INFLUENCE OF ENVIRONMENT ON STREAM MICROBIAL DYNAMICS

By

S. M. Morrison and J. F. Fair

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ABSTRACT

A study was conducted on the Cache la Poudre River in Larimer County, Colorado between June 1963 and September 1964 to determine the causes of variation in the bacterial quality of unpolluted surface waters.

Water samples were analyzed daily during the summer and two to three times per week during the winter for the presence of coliforms and total bacteria by the membrane filter procedure. Selected environmental variables including water and air temperatures, precipitation, pH, orthophosphate, and ammonia nitrogen were monitored.

Analysis of the collected data indicated that overland flow resulting from summer rainstorms was the most important single factor involved in variation of coliform and total bacterial counts, and the chemical variables studied. By continuous sampling during periods of increasing streamflow resulting from precipitation, it was determined that the bacterial and chemical variables showed an immediate increase at the beginning of overland flow, followed by a decrease near the crest of the storm hydrograph. Evidence was obtained which indicated that during the early portion of the streamflow increase, both bacteria and the chemical compounds studied were being deposited in the groundwater associated with the stream. Elution of bacteria and the chemical compounds from the groundwater was observed during the streamflow recession.

During periods of stable streamflow, it was shown that increases in coliform counts occurring between adjacent sampling sites were a function of wetted stream perimeter, dilution and stream velocity.

Small changes in water temperature were observed to be related to variation of coliform and total bacterial counts during the winter, the highest counts occurring at water temperatures of 3 to 4°C.

Cattle, grazing in a marshy, subirrigated meadow adjacent to the stream studied, caused increases in coliform and total bacterial counts of the stream.

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S. M. Morrison[1] and J. F. Fair[2]

CHAPTER I

INTRODUCTION

Limnologists have expended a great deal of effort in describing the interrelationships between aquatic organisms and the manner in which these organisms affect or are affected by the physical and chemical environment. Such investigation has resulted in a highly refined concept of microbial transformations of dissolved materials and of plankton community dynamics within the aquatic environment. It is surprising therefore to find that the available information concerning the dynamics of bacterial populations in fresh water is fragmentary and limited largely to bacteria other than those of sanitary significance. The sanitary bacteriologist has obtained much valuable information about the dynamics of bacteria of sanitary significance, particularly as regards the death or survival of coliforms, through the study of rivers polluted with domestic sewage. However, no particular significance has been attached in these studies to the specific chemical and physical variables which influence the dynamics of the bacteria involved.

The relationships between bacteria and the physical and chemical aquatic environment is of particular importance in evaluating quality of waters which normally contain low bacterial numbers. There is no question that coliform organisms found in water below a sewage outfall are largely of fecal origin and that their numbers are indicative of the extent of the pollution. It is not clear however, if coliforms encountered in water of normally good quality are in fact indicative of recent fecal contamination. It is logical to suspect that variations in the bacterial densities of a generally high quality water source, in the absence of continuing fecal pollution, are due to the currently undefined favorable or unfavorable influences of the stream environment. It is well known that significant short term variation in the bacterial quality of water sources occur, and to such an extent, as was pointed out by Kittrell and Furfari (1963), that sanitarians often lose confidence in any means of estimating bacterial quality. It is unfortunate that existing methods for detecting bacterial pollution do not and cannot take into account normal, environment induced variation in bacterial composition of surface waters. This situation can be improved only through continuing studies which relate bacterial dynamics to environmental variables.

Much interest has recently developed in ascertaining the effects on water quality of changing land use, population relocation and watershed protection. As was concluded by Teller (1963), there is insufficient information to define just what the natural quality of water should be, and to what extent one may consider fluctuations in bacterial numbers as being due to natural, rather than man-made causes. Such information can be gained only through the elucidation and quantitation of the effects which natural chemical, physical, and biological variables have on the bacterial content of a water source.

This study is devoted to defining the causes of variation in the bacterial quality of a high quality water source in the absence of obvious sources of pollution, with emphasis upon the effects of specific environmental variables.

The principal objectives of this study are as follows:

1. To quantitatively measure specific environmental variables of a high quality mountain stream which may influence or be related to the bacterial composition of the stream.
2. To determine the nature and extent of fluctuations in numbers of coliforms and total bacteria in a high quality mountain stream.
3. To develop specific relationships between the environmental and bacterial variables which will provide a basis for detailed mathematical analysis of future studies.

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CHAPTER II

REVIEW OF LITERATURE

Previous investigators who have attempted to define the relationships between aquatic microorganisms and the aquatic environment have generally concluded that the complexity of the correlations prohibits quantitation of the effect of any specific variable (Pennak, 1946; Teller, 1963). Most investigations have been resolved to a seasonal pattern of variation of microbial numbers with no intent for direct correlation to any specific environmental variable or combination of variables. Sufficient evidence has accumulated however to indicate that certain environmental variables are responsible to a large extent for the observed variations in microbial numbers. The strong influence of precipitation and temperature have been most frequently mentioned.

Collins (1960) attributed observed seasonal variation of gram-negative bacteria other than Enterobacteriaceae in lakes to progressive nutrient limitation occurring during precipitation-free periods. Although Collins considered the observed effect of precipitation to be the result of addition of nutrients to the water from surrounding land surfaces, she also pointed out that increases in bacterial numbers after heavy rainfall might be due to washed-in bacteria. Ritter and Hausler (1961) described a marked seasonal variation in coliform densities in wells, lowest values being observed in March, followed by a rapid increase in numbers following heavy spring rains. Voelker, Heukelekian, and Orford (1960) similarly observed a seasonal variation of coliform numbers in wells, and attributed the observed fluctuation to both precipitation and water temperature. Teller (1963) reported a regular seasonal fluctuation of coliform numbers in rivers serving as domestic water sources in the Pacific Northwest. In this study it was observed that seasonal lows occurred in June or July followed by maximum counts in mid-winter. Teller stated that a definite precipitation effect existed but that its correlation with coliform densities could be established only when considered on a long term basis. Teller's data suggest that variation in coliform densities correlates best with variations in air and water temperature. Taylor (1958), by summarizing a large volume of data collected by other investigators, concluded that a definite seasonal coliform pattern exists in most surface waters, and that on the average, maximum densities are observed during the winter months; spring and summer peaks are of short duration which would be suggestive of a precipitation effect. Petersen and Boring (1960) concluded from a small number of samples collected from a mountain stream in Colorado that surface runoff contributed significantly to the coliform content of the stream. In a literature review on coliform densities in a large, deep, polluted stream, Kittrell and Furfari (1963) cautioned that following precipitation, large numbers of the coliforms present may not be attributed to sewage. In contrast to the previous citations, Geldreich et al. (1962) concluded that the possibility of soil contributing significant numbers of coliforms to surface waters during surface runoff was purely speculative. Geldreich, Kenner, and Kabler (1964) later concluded that plants did not contribute coliforms to surface runoff. However Taylor (1942) demonstrated a marked correlation between the numbers and types of bacteria found in soil and water and postulated that soil was a potential source of coliforms found in surface waters.

Bigger (1937) suggested that the reproduction of coliforms in water may be a contributing factor in seasonal fluctuations of coliform densities as well as explaining the presence of coliforms in high quality water sources. It was established by Bigger that coliforms are able to reproduce in river water, the most favorable temperature for growth and survival being about 22 C, far below that which is normally considered as optimal for these organisms.

Wuhrmann (1964) stated that streamflow, independently of all other variables, is known to have a strong influence on the presence of vertebrate and invertebrate water animals, but that information is meager as to its effect on bacteria. Wuhrmann's statement, it must be noted, was referring to streamflow as an entity independent of the effects of precipitation upon it. Streamflow in this context has been reported by Dorris, Copeland, and Lauer (1963) to have an overriding influence on most chemical and physical stream variables; however, Greenburg (1964) observed only slight flow effects upon plankton communities. Kittrell and Furfari (1963) postulated that the physical characteristics of the stream channel may be a factor in determining coliform densities. These authors considered the presence or absence of riffle areas as being an important factor in stream self-purification, due to the action of attached predators. Variation in the chemical state of surface water courses has received considerable attention in the literature; however, few attempts have been made to correlate chemical factors with bacterial quality. A large number of studies conducted by water pollution control agencies have reported data obtained simultaneously upon chemical and biological factors (Ohio River Valley Water Sanitation Commission, 1957; U. S. Public Health Service, 1950), but the data has not been analyzed in such a manner that correlative trends may be observed.

Gorham (1961) presented a comprehensive review of the literature dealing with the source of inorganic ions found in surface waters. He concluded that weathering of rocks was the most important single source, but that the atmosphere contributed significant amounts of ions to water, chiefly through dry fallout. Gorham also observed that groundwater, directly connected to surface streams, supplied about 76 percent of the total dissolved solids found in streams when the total groundwater contribution to the flow was only 35 percent. Wiebe (1931), in a study conducted on the Mississippi River, observed that orthophosphate concentrations ranged from 0.065 to 0.000 mg/l while ammonia nitrogen levels varied from 0.012 to 0.221 mg/l. Wiebe stated that there is a general trend for orthophosphate and ammonia nitrogen concentrations to correlate when considered over long periods of time. Hem (1959) stated that the major supply of soluble phosphate in surface waters is due to weathering of igneous rocks containing apatite, while ammonia nitrogen is largely the result of biological activity. Taylor (1958) related phosphate concentrations in rivers to algal growth, lowest concentrations occurring whenever algal life is dormant. Hutchinson (1957) presented a definitive review of the phosphorus, nitrogen and carbon cycles in surface waters and how they relate to microbial activity. Of particular interest is Hutchinson's observation that ammonia nitrogen in lake waters may be bound to suspended or sedimented collodial materials, the extend of the binding being a function of pH.

CHAPTER III

RESEARCH DESIGN

This study was carried out during the period September 1, 1963 to September 15, 1964 on the Cache la Poudre River in Larimer County, Colorado (Fig. 1). The portion of the river studied extends from a point 13 miles northwest of Fort Collins, Colorado near the junction of Colorado Highway 14 and U. S. Highway 287, to a point 45 miles west of Fort Collins on Colorado Highway 14. The altitude of the river reach varies from 5,300 feet to 7,600 feet and drains a total area of 1,055 square miles. The natural flow of the Cache la Poudre River is augmented during the summer irrigation season by transbasin diversions and stored reservoir water. The principal diversions are from the Laramie River watershed through the Laramie-Poudre Tunnel and through the Skyline Ditch to Chambers Lake, a storage reservoir tributary to the Cache la Poudre River (Fig. 1). The study area is sparsely populated during the winter, but receives large numbers of tourists during the summer. Two communities, Poudre Park and Poudre City, represent the only areas of concentrated population, both having maximum populations of less than 150. However, numerous improved picnic and camping areas on the stream contribute much to the population density during the summer.

There are no known additions of domestic sewage to the stream, all of it being disposed of in vaults or septic tanks. The only significant commercial operation on the river which adds any foreign material to the stream is a trout rearing station which diverts a small quantity of water through a series of shallow ponds before returning it to the river.

Eleven sample collection sites were selected to provide a variety of terrain and population density, with river conditions ranging from large deep pools to shallow riffle areas. During the period September 1, 1963 to June 1, 1964 all sites were sampled approximately twice each week. During the period June 1, 1964 to September 15, 1964 sites 7A1, 7B, 7C, 8, 8A, 8B, and 9 (Fig. 1) were sampled daily, the remaining four sites being sampled three times each week. In order to maintain the sampling on a daily basis during the summer, it was necessary to establish a field laboratory which was located at site 9. The field laboratory was housed in a 15-foot house trailer which was equipped to make possible the performance of complete water analyses in the field. Samples collected during the period September 15, 1963 to May 31, 1964, and those samples collected during the summer from sites 1, 4, 6A and 7A (Fig. 1), were transported to Fort Collins where analyses were performed.

In addition to the comprehensive study performed during the period September 1, 1963 to September 15, 1964, a preliminary investigation was performed between June 1 and August 31, 1963, the data from which were used to develop sampling techniques and to provide a procedural basis for subsequent studies. Sites 8, 8A, 8B, 8C (not included in subsequent studies), and 9 were sampled daily, samples being analyzed for coliform and total count densities, water temperature, precipitation and stream discharge as described below. At the time of this preliminary study the field laboratory consisted of a small tent and minimal equipment which did not permit more thorough analysis of samples.

Water samples were collected in one-half gallon polyethylene narrow-mouthed bottles which were thoroughly rinsed in the water at the sampling site before a collection was made. Samples were collected as far from the shore as wading permitted, at a depth of about 1 foot. At the time of sampling, air and water temperatures at the sample site were determined by means of a Weston dial thermometer. Samples were then transported to either the field laboratory or to Fort Collins for analysis. Samples were analyzed in the field within one hour of collection, three hours being required before analysis was performed on those samples transported to Fort Collins. During transportation to Fort Collins, samples were maintained within 2 C of the water temperature at time of collection.

Standard Methods procedures (American Public Health Association, 1960) were followed for the determination of coliform densities by the membrane filter technique. Total bacterial counts were determined by the membrane filter technique using m-Plate Count Broth (Difco) as the cultivation medium with incubation being carried out for 24 hours at 35 C. Ammonia nitrogen and orthophosphate concentrations were colorimetrically determined on each sample collected from site 9 during the period June 1 to September 15, 1964 by means of the direct nesslerization and ammonium molybdate procedures respectively (American Public Health Association, 1960) using an Evelyn filter photometer as the color measuring device. An estimation of sample turbidity was made by measuring percent transmittance at 420 mu on an Evelyn filter photometer. Measurement of pH was performed potentiometrically by means of a battery powered Beckman Model N pH meter for field analyzed samples and by means of a Beckman Zeromatic[1] meter for samples analyzed in the Fort Collins laboratory.

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[1] Registered trademark, Beckman Instruments, Inc., South Pasadena, California.

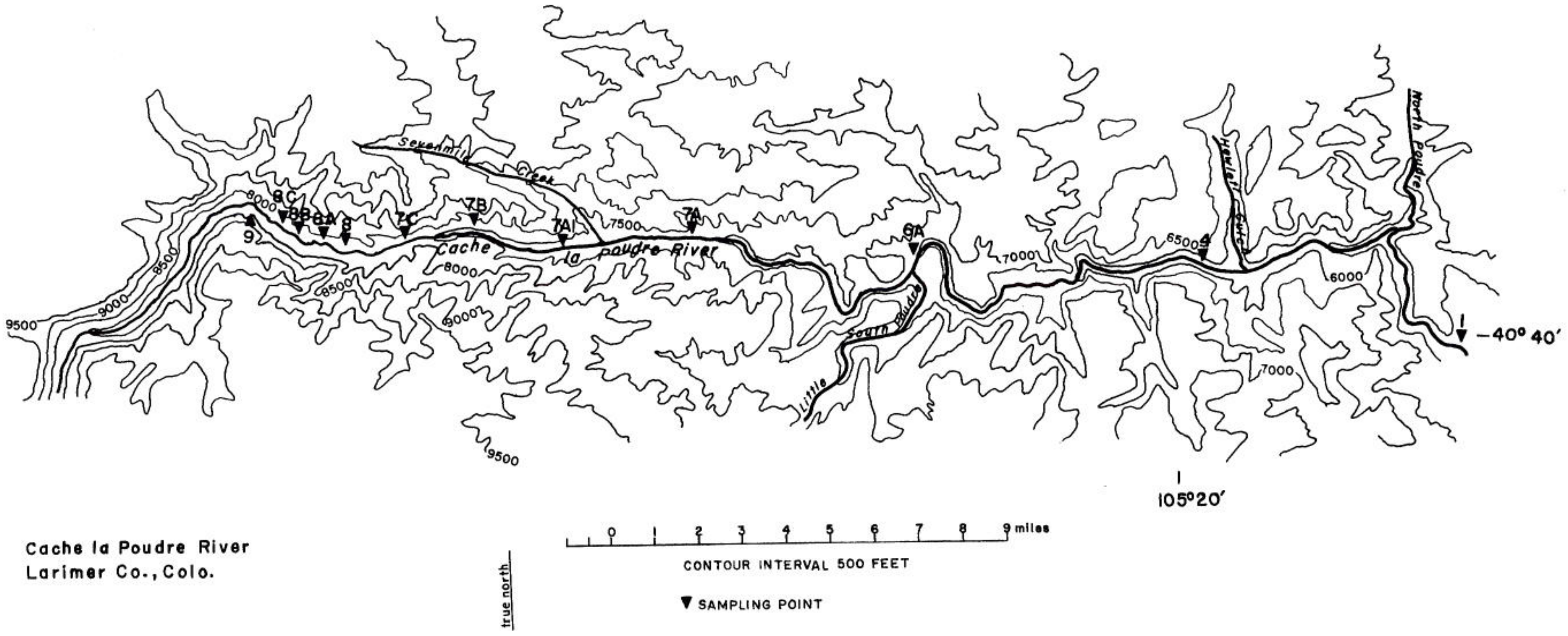

Fig. 1 Cache la Poudre River drainage study area.

Observations on the physical environment were routinely made at site 9 during the period June 1 to September 15, 1964. Precipitation was measured by means of a three inch rain gauge located in an open field about 150 yards from the stream channel. Maximum-minimum Taylor thermometers were immersed in the stream and read daily to provide 24 hour temperature extremes. A continuous recording Bachrach immersible probe thermometer was used to supplement the maximum-minimum water temperature data. A Bachrach air temperature recorder, housed in a standard white wood instrument shelter, provided continuous monitoring of air temperatures in the same location as described for the rain gauge.

Streamflow (discharge) data was obtained from Stevens A-35 water level recorders located at site 8 and about two miles downstream from site 1. Both recorders are maintained by the U. S. Geological Survey and are frequently calibrated by current meter measurements.

For certain aspects of this study discharge measurements and hydrograph characteristics were required at site 9. Because of the proximity of the recording station it was assumed that flow at site 9 was equivalent to that recorded at site 8. A staff gauge and a simple pneumatic water stage recorder of the author's own design were installed at site 9. Stage-discharge relations were established for the staff gauge and pneumatic recorder by plotting stage at site 9 versus instantaneous discharge at site 8. In order to remove the effects of water travel time between sites 8 and 9, the stage-discharge relationship was derived from data obtained during periods of stable flow. By use of the stage-discharge relationship and the water stage tracing of the pneumatic recorder, it was possible to construct approximate hydrographs for site 9.

CHAPTER IV

EXPERIMENTAL RESULTS

The data collected in this study of the Cache la Poudre River bacterial dynamics and environmental interrelationships have, in most cases, been grouped according to time of year and sampling site from which the data were obtained, followed by reduction of the grouped data to arithmetic means. The arithmetic mean was chosen over other estimates of central tendency because of the lack of sufficient numbers of extreme data values to bias the estimate. The mean values of the variables are presented in the form of line graphs which relate the variables to the base parameters of time of year and sampling site. This method of analysis, in addition to elucidating seasonal and locational effects, makes it possible to arrive at certain relationships between variables by inspecting the graphed data for trend similarities.

The results of this study are presented under the following headings: Preliminary Study Summer 1963, Twelve Month Study September 1963 through August 1964, Summer 1964 Study, Specific Variable Interrelationships.

<u>Preliminary Study Summer 1963</u> -- The results of the preliminary study are presented in Figs. 2, 3, and 4. Bacteriological observations from sites 8, 8A, 8B, 8C (not included in later studies) and 9 were pooled in groups representing data collected during consecutive seven-day periods for which mean values of the data were then plotted by time periods against the mid-point of the seven-day period (Fig. 4). The environmental data presented in Fig. 3 were recorded at site 9 only, and are also presented as mean values for consecutive seven-day periods. Because of extenuating circumstances, mean values for the data presented in Figs. 2, 3 and 4 during the periods of July 2, August 6, and August 27 were based on five observations. Mean values of the bacteriological data for each site were determined for the entire summer period and were then plotted against sampling site (Fig. 2).

Both coliform and total bacterial counts (Fig. 4) varied considerably during the study period, although a general increase in both variables was

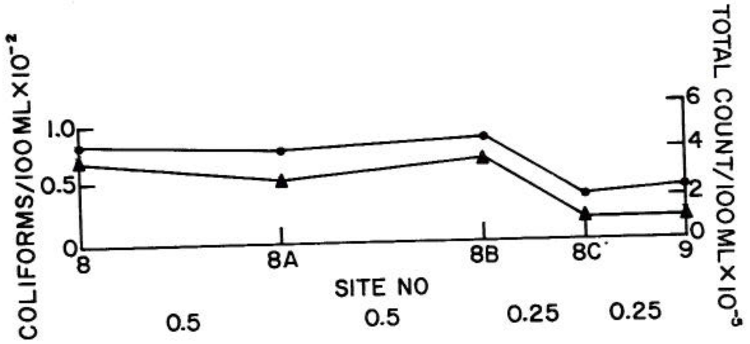

Fig. 2 Mean coliform (●) and total bacterial counts (▲) for summer 1963 study distributed according to sampling site.

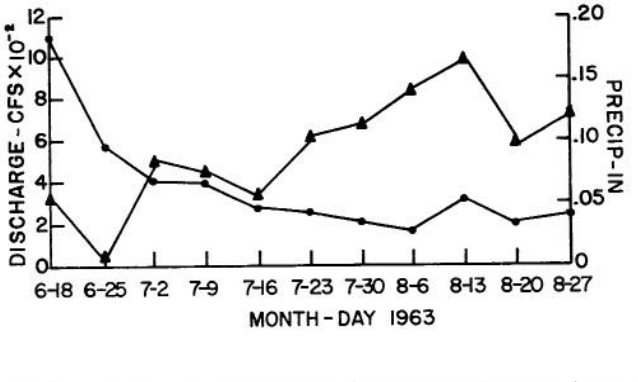

Fig. 3 Mean precipitation (▲) and stream discharge (●) for summer 1963 study distributed according to consecutive seven-day periods.

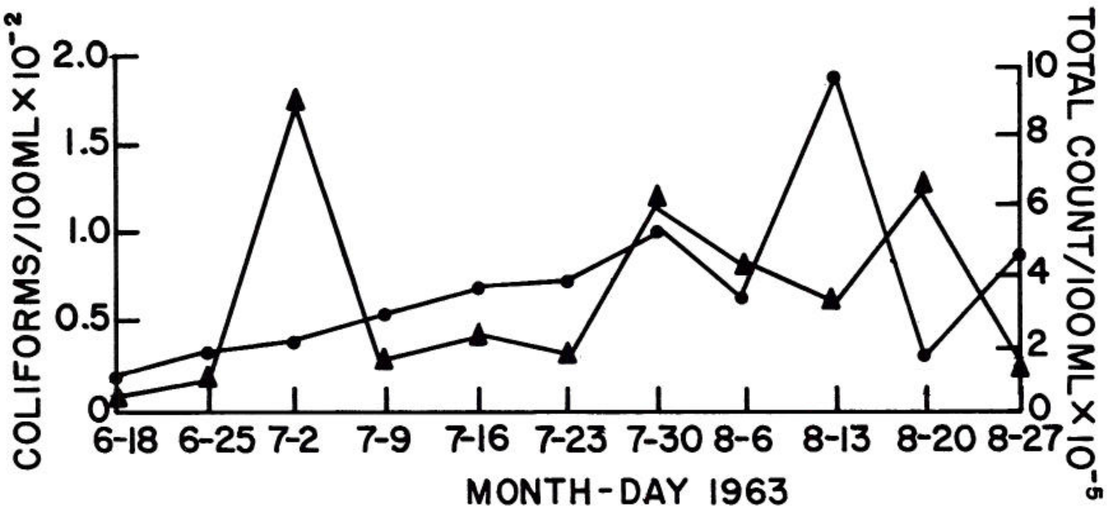

Fig. 4 Mean coliform (●) and total bacterial counts (▲) for summer 1963 study distributed according to consecutive seven-day periods.

evidenced as the summer period progressed. Both coliforms and total bacteria presented a similar pattern when considered on the basis of sampling site, (Fig. 2), highest counts being obtained at sites 8B and 8.

Stream discharge (Fig. 3) showed a typical recession pattern during the summer period, although two distortions in the recession were observed during the weeks of July 9 and August 13. Measurable precipitation was recorded at site 9 during each seven-day period (Fig. 3) the largest amount being recorded during the August 13 period.

Twelve Month Study September 1963 through August 1964 -- The total number of samples collected between September 1, 1963 and August 31, 1964, from sites 1, 4, 6A, 7A, 7B, 8, 8A, 8B, and 9 are presented in Table 1 arranged according to month and site of collection. Figures 5 through 9, which show the variation in coliforms, total count, pH, air and water temperature and stream discharge for the same twelve month period, are based on the number of samples shown in Table 1, the number of samples being equivalent to the number of observations on each variable.

TABLE 1

NUMBER OF SAMPLES COLLECTED DISTRIBUTED ACCORDING TO MONTH OF COLLECTION AND SAMPLING SITE

Site No.	Month-Year												
	9-63	10-63	11-63	12-63	1-64	2-64	3-64	4-64	5-64	6-64	7-64	8-64	Total
1	2	5	4	2	3	3	3	3	2	8	12	4	51
4	2	5	4	2	3	3	3	3	2	8	12	4	51
6A	2	5	4	2	3	3	3	3	2	8	12	4	51
7A	2	5	4	2	3	3	3	3	2	8	12	3	50
7B	2	5	4	2	1	0	2	3	2	25	27	26	99
8	5	5	4	2	3	3	3	2	2	25	27	26	107
8A	5	5	4	2	3	3	3	3	2	25	27	26	108
8B	5	5	4	2	3	3	3	3	2	25	27	26	108
9	5	5	4	2	2	0	3	2	2	25	27	26	103
Total	30	45	36	18	24	21	26	25	18	157	183	145	
												Total	728

A marked seasonal variation in coliform counts was evidenced (Fig. 8), peak values being observed in October, February, and August. Figure 5 shows that sites 8B and 7B produced the highest coliform counts and some evidence that there was a downstream decrease in coliform numbers.

The seasonal variation in total bacterial counts (Fig. 8) appeared to be similar to that of coliforms especially during the period May through August. On the basis of sampling site, total bacterial counts (Fig. 5) did not appear to be closely related to coliform counts although there was a general downstream decrease in total bacteria.

Mean sample pH showed wide variation during the twelve month period (Fig. 7), the highest values being observed in May after a rapid increase beginning in February. As shown in Fig. 6, there was a definite increase in pH in a downstream direction, excepting the decrease between sites 9 and 8B.

The seasonal variation in water temperatures at the time of sample collection, shown in Fig. 7 is typical of a high altitude area. Of particular significance to this study was the observation that water temperatures at sites 7A through 9 were essentially equal (Fig. 6). This situation has made it possible to remove water temperature as a factor in variation of coliform and total bacterial counts at these sites.

The variation in air temperatures at time of sample collection, arranged according to month (Fig. 7) and site (Fig. 6), closely approximated the water temperature pattern previously described.

Seasonal variation in stream discharge for sites 1 and 8, where continuous recording stations were in operation, is typical of high altitude, snow-fed streams (Fig. 9). Between October and April the low flow level was essentially constant; peak discharges occurred in early summer. The early summer increase in stream discharge closely followed the air and water temperature trends shown in Fig. 7. It appears that the period of increasing runoff was accompanied by increases in coliform and total bacterial counts (Fig. 8) however, the subsequent decrease in runoff was not accompanied by decreasing bacterial numbers.

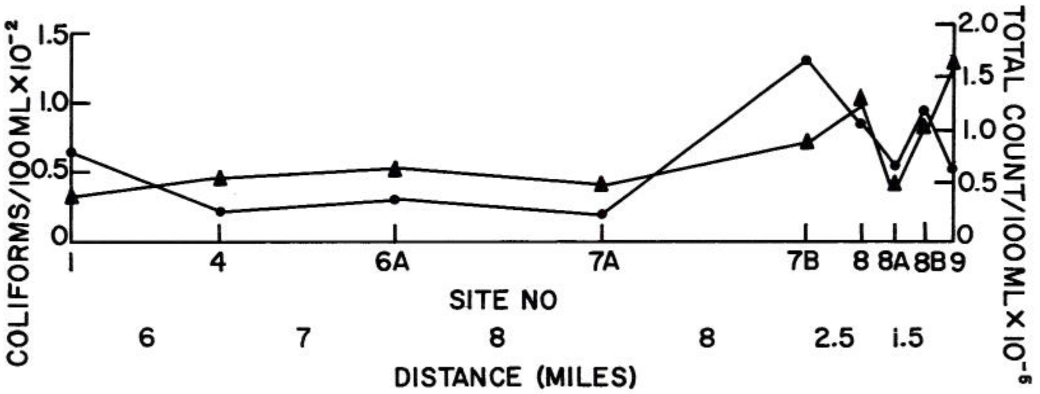

Fig. 5 Mean coliform (●) and total bacterial counts (▲) for twelve month study distributed according to sampling site.

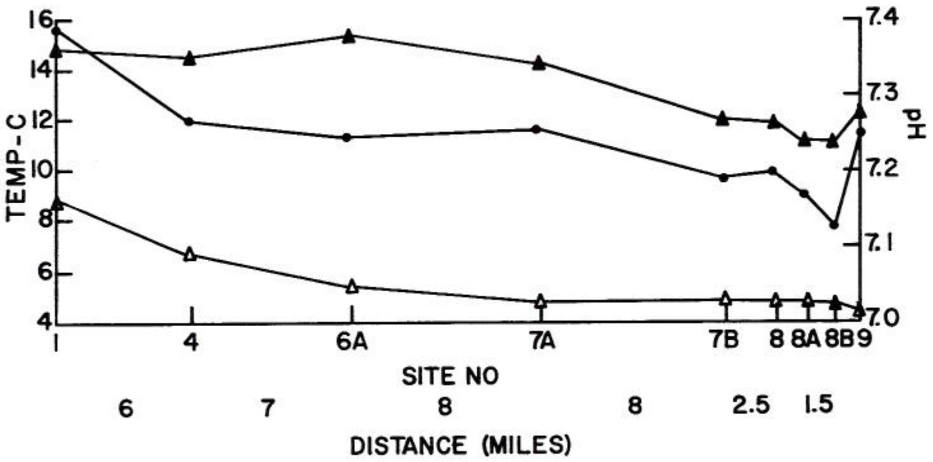

Fig. 6 Mean air temperature (▲), water temperature (△) and pH (●) for twelve month study distributed according to sampling site.

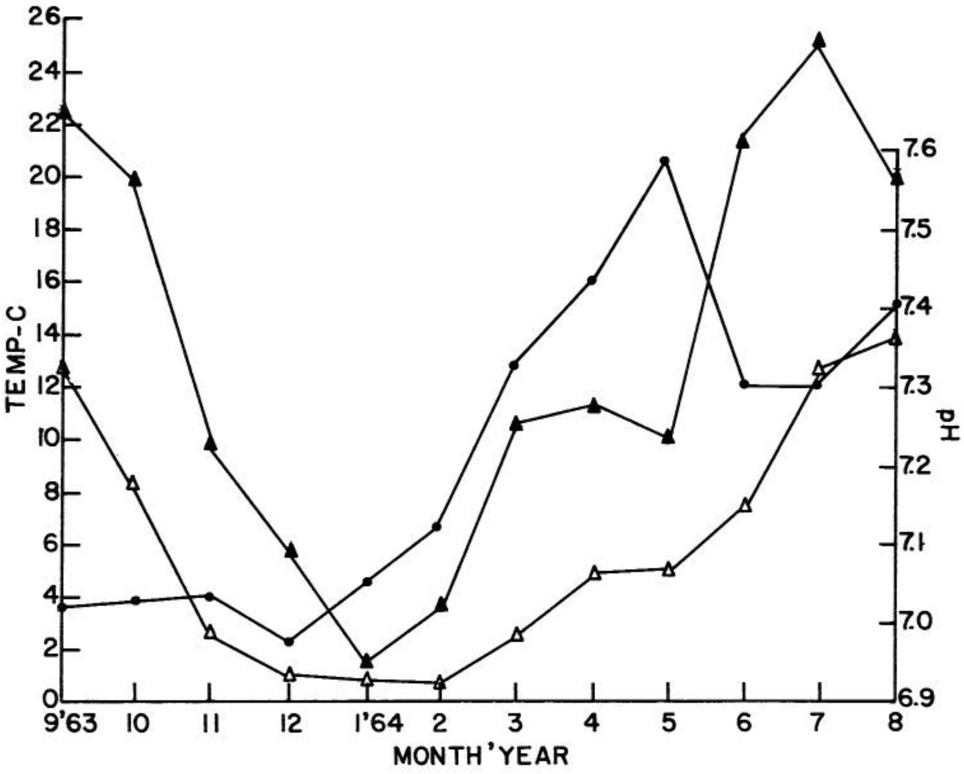

Fig. 7 Mean air temperature (▲) and water temperature (△) and pH (●) for twelve month study distributed according to month.

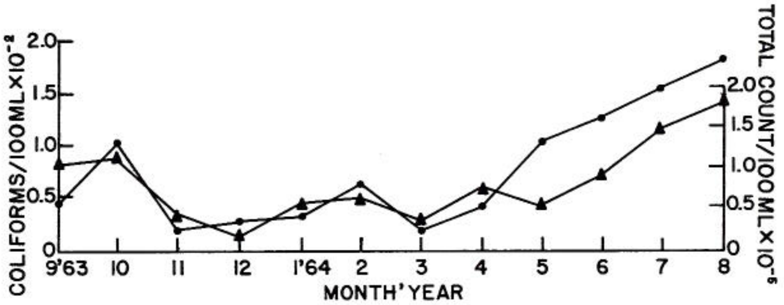

Fig. 8 Mean coliform (●) and total bacterial counts (▲) for twelve month study distributed according to month.

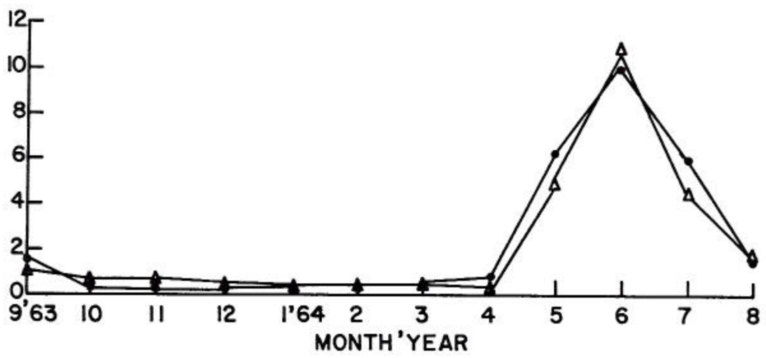

Fig. 9 Mean stream discharge for twelve month study at sites 8 (●) and 1 (△) distributed according to month.

Summer 1964 Study -- The intensive sampling at sites 7A1, 7B, 7C, 8, 8A, 8B, and 9 during the summer of 1964 made it possible to more closely investigate short term fluctuations in the variables. For the 1964 summer period the data were grouped according to site of collection and according to consecutive seven-day periods in the same manner as described for the preliminary study.

It was observed that coliform counts showed a wide variation during the summer season (Fig. 12), maximum counts being obtained in August. There appeared to be a tendency for coliform counts to increase in a downstream direction (Fig. 10).

Although total bacterial counts showed an increase during the summer period (Fig. 12), they did not appear to behave in the same manner as noted for the coliforms. Total bacterial counts did, however, show an increase in a downstream direction (Fig. 10).

Because orthophosphate and ammonia nitrogen were measured at site 9 only, coliform and total count data are presented for site 9 (Figs. 13 and 14) in order to provide a direct comparison between the environment and the bacteria without the necessity of assumptions regarding the constancy of the environmental data at the different sites studied. It was observed that coliform and total count patterns at site 9 were very similar to those observed over the whole study area (Fig. 14). The effect of precipitation upon streamflow did not appear to be great (Fig. 11). A strong inverse relationship between sample pH and ammonia nitrogen is suggested in Fig. 13. Orthophosphate and ammonia nitrogen concentrations appeared to be inversely related up to the August 9 period and directly related thereafter (Fig. 13).

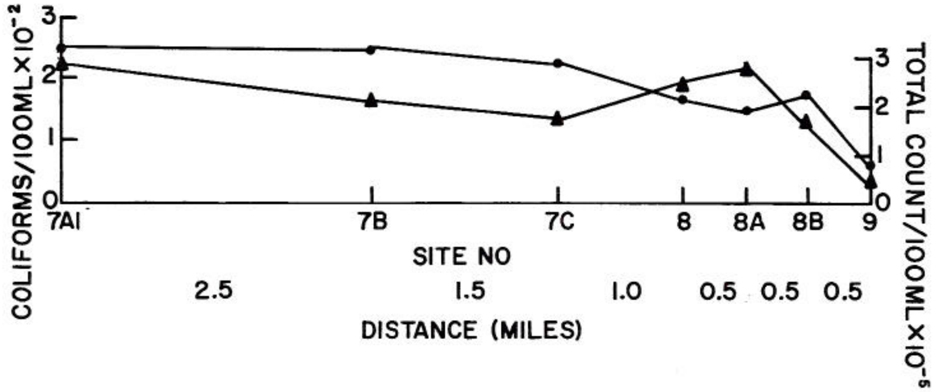

Fig. 10 Mean coliform (●) and total bacterial counts (▲) for summer 1964 study distributed according to sampling site.

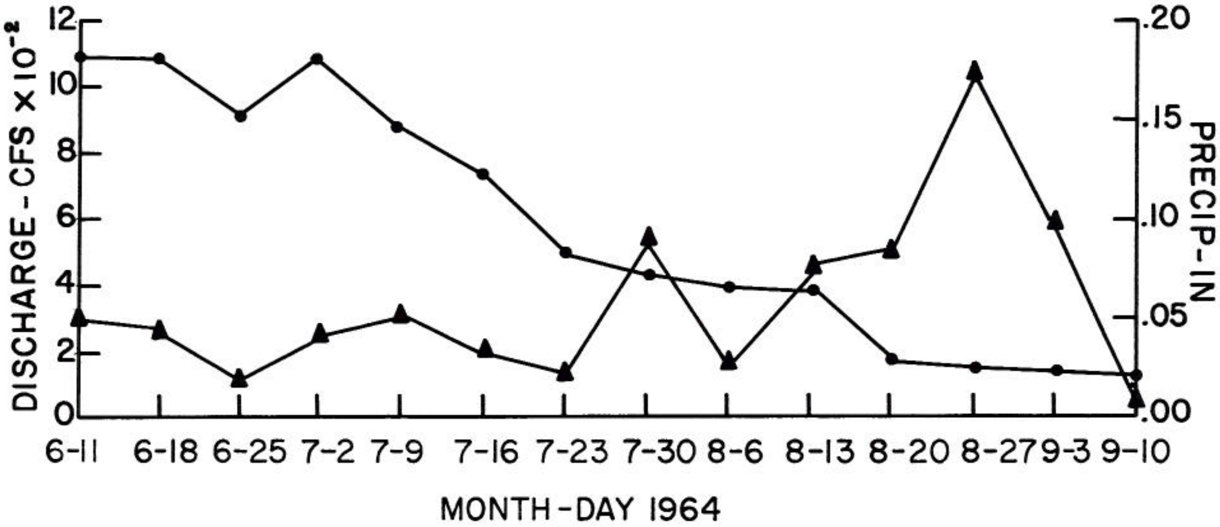

Fig. 11 Mean precipitation (▲) and stream discharge (●) for summer 1964 study distributed according to consecutive seven-day periods.

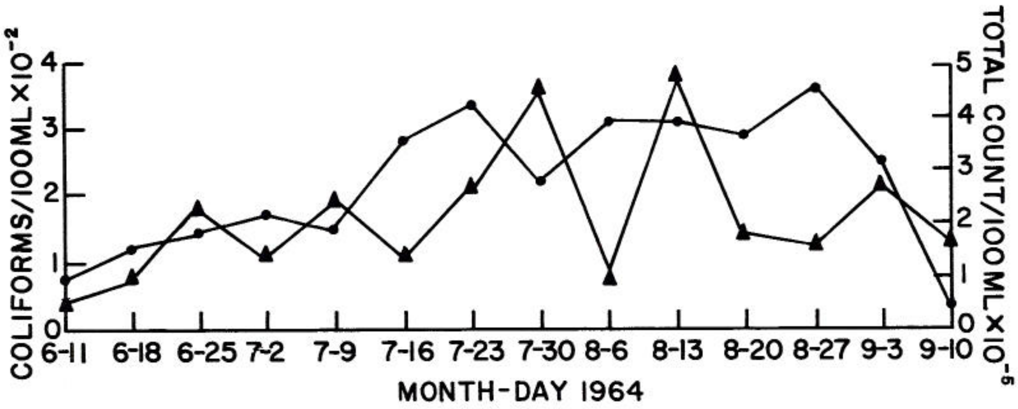

Fig. 12 Mean coliform (●) and total bacterial counts (▲) for summer 1964 study distributed according to consecutive seven-day periods.

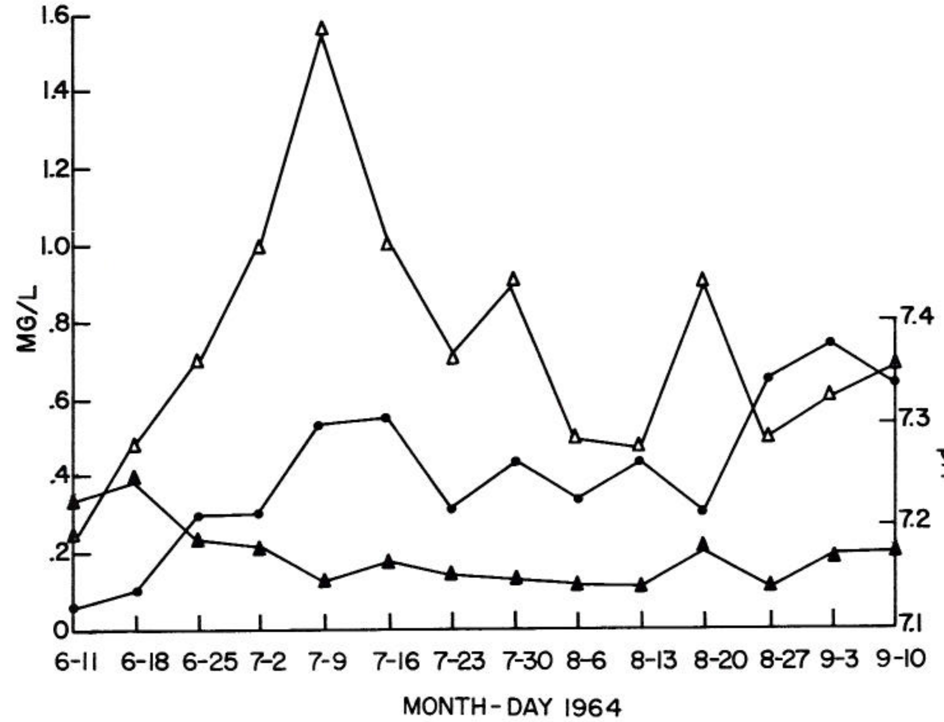

Fig. 13 Mean pH (●), orthophosphate (△) and ammonia nitrogen (▲) at site number 9 for summer 1964 study distributed according to consecutive seven-day periods.

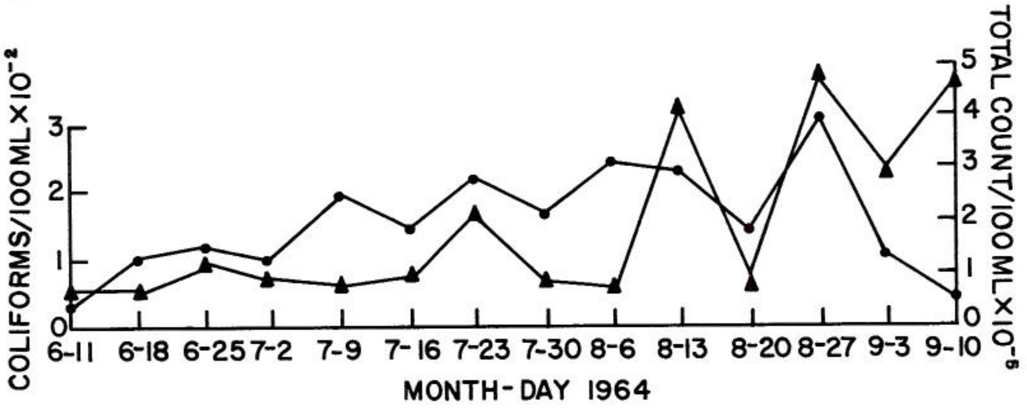

Fig. 14 Mean coliforms (●) and total bacterial counts (▲) at site number 9 for summer 1964 study distributed according to consecutive seven-day periods.

Specific Variable Interrelationships -- In an attempt to determine the specific effect of water temperature upon coliform and total bacterial counts, data collected during the period November 1963 to March 1964 were grouped according to water temperature at the time of sampling and mean bacterial counts at the various temperatures determined. This period was chosen for analysis because of the lack of surface runoff and the relatively constant flow. Figure 15 shows a well defined spiked distribution pattern of coliform counts as the water temperature increased, maximum counts being observed at a water temperature of 3 C. The relationship of total counts to temperature (Fig. 15) was less clearly defined, two distinct peaks occurred at 3 and 4 C.

In order to investigate the effects of surface runoff and flow variations upon the bacterial, chemical and physical composition of the stream, intensive sampling was performed at site 9 during periods of changing flow resulting from precipitation. Two storms, August 27 and August 29 (Fig. 16) resulted in well defined hydrographs and are presented here. Each of the storms showed an immediate rise in coliform and total bacterial counts which coincided with the beginning of runoff, followed by a period of decreasing counts during the period of maximum flow. In both storms, an increase in coliform and total bacterial counts was observed during the recession. The physical and chemical data for the storms, in general, showed the same patterns as the coliform and total count data. A particularly large increase in orthophosphate concentrations was observed between the beginning of overland flow and the hydrograph crest.

The Cache la Poudre River, because of the controlled addition of foreign and stored reservoir water, exhibits periods of essentially stable flow. This situation made it possible to investigate the effects of flow upon bacterial and chemical variables in the absence of a precipitation influence. For the purposes of this study, stable flow periods were defined as periods during which the instantaneous value of the stream discharge at any time during a 24 hour period did not fluctuate more than 5 percent above or below the mean discharge for the period. Coliform and total count data obtained at site 8 (located adjacent to a U. S. Geological Survey water stage recorder), during stable flow periods, are presented in Fig. 17 plotted against the instantaneous discharge. Coliform and total bacterial counts behaved in a very similar manner, counts increased up to flow values of about 200 cubic feet per second, and decreased thereafter Coliform and total bacterial counts obtained during stable flow periods occurring during the 1963 preliminary study are not presented here but followed the same pattern as shown for the summer 1964 study.

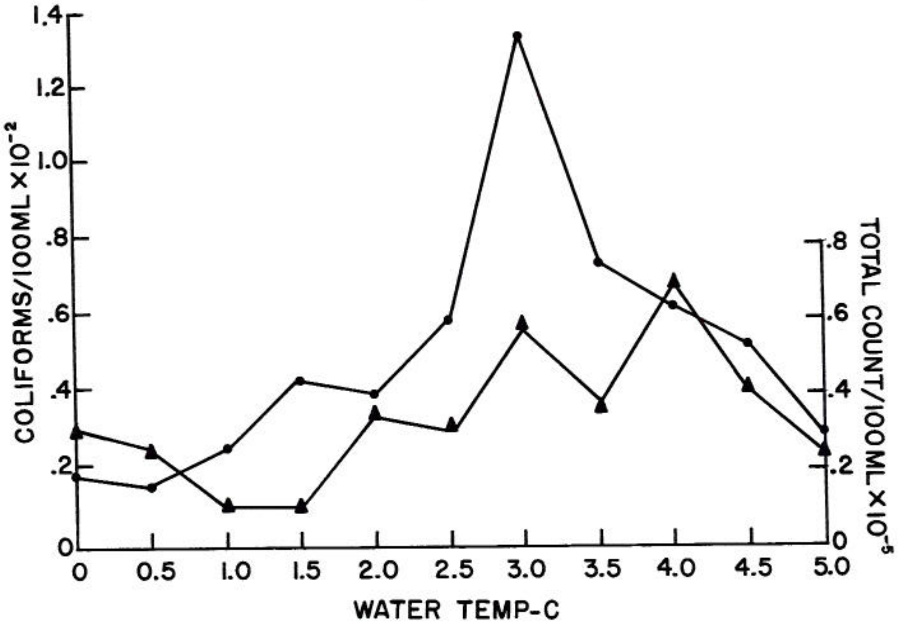

Fig. 15 Mean coliform (●) and total bacterial counts (▲) distributed according to water temperature at time of sampling.

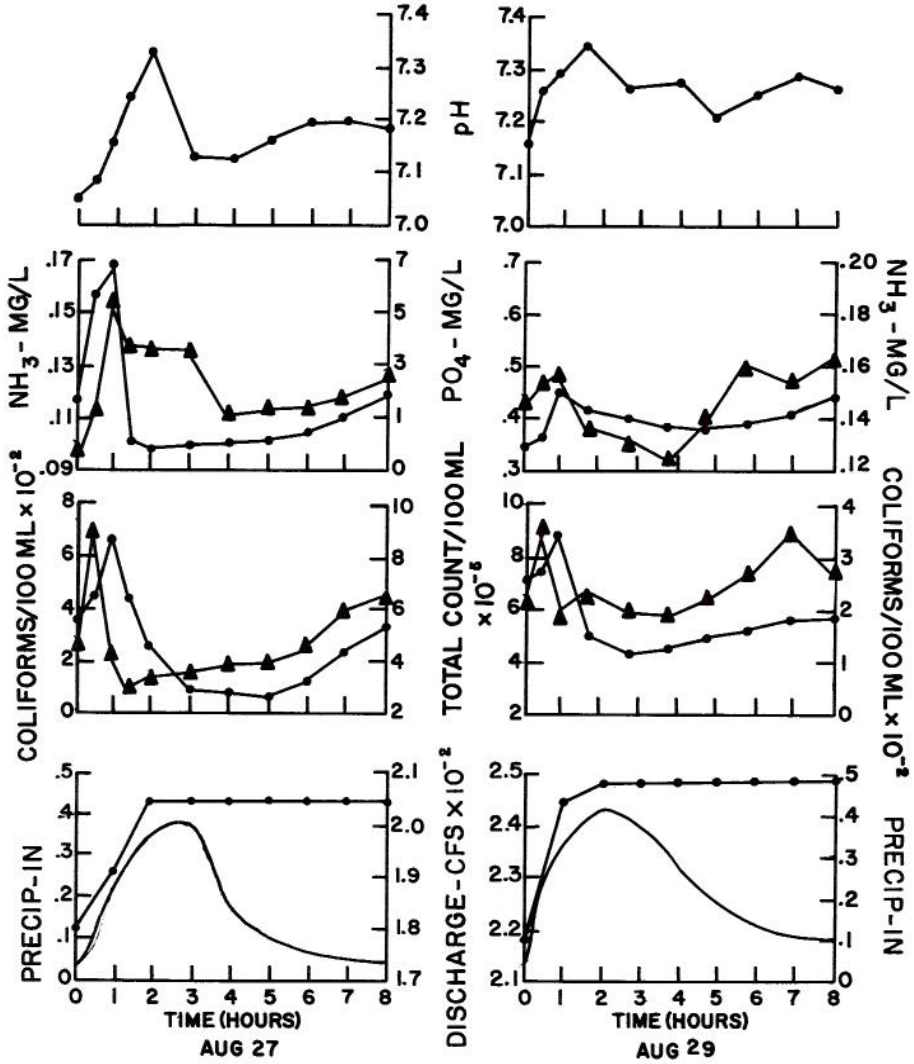

Fig. 16 Variation of coliform (●) and total bacterial counts (▲), ammonia nitrogen (▲), orthophosphate (●), and pH (●) with precipitation (●) and stream discharge (▬) during the storms of August 27 and 29, 1965.

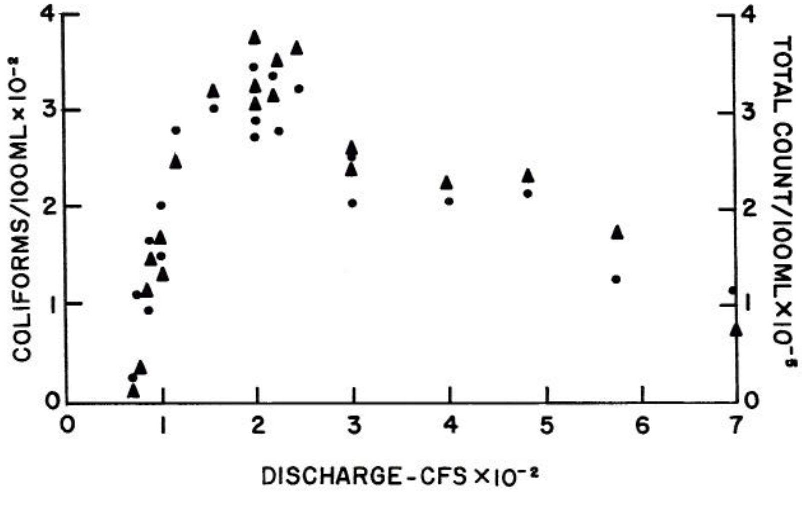

Fig. 17 Coliform (●) and total bacterial counts (▲) at site 8 during stable flow periods occurring during the summer of 1964 distributed according to instantaneous stream discharge.

CHAPTER V

RESEARCH IMPLICATIONS

Although a large number of observations on selected chemical and physical variables of the Cache la Poudre River have been compiled in this study, it is apparent that other uninvestigated variables may also be related to the bacterial dynamics of the stream. The emphasis placed upon the dynamics of coliform and total bacterial counts in this study does not exclude the possibility of other groups of bacteria being similarly influenced by environmental changes. The environmental variables selected for study are those which would be expected to be closely related to changes in bacterial numbers, not only in the river studied, but also in other surface waters.

The 1963 preliminary summer study provided the first evidence that surface runoff resulting from precipitation (rainfall) is related to variation of coliform and total bacterial counts of the stream. The expected effect of precipitation would be to increase both coliform and total bacterial counts due to the washing of bacteria into the stream from the land surface of the watershed. The coliform pattern observed during the summer of 1963 (Fig 4) is very similar to the precipitation pattern (Fig. 3), the two variables apparently being directly related; total counts (Fig. 4) however show a pattern with respect to precipitation which suggests an inverse relationship. During the more intensive 1964 summer study, the behavior of coliform and total bacterial counts (Fig. 12) with respect to precipitation (Fig. 11) is essentially the same as that observed during the preliminary study.

Since precipitation was measured at one location only during both summer periods it was not expected that there would be a clearly defined or consistent relationship between precipitation and bacterial counts. A large number of factors would be expected to be involved in precipitation effects namely, amount, duration, distribution, and intensity of individual storms. Although the data recorded is insufficient to provide interpretation on the basis of all these factors, there is some evidence that the distribution of precipitation may be related to the variation in coliform and total bacterial counts during periods of heavy precipitation.

Investigation of the precipitation data recorded during the summer of 1963 shows that the greatest amounts occurred during the August 13 period. An increase was also observed in coliform counts but not in total bacterial counts. On the basis of personal observations, streamflow patterns, and information supplied by residents of the area, there is no question that precipitation during this period was widely distributed over much of the watershed area.

During the 1964 summer study the greatest precipitation amounts were recorded during the August 27 period. Both coliform and total bacterial counts increased at site 9, but no increase in total bacterial counts was observed over the entire sampling area. Investigation of the raw data collected during this period revealed that both coliform and total bacterial counts increased at sites 9, 8B, 8A, 8, and 7C, but that the decreases in total counts at sites 7B and 7A1 were so great that the mean values for the river reach showed little net change. Information from residents of the area indicated that precipitation was light during the period near site 7A1. This information, in conjunction with the observation that there was no increase in streamflow, indicates that the precipitation was localized in the site 9 area. Although the exact reasons are not clear, it appears that the difference in behavior of coliform and total bacterial counts during periods of heavy precipitation may be related to whether the precipitation is localized in the sampling area or widely distributed over the watershed.

Because of the short duration of most storms occurring in the sampling area, period mean coliform and total bacterial counts are not highly indicative of the behavior of bacterial counts during individual storms. This study is the first to report the results of intensive sampling carried out during specific storm periods and to relate the bacterial numbers during such storms to changes in streamflow. As will be seen in the following discussion, the relationship between coliform and total bacterial counts depends heavily upon the portion of storm hydrograph during which samples are collected.

Bacteriological data collected during the storms of August 27 and August 29 (Fig. 16) shows that an immediate increase in both coliforms and total bacterial counts occurs during the early portion of the streamflow rise that results from overland flow in the immediate vicinity of the stream-gauging and sampling site. Near the crest of both storms it is observed that both coliform and total bacterial counts decrease. The explanation for these observations is not apparent, although it would appear that dilution is playing a role near the crest of the storms. It is observed that the relative initial increase in total counts is somewhat greater than that for coliforms. The lesser increase in coliforms as compared to total bacteria near the beginning of the storm may be indicative of the relative availability of coliforms and the organisms measured by total count technique on the watershed surface. In accordance with theory, coliforms should be sparsely distributed over the watershed, confined primarily to fecal masses or to soil areas recently contaminated with fecal material. The organisms represented by the total bacterial count (spore formers, Pseudomonas, Proteus, etc.) however, would be expected to be found uniformly distributed over the watershed surface because of the close association many of the bacteria have with the soil habitat.

Associated with precipitation effects upon coliform and total bacterial counts are observations made upon the numbers of these organisms during streamflow recessions following the storms of August 27 and August 29. It is observed that in both storms, an increase in both coliform and total count occurs during a portion of the recession. The portion of the hydrograph during which the increases in counts were observed is composed to a large extent of groundwater stored during the rising limb of the hydrograph. These observations strongly suggest that bacteria are being released in the groundwater after having been deposited there during the high count period of the storm. The behavior of chemical components of the stream, which will be discussed later, appears to substantiate this hypothesis.

The late spring increase in coliform and total bacterial counts and streamflow (Fig. 8) is also evidence of addition of organisms to the stream from the land surface of the watershed. During this period, the water picks up much foreign material from the banks of the stream as it rises. The earliest portion of the spring runoff, however, is made up largely of melting ice and snow which accumulates along the stream banks during the fall. If there is any significant survival of bacteria in ice and snow during the winter period these organisms could make a contribution to the counts observed during the early spring. It is observed that the first rise in coliform and total bacterial counts occurred in April and coincided with a slight increment in flow (Fig. 9) which was largely due to melting of channel ice. This may be indicative that certain organisms do survive the winter in ice and snow along the banks. It was observed that the increase in total counts was proportionately greater than coliforms, which might be expected if winter survival were a factor. It has been demonstrated in our laboratory (S. M. Morrison, 1965, Personal Communication) that coliforms generally exhibit a very low survival rate in ice and can only infrequently be isolated from ice collected in the study area. Spore formers and Pseudomonas, which would be detected by the total bacterial counts, survive in ice much better than coliforms, and have been frequently isolated from ice in the study area.

Streamflow, independent of precipitation effects, appears to have a distinct effect upon the bacterial composition of the stream. The relation between coliform and total bacterial counts and streamflow during periods of stable flow as shown in Fig. 17 shows an increase of both groups of organisms up to flow values of about 200 cubic feet per second and a gradual decrease thereafter. This relationship between bacterial numbers and stream discharge during periods of stable flow is believed to be due to the elution of bacteria which are attached to the zoogleal film which covers the stream bottom. In the absence of precipitation or other variables which would add bacteria to the stream from the watershed surface, the stream bottom would be the only logical source of the organisms. Observations made over the entire river reach indicate that at low flow levels, the ratio of the wetted stream perimeter to stream discharge is quite large. It is therefore possible that the increases in bacterial numbers shown in Fig. 17 over the range of 100 to 200 cfs could be due to elution of organisms from the stream bottom. Subsequent decreases in numbers could conceivably be the result of dilution. This proposed model can be evaluated by observations made on the increases in bacterial numbers occurring between two adjacent sampling sites during periods of stable flow. Assuming that there is no die-off of bacteria between the two sampling sites and that there is a constant number of bacteria present on the wetted perimeter of the stream the following relationships would be expected to hold:

$$N \alpha WA$$

where N is the number of organisms in the river reach between the two sampling sites and WA is the wetted stream surface area between the two sites. The number of organisms present would therefore be

$$N = k.WA \qquad (1)$$

where the proportionality constant k is the number of organisms per unit area which are available for elution. The concentration of organisms (C) per unit volume (V) then becomes

$$C = \frac{k\,WA}{V} \qquad (2)$$

The wetted stream area is

$$WA = WP\,L \qquad (3)$$

where WP is the wetted stream perimeter and L is the distance between the sampling points. The volume of water between the two points is

$$V = A\,L \qquad (4)$$

where A is the cross sectional area of the stream. For any two fixed sampling sites the bacterial concentration difference between the two sites may be obtained by substituting equations 3 and 4 into equation 2 yielding

$$C = \frac{k\,WP}{A} \qquad (5)$$

It is probable that the velocity of streamflow should be included in equation 5 in that increased velocities would be expected to increase the elution of bacteria from the wetted stream surfaces. Equation 5 would then become

$$C = k \frac{WP}{A} f(v) \qquad (6)$$

where $f(v)$ is some unknown function of the velocity.

In order to test the validity of equation 6 as a means of predicting the change in bacterial numbers between two sampling sites, the coliform counts obtained at sites 8 and 8A during periods of stable flow have been analyzed. In order to obtain information regarding the nature of the stream channel between these two sampling points stream cross section profiles were established at four different locations between the two sampling sites. The mean cross sectional area and wetted perimeter were then computed for the river reach at 100 cfs discharge intervals. The mean stream velocity was then computed from the mean discharge and cross sectional area. The obtained values of wetted perimeter and cross sectional area along with the observed average coliform counts at the corresponding discharge values were then substituted in equation 5 and the equation solved for the value of k (Table 2). It can be seen that the value of k is not a constant as predicted but rather that it decreases with increasing discharge, indicating that another variable associated with discharge is involved. It was considered that this deviation of k from a constant value might be decreased by incorporating into the relationship some function of velocity as proposed in equation 6. Of the various functions of velocity which were applied to the evaluation of k in equation 6, it was observed that the first power of the velocity resulted in the most nearly constant k (Table 2). It is believed that the results presented in Table 2 are adequate to substantiate the proposed source of bacteria in a stream in the absence of addition from extraneous sources.

There are undoubtedly variables other than wetted perimeter, cross sectional area and velocity which should be taken into account in equation 6. Proper inclusion of temperature, pH and the chemical composition of the stream would likely improve the fit of the observed data with theory. Furthermore, the precision of measurement of the variables included in equation 6 would determine the goodness of fit of observation with theory. It seems that further studies of this nature would be both desirable and informative.

Fluctuations in water temperature would be suspected as being a major factor in determining variation in bacterial types and numbers in a surface water course. It appears that the previously described precipitation and flow effects, because of their magnitude, would make it impossible to determine the isolated effect of water temperature. The summer water temperature patterns observed in this study do not show a relationship to the variation in bacterial numbers. In order to study the effect of water temperature it was believed necessary to study fall and winter periods when flow was essentially constant and precipitation and surface runoff negligible. These restrictions resulted in a study over a very limited temperature range which is in no manner typical of the summer seasons, but which is quite typical of winter temperatures. The data presented for coliforms and total bacterial counts in Fig. 15 show a very definite temperature-count pattern. It is observed that coliform counts were the greatest during this restricted period at a water temperature of 3 C. Because of the low temperature involved it does not seem reasonable that this peak is the result of growth of the organisms, but rather a result of increased survival. The total count pattern shows peaks at both 3 and 4 C indicating that possibly two major groups of organisms with unique temperature relationships are involved.

It was anticipated that the location of the sampling sites would play a role in determining bacterial numbers in the river. Obviously the previously discussed precipitation, flow and temperature effects and all other variables of significance are evidenced as a single effect at any given site. There are, however, some variations between sites in this study which can be attributed directly to a single and unique characteristic of the sampling site.

An increase in coliform and total counts was observed at site 8B during both the preliminary study (Fig. 2) and the summer 1964 study (Fig. 10). The sampling site is located about 300 yards downstream from the outfall of the trout rearing station and it was therefore suspected that the organisms were being added by its effluent. During the summer of 1963 a total of 18 samples were collected from the pond outfall which resulted in a mean coliform count of 400 per 100 ml and a total bacterial count of 500,000 per 100 ml. The bacterial numbers in the trout rearing station effluent are therefore great enough to have caused the increase in counts observed at site 8B.

The river reach in which sites 8, 8A, and 8B are located is surrounded by a large subirrigated meadow which is used for cattle grazing during the summer. The river reach is fenced at both the upstream end (site 8B) and at the downstream end just above site 8. The fences prevent upstream or downstream movement of the cattle, however on one side of the stream they are free to wander laterally from the river. Each day during the summer periods, notes were made as to the location and approximate numbers of cattle in the immediate vicinity of the stream between the three above mentioned sites. During the preliminary 1963 summer study it was observed that the heaviest grazing activity occurred between sites 8 and 8A. As shown in Fig. 2 an increase in both coliform and total bacterial counts was observed between sites 8A and 8 during the 1963 summer study. During the 1964 summer study, the grazing activity was considerably less than during the summer of 1963, and was largely confined to the river reach between sites 8B and 8A. It was observed during the summer 1964 study that total bacterial counts increased between sites 8B and 8A and that coliform counts (Fig. 10) remained essentially constant between the two sites. It is apparent that the presence of cattle in the area contributed to the bacterial counts observed in the meadow area.

TABLE 2

RELATIONSHIP BETWEEN COLIFORM COUNT INCREASES BETWEEN SITES 8A AND 8 AND THE STREAM CHANNEL CHARACTERISTICS

Discharge ft^3/sec	Count Increase Coliforms/ft^3	Stream Cross Sectional Area ft^2	Wetted Perimeter ft	Mean Velocity ft/sec	k	k x v
100	53×10^4	64	38	1.58	90×10^4	142×10^4
200	36×10^4	102	49	1.95	75×10^4	145×10^4
300	32×10^4	134	59	2.26	73×10^4	165×10^4
400	22×10^4	163	64	2.45	57×10^4	140×10^4
500	18×10^4	201	70	2.49	51×10^4	127×10^4
600	15×10^4	222	73	2.70	45×10^4	122×10^4

Although it is possible that the cattle dislodged enough foreign material from the stream bank to have caused the count increases, it was observed that whenever cattle were not present in the area the counts tended to remain high. There were periods during both the summer of 1963 and 1964 during which no cattle were present in the immediate vicinity of the stream. For unknown reasons, the cattle would suddenly move back into the hills bordering the meadow and remain for three to five days. Investigation of the raw data collected during these periods shows that a decrease in counts did not occur until two to three days after the cattle had left the area, and that after the cattle returned, three to five days were required before the counts again increased. These observations do not suggest that direct addition of bacteria is of great significance. It is possible that the organisms were reaching the stream through the groundwater which underlies most of the meadow. This would be consistent with the lag in decrease or increase in counts associated with movement of cattle out of or into the meadow. It is doubtful if direct surface runoff from the meadow was involved since most of the grazing area slopes away from the river and drains into a large marsh.

Although this study was not designed to determine the ecological relationships between the bacteria and higher protists, an observation of value has been made regarding the influence of algal blooms upon the pH of the river. It was expected that pH would be a factor in determining fluctuation of bacterial numbers in the river studied, however within the pH range exhibited by the river during the study no pH effect could be demonstrated. This does not exclude the possibility of a pH effect which could be masked by the effects of flow, precipitation and temperature. The major pH changes observed in this study have been connected with algal blooms occurring over the river reach. A marked pH increase was observed between December and May of the twelve month study (Fig. 6). Several inspections of the river reach during the period of highest pH values (April and May) revealed the presence of large masses of an attached alga which was subsequently identified as a species of Vaucheria. The alga was most often found concentrated in small areas, particularly along the bank and where the water was shallow. It is not known if the pH rise occurring up to the time of visible observation of the alga was caused in the same manner, however, there were periods during which ice was absent at lower altitude sampling sites and growth could have occurred if nutrient and temperature conditions were favorable. The tendency for pH values to increase during the latter part of the 1964 summer study has also been related to algal growth, although it is not believed that Vaucheria was involved. It is observed that this increase occurred during low flow periods which would be conducive to attachment of algae and would provide improved light penetration for growth.

The behavior of pH during periods of changing flow resulting from precipitation shows essentially the same pattern in both storms analyzed. The increase in pH during the rising limb of the storms is suggestive of the addition of additional buffering salts as the result of overland flow. The increase in pH during the hydrograph recessions is consistent with that observed for the bacteriological data, suggesting a retention of buffering material in the groundwater.

Analysis for ammonia nitrogen was included in this study because of its association with bacterial metabolism. Most literature sources agree that increases in ammonia nitrogen is a good indication of microbial activity, since it is both an end product of protein decomposition and the reduced product liberated by many bacterial species from simple inorganic and organic nitrogen compounds. It has not been possible to show any direct relationship between ammonia nitrogen and bacterial numbers in this study except in the data gained from storm analyses. This relationship is likely not indicative of bacterial growth but rather of the simultaneous addition of both bacteria and ammonia from the watershed surface.

An unusual relationship was observed between ammonia nitrogen and pH, ammonia nitrogen levels apparently decreasing with increasing pH (Fig. 13). The apparent contradiction presented by this observation with chemical theory is of no significance since the nesslerization method used for measurement of ammonium nitrogen measures ammonia nitrogen in all its forms (NH_4^+, NH_3, and undissociated NH_4OH). A discussion presented by Hutchinson (1957) concerning the ability of organic material to adsorb ammonia may provide an explanation for the phenomena observed. In Hutchinson's system, ammonia is adsorbed either to suspended colloidal material, or larger particulate organic materials, the extent of adsorption decreasing with increasing pH. The net result of this phenomena would be the binding of much of the ammonia nitrogen to settleable or attached organic material, which would result in an uneven distribution of the ammonia in the stream cross section. If Hutchinson's system is operative, it is unlikely that the sampling procedure used in this study provided ammonia values which are characteristic of the stream.

Orthophosphate determinations were included in this study in an attempt to determine if a naturally occurring chemical component of the watershed could be related to variation in bacterial numbers in the stream, particularly during periods of surface runoff and precipitation. It can be observed in Figs. 13 and 14 that, during the early portion of the summer period when precipitation was slight and flow high, orthophosphate appears to be related to coliform counts and to a lesser extent to total counts. This may indicate that, when runoff is confined to a relatively constant area such as from melting snow fields, phosphate concentrations may be related to the washing off of bacteria from the land surface. Particularly large increases in orthophosphate were observed during the storms sampled. Of particular interest is the observation that in the storm of August 27 phosphate continued to increase up to the crest of the hydrograph, as contrasted with coliform counts which dropped before the crest was reached. If coliforms were being added to the stream in the same fashion as phosphate and were able to maintain their viability, it would be expected that coliforms would also increase up to the hydrograph crest. The drop in bacterial numbers is therefore suggestive that during a period of precipitation over a given land surface, that decreasing numbers of coliforms are added as the surface runoff progresses, which could logically result from surface washing and dilution. It is possible also that in the period of time required for water to reach the sampling point from the most distant point of the watershed, considerable bacterial death could occur.

Teller (1963) indicated that there was a definite precipitation (rainfall) effect upon coliform numbers but that the correlation could be established only on a long term basis. As pointed out in this study, precipitation effects are of extreme complexity and therefore it would be expected that correlations could be established only through intensive, long-term sampling and definition of all variables associated with precipitation. The greatest problem associated with establishment of a precipitation-bacteria correlation would appear to be the estimation of precipitation intensity and distribution over relatively large watersheds with adequate accuracy. Accordingly, it is invalid to attempt to establish statistical relationships between precipitation and bacterial numbers until the mechanisms are more thoroughly understood. This study has revealed that the most fruitful approach to the study of precipitation effects and determination of the mechanisms involved is probably through the intensive analysis of bacterial changes occurring during specific periods of precipitation.

Streamflow effects upon bacteria in the absence of precipitation, as presented in this study, have not previously been reported. Likewise the observation that bacteria may be released from groundwater intimately associated with a surface stream has not been reported previously, although a number of workers have evidenced suspicion that bacteria are capable of permeating both sand and other soil materials (Voelker and Heukelekian, 1960). These observations may contradict currently held views about the filtering capacity of sand and soil, and must be more clearly defined. Much information of value might be obtained from further investigation of this phenomenon because of its relationship with groundwater flow. At present there is no completely satisfactory method of determining with certainty what portion of a hydrograph is attributable to a groundwater flow. It is possible that bacteriological and chemical data such as has been presented here would provide a better method for hydrograph separation.

CHAPTER VI

SUMMARY AND CONCLUSIONS

This study, conducted on the Cache la Poudre River in Larimer County, Colorado, was designed to determine the causes of variation in bacterial numbers (coliform and total bacterial counts) of a small, unpolluted stream, with emphasis upon the effects of selected chemical and physical variables. On the basis of the data compiled in this study, the following conclusions may be drawn:

1. The addition of bacteria to the stream from the land surfaces of the watershed during short duration summer rainstorms is the most important cause of variation in bacterial numbers in the stream studied.

2. The chemical variables studied (pH, ammonia, and orthophosphate) also vary with precipitation and therefore cannot be directly related to bacterial numbers.

3. During periods of stable streamflow, and in the absence of precipitation, bacterial numbers can be related to the size of the water-stream bed contact surface.

4. During periods of increasing streamflow resulting from precipitation, both the bacteria and chemical compounds studied are deposited in the groundwater which is closely associated with the stream, and are later released during the streamflow recession.

5. Small changes in water temperature in the 0 - 5.5 C range are responsible for variation in bacterial numbers during the winter.

6. Cattle, located in marshy, subirrigated meadow areas adjacent to the stream studied, contribute to the bacterial density of the stream.

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Key Words: Hydrology, Microbiology, Fresh Water, Environment, Stream, Bacterial Fluctuation.

Abstract: A study was conducted on the Cache la Poudre River in Larimer County Colorado between June 1963 and September 1964 to determine the causes of variation in the bacterial quality of unpolluted surface waters. Water samples were analyzed daily during the summer and 2 to 3 times per week during the winter for the presence of coliforms and total bacteria by the membrane filter procedure. Selected environmental variables including water and air temperatures, precipitation, pH, orthophosphate, and ammonia nitrogen were monitored. Analysis of the data indicated that overland flow resulting from summer rainstorms was the most important factor involved in variation of coliform, total bacterial counts and chemical variables studied. Continuous sampling during periods of increasing streamflow resulting from precipitation determined that the bacterial and chemical variables showed an immediate increase at the beginning of overland flow, followed by a decrease near the crest of the storm hydrograph. During the early portion of the streamflow increase, both bacteria and the chemical compounds studied were being deposited in the groundwater associated with the stream. Elution from the groundwater was observed during the streamflow recession. During periods of stable streamflow, it was shown that increases in coliform counts occurring between adjacent sampling sites were a function of wetted stream perimeter, dilution and stream velocity. Small changes in water temperatures were observed to be related to variation of coliform and total bacterial counts during the winter. Cattle, grazing in a marshy, subirrigated meadow adjacent to the stream studied, caused bacterial increases in the stream.

Reference: Morrison, S. M., and J. F. Fair, Colorado State University (Microbiology), Hydrology Papers No. 13 (April 1966), "Influence of Environment on Stream Microbial Dynamics."

Key Words: Hydrology, Microbiology, Fresh Water, Environment, Stream, Bacterial Fluctuation.

Abstract: A study was conducted on the Cache la Poudre River in Larimer County Colorado between June 1963 and September 1964 to determine the causes of variation in the bacterial quality of unpolluted surface waters. Water samples were analyzed daily during the summer and 2 to 3 times per week during the winter for the presence of coliforms and total bacteria by the membrane filter procedure. Selected environmental variables including water and air temperatures, precipitation, pH, orthophosphate, and ammonia nitrogen were monitored. Analysis of the data indicated that overland flow resulting from summer rainstorms was the most important factor involved in variation of coliform, total bacterial counts and chemical variables studied. Continuous sampling during periods of increasing streamflow resulting from precipitation determined that the bacterial and chemical variables showed an immediate increase at the beginning of overland flow, followed by a decrease near the crest of the storm hydrograph. During the early portion of the streamflow increase, both bacteria and the chemical compounds studied were being deposited in the groundwater associated with the stream. Elution from the groundwater was observed during the streamflow recession. During periods of stable streamflow, it was shown that increases in coliform counts occurring between adjacent sampling sites were a function of wetted stream perimeter, dilution and stream velocity. Small changes in water temperatures were observed to be related to variation of coliform and total bacterial counts during the winter. Cattle, grazing in a marshy, subirrigated meadow adjacent to the stream studied, caused bacterial increases in the stream.

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