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Biogeosciences Discussions

Interactive comment on "Marine viral populations detected during a nutrient induced phytoplankton bloom at elevated pCO_2 levels" *by* J. B. Larsen et al.

J. B. Larsen et al.

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Answer to comment 1) and 3) by Anonymous referee # 2: Our results show that the CO2 treatment affected EhV and HFV as the concentration of both these viral populations decreased with increasing CO2 (and decreasing pH). Their host (E. huxleyi for EhV) or possible host (nanoeukaryotes for HFV) organisms reacted the other way around - by a slight increase in abundance with increasing CO2/decreasing pH (Paulino et al. this issue). Under identical conditions high host concentrations produce more virus than low, and reduced viral concentrations are therefore likely a direct effect on the host-virus interaction rather than an indirect effect via their hosts (as discussed in the paper (p.3975)). In order to calculate burst sizes both host and virus concentrations are needed and the only host/virus pair of which both these numbers are known

is Eh/EhV. EhV burst sizes for the 1x, 2x and 3xCO2 treatments were 2890(+/-593), 2495 (+/-581) and 2032 (+/- 339), respectively. Although not significant, the decrease in burst size with increasing CO2 concentration support the idea that increased CO2 resulting in lowered pH may result in less effective phytoplankton viruses. Although some early works demonstrated that virus infecting animal cells can be stable and that phage production may be unaffected over a wide pH range (Krueger & Fong 1937, Weil et al. 1948) it is by now recognized that entry of viruses infecting animal cells can be both pH dependent and pH independent (e.g. Jin et al. 2005). One is also well aware that some are acid stable, surviving pH3 or even lower, whereas others are labile at pH $<$7. Such difference in pH stability is believed to represent adaption to a way of life (Rueckert 1996). Brussaard et al. (2004) demonstrated loss of infection for MpRNAV-01B (which infects the eukaryotic algae Micromonas pusilla) following treatment with acidic pH equal or less than 5, but as the pH in our mesocosms never dropped below 7.6 and the maximal difference in pH between the treatments was 0.47 pH units (7.64 (in 3xCO2) and 8.11 (in 1xCO2 initially (Bellerby et al. this issue) their study is of limited relevance. As reviewed by Weinbauer (2004) however, pH can affect adsorption of phages in freshwater, whereas marine bacteriophages are typically only affected by pH values deviating from that of seawater. We are not aware of additional studies that investigate pH effects on marine phytoplankton viruses but from the above it is not inconceivable that coupling between marine virus and their respective phytoplankton hosts have a limited tolerance to pH changes and that ocean acidification when taken to an extreme, may affect the marine microbial food web at the viral level.

We will take into account concern 1 and 3 put forward by Anonymous Referee #2 and rewrite this part of the discussion according to the above in the revised version of the manuscript.

Answer to comment 2 by Anonymous Referee #2): Allgaier et al. (this issue) report that bacterial production of free-living bacteria increased from day 6 on and that a pronounced maximum of cell specific bacterial production of free-living bacteria occurred

BGD
4, S2024–S2027, 2007

EGU

on days 6-8. We argue that LFV consist mainly of bacteriophages (p. 3972) and as this group increased from day 6 the coupling to bacterial production (as well as to bacterial biomass (Allgaier et al., Paulino et al. this issue) is apparent. We did not observe any difference in LGV according to the different CO2 treatments which is in congruency with no difference in bacterial abundance nor in BPP (Allgaier et al. this issue). Size fractionated primary production was also measured (Egge et al. this issue). This is a separation technique with limited resolution. Osmotroph organisms of different function (e.g. phytoplankton and heterotrophic bacteria) may overlap in size, and organisms may end up in wrong size classes due to aggregation and/or particle attachment. It is therefore a difficult/impossible (?) task to relate the primary production of the various size groups to specific viral populations. There is a however, a tendency of higher primary production at high CO2 levels in size group 5-10 μm (Egge et al. this issue) around day 10 which is when the increase of EhV and HFV start, which could perhaps be related to these viral groups, and thus may support our arguments (see above) of a real effect of CO2/pH on the phytoplankton virus/host relationship.

These aspects will also be included a revised version of the manuscript.

Answer to 4 by anonymous Referee #2): For detection and enumeration by flow cytometry we sampled all 9 bags. There is however no apparent statistical analyses that suit this kind of dataset (Trygve Nilssen, Department of Mathematics, UiB pers. comm.) and we have therefore presented the data as means, with standard deviations (Fig. 2). Whenever standard deviations do not overlap the treatments are believed to have a clear effect on the population under consideration. The analyses that were sampled for in only 3 of the 9 mesocosms are of a qualitative rather than a quantitative character.

Anonymous Referee #2, comment: Figure 2 is very small and the single plots are very hard to read!

Anonymous Referee #2, minor comments: Abstract: PeECE III mesocosm: Will be corrected Introduction, p3963, line 15-17 please also cite Riemann and Middelboe 2002,

BGD
4, S2024–S2027, 2007

EGU

Ophelia 56: 57-68: We agree that Riemann & Middelboe 2002 which describes implications of viral activity on organic matter cycling and bacterial composition should be cited and the paper will thus be included in the revised version. Mat and Met, p3965, l10 please explain SSC (has been done in the figure legend): SSC will be written fully (as side scatter signal) in the revised version. p3967, line 3: the primers...were ... p3970, line 18 (own unpublished work) which tests have been performed to check for reliability in detection of your newly designed primers?: The design and use of the primers for phylogenetic inferring of the Phycodnaviridae virus family is reported in a manuscript presently in review in the Journal of Applied and Environmental Microbiology. We cloned and sequenced nine phycodna OTU’s from seawater samples, and obtained sequences from seven isolated viruses previously putatively assigned to this family. Phylogenetic analysis of the MCP protein of currently sequenced genomes from members of the Phycodnaviridae family indicated a large divergence. The primers are designed to amplify Chloroviruses,Pprymnesioviruses and Raphidoviruses but not members of the Coccolitho- and Phaeovirus genera (EhV viruses, and FirrV-1 and EsV-1). Discussion: p3971, line 22: ...around 1:10... : Will be corrected p3975, lines 2ff please state whether you are dealing with direct or indirect of pCO2 increases and subsequent reduction in seawater pH (see above): See answer to 1) and 3) above p3976, line 2:...of masked effects...: Will be corrected

Interactive comment on Biogeosciences Discuss., 4, 3961, 2007.