Environ. Sci. Technol. **2009,** *43,* 8046–8052

Covariation and Photoinactivation of Traditional and Novel Indicator Organisms and Human Viruses at a Sewage-Impacted Marine Beach

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Received May 22, 2009. Revised manuscript received August 27, 2009. Accepted September 6, 2009.

Sunlight modulates concentrations of *Escherichia coli* and enterococci in marine waters. However, the mechanism of photoinactivation is poorly understood. Additionally, little is known about photoinactivation of other fecal indicators and human viruses in recreational waters. We sampled nearshore waters at Avalon Beach, California hourly for 72 h for reactive oxygen species (ROS), traditional indicator bacteria (*E. coli* and enterococci, and QPCR-based detection of enterococci), F+ (DNA and RNA) and somatic coliphages, the human-specific marker in *Bacteroidales* (HF marker), human enterovirus, and human adenovirus. *E. coli* and enterococci (regardless of measurement technique) covaried with each other and the coliphages suggesting similar sources and fates. The occurrence of the HF and enterovirus markers was correlated, but their occurrence was not positively correlated with the other indicators. Lower concentrations or occurrence of all microbes, excluding the HF and enterovirus markers, were observed during sunlit as opposed to dark hours, pointing to the importance of photoinactivation. Empirical-deterministic models for a subset of microbial indicators were created to determine field-relevant sunlight inactivation rates while accounting for time dependent sources and sinks. Photoinactivation rates of enterococci and *E. coli*, enterococci measured by QPCR, and somatic coliphage were estimated at 7, 6, 3, and 28 d^{-1} I^{-1}, respectively, where I is UVB intensity in W/m^2. Average H_2O_2 was 183 nM and the maximum singlet oxygen steady state concentration was 6.6 fM. Given the clarity of the water, direct genomic damage of bacteria and coliphage, as well as indirect endogenous damage of bacteria, were likely the most important inactivation mechanisms, but we cannot rule out a contribution by indirect mechanisms involving the H_2O_2 and singlet oxygen produced exogenously.

Introduction

Fecal indicator bacteria *Escherichia coli* and enterococci are used worldwide to assess the safety of swimming waters (*1*). Their presence in waters polluted with urban runoff and effluent from publicly owned treatment works has been quantitatively linked to recreational waterborne illnesses in epidemiology studies (*2*). In the U.S. during 2007, elevated levels of fecal indicator bacteria triggered nearly 16 000 of the total 22 000 swimming advisories and closures at recreational beaches (*3*).

In the U.S., the Federal Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000 amended the Clean Water Act (*4*). The BEACH Act requires a transition to recreational water quality criteria that employ novel fecal indicators and rapid measures of indicator organisms. These requirements were motivated by findings that changes in recreational water quality occur faster than analytical results can be obtained using traditional microbial assays (*5*), and that *E. coli* and enterococci are not exclusively enteric organisms and might grow in environmental reservoirs like beach sands (*6*). A rapid method showing promise is quantitative polymerase chain reaction (QPCR) detection of *Enterococcus*. Novel indicators that are being considered for new criteria include coliphage, the human specific marker in *Bacteroidales*, and human viruses (*7*). However, little is known about the fate of these novel indicators in natural waters. A more thorough understanding of their fate is needed to properly inform recreational water quality policy.

Field observations at marine and freshwater beaches indicate that sunlight is a major factor affecting the concentrations of *E. coli* and enterococci (*8, 9*). *E. coli* and enterococci concentrations are highest in the dark and can fall to undetectable levels during the day. Sunlight can damage microorganisms through at least three different mechanisms. The first mechanism involves the direct damage of cellular and viral components by sunlight in the ultraviolet (UV) region (*10*). The second and third mechanisms, endogenous and exogenous photoinactivation, are indirect processes initiated by reactive oxygen species (ROS) which are formed during photochemical reactions between sunlight and sensitizer molecules located inside or outside of the organisms, respectively (*11*).

This study investigates diurnal variations of fecal indicator bacteria, novel indicators, and pathogens in natural waters. A field study was carried out at a sewage-polluted enclosed marine beach (*12*). We simultaneously measured sunlight intensity, reactive oxygen species (ROS), microbial pollutants, including fecal indicator bacteria (*E. coli* and enterococci), fecal indicator viruses (somatic and F+ coliphage), human viruses (enterovirus and adenovirus), and human specific marker in *Bacteroidales*, hourly for 72 h. We were able to compare the variability of different indicator organisms, document the effect of sunlight on novel indicators, and develop empirical-deterministic models of indicator concentrations to determine field-relevant sunlight inactivation rates for microbial indicators.

Materials and Methods

Field Site. Avalon Beach is located on Santa Catalina Island in the Southern California Bight. Water quality is impacted

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10.1021/es9015124 CCC: $40.75
Published on Web 09/23/2009

by leaking sewage lines located within 10 m of the land-sea interface (*12*). The leaking sewage, which has high salinity since ocean water is used for toilet flushing, is transported through the beach aquifer and discharges to the surf zone via submarine groundwater discharge. Another potential reservoir of microbial pollutants is the sand, which contains *E. coli* and enterococci (J. A. Jay, personal communication, 2009).

Water Sampling. Water was collected every hour from 0400 h 19 August to 0300 h 22 August 2008 10 m north of the Pleasure Pier (33°20.9′ N, 118°19.5′ W). Samples were collected at ankle depth in an ethanol-sterilized, triple-rinsed bucket and transferred to a 20 L 10% hydrochloric acid washed, triple-rinsed cube-container. The water was stored in the dark in a cooler and was processed within 6 h of collection.

Irradiance Measurements. Simple model of the atmospheric radiative transfer of sunshine (SMARTS) (*13*) was used to estimate total solar and UVB intensity each hour of the study. A field sensor was also used to record solar intensity (data not shown). Agreement between the two was good (Pearson's $r = 0.99$, $p < 0.01$), so SMARTS was used for UVB intensity.

Measurement of ROS Concentrations. Singlet oxygen ($^1O_2$) production rates were determined through laboratory photolysis experiments with furfuryl alcohol (FFA) as a probe compound (*14*) on 9 of the 72 water samples. Briefly, observed rate constants (k_{obs}) were measured for reaction of $^1O_2$ produced by 0.7 μm-filtered seawater samples with FFA. Samples were filtered in the field and then archived at 4 °C until analysis (within 2 weeks). The nine k_{obs} values were indistinguishable from the rate of direct FFA degradation, indicating the samples contained minimal sensitizers for $^1O_2$ production. Therefore, we report the upper limit of $^1O_2$ steady-state concentrations, which were calculated using the maximum $^1O_2$ quantum yields. It should be noted that effect of sample storage time on these measurements has not been determined. Hydrogen peroxide concentrations were measured in seawater with a flow injection analysis instrument in our field lab (Waterville Analytical, Waterville, ME) using an acridinium ester chemiluminescence-based method (*15*). H_2O_2 measurements were made within 1 h of collection. Further details including methods for calculating absorbance are in the Supporting Information (SI).

Culture-Based Microbiological Measurements. *E. coli* and enterococci were measured using membrane filtration (MF) with mTEC and mEI media (USEPA methods 1603 and 1600) and defined substrate assays (Colilert-18 and Enterolert, IDEXX, Westbrook, ME). All analyses were conducted within 6 h of sample collection in the field laboratory. Coliphages were concentrated from 1 L water samples by MF (*16*). Two filters were prepared for each sample, one analyzed for F+ coliphages (*E. coli* F_{amp} host, ATCC 700891), and the other was analyzed for somatic coliphages (*E. coli* CN13 host, ATCC 700609). Filters were stored at −80 °C until analysis. F+ coliphages were grouped into F+ RNA or F+ DNA coliphages using an RNase test (*17*). (Details in the SI).

Total Enterococci and Human Specific Bacteroidales by QPCR. Total enterococci (ENT-QPCR) and human specific *Bacteroidales* (HF marker) were quantified in 100 mL of sample water using the Taqman QPCR assays targeting 23S (*6, 18*) and 16S rRNA (*19*) genes, respectively. Water was filtered in the field lab, and filters were stored at −80 °C until analysis. Reported concentrations were corrected for inhibition (both assays) and extraction efficiency (ENT-QPCR only) as described by Yamahara et al. (*6*). Whole genomic DNA from *E. faecium* (ATCC 19434) and human-specific *Bacteroidales* rRNA gene plasmid DNA were used to generate standard curves for QPCR assays. The limit of detection was 37 cell equivalents (CE)/100 mL for both targets. (Details are in the SI).

Human Viruses. Adenoviruses and enteroviruses were co-concentrated from 1 L water samples by MF using HA filters (Millipore, Billerica, MA) (*20, 21*) and stored at −80 °C until analysis. DNA and RNA were extracted from filters using the AllPrep DNA/RNA micro kit (Qiagen, Valencia, CA). Enteroviruses and adenoviruses were detected using RT-PCR (*22*) and nested PCR (*23*), respectively. The detection limits for the assays were ∼50 adenovirus genomic units and 75 enterovirus genomic units per liter water. It should be noted that this assay does not ensure detection of only infectious viruses. (Details in the SI).

Ancillary Measurements. Dissolved nitrate and silicate were detected in each water sample using flow-injection on a Lachat QuikChem 8000 (Zellweger Analytics, Lincolnshire, IL). Dissolved organic carbon (DOC) and chlorophyll *a* (chl *a*) were used to describe the potential for sensitizers to be present. DOC was measured using the method of nonpurgeable organic carbon on a TOC analyzer (Shimadzu, Columbia, MD). Chl *a* was measured using fluorometry on a Turner 10 AU fluorometer (Turner Designs, Sunnyvale, CA). These analyses were carried out on archived samples. (Further details are in the SI).

Statistical Analysis. All microbial concentrations were $\log_{10}$ transformed to achieve normality. When concentrations were below the detection limit, one-half of the detection limit was used. Pearson's correlation coefficients (r_p) between microbial concentrations and/or occurrence data were computed. Pearson's chi-square test was used to determine whether occurrence of microbes was more common during day or night, ebb or flood tide, and high or low tide (definitions of tides are in the SI). Statistical tests were considered significant for $p < 0.05$. Analyses were carried out using SPSS 16.0.1 (Chicago, IL).

Model Formulation. An unsteady one-dimensional mass balance model of the nearshore was constructed to estimate groundwater discharge rates and calculate empirical photoinactivation rates of target microbes (Figure 1) In brief, a well-mixed nearshore prism changes volume with the rising and falling tide due to cross-shore tidal-driven exchange Q into (positive) and out of (negative) the surf zone. Q is calculated from a mass balance of water in the surf zone (see the SI). Alongshore transport into and out of the prism is assumed to be unimportant relative to cross-shore exchange. This is consistent with field observations of drogues during the study, which did not move significantly alongshore (data not shown).

Possible sources of constituents to the model domain include the beach sands via "beach washing" (*24*), submarine groundwater discharge (*12*), and waters outside the surfzone which enter the prism during flood tides. Beach washing moves target from sand into the surfzone during flood tides (*24*). It was assumed sand had *a* target per g and this was removed from a volume (V_{sand}) 5 cm deep, a unit long, and a width equal to the length of sand submerged by the flood tide between each model time step. We assumed the discharge of groundwater, Q_{gw}, varied between a maximum value Q_{gwmax} and 0 L/min/m in phase with the tides. Q_{gwmax} was determined to be 3 L/min/m of shoreline by fitting the model to measured silicate concentrations, and further confirmed using nitrate concentrations assuming the only source of these tracers was submarine groundwater discharge (see the SI). It was assumed that the groundwater had a concentration of constituent C_{gw}. Waters outside the prism were assumed to contain no microbial constituents ($C_{off} = 0$) (*12*). It is important to note that we did not include a source term for the possible recovery of photodamaged bacteria (*25*).

Sinks of constituents include discharge from the model domain during ebb tides, photoinactivation described by decay constant k_{sun} (units of $d^{-1}I^{-1}$ where I is the intensity

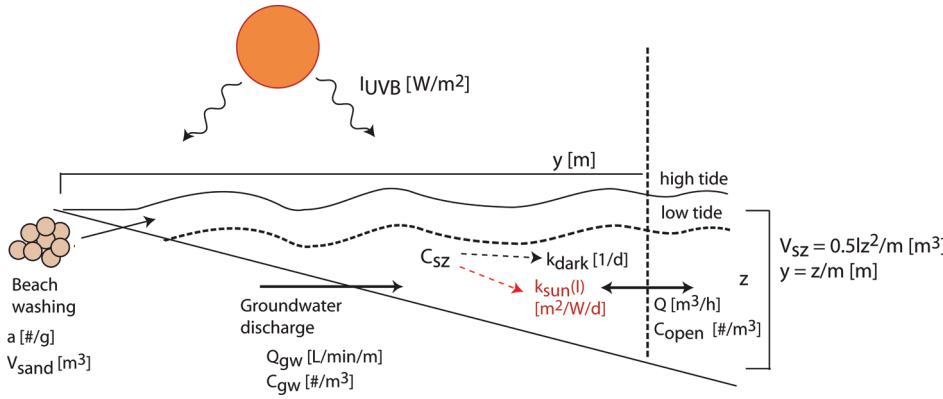

FIGURE 1. Mass balance for the surf zone. *a* is the concentration of target per g of beach sand contributed to the nearshore during beach washing, V_{sand} is the volume of sand contributing *a*, Q_{gw} is the discharge of groundwater, C_{gw} is the concentration of target, C_{sz} is the concentration of target in the surf zone, k_{dark} and k_{sun} are the inactivation of target attributable to dark and sunlit processes, *Q* is the discharge out of and into prism during food and ebb tides, respectively, *z* is the depth of the water column, *y* is the distance to the offshore edge of the prism, and *m* is the slope of the beach. Units are provided in brackets.

of UVB in W/m^2 from SMARTS), and first order decay via "dark" mechanisms (k_{dark} with units d^{-1}). UVB is used as a proxy for sunlight intensity and its use is not meant to imply that indirect photoinactivation with longer wavelengths is not important or that UVB is the only portion of the spectrum that contributes to photoinactivation. Dark inactivation represents inactivation by all processes unrelated to sunlight.

The full material balance equation for microorganism *M* is given by

$$M_{sz}(t) \approx M_{sz}(t-1) + C_{sz}(t-1)Q(t-1)\delta_{x,ebb}\Delta t + C_{off}(t-1)Q(t-1)\delta_{x,flood}\Delta t + Q_{gw}(t-1)C_{gw}\Delta t + aV_{sand}\delta_{x,flood}\Delta t - (k_{sun}I(t-1) + k_{dark})M_{sz}(t-1)\Delta t \quad (1)$$

where $\delta_{x,ebb}$ and $\delta_{x,flood}$ are Kronecker deltas and x is "ebb" and "flood" during ebb and flood tides, respectively. The model was iterated in 1 h time steps except in the case of somatic coliphage where 15 min time steps were needed. The parameters, a, k_{sun}, k_{dark}, and C_{gw} were used as fitting parameters. a and k_{dark} were set to 0 unless they significantly improved the model. The parameter values that provided the best fit to the microbe models were determined by minimizing the root-mean-square error of the log-transformed predicted and measured concentrations of microbes (log-RMSE). It should be noted that the models were not validated using an independent data set, thus they are not suited for prediction or forecasting. (More model details can be found in the SI).

Results

Performance of Microbiological Assay Controls. All method blanks were negative indicating no cross contamination during filtrations, nucleic-acid extractions, or PCR. QPCR-based detection of ENT and the human marker in *Bacteroidales* (hereafter referred to as HF marker) were corrected for inhibition. (Further information in the SI).

Co-Occurrence of Indicators and Pathogens. With the exception of adenovirus, all microorganisms were detected during the 72 h experiment (Figure 2, SI Figure S3). There was a high degree of correlation between concentrations of microbes (SI Table S1). ENT measured using MF (ENT-MF), IDEXX (ENT-IDEXX), and QPCR (ENT-QPCR) were highly correlated ($0.65 \le r_p \le 0.75$, $p < 0.05$). ENT-IDEXX was on average 1.3 times ENT-MF, and ENT-MF was on average 4% of ENT-QPCR. EC measured by IDEXX (EC-IDEXX) and MF (EC-MF) were highly correlated ($r_p = 0.75$, $p < 0.05$) with EC-IDEXX, on average, 1.4 times higher than EC-MF. The concentrations of both somatic and F+ coliphage were

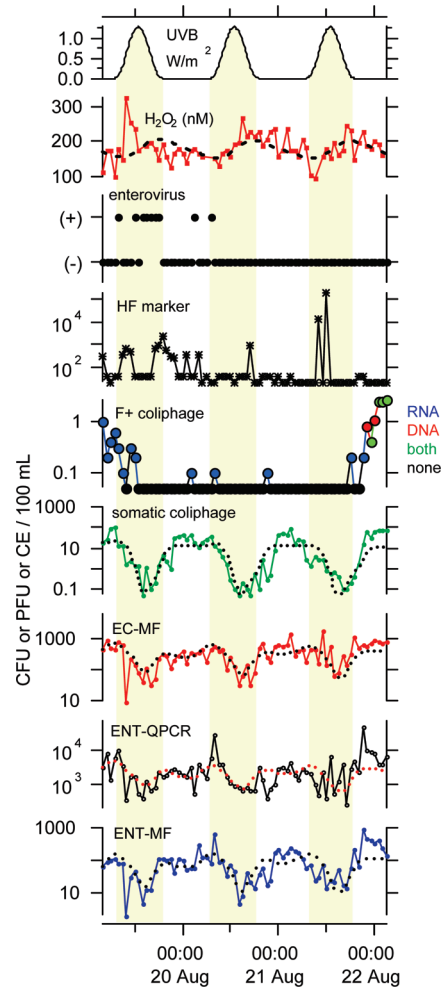

FIGURE 2. Concentrations of organisms observed in our study. Units are CFU/100 mL for EC-MF and ENT-MF, PFU/100 mL for somatic and F+ coliphage, and CE/100 mL for ENT-QPCR and HF. In the F+ coliphage panel, the color of the symbol indicates whether just F-RNA (blue), just F-DNA (green), or both types of F+ phage (red) were detected. Time series of ENT-IDEXX and EC-IDEXX are provided in SI Figure S3. Tide level is provided in SI Figure S4. Dotted lines in bottom four panels, and H_2O_2 panel are best-fit model predictions.

significantly correlated to the traditional indicators ENT-MF and EC-MF ($r_p = 0.69$ and 0.63, $p < 0.05$ between somatic coliphage and ENT-MF and EC-MF, respectively; $r_p = 0.40$

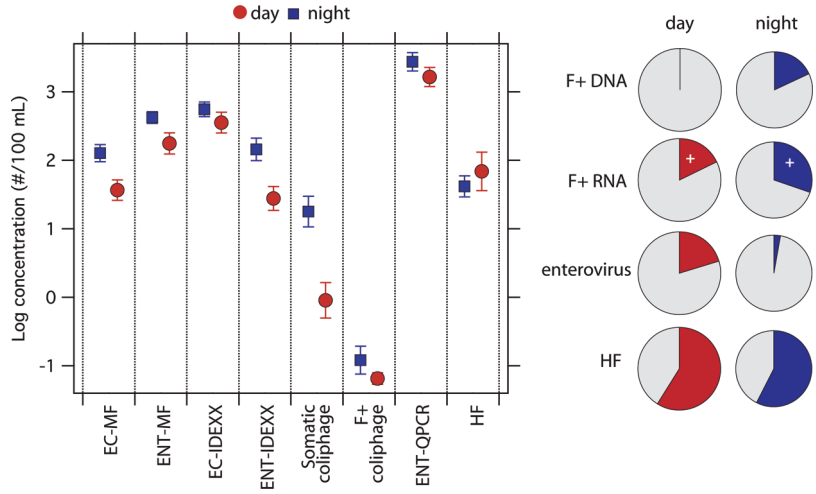

FIGURE 3. Concentration and occurrence of target microbes during the 72 h study. For the concentration data, the error bars represent 95% confidence intervals about the log mean. Units are CFU/100 mL for EC-MF and ENT-MF, MPN/100 mL for EC-IDEXX and ENT-IDEXX, PFU/100 mL for somatic and F+ coliphage, and CE/100 mL for ENT-QPCR and HF. For the occurrence data, gray and color represent the proportion of samples that are negative and positive, respectively. Pie size is proportional to the number of samples collected (n = 39 and 33 in day and night, respectively).

TABLE 1. Best Fit Model Parameters

model	C_{gw}[b] (no./100 mL)[f]	k_{sun}[c] (d^{-1} I_{UVB}^{-1})	k_{dark}[c] (d^{-1})	a (no./g)	log-RMSE (log no./100 mL)	% within 10×[d]	% within 2×[d]
ENT-MF	3000	7.0 ± 0.6	1.3 ± 0.3	0	0.40	99	58
EC-MF	1000	6.0 ± 0.4	0.8 ± 0.3	0	0.35	99	69
ENT-QPCR	80 000	3.0 ± 0.8	1.8 ± 0.4	0	0.38	98	59
Somatic coliphage	35	28[e]	0	4×10^5	0.58	93	42

[b] Variable definitions are in the text. [c] Errors for bacterial models were derived from a sensitivity analysis holding C_{gw} and a constant while varying inactivation rates (SI Figure S6). [d] The two most right columns show the percent of predictions within 10 times or 2 times the measured concentrations. [e] High uncertainty associated with this value (see SI Figure S6). This value represents the lowest estimate. [f] No. is CFU, PFU, or CE for cultured bacteria, coliphage, or ENT-QPCR, respectively.

and 0.38, $p < 0.05$ between F+ coliphage and for ENT-MF and EC-MF, respectively). The concentrations of coliphages were also significantly correlated to ENT-QPCR, ENT-IDEXX, and EC-IDEXX (SI Table S1). The concentration of the HF marker was negatively, significantly correlated to EC-MF (r_p = −0.31, $p < 0.05$), ENT-QPCR (r_p = −0.30, $p < 0.05$), and ENT-IDEXX (r_p = −0.33, $p < 0.05$). When expressed as occurrence data, the presence of the HF marker was positively correlated with the presence of enterovirus (r_p = 0.23, $p <$ 0.05) and negatively correlated with the presence of F+ DNA coliphage (r_p = −0.36, $p < 0.05$). The occurrence of the HF marker was also negatively correlated with EC and ENT by both MF and IDEXX. Enterovirus was negatively correlated with EC-MF and EC-IDEXX (r_p = −0.24 and r_p = −0.26, $p <$ 0.05, respectively).

Effect of Sunlight. The log-mean concentrations and the occurrence of each organism during day and night are shown in Figure 3. Thirty-three samples were collected in the dark (between 2000 and 600 h) and 39 in sunlit conditions (700 to 1900 h). The concentration of all microbes tested quantitatively, except for the HF marker, was higher during the night compared to the day ($28.25 \le F \le 4.0$, $p < 0.05$, SI Table S2), although some relatively high concentration of EC and ENT were observed during the day, particular during the final day of the experiment. The occurrence of F+DNA coliphage was greater during the night than the day (χ^2 = 7.74, $p = 0.005$), but the occurrence of F+RNA phage was not different between night and day (χ^2 = 1.51, p = 0.219). The occurrence of the HF marker was not different between night and day (χ^2 = 0.014, p = 0.904). Interestingly, enterovirus was more likely to be detected during the day compared to the night (χ^2 = 5.00, p = 0.025).

We also examined the effect of the tides on measured microbe concentrations (SI Figure S4). Only ENT-MF showed significant differences between high and low tides (ENT-MF higher during low versus high tides by 0.2 log units). Only EC-MF showed significant differences between flood and ebb tides with EC-MF higher during flood compared to ebb tides (by 0.2 log units). (Further details are in the SI).

Reactive Oxygen Species (ROS). Sensitizers were present in the water column which could facilitate the production of ROS: average DOC was 7.0 mg/L (range 0.7−40.8 mg/L) and average chl a was 0.7 μg/L (range of 0.4 μg/L to 1.0 μg/L). Average absorbance (a_{300}) was low at 0.584 m^{-1} (range of 0.398−0.941 m^{-1}). Average H_2O_2 was 183 nM (range of 93 to 329 nM) (Figure 2). There was not a significant difference in measured H_2O_2 between day and night (F = 0.195, p = 0.660, ANOVA), however; H_2O_2 is autocorrelated at a lag of 27 h (r = 0.234, $p < 0.05$) indicating diurnal variation out of phase with the solar cycle. A simple H_2O_2 model (methods in the SI) provided a formation rate of 0.026 nM h^{-1} $I^{-1}_{(UVB+UVA+PAR)}$ and a decay rate of 0.024 h^{-1}, where I is from SMARTS in W/m^2. The net hydrogen peroxide production rate (NHPPR) was 9.5 nM h^{-1} (*26*). An upper limit of 6.6 fM was calculated for $^1O_2$ steady state concentrations using the solar irradiance and an estimated upper limit for the $^1O_2$ production quantum yield (0.03). It should be noted that this upper limit for Avalon waters is the lower detection limit of the assay.

Model-Derived Photoinactivation Rates. We modeled concentrations of ENT-MF, ENT-QPCR, EC-MF, and somatic coliphage to empirically determine sunlight inactivation rates while considering unsteady sources and sinks. The best-fit model parameters are provided in Table 1. Beach washing was not necessary for the models of bacterial indicators;

discharge of polluted groundwater was the only necessary bacterial source. C_{gw} was 3000 CFU/100 mL, 1000 CFU/100 mL, and 80000 CE/100 mL for ENT-MF, EC-MF, and ENT-QPCR, respectively; k_{sun} was 7.0, 6.0, and 3.0 d^{-1} $I_{UVB}{}^{-1}$ where I is in W/m^2, respectively; and k_{dark} was 1.3, 0.8, and 1.8 d^{-1}, respectively. The bacterial models had log-RMSE of approximately 0.4 log CFU or CE /100 mL (Table 1).

We included beach washing and groundwater as sources in the somatic coliphage model to obtain the best fit. The model required C_{gw} of 35 PFU/100 mL, a beach sand concentration of 4×10^5 PFU/g, and $k_{sun} = 28$ d^{-1} $I_{UVB}{}^{-1}$ where I is in W/m^2. We set $k_{dark} = 0$ as it did not improve the model. The decline of somatic coliphage once the sun came out was so rapid, the model had to be run in 15 min time steps. The log-RMSE was 0.58 log PFU/100 mL (Table 1).

A sensitivity analysis examined how log-RMSE changed by varying k_{sun} and k_{dark} while holding C_{gw} and a constant in the bacterial models (SI Figure S6). We found local minima in log-RMSE space that spanned an area defined by a range of k_{sun} and k_{dark}. Using this area, we defined an error for k_{sun} and k_{dark} that describe how much they can vary while log-RMSE remains at a minimum (Table 1). A similar sensitivity analysis was not relevant for the somatic coliphage model since k_{dark} was set to 0, so log-RMSE was examined as a function of k_{sun}. It should be emphasized values of k_{sun} were derived using modeled UVB intensities (SMARTS), rather than direct field measurements, and the values are thus sensitive to the accuracy of the modeled values for UVB. However, the relative differences between organism-specific k_{sun} are not.

Discussion

ENT, EC, and Coliphage Concentrations, Regardless of Measurement Method, Were Positively Correlated to Each Other. This result is consistent with, but not proof of, the organisms having similar sources and/or loss mechanisms in the environment. Similar results have been reported in European bathing waters (*27, 28*). It should be noted that the observed correlation may be "source" specific. At Avalon, the source of pollution is leaking sewage lines. At beaches where sources are predominately wildlife, nonpoint, or mixed, correlations may break down. For example, Jiang and Chu (*29*) found that in urban runoff in southern California, F+ coliphage covaried with fecal coliform, but not ENT while somatic coliphage did not covary with either.

The HF Marker and Enterovirus Were Positively Correlated to Each Other, And Negatively Correlated to Concentrations of ENT and EC. A lack of correlation between the HF marker and traditional indicators was also reported at Huntington Beach, CA where the source of pollution is urban runoff (*30*). However, Sercu et al. (*31*) reported correlations between traditional indicators and the HF marker in urban runoff in Santa Barbara, CA. A lack of positive correlation between human virus and traditional indicators is well documented in the literature (*22, 32*) and has been attributed to differential sources and persistence of bacteria and viruses, as well as the potential low occurrence of viruses during nonoutbreak conditions.

Adenoviruses Were Not Detected. Low occurrence (2−4% of samples) of adenovirus has also been reported in other areas in southern CA (*33, 34*). Given the small population living on Catalina Island (3700 people) and our small sample size, an explanation for the result is low occurrence of adenoviruses in Avalon's sewage. Other explanations include low survivability of the virus in the environment, or inhibition of the PCR.

Lower Mean Concentrations or Occurrences of All Targets Excluding the HF and Enterovirus Markers Were Observed during Sunlit As Opposed to Dark Hours. This result is not driven by the tides, which are sometimes in phase with the solar cycle, as evidenced by the lack of tidal correlations. The result suggests that photoinactivation affects the persistence of indicator bacteria and viruses under field conditions. While studies have documented the effect of sunlight on ENT-MF, ENT-IDEXX, EC-MF, and EC-IDEXX in the field (*8, 9*), this is the first time to our knowledge that a diel cycle in indicator viruses and ENT-QPCR at an impacted beach has been reported. The results underscore the importance of considering photoinactivation when conducting routine water quality monitoring, measuring indicators during epidemiology studies, and modeling concentrations in the environment.

Evidence of Photoinactivation of the HF and Enterovirus Markers Was Lacking. However, if the source of the HF and enterovirus markers were intermittent, like bather shedding (*35*), or if concentrations were too low, then we would perhaps not be able to observe photoinactivation. While the lack of a diurnal cycle in the HF marker has been reported in two microcosm studies (*36, 37*), one other microcosm study (*38*) and a field study (*32*) provide evidence of HF marker photoinactivation. Enterovirus was preferentially detected in the day relative to night, as might be expected if bather shedding were an important source. Work is underway in our laboratories to further understand photoinactivation of these targets.

The Model-Derived Apparent Second-Order Sunlight Decay Rate of ENT-MF Was over Twice That of ENT-QPCR (Table 1). This difference is probably a result of QPCR measuring nonviable and dead organisms in addition to the viable ones measured by membrane filtration. The fact that we observed a diurnal variation in the QPCR-ENT signal suggests that either the QPCR DNA target is damaged by sunlight directly such that it cannot be amplified, or that photoinactivation causes some cell lysis and released ENT DNA is subsequently degraded. The field observations disagree with results from a multiday seawater microcosm experiment that showed no effect of sunlight on ENT-QPCR degradation (*38*). Controlled laboratory studies are needed to further understand these results.

Given the Clarity of the Water, Direct Genomic Damage of Bacteria and Coliphage by UVB, as Well As Indirect Endogenous Damage of Bacteria, Were Likely the Most Important Mechanisms of Photoinactivation during Our Study. Short wavelengths are known to be the most germicidal (*39, 40*), and the average intensity of 300 nm light over the entire depth of the water column at the deepest point of the nearshore prism (Figure 1) was at least 20% of the surface intensity. Exogenously produced ROS were measured to determine their potential role in inactivating coliphage and bacteria. Measured H_2O_2 (Figure 2), calculated NHPPR, and derived decay rates are similar to those observed in other coastal seawater sites (*26, 41*). Interestingly, absorbance at 300 nm (a_{300}) is on the low end of reported marine values, indicating that photoexcited colored dissolved organic matter (CDOM) may not be the only source of H_2O_2; other potential sources include beach sands and wrack (*42*). More work will need to be done to understand the dynamics of H_2O_2 in the surf zone. Although measured H_2O_2 levels were much too low to cause bacterial lysis, it is possible H_2O_2 contributed to oxidative stress (*43*). Furthermore, H_2O_2 and sunlight may act synergistically on bacteria (*44*) and F+ coliphage (*45*).

Singlet oxygen is known to be produced by energy transfer from photoexcited CDOM to dissolved oxygen (*46*). Consistent with previous studies (*47*), the low CDOM content of Avalon waters, inferred from the low absorbance values, resulted in low steady-state $^1O_2$ concentrations. Steady-state bulk $^1O_2$ concentrations were estimated to be, at most 6.6 fM, 1 order of magnitude lower than those reported for seawater collected near Long Island, New York (*47*). Even these low levels of $^1O_2$ may be sufficient to contribute to inactivation of viruses such as MS2, especially if the viruses

are in close proximity to sensitizer molecules where concentrations can be much higher (*48*). Thus, given the presence of H_2O_2 and $^1O_2$, and the potential synergistic effects of ROS and sunlight, it is not possible to rule out a contribution from exogenous photoinactivation at the field site.

The Photoinactivation Rate of Somatic Coliphage Was Estimated to Be Four Times Higher than the Rates for the Indicator Bacteria (Table 1). This result contradicts previous research, in which inactivation of fecal coliforms and enterococci was faster than somatic coliphage in seawater (*49*). Many factors may contribute to these observed differences, including strain diversity and differential susceptibility to the dominant inactivation mechanism(s).

The Models Developed for ENT-MF, EC-MF, ENT-QPCR, and Somatic Coliphage Rationalized the Dynamics of Indicator Concentrations in the Nearshore. The models required inputs of bacteria and viruses from groundwater discharging across the beach face in phase with the tides. The ENT-MF and EC-MF concentrations required by the models (Table 1) are within the range of values measured previously in Avalon groundwater within the beach aquifer (*12*), however, ENT-QPCR and somatic coliphage have not been measured in the aquifer, so these parameters cannot be verified. The somatic phage model required an additional input from the beach sand. Although it has been shown that ENT-MF can enter nearshore waters via beach washing (*24*), the importance of beach washing in contributing coliphage to the nearshore has not been directly verified. Bonilla et al. (*50*) found a high prevalence of somatic coliphage in Florida beach sands suggesting sands may serve as a coliphage reservoir. Future work should verify our findings on the relative importance of beach washing for somatic coliphage, ENT, and EC at Avalon.

Our models represent simplifications of a complex system. We did not consider the potential for photodamaged bacteria to undergo repair in the afternoon or in the dark. We parametrized dark inactivation as a microbe-specific first-order constant, but it could vary as a function of many biotic and abiotic parameters. We assumed alongshore transport was negligible. We parametrized groundwater discharge and beach washing based on our experiences at other beaches; these parametrizations should be tested and further refined. There may be other sources we did not consider. Further work will be needed to refine the model of the Avalon nearshore. In particular, the collection and testing of a validation data set would lend credence to the models. Regardless, the models served an important purpose of providing estimates of photoinactivation rates while controlling for time dependent sources and sinks.

Acknowledgments

This work was supported by a UPS Foundation grant to A.B.B., K.M., and K.L.N., and NSF CAREER award (A.B.B., K.M.Y.) (BES-0641406). We thank Sarah Kliegman, Kristen Thoreson, Nick DeSieyes, and Jee Yeon Kim for help with sampling and laboratory analysis during the study. We thank the City of Avalon, John Griffith, and Steve Weisberg for support.

Supporting Information Available

Detailed analytical methods and model parametrization, descriptions of inhibition, tidal variation, H_2O_2 model and nutrient model results, input and output files from SMARTS, Figures S1−S7, and Tables S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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ES9015124