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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 1

We thank anonymous referee 1 for the positive and constructive review and we appreciate the comments about the usefulness of this study. We are pleased that the referee believes the manuscript broadens our understanding of lipid content and behavior in algal virus infection.

Comments and reply (reply starts with *):

This is a useful report in that it shows that GSLs may not be active components in P.

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globosa infection even though PgV-07T, like the *E. huxleyi* viruses, is surrounded by a lipid envelop.

* One important point from our manuscript is that it seems that PgV-07T, to our knowledge similar to most other known NCLDV from which the lipid membranes have been studied, does not contain a lipid envelope, but a membrane spanning the inside of the capsid.

This study is limited, as it documents only one additional Prymnesiophyte species and only used one model host and virus strain. The manuscript is well written, easy to understand and broadens our understanding lipid content and behavior in algal virus infection. It is relevant to the Biogeosciences readership, but is really the bare minimum data set for publication and lacks breadth for making broad conclusions about Prymnesiophytes as a whole. Is it possible that *E.huxleyi* is more representative and that *P. globosa* is an outlier.

* The reviewer is correct that *P. globosa* could be an exception rather than *E. huxleyi*. However, in comparison to other known virus-host systems, EhV-86 is a virus that exhibits an infection strategy different from all other known NCLDVs. This was previously highlighted by Mackinder et al (2009 – ‘A unicellular algal virus, *Emiliana huxleyi* virus 86, exploits an animal-like infection strategy’). Importantly, EhV-86, is as far as we know the only known phytoplankton infecting NCLDV that has a lipid envelope (as outer membrane). However, we understand the reviewers comment that more data is needed to strengthen this point. Therefore, in the revised manuscript, we will modify the text proposing that PgV might show more general features than EhV, and mention the necessity for more data on this topic.

Section 2.6 Why were the filters containing cells freeze-dried? It seems that one of the benefits of the single phase liquid extraction method is that frozen filters can be extracted quickly with minimal sample preparation that could affect lipid distributions. Pitcher et al. (2011) was referenced for the extraction protocol, but their manuscript

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did not justify freeze-drying, only reported it. Pitcher et al. (2011) referenced Schouten et al. (2008) for the extraction protocol, but the 2008 manuscript did not report freeze-drying.

* We followed the regularly applied IPL extraction method from our laboratory, which involves first freeze drying our samples in order to eliminate an unknown quantity of water being introduced into the single phase liquid extraction solution. We are not aware of a study which has demonstrated a better recovery of intact polar lipids when the filter papers are not freeze dried.

Section 3.2 Can the authors provide any details at all about the GSLs that were detected in *P. globosa*? Vardi et al. (2012) provided detection parameters for a host GSL in *E. huxleyi* with some distinctive mass spectra. Did the authors detect hGSL or some other GSL class? In Fig. 4 a GSL peak is noted in all four chromatograms. Is that always the same compound(s)? Is there any change in ion m/z distributions during infection? As Maat and coauthors targeted GSLs for this manuscript, it would be nice to see a description of what they detected.

* We agree that this information would be a valuable addition to the manuscript and will describe the GSLs detected in more detail in section 3.2. However as the glycosphingolipids were not found to be infection-specific compounds, full structural elucidation was not within the scope of this work.

Section 4.1 The authors state that no data have been published on the fatty acids of infected *P. globosa*. Using their mass spec data, can the authors at least predict total numbers of carbon atoms and double bonds on the fatty acid moieties for the different species of IPLs? This could be provided as a supplement if it is not deemed useful to their lipid description in the manuscript.

* We agree this would be a valuable addition to the manuscript and will describe IPL-FAs in the revised manuscript. We will add tables to the manuscript that describe the fatty acid composition of the IPLs.

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Section 4.2 The authors used chloroform and diethyl ether to remove the lipid envelope from PgV-07T then reported that the virus was no longer infective, concluding that the membrane was crucial for infection. Please include a citation for this treatment or experimental data showing that other, non-enveloped virus that have been treated with chloroform and diethyl ether remain infective.

* We don't think that PgV-07T contains a lipid envelope such as found for EhV-86. As explained in section 4.2, we believe that PgV-07T possesses a membrane that spans the inside of the capsid, such as found for PBCV-1 and PpV01. We will add an appropriate reference relating to this virological characterization method to our manuscript (Feldman H.A., Wang S.S. Sensitivity of various viruses to chloroform. Proc Soc Exp Biol Med. 1961 106:736–738) and two other references that together show the inactivation by chloroform of a bacteriophage with an inner-membrane (Olsen RH, Siak JS, Gray RH (1974). characteristics of prd1, a plasmid-dependent broad host range dna bacteriophage. J Virol 14: 689-699 & Bamford DH, Caldentey J, Bamford JKH (1995). bacteriophage prd1 - a broad-host-range dsdna tectiviruses with an internal membrane. Advances in Virus Research, Vol 45 45: 281-319).

Table 1. How similar are the ionization efficiencies of the IPLs? For example, can it be concluded that SQDG is always less abundant than MGDG and DGDG in the experiments, or is it just more difficult to detect using ESI-MS? Please provide a statement on relative ionization efficiencies to make this limitation clear to readers not familiar with quantification by MS.

* We realize we have not been sufficiently explicit in explaining the qualitative nature of the data. We will highlight in section 2.6 of the revised manuscript the fact that the IPLs have different ionization efficiencies and 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.

Fig. 4. Based on relative retention times, the MGDG distributions in infected and

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noninfected cultures appear to be a little different. Is this significant? Is there a shift in fatty acids?

* We will insert an explanation in section 3.2 that the MGDG-FA distribution does not change significantly in the control cultures over the 48 h experiment. However in the infected cultures there was a relative increase in the C32:1 and C34:1 MGDG with a concomitant decrease or little change in the polyunsaturated MGDG-FAs and no change in the saturated MGDG-FAs. As mentioned in the previous comment we will describe IPL-FA dynamics, where relevant, in the revised manuscript.

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