Vol. 262: 71–80, 2003 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published November 7

Effects of virus infection on respiration rates of marine phytoplankton and microplankton communities

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ABSTRACT: The possible influence of viral infection on respiration rates in marine microbial pelagic communities was assessed by means of 3 experiments on respiration rate with viral concentrate addition on single-species cultures of *Mantoniella* sp. and *Micromonas pusilla* and another 3 on natural microplankton communities (organisms <200 µm) from the Kattegat Sea (Åstol) and the Baltic Sea. Coastal surface seawater samples were taken during cruises of the RVs 'Ancylus' and 'Argos' during winter and spring 2000. Approximately 50 to 70 l of seawater were concentrated by ultrafiltration. The experiments were started by adding a viral particle concentrate to a container with algae or a natural microplankton community; a control container was kept free of the viral concentrate addition. Oxygen concentration determinations were carried out on each treatment and control to measure respiration rates throughout the incubation period. The *in vivo* chlorophyll *a* fluorescence was also monitored as an indication of algal infection. The rates of respiration indicated that the addition of the viral particle concentrate affected the respective metabolisms of the *Mantoniella* sp. and *Micromonas pusilla* cultures as well as natural microplankton communities. Viral infection decreased the *Mantoniella* sp. respiration rate (by 96%) and increased the *Micromonas pusilla* respiration rate (by 235%). Hence, if our results can be extrapolated to nature, then, at least in a bloom situation, the fate of primary production and carbon fluxes could be strongly modulated by viral infection. The addition of a viral particle concentrate to the microplankton community generated complex responses in terms of respiration rates, which increased (by 84%) or remained similar to the controls. Our results suggest that viral infection of microplanktonic organisms could be one of the factors significantly modifying pelagic carbon fluxes.

KEY WORDS: Respiration · Marine viruses · Microalgae · Virus infection · Kattegat · Baltic Sea

INTRODUCTION

Viruses or virus-like particles (VLP) may be numerically the most dominant life form in the oceans, with concentrations from 10^6 to $>10^8$ viruses ml^{-1} (e.g. Bergh et al. 1989, Proctor & Fuhrman 1990, Hara et al. 1991, Wommack et al. 1992). Viral infection may cause about 30% of total mortality in marine cyanobacteria and up to 60% in heterotrophic marine bacteria in coastal and offshore environments (Proctor & Fuhrman 1990). Furthermore, several studies have demonstrated that natural virus populations can consist of different viruses infective to many algal groups, such as coccolithophorids (Bratbak et al. 1995), picoflagellates (Chen & Suttle 1995, Cottrell & Suttle 1995, Suttle & Chan 1995), and dinoflagellates (Tarutani et al. 2001), and that viruses seem to be very specific to algal species or strains (Sahlsten 1998, Cottrell & Suttle 1991).

Experimental studies have shown that the addition of viral concentrates to a variety of important marine primary producers can reduce primary production by as much as 78% (Suttle et al. 1990, Suttle 1992), indi-

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cating that, in addition to grazing and nutrient limitations, virus infections could be a factor regulating the phytoplankton community structure and primary productivity in the oceans (Suttle et al. 1990). In fact, if viruses regulate primary production, they could also affect the inverse process, i.e. respiration, not only of phytoplankton but also of heterotrophic organisms. Accordingly, if viruses affect both processes, then they could be a significant factor in modulating total metabolism in the pelagic system.

Here, we present results from a study in which we determined whether respiration rates of single phytoplankton species and natural microplankton communities are affected by the addition of viral concentrates.

Table 1. Characteristics of the viral particle concentrate (VC) and microplankton communities (M) for each of the experiments conducted. The last column shows the number used in the text to identify each of the experiments conducted

Sampling location	Sample type	Sampling date (2000)	*In situ* *T* (°C)	*In situ* salinity	Expt
Kattegat Sea (Morups Bank)	VC	10 Feb	3.4	23.9	4
Kattegat Sea (Åstol)	M	8 Mar	3.4	24.5	4
Saltholmen Bay	VC	28 Mar	2.7	19.0	5
Kattegat Sea (Åstol)	M	5 Apr	4.3	16.0	5
Baltic Sea (Stn BY38)	VC	28 Apr	5.9	7.1	1, 6
Baltic Sea (Stn BY38)	M	15 Jun	11.4	7.1	6

MATERIALS AND METHODS

Sampling. Coastal surface seawater samples were taken during a cruise of the RV 'Ancylus' and RV 'Argos' during winter and spring 2000 (Table 1, Fig. 1). The samples were collected using a water pump previously cleaned by flushing with seawater. The samples were then kept in polypropylene containers (~30 l) that had been cleaned by pre-soaking with 0.1 N HCl and then rinsed several times with distilled and ultrapure water. Some of the seawater sampled was used for concentrating viruses and some was used as natural microplankton assemblages in the experiments (Table 1).

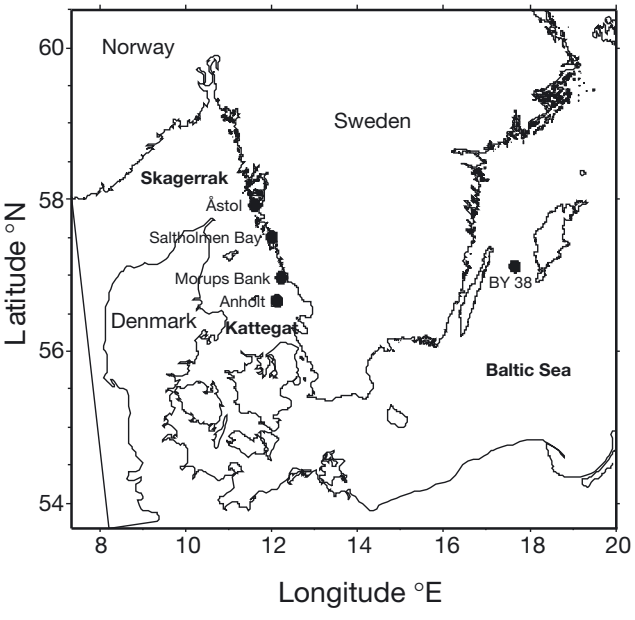

Fig. 1. Map showing the stations where the samples were taken

Concentrating viruses from seawater. In order to remove all plankton cells and most of the bacterioplankton, approximately 50 to 70 l of surface seawater were pre-screened and filtered (vacuum pressure <130 mm Hg) through two 142 mm diameter filters allocated in series: (1) Whatman GF/C glass-fibre filter, (2) 0.45 µm Durapore filter (Millipore). The filtrate was concentrated approximately 250-fold by ultrafiltration using a 30 000 MW cut-off ultrafilter (Amicon Spiral Cartridge Model S1Y30) as described by Suttle et al. (1991). Viral concentrates were checked qualitatively using transmission electron microscopy to make sure that there was no significant bacterial contamination.

Algae cultures. Algal cultures of *Mantoniella* sp. (Prasinophyceae), *Micromonas pusilla* (Prasinophyceae), *Chaetoceros muelleri* (Bacillariophyceae) and *Prorocentrum minimum* (Dinophyceae) were obtained from the phytoplankton culture collection of the Department of Marine Botany at the University of Göteborg. The cells were grown in a medium prepared with autoclaved artificial seawater (Harrison et al. 1980) enriched with f/2 nutrients (Guillard 1975) and modified by the addition of 5 mM Tris-HCl (pH = 7.7) and 10 nM Na_2SeO_3 (Cottrell & Suttle 1991). The cultures were incubated at approximately 20°C under a 12:12 h light:dark cycle using artificial light ('daylight fluorescent tubes', 80 µE s^{-1} m^{-2}).

Test for viral infection and viral isolation. To carry out single-alga respiration experiments, it was necessary to find an alga-host to lytic–virus relationship. An inoculate of viral particle concentrate obtained from the Baltic Sea (Stn BY38, Fig. 1) was added to 4 algal species (*Chaetoceros muelleri*, *Micromonas pusilla*, *Mantoniella* sp., and *Prorocentrum minimum*) to investigate possible lytic virus infections in any of the algae species. The algae cultures were grown in 10 ml polypropylene screw-capped glass tubes under the conditions described above; 5 tubes of exponentially growing cultures were inoculated with 0.5 ml of viral

particle concentrate, and 5 tubes were kept as controls. Algae growth was monitored by daily measurements of *in vivo* chlorophyll *a* fluorescence with a fluorometer (Turner Model 111) equipped with a blue (Corning 560) primary filter and a red (Corning 2-64) secondary filter. It was considered that a viral infection, indicated by lysis of the algae, had taken place when a drastic decrease of *in vivo* fluorescence, reaching values close to zero, was observed while the fluorescence of the controls stayed high. Cultures that had not lysed after 18 d were considered to be unsuitable hosts for the viruses.

After viral infection was detected, 1 ml of each of the lysed cultures was filtered through 0.20 µm membrane filters (MiniSart sterile, Sartorius) and used to inoculate a new set of exponentially growing cultures. This procedure was repeated to enrich and isolate the specific algal virus that was infective to the alga species. The enriched viruses, called stock viruses, were stored in the dark at 4°C until use. The infectivity of the stock virus sample was checked before each experiment. The host-specificity of each isolated virus was also checked for each of the 4 algae species used. However, it is important to note that the stock virus samples were not completely pure, as the infective viral particles were not isolated and purified.

Transmission electron microscope (TEM) and flow cytometric analyses. Samples were stored at 4°C before analysis and prepared for TEM observation as described by Bratbak & Heldal (1993). Particles were harvested onto electron microscope grids (Ni, 400-mesh carbon-coated formvar film) by centrifugation for 30 min at 200 000 × *g* in a Beckman SW 41 swing-out rotor. The grids were positively stained with 2% uranyl acetate and viewed in a JEOL 100CX TEM at 30 000× magnification. Flow cytometric analysis was performed as described by Marie et al. (1999). A FACSort flow cytometer (Becton Dickson) equipped with an air-cooled laser providing 15 mW at 488 nm and with the standard filter setup was used. For enumeration of virus and bacteria, the sample was diluted up to 1000-fold, the discriminator was set to green fluorescence, and the sample was analysed by flow cytometry for 1 to 4 min at a delivery rate of 50 µl min^{-1}.

Experimental design for single-species cultures. The effect of virus addition on respiration rates was assessed using single-species cultures of *Mantoniella* sp. and *Micromonas pusilla*: 2 experiments were carried out on the *Mantoniella* sp. cultures and 1 on the *M. pusilla* cultures. Prior to each experiment, all material used was carefully cleaned with ultra-pure water and then sterilised by autoclaving at 120°C. In addition, bacterioplankton communities were sorted out by gently filtering (<130 mm Hg) the algae culture, first through a glass-fibre filter (Whatman GF/C) and then through a 1 µm pore-size polycarbonate filter (Nuclepore). The filtrate was used operationally as the 'bacterioplankton community'.

Table 2. *In situ* and mean ± SD experimental incubation temperatures (°C)

Expt	*In situ*	Incubation	Mean difference
1	21.6	21.7 ± 1.0	0.8
2	22.2	22.3 ± 0.5	0.4
3	18.3	18.3 ± 0.4	0.4
4	3.3	15.5 ± 1.1	12.2
5	4.3	17.2 ± 1.6	12.9
6	11.4	21.5 ± 1.6	10.2

The experiment was started by adding 50 ml of viral stock to 2 carboys containing 20 l of the algae culture and bacterioplankton community respectively. In Expt 1, crude viral concentrate from Stn BY38 was used (Table 1). In Expts 2 and 3, the stock corresponded to enriched *Mantionella* sp. and *Micromonas pusilla*-specific viruses respectively. As controls we kept 20 l of both fractions without any virus addition. The algae cultures with and without viruses were incubated under a 12:12 light:dark cycle while the temperature was kept at 21.4 ± 1.6°C (Table 2). The bacterioplankton community was incubated in the dark. Oxygen samples were taken from each carboy to measure respiration rates throughout the incubation period. The *in vivo* chlorophyll *a* fluorescence was monitored during every experiment for evidence of infection (Sequoia-Turner Fluorometer Model 450).

Experimental design for natural microplankton assemblages. We carried out 3 microplanktonic experiments to determine the effect of viral infection on respiration rates of natural microplankton assemblages (organisms <200 µm).

The experimental design was nearly the same as that used in the single-alga experiments. First, the bacterioplankton was separated from the microplankton community by filtering through a 1 µm Nuclepore polycarbonate filter and keeping the filtrate. Then 15 to 20 l of the microplankton community as well as 15 to 20 l of the bacterioplankton community were infected with 40 to 50 ml of the natural viral particle concentrates (Table 1). We kept 20 l of both fractions without any virus addition as an experimental control. The microplankton communities — with and without viruses — were incubated under the conditions described above for single-species experiments. It was not possible to maintain the incubation temperature very close to the *in situ* temperature (Table 2). Oxygen samples were taken from each carboy to measure respiration rates throughout the incubation period. The *in vivo* chlorophyll *a* fluorescence was monitored (Sequoia-Turner

Fluorometer Model 450) during each experiment for evidence of phytoplankton infection.

Respiration rates. Dissolved oxygen measurements were carried out using the Winkler method (Carpenter 1965) and the analytical procedures suggested by Knap et al. (1993) and by Culberson (1991). The variation coefficient of the dissolved oxygen measurements was 0.1%.

As zero samples (t_o), we fixed 5 replicates of each treatment. The remaining bottles (5 replicates per control and 5 per virus treatment) were incubated for 21 to 24 h in the dark in glass oxygen bottles until fixation at the final time (t_f). Microplanktonic respiration is estimated as the difference of dissolved oxygen between t_o and t_f. In addition, 5 oxygen samples of the control or virus-infected treatments were fixed after 10 to 12 h incubation to check the linear decay of oxygen during the incubation period.

The standard error was used to statistically compare respiration rates between the controls and the treatments (i.e. with viral concentrate addition), and was calculated according to Zar (1996). Respiration rates were considered to be different when the standard errors of the measurements did not overlap.

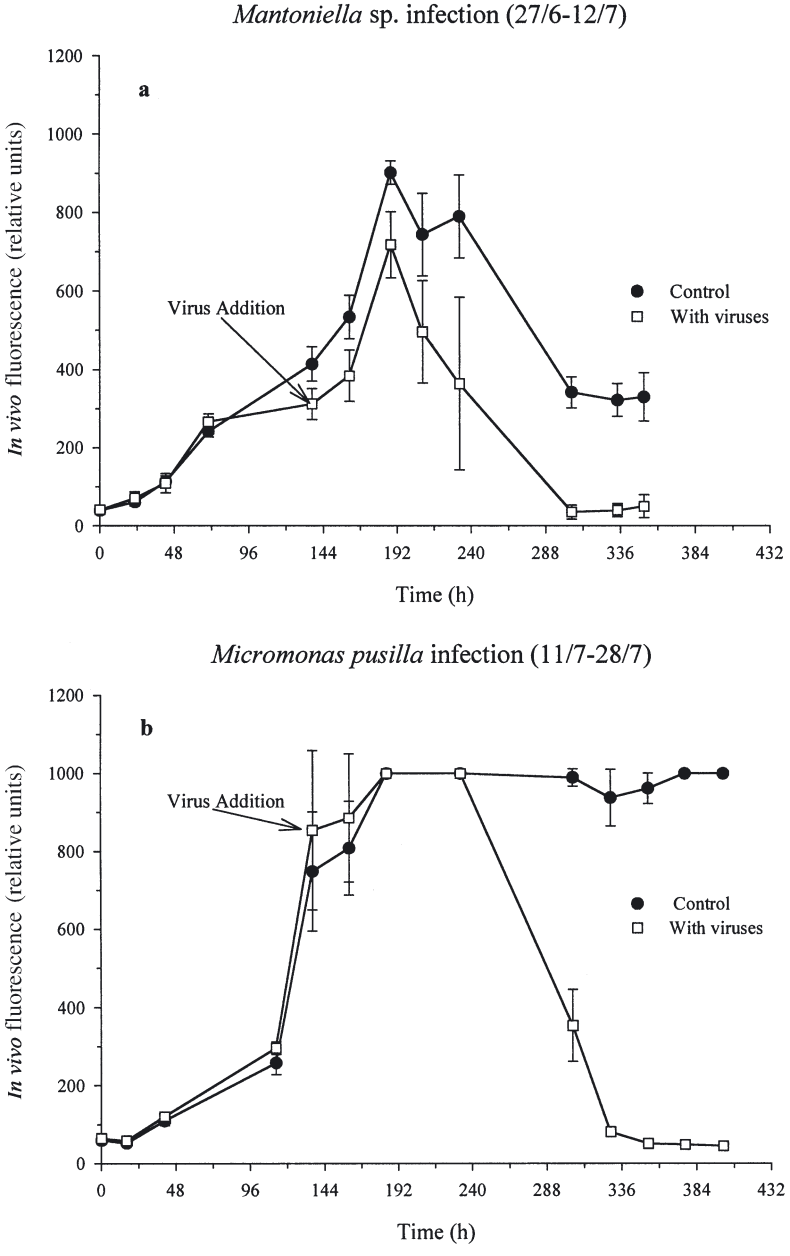

Fig. 2. *Mantoniella* sp. and *Micromonas pusilla*. Effect of virus infection on *in vivo* fluorescence

RESULTS

Viral infection of single-species cultures

From the 4 species inoculated with the viral particle concentrate from Stn BY38 (Fig. 1), only *Mantoniella* sp. (Fig. 2a) and *Micromonas pusilla* (Fig. 2b) were infected after 51 and 96 h of viral addition respectively (i.e. 188 and 232 h of incubation time respectively). Isolates of the viruses able to infect *Mantoniella* sp. and *M. pusilla* did not infect *Chaetoceros muelleri* or *Prorocentrum minimum*.

Mantoniella sp. virus

The lysate (i.e. viruses freed from cells) of *Mantoniella* sp. obtained from the first experiment was examined by transmission electron microscopy. The estimated size of the viruses ranged from 100 to 133 nm and the size of the *Mantoniella* sp. scales ranged from 200 to 280 nm in diameter (Fig. 3). This virus did not present a tail and was hexagonal in shape, indicating icosahedral symmetry. The same viral sample examined by flow cytometry presented 1.2×10^8 virus particles ml^{-1} and 1.5×10^8 bacteria ml^{-1}.

Respiration in single-species cultures

During both experiments with *Mantoniella* sp., respiration rates always differed between the virus-treated culture and the untreated control (Fig. 4a,b), the difference

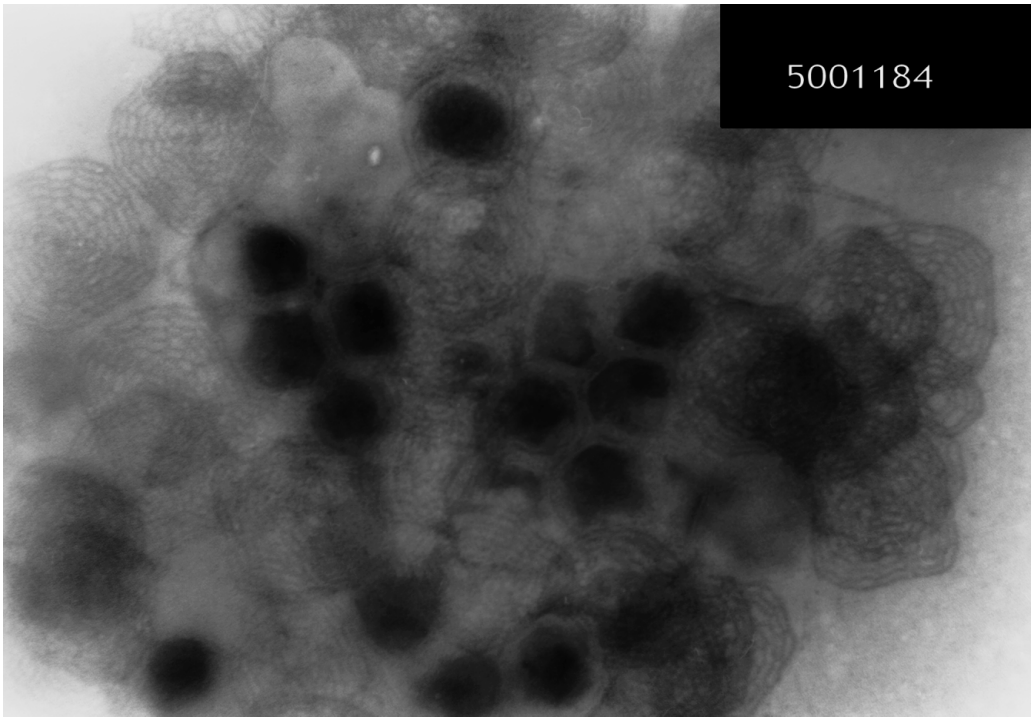

Fig. 3. *Mantoniella* sp. Electron microscope photograph showing viruses and scales (supplied by M. Heldal)

being as high as 96%. The bacterioplankton respiration rates with and without viral addition were consistently much lower than those of *Mantoniella* sp. and they did not present changes during the incubation period (Fig. 4a,b).

During Expts 1 and 2, the *in vivo* fluorescence of the virus-treated *Mantoniella* sp. culture strongly decreased after 112 and 15 h of viral addition respectively. The *in vivo* fluorescence of the infected cultures corresponded, for Expts 1 and 2, to 2 and 0.6% of the fluorescence of the controls (Fig. 4d,e). The respiration rates of *Micromonas pusilla* with and without viral addition differed, with a comparative increment of the respiration rate as high as 235% in the virus-treated *M. pusilla* culture (Fig. 4c). The *in vivo* fluorescence started to decrease in the virus-treated fraction after 144 h incubation; the *in vivo* fluorescence of the infected culture was 18% of the fluorescence of the control at the end of the experiment (Fig. 4f).

Respiration in natural microplankton assemblages

We carried out 3 respiration experiments to verify the possible effect of viral infection on microplankton and natural bacterioplankton communities.

During Expt 4 (Fig. 5a), there was no difference in respiration rates between the microplankton community subjected to the addition of the viral particle concentrate (MV) and the control without the added viral particle concentrate (MC). The respiration rate of the bacterioplankton control community (BC) was higher (58%) than that of the bacterioplankton with the added viral particle concentrate (BV).

In Expts 5 (Fig. 5b) and 6 (Fig. 5c), MV presented a maximum respiration difference over MC of 67% (180 h) and 84% (255 h) respectively. Moreover, the MV respiration rates in Expt 6 showed a maximum decrease of about 31% in comparison with MC. BC respiration rates were as much as 40 and 45% higher than BV in Expts 5 and 6 respectively.

The MC *in vivo* fluorescence did not differ from that of the MV during Expt 4 (Fig. 5d) and an increase in the *in vivo* fluorescence was seen during the incubation period. During Expt 5 (Fig. 5e), the *in vivo* fluorescence of MV and MC increased and the MV fluorescence was higher than that of the MC until 246 h after the addition of the viral particle concentrate. In Expt 6, there was no apparent effect of the viral particle concentrate addition on the *in vivo* fluorescence (Fig. 5f).

DISCUSSION

Our results provide evidence that the addition of a viral particle concentrate can affect respiration rates of

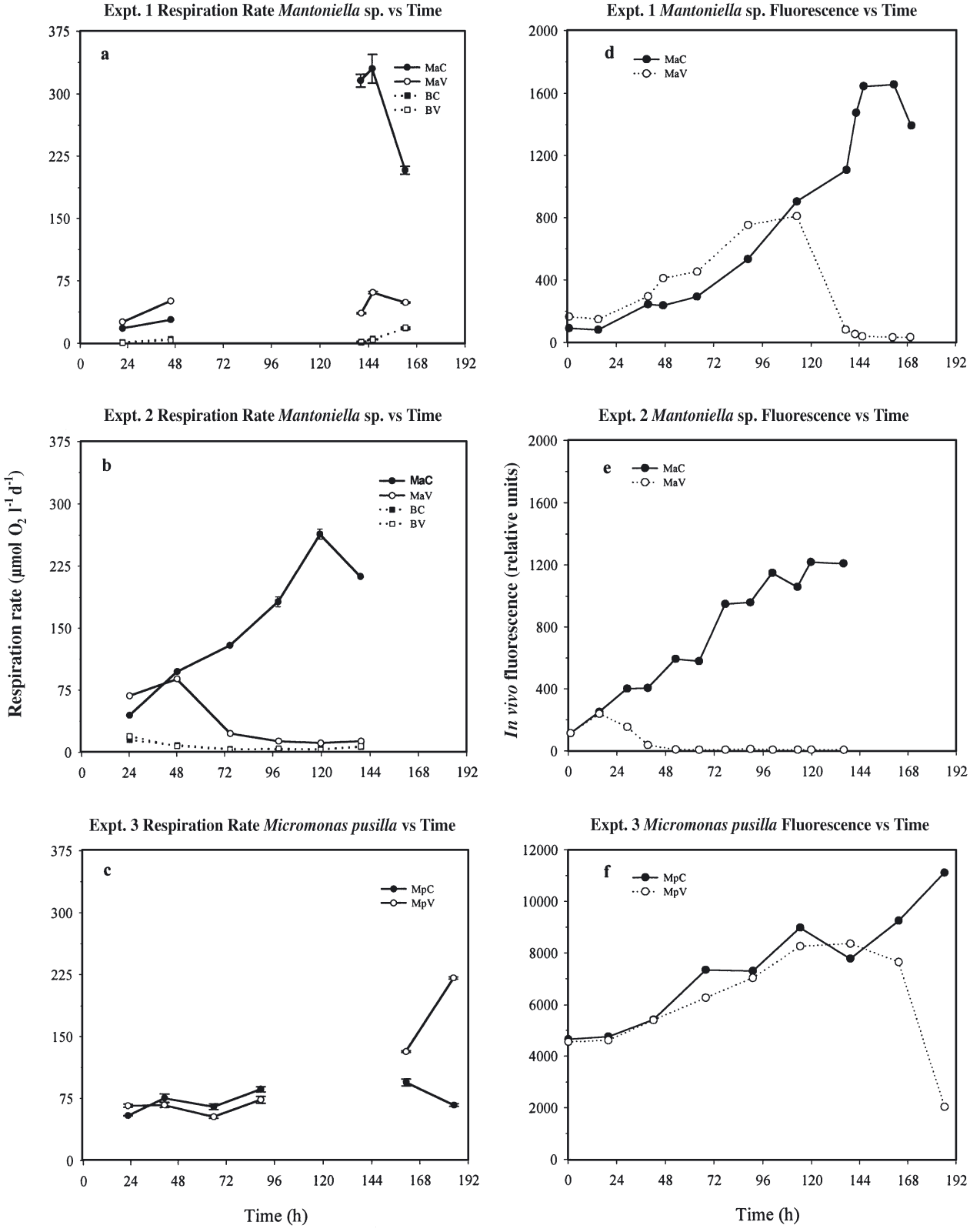

Fig. 4. *Mantoniella* sp. and *Micromonas pusilla* culture. Respiration rates and *in vivo* fluorescence (relative units) as a funtion of incubation time for single-alga treatments (Expts 1 to 3); 2 experiments were carried out for *Mantoniella* sp., 1 for *Micromonas pusilla*. MaC, MaV: *Mantoniella* sp. control and virus-added experiments respectively; BC: bacterioplankton control; BV: bacterioplankton with added virus; MpC, MpV: *Micromonas pusilla* control and virus-added experiments repectively

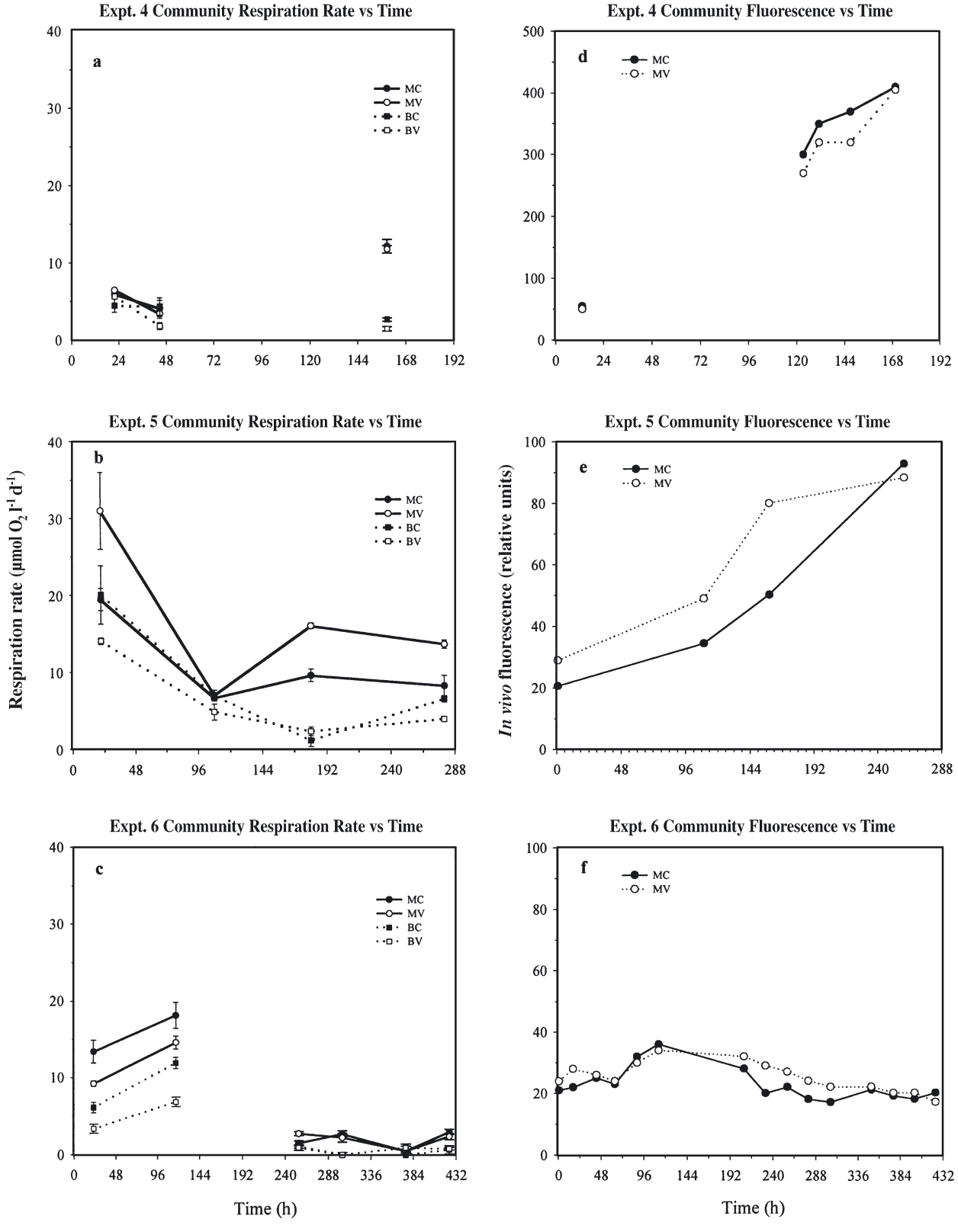

Fig. 5. Microplankton community respiration rate and *in vivo* fluorescence (relative units) as a function of incubation time for natural microplankton assemblages (Expts 4 to 6) in 3 replicate experiments. MC, MV: control and virus-added experiments respectively; BC: bacterioplankton control; BV: bacterioplankton with added virus

natural microplankton communities as well as single-species algae cultures. Nevertheless, the response is far from simple. In the case of the experiments on *Mantoniella* sp., the respiration rate decreased by as much as 96% after inoculating the culture with a viral particle concentrate, while in the case of the *Micromonas pusilla* culture, the respiration rate increased by as much as 235%.

We found that viruses infecting *Mantoniella* sp. and *Micromonas pusilla* are species-specific and co-exist in the same environment as their host. The size and shape of the *Mantoniella* sp. virus (Fig. 3) was very similar to an earlier virus found by Sahlsten & Shaohong (unpubl. data) and to a *Micromonas pusilla* virus described by Cottrell & Suttle (1991) and Sahlsten (1998). Viruses infecting *Mantoniella* sp. and *Micromonas pusilla* seem to be abundant in the environment (Cottrell & Suttle 1991, Sahlsten unpubl. data), reflecting the abundance and wide distribution of their hosts.

The effect of viral addition on respiration rates was consistent in *Mantoniella* sp. experiments even when different assemblages of viruses (i.e. Stn BY38 and enriched *Mantoniella* sp.-specific viruses) were used to infect the algae. The ratio between the number of *Mantoniella* sp.-specific viruses and the number of *Mantoniella* sp. cells was much higher when a stock virus sample was added than when the algal culture was inoculated with a concentrate from a natural viral assemblage. The different specific virus:host ratios used in the 2 experiments with *Mantoniella* sp. could explain the difference in time-lag before any infection was detected.

On the other hand, we do not know the causes for the slower viral infection rate observed in the *Micromonas pusilla* experiment in comparison to others described in the literature (Cottrell & Suttle 1991), but it is possible that the viral concentrate had diminished its infectivity capacity due to storage (Cottrell & Suttle 1995). Unfortunately, we do not have more information about this virus and it may differ from other viruses previously described for *M. pusilla* (Cottrell & Suttle 1991, 1995, Sahlsten 1998).

The only information published to date about the effect of viruses on respiratory rates of marine algae is the work of Robledo et al. (1994) on the pluricellular filamentous brown-algae *Feldmannia irregularis* and *F. simplex* (Phaeophyceae). Robledo et al. (1994) showed that viral infection slightly decreased the respiration rates of both algae species. This trend is similar to the effect on the culture of *Mantoniella* sp. On the other hand, respiration rates of plants may increase or decrease depending on the kind of viral infection developed (Matthews 1981, 1991). The *Micromonas pusilla* experiment showed an increment in respiration rates when viral concentrates were added, suggesting that virus infection increased cell metabolism before lysis.

In spite of the significant decrease in respiration rates due to massive cell lysis observed after 112 and 15 h of incubation in Expts 1 and 2 respectively (Fig. 4a,b), there was always detectable respiratory activity in the virus-treated *Mantoniella* sp. culture, indicating the presence of cells surviving the virus infection. In fact, we observed the recovery of the cultures approximately 1 wk after lysis (results not shown).

Our results show that the respiration rate of *Mantoniella* sp. can decrease between 5 and 26 times due to viral infection, suggesting that, during a bloom of this species, the fate of primary production and carbon fluxes may be strongly modulated by viral infection on a weekly time scale. Whether viruses may be a potential modulating factor for the biogeochemical fluxes in other species remains open.

The results of virus infection experiments with natural microplankton communities may be influenced by viral host-specificity. There are many reports in the literature indicating that viruses are host-specific (Milligan & Cosper 1994, Jacobsen et al. 1996) and even strain-specific in the marine environment (Suttle & Chan 1993, Nagasaki & Yamaguchi 1997, Sahlsten 1998). For instance, Sahlsten (1998) found that 10 isolated viruses were species-specific to *Micromonas pusilla* and even strain-specific to 1–3 of the 6 strains of *M. pusilla*. A recognisable effect of viral infection on community respiration rates can only be detected if those affected are very abundant microplankton species.

Respiration rates of natural microplankton communities reacted to the addition of viral concentrates by increasing (84%, Expts 5 and 6), decreasing (31%, Expt 6), or remaining equal (Expt 4). The causes of these responses are not clear, but at least 2 hypotheses emerge: (1) since viruses are host-specific, it is possible that there was no infection at all during the incubation, or that just 1 or a few non-abundant species were infected and, accordingly, the effect on community respiration rates would not be detectable; or (2) after virus infection, the metabolism of the host species increased before lysis took place. The first hypothesis seems most likely, as fluorescence followed the same trend and even increased both in the controls and in the virus-added communities in 2 of the experiments (Fig. 5d,e), while in the last experiment the fluorescence decreased slightly (Fig. 5f).

Other possible explanations for the absence of a noticeable change in respiration rates after the viral concentrate addition, are: (1) the viral concentrate could have been too dilute, diminishing the virus–host encounter rate and, therefore, not allowing infection to take place; (2) low infective efficiency of the viruses

could be due to the effect of exposure during incubation to light and particulate organic matter (Wommack & Colwell 2000); (3) virus concentrates may have degraded during storage so that the rates of viral impact were underestimated.

In Expt 5, the respiration rate of the microplanktonic community seemed to be affected by viral addition, but fluorescence did not decrease, suggesting that the viruses may have infected only heterotrophic organisms. There is evidence of viral lysis in marine heterotrophic nanoflagellates (Nagasaki et al. 1993, Garza & Suttle 1995) and the presence of lytic viruses in marine heterotrophic nanoflagellates (Nagasaki et al. 1995). Thus, it is possible that, in the viral community concentrated from the Baltic Sea, there may have been viruses present capable of infecting heterotrophic microorganisms of the microplanktonic community. In this case, the viral infection might have had the effect of increasing cellular metabolism, reflected in an increment of respiration rates of the infected organisms. Plant hosts have been known to experience virus-induced synthesis of new proteins, some of which are biologically active substances (enzymes, toxins, etc.) able to interfere with the normal metabolism of the host (Agrios 1970). A similar example is the effect produced by oncogenic viruses in heterotrophic cells of mammals (Murray et al. 1997).

Fuhrman & Suttle (1993) indicated that bacterial respiration can increase 33% because of the enhanced effect of bacterial lysis on the availability of dissolved organic matter due to viral infection. There may have been a similar effect in the *Micromonas pusilla* experiment (Fig. 4c,f) where the respiration rate increased at the time when the fluorescence showed a drastic drop. The rise in the respiration rate might be explained by a few remaining uninfected cells being stimulated by nutrient addition from the lysed cells. In contrast, our results suggest that bacterioplankton respiration rates treated with viral concentrate addition may decrease by about 58% and, therefore, the loss terms (i.e. bacterial mortality/lysis, depression of respiration rates) could be much higher than the gain terms (i.e. metabolic increase due to more availability of energetic substrates).

Unfortunately there was an important temperature difference (10.2 to 12.9°C) between *in situ* and incubation conditions during the experiments (Table 2). However, our goal was to assess whether virus infection affects respiration rates and not to estimate the respiration rate at the *in situ* temperature. The critical comparison was that between respiration rates of microplankton communities treated with, and those not treated with, viral concentrate addition.

In terms of carbon fluxes, our results show that viral infection can affect respiration rates as much as primary productivity (i.e. decrease in 78%; Suttle et al. 1990). However, in the case of the viral effect on respiration rates, the infection can increase or decrease respiration rates by as much as 84 and 31% respectively. Recently, Eissler & Quiñones (2003) observed similar trends whilst conducting experiments to test the effect of viral concentrate addition on respiration rates of microplankton communities from a shallow bay in the Humboldt Current System. Thus, viral infection on microplanktonic organisms could be one of the factors significantly modifying pelagic carbon fluxes, especially by altering the gross production-to-community respiration ratio (P/R). Further research is needed to understand the role of viral infection in both marine photoautotrophic and microheterotrophic organisms and their mediated biogeochemical fluxes in order to attain a more complete picture of the microbial food web and, consequently, a better understanding of the paths of matter and energy in the pelagic ecosystem.

Acknowledgements. We wish to thank Gunnar Bratbak and Mikal Heldal (Department of Microbiology, University of Bergen, Norway) for Y.E.'s training and access to transmission electron microscopy and flow cytometry. We thank Monica Appelgren and Bengt Karlson (Department of Botany, Göteborg University) for providing the algal cultures. We also acknowledge the personnel of the Swedish Meteorological and Hydrological Institute (SMHI) Oceanographic Laboratory for assistance in the field. The SMHI and the Department of General and Marine Microbiology of the Göteborg University (Sweden) facilitated the installations and equipment necessary for conducting the experiments. This research was funded by the 'VIRTUE Project: host specificity and algal-virus interaction' (Swedish Wallenberg Foundation) and by the FONDAP Program (CONICYT, Chile). Y.E. thanks the Swedish Institute for giving her a fellowship to conduct research in E.S.'s laboratory.

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Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

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Submitted: October 11, 2001; Accepted: August 26, 2003
Proofs received from author(s): October 27, 2003