Role of viruses in controlling phytoplankton blooms

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INTRODUCTION

Increased awareness of global environmental changes fostered research on the role of aquatic microorganisms in the geochemical cycling of energy. Despite the considerable knowledge on the (whole community) dynamics of microorganisms, many aspects of their specific ecology and physiology are still poorly understood. For example, very little is known about the actual *in situ* growth and loss rates of microorganisms on a species level. The problems in quantifying species-specific growth- and loss rates in a natural plankton assemblage are in part due to the multitude and complexity of processes involved.

The presence of viruses in marine environments has been acknowledged for many years, and it is by now well established that viruses are extremely abundant in aquatic ecosystems (Bergh *et al.*, 1989; Fuhrman and Suttle, 1993; Proctor, 1997; Wilhelm and Suttle, 1999; Wommack and Collwell, 2000). They are regarded as active and important members of the microbial food web (Wilhelm and Suttle, 1999; Fuhrman, 1999). Viruses are known to infect a wide spectrum of hosts, infecting not only bacteria but also eukaryotic primary producers (Suttle *et al.*, 1990; Van Etten *et al.*, 1991; Bratbak *et al.*, 1993; Peduzzi and Weinbauer, 1993; Brussaard *et al.*, 1996; Weinbauer and Höfle, 1998). Over the last decade it has been shown that viruses are important regulating factors in marine ecosystems. There is now the notion emerging that viruses influence species diversity, dynamics, population, the fluxes of organic carbon and nutrients, as well as gene transfer. Unicellular primary producers are a major group of organisms in natural aquatic communities and viruses have been shown to be an important source of mortality for phytoplankton (Van Etten *et al.*, 1991; Reisser, 1993; Proctor, 1997). Viruses or virus-like particles have been reported in all major taxa of eukaryotic algae, varying from unicellular to multicellular, swimming to non-swimming, bloom-forming to non-bloom-forming, free-living to symbionts.

VIRUS ENUMERATION

Because of the relatively short infection cycles, virus dynamics are often highly variable and rapid changes in both total abundance and diversity are reported (Suttle and Chan, 1994; Cottrell and Suttle, 1995; Castberg *et al.*, 2001; Larsen *et al.*, 2001; Wilson *et al.*, 2002a). Assays for counting viruses with high precision and fast analysis are, therefore, beneficial for the study of viral ecology.

In any field of research, the development of new methods stimulates research; either by improving speed or accuracy, or by allowing the detection or quantification of parameters not

measurable before. More traditionally, diverse culturing (plaque counts and most-probable-number assays) and transmission electronmicroscopy were used to estimate the number of viruses in natural waters. These techniques were either selective for viruses infective for a specific host, or very time-consuming. The use of very sensitive fluorescent nucleic acid-specific stains allowed faster detection of aquatic viruses, infective as well as non-infective, with epifluorescence microscopy (Hennes and Suttle, 1995; Noble and Fuhrman, 1998). With the recent introduction of flow cytometric detection and enumeration of free viruses by Marie *et al.* (1999a), speed of analysis and accuracy of counting improved largely. Brussaard *et al.* (2000) showed that a wide range of viruses, differing in morphology and genome size, could be detected flow cytometrically. Flow cytometry, applying SYBR Green I as the nucleic acid-specific fluorescent stain, has been used successfully to count viruses from laboratory experiments (Brussaard *et al.*, 1999, 2001), as well as from natural marine environments (Marie *et al.*, 1999a; Larsen *et al.*, 2001; Castberg *et al.*, 2001; Li and Dickie, 2001; Chen *et al.*, 2001; Wilson *et al.*, 2002a).

Studies using flow cytometry to count free viruses in natural samples are not uniform in the method used (Marie *et al.*, 1999b; Brussaard *et al.*, 2000; Chen *et al.*, 2001; Wilson *et al.*, 2002a). Working closely at the limits of staining methodology and instrumentation at present, however, the level of GFL is of importance for optimization of the detection of free viruses (Brussaard *et al.*, 2000). The few reports on the methodology of flow cytometric analysis of virus samples suggest that optimal detection of the free virus particles depends on various factors. A very recent evaluation of ours of the effects from a broad range of variables on the staining specifications of aquatic viruses (various specific cultured phytoplankton viruses and bacteriophages, but also natural marine samples) indicates that large variations in reactions between the different viruses were found. When dealing with unknown mixed virus communities, one specific set of variables seems to provide the best results, involving storage of fixed samples in liquid nitrogen, the use of SYBR Green I as fluorescent dye at low concentration, and heating of the sample before flow cytometric analysis. Further, comparison between epifluorescence microscopy and flow cytometry showed that the latter method to count free viruses is more sensitive and can be applied more generally (marine *vs.* freshwater, eutrophe *vs.* oligotrophe, shallow *vs.* deep water layers) than the epifluorescence microscopy method.

In general, many of the phytoplankton viruses exibit a relative high green fluorescent signals after staining (Fig. 1), allowing a convenient discrimination of these viruses from the total virus fraction (mainly bacteriophages). As long as there are no specific probes for the viruses of interest, such can be very useful for research on phytoplankton virus ecology under natural conditions.

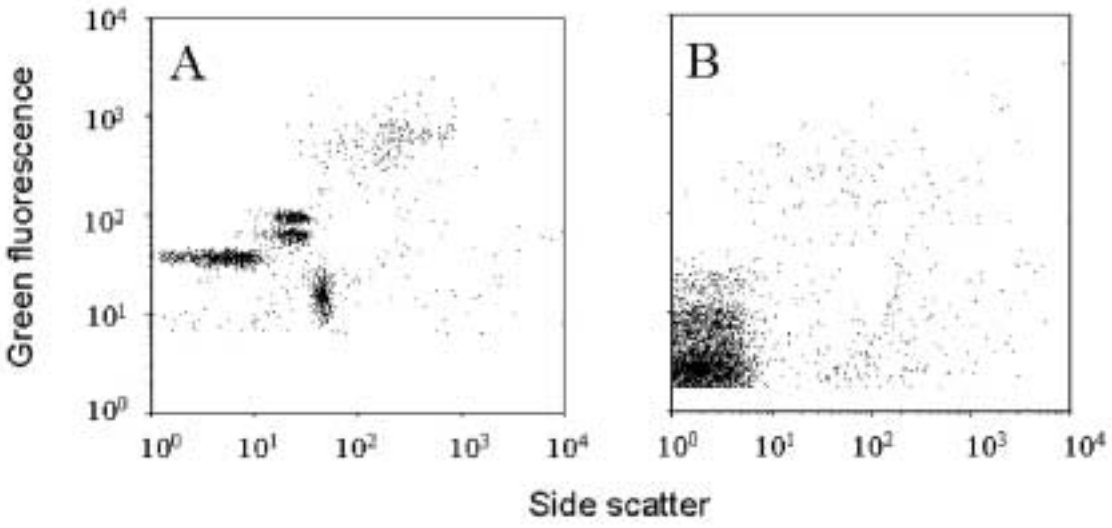

Fig. 1. Cytograms of green fluorescent signal of stained viruses vs side scatter signal. A mixture of 4 different algal viruses **(A)** and **(B)** a natural virus sample from coastal waters was analyzed according to Brussaard *et al.* (2002)

ROLE OF VIRUSES ON MICROBIAL FOOD WEB

With phytoplankton being the base of the marine food web, extensive research has been conducted on the factors affecting succession and biodiversity. Traditionally these studies concentrate on the ability of the species to deal with growth limiting conditions, but lately studies have shown that cell lysis can have a strong impact on phytoplankton population dynamics as well (Brussaard *et al.*, 1995, 1996a; Riegman *et al.*, 2001). Field studies showed, using the dissolved esterase activity assay, that the specific lysis rates of the algal community varied widely in time and space. Cell lysis was found to be a significant, and regularly the major, mortality factor for phytoplankton as compared to other loss factors of phytoplankton studied during these field campaigns. Inclusion of algal cell lysis into mathematical ecosystem models often improves the match between simulation and measured data. Lysis appeared essential for optimal simulation of algal dynamics, species succession and regeneration of inorganic nutrients in ecosystem modelling. Whether phytoplankton sink out, are grazed upon or die due to viral induced cell lysis has major implications for the flow of material and energy cycling in the marine pelagic food webs. The primary result of cell lysis is the release of dissolved organic matter and nutrients into the surrounding water, thereby directly promoting bacterial production (Brussaard *et al.*, 1995, 1996a). The production of DOM is the first step in the microbial food web; DOM is being utilized by heterotrophic bacteria which in turn are controlled by protozoa with the eventual result of linking the microbial food web with the classical food web. Knowledge on processes leading to the production of DOM is crucial for a good understanding of the global carbon cycle, since cell lysis forces the food web towards a more regenerative system (Gobler *et al.*, 1997; Fuhrman, 1999; Wilhelm and Suttle, 1999). Ecosystem models including viral mediated lysis (Fuhrman, 1999; Wilhelm and Suttle, 1999) show that up to 26% of the organic carbon flows through the viral shunt, with bacterial production and respiration increased by 33%.

Especially during phytoplankton blooms, when the high algal cell concentration will enhance the virus-host contact rates, viruses can have a significant impact on the development of these blooms (Bratbak *et al.*, 1993; Brussaard *et al.*, 1996b). *Phaeocystis globosa* and *Emiliania huxleyi* (Prymnesiophyceae), for example, can produce very dense blooms in temperate waters, and are considered very important in biogeochemical cycling of organic carbon and nutrients, as well as climate-relevant elements (e.g. by contributing strongly to DMS fluxes). With the high amount of particulate organic matter and nutrients stored in algal blooms, viral lysis of a single dominating phytoplankton population has been shown to significantly affect the transfer and cycling of energy and matter within the pelagic food web (Bratbak *et al.*, 1998; Brussaard *et al.*, 1996b; Gobler *et al.*, 1997; Fuhrman, 1999; Wilhelm and Suttle, 1999). Differences in the magnitude of blooms of HAB-species *Phaeocystis globosa* were indeed reflected in the structuring impact of phytoplankton cell lysis on the microbial pelagic food web. As a result of the remineralized nutrients, virus infection can also be expected to indirectly account for changes in population interactions such as phytoplankton competition and succession.

High total phytoplankton lysis rates (up to 30 % d^{-1}) were, however, not only recorded during the decline of algal blooms (Brussaard *et al.*, 1995, 1996b) but also occur in the open ocean (Agusti *et al.*, 1998). Comparative studies on the ecological role of virus-induced mortality of phytoplankton in ecosystems with contrasting trophic status are of great importance because of the differences in food web structure of eutrophic *versus* oligotrophic ecosystems. With ecosystems along a large trophic gradient being very different in their environmental controlling factors (nutrients, UV), the mechanisms behind phytoplankton cell death are likely to be different too. Generally, in oligotrophic waters the import rate of the controlling nutrient is low and regeneration of the limiting nutrient by members of the microbial food web is found important to sustain high productivity. Picophytoplankton dominate the photoautotrophic community due to their good competitive growth characteristics. As a result of rapid numerical response by heterotrophic nanoflagellates and microzooplankton, phytoplankton biomass is size-selective grazer controlled (Riegman *et al.*, 1993). Although this highly dynamic regenerative system is present in any euphotic zone (Thingstad and Sakshaug, 1990), at increasing import rates of the controlling nutrient (eutrophic waters) microzooplankton set limits to the biomass of the small algae. The primary effect is an increase in the biomass of larger algae escaping the size-selective grazing by

microzooplankton. Control of these larger phytoplankton by mesozooplankton is relatively low due to the relatively long generation times of these grazers. Furthermore, non-edible algal species will be able to increase in concentration, forming temporal algal blooms (e.g. diatoms, *Phaeocystis* spp.). Lysis of an unialgal bloom will affect population interactions (competition, commensalism, antagonism) and may lead to a distortion of the "classical" strucure of the ecosystem. The temperate eutrophic ecosystems seem more likely to be controlled by viruses. Infection of the host organism with lytic viruses leads to cell death while releasing numerous progeny viruses. Infection with lytic viruses is expected to be most prominent in environments with high abundance of the algal host (e.g. algal blooms) because of the increased host-virus contact rates. Phytoplankton at low abundance are not expected to be easily infected, but they may contain temperate viruses. Lysogeny (the viral genome is maintained in the host cells as a prophage without producing progeny viruses) might act as a survival strategy for the virus specific for low abundant algal species. The shift to the lytic mode may occur spontaneously, but at low rate or may be induced by environmental variables such as UV-light, temperature changes, nutrient depletion.

Differences between ecosystems can include variation in nutrient loadings, and it is, therefore, of interest to study the effect of nutrient depletion and different N:P ratios on viral lysis of phytoplankton. Nutrient depletion was sometimes found to affect host-virus interactions (Bratbak *et al.*, 1993; Wilson *et al.*, 1996). Our indoor mesocosm study on *P. globosa* (flagellated single cells and colonial cells) under different N:P ratios did not show obvious differences in their overall population dynamics. This implies a minor role of the macro nutrients (N and P) on the growth dynamics and loss rates of *P. globosa*. However, it was for the first time that viruses were found that infected this HAB-species. Blooms developed in one week, with enhanced rates of total phytoplankton cell lysis during the decline of the *P. globosa* blooms. Viral induced mortality of *P. globosa* caused the decline of the bloom. Viruses seemed an important controlling agent, having strong implications for our understanding of the impact of viruses on the temporal dynamics of *P. globosa*. Microbial dynamics and diversity were strongly influenced by viral induced lysis of phytoplankton, as was found for other mesocosm studies in Norwegian fjords (Castberg *et al.*, 2001).

ROLE OF VIRUSES FOR PHYTOPLANKTON HOST DIVERSITY

Over the last decade, about a dozen model systems of virus and photosynthetic host were isolated and brought into culture. Studies of these systems showed that viral-induced lysis of the entire algal host population usually occurs within one day, thus indicating that viruses can have a major impact on phytoplankton population dynamics (Suttle and Chan, 1993; Bratbak *et al.*, 1998; Brussaard *et al.*, 2001; Sandaa *et al.*, 2001).

In the field, phytoplankton communities differ in species composition through space and time. Especially in temperate coastal waters, a strong succession of phytoplankton species can be observed on a annual base. Similarly, spatial and temporal variations in the composition of the virioplankton community occur as well (Suttle and Chan, 1993; Cottrell and Suttle, 1995; Wommack *et al.*, 1999a; Castberg *et al.*, 2001; Larsen *et al.*, 2001; Short and Suttle, 2002). The use of molecular techniques such as DGGE and PFGE showed the appearance of different virus species after an almost-complete lysis of the blooming photosynthetic host population (Wommack *et al.*, 1999; Steward *et al.*, 2000; Castberg *et al.*, 2001; Larsen *et al.*, 2001; Short and Suttle, 2002). Since viruses have a restricted host range, infection by a particular phytoplankton virus does not act on the total phytoplankton community but will be limited to specific algal species or even different strains. Coexistence of competing phytoplankton species is therefore ensured by the presence of viruses that "kill the winner" (Thingstad, 2000b). Besides this direct role of viruses on interspecies phytoplankton competition, virally regenerated nutrients might stimulate the growth of other algal species (indirect impact; Gobler *et al.*, 1997). The number of studies on this topic is, however, still very limited.

Furthermore, there is increasing awareness that a specific geographical population of one phytoplankton species is not morphologically and genetically homogeneous, but can be rather diverse (Barker *et al.*, 1995; Rynearson and Armbrust, 2000). Thus, besides species-specific control of phytoplankton diversity by viruses, one can also expect viruses to affect phytoplankton

diversity and competition on an intraspecies level. Studies on phytoplankton viruses have revealed strain-specific viral infection in the laboratory and in natural environments (Sandaa *et al.*, 2001; Suttle and Chan, 1993; Cottrell and Suttle, 1995; Sahlsten, 1998; Tarutani *et al.*, 2000). An essential advantage of high diversity in immunity to virus infection and/or in host specificity would be that the potentially regenerated nutrients are not necessarily utilized by other competing phytoplankton species, but are taken up by individuals of the same species. Assuming comparable competitive capacity between the different algal strains, the dominant phytoplankton species will only be reduced in the number of cells of one particular strain and not necessarily in its overall abundance. This suggests the existence of complex and constantly varying host – virus relationships.

In addition to highly diverse individual cross reactions between an algal strain and a virus clone, the challenging issue of the impact of virus infection on competition between non-resistant and resistant phytoplankton strains should be addressed. Very recently, a few studies, including our own, noted recovery of phytoplankton populations upon viral-induced lysis (Thyrhaug *et al.*, 2002). The mechanisms for coexistence of phytoplankton host and virus require further investigation since they are still unknown. It can be hypothesized that resistant host cells may have a reduced competitive fitness as compared to sensitive viruses (Middelboe *et al.*, 2001).

Finally, there are only a few studies that examined the morphologic and genetic diversity of virus strains infecting the same phytoplankton strain (Cottrell and Suttle, 1991; Lu *et al.*, 2001). Diversity in particle size, latent period, genome size, host range, and restriction fragment pattern of the viral DNA was found amongst virus isolates infecting identical host strain, indicating that these viruses are different from eachother and that they are likely competing with eachother to infect the phytoplankton host cells. The factors regulating the competitive fitness of such viruses are largely unknown. Interestingly, despite the differences in basic virological characteristics, a close molecular relationship among the *P. globosa*-infecting viruses has ben recorded.

So far, almost all isolated phytoplankton viruses have a dsDNA genome. Recently, there have been reports by our group and others of ssRNA and dsRNA viruses infecting phytoplankton species (Tai *et al.*, in press; Tomaru, pers. comm.). The dsRNA virus infecting Micromonas pusilla has a relative long latent period (36 h), but was still able to coexist with a dsDNA virus infecting the identical strain of *M. pusilla* with a latent period of only 14 h (15). Nothing is known yet about the interactions between these two different types of viruses. Virus competition is an unexplored field of ecological research, deserving attention since it has the potential to contribute to a comprehensive understanding of phytoplankton successions in marine ecosystems.

CONCLUSIONS

This brief, and incomplete, overview is an attempt to highlight what we presently know and do not know about some important processes in phytoplankton virus ecology. The following issues deserve particular consideration:

- Information on the quantitative significance of the different phytoplankton mortality processes is still lacking. Method that allow the quantification of viral lysis rates specifically phytoplankton under natural conditions need to be developed.
- Insight in the ecological importance of viruses for phytoplankton in ecosystems with different trophic level, as well as the influence of important environmental variables on the interactions between virus and algal host cells.
- Viruses can keep the host at non-blooming concentrations (Larsen *et al.*, 2001), and are often found to be a major cause for phytoplankton decline (Bratbak *et al.*, 1993; Nagasaki *et al.*, 1994; Brussaard *et al.*, 1996). However, blooms do occur and so viruses are unable to prevent blooms at all times. To understand the mechanisms behind this, we need to know what factors are important regulators.
- To allow a proper study of viral diversity under natural conditions, on a spatial and temporal scale, and to obtain insight into the impact of different viruses on phytoplankton dynamics and diversity, virus-specific probes need to be developed.

- Despite the considerable knowledge on phytoplankton dynamics and the increasing number of studies on viral ecology, many aspects of the interactions between virus and phytoplankton host are still poorly understood (only one example is the importance of more than one type of virus in a phytoplankton cell, Brussaard *et al.*, 1996). In awareness of these gaps in knowledge, the ecological role of virus competition and clonal variation on phytoplankton host population dynamics should receive more attention.
- The use of mathematical ecosystem models including a thoroughly tested virus module, and validated against actual data, will add to our understanding of the regulatory role of viruses on phytoplankton dynamics and their impact within the pelagic food web.
- With more virus-phytoplankton host model systems being isolated and brought into culture, we will likely also find “new” virus types. Some of these viruses have been hard to isolate or keep in culture. As was found for the 'non-culturable' bacteria, they might be of significant ecological importance. Comparative studies on the ecological relevance of these viruses is recommended.

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