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Interactive Comment

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.

Anonymous Referee #4

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Ghiglione et al. (2008) Role of environmental factors for the vertical distribution (0-1000 m) of marine bacterial communities in the NW Mediterranean Sea. Biogeosciences Discuss. 5:2131-2164

Using a combination of microbial community profiles and a complex environmental dataset this manuscript describes the usefulness of direct gradient multivariate ordination analyses in order to determine the driving force behind bacterial community structure shifts in the water column of the NW Mediterranean Sea.

Microbial community analyses were based on CE-SSCP fingerprints and as environmental parameters nutrients, lipid biomarkers and phytoplankton pigments were deter-





mined. Relationships between bacterioplankton community structure and environmental parameters were further investigated by different ecological statistical analyses.

It is for sure that the determination of factors influencing microbial community compositions is important in microbial ecology. The approach presented in this manuscript is a promising tool to get new insights into this field but, in fact, has also been applied elsewhere already (e.g. Hannig et al., FEMS Microbial Ecol 2006 for the vertical distribution of denitrifying microorganisms in the central Baltic Sea).

Anyway, first of all I have to admit that I am not really able to evaluate the usefulness of most of the statistical analyses which have been done here. Therefore I will focus on the datasets generated for environmental parameters on the one and bacterioplankton on the other side:

Determination of physico-chemical parameters (as nitrate, nitrite, phosphate or silicate), lipid biomarkers, as well as phytoplankton pigments is based on well established procedures. Thus, and despite the fact that I am missing the presentation of a comprehensive dataset at least for one representative vertical profile, these datasets should be robust.

The determination of the bacterioplankton structure is only based on CE-SSCP. The authors mentioned themselves already that this part of the study could be influenced by nucleic acid extraction procedures or PCR biases, but this has not been discussed consistently. In my opinion the most important bias is the selectivity of the primers used. With w49 and w34 the combination of a bacterial and a universal primer was chosen. This primer pair is probably unable to differentiate between bacterioplankton and phytoplankton. The appearance of cyanobacteria or chloroplasts in more general fingerprints is a pretty well known phenomenon. Because CE-SSCP excludes sequencing and identification of the peaks, the authors could have followed the vertical distribution of marine phytoplankton based on CE-SSCP profiles, at least in chlorophyll maximum zones. As a consequence, further statistical analyses in order to determine

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the influence of phytoplankton (based on lipid or pigment analyses) on bacterioplankton diversity is potentially insufficient.

In conclusion, at least the possibility exists that the most important parameter bacterioplankton diversity is not robust, potentially leading to corrupt statistical analyses. The authors have to exclude this possibility. Therefore, additional analyses concerning the identification of the CE-SSCP peaks (e.g., by cloning, comparable to T-RFLP) have to be done and cyanobacterial or chloroplast peaks excluded from further analyses.

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