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NOTE

Regeneration of dissolved organic matter by viral lysis in marine microbial communities

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ABSTRACT: The influence of viruses on bacterial net growth and respiration was investigated in batch cultures with natural assemblages of marine bacterioplankton, which were manipulated with respect to abundance of natural virioplankton. In 1 set of cultures (–virus), a virus-free water sample (0.02 µm filtered) was inoculated with a bacterioplankton concentrate, and in a parallel set of cultures (control) a virus-containing water sample (0.2 µm filtered) was inoculated with the bacterioplankton concentrate. The 0.02 µm filtration procedure reduced viral abundance by 62 to 92% in the –virus cultures relative to the parallel control cultures with the natural density of viruses (i.e. the fraction of natural viruses <0.2 µm). This approach allowed us to examine the effects of reduced viral densities on the production of natural assemblages of bacteria and viruses and on the distribution of added ^{3}H-thymidine into size fractions (the bacterial size fraction, viral size fraction, dissolved size fraction and respired fraction). The results showed significantly higher bacterial net growth and growth efficiency in cultures with a reduced abundance of viruses relative to control cultures with natural viral abundance, and indicated viral regulation of bacterial abundance in the control cultures. We suggest that viral lysis significantly affected the bacterial carbon cycling in the cultures by liberating a fraction of the organic matter already taken up by the bacteria, thus stimulating recycling of bacterial carbon and reducing the net bacterial production. The implications of such regeneration of dissolved organic matter by viral lysis for pelagic carbon cycling and for measurements of bacterial production are discussed.

KEY WORDS: Viruses · Bacterioplankton · Recycling · Dissolved organic matter

It is well established that viral lysis may be a significant bacterial loss factor (e.g. Fuhrman 1999) and contribute to 20 to 50% of bacterial mortality in pelagic systems (e.g. Fuhrman & Noble 1995). However, the influence of viruses on microbial food web dynamics is not only due to their role as agents of bacterial mortality but also the result of other processes associated with viral lysis. On one hand, viruses cause the death of their host cells (e.g. Fuhrman & Noble 1995, Steward et al. 1996), while on the other hand, viral activity produces dissolved organic carbon (DOC) from lysis of particles and thereby promotes the recycling of carbon and nutrients by bacteria in the water column (Middelboe et al. 1996, Gobler et al. 1997, Noble et al. 1999). While there is consensus regarding the effects of viruses on bacterial mortality, the role of viruses in pelagic carbon and nutrient cycling is poorly understood. It has been shown that DOC released by viral lysis of a bacterial host population can be an important substrate source stimulating the growth of non-infected bacterial populations (Middelboe et al. 1996). Consequently, viral activity tends to retain heterotrophic production and respiration in the bacterial size fraction and, thus, reduce the transfer of organic carbon to higher trophic levels. It is generally assumed that 10 to 30% of the bacterioplankton production is lost daily as a result of viral lysis (e.g. Fuhrman 1999); hence, a large fraction of the bacterial production may be released as dissolved organic matter (DOM). It is likely, therefore, that the bacterial production is in part sustained by recycling of viral lysates and that a significant fraction of the total heterotrophic respiration in the marine plankton is the result of such recycling of viral lysates by bacteria (Fuhrman 1992, 1999). In an attempt to examine the potential impact of viral lysis on planktonic community respiration, Fuhrman (1992) modeled the carbon flux in the microbial food web in 2 hypothetical marine systems with and without viruses. In the model with viruses, viral lysis resulted in a 27% increase in bacterial respiration and production rates and a 37% decrease in the carbon export to protozoan grazers relative to the virus-free model. The effects of viral lysis were (1) increased levels of bacterial activity

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and (2) reduced transfer of organic matter to higher trophic levels. Experimental evidence of such effects of viruses on the flux of DOM was presented by Middelboe et al. (1996), who found increased respiration and reduced growth efficiency for bacterial growth supported by viral lysates.

Thus, it seems that viruses have a significant influence on the fate of the organic matter in the sea and consequently play an important role in the turnover of organic matter in the global ocean (Wilhelm & Suttle 1999). However, there are no direct measurements of the quantitative importance of viruses for bacterial carbon turnover. It is essential to obtain such experimental data on the effects of viruses on bacterial carbon turnover to provide estimates of the influences of viruses on global carbon cycling.

In the present study we examined how a reduction in viral abundance in batch culture experiments with natural bacterial assemblages affected bacterial net production and respiration. Our results show that bacterial net growth and growth efficiency increased in cultures with a reduced abundance of viruses. We suggest that viral lysis increased bacterial recycling of DOM in the microbial assemblages resulting in reduced bacterial net production.

Materials and methods. ***Approach:*** The effects of viruses on bacterial growth and respiration were studied in 3 batch culture experiments with natural assemblages of viruses and bacteria, which were manipulated with respect to the density of natural virioplankton. In 1 subset of cultures (control), a virus-containing (0.2 µm filtered) seawater was inoculated with a concentrated bacterioplankton assemblage, and in a parallel set of cultures a virus-free (0.02 µm filtered) water sample was inoculated with the concentrated bacteria, thereby reducing the viral abundance in the cultures relative to the control cultures by 62 to 92%. The influence of reduced viral abundance on bacterial growth was investigated by measurements of bacterial and viral production and by examining the distribution of added ^{3}H-thymidine into different pools of organic matter and ^3H_2O in the –virus and control cultures.

Experimental design: In each of 3 experiments (Expts 1 to 3), a 2 to 4 l water sample from the southern Kattegat (inner Danish waters) was GF/C filtered (Whatman) and bacteria in the GF/C filtrate were concentrated with a 0.2 µm polycarbonate filter (MSI) to a final volume of about 50 ml. The 0.2 µm filtrate was split into 2 subsamples, and 1 of the subsamples was 0.02 µm filtered (Anodisc, Whatman) to remove viruses. The resulting 0.2 µm filtered and 0.02 µm filtered subsamples were transferred to 1 l culture bottles. The bacterial concentrate was also split into 2 subsamples and inoculated into 0.2 µm filtrate and the 0.02 µm filtrate culture bottles, respectively. The 2 bacterial suspensions were each distributed into 2 replicate cultures. The resulting duplicate cultures of the natural bacterial assemblage incubated with the natural density of viruses <0.2 µm (control) and the natural bacterial assemblage incubated with a reduced density of the natural viral assemblage <0.2 µm (–virus) were incubated in the dark at room temperature. The initial density of viruses in –virus culture was reduced to 38, 30 and 8% of the density in control cultures in Expts 1, 2, and 3, respectively. In Expt 1, the cultures were enriched with 1 ml Zobell medium (5 g peptone l^{-1}, 1 g yeast extract l^{-1}, 80% filtered, autoclaved seawater, 20% distilled water) l^{-1} of culture to stimulate bacterial activity.

Sampling: In all the experiments, samples were taken for determination of bacterial and viral abundance, and in Expts 1 and 2 we also measured bacterial thymidine uptake (see below) to establish conversion factors for calculation of cell production from thymidine incorporation in the different treatments. In Expt 3, 0.1 µCi ml^{-1} of ^{3}H-thymidine (1.2 nM final concentration) was added at time zero and the distribution of the labeled ^{3}H in the bacterial size fraction (>0.2 µm), the viral size fraction (<0.2 µm, >0.025 µm), the dissolved size fraction (<0.025 µm) and the respired fraction (^3H_2O) was followed over time by filtration of subsamples through duplicate nitrocellulose filters (Millipore). The amount of respired ^{3}H was determined by distillation of 3 to 5 ml subsamples, which were heated to 80°C and distilled under a vacuum. The filters and distillates were transferred to 6 ml scintillation vials and radio assayed. The amount of radioactivity associated with DOM (<0.025 µm) was estimated by subtraction of the respired activity from the total activity in the <0.025 µm fraction. A parallel killed control culture was used to obtain blank values for each ^{3}H measurement.

Bacterial and viral abundance: Viral and bacterial abundances were measured by epifluorescence microscopy after SYBR-Green staining according to Noble & Fuhrman (1998). Duplicate samples of 500 to 2000 µl were added to 2 ml of a 0.02 µm filtered (Anodisc, Whatman) seawater sample to increase the filtration volume, and the samples were filtered onto 25 mm 0.02 µm filters (Anodisc, Whatman). The filters were placed on a drop of 0.25% SYBR Green I (Molecular Probes) and stained for 15 min. Filters were rinsed with 0.02 µm filtered, distilled water and mounted with an anti-fade mounting solution (Noble & Fuhrman 1998). In all experiments, filters were screened for the presence of heterotrophic flagellates, and the abundance of flagellates never exceeded the detection limit (i.e. a few hundred cells ml^{-1}) within the incubation period. In Expt 2, the abundance of colony-forming bacteria

was determined by plating 100 µl of undiluted and 10×, 100× and 1000× diluted subsamples on duplicate Zobell agar plates.

Conversion factors for bacterial production: Ten milliliter subsamples were incubated for 1 h to measure bacterial incorporation of ^{3}H-thymidine into bacterial DNA (Fuhrman & Azam 1980). Samples were fixed with formaldehyde (2% final concentration), and bacteria were collected on 0.2 µm nitrocellulose filters (Millipore) and washed 10 times with 5% ice-cold trichloroacetic acid. Filters were transferred to 5 ml scintillation vials and allowed to dissolve in scintillation liquid (Packard) for 3 d before the radioactivity was measured with a liquid scintillation counter. The conversion of ^{3}H-thymidine incorporation to cell production was calculated from the integrated ^{3}H-thymidine incorporation and the net production of cells during the incubation period (Fuhrman & Azam 1980).

Results. In all 3 batch culture experiments, the increase in bacterial abundance was significantly higher ($p < 0.05$) in cultures from which viruses had been removed (–virus) than in control cultures with the natural viral abundance (control) (Figs. 1A, 2A & 3A). In Expt 1, bacterial abundance reached 2.6×10^7 cells

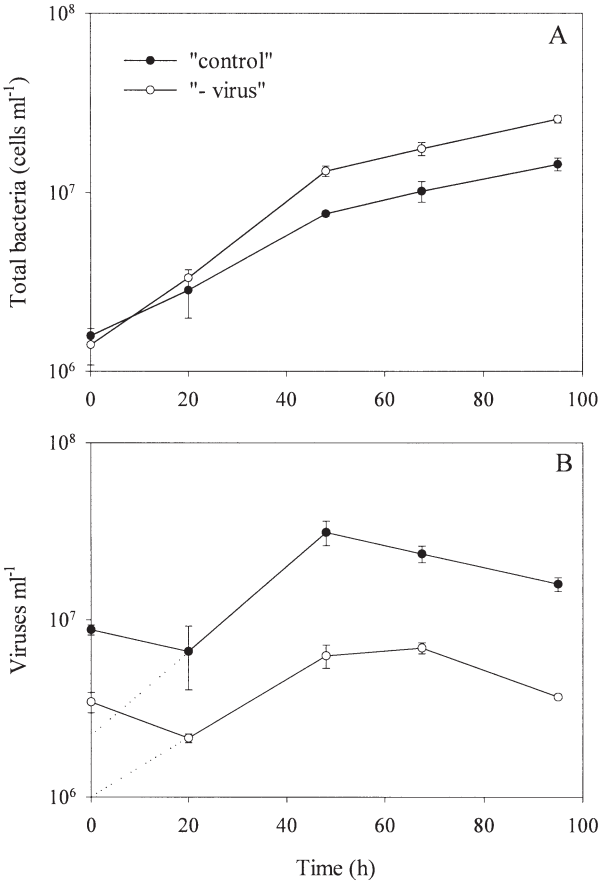

Fig. 1. Expt 1: (A) Bacterial abundance and (B) viral abundance in control and –virus cultures. Dotted lines in (B) indicate the linear regression lines for the increase in viral abundance assuming exponential growth of viruses during the first 48 h of incubation. Equations for regression lines: log viruses = 0.024 time + 6.3 (control) and log viruses = 0.017 time + 6.0 (–virus)

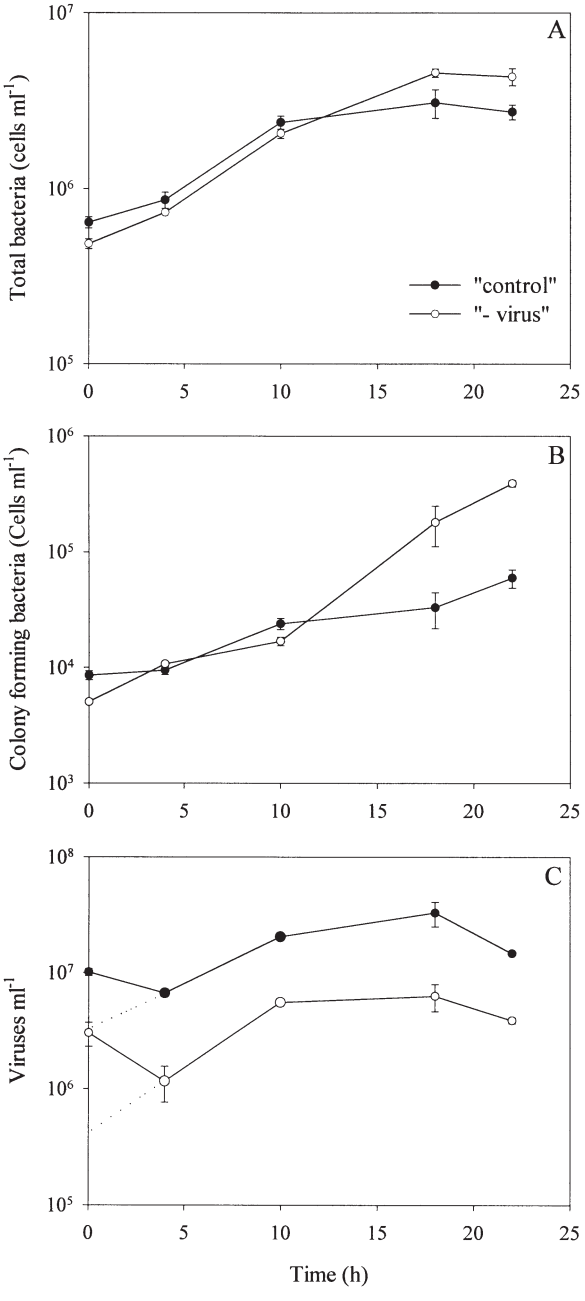

Fig. 2. Expt 2: (A) Total bacterial abundance, (B) abundance of colony-forming bacteria and (C) viral abundance in control and –virus cultures. Dotted lines in (C) indicate the linear regression for the increase in viral abundance assuming exponential growth of viruses during the first 10 h of incubation. Equations for regression lines: log viruses = 0.08 time + 6.5 (control) and log viruses = 0.11 time + 5.6 (–virus)

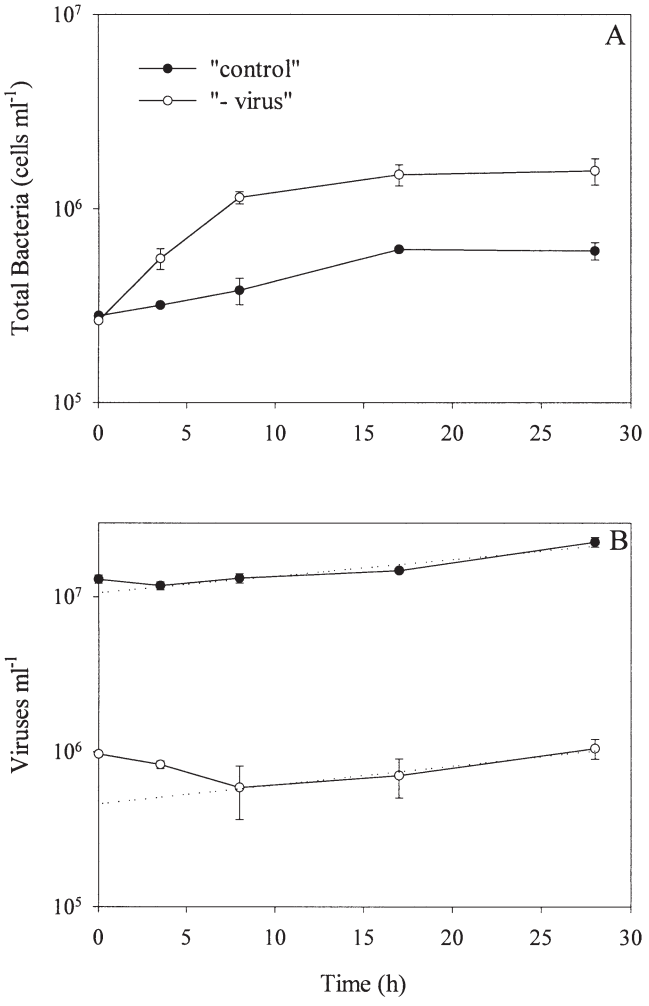

Fig. 3. Expt 3: (A) Bacterial abundance and (B) viral abundance in control and –virus cultures. Dotted lines in (B) indicate the linear regression for the increase in viral abundance during the incubation. Equations for regression lines: log viruses = 0.011 time + 7.0 (control) and log viruses = 0.013 time + 5.6 (–virus)

ml^{-1} in the virus-reduced cultures while only 1.5×10^7 cells ml^{-1} were produced in the control culture (Fig. 1A). The relative changes in viral abundance showed a similar pattern in the 2 treatments, whereas the actual abundance of viruses in the control cultures was approximately an order of magnitude higher than in the –virus cultures. After an initial decrease in abundance, the number of viruses increased by a factor of 5, reaching densities of 3.2×10^7 and 6.3×10^6 ml^{-1} in the control and –virus cultures, respectively (Fig. 1B). Following this increase, there was an exponential decrease in viral abundance in the control culture with a viral decay rate of 0.011 h^{-1}. Assuming that the difference in bacterial abundance between the 2 treatments was a result of viral lysis, a rough average burst size estimate of 6 viruses per lysed cell was estimated from the observed net production of viruses from 20 to 48 h incubation. Since the calculation is based on only 2 time points and does not consider decay of viruses during incubation, it should be regarded as a minimum value of virus burst size.

The development in bacterial and viral abundance in Expt 2 was similar to that in Expt 1, and the bacterial abundance reached maximum values of 4.3×10^6 and 2.7×10^6 cells ml^{-1} in the –virus and control cultures, respectively (Fig. 2A). The abundance of colony-forming bacteria constituted approximately 1% of the total bacterial abundance at the beginning of the experiments (Fig. 2B). During the incubation, the fraction of culturable bacteria increased to 9% in –virus cultures and 2% in control cultures, and the total production of colony-forming bacteria was 3.9×10^5 and 6.0×10^4 cells ml^{-1}, respectively, in the 2 cultures (Fig. 2B). As in Expt 1, there was a 5-fold increase in viral abundance in both cultures followed by a decay of the viral assemblage (Fig. 2C). As for Expt 1, we calculated the average virus burst size from net production of viruses in control cultures, resulting in an estimated virus burst size of 16 viruses per lysed cell.

In both Expts 1 and 2, bacterial thymidine uptake was higher in the –virus cultures than in control cultures, corresponding to the increase in bacterial abundance in these cultures. The calculated ^{3}H-thymidine conversion factor was, therefore, not significantly different in the 2 treatments and the average conversion factors were 7.4×10^{18} and 6.0×10^{18} cells mol^{-1} thymidine in Expts 1 and 2, respectively.

In Expt 3, we observed patterns of bacterial and viral abundance similar to those in the previous experiments (Fig. 3A,B). In this experiment the removal of viruses from –virus cultures was more efficient, however, and the relative effect on bacterial net production was larger than in Expts 1 and 2. Bacterial net production was 6.1×10^5 cells ml^{-1} in the control cultures and almost 3 times higher (1.6×10^6 cells ml^{-1}) in –virus cultures. Since the net production of viruses in the control cultures was 1.1×10^7 viruses ml^{-1} (Fig. 3B), the estimated average burst size was 11 viruses produced per lysed bacteria.

The distribution of radioactivity in the size fractions during Expt 3 confirmed and elaborated the information provided by bacterial and viral counts in the 3 experiments (Fig. 4). During the first 8 h of incubation, the bacterial size fraction (>0.2 µm) accumulated 36 000 ± 900 dpm ml^{-1} in the –virus culture (Fig. 4A). At the same time, the respired activity constituted 11 000 ± 1200 dpm ml^{-1}, while ^{3}H incorporation in the viral size fraction (>0.025 µM) was insignificant (Fig. 4B). The corresponding decrease in ^{3}H-labeled DOM (<0.025 µM DOM fraction) was 56 000 ± 8000 dpm ml^{-1} (Fig. 4A).

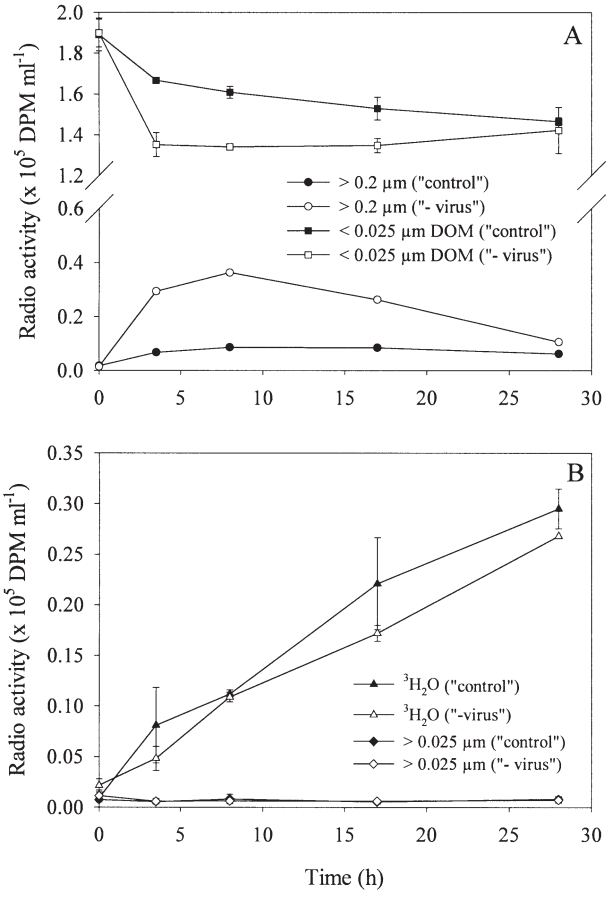

Fig. 4. Expt 3: Distribution of ^3H, added as ^3H-thymidine at time zero, (A) into the bacterial size fraction and the dissolved organic size fraction (dissolved organic matter [DOM]) and (B) into the viral size fraction and the respired fraction (^3H_2O) in control and –virus cultures

In the control culture, on the other hand, the radioactivity in the bacterial fraction (Fig. 4A) and the respired fraction (Fig. 4B) reached 8600 ± 600 and 11 000 ± 400 dpm ml^{-1}, respectively, during the same period. As in the –virus culture, there was no significant accumulation of ^3H in the viral size fraction (Fig. 4B). The decrease in ^3H-labeled DOM accounted for 29 000 ± 9000 dpm ml^{-1} during the same period (Fig. 4A). The efficiency of bacterial conversion of the assimilated ^3H-thymidine into bacterial biomass was calculated from the activity in the bacterial size fraction and the respired activity as follows: conversion efficiency = Δ > 0.2 µm fraction/(Δ > 0.2 µm fraction + Δ respired fraction). The conversion efficiency was 0.44 and 0.77 for the bacterial uptake of ^3H-thymidine in the control and –virus cultures, respectively, during the initial 8 h of incubation (Fig. 4).

After the initial rapid uptake of ^3H-thymidine and accumulation of ^3H in bacterial biomass, the amount of radioactivity in the bacterial size fraction decreased concomitant with an increase in the amount of respired activity (Fig. 4), and after 28 h the distribution of ^3H was similar in the 2 cultures. Total bacterial uptake of ^3H (i.e. respired label + incorporated label) accounted for 84 and 79% of the measured decrease in ^3H-labeled DOM in control and –virus cultures, respectively, and 83 and 72% of total bacterial ^3H uptake had accumulated as ^3H_2O, respectively, in the 2 treatments (Fig. 4).

The relative changes in viral abundance during the incubations were similar in all the experiments despite the large differences in absolute numbers between the treatments (Figs. 1B, 2C & 3B). After an initial decrease in viral abundance, the number of viruses increased again, and in Expts 1 and 2 this was followed by a second phase of decreasing viral abundance. Assuming that the increase in viral abundance reflected exponential growth of a subset of the viral assemblage, we could extrapolate this exponential production phase back to time zero, hence estimating the fraction of the viral assemblage that was able to reproduce at the given conditions in the cultures (Figs. 1B, 2C & 3B, dotted lines). Given that these assumptions are correct, the fraction of infective viruses at the beginning of the experiments was 25 and 30% in Expt 1, 31 and 14% in Expt 2, and 80 and 50% in Expt 3 in the control and –virus cultures, respectively. It should be noted, however, that the regressions are based on only a few time points, and that calculation of viral production rates and the initial abundance of infective viruses based in these equations, therefore, is associated with some uncertainty.

DISCUSSION

The enhanced bacterial growth at reduced viral densities in –virus cultures, relative to the control cultures with the natural concentration of viruses, strongly indicated that viruses suppressed the net production of bacteria (Figs. 1 to 3). Removal of viruses increased the maximum bacterial abundance by a factor of 1.6 to 2.5 in the 3 experiments ($p < 0.05$), suggesting that viral lysis in the control cultures had a significant negative effect on the total number of bacteria produced in the cultures. One explanation for the apparent viral control of bacterial production could be that viruses exerted a top-down control of the total bacterial abundance. In that case, removal of viruses resulted in a reduced infection pressure from the viruses, leaving room for increased bacterial growth in these cultures. The increase in abundance of colony-forming bacteria in the –virus cultures (Fig. 2B) suggested that the subset of the bacterial population that was culturable on Zobell medium was indeed

controlled by viral lysis, since removal of viruses allowed growth of these particular populations. The loss of culturable bacteria by viruses accounted for 20% of the total bacterial loss in the control cultures, indicating that direct viral control of specific bacterial populations may affect the total bacterial abundance obtained in such batch culture experiments. It seems unlikely, however, that the viral population exerted an overall control over the total bacterial assemblage similar to a grazer control by protozoans, given a high specificity of viral infection. Assuming that viral populations are specific for individual bacterial populations, viral lysis would be expected to affect the composition of the bacterial community rather than the total bacterial abundance (e.g. Thingstad & Lignell 1997, Wommack & Colwell 2000), since non-infected bacterial populations (i.e. virus resistant strains or strains in which specific viruses were not present in significant densities) may replace virus-lysed populations. In the absence of a continuous substrate input (which was the case in these batch culture experiments), the loss of cells due to viral lysis and the recycling of lysates by other bacteria would, however, reduce the net production of cells in the cultures, which may be part of the explanation for the significant negative impact of viruses on the total number of cells produced in the cultures (Figs. 1 to 3).

The distribution of ^{3}H into size fractions supported the idea that viral lysates were recycled during the incubation. The incorporation of ^{3}H-thymidine into the bacterial size fraction (Fig. 4) confirmed that the presence of viruses reduced the net bacterial production in the control cultures. The ^{3}H balance showed that total ^{3}H uptake during the whole experiment was similar in the 2 cultures; however, during the first 17 h of incubation, the fraction of ^{3}H that accumulated in bacterial biomass was significantly lower ($p < 0.01$) and the fraction that was found in the DOM was significantly higher ($p < 0.05$) in the virus-containing cultures than in the virus-free cultures (Fig. 4A). Consequently, the bacterial efficiency in the utilization of the assimilated ^{3}H-thymidine was reduced from 77% in the –virus culture to 44% in the control culture after 8 h of incubation (Fig. 4). These results suggested that the presence of viruses did not affect total DOC uptake but had substantial impact on the bacterial growth efficiency, not by increasing bacterial respiration directly but by causing release of part of the organic matter that was already taken up. Since the total respiration (i.e. accumulation of ^{3}H-H_2O) was similar in the 2 cultures, it is possible that the total metabolism (i.e. organic carbon production and respiration) was also the same in the 2 treatments, and that the reduced bacterial biomass was caused only by loss of already produced bacterial carbon. We suggest that release of DOM by viral lysis enhanced the recycling of organic matter within the bacterioplankton and resulted in a reduced net bacterial production, and therefore a reduced carrying capacity of the given substrate.

Consequently, while substrate availability was the overall limiting factor for bacterial growth in both types of cultures as they reached stationary growth phase, viral activity reduced the efficiency of the conversion of substrate to cell production during the incubation. At the end of the incubations, the production of viruses ceased in all cultures, and viral abundance in the –virus cultures, therefore, did not reach the same densities as in the control cultures.

In the interpretation of these data, we have assumed that the observed differences in bacterial activity between the 2 sets of cultures were caused by the removal of viruses and not by some side effects of the filtration procedure. Removal of viruses by 0.02 µm filtration may change the bacterial substrate conditions, since the filtration may remove a potential bacterial substrate in the size fraction between 0.02 µm and 0.2 µm (large molecules and colloids). However, this is not a likely explanation for the increased cell production in the 0.02 µm filtered culture. Rather, removal of a potential bacterial substrate would be expected to reduce bacterial growth, unless this size fraction contained some inhibiting factor for bacterial production other than viruses. However, we are not aware of studies that have demonstrated inhibiting effects of bacteria by DOM molecules and infer, therefore, that the differences in bacterial activity between the 2 culture sets were caused by viral activity.

Implications for bacterial production measurements

Apart from having large implications for our understanding of pelagic carbon flow in general (see below), the observed effects of the distribution of ^{3}H-thymidine during the incubation also have some implications for the interpretation of measurements of bacterial production. After only 4 h of incubation in the –virus cultures, the bacterial fraction contained 4 times more ^{3}H than was found in bacteria in the control culture, and the amount of radioactivity in the dissolved fraction was correspondingly lower in the –virus than in control cultures (Fig. 4). If these incubations had been performed to measure bacterial production from incorporation of ^{3}H-thymidine, we would therefore have underestimated the true bacterial production significantly, since a large fraction of the produced cells would have been lost again during the incubation. Ideally, the true bacterial production should, therefore, be measured in the absence of viruses, and since viruses were not completely removed from the –virus

cultures, these incubations may also have underestimated bacterial production. Incubations for measurements of bacterial production by use of ^{3}H-thymidine are usually shorter (1 to 2 h) than the 4 h that passed between the first 2 sampling points in this experiment. However, there may still be a significant lysis of bacteria that have taken up the added isotope even during such short incubations. Bacterial production may, therefore, be underestimated by the traditional radioisotope uptake incubations and using the traditional conversion factors between cell production and isotope uptake. The measured conversion factors between ^{3}H-thymidine incorporation and net cell production (i.e. cell production divided by the accumulated ^{3}H incorporation) did not differ between the experiments. Moreover, there were no systematic differences in the specific bacterial thymidine uptake (i.e. thymidine uptake per cell; data not shown) between experiments, indicating that bacterial growth rates were not significantly affected by viral lysis. Thus, reduced net bacterial cell growth in the presence of viruses resulted in a correspondingly reduced accumulation of ^{3}H-thymidine during the incubations. Hence, we cannot just apply a conversion factor determined in the absence of viruses to determine the true bacterial production in incubations where viruses are present.

Our results have shed some light on the potential influence of viral activity on estimates of bacterial production; however, more work is needed to evaluate the quantitative importance of viruses in underestimating bacterial production measurements and to what extent the used conversion factors should be changed to correct for the loss of cells during incubations. Since viral activity may vary between water samples, it is not obvious how to apply a general correction for viral loss during bacterial production measurements. Despite the indications in this study that viral lysis may affect bacterial net production even during short term incubations for bacterial production, the effect of viruses may still be overshadowed by the general uncertainty that is implied in the use of a given conversion factor used between thymidine incorporation and cell production.

Implications for pelagic carbon flow

The observed reduction in bacterial net production in the presence of viruses supports the suggestions presented previously that a significant fraction of heterotrophic bacterial production is recycled back to DOM by viral lysis (Middelboe et al. 1996, Wilhelm & Suttle 1999). The data also support model predictions by Fuhrman (1992), which showed that bacterial respiration increased significantly in the presence of viruses compared to an otherwise identical system but without viruses. Although it is not given that the observed effects of carbon recycling by viral lysis can be directly extrapolated to natural systems, the assumption that 10 to 30% of the daily bacterioplankton production is lost as a result of viral lysis (e.g. Fuhrman & Noble 1995, Wommack & Colwell 2000) suggests that this process has a significant influence on bacterial carbon turnover in the global ocean.

In the present study we suggested that the increased respiration was the result of recycling of organic matter by viral lysis, thus decreasing the output of bacterial biomass from a given substrate uptake. The overall consequence of these observations is that viruses can have a significant impact in the pelagic carbon flow, and that high viral activity tends to increase the importance of bacteria for organic carbon turnover and community respiration. Viral lysis, thus, tends to keep the organic matter in a dissolved form and therefore reduces the transfer of nutrients to higher trophic levels. Temporal and spatial differences in the relative importance of viruses as agents of bacterial mortality, as indicated in a number of studies (e.g. Weinbauer et al. 1993, Guixa-Boixareu et al. 1996), may, therefore, have a large influence on the role of the bacterial processing of organic carbon in pelagic marine systems.

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LITERATURE CITED

Fuhrman JA (1992) Bacterioplankton roles in cycling of organic matter: the microbial food web. In: Falkowski PG, Woodhead AD (eds) Primary productivity and biogeochemical cycles in the sea. Plenum Press, New York, p 361–383

Fuhrman JA (1999) Marine viruses and their biogeochemical and ecological effects. Nature 399:541–548

Fuhrman JA, Azam F (1980) Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. Appl Environ Microbiol 39: 1085–1095

Fuhrman JA, Noble RT (1995) Viruses and protists cause a similar bacterial mortality in coastal seawater. Limnol Oceanogr 40:1236–1242

Gobler CJ, Hutchins DA, Fisher NS, Cosper EM, Sanudo-Wilhelmy S (1997) Release and bioavailability of C, N, P, Se, and Fe following viral lysis of a marine chrysophyte. Limnol Oceanogr 42:1492–1504

Guixa-Boixareu N, Calderón-Paz JI, Heldal M, Bratbak G, Pedrós-Alió C (1996) Viral lysis and bacterivory as prokaryotic loss factors along a salinity gradient. Aquat Microb Ecol 11:215–227

Middelboe M, Jørgensen NOG, Kroer N (1996) Effects of viruses on nutrient turnover and growth efficiency of noninfected marine bacterioplankton. Appl Environ Microbiol 62:1991–1997

Noble RT, Fuhrman JA (1998) Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat Microb Ecol 14:113–118

Noble RT, Middelboe M, Fuhrman JA (1999) The effects of viral enrichment on the mortality and growth of heterotrophic bacterioplankton. Aquat Microb Ecol 18:1–13

Steward GF, Smith DC, Azam F (1996) Abundance and production of bacteria and viruses in the Bering and Chukchi Seas. Mar Ecol Prog Ser 131:287–300

Thingstad TF, Lignell R (1997) Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. Aquat Microb Ecol 13:19–27

Weinbauer MG, Fuks D, Peduzzi P (1993) Distribution of viruses and dissolved DNA along a coastal trophic gradient in the northern Adriatic Sea. Appl Environ Microbiol 59:4074–4082

Wilhelm SW, Suttle CA (1999) Viruses and nutrient cycles in the sea. Bioscience 49:781–788

Wommack KE, Colwell RR (2000) Virioplankton: viruses in aquatic environments. Microbiol Mol Biol Rev 64:69–114

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