

Revision of the manuscript L&O 13-159 entitled “Bacterial survival governed by organic carbon release from senescent oceanic phytoplankton” by Sébastien Lasternas and Susana Agustí

## General comments

The manuscript discusses the coupling between heterotrophic bacterial survival and the release of recently photosynthesized carbon, mostly through phytoplankton cell lysis, in three distinct oceanic regions (upwelling, intermediate and oligotrophic) of the NE subtropical Atlantic. The authors suggest a link between phytoplankton cell death, extracellular carbon release and a subsequent increase in the percentage of living heterotrophic bacteria cells.

The manuscript is well written and well structured, and the subject is of great interest for L&O readers.

However, in its present state there are some points that need some clarification, such as the methodology to determine phytoplankton cellular dead. The Nucleic Acid Staining Protocol also needs some clarification, as some studies preconize a simultaneous addition of the 2 staining solutions, and that was not the case here. Also, the PER rates seem very high, compared to literature data. The authors relate bacterial survival to the PER. These results should be taken more carefully as other factors can have affected the % of living bacterial cells. There might be different grazing or viral infection rates in the different oceanic regions, and the bacterial survival might be unrelated do PER.

I also noted the lack of objectives and hypotheses clearly stated on the manuscript. If the goal was to test the hypothesis that bacterial survival is actually governed by DOC released from senescent oceanic phytoplankton (stated in the title), the authors failed to demonstrate the mechanisms that drive those survival rates. The authors found a positive correlation between the %PER and the %living bacterial cells, but it was not possible to exclude other possible factors that influence bacterial survival rates (grazing, virus, nutrient limitation, DOC quality, etc...).

## Detailed comments

L38 – “been” is not correct

L52 – “PDOC” recent papers have used DOCp as an abbreviation for extracellular release or production of dissolved organic carbon, I think it is more intuitive than PDOC

L58 – “labeled carbon”? do not understand

L105 – Rephrase “delivered”

- L131 – Falconi et al 2008 (*Applied Environ. Microbiol.* 74, 1767-1779) say that it is important to add the 2 staining solutions simultaneously. This was not the case here. Any comments? Are these results valid?
- L167 – A more detailed description of this method would be welcome. Is this method validated? Is it been widely used by other research groups? The concentration method certainly causes some cell losses, any comments? Is there the possibility of grazing or phytoplankton cell division going on during the incubation? How can you control that? It is important to clarify those points.
- L181 – Why is it necessary to group PER? Why not using the continuous variable PER against the continuous variable %LHB? In which figure are the PER results grouped in classes?
- L181 – Decide if you use the abbreviation %LHB or not and apply it throughout the manuscript. Personally I prefer to avoid as much as possible having too many abbreviations, especially when they are not used very often (this comment is valid for other abbreviations).
- L198 – Fig. 4 show PER up to 90%. This is much higher the values reported in the literature (up to 45%, Baines, S.B. & Pace, M.L. (1991) *L&O*, 36, 1078-1090.; ~20% Maranon, E et al. (2005) *MEPS*, 299, 7-17). Any comments?
- L216 – Is it really 103 cells ml<sup>-1</sup>? Exponential is missing
- L260 – The authors should be more careful here and throughout discussion as other factors may affect the % of living bacterial cells. It can not be excluded that the different oceanic regions had different grazing or viral infection rates or other factors than PER, affecting bacterial mortality. Actually, bacterial survival rates might well be unrelated do PER. The authors should provide more arguments to this statement. There is no direct observation of the processes that enable bacterial survival rates at higher PER rate in the results presented here.
- L292 – The lability of the released compounds may also change depending on the phytoplankton composition, and that affects bacterial response. There are some recent papers showing that: Sarmiento, H. & Gasol, J.M. (2012) *Environ. Microbiol.*, 14, 2348-2360; Nelson, C.E. & Carlson, C.A. (2012) *Environ. Microbiol.*, 14, 1500–1516; Sarmiento, H. et al. (2013) *L&O*, 58, 1123-1135
- L300 – Again, the authors should be more careful here and throughout the text as healthy phytoplankton cells also release DOC, not only dead cells. By the way, I miss this landmark publication in this manuscript: Baines, S.B. & Pace, M.L. (1991) *L&O*, 36, 1078-1090.
- L306 - Explain how DOC per bacterial cell was calculated. How accurate is this variable? By the way, in some superficial samples is was hard to differentiate *Prochlorococcus* from HB in the flow cytometry counts with Sybr-Green. Explain how do you deal with this. Were the *Prochlorococcus* subtracted from HB?
- L313 – “higher flux of PDOC per bacterial cell”: living cell? Or both living and dead cell? How was this flux calculated?

- L315 – “while bacterial carbon demand was not related to algal PDOC in coastal and productive systems”: could be a question of DOC quality (see references suggested above)
- L319 – This sentence is speculation, should be removed in my opinion.
- L324 – More DOC release could be related to more DOC exudation, not only to phytoplankton cell death. Is it possible to estimate the contributions of each?
- L326 – “...high phytoplankton cell death in the open oligotrophic areas of the NE Atlantic results in a large release of DOC relative to primary production, providing a significant flux of labile carbon, that results in high heterotrophic bacteria survival,...”: Again, bacterial survival can be affected by many other things other than higher PER.