

Interactive comment on “Short-term temporal variations of heterotrophic bacterial” by G. Mével et al.

Anonymous Referee #2

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The paper reports short-term variations of bacterial abundance and production between 0 and 1000 m at a fixed station in the NW Mediterranean Sea. This study was done within the frame of the DYNAPROC-2 (DYNAMics of the rapid PROCesses in the water column) project. To investigate the vertical and temporal dynamics of both free-living and particle-attached bacteria in offshore waters, the authors used two different time scales of sampling (i.e. daily and hourly) over 5 weeks during a transient period from summer to autumn. Because of this intensive effort, the authors obtained a large dataset of bacterial abundance and production that I think is unique in the NW Mediterranean Sea. Below I mention some concerns that need to be addressed.

A similar study was done at the DYFAMED site and published by Ghiglione et al. (2007). Drs. Mével and Ghiglione are involved in the paper of Ghiglione et al. (2007).

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I assume that these two studies are related. I therefore suggest that the authors summarize what Ghiglione et al. (2007) found and mention which question/hypothesis the authors wanted to answer/test in this study in the Introduction section.

The authors defined particle-attached bacteria as bacteria retained on 0.8 μm pore-size polycarbonate filters. Bacterial cells retained on 0.8 μm pore-size filters are not necessarily particle-attached. Some free-living bacterial cells can be found on the filters because of filter clogging or because of cell size (e.g. longer than 0.8 μm). Did the authors confirm before or during this study that bacterial cells on 0.8 μm pore-size filters were mostly particle-attached under microscope? The authors filtered 5 ml samples through 0.8 μm pore-size filters to count particle-attached bacteria. For particle-attached bacterial production, they used 10 to 30 ml samples. Abundance of particles attached by bacteria can be variable spatially (0-1000 m) and temporally (5 weeks). I want to see if difference in sample volume can result in significant difference in abundance and production of particle-attached bacteria. The authors used the measurement of bacterial production in 1.5 ml samples as total bacterial production. In addition, they used 10-30 ml samples to measure another total bacterial production and free-living bacterial production. The authors should clarify (1) if there were differences in total bacterial production between 1.5ml and 10-30 ml samples and (2) if the production in free-living fraction ($<0.8 \mu\text{m}$) was always equal to or smaller than the total production.

The authors show the data on total bacterial biomass, total bacterial production, and total specific activity in March and June 2004 in the Results section and Table 1. But they give no explanation of these data in the Introduction and the Materials and methods section. I have learned from the reference list that these data are published in Ghiglione et al. (2007). Such a presentation is very confusing and should be avoided. I suggest the authors to mention how the data in March and June 2004 were obtained and to do the data comparison between the previous study and this study in the Discussion section.

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The authors can improve the interpretation of how much (or less) vertical and temporal changes of bacterial abundance and production were influenced by physical and biological factors. The authors mention that they did not find any diel periodicity on the 0-150 m integrated bacterial abundance and production. If bacterial abundance and production depend on dissolved organic matter produced by phytoplankton, one can expect that temporal variations of these parameters are related to those of phytoplankton primary production (i.e. diel periodicity). But it should be noted that bacterial abundance is generally controlled by predation and viral infection. In this case, bacterial abundance can show Lotka-Volterra type oscillations (e.g. Fenchel 1982, MEPS 9: 35-42). If top-down control is significant, one can not expect to find clear diel periodicity of bacterial abundance and production.

The authors mention that the intrusion of low salinity water mass happened between 40 and 80 m twice, and the strong wind event happened three times. These physical forcing could give direct influence to limited depth layers and possibly indirect influence to neighboring depth layers. It has been reported that bacterial growth in the photic zone can be limited by different substrates depending on depth during the stratified period in the Mediterranean Sea (Van Wambeke et al. 2002, Microbial Ecology 43: 119-133). In this situation, the integrated data may mask vertical variations of bacterial dynamics. The authors can apply the dataset integrated for different depth layers (e.g. surface mixed layer, chl_a max layer) to correlation analysis. Similarly, it is important to mention whether or not there was significant intrusion of different water mass into the mesopelagic layer during the study period.

Specific comments

1. The title: The authors can improve the title. They measured short-term variations of bacterial abundance and production down to 1000 m. As they emphasize in the paper, such data are original. But this is not reflected in the title.
2. Abstract: The authors should include the results and conclusion of bacterial dynam-

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ics in the mesopelagic layer.

3. Page 1901, Lines 9-12: Please give reference(s) for the network of Microbial Observatories.

4. Page 1901, Lines 16-17: I agree that less information of bacterial abundance and activity is available in oceanic systems. However, for example, Ducklow and Carlson (1992) already review the data on bacterial abundance and activity in the oceans (see also Page 1914, Lines 8-9). I think that the references (Wikner and Hagström 1999, Lemée et al. 2002) are not proper here. In case these two studies are remarkably different from previous studies, the authors should specify the reason to cite these two papers.

5. Page 1902, Lines 17-18: Previous studies have reported the contribution of particle-attached bacteria in the NW Mediterranean Sea (e.g. Harris et al. Deep-Sea Res. 1 48: 2631-2644; Turley and Stutt 2000 Limnol. Oceanogr. 45: 419-425). The authors may be able to summarize what these studies did and what the authors wanted to investigate this aspect further.

6. Page 1902, Line 23: Dyfamed should be DYFAMED.

7. Page 1903, Lines 3-6: An offshore station located near is vague. Give the sampling position (latitude and longitude).

8. Page 1903, Line14: In page 1911, line 5, the authors mention that the 0-1000 m profile was taken 12 times. If this is true, there is no reason not to mention 12 times here. The phrase several times is vague.

9. Page 1903, Lines 18-19: The authors refer the linear regression equations here. Coefficient of regression should be R^2 , R is used for coefficient of correlation.

10. Page 1905, Lines 10-13: A significant linear regression with a slope close to 1 and an interception close to zero can support that both epifluorescence microscope method and flow cytometry method show similar results.

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11. Page 1906, Lines 28-29: While the bacterial production in volumetric scale is shown as $\text{ngC l}^{-1} \text{h}^{-1}$, that in aerial scale is shown as $\text{mmol C m}^{-2} \text{d}^{-1}$. I think that this is confusing and thus suggest using the same unit (weight or mole) throughout the paper.
12. Page 1907, Line 12: Dyanproc-2 should be DYNAPROC-2. See page 1903, line 3.
13. Page 1909, Line 7: r should be R.
14. Page 1909, Lines 18-21: The authors applied a linear regression to test if there was significant effect of time on the 0-150 m integrated parameters. I have two problems here. (1) Which parameter did the authors use for this analysis? (2) They did not give any hypothesis or assumption that these parameters change linearly with time.
15. Page 1909, Line 27: P index should be defined in the section of Materials and methods.
16. Page 1911, Lines 13-15: The authors should explain what kind of t-test was used and how the data were compared (each depth for the entire study period? All depths for the entire study period?) and statistical results. The same for page 1911, line 26-page 1912, line 1.
17. Page 1913, Line 15: The referred paper Lemée et al. (2002) is not based on the field study in 2003.
18. Page 1914, line 2: phytoplankton nutrients is not clear.
19. Page 1914, line 16: Please specify the six upper layers.
20. Page 1914, Lines 19-24: Please show the result of linear regression analysis (slope, coefficient of regression, statistical significance of slope, number of data). It is evident that there are both bottom-up and top-down controls on bacteria in the euphotic layer. A more interesting question to be answered is which controlling factor is dominant and how these factors change in space and time.

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21. Page 1915, Lines 3-4: Ghiglione et al. (2007) studied at the DYFAMED station. This is not the same site as in this study.

22. Page 1915, Lines 14-17: The suggestion here is not clear for me. Do the authors want to suggest that the low salinity reduced bacterial abundance and production? Can they suggest which mechanisms are behind to explain this relationship?

23. Page 1915, Lines 23-26: The Mediterranean surface waters are usually limited by P during the stratified period. This study was done in the Mediterranean Sea. The authors can specify that an increased NO₃ availability could result in changes of total bacterial abundance and production in the system limited by N availability.

24. Page 1915, Line 19, Line 28: found should be find.

25. Page 1916, Lines 12-15: The authors mention that a strongest relationship was observed between total bacterial abundance and production ($R=+0.59$, $p<0.01$). However the relationship between the percentage of HNA in the total bacterial population and the total bacterial production appears to be stronger ($R=+0.75$, $p<0.01$).

26. Page 1917, Line 5: The phrase organization of bacterial diversity with depth is not clear.

27. Page 1917, Lines 15-24: Tanaka and Rassoulzadegan (2004) report the slopes in the 110-1000 m layer but not the 0-1000 m layer. Their study is based on seasonal sampling and mention that the mean depth-dependent decrease of bacterial abundance was slightly smaller in 2001-2002 than in 1999-2000. In contrast, the authors measured vertical profiles of bacterial abundance and production in the mesopelagic layer at short intervals in the summer-autumn transient period. It is also interesting to argue short-term variations by presenting the range of slopes (min-max) rather than the mean, SD and CV.

28. Page 1917, Line 23: Given the mean SD of triplicate BP measurements (12.5%), it seems to be strange that the mean SD of bacterial production is shown as percentage.

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29. Page 1917, Line 26: excretion by metazoan, generally means regeneration of inorganic nutrients such as N and P. In deeper layers, concentrations of inorganic nutrients are high. Regeneration of inorganic nutrients is not an important factor to stimulate bacterial biomass production in mesopelagic layers.

30. Page 1918, Line 8: I do not think that Marty et al. (2002) worked on particle-attached bacteria using GF/C filters.

31. Page 1922, Line 22: Add pages.

32. Page 1926, Table 1: Reference(s) should be given for the data obtained in March, 2003 and June, 2003. the Dyfamed site should be the DYFAMED site. The data in this study (September-October 2004) were not obtained at the DYFAMED site.

33. Page 1929, Figure 3 legend: Give a brief explanation of P index with the data source of P index.

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5, S718–S724, 2008

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