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Estimating Environmental Conditions Affecting Protozoal Pathogen Removal in Wetland Systems Using a Multi-Scale, Model-Based Approach

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**Estimating Environmental Conditions Affecting Protozoal Pathogen
Removal in Wetland Systems Using a Multi-Scale, Model-Based
Approach**

A Thesis Presented to the Faculty of the Division of Science and Environmental
Policy
California State University of Monterey Bay

In Partial Fulfillment of the Requirements for the Degree Master of Science
in
Coastal and Watershed Science and Policy

by

Miles Eric Daniels

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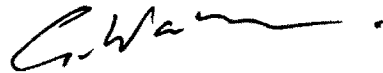
The Undersigned Faculty Committee Approves the

Thesis of Miles Eric Daniels:


Estimating Environmental Conditions Affecting

Protozoal Pathogen Removal in Surface Water Wetland Systems

Using a Multi-Scale, Model-Based Approach



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Abstract

Cryptosporidium parvum, *Giardia lamblia*, and *Toxoplasma gondii* are waterborne protozoal pathogens distributed worldwide and empirical evidence suggests that wetlands reduce the concentrations of these pathogens under certain environmental conditions. The goal of this study was to evaluate how protozoal removal in surface water is affected by the water temperature, turbidity, salinity, and vegetation cover of wetlands in the Monterey Bay region of California. To examine how protozoal removal was affected by these environmental factors, we conducted observational experiments at three primary spatial scales: settling columns, re-circulating wetland mesocosm tanks, and an experimental research wetland (Molera Wetland). Simultaneously, we developed a protozoal transport model for surface water to simulate the settling columns, the mesocosm tanks, and the Molera Wetland. With a high degree of uncertainty expected in the model predictions and field observations, we developed the model within a Bayesian statistical framework. We found protozoal removal increased when water flowed through vegetation, and with higher levels of turbidity, salinity, and temperature. Protozoal removal in surface water was maximized ($\sim 0.1 \text{ hr}^{-1}$) when flowing through emergent vegetation at 2% cover, and with a vegetation contact time of ~ 30 minutes compared to the effects of temperature, salinity, and turbidity. Our studies revealed that an increase in vegetated wetland area, with water moving through vegetation, would likely improve regional water quality through the reduction of fecal protozoal pathogen loads.

Key Words

Pathogens, Protozoa, Wetlands, Bayesian, Vegetation, *Cryptosporidium*, *Giardia*, *Toxoplasma*

Title

Estimating Environmental Conditions Affecting Protozoal Pathogen Removal in Surface Water Wetland Systems Using a Multi-Scale, Model-Based Approach

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1. Introduction

Protozoal fecal pathogens pose significant human health concerns worldwide and are estimated to be a main cause of the four billion cases of diarrhea that occur globally each year (Baldursson and Karanis, 2011; Kotloff et al., 2012). A sub-group of protozoal pathogens, oocysts from *Cryptosporidium parvum* and *Toxoplasma gondii*, and cysts from *Giardia lamblia*, are important to public health as they can cause severe gastrointestinal illness and can be fatal in immunocompromised individuals (O'Connor et al., 2011). During the environmental stage of these parasites (oocyst and cyst stages), these organisms are resistant to most conventional methods of wastewater treatment and, therefore, can bypass measures meant to protect the public from pathogen exposure. (Carpenter et al., 1999; Chauret et al., 1999; Payment et al., 2001; Robertson et al., 1992; Wainwright et al., 2007). These parasites can also remain infectious for months to years in water and can be transported great distances from sources, where fecal contamination may not be as apparent (Fayer et al., 1998; Lindsay et al., 2003; Yilmaz and Hopkins, 1972). Lastly, oocysts and cysts can cause disease at relatively low doses, resulting in disease risk even with minimal exposure, such as accidental ingestion during short contact times with water having low parasite loads (Dubey et al., 1996; DuPont et al., 1995; Okhuysen et al., 1999).

The Clean Water Act of the United States requires that all waterbody impairments be identified and placed on a list referred to as the “303(d) list”. Many of the waterways in the central coast region of California are on the 303(d) list due to elevated levels of fecal indicator bacteria (FIB), a proxy for fecal pathogens (CCRWQCB, 2010a). Regional sources of pathogen pollution are likely diverse, and include feces from wild and domestic animals, as well as sewage from human sources (CCLEAN, 2011; Oates et al., 2012). While direct monitoring of pathogens in water is limited in comparison to FIB monitoring, studies have found protozoal pathogens in fresh and nearshore waterways in the Monterey Bay region of California (CCLEAN, 2011).

Wetlands are often used to improve water quality, and while previous studies have demonstrated the abilities of wetlands to remove protozoal pathogens (Gerba et al., 1999; Hogan et al., 2012; Hogan et al., 2013; Morsy et al., 2007; Nokes et al., 2003; Reinoso et al., 2008; Shapiro et al., 2010a; Thurston et al., 2001), specific mechanism of pathogen removal need clarification. For example, in many studies the advection and dispersion rates of a wetland are not known (Gerba et al., 1999; Reinoso et al., 2008). Without determining the advection and dispersion rates, it is impossible to accurately assess pathogen removal rates by re-sampling a water parcel as it moves through a wetland (Lagrangian sampling). As a result, studies comparing unmatched upstream and downstream concentrations have increased potential for confounding, which can lead to inaccurate conclusions when input concentrations have considerable variability, as demonstrated by Krone-Davis et al. (2013).

Determining protozoal net removal dynamics and how environmental factors affect removal is also crucial for developing effective strategies to reduce protozoal loads, but further research is needed. For example, while protozoal settling and sorption to wetland vegetation and substrate are postulated as removal mechanisms in aquatic systems (Searcy et al., 2005; Shapiro et al., 2010a), little is known about protozoal resuspension or de-sorption, with most studies focusing on FIB rather than protozoa (Dorner et al., 2006; Muirhead et al., 2004). Additionally, few studies estimate protozoal removal rates attributed to particular environmental processes by manipulating environmental factors in wetland field studies, such as vegetation coverage and configuration, and then observing the response. Rather, most experimental studies take place in laboratory settings (Dai and Boll, 2006; Searcy et al., 2005). While laboratory studies might allow for better control of experimental factors, their results might not reflect additional dynamics of field wetland ecosystems.

In the United States and worldwide, wetlands have been destroyed or significantly altered since the beginning of the 1900s. Throughout California and much of Monterey Bay, much of the historical wetland habitat has been converted or altered due to

agricultural and urban uses (CCRWQCB, 2010b; Gennet et al., 2013). This wetland loss and degradation may be exacerbating fecal pollution in the Monterey Bay region as beneficial ecosystem functions are reduced (Shapiro et al., 2010a).

The goal of our research was to determine how water temperature, salinity, turbidity, and vegetation affect the removal of protozoa from surface water flowing through wetlands within Monterey Bay watersheds and to predict removal efficiencies of wetlands in the region. To achieve this goal, we developed a simulation model capable of being used at multiple temporal and spatial scales so that results from smaller scale experiments could help validate model components used to predict the fate and transport of pathogenic protozoa in larger scale wetland settings. We then used laboratory and field data collected at three temporal and spatial scales combined with a Bayesian statistical approach to calibrate the parameters of our model and infer how changing environmental conditions affect protozoal transport in surface waters. Specifically, we derived two results: (1) inferences regarding specific relationships between protozoal removal and environmental characteristics; (2) the ability to predict protozoal removal from surface waters given environmental characteristics.

2. Methods

2.1. Simulation modeling

We used a one-dimensional advection-dispersion-decay model to simulate protozoal transport. The conceptual design of the model simplified a wetland channel into a series of sections with uniform dimensions. Each section was assumed to be completely mixed and was described by model parameters representing environmental processes and characteristics. To simulate transport between sections, we used a partial differential equation commonly used in transport modeling (Clark, 2012):

$$\frac{\partial c}{\partial t} = -U \frac{\partial c}{\partial x} + E \frac{\partial^2 c}{\partial x^2} - kc \quad (1)$$

where for each of the n sections linked along the length, x , of a channel, c (L^{-3}) was the concentration of the constituent being modeled, U ($L T^{-1}$) was the channel advection rate, E ($L^2 T^{-1}$) the turbulent diffusion coefficient, $k(t)$ (T^{-1}) a inactivation/decay rate, with L and T representing generic units for length and time.

We further defined the inactivation/decay rate (k) of Eq. 1 to explicitly account for the effect vegetation, salinity, turbidity, and temperature have on the decay rate of protozoal oocysts and cysts, such that:

$$k = k_0 + k_{veg} \times V + k_{sal} \times S + k_{turb} \times N + k_{temp} \times T \quad (2)$$

where k_0 is the removal rate in a wetland not attributed to the environmental factors examined in this study, k are model parameters expressing the increase in the removal rate due to the presence of specific environmental factors expressed as discrete increases in; vegetation (V) percent cover, salinity (S) in ppt, turbidity (N) in NTU, and temperature (T) in degrees Celsius.

Discrete approximation was used to solve Eq. 1, such that variations in concentration over space and time were represented as discrete elements in our model. A matrix approach was used in the R modeling environment (R Development Core Team, 2013) to implement the discrete approximation to the advection-dispersion-decay equation using methods described in Appendix A.

2.2. Inference

We derived inference about the existence and strength of each potential influence on removal using a Bayesian approach. Specifically, we estimated the posterior probability density function (PDF) of each model parameter given the model and our observations (Beven, 2008) using Metropolis Markov Chain Monte Carlo (MCMC) sampling (Givens and Hoeting, 2012; Metropolis et al., 1953). This approach requires specification of a likelihood function, for which we assumed that variation of observations from model predictions was gamma distributed, since the gamma distribution is non-negative, continuous, and can be skewed. Thus, we assumed:

$$c' \sim \text{Gamma}(c; \beta, \alpha)$$

$$\text{Gamma}(c; \beta, \alpha) = \beta^\alpha \frac{1}{\Gamma(\alpha)} c^{\alpha-1} e^{-\beta c} \quad (3)$$

where c' are observed concentrations, c is a model-predicted concentration (from Equation 1), α is the shape parameter of the gamma distribution, β is the rate parameter of the distribution, and Γ is the gamma function. Each MCMC run comprised three chains of 10 to 20 thousand iterations with random starting points, with convergence determined using the Gelman and Rubin diagnostic in the BOA package for R (Brooks and Gelman, 1998; Gelman and Rubin, 1992; Smith, 2007).

We inferred the importance of environmental processes represented by model parameters using 95% Bayesian credible intervals (CI) based on the posterior PDF; calculated using the highest probability density function in the BOA package (Smith, 2007). If a CI did not encompass a null value, such as zero, we inferred that the

parameter was important in describing protozoal transport. We inferred the degree of uncertainty in our understanding of a process represented a parameter by the CI width, where a wider CI represented a higher degree of uncertainty and thus a low degree of knowledge regarding the importance of the parameter and the process it represented.

Hogan et al. (2013) analyzed many of the same data using classical statistical inference (longitudinal negative binomial regression and generalized estimating equations). Here we used simulation modeling instead of regression modeling so that we could explicitly account for advection and dispersion processes in a predictable manner, and so that we could develop an analytical framework that intrinsically integrated understanding across multiple spatial scales from settling columns to wetlands. We used Bayesian inference instead of classical frequentist inference to allow small-scale results to constrain larger-scale analyses through the use of informative priors generated by the small-scale studies, and to achieve a final set of model posteriors that could be used to generate predictions with known uncertainty.

2.3. *Observational experiments*

A series of observational experiments at three different spatial scales were conducted to generate data for model fitting. We started with small-scale laboratory experiments, examining the depositional dynamics of oocysts in a still water column, then moved up to recirculating mesocosm tanks, and finally to continuous release studies at a research-scale wetland. For each experiment we monitored heat-inactivated *Cryptosporidium parvum* oocysts, *Giardia lamblia* cysts, or surrogate microspheres (Dragon Green (DG) and Glacial Blue (GB)) previously shown to approximate *Toxoplasma gondii* surface properties and behavior in water (Hogan et al., 2013; Shapiro et al., 2009). In the observational experiments we manipulated environmental factors by increasing or decreasing their levels in a blocked experimental design, allowing us to observe protozoal and microsphere transport dynamics in surface water under controlled environmental treatments. A low level treatment reflected the absence of the environmental factor, while a high level treatment reflected the presence of the environmental factor and its effect on

protozoal removal in surface waters. Figure 1 is a diagram representing the progression of experiments used to successively refine knowledge about processes in the form of PDFs, resulting finally in inference directly from the PDFs, and also predictions with known uncertainty.

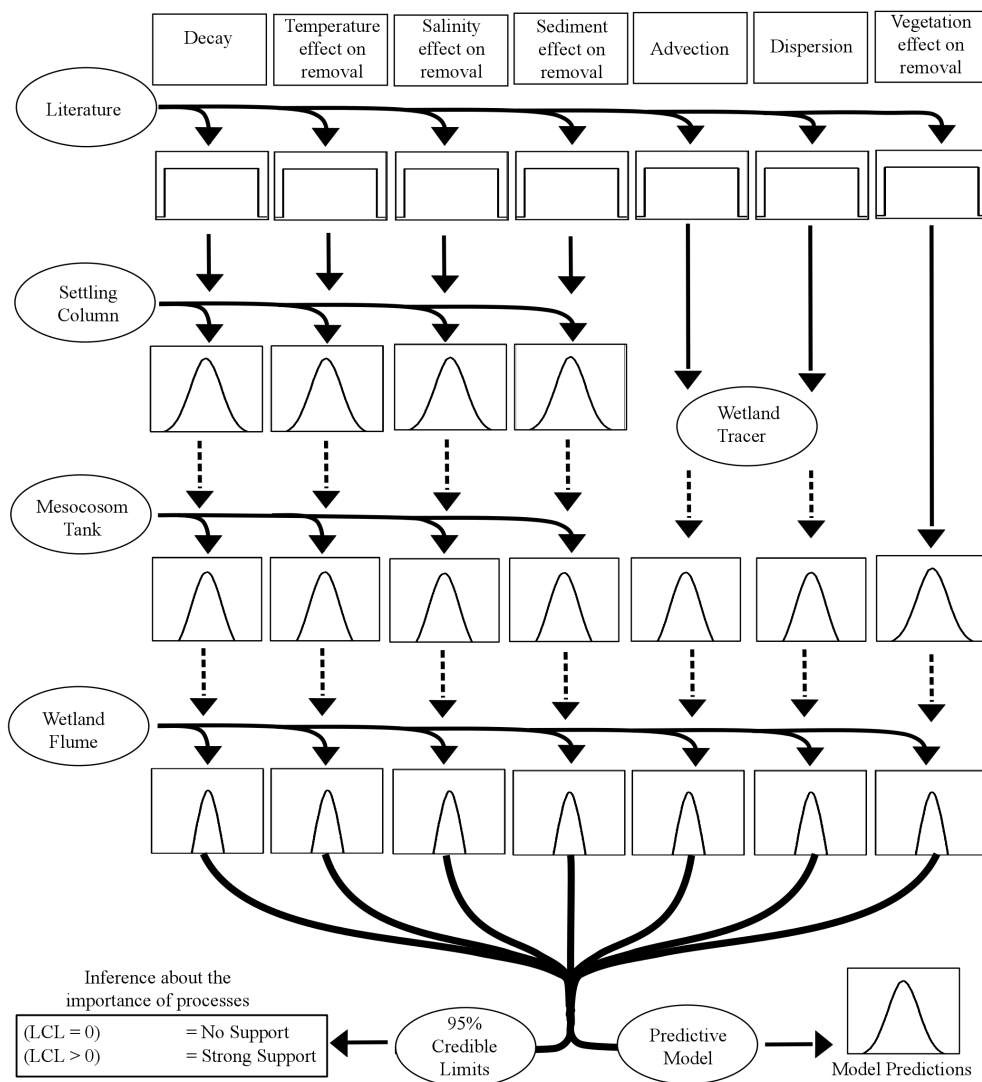


Fig. 1: Conceptual illustration of the progressive identification of model parameter distributions from literature and experiments at multiple scales. Parameter PDFs are indicated by bell-shaped or uniform curves. Rectangles represent the parameters of the model. Ovals represent sources of data, either from the literature or experiment. Solid arrows signify information added to PDFs from experimental and literature sources. Dashed arrows represent information added to PDFs from previous experiments in the form of informative uniform priors. The bottom of the figure depicts the final set of posterior PDFs being utilized to both infer the importance of specific environmental conditions, and to extend predictions beyond our experiments

2.3.1. *Settling column*

Settling column experiments were used to estimate settling behaviors of oocysts, cysts, and surrogate microspheres in still surface waters, similar to others who have investigated protozoal deposition (Medema et al., 1998; Searcy et al., 2005) and as described by Hogan et al. (2013). For each experiment, a one-liter water matrix was spiked with one million each of inactivated *Cryptosporidium parvum* oocysts, *Giardia lamblia* cysts, and both DG and GB microspheres. The water matrix was homogenized in a one-liter graduated cylinder (45 cm length, 6 cm diameter) and water samples were collected over a period of 48 hours at depths of 10 and 30 cm below the water surface using side ports. An 18-gauge syringe was injected into rubber side ports and used to extract approximately 1 mL of sample at each pre-determined sampling time point. Samples were air dried on a slide and stained with a direct fluorescent antibody (DFA) test (Aqua-Glo G/C Direct, Waterborne Inc., New Orleans, LA). Parasites and microspheres were enumerated under FITC and DAPI fluorescent microscopy. Experiments occurred in triplicate pairs. The environmental factors that we altered in experiments were turbidity (low = ~0.01 NTU or high = ~ 80 NTU), salinity (low = 0.01 ppt or high = 30 ppt), and water temperature (low = 4° C or high = 27° C).

To estimate the loss of protozoa and surrogate microspheres in the settling column, our transport model was fit to the time series of concentration measurements from each triplicate experiment (n = 95). The starting concentration was the mean concentration from all experiments at time zero. As a homogenous closed system, advection and dispersion had no effect on protozoal surface water concentration reduction in the settling columns and so we excluded these terms in our model. The simplified model had a single decay parameter, which was decomposed to Eq. 2. The decay parameter represented the water concentration reduction due to settling of protozoa and microspheres out of a water matrix and the effect environmental factors had on increasing or decreasing removal. Non-informative uniform priors were used for each

parameter with a range from 0-1, as previous literature has suggested that settling may be affected by each of the environmental conditions examined.

2.3.2. *Re-circulating tank*

We conducted wetland mesocosm tank experiments as described by Hogan et al. (2013) to estimate how the presence of vegetation influenced the removal dynamics of oocysts, cysts, and surrogate microspheres in an advective-dispersive surface water environment. The tanks were rectangular (3 m long, by 0.5 m wide, by 1.5 m high), constructed from polypropylene material, and water was re-circulated via centrifugal pumps. For each experiment the tanks were filled with water collected from a local waterway to a depth of 30 cm, and coarse-grained sand was applied to the channel bottom at a depth of 1 cm in a uniform fashion. The flow rate of the tanks was set to a desired rate using inline control valves prior to starting an experiment, and was held constant throughout each experiment.

Release studies consisted of injecting inactivated oocysts and cysts (*Cryptosporidium* and *Giardia*) and DG surrogate microspheres for *Toxoplasma* as a spike pulse 10 cm from the most upstream section the tanks at a depth of 10 cm. The spike consisted of ten million particles of each particle type. Water samples were collected 76 cm and 226 cm downstream from the start of the tanks, in the center of the channel, and 15 cm below the water surface over a period of three days at pre-determined time points (0, 5, 10, 15, 30, 60, and 90 min and 2, 4, 6, 24, 48, and 72 hours). We collected 50 mL of water using pipettes for later analysis following the methods described by Hogan et al. (2013) and (Shapiro et al., 2010b) and similar to settling column experiments. Experiments occurred in triplicate pairs and manipulated the effect of salinity (low = 0.01 ppt or high = 30 ppt), and vegetation (absent or present as a buffer at ~0.2% cover) on protozoal and microsphere removal in surface waters, with temperature and turbidity monitored.

To model the protozoal and microsphere concentration data, we took the same modeling approach as when fitting the settling column data. Since the tanks were recirculated, we assumed that advection and dispersion had no effect on surface water concentration reduction once the tanks were well mixed; thus, we assumed all removal was attributed to decay/loss of particles and not dilution or transport. Uniform PDFs were used as priors for each parameter. For temperature, salinity, and turbidity (the environmental factors previously examined in the settling columns) the prior PDFs were informative as their ranges were set based off the settling column's posterior PDFs extended by 25%, while always having a distribution that included zero. The average of samples at both locations was used to fit our transport model to a time series of concentrations ($n = 90$), with the initial state of the model being the average concentration at the one-hour mark after injecting the pulse of particles.

We tested our assumption that the tanks mixed rapidly to a homogenous state using a conservative water tracer. On three occasions, after completing a release study so as not to alter water chemistry prior to the experiment, we injected a known concentration of sodium bromide in solution as a spiked pulse into the most upstream section of a tank. To measure the bromide concentration, an ion-specific probe was placed 90 cm downstream from the injection point at center depth and width. The bromide concentration stabilized within 15 minutes across all experiments, representing a completely mixed tank.

2.3.3. *Flume with wetland*

To examine protozoal and surrogate microspheres removal in an open surface water system with advection and dispersion, we used a 12 m long by 0.1 m wide flume, with an open top and bottom. The flume was placed in a section of a surface water research wetland (Molera Wetland) characterized by a sinuous engineered channel with a mean length, width, and depth of 280 m, 6 m, and 0.3 m respectively, and described previously by Krone-Davis et al. (2013). A continuous supply of water was delivered at a controlled and monitored rate to the inlet of Molera Wetland during experiments via an electric

centrifugal pump pulling water from an adjacent waterway, which drains natural and developed lands. The flume was positioned ~100 m downstream from the inlet of Molera Wetland in the center of the channel in an area free of vegetation.

A conservative water tracer test was conducted in the flume, prior to the release of DG microspheres, to generate prior PDFs for the advection and dispersion terms from Eq. 1 and to validate our assumption of unidirectional flow in the flume. We injected a known concentration of Rhodamine dye as a spiked pulse one meter downstream from the start of the flume. We measured Rhodamine concentration in the flume using a fluorometer placed 10 m downstream from the start of the flume at center depth and width. Results (not shown) of the tracer experiment indicated unidirectional flow as a pulse.

Release studies at the flume consisted of injecting DG microspheres for the duration of the experiments one meter downstream of the start of the flume at center width and depth. Only microspheres were used in these experiments due to the concern of releasing potentially infectious organisms into an open environment. The injection rate of microspheres was 2 mL min^{-1} and was delivered to the flume using low-volume peristaltic pump. The pump drew the microsphere dose from a well-mixed holding reservoir containing microspheres in de-ionized water at a concentration of $\sim 160,000 \text{ N L}^{-1}$. Water samples were collected at two locations from the surface water downstream from the start of the flume (1.5 m and 10.5 m) at three depths (10, 15, and 20 cm) below water level. We collected 50 mL water samples using pipettes while standing on footbridges suspended above the flume and wetland channel to avoid disturbing the water. Microspheres in water samples were enumerated via direct filtration and FITC fluorescent microscopy (Shapiro et al., 2010b). Sampling occurred four times a day and was spaced apart by two hours over three to four days. Experiments occurred in duplicate and isolated the effect of vegetation (absent or present) on microsphere removal.

Vegetation was manipulated by transplanting culms of California bulrush (*Schoenoplectus californicus*) into the channel bed of the flume, resulting in culms emerging from the water column. The number and diameter of the culms were uniform between duplicate experiments. With this design, the wetland had a total of 2% cover and a buffer length of one meter in the center of the flume and every parcel of water was forced to move through these buffers

The complete version of our transport model (Eq. 1) was used to simulate protozoal transport in the surface waters of Molera Wetland. Our model input was the mean concentration measurements collected 1.5 m downstream from the start of the flume that were smoothed with respect time using the LOESS function in R with a span value of 0.4 (R Development Core Team, 2013). We fit our transport model to the time series of mean outlet concentrations ($n = 59$) collected 10.5 m downstream from the start of the flume. Prior PDFs for decay parameters were informative priors with their ranges set according to the re-circulating tank's posterior PDFs extended by 25%, while always having a distribution that included zero (Fig. 1). Prior PDFs for advection and dispersion terms were based off posterior PDFs from the Rhodamine dye tracer studies (Fig. 1).

To estimate the amount of microsphere adhering to the sides of the flume or those that moved upstream from the injection point, we used removable pieces of flume material (coupons), similar to Searcy et al. (2006), and upstream samples. Removable coupons were attached to the sides of the flume to estimate the amount of microspheres stuck to the flume walls. Coupons were composed of the same material as the flume walls and were 5 cm by 40 cm sheets affixed to both sides of the flume at three locations downstream from the injection point (0.5 m, 4.2 m, and 8.5 m). Sections of the coupons at depths of 10, 15, and 20 cm below the water surface were analyzed for microsphere concentration using methods described in Searcy et al. (2006). Water samples were also collected three times daily upstream from the injection point at center width and depth during experiments to estimate if microspheres had moved upstream and bypassed the downstream section of the flume. Results from the coupons and upstream samples

revealed that less than 0.0001% of injected DG microspheres were lost from moving upstream or sticking to sides of the flume.

2.4. Longitudinal predictions

To estimate how efficiently wetlands in the region may reduce fecal protozoal loads in surface waters and how environmental factors contribute to removal, we extrapolated the results from our flume experiments out to 250 m of the Molera Wetland where the flume studies occurred. We made predictions of removal with quantified uncertainty by randomly sampling 1000 sets of parameter values from the posterior PDFs of the flume experiments and used these as parameter inputs. We ran simulations using a model structure with a constant input of parasites to estimate percent removal rates and configured the model to simulate and record output along 5 m increments of the wetland channel. All simulations included a background decay parameter as a reference condition, which for our purposes was the decay not attributed to temperature, salinity, turbidity, or vegetation. Then, to isolate the effect of a particular environmental factor, such as vegetation, we added the vegetation parameter while excluding parameters for temperature, salinity, and turbidity. Using the simulated results of concentration over distance along the wetland channel, we calculated 95% credible intervals of removal along the entire channel length to estimate removal efficiencies under different environmental conditions, i.e. with and without vegetation present in the water column.

3. Results

3.1. Settling column

There was strong evidence that all protozoa and surrogate microspheres settled in the settling columns when temperature, salinity, and turbidity were all at increased levels, as indicated by lower credible limits (LCLs) above zero (Fig. 2E). Increased turbidity had the greatest effect on increasing settling for all particles examined, with the highest mean removal rates (Fig. 2D). *Giardia* cysts had the highest degree of settling, while *Cryptosporidium* oocysts had the lowest degree of settling.

While all protozoa and microspheres settled at greater rates under increased levels of salinity, turbidity, and temperature, not all environmental factors had strong support for enhancing settling on their own, with some LCLs including a value of zero. There was strong evidence that higher turbidity levels, controlling for temperature and salinity, increased the settling of *Giardia* and GB Microspheres with LCLs above zero (Fig. 2D). We also found strong evidence that more saline conditions increased the settling of *Giardia* and GB and DG microspheres (Fig. 2C). However, unlike turbidity and salinity, we did not find strong evidence that higher temperatures increased the settling of any protozoa or surrogate microsphere as the LCLs of the temperature parameter included zero (Fig. 2B).

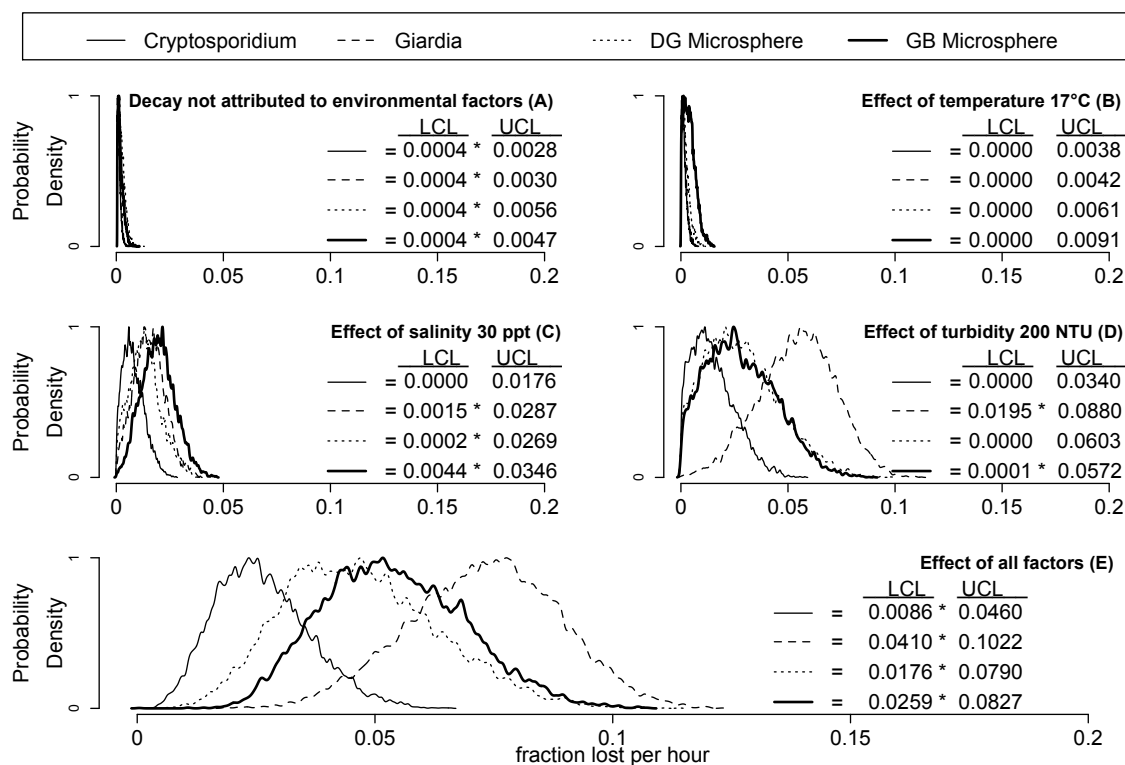


Fig. 2: Posterior PDFs (lines) and Bayesian 95% lower (LCL) and upper credible limits (UCL) for each particle type and each environmental condition examined in the settling columns experiments. Plots A-D depict the fraction lost per hour (x-axis) attributed to each environmental condition listed in the top right corner, and the respective probability densities (y-axis). Plot E represents the total loss per hour under the combination of the specified environmental conditions. Posteriors with a LCL > 0 are denoted with * to represent strong support for an association with settling.

3.2. Re-circulating tank

From our studies monitoring spiked pulses of protozoa and surrogates in re-circulating tanks, we found strong evidence that *Cryptosporidium*, *Giardia*, and the DG microspheres were removed from surface waters when all environmental conditions were present at high levels, as indicated by LCLs above zero (Fig. 3F). The DG microspheres had the highest removal rate, while *Cryptosporidium* and *Giardia* had lower removal rates that were similar to each other.

The two environmental factors with strong evidence for removal were temperature and vegetation. The presence of vegetation in the water column had the greatest effect on the removal of all particle types examined, as indicated by a LCL clearly above zero (Fig. 3E). While higher water temperatures enhanced removal of all particle types with LCLs above zero, the removal rate was less than the effect of vegetation (Fig. 3B). In comparison to the effect of vegetation and temperature, we did not find strong evidence that higher levels of salinity or turbidity alone, without the combined effects of vegetation and temperature, increased the removal rate of protozoa or DG microspheres as the LCLs of these factors included zero (Fig. 3C & 3D).

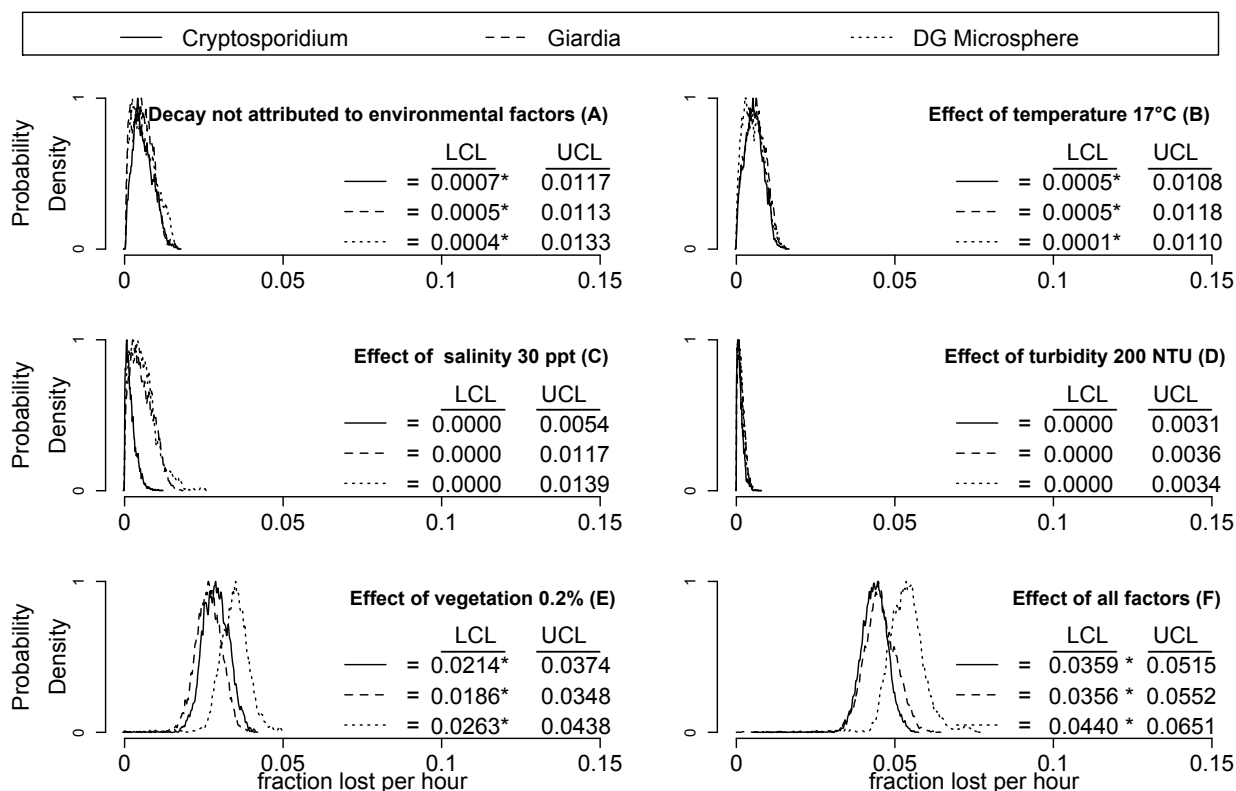


Fig. 3: Posterior PDFs (lines) and Bayesian 95% lower (LCL) and upper credible limits (UCL) for each particle type and each environmental condition examined in the recirculating tank experiments. Plots A-E depict the fraction lost per hour (x-axis) attributed to each environmental listed in the top right corner, and the respective probability densities (y-axis). Plot F represents the total loss per hour under the combination of the specified environmental conditions. Posteriors with a LCL > 0 are denoted with * to represent strong support for an association with removal.

3.3. Flume within wetland

From our continuous release studies in a flume, we found strong evidence that DG microspheres were removed from the water column when all environmental conditions examined were present at high levels, with LCLs greater than zero (Fig. 4F). In the flume experiments, vegetation cover was the only environmental condition whose effect on removal was strongly supported, with a LCL greater than zero (Fig. 4E). The most probable effect of vegetation was to increase the removal rate of DG microspheres by

0.1 hr⁻¹ (95% CI: 0.0007, 0.2595). Temperature, salinity, and turbidity all lacked strong support for increasing the removal rate on their own of DG microspheres (Fig. 4B, 4C, & 4D).

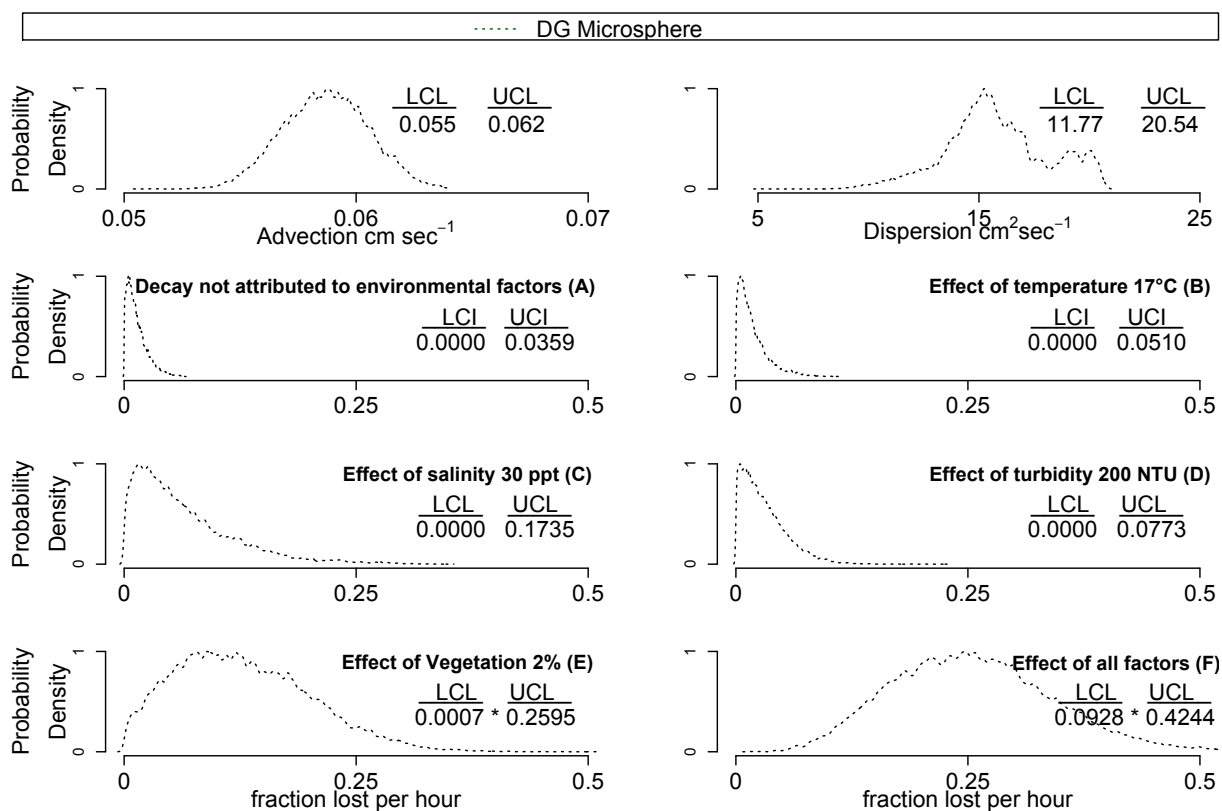


Fig. 4: Posterior PDFs (lines) and Bayesian 95% lower (LCL) and upper credible limits (UCL) for DG microspheres and each environmental condition examined in the flume experiments as well as the advection and dispersion characteristics. Plots A-E depict the fraction lost per hour (x-axis) attributed to each environmental listed in the top right corner, and the respective probability densities (y-axis). Plot F represents the total loss per hour under the combined environmental conditions. Posteriors with a LCL > 0 are denoted with * to represent strong support for an association with removal.

3.4. Longitudinal predictions

Longitudinal predictions revealed that removal of DG microspheres was enhanced by any of the postulated environmental influences (higher temperature, higher salinity, higher turbidity, or higher emergent vegetation cover), and removal was optimized when all of these conditions occurred simultaneously (Fig. 5). Specifically, when the wetland channel had low temperature, vegetation, turbidity, and salinity (Fig. 5A), ~70% of DG microspheres were predicted to be removed on average after traveling ~250 m over a period of 4.7-5.3 days. When a single condition, such as temperature (Fig. 5B), salinity (Fig. 5C), turbidity (Fig. 5D), or vegetation cover (Fig. 5E) was increased, the removal rate increased to ~95% or greater after traveling ~250 m with the same residence time. Of the four conditions (temperature, salinity, turbidity, and vegetation), vegetation was found to enhance removal the most. When all the conditions were increased at the same time (Fig. 5F), optimal removal was achieved and >99% of particles were removed after traveling just 100 m over a period of 1.8-2.1 days.

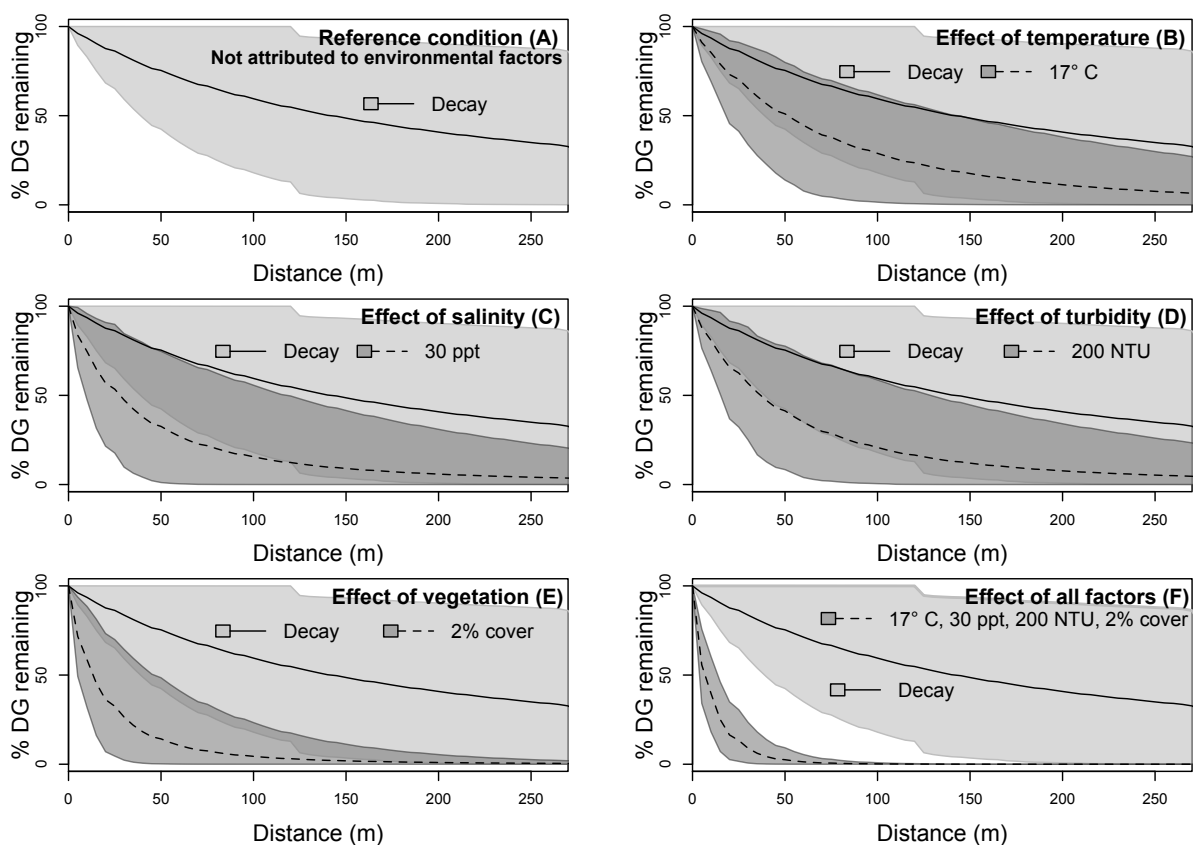


Fig. 5: Plot of 95% Bayesian credible intervals for the predicted percent removal of DG microspheres along the length of a surface water research wetland, where the distance of 250 m corresponds to a residence time of $\sim 4.7 - 5.3$ days. A solid line shows the mean percent removal for background decay, while a dashed line shows the mean percent removal for a given environmental condition.

4. Discussion

Protozoal removal rates in surface waters in our wetland studies increased by 0.1 hr^{-1} when protozoa were pushed through a water column with 2% emergent vegetation cover, compared to a water column with no vegetation cover. With an advection rate of $\sim 0.06 \text{ cm sec}^{-1}$ and vegetation buffer length of 1 m, this corresponded to a water-vegetation contact time of ~ 30 minutes. Therefore, planting emergent wetland vegetation across a wetland channel to force water through the emergent vegetation and enhance water-vegetation contact time is an important environmental factor to consider when optimizing wetland design to remove protozoal pathogens. Vegetation also enhanced protozoal removal the most in comparison with the other environmental factors examined in this study (water temperature, salinity, or turbidity). Finding that vegetation is an important environmental factor for protozoal removal aligns with previous research that found protozoal removal increased in vegetated tidal wetlands in comparison to non-vegetated tidal mudflats (Shapiro et al., 2010a).

With the similar removal rates across particle types observed in this study, we suggest that the surrogate microspheres used in our studies approximate the surface water transport properties of not only *Toxoplasma*, but *Cryptosporidium* and *Giardia* as well. Previous studies have suggested that surrogates are unable to mimic the complex interactions of actual parasites in an environmental matrix (Dumètre et al., 2012). Shapiro et al. (2009), however, reported that both DG and GB microspheres mimic surface properties of *Toxoplasma* and possibly other protozoal oocysts and cysts of similar sizes, densities, and electrophoretic motilities (i.e. \sim surface charge), important factors governing transport of fine-scale particles (Dumètre et al., 2012). Results from this study and Shapiro et al. (2009) support the use of surrogate microspheres to study parasite dynamics in natural environments without the associated risk of releasing infectious organisms.

The effect of increased turbidity on pathogen removal in surface waters varied between experiments, indicating that the effect of turbidity may depend on other environmental conditions, such as turbulence. In the low turbulent settling columns the turbidity parameter had the highest upper credible limit for all particle types, suggesting increased turbidity levels enhances settling. This finding aligns with other research that postulate protozoa settle at faster rates in more turbid conditions (Medema et al., 1998; Searcy et al., 2005). Increased settling under more turbid conditions also aligns with Stoke's law and flocculation theory, which suggests particles have more chances to interact in higher particle concentration settings and form aggregates, which increases settling rates (Gregory, 2004). In the re-circulating tanks studies, however, where water velocities were between 0.1 and 1 cm sec⁻¹, a turbidity effect was less apparent and possibly due to higher turbulence (Reynolds number ~ 9,000) impairing flocculation or settling. This turbidity-turbulence interaction was further supported by the flume studies where the water velocity was ~0.06 cm sec⁻¹, conditions were less turbulent (Reynolds number ~ 900) than in the re-circulating tanks, and the CI of the turbidity parameter for DG microspheres (95% CI: 0.0, 0.08) was similar to the CI of the turbidity parameter in the settling columns (95% CI: 0.0, 0.06).

The increase in protozoal removal in surface waters under more turbid environmental conditions has important consequences for wetlands hydraulically connected to or drawing water from streams and rivers in Monterey Bay watersheds. Typically, turbidity levels and FIB concentrations in waterways are higher during precipitation events in California as sediments and other material from land are transported via overland flow into waterways (Ackerman and Weisberg, 2003; Ahearn et al., 2004; CCRWQCB, 2010b; Walters et al., 2011). Although FIB and protozoal pathogen levels do not always correlate, prior research has demonstrated higher concentrations of *Cryptosporidium* and *Giardia* from water samples collected during storm events, when compared to non-storm events in the Monterey Bay region (CCLEAN, 2011). Therefore, it is reasonable to assume that rivers and streams, following heavy precipitation events, may transport a large fraction of protozoal pathogen

loads. At the same time, if higher levels of turbidity increase the removal rate of pathogens in wetlands, as suggested by our results, then wetlands receiving these waters may have increased protozoal removal rates during these higher loading events, i.e. during high turbidity storm conditions.

In addition to the effects of turbidity and vegetation, we found that increased salinity of surface waters may enhance pathogen removal capacity of coastal wetlands. Previous work has suggested (Shapiro et al., 2009) and confirmed that flocculation of fecal pathogens increases in saline water conditions as repulsion forces between particles are reduced (Shapiro et al., 2013; Shapiro et al., 2012). Our settling column data confirmed this effect for all particles except *Cryptosporidium*. We also simulated the effect of increased salinity along a 250 m length of wetland and predicted a mean removal increase of 25%, compared to the same system without saline conditions. If future studies confirm this effect of salinity on protozoal removal, it will have important implications tied to freshwater coastal wetlands that are tidally influenced by increasing salinity-induced settling of pathogens in these systems.

A primary benefit of our study was the use of a multi-scale, model-based approach. The multi-scale, model-based approach allowed us to progressively build-up information on the effect of various wetland characteristics through a series of experiments at multiple scales. For example, using the posterior PDFs of the advection and dispersion parameters from the flume tracer experiment as prior PDFs for the flume microsphere release studies allowed for a better-specified model and ultimately more accurate estimates of the effects of vegetation and other characteristics on wetland performance. Had we not utilized this information, the alignment between inlet and outlet concentrations in the flume may have been misspecified and led to a model that fit the data well, but was not likely according to realistic advection and dispersion rates in the system. For example, Antweiler and Murphy (2014) found that even when conducting Lagrangian sampling, misspecifying the alignment of upstream and downstream samples by a few hours resulted misspecified estimates of a conservative water tracer by as much

50%. Therefore, it is reasonable to assume that non-Lagrangian sampling can result in misspecified water constituent estimates of greater than 50%.

Comparing our Bayesian statistical analysis of the settling column and mesocosm tank studies to the frequentist statistical analysis conducted by Hogan et al. (2013) with the same data yielded similar conclusions and further bolsters the findings from this study. In the settling columns, both types of statistical analysis found settling of protozoa and microspheres increased when turbidity or salinity was increased, but that settling did not increase under higher water temperatures. In the mesocosm tanks both types of analysis found that the removal of *Giardia* and DG microspheres increased as vegetation levels increased. Although Hogan et al. (2013) was unable to reject the null hypothesis that vegetation increased the removal of *Cryptosporidium* with a p-value ~ 0.1 , the Bayesian analysis found strong support that vegetation enhanced the removal of *Cryptosporidium*. Concentration data from the flume within wetland studies were not analyzed by Hogan et al. (2013), so no comparisons were made between Bayesian and frequentist approaches to data analysis for those experiments.

While we did find strong evidence that wetlands remove protozoal pathogens from the waters moving through them, there were limitations to this study. We used heat-deactivated pathogens and surrogate microspheres in our experiments. These particles may react differently to environmental conditions compared to living pathogens (Dumètre et al., 2012; Sinclair et al., 2012). However, because releasing live, potentially infectious, organisms in field studies carries risk, we see this as a justified limitation. DG microspheres have also been demonstrated to have higher removal rates in environmental water compared to *Toxoplasma* (Shapiro et al., 2012) and therefore our estimated removal rates should be interpreted with this information in mind. Our simulation model was also one-dimensional and, therefore, we assumed the water column was not stratified by environmental factors, such as water temperature, salinity, and turbidity. To minimize the effect of this assumption in the flume experiments, we monitored water temperature, salinity, and turbidity in the center depth of the water column and collected water

samples at three-equally spaced depths in the water column. Additionally, our results do not address the long-term storage potential of wetlands, as we were also unable to determine if particles removed in the flume studies were only lost to the system temporarily and resuspended or desorbed from vegetation or other matter at a later time.

5. Conclusion

We found strong evidence that wetlands remove protozoal pathogens from the waters moving through them. We estimate that when water carrying pathogens spent 1/9th of the travel time moving through emergent vegetation at 2% cover, and when turbidity (200 NTU), salinity (30 ppt), and water temperature (17° C) were high, that >99% of protozoal pathogens would be removed 100 m downstream from a wetland channel, corresponding to a residence time of 1.8-2.1 days and water depth of 30 cm. Vegetation had the clearest effect on removal of *Cryptosporidium*, *Giardia*, and DG surrogate microspheres for *Toxoplasma*. Turbidity, salinity, and temperature were also found to enhance removal, but to a lesser extent compared to vegetation. With our reported removal rates, we conclude that wetlands in Monterey Bay watersheds represent a viable approach to reducing protozoal concentrations in downstream waters, but that further research into protozoal removal is needed. For example, studies examining the long-term storage potential of wetlands are necessary to validate their use as a water quality improvement practice, such as determining resuspension and desorption rates. Additionally, our study focused on a select set from the many environmental factors that might affect protozoal removal and transport. Additional factors that might be important to protozoal removal include biochemical processes that alter the surface properties of oocysts and cysts, disinfection from ultraviolet and solar radiation, as well as predation from filter feeding and grazing organisms such as zooplankton, protozoa, and snails.

Appendix A

5.1. Discrete Approximation of the Advection Dispersion Equation

The R environment (R Development Core Team, 2013) was used to implement a discrete approximation to the advection and dispersion equation (Equations 1 & 2 in the main text).

The discrete approximation used a forward-time forward-space approach (Chapra 1996):

$$c_i^{j+1} = c_i^j + \Delta t \left(-U \frac{c_i^j - c_{i-1}^j}{\Delta x} + E \frac{c_{i+1}^j - 2c_i^j + c_{i-1}^j}{\Delta x^2} - K c_i^j \right)$$

where c_i^j denoted the concentration at location j and iteration i , Δt and Δx denoted the simulation time step and space step respectively, and U , E , and K denoted advection, dispersion, and decay rates as defined in Equation 1. A matrix approach was used to implement the simulation. The concentrations along the length (l) of the channel were represented at iteration i as a vector, C_i :

$$C_i = \begin{bmatrix} c_i^1 \\ c_i^2 \\ \vdots \\ c_i^n \end{bmatrix} \quad (\text{A.1})$$

Advection, dispersion, and decay processes were represented by a matrix, A , that multiplied the current concentration vector at each iteration:

$$C_{i+1} = A C_i \quad (\text{A.2})$$

$$A = \begin{bmatrix} b_2 & b_1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ b_1 & b & b_3 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & b_1 & b_2 & b_3 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \ddots & \ddots & \ddots & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \ddots & \ddots & \ddots & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \ddots & \ddots & \ddots & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & \ddots & \ddots & \ddots & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \ddots & \ddots & b_3 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & b_1 & b_2 \end{bmatrix} \quad (\text{A.3})$$

where matrix elements were provided by a 3-element ‘kernel’ vector, B , that was repeated along and adjacent to the diagonal of the elements of the matrix:

$$B = \begin{bmatrix} b_1 \\ b_2 \\ b_3 \end{bmatrix} \quad (\text{A.4})$$

The elements of B are derived by first considering their role in the matrix multiplication:

$$c_{i+1}^j = b_1 c_{i-1}^j + b_2 c_i^j + b_3 c_{i+1}^j \quad (\text{A.5})$$

with zeroes beyond the boundaries of C .

Collecting the terms of the advection dispersion equation and factoring out the concentrations, provides an equation for each element of B :

$$\begin{aligned} c_i^{j+1} &= c_i^j - U \frac{c_i^j - c_{i-1}^j}{\Delta x} \Delta t + E \frac{c_{i+1}^j - 2c_i^j + c_{i-1}^j}{\Delta x^2} \Delta t - kc_i^j \Delta t & (\text{A.6}) \\ c_i^{j+1} &= U \frac{c_{i-1}^j}{\Delta x} \Delta t + E \frac{c_{i-1}^j}{\Delta x^2} \Delta t + c_i^j - U \frac{c_i^j}{\Delta x} \Delta t - E \frac{2c_i^j}{\Delta x^2} \Delta t - kc_i^j \Delta t + E \frac{c_{i+1}^j}{\Delta x^2} \Delta t \\ c_i^{j+1} &= c_{i-1}^j \Delta t \left(\frac{U}{\Delta x} + \frac{E}{\Delta x^2} \right) + c_i^j \left(1 - \Delta t \left(\frac{U}{\Delta x} + \frac{2E}{\Delta x^2} + k \right) \right) + c_{i+1}^j \Delta t \frac{E}{\Delta x^2} \end{aligned}$$

Thus:

$$B = \begin{bmatrix} \Delta t \frac{E}{\Delta x^2} \\ 1 - \Delta t \left(\frac{U}{\Delta x} + \frac{2E}{\Delta x^2} + k \right) \\ \Delta t \left(\frac{U}{\Delta x} + \frac{E}{\Delta x^2} \right) \end{bmatrix} \quad (\text{A.7})$$

The kernel vector B only needed to be computed once, and was then used to construct the matrix A, which then provided an efficient method of applying the advection dispersion model to the concentration vector C iteratively over time.

5.2. Numerical Dispersion

Numerical dispersion occurs whenever the advection differs from the quotient of space step and time step. To eliminate this, we set the time step to be the quotient of space step and advection. If dispersion was simulated at this time step, numerical oscillation or other instabilities would occur. So two time steps were used: one for advection, and one for dispersion and decay. To achieve this, the kernel was further decomposed into separate kernels for advection, and for dispersion and decay.

The decomposed kernels were:

$$B_{Adv} = \begin{bmatrix} 0 & \\ 1 - \Delta t_A \frac{U}{\Delta x} & \frac{U}{\Delta x} \\ \Delta t_A \frac{U}{\Delta x} & \end{bmatrix} \quad (\text{A.8})$$

$$B_{DD} = \begin{bmatrix} \Delta t_{DD} \frac{E}{\Delta x^2} & \\ 1 - \Delta t_{DD} \left(\frac{2E}{\Delta x^2} + k \right) & \\ \Delta t_{DD} \frac{E}{\Delta x^2} & \end{bmatrix} \quad (\text{A.9})$$

The advection time step was set to:

$$\Delta t_A = \frac{\Delta x}{U}$$

and the dispersion time step was set to be the largest value that divided into the advection time step an integral number of times and that satisfied the following condition (Ramaswami et al., 2005):

$$\Delta t_{DD} \leq \frac{\Delta x^2}{3E}$$

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References

- Ackerman D, Weisberg S. Relationship between rainfall and beach bacterial concentrations on Santa Monica Bay beaches. *J Water Health* 2003; 1: 85-89.
- Ahearn DS, Sheibley RW, Dahlgren RA, Keller KE. Temporal dynamics of stream water chemistry in the last free-flowing river draining the western Sierra Nevada, California. *Journal of Hydrology* 2004; 295: 47-63.
- Antweiler RC, Murphy SF. Evaluation of wastewater contaminant transport in surface waters using verified Lagrangian sampling. *Science of The Total Environment* 2014; 470: 551-558.
- Baldursson S, Karanis P. Waterborne transmission of protozoan parasites: Review of worldwide outbreaks – An update 2004–2010. *Water Research* 2011; 45: 6603-6614.
- Beven K. *Environmental modelling: an uncertain future?*: Taylor & Francis, 2008.
- Brooks SP, Gelman A. General methods for monitoring convergence of iterative simulations. *Journal of computational and graphical statistics* 1998; 7: 434-455.
- Carpenter C, Fayer R, Trout J, Beach MJ. Chlorine disinfection of recreational water for *Cryptosporidium parvum*. *Emerging infectious diseases* 1999; 5: 579.
- CCLEAN. *Monitoring and Mitigation to Address Fecal Pathogen Pollution along California Coast*. Central Coast Long-term Environmental Assessment Network, San Luis Obispo, California, 2011.
- CCRWQCB. *Conditionally Waiving Individual Waste Discharge Requirements for Dischargers from Irrigated Lands*. Central Coast Water Quality Control Board, San Luis Obispo, California, 2010a.

- CCRWQCB. Total Maximum Daily Load for Fecal Coliform for the Lower Salinas River Watershed, Monterey County, California. Central Coast Water Quality Control Board, San Luis Obispo, California., 2010b.
- Chauret C, Springthorpe S, Sattar S. Fate of *Cryptosporidium* oocysts, *Giardia* cysts, and microbial indicators during wastewater treatment and anaerobic sludge digestion. *Can J Microbiol* 1999; 45: 257-62.
- Clark MM. Transport modeling for environmental engineers and scientists: John Wiley & Sons, 2012.
- Dai X, Boll J. Settling velocity of *Cryptosporidium parvum* and *Giardia lamblia*. *Water Res* 2006; 40: 1321-5.
- Dorner SM, Anderson WB, Slawson RM, Kouwen N, Huck PM. Hydrologic modeling of pathogen fate and transport. *Environmental science & technology* 2006; 40: 4746-4753.
- Dubey JP, Lunney JK, Shen SK, Kwok OCH, Ashford DA, Thulliez P. Infectivity of low numbers of *Toxoplasma gondii* oocysts to pigs. *The Journal of parasitology* 1996: 438-443.
- Dumètre A, Aubert D, Puech P-H, Hohweyer J, Azas N, Villena I. Interaction forces drive the environmental transmission of pathogenic protozoa. *Applied and environmental microbiology* 2012; 78: 905-912.
- DuPont HL, Chappell CL, Sterling CR, Okhuysen PC, Rose JB, Jakubowski W. The infectivity of *Cryptosporidium parvum* in healthy volunteers. *New England Journal of Medicine* 1995; 332: 855-859.
- Fayer R, Trout JM, Jenkins MC. Infectivity of *Cryptosporidium parvum* oocysts stored in water at environmental temperatures. *The Journal of parasitology* 1998: 1165-1169.

- Gelman A, Rubin DB. Inference from iterative simulation using multiple sequences. *Statistical science* 1992; 457-472.
- Gennet S, Howard J, Langholz J, Andrews K, Reynolds MD, Morrison SA. Farm practices for food safety: an emerging threat to floodplain and riparian ecosystems. *Frontiers in Ecology and the Environment* 2013; 11: 236-242.
- Gerba CP, Thurston JA, Falabi JA, Watt PM, Karpiscak MM. Optimization of artificial wetland design for removal of indicator microorganisms and pathogenic protozoa. *Water Science and Technology* 1999; 40: 363-368.
- Givens GH, Hoeting JA. *Computational statistics*. Vol 708: John Wiley & Sons, 2012.
- Gregory J. *Particles in water: properties and processes*: CRC Press, 2004.
- Hogan JN, Daniels ME, Watson FG, Conrad PA, Oates SC, Miller MA, et al. Longitudinal Poisson Regression To Evaluate the Epidemiology of *Cryptosporidium*, *Giardia*, and Fecal Indicator Bacteria in Coastal California Wetlands. *Applied and environmental microbiology* 2012; 78: 3606-3613.
- Hogan JN, Daniels ME, Watson FG, Oates SC, Miller MA, Conrad PA, et al. Hydrologic and vegetative removal of *Cryptosporidium parvum*, *Giardia lamblia*, and *Toxoplasma gondii* surrogate microspheres in coastal wetlands. *Applied and environmental microbiology* 2013; 79: 1859-1865.
- Kotloff KL, Blackwelder WC, Nasrin D, Nataro JP, Farag TH, van Eijk A, et al. The Global Enteric Multicenter Study (GEMS) of diarrheal disease in infants and young children in developing countries: epidemiologic and clinical methods of the case/control study. *Clinical infectious diseases* 2012; 55: S232-S245.

- Krone-Davis P, Watson F, Los Huertos M, Starner K. Assessing pesticide reduction in constructed wetlands using a tanks-in-series model within a Bayesian framework. *Ecological Engineering* 2013; 57: 342-352.
- Lindsay D, Collins MV, Mitchell SM, Cole RA, Flick GJ, Wetch CN, et al. Sporulation and survival of *Toxoplasma gondii* oocysts in seawater. *Journal of Eukaryotic Microbiology* 2003; 50: 687-688.
- Medema GJ, Schets FM, Teunis PFM, Havelaar AH. Sedimentation of Free and Attached *Cryptosporidium* Oocysts and *Giardia* Cysts in Water. *Applied and Environmental Microbiology* 1998; 64: 4460-4466.
- Metropolis N, Rosenbluth AW, Rosenbluth MN, Teller AH, Teller E. Equation of state calculations by fast computing machines. *The journal of chemical physics* 1953; 21: 1087.
- Morsy E, Al-Herrawy A, Ali M. Assessment of *Cryptosporidium* Removal from Domestic Wastewater Via Constructed Wetland Systems. *Water, Air, and Soil Pollution* 2007; 179: 207-215.
- Muirhead RW, Davies-Colley RJ, Donnison AM, Nagels JW. Faecal bacteria yields in artificial flood events: quantifying in-stream stores. *Water research* 2004; 38: 1215-1224.
- Nokes R, Gerba C, Karpiscak M. Microbial Water Quality Improvement by Small Scale On-Site Subsurface Wetland Treatment. *Journal of Environmental Science and Health, Part A* 2003; 38: 1849-1855.
- O'Connor RM, Shaffie R, Kang G, Ward HD. Cryptosporidiosis in patients with HIV/AIDS. *Aids* 2011; 25: 549-560.

- Oates SC, Miller MA, Hardin D, Conrad PA, Melli A, Jessup DA, et al. Prevalence, environmental loading, and molecular characterization of *Cryptosporidium* and *Giardia* isolates from domestic and wild animals along the Central California Coast. *Appl Environ Microbiol* 2012; 78: 8762-72.
- Okhuysen PC, Chappell CL, Crabb JH, Sterling CR, DuPont HL. Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults. *Journal of Infectious Diseases* 1999; 180: 1275-1281.
- Payment P, Plante R, Cejka P. Removal of indicator bacteria, human enteric viruses, *Giardia* cysts, and *Cryptosporidium* oocysts at a large wastewater primary treatment facility. *Canadian Journal of Microbiology* 2001; 47: 188-193.
- R Development Core Team. R: A language and environment for statistical computing. . R Foundation for Statistical Computing, Vienna, Austria, 2013.
- Ramaswami A, Milford JB, Small MJ. Integrated environmental modeling: pollutant transport, fate, and risk in the environment: J. Wiley, 2005.
- Reinoso R, Torres LA, Becares E. Efficiency of natural systems for removal of bacteria and pathogenic parasites from wastewater. *Sci Total Environ* 2008; 395: 80-6.
- Robertson LJ, Campbell AT, Smith HV. Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. *Applied and Environmental Microbiology* 1992; 58: 3494-3500.
- Searcy KE, Packman AI, Atwill ER, Harter T. Association of *Cryptosporidium parvum* with suspended particles: impact on oocyst sedimentation. *Appl Environ Microbiol* 2005; 71: 1072-8.

- Searcy KE, Packman AI, Atwill ER, Harter T. Deposition of *Cryptosporidium* oocysts in streambeds. *Appl Environ Microbiol* 2006; 72: 1810-6.
- Shapiro K, Conrad PA, Mazet JA, Wallender WW, Miller WA, Largier JL. Effect of estuarine wetland degradation on transport of *Toxoplasma gondii* surrogates from land to sea. *Appl Environ Microbiol* 2010a; 76: 6821-8.
- Shapiro K, Largier J, Mazet JA, Bernt W, Ell JR, Melli AC, et al. Surface properties of *Toxoplasma gondii* oocysts and surrogate microspheres. *Appl Environ Microbiol* 2009; 75: 1185-91.
- Shapiro K, Mazet JAK, Schriewer A, Wuertz S, Fritz H, Miller WA, et al. Detection of *Toxoplasma gondii* oocysts and surrogate microspheres in water using ultrafiltration and capsule filtration. *Water Research* 2010b; 44: 893-903.
- Shapiro K, Miller WA, Silver MW, Odagiri M, Largier JL, Conrad PA, et al. Research commentary: association of zoonotic pathogens with fresh, estuarine, and marine macroaggregates. *Microbial ecology* 2013; 65: 928-933.
- Shapiro K, Silver MW, Largier JL, Conrad PA, Mazet JAK. Association of *Toxoplasma gondii* oocysts with fresh, estuarine, and marine macroaggregates. *Limnol. Oceanogr* 2012; 57: 449-456.
- Sinclair RG, Rose JB, Hashsham SA, Gerba CP, Haas CN. Criteria for selection of surrogates used to study the fate and control of pathogens in the environment. *Appl Environ Microbiol* 2012; 78: 1969-77.
- Smith BJ. boa: an R package for MCMC output convergence assessment and posterior inference. *Journal of Statistical Software* 2007; 21: 1-37.
- Thurston JA, Foster KE, Karpiscak MM, Gerba CP. Fate of indicator microorganisms, *Giardia* and *Cryptosporidium* in subsurface flow constructed wetlands. *Water Research* 2001; 35: 1547-1551.

- Wainwright KE, Lagunas-Solar M, Miller MA, Barr BC, Gardner IA, Pina C, et al.
Physical inactivation of *Toxoplasma gondii* oocysts in water. Applied and environmental microbiology 2007; 73: 5663-5666.
- Walters SP, Thebo AL, Boehm AB. Impact of urbanization and agriculture on the occurrence of bacterial pathogens and *stx* genes in coastal waterbodies of central California. Water research 2011; 45: 1752-1762.
- Yilmaz SM, Hopkins SH. Effects of different conditions on duration of infectivity of *Toxoplasma gondii* oocysts. The Journal of parasitology 1972; 58: 938.