Virus Decay and Its Causes in Coastal Waters

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Recent evidence suggests that viruses play an influential role within the marine microbial food web. To understand this role, it is important to determine rates and mechanisms of virus removal and degradation. We used plaque assays to examine the decay of infectivity in lab-grown viruses seeded into natural seawater. The rates of loss of infectivity of native viruses from Santa Monica Bay and of nonnative viruses from the North Sea in the coastal seawater of Santa Monica Bay were determined. Viruses were seeded into fresh seawater that had been pretreated in various ways: filtration with a 0.2-μm-pore-size filter to remove organisms, heat to denature enzymes, and dissolved organic matter enrichment to reconstitute enzyme activity. Seawater samples were then incubated in full sunlight, in the dark, or under glass to allow partitioning of causative agents of virus decay. Solar radiation always resulted in increased rates of loss of virus infectivity. Virus isolates which are native to Santa Monica Bay consistently degraded more slowly in full sunlight in untreated seawater (decay ranged from 4.1 to 7.2% h^{-1}) than nonnative marine bacteriophages which were isolated from the North Sea (decay ranged from 6.6 to 11.1% h^{-1}). All phages demonstrated susceptibility to degradation by heat-labile substances, as heat treatment reduced the decay rates to about 0.5 to 2.0% h^{-1} in the dark. Filtration reduced decay rates by various amounts, averaging 20%. Heat-labile, high-molecular-weight dissolved material (>30 kDa, probably enzymes) appeared responsible for about 1/5 of the maximal decay. Solar radiation was responsible for about 1/3 to 2/3 of the maximal decay of nonnative viruses and about 1/4 to 1/3 of that of the native viruses, suggesting evolutionary adaptation to local light levels. Our results suggest that sunlight is an important contributing factor to virus decay but also point to the significance of particles and dissolved substances in seawater.

There is ample evidence that viruses play a large role in the microbial food web. Recent investigations have demonstrated high viral abundances in marine ecosystems (2, 7, 25). It has been shown that viruses are significant agents of mortality of prokaryotes in the oceans and in certain marine environments, e.g., in coastal areas, viruses and protists can contribute equally to bacterial mortality (11, 25). Models show that viruses may increase the cycling of dissolved organic matter (DOM) within the heterotrophic bacterial population, lowering the amount of matter and energy that is passed on to higher trophic levels (7, 10, 24). This occurs when DOM is released (by cell lysis due to viral infection) into the water column and is assimilated by noninfected bacterioplankton (19). The importance of cell lysis is further sustained by the hypothesis that bacteria are the major hosts of marine viruses and that lytic infection is likely to be the dominant type of interaction between host and bacteriophages in the eutrophic ocean (31). In addition, high rates of removal of virus particles in seawater imply high rates of viral production (14) and, therefore, significant bacterial mortality due to virus infection. A small percentage of bacteria and cyanobacteria sampled from surface waters consistently contain many (more than five) mature intracellular virus-like particles and indicate the prevalence of virus infection (25, 26, 30).

In order to fully understand the quantitative significance of viruses in marine food webs, we must learn the rates at which viruses decay and the processes causing that decay. For processes that depend upon viral infectivity, the decay of interest is the loss of infectivity. This study focused upon the decay of viral infectivity in laboratory-isolated marine host-virus systems under various conditions. The decay of infectivity, rather

than rates of disappearance of virus particles, was monitored, as many of the visible viruses could be inactive. Because infectivity cannot be determined via natural population studies (where hosts are unknown and often unculturable), lab-cultured virus systems are needed to ascertain the relative loss of infectivity within the water sample. In one report (29), decay rates for marine phages were similar to previously reported decay rates for enteroviruses in nonpolluted seawater, typically losses of a few percent per hour (3, 4). In this study, decay of infectivity has been researched with both native and nonnative bacteriophage isolates, manipulated under various conditions. Studies of virus decay in conjunction with information about natural populations will allow us to determine the controlling factors of the dynamics of viruses within the marine microbial food web.

MATERIALS AND METHODS

Marine bacteria and bacteriophages. The bacteria PR1, PR2, PR3, and PR4 and their respective bacteriophages PR1/1, PR2/1, PR3/1, and PR4/1 were isolated from coastal waters of Santa Monica Bay. All were isolated from seawater collected from the end of the jetty in Playa del Rey, Calif., at 34°N, 118°30′W. The host bacteria were isolated as CFU from concentrated seawater spread onto Moebus seawater (MSW) agar plates (0.1% yeast extract and 0.5% peptone in 80% glass fiber-filtered seawater) according to the method of Moebus (21). The bacteriophages were isolated from 2 liters of natural seawater by tangential flow ultraconcentration (30-kDa molecular mass cutoff, spiral cartridge; Amicon) as outlined by Proctor and Fuhrman (24). Forty microliters of concentrated seawater was added to 500 μl of exponentially growing host cells and allowed to adsorb for 30 min. This mixture was added to 3 ml of MSW low-melting-point top agarose (0.7%; SeaPlaque; FMC BioProducts) at 29°C and poured over an MSW agar plate at room temperature. These plates were screened the following day for plaque formation, and bacteriophage isolates were obtained by the protocols of Sambrook et al. (27). In short, 25 ml of liquid MSW media was added to plates that had plaques. The plates were shaken for 12 h at room temperature. The liquid was then poured off and centrifuged at 4,000 × g for 20 min to remove large bacterial debris. The supernatant fluid was then filtered with a 0.2-μm-pore-size filter (polycarbonate Nuclepore) and stored at 4°C until use. In an attempt to compare and contrast the rates of decay of infectivity for indigenous

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TABLE 1. Summary of experiments

Date (mo/day/yr)	Virus(es) tested	Incubation condition(s)[a] for indicated seawater treatment				Total solar irradiance (avg [μW/m²])
		Untreated	0.2-μm filtered[b]	Heat treated	Heat treated + DOM	
3/13/92	H40/1	A, B, C				13,000
4/16/92	H40/1, H85/1	D	D	D		Low ambient lab light
5/6/92	H11/1, H85/1	A, C		A, C		18,500
7/15/92	H40/1, H85/1	A, B, C				ca. 18,000
3/27/95	H2/1, H11/1, H40/1, H85/1, PR1/1, PR2/1, PR3/1, PR4/1	A, C	A, C	A, C		16,000
9/8/95	PR1/1, PR2/1, PR3/1, PR4/1, H2/1	A	A	A	A	18,000

[a] Incubation conditions: A, full sunlight; B, glass covered; C, dark; D, laboratory.
[b] 0.2-μm filtered, filtered with a 0.2-μm-pore-size filter.

and nonnative bacteriophages, two sets of host-virus systems were used; the previously mentioned PR series and H2/1, H11/1, H40/1, and H85/1, which were isolated from the North Sea (21).

Decay experiments. Lab-cultured viruses were produced by adding 100 μl of high-titer virus stock (ca. 10^{10} PFU ml^{-1}) into 250 ml of host bacteria at mid-log phase. The cultures were allowed to clear, and the viral lysate was centrifuged at $4,000 \times g$ to remove large bacterial debris. The viral lysate was then filtered (pore size, 0.2 μm) and subsequently concentrated with Centriprep 30 concentration units (Amicon). The concentrated viral lysate was reconcentrated and resuspended three times with filtered (pore size, 0.02 μm, Anopore; Whatman) seawater to remove any remaining media. The lab-cultured viruses were then sonicated with a Branson ultrasonic cleaner for 20 s (to disperse clumped viruses) and seeded into freshly collected Santa Monica Bay seawater. Rates of decay of infectivity were examined via the addition of lab-grown viruses to duplicate treated or untreated 350-ml seawater samples (obtained in the early morning of each experiment from the Santa Monica Pier) in triple-rinsed polyethylene Whirl-Pak bags, at initial concentrations of ca. 10^4 ml^{-1}. At this concentration, the added phages remain a tracer (<1%) of the natural populations yet are at a high enough concentration that the decay of infectivity (measured by PFU) can be monitored over a reasonable time course. The Whirl-Pak bags were then incubated at ambient seawater temperatures.

At each time interval, 200 μl of each serially diluted 1-ml seawater subsample was added to 200 μl of host bacteria (grown to mid-log phase in liquid MSW) in 1.5-ml Eppendorf tubes and incubated at room temperature for 20 min to allow phage adsorption. This mixture was then added to 3 ml of 0.7% MSW low-melting-point top agarose at 29°C, vortexed for 5 s, and plated onto MSW agar plates. The plates were incubated at room temperature overnight, and plaques were enumerated the following morning. By adding two or more types of bacteriophages (each with a different specific bacterial host) to the same sample containers and performing plaque titers for each, we were able to compare their loss of infectivity while ensuring that all other conditions remained consistent. The effect of sunlight on infectivity was determined by incubating the samples at ambient seawater temperatures in Whirl-Pak bags exposed to full sunlight on the roof of the Allan Hancock Foundation building at the University of Southern California and comparing them with samples incubated under the same light and temperature conditions but incubated under a glass beaker or in the dark (triple-wrapped in aluminum foil). Attenuation of light by Whirl-Pak bags and glass was determined by a 190- to 700-nm scan with a Kontron spectrophotometer. Heat treating the seawater samples in these experiments consisted of boiling for 1 min in a 700-W microwave oven. Also, in some experiments, one of the seawater samples was filtered with a 0.2-μm-pore-size filter (Nuclepore) before addition of bacteriophages. High-molecular-weight DOM larger than 30 kDa but smaller than 0.2 μm was concentrated by filtration of a natural seawater sample with a 47-mm-diameter, 0.2-μm-pore-size Nuclepore filter and subsequent centrifugation with Centriprep 30 ultraconcentration units. The concentrate was then added back to previously heat-treated seawater samples at the original concentration (assuming no loss during concentration). This 30 kDa-to-0.2-μm concentrated material was spiked back into the seawater samples in an attempt to reconstitute the loss of active degradative materials incurred by heat treatment. Treatments and dates of experiments are summarized in Table 1.

All experiments were performed in duplicate. Experiments which measured the decline in the number of PFU were plotted as PFU per milliliter versus time, and the exponential decay rate constant was calculated from the linear regression of log-transformed data. Therefore, abundance would follow the model $n_t = n_o e^{kt}$, where n_o is the abundance of PFU at time zero, n_t is the abundance of PFU at time t (in hours), and k is the decay constant (unit used: per hour). On a semilog plot of ln PFU versus time, the slope of the line is the decay constant and the reciprocal is the turnover time of the virus population. If the decay constant is multiplied by 100, the decay rate is expressed as a percent per hour. Negative controls were performed with each experiment by incubating 200 μl of the original seawater with the host bacteria and subsequently performing the plaque assay. No plaques were found.

Direct virus and bacterial counts. Viruses were counted by ultracentrifugation ($120,000 \times g$, 3 h, 20°C) of 4-ml seawater samples (2% Formalin preserved) onto carbon-stabilized Formvar-coated 200-mesh copper grids (Ted Pella, Inc.) (6, 8). The grids were then stained with 1% uranyl acetate for 30 s. Viruses were enumerated on a JEOL 100 CXII transmission electron microscope (TEM). Taper corrections were implemented into the final calculations (18, 28). Viruses were counted at a magnification of ×27,000, and bacteria were counted at a magnification of ×10,000 (80 keV). Direct counts of bacteria were also performed by epifluorescence microscopy with acridine orange stain (0.1%, wt/vol) (16). Virus characterization used a procedure similar to that outlined above. However, concentrates of virus isolates were spotted onto 200-mesh copper electron microscopy grids for 30 min instead of using ultracentrifugation. Nucleic acid composition of the isolated viruses was tentatively determined by staining the high-titer viral lysates with 20 μg of 4,6-diamidino-2-phenylindole (DAPI) per ml for 5 min, filtering onto 0.02-μm-pore-size Anopore filters, rinsing the filters with 5 ml of filtered (pore size, 0.02 μm) fresh seawater, and analyzing the filter under UV excitation for the presence of stained virus particles (13).

Solar irradiance. Solar irradiance was measured with the use of a light sensor attached to a mooring at 33°32′N, 118°17′W (18 miles from the site of the experiment) maintained by Richard Pieper. Thirty light measurements in microwatts per square meter were made and averaged at each 30-min interval. The average daily surface irradiance was calculated for the day of each experiment to provide an estimate of incoming solar energy. On days for which there were no available data, the average surface irradiance was estimated from data for the same day from another year between 1992 and 1995 (Table 1). Each day on which an experiment was performed was cloudless.

RESULTS

Direct counts and characterization. Direct bacterial and viral counts were performed for all experiments to monitor changes in bacterial populations due to addition of the lab-cultured viruses into seawater. A comparison of these bacterial counts via electron microscopy, with the direct epifluorescence method (acridine orange), showed that the ratio of TEM counts to epifluorescence counts was 1.04 ± 0.09 ($\bar{x} \pm$ standard error of the mean, $n = 14$). Direct counts of the supernatant fluid, after ultracentrifugation, also demonstrated that <1% of the bacteria remained. In a typical experiment, e.g., on 6 May 1992, total viral and bacterial direct counts varied over time, with viruses roughly 10 times more abundant than bacteria (Fig. 1). Direct counts were performed for all other experiments, and there was no indication of enrichment of the seawater samples due to the addition of the bacteriophage isolates, i.e., no drastic changes in the abundances of bacteria in the beginning of the experiment (data not shown).

Bacteriophages were analyzed for probable nucleic acid composition, head diameter and tail length (to the nearest nanometer), and head morphology (Table 2). All of the phages were icosahedral and appeared to contain DNA, with head sizes ranging from 41 to 86 nm (Table 2). A known RNA phage (MS2) was used as a negative control. Of the PR series phages, only PR2/1 was tailed. The H series phages were previously characterized by Frank and Moebus (9) and were all tailed, ranging in head size from 57 to 64 nm. Generally, both PR
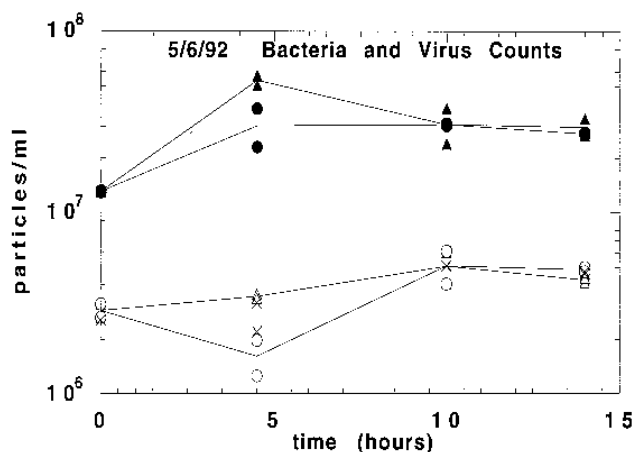
FIG. 1. Bacteria and virus counts by TEM on 6 May 1992 for an untreated seawater sample from Santa Monica Bay. Shown are abundances of marine viruses in seawater incubated in full sunlight (▲) and in the dark (●) and those of bacteria incubated in full sunlight (△) and in the dark (○). Direct bacterial counts (seawater incubated in sunlight) by epifluorescence are also shown (X).

series and H series phages have the typical size and morphology of other reported marine viruses (Table 2) (5).

Loss of infectivity of cultured viruses. When calculating the decay constant for a time series, we used the initial 12 to 15 h. Thereafter, the decay constant typically tapered off appreciably. Thus, half-lives calculated from endpoint concentrations of longer incubations would have usually underestimated initial decay rates. Correlation coefficients were mostly greater than 0.90, as the loss of infectivity versus time (plotted semilogarithmically) was close to linear.

Whirl-Pak bags allowed 67% transmittance (averaged over wavelengths of 290 to 320 nm) of UV-B and 75% transmittance of UV-A (320 to 400 nm). Covering a sample with glass excludes transmittance of those wavelengths less than 320 nm and slightly attentuates wavelengths of 320 to 330 nm (data not shown). Even though temperature has been shown to be an important factor in virus persistence in all types of water (32), variations in virus decay due to fluctuations in seawater temperature should represent a minor fraction of the rates of total decay observed in these experiments, as the range of ambient ocean temperatures used for the in situ incubations was a total of 3°C (from 16.5 to 19.5°C).

Decay of nonnative bacteriophages. In mid-March 1992, the decay of bacteriophage H40/1 was rapid in full sunlight at $11.1\% \pm 2.8\%$ h^{-1} ($\bar{x} \pm$ standard deviation, $n = 8$) and in the dark was about half ($5.4\% \pm 0.6\%$ h^{-1}, $n = 8$). H40/1 decayed at an insignificantly lower rate when incubated under glass than when incubated in full sunlight (Fig. 2).

In mid-April 1992, two different types of bacteriophages were seeded into seawater samples (at ca. 1.8×10^4 PFU ml^{-1}) which had been previously manipulated as follows: (i) untreated, (ii) filtered with a 0.2-μm-pore-size filter (removes all bacteria and protists, etc.), and (iii) heat treated (denatures enzymes, DNA, and other heat-labile molecules). This experiment was performed in an incubator at 18°C in the dark. The two viruses responded similarly to the various seawater treatments (Fig. 2). With both H40/1 and H85/1, samples which were incubated in the dark without prior filtration or heat treatment showed the highest rates of decay at $4.0\% \pm 1.4\%$ h^{-1} ($n = 6$) and $4.7\% \pm 0.9\%$ h^{-1} ($n = 6$), respectively. With H40/1, the rate of decay was significantly lower in filtered (pore size, 0.2 μm) seawater, at $1.0\% \pm 0.7\%$ h^{-1} ($n = 6$, $P < 0.001$),

and a similar rate of decay occurred in heat-treated seawater ($0.9\% \pm 0.2\%$ h^{-1}, $n = 6$). With H85/1, the rate of decay in the filtered (pore size, 0.2 μm) seawater was about twice that of the heat-treated samples, at $3.2\% \pm 0.2\%$ ($n = 6$) (Fig. 2).

On 6 May 1992, we observed a high rate of decay of infectivity in full sunlight in both H11/1 and H85/1 at $7.1\% \pm 1.7\%$ and $7.1\% \pm 0.4\%$ h^{-1} ($n = 8$), respectively (Fig. 3). In addition, H11/1 and H85/1 phages which were seeded into heat-treated seawater and were dark incubated decayed at very similar rates: $1.3\% \pm 0.7\%$ and $1.8\% \pm 0.2\%$ h^{-1} ($n = 8$), respectively (Fig. 2 and 3).

In an experiment performed on 15 July 1992, the rate of decay of infectivity in samples incubated in full sunlight was about twice that of the other treatments. In addition, two of three samples incubated under glass had insignificantly greater rates of decay than samples incubated in the dark, e.g., for H40/1, glass covered, the rate of decay was $3.4\% \pm 0.8\%$ h^{-1} ($n = 8$), and for H40/1, dark incubated, the rate of decay was $3.3\% \pm 0.7\%$ h^{-1} ($n = 8$) (Fig. 2).

In the experiment performed on 27 March 1995, we measured the degradation of all of the bacteriophages considered in this study. Rates of decay for the H series bacteriophages ranged from 6.6 to 7.5% h^{-1} ($n = 12$) in untreated samples which were incubated in full sunlight (Fig. 4). When incubated in the dark, all H series phages decayed about 50% slower than in full sunlight. Also, phages incubated in the dark in filtered (pore size, 0.2 μm) seawater always decayed at a significantly higher rate than those which were incubated in the dark in heat-treated seawater ($P < 0.05$). Decay in sunlight in heat-treated seawater was similar to that in the dark in untreated water for all phages (Fig. 4).

Decay of native bacteriophages. On 27 March 1995, the rates of decay of infectivity of viruses PR1/1, PR2/1, PR3/1, and PR4/1 ranged from 4.1 to 4.6% h^{-1} ($n = 12$) in untreated seawater that was incubated in full sunlight (Fig. 4). However, as a group these bacteriophages also decayed in the dark at rates which were only 1.3 to 1.8% h^{-1} lower (Fig. 4). PR series phage which were incubated in the dark in filtered (pore size, 0.2 μm) seawater always degraded significantly faster than those in heat-treated seawater. The rate of decay of PR1/1 in heat-treated dark-incubated seawater was the lowest, at 1.2% $\pm 0.2\%$ h^{-1} ($n = 12$). This rate of decay was similar to the background rates of decay of all of the native viruses in this experiment.

On 8 September 1995, the rates of decay for all of the native phages (PR series) in samples that were filtered (pore size, 0.2 μm), were approximately equal to those which were heat treated and then enriched with the DOM concentrate (data not shown). In this experiment, PR1/1, PR2/1, PR3/1, and PR4/1 all decayed at a higher rate in full sunlight in untreated seawater than in previous experiments (rates ranged from 5.1

TABLE 2. Characterization of bacteriophage morphologies

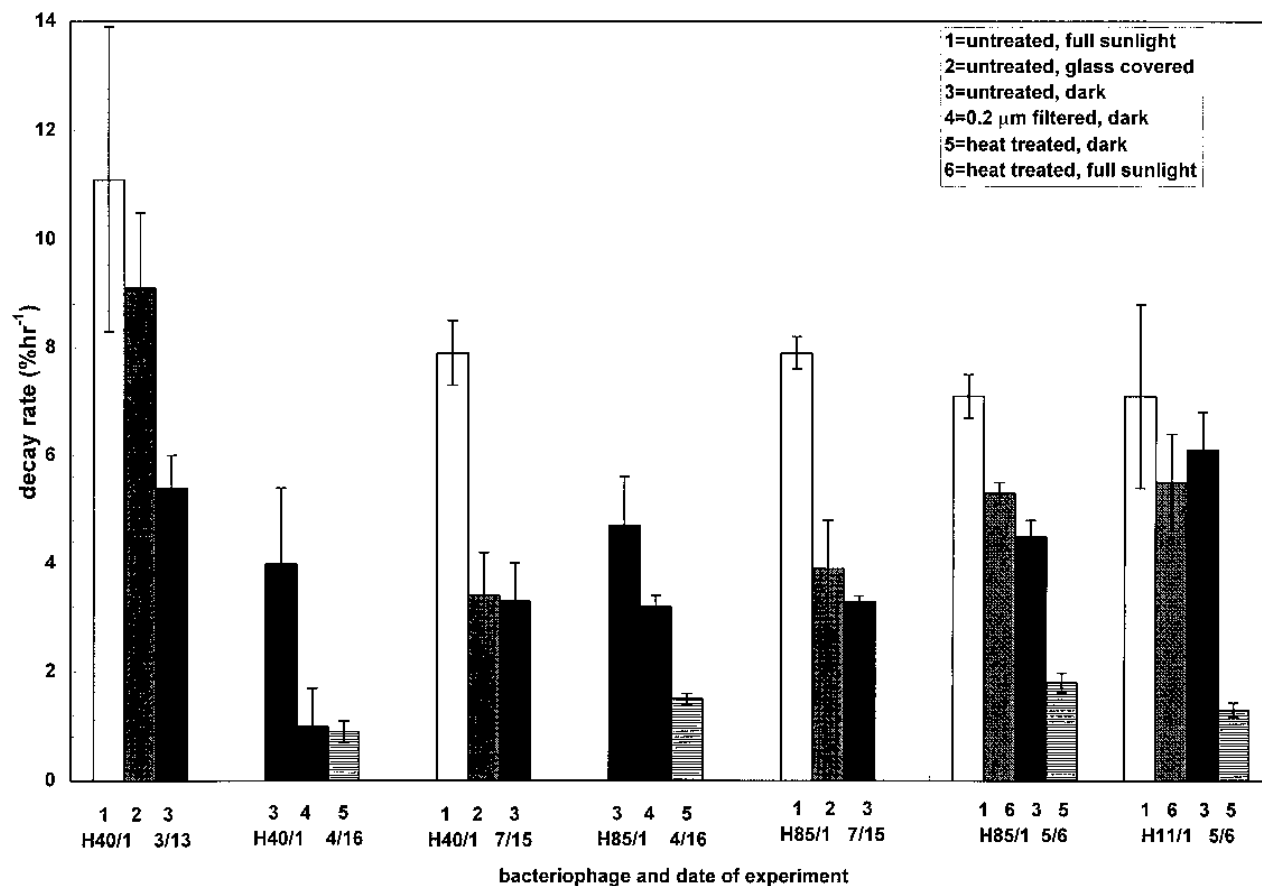| Bacteriophage type	Head diameter (nm)	Tail length (nm)
H2/1	64	71
H11/1	62	75
H40/1	62	117
H85/1	57	120
PR1/1	41	—[a]
PR2/1	86	142
PR3/1	42	—
PR4/1	52	—

[a] —, no tail.

FIG. 2. Rates of decay of infectivity in H series phages in experiments performed in 1992. Error bars represent 95% confidence intervals of the slope of the line. The date (month/day) of experiment is reported with each phage type. The numbers (1 through 6) under the bars are defined in the upper right corner.

to 7.7% h^{-1}). In addition, H2/1 (the only nonnative phage tested) decayed at a significantly higher rate, at 10.1% ± 2.0% h^{-1} ($n = 8$) than the PR series phages.

DISCUSSION

This study indicates that many causative agents, such as solar radiation, extracellular enzymes, and particles larger than 0.2 μm, contribute to the decay of infectivity of marine bacteriophages in coastal waters. We are not aware of any previous study which compares rates of decay of infectivity between groups of native and nonnative natural marine viruses in seawater. In addition, we have provided a direct assessment of the effects of heat-labile DOM on loss of infectivity. Previous studies showed that, without considering the added influence of sunlight, degradation of viruses occurred by physical forces, adsorption onto particles, extracellular nucleases, and proteases (20, 28). It has recently been suggested, however, that degradation of marine bacteriophages is primarily dependent upon solar radiation (29).

UV light. In Fig. 2, H40/1, H85/1, and H11/1 demonstrate the differential susceptibility of marine bacteriophages to solar radiation. In the experiments performed on 13 March 1992 and 15 July 1992, differences in the rates of decay of infectivity are seen between those samples which were incubated in full sunlight and those which were incubated under glass (excludes wavelengths of less than 320 nm, UV-B). In the 15 July 1992 experiment, it is apparent that UV-B is responsible for about 2/3 of the maximum decay of bacteriophages H40/1 and H85/1,

with UV-A and visible light contributing very little (Fig. 2). However, on 13 March 1992, the decay of H40/1 appears to be attributable to a combination of visible and/or UV-A plus, possibly, UV-B. The damaging effects of UV solar radiation have been documented in the past (15, 22, 23). Both UV-A and UV-B are capable of penetrating to tens of meters or more in seawater. In eutrophic coastal waters, the combined effects of solar radiation and particulate and dissolved causative agents of degradation are seen. It has been reported that UV-B may slow the growth of bacterioplankton in the surface waters of the ocean and thus reduce the amounts of extracellular enzymes secreted (15). In addition, UV-B solar irradiation can cause the breakdown of extracellular enzymes, possible reducing their degradative effects on bacteriophages and contributing to the pool of available nutrient sources for other bacterioplankton (1, 17). On 6 May 1992, the untreated samples which were incubated in full sunlight demonstrated rates of decay of infectivity for H85/1 and H11/1 which ranged from 6.6 to 7.9% h^{-1} (Fig. 3). However, compared to the results of previous studies (29), these rates of decay are 50% as high as the rate of decay of bacteriophages isolated from euphotic waters of the Gulf of Mexico. Trends of degradation in these two North Sea viruses H85/1 and H11/1 are very similar (Fig. 2 and 3). Decay is also rapid in the heat-treated samples, revealing just the direct effects of solar radiation. Conversely, the dark untreated samples still demonstrate rapid declines in infectivity, even without the effects of sunlight. This demonstrates the degradative effects of agents such as extracellular
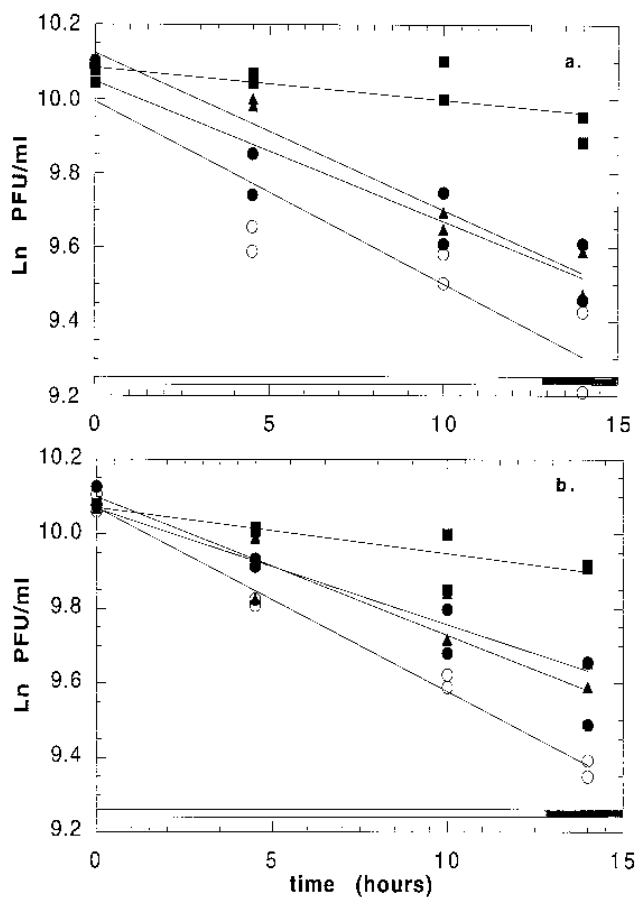
FIG. 3. Decay of infectivity in H series bacteriophages H11/1 (top panel) and H85/1 (bottom panel) on 6 May 1992. Shown are PFU declines for samples incubated as follows: untreated, full sunlight (○), heat treated, full sunlight (●), untreated, dark (▲), heat treated, dark (■). The open bar above the x axis represents daylight, and the dark bar represents darkness during the experiment.

proteases and nucleases, presumably from marine organisms. Heat-treated samples incubated in the dark demonstrate a much more gradual decline in infectivity for both bacteriophages. This is consistent with the hypothesis that the degradative agents are proteins.

High-molecular-weight DOM. On 27 March 1995, all of the PR series phages degraded less rapidly in full sunlight than the H series phages (Fig. 4). On 8 September 1995, seawater samples which had previously been either filtered (pore size, 0.2-μm) or heat-treated and reconstituted with active DOM (30 kDa to 0.2 μm) had rates of decay which were not significantly different, although heat treatment alone resulted in much slower decay. The response indicates that heat-labile, high-molecular-weight molecules or colloids between 30 kDa and 0.2 μm are responsible for about 20 to 25% of the maximal degradation of these isolates. In addition, phages have a uniform response to heat-treated seawater when incubated in the dark, as they decay at rates of ca. 1 to 2% h^{-1}.

Overview of decay of infectivity. It is apparent throughout all the experiments that both native and nonnative marine bacteriophages have a natural background rate of decay that is typically about 1% h^{-1}, in the absence of light, particles larger than 0.2 μm, or heat-labile substances. This is demonstrated in experiments which involve incubation of the bacteriophages in heat-treated water in the dark (Fig. 2, 3, and 4). In experiments

which involved incubation of phages in filtered (pore size, 0.2 μm) water in the dark (Fig. 2, 3, and 4), the observed rate of decay was almost always (93% of the time) significantly higher than the respective phages' background decay (dark, heat treated). This increased rate of decay indicates susceptibility of the tested viruses (both native and nonnative) to heat-labile DOM, including extracellular nucleases and proteases.

The native and nonnative phages decayed differently in relation to solar radiation and darkness. Not surprisingly, phages which were incubated in untreated seawater in the dark always demonstrated a significantly lower rate of decay than those incubated in full sunlight. However, as a group, the native bacteriophages (PR series) decayed only 35% slower in the dark, whereas the nonnative bacteriophages (H series) decayed about 50% slower (Fig. 2, 3, and 4). The H series phages consistently demonstrated relatively higher decay rates than those seen in the PR series phages. The nonnative bacteriophages (H series) had an average decay rate of 7.8% ± 0.8% h^{-1} (average turnover time of 12.8 h) in full sunlight. The native bacteriophages (PR series) decayed at an average rate of 5.4% ± 0.5% h^{-1} (turnover time of 18.5 h) in full sunlight. Phages incubated in untreated seawater in full sunlight always decayed at the highest rate. While solar radiation and other causative agents are important in inactivating viruses over relatively short-term time periods, it seems that as time proceeds, the degradative effects are not as pronounced. Decay rates in all experiments dropped off significantly after the first 15 h. This may occur because certain viruses, even in a clonal population, may be more resistant to degradation than others or because of variable protection by adsorbed material from seawater.

Implications. Sunlight increases viral degradation rates significantly, no matter what the origin of the bacteriophages. Moreover, it is apparent that the rate of decay of infectivity is nearly always significantly reduced if the seawater has been previously filtered (pore size, 0.2-μm) or heat treated. It has long been known that inactivation of viruses by sunlight is a direct and important effect, but compound effects are observed, e.g., particles larger than 0.2 μm and heat-labile substances in seawater often appeared to affect infectivity just as much as sunlight alone (e.g., compare data for samples heat treated in the sun with those for samples untreated in the dark [Fig. 4]). Sunlight had a less degradative effect on native viruses than it did on viruses isolated from the North Sea, both on an absolute basis and in proportion to their rates of decay in the dark. This may represent some adaptation made by the bacteriophages indigenous to this area to relatively high levels of solar radiation. The rates of decay in full sunlight observed in these experiments with both native and nonnative marine bacteriophages are much lower (typically by less than half) than those reported in previous virus decay studies (29). For comparison, it has been suggested that the rate of decay of infectivity of viruses isolated from the coastal waters of Texas is primarily dependent upon solar radiation (29). We demonstrate, however, that the phages from both Santa Monica Bay and the North Sea, when incubated in the dark in untreated seawater, decay at rates higher than (sometimes more than double) reported previously (29). In addition, phages in all experiments demonstrated a significant susceptibility to degradation by heat-labile substances in the dark, as demonstrated by the difference in the relative rates of decay in heat-treated and untreated seawater. The combination of slower decay in sunlight with faster decay in the dark would greatly change the conclusion that sunlight is the major factor controlling virus decay. Consequently, it is clear that models which use rates of virus decay need to be reexamined and should consider the
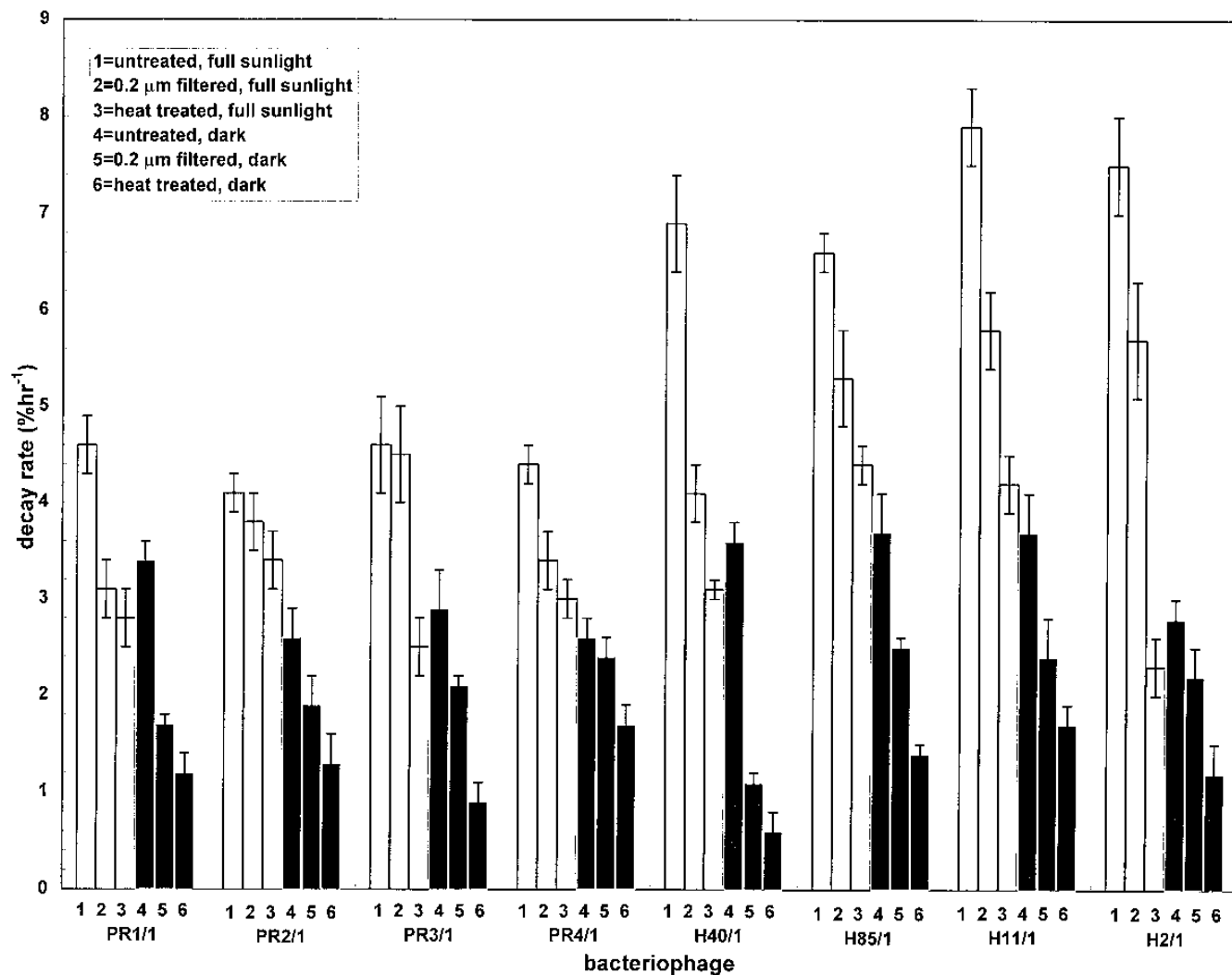
FIG. 4. Rates of decay of infectivity of all isolates tested on 27 March 1995. Error bars represent 95% confidence intervals of the slope of the line. White bars, full sunlight; black bars, dark incubation. The numbers (1 through 6) under the bars are defined in the upper left corner; the treatments are presented in the same order for each bacteriophage.

variations between different groups of bacteriophages (12). The differences in degradation patterns of lab-cultured viruses under identical conditions demonstrate that various viruses degrade at different rates and by different mechanisms. Future studies with more isolates from all types of marine systems will allow us to further characterize the mechanisms of virus degradation and the relative rates of decay of specific virus groups.

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