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Interactive comment on "Bio-optical characterization of subsurface chlorophyll maxima in the Mediterranean Sea from a Biogeochemical-Argo float database" *by* Marie Barbieux et al.

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We thank Reviewer #1 for his/her comments, which are displayed in blue font in this document. Our responses and description of any action taken in the revised manuscript follow each comment in black font.

1) Although SCMs have been assessed before in different regimes, a key strength of the paper is that a gradient across contrasting regimes is presented. The use of schematic diagram Fig12 was very helpful to illustrate how the vertical profiles vary

across this gradient. However, of key importance to driving the SCM dynamics across the different regimes is the physical forcing, and thus physical structure of the water column, and I felt the physical context was somewhat neglected in the data analysis and interpretations. Some comment is included in the later discussion (Fig 10) but I would urge the authors to consider adding a few sentences or short paragraph on the underlying physical controls in the different regimes and, in particular, consider adding the thermocline (or MLD?) to the schematic (Fig 12). It could also be useful to add to Fig 7 and/or 9 as well. Placing the observations into the physical context would provide a more complete explanation of the data presented and would help apply what is learnt to other regions globally.

Response: We agree with the Reviewer that the underlying physical controls of the SCM dynamics were not sufficiently considered in our analysis. Therefore we made substantial modifications to our manuscript in order to account for Reviewer #1 and #2's comments. We chose to consider the Mixed Layer Depth (MLD) as it seems to be a more complete indicator of the physical processes than the thermocline. In addition both the MLD and the thermocline had very similar temporal evolution hence we decided to represent only the MLD on the different figures for a better readability. We represented the value of the Mixed Layer Depth (MLD) on Figure 5, on Figure 7 and on the schematic representation of the different situations of SCMs in the Mediterranean Sea during the oligotrophic summer period shown in Figure 12. We also analysed the difference between the MLD and the nutricline depth and reported this information on Figure 6e. Our results indicate that the summer MLD exhibits very similar values among the considered regions and that, on the opposite, the winter MLD shows significantly different values between the Western and Eastern Basins. Hence, we suggest that the different mixing regimes and subsequent nutrient supply to the surface layer of the ocean may explain the seasonal succession and the amount of typical shapes of SCMs in the various regions of the Mediterranean Sea. For example, in the Northwestern region of the Mediterranean Sea, substantial mixing occurs during the winter period (MLD deeper than the nitracline) inducing a seasonal renewal of the nutrients available in the surface and subsurface layers. In this region, 4 types of profiles of Chla and bbp are retrieved along the annual cycle and an SBM is observed during the oligotrophic period. On the opposite, in the Levantine Sea, the MLD is significantly shallower than the nitracline all year long, the upward diffusive flux of nitrates is weak and a SCM is systematically observed during the summer season.

To account for this comment, we modified the text in Section 3.1.3 (line numbers refer to the revised manuscript) as follows:

Âń To explore the light-nutrient regime within the SCM layer, a monthly climatology of the isolume and nitracline in the different considered regions was represented along with the depth of the Subsurface Chla and bbp Maxima (i.e. SCM and SbbpM, respectively). The MLD was also superimposed in order to illustrate physical forcings (Figure 5).

In the Western Basin, the isolume 0.3 mol quanta m-2 d-1, the nitracline 1 μmol, the SbbpM and the SCM were all located at a similar depth during the oligotrophic period (maximum depth difference < 20 m; Figures 5a-c). In accordance with previous findings (e.g. Pasqueron de Fommervault et al., 2015a), our results suggest that in the NW region of the Mediterranean Sea, the winter deepest climatological mixed layer depth reached the nutricline, thus likely inducing nutrient input to the surface layer.

In the TYR region, the MLD was always shallower than the nutricline during the winter season but the difference between the MLD and the nutricline remained very small all year long. Hence, in the Western Basin of the Mediterranean Sea both light and nutrient resources may be available at the level of the SCM to support an actual increase in phytoplankton biomass. In the Northwestern part of the Mediterranean Sea, the MLD was deeper than the nutricline $\sim$20% of the time during an annual cycle (Figure 6e) essentially during the winter season (Figure 5 a-c). The shallowest (median of 61 m; Figure 6c) and the steepest (slope of 90 μmol m-4; Figure 6d) nitraclines were also recorded in this region, thus confirming an important upward diffusive flux of nitrates

available to sustain phytoplankton biomass and eventually allowed the occurrence of a Subsurface Biomass Maximum.

In contrast, in the ION and LEV regions, the isolume 0.3 mol quanta m-2 d-1, nitracline 1 μmol, SCM and SbbpM were not collocated in the water column (Figures 5d-e). The SCM was located $\sim$50 m above the nitracline during the stratified period (Figures 5d-e and 6a) and the SbbpM was shallower than the SCM (by $\sim$40 m), suggesting that the standing stock of carbon is maintained at a higher concentration above the depth of the SCM. In the Eastern Basin (Ionian and Levantine Sea), the MLD almost never reached the nutricline even during the winter period as it was deeper than the nutricline only <3% of the time during an annual cycle (Figure 6e).Âż (p. 17-18, l. 396-423)

We modified the text in Section 3.2 as follows:

Âń The mixed shape was characterized by a homogeneous distribution of Chla and bbp (as suggested by the deep mean MLD associated with this type of profile; Figures 7a-b) and showed occurrence exceeding 60% from December to March (Figure 8a). Âż (p. 21, l. 501-504)

Âń In the Levantine Sea, only two distinct shapes were encountered, i.e. the SCMaZeu and the SCMbZeu shapes and associated with shallow MLDs (Figures 7i-j). Âż (p. 23, l. 549-550)

We also modified the text in Section 4 as indicated below:

Âń 1) The SBMaZeu is a Subsurface Biomass Maximum that settles above the euphotic zone in the Northwestern Mediterranean Sea (NW). It is the thinnest ($\sim$40m) and shallowest ($\sim$60 m) biomass maximum. It is also the most intense, probably because it benefits from adequate light and nutrient resources, with the deep mixed layer occurring in this region during the winter period probably inducing a seasonal renewal of the nutrients in the surface layer.

2) The SBMbZeu establishes below the euphotic zone in the NW. As well as the SBMs

of the Southwestern Mediterranean Sea (SW) and Tyrrhenian Sea (TYR), less intense than the SBMaZeu probably because nutrients conditions are less favourable than in the NW region as the winter MLD is close to, but never reaches the nutricline.

3) The SCM of the SW and TYR as well as the SCMaZeu (i.e. settling above the euphotic depth) of the Ionian (ION) and Levantine (LEV) Seas are not biomass subsurface maxima, but reflect Chla maxima resulting from photoacclimation. Moving from the SW to LEV region, the amplitude of the SCM decreases while its thickness increases.

4) The SCMbZeu of the ION and LEV settle below the euphotic depth and are deeper (∼95 m) than all the other subsurface maxima. They are most probably the consequence of a decoupling of the MLD and the nutricline and represent the oligotrophic end-member type of subsurface maxima in the Mediterranean Sea.Âż (p. 27-28, l. 644-660)

2) Line 29: suggest change "to understand which parameter controls the SCMs" to "to understand the main controls on the SCMs".

Response: We modified the sentence as suggested by Reviewer #1: Âń Finally, a case study was performed on two contrasted regions and the environmental conditions at depth were further investigated to understand the main controls on the SCMs. Âż (p. 1 l. 27-29)

3) Line 62: "their contributions to the depth integrated-production [. . .] remains largely unknown [. . .]". The use of the Arctic example here is a bit of an odd choice, other examples could be added, for example the contribution is >40% in the oligotrophic Atlantic (Perez et al. 2006 Deep Sea Res 53:1616), 40-50% in the Celtic Sea (Hickman et al. 2012 MEPS 463:39); 58% in the North Sea (Weston et al. 2005 JPR 27:909). (The paper by Perez et al. is shows nicely the decoupling of Chl -a maxima, carbon maxima (idea of Chl:C), thermocline, nitracline and 1% light depth in oligotrophic regimes that could be relevant to other statements about oligotrophic conditions as well).

Response: Following the Reviewer's advice, we modified the sentence (line 62) giving examples of the underestimated production associated with the SCM in different regions of the global ocean: Âń Their contribution to the depth-integrated primary production has been assessed for a limited number of regions and remains largely unknown. It has been reported to be underestimated from 40 to 75% in the Arctic Ocean (Ardyna et al, 2013; Hill et al, 2013), to more than 40% in the oligotrophic Atlantic (Perez et al., 2006), 40-50% in the Celtic Sea (Hickman et al., 2012) and about 58% in the North Sea (Weston et al., 2005). Âż (p. 3 l. 60-65)

4) Lines 73-81. "[. . .] Hence, this "miniature ocean" presents SCMs that may be encountered in both temperate environments and stratified waters of the global ocean". I found contrasting "temperate" and "oligotrophic" and/or "stratified" a bit confusing (many temperate regions are stratified), as it's not quite clear which properties of these different regimes are the relevant ones (seasonality? stratification? nutrient status? Maybe all of these?). Using 'seasonally stratified' vs 'permanently stratified' would be more precise?

Response: We thank Reviewer #1 for this comment. Accordingly the terms 'seasonally stratified' vs. 'permanently stratified' are now used in the whole manuscript in order to avoid confusion: Âń Hence, this "miniature ocean" presents SCMs that may be encountered in both seasonally stratified environments and permanently stratified waters of the global ocean. Âż (p. 3 l. 82-83). We also modified the sentence: Âń In permanently stratified oligotrophic ecosystems, [. . .] Âż (p. 28 l. 666-667).

5) Methods Section: Please describe what (if any) correction for non-photochemical quenching was applied to the Chl-a fluorescence data.

Response: We added a sentence in Section 2.2 accordingly to the Reviewer's comment: Âń This procedure included a correction of non-photochemical quenching for Chla following Xing et al. (2012) method. Âż (p. 6 l. 142-143).

6) Line 146. Please give a reference for the quoted regional correction factors, or

describe how they were obtained.

Response: Following this comment and in order to better describe the Roesler et al. (2017) correction factor, we added the following sentences to Section 2.2:

Âń In addition, we applied a correction factor to Chla fluorescence measurements from the BGC-Argo floats, following the recommendation of Roesler et al. (2017). Comparing estimates of Chla from the WET Labs ECO fluorometers (used on BGC-Argo floats) with Chla estimates from other methods, these authors evidenced a bias varying according to the region sampled. In order to quantify this bias, they calculated the slope of the relationship between the Chla values from the ECO fluorometers and those estimated independently using HPLC analyses. This bias was further confirmed using optical proxies such as in situ radiometric measurements (Xing et al. 2011) or algal absorption measurements (Boss et al. 2013; Roesler and Barnard et al. 2013). At a global scale, Roesler et al. (2017) evidenced an overestimation of the Chla concentration by a factor of 2, on which regional variations of the fluorescence-to-Chla ratio are superimposed. Âż (p. 6 l. 143-154).

7) Line 153: "0.03 kg m-3 density criterion", please describe what the criterion is.

Response: In response to the Reviewer's comment we added the following sentence: Âń After binning the data at a 1-m resolution, the mixed layer depth (MLD) was derived from the CTD data using the density criterion of de Boyer Montégut (2004). The MLD was calculated as the depth where the density difference compared to the surface (10 m) reference value is 0.03 kg m-3.Âż (p. 7 l. 164-167).

8) Line suggest changing "Occidental" and "Oriental" to a description more geo/oceanographic.

Response: We used "western" and "eastern" instead of "Occidental" and "Oriental" according to the Reviewer's suggestion: Âń Similarly, the seasonal cycle of bbp in the SCM was more pronounced in the Western part of the Mediterranean Sea than in the

Eastern Basin. Âż (p. 15 l. 358-359).

9) Line 375: Suggest change "and presents an actual increase in phytoplankton biomass" to "that we propose supports an actual increase in phytoplankton biomass".

Response: We modified the sentence accordingly: Âń Hence, in the Western Basin of the Mediterranean Sea both light and nutrient resources seem to be available and probably support an actual increase in phytoplankton biomass (Figures 5 and 6a-b). Âż (p. 17, l. 408-410).

10) Line 384: Suggest change "suggesting no accumulation of carbon at the SCM". It's unclear what you mean by accumulation here (implies sinking?), also carbon at a subsurface bbp peak isn't necessarily accumulating. There is likely some generation and turnover of carbon at all depths in the water column, but the standing stock of biomass is maintained at a higher concentration at the depth of the bbp peak than the depth of the SCM. Suggest using more precise wording here.

Response: We agree with Reviewer #1 that the wording "accumulation of carbon" is not precise enough and, hence, modified the sentence accordingly: Âń The difference between the depths of the SCM and nitracline was $\sim$50 m during the stratified period (Figures 5d-e and 6a) and the SbbpM was shallower than the SCM (by $\sim$40 m), suggesting that the standing stock of carbon is maintained at a higher concentration above the depth of the SCM. Âż (p. 18 l. 418-422)

11) Line 393: please change "is, thus, limited by both the availability of light and nutrients" to "is, thus, likely to be limited by both light and nutrients". No measurements were made to assess whether phytoplankton were light or nutrient limited.

Response: We modified the sentence as follows: Âń The development of the SCM in this system is, thus, likely to be limited by the availability of both light and nutrients. Âż (p. 18 l. 432-434).

12) Line 412-426. I found this section a little jumbled. The section on vertical species

distributions and low light ecotypes seemed a bit out of place and it wasn't clear how it linked to the results presented. I suggest moving Lines 420-425 (which seem to provide the link) further up in this section, and re-consider the wording elsewhere to make the discussion easier to follow. The key points are there: that different phytoplankton species or ecotypes are likely to have different depth and magnitude of C and Chl maxima, different Chl:C, and different bbp properties; gradients in taxa are likely (expected?) in stratified water columns, including through SCMs; and there are vertical gradients in the non-phytoplankton particles that contribute to bbp as well. Consequently, the overall Chl, C, and bbp profiles are the result of all taxa present, their bio-optical properties and their physiology, but it is not possible to tease these apart with the data. This is contained in the existing text, but could be clarified.

Response: We modified Section 3.1.4 to clarify the discussion on this point:

Âń We have seen that the SCM of the Western Basin benefits from both light and nutrient resources. In these conditions, the observed simultaneous increase in Chla and bbp at the SCM most likely represents an actual development of phytoplankton biomass, as indicated by the concordance between the depths of the SCM and the SbbpM (Figure 5). On the opposite, in the Eastern part of the Mediterranean Sea, the maxima of Chla and bbp are not co-located. This result suggests that environmental conditions, typically the light conditions, might inhibit the increase in phytoplankton biomass.

In the Eastern Basin of the Mediterranean Sea, the microorganisms are, most probably, acclimated or even adapted to the environmental conditions. While photoacclimation is defined as a short-term acclimation of a photosynthetic organism to changing irradiance, photoadaptation refers to the long-term evolutionary adaptation of photosynthetic organisms to ambient light conditions, through genetic selection. SCM species are known to use different strategies such as photoacclimation to low light (i.e. increase in the intracellular pigment content), mixotrophy or small-scale directed movements towards light (Falkowski and Laroche, 1991; Geider et al., 1997; Clegg et al.,

2012). Phytoplankton species are also likely to have different carbon-to-chlorophyll ratio (Falkowski et al., 1985; Geider, 1987; Cloern et al., 1995; Sathyendranath et al., 2009) and bbp properties (Vaillancourt et al., 2004; Whitmire et al., 2010), and a vertical shift toward species photoadapted to the particular environmental conditions prevailing in the SCM layer is a well-known phenomenon (e.g. Pollehne et al., 1993; Latasa et al., 2016). For example, two ecotypes of Prochlorococcus, characterized by different accessory pigment contents are known to be adapted to either low-light or high-light conditions and to occupy different niches in the water column (Moore and Chisholm, 1999; Bouman et al., 2006; Garczarek et al., 2007). In particular, the low-light ecotype, characterized by increased intracellular pigmentation, has been frequently observed at the SCM level in the Mediterranean, especially in the Eastern part (Brunet et al., 2006; Siokou-Frangou et al., 2010). A west-to-east modification in the composition of phytoplankton communities in the SCM toward a dominance of picophytoplankton species adapted to recurring light limitation, has been observed (Christaki et al., 2001; Siokou-Frangou et al., 2010; Crombet et al., 2011). A vertical decoupling between bbp and Chla could thus illustrate either a photoacclimation of phytoplankton cells or the occurrence of specific phytoplankton communities adapted to the conditions prevailing in the SCM layer.

Although photoacclimation seems to be a widespread hypothesis in numerous studies to explain the vertical decoupling of Chla and bbp (e.g. Brunet et al., 2006; Cullen, 1982; Mignot et al., 2014), it should yet be reminded that this decoupling could also result from a change in the nature or size distribution of the entire particle pool. Small particles are, for example, known to backscatter light more efficiently than large particles (Morel and Bricaud, 1986; Stramski et al., 2004). A higher proportion of nonalgal particles in the Eastern compared to the Western Basin could thus explain the decoupling between bbp and Chla. The nonalgal particles compartment is defined as the background of submicronic living biological cells (i.e. viruses or bacteria) and non-living particles (i.e. detritus or inorganic particles) and is typically known to represent a significant part of the particulate assemblage in oligotrophic ecosystems (Morel and

Ahn, 1991; Claustre et al., 1999; Stramski et al., 2001).

Finally, photoacclimation processes as well as vertical gradients in phytoplankton species or in the non-phytoplankton particles, also contributing to bbp, could explain the vertical decoupling of bbp and Chla we observed in the Eastern Basin. The different types of Chla and bbp vertical profiles depends on both the nature of the particles present in the water column, the physiology of phytoplanktonic cells and their related bio-optical properties, but yet our dataset did not allow us to conclude on the dominance of one process compared to the other.Âż (p. 18-20 l. 437-485)

13) Line 464: I suggest a very short description of what the “light driven hypothesis” is here.

Response: We added a few sentences to address this comment: Âń These authors observed that the seasonal variation of the depth of the SCM depicts the same displacement as the isolumes and consequently suggested that the SCM depth displacement is light-driven. Âż (p. 22 l. 516-518)

14) Line 581: “(1) SCMs arising from an actual increase in carbon biomass at depth (or SBMs) and benefitting from both light and nutrients”. I think you have to be a little careful here because the data didn’t unequivocally show that phytoplankton biomass increased (the bbp max could be due to non-phytoplankton particles). Throughout the rest of the paper great care has been taken not to overinterpret bbp as phytoplankton carbon and to make careful statements about Chl-a:C (photoacclimation) with due consideration of non-phytoplankton particles contributing to the bbp signal. So, I suggest it’s worth making sure this summary statement is equally precise. If by ‘carbon biomass’ you are being more general to include all plankton then say so, and distinguish from ‘benefitting from both light and nutrients’.

Response: We thank Reviewer #1 for this comment and modified the sentence accordingly:

Âń (1) SCMs arising from an actual increase in carbon biomass most probably reflecting an increase in phytoplankton biomass benefiting from both light and nutrient resources (SBMs) with a potentially non negligible contribution of non-phytoplankton particles at depth Âż (p. 26-27 l. 633-636).

15) Throughout: the use of term “in the SCM layer” is often ambiguous as to whether you mean “at the SCM peak” or “integral within the SCM layer”. For example, in the figure caption of Figure 3 and 4 it is not clear whether what’s plotted is the Chl-a concentration at the SCM peak or an integrated Chl-a concentration through the SCM layer. The units (mg m-3) indicate the peak magnitude, but the words “in the SCM layer” imply the integral.

Response: To clarify the term “in the SCM layer”, we added a section of explanation in Data and Methods:

Âń 2.5. Definition of the SCM Layer To study specifically the dynamics of the bio-optical properties in the SCM layer, we adjusted a Gaussian profile to each vertical profile of Chla of the database that presented a subsurface Chla maximum and computed the width of this SCM. This parameterizing approach proposed by Lewis et al. (1983) has been widely used to fit vertical profiles of Chla (e.g., Morel & Berthon, 1989; Uitz et al., 2006) such as:

$c(z)=c\_max\ e\hat{}(-(((z-z\_max)/\Delta z)\hat{}2))$ (5)

where c(z) is the Chla concentration at depth z, cmax is the Chla concentration at the depth of the SCM (zmax), and Δz, the unknown, is the width of the SCM. In order to retrieve Δz, the unknown parameter, we performed an optimization of equation (5) with a maximum width set at 50 m so only the profiles with a relatively pronounced SCM are kept. Finally, in this study, the different biogeochemical variables are averaged in this SCM layer (cf. Figures 3, 4, 6 and 11)Âż (p. 12 l. 300-312).

16) Throughout: check that any abbreviations for Mediterranean Sea are used appro-

priately (Mediterranean Sea is used at the beginning but after a point “Med Sea” is used, e.g. Line 485).

Response: Throughout the text we replaced the abbreviation “Med Sea” by “Mediterranean Sea”.

In addition to our responses to Reviewers #1 and #2, we modified Figure 1 for a better presentation of the BGC-Argo dataset. We underlined in black the trajectories of the BGC-Argo float of the Gulf of Lions and Levantine Sea that are used in Figure 10 and 11. We also modified the scale in Figure 9, 10 (a and c) and 11 for a better clarity. In Figure 9, we systematically adjusted the abscise axis between 0 and 0.8. In Figure 10, we modified the legend of the time scale of the float trajectories (Figure 10 a and c). In Figure 11, we adjusted the abscise axis from 0 to 0.16 (Figure 11a) and from 0 to 0.04 (Figure 11b).

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