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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.

Anonymous Referee #3

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General This manuscript presents data on the activity of planktonic heterotrophic prokaryotes measured as leucine (Leu) incorporation rates at a few open ocean Mediterranean sites during summer. Its interest lies in the splitting of bulk Leu incorporation between different groups of bacterioplankton as distinguished by current flow cytometry (FC) techniques. The easy and widespread recognition of at least two major subgroups of bacteria of high (HNA) and low nucleic acid (LNA) content makes contributions such as this study relevant for microbial ecologists, although it is not the first time that this task is attempted. Some of the results of Talarmin and co-workers are not new (e.g., that HNA bacteria show higher cell-specific activity than LNA cells, or that LNA cells can be active rather than dormant or dead). There are some novel

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results [e.g., the joint consideration of heterotrophic and autotrophic (*Prochlorococcus*) prokaryotes, or that cell-specific activities of all groups considered are more similar in the vicinity of the deep chlorophyll maximum] 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.

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. The objective of assessing their possible change with the degree of oligotrophy is thus not achieved in this piece of work. Nor is the exploration of “cell-activities of HNA and LNA cells over different basins in the Mediterranean Sea”. Such a statement needs at least duplicate stations per basin, which is not the case.

The separation of high nucleic acid content bacteria into three different clusters: HNA-Is, 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.

The text needs thorough revision for language use and re-writing at many parts. The text is frequently packed with too many details making its reading difficult. 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. There is no point, for instance, in making point-per-point comparisons with the results found by other authors in other oceanic regions, or repeating their findings with so much detail (e.g. p. 6563 and 6564)

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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.

Specific

p. 6548, 5. Please include a statement that the paper is addressing aquatic or marine heterotrophic prokaryotes

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.

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”.

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).

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.

The discussion on differences in temperature or chl a concentrations in Oregon or

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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.

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

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.

The text should be checked for verbosity. There is absolutely no need to continuously repeat "during the period of study" or the depth range in which the samples were taken.

There is confusion throughout the text between variables and parameters. Abundance is a variable, not a parameter.

Table 1. How was BP estimated? Please provide details about the leucine to carbon conversion factors used.

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. A noticeable loss of activity in the summed cell-sorted populations is evident in panel (b).

Fig. 7. Changes in relative contributions to activity and abundance in the lower panels should be accompanied by statistical significance.

Technical The manuscript needs a thorough revision of English usage. The list of instances in which poor syntax or incorrect wording appear is too long so as to include it here. To give just a couple of examples, please check the unnecessary presence of "the" before nouns. Also, only in page 6548 terms such as "advent... in the past 20 years", "contrasted ocean areas", "gather", "validated", "in all results", "various conclusions", are incorrect and should be re-written. I have preferred not to go with the rest of the main text but this is a serious shortcoming of the paper.

- What are "neoproducts"???

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- Scharek and Latasa (2007) do not support the authors' statement. This is just an example of much loose interpretations of the extant literature.

- The codes of the 5 stations sampled are confusing. Could the authors use a more logical naming?

- The first 4 sentences of section 2.2. could be deleted.

- Justification and details on the separation of the different HNA groups must be included in the 2.4.1 sub-section.

- The r value is the coefficient of correlation, not a regression coefficient! Please correct.

- p. 65558. Please delete or substantially reduce the first 9 lines of section 3.2.2.

- p. 6566. The statement about nutrient conditions in the Mediterranean should be better explained. Why not showing nutrient concentrations for the 5 stations?

- Please check the number of significant features in correlation coefficients.

- p. 6564. line 5-7. Please include reference.

- p. 6565-6566. I do not follow the reasoning of the references to the work of Lebaron et al. (2001) and Longnecker et al. (2006b) in the next paragraph. Please re-write.

- 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.

Table 2 legend is wrong. Not only the characteristics of LNA population are given here.

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

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