

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

G. Mével et al.

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Answer to 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 revised version of manuscript (together with number of the corresponding line in the original manuscript (BG numbers), when necessary):

Answer to general comments

Answer to general comments from “The paper reports short-term variations of ...” until “...in this study in the Introduction section.” The relation between our previous investigation at the DYFAMED site (Ghiglione et al., 2007) and this study has been clarified in the Introduction section on Page 4 line 23 to page 5 line 14 (BG page 1902, lines 15 to

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25): “The vertical and temporal dynamics of heterotrophic bacteria (Van Wambeke et al., 2001; Lemée et al., 2002; Tanaka and Rassoulzadegan, 2004; Mistic and Fabiano, 2006) and the contribution of particle-attached bacteria (Turley and Stutt, 2000; Harris et al., 2001) have already been reported in the NW Mediterranean Sea. In a previous study, we have shown that the contribution of attached bacteria to total bacterial activity can reach until 83% under mesotrophic condition, then reinforcing the biogeochemical role of this fraction in the cycling of particulate organic carbon in the NW Mediterranean Sea (Ghiglione et al., 2007). However, the temporal scales at which bacterial abundance and production vary through the water column are still missing. A companion paper (Ghiglione et al., 2008) explored the community composition of the bacterioplankton along the depth gradient with molecular biology tools but do not investigate the effect of episodic events because of methodological considerations. In this study, we provide a large set of data on the vertical (0-1000 m) and temporal (from hour to week scales, during 5 weeks) dynamics of total vs. particle-attached bacterial abundance and activity under summer-autumn transition at a site very close to the JGOFS-DYFAMED station in NW Mediterranean Sea. The seasonal pattern was investigated by comparison with results previously obtained at the station DYFAMED (Ghiglione et al., 2007).”

Answer to general comments from “The authors defined particle-attached bacteria...” to “...were mostly particle-attached under microscope?” As it has been explained in the discussion section (page 24, lines 4-16 or BG page 1918, lines 3 to 8): “We defined the terms “free-living” and “particle-attached” bacteria on the basis of a 0.8- μm -pore-size filter fractionation. This barrier is mainly operational and should include most microscopic and macroscopic aggregates (Simon et al., 2002). A similar cut-off was used by several authors (Hollibaugh et al., 2000; Ghiglione et al., 2007) but some authors used also GF/C filters with a nominal pore size of 1.2 μm (Gasol and Morán, 1999).” We agree with reviewer #2 that the nominal size of the filters cannot be considered as an absolute standard, and a fraction of free-living bacteria (some copiotrophic species) may be retained on these filters, even in oligotrophic waters (Gasol et al., 1999). Free-

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living bacteria also can be retained as a consequence of clogging and it is generally assumed that the fraction of retained free-living bacteria increases with the amount of filtered water (Lee et al. 1995). Special care has been taken about this aspect, as depicted in the Material and Methods section that directly answer reviewer comment: Page 8, lines 18-23; BG page 1905, lines 9 to 13: “The microscopic observations have shown a good dispersion of bacteria and tolerance to sonication process and confirmed that bacterial cells on 0.8 μm pore-size filters were mostly particle-attached. In addition, results obtained by the two methods showed no significant difference (mean=5.9 \pm 1.8 and 6.8 \pm 1.3 $\times 10^5$ cells ml $^{-1}$ by epifluorescence and flow cytometry, respectively. n=44) and were closely correlated (R=+0.68, p<0.01, n=44) that confirmed the validity of using sonication before flow cytometric enumerations as shown previously (Riemann and Winding, 2001; Worm et al., 2001).”

Answer to general comments from “The authors filtered 5 ml samples...” to “...was always equal to or smaller than the total production.” To make these points clearer, two sentences have been added in the text: -Page 10, line 12 (BG page 1906, ligne 20): “The production in <0.8 μm fraction was always smaller than the total production.” -Page 10, lines 17 to 20 (BG Page 1906, ligne 26): “The TBP measured in 84 subsurface samples by using the both methods showed no significant difference (mean TBP=22.52 \pm 5.50 and 20.01 \pm 4.85 ngC l $^{-1}$ h $^{-1}$ with centrifugation and filtration method, respectively).”

Answer to general comments from “The authors show the data on total bacterial biomass...” to “...and this study in the Discussion section.” The results presented here are related to our previous work in March and June 2003 in the same zone (Ghiglione et al., 2007). As pointed out by the reviewer, it is interesting to compare these data apart from one year and we agree that it may confuse the reader about the origin of the samples. This point was already clarified in the discussion section of the original manuscript: -Page 18, lines 10-13 (BG 1913, lines 12-16): “To evaluate the dynamics of total heterotrophic bacterial abundance and activity at a seasonal scale, data

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from this study (summer-autumn transition) were compared with previous data from two cruises led at the same site under spring bloom and summer stratification conditions (Ghiglione et al., 2007) (Table 1)."

To make this point even clearer, references to this article and how samples were compared have been added: -Page 5, lines 12-14: "The seasonal pattern was investigated by comparison with results previously obtained at the station DYFAMED (Ghiglione et al., 2007)." -Page 13, lines 1-3 (BG page 1908, line 10): "The results we obtained during the September-October 2004 sampling period were compared to those obtained for two previous cruises conducted at the same site in March and June 2003 (Ghiglione et al., 2007) (Table 1)." -Table 1 legend (page 36, lines 4-5; BG page 1926): "March and June 2003 data originated from previous cruises at the station DYFAMED (see Ghiglione et al., 2007)."

Answer to general comments from "The authors can improve the interpretation..." to "...to find clear diel periodicity of bacterial abundance and production." Bacterial to autotrophic biomass ratio as well as links between bacterial abundance and production variation with depth and time gave information about top-down or bottom-up control. This has been discussed in: Page 19, line 2 to page 20 line 2 (BG 1914, lines 8-24): "Integrated 0-150 m depth values of bacterial biomass to autotrophic biomass ratios remained relatively constant and always >1 (Fig. 2). Such domination of bacterial biomass relative to autotrophic biomass is in accordance with other results in open Ocean (Ducklow and Carlson, 1992). These authors suggested that such situation occurs when phytoplankton biomass is low ($\text{Chla} < 1 \text{ mg m}^{-3}$) and when bacteria are sustained by phytodetritus or by-products from grazing. For the entire study period, we found a significant correlation between 0-150 m integrated bacterial biomass and Chla concentration ($R=+0.44$, $p<0.05$, $n=54$) but this relation was more significant in the 40-80 m layer ($R=+0.67$, $p<0.01$, $n=54$) compared to 0-20 m layer ($R=+0.13$, $p<0.05$, $n=54$). In a same way, bacterial abundance and bacterial production measured into the Chla maximum layer (40-80 m) were also strongly linked during the whole sam-

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pling period ($R=+0.65$, $p<0.01$, $n=162$) when no significant relation was observed in the surface mixed layer (0-20 m). These results showed vertical variations of bacterial dynamics and suggest that bacterioplankton living in the Chla maximum layer was regulated by C-compounds derived from phytoplankton activity. In addition, linear regression analysis calculated in the 40-80 m layer between integrated BB and Chla concentration ($Y=0.24X+5.25$, $R^2=0.65$, $n=54$) showed a slope <1 suggesting that bacterial responses to resource availability tend to be attenuated by predation or viral pressure (Ducklow and Carlson, 1992; Dufour and Torretton 1996; Christaki et al., 2004). Nanoflagellates and ciliates populations have been previously observed in our study site and predation over bacterioplankton considered (Tanaka and Rassoulzadegan, 2002). Thus, in our conditions both bottom-up and top-down processes were controlling bacterial populations inhabiting the 0-150m upper layer but, further grazing experiments are needed to estimate the grazing rates and the relative importance of bottom-up and top-down control in such conditions.”

Another sentence has been added: Page 20, lines 9-12 (BG 1915, line 3): “These results together with hypothesis mentioned above suggest that bacterial abundance and production was not directly linked to the diel changes in dissolved organic matter produced by phytoplankton (bottom-up control), thus reinforcing the role of top-down control by predation or viral lysis in our sampling conditions.”

Answer to general comments from “The authors mention that the intrusion of low salinity water mass...” to “...different water mass into the mesopelagic layer during the study period.” In accordance to reviewer comment, this point has been clarified on Page 19, lines 9 to 24 (BG Page 1914, line 13): “For the entire studied period, we found a significant correlation between 0-150 m integrated bacterial biomass and Chla concentration ($R=+0.44$, $p<0.05$, $n=54$) but this relation was more significant in the 40-80 m layer ($R=+0.67$, $p<0.01$, $n=54$) compared to 0-20 m layer ($R=+0.13$, $p<0.05$, $n=54$). In a same way, bacterial abundance and bacterial production measured into the Chla maximum layer (40-80 m) were also strongly linked during the whole sampling period

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Answer to specific comments 1. The title has been changed into: "Seasonal to hour variation scales in abundance and production of total and particle-attached bacteria in the open NW Mediterranean Sea (0-1000 m)"

2. Page 2, lines 16-17 (BG 1900, line 17): "In the mesopelagic layers, bacterial abundance and production linearly decreased with depth, except some production peaks between 400-750 m."

3. Reference has been added on Page 3, line 17 : "(www.nsf.gov/bio/pubs/awards/mo03.htm)"

4. Page 3, line 22: The references Wikner and Hagström (1999) and Lemée et al. (2002) have been removed and replaced by Ducklow and Carlson (1992).

5. Answer to this specific comment correspond to changes already mentioned in the General comments. It correspond to changes in the introduction section on Page 4 line 23 to page 5 line 14 (BG page 1902, lines 15 to 25): see above.

6. Page 5, line 13: "Dyfamed" has been changed into "DYFAMED".

7. Page 6, lines 5-6: "... at an offshore station (28 miles offshore, $43^{\circ}25'4N$, $8^{\circ}00'5E$) located near the permanent DYFAMED station ($43^{\circ}25'2N$, $07^{\circ}51'8E$)..."

8. Page 6, line 14: "several times" has been changed into "12 times at day and night"

9. Page 6, line 24 and Page 7, line 1: “R” has been changed into “R2”.
10. This point has been already discussed in the General comments, and refer to changes done on Page 8, lines 18-23.
11. The same unit is now used throughout the paper. Changes have been done throughout the text and in table 1, figure 2 and figure 3.
12. Page 12, line 3: “Dynaproc-2” has been changed into "DYNAPROC-2"
13. Page 13, line 22: “r” has been changed into “R”
14. This point has been clarified in Page 14, lines 8-10: “These four parameters (TBB, TBP, HNA and TBB/AB) fitted to a linear regression with time showed no long-term trend during the one-month sampling period ($p > 0.05$, variance analysis F-test).”
15. P index has been defined in the Materials and methods section on Page 6, lines 16-22: “Low salinity water masses (LSW) percentage in the water column was depicted by the P index, based on average salinity S in the 40-70 m water layer, according to the following formula : $P \text{ index} = (S_{\text{max}} - S(\text{sta})) / (S_{\text{max}} - S_{\text{min}})$; S(sta) is the average salinity in the 40-70 m layer, Smin is that of the water taken as reference for dessalted water (coastal waters in our case), Smax is that of the salinity waters without anomaly (see <http://www.obs-vlfr.fr/proof/vt/op/ec/peche/pec.htm> - data DYNAPROC log & basic files - for more detailed explanation).”
16. These points have been clarified on Page 16, line 1-7: “The abundance of particle-attached bacteria followed a similar pattern with a strong vertical gradient (Fig.5). The main contribution of the attached bacteria to the TBA was observed in the upper 150 m layer with maximal values ranging from 14 to 25% of the TBA at the DCM, whereas their contribution was very low in the mesopelagic layers (<4% of the TBA). The day and night samples were compared for total and attached bacterial abundance (each depth for the twelve 0-1000 m profiles) and Student’s t-test showed no significant difference between noontime and midnight.” Page 16, lines 13-20: “The contribution of

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the particle-attached fraction on the TBP varied also drastically with depth and showed a similar vertical gradient as TBP (Fig. 5). The maximal contribution of the attached fraction reached values up to 49% of TBP, and generally situated between 20-40 m (just above DCM layer). In the mesopelagic waters, this contribution was generally negligible (<2%), with some exceptions found between 400 and 750 m depths (from 8 to 15% of TBP). As observed for the abundance parameter, the day and night samples were compared for total and attached bacterial production and Student's t-test also showed no significant changes between day and night.”

17. Page 18, line 12: “but one year before and” has been removed.

18. Page 18, line 25: “phytoplankton nutrients” has been changed into “nutrients from phytoplankton origin”

19. This sentence has been changed in accordance to General comments. See above page 19, line 9 to page 20, line 1.

20. This point has been changed as described in the answer to General comments. See above page 19, line 9 to page 20, line 1.

21. Page 20, line 13: “At the studied station” has been changed into “At the DYFAMED station...”

22. To clarify this point, a sentence has been added: Page 21, lines 1-3: “This observation suggests that these desalted waters were characterized by a lower bacterial populations compared to surrounding waters.”

23. Page 21, lines 12-14: “Mediterranean surface waters being limited by P and N during the stratified period, an increased NO₃- availability could result in changes of bacterial abundance and production.”

24. Page 21, lines 4 and 16: “found” has been changed into “find”

25. Page 22, line 8: “and a strongest” has been changed into “while a weaker”

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26. This sentence has been changed: Page 22, line 22 to 24: “In parallel, clear changes in bacterial diversity by vertical zonation in three layers, above, in or just below the chlorophyll maximum and deeper, that remained stable during the entire sampling period was also observed by Ghiglione et al. (2008).”

27. This point has been clarified on Page 23, lines 9-21: “The magnitude of depth-dependent decrease of total bacterial abundance was relatively constant for our study period. The calculated slopes of log-log linear regression varied from -0.47 to -0.62 and the mean slope ($=-0.50 \pm 0.07$, $n=12$) was slightly smaller compared to those previously described in the same zone ($=-0.66 \pm 0.13$, $n=10$ in Tanaka and Rassoulzadegan, 2004). In contrast, the slopes relative to total bacterial production fluctuated for the one-month study period (from -0.70 to -1.38) suggesting short-term variations in depth-dependent decrease of total bacterial production during the summer-autumn transient period. Moreover, compared to slopes previously described by Tanaka and Rassoulzadegan (2004) in their study based on seasonal sampling (mean slope $=-1.15 \pm 0.30$, $n=5$), our mean slope for TBP (mean slope $=-0.73 \pm 0.49$, $n=12$) is clearly lower suggesting here a seasonal variation in the vertical distribution of bacterioplankton. In addition, the high variability of TBP slopes can be due to episodic deep peaks of production observed between 400 to 750 m depths.”

28. Page 23, line 22: “SD” has been changed into “CV”.

29. Page 23, line 25: “excretion by metazoan” has been removed.

30. Page 24, line 7: the reference Marty et al. (2002) has been removed.

31. Page 30, line 25: “1963-1964” has been added in the reference.

32. As it has been proposed in the answer to general comments (see above), additional information has been added in Table 1 legend (page 36, lines 4-5): “March and June 2003 data originated from previous cruises at the station DYFAMED (see Ghiglione et al., 2007).”

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33. Page 35, lines 9-10: legend of figure 3: “P index (a proxy of salinity anomalies)”

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