

Supporting Information

Sunlight inactivation of viruses in open-water unit process treatment wetlands: Modeling endogenous and exogenous inactivation rates

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MS2 and poliovirus type 3 propagation, purification and enumeration. MS2 (ATCC 15597-B1) was propagated by broth enrichment using tryptic soy broth, *E. coli* F_{amp} (ATCC 700891) host, and 1X ampicillin and streptomycin antibiotics (amp/strep, 0.015 g/L, w/v, each).

Poliovirus type 3 (PV3; ATCC VR-300) was propagated on 90% confluent HeLa cells (ATCC CCL-2) in T-150 flasks: cells were first washed with 30 mL of 1X Dulbecco's Modified Eagle Medium (DMEM; Invitrogen), then 2 mL PV3 was added to the flask (MOI between 0.01 and 0.1); flasks were incubated at 37 °C and 5% CO₂ for 1 h, and rocked every 15 min. After 1 h, 30 mL of 1X DMEM amended with 1X penicillin and streptomycin antibiotics (pen/strep, 100 U/mL, each) and 10% (vol/vol) fetal bovine serum (FBS) was added to the flask, which was then incubated for 4 d. After incubation,

viruses were released from cells by three freeze-thaw cycles using an ethanol-dry ice bath and 37 °C water bath.

To purify crude virus stocks, MS2 and PV3 stocks were first chloroform extracted to remove cell debris: chloroform was added at a 1:3 (vol/vol) concentration, the solution was vortexed for 2 min, then centrifuged at 2000 ×g for 10 min. To remove broth constituents, the supernatant was polyethylene glycol (PEG) precipitated overnight at 4 °C (9% PEG, 0.3 M NaCl), centrifuged at 20,000 ×g for 15 min to produce virus pellets that were resuspended in phosphate buffered saline (PBS; 10 mM NaCl, 20 mM phosphate), chloroform extracted as above and filtered through a 0.22 µm filter. Virus stocks were stored at -80 °C.

MS2 were enumerated using the double agar layer (DAL) plaque assay with 100 µL sample inocula, modified Luria Bertani (LB) top and bottom agars, and 1X amp/strep. Modified LB consists of: bacto agar [0.75% (top) or 1.5% (bottom) wt/vol; BD], 10 g/L bacto tryptone (BD), 0.137 M NaCl, 1 g/L yeast extract (EMD Chemicals), 0.0055 M dextrose (EMD Chemicals), 0.002 M CaCl₂. DAL plates were incubated at 37 °C for 18-24 h, after which plaque forming units (PFU) were counted.

PV3 plaque assays were performed in duplicate on 6-well plates of 90% confluent HeLa cells with 100 µL sample inocula and an agar overlay [1.5% wt/vol low melting point agarose (Fisher Scientific), 1X DMEM, 1X pen/strep, and 10% FBS]. Plates were incubated at 37 °C and 5% CO₂ for 3 d. After incubation, agar overlays were removed and cells stained with crystal violet in order to count PFUs.

F+ and somatic coliphage enumeration. Water samples were concentrated for coliphage enumeration using membrane filtration with 47-mm diameter, 0.45-µm pore size, mixed cellulose ester HA filters (Millipore). Before filtration, water samples were amended with MgCl₂ (0.05 M final concentration) and held for 5 min to facilitate virus adsorption to filters.¹ Between 1 and 300 mL of sample was filtered (filtration rate <30 mL/min), depending on expected virus concentrations. Filters were preserved until elution by freezing at -20 °C on 300 µL of 50% glycerol (1:1 vol/vol with PBS). Separate

filters were prepared for F+ and somatic coliphage.

Coliphages were eluted from filters by adding 2 mL of 3% Beef Extract (pH 9; 30 g/L Beef Extract, 30 mL/L Tween 80, 0.3 M NaCl) and swirling for 10 min. Filter eluent was assayed for coliphage using the DAL method with 1-mL sample inoculums, a modified LB top agar (0.75% wt/vol) and bottom agar (1.5% wt/vol), and appropriate hosts and antibiotics. F+ coliphage were assayed using *E. coli* F_{amp} host bacteria with ampicillin and streptomycin antibiotics (0.0015 g/L of each); somatic coliphage were assayed using *E. coli* CN13 host with nalidixic acid (0.01 g/L). Eluted filters were plated, face down, on top agar augmented with 0.3% Tween 80. Plates were incubated at 37 °C for 18-24 h and enumerated as PFUs. Total phage concentrations were calculated by adding counts from the DAL and filter plates.

A subset of F+ coliphage samples were treated with RNase A (Boehringer) to quantify F+DNA coliphage (40 mg/L final concentration added to top agar). After zero F+DNA coliphage were found in initial samples, RNase A treatment was discontinued, and it was assumed that all F+ coliphage found in this system were F+RNA coliphage.

Solar simulator experiments in the laboratory. Sunlight inactivation experiments were conducted using a 1000-W solar simulator (Oriel #91194) with an ozone-free Xe bulb (Newport #6271), a 1.5:G:A global airmass filter (Newport #81388) and either an atmospheric attenuation filter (Newport #81017) to mimic the solar spectrum or a UVB-blocking filter (Newport #81050) to exclude endogenous inactivation. Bulb irradiance was measured at the start of each experiment using a spectroradiometer (EPP2000C-SR-100 with CR2 cosine receptor, Stellarnet), and the power was adjusted to ensure a consistent light spectrum across experiments (average spectra are provided in Figure S6). Average total irradiance (280–700 nm) with the atmospheric filter was 237 W/m² [UVB irradiance (280–320 nm): 1.91 W/m²; UVA (320-400 nm): 28 W/m²]. For UVB-blocked light, the average total irradiance was 235 W/m² (UVB: 0.2 W/m²; UVA: 21 W/m²).

Experimental reactors consisted of open-top glass beakers painted black on the outside; reactors were constantly mixed using sterilized magnetic stir bars, and located in a water

bath cooled by a recirculating chiller to maintain a temperature of 20 °C. MS2 and PV3 inactivation rates were investigated in separate experiments, which were conducted in duplicate reactors. The average initial virus concentrations were 5.1×10^4 PFU/mL for MS2 and 8.5×10^4 PFU/mL for PV3. During experiments, 0.5 mL sub-samples were removed from reactors every 1-2 h, immediately placed on ice and assayed within 6 h of collection.

As mentioned in the main manuscript, three sets of experiments were conducted: (1) experiments to determine β , (2) experiments to determine k_2 values for MS2 and PV3 that were specific to water from the Discovery Bay open-water cell, and (3) experiments to measure k_{obs}^L , to determine if modeling equations were able to approximate inactivation rates. See main text for details.

Measurement of steady-state singlet oxygen concentrations. Bulk-phase, steady-state singlet oxygen concentrations ($[{}^1\text{O}_2]_{\text{ss,bulk}}$) were determined through photolysis experiments using furfuryl alcohol (FFA; $k_q = 1.2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$) as a probe compound.² Photolysis experiments were conducted in a similar manner as virus inactivation experiments, but with FFA added to each reactor at an initial concentration of 50 µM. Reactors were irradiated with simulated sunlight for 6 h and sub-samples were collected every 1 to 2 h. Sub-samples were analyzed for FFA depletion (UV-detection at 215 nm) by HPLC (Gynkotek) with a reverse-phase C18 column (Cosmosil C18-5-MS-II, 100×3 mm Nacalay Tesque) equipped with a guard column and a pre-filter, and eluted with 85% phosphoric acid buffer (pH 2) and 15% acetonitrile. Observed first order FFA decay constants ($k_{\text{obs,FFA}}$) were calculated as the negative slope of $\ln([FFA]_t/[FFA]_0)$ versus time, following the equation:

$$\ln \frac{[FFA]_t}{[FFA]_0} = -k_{\text{obs,FFA}} \cdot t \quad (\text{SI 1})$$

where $[FFA]_0$ is the initial FFA concentration and $[FFA]_t$ is the concentration of FFA at time t . The slope of the graph was determined by linear regression.

$[{}^1\text{O}_2]_{\text{ss,bulk}}$ was calculated according to the equation:

$$[{}^1\text{O}_2]_{\text{ss,bulk}} = \frac{k_{\text{obs,FFA}}}{k_q} \quad (\text{SI } 2)$$

where k_q is the reaction rate constant between FFA and singlet oxygen.

Modeling light attenuation. Light attenuation was modeled using the following relationship.³

$$E_0(z, \lambda) = E_d(z, \lambda) = E_d(0, \lambda) e^{-\psi \cdot K_d(\lambda) \cdot z} \quad (\text{SI } 3)$$

where $E_0(z, \lambda)$ is the scalar irradiance at depth z , $E_d(z, \lambda)$ is the downward irradiance at depth z , $E_d(0, \lambda)$ is the irradiance just below the water surface, $K_d(\lambda)$ is the vertical attenuation coefficient for downward irradiance, and ψ is a pathlength correction factor used to correct z for light-path geometry (see main manuscript text). $E_0(z, \lambda)$ was assumed to be equal to $E_d(z, \lambda)$ because scattering was previously found to be negligible.^{3,4} To estimate average light transmitted over a well-mixed water column of depth z [$\langle E_0(z, \lambda) \rangle$], Equation SI 3 was integrated from 0 to z and divided by z :

$$\langle E_0(z, \lambda) \rangle = \frac{1}{z} \int_0^z E_d(0, \lambda) e^{-\psi \cdot K_d(\lambda) \cdot z} dz = E_d(0, \lambda) \left(\frac{1 - e^{-\psi \cdot K_d(\lambda) \cdot z}}{\psi \cdot K_d(\lambda) \cdot z} \right) \quad (\text{SI } 4)$$

We assume no reflection or refraction at the water surface; scattering was previously found to be negligible⁴ and was not included. Therefore, $K_d(\lambda)$ was assumed to be equal to the naperian absorbance [$a(\lambda)$].⁴ $a(\lambda)$ is equal to $2.303 \times$ decadic absorbance [$\alpha(\lambda)$], resulting in the following equation:

$$\langle E_0(z, \lambda) \rangle = E_d(0, \lambda) \left(\frac{1 - e^{-\psi \cdot 2.303 \cdot \alpha(\lambda) \cdot z}}{\psi \cdot 2.303 \cdot \alpha(\lambda) \cdot z} \right) \quad (\text{SI } 5)$$

Using the model to design treatment wetlands – calculations. To estimate the open-water wetland surface areas needed for 1-, 2- and 3-log virus inactivation for a flow rate of 1 million gallons per day (MGD), we used an approach similar to that employed by Jasper and Sedlak.⁵ As described in the main manuscript text, inactivation in the open-water cell was modeled using the Wehner-Wilhelm equation:⁶

$$\left[\frac{C_{\text{outlet}}}{C_{\text{inlet}}} \right] = \frac{4a \exp\left(\frac{1}{2d}\right)}{(1+a)^2 \exp\left(\frac{a}{2d}\right) - (1-a)^2 \exp\left(-\frac{a}{2d}\right)} \quad (\text{SI } 6)$$

$$a = \sqrt{1 + 4 k_{\text{tot}}^W \cdot \theta \cdot d} \quad (\text{SI } 7)$$

where C_{inlet} and C_{outlet} are the virus concentrations at the open-water cell inlet and outlet, respectively; d is the dispersion number (equal to the inverse of the Péclet number); k_{tot}^W (d^{-1}) is the virus inactivation rate calculated using model Equations 8 to 11 in the main text; and $\theta(d)$ is the hydraulic residence time. To calculate the hydraulic residence time needed to treat 1 MGD of wastewater, Solver (Microsoft Excel) was used to determine the value of θ for a given value of $C_{\text{outlet}}/C_{\text{inlet}}$ (i.e., 0.1, 0.01, and 0.001 for 1-, 2-, and 3-log inactivation, respectively).

To calculate the corresponding theoretical surface area (A ; ha) needed to treat 1 MGD of wastewater, the following equation was used:

$$\begin{aligned} A \text{ (ha)} &= \frac{\theta(d) \cdot Q \text{ (MGD)}}{z \text{ (m)}} \cdot \frac{\text{ha}}{10,000 \text{ m}^2} \cdot \frac{3785 \text{ m}^3 \text{ d}^{-1}}{\text{MGD}} \\ &= \frac{\theta(d) \cdot Q \text{ (MGD)}}{z \text{ (m)} \cdot 2.64 \text{ (MGD d m}^{-1}\text{ha}^{-1})} \end{aligned} \quad (\text{SI } 8)$$

where Q is the flow rate in MGD and z is the depth in m (the same value of z was input in Equations 8 to 11 in the main text to calculate k_{tot}^W).

Calculation of hydraulic residence time and dispersion number from tracer test

results. Characteristics of the water flow in the open-water wetland cell were determined using a Rhodamine-WT tracer test (Figure S16). The total hydraulic residence time (HRT) was estimated from the concentration distribution curve, as follows:⁷

$$\text{HRT} = \frac{\sum t_i F_i \Delta t_i}{\sum F_i \Delta t_i} \quad (\text{SI } 9)$$

where F_i (mg/L) is the Rhodamine-WT fluorescence signal in the water sample collected at the outlet at time t_i (h); and $\Delta t_i = (t_{i+1} - t_i)$. The estimated total HRT was equal to 1.12 d.

The dispersion number (d) was estimated from the dimensionless variance (σ_{HRT}^2):⁷

$$\sigma_{\text{HRT}}^2 = \frac{\sigma^2}{(\text{HRT})^2} = 2d - 2d^2 \left(1 - \exp \left(-\frac{1}{d} \right) \right) \quad (\text{SI } 10)$$

where the variance σ^2 was estimated using:⁷

$$\sigma^2 = \frac{\sum t_i^2 F_i}{\sum F_i} - \left(\frac{\sum t_i F_i}{\sum F_i} \right)^2 \quad (\text{SI } 11)$$

The calculated value for d was equal to 0.08.

Sensitivity of the Wehner-Wilhelm equation to HRT and dispersion number. The calculation of monthly coliphage k_{obs}^W from monitoring data depended on measurements of total HRT and d , which were used as inputs to the Wehner-Wilhelm equation. To determine the sensitivity of calculated k_{obs}^W to each of these variables, we conducted a simple sensitivity analysis where F+ coliphage k_{obs}^W values were calculated with total HRT and d values that were individually increased and decreased by 50%.⁸

The calculation of k_{obs}^W was more sensitive to changes in total HRT than changes in d (Figure S1). A 50% increase in total HRT led to a 33% decrease in calculated k_{obs}^W for all

months, and a 50% decrease in total HRT led to a doubling of k_{obs}^W for all months.

Conversely, there was less than a 10% difference in k_{obs}^W when d was adjusted by 50%.

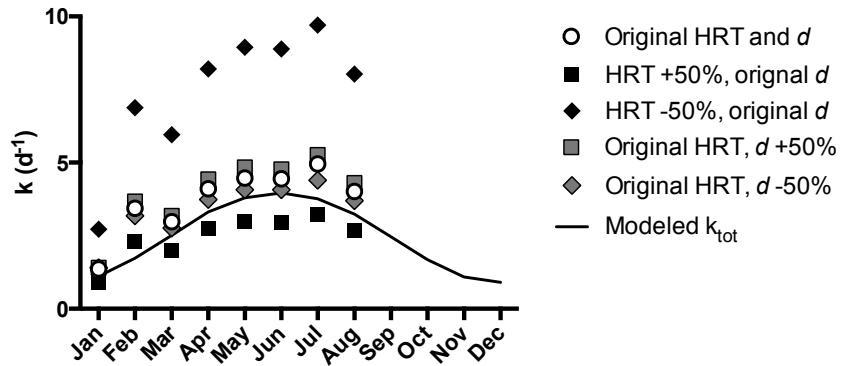


Figure S1. Sensitivity of the Wehner-Wilhelm equation to changes in total HRT and d . k_{obs}^W values were calculated with original HRT and d (white circles), with total HRT increased and decreased by 50% (black symbols), and with d increased and decreased by 50% (grey symbols). Modeled k_{tot}^W (black line) is provided for comparison, and is the same data as in Figure 3 of the main manuscript.

Sensitivity analysis of the MS2 inactivation rate model. A simple analysis was undertaken to determine the sensitivity of the MS2 inactivation rate model to each of its inputs. Each input was individually varied to values 50% greater and 50% less than its original value.⁸ The resulting k_{tot}^W values were calculated for each month, then averaged over twelve months to determine which inputs make the most impact on the predicted inactivation rate.

For MS2, varying the input values (i.e., z , $E_d(0,\lambda)$, $a(\lambda)$, $[{}^1\text{O}_2]_{\text{ss,bulk}}$, k_2 , and the thickness of the biomat) had a larger effect on k_{exo}^W than k_{endo}^W due to the importance of the exogenous mechanism on overall inactivation rates (Figure S2). The inactivation rate models were most sensitive to inputs of $E_d(0,\lambda)$, $[{}^1\text{O}_2]_{\text{ss,bulk}}$, and k_2 . β is only included in the $k_{\text{endo,tuvb}}$ model; adjusting the value of β by 50% affected the total inactivation rate predicted with the tUVB model by only 7.5%.

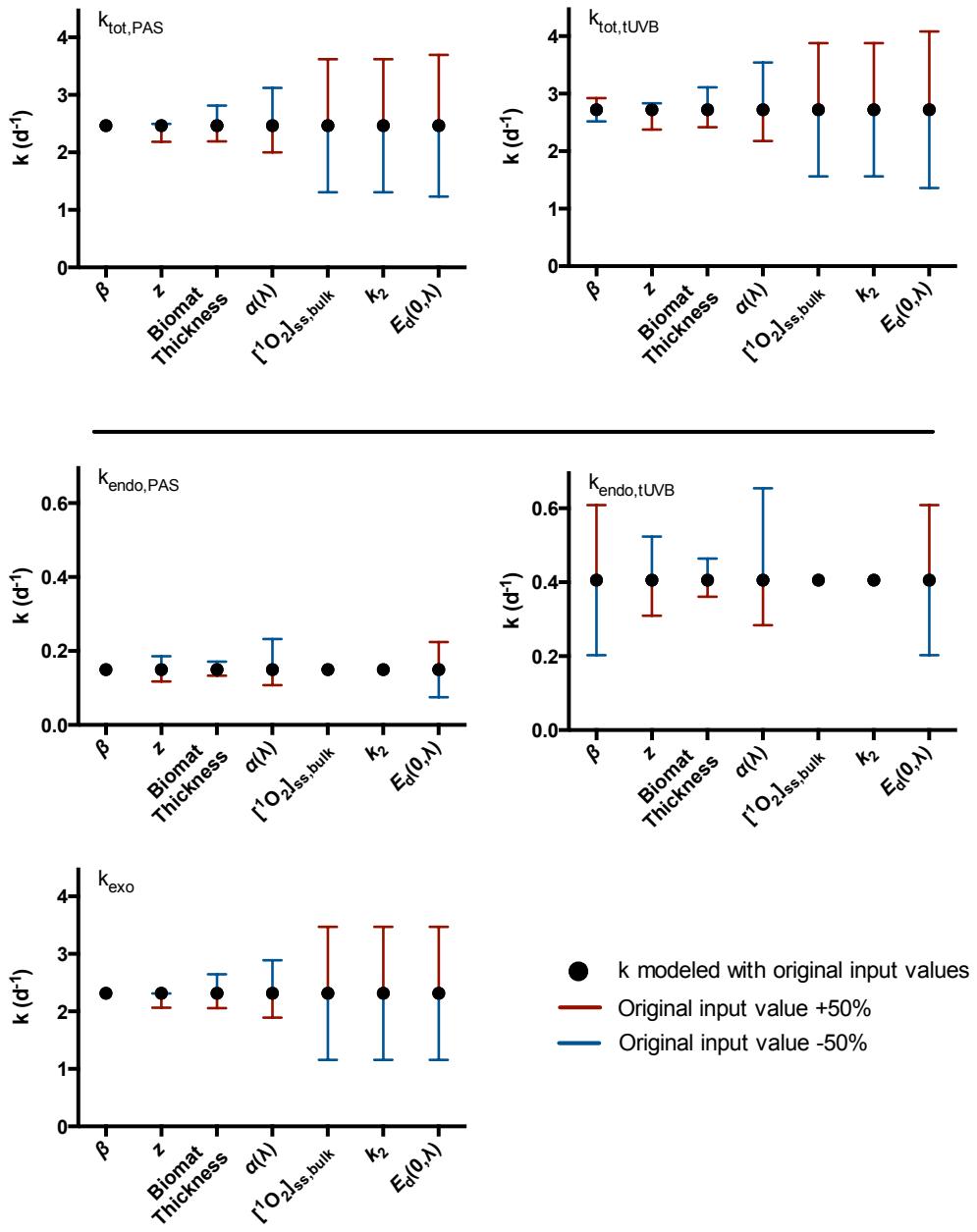


Figure S2. Sensitivity of the MS2 inactivation rate model to its inputs. Inactivation rates (k_{tot}^W) were approximated in the wetland for each month of the year, and 12-month average values are presented. Black circles denote inactivation rates calculated with the original model inputs. Red and blue bars designate the range of inactivation rates predicted by individual varying inputs by +50% and -50% of their original value, respectively (varied inputs are listed on the y-axis). Note different y-axis scale on $k_{endo,PAS}$ and $k_{endo,tUVB}$ graphs.

Measured F+ and somatic coliphage inactivation rates in the open-water wetland.

There was no significant difference between F+RNA and somatic coliphage inactivation rates based on three months of data (Table S4 provides a summary of inactivation rate constants; F+RNA data are presented in Figure 2 of the main manuscript, somatic data are presented in Figure S3 below). Previous researchers have shown these broad groups to differ in their susceptibility to sunlight mechanisms: while F+RNA coliphage were inactivated more slowly than somatic coliphage in clear water,⁹ the inactivation rates of the two groups were more similar in the presence of sensitizers, due to the susceptibility of F+RNA coliphage to exogenous inactivation.¹⁰ The average F+RNA and somatic coliphage concentrations at the inlet were 60 PFU/100 mL (n=24) and 2,400 PFU/100 mL (n=11), respectively. The low concentrations of F+RNA coliphage detected in inlet water could make this coliphage challenging to use as an indicator virus.

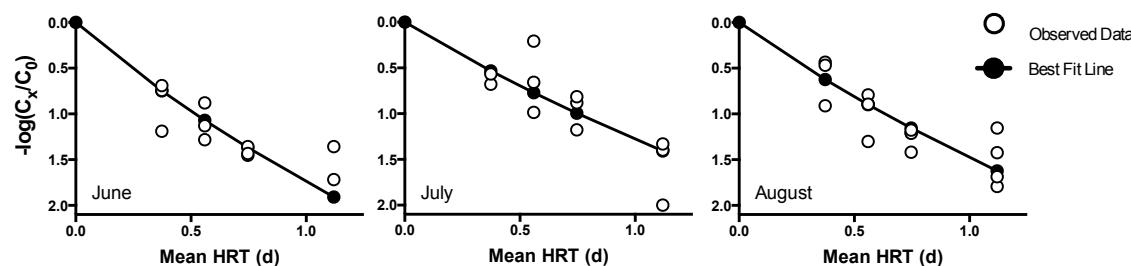


Figure S3. Somatic coliphage monitoring data from the Discovery Bay wastewater treatment wetland open-water cell. Best-fit lines were plotted using the Wehner-Wilhelm equation and the best-fit k_{obs}^W for each month. F+RNA coliphage data are presented in Figure 2 of the main manuscript.

Summary of Model Terms

Inputs to Model Equations

Term	Definition	Source for Laboratory Modeling	Source for Wetland Modeling
z	Water depth (cm)		Measured
$E_d(0,\lambda)$	Wavelength-specific irradiance incident on the water surface (W m^{-2})	Measured by spectroradiometer	Predicted using the Simple Model of the Atmospheric Radiative Transfer of Sunshine (SMARTS; global horizontal irradiance) ¹⁵
γ	Solar zenith angle	Assumed to be 0	Predicted using SMARTS ¹⁵
$\alpha(\lambda)$	wavelength-specific decadic absorbance (cm^{-1})		Measured by UV-visible spectrophotometer
$[{}^1\text{O}_2]_{\text{ss,bulk}}$	Bulk-phase, steady-state singlet oxygen concentration (M)	Measured using FFA probe	Modeled – see equation for $[{}^1\text{O}_2]_{\text{ss,bulk,mod}}$ below
k_2	Apparent reaction rate constant between each virus and $[{}^1\text{O}_2]_{\text{ss,bulk}}$ ($\text{M}^{-1} \text{h}^{-1}$)		Measured through inactivation experiments using the UVB-blocking filter
[DOC]	Dissolved organic carbon concentration		Measured by TOC analyzer (average value used)

Model Terms

$k_{\text{obs}}^{\text{L}}$ First-order, observed inactivation rate measured during laboratory experiments (h^{-1})

$k_{\text{obs}}^{\text{W}}$ First-order, observed inactivation rate measured in the wetland (h^{-1})

$k_{\text{tot}}^{\text{L}}$ Total inactivation rate modeled during laboratory experiments (h^{-1}). This value is calculated as the sum of $k_{\text{endo}}^{\text{L}}$ and $k_{\text{exo}}^{\text{L}}$

$k_{\text{endo,PAS}}^{\text{L}}$ Endogenous inactivation rate modeled during laboratory experiments using the photoaction spectrum (PAS) approach (h^{-1})

$k_{\text{endo,tUVB}}^{\text{L}}$ Endogenous inactivation rate modeled during laboratory experiments using the total UVB (tUVB) approach (h^{-1})

$k_{\text{exo}}^{\text{L}}$ Exogenous inactivation rate modeled during laboratory experiments (h^{-1})

k_{tot}^W	Total inactivation rate <u>modeled</u> in the wetland (d^{-1}). This value is calculated as the sum of k_{endo}^W and k_{exo}^W
$k_{\text{endo,PAS}}^W$	Endogenous inactivation rate <u>modeled</u> in the wetland using the photoaction spectrum (PAS) approach (d^{-1})
$k_{\text{endo,tUVB}}^W$	Endogenous inactivation rate <u>modeled</u> in the wetland using the total UVB (tUVB) approach (d^{-1})
k_{exo}^W	Exogenous inactivation rate <u>modeled</u> in the wetland (d^{-1})
$\langle E_0(z,\lambda) \rangle$	Average scalar light irradiance over a well-mixed depth z (W m^{-2})
$a(\lambda)$	Naperian absorbance (cm^{-1})
ψ	Pathlength correction factor used to correct z for light-path geometry
n	Index of refraction (~1.34 for water)
$P(\lambda)$	Spectral sensitivity coefficient – wavelength- and virus-dependent ($\text{m}^2 \text{W}^{-1} \text{h}^{-1}$)
$\Delta\lambda$	Bin size over which $P(\lambda)$ is defined (equal to 3 nm in the current model)
β	Correlation factor between total UVB irradiance and k_{endo} ($\text{m}^2 \text{W}^{-1} \text{h}^{-1}$)
$[{}^1\text{O}_2]_{\text{ss,bulk,mod}}$	Bulk-phase, steady-state singlet oxygen concentration modeled in the wetland (M)

Data Analysis Terms

t	Time (h)
C_0	Initial virus concentration (at $t = 0$ for laboratory experiments, and at the inlet for wetland monitoring)
C_t	Virus concentration at time t
C_x	Virus concentration at sampling location x in the wetland
θ_x	Mean hydraulic residence time (HRT) at sampling location x
d	Dispersion number

Summary of Model Equations (**bold variables** are inputs)

Light Attenuation

$$\langle E_0(z, \lambda) \rangle = \mathbf{E_d}(\mathbf{0}, \lambda) \left(\frac{1 - e^{-\psi \cdot 2.303 \cdot \alpha(\lambda) \cdot z}}{\psi \cdot 2.303 \cdot \alpha(\lambda) \cdot z} \right)$$

$$\psi = \left(\sqrt{1 - (n^{-1} \sin \gamma)^2} \right)^{-1}$$

Inactivation in the Laboratory

$$k_{\text{tot}}^L = k_{\text{endo}}^L + k_{\text{exo}}^L$$

$$k_{\text{endo,PAS}}^L = \sum_{\lambda} \langle E_0(z, \lambda) \rangle \cdot P(\lambda) \cdot \Delta \lambda$$

$$k_{\text{endo,tUVB}}^L = \beta \cdot \sum_{\lambda=280}^{320} \langle E_0(z, \lambda) \rangle$$

$$k_{\text{exo}}^L = \mathbf{k}_2 \cdot [{}^1\text{O}_2]_{\text{ss,bulk}}$$

Inactivation in the Environment

$$k_{\text{tot}}^W = k_{\text{endo}}^W + k_{\text{exo}}^W$$

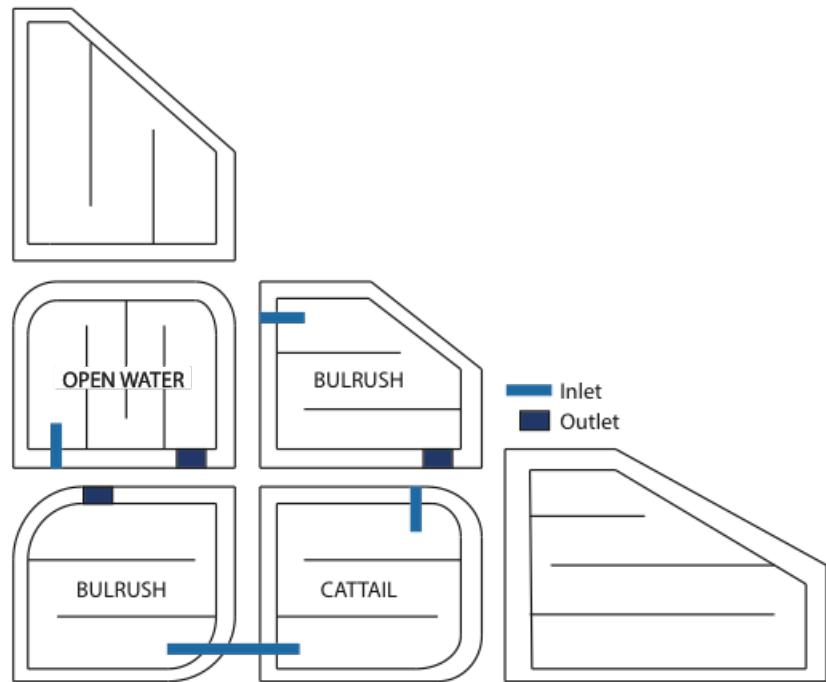
$$k_{\text{endo,PAS}}^W = 24 \cdot \sum_{\lambda} \langle E_0(z, \lambda) \rangle_{24 \text{ h avg}} \cdot P(\lambda) \cdot \Delta \lambda$$

$$k_{\text{endo,tUVB}}^W = 24 \cdot \beta \cdot \sum_{\lambda=280}^{320} \langle E_0(z, \lambda) \rangle_{24 \text{ h avg}}$$

$$k_{\text{exo}}^W = 24 \cdot \mathbf{k}_2 \cdot [{}^1\text{O}_2]_{\text{ss,bulk,mod}}$$

$$[{}^1\text{O}_2]_{\text{ss,bulk,mod}} = 1 \times 10^{-14} \cdot [\mathbf{DOC}] \cdot \frac{\langle E_0(z, \lambda = 410) \rangle_{24 \text{ h avg}}}{\mathbf{E_d}(\mathbf{0}, \lambda = 410)_{\text{June-noon}}}$$

Figures



Open Water Cell



Figure S4. Top: diagram of the Discovery Bay wastewater treatment wetland. Bottom: photograph of the open-water cell with flow direction and sampling points denoted (i.e., inlet, outlet, and turns 1, 2, and 3).

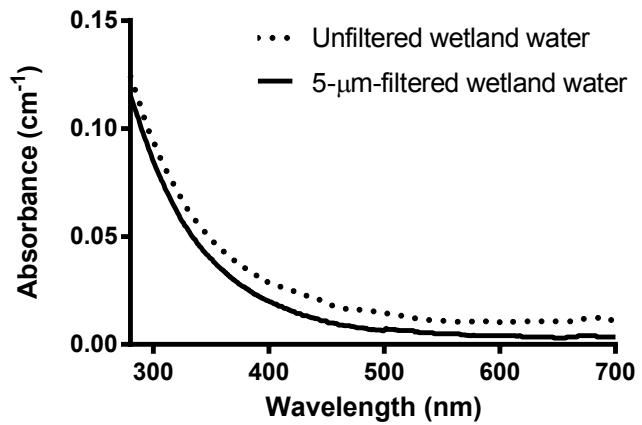


Figure S5. Decadic absorbance spectra [$\alpha(\lambda)$] of filtered and unfiltered water from the Discovery Bay open-water wetland cell; $l = 1$ cm. Average [DOC] was 8 mg/L-C.

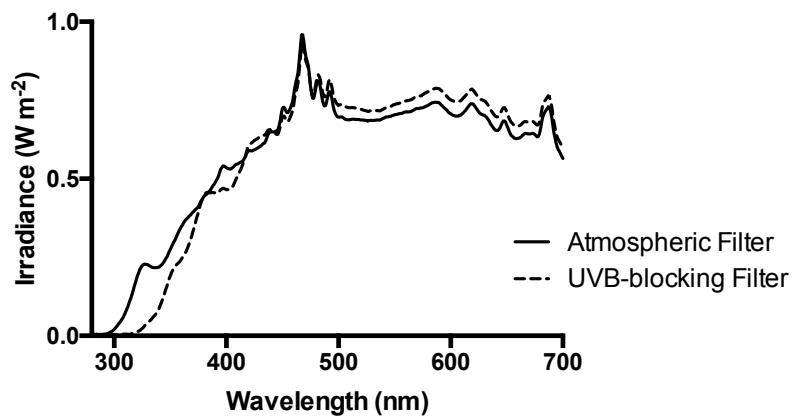


Figure S6. Irradiance of simulated sunlight used in laboratory experiments.

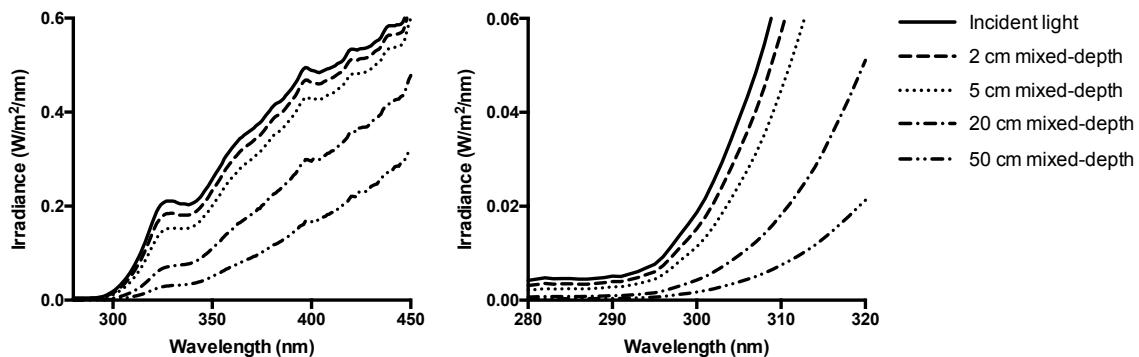


Figure S7. Measured simulated sunlight irradiance incident on the water surface [$E_d(0,\lambda)$; solid line], and modeled average scalar irradiance transmitted through well-mixed columns of Discovery Bay wetland water [$\langle E_0(z,\lambda) \rangle$; 5- μm filtered]. Both panels present the same data; the right panel is a close-up of the UVB range.

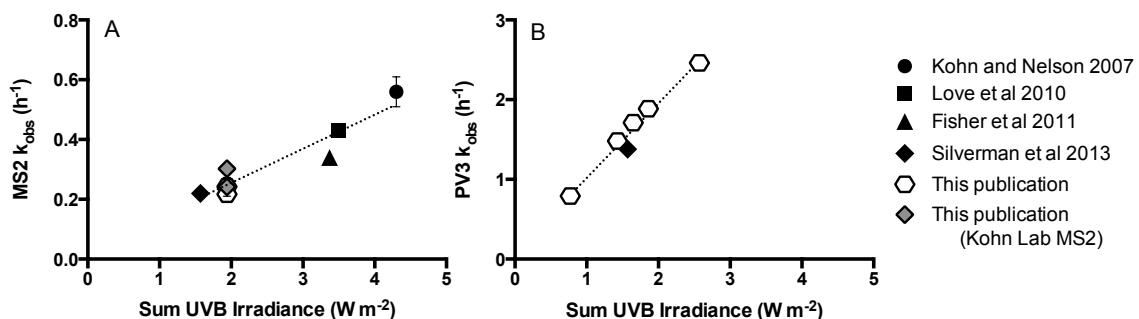


Figure S8. Relationship between total UVB irradiance and k_{obs} for MS2 (A) and PV3 (B) in clear, sensitizer-free solution (PBS); this data was used to calculate β . Dotted lines are the linear regression lines (MS2: slope = $0.12 \pm 0.02 \text{ m}^2 \text{ W}^{-1}\text{h}^{-1}$; $R^2 = 0.89$. PV3: slope = $0.94 \pm 0.08 \text{ m}^2 \text{ W}^{-1}\text{h}^{-1}$; $R^2 = 0.97$). Error bars report the standard error; some error bars are smaller than the symbols. Note the different y-axis scales in panels A and B. While all experiments used to construct the MS2 curve were conducted using the same solar simulator, they were conducted by four different investigators,^{9,11-13} over a 6-year period and included MS2 stocks that were propagated and purified in both our laboratory and the laboratory of Professor Tamar Kohn at École Polytechnique Fédérale de Lausanne.

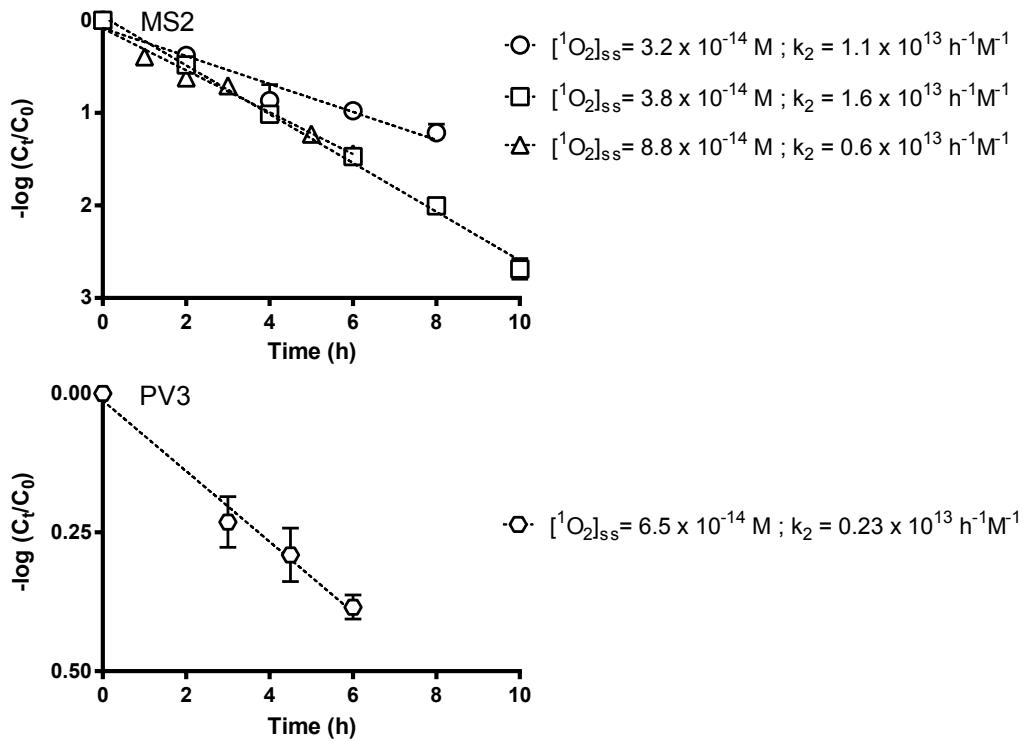


Figure S9. Virus inactivation data from experiments used to calculate k_2 for Discovery Bay open-water wetland effluent. Experiments were conducted at different times, with wetland water collected on different days (in March, September, and October). The UVB-blocking filter was used for all experiments. Error bars report standard error.

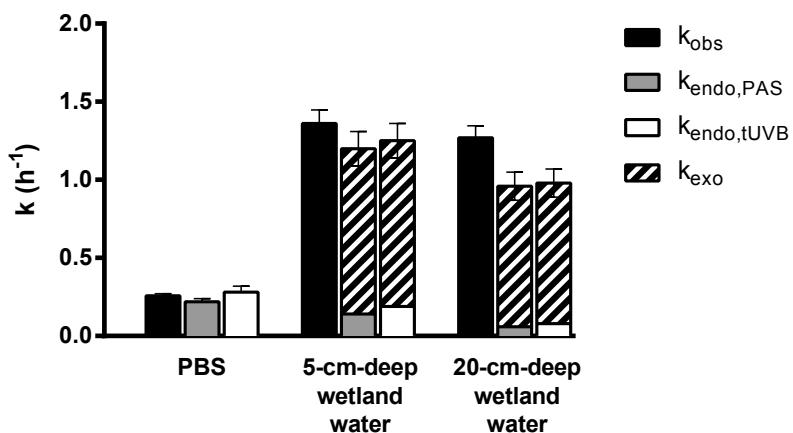


Figure S10. Comparison between MS2 $k_{\text{endo}}^{\text{L}}$ predicted by the PAS and tUVB models during laboratory experiments.

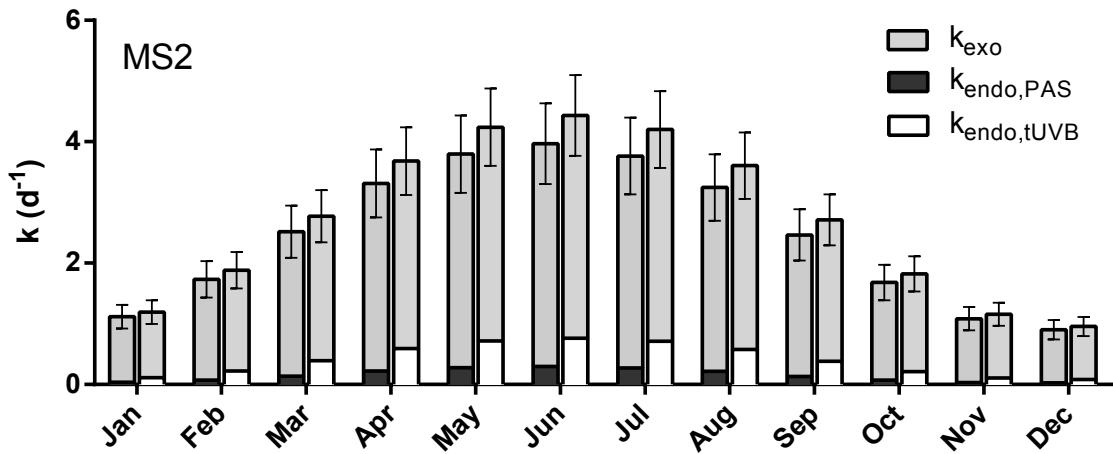


Figure S11. Comparison between MS2 k_{endo}^W predicted by the PAS and tUVB models for inactivation in the Discovery Bay wetland open-water cell. Error bars are standard error of the total modeled inactivation rate.

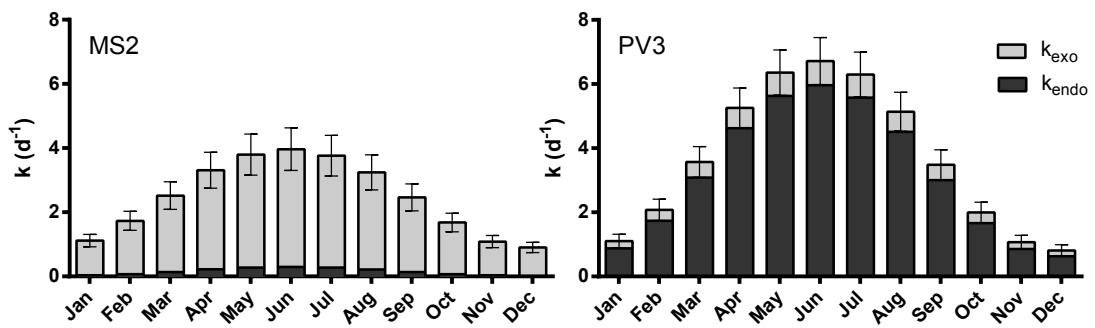


Figure S12. Modeled MS2 and PV3 inactivation rates in the Discovery Bay open-water wetland cell over the course of the year. The PAS modeling approach was used to calculate MS2 k_{endo}^W , and the tUVB approach was used for PV3. Error bars are standard error for the total modeled inactivation rate.

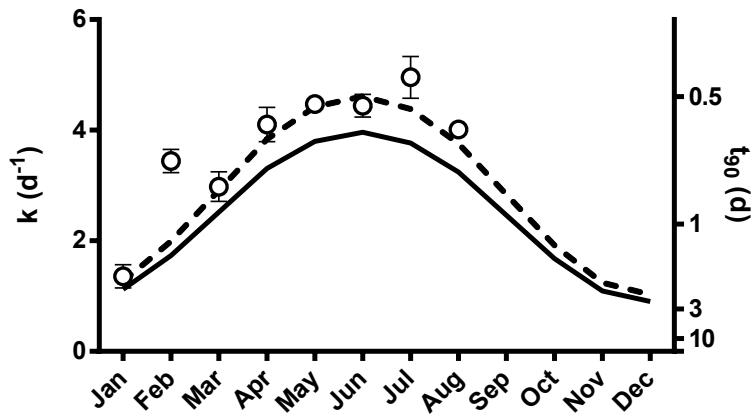


Figure S13. Measured F+ coliphage $k_{\text{obs}}^{\text{W}}$ (white dots) and modeled MS2 $k_{\text{tot}}^{\text{W}}$ (PAS model) in the Discovery Bay wastewater treatment wetland open-water cell. Modeled values were either corrected (black line; same data as Figure 3 of the main manuscript) or not corrected (dashed line) for the effect of the biomat; for the uncorrected data, $z = 20$ cm and no correction was made for shading within the biomat. Error bars on $k_{\text{obs}}^{\text{W}}$ are root-mean-square errors.

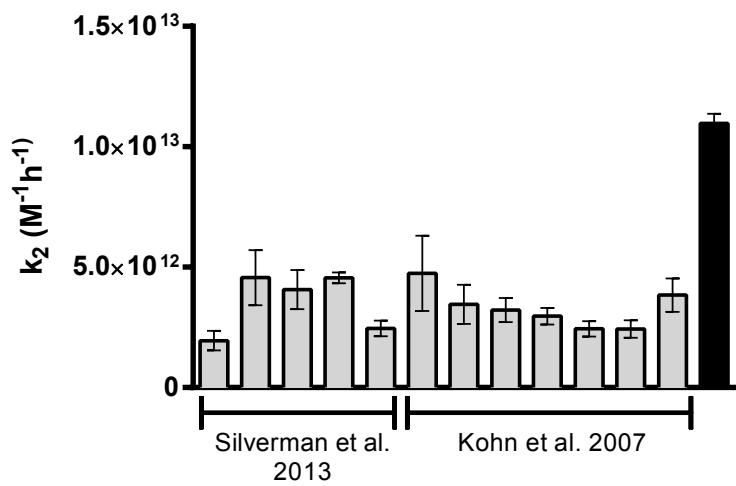


Figure S14. Summary of k_2 values for MS2 and ${}^1\text{O}_2$ determined for a range of surface waters (grey bars).^{12,13} The black bar is k_2 determined in this study for water collected from the Discovery Bay open-water wetland cell.

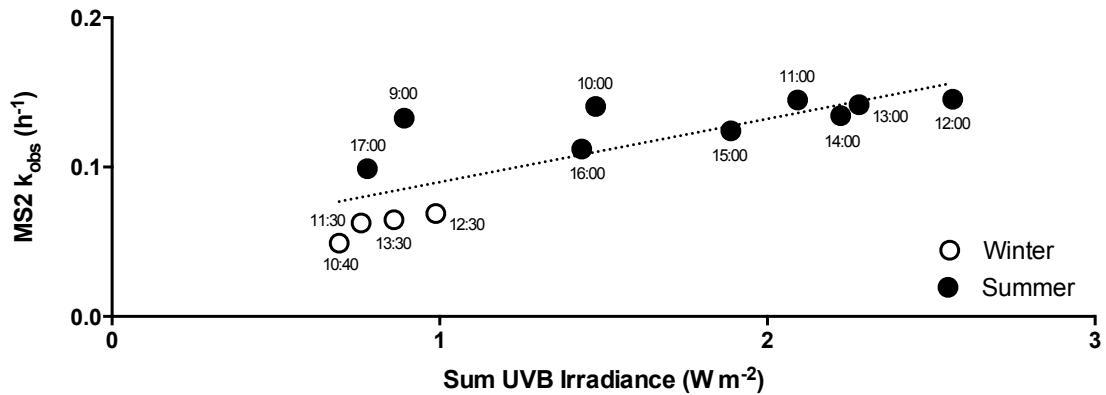


Figure S15. Data from Nguyen et al.⁴ relationship between total UVB irradiance and MS2 k_{obs} in clear, sensitizer-free solution (PBS) for one-hour intervals with exposure to natural sunlight in Berkeley, California, USA. Dotted line is the linear regression line (slope = $0.04 \pm 0.01 \text{ m}^2 \text{ W}^{-1}\text{h}^{-1}$; $R^2 = 0.64$). Labels next to data points designate the time of day the sample and irradiance spectrum were collected.

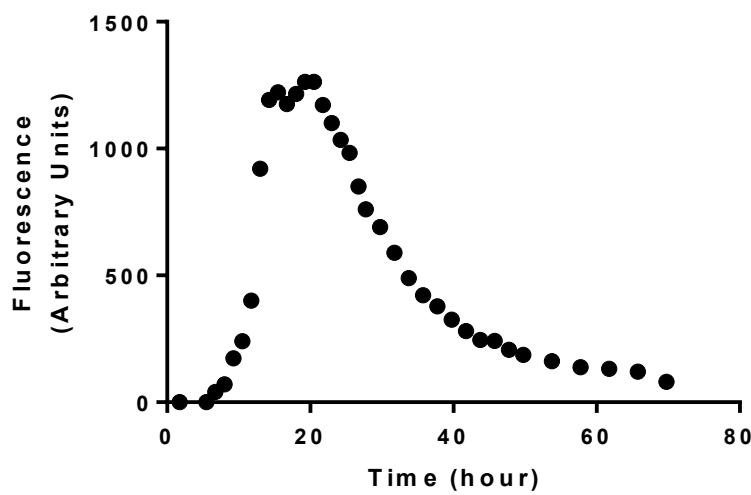


Figure S16. Breakthrough curve of Rhodamine-WT tracer test in the open-water wetland.¹⁴

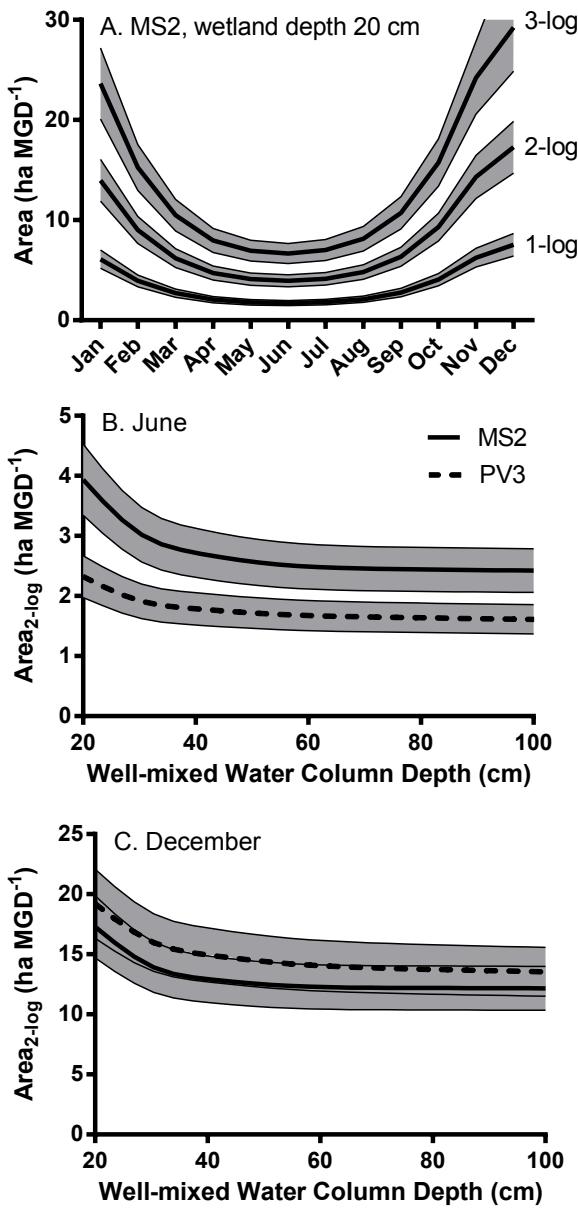


Figure S17. Data is the same as presented in Figure 4 of the main manuscript. (A) Open-water wetland surface areas required to treat 1 million gallons of wastewater per day (MGD) to the specified MS2 treatment objectives (i.e., 1-, 2- or 3-log inactivation), given a well-mixed water column depth of 20 cm. (B,C) Surface areas required per MGD to achieve 2-log inactivation of MS2 and PV3 for a range of well-mixed water column depths in June and December. The sunlight spectra used to model k_{tot}^W were for 40°N latitude. Grey bands illustrate uncertainty (set as 15% of calculated areas).

Tables

Table S1. Spectral sensitivity coefficients, $P(\lambda)$ ($\text{m}^2 \text{ W}^{-1} \text{ h}^{-1}$), for the MS2 photoaction spectrum.¹¹

λ (nm)	$P(\lambda)$ ($\text{m}^2 \text{ W}^{-1} \text{ h}^{-1}$)	λ (nm)	$P(\lambda)$ ($\text{m}^2 \text{ W}^{-1} \text{ h}^{-1}$)	λ (nm)	$P(\lambda)$ ($\text{m}^2 \text{ W}^{-1} \text{ h}^{-1}$)
281	2.88	320	0	359	0
284	1.28	323	0	362	0
287	0.320	326	0	365	0
290	0	329	0	368	0
293	0	332	0	371	2.65×10^{-6}
296	0.0669	335	0	374	0.00096
299	0.183	338	0	377	0.00192
302	0.299	341	0	380	0.00287
305	0.281	344	0	383	0.00191
308	0.165	347	0	386	0.000956
311	0.0491	350	0	389 - 496	0
314	0	353	0		
317	0	359	0		

Table S2. Inputs to SMARTS.^{4,15}

Parameter	Input	Unit
Site pressure	1005.035	millibar
Altitude	0.02	km
Height	0.00	km
Atmosphere*	US standard atmosphere 1976	
Water vapor	Calculated from reference atmosphere and altitude	
Columnar ozone abundance	Default from reference atmosphere	
Gaseous absorption and pollution	Default from reference atmosphere	
Carbon dioxide	370	ppmv
Extraterrestrial spectrum*	Gueymard 2004	
Aerosol model*	Shettle & Fenn Urban	
Atmospheric turbidity	130 Meterological range (km)	
Regional albedo	Water: Water or calm ocean	
Tilted surface and local albedo	Bypass tilt calculations	
Spectral range	280-700	nm
Solar constant	1366.1	W m ⁻²
Circumsolar calculations	Bypass	
Extra scanning/smoothing	Bypass	
Extra illuminance calculations	Bypass	
Extra UV calculations	Bypass	
Solar position and air mass	Latitude: 37.908611 Longitude: -121.600278 Time zone: -8	

* Suggested by SMARTS

Table S3. Measured ($k_{\text{obs}}^{\text{L}}$) and modeled ($k_{\text{tot}}^{\text{L}}$) sunlight inactivation rates for MS2 and PV3 during laboratory experiments. Values are presented as inactivation rates \pm standard error; n = 2 for all, unless otherwise noted. k_{dark} is the dark inactivation rate in wetland water.

	MS2 $k_{\text{obs}}^{\text{L}}$ [h ⁻¹] (R^2)	MS2 $k_{\text{tot,PAS}}^{\text{L}}$ [h ⁻¹]	MS2 $k_{\text{tot,tUVB}}^{\text{L}}$ [h ⁻¹]	PV3 $k_{\text{obs}}^{\text{L}}$ [h ⁻¹] (R^2)	PV3 $k_{\text{tot,tUVB}}^{\text{L}}$ [h ⁻¹]
Sensitizer-free water (PBS)	0.23 \pm 0.01 (0.96)	0.22 \pm 0.02	0.28 \pm 0.05	1.89 \pm 0.04 (0.99)	1.77 \pm 0.15
5 cm deep wetland water	1.36 \pm 0.09 (0.96)	1.20 \pm 0.11	1.25 \pm 0.11	1.42 \pm 0.05 (0.99)	1.40 \pm 0.11
20 cm deep wetland water	1.27 \pm 0.08 (0.96)	0.96 \pm 0.09	0.98 \pm 0.09	0.77 \pm 0.05 (0.96)	0.68 \pm 0.05
Wetland water k_{dark}	0.13 \pm 0.03 (n = 5)	-	-	0.08 \pm 0.06 (n = 4)	-

Table S4. $k_{\text{obs}}^{\text{W}}$ for F+RNA and somatic coliphage in the Discovery Bay treatment wetland open-water cell. MS2 $k_{\text{tot}}^{\text{W}}$ was calculated using the PAS approach for k_{endo} .

	F+RNA coliphage $k_{\text{obs}}^{\text{W}}$ [d ⁻¹] (R^2)	MS2 $k_{\text{tot}}^{\text{W}}$ [d ⁻¹]	% difference between F+ coliphage $k_{\text{obs}}^{\text{W}}$ and MS2 $k_{\text{tot}}^{\text{W}}$	Somatic coliphage $k_{\text{obs}}^{\text{W}}$ [d ⁻¹] (R^2)
January	1.4 (0.45)	1.1	18%	-
February	3.4 (0.82)	1.7	50%	-
March	3.0 (0.59)	2.5	16%	-
April	4.1 (0.69)	3.3	19%	-
May	4.5 (0.98)	3.8	15%	-
June	4.4 (0.88)	4.0	11%	5.2 (0.89)
July	5.0 (0.59)	3.8	24%	3.5 (0.78)
August	4.0 (0.97)	3.2	19%	4.3 (0.89)

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