Biogeosciences
Discussions

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

Anonymous Referee #1

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General comments This manuscript presents data on flow cytometric sorting of radiolabeled samples from the Mediterranean. The methods used and the questions raised by the manuscript are not new, and the contribution of the authors is to expand this type of research to more stations in the Mediterranean. The authors do consider their data in a slightly different light than previous studies using this technique and that might be of interest to some readers. However, I think that the manuscript needs substantial revisions before it would be appropriate for publication in BG. I also feel that at least some example of the depth profile of cell-specific incorporation rates should be presented. By only presenting averages (Table 2,3), ratios (Figure 3), and summed data (Figures 4,5) the reader is left wondering about the variability in cell-specific leucine incorporation

ratios with depth. In the technical corrections section which follows, I have also listed cases where I observed differences between values presented in the text and values in tables/figures. Since there is more than one case where this occurs, I had a hard time deciphering if the patterns presented by the authors are real or are what appears when selected samples are considered. Specific comments The manuscript should be edited throughout to improve its readability. I have made some comments, but I eventually stopped trying to edit the manuscript during the course of my review. The manuscript is quite long. The introduction and the discussion sections include paragraphs unrelated to the topic, and the discussion in particular should be shortened. The results section repeats the contents of entire tables and figures, and is therefore a bit redundant. This redundancy gets even more annoying when the results are presented again in the discussion section. The manuscript also suffers from discrepancies between what is presented in the text and data in tables and figures. Furthermore, presentation of the data and the terms used to describe the different measurements are so vague that it is difficult to follow what is being presented. I will describe both of these concerns below in more detail. The distinction between HNA-hs and HNA+ seems somewhat arbitrary. Was there an SSC cutoff used to decide if a cell was one or the other? Is there some reason to not just lump the two groups together? I realize that the sorts are done and cannot be repeated, but I do not think that sufficient reason is given to consider the two groups differently in the manuscript. One sentence describing the differences between Figure 2b and 2c would be sufficient. The different terminology used to describe activity in the manuscript is confusing. The definitions given the methods section are good, and I would use the following terms: 'cell-specific leucine incorporation', 'volumetric leucine incorporation', and 'bulk leucine incorporation rates'. I would suggest using those terms throughout the manuscript and eliminate the other terms used: 'contributing activity', 'Hprok activity', 'bulk activity', 'leucine incorporation activity', 'specific activity', 'bulk heterotrophic prokaryotic production', 'cell-specific leucine incorporation'...I am quite familiar with this literature and I had a hard time following the different terms. Anyone even slightly outside the field will be completely

lost. Also, you should define the 'summed volumetric leucine incorporation rates' in the methods rather than leaving that information elsewhere in the text. I am little confused about which stations actually had samples sorted. The methods section discusses five stations (A, B, C, 21, 25). However, only stations A, B, C are included in figures 3 and 5. Since at no point are individual data from the five stations shown, I can't figure out if the remaining tables and figures include data from all five stations, or only a subset of the stations. Finally, with respect to the enrichment experiment conducted at 85 m at station 21. Since the control sample was apparently lost (though it is mentioned in the text on page 17, line 25 which I do not understand), the reader cannot assess the changes in the treatments. Therefore, I think that the dataset from 85 m should be eliminated because no inferences can be made about the data. There are methodological details missing from the manuscript. In particular, I cannot determine how many samples were resorted to assess the variability in the sorts. The data on page 10, line 21 seem to suggest that 24 samples were sorted multiple times. If that is the case, then error bars should be presented on the data in tables 2,3 and figures 3,5,6,7. Also, were the gates defining the different groups in the flow cytometer moved for each sample, or was the same set of gates used for the whole sample set? Technical corrections The title is a bit long and contains unnecessary information. Furthermore, the paper discusses both cell-specific and volumetric leucine incorporation rates. I would suggest: 'flow cytometric assessment of leucine incorporation in the Mediterranean' . . .or some variant of those points. SYBR Green I (not SyBR) There is no information in the methods on how the data on chlorophyll a or DOC concentrations were obtained. If these data came from collaborators on the cruise, the authors should at least state that. Page 2, line 4: please be consistent when presenting the bulk leucine incorporation data. The text only mentions integrated values, so having pmol leucine/l/hr in the abstract is confusing. Page 2, line 12 'bulk activity' is too vague. Also, I can't figure out where the 17-55% number for LNA activity comes from. Table 2 would seem to indicate that LNA cells contribution of bulk leucine incorporation ranges from 9-43%, or LNA cells contribution to the summed hprok leucine incorporation ranges from <1 to 58%. Page

2, line 15: 'HNA-hs was mostly responsible for the leucine incorporation activity' is this referring to cell-specific or volumetric leucine incorporation? Page 2, line 16: I also can't reconcile the 0.9 to 54.3 . . .rate given for HNA-hs samples with the data actually presented in table 2; HNA-hs rates in the table range from 2.3 to 54.3 which is higher than the 0.9 rate listed in the abstract. Page 2, 'at the opposite' – too vague because it is not clear what opposite you are talking about. Page 4, line 29: 'comparable diversity picture' is awkward Page 5, lines 4-12: this list of papers using the technique doesn't add to the introduction and can be eliminated. Page 5, line 21: after (Bouvier et al., 2007): this phrase is awkward and incomplete. Page 6, line 2: it would be nice if you could convert the ng c/l/hr units to whatever units are used in the present manuscript. Page 7, line 15: no need to mention the enrichment experiments at stations A and C in the methods if no data from them are presented in the manuscript. Page 7, line 17: Also, the manuscript mentions 20L polycarbonate carboys in one place and the 60L polycarbonate carboys here. I am reasonably certain this is experiment was done in a 20L carboy, but either way the difference should be clarified. Page 7, line 22: delete the phrase 'among many other parameters' because if they are mentioned in this manuscript, they are irrelevant. Also, when describing the two different incubations experiments please be consistent and either explain the duration in hours or days, but not one for each experiment. Page 8, line 19: 'incubation durations were enlarged' . . .I think this means the incubations were longer. However, it would be better to give the actual duration of the incubations rather than the vague 'enlarged' term. Page 8, line 22: just give the final concentration of PFA and don't make the reader have to do the math to figure that number. Page 9, line 22: this first sentence describing the sorts is confusing because it is a mix of complete sentences and partial phrases in list format. Page 10, line 4: change unfreezing to thawing. Page 10, line 5: what was the target number of cells? Page 10, line 23: 'equal sorts, n = 24' Does this mean that 24 samples were sorted multiple times? How many replicate sorts are there for each sample? Page 11, line 6: Trucount beads (not tubes). However, not clear when this was needed because the authors state that the concentrations were obtained by getting the exact

volumes before and after sorting. Page 11, line 18: '...was discarded...' Discarded is not the correct word here because it sounds like the sample was not used. Page 11, line 17: what were the conversion factors used to go from pmol leucine to carbon? This should be clarified since the authors presented the bulk leucine incorporation data in carbon units. Page 12, line 2: data are presented as average/standard deviations? Uh...everything I found seemed to be a range and mean and completely lacked standard deviations. I don't recall discussion of any non-parametric tests nor do I recall any model I regressions... these should be removed from the methods. Which model II regression? There are several types. Page 12, line 10: not clear which stations would be to 150m and which would be to 200 m depth, 'according to stations' is too vague. Page 12, line 5: Fisher's LSD test is inappropriate because it does not protect against multiple comparisons Page 12, line 17: for station 25, the text lists a value of 38 mg/m2 for the upper 150 m, but Table 1 shows a value of 55 mg/m2 which is also apparently for the upper 150 m. Why is there a discrepancy between these two values? Also, Table 1 refers to 'mixed layer temperature' while the text discusses 'sea surface temperature'...please clarify if these are different measurements or the same measurements. To me, the 'sea surface temperature would be the temperature at the shallowest depth sampled, while 'mixed layer temperature' would be an average of all the depths sampled within the mixed layer. I am further confused because the sea surface temperature in the text for station 25 is listed as 21.4, the same value as the mixed layer temperature in table 1. However, for station B, the sea surface temperature is 26.9, but the mixed layer is 26.8? I also think that discussion of the mixed layer depth can be removed because the manuscript focuses on the DCM. Same goes for the later mention of the euphotic zone. Page 13, lines 1-4: mostly repeating the methods section and can be removed from the results section. Page 13, line 4: this should be a reference to figure 2b. Page 13, line 9: the percent of LNA cells is not in table 2. Page 13, line 10: confusing because of the use of the term 'subsurface' when referring to 'surface' samples. Page 13, line 16: I don't understand how the 50m fits. The table seems to indicate data were integrated to 150/200 m, and the value given

has the units to be appropriate for integrated data. Page 14, line 6: the correlations between the cell-specific rates for the different sorted groups is not discussed further and can be removed from the results section. Page 14, lines 11-18: this is redundant since the pattern with depth was already discussed on page 13. However, I don't understand how the ratio of HNA-hs/HNA-ls in the text is given as 1.4-13.3, but the figure shows the highest value of that ratio is less than 12? I have a similar concern for the HNA-hs/LNA ratio where the values listed in the text do not match what is in figure 3. Page 14, lines 20-25. I think that one sentence indicating that detection limits for Syn and Pic were below detection would be sufficient. Page 15, line 13: this seems to be the first mention of a single sort for the whole hprok population. It would be better if this were first mentioned in the methods section and not in the results. Also, as for the cell-specific leucine activity, I don't think the correlations between the different sorts is worth mentioning because they are not discussed in the final section of the manuscript. Page 15, line 17: please test if the slopes were significantly higher than one. Page 15, line 21: Bpk? This appears to be a new abbreviation. I see it in Table 3, but it should be described in the text as well. Page 16, line 4: I don't understand how the HNA-hs cells 'contribution to leucine incorporation' was depth dependent since the data span the full range of measured activities. Page 16, line 6: where does the <1 to 11% number come from? Page 17, line 19: The text does not discuss an NPG addition in the station B experiments, so I am confused how there are data available from such an addition. Page 18, lines 3-24: this whole paragraph is just repeating what is in the figure and can therefore be shortened. Page 18, lines 25-30: you have already discussed the abundance trends on page 17, line 7. Please only discuss each trend once rather than repeating the same ideas. Page 19, line 9: where does the 3-20 times blank value number come from, and why is it being presented for the first time in the discussion? Even better would be to eliminate the results already mentioned in the results section (lines 10-13 are just reiterating data already presented in the results). Page 20, line 2: I don't consider 200 m 'deep water' Page 20, lines 5-10: this needs a citation as it clearly isn't coming from the present manuscript. Also the referring the 'latter' manuscript is

confusing because it isn't clear to the reader which manuscript is now being discussed. Please be specific. Page 20, line 12: the pearson's r values are already in the results section and don't need to be repeated in the discussion. However, I am not clear why slightly different r values are presented for apparently the same correlation. Page 23, line 16: 'probable at stn 21' can't be concluded from the authors' data because, as they note, they didn't make that measurement. Page 24, line 22: why is the percent of Proc. different than what is presented in table 3?

Figure 1: The stations not discussed in the present manuscript should be eliminated from the figure because they only clutter the figure. Figure 2: what is the dashed line in figure 2b? I would imagine this is the 'single sorts' done for the total population of heterotrophic prokaryotes, but this should be stated as such. Also, the legend is confusing since it starts out describing 'phototrophic' populations when only panel A shows phototrophs. 'natural fluorescence' is also inappropriate because the term means something quite specific to phytoplankton ecologists and is not what is being studied in the present manuscript. Figure 4 can be eliminated and just left as a text in the manuscript. The different regions (surface, DCM, deep) are not discussed with respect to this figure. Therefore the figure does not add to the conclusions in the manuscript. Figure 6: The different color symbols for the surface samples should all be one color. Since blue and green are used for deep and DCM respectively, having a combinations of red and yellow or yellow for the surface is confusing. Also, please clarify how the 'summed activities' was calculated . . . I imagine this is the sum of the volumetric leucine incorporation for HNA-hs, HNA-ls, and LNA; but what is the denominator? bulk leucine incorporation? the volumetric leucine incorporation of the 'single hprok' sort? Figure 7. Using red-green as a color palette is problematic for people who are colorblind. I would suggest using a different set of colors for the figure because it all appears grey to anyone who is red-green colorblind. Also, I would just define the different abbreviations in the methods and then refer to the methods in the figure legend rather than repeating all of the abbreviations in the legend. Figure 7: the last row of the figure isn't discussed in the manuscript and therefore should be eliminated

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from the figure. Table 1 has too many significant digits for many of the variables. See Sokal and Rolf, Biometry for a good rule of thumb. Table 1: what criteria were used to decide the depth of the DCM? In figure 3, the DCM for station B looks more like 140 m. Table 1: is the mixed layer temperature data the average over the mixed layer? And if so, why bother presenting this value since everything else in the paper is presented relative to the DCM. Table 2: the beginning of the legend for this table is misleading because the table encompasses more than LNA cells. Also, the values appear to be ranges with means in parentheses; please state this in the legend. Finally, the table has more columns than are described in the legend. Table 2: Why is n = 6 for the deep samples in Figure 6 and n = 7 in this table? Table 2: I do not understand why the range of 'contribution to summed hprok leucine incorporation for HNA-hs cells is 11-65 in the table, and appears to be 20-65 in figure 6. Table 3 can be eliminated and the data incorporated into the text.