SAMPLING BACTERIA IN A MOUNTAIN STREAM

By

Samuel H. Kunkle and James R. Meiman

March 1968

HYDROLOGY PAPERS
COLORADO STATE UNIVERSITY
Fort Collins, Colorado

28

Several departments at Colorado State University have substantial research and graduate programs oriented to hydrology. These Hydrology Papers are intended to communicate in a fast way the current results of this research to the specialists interested in these activities. The paper will supply most of the background research data and results. Shorter versions will usually be published in the appropriate scientific and professional journals, or presented at national or international scientific and professional meetings and published in the proceedings of these meetings.

The College of Forestry and Natural Resources of Colorado State University is involved in comprehensive studies of the natural resources of the Little South Fork of the Cache la Poudre Watershed in the Colorado Front Range. The research reported here is a part of this program. Many organizations are involved in these cooperative studies including the U. S. Forest Service, U. S. Geological Survey, U. S. Bureau of Reclamation, Colorado Water Conservation Board, and other colleges within the University. The cities of Colorado Springs and Greeley, Colorado, have contributed financial assistance.

This research is part of a continuing project under the McIntire-Stennis Cooperative Forestry Research Program. The present report further elaborates material presented earlier in Hydrology Paper No. 21 (June 1967) and is published with the approval of the Director of the Colorado Agricultural Experiment Station as General Series Paper No. 863. The material presented in this report is essentially that submitted to the Graduate School of Colorado State University in fulfillment of the thesis requirement for the Doctor of Philosophy degree for the senior author.

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ABSTRACT

Three pollution-indicating bacteria groups--the coliforms, fecal coliforms (FC), and fecal streptococci (FS)--were used to investigate bacteria fluctuations in a small, high-elevation stream in the Colorado Rocky Mountains in 1966-67. A total of 3102 observations were made at two sites. The upper site was located to sample water flowing from an uninhabited, forested catchment, while the lower site was 1.5 miles downstream, below a grazed meadow irrigated by the creek. The primary objectives of the study were to describe bacteria concentrations and variability at the natural and cattle-contaminated stream sites and to investigate bacteria cycles. Secondary objectives of the study were to examine relationships between bacteria counts and stream stage, water temperature, and insolation, and to describe the relative sensitivity of the three groups to the pollution.

Statistical analyses revealed: (1) The analytical error is an important source of variation; a coefficient of variation of about 0.5 was common for coliform replicates taken from one bottle. (2) Two bottles collected simultaneously were very similar in bacteria counts. (3) More variation occurred on a day-to-day basis than within a day. (4) Variability was highest when concentrations were lowest.

A daily cycle was found for all groups and sampling weeks 95% of the time. Evening maximums in concentrations followed afternoon minimums, while morning bacteria counts usually fell between the two. The cycle was apparently related, among other factors, to rising stream stages of early evening, whereby streambank "flushing" took place. Seasonally, the coliform and FC attained maximum values in the spring "flushing" period of rising stages at the cattle-influenced site; these groups showed highest counts at the upper site during low-dilution flows of mid-summer. The FS indicated seasonal maximums for both sites during mid-summer.

Very high bacteria counts occurred during summer storm stage rises, however counts on receding limbs of these hydrographs were comparable to pre-storm values, or even lower.

Water temperature was inversely related to bacteria counts, however distinct separation of water temperature and insolation effects was not possible. Comparison of shaded and unshaded containers suspended in the stream (at stream temperature) indicated extreme die-off of coliform bacteria in only 1-2 hours exposure to sunlight--another possible explanation for the afternoon low counts.

The cattle-contaminated site always showed higher bacteria concentrations than the upstream site. The FC were slightly more sensitive in detecting the pollution than the coliforms and far more sensitive than the FS. The FC/FS ratio was always less than 1.0 at the upper site, but ranged from 1.70 to 5.45 at the lower site.

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INTRODUCTION

High elevation mountain watersheds, where snowmelt contributes a large portion of the runoff, provide much of the water supply for municipal, agricultural, industrial, and recreational needs. As these high elevation watersheds are managed for a multitude of uses, water quality in the streams is commonly affected.

In many areas of the United States, for example, much interest exists regarding timber management, grazing, recreation, and other land use activities and the impact of these activities on streams. In order to arrive at management decisions regarding these land uses, a "yardstick" is needed, whereby the impact which a particular land use has on the streams may be evaluated. Many pollution indicators are presently in common use in heavily-polluted urban areas, and some of the indicators are no doubt applicable to the less-contaminated conditions of certain mountain areas. However, any pollution measuring system needs to first be field tested before it may be assumed that the system produces valid results under the field conditions at hand.

In order to provide needed information to land managers about water quality of mountain watersheds and the influence of land management on streams, the Department of Recreation and Watershed Resources of Colorado State University is conducting a 10-year water quality research program. The research began in 1964. This paper reports findings of the 1966-67 phase of the overall investigations. It is hoped that the information provided will aid in better measurement of land use impact on mountain streams. The observations made in this report apply to the conditions encountered and the information presented will, of course, not be applicable to streams of every geographic location. The results should, however, be meaningful in other lower-pollution areas of relatively cold, well-aerated streams, especially those of snowmelt origin.

Frequently bacterial indicators are used to evaluate the biological water quality of streams. These organisms--especially when isolated by the membrane filter technique--provide a relatively simple, fast, and inexpensive index of pollution. Yet, there is a paucity of information regarding use of bacterial indicators to assess the impact of mountain land use on water quality. Essential knowledge is missing regarding cycles and variability of bacteria in mountain streams and the relationships of bacteria to physical environmental factors. This basic information is requisite to the development of sampling procedures for use of bacterial indicators in low-pollution areas.

Geldreich et al., (1962) studied the relationship between land use and fecal coli-aerogenes bacteria concentrations by testing 251 soils, including creek banks, pastures, forests, an alpine area, and irrigated farm land. They found that fecal coli-aerogenes bacteria are usually absent in undisturbed soils (for example in a "virgin" forest), or are present only in comparatively small numbers. Conversely, there was a sharp increase in the numbers of these bacteria types from soils known to be polluted, for instance, in pasture areas. Geldreich (1966) asserts that the presence of fecal coliform organisms in untreated water is an indication of recent fecal pollution, while coliform organisms may indicate soil pollution or less frequent fecal contamination. He reports:

> Our findings support the current interpretation that fecal coliforms in surface waters are largely, if not completely, derived from fecal pollution of animal origin.

Teller (1963) conducted an extensive investigation of coliform concentrations on several watersheds of the Northwest, using available data from records of municipal watersheds. He described broad seasonal trends for the coliforms and found evidence of relationships between coliform counts and certain physical environmental factors, for example, streamflow and air temperature. No increase in coliform densities was seen to coincide with large increases in logging and stream turbidity over a period of years. (It should be noted that the logging was on municipal watersheds and probably carefully controlled).

Reigner (1965) studied the impact of recreational land use on water quality; he notes that the sampling and testing methods for detecting pollution need to be strengthened so that land use impact may be accurately evaluated.

Morrison and Fair (1966), studying a Colorado stream, observed that surface runoff washes bacteria directly into the stream. During rising stages of the spring runoff, they surmised that water washes material into the stream and picks up foreign material from streambanks. Geldreich (1966) states that storm water is the major intermittent source of bacterial pollution entering our nation's waterways.

With ability to detect pollution sources, land management activities and human activities could be better controlled in order to minimize the detrimental effect which land use may have on water quality.

Forerunning Investigations

In 1964-65 the Department of Recreation and Watershed Resources at Colorado State University carried out a study of water quality on a mountain watershed in north-central Colorado. A literature review was completed in 1965. The results of the first two years of study were published in June, 1967 (Kunkle and Meiman, 1967). Among other observations the study determined that:

1. High bacteria concentrations associated with grazing and irrigation impact appear to depend on the "flushing effect" of the flooding. This flushing effect also occurs during spring snowmelt and summer storm runoff periods.

2. Considering nine sampling sites located on several streams, broad seasonal trends for the coliform,

FC, and FS bacteria groups were similar: (a) low winter counts prevailed while the water was 0°C; (b) high concentrations appeared during the peak flows of June; (c) a short "post-flush" lull in counts took place as the hydrograph declined in mid-summer; (d) high concentrations were found again in the late summer period of warmer temperatures and low flows; and (e) counts declined with the arrival of autumn. The FS bacteria showed higher counts in April-May than the other two groups. The coliforms and FC were most similar in seasonal trends.

Research Needs

Following the 1964-65 investigations, there were still many obscurities regarding sampling procedures for use in appraising mountain water quality by bacterial indicator methods. Some of the questions raised by the study were:

1. What variation in bacteria counts occurs (a) hourly, (b) daily, (c) within a week, or (d) seasonally?

2. Are the bacteria variations related to bacteria concentrations, for example, is variation at a cattle contaminated site greater than at an undisturbed natural stream site?

3. What is the sampling error inherent in a water sample taken from a stream?

4. Is there a daily cycle in bacteria counts? If such a cycle exists, does it occur at both cattle-contaminated and natural locations in the stream?

5. Can the relationship between bacteria counts, stream stage, temperature, or insolation be detected on a daily basis?

6. How much do bacteria counts increase during or following summer storm runoff, and how long are these elevated concentrations maintained?

7. How do the variations, errors, and cycles enumerated above compare for the three bacterial indicators--coliforms, FC, and FS?

A limited number of duplicate samples taken within 24-hour periods during the 1964-65 studies gave a hint to the tremendous variations in stream bacteria concentrations within replicates and within a period of a few hours. Some sources of variation or cycles in concentrations are surmised to be:

1. Difference in location within the cross section of the stream from which the sample is taken.

2. Diurnal fluctuations in concentrations, either randomly or because of a daily cycle.

3. Day-to-day and seasonal variations in bacteria counts.

4. Changes in bacteria concentrations as the degree of land use varies.

5. Die-off or multiplication of organisms in the stream.

6. The size of the aliquot used in analysis.

7. Difference between duplicate bacteria plates (replicates) resulting from pipetting errors, counting errors, and real differences in bacteria numbers.

8. Incubator temperature fluctuations, changes in media composition, accidental contamination of laboratory equipment, and other errors stemming from laboratory technique.

Research Objectives

Based on the research needs cited above, the research objectives for the 1966-67 study were established as:

MAJOR OBJECTIVES

1. To measure variability in bacteria concentrations arising from field laboratory techniques.

2. To evaluate the sampling error inherent in bacteria samples taken from a mountain stream under natural as opposed to contaminated conditions.

3. To investigate hourly, daily, weekly, and seasonal cycles in bacteria concentrations in the undisturbed as opposed to impacted section of the stream.

SECONDARY OBJECTIVES

4. To examine relationships in the stream between bacteria counts and stream stage changes, water temperature, and insolation.

5. To describe the relative sensitivity of three bacteria groups--the coliforms, fecal coliforms and fecal streptococci--to cattle grazing and irrigation impact in a mountain stream.

Chapter II

RESEARCH DESIGN AND METHODS

A stream was selected for the study whereby a distinct land use impact could be measured and studied. This chapter discusses the study area and reasons for its selection; the parameters measured; sampling sites, schedules, and intensity; the equipment used; and analysis of the data.

The Parameters Measured

Three bacteria groups were tested: the coliforms, fecal coliforms (FC) and fecal streptococci (FS)[1]. Stream stage and water temperature recorders were in operation throughout the study. Stream stage measurements were used to define rising and falling segments of the daily and seasonal hydrograph and to isolate storm runoff periods. Because of interest in "stream flushing" by rising stages, stage measurements are used in the analysis without converting to discharge (volume of flow per se being of secondary interest). For information regarding flow volume, the 1966 hydrograph is presented in Figure 3. The year 1967 had flows perhaps 50 percent greater than in 1966; calibration of the gage is still in process at this time and 1967 flow records are not given. A stream thermograph was operating at the upper site during the study.

The Study Area

Within the Little South Fork of the Cache la Poudre watershed of the previous investigations, a subwatershed was selected for the research (Fig. 1). This

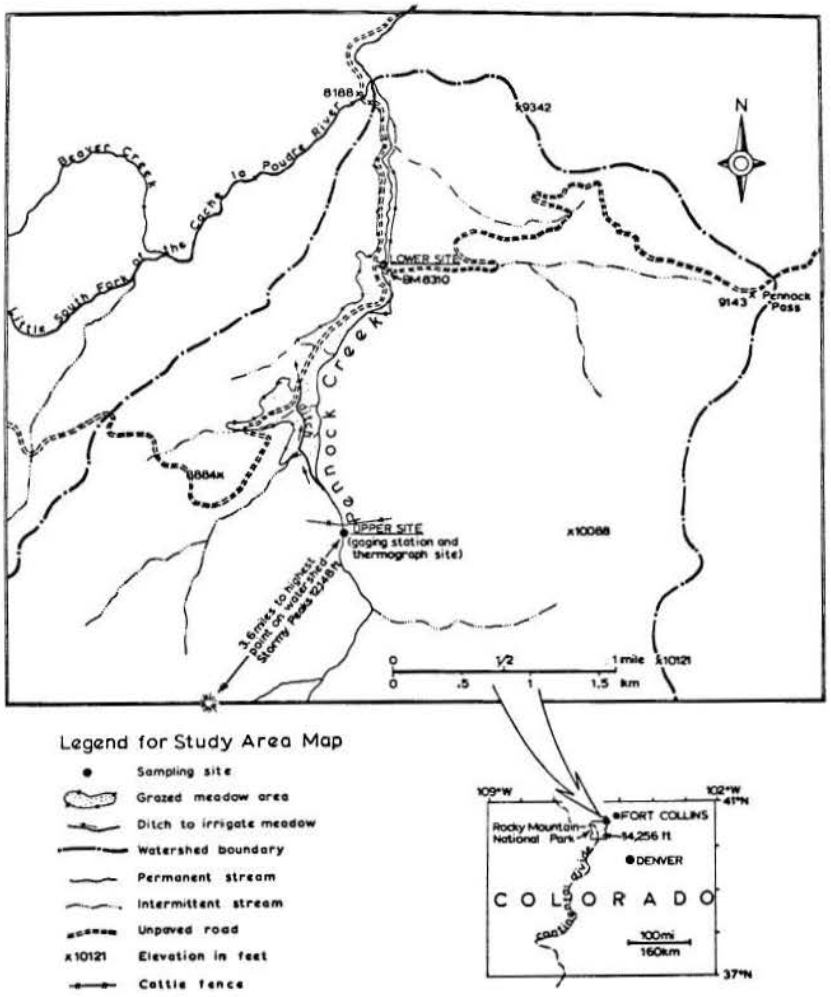


Figure 1. Map of the study area

[1]Also known as the enterococci.

catchment, Pennock Creek, has several advantages which make it particularly suited for such a study:

1. The stream offers a superb opportunity to compare undisturbed to contaminated conditions of a stream, all within a distance of about one mile. The first two miles of the creek are in "virgin" condition, flowing out of the northeastern corner of Rocky Mountain National Park. In contrast to this "clean" stream situation, the lower portion of the creek is partially diverted for the flooding of a small, grazed meadow (Fig. 2). About 75 head of cattle graze throughout the summer.

2. The stream is close to the Pingree Park field laboratory, making frequent sampling possible.

3. A stream gage and thermograph are located at the upper end of the grazed area.

4. Flow continues throughout the summer, although the stream is small enough for the grazing-irrigation impact to be distinct. Flow during the summers was from 5 to 15 cfs.

Figure 2. The irrigated grazed meadow of the study, showing the irrigation ditch in the foreground.

Pennock Creek originates at an elevation of approximately 10,000 feet and flows to 8,200 feet where it empties into the Little South Fork of the Cache la Poudre River (Fig. 1).

Sampling Sites

Two sites were sampled. The upper site was located above the cattle impact area in order to sample the "uncontaminated" water flowing from Rocky Mountain National Park. The lower site was placed near the lower end of Pennock Creek, to sample below the cattle grazing and irrigation.

Sampling Schedule

The study was conducted during two runoff seasons. There were four seven-day periods of sampling in 1966 ("Weeks I-IV") and one in 1967 ("Week V"). The sampling design for these five weeks was identical. An intensive three-day and four-day study, an intensive storm sampling, and a two-day study of insolation (incoming solar radiation) effects on bacteria were also completed in 1967. Dates of the sampling periods were:

1966	Week I	June 3-9
	Week II	June 23-29
	Week III	July 29 - August 4
	Week IV	August 24-30
1967	"3-day study"	June 7-9
	Week V	June 15-21
	Storm study	June 21
	"4-day study"	August 2-5
	Insolation study	August 4-5

The location of Weeks I through IV in relation to the 1966 hydrograph appears in Figure 3. Week V was on the rising limb of the 1967 hydrograph, similar to Week I of 1966. The "3-day study" and "4-day study" were comparable to Weeks I and III of 1966, respectively, in regard to hydrograph position.

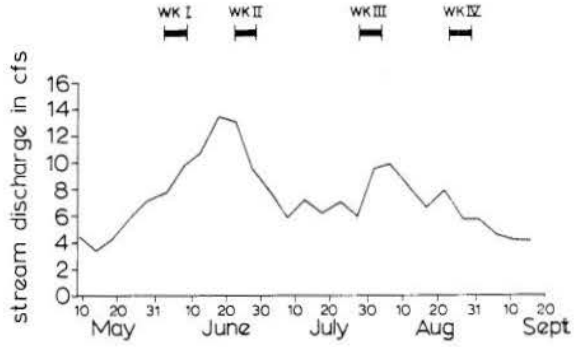


Figure 3. The sampling weeks in 1966 (Weeks I-IV) shown in relation to the seasonal hydrograph

Sampling Intensity

During Weeks I-V, sampling was as shown in Figure 4. Three times during the day, two bottles were collected from each site, upper and lower. Two aliquots were taken from each bottle. The sampling times are referred to as "morning", "afternoon", and "evening".

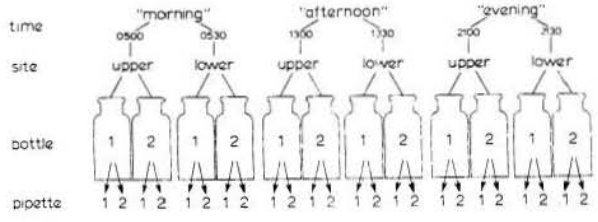


Figure 4. The sampling design used in the Weeks I-V phase of the study. Two pipettes were taken from each bottle, two bottles were taken at each site three times a day, and seven days were sampled for each week, for each of the bacteria groups.

The earliest time shown on the diagram is for the upper site, while the lower site was always sampled approximately one-half hour later, for example, the "morning" sample was taken at the upper site at 0500 hours and at the lower site at 0530.

In summary, the samples and replicates taken for Weeks I-V (for each bacterial indicator group) were:

2 pipettes from each bottle
2 bottles from each site
2 sites (upper and lower)
3 times per day (morning, afternoon, evening)
7 days per week
168 observations for each week-long period

(x 5 weeks = 840 observations)

The "3-day study" and "4-day study" were designed to further identify bacteria variations and trends during the afternoon and evening period. In these two studies only coliform bacteria were isolated. One bottle was collected every two hours at the lower site, from noon until 2:00 a.m. (8 samples per day). Six replicates were taken from each bottle during the "3-day study", nine during the "4-day study".

In the insolation study, six containers were exposed to the sun, six were not. One coliform sample was taken from each container several times during the day.

The one storm sampling period was very intensive as shown in Figure 24.

The following observations were made for the various special studies:

"3-day study"

6 replicates per sample
8 samples per day (every two hours--
3 days 12 noon to 2 a.m.)
144 coliform observations

"4-day study"

9 replicates per sample
8 samples per day
4 days
288 coliform observations

"storm study"

14 individual observations of each bacteria group during a 7 hour period = 42 observations

"insolation study"

6 replicates (individual containers)
2 treatments (sun vs shade)
9 observation times
108 coliform observations during a 2 day period

For all phases of the research there were 1394 coliform, 854 FC, and 854 FS individual observations, or a total of 3102 observations.

[1]Beckman Scientific Instruments, Fullerton, California

[2]Foxboro Company, Foxboro, Massachusetts

Laboratory and Field Equipment

The bacteria were analyzed by the membrane filter technique, using a portable incubator, water bath, portable autoclave, and a stainless steel suction arrangement similar to that described in Standard Methods (American Public Health Association, 1965). The pH measurements were made on a Beckman Model N pH meter[1]. Temperatures were recorded by a Foxboro thermograph[2], accurate to about ± 1°F. Stages were recorded on a Stevens A-20 recorder[3] attached to a Servo Manometer "bubble" gage[4]. Both recording instruments were located at the upper site. Temperatures at the lower site were measured by a pocket thermometer, accurate to about ± 1°F. Samples for Weeks I-V and the "3-day" and "4-day" studies were collected in gallon-size collecting bottles, randomly selected and marked before each sampling trip (morning, afternoon, and evening). Samples for storm observations were collected in 500 ml polyethylene bottles. Bottles were rinsed in boiling water after each use. Pipettes, funnels, filters, and other supplies and equipment were sterilized before use.

To observe the effect of solar radiation, twelve wide-top, two-quart, ice cream containers were supported in a rack in the stream. The rack allowed stream water to pass under and around the bottom half of the vessels, maintaining all container temperatures at nearly the same level (Fig. 5). Six of the receptacles were shaded with a black felt paper, while still allowing air to pass over them; six were left open to sunlight. Samples were pipetted from the containers into 250 ml polyethylene bottles for transport to the laboratory.

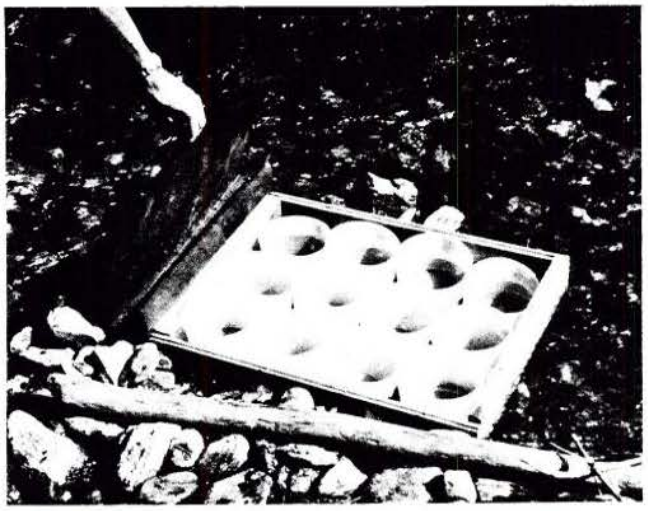


Figure 5. The arrangement used in the insolation study. The black felt paper provided shade for six of the containers, while the others remained exposed to sunlight.

Data Analysis

Data for Weeks I-V were entered on standard punch cards and analyzed on IBM 1401 and CDC 6400 computers. Data for the 3-day and 4-day studies were

[3]Leupold and Stevens Instruments, Inc., Portland, Oregon

[4]Exactel Instrument Company, Mountain View, California

analyzed by desk calculator. A 2 x 3 x 7 factorial analysis of variance was carried out (2 sites, 3 times, and 7 days); results showed extreme upper to lower site contrasts in bacteria, and, therefore, subsequent analyses were calculated by separate sites. A 3 x 7 factorial analysis of variance (3 times and 7 days) was then computed; the time of day and days were found to be significantly different. Thereafter, individual analyses of variance were carried out between morning-afternoon, morning-evening, and afternoon-evening.

Pairs of pipettes from within the same bottle and bottles from the same site were compared by using a paired t-test.

Scattergrams between the coliform-FC, coliform-FS, and FC-FS groups were drawn, but the scatter was so extreme that further analysis was deemed purposeless.

The major components of variance were broken out by use of a factorial analysis of variance using factor one as day of the week, factor two as time of the day, and factor three as bottle, with two replicates taken from each bottle. The sources of variation are shown in Tables A-C, Appendix.

Chapter III

BACTERIA VARIATIONS

The variation inherent in replicates of individual bacteria samples and the variability occurring during periods of a day, week, and season are major factors to consider in sampling design. These components of variance are evaluated and compared in this chapter.

A factorial analysis of variance was carried out for each bacteria group by site and week. Initial analysis revealed obvious and drastic differences between sampling stations, the lower site always showing much higher concentrations. For this reason, all further analyses of the individual bacteria groups were carried out by separate site. A factorial analysis of variance was calculated, using three factors:

factor 1: day of the sampling week
factor 2: time of the day
factor 3: bottle 1 or 2
(with 2 replicates taken from each bottle) .

As an aid to sampling design, a tabulation of variance components, taken from the factorial analysis of variance, is presented in Tables A-C of the Appendix. The components of variance considered in the breakdown of the tables are:

1 - analytical error (as shown by the difference in two pipettes taken from one bottle)

2 - "bottle" (as illustrated by the difference of two bottles collected simultaneously)

3 - day x time interaction

4 - time of day

5 - day-to-day.

Each of these components will be considered in some detail in the folowing sections; in comparing them (Tables A-C, Appendix) it is noted in general that:

1. The analytical error is usually one of the most important sources of variance.

2. Little variation appears to be coming from the "bottle" component, i.e., two bottles taken from the stream simultaneously are extremely similar.

3. At the lower site, the day component is generally of more importance than time (for all organisms). The upper site has little difference in time or day variation, except in the case of the coliforms, where the time shows greater variation than day.

4. In many cases there is a strong day x time interaction, indicating that the daily cycle is not independent of day-to-day variation.

A sampler may use variance values from the tables in conjunction with an appropriate variance formula for general guideposts, so that he may best invest his samples by sampling more intensively those factors having the greater variance.

From a practical standpoint, an investigator may need to compromise in the allocation of his samples. For example, although it may lower total variation in the data very effectively for the researcher to sample several days during the week, transportation costs may overrule such plans. At the same time the sampler may be able to reduce the analytical error, another important component of the overall variance, by increasing the number of replicates taken from each bottle and thereby still use the resources at his command to improve the estimate of bacteria counts.

Error in Analytical Techniques

One purpose of the study is to define the error inherent in laboratory technique. During a total of seven days (the 3-day and 4-day studies), bottles were collected at the lower site, with several coliform replicates taken from each bottle. The insolation study used six replicates in each sample. Based on these data, the error in laboratory technique may be estimated.

Several Replicates from a Bottle

Using data of the 3-day and 4-day studies of 1967, the variation within one sample can be described. Eight coliform samples were taken daily during the total seven days, each sample including either six or nine replicates (details of the sampling scheme appear in Chapter II). The coefficients of variation for the samples range from 0.14 to 1.06 for the six replicate samples and 0.21 to 1.64 for the samples made up of nine replicates, with a mean CV of 0.51 for the six-replicate (3-day) study and 0.59 for the nine-replicate (4-day) investigation (Tables 1 and 2). The high coefficients of variation indicate that replicates from a single bottle (at one time) vary tremendously about the mean of the bottle, i.e., that a single pipette would be a poor estimate of the bottle mean. In brief, the analytical error is very high. The lowest CV, 0.14, implies that there is a 95 percent chance that a pipette taken from a bottle will estimate the mean coliform concentration by an error of about ± 28 percent. An error of ± 100 percent (CV = 0.50) would be more common, while an error as large as ± 328 percent might be expected at some times (CV = 1.64).

Plotting all sample means against coefficients of variation for the two studies produces the inverse relationship of Figure 6. There is a general increase in the CV of replicates within a sample as the mean of the population sampled decreases, i.e., the error in analytical technique appears to be absolute and thus becomes relatively more important as low-concentration bacteria samples are analyzed.

Insolation Study Replicates

The insolation study of August 4-5, 1967, made use of six replicates, taken in a slightly different

Table 1-- Means, standard deviations, and coefficients of variation for each individual bottle during the 3-day study. Six replicates (pipettes) were taken from each sample (bottle); the s and CV values below describe variation of the replicates about the mean of the sample. Means are in coliform colonies per 100 ml.

"3-DAY STUDY"

	Time	Values for the 6 replicates (coliforms) $\bar{x}$	s	CV
	12	131.7	45.4	0.35
	14	168.3	55.3	0.33
	16	213.3	73.1	0.34
June 7,	18	175.0	96.5	0.55
1967	20	120.0	71.3	0.59
	22	211.7	108.7	0.51
	24	90.0	63.3	0.70
	2	173.3	66.5	0.38
	12	140.0	74.3	0.53
	14	158.3	50.4	0.32
	16	186.7	64.4	0.34
June 8	18	200.0	89.2	0.45
	20	183.3	105.6	0.58
	22	181.7	83.3	0.46
	24	125.0	132.2	1.06
	2	150.0	133.9	0.89
	12	180.0	67.8	0.38
	14	115.0	67.2	0.58
	16	235.0	130.7	0.56
June 9	18	140.0	101.4	0.72
	20	106.7	70.9	0.66
	22	143.3	98.1	0.68
	24	375.0	52.1	0.14
	2	243.3	63.8	0.26

Table 2-- Means, standard deviations, and coefficients of variation for each individual bottle during the 4-day study. Nine replicates (pipettes) were taken from each sample (bottle); the s and CV values below describe variation of the replicates about the mean of the sample. Means are in coliform colonies per 100 ml.

"4-DAY STUDY"

	Time	Values for the 9 replicates (coliforms) $\bar{x}$	s	CV
	12	33.3	35.4	1.06
	14	22.2	36.3	1.64
	16	83.3	86.6	1.04
Aug. 2,	18	72.2	117.6	1.63
1967	20	66.7	55.9	0.84
	22	894.4	686.7	0.77
	24	1016.7	533.3	0.52
	2	261.1	235.6	0.90
	12	324.4	123.2	0.38
	14	353.3	130.0	0.37
	16	368.9	111.4	0.30
Aug. 3	18	233.3	154.0	0.66
	20	535.6	422.0	0.79
	22	155.6	84.1	0.54
	24	315.6	239.1	0.76
	2	473.3	260.6	0.55
	12	377.8	145.4	0.38
	14	466.7	167.0	0.36
	16	368.9	87.2	0.24
Aug. 4	18	266.7	85.4	0.32
	20	433.3	150.3	0.35
	22	335.6	117.0	0.35
	24	404.4	86.5	0.21
	2	295.6	76.0	0.26
	12	240.0	157.5	0.66
	14	368.9	96.5	0.26
	16	180.0	122.1	0.68
Aug. 5	18	246.7	78.7	0.32
	20	73.3	52.0	0.71
	22	173.3	64.8	0.37
	24	120.0	34.7	0.29
	2	151.1	74.2	0.49

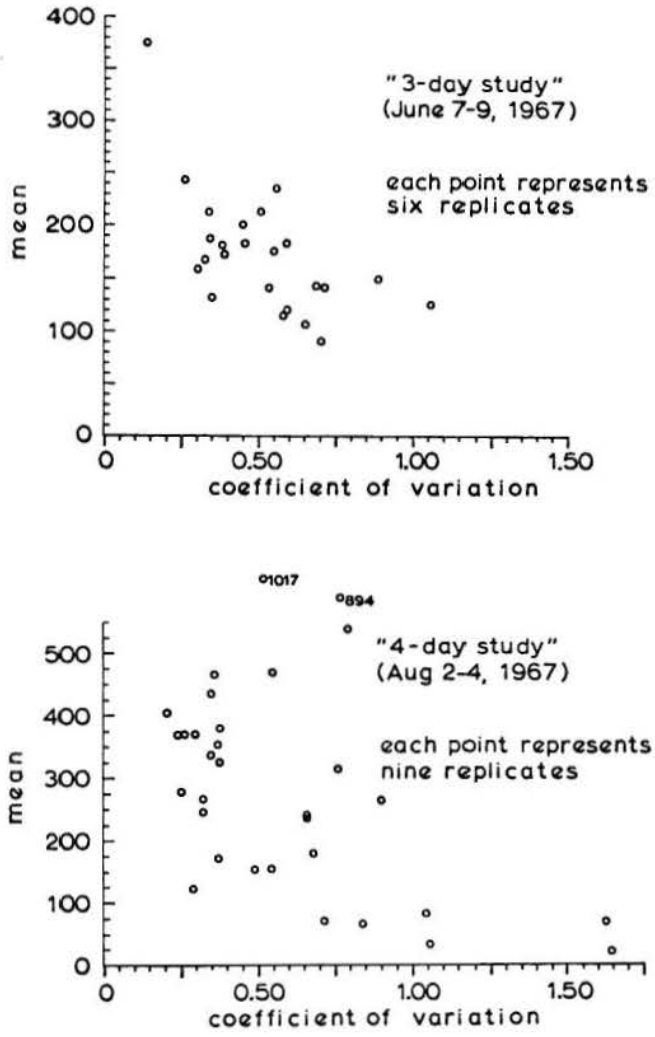


Fig. 6. Coefficients of variation (CV) vs means for individual coliform samples during the 3-day and 4-day studies. CV represents the variation found within one sample (containing several replicates).

manner than those replicates described on the preceding page for the 3-day and 4-day studies. In the six open containers of the insolation study, the water was mixed back and forth in all vessels at the initiation of the testing, so as to have the "same" water in all containers. One pipette was taken from each receptacle, each time, for a total of six replicates per sample. The means, standard deviations, and coefficients of variation for six replicates taken in this fashion are presented in Table 3. The coefficients of variation for the six replicates average 0.34 for August 4, 0.39 for August 5.

Two Bottles Taken Simultaneously

During Weeks I-V, two pipettes were taken from each bottle and two bottles were collected from each site, as illustrated in the sampling design of Figure 4, Chapter II. According to the components of variance breakdown of Appendix Tables A-C, there appears to be very little benefit in taking more than one bottle of water at a given sampling time. The bottle source of variation is the lowest or nearly the lowest component

Table 3. Means, standard deviations and coefficients of variation for the six replicates of the "shaded" samples in the insolation study. Means are in coliform colonies per 100 ml.

	Time	$\bar{x}$	s	CV
8-4-67	1045	220.0	96.3	0.44
	1230	263.3	42.7	0.16
	1420	210.0	67.8	0.32
	1720	673.3	332.9	0.49
8-5-67	0920	166.6	62.8	0.38
	1045	170.0	76.7	0.45
	1210	353.3	151.6	0.43
	1400	146.7	77.6	0.53
	1545	140.0	28.3	0.20

of variance in nearly every case for the three bacteria groups and the two sites. It would, therefore, be better to invest the sampling effort into additional replicates from the same bottles or more samples over time.

Because a greater possibility exists for consistent contamination of the second pipette from a bottle or the second bottle from a site, paired t-tests were carried out to test for significant differences between pipettes or between bottles. For the bottle t-tests, a value was derived from the average of the two pipettes in a bottle and this value represented the single bottle. As shown in Table 4-a through 4-c, there is no significant difference between the two pipettes. Similar tests for the bottles showed they also were not significantly different.

Technique Error

The membrane filter technique would probably be more accurate when used in a more elaborately equipped laboratory than that of the study. However, users of the method will likely utilize equipment comparable in refinement to that of the study when sampling mountain streams. It is possible to take several replicates from each bottle to improve the estimate of bacteria concentrations. The merit of taking several pipettes from each bottle increases as the population sampled decreases. The sampler may wish to improve his estimate of bacteria concentrations within a certain time span and, therefore, prefer to spread more observations over time instead of replicating. In any event, inferences made from the bacteria data should be with due consideration of the extremely large error inherent in the field laboratory technique. As shown by the components of variance breakdown (Appendix Tables A-C) the variance due to analytical technique is consistently one of the major sources of variation. Despite such large variation, distinct land use impact may be detected, as evidenced by the extremely consistent upper to lower site comparison (Chapter 5).

Variations Within a Day

The components of variance, Tables A through C, Appendix, show there is generally large variation in bacteria concentrations within a day (the "time" component of the tables). The tables illustrate that this time variation, however, is often less than the fluctuations on a day-to-day basis, one exception being the coliform group of the upper site.

Table 4-a-- Means, standard deviations, and coefficients of variation for individual pipettes by week and site for the coliform bacteria. Values for paired t-test comparisons between pipettes 1 and 2 are shown.

COLIFORMS - UPPER SITE

Week	Pipette 1 $\bar{x}$	Pipette 1 s	Pipette 1 CV	Pipette 2 $\bar{x}$	Pipette 2 s	Pipette 2 CV	t-test	df
I	5.28	8.45	1.60	5.83	9.96	1.71	0.285	35
II	26.43	19.73	0.75	24.05	26.60	1.11	0.548	41
III	99.44	126.90	1.28	116.67	135.94	1.17	0.895	35
IV	79.52	77.11	0.97	87.86	90.11	1.03	0.610	41
V	16.00	29.53	1.85	14.93	24.19	1.62	0.692	29
Average			1.29			1.33		
				1.31				

COLIFORMS - LOWER SITE

Week	Pipette 1 $\bar{x}$	Pipette 1 s	Pipette 1 CV	Pipette 2 $\bar{x}$	Pipette 2 s	Pipette 2 CV	t-test	df
I	1549.7	1529.1	0.99	1584.2	1447.6	0.91	0.111	35
II	993.8	608.6	0.61	881.0	571.2	0.65	0.963	41
III	458.6	359.0	0.78	470.8	433.8	0.92	0.238	35
IV	352.1	357.7	1.02	297.6	289.7	0.97	1.369	41
V	466.7	382.1	0.82	499.7	381.0	0.76	1.295	29
Average			0.84			0.84		
				0.84				

Table 4-b-- Means, standard deviations, and coefficients of variation for individual pipettes by week and site for the fecal streptococcus bacteria. Values for paired t-test comparisons between pipettes 1 and 2 are shown.

FS - UPPER SITE

Week	Pipette 1 $\bar{x}$	Pipette 1 s	Pipette 1 CV	Pipette 2 $\bar{x}$	Pipette 2 s	Pipette 2 CV	t-test	df
I	2.25	5.01	2.23	1.86	4.73	2.54	0.868	35
II	3.81	3.23	0.85	4.36	5.34	1.22	0.844	41
III	12.83	16.33	1.27	12.44	10.42	0.84	0.213	35
IV	2.79	2.63	0.94	2.86	2.10	0.73	0.161	41
V	2.89	6.17	2.13	3.56	5.31	1.49	1.323	35
Average			1.48			1.36		
				1.42				

FS - LOWER SITE

Week	Pipette 1 $\bar{x}$	Pipette 1 s	Pipette 1 CV	Pipette 2 $\bar{x}$	Pipette 2 s	Pipette 2 CV	t-test	df
I	27.3	38.9	1.42	27.3	39.8	1.46	0.014	35
II	30.0	42.3	1.41	29.2	34.8	1.19	0.252	41
III	33.8	32.2	0.95	45.0	40.8	0.91	2.687	35
IV	5.0	7.0	1.40	5.4	9.3	1.72	0.461	41
V	40.8	21.1	0.52	38.3	22.2	0.58	0.833	35
Average			1.14			1.17		
				1.16				

Table 4-c-- Means, standard deviations, and coefficients of variation for individual pipettes by week and site for the fecal coliform bacteria. Values for paired t-test comparisons between pipettes 1 and 2 are shown.

FC - UPPER SITE

Week	Pipette 1 $\bar{x}$	Pipette 1 s	Pipette 1 CV	Pipette 2 $\bar{x}$	Pipette 2 s	Pipette 2 CV	t-test	df
I	0.31	1.09	3.52	0.08	0.37	4.63	1.187	35
II	1.91	4.58	2.40	1.71	3.78	2.21	0.500	41
III	0.56	1.40	2.50	0.72	1.80	2.50	0.443	35
IV	0.07	0.34	4.86	0.10	0.37	3.70	0.571	41
V	6.22	25.22	4.05	1.44	4.16	2.89	1.121	35
Average			3.47			3.19		
				3.33				

FC - LOWER SITE

Week	Pipette 1 $\bar{x}$	Pipette 1 s	Pipette 1 CV	Pipette 2 $\bar{x}$	Pipette 2 s	Pipette 2 CV	t-test	df
I	139.4	148.1	1.06	134.4	155.1	1.15	0.478	35
II	85.2	54.3	0.64	87.6	48.0	0.55	0.553	41
III	61.6	87.1	1.41	72.1	94.8	1.31	0.990	35
IV	30.2	35.9	1.19	26.5	34.9	1.32	1.064	41
V	202.3	136.4	0.67	228.1	173.8	0.76	1.162	35
Average			0.99			1.02		
				1.01				

The factorial analyses of variance demonstrate a significant difference between the three times of the day (0500, 1300, 2100 hours), especially at the lower site. An exception to this is for the FC, upper site, however concentrations are so often zero for FC at the upper site that the time of day comparisons are difficult to make.

A daily cycle probably accounts for most of the variation within a day, therefore, the amount of fluctuation for a day is perhaps best shown by description of the daily trend. This cycle is dealt with in detail both graphically and statistically in Chapter IV.

Variations Within a Week

Tables 4-a through 4-c give means, standard deviations, and coefficients of variation for Weeks I-V, by site, bacteria groups, and either pipette one or two. The analyses do not include "storm days" (where a distinct rise in stage following a storm is associated with an upward surge of bacteria counts). One outstanding feature of the table is the large variability found over a period of one week, as evidenced by the coefficients of variation for the individual weeks. For pipette one, the values are plotted in Figure 7,

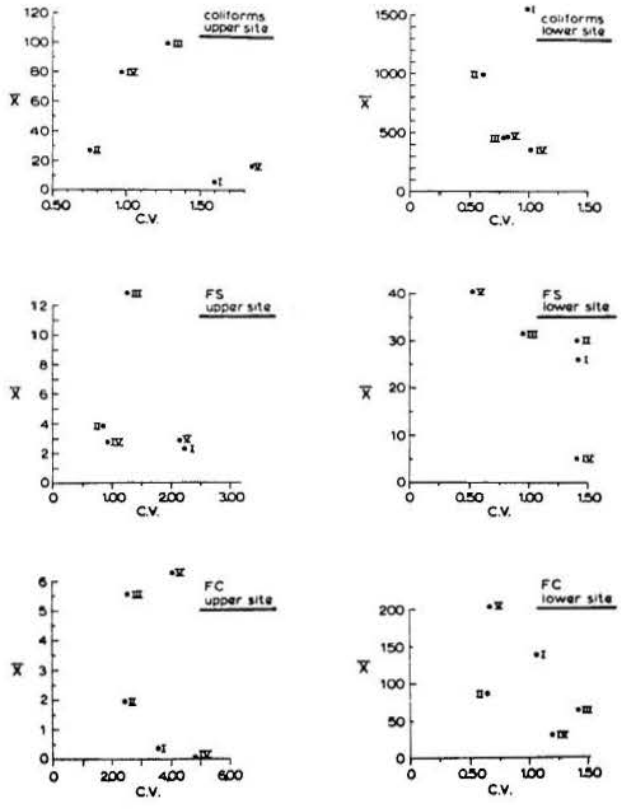


Figure 7. Coefficients of variation (CV) vs means for individual weeks (numbered), by site and bacteria group, during 1966-67. CV represents the variation found over the period of a week.

for all sites, bacteria groups, and Weeks I-V. The lowest CV for any week is 0.61, still a very high degree of fluctuation. These values of CV include all possible sources of variation, pipette differences, bottle comparisons, hour-to-hour fluctuations, and day-to-day variation, during the week. Figure 7 compares the coefficients of variation between sites and among bacteria groups. It is clear that CV values are greatest where the mean bacteria concentrations are lowest--the upper site. The lower site coefficients of variation are smaller than CV values for the upper site in 12 out of the 15 comparisons (three bacteria groups x five weeks) of Figure 7. Two of the three exceptions to this trend are for the FS bacteria, and, as will be pointed out in Chapter V, the FS show the least contrast between upper and lower site concentrations.

Variations within a week for the individual times of the day are shown in Tables 5-a through 5-c in terms of morning, afternoon, and evening (M, A, and E) variations throughout the week. Each time of the day is represented by one value, made up of the mean of the four replicates taken at that time (two pipettes x two bottles). Each time is then used to calculate the amount of variation taking place from day-to-day during the week, for the one time of the day only. The coefficients of variation given would not include the "within-day" variation, because only one time of the day is considered.

Taking the medians of the values of CV from Table 5, to have some relative comparison of the day-to-day variation for the individual times of the day, the following values are obtained:

	Median CV For Weeks I-V			
	Upper Site		Lower Site	
Coliforms	M	0.46	M	0.50
	A	1.20	A	0.54
	E	0.59	E	0.59
FS	M	0.69	M	0.54
	A	1.00	A	0.73
	E	0.92	E	0.48
FC	M	1.64	M	1.02
	A	1.94	A	0.78
	E	1.94	E	0.53

Afternoons show the highest CV in 4/6 of the comparisons and in no case exhibit the lowest CV of the three times. Mornings and evenings alternate in having the lowest values of CV. This is again in agreement with findings that variability is greatest where means are lowest; afternoons often show daily minimum bacteria concentrations, as will be illustrated in discussion of the daily cycle in Chapter IV.

The components of variance tables (Tables A-C, Appendix) illustrate that a strong day x time interaction exists in many of the weeks for both sites. The FS and FC show this component to be relatively larger than do the coliforms. The interaction indicates that the daily cycle of bacteria counts depends also on day-to-day changes.

Seasonal Variation

Ranges of bacteria variations during the entire sampling season of 1966 (Weeks I-IV) may be seen in

Table 5-a-- The day-to-day variation in coliform counts by sites taking place during a week, as shown by analysis of individual times of the day. Each time for each day is the mean of 4 observations (2 pipettes x 2 bottles). No storm days are included.

Week	Time	n	x̄	s	CV
COLIFORMS - UPPER SITE					
	M	6	5.42	3.68	0.68
I	A	6	2.92	4.01	1.37
	E	6	8.33	8.17	0.98
	M	7	20.36	12.78	0.63
II	A	7	13.21	11.61	0.88
	E	7	42.14	11.85	0.28
	M	6	32.50	43.33	1.33
III	A	6	100.83	117.87	1.17
	E	6	190.83	112.58	0.59
	M	7	72.50	33.54	0.46
IV	A	7	63.57	76.50	1.20
	E	7	115.00	75.77	0.66
	M	5	2.90	3.19	1.10
V	A	5	3.00	4.11	1.37
	E	5	40.50	23.55	0.58
COLIFORMS - LOWER SITE					
	M	6	1865.83	882.58	0.47
I	A	6	1403.75	1108.50	0.79
	E	6	1431.25	1004.80	0.70
	M	7	757.86	242.02	0.32
II	A	7	1016.07	273.98	0.27
	E	7	1038.21	164.64	0.16
	M	6	335.00	363.51	1.08
III	A	6	378.33	147.53	0.39
	E	6	680.83	400.37	0.59
	M	7	168.21	83.80	0.50
IV	A	7	178.57	98.41	0.55
	E	7	627.86	309.96	0.49
	M	5	300.50	325.36	1.08
V	A	5	444.50	240.72	0.54
	E	5	704.10	456.92	0.65

Table 5-b-- The day-to-day variation in fecal streptococcus counts by sites taking place during a week, as shown by analysis of individual times of the day. Each time for each day is the mean of 4 observations (2 pipettes x 2 bottles). No storm days are included.

Week	Time	n	x̄	s	CV
FECAL STREPTOCOCCI - UPPER SITE					
	M	6	2.25	2.16	0.96
I	A	6	0.42	0.49	1.17
	E	6	3.50	5.48	1.57
	M	7	3.39	1.54	0.45
II	A	7	2.32	1.05	0.45
	E	7	6.54	5.81	0.89
	M	6	8.50	5.85	0.69
III	A	6	9.42	5.58	0.59
	E	6	20.00	18.38	0.92
	M	7	2.39	1.25	0.52
IV	A	7	2.68	2.69	1.00
	E	7	3.39	1.06	0.31
	M	6	1.83	1.69	0.92
V	A	6	0.92	0.92	1.00
	E	6	6.92	8.56	1.24
FECAL STREPTOCOCCI - LOWER SITE					
	M	6	41.42	66.83	1.61
I	A	6	14.92	13.53	0.97
	E	6	25.63	12.43	0.48
	M	7	38.89	64.31	1.65
II	A	7	19.32	10.54	0.55
	E	7	30.68	13.58	0.44
	M	6	33.83	15.67	0.46
III	A	6	33.33	31.55	0.95
	E	6	51.00	45.67	0.89
	M	7	4.18	2.24	0.54
IV	A	7	3.00	2.19	0.73
	E	7	8.39	12.57	1.50
	M	6	40.67	20.61	0.51
V	A	6	34.17	21.26	0.62
	E	6	48.83	14.12	0.29

Table 5-c-- The day-to-day variation in fecal coliform counts by sites taking place during a week, as shown by analysis of individual times of the day. Each time for each day is the mean of 4 observations (2 pipettes x 2 bottles). No storm days are included.

Week	Time	n	x̄	s	CV
FECAL COLIFORMS - UPPER SITE					
	M	6	0.50	0.79	1.58
I	A	6	0.08	0.20	2.50
	E	6	0.00	0.00	-
	M	7	3.32	5.95	1.79
II	A	7	0.43	0.93	2.16
	E	7	1.68	2.99	1.78
	M	6	0.67	1.17	1.75
III	A	6	0.83	1.13	1.36
	E	6	0.42	0.49	1.17
	M	7	0.11	0.14	1.27
IV	A	7	0.00	0.00	-
	E	7	0.14	0.37	2.47
	M	6	2.58	4.22	1.64
V	A	6	1.92	3.29	1.71
	E	6	7.00	14.74	2.10
FECAL COLIFORMS - LOWER SITE					
	M	6	178.75	224.36	1.25
I	A	6	46.58	42.22	0.91
	E	6	185.42	96.69	0.52
	M	7	76.89	38.88	0.51
II	A	7	61.93	17.17	0.28
	E	7	120.50	63.59	0.53
	M	6	61.93	77.05	1.25
III	A	6	43.83	38.46	0.88
	E	6	95.25	116.94	1.23
	M	7	12.93	13.18	1.02
IV	A	7	45.79	33.21	0.72
	E	7	26.32	38.86	1.48
	M	6	261.42	160.09	0.61
V	A	6	137.00	106.59	0.78
	E	6	247.17	126.89	0.51

Figure 7 by noting the location of the respective weeks on the graphs.

Week II has the lowest coefficient of variation in five out of six of the groups in Figure 7. The relatively stable bacteria concentrations of Week II are possibly associated with the stable stream stage and temperature patterns for the week (see Figs. 10-11, Chapter IV). Weeks I, III, and V all contain storms and generally display more erratic stage readings. Week IV is consistent in stage values, similar to Week II, but shows higher coefficients of variation. Because Week IV has a lower mean value than Week II in five out of the six cases, it is reasonable that Week IV would have higher CV values, in line with other evidence that higher coefficients of variation accompany lower concentrations.

Yearly Variation

Some indication of the amount of variation from year to year is given by comparing Weeks I and V in Figure 7. In 4/6 of the graphs Weeks I and V are similar in variability as expressed by the coefficients of variation. In only 1/6 of the graphs are the two weeks noticeably dissimilar. This suggests that hydrologically similar weeks in two years may exhibit about the same amount of variability.

Chapter IV

FLUCTUATIONS IN BACTERIA CONCENTRATIONS

Bacteria concentrations fluctuate cyclically on a daily basis according to results of the study. These cycles are of vital importance in both sampling design and data analysis. For example, in analyzing data, are two sites in question really different in bacterial content, or is the apparent difference a result of the routine sampling schedule's relation to the daily cycle of bacteria counts? Seasonal trends likewise must be considered in both the planning and analysis stages of research. This chapter describes the fluctuations observed in the mountain stream of the study.

Daily Cycle

Graphical Plots

The daily fluctuations of all three organisms are presented in Figures 8-17. Bacteria counts for Weeks I-V for many days are higher at 2100 hours (the evening sample) than at 1300 hours (the afternoon sample). Figure 15, for example, demonstrates clearly an evening versus afternoon contrast for the coliform bacteria, evening values being higher than afternoon values for all seven days of Week IV, lower site. This cycle is common for all weeks, bacteria groups, and sites.

Data for all bacteria groups were replotted as shown in Figures 18 and 19; only the coliforms from this set of graphs are presented, because the daily cycles for the other two bacteria groups are very similar. Figures 18 and 19 illustrate that within a single day, evening counts are often higher than concentrations for morning or afternoon.

Analysis of all the individual days in Weeks I-V, as plotted in Figures 8-17, produces the tabulation of Table 6. In the table, each time is rated as to the percent of individual days when the particular time (morning, afternoon, or evening) contains the daily maximum. No "storm days" are included. Bacteria counts for all groups are highest at the evening sampling time for the majority of the individual days. The Table 6 tabulation of maximum values for individual days does not describe the magnitude of differences between times of the day, rather merely enumerates the times of maximum values. Statistical and graphical analyses based on means in the following sections evaluate the extent of the differences between time of the day and point out that morning values are greater than afternoon values, and not as similar as Table 6 indicates.

Table 6. The percentage of days during Weeks I-V when a particular time of the day (morning, afternoon, or evening) shows the maximum value in bacteria counts. Breakdown is by bacteria group and site.

	Morning %	Afternoon %	Evening %
	Coliforms		
Upper	6.4	9.6	83.8
Lower	16.1	22.5	61.2
	FS		
Upper	24.1	3.4	72.4
Lower	28.1	18.8	53.1
	FC		
Upper	(not tabulated because of zero counts)		
Lower	24.2	21.2	54.6

Statistical Tests

To test for a daily cycle, analyses of variance were calculated for morning-afternoon, afternoon-evening, and morning-evening comparisons, using all data of Weeks I-V, analyzing separately by sites and weeks. No storm days were included in the analyses. Tables 7, 8, and 9 give results of the tests. The evening-afternoon contrasts are the greatest, being significant in 23/30 of the comparisons (Table 9), evening being largest (note that many of the differences are significant at the one percent level). The evening-morning differences are significant in 17/30 of the tests, evening also being higher than morning. Finally, the morning-afternoon contrasts show significant differences in only 8/30 of the cases, morning counts being greater. In summary, the statistical analyses of variance for the three times of the day show:

evening >> afternoon
evening > morning
morning > afternoon

Summary Graphs

All of the time of day comparisons of Weeks I-V are presented in capsule form by the bar graphs of Figure 20. The daily cycle is especially well-defined in all weeks of data, with an evening versus afternoon contrast present in 27 of the 30, or 90 percent of the bar graphs (5 weeks x 2 sites x 3 bacteria = 30 graphs). This 90 percent value is based on 840 observations of each bacteria group or a total of 2520 individual observations. The FC bacteria at the upper site make up two of the three exceptions to the trend for evening-afternoon differences. FC upper site counts are by far the most variable, the isolations containing many zero counts; therefore, two-thirds of the exceptions are in the group having results on which the least confidence may be placed.

Also apparent in the summary graphs is the morning-afternoon drops in counts. The summary graphs emphasize a daily cycle in agreement with the statistical findings, namely:

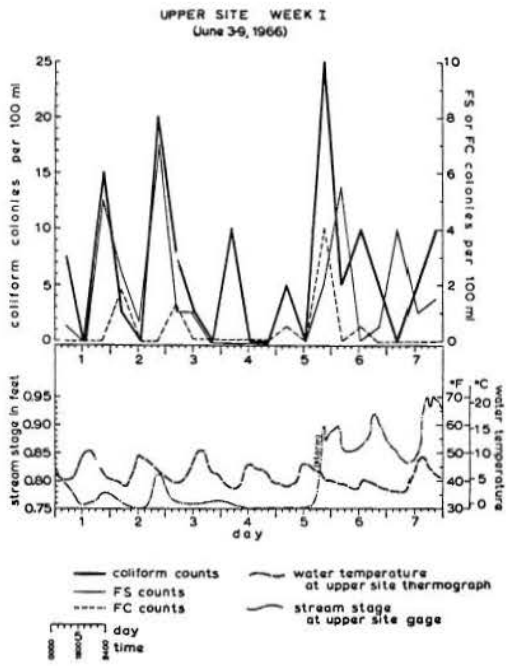


Figure 8-- Coliform, fecal coliform (FC), and fecal streptococcus (FS) bacteria counts for morning (0500 hours), afternoon (1300 hours), and evening (2100 hours), with corresponding stream stage and temperature.

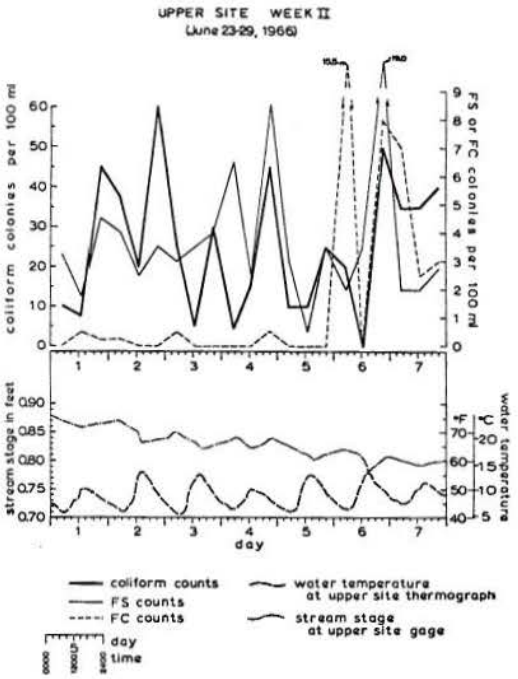


Figure 10-- Coliform, fecal coliform (FC), and fecal streptococcus (FS) bacteria counts for morning (0500 hours), afternoon (1300 hours), and evening (2100 hours), with corresponding stream stage and temperature.

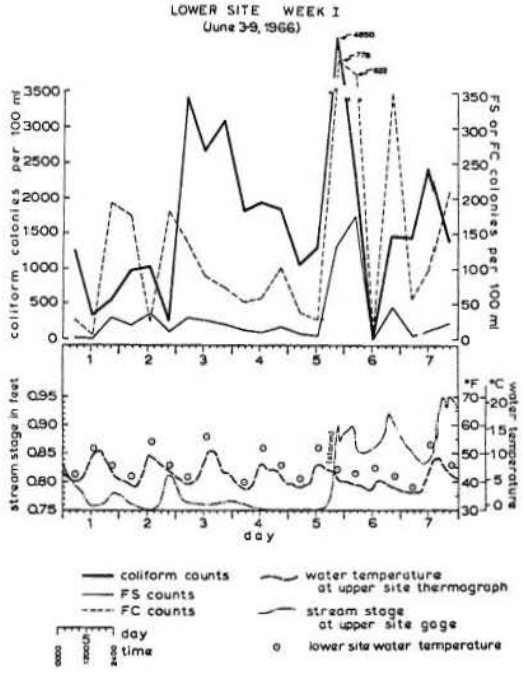


Figure 9-- Coliform, fecal coliform (FC), and fecal streptococcus (FS) bacteria counts for morning (0500 hours), afternoon (1300 hours), and evening (2100 hours), with corresponding stream stage and temperature.

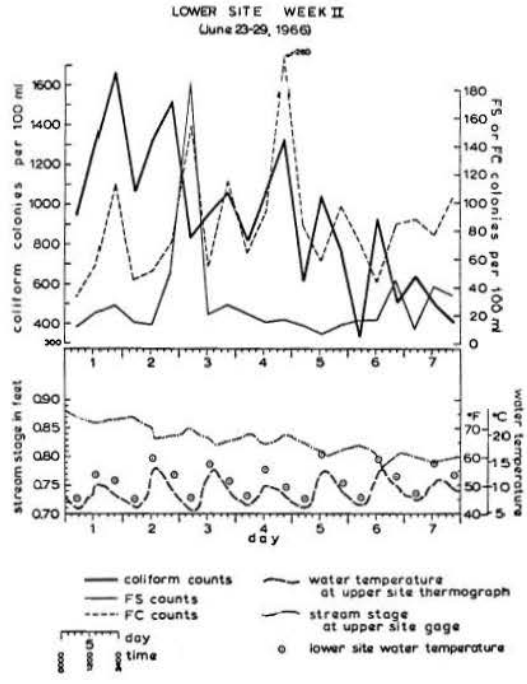


Figure 11-- Coliform, fecal coliform (FC), and fecal streptococcus (FS) bacteria counts for morning (0500 hours), afternoon (1300 hours), and evening (2100 hours), with corresponding stream stage and temperature.

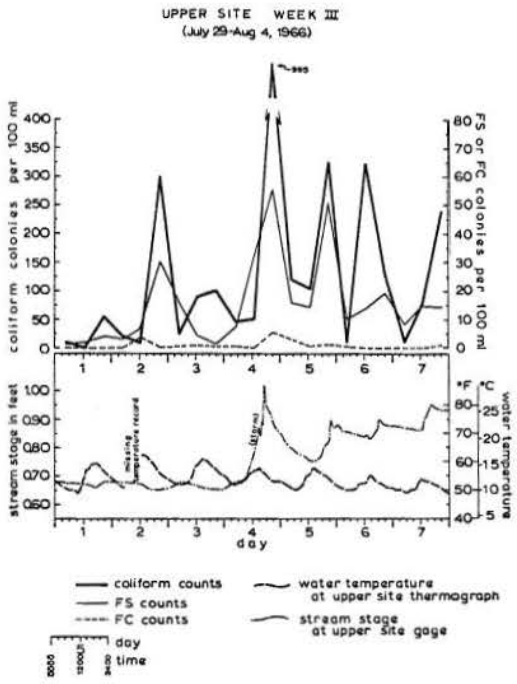


Figure 12-- Coliform, fecal coliform (FC), and fecal streptococcus (FS) bacteria counts for morning (0500 hours), afternoon (1300 hours), and evening (2100 hours), with corresponding stream stage and temperature.

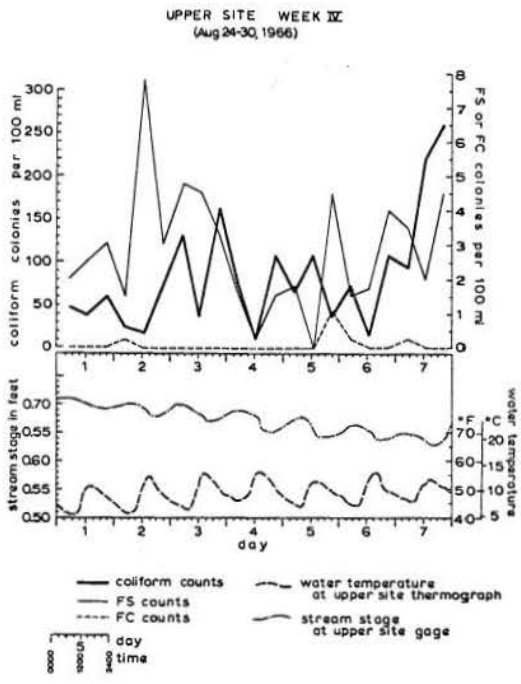


Figure 14-- Coliform, fecal coliform (FC), and fecal streptococcus (FS) bacteria counts for morning (0500 hours), afternoon (1300 hours), and evening (2100 hours), with corresponding stream stage and temperature.

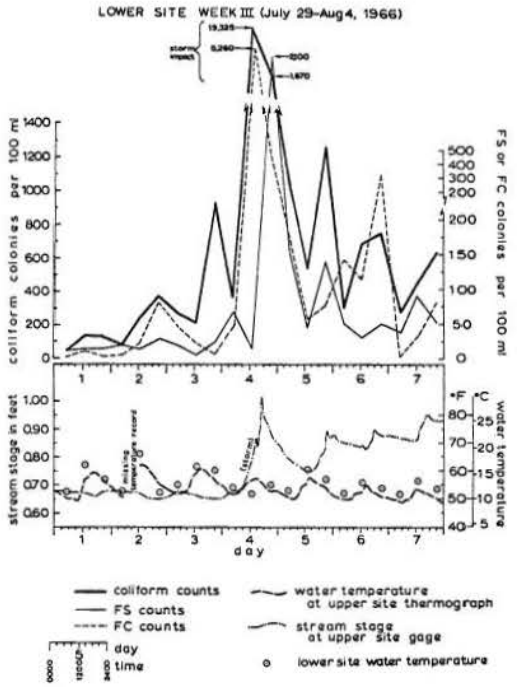


Figure 13-- Coliform, fecal coliform (FC), and fecal streptococcus (FS) bacteria counts for morning (0500 hours), afternoon (1300 hours), and evening (2100 hours), with corresponding stream stage and temperature.

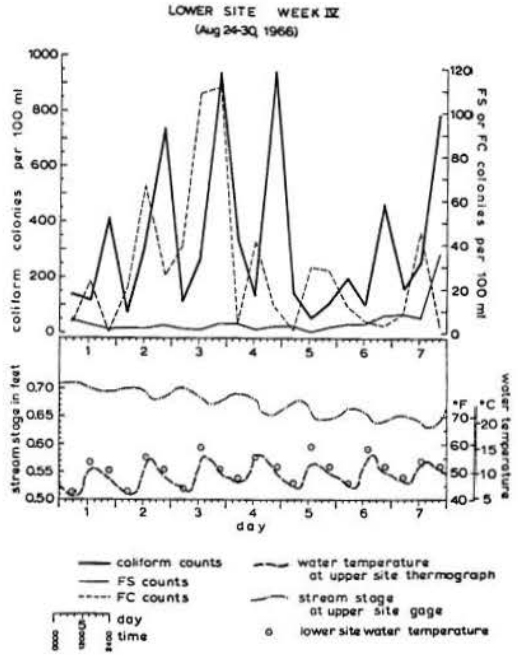


Figure 15-- Coliform, fecal coliform (FC), and fecal streptococcus (FS) bacteria counts for morning (0500 hours), afternoon (1300 hours), and evening (2100 hours), with corresponding stream stage and temperature.

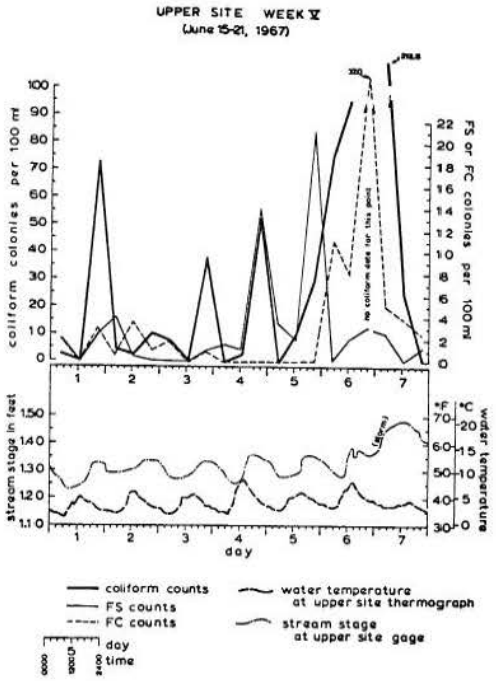


Figure 16-- Coliform, fecal coliform (FC), and fecal streptococcus (FS) bacteria counts for morning (0500 hours), afternoon (1300 hours), and evening (2100 hours), with corresponding stream stage and temperature.

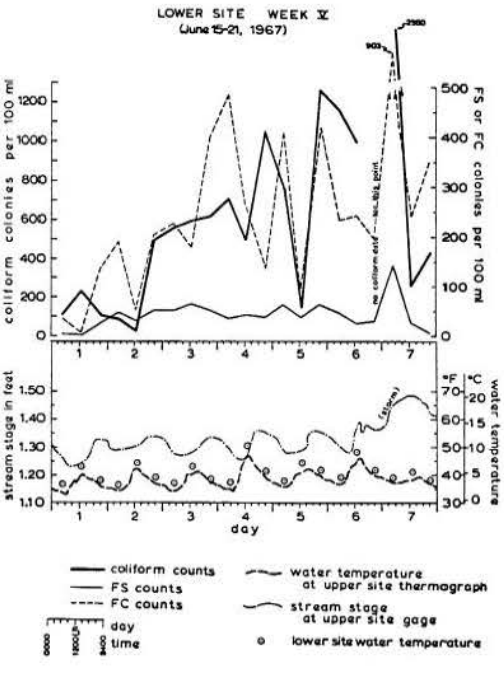


Figure 17-- Coliform, fecal coliform (FC), and fecal streptococcus (FS) bacteria counts for morning (0500 hours), afternoon (1300 hours), and evening (2100 hours), with corresponding stream stage and temperature.

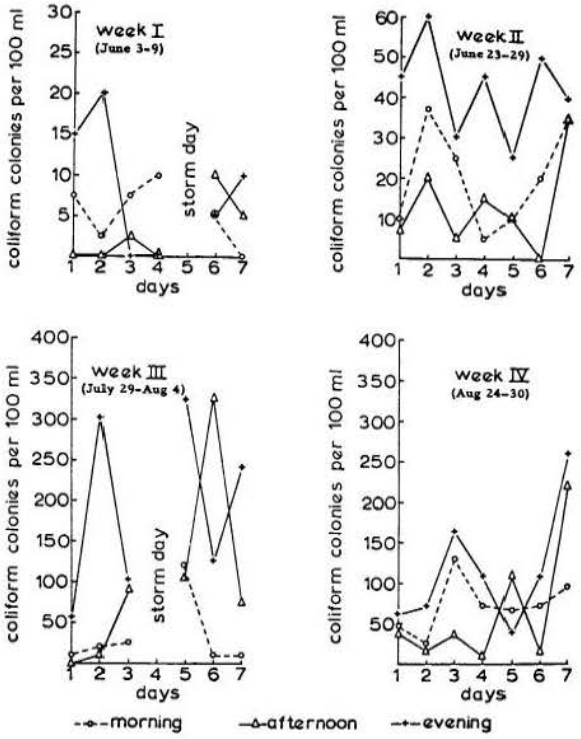


Figure 18-- Morning (0500 hours), afternoon (1300 hours), and evening (2100 hours) coliform concentrations in 1966 at the upper site.

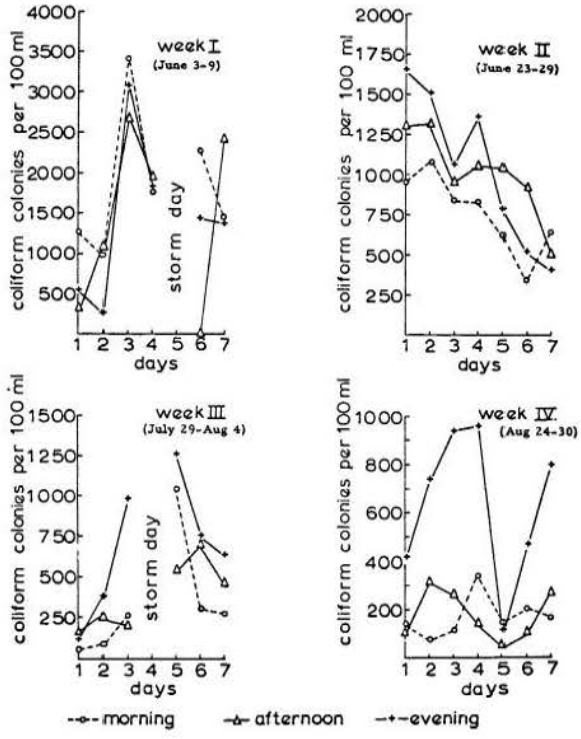


Figure 19-- Morning (0500 hours), afternoon (1300 hours), and evening (2100 hours) coliform concentrations in 1966 at the lower site.

Table 7-- Analysis of variance results for morning (0500 hours) vs afternoon (1300 hours) values for Weeks I-V, showing means, F-values, degrees of freedom, and significance.

MORNING vs AFTERNOON

		UPPER SITE					LOWER SITE				
		Means					Means				
Bacteria	Week	(Morn.)	(Aft.)	F	df	%	(Morn.)	(Aft.)	F	df	%
	I	5.42	2.92	1.42	35	NS[1/]	1865.83	1403.75	1.96	35	NS
	II	20.36	13.21	3.43	41	NS	757.86	1016.07	2.72	41	NS
COLIFORMS	III	32.50	100.83	17.51	35	1	335.00	378.33	0.99	35	NS
	IV	72.50	63.57	0.32	41	NS	168.21	178.57	0.17	41	NS
	V	14.92	18.33	0.05	35	NS	565.00	417.08	13.07	35	1
	I	2.25	0.42	9.31	35	1	41.42	14.92	150.03	35	1
	II	3.39	2.32	3.52	41	NS	38.89	19.32	69.20	41	1
FS	III	8.50	9.42	0.40	35	NS	33.83	33.33	0.02	35	NS
	IV	2.39	2.68	0.46	41	NS	4.18	3.00	2.20	41	NS
	V	1.83	0.92	2.57	35	NS	40.67	34.17	2.53	35	NS
	I	0.50	0.08	2.46	35	NS	178.75	46.58	269.51	35	1
	II	3.32	0.43	25.81	41	1	76.89	61.93	8.98	41	1
FC	III	0.67	0.83	0.12	35	NS	61.42	43.83	2.37	35	NS
	IV	0.11	0.00	3.03	41	NS	12.93	45.79	56.82	41	1
	V	2.58	1.92	0.13	35	NS	261.42	137.00	34.21	35	1

1/ NS = level of significance greater than 5%

Table 8-- Analysis of variance results for morning (0500 hours) vs evening (2100 hours) values for Weeks I-V, showing means, F-values, degrees of freedom, and significance.

MORNING vs EVENING

		UPPER SITE					LOWER SITE				
		Means					Means				
Bacteria	Week	(Morn.)	(Eve.)	F	df	%	(Morn.)	(Eve.)	F	df	%
	I	5.42	8.33	1.26	35	NS[1/]	1865.83	1431.25	1.18	35	NS
	II	20.36	42.14	15.61	41	1	757.86	1038.21	4.16	41	5
COLIFORMS	III	32.50	190.83	40.41	35	1	335.00	680.83	25.73	35	1
	IV	72.50	115.00	7.18	41	5	168.21	627.86	63.72	41	1
	V	2.90	40.50	30.24	29	1	445.00	704.10	26.17	29	1
	I	2.25	3.50	0.81	35	NS	41.42	25.63	34.49	35	1
	II	3.39	6.54	16.67	41	1	38.89	30.68	3.90	41	NS
FS	III	8.50	20.00	23.53	35	1	33.83	51.00	6.17	35	5
	IV	2.39	3.39	4.11	41	5	4.18	8.39	12.58	41	1
	V	1.83	6.92	45.53	35	1	40.67	43.83	0.60	35	NS
	I	0.50	0.00	3.93	35	NS	178.75	185.42	0.32	35	NS
	II	3.32	1.68	6.54	41	5	76.89	120.50	72.21	41	1
FC	III	0.67	0.42	0.73	35	NS	61.42	95.25	4.41	35	5
	IV	0.11	0.14	0.12	41	NS	12.93	26.32	9.13	41	1
	V	2.58	7.00	0.55	35	NS	261.42	247.17	0.24	35	NS

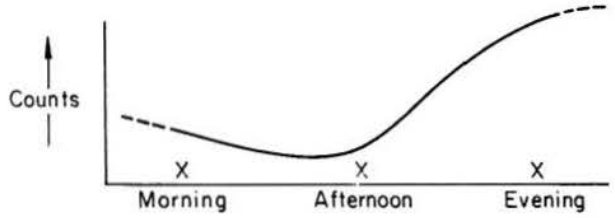
1/ NS = level of significance greater than 5%

Table 9-- Analysis of variance results for afternoon (1300 hours) vs evening (2100 hours) values for Weeks I-V, showing means, F-values, degrees of freedom, and significance.

AFTERNOON vs EVENING

		UPPER SITE					LOWER SITE				
		Means					Means				
Bacteria	Week	(Aft.)	(Eve.)	F	df	%	(Aft.)	(Eve.)	F	df	%
	I	2.92	8.33	4.92	35	5	1403.75	1431.25	0.01	35	NS[1/]
	II	13.21	42.14	28.82	41	1	1016.07	1038.21	0.02	41	NS
COLIFORMS	III	100.83	190.83	11.47	35	1	378.33	680.83	16.25	35	1
	IV	63.57	115.00	9.23	41	1	178.57	627.86	57.95	41	1
	V	3.00	40.50	30.63	29	1	300.50	704.10	91.89	29	1
	I	0.42	3.50	5.78	35	5	14.92	25.63	20.99	35	1
	II	2.32	6.54	30.67	41	1	19.32	30.68	8.45	41	1
FS	III	9.42	20.00	16.88	35	1	33.33	51.00	7.39	35	1
	IV	2.68	3.39	1.73	41	NS	3.00	8.39	18.63	41	1
	V	0.92	6.92	85.46	35	1	34.17	43.83	6.63	35	5
	I	0.08	0.00	1.00	35	NS	46.58	185.42	128.96	35	1
	II	0.43	1.68	15.38	41	1	61.93	120.50	125.93	41	1
FC	III	0.83	0.42	0.71	35	NS	43.83	95.25	13.21	35	1
	IV	0.00	0.14	3.01	41	NS	45.78	26.32	11.65	41	1
	V	1.92	7.00	0.66	35	NS	137.00	247.17	16.97	35	1

1/ NS = level of significance greater than 5%



Physical Factors and the Daily Cycle

The daily cycle of bacteria counts in the stream is very likely associated with the physical factors of the stream environment, for example, stream stage, water temperature, and incoming solar radiation. In the preceding section, the observed daily cycle was described. This section discusses possible reasons for the daily cycle in terms of the observed physical factors, in line with the secondary objectives of the study defined in Chapter I. The relationships considered here are necessarily simplified, with no attempt being made to account for all possible causative factors of the daily cycle. For convenience of discussion, stream stage, for example, is discussed under a separate subheading from incoming solar radiation, water temperature, or other factors. These physical factors are probably interrelated in a complex fashion and in reality the influence of a single factor on bacteria counts cannot easily be isolated, if at all. The biological cycles of micro-organisms other than bacteria in the stream, daily migratory activities of wildlife about the stream, and numerous unknown factors are also probably related to the daily cycle of bacteria in the stream.

Stream Stage

In Figures 8-17, many of the weeks demonstrate an afternoon to evening stream stage rise. In a stream fed by melting snow from the "high country", daily peak runoff at lower sites lags behind the peak melt period at the higher elevations. Streambank "flushing" by rising stages of early evening would be one possible explanation for the observed evening bacteria count increase. The phenomenon of streambank "flushing" was also observed during the 1964-65 investigations on the same watershed on both a seasonal basis and during storms (Kunkle and Meiman, 1967). The concept of

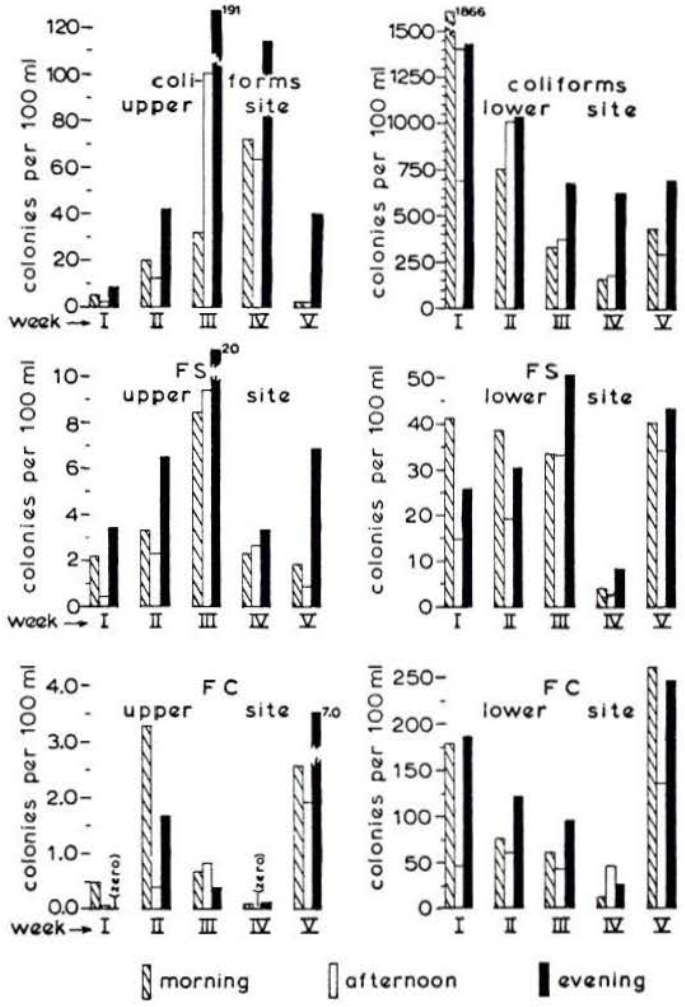


Figure 20. Morning (0500 hours), afternoon (1300 hours), and evening (2100 hours) bacteria concentrations for the three indicator groups for Weeks I-V, 1966-67, by grand means for the weeks.

streambank "flushing" is an oversimplified hypothesis, since much more is probably involved than the simple physical process of washing bacteria into the stream. Perhaps nutrients being washed into the stream are quite important. Cycles of micro-organisms other than bacteria may be involved. It has yet to be shown that a definite cause-and-effect relationship exists between stream stage rise and bacteria count rise, however the relationship is strong.

The 3-day and 4-day studies, discussed in Chapter III, were designed (among other things) to further describe the stage-bacteria relation, however the two studies did not exemplify the relationship, possibly because of the particular days selected. The 4-day study displays extremely stable, even "atypical" stage patterns (Fig. 21). The 3-day study shows more typical afternoon-evening stage rises, however does not clearly indicate an evening bacteria rise (Fig. 22). The tremendous degree of variation evident in the graphs of the two special studies could very possibly mask relationships. Further evidence of the bacteria-stage relationship is presented in the section on storm sampling.

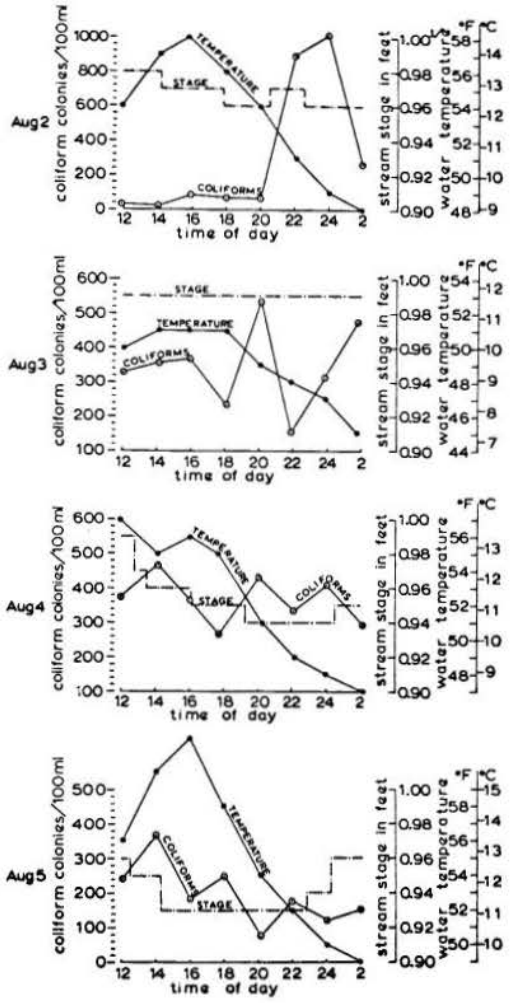


Figure 21. Four-day study of August 2-5, 1967

Insolation

A two-day investigation of incoming solar radiation (insolation) impact on bacteria was carried out,

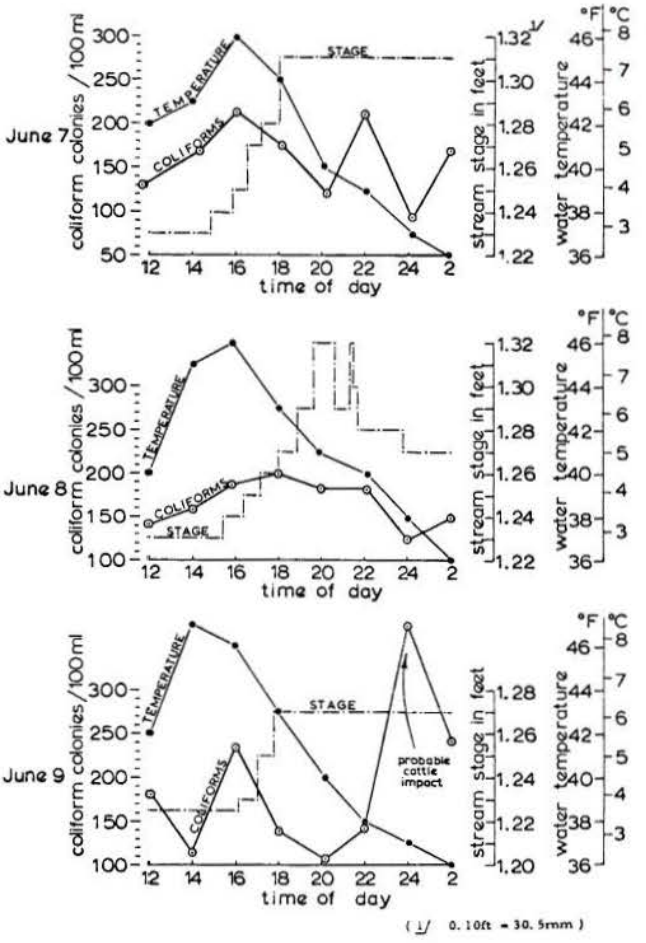


Figure 22. Three-day study of June 7-9, 1967

as detailed in Chapter II. Six open containers were exposed to sunlight, six were shaded. All vessels were standing partially immersed in the running stream so that temperatures in the containers remained close to the stream temperature (within 1°C). Hydrogen ion activity (pH) showed a slight drop in all receptacles as the study progressed, but pH change for all twelve containers was identical.

Results of the study appear in Figure 23. The first point plotted in both graphs is the control, i.e., concentrations found in the containers immediately after filling from the stream and just prior to lowering the shade over six of the vessels. Coliform counts in the sun-exposed pails dropped drastically below levels found in the shaded containers in both days of testing. All bacteria apparently were killed in a three to four hour period, while one hour of exposure caused extreme bacteria die-off. Standard deviations for the six values which make up the individual points on the graph appear in Table 3. Values for the "shaded" samples only are given since the samples exposed to the sun were in most cases zero or very low.

The sterilization potential of ultraviolet radiation is a well-known phenomenon, sometimes applied as a water purification technique (Klein, 1962). The bacteria evening-afternoon cycle in concentrations could reasonably be related to sunlight, with die-off of organisms proceeding at a higher rate in the afternoon periods.

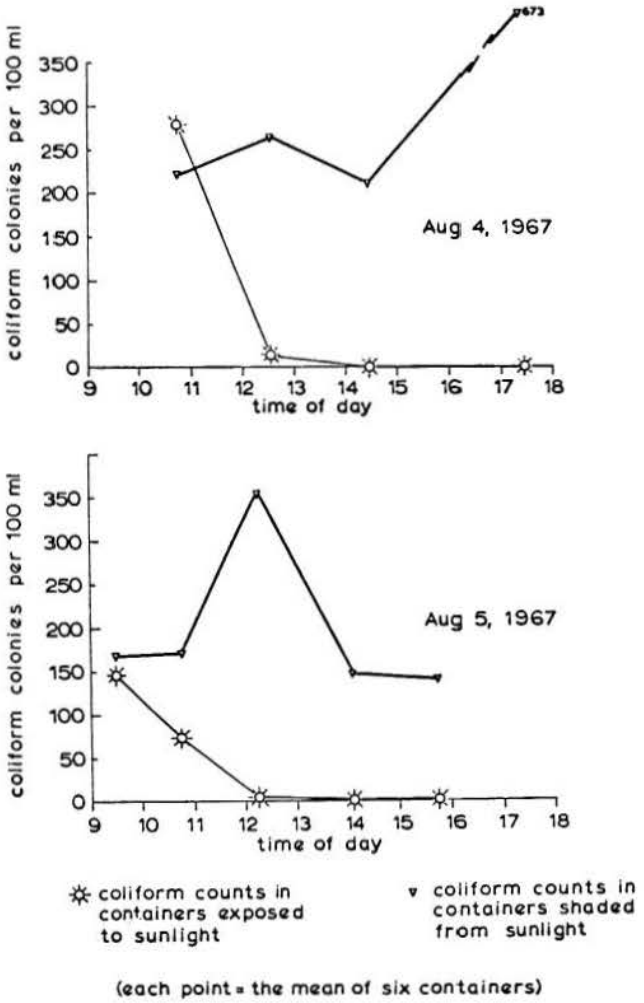


Figure 23. Insolation study, comparing coliform counts of sun-exposed containers to shaded ones

This radiation effect could very possibly work in conjunction with the "flushing" process discussed in the preceding section, since stages commonly rise at about the time insolation decreases.

Obviously the small tubs do not imitate the stream environment with much sophistication, however, the drastic sunlight impact on bacteria counts is emphasized.

Water Temperature

Bacteria concentrations in a stream are probably associated to some extent with water temperature. Since peak water temperatures are usually attained at approximately the same time as the "afternoon" minimum bacteria concentrations (Figs. 8-17), one might speculate that water temperature rises increase organism die-off. The "insolation study" (preceding section) would imply, however, that if such a temperature influence exists, it is minor compared to the radiation effect; water temperatures remained identical in both exposed and shaded containers in the insolation study, yet there was a drastic die-off in the sun-exposed containers. In regard to warm water increasing die-off, it should be noted that the "warmest" time of the day in the study stream is still very cold (Figs. 8-17), daily maximum water temperatures attaining only about 8-14°C and often less than 10°C.

On the other hand, water temperature is related to the reproduction rate of the organisms, so that in a cold stream, "warmer" temperatures could possibly raise bacteria counts by increasing multiplication. The high coliform concentration in the last sample of the August 4 insolation study (Fig. 23), for example, could possibly be due to bacteria reproduction, since no new coliforms were injected during the observation period. Potter (1963) observed bacteria populations to multiply in stored water samples.

Burman (1961) describes temperature effect on stream bacteria and the optimum temperatures for organism concentrations in relation to reproduction and die-off rates. Greenberg (1964) found a strong correlation between plankton and temperature in the upper reaches of the Sacramento River.

In streams of mostly snowmelt origin, warming air temperatures normally result in both rising water temperatures and rising stream stages, making separation of the effects of the two physical factors difficult. Effects of insolation and water temperatures on bacteria are also difficult to separate. Probably bacteria concentrations of the stream are related to water temperatures on a daily basis, however, the relationship is not easily defined.

Water temperature is considered again in the section on seasonal trends.

Storm Sampling

Early in the morning of June 21, 1967, a rain storm began. The first rise of stream stage occurred at approximately 3:00 a.m. Sampling for coliform, FS, and FC groups was underway at 0530 hours, and frequent samples, 14 of them, were collected during the morning to early afternoon period. Results of these samples are presented in Figure 24. The initial burst of run-off was not sampled, according to the stage information (at the bottom of the graph), however the graphs imply that peak bacteria concentrations of the storm were sampled, since counts on either side of approximately 0545-0600 hours are lower.

The rain persisted throughout the morning, with the stage remaining high until early afternoon. Although the stage retained a high level, bacteria concentrations began to decline. Geldreich (1966) observed the same pattern of reducing bacteria counts after rainfall continues for a period of time.

Coliform counts rose to six times the pre-storm value, FC to four times, and FS to about eight times. The individual days of storms shown in Figures 8-17 (Day 5, Week I; Day 4, Week III; Day 7, Week V) exhibit similar upward bursts of bacteria counts related to storm runoff. The lower site of Week III (Fig. 13) shows the most extreme storm impact.

One phenomenon was observed at the lower site, Week I (Fig. 6). Bacteria counts following the storm of Day 5 did not return to pre-storm values after the storm period, but dropped to zero, as if "flushing" had momentarily rid the stream of bacteria.

All observations of storms support the hypothesis that "flushing" by rising stages increases bacteria counts.

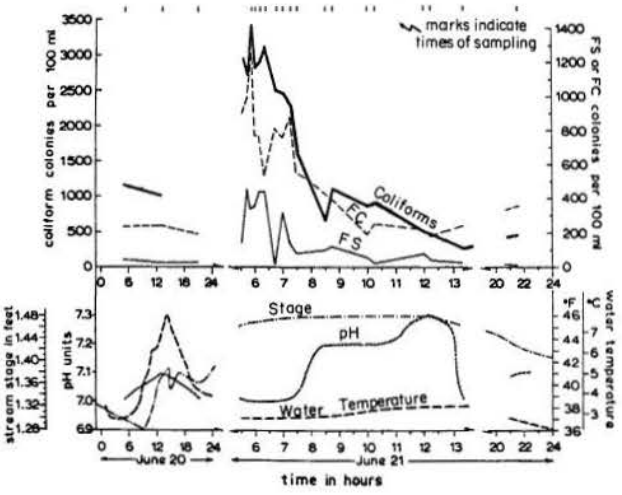


Figure 24. Special storm sampling, showing coliform FC, and FS bacteria counts with corresponding values of stream stage, pH, and temperature.

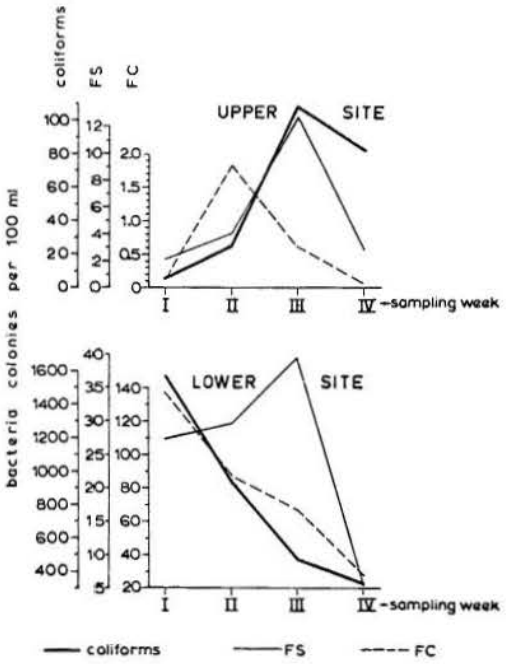


Figure 25. Seasonal trends of bacteria counts for the three indicator groups in 1966, at the upper and lower sampling sites. Each point represents the grand mean of all samples taken during the week.

Seasonal Trends

In Figure 25, there is indication of a seasonal trend for the bacteria groups:

1. The coliform and FC attain maximum values for the season in the "flushing" period of Week I for the lower, cattle and irrigation influenced site, while maximum values for the season at the upper site occur during the post-flush, warmer water period of Weeks II-III.

2. The FS exhibit a cycle at both sites similar to the upper site trend for the other two bacteria groups. Maximums are reached during Week III.

The general similarity of the FS bacteria at both sites to the upper site trend for the other two groups may be related to the selectivity of the groups in question. Since the FS demonstrate much less contrast in bacteria counts between upper and lower sites than the other two groups (discussed in Chapter V), it is reasonable that the FS might differ in seasonal trend from the coliforms and FC. The difference in FS from the other groups will be discussed further in Chapter V.

It is notable that two of the three bacteria groups at the upper site show maximums during Week III. Referring to Figures 8-17, Week III is also seen to be the week of maximum temperatures for the year. There is possibly an increase in bacteria concentrations in the upper site as water temperatures rise and allow increased reproduction of the organisms. The tremendous "flushing" of cattle manure, soil particles and other surface matter in the irrigated meadow, especially in Weeks I-II, would probably overshadow any bacteria-water temperature relations for the lower site. The FS, the bacteria group least sensitive to the cattle pollution, show maximums in Week II for the lower site.

Chapter V

BACTERIAL INDICES OF POLLUTION

One reason for selection of the study area was the extremely distinct and measurable pollution impact. From a land management point of view, it is desirable to evaluate the relative merits of the three groups--the coliforms, fecal coliforms, and fecal streptococci, in regard to their ability to measure the grazing-irrigation impact throughout the season.

Contrast Between Upper and Lower Sites

Range of Contrasts

The pollution impact at the lower sampling station is extremely obvious. At any time of the day, any week of the season, for either year, or for any bacteria group, bacteria counts at the lower site exceeded those of the upper site (Fig. 25 and Figs. 8-17).

Table 10 presents contrasts between sites in terms of a "lower site to upper site ratio," L/U ratio, which is simply the number of times greater lower site concentrations are than those at the upper site. For Weeks I-V, all bacteria groups show a L/U ratio range of:

Group	Range
FC	47.8 to 720.6
Coliforms	3.9 to 281.8
FS	1.8 to 13.3

The mean and median values of these ranges are:

Group	Mean	Median
FC	384.2	336.4
Coliforms	142.9	139.0
FS	7.6	5.7

The FS show a far lower difference between the two sites than the other two indicator groups. The coliforms and FC are more similar in their response to the cattle impact. The FC was the most "sensitive" group to cattle pollution under conditions of the study; they retained the greatest L/U ratio on a consistent basis, never displaying a ratio of less than 47.8.

The FC and FS

As noted in preceding sections, the FC counts are lower than FS counts at the upper, "natural" site, while at the lower site the reverse is true. This is shown clearly for the individual weeks in the comparison of upper and lower sites in Figures 8-17, where FS and FC concentrations are graphed on the same scale. The pattern implies that the FC are much more "sensitive" to the cattle impact than the FS.

Seasonal Trend and Pollution Detection

Week I of the four weeks in the 1966 season is distinctly the period showing the greatest L/U ratio or site contrast for all three bacterial indicator groups. It is assumed that the initial spring "flushing" would be closely related to the strong upper-to-lower site difference of Week I. Week V, 1967, and

Table 10. Contrasts between sampling sites by "lower site to upper site ratio," L/U ratio. The ratio is the number of times greater lower site concentrations are than those at the upper site. Means are derived from all observations during a week excluding "storm days".

	BACTERIA GROUP								
	COLIFORM			FS			FC		
Week	Upper	Lower	L/U	Upper	Lower	L/U	Upper	Lower	L/U
I	5.56	1566.94	281.8	2.06	27.32	13.3	0.19	136.92	720.6
II	25.24	937.38	37.1	4.08	29.63	7.3	1.81	86.44	47.8
III	108.06	464.72	4.3	12.64	39.39	3.1	0.64	66.83	104.4
IV	83.69	324.88	3.9	2.82	5.19	1.8	0.08	28.35	354.4
V	15.47	483.20	31.23	3.53	40.60	11.5	0.87	213.10	244.9

Week I, 1966, which are similar in location on the hydrograph are also similar in that both have relatively high L/U ratios. The years 1966 and 1967 were not very similar in flow as noted in Chapter II, therefore some differences in counts would be expected.

During all weeks of both seasons (Weeks I-IV, 1966 and Week V, 1967), the L/U ratios for the FC are clearly higher than L/U ratios for the other two bacteria groups. This is in agreement with findings in the earlier studies of 1964-65, where the FC group defined grazing impact much better than the FS and slightly better than the coliforms (Kunkle and Meiman, 1967).

"FC to FS Ratio"

Geldreich et al., (1964) describe development of an FC/FS ratio method used in a bacteriological study of a waste stabilization pond, whereby it is possible to distinguish human from farm animal contamination. Geldreich (1966) applied the same index in distinguishing human from domestic animal and wildlife contamination in storm runoff from urban areas. The ratio in the case of livestock and wildlife was < 0.6, while human contamination displayed ratios of 4.4.

Applying the FC and FS values from Table 10, the FC/FS ratios in all weeks would be less than 1.0 at the upper "clean" site, where the very slight contamination is probably the wildlife or soil origin, in agreement with Geldreich's findings.

Week	FC/FS Ratios at the Upper Site	
I	0.19/ 2.06	0.09
II	1.81/ 4.08	0.44
III	0.64/12.64	0.05
IV	0.08/ 2.82	0.03
V	0.87/ 3.53	0.25

At the lower site, however, where the contamination is almost without doubt a result of the grazing-irrigation impact, the ratio becomes 4.4 or greater in 3/5 of the weeks, while in every week the ratio is much greater than 0.6.

Week	FC/FS Ratios at the Lower Site	
I	136.92/27.32	5.01
II	86.44/29.63	2.92
III	66.83/39.39	1.70
IV	28.35/ 5.19	5.46
V	213.10/40.60	5.25

Communications[1] with the Taft Center at Cincinnati indicate they use a KF-Streptococcus Agar for their FS determinations whereas this study utilizes the m-Enterococcus Agar to isolate the FS bacteria. According to the Taft Center, their medium is more sensitive to Streptococcus bovis. This could account for the apparent contradiction of results.

Although the L/U ratios of Week I, 1966 and Week V, 1967--the two "flushing" weeks--are not very similar, it is very notable that the FC/FS ratios are nearly identical. The highest FC/FS ratio is only 5.46. In view of the large variation in counts, it is worth noting how similar the FC/FS ratios are in Weeks I, IV, and V, namely the two "flushing" weeks and the week of lowest flows or least dilutions. Perhaps a modified FC/FS ratio can be developed that would be applicable under cold, mountain stream conditions.

[1] Personal communication with B. A. Kenner

Chapter VI

SAMPLING RECOMMENDATIONS

The most useful data for sampling design is the summation of the variance components presented in Tables A-C of the Appendix. An investigator may wish to use the variance values of the table in conjunction with an appropriate variance formula[1] to attain general guideposts whereby he may best invest his samples and replicates.

Recommendations are developed from much of the information in Chapters II and III regarding bacteria variations for organisms, sites, weeks, and times.

The following paragraphs attempt to highlight some of the findings of the study pertinent to sampling design for streams similar to that of the study.

1. An analytical error represented by a coefficient of variation of from 0.4 to 0.6 would be an average "working" approximation for field laboratory measurements of coliform bacteria, assuming of course, reasonable care and experience on the part of the technician; occasionally the CV may reach as low as 0.15 and as high as 1.65. There is a general decrease in the CV of replicates within a sample as the mean of the population sampled increases, i.e., the error in analytical technique becomes a more important portion of the total variance as low-concentration bacteria samples are analyzed. When concentrations of organisms are low, a sampler may wish to invest relatively more effort in replicating technique as opposed to taking more samples from day-to-day and within days.

2. There appears to be very little benefit from taking more than one bottle of water at a given sampling time; it is better to invest the sampling effort over time and in technique.

3. Afternoon lows in bacteria concentrations followed by relatively high evening concentrations represent the cycle common to all bacteria groups and both sites. Highest coefficients of variation are associated with the afternoon lows; evenings show lowest CV values at the grazing-irrigation polluted sites, while mornings are lowest at natural sites.

4. When sampling a network of stations, it is normally impossible to sample all sites at the same time, therefore enough sampling days should be included to enable varying the time of sampling during the day for each site. The sampler should be aware that the daily bacteria cycle will likely introduce bias into the data if a routine sampling route is consistently used for a network of sites.

5. Land use impact on stream water quality appears to be most drastic during periods of stream rises ("flushing") associated with snowmelt runoff and storm rises in the hydrograph. Sampling at these times gives the best chance of detecting land use impacts.

6. Although bacteria counts are exceptionally high during the rising limb of a storm hydrograph and during spring rises in flow, bacteria concentrations during the receding limbs of the same hydrographs may be exceptionally low, even lower than the pre-storm values.

7. The fecal coliform bacteria group appears to be the best indicator for detecting the combined grazing-irrigation impact. The coliform group is somewhat less sensitive to such pollution, while the fecal streptococci are far less capable of indicating the impact.

8. The coliforms generally show the lowest coefficients of variation of the three bacteria groups at both natural and impacted sites, primarily because coliform concentrations from a sample are nearly always higher than counts of the other two groups. The coliforms especially exhibit lower coefficients of variation than either the FS or FC at the upper site where many zero counts of FS and FC occur.

9. Any sampling scheme will, of course, depend on objectives and resources available, however seasonal and daily trends along with the magnitude of the analytical error should be carefully considered.

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[1] For example optimum allocation of samples is discussed in Cochran, W. G., 1963, Sampling Techniques. Wiley & Sons, pp. 270-290.

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APPENDIX

Table A-- Partitioning of variance into components of day, time, day-time interaction, bottle, and analytical technique (pipette) for the coliform bacteria. Number of days used in the analysis for individual weeks is shown under "n".

COLIFORM BACTERIA
COMPONENTS OF VARIANCE

Site	Week	n	Day	Time	Day x Time Interaction	Bottle	Analytical Technique
	I	6	0.00	4.34	24.38	2.78	66.67
	II	7	60.48	217.82	31.30	0.00	390.48
UPPER	III	6	2,813.15	60,622.68	7,805.28	0.00	6,627.78
	IV	7	2,509.66	745.34	1,373.76	0.00	3,863.10
	V	5	0.00	426.26	0.00	262.13	350.67
	Average		1,076.66	12,403.29	1,846.94	52.98	2,259.64
	I	6	518,207.13	0.00	0.00	29,994.45	1,676,894.44
	II	7	72,921.76	13,267.05	40,000.79	10,344.05	288,071.43
LOWER	III	6	81,123.89	33,270.83	27,435.00	3,552.78	46,333.33
	IV	7	14,898.58	67,583.50	20,506.75	1,054.76	33,989.29
	V	5	80,497.35	40,032.94	51,933.05	12,888.14	9,918.26
	Average		173,529.74	30,830.86	27,975.12	11,566.84	411,041.35

Table B-- Partitioning of variance into components of day, time, day-time interaction, bottle, and analytical technique (pipette) for the FS bacteria. Number of days used in the analysis for individual weeks is shown under "n".

FS BACTERIA
COMPONENTS OF VARIANCE

Site	Week	n	Day	Time	Day x Time Interaction	Bottle	Analytical Technique
	I	6	0.50	0.73	1.64	18.29	3.58
	II	7	5.06	4.68	10.78	0.00	8.77
UPPER	III	6	63.14	38.38	84.77	0.52	58.28
	IV	7	0.44	0.45	2.63	0.00	3.96
	V	5	11.99	13.21	24.32	1.34	4.80
	Average		16.23	11.49	24.83	4.03	15.88
	I	6	550.07	174.55	1,548.54	4.81	66.29
	II	7	472.63	91.82	1,456.69	0.00	199.94
LOWER	III	6	735.35	80.84	458.40	59.95	368.22
	IV	7	28.66	7.51	35.30	0.00	17.81
	V	5	223.19	19.23	74.75	23.87	160.40
	Average		401.98	74.79	714.74	17.73	162.53

Table C-- Partitioning of variance into components of day, time, day-time interaction, bottle, and analytical technique (pipette) for the FC bacteria. Number of days used in the analysis for individual weeks is shown under "n".

FC BACTERIA
COMPONENTS OF VARIANCE

Site	Week	n	Day	Time	Day x Time Interaction	Bottle	Analytical Technique
	I	6	0.03	0.05	0.14	0.00	0.64
	II	7	8.84	1.90	7.16	1.42	2.96
UPPER	III	6	0.13	0.00	0.56	0.00	2.50
	IV	7	0.00	0.00	0.00	0.10	0.04
	V	5	0.62	0.00	1.22	0.00	2.00
	Average		1.92	0.39	1.82	0.30	1.63
	I	6	8,805.93	6,085.99	17,118.72	0.00	1,884.39
	II	7	809.04	914.71	1,592.59	0.00	391.06
LOWER	III	6	4,678.05	558.39	2,407.33	471.89	2,045.00
	IV	7	696.98	255.04	159.72	127.39	247.15
	V	5	11,150.83	70,103.27	13,458.49	0.00	10,033.40
	Average		5,228.17	15,583.48	6,947.37	119.86	2,920.20

Key Words: Water quality, Bacteria, Mountain watersheds

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