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Mechanisms and Rates of Decay of Marine Viruses in Seawater†

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Loss rates and loss processes for viruses in coastal seawater from the Gulf of Mexico were estimated with three different marine bacteriophages. Decay rates in the absence of sunlight ranged from 0.009 to 0.028 h^{-1}, with different viruses decaying at different rates. In part, decay was attributed to adsorption by heat-labile particles, since viruses did not decay or decayed very slowly in seawater filtered through a 0.2-μm-pore-size filter (0.2-μm-filtered seawater) and in autoclaved or ultracentrifuged seawater but continued to decay in cyanide-treated seawater. Cyanide did cause decay rates to decrease, however, indicating that biological processes were also involved. The observations that decay rates were often greatly reduced in 0.8- or 1.0-μm-filtered seawater, whereas bacterial numbers were not, suggested that most bacteria were not responsible for the decay. Decay rates were also reduced in 3-μm-filtered or cycloheximide-treated seawater but not in 8-μm-filtered seawater, implying that flagellates consumed viruses. Viruses added to flagellate cultures decayed at 0.15 h^{-1}, corresponding to 3.3 viruses ingested flagellate^{-1} h^{-1}. Infectivity was very sensitive to solar radiation and, in full sunlight, decay rates were 0.4 to 0.8 h^{-1}. Even when UV-B radiation was blocked, rates were as high as 0.17 h^{-1}. Calculations suggest that in clear oceanic waters exposed to full sunlight, most of the virus decay, averaged over a depth of 200 m, would be attributable to solar radiation. When decay rates were averaged over 24 h for a 10-m coastal water column, loss rates of infectivity attributable to sunlight were similar to those resulting from all other processes combined. Consequently, there should be a strong diel signal in the concentration of infectious viruses. In addition, since sunlight destroys infectivity more quickly than virus particles, a large proportion of the viruses in seawater is probably not infective.

High concentrations of viral particles are now recognized as being a ubiquitous component of surface seawaters (1, 5, 20, 23, 32, 35). Furthermore, a relatively large proportion of bacteria and cyanobacteria in surface waters contains virus-like particles, suggesting that infection by viruses may be a significant loss process for microbes in the sea (10, 23). Yet, despite the abundance and potential importance to nutrient and energy cycling of viruses, little is known about the processes responsible for regulating virus concentrations in the sea.

Observations of high concentrations of viruses in the sea are recent; nevertheless, interest in the fate of viruses in marine systems is not. In fact, a great deal of effort has centered on the survival of viruses in seawater (3, 17, 18), but these studies have largely focused on human pathogens and coliphages. Data from these model systems suggest that virus infectivity decays significantly over periods of days and that the decay is primarily biologically mediated, although chemical effects and effects from solar radiation have also been implicated (3, 18). In addition, colloids and particulate material have been shown to adsorb and rerelease coliphages in seawater (4). Most recently, it was reported that the disappearance of natural communities of virus particles from seawater was extremely rapid, with measured decay rates of up to 1.1 h^{-1} (10). The agents responsible for the disappearance were not identified; however, unlike the case in other investigations, high decay rates persisted when particulate material was removed by centrifugation or when cyanide was added to the seawater.

Ultimately, a number of mechanisms is likely responsible for the natural removal of viruses from seawater. In the present study, we examined the processes responsible for the decay of infectivity of marine bacteriophages in seawater. Infectivity rather than the abundance of virus particles was monitored, since infection is the process by which most virus-mediated effects occur. Our use of indigenous marine bacteriophages rather than exotic viruses is an important distinction differentiating our studies from those of others, given the recent interest in the role of viruses in marine ecosystems. We also extend the observations of previous work by examining the effect of solar radiation and microflagellate grazing on decreases in viral infectivity as well as determining which size fractions are associated with the decay of viruses in seawater. The relative importance of these processes is considered with a simple model.

MATERIALS AND METHODS

Marine bacteriophages and their hosts. The viruses (LMG1-P4, PWH3a-P1, and LB1VL-P1b) and bacteria (LMG1, PWH3a, and LB1) used for these studies were isolated from the coastal waters of Texas. LMG1-P4 and its host (LMG1) were isolated from a large, typically hypersaline lagoon (Laguna Madre), whereas PWH3a and LB1 and the viruses infecting them were isolated from seawater collected from the Marine Science Institute pier at Port Aransas, Tex. The taxonomic statuses of LMG1 and PWH3a are currently unknown. They were isolated as CFU from seawater spread on 1% agar plates made up with natural seawater enriched with 0.05% peptone and 0.05% Casamino Acids. LMG1 is red and was isolated from Laguna Madre; PWH3a forms milky colonies and was isolated from pier water. LB1 is a bioluminescent bacterium tentatively identified as *Photobacterium leiognathi*. It was also isolated from pier water with the agar-solidified medium described above,

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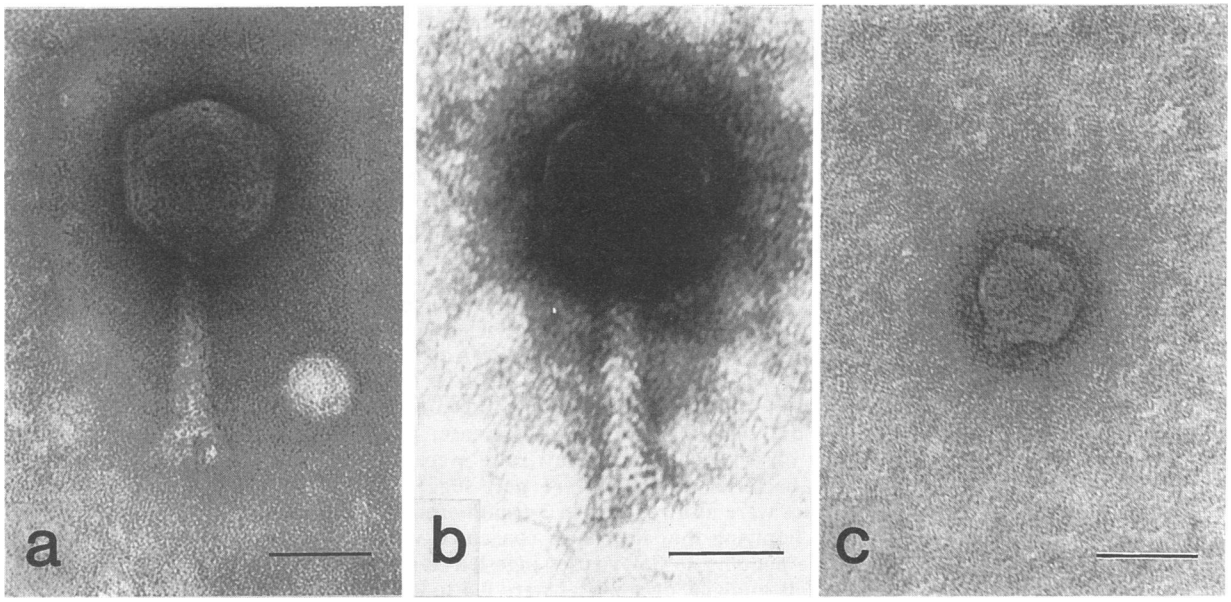

FIG. 1. Electron micrographs of the bacteriophages used in the decay experiments. (a) LMG1-P4; (b) PWH3a-P1; (c) LB1VL-P1b. Scale bar, 50 nm.

supplemented with 0.05% yeast extract and 3 ml of glycerol liter^{-1}.

The viruses LMG1-P4 and PWH3a-P1 were isolated from natural virus communities concentrated by ultrafiltration from water collected from Laguna Madre and the Marine Science Institute pier (33). One milliliter of exponentially growing host cells was combined with 20- or 40-μl aliquots of the virus communities, and the mixtures were allowed to adsorb for 30 min. These mixtures were combined with 2.5 ml of nutrient-enriched 0.6% molten agar, poured over a 1% agar underlay, and screened for plaque formation. The virus-infecting *P. leiognathi* was isolated from a culture in which a 2.5-ml culture of the bacterium and 25 ml of the enriched seawater medium had been added to 100 ml of seawater. After 48 h, aliquots of the culture were plaque assayed and viruses were detected. Clonal isolates of all of the viruses were obtained by standard plaque purification procedures (16).

Characterization of the viruses. Each of the viruses was characterized by electron microscopy. Samples from amplified stocks were spotted onto 400-mesh carbon-coated copper grids and allowed to adsorb for 30 min. The adsorbed viruses were rinsed through several drops of deionized, distilled water to remove salts, stained with 1% (wt/vol) uranyl acetate, and observed by transmission electron microscopy.

Evidence for the nucleic acid composition of the viruses was obtained by staining them with the DNA-specific fluorochrome DAPI (4′,6-diamidino-2-phenylindole) and examining them by epifluorescence microscopy. Further details on procedures that were used to isolate, purify, and visualize the viruses are described elsewhere (31, 33).

Plaque assays. The concentrations of viruses in amplified stocks and seawater samples to which viruses had been added were determined by assaying for the number of PFU. A colony from a plate of host cells was transferred to sterile medium in the late afternoon, and the cells were shaken overnight. In the morning, a 5 to 10% inoculum was trans-

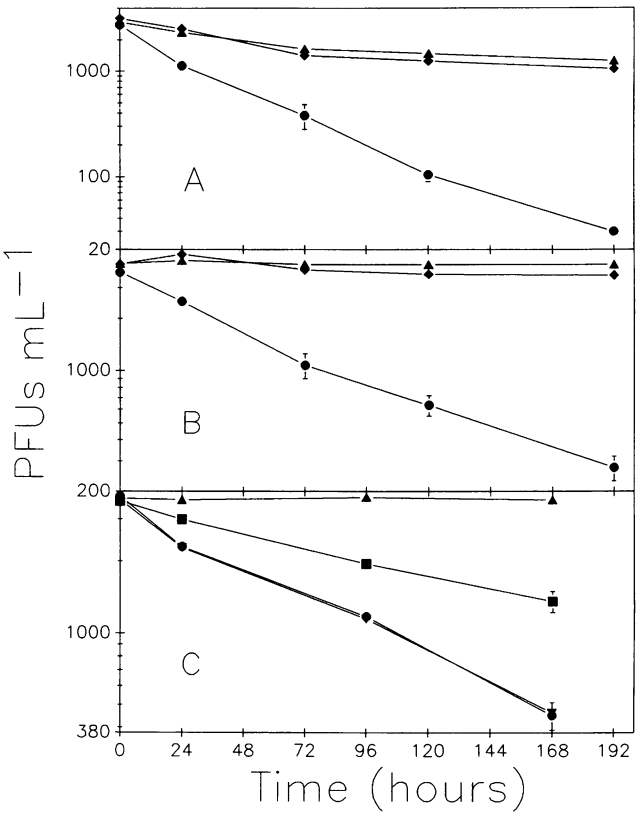

FIG. 2. Decay of infectivity of the marine viruses LB1VL-P1b (A) and PWH3a-P1 (B and C) in untreated natural seawater samples (●) and seawater samples that were autoclaved (◆) or filtered through 0.2 (▲)-, 3.0 (■)-, or 8.0 (▼)-μm-pore-size filters. When error bars are not shown, 1 standard deviation was less than the width of the symbols. Data are means from duplicate experiments.

TABLE 1. Decay rates and turnover times for PWH3a-P1 and LB1VL-P1b in natural seawater

Date	Value for PWH3a-P1				Value for LB1VL-P1b			
	k (h^{-1})	TT[b] (day)	r^2	n[a]	k (h^{-1})	TT[b] (day)	r^2	n[a]
22 May 1991	0.012	3.4	0.994	5	0.023	1.8	0.983	5
22 May 1991	0.014	2.9	0.982	5	0.023	1.8	0.992	5
24 May 1991	0.011	3.7	0.988	4	0.028	1.5	0.974	4
24 May 1991	0.013	3.2	0.983	4	0.025	1.6	0.932	4

[a] Number of time points used in decay rate determinations.
[b] Turnover time.

ferred to fresh medium, and 4 to 5 h later the exponentially growing culture was suitable for plaque assays. This protocol ensured that the number of nonviable host cells to which phage could attach was extremely small. Aliquots (20 to 500 μl each) of each virus sample to be assayed were combined with 500 μl from the culture of host cells and adsorbed for 30 min (for LMG1 and PWH3a). A shorter adsorption time (10 min) was found to be optimal for *P. leiognathi*. The culture containing the adsorbed virus was then combined with 2.5 ml of molten agar (44 to 46°C) to make a final concentration of 0.5%, and the mixture was immediately poured over a 1% agar underlay. Plaques would begin to appear 12 to 16 h later on plates that were incubated at 23 to 25°C. Triplicate plaque assays were done on each culture for every time point. In the event that it was necessary to dilute the concentrations of viruses before assaying them, the dilutions were always done in triplicate and independently of each other with autoclaved, artificial (9), or ultrafiltered (33) seawater.

Virus decay experiments. The seawater for the decay experiments was collected from the Marine Science Institute pier during incoming tides when the seawater was relatively clear and representative of water farther offshore (salinity, typically 30 to 35 ppt). Experiments were initiated when treatments to the seawater were completed. The rate of decay of infectivity of each of the marine bacteriophages was assayed in whole seawater and seawater that had been treated by autoclaving (121°C for 20 min), centrifugation in swinging-bucket rotors (16,000 or 121,000 × *g* for 20 or 300 min, respectively), or gentle filtration (<120 mm Hg [ca. 16,000 Pa]) through polycarbonate filters (Poretics; 0.2-, 0.8-, 1.0-, 3.0-, or 8.0-μm pore sizes). A tracer level (ca. 10^3 PFU ml^{-1}) of each of the viruses was introduced into duplicate treated or untreated seawater samples contained in polycarbonate Erlenmeyer flasks or borosilicate glass bottles, the mixture was incubated at 23 to 25°C, and the decay of infectious units was monitored.

In addition, decay rates in samples in which biological activity was stopped by the addition of 2 mM $NaCN^-$ were measured. The effect of the cyanide on microbial activity was determined by measuring thymidine incorporation. Tritiated thymidine ([*methyl*-^{3}H]thymidine; 3.7 × 10^5 Bq) was added to poisoned and nonpoisoned seawater subsamples (35 ml each) at 0, 22, 42, 114, and 159 h after the start of the experiment. The mixtures were incubated for 60 min and filtered through 0.2-μm-pore-size polycarbonate membranes (Poretics) that were overlaid on glass fiber filters. The amount of radioactivity trapped on the filters was determined by liquid scintillation counting.

For the grazing experiments, 5 × 10^7 PFU of PWH3a-P1 ml^{-1} were introduced into cultures containing 6 × 10^5 cells of a marine phagotrophic nanoflagellate (strain E4) ml^{-1} and into culture filtrate in which grazers and bacteria were removed by filtration through 0.8- and 0.2-μm-pore-size filters, respectively. The densities of viruses approximated concentrations in the field, while the abundance of flagellates ensured measurable decay rates over the duration of the experiment. In another experiment, cycloheximide (200 mg $liter^{-1}$), an inhibitor of protein synthesis in eukaryotes, was added to natural seawater to which which 1.7 × 10^4 PFU of PWH3a-P1 ml^{-1} had been added. The virus decay rates were compared with rates in seawater filtered through a 0.2-μm-pore-size filter (0.2-μm-filtered seawater) and seawater to which no cycloheximide was added.

The effect of solar radiation on the decay of viruses was investigated by adding ca. 2 × 10^3 PFU of each virus ml^{-1} into 10 glass dishes (19 cm wide by 7 cm deep) containing 1.5 liters of natural seawater. We had previously determined that each of the viruses could not cross-infect any of the

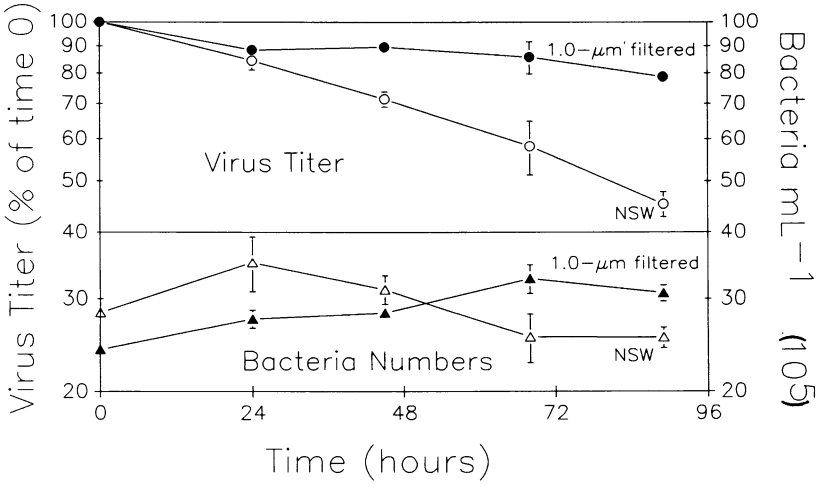

FIG. 3. Titers of PWH3a-P1 (upper panel) and changes in bacterium numbers (lower panel) in natural seawater (NSW [open symbols]) and in seawater that was filtered through 1.0-μm-pore-size polycarbonate filters (closed symbols). When error bars are not shown, 1 standard deviation was less than the width of the symbols. Data are means from duplicate experiments.

TABLE 2. Decay rates of marine viruses in filtered and unfiltered natural seawater[a]

Date	Virus	Decay rate in natural seawater filtered through the following pore size (h^{-1}):				Decay rate in unfiltered seawater (h^{-1})
		0.2 μm	0.8 μm	3.0 μm	8.0 μm	
24 May 1991	PWH3a-P1	0.000 (0.0001)		0.005 (0.0000)	0.011 (0.0000)	0.012 (0.0005)
24 May 1991	LB1VL-P1b	0.004 (0.0004)		0.020 (0.0012)	0.028 (0.0001)	0.027 (0.0014)
25 July 1991	PWH3a-P1	0.011 (0.0009)	0.011 (0.0015)			0.023 (0.0014)
30 July 1991	PWH3a-P1	0.004 (0.0001)	0.007 (0.0007)			0.015 (0.0005)
27 January 1992	PWH3a-P1	0.003 (0.0001)	0.002 (0.0004)[b]		0.012 (0.0008)	0.008 (0.0002)

[a] Rates determined from a minimum of four time points (standard deviations of duplicate determinations in parentheses).
[b] Filtered through a 1.0-μm-pore-size filter.

other hosts. The dishes were placed in a trough of flowing water to maintain constant in situ seawater temperatures, and dark controls were compared with treatment groups that were incubated for 4.5 h in full sunlight or in sunlight that was reduced by 80 or 53% by neutral-density screening. In addition, 6-mm-thick glass covers were used to exclude wavelengths of <320 nm from two full-sunlight treatments. The percentages of light transmitted by the glass at the following wavelengths were as follows: 450 nm, 88%; 400 nm, 86%; 370 nm, 78%; 360 nm, 71%; 350 nm, 58%; 340 nm, 36%; 330 nm, 12.3%; 320 nm, 1.5%; and 310 nm, <0.3%. All treatments were duplicated. Samples for virus titer were taken at time zero and at 1.5-h intervals thereafter. Irradiance was measured with a Licor Li-1000 quantum data logger and cosine collector that measured photosynthetically active radiation.

The decay rate constants were calculated by fitting a least-squares method linear regression to a plot of the natural logarithm of PFU per milliliter versus time. The slope of the line is the decay constant (per hour), and the reciprocal is the turnover time of the virus population.

RESULTS

Characterization of the viruses. Two of the bacteriophages used in these studies were very similar in size and appearance (Fig. 1a and b). LMG1-P4 and PWH3a-P1 are approximately 78 and 83 nm in diameter and have rigid tails that are about 97 and 104 nm in length, respectively. In contrast, LB1VL-P1b is smaller (50 nm in diameter) and has a very short tail (Fig. 1c). The DAPI-stained viruses could be visualized by epifluorescence microscopy, suggesting that they contained double-stranded DNA.

Decay rates in seawater in the absence of solar radiation. Viruses generally did not decay or decayed very slowly in seawater that was autoclaved or filtered through 0.2-μm-pore-size polycarbonate membrane filters (Fig. 2). In untreated natural seawater, the disappearance of PFU was typically linear ($r^2 > 0.95$) when plotted against time on a semilogarithmic axis (Table 1; Fig. 2 and 3). The range of measured decay rates in natural seawater was 0.008 to 0.023 h^{-1} for PWH3a-P1 and 0.023 to 0.028 h^{-1} for LB1VL-P1b (Tables 1 to 3). This corresponds to turnover times for the virus populations that ranged between 1.5 and 5.2 days. Experiments run with the same virus on the same day resulted in very similar decay rates (Table 1); in paired experiments, however, the infectivity of LB1VL-P1b decayed at about twice the rate of PWH3a-P1 (t test; $P < 0.0001$, $n = 8$). When particles >3 μm in size were removed, both LB1VL-P1b and PWH3a-P1 decayed more slowly (paired t test; $P < 0.001$, $n = 4$) but there was no difference in decay rates of viruses in 8.0-μm-filtered and unfiltered seawater (Table 2 and Fig. 2) (paired t test; $P = 0.256$, $n = 6$). Likewise, removal of particles by centrifugation at 16,000 × g for 20 min resulted in reduced decay rates that were similar to those of 0.2-μm-filtered seawater; however, there was no detectable decay in viruses that were added to ultracentrifuged seawater (Table 3). Nonliving particles were partially responsible for the decay, since decay continued in cyanide-treated seawater (Table 3) even though there was no detectable microbial activity over the 7 days of the experiment (data not shown). Furthermore, when particles >0.8 or >1.0 μm in size were removed, decay rates were substantially reduced (Table 2 and Fig. 3) (paired t test; $P < 0.001$, $n = 4$); however, bacterial abundances remained high. Nonetheless, biological processes were also involved, since decay rates were reduced by the addition of cyanide.

Since a significant amount of decay was attributable to material in the 3.0- to 8.0-μm size fraction and since decay rates were reduced by the addition of cycloheximide (Table 3) (t test; $P < 0.03$, $n = 4$), it indicated that protozoan grazers might be responsible for some of the observed decay. Addition of PWH3a-P1 to cultures of a phagotrophic marine nanoflagellate resulted in accelerated decay rates (Fig. 4) relative to viruses that were added to filtrate from a 0.2- or 0.8-μm-filtered flagellate culture.

Effect of solar radiation on virus decay rates. The average surface irradiance during the decay experiment was 1,872

TABLE 3. Effect of selective inhibitors and centrifugation on decay rates of PWH3a-P1 in treated and untreated natural seawater[a]

Date	Decay rate with the following treatment (h^{-1}):					Decay rate in untreated seawater (h^{-1})
	0.2-μm-pore-size filtration	Cycloheximide[b]	$NaCN^{-}$[c]	Centrifugation[d]	Ultracentrifugation[e]	
30 July 1991	0.004 (0.0001)	0.011 (0.0007)		0.005 (0.0008)	0.000 (0.0007)	0.015 (0.0005)
19 June 1992	0.001 (0.0001)		0.005 (0.0003)			0.012 (0.0003)

[a] Rates determined from a minimum of four time points (standard deviations of duplicate determinations in parentheses).
[b] Final concentration, 2 mM.
[c] Final concentration, 200 mg $liter^{-1}$.
[d] Centrifuged at 16,000 × g for 20 min.
[e] Centrifuged at 121,000 × g for 300 min.

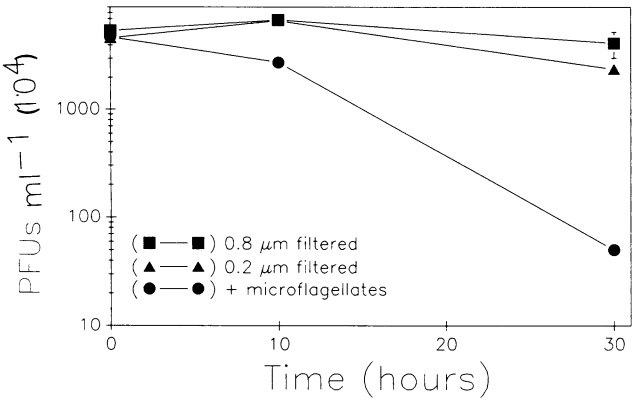

FIG. 4. Titers of PWH3a-P1 in cultures of a marine phagotrophic nanoflagellate (6×10^5 cells ml^{-1}) and in 0.2- and 0.8-μm-filtered culture filtrate. Data are means from duplicate cultures.

μmol of quanta m^{-2} s^{-1} but ranged between 1,288 and 2,228 μmol of quanta m^{-2} s^{-1} during the 15-min periods over which the irradiance was integrated. The decay rates of the viruses were very sensitive to solar radiation. In fact, as little as 20% of the surface irradiance resulted in decay rates for PWH3a-P1 that were 3.2 to 8.9 times those measured in natural seawater not exposed to solar radiation; for LB1VL-P1b, the range was about 3.9 to 4.8 (Fig. 5 and Tables 1 to 3). Similar decay rates were obtained if the viruses were added to ultrafiltered seawater and exposed to sunlight (data not shown); hence, the presence of phytoplankton, bacteria, or other particulate material was not required for sunlight to cause decay of infectivity. The decay rates were proportional to the amount of radiation received (Fig. 6). For example, eliminating 80% of the incident irradiance resulted in decay rates that were 20% of those in full sunlight. Similar experiments were done on three other occasions with comparable results. When wavelengths of <320 nm (UV-B radiation) were eliminated, decay rates were still very high relative to those of the dark controls and were similar to those of the treatment group in which 80% of the sunlight was removed (Fig. 5). The loss of infectivity was not reversible by overnight incubation in the dark.

Given that the decay rate of infectivity was directly related to the amount of radiation received (Fig. 6), it was possible to approximate the light-related decay of viruses in seawater from the attenuation coefficient (k) for damaging radiation. Hence, the decay rate at any depth (z) due to solar radiation (d_r) can be approximated from $d_{r0} \times e^{-kz}$, where d_{r0} is the decay rate at the surface. Using a range of attenuation coefficients for biologically damaging radiation in seawater (0.15 to 5.0 m^{-1}) and the average decay rate of infectivity at the surface for the three sunlight-exposed viruses (0.5 h^{-1}), we calculated the decay rates of infectivity at different depths for an average surface irradiance of 1,872 μmol of quanta m^{-2} s^{-1} (Fig. 7).

DISCUSSION

This study provides the first data on the decay of infectivity of natural marine viruses in seawater of which we are aware. Our results indicate that a number of factors contribute to the decay of virus infectivity in seawater, including solar radiation, living and nonliving particulates, and grazing by protozoa.

Characterization of viruses. The marine bacteriophages used in our studies are tailed icosahedrons. They are morphologically similar to other bacteriophages which have been isolated from seawater (8, 11, 12) and resemble many of the viruses that are observed in electron micrographs of marine virus communities (1, 23, 35). It is reasonable to expect that the mechanisms and rates of decay of these viruses are representative of those experienced by the rest of the marine bacteriophage community.

Effect of solar radiation on viral decay. Sunlight greatly accelerated the decay of viral infectivity. This was not unexpected, since viruses are very sensitive to UV irradiation. More surprising was that decay rates were 2 to 10 times higher than those of dark controls when wavelengths of <320 nm were removed or when 80% of the incident irradiance was excluded (Fig. 5). Biologically damaging effects of UV-A radiation (wavelength, 320 to 400 nm) and even longer wavelengths have been documented elsewhere (21). Unlike the effects of shorter-wavelength UV, the damage is not inflicted by direct interaction of the radiation with DNA and is thought to be the result of non-DNA photosensitizers and reactive species of oxygen (22). For example, thymine dimerization and single-strand breaks in DNA occur when intact bacteriophage T4 is irradiated at a wavelength of 365 nm (36), whereas isolated phage DNA from X174 was unaffected by wavelengths of >320 nm (19).

The sensitivity of marine viruses to solar radiation has major implications in terms of the persistence of virus infectivity in seawater. Significant amounts of UV-B (290- to 320-nm) and UV-A (320- to 400-nm) radiation, which directly and indirectly damage DNA, penetrate to considerable depth in seawater. For example, estimated attenuation coefficients for 310-nm-wavelength radiation range from 0.15 m^{-1} in clear oceanic water to 0.86 m^{-1} in moderately productive, coastal waters containing high concentrations of dissolved organic matter (27). Hence, 22 and 2%, respectively, of the surface irradiance would remain at 10 m. At the bacteriophage-damaging wavelength of 365 nm (36), the attenuation coefficient is even lower (k = 0.040 for clear ocean water) and continues to decrease at longer UV-A wavelengths (28).

Radiation will affect decay rates to significant depths over a wide range of attenuation coefficients (Fig. 7). Even at a coefficient of 1 m^{-1}, the decay rate of viruses at 3 m resulting from radiation (0.025 h^{-1}) would be similar to the highest decay rates that we measured in the absence of sunlight (Tables 1 to 3). If decay rate is integrated $[(d_{r0}/-k)(e^{-kz} - 1)]$ to determine the average decay rate in the water column, then it is not until 30 m that the mean decay attributable to solar radiation is equal to the average rate measured in the absence of sunlight (0.017 h^{-1}). For coastal waters with a higher attenuation coefficient (2.0 m^{-1}), the depth at which they would be equal is 15 m. In clear oceanic waters (k = 0.15 m^{-1}), only at depths exceeding 200 m would the average decay rate over the water column resulting from radiation be exceeded by the average decay rate measured in the absence of sunlight (0.017 h^{-1}). In offshore waters, the relative importance of solar radiation to decay is probably even more important than that indicated here, since loss rates resulting from other processes (e.g., attachment to particulate material) are probably greatly reduced. The significance of solar radiation is supported by data documenting that the survival of repair-deficient mutants of *Escherichia coli* was affected by sunlight at depths of 10 m in Antarctic waters (13).

Since the rate of decay of infectivity is proportional to the radiation received (Fig. 6), the average decay rate at the surface as a result of radiation can be estimated from the rate

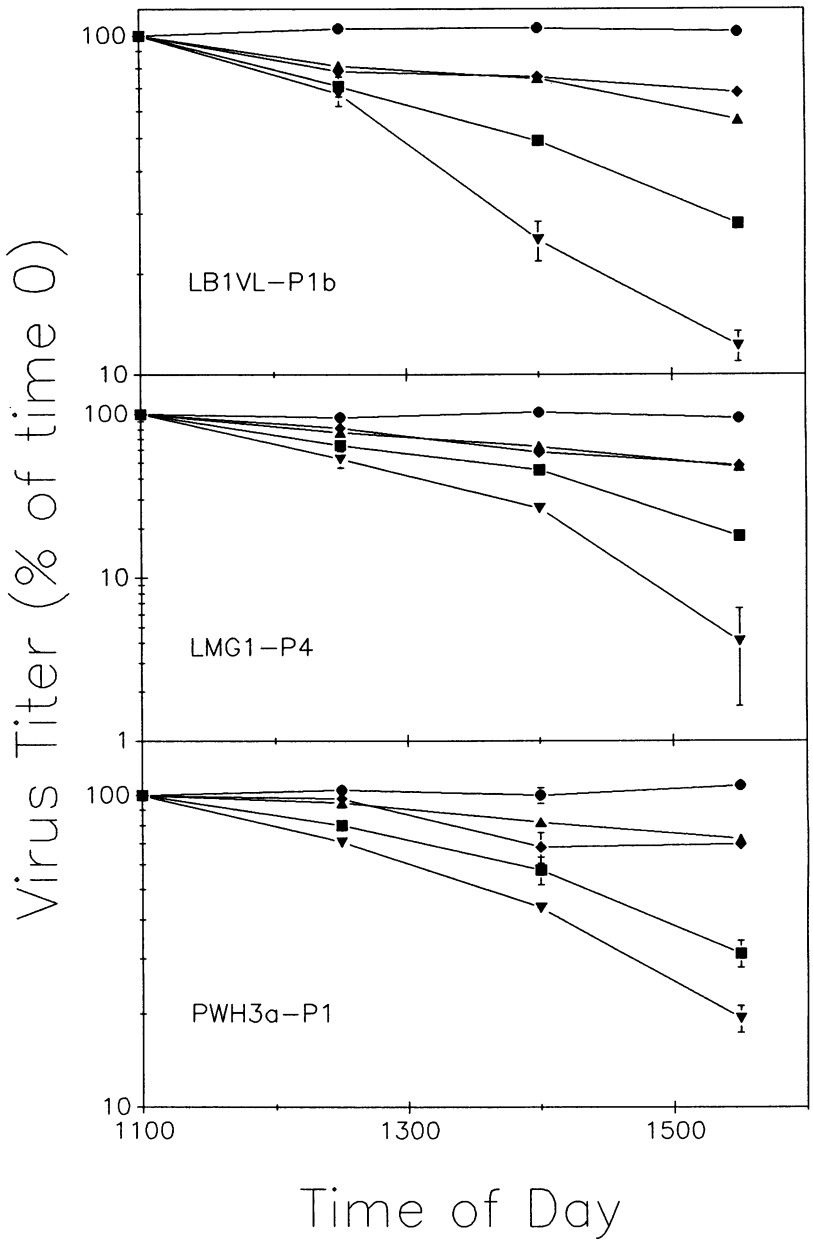

FIG. 5. Effect of solar radiation on the titers of three different marine viruses (LB1VL-P1b, LMG1-P4, and PWH3a-P1) on 29 May 1991. Treatment groups: ●, dark controls; ▲, no UV-B radiation; ◆, ■, and ▼, 20, 53, and 100% of incident irradiance, respectively.

of incident radiation averaged over 24 h. For example, on the date of the sunlight decay experiments (Fig. 5), the average quantum flux (photosynthetically active radiation) at Port Aransas, Tex., over 24 h, was 521 μmol of quanta m^{-2} s^{-1}. By the regression equation relating decay rate to irradiance (Fig. 6; $y = 0.2646x + 0.01938$), the average daily decay rate at the surface caused by radiation would be 0.16 h^{-1}, approximately 10-fold greater than the average loss of virus infectivity in the absence of sunlight (Tables 1 to 3). In the upper 60 m of clear oceanic water ($k = 0.15$), the estimated daily decay rate attributable to radiation (0.018 h^{-1}) exceeds the average rate measured without sunlight. In coastal water ($k = 1.0$ m^{-1}), sunlight would be responsible for most of the total daily decay in the upper 10 m. Clearly, solar radiation is a major factor responsible for the decay of virus infectivity in marine waters.

An important consequence of decay caused by radiation is that the viruses may not be removed. Therefore, in sunlight-exposed surface waters a large proportion of the viruses is probably not infective. Consequently, estimates of abundance made by electron microscopy probably greatly overestimate the number of infective viruses. A caveat is that photoreactivation and cell-mediated UV reactivation (for a brief review, see reference 2) can repair radiation-damaged phage DNA and restore virus infectivity. The significance of these processes in nature remains to be evaluated.

Virus decay in the absence of solar radiation. Decay rates in the absence of sunlight ranged from 0.008 to 0.028 h^{-1},

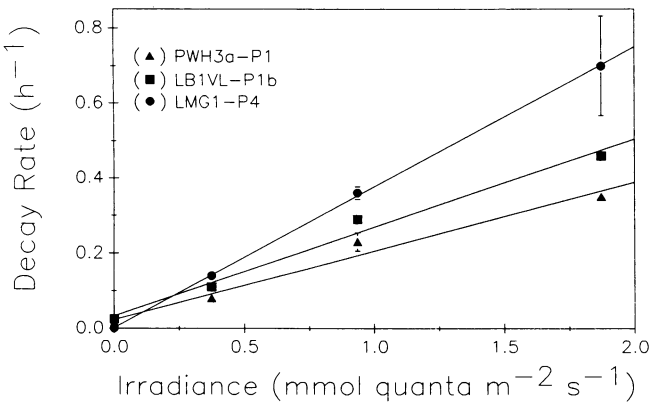

FIG. 6. Effect of solar radiation on the decay rates of LMG1-P4 ($y = 0.3746x + 0.0019$; $r^2 = 1.000$), LB1VL-P1b ($y = 0.2363x + 0.0332$; $r^2 = 0.992$), and PWH3a-P1 ($y = 0.1830x + 0.0230$; $r^2 = 0.987$). When error bars are not shown, 1 standard deviation was less than the width of the symbols. The regression equation describing all of the bacteriophage data is $y = 0.2646x + 0.0194$ ($r^2 = 0.918$).

corresponding to turnover times of between 1.5 and 5.2 days (Tables 1 to 3). The kinetics were first order and well described by linear regression of logarithmically transformed data, suggesting that most of the decay was attributable to a single rate process. The viruses should have served as good tracers, since they were added at low concentrations (ca. 10^3 ml^{-1}) relative to the number of virus particles typically present (ca. 10^7 ml^{-1}). The decay rates that we report are similar to those observed by others (3, 4, 26) for enteroviruses in nonpolluted seawater. In contrast, recently reported disappearance rates for viruses in seawater, determined by electron microscopy, were 9 to 39 times more rapid (0.26 to 1.1 h^{-1}) than the highest rates we observed (10). These observations are difficult to reconcile, since decreases in infectivity should provide more conservative estimates of virus decay rates than disappearance of virus particles. Moreover, Heldal and Bratbak (10) found that centrifugation of seawater did not reduce virus disappearance rates, whereas in our studies and those of others, decay rates decreased in seawater that was centrifuged to the same degree (our studies) or a lesser extent (3).

In our studies, the 0.8- to 8.0-μm size fraction was associated with much of the virus decay (Table 2; Fig. 2 and 3). A decrease in decay rates associated with the removal of particles has generally been attributed to removal of bacteria (18, 26). However, decay rates were significantly reduced by removal of particles >0.8 or >1.0 μm (Table 2; Fig. 2 to 4) in size, even though bacterial numbers were not. Nevertheless, bacteria or other organisms were involved in virus decay, since rates were reduced in the presence of cyanide.

Considerable decay was also associated with the 3.0- to 8.0-μm size fraction. Moreover, since decay rates were reduced by the addition of cycloheximide (Table 3) and marine flagellates have been observed to consume viruses (8a), it suggested that some of the decay might result from grazing. Decay rates in the presence of flagellates were accelerated; however, the consumption rate was relatively modest (3.3 viruses $flagellate^{-1}$ h^{-1}) when the viruses were added at a natural concentration (5×10^7 viruses ml^{-1}). Consequently, even if flagellates were present at 10^4 ml^{-1}, they could consume only about 0.1% of the virus community h^{-1} and could not account for the decay attributable to the >1-μm size fraction.

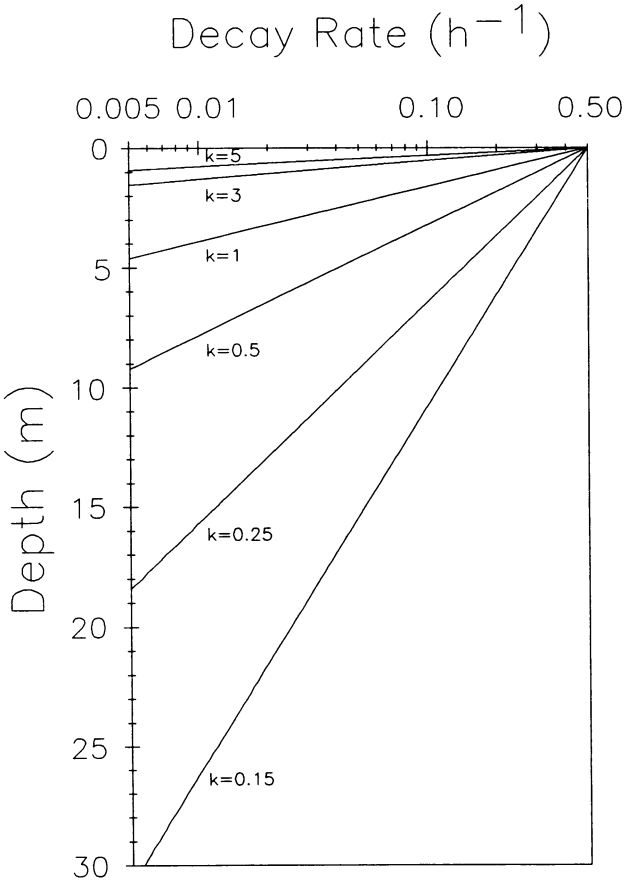

FIG. 7. The effect of solar radiation on the decay rate of marine viruses with attenuation coefficients (k) ranging from that for clear oceanic water ($k = 0.15$) to that for turbid coastal waters ($k = 5$). The surface decay rate is based on the averaged decay rate of LB1VL-P1b, LMG1-P4, and PWH3a-P1 during a 4.5-h incubation, during which the average irradiance was 1,872 μmol of quanta m^{-2} s^{-1} (photosynthetically active radiation).

The element responsible for decay in the absence of sunlight was heat labile and could be greatly reduced by filtration or centrifugation but was only partially attributable to bacteria or protists. Lycke et al. (15) found that viriocidal material in seawater was destroyed by heating to as low as 45°C. They interpreted their results as evidence that nonliving organic material is responsible for the decay of virus infectivity but offered no explanation as to its nature. We suggest that viruses irreversibly bind to heat-labile, loosely associated aggregates and in the process loose their infectivity. This hypothesis is supported by observations that fluorescence-stained viruses rapidly adsorb onto microscopic particulates in seawater (8a). The origin of the particles is unknown, but their appearance and the variable efficiency with which they can be removed by filtration suggest that they are loosely associated microscopic aggregates. Although viruses reversibly bind to a number of organic and inorganic materials (4, 25), we were unable to recover a significant number of infectious units with a number of standard protocols for desorption or by using gentle sonication to break up aggregates (data not shown). Enteroviruses can also strongly adsorb to natural particles in estuarine waters and only be desorbed with low efficiency (14). Our

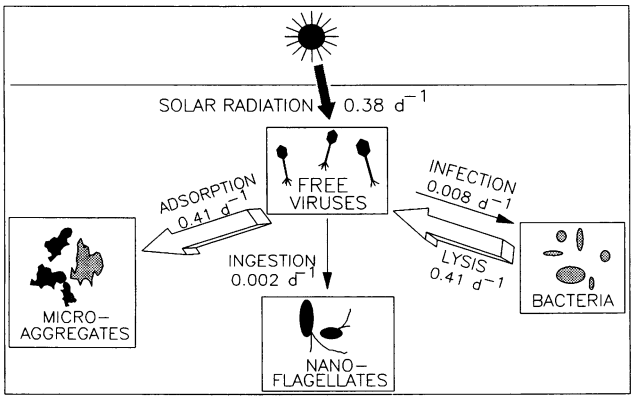

FIG. 8. A simple model depicting the estimated daily rate constants for the decay and production of marine viruses, averaged over a 10-m coastal water column, for a daily averaged surface irradiance of 45.052 mol of quanta m^{-2} and an attenuation coefficient of 1.0 m^{-1}. Arrows represent loss and production of viral particles, except for the hatched arrow, which represents the destruction of virus infectivity resulting from solar radiation.

results suggest that attachment to particles removes both infective and noninfective viruses from seawater, since, once adsorbed, the viruses will be lost by sedimentation or potentially via zooplankton grazing. Studies have described large numbers of virus particles affiliated with material collected from sediment traps (24) and viruses embedded in slime associated with a collapsing diatom bloom (6).

Implications. Our study demonstrated that solar radiation is likely the primary mechanism causing decay of virus infectivity in surface waters. Even when averaged over 24 h and integrated over the upper 30 m of the water column, the estimated destruction rate of infectivity by solar radiation in clear ocean water (0.033 h^{-1}) exceeded the greatest loss rates that we measured for coastal waters in the absence of sunlight (Tables 1 to 3). In addition, since infectivity is probably destroyed much more quickly than viruses are physically removed, it implies that a large proportion of the viruses that are counted by electron microscopy are noninfective.

Abundance and infectivity of viruses in seawater are controlled by several processes. The most important of these are production of viruses via cell lysis, destruction of infectivity by solar radiation, removal of viruses through adhesion to particulates, and perhaps digestion by bacterial enzymes (Fig. 8). The relative importance of solar radiation will vary as a function of insolation and water transparency. In near-surface and shallow coastal waters, sunlight can destroy the infectivity of most of the virus community in a few hours. In addition, unlike adsorption to particles or grazing by protists, sunlight will not remove viruses. Hence, virus production needs only to balance the removal of virus particles to maintain a constant number. Average rates of loss of infectivity in our studies in the absence of sunlight averaged 0.41 day^{-1} (range, 0.22 to 0.67; Tables 1 to 3). This implies similar production rates, assuming that these decay rates reflect the removal of free virus particles. In order to support this production and with an average burst size for marine bacteria of 50 viruses assumed (10), the loss rate from the virus community required to support this production, via infection of bacteria, would be approximately 2% of the viral production rate (range, 0.004 to 0.013 day^{-1}). For typical viral and bacterial abundances of 10^7 and 10^6 ml^{-1}, respectively, this would require about 4 to 13% of the bacteria to be infected daily, assuming that lysogenic cells are a minor source of virus progeny. The proportion of the bacterial mortality that this represents depends strongly on bacterial growth rates, having the greatest effect on bacterial mortality when growth rates are lowest. These values seem reasonable, given that 0.7 to 16% of marine bacteria have been estimated to contain mature phage (10, 23, 24). The virus production and removal rates are similar to the decay rates that would be attributable to sunlight in coastal waters with an attenuation coefficient of 1.0 m^{-1}. The average daily decay rate of virus infectivity for a 10-m water column would be 0.38 day^{-1}. Hence, almost all of the daily virus production would be made noninfective during a sunny day. This suggests that there is a strong diel signal in the number of infectious viruses.

The effect of solar radiation on virus infectivity may also explain a paradox. It is puzzling that despite the great abundance of viruses in seawater and the numerous host-virus systems that have been isolated, the concentration of infectious viruses for a given host (except cyanobacteria) is generally low, ranging from below detection limits (or a fraction of an infectious unit ml^{-1}) to 10^4 infectious viruses ml^{-1} (7, 29, 30, 34). Possible explanations for this observation are that (i) the host-pathogen systems that have been isolated are not representative of those occurring most abundantly in nature; (ii) the viruses are extremely host specific, and there are 10^3 to 10^4 different hosts in each milliliter of seawater; or (iii) a large proportion of the viruses in seawater is not infective. Given the potentially strong impact of solar radiation on virus infectivity, it seems that the last hypothesis is likely correct.

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