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Solar Inactivation of Enterococci and *Escherichia coli* in Natural Waters: Effects of Water Absorbance and Depth

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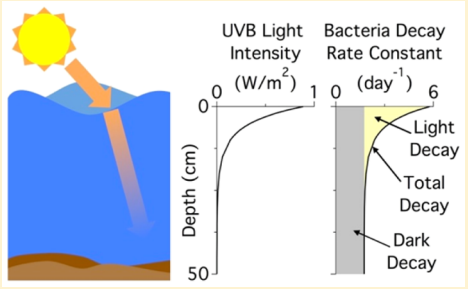
Supporting Information

ABSTRACT: The decay of sewage-sourced *Escherichia coli* and enterococci was measured at multiple depths in a freshwater marsh, a brackish water lagoon, and a marine site, all located in California. The marine site had very clear water, while the waters from the marsh and lagoon contained colored dissolved organic matter that not only blocked light but also produced reactive oxygen species. First order decay rate constants of both enterococci and *E. coli* were between 1 and 2 d^{-1} under low light conditions and as high as 6 d^{-1} under high light conditions. First order decay rate constants were well correlated to the daily average UVB light intensity corrected for light screening incorporating water absorbance and depth, suggesting endogenous photoinactivation is a major pathway for bacterial decay. Additional laboratory experiments demonstrated the presence of colored dissolved organic matter in marsh water enhanced photoinactivation of a laboratory strain of *Enterococcus faecalis*, but depressed photoinactivation of sewage-sourced enterococci and *E. coli* after correcting for UVB light screening, suggesting that although the exogenous indirect photoinactivation mechanism may be active against *Ent. faecalis*, it is not for the sewage-source organisms. A simple linear regression model based on UVB light intensity appears to be a useful tool for predicting inactivation rate constants in natural waters of any depth and absorbance.



INTRODUCTION

Recreating in surface waters with high concentrations of fecal indicator bacteria (FIB) from wastewater and urban runoff has been linked to increased risk of gastrointestinal and respiratory symptoms in swimmers.[1,2] In an effort to protect the health of swimmers, surface waters are routinely monitored for *Escherichia coli* and enterococci.[3,4] When *E. coli* and enterococci concentrations exceed certain thresholds, a water advisory or closure is issued. In the United States, when FIB concentrations are persistently elevated, waters can be deemed impaired and added to the Clean Water Act 303(d) list. Currently, 25% of the ∼43 000 waterbody segments on States' 303(d) list are impaired with respect to microbial pollutants, the greatest percentage attributed to any single contaminant.[5]

Mitigation of microbial pollution in surface waters has proven challenging because there are many potential point and nonpoint sources,[6] and there is imperfect knowledge about the factors that modulate the abundance and distribution of microbial pollutants once they are introduced into the environment.[7,8] Science that yields insight into the factors controlling the abundance and distribution of microbial pollution in surface waters is needed and of both national and international importance. Such knowledge would guide remediation efforts and assist in the identification of safe and dangerous conditions for swimming.

Both field observations and laboratory experiments have highlighted the importance of sunlight in modulating and reducing concentrations of FIB in surface waters.[7,9,10] Sunlight can damage and inactivate bacteria via three routes: (1) directly, (2) indirectly via endogenous photosensitizers, and (3) indirectly via exogenous photosensitizers.[11,12] Via the first and second modes, sunlight penetrates the bacterial cell and either damages a vital cellular component directly[13,14] or is absorbed by endogenous photosensitizers, such as porphyrins, bilirubin or chlorophyll, and generates reactive species that then damage a vital cellular component.[15] Via the third mode, sunlight outside the cell may be absorbed by external photosensitizers, such as dissolved organic matter and humic acids, which then generate reactive species that damage vital cellular components.[16,17] In practice, we may attribute FIB photoinactivation in clear water solely to endogenous processes (direct and

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Table 1. Field Deployment Information and Physical Properties of Site Water (Mean ± Standard Deviation) For Each Location and Phase[a]

deployment site and sewage source	phase	deployment period	shading? yes/no	depth [cm]	temp. [°C]	salinity [ppt]	turbidity [NTU]	NPOC [mg/L]
Pillar Point Harbor								
Palo Alto Regional Water Quality Control Plant	Summer I	8–18 Sept. 2014	no	5	17.6 ± 0.4	33.5 ± 0.2	3.0 ± 1.8	2.2 ± 0.6
	Summer I	8–18 Sept. 2014	no	99				
	Winter I	13–20 Feb. 2015	no	5	15.1 ± 0.2	32.9 ± 0.5	2.8 ± 0.5	1.8 ± 0.4
	Winter I	13–20 Feb. 2015	no	18				
	Winter I	13–20 Feb. 2015	no	99				
San Joaquin Marsh								
Orange County Sanitation District	Summer I	18–28 Aug. 2014	no	15	25.0 ± 1.2	1.4 ± 0.5	24.5 ± 7.9	12.3 ± 4.4
	Summer I	18–28 Aug. 2014	yes	15				
	Winter I	9–19 Jan. 2015	no	15	13.6 ± 1.3	1.0 ± 0.1	16.9 ± 6.4	9.2 ± 0.5
	Summer II	17–22 Sept. 2015	no	2	23.4 ± 1.0	1.2 ± 0.1	13.9 ± 6.4	10.7 ± 0.2
	Summer II	17–22 Sept. 2015	no	6				
	Summer II	17–22 Sept. 2015	no	15				
Arroyo Burro Lagoon								
El Estero Wastewater Treatment Plant	Summer I	8–18 Sept. 2014	no	15	23.8 ± 0.8	10.2 ± 2.2	2.2 ± 0.9	7.5
	Summer I	8–18 Sept. 2014	yes	15				
	Winter I	2–12 Feb. 2015	no	15	16.6 ± 0.5	19.5 ± 5.3	4.4 ± 2.8	4.7 ± 0.5

[a]The NPOC at Arroyo Burro Lagoon during the Summer I deployment is from a single sample collected on the day of deployment. Salinity at Arroyo Burro Lagoon was calculated from the mean temperature and conductivity.

indirect), while photoinactivation in colored water containing photosensitizers may occur via all three mechanisms.

To date, a unifying model for determining bacterial photoinactivation rates in ambient surface waters does not exist. Photoinactivation rates should depend on solar radiation present in the water column, which in turn depends on light incident on the water column, water clarity, presence of exogenous sensitizers, and depth,[11,12] as well as characteristics of the bacterial cell (e.g., presence of endogenous sensitizers and presence of peptidoglycan in the cell wall).[11,18]

This study develops a model for photoinactivation of *E. coli* and enterococci in ambient surface waters. First, field studies were carried out at three locations broadly representing ambient surface waters typical of California (a marine site, a brackish water site, and a freshwater site) during both winter and summer months. Decay of *E. coli* and enterococci was measured at multiple depths for 6–11 days, from which a model was developed for predicting photoinactivation rate constants using incident sunlight intensity, water clarity, and depth. Additional laboratory experiments were carried out to better understand whether exogenous photoinactivation should be considered in the model.

EXPERIMENTAL MATERIALS AND METHODS

Field Sites. Field experiments were conducted at three locations in three separate phases (Table 1). The three locations broadly represented ambient surface waters typical of California and included a representative marine site at Pillar Point Harbor (PPH, 37° 30′ 9.24″ N, 122° 29′ 1.78″ W) in Half Moon Bay, CA, a freshwater site located within San Joaquin Marsh (SJM, 33° 40′ 9.84″ N, 117° 49′ 23.16″ W) receiving urban runoff in Irvine, CA, and the brackish Arroyo Burro Lagoon (ABL, 34° 24′ 16.26″ N, 119° 44′ 26.14″ W) near the land sea interface in Santa Barbara, CA. The salinity, temperature, nonpurgeable organic carbon, turbidity, and ambient water absorbance were measured during the experiments (see Supporting Information (SI) for detail).

Field Experiments. The first phase (Summer I) and second phase (Winter I) were performed at all three field sites at roughly the same time, while the third phase (Summer II) was only performed at SJM (Table 1). Each experimental phase lasted between 6 and 11 days. Field experiments deployed dialysis bags (6–8 kDa pore size, 120 mm diameter Spectra/Por 1 RC Tubing, Spectrum Laboratories Inc., Rancho Dominguez, CA) containing 95% by volume raw environmental water mixed with 5% by volume locally sourced raw sewage (Table 1). Environmental water and raw sewage were collected the day of or day before the initiation of the experiment. The dialysis bags were filled to a total volume of 1 L for the Summer I and Winter I phases, and 250 mL for the Summer II phase. The volume of the dialysis bags was reduced for the Summer II phase to allow placement at shallower depths. Bags were oriented in the horizontal direction in floating deployment modules that held the bags at a fixed depth in the water column (Figure S1). The pore size of the dialysis bags allowed the passage of nutrients and water, but prevented the passage of bacteria.[19]

Dialysis bags were placed at different depths in the water column to vary the light incident on the bag contents. At the marine site (PPH), dialysis bags were deployed at depths in the water column ranging from 5 to 99 cm. At the wetland (SJM) and lagoon sites (ABL) dialysis bags were deployed at various depths in the water column or at fixed depths with and without shading (Table 1). To simulate shading, a shade cloth (Heavy Black Sun Screen Fabric #75020, Easy Gardener, Waco, TX) was placed at the top of the floating deployment module above the water surface. Each day of the experiments, two dialysis bags representing biological replicates were destructively sampled at sunrise. Dialysis bags were transported to a laboratory where the water was aseptically poured into a sterile

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bottle, stored in the dark on ice or at 4 °C, and processed within 6 h of collection.

Bacteria Enumeration. Enterococci and *E. coli* were enumerated using a colorimetric-liquid-defined substrate assay (Enterolert and Colilert, respectively; IDEXX, Westbrook, ME). Samples were decimally diluted and multiple dilutions were assayed. In some cases, dilutions were assayed in replicate. All dilutions that yielded measurements within the assay range of quantification (ROQ) were averaged to find the concentration of each biological replicate. If all replicates for a particular biological replicate were below the assay detection limit (BDL, 10 most probable number (MPN)/100 mL), then <10 MPN/100 mL was retained.

Estimation of Light Irradiance. The UVB intensity of light incident on the surface of the water at each site was obtained in 30 min intervals for the duration of applicable experimental phases using the Simple Model of the Atmospheric Radiative Transfer of Sunshine (SMARTS, Table S1).[20] SMARTS does not incorporate cloud cover into the calculation of the light intensity; therefore, variation in the cloud cover between locations and days within each deployment may confound the calculated light intensities. There was little variation in the incident UVB between days of each experiment (data not shown) so data from the middle day of each experiment was used to represent each day of the experiment. Incident UVB was used to find the UVB transmitted to the middle of the deployed dialysis bags within the water column. The calculation used the membrane transmittance (Figure S2), site-specific water absorbance, and the solar zenith angle (see SI). The absorbance spectrum of the membrane was measured with an Uvikon XL Spectrophotometer (BioTek Instruments, Winooski, VT), while the absorbance of the ambient PPH, SJM, and ABL water was measured as stated in the SI. Water spectra from each field site were averaged across experimental phase and the average was used in the calculations; there was little variability between site water spectra across all experimental phases (data not shown). The daily average UVB transmitted to the experiments was calculated across the entire day, including the time of day when it was dark. Light passing through the shade cloth used in some of the field deployments (Table 1) was measured and compared against unobstructed light. The shade cloth uniformly reduced light intensity by 75% regardless of wavelength. Subsequently, the daily average UVB light intensity for shaded modules was calculated as a quarter of the intensity of the unshaded modules.

The relative importance of scattering and absorption to vertical light attenuation was approximated from the water absorbance and turbidity at each site and phase (see SI). For all waters tested, scattering had a negligible effect on the light attenuation relative to the absorption (see SI), consistent with past studies with similar surface waters.[21,22] Therefore, we did not consider light scattering in the estimations of light irradiance.

Data Analysis. The average concentration from the two biological replicates was calculated for each time point if they were within the ROQ. If one of the biological replicates was BDL (i.e., <10 MPN/mL), then BDL was replaced with half the limit of detection (5 MPN/100 mL) and used in the average. The decay curve was then fit with a first order decay model:

$$\frac{C(t)}{C_0} = e^{-kt} \quad (1)$$

where t is time (day), C (MPN/100 mL) is the measured concentration at time t, C_0 is the measured concentration at time 0, and k [day^{-1}] is the rate constant. All points up to and including the first time point when both replicates were BDL or the last time point before measurements leveled off or began to increase (possibly due to tailing or growth) were used to calculate k (Figures S3 and S4). For *E. coli* at SJM and ABL, the initial time point (t = 0) was excluded from the regression as its concentration was lower than the concentration at t = 1 d (perhaps due to an initial growth of cells or disaggregation of wastewater bacteria in the experimental device).

k was plotted against the corresponding estimated daily average UVB light intensity, I_{UVB} [Wm^{-2}], for each experiment (defined by location, time, and depth) and fitted with a linear model,

$$k = k_{dark} + k_{sun} I_{UVB} \quad (2)$$

where k_{dark} is a first order dark inactivation rate constant [d^{-1}] and k_{sun} is a pseudo second order photoinactivation rate constant [$m^2/W/d$]. In the linear regression, k_{dark} is the y-intercept and k_{sun} is the slope. In eq 2, $k_{sun} I_{UVB}$ represents the contribution of photoinactivation to k and k_{dark} represents the inactivation due to stresses other than light, such as osmotic stress, predation, and starvation.[11]

Predicting Photoinactivation in Well-Mixed and Unmixed Water Columns at PPH, SJM, and ABL. We used SMARTS to calculate the I_{UVB} incident on the surface of the water column and solar zenith angle at PPH, SJM, and ABL on 8 September 2014 (as previously described) as a representative summer day. Using the average water absorbance from Summer I phase at each of the three sites, we calculated $I_{UVB}(z)$ for 0 < z < 1 m, as well as the depth-averaged $<I_{UVB}>(z_{tot})$ for a well-mixed water column of total depth z_{tot} ranging from 0 to 1 m deep (see SI). These were subsequently used in eq 2 to calculate $k(z)$ and depth averaged $<k>$ (z_{tot}) for enterococci and *E. coli*.

Laboratory Experiments. Microcosm photoinactivation experiments were performed with laboratory strain *Enterococcus faecalis* (ATCC 19433) and sewage-sourced *E. coli* and enterococci (Table S2) to investigate the relative importance of endogenous and exogenous photoinactivation in the raw waters from the field sites. Raw waters had to be spiked with organisms for the experiments as background levels present in the water were too low to quantify during photoinactivation experiments (<887 MPN/100 mL *E. coli*, <284 MPN/100 mL enterococci, data not shown). All experiments were performed in triplicate.

Laboratory strain *Ent. faecalis* were grown in a chemostat, pelleted and resuspended in carbonate buffer saline (CBS) to act as the bacterial seed[23] (see SI). 0.5 mL of the *Ent. faecalis* bacterial seed was diluted into 50 mL of PPH, SJM, or ABL water and placed in a 100 mL sterile beaker resulting in a final concentration of ~10^6 CFU/ml. The solution was 2.48 cm deep. The PPH, SJM, and ABL waters used in these experiments were collected during the Winter I phase, stored in the dark at 4 °C, and used 4−6 months after collection, and were not autoclaved or sterilized[24]

Sewage was concentrated from 900 to 10 mL using centrifugation to act as a general inoculant (see SI). Ten mL of concentrated sewage inoculant (which contained sewage-sourced *E. coli* and enterococci as well as other sewage microbes) were diluted into 530 mL of either PPH or SJM water and placed in a wide mouth (16.5 cm diameter) sterile

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beaker, resulting in a final concentration of ~10^4 and ~10^5 MPN/ml for enterococci and *E. coli*, respectively (Table S3). Larger beakers were necessary for the sewage-sourced bacteria experiments because the initial starting concentrations were lower (and thus larger volumes were needed for enumeration), yet we wanted to ensure that the depth of the water was consistent (2.48 cm) between these and the laboratory strain *Ent. faecalis* experiments and that the depth did not change by more than 10% over the course of the experiment. The PPH and SJM waters spiked with the sewage inoculant were collected during the Winter I and Summer II phases, respectively, and were not autoclaved or sterilized. Prior to use, the PPH and SJM waters had been stored in the dark at 4 °C and used 8 months and 1 month after collection, respectively[24] ABL water was not used in the sewage experiments—as will be shown in the results, it did not appear to contain photosensitizers active against the bacterial targets. It should be noted that there was little variation in site water absorbance across time, so waters collected in summer versus winter, for example, had nearly the exact same absorbance characteristics.

Beakers were placed in a recirculating water bath set at 15 °C in a solar simulator (Altas Suntest CPS+ Linsengericht-Altenhaßlau, Germany). They were wrapped in black electrical tape to avoid reflected light and continuously stirred. The solar simulator was set at an irradiance of 400 W/m^2 (for all wavelengths) and was equipped with a coated quartz filter and a UV special glass filter to block the transmission of wavelengths below 290 nm and simulate natural sunlight (passing wavelength, 290 nm < λ < 800 nm).[25] The resulting light spectrum as measured using a spectroradiometer (ILT950; International Light, Peabody, MA) compared favorably to the light intensity at the ground level (17 m above sea level) in Palo Alto, CA (37.4292° N, 122.1381° W) at midday in the summer (Figure S5). Dark controls, seeded with the same bacterial and water sources as the experimental reactors, were run in parallel unstirred in the dark at 15 °C.

Upon exposure to the solar simulator light source, 0.5 mL samples were aseptically withdrawn from the 50 mL beakers and 0.1−10 mL samples from the 530 mL beakers every 15−30 min for a total of 1−3 h. Samples from the 50 mL beaker, containing the laboratory strain *Ent. faecalis*, were serially diluted with CBS, and the colonies were enumerated by membrane filtration of appropriate dilutions in duplicate on mEI agar[26] (see SI). Concentrations were calculated using counts from all plates with between 10 and 400 colonies after accounting for the dilution and volume applied to the agar. Samples from the 530 mL beakers, containing sewage-sourced enterococci and *E. coli*, were enumerated using IDEXX colorimetric-liquid-defined substrate assay as described previously. The different enumeration method was used for sewage-sourced bacteria to best replicate the methods performed at the field sites. Method comparison studies show that the two methods (EPA method 1600 with mEI and IDEXX Enterolert) generally agree very well.[27,28] Sterile laboratory techniques were used throughout the study (see SI). Variations between replicates were considered small relative to the large decreases in bacterial concentration over the course of the microcosm experiments.

The inactivation data obtained from three replicate experiments were aggregated and fit using a shoulder-log linear model:[29]

$$\frac{C(t)}{C_0} = e^{-kt}\left(\frac{e^{kS}}{1 + (e^{kS} - 1)e^{-kt}}\right) \quad (3)$$

where t is time (min), C (CFU/ml or MPN/ml) is the measured concentration at time t, C_0 is the measured concentration at time 0, S (min) is the shoulder or lag time over which there is minimal inactivation of the bacteria, and k (min^{-1}) is the rate constant for the log linear portion of the inactivation curve after completion of the lag time. All fitted values are non-negative. If the 95% confidence interval of the shoulder length, S, included zero, then the inactivation data were refit using a simpler first order decay model (eq 1), but with t in min, C and C_0 in CFU/mL or MPN/mL, and k in min^{-1}.

Fit parameters, and their standard deviations and 95% confidence intervals were obtained using IGOR PRO (WaveMetrics Inc., Lake Oswego, OR). A χ^2 value was generated for each model (eq 1 and 3) and the Pearson's χ^2 test for goodness of fit was used to determine the P value, with the assumed null hypothesis that the model predicted the experimental data. Model parameters k and S were corrected for light screening of UVB photons (280−320 nm) as described in the SI, and are hereon referred to as $\hat{k}$ and $\hat{S}$. Light screening incorporated light absorbance by water constituents. Therefore, a correction for light screening resulted in a fluence of the light incident on the bacteria.

Statistical Methods. $\hat{k}$ values from the laboratory experiments were compared across experimental treatments using a t test with a null hypothesis that the $\hat{k}$ values were the same. A P value < 0.05 was deemed significantly different.

Chemical Probes. The steady-state bulk concentrations of singlet oxygen ($^1O_2$) were indirectly measured in PPH, SJM, and ABL waters collected during Summer I phase by monitoring the decay of probe compound furfuryl alcohol (FFA)[30,31] upon exposure to simulated sunlight via the solar simulator (see SI). Singlet oxygen experiments were performed in duplicate.

Estimating the Exogenous Photoinactivation Rate Constant in the Field. The exogenous photoinactivation rate constant, $\hat{k}_{exo}$, was translated from the laboratory to the field following a previously published approach[32] (see SI). In brief, if $\hat{k}$ in colored water was greater than $\hat{k}$ in clear PPH water, the difference was defined as $\hat{k}_{exo}$. Then, an apparent second-order rate constant for the reaction between singlet oxygen and the bacteria was calculated using $\hat{k}_{exo}$. k_{exo} expected under field conditions was then determined by multiplying the bulk phase singlet oxygen concentrations in the environmental waters (estimated from a model that uses DOC concentrations and irradiance at 410 nm as inputs[31,33]) by the second-order rate constant.

RESULTS

Physical and Chemical Properties of the Site Waters. The average temperature and salinity for each phase and location is listed in Table 1. The NPOC concentrations and turbidities averaged across all phases were 10.7, 6.1, and 2.0 mg/L and 18.4, 3.3, and 2.9 NTU, respectively, for SJM, ABL, and PPH waters, respectively (Table 1). The water absorbance values from each field site were extremely similar across the experimental phases (data not shown) and thus the average absorbance across experimental phase was used in light

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penetration calculations (Figure 1). The absorbance from 280 to 700 nm was highest in the SJM water, followed by the ABL

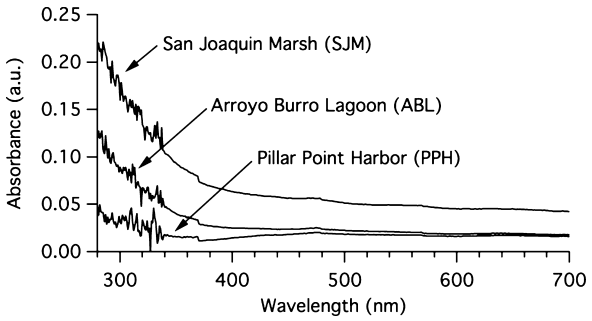

Figure 1. Measured absorbance spectra for water from each field site, averaged across experimental phases.

water then PPH water. The bulk phase, steady state singlet oxygen concentrations from Summer I phase were highest in the SJM water (127×10^{-15} M), second highest in the ABL water (60×10^{-15} M), and lowest in the PPH water (11×10^{-15} M). The UVB light intensity in the solar simulator was 1.59 W/m^2, which is above the daily average UVB light intensity (0.34−1.00 W/m^2) but below the peak UVB light intensity (1.64−3.57 W/m^2) incident to the water surface on any day of the field deployments; therefore, the singlet oxygen concentrations are representative of those that could be generated in the environment.

Field Experiments. Enterococci and *E. coli* decay profiles from the three field sites during the different experimental phases were fit with first order decay models (Figures S3 and S4). k ranged from 0.79 to 4.17 day^{-1} for enterococci and 0.69 to 4.18 day^{-1} for *E. coli* (Table S4). The daily average UVB light intensity within the dialysis bags, I_{UVB}, ranged from 0.0003 W/m^2 (SJM during Winter I phase) to 0.34 W/m^2 (PPH during Summer I phase at 5 cm depth) (Table S4).

k values obtained for microbes deployed in dialysis bags at depth and in colored waters were generally smaller than those deployed near the surface and in clear water, respectively. We posit that this trend is due to the different light intensities incident on the experiments. k values were thus plotted against the corresponding daily average I_{UVB} (Table S4), a linear regression was applied, and the y-intercept and slope of the best fit line were interpreted as k_{dark} and k_{sun}, respectively, according to eq 2 (Figure 2). R^2 of the curve fit was 0.69 ($p < 0.05$) for enterococci and 0.66 ($p < 0.05$) for *E. coli*. k_{dark} was 1.74 d^{-1} for enterococci and 1.40 d^{-1} for *E. coli*. k_{sun} was 7.47 m^2/(Wd) for enterococci and 7.01 m^2/(Wd) for *E. coli*. k_{sun} for enterococci and *E. coli* can also be expressed as 86.5 and 81.2 m^2/MJ, respectively, and be interpreted as $\hat{k}$, which is k corrected for UVB light screening. The root-mean-square error between the modeled k (eq 2) and actual k is 0.28, 0.61, and 0.52 d^{-1} for enterococci and 0.41, 0.64, and 0.16 d^{-1} for *E. coli* at PPH, SJM, and ABL field sites. An analysis considering both UVB and UVA is included in the SI.

Photoinactivation Rate Constant Predictions for Diverse Water Columns. k for enterococci and *E. coli* as a function of depth was modeled using k_{sun} and k_{dark} from eq 2 and $I_{UVB}(z)$ for a representative summer day (8 Sept 2014) at the PPH, SJM, and ABL deployment sites (Figure 3). The depth-averaged $<I_{UVB}>(z_{tot})$ and depth-averaged $<k>(z_{tot})$ in a well-mixed system were modeled as well (Figure 3). The resulting light intensities and k values were highest for the PPH water, second highest for the ABL water, and lowest for the SJM water. In an unmixed water column, photoinactivation, or $k_{sun}{*}I_{UVB}$, makes up a significant fraction of the overall inactivation, k, in waters as deep as 10 cm in colored SJM water and as deep as 70 cm in clear PPH water. In well-mixed water columns that are colored like SJM, photoinactivation makes up a significant fraction of the overall photoinactivation, $<k>$, up to z_{tot} = 200 cm deep, whereas this depth increases to 1000 cm for clear waters like those at PPH.

Laboratory Experiments. First order decay rate constants of the organisms in the dark controls did not differ from zero ($p < 0.05$, data not shown). Aggregated inactivation data from the replicate laboratory experiments (Figure S6) were fit using either a shoulder-log linear model or first-order decay model (Table S5). The Pearson's χ^2 test values for all 7 fitted curves were above 0.05, indicating that model fit was acceptable.

For five of the seven microcosm experimental treatments, there was a shoulder $\hat{S}$ ranging from 0.0012 to 0.0022 MJ/m^2. All decay curves from experiments with the laboratory strain

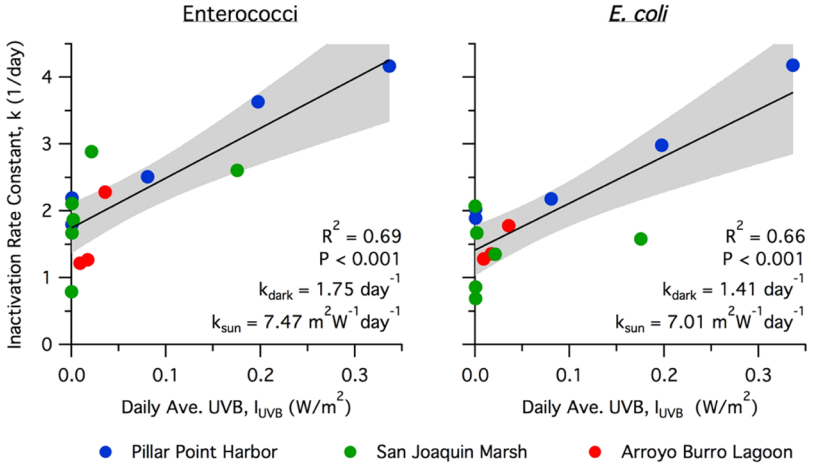

Figure 2. Observed inactivation rate constants of enterococci and *E. coli* from field deployments at Pillar Point Harbor, San Joaquin Marsh, and Arroyo Burro Lagoon plotted against the corresponding daily average UVB intensity. The data are fit with a linear regression function (solid, parameters on bottom right of windows), with the 95% confidence interval (gray background). The resulting coefficient of determination, P value of the linear regression, and fitted k_{dark} and k_{sun} parameters are displayed on the bottom right of each graph.

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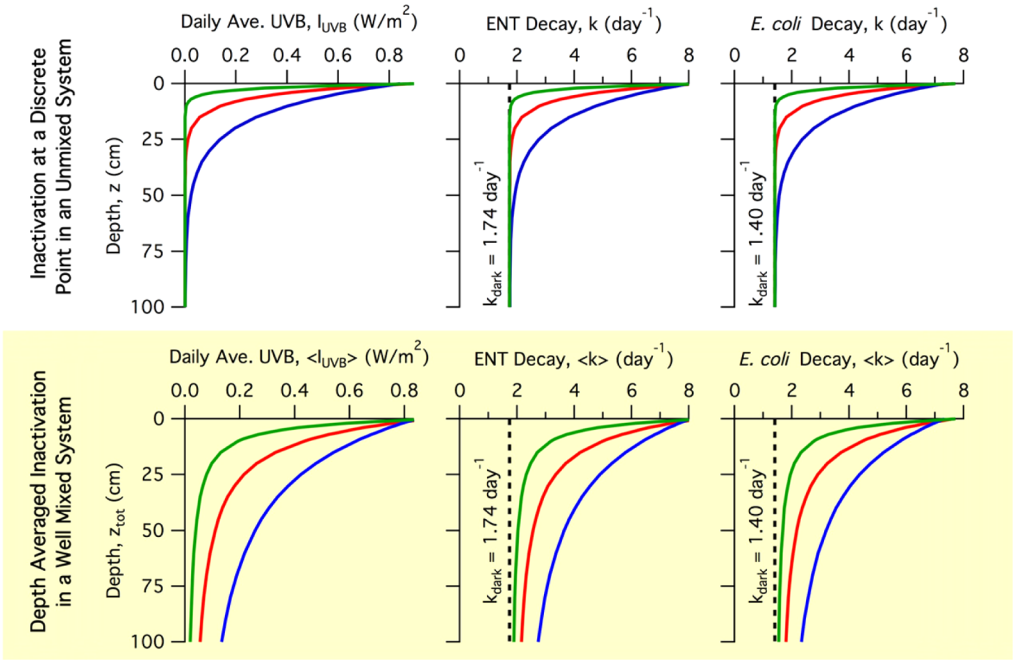

Figure 3. (Top Row) $I_{UVB}(z)$, $k(z)$ for enterococci, and $k(z)$ for *E. coli* in an unmixed system and (Bottom Row) the depth-averaged $<I_{UVB}>(z_{tot})$, depth-averaged $<k>(z_{tot})$ for enterococci, and depth-averaged $<k>(z_{tot})$ for *E. coli* in a well mixed system in San Joaquin Marsh (green line), Arroyo Burro Lagoon (red line), and Pillar Point Harbor (blue line) waters. The total depth used for the depth-averaged calculations was from the water surface to the depth listed on the *y*-axis of each graph. *k* was modeled using k_{sun} and k_{dark} from eq 2 for a representative summer day (8 Sept 2014). The k_{dark} is represented as the vertical dashed line at 1.75 day^{-1} for enterococci and 1.41 day^{-1} for *E. coli*.

Ent. faecalis displayed a better fit using the shoulder model. Curves of sewage-sourced enterococci in SJM water and sewage-sourced *E. coli* in PPH water displayed better fit with no shoulder.

$\hat{k}$ values are listed in Table S5 and shown graphically in Figure 4. $\hat{k}$ of the laboratory strain *Ent. faecalis* in SJM water was significantly higher than in PPH or ABL water ($P < 0.05$). However, $\hat{k}$ of sewage-sourced enterococci was lower in SJM water than PPH water ($P < 0.05$); the same trend was observed for sewage-sourced *E. coli* ($P < 0.05$). Comparing between bacterial sources, $\hat{k}$ of the laboratory strain *Ent. faecalis* was significantly higher than the sewage-sourced enterococci in SJM water. Conversely, the $\hat{k}$ of the laboratory strain *Ent. faecalis* was significantly lower than the $\hat{k}$ of sewage-sourced enterococci in PPH water.

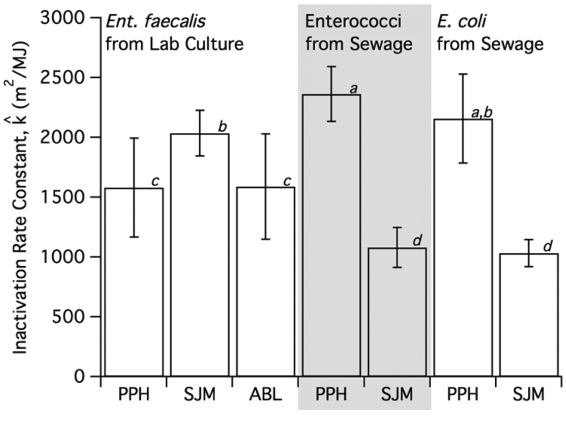

Figure 4. Photoinactivation rate constants corrected for UVB light screening, $\hat{k}$, from microcosm tests of laboratory strain *E. faecalis* suspended in three natural waters (Pillar Point Harbor (PPH), San Joaquin Marsh (SJM), Arroyo Burro Lagoon (ABL)), and sewage-sourced *E. coli* and enterococci suspended in two natural waters (PPH, SJM). Error bars represent 95% confidence intervals. $\hat{k}$ values marked with the different letter (a, b, c, and/or d) are significantly different from each other as indicated by a *t* test ($P < 0.05$).

Estimation of Exogenous Photoinactivation Rate Constants in the Field. Because $\hat{k}$ of the laboratory strain *Ent. faecalis* in colored SJM water was significantly higher than in clear PPH water, k_{exo} of enterococci was estimated for the SJM water only. $\hat{k}_{exo}$ for *Ent. faecalis* in SJM water found in the laboratory was 457 m^2/MJ. k_{exo} in the field was estimated to range from 0.2–0.8 day^{-1} for enterococci at 15 cm depth in shaded and unshaded modules over the three phases at SJM, to as high as 5.5 day^{-1} at a depth of 2 cm in the Summer II phase (see SI).

DISCUSSION

First order decay rate constants for culturable enterococci and *E. coli* in natural waters are well correlated to the daily average UVB light irradiance in the water column. The I_{UVB} at the experimental depth was calculated considering incident light intensity modeled using SMARTS, water and membrane absorbance, and water column depth and thus considered light screening by the water column. The model indicates that decay rate constants of both enterococci and *E. coli* are between 1 and 2 per day under low light conditions and can be as high as 4 per day under high light conditions within the dialysis membranes. After accounting for membrane absorbance, this is equivalent to 1 and 2 per day under low light conditions and as high as 6 per day under high light conditions. It is difficult to directly compare these rate constants with those reported in other experiments as rate constants depend on the experimental system including photon source and spectrum and reactor size.[34] A previous study reported k_{dark} and k_{sun} for

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enterococci and *E. coli* in clear marine water of 0.8−1.3 d^{-1} and 6−7 m^2/(Wd) considering UVB, respectively, but they used UVB incident on the water column instead of accounting for light screening within the water.[35] Regardless, those results compare favorably to those reported herein. UVB wavelengths are those most damaging to bacterial cells and mechanistically are considered the main cause of endogenous photoinactivation.[11] One interpretation of the good correlation between k and UVB intensity is that endogenous photoinactivation is the main cause of inactivation in the field studies. Including UVA, which has also been implicated in the endogenous photoinactivation pathway,[36] in the calculation of light intensity also resulted in good correlations between *E. coli* and enterococci k and light intensity (Figure S7).

The model was the least successful at predicting k at SJM as root-mean square errors were the highest for SJM rate constants. SJM was the site with the most colored water and the greatest potential for generation of reactive oxygen species. Previous research in a wetland system indicated the importance of the exogenous indirect photoinactivation mechanism,[32] which could not be determined for this study from the field results alone. We undertook laboratory experiments to determine whether or not the exogenous indirect photoinactivation mechanism could be active and thus contribute to the observed divergence from the model.

Laboratory experiments with raw field water seeded with laboratory grown *Ent. faecalis* suggested that the exogenous indirect photoinactivation mechanism was active in SJM water. The first order rate constant of *Ent. faecalis*, corrected for UVB light screening in the experimental reactor, was higher in the colored SJM water than in PPH water. PPH water is clear and thus has less potential to generate reactive species like singlet oxygen. Interestingly, even though ABL water was also colored, decay of *Ent. faecalis* suspended in ABL was similar to decay in PPH water, once corrected for light screening. These results suggest that the colored dissolved organic matter (CDOM) present in SJM water, as opposed to CDOM present in ABL water, is an effective photosensitizer for *Ent. faecalis*. In our study, the exogenous indirect photoinactivation rate constant for *Ent. faecalis* at SJM, translated from the laboratory to the field, was estimated to be anywhere from 0.2 day^{-1} at low light conditions to as high as 5.5 day^{-1} at high light conditions. Assuming that exogenous and endogenous photoinactivation rate constants are additive,[32] and that the results for *Ent. faecalis* are extendable to all enterococci present in the field experiments. These results suggest that a model that only considers endogenous photoinactivation, such as the model derived from the field experiments, would under-predict k of enterococci at SJM. However, we did not observe that the field model consistently underpredicted k at SJM. This suggests that the exogenous indirect photoinactivation observed in the laboratory for *Ent. faecalis* does not translate to the field. This may be in part due to our use of a laboratory pure culture of *Ent. faecalis*.[34] Previous work[34] suggests that decay characteristics of sewage-sourced enterococci can diverge from laboratory grown pure cultures. We therefore repeated the laboratory experiments using sewage as the inoculant to obtain more field-relevant results.

When laboratory experiments were conducted with field water seeded with sewage-sourced enterococci and *E. coli*, there was no evidence of exogenous indirect photoinactivation in SJM water. We observed that photoinactivation was depressed in colored SJM water relative to clear PPH water, even after correcting for UVB light screening. A slowing of photoinactivation after correcting for light screening in the presence of CDOM has been observed previously and attributed to the shielding of susceptible targets to photoinactivation[37] or, in the case of the photolysis of chemicals, organic matter acting as a sink for the reactive species.[38−42] The diverging results between lab-sourced *Ent. faecalis* and sewage-sourced enterococci suggest that the lab-sourced *Ent. faecalis* strain is more susceptible to the exogenous indirect photoinactivation mechanism than the sewage-sourced enterococci. This could occur if the sewage sourced enterococci included pigmented enterococci which are less susceptible to photoinactivation than nonpigmented enterococci,[32,43] or if the sewage-sourced organisms were better equipped to deal with oxidative stress than the laboratory strain. Given the lack of clear evidence of the importance of exogenous indirect photoinactivation mechanism in field waters, we determined that accounting for it mechanistically in a decay model for enterococci and *E. coli* was not justifiable or warranted. We investigated whether accounting for the observed depression in photoinactivation in SJM water would improve model fit, but it did not (see Figure S8). These results highlight the complex role that natural CDOM may play in bacterial photoinactivation pathways.

Interestingly, all of the laboratory experiments overpredicted $\hat{k}$ relative to what was observed in the field. The field model gives k_{sun} (which is equivalent to $\hat{k}$) of 7−8 m^2/(Wd) or 80−90 m^2/MJ. This is ∼1/20th of what was observed in the laboratory experiments (between 1000 and 2400 m^2/MJ). In other words, for the same apparent dose of UVB, there is much more inactivation in the laboratory compared to the field. A discrepancy between laboratory and field-based bacterial photoinactivation rate constants has been reported previously where laboratory experiments yielded rate constants 2−13 times those observed in the field.[32] The discrepancy could be due to the solar simulator producing more high energy UVB photons than the sun, diurnal fluctuations in natural solar irradiance, cloud cover in the field, and the different concentrations of dissolved oxygen entrained in the experimental water due to mixing.[12] This highlights the complexities in extending laboratory-derived photoinactivation rate constants to the field.

We used the field model ($k = k_{dark} + k_{sun}{*}I_{UVB}$) to predict k in PPH, SJM, and ABL waters on a typical summer day. Most recreation surface waters are nearshore and well mixed. $\langle k \rangle$ is the appropriate parameter to include in FIB modeling efforts for those systems, and it varies as a function of total water column depth as well as water absorbance characteristics, with $\langle k \rangle$ smaller in deeper waters and colored waters. This model can be extended to other water columns given their absorbance spectrum and the incident sunlight at the surface of the water column.

There are several limitations to this study. When estimating the incident sunlight intensity through SMARTS, we could not account for cloud cover. Overestimating I_{UVB} would then cause us to underestimate k_{sun}. Additionally, the model does not account for other factors that could contribute to decay, such as salinity or temperature. Experiments on the effects of salinity on FIB decay are equivocal with some studies showing an effect and others not,[44−46] while temperature has been correlated to the photoinactivation of both enterococci and *E. coli* in natural waters with a relatively small effect size (∼<1 d^{-1}).[47,48] Lastly, for the laboratory experiments, site water was stored up to eight months prior to use. We noted little change in water

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absorbance over time consistent with prior studies,[24,49] which alleviated concerns over the possibility of changing optical water characteristics. Nonetheless, the long-term storage may have introduced some error in the measured photoinactivation rate constants and certainly changed the biological characteristics of the waters (natural biota).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b00505.

Includes additional experimental details, calculations for light intensity estimations, light screening, ROS concentrations, and estimated exogenous photoinactivation rate constants, inputs to SMARTS, tables of experimental details, field deployment information, and results of model fittings and statistical analysis, diagrams of the field deployment modules, and graphs of the bacterial decay during field deployment, bacterial decay during laboratory experiments, natural and simulated light spectra, and additional graphs exploring the use of alternative light spectra (PDF)

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Notes

The authors declare no competing financial interest.

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