

SURVEILLANCE OF ZONOTIC PARASITES IN SEDIMENT, WATER, AND INVASIVE BIVALVES AT THE LAND-WATER INTERFACE

By

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ABSTRACT

Climate-driven shifts in precipitation, paired with human activity, increasingly mobilize terrestrial pathogens into aquatic systems, where filter-feeding bivalves such as *Corbicula fluminea* can acquire and retain waterborne contaminants. Because the parasites *Toxoplasma gondii* and *Cryptosporidium* spp. persist in the environment and are closely tied to hydrologic variability, the Tar-Pamlico River Basin offers an ideal system for examining how precipitation-driven transport shapes pathogen presence across soil, water, and clam tissue. To investigate this, soil, surface water, and *C. fluminea* tissue were collected seasonally from five sites spanning a freshwater-to-brackish environmental gradient, alongside USGS (US Geological Survey) stream gage height data used to characterize hydrologic variability. Samples were screened for *T. gondii* and *Cryptosporidium* spp. the molecular method quantitative PCR (qPCR), and pathogen occurrence was estimated using bias-reduced logistic regression and generalized linear mixed-effects models to assess how sample type, hydrology, and water quality predicted detection. *Cryptosporidium* spp. was detected frequently in soil (up to 97.8%) and water (up to 93.3%) across all seasons, but rarely in clam tissue, while soil and water samples were significantly more

likely to test positive than clams (OR = 92.65 and 26.59, respectively). *T. gondii* was detected far less often overall and never in clams. Nonetheless, both parasites showed positive associations with stream gage height and salinity, with this relationship considerably stronger for *Cryptosporidium* than *T. gondii*. This contrast suggests that although parasites are clearly present and mobilized within the watershed, their environmental availability does not consistently lead to bivalve uptake. The rarity of detections in *C. fluminea* likely suggests intermittent, low-level exposure, behavioral constraints on filtration, and the limits of whole-tissue qPCR rather than true parasite absence.

SURVEILLANCE OF ZOONOTIC PARASITES IN SEDIMENT, WATER, AND INVASIVE
BIVALVES AT THE LAND-WATER INTERFACE

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BACKGROUND

Climate change and human activity can be linked to the emergence and persistence of infectious diseases (Baker et al., 2022). By engaging in activities such as agriculture, urbanization, and deforestation, among others, humans have altered global-scale precipitation patterns and now face the consequences of these actions (Cable et al., 2017; Patz et al., 2000). Specifically, increasing urbanization and more intense and frequent storm events worsen contaminant transport from terrestrial to aquatic ecosystems. Furthermore, globalization introduces new species into non-native habitats, which can dominate without native predators, become invasive, and outcompete native species, ultimately leading to declines in biodiversity (Gomes et al., 2018).

Therefore, studying how compounding abiotic (precipitation) and biotic (invasive species) stressors influence disease dynamics is important, particularly at the interface of terrestrial and aquatic environments. These land-water interfaces are ecologically dynamic sites of physical, chemical, and biological exchange. Changes in precipitation can alter runoff volume, peak flow, and the transport of nutrients and pathogens into streams, thereby directly influencing pathogen transport and survival. Consequently, increased precipitation can enhance stormwater-driven mobilization of pathogens from terrestrial to aquatic systems (Patz et al., 2000; Baker et al., 2022; Cable et al., 2017).

The Tar-Pamlico River Basin in eastern North Carolina, USA, provides an ideal system for examining the interaction of hydrologic variability and contaminant transport at land-water interfaces. The watershed spans approximately 6,400 square miles and is characterized by a mosaic of agricultural and urban land uses, which together increase the potential for pollutant

and pathogen inputs from agricultural runoff, septic systems, and urban development (Tapas et al., 2024; Tran et al., 2024).

Hydrologic variability within the basin is strongly driven by storm events and seasonal precipitation, which influence runoff volume, peak discharge, and downstream transport processes. These precipitation-driven pulses elevate nutrient and biological contaminant inputs from urbanized areas, while agricultural sub-watersheds contribute higher nitrate and sediment loads, collectively driving spatial and temporal variation in water quality parameters such as nutrient concentrations, conductivity, and pH (O'Driscoll et al., 2024; Tapas et al., 2024; Tran et al., 2024). Episodic storm events can mobilize terrestrial contaminants, including pathogens, allowing them to enter streams and rivers via surface runoff (VanWormer et al., 2016). The resulting hydrologic and abiotic gradients across the Tar-Pamlico River Basin create a dynamic environmental context for examining how aquatic organisms respond to variation in precipitation, runoff, and water quality conditions throughout the year.

Once introduced into aquatic systems, these contaminants may persist, settle, or be redistributed across benthic and pelagic habitats, potentially increasing exposure risk for aquatic organisms and influencing local ecosystem health (Shapiro et al., 2019; Graczyk et al., 2003). Suspended solids and nutrients can reduce water quality; however, filter-feeding bivalves such as oysters and clams can remove suspended particles through suspension feeding and are therefore considered effective biofilters (Gomes et al., 2018; Graczyk et al., 2003; Miller et al., 2005; Fung et al., 2021). This feeding strategy allows bivalves to remove suspended particles, nutrients, algae, and microbial contaminants, including pathogens, from the water column. However, retention and accumulation of pathogens within bivalve tissues is not universal and depends on factors such as filtration rate, particle size, pathogen viability, and retention efficiency.

In some cases, filter-feeding bivalves have been shown to retain infectious stages of waterborne pathogens following exposure. For example, freshwater bivalves including *Corbicula fluminea* and zebra mussels have been documented to concentrate protozoan parasites such as *Cryptosporidium* and *Giardia* from contaminated waters, with oocysts detected in tissues or biodeposits after exposure (Graczyk et al., 2003; Miller et al., 2005). Similarly, *Toxoplasma gondii* oocysts have been detected in marine and freshwater bivalves following environmental exposure, indicating that bivalves can serve as reservoirs of pathogen material in aquatic systems (Shapiro et al., 2019; Fung et al., 2021; Kim et al., 2023). Viral pathogens, including avian influenza virus, have also been shown to persist within bivalve tissues and remain infectious after uptake (Faust et al., 2009; Huyvaert et al., 2012). Therefore, rather than functioning as a biological host, bivalves may therefore act as transport hosts, passively acquiring and retaining pathogens through filtration and potentially facilitating redistribution or exposure within aquatic food webs (Graczyk et al., 2003; Miller et al., 2005). This transport role does not sustain pathogen reproduction within the host but may nonetheless influence pathogen persistence and transmission dynamics in aquatic ecosystems (Shapiro et al., 2019).

Within this conceptual framework of bivalves as transport hosts that passively acquire and retain pathogens through filter feeding, we focused on a dominant freshwater bivalve in the Tar-Pamlico River Basin: *Corbicula fluminea*, commonly known as the Asian or golden clam. *Corbicula fluminea* is an abundant invasive species throughout the basin and serves as the model host organism in this study. Originally introduced to the United States in the early 1900s, *C. fluminea* has rapidly expanded across freshwater systems nationwide, with particularly dense populations documented in North Carolina waterways (Cooper, 2007; Rosa et al., 2012; Weber & Cibulka, 2022).

Corbicula fluminea exhibits broad environmental tolerance, surviving across a temperature range of approximately 2-36 °C and tolerating short-term salinity fluctuations, which enables year-round persistence and sampling even during extreme precipitation events (Rosa et al., 2012; Weber & Cibulka, 2022; Crespo et al., 2015; Sousa et al., 2008). These characteristics compliment the hydrologic variability of the Tar-Pamlico River Basin. The basin's hydrologic variability driven by storm events and seasonal precipitation strongly influences nutrient concentrations, conductivity, and pH (O'Driscoll et al., 2024; Tapas et al., 2024).

The reproductive strategy, rapid growth, and physiological plasticity of *C. fluminea* allows it to persist across diverse freshwater environments characterized by fluctuations in temperature, nutrient availability, conductivity, and dissolved oxygen (Crespo et al., 2015; Rosa et al., 2012). Numerous studies have documented the presence of human and wildlife pathogen DNA including *Cryptosporidium parvum*, *Giardia* spp., and avian influenza viruses, within the soft tissues of *C. fluminea* following exposure to contaminated waters (Graczyk et al., 2003; Miller et al., 2005; Faust et al., 2009; Huyvaert et al., 2012). While evidence for active transmission to subsequent hosts remains limited, these findings demonstrate that *C. fluminea* can efficiently acquire and retain pathogen material through filtration, supporting its role as a transport host.

Filtration rates for *C. fluminea* have been quantified to range from approximately 0.5 to 100 mL g⁻¹ h⁻¹ under laboratory conditions, enabling efficient concentration of suspended particles and microbial contaminants from surrounding water (Gomes et al., 2018; Rosa et al., 2012; Sousa et al., 2008). Because *C. fluminea* inhabits shallow stream margins and benthic zones where terrestrial runoff and flood pulses first enter aquatic systems, individuals are directly exposed to pathogen inputs originating from agricultural and urban land use (Graczyk et al.,

2003; Miller et al., 2005; Tapas et al., 2024). Together, these traits highlight *Corbicula fluminea*'s ability to retain pathogens from surrounding water over time and provides a useful lens for examining how hydrologic variability influences the environmental presence of waterborne parasites.

Therefore, pathogens with environmentally resistant life stages and strong links to terrestrial runoff are particularly well suited to this approach. One such pathogen is *Toxoplasma gondii*, a zoonotic protozoan parasite whose oocysts are shed on land and transported into aquatic systems during precipitation events. *T. gondii*'s definitive hosts (i.e., organisms that support the adult stage and sexual reproduction of the parasite) are the felids (domestic and wild cats). Infected cats disperse millions of environmentally resistant oocysts through their feces, which can remain infectious in the environment for months (Shapiro et al., 2019; Dubey, 2001). *T. gondii* oocysts' persistence, along with precipitation-driven runoff in the Tar-Pamlico River Basin, creates the potential to study how climate-induced precipitation changes can influence pathogen transmission and public health concerns (Cable et al., 2017; Egorov et al., 2018). Humans and animals may be exposed to oocysts in the environment or indirectly by consuming intermediate hosts and reservoirs, which maintain and support pathogen survival and reproduction, which can lead to toxoplasmosis. In healthy individuals, toxoplasmosis usually results in mild or asymptomatic infections. However, toxoplasmosis in immunocompromised individuals can result in vision loss and neurological disease, including encephalitis (brain inflammation) (Bahia-Oliveira et al., 2019). It can also result in severe disease for pregnant women if the parasite enters the placenta, causing congenital toxoplasmosis, which results in miscarriage, stillbirth, and fetal disorders like hydrocephalus, microcephaly, and chorioretinitis (Bahia-Oliveira et al., 2019). *Toxoplasma gondii* is of relevance in North Carolina due to

documented associations between environmental exposure and regional hydrologic conditions. Egorov et al. (2018) reported that *T. gondii* seroprevalence among residents of central North Carolina was significantly associated with soil moisture and runoff potential, suggesting that precipitation-driven transport plays a key role in exposure risk. These findings highlight how terrestrial contamination sources, coupled with regional hydrology, may facilitate the movement of oocysts into aquatic systems where they can persist and be intercepted by filter-feeding organisms.

In addition to *Toxoplasma gondii*, other environmentally persistent protozoan parasites are routinely mobilized into freshwater systems through fecal contamination and precipitation-driven runoff including species within the genus *Cryptosporidium*. Species within the genus *Cryptosporidium* are zoonotic protozoan parasites that infect a wide range of vertebrate hosts, including humans, livestock, and wildlife (Gerace et al., 2019). *Cryptosporidium* spp. produce environmentally robust oocysts that are shed in feces and can persist in surface waters under diverse conditions, including chlorination and variable temperatures, for extended periods ranging from weeks to months (Carey et al., 2004; Gerace et al., 2019; Hemphill et al., 2019; Reinoso et al., 2008). Human infection occurs through ingestion of oocysts from contaminated food or water, with as few as one oocyst capable of causing cryptosporidiosis. Symptoms in healthy individuals typically include watery diarrhea, nausea, abdominal cramps, and fever, while infections in young children and immunocompromised individuals can become severe or life-threatening (Carey et al., 2004; Gerace et al., 2019; Hemphill et al., 2019). *Cryptosporidium* spp. are also well suited for examining pathogen transport in freshwater systems due to their environmental persistence and frequent detection in surface waters impacted by agricultural and urban runoff. Previous studies have documented *Cryptosporidium* oocysts in watersheds

influenced by livestock operations, wastewater inputs, and stormwater runoff, conditions that are characteristic of many regions in North Carolina, including the Tar-Pamlico River Basin (Reinoso et al., 2008; Carey et al., 2004). The ability of *Cryptosporidium* oocysts to remain viable under a range of environmental conditions makes them useful indicators for assessing how hydrologic variability influences pathogen presence in aquatic ecosystems.

This study examined how precipitation-driven hydrologic processes influence the environmental presence and transport of *T. gondii* and *Cryptosporidium* spp. across soil, surface water, and the freshwater clam, *C. fluminea* within the Tar-Pamlico River Basin. By integrating field-based sampling across seasons and sample types, this work evaluated pathogen detection at the land-water interface and assessed the role of hydrologic variability in shaping environmental exposure pathways. Specifically, this study addressed the following research questions: (1) How does pathogen presence compare among soil, surface water, and *C. fluminea*? and (2) how do precipitation patterns and runoff influence the detection of *T. gondii* and *Cryptosporidium* spp. in these environmental compartments? In the absence of a known point source, this study further evaluated whether passive environmental surveillance could detect *T. gondii* and *Cryptosporidium* spp. across soil, water, and clam tissues. We hypothesized that (1) *T. gondii* and *Cryptosporidium* spp. would be detected across all three sample types, reflecting precipitation-driven transport across the land-water interface, and (2) increased precipitation would increase the likelihood of pathogen detection in the Tar-Pamlico River Basin by mobilizing terrestrial contaminants into aquatic systems. Taken together, these objectives aimed to characterize pathogen presence across the land-water interface and assess the influence of precipitation-driven transport on environmental pathogen detection, with *C. fluminea* serving as a transport host that reflects hydrologic connectivity rather than active biological amplification.

METHODS

Sampling Location

Field sampling was conducted across four seasonal periods. Summer 2025 sampling occurred on May 28, June 9, July 9, and July 25, 2025. Fall 2025 sampling occurred on December 11 and 13, 2025. Winter 2026 sampling occurred on February 16 and 21, 2026, and spring 2026 sampling occurred on March 30 and April 1, 2026. Sampling was conducted at sites located within the Tar-Pamlico River Basin in North Carolina, USA (Figure 1), which spans approximately 6,400 square miles.

To capture a gradient from freshwater to brackish conditions, five sampling sites were selected in Greenville and Washington, North Carolina. These sites included Green's Mill Run (Greenville, NC), River Park North (Greenville, NC), Town Commons (Greenville, NC), Dinah Landing at Goose Creek State Park (Washington, NC), and Jack's Creek (Washington, NC) (Figure 1). Each site was sampled seasonally to capture variation in precipitation, stream flow, and temperature, which influence pathogen transport from terrestrial to aquatic systems. These seasonal conditions may also indirectly influence bivalve filtration activity and pathogen exposure opportunities (Patz et al., 2000; Cable et al., 2017). Sampling sites were selected based on known presence of the non-native golden clam, as identified through iNaturalist sightings, ongoing water quality monitoring by the ECU Water Resources Center, and records from the USGS Nonindigenous Aquatic Species database.

Sampling Design

At each site, water quality parameters including water temperature, dissolved oxygen, salinity, and conductivity were measured using a YSI ProDSS multiparameter probe. Previous studies have shown that water quality conditions influence pathogen transport and persistence, and that precipitation events affect environmental transmission and hydrological conditions

relevant to oocyst survival (Shapiro et al., 2019; Reinoso et al., 2008; Egorov et al., 2018).

Following assessment of stream flow conditions, samples were collected along a transect representing upstream, midstream, and downstream locations at each site. At each transect location, 1 L of surface water was collected. Sediment samples were also collected from three locations at each site: the center of the channel, the left bank, and the right bank. If streams were too deep or wide to safely access the channel center, sediment was instead collected along the stream edge at foot, mid-calf, and knee depths.

Sediment samples were homogenized by mixing and subsampling 5-15 mL of sediment into sterile 50-mL tubes and Whirl-Pak bags for downstream analyses. Live clams were collected at each site when present. Not all sites yielded clam samples, as some locations lacked environmental conditions suitable for clam habitation. During summer 2025, 12 water samples were lost due to a processing error involving the use of an incorrect filter type. All samples were transported on ice to the laboratory for molecular analysis. Sediment and clam samples were stored at -20°C until DNA extraction, while water samples were stored at 4°C until filtration and subsequently stored at -20°C prior to DNA extraction.

Additional Data Collection

In addition to field-collected data, publicly available environmental data were obtained from the U.S. Geological Survey (USGS) to characterize precipitation, stream stage, and streamflow. Hourly gage height data were downloaded for the Tar River at Greenville, NC (USGS station 02084000), and the Pamlico River at Washington, NC (USGS station 02084472). Gage height represents the height of the water surface above a standardized USGS gage datum and is used as a measure of stream water level. These data were used to evaluate storm events, runoff pulses, and precipitation patterns.

DNA Extraction

Approximately 100 mL of each water sample was filtered onto a sterile 0.22- μ m PES membrane filter using a vacuum pump. Filters were transferred to sterile 2-mL screw-cap tubes and stored at -20°C until extraction (Kim et al., 2023). DNA was extracted from water samples using the Zymo Quick-DNA/RNA Water Kit following the manufacturer's Water Filters workflow. Next, clams were counted, and shell length and width were measured prior to processing. Due to the small size of individual clams, tissue from two to four clams was pooled into each 2-mL microcentrifuge tube and stored at -20°C . All clam samples underwent a freeze-thaw step, during which samples were stored at -20°C for a minimum of 48 hours and then thawed prior to extraction to promote oocyst rupture and improve DNA recovery (Rousseau et al., 2019). DNA was extracted using the Zymo DNA Mini Prep Plus Kit following the Solid Tissues workflow. Lastly, DNA was extracted from sediment samples using the Qiagen PowerSoil DNA Extraction Kit following the manufacturer's protocol. All extracted DNA samples were stored at -20°C prior to downstream analyses.

Parasite Concentration Analyses

Prior to quantitative PCR (qPCR), DNA concentration and purity were assessed using Qubit fluorometry. Detection of *Toxoplasma gondii* was performed using the protocol described by Belaz et al. (2015). Each 25- μ L reaction contained 2.5 μ L of DNA template, 12.5 μ L of 2 $\times$ Master Mix, 1.0 μ L of bovine serum albumin (2 μ g/mL), 0.5 μ L each of REP529 forward and reverse primers (10 μ M), 0.4 μ L of probe (10 μ M), and 7.6 μ L of molecular-grade water. Thermal cycling conditions included an initial incubation at 50°C for 2 min, denaturation at 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. A *T. gondii* gBlock DNA fragment was used as a positive control, and molecular-grade water served as the negative control.

Genus-specific detection of *Cryptosporidium* spp. was conducted using the qPCR protocol described by Raja et al. (2021). Each 20- μ L reaction contained 2.0 μ L of DNA template, 10.0 μ L of 2 $\times$ Master Mix, 0.5 μ L each of Crypto18 forward and reverse primers (10 μ M), 0.2 μ L of probe, and 6.8 μ L of molecular-grade water. Thermal cycling conditions included incubation at 50 °C for 2 min, denaturation at 95 °C for 3 min, and 45 cycles of 95 °C for 15 s and 55 °C for 1 min. DNA extracted from a confirmed *Cryptosporidium* sample was used as a positive control, and molecular-grade water served as the negative control.

Statistical Analysis

All statistical analyses were conducted in the R statistical environment (RStudio 2026.04.0+526; R version 4.5.2) (Posit Team, 2026). Parasite occurrence patterns were summarized descriptively to characterize the distribution of *Cryptosporidium* spp., and *Toxoplasma gondii* across sample types, seasons, and sampling sites. Prevalence was calculated as the proportion of positive samples relative to the total number of samples for each sample type and season, and 95% confidence intervals were computed using the binom package (Dorai-Raj, 2022).

To evaluate the effect of sample type on parasite detection, logistic regression models with a binomial error distribution were fitted. Because preliminary analyses indicated evidence of (quasi-)complete separation, which can result in unstable parameter estimates, bias-reduced logistic regression was implemented using the brglm2 package (Kosmidis & Firth, 2021; Kosmidis, 2025). Model coefficients were reported as log-odds and converted to odds ratios with 95% confidence intervals. Statistical significance was assessed at $\alpha = 0.05$.

To evaluate hydrological predictors of *Cryptosporidium* detection, generalized linear mixed-effects models (GLMMs) were fitted using the glmmTMB package, with sampling site

included as a random effect. Candidate models included combinations of hydrologic predictors, water quality variables, sample type, and season. Model selection was based on Akaike's Information Criterion corrected for small sample size (AICc), and the model with the lowest AICc was selected for interpretation. Model coefficients were reported as log-odds with corresponding standard errors, z-values, and p-values, and were converted to odds ratios with 95% confidence intervals for visualization in forest plots. Statistical significance was assessed at $\alpha = 0.05$.

To further evaluate the relative importance of hydrologic and environmental predictors of *Cryptosporidium* detection, a set of four candidate GLMMs was constructed incorporating different combinations of hydrologic predictors, water quality variables, sample type, and season. Model 1 included mean gage height as the sole predictor. Model 2 included mean gage height and salinity, Model 3 included mean gage height and conductivity, and Model 4 included mean gage height, conductivity, salinity, sample type, and season. The best-supported model was identified as the model with the lowest Akaike's Information Criterion corrected for small sample size (AICc) and was used for interpretation. Model coefficients were reported as log-odds with corresponding standard errors, z-values, and p-values, and were converted to odds ratios with 95% confidence intervals for visualization in forest plots. Statistical significance was assessed at $\alpha = 0.05$.

RESULTS

Descriptive statistics

We evaluated the presence of *Toxoplasma gondii* and *Cryptosporidium spp.* across soil, water and clams in the Tar-Pamlico River Basin. A total of 269 samples were included in the analyses, comprising 177 soil samples, 48 water samples, and 44 clam samples. Most clam samples were collected in the fall (57%), with smaller proportions collected in the other seasons. Clam sampling was attempted at all study sites; however, clams were only consistently found at Greensprings Park. There was limited availability at other locations resulted in only one clam sample being collected at Town Commons (Table 1).

Cryptosporidium spp. was detected in clam, soil, and water samples across all four seasons (Table 2; Figure 2). Among clam samples, prevalence ranged from none [0 - 13.3%] in fall and summer to 28.6% [8.2 - 64.1%] in winter. One clam sample tested positive in spring, corresponding to a prevalence of 16.7%. Confidence intervals for clam samples were relatively large, reflecting small seasonal sample sizes. Soil samples exhibited consistently high *Cryptosporidium spp.* prevalence across seasons. Prevalence exceeded 85% during fall, spring, and winter, with winter showing the highest observed prevalence (97.8% [88.4 - 99.6%]). Lower prevalence was observed during summer (48.9%) compared to the other seasons. Water samples also showed frequent detection of *Cryptosporidium spp.* Prevalence ranged from 53.3% [0.30.% - 75.2%] in fall to 93.3% [70.2 - 98.8%] in spring. Summer prevalence was 66.7% [20.8% - 93.9%]; however, only two of three samples were positive, resulting in the largest confidence intervals among the seasons and reflecting the limited sample size (Table 2; Figure 2).

Toxoplasma gondii was infrequently detected across all seasons and sample types (Table 3). No clam samples tested positive for *T. gondii* in any season. Among soil samples, positive

detections were observed in summer and winter, with prevalences of 2.2% [4 - 11.6%] and 4.4% [1.2 - 14.8%], respectively (Table 3; Figure 3). No positive detections in soils were observed in fall or spring. No *T. gondii* were detected in water samples during any season. Across all sample types, confidence intervals were low or included zero, reflecting the small number of positive samples and the overall rarity of parasite detection in this study (Table 3; Figure 3).

Parasite presence across Corbicula fluminea, soil, and water

The association between parasite detection and sample type was evaluated using logistic regression model with Firth correction to account for quasi separation, particularly among clam samples. Sample type and season were included as predictors, with clam samples and the fall season used as reference categories. Results from the logistic regression model highlighted a significant association between sample type and *Cryptosporidium spp.* detection (Table 4; Figure 4). Relative to the clam samples, soil samples had substantially higher odds of testing positive for *Cryptosporidium* (OR = 92.65 [25.79 - 332.84]), while water samples also showed higher odds of detection (OR = 26.59 [7.08 - 99.87]). Season was also significantly associated with *Cryptosporidium* detection. Compared to fall, the odds of parasite detection were higher in spring (OR = 4.47 [1.52 - 13.15]) and winter (OR = 4.33 [1.49 - 12.55]), whereas summer samples had significantly lower odds of detection (OR = 0.24 [0.10- 0.58]).

In contrast to *Cryptosporidium spp.*, the *Toxoplasma gondii* logistic model did not identify significant associations between sample type or season and parasite detection (Table 5; Figure 5). Relative to the clam tissue samples, neither soil samples (OR = 1.25 [0.09 - 17.50]) nor water samples (OR = 0.71 [0.02 - 23.81]) exhibited significantly different odds of detection. No seasonal effects were statistically significant. Although odds ratios for spring, summer, and

winter were greater than one relative to fall, confidence intervals were wide and included the null value (Figure 5).

Association of precipitation patterns with parasite presence in soil, water and clam tissues

Next, the association between hydrological variables and parasite detection was evaluated using regression models that accounted for sample type and season. Clams did not consistently accumulate parasites in this system; therefore, Question 2 focuses on environmental matrices (water and soil) to examine if hydrological variables influence pathogen transport and detection.

A generalized linear mixed-effects model (GLMM) with a binomial distribution was used to assess hydrologic related predictors for *Cryptosporidium spp.* detection, with sample site included as a random effect. A logistic regression model with Firth correction was used for *Toxoplasma gondii* detection due to the small sample size. Four candidate models were run, and AICc values were compared to determine the best fit (Table 6). M4 (mean gage height + water temperature + dissolved oxygen + conductivity + salinity + sample type + season) had the lowest AICc and was used for interpretation.

Results from the GLMM indicated that hydrologic and water quality variables were significantly associated with *Cryptosporidium spp.* detection across sample types (Table 7; Figure 6). Mean stream gage height was positively associated with *Cryptosporidium spp.* detection ($\beta = 0.71$, $p = 0.042$), indicating increased odds of detection at higher water levels. Water salinity was also positively associated with detection ($\beta = 1.77$, $p = 0.002$). Seasonal effects were examined, with significantly lower odds of detection during summer relative to fall ($\beta = -6.64$, $p = 0.003$), while spring and winter did not differ significantly from fall. Including sample site as a random effect helped account for the non-independence of samples collected from the same location.

A logistic regression model was used to assess hydrologic and water quality related predictors for *Toxoplasma gondii* detection. Four candidate models were run, and AICc values were compared to determine the best fit (Table 8). Model 2 (mean gage height + salinity) had the lowest AICc and was used for interpretation.

Results from the logistic regression model evaluating hydrological variables and *Toxoplasma gondii* detection revealed no significant associations (Table 9; Figure 7). Mean stream gage height was positively associated with parasite detection ($\beta = 2.26$, $p = 0.037$), highlighting higher streamflow increases the likelihood of *T. gondii* detection. Water salinity also showed significant positive effect ($\beta = 0.60$, $p = 0.020$).

DISCUSSION

This study evaluated parasite presence across environmental and biological compartments, soil, water, and *Corbicula fluminea* tissue, within the Tar-Pamlico River Basin to better understand how hydrologic processes influence parasite dynamics at the land-water interface. Overall, parasite detection was highest in soil and water samples, while detection in clam tissue was rare for both *Cryptosporidium* and *Toxoplasma gondii*. These results highlight that although parasites are present and transported throughout the watershed, their occurrence is not uniformly observed across environmental compartments. Specifically, *C. fluminea* did not show consistent qPCR-positive detection of parasite DNA in whole-organism samples, despite confirmed parasite presence in environmental samples.

Findings from this study are consistent with previous work in natural aquatic systems, which has shown that qPCR detection of protozoan parasites in bivalve tissues is often infrequent under environmentally relevant exposure concentrations (Fung et al., 2021; Shapiro et al., 2019; VanWormer et al., 2016; Egorov et al., 2018). One explanation for this pattern is that parasite inputs in natural systems are often intermittent or dilute, resulting in exposure levels below the threshold required for consistent detection in bivalve tissues. In contrast, manipulative experimental and field studies conducted under high and sustained parasite exposure conditions have demonstrated that bivalves can accumulate detectable protozoan parasite loads, particularly in systems subject to chronic fecal contamination from wastewater discharge or intensive agricultural practices (Graczyk et al., 2003; Miller et al., 2005). Collectively, these findings suggest that parasite detection in bivalves is most reliable under conditions of sustained parasite loading rather than sporadic or low-level environmental inputs.

This pattern of low parasite detection in clams is consistent with broader ecological knowledge of how filter-feeding organisms interact with resources. Bivalves integrate suspended particles over time, but to reflect environmental loads consistently depends on exposure frequency of bivalves in the water column (Atkinson et al., 2013). In flowing water systems, resource availability is highly variable due to hydrological pulsing, which results in intermittent rather than continuous exposure to pathogens and food resources. Additionally, filter-feeding bivalves such as *C. fluminea* may further decouple environmental exposure from actual uptake due to behavioral and habitat-related constraints. For example, filtration rates and feeding activity are sensitive to environmental conditions such as temperature, dissolved oxygen, and salinity, with stressors reducing filtration efficiency or inducing temporary inactivity (Rosa et al., 2012; Sousa et al., 2008). Feeding activity can also be influenced by food quality and suspended particulate composition, which affects ingestion rates and energy allocation (Atkinson et al., 2013).

Moreover, burrowing behavior further modifies exposure by limiting direct contact with the water column, particularly when clams are partially or fully embedded in sediments, thereby reducing filtration of suspended particles where pathogens may be more concentrated (Vaughn & Hakenkamp, 2001; Gomes et al., 2018). This behavior may therefore limit effective exposure and accumulation of waterborne pathogens, even during periods of elevated environmental concentrations. In addition, seasonal reductions in metabolic activity during cooler periods may further limit uptake when peak pathogen transport does not coincide with periods of active filtration (Weber & Cibulka, 2022).

Methodological constraints can further influence observed detection patterns. Molecular detection using qPCR is highly sensitive but may underestimate parasite presence when

concentrations fall below detection limits. As a result, non-detection does not necessarily indicate true absence of the target (Bahia-Oliveira et al., 2019). Recovery efficiency is further influenced by sample matrix, organic content, and processing techniques, all of which can reduce detection sensitivity in environmental and biological samples (Reinoso et al., 2008). In addition, previous studies have demonstrated that filter-feeding bivalves, including *C. fluminea*, can transiently accumulate protozoan parasites from surrounding waters; however, detections may reflect either short-term retention within gut contents or longer-term association with tissues, and these pathways are not always distinguishable using whole-tissue qPCR approaches (Graczyk et al., 2003; Miller et al., 2005; Kim et al., 2023). Consequently, even if clams were intermittently exposed to parasites, such exposure may not have resulted in detectable parasite DNA within clam tissues at the time of sampling.

Findings from this study highlight low parasite detection in clam tissue is not unexpected in natural systems and likely reflect the combined effect of spatial and temporal pathogen loading, low environmental concentrations, behavioral and ecological constraints and methodological limitations in environmental detection.

Beyond biological compartments, hydrologic variability played a key role in shaping parasite presence in environmental samples. *Cryptosporidium* occurrence was significantly associated with stream gage height, salinity, and conductivity, indicating sensitivity to precipitation-driven transport and hydrologic mixing. These findings align with extensive evidence that runoff events mobilize fecal contaminants from terrestrial sources and enhance pathogen delivery to aquatic systems, particularly in agricultural and urbanized watersheds (Patz et al., 2000; Cable et al., 2017; VanWormer et al., 2016). Because *Cryptosporidium* is primarily transmitted through ingestion of contaminated water or food and exhibits prolonged

environmental persistence because of oocyst structure, it can be mobilized through runoff and hydrologic flow after precipitation events (Carey et al., 2004; Gerace et al., 2019). In comparison, *T. gondii* showed weaker but detectable associations with hydrologic variables. The best-supported statistical model identified positive relationships between stream gage height and salinity with parasite detection, suggesting that increased streamflow and hydrologic mixing may enhance transport of oocysts into aquatic environments (O'Driscoll et al., 2024; VanWormer et al., 2016). However, detection frequency remained low and confidence intervals were large, reflecting limited statistical power and indicating that *T. gondii* inputs are likely episodic rather than continuous (Shapiro et al., 2019). This is likely because *T. gondii* oocysts enter aquatic systems primarily through felid feces, parasite loading is expected to be spatially and temporally heterogeneous, driven by localized deposition and stochastic runoff events (Dubey, 2001; Egorov et al., 2018; Shapiro et al., 2019; VanWormer et al., 2016).

Across both parasites, results indicate hydrologic conditions are associated with environmental detection patterns at the land-water interface. While precipitation was not directly measured, stream gage height serves a proxy for hydrologic response to precipitation events (O'Driscoll et al., 2024; Tapas et al., 2024; Tan et al., 2024). Previous studies have shown rainfall enhances runoff volume, accelerates surface transport, and increases connectivity between terrestrial pathogen reservoirs and aquatic ecosystems (Patz et al., 2000; Cable et al., 2017). In human-modified watersheds such as the Tar-Pamlico River Basin, these effects are amplified by agricultural land use, urban stormwater infrastructure, and hydrologic alteration, which can modify pathogen transport pathways (Tapas et al., 2024; Tran et al., 2024).

FUTURE DIRECTIONS

Future research can build on these findings by improving temporal resolution, expanding spatial coverage, and refining methodological approaches to better characterize parasite dynamics in freshwater systems. First, improving temporal resolution is critical for capturing the short-term pulses of parasite transport that are likely missed by routine seasonal sampling. Event-based sampling during or immediately after precipitation events would allow researchers to observe hydrologic mobilization of oocysts as it occurs, providing clearer insight into the timing and magnitude of pathogen runoff. Second, expanding spatial coverage would help clarify how land use and contamination sources influence parasite occurrence. Sampling across agricultural, urban, and forested gradients would allow for direct comparison of how human activity alters pathogen loading. Additionally, targeting areas with high densities of domestic or feral cats would help identify drivers of *T. gondii* inputs, given the species' reliance on felid hosts. Last, improving detection sensitivity is also essential for understanding the role of bivalves in pathogen transmission pathways. Increasing sample volumes, enhancing concentration and extraction methods, and increasing replication across sample types would improve statistical power and reduce uncertainty associated with low detection frequencies. Furthermore, controlled laboratory experiments examining retention time, filtration efficiency, and minimum exposure thresholds for *C. fluminea* would help distinguish between true absence of bioaccumulation and methodological limitations imposed by low environmental concentrations or detection thresholds. Together, these approaches would provide a more comprehensive framework for understanding parasite dynamics in freshwater systems and clarify how climate-driven changes in precipitation and runoff influence pathogen transport, exposure risk, and ecosystem health.

CONCLUSION

This study demonstrates that protozoan parasites at the land-water interface are shaped by environmental reservoirs and hydrologic variability. *Cryptosporidium* spp. and *Toxoplasma gondii* were detected in soil and water, while detection in *Corbicula fluminea* tissue was rare. This suggests the presence of parasites in the environment, but their uptake by bivalves is limited by low environmental concentrations, episodic loading and biological constraints on filtration and exposure. Furthermore, hydrologic conditions are key drivers of parasite presence. *Cryptosporidium* detection was influenced by stream gage height, conductivity, and salinity, indicating strong sensitivity to runoff-driven transport and hydrologic mixing, while *Toxoplasma gondii* showed weaker but still positive associations with gage height and salinity, consistent with its more sporadic and localized inputs. In conclusion, these findings demonstrate that parasites transport at the land-water interface is dynamic, event driven and shaped by watershed connectivity and land use.

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APPENDIX

Table 1. Clam characteristics by season and sample site. Presented as number of samples (n) and proportion of sample represented by season or sample site (%).

Characteristic	Clam (n = 44)
Season	N/A
Fall	25 (57%)
Spring	6 (14%)
Summer	6 (14%)
Winter	7 (16%)
Sample site	N/A
Dinahs Landing	0 (0%)
Greensprings Park	43 (98%)
Jacks Creek	0 (0%)
River Park North	0 (0%)
Town Commons	1 (2.3%)

Table 2. The total number of samples, positives samples and prevalence of *Cryptosporidium* spp. by sample type and season.

Sample Type	Season	n	Positives	Prevalence
Clam	Fall	25	0	0.000 [0, 0.133]
Clam	Spring	6	1	0.167 [0.030, 0.564]
Clam	Summer	6	0	0.000 [0, 0.390]
Clam	Winter	7	2	0.286 [0.082, 0.641]
Soil	Fall	42	36	0.857 [0.722, 0.933]
Soil	Spring	45	42	0.933 [0.821, 0.977]
Soil	Summer	45	22	0.489 [0.350, 0.630]
Soil	Winter	45	44	0.978 [0.884, 0.996]
Water	Fall	15	8	0.533 [0.301, 0.752]
Water	Spring	15	14	0.933 [0.702, 0.988]
Water	Summer	3	2	0.667 [0.208, 0.939]
Water	Winter	15	11	0.733 [0.480, 0.891]

Table 3. The total number of samples, positives samples and prevalence of *Toxoplasma gondii* by sample type and season.

Sample Type	Season	n	Positives	Prevalence [95% CI]
Clam	Fall	25	0	0.000 [0, 0.133]
Clam	Spring	6	0	0.000 [0, 0.390]
Clam	Summer	6	0	0.000[0, 0.390]
Clam	Winter	7	0	0.000 [0, 0.354]
Soil	Fall	42	0	0.000 [0, 0.084]
Soil	Spring	45	0	0.000 [0, 0.079]
Soil	Summer	45	1	0.022 [0.004, 0.116]
Soil	Winter	45	2	0.044 [0.012, 0.148]
Water	Fall	15	0	0.000 [0, 0.204]
Water	Spring	15	0	0.000 [0, 0.204]
Water	Summer	3	0	0.000 [0, 0.561]
Water	Winter	14	0	0.000 [0, 0.215]

Table 4. Logistic regression results for *Cryptosporidium* spp. detection across sample type and seasons. Values represent odds ratio with 95% confidence interval and p-value. Odds ratios are expressed relative to clams (sample type) and fall (season reference).

Predictor	Odds Ratio [95% CI]	p-value
Soil vs Clam	92.65 [25.79, 332.84]	<0.001
Water vs Clam	26.59 [7.08, 99.87]	<0.001
Spring vs Fall	4.47 [1.52, 13.15]	0.007
Summer vs Fall	0.24 [0.10, 0.58]	0.001
Winter vs Fall	4.33 [1.49, 12.55]	0.007

Table 5. Logistic regression results for *Toxoplasma gondii* detection across sample type and seasons. Odds ratios are expressed relative to clams (sample type) and fall (season reference).
Odds ratios are expressed relative to clams (sample type) and fall (season reference).

Predictor	Odds Ratio [95% CI]	p-value
Soil vs Clam	1.25 [0.09, 17.50]	0.869
Water vs Clam	0.71 [0.02, 23.81]	0.851
Spring vs Fall	1.07 [0.03, 36.93]	0.968
Summer vs Fall	3.39 [0.18, 62.83]	0.412
Winter vs Fall	5.48 [0.36, 82.61]	0.219

Table 6. AICc values for candidate generalized linear mixed-effects models evaluating hydrological and water quality predictors of *Cryptosporidium* detection. Lowest AICc indicates the better fit model and was selected as final model for interpretation.

Model	AICc
Model 4 (gage + temp + DO + cond + sal + type + season)	191.4267
Model 3 (gage + temp + DO + cond + sal)	220.1895
Model 1 (gage)	231.0880
Model 2 (gage + type)	231.3722

Abbreviations in parentheses: mean gage height (gage), water temperature (temp), dissolved oxygen, (DO), conductivity (cond), and salinity (sal), sample type (type), and season.

Table 7. Summary table of generalized linear mixed model (GLMM) results for hydrological predictors of *Cryptosporidium* detection across hydrologic variables, water quality parameters, season and sample type. Parameter estimates, standard error, z-value and p- value for each fixed effect is expressed. Sampling site served as random effect.

Predictor	Estimate (β)	Std. Error	z-value	p-value
Mean gage height	0.71	0.35	2.04	0.042
Water temperature (°C)	0.15	0.11	1.37	0.171
Dissolved oxygen (mg/L)	-0.35	0.26	-1.35	0.178
Conductivity (μ S/cm)	-0.001	0.0005	-2.12	0.034
Salinity (ppt)	1.77	0.79	2.24	0.025
Water vs Soil	-1.49	0.48	-3.11	0.002
Spring vs Fall	-0.20	1.20	-0.17	0.865
Summer vs Fall	-6.64	2.23	-2.98	0.003
Winter vs Fall	0.71	0.65	1.10	0.274

Table 8. AICc values for candidate generalized linear mixed-effects models evaluating hydrological and water quality predictors of *Toxoplasma gondii* detection. The lowest AICc value indicates the best fit model and was selected as final model for interpretation.

Model	AICc
Model 2 (gage + sal)	30.19579
Model 3 (gage + cond)	31.48081
Model 1 (gage)	35.63748
Model 4 (gage + cond + sal + type + season)	41.06949

Abbreviations in parentheses: mean gage height (gage), water temperature (temp), dissolved oxygen, (DO), conductivity (cond), and salinity (sal), sample type (type), and season.

Table 9. Logistic regression assessed association between hydrologic predictor relative to sample type (reference is soil) and season (reference is fall) for *Toxoplasma gondii* detection. Expressed estimate, standard error, z-value and p-value are presented.

Predictor	Estimate (β)	Std. Error	z-value	p-value
Mean gage height	2.26	1.08	2.09	0.0369
Salinity	0.60	0.26	2.32	0.0204

Sampling Sites in the Tar-Pamlico River Basin

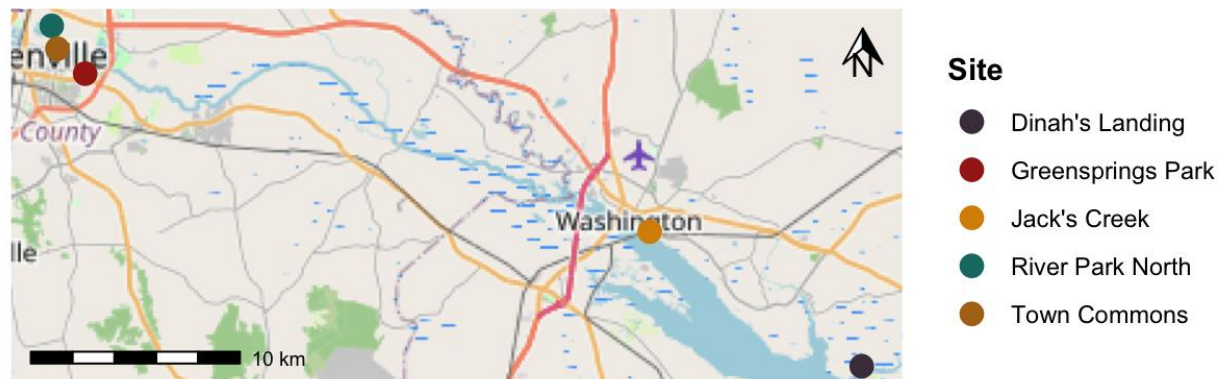


Figure 1. The five sample sites where seasonal sampling took place. Sites are situated in Greenville, NC, USA or Washington, NC, USA. Color represents site location.

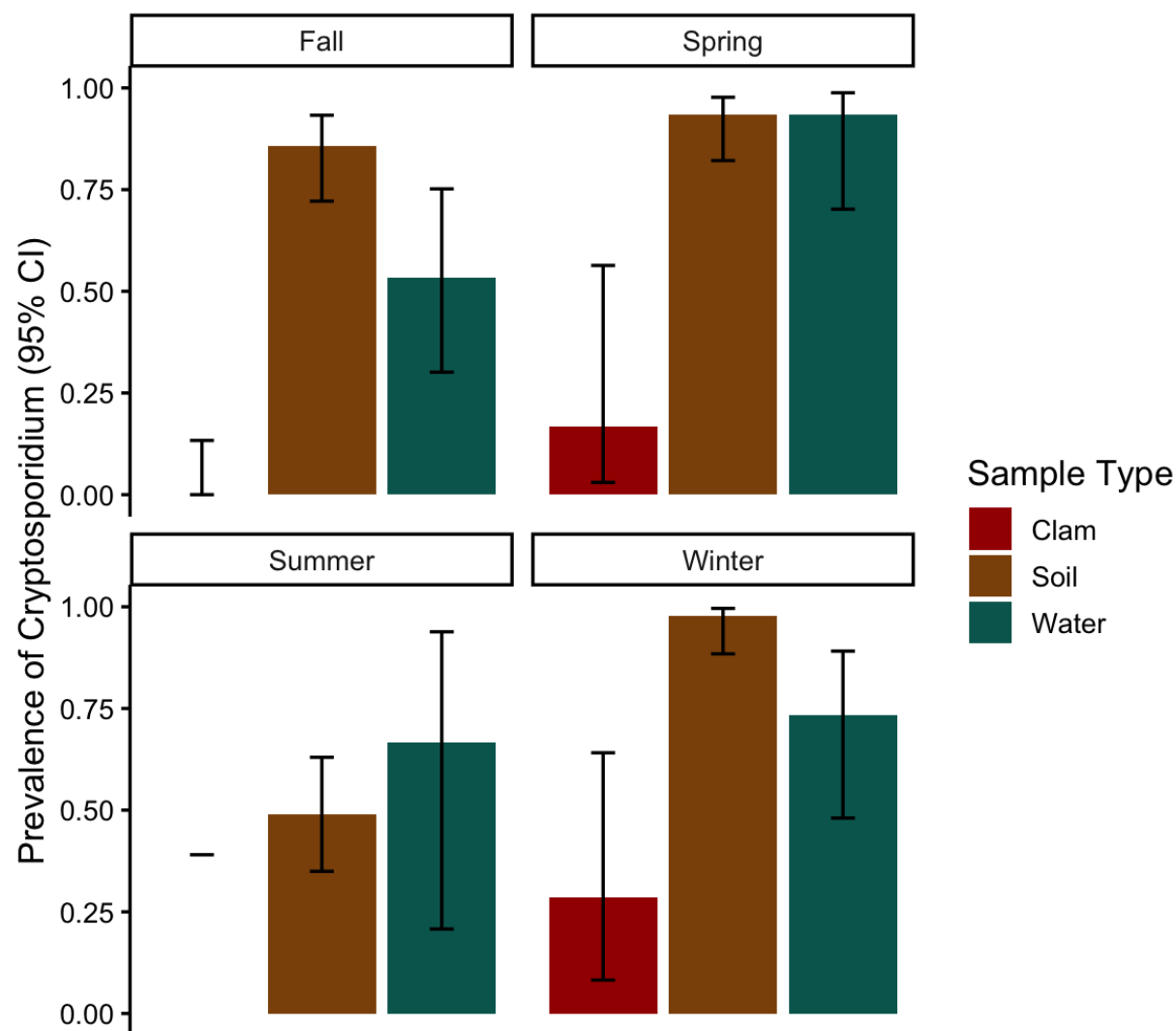


Figure 2. Summary bar graph displaying prevalence of *Cryptosporidium* across sample type and season, along with 95% confidence intervals. Color represents sample type.

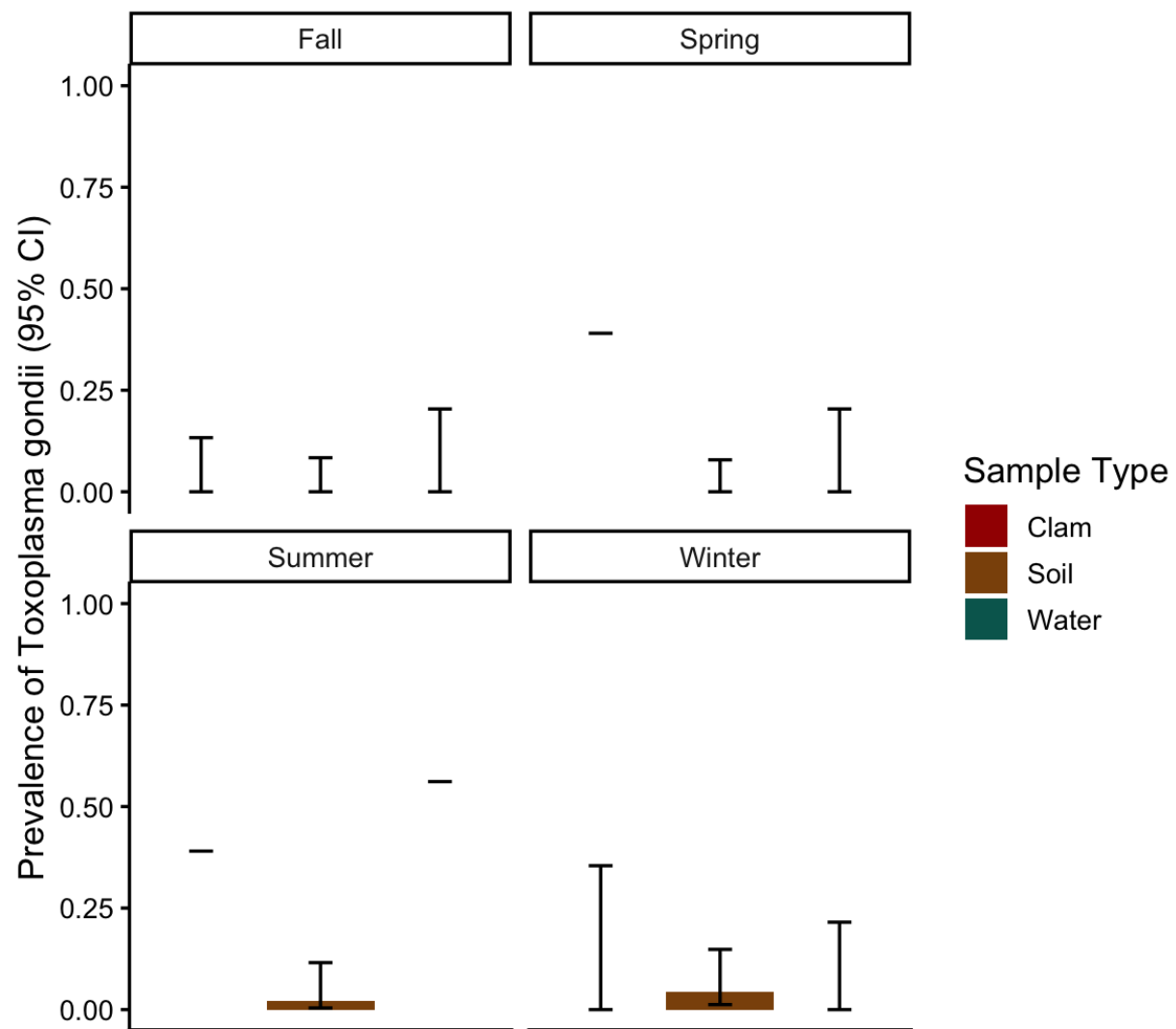


Figure 3. Summary bar graph displaying prevalence of *Toxoplasma gondii* across sample type and season, along with 95% confidence intervals. Color represents sample type.

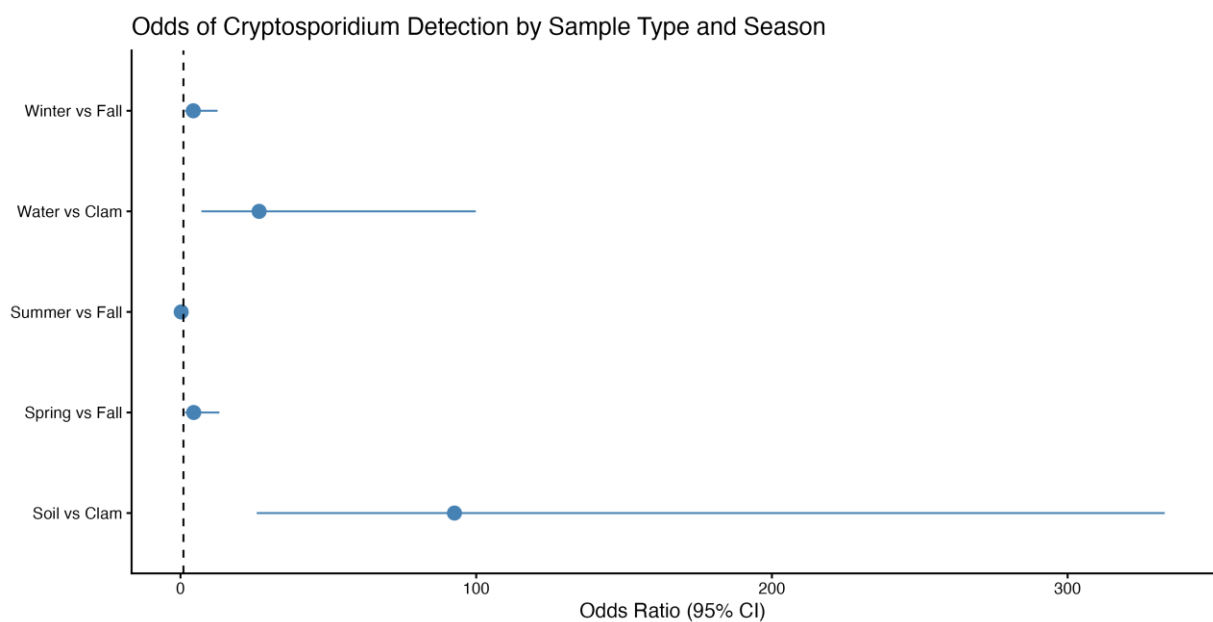


Figure 4. Forest plot of logistic regression showing the differences in *Cryptosporidium* detection across sample type and season. Odds ratios are expressed relative to clams (sample type reference) and Fall (season reference). Only significant model comparisons are shown.

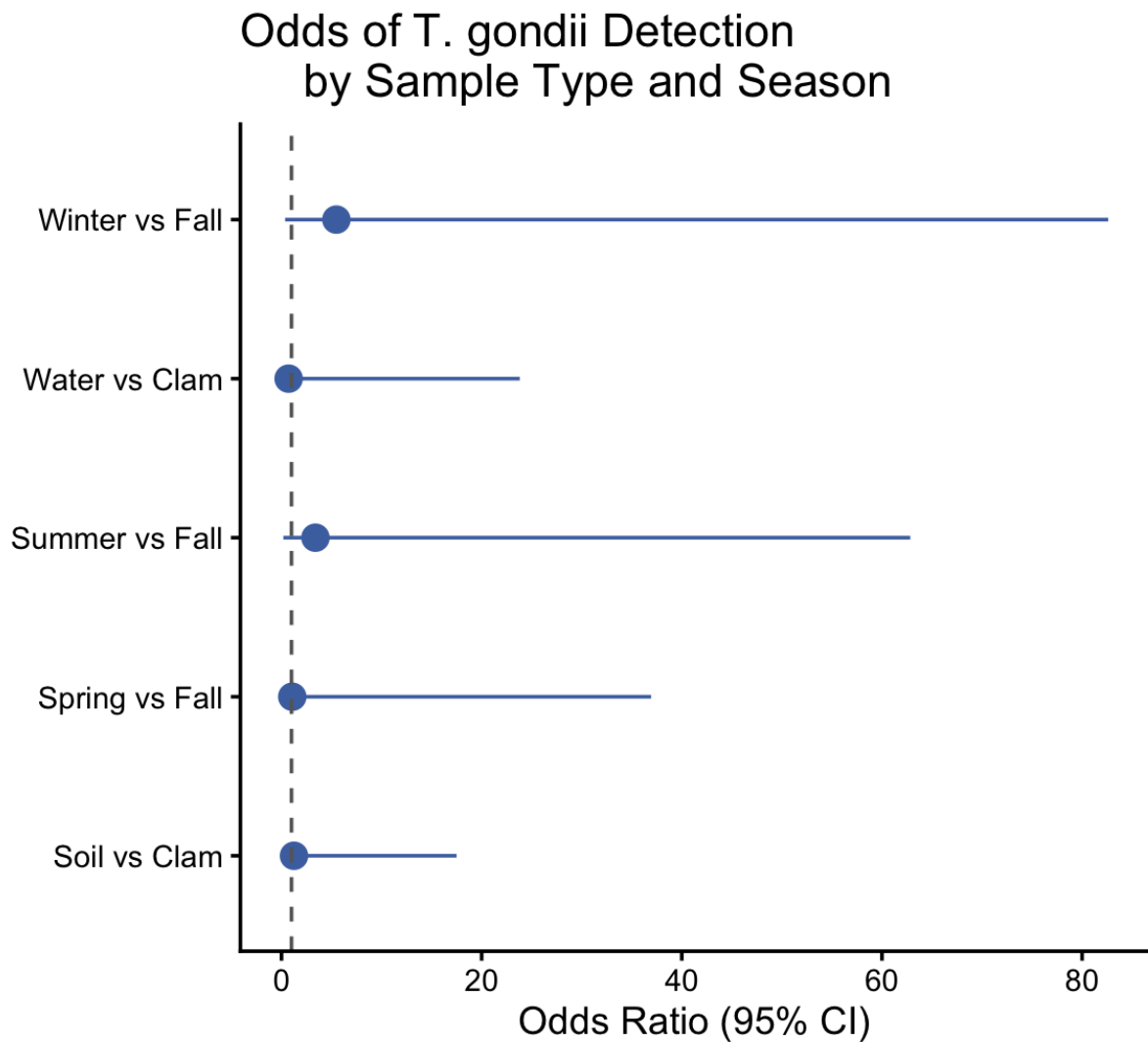


Figure 5. Forest plot of adjusted odds ratios from the logistic regression showing the difference in *Toxoplasma gondii* detection across sample type and season, relative to clams and Fall (references). Only significant model comparisons are shown.

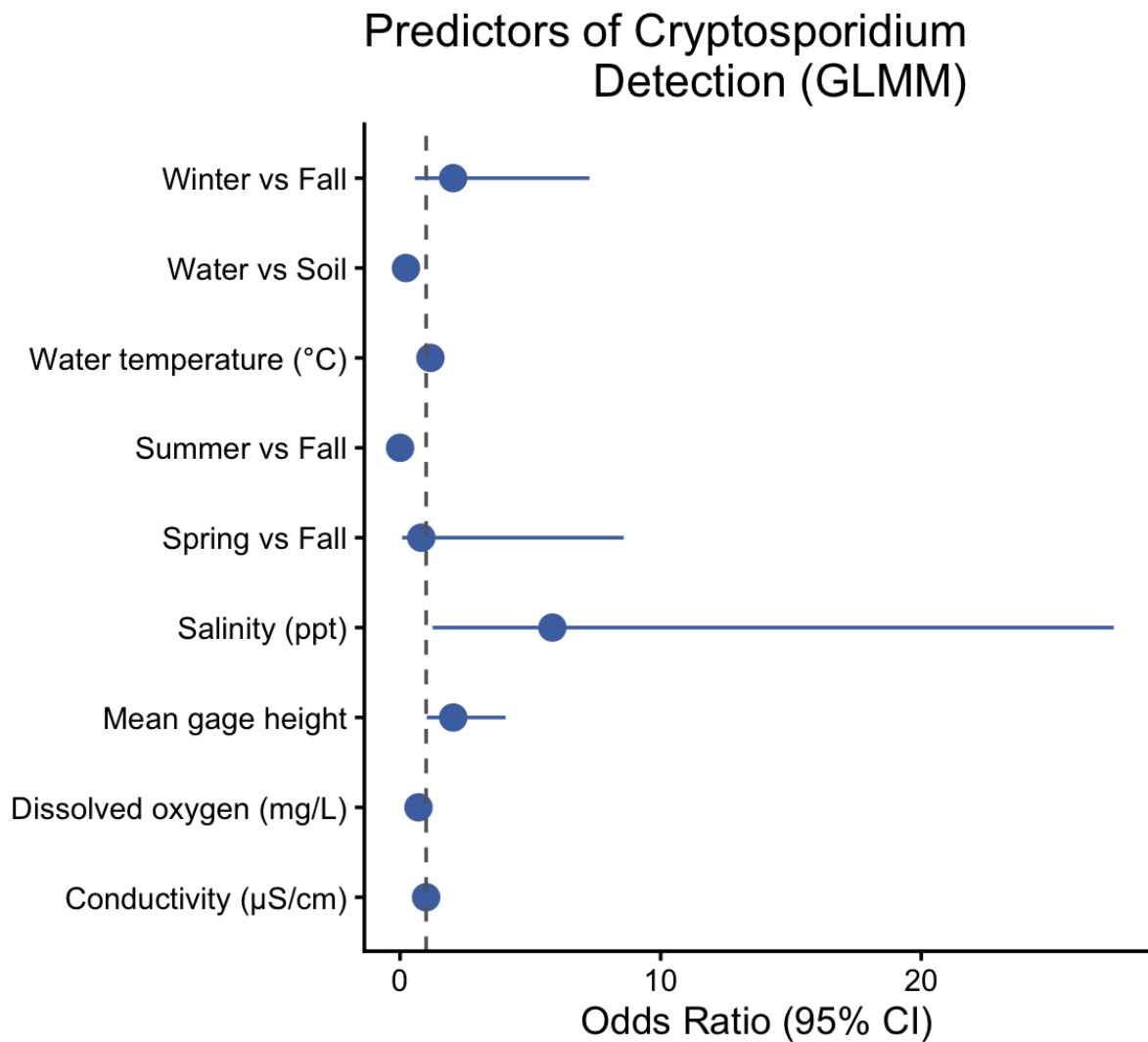


Figure 6. Forest plot of adjusted odds ratios evaluating environmental, hydrologic, seasonal and sample type predictors of *Cryptosporidium* detection based on GLMM. Each point represents the odds ratio from model's estimates and horizontal lines represent the 95% confidence interval

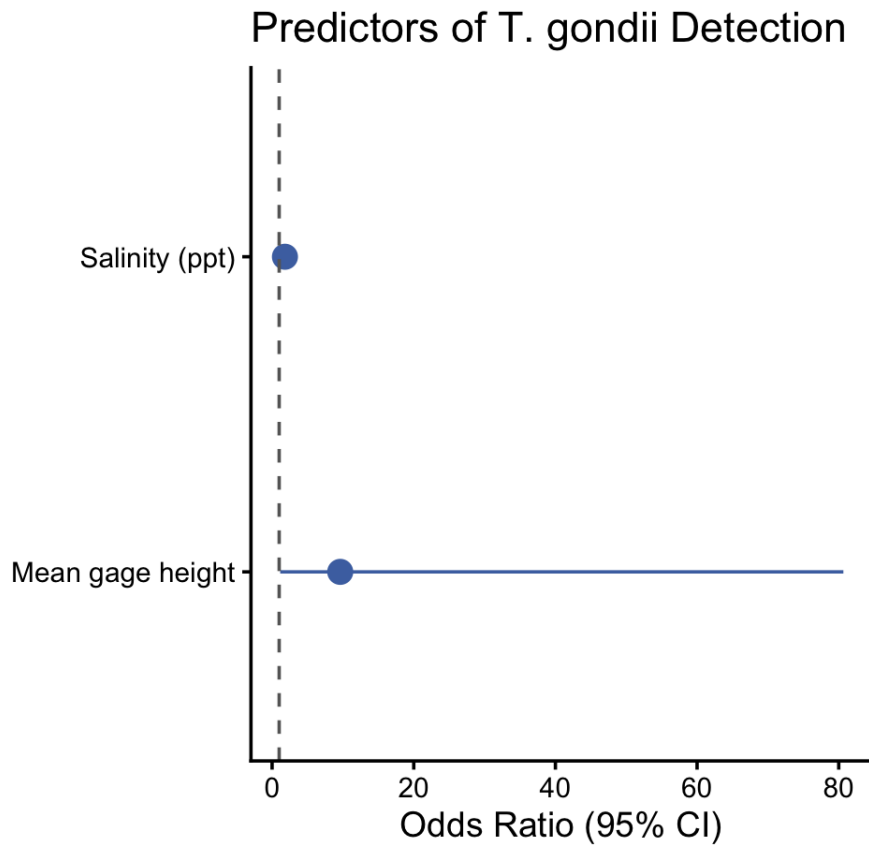


Figure 7. Forest plot of adjusted odds ratios from the logistic regression showing the difference in *Toxoplasma gondii* detection across hydrologic variables. Each point represents the odds ratio from model's estimates and horizontal lines represent the 95% confidence interval.