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Biogeosciences Discussions

Interactive comment on "Distribution of micro-organisms along a transect in the South-East Pacific Ocean (BIOSOPE cruise) from epifluorescence microscopy" *by* S. Masquelier and D. Vaulot

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We have prepared a revised version of the paper taking into account the comments by the referees (see below, referee comments appear in bold and our response is below). One of the major changes we introduce is to estimate the carbon contribution of the different populations using conversion factors (Worden et al., 2004). We also discuss our data in the light of other papers recently submitted to the special BIOSOPE issue of Biogeosciences.

Response to Anonymous Referee #1

Received and published: 12 September 2007

General comments

This paper reports some very interesting data set of several different types of microorganisms along a transect in the South-East Pacific Ocean that across a variety of trophic conditions, from the hyper-oligotrophic South-East Pacific gyre to the eutrophic Chile upwelling. It will be very valuable and important to compare the microbial food web structure under different environmental conditions and its implication to oceanic primary production and carbon cycling.

Our data do not allow to estimate carbon cycling. However calculation of the contribution of PE cyanobacteria, autotrophic picoeukaryotes and heterotrophic picoeukaryotes to particulate organic carbon at each station allows comparison of our data set with other data acquired during the cruise (Grob et al., 2007).

However, there are some serious problems in methodology, and the authors admitted that in their articles.

First of all, using 0.8 um filter will cause the lost of the majority of *Prochlorococcus* and *Synechococcus*. Therefore, the conclusion that up to 50% of picocyanobacteria is in the form of colonies is a dramatic overstate.

We have tried to make these limitations as clear as possible in the revised version.

1. *Prochlorococcus* cannot be counted reliably by epifluorescence microscopy. Therefore, “picocyanobacteria” refer only to “phycoerythrin containing picocyanobacteria”. This is now made explicit everywhere in the text.

2. It is possible that a small fraction of the *Synechococcus* can pass through a 0.8 μm filter. Since comparison with the flow cytometry data show that at very least 33 % of the *Synechococcus* are retained by the 0.8 μm filter (assuming that no

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cells are lost because of sample fixation and conservation, Fig. 5), our estimate is not such a “dramatic overstate” since colonial PE cyanobacteria would represent at the very least 15% of all PE cyanobacteria. We have explicated this caveat in the revised text.

Second, long storage of slides (>1 year) will cause cell loss and fluorescence reduction, especially if the slides have been thawed during transportation. Further more, according what said in the method and materials, dinoflagellate and ciliate concentrations were as low as 1.5 cells per liter. Given that only 100 ml were filtered and 50 fields were counted using 100x objectives, it is likely that only a few cells of dinoflagellates and ciliates were counted, which is insufficient. Also, ciliates are likely severely underestimated as it is not fixed by Lugol's solution. Based on above reason, I believe the quality and reliability of the data is compromised. In fact, the authors were aware of most of the points I have raised and discussed them in the paper.

We are well aware of the limitation of our data as pointed by this referee and we have tried to detail them in the paper. However, microscopy counts can provide information not obtained by other techniques, for example percentage of eukaryotes that are heterotrophic or evidence of colonial picocyanobacteria.

Specific comments

Page 2669, line 8: Actually nanoflagellates are the most important grazer of picoplankton (e.g., Christaki et al. 2001, 2002, Guillou et al. 2001).

This has been changed in the paper

Page 2671, line 22: how can DAPI staining separate prokaryotes and eukaryotes?

DAPI stains DNA and allows visualization of eukaryotic cell nucleus that appears a separate blue organelle under UV light. For prokaryotes, no nucleus is visible and cells are uniformly stained.

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Page 2673, line 5: what is the rational to only test the correlation for eukaryotes below 40-60 m?

In fact, we computed correlations both for all samples and then for those below 40-60 m depth. The rationale for such specific analysis was the fact that during counting, we observed that the fluorescence of organisms from samples below 40-60 m was brighter and faded more slowly than for the upper samples. For these deep samples, organisms were easier to distinguish from heterotrophic organisms. Therefore, we expected to have a correlation slope closer to 1 between counts made on samples below 40-60 m and abundances obtained by flow cytometry, which is indeed the case (Fig. 5A).

Also throughout the manuscript, "picocyanobacteria" is used in many places, but it really means "PE containing picocyanobacteria" as *Prochlorococcus* is not included.

See above.

Page 2674, line 13-14 stated that heterotrophic eukaryotes accounted for quite high percentage of total eukaryotes cells, but on line one of the same pages it is stated that autotrophic cells were much more abundant than the heterotrophic ones. This appears to be contradictory.

In fact, these sentences refer to different regions. We rephrased the sentences to make this clearer in the revised text.

Page 2678, line 15-18: Are you talking about the contribution of $>$20 um dino to total dino or to total eukaryotes? If it is the former, the numbers ($<$1 to 2%) are extremely low.

We are talking about the contribution of >20 μm dino to total dinoflagellates in terms of abundances and not in terms of biomass. Indeed these numbers are quite low and small dinos are much more prevalent in these waters than larger ones.

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Fig. 5 caption did not indicate what the circles and squares represent.

Circles correspond to data from surface to 40-60 m depth depending on samples. Squares correspond to data from 40-60 m depth to 300 m depth depending on samples. This has been added in the paper.

Several typos: P2678, line 7: delete "station"; p2679, line 11: "iin" should be "in";

p2680, line 8: "if" should be "of"

This has been changed in the paper.

Response to Anonymous Referee #2

Received and published: 4 October 2007

General comments

This study reported the distribution of different microbial groups (picocyanobacteria, picoeukaryotes, dinoflagellates and ciliates) based on the epifluorescence microscopy work. Although the methodology itself is quite challenging and very labor-intensive, it has its own merits compared to some existing methods like flow cytometer. The idea of differentiating microbes into functional groups is novel. The findings of large proportion of colonial picocyanobacteria in the high nutrient region and large contribution of green fluorescing dinoflagellates are interesting. Unfortunately, all the samples were stored for more than one year before the counting took place. The authors discussed the potential artifacts due to the delayed counting. These problems (i.e. using 0.8 um pore-size filter; long storage time; and pre-staining with DAPI) are not trivial and are the main concern for this manuscript. I understand it is unrealistic for them to redo the counting. However, I would recommend to re-stain the samples with DAPI right before counting if they will continue this work. Many nucleic acid stains like DAPI are not very stable in low concentration.

In general, nuclei were clearly visible during our observation and therefore re-staining with DAPI may not have improved our counts. The main problem encountered was the low and fast fading pigment fluorescence of organisms from surface samples.

Specific comments

P2676, L20-30: Appearance of colonial cyanobacteria here could simply be the adaptation of cyanobacteria to high nutrient waters.

Adaptation to high nutrient water is probably only one of the factor involved because for example we observed only 1% of colonial picocyanobacteria in the Chile upwelling which is very nutrient rich. In the light of our observations, it would be probably interesting to extend such counts to other oceanic regions to better understand how the fraction of colonial cyanobacteria varies with oceanographic conditions.

Change "from" in the title to "using"

This has been changed in the paper.

P2673, L11, add "profile" to "vertical".

This has been added in the paper.

P2673, L14: delete "percent"

This has been deleted in the paper.

P2675, L21: change "estimates" to "estimated"

This has been changed in the paper.

P2675, L22: change "cells smaller than" to "picocyanobacteria"

This has been changed in the paper.

P2678, L20: add "This is in agreement. . . " and make this long sentence into

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Interactive Comment

two.

This has been done in the paper.

P2679, L6: remove "."

This has been removed in the paper.

P2679, L11: change "iin" to "in"

This has been changed in the paper.

P2679, L24: remove "."

This has been removed in the paper.

P2680, L8: change "if" to "of"

This has been changed in the paper.

P2680, L11: remove "up"

This has been removed in the paper.

References cited

Grob, C., Ulloa, O., Claustre, H., Huot, Y., Alarcon, G., and Marie, D.: Contribution of picoplankton to the total particulate organic carbon concentration in the eastern South Pacific. Biogeosciences, 4, 837-852, 2007.

Worden, A. Z., Nolan, J. K., and Palenik, B.: Assessing the dynamics and ecology of marine picophytoplankton: The importance of the eukaryotic component. Limnol. Oceanogr., 49, 168-179, 2004.