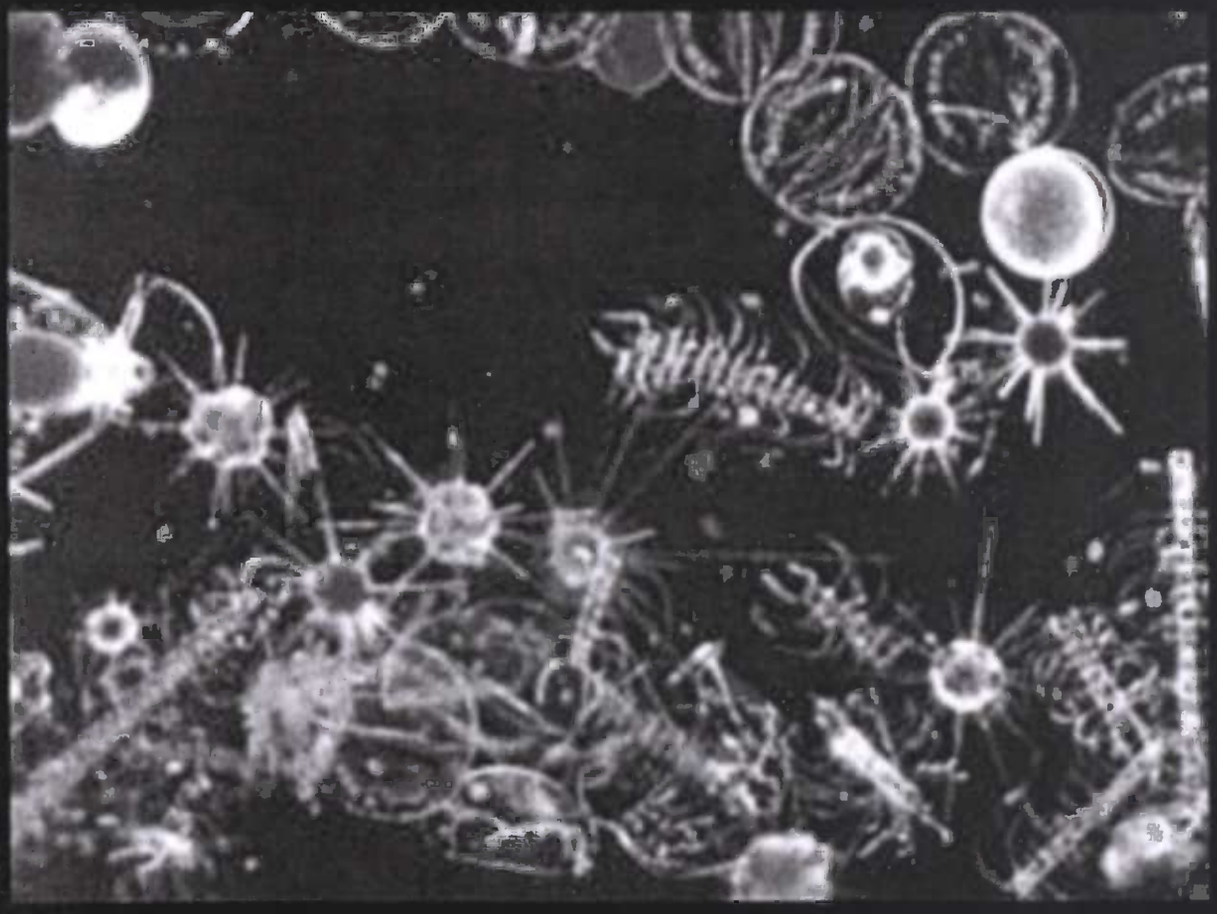

The role of phages and viruses in oceanic primary production

Reinout Havinga

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Reinout Havinga
RuG essay, february 2005
Supervision: Anouk Piquet
University of Groningen

De rol van bacteriofagen en virussen in de primaire productie van de oceanen

Reinout Havinga
RuG scriptie, februari 2005
Begeleiding: Anouk Piquet
Rijksuniversiteit Groningen

Cover image. Close-up impression of eukaryotic phytoplankton.

Summary

Phages and viruses are currently becoming recognised as important actors in the oceanic food-web. They have not only shown to be capable of controlling plankton crops by infecting cells and inflicting lysis upon them. By breaking down live microalgea and cyanobacteria, they also add organic material to the seawater debris and DOC pools. This again serves as a food-source for heterotrophic bacteria. In effect, marine viruses are cutting-short in the nutrient and energy chain, bypassing the higher trophic levels occupied by creatures like krill, fish, seabirds and cetaceans. This results in what is called the 'microbial loop': materials and energy are circulating around between microbes without being exported upward to bacterivores, krill, fish etc.
Although we now have come to the agreement that for some organisms it is a good thing that phages and viruses - who are considered to be host-specific - are around, so far it is still bad news for the infected ones. However, some cyanobacteria and microalgea seem to benefit from respectively phage or virus infection. They seem to be better able to cope with extreme light intensities when infected. Some phages have proven able of mending the damage of excess light on their cyanobacteria hosts.

Samenvatting

Aan bacteriofagen en virussen als spelers in het mariene voedselweb wordt de laatste tijd steeds meer belang toegekend. Ze blijken het planktonbestand te kunnen beïnvloeden en bovendien voorzien ze het zeewater van grote hoeveelheden organisch materiaal en opgeloste organische koolstofverbindingen; het slachtafval van hun gastheercellen. Dit substraat dient vervolgens weer als voedselbron voor heterotrofe marine bacteriën. Het komt er eigenlijk op neer dat de virussen het verloop van stoffen en energie door de gehele voedselketen ergens bij het begin afsnijden, waardoor het niet eerst helemaal door de garnaal, de haring en de jan-van-gent heen moet voordat het weer als afval beschikbaar komt voor de bacteriën. Dit resulteert in wat men noemt de 'microbiële kringloop': energie en materialen gaan in een kringetje rond tussen bacteriën zonder dat ze beschikbaar komen voor de hogere trofische niveau's.
Hoewel het nu duidelijk is dat sommige organismen - de heterotrofe bacteriën - profiteren van het werk van virussen en bacteriofagen, het is nog steeds nadelig voor een organisme om zelf geinfecteerd te zijn. Toch schijnen sommige cyanobacteriën en microalgen te kunnen profiteren van een infectie. Ze zijn dan namelijk beter in staat om bij overmatig zonlicht door te groeien. Dit komt waarschijnlijk doordat sommige virussen de moleculaire schade van overmatig zonlicht op hun gastheercel kunnen repareren.

Table of contents

Introduction

While the concept of eukaryotic virusses was already acknowledged, bacteriophages were first described in 1915 (Duckworth 1987). Bacteriophage litteraly means 'eater of bacteria'. The first implication of the knowledge of phages was the medical application of phages as a method for curing bacterial infections in humans. In Western Europe and the US however, phage therapy was abandoned after the discovery of antibiotics. A revival of phage therapy can nevertheless be observed since the recent increase in antibiototic-resistant bacteria (Weinbauer 2004).
Although it was hypothesised that phages might have a regulating effect on bacterial populations as early as 1968 (Wiebe and Liston 1968), their possible role was ignored when the first microbial food web models (figure 1a) were developed. One decade later high numbers of phages (>10^4 ml^{-1}) in the ocean were discovered for the first time (Torrella and Morita 1979), making it more accepted that they might be involved in the regulation of bacteria in natural environments. Since 1990 (Proctor and Fuhrman 1990) this trophic model is finally being revised, and for sensible reasons.

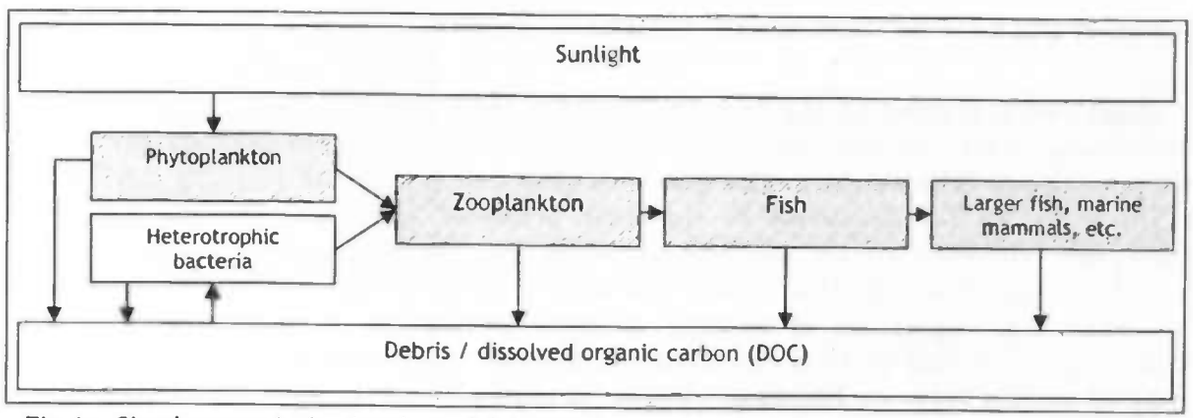

Fig 1a. Simple oceanic food web model in which microbial activity is recognised (heterotrophic bacteria). The pathways adding to the debris/DOC pool are not specified. The shaded boxes represent the classical concept of upward transport in the food-chain.

Cyanobacteria and algae are the main contributors to carbon fixation in the oceanic food web (Mann 2003). In the earliest food-web models this carbon is solely transported upward into the food chain, as the phytoplankton is consumed by zooplankton, which in its turn is consumed by small fish, all the way up to the top predators (shaded part of figure 1a). It is shown that viral lysis of phytoplanktonic cells deprives higher trophic levels of 5-25% of the carbon produced by primary producers (Wilhelm and Suttle 1999). This recycling of dissolved organic material (DOM) back to microbial production is called 'the planktonic viral loop' (Fuhrman 1999). A virus-oriented model of the oceanic food web is shown in figure 1b.

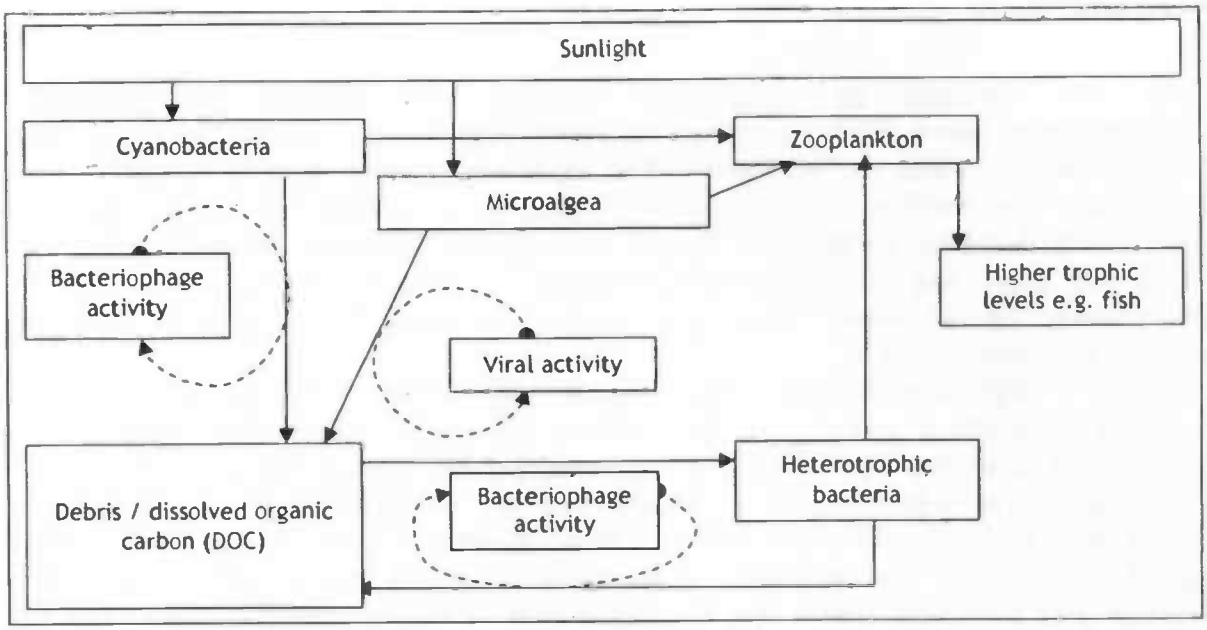

Fig 1b. A more coplex oceanic foodweb model, with special emphasis on the role of viruses in adding to the debris/DOC pool. Note that viruses and bacteriophages rely on the same trophic groups as does the zooplankton. See also figure 5.

In oligotrophic regions of the oceans, cyanobacteria are the main carbon fixators (Weinbauer 2004). In the meso- and eutrophic seas, the main primary producers are microalgea (eukaryotes). The greatest effect of phages on primary production is therefore to be expected in the vast areas of the open ocean. However, eukaryotic viruses can be considered important factors in controlling biomass growth in coastal areas. This review will mainly be on phage-bacteria interactions, but when the regulation of oceanic primary production is reviewed, also viral control of microalgal activity has to be considered. When the word 'phage' is used, this will always refer to bacterial viruses, whereas 'virus' can be either eukaryotic or bacterial virus.

The study of phage ecology was initiated with the description and characterisation of phages and hosts. This will also be the starting point in this review, followed by a short technical essay of the infection strategies used by phages. There appears to be more to it than just infection followed by cell lysis and dispersion within a time period that depends on the metabolical capacities of the host cell only. Some phages are capable of extending their residence time in host cells, and this delayed proliferation strategy obviously has some evolutionary benefits.
After reviewing the biology of phages and hosts I will concentrate on some interesting issues in the ecology of virus- (pico-) phytoplankton interactions like phytoplankton population regulation, nutrient cycles, dial patterns of infection and lysis of host cells and a presumed mutualistic relationship between parasite and host. The latter refers to indications that viruses and hosts may benefit from infection as it offers protection to both organisms against damage by high light intesities.

Phages & Hosts

The Phages

Phages, also called bacteriophages or prokaryotic viruses, are individual strands of genetic material, parasitising the cytoplasm and metabolism of bacterial cells for proliferation purpose (Weinbauer 2004). Viruses rely on tiny genome containers with diameters from 20 through 200 nm (Fuhrman 1999), as dispersion morphs. Phages are extremely abundant in the oceanic environment. Numbers range between 10^4-10^8 virus particles per ml^{-1} in aquatic systems (Wommack and Colwell 2000), with highest densities found in estuaries, intermediate in offshore surface waters and lowest values found in deep-sea waters. These high numbers show that viruses are one of the most abundant organisms in the oceans.

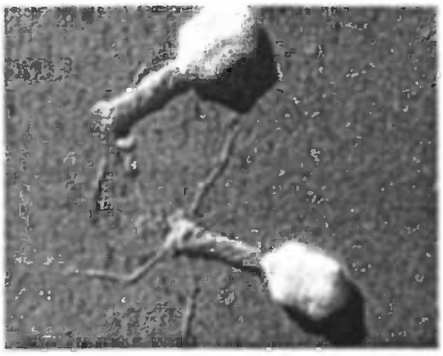

Fig 2. T4 Bacteriophage. In this image from an electron microscope, a complex virus known as a bacteriophage ("eater of bacteria") can be seen with its bulging head and tubular tail. Secured to its victim by the stringy fibers visible here, the tail acts like a needle in piercing the bacterium's cellular wall. The head then passes its reservoir of DNA down the tail and into the host. Within 25 minutes the dying cell is teeming with about 100 fresh copies of the virus, which are created by the rearrangment of the bacterium's own genetic contents (Encarta Encyclopedia 2004).

By 2000 more than 5100 phages have been described, of which 96% percent were tailed phages (Ackermann 2001). Tailed phages are called after the morphology of their protein container in the dispersion phase. Attached to the main genome-containing 'body', there is a tail-shaped tube, which is used as a duct through which the phage inserts its genome into a host cell at an infection event (figure 2). Almost all viruses known to infect phytoplankton have double stranded DNA (dsDNA) genomes and belong to the virus family of Phycodnaviridae (Pringle 1999). More recently however, plankton-infecting viruses with ssRNA (Nagasaki *et al.* 2004) and dsRNA (Brussaard et al. 2004) genomes have been found as well. These genomic differences might very well have come into being due to different evolutionary pathways along which viruses evolved, and it may be quite likely that these differences determine the molecular behaviour of a virus when infecting a cell. They should therefore be taken into account when studying virus ecology. However in this review I will not cut down to the level at which these differences become apparent.

A concise listing of methods used to count phages (Bettarel *et al.* 2000;Ferris *et al.* 2002) includes transmission electron microscopy (TEM) (Weinbauer *et al.* 2002), epifluorescence microscopy (Wen *et al.* 2004), flow cytometry (Brussaard 2004), plaque assay (solid medium) and most probable number method. A general drawback in practically any method is the underestimation of numbers of virus-like particles (VLP's). Therefore, a distiction is usually made between observed and estimated numbers of VLP's.

The Hosts

In the open oceans, nearly all of the CO_2 fixation comes to the account of marine cyanobacteria (Weinbauer 2004). Especially the picophytoplankton genera of *Synechococcus* and *Prochlorococcus* cyanobacteria are ubiquitous. Together they account for up to 89% of the primary production in oligotrophic regions of the oceans (Goericke and Welschmeyer 1993;Li 1995;Liu *et al.* 1997;Veldhuis *et al.* 1997), consequently these two genera of cyanobacteria are at quantities subject to viral activity and rather well studied. *Prochlorococcus* distinguishes itself from *Synechococcus* by being typically ten times as abundant, by being confined to warmer waters (>10°C) and by using another light-capturing molecule, namely a chlorophyll a_2/b_2 complex (Chisholm *et al.* 1992). *Synechococcus* grows at temperatures as low as 2°C (Partensky *et al.* 1999) and the species of this genera use a phycobilisome such as phycoerythrin (MarineCluster-A) (Johnson and Sieburth 1979;Waterbury *et al.* 1979) or phycocyanin (MC-B) (Waterbury and Rippka 1989) for light capturing. Molecular tools have shown useful in analising ecological parameters as community structure, growth rate and nutrient status at the cellular level. This way biotic and abiotic factors controlling the productivity of specific genotypes, can be found, resulting in the definition of niches for several types of *Prochlorococcus* and *Synechococcus* (Scanlan and West 2002). Cyanobacterial growth is considered to be limited by nutrient availability, photoinhibition and viral infection (Mann *et al.* 2003).
Also eukaryotic microalgae are active primary producers that are notoriously being harassed by viruses, but they are confined to richer seas as continental shelf waters and estuaria. However the biology of viruses infecting eukaryotic cells must be quite different from that of bacteriophages, as the stucture of the target genome differs between eukaryotes and prokaryotes.
Some species of microalgae tend to start growing in massive quantities at a time. Such an explosion of microalgal growth is called a 'bloom' and these usually consist of only one species. Several studies have looked at the effect of viruses on algal blooms (e.g. Jacquet and Bratbak 2003;Onji *et al.* 2003). Clearly, viral lysis of this phytoplankton from the highly poductive marine regions can be expected to strongly influence the overall global effect of viral activity on marine primary production.

Phage Life Styles

Bacteriophages may be either obligatory lytic or temperate. The first type always starts the lytic cycle immediately after infection of a cell. The second type can induce lysogeny, where cell lysis is postponed and viral DNA is integrated into the genome of the host-cell (Mann 2003). The integration of such phage-DNA into the bacterial genome is called a prophage. The prophage will be replicated along with its host, and consequently be present in several new host cells. Finally, a subordinate of obligate lysis is recognised as a pseudolysogen: "a phage-infected cell that grows and divides even though its virus is pursuing a lytic infection"(Birge 2000). This implies that a pseudolysogen is an infected cell, which is able to resist

viral lysis even though the infectant is persuing to induce a lytic cycle upon its host.

Two major selective advantages for temperate phages are recognised (Stewart and Levin 1984). At first, lysogenised cells are immune to lytic infections by the same strain of phages. The infected cell is then, although not lysed instantly, 'booked' by the infecting phage for proliferation purposes at a perhaps more appropriate moment in time. Secondly, lysogenised cells proliferate the phage DNA through cell division and the virus thus replicates itself without going through the risky dispersion-infection phases. Especially in nutrient-poor conditions where host-cell densities are low, this might be a more effective replication strategy than the dispersion of phage particles and reinfection of host cells.

This leads to the consideration that phages in oligotrophic environments like the open sea, where host cells are scarce, are more likely to induce lysogeny than their counterparts in eu- or mesotrophic environments like continental shelves and coastal or delta areas, where hosts are abundant. Mittler (1996) however, shows in a theoretical study that when an equable environment is rich in both cells and resources, a phage will benefit from having both low probabilities of lysogenisation, in order to maintain efficient infectious transfer, as well as a low induction rate. In an unequable environment on the contrary, phages will benefit from a high induction rate and a high probability of lysogenisation. The authors suggest that temperate phages have thus evolved in habitats where numbers of sensitive hosts might have been close to zero for a long period, with every now and then a large number of cells entering the habitat, allowing for new rounds of lysis and lysogenisation.

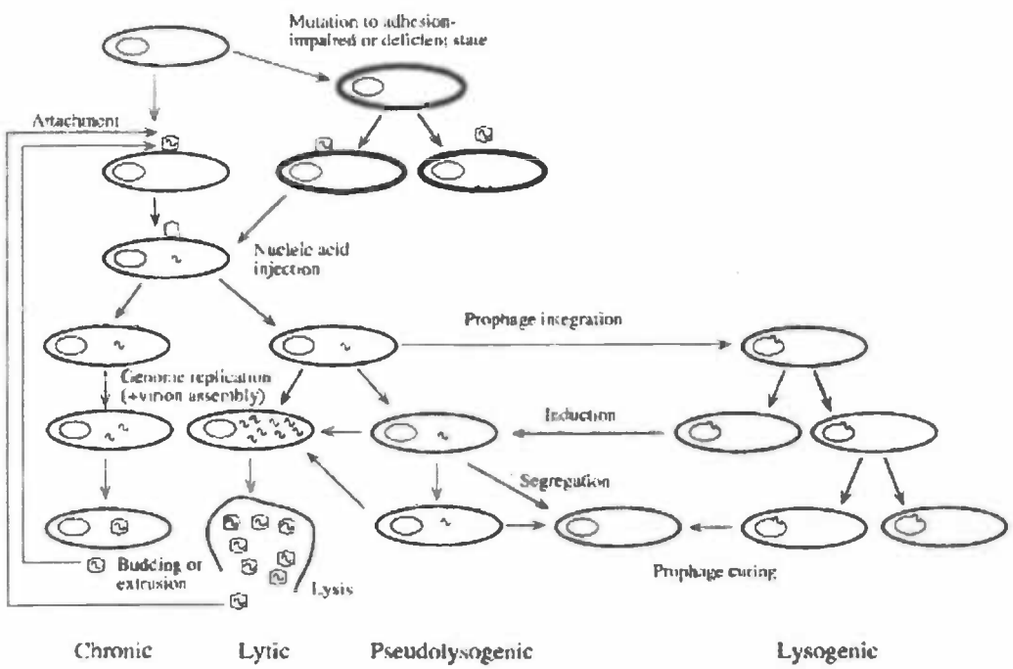

Fig 3: Types of viral life cycles. The model is adopted from Paul and Jiang (2001), with some modifications and expanded by chronic infection (Weinbauer 2004).

Ecological Interactions

Viral Loop

In the North Water, an ecologically important polynya between Ellesmere and Northwest Greenland (Deming *et al.* 2002), a large amount of organic carbon generated during spring bloom was eventually consumed by bacteria rather than by zooplankton, thus entering the microbial food web. 6%-28% of bacterial production in its turn was found to be 'lost' by viral lysis (Middelboe *et al.* 2002), thus contributing to the amount of DOM which again counts as a food source to heterotrophic microbes. In the same study, organic carbon was considered to be the limiting resource for bacterioplankton. This implies that in situ bacterial growth is being pushed forth partly due to viral activity.
Winter *et al.* (2004) suggest that in rich systems such as the North Sea, a dial cycle of infection and lysis would enhance the microbial loop by adding DOC to the nutrient pool, to the costs of the availability of organic carbon to higher trophic levels beginning with bacterivorous zooplankton. This is a sensible suggestion when viral lysis of phytoplankton comes at once in a reaction to high growth rates in the afternoon, transferring organic carbon to the DOC pool at the same rythm as it is collected from CO_2 by phytoplankton. This does not necessarily mean the carbon is transferred at the same amounts, but the differences in release of organic carbon from viral lysis are forthwith leading back to the differences in CO_2-uptake by phytoplankton. Gobler *et al.* (1997) found an inverse correlation between prokaryotic and eukaryotic plankton densities as a result of increased levels of dissolved nutrients due to viral activity. This discovery strongly supports the idea that the viral loop deprives the higher trophic levels of nutrients and organic carbon sources, to the benefit of the microbial community. This is probably due to the fact that heterotrophic bacteria are capable of feeding on debris and dissolved carbon in the environment, both of which levels are partly determined by the products of viral cell lysis. Heterotrophic eukaryotes on the contrary, feed on the same live prey organisms as do the viruses. They therefore meet in a virus a competitor rather than a facilitator (see also figure 1b).
Guixa-Boixereu et al. (2002) found that in Antarctic waters, on prokaryotic survival, the effect of phages is significantly greater than the impact of predation by bacterivores. This corresponds to extremely low values of bacterivory found in Antarctic waters (Vaque *et al.* 2002). Bearing in mind the 1:1 connection between the activity of the host and the parasite, the authors therefore suggest the following interesting hypothesis: "In cold environments, viruses could cause a higher impact on prokaryotic mortality than bacterivores, at temperatures where bacterivores show a lower activity than bacteria (Guixa-Boixereu *et al.* 2002)." If such a hypothesis holds true, it would mean a constitutional difference in the way and the extent at which viruses promote the microbial loop between polar and temperate or tropical waters. In the North Sea phages were considered to be outcompeted by zooplankton grazing on 'their' host-cells (Wilson *et al.* 2002a). This advocates that the relative importance of viral control of bacteria decreases when temperatures increase. However, data from the arctic Bering and Chukchi

Table 1. Share of viruses in control of primary production

author	Primary production 'grazed' by viruses	Primary production grazed by zooplankton	location	Conditions
Steward 1996	23%	idem	Bering sea	Natural
Steward 1996	10%	idem	Chukchi sea	Natural
Wilson 2002a	< zooplankton	> viruses, 22%-39%	North sea	Natural
Guixa-Boixereu 2002	> zooplankton	< viruses	Antarctica	Natural

sea's (Steward *et al.* 1996), where bacterivory and viral lysis of prokaryotes were found to be equally important, are not supporting this concept.
It might not be possible to deduce a general rule from the hypothesis as mentioned above, yet it is an interesting thought which could help understand the ecological framework phages are in when competing with other organisms for the same food source.
An overview of phage impact as compared to grazer impact on primary production from the studies mentioned above is given in table 1.

Blooms (figure 4) of marine microalgae species like *Emiliania huxlei* are characterised by rapidly growing numbers of individuals reaching a population summit, which eventually crashes rather suddenly. Recently it has been accepted that these crashes are due to viral activity (Jacquet *et al.* 2002;Wilson *et al.* 2002b). Singh *et al.* (2004) propose an oscillation model in which viruses play a key role in a predator-prey (zooplankton-phytoplankton) dominated system of

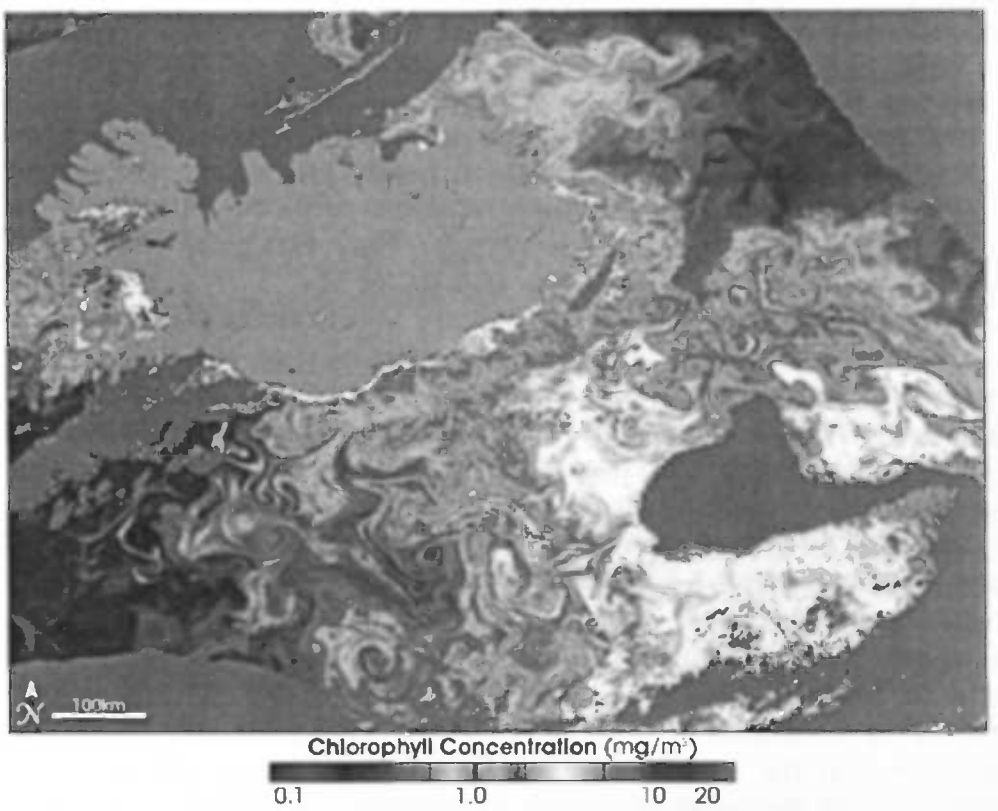

Fig 4. Bloom between Iceland and the Faroes. The 'black holes' are clouds through which the satellite is not able to record any data from below (Nasa Earth Observatory 2004).

planktonic bloom cycles. Their key assumption is that infected phytoplankton cells are more vulnerable to predation by zooplankton than are not-infected cells. At high infection frequencies the predation pressure will consequently increase. Within a range of infection frequencies, blooms then rise and fall as a result of simple, plausible sudden biological events like a variation in the rate of infection. Differences in observed natural bloom patterns can with this model be explained by differences in infection frequencies.

Nutrient Dynamics

Schroeder *et al.* (2003) suggest that nutrients do not have an effect on virus-host interactions in the coccolithophoroid *Emiliania huxlei*. Their experiment started off by exposing a bloom to a high diversity of viruses, but the termination of the bloom was always due to a single virus species. In different replicates, the same virus strain killed the bloom, irrespective the availability of nutrients P and N. They argue that if the nutrient conditions had affected host-virus interactions, one would expect that (a) different virus (-es) would be selected to succeed at different nutrient concentrations". This is not the case. Changes in nutrient concentrations however might influence the severity of an infection or other fluctuations within the same host-virus system. A similar conclusion was drawn by Jaquet *et al.* (2002) when they found that virus production by *E. huxlei* was delayed in N-depleted enclosures. The latter finding makes sense, as viral activity is invariably connected to host cell metabolism, which logically reacts to changing nutrient levels.

Corinaldesi *et al.* (2003) made an extensive survey concerning phage-host interactions in the Adriatic Sea. The density of sampling sites increased from the middle Adriatic towards the more eutrophic waters of the inner Gulf of Triest. Parameters were viral abundance, bacterial density, natural fluorescence, bacterial carbon production and nutrient concentrations (N, P) among others. Fluorescence indicated chlorophyll-a levels and may therefore be considered a measure for several species of algae and *Prochlorococcus*. The authors made an effort to correlate these many parameters in order to understand viral distribution. They conclude that "viral abundance depends on bacterial activity and on host-cell abundance." Their data probably contain excellent material to also say something about what factors may influence bacterial carbon production in the Adriatic Sea, and how viral abundance relates to this. Although the authors only explain the correlation's they found as effects of measured parameters on phages, their statistical approach doesn't exclude opposite causal connections. I would therefore like to be equally bold and use the same results to underpin the concept that bacterial activity increases as a result of viral abundance that speeds up the nutrients and organic carbon cycle by cell-lysions. This concept has been indicated before, e.g. by Gobler *et al.* (1997). They found that N and P levels increased as result of viral culling of a microalgal bloom. This increase consequently relieved diatom nutrient limitation.

As for bacteria, it has been shown that virus-bacteria ratio's (VBR) are higher in nutrient-rich environments (Bratbak and Heldal 1995;Wommack and Colwell 2000), suggesting an effect of nutrients. Both positive (Middelboe *et al.* 2002) and negative (Wilson *et al.* 2002a) correlation's between bacterial production and VBR have been found. Most remarkably, both results were explained as clues for a

beneficial effect of productivity on viral activity: the positive correlation assumes all life cycle stages, including free virus particles, to increase when livelyhoods amend. However the negative correlation was explained as due to a higher rate of viral infection and therefore lower numbers of free virus particles. In general, the relevance of viruses in aquatic systems is considered to increase with productivity (Danovaro *et al.* 2003;Guixa-Boixereu *et al.* 2002).
Winter et al (2004) found an indication that in the two more coastal of their three North Sea sampling sites, bacterial abundance might be higher in the light period, whereas in the more off-shore location, bacterial abundance hardly seems to fluctuate during the 24 hours period. Although these differences statistically appeared undetectable, they might nevertheless have been a result of the principle of increased viral significance in resource-rich environments. It would be logical to find a distinct decrease of bacterial abundance during the night when the main lytic events take place, in sites where viral impact on bacterial populations is greatest.

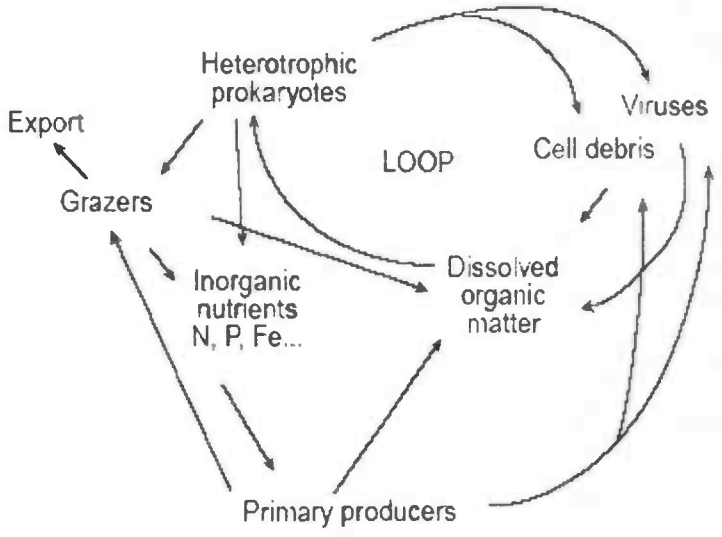

Fig 5.The planktonic viral loop. Schematic diagram of an aquatic food web, emphasizing the semi-closed loop connecting prokaryotes, viruses and DOM (curved lines). Note that the loop has the net effect of converting organic matter into dissolved inorganic nutrients. The viruses and cell debris are separated for illustration, but are often defined operationally as DOM (from: Fuhrman 1999).

The question remains whether this effect is direct or indirect. As mentioned earlier, some studies claim that viral activity is primarily stimulated by bacterial activity rather than by productivity (Corinaldesi *et al.* 2003), some argue that viral activity is inherently dependent on the activity of its host (Guixa-Boixereu *et al.* 2002). Winter *et al.* (2004) found that estimated burst size increased with increasing productivity. Clearly, one would therefore expect bacterial activity to be positively correlated to productivity.

Diurnal Patterns

An implication of lysogeny in oligotrophic environments could be that temperate phages in a lysogenic state first induce the lytic cycle when growth conditions for

cyanobacteria are at or around an optimum. Winter *et al.* (2004) showed that viral lysis of bacterioplankton in the north sea took place during noon and afternoon, whereas infection mainly took place during the night. Lysis was assessed by the frequency of infected cells (FIC), which was considered to be at a minimum after a major lysis event. FIC showed to be negatively correlated to bacterial activity, indicating that the temperate viruses induce the lytic cycle when the activity of their host is at an optimum, which would increase the number of progeny. Obviously for cyanobacteria, this is during the daytime. These results are in contrast to the earlier ideas that favoured viral infection, rather than lysis, at the bacterial physiological optimum (Wommack and Colwell 2000). More active bacterial cells may be more susceptible to viral infection than less active ones, and thus a more active bacterial community may sustain higher viral abundance (Corinaldesi *et al.* 2003;Steward *et al.* 1996).
Apart from host-cell growth conditions, also sunlight-induced DNA damage might well be an explanation for the dial patterns in infection and lysis events. The highest concentrations of phages are found in the surface layer (Wilson *et al.* 2002a) and one of the factors regulating viral abundance is genome damage by sunlight. Prophages have the benefit of having the host metabolism at their disposal, which can facilitate DNA-repair. A lysogenic state during daytime therefore protects the phage from being destroyed by sunlight. At night, when 'the coast is clear' and viral numbers have increased within their hosts by high cell activity and replication, the lytic cycle is induced and new virus particles spread out in the plankton to find new hosts.
Understanding phage dynamics as a function of light intensity in the (ant-) arctic, where variation in light intensity follows an annual rather than a diurnal pattern, is a whole different ball game. In two of three Antarctic sampling sites where dial examinations were carried out (Guixa-Boixereu *et al.* 2002), VLP (virus-like particle) abundance was lowest at maximal light intensities. This might indicate a high FIC, where viruses 'hide' and proliferate as a part of their host genome, awaiting more suitable light intensities for dispersion. Both dial and annual fluctuations were rather low.
It was also shown for some microalgae (e.g. *Phaeocystis pouchetti* & *Micromonas pusilla*) to be less sensitive to UVB radiation when grown in culture together with viruses (Jacquet and Bratbak 2003). Apparently both parties of the parasitic pair may benefit from infection by protection against excessively high light intensities. In nature, high FIC values coincide with the highest light intensities over a given time period. It thus seems that, if the costs of infection (Juneau *et al.* 2003) are lower than those of photodamage to phytoplankton, phages and photosynthesists can be involved in a mutualistic relation to survive the, at times blindingly luminous, habitat of the open sea. Whatever the mechanisms cause the dial pattern of virus dynamics, it has to be taken into consideration when sampling the ocean for it. Ignoring the diurnal shifts in abundance, infection rates etc. might seriously blur your data (Winter *et al.* 2004).

Photorehabilitation

One of the constraints to cyanobacterial growth in open oceans is light (Mann 2003). This might seem a bit paradoxal, as light is also the immediate life and growth giving factor for these organisms. That is, when it comes at appropriate

levels. At extreme intensities sunlight can turn in to a peril for cyanobacteria. When irradiance levels are too high, the light-capturing molecule of some cyanobacteria is destroyed, resulting in a decreased growth rate and less assimilation of CO_2 into carbohydrates. At least one temperate phage is now known to contain DNA that encodes for the sections of the light-capturing complex of its host that are destroyed by a surplus of UV-radiation leading to photoinhibition (Mann *et al.* 2003). The light-affected sections of the complex are called D1 & D2 and are part of photosystem II. After lysogenisation, the part of the prophage genome that encodes for these sections is transcribed in addition to the usual amount of host-cell genome transcribed, producing a surplus of D1 & D2 proteins. The prophage thus reduces photoinhibition by 'outnumbering' the UV-damage to the host cell. As a result the host cell continues to grow to the benefit of the virus.
It will be tempting to argue that this way viral infection could enhance picophytoplankton growth, primary production and carbon fixation. But one then assumes that a higher growth rate due to phage infection is beneficial to the bacteria as well. It would be odd if the cyanobacterium has not evolved to overcome photoinhibition likewise by its own means. We should therefore expect there to be a cost to permanently transcribing more D1 & D2 than is the case for the uninfected bacterium, or perhaps to avoiding photoinhibition as such. Likewise, the bacteria might have co-evolved in presence of the phage, in circumstances where infection rates were perhaps so high that the extra bacterial D1 & D2 genes were 'traded off' for something more urgent.
One study (Juneau *et al.* 2003) revealed that although the overall effect of viral infection impaired photosynthetic activity in the bloom-forming microalga *Heterosigma akashiwo*, the activity of photosystem II was least affected of all the enzymes involved in photosynthesis. In this study PAM (pulse-amplitude-modulated)-fluorescence was used to assess phytoplankton photosynthetic activity. Note that this study was performed on a eukaryote, whereas the discovery of the viral photosynthesis genes concerned a prokaryotic phage-host system. Nevertheless it is a striking match, not in the least because both infections relieve photosystem II from photoinhibition.

Host Specifity

Several studies stress the host-specific nature of viruses. Schroeder *et al.* (2003) revealed that, although several virus genotypes were present at the start of a bloom of *Emiliania huxleyi*, only a few were considered responsible for the final killing of the bloom. An opposite experiment, involving several hosts but only one infection agent, also advocated host-specificity: Of 8 strains of phytoplankton only one, *Gymodinium mikimot*, was suppressed in its growth in the presence of a single type of VLP (Onji *et al.* 2003). These complementary findings could support a concept of specific natural host-parasite couples (figure 6). This might also enhance the possibility of coevolution of hosts and viruses when genomes are optimally adapted to infection by/of the well-known organism, optimising the benefits to the viruses and minimising the costs to the hosts. One should also bear in mind that infected cells are not susceptible to infection anymore. If these hypotheses hold true, it will be extremely difficult for a mutant virus to establish a population, even though it might technically very well be able to infect the host from an existant host-virus couple. The mutant virus will not reproduce at the

same efficiency as does the virus species that already is involved with the host for some time. In addition, the new virus might cause quicker death upon its host, thus depriving itself from its own resource. On the other hand, however, a new mutant virus might just as well have an enormous evolutionary benefit over its host-specific collegue, as the infectee does not have a defense mechanism set out to fight the brand new infecter. If not a hypothetical situation like this is an evolutionary stable alternative strategy, it might well be the precedent to another new host-parasite system under construction.

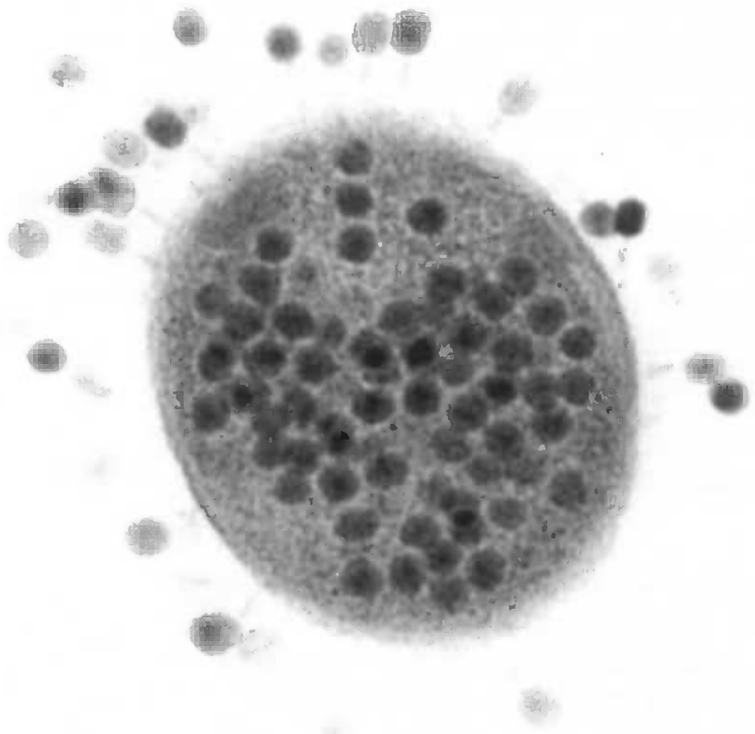

Fig 6. Hosts and parasites: Thin section of T4 phages hitting a microcolony of E. coli K-12 (Wertz 2004)

Conclusion

In temperate seas phages appear to have less impact on picophytoplankton than do eukaryote grazers, whereas in the polar regions there are indications that phages might be the main regulating organisms or at least equal to grazers. A possible explanation is that regeneration time of eukaryote grazers in the cold seas might be lower than that of its prokaryotic prey, making it hard to anticipate on prey population oscillations, whereas phage regeneration time is directly coupled to that of its host.

Furthermore there seems to be an indirect coupling between system productivity and phage activity. Phage activity is considered to be dependent on bacterial activity, which in its turn may be expected to be dependent on productivity. It has been shown that the effect of viral lysis on bacterial populations increases with productivity. However one might also expect phage activity to affect productivity, as lysis products (DOC, DOM, nutrients) are released back into the environment and made available again to biological assimilation.

Phototrophic plankton species, prokaryotic as well as eukaryotic, and viruses seem to be troubled by excess UV-radiation. Yet it is shown for a cyanobacterium species infected with a specific phage to have compensated photoinhibition damage. Moreover, the infected cell increased its production. Also several eukaryotic algae seem to benefit from viral infection when exposed to excessive light regimes. Lysogenic viruses on the other hand find themselves protected by cytoplasm metabolism, including DNA-repair mechanisms, at high levels of UV radiation. These findings suggest a mutualistic relationship between phages and photosynthetic plankton.

In natural systems it seems that temperate phages 'hide' from light stress by residing in a host cell during the most illuminated time periods. This was found in the arctic as well as in temperate waters. The vulnerable dispersion and reinfection phase of phages following cell-lysis appears to be bound to the night, when light intensities are low. This results in a distinct diurnal cycle of lysogenisation and lysis.

Host specifity is found from the perspective of both hosts and parasites. It is likely that viruses and hosts can be categorised into fixed host-parasite couples, but so far this idea remains a concept.

The discussion on the role of the viral loop in primary production covers several overlapping aspects in the planktonic ecosystem. At first, there are two major groups of primary producers: cyanobacteria (prokaryote) and microalgae (eukaryote). Secondly, bacteriophages infect not only photosynthetic bacteria, but also heterotrophic bacterioplankton. Finally, DOC derived by viral lysis from heterotrophic marine bacteria can be considered recyclable organic carbon as it can be reassimilated by the same trophic group, but this is not the case for cyanobacteria-derived DOC. This line of reasoning would suggest a purely predatory effect of phages on any kind of phytoplankton, unless photoautotrophic plankton switches to organic carbon sources during the night, nutrients derived from viral

lysis enhance phytoplankton growth, or levels of dissolved CO_2 increase when growth of heterotrophic bacteria is boosted by increased levels of DOC from viral lysis. The last possibility involves an additional step in the recycling of carbon.
Bearing this in mind, there is a lot more research required to clear up the oceanic carbon and nutrient flows.

Glossary

Burst size: number of virus particles released at lysis
Coccolithophore: small motile golden algae (Chrysophyta or Haptophyta), having calcareous plates (coccoliths) covering the cells
Cyanobacteria: aquatic prokaryote photosynthesists
Diatom: group of algea characterised by thin double shells of silicia
DOC: dissolved organic carbon
DOM: dissolved organic material
dsDNA: double-stranded deoxyribonucleic acid
Eutrophic: nutrient-rich
FIC: frequency of infected cells
Lysis: see 'viral lysis'
Lysogen: infected cell with phage in dormant state (prophage)
Lytic cycle: cycle of infection, phage or virus proliferation, cell burst and VLP-dispersion
Microalgea: aquatic eukaryote photsynthesists
Oligotrophic: nutrient-poor
Picoplankton: prokaryote plankton, including cyanobacteria and heterotrophs
dsRNA: double-stranded ribonucleic acid
ssRNA: single-stranded ribonucleic acid
Phage: bacteriophage, bacterial virus
Phycobilisome: small particle present present on photosynthetic lamellae of some red algae and cyanobacteria, and which contain phycobilin like phycocyanin and phycoerythrin
Phycocyanin: accessory photosynthetic pigment
Phycoerythrin: accessory photosynthetic pigment
Phytoplankton: unicellular photosynthetic plankton, including microalgea and cyanobacteria (Henderson's 1995)
Polynya: year-round ice-free surface water within pack-ice zone. Arctic 'hole-in-the-ice' due to subsea currents
Primary production: generation of organic substance from inorganic material. E.g. phototrophs turn carbon-dioxide into carbohydrates
Prophage: viral genome in the lysogenic state that is incorporated into the host cell genome
VBR: virus-bacteria ratio
Viral lysis: cell lysis (death) as a result of viral infection. Cell contents, including virus progeny, are released into the environment
VLP: virus-like particle
Zooplankton: aquatic heterotrophic organisms of the surface waters

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