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 #2

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This manuscript presents large amounts of data on distribution patterns of prokaryote cells and their single cell characteristics in the Mediterranean Sea. One anonymous reviewer has already provided critical comments on this manuscript, pointing out the need of revisions. After careful examination of the manuscript and the review, I reached the conclusion that I mostly share the opinion with the other referee. In order to minimize repetition, the following review will focus on the points that were not mentioned or not adequately stressed by the other referee.

1. Testable hypotheses (research questions) should be formulated in order to contribute to the progress of science. Introduction states two aims. One is to explore the factors that determine HNA-LNA cell variability in the Mediterranean Sea. This sort of "aim" would have been justifiable in 80-90's when the flow cytometric technique in marine science was relatively new. However, given that extensive amounts of data on single-cell characteristics of marine prokaryotes have been published during the past decade, this naïve formulation of research question cannot be acceptable. The second stated aim (to explore HNL-LNA connections) appears to be more specific, yet I could not figure out what "HNA-LNA connections" means at all on the basis of the information provided in Introduction. It was unfortunate that what this question (HNA-LNA connections) means was unclear even after reading the entire text including the relevant section in Discussion (Section 4.4). My suggestion is that the manuscript be reorganized to address specific (and meaningful) hypotheses regarding single cell properties (biochemistry and ecology, not just "cytometric characteristics") of marine prokaryotes. What was the question? What was the answer? What was the news?

2. Need more examination regarding prokaryote-environment relationships. It was rather disappointing that the authors did not explore potentially interesting questions regarding prokaryote-environment relationships in the Mediterranean Sea. In fact, the environmental factor that was dealt with by this study was only chlorophyll. I suspect that there were gradients in nutrient concentrations and stoichiometry along the transect and over depth. Mediterranean ecosystem in general is known to be severely limited by P, but the extent of P-limitation may vary depending on regions and depths. Also, there is a vertical gradient in temperature but Mediterranean is unique with relatively warm waters at great depths. How these unique environmental settings of the system examined by this study may affect prokaryote single cell properties?

3. SSC-FL diagrams should be presented. Definitions of HNA and LNA differ among studies using different instrument and/or conditions. Since SSC and FL variations are the focus of this study, it is important to show representative diagrams.

Specific comments

Section 3.2 As pointed out by the other referee, this section and related tables and figures can be substantially reduced or eliminated.

The increase in %HNA with depth has been ascribed to the alleviation of P-limitation (Nishimura et al. 2005 AEM) and this notion has been theoretically considered under the framework of temperature-resource acquisition trade offs (Hall et al. 2008 ISME J. 2:471-). I would recommend that the authors mention this.

The first paper that described %HNA-chl relationship in the ocean is Li et al. (1995 L&O). I would recommend that this paper be cited.

Although the authors considered only bottom up forces as determinants of single-cell properties of prokaryotes, there is ample evidence in support of the notion that selective grazing and viral infection may also affect size and surface properties of cells.

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