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Interactive comment on “Is the distribution of *Prochlorococcus* and *Synechococcus* ecotypes in the Mediterranean Sea affected by global warming?” by D. Mella-Flores et al.

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General comments Reviewer: The manuscript presents cyanobacterial abundance and molecular diversity data from a research cruise in the Mediterranean Sea and compares the results from a previous occupation several years prior. In general the data and analyses are of high quality and the results of the study are quite interesting and the manuscript is well-written. It is apparent that the authors spent a lot of time collecting, processing and analyzing the data and the results highlight that. The Mediterranean Sea is a particularly interesting region oceanographically so the results presented here, are important contribution to the field – and the fact that the results are

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somewhat counter-intuitive makes them even more important to distribute. Although the data and analyses are interesting and point to future areas of research, I am somewhat confused by how the study has been framed and some of the specifics of the interpretation of the results, which are discussed in the specific comments section. Taking into account those comments below and others' and based on your results and interpretation already, you'll have a solid contribution.

Response: There is no response to provide here since we address all referee's concerns in the specific comments part below.

Specific Comments:

Reviewer: (1) The framing of the results. I'm not convinced of the temporal comparisons of the datasets or of the "global warming" comparison. Although conceptually interesting, the reality is that most of the stations and transects are different (fig 1). Further, based on the time-series studies that you cite (BATS, HOT) there is substantial variability even at a given station location over a year so two end points are less convincing. If you do head down this path, you'll have to make a more statistically convincing argument on how the environmental variables have changed (or not) and how these influence populations. For example, is the temperature increase (at most 1 C for station C) over the last 25 years really expected to cause changes to the cyanobacteria. A more plausible effect would be changes to nutrient inputs or top down effects.

Response: The referee is right to say that most of the stations and transects are different between PROSOPE and BOUM and that the two cruises were done at different times of the year, and thus are not fully comparable, even though hydrological conditions were broadly similar since in both cases the water column was strongly stratified and the upper mixed layer was (in general) highly oligotrophic and exhibiting comparably high sea surface temperature (SST). To answer the referee's critics, we have, however, put much less emphasis on the temporal comparison between the two cruises in the revised version of the text, i.e. we now avoid to suggest that they represent

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two stages of the Mediterranean Sea at different times of the global warming process. Thus, a number of modifications in the initial text have been made including in the abstract, at the end of the introduction and in the discussion part (end of paragraph 4.1).

Still, we maintain that the question of an eventual effect of global warming on cyanobacterial population structure is quite relevant. Firstly, the trend in SST anomaly that was observed in the Levantine basin over the last 25 years is highly significant (at $P < 0.01$) and if it continues at this rate, this would correspond to a 5°C increase per century, in line with worst case scenarios of the effects of global warming on ocean temperature (Coll et al., 2010; Hertig and Jacobeit, 2008; Somot et al., 2006). Secondly, recent literature on the distribution of *Prochlorococcus* HLI and HLII ecotypes (e.g. Johnson et al. 2006, Zinser et al. 2007) clearly identifies seawater temperature as the main factor explaining the different latitudinal distribution of these two ecotypes. The same is true for *Synechococcus* clades I and IV (temperate clades) with regard to clade II (tropical clade; Zwirgmaier et al., 2007, 2008). So, it was important to raise the question of whether there have been changes in the distribution of these biomarkers (and our response is clearly: so far, no!) and whether such change may occur in future. It is quite possible, as the referee argues, that changes in nutrient inputs may occur (or have already occurred) in the Mediterranean Sea and have effects on the composition of cyanobacterial communities, and we evoke this possibility in the revised discussion. But we chose to keep our initial focus on SST for which we have solid background data with high temporal and spatial resolution over a long period of time, including those from PROSOPE and BOUM cruises.

Reviewer: (2-a) The discussion on page 4300 surrounding the dominance of *Synechococcus* is confusing to me given the data in Table 2 and Figures 2, 4 and 5 that show that *Prochlorococcus* is the numerically dominant cyanobacteria. If you measured mono-vinyl chlorophyll a, you could estimate the percentage contribution of *Prochlorococcus* using the divinyl portion, which could help to solidify your position

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(or refute it).

Response: North of the western basin, both populations could be discriminated by flow cytometry up to the surface, so we are sure that they were either co-dominating (Sta. 27) or that *Synechococcus* was the dominant organism (Sta. 25). This is actually consistent with the integrated concentrations reported in Table 2 for these two stations. However, the referee is right that given the absence of reliable counts for *Prochlorococcus* in the upper layer of the more oligotrophic waters located further South and East, we cannot be sure that *Synechococcus* actually "dominated" (i.e. was more abundant than *Prochlorococcus*) in this layer. So we modified the original sentence to make clearer these points, as follows (the underlined text has changed): "In the northern part of the Algero-Provencal basin, both genera formed subsurface abundance maxima and they either exhibited comparable cell densities (e.g. Sta. 27) or the latter genus outnumbered the former one (e.g. at Sta. 25 at all depths). This type of distribution is typical of mesotrophic areas (Partensky et al., 1996; Partensky et al., 1999). In the southern part of the Algero-Provencal basin, the Sicily Strait and the eastern Ionian Sea, *Prochlorococcus* was the dominant group at depth, while *Synechococcus* was most abundant in the upper mixed layer, with abundances usually above 104 cells ml⁻¹. However, because *Prochlorococcus* cells could not be precisely enumerated in the top 50 m or so (see methods), we cannot ascertain which of the two picocyanobacterial genera numerically dominated in this layer."

Note that contrary to the referee's suggestion, it is not possible to use the proportion of mono- and divinyl chlorophyll to firmly establish which organism dominates in the upper layer because not all the monovinyl Chl is attributable to *Synechococcus*.

Reviewer (2-b) Putting Figure 2 D and 2E on the same scale would help the reader make a better comparison between the populations, at least in terms of biomass.

Response: The use of a same scale for Fig. 2D and 2E would indeed facilitate the comparison between *Prochlorococcus* and *Synechococcus* populations but it make it

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impossible to discern local patches of *Synechococcus* abundance in the oligotrophic waters. With a scale set as for *Prochlorococcus* concentration, all *Synechococcus* data are shown in pink along most of the BOUM transect. Thus, we chose to keep distinct scales for both picocyanobacteria genera. However, in the revised version, we now draw the reader attention to this point by adding the following sentences to the legend: "Note that to better emphasize the differential distributions of *Prochlorococcus* and *Synechococcus* along the BOUM transect, different scales have been used in Fig. 2D and 2E"

Reviewer: (2-c) Also, I don't really follow the low-P argument for the "dominance" of *Synechococcus*. I think a more likely possibility is Cu toxicity, at least near the surface (Mann et al. 2002).

Response: To answer this comment and also because the low-P argument did not convince the other referee either, we reworked the discussion part of the revised manuscript and considerably reduced our arguments on P. Also, we now cite the work by Mann et al. (2002) in the revised text, as follows: "These atypical vertical profiles might in part be explained by other factors than macronutrient depletion, and in particular by copper toxicity, since the annual Cu deposition rate is quite high in the eastern Mediterranean Sea (1-2 mg m⁻² yr⁻¹; Paytan et al., 2009). Indeed, *Prochlorococcus* growth was shown to be strongly affected by fairly low concentrations of this element, while *Synechococcus* cells can usually withstand higher concentrations (Mann et al., 2002). A more recent report claimed that some copper-rich Saharan dust had a more severe toxic effect on the growth of *Synechococcus* in comparison to *Prochlorococcus* growing in the Red Sea (Paytan et al., 2009). It is therefore possible that, after events of dust deposition, abundances of both picocyanobacteria in the upper mixed layer of the eastern Mediterranean Sea may strongly vary over short time scales, in a direction depending on the nature and origin of aerosols."

Yet, we don't want to put more focus than is reasonable on the eventual role of copper because i) no measurements of copper concentrations were made during any of the

two cruises reported here and ii) there are contradicting data on the effect of copper on *Synechococcus* in the literature, some studies having concluded that *Synechococcus* is resistant to Cu (at least more than *Prochlorococcus*, Mann et al. 2002) and others that *Synechococcus* is particularly Cu sensitive (Paytan et al. 2009). Furthermore, the latter authors suggested from modeling that fluxes of copper deposition into the Mediterranean Sea did not change significantly since the pre-industrial era.

Reviewer: (3) One of the more interesting findings from this study is the dominance of the HLI *Prochlorococcus* clade following the work of Garczarek et al. 2007. Perhaps this can be brought out more by exploring its relationship to environmental variables a bit more. This finding as well as the refining of niches of some of the *Synechococcus* clades is most compelling part of the observations.

Response: We are already discussing in detail in section 4.2.1 the dominance of HLI in this region and especially in the context of why HLII ecotypes are so scarce.

Technical Corrections: Reviewer: Pg 4286 line 6: do you really mean “global warming.” Shouldn’t this be “global climate change” or may be just “environmental change.” No doubt a host of environmental variables have or could have changed over this period.

Response: Change made as suggested

Reviewer: Pg 4287 line 3: quantify “gentle filtration”

Response: We preferred to simply remove the term "gentle" which is highly imprecise and depends on multiple factors, such as pump characteristics, pump speed set up, tube and filter characteristics, etc. This would add a lot of useless details which are not so useful for the experiment.

Reviewer: Pg 4290 line 21: The real sequence accession numbers need to be added.

Response: Change made as suggested

Reviewer: Pg 4292 line 22: It would be good to remind us when the PROSOPE cruise

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occurred.

Response: It is mentioned in this sentence that the cruise was done in late summer-early fall and the exact dates are given in the Methods section just a few paragraphs above. So we think it is really redundant to give the dates again here.

Reviewer: Pg 4300 lines 19-25: There has also been similar work done with *Prochlorococcus* (Mann et al. 2002) and this should be referenced and fully considered as this seems like it could explain some of the findings here.

Response: The work by Mann et al. (2002) is now mentioned twice in the revised text, but later in the discussion where we think it was more relevant. Indeed the part mentioned by the referee is focused on macronutrients.

Reviewer: Pg 4322, Table: Please list the integration depth (or feature).

Response: The deeper samples counted by flow cytometry were taken at 200 m at all stations, so we added "over the top 200 m " in the legend of Table 2.

Reviewer: Pg 4325 Figure 3. You can delete the full equations and just put the slope with standard deviation for the estimate. You should also say if this slope is statistically different from zero.

Response: Change made as suggested. The slope was not statistically different from zero at Sta. A ($P=0.435$, i.e. $P>0.05$). in contrast, at Sta. B. and C, it was significantly different from zero with P-values of 0.026 (i.e. <0.05) and 0.0000 (i.e. <0.01), respectively. We also noticed that we did a mistake when plotting the trend for August SST at Sta. A in Fig. 3A. So the revised Fig. 3A and corresponding text have been corrected accordingly. We also somewhat shortened this part of the results section which was a bit lengthy.

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