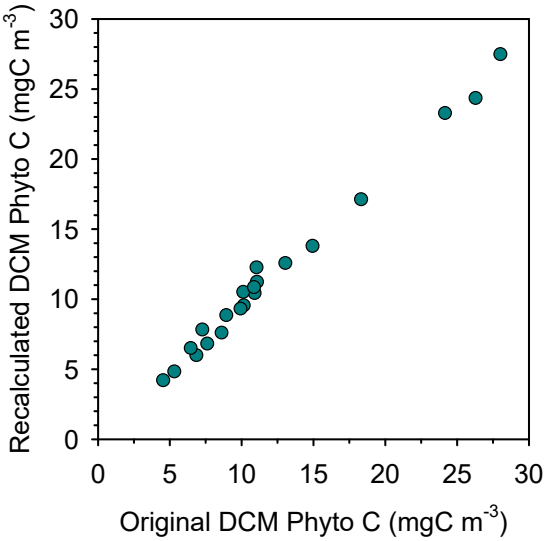
As explained in the Methods section, C biomass estimates for small phytoplankton (measured with flow cytometry) assumed constant C content for each group of cells. Although estimates of cell biovolume based on the side scattering (SSC) signal were not routinely available for the cruise, we have examined 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:



On the other hand, while calculating Fucoxanthin to total chlorophyll a ratio calculation, I find authors have ignored that besides diatoms, Phaeocystis spp. are also potential sources of Fucoxanthin (see Latasa and Bidigare, 1998) instead accounted to diatom community.

To examine the possibility that the increase in fucoxanthin with depth may have reflected an increased abundance in other fucoxanthin-containing groups such as haptophytes and pelagophytes, we have calculated the fucoxanthin:(19'hex-fuco+19'but-fuco) ratio. We found that 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.

Furthermore, the presence of divinyl chl a, a marker for prochlorophyte, seems to have ignored and accounted for diatoms. Suggest authors revisit HPLC based pigment (depth-wise) analyses to rule out Prochlorococcus community is not missed out. In my opinion, low light-adapted Prochlorococcus at the DCM may be sizably contributing to the DCM community.

Following the reviewer's advice, we have assessed the potential contribution of *Prochlorococcus* to total phytoplankton biomass by examining previously unused data obtained with flow cytometry. Applying a cell C content of 0.06 pgC $cell^{-1}$ (based on Buitenhuis et al. 2012), the typical *Prochlorococcus* abundances measured at the DCM (ca. 40,000 cell mL^{-1}) represent a C biomass of ca. 2.4 mgC m^{-3}. By contrast, *Prochlorococcus* was undetected in the upper layers (0-30 m). In the revised version of the manuscript, total phytoplankton biomass will be recalculated taking into account also the contribution of *Prochlorococcus.*

References

Marañón (2015) Cell size as a key determinant of phytoplankton metabolism and community structure. Annual Review of Marine Science, 7, 241-264. doi: 10.1146/annurev-marine-010814-015955

Huete-Ortega et al. (2011) Isometric size-scaling of metabolic rate and the size abundance distribution of phytoplankton. Proceedings of the Royal Society B, 279, 1815-1823. doi:10.1098/rspb.2011.2257.