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Biogeosciences Discussions

Interactive comment on "Diel variability of heterotrophic bacterial production and UV doses in the South East Pacific" *by* F. Van Wambeke et al.

F. Van Wambeke et al.

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Response to comments

Anonymous Referee 1

It is not clear from the Ms what exactly the authors did as the material and method section is very poor and unbalanced, with very little explanation on sampling, depths, incubations, determination of BP (about a page for all these topics) and a lot explanations on the measurements and calculations of UVR (3 pages).

Yes, we did not give many details on bacterial production measurements because this methodology is detailed in another paper of the BIOSOPE special issue (Van Wambeke et al, 2008b). We added more details in the revised MS about the sampling strategy (MM section 2.1 Strategy of sampling). Note also that the exact sampling

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periods and frequencies were described in the legend of figure 2 of the BGD version (which is now figure 3).
At the opposite, the estimation of the mean UV doses within the mixed layer (H_m) is an original topic which needed to be developed. Indeed, little is reported in the literature about H_m data. However, it is clear that H_m is a useful parameter to asses the actual light dose received by organisms in the mixed layer (Jeffrey et al., 1996; Boelen et al., 2000). In this study, we provide a detailed description of the H_m calculation from in situ radiometric measurements. We explain step by step how we obtained all the parameters: Z_m, H_{0-}, ocean surface albedo, solar zenith angle, correction factor for K_d.To our knowledge, there is no other study providing this degree of details for the determination of H_m.

As the author clearly stated, the cells are moving within the UML and thus the mean irradiance is adequate to evaluate the effects of UVR. However, their data seems not to support this idea and they show higher variations at 5 m and not in the UML. The author should consider why this is happening? One of the potential explanation is the cells are moving within the UML al a lower speed than their sampling frequency. That means the a complete circulation within the UML takes longer than the three hours between their sampling, so they are sampling cells with different light acclimation history (this is clearly seen in the profiles presented in Figure 4, at least for MAR and EGY).
For measuring bacterial production, samples were incubated for 2 hours in the dark. Because of confinement in a small volume (1.5 ml), this incubation process encloses bacteria in a surrounding environment which might not completely reflects the in situ one. For instance light and continuous supply of high-sized organism are not well represented in such a small volume. Nevertheless, we were able to observe significant cycling of BP, and thus, we suppose that both light and DOM availability at sampling time influenced activity during the 2 following hours of incubation in the dark.
Turbulence was not measured during this cruise. Theoretically, in an ideal mixed layer,

BGD
5, S562–S571, 2008

a bacterial cell would have the time, in three hours, to go up and down through the mixed layer. We should consider that this " ideal" mixed layer rarely occurs in nature, because bacterial production (data of our ms) clearly showed higher variations at 5 m than at deeper layers. However, at MAR, there was a significant correlation between BP at 5 m and BP at 10 m and BP at 15 m; with deeper layers, the correlation progressively decreased. We added information about this in the ms (page 9). At MAR there was a significant positive correlation between the daily trend seen at 5 m depth and integrated data down to Z_e (r=0.61, p<0.05) but with our new way to consider varying values of Z_m according the time of the day (see below), the correlation between BP at 5m and BP integrated to Z_m at MAR is now insignificant. This difference between the results presented in our previous version of ms in BGD is probably due to the fact that depth of Z_m increased significantly at MAR during the diel cycle (15 m per day, see diel variation of mixed layer depth, in figure 3). At GYR and EGY, although the way to integrate BP to Z_m has also been changed , there is still significant correlations with BP at 5 m and BP integrated to Z_m, but with lower r values than in the BGD version (now values are r = 0.62, p<0.01 at GYR and r = 0.73, p<0.05 at EGY, Table 2). Hence yes, we believe that the reasons imposing BP to vary at 5 m depth imposed also variation within the upper mixed layer, but not necessarily down to Z_m at MAR site. It is also worth to notice that some specific layers inside the mixed layer exhibited a clear diel variability for other bio-optical parameters determined without the need of an 8220;incubation8221; period, like distribution of particles (Steemann et al., 2008), and particle attenuation coefficient (Claustre et al., 2008b). Clearly, more sophisticated estimates of all physical forces governing vertical motion of floating micro-organisms (Ekman pumping, internal waves, Langmuir circulation, micro-turbulence, turbulent advection by eddies, double diffusive process) are necessary in that field of research.

In addition, and looking at Table 3, the depth of the UML was highly variable at GYR, so a mean value for this depth is not good. So a different approach should be used to present their data.

BGD

5, S562–S571, 2008

For each cast and at all sites, Z_m was calculated as the average Z_m of 4 data calculated following 4 different criterions (see MM section 2.2.). According to the criterions, Z_m could exhibit large variability for a given CTD cast. It was particularly true at GYR where the density profiles showed succession of pycnoclines not very well contrasted (see Figure 2 in the revised version). In addition, slight upper-surface increases of temperature during the evening created micro-stratification within subsurface layers, making Z_m as shallow as 10-15 m at some CTD casts, which largely participated to the large Z_m diel variability at GYR presented in the previous version.
Thus, we reconsidered mixed layer estimates for these particular CTD casts keeping only a citerion of 0.1 kg m^{-3} difference with surface, and neglecting the first 10 m when micro-stratification due to temperature was too high. This provided less variable depths for Z_m. Now the average Z_m at GYR is 61 $\pm$ 9 m (instead of 46 $\pm$ 27 m). However, to be consistent with UV doses estimates, and in order to consider daily variability in Z_m, bacterial production integrated to Z_m was recalculated for each CTD cast, considering a Z_m equivalent to the average of Z_m estimated from the 3 h-previous period (for instance Z_m data for BP integrated at 12:00 was based on the average Z_m calculated from the two Z_m obtained from the 12:00 and 9:00 CTD casts). We are aware of problems linked with considering a theoretical ideal mixed layer at GYR. However we kept the information in the revised version to be consistent and to allow comparison with other sites. In addition, we provided Z_m values which are now plotted in figure 3.

Specific comments: Abstract:
1) BP is expressed in %, it is also necessary to include real values as Carbon, so the reader will have and idea of the BP rates.
Average values of BP at 5 m depth and integrated rates down to Z_e and Z_m, expressed in carbon, are presented Table 1. However, as requested by the reviewer, average integrated values of BP at the sites investigated are now also added in the abstract.

BGD
5, S562–S571, 2008

2) The authors measured leucine incorporation, and they made a relation to CPD as if this was obvious and routinely. I disagree with this as they involve two different targets for UVR as well as different metabolic and timescales. It is possible to infer a indirect relation, but not as the author did assuming from BP and UVR levels the amount of CPDs.

It is strange that this comment is cited from the abstract. In the abstract, we discussed on one hand variability of BP, and on another hand variability of UVA and UVB doses that could be considered as "proxies" for CPD and PER. We only used a theoretically linear relationship from the literature between UV doses and production of CPD in the discussion, because as we clearly write in the paper (page 13), we did not measured CPDs during our cruise. We agree with the referee that the growth process measured with the leucine technique is protein synthesis rates, whereas the target affected by UV is mainly DNA. BP was not used to determine CPDs, but only UV doses.

Material and methods

3) It is not clear here that they sampled every depth and what were the depths, it only seems so after looking at figure 4.

We added information about sampled levels in the MM section.

4) As mentioned above this section is poorly described and the author devote a lot of work explaining the measurements of UVR, etc.

See above

5) There is NO statistics with the exception of few correlations that are poor and in the end the authors present more variability with depth and at 5 m that in relation to the UML and 7) Through the text the author used words like "great magnitude, variations, very low ", but most important the reader would like to know if the observed variations were significant or not (statistically).

BGD

5, S562–S571, 2008

We corrected some of these sentences and gave statistical results when necessary.

6) The results section is confusing as the author mix results with discussion
We removed some repetitive sentences in the results and discussion sections.

8) One of the y-axis in Figure 2 is wrong or have the wrong units as maximum solar PAR at noon is ca 500 W m^{-2}.
We apologize for this error. Units presented are now μmole quanta m^{-2}s^{-1} which is correct and in accordance with other articles (Claustre et al., 2008b).

9) In the figure caption the authors state that the bars are standard deviation, however, in MM they mentioned only duplicate samples and half difference between samples.
We partially agree with the referee. On figure 2 it was variability within duplicate samples, but on figure 4 (mean profile along diel cycle) it was really standard deviation. The legend of Figure 2 was modified accordingly.

10) Figure 3 seems to be based on the actual profiles of BP, such as the ones presented in Figure 4, so the authors should present first the real profiles and then the contour plots. Even though contour plots are nice to see, they suffer from the potential variability according to the way used to calculate them. It is often see that differences arise from the power or method used for the gridding, so more explanations should be available to the reader.
We agree with the reviewer that this figure is providing some redundant information compared to Figure 2 and 4 of the BGD version. However, we felt interesting to keep it in order to see that influence of diel cycle was not visible only at 5 m depth. On this figure, dots represented all samples. More details about the software and gridding criterions were added in the legend. Figure 4 presenting mean BP profiles is now cited

BGD
5, S562–S571, 2008

before the figure showing contour plots. Moreover,we added density profiles on this figure.

11) There are a lot of repetitions between the text and what is shown in the Tables.
We did it intentionally to help the reader.

Discussion
12) This section is highly speculative and many parts are repetition of the results.
We removed some repetitions in the text.

13) What do you mean by "volumetric surface"? One can not consider the 5m sample as surface sample.
"Volumetric surface" was a term used to make a clear distinction between this variable and "integrated data" down to Z_e or Z_m. 5 m layer is generally taken as a reference for "surface" waters not influenced by the sea-surface microlayer (the Niskin bottle is closed under the sea surface, even when there is ship movement). Practically, this "5 m" layer was sampled according CTDs casts between 4.0 and 6.2 m depth, as shown by the pressure sensor.

14) Please see above my comments for BP and CPDs.
We agree with the referees that the leucine technique measures protein synthesis whereas the targets for UV damages are nucleic acids. The leucine technique, used for estimating bacterial production, is commonly used even in studies of daily cycle of BP or in experiments testing UV radiation effects on bacterial activity. When discussing daily variability of BP, we indicated that the results obtained using leucine incorporation rates into proteins or thymidine incorporation rate into DNA are not necessarily the same.

BGD
5, S562–S571, 2008

15) The authors tried to evaluate the effect of UVR by taking samples and they relating their response to the UV R levels measured during a 3-hour period. This could be an interesting approach, but it is not clear what the authors related, for example, what dose did they use for the calculations, the actual dose occurring during the incubation or the previous one so they also account for the previous light history? In addition, this was calculated for every depth or just integrating the effect in the UML?
We considered previous light history and used the dose integrated during the 3 hours before the CTD cast. This was made for UVB and UVA doses, but not for integration of BP down to Z_m (in the BGD version we used an average Z_m for the whole diel cycle). In this revised version we used the average Z_m for the preceding period (see above), so that the same criterion for Z_m estimate was used for UV doses and integrated BP. In such a way, diel variability of Z_m was taken into account.

16) The final statement or conclusion is highly speculative.
We agree with the reviewer that direct measurements of DNA damage as well as DOC production by phytoplankton are important missing data. We added a sentence on this in the conclusion page 14. But the abundant observations cumulated by ourselves and our colleagues provide indirect evidence that dual effect of phytoplankton-bacteria and UV radiation influence daily BP variability.

Anonymous Referee 2

The authors describe the investigation of heterotrophic bacteria production in the South East Pacific during a cruise in 2002. This is certainly of interest since biomass production and turnover is important for CO2 uptake and global climate change. I wonder if the authors verified that their method (measuring leucine incorporation)

BGD
5, S562–S571, 2008

is really a good measure for bacterial frequency. At least this should be proven by standard fluorescence counting methods.
The leucine technique is currently used to determine bacterial production in seawater (Ducklow, 1992) since the pioneer studies on that field by Kichman et al. (1986). Bacterial abundance was also determined by flow cytometry (Grob et al., 2007; Van Wambeke et al., 2008b). However bacterial abundance was not sampled on a high frequency during diel cycles and so cannot be presented in this ms focusing on diel cycles.

I am disappointed by the statistics. Of course you can calculate a SE from two data points, but this is mathematically incorrect, since in the equation you divide by (n-1) which gives you 1 degree of freedom. Thus at least three measurements are required to do meaningful statistics.
We agree. Bacterial production estimate only result from duplicate measurements. This was specified in MM section of the BGD ms page 440 line 22. The variability within two duplicate samples was just drawn on this figure 2, not standard error. There was an error in the legend of this Figure 2 which was corrected. Variability within duplicate samples was just used to plot the data, not for statistics.

There is something wrong with the calibration of the light sensor. Values aroung 1000 W m^{-2} for the PAR range sound excessive.
Yes, we apologize for the error; this was corrected on the Figure 2. Maximum values reached within the GYR site are now around 2000 μmole quanta $m^{-2}s^{-1}$.

There are numerous repetitions between the results and discussion section which should be eliminated.
We removed repetitions as suggested.

BGD
5, S562–S571, 2008

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Interactive comment on Biogeosciences Discuss., 5, 435, 2008.