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Comment

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.

Anonymous Referee #2

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General Comments

This discussion paper by Mella-Flores et al. addresses an interesting and timely question as to whether the composition of the picocyanobacteria assemblages in the Mediterranean Sea have been impacted by rising temperatures over the past decade. To do this the researchers assessed the total population size of both *Synechococcus* and *Prochlorococcus* using flow cytometry as well as ecotype composition using dot blot hybridization with clade-specific probes on cruises 9 years apart. The first was in September 1999 and the second in July 2008. The methodology used is generally appropriate and was well implemented. The findings are interesting and perhaps even

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a little surprising, that despite an increase in water temperatures between the two sampling periods: that the ecotype composition is generally similar between the two cruises and ecotypes known/thought to be more adapted to higher temperatures have not significantly increased in their relative abundance. The authors therefore conclude that, at least so far, increased temperatures have not led to changes in picocyanobacterial composition in the Mediterranean Sea. The manuscript is generally well written and the major conclusions valid. I have mainly minor comments. However, one aspect that complicates interpretation of the findings, mainly for the differences that were found for *Synechococcus* assemblages, is the different seasons in which the cruises took place. This coupled with the lack of seasonal analysis to assess whether seasonality could explain some of these differences, and lack of multi-year analysis to ascertain the reproducibility of the findings year after year, renders some of the discussion overstated. Furthermore, some of the Discussion is not sufficiently backed up by data or focused enough, and occasionally also contradicts itself (see Specific Comments for details). Therefore, besides some minor changes to the manuscript, the Discussion needs to be shortened and focused and some of the arguments revisited.

Specific Comments

Methods:

It should be noted here that surface populations of *Prochlorococcus* can not be accurately enumerated by flow cytometry. Alternatively, this could be mentioned in the Results when *Prochlorococcus* abundances are first mentioned, on page 4294. Right now this is stated in passing in the Discussion only on page 4301 line 13.

P4289, line 14-20: The normalization procedure needs to be clarified. The authors state that relative hybridization of the clades is to total oxygenic phototrophs yet a general eubacterial probe was used for this. It seems to be explained in a clearer manner in the figure legend of Figure S1 and perhaps an explanation similar to this should be included in the Methods.

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Results:

P4293, line24. It should be clarified in the text that the analyses being discussed are total genus abundances as determined by flow cytometry (my assumption) or combined hybridization blots (if this is actually the case).

P4294, lines 18-29. It would be nice if maximum and minimum integrated numbers for Prochlorococcus would be presented in a similar way in which these were provided for Synechococcus.

P4294, line28. State how it was determined that the increase in dvChla was due to strong photoacclimation, with “as determined from. . .” Was chla per cell assessed by flow cytometry and found to increase with depth? If not, then please qualify statement with “probably due to. . .”

P4296, lines 12-21. Isn't it true that clade I was more abundant than clade IV at Sta 5 on both cruises? Please change wording to clarify this.

P4296, lines 26-28. According to Fig. 6, clade IV was considerably more abundant than clade III in the Alboran Sea (assuming that the scale for clade IV for this figure is accurate – see comment below).

P4298, line 20. No Synechococcus or no clade I Synechococcus? Please clarify.

Tables and Figures

Table 1: It is not so clear what the reference sequence refers to despite the footnote. That used to design the probe? In fact the meaning of the footnote is not clear and I could not find what the authors are referring to in the Methods on page 4289. It would be more useful for the reader if, rather than the actual reference sequences used to design the probes, strain names representative of the clades that have the most meaning to the reader be used. I propose that these be the fully sequenced strains for Synechococcus that are published in Dufrense et al. 2008 and Scanlan et al. 2009. For example this would include CC9605 and WH8109 instead of RS9903, WH8102 instead

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of WH8103, CC9902 and BL107 for clade IV and so on. For *Prochlorococcus* these should correspond to the representative ecotypes discussed in the introduction and presented in the legend of figure 4. For example, use MIT9312, MED4 and MIT9313. If the reference sequence itself is important to retain in the Table, then this information should be included as an additional column of the table. There appears to be a typo (a number: 5 x 15) instead of a reference sequence strain for the SYN635 probe.

Figure 2: Please clarify in the 4th line of the legend that the x-axis represents the cumulative distance towards to the east from the mentioned site.

Figure 5: It would be very helpful to the reader if a clade strain representative of each clade be presented in the legend as is done for *Prochlorococcus* in the Figure 4 legend. I suggest these strains be those that have had their genomes fully sequenced (see comment regarding Table 1).

Fig. 5 and 6: Is the scale for clade IV in figure 5 and figure 6 supposed to be different – 12

Figure 7: The type is too small to see in a printed version of the figure. It needs to be increased significantly or else the entire figure needs to be larger to make the print readable. Also on a screen it is necessary to increase the figure to a very large size to read the print easily, which prevents visualizing the entire tree all at once. Please provide a key for the level of shading on the right side of the figure. Legend should read 0.07 substitutions rather than substitution.

Figure S2: These trees are also impossible to read with the font size used. Placing fewer trees on the page and increasing their size will help.

Discussion:

P4300, lines 10-13: Are the abundances of *Synechococcus* really higher than *Prochlorococcus* in the surface waters in the southern part of the Algero-Provencal basin, Sicily Strait and Ionian Sea when one considers that flow cytometry cannot de-

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fect surface populations of *Prochlorococcus*? Are these findings also borne out from the dot blot hybridizations? This does not seem to be the case for these waters when comparing figure 4 and 5, assuming that both *Synechococcus* and *Prochlorococcus* are normalized to the same parameter in the same way. This should also be double-checked for the northern Algero-Provençal basin (discussed in lines 5-8), although it seems to be possible from the dot blots, but it is hard to ascertain because of differences in scale in Figure 4 and 5. and the need to combine a number of *Synechococcus* clades.

P4300, lines 13-18. Near surface maxima for *Synechococcus* occurs during blooms soon after stratification of the water column (see Lindell and Post 1995, DuRand et al. 2001). Could this be the situation here? Is much known about the water column conditions at these stations in the month or so prior to sampling?

P4300, lines 19-21: I don't think sufficient information is available to state that there is maintenance of high *Synechococcus* cell densities in surface waters. Is this the case year round? And from year to year? This is not so clear from the results regarding the Proscope cruise.

The ensuing Discussion from P4300 line 20 to P4301 line needs to be revisited in light of the responses to the above questions and written in a more focused and concise manner.

P4302, line 7: Do the authors mean HL (i.e. either HLI or HLII)?

P4302, line 12, 17: I wonder if the term "true LL" ecotype is appropriate. The LL nature of the ecotypes is related to their adaptation to LL levels, not to their ecological position in the water column. As such these could be "true LL" adapted strains that are found higher up in the water column. According to Malmstrom et al. (2010) it is not that the LLI (eNATL) ecotype is less adapted to LL than the other LL ecotypes, rather that the LLI ecotype can better withstand light shock and thus may be capable of residing higher in the water column at depths subjected to mixing events.

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P4304 line 15 to P4305 line 6: The P depletion arguments seems very unlikely to explain the lack of HLII ecotype in these waters for the very reasons stated by the authors. In addition to the arguments mentioned by the authors Coleman Chisholm (2010) found that there are more HLII types than HLI types (Table S2) at both HOT and BATS (with BATS being considered more P-deplete) despite the larger set of P uptake genes in the HLI MED4 strain, making the presence of such genes unlikely to be the reason for more HLI types than HLII types in the Mediterranean Sea. Therefore there does not seem to be a valid reason to invoke P depletion as a reason for the lack of HLII types in the Mediterranean Sea.

P4309, lines 1-3. Once again, giving the reason of low P levels for the lack of clade II Synechococcus strains does not sit well with their relative high abundance in oceanic regions with low P levels (Fuller et al. 2005, Zwirgmaier et al. 2008). Overall section 4.2.1 and 4.2.2 should be written more concisely.

P4310, line 17: It would be helpful to briefly restate the major generalizations the authors are referring to here.

P4310, line 23-25. Such methodologies, such as quantitative PCR, have already been used to differentiate between distinct Prochlorococcus and Synechococcus ecotypes (see publications from the Chisholm and Palenik labs (such as Ahlgren et al. 2006 EM, Tai Palenik 2009 etc). This should be mentioned here.

P4311, line 1-2: This statement is not based and should be clarified. In what way does this study point out the need for long term temporal studies for the Mediterranean Sea in relation to global warming, if so far no significant effect has been seen? Because there might be change once greater temperature differences occur? So that there is a better baseline from which to measure potential differences in the future? Or simply because it is important to understand the temporal difference occurring in the Mediterranean Sea irrespective of the impacts of global warming?

P4311, lines 2-6: The statement that following HLII Prochlorococcus and clade II Syne-

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chococcus is important for monitoring global warming seems to contradict one of the major conclusions arrived at in the Discussion: that the low levels of these two clades are probably explained by factors other than temperature in the Mediterranean Sea.

Technical Comments

P4290, line 17. “from the analysis” instead of “for the analysis”

P4290, line 21. The accession numbers have not been provided.

P4291, line 10. see the Supplementary Information (?)

P4295, line 20. “translating” seems to be strange terminology in this context. Do the authors mean “probably due to. . .” ?

P4296, line 1. Use of the word “whereas” makes the statement a little ambiguous as it is not clear if this whereas relates to differences between HLI and HLII or between the two cruises. Assuming that relatively high numbers of HLII types were found at Sta 5 in both cruises, then “and” would be clearer.

P4301, line 5. “sustain” seems inappropriately used here. Do the authors mean: maintain numbers under sustained P depletion?

P4306, line 10. “where” instead of “but” seems more appropriate if I understand the meaning here.

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8, C2124–C2130, 2011

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