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***Interactive comment on* “Role of environmental factors for the vertical distribution (0–1000 m) of marine bacterial communities in the NW Mediterranean Sea” by J. F. Ghiglione et al.**

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General comments

Ghiglione et al report on a comprehensive statistical analysis of environmental meta-data and microbial community profiling in order to understand vertical stratification of marine bacteria in the Mediterranean Sea. Most of the paper is well written and the main outcome is that it is not a single factor that shapes these microbial communities but rather a synergistic complex interaction of multiple factors. The statistical analysis seems to be sound, but I am far from being a specialist in statistics and others should jump in here, make best use of the open discussion, and evaluate the approaches. I

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have several main points of criticism, which I list below.

Specific comments

First of all, several very important papers on the topic are not cited, and I will list them here in my review.

Secondly, the microbial community profiling method: The authors argue on page 2135 that CE-SSCP is the best profiling method and that it is better than DGGE and T-RFLP. Well, I would argue it always depends on what you want to say, and think that this present paper is the best example to show that. What I was expecting from the paper was to shed some light on the reasons why many marine heterotrophic microbial taxa have a distinct vertical distribution and which environmental factors are driving that. I was also hoping to see additional information on the vertical distribution of ecotypes of certain phylogenetic taxa similar to previously published papers (e.g. SAR11 Morris et al. 2005, LO or Prochlorococcus Johnson et al. 2006 Science). What I am greatly missing in this manuscript is 16S rRNA gene sequence information and the actual species names of taxa. With CE-SSCP, you can just compare peaks whereas with T-RFLP you can relate them back to sequences, if you have a clone library, and with DGGE you can cut and sequence the bands. So much said, I think that clone libraries from the different depths would bring this paper on a different level and should be performed and included at all means.

The main criticism I have is on pretty much ignoring the fact that it is well known that in marine environments protists and viruses are among the most important factors shaping microbial communities. Not only bottom-up but also top-down effects have to be considered when talking about factors shaping microbial communities. The authors mention predation by viruses and protists in their very last sentence but, obviously, this does not suffice. For references on the viral part, I refer to Jed Fuhrman's chapter in "Microbial Ecology of the Oceans"; (David Kirchman, ed., 2001) and for references on the grazing part to Ev and Barry Sherr's review (Significance of predation by protists in

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aquatic microbial food webs. 2002. *Antonie Van Leeuwenhoek* 81(1-4):293-308) and Jakob Pernthaler's review (Predation on prokaryotes in the water column and its ecological implications. 2005. *Nature Reviews Microbiology* 3(7):537-546). I understand that it is easier to measure nutrient concentrations, but these two factors have at least to be discussed properly. In this context, I would also like to refer to Strom 2008 (Microbial ecology of ocean biogeochemistry: a community perspective. 2008. *Science* 320(5879):1043-5 and Ed DeLong and Dave Karl's paper on metagenomics of stratified marine microbial communities (Community genomics among stratified microbial assemblages in the ocean's interior. 2006. *Science* 311(5760):496-503. These papers are the basis for what is presented and analyzed here in the present manuscript and should be discussed properly.

I might be wrong on this one, but the authors are not mentioning Archaea once. Archaea can be a major part of marine microbial communities (see Fuhrman et al. 1992. Novel major archaeobacterial group from marine plankton. *Nature* 356:148-9 and DeLong. 1992. Archaea in coastal marine environments. *PNAS* 89:5685-9) and I'm not sure whether the used primer set would get them or not. Either way, the authors should discuss this issue as well.

Lastly, the discussion does not read well and is merely a brief summary of the results. This part definitely needs work and all the papers I mentioned above could get integrated. The results should be combined and put in perspective of other work in the same field in order to work out what we have actually learned from this study.

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