Assessing the microbial quality of a tropical watershed with an urbanization gradient using traditional and alternate fecal indicators

Tasha M. Santiago-Rodriguez, Gary A. Toranzos and Javier A. Arce-Nazario

ABSTRACT

Urbanization affects the microbial loading into tropical streams, but its impact on water quality varies across watersheds. Rainfall in tropical environments also complicates microbial dynamics due to high seasonal and annual variations. Understanding the dynamics of fecal contamination in tropical surface waters may be further hindered by limitations from the utilization of traditional microbial indicators. We measured traditional (*Enterococcus* spp. and *Escherichia coli*), as well as alternate (enterophages and coliphages) indicators of fecal contamination in a tropical watershed in Puerto Rico during a 1-year period, and examined their relationship with rainfall events across an urbanization gradient. *Enterococcus* spp. and *E. coli* concentrations were 4 to 5 logs higher in non-urbanized or pristine sites when compared to enterophages and coliphages, suggesting that traditional fecal indicator bacteria may be natural inhabitants of pristine tropical waters. All of the tested indicators were positively correlated with rainfall and urbanization, except in the most urbanized sites, where rainfall may have had a dilution effect. The present study indicates that utilizing novel indicators of microbial water quality may improve the assessment of fecal contamination and pathogen risk for tropical watersheds.

Key words | coliphages, *Enterococcus* spp., enterophages, *Escherichia coli*, rainfall, urbanization

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INTRODUCTION

The microbial quality of tropical watersheds is traditionally monitored using *Enterococcus* spp. and *Escherichia coli*. These two indicators have an extensive history of being used for water quality regulatory purposes under United States laws, especially the Safe Drinking Water Act (NRC 2004). While these bacteria are found in the gut of warm-blooded animals and may indicate fecal contamination, they are also natural inhabitants of pristine waters and originate from non-fecal sources; thus, their detection may not always indicate fecal contamination (Fujioka *et al.* 1988, 1998; Solo-Gabriele *et al.* 2000; Noble *et al.* 2010; Byappanahalli *et al.* 2012; Boehm & Sassoubre 2014). In addition, traditional indicator bacteria-culturing methods provide results at 18–24 h and additional confirmation steps are required, preventing immediate actions to be taken to prevent potential risks to public health (Wade *et al.* 2003; Noble & Weisberg 2005). For these reasons, alternate indicators of fecal contamination may exhibit a higher source-specificity and may be more cost- and time-efficient (Sinton *et al.* 1998; Griffin *et al.* 2001; Bonilla *et al.* 2010; Purnell *et al.* 2011; Kent *et al.* 2014; Rusinol *et al.* 2014). For instance, polymerase chain reaction (PCR)-based methods, such as quantitative PCR (qPCR), are increasingly being utilized to determine the microbial quality of waters. PCR-based methods have several advantages, including high-specificity and the reduced time to obtain results (Wade *et al.* 2006), but there are significant drawbacks. These still primarily target

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microorganisms whose reliability as indicators of fecal contamination has been questioned, including *Enterococcus* spp. and *E. coli* (Noble *et al.* 2010). While qPCR assays have been developed to target microorganisms originating from the gut of specific warm-blooded animals, such as *Bacteroides* spp. (Reischer *et al.* 2013), these assays cannot be performed at all laboratories. Another drawback is that 16S or 23S rRNA gene amplification usually cannot demonstrate viability due to the possible amplification of naked nucleic acids or those originating from dead cells.

Bacteriophages have also been proposed and tested as alternate indicators of fecal contamination (Savichtcheva & Okabe 2006). *E. coli* phages or coliphages have been proposed as possible indicators of specific sources of fecal contamination including those originating from dogs, pigs, and humans (Osawa *et al.* 1981; Rose *et al.* 1997; Kirs & Smith 2007; Sundram *et al.* 2007; Muniesa *et al.* 2012); however, while coliphages are rarely detected in pristine waters and are present in the gut of warm-blooded animals, conflicting data exist suggesting that these may not be limited to a single source (Cole *et al.* 2003). Bacteriophages infecting *Enterococcus faecalis*, or enterophages, have also been shown to possess several characteristics of an indicator of human fecal contamination. For instance, enterophages are prevalent in raw and treated domestic sewage across diverse geographical regions, exhibiting concentrations of up to 10^3 PFU/100 mL, and have not been detected in the feces of domestic or wild animals. Enterophages also exhibit survival times and inactivation rates similar to enteric pathogenic viruses in different water types (Santiago-Rodriguez *et al.* 2010, 2013). These enterophages are strictly lytic phages that infect a specific *E. faecalis* type strain, and this specificity has made the enterophage method simple to replicate in different laboratories (Santiago-Rodriguez *et al.* 2010). Enterophages have also been tested as alternate indicators of human fecal contamination in tropical watershed systems (Santiago-Rodriguez *et al.* 2012). Both the enterophage and coliphage methods represent relatively rapid approaches to infer human fecal contamination, as results are produced 4–6 h post-processing, making the technique comparable to emerging molecular techniques for determining any potential risks to public health (Purnell *et al.* 2011). Additionally, detection of coliphages and enterophages using culturing methods also indicates pathogen infection risk in a short period of time.

Determining the microbial quality of watershed systems and the possible sources of fecal contamination may also be challenging because a variety of land-use patterns in the watershed may result in point and non-point sources of fecal contamination (Mallin *et al.* 2000; Gellis 2013). Land-use changes such as deforestation for agriculture can change the temperature, topography, and local hydrology, which can strongly influence the concentration and movement of microorganisms throughout the watershed (Liang *et al.* 2013; Vereen *et al.* 2013). This complexity increases due to environmental perturbations such as rainfall (Bolca *et al.* 2007; Coulliette & Noble 2008; Rowny & Stewart 2012; Santiago-Rodriguez *et al.* 2012; Lee *et al.* 2014). Microorganisms are carried from soils by surface runoff, within rivers by resuspension of sediments and by sewage overflows, increasing pathogen risk to human health (Shehane *et al.* 2005). A spatially and temporally broad sampling of tropical watersheds, which usually exhibit varying degrees of urbanization as well as rainfall events that vary throughout the year, could provide information on both the dynamics of fecal indicators and on the true extent of the fecal contamination (Bolca *et al.* 2007; Coulliette & Noble 2008; Hathaway *et al.* 2010; Viau *et al.* 2011; Rowny & Stewart 2012; Santiago-Rodriguez *et al.* 2012; Liang *et al.* 2013; Vereen *et al.* 2013; Lee *et al.* 2014). Little is known about the dynamics of contamination in tropical streams and the impact of land-use and rainfall events on these systems (Shibata *et al.* 2004). Therefore, the present study aimed to determine: (i) if the coliphage and enterophage methods can be utilized to determine the microbial quality of tropical watersheds in 4 h; (ii) how rainfall correlates to the microbial quality of tropical streams measured using enterophages and coliphages as alternate indicators, and *Enterococcus* spp. and *E. coli* as traditional indicators; and (iii) how varying degrees of urbanization affect the microbial dynamics measured using these traditional and alternate indicators of fecal contamination.

MATERIALS AND METHODS

Sample collection and sampling sites

A total of 15 sampling sites were selected within the La Plata River Watershed in Puerto Rico (Figure 1). The sites were selected because they exhibited a gradient of urban

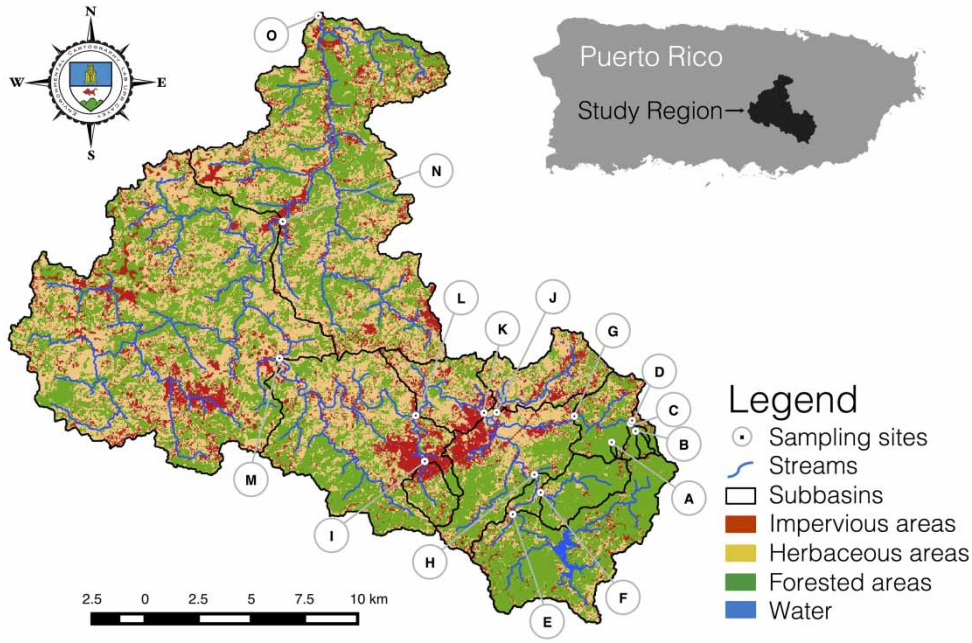

Figure 1 | Sampling sites in the present study. Sampling sites included pristine sites and sites that followed a gradient of urbanization.

development within the watershed. Two of the most forested sampling sites were located within the Carite National Forest Reserve, while the most urbanized sites were located in the town of Cayey. The sites were also selected to allow safe water collection even during extreme precipitation events and to allow the samples to be transported to the lab for analysis within 6 h of collection. Sampling started in June 2013 and ended in June 2014. In general, sampling tours would be organized on a weekly basis to sample the eight sites that are located upstream of the densely urbanized town center, and the seven sites which are located downstream of the town center. Sampling was done by hand at small streams with foot access using sterile 500 mL bottles. Collection points located over high bridges were sampled using a 1-liter NASCO bag, and collection points at sites with low bridges were sampled using a 1-liter sterile bottle connected to a pole.

Collection of precipitation databases

Precipitation data were collected with a RainWise MK-III station located at 18°07′06″N 66°09′45.0″W, at the University of Puerto Rico at Cayey. The average annual rainfall for the entire watershed study area is 1,625 mm, and the pluviometer is located in an area whose annual precipitation is 1,429 mm (Daly *et al.* 2003). The data collected by the station were compared for accuracy to data collected by other USGS pluviometers and to pluviometers deployed by the research team.

Enumeration of alternate and traditional indicators

For the enumeration of the alternate indicators, 50 mL of fresh water sample was processed using the single-layer method as described (Bonilla *et al.* 2010; Santiago-Rodriguez *et al.* 2010). The type strains used for phage enumeration were *E. faecalis* (ATCC 19433) for enterophages, and *E. coli* (ATCC 15597) for coliphages. Plates were incubated at 37 °C and enumerated at 4 and 24 h as plaque forming units (PFU)/100 mL. *Enterococcus* spp. and *E. coli* were measured using Enterolert® and Colilert®, respectively. Samples were diluted 1:10 in sterile distilled water and incubated in 97-well most probable number (MPN) Quanti-tray®/2000 at the recommended temperatures and times according to the manufacturer's instructions. At 24 h, positive wells were counted and the results were transformed to MPN values/100 mL using the IDEXX Quanti-Tray®/2000 MPN table.

Spatial analysis

The basins for each sampling site were defined using the SRTM 1-ARC elevation model of Puerto Rico, GPS measurements of the sampling site locations, and GRASS hydrology methods. The resulting basin areas were visually inspected and edited if necessary to require that the modeled basins matched the topography observed in the 2010 high resolution orthoimagery collection of Puerto Rico. The area covered by roads is strongly correlated to the total number of housing units within a watershed (Claggett *et al.* 2013), and is a good surrogate for urbanization density. We constructed the ratio of total road length to basin area, $_{LR/AB}$, calculated by measuring the total length of roads within each basin in kilometers and dividing that length by the area of the basin in hectares. The road data were obtained from the 2014 US Census TIGER Shapefile lines (http://www.census.gov/geo/maps-data/data/tiger.html). The resulting basin areas varied significantly in size and fraction of road cover (Figure 1).

Statistical analysis

For the statistical analysis, MPN and PFU values per 100 mL were normalized by a $\log_{10}$-transformation, with non-detect data assigned a $\log_{10}$ normalized value of 0.0. In 8% of the samples, the maximum detection levels of traditional indicators were measured and these were used without modification; discarding these values instead had qualitatively little effect on the resulting analysis. To quantify rainfall, total precipitation in the 48 h previous to sampling (P_{48}) was calculated for every sampling day. The 48 h interval was chosen over a 24 h interval based on extensive studies of the impact of rain on water quality in Puerto Rico (Uriarte *et al.* 2011) over a range of basin sizes from 775 ha to 66,500 ha. Urbanization was quantified using the measure $_{LR/AB}$ defined above. The values of precipitation (P_{48}) are not normally distributed (Shapiro-Wilk test $W = 0.618$; $P < 0.001$), but this feature does not affect our interpretation of the statistical analysis. The coefficient of determination R^2 in the simple linear regression tests, and the regression coefficients in the multi-linear analysis, were interpreted as measures of correlation. R^2 values ≥ 0.7 were considered a strong correlation, values of 0.7–0.4 were considered a moderate correlation and values <0.4 were considered a weak correlation. All statistical analysis was performed using R version 3.1.1.

Because the degree of correlation between the plaque counts measured at 4 and 24 h would indicate whether the shorter incubation time could be a useful substitute for the longer period, the coefficient of determination between the plaque counts at 4 versus 24 h was calculated using a simple linear regression model. To better understand the combined effects of rainfall and urbanization on each indicator in a tropical watershed, the effect of rainfall on the microbial quality was analyzed at each sampling site, and averaged across all sites. For each sampling site, a simple linear regression was performed for each fecal indicator as a dependent variable of P_{48}. The P_{48} values, divided into quartiles, were considered as factors explaining the effect of rainfall on the mean value of fecal indicators across all sites using a one-way analysis of variance (ANOVA) with Bonferroni post-hoc comparisons. A simple linear regression was performed for each fecal indicator as a dependent variable of urbanization. A multiple linear regression was also carried out for each indicator using standardized measures of both $_{LR/AB}$ and P_{48} as explanatory variables, to compare the strength of the correlations to urbanization and rainfall for the considered fecal indicators.

RESULTS

Correlation of enterophages and coliphages detected at 4 and 24 h

A total of 366 and 348 samples were processed for the detection of enterophages and coliphages, respectively. PFU/100 mL data detected at 4 h and 24 h for enterophages were strongly correlated ($R^2 = 0.959$) (Figure 2(a)), and for coliphages there was a weak correlation ($R^2 = 0.264$) (Figure 2(b)). These correlations suggest that data obtained at 4 h could be similar in utility to the data obtained at 24 h in terms of determining the extent of the fecal contamination.

Correlation of alternate and traditional indicators with rainfall and urbanization

A total of 388 samples were processed for the detection of the traditional indicators. We found a positive correlation

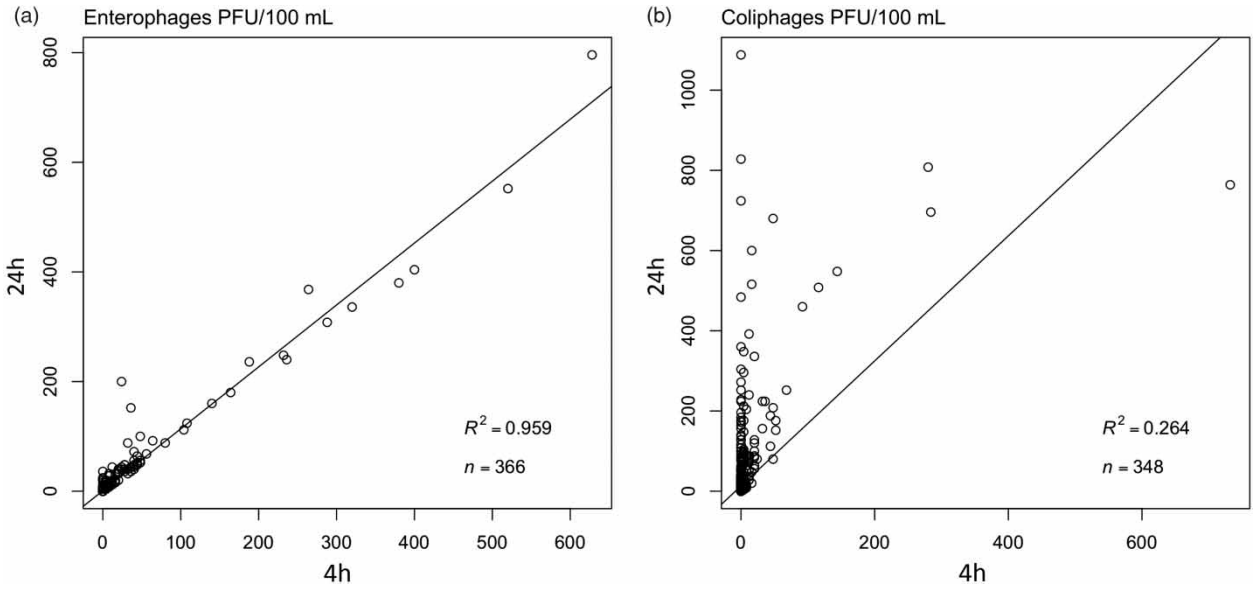

Figure 2 | Correlation of enterophages (a) and coliphages (b) PFU/100 mL detected at 4 and 24 h. R^2 and n values are shown for each indicator.

between each of the indicators tested and P_{48} in the majority of the sampling sites (Table 1). Enterophages, *Enterococcus* spp., and *E. coli* were positively correlated with rainfall in 14 of the 15 sampling sites, and coliphages were positively correlated with rainfall in 13 of the 15 sampling sites. Interestingly, all of the indicators were inversely correlated with rainfall in the most urbanized site (Basin I). The correlations between the fecal indicators and P_{48} were statistically significant in most of the sampling sites (Table 1).

The correlation of the fecal indicators with rainfall reported 48 h prior to sample collection presented the opportunity to determine whether there is a specific threshold of rainfall necessary for an increase in the numbers of the fecal indicators. This analysis was performed because tropical watersheds, like those in Puerto Rico, often receive frequent rainfall episodes (Figure 3(a)), and so the effect on contamination may be nonlinear. We separated the precipitation data into quartiles with lower bounds of 0.00, 2.79, 8.13, and 8.38 mm of rain, each containing 25% of the samples studied. The ANOVA tests using rainfall quartiles as factors to explain the difference in indicator mean values gave statistically significant results for all indicators: enterophages ($F = 30.23$; $P < 0.001$), coliphages ($F = 11.35$; $P < 0.001$), *Enterococcus* spp. ($F = 38.81$; $P < 0.001$), and *E. coli* ($F = 41.8$; $P < 0.001$). Rainfall thresholds had a noticeable effect on the detection of enterophages, where >8.38 mm of rain was necessary to see a significant ($P < 0.001$) increment in the mean (Figure 3(b)). The effect of different rainfall on the coliphages was not as noticeable, where a 0.7 log increase resulted from a threshold of >8.13 mm of rain (Figure 3(c)), but the difference in samples after more than 8.13 mm of rain was statistically significant ($P < 0.05$). *Enterococcus* spp. numbers increased 1.0 log in samples with >8.38 mm of rain (Figure 3(d)), and an increase of 1.5 logs for *E. coli* was noted with a threshold of >8.38 mm of rain, which also met statistical significance ($P < 0.001$) (Figure 3(e)).

Urbanization was positively correlated with both traditional and alternate indicators. Enterophages exhibited a moderate correlation ($R^2 = 0.641$; $P < 0.001$), while coliphages exhibited a weak correlation ($R^2 = 0.337$; $P < 0.01$) with urbanization. *Enterococcus* spp. ($R^2 = 0.318$; $P < 0.05$) showed weak correlation, and *E. coli* ($R^2 = 0.461$; $P < 0.01$) showed moderate correlation with the degree of urbanization. Enterophages and coliphages PFU/100 mL were as low as 0.5 log in pristine sites, and as high as 3.5 logs in more urbanized regions. *Enterococcus* spp. and *E. coli* concentrations were several logs higher in non-urbanized (4.0 to 5.0 logs) sites compared to the enterophages and coliphages.

Table 1 | Correlation of enterophages, coliphages, *Enterococcus* spp., and *E. coli* with rainfall reported 48 h prior to sample collection

				Enterophages			Coliphages			*Enterococcus* spp.			*E. coli*		
Basin	**Elevation (meters)**	**Area (ha)**	**LR/AB (meters/ha)**	**Slope**	**R^2**	***P*-value**	**Slope**	**R^2**	***P*-value**	**Slope**	**R^2**	***P*-value**	**Slope**	**R^2**	***P*-value**
A	618	13	0	1.117	0.446	0.001	2.570	0.651	0.001	2.082	0.345	0.001	1.692	0.484	0.001
B	546	56	4	2.362	0.634	0.001	3.241	0.517	0.001	2.629	0.340	0.010	2.253	0.492	0.001
C	518	165	27	3.391	0.500	0.001	1.742	0.146	0.050	2.688	0.381	0.001	2.432	0.405	0.001
D	509	84	59	3.112	0.613	0.001	2.287	0.240	0.050	1.819	0.221	0.050	1.381	0.232	0.050
E	476	3,367	30	0.932	0.150	0.050	2.404	0.445	0.001	1.642	0.143	0.050	2.739	0.527	0.001
F	411	900	28	2.236	0.528	0.001	1.360	0.112	0.076	1.665	0.244	0.010	1.045	0.369	0.001
G	401	1,173	43	3.285	0.486	0.001	1.706	0.124	0.057	1.894	0.218	0.010	2.884	0.543	0.001
H	398	4,470	29	2.119	0.442	0.001	1.648	0.176	0.050	2.438	0.386	0.001	2.416	0.719	0.001
I	389	486	120	−0.332	0.001	0.869	−5.242	0.417	0.010	0.991	0.149	0.083	0.323	0.007	0.728
J	375	1,385	76	2.021	0.230	0.050	1.127	0.085	0.212	1.356	0.054	0.312	2.418	0.251	0.050
K	369	9,601	47	2.296	0.216	0.050	−0.353	0.005	0.761	1.329	0.060	0.249	1.129	0.109	0.115
L	333	10,807	51	3.706	0.326	0.010	1.308	0.041	0.369	3.793	0.426	0.001	5.571	0.662	0.001
M	260	16,280	56	4.443	0.638	0.001	4.815	0.449	0.010	4.451	0.336	0.010	4.576	0.389	0.010
N	196	29,499	53	2.405	0.114	0.170	3.132	0.148	0.104	5.047	0.375	0.010	4.613	0.396	0.010
O	52	39,981	52	4.785	0.772	0.001	1.245	0.197	0.112	5.774	0.740	0.001	7.022	0.837	0.001

Shown are the sampling site elevation (m), the basin area (ha), the ratio of total road length to basin area (meters/ha) and the slopes, R^2 and *P*-values for each indicator at each of the sampling sites.

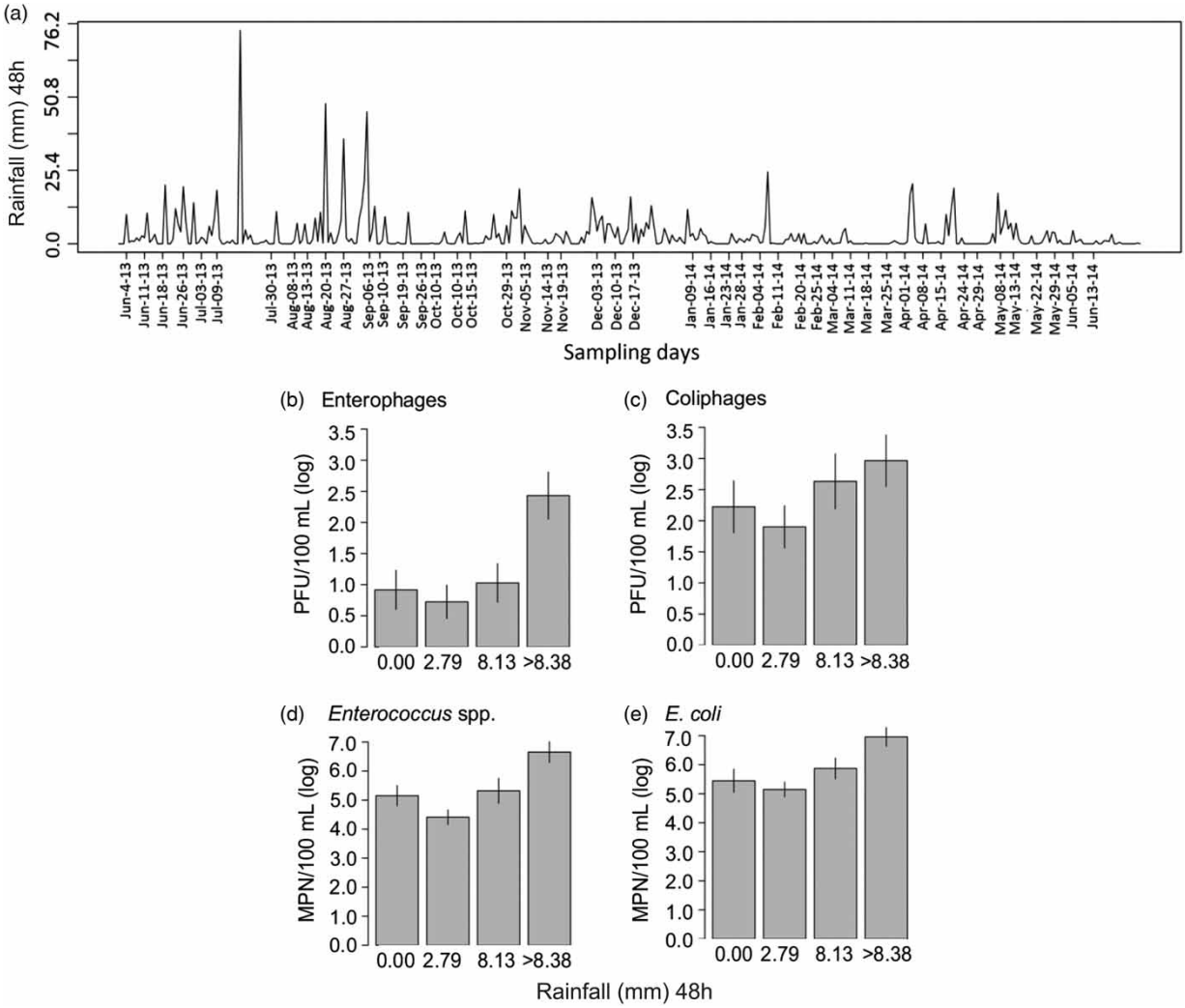

Figure 3 | Rainfall events during the sampling period and the effect of rainfall thresholds on measured fecal indicators. (a) Rain reported (mm) 48 h prior to sample collection during the study period. The effect of specific rain (mm) thresholds for the enterophages (b), coliphages (c), *Enterococcus* spp. (d), and *E. coli* (e) are shown.

Multi-linear regression was used to compare the importance of rainfall and urbanization in determining the microbial dynamics of each indicator (Table 2). The alternate indicators showed a stronger correlation with rainfall than the traditional indicators. The R^2 values in each case were relatively small (0.242–0.420), suggesting that basin size and other factors might also influence the microbial dynamics in tropical watersheds. To visualize possible effects beyond a simple multi-linear model for each considered indicator, we compared the distribution of fecal indicators vs. rainfall in the most urbanized site and the site within the Carite Reserve most similar in size to the

Table 2 | Multiple linear regression analyses for enterophages, coliphages, *Enterococcus* spp., and *E. coli*, and rainfall and urbanization

Indicator	Intercept (std. error)	Coefficient, rainfall (std. error)	Coefficient, urbanization (std. error)	R^2
Enterophages	1.32 (0.07)	0.96 (0.07)	0.77 (0.07)	0.420
Coliphages	2.42 (0.09)	0.77 (0.09)	0.74 (0.09)	0.242
Enterococcus spp.	5.40 (0.09)	0.81 (0.09)	0.61 (0.09)	0.232
E. coli	5.88 (0.07)	0.83 (0.07)	0.85 (0.07)	0.363

Coefficient values for each factor and R^2 are shown; all the coefficients were statistically significant ($p < 0.001$).

most urban site (Figure 4). In the most pristine sites (white bars), heavy rainfall (>8.38 mm) increased the detection of the enterophages (Figure 4(a)), coliphages (Figure 4(b)), *Enterococcus* spp. (Figure 4(c)) and *E. coli* (Figure 4(d)) by approximately 1.0 to 2.0 logs. In the most urbanized sites (dark gray bars), recent rainfall did not have a clear effect on the contamination measured through enterophages (Figure 4(a)), *Enterococcus* spp. (Figure 4(c)), or *E. coli* (Figure 4(d)); yet, data showed that rainfall had a clear dilution effect on the coliphages (Figure 4(b)).

DISCUSSION

While several studies have analyzed the microbial quality of tropical watersheds (Carrillo *et al.* 1985; Shibata *et al.* 2004; Viau *et al.* 2011; Santiago-Rodriguez *et al.* 2012; Toledo-Hernandez *et al.* 2013), very few have tested both enterophages and coliphages as alternate indicators of the microbial quality and compared them to traditional fecal indicators. Enterophages and coliphages were tested as alternate indicators because these have been shown to be source-specific (Hernández-Delgado *et al.* 1991; Espinosa *et al.* 2009; Santiago-Rodriguez *et al.* 2012). The present study tested these indicators as a rapid alternative to traditional indicators of fecal contamination. Reducing the 18–24 h period required to obtain results from traditional fecal indicators could be advantageous to public health (Grabow *et al.* 1984; Santiago-Rodriguez *et al.* 2013). Although our comparisons did not utilize a quantitative method for the detection of the traditional indicators, such as membrane filtration, Enterolert® and Colilert® are US EPA-approved methods to determine the microbial quality of waters, and are being used in field studies (González &

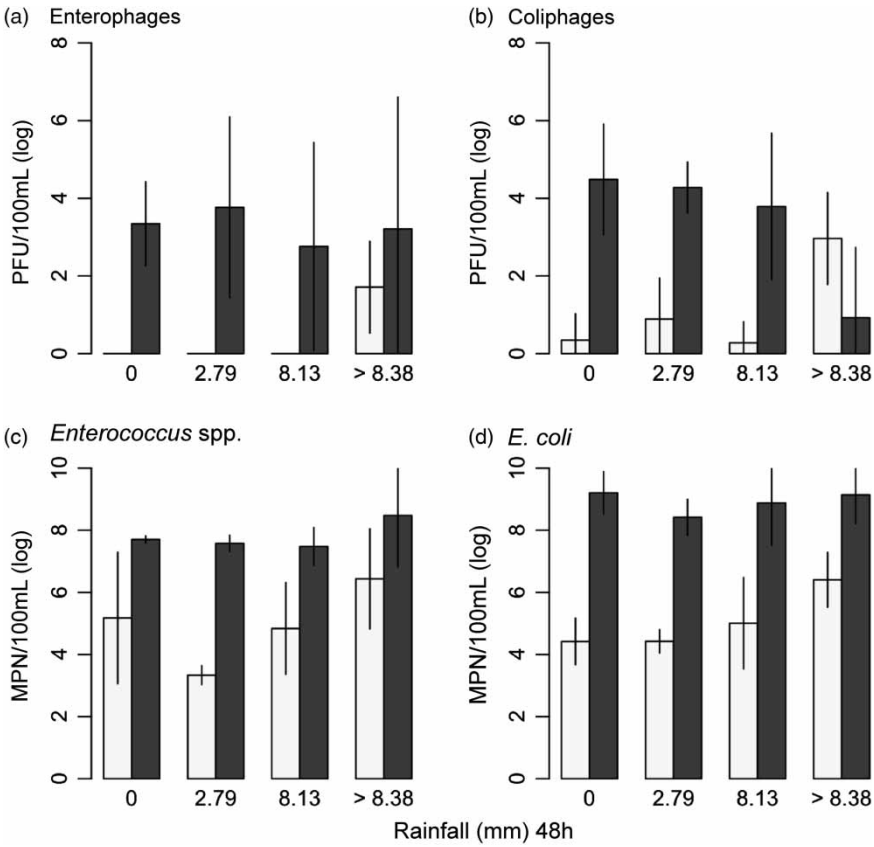

Figure 4 | Rainfall at 48 h versus mean value of enterophage PFU/100 mL (a), coliphages PFU/100 mL (b), *Enterococcus* spp. MPN/100 mL (c), and *E. coli* MPN/100 mL (d) in the most urban site (dark gray bars, $n = 20$) and a highly forested site (white bars, $n = 26$) within the Carite Reserve most similar in size to the most urban site. The average concentration (log) for each fecal indicator is shown with 95% confidence intervals.

Noble 2014). The significant correlation we found between 4 and 24 h enterophage and coliphage data suggests that fecal contamination could be promptly inferred from short duration incubation periods.

Our analysis also suggests that enterophage and coliphage methods may be especially useful in tropical ecosystems, since traditional fecal indicators may not show the true extent of fecal contamination in tropical regions (Hazen & Toranzos 1990). There is strong evidence that tropical soils support survival of traditional indicator bacteria long after the contamination event, suggesting that their presence in tropical waters does not always translate into pathogen risk for water users (Byappanahalli & Fujioka 1998; Fujioka *et al.* 1998; Byappanahalli & Fujioka 2004). Also, traditional indicators are known to persist up to >20 days in fresh waters with temperatures similar to those in tropical regions (Fujioka 2001; An *et al.* 2002; Isobe *et al.* 2004; Fries *et al.* 2006; Ahmad *et al.* 2014). Once introduced into water sources, fecal indicator bacteria persistence may not necessarily indicate a recent contamination event. In contrast, bacteriophages, including enterophages and coliphages, survive 3 to 5 days, and 4 to 8 days in fresh waters at 22 °C, respectively (Santiago-Rodriguez *et al.* 2013). Bacteriophages are also rarely detected in pristine water sources (Bermúdez & Hazen 1988; Rivera *et al.* 1988; Hernández-Delgado *et al.* 1991), and enterophages and coliphages both had very low detection levels in the least urbanized watersheds, suggesting that these alternative indicators limit false positive results when testing fecal contamination in tropical environments.

Correlations between fecal indicators and rainfall events are well established in temperate and subtropical watershed systems (Mallin *et al.* 2000; Kistemann *et al.* 2002; Jamieson *et al.* 2004; Wilkes *et al.* 2009; Rowny & Stewart 2012). Our study found that correlations with recent rainfall were the strongest with the alternate indicators, suggesting that for tropical watersheds, alternate indicators may be better than others in tracking contamination dynamics directly linked to light or moderate rainfall events. Previous studies have demonstrated that rainfall contributes to fecal indicator dynamics in subtropical watershed systems (Lipp *et al.* 2001; Shehane *et al.* 2005); however, the duration and intensity of rainfall episodes have differing patterns in tropical regions, so it is important to understand their role in fecal indicator dynamics in these watersheds. The positive correlation of rainfall with both the traditional and alternate indicators in both pristine sites and the majority of the urbanized sites indicate that rainfall may introduce traditional and alternate indicators into surface waters from runoff, sewage overflows, and/or sediments. Dilution in the most urbanized site after rainfall, with the coliphages exhibiting the greatest effect, may suggest that point- and nonpoint-sources of fecal contamination may have differing effects on the dynamics of fecal indicators in watershed systems. Our data also indicate that land cover adds to the complexity of fecal indicator dynamics in tropical watershed systems, and that it would also need to be considered when assessing the microbial quality of watersheds. Alternate indicators of fecal contamination may improve the assessment of fecal contamination and pathogen risk for tropical watersheds with an urbanization gradient.

CONCLUSIONS

The enterophage and coliphage culture methods represent rapid approaches for assessing the microbial quality of tropical watersheds. Like traditional indicators, alternate indicators of fecal contamination were positively correlated with urbanization. Rainfall affects the dynamics of alternate and traditional fecal indicators in pristine and urbanized sites in tropical watersheds. With the increasing degree of urbanization taking place in tropical watershed systems, and the impact rainfall exhibits on their microbial quality, it is important to find indicators and models leading to an improved assessment of fecal contamination and pathogen risk for tropical watersheds to better protect public health.

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