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# Drivers of Water Quality Variability in Northern Coastal Ecuador

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Microbiological safety of water is commonly measured using indicator organisms, but the spatiotemporal variability of these indicators can make interpretation of data difficult. Here, we systematically explore the variability in *Escherichia coli* concentrations in surface source and household drinking water in a rural Ecuadorian village over one year. We observed more variability in water quality on an hourly basis (up to 2.4 log difference) than on a daily (2.2 log difference) or weekly basis (up to 1.8 log difference). *E. coli* counts were higher in the wet season than in the dry season for source (0.42 log difference, $p < 0.0001$) and household (0.11 log difference, $p = 0.077$) samples. In the wet season, a 1 cm increase in weekly rainfall was associated with a 3% decrease ($p = 0.006$) in *E. coli* counts in source samples and a 6% decrease ($p = 0.012$) in household samples. Each additional person in the river when source samples were collected was associated with a 4% increase ($p = 0.026$) in *E. coli* counts in the wet season. Factors affecting household water quality included rainfall, water source, and covering the container. The variability can be understood as a combination of environmental (e.g., seasonal and soil processes) and other drivers (e.g., human river use, water practices, and sanitation), each working at different time scales.

## Introduction

Microbiological safety of recreational and drinking waters is commonly measured using fecal indicator bacteria, with the ultimate goal of preventing the transmission of waterborne diseases. However, concentrations of indicator organisms are spatiotemporally variable, and most sampling is too infrequent to transcend this granularity, making interpretation of data difficult (*1*). For example, in developing countries, large variations in concentrations of indicator organisms have been observed at different times of the year, especially for in-house drinking water storage containers (*2*). In the United States, indicator concentrations have been observed to be highly variable in near coastal waters on time scales ranging from minutes to decades (*3*). This variability presents challenges to the use of indicator organism concentrations to guide water quality interventions and regulations.

Seasonal changes are known to be a major source of variability in indicator data. Peaks of microbial contamination in waterways have been associated with rainfall in the nearshore marine environment (*3*), with rainfall and soil moisture content in subtropical rivers (*4*), with rainfall and suspended solid content in temperate streams (*5*), and with peak rainfall, peak streamflow events, and peak turbidity measurements in rivers in northern latitudes (*6*). Higher levels of fecal contamination of drinking water sources have been correlated with the rainy season or increased rainfall in Uganda (*7*), Malawi (*8*), and Gambia (*9*). In other locations, such as Nicaragua (*10*) and Sierra Leone (*11*), deteriorations in source water quality have been observed in the dry season, or in the transition from the dry season to the wet season (Sierra Leone (*11*) and Nigeria (*12*)).

Seasonality is also known to be an important factor in determining the incidence of infectious diseases (*13*). Waterborne disease outbreaks have been associated with peak rainfall events in the United States (*14*), Canada (*15*), and Guatemala (*16*), and with both high and low extremes of rainfall in Fiji (*17*). Increased waterborne diseases have been associated with major floods in Bangladesh and elsewhere (*18*–*20*).

In addition, variability in water quality measurements has been noted on hourly, daily, weekly, and other time scales. For example, Boehm et al. (*3*) found that the concentration of fecal indicator bacteria in the nearshore marine environment in Southern California varied over time scales ranging from minutes to decades, determined by pulse contamination events, tidal processes, diurnal sun movements, lunar cycles, seasonal dynamics, El Niño dynamics, and changing human practices over time. Assessing microbial contamination at these different time scales helps us distinguish true patterns from noise in water quality data. Yet, this same type of study has not been undertaken in freshwater sources of drinking water or in a developing country.

To interpret the data provided by indicator organisms, it is important to understand and quantify sources of variability in their measurement in both space and time. Understanding this variability has implications for when and how often to collect water samples, for providing recommendations on whether water is safe for drinking or bathing, and for distinguishing true variability from uncertainty in measurement caused by limitations of testing technology. With a better grasp of the inherent variability in water quality samples, one can ask more informed questions about how environmental drivers of water quality such as seasonal and soil processes interact with human practices such as water use, water storage, and community sanitation.

In this paper, we systematically examine sources of variability in water quality measurements in source and household water samples at varying time scales over the course of one year in a rural Ecuadorian village, where high rates of diarrheal disease have been observed (*21, 22*). Specifically, we explore the environmental drivers of seasonal, day-to-day, and hour-to-hour variability in water quality measurements of surface waters used as drinking water

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sources. We also examine seasonal and other drivers of variability in water quality in the home.

## Experimental Section

**Study Site.** Water samples were collected in the village of Colon Eloy, a town of approximately 700 inhabitants living in 170 houses in the northwestern Ecuadorian province of Esmeraldas. Villagers use unimproved surface water from the Estero Maria, a small stream, as their primary source of drinking water. Our field staff carried out basic surveys about sanitation and water usage practices in the village. According to these surveys, 71% of the people interviewed report using the stream as their primary water source, and more than half (54−69%, depending on the season) of these people drink their water without treating it. Alternate sources of drinking water include harvesting of rainwater and use of private unprotected hand-dug wells (reported as the primary drinking water source by 10% and 13%, respectively, of people surveyed). Some community members also import water from the larger Santiago River nearby during the dry season, when reduced flows are experienced in the Estero Maria. Inadequate sanitation infrastructure exists in this community, and only 65% of houses have private latrines.

The difference in the mean air temperature between the wet and dry seasons is minimal, with an average in the wet season of 27.2 °C (range of 23.8−29.9 °C) and in the dry season of 26.6 °C (range of 24.1−29.1 °C). Average daily precipitation, on the other hand, varies quite a bit between the wet (0.61 cm, range of 0−18 cm) and dry seasons (0.34 cm, range of 0−6 cm).

**Water Sample Collection.** Between January 2005 and March 2006, weekly samples were collected from five sites along the Estero Maria (Figure 1), one just upstream of the village (site 1), one upstream of the major population center (site 2), two in the center of town (sites 3 and 5), and one at the downstream end of the village (site 6). One day per month samples were taken three times on the same day at the three middle sites (sites 2, 3, and 5). Weekly samples were also collected from seven households randomly selected using a block randomization scheme to ensure a distribution of houses throughout the village (Figure 1). To assess day-to-day variability, daily samples were collected for 11 consecutive days in the dry season from four stream sites at the same time each day at each particular site. Samples from sites 1, 2, and 4 were collected between 0900 and 1200 h, and samples from site 6 were collected between 1400 and 1600 h. To assess hour-to-hour variability, samples were collected every 90 min between 0500 and 2300 h and every 180 min between 2300 and 0500 h for four consecutive days, once in the wet season and once in the dry season, at site 4. These different sampling schemes are summarized in Table 1.

**Water Quality Testing.** All samples were collected in Whirl-Pak bags (Nasco), immediately placed on ice, and processed within 24 h. Petrifilm coliform−*Escherichia coli* count plates (3M) were used to detect and quantify *E. coli* colonies in the samples (*23*). A previous study comparing petrifilms to other established assays for *E. coli* found comparable results when 1 mL samples were consistently used (*24*). Petrifilms were inoculated with 1 mL of water and, because of logistical limitations of the study context, were incubated at ambient temperatures (27 ± 3 °C) for 24 h. If a sample was suspected of being particularly clean (rainwater or treated drinking water), the test was carried out in triplicate, and the results of the three tests were summed and divided by 3 mL; this occurred in 15% of the weekly samples and none of the daily or hour-to-hour samples. Nondetects were included in the analysis as one-half of the lower detection limit. The highest plate count recorded for the weekly samples over the course of the year (processed by one researcher) was 100 colony-forming units (CFU)/plate, and the highest value for the samples collected on a day-to-day and hour-to-hour basis (processed by a different researcher) was 450 CFU/plate. The plates that had too many colonies to count were therefore assigned a value of 100 CFU/plate for the weekly samples collected over the course of the year and a value of 450 CFU/plate for the samples collected on a day-to-day and hour-to-hour basis. The number of samples above and below the detection limit for each of the sampling schemes is summarized in Table 1. In all analyses described herein that combine results from samples collected by the two different researchers, an upper limit of 100 CFU/plate was assigned. The number of *E. coli* colonies/plate was multiplied by 100 to get a standardized total count per 100 mL. Possible results therefore ranged from 16.7 or 50 CFU/100 mL (halfway between zero and the lower detection limit of 33 or 100 CFU/100 mL) to 10000 or 45000 CFU/100 mL, depending on the analysis. These values were $\log_{10}$-transformed for use in the analysis because we were interested in the order-of-magnitude scale variability in indicator counts.

**Data Analysis.** All analysis was carried out using STATA 9.0 (StataCorp LP, College Station, TX). Generalized estimating equations (GEE) were used to estimate the association between water quality (*E. coli* concentration, CFU/100 mL) and various explanatory variables, controlling for correlation within sampling sites and within households and using a log-linear (Poisson regression) model (*25*). Robust standard errors were specified to protect the inference against misspecification of this model. Explanatory variables were first modeled individually, and then combined into a multivariate model to produce adjusted coefficients.

For the source samples, environmental covariates included weekly rainfall (centimeters of rainfall summed over the calendar week), river height and river clarity (both measured weekly at sampling site 1), pH, electroconductivity (mS/cm), water temperature (°C), and number of people in the river at the time of sample collection. Water temperature, pH, and electroconductivity were measured at source waters at the time of collection using a hand-held device (Hanna Instruments, Ann Arbor, MI). The number of people in the river was also noted. River level and water clarity were measured each week at the most upstream location in the village (site 1). The distance from a fixed point above the river (a cement bridge) to the water level was measured to calculate river height, and a Secchi disk lowered into the water from the same point was used to determine water clarity. Values for precipitation were taken from a rain gauge (Onset Computer Corporation, Bourne, MA) in the town of Borbón, 12 km away.

The association of weekly rainfall, river height, and river clarity with water quality was tested with no lag and with a lag of one week in the univariate analysis, and the effect of rainfall with no lag was used in the final multivariate models. Because river level and river clarity lie in the causal pathway between rainfall and water quality, these variable were omitted from the multivariate analysis. The models were stratified by season to assess any interacting effects of season and rainfall. On the basis of rainfall patterns for the time period of collection, we considered July−December the dry season and January−June the rainy season.

For household samples, in addition to weekly rainfall, covariates included observed container type (small versus large mouth), reported duration of storage time (h), reported water source (rain, well, Estero Maria, or Santiago River), reported treatment (none, boiled, chlorinated, or left to settle), and whether the container was covered at the time of collection. Container mouths were classified as <8 cm (plastic soda bottles and jerry cans) versus >8 cm (buckets, large water barrels, and cooking pots). Additionally, whether the stored water was used as drinking water in the home was

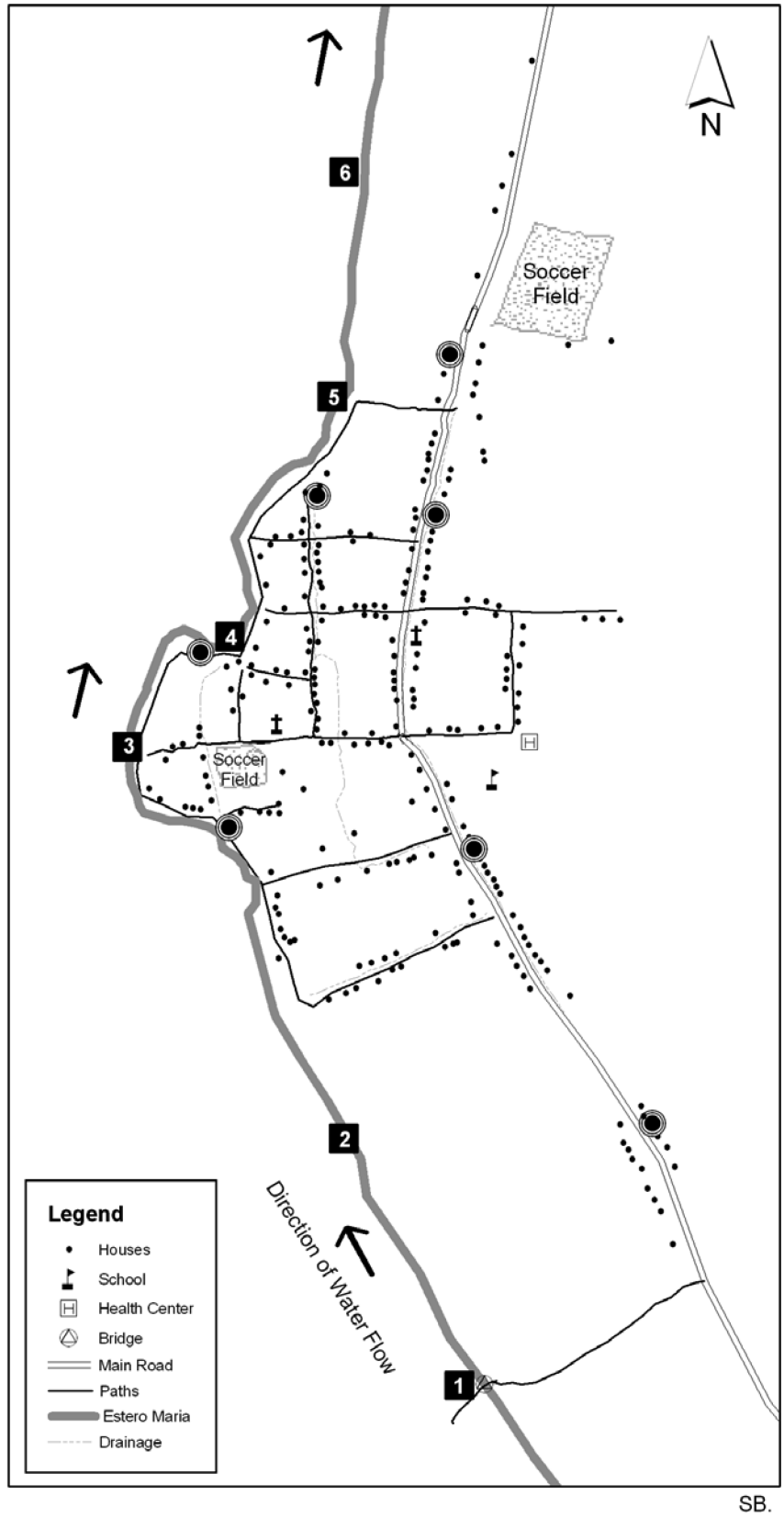


**FIGURE 1. Map of Colon Eloy indicating water collection sites. Source sampling sites are numbered and indicated in black boxes. Household sites are indicated with a large circle. Note the location of household sites is slightly offset in order to protect the identity of human subjects. Weekly samples were collected at sites 1, 2, 3, 5, and 6. Daily samples were collected for 11 consecutive days at sites 1, 2, 4, and 6. Hourly samples were collected for four consecutive days in the wet and dry seasons at site 4. Arrows indicate direction of river flow.**

tested univariately. Because this variable is not a driver of water quality, and because this question was only asked for half of the samples, it was not included in the multivariate analysis. Information on household samples was either observed directly or reported by a key informant in the household. The Institutional Review Boards at the University

**TABLE 1. Summary of Data Sets Used in Analyses[a]**

| data set | sampling locations | sampling dates | sampling frequency | number of samples analyzed | lower detection limit | number of results below xdetection limit | upper detection limit | number of results above detection limit |
|---|---|---|---|---|---|---|---|---|
| hour-to-hour: dry season | site 4 | 9/1/05−9/4/05 | every 90 min[b] | 82 | 50 | 0 | 45000 | 6 (7.3%) |
| hour-to-hour: wet season | site 4 | 1/27/06−1/30/06 | every 90 min[b] | 76 | 50 | 0 | 45000 | 5 (6.6%) |
| day-to-day | sites 1, 2, 4, 6 | 8/12/05−8/22/05 | daily | 51 | 50 | 1 (2.0%) | 45000 | 0 |
| week-to-week: source | sites 1, 2, 3, 5, 6 | 1/28/05−3/2/06 | weekly | 306 | 16.7 | 20 (6.5%) | 10000 | 50 (16.3%) |
| week-to-week: household | 7 households | 1/28/05−3/2/06 | weekly | 980 | 16.7 | 349 (35.6%) | 10000 | 53 (5.4%) |

[a] Detection limit units are number *E. coli* colony-forming units per 100 mL. [b] Between 2300 and 500 h samples were collected only every 180 min.

of California, Berkeley and the Universidad San Francisco de Quito approved protocols for interaction with human subjects.

## Results

Geometric means for all samples, source samples and household samples, stratified by season are listed in Table 2. Further details for each sampling site and household are provided in Table S1 of the Supporting Information. Water contamination was approximately 0.2 log higher in the wet season than in the dry season ($p = 0.0025$). A stronger seasonal difference was seen in source samples (0.42 log difference, $p < 0.0001$) than in household samples (0.11 log difference, $p = 0.077$). An overall trend of increasing contamination from sampling site 1 (upstream of the main human settlement) to sampling site 6 (downstream end of the village) was also observed (nonparametric test for trend across ordered groups of $p < 0.0001$).

The variability at different time scales is shown in Figure 2. We observed seasonal differences, with overall lower counts in the dry season, but more variability occurred on an hour-to-hour basis than on a day-to-day or week-to-week basis (Figure 2a). The week-to-week sampling series ranged over a 1.5 log difference in the wet season and a 1.8 log difference in the dry season, from 164 to >10000 CFU/100 mL in the dry season (mean = 1859, $\sigma = 2792$) and from 308 to >10000 in the wet season (mean = 2354, $\sigma = 1744$). In the day-to-day sampling series at four sites along the Estero Maria, ranges spanned a 2.2 log difference, from 17 to 2800 CFU/100 mL (mean = 477, $\sigma = 502$) (Figure 2b). Within any one given site, 1.0−2.2 log differences occurred from one day to the next. Counts of *E. coli* varied over the course of a single day even more than the day-to-day measurements taken at the same time each day. The hour-to-hour sampling series ranged over a 2.15 log difference in the wet season and a 2.35 log difference in the dry season. Mean counts for the hour-to-hour sample series were similar in both seasons and ranged from 200 to >45000 CFU/100 mL (mean = 5383, $\sigma = 11363$) in the dry season and from 300 to >45000 CFU/100 mL (mean = 6614, $\sigma = 11401$) in the wet season (Figure 2c).

The stream overtopped its banks during the four-day sampling period in the wet season. During the flood, *E. coli* counts were high, despite its occurrence during nighttime hours when lower counts are usually experienced (Figure S1 of the Supporting Information).

The impact of various explanatory variables on water quality for source and household samples is listed in Table 3. A more complete table showing univariate analyses for all variables considered in the model selection process is listed in Table S2 of the Supporting Information. As mentioned above, river level and river clarity are not included in the multivariate model because they are in the causal pathway between rainfall and water quality. Correspondence of these three variables can be seen in Figure S2 of the Supporting Information. Controlling for the time of day when the sample was collected did not affect the outcome of the model and was therefore not included.

When both seasons were combined, the adjusted coefficient of −0.03 for weekly rainfall suggests that a 1 cm increase in rainfall was associated with a 3% decrease in *E. coli* counts (CFU/100 mL) in source waters ($p = 0.006$). This effect was not seen in the dry season ($\beta = 0.01$, $p = 0.96$) but was evident in the wet season ($\beta = -0.03$, $p = 0.006$). Spikes of up to 25 cm in weekly rainfall occur during the wet season (Figure S2 of the Supporting Information). The number of people in the river at the time of sample collection was associated with significantly elevated *E. coli* counts when both seasons were considered together ($\beta = 0.04$, $p = 0.004$), but again the stratified analysis revealed that this effect was

**TABLE 2. Geometric Mean Values (*E. coli* CFU/100 mL) by Season for Weekly Samples[a]**

| | overall | dry season | wet season | $\log_{10}$ difference | *p* value |
|---|---|---|---|---|---|
| all weekly samples | 375 ($n = 1251$) | 293 ($n = 541$) | 451 ($n = 710$) | 0.19 | 0.0025 |
| weekly samples: sources only | 1182 ($n = 238$) | 680 ($n = 106$) | 1775 ($n = 132$) | 0.42 | <0.0001 |
| weekly samples: households only | 260 ($n = 1009$) | 224 ($n = 430$) | 291 ($n = 579$) | 0.11 | 0.0770 |

[a] Samples were collected over 25 weeks in the dry season and 40 weeks in two wet seasons. $\text{Log}_{10}$ differences are calculated from the difference between the geometric mean values. Reported *p* values are for the Mann−Whitney nonparametric test of equality of distribution between seasons.

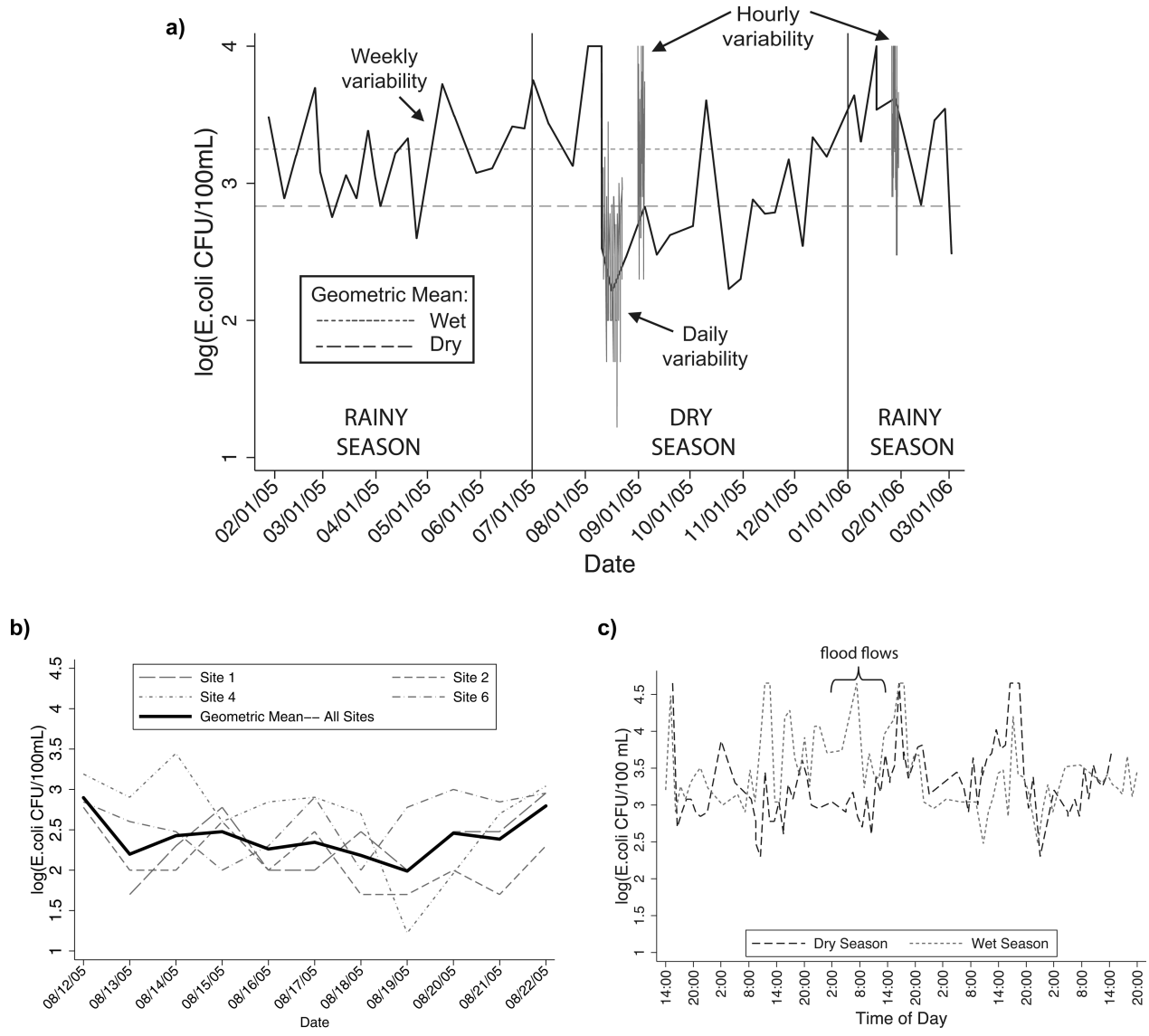


**FIGURE 2. (a) Week-to-week, (b) day-to-day, and (c) hour-to-hour variability in source water quality, as measured by *E. coli* concentrations. (a) Geometric mean of weekly samples collected at five stream sampling sites, with results of daily and hourly sampling indicated. Note the maximum value of log *E. coli* concentrations was capped at 4.0 for all sampling series. Geometric mean values for each season are shown with horizontal lines. (b) Daily samples collected during the dry season over the course of 11 days (August 12−22, 2005) for individual sites and overall geometric mean for all sites (shown with a thick solid line). Sites 1, 2, and 4 were sampled between 0900 and 1200 h each day, and site 6 was sampled between 1400 and 1600 h. (c) Samples collected on an hour-to-hour basis over the course of four consecutive days at sampling site 4 in the dry season (September 1−4, 2005) and in the wet season (January 27−30, 2006). Note in the wet season the stream flooded; the time for which it flowed at greater than bankful flow is indicated.**

only significant in the wet season ($\beta = 0.04$, $p = 0.026$) and not in the dry season ($\beta = 0.05$, $p = 0.315$). This suggests a 4% increase in *E. coli* counts in source waters for every additional person in the river.

Household and source water quality were loosely correlated over time (regression of weekly geometric means across sampling sites and households is $r^2 = 0.15$, $p = 0.006$) (Figure 3), although on average approximately 0.7 log less contamination (dry season is 0.5 log, wet season is 0.8 log) was observed in the household samples than in the source samples (Table 2, Figure 3). In the household samples, rainfall was also associated with reduced *E. coli* counts ($\beta = -0.03$,

**TABLE 3. Results of GEE Regressions Testing the Effect of Various Covariates on Counts of *E. coli*/100 mL[a]**

**Source Samples**

| | both seasons | | | dry season | | | wet season | | |
|---|---|---|---|---|---|---|---|---|---|
| variable | coefficient | *p* value | 95% CI | coefficient | *p* value | 95% CI | coefficient | *p* value | 95% CI |
| pH | 0.04 | 0.770 | −0.25 to 0.34 | 0.09 | 0.722 | −0.41 to 0.60 | −0.16 | 0.617 | −0.78 to 0.46 |
| electroconductivity (mS/cm) | −1.88 | 0.663 | −10.33 to 6.57 | 0.36 | 0.962 | −14.09 to 14.80 | 7.89 | 0.108 | −1.73 to 17.50 |
| water temperature (°C) | −0.04 | 0.301 | −0.11 to 0.03 | 0.04 | 0.720 | −0.18 to 0.26 | −0.01 | 0.468 | −0.05 to 0.02 |
| number of people in river | 0.04 | **0.004** | 0.01−0.06 | 0.05 | 0.315 | −0.04 to 0.14 | 0.04 | **0.026** | 0.01−0.07 |
| weekly rainfall (cm) | −0.03 | **0.006** | −0.05 to −0.01 | 0.01 | 0.961 | −0.31 to 0.33 | −0.03 | **0.006** | −0.05 to −0.01 |

**Household Samples**

| | both seasons | | | dry season | | | wet season | | |
|---|---|---|---|---|---|---|---|---|---|
| variable | coefficient | *p* value | 95% CI | coefficient | *p* value | 95% CI | coefficient | *p* value | 95% CI |
| container: large mouth | **−0.02** | **0.899** | **−0.38 to 0.33** | **0.35** | **0.300** | **−0.31 to 1.01** | **−0.32** | **0.094** | **−0.70 to 0.06** |
| storage time (h) | **0.00** | **0.186** | **−0.01 to 0.00** | **0.00** | **0.897** | **−0.01 to 0.01** | **−0.01** | **0.111** | **−0.02 to 0.00** |
| source: Estero Maria (baseline) | | | | | | | | | |
| source: rainwater | **−0.62** | <0.0001 | **−0.95 to −0.28** | **−1.10** | <0.0001 | **−1.46 to −0.75** | **−0.80** | <0.0001 | **−1.08 to −0.52** |
| source: well water | **−0.93** | 0.001 | **−1.49 to −0.37** | **−2.49** | <0.0001 | **−3.89 to −1.12** | **−0.01** | **0.981** | **−0.71 to 0.69** |
| source: Santiago River | **−0.27** | **0.390** | **−0.88 to 0.34** | **−0.13** | **0.772** | **−0.98 to 0.73** | | | |
| treatment: none (baseline) | | | | | | | | | |
| treatment: boiled | **0.16** | **0.211** | **−0.09 to 0.41** | **0.11** | **0.652** | **−0.38 to 0.61** | **0.14** | **0.446** | **−0.22 to 0.49** |
| treatment: chlorinated | **0.14** | **0.642** | **−0.46 to 0.74** | **0.43** | **0.102** | **−0.09 to 0.95** | **−0.19** | **0.723** | **−1.25 to 0.86** |
| teatment: left to settle uncovered (baseline) | **−0.02** | **0.981** | **−1.32 to 1.29** | | | | **−0.44** | **0.609** | **−2.13 to 1.25** |
| covered | **0.46** | 0.003 | **0.16 to 0.76** | **0.88** | <0.0001 | **0.45 to 1.31** | **0.20** | **0.242** | **−0.14 to 0.54** |
| weekly rainfall (cm) | **−0.03** | 0.049 | **−0.06 to 0.00** | **0.11** | **0.273** | **−0.09 to 0.32** | **−0.06** | 0.012 | **−0.10 to −0.01** |

[a] Coefficients report results of multivariate analyses, controlling for correlation within sampling sites (source samples) and within households (household samples). Results of univariate analyses for the full set of variables tested can be found in Table S2 of the Supporting Information. Results are shown for the entire year (source samples of $n = 291$, household samples of $n = 874$) and also stratified by season [source samples of $n = 119$ (dry season) and $n = 172$ (wet season), household samples of $n = 382$ (dry season) and $n = 492$ (wet season)].

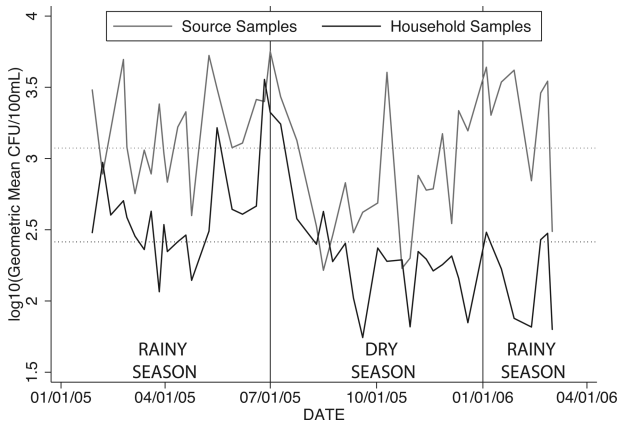


**FIGURE 3. Variation in geometric mean counts of *E. coli* in water from surface source waters and household containers over the course of the year. The geometric mean value for each type of sample is shown with a horizontal line. For log geometric mean counts of *E. coli,* the upper limit of detection is 4.0 (10000 CFU/100 mL) and the lower limit of detection is 1.23 (17 CFU/100 mL).**

$p = 0.049$). The significance and magnitude of this effect increased in the wet season ($\beta = -0.06$, $p = 0.012$) but was not observed in the dry season ($\beta = 0.11$, $p = 0.273$). This analysis suggests that a 1 cm increase in rainfall was associated with a 3% decrease in *E. coli* counts in water stored in the household (6% decrease in the rainy season only).

Other factors affecting household water quality included the source of the water (rainwater and well water were protective in the dry and wet season, whereas only rainwater was protective in the wet season) and whether or not the container was kept covered, which predicted an increase in contamination in the dry season. A significantly higher percentage of households reported treating their water during the dry season (mean = 46%, range of 24−64%) than during the wet season (mean = 31%, range of 0−71%) (one-sided *t* test, $p = 0.0003$).

## Discussion

**Explaining Variability in Surface Source Water Quality.** We observed large variations in concentrations of *E. coli* at different times of the day, week, and year in this rural Ecuadorian village. More variability was seen in water quality on an hour-to-hour basis (Figure 2c) than on a day-to-day (Figure 2b) or week-to-week (Figure 2a) basis. Despite this variability, seasonal, diurnal, and spatial patterns can be discerned and explained by environmental (e.g., seasonal weather and soil processes) and other drivers (e.g., human river use and water practices), suggesting that weather variables can interact with human practices to determine water quality at the source and in households in this village.

Strong seasonal differences in microbial contamination were seen in source waters, with significantly higher than average *E. coli* counts in the wet season than those in the dry season (Table 2). Interestingly, despite higher contamination levels in the wet season, increased weekly rainfall was significantly associated with reduced contamination levels in the wet season (Table 3). This seemingly inconsistent result can be understood via consideration of microbial processes related to soil moisture conditions, as well as an examination

of the effects of rainfall at different time scales. We explore three mechanisms for how weather affects variability in microbial contamination of source waters—the runoff effect, the dilution effect, and the concentration effect—and the time scales over which they act.

The first way in which weather and season might affect *E. coli* counts in source waters is through what we call the "runoff effect". This effect is characterized by increased microbial contamination associated with wet conditions as caused by direct flushing of fecal material from the surrounding environment into the stream following heavy rainfall and/or mobilization of bacteria resident in the soil. These phenomena can act at seasonal and short-term (hourly) time scales. *E. coli* counts in source water samples were on average 0.4 log higher in the wet season than in the dry season. We posit that the higher overall contamination levels observed in the rainy season can be explained by transport of *E. coli* via shallow subsurface flows and scouring of riverbank soils due to variable river flows in the wet season that may release bacteria that are associated with sediments or have survived or grown in the soil.

Several authors have found that *E. coli* and other indicators of fecal contamination can survive and regrow in tropical (*4, 26*−*28*) and even temperate soils (*29*), and the ability of *E. coli* to multiply in the soil is dependent on soil moisture content (*4, 27*). While this microbial contamination may not all be human sourced, poor sanitation in the village suggests the potential for at least some of it to originate from human fecal material, and more work on source tracking is necessary to establish the human health risk.

We also observed some evidence of the runoff effect on the shortest time scale measured (hours), with the immediate impact of a heavy pulse of rainfall. During the four day intensive (hour-to-hour) sampling, we observed increased levels of microbial contamination at the onset of the flood event during the hours of the day that normally experience lower contamination. This spike in contamination was followed by a decrease before the flood had subsided, suggesting that existing contamination might have been flushed out of the system in the short term (Figure 2c).

The effect of flushing by rainfall is also seen in the results of the GEE analysis. This comprises the second effect we believe to be working in this system, which we term the "dilution effect", whereby higher overall water flows dilute the concentration of *E. coli* in the stream. This effect occurs at an intermediate time scale on the order of days or weeks. Despite the overall higher contamination levels in the rainy season, we observed a significant inverse relationship between rainfall and *E. coli* counts in source water samples. For every centimeter of weekly rainfall in the wet season, a 3% decrease in *E. coli* counts in source waters was observed. Thus, while soil moisture content coupled with subsurface transport may lead to overall higher levels of *E. coli* in the stream, rainfall pulses still have the effect of diluting the concentrations of these microorganisms.

Flushing is a common phenomenon in hydrology, describing a situation where solutes or other substances undergo a period of accumulation, followed by a period of flushing by a snowmelt or rainfall event. What distinguishes the processes in this system is the constant source of fecal material available in the environment due to poor sanitation. As opposed to a "first flush" effect, the runoff and dilution effects in this village are not limited to the first heavy rains of the year. The collective flushing effects observed here are thus more transport limited than supply limited (*30*).

Lastly, another competing but not mutually exclusive phenomenon is the "accruing input effect" (aka, the "concentration effect"), whereby dry conditions cause an increase in the density of microorganisms due to a lack of dilution by higher flows during storm events (*11*). This effect occurs at a seasonal time scale. These bacteria may originate from poor sanitation, from survival and/or growth in riverbank soils, or from people bathing and washing in the stream. The concentration effect has been used to explain cases of dry season epidemics of diarrheal disease (*31*). We observe some evidence of this effect in the dry season because microbial contamination is not associated with rainfall (Table 3). During the dry season, when it still rains periodically, peaks in contamination are therefore independent of rainfall pulses and likely driven by local contamination events that do not get flushed out of the river, as they would during higher wet rains (on a shorter time scale). *E. coli* does not regrow in the soil or get transported through subsurface flows to the extent that it occurs during the wet season. Further research quantifying the dynamics of *E. coli* populations in the soil and subsurface flows during wet and dry seasons is needed to test these hypotheses.

Interestingly, we found a significant positive association between the number of people in the river at the times of sample collection and *E. coli* counts in the wet season only. People in the river may contribute localized pulses of fecal contamination through bathing and washing in the stream, and if the accruing input (concentration) effect were at play in this system, we would expect to observe these impacts in the dry season. However, more people use the river for bathing and washing in the wet season than they do in the dry season, and during this particular period of study, a community-enforced ban on bathing and washing in the river was put into place in the sampling months when the river was extremely low (mid-July through mid-November). The mean number of people in the river at the time of weekly sample collection in the dry season was 0.79 ($\sigma = 1.95$) versus 2.15 in the wet season ($\sigma = 3.62$) ($\chi_2$, $p = 0.02$), which may explain why we did not observe a significant effect from people in the river during the dry season. Still, the significant relationship between people in the river and contamination levels in the wet season suggests that bathing and washing clothes in the river are likely primary sources of fecal contamination in these surface waters. This effect occurs on a short (hourly) time scale and is supported by our observation that peak *E. coli* counts occurred during daytime hours (Figure S1 of the Supporting Information), despite the fact that solar radiation is known to reduce the concentration of fecal indicator bacteria (*3*).

The interaction of environmental drivers and the water quality of source samples can therefore be understood as a complex interaction of various effects under different conditions at different time scales. The runoff, dilution, and concentration effects are all likely contributing to the patterns observed. It is important to note that the runoff and accruing input (concentration) effects are exacerbated in areas with poor sanitation, where a constant input of fecal material is available for environmental transport. Often areas with poor sanitation also have inadequate protection of drinking water sources such as surface water and shallow wells, which increases the impact of storm flows on drinking water quality.

**Explaining Variability in Stored Household Water Quality.** For household water samples, both seasonality (which represents temporal variability) and household-level factors (which represents a sort of spatial variability) appear to affect the variability in water quality.The water source, container type (large or small mouth), and whether the container was kept covered were consistently significant factors affecting household water quality in both seasons, whereas rainfall was significant in the wet season but not in the dry season.

Treatment of water by boiling, chlorination, or settling did not show a significantly protective effect against microbial contamination in the samples taken from the seven households surveyed (Table 3). In fact, chlorination was actually associated with an increase in *E. coli* concentrations. This

inverse association may be due to misclassification due to respondent error because treatment practices were recorded as reported by key informants in the household, poor post-treatment storage practices leading to recontamination of water after treatment (*32*), or in the case of chlorine, incomplete disinfection due to rapid dissipation of the chlorine residual due to reactions with other compounds (e.g., organic matter) (*33*). In another study in the same village, the concentrations of indicator organisms in stored water of households chlorinating their drinking water were not significantly lower than in households that did not treat their water (unpublished). Boiling has also been shown to be an ineffective form of treatment, likely due to recontamination during water handling (*34*).

Household water samples were approximately 0.7 log less contaminated than source water samples, both in the wet season and in the dry season (Table 2), and we saw a loose correlation over time between household and source water quality (Figure 3). This correlation was likely weakened by the fact that drought conditions led some villagers to import their drinking water from the nearby Santiago River, a larger and faster flowing water source, during the dry season period of study. While the use of Santiago River water in the dry season may affect the comparison of source versus household water, the result seen here is also similar to what we have observed throughout this study region, where we have recorded significantly higher concentrations of indicator organisms in source waters than at its point-of-use (*35*). This decrease may be due to settling or die off of bacteria in turbid source waters. This result contradicts other studies that have found water stored in the home to be significantly more contaminated than the source water (*32*). However, most studies of contamination between source and point-of-use have been carried out in areas with improved water supplies. In locations with highly contaminated source waters, this result is less apparent (*36*−*38*).

We observed rainfall to be associated with reduced contamination in the home in the wet season. This effect may be due to reduced microbial contamination in original source waters, a higher turnover rate of water stored in the home in the wet season, which would limit the opportunity for recontamination, or a combination of both. Interestingly, covering the storage container was associated with increased levels of contamination, especially in the dry season. This might also be related to lower turnover rates in the dry season, increasing the probability of recontamination during storage. Unfortunately, we did not collect data on the frequency with which households refill storage containers in the wet and dry seasons, so we cannot comment definitively. Another possibility is that households may rely more on rain water and well water in the wet season, both of which were significantly protective against microbial contamination (Table 3) in the wet season. Data on water treatment trends across the village show that a higher percentage of households treat their water during the dry season than they do during the wet season. However, as mentioned above treatment is not always sufficient, which might explain why treatment did not improve water quality in the home during either season. Additionally, the correlation between environmental factors and household water samples may have been biased by the importation of water from the larger Santiago River, as mentioned above.

**Uncertainty in Measurement.** While much of the variability in contamination levels in the home and at the source can be explained by temporal variability, environmental factors, and water storage and usage practices, a large amount of uncertainty in measurement limits the conclusions drawn by any study using indicator organisms.

Measurement error may be due to the methods used to culture *E. coli*. Each petrifilm plate tests a 1 mL water sample, which limits the precision of the analysis. This limitation was especially important in assessing the lower contamination levels in the household samples (Table 1). However, studies have shown that results with petrifilms were comparable to those of standard methods when 1 mL samples were consistently used (*24*). Additionally, we found a high degree of variability in results when we quantified *E. coli* concentrations in the hour-to-hour (up to a 1.24 log difference) and day-to-day (up to a 1.88 log difference) samples using 10 mL sample volumes and processed the samples using membrane filtration with MI agar (BD Difco) (Figures S3 and S4 of the Supporting Information). We did not use these techniques on the samples collected on a weekly basis.

In this study, weekly samples were not collected at a consistent time each day because at the time we designed the study, we did not consider the potential impact of the time of day of sample collection on results. Our methods were also limited by the logistical constraints of our field study site, which constrained us to incubating *E. coli* at ambient temperatures.

**Applications.** The results reported here have several practical applications. First, in measuring water quality, our results suggest that an analysis of more than one grab sample is needed to provide an accurate assessment of water quality at any given time. In the time period of this study, we might have come to drastically different conclusions about the quality of source waters depending on the time, day, or season when sampling occurred. From an examination of Figure 2a, it appears that the day-to-day sampling series took place during a dip in the levels of contamination during the dry season, whereas the hour-to-hour sampling series in the dry season occurred during a point of higher overall contamination. This may also be due to the consistent hour of day at which the day-to-day sampling occurred (in the morning, in all but one site).

As suggested by Jensen et al. (*2*), the relatively low sampling frequency of water quality in epidemiological studies may fail to provide accurate estimates of the varying levels of fecal contamination to which people are exposed through drinking water. This factor may explain in part why indicators of water quality are rarely correlated with diarrheal disease, even when interventions to improve water quality are seen to be effective in preventing diarrheal disease (*39*). We agree with Boehm et al. (*3*), who suggest that the geometric mean standard, in which the geometric mean of multiple samples is collected over a specified window of time, is preferable to single samples for evaluation of water quality.

When using indicator organisms to characterize fecal contamination, it is important to characterize the variability inherent in the system in order to sample at a frequency in both space and time that provides the appropriate resolution to detect true differences between samples. For example, we were able to detect differences between seasons using our weekly data collected over the course of a year, but these seasonal patterns were not apparent when only comparing hour-to-hour data collected over the course of four days in two different seasons because of the high degree of variability inherent in this system. Additionally, the seasonal, diurnal, and spatial patterns we observed suggest that some sources of variability can be reduced to focus on the variables of interest (e.g., samples can be collected at the same time of day, at the same location, or within a single season).

Second, while the results of this study show that water can potentially be contaminated at any time of day, there were certain hours of the day and conditions under which water was more likely to be contaminated. In the absence of more comprehensive interventions involving in-home water treatment, safe storage devices, and sanitation improvements, community health workers could encourage

people to collect their water in the morning, before the onset of extensive human activity in the river, and also encourage the use of rainwater when available. However, in this and other villages with inadequate sanitation that depend on untreated surface water, more significant interventions are clearly needed to prevent consumption of microbially contaminated drinking water in the village.

This study illustrates the importance of seasonal drivers on water quality at both the household and village scales. Heavy rainfall events, flooding, and increased temperature associated with global climate change are all expected to affect the incidence of waterborne diseases (*40*), and climate change is expected to increase the variability of rainfall. While we did not characterize the direct effects of sanitation on water quality, the main source of contamination in this village is likely feces due to poor sanitation and use of the river for bathing and washing. Factors such as water supply and sanitation have the potential to modify the effect of seasonal weather differences on water quality in the home and should therefore be considered when predicting the impact of climate change on diarrheal disease in areas with poor sanitation infrastructure. The effect of rainfall in other regions has been shown to vary depending on water source (*10, 16*). We believe that sanitation is likely interacting with rainfall in causing increased microbial contamination in unprotected wells and source waters and should continue to be considered alongside other intervention strategies in efforts to reduce waterborne illnesses.

These results can also be considered in the larger context of anthropogenic changes in the landscape of this region. In 1996, a new highway was completed along the northern Ecuadorian coast to facilitate logging in the region, which is now considered one of the top 10 deforestation fronts in the world (*41*). Deforestation is known to affect local climatic and hydrological patterns (*42, 43*), and decreased vegetative cover may lead to flashier conditions, with higher incidence of both flooding and drought in the stream. In this region, higher rates of diarrheal disease, especially those with bacterial etiologies, have been observed in villages closer to the new road (*21*), which correspond to the villages with the greatest loss of surrounding forests. The village of Colon Eloy, situated along a secondary road that branches off of this main road, has suffered high amounts of forest clearing, and this loss of forest cover may indirectly affect water quality by increasing variability of water flow patterns in the Estero Maria. In considering the interactions among seasonality, water quality, and human health, such upstream effects merit further investigation.

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## Supporting Information Available

Table S1 is an extended version of Table 2. Note that sample site 4 was not sampled on a weekly basis and is therefore not included here. Table S2 is an extended version of Table 3. Unadjusted coefficients report results of univariate analyses, whereas adjusted coefficients report the results of multivariate analyses. Results are shown for the entire year and are also stratified by season. Figure S1 shows $\log_{10}$ geometric mean counts of *E. coli* by hour of day for the (a) dry season and (b) wet season. Upper limit of detection is 4.65 (45000 CFU/100 mL), and lower limit of detection is 1.70 (50 CFU/100 mL). Geometric mean value for the season is shown with a dotted line. Figure S2 is the correspondence of variations in weekly measurements of rainfall, river level, and river clarity over the period of study. Figure S3 is the hour-to-hour variability in *E. coli* concentrations over the course of four days in the dry season and wet season, as measured by membrane filtration using MI agar (BD Difco). Figure S4 is the day-to-day variability in *E. coli* concentrations at four stream sampling sites on Estero Maria over the course of 11 days, as measured by membrane filtration using MI agar (BD Difco). This material is available free of charge via the Internet at http://pubs.acs.org.

## Literature Cited

(1) Committee on Indicators for Waterborne Pathogens, Board on Life Sciences, Water Science and Technology Board, Division on Earth and Life Studies, National Research Council. Introduction and Historical Background. *Indicators for Waterborne Pathogens*; The National Academies Press: Washington, DC, 2004; pp 17−52.

(2) Jensen, P. K.; Jayasinghe, G.; van der Hoek, W.; Cairncross, S.; Dalsgaard, A. Is there an association between bacteriological drinking water quality and childhood diarrhoea in developing countries. *Trop. Med. Int. Health* **2004**, *9* (11), 1210–1215.

(3) Boehm, A. B.; Grant, S. B.; Kim, J. H.; Mowbray, S. L.; McGee, C. D.; Clark, C. D.; Foley, D. M.; Wellman, D. E. Decadal and shorter period variability of surf zone water quality at Huntington Beach, California. *Environ. Sci. Technol.* **2002**, *36* (18), 3885–3892.

(4) Solo-Gabriele, H. M.; Wolfert, M. A.; Desmarais, T. R.; Palmer, C. J. Sources of *Escherichia coli* in a coastal subtropical environment. *Appl. Environ. Microbiol.* **2000**, *66* (1), 230–237.

(5) George, I.; Anzil, A.; Servais, P. Quantification of fecal coliform inputs to aquatic systems through soil leaching. *Water Res.* **2004**, *38*, 611–618.

(6) Dorner, S. M.; Anderson, W. B.; Gaulin, T.; Candon, H. L.; Slawson, R. M.; Payment, P.; Huck, P. M. Pathogen and indicator variability in a heavily impacted watershed. *J. Water Health* **2007**, *5* (2), 241–257.

(7) Howard, G.; Pedley, S.; Barrett, M.; Nalubega, M.; Johal, K. Risk factors contributing to microbiological contamination of shallow groundwater in Kampala, Uganda. *Water Res.* **2003**, *37* (14), 3421–3429.

(8) Lindskog, R. U. M.; Lindskog, P. A. Bacteriological contamination of water in rural areas: an intervention study from Malawi. *J. Trop. Med. Hyg.* **1988**, *91*, 1–7.

(9) Barrell, R. A. E.; Rowland, M. G. M. The relationship between rainfall and well water pollution in a West African (Gambian) village. *J. Hyg. (London)* **1979**, *83*, 143–150.

(10) Sandiford, P.; Gorter, A. C.; Davey Smith, G.; Pauw, J. P. C. Determinants of drinking water quality in rural Nicaragua. *Epidemiol. Infect.* **1989**, *102*, 429–438.

(11) Wright, R. C. The seasonality of bacterial quality of water in a tropical developing country (Sierra Leone). *J. Hyg. (London)* **1986**, *96*, 75–82.

(12) Blum, D.; Huttly, S. R. A.; Okoro, J. I.; Akujobi, C.; Kirkwood, B. R.; Feachem, R. G. The bacteriological quality of traditional water sources in north-eastern Imo State, Nigeria. *Epidemiol. Infect.* **1987**, *99*, 429–437.

(13) Altizer, S.; Dobson, A.; Hosseini, P.; Hudson, P.; Pascual, M.; Rohani, P. Seasonality and the dynamics of infectious diseases. *Ecol. Lett.* **2006**, *9*, 467–484.

(14) Curriero, F. C.; Patz, J. A.; Rose, J. B.; Lele, S. The association between extreme precipitation and waterborne disease outbreaks in the United States, 1948−1994. *Am. J. Public Health* **2001**, *91* (8), 1194–1199.

(15) Thomas, M. K.; Charron, D. F.; Waltner-Toews, D.; Schuster, C.; Maarouf, A. R.; Holt, J. D. A role of high impact weather events in waterborne disease outbreaks in Canada, 1975−2001. *Int. J. Environ. Health Res.* **2006**, *16* (3), 167–180.

(16) Shiffman, M. A.; Schneider, R.; Turner, A. G.; Helms, R. W. Seasonality in water related intestinal disease in Guatemala. *Int. J. Biometeorol.* **1976**, *20* (3), 223–229.

(17) Singh, R. B. K.; Hales, S.; de Wet, N.; Raj, R.; Hearnden, M.; Weinstein, P. The influence of climate variation and change on diarrheal disease in the Pacific islands. *Environ. Health Perspect.* **2001**, *109* (2), 155–159.

(18) Kondo, H.; Seo, N.; Yasuda, T.; Hasizume, M.; Koido, Y.; Ninomiya, N.; Yamamoto, Y. Post-flood infectious diseases in Mozambique. *Prehosp. Disaster Med.* **2002**, *17* (3), 126–133.

(19) Schwartz, B. S.; Harris, J. B.; Khan, A. I.; Larocque, R. C.; Sack, D. A.; Malek, M. A.; Faruque, A. S.; Qadri, F.; Calderwood, S. B.;

Luby, S. P.; Ryan, E. T. Diarrheal epidemics in Dhaka, Bangladesh, during three consecutive floods: 1988, 1998, and 2004. *Am. J. Trop. Med. Hyg.* **2006**, *74* (6), 1067–1073.
(20) Qadri, F.; Khan, A. I.; Faruque, A. S.; Begum, Y. A.; Chowdhury, F.; Nair, G. B.; Salam, M. A.; Sack, D. A.; Svennerholm, A. M. Enterotoxigenic *Escherichia coli* and *Vibrio cholerae* diarrhea, Bangladesh, 2004. *Emerg. Infect. Dis.* **2005**, *11* (7), 1104–1107.
(21) Eisenberg, J. N. S.; Cevallos, W.; Ponce, K.; Levy, K.; Bates, S. J.; Scott, J. C.; Hubbard, A.; Vieira, N.; Endara, P.; Espinel, M.; Trueba, G.; Riley, L. W.; Trostle, J. Environmental change and infectious disease: How new roads affect the transmission of diarrheal pathogens in rural Ecuador. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103* (51), 19460–19465.
(22) Vieira, N.; Bates, S. J.; Solberg, O. D.; Ponce, K.; Howsmon, R.; Cevallos, W.; Trueba, G.; Riley, L.; Eisenberg, J. N. S. High prevalence of enteroinvasive *Escherichia coli* isolated in a remote region of northern coastal Ecuador. *Am. J. Trop. Med. Hyg.* **2007**, *76* (3), 528–533.
(23) Petrifilm *E. coli*/Coliform Count Plate Interpretation Guide. [Online]; 3M: St. Paul, MN, www.3m.com/microbiology(accessed May 1, 2007).
(24) Vail, J. H.; Morgan, R.; Merino, C. R.; Gonzales, F.; Miller, R.; Ram, J. L. Enumeration of waterborne *Escherichia coli* with Petrifilm plates: Comparison to standard methods. *J. Environ. Qual.* **2003**, *32*, 368–373.
(25) Liang, K.; Zeger, S. L. Longitudinal data analysis using generalized linear models. *Biometrika* **1986**, *73* (1), 13–22.
(26) Byappanahalli, M.; Fujioka, R. Indigenous soil bacteria and low moisture may limit but allow faecal bacteria to multiply and become a minor population in tropical soils. *Water Sci. Technol.* **2004**, *50* (1), 27–32.
(27) Desmarais, T. R.; Solo-Gabriele, H. M.; Palmer, C. J. Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Appl. Environ. Microbiol.* **2002**, *68* (3), 1165–1172.
(28) Fujioka, R.; Hardina, C. Soil: The environmental source of *Escherichia coli* and enterococci in Hawaii's streams. *Environ. Toxicol. Water Qual.* **1995**, *6*, 185–195.
(29) Ishii, S.; Ksoll, W. B.; Hicks, R. E.; Sadowsky, M. J. Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. *Appl. Environ. Microbiol.* **2006**, *72* (1), 612–621.
(30) Burns, D. What do hydrologists mean when they use the term flushing. *Hydrol. Process.* **2005**, *19*, 1325–1327.
(31) Drasar, B. S.; Tomkins, A. M.; Feachem, R. G. Seasonal aspects of diarrhoeal diseases. In *Seasonal Dimensions to Rural Poverty*; Chambers, R., Ed.; Frances Pinter Publishers, Ltd.: London, 1981.
(32) Wright, J.; Gundry, S.; Conroy, R. Household drinking water in developing countries: A systematic review of microbiological contamination between source and point-of-use. *Trop. Med. Int. Health* **2004**, *9* (1), 106–117.
(33) LeChevallier, M. W.; Evans, T. M.; Seidler, R. J. Effect of turbidity on chlorination efficiency and bacterial persistence in drinking water. *Appl. Environ. Microbiol.* **1980**, *42* (1), 159–167.
(34) Oswald, W.; Lescano, A.; Bern, C.; Calderon, M.; Cabrera, L.; Gilman, R. Fecal contamination of drinking water within periurban households, Lima, Peru. *Am. J. Trop. Med. Hyg.* **2007**, *77* (4), 699–704.
(35) Levy, K.; Nelson, K.; Hubbard, A.; Eisenberg, J. Following the water: A controlled study of drinking water storage in northern coastal Ecuador. *Environ. Health Perspect.* **2008**, *116* (11), 1533–1540.
(36) Musa, H. A.; Shears, P.; Kafi, S.; Elsabag, S. K. Water quality and public health in northern Sudan: A study of rural and per-urban communities. *J. Appl. Microbiol.* **1999**, *87*, 676–682.
(37) VanDerslice, J.; Briscoe, J. Environmental interventions in developing countries: Interactions and their implications. *Am. J. Epidemiol.* **1995**, *141* (2), 135–144.
(38) Verweij, P. E.; van Egmond, M.; Bac, D. J.; van der Schroeff, J. G.; Mouton, R. P. Hygiene, skin infections and types of water supply in Venda, South Africa. *Trans. R. Soc. Trop. Med. Hyg.* **1991**, *85*, 681–684.
(39) Gundry, S.; Wright, J.; Conroy, R. A systematic review of health outcomes related to household water quality in developing countries. *J. Water Health* **2004**, *2* (1), 1–13.
(40) Hunter, P. R. Climate change and waterborne and vector-borne disease. *J. Appl. Microbiol.* **2003**, *94*, 37S–46S.
(41) Myers, N. Tropical forests: The main deforestation fronts. *Environ. Conserv.* **1993**, *20*, 9–16.
(42) Gentry, A. H.; Lopez-Parodi, J. Deforestation and increased flooding of the Upper Amazon. *Science* **1980**, *210*, 1354–1356.
(43) Neill, C.; Deegan, L. A.; Thomas, S. M.; Haupert, C. L.; Krusche, A. V.; Ballester, V. M.; Victoria, R. L. Deforestation alters the hydraulic and biogeochemical characteristics of small lowland Amazonian streams. *Hydrol. Process.* **2006**, *20*, 2563–2580.

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