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ORIGINAL RESEARCH

# Fecal Indicator Bacteria Dynamics in a Surface Flow Constructed Wetland in Southwestern Illinois, USA

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**Abstract** Constructed wetlands have been used to treat wastewater because of their efficiency at removing fecal indicator bacteria (FIB), their low cost, and their ease of maintenance. This study investigates the ability of a surface-flow wetland (0.03 ha in size), constructed in the rural village of Elsah, Illinois, to treat FIB pollution. The objectives of this study were to: 1) compare mean FIB concentrations (specifically thermotolerant coliforms [TTC] and enterococci [ENT]) in the wetland during low versus high precipitation conditions; 2) compare mean FIB levels among different sampling locations along the wetland's treatment gradient; and 3) determine whether FIB and other environmental variables were significantly correlated. Both TTC and ENT levels increased during storm events, likely due to increased mobilization of sediment. Both TTC and ENT were significantly lower in zones located further from the inflow point. The strongest correlation was observed between TTC and ENT, and both parameters were strongly correlated with precipitation.

**Keywords** Fecal indicator bacteria · Constructed wetlands · Bioremediation · Septic pollution

## Introduction

Although nonpoint source pollution is the most well-known pollution problem in the Mississippi River watershed (Karr and Dudley 1981; Young et al. 1989; Pereira and Hostettler 1993), point source pollution remains a concern, despite significant progress since the passage of the 1972 Clean Water Act (Sparks 2010). Even today, the Mississippi River has not reached "swimmable" and "fishable" levels (National Park Service 2014), in part due to contamination by point-source pathogens (Russell and Weller 2013). The exact sources of these pollutants is still being determined (National Park Service 2014), but it is known that bacterial pollution generally comes from fecal matter, which can originate from malfunctioning septic systems (Russell and Weller 2013).

Large river systems are difficult to study (Mihuc and Feminella 2001) and their problems are difficult to change (Gore and Shields 1995), so some studies have examined small tributaries to major river systems as indicators of how to solve pollution problems in the larger river system (Royer et al. 2006). Small streams are also valuable in their own right, as they provide ecosystem services such as linkages to downstream ecosystems, floodwater and groundwater storage, nutrient and sediment removal (Cappiella and Fraley-McNeal 2007), and wildlife habitat (Cappiella and Fraley-McNeal 2007; Pracheil et al. 2013). As the site of one such small tributary (Elsah Creek), Elsah, Illinois has the potential to contribute to pollution remediation in the larger Mississippi watershed.

A recently constructed wetland in Elsah was expected to provide water quality benefits for runoff inflows (List 2014). Prior to construction, runoff from Elsah village flowed down steep hills directly into Elsah Creek, a small tributary of the Mississippi River. Students and professors at Principia College have analyzed the water quality of Elsah Creek since

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1994, and their data suggest that the creek's water is highly contaminated by fecal indicator bacteria (FIB) (J. Cornelius, pers. comm. 2014). Because Elsah lacks a sewage treatment plant, and septic tanks are the primary option for sewage treatment in Elsah, it is generally assumed that high FIB levels in Elsah Creek are caused by malfunctioning or otherwise inadequate septic systems (J. Cornelius, pers. comm. 2014). As a result of its location between these FIB sources and Elsah Creek, the wetland ("Elsah Wetland" from this point on) has potential to filter out FIB before they flow into the creek and eventually the Mississippi.

The objectives of this study were to: 1) compare mean FIB concentrations (specifically thermotolerant coliforms [TTC] and enterococci [ENT]) in the wetland during low versus high precipitation conditions; 2) compare mean FIB levels among different sampling locations along the wetland's treatment gradient; and 3) determine whether FIB and other environmental variables were significantly correlated. It was hypothesized that: 1) FIB concentrations would increase during periods of high rainfall (stormflow) as compared to low rainfall (baseflow); 2) FIB concentrations would decrease with distance from the wetland's inflow point; and 3) there would be an association among environmental variables and FIB. These objectives were testing using a variety of precipitation, water level, and water quality monitoring techniques during the fall of 2014.

## Methods

### Site Description

Elsah Wetland is classified as a surface-flow constructed wetland (Mitsch and Gosselink 2007) or a free water surface (FWS) wetland (Kadlec and Wallace 2009), and is located in Elsah, Illinois, USA. The wetland's surface area is approximately 0.03 ha, with a width of approximately 15 m and a length of approximately 25 m. Average depth in the deepest part of the wetland was approximately 24.4 cm during the study period. Retention time was not quantified, but was estimated to vary between less than 1 day to a few weeks. Runoff from Elsah Village flows down steep hills and through a concrete drainage ditch that terminates in the wetland. The wetland's location at the end of this runoff stream, and immediately adjacent to Elsah Creek, makes it a potential filter for runoff before it flows into the Mississippi River.

Prior to the start of sampling, Elsah Wetland was divided into four "zones" from which samples were collected, each successive zone further from the inflow point. Zone 1 was located nearest the inflow stream; Zone 2 was located after the transition point between the inflow stream and wetland, where topography leveled and emergent plants were noticeable; Zone 3 was located in the middle of the wetland; and Zone 4 was located in the outflow area bordering the creek (Fig. 1). During baseflow conditions, the barrier at the wetland's outflow point prevented wetland water from entering the creek (Fig. 1).

### Sampling Methods

From 18 September to 7 December, 2014, the wetland was sampled five times during baseflow conditions (little to no precipitation 24–48 h before sampling) and five times during stormflow conditions (precipitation 24–48 h before sampling), for a total of ten sampling events. Each sampling event produced four grab samples of water collected from the four respective zones. Grab samples were kept at 4 °C and analyzed within 24 h of collection. Indicator bacterial concentrations undergo diurnal variation, so while not all grab samples were collected at the same time each day due to timing constraints, all samples were obtained between the hours of 11 am and 5 pm, when little variation in bacterial concentration should have occurred (Kim et al. 2009). Each sampling event included zone-by-zone measurements of water temperature, conductivity, total dissolved solids (TDS), dissolved oxygen (DO), pH, and oxidation-reduction potential (ORP) with a multiprobe (Model 556, YSI, Inc., Yellow Springs, OH). Water level was recorded from a staff gauge installed between Zone 2 and Zone 3.

### Water Sample Analyses

Sterile 120 mL IDEXX bottles containing sodium thiosulfate were used to collect grab samples, and four additional, identical bottles were designated for making dilutions of each grab sample. For the purpose of thermotolerant coliform (TTC) detection, two of the four bottles received IDEXX Colilert®-18 indicator, and each of the two received a different water sample dilution (1/10 and 1/100, respectively). For the purpose of enterococci (ENT) detection, the remaining two bottles received IDEXX Enterolert® indicator and the same respective sample dilutions.

Each dilution was transferred to an IDEXX Quanti-Tray®/2000 and incubated according to IDEXX procedures: trays containing Colilert®-18 were incubated in a Binder® oven for 18 h at 44.5 °C, while trays containing Enterolert® were incubated for 24°h at 41 °C. Blanks for Colilert®-18 and Enterolert® were included for each round of incubation. After incubation, Most Probable Numbers (MPNs) for TTC and ENT were determined using the IDEXX MPN table.

### Statistical Analyses

Precipitation 24 and 48 h prior to each event was obtained from the nearest USGS weather station (USGS 05587450 Mississippi River at Grafton, IL, waterdata.usgs.gov), which

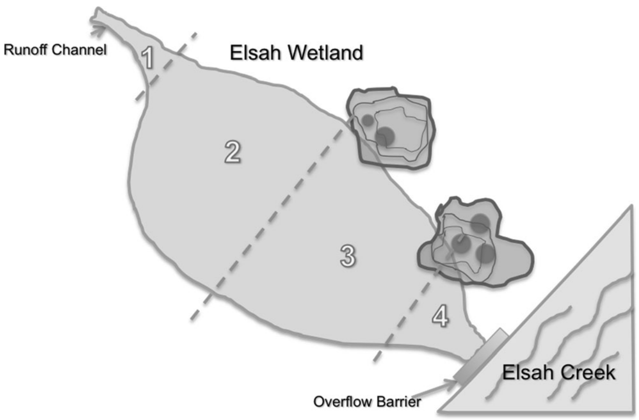


**Fig. 1** Aerial view of Elsah Wetland. Borders between Zone 2 and 3 and Zone 3 and 4 were determined by tree cluster location. The border between Zone 1 and Zone 2 coincided with a change from steep to flat topography, a broadening of the wetland, and the presence of emergent aquatic macrophytes beginning in Zone 2

was located about 8 km from the wetland. Two-way ANOVAs (IBM SPSS Statistics software, Version 19, IBM, New York, NY) were applied to the FIB (TTC and ENT) data, to determine whether FIB concentrations were related to position in the wetland (represented by "Zone"), and/or to precipitation (represented by "FlowRegime"). FIB data was log-transformed to meet ANOVA requirements, and although the log-transformed ENT data missed the normality cutoff according to the Kolmogorov-Smirnov test, ANOVA was used because it is robust to departures from normality, especially when sample sizes are large ($n > 30$). Both the Zone and FlowRegime ANOVA models were significant, but because there was no interaction between zone and flow regime, data was pooled for mean comparison among zones and between flow regimes (Fig. 3). A post-hoc Tukey test was used to compare means pooled by zone. Because flow regime only had two categories (baseflow and stormflow), flow regime data was not analyzed with a Tukey test, which requires that a parameter have at least three categories. Instead, flow regime mean comparison was computed with a Student's *t*-test ($n < 30$) in Microsoft Excel. Associations between FIB and other environmental variables were calculated using Spearman Correlation in SPSS.

## Results

### Precipitation and Water Levels

Precipitation showed high temporal variability. During the first stormflow sampling event (10/2/14), 9.5 cm of rainfall accumulated in the 24 h prior to sampling (Fig. 2), while mean rainfall for the other four storm events was only 1.07 cm. During the fourth stormflow event (10/30/14), only 0.058 cm of rain accumulated (Fig. 2). Water level also showed high temporal variability, but water level did not necessarily correspond to rainfall. For instance, although peak water level coincided with peak rainfall during the heaviest storm on 10/2/14, water level on 9/25/14 was very high, at 33 cm, despite an absence of antecedent rainfall (Fig. 2).

### Fecal Indicator Bacteria

Two-way ANOVAs for both Log(TTC) and Log(ENT) were statistically significant ($p < 0.1$). For Log(TTC), there was no interaction between zone and flow regime (Zone*FlowRegime $p = 0.69$), but taken separately, zone and flow regime had significant effects ($p = 0.00$ and 0.01, respectively). For Log(ENT), there was also no interaction between zone and flow regime (Zone*FlowRegime $p = 0.77$), but as with TTC, zone and flow regime taken separately had significant effects ($p = 0.06$, 0.01, respectively). Post-hoc Tukey tests showed significant differences between Zones 1 and 3, 1 and 4, 2 and 4, and 3 and 4 in terms of mean TTC concentration and a significant difference between Zone 1 and Zone 4 in terms of mean ENT concentration (Fig. 3). For both TTC and ENT, Zone 4 concentrations were low compared to those of other zones (Fig. 3). Student *t*-tests revealed significant differences between stormflow and baseflow FIB concentrations: water had significantly higher mean TTC and ENT concentrations during stormflow conditions (Fig. 3).

Spearman correlation analyses revealed many significant relationships among FIB and environmental variables (Table 1). For TTC, the highest correlation was observed with ENT (0.69), followed by Precip 48 (0.53) and Precip 24 (0.47). ENT also had the highest correlations with Precip 48 (0.65) and Precip 24 (0.51), as well as a significant negative relationship with pH (-0.41) (Table 1).

## Discussion

### Hydrology and Fecal Indicator Bacteria

The hypothesis that FIB concentrations would vary from baseflow to stormflow was supported in the case of both TTC and ENT. As key contributors to wetland function, precipitation and water level have potentially large impacts on a wetland's ability to remove contaminants such as FIB. In Elsah Wetland, mean concentrations for both TTC and ENT were significantly higher during stormflow conditions (Fig. 3). Additionally, precipitation, both 24 and 48 h before sampling, was significantly correlated to TTC (0.47 and 0.53, respectively) and ENT (0.51 and 0.65, respectively) (Table 1).

While some sources suggest that rainfall dilutes contaminants (Kadlec 1989; US EPA 1999; Strauch et al. 2014), most hydrology studies have observed increases in FIB concentrations with increases in rainfall. This relationship has been

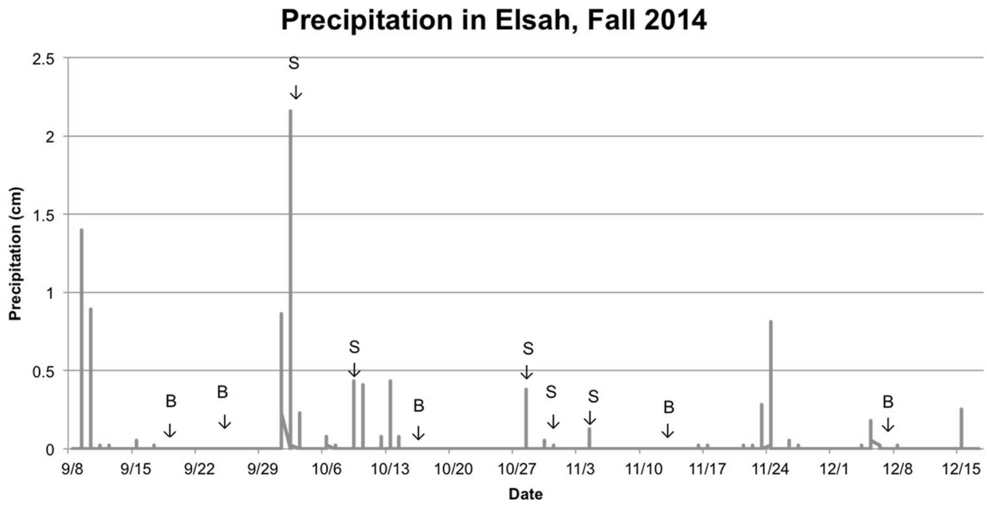


**Fig. 2** Precipitation data from the Grafton Weather Station. Arrows represent sampling events. "B" indicates a baseflow event, while "S" indicates a stormflow event

observed in studies of freshwater creeks and rivers (Crabill et al. 1999; Dorner et al. 2006; Rowny and Stewart 2012; Lee et al. 2014) as well as coastal waters (Evanson and Ambrose 2006; Fries et al. 2006; Lee et al. 2006; Fries et al. 2008; Dwight et al. 2011; Walters et al. 2011). Some of these studies specifically note that this positive relationship is generally caused by storm events re-suspending sediment-bound bacteria into the water column (Crabill et al. 1999; Dorner et al. 2006; Fries et al. 2006; Lee et al. 2006; Fries et al. 2008; Walters et al. 2011). The FIB-rainfall relationship appears less frequently in the wetland literature, although Birch et al. (2004) found that rainfall reduced a treatment wetland's TTC removal efficiency (with the most intense storms causing a large drop in TTC removal efficiency), and that elevated TTC concentrations coinciding with rainfall were likely due to particle re-suspension. Mitsch and Gosselink (2007) confirm this effect, noting that intense storms generally cause sudden outflows of contaminants in wetlands. In Elsah, an unusually intense storm surrounding the 10/2/14 sampling event corresponded to very high FIB concentrations, and may have skewed overall FIB data for storm events (Fig. 3).

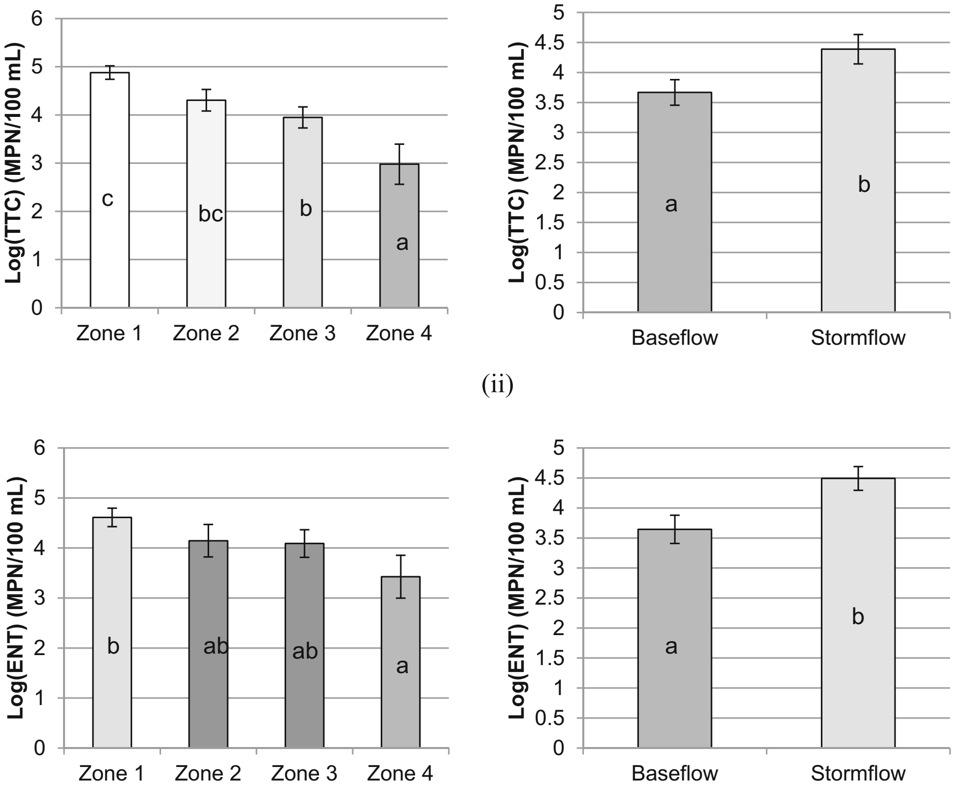
Water level did not always increase in proportion to precipitation, probably because water level was manipulated as part of an adaptive management plan, for which the wetland was either partially drained or supplemented with water from Elsah Creek to deal with herbivory from a muskrat (*Ondatra zibethicus*) (M. Rhaesa, personal communication, December 7, 2014). However, precipitation and water level were significantly correlated (Table 1), which implies that the adaptive management plan did not completely counteract the effects of precipitation. Water level may have increased with storm events as increased rain caused the creek to overflow into the wetland, thereby increasing water level.

Although ENT were positively correlated with water level, TTC had no significant correlation with water level (Table 1). One explanation for this result is that ENT and TTC may have originated from different sources. A study of bacterial markers by Flood et al. (2011) suggests that ENT is more closely related to animal waste sources than to human sewage. Given that Elsah Creek passes through rural areas, presumably with wildlife exposure, wildlife may have contributed to elevated ENT levels in the creek. Viau et al. (2011) suggested that ENT may come from agricultural land, which would support the idea that Elsah Creek contains high ENT, as the creek passes through an agricultural watershed. If high ENT concentrations occurred in Elsah Creek, and wetland water level increased with creek overflow, then increases in water level solely due to creek water may have coincided with elevated ENT concentration regardless of the factors increasing TTC concentration.

## Zone and Fecal Indicator Bacteria

The hypothesis that bacteria levels would vary among the four zones was supported. The two-way ANOVA showed that Zone was a significant factor affecting both TTC and ENT, with the lowest TTC and ENT concentrations observed in Zone 4 (Fig. 3). Most constructed wetland studies, instead of comparing FIB concentrations among zones, have focused exclusively on comparing FIB concentrations in influent and effluent, and have noted a decrease in FIB concentration once water passes through the wetland (Davies and Bavor 2000; Coleman et al. 2001; Knowlton et al. 2002; Steer et al. 2002; Arias et al. 2003; Hench et al. 2003; Karathanasis et al. 2003; Solano et al. 2004; Ou et al. 2006). The overall water quality improvement observed in Elsah Wetland is therefore consistent with the general scientific consensus.

Hydrology and wetland studies generally assume that FIB bind to soil particles in the water and are immobilized as soil particles settle (Davies and Bavor 2000; Vymazal 2005; Hathaway et al. 2011). Zone 4 may have experienced



(i) (ii)

(iii) (iv)

**Fig. 3** Mean TTC comparisons among zones (i) and between flow regimes (ii), and mean ENT comparisons among zones (iii) and between flow regimes (iv). Figures reflect the results of post-hoc Tukey tests ((i) and (iii)) and Student's *t*-tests ((ii) and (iv)). *Error bars* represent the standard error of the mean. Different letters indicate a statistically significant difference among means

particularly high rates of sedimentation, and therefore FIB immobilization. Zone 4 was substantially narrower and shallower than other zones (the only exception being Zone 1, the runoff channel itself, which is not part of the wetland). Because particles settle out of the water column more easily when the basin cross-section is small (O'Green and Bianchi 2015), Zone 4's small cross section would have encouraged FIB-containing particles to settle and escape detection by water sample analysis. Increased exposure to UV radiation may have contributed to low FIB concentrations in Zone 4. UV radiation is a major cause of FIB mortality in water (Manios et al. 2006; Boukef et al. 2010; Cho et al. 2010), including wetlands (Richter and Weaver 2003). Because water was shallowest in Zone 4, UV radiation may have reached FIB associated with submerged biofilms and sediment most easily. Confirming this effect was beyond the scope of this study, and is recommended as a topic for future research. Bacterial adsorption to biofilms, an important removal mechanism for FIB in constructed wetlands (Stott and Tanner 2005; Osem et al. 2007; Kalibbala et al. 2008; Lohay et al. 2012; Mulling et al. 2013; Morató et al. 2014), may also have increased FIB removal in Zone 4 due to high plant densities there.

**Table 1** Spearman correlation coefficients for significant ($p$-value > 0.1) correlations among fecal indicator bacteria and environmental variables

| | Fecal Coliform (MPN 100 mL$^{-1}$) | Enterococci (MPN 100 mL$^{-1}$) | Temperature (°C) | Conductivity (mS cm$^{-1}$) | TDS | DO (Mg L$^{-1}$) | pH | ORP | Precip 24 (cm) | Precip 48 (cm) |
|---|---|---|---|---|---|---|---|---|---|---|
| Enterococci | 0.69 | | | | | | | | | |
| Temperature | NS | NS | | | | | | | | |
| Conductivity | NS | −0.29 | −0.36 | | | | | | | |
| TDS | NS | −0.28 | −0.36 | 1.0 | | | | | | |
| DO | NS | NS | NS | NS | NS | | | | | |
| pH | NS | −0.41 | 0.35 | 0.29 | 0.29 | NS | | | | |
| ORP | 0.33 | NS | 0.52 | −0.45 | −0.46 | NS | NS | | | |
| Precip 24 | 0.47 | 0.51 | NS | −0.63 | No Data | NS | NS | 0.30 | | |
| Precip 48 | 0.53 | 0.65 | NS | −0.59 | No Data | NS | NS | 0.44 | 0.82 | |
| Water Level | NS | 0.39 | 0.40 | −0.43 | No Data | NS | NS | NS | 0.47 | 0.41 |

"NS" means "not significant." (*TDS* Total Dissolved Solids, *DO* dissolved oxygen, *ORP* oxidation reduction potential, Precip 24: precipitation amount in the last 24 h, Precip 48: precipitation amount in the last 48 h)

Zone 4 likely experienced greater treatment due to its position along the wetland's treatment gradient. As hydraulic residence time (HRT) increases, bacteria exposure to treatment also increases (Arias et al. 2003; Karathanasis et al. 2003; Ou et al. 2006; O'Green and Bianchi 2015). While this study did not quantify retention time, estimates of approximate residence time in each zone were made based on observation. Water movement in the wetland was sluggish to nonexistent during baseflow conditions, as Zone 1 inflows are shallow or intermittent, but more consistent water movement in Zone 1, triggered by storm events, presumably forces water down the mild elevation gradient to Zone 4. In this case water in Zone 4 represents the longest HRT in the wetland; water must take a longer path to get to Zone 4, and is unlikely to escape due to the overflow barrier, especially once the wetland reverts to baseflow conditions (Fig. 1). Periodic diversion of water into the wetland from Elsah Creek also triggered water movement towards Zone 4. Ripples on the wetland's surface from these inputs were observed as far along the gradient as Zone 3, but Zone 4 always appeared quiescent, probably because of its narrow basin morphology and dense vegetation. This lack of disturbance in Zone 4 likely contributed to low FIB concentrations, as sediment-bound FIB were not kept in suspension by fast-moving creek diversions.

### Other Environmental Variables and Fecal Indicator Bacteria

The hypothesis that there would be an association between FIB and environmental variables was partially supported. Thermotolerant coliforms were positively correlated to ORP; ENT were negatively correlated with pH; and TTC and ENT were positively correlated to each other (Table 1). A number of studies have found similar results. For example, studies of river water quality found positive relationships between TTC and ENT (Cabral and Marques 2006; Suzuki et al. 2012), and the positive correlation observed between TTC and ORP (Table 1) aligns with Belmont et al.'s (2004) results, in which Eh (related to ORP) decreased as water moved through a wetland.

Several other studies obtained results that differ from those observed in Elsah Wetland. The lack of correlation between FIB and temperature in Elsah Wetland, the opposite of the trend observed in other studies (Alcalde et al. 2003; Walters et al. 2011), was likely due to the short sampling period, which covered only the fall season. Other studies found that FIB removal in wetlands changed with the seasons (Shellenbarger et al. 2008; Papadopoulos et al. 2011; Pan and Jones 2012), so future studies should examine Elsah Wetland over an entire year or multiple years. Belmont et al. (2004) and Ou et al. (2006) observed a reduction in constructed wetland pH when comparing influent to effluent, the opposite trend of that observed in Elsah Wetland, in which pH increased with ENT reductions as water moved through the wetland (Table 1). pH is a complex parameter to study as many variables interact with pH, so the significance of this discrepancy should be examined further by follow-up research. Studies have shown an increase in DO as water moves through a treatment wetland system (Hench et al. 2003; Belmont et al. 2004; Ou et al. 2006). In Elsah Wetland, however, no correlation between DO and FIB was observed (Table 1). Future research could approach this issue by examining the wetland's hydrologic dynamics in more detail. Suter et al. (2011) found that turbidity was positively correlated to ENT, so the negative correlation between TDS (a component of turbidity) and ENT observed in Elsah Wetland was unexpected (Table 1). Because turbidity and TDS measure different aspects of water quality, future research should measure turbidity directly, especially given the importance of turbidity for predicting pollution discharge following storm events (Struck et al. 2008).

## Limitations and Conclusions

The specifics of constructed wetland FIB removal mechanisms are not well understood (Stottmeister et al. 2003), and constructed wetlands have been compared to "black box" systems involving unknown biogeochemical transformations (Haberl et al. 2003). This study seeks to shed light on the "black box" as it applies to small, surface-flow constructed wetlands, by going beyond inflow versus outflow comparisons to address changes in FIB and other water quality parameters along a treatment gradient. However, as evidenced by the complex results obtained in the correlation analysis, future studies should continue to examine the biogeochemistry and hydrology of the wetland in-depth, and should consider alternative testing methodologies that could provide data on precise movements and sources of FIB. IDEXX methodology is EPA-approved (US EPA 2003) and is easy to use, making it an ideal method for a preliminary study such as this one. However, alternative methods may be preferable for future research (see Baker and Herson 1999). For instance, the microbial source tracking approach uses genetic markers to track specific contaminants as they move through water (e.g. Flood et al. 2011; Lee et al. 2014; Morató et al. 2014; Ridley et al. 2014), and could be applied to tracking the movement of FIB.

From a management perspective, further research is recommended to address the consistent failure of Elsah Wetland to meet water quality standards. Even in low-FIB Zone 4, ENT MPNs often exceeded the recommended surface water standard of 33 MPN/100 mL (US EPA 1986), while TTC concentration often exceeded Mitchell and Stapp's (1997) recommendation that treated sewage effluent contain no more than 200 TTC colonies/100 mL. Constructed wetlands' failure to meet water purification guidelines is documented in the literature (e.g. Belmont et al. 2004), highlighting the fact that most treatment wetlands are designed to "polish" wastewater rather than remove high concentrations of sewage contamination (Solano et al. 2004). Additionally, Mitsch and Gosselink

(2007) caution that constructed wetlands using natural systems for treatment may have unpredictable results. Elsah Wetland was no exception, as FIB levels fluctuated among sampling events. Given this water quality variability, a management plan for Elsah Wetland should utilize a model to predict FIB concentration, such as the commonly used first-order decay model (see Wong and Geiger 1997; Struck et al. 2008). Despite its limitations, Elsah Wetland is an important addition to a community for which wastewater treatment and septic tank monitoring is currently unfeasible. Given its status as a constructed-created wetland hybrid, with few controls on its operation, the pollution remediation observed in Elsah Wetland suggests that relatively low-cost treatment wetlands can be effective. Installing an additional wetland along the same inflow stream or elsewhere in the village may provide sufficient FIB removal to enable Elsah Wetland outflows to meet EPA standards.

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