Hydrologic and vegetative removal of Cryptosporidium parvum, Giardia lamblia, and Toxoplasma gondii surrogate microspheres in coastal wetlands

Running Title: Hydrologic and vegetative removal of protozoa in coastal wetlands.

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ABSTRACT

Constructed wetland systems are used to reduce pollutants and pathogens in wastewater effluent, but comparatively little is known about pathogen transport through natural wetland habitats. Fecal protozoans including Cryptosporidium parvum, Giardia lamblia, and Toxoplasma gondii are waterborne pathogens of humans and animals, which are carried by surface waters from land-based sources into coastal waters. This study evaluated key factors of coastal wetlands for the reduction of protozoal parasites in surface waters using settling column and re-circulating mesocosm tank experiments. Settling column experiments evaluated the effects of salinity, temperature, and water type (“pure” versus “environmental”) on the vertical settling velocities of C. parvum, G. lamblia, and T. gondii surrogates, with salinity and water type found to significantly affect settling of the parasites. The mesocosm tank experiments evaluated the effects of salinity, flow rate, and vegetation parameters on parasite and surrogate counts, with increased salinity and presence of vegetation found to be significant factors for removal of parasites in a unidirectional transport wetland system. Overall, this study highlights the importance of water type, salinity, and vegetation parameters on pathogen transport within wetland systems, with implications for wetland management, restoration efforts, and coastal water quality.

Keywords

Cryptosporidium, Giardia, salinity, Toxoplasma, transport, vegetation, wetlands
INTRODUCTION

Natural and constructed wetlands have the capacity for waterborne pollutant and pathogen reduction and removal. Construction of wetlands near dairies, farms, and wastewater treatment facilities may help reduce pollutant loads entering adjacent surface waters (10, 12, 26). Few published studies have focused on the efficacy of naturally occurring coastal wetlands to filter polluted water (26, 30). As a conduit for microbial transport from land to sea, natural wetlands may have an ameliorating effect on concentrations of potential pathogens including Cryptosporidium parvum, Giardia lamblia (syn. G. intestinalis and G. duodenalis), and Toxoplasma gondii, reducing pathogen contamination in downstream waters that may be used for recreation, drinking water, food harvest, and wildlife habitat (18).

Waterborne protozoa including C. parvum, G. lamblia, and T. gondii are important human and animal pathogens in coastal ecosystems (6, 19). The environmentally resistant oocyst and cyst stages of C. parvum and G. lamblia, respectively, facilitate parasite survival, rendering them potentially infectious to susceptible hosts as they are transported through waterways (35). The presence of these protozoal parasites in surface waters that are used for human recreation, drinking, or food harvest is of significant public health interest, because infectious doses are relatively low. Both C. parvum and G. lamblia are transmitted through fecal-oral routes and can cause severe gastrointestinal illness (7, 9, 27). Toxoplasma gondii oocysts are shed in the feces of cats, the only known definitive host of the parasite; humans and other warm-blooded animals are intermediate hosts (4). Toxoplasma gondii can adversely affect the fetus if a pregnant woman acquires the parasite, and immunocompromised individuals may suffer severe, even fatal, infections (23). Toxoplasma gondii can also infect and cause disease in marine mammals, including the threatened California sea otter (Enhydra lutris nereis) that ranges along the...
California coast. Otters may become infected following exposure to oocysts that are transported from land-to-sea through contaminated freshwater runoff (14, 19).

Previous studies have examined parasite transport through surface waters to identify hydrologic factors that result in protozoal reduction. For example, *C. parvum* counts were reduced more quickly in water flumes containing sediment when compared to those without (29), which is consistent with results from studies examining the vertical settling velocity of *C. parvum* and *G. lamblia* (2, 28). Wetland vegetation has also been demonstrated to reduce water pollution and prevent pathogen run-off near dairies through application of various vegetation types and configurations in constructed wetlands (1, 3, 11). For example, in a study of natural estuarine wetlands, vegetation-lined waterways significantly reduced transport of *T. gondii* surrogates, when compared with non-vegetated mud flats (30).

Our aim was to establish a more robust model of estuarine wetland ecology and its role in removing waterborne pathogens of public health significance. Our objectives were to: 1) compare the effects of physical factors (salinity, temperature, and water type) on the vertical settling velocities of *C. parvum*, *G. lamblia*, and *T. gondii* surrogates; and 2) compare the effects of hydrologic factors (salinity and flow rate) and vegetation (presence, species, and configuration) on removal of these parasites from environmental surface water using wetland mesocosm tanks.

**MATERIALS AND METHODS**

**Source of protozoa and surrogates.** Heat inactivated *C. parvum* oocysts and *G. lamblia* cysts were obtained from the Wisconsin State Laboratory of Hygiene (Madison, WI) and Waterborne Inc. (New Orleans, LA), respectively. Oocysts and cysts were obtained no more than
one week prior to use for both settling column and mesocosm tank studies. Dragon Green (DG) microspheres (Product No. FC07F/5493, Bangs Laboratories, Inc., Fishers, IN) and Glacial Blue (GB) microspheres (Product No. PC06N/8319, Bangs Laboratories, Inc., Fishers IN) have been previously evaluated as surrogate particles for *T. gondii* oocysts based on their surface properties (31).

**Settling column experiments.** To investigate the effects of hydrologic factors on settling velocities, experiments were conducted in vertical settling columns (Figure 1). Each settling column held 1 L of water with sampling ports at 10cm (T) and 30cm (B) from the top of the water column. Protozoan and microsphere counts at the two ports over time were used to assess differences in settling velocities for the various water conditions that were evaluated. Eight treatment conditions in a complete blocked design were evaluated in triplicate to measure the effects of water type, salinity level, and water temperature on settling velocity of parasites and microspheres. Two water types were evaluated: reverse osmosis purified distilled water from a Milli-Q® water system (“pure”) and environmental water collected from Tembladero Slough near Castroville, CA (“environmental”). Two salinity levels were evaluated to parameterize the variability in salinity levels in coastal wetlands from freshwater to marine influence: “low salinity” at baseline saline levels (0 ppt in Milli-Q® water and 0.1 ppt in environmental water) and “high salinity” at 30 ppt, which was achieved through the addition of salt (Coralife Scientific Grade Marine Salt®, Franklin, WI). Two water temperatures, 4°C and 27°C, were also evaluated for each water type and salinity level to assess the effect of water temperature on protozoan and microsphere settling properties.

Immediately prior to each experiment, 1x10⁶ each of *C. parvum* oocysts, *G. lamblia* cysts, and *T. gondii* oocyst surrogates (DG and GB) were mixed together into a 1 L container of
water and homogenized by shaking. Once mixed, water was poured into the settling column and
subsamples were taken at 0, 5, 15, 30, 60, and 90 min, and 2, 4, 8, 24, and 48 hrs. At each sample
point, 1 mL of water was removed from the column using an 18 gauge needle and 100 µL was
expressed onto a microscope slide and air-dried. Slides were stained with direct fluorescent
antibody (DFA) for oocyst and cyst identification using Aqua-Glo™ G/C Direct (Waterborne,
Inc., New Orleans, LA, USA) and parasites were enumerated under epifluorescent illumination
using FITC and DAPI emission filter sets. Particles with apple-green fluorescence in an oval or
spherical shape (3-7 µm in diameter) with bright, highlighted edges under FITC and light blue
internal staining with a green rim or up to four distinct, sky-blue nuclei under DAPI were
classified as *C. parvum* oocysts. Particles with apple-green fluorescence in a round to oval shape
(6-15 µm in diameter) with bright, highlighted edges under FITC and light blue internal staining
with a green rim or up to four distinct, sky-blue nuclei under DAPI were classified as *G. lamblia*
cysts. Surrogates were enumerated using epifluorescent microscopy as previously described (32).

Settling velocities were calculated by linear regression using the equation derived by Dai
and Boll (2006):

\[
\ln \left( \frac{N_0}{N_t} \right) = \frac{v_s}{H} t
\]

where \( N_0 \) is the number of parasites or microspheres enumerated from a single port at time 0, \( N_t \)
is the number of parasites or microspheres enumerated at time \( t \), \( H \) is the height (mm) from the
top of the water column to the sampling port, and \( v_s \) is the settling velocity expressed as mm/hr.

These settling velocities were converted to µm/sec. Any settling velocities estimated using
regression to be below zero were truncated to 0 µm/sec, and were classified as NS, “no settling”.

Regression on Ordered Statistics (ROS) analysis was used to determine mean settling velocity
and the 95% Confidence Interval for each experimental condition by adjusting for the truncated
values (34). Given the non-normal distribution of residual errors observed around the predicted mean with a linear regression model, the non-parametric Kruskall-Wallis test was used to evaluate if there were differences between any of the treatment groups or combinations.

**Wetland mesocosm release experiments.** To investigate the effect of wetland factors (salinity, flow rate, and vegetation) on reduction of particle numbers, experiments were completed in re-circulating wetland mesocosm tanks (Figure 2A). Three tanks were used in this study, and each was a closed system measuring 3 m by 0.5 m. A depth of one cm of commercially available Monterey Beach silica sand was used on the bottom of the tank as substrate. Water for the tanks was obtained from Tembladero Slough in Castroville, CA. Each tank was filled with 450 L of water to achieve a depth of 30 cm. Ten treatment conditions were compared: non-vegetated tanks at high and low salinity, California bulrush (*Schoenoplectus californicus*) at low salinity in two configurations, and slough sedge (*Carex obnupta*) at low salinity. Each of these conditions was tested at fast and slow water flow rates. The inflow velocity of water was manipulated to compare flow rates of 0.1 cm/sec (slow) and 1 cm/sec (fast), which approximate water flow rates observed in regional natural and reconstructed coastal wetlands (30).

In non-vegetated tanks, salinity was increased from baseline (0.1 ppt; low) to 30 ppt (high) through addition of Coralife Scientific Grade Marine Salt® to the tank. For experimental conditions requiring vegetation, wild California bulrush and slough sedge were transplanted from Molera Constructed Wetland near Castroville, CA and placed in tanks at 150 cm from the water inflow and between the two sampling locations. Both species of California bulrush and slough sedge were placed such that water would flow through stalks emerging from a 15cm wide piece of rhizome. This was stabilized by gravel at the base of the rhizome and rootstock as well as
through securing the top of the stalks with twine. Furthermore, vegetation was placed in either of two configurations across the width of the tank: buffer or channel. The buffer configuration consisted of stands of bulrush being placed across the width of the tanks (Figure 2B). The root and substrate mass reached a height of 15 cm, and plant stalks continued past the top of the water. The channel configuration was similar to the buffer configuration, but an opening 25 cm wide was left between two stands of bulrush (Figure 2C). For slough sedge, only the buffer configuration was used. Three tank replicates were evaluated for each combination of salinity, flow rate, and vegetation.

At the beginning of each experiment, aliquots of $4.5 \times 10^6$ each of *C. parvum*, *G. lamblia*, and DG *T. gondii* surrogates were homogenized into a 5 mL volume of phosphate buffered saline (PBS). Prior to particle injection, baseline water samples were taken to determine prevalence of naturally occurring *C. parvum* or *G. lamblia*. Particles were then injected as a bolus at a depth of 10 cm and a distance of 10 cm from the inflow. Fifty mL samples were collected downstream at a 15 cm depth at two locations: 76 cm and 226 cm from the inflow, at post-injection times of 1, 5, 10, 15, 30, 60, 90 min, and 2, 4, 6, 24, 48, and 72 hr. Water quality data, including pH, temperature, salinity, conductivity, turbidity, and dissolved oxygen, were also collected for each tank once at the start of the experiment.

After collection, samples were centrifuged at 1000 g for 15 min and the upper 45 mL of supernatant was discarded. The remaining pellet was stored at 4º C and processed within 48 hr. *Cryptosporidium parvum* and *G. lamblia* were concentrated using immunomagnetic separation (IMS) with a half dose of magnetic beads (20) (Dynabeads® Invitrogen Life Sciences, Carlsbad, CA, USA) and visualized using DFA (Easy Stain™, BTF Bio, Pittsburgh, PA, USA) as described above. Results were expressed as the (oo)cyst count per 50 mL of water and were not
adjusted for assay percent recovery. Supernatant from the IMS process was directly filtered through a 5µm pore size membrane filter and *T. gondii* surrogates trapped on the filter surface were enumerated under epifluorescence microscopy (32).

To estimate the time for water to fully circulate within the tank and allow complete dispersion, concurrent tracer experiments were conducted for each treatment condition. Using a single bolus injection of sodium bromide (NaBr), the salt reached equilibrium in each tank after 15 min, so samples taken at 15 min or earlier were excluded from subsequent analyses in order to focus on post-dispersion parasite counts.

Mesocosm tank experimental data were analyzed using longitudinal negative binomial (NB) regression models to determine associations between cyst counts and key factors including sample location, flow rate, salinity, vegetation presence, vegetation species (bulrush versus sedge), configuration of bulrush (buffer versus channel), and time post-particle injection. The models were fitted using generalized estimating equations (GEE) because this approach inherently allows for temporal autocorrelation, which we expected to be present in our data by virtue of repeated sampling of each tank; a first-order autoregressive correlation structure was specified in the GEE formulation after an initial examination of the covariance structures (15). Negative binomial variance was specified in the models because the descriptive statistics revealed that the mean counts for all three parasites were over-dispersed, with the variances far exceeding the means. A logarithmic link function was employed in order to assure that modeled mean counts were non-negative, as is typical for negative binomial regressions (36). Predictors were included for each variable assessed in the study, including time post-particle injection. The importance of each predictor was inferred from its incident rate ratio (IRR), which in turn was calculated by exponentiation of the estimated parameter value corresponding to that predictor.
The effect of individual covariates was modeled first, and those determined to be significant were then used to build models with multiple covariates. Quasi-likelihood under the Independence model Criterion (QIC) values were used to determine model fit. Statistical analyses were conducted using SAS software, Version 9.2 (SAS Institute Inc., Cary, NC, USA, PROC GENMOD, with options REPEATED, CORR=AR(1), and DIST=NEGBIN), and p < 0.05 was considered statistically significant.

RESULTS

Settling column experiments. A total of eight conditions (evaluating water type, temperature, and salinity) were examined in settling column experiments, each run in triplicate, and 48 individual settling velocities were determined, which included one for each condition per replicate and sampling port. The overall mean settling velocities (± standard error) were 0.44 (±0.13) µm/sec for C. parvum, 0.84 (±0.16) µm/sec for G. lamblia, and 1.05 (±0.19) µm/sec and 1.14 (±0.19) µm/sec for DG and GB T. gondii surrogates, respectively. No statistical difference in settling velocities was observed between the top and the bottom ports within a single column, so data from both ports was pooled for subsequent analysis.

Settling velocity means and standard errors for C. parvum, G. lamblia, and both T. gondii surrogates stratified by water type and salinity are shown in Table 1. No direct comparisons were made between C. parvum, G. lamblia, and T. gondii surrogates due to potential variation in percent recovery across assays. When the settling velocities were compared across individual predictor variables of water type, salinity, and temperature, the DG surrogates’ settling velocities were significantly different between the pure and environmental water matrices (Kruskall-Wallis p < 0.05). When these predictor variables were examined in combination, significant differences
were found for the settling velocities of both *C. parvum* and *G. lamblia*: in pure water, increased salinity led to faster settling; however, in environmental water, increased salinity led to slower settling (Kruskall-Wallis *p* <0.05, for both parasites). No significant differences were found between settling velocities at high and low temperatures.

**Wetland mesocosm release experiments.** Based on the significant effect of salinity on the settling velocities of protozoa in the settling column experiments, salinity was also examined in the subsequent mesocosm release experiments using environmental waters. With the NB regression model evaluating the effects of individual predictors (Table 2), the *C. parvum* oocyst concentration ratio for the effect of salinity was 1.33 (*p* <0.05), indicating that the IRR of oocysts per 50 mL was 1.33-fold higher in the higher saline water compared to the concentration of oocysts in lower salinity water. No significant difference was observed between slow and fast flow rates for either parasite or *T. gondii* surrogate counts with the NB model (Table 2). There was no significant difference between sampling locations or flow rates in the non-vegetated system in the NB analyses.

Both California bulrush and slough sedge significantly enhanced removal of *G. lamblia* and *T. gondii* surrogates from the surface water in the wetland mesocosm tanks, compared to experimental replicates where no vegetation was used. Figure 3 shows the mean parasite count changes over time for *C. parvum*, *G. lamblia*, and *T. gondii* surrogates in surface water from the vegetated and non-vegetated tanks. To illustrate the effect that individual variables had on parasite and surrogate counts, Table 2 shows the IRRs for individual vegetation parameters. For the *T. gondii* surrogate counts, the presence of any vegetation (regardless of species or configuration) was significant (OR = 0.51; *p* <0.05), indicating that fewer surrogates were
recovered from the water column of mesocosm tanks with vegetation, when compared to mesocosm tanks without vegetation.

Individual vegetated conditions also showed significant trends in parasite or surrogate recovery, as shown in Table 2. Recovery of *T. gondii* surrogates from all three vegetated conditions was significantly lower than recovery counts from the non-vegetated replicates, with IRRs of less than 1 (*p* < 0.01). The effect of vegetation species and California bulrush configuration comparisons varied between the parasites and surrogates (Table 2). When comparing buffer and channel configurations of California bulrush, a significant effect on recovery counts occurred for *T. gondii* surrogates only, with an IRR of 0.75 (*p* < 0.001), indicating that the buffer strip configuration removed more surrogates than the channel configuration. Conversely, there was no difference in effect of channel or buffer configurations of California bulrush on counts of either *C. parvum* or *G. lamblia*. When comparing vegetation species in a buffer configuration, significant differences were noted for both *G. lamblia* and *T. gondii* surrogates (*p* < 0.001) with IRRs of 0.45 and 0.7, respectively, which suggests that wetland mesocosm tanks containing bulrush removed more parasites than those containing sedge.

Evaluation of the combined effect of the hydrologic and vegetation parameters on the rate of removal in the mesocosm tanks further emphasizes the role of vegetation in removing *G. lamblia* and *T. gondii* surrogates from surface water. Using longitudinal NB multiple regression analysis (Table 3), parameters significant to the final model included the effects of either vegetation type when compared to absent vegetation, plus the effect of increasing salinity for *C. parvum* and *G. lamblia*. For *C. parvum*, both bulrush in the channel configuration and slough sedge had significant effects on parasite counts, with IRRs of 1.5 and 1.6, respectively (*p* < 0.01),
as compared to absent vegetation. The inclusion of salinity was also significant with an IRR of 1.8 ($p < 0.0001$), indicating that at increased salinity more $C. \text{parvum}$ oocysts were retained in surface water than at lower salinity. For $G. \text{lamblia}$, bulrush in the buffer configuration was significant with an IRR of 0.56 ($p < 0.05$), indicating that when California bulrush is present, fewer cysts were recovered. For $T. \text{gondii}$ surrogates, all three vegetation conditions had IRRs of less than 1 ($p < 0.05$), indicating that when vegetation is present, fewer surrogates are recovered. In summary, addition of vegetation significantly enhanced removal of $G. \text{lamblia}$ cysts and $T. \text{gondii}$ surrogates, while decreased water salinity enhanced removal of $C. \text{parvum}$ oocysts in wetland mesocosm tanks.

**DISCUSSION**

Wetlands reduce the concentration of numerous water pollutants and pathogens in effluent waters, but the mechanisms responsible for pathogen removal vary by wetland and pathogen (8, 26). This study aimed to better characterize the physical and hydrological conditions that enhance removal of $\text{Cryptosporidium}$, $\text{Giardia}$, and $\text{Toxoplasma}$ in fecal pathogen-polluted surface water that enters coastal wetlands. We determined that salinity, water type, and vegetation type and configuration are key factors for reduction of suspended protozoal parasites in water. Although field investigations in the Tembladero Slough wetland system have also been conducted to evaluate its role in pathogen transport (8), experimental laboratory approaches in simulated natural wetlands conditions utilized during this study enabled examination of key factors not easily controlled in the field setting.

At low salinity, faster settling of all four particles ($C. \text{parvum}$ oocysts, $G. \text{lamblia}$ cysts, DG and GB $T. \text{gondii}$ surrogates) was observed in environmental water as compared to pure
water, a finding that is consistent with previous studies (17, 28). Higher salinity correlated with faster settling of *Cryptosporidium* oocysts and *Giardia* cysts in pure water, but higher salinity correlated with slower settling of *Giardia* cysts in environmental water. This latter observation was surprising in light of recent findings that increased numbers of *T. gondii* oocysts and surrogate microspheres were recovered from settled aggregates in environmental waters with increased concentrations of salt (33). The effect of salinity on aggregation and subsequent particle settling may be countered by increased buoyancy of particles when suspended in higher density aquatic solutions. In addition, the experimental setup described here was conducted on spiked parasites and surrogates added directly to settling columns, without incubation or rolling conditions that enhance formation of aggregates (33). Thus, the apparent impact of salinity on pathogen settling may be confounded by experimental methodology, altered surface properties of the organism, and/or interactions between the protozoa and suspended sediments (5), perhaps due to the use of heat-inactivated protozoa which may have altered surface properties compared to live protozoa.

Previous studies aiming to quantify the settling velocities of *C. parvum* and *G. lamblia* in pure and environmental waters at low salinity have demonstrated a wide range of results (2, 17, 28). Our study showed that salinity has a significant effect on the vertical settling of *Cryptosporidium, Giardia* and *Toxoplasma* surrogates and that water type also contributes significantly to variation in settling properties, thus accounting for the wide range of results in prior studies. The impact of environmental water salinity on parasite transport was further evaluated in the mesocosm tank studies. For *Giardia* and *T. gondii* surrogates in the settling columns, increased salinity in environmental water correlated with slower settling velocities, and in the mesocosm tanks increased salinity correlated with slower removal of *Cryptosporidium*.
The impact of salinity is relevant for coastal wetlands, which are greatly affected by tidal influence. Beyond the interaction of water type and salinity affecting the settling of protozoa, the presence or absence of vegetation was also shown to be a critical factor in protozoal removal. Wetlands can reduce microbial pollutant loads through several processes, including adsorption, sedimentation, and vegetative uptake (10, 11, 13). When vegetation was included in the experimental wetland mesocosm tanks, *Giardia* and *T. gondii* surrogate counts were significantly lower in the surface water as compared with mesocosm tanks without vegetation, suggesting that the presence of vegetation can reduce the concentrations of parasites that remain suspended in surface waters.

The utility of vegetation for microbial removal has been demonstrated in prior studies, which have shown that vegetated buffer strips can effectively remove microbial pathogens from contaminated run-off (1, 21, 22, 37). However, different aquatic plants may vary in their ability to remove parasites due to distinct surface properties, unique biofilms, and differential effects on water flow and drag (16, 24). Therefore, plant selection is an important consideration for wetlands managers in understanding how to best restore degraded wetlands or conserve existing ones (18, 30, 38). This study compared California bulrush, a dense, reed-like plant, to slough sedge, a more grass-like plant. Both are found in California coastal wetlands and are commonly used in landscape restoration. The presence of bulrush was more likely to reduce parasite counts for both *Giardia* and *T. gondii* surrogates; however no difference was seen between the two vegetation treatment groups for *Cryptosporidium*. A further consideration for wetlands managers is how to best integrate vegetation into the landscape, as this can also affect the flow and drag of water (24). Interestingly, the channel and buffer configurations of California bulrush in the
mesocosm tanks showed no significant difference in removal of Cryptosporidium or Giardia, despite the presence of a 15 cm gap between stands of bulrush in the channel configuration. Although wider gaps still need to be studied, our results support the use of either configuration in restoration efforts, particularly if the waterway is narrow.

In coastal habitats where land-to-sea transport of parasites such as Cryptosporidium, Giardia, and Toxoplasma is of concern for the commercial and sport harvest of invertebrates for human consumption, recreational water use, and marine wildlife health, the comparatively low-cost water purification services provided by wetlands should not be discounted. The ability of wetland habitats to reduce outflow of parasites and other fecal pathogens to receiving coastal waters could play a key role in preserving or restoring adequate water quality that will promote human and wildlife health. Salinity and the presence of vegetation play an important role in moderating wetlands’ capacity to remove parasites through adsorption, sedimentation, and uptake (13). Because much of the coastal wetlands in the United States have been degraded, there are numerous efforts currently underway to restore these habitats; the inclusion of vegetation within wetland restoration projects will not only promote healthier ecosystems, but will also enhance parasite removal from surface waters.

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REFERENCES


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Table 1: Settling velocities (µm/sec) as measured in settling column experiments utilizing *Cryptosporidium parvum* oocysts, *Giardia lamblia* cysts, and *Toxoplasma gondii* surrogate microspheres (DG-Dragon Green; GB-Glacial Blue), stratified by water type and relative salinity

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<tr>
<th>Water Type</th>
<th>Salinity (ppt)</th>
<th>n</th>
<th>Mean</th>
<th>SE</th>
<th>Mean</th>
<th>SE</th>
<th>Mean</th>
<th>SE</th>
<th>Mean</th>
<th>SE</th>
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<tbody>
<tr>
<td>Pure Low (0)</td>
<td>6</td>
<td>0.08</td>
<td>0.04</td>
<td>0.14</td>
<td>0.04</td>
<td>0.42</td>
<td>0.13</td>
<td>0.84</td>
<td>0.26</td>
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<tr>
<td>Pure High (30)</td>
<td>6</td>
<td>0.93</td>
<td>0.44</td>
<td>1.04</td>
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<td>0.84</td>
<td>0.50</td>
<td>0.81</td>
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<td>Environmental Low (0)</td>
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<td>1.48</td>
<td>0.49</td>
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<td>1.15</td>
<td>0.23</td>
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NS=No Settling
Table 2: Effect of individual hydrologic or vegetation parameters on counts of Cryptosporidium parvum oocysts, Giardia lamblia cysts, and Dragon Green (DG) Toxoplasma gondii surrogate microspheres in wetland mesocosm tanks using longitudinal negative binomial regression.

<table>
<thead>
<tr>
<th>Predictor</th>
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<th>Giardia</th>
<th>T. gondii surrogates</th>
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<td></td>
<td>IRR</td>
<td>p</td>
<td>IRR</td>
<td>p</td>
</tr>
<tr>
<td>Salinity-Low</td>
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<td>1.33</td>
<td>0.0167*</td>
<td>0.79</td>
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<td>Salinity-High</td>
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<td>1</td>
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<tr>
<td>Flow-Slow</td>
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<td>1.02</td>
<td>0.8796</td>
<td>1.12</td>
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<tr>
<td>Flow-Fast</td>
<td>300</td>
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<td>1.00</td>
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<td>Tank Location 1</td>
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<td>1</td>
<td></td>
<td>1</td>
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<tr>
<td>Tank Location 2</td>
<td>300</td>
<td>0.99</td>
<td>0.8384</td>
<td>1.00</td>
</tr>
<tr>
<td>Time since injection</td>
<td>600</td>
<td>0.97</td>
<td></td>
<td>0.97</td>
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<tr>
<td>(continuous)</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Vegetation absent</td>
<td>240</td>
<td>1.04</td>
<td>0.7114</td>
<td>0.97</td>
</tr>
<tr>
<td>Vegetation present</td>
<td>360</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Vegetation Absent</td>
<td>240</td>
<td>1.04</td>
<td>0.7114</td>
<td>0.97</td>
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<tr>
<td>Buffer</td>
<td>120</td>
<td>0.92</td>
<td>0.5737</td>
<td>0.66</td>
</tr>
<tr>
<td>Bulrush Channel</td>
<td>120</td>
<td>1.05</td>
<td>0.7271</td>
<td>0.82</td>
</tr>
<tr>
<td>Sedge Buffer</td>
<td>120</td>
<td>1.14</td>
<td>0.3366</td>
<td>1.46</td>
</tr>
<tr>
<td>Bulrush Configuration</td>
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<td></td>
<td>1</td>
</tr>
<tr>
<td>Channel (reference)</td>
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<td>1</td>
<td></td>
<td>1</td>
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<td>Buffer</td>
<td>120</td>
<td>0.88</td>
<td>0.4504</td>
<td>0.81</td>
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<tr>
<td>Vegetation Species</td>
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<td>1</td>
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<tr>
<td>Sedge (reference)</td>
<td>120</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Bulrush</td>
<td>120</td>
<td>0.81</td>
<td>0.1225</td>
<td>0.45</td>
</tr>
</tbody>
</table>

*pEffect of factor is significant with p<0.05

IRR = incident rate ratio = $\exp(\beta)$

n = number of water samples included in analysis for factor,
Table 3: Effect of multiple hydrologic and vegetation parameters on counts of Cryptosporidium parvum oocysts, Giardia lamblia cysts, and Dragon Green (DG) Toxoplasma gondii surrogate microspheres in wetland mesocosm tanks using longitudinal negative binomial regression.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>n</th>
<th>Cryptosporidium</th>
<th>Giardia</th>
<th>T. gondii surrogates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity-Low (ref)</td>
<td>480</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Salinity-High</td>
<td>120</td>
<td>1.8 &lt;0.0001*</td>
<td>0.69</td>
<td>0.2922</td>
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<tr>
<td>Vegetation</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Absent (ref)</td>
<td>240</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bulrush Buffer</td>
<td>120</td>
<td>1.3 0.1381</td>
<td>0.56</td>
<td>0.0167*</td>
</tr>
<tr>
<td>Bulrush Channel</td>
<td>120</td>
<td>1.5 0.0075*</td>
<td>0.69</td>
<td>0.1445</td>
</tr>
<tr>
<td>Sedge Buffer</td>
<td>120</td>
<td>1.6 &lt;0.0001*</td>
<td>1.23</td>
<td>0.3045</td>
</tr>
</tbody>
</table>

*Effect of factor is significant with p<0.05

IRR = incident rate ratio = e^β

n = number of water samples included in analysis for factor
Figure Captions

Figure 1: Schematic diagram of an experimental settling column where T is top sampling port and B is bottom sampling port.

Figure 2: Schematic diagrams of an experimental wetland mesocosm tank where 1 and 2 indicate sampling locations, and “inj” indicates the injection point for Cryptosporidium parvum, Giardia lamblia and DG Toxoplasma gondii surrogates. (A) Entire mesocosm tank with water flow directionality; (B) Mesocosm tank view from above with vegetation (v) in buffer strip configuration; (C) Mesocosm tank view from above with vegetation (v) in channel configuration.

Figure 3: Concentrations of Cryptosporidium parvum, Giardia lamblia, and Dragon Green Toxoplasma gondii surrogates per 50 mL volume of surface water in mesocosm tanks plotted over time. Three replicates of each wetland condition were tested to evaluate the effect of vegetation presence and species on removal of parasites from water column. (A) Cryptosporidium, (B) Giardia, and (C) Toxoplasma surrogates.
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