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Interactive comment on "Acquisition of intact polar lipids from the Prymnesiophyte *Phaeocystis globosa* by its lytic virus PgV-07T" *by* D. S. Maat et al.

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Response to comment of referee 2

We thank anonymous reviewer 2 for reviewing our manuscript in such constructive way. We appreciate the positive comments ('The manuscript is well written and the topic is certainly interesting to the readers of Biogeosciences') and agree that by applying the reviewers suggestions we will improve the quality of the revised manuscript.

Comments and reply (reply starts with *):

The results of this study are only of limited significance because only one additional

virus-algal host system has been analyzed. In light of this, I do not agree with the general conclusion that "the absence of viral sphingolipids as shown in the current study might be a more general feature" (page 11718, line 3). We have one system producing vGSLs and another that does not, so I suggest to not jump to early conclusions until a variety of other species have been studied (also answering the later comment: 'P18, line 3-8: see general comment above. We don't have enough data to generalize').

* We think have been careful in stating our conclusions but we see the point made by the referee. We still like to highlight the exceptionality of EhV-86 amongst the other known phytoplankton NCLDVs, but will revise our statement (section 4.3) and stress the need for more data to allow more general conclusion. See also our reply to comment on the topic by referee 1.

The current manuscript will thus greatly benefit from a more detailed description of the IPL composition, which also directly translates into increased relevance for future studies of other viral-algae systems where robust data for comparison is mandatory. With little additional work, the improvements outlined below will strengthen the manuscript considerably and raise the impact from "smallest publishable unit" to "good research paper": 1) A more detailed description of the detection limit for the individual IPL classes. Some IPLs were below detection limit, where is the cutoff – what is significant?

* We appreciate the suggestion by the referee to strengthen the manuscript by the addition of extra information. We will revise the manuscript accordingly and will give a more detailed explanation of how we will do that below. We understand that by using the term 'detection limit' we have given a false sense of quantification. We will revise this and instead state that these compounds were not detected.

(2) The use of response factors for quantification of relative IPL distribution, which is also directly relevant to a question raised by referee #1. It is known, that different ionization efficiency of different IPL classes can lead to apparent increase/decrease

of the relative proportion of certain IPL groups when not considered. Quite often IPL standards are not commercially available and can thus not be used for quantification. In this case, however, all the required IPL standards to determine the relative response are available in the lab (P11712, lines 8-12) (also answering later comments: 'P13, line 22: state the detection limit' and 'P12, line 12: here the description of response factor and detection limit determination can be inserted').

* We realize that we have not been sufficiently explicit in explaining the qualitative nature of the data, as we have also described in the response to referee 1. We will highlight in section 2.6 of the revised manuscript the fact that the IPLs have different ionization efficiencies and that, therefore, we are comparing the apparent abundance of the IPLs in the chromatograms, which factually may not reflect the actual relative concentrations of the different IPL groups in the cells.

(3) Most importantly, a more detailed description of the variation of fatty acids within each IPL class is necessary. The base peak plots in Fig. 4 show clear evidence of in-class variation of fatty acids in the IPLs, especially for the phosphocholine lipids (also answering later comments: 'P17, line 8-11: maybe some IPL-group-fatty acid compositions are specific for the infected culture or the virus?' and 'P13, line 12-16: there are clear differences in Fig. 4 not only regarding the abundance, but also regarding the fatty acid pattern (see general comment above). This needs to be addressed').

* We will extend our results section 3.2 to describe the changes in the fatty acid composition of IPLs, as determined from MS fragmentation patterns, between the infected and control cultures at 48 h. Two classes showed differences and will be presented in more detail: i.e. the PCs and the MGDGs. Also, differences in FA composition were found between the host and the virus. Specifically, the viral MGDGs, DGDGs and DMPEs showed a reduced complexity in FA composition as compared to the host, while on the other hand some PC FAs that were present in the virus, were not detected in the host. This too will be clarified in the results section (3.2) of our revised manuscript.

As the authors report (page 11714, line 23), the saturation state within IPL classes can be affected by viral infection (Evans et al., 2009). I strongly suggest that such data be included in the revised version of the manuscript. The good news is, that a lot of the required data are already available from the MS2/MS3 fragmentation pattern already acquired and that only four additional analyses in negative ionization mode are necessary for the remaining IPL species. Alternatively, as bare minimum, the authors could include information about the total number of carbon atoms and double bonds combined in both fatty acid chains; this information is available in the MS full scan data. The former option, however, is more desirable because it allows direct comparison with existing and future fatty acid data ontained by gas chromatography (also answering later comments: 'P14, line 5-6: the relative abundance of polar headgroups might be similar, but what about the fatty acids (see above)' , 'P14, line 22-paragraph end: here the authors suggest that the fatty acid composition within each IPL class might be different and they state the relevance of unsaturations (Evans et al., 2009). Since fatty acid composition data has already been generated or can be recorded with little extra work, it should be included in the discussion here (see general comment above)' and 'As a result of the modifications, Table 1 needs to be updated to include the relative composition of different IPL groups in % and the additional in-class fatty acid data').

* We will follow the first suggestion of the referee and will extend the manuscript with supplementary tables depicting the fatty acid combinations of the IPLs, based on MS2/MS3 fragmentations. However for some IPLs, the individual FA make-up could not be deduced. In these cases only the combined fatty acid combinations are presented. In the results section (section 3.2) we will then describe the general changes in the distribution and will follow up on this by adding the topic of varying FAs in the discussion section. The reason we choose for a supplementary table rather than an addition to Table 1, is because we want to keep the main focus of the manuscript on the IPL classes themselves and not affect the readability of the manuscript.

Detailed comments: P09, line 24: what was the filter pore size? P09, line 7: I believe it

should be “4100 x g” P10: line 4: filter pore size?

* We will make the requested edits and provide the additional information. The nominal filter pore size of GF/F (P09, line 2) is 0.7μm and of GF/C ((P10, line 4) is 1.0 μm.

Section 4.2: the discussion of similarity of IPL patterns of the algal host and the virus would also benefit greatly from a more detailed comparison of the fatty acid composition. I am sure, that different IPL sources within the cells (cytoplasm, cell membrane, endoplasmatic reticulum, chloroplasts etc) might lead to different fatty acid compositions this should be considered in the discussion. I wonder, if there might be some carry-over of lipids from the host lysate to the purified virus solution during the separation outlined in section 2.4. Is it possible, that some of the lipids found in the virus might be derived from incomplete separation? This could be tested by adding a synthetic standard to the lysate before isolation of the virus and subsequent testing for the presence of the synthetic compound in the virus extract. Has this been considered? Could fatty acid patterns help to exclude this possibility, too?

* We appreciate highly the reviewers' constructive thoughts on this matter. Unfortunately, only little data is available on the specific IPL and FA characteristics of different cellular compartments. In our case the IPL composition has shed some light on the matter since certain IPLs are considered to be typical for certain cellular compartments. The literature on FAs that could be used to clarify, is unfortunately sparse and not specific enough to use for comparison to our study. We did not use a synthetic standard to test the possible contamination of the viral isolate by host material. The method we applied for the purification of the virus, i.e. iodixanol density gradient separation, has been proven to be highly effective to separate viruses from host material (Moller-Larsen A, Christensen T (1998). Isolation of a retrovirus from multiple sclerosis patients in self-generated Iodixanol gradients. J Virol Methods 73: 151-161). The viruses were concentrated in a clearly visible sharp white band in the Optiprep gradient, to us confirming that this was not a mixture of different particles.

Fig. 3: it occurs to me that some of the structures are negatively charged while others are positively charged or in a neutral zwitterionic state. Perhaps this can be made consistent (all with a net neutral charge).

* We will modify the figure such that all compounds are shown with a net neutral charge, with the exception of the PC, DGTA and DGCC which are quarternary ammonium cations and thus have intrinsic positive charges.

Fig. 4: can the figure be expanded a bit?

* We will enlarge the figure in the revised manuscript.

Interactive comment on Biogeosciences Discuss., 10, 11705, 2013.