Hydrologic and Vegetative Removal of Cryptosporidium parvum, Giardia lamblia, and Toxoplasma gondii Surrogate Microspheres in Coastal Wetlands

Jennifer N. Hogan, Miles E. Daniels, Fred G. Watson, Stori C. Oates, Melissa A. Miller, Patricia A. Conrad, Karen Shapiro, Dane Hardin, Clare Dominik, Ann Melli, David A. Jessup, Woutrina A. Miller

Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, California, USA; Division of Science and Environmental Policy, California State University, Monterey Bay, Seaside, California, USA; Marine Wildlife Veterinary Care and Research Center, California Department of Fish and Game, Santa Cruz, California, USA; Applied Marine Sciences and Central Coast Long-Term Environmental Assessment Network, Santa Cruz, California, USA

Constructed wetlands 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 recirculating mesocosm tank experiments. Settling column experiments evaluated the effects of salinity, temperature, and water type (“pure” versus “environmental”) on the vertical settling velocities of Cryptosporidium parvum, Giardia lamblia, and Toxoplasma 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 the 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 for pathogen transport within wetland systems, with implications for wetland management, restoration efforts, and coastal water quality.

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 (1–3). Few published studies have focused on the efficacy of naturally occurring coastal wetlands to filter polluted water (3, 4). 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 (5).

Waterborne protozoa, including C. parvum, G. lamblia, and T. gondii, are important human and animal pathogens in coastal ecosystems (6, 7). 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 (8). 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 (9–11). 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 (12). Toxoplasma gondii can adversely affect the fetus if a pregnant woman acquires the parasite, and immunocompromised individuals may suffer severe, and even fatal, infections (13). Toxoplasma gondii can also infect and cause disease in marine mammals, including the threatened California sea otter (Enhydra lutris nereis), which ranges along the California coast. Otters may become infected following exposure to oocysts that are transported from land to sea through contaminated freshwater runoff (7, 14).

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 than in those without (15), which is consistent with results from previous studies examining the vertical settling velocity of C. parvum and G. lamblia (16, 17). Wetland vegetation has also been demonstrated to reduce water pollution and prevent pathogen runoff near dairies through application of various vegetation types and configurations in constructed wetlands (18–20). For example, in a previous study of natural estuarine wetlands, vegetation-lined waterways significantly reduced the transport of T. gondii surrogates, compared with nonvegetated mud flats (4).

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 (i) compare the effects of physical factors (salinity, temperature, and water type) on the vertical settling velocities of Cryptosporidium parvum, Giardia lamblia, and Toxoplasma gondii surrogates and (ii) 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 1 week prior to use for both settling column and mesocosm tank studies. Dragon Green (DG) microspheres (product no. PC07F/5493; Bangs Laboratories, Inc., Fishers, IN) and Glacial Blue (GB) microspheres (product no. PC06N/8319; Bangs Laboratories, Inc., Fishers, IN) were previously evaluated as surrogate particles for *T. gondii* oocysts based on their surface properties (21).

**Settling column experiments.** To investigate the effects of hydrologic factors on settling velocities, experiments were conducted in vertical settling columns (Fig. 1). Each settling column held 1 liter of water, with sampling ports at 10 cm (top) and 30 cm (bottom) 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 under the various water conditions that were evaluated. Eight treatment conditions in a complete block 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 military-purified distilled water from a Milli-Q water system (“pure”). 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 (scientific-grade marine salt; Coralife, 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, 1 × 10⁶ each of *C. parvum* oocysts, *G. lamblia* cysts, and *T. gondii* oocyst surrogates (DG and GB) were mixed together into a 1-liter 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 h. At each sampling point, 1 ml of water was removed from the column by 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), and parasites were enumerated under epifluorescent illumination using fluorescein isothiocyanate (FITC) and 4’6-diamidino-2-phenylindole (DAPI) emission filter sets. Particles with apple-green fluorescence in an oval or spherical shape (3 to 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 to 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 by using epifluorescence microscopy as previously described (22).

Settling velocities were calculated by linear regression using the following equation derived previously by Dai and Boll (16):

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

where *N₀* is the number of parasites or microspheres enumerated from a single port at time zero, *Nₜ* is the number of parasites or microspheres enumerated at time *t*, *H* is the height (millimeters) from the top of the water column to the sampling port, and *vₜ* is the settling velocity expressed as millimeters per hour. These settling velocities were converted to Any settling velocities estimated by regression to be below zero were truncated to 0 µm/s and were classified as NS (“no settling”). Regression on ordered statistics (ROS) analysis was used to determine the mean settling velocity and the 95% confidence interval under each experimental condition by adjusting for the truncated values (23). Given the nonnormal distribution of residual errors observed around the predicted mean with a linear regression model, the nonparametric Kruskal-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 recirculating wetland me-
socosm tanks (Fig. 2A). Three tanks were used in this study, and each tank was a closed system measuring 3 m by 0.5 m. A depth of 1 cm of commercially available Monterey Beach silica sand was used on the bottom of the tank as the substrate. Water for the tanks was obtained from Tembladero Slough in Castroville, CA. Each tank was filled with 450 liters 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 obtusa) at low salinity. Each of these conditions was tested at high and low water flow rates. The inflow velocity of water was manipulated to compare flow rates of 0.1 cm/s (low) and 1 cm/s (high), which approximate water flow rates observed for regional natural and reconstructed coastal wetlands (4).

In nonvegetated tanks, salinity was increased from baseline (0.1 ppt (low)) to 30 ppt (high) by the addition of Coralline scientific-grade marine salt to the tank. For experimental conditions requiring vegetation, wild California bulrush and slough sedge were transplanted from the Molera Constructed Wetland near Castroville, CA, and placed into 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 15-cm-wide piece of rhizome. This was stabilized by gravel at the base of the rhizome and rootstock as well as by 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 (Fig. 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 each species of California bulrush and slough sedge. To control for plant density, all cropped plants were left in the channel, and 28 plants were left in the buffer configuration of bulrush (buffer versus channel), and time after particle injection. The models were fitted by 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 the 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 (25). Negative binomial variance was specified in the models because the descriptive statistics revealed that the mean counts for all three parasites were overdispersed, with the variances far exceeding the means. A logarithmic link function was employed in order to ensure that modeled mean counts were nonnegative, as is typical for negative binomial regressions (26). Predictors were included for each variable assessed in the study, including time after 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 (27). 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 (PROC GENMOD, with options REPEATED, CORR = AR(1), and DIST = NEGBIN; SAS Institute, Inc., Cary, NC), and a P value of <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 errors) were 0.44 (±0.13) μm/s for C. parvum, 0.84 (±0.16) μm/s for G. lamblia, and 1.05 (±0.19) μm/s and 1.14 (±0.19) μm/s 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 were 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 (P < 0.05 by Kruskal-Wallis test). 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 (P < 0.05 for both parasites by Kruskal-Wallis test). No significant differences between settling velocities at high and low temperatures were found.

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 concentration of oocysts per 50 ml was 1.33-fold higher in the higher-saline water than the concentration of oocysts in lower-salinity water. No significant difference was observed between low
and high 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 nonvegetated 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 nonvegetated 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 (odds ratio [OR] = 0.51; *P* < 0.05), indicating that fewer surrogates were recovered from the water column of mesocosm tanks with vegetation than from mesocosms without vegetation.

Individual vegetated conditions also showed significant trends in parasite or surrogate recovery, as shown in Table 2. Recovery counts of *T. gondii* surrogates under all three vegetated conditions were significantly lower than recovery counts from the nonvegetated 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

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**TABLE 1**  
Settling velocities measured in settling column experiments utilizing *Cryptosporidium parvum* oocysts, *Giardia lamblia* cysts, and *Toxoplasma gondii* surrogate microspheres (DG and GB), stratified by water type and relative salinity

<table>
<thead>
<tr>
<th>Water type</th>
<th>Salinity (ppt)</th>
<th>No. of samples</th>
<th>Cryptosporidium</th>
<th>Giardia</th>
<th>DG surrogates for <em>T. gondii</em></th>
<th>GB surrogates for <em>T. gondii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure</td>
<td>Low (0)</td>
<td>6</td>
<td>0.08 ± 0.04</td>
<td>0.14</td>
<td>0.42 ± 0.13</td>
<td>0.84 ± 0.26</td>
</tr>
<tr>
<td>Pure</td>
<td>High (30)</td>
<td>6</td>
<td>0.93 ± 0.44</td>
<td>1.04</td>
<td>0.84 ± 0.50</td>
<td>0.81 ± 0.51</td>
</tr>
<tr>
<td>Environmental</td>
<td>Low (0)</td>
<td>6</td>
<td>0.86 ± 0.28</td>
<td>1.48</td>
<td>1.82 ± 0.48</td>
<td>1.95 ± 0.48</td>
</tr>
<tr>
<td>Environmental</td>
<td>High (30)</td>
<td>6</td>
<td>NS</td>
<td>0.79</td>
<td>1.11 ± 0.30</td>
<td>1.15 ± 0.23</td>
</tr>
</tbody>
</table>

*NS, no settling.*

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**TABLE 2**  
Effect of individual hydrologic or vegetation parameters on counts of *Cryptosporidium parvum* oocysts, *Giardia lamblia* cysts, and DG *Toxoplasma gondii* surrogate microspheres in wetland mesocosm tanks using longitudinal negative binomial regression

<table>
<thead>
<tr>
<th>Predictor</th>
<th>No. of water samples</th>
<th><em>Cryptosporidium</em></th>
<th><em>Giardia</em></th>
<th><em>T. gondii</em> surrogates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IRR</td>
<td>P value</td>
<td>IRR</td>
</tr>
<tr>
<td>Low salinity</td>
<td>480</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>High salinity</td>
<td>120</td>
<td>1.33</td>
<td>0.0167*</td>
<td>0.79</td>
</tr>
<tr>
<td>Low flow rate</td>
<td>300</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>High flow rate</td>
<td>300</td>
<td>1.02</td>
<td>0.8796</td>
<td>1.12</td>
</tr>
<tr>
<td>Tank location 1</td>
<td>300</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<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 (continuous)</td>
<td>600</td>
<td>0.97</td>
<td>&lt;0.0001*</td>
<td>0.97</td>
</tr>
<tr>
<td>Vegetation absent</td>
<td>240</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Vegetation present</td>
<td>360</td>
<td>1.04</td>
<td>0.7114</td>
<td>0.97</td>
</tr>
<tr>
<td>Vegetation Absent (reference)</td>
<td>240</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Bulrush 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>
<td>Channel (reference)</td>
<td>120</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bulkrush Buffer</td>
<td>120</td>
<td>0.88</td>
<td>0.4504</td>
<td>0.81</td>
</tr>
<tr>
<td>Vegetation species</td>
<td>Sedge (reference)</td>
<td>120</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bulkrush</td>
<td>120</td>
<td>0.81</td>
<td>0.1225</td>
<td>0.45</td>
</tr>
</tbody>
</table>

* effect of the factor is significant, with a *P* value of <0.05; IRR, incident rate ratio (exp).
the buffer strip configuration removed more surrogates than the channel configuration. Conversely, there was no difference in the 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 did 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 for the final model included the effects of either vegetation type 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), compared to absent vegetation. The inclusion of salinity was also significant, with an IRR of 1.8 (*P* < 0.0001), indicating that more *C. parvum* oocysts were retained in surface water at increased salinity than at lower salinity. For *G. 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 are recovered. For *T. 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. lamblia* cysts and *T. gondii* surrogates, while decreased water salinity enhanced removal of *C. 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 (3, 28). This study aimed to better characterize the physical and hydrological conditions that enhance removal of *Cryptosporidium*, *Giardia*, and *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 the amount 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 (28), experimental laboratory approaches under simulated conditions provide valuable insights into the efficiency of wetland-based pathogen removal. The results of this study highlight the importance of vegetation and salinity conditions in the effectiveness of wetland mesocosm tanks for pathogen removal.

**TABLE 3** Effect of multiple hydrologic and vegetation parameters on counts of *Cryptosporidium parvum* oocysts, *Giardia lamblia* cysts, and Dragon Green *Toxoplasma gondii* surrogate microspheres in wetland mesocosm tanks using longitudinal negative binomial regression

<table>
<thead>
<tr>
<th>Predictor</th>
<th>No. of water samples</th>
<th><em>Cryptosporidium</em></th>
<th><em>Giardia</em></th>
<th><em>T. gondii</em> surrogates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IRR</td>
<td><em>P</em> value</td>
<td>IRR</td>
</tr>
<tr>
<td>Low salinity (reference)</td>
<td>480</td>
<td>1</td>
<td>&lt;0.0001*</td>
<td>1</td>
</tr>
<tr>
<td>High salinity</td>
<td>120</td>
<td>1.8</td>
<td>&lt;0.0001*</td>
<td>0.69</td>
</tr>
<tr>
<td>Vegetation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent (reference)</td>
<td>240</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Bulrush buffer</td>
<td>120</td>
<td>1.3</td>
<td>0.1381</td>
<td>0.56</td>
</tr>
<tr>
<td>Bulrush channel</td>
<td>120</td>
<td>1.5</td>
<td>0.0075*</td>
<td>0.69</td>
</tr>
<tr>
<td>Sedge buffer</td>
<td>120</td>
<td>1.6</td>
<td>&lt;0.0001*</td>
<td>1.23</td>
</tr>
</tbody>
</table>

* *P* value of <0.05; IRR, incident rate ratio. 

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**FIG 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 under each wetland condition were tested to evaluate the effect of vegetation presence and species on removal of parasites from the water column. (A) *Cryptosporidium*; (B) *Giardia*; (C) *Toxoplasma* surrogates.
natural wetland 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. parvum oocysts, G. lambilla cysts, and DG and GB T. gondii surrogates) was observed in environmental water than in pure water, a finding that is consistent with previous studies (17, 29). 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. The 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 (30). The effect of salinity on aggregation and subsequent particle settling may be countered by the 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 (30). 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 (31), 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. lambilla in pure and environmental waters at low salinity demonstrated a wide range of results (16, 17, 29). 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 previous 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 lower 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 (1, 20, 32). When vegetation was included in the experimental wetland mesocosm tanks, Giardia and T. gondii surrogate counts were significantly lower in the surface water than in 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 was demonstrated in previous studies, which showed that vegetated buffer strips can effectively remove microbial pathogens from contaminated runoff (18, 33–35). 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 (36, 37). Therefore, plant selection is an important consideration for wetlands managers in understanding how to best restore degraded wetlands or conserve existing ones (4, 5, 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 for 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 (37). 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 harvesting 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 (32). Because much of the coastal wetlands in the United States have been degraded, there are numerous efforts under way to restore these habitats; the inclusion of vegetation within wetland restoration projects will not only promote healthier ecosystems but also enhance parasite removal from surface waters.

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