**Biogeosciences
Discussions**

Interactive comment on "Relationships between cytometric characteristics of high and low nucleic-acid bacterioplankton cells, bacterial production and environmental parameters along a longitudinal gradient across the Mediterranean Sea" *by* F. Van Wambeke et al.

Anonymous Referee #1

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GENERAL COMMENTS

This manuscript presents data on the abundance of different bacterial groups (as defined by flow cytometry) and bacterial production. The data were apparently collected as part of a larger project (BOUM) which is the topic of a special issue in Biogeosciences. A large number of samples were collected and processed. While I understand that a large amount of effort went into the data, I think that the manuscript needs

to be revised in order to clearly present the key points and not bury the reader in repetitive correlations and dot plots. By the time I reached the end of the paper, I had completely lost track of which depth layer was important for which correlations. Also there are multiple places in the discussion where the results are just repeated rather than allowing the discussion to focus on the data that has already been presented.

SPECIFIC COMMENTS:

I understand that studying water masses above/within/below the DCM is common, and I do not have a problem with those distinctions. However, I think that considering three layers within the water column should be sufficient. The division below the DCM is apparently arbitrary. Furthermore, the authors used two different methods to define bacterial production: one method above 200m and a second method below that point. Yet, the authors then discuss data down to 250 m as separate from samples below 250 m. Indeed, the authors begin their own discussion (page 8257, line 23-25) with a description of three water column layers.

My confusion about what the important points of this manuscript are was exacerbated when the same data are repeatedly presented in tables, in the text, and in figures. I would delete all of Table 3 and only show the data in Figure 6. The text can then present the correlations most relevant to the points the authors are trying to make. In addition, I think that table 1 can be shortened to only include the data on the different layers without the upper part of the table showing n, min, max, and so on. The boxplot in Figure 5 is a nice way to clearly show what happened at the different depths for the different variables. I think that much of the discussion in the paper could focus on this figure.

The data in table 2 appear to only be considered briefly in the results where the authors compare their data to previous work and conclude that variability in bottom-up control as important. However this idea does not get expanded up in the discussion, and is out of context for the rest of the paper. Furthermore, table 2 should only include the Model

II data. While I understand that previous work has used Model I (inappropriately as correctly noted by the authors), I do not think it is necessary to devote so much room to data incorrectly analyzed.

In places in the discussion, the authors go a little too far past their own data with the conclusions they reach. For example, the idea that the level of variability in green fluorescence is a direct link to adaption to response to the environment (page 8261, line 23) is a bit of a stretch. The authors go even further and bring up the switch from nutrient limitation to carbon limitation as a factor controlling SSC and green fluorescence (page 8262, line 13) – yet the manuscript has no data either carbon or nutrient limitation. Then the discussion makes the leap to membrane physiology and SSC (page 8263, line 12) which I think is stretching their data way too far.

The authors' appear to have no killed controls for the bacterial production data. Since this is a standard part of the protocol in measuring bacterial production, the authors need to provide a strong justification for its omission and the potential impact on their results.

TECHNICAL CORRECTIONS

Page 8247, line 7: 'contrarily' is a bit awkward, how about 'contrary'?

Page 8248, line 19: '...showed that these characteristics changed...' I would specify 'cytometric characteristics' because otherwise the sentence could be interpreted as referring to BP and chl a.

Page 8249, line 2: '...using an unique procedure/instrument...' this study is presenting standard flow cytometry and bacterial production data, so I don't think the description of 'unique' methods is appropriate

Page 8249, line 9: 'connexions' should be 'connections'

Page 8249, lines 9-11: 'were examined according to the distribution of chlorophyll by dividing vertical layers' ?? This needs to be rephrased because it is awkward and it is

not clear what is mean by dividing vertical layers.

Page 8249, line 20: '...represented the majority of the area were occupied briefly...' not clear what is meant by majority of the area.

Page 8249, line 23: 'only one over two' ? not clear what this means.

Page 8252, lines 11-15: please define 'dcm' before using it to describe how the water layers were partitioned. Also, since the definition of the dcm is a key part, please specify how the depth of the dcm was actually determined.

Page 8253, line 3-5: Looking at figure 4, I don't see surface water temperatures down to 17degC.

Page 8254, lines 13-15: 'Box plot distributions of HNA and LNA cell abundances relating to layer ("surface", "dcm", "below dcm", "deep") were similar (Fig. 5a, b)' ... this sentence is too vague, so I can't figure out what the authors think is similar in subplots 5a and 5b. I think the sentence can be removed since the authors go into detail in the following paragraphs.

Page 8258-8259: if the %HNA with depth is the most striking feature in the dataset, this point should not be buried in the results section. Also, the statement about LNA cells decreasing with depth faster cannot be seen in the way the data in figure 4 are currently plotted. Furthermore, while the authors do not need to repeat their results in the discussion section, they should check as to why the discussion has n = 55 but the results have n = 53 for what appears to be the same conclusion.

Page 8259, lines 9-28: The extended discussion on factors other than size which affect SSC can be shortened because the authors' data really did not cover those points.

Page 8260, line 28: '...along the different sub-groups of chlorophyll categories...' not clear what this means – perhaps the different layers of the water column being considered?

Page 8261, line 3: '...the correlation was significant...' not clear which correlation is being discussed, HNA cells? LNA cells? Also: 'The slope of the regression of abundance versus chlorophyll was slightly higher for HNA than for LNA cells within the "dcm" layer, suggesting that the HNA cells were very responsive to changes in phytoplankton stocks in the "dcm" layer....since the reader doesn't know the slopes for the data only within the dcm layer, this statement is hard to evaluate. Also, there are statistical tests to compare slopes and they should be done if the data are that important (see Zar, Biostatistical Analysis for one book with an excellent description of how to determine if two slopes are in fact significantly different)

Page 8261, line 21: 'hypothesize' not 'hypothesise'

There appear to be references which are not cited in the text, but do appear in the references section (Schlitzer for example. I did not look for others, but that one stood out).

Table 1: in the column for temperature, does the 'pot' indicate that potential temperature was used? And if so, why? Also, I am not clear about what '...for the data set used for comparison of abundance and cytometric characteristics of HNA & LNA cells' means – the statement seems to imply there are other data not being presented in the manuscript.

Table 2: please define 'ns' in the legend.

Figure 1: please indicate what the colors in the map are. I would imagine they are depth, but that should be indicated.

Figure 2: please specify what the vertical bar is around 1000 km. Also, even though you do not have bacterial production data for all of the stations, please make the plots with the same x-axis to allow for easier comparison between the two variables.

Figure 3: the points would be easier to distinguish if only one circle were used, and the other circles were replaced with non-circle and non-square shapes.

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Figure 4: The LNA and HNA abundances overlap enough that I would separate them into two figures or add some color so that the reader can see any possible patterns between LNA and HNA abundances. Also, the figure legend indicates that the scales are different for 4b and 4f; however, there also appears to be a difference scale for bacterial production (0-50 in the upper plot an d0-5 in the lower plot).

Figure 5: please make the letters for each of the subplots the same in order to prevent confusion between the letters indicating statistical significance and the letters for each of the subplots.

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