# Effects of bathing intensity, rainfall events, and location on the recreational water quality of stream pools in southern Ecuador

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HIGHLIGHTS:

- Poor recreational water quality can impact public health and ecotouristism
- Bathers, rainfall, and location determine recreational water quality
- Bathing diminishes microbiological water quality from upstream to downstream pools.
- Regulations and land-use practices can enhance recreational water quality.

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# Abstract

Bathing in natural waters is a highly demanded recreational activity in tropical countries because of climatic conditions and availability of bathing sites; but, do users know the water quality of these sites? We determined the physicochemical and microbiological water quality of a highly used bathing site in southern Ecuador. We assessed how bather attendance, rainfall events, and pool location alters the recreational water quality (RWQ). Most of the parameters measured in the stream pools did not accomplish the Ecuadorian and international regulations for recreational water quality. Microbiological water quality diminishes from upstream to downstream pools because of human activities and bathing intensity having potential effects on bather health and eco-touristic development. We found that an increase of bathers is strongly associated with a growing concentration of *Escherichia coli*. It is suggested better land-use practices and review thoroughly the Ecuadorian regulation to assure a healthy RWQ. Further efforts are needed to identify more risky bathing sites, determine pollution sources, and establish a long-term monitoring program to support the touristic development in countries looking for diversifying their economy.

# Key words

Recreational water pollution; Fecal coliform; *Escherichia coli*; gastrointestinal illness; Tropical dry forest; Ecotourism

# 1 Introduction

Ecuador has a very rainy climate and is one of the South American countries with more abundant water resources: 43,500 $m^3 inhab^{-1} year^{-1}$. The availability of water is very different across the Ecuadorian Andes due to the differences in rainfall. For instance, the eastern slopes (Amazon basin) have a water availability of 82,900 $m^3 inhab^{-1} year^{-1}$, while in the western slopes (Pacific basin) diminishes to only 5,200 $m^3 inhab^{-1} year^{-1}$ (SENAGUA, 2012). The abundant rainfall, geology and the steep relief of the Ecuadorian Andes provide the necessary conditions to form pools along rivers and streams. Those pools are used for recreational activities contributing to the ecotourism industry, becoming an important income for rural population living around protected areas in Ecuador (López-Rodríguez and Rosado, 2017), and promoting the conservation of one of the most biodiverse countries in the world (Myers et al., 2000).

On the other hand, when precipitation falls over agricultural land, runoff can wash pathogens from the surface indirectly decreasing the microbiological water quality of the streams. For instance, runoff from manure-fertilized pastures (Iñiguez-Armijos et al., 2014). Moreover, sewage effluents can directly add more pathogens to streams (Knee and Encalada, 2014). In both cases, such an impoverishing of the microbial water quality may contribute to an increase of pathogens in recreational waters downstream becoming a public health problem (Santiago-Rodriguez et al., 2012). The quality of recreational water can also be affected by internal sources of pollution. Bathers act as a source of microorganisms, principally by shedding microorganisms from their bodies and by resuspension of polluted sand and sediment (Fewtrell and Kay, 2015; Phillip et al., 2009). This phenomenon becomes more intense when there are many bathers, and the pools are narrow, deep and with little water renewal (Kistemann et al., 2016).

This situation led developed countries and international institutions to write guidelines to ensure the quality of water for recreational use and the health of its users. Examples are the European Union with the Bathing Water Directive 2006/7/EC (European Council, 2006), the United States of America (United States Environmental Protection Agency, 2012) or the United Nations (World Health Organization, 2003). Later, developing countries, like Ecuador, drafted their regulations for recreational water quality (Ministerio del Ambiente del Ecuador, 2015). However, the regulations are rarely enforced because the public administrations lack the necessary resources to assure the quality of all types of water, prioritizing potable water.

Once in the water, microorganisms can infect human beings by the ingestion of contaminated water and bathing also, since microorganisms can enter the body through the ears, nose, eyes or a skin wound (World Health Organization, 2003). Pathogenic microorganisms are very diverse and include bacteria, viruses, and protozoans. For this reason, it is common to use fecal indicator bacteria (FIB) whose presence is correlated with the set of pathogenic microorganisms and their determination is easier, faster and cheaper (Dhondia et al., 2014; Jacob et al., 2015; Sunger and Haas, 2015).

Several studies have concluded that the presence of pathogenic microorganisms in recreational water increases the risk of gastrointestinal and dermatological diseases, in addition to respiratory and ear and eye related diseases (Eregno et al., 2016; Fewtrell and Kay, 2015). Epidemiological studies show a greater number of gastrointestinal diseases among frequent bathers than among non-bathers (Kistemann et al., 2016). In developing countries, diarrhea caused by microbiologically contaminated water are common and cause 2.2 million deaths per year (Montgomery and Elimelech, 2007).

The most common FIB are total coliforms, fecal coliforms, and *Escherichia coli*. Total coliforms are defined as all facultative aerobic and anaerobic bacillus, Gram negative, non-spore forming and fermenting lactose forming a gas at 35$^o$C. They are not a health threat itself, but an indicator of whether other potentially harmful bacteria may be present (American Public Health Association, 2012). Total coliforms refer to the entire group, and their origin does not have to be pathogenic, whereas fecal coliforms only include bacteria of fecal origin (Okafor, 2011). Fecal coliforms are defined as gram-negative, non-sporulating bacilli fermenting lactose with acid and gas production at 44.5°C. Fecal coliforms are an indicator of possible contamination by sewage or other decaying debris. The most representative species of the fecal coliform group is *E. coli*, that stands out as an indicator since it can cause cystitis, peritonitis, meningitis, infections of the excretory apparatus, mastitis, septicemia and pneumonia (Sunger and Haas, 2015). This fact has led the US Environmental Protection Agency and the European Union to recommend the use of *E. coli* (fecal coliform group) to analyze the microbiological quality of recreational waters (Mwanamoki et al., 2014).

For these reasons, pathogenic microorganisms in recreational waters should be permanently monitored. This type of monitoring ensures the health of users, promotes tourism and contributes to sustainable management (Phillip et al., 2009). With all of this in mind, this study aims to assess how recreational water quality of natural pools in a tropical dry forest stream is modified along the longitudinal gradient, by bathers, and during rainfall. We hypothesized that microbiological water quality decreases up-to-downstream pools, with higher bathing intensity, and during rainfall events. We also determined the relationships between the microbiological water quality and bathers in the stream pools, expecting an increase in *E. coli* concentration with a higher number of bathers. Finally, we evaluated the compliance to environmental regulations aimed to assure the recreational water quality in a country like Ecuador, which is looking to strengthen the tourism industry.

# 2 Materials and methods

## 2.1 Study site

We conducted this study in a second order stream (Papalango stream) of the Catamayo-Chira river basin located in the dry forest of southwestern Ecuador, 217 km far from the city of Loja (Fig. 1). The climate of this region is influenced by dry air masses coming from the Tumbesian Dry Region and by a low precipitation regime (Rollenbeck and Bendix, 2011). The annual mean temperature and precipitation in the study site are 23°C and 500 mm respectively, making the stream one of the most favorite natural places for bathing and outdoor recreation. For that reason, around 14,000 people visit this stream annually (Sánchez and Villareal, 2016). Papalango is a clear water stream presenting shallow fast-flowing riffles in the upper section and several deep slow-flowing pools in the middle and lower sections of the catchment. Because of size and accessibility, the pools located in the lower section are the favorite sites for recreational use.

The native vegetation of the study area is represented by scrubland and seasonally dry forest. However, this area holds an intensive agriculture such as most of southwestern Ecuador (Tapia-Armijos et al., 2015). The upper section of the Papalango’s catchment shows a mixed land use of native vegetation, pastureland, and cropland. The middle section is covered by native vegetation and a variety of crops, while the lower section is devoted to cropland, mostly maize.

At the lower section of the Papalango’s catchment, we selected four pools having different intensity of recreational use (Fig. 1). The four pools were labeled from A to D, being the pool A the most upstream site and pool D the most downstream site respectively. At pool A, no recreational activities are carried out because of difficult access and dense vegetation. The pool B is surrounded by low vegetation and access is only possible on foot, for this reason, the recreational use of the site is moderate. The pool C shows the highest use by bathers thanks to its easy access by car and new touristic infrastructure. The pool D has the lowest recreational use since it is the furthermost bathing site of the study area.

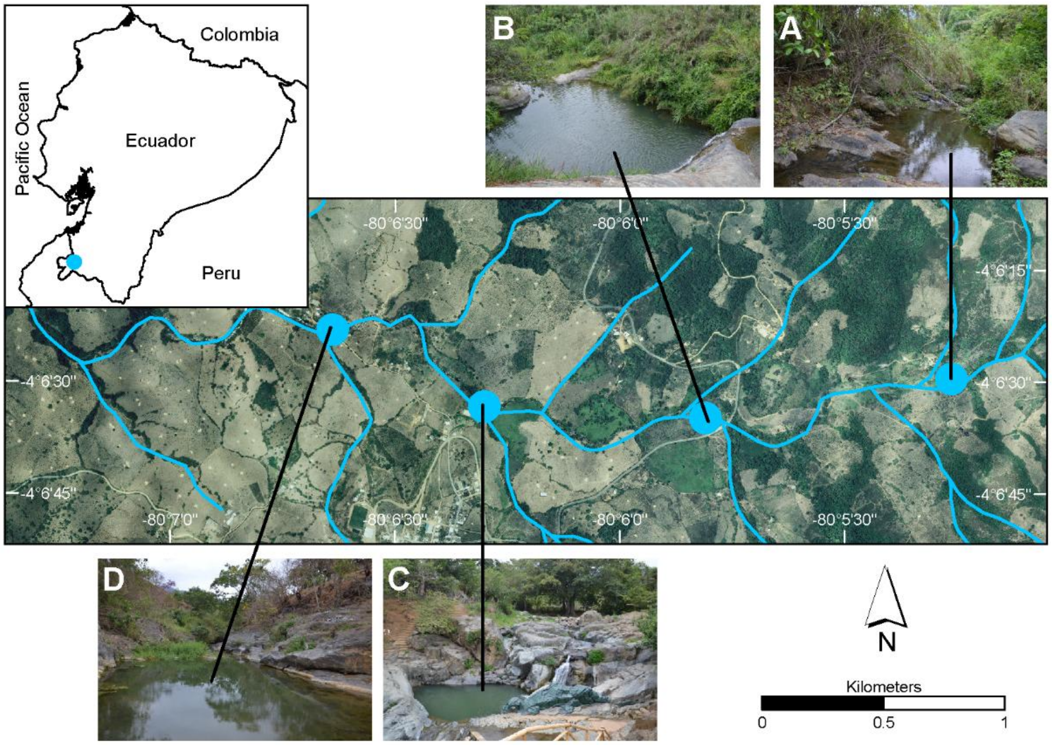

**Fig. 1.** Study area and location of the sampled pools. Photographs indicate the four studied natural pools located from up (A) to downstream (D) reaches along a tropical dry forest stream in southwestern Ecuador.

## 2.2 Field procedures

Sampling was conducted over eight dates before, during, and after Carnival celebration from February to March in 2015. Sampling dates were divided equally between the high (weekends and carnival days) and low (business days) attendance days, and between days with and without rainfall events. On each sampling date, the pools were sampled three times during the high demand period between 14:00 and 18:00 h. In total, each pool was sampled 24 times during this study ($n = 96$). *In situ*, we determined water temperature, pH, conductivity (Oakton PCTestr 35, USA), and dissolved oxygen (Sper Scientific DO Pen 850045, USA) using different portable probes. Water samples were taken in two sterilized plastic containers from each pool at 30 cm depth. One sample (500 ml container) was used for turbidity, phosphate, and nitrate determinations; while the other one (125 ml container) was used for determining the concentration of total and fecal coliforms, and *E. coli*. Water samples were transported in a dark cooler at 4°C to the laboratory and analyzed within two hours of sample collection. Additionally, we quantified the number of bathers at each pool during the field sampling.

## 2.3 Laboratory procedures

Water analyses were carried out in a laboratory located at 20 km far from the study site. The infrastructure, equipment, and reagents of the laboratory are managed by a group of municipalities situated in the dry forest region (Mancomunidad del Bosque Seco) with the aim of monitoring water quality in this region.

Turbidity was measured according to the nephelometric method with a turbidimeter (Hach 2100N, USA) following the standard methods protocol 2130 B (APHA, 2012). The turbidimeter was calibrated with standards supplied by the manufacturer. Turbidimeter cells were rinsed three times with water of the sample. Cells with the sample were immersed in an ultrasonic bath for 2 min before measurement to remove bubbles.

Phosphate measurements were carried out following the ascorbic acid method as described in protocol Hach 8048, similar to method 4500-P-E of standard methods (APHA, 2012). A Hach DR2800 spectrophotometer was calibrated at 880 nm with standard solutions supplied by the manufacturer. 10 ml cells were filled with the samples in duplicate, Phosver 3 reagent (Hach, USA) was added in only one of the cells containing every sample leaving the other duplicate as a blank. Cells were stirred for 30 s and allowed to stand for 2 min. Next, blanks were inserted into the spectrophotometer followed by the reagent samples to determine the concentration of phosphate.

Nitrate was determined using the Hach 8171 protocol, similar to method 4500-$NO_3$ E of standard methods (APHA, 2012). The spectrophotometer was calibrated at 400 nm with standards supplied by Merck (Germany). One cell of 25 ml was filled with deionized water and other 25 ml cell with the sample. Then, the reagent Nitraver 5 (Hach, USA) was added, and cells were stirred. Zero was set in the spectrophotometer with the blanks and samples were measured.

Fecal coliforms were determined following the protocol 9222 E (APHA, 2012). 100 ml of the sample was filtered. The filter was placed in a chromogenic agar for coliform bacteria. The filter was incubated at 44°C for 24 hours. Positive colonies to β-galactosidase and d-β-d-glucuronidase were counted.

Total coliforms and *E. coli* were determined following the protocol 9222 H (APHA, 2012). 100 ml of the sample was filtered. The filter was placed in a chromogenic agar (m-

ColiBlue24 Broth, Hach, USA) for both total coliforms and *E. coli*. The filter was incubated at 35°C for 24 hours. Positive colonies in red (total coliforms) and blue (*E. coli*) were counted.

## 2.4 Data analysis

The IDAHO water quality index, designed by Said and Stevens (2004), was calculated to summarize in a single value the water quality at each pool based on four physicochemical parameters (dissolved oxygen in %saturation, total phosphorus, specific conductance and turbidity) and one microbiological (fecal coliforms) analyzed in this study (Table 1). IDAHO index was calculated using the ICATest v1.0 software developed by Fernández et al. (2001).

The mathematical expression for the IDAHO index is as follows

$$\text{IDAHO index} = \log \frac{(\mathrm{DO})^{1.5}}{(3.8)^{\mathrm{TP}}\ (\mathrm{Turb})^{0.15}(15)^{(\mathrm{FCol}/10000)} + 0.14(\mathrm{SC})^{0.5}}$$

where DO is the dissolved oxygen (% oxygen saturation), Turb is the turbidity (Nephelometric turbidity units, NTU), TP is the total phosphates ($\mathrm{mg/L^{-1}}$), FCol is the fecal coliform bacteria ($\mathrm{CFU \cdot 100\ m/L^{-1}}$) and SC is the specific conductivity ($\mathrm{\mu S/cm^{-1}}$ at 25°C). The IDAHO index ranges from 0 to 3. The maximum quality value corresponds to 3 and the minimum to 0. The value of the index is 3 in good quality waters with 100% DO, turbidity less than 1 NTU, no TP, no FCol, and SC less than 5 $\mathrm{\mu S/cm^{-1}}$. Values from 3 to 2 indicate an acceptable water quality. An IDAHO index below 2 points out that remediation is needed and that one or two variables have deteriorated. Finally, the index is less than 1 if most of the variables have deteriorated and water quality is poor (Said and Stevens, 2004).

To assess differences in the microbiological water quality, we constructed a matrix with the values of each microbiological variable determined in the laboratory. The data were arranged by the attendance of bathers (high *vs.* low), rainfall event (yes *vs.* no), and spatially based on pools location (upstream *vs.* downstream). All analyses were performed in the R environment (R Development Core Team, 2018).

We screened collinearity between microbiological variables using the Pearson’s correlation coefficient ('stats' package; R Development Core Team, 2017). Nonetheless, we did not detect intercorrelated variables ($r$ <60). Mixed-effect models (‘nlme’ package; Pinheiro et al. 2014) were used to assess variations in the microbiological variables. Thus, the data matrix was analyzed in a nested design of three factors, and the sources of variation were attendance (2 levels), rainfall event (2 levels), pool location (4 levels), and their interactions. Attendance, rainfall event, and pool location were treated as fixed factors, while sampling date was treated as a random factor. Mixed-effect models were fitted by generalized least squares (GLS) using restricted maximum likelihood (REML) procedures. Residuals were assessed by applying the Shapiro-Wilk test, and data were log-transformed if needed (i.e. if the non-normal distribution was detected).

To assess the relationship between microbiological water quality and bathers, we pooled the data and compared the concentrations of total and fecal coliforms, and *E. coli* against the number of bathers by applying simple linear and nonlinear regressions ('stats' package; R Development Core Team, 2017). Similarly, these three variables were analyzed against the physicochemical parameters to determine further interactions. For all models, residuals were assessed, and data were log-transformed if needed.

# 3 Results and discussion

## 3.1 Physicochemical parameters and IDAHO index

The physicochemical parameters and, therefore, the IDAHO index, are similar in the four pools analyzed (Table 1). The index ranged from 1.488 to 1.519 (maximum = 3) in the four pools analyzed, suggesting an intermediate water quality and that one or two variables have deteriorated (e.g. SC and fecal coliform).

**Table 1** Physicochemical and microbiological parameters (mean ± SD) and IDAHO index measured in four natural pools of a tropical dry forest stream in southwestern Ecuador. The values for recreational waters limits according to the Ecuadorian regulation are indicated to the right of each parameter.

| Variable | Pool A | Pool B | Pool C | Pool D | Recreational Water Quality Limit |
|---|---|---|---|---|---|
| Water temperature (°C) | 22.4 ± 0.6 | 22.4 ± 0.6 | 23.3 ± 0.7 | 23.8 ± 1.0 | - |
| pH | 8.5 ± 0.1 | 8.5 ± 0.1 | 8.5 ± 0.3 | 8.7 ± 0.1 | 6.5-8.3 |
| Specific conductivity ($\mu S/cm^{-1}$) ‡ | 173.6 ± 34.0 | 173.7 ± 35.4 | 197.2 ± 45.1 | 202.0 ± 46.1 | - |
| $O_2$ ($mg/L^{-1}$) | 6.4 ± 0.6 | 6.4 ± 0.5 | 6.2 ± 0.7 | 6.2 ± 0.6 | - |
| $O_2$ (%) ‡ | 80 ± 7.5 | 80 ± 6.3 | 76 ± 8.8 | 76 ± 7.5 | 80 |
| Turbidity (NTU) ‡ | 21.7 ± 18.4 | 22.7 ± 18.6 | 27.2 ± 15.6 | 27.9 ± 14.8 | - |
| Phosphate ($mg/L^{-1}$) ‡ | 0.5 ± 0.5 | 0.4 ± 0.3 | 0.4 ± 0.4 | 0.4 ± 0.2 | - |
| Nitrate ($mg/L^{-1}$) | 0.3 ± 0.1 | 0.3 ± 0.4 | 0.2 ± 0.2 | 0.2 ± 0.2 | - |
| Total coliforms ($CFU \cdot 100\ mL^{-1}$) | 3,025 ± 982 | 3,133 ± 1,517 | 3,499 ± 841 | 3,408 ± 801 | 2,000 |
| Fecal coliforms ($CFU \cdot 100\ mL^{-1}$) ‡ | 219 ± 123 | 273 ± 164 | 569 ± 515 | 517 ± 349 | 200 |
| *Escherichia coli* ($CFU \cdot 100\ mL^{-1}$) | 171 ± 73 | 160 ± 64 | 389 ± 159 | 286 ± 91 | - |
| IDAHO index | 1.49 | 1.52 | 1.46 | 1.46 | |

‡ Indicates the five variables used to calculate the IDAHO index.

Few physicochemical parameters are considered in recreational water regulations, which is normal considering that the most important water quality parameters for recreational waters are microbiological (Bedri et al., 2016). As shown in Table 1, the Ecuadorian regulation of recreational waters includes limits only for pH and $O_2$ (% sat), but exclude temperature, conductivity, turbidity, nitrates or phosphates. Only recommends the total nitrogen and total phosphorus ratio of 15:1. Nitrates and phosphates are not regulated because they are relatively innocuous compounds for living beings. However, they can cause eutrophication and remove oxygen from water when are present in large amounts and the right proportion. Some global regulators, such as the EU or the USEPA, do not set limits for the physicochemical parameters described in Table 1 for recreational waters (European Council, 2006; United States Environmental Protection Agency, 2012).

The analyzed pools did not comply with the pH and $O_2$ (% sat) value limits, 8.3 and 80%, respectively, given in the Ecuadorian regulation of recreational waters, i.e. they are unsuitable for bathing (Table 1), although the variability in pool C for pH was higher. The above pH limit was exceeded by a few tenths and showed an increase from up-to-downstream pools. For this reason, monitoring the pH is recommended to ensure the quality of bathing water and the safety of the bathers. Regarding $O_2$ (% sat), pools C and D were below this threshold and pools A and B were on average exactly on the limit (80%). So, they are considered uncompliant since many of the measures used to calculate that average were below the limit given in the regulation.

Due to the lack of thresholds for physicochemical parameters in the regulation of recreational waters, specific conductivity, turbidity, and nitrate were compared to Ecuadorian, EU, and USEPA drinking water standards. Phosphate is not controlled in any of these regulations for the reasons cited above and, therefore, no comparison is established. Nitrate complied with the three drinking water standards, i.e. below 50 $mg/L^{-1}$

in the Ecuadorian, 50 mg/L$^{-1}$ in EU, and 10 mg/L$^{-1}$ in US regulations. Specific conductivity, compared to the unique reference of the EU regulation, is considered of excellent quality which is below 2,500 µS/cm$^{-1}$. On the other hand, turbidity did not comply with the norms of potable water according to the single reference in the Ecuadorian regulation of 5 NTU, in which it must be negligible. Despite this, these values are normal for natural surface waters, and the difference with the regulations is explained because this is a critical parameter for the consumer and treatments dedicated to the reduction of turbidity are carried out in the water treatment plants. These values are very difficult to reach without these treatments. Also, turbidity is not a dangerous parameter for the health of people, so it does not pose a risk as microbiological parameters do.

## 3.2 Microbiological parameters

Results suggested that the microbiological water quality of the pools is not suitable for bathing according to the Ecuadorian regulations of recreational waters (Ministerio del Ambiente del Ecuador, 2015). According to Table 1, the maximum permissible limit of 2,000 CFU·100 mL$^{-1}$ of total coliforms was exceeded up to 3-fold in all the pools (Fig. 2), and in most cases, exceeded the 200 CFU·100 mL$^{-1}$ of fecal coliforms (Fig. 3). Also, *E. coli* concentration in all pools (Fig. 4) was not suitable for bathing according to the USEPA regulations (United States Environmental Protection Agency, 2012), and the same happened in pools C and D according to European regulations (European Council, 2006).

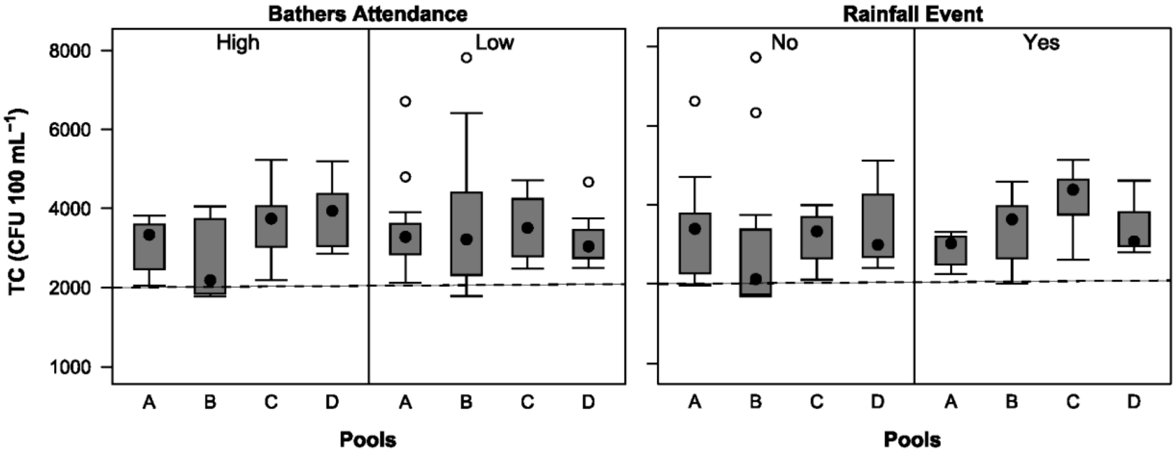

**Fig. 2.** Boxplot of the concentrations of total coliforms between bathers' attendance (high *vs.* low) and between rainfall events (no *vs.* yes) in natural pools located from up (A) to downstream (D) reaches along a tropical dry forest stream in southwestern Ecuador. Dashed horizontal line indicates the maximum permissible value for recreational waters according to the Ecuadorian regulation.

As with physicochemical parameters, the microbiological parameters included in the Ecuadorian, European and US regulations for recreational waters differ. Within the parameters analyzed, Ecuadorian regulations only contain limits for total and fecal coliforms, while European and US regulations only set a limit for *E. coli* as a bacteria with greater health risk within coliforms.

The results of microbiological parameters were disaggregated according to the attendance of bathers, rainfall events, and pool location (Table 2). The concentration of total coliforms did not differ significantly between the three factors assessed. However, significant interactions were observed between the attendance of bathers and pool location on the concentration of total coliforms. As shown in Fig. 2, all measured values of total coliforms ranged from 2,000 to 4,500 CFU·100 mL$^{-1}$ exceeding the Ecuadorian regulation for recreational waters. The values are very similar in all pools despite differences in the attendance of bathers and rainfall events. These data suggest that bathers are not the cause of the high values of total coliforms in this stream. More research is needed to identify the sources of such number of coliforms or the factors causing this phenomenon.

**Table 2** Summary table of the mixed-effect models performed on concentrations of total and fecal coliforms and *Escherichia coli* in water samples from natural pools of a tropical dry forest stream in southwestern Ecuador. Water samples were analyzed between bather attendance (high and low), rainfall event (yes and no), pool location (from A to D). Numerator degrees of freedom (numdf), denominator degrees of freedom (dendf), *F*-statistic and *p*-values are shown (significant difference is indicated in bold).

| Source of variation | numdf | dendf | *F* | *p* |
|---|---|---|---|---|
| *Total coliforms* | | | | |
| Intercept | 1 | 62 | 764.88 | <0.001 |
| Bather attendance (BA) | 1 | 62 | 0.72 | 0.401 |
| Rainfall event (RE) | 1 | 62 | 0.4 | 0.528 |
| Pool location (PL) | 3 | 62 | 0.43 | 0.733 |
| BA × RE | 1 | 62 | 0.01 | 0.939 |
| BA × PL | 3 | 62 | 1.64 | 0.188 |
| RE × PL | 3 | 62 | 2.21 | 0.096 |
| BA × RE ×PL | 3 | 62 | 1.59 | 0.200 |
| *Fecal coliforms* | | | | |
| Intercept | 1 | 62 | 120.23 | <0.001 |
| Bather attendance (BA) | 1 | 62 | 20.55 | **<0.001** |
| Rainfall event (RE) | 1 | 62 | 5.9 | **0.018** |
| Pool location (PL) | 3 | 62 | 5.91 | **0.001** |
| BA × RE | 1 | 62 | 0.02 | 0.958 |
| BA × PL | 3 | 62 | 3.54 | **0.020** |
| RE × PL | 3 | 62 | 1.16 | 0.331 |
| BA × RE ×PL | 3 | 62 | 0.37 | 0.773 |
| *Escherichia coli* | | | | |
| Intercept | 1 | 62 | 266.95 | <0.001 |
| Bather attendance (BA) | 1 | 62 | 25.06 | **<0.001** |
| Rainfall event (RE) | 1 | 62 | 9.16 | **0.004** |
| Pool location (PL) | 3 | 62 | 29.09 | **<0.001** |
| BA × RE | 1 | 62 | 8.34 | **0.005** |
| BA × PL | 3 | 62 | 3.2 | **0.029** |
| RE × PL | 3 | 62 | 1.71 | 0.175 |
| BA × RE ×PL | 3 | 62 | 1.14 | 0.340 |

Around 10-30% of the total coliforms found in the stream pools are fecal coliforms (≈ 200-1,000 CFU·100 m/L$^{-1}$). As shown in Fig. 3, a large part of the fecal coliforms exceeds the Ecuadorian regulation for recreational waters. Also, the concentration of fecal coliforms was significantly higher during high attendance days and periods without rainfall events and increased significantly from pool A to pool D. The interaction between attendance of bathers, rainfall events and pool location on the concentration of fecal coliforms was significant. These results suggest that bathers may be increasing the concentration of fecal coliforms by removing sediments, having a similar outcome in those found in a small tropical stream in the Caribbean island of Trinidad, ranging 500-700 CFU·100 mL$^{-1}$ (Phillip et al., 2009). However, the authors reported higher numbers of fecal coliforms in the rainy season than in the dry season in contrast to this study. This situation can be explained by the fact that a higher number of bathers and the smaller size of the stream may potentially increase the concentration of fecal bacteria during the dry season.

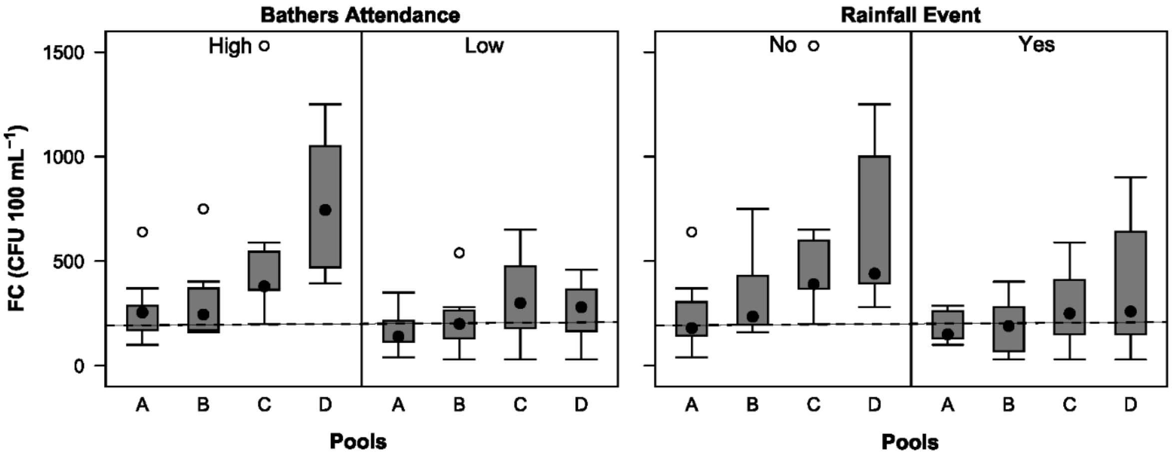

**Fig. 3.** Boxplot of the concentrations of fecal coliforms between bathers' attendance (high *vs.* low) and between rainfall events (no *vs.* yes) in natural pools located from up (A) to downstream (D) reaches along a tropical dry forest stream in southwestern Ecuador.

Dashed horizontal line indicates the maximum permissible value for recreational waters according to the Ecuadorian regulation.

The concentration of *E. coli* was significantly higher during high attendance days and periods without rainfall events, and much higher in the pool C than in the others. There was only one significant interaction between attendance of bathers and rainfall events on *E. coli* concentration. As observed in Fig. 4, *E. coli* values are in the range of 50-550 CFU·100 mL$^{-1}$, but also, they are showing more variability across the pools reaching the highest concentration in pool C which is the most used for bathers. According to the EU and US regulations, it is required calculating the 90th percentile of *E. coli* of the measurements recorded in each pool and its standard deviation to determine the recreational water quality. Once this percentile has been known, it was compared to the threshold of sufficient quality of the European regulation of 900 CFU·100 mL$^{-1}$, and the USEPA regulation for 36 estimated illness per 1,000 bathers. Following this procedure, none of the pools comply with USEPA limit, while pools C (of 1,245 CFU·100 mL$^{-1}$) and D (965 CFU·100 mL$^{-1}$) do not meet the EU limits. These values assume that the risk of diseases associated with *E. coli* is high in this natural bathing site. *E. coli* was reported as the best predictor of gastrointestinal illness, above enterococci and other bacterial indicators (Wade et al., 2003).

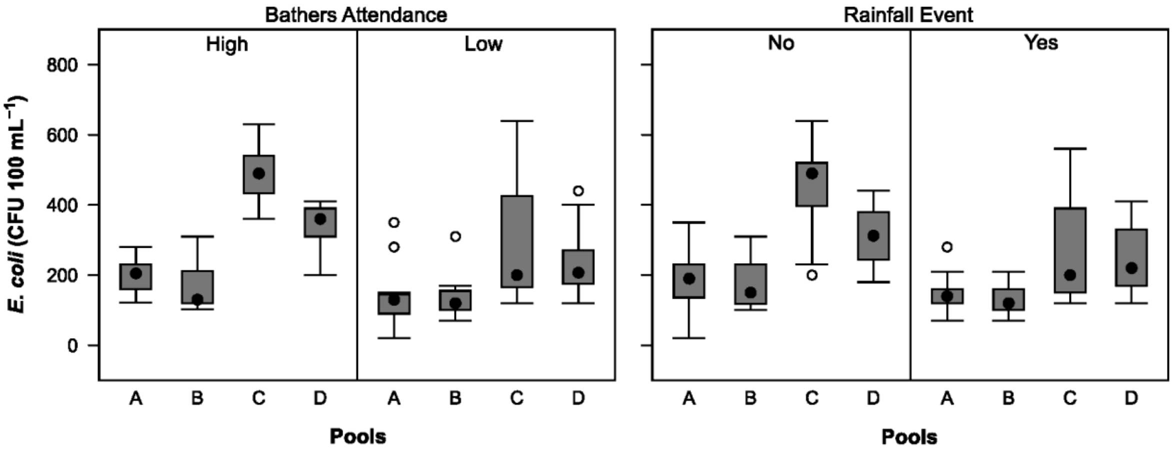

**Fig. 4.** Boxplot of the concentrations of *Escherichia coli* between bathers' attendance (high *vs.* low) and between rainfall events (no *vs.* yes) in natural pools located from up (A) to downstream (D) reaches along a tropical dry forest stream in southwestern Ecuador. Dashed horizontal line indicates the maximum permissible value for recreational waters according to the European and US regulations. Ecuadorian regulation not applicable.

## 3.3 Bacteria origin

The bacteria studied in recreational waters can have different origins. One of them is the presence of bathers in the pools, which would explain the significant differences of *E. coli* between pools and attendance of bathers. It could also explain the difference between rainfall events if considered that during the dry periods there were more bathers and streamflow was lower, consequently that a smaller amount of *E. coli* could generate the same concentrations. However, the differences found between rainfall events could not be explained by other factors such as the rainfall-related runoff (Bedri et al., 2016), which can drag microorganisms from livestock and agriculture in the Papalango catchment, since during days without precipitation the concentrations of *E. coli* were greater than in rainy days.

Fig. 4 shows an increase in the concentration of *E. coli* from pool A to B, and B to C, followed by a decrease to pool D. This pattern behaves similarly to the intensity of recreational use in these pools, suggesting that bathers are a potential source of this bacteria. In this regard, we regressed the concentration of *E. coli* against the number of bathers at the most used stream pools (i.e. B and C), showing a strong relationship between both variables (Fig. 5). However, further research is recommended to determine the *E. coli* variety (e.g. Enteropathogenic, Epec, Enterotoxigenic, Etec, enterohaemorrhagic, Ehec, enteroinvasive, eiec, enteroaggregative, EAggEC, and diffuse adherent, Daec) and to clarify its origin and possible health effects (American Public Health Association, 2012).

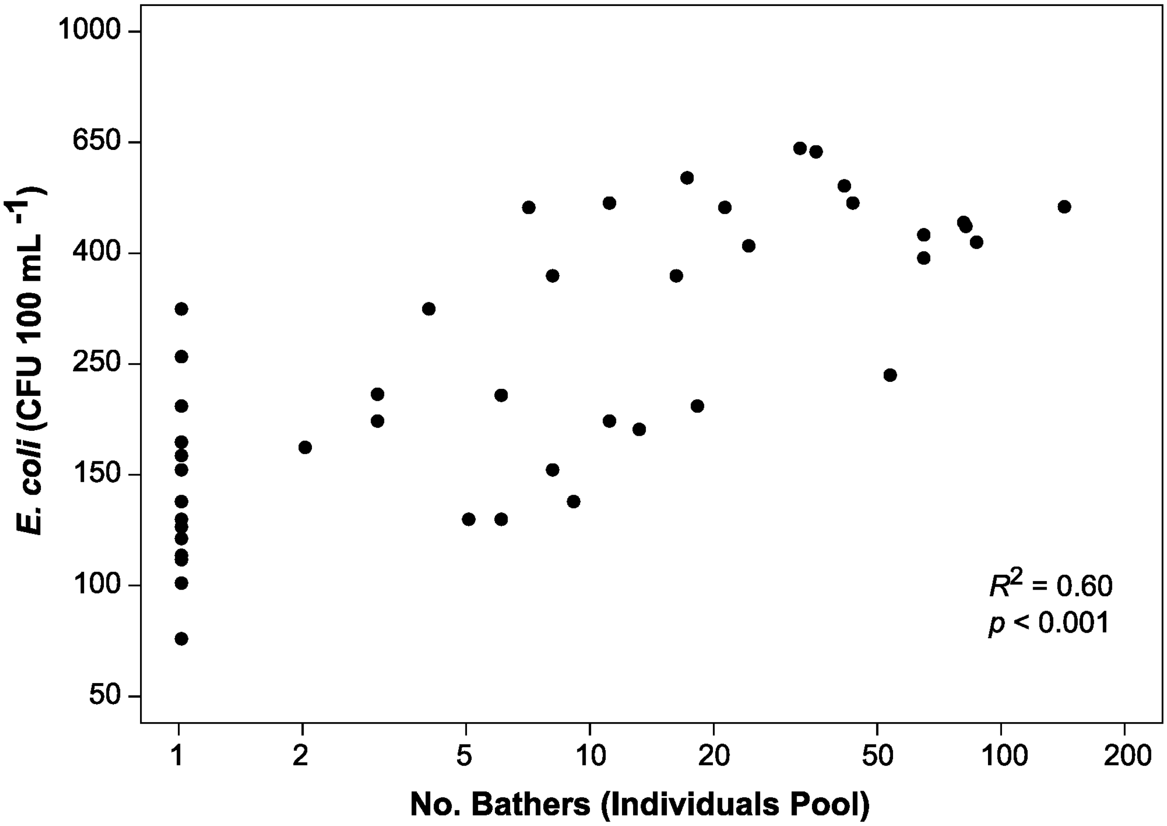

**Fig. 5.** Regression analysis between the concentration of *E. coli* and number of bathers at the most used pools (B and C). The coefficient of determination ($R^2$) and significance (*p*-value) of the regression are shown. Values for both variables are logarithmically represented.

Luo et al. (2018) found a similar relationship in the National Nature Reserve for the Chinese Giant Salamander in Zhangjiajie, Hunan Province, China. The authors suggested that the rise in *E. coli* might be produced by increased organic matter availability due to lint and other organic materials brought in by visitors, as also suggested by others (Ikner et al., 2007). The same pattern was found by Phillip et al. (2009) in a small tropical stream in the Caribbean island of Trinidad. The authors suggested that the increase in bacteria in the areas with bathers could be due to the resuspension of bacteria attached to bottom sediment by the bathers. However, *E. coli* is a bacteria known to have a high concentration in human feces, which could also be a source in recreational waters (González-Leal, 2012).

Although fecal coliforms showed significant differences based on the three variables studied (attendance of bathers, rainfall events, and pool location), results suggested that bathers may not be the main source of bacteria, unlike with *E. coli*. In fact, the relationship between the number of bathers and fecal coliforms was significantly week ($R^2$ =0.12), despite the concentration of fecal coliforms always increased from up to downstream pools. Therefore, further research is needed to elucidate whether other sources of microorganisms are modifying the concentration of fecal coliforms.

In the case of total coliforms, no significant differences were found regarding attendance of bathers, rainfall events, and pool location, suggesting that the origin of total coliforms seems to be in some more stable over time sources located upstream of pool A (control). This phenomenon would be possible with the pH (8.5-8.7) and temperature (22-24 °C) of water, which are favorable for the growth of microorganisms.

There are diffuse sources along the Papalango catchment that could add pathogen microorganisms to water. In this area, direct discharge of untreated sewage and effluents from septic tanks to the streams are common, due to the small size of urban centers and the lack of economic resources. These spills are well-known sources of coliforms and are more stable over time (Santiago-Rodriguez et al., 2012). Therefore, the identification of sources and remediation need immediate attention.

With regard to the interactions of bacteria with physicochemical parameters, we only found a weak, but positive, relationship ($R^2 = 0.28$; $p < 0.001$) between *E. coli* and water temperature. Higher growth rates of *E. coli* have been found to be positively correlated to higher temperatures (Jang et al., 2017; Vital et al., 2008), indicating that warmer waters may be an advantage for the proliferation of pathogenic organisms.

# 4 Conclusions

This study indicates that the recreational water quality of stream pools is associated with multiple factors within a dry tropical landscape. Climate and bathing intensity alter the concentrations of fecal bacteria reducing the microbiological water quality of the bathing sites. Additionally, our findings suggest that human activities (e.g. farming and ranching) along streams may lead into a top-down decreasing of the recreational water quality having potential effects on bather's health and eco-touristic development. Nevertheless, the water quality of streams can be enhanced or maintained by increasing forest cover, improving land-use management, and protecting riparian vegetation (Iñiguez-Armijos et al., 2014). For instance, sediments, nutrients, and pathogens washed away by runoff can be trapped in the riparian vegetation. Livestock exclusion along streams can significantly reduce direct bacteria inputs to water.

In this study, we also detected a deficiency in the Ecuadorian regulations to control and monitoring recreational water quality. The inclusion of *E. coli* and other pathogens in the national regulations is a pressing task to support local governments when mitigation strategies are applied.

We believe that the problems showed here might occur in other bathing sites in Ecuador (or other tropical countries). Identify the problematic bathing sites, determine the pollution sources and a long-term monitoring program are needed to sustain and enhance the touristic development in countries looking for diversifying their economy.

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