**Biogeosciences
Discussions**

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.

J. F. Ghiglione et al.

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Answer to Anonymous Referee #2:

Answers to referee #2 are reported point by point. Changes in the text are located by the number of the corresponding line in the original manuscript.

Answer to general comments: -We propose to remove the last sentence of the abstract (page 2133, lines 24-26: "This study is probably the first example of an analysis employing a complex environmental dataset in combination with microbial community profiles to unravel the mechanisms underneath bacterial assemblages in marine systems.") that may overestimate the originality of our study compared to the existing literature. -We also propose the following modifications throughout the manuscript: Page

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

2133, line 4: "Here we show the explanatory power of multivariate statistical analysis" will be changed into "Here we use the explanatory power of direct multivariate gradient analysis". Page 2134, line 21: "Although indirect gradient multivariate statistical analyses have been used to link microbial community profiling to environmental parameters (Roeling et al., 2001; Edlund et al., 2006), the use of direct gradient analyses like CCA in combination with high-throughput molecular technologies is scarce in spite of the power of this method for this purpose (Ramette, 2007; Rooney-Varga et al, 2005)." Page 2149, lines 5-7: the sentence "To our knowledge, however, no studies have demonstrated the direct influence of environmental parameters on the bacterial community structure of natural environmental gradients" will be removed because it is confusing as the "direct" gets lost in this context. Page 2151, line 15: "Our study is perhaps the first example of such complex biogeochemical dataset," will be changed into "Our study propose a complex biogeochemical dataset," We already acknowledge in the original manuscript that other studies are already published using direct gradient analysis in several environments (see page 2148, lines 11-13: "from marine (Cordova-Kreylos et al., 2006; Klaus et al., 2007; Sapp et al., 2007), lake (Yannarell and Triplett, 2005) and soil (Salles et al., 2004) systems"). As proposed by referee #4, reference to Hannig et al. (2006) will be added in the manuscript (page 2148, line 11) and in the reference section (Hannig, M., Braker, G., Dippner, J., and Jürgens, K.: Linking denitrifier community structure and prevalent biogeochemical parameters in the pelagial of the central Baltic Proper (Baltic Sea), FEMS Microbiol. Ecol., 57, 260-271, 2006.).

We also propose to add new information about CE-SSCP: Page 2135, line 14: "In a recent paper (Rodriguez et al., in press), we show that CE-SSCP does not miss any known major group detected by a clone library of the same sample and that this technique is a reasonably quantitative measure of the relative abundance of the only taxon we could independently measure (Prochloroccocus, by flow cytometry)." Page 2147, line 9: "Molecular fingerprinting techniques in general may be restricted in giving information about microbial diversity, since they results in 30 to 40 peaks (or bands) in marine waters. This is due to co-migration of PCR-products from different species within

the same peak (or band), as depicted by several authors (Wintzingerode et al., 1997; Schmalenberger and Tebbe, 2003). Similarly, Schauer et al. (2000) mentioned the subjectivity in deciding whether a very weak DGGE band is a real band or a background artefact. In our study, this problem was, at least partly, overcome by taking into account the presence or absence of individual CE-SSCP peaks and the relative contribution of each peak to the total surface area of the pattern. The advantage of analysis finger-printing data this way has been recently enlightened by numerical simulation (Loisel et al., 2006). This study underlines that fingerprinting patterns contains extractable data about diversity although not on the basis of a number of bands or peaks, as is generally assumed to be the case, and can be considered as an 'image' of the whole microbial ecosystem free of inventory (cloning) limitation."

Schauer M, Massana R, Pedrós-Alió C (2000) Spatial differences in bacterioplankton composition along the Catalan coast (NW Mediterranean) assessed by molecular fin-gerprinting. FEMS Microbiol Ecol 33:51-59

The vertical distribution of bacterial communities has been discussed in more details with regards to reviewer comments. Page 2148, line 3: This is also consistent with genotypic and phenotypic properties of stratified Prochlorococcus "ecotypes" for ex-ample, that are suggestive of depth-variable adaptation to light intensity and nutrient availability (Rocap et al., 2003). However, none of these studies tested for the statisti-cal significance of vertical trends (autocorrelation), nor did they test for the predictability of vertical pattern from environmental parameters, so we do not know whether these studies show spatial patterning or predictability comparable to what we observed. Re-cent metagenomic approaches showed that vertical zonation of taxonomic groups was concomitant with functional gene repertories and metabolic potential (DeLong et al., 2006) and future efforts are needed to interpret the observed genomic pattern as a function of contextual environmental parameters (Ramette, 2007).

References to be added in the revised manuscript: DeLong, E.F., Preston, C.M., Min-cer, T., Rich, V., Hallam, S.J., Frigaard, N.U., Martinez, A., Sullivan, M.B., Edwards,

R., Brito, B.R., Chisholm, S.W., and Karl, D.M.: Community genomics among stratified microbial assemblages in the ocean's interior. Science, 311, 496-503, 2006.

Loisel, P., Harmand, J., Zemb, O., Latrille, E., Lobry, C., Delgenès, J.P., and Godon, J.J. : Denaturing gradient electrophoresis (DGE) and single-strand conformation polymorphism (SSCP) molecular fingerprintings revisited by simulation and used as a tool to measure microbial diversity, Environ. Microb., 8, 720-731, 2006.

Rocap, G., Larimer, F.W., Lamerdin, J., Malfatti, S., Chain, P., Ahlgren, N.A., Arellano, A., Coleman, M., Hauser, L., Hess, W.R., Johnson, Z.I., Land, M., Lindell, D., Post, A.F., Regala, W., Shah, M., Shaw, S.L., Steglich, C., Sullivan, M.B., Ting, C.S., Tolonen, A., Webb, E.A., Zinser, E.R., Chisholm, S.W.: Genome divergence in two Prochlorococcus ecotypes reflects oceanic niche differentiation. Nature, 424, 1042-1047, 2003.

Rodríguez-Blanco, A., Ghiglione, J.F., Catala, P., Casamayor, E.O., and Lebaron, P.: Spatial comparison of total vs. active bacterial populations by coupling genetic fingerprinting and clone library analyses in NW Mediterranean Sea, FEMS Microb. Ecol., in press.

Wintzingerode, F.V., Göbel, U.B., and Stackebrandt, E.: Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis, FEMS Microbiol. Rev., 21, 213-229, 1997.

Answer to Specific comments: Specific comment 1: The detection limits of the technique have been added in the Material and Methods section: Page 2136, line 19: "Detection limits were 3 nmol l^{-1} for nitrate and nitrite, 0.02 μmol l^{-1} for phosphate and 5 nmol l^{-1} for ammonium. To ensure reproducibility in nutrient measurements between analyses, an unique type of in-house standards was used, which was regularly compared to commercial products (OSIL). Precision was also tested, through the participation in the European inter-calibration exercise QUASIMEME (http://www.quasimeme.marlab.ac.uk/)."

Specific comment 2: The ranges of physico-chemical concentrations as well as the ranges of lipid concentrations have been added in the Results section: Page 2143, line 9: "The upper layer (0-40m) was nutrient depleted with nitrate and phosphate concentrations close to the detection limit. Concentrations increased along a nitracline up to maxima (7 and 0.2 μM, respectively) around 80 m depth. Silica varied from 1.0 μM at the surface to 13.5 μM) at 1000 m depth. DOC concentrations varied from 72.8 μM to 38.8 μM from surface to depth." Page 2144, line 16: "Total dissolved lipids varied within a range of concentrations (0.4-4 μM). Detailed analysis of this fraction of the organic matter is given in Goutx et al. (this issue). Chloroplast lipids (LC) dominated the lipid pool (38.7$\pm$8.5% on average, n=166), which indicated a phytoplankton source for DOM. Triglycerides (TG) were minor lipids ($<$10 % of total lipids)."

Goutx, M., Guigue, C. , Aritio, D. , Ghiglione, J.F., and Andersen, V.: Short term variability of dissolved lipid classes during summer to autumn transition in the Ligurian sea (NW Mediterranean), submitted in this issue.

Specific comment 3: The suggestion of the referee #2 has been taken into account by adding the following sentences: Page 2149, lines 5-7: "Our results are consistent with Fuhrman et al. (2006) that reported high predictability and significant influence of physico-chemical parameters (temperature, oxygen, salinity, dissolved nitrite and dissolved silicate) in annually reoccurring surface marine bacterial communities. The originality of the present study is to unravel such question in spatial (0-1000 m depth) gradients and we demonstrate that physico-chemical parameters are acting in synergy with nearly equal contribution to the bacterial vertical stratification."

Specific comment 4: As depicted in the Materials and Methods section (page 2142, lines 13-15), we have performed Spearman's rank pairwise correlations between the environmental variables in order to determine their significance for further ecological analysis. For example, a strong Spearman's rank pairwise correlation between nitrate and silicate (R=0.91, p$<$0.01) was found and it allowed us to use nitrate as a proxy of silicate to perform CCA together with the rest of physico-chemical parameters

(see results section page 2143, lines 13-16). Such test avoided co-linear variables when performing CCA (see discussion section on page 2148, lines 17-18). For instance, we confirm that the statistical analysis performed does demonstrate on its own that all the physico-chemical variables are acting in synergy.

Specific comment relating to page 2150, lines 15-17: We propose to add information about "bottom-up" and "top-down" control in the introduction and in the discussion sections: Page 2134, line 21: "More generally, theoretical models and empirical surveys indicate that microbial growth or population size are controlled by resource availability, including nutrient and energy sources ("bottom-up" control) and microbial biomass is controlled by mortality, including factors such as predation or viral lysis ("top-down" control) (Pernthaler, 2005). The relative importance of "top-down" and "bottom-up" controls on bacteria have been poorly investigated (Fuhrman and Hagström, 2008), and our knowledge on the interplay between the different factors involved within "top-down" and "bottom-up" controls is still limited in the field. Protistan predation may often be most influential in limiting the total abundance and biomass of the bacterioplankton, whereas viruses are considered to more profoundly affect prokaryotic community diversity (Pernthaler, 2005). On the other hand, clear bottom-up effects on bacterial community structure have been observed in controlled laboratory conditions (Pinassi et al., 1999; Carslon et al., 2002) but few studies demonstrated statistically robust, predictable patterns in the field (Korona et al., 1994). Such complex ecological questions may be addressed by robust statistical analysis. A recent review (Ramette, 2007) underlines that hypothesis-driven technique such as redundancy analysis, canonical correspondence analysis (CCA), or Mantel tests are rarely used by microbial ecologists, despite the fact that high-throughput molecular technologies are now available at the field. For example, this approach allows better understanding of the linkage between bacterioplankton and phytoplankton dynamics in marine coastal environment (Rooney-Varga et al., 2005)."

We propose to remove section page 2134, lines 21-27.

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

Page 2152, line 5: "In our conditions, resources ("bottom-up" control) as well as other factors such as pressure, temperature, light, O2 and PAR controlled simultaneously the vertical zonation of bacterial communities in the water column. Unfortunately, the parameters measured in our study do not permit to evaluate the effect of predation by viruses or protists ("top-down" control). There are indications in the literature that bacteria are tightly controlled by protistan predation in highly oligotrophic systems, whereas their growth is limited by competition for nutrients in more productive waters (Strom, 2000; Gasol et al., 2002). On the other hand, virus-induced mortality seems more substantial when bacterial productivity is enhanced or in anoxic conditions (Weinbauer et al., 2004). "Sideways controls" such as competition for resource, allelopathy or syntrophy may also influence bacterial community structure in marine environments and the new insights given by metagenomics approach should allow more thorough investigation of this subject in the near future (Fuhrman and Hagström, 2008; Strom et al., 2008). Numerous factors can regulate microorganism population dynamics, often simultaneously, and our study together with other evidences found in the literature (Fuhrman et al., 2006) underline the importance of relevant statistical analysis for further multidisciplinary investigations to investigate the relative importance of environmental factors in predicting the bacterial community dynamics in marine systems."

We propose to remove section page 2152, lines 5-8.

References to be added in the revised manuscript:

Carlson, C.A., S.J. Giovannoni, D.A. Hansell, S.J. Goldberg, R. Parsons, M.P. Otero, K. Vergin, and B.R. Wheeler. The effect of nutrient amendments on bacterioplankton production, community structure and DOC utilization in the northwestern Sargasso Sea. Aquat. Microb. Ecol., 30, 19 - 36, 2002.

Fuhrman, J.A., and Hagström Å.: Bacterial and Archaeal community structure and its patterns. In: Microbial Ecology of the Oceans (2nd edition), edited by Kirchman, D. L., Wiley-Liss, Inc., New York, 45-90, 2008.

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

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Gasol, J. M., Pedros-Alio, C., and Vaqué, D. Regulation of bacterial assemblages in oligotrophic plankton systems: results from experimental and empirical approaches. Antonie Van Leeuwenhoek, 81, 435-452, 2002.

Korona, R., Nakatsu, C.H., Forney, L.J., and Lenski R.E. Evidence for multiple adaptive peaks from populations of bacteria evolving in a structured habitat. Proc. Natl. Acad. Sci., USA, 91, 9037-9041, 1994.

Pernthaler, J.: Predation on prokaryotes in the water column and its ecological implications, Nature, 3, 537-546, 2005.

Pinhassi, J., Azam, F., Hemphälä, J., Long, R.A., Martinez, J., Zweifel, U.L., and Hagström, Å.: Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. Aquat. Microb. Ecol., 17, 13-26, 1999.

Rooney-Varga, J.N., Giewat, M.W., Savin, M.C., LeGresley, M., and Martin, J.L. Links between phytoplankton and bacterial community dynamics in a coastal marine environment. Microb. Ecol., 49, 163-175, 2005.

Strom, S.L. : Bacterivory: interactions between bacteria and their grazers. In: Microbial Ecology of the Oceans, edited by Kirchman, D. L., Wiley-Liss, Inc., New York, 351-386, 2000.

Strom, S.L.: Microbial ecology of ocean biogeochemistry: a community perspective. Science, 320, 1043-1045, 2008.

Weinbauer, M.G., and Rassoulzadegan, F.: Are viruses driving microbial diversification and diversity? Environ. Microbiol. 6, 1-11, 2004.

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Full Screen / Esc

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Interactive Discussion

Discussion Paper