

## ***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.

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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  $\text{pmol leu l}^{-1} \text{h}^{-1}$  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

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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.

Anonymous Referee #2 Specific comments

"1) One of the main interest of this work is the large range of samples collected along the transect of stations encompassing a large longitudinal gradient in the Mediterranean Sea. This point could be emphasized a bit more in the text showing the originality of the work". Because we had only 5 stations sampled, in fact we focus more on the vertical than on the longitudinal variability and this has been considered in the new version.

"2) Has any phylogenetic characterization of the LNA and HNA populations been done, at least for a few samples, using for example *in situ* hybridization after cell sorting? It would have been interesting for the discussion to have an idea of the diversity found in these populations, how this diversity varies across the Mediterranean Sea and with depth, and how it differs/compares with already known HNA/LNA populations from other environments. Similar metabolic relevance of the LNA population in the Atlantic ocean (for example, i.e. Schattener et al., EM, 2009 or Mary et al., AME, 2006 mentioned in the introduction but wrong reference used in the Reference section, should be changed) for a large range of samples along a transect has also been shown using similar method. This population was mainly composed of SAR11 cells. It would be then interesting to see if such a dominance is found in the Mediterranean Sea." Unfortunately, we have no volume left over to filter sorted samples for phylogenetic analysis. We are aware that the lack of information on the identity of the cells composing the

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different groups is the main drawback of our study. Maybe most of the LNA group were composed of SAR11 cells and we discuss this hypothesis in the discussion (page 11 lines 24-28).

"3) Material and methods. Not sure it is necessary to mention the 3 enrichment experiments (A, B, C) if only one was used for this work. Just describe the experiment B. Is there any references for the TCA method that could be quoted to simplify the text?" Stations A and C are not stated in the revised manuscript.

4) "In this work, labeled amino acids were added at saturating concentrations. How does this compare with nonperturbing tracer concentrations used in other works mentioned in the manuscript? It is known that the use of high concentration of leucine can as a result, elevate the ambient concentrations of amino acids in seawater and the activities of some groups of bacteria could be overestimated because of their ability to use the added compound as an alternative nutrient." The initial objective was to compare bacterial production among different groups sorted and adding leucine at saturating concentration is necessary in the protocol of BP measurement. It is thus possible that assimilation by mixotrophs should be lower *in situ* where natural leucine concentration is about 0.5 nM (Mary et al 2008b). Michelou et al. (2007) observed in North Atlantic that Proc contributed up to 24 % of the total leucine assimilation (added at 20 nM) and only up to 10 % of assimilation of an amino acid mixture (added at 0.5 nM concentration), but the opposite trend was found for Syn. Significant assimilation of methionine and leucine by Proc have been also demonstrated when adding 0.5 to 1 nM concentrations (Zubkov et al., 2004; 2006; Mary et al., 2008b). Thus we cannot rule out that the contribution of Proc facing *in situ* concentration of leucine would have been lower. We added a sentence on this in the discussion section 4.4 (page 14 lines 10-17).

"5) Again, has any molecular characterization of the HNA+ population been done? Thus, it would very interesting to discuss the results, especially in terms of nutrient response and distribution." We agree about the added value of phylogenetic charac-

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terization of the cytometric groups, which we could not make. If we had to chose one probe, we might use a high-level targeting probe such as CF319a probe for Bacteroidetes detection or Gammaproteobacteria, as they are expected to rapidly respond to favourable trophic conditions, rather than SAR11.

"Technical corrections 6) p6554: SyBR Green commercial solution is usually 10000x concentrated so the dilution is probably more." This was corrected. In the old version of the manuscript, we referred to a dilution of the working solution, not the stock solution.

7) "p6559: "15+/-31%" Is that correct?" After the reviewers' comments, we checked all data, and this section was modified in the revised manuscript (end of section 2.4.2 and section 3.3.2)

8) "P6569. Line 3: Should it be "incubation in the light" rather than in the dark?" Yes, we apologize for the typo, this is what we meant.

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Interactive comment on Biogeosciences Discuss., 7, 6545, 2010.

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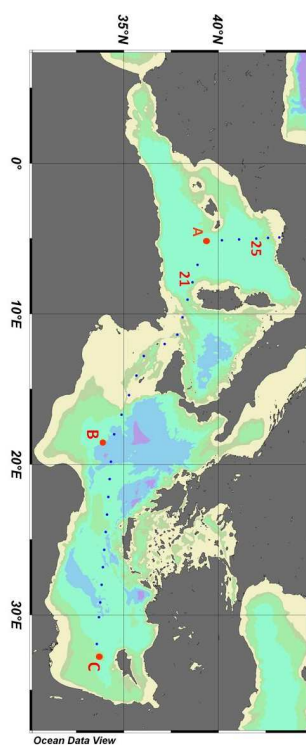


Fig. 1.

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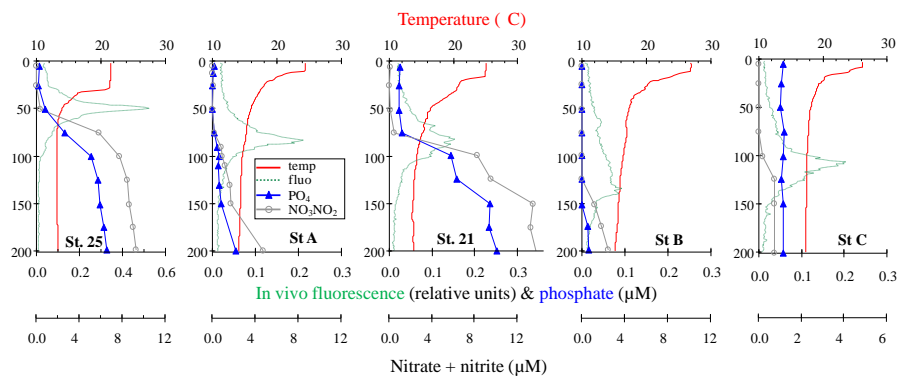


Fig. 2.

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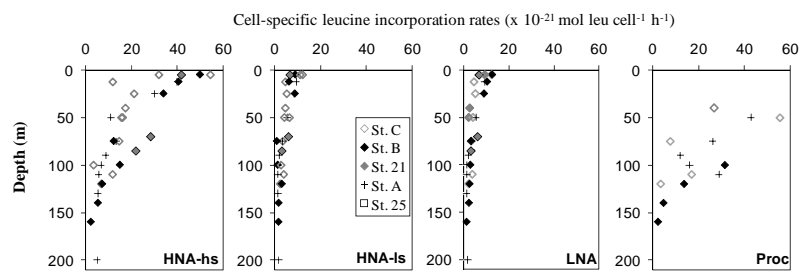


Fig. 3.

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	Int Chl a (mg m <sup>-2</sup> )	Number of data	Depths	Proc (x 10 <sup>4</sup> ml <sup>-1</sup> )	Syn (x 10 <sup>3</sup> ml <sup>-1</sup> )	Pic (x 10 <sup>3</sup> ml <sup>-1</sup> )	Hprok (x 10 <sup>5</sup> ml <sup>-1</sup> )	Bulk rate (pmol l <sup>-1</sup> h <sup>-1</sup> )
St. 25	55	3	5, 40, 50m	ld-2.9	0.07-77	0.05-5.6	6.6-10.1	11-17
St. A	16	9	12-200m	ld-8.1	0.03-6.4	0.04-1.1	1.8-5.0	0.6-8.9
St.21	21	3	5, 70, 85m	ld-3.4	1.6-7.1	ld-0.69	5.0-5.6	4.7-9.4
St.B	16	8	5-160m	ld-8.2	ld-6.6	0.32-1.0	2.0-5.6	0.8-9.5
St.C	23	7	5-120m	ld-5.7	0.13-16	0.43-1.0	2.5-3.5	1.5-4.9

**Fig. 4.**

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