

## Answers to Pomati's suggestions

**Interactive comment on “Dynamics of auto- and heterotrophic picoplankton and associated viruses in Lake Geneva” by A. Parvathi et al.**

**F. Pomati (Referee)**

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### GENERAL COMMENTS

I think that the manuscript reports important and interesting findings on a subject that is definitely understudied in freshwater ecosystems, and therefore merits publication. There are several comments however than need to be addressed before the article is ready for publication.

We would like to thank Dr Pomati for his encouraging comments. We agree with all his comments and did our best to improve upon the presentation of the results following his recommendations. All changes are in red (both in our answers below and in the revised article).

- there are several grammar mistakes, looks like a rather early draft of the manuscript. It needs revision of the English language, text and structure. - I have doubts about the methods used for quantification of picoplankton and about the statistical analyses.

English was checked by a native English speaker and effort was made to propose a more structured version. We also tried to give better explanation on methods.

- I think that there are too many figures, number 3 and 4 can be provided as supplementary material, possibly also number 7.

We agree that Figures 3 and 4 could only be provided as supplementary material. It is suggested however to keep Figure 7.

### SPECIFIC COMMENTS:

1. Abstract - line 1: "dynamics have", not has. –

This was fixed.

2. line 15: define interplay

The term interplay was used as a synonym of interaction. We changed this and used “complex interactions”.

3. be more specific. - line 16 to end: it looks like these are all conclusions. maybe better to be more specific with the results and leave just one sentence of concluding remarks.

We followed the suggestion and proposed a new abstract ending as follows:

“The present study suggests that temperature and top-down control by viruses are important factors regulating the picocyanobacterial dynamics in this lake. More generally speaking, our results add strength to the growing evidence that viruses are an important actor of freshwater microbial dynamics and more globally of the functioning of the microbial food webs.”

Introduction - line 12: I think that viral control should be mentioned as bottom up, rather than top-down (more generally associated with zooplankton) control - page 4

Dr Pomati is right when saying that using top-down for viruses is not really appropriate. However bottom-up is never used for the viruses. By past, some authors proposed to use “side effects” to show that viral effect is indeed different from zooplankton pressure. However this term is never used and top-down remained. It is thus proposed not to change here what was initially written.

line 11: sentence not clear, please rephrase.

This sentence was modified as “The dynamics of picoplankton in aquatic ecosystems are not only controlled by abiotic factors (temperature, light and nutrients), but also by biotic factors such as natural death, viral lysis, predation and parasitism”

line 18: please re-write being more explicit in "various", bring out is also not appropriated.

This was fixed by changing the words and being more explicit. This is changed as “Through their lysis activity, viruses play an important role in regulating carbon and nutrient fluxes, food web dynamics and microbial diversity in aquatic systems”

Methods - chapter 2.1: details of the size and volume of the lake are not relevant for this study –

This was provided to state the importance and significance of the study area. It is also a way of introducing the ecosystem to HESSD readers which are probably not very familiar with Lake Geneva. We chose to retain it.

Chapter 2.2: it is not clear what was sampled (chapter 2.1) at discrete depths, since phyto and zooplankton were collected as integrated samples. Please be clear –

This has been clarified in the section 2.2. It is modified as follows:

“Samples collected at discrete depths (i.e. 2.5, 5, 7.5, 10, 15, 20, 30, 50 m) were analysed for nutrients namely nitrate (N-NO<sub>3</sub>), nitrite (N-NO<sub>2</sub>), total nitrogen (N tot), phosphate (PO<sub>4</sub>), total phosphorous (P tot) and silicate (SiO<sub>2</sub>) using standardised methods (Anneville et al., 2005). Raw water samples for the phytoplankton analysis, production assays and cyanophage abundances were taken with a patented integrating instrument developed by Pelletier and Orand (1978) integrating the 0-18 m

upper water layer and fixed with a few drops of Lugol's solution for phytoplankton and zooplankton analysis.”

chapter 2.3, line 13: do you mean filtered lake water instead of TE? Please correct. Why lake water instead of buffer?

This has been corrected in the present version. Heterotrophic bacteria are indeed analyzed by diluting the sample with the same lake water filtered through 0.02  $\mu\text{m}$  to remove any particle. For the viruses the dilution is made with  $<0.02 \mu\text{m}$  TE. A battery of tests was made to find the best diluting liquid to analyse either bacteria or viruses for the peri-alpine lakes, with our FACSCalibur FCM, and with also different possible fixatives and dyes. This will be published in a methodological chapter in Nova Scientific book soon (Jacquet et al. in press). Briefly, it was shown that to analyse viruses we need TE buffer while it is filtered lake water for the bacteria. The reference below is now added to the revised form.

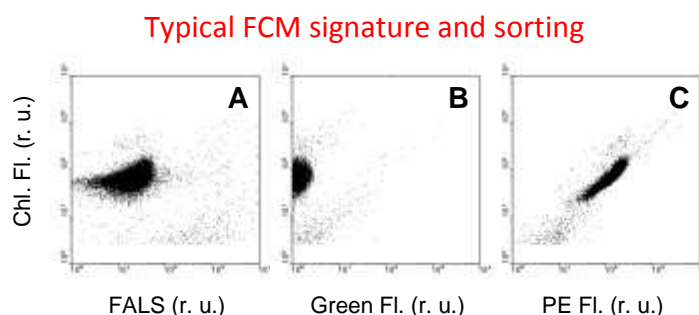
**Jacquet, S., U. Dorigo and S. Personnic. 2013. A few tests prior to flow cytometry and epifluorescence analyses of freshwater bacterio- and viroplankton communities. In: *Flow Cytometry: Principles, Methodology and Applications*. Related Nova publication (in press)**

Did you filter out phytoplankton larger than 3  $\mu\text{m}$  for the pico-cyano analysis? how did you distinguish / count pico-cyanobacteria with FCM? size classes can be tricky to define based on total scattering or FL. –

This is indeed an important aspect raised by Dr Pomati. The flow cytometric signature obtained for picocyanobacteria allows discriminating this community from other phytoplanktonic groups without any ambiguity. It is in fact the only community for which we are sure in terms of identity. It is noteworthy however that such identity was verified. Our FCM can also be used as a cell sorter and during our lake surveys, picocyanobacteria have been sorted, cultured and both genetic affiliation and size were analyzed. All cultured strains belonged to phycoerythrin-rich (PE) *Synechococcus*-like populations and the PE-rich picocyanobacteria of the French sub-alpine lakes vary in size between 1.5 and 2.5  $\mu\text{m}$ . This part is now included in the present version as follows:

“During previous experiments or surveys in peri-alpine lakes (e.g. Annecy, Bourget and Geneva), some picocyanobacteria were sorted with flow cytometry, cultured and both genetic affiliation and size were analyzed in order to confirm their identity. All cultured strains isolated so far with typical phycoerythrin-rich (PE) picocyanobacteria FCM signatures belong to *Synechococcus*-like populations and these PE-rich picocyanobacteria for the French sub-alpine lakes varies in size between 1.5 and 2.5  $\mu\text{m}$  (Jacquet unpublished).”

Example of analyses for culture PE-rich *Synechococcus* strains:





chapter 2.4: this paragraph is not clear, the procedure is not well explained. what do you mean with over washed? please re-write the description of the selective filtration steps, it is not understandable. –

We rewrote this section in order to make it clearer:

“Size - fractionated primary production at five discrete depths (2.5, 7.5, 10, 15 and 20 m) was determined by *in situ* incubations with the isotope  $^{14}\text{C}$ . <200- $\mu\text{m}$  water samples from each depth were filled into three 250 ml glass bottles (two “light” and one “dark” bottle). These bottles were inoculated with 1 ml of radiolabeled  $\text{NaH}^{14}\text{CO}_3$  ( $5 \mu\text{Ci ml}^{-1}$ ) and subsequently incubated for 5 h at respective depths where the water was sampled. At the end of the incubation, samples were sequentially filtered through 20- $\mu\text{m}$  nylon mesh and 3.0 and 0.2  $\mu\text{m}$  polycarbonate filters. The phytoplankton cells concentrated in the 20  $\mu\text{m}$  mesh were washed with filtered lake water and again concentrated on GF/F filters. This corresponded to the microphytoplankton fraction, whereas the 3.0 and 0.2  $\mu\text{m}$  represented the nano- and picophytoplankton fractions, respectively. The filters were used for subsequent analysis after removing excess dissolved inorganic carbon ( $\text{DI}^{14}\text{C}$ ) by exposing to concentrated hydrochloric acid fumes for one minute. The filters were then placed in scintillation vials and a 5 ml scintillation cocktail was added. Radioactivity was measured using a liquid scintillation counter (Beckman coulter, USA). Production rate was calculated based on the photo-period of each day and expressed as  $\mu\text{g C L}^{-1} \text{d}^{-1}$ . Other details can be found elsewhere (Anneville et al., 2002; Tadonl  k  , 2010)”.

ch 2.5, line 16: why you say *in situ*? aren't water samples in the lab?

The incubation was indeed realised in the lab and this aspect has now been corrected in the revised version as follows:

“Bacterial production was determined by incorporation of the nucleoside  $^3\text{H}$ -Thymidine into bacterial DNA (Tadonl  k  , 2005). Briefly, a thirty milliliter water sample (in triplicates) along with trichloroacetic acid (TCA) killed control (1% final concentration) was incubated with  $^3\text{H}$  Thymidine ( $^3\text{H}$ -TdR) at a final concentration of 10 nM in the dark for 1 hour at ambient temperature in the laboratory.”

ch 2.8: what is month wise stat analysis?

The sentence was changed as “The statistical analysis was carried out on monthly data”

log-transformation is not sufficient to get rid of multicollinearity, as you actually found out.

This has been corrected in the revised version. To get rid of multi-collinearity principal component regression (PCR) an alternative method of estimation has been used.

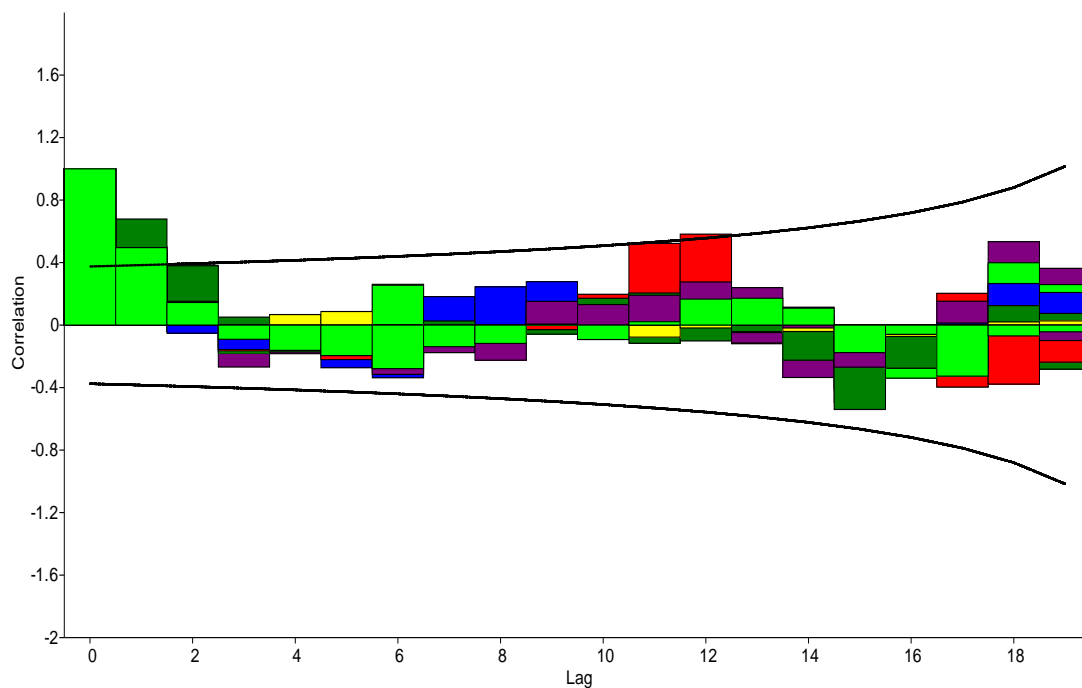
Also, how did you deal with serial autocorrelation of temporal data? this is crucial because it affects all your stats.

We agree that temporal auto-correlation is a very common phenomenon in ecology.

Autocorrelation analysis was carried out in on separate column(s) of evenly sampled temporal data and was done using Past. Lag times  $\tau$  up to  $n/2$ , where  $n$  is the number of values are shown along the x axis (. A predominantly zero autocorrelation signifies random data - periodicities turn up as peaks. The "95 percent confidence interval" lines were drawn at

$$\pm 1.76 \sqrt{\frac{1}{n - \tau + 3}}$$

The analysis of our data shows that our data are not completely random and revealed autocorrelations that were insignificant. There was a couple of points where the autocorrelation was however observed (above the 95% confidence limit), and as recommended, ignored in the statistical analysis, as we did not use the data for stochastic modeling. We performed a regression analysis to see the influencing factors determining the dynamics of different planktonic communities in the Lake. We are sure that you would agree that in standard regression analysis, which is designed for fitting the equation to a given set of data, the autocorrelation of factors does not create any problems.



Analysis of autocorrelation using PAST software with 95% confidence limits drawn.

Davis, J.C. 1986. Statistics and Data Analysis in Geology. John Wiley & Sons problem).

Results - figures should be numbered according to their appearance in the text. here figure 6 is mentioned before figure 3 and 4, etc. –

Figures 3 and 4 have been removed so that figures have been now renumbered.

Table 3: how does temporal autocorrelation affect the calculation of coefficients in your analysis? –

This aspect is mentioned in the previous section.

Table 4: significant at what p-value? –

The p value was  $<0.01$ . It is now mentioned in the modified version.

Figure 1: what type of interpolation was used to account for missing temporal datapoints in the graphs? you don't really know what happens in between

We agree with this comment and the interpolation is directly proposed by the software Sigmaplot as the best fit to the data. We agree however that this is not perfect and that some information is probably missing between dots. We think however that such representation is a satisfactory representation of distribution of the biological components. If Dr Pomati has some suggestions to improve the Figure, we can try to follow them.

## Answers to anonymous reviewer #2

### Anonymous Referee #2

Received and published: 29 August 2013

#### General comments.

This study is providing important information regarding the dynamics of Microorganisms in Lake Geneva, ranging from phytoplankton to viruses. They measure abundance and production of the different organisms and try to correlate this to environmental parameters. The sampling period extends from July to November and commonly measurements are made at different depths in the water column. In that sense the manuscript provides important and novel information regarding a subject that is vastly understudied. However, the result and discussion part is not written to satisfaction. In the results part, the different sections are difficult to follow and also their references to different figures and tables is confusing. I would also prefer that the data was more directly shown as individual points in a table, not only min, max, and mean, which regarding to what this study want to show is quite irrelevant (preferably as supplementary material). Throughout it is not clear what depths and seasons you are using for e.g. statistics work. Also the discussion is difficult to follow and I think the authors draw to big conclusions compared to what the data is showing. Further, overall the English need to be worked on and abbreviations are sometimes used and sometime not, please be consistent.

We are grateful to the reviewer who read this article and proposed to help us to improve it. We tried to follow his/her recommendations in order to propose a better and improved manuscript.

#### Specific comments.

In methods you say you see 3 VLPs (VLP1, VLP2, and VLP3) in flow cytometer but in results only 2? Please correct.

We could indeed observe 3 groups but not all the time so that we decided to only present VLP1 and VLP2. To get rid of this confusion we proposed to improve the section in the M&M as follows:

“FCM discriminated at least 3 sub groups of viruses, designated as VLP1, VLP2 and VLP 3 (virus like particles, group 1, 2 and 3) but only VLP1 and VLP2 could be observed throughout the period of analysis and were shown thereafter”.

8723: Might not want to use PCR as a abbreviation as the majority of micro- and molecular biologists think you are talking about Polymerase chain reaction. But it is up to you.

The reviewer is right and we removed the abbreviation along the text.

Page 8725: Your figure numbers are scrambled in your text (fig 1, 6, 2, 5, 3, 4, 7). Should it not be 1, 2, 3, 4, 5, 6, 7?



The reviewer is right and we corrected this.

Note that we removed 2 figures to follow Dr Pomati's recommendation.

Page 8726: I do not find any information regarding what depth bacterial and viral production was measured at. Was it only on one depth, which?

The reviewer is right. This is now added. It was on the 0-18 m integrated water sample to have a global view of production from the upper lit layer.

Page 8726-8727: I assume the correlations you discuss in the first paragraph of Statistical analyses are the one you show in table 3. However, in table 3, every number except the last row are bold, meaning that everything except Chl a is significant. However, in the text you write that you have several other not significant correlations (e.g. VLP1 and bacterial abundance). Overall, I am not sure from your text what correlations you are showing in table 3. Please explain and correct.

This is taken care of in the revised version.

Page 8727, Line 14-21: You write you do a PCR analyses for picocyanobacteria and VLP's, but I do not find a graph showing this. Please include.

This was published and discussed elsewhere. The reference is provided.

Page 8730, line 23-24: I do not understand the following sentence or it's connection to figure 6 : "In the case of VLPs host abundances (bacteria, picocyanobacteria and other phytoplankton) also played important role in determining their variations (Fig. 6)."

A coma was missing after VLP but the sentence was also rewritten as follows:

"In the case of VLPs, host abundances (i.e. bacteria, picocyanobacteria and potentially all other phytoplanktonic cells) also played an important role in determining the variations observed for viral abundances."

Page 8730, Line 24-27: You write about that PCR revealed different parameters that attributed to variability for the different VLP-groups, but you do not show that anywhere in your results.

This was fixed.

Table 2. It is not clear what the abbreviations PCGM, PCVM, BGM, and BVM stands for.

This was fixed as follows:

"PCGM refers to picocyanobacterial mortality due to grazing while PCVM refers to the virus-mediated mortality of this community. BGM and BVM are as above but for the heterotrophic bacteria."

Table 3. It says that abbreviations are explained in the text. I guess that means the main text, in which I do not find it.

The reviewer is right and the term “main” was added.