

## Estimation of Biologically Damaging UV Levels in Marine Surface Waters with DNA and Viral Dosimeters<sup>¶</sup>

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### ABSTRACT

We have surveyed the biologically harmful radiation penetrating the water column along a transect in the western Gulf of Mexico using dosimeters consisting of intact viruses or naked calf-thymus DNA (ctDNA). The indigenous marine bacteriophage PWH3a-P1, which lytically infects the heterotrophic bacterium *Vibrio natriegens* (strain PWH3a), displayed decay rates for infectivity approaching  $1.0 \text{ h}^{-1}$  in surface waters when deployed in a seawater-based dosimeter. The accumulation of pyrimidine dimers in ctDNA dosimeters provided a strong correlation to these results, with pyrimidine dimers representing more than 0.3% (up to ca 3800 dimers  $\text{Mb}^{-1}$  DNA) of the total DNA in dosimeters exposed to sea surface levels of solar radiation. The results demonstrate a strong correlation between the dimer formation in the DNA dosimeters, the decay rates of viral infectivity and the penetration of UVB radiation into the water column. The decay of viral infectivity attenuated with depth in a manner similar to the decay of solar radiation and was still significant at 10 m in offshore oligotrophic water and at dimer frequencies less than 0.1% (ca 200–300 dimers  $\text{Mb}^{-1}$  DNA).

### INTRODUCTION

Increasing evidence suggests that stratospheric ozone ( $\text{O}_3$ ) levels are decreasing because of the presence of anthropogenically derived chlorofluorocarbons in the upper atmosphere (1,2). Stratospheric ozone functions as the main atmospheric attenuator of ultraviolet (UV) radiation, and thus ozone decay is predicted to lead to increased levels of incident UV radiation at the earth's surface (3). Because of

the direct deleterious effects of UV on living organisms, it is important to develop indicators of UV exposure that would measure biologically relevant UV damage (4,5).

Quantifying the exposure to UV radiation is difficult and expensive. Spectroradiometers are commonly used to measure the energy of discreet wavelengths but are costly and limited in their ability to integrate over long periods of exposure and to account for sporadic anomalies such as cloudiness (6,7). Chemical indicators of UV exposure are more cost-effective but are limited by the wavelengths of their specific chemical reactions (8,9). Given these considerations, it is important to develop UV detection systems that integrate dosage over time, respond to all wavelengths of solar radiation and provide biologically relevant results in a cost-effective manner.

Recently, DNA dosimeters, consisting of naked DNA within quartz enclosures, have been used to estimate the damaging UV radiation on the basis of the formation of *cis-syn* cyclobutane pyrimidine dimers (CPD) in the DNA (6,10). Although the dimer formation within DNA is most efficient at 254 nm (11), wavelengths less than 280 nm do not reach the earth's surface, leaving wavelengths of ca 305 nm to be the most biologically effective (12). UVB radiation induces dimer formation that is ca 90 times less effective than at 254 nm but remains four orders of magnitude more damaging than light at 360 nm (13). The UVB (290–320 nm, as defined by the American Society for Photobiology) component of the solar spectrum can directly damage DNA through the formation of CPD as well as induce the formation of pyrimidine [6-4] pyrimidone photoproducts (14). Both types of damage are inhibitory to cellular replication processes (15) and have been examined in viruses in marine systems (16,17). But pyrimidine [6-4] pyrimidone photoproducts can be converted to the Dewar isomer by longer-wavelength irradiance (18), making them more difficult to be accurately estimated *in situ* (16).

Viruses are pervasive within aquatic environments at titers of  $10^5$ – $10^9$  infective units per milliliter (19,20). Viral particles demonstrate host-specific relationships with many aquatic organisms, including bacteria, cyanobacteria and eukaryotic plankton. The cosmopolitan distribution of viruses and their sensitivity to solar radiation suggest that they may

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**Abbreviations:** CPD, cyclobutane pyrimidine dimers; ctDNA, calf-thymus DNA; PAR, photosynthetically active radiation; PFU, plaque-forming units; PVC, polyvinyl chloride; RIA, radioimmunoassay; UV, ultraviolet.

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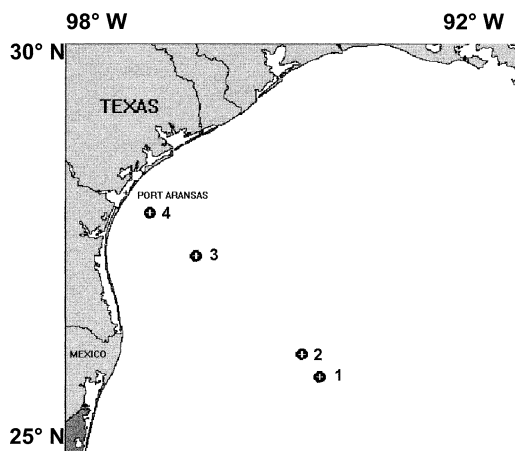


Figure 1. Sample sites in the western Gulf of Mexico.

provide excellent indicators of biologically harmful radiation in aquatic systems (21–24).

The use of viruses as dosimeters of DNA damage has previously been studied using the coliphages T1 (25) and T7 (4,26) as well as the naked DNA from the coliphage ΦX174 (6). Quantifying the bacteriophage infectivity, which is destroyed upon exposure to UV, is cost effective relative to the previously mentioned methods for monitoring UV. Plaque assays on lawns of host cells can be used to determine the abundance of plaque-forming units (PFU), which provide an estimate of the total abundance of infective viruses in a given aliquot. With incubation times ranging from 8 to 24 h, plaque assays provide for the rapid overnight estimate of the abundance of infective centers. The decay of viral infectivity in marine systems caused by solar radiation has been documented, with rates shown to decrease with depth similar to the attenuation of light in the water column (21,27).

In this study we have used the viral dosimeters containing the marine bacteriophage PWH3a-P1, the dosimeters containing calf-thymus DNA (ctDNA) and a profiling spectroradiometer to estimate the biologically harmful solar radiation at different depths along a transect in the Gulf of Mexico and to demonstrate the phenomenon of internal solar reflectance in the water column. This marine phage provides the advantage of being stable when added to seawater, accounting for any UV-attenuating effects of the native dissolved organic material. The results demonstrate the efficacy of these dosimeters and suggest that viral dosimeters may provide an adequate proxy for estimating the damaging UV when more expensive and technically challenging methods are not practical or available.

## METHODS AND MATERIALS

**Study sites and physical characteristics.** Research was carried out on the RV *Longhorn* on a transect from the western Gulf of Mexico to its homeport in Port Aransas, TX. Four lagrangian stations were occupied on cloudless days from 22 to 29 June 1995 and from 23 to 30 July 1996 (Fig. 1). The attenuation of light penetration at 305, 320, 340 and 380 nm and photosynthetically active radiation (PAR) through the water column was determined with a Biospherical PUV-500 profiling radiometer (bandwidths  $\cong$  10 nm). Measurements were integrated over depth and attenuation coefficients used to calculate irradiance at experimental depths. To examine the correlation between dimerization in ctDNA dosimeters and UVB fluence, experiments

were also performed in Santa Rosa Sound, FL (30°20.334'N, 87°09.373'W) and in the Gerlache Strait, Antarctica (64°13.26'S, 61°46.94'W). Dosimeters were placed flush with the photocollector of the PUV-500 radiometer and were held 10 cm below the seawater surface.

**Viruses and bacteria.** The bacteriophage PWH3a-P1, which infects a marine bacterium (strain PWH3a) that (based on sequence analysis and biochemical tests) is closely related to *Vibrio natriegens*, was previously isolated from the Gulf of Mexico (21). Host populations of *Vibrio* strain PWH3a were maintained in ultrafiltered (30 kDa cutoff) seawater amended with peptone, Casamino acids, and yeast extract (0.05% wt/vol each), and glycerol (0.3% vol/vol). Axenic bacteria were maintained on agar (1.0%) plates until transfer to liquid cultures for experiments. Viruses were amplified in 50 mL cultures of exponentially growing cells in 250 mL polycarbonate flasks. Lysates ( $ca$   $10^8$  infective viruses per milliliter) were rendered free of cellular debris by centrifugation and subsequent filtration through 0.22  $\mu$ m pore size polyvinylidene difluoride Durapore filters (Millipore). Viral stocks were maintained in the dark at 4°C until use.

**Dosimeters.** Viral dosimeters were prepared by diluting concentrated viral stocks to  $ca$   $10^5$  PFU mL<sup>-1</sup> with either filtered (Poretics: 0.20  $\mu$ m pore size polycarbonate filters) or whole seawater collected from each station. Diluted viral stock was then dispensed (50 mL) and heat sealed into 250 mL polyethylene sample bags (Fisherbrand; 320 nm radiation attenuated by  $ca$  9%, 305 nm radiation attenuated by  $ca$  12%). DNA dosimeters consisting of 100  $\mu$ g mL<sup>-1</sup> of ctDNA in quartz tubes (1 cm diameter) were attached to the polyvinyl chloride (PVC) racks (see below) in parallel with the viral dosimeters.

**Deployments.** *In situ* samples were attached to a series of PVC racks that were suspended at fixed depths (1, 5, 10 and 15 m) from a buoy (28,29). Ballast (20 kg) was attached to the bottom of each deployment. In addition, a floating rack at the surface was tethered to the main buoy by 5 m of nylon rope. Samples were deployed before sunrise and retrieved at dusk (after 12 h of incubation) in order to expose them to 1 complete solar day. Three independent racks were deployed at each station to provide triplicate samples at each depth.

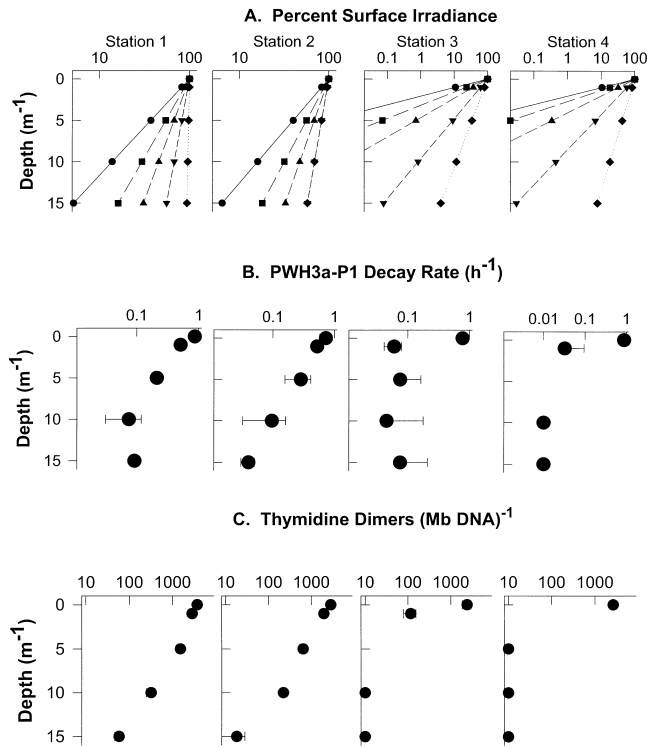
Whole-seawater dosimeters containing PWH3a-P1 were deployed in a series of floating attached racks that were retrieved 4, 6, 9 and 12 h after deployment (sunrise) to examine the *in situ* surface-enhanced UV dose. Parallel samples were maintained on deck in an open-top flowing-seawater incubator to allow for the measurements of decay without the influence of upwelling or internally reflected radiation. The incubator was lined with black plastic to reduce any internal reflection of light, and water levels were maintained near the top to prevent any potential side shading. To compare the influence of different wavelengths of sunlight on viral infectivity, PWH3a-P1 dosimeters were deployed in the on-deck incubators under different filters: Mylar D (excluding wavelengths of <312 nm) and Acrylite 436-4 (Cryo Industries [Manchester, NH]; excluding wavelengths of <550 nm). Dark controls for all experiments were maintained in several layers of black plastic in an on-deck flowing-seawater incubator. Temperature was monitored during these incubations to ensure that it did not vary from the ambient surface temperature.

The abundance of infective PWH3a-P1 in each sample was determined by plaque assay using *Vibrio* sp. strain PWH3a as the host bacterium (21). Plaque assays were carried out under low irradiance (<15  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) to reduce any potential photoreactivation of damaged viruses by bacterial photolyases (30). Decay rates were determined from the equation

$$\text{Decay rate} = [(\ln 100) - \ln(100 \times (P_L/P_D))]/t$$

where  $P_L$  is the concentration of PFU in the light treatment,  $P_D$  is the concentration of PFU in the dark treatment and  $t$  is the length of time of the deployments (12 h). This treatment of the data accounts for any light-independent destruction of viral infectivity (17).

**Assessment of DNA damage.** A competitive radioimmunoassay (RIA) (14) was used to quantify the CPD in the DNA dosimeters. The antiserum for CPD was raised against Salmon testes DNA in 20 mM acetophenone (a triplet sensitizer that promotes the production of CPD but not of pyrimidine [6-4] pyrimidone photoproducts) and was irradiated with a UVB source (Westinghouse FS20; 75 kJ m<sup>-2</sup> at 280 nm). For the RIA, 2–5 mg of heat-denatured sample DNA was incubated with 5–10 pg of poly(dA):poly(dT) probe (labeled to  $>5 \times 10^8$  cpm  $\mu$ g<sup>-1</sup> by nick translation with <sup>32</sup>P-dTTP) in



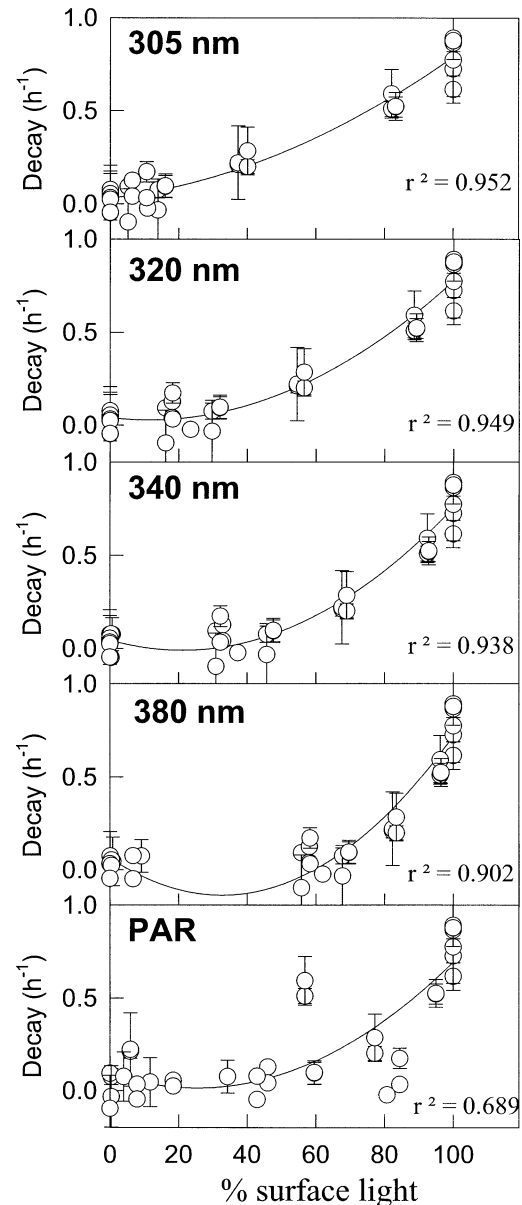
**Figure 2.** Estimates of the penetration of solar radiation into the western Gulf of Mexico. A: Penetration of 305 nm (●), 320 nm (■), 340 nm (▲), 380 nm (▼) radiation as well as PAR (◆) into the water column. B: Rates of infectivity decay for bacteriophage PWH3a-P1 in whole seawater ( $n = 3$ ,  $\pm$ SD). C: Concentrations of pyrimidine dimers ( $\text{Mb}^{-1}$  DNA) in ctDNA dosimeters ( $n = 2$ ,  $\pm$ range of replicates).

a total volume of 1 mL buffer (pH 7.8) (10 mM Tris, 1 mM ethylenediaminetetraacetic acid, 150 mM NaCl, 0.2% gelatin [Sigma, St. Louis, MO]). Antiserum was added to the reaction mixture at a dilution that yields 30–60% binding of the labeled ligand. After incubation for 3 h at 37°C, the immune complex was precipitated with goat anti-rabbit immunoglobulin and carrier serum from non-immunized rabbits (both from Calbiochem, La Jolla, CA). After centrifugation and removal of the supernatant, the pellet was dissolved in tissue solubilizer ([NCS]; Amersham, Piscataway, NJ), and the sample was transferred to a scintillation vial containing Scintisafe 30% LCS cocktail and counted in a Packard 1600TR scintillation counter. DNA photoproducts in the sample DNA were determined by the reduction in counts as a result of competition for antibody between the sample DNA and the radiolabeled poly(dA):poly(dT) probe. Each assay was standardized with DNA of known photoproduct content and analyzed in duplicate.

## RESULTS

Significant levels of UVB penetrated as deep as 15 m in the water column at Stations 1 and 2 (Fig. 2A). Short-wavelength light was attenuated more quickly at the more productive nearshore stations, with 305 nm light being diminished by approximately 90% at 1 m.

The rate of decay of infective viruses in the dosimeters varied with the depth of the deployment and was similar to the attenuation of 305 nm light (Fig. 2B). There was no significant difference ( $P < 0.05$ ) between the decay rates for deployments in filtered (not shown) and unfiltered seawater, suggesting that UV-mediated decay was the factor most significant in regulating the destruction of viral infectivity. The

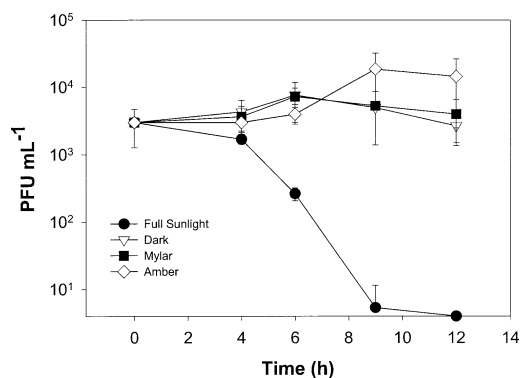


**Figure 3.** Correlations between the decay rate of infectivity ( $n = 3$ ,  $\pm$ SD) in viral dosimeters and the penetration of light into the water column. Second-order regressions were used to generate curves to predict decay rates of infectivity on the basis of light penetration.

decay rates of viral infectivity for Stations 1 and 2 decreased to near 0 by 1 m, consistent with the penetration of 305 nm light at these stations.

At all stations the concentration of photoproducts in the ctDNA dosimeters decreased with depth (Fig. 2C). Dimerization was highest at the surface and occurred at greater depths at the offshore stations (Stations 1 and 2) than at the nearshore stations (Stations 3 and 4). Samples from 5 m depth at Station 3 and 1 m depth at Station 4 were lost during the deployment.

The data for destruction rates for viral infectivity at all stations were combined and compared with those for light penetration (Fig. 3). Second-order regressions fit to the data demonstrated that the decay rates of infectivity correlated best



**Figure 4.** Wavelength-dependent destruction of viral infectivity. Viral infectivity was monitored in the absence of radiation of  $<312$  nm as well as  $<550$  nm and compared with the rates of destruction in full sunlight and in the dark ( $n = 3$ ,  $\pm$ SD).

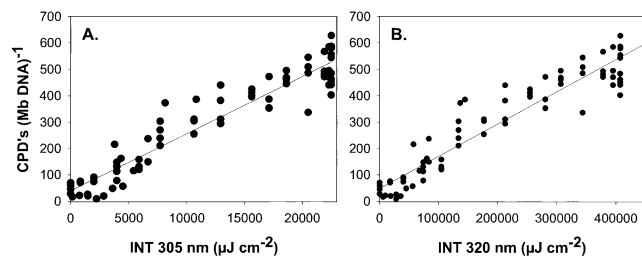
with the penetration of shorter-wavelength radiation. Also, the destruction of viral infectivity in on-deck incubators was the result of wavelengths that were removed by Mylar D (Fig. 4). The abundance of infectious units decreased by more than two orders of magnitude after 9 h of exposure to full sunlight as compared with no significant decrease in samples shielded with Mylar D or maintained in the dark. An increase in titer, possibly caused by viral production, occurred in dosimeters maintained under the Acrylite filter.

At both the Santa Rosa Sound and Antarctica sites, the accumulation of CPD in the dosimeters was predicted by the radiation at 320 nm (Fig. 5). Dimerization also correlated well with the loss of infectivity in the viral dosimeters at the Gulf of Mexico sites (Fig. 6) and is best described by the following relationship: decay rate =  $2.346 \times 10^{-4}$  (CPD Mb<sup>-1</sup> DNA) + 0.025 ( $r = 0.93$ ).

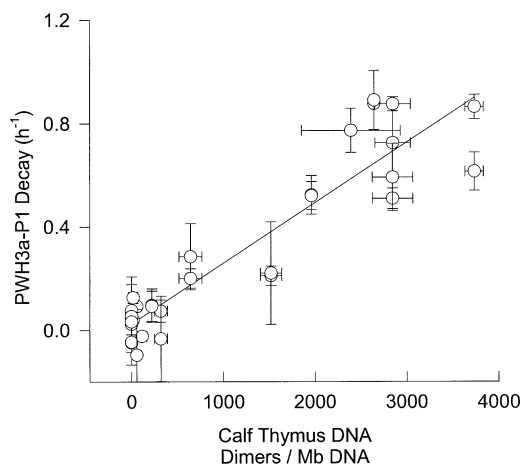
Potential evidence of enhancement of UV fluence caused by the upwelling of UV (*aka* surface-enhanced dose) is presented in Fig. 7. Viral infectivity in dosimeters at the sea surface was almost completely destroyed within 6 h but did not occur until 9 h in the on-deck samples.

## DISCUSSION

The development of a sensitive, yet biologically relevant, UV dosimeter requires the consideration of several important factors. Primarily, the dosimeter must be sensitive to 100% of the damaging radiation that strikes it and must respond to these wavelengths with a biologically weighted function

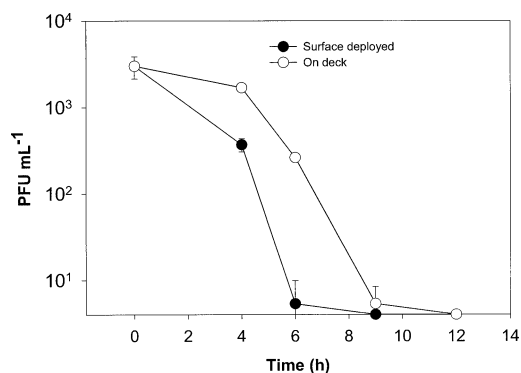


**Figure 5.** Correlation between CPD formation in ctDNA dosimeters and UV fluence at 305 nm (A) and 320 nm (B). Results are a combination of data from both Santa Rosa Sound and the Gerlache Straits.



**Figure 6.** Mean correlation ( $r = 0.93$ ) between the formation of pyrimidine dimers and the destruction of infectivity between ctDNA and viral dosimeters. For each symbol,  $n = 3$  for the decay rate ( $\pm$ SD) and  $n = 2$  for the DNA damage estimates ( $\pm$ range).

rather than with a normal distribution (28). The development of bioassays for UV exposure provides for direct information on the biological effects of the dosage received by the dosimeter. The use of naked DNA to measure dimerization provides relevant biological information, but the process for measuring dimerization within samples requires considerable material and technical ability (14). Assays with repair-deficient prokaryotes have also been developed (28), but the widespread use of such assays requires strict control over other growth conditions of the organisms because the environment changes (nutrient status, temperature, *etc.*). Intact viruses do not respond to these environmental variances and maintain infectivity for relatively long periods in the absence of light. Further, intact viruses can be added directly to *in situ* water samples because the capsid offers resistance to endogenous exozymes. Viral dosimeters can also be uniformly prepared before the deployment and used as consistent standards to compare UV levels over different time periods. Moreover, assessment of viral infectivity is relatively cost-effective, with only a minimal investment in training, materials and equipment required. Unlike some previous studies the use of this virus system avoids the requirements



**Figure 7.** Comparison of *in situ* deployments with on-deck deployments to resolve the surface-enhanced UV effect on viral dosimeters ( $n = 3$ ,  $\pm$ SD).

**Table 1.** The enhancement of the subsurface UV dose caused by internal reflection for 305 and 320 nm radiation and calculated doses (based on our equations). Results are presented only for the 0–4 and 4–6 h time periods because too much infectivity had been lost for accurate estimates after these periods

Time (h)	Decay rate of infectivity (h <sup>-1</sup> )		Estimated dosage (J cm <sup>-2</sup> )				Subsurface enhanced dose (J cm <sup>-2</sup> )	
			<i>In situ</i>		On deck			
	<i>In situ</i>	On deck	305 nm	320 nm	305 nm	320 nm	305 nm	320 nm
0–4	0.52	0.14	0.08	1.76	0.01	0.14	0.07 (8-fold)	1.62 (12.6-fold)
4–6	2.12	0.93	0.39	8.55	0.16	3.49	0.23 (2.4-fold)	5.06 (2.4-fold)

of generating spheroplasts from host cells before titering viral infectivity (6).

### Correlation of viral infectivity, ctDNA damage and light penetration

In our experiments bacteriophage decay rates correlated well with the level of DNA dimerization within the ctDNA dosimeters (Fig. 6). The results from different stations were well distributed along the relationship, with no specific station dictating or altering the trend. Variance in the relationship was most likely caused by the loss of viral infectivity by damage other than DNA dimerization. Although the formation of pyrimidine [6-4] pyrimidone photoproducts occurs with a spectral response similar to that of CPD formation (32), the damage of viral coat proteins by longer-wavelength radiation will increase the rate of loss of viral infectivity while not increasing the level of DNA dimerization significantly. As such, it is possible that the variation associated with our data is, in part, attributable to the damage to viral particle structure.

Previous examinations of UVB in aquatic systems have detected its presence as deep as 10 m in Antarctic waters and its effects up to 20–30 m depth (28). The results of this study demonstrate that biologically harmful radiation penetrates to depths of at least 10 m in the central Gulf of Mexico. These data do not, however, account for the transient variations in the effect of UV caused by mixing in the water column.

Our results demonstrate that the destruction of viral infectivity is consistent with the formation of pyrimidine dimers in DNA dosimeters, suggesting that the action spectra for the formation of dimers are similar to the action spectra for the removal of viral infectivity. Regardless of the mechanisms, these results demonstrate how viral dosimeters may provide a cost-effective, yet accurate, sensitive and rapid mechanism for the estimation of biologically harmful radiation in aquatic systems.

From a comparison of dimers and infectivity as well as dimers and light intensity at 305 and 320 nm (Fig. 5), we can predict (assuming that the 305:320 nm ratio remains constant) the exposure of viruses to biologically damaging radiation from decay rates:

$$\text{Decay rate} = 5.16 \times 10^{-6}(\text{INT } 305 \text{ nm}) + 0.09$$

$$\text{Decay rate} = 2.35 \times 10^{-7}(\text{INT } 320 \text{ nm}) + 0.11$$

where INT XXX nm represents the energy ( $\mu\text{J cm}^{-2}$ ) for either 305 or 320 nm radiation. Given these equations, we can then predict that the decay rate of 1.0 h<sup>-1</sup> seen in the

surface waters of the Gulf of Mexico correlates to exposures of 0.19 J cm<sup>-2</sup> at 305 nm and 4.1 J cm<sup>-2</sup> at 320 nm.

### Wavelength-specific removal of viral infectivity and surface-enhanced UV effects

In clear marine surface waters with high levels of solar radiation, the destruction rates of viral infectivity are such that, on a daily basis, all viral infectivity should be destroyed (27,31). The results from the on-deck deployments demonstrate that UVB radiation (in this case, wavelengths of <312 nm) is responsible for most of the sunlight-mediated destruction of infectivity for this virus. Previous studies have demonstrated that longer-wavelength radiation (UVA, PAR) can account for a significant proportion of the decay of viral infectivity attributable to solar radiation (21,23). Suttle and Chen (21) reported high decay rates of viral infectivity with three phages (including PWH3a-P1) when wavelengths of <320 nm were excluded. Similarly, Noble and Fuhrman (23) demonstrated that, in some experiments, most of the decay of viral infectivity for phage H40/1 is caused by UVA and visible light. But both the PWH3a-P1 and H40/1 phage–host systems are known to have significant potentials for the photoreactivation of damaged viral DNA by host mechanisms (17), suggesting possible confounding results if photorepairing conditions were transient during the assessment of infectivity.

Results from the surface-enhanced dose experiments indicate that upwelled or reflected radiation may have a significant effect on viral infectivity. It is not clear from the previous studies whether the exclusion of UV from the solar spectrum was accounted for in all directions or simply from above (as is commonly done). The results of the current study stress the importance of controlling light from all directions in future studies and call into question the potential impact of longer-wavelength light on viral infectivity, suggesting that it is at its most minimal.

Surface-enhanced effects of UV radiation on marine microbial communities have been shown in previous studies (6). In most cases these effects are identified by nonlinear increases in UV effects at the surface. To more directly measure these effects, we deployed viral dosimeters *in situ* as well as in on-deck incubators. In the on-deck incubators, having black plastic immediately below the dosimeters reduced internal UV reflection (Table 1). Although preliminary, the results of this experiment suggest that the enhanced effects of solar radiation observed in surface waters are attributable to photon accumulation resulting from internal reflection (*i.e.* upwelling irradiance). Decay rates from these

treatments suggest that internal reflectance increases the perceived UV dose eight-fold (for 305 nm radiation) and 12.6-fold (for 320 nm radiation) in the oceanic subsurface. Although the absolute doses are higher for the 4–6 h exposure period (approximately 1000–1200 h local sun time), the magnitude of this enhancement (2.4-fold for both 305 and 320 nm radiation) is lower. This is most likely because internal reflectance is a function of the incident solar angle. Regardless of the cause, the results of this emphasize that photobiologists looking for surface UVB marine effects must consider subsurface enhancements as real and important phenomena.

Recent evidence has suggested that direct impacts of increased levels of UVB caused by stratospheric ozone depletion may not be critical in marine environments because phenomena such as mixing and DNA photorepair can effectively negate much of these effects. Although direct effects of UV radiation on marine organisms have received significant attention, secondary effects, such as those on nutrient and carbon remobilization, marine chemistry, community structure and distribution, require further investigation and estimates of UV burdens in the system. The use of either viral or naked DNA dosimeters should alleviate this requirement in situations where other dosimeters are impractical or unavailable.

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