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The role of viruses in marine phytoplankton mortality

Baudoux, Anne-Claire

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Synthesis

The synthesis of the field studies presented in this thesis focuses on the extent to which viral mediated mortality of phytoplankton varies across marine systems with contrasting trophic status and what these results may imply for the organic carbon cycling.

1. Methodological insights

Prior to a more general analysis, some methodological issues should be considered. As emphasized in the previous chapters, the lack of appropriate methodology has long constrained advances in viral ecology. Several approaches have been developed to determine virally mediated mortality and all of them suffer from limitations (Brussaard 2004, Suttle 2005). Although not shown in this thesis, the utility of virally mediated mortality methods other than the modified dilution assay were examined.

Among these, we attempted to use transmission electron microscopy (TEM) to determine the frequency of visibly infected cells (FVIC) that can be converted to estimate of virally mediated mortality. However, several methodological caveats have constrained the reliability of our analysis. Firstly, TEM sample preparation includes multiple steps of fixation and centrifugation that lead to inevitable losses of material. The extent of these losses was particularly problematic when processing samples with either low algal abundance or small-sized phytoplankton. Secondly, the discrimination of the algal host species of interest in the TEM thin-sections is essential but nearly impossible for the smaller-sized algal species (e.g. flagellated picophytoplankton) in natural samples. Thirdly, this methodology relies on the use of different conversion factors to covert the FVIC to absolute estimate of virally mediated mortality. However, the estimation of the value of the conversion factors is fraught with problems and therefore may distort the clear picture (Proctor et al. 1993, Waterbury & Valois 1993, Binder 1999). Finally, the sample processing on board of a research vessel, the access to a TEM and laborious analysis are other practical constraints.

Different adaptations of the phage production approaches from Wilhelm et al. (2002) and Winter et al. (2004) were, furthermore, tested and compared to the dilution-based phytoplankton viral lysis rates. In brief, a natural phytoplankton concentrate was

resuspended in virus-free diluent and the algal virus abundance was monitored in parallel light and dark incubations for 24 h. The reduction in viral abundance prior to incubation lowered the incidence of new infection; hence any increase in viral abundance is assumed to result from an earlier infection event. Using an empirical burst size, the algal virus production can be converted to virally mediated mortality. Although routinely used for the determination of viral impact in bacterial communities, the application of these methodologies to phytoplankton was more difficult. Indeed, the extensive sample handling could alter phytoplankton physiology, particularly for the large-sized phytoplankton. Furthermore, the initial phytoplankton concentration step occasionally led to concentrate viruses along, which violates the critical assumption of this method, i.e., preventing the incidence of new infection during the incubation. Finally, the generally low abundance of putative algal viruses discriminated from the rest of the marine viruses (based on flow cytometry analysis) limited in most cases the accuracy of the measurements. In contrast, we obtained statistically valid viral lysis rates using the modified dilution technique. Keeping in mind the constraints of the modified dilution technique (Chapters 2, 4, and 5) but realizing its higher resolution and the possibility to accurately discriminate different phytoplankton groups, we considered this approach as the most appropriate for determining viral lysis rates during our field studies.

Potential limitations of the dilution method have indeed been addressed in this dissertation, but as stated earlier, all existing assays determining rates of viral lysis has its restrictions. The adapted dilution technique is only applicable to the numerically dominating phytoplankton, the use of flow cytometry restricts the analysis to the cells smaller than 20-30 μm in diameter, and the detection limit of the dilution technique is unclear (Chapter 5). Thus, the here reported results are likely conservative estimates of virally induced mortality. Nonetheless, the consistency of the dilution-based estimates with independent assessment of total cell lysis (Chapter 2), specific (or putative) algal virus abundance (Chapters 2, 4, and 5), and specific algal virus production (Chapter 2) strengthen our results.

2. Extent of viral lysis in eutrophic vs. oligotrophic conditions

Our results showed that algal viruses imposed substantial mortality rates to specific phytoplankton populations in eutrophic (range $0.01 - 0.35$ d^{-1}, Chapter 2) as well as in oligotrophic environments (range $0 - 0.20$ d^{-1} in surface and $0 - 0.80$ d^{-1} in DCM waters as shown in Chapters 4 and 5, respectively). In order to compare the extent of viral lysis among the different areas, we computed the total virally mediated carbon losses and related them to phytoplankton carbon production (CP) using the adapted formulas by Landry et al. (2000; Chapters 4 and 5). Phytoplankton carbon production is an important biological process in the ocean because it starts off the organic carbon cycle. Thus, expressing viral and grazing mediated algal mortality as the proportion of

phytoplankton CP is a relevant manner to evaluate the impact of these loss factors on the organic carbon flux. The absolute amount of carbon released by phytoplankton viral lysis varied widely among the studied areas (Table 1).

Table 1. *Summary of carbon losses due to viral lysis and microzooplankton grazing and corresponding fraction of phytoplankton carbon production removed. Values between brackets indicate the mean estimate per site and the number of studied stations (n).*

	Eutrophic [a,b]	Oligotrophic	
		surface [c,e]	DCM [d,e]
Carbon losses due to viral lysis (μg C L^{-1} d^{-1})	0.8 – 43 (14, n = 9)	0.0 – 2 (0.5, n = 5)	0.1 – 0.5 (0.2, n = 6)
Carbon losses due to grazing (μg C L^{-1} d^{-1})	3 – 79 (27, n = 9)	1 – 6 (3, n = 5)	0.1 – 1 (0.5, n = 6)
CP lost by viral lysis (% d^{-1})	2 – 280 (47, n = 9)	0 – 16 (4, n = 5)	11 – 32 (24, n = 6)
CP lost by grazing (% d^{-1})	9 - 217 (69, n = 9)	16 – 89 (44, n = 5)	17 – 65 (44, n = 6)

[a] *Southern North Sea, 2m, spring 2003 and 2004 (Chapter 2)*
[b] *Calculation restricted to Phaeocystis globosa single cells that numerically dominated the community of phytoplankton < 20 µm.*
[c] *North Sea, 5m, July 2003 (Chapter 5)*
[d] *Subtropical northeastern Atlantic, 60 – 100 m, October 2002 (Chapter 4) and North Sea, 37 m, July 2003 (Chapter 5)*
[e] *Calculation including the four picophytoplankton groups numerically dominating the studied area. The detailed calculation of this parameter is given in Chapters 4 and 5.*

The highest value, on average 14 ± 16 µg C L^{-1} d^{-1} (about 47 ± 90 % of CP d^{-1}), were obtained in the eutrophic environment during the *Phaeocystis globosa* spring bloom. Such a substantial impact of viruses on phytoplankton CP during the *P. globosa* bloom was expected as the probability of successful infection increased during the development of the bloom. By comparison, viral lysis led to a relatively lower carbon release of 0.2 - 0.5 µg C L^{-1} d^{-1} (on average) in the oligotrophic environments. This comprised, however, a considerable fraction of the phytoplankton CP, particularly in the DCM water layer (on average 24 ± 12% d^{-1} as compared to 4 ± 6% d^{-1} of the CP in surface waters).

The virally mediated mortality rates from the surface waters of the oligotrophic waters (4 ± 6% d^{-1}) matched closely the few estimates reported for specific

phytoplankton in the literature (Suttle & Chan 1994, Cottrell & Suttle 1995). This result furthermore supports the value of 2 - 10% of the algal production lost by viral lysis in steady-state pelagic systems as predicted in the revised model of Jumars et al. (1989) described by Wilhelm & Suttle (1999). However, this predicted estimate is considerably lower than the $24 \pm 12\%$ of the CP lost by viral lysis in DCM oligotrophic waters. These observations suggest that the impact of virus on phytoplankton may be higher in DCM than in surface waters. It is important to realize that the DCM and surface viral lysis estimates were obtained at different oligotrophic sites. We should, therefore, be cautious when extrapolating these results. The algal group specific effect of irradiance on virally induced algal mortality (Chapter 6) confirms such need for caution. Nonetheless, the prevalence of up to 24% of the CP removed daily by viruses suggests that the impact of viruses on marine phytoplankton in steady-state pelagic environments might be higher than previously assumed.

Interestingly, we noted that the picophytoplankton groups prone to the highest rates of viral lysis in DCM waters of the oligotrophic subtropical northeastern Atlantic Ocean were also suggested to be responsible for the release of Fe-organic ligands (Gerringa et al. 2006). Recently, Fe released by virally mediated lysis was shown to be highly bioavailable to marine plankton (Poorvin et al. 2004). Our observation supports the idea that viruses may be involved in the Fe cycling and adds to it that specific phytoplankton groups may play an essential role in this process. Given that large parts of the world's ocean are thought to be Fe-limited (Moore et al. 2002), this finding is of prime importance and therefore requires further investigation.

In summary, the presented results indicate that, next to microzooplankton grazing, viral lysis significantly influences the flow of organic carbon. The trophic status of the ecosystem, and arguably relevant environmental variables, seems to affect the extent of viral lysis. The relatively low rates in the open oligotrophic waters appeared to be rather constant in contrast to the viral lysis during spring algal blooms in eutrophic waters. This may result in a steady amount of photosynthetically fixed carbon shunted towards the regenerative food web. Our results provide one of the first data sets on actual viral lysis rates of natural phytoplankton in different ecosystems; data that are urgently needed for a better understanding of global biogeochemical cycling. The high viral lysis rates at the DCM of oligotrophic waters, and the algal group specificity of virally mediated mortality rates are other interesting findings in this thesis that actually strengthen the present call for more detailed studies on the role of viruses in the ocean.

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