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Removal of fecal indicator bacteria and antibiotic resistant genes in constructed wetlands

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Abstract

Wastewater discharge evidently increased bacterial diversity in the receiving waterbodies. The objective of this study was to evaluate the effectiveness of a constructed wetland in reducing fecal indicator bacteria (FIB) and antibiotic resistant genes (ARGs). We determined the prevalence and attenuation of fecal indicator bacteria including *Escherichia coli* and enterococci, along with ARGs, and human-associated *Bacteroidales* (HF183) markers by quantitative polymerase chain reaction (qPCR) method. Three types of water samples (inlet, intermediate, and outlet) from a constructed wetland were collected once a month from May to December in 2013. The overall reduction of *E. coli* was 50.0% based on culture method. According to the qPCR result, the overall removal rate of *E. coli* was only 6.7%. Enterococci were found in 62.5% of the wetland samples. HF183 genetic marker was detected in all final effluent samples with concentration ranging from 1.8 to 4.22 $\log_{10}$ gene copies (GC)/100 ml. Of the ARGs tested, erythromycin resistance genes (*ermF*) were detected in 79.2% of the wetland samples. The class 1 integrase (*intI1*) was detected in all water samples with concentration ranging from 0.83 to 5.54 $\log_{10}$ GC/100 ml. The overall removal rates of enterococci, HF183, *intI1*, and *ermF* were 84.0%, 66.6%, 67.2%, and 13.1%, respectively.

Keywords Constructed wetlands · Fecal indicator bacteria · qPCR · ARGs · Water reuse

Introduction

Wastewater is increasingly recognized as another option that can be treated and then used as a resource (Greenway 2005). One major issue with the use of this wastewater for potable reuse is the presence of both chemical and microbial pollutants that would adversely affect human health (Tran et al. 2015, 2016). The removal of pathogenic bacteria and antibiotic resistance genes (ARGs) from municipal wastewater treatment processes such as membrane bioreactor systems and conventional activated sludge systems, as well as more advanced Bardenpho treatment, has been well documented (Chen et al. 2010, 2016; Le et al. 2018; Xue et al. 2019). However, there are a few disadvantages of these technologies, such as expensive, not entirely feasible for application in rural areas, and unwanted chemical by-products (Guo et al. 2008;

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Wu et al. 2015; Waller and Bruland 2016; Lu et al. 2016). Compared with wastewater treatment technology, constructed wetlands, on the other hand, have been recognized as environmental-friendly and eco-technology due to their low-cost and easy operation (Faulwetter et al. 2009; Tu et al. 2014; Rachmadi et al. 2016; Chen et al. 2016), although it has disadvantages such as requires large area and the removal efficiency may be affected by environmental factors (Ramirez et al. 2005).

Constructed wetlands are artificial designed and constructed to simulate natural processes to treat domestic wastewaters. As wastewater passing through natural wetlands, it is filtered through sediments and finally replenishes the aquifer, providing clean water to the city and other municipal sources (Gelt 1997). Previous study suggested that constructed wetlands were able to remove organics, total carbon, and nutrients effectively (Tu et al. 2014). It was also reported that fecal indicator bacteria removal in constructed wetland was ranged from 97 to 99%, such as coliforms and *Escherichia coli* (Quiñónez-Dìaz et al. 2001; Wu et al. 2010). In addition to removal of bacteria, previous researches demonstrated that mesocosm-scale constructed wetlands are capable of removing 18 ARGs varied between 50 and 85.8% (Chen et al. 2010, 2016). However, these experiments were conducted by using mesocosm-scale constructed wetlands. In 2017, Yi et al. (2017) found several ARGs (*intl1*, *sul1*, *sul2*, and *qnr*A) from landfill leachate could be effectively removed by one hybrid constructed wetlands system. So far, there is limited data on removal of ARGs in treated wastewater by full-scale constructed wetlands system. More importantly, unlike other water treatment facilities, physicochemical and biological factors may affect fecal indicator bacteria removal in constructed wetlands, such as seasonal fluctuations, vegetation, sedimentation, and water composition (Kadlec and Wallace 2008; Wu et al. 2016). Increased water temperature, pH, and retention time are known to increase treatment efficiency, while increased turbidity is associated with decreased pathogen removal (Maiga 2017).

In the present study, quantitative polymerase chain reaction (qPCR) results can be used to determine the effectiveness of the wetlands in removing pollution from water. The concentration of human-associated *Bacteroidales* marker (HF183) (Bernhard and Field 2000; McLellan and Eren 2014) and genetic markers targeting fecal indicator bacteria (FIB) were determined in inlet, intermediate, and outlet water samples. QPCR were applied to understand the sources of fecal contamination by targeting host-associated molecular markers (Xue et al. 2017, 2018a, b, c; Tandukar et al. 2018). When compared to other culture-based methods, PCR-based methods are able to provide source information for a water sample that has been recently contaminated as they are obligate anaerobes that only are able to

survive for short periods of time and have proven to be more successful at accurately detecting DNA and provided more specific and accurate readings (Aly et al. 2012; Kapoor et al. 2015; Xue et al. 2017, 2018c). Antibiotics are used in medicine and livestock animals for prophylactic, therapeutic, and growth-promoting purposes (Davies and Davies 2010; Hijosa-Valsero et al. 2011). Antibiotic residues can be discharged into environments though feces or urine, posing risks to not only human health but also the environment (Costanzo et al. 2005; Kotzerke et al. 2008; Liu et al. 2009; Underwood et al. 2011). The increase in antibiotic usage in humans and livestock leads to spread of occurrence of antibiotic-resistant bacteria (ARB) and ARGs in the environment (Pruden et al. 2006; Tao et al. 2010; Su et al. 2012), which may lead to source of resistant bacteria in humans. We are particularly interested in ARGs of *intI1* and *ermF* that are responsible for resistance to class I integrase and erythromycin antibiotics, respectively. Class 1 integrons have been considered to be the key of worldwide antibiotic resistance problem, and *intI1* gene has been known to be a proxy for horizontal gene transfer of ARGs (Gillings et al. 2008; Le et al. 2016). The erythromycin treatment failure has been noted in the past few years and the erythromycin resistance might because of the presence of the *ermF* genes in the bacterial genome (Chung et al. 2002; Xing et al. 2015).

In the present study, we evaluated the removal efficiency of FIB, HF183 genetic marker, as well as *intI1* and *erm*(F) in surface flow of the constructed wetlands and explore the relationship between FIB and HF183 genetic marker concentrations. We compared various physicochemical water quality parameters such as temperature, pH, and turbidity to investigate whether those factors have any effect or correlation on the prevalence of the bacteria and ARGs. In addition, we analyzed the inlet, intermediate, and outlet samples to compare the efficiency rates among each step of the treatment processes.

Materials and methods

Description of the constructed wetlands studied

This study was conducted in the Sweetwater Wetlands located in the city of Tucson, Arizona. The Sweetwater Wetlands has been operated for about 20 years and is one of the most important functional, environmental, and educational components of Tucson's reclaimed water system. It provides additional treatment of the treated wastewater from the Roger Road Water Reclamation Facility as well as backwash water from mixed media filters at the City of Tucson Water Reclamation Plant. This wetland is approximately 0.03 km^2 in size with a

retention time of 7 days (Vidales-Contreras et al. 2006; Rachmadi et al. 2016). Major vegetations include cattails, bulrushes, large cottonwood, and willow trees. The recovered wastewater is distributed for reuse in Tucson's golf courses, parks, schools, and other large turf irrigation areas (Fig. 1) (https://www.tucsonaz.gov/water/about-sweetwater-wetlands-and-access).

Sample collection

In total, 24 surface water samples were collected from the Sweetwater Wetlands by using sterile 1-l plastic containers over a period of 8 months. The water samples were then kept on ice and delivered to Dr. Gerba's laboratory at the University of Arizona within 6 h. The parameters of water temperature, dissolved oxygen, salinity, specific electrical conductivity, and turbidity were measured in situ by using the PCSTEst35 (Eutech Instruments, Singapore) through inlet, intermediate, and outlet samples.

DNA extraction from water samples

On the day of sample arrival, 500 ml from each sample was filtered through a 0.45 μm-pore-size membrane filter and 47-mm-diameter nitrocellulose membrane (Thermo Fisher Scientific, Waltham, MA) under vacuum. After filtration, sterile forceps were used to aseptically fold each of the membrane filters and placed in separate 50-ml conical tubes and stored at −20 °C until the DNA extraction. Genomic DNA was extracted from membrane filters using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. Membrane filters were cut into small pieces with sterile scissors to maximize DNA extraction efficiency, and the extracted DNA was quantified with

Fig. 1 Sampling sites in Sweetwater Wetlands in the City of Tucson, Arizona, USA

a NanoDrop ND-2000 UV spectrophotometer (Thermo Fisher Scientific, Walkham, MA). The DNA samples were stored at −20 °C prior to use.

Quantitative real-time PCR

Quantitative PCR assays targeting *E. coli*, enterococci, human-associated *Bacteroidales* (HF183), and ARGs of *erm*(F) and *intI1* were conducted (Table 1). For *E. coli*, enterococci, the PCR reaction mixture (20 μl) contained 1× PerfeCTa qPCR ToughMix (Quanta Biosciences, Beverly, MA), 0.5 μM of each primer, 0.5 μM of probe, and 2.5 μl of the template DNA. For HF183, *erm*(F), and *intI1*, the reaction mixture (15 μl) contained 1× SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA), 0.2 μM of each primer, and 2.5 μl of the template DNA. All PCR reactions were performed in duplicate using the Applied Biosystems StepOne™ Real-Time PCR system (Thermo Fisher Scientific). The amplification conditions consisted of a hold at 95 °C for 10 min, followed by 40 cycles of 95 °C 15 s, 60 °C 30s, and 72 °C 30s. A calibration curve with concentrations spanning the range from 10 to 10^6 gene copies per reaction with two replicates was generated. Duplicate no-template controls were included in each run. The calibration standard curves covering the range from 10 to 10^6 gene copies per reaction were prepared using serial dilutions of plasmid DNA or commercial genomic DNA purchased from ATCC (700926DQ for *Escherichia coli* and ATCC 29212Q-FZ for enterococci; Manassas, VA, USA). For SYBR Green-based qPCR assays (HF183), plasmid DNA obtained from Dr. Feng's lab at Auburn University, Alabama. For ARGs SYBR Green-based qPCR assays, *intI1* environmental *E. coli* isolate and *erm*(F) erythromycin-resistant environmental *Staphylococcus* spp. isolates were obtained from Dr. John Brook's lab from USDA-ARS at Mississippi State. Sequencing results were confirmed by consulting the NCBI website using the nucleotide Basic Local Alignment Search Tool (BLAST). Plasmid DNA concentration was measured with a NanoDrop ND-2000 UV spectrophotometer (Thermo Fisher Scientific), and the gene copy numbers were calculated.

Data analyses

Statistical analyses were performed using SAS® 9.3 software. A Spearman's test was used to identify relationships between the environmental parameters and the presence of *E. coli*, enterococci, HF183, and ARGs, if any. The coefficient correlation (*r*) value for each regression model was used to provide an indication of
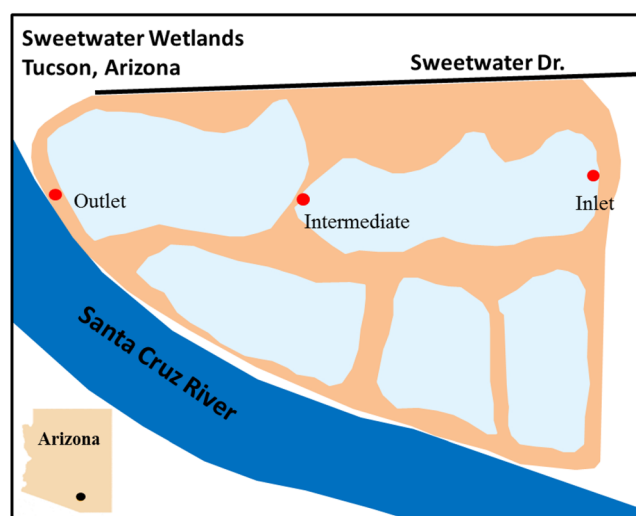
Table 1 Summary of qPCR assay conditions in this study

qPCR assays	Primer/probe	Target organism	Sequence (5′–3′)	Annealing T (°C)	Size (bp)	Reference
TaqMan-based qPCR	784F 866R Ec807	E. coli	GTG TGA TAT CTA CCC GCT TCG C AGA ACG GTT TGT GGT TAA TCA GGA FAM-TCG GCA TCC GGT CAG TGG CAG T-TAMRA	60	83	Frahm and Obst 2003
	EnteroF1A EnteroR1 Entero. probe	Enterococcus spp.	AG AAA TTC CAA ACG AAC TTG CAG TGC TCT ACC TCC ATC ATT FAM-TGG TTC TCT CCG AAA TAG CTT TAG GGC TA-TAMRA	60	90	Ludwig and Schleifer 2000
SYBR green–based qPCR	HF183F HF265R	Human Bacteroidales	ATC ATG AGT TCA CAT GTC CG TAC CCC GCC TAC TAT CTA ATG	60	82	Bernhard and Field 2000
	erm(F)-189F erm(F)-497R	Erythromycin-resistant gene	CGA CAC AGC TTT GGT TGA AC GGA CCT ACC TCA TAG AGA AG	56	309	Chen et al. 2007
	HS463a HS464	Integrase gene	CTG GAT TTC GAT CAC GGC ACG ACA TGC GTG TAA ATC ATC GTC G	60	473	Stokes et al. 2006

goodness of fit. An ANOVA test was used to identify any seasonal differences between site-specific data.

Results

Physicochemical and biological water quality parameters

Twenty-four water samples were collected from the Sweetwater Wetlands from the months of May to December in 2013. In our study, the correlation between physicochemical parameter and FIB as well as ARGs was studied. Turbidity of the water samples (Table S1) was highest in the inlet water, at a value of 30.2 NTU. *E. coli* was found in 100% of water samples from inlet, intermediate, and outlet locations. The inlet water samples had consistently high *E. coli* concentrations, with the highest concentration was found in November of 3.74 $\log_{10}$ of MPN/100 ml over the sampling period (Fig. S1). The geometric mean of *E. coli* concentration over the study period in outlet water samples was decreased by 50.0% compared with inlet water samples (Fig. 2).

Occurrence and reduction of *E. coli*, enterococci, and HF183 determined by qPCR

E. coli was found in 100% of the water samples during the study period with concentrations ranging from 0.68 Log_{10} GC/100 ml (intermediate sample of September) to 1.89 Log_{10} GC/100 ml (outlet sample of December) (Table 2). Except for December samples, the reduction of *E. coli* concentrations from inlet to intermediate samples ranged from 31.3% in May to 70.2% in October. Compared with intermediate water samples, the geometric mean of *E. coli* concentrations in outlet water samples increased by 65.8% (Fig. 2). *E. coli* concentrations in outlet water samples collected in September and December increased by 96.3% and 293.9%, respectively. The geometric mean of *E. coli* concentration over the study period in outlet water samples decreased by 6.7% compared with inlet water samples.

The enterococci markers were found in 62.5% (15/24) of water samples (Table 2), with concentrations ranging from 0.81 Log_{10} GC/100 ml (Intermediate sample of July) to 3.88 Log_{10} GC/100 ml GC/100 ml (inlet sample of May). From inlet to intermediate water samples in all months except for December, the removal rates of enterococci ranged from 70.0% in June to 99.0% in July. The overall concentration of enterococci in outlet water samples decreased by 84.0% compared with inlet water samples.

Human-associated *Bacteroidales* (HF183) was detected in 95.8% (23/24) of water samples, with concentrations ranging from 1.80 Log_{10} GC/100 ml (outlet water sample in Oct.) to 4.22 Log_{10} GC/100 ml (outlet sample

Fig. 2 Removal rate of FIB, HF183, and ARGs from inlet to intermediate and to outlet in Sweetwater constructed wetland. Positive value indicates the concentration of tested indicators reduced during the treatment. Negative value indicates the concentration of tested indicators increased during the treatment
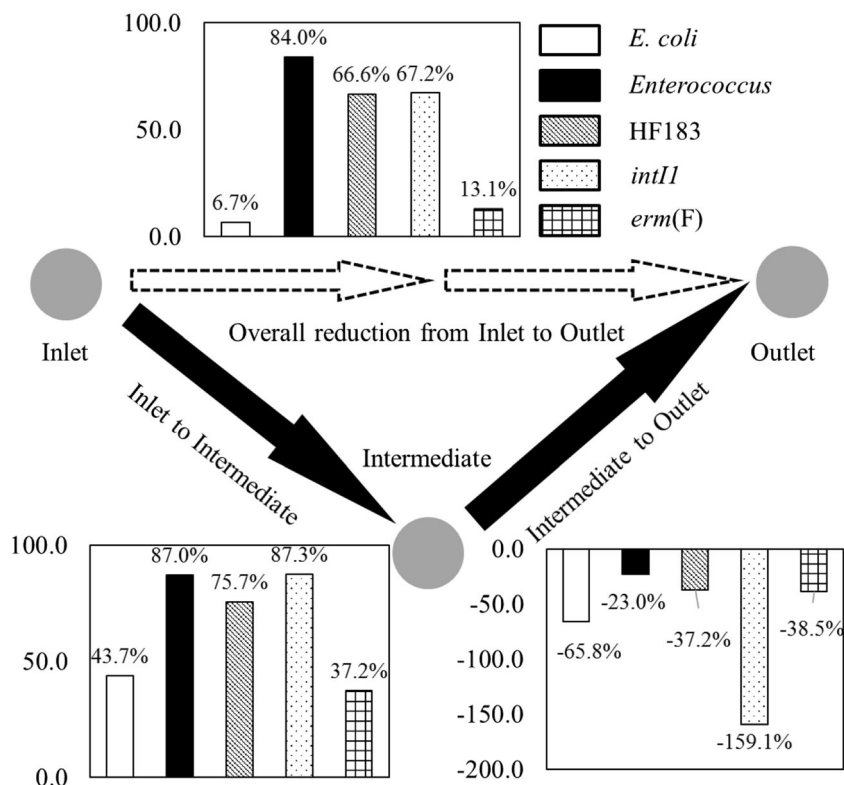
of December). Except for June and September, the reduction rates of HF183 from inlet to intermediate water samples ranged from 63.0% (in August) to 91% (in July). The HF183 concentrations increased from inlet to intermediate in June and September, with increasing rate of 90% and 3%, respectively. Compared with intermediate water samples, the geometric mean of HF183 concentrations in outlet water samples increased by 37.2%. Except for a decrease of 64.7% from the inlet to intermediate sample in December, the geometric mean of HF183 in the outlet samples decreased by 66.6% when compared to inlet samples (Fig. 2).

Occurrence and reduction of ARGs determined by qPCR

In addition to FIB and HF183, the occurrence of ARGs, such as intI1 and erm(F), was also determined using qPCR method in the present study. All Sweetwater samples tested positive for intI1 marker. The concentrations of intI1 marker ranged from 0.83 Log_{10} GC/100 ml to 5.54 Log_{10} GC/100 ml. The removal rate of intI1 in intermediate samples ranged from 3.4% to 99.6% compared with inlet samples. The geometric mean of intI1 for outlet samples increased by 159.1% compared with intermediate samples. The overall removal rate of

Table 2 qPCR results for FIB, HF183, and ARG genetic markers during sampling month

Sampling month	E. coli (Log_{10} of GC/100 ml)			Enterococci (Log_{10} of GC/100 ml)			HF183 (Log_{10} of GC/100 ml)			intI1 (Log_{10} of GC/100 ml)			erm(F) (Log_{10} of GC/100 ml)		
	Inlet	Int.	Outlet	Inlet	Int.	Outlet	Inlet	Int.	Outlet	Inlet	Int.	Outlet	Inlet	Int.	Outlet
May	1.63	1.46	1.49	3.88	1.89	ND	3.44	ND	2.27	5.54	3.66	2.12	ND	ND	2.33
June	1.43	1.12	1.13	2.17	1.65	2.02	2.61	2.89	1.80	4.41	4.40	4.53	2.46	2.44	2.86
July	1.52	1.28	1.38	2.81	0.81	ND	3.14	2.09	2.19	4.94	4.15	3.25	3.37	2.61	2.40
August	1.43	1.25	1.43	ND	ND	1.94	2.27	1.84	1.81	3.31	3.27	4.85	2.35	2.31	2.75
September	1.13	0.68	1.42	ND	ND	0.45	2.06	2.08	2.04	1.82	0.83	2.88	2.27	ND	2.36
October	1.53	1.01	1.00	2.79	ND	0.39	3.15	2.20	1.78	4.64	2.20	1.83	2.70	2.28	ND!
November	1.35	1.16	1.35	1.86	ND	0.05	2.94	1.91	2.26	3.48	2.64	3.07	ND	2.35	2.37
December	1.29	1.37	1.89	ND	2.22	3.49	2.56	2.11	4.22	3.28	3.08	5.02	2.35	2.32	2.61

Int. intermediate water samples of the wetland, *ND* not detected

intI1 from inlet to outlet was 66.6%. The *erm*(F) marker was detected in 79.2% (19/24) of Sweetwater samples, with concentration ranged from 3.37 Log_{10} GC/100 ml (inlet water sample in July) to 2.28 Log_{10} GC/100 ml (inlet sample of Sept). From inlet to intermediate water samples, the reduction rate ranged from 5.7% in June to 83% in July. From inlet to outlet samples, the overall removal rate was 13.1%.

Discussion

It should be noticed that data on the water physicochemical and biological (*E. coli* IDEXX) parameters was recently published (Rachmadi et al. 2016). However, data included in the present study was to gain insight of removal efficiency and co-occurrence patterns between these parameters and ARGs. During the 8 months sampling period, *E. coli* was found in all water samples (24 samples) based on both IDEXX (culture method) and molecular qPCR (molecular method) results (Fig. S1; Table 2). Sweetwater Wetland water temperature ranged from 11.5 to 30.9 °C (Table S1), and a significant negative correlation ($r = 0.64$, p value < 0.01) was observed between *E. coli* (IDEXX) concentration and water temperature (Fig. S2). *E. coli* is likely to survive and regrow in aquatic environment (water and sediment) and can be affected by a number of environmental factors, such as water temperature, bacterial growth, sedimentation rate, and pollution sources (Solo-Gabriele et al. 2000; Karimi et al. 2014; Morató et al. 2014; Howitt et al. 2014; Nguyen et al. 2015). There are several reasons for this phenomenon: First, since we pooled *E. coli* results from all sampling sites together, it is possible that the reduction of *E. coli* by wetland treatment was higher in summer compared to winter due to different field condition in different seasons (more plants in summer). Previous study suggested that constructed wetlands with vegetation are more effective in removal of bacteria than without the vegetation (Maiga 2017); second, the *E. coli* concentration in Sweetwater Wetland can be affected by its water source, since the receiving water is treated wastewater. Previous studies have demonstrated that temperature is one of the most important factors driving the microbial community composition in wastewater treatment which will affect the bacterial removal rates (Ebrahimi et al. 2010; Wang et al. 2014; Lu et al. 2015; Meerbergen et al. 2016). In that case, higher temperature during summer increased the *E. coli* removal rates which is also observed in Fig. S1. In addition, pH has been studied extensively and was suggested affect the growth of bacteria in water, for example, microbes survive best at pH between 5.5 and 7.5 (McFeters and Stuart 1972; Blaustein et al. 2013). Previous study suggested that increased pH is linked to higher bacterial pathogen removal in constructed wetlands and that most bacteria are sensitive to high pH levels (Maiga 2017). The small change in pH among inlet, intermediate, and outlet

samples has an insignificant effect on the occurrence of *E. coli*.

An interesting finding in the present study was that the concentration of *E. coli*, enterococci, and HF183 genetic markers decreased from inlet to intermediate and then increased back from intermediate to outlet (Fig. 2). The reason to explain this phenomenon was probably due to the fluctuation of turbidity during the treatment process. Water turbidity was found decreasing from inlet to intermediate, and then increased from intermediate to outlet (Table S1). Significant positive correlations between turbidity level and qPCR results for *E. coli* and HF183 were observed (Fig. 3). A weak positive correlation between turbidity and enterococci was also observed, even though it is not significant. The adsorption of DNA molecules on sediments have been studied extensively (Crecchio et al. 2005; Cai et al. 2006a, b; Yu et al. 2013; Xue and Feng 2018). It is reasonable to assume that the increase of water turbidity is a source of fecal indicator found in the water column. Increasing evidence suggests that sediments serve as a reservoir for pathogenic microorganisms of fecal origin (Wheeler Alm et al. 2003; Badgley et al. 2010). However, no significant correlation between turbidity and *E. coli* (IDEXX) was found, as qPCR is capable of detecting DNA from culturable cells, nonviable intact cells, viable but non-culturable (VBNC) cells, and extracellular free DNA (Levy-Booth et al. 2007; Rogers et al. 2011; Staley et al. 2012; Xue et al. 2017). Given the fact that qPCR-based MST methods target genetic materials rather than viable organisms, it is more likely that sorption characteristics of DNA molecules may be different compared with intact bacterial cells (Kim et al. 2011; Staley et al. 2012; Shelton et al. 2014).

Previous study evaluated the effectiveness of constructed wetland treatment on pathogen removal (Greenway 2005). This wetland showed a 95% removal rate of pathogens and fecal indicator organisms but also still contained fecal-coliform concentrations in the final effluent. Other constructed wetlands find that all pathogens are expected to be removed but are highly variable on the physicochemical factors (Chen et al. 2016; Le et al. 2016; Yi et al. 2017). The Sweetwater Wetland also had similar findings, where the overall fecal bacteria decreased, but was still prevalent in the final water sample. These studies of constructed wetlands all suggest a similar trend and efficacy of the wetland removal system: while the outlet samples still contain some pathogen bacteria, they are effective in reducing their concentrations. This could be due to extraneous influences, such as temperature, vegetation, sunlight or precipitation, or alternatively, an impact from high retention time to receive samples (Rachmadi et al. 2016). The correlation between environmental factors and bacteria removal efficiency have been studied. For instance, Karimi et al.'s research observed a negative correlation between the content of dissolved oxygen and microorganisms (Karimi et al. 2014). However, our study did not observe any
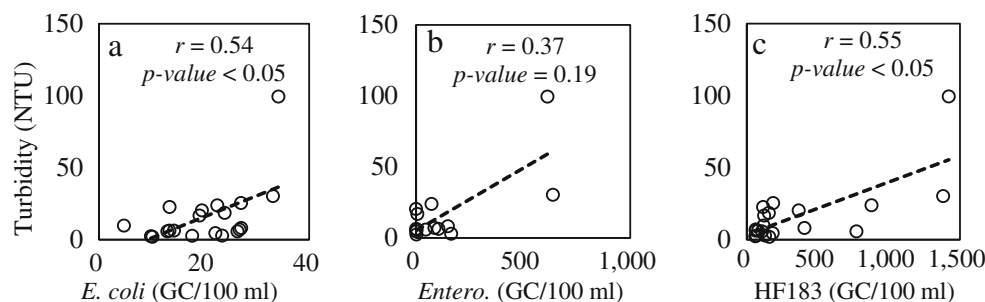
Fig. 3 Correlation between turbidity level and qPCR results for three genetic markers: **a** *E. coli*, **b** *Enterococcus*, and **c** HF183. The outlet sample result in December has been removed from this data analysis due to extremely high value for those markers

correlation between physicochemical parameters and the removal effectiveness of the wetlands. It could possibly be due to the limited sample size in the present study. Future research is needed to look into the insight of the connection between these parameters and the performance of Sweetwater Wetland.

In addition, our results suggested that constructed wetlands are better at reducing enterococci concentrations than the other two indicators. In comparison, enterococci concentrations decreased by 84.0%, whereas HF183 genetic marker concentrations decreased by 66.6% and *E. coli* by 6.7%. The findings for HF183 are significant because this marker is an indicator of fecal pollution which may pose human health at risk due to the presence of enteric pathogens in fecal matter (Webster et al. 2013; Nayak et al. 2015). Regulation for *E. coli* and enterococci in drinking water is zero, so the prevalence of fecal indicator bacteria and HF183 in the final outlet water sample is not safe to be released to the public (EPA). As we mentioned previously, the reduction of *E. coli* can be affected by a number of environmental factors; we are not clear about why there was such a minimal decrease in *E. coli* compared to the other targets. Additionally, *E. coli* could have a slower decay rate than HF183 and enterococci and therefore have a higher prevalence than the other two indicators (Ahmed et al. 2016).

The removal of ARGs by constructed wetlands has been studied and results varied accordingly. The removal rates of ARGs in Chen et al.'s study (2016) ranged from 50.0 to 85.8%. In comparison, the overall removal efficiencies of ARGs in Yi et al.'s research varied significantly from − 145.6 to 98.9% depending on the type of gene (2017). It is noteworthy that even after wastewater treatment process, several ARGs were still found in final effluent at significant higher concentration (10^3 copies/ml) (Vaz-Moreira et al. 2014; Le et al. 2018). The removal of ARGs in Sweetwater Wetland is comparable to previous studies. Our results showed that Sweetwater Wetlands are less efficient in removing *ermF* than *intI1*. For example, the *ermF* concentrations were reduced by only 13.1% and *intI1* markers had reached a 67.2% decrease. Although previous study suggested that the removal rates of ARGs in winter is higher than that in summer

(Fang et al. 2017), we did not observe such pattern in our study due to the smaller sample size. However, we did observe the highest concentrations of both ARGs in the months of May and August, where water temperatures are higher (Table 2).

The molecular methods are more accurate than culture-based methods for detecting bacteria (Wade et al. 2010; Xue et al. 2018b) and have been essential to our study at providing information about prevalence of ARGs. Interestingly, we did not observe any correlation between qPCR and IDEXX results for *E. coli*. The difference between IDEXX and qPCR results reflects differences in natures of analyses as well as differences in persistence of live *E. coli* compared to DNA molecules in aquatic environment. As we mentioned before, qPCR is capable of detecting DNA from live cells, VBNC cells, and extracellular free DNA. The potential of *E. coli* regrow in aquatic environment may also alter the results. Previous studies have demonstrated that *E. coli* are likely to survive and regrow in sediment (Desmarais et al. 2002; Anderson et al. 2005). As the water level in Sweetwater Wetland is shallow, we therefore assume turbulences caused by water flow may resuspend sediments and subsequently release *E. coli* from sediment. Another study also suggests that when comparing *E. coli* concentrations using IDEXX and qPCR methods, qPCR is the more suitable method to use due to the potential loss of *E. coli* cells during sample processing while using the IDEXX method (Noble et al. 2010).

Conclusions

In our study, we investigated the occurrence of fecal indicator bacteria in the Sweetwater Wetlands, which allowed us to compare the bacterial resistance in a constructed wetland after wastewater treatment. Looking towards the qPCR results when compared with the inlet samples, the outlet samples all showed a decreased prevalence of bacteria. There is no correlation between temperature and FIB. Turbidity affected our qPCR results and had a positive correlation only to *Enterococcus*, HF183, and *E. coli*. Overall reduction rates

for *intl1* and *erm*(F) were 67.2% and 13.1%, respectively. *E. coli* rates decreased by 6.7% from inlet to outlet. *Enterococcus* and HF183 decreased by 84.0% and 66.6%, respectively. The prevalence of FIB even after treatment suggests that there could be additional factors to resilience, such as ARGs that should be further investigated to understand completely. Future study is needed to explore the pattern of removal efficiencies of ARGs and FIB throughout the entire year. The findings of our study, however, are promising and suggest that constructed wetlands are efficient in decreasing ARGs and fecal indicator bacteria in the water.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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