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The Effect of Cyanophages on the Mortality of *Synechococcus* spp. and Selection for UV Resistant Viral Communities

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ABSTRACT

Viruses that cause lysis of *Synechococcus* spp. are present throughout the year in the western Gulf of Mexico. The effect of sunlight on loss rates of cyanophage infectivity was determined by incubating natural cyanophage communities and cyanophage isolates (strains S-PWM1 and S-PWM3) in UV-transparent bags at the surface, and at depth, on several occasions throughout the year. Decay rates of infectivity of natural cyanophage communities at the surface, at Port Aransas, Texas, USA, ranged from undetectable to 0.335 h^{-1}, with the highest rates occurring during the summer. During the spring and winter, decay rates of cyanophage isolates and natural cyanophage communities were generally similar, but during summer, decay rates of isolates were as much as twofold higher than the natural communities. In situ incubations at two offshore stations during a bloom of *Synechococcus* spp. produced decay rates of 0.53 and 0.75 d^{-1}, integrated over the mixed layer and averaged over 24 h. Based on a burst size of 81 viruses produced per lysed cell (measured for natural cyanobacterial communities in the Gulf of Mexico), cyanophages imposed mortality rates of 1 and 8%, respectively, on *Synechococcus* spp. In contrast, in nearshore incubations in the winter and spring, cyanophages were responsible for removing <1% of the *Synechococcus* cells on a daily basis. Only an estimated 2 to 3% of contacts led to viral infections (based on theoretical contact rates between host cells and cyanophages, and estimates of cyanophage mortality), regardless of the time of year or concentrations of viruses and hosts. These results indicate that natural cyanophage communities tolerate damage by solar radiation better in summer than in winter. Moreover, net decay rates of cyanophage infectivity in sunlight were similar, whether host cells were present or not, indicating that detectable cyanophage production did not occur during daytime in situ incubations.

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Introduction

Viruses are an abundant and dynamic component of marine microbial communities and range in concentration from $<10^5$ ml^{-1} to $>10^8$ ml^{-1} [3, 4, 10]. The range in viral abundances reported for the Gulf of Mexico is similar to the highest levels associated with the most productive environments [2, 13, 26, 27, 39]. Not only are viruses abundant, but they are significant agents of microbial mortality. Estimates using a variety of approaches indicate that, on average, ca. 10–20% of the bacterial community and 5–10% of *Synechococcus* cells are infected by viruses at any given time [4, 10, 34]. In some cases, viral-mediated mortality on microorganisms is comparable to that imposed by flagellate grazing [9, 33]. Consequently, viral lysis may alter carbon flow to higher trophic levels [8, 23].

The realization that viruses are abundant and important agents in the mortality of aquatic microorganisms has led to studies to determine the factors responsible for, and the rates of, viral removal in marine waters [12, 38], with the idea that viral removal rates must be balanced by viral production. Not surprisingly, marine viruses are susceptible to a suite of destructive processes, including adsorption to particles [38], elevated temperature [7], exposure to solar radiation [25, 38, 47], and grazing by heterotrophic nanoflagellates [11]. Viruses are particularly sensitive to solar radiation. Calculations suggest that, in some environments, sunlight can destroy the infectivity of viruses in the surface mixed layer daily [7, 38]. Recent evidence, however, indicates that much of this damage may be repaired by photoreactivation [43]. The decay of viral infectivity does not imply the destruction of viral particles, as measured destruction rates of viral particles are typically much less than decay rates of infectivity [40, 47]. Clearly, the fate of infectious particles is ecologically most important, as infectivity is the mechanism through which viruses exert ecological and biogeochemical influence. However, the lack of suitable host systems precludes measurement of the total number of infectious viruses in natural viral communities. Consequently, the approach that a number of investigators have taken is to use marine viral isolates as model systems to trace the fate of infectious viruses in nature [25, 38, 47]. Because there is considerable variation among isolates in their sensitivity to environmental conditions [22, 25, 38] and in the extent to which host cells can restore infectivity to UV-damaged viruses [45], the concern remains whether loss rates of infectivity in viral isolates are representative of those in natural viral communities. Clearly, the best approach is to follow indigenous populations of viruses that infect specific hosts, as has been done for viruses which infect the photosynthetic picoplankter *Micromonas pusilla* [7].

Another group of viruses that is potentially useful for inferring dynamic processes in natural viral communities is that which infects cyanobacteria of the genus *Synechococcus*. These viruses can occur at concentrations in excess of 10^5 ml^{-1} [35] and are diverse and dynamic components of natural marine viral communities [36, 37, 42, 46]. Moreover, unlike viruses infecting *M. pusilla* (which are large icosahedrons belonging to the Phycodnaviridae) [5, 6], cyanophages are morphologically similar to the three families of tailed bacteriophages that are abundant in marine viral communities [36, 42]. Therefore, processes affecting these viruses should be similar to those affecting other bacteriophages, making cyanophages excellent models for determining the mechanisms associated with viral production and destruction in nature (without the need to follow the dynamics of added surrogate viruses).

In this study, the rates of destruction of natural cyanophage communities and cyanophage isolates in the western Gulf of Mexico were examined. There were two objectives of this study. The first was to measure the in situ destruction and production rates of natural communities of cyanophages which infect *Synechococcus* spp. This was done to determine if production rates of infectious viruses during the day were adequate to balance measured rates of viral destruction. The data also permit one to infer the impact of cyanophages on mortality rates of *Synechococcus* spp. The second objective was to determine if the decay rates of cyanophage isolates are representative of natural cyanophage communities.

Materials and Methods

Sampling and Sampling Locations

On eight dates between November 1994 and November 1995, 20-liter water samples containing natural populations of *Synechococcus* spp. and cyanophages were collected near dawn, just below the surface, from the University of Texas Marine Science Institute boat harbor (27°50′N, 97°02′W) or nearby Island Moorings Marina (27°48′N, 97°06′W), placed in polyethylene carboys, and transported to the laboratory. In addition, on 26 and 28 June 1995, a submersible pump was used to collect predawn samples from 1 m depth at Station E (27° 00′ N, 96° 14′ W) and Station F (27° 32′ N, 96° 45′ W) in the western Gulf of Mexico. Seawater was left whole or filtered through either a 0.22-µm pore-size polyvinyl difluoride membrane filter (Durapore, Millipore, Ltd.) (boat harbor) or a 0.2-µm pore-size Nuclepore polycarbonate cartridge filter (Stations

E and F), to remove zooplankton grazers, bacteria, and host cells, while leaving the natural viral communities intact.

During experiments, surface photosynthetically active radiation (PAR; 400–700 nm) was measured every 5 s and integrated over 15 min intervals with a Licor Li-1000 datalogger equipped with a cosine collector. At Stations E and F, attenuation coefficients of 305-, 320-, 340- and 380-nm radiation, as well as PAR, were determined with a Biospherical PUV-500 profiling radiometer.

Synechococcus *spp. and Cyanophages*

The cyanobacterium *Synechococcus* sp. strain DC2 (= WH7803) was obtained from the Center for Culture of Marine Phytoplankton at Bigelow Laboratory and grown at 25°C in artificial seawater under 5–20 µmol quanta $m^{-2}s^{-1}$ continuous photosynthetically active radiation [36]. The cyanophage isolates S-PWM1 and S- PWM3 were previously isolated from the Gulf of Mexico, using *Synechococcus* sp. strain DC2 as the host organism [36]. The viruses were amplified by adding cyanophage S-PWM1 or S-PWM3 to a 1–l culture of exponentially growing *Synechococcus* sp. DC2, at a virus to host-cell ratio of 1. After complete lysis (usually 1 to 2 days post-infection) lysates were filtered through a 0.45-µm pore-size polyvinyl difluoride membrane filter (Durapore, Millipore), to remove host-cell debris, and stored at 4°C. Viral titer in the lysate was measured by MPN assay (see below) and was ca. 10^8 infectious units ml^{-1}.

Enumeration of Synechococcus *and Infectious Cyanophages*

Synechococcus spp. was enumerated by direct count, using an Olympus IMT-2 epifluorescence microscope equipped with a 100 W mercury lamp and a green excitation filter set. Seawater samples (10–20 ml) were gently filtered (<17 kPa vacuum) onto 0.2-µm pore-size black polycarbonate membrane filters, then mounted on glass slides with a drop of immersion oil. A minimum of 20 fields and 200 cells were counted for each filter.

The titer of naturally occurring cyanophages and viral isolates was estimated by a most-probable-number (MPN) assay using *Synechococcus* sp. DC2 as the host organism [36]. Titer was determined by adding 200 µl of exponentially growing cells to each well of a microwave-sterilized [20], 96-well microtiter plate (Corning #25803). Samples to be titered were subjected to a 7-step, 10× dilution series, using artificial seawater as the diluent [6]. A 100-µl aliquot from each step of each dilution was added to each of the 16 wells on the microtiter plate. The number of wells in which lysis occurred was scored and the viral titer was determined using a BASIC program [15]. Data are presented as means and standard deviations of triplicate samples.

Decay and Production Rates of Natural Cyanophage Communities

Decay rates of natural cyanophage communities in the dark were determined for triplicate seawater samples collected from Aransas Pass, adjacent to the boat harbor. Unfiltered seawater (225 ml) was added to sterile, 250-ml polycarbonate flasks and kept in the dark at 4, 14, 18, and 25°C for 35 days. On five occasions, a 1-ml aliquot was withdrawn from each sample and cyanophage titer was assayed. The decay rate of infectivity at each temperature was determined from the slope of the linear regression between the natural logarithm of cyanophage titer and time [7].

In situ decay rates of natural viral communities and cyanophage isolates were determined in triplicate samples collected before dawn from the boat harbor on five dates between October 1994 and November 1995, and at Stations E and F during June 1995. Seawater samples were gently filtered (<17 kPa vacuum) through 0.22-µm pore-size polyvinyl difluoride filters, or left whole. Aliquots (30 ml) of either filtered or whole seawater were placed in thrice-rinsed, 250-ml, UV-transparent polyethylene Fisherbrand bags (320 and 305 nm radiation attenuated by 9 and 12%, respectively). The filtered and unfiltered samples were heat-sealed in the centers of the bags, then attached to PVC frames [19]. The frames were floated at the surface and suspended at several depths (Fig. 1), from dawn to dusk, on sunny days. Cyanophage isolates (ca. 10^4 infectious units ml^{-1}) were prepared in a similar manner and incubated, in parallel, in filtered and unfiltered seawater. Dark controls were kept at in situ temperature for the duration of the incubation. Following incubation, samples were kept in the dark at 4°C until the abundance of cyanophages was titered (within 6 h).

Viral decay rate (K) at any depth (z) was calculated as: $K_z = [\ln (C_{t0}) - \ln (C_{tE}) / t$, where C_{t0} and C_{tE} are the cyanophage titers at the start and end of the incubation, respectively, and t is the duration of the incubation in hours. Light-mediated decay rates were normalized to changes in viral titer in the dark [38].

Decay rates with depth for natural cyanophage communities were better described using an equation comprising two exponential functions, $K_z = ae^{(-k_1 z)} + be^{(-k_2 z)}$ (Stations E and F), where K is the viral decay rate (h^{-1}) at depth (z), and a and b are the decay rates at the surface which are attributed to wavelengths with attenuation coefficients in the water column of k_1 and k_2, respectively.

The Impact of Viral Infection on Synechococcus

The impact of cyanophage infection on the mortality of *Synechococcus* spp. was estimated by measuring the decay of infectious cyanophages and assuming that viral production balanced removal [36]. Dividing the estimated viral production by the measured burst size of 81 cyanophages per lytic event (measured for natural *Synechococcus* communities in the Gulf of Mexico; Weinbauer, unpubl. data) yielded the number of host cells in which lysis must have occurred in order to balance the measured viral removal. These rates were compared to the calculated contact rates between *Synechococcus* spp. and cyanophages (see below). As the infection rate cannot exceed the encounter rate between viruses and host cells, the calculated contact rates are an estimate of the maximum infection rate.

Contact rates between *Synechococcus* and cyanophages were calculated as in Suttle and Chan [37], using the model of Murray and Jackson [24]. Briefly, contact rates R (encounters ml^{-1} d^{-1}) were calculated as: $R = (\mathrm{Sh}\ 2\pi\ d\ D_v)\ V\ P$, where Sh is the Sherwood

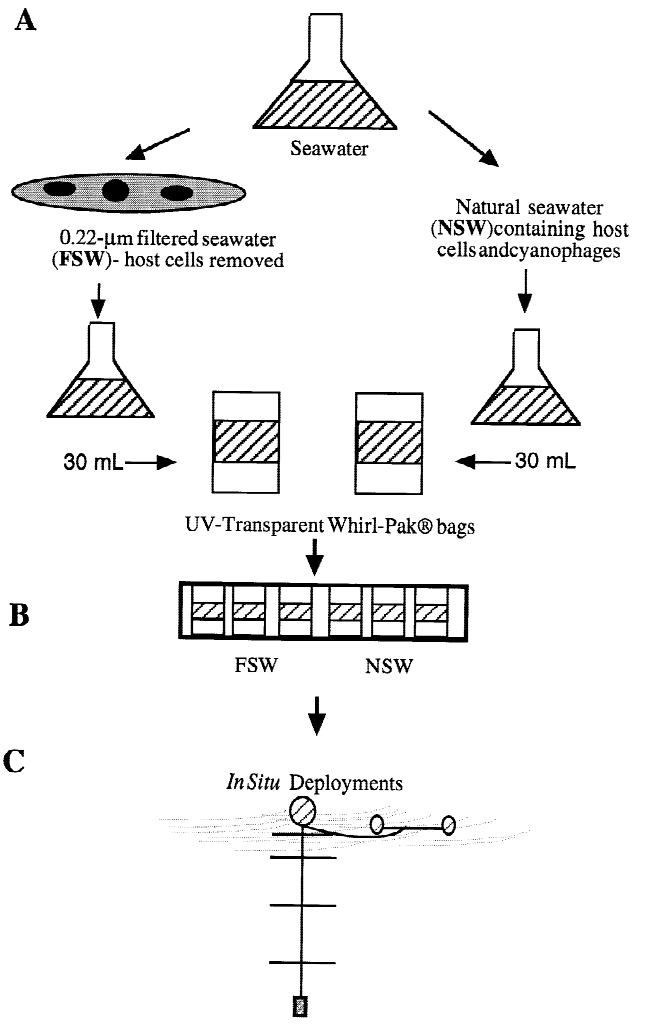

Fig. 1. (A) Experimental procedure for measuring the production and decay of natural cyanophage communities in situ. Approximately 1 liter of seawater was collected and either filtered (FSW) to remove host cells or left whole (NSW). Filtrate and unfiltered samples were heat-sealed in UV-transparent, polyethylene bags, which were attached to PVC frames (B) and deployed in situ during the day (C).

number of *Synechococcus* (1.01; dimensionless), d is cell dia (1.5×10^{-4} cm), D_v is diffusivity of the cyanophages (3.456×10^{-3} cm^2 d^{-1}), V is the concentration of cyanophages (ml^{-1}), and P is the concentration of *Synechococcus* (ml^{-1}). The contact rates between cyanophages and *Synechococcus* spp. represented by this model are the maximum values, determined by diffusive processes [24].

Results

Seasonal Changes in the Decay Rates of Natural Cyanophage Communities and Viral Isolates

In near-surface incubations, the decay rates of infectivity for cyanophage isolates and natural communities varied seasonally; they were generally lower in the winter and spring than in the summer (Fig. 2). The changes were more pronounced for the cyanophage isolates than for natural cyanophage communities. Seasonal changes in decay rates for both groups were ca. 0.1 h^{-1} during the fall through spring; the decay rates of the isolates in summer were considerably higher than those of the natural viral communities. This is shown clearly when the decay rates of the cyanophage isolates and natural cyanophage communities, determined at the same time, are plotted (Fig. 3). Although the light-dependent decay rates at the surface clearly varied seasonally, they were not strongly correlated with PAR (r = 0.62, data not shown).

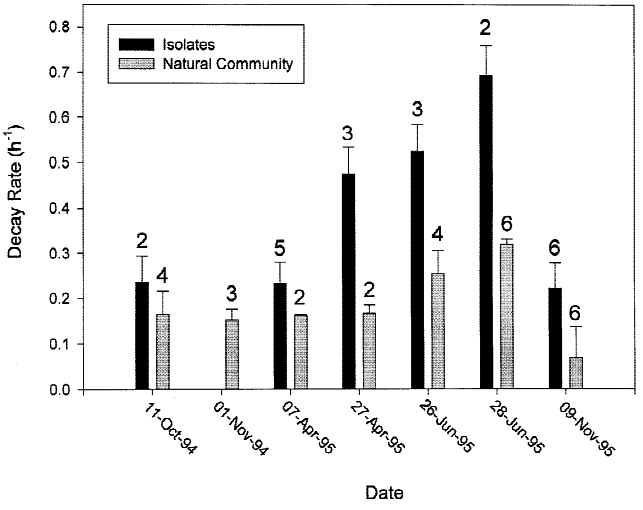

Fig. 2. Decay rates of natural cyanophage communities and cyanophage isolates S-PWM1 and S-PWM3 at the surface. Error bars represent 1 standard deviation; values associated with each bar indicate the number of replicate incubations. Data for filtered and unfiltered natural viral communities were combined, as were data from the viral isolates, since there were no significant differences in decay rates between the combined groups.

The Effect of Temperature on Cyanophage Decay Rates

Decay rates of infectivity of a natural cyanophage community in the dark ranged from 0.034 d^{-1} at 4°C to 0.126 d^{-1} at 25°C (Fig. 4), corresponding to turnover times of 29 and 8 days, respectively. Decay rates (y) were similar at 4 and 14°C, but increased sharply at higher temperatures (T). Changes in decay rate with temperature were well described by a second-order polynomial equation: $y = 2.99 \times 10^{-4}(T)^2 - 4.5 \times 10^{-3}(T) + 0.051$, ($r^2$ = 1.0). The equation was used to estimate dark decay rates when they were not determined, or when they were too slow to be measurable over a diurnal incubation.

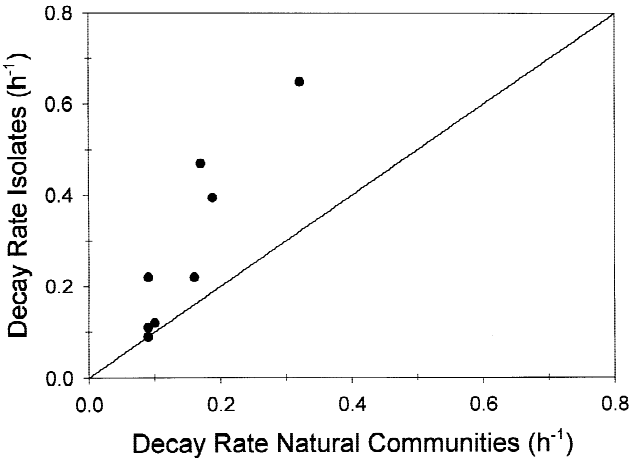

Fig. 3. Relationship between the decay rates of natural cyanophage communities and isolates. The solid line represents a 1:1 ratio where the decay rates of isolates and the natural community would be the same. Data were collected over the course of the year from side-by-side incubations.

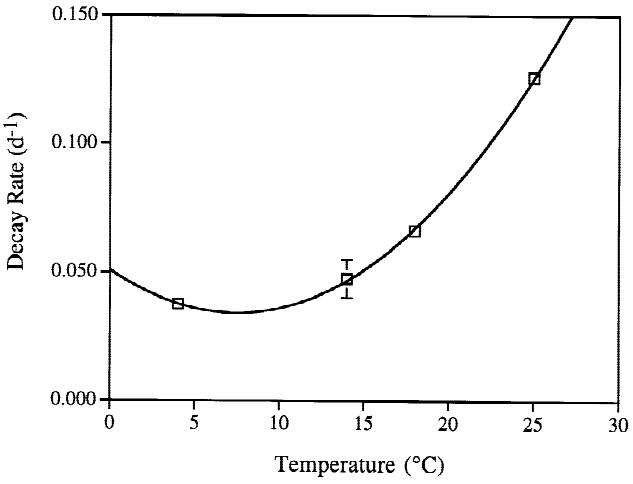

Fig. 4. Dark decay rates (*K*d) of natural cyanophage communities versus temperature (*T*). Data represent means and standard deviations for natural cyanophage communities taken from triplicate water samples. Where error bars are not shown, the standard deviation was less than the width of the symbol. The equation for the regression is $K\text{d} = 2.99 \times 10^{-4}\ (T^2) - 4.5 \times 10^{-3}\ (T) + 0.051$, $r^2 = 1.00$, where T is temperature (°C).

Viral Production and Decay

During in situ diurnal experiments in the boat harbor, changes in the abundances of viruses infecting *Synechococcus* strain DC2 were only detected for the filtered seawater treatment at one depth on 24 January. They were undetectable at all depths for the filtered and unfiltered treatments on 21 March (data not shown). The abundance of *Synechococcus* spp. was similar (ca. 2×10^4 ml^{-1}) on both dates, whereas cyanophages were more abundant in January (9.8×10^3 ml^{-1}) than March (2.4×10^3 ml^{-1}) (Table 1). Although decay rates were too slow to be measured, estimates based on temperature were 0.048 d^{-1} and 0.068 d^{-1} for January and March, respectively.

In contrast to experiments in the boat harbor, changes in cyanophage abundance were detected during experiments in June at Stations E and F (Table 1). A monospecific bloom (based on light and epifluorescence microscopy) of *Synechococcus* (ca. 10^5 ml^{-1}) was present at Station E; cyanophages were even more abundant (ca. 10^6 ml^{-1}). The abundance of *Synechococcus* at Station F was similar to that at Station E, but cyanophages were less numerous (ca. 1.7×10^5 ml^{-1}).

At Station E, solar radiation was responsible for significant cyanophage mortality, destroying more than 90% of the original titer in the natural cyanophage community at the surface during diurnal incubations (Fig. 5). Cyanophage titers were not significantly different in parallel incubations using either whole seawater or seawater in which *Synechococcus* cells were removed by filtration, indicating no measurable cyanophage production during the incubations. Station F incubations showed a similar pattern, with >90% of the initial titer lost in sunlight-exposed samples.

Light-mediated decay rates of infectivity in natural cyanophage communities, averaged over the incubation period, decreased from 0.28 h^{-1} and 0.33 h^{-1} at the surface to 0.09 h^{-1} and 0.08 h^{-1} at 15 m (Stations E and F, respectively) (Fig. 5). The decreases were described by an equation that included a component that was responsible for most of the surface decay (0.17 and 0.22 h^{-1}) and had attenuation coefficients of 0.67 and 1.33 m^{-1} (Stations E and F, respectively). It was similar to that for UV-A (320–400 nm) (Table 2). A second component that was responsible for less of the decay at the surface (0.12 and 0.14 h^{-1}, at Stations E and F, respectively) had a much lower attenuation coefficient (0.03 m^{-1}). At Station E, decay rate with depth was described by $0.17e^{(0.67 \times -z)} + 0.12e^{(0.03 \times -z)}$, where 0.17 and 0.12 are decay rates at the surface attributable to damaging radiation with attenuation coefficients of 0.67 m^{-1} and 0.03 m^{-1}, respectively (Table 2). Similarly, at Station F, the decrease in decay rate with depth was described by $0.22e^{(1.33 \times -z)} + 0.14e^{(0.03 \times -z)}$.

Decay rates attributable to solar radiation, averaged over 24 h and integrated over the surface mixed layer ($a/-k_1(e^{(-k_1 z)} - 1)) + (b/-k_2\, e^{(-k_2 z)} - 1)$), were 0.75 and 0.53 d^{-1} at Stations E and F, respectively (Table 1). The higher

Table 1. Description of experimental sites including biological and physical characteristics

1995	Location	Mixed depth (m)[a]	T (°C)	Cyanophages (ml^{-1})	*Synechococcus* spp. (ml^{-1})	Cyanophage decay rate (d^{-1})[b]	% *Synechococcus* spp. lysed (d^{-1})[c]	R (encounters ml^{-1} d^{-1})	% *Synechococcus* spp. encountered (d^{-1})[d]	Efficiency of infection (% of encounters leading to infection)[e]
24 Jan	Boat harbor (27°50′ N, 97°02′ W)	2	14.7	9.80×10^3	2.44×10^4	0.048	0.02	578	2.37	1.01
21 Mar	Boat harbor (27°50′ N, 97°02′W)	2	18.5	2.35×10^3	1.95×10^4	0.068	0.01	111	0.57	1.78
26 Jun	Gulf of Mexico (Stn. E) (26°47′ N, 96°38′ W)	10.4	21.4	1.05×10^6	1.20×10^5	0.75	8.09	304,789	254	3.18
28 Jun	Gulf of Mexico (Stn. F) (27°35′ N, 96°71′ W)	28.2	23.7	1.68×10^5	1.00×10^5	0.53	1.11	40,638	41	2.72

[a] Defined as the pycnocline from multiple CTD casts.

[b] Decay rate for cyanophage infectivity, averaged over 24 h and integrated for the surface mixed layer. Rates are corrected for dark decay rates. Where dark decay rates were too slow to be measured, they were estimated from temperature using the equation $y = 2.99 \times 10^{-4} (T)^2 - 4.5 \times 10^{-3} (T) + 0.051$, where T = temperature (see text).

[c] Assuming a burst size of 81 (measured empirically by Weinbauer, unpubl. data).

[d] % of cells encountered was determined as *R* divided by *Synechococcus* spp. abundance.

[e] Efficiency of infection was determined as % of *Synechococcus* spp. lysed per day divided by % of *Synechococcus* spp. contacted per day.

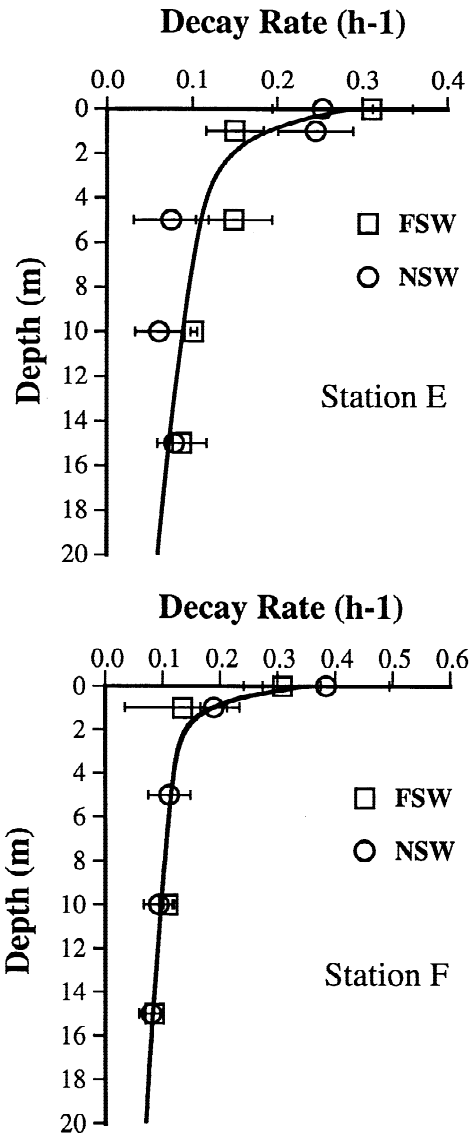

Fig. 5. Depth profile of light-mediated decay rates of natural cyanophage communities averaged over the entire incubation at Stations E and F. Data represent mean and standard deviation of triplicate samples. The curves represent the best fits through the combined data for 0.22-µm filtered (FSW) and untreated natural seawater (NSW) and are derived from an equation comprising two attenuation coefficients for damaging radiation (see text).

average decay rate at Station F was largely because the pycnocline was deeper at Station F (ca. 28 m) than at Station E (ca. 10 m).

The Effect of Viruses on the Mortality of Synechococcus *spp.*

In order to balance the measured loss rates of infectious cyanophages, the percent of the *Synechococcus* population in which lysis occurred was estimated to range from 0.01% at the boat harbor to 8.09% at Station E (Table 1). For example, on 21 March and 26 June, cyanophage decay rates were 0.068 and 0.75 d^{-1}, respectively. This corresponded to the loss of 160 and 786,000 cyanophages ml^{-1} d^{-1}. Assuming a burst size of 81 (measured for cyanobacteria in the Gulf of Mexico, Weinbauer, unpubl. data), balancing viral decay rates would require lysis of 2 and 9,700 cells ml^{-1} d^{-1}, or 0.01% and 8.09% of the *Synechococcus* population, respectively.

Calculated encounter rates for 21 March and 26 June revealed that ca. 0.6% and 254% of the *Synechococcus* cells were contacted daily by infectious cyanophages. Therefore, contact rates were adequate to support the inferred infection rates. In fact, on average, only about 2.2% of the encounters resulted in infection (Table 1), implying that most of the *Synechococcus* population was resistant to infection or that the efficiency of infection was low.

Discussion

This study shows that the infectivity of natural communities of viruses which infect *Synechococcus* sp. (Strain DC2) is destroyed by light-dependent and light-independent processes. Loss rates of infectivity for cyanophage isolates and natural communities were greatest in summer and early fall, when insolation was highest. However, the sensitivity of natural cyanophage communities to solar radiation, (relative to that of individual isolates) also decreased when insolation was greatest, implying selection for UV-tolerant cyanophage communities. In addition, the destruction of cyanophage infectivity was not balanced by viral production during dawn-to-dusk incubations, suggesting that viral production was occurring at night. These findings are considered in detail below.

Seasonal Changes in Tolerance of Natural Viral Communities to Solar Radiation

Decay rates of marine phage isolates are proportional to solar radiation [36] and can be used as a standard against which decay rates of natural communities can be compared. The data in this study show that during summer the decay rates of natural cyanophage communities were as little as one-half of those measured for cyanophage isolates; during winter the decay rates between natural communities and isolates were similar. Seasonal changes in the tolerance to

Table 2. Attenuation coefficients (m^{-1}) for light and cyanophage decay at Stations E and F in the Gulf of Mexico[a]

Location (Gulf of Mexico)	Attenuation coefficients of ligh (m^{-1})					Attenuation coefficients of decay (m^{-1})[b]	
	PAR (400–700 nm)	380 nm	340 nm	320 nm	305 nm	K_1	K_2
Station E (26°47′N, 96°38′W)	0.21	0.48	0.99	1.45	2.22	0.67	0.03
Station F (27°C35′N, 96°71′W)	0.17	0.54	1.13	1.70	2.24	1.33	0.03

[a] Visible and UV light was measured with a Biospherical PUV-500 submersible radiometer.
[b] The coefficients are for the equation describing the attenuation of decay with depth (see Materials and Methods).

solar radiation of the natural cyanophage communities, relative to that of the isolates, reflects changes in the natural viral communities, as the same stock of viral isolates (S-PWM1 and S-PWM3) were used in all experiments. Furthermore, in all cases *Synechococcus* strain DC2 (WH7803) was used to determine the titer of infectious cyanophages, under similar conditions of light and temperature.

There are two major implications of finding that the tolerance of natural cyanophage communities to UV radiation changes during the year. The first is that the decay rates of viral isolates added to seawater are not necessarily representative of those in the natural community. Although it is recognized that viruses differ in their sensitivity to solar radiation [7, 38], it was assumed that the relative decay rates between isolates and natural communities would remain similar. If decay rates of isolates were assumed to be representative of the natural community throughout the year, viral-mediated mortality in the summer would have been overestimated by as much as a factor of 2 (e.g., 16% vs. 8% per day). During the winter, however, decay rates of the isolates were close to those of the natural community. Although seasonal changes in UV sensitivity can affect the accuracy of estimates of decay rates of natural viral communities, inferred from measured decay rates of viral isolates, it is encouraging that decay rates of infectivity in natural communities and isolates were within a factor of 2 of each other.

Higher loss rates of infectivity in natural cyanophage communities in the summer were likely caused by seasonal increases in insolation. UV-A (320 to 400 nm), UV-B (290 to 320 nm) and even longer wavelengths directly and indirectly destroy infectivity [28, 47]. Moreover, sunlight is a major factor causing loss of infectivity in marine bacteriophages [38] and algal viruses [7]. In clear oceanic waters, sunlight can be responsible for most of the decay of infectivity integrated over a 200-m water column [38]. In coastal environments, where light attenuation is higher, sunlight can still be responsible for most of the decay when mixed depths are <10 m. The extent and severity of the damage to viruses caused by sunlight depends on several variables, including the destructive potential of each wavelength and the penetration depth of damaging radiation. In addition, recent studies have shown that much of the loss of infectivity in bacteriophages caused by sunlight can be restored by host cells via photoreactivation [43–45].

The mechanism responsible for increased resistance of cyanophages to solar radiation is unknown, but could involve modifications leading to increased stability of the viral DNA. As viral replication is rapid and exponential, nonlethal, beneficial mutations are quickly propagated through a population [21]. Conversely, viruses which are most sensitive to solar radiation will be selectively destroyed or inactivated when insolation is high. Given the small size of viral particles, it is unlikely that selection could result in a mechanism to decrease the penetration of damaging radiation through viral capsids. An alternative hypothesis is that selection has resulted in cyanophage communities that encode additional host-cell-mediated repair mechanisms during seasons when insolation is greatest.

Decay Rates of Natural Cyanophage Communities

Sunlight-dependent decay rates of natural cyanophage communities incubated at the surface varied throughout the year, but were highest in summer and early fall (ca. 0.35 h^{-1}; Fig. 2). They were undetectable during January and March. As UV is responsible for most of the viral decay caused by sunlight [38], one would expect to see less of an impact during the winter. The strong seasonal variation likely resulted from changes in light quality, as well as quantity. Seasonal changes in UV reaching the earth's surface are greater than those of longer wavelengths due to higher atmospheric attenuation of UV as the solar angle increases [14,

17]. This may be why seasonal changes in the decay rates of the viral isolates were not well correlated with the amount of PAR received (r = 0.62, data not shown).

The effect of solar radiation on decay rates of natural cyanophage communities was also examined offshore (Stations E and F). The decrease in decay rates with depth was not adequately described by a single attenuation coefficient for damaging radiation, but by an equation that included two attenuation coefficients (Fig. 5). Most of the changes in decay rates with depth were explained by an attenuation coefficient that was similar to that of UV-A (320–400 nm) (Table 2). However, another component involved in the decay decreased much more gradually with depth. The penetration of UV-B during these experiments was shallow, with >99% of 305 nm radiation attenuated by 5 m, consistent with the rapid decrease in decay rates between the surface and 5 m (Fig. 5). Although other studies have found that the decay of viral infectivity with depth can be explained by a single coefficient which varies with water type [30, 40], wavelengths longer than UV-B can contribute significantly to the decay of viral infectivity [38]. The difference between this study and others may have resulted from changes in water quality with depth because of changes in attenuation coefficients caused by the surface *Synechococcus* bloom and the complex physical structure of the water column (data not shown).

Decay rates of natural cyanophage communities in the dark were found to range from 0.034 d^{-1} at 4°C to 0.126 d^{-1} at 25°C (Fig. 4), corresponding to turnover times of 30 to 8 d, respectively. Consequently, light-independent decay rates increased ca. 4-fold from 0.04 d^{-1} in winter to 0.16 d^{-1} during summer, in response to seasonal changes in water temperature from about 10 to 28°C (data not shown). Light-independent decay of infectivity has been shown for marine bacteriophages [22, 38, 47] and *Micromonas pusilla* virus (*Mp*V) communities [7], with different viruses decaying at different rates. The turnover times measured for the natural cyanophage communities are similar to those reported for natural *Mp*V communities over the same temperature range (17 to 8 d) [7], but are lower than the average of 2 d reported for a variety of marine and nonmarine bacteriophage isolates [34].

It is interesting that the temperature-dependent decay rates of natural communities of cyanophages and *Mp*V are similar, even though the two groups of viruses are morphologically different, while cyanophage communities decay more slowly than morphologically similar bacteriophage isolates. Such observations further suggest that natural viral communities can be more resistant to decay than viral isolates. The virucidal activity of seawater involves bacteria [18, 22], adsorption to heat-labile particles [38], and grazing by flagellates [11]. Consequently, seasonal and spatial variation in these factors, as well as in temperature and salinity, would also affect light-independent decay rates. This is evident in the data of Moebus [22], which show considerable variation in decay rates of marine phage isolates added to seawater collected from different locations and at different times.

Impact of Viral Mortality on Synechococcus *Communities*

In the present study, the estimated cyanophage-mediated mortality for natural communities of *Synechococcus* spp. ranged from <1 to ca. 8% of the *Synechococcus* community per day, similar to previous reports [37, 42]. Investigators have estimated the impact of viruses on marine microbial mortality by determining the number of visibly infected bacteria and cyanobacteria [29], the destruction rates of viral particles [9, 12] and infectivity [7, 38], and the production rates of viral nucleic acids [32]. Estimates indicate that, on average, viruses are responsible for the mortality of ca. 3% of the standing stock of *Synechococcus* and 10–20% of bacterial biomass on a daily basis [34].

During dawn-to-dusk incubations, significant differences were not observed between decay rates of infectious cyanophages in unfiltered seawater and in seawater in which host cells were removed by filtration (Fig. 5), indicating that detectable production of infectious cyanophages was not occurring. A possible explanation is that viral production occurred at night. The marine cyanophage S-BBS1 has a lytic cycle of ca. 12 h under continuous irradiance in artificial seawater [36]. In addition, the lytic cycle of viruses that infect freshwater cyanobacteria is prolonged in the dark [31]. If these observations are also true for natural cyanophage communities, host cells infected during the night would not produce new viruses until about 12 h after sunrise. They would not have been detected in these experiments. Timing cyanophage production to occur at the onset of darkness makes sense from an evolutionary perspective, given that cyanophage infectivity is very sensitive to sunlight. Viruses that are produced at dusk will have the entire night to contact a suitable host. Moreover, once within the host, the viral genome will be protected from damaging solar radiation by the DNA repair mechanisms of the host cell. Evidence of cyanophage production at night was obtained during diel studies of in situ cyanophage abundance that were conducted during the *Synechococcus* bloom at Station E [41].

If it is assumed that the decay of viral infectivity that occurred during daylight must be balanced by production over a diel cycle, then the estimated 24 h production rates of infectious cyanophages, integrated over the mixed layer, were 0.75 and 0.53 d^{-1} at stations E and F, respectively (Table 1). The percent of the *Synechococcus* community that had to be lysed on a daily basis in order to balance these loss rates were 8% at Station E and 1% at Station F. These estimates assume that the measured burst size, at Station E, of 81 viruses produced for each *Synechococcus* cell lysed (Weinbauer, unpubl. data) is accurate, and that all of the viruses produced were infectious.

The inferred infection rates in this study are much less than the calculated encounter rates between *Synechococcus* spp. and infectious cyanophage. At Station E, *Synechococcus* cells were estimated to encounter an infectious cyanophage more than twice a day (Table 1). Yet, in order to balance the measured cyanophage destruction rate, only ca. 3% of these encounters would have needed to result in infection (Table 1). At Station F, the percent of *Synechococcus* encountered daily by cyanophages was considerably less than at Station E. Nonetheless, only 2.7% of the encounters would have had to result in infection in order for viral production to have balanced decay. In all experiments, regardless of time and location, the encounters that resulted in infection ranged from ca. 2 to 3.7% (Table 1). These results imply that most *Synechococcus* were resistant to infection by most cyanophages, or that the efficiency of infection was very low (i.e. many encounters were required before infection was successful).

The Paradox of High Cyanophage Concentrations in Surface Waters

High concentrations of infectious cyanophages in surface waters in the presence of high levels of solar radiation has been termed a paradox [40]. This is because the high mortality rates of *Synechococcus* that would be required to balance the loss rates of viral infectivity would be difficult to sustain. For example, in this study, balancing the viral decay rates measured at the surface (ca. 0.3 h^{-1}) with viral production at the surface would require lysis of ca. 37% of the *Synechococcus* cells on a daily basis. In other situations, when infectious cyanophages are similarly abundant but concentrations of *Synechococcus* are considerably less [37], balancing decay and production at the surface is untenable. Recent observations have shown that restoration of infectivity to UV-damaged viruses by host-cell-mediated photoreactivation is an important mechanism for maintaining high bacteriophage concentrations in surface waters [42, 43, 44]. Photoreactivation is also likely to be important for maintaining high cyanophage abundances, as cyanobacteria are able to repair damaged DNA by this mechanism, as well [1, 16]. However, the decay rates that were measured in this study, as well as in other studies, would include repair by photoreactivation as the abundance of infectious cyanophages was titered under photoreactivating conditions. When decay is integrated over the water column, the mortality imposed on the *Synechococcus* community in the water column is relatively modest. This emphasizes that mixing of host and viral populations from deeper waters is an important factor allowing for the persistence of high concentrations of viruses at the surface.

Results from this study demonstrate that the infectivity of natural cyanophage communities is sensitive to both solar radiation and light-independent mechanisms. As the turnover times of viruses must be supported by the lysis of *Synechococcus* cells, viral infection and cell lysis are important factors in the ecology of *Synechococcus* communities in nature. Further, seasonal effects influence the light environment. As a result, so will the relative importance of light-dependent and light-independent decay processes. This is further complicated by the finding that natural cyanophage communities are more resistant to solar radiation in the summer than in winter. The balance that exists between populations in nature, and the viruses which infect them, are complex. Understanding them is essential in order to determine the role of viruses in planktonic communities. This is clearly a worthwhile goal as it becomes more and more apparent that viruses mediate a quantitatively important pathway of nutrient cycling in the sea.

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References

1. Amla DV (1979) Photoreactivation of ultraviolet irradiated

blue-green alga Anacystis nidulans and Cyanophage AS-1. Arch Virol 59:173–179
2. Boehme J, Frischer ME, Jiang SC, Kellogg CA, Pichard S, Rose JB, Steinway C, Paul JH (1993) Viruses, bacterioplankton, and phytoplankton in the southeastern Gulf of Mexico: distribution and contribution to oceanic DNA pools. Mar Ecol Prog Ser 97:1–10
3. Børsheim KY (1993) Native marine bacteriophages. FEMS Microbiol Ecol 102:141–159
4. Bratbak G, Thingstad T, Heldal M (1994) Viruses and the microbial loop. Microb Ecol 28:209–221
5. Chen F, Suttle CA (1996) Evolutionary relationships among large double-stranded DNA viruses that infect microalgae and other organisms as inferred from DNA polymerase genes. Virology 219:170–178
6. Cottrell MT, Suttle CA (1991) Wide-spread occurrence and clonal variation in viruses which cause lysis of a cosmopolitan, eukaryotic marine phytoplankter, *Micromonas pusilla.* Mar Ecol Prog Ser 78:1–9
7. Cottrell MT, Suttle CA (1995) Genetic diversity of algal viruses which lyse the photosynthetic picoflagellate *Micromonas pusilla* (Prasinophyceae). Appl Environ Microbiol 61:3088–3091
8. 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, pp 361–383
9. Fuhrman JA, Noble RT (1995) Viruses and protists cause similar bacterial mortality in coastal seawater. Limnol Oceanogr 40:1236–1242
10. Fuhrman JA, Suttle CA (1993) Viruses in marine planktonic systems. Oceanography 6:51–63
11. González JM, Suttle CA (1993) Grazing by marine nanoflagellates on viruses and virus-sized particles: Ingestion and digestion. Mar Ecol Prog Ser 94:1–10
12. Heldal M, Bratbak G (1991) Production and decay of viruses in aquatic environments. Mar Ecol Prog Ser 72:205–212
13. Hennes KP, Suttle CA (1995) Direct counts of viruses in natural waters and laboratory cultures by epifluorescence microscopy. Limnol Oceanogr 40:1050–1055
14. Holm-Hansen O, Lubin D, Helbling EW (1993) Ultraviolet radiation and its effects on organisms in aquatic environments. In: Young AR (ed) Environmental UV Photobiology, Plenum Press, New York, pp 379–426
15. Hurley MA, Roscoe ME (1983) Automated statistical analysis of microbial enumeration by dilution series. J Appl Bacteriol 55:159–164
16. Hwang-Lee L, Cohn G, Cosowsky L, McGowan RE, Blamire J (1985) DNA metabolism during infection of *Anacystis nidulans* by cyanophage AS-1. VII. UV-induced alterations of the AS-1/*A. nidulans* lytic cycle. Microbios 43:277–295
17. Iqbal M (1983) An Introduction to Solar Radiation. Academic Press, Toronto
18. Kapuscinski RB, Mitchell R (1980) Processes controlling virus inactivation in coastal waters. Water Res 14:363–371
19. Karentz D, Lutze LH (1990) Evaluation of biologically harmful ultraviolet radiation in Antarctica with a biological dosimeter designed for aquatic environments. Limnol Oceanogr 35:549–561
20. Keller MD, Bellows WK, Guilland RRL (1988) Microwave treatment for sterilization of phytoplankton culture media. J Exp Mar Biol Ecol 117:279–283
21. Lin J (1993) Evolution of bacteriophage T7 in a growing plaque. J Bacteriol 75:1272–1277
22. Moebus K (1992) Laboratory investigations on the survival of marine bacteriophages in raw and treated seawater. Helgol Meeresunters 46:251–273
23. Murray AG, Eldridge PM (1994) Marine viral ecology: incorporation of bacteriophage into the microbial planktonic food web paradigm. J Plankton Res 16:627–641
24. Murray AG, Jackson GA (1992) Viral dynamics: A model of the effects size, shape, motion and abundance of single–celled planktonic organisms and other particles. Mar Ecol Prog Ser 89:103–116
25. Noble RT, Fuhrman JA (1997) Virus decay and its causes in coastal waters. Appl Environ Microbiol 63:77–83
26. Paul JH, Jiang SC, Rose JB (1991) Concentration of viruses and dissolved DNA from aquatic environments by vortex flow filtration. Appl Environ Microbiol 57:2197–2204
27. Paul JH, Rose JB, Jiang SC, Kellogg CA, Dickson L (1993) Distribution of viral abundance in the reef environment of Key Largo, Florida. Appl Environ Microbiol 59:718–724
28. Peak JG, Peak MJ, Sikorski RS, Jones CA (1985) Induction of DNA-protein crosslinks in human cells by ultraviolet and visible radiations: action spectrum. Photochem Photobiol 41: 295–302
29. Proctor LM, Fuhrman JA (1990) Viral mortality of marine bacteria and cyanobacteria. Nature 343:60–62
30. Rontó G, Gáspár S, Gróf P, Bérces A, Gugolya Z (1994) Ultraviolet dosimetry in outdoor measurements based on bacteriophage T7 as a biosensor. Photochem Photobiol 59:209–214
31. Sherman LA, Haslekorn R (1971) Growth of the blue-green algae virus LPP-1 under conditions which impair photosynthesis. Virology 45:739–746
32. Steward GF, Wilkner J, Cochlan WP, Smith DC, Azam F (1992) Estimation of virus production in the sea: II. Field results. Mar Microb Foodwebs 6:79–90
33. 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
34. Suttle CA (1994) The significance of viruses to mortality in aquatic microbial communities. Microb Ecol 28:237–243
35. Suttle CA (1999) Cyanophages and their role in the ecology of cyanobacteria. In: Whitton BA, Potts M (eds) The Ecology of Cyanobacteria: Their Diversity in Time and Space. Kluwer Academic Publishers, Boston
36. Suttle CA, Chan AM (1993) Marine cyanophages infecting oceanic and coastal strains of *Synechococcus:* Abundance, morphology, cross-infectivity and growth characteristics. Mar Ecol Prog Ser 92:99–109

37. Suttle CA, Chan AM (1994) Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. Appl Environ Microbiol 60:3167–3174
38. Suttle CA, Chen F (1992) Mechanisms and rates of decay of marine viruses in seawater. Appl Environ Microbiol 58:3721–3729
39. Suttle CA, Chan AM, Cottrell MT (1990) Infection of phytoplankton by viruses and reduction of primary productivity. Nature 347:467–469
40. Suttle CA, Chan AM, Chen F, Garza DR (1993) Cyanophages and sunlight: A paradox. In: Guerrero R, Pedrós-Alió C (eds) Trends in Microbial Ecology. Span Soc Microbiol, Barcelona, pp 303–307
41. Suttle CA, Chan AM, Rodda KM, Short SM, Weinbauer MG, Garza DR, Wilhelm SW (1996) The effect of cyanophages on *Synechococcus* spp. during a bloom in the western Gulf of Mexico. Eos 76(suppl):OS207–OS208
42. Waterbury JB, Valois FW (1993) Resistance to co-occurring phages enables marine *Synechococcus* communities to coexist with cyanophages abundant in seawater. Appl Environ Microbiol 59:3393–3399
43. Weinbauer MG, Wilhelm SW, Suttle CA, Garza DR (1997) Photoreactivation compensates for UV damage and restores infectivity to natural marine viral communities. Appl Environ Microbiol 63:2200–2205
44. Wilhelm SW, Weinbauer MG, Suttle CA, Jeffrey WH (1998) The role of sunlight in the removal and repair of viruses in the sea. Limnol Oceanogr 43:586–592
45. Wilhelm SW, Weinbauer MG, Suttle CA, Pledger RJ, Mitchell DL (1998) Measurements of DNA damage and photoreactivation imply that most viruses in marine surface waters are infective. Aquat Microb Ecol 14:215–222
46. Wilson WH, Joint IR, Carr NG, Mann NH (1993) Isolation and molecular characterization of five marine cyanophages propagated on *Synechococcus* sp. strain WH7803. Appl Environ Microbiol 59:3736–3743
47. Wommack KE, Hill RT, Muller TA, Colwell RR (1996) Effects of sunlight on bacteriophage viability and structure. Appl Environ Microbiol 62:1336–1341