Interactive comment on “Specific rates of leucine incorporation by marine bacterioplankton in the open Mediterranean Sea in summer using cell sorting” by A. Talarmin et al.

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General reply to the reviewers

We greatly acknowledge the dense reviews and many comments given by the reviewers which greatly helped us to improve this manuscript. In this response, we focus on the main corrections and general scientific points raised. You will find figures 1, 3 & 4 from the revised manuscript, as well as Table 1, as attached files. A point-by-point reply follows the general reply.

The manuscript has been shortened from 21 to 16 pages. The map (Figure 1) was simplified, only the studied stations remain.
A new figure shows the main environmental parameters at the 5 studied stations (new figure 3) so that the data description in this paper is completely independent of other manuscript of the special issue BOUM. However, because code stations are common to many papers of the special issue, we decided not to change the codes here.

A new figure shows vertical profiles of cell-specific incorporation rates (new figure 4). This figure shows the whole data set: n=30 data for HNA-hs, HNA-ls and LNA cells, and n=14 data for Proc.

Table 1 was modified: mixed layer depth and temperature ranges were removed because now they appear on the new Figure 3. Instead, we show the number of samples collected per profile for cell sorting, as well as the ranges of population abundance and bulk leucine incorporation rates in these samples. Note that all data of rates are now expressed in pmol leu l-1 h-1 and the conversion factor asked by referees for BP estimate is not necessary anymore.

Only 3 and 4 depths were sampled at St. 21 and St. 25, respectively, so we deliberately show only 3 profiles of St A, B and C on Figure 5 (volumetric rates), where more data are displayed. Old Table 3 and old Figures 3 and 4 were removed and their contents are now simply described in the text.

Graphs showing relative activities and contributions were removed from old figure 7 (new figure 7). Because of the missing control sample, data from St. 21 85 m were removed too.

Color codes in Figure 6 were changed for easier reading; such modifications were also done for other figures, so that codes could easily be discriminated in black and white prints. We re-checked the consistency between data in tables, figures and text. We defined the terms of volumetric rates and cell specific rates in the M&M section and then used this terminology along the whole manuscript. The term "population" was removed and we always referred to "cytometric groups" or "groups" for LNA and HNA cells. We insisted more on vertical than on longitudinal variability in the revised
manuscript and removed parts dealing with longitudinal variability, particularly in the objectives because, as stated by the referees, we had only one complete profile per basin.

In the following text, reviewers' comments and suggestions are in italics. New figures included from the revised manuscript are annotated as they are in the revised version, while additional figures are named with letters.

Table 3 was removed.

Reply to anonymous Referee #3

General 1) "... but the biogeochemical implications of the study are virtually absent. This is something the authors should emphasize before the paper can be eventually published in Biogeosciences. I suggest accommodating this point as early as in the title. In its present form the manuscript is not very appealing for biogeochemists." The title was modified. We agree that this paper deals mainly with activity measurements of functional groups. For appealing biogeochemists, we insist on surface - dcm layers differences, and we pointed out the Proc mixotrophy.

2) "Although the apparent geographical scale is large, spanning the Mediterranean east west range of increasing trophic state, the sampling of only one site per “main” (sic) basin is not representative enough so as to significantly improve our knowledge of the response of LNA and HNA bacteria in open-ocean Mediterranean waters...". We agree and insist on vertical trends rather than longitudinal trend on the revised version of the manuscript.

3) "The separation of high nucleic acid content bacteria into three different clusters: HNAls, HNA-hs and HNA+, is not sufficiently explained given the extensive discussion of among-group differences made by the authors. Where do the HNA+ come from? On page 6568 the authors apparently suggest that this is a completely new flow cytometric subpopulation". See response to referee 1 above on this point.
4) "...To increase readability I suggest a substantial reduction of the Results section aiming at showing only the most relevant results (for instance section 3.4 is way too long). The authors should also especially avoid repetitions of facts in the Discussion section, as well as a minimum general shortening of 30% in its length. " The text has been shortened from 21 to 16 pages and we simplified some sections as suggested by the referee.

5) "In the conclusions the authors suggest a positive correlation of the decrease in heterotrophic bacterial activity with depth but they do not show any analysis. I doubt that temperature rather than substrate availability explains this pattern. The statement should be either fully supported or eliminated from this section. The paper would greatly benefit from further analysis trying to explain the observed patterns. The present version is merely descriptive". In the results we show correlations with depth, temperature and Tchl a. We agree that there are autocorrelations between such variables along depth. Thus is the discussion section we focus on the two layers "surface" and "dcm" where Hprok are not under the same controlling factors and environmental conditions.

Specific 6) "p. 6548, 5. Please include a statement that the paper is addressing aquatic or marine heterotrophic prokaryote" This is stated in the beginning of the abstract.

7) "p. 6549. The list of biotic and abiotic factors “governing the dynamics” of HNA and LNA cells is rather irrelevant. The authors should better focus what is already known and which their contribution is." This paragraph is modified (page 3 lines 17-27)

8) p. 6549. The paragraph “Both populations. . ..” makes absolutely no sense to me. What do the authors mean here? The misuse of the term “population” is frequent. HNA and LNA are not populations in the ecological sense; they can only be properly referred to here as “flow cytometric populations”. We agree. This is modified in the whole manuscript

9) "The claimed “good recovery” of the radiolabel in the sorted bacterial groups needs
further support. Fig. 5 shows several examples of really “bad recovery”. Similarly, Prochlorococcus was insufficient in some cases to compensate the “unrecovered activity” (page 6568, lines 26-27)." We agree. This is modified in the section 3.3.2 where we added a sentence on the unrecovered activity.

10) There is a clear contradiction in the statement on dark enhancement of Leu uptake by Prochlorococcus at the beginning of page 6569. Do the authors mean that by incubating their samples under natural irradiance conditions Leu uptake by this cyanobacterium would be higher? Please explain." It was a typo mistake. Of course we meant "light enhancement". This was corrected.

11) "The discussion on differences in temperature or chl a concentrations in Oregon or the BOUM cruise can be safely eliminated. I suggest to delete most if not all of the excessively detailed references to other authors’ work in the Discussion." This is done. We simplified this part

12) "Truisms such as those on page 6564, lines 12-16, or page 6567, lines 4-8 should be deleted". This is done.

13) "p. 6567. Inclusion of a discussion about differences in the quality of DOM, e.g. recently produced by phytoplankton or semi-labile DOM would perhaps be useful here." We discuss changes of environmental conditions between "surface" and "DCM" layers merely based on change of limiting factors and changes of taxonomic populations referring on literature.

14) "Table 1. How was BP estimated? Please provide details about the leucine to carbon conversion factors used." All data are now cited in terms of leucine incorporation rates and thus the conversion factor is not necessary anymore.

15) "Fig. 4 is not sufficiently explained. Details on what Tessier’s slope represent are needed. Also, the intercept of the regressions should be given and discussed." Figure 4 has been removed and results of Figure 4a have been inserted in the M&M section.
Slope and intercept have been discussed. We do not discuss anymore of the Figure 4b because its interpretation is redundant with the discussion in section 3.3.2 about proc contribution to bulk rates and unrecovered activities.

16) "Fig. 7. Changes in relative contributions to activity and abundance in the lower panels should be accompanied by statistical significance." This part of figure 7 on relative contributions was removed.

Technical 17) "The manuscript needs a thorough revision of English usage". We tried to do this.

18) "What are neoproducts?" We were discussing about phytoplankton by release (through excretion, grazing, lysis). We modify the sentence. 19) "Scharek and Latasa (200/) do not support the authors’ statement. This is just an example of much loose interpretations of the extant literature." We checked the appropriateness of all references.

20) "The codes of the 5 stations sampled are confusing. Could the authors use a more logical naming?" It is not possible to be kept consistent with all papers of the special issue 'BOUM'. However the figure 1 has been simplified to show only the 5 stations under interest.

21) "Justification and details on the separation of the different HNA groups must be included in the 2.4.1 sub-section" We prefer to define them in the section 2.4.2 when describing cell sorting gates.

22) "p. 6566. The statement about nutrient conditions in the Mediterranean should be better explained. Why not showing nutrient concentrations for the 5 stations?" We add a figure describing temperature and nutrients (new figure 3).

23) “p. 6564. line 5-7. Please include reference.” This is done (page11 line 17).

24) "p. 6566. To the best of my knowledge, Scharek and Latasa (2007) provide another possible explanation for HNA cells showing higher specific growth rates towards the
surface. “Fluvial water” is not a proper term here.” We read again this paper and did not find another explanation for HNA cells showing higher specific growth rates towards the surface. We used the term fluvial waters because it was used by Sharek and Latasa themselves. However, we use now the term "Rhone River influence".

25) "Table 2 legend is wrong. Not only the characteristics of LNA population are given here." The legend of table 2 has been modified.

26) "Table 3. Probably a new table for Prochlorococcus is not needed and information could be given in the main text." Table 3 is removed.

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Fig. 2.
Fig. 3.

Cell-specific leucine incorporation rates (x 10^{-21} mol leu cell^{-1} h^{-1})

- St. C
- St. B
- St. 21
- St. A
- St. 25

- HNA-hs
- HNA-ls
- LNA
- Proc
<table>
<thead>
<tr>
<th>Location</th>
<th>ID</th>
<th>Data Points</th>
<th>Depths</th>
<th>Int Chl a (mg m⁻²)</th>
<th>Number of data</th>
<th>Proc (x 10⁴ ml⁻¹)</th>
<th>Syn (x 10³ ml⁻¹)</th>
<th>Pic (x 10³ ml⁻¹)</th>
<th>Hprok (x 10⁵ ml⁻¹)</th>
<th>Bulk rate (pmol l⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. 25</td>
<td>25</td>
<td>3</td>
<td>5, 40, 50m</td>
<td>2.9</td>
<td>55</td>
<td>0.07-0.77</td>
<td>0.05-5.6</td>
<td>6.6-10.1</td>
<td>11-17</td>
<td></td>
</tr>
<tr>
<td>St. A</td>
<td>16</td>
<td>9</td>
<td>12-200m</td>
<td>8.1</td>
<td>16</td>
<td>0.03-6.4</td>
<td>0.04-1.1</td>
<td>1.8-5.0</td>
<td>0.6-8.9</td>
<td></td>
</tr>
<tr>
<td>St. 21</td>
<td>21</td>
<td>3</td>
<td>5, 70, 85m</td>
<td>3.4</td>
<td>21</td>
<td>1.6-7.1</td>
<td>1.0-0.69</td>
<td>5.0-5.6</td>
<td>4.7-9.4</td>
<td></td>
</tr>
<tr>
<td>St. B</td>
<td>16</td>
<td>8</td>
<td>5-160m</td>
<td>8.2</td>
<td>16</td>
<td>1.6-6.6</td>
<td>0.32-1.0</td>
<td>2.0-5.6</td>
<td>0.8-9.5</td>
<td></td>
</tr>
<tr>
<td>St. C</td>
<td>23</td>
<td>7</td>
<td>5-120m</td>
<td>5.7</td>
<td>7</td>
<td>0.13-16</td>
<td>0.43-1.0</td>
<td>2.5-3.5</td>
<td>1.5-4.9</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 4.**