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Dynamics of a lytic virus infecting the photosynthetic marine picoflagellate *Micromonas pusilla*

Matthew T. Cottrell[1] *and Curtis A. Suttle*
Marine Science Institute, University of Texas at Austin, Port Aransas, 78373-1267

Abstract

The impact of *Micromonas pusilla* virus (*Mp*V) on *Micromonas pusilla* was inferred from measurements of the abundance of *Mp*V, the kinetics of *Mp*V adsorption to host cells, and the estimated in situ decay rate of *Mp*V infectivity. The viral production rate was calculated to balance the estimated in situ decay rate of *Mp*V infectivity. In inshore water of the Texas coast, the abundance of infective *Mp*V was high and decreased from 1.3×10^5 ml^{-1} in January 1993 to 2.1×10^3 ml^{-1} at the end of April 1993. Decay rates of *Mp*V infectivity in seawater incubated in the dark ranged from 0.06 d^{-1} at 4°C to 0.09 d^{-1} at 25°C. In unattenuated sunlight, decay rates of infectivity were much higher, ranging from 6.9 to 7.1 d^{-1}. Sunlight-mediated decay rate of viral infectivity was depth-dependent, with an attenuation coefficient estimated to equal 0.73 m^{-1}. The *Mp*V production rate was 0.79 d^{-1}, equal to a turnover time of 1.3 d. *Mp*V abundance changed slowly relative to its turnover time, suggesting a stable coexistence of *M. pusilla* and the lytic virus. The adsorption coefficient for *Mp*V-SP1 and host strain Plymouth 27 was 1.40×10^{-9} ml min^{-1}. Using this coefficient, we calculated that from 2 to 10% of the *M. pusilla* population was lysed per day (avg, 4.4% d^{-1}). These results suggest that lysis of phytoplankton by viruses is a process that needs to be incorporated into models of nutrient and energy cycling in aquatic food webs.

The presence of bacteriophages in seawater has long been recognized (e.g. Kriss and Rukina 1947; Spencer 1955); however, recent observations that viruses are very abundant in seawater (Bergh et al. 1989; Proctor and Fuhrman 1990) have stimulated investigations into the role viruses play in marine ecosystems. A number of independent approaches suggest that on average, ~10–20% of the heterotrophic bacterial community and 3% of *Synechococcus* spp. populations may be lysed daily by viruses (Suttle 1994). These include the enumeration of visibly infected bacteria and cyanobacteria (Proctor and Fuhrman 1990; Heldal and Bratbak 1991; Weinbauer et al. 1993), removal of viral particles (Heldal and Bratbak 1991; Bratbak et al. 1992), decay of viral infectivity (Suttle and Chen 1992), and direct measurements of viral production rates (Steward et al. 1993). However, it has been more difficult to obtain evidence with which to evaluate the importance of viruses as mortality agents of eucaryotic phytoplankton.

Several studies have suggested a relationship between the abundance of viruses and the hosts they infect. In the Southern California Bight, the total viral abundance enumerated with transmission electron microscopy (TEM) was highest in coastal and surface waters, and the variability in viral abundance was largely explained by the abundance of bacteria (Cochlan et al. 1993). Similarly, in Tampa Bay viral abundance was highest in summer, lowest in winter, and positively correlated with chlorophyll *a* concentration and abundance of bacteria (Paul et al. 1991; Jiang and Paul 1994).

Studies of cyanophages that infect marine *Synechococcus* spp. have demonstrated that the abundance of cyanophages is related to the abundance of *Synechococcus* spp. The abundance of cyanophages in the western Gulf of Mexico (Suttle and Chan 1993, 1994) and in Woods Hole Harbor (Waterbury and Valois 1993) covaried with both the abundance of *Synechococcus* spp. and water temperature. In the western Gulf of Mexico, cyanophage abundance decreased along an onshore–offshore transect paralleling the distribution of *Synechococcus* (Suttle and Chan 1993, 1994). These results support the idea that cyanophage abundance is influenced by the abundance of *Synechococcus*.

Through studies such as these, we are beginning to understand the factors responsible for regulating bacteriophages and cyanophages in nature and the roles these viruses play in marine planktonic ecosystems. Much less is known about viruses that infect eucaryotic phytoplankton. Our understanding of these viruses comes primarily from the study of *Micromonas pusilla* viruses (*Mp*V)—a group of lytic, double-stranded DNA viruses specific for the marine photosynthetic picoflagellate *Micromonas pusilla* (Prasinophyceae) (Mayer 1978; Mayer and Taylor 1979). Given that it is a cosmopolitan and abundant component of marine phytoplankton communities (e.g. Manton and Parke 1960; Furuya and Marumo 1983; Hoepffner and Haas 1990) and that the viruses infecting it are also widespread and abundant (Cottrell and Suttle 1991), *M. pusilla* is an excellent model system for examining algal viruses and their impact on phytoplankton.

[1] Present address: College of Marine Studies, University of Delaware, Lewes 19958-1298.

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There were three major components of this study. First, we developed suitable procedures for enumerating *Mp*V in the field and in the laboratory. Second, we followed the dynamics of *Mp*V populations in nature and related these dynamics to changes in sunlight-mediated and sunlight-independent decay rates of viral infectivity. Third, we estimated the proportion of *M. pusilla* that must be lysed daily to support the inferred production rates of *Mp*V in field populations.

Materials and methods

Origin of isolates—*M. pusilla* (strain Plymouth 27) obtained from the Culture Collection of Algae at the University of Texas at Austin (Starr and Zeikus 1993) was used for all studies. *Mp*V strain SP1 (*Mp*V-SP1) was isolated from water collected from the Scripps pier in La Jolla, California (Cottrell and Suttle 1991).

Enumeration of Mp*V with a most probable number assay*—*Mp*V abundance was estimated with a most probable number (MPN) method (Taylor 1962). Aliquots (200 μl) from an exponentially growing culture of *M. pusilla* were added to each well of 96-well microtiter plates (Corning Glass Works, No. 25860). Samples to be assayed for abundance of *Mp*V were diluted with culture media (ESAW) (Harrison et al. 1980 modified with both selenium and Tris-HCl addition as by Cottrell and Suttle 1991) in a series of 10-fold dilution steps. Aliquots (0.1 ml) of each dilution were added to 8 wells in the microtiter plates containing *M. pusilla*. Viruses were not added to controls. Seawater samples were passed through 0.45-μm pore-size polyvinylidene difluoride Durapore filters (Millipore) to remove grazers and algae before preparation of the dilution series. The cultures in microtiter plates were incubated under a continuous quantum flux of 50–70 μmol quanta m^{-2} s^{-1} of photosynthetically active radiation (PAR) at 18°C and were checked daily for 7–9 d for the occurrence of culture lysis. The number of culture wells in which lysis occurred was scored, and the abundance of viruses was determined with a BASIC program (Hurley and Roscoe 1983).

Plaque assay of Mp*V*—The plaque assay was modified from the agar overlay method of Adams (1959). The bottom and top layers consisted of ESAW solidified with 0.2% Phytagel (Sigma, No. P-8169) and 0.4% purified agar (Sigma, No. A-7049) respectively. The top agar was autoclaved, and aliquots (2.5 ml) were dispensed into sterile 13 × 100-mm glass test tubes and placed in a 40°C heat block. An exponentially growing culture (1 liter) of *M. pusilla* was grown to half of maximum cell yield (2×10^7 cells ml^{-1}) and concentrated by centrifugation (92,600 × *g*) for 10 min. The supernatant was poured off, and the cells were resuspended in 2 ml of the supernatant at a final concentration of $\sim 1 \times 10^9$ cells ml^{-1}. An aliquot (200 μl) of the resuspended cells was transferred to a sterile microfuge tube, and a 10-μl sample of the virus solution to be titered was added. After 30 min of adsorption, the cells were transferred to the 40°C top agar, mixed, and poured over the bottom Phytagel. The plates were incubated at 18°C and 50 mol quanta m^{-2} s^{-1} PAR for >9 d.

Comparison of MPN assay, plaque assay, and direct microscopic count—We used three replica dilution series of culture lysate to compare the abundance of *Mp*V enumerated by MPN assay, plaque assay, and direct fluorescence microscopic count. Viruses were stained for fluorescence microscopy with 4′-6-diamidino-2-phenylindole (DAPI) at a final concentration of 1 μg ml^{-1} for >10 h. A 5-μl drop of the stained sample was sandwiched between a glass slide and a cover slip, and 20–200 particles were counted in 20 fields at a magnification of 1,000×. The volume of sample counted was calculated as the volume mounted on the slide multiplied by the area of the counting field divided by the area of the cover slip (Suttle 1993). Triplicate counts made with this procedure had a C.V. of 27%.

Adsorption of Mp*V-SP1 to* M. pusilla—The adsorption coefficient (equivalent to the volume clearance rate) was determined by adding 5 ml of a stock solution of *Mp*V-SP1 (8×10^7 viruses ml^{-1}) to triplicate 40-ml exponentially growing cultures of *M. pusilla* (5×10^6 cells ml^{-1}) held under the same conditions of light and temperature used for growth of the algal cultures. After virus addition, samples (3 ml) were taken every 20 min for 140 min and centrifuged at 7,000 rpm (5,857 × *g*) for 2 min. A 2-ml sample of the supernatant was fixed with 50% biological-grade glutaraldehyde at a final concentration of 1%. The unabsorbed virus particles were counted by epifluorescence microscopy by means of a modification of the protocol reported by Suttle (1993). DAPI (100 μg ml^{-1}) was added to each fixed sample at a final concentration of 1 μg ml^{-1}, and the samples were then incubated in the dark at 4°C for ≥10 h. The stained samples were filtered onto 25-mm-diameter 0.02-μm pore-size Anodisc 25 inorganic membrane filters (Whatman) at a vacuum of <13 cm of Hg. The filters were mounted on a glass slide with a drop of low-fluorescence immersion oil and covered with another drop of immersion oil and a cover slip. A minimum of 20–100 DAPI-stained viruses were counted in 20 fields at a magnification of 1,000×.

The adsorption coefficient (C_d) was calculated from the natural logarithm of the remaining fraction of free viruses plotted against the sampling time. C_d was calculated as $C_d = a/N$, where C_d is in ml min^{-1}, N (No. ml^{-1}) is the abundance of cells, and a (min^{-1}) is the slope determined by linear regression of the natural logarithm of the remaining fraction of free viruses plotted against sampling time. C_d was estimated from the average of triplicate cultures.

Field study—Between January and April 1993, *Mp*V abundance was determined in surface water samples collected weekly from three sampling sites separated by ~10 m located in a private marina of ~70,000 m^2 (Island Moorings, 27°48′N, 97°06′W) near the Marine Science Institute on the north end of Mustang Island, Texas. This

basin is essentially an enclosed body of water that has very limited exchange with the Corpus Christi ship channel, which is connected to the marina by a 2.8-km channel. Seawater temperature was measured with a mercury thermometer, and the attenuation coefficient for PAR was measured with a digital scalar irradiance meter (model QSP 170B with a 200D 4π sensor, Biospherical). Daily quantum flux was measured with a Li-100 quantum datalogger with a cosine collector that measured PAR (LiCor); the datalogger was mounted on the roof of the Marine Science Institute.

Dark decay rates of Mp*V infectivity*—The effect of incubation volume on the measurement of dark decay rate was tested with three 20-liter surface water samples collected adjacent to the Marine Science Institute on 24 March 1991. Three polycarbonate containers (0.25, 4, and 10 liters) were immediately filled with unfiltered water from each sample and incubated in the dark at 18°C. A 1-ml sample was taken from each container for the measurement of naturally occurring *Mp*V abundance by MPN assay after 0, 2, 5, 15, and 36 d. Dark decay rates (k_d) were calculated from the slope of the linear regression of the natural logarithm of viral abundance plotted against sampling time.

The effect of temperature on dark decay rates was examined with seawater collected from the Island Moorings marina on 22 February 1993. The same experimental design was used, except that the seawater was incubated at 4, 10, 18, and 25°C in 250-ml volumes. The relationship between dark decay rate and temperature was established by fitting a second-order polynomial to the decay rates plotted against temperature. In another experiment, 250-ml incubations were conducted at 18°C with surface seawater collected from the Island Moorings marina on 5 January 1993.

Sunlight-mediated decay rate of Mp*V-SP1*—Triplicate samples of *Mp*V-SP1 (25 ml) in ESAW were incubated at the air–water interface in UV-transparent polyethylene bags in unattenuated sunlight from ~0900 to 1800 hours. Incubations were kept at 25°C by means of a water bath. The rate of sunlight-mediated decay of *Mp*V infectivity (k_s) was calculated as the slope of the natural logarithm of the remaining fraction of infectious virus plotted against incubation time. Suttle and Chen (1992) demonstrated that the sunlight-mediated decay of viruses is proportional to the solar quantum flux and that the constant of proportionality between sunlight-mediated decay rate and solar quantum flux varies among viral taxa. Therefore, we estimated the proportionality constant for the relationship between sunlight-mediated decay rate and solar quantum flux for *Mp*V by dividing the observed sunlight-mediated decay rate by the average solar quantum flux during the incubation. Sunlight-mediated decay rates were measured in three experiments conducted on 16 March, 31 March, and 22 April 1993.

Attenuation coefficient of sunlight-mediated decay of viral infectivity—We used bacteriophages enumerated with a high-precision plaque assay rather than *Mp*V enumerated with an MPN assay with much lower precision to determine the attenuation coefficient of sunlight-mediated decay of *viral* infectivity. Duplicate samples of bacteriophages PWH3a-P1 and LB1VL-P1b (Suttle and Chen 1992) in autoclaved, ultrafiltered (<30,000 mw) seawater were incubated in UV-transparent polyethylene bags suspended in the water column at six depths (0, 0.2, 0.5, 1.0, 1.5, and 2 m) for 12-h daytime incubations. The abundance of infective phages was determined at the start and the end of the incubations with plaque assays (Suttle and Chen 1992). Four experiments were conducted (two for each phage) during fall 1992 in the Marine Science Institute boat basin, where the attenuation coefficient for PAR (k_{PAR}) measured with the digital scalar irradiance meter was similar to k_{PAR} in the Island Moorings marina. The rate of sunlight-mediated decay of viral infectivity (k_s) at each depth was calculated as the slope of the natural logarithm of the fraction of infectious virus remaining at the end of the incubation plotted against the incubation time. The attenuation coefficient of the solar radiation causing the sunlight-mediated decay of infectivity (k_{SMD}) was calculated as the slope of the linear regression of the natural logarithm of k_s at each depth plotted against the incubation depth. The average ratio of k_{SMD} to k_{PAR} was determined for the four experiments. The k_{SMD} in the Island Moorings marina was estimated by multiplying k_{PAR} by the average ratio $k_{SMD}:k_{PAR}$.

Estimating the in situ sunlight-mediated decay rate of Mp*V*—The in situ sunlight-mediated decay rate at the seawater surface was estimated by multiplying the solar quantum flux by the proportionality constant between *Mp*V sunlight-mediated decay rate and solar quantum flux. The average sunlight-mediated decay rate for the water column was calculated by integrating the sunlight-mediated decay rate from the surface to the bottom (2.5 m) and dividing by 2.5 m. The integration of sunlight-mediated decay rate was done with the k_{SMD} obtained by multiplying the average k_{PAR} measured in Island Moorings on three dates in January and April 1993 by $k_{SMD}:k_{PAR}$.

Calculation of production rate of Mp*V*—The production rate of *Mp*V during February and March was calculated by subtracting the rate of change of infective *Mp*V abundance from the sum of the dark decay rate and the sunlight-mediated decay rate. The dark and sunlight-mediated decay rates were calculated from the average in situ seawater temperature and average solar quantum flux during February and March, respectively. The rate of change of *Mp*V abundance was calculated as the slope of the linear regression of the natural logarithm of *Mp*V abundance plotted against sampling date for the months of February and March.

Calculation of infection rate, abundance of host required to support viral production rate, and the fraction of host population lysed—The infection rate expressed as the

number of host cells lysed per milliliter per day was calculated from

$$R = V \times P \times (B)^{-1}. \quad (1)$$

R is the infection rate (No. ml^{-1} d^{-1}), V the viral abundance (No. ml^{-1}), P the viral production rate (d^{-1}), and B the burst size (dimensionless).

We assumed that R was equal to adsorption rate in order to calculate the abundance of host cells needed to support the viral production rate:

$$N = P \times (B \times C_d)^{-1} \quad (2)$$

where N is the host abundance (No. ml^{-1}).

The fraction of the host population infected was calculated by dividing N by R.

Results

Enumeration of Mp*V*—The highest estimate of *Mp*V abundance in culture lysate was obtained by fluorescence microscopy, intermediate with the MPN assay, and lowest with the plaque assay (Table 1). The abundance of *Mp*V enumerated by MPN was ~20% of the estimate obtained by fluorescence microscopy but ~8-fold greater than that obtained by plaque assay. The precisions of the fluorescence microscopy, MPN, and plaque assays expressed as coefficients of variation (SD/mean × 100) were similar (27, 28, and 20%, respectively). Plaques were visible after 4 d and reached maximum numbers after 9 d. The precision of the plaque assay was similar to that of the MPN assay, although the number of infective units detected by the plaque assay was considerably less.

Adsorption coefficient of M. pusilla—*M. pusilla* strain Plymouth 27 rapidly cleared *Mp*V-SP1 from the media, with ~35% of the free virus remaining after 125 min (Fig. 1). The C_d for *M. pusilla* was $1.40 \times 10^{-9} \pm 0.42 \times 10^{-9}$ ml min^{-1} (mean ± SD, $n = 3$).

Field study—The abundance of *Mp*V decreased by a factor of ~60 from a maximum of 1.3×10^5 ml^{-1} at the end of January to a minimum of 2.1×10^3 ml^{-1} at the end of April 1993 (Fig. 2). However, the change in abundance was slow enough that differences between weekly samplings were not resolved because their standard errors generally overlapped. We attributed the change in *Mp*V abundance to production and decay because the marina is essentially an enclosed body of water. *Mp*V abundance decreased as seawater temperature increased from 14 to 23°C, except that there was a transient change in temperature between 17 and 14°C in the middle of March associated with the passage of a cold front. During February and March, the average solar quantum flux was 0.329 mmol quanta m^{-2} s^{-1} and seawater temperature was 17°C. The k_{PAR} was 1.1 m^{-1} on 1 January 1993, 0.59

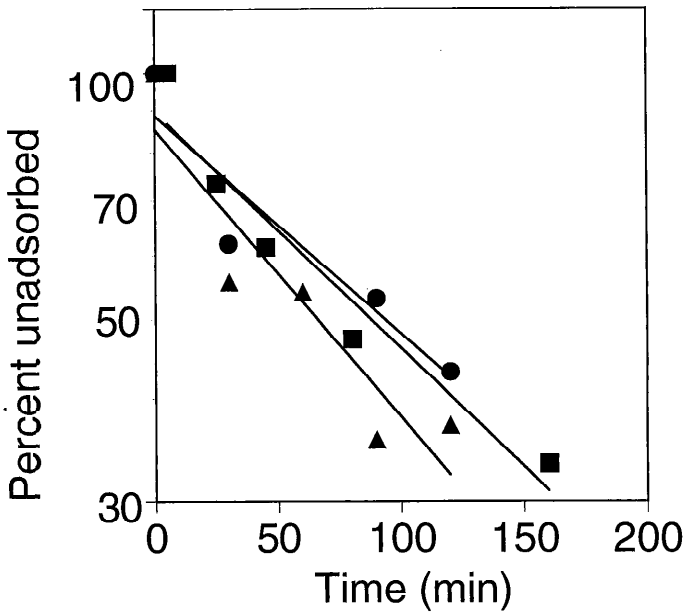

Fig. 1. Adsorption of *Mp*V-SP1 to *Micromonas pusilla* (Plymouth 27). The y-axis is a log scale, and different symbols represent individual cultures. Average adsorption rate constant C_d of the three replicate cultures is 1.40×10^{-9} ml min^{-1}.

Table 1. Estimates of the concentration of *Mp*V-SP1 in two culture lysates from MPN assay, fluorescence microscopy, and plaque assay. (Not determined—nd; coefficient of variation—C.V.)

Culture lysate	Dilution series	MPN	Microscopy	Plaque	Microscopy/MPN	Plaque/MPN
			(×10^7 ml^{-1})			
A	1	1.6	13	nd	8.1	nd
	2	3.7	14	nd	3.8	nd
	3	3.1	9.3	nd	2.9	nd
		C.V. = 38%	C.V. = 27%		mean = 5.0	
B	1	5.9	nd	0.59	nd	0.10
	2	5.9	nd	0.83	nd	0.14
	3	4.2	nd	0.61	nd	0.15
		C.V. = 18%		C.V. = 20%		mean = 0.13

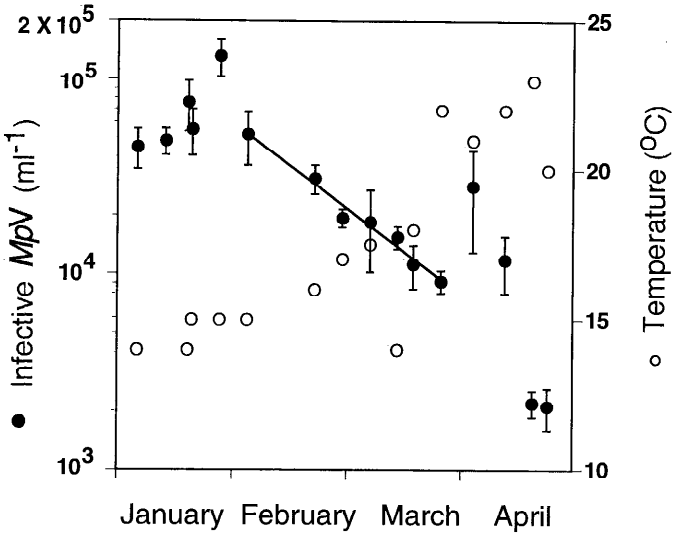

Fig. 2. Seawater temperature and *Mp*V abundance (SE, n = 3) measured by MPN assay with *Micromonas pusilla* (Plymouth 27), January–April 1993. The slope of the regression line through the February and March virus abundance is 0.035 d^{-1}.

on 2 January, and 0.86 on 22 April, with an average value of 0.85 m^{-1}.

The change in abundance of *Mp*V between January and April occurred in three phases. During January, *Mp*V abundance was relatively constant, except for an apparent peak at the end of the month. Subsequently, *Mp*V abundance decreased exponentially in February and March at a rate of 0.035 d^{-1} (r^2 = 0.85), increased at the beginning of April, and decreased until the end of April.

Attenuation coefficient of sunlight-mediated decay of viral infectivity—The sunlight-mediated decay rate of infectivity (k_s) for bacteriophages PWH3a-P1 and LB1VL-P1b decreased exponentially with increasing depth of incubation in the water column, and the square of the correlation coefficient between the natural logarithm of k_s and depth was >0.86 (Table 2). The attenuation coefficient for sunlight-mediated decay of viral infectivity (k_{SMD}) determined from the slopes of the regression of k_s against depth ranged from 0.63 to 2.2 m^{-1}. The k_{PAR} during these incubations ranged from 0.96 to 2.1 m^{-1}, which overlapped k_{PAR} measured at the Island Moorings marina, and k_{SMD} : k_{PAR} was 0.85±0.10 (mean ± SE, n = 4). The estimate of k_{SMD} at Island Moorings, obtained by multiplying the k_{PAR} at Island Moorings by the average k_{SMD} : k_{PAR} observed at the Marine Science Institute boat basin, was 0.73 m^{-1}.

Dark and sunlight-mediated decay rates of Mp*V infectivity*—The decrease of naturally occurring *Mp*V abundance in 4, 10, 18, and 25°C incubations was linear when plotted as the logarithm of the fraction of infective viral remaining vs. incubation time ($r^2 \geq 0.82$) (Fig. 3). Decay rates calculated as the slopes of the semilogarithmic plots increased with temperature and ranged from 0.058 d^{-1} at 4°C to 0.085 d^{-1} at 25°C. To predict dark decay rate from in situ seawater temperature, we fit the relationship between temperature and dark decay rate by the equation k_d (d^{-1}) = 6.44×10^{-5} T^2 (°C) − 5.47×10^{-4} T (°C) + 5.88×10^{-2} (R_2^2 = 0.99, R_1^2 = 0.92, R_0^2 = 1.00) (Fig. 4). The temperature coefficient of the dark decay rate was low [$Q_{10(5-15)}$ = 1.1, $Q_{10(15-25)}$ = 1.3], indicating that over this environmental range temperature would have a relatively small effect on dark decay rates. The small difference between these temperature coefficients was likely not significant. Dark decay rates of *Mp*V measured in two seawater samples taken from the Island Moorings marina on 5 January and 22 February 1993 and incubated at 18°C were not significantly different, and dark decay rates were not significantly different when measured in 0.25-, 4-, and 10-liter incubations at 18°C (Table 3).

The infectivity of *Mp*V was sensitive to solar radiation. The abundance of infective *Mp*V remaining after 0.25–0.31-d exposure to sunlight ranged from 10.1 to 17.9% (Table 4). Decay constants ranged from 4.9 to 5.3 d^{-1} (mmol quanta m^{-2} $s^{-1})^{-1}$ (5.0±0.13; mean ± SE, n = 3). The average sunlight-mediated and dark decay rates during February and March were estimated to be 0.76 and 0.068 d^{-1}, respectively.

Table 2. Attenuation coefficient for sunlight-mediated decay of virus infectivity (k_{SMD}) and its relationship to the attenuation coefficient for PAR (k_{PAR}). Variables in the linear regression are natural logarithm of the rate of sunlight-mediated decay of virus infectivity (y) and depth (x). k_{SMD} is equal to the slope of the linear regression.

		Linear regression analysis					
1992	Phage	Slope	Intercept	r^2	k_{SMD} (m^{-1})	k_{PAR} (m^{-1})	k_{SMD} : k_{PAR}
5 Aug	PWH3a-P1	2.20	−1.02	0.96	2.2	2.1	1.0
18 Aug	LB1VL-P1b	1.44	−0.34	0.88	1.4	2.1	0.67
17 Sep	LB1VL-P1b	1.48	−0.76	0.93	1.5	1.5	1.0
9 Dec	PWH3a-P1	0.63	−1.66	0.86	0.63	0.96	0.66
							mean = 0.83 SE = 0.10 n = 4

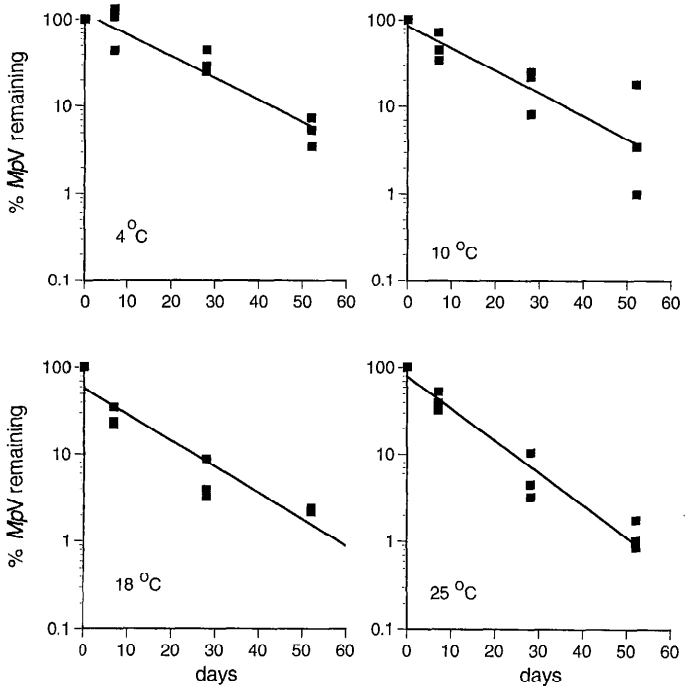

Fig. 3. Decay of *Mp*V infectivity in seawater incubated in the dark at 4, 10, 18, and 25°C. The dark decay rate (k_d) at each temperature is equal to the slope of the regression equations $y = -0.0576x + 10.5$, $r^2 = 0.90$ (4°C), $y = -0.0599x + 10.2$, $r^2 = 0.82$ (10°C), $y = -0.0679x + 9.79$, $r^2 = 0.87$ (18°C), and $y = -0.0854x + 10.1$, $r^2 = 0.95$ (25°C). Each point represents a measurement made from a single bottle.

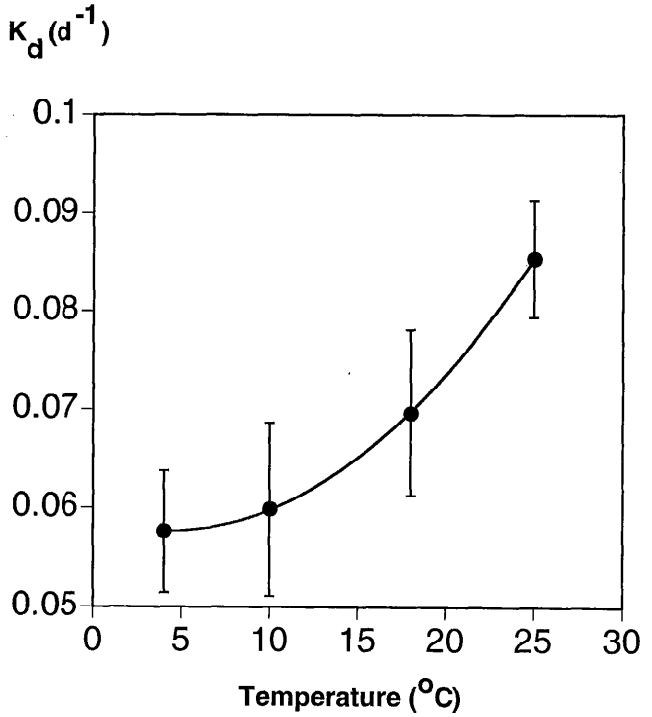

Fig. 4. Dark decay rate of *Mp*V infectivity (k_d) in seawater incubated in the dark at temperatures ranging from 4 to 25°C. Error bars represent 1 SE of the mean, $n = 3$. The equation of the regression is given in the text.

Viral production rate, host abundance required to support viral production rate, and the fraction of host population lysed—The difference between the net decrease of *Mp*V abundance (0.035 d^{-1}) and the sum of the sunlight-mediated and dark decay rates (0.82 d^{-1}) yielded an estimate of the *Mp*V production rate during February and March equal to 0.79 d^{-1} (turnover time = 1.3 d).

We calculated that the infection rate decreased from 570 to 100 cells ml^{-1} d^{-1} between 5 February and 26 March (Table 5). The estimated host abundance required to support the production rate and the fraction of the host population lysed were dependent on the value of the adsorption coefficient. Using the maximum theoretical adsorption coefficient (7.03×10^{-9} ml min^{-1}), we estimated *M. pusilla* abundance to be 1,100 ml^{-1} and 9–51% lysed per d. We estimated that *M. pusilla* abundance was 5,400 ml^{-1} and 2–10% lysed per d using the C_d measured in this study (1.40×10^{-9} ml min^{-1}).

Table 3. Dark decay rates (k_d) of naturally occurring *Mp*V incubated in unfiltered seawater in the dark.

	Incubation temp. (°C)	Incubation vol. (liter)	k_d (d^{-1})	SE
24 Mar 91*	18	0.25	0.122	0.007
	18	4	0.117	0.010
	18	10	0.114	0.007
5 Jan 93†	18	0.25	0.095	0.014
22 Feb 93†	4	0.25	0.058	0.007
	10	0.25	0.060	0.004
	18	0.25	0.070	0.008
	25	0.25	0.085	0.008

* Samples collected from the Marine Science Institute boat basin.
† Samples collected from the Island Moorings marina.

Table 4. Sunlight-mediated decay rates of *Mp*V-SP1 infectivity and the constant of proportionality between sunlight-mediated decay (k_s) and average solar quantum flux (PAR).

1993	(%) Infective virus remaining	Incubation time (d)	Avg quantum flux I (mmol quanta m^{-2} s^{-1})	Decay rate k_s (d^{-1})	k_s/I
16 Mar	17.9	0.25	1.3	6.9	5.3
31 Mar	17.7	0.25	1.4	6.9	4.9
22 Apr	10.1	0.31	1.5	7.4	4.9
					mean = 5.0
					SE = 0.13
					$n = 3$

Table 5. Infection rate, abundance, and fraction of *Micromonas pusilla* lysed per day. *Mp*V production rate, 0.79 d^{-1}. The rates were calculated assuming a burst size of 72 (Waters and Chan 1982) and using adsorption coefficients of 7.03×10^{-9} ml min^{-1} (Waters and Chan 1982) and 1.40×10^{-9} ml min^{-1} (this study).

			$C_d = 7.03 \times 10^{-9}$ ml min^{-1}		$C_d = 1.40 \times 10^{-9}$ ml min^{-1}	
1993	*Mp*V (ml^{-1})	Infection rate (cells $ml^{-1}d^{-1}$)	*M. pusilla* (ml^{-1})	% *M. pusilla* lysed d^{-1}	*M. pusilla* (ml^{-1})	% *M. pusilla* lysed d^{-1}
5 Feb	52,000	570	1,100	51	5,400	10
22 Feb	31,000	340	1,100	31	5,400	6
1 Mar	19,000	210	1,100	19	5,400	4
8 Mar	19,000	210	1,100	19	5,400	4
15 Mar	16,000	180	1,100	16	5,400	3
19 Mar	11,000	120	1,100	11	5,400	2
26 Mar	9,200	100	1,100	9	5,400	2

Discussion

Enumeration of Mp*V*—There are two nonmutually exclusive possible reasons that only a portion of the viruses visible by epifluorescence microscopy were detected by the MPN assay: either a portion of the virus particles was infective or the MPN assay detected only a portion of the infective particles. The MPN assay would underestimate the infective viral abundance if infective viruses adsorb to material other than living host cells. This would include nonviable cells, other particulate material, and the surface of the culture vessel. In an exponentially growing culture, the particulate material would be dominated by living host cells; hence, adsorption to nonliving particulate material is probably of minor importance. Therefore, the MPN assay probably reflects the abundance of infective *Mp*V in culture lysate and in seawater.

The reason only a fraction of the *Mp*V in lysed cultures is infective is likely that viruses adsorb to cell debris produced during cell lysis. Inactivation of bacteriophages occurs because of adsorption of viruses to cell debris, and this inactivation occurs to a lesser extent in lysates produced from cultures with low concentrations of cells (Sagik 1954). The *Mp*V lysates used in this study were produced from dense cultures; therefore, adsorption of *Mp*V to host cell debris probably accounts for the difference between estimates of *Mp*V abundance made by microscopic counts and MPN assay. Because MPN estimates were consistent with limiting dilutions of seawater that produced clonal *Mp*V isolates (Cottrell and Suttle 1991), the MPN assay probably detected all of the infective MPV in seawater.

The agent used to solidify media in the plaque assay was an important variable influencing the growth of *M. pusilla* as lawns. Agar-solidified media laid over phytagel-solidified media produced denser lawns than agar-solidified media laid over agar-solidified media. Plaques were visible after 4 d and reached maximum numbers after 9 d. The precision of the plaque assay was similar to that of the MPN assay, but the number of infective viruses detected by the plaque assay was considerably smaller (Table 1). Given the lower efficiency of the plaque assay, it is probably best suited for purification of clonal isolates of *Mp*V rather than for enumerating infective *Mp*V.

Adsorption coefficient of M. pusilla—An estimate of C_d was needed to estimate the abundance of *M. pusilla* and the fraction of *M. pusilla* lysed per day. Two approaches can be used: C_d can be calculated from the diffusive transport of viruses to cells assuming all contacts between hosts and viruses yield adsorption (Murray and Jackson 1992), or it can be measured in culture. Murray and Jackson (1992) calculated the C_d for *M. pusilla* and *Mp*V to be 7.01×10^{-9} ml min^{-1}, which agreed with 7.03×10^{-9} ml min^{-1} measured in culture by Waters and Chan (1982). However, our C_d for *M. pusilla* and *Mp*V-SP1 was 1.40×10^{-9} ml min^{-1} (Fig. 1). The difference between our results and those of Waters and Chan is probably related to the manner in which the viruses used for the experiments were isolated. They used a clone of *Mp*V (*Mp*V1) that was isolated from a lysate; the *Mp*V-SP1 we used was cloned directly from seawater. Amplifying *Mp*V before cloning probably selected for a strain that adsorbed and amplified efficiently. In contrast, *Mp*V-SP1 cloned directly from seawater is more likely representative of naturally occurring *Mp*V populations. Nevertheless, these results emphasize the possibility that a large range in adsorption kinetics may exist in natural populations of *Mp*V.

Dark and sunlight-mediated decay rates of Mp*V infectivity*—A number of variables may influence the dark decay rate of viral infectivity. The highest dark decay rates for *Mp*V were lower than those measured for marine bacteriophages (0.216–0.672 d^{-1}) incubated in seawater in the dark at 23–25°C (Suttle and Chen 1992), suggesting that dark decay rates vary among viral taxa. The dark decay rate of *Mp*V in seawater collected from the Marine Science Institute boat basin on 24 March 1991 and incubated at 18°C was significantly ($P < 0.05$) higher (1.7-fold) than the rate in Island Moorings on 22 February 1994, indicating that dark decay rates may vary between locations and (or) times. In contrast, the dark decay rates measured at 18°C for *Mp*V collected from the Island Moorings marina on 5 January and 22 February 1993 (Table 3) were not significantly different, suggesting that the dark decay rates we used in our calculations were representative of this location in early spring 1993.

Sunlight-mediated decay rates also vary among viral taxa and water types. The decay rate of *Mp*V-SP1 in natural sunlight [5.0 d^{-1} (mmol quanta m^{-2} $s^{-1})^{-1}$] was 80% of the average for bacteriophages and cyanophages [6.26 d^{-1} (mmol quanta m^{-2} $s^{-1})^{-1}$, Suttle and Chan 1994]. The attenuation coefficient for sunlight-mediated decay of viral infectivity varies greatly with water type (i.e. 0.36 m^{-1} in the Adriatic Sea and 7.27 m^{-1} in the Danube River, Rontó et al. 1994). A significant strength

of our study is the use of an attenuation coefficient for sunlight-mediated decay (0.73 m^{-1}) obtained from experiments conducted in a water type similar to that in the Island Moorings marina.

Relative to the depth of the water column, the solar radiation causing sunlight-mediated decay of viral infectivity penetrated deeply. From the estimate of the attenuation coefficient for sunlight-mediated decay of viral infectivity (0.73 m^{-1}), the sunlight-mediated decay rate at the bottom (2.5 m below the surface) was 16% of the surface rate.

Viral production rate—A number of approaches have been used to estimate viral production rates, including the enumeration of bacteria containing mature virus particles (e.g. Proctor and Fuhrman 1990; Proctor et al. 1993; Weinbauer and Peduzzi 1994), the removal rate of virus particles (Heldal and Bratbak 1991; Bratbak et al. 1992), the decay rate of infectivity of viruses added as tracers (Suttle and Chen 1992; Suttle and Chan 1994), and the direct measurement of viral production by a radiotracer-based method (Steward et al. 1993). There are significant uncertainties associated with these production rates: decay of infectivity varies among viral taxa and those used as tracers may not be representative of those in a natural community; conversion factors used to calculate the total number of infected cells from the number visibly infected are obtained from bacterial isolates and culture conditions likely not representative of those in nature; and estimates based on the decay of virus particles are extremely rapid and cannot be balanced by measured bacterial production rates.

In this study we used the decay of viral infectivity to measure viral production in a single host-specific viral system, which alleviated the need to extrapolate from potentially unrepresentative systems to the system under investigation. We assumed that during February and March, *Mp*V production and decay were approximately balanced, the difference between them producing the observed net change in infective *Mp*V abundance (0.035 d^{-1}). Subtracting the net decrease of infective *Mp*V abundance (0.035 d^{-1}) from the sum of the sunlight-mediated and dark decay rates (0.82 d^{-1}) yielded an estimated *Mp*V production rate during February and March of 0.79 d^{-1} (turnover time, 1.3 d).

From the maximum adsorption coefficient measured by Waters and Chan (1982), the *M. pusilla* abundance required to support the estimated *Mp*V production rate was 1,100 ml^{-1}; it was 5,400 ml^{-1} when we used the lower adsorption coefficient measured in this study (Table 5). In situ adsorption coefficients are unknown and may be less than the rate measured by Waters and Chan and the maximum theoretical value calculated from diffusive transport in which every contact between host and virus yields adsorption (Murray and Jackson 1992). Rates lower than the theoretical maximum might explain the paradoxical presence of virus-infected cells during a persistent and dense bloom ($\sim 10^6$ cells ml^{-1}) of *Aureococcus anophagefferens* (Chrysophyceae) (Sieburth et al. 1988) or cells in the presence of high viral abundance (Suttle and Chan 1994).

Our results suggest that *Mp*V was more abundant than *M. pusilla*. This seems counterintuitive; however, similar observations have been made for the concentration of cyanophages which infect *Synechococcus* and the abundance of *Synechococcus* spp. (Suttle and Chan 1994). Ultimately, the production of lytic viruses is dependent on the adsorption of viruses to host cells. Because the efficiency of adsorption and infection can vary (i.e. not all contacts must result in adsorption and infection), the co-occurrence of host cells with higher concentrations of infective virus is consistent with our model and with empirical results. When viral production is not dependent on virus adsorption (e.g. the induction of lysogens), factors which induce lysogens (e.g. cause DNA damage) or influence the frequency of spontaneous induction may influence the viral production rate and yield different virus-host dynamics.

Impact of viruses on mortality—The key to determining the role of lytic viruses in aquatic systems is knowing what fraction of phytoplankton and bacterioplankton mortality is caused by viral lysis. Mortality rates inferred from the fraction of cells infected daily have been estimated with the same approaches used to estimate viral production rates, as discussed above. Enumerating visibly infected cells with TEM yields a relatively direct estimate of the fraction of infected host cells. However, this estimate is sensitive to uncertainty in the fraction of the infection cycle during which mature viruses are visible. Less directly, production estimates based on the loss of virus particles or the decay of viral infectivity can be divided by estimates of burst size and host abundance to obtain an estimate of the fraction of cells infected daily.

The fraction of *M. pusilla* lysed ranged from 9 to 51% per day when we used the maximum C_d measured by Waters and Chan (1982) (Table 5). *M. pusilla* doubling once per day could support infection rates as high as 50% per day, which suggests that the maximum C_d could have been operating in situ. In contrast, the maximum C_d is not necessary to support the turnover of *Mp*V and could be offset by higher *M. pusilla* abundance. If the C_d was equal to the lower rate we measured, then infecting 2–10% of *M. pusilla* per day with a 5-fold higher abundance would have supported the turnover of *Mp*V (Table 5).

The lower estimates of the fraction of the *M. pusilla* population lysed (2–10% d^{-1}) are likely the more reasonable because they are based on an estimate of the adsorption coefficient obtained from a clone of *Mp*V obtained directly from seawater. These lower estimates are similar to estimates based on contact rate of the proportion of *Synechococcus* spp. infected daily by cyanophages in coastal waters of the Gulf of Mexico (Suttle and Chan 1994) and Woods Hole Harbor (Waterbury and Valois 1993) as well as estimates based on the proportion of *Synechococcus* spp. cells containing visible cyanophages (Proctor and Fuhrman 1990). Moreover, Suttle (1994) suggested that rates of primary productivity would be 2–3% higher in the absence of the viral size fraction.

Implications of the impact of viruses on M. pusilla—The maintenance of a high abundance of viruses that lyse *M. pusilla* ($\sim 10^3$–10^5 ml^{-1}) with a rapid turnover time (1.3 d) implies that viruses could be responsible for a moderate amount of mortality in *M. pusilla* populations. Calculations indicate that *M. pusilla* must be infected at the rate of hundreds of cells ml^{-1} d^{-1} in order to support the loss of viral infectivity. In the inshore environment we studied, *Mp*V probably infected a moderate fraction of *M. pusilla* (avg, 4% d^{-1}; range, 2–10% d^{-1}). The slow change in *Mp*V abundance relative to its turnover time suggests that *Mp*V maintained a stable coexistence with *M. pusilla* rather than causing rapid declines in *M. pusilla* abundance.

Other studies have demonstrated that viruses can be important agents of mortality in natural communities of heterotrophic bacteria (e.g. Proctor and Fuhrman 1990; Heldal and Bratbak 1991; Weinbauer et al. 1993) and cyanobacteria (Proctor and Fuhrman 1990; Suttle and Chan 1994). Our observations extend these results and demonstrate that viruses can be a significant source of mortality for populations of eucaryotic phytoplankton. Hence, viral-mediated mortality of phytoplankton is a process that needs to be understood and incorporated into models of nutrient and energy cycling in aquatic systems.

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