

## **Actions taken to accommodate the comments of Reviewer#1**

**Reviewer # 1:** I find this ms original and interesting, the whole data set and variety of methodologies used merits publications. However, an effort should be done to be more rigorous in the description of results, particularly considering statistics (text and figures says sometimes the opposite), and the way to estimate the average percentages and their associated errors. Because results are most of the time pooled, we have not information on evolution along depth, just general trends along sites, and this could give also interest in this ms, because PER is very sensitive to light and nutrient stress.

**Author's comment:** We thank the reviewer for the useful comments. We followed his/her advise in preparing the revised version, which is now much improved.

**Reviewer # 1:** *P 16975. Line 25. It should be better emphasized what are the topic and originality of the two papers (Agustí and Duarte 2013, Lasternas et al 2013), compared to this one. Not clear the end of the sentence line 27 verb of comma missing?*

**Author's action:** We specified the topic of the two papers. The text now reads: "Recent studies, exploring the release of DOC by phytoplankton (Agustí and Duarte, 2013; Lasternas et al., 2013), provided evidence that increasing phytoplankton mortality in oligotrophic waters led to increasing DOCp among senescent or dying natural populations and accounts thus for a large fraction of the photosynthetic carbon channeled through bacteria characteristic of oligotrophic marine communities. In a comparison of contrasting oceanic systems, Agustí and Duarte (2013) found that percent extracellular organic carbon release (PER) was related to phytoplankton lysis rates, with the largest PER observed at the oligotrophic waters. Lasternas et al. (2013) found that oligotrophication forced by anticyclonic eddies (AE) formation in the Eastern North Atlantic resulted in increased phytoplankton cell mortality and in increased PER within AE waters."

**Reviewer # 1:** *16976. Line 2 The status of bacterial cells. . .*

**Author's action:** Text has been changed and reads: "The status of bacterial cells."

**Reviewer # 1:** *Line 22 . was Feb 2007 a bloom? pre bloom period? due to the delay for sampling all stations, was the seasonal situation of the bloom equal at all the sites? (the ship could be in pre bloom in one zone, and post bloom in the other one)*

**Author's comment:** The differences encountered here were associated to the waters characteristics (nutrients supply in the vicinity of the upwelling). Zone 2 was sampled at the beginning and end of the cruise, so its characteristics were associated to the zone more than to the delay in sampling. Moreover, the upwelling dynamics and

phytoplankton responses in the area, operates at larger time scale than our cruise sampling interval.

**Reviewer # 1:** *Line 23. 9+8+8 is 25 stations, not 24. I could see only 23 dots on the map of Figure 1.*

**Author's comment:** Reviewer is true, 6 stations were sampled in zone 3 and not 8. So 23 stations were sampled.

**Author's action:** We corrected the text, that now reads: "A total of 23 stations were sampled, nine stations in the north Atlantic gyre area (Zone 1), eight placed in the vicinity of the Canary Current region (Zone 2) and six stations in the area influenced by the Mauritania's upwelling (Zone 3; Fig 1)."

**Reviewer # 1:** *P 16977. Incubation for primary production lasted 4h. From which day time to which day time? Could 4h of incubation be considered as a gross or net release for DOC production? Is there a correction of bacterial re-use of DOC released during the incubation time? If the sampling time for PP was not always the same, does it influence %DC? PER?*

**Author's comment:** The 4 hour incubations were performed at the same time of the day, always including noon so the comparison was established without bias.

As described by Morán and Estrada (2002), and other authors, a short incubation period should be selected when measuring DOC<sub>p</sub> to match a compromise between the time needed to obtain a significant signal in the PP phase, but at the same time, minimize the loss of <sup>14</sup>C-labeled dissolved organic carbon (DOC) due to assimilation by heterotrophic prokaryotes. We then used short-time incubations as recommended to optimise the measurements and minimise the contribution of trophic-related processes to DOC production

**Author's action:** We mentioned the time of the day in the manuscript. Text reads: "Inoculated bottles were set up at respective depths along a mooring buoy and incubated in-situ for 4 hours, at the same time of the day (from 12.00 to 4.00 p.m), always including noon. As recommended by Morán and Estrada (2002), a short incubation period was used to optimise the measurements and minimise the contribution of trophic-related processes to DOC production."

**Reviewer # 1:** *Line 15. It is not clear how the obscure sample is treated. Are their respective dpm subtracted form particulate? From total of the light samples?*

**Author's comment:** Yes, the obscure samples were treated the same way as the light samples. Their respective dpms were measured with a scintillation counter

(EG&G/Wallac).

**Author's action:** The paragraph was modified as follow: “.. with a scintillation counter (EG&G/Wallac). Dpms from dark incubations from particulate and total samples were subtracted from light particulate and total samples respectively.”

**Reviewer # 1:** *P 16977 Line 1 What could be the error of Pdoc and then PER based on a difference between total primary production (based on 5 ml water volume) and particulate primary production (based on 150-2x5=140 ml water volume filtered)?*

**Author's comment:** The error would be scarce as this technique (and volume used for) allows the accurate measurements of low <sup>14</sup>C incorporation as total or particulate production. The possible errors, that might have caused the differences in volume between the total and particulate samples, was resolved by proceeding longer counts reading of the low volume samples.

**Reviewer # 1:** *Line 18 Does PI technique allow to discriminate % viability between HNA and LNA cells?*

**Author's comment:** Since the discrimination of High Nucleic Acid (HNA) and low Nucleic Acid (LNA) bacteria is established according to their green fluorescence, the use of another dye (such as PI) do not allow a proper discrimination. So the percentage viability among HNA and LNA bacteria was not explored.

**Reviewer # 1:** *P 16979 line 26 . It s not absolutely clear that the cell digestion assay was made on unfixed cells and in which portion. Total water? 70 ml concentrate? Filter? Is there an influence of the DNase and trypsin concentrations and/or on the incubation time of the enzyme cocktail on the different types of species affected? i.e is there a different resistance to the cocktail according to the phytoplankton species targeted? For instance I would imagine cell walls of Bacteria (Syn and Prok) more resistant than that of eukaryotes? And inside eukaryotic cells, is a cocolithophore or a diatom more resistant than a flagellate?*

**Author's comment:** In the revised manuscript, we extended the description of the CDA method to increase clarity. The digestion of compromised cells by the enzymes is very efficient and after the incubation time is complete for all the phytoplankton groups as diatoms, cyanobacteria, dinoflagellates, this has been tested in different studies: Agustí & Sanchez (2002), Agustí et al. (2006) and Llabres & Agustí (2008). The CDA was applied on fresh (unfixed) samples. For the larger species, the assay was made on 10 ml of the concentrated 70 ml sample. This concentration system has been used in previous studies (Alonso-Laita and Agustí, 2006; Lasternas et al., 2010; Lasternas and Agustí, 2010) with accurate results for microphytoplankton, and no effect on the viability or other cell properties (i.e. movement for flagellated cells, integrity of frustules, etc.) .

**Author's action:** The revised version includes a more extended description in the methods section: "The CDA was applied on fresh (unfixed) samples. For the larger species, the assay was made on 10 ml of the concentrated 70 ml sample. This concentration system has been used in previous studies (Alonso-Laíta and Agustí, 2006; Lasternas et al., 2010; Lasternas and Agustí, 2010) with accurate results for microphytoplankton, and no effect on the viability or other cell properties (i.e. movement for flagellated cells, integrity of frustules, etc.). For the picophytoplankton groups, the CDA was run on duplicated 1 ml fresh samples."

**Reviewer # 1:** *P 16980 Line 7: The percentage of dead cells was calculated for the different phytoplankton groups It should be written there.*

**Author's action:** We modified the sentence and the text in the revised manuscript now reads: "The percentage of dead cells was calculated for the different phytoplankton groups from the ratio between the concentration of dead cells (total concentration minus the concentrations of living cells) and total population abundance, which includes both living and death cells (Agustí and Sanchez, 2002)."

**Reviewer # 1:** *Line 10-15. Student t test is only applicable for normalized data.*

**Author's comment:** Reviewer is true. We reanalyzed the data using the Mann-Whitney Wilcoxon Test. The new results however showed the same significance levels at the critical p-value of 0.05.

**Author's action:** The revised manuscript now reads: "The statistical significance of the differences between average values was tested using the Mann-Whitney Wilcoxon Test, with a critical p-value of 0.05."

**Reviewer # 1:** *P 16981. line 3. In the text it seems that primary production is different between the 3 sites but from statistics on table 1 it is not.*

**Author's comment:** We agree with reviewer#1. The average values were not significantly different between zones. However maximum values were found at the waters influenced by the upwelling. In any case we rewrite the paragraph.

**Author's action:** Text now reads: "Maximum total primary production (TPP) was found at the waters influenced by the upwelling ( $2.68 \pm 0.03 \text{ mg C m}^{-3} \text{ h}^{-1}$  at station #8 - 21°49N / 20°52W) and the lowest values at the oligotrophic zone, although differences in the averaged values were not significant between the three zones (Table 1)."

**Reviewer # 1:** *Line 5-8. It would be interesting to know if this relationship is driven by depth, or by differences between stations. Adding a figure should be nice.*

**Author's comment:** DOCp values did not show a significant trend with depth in our study, and variability was better related to the oceanographic zones.

**Reviewer # 1:** *P 16982. line 12 'heterotrophic bacteria. . .across communities' which communities? Was this test made on the whole data set? Was it a paired comparison? Or based on averages of both groups? If the whole phytoplankton group is considered, its % of dead cells average is necessarily related to the most abundant phytoplankton group's one.*

**Author's comment:** The test has been made only for the bacterial community, so as reviewer#1 suggested we changed the sentence to better explained this data.

**Author's action:** We followed reviewer recommendation and change the sentence. Text reads: "Bacterial viability varied between 60 to 95 percent of living cells and the averaged % of living heterotrophic bacteria cells was higher than that of autotrophic picoplankton (Mann-Whitney Wilcoxon Test,  $P < 0.0001$ )."

**Reviewer # 1:** *Line 13-14. It seems from this sentence that the authors compared the % of living heterotrophic bacteria (with PI technique) with the percentage of living phytoplankton (I assume 100 - % of dead phytoplankton, with the cell digestion assays). Thus it seems that both methods give equivalent results in terms of '% dead cells'. Is it really the case? Because the authors counted by epifluorescence prok and syn on filters after the cell digestion assay, they could also count heterotrophic bacterial cells after staining with a DNA probe. Did they check that PI and CDA techniques give the same results on heterotrophic bacteria cells?*

**Author's comment:** As it is explained in the M&M section, the % of living and dead cells for picophytoplankton and heterotrophic bacteria were both counted using flow cytometry so the methods may give equivalent results in terms of '% dead cells variability'.

**Reviewer # 1:** *Line 14. There are no heterotrophic bacterial abundance data on Table 2 or on Figure 5.*

**Author's comment:** We agree.

**Author's action:** We changed the sentence in order to avoid any misunderstanding. Text reads: "While bacterioplankton presented the highest abundance in the upwelled waters, the percentage of heterotrophic living bacteria was lowest in the most productive waters and increased towards more oligotrophic waters (Fig. 5, Table 1)"

**Reviewer # 1:** *Line 19. Was the flux divided by total abundance or only by living cells?  $h^{-1}$  is missing after pgC.bacterial cell $^{-1}$*

**Author's comment:** We calculated the flux by the total abundance. We change the text to include this aspect and also add the  $h^{-1}$  missing.

**Author's action:** Text reads:” By dividing the production of dissolved organic carbon by phytoplankton and the bacterial total abundance, we obtained the flux of P<sub>DOC</sub> per bacterial cell (pg C. bacterial cell<sup>-1</sup>h<sup>-1</sup>).”

**Reviewer # 1:** *Line 21” .. than at the other zone. . .” No, as shown by statistics, there is no difference with intermediate zone.*

**Author's comment:** We agree, Fig. 5 shows significant differences between oligotrophic and intermediate waters with the upwelled waters.

**Author's action:** We revised the paragraph to avoid confusion, and modified as follows “...and could appreciate that availability in DOC for heterotrophic bacteria were higher in the oligotrophic and intermediate waters (Fig. 5) than at the ones influenced by the upwelling.”

**Reviewer # 1:** *P 16983 lines 5-10. It should have been interesting to see the plots of PDOC with PPP and PDOC with bacterial production.*

**Author's comment:** We agree, however we have no data of bacterial production during this cruise to work with. Further studies would be needed.

**Reviewer # 1:** *Line 12 There must be other references dealing on pDOC in Mediterranean sea.*

**Author's comment:** As mentioned by reviewer#1, our assumption on the relationship between PDOC and the bacterial production was not supported by any reference for the NW Mediterranean Sea, we modified the text, removing the Mediterranean Sea.

**Author's action:** Text reads: “In open ocean sites, bacterial production and dissolved primary production (DPP) are often tightly linked (Morán et al., 2001; Antarctic off shore waters), while in coastal (Morán et al. (2002a) NE Atlantic coastal system and Morán et al. (2002b)) or eutrophic sites (Morán et al., 2002b Antarctic coastal) persists a lack of linkage.”

**Reviewer # 1:** *Lines 25-30. The authors mostly considered averages between stations (like on figure 4 for example) or all over a group of stations (like on table 1). Was there an effect of depth as irradiance, nutrients, temperature, and phytoplankton populations, all probably changed with depth in stratified conditions. This is partly discussed p 16984. So why choosing averages between stations? because the trend is higher with trophic conditions?*

**Author's comment:** As mentioned before, we were not able to stress direct effects of irradiance, nutrients or temperature. The relationship was indeed driven by geographical zone and trophic conditions.

**Reviewer # 1:** *p 16984 line 1-3. Why % of dead cells could vary proportionally to mortality rates?*

**Author's comment:** We do not follow well the question. Both measurements inform about cell mortality. Cell lysis is a rate and %DC inform about the health of the population, and we expect both processes to be related when considering the dynamics of the population. In phytoplankton cultures, the proportion of death cells is minimum at the exponential growth phase, when lysis rates are minimum, and the proportion of death cells increase as the culture slow the growth rate, when cell lysis rates also increased (Agustí et al. L&O, 1998). In any case, we revised the text to increase clarity.

**Reviewer # 1:** *line 21 ' the magnitude... was higher, because...' No it is not the case, as shown by table 1, the average DOC production rate by phytoplankton rate is not statistically different among the three zones. lines 27-28. Again, statistics of table 1 don't confirm this. the PDOC flux per bacterial cell is lower in upwelling zone and not statistically different than in intermediate zone*

**Author's comment:** We agree with the reviewer that the paragraph was confusing. The values were not significantly different between the areas.

**Author's action:** We agree and removed the paragraph in the revised version. Text now reads: "In agreement with previous studies, communities in unproductive oligotrophic waters tended to release as DOC a higher fraction of their total primary production compared to more productive, nutrient-rich upwelling waters (Teira et al., 2001; Morán et al., 2002a). Moreover, the larger phytoplankton mortality lead to a higher release of primary production as dissolved organic carbon, which, in turn, can support a larger biomass and carbon flux through bacteria in the upwelling zone compared to the oligotrophic or intermediate waters."

**Reviewer # 1:** *p 16985 lines 3. No, bacterial abundance are not higher in the upwelling, as I can see from figure 2. line 5 No, Pdoc do not decrease toward oligotrophic zone, as I can see from table 1*

**Author's comment:** We agree and have revised the paragraph. DOC production by phytoplankton in the oligotrophic zone was significantly higher than in the upwelling zone.

**Author's action:** We re-wrote the paragraph and text reads now: "In our study, we found higher bacterial abundance in intermediate and oligotrophic waters offshore, consistent with the higher release of DOC from phytoplankton, declining towards the waters influenced by the upwelling".

**Reviewer # 1:** *Table 1. it is not clear how all means and se are calculated. For nutrients in upwelling for instance, 9 stations x 7 depth layers = 63 data? and for PER? mean of each PER (9 stations x 5 layer depths=45 data) or PER based on averages of PDOC and*

*PPP fluxes? and for phytoplankton dead cells? average of %DC for all data (9 stations x 2 depths x 5 phytoplankton categories (diatoms, syn, prok, nanomicrophyto, picophyto), or ratio of mean total abundance of living cells to total abundance of dead cells?*

**Author's comment:** All the means and SE were calculated using the whole data set for each parameter. As it was not clear, we added a sentence in the statistics section of the M&M.

**Author's action:** Text reads: "The averages and standard error were calculated on the whole dataset of each parameter."

**Reviewer # 1:** *Fig 2. , Fig 5 Pdoc per cell. Because the data are presented on log scale, I wonder if statistics for comparison of data were made on log-transformed values or not.*

**Author's comment:** Statistics comparing the data were made in not-transformed data.

**Reviewer # 1:** *Figure 4 Only 10 dots for 25 stations.why ? Again, how are calculated percentages? how is calculated the error bar?*

**Author's comment:** The total number of stations was 23, but the data of Fig. 4 match for 10 stations. Incubations of primary production were made in situ, but some incubations were not recovery properly due to rough sea or buoys were not deployed.

**Author's action:** We added more precision in the M&M section. Text reads: "In situ total and particulate primary production (TPP and PPP) was successfully measured using <sup>14</sup>C additions (Steemann-Nielsen 1952) at 10 of the 23 stations of the study. Indeed, the rough sea and technical issues that occurred during the cruise prevented us to properly deploy the buoys for incubations at all the stations.

**Reviewer # 1:** *Figure 6. Is the relationship on all data points also significant? I don't understand why the black points are not equally distributed every 20% PER. X axis correct for 'percent extracellular release'*

**Author's comment:** There was a mistake; bins were done on 10% PER so the black dots correspond to the averaged data of 10% PER binned. Moreover the relationship between PER and % of heterotrophic living bacteria, based on the whole dataset, was significant ( $R^2 = 0.35$ ,  $P < 0.0001$ ).

**Author's action:** We corrected in the revised the error, indicating that PER data was binned each 10%. We added the results of the relationship obtained for all the data set as well.

**Reviewer # 1:** *Pico eucaryotes are used Fig 2, and picophytoplankton figure 3. Be homogeneous.*



**Author's action:** We revised Fig 3 and replaced Picophytoplankton by Picoeukaryotes.

## **Actions taken to accommodate the comments of Reviewer#2**

**Reviewer # 2:** 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 BG 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...).

**Author's comment:** We acknowledge the reviewer for his/her useful comments that we followed carefully in preparing the revised version of the manuscript. Our study analysed the relationships between in situ PER and phytoplankton and bacterial survival. We studied bacterial cell survival at the physiological level, not survival rates, and the title of the manuscript is reflecting the major result found. We agree, PER did not explain all the variability of % living bacteria, and the relationship we found do not explain the 100% of the variability in bacterial survival. We also agree that we are not studying the mechanisms, but the relationships, and our results may not apply to other areas of the ocean.

**Reviewer # 2:** *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.*

**Author's comment:** The method was first described by Gregori et al. (2001), and

we followed the protocol described by these authors. Falcioni et al., (2008) used a simultaneous addition of the PI and SyBR green. But we tested both procedures and found not difference between the two procedures in our samples (Lasternas et al., 2010; 2013). We add more information below.

**Reviewer # 2:** *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...)*

**Author's comment:** PER are within the range of literature values (see a more detailed answer below). As we mentioned in our introduction, we totally agree with reviewer#2 that it is indisputable that bacterial survival could be governed by different factors, such as grazing, viral infection as mentioned by reviewer#2 and by resources as nutrients limitation and DOC availability and quality. In any case, in our study we are analysing “cell survival” at the physiological level, with most studies analysing rates of survival and changes in abundance per unit time. Other factors may influence the % of living bacteria, but there are not yet publications relating the % of living bacteria to those factors (grazing or virus) in natural waters. There are some publications for phytoplankton, but not for bacteria.

In any case, we revised the manuscript to increase the clarity of these aspects.

**Reviewer # 2:** *L38 – “been” is not correct*

**Author's action:** Text changed.

**Reviewer # 2:** *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*

**Author's action:** We revised the manuscript and we changed “P<sub>DOC</sub>” to DOCp as suggested by reviewer#2.

**Reviewer # 2:** *L58 – “labeled carbon”? do not understand*

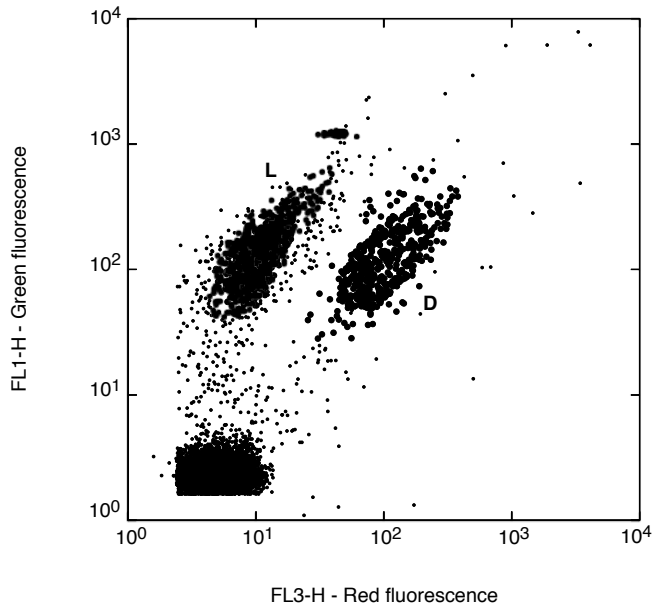
**Author's action:** Text changed and reads: “incorporated carbon”

**Reviewer # 2:** L105 – Rephrase “delivered”

**Author’s action:** Text changed and reads: “poured”

**Reviewer # 2:** 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?

**Author’s comment:** As we commented above, the simultaneous or separated addition of the staining solutions have no effect on the efficiency of the technique. Hereafter, we include a figure that indicates that our signals perfectly matched with that of Falcioni et al. 2008.



Flow cytometer dot plot of red (FL3) versus green (FL1) fluorescence of bacteria stained with SG1 and PI (NADS method). The living cells (indicated as L), showed high green fluorescence but low red fluorescence, and differentiated well from dead cells, (indicated as D) which showed lower green fluorescence.

**Reviewer # 2:** 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.

**Author’s comment:** We agree that the description of the method was very limited. We summarized as all these aspects have been addressed extensively in previous studies.

Results using this method were published in 24 peer reviewed publications, including those from other research groups. It has been validated several times against the results using other membrane permeability tests (vital stains) as FDA (Agustí & Sanchez 2002), Bac-light Kit (Agustí et al. 2006), both FDA and Bac-light Kit (Llabres and Agustí 2008). The concentration method does not induce significant cell losses, as we tested in both cultures and natural communities. Also, the blank and the treatment sample are both concentrated. The incubation time is short, 45 minutes, is close to the time used when staining (e.g. FDA is incubated for 20-30 minutes), but grazing effects on phytoplankton are negligible for that short time. Also, we checked in blank samples that the incubation does not reduce the number of cells (Agustí 2004).

In any case, we revised the manuscript and included more information about the CDA method.

**Author's action:** We revised and extended the description of the CDA method in the revised manuscript, that now reads:” The CDA was applied on fresh (unfixed) samples. For the larger species, the assay was made on 10 ml of the concentrated 70 ml sample. This concentration system has been used in previous studies (Alonso-Laita and Agustí, 2006; Lasternas et al., 2010; Lasternas and Agustí, 2010) with accurate results for microphytoplankton, and no effect on the viability or other cell properties (i.e. movement for flagellated cells, integrity of frustules, etc.).”

And “For microphytoplankton counts and viability test, samples of 2-3 liters were concentrated into 50-70 ml samples using a Millipore cell concentration chamber. This concentration system has been used in previous studies (Alonso-Laíta and Agustí, 2006; Lasternas et al., 2010; Lasternas and Agustí, 2010) with accurate results for microphytoplankton, and no effect on the viability or other cell properties (i.e. movement for flagellated cells, integrity of frustules, etc.). 10 ml aliquots (duplicates) of the concentrated sample were used as blanks for cell abundance quantification, and duplicated 10 ml aliquots of the cell concentrate were used to apply the CDA by adding 2 ml of DNase I solution (400 µg ml<sup>-1</sup> in HBSS (Hanks' Balanced Salts), followed by 15 minutes incubation at 35°C in a Digital Dry Bath. After this time, 2 ml of Trypsine solution (1% in HBSS) were added, followed by 30 minutes incubation at 35°C. At the end of this time samples were placed in ice in order to stop the enzymatic cell digestion process. Both 10 ml aliquots (duplicates) from blanks and CDA samples were filtered onto 2 µm pore-size black polycarbonate filters, with the samples from CDA washed several times with filtered seawater to remove the enzymes, then fixed with gluteraldehyde (1% final concentration), and stored frozen at -80°C until counting.”

And “The incubation time and concentration of enzymes was tested to be sufficiently efficient for the digestion of different phytoplankton groups (Agustí and Sanchez 2002). Also, it was checked in blank samples that the incubation procedure does not reduce the number of cells (Agustí and Sanchez 2002, Agustí 2004). “

**Reviewer # 2:** 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?*

*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).*

**Author's comment:** Figure 6 represents the relationship between the individual and average ( $\pm$  SE) percentage of living bacteria, binned by 10% PER intervals and the percent extracellular dissolved organic carbon release. While the fitted regression equation does not change using the whole dataset or the averages binned by 10%PER ( $\% \text{ Viable Bacteria} = 71.6 + 0.157 \text{ PER}$ ), the utilization of averaged values binned by 10%PER helped in highlighting the relationship between the bacterial viability and production in DOCp. Moreover the relationship between PER and % of heterotrophic living bacteria, based on the whole dataset, was significant ( $R^2 = 0.35$ ,  $P < 0.0001$ ).

**Author's action:** We revised the manuscript and changed %HLB to percentage of living bacteria cells. We add the results of the relationship obtained for all the data set as well.

**Reviewer # 2:** 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?*

**Author's comment:** Mykkestad (2000, Dissolved Organic Carbon from Phytoplankton, In: The handbook of environmental chemistry) compiled PER values up to 80% for natural phytoplankton populations, as we found in our study. Differences with other studies could be due by several reasons. Our study is including oceanic oligotrophic waters, probably more oligotrophic than those examined elsewhere: Maranon et al. (2005) studied coastal eutrophic waters, so as expected PER showed low values, and in the compilation of Baines and Pace (1991) all marine sites were coastal waters which tend to be more eutrophic. Also, another reason could be methodological, due to the generalized use of glass fiber filters. Karl et al. (1998 L&O, cited in the manuscript) indicated that PDOC could be underestimated by up to 30% as a result of  $^{14}\text{C}$ -labeled dissolved organic carbon ( $^{14}\text{C}$ -DOC) adsorption onto the glass fiber filters. He recommends the use of membrane filters as the cellulose membrane filters we used here.

**Reviewer # 2:** L216 – *Is it really 103 cells ml-1? Exponential is missing.*

**Author's action:** Text changed

**Reviewer # 2:** 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.*

**Author's comment:** As we responded above to reviewer#2, we do not questioned the influence of others factors but we provide evidences of a significantly positive relationship between the release of DOC by phytoplankton and the bacterial cell health or survival at the cellular level, in the area studied.

We strongly agree that the rates of losses of bacteria populations are influenced by viruses and grazing. We revised the text to avoid misunderstanding.

**Reviewer # 2:** 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 –*

**Author's comment:** We agree with the reviewer. This is a relevant aspect. However, although relevant to our results, none of these studies examined the effect of the different compounds in the % of living bacterial cells.

**Author's action:** We discussed this aspect in the revised manuscript. The discussion now reads: “Moreover, the lability of the released compounds by phytoplankton may also change depending on the phytoplankton composition and that would differentially affect bacterial responses (Nelson and Carlson, 2012). Indeed, recent studies showed that community structure of heterotrophic prokaryotes tend to respond to differences in the quality of organic matter released among microalgal species (Sarmiento et al., 2013). In our study, while we were not able to specify the quality of the DOCp released by the phytoplankton community, our results address the positive response of the bacterial viability to the increasing in DOCp production by phytoplankton“.

**Reviewer # 2:** 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.*

**Author's comment:** Even though we were not able to analyze whether other processes as cell exudation may be involved in the DOC production by phytoplankton, we stressed that DOCp production was significantly related to phytoplankton cell mortality.

**Author's action:** We cited the publication in the discussion of the revised manuscript.

**Reviewer # 2:** L306 - *Explain how DOC per bacterial cell was calculated. How accurate is this variable? "higher flux of PDOC per bacterial cell": living cell? Or both living and dead cell? How was this flux calculated?*

**Author's comment:** We explained this in the results section: "By dividing the production of dissolved organic carbon by phytoplankton and the bacterial total abundance, we obtained the flux of P<sub>DOC</sub> per bacterial cell (pg C. bacterial cell<sup>-1</sup>h<sup>-1</sup>)."

**Reviewer # 2:** *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?*

**Author's comment:** We did not have major problems in differentiate *Prochlorococcus* from HB in our samples.

**Reviewer # 2:** L319 – *This sentence is speculation, should be removed in my opinion.*

**Author's comment:** We removed this sentence from the revised manuscript.



### **Actions taken to accommodate the comments of Reviewer#3**

**Reviewer # 3:** The manuscript "Bacterial survival governed by organic carbon release from senescent oceanic phytoplankton" deals with the highly interesting topic if additional extracellular organic matter release by senescent autotrophic organisms secures the survival of heterotrophic prokaryotes. The manuscript is well written and adds valuable information on bacterioplankton dynamics to the existing literature.

Comments:

**Reviewer # 3:** *P. 16975, l. 9: It's arguable if the word "phytoplankton" includes cyanobacteria – so it would make it easier for the reader if this information is added.*

**Author's comment:** We understand reviewer#3's preoccupation. However, since cyanobacteria (*Synechococcus sp.* and *Prochlorococcus sp.* in our study) are photosynthetic organisms and a source of DOCp, we must include them within the phytoplankton group. We think that it would be confusing here to mention cyanobacteria in the sentence. Text would read: "Phytoplankton including cyanobacteria, in turn, is the main source of DOC to support bacterial dynamics, linking phytoplankton and bacterial dynamics in the ocean."

**Reviewer # 3:** *P. 16975, l. 19: Not sure why it is only "recently labelled" carbon.*

**Author's comment:** We agree.

**Author's action:** We removed "recently".

**Reviewer # 3:** *P. 16976, ll. 5-9: The integrity of the prokaryotic cell membrane is often used as a proxy for living (or even active) and dead cells. However, it should be mentioned that this is not completely true and that there are many discussions about this point. As the results are strongly dependent on the amount of chemicals used the authors should also describe all their controls.*

**Author's comment:** We agree, for heterotrophic bacteria the use of these techniques contains more uncertainties than for other cells. There are uncertainties due to the efficiency in the use of different vital DNA stains, but also in comparisons with active cells that may consider that dead cells are still active in some degree. In phytoplankton, it has been shown that dead cells are still active, with photosynthesis rates decreasing as cell degradation progress.

However, Falcioni et al. (2008) tested that the number of living bacteria obtained with the NADS method, the method we used, matched well with the number of active bacteria, and the number of live cells declined concurrently with that of actively respiring cells (CTC positive) and also with total bacterial production measured by leucine incorporation.

**Author's action:** We included this aspect in the revised manuscript in the

introduction: “While the discrimination between active and viable bacteria is still matter of controversy, the analysis of bacterial cell-membrane integrity allows a reliable discrimination between living and dying cells (Falcioni et al., 2008).”

**Reviewer # 3:** *P. 16977, 2.2: Was the same CTD cast used to get the samples for primary production as for the other samples? At which day time were the samples incubated? Did the day time differ between samples? Was the light measured at incubation depths over time?*

**Author’s comment:** The 4 hour incubations were performed at the same time of the day, always including noon so the comparison was established without bias.

**Author’s action:** In the methods section, we included information this information. Text reads: “Inoculated bottles were set up at respective depths along a mooring buoy and incubated in-situ for 4 hours, at the same time of the day (from 12.00 to 4.00 p.m), always including noon.”

**Reviewer # 3:** *P. 16978, 2.3: At which depths were the samples taken? Corresponding to the primary production samples? Why are the authors sure to have only bacterioplankton and no Archaea?*

**Author’s comment:** Yes, HB samples were taken at the same depths of primary production. We did not discriminate between bacteria and archaea, both heterotrophic prokaryotes, in our study.

**Reviewer # 3:** *P. 16979, 2.4: Same depths as before? Same CTD cast?*

**Author’s comment:** Samples for the quantification of phytoplankton abundance and biomass (as chlorophyll) were performed at the same depth and same cast as before.

**Author’s action:** We specified this information in the manuscript. Text reads now: “Seawater from the same CTD casts were used for the various samples performed during the cruise.” “...abundance was sampled at the surface (5 m) and the deep chlorophyll maximum (DCM) at each station, at the same casts as for the other parameters”. “At each station, the proportion of living heterotrophic bacteria was quantified from seawater sampled at up to 7 depths, at the same CTD casts as before.”

**Reviewer # 3:** *P. 16980, l. 15: Please, introduce abbreviations as LHB before first use.*

**Author’s comment:** The text has been changed and “living heterotrophic bacteria” in the whole manuscript now replaced the “LHB” abbreviations.

**Reviewer # 3:** *P. 16980, 2.5: Which samples were not normally distributed??*

**Author’s comment:** Some of the biological parameters (such as abundance or % of dead cells) were not following normal distribution. In order to be rigorous in our statistical analysis, we decided to use non-parametric statistical tools.

**Reviewer # 3:** *P. 16982, l. 17: Only net production is measured...*

**Author's comment:** The  $^{14}\text{C}$  method do not measure net production, but measures something between net and gross, depending on many factors.

**Reviewer # 3:** *P. 16982, l. 23: At least in the version I got, there is no figure 6.*

**Author's comment:** There is a figure 6 in the manuscript. The relationship between the percentage of living heterotrophic bacteria and the percent extracellular dissolved organic carbon release.

**Reviewer # 3:** *Table 2: Please, indicate significant differences.*

**Author's comment:** We did not observe any significant statistical difference for the large phytoplankton by zones in our study, certainly because of the small size of the statistical samples.

**Reviewer # 3:** *Fig. 2: Why are the significant differences given in brackets? Same axes would make this figure better comparable.*

**Author's comment:** We agree.

**Author's action:** We deleted the brackets in figures 2, 3 and 5.

**Reviewer # 3:** *Fig. 3: Level of significance would be a good addition.*

**Author's comment:** We run again the statistic test and specified in the figure legend the level of significance.

**Author's action:** Legends read now: The boxes connected by same letter are not significantly different ( $p < 0.005$ ).

**Reviewer # 3:** *Fig. 5: Please, explain better what's what, e.g. Percer, HLB etc.*

**Author's action:** Legends reads: "Box plots showing the distribution of the percentage of living bacteria cells, the distribution of PER (Percentage extracellular release) and the variation of fluxes of DOCp per bacteria at the three sampled zones. The boxes present the lower and upper quartiles, median, minimum and maximum values, and outliers. The boxes showing the same letter do not have significantly different mean values (Mann-Whitney Wilcoxon,  $p < 0.005$ )".

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