A comparative assessment of water quality in tropical streams located in primary and secondary rainforest.

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Abstract

E. coli and other fecal coliforms can enter stream water through the use of the stream by animals and people. Various nutrients can also be added to the water by animals or soil runoff. The levels of *E. coli*, other coliforms, and nutrients were analyzed, along with other measures of water chemistry, including pH, conductivity, and dissolved oxygen, to determine the quality of water in the Baru River and streams running through primary and secondary tropical rainforest. This study is part of a long term investigation of the water at the Firestone Center for Restoration Ecology and the surrounding area. Water samples were taken at the Firestone Center, which contains secondary growth forest, and Hacienda Baru National Wildlife Refuge, which contains primary rainforest. This comparison between primary and secondary rainforest allows for an assessment of the success of restoration at the Firestone Center. The levels of *E. coli* were higher at Hacienda Baru and during the dry season, but the levels of other coliforms were higher at Firestone, and there was no significant variation between seasons. The levels of nitrate were higher at Hacienda Baru and during the dry season at Hacienda Baru. The levels of nitrite did not vary significantly either by site or by season. Phosphate levels were also not significantly different between sites. These data can be used in conjunction with data taken during other years and season to asses the quality of the water and the health of the surrounding environment.

Introduction

. Measuring the levels of *Escherichia coli* and other fecal coliforms in streams provides an indication of the quality of the water and also provides information regarding the surrounding environment. The levels of *E. coli* in stream water can be affected by multiple factors. *Escherichia coli* and other fecal coliforms are bacteria found in the intestines of endothermic animals, and they enter the water through animal usage of the stream. Therefore, areas with larger mammal populations would be expected to have higher levels of fecal coliforms. The amount of bacteria in streams has also been shown to be affected by temperature. Because the optimum temperature for *E. coli* is around 35ºC, warmer streams would be expected to support larger *E. coli* populations. Indeed, in a previous study, Ksoll *et al.* (2007) found a significant positive correlation between *E. coli* and other fecal coliform concentrations and water temperature.

The levels of nutrients in stream water provide another indication of stream health. Phosphorus and nitrogen are two nutrients found in compounds dissolved in water that are essential macronutrients for plants. The levels of these nutrients in both sediment and in the water column affect the growth and balance algae and higher plants in around the streams, which in turn can affect the animals that rely on these plants for survival. Phosphorus can enter the streams from a variety of sources, including soil run-off, livestock excrement, and inorganic fertilizers (Mainstone and Parr, 2002). Nitrogen enters the soil, typically in the form of ammonia, from plant and animal wastes, where it is then converted into nitrate and nitrite through nitrogen fixation (Chang, 2005). Nitrogen can also enter the stream in the form of nitrates from agricultural run-off. The levels of nutrients can also be influenced by the vegetation in and surrounding the streams. Plants absorb the nutrients through their roots, prevented them from being washed out of the soil and into the streams. An ecosystem with a larger plant would have a greater uptake of nutrients which would decrease the levels of nutrients in the water.

This study investigated the levels of bacteria and nutrients in streams at Hacienda Baru and the Firestone Center for Restoration Ecology (FCRE). Both properties were formerly used as pastureland for cows, and are currently being restored to natural rainforest. However, Hacienda Baru also contains primary rainforest and the restoration process began twenty seven years ago, whereas FCRE began restoration approximately fifteen years ago. As a result, Hacienda Baru is expected to have a thicker, more developed canopy than FCRE, as well as a more substantial mammal population. Hacienda Baru, having a greater mammal population, would be expected to have higher levels of fecal coliforms in the streams. On the other hand, Hacienda Baru would also be expected to harbor colder streams due to shading from a thicker canopy.   This would suggest that FCRE, with the warmer water, would contain higher levels of fecal coliforms in its streams. Samples were also taken from the larger Baru River near the town of Dominical. These samples were taken to demonstrate the effects of complete disturbance and frequent human contact.

Methods and Materials

Sites

The samples were taken in 2007 between the dates of 11 June and 26 July, which is in between the rainy and dry seasons in Costa Rica. A total of fourteen sites were sampled, six at Hacienda Baru, six at the FCRE, and two at the Baru River near the town of Dominical.

At FCRE the uppermost sites were Terciopelo Spring and North Creek Falls. Terciopelo Spring was located at the source of Terciopelo Creek, which flows down to the mountain and joins Cacao Stream. Another sample was taken just upstream of the colvert before Terciopelo joins Cacao. North Creek also flows into Cacao Stream farther upstream from Terciopelo Creek. Three sites were also samples along Cacao stream. The first was at the base of Cacao waterfall. The second was midway along the stream just after two smaller streams joined Cacao. The last was taken at the colvert before Cacao flowed under the road and exited the property. Cacao Stream then flowed through another property before flowing into the Baru River.

At Hacienda Baru, the first site was located at the stream head, in primary rainforest. The second was downstream at the mouth of a pipe taking water to the overnight camp. Another sample was taken just below the camp, and another farther downstream below a giant ceibo tree. The fifth site was located at the boundary between the primary and secondary forest on Hacienda Baru. The final sample was taken just before the stream flows under the main road. It was located in secondary rainforest. Two samples were also taken in the Baru River. The first was taken underneath the bridge connecting Baru and Dominical. The second was taken where the Baru River met the Pacific Ocean.

Sampling and Procedure

Before sampling, Nalgene water bottles were autoclaved in a pressure cooker to kill any bacteria already present in the bottles. The bottles used for nutrient sampling were also rinsed with deionized water to remove traces of chemicals left from previous sampling. The samples were taken at each site by submerging the bottles, opening them underwater, and closing them while still underwater to prevent contamination from the air. It is recommended by the EPA that the samples be refrigerated if they cannot be filtered immediately. However, due to the remote locations of the sites, it was not possible to refrigerate the samples, and they were often not filtered until several hours after they were taken. At the sample sites, several other properties of the water were also measured. A YSI 85 meter was used to measure conductivity, dissolved oxygen, salinity, and temperature, and the pH was measured with a pH meter.

The samples for measuring bacteria were filtered in the lab following the procedure outlined in the EPA Method 1604: Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium).

All equipment was sterilized in a pressure cooker before use. Using sterile pipettes, 5 mL and 2 mL samples were filtered. The filter paper was then placed directly onto agar plates and incubated at 35ºC for 24 hours. The *E. coli* colonies were then counted under white light, and the other coliforms were counted under UV light.

The water from the nutrient sample bottles was used to measure both the nutrients and the turbidity. The turbidity was measured using a LaMotte turbidity meter. The levels of phosphate, nitrate, nitrite, and ammonia were determined using HACH water testing kits. A HACH saltwater test kit was used when analyzing samples taken at the Baru River mouth during high tide.

Statistics

The statistical analyses were conducted with SAS. T-tests were used to compare Hacienda Baru and Firestone. Two-way ANOVAs were used to compare the results from different seasons, using site as the second independent variable to control for variation between sites.

Results

The amounts of bacteria in the streams varied between Hacienda Baru and FCRE. Hacienda Baru had significantly higher levels of *E. coli* (t-test, $p=0.0278$). However, FCRE had significantly higher levels of other fecal coliforms (t-test, $p=0.0287$), and the level of total coliforms did not differ significantly between the two reserves (t-test, $p=0.1209$). (Fig. 1).

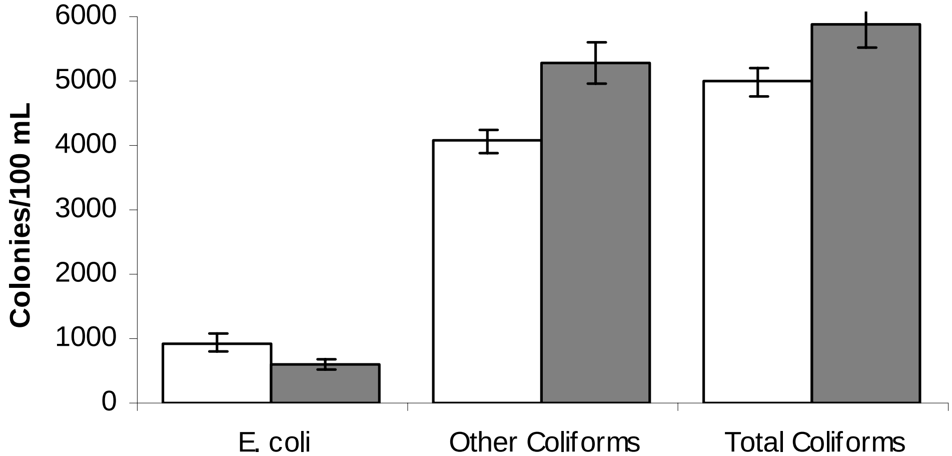

Figure 1. Number of colonies of bacteria per 100 mL (mean ± SE, n=6) at Hacienda Baru as compared to FCRE. White= Hacienda Baru, Grey= FCRE.

Hacienda Baru had significantly higher levels of nitrate in the streams (t-test, $p<0.001$). (Fig. 2). Both locations had very low concentrations of nitrite, but the concentrations were not significantly different between the two reserves (t-test, $p=0.3174$). (Fig. 3). There was also not a significant difference in the levels of phosphate between sites (t-test, $p=0.5451$), though both reserves had very high concentration of phosphate in their waters (Fig. 4). Neither reserve was found to have any detectable amounts of ammonia at any date (data not shown).

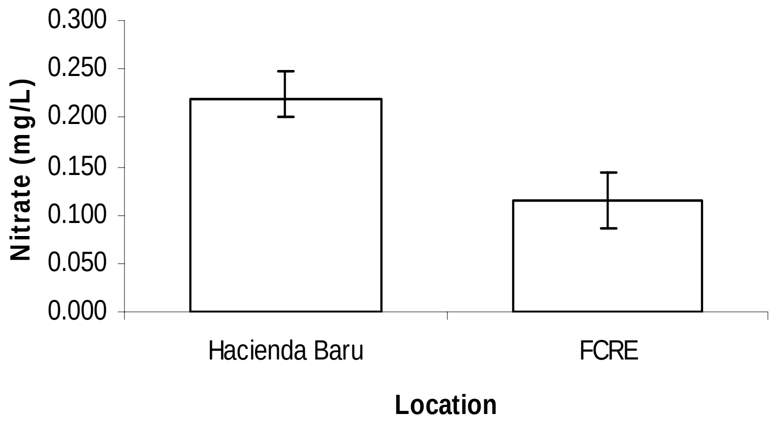

Figure 2. Levels of nitrate (mean ± SE, n=6) in stream water at Hacienda Baru and FCRE.

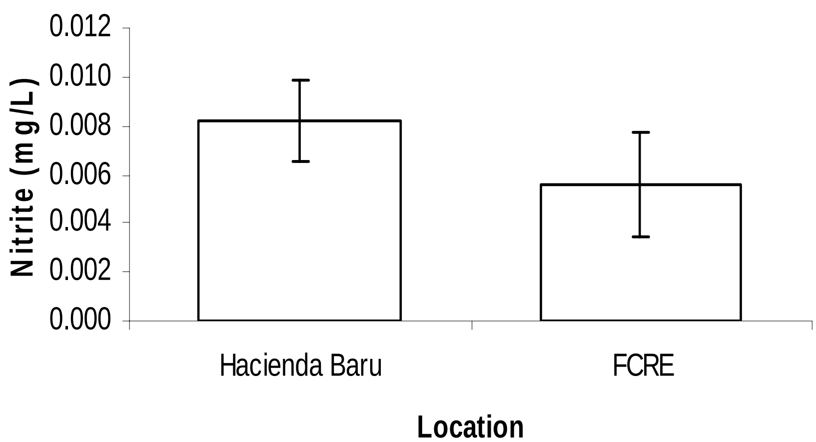

Figure 3. Levels of nitrite (mean ± SE, n=6) in stream water at Hacienda Baru and FCRE.

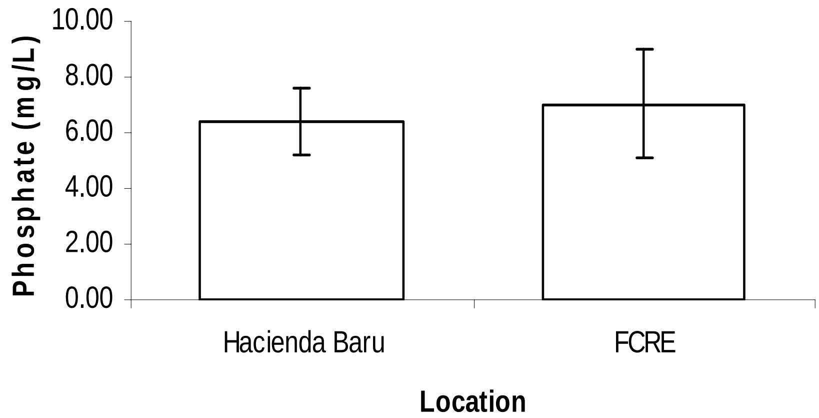

Figure 4. Levels of phosphate (mean ± SE, n=6) in stream water at Hacienda Baru and FCRE.

The temperature of the water at FCRE was significantly higher than the water at Hacienda Baru (t-test, $p<0.001$), the mean temperatures being one degree apart (Fig. 5).

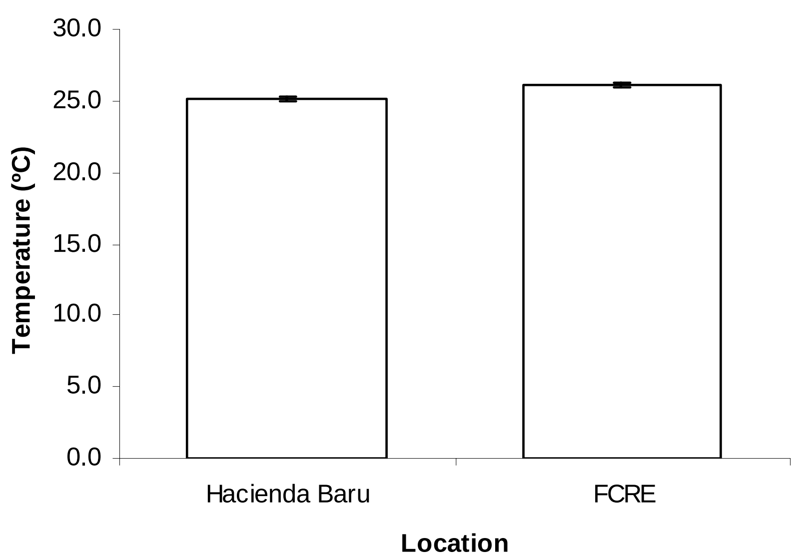

Figure 5. Temperature of stream water (mean ± SE, n=6) at Hacienda Baru and FCRE.

With regards to levels of *E. coli*, there was a significant interaction effect between season and site (Two-way ANOVA, p=0.0117; Fig. 6). At Hacienda Baru, the levels of *E. coli* during the dry season were about nine times higher than the levels during the almost season and about 20 times higher than the levels during the rainy season (p= 0.0069 and p=0.0255, respectively). There was no significant difference between the levels during the rainy and almost rainy season (p=0.8480). A FCRE, the dry season had levels about nine times higher than the levels during the almost rainy season and approximately 25 times higher than the levels during the rainy season (p=0.0033, p=0.0205, respectively). There was no significant difference in levels between the rainy and almost rainy seasons (p=0.8305). The levels of bacteria at Dominical during were almost 22 times higher during the dry season than the almost rainy season (p<0.0001) and 11.5 times higher during the dry season than the rainy season (p<0.001). No significant difference was observed between the levels during the rainy and almost rainy season at Hacienda Baru (p=0.8069). (Fig. 6).

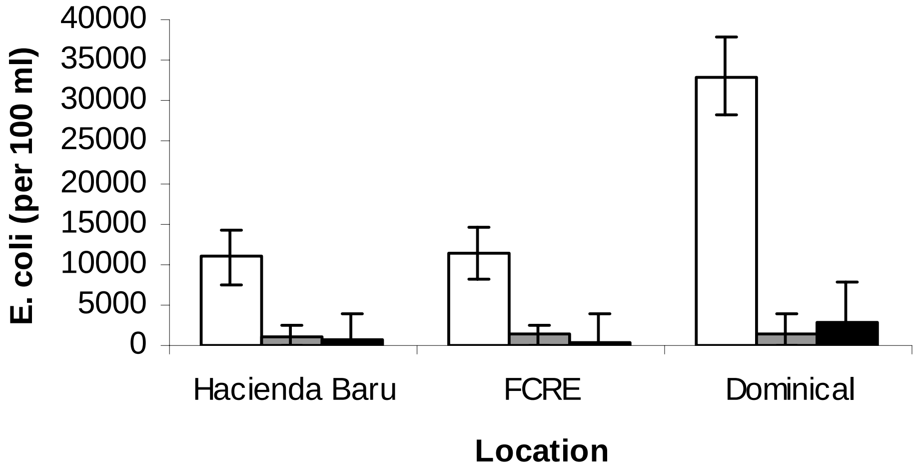

Figure 6. Number of *E. coli* colonies per 100 mL (mean ± SE) at all sites during the each season. White= Dry, Grey= Almost Rainy, Black= Rainy

There was no significant difference in levels of other coliforms between the seasons at any of the three site (Two-way ANOVA, p=0.7987). The interaction effect was not significant (Two-way ANOVA, p= 0.5368). (Fig. 7).

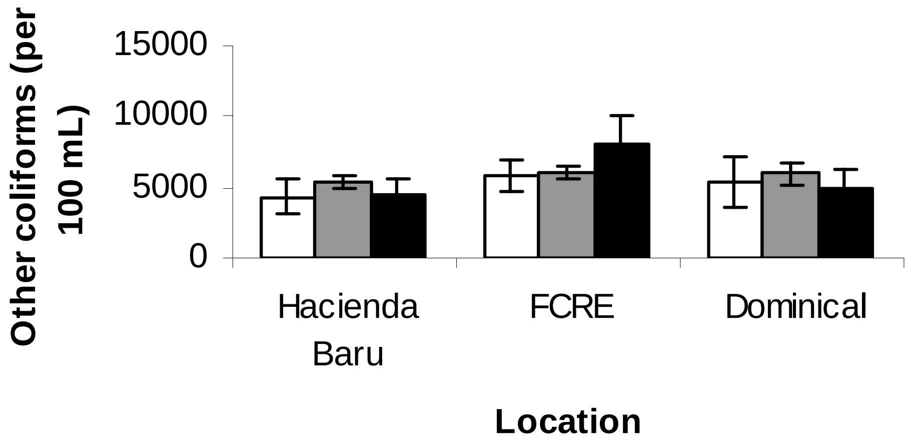

Figure 7. Number of other coliform colonies per 100 mL (mean ± SE) at all sites during the different seasons. White= Dry, Grey= Almost Rainy, Black= Rainy

In the levels of total coliforms, there was no significant interaction between site and season (Two-way ANOVA, p=0.6153; Fig. 8). Overall there was a significant effect of season on the concentration of total coliforms (Two-way ANOVA, p=0.0090). The levels of total coliforms during the dry season were significantly higher than the levels during both the almost rainy and rainy seasons (p=0.0067 and p=0.0044, respectively). There was no significant difference in total coliform level between the almost rainy and rainy seasons (p=0.3133). (Fig. 8).

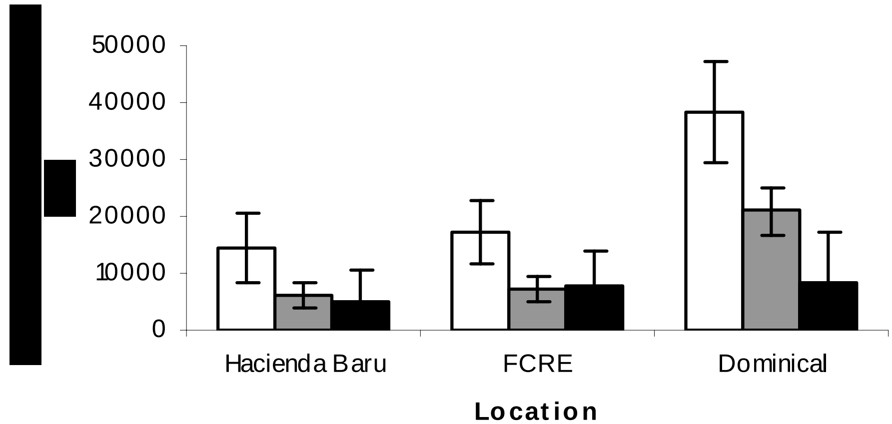

Figure 8. Number of total coliform colonies per 100 mL (mean ± SE) at all sites during the different seasons. White= Dry, Grey= Almost Rainy, Black= Rainy

There was a significant interaction between site and season in terms of the nitrate concentrations (Two-way ANOVA, $p<0.0001$; Fig. 9). At Hacienda Baru, the nitrate levels were significantly higher during the dry season than during the almost rainy and rainy seasons ($p<0.0001$ and $p<0.0001$, respectively). There was no significant difference in nitrate between the almost rainy and rainy seasons ($p=0.0834$). At FCRE, there were no significant differences between the dry and rainy seasons ($p=0.4119$), the dry and almost rainy seasons ($p=0.6841$), or the almost rainy and rainy seasons ($p=0.5057$). Similarly, at Dominical, there were no significant differences between the dry and rainy seasons (p=0.0.3479), the dry and almost rainy seasons ($p=0.2159$), or the almost rainy and rainy seasons ($p=0.9541$). (Fig. 9).

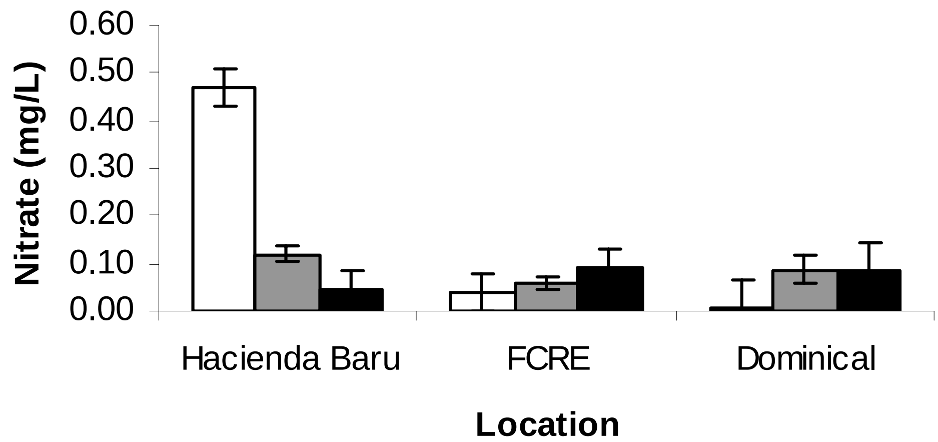

Figure 9. Levels of nitrate (mean ± SE) at all sites during the different seasons. White= Dry, Grey= Almost Rainy, Black= Rainy

There was no interaction between site and season regarding the levels of nitrite (Two-way ANOVA, p=0.8894; Fig. 10). There was a significant difference in nitrite levels between seasons (Two-way ANOVA, p=0.0200). There were significantly lower levels of nitrite during the dry season than during the almost rainy season (p=0.0146). However, there was no significant difference between the rainy season and the dry season (p=0.2209), nor between the rainy season and almost rainy season (p=0.6898). (Fig. 10).

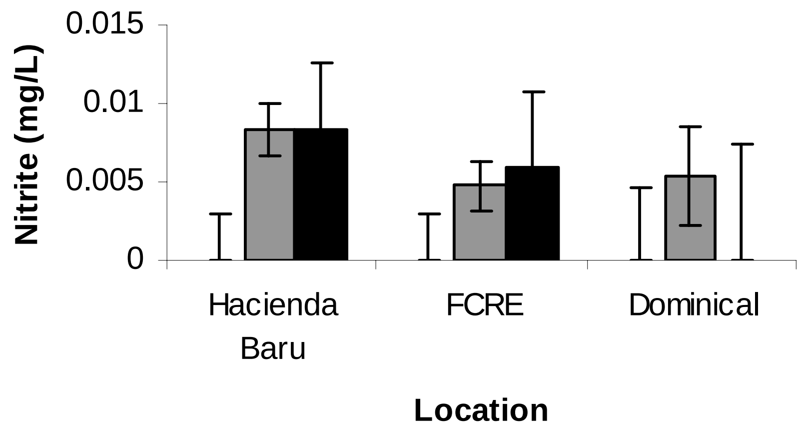

Figure 10. Levels of nitrite (mean ± SE) at all sites during the different seasons. White= Dry, Grey= Almost Rainy, Black= Rainy

There was a significant interaction between site and season in their effect on temperature (Two-way ANOVA, p<0.0001; Fig. 11). At Hacienda Baru, there were no differences in temperature between the dry and rainy seasons (p=0.2269), the dry and almost rainy seasons (p=0.4830), or the almost rainy and rainy seasons (p=0.3833). At FCRE, there were also no differences in temperature between the dry and rainy seasons (p=0.4217), the dry and almost rainy seasons (p=0.2503), or the almost rainy and rainy seasons (p=0.9493). However, in Dominical, the temperature of the streams was significantly higher during the dry season than during both the rainy and almost rainy seasons (p<0.0001 and p<0.0001, respectively). There was no difference in temperature between the rainy and almost rainy seasons (p=0.5752). (Fig. 11).

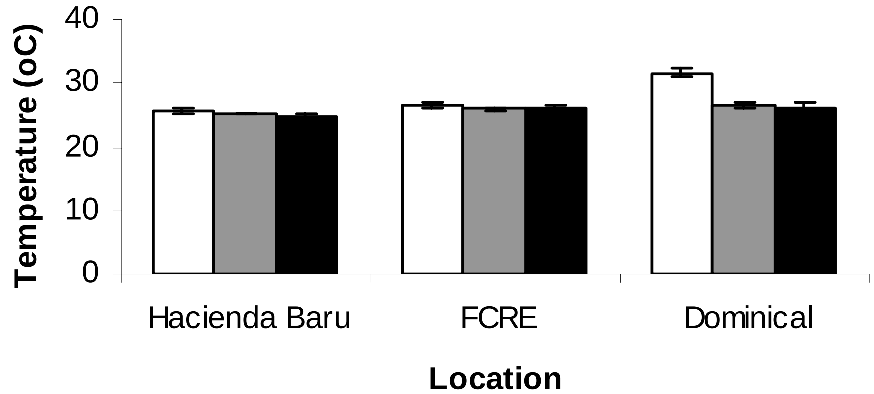

Figure 11. Temperature (mean ± SE) at all sites during the different seasons.

White= Dry, Grey= Almost Rainy, Black= Rainy

Discussion

It was found that there were greater amounts of *E. coli* in the streams at Hacienda Baru than in the streams at FCRE. However, other coliforms were more prevalent at FCRE than at Hacienda Baru. These results were somewhat unexpected because both *E. coli* and other coliforms can enter stream water from the same source, namely the intestines of endothermic animals. The higher levels of *E. coli* at Hacienda Baru could be explained by the more substantial mammal population. Other coliforms are found naturally in the soil, vegetation, and water as well as in the intestines of animals (Environmental Protection Agency, 2002). Because the method used to identify the coliforms could not identify them down to the species level, it is impossible to say what proportion came from animals and soil. If a sizeable proportion came from the soil this provides a possible explanation for the higher levels at FCRE. Bacteria from the soil would get washed into the streams during rain events. A greater number of samples at

FCRE were taken during and after rain events than at Hacienda Baru, which could lead to elevated levels of coliforms in the FCRE samples.

This explanation is also supported by seasonal data. There were higher concentrations of *E. coli* in samples taken during the dry season. During the dry season, when rainfall is very low, the levels of the rivers are much lower than the levels during the dry season. If animals use the streams with equal frequency throughout the year, and deposit equal amounts of bacteria into the streams, it would be expected that the concentrations would higher if there is less water in the streams. However, this same pattern of higher concentrations during the dry season is not observed with regards to the other coliforms. There was not a significant difference in the levels of other coliforms between the seasons. If some of the coliforms were entering the streams from the soil, greater amounts of these coliforms would enter the streams from runoff during the rainy season. This could counter the affect of higher water levels diluting the bacteria, leading to equal levels of bacteria across seasons.

It was expected that temperature would have an effect on the levels of bacteria in the streams. Although FCRE did have significantly higher temperatures than Hacienda Baru, it was difficult to see this difference reflected in the bacteria data because there were other variables, such as mammal population size, contributing to the levels of bacteria. However, the seasonal data does present some evidence of an effect of temperature on bacteria concentration. The Dominical sites had a greater difference in the levels of *E. coli* between the dry season and the other two seasons than did the sites at Hacienda Baru. Dominical was also the only site that showed significantly higher temperatures during the dry season. This temperature difference is one possible explanation for the great increase in bacteria concentration at the Dominical sites during the dry season.

The levels of phosphate did not differ significantly between Hacienda Baru and FCRE. The majority of phosphorus in the streams comes attached to soil particles in surface run-off. Because Hacienda Baru and FCRE are adjacent properties, it is possible that they have similar phosphorus contents in there soils, which would lead to similar phosphorus contents in the streams. The similar phosphorus levels in soil seem to outweigh the effects of a larger animal population at Hacienda Baru.

The concentration of nitrate was significantly higher at Hacienda Baru than at FCRE. As with the *E. coli* data, this could be explained by the more developed animal population at Hacienda Baru. With more animals using the stream, there should be higher concentrations of nitrate. The levels of nitrate were also higher during the dry season at Hacienda Baru. This could be largely due to the lower levels of water caused by lack of rainfall. If the animals utilize the stream with the same frequency throughout the year, the concentration of nitrate will be higher when there is less water in the streams.

There was no significant difference in nitrite levels between the sites. This was likely due to the large amount of variation in the data, which overwhelmed any difference which might have been seen. The concentrations of nitrite were very low in the streams at both sites. A more sensitive test of nitrite concentration would probably be needed to observe any pattern in nitrite concentration.

In the future it would be beneficial to make an effort to collect more samples during and after rain events. This would allow comparisons to be made between wet and dry times within one season, which could provide insight into whether rain in fact does have differing effects on *E. coli* and other coliforms. A long term study monitoring stream health would also be useful in tracking the progress of restoration at the Firestone Center for Restoration Ecology.

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