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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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Authors answer: The referee would have liked to see the phytoplankton data in order to help illustrate if the virus response was simply caused by the changes in host concentrations. The phytoplankton data have already been published in the same special issue (Paulino et al. 2007) and are thus easily accessible to the readers of the current paper. Besides, all authors publishing in the current special issue have agreed not to duplicate figures to a too great extent. For these two reasons we still prefer to refer to the mentioned paper only. We also believe that the Paulino et al. paper, combined with rewritten part of the discussion of the current paper (as a response to referee #2) in which we will underline that the host (E. huxleyi for EhV) or possible host (nanoeukaryotes for HFV) organisms reacted opposite to their viruses by a slight increase

in abundance with increasing CO2/decreasing pH, should be sufficient for the readers to judge whether the drop in viral concentrations seem to be due to a correspondingly drop in possible host cell concentrations (as it does not). We agree that the mechanism behind this is an interesting and deserves to be looked further into. We think, however, it is beyond the scope of this paper, with the data available from the current experiment, to find the exact reason for the drops in viral concentrations. Looking for such mechanisms probably call for laboratory experiments with one specific virus-host system at the time rather than experiments in which the whole community with both identifiable and non-identifiable algal host virus systems are included.

Referee minor comments On the primer sets used: P3967: lines 1-5, where did these primer sequences come from? Were they developed in house? Why did not the authors use published EhV MCP primer sequences? Did these primers amplify all phycodnaviridae in the EhV (or CeV) sized bands or just EhV (or CeV) ie. What was the specificity of the primers? L6: Degenerate Phycodnaviridae primers. There is a lot of information missing on the design, sequence alignments optimisation etc in the development of this primer set (probably worthy of supplementary information or even a seperate paper), we have to take the authors at their word on this data (I have no doubts their "word" is sincere!). For example there is no explanation for the different sized amplicons, this seemed a bit odd for a "universal" set of primers. How do they know they are amplifying the MCP gene?

Authors answers: The EhV primers reported here were designed in house using the published sequences of the EhV-86 and 163 genome and the EhV-99B1 previously unpublished. The MCP nucleotide sequence of these viruses is very conserved. The primers cover regions that are conserved in all the three viruses. We have however not tested the primers beyond this point except on virus concentrate from mesocosms and isolated viruses.

The CeV primers were designed based on the MCP gene sequence from the CeV-01 virus. The MCP gene of this virus also seems to be conserved at the nucleotide level, at

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least in the region amplified using the degenerated primers. By applying these degenerated primers we have successively over a range of 7 years obtained sequences from concentrated seawater samples, 100% identical to CeV-01. Although, this not necessarily proves that the CeV MCP primers covers all genotypes of CeV, they adequately amplifies the sequence of this virus for the current study.

The design and application of the degenerated primers are described in the ms by JB Larsen et al.: "Phylogenetic analysis of members of the Phycodnaviridae virus family using amplified fragments of the major capsid protein gene" currently in review in Applied and Environmental Microbiology. The ms includes a comparison of the MCP sequence of currently sequenced phycodnaviruses and the mimivirus, and describes significant differences between coccolithoviruses + phaeoviruses, and the remaining currently sequenced phycodnaviruses + the mimivirus. Due to these differences the primers could not be designed to include the EhV viruses. Instead they were designed to amplify from Chloroviruses, Raphidoviruses, Prymnesioviruses, as well as viruses presently not assigned to any genera (CeV-01 (host- Chrysochromulina ericina), PpV-01 (host- Phaeocystis pouchetii), PoV-01 (host- Pyramimonas orientalis), and PgV (host- Phaeocystis globosa). The primers target the II and IV conserved region out of eight identified in the protein. Since the gene varies considerably in regard to both homology of sequence and inserts/deletions outside these regions, the amplification products differ in size. For instance amplicons of P. orientalis range between 300-400 bp, while CeV, and PpV viruses produces products of a constant size of 518 and 500 bp. The product is recognized as MCP OTUs by sequencing, and amplification from the MCP gene seems very specific, with no nested PCR or secondary amplification necessary to obtain sequences from concentrated seawater samples or viral lysate.

A short discussion around the size of the amplicons will be included in the revised version of the manuscript.

Referee comment: P3974 L3: Actually there are 14 core genes, for more information see: Allen et al.(2006) Evolutionary history of the Coccolithoviridae. Molecular Biology

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and Evolution, 23(1), 86-92.

Authors answer: Will be corrected in the revised version of the manuscript.

Referee comment: P 3975 L6: Another explanation could be simply a change in host genotype which dominates in a lower pH with consequent changes in virus production.

Authors answer: Although it seems from previously published papers that EhV is not strain specific (Castberg et al. 2002, Isolation and characterization of a virus that infects Emiliania huxleyi (Haptophyta). J. Phycol. 38: 767) we agree that the viral production could differ from host strain to host strain. We will include a comment on this in the revised version of the manuscript.

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