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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.

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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

BGD

7, C3990–C4011, 2010

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 #1

General comments

". . .. 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. . ."

A new figure shows vertical profiles of cell-specific incorporation rates (new figure 4).

"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".

We checked consistency between tables, graphs and citations in the text. The number of data collected in situ are now indicated in the text (page 8 line 21) and Tables 1and 2.

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

BGD

7, C3990–C4011, 2010

course of my revie"w.

We conscientiously worked on the clarity and readability of the text in the revised manuscript, in order to avoid possible misunderstandings and incoherencies.

"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".

We reduced the length of our manuscript, by simplifying the results section, and reducing the discussion.

"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" "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'.. . .." .

As we explained earlier, data from figures, text, and tables were checked to discard incoherencies. The numbers of data are now presented in the text, tables and the whole data set is visible on the new figure 4. The terms of "cell specific rates", "volumetric rates" and "summed Hprok volumetric rates" were defined in the M&M section (page 7 lines 15 17, 23) and used throughout the manuscript. We removed BP data, and used "bulk rate", expressed in pM leucine h-1 instead, as stated in the revised 2.5 section.

"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

BGD

7, C3990–C4011, 2010

repeated, but I do not think that sufficient reason is given to consider the two groups differently in the manuscript."

HNA-hs and HNA-ls gates were designed in a protocol applied to in situ samples. During Hprok sorts, gates did not have to be moved, because there were very little changes in SSC and green fluorescence signals. Discrimination in HNA-hs, HNA-ls and HNA+ help to explain variability of cell-specific activity within HNA group, and then it is always possible (like we did in the discussion page 12 line 26) to examine cytometric characteristics of HNA group a posteriori. Dealing on the enrichment experiment, we felt more logical to keep a HNA window corresponding to the HNA-ls + HNA-hs gates of in situ sample, and thus the cut off criterion for HNA+ was just to include all these very high SSC cells out of the HNA window (HNA+ were observed in only 1 enrichment experiment).

"One sentence describing the differences between Figure 2b and 2c would be sufficient".

New section 2.4.2 is more precise and the legend of figure 2 is modified.

"Also, you should define the 'summed volumetric leucine incorporation rates' in the methods rather than leaving that information elsewhere in the text". This was done (p7 line 23), as well as the results of the comparison between the summed Hprok volumetric rates and the volumetric rate of the total (single) Hprok sort which we presented at the end of section 2.4.2 (data of old figure 4a, which was removed in this version).

"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".

The data set includes data from all 5 stations. However, only 3 of these (A, B and C)

BGD

7, C3990–C4011, 2010

included more than 3 depths. This is the reason why they were chosen as an illustration of profile data of volumetric rates on figure 5. But another figure showing all data of cell specific activities has been made in this manuscript (new figure 4).

"Finally, with respect to the enrichment experiment conducted at 85 m at station 21. Since the control sample was apparently lost.... Therefore, I think that the dataset from 85 m should be eliminated because no inferences can be made about the data".

Indeed, the control tube could not be found at the return to the lab. We thought it could be interesting to maintain it because it showed stimulation of Hprok by glucose at the vicinity of the DCM. However, we agree with the comment of the reviewer and removed this experiment from the data set. Thus, enrichment experiments in the revised manuscript includes only experiment from St.21 5m, and from St. B 8m, as shown in the new Figure 7.

"I cannot determine how many samples were resorted to assess the variability in the sorts. The data on page 10, line 21 seems 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".

On different occasions, we sorted triplicate samples of the same cytometric groups, with equal and/or different quantities of cells. The coefficient of variation was obtained by comparing the different activity per cell obtained in these replicates. Such tests were made on 24 different samples. We modified the text to be clearer page 7 lines 18-20. However, we choose not to show the error bars in Figure 4 for clarity.

"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?"

As we explained earlier, the same cytometric protocol was applied to all samples, and no changes were made to the protocol for stained samples (Hprok cells). The case was different in unstained samples, because along the water column, there were major

BGD

7, C3990–C4011, 2010

variations in the natural fluorescence of phototrophic cells. Then, along a profile, gates were moved to better fit different groups. The variation of SSC signals was negligible compared to the variation of fluorescence signals. See attached Figure A, showing the gates drawn at 2 different depths from St. 21. This figure does not appear in the revised version of the manuscript.

Technical corrections "The title is a bit long and contains unnecessary information. . .".

We changed it for “Flow cytometric assessment of specific leucine incorporation in the open Mediterranean”.

"There is no information in the methods on how the data on chlorophyll a or DOC concentrations were obtained..."

We now refer to companion papers for methodology (presently submitted to the BOUM special issue and accessible in biogeosciences discussion) in the revised manuscript. Those can be found at the following web addresses: Chlorophyll: Crombet et al. 2010, http://www.biogeosciences-discuss.net/7/6789/2010/bgd-7-6789-2010.pdf DOC and nutrients: Pujo-Pay et al. 2010, http://www.biogeosciences-discuss.net/7/7315/2010/bgd-7-7315-2010.pdf

"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".

Only volumetric values are presented in the new manuscript (abundances in cells ml-1, bulk leucine incorporation rates in pmol leucine l-1 h-1, see new Table 1), except for total chlorophyll concentrations (and the Table 1 has been modified accordingly).

"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%".

BGD

7, C3990–C4011, 2010

For most of the following questions, refer to the new Table 2 in the revised manuscript. The revised version of the text is now based on these values that were cross-checked several times and expect we are now consistent between text, figures and tables.

"Page 2, line 15: 'HNA-hs was mostly responsible for the leucine incorporation activity' is this referring to cell-specific or volumetric leucine incorporation?"

In the context of this sentence, it was referring to HNA-hs volumetric rates compared to the bulk rates, because the highest contribution was measured in HNA-hs group (cf Table 2). The abstract was re-written.

"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".

The Introduction was simplified and we checked all references cited.

"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".

After this comment, we expressed all fluxes (bulk, cell specific and volumetric rates) in terms of leucine incorporation rates and thus removed the use of BP in terms of carbon units in the 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".

Stations A and C are no longer mentioned in the revised manuscript.

"Page 7, line 17: Also, the manuscript mentions 20L polycarbonate carboys in one place and the 60L polycarbonate carboys here..."

We apologize for this typo; at St. 21, the enrichment experiment was made in 60 ml carboys, and in 20 l carboys at St. B. This was corrected.

BGD

7, C3990–C4011, 2010

"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."

In the revised manuscript we removed the irrelevant sentence and kept amended incubation durations in days (4 days at St. B and 1.5 days at St. 21). See section 2.2.

"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."

Incubation lasted up to 5 hours (in the linear phase of incorporation) to ensure sufficient labeling before flow sorting. This was added to the M&M section (page 5 line 22).

"Page 8, line22: just give the final concentration of PFA and don’t make the reader have to do the math to figure that number".

2% final concentration. This was modified (page 5 line 12).

"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".

The sentence was modified (page 6 lines 10-12).

"Page 10, line 5: what was the target number of cells?"

It is the number of cells that we chose to sort. Using the 4-way sorting mode, at least one threshold (determined number of cells) was set for a targeted group.

"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".

BGD

7, C3990–C4011, 2010

Yes, we agree. On the FACSAria sorter, beads were not used for determining concentrations but to have an idea of the HNA+ size.

"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".

In the revised manuscript, there are no data in carbon units anymore.

"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".

Finally we used model I to be able to have slopes and intercept values with their associated errors (the figure 3 have been removed and results presented in M&M section). In the text and discussion, we use ranges (minimum-maximum), means and standard deviations, as well as Pearson's correlation coefficients. The M&M section has been modified accordingly (page 8 lines 6-8)

"Page 12, line 5: Fisher's LSD test is inappropriate because it does not protect against multiple comparisons"

A posteriori tests are no longer needed in the revised manuscript.

"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?"

It was a mistake in the text, for St 25 the correct value was that in the table: 55 mg/m2. All data of Tchla are integrated between 0 and 150m, which is specified in the legend of Table 1.

BGD

7, C3990–C4011, 2010

"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. .."

We agree with the referee, this was confusing. In the old Table 1, we presented mixed layers depths but sea surface temperatures. In the new version of the manuscript these data are removed from the table because a new figure describing temperature, nutrients and in vivo fluorescence was added (new figure 3). Sea surface temperature ranges are cited in the text section 3.1 (page 8 lines 16-17).

"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."

We agree and removed this redundancy, and thus the reference to Fig. 2.

"Page 13, line 9: the percent of LNA cells is not in table 2".

We removed citation of table 2 in this sentence.

"Page 13, line 10: confusing because of the use of the term 'subsurface' when referring to 'surface' samples".

The term 'subsurface' is not employed in the revised manuscript, to avoid ambiguous interpretation of the results. The three layer categories used in the manuscript are defined in the legend of Table 2.

"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."

This was a copy-paste mistake and is now corrected.

"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."

BGD

7, C3990–C4011, 2010

This result was eliminated.

"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."

The former figure 3 showed a HNA-hs/HNA-ls ratio of 13.3 (St. B). Mistakes were made in the text, while the figure was correct, after thorough verification. A new figure showing cell-specific activities was inserted in the manuscript (new Figure 4) and is described in the text (section 3.2.1).

"Page 14, lines 20-25. I think that one sentence indicating that detection limits for Syn and Pic were below detection would be sufficient."

This section was shortened (page 9 lines 14-21).

"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".

We agree and did this. This part is now in the M&M at the end of section 2.4.2 (page 7) and not further discussed.

"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."

In the revised manuscript, we don't use this term, so as to limit confusion about the groups involved.

BGD

7, C3990–C4011, 2010

"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?".

Section 3.3.1 has been corrected (end of page 9).

"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."

There was no NPG addition in the mesocosm experiment from St. B. Detailed nutrient combinations are stated in the M&M (section 2.2).

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. "

The length of this paragraph was considerably diminished in the revised manuscript.

"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)."

In the discussion, we often introduced comparisons (mostly ratios) based on the data presented in the Results section. This way, information is conserved, but the approach is less focusing on absolute values.

"Page 20, line 2: I don't consider 200 m 'deep water' "

This term was removed and instead we refer to "below dcm" layer as defined in the legend of table 2.

"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

BGD

7, C3990–C4011, 2010

to the reader which manuscript is now being discussed. Please be specific".

The latter study refers to Longnecker et al., 2006. Precision was added to the revised text (page 11 lines 5-13)

"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"

Such redundancies with the results section were eliminated from Discussion.

"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."

This assumption was removed.

"Page 24, line 22: why is the percent of Proc. different than what is presented in table 3?"

The revised manuscript indicates all details for Proc in the text in section 3.3.2 (page 10 lines 9-18), Table 3 has been removed.

"Figure 1: The stations not discussed in the present manuscript should be eliminated from the figure because they only clutter the figure."

This is done.

"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."

The legend of this figure was modified (page 22).

BGD

7, C3990–C4011, 2010

"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".

We remove this figure and we include its interpretation in the text (sections 2.4.2. and 3.3.2 of the revised 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?"

The legend of this figure was modified. The denominator for both contributions is the summed abundance or volumetric rate. For example, abundance contribution of HNA cells = 100 x NHNA / NHNA-hs+NHNA-ls+NLNA), where N is the abundance of a group.

"Figure 7. Using red-green as a color palette is problematic for people who are color-blind. 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. "

The color code is modified on many figures, to be readable even from a black and white print.

"Figure 7: the last row of the figure isn't discussed in the manuscript and therefore should be eliminated from the figure. "

This row doesn't appear anymore in the revised version of the Figure 7.

"Table 1 has too many significant digits for many of the variables. See Sokal and Rolf,

BGD

7, C3990–C4011, 2010

Interactive Comment

Biometry for a good rule of thumb. "

This is checked and modified in both Tables

"Table 1: what criteria were used to decide the depth of the DCM?"

It is the depth where maximum in vivo fluorescence was detected, because precision is better with the sensor of the CTD cast (one value every m) instead of that using discrete sample of total chlorophyll a. These values are removed from Table 1 and described in the results section when referring to the new figure 3 (page 8 lines 14-15).

"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".

No, it was the sea surface temperature which was presented. However this information is now removed from Table 1, and now appears in the text of the revised manuscript when describing the new figure 3 (section 3.1 page 8).

"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."

The legend of Table 2 is modified.

"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".

There was a missing data in the Figure, the correct range for HNA-hs is 11 – 65 % of the Hprok incorporation (see new Table 2).

"Table 3 can be eliminated and the data incorporated into the text".

BGD

7, C3990–C4011, 2010

We incorporated data from Table 3 in the text and eliminated this table.

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

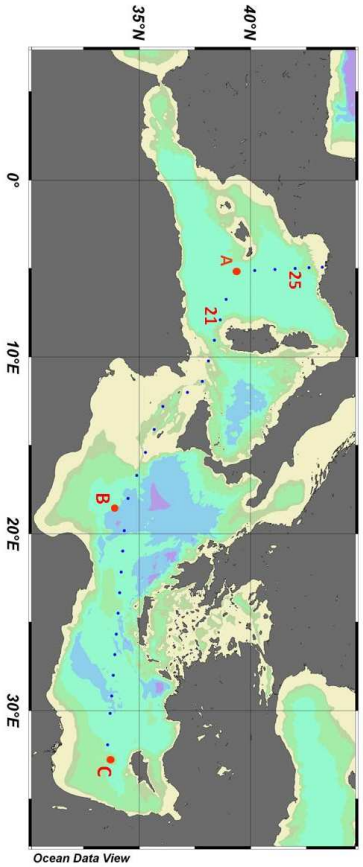

Fig. 1.

BGD
7, C3990–C4011, 2010

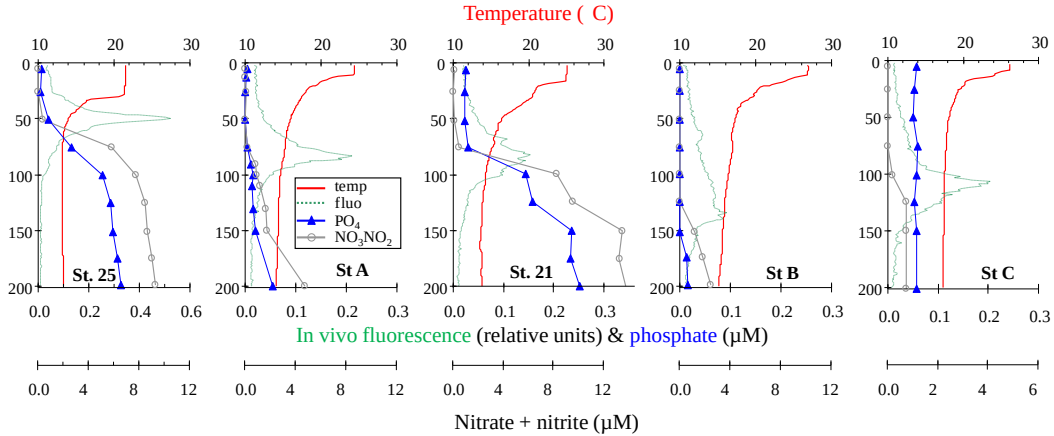

Fig. 2.

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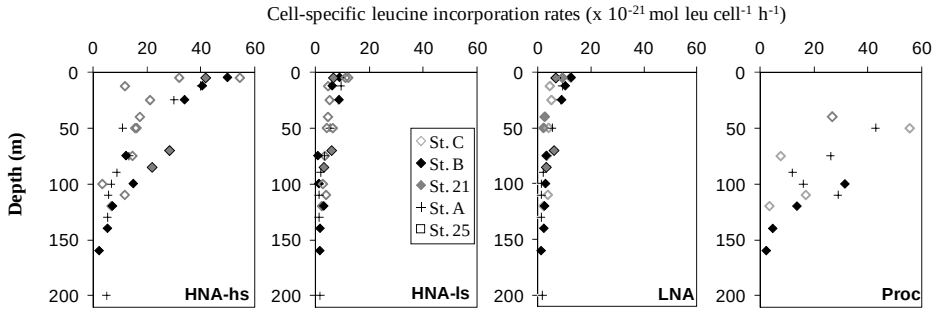

Fig. 3.

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	Int Chl a (mg m^{-2})	Number of data	Depths	Proc (x 10^4 ml^{-1})	Syn (x 10^3 ml^{-1})	Pic (x 10^3 ml^{-1})	Hprok (x 10^5 ml^{-1})	Bulk rate (pmol l^{-1} h^{-1})
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.

BGD
7, C3990–C4011, 2010

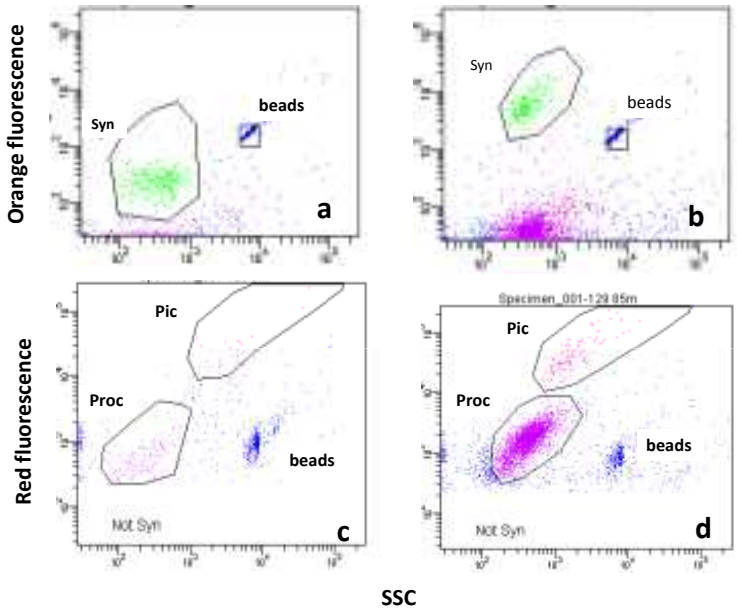

Figure A. Cytograms showing phototrophic cells at St. 21 5m (left panels) and 85m (right panels): a, b) orange fluorescence of Syn versus SSC, and c, d) red fluorescence of Proc and Pic cells versus SSC. Decadal scales are similar on both axes of all cytograms, from 10^2 to 10^5.

Fig. 5.

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