APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1990, p. 1400–1405
0099-2240/90/051400-06$02.00/0

Vol. 56, No. 5

Viruses as Partners in Spring Bloom Microbial Trophodynamics

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Received 11 December 1989/Accepted 12 February 1990

Population sizes of algae, bacteria, heterotrophic flagellates, and viruses were observed through the 1989 spring diatom bloom in Raunefjorden in western Norway. The culmination of the diatom bloom was followed by a peak in the concentration of bacteria and an increase in the concentration of heterotrophic flagellates, a pattern consistent with the concept of a food chain from photosynthetically produced organic material, through bacteria, to bacterivorous flagellates. The concentration of viruses varied through the spring bloom from 5×10^5 in the prebloom situation to a maximum of 1.3×10^7 viruses ml^{-1} 1 week after the peak of the diatom bloom. Coinciding with the collapse in the diatom bloom, a succession of bacteria and viruses was observed in the mucous layer surrounding dead or senescent diatoms, with an estimated maximum of 23% of the total virus population attached to the diatoms. The dynamic behavior observed for the virus population rules out the possibility that it is dominated by inactive species, and the viruses are suggested to be active members of the microbial food web as agents causing lysis in parts of the bacterial population, diverting part of the bacterial production from the predatory food chain.

In the last decade, a lot of effort has been put into the development of a detailed concept of how carbon and nutrients flow between the different components in marine microbial food webs. In general discussions (4, 17, 31), the groups of microbial organisms considered as important trophodynamic partners are bacteria, protozoans, and phytoplankton. Additional complexity has been added to the concept of the microbial food web by the inclusion of pico-sized phytoplankton (12, 23) and by the possible importance of phagotrophic algae (14, 26).

Many studies have confirmed a temporal distribution of microorganisms in aquatic ecosystems, with bacteria succeeding phytoplankton blooms and with protozoans succeeding the bacteria (2, 3, 6, 20). These successional patterns support a concept by which bacteria live on particulate or dissolved organic material which either originates from phytoplankton directly or is released by activities closely connected to the phytoplankton, while the protozooplankton feed on small-sized organisms. These small-sized organisms may be heterotrophic bacteria, cyanobacteria, or small eucaryotic cells.

The completeness of this concept, however, may be questioned as recent reports show that the abundance of viruses in marine environments is much higher than thought previously (5, 25, 30). The concentration of viruses may in principle be caused by a mechanism whereby long-lasting viruses rarely interact with the cellular organisms of the system. In this case, a high viral number would be of minor consequence to the population dynamics and the material flows in the microbial ecosystem. If, however, large temporal fluctuations in the virus population are correlated to other fluctuations in the microbial community, this would indicate the existence of a strong dynamic coupling between the viruses and the cellular organisms in the microbial food web. A further question would then be whether this is a one-way relationship only, with fluctuations in the system of cellular organisms driving the fluctuations in viruses, or a two-way relationship in which the frequency of viral infections is sufficiently large to influence the dynamics quantitatively and the material flows between the cellular organisms.

To test the hypothesis of large dynamic variations in the density of pelagic viruses, we have taken advantage of the spring diatom bloom which occurs regularly in Norwegian waters (27), causing large natural fluctuations in the populations of cellular organisms in the microbial food web.

MATERIALS AND METHODS

The samples were taken at 1.5-m depth with a Ruttner water sampler in Raunefjorden (60°16.2'N, 5°12.5'E), south of Bergen, Norway (Fig. 1).

The samples were preserved with 2.5% glutaraldehyde (final concentration). Counts of eucaryotic and procaryotic organisms were obtained by epifluorescence microscopy after double staining with 4',6-diamidino-2-phenylindole (24) and primulin (11). This technique, by which the cellular material is stained with primulin, allows eucaryotic organisms to be identified by the presence of a 4',6-diamidino-2-phenylindole-stained nucleus, autotrophs to be identified on the basis of autofluorescence from chlorophyll, and phycoerythrin-containing cyanobacteria to be identified on the basis of phycoerythrin autofluorescence. Phototrophs were classified as diatoms, phycoerythrin-containing cyanobacteria, or "other autotrophs." Bacteria (i.e., nonphotosynthetic) were recognized by their size and shape. Viruses were counted in a transmission electron microscope (TEM) as described by Bergh et al. (5). Water samples were filled in centrifuge tubes with plastic-molded flat bottoms covered by cellulose nitrate filters (Sartorius). Electron microscope grids with carbon-coated Formvar film were then dropped through the water column onto the filters, and particles in the samples were collected on the grids by centrifugation for 2.5 h at $100{,}000 \times g$ in a swing-out rotor. After sucking off the water, the grids were removed from the centrifuge tubes by pulling out the filters with a forceps. The concentration of particles (i.e., bacteria and viruses) in the water sample was calculated by dividing the number of particles counted per unit area on the grid by $(r_{max}^2 - r_{min}^2)/2r_{max}$, where r_{max} is the maximum and r_{min} is the minimum radial distance of the water sample during centrifugation. Bacteria and viruses were sized in the TEM, and their volumes were calculated as

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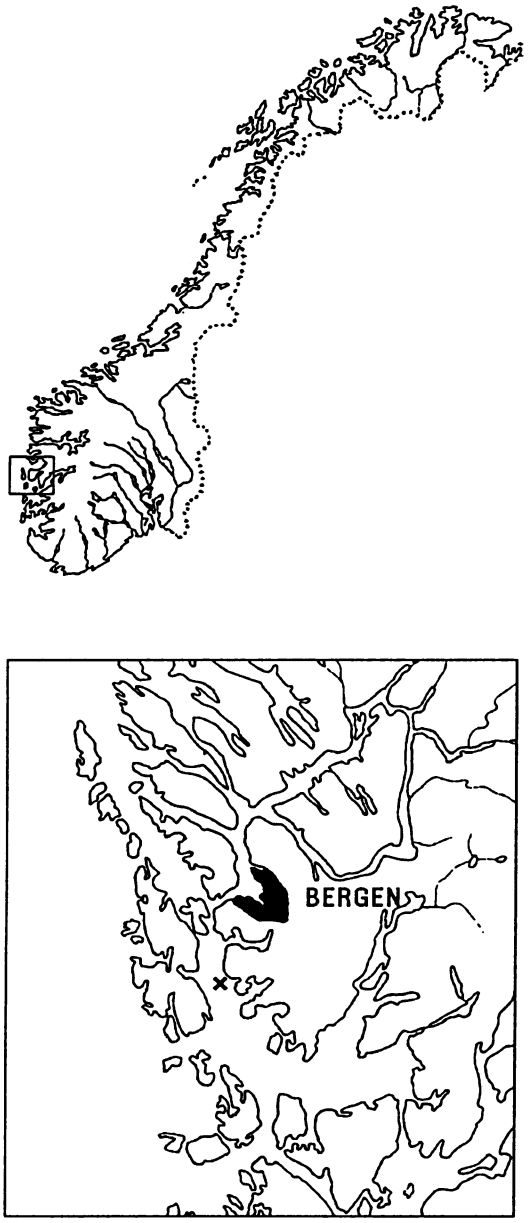

FIG. 1. Map showing the location of the sampling station (×) in Raunefjorden in western Norway.

spheres and as cylinders with hemispherical ends. The relative distribution of bacteria, classified as rods, curved rods, or cocci, and the frequency of dividing cells were recorded in the TEM.

The uncertainties of the concentrations of viruses and cellular organisms given in the text are based on counting statistics.

RESULTS

Spring bloom plankton dynamics. The diatom bloom (hereafter called "the bloom") commenced in early March with a near-exponential increase in cell number until a level of (11.4 ± 0.9) × 10^3 cells ml^{-1} was reached on 17 March (Fig. 2a).

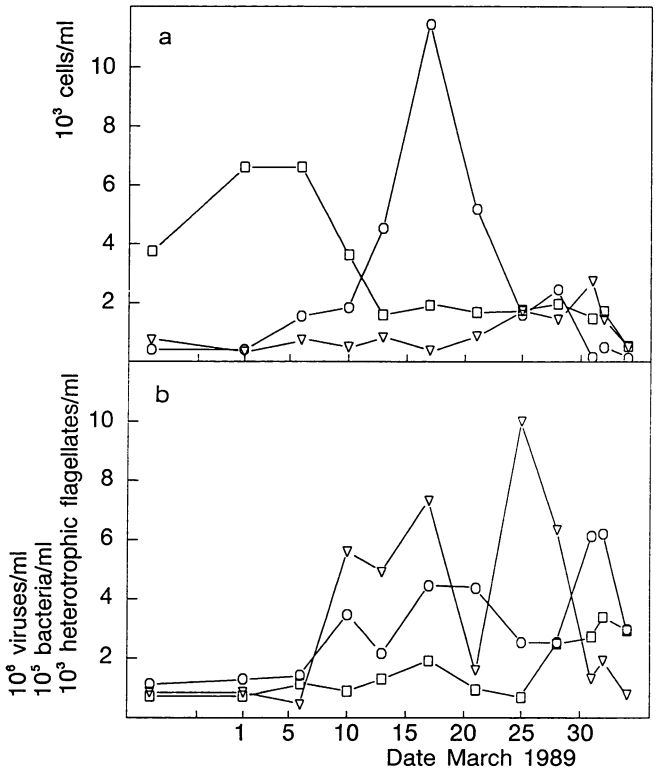

FIG. 2. Autotrophic organisms (a) and heterotrophic organisms and viruses (b) in spring bloom. (a) Diatoms (○); phycoerythrin-containing cyanobacteria (▽), other autotrophs (□). (b) Bacteria (○); heterotrophic flagellates (□); and free viruses (▽).

The population was then strongly dominated by *Skeletonema costatum*, but other diatoms including *Chaetoceros* sp., *Arcocellulus cornucervis*, and several unidentified pennate forms were also present. A rapid subsequent decline brought the diatom concentration back to prebloom levels at the end of March. The population of other autotrophs declined during the bloom from a level of about 7 × 10^3 ml^{-1} to about 2 × 10^3 ml^{-1} during and after the bloom. The population of phycoerythrin-containing cyanobacteria was small (about 0.5 × 10^3 ml^{-1}) until the collapse of the bloom, after which it increased and reached a low maximum of (2.7 ± 0.4) × 10^3 ml^{-1} at the end of March.

Apart from minor fluctuations, the population density of bacteria (Fig. 2b) increased during and after the bloom with one maximum ([4.4 ± 0.3] × 10^5 ml^{-1}) coinciding with the culmination of the bloom on 17 March and a second maximum ([6.2 ± 0.3] × 10^5 ml^{-1}) on 1 April. Classification of the bacterial population into morphological groups (Fig. 3) revealed that most of the variation in bacterial numbers was due to variation in the number of rods and cocci. The number of curved rods showed little variation during the observed period (Fig. 3). The average bacterial cell volume was about 0.06 μm^3 during the whole bloom period but increased as the bloom culminated and reached a maximum of 0.17 μm^3 on 28 April, i.e., before the second maximum in number of bacteria. The biomass of the bacterial population was, in terms of biovolume, relatively constant during the bloom (about 2 × 10^4 μm^3 ml^{-1}). A single maximum in bacterial biomass (Fig. 4) (7.8 × 10^4 μm^3 ml^{-1}) coincided with the second maximum in number of bacteria (Fig. 2b).

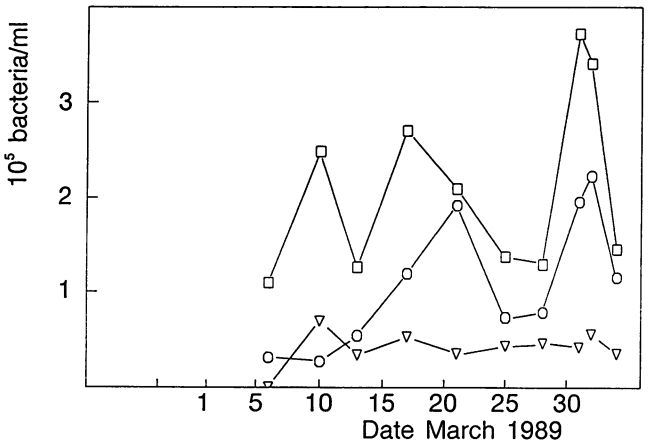

FIG. 3. Number of different morphological forms of bacteria: cocci (○), rods (□), and curved rods (▽).

The frequency of dividing rods increased during the bloom from <10% to a maximum value of 28% on 21 March and then decreased to 10 to 15% at the end of the month. The maximum frequency of dividing cells was thus observed between the first and second maxima in bacterial numbers (Fig. 2b). It should also be noted that the frequency of dividing cells for rods was increasing while the number of rods was decreasing between 17 and 21 March (Fig. 3).

From the low prebloom density ([0.7 ± 0.2] × 10^3 ml^{-1}), the population of heterotrophic flagellates (Fig. 2b) increased slightly during the bloom and more rapidly from 25 March on, as the bloom had collapsed, with a maximum observed density of (3.4 ± 0.4) × 10^3 ml^{-1} on 1 April.

From low values in the range 5 × 10^5 to 9 × 10^5 ml^{-1} in the prebloom situation, the density of free viruses (Fig. 2b) increased with about 1 order of magnitude between 6 and 10 March, i.e., in the early phase of the bloom, reaching (7.3 ± 0.7) × 10^6 ml^{-1} on 17 March, the day of the observed maximum diatom concentration. Following a low value on 21 March, the maximum number of free viruses of (1.0 ± 0.1) × 10^7 ml^{-1} was reached on 25 March, coinciding with the observation of dense populations of viruses in the slime surrounding dead or senescent diatoms (see below). The number of possible host organisms present in the system suggests to us that the viruses mainly were bacteriophages.

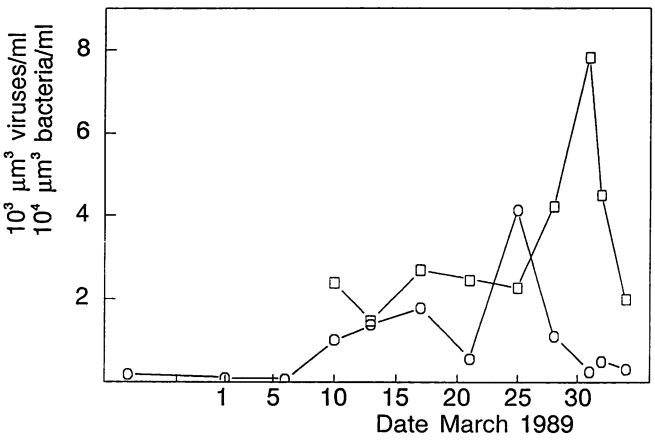

FIG. 4. Biovolume of bacteria (□) and viruses (○).

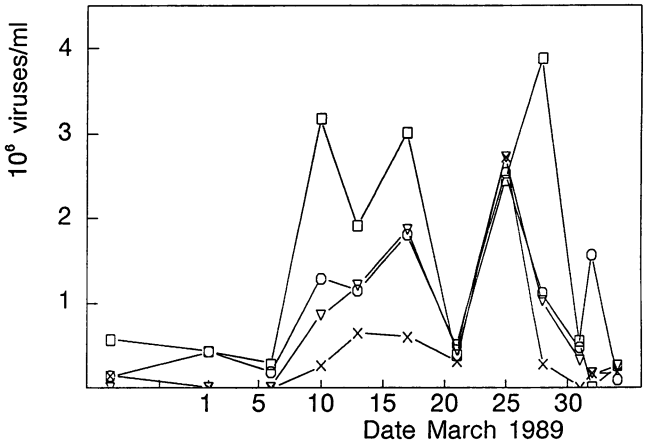

FIG. 5. Number of viruses in different size groups: head length and width of <60 nm (□), 60 to 80 nm (○), 80 to 100 nm (▽), >100 nm (×). The average headsizes in the smallest and in the largest size groups were about 50 and 120 nm, respectively.

Morphologically, phages without visible tails (Bradley group E) were dominating, but phages with short tails (Bradley group C), long noncontractile tails (Bradley group B), and long contractile tails (Bradley group A) were also present (9). As a general pattern, the virus population was dominated by smaller forms, although exceptions were found on some dates (Fig. 5).

The biovolume of the virus population was generally between 1 and 2 orders of magnitude less than that of bacteria (Fig. 4). A single maximum of 4 × 10^4 μm^3 ml^{-1} was found on 25 March, coinciding with the maximum number of viruses. On this date, the virus biovolume was 18% of the bacterial biovolume but only 5% of the maximum bacterial biovolume (31 March).

Diatom slime subsystem. The diatom population appeared to be healthy when the bloom culminated. Four days later (21 March), as the bloom collapsed, we could observe in both the epifluorescence microscope and the TEM that the diatoms were colonized by bacteria. The bacteria appeared to be immobilized in an extracellular slime layer surrounding the diatoms (Fig. 6a). The number of bacteria in the slime surrounding the *Skeletonema* cells was estimated to be 60 bacteria per cell. The bacteria were classified in three morphological groups: rods (48%), curved rods (13%), and cocci (39%).

The bacterial colonization of dead or senescent diatoms was observed in the TEM to be succeeded by a dense population of viruses also located in slime surrounding the diatoms (25 March) (Fig. 6b, c, and d). Some 70% of the *Skeletonema* population had extracellular slime, and the slime contained 40 bacteria and approximately 2,500 bacteriophages per *Skeletonema* cell. The decrease in number of bacteria in the slime was due to a decimation of the cocci population alone as the number of rods and curved rods per *Skeletonema* cell was unchanged. The phage population was homogeneous in morphology and size (Fig. 6b); all had long noncontractile tails and could be assigned to Bradley group B (9). The length and width of phage heads and tail lengths were 119 ± 10, 91 ± 8, and 142 ± 16 nm (mean ± standard deviation). These results indicate that a single phage-host system was dominating and that the host bacteria were cocci.

Extracellular slime containing bacteria and bacteriophages

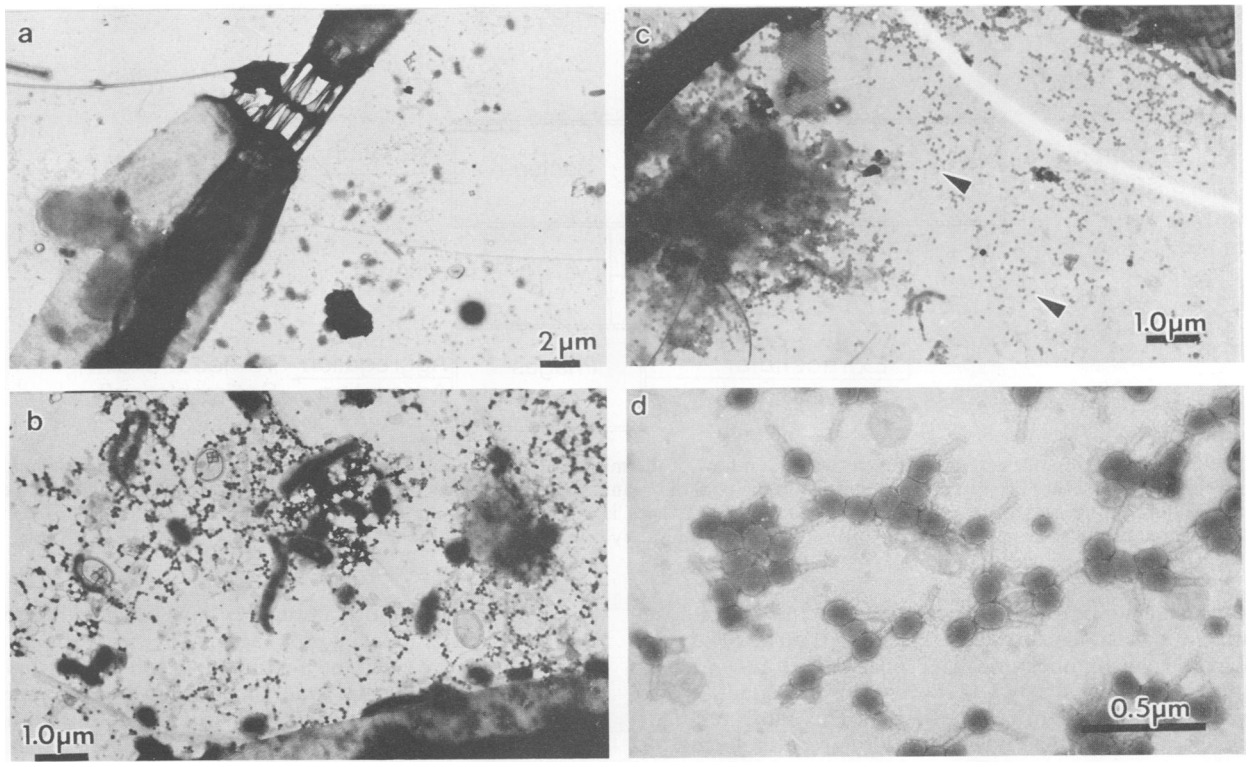

FIG. 6. Diatom slime subsystem. (a) *S. costatum* with slime layer infected with bacteria (21 March). (b) *S. costatum* slime layer with bacteria and viruses (25 March). (c) *Chaetoceros* sp. slime layer with viruses (arrowheads) (25 March). (d) Viruses in *Skeletonema* slime layer showing details of heads and tails (25 March).

could be observed for all diatom species (Fig. 6c and d). The phages involved were similar in size and morphology to the phages described for the *Skeletonema* cells (Fig. 6b). Flagellates (photo- or heterotrophic), however, had no apparent extracellular material and they were not colonized by bacteria.

From knowledge of the concentration of diatoms based on epifluorescence counts and the number of attached viruses per diatom obtained from electron micrographs, we estimate the number of particle-bound viruses to be 0.3×10^7 ml^{-1} on 25 March. The total number of viruses (free plus attached) was thus 1.3×10^7 ml^{-1} on this date.

On 6 April, samples were collected at several depths down to 30 m to track the remains of the spring bloom. In these samples, we found only empty diatom frustules with signs of neither extracellular slime nor attached bacteria or viruses.

DISCUSSION

The main impression obtained from the successional pattern observed for cellular microorganisms was that of a conventional spring bloom with some bacterial growth occurring parallel to the growth of the diatoms and then a succession of bacteria and heterotrophic flagellates following the rapid decline of the diatom population. During the time span of approximately 1 month during which this spring bloom was observed, the number of free viruses varied by a factor of >30. Similar large relative variations in number of phages, as determined by PFU using *Agrobacterium stellulatum* as host, have been observed in Kiel Bay by Ahrens (1). In our opinion, the data therefore strongly suggest a close dynamic coupling from the food web of cellular microorganisms to the virus population. The alternative possibility, that the variation in the virus population was caused by drifting of different water masses, is not supported by concomitant changes in the populations of cellular organisms.

The number of small (1 to 6 µm in diameter) autotrophic and heterotrophic organisms was possibly underestimated as parallel flow cytometric countings of unfixated samples (data not shown) gave significantly higher numbers. This observation is in agreement with studies demonstrating that fixation of water samples may cause serious loss of cells (7, 19). Our results, as well as others based on counting of fixated samples, should thus be interpreted with care.

From 21 to 25 March, there was a reduction in the population of free bacteria of 1.8×10^5 bacteria ml^{-1}, accompanied by an increase in free viruses of 8×10^6 viruses ml^{-1}, from which an apparent burst size of about 50 can be estimated. From counts of cocci and phage in the extracellular slime, we have estimated a burst size of approximately 120 phages per bacterium. Both of these values are reasonable compared with burst sizes of cultured phages (1, 22, 33). It is obvious that any attempt to estimate burst sizes of free viruses is subject to uncertainties: loss rates of viruses are unknown, and the bacterial population density may, in addition to lysis, also have changed due to growth and predation. The burst size estimated for the cocci in the extracellular slime is probably more reliable, as the number of rods and curved rods in the slime was constant, indicating that the decrease in number of cocci was caused by lysis only.

The average volumes of phage and cocci in the slime were

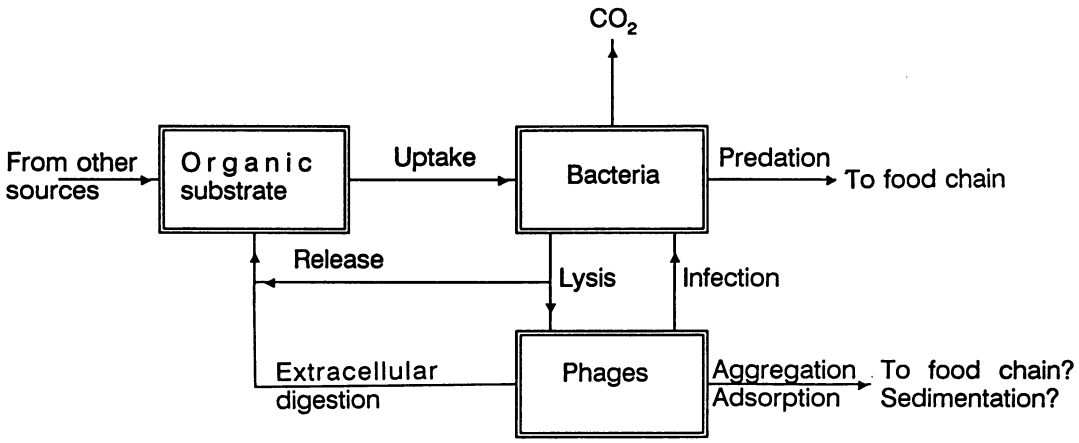

FIG. 7. Potential role of bacteriophages in the carbon flow of the microbial food web as discussed in the text. Organic material and cell debris released by lysis are proposed to be returned to the pool of organic substrates for bacteria together with material from phages digested by extracellular enzymes. Apart from digestion, removal of phages is proposed to occur through aggregation or adsorption to nonhost particles. Some of the viral nucleic acid is also returned to bacteria by reinfection.

6×10^{-4} and 0.05 μm^3, respectively, and the phage-host volume ratio was thus about 1%. This ratio is 50 to 200 times the phage-host volume ratio of T phages and *Escherichia coli*, having volumes of 0.5×10^{-4} to 2×10^{-4} (data from reference 22) and 1 μm^3, respectively. This indicates that phages parasitizing small marine bacteria in natural ecosystems may have a much higher production efficiency than phages growing on large bacteria under laboratory conditions. For calculation of phage volume, we included the phage head only, as the tails, by volume, make up <20% of the head (data from reference 22).

From the burst size and the average volume of bacteria (on 21 March) and phages (on 25 March), we may calculate that the volume of the phages produced in the slime was 1.4 times the volume of the cocci disappearing. In terms of dry matter, this production factor increases to 1.8 if the bacteria and the phages are assumed to have a density of 1.1 and 1.4 g cm^{-1}, respectively (10, 21). With an average DNA content of 2.6 fg in the bacteria (16) and 0.08 fg in phages (8), we may calculate that the phages produced in the slime have a DNA content that is 3.8 times that of the cocci disappearing. The corresponding factors that may be calculated for free bacteria and phages are 0.34 (volume), 0.43 (dry-matter content), and 1.4 (DNA). These results are in accordance with laboratory experiments showing that most of the material used for phage DNA and protein production is assimilated by the bacteria after infection (28). A substantial fraction of the original bacterial biomass may thus be released as dissolved material during lysis, in addition to the newly synthesized phage biomass.

The volume of the free phage population increased by about 3,600 μm^3 ml^{-1} between 21 and 25 March, while the volume of the bacterial population only decreased by about 1,900 μm^3 ml^{-1} (Fig. 4). The reason for the small decrease in volume of the bacterial population, compared with the increase in the phage population, is that the average cell volume of the bacteria increased and in part replaced the volume lost as the number of bacteria decreased by about 40%.

The first increase in viruses between 6 and 10 March coincided with the initiation of the bloom (Fig. 2a and b). It is not reasonable to assume any cause-effect relationship between these two events. A common cause may exist, however; increased irradiation may have initiated the spring bloom as well as induced prophages. Whether this also may explain the decrease in the population of other autotrophs in this period is uncertain. One should note that there is no decline in bacterial concentration in conjunction with the first increase in viruses between 6 and 10 March. Also, the rapid decline of rods and cocci towards the end of the sampling period was not accompanied by any increase in virus concentration (Fig. 2b). This decline in bacteria is thus more easily explained as a result of predation from the then more numerous population of heterotrophic flagellates.

On six occasions throughout this study we observed that the virus population was decreasing (Fig. 2b). The rate constant (k) for each decrease was calculated as $k = [\ln (C_t/C_0)]/t$, where C_0 is the initial virus concentration and C_t is the virus concentration at time t; they were found to range from 0.04 to 0.5 day^{-1}, with a mean value of 0.28 day^{-1}. These rates are minimum estimates as the simultaneous virus production rate is unknown. They are comparable, however, to the rates of decrease in total count of viruses observed when natural seawater is incubated in the laboratory (range, 0.02 to 0.89 day^{-1}; mean, 0.33 day^{-1}) (8). At present, we do not know the fate of the virus particles. Loss of structural integrity because of physical or chemical factors or extracellular enzymatic activity is one possibility. It is also possible that they form aggregates or adsorb nonspecifically to different particles sufficiently large to sediment out of the water column or so rare that they are not included in our counting. These and similar models assuming physical interaction between particles, however, cannot explain the very low total number of viruses observed in some waters (5).

The rate of inactivation of bacteriophages in seawater determined on the basis of PFU for different phage-host systems is reported in the literature to be in range of 0.1 to 2.3 day^{-1}, with a mean of 0.8 day^{-1} (1, 32; data reviewed in reference 18). The physical, chemical, and biological factors possibly controlling virus inactivation in seawater include temperature, solar radiation, adsorption, heavy metals, salinity, organic chelators, bacterial activity, algae, and protozoa (18). The rate of inactivation (i.e., ability to infect and form plaques) appears on the average to be about three times as high as the rate of disappearance (i.e., becoming unde-

tectable in the TEM). This comparison is uncertain, however, as the phages for which the rate of inactivation in seawater is determined in most cases are allochthonous to the marine environment and thus may have a rate of inactivation different from that of autochthonous marine phages. The conclusion, nevertheless, is that phages in marine waters are not very persistent as free particles. With the fact in mind that >90% of all known phages are temperate (15), this suggests that marine phages in general are temperate and not lytic.

With the proposed mechanism of phages causing cell lysis and changes in the bacterial abundance, the effects on microbial trophodynamics may be a kind of short circuit of the bacterial production (Fig. 7). A substantial fraction of the bacterial biomass production will not be available to phagotrophic predators if released into the surrounding medium when the cells lyse. Bacterial cytoplasm and cell debris released during lysis may enter to the pool of dissolved organic material utilized by the bacteria. The organic material of the phages released may possibly also enter the pool of dissolved organic material after digestion by extracellular enzymes. By recycling organic material in a bacteria-phage-dissolved organic material loop, bacterial production may become very high and theoretically even exceed primary production (29). Observations of rapid bacterial growth and assimilation of organic material, combined with no transfer of this material to organisms in larger-size fractions (13), can be explained by this model. Phages may also be removed by aggregation and by adsorption to nonhost particles, and the phage biomass may thus become available to detritus feeders or sink out of the water column (Fig. 7). Some of the viral nucleic acid may also be returned to bacteria by reinfection. Adding the possibility that synthesis of viral DNA potentially may interfere with estimates of bacterial production based on thymidine incorporation, the proposed scenario is of great potential importance to the interpretation of bacterial production estimates.

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