

CEPR-7-200: "Effect of Sunlight and Other Factors on the Population Dynamics of Coliform Bacteria" by Cindy Ginés Sanchez. Terrestrial Ecology Division, Center for Energy and Environment Research. Submitted to the School of Environmental Health, University of Puerto Rico, Medical Sciences Campus in partial fulfillment of the requirements for the Master of Science Degree. December 10, 1984. CENTER FOR ENERGY AND ENVIRONMENT RESEARCH.

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Bobby, Sandra, and Kenny.

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LIST OF ABBREVIATIONS

1. V0 - Dissolved Oxygen
2. Fe - Fecal Coliforms
3. Logec - Logarithm of the Fecal Coliforms
4. Logtc - Logarithm of the Total Coliforms
5. TC - Total Coliforms
6. Tene - Water Temperature
7. STP - Sewage Treatment Plant

ABSTRACT

Effect of Sunlight and Other Factors on the Population Dynamics of Coliform Bacteria: Francisco Folch Castañer, Ph.D., Principal Advisor; William H. McDowell, Ph.D., Associated Advisor; Iraida Robledo, M.S., Associated Advisor.

The effect of sunlight on the population dynamics of coliform bacteria was studied in a stream receiving the effluent from Caguas' Sewage Treatment Plant (STP). The effects of nutrients and chlorine were also studied. Five stations were established in El Caño (into which the STP discharges) and Rio Caguitas (which merges with El Caño approximately 0.5 kilometers downstream from the STP discharge). Samples were taken during the daytime and nighttime, and dissolved oxygen, water temperature, light intensity, and chlorine were measured in the field."

Samples were taken from the field back to the laboratory for analysis of fecal and total coliforms, nitrates and nitrites, and ammonia. The results of this study show that the water quality of these streams is extremely poor. Both the fecal coliforms and dissolved oxygen had a high rate of non-compliance with the water quality regulations. Moreover, Rio Caguitas, which does not receive a discharge from a Sewage Treatment Plant (STP), usually had a higher density of coliform bacteria than El Cano, whose waters are composed almost solely of the effluent from an STP. A multiple regression-correlation analysis demonstrated that there was no significant effect of either the nutrients or chlorine on the density of either coliform group. An ANOVA analysis showed that the stream segment significantly affected the change in the density of coliforms between adjacent

stations, but the hour of sampling did not. This result shows that segments of the streams had different rates of increase or decrease in the density of bacteria, likely due to the fact that Rio Caguitas had a higher density than El Cano. The fact that the hour of sampling did not have a significant effect shows that there was no difference between daytime and nighttime samplings, therefore suggesting that sunlight did not cause substantial coliform die-off in these streams.

INTRODUCTION

The use of non-pathogenic bacteria as indicators of pollution with pathogenic bacteria has generated much interest in the population dynamics of coliforms once they are released into streams, lakes or the ocean. Public health officials need to be able to predict the population dynamics of these bacteria in the environment in order to determine what uses can be given to a body of water that receives fecal pollution. In Puerto Rico, it is necessary to predict coliform population dynamics because the high human population density creates a water pollution problem that is increased by industrial waste discharges. The small number of water bodies fit to serve as water supply sources and as

Recipients of wastes make water reuse a necessity, which further amplifies the problem. Based on a review of the literature, Chamberlin and Mitchell (1978) concluded that sunlight is the most important factor in the mortality of coliform bacteria released into the ocean. Exposure to sunlight can kill over 90% of a population of total coliforms in 24 hours (Grigsby and Calkins, 1980). Therefore, sunlight appears to be an effective bactericidal agent that is not being optimally utilized to disinfect sewage effluents that are released into the environment.

However, other factors have also been found to affect coliform survival. The chlorine applied to sewage treatment plant (STP) effluent kills a large proportion of bacteria in the effluent (Camper and McFeters, 1979), and the density of these bacteria can also be affected by the concentration of nutrients in the water (Hazen and Aranda, 1980). Therefore, it is clear that the survival of bacteria released into the environment may be determined by more than one factor. This experiment was conducted to assess the effect of sunlight on the survival of fecal and total coliform bacteria (FC and TC, respectively) released by an STP into a small, tropical stream in Puerto Rico. Samplings were carried out during the day and at night to determine the effect of sunlight on their mortality pattern. The effect of other factors, specifically chlorine and nutrients, was also examined.

OBJECTIVES

The purpose of this study is to assess the effect of sunlight on the population dynamics of total and fecal coliform bacteria, and to examine the effect of other factors, specifically chlorine and nutrients.

LITERATURE REVIEW

Coliform Population Dynamics Measurement

The magnitude of the die-off of a population of bacteria once it is released into a body of water is

measured using Chick's decay coefficient, k (hour), which is a function of the initial density of coliforms (j_0), the final density (B), and the time elapsed.

(Chamberlin and Mitchell, 1978): $\log(B/B_0) \propto t$. The final density of bacteria must be corrected for dilution of the plume, and this procedure is explained in John (1978) and Hubbard, et al. (1982). This equation is based on the assumption that bacteria released into an unfavorable environment will experience first-order die-off. This die-off pattern is characterized by the death of an equal proportion of the remaining population of bacteria in a given time period (See Fig. D). However, bacteria populations do not always follow this pattern.

Population Dynamics in Temperate Regions and Lakes

Studies carried out in temperate areas show a great variability in k values. In their review of the literature, Chamberlin and Mitchell (1978) report that k values range from 0.010 to 1.0 and generally fall between 0.015 and 0.20 per hour. Another author (Velz, 1970) reports that during the warm weather period, k values for temperate rivers ranged from 0.46 to 0.96. The discrepancy between these authors is not easily explained. One possible reason is that Chamberlin and Mitchell (1978) corrected the k coefficient for the dilution of the effluent, but Velz (1970) apparently did not. The most likely explanation is that the studies they cited lasted different time periods. The studies used by Velz (1970) lasted 1 to 2 days and were carried out during the summer.

Fig. 1. Patterns of bacteria mortality. "1st" indicates first-order and "3rd" indicates third-order die-off. Adapted from Mancéni (1978).

Chamberlin and Mitchell (1978) do not state how long the studies they reviewed lasted or in what season they were done. Based on a review of the literature and on his own sampling, Velz (1970) concluded that the most common pattern of coliform mortality is third-order die-off. This pattern consists of rapid initial die-off followed by much slower mortality (See Fig. 1). He found that 99.5% of a coliform population had a half-life of 0.64 days, and the other.

Bacteria mortality is based on studies of coliforms.

Factors That Affect Coliform Population Dynamics

Sunlight

Based on an extensive review of the literature, Chamberlin and Mitchell (1978) concluded that sunlight is the most important factor in the mortality of indicator bacteria released into the ocean. They found a significant linear relationship between the decay rate of coliforms and the depth-corrected intensity of sunlight. Evidence of sunlight's lethal action to bacteria in freshwater was provided by Grigsby and Calkins (1980) who placed samples of effluent from a sewage treatment plant in tanks exposed to the sun. They found that the density of TC decreased steadily

during the day and increased slightly at night (See Fig. 2). Several other in vitro experiments have demonstrated that sunlight decreases the density of coliforms (Chojnowski, et al., 1979; Fujioka and Narikawa, 1982; Tuveson and March, 1980). The bar in Fig. 2 represents daytime (empty) and nighttime (filled) exposure. This data is adapted from Grigsby and Calkins (1980).

Sunlight causes a decrease in the density of coliforms. However, there is widespread evidence to suggest that sunlight causes mortality in vitro. To our knowledge, there have been no experiments that test the effect of sunlight on coliform mortality in sewage plumes. Proving this effect may be difficult because the turbulence and turbidity of plumes may attenuate sunlight's lethal action. However, Fattal, et al. (1983) presented indirect evidence, detecting a negative correlation between the mean monthly FC density in a sewage plume and the mean monthly duration of sunlight.

The ultraviolet (UV) component of sunlight is at least partially responsible for its lethal effect. All three components of UV light - near ($\lambda = 320-400$ nm), mid ($\lambda = 290-320$ nm), and far ($\lambda = 200-290$ nm) UV - can kill bacteria (Peak, 1983). Their modes of action are different. The mid UV component causes mixed results, but the far and near UV cause the same.

End result: the death of bacteria due to DNA damage (Peak, 1983). Near UV can also cause sub-lethal damage to the permeability of the cell membrane and protein synthesis apparatus (Jagger, 1961).

Chlorination

Chlorine is the most commonly used disinfectant of wastewater effluents. It rapidly reduces coliform densities by as much as 90% (Camper and McFeters, 1979). However, recent experiments have shown that this decline is only temporary and, after a lag period, the density of coliforms begins to increase. Following exposure to chlorine, the density of indicator bacteria decreases for an hour (Camper and McFeters, 1979). But, after this initial decline, the density can increase for as long as five days (Kinney, et al., 1978).

The explanation of this "regrowth" lies in chlorine's mode of action. A large proportion of the bacteria exposed to chlorine are not killed; they are injured sub-lethally. Although chlorine damages the cell's genetic material and protein synthesis apparatus (Haas and Engelbrecht, 1980), the greatest damage occurs in the cell membrane. Pores are opened in the membrane that disrupt active transport and allow low molecular weight material to escape the cell (Haas and Engelbrecht, 1980). The net result is that the bacteria are not dead but are injured to such an extent that they are unable to grow on selective media such as m-FC and m-endo.

Proof of this hypothesis was provided by an experiment in which the absorbance of a suspension of chlorine-injured coliforms was measured simultaneously with plate counts. The former remained unchanged while the latter decreased (Camper and McFeters, 1979). Given time, the cells repair the damage and are again able to grow on the plating media, creating the illusion of regrowth.

All research involving indicator bacteria must take into consideration the fact that the most commonly used plating media do not detect these sub-lethally injured cells. m-endo can recover 95% of the uninjured and 66% of the chlorine-injured.

Cells and n-PC can recover by 1058 and 7%, respectively (McFeters, et al., 1982). Little emphasis

has been placed on the role of nutrients in the population dynamics of coliforms in the environment. In their review of factors influencing coliform mortality, Chamberlin and Mitchell (1978) concluded that nutrients could explain only a small fraction of coliform die-off. However, several experiments have demonstrated that a correlation can exist between nutrients and coliform density.

Research conducted in a Swedish estuary showed that the density of FC was positively correlated with total nitrogen and nitrate (Hirn, et al., 1980). Razen and Aranda (1980) found that the density of FC in several stations along a Puerto Rican stream was positively correlated with ortho-phosphate and nitrate concentrations. A study of the population dynamics of bacteria in a plume of organic-rich industrial effluent dumped into the sea showed that the density of the bacteria *Acreosnes hydrophila* was positively correlated with total phosphorous, total organic carbon, and orthophosphate; whereas the density of FC correlated only with conductivity (Biam'n and Hazen, 1983).

Since a relationship between bacteria density and nutrients does not always exist, and since the nutrients involved vary, it is possible that the effect of nutrients is indirect or only one of many. One possibility is that phytoplankton may be modifying the effect of nutrients on coliforms (Hazen and Aranda, 1980).

Various other factors have been proposed to cause coliform mortality, including predation. In one experiment, predation was found to remove 107 coliforms in 10 days (McCambridge and McMeekin, 1979), but in another experiment, predation did not cause significant die-off (DeLeval and Remacle, 1979). This discrepancy is likely due to the different methodologies used. The latter experiment removed the protozoans by filtering the water through a 0.8m membrane, but the former experiment demonstrated that it is this component of

The fauna that is most active in predation, sedimentation could also affect coliform population dynamics. *E. coli* and *Vibrio cholera* can survive for considerable periods of time and even reproduce in sediments (Hood and Ness, 1982). Furthermore, activities such as dredging the bottom of a stream can resuspend coliforms bound to the sediment and increase their density in the water column (Grimes, 1980). Therefore, sediments may act as a sink or as a source of coliforms.

Still, other factors have been proposed, including heavy metals, bacteriophages, algae, and bacterial toxins. However, their effect on the population dynamics of bacteria is deemed to be minimal (Chamberlin and Mitchell, 1978).

MATERIALS AND METHODS

Location

The source of fecal bacteria for this study was the city of Caguas' Sewage Treatment Plant (STP). The trickling filter plant received mostly domestic wastes at an average flow of 6.9 MGD (PRASA, 1984). The plant discharged the treated effluent into a channel locally called El Caño (See Fig. 3). During stable flow conditions, the plant's effluent makes up the entire flow of El Caño, which runs through 0.5 km of pasture before merging with Rio Caguaitas. Downstream from the point of merger

with El Caño, Rio Caguitas flows by a sand and gravel quarry; however, this activity does not seem to affect the river's flow significantly. Both Rio Caguitas upstream from El Caño, and the STP's effluent have highly variable water quality. At times both the river and the plant's effluent bear almost raw sewage and at other times the water they bear is moderately clean. For other studies of this river see Quiñones-Marquez (1980) and Font, et al. (1972).

Sampling Procedure

Five stations were established in a 1-km stretch of the Caguitas and El Caño (See Fig. 3). Station 1 was

Fig. 3. Location of the sampling stations. Caguas Sewage Treatment Plant (STP in the map) is located on the eastern edge of.

The city, adapted from USGS, begins at a page break. It is located at the point of discharge of the STP into El Caño. Station 3 was located in Rio Caguitas upstream from its merger with El Caño and is equivalent to the Geological Survey's water quality station number 50055250 (Curtis, et al., 1984).

Samples were taken once a week beginning in March 1984. Five daytime samplings were followed by three nighttime samplings. All samplings were carried out in stable flow conditions beginning at 9 a.m. and 9 p.m. respectively. The order in which the stations were sampled varied but was generally: 3, 1, 2, 4, 5. Two samples were taken at each station.

A sample for microbiological analysis was taken in a sterile bottle with 1 ml of 10% sodium thiosulfate to neutralize residual chlorine (APHA, 1980). A sample for chemical analysis was taken in a plastic bottle. The samples were taken simultaneously 10 cm from the water surface and were protected from sunlight immediately. All samples were placed on ice as soon as possible (but never more than five minutes after sampling) and were kept on ice until they were analyzed.

Several other parameters were measured at each station at a depth of 10 cm. Dissolved oxygen (DO) and water temperature (Temp) were measured using a Model 57 Meter (Yellow Springs Inst. Co.). Light intensity was measured using an underwater photometer (Protomatic). Total Chlorine was measured in Station 1 with a Model Ch-66 meter (Hach Co.).

After another page break, we discuss coliform enumeration. Coliform densities in the samples taken for microbiological analysis were determined using the membrane filtration method as described in Standard Methods (APHA, 1980). M-endo and M-Fc broths (Difco) were used to determine total coliform and fecal coliform densities respectively.

These media were filter sterilized through 0.20 μm pore diameter membranes (Type SM, Sartorius Co.) and dispensed into sterile petri dishes with absorbent pads (Millipore Co.). A sample was chosen randomly for analysis. Using sterile phosphate buffer (APHA, 1980), two...

Dilution series (one for each medium) were made. The final filtration volume was always 10 mL, and these dilutions were filtered in less than 30 minutes. A sterile filtration unit and 0.45 μm pore diameter membranes (Type HA, Millipore Co.) were used. The four dilutions for one medium were

filtered beginning with the most dilute. The filter was then sanitized by rinsing it with ethanol, allowed to air dry, and was rinsed with sterile buffer before beginning the remaining filtrations for that station. The same procedure was followed for each station, and the maximum time elapsed between collection and examination of the samples was 8 hours. TC were incubated at 35°C for 24 hours, and red colonies with green metallic sheen were counted. FC were incubated at 44.5°C for 24 hours, and blue colonies were counted.

Chemical Analysis Samples were analyzed for nitrite and nitrate, ammonia, and chloride, using automated analysis (AutoAnalyzer II, Technicon Instrument Co.) as described in Standard Methods (APHA, 1980). The samples were prepared by filtering them through Whatman filter paper (W & R Balston Co.) and then through 0.45 µm pore diameter membranes (Type BA, Millipore Co.). The filtered samples were diluted 1 in 10 using distilled-deionized water (Nanopure, Barnstead Co.) before analysis. A portion of each diluted sample was stored at 4°C for determination of nitrogen as nitrate and nitrite (NO₂⁻/NO₃⁻) and chloride ion (Cl⁻) (holding time < 5 days, and 3 weeks, respectively). A second portion was acidified by adding concentrated sulfuric acid and stored at 4°C for analysis of nitrogen as ammonia (NH₃) (holding time < 6 days).

Data Analysis was done using the SPSS statistical package (Nie, et al., 1975).

Results: Water Quality

A summary of the values obtained for several indicators of the water quality of Rio Cagilitas and El Cao appears in Table 1. As expected, the high densities of FC and TC, low concentrations of DO, and extremely high concentrations of...

Nig*, confirmed that both streams receive sewage effluent. Two of these parameters (FC and DO) had a high rate of non-compliance with the applicable water quality regulations (EQB, 1983). The standard stipulates that no more than 20% of all samples taken from a given location should contain more than 40 CFU/mL of fecal coliforms. However, 80% of the samples in this study exceeded that value. Above 90% of all samples analyzed for DO had a concentration lower than the 4.0 ppm standard. On the other hand, both Cl⁻ and NO₂⁻/NO₃⁻ had 100% compliance. All values obtained in this study are presented in Table 4 in the Appendix.

Table 1 displays a water quality summary for Rio Cagilitas and El Cafic, which are based on all samples taken during this study.

A multiple regression-correlation analysis was performed to detect any correlations between the logarithm of either coliform group and the physico-chemical factors studied. The correlation coefficients are presented in Table 2, but no significant multiple correlation was found with the logarithm of the bacteria ($p=0.05$). Additionally, the logarithm of neither coliform group in the STP was significantly correlated with the concentration of chlorine in the STP (See Fig. 4).

Coliform Population Dynamics

Although the number of bacteria fluctuated greatly with each sampling, a prevalent population trend was evident (See Fig. 5 and 6). In general, there was a decrease in density between the STP and

Station 2, followed by an increase between Stations 2 and 3, and a moderate decrease between Stations 4 and 5. However, contrary to the expected decrease in density between the first (Station 1) and last stations (Station 5), there was a slight increase. This increase may be attributed to the fact that Rio Caglitas (Station 3) usually had a much higher density of coliforms than El Cafic.

Therefore, the density greatly increased in Stations 4 and 5 (See Fig. 5 and 6). Several other trends could also be detected. As expected, the total coliforms had a higher density of 21.

Table 2 outlines the coefficients for the correlation of the physico-chemical parameters with the logarithm of fecal or total coliforms. A stepwise multiple regression-correlation analysis with forward inclusion proved that none of the parameters were significantly correlated to either coliform group ($p=0.05$).

Dependent Variable	Logarithm	Logarithm
Independent Variable	Fecal Colif.	Total Colif.
Dissolved Oxygen	-0.60	0.48
Temperature	0.83	0.37
Light	-0.03	0.12
8027/03"	0.44	0.64
Way	-0.50	-0.68
Discharge	-0.34	0.55
Chlorine	-0.62	-0.50

Fig. 4 illustrates the correlation of chlorine with the logarithm of coliforms in the Sewage Treatment Plant outfall. Neither correlation was significant ($p=0.08$).

Figure 5 depicts the total coliform density in the Coguitas River and El Cabs. Values represent the average for each sampling station with one standard deviation for daytime (circle) and nighttime (square) samplings.

Figure 6 shows the fecal coliform density in the Caguitas River and #1 Cyl. Values represent the average for each sampling station with one standard deviation for daytime (circle) and nighttime (square) samplings.

Contrary to what was expected, the average density of both groups of bacteria was greater during the daytime samplings than during the nighttime samplings. An ANOVA analysis was carried out to determine if the stream segment, the group of bacteria, or the hour of sampling affected the change in the logarithm of coliform bacteria between adjacent stations (See Table 3). The patterns that were observed will be discussed further.

The differences were apparent in the graphs, however, only the difference between stations was statistically significant ($p<0.05$). This proves that the increases or decreases in the density of coliforms were not the same for all reaches of the river. Since there was no effect of the hour of

sampling, the apparent differences between daytime and nighttime samplings that could be observed in Fig. 5 and 6 were not statistically significant. Likewise, the apparent differences in the change in the number of bacteria measured by the two coliform groups were not substantial. As expected, the logarithms of the two groups of bacteria (PC and TC) were highly correlated (See Fig. 7). The logarithm of TC accounted for 64.5% of the variation of the logarithm of Fc.

Table 3. ANOVA used to examine the effect of the stream segment, the hour of sampling, and the group of bacteria on the change in the logarithm of coliform bacteria between adjacent stations. The stream segments were classified into the areas between Station 1 and 2, 2 and 3, 3 and 4, and 4 and 5. The hour of sampling was classified into daytime and nighttime, and the group of bacteria was classified into total and fecal coliforms. The dependent variable was the difference in the logarithm of coliforms between these stations.

Source of Variation	Sum of Squares	DF	Mean square	F	Significance of F
Hour	0.69	1	0.69	0.63	0.432
Segment	29.00	3	9.67	8.78	0.000
Group	0.12	1	0.12	0.74	0.746
Two-way Interactions:					
Segment and Hour	3.19	3	1.06	0.97	0.416
Segment and Group	1.74	1	1.74	0.53	0.665
Hour and Group	0.78	1	0.78	0.27	1.403
Three-Way Interaction:					
Segment, Hour and Group	0.10	3	0.03	0.03	0.992
Residual	52.83	48	1.10		
Total	68.45	63	1.09		

DISCUSSION

Water Quality

The results of this study agree with the findings of another study carried out in Rio Caguitas (Quinones-Miequez, 1980). The

The values of dissolved oxygen, chloride, and total and fecal coliforms detected in both studies were in the same range. However, the values obtained for NO₂/103 and especially for NH₃ were much higher than the values reported by Quinones-Marquez. The averages obtained in this study were 0.41 and 1350 ppm, respectively, whereas the averages obtained by Quinones-Marquez were approximately 2.2 and 4 ppm, respectively. This discrepancy could be due to the fact that they only sampled the Caguitas upstream from its merger with El Cafe (equivalent to station 3 in this study). This station generally had a lower concentration of NH₃ than the other stations. Another possible explanation might be found in the large fluctuation in these parameters with each sampling (See Table 4). Whereas in one sampling the NH₃ concentration was 87 ppm, in the next sampling it was 2290 ppm. With such large fluctuations, the discrepancy could be explained. Of course, the best explanation may be that the quality of the river has worsened since Quinones-Marquez studied it. The high concentrations of nutrients, low concentrations of dissolved oxygen, and the high densities of coliforms found in Rio Caguitas and El Caño corroborate Quinones-Marquez's (1980,

p.51) conclusion that "... the quality of its water ranks among the worst in Puerto Rico." The similarity in the values obtained in both studies proves that the quality of the river has not improved in four years despite a doubling of the STP's capacity in 1963. In fact, it appears to have worsened as far as NH_3 and NO_2/NO_3 are concerned. A disturbing conclusion that can be drawn from these data is the fact that Rio Caguitas, which does not receive STP effluent, is equally as polluted as El Caño, whose waters are composed almost solely by the effluent from a sewage treatment plant.

Coliform Population Dynamics The two groups of coliforms, the fecal and total coliforms, behaved as expected in detecting fecal pollution (APIA, 1960). A lower number of bacteria grew on

The fecal coliform medium has more selectivity than the total coliform medium. On the other hand, the number of bacteria detected by the two plating media were always equivalent because they both measure fecal pollution.

Although a significant effect of the stream segment was observed on the density of coliforms detected, it was impossible to detect a definitive die-off or regrowth of the bacteria. The decrease in the density of coliforms between Stations 1 and 2 suggests that the bacteria released by the Sewage Treatment Plant (STP) experienced die-off. However, the overall trend in these streams was an increase in the density of bacteria, as indicated by the increase between the STP and Station 5. An ANOVA showed that the stream segment significantly affected the change in coliform bacteria, as seen in Table 3. This result is probably due to the fact that Rio Caguitas had a significantly higher density of bacteria than El Caño, resulting in a change in density between Stations 2 and 3 that was significantly different from the other segments. Therefore, it is impossible to tell whether the increase between Stations 1 and 5 was due to the addition of bacteria by Caguitas or the regrowth of chlorine-injured cells. To determine if the bacteria released by the STP experienced die-off or regrowth, a mathematical calculation must be carried out to correct for the number of bacteria added by Rio Caguitas (Velz, 1970). This study attempted to carry out this correction using the chloride naturally found in these waters as a conservative tracer, but the calculation was not successful, apparently due to the addition of subsurface flow that diluted the chloride.

Effect of Nutrients and Chlorine

Neither NH_4^+ , nor $\text{NO}_2^-/\text{NO}_3^-$, nor chlorine affected the density of the coliform bacteria. This result does not agree with a study conducted in Puerto Rico that found a correlation between $\text{NO}_2^-/\text{NO}_3^-$ and fecal coliforms (Hazen and Aranda, 1980). However, the authors hypothesized that this

The relationship is indirect and is due to a direct symbiosis between algae and the coliforms. Their hypothesis is corroborated by other studies (Cole, 1982). Hazen and Aranda studied a clear mountain stream where there should be an abundant population of algae. Rio Caguitas and El Caño, on the other hand, which carry a heavy load of suspended and dissolved solids (Curtis, et al., 1984), filter out sunlight and may inhibit algae from growing. Therefore, the correlation of nutrients with coliform density should not be expected in these streams.

A surprising result is the lack of an effect of chlorine on the density of the coliforms. It was not correlated with the density of either group of bacteria in the STP outfall. The lack of correlation shows that the plant's operators applied excessive quantities of chlorine to the effluent. The

concentration of total chlorine in the outfall surpassed 3.0 ppm more than once. This excess did not cause a proportionate decrease in the density of coliforms but instead may have caused damage to the stream's biota (Esvelt, et al., 1973).

Effect of Sunlight

Sunlight did not affect the dynamics of the coliforms in this stream. The non-significant effect of the hour of sampling on the change in the density of the bacteria between stations indicates that there was no difference between day and night samplings (see Table 3). In addition, the average density of both groups was higher during the daytime than during the nighttime (See Fig. 5 and 6). If sunlight had acted as a bactericidal agent as suggested by Grigsby and Calkins (1980), then the coliform density should be lower during the daytime. Therefore, sunlight was not an effective bactericidal agent in this stream.

Sunlight's bactericidal action has only been proven in highly controlled experiments so it is not too surprising that no effect was found in this experiment. The turbidity and turbulence of the stream's water may attenuate the effect of sunlight by filtering out the UV rays and moving the bacteria around.

Bacteria settle at the bottom of the stream where UV radiation does not penetrate. Tuveson and Hareh (1980) found that clear water filtered out UV rays and attenuated its lethal effect. The reduction of UV lethality should be even greater in those streams that carry a heavy load of dissolved and suspended solids (Curtis et al., 1984). This finding demonstrates that the hypothesis put forth by Chamberlin and Mitchell (1978) that sunlight is the most important factor in the mortality of coliform bacteria released into the ocean cannot be extrapolated to all streams. El Caño and Rio Caguitas do not behave as expected, probably due to the high degree of turbidity of their waters.

CONCLUSIONS AND RECOMMENDATIONS

The results of this study show that there was no significant effect of sunlight on the population dynamics of coliform bacteria released into El Caño. There was also no effect of nutrients or of chlorine. In addition, the water quality of these two streams was extremely poor. It would be useless to suggest ways to enhance the bactericidal effect of sunlight when the water quality of both streams is so poor. Sunlight-induced mortality should be used as a second line of defense against fecal pollution; the first line of defense must be the efficient functioning of the sanitary system. Not only is the sewage treatment plant not functioning correctly, but Rio Caguitas receives pollution from sources other than the STP, resulting in a higher degree of fecal pollution than El Caño. The first step in correcting the gross pollution of this area is to identify and eliminate the source or sources of fecal pollution in Rio Caguitas. The next step must be to increase the efficiency of Caguas' STP, which currently barely provides primary treatment. Only when this plant removes the high load of solids in its effluent can sunlight begin to be a contributing factor in the control of indicator and pathogenic bacteria.

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APPENDIX

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Table 4a. Sampling results. NA represents missing value. Date 3/21/84-Diurnal STP Discharge (m³/hr) 900 Chlorine Applied (kg/hr) 7.56 Parameter Time (ppm) 52.0 | 714 | 9.8 | 18.8 | 38.2

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Table 4b. Sampling results. NA represents missing value. Date 3/28/84-Diurnal STP Discharge (m³/hr) 792 Chlorine Applied (kg/hr) 9.45 Parameter Time.

The text appears to be a series of data tables for scientific results. It seems the text has been corrupted with various symbols and misplaced values. Here's an attempt at fixing the information to the best of my understanding based on the context. However, the text is quite disordered and some parts are ambiguous, hence the corrections might not be totally accurate:

Periods 31 #7 5 — 1 1 1 1 1 1 1 1 Time 12010 | 1130 | 0945 | 1215 | 1045 | Fecal Coli. (CFU/mL) 1330 | 110 | 128 | 170 | Log Fecal Coli. 12.52 | 11.00 | 12.45 | Total Coli. (CFU/mL) 10000 | 175 | 250 | 200 | 2400 | Log Total Coli. 13.92 | 11.88 | 2.40 | 3.38 | Dissolved Oxygen (ppm) 13.50 | 3.40 | 3.40 | 1.80 | 1.80 | Temperature (°C) 28.3 | 29.2 | 28.0 | 28.9 | 26.2 | Oxygen Saturation (%) 44 | 44 | 23 | 23 | Light Intensity (Candle/ft²) 1800 | 78 | 570 | 2900 | 2700 | NO₃⁻ (ppm) 0.83 | 0.51 | 0.31 | 0.27 | N (ppm) 183.6 | 34.2 | 14.8 | 26.1 | Cl⁻ (ppm) 173 | 147 | 169 | Chlorine (ppm) 11.00

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Table 4c. Sampling results. NA represents missing values. Date 4/8/84-Diurnal STP Discharge (m³/hr) 890 Chlorine Applied (kg/hr) 4.54 Parameter to r T 1 riatbobels epee ds ng (ppm) 93.0 | 73.0 | 110.0 | 150.0 | 68.0 |

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Table 4d. Sampling results. WA represents missing values. Date 5/2/84-Diurnal STP Discharge (m³/hr) 792 Chlorine Applied (kg/hr) 5.67 Parameter NH₄⁺ (ppm) 1140.0 | 150.0 | 280.0

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Table 4e. Sampling results. Date 5/9/84-Diurnal STP Discharge (m³/hr) Chlorine Applied (kg/hr) Parameter Time Fecal Coli. (CFU/mL) Log Fecal Coli. Total coli. (CFU/mL) Log Total Coli. Dissolved Oxygen (ppm) Temperature (°C) Oxygen Saturation (%) Light Intensity (Candle/ft²) NO₃⁻/NO₅⁻ (ppm) N (ppm) Cl⁻ (ppm) Chlorine (ppm) NA represents missing values.

Times 1030 | 1008 | 0900 | 1036 | 2045 | Fecal Coli. (CFU/mL) 11500 | 8700 | 560 | 2200 | 300 |
Log Fecal Coli. 14.00 | 3.94 | 3.75 | 13.34 | 2.48 | Total Coli. (CFU/mL) 43000 | 22000 | 2300 |
12000 | 3000 | Log Total Coli. 14.63 | 4.34 | 3.36 | 4.08 | 3.48 | Dissolved Oxygen (ppm) NA | NA | NA |
NA | NA | NA | Temperature (°C) NA | NA | NA | NA | NA | Oxygen Saturation (%) NA | NA | NA |
NA | NA | Light Intensity (Candle/ft²) 1300 | 2000 | 3000 | 3500 | 1000 | NO₃-/NO₅- (ppm) 10.64 |
6.61 | 0.12 | 0.23 | 0.08 | N (ppm) 148.0 | 150.0 | 38.0 | 100.0 | Cl- (ppm) NA | NA | NA | NA | NA |
Chlorine (ppm) 5.00

Note: NA represents missing values.

Here is your corrected text:

Table 4f. Sampling results. NA represents missing values.

Date: 6/20/84, Nocturnal STP
Discharge (m³/hr): 890
Chlorine Applied (kg/hr): 8.98

Parameter Time: ta0s2
Fecal coli. (cfu/m): 14720
Log Fecal Coli.: ta.aa
Total count (cfu/m): 18500
Log Total coli.: 3.93
Dissolved Oxygen (ppm): NA
Temperature (°C): NA
Oxygen Saturation (%): NA
SO₂/1105 (ppm): NA
Tent fag (ppm): 185.0
Cl- (ppm): NA
Chlorine (ppm): NA

Table 4g. Sampling results. NA represents missing values.

Date: 6/28/84, Nocturnal STP
Discharge (m³/hr): 1003
Chlorine Applied (kg/hr): 3.92

Parameter: NA

Table 4h. Sampling results, NA represents missing values.

Date: 7/2/84, Nocturnal STP
Discharge (m³/hr): 1003
Chlorine Applied (kg/hr): 3.92

Parameter: NA