



A microbiological and chemical investigation of the effects of multiple use on water quality of high mountain watersheds
by Gary Kent Bissonnette

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Microbiology
Montana State University
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Abstract:

During the summers of 1969 and 1970 bacteriological determinations of coliform, enterococcal, and standard plate counts were performed on two high mountain drainage systems: Hyalite, a watershed open for public use and Mystic, a watershed that had been closed from 1917 until its opening for limited human activity in the spring of 1970.

The 1969 bacteriological results agreed with previous studies in that coliform densities were found to be greater in the closed watershed than found in the open watershed. In 1970 coliform densities decreased considerably to values that were quite similar to numbers observed in the open watershed. Coliform densities were found to be high in the South Fork of the Bozeman Creek in 1969, while these densities decreased considerably in 1970.

Chemical and physical analyses included air temperature, water temperature, pH, conductivity, turbidity, calcium, magnesium, sodium, potassium, bicarbonate, sulfate, chloride, nitrite, nitrate, and orthophosphate. These analyses indicated that the chemical and physical make-up of the two drainages did not adequately account for differing bacterial densities.

Serological studies on *Escherichia coli* isolated from water and wild animal (bear and elk) fecal droppings indicated the strong influence that wild game animals had on determining bacterial densities in the closed watershed.

It was concluded that the cause of significant changes in the closed watershed were a direct result of the influences of its main tributary, the South Fork. Wild game animal populations which inhabited the South Fork area in 1969 were the primary cause of the high bacterial contamination. The opening of the closed watershed for limited public use and an extensive logging operation in 1970 coincided with decreasing bacterial densities in this drainage. The influence exerted by the South Fork on bacterial numbers in the closed (Mystic) watershed was a result of its direct entrance into the Bozeman Creek below the Mystic reservoir.

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A MICROBIOLOGICAL AND CHEMICAL INVESTIGATION OF THE
EFFECTS OF MULTIPLE USE ON WATER QUALITY
OF HIGH MOUNTAIN WATERSHEDS

by

GARY KENT BISSONNETTE

A thesis submitted to the Graduate Faculty in partial
fulfillment of the requirements for the degree

of

MASTER OF SCIENCE


in

Microbiology

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Bozeman, Montana

June, 1971

ACKNOWLEDGEMENTS

The author would like to thank Dr. David G. Stuart for his guidance and assistance throughout the course of this study. Sincere thanks are also due Drs. James J. Jezeski, Richard J. Graham, and William G. Walter for their careful review of the manuscript and assistance in its preparation.

The cooperation of Thomas D. Goodrich in the collection of field samples and laboratory assistance is gratefully acknowledged. Thanks are also due Sandra Hanley for her assistance in laboratory bacteriological analyses.

This investigation was supported by Montana University Joint Water Resources Research Center (Helmer Holje, Director) Grant

OWRR A-027 Mont.

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ABSTRACT

During the summers of 1969 and 1970 bacteriological determinations of coliform, enterococcal, and standard plate counts were performed on two high mountain drainage systems; Hyalite, a watershed open for public use and Mystic, a watershed that had been closed from 1917 until its opening for limited human activity in the spring of 1970.

The 1969 bacteriological results agreed with previous studies in that coliform densities were found to be greater in the closed watershed than found in the open watershed. In 1970 coliform densities decreased considerably to values that were quite similar to numbers observed in the open watershed. Coliform densities were found to be high in the South Fork of the Bozeman Creek in 1969, while these densities decreased considerably in 1970.

Chemical and physical analyses included air temperature, water temperature, pH, conductivity, turbidity, calcium, magnesium, sodium, potassium, bicarbonate, sulfate, chloride, nitrite, nitrate, and orthophosphate. These analyses indicated that the chemical and physical make-up of the two drainages did not adequately account for differing bacterial densities.

Serological studies on Escherichia coli isolates from water and wild animal (bear and elk) fecal droppings indicated the strong influence that wild game animals had on determining bacterial densities in the closed watershed.

It was concluded that the cause of significant changes in the closed watershed were a direct result of the influences of its main tributary, the South Fork. Wild game animal populations which inhabited the South Fork area in 1969 were the primary cause of the high bacterial contamination. The opening of the closed watershed for limited public use and an extensive logging operation in 1970 coincided with decreasing bacterial densities in this drainage. The influence exerted by the South Fork on bacterial numbers in the closed (Mystic) watershed was a result of its direct entrance into the Bozeman Creek below the Mystic reservoir.

Chapter 1

INTRODUCTION

A large amount of the water supply for municipal, agricultural, industrial, and recreational purposes comes from high mountain watersheds that are relatively unused at present. With increasing demands for water, it is important that adequate knowledge of the natural characteristics of these supplies be obtained. High mountain watersheds, such as those in the northwestern United States, contain much of the water considered to be in a near virgin state. In the past, the principal investigations of water quality have been concerned with surface water that was considered to be definitely polluted; there is, however, limited knowledge concerning the composition of high quality waters. Perhaps more importantly, there is little known about what actually does constitute "high quality water."

Since there is increasing pressure for the use of watersheds for timber, mining, grazing, recreation, etc., it is of extreme importance to obtain a better knowledge of the natural characteristics of these water supplies. Specifically, a thorough knowledge of natural, pristine watersheds must be obtained in order to understand the impact of later land use on water quality.

Recently, much interest has developed concerning the impact of land use on water quality. In 1963, Teller³⁷ concluded that there was insufficient information to determine what the natural

quality of water should be, and to what extent fluctuations in bacterial numbers in a stream can be attributed to natural or man-made causes. Van Nierop³⁸ has shown that public use of reservoirs and municipal watersheds is possible without drastically affecting water quality, provided that proper sanitary practices are strictly observed. Carswell et al.⁷ have examined the arguments both for and against the use of public watersheds for recreational purposes. In their study of five watersheds they concluded that little or no deterioration in bacterial water quality occurred when recreation was permitted in or around water supplies. Also, they state that even when a rise in indicator organisms did occur, the bacterial content was within limits that permit removal by existing water treatment technology. Geldreich¹³ has examined bacteriological parameters which may be used in quantitating effects of recreational use of water supplies and has attempted to establish the sanitary significance of total coliforms, fecal coliforms, and fecal streptococci.

The subject of this thesis involves the study of two watersheds serving the city of Bozeman, Montana. Specifically, the investigation concerns water quality of two high mountain watershed areas: Middle Creek drainage (Hyalite), an open watershed that is used extensively for numerous recreational activities, and the Bozeman Creek drainage (Mystic) which has been closed to public entry from 1917 until the spring of 1970. In March of 1970, the Mystic watershed area was.

opened for limited activities. This unique situation permits a comparison of the "natural quality" of two different sources of mountain water, i.e., a watershed used for recreation and a watershed protected from human use. In essence, this study allows for possible conclusions about the effect of man's activities, such as logging and recreation, on water quality. Equally important, it enables an evaluation of the effect of wild game animals on the composition of waters from high mountain elevations.

Statement of Purpose

In a previous study by Walter and Bottman,³⁹ results indicated that the Mystic area (closed watershed) had higher coliform and enterococcal counts than the Hyalite area (open watershed). In light of these findings, the purposes of the present study are many-fold:

1. To gain a better knowledge of what actually constitutes natural quality water of high elevation mountain watersheds, both chemically and bacteriologically.
2. To postulate a possible explanation as to the reason for the existence of higher bacterial numbers in the closed watershed.
3. To examine the possibility of tracing microbial pollution to its source by means of serological methods.
4. To gain an insight as to the effects of logging, recreation, and wild animals on water quality in mountain watersheds.

5. To determine whether there are statistical relationships between bacterial numbers and the physical and chemical aquatic environment in these two high elevation mountain watersheds.

Chapter 2.

LITERATURE REVIEW

In determining quality of waters which normally contain low bacterial numbers it is of special importance to examine the relationships between bacteria and the physical and chemical environment. The determination of bacterial and chemical indicators of pollution in water has resulted in qualitative and quantitative standard methods.^{1,10,23,35} The evaluation of results obtained by using these bacteriological methods has been extensively examined by a number of different researchers as described below.

Bacteria are introduced into waters both naturally and by man and his activities. The coliform organisms have been used as one of the primary indicators of pollution. According to the Standard Methods for the Examination of Water and Wastewater,¹ the coliform group includes all of the aerobic and facultatively anaerobic, Gram-negative, nonsporeforming, rod-shaped bacilli which ferment lactose with gas formation within 48 hours. These coliform organisms are present in soil, on plants, and in the feces of many warm-blooded animals. Schuettpelz³⁴ states that coliform bacteria have the following advantages for use as indicator organisms: (1) coliforms are constantly found in the human intestine in large numbers; (2) the fate of the coliform organism in water reasonably reflects that of pathogenic bacteria, although the coliform bacteria will normally

live longer than intestinal pathogens; (3) the coliform organism is easy to isolate and enumerate in the laboratory; and (4) coliforms are not normally pathogenic and are easy to handle. Schuettpelz further states that the specific group called fecal coliforms indicates a much better relation to true contamination than that of total coliforms. Geldreich et al.^{12,13,15,17} states that fecal coliforms may be the best tool to detect evidence of fecal pollution from warm-blooded animals in polluted water. Kunkle²⁶ also concluded that the fecal coliform group was the best index for pollution surveillance in an agricultural watershed.

The use of enterococci as a bacterial pollution indicator has become accepted as a standard method. Winslow et al.,⁴⁰ as early as 1902, reported observing that streptococci were present consistently in the feces of all warm-blooded animals and in the water associated with such animal discharges. However, the true sanitary significance of fecal streptococci has been confused by controversies concerning procedures for quantitation, definition of the group, and differing concepts as to their occurrence in the water environment and in warm-blooded fecal discharges. Geldreich et al.¹⁸ questions the sanitary significance of Streptococcus faecalis var. liquifaciens and atypical Streptococcus faecalis and implies that the detection of S. bovis and S. equinis, which are not found in human feces but are specific indicators of non-human animal pollution, may be a more sensitive test.

of sanitary significance. It is also stated that a valuable application of the fecal streptococci indicator system is through fecal coliform to fecal streptococci ratios which would aid in the determination of sources of fecal discharge into streams. A high ratio indicates human origin, while a low ratio indicates animal origin.

The sanitary significance of fecal streptococci was also examined by Burman⁶ who additionally submitted evidence of the relatively greater ability of fecal streptococci than Escherichia coli to survive in various natural and antagonistic environments. In a study concerning bacterial survivability by Benson⁴ the results indicated that Streptococcus faecalis was as good, but not necessarily a better indicator of recent and dangerous pollution in a cold, fresh water environment. Halton et al.²⁵ also examined survivability of coliforms concluding that low sea water temperatures favor the survival of large numbers of E. coli.

Sources of bacterial indicators of pollution, such as coliforms and enterococci, are extremely diverse. Mundt³² determined the presence of enterococci in a truly wild environment, the Great Smoky Mountains National Park. Enterococci were isolated from most specimens of bats and from carnivorous mammals, such as fox, bear, racoon, boar, and skunk. The distribution of coliform bacteria in the feces of such warm-blooded animals as humans, cows, pigs, sheep, chickens, turkeys, and ducks has been investigated by Geldreich et al.¹⁵ Examination of wild animal fecal droppings (elk, moose, bear) by Goodrich et al.²⁴

in a high mountain watershed indicated fecal pollution was primarily from a non-human source, including both fecal coliforms and fecal streptococci.

Bacterial indicators of pollution are also found in soil and on vegetation. Geldreich et al.¹⁶ surveyed the fecal coli-aerogenes flora of soils from various geographical areas. The occurrence of enterococci on plant materials, in spite of their sanitary significance, indicated to Mundt et al.³³ that enterococci do occur naturally on plant surfaces in an agricultural and an inhabited environment, as well as in soils under cultivation or in the vicinity of cultivation. Geldreich et al.¹⁷ conducted a study considering the sanitary significance of coliforms, fecal coliforms, and fecal streptococci isolated from a number of species of plants and a variety of samples of insects. Their findings supported the use of the fecal coliform test for surface water quality evaluations.

An important criterion for the existence of poor quality water is through the recovery of bacterial pathogens from supposedly high quality water. An investigation in a high quality mountain stream by Fair and Morrison⁹ resulted in the isolation of enteric pathogens, specifically eleven isolates of the genus Salmonella and 51 isolates of organisms belonging to the Arizona group. The authors state that the isolation of potentially pathogenic bacteria in waters of remote mountain regions indicates that naturally occurring potable surface

water does not exist. They also postulate that the presence of these potentially pathogenic bacteria may be the result of contamination by wild or domestic animals in the watershed area.

Although coliform organisms indicate the possibility of the presence of pathogens, Gallagher and Spino¹¹ showed little apparent correlation between levels of total or fecal coliforms and the isolation of salmonellae. The authors reason that salmonellae are persistent under conditions which may be adverse to survival of fecal coliforms. In the Northwest Watershed Project³⁶ pathogenic enterobacteriaceae were found in 28% of the samples collected at the most downstream sampling station although the fecal coliform density was always less than 100/100 ml.

A common problem encountered in bacteriological studies of aquatic systems is to definitely identify the source of bacterial pollution. In recent years Glantz and others^{19,20,21,22} have demonstrated the value of serological typing procedures for tracing the source of bacterial pollution. Specifically, Glantz²¹ isolated different E. coli serogroups at various sampling points on a stream and used this information to trace these serogroups to their probable upstream source. Support of serological typing procedures for determining microbial pollution was also performed by Bissonnette et al.⁵ in the examination of high mountain watersheds. Similar serological reactions were observed in E. coli isolates obtained both

from water and wild animal (bear and elk) fecal droppings in the watershed areas, indicating that the microbial pollution might possibly be traced to wild animals inhabiting the surrounding area of the streams.

Water quality in high elevation mountain watersheds is affected by recreation, grazing, and timber management. As these watersheds are developed for a variety of uses, water quality of the streams is commonly affected. However, there is a dearth of knowledge regarding cycles and variability of bacteria in mountain stream environments. Equally lacking is information concerning the relationships of the microbiology to physical and chemical environmental factors. Also, it is not clear whether the presence of coliforms encountered in water of normally good quality (such as high mountain streams) is in fact an indication of recent fecal contamination.

The environmental influences on stream microbial dynamics have been extensively examined by Morrison and Fair.³¹ They determined the causes of variation in bacterial numbers of an unpolluted mountain stream, with emphasis upon the effects of selected chemical and physical variables. They concluded that summer rainstorms washing bacteria into the stream caused the greatest variations in bacterial numbers. Also, the chemical factors (pH, ammonia, and orthophosphate) varied with precipitation and therefore cannot be directly related to bacterial numbers. Differences in bacterial

numbers during the winter were attributed to small changes in water temperature in the 0 - 5.5 C range.

Proper sampling techniques and interpretation of data from high quality mountain water have been provided by Kunkle and Meiman.²⁸ They observed a daily cycle for indicator organisms; evening maximums in concentrations preceded by afternoon minimums, while morning bacterial counts usually fell between the two. It was postulated that rising stream stages of early evening caused stream bank "flushing" to account for evening maximums. Also, maximum coliform and fecal coliform numbers were observed in the spring "flushing" or runoff period as well as during summer storm stages. Additionally, water temperature was inversely related to bacterial counts. High bacterial yields from a rural watershed were also attributed to storm runoff by Kunkle²⁶ in a Vermont stream study. Schuettpelz³⁴ found that coliform bacteria are especially common during periods following rainfall when there are large amounts of surface runoff. Geldreich et al.¹⁴ have also examined the bacteriological aspects of storm water runoff and found similar results.

Kittrell and Furfari²⁹ postulated that physical characteristics of a stream may be a prime factor in determining coliform densities. They agree that high densities of coliform bacteria in streams usually follow runoff from rainfall. They also conclude that there is seasonal variance of coliform numbers with temperature, as well as the fact

that turbidity appears to affect rates of bacterial decrease through sedimentation. These authors place much emphasis on the presence or absence of riffle areas as being an important factor in stream self-purification, due to the action of attached predators.

A water quality investigation of mountain watersheds in Colorado by Kunkle and Meiman²⁷ indicated that physical parameters of the stream were closely related to bacterial numbers. Bacterial groups were especially dependent upon the "flushing" effect of the runoff from snowmelt and rain, summer storms, or irrigation. Observations of surface runoff during thunderstorms indicated most of the storm sediment was contributed by roads in the watershed area. Additionally, there was no indication that the level of human use in campgrounds, picnic areas, or cabin sites increased sediment in the streams. The authors observed numerous significant correlations of bacterial groups to pH, turbidity, and suspended sediment. The coliforms, fecal coliforms, and fecal streptococci were positively related to flow, turbidity, and suspended sediment and negatively related to pH at most sites on the watershed.

Interesting results were provided by Lee et al.³⁰ concerning a study of three northwestern United States watersheds. They observed that during periods of high flow, indicator organism densities were lower and that they reached their peaks during low flow. They concluded that, although some indicator organisms may be washing into the stream during times of runoff, the bacterial densities were

actually being diluted during periods of high streamflow. Additionally, peak turbidities occurred during times of high streamflow, but the indicator organism densities were low at this time. The dominant factor contributing to fecal coliform densities was attributed to the presence of a large animal population in all three watersheds.

Chapter 3

DESCRIPTION OF THE STUDY AREA

Two high mountain watersheds provide a major portion of the municipal water supply for the approximately 18,000 people of Bozeman, Montana. The watershed areas are located about ten miles south of the city (Figure 1). This study involves the examination of these two watersheds - an open watershed in the Hyalite area, and a closed watershed in the Mystic area.

Separated by a single mountain ridge, Bozeman Creek (Mystic) and Middle Creek (Hyalite) provide about 90% of Bozeman's water supply and are among the principal tributaries of the East Gallatin River.

The Hyalite reservoir receives water draining 5,760 acres and stores 8,000 acre-feet of water. The entire watershed covers 30,080 acres and is completely open to the public for recreational purposes, including boating, swimming, fishing, hunting, camping, and mechanized vehicular travel. Logging has been conducted in the area for several years.

With a total watershed area of 28,160 acres, the Mystic reservoir receives water from 2,880 acres and stores 675 acre-feet of water. This watershed has been closed to the public since 1917 but was opened to foot and horseback travel in March of 1970, as well as for camping, fishing, and hunting. However, extensive logging has taken place in

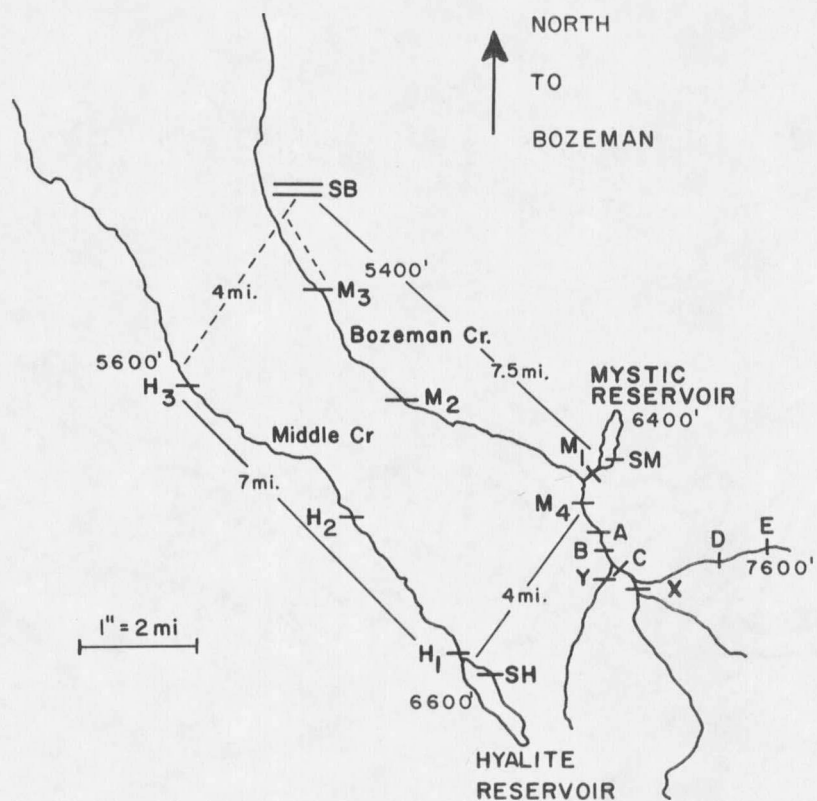


Figure 1. Elevations and Sampling Sites of Mystic (M) and Hyalite (H) Watersheds, Surface of Reservoir (S), Reservoir Outlet (1) Halfway Point (2), Diversion Dam (3), Settling Basin (SB), and the South Fork Sites: M₄, A, B, C, D, E, and South Fork Tributaries X and Y

recent years and mechanized vehicular travel is permitted for this purpose. Most of the present logging activity is in the South Fork of Bozeman Creek.

Being adjacent mountain watersheds, the Hyalite and Mystic streams are similar in many respects: viz., they originate in high elevation snowmelt areas, are impounded to form mountain reservoirs, and the water for the municipal water supply is drawn off at a diversion dam just before the stream leaves the mountain canyon and flows out onto the valley floor.

The various sampling sites of both watersheds are indicated in Figure 1 and are designated as follows:

Site SM - Surface of the Mystic reservoir

Site M₁ - Mystic reservoir outlet

Site M₂ - Halfway point of Bozeman Creek

Site M₃ - Diversion dam of Bozeman Creek

Site SH - Surface of the Hyalite reservoir

Site H₁ - Hyalite reservoir outlet

Site H₂ - Halfway point of Middle Creek

Site H₃ - Diversion dam of Middle Creek

Site SB - Settling basin

Sites M₄, A, B, C, D, E, X, and Y - sampling points on the South Fork drainage of Bozeman Creek.

Chapter 4

METHODS AND MATERIALS

Sampling - Bacteriological

Weekly samples were collected in two-liter sterile nalgene bottles from sites shown in Figure 1 during the summer months of 1969 and 1970. Additionally, periodic sampling of water from the two diversion dams and the settling basin was carried out from January 1970 through May 1970, as well as during October and November of 1970. The sampling of the ten sites in the Mystic and Hyalite watersheds (Figure 1) was performed on one day, while on the following day sampling was from eight sites on the South Fork drainage of Bozeman Creek. Routinely, the first sample was collected from the surface of Mystic reservoir about 9 a.m. and the others subsequently at about the same time on each occasion. The samples were always taken at the same sites in the stream. When sampling the South Fork area, the first sample was taken at site E and subsequently downstream to site M₄. The samples were returned by 1 p.m. to the University laboratory for testing and beginning of analyses. All samples were generally tested within four hours of collection. All samples were stored in a Coleman cooler immediately after collection and held at approximately 5-10 C until testing.

Standard Plate Count

The procedures recommended in the 1965 edition of Standard Methods for the Examination of Water and Wastewater¹ were followed. Dilutions used for inoculation of standard petri dishes (100 X 15 mm) included 10^{-2} , 10^{-1} , and 10^0 . In addition, water and agar controls were prepared. The medium of choice was tryptone glucose extract (TGE) agar (Difco). After solidification of the agar, the plates were inverted and incubated at 35 C for 48 hr. during the summer of 1969 analysis and at 20 C for five days during the 1970 analysis. Plates were then counted with the aid of a New Brunswick Scientific Colony Counter and reported as SPC/ml.

Coliforms

The membrane filter technique as described in Standard Methods¹ was used in determining coliform numbers. All samples were thoroughly shaken before withdrawing 50, 10, or 1 ml of water for filtration. The 1 ml samples were placed in a 99 ml sterile phosphate buffer dilution blank before pouring through the sterile membrane filters (Millipore Filter type HAWG 047 S0 with a pore size of 0.45 micron). After filtration, the membrane filter was aseptically rolled onto pads (Millipore) that had been previously saturated with 2.0 ml of m-coliform broth (BBL) in disposable 50 X 12 mm, sterile, plastic petri dishes (Falcon Plastics). Filter and water controls were also performed.

The dishes were inverted and incubated at 35 C for 40-48 hr. All organisms which produced a dark purple-green colony with a metallic sheen within the incubation period were considered to be members of the coliform group. A viewing scope and incident light were used to facilitate counting of the coliforms. These were reported as numbers of coliform bacteria/100 ml.

To confirm the presence of coliforms, green metallic colonies were transferred from the m-coliform medium to brilliant green lactose bile (BGLB) broth (Difco) and considered positive if gas was produced within 48 hr. at 35 C. One-half the number of metallic green colonies counted on the 10 or 50 ml plate, up to a five per plate maximum, were used to inoculate the BGLB broth. Tubes exhibiting gas production were then streaked for differentiation and isolation on eosin methylene blue (EMB) agar (Difco) plates, inverted, and incubated at 35 C for 24 hr. Representative colonies were then transferred to 0.5 ml sterile phosphate buffered water to form a dense suspension of cells. This was inoculated into EC medium (Difco) for incubation at 44 ± 0.1 C for 24 hr. and into IMViC media (Difco). Cultures producing gas from the EC medium were considered to be fecal coliform bacteria. The IMViC tests were all incubated at 35 C for the times required.¹ These tests allowed for differentiation among E. coli, Enterobacter (Aerobacter) aerogenes, and intermediates. Interpretation of results was determined according to Standard Methods.¹

Enterococci

The membrane filter technique was also used in determining enterococcal counts as described in Standard Methods.¹ After filtration of the appropriate volume of water (100 to 500 ml), the membrane filters (Millipore) were aseptically placed in 60 X 15 mm disposable sterile, plastic petri dishes (Falcon) containing m-enterococcus agar (Difco). Filter and sterile water controls were also prepared. The plates were inverted and incubated at 35 C for 40-48 hr. Typical dark red to pink colonies were counted using a viewing scope and incident light. Counts were reported as numbers of enterococci/100 ml.

Representative colonies - one-half the number of colonies counted per plate, up to a five per plate maximum - were inoculated into 10 ml of ethyl violet azide (EVA) broth (Difco) and observed for a purple button and/or turbidity after 48 hr. of incubation at 35 C. Cultures giving positive reactions in EVA broth were streaked onto m-enterococcus agar for isolation, inverted, and incubated at 35 C for 48 hr.

Differentiation of enterococci to species was based on a schema presented by Ayres et al.² Isolated colonies from m-enterococcus agar were inoculated into 7 ml of peptone broth (Difco) and incubated for 5 days at 35 C. Production of ammonia from arginine was determined by the spot plate method using Nessler's reagent. If the test were negative, the culture was inoculated into 7 ml of lactose broth, consisting of nutrient broth (BBL), 0.5% yeast extract, 1% lactose

(Difco), and 0.0015% bromo cresol purple. Those cultures showing production of ammonia were inoculated onto tryptic soy agar (Difco) plates containing 1.5% gelatin (Difco) for 6 days, potassium tellurite agar plates for 48 hr. (TGE agar with the addition of 0.4% glucose and 0.04% potassium tellurite), mannitol broth for 48 hr. (nutrient broth with the addition of 0.5% yeast extract, 1% mannitol, and 0.0015% bromo cresol purple), 5% horse blood agar for 24 hr., and nutrient agar (Difco) slants for 24 hr. All were incubated at 35 C.

Animal Dropping Examination from Closed Watershed

Periodic sampling of animal droppings from bear, elk, moose, and deer were made in the closed Mystic area, especially in the South Fork drainage area. The use of trail bikes in 1969 enabled access into remote areas to obtain fresh droppings. Fecal samples were collected with sterile applicator sticks and placed in 35 ml vials containing four types of media respectively: BGLB broth for detecting coliform bacteria; azide dextrose broth (Difco) for enterococci; selenite broth (Difco) for isolating salmonellae, shigellae, and other Gram-negative enteric bacteria; and lactose broth for enrichment of enterobacteria. After overnight incubation at 35 C, the samples were subcultured into tubes of appropriate fresh media.

All BGLB broth tubes showing fermentation were streaked onto EMB agar for 48 hr. incubation at 35 C. The IMViC reactions were used for final identification and differentiation of the coliform organisms.

All azide dextrose broth tubes showing cloudiness were treated as previously described for enterococci beginning with isolation on m-enterococcus agar.

All selenite broth tubes showing marked turbidity were streaked onto both MacConkey (Difco) and EMB agar, inverted, and incubated at 35 C for 24 hr. Isolated colonies from MacConkey agar were transferred to Kliger iron agar (Difco) slants and examined after 12 and 24 hr. at 35 C incubation. Urea broth (Difco) was inoculated from Kliger iron agar and read at 8 and 24 hr. at 35 C incubation. Also, dulcitol broth (nutrient broth with 1% dulcitol and 0.0015% bromo cresol purple) and lysine decarboxylase medium (Difco) were inoculated and incubated at 35 C. Dulcitol broth tubes were read after 48 hr. and lysine decarboxylase after 24 hr.

All lactose broth tubes showing growth were first subcultured into selenite broth and subsequently treated as previously described with MacConkey agar, EMB agar, Kliger iron agar, urea broth, dulcitol broth, and lysine decarboxylase medium.

Serological Examination

Serological examination of E. coli from water samples and isolates from animal droppings (bear, elk, and moose) were performed during the summer of 1969. Additionally, suspected Salmonella and Shigella-like organisms isolated from animal droppings were also reacted with

corresponding antisera. The techniques used for the determination of E. coli OB and OK antigens were those advocated by the manufacturer of the antisera (Difco).

The E. coli cultures from both water and fecal samples were first transferred from stock culture agar to veal-infusion agar (Difco) slants for 24 hr. incubation at 35 C. Dense suspensions of E. coli were then prepared by mixing the growth from veal-infusion agar slants in 0.5 ml of 0.85% saline. Each suspension was tested using three polyvalent antisera (A, B, C) employing the slide agglutination technique. If agglutination occurred, a portion of the suspension was boiled for one hr. Both heated and unheated suspensions were tested on the OB and OK individual antisera (which comprised the polyvalent antiserum). The Difco antisera employed for E. coli are those shown in Tables 7, 8, 9, 10, 11, and 12.

A similar procedure was used for the serological examination of Salmonella and Shigella-like organisms. Sera employed for Salmonella were: Poly. A-1; Group A Factor 2; Group B Factors 4, 5; Group C₁ Factor 7; Group C₂ Factor 8; Group D Factor 9; Group E₁ Factor 10; Group E₂ Factor 15; Group E₄ Factor 19; Group F Factor 11; Group G Factors 13, 22; Group H Factors 14, 24; Group I Factor 16; and Vi. Shigella sera employed were polygroups A, A₁, B, C, C₁, C₂, D, and Alkalescens-Dispar group.

Sampling - Chemical

The water remaining after performing the bacteriological tests was used for chemical analyses; however, an additional sample was also taken at each site for determining orthophosphate, total alkalinity, nitrate, and nitrite. This involved rinsing a 250 ml pyrex glass stoppered bottle in the water and then filling to overflow before inserting the stopper. Special precautions were taken so as to not enable the incorporation of gas bubbles within the bottles.

At the time of collection, water and air temperature were recorded. A portable Sargent-Welch pH meter was used with a thermocompensator for on-site pH readings. Additionally, conductivity measurements were recorded in the natural water at the time of collection or upon return to the laboratory using a Lab Line Lectro MHO-meter (Model MC-1, Mark IV).

Water Chemistry Analyses

In the laboratory, a 100 ml sample was taken from the glass bottle for a total alkalinity determination according to Standard Methods.¹ The remaining water in the glass bottles was then filtered through membrane filters (Millipore) and used for orthophosphate, nitrate, and nitrite determinations.

Total hardness, calcium, magnesium, chloride, sulfate, and

turbidity were also determined as described by the American Public Health Association.¹

The colorimetric equipment used in the various analyses was either a Bausch and Lomb "Spectronic 20", Beckman Model B Spectrophotometer, or a Klett-Summerson colorimeter.

Potassium and sodium were determined by flame emission utilizing a Beckman DU Flame Spectrophotometer, following the procedures given in the Beckman Instruction Manual #334-A (March, 1957).

Total alkalinity, orthophosphate, nitrate, and nitrite determinations were made within 8 hr. after collection of samples. The remaining analyses were routinely performed within the following 72 hr.

Chapter 5

RESULTS

Quantitative Bacteriological Studies of Water Samples

The numbers of bacteria obtained from water samples collected during the summers of 1968, 1969 and 1970 from the Mystic and Hyalite watersheds are summarized in Tables 1, 2, and 3. Ranges and geometric means are given for coliform, enterococcal, and standard plate counts at each site. Geometric means were used in order to eliminate the large variations that occurred throughout the summer months. These geometric means were then used to produce a "bacteriological profile" of the streams (Figures 2, 3, and 4). These profiles were based on eight, nine, and thirteen weekly collections for the respective years.

During 1968 and 1969 no great difference was observed between the two watersheds with regard to standard plate counts. In 1970, a 5 to 10-fold increase in total organisms was obtained at each site. This increase can be attributed to incubating plates at 20 C for five days, whereas plates were incubated at 35 C for 48 hours during the 1968 and 1969 seasons. The lower temperature (20 C) was used after it had been determined that this procedure gave more realistic counts, since the water temperature of these mountain streams was quite cold. Once again, it was observed that the standard plate counts were essentially the same in both watersheds during 1970.

The coliform "profiles" for 1968 and 1969 indicate greater numbers

Table 1. Comparison of Numbers of Bacteria Obtained from 8 Weekly Water Sample Collections at Different Sites in Mystic (Closed) and Hyalite (Open) Watersheds During the Summer of 1968

SITE*	MYSTIC		HYALITE	
	RANGE	GEOMETRIC MEAN	RANGE	GEOMETRIC MEAN
COLIFORMS/100 ML				
S	0-570	26	0-4600	5
1	0-160	12	10-80	18
2	0-280	76	8-130	42
3	67-540	227	6-220	63
SB	10-270	35		
ENTEROCOCCI/100 ML				
S	0-13	5	0-16	2
1	0-15	2	0-3	1
2	3-38	12	3-24	10
3	9-87	27	5-26	13
SB	3-116	13		
**SPC/ML @ 35 C				
S	32-6190	291	31-450	199
1	14-325	82	27-415	95
2	42-640	93	34-151	88
3	51-294	102	49-159	82
SB	10-283	85		

* S = surface of reservoir; 1= reservoir outlet; 2= halfway point
3 = diversion dam

**SPC = Standard Plate Count

Table 2. Comparison of Numbers of Bacteria Obtained From 9 Weekly Water Sample Collections at Different Sites in Mystic (closed) and Hyalite (open) Watersheds During the Summer of 1969.

SITE*	MYSTIC		HYALITE	
	RANGE	GEOMETRIC MEAN	RANGE	GEOMETRIC MEAN
COLIFORMS/100 ML				
S	0-170	7	0-60	1
1	0-70	1	0-60	2
2	40-540	121	10-130	65
3	90-930	217	10-310	63
SB	0-200	25		
ENTEROCOCCI/100 ML				
S	0-998	6	0-48	1
1	0-3	1	0-2	1
2	1-135	15	4-65	13
3	4-117	32	4-99	39
SB	1-101	19		
**SPC/ML @ 35 C				
S	39-860	144	65-8000	447
1	3-62	23	10-5870	69
2	15-308	77	26-1560	113
3	29-277	76	26-760	124
SB	26-446	136		

* S = surface of reservoir; 1 = reservoir outlet; 2 = halfway point
3 = diversion dam

**SPC = Standard Plate Count

Table 3. Comparison of Numbers of Bacteria Obtained From 13 Weekly Water Sample Collections at Different Sites in Mystic (closed) and Hyalite (open) Watersheds During the Summer of 1970

SITE*	MYSTIC		HYALITE	
	RANGE	GEOMETRIC MEAN	RANGE	GEOMETRIC MEAN
COLIFORMS/100 ML				
S	0-1300	7	0-230	36
1	0-200	8	0-100	13
2	0-270	41	10-220	56
3	10-390	91	10-290	85
SB	10-350	69		
ENTEROCOCCI/100 ML				
S	0-179	9	0-71	5
1	0-18	3	0-17	2
2	1-87	14	0-141	12
3	0-140	23	10-239	23
SB	1-71	15		
**SPC/ML @ 20. C				
S	190-3200	936	170-3800	667
1	30-4100	294	0-2500	373
2	300-2400	781	80-6500	563
3	370-5600	1033	230-2100	656
SB	150-4100	797		

* S = surface of reservoir; 1 = reservoir outlet; 2 = halfway point
3 = diversion dam

** SPC = Standard Plate Count

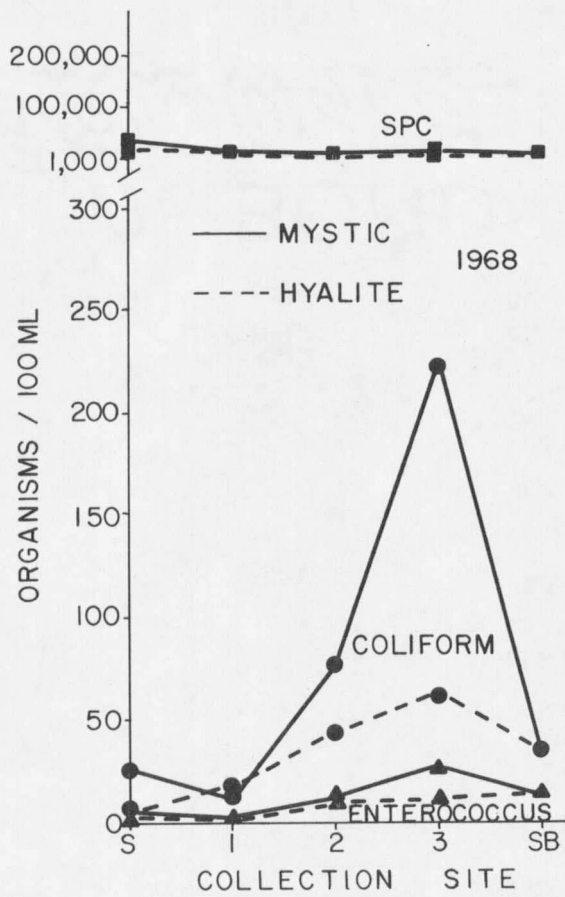


Figure 2. Bacteriological Profile of the Bozeman (Mystic) Creek and Middle (Hyalite) Creek Showing Geometric Means of Organisms/100 ml. SPC (■), Coliform (●), and Enterococcus (▲) Counts Geometrically Averaged From Eight Weekly Collections During the Summer of 1968

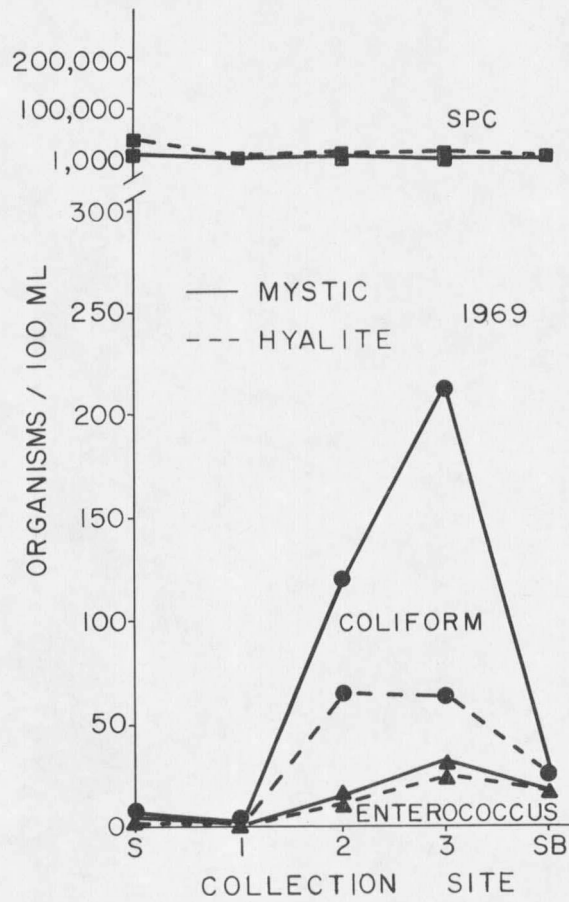


Figure 3. Bacteriological Profile of the Bozeman (Mystic) Creek and Middle (Hyalite) Creek Showing Geometric Means of Organisms/100 ml. SPC (■), Coliform (●), and Enterococcus (▲) Counts Geometrically Averaged From Nine Weekly Collections During the Summer of 1969

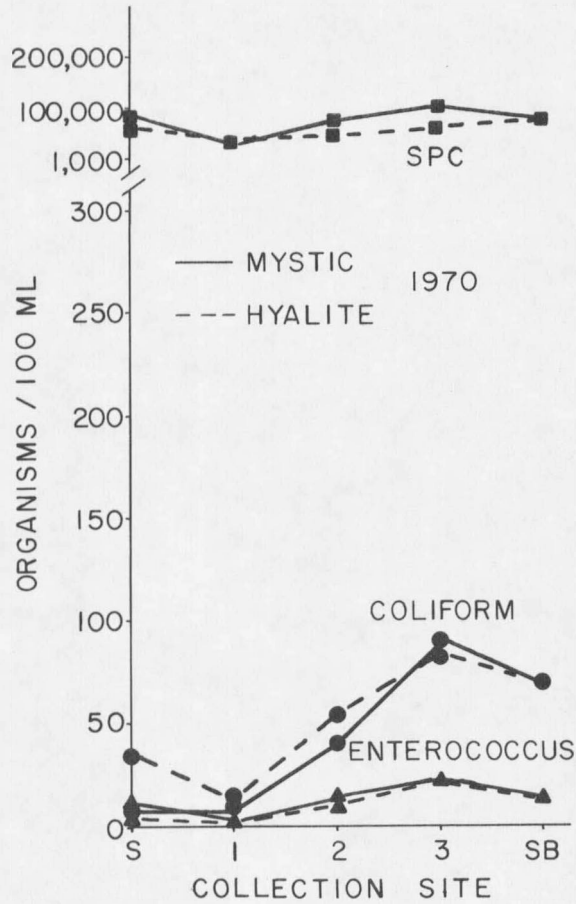


Figure 4. Bacteriological Profile of the Bozeman (Mystic) Creek and Middle (Hyalite) Creek Showing Geometric Means of Organisms/100 ml. SPC (■), Coliform (●), and Enterococcus (▲) Counts Geometrically Averaged From Thirteen Weekly Collections During the Summer of 1970

at the halfway point and diversion dam (sites 2 and 3) in the Mystic (closed) watershed than found in the Hyalite (open) watershed. Additionally, the coliform numbers increase as the water flows downstream from the spillway resulting in geometric means of over 200/100 ml in Mystic compared to about 65/100 ml in Hyalite at the diversion dams (site 3). Essentially, the geometric means were approximately equal at the surfaces (site 5) and spillways (site 1) of both watersheds; however, the halfway point (site 2) and diversion dam (site 3) of Mystic gave higher counts. Comparison of the two curves (Figures 2 and 3) for each watershed indicates excellent correlation for all stream sites.

The coliform profile for the Hyalite area in 1970 (Figure 4) is similar to those for 1968 and 1969. In contrast, the Mystic area shows a decrease in coliform numbers at site 3. Whereas the geometric means were over 200 coliforms/100 ml at the Mystic diversion dam in 1968 and 1969, the 1970 mean is about half or 91/100 ml. In addition, there is a definite decrease at site 2, even below that in the open watershed (56/100 ml) as compared to the closed Mystic watershed (41/100 ml).

Examination of the enterococcal counts in 1968 and 1969 also reveals a similar picture of greater contamination in the closed Mystic area, although not as profound as the coliform profile. The contamination once again increased in both watersheds as the water

flowed downstream. An adequate explanation of this increase has yet to be determined. The enterococcal counts in 1970 were nearly identical at all sites in both watersheds.

In 1969, a study of the South Fork (Figure 1) was undertaken to determine the water quality of the major tributary of Bozeman Creek draining the upper basin of the Mystic watershed. The ranges and geometric means for bacteriological counts during 1969 and 1970 are shown in Table 4. The bacteriological profiles for the two years (Figures 5 and 6) reflect essentially straight lines for the standard plate counts.

The coliform profiles were quite different. In 1969, the geometric means progressed from a low at E (62/100 ml) to a peak at C (219/100 ml) and subsequently decreased in numbers as the water flowed downstream to M₄ (146/100 ml). In 1970, there was a definite decrease in coliform densities with a minimum of 22/100 ml at E and only 49/100 ml at C, representing a fourfold decrease at the latter site.

In 1970, samples were taken from two small tributaries (designated as sites X and Y in Figure 1) of the South Fork in hope of determining what effect they might have on resulting bacterial densities further downstream in the South Fork. The geometric mean at X was 20 coliforms/100 ml and at Y was 52 coliforms/100 ml. A proper interpretation of these results is not yet possible.

The enterococcal profiles of the South Fork were essentially the

Table 4. Comparison of Numbers of Bacteria Obtained From Water Samples Collected at Different Sites in the South Fork of Mystic (Closed) Watershed During the Summers of 1969 (7 Weekly Collections) and 1970 (13 Weekly Collections)

1969 DETERMINATION						
SITE	Coliforms/100 ml		Enterococci/100 ml		*SPC/ml @ 35C	
	Range	Geometric Mean	Range	Geometric Mean	Range	Geometric Mean
E	10-100	50	7-35	15	1-25	8
D	20-160	72	12-57	36	6-40	22
C	100-450	219	2-70	17	17-22	21
B	120-480	203	3-60	14	8-25	14
A	70-370	167	1-69	21	12-43	19
M ₄	60-430	146	1-81	21	14-35	21
1970 DETERMINATION						
SITE	Coliforms/100 ml		Enterococci/100 ml		*SPC/ml @ 20C	
	Range	Geometric Mean	Range	Geometric Mean	Range	Geometric Mean
Y	0-240	52	1-88	10	90-780	329
X	0-270	20	0-56	8	73-850	189
E	0-800	22	0-199	12	76-3260	266
D	0-3000	38	0-300	8	85-7500	329
C	0-1210	49	0-239	11	92-4380	329
B	0-470	39	0-151	9	99-1810	317
A	0-900	35	0-159	9	118-3390	346
M ₄	0-700	43	0-83	11	109-2260	376

* SPC = Standard Plate Count

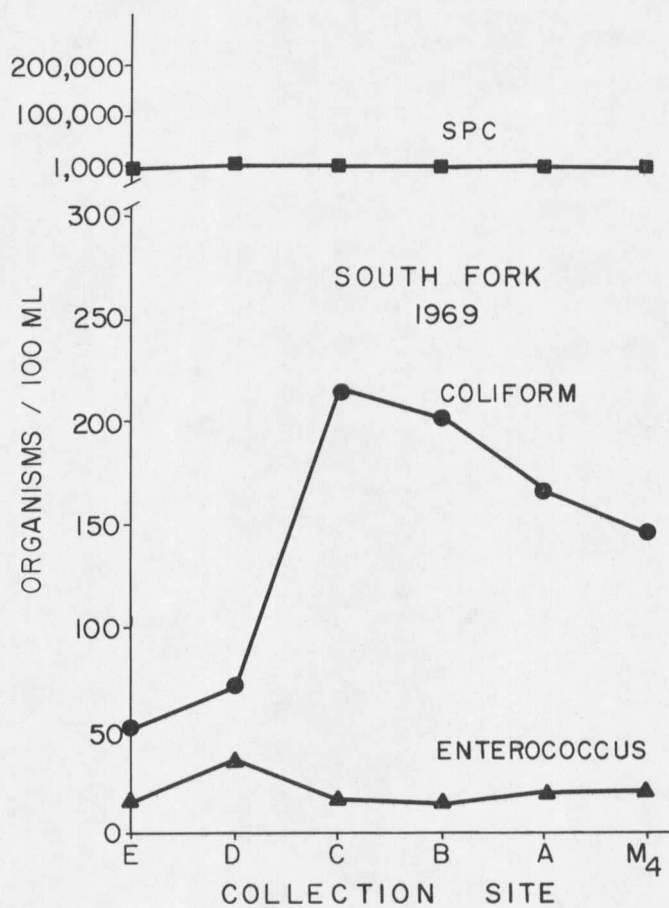


Figure 5. Bacteriological Profile of the South Fork of Bozeman (Mystic) Creek During the Summer of 1969 Showing Geometric Means of Organisms/100 ml. SPC (■), Coliform (●), and Enterococcus (▲) Counts Geometrically Averaged From Seven Weekly Collections at Sites E Through M₄ as Shown in Figure 1

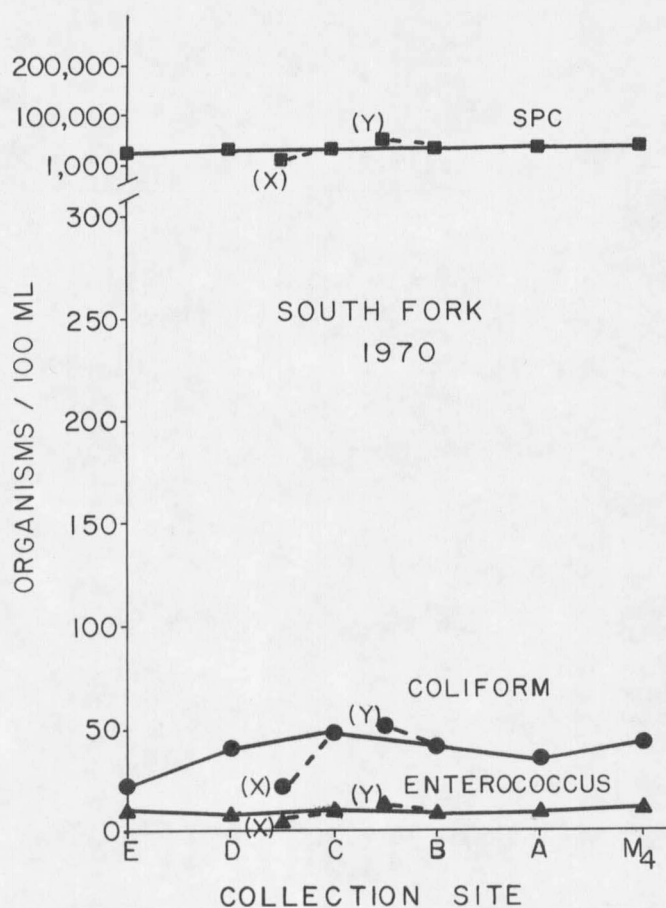


Figure 6. Bacteriological Profile of the South Fork of Bozeman (Mystic) Creek During the Summer of 1970 Showing Geometric Means of Organisms/100 ml. SPC (■), Coliform (●), and Enterococcus (▲) Counts Geometrically Averaged From Thirteen Weekly Collections at Sites E Through M₄ and Sites X and Y (Tributaries of the South Fork) as Shown in Figure 1

same in 1969 and 1970. However, a slight peak was observed at D (36/100 ml) in 1969. No peaks were observed in 1970, the profile being basically a straight line.

Qualitative Bacteriological Studies of Water Samples

Coliforms. A comparison of the differentiated coliform bacteria obtained from the two watersheds in 1968, 1969, and 1970 is shown in Table 5. The percentage of Escherichia coli was higher in Mystic than Hyalite for all three years. Also, the percentages of fecal coliforms were higher in the Mystic area. An even greater percentage of fecal coliforms was obtained from the South Fork area than from the lower Bozeman Creek in 1969 and 1970.

Enterococci. Differentiation of the enterococci to species resulted in the following: Streptococcus faecium, S. faecium var. durans, S. faecalis var. liquefaciens, and S. bovis. Most of the enterococci were found to be S. faecalis var. liquefaciens.

Seasonal Variation of Coliforms and Enterococci

In an effort to determine bacterial fluctuations with seasons, sampling was performed periodically during all of 1970 at both diversion dams and the settling basin. The coliform and enterococcal counts obtained on 36 sampling dates during 1970 are presented in Figures 7, 8, and 9. Highest coliform densities were generally obtained from early August to mid-October at all three sites. A smaller peak

Table 5. Percentage Distributions of Escherichia coli, Enterobacter aerogenes, Intermediates and Fecal Isolates Obtained at Different Sites

SUMMER 1968				
DETERMINATION	Mystic	Hyalite	Settling Basin	South Work
<u>Escherichia coli</u>	33	25	46	-
<u>Enterobacter aerogenes</u>	23	43	0	-
Intermediates	44	32	54	-

Fecal origin	32	29	46	-
SUMMER 1969				
<u>Escherichia coli</u>	47	38	31	41
<u>Enterobacter aerogenes</u>	42	41	46	46
Intermediates	11	21	23	13

Fecal Origin	64	52	38	70
SUMMER 1970				
<u>Escherichia coli</u>	35	12	31	47
<u>Enterobacter aerogenes</u>	10	24	4	15
Intermediates	55	64	65	38

Fecal origin	42	29	48	54

- not determined

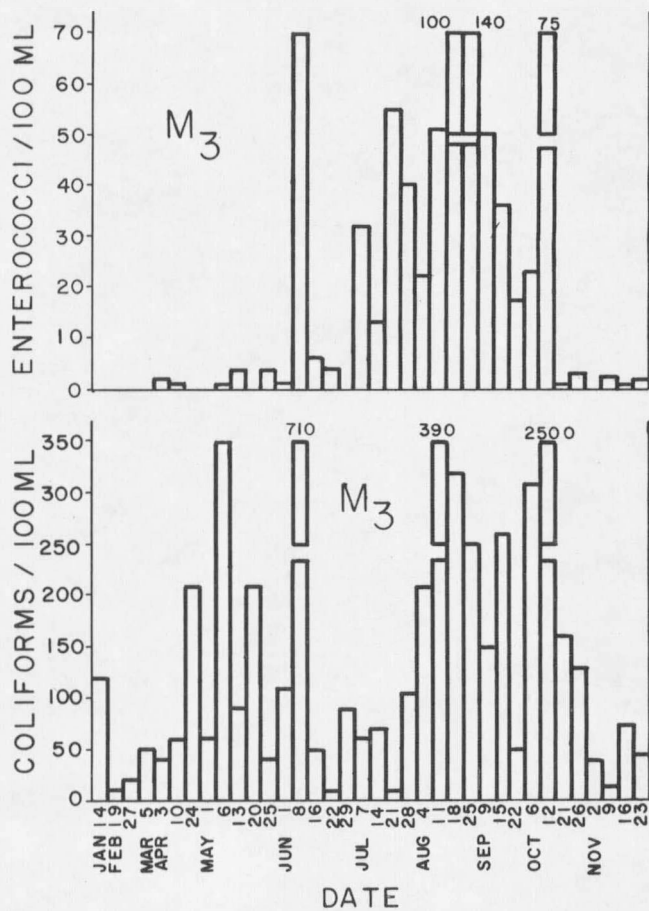


Figure 7. Numbers of Coliforms/100 ml and Enterococci/100 ml of Water Obtained at the Bozeman (Mystic) Creek Diversion Dam (M_3) on 36 Sampling Dates in 1970

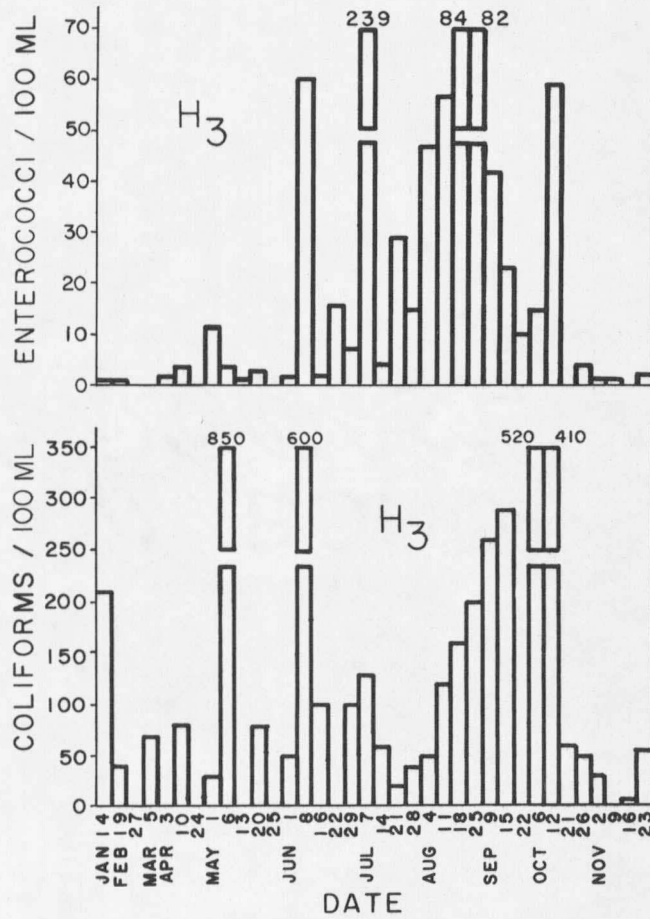


Figure 8. Numbers of Coliforms/100 ml and Enterococci/100 ml of Water Obtained at the Middle (Hyalite) Creek Diversion Dam (H₃) on 36 Sampling Dates in 1970

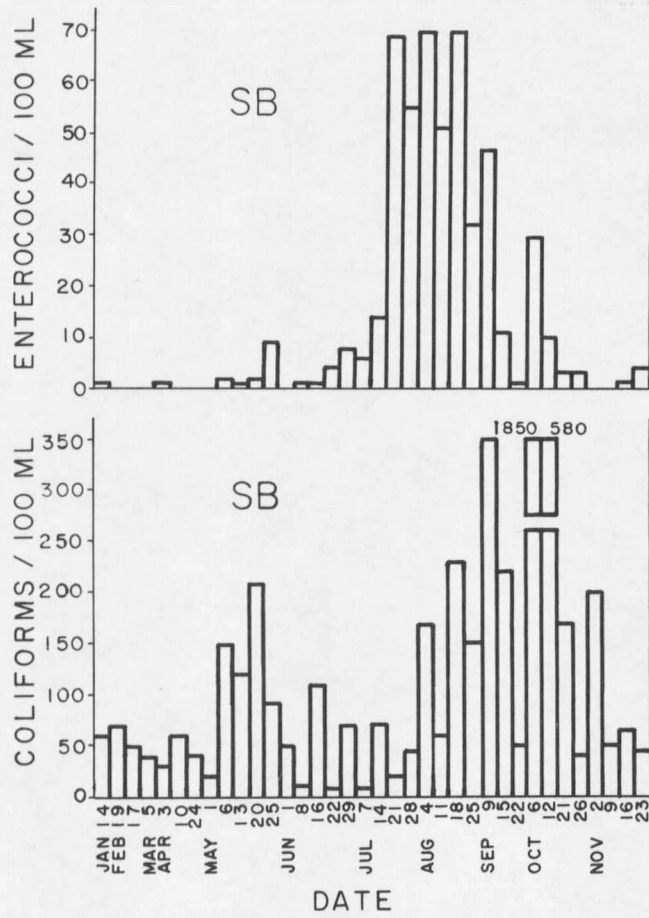


Figure 9. Numbers of Coliforms/100 ml and Enterococci/100 ml of Water Obtained at the Settling Basin (SB) on 36 Sampling Dates in 1970

period is indicated from early May to mid-June. Lowest coliform numbers occurred from October through April and mid-June to early August. The enterococci reached only one peak density in early July and remained high until early October. From mid-October through June, enterococci remained quite low.

Bacteriology of Animal Droppings

Animal droppings from elk, moose, bear, deer, and horse were collected from the closed Mystic area, especially in the South Fork drainage area. Different genera of Gram-negative organisms including Escherichia, Enterobacter, Proteus, Salmonella-like, and Shigella-like as well as various streptococcal species were isolated from these fecal droppings and differentiated using physiological tests (Table 6).

Serology of Organisms Isolated from Water and Animal Droppings

Serological procedures were performed on water and fecal dropping isolates of Escherichia, Salmonella-like, and Shigella-like organisms obtained during 1969. None of the Salmonella-like nor the Shigella-like organisms reacted with the sera employed.

According to the IMViC results, of 202 coliforms isolated during the summer of 1969, 86 were classified as E. coli. Upon serological examination of these 86 strains, 39 agglutinated with one of the polyvalent antisera and nine cross-reacted among the antisera (Table 7).

The resultant reactions of unheated cultures with individual OB

Table 6. Enterobacteria and Enterococci Found in Animal Droppings

ENTEROBACTERIA							
ORIGIN	<u>Escherichia coli</u>	<u>Enterobacter aerogenes</u>	Intermediates	Proteus	Salmonella-Like	Shigella-Like	Providencia
Elk	X	X	X	X	X	X	X
Moose		X					X
Bear	X	X	X				X
Deer	X	X	X				X
Horse	X	X		X	X		X

ENTEROCOCCI				
ORIGIN	<u>Streptococcus faecium</u>	<u>Streptococcus faecium var. durans</u>	<u>Streptococcus faecalis var. liquefaciens</u>	<u>Streptococcus faecalis</u>
Elk	X	X	X	X
Moose		X		
Bear	X			
Deer		none		
Horse	X	X		

Table 7.* Reactions of 86 E. coli Isolates with Polyvalent Antisera

		Polyvalent Antisera						
		A		B		C		
A	6% A	1302 1516 1520	6% AB	1315 1318 1522	2% AC	1125		
				814 937 985 1059 1061 1066 1067	1222 1224 1370 1422 1423 1424 1426			
			B	50% B	1068 1072 1073 1074 1076	1427 1518 1519 1521 1525	6% BC	1275 1428 1527
							801 1280 805 1297	
<u>Additional cross reactions</u>						C	25% C	972 1429 1223 1517 1274 1524 1279 1526
	A B C	1271 1273	5%					

* Data in these tables are presented in a "Punnett square" fashion. Antisera used are shown vertically and horizontally. Culture numbers are displayed at the intersection of sera with which the culture reacted. Homologous reactions are located on the outside diagonal while cross reactions occur within the system. NR = no reaction.

antisera comprising the polyvalent A set are indicated in Table 8. The B antigen masks the O antigen and is inactivated by boiling for one hour. The O antigen is heat stable. It was noted that none of the heated cultures reacted with individual A antisera.

The resultant reactions of unheated and heated cultures with individual OB antisera comprising the polyvalent B set are shown in Tables 9 and 10. Clustering of serotypes can be observed in various sections of these tables, e. g., a large cluster was found with 0119:B14. Several unexplainable cross reactions were also observed.

The results of reacting unheated and heated cultures with individual OK antisera comprising the polyvalent C set are given in Tables 11 and 12. A distinctly predominant clustering was observed with antiserum 018:B21.

Physical and Chemical Results of Water Analyses

Air and water temperatures for the summers of 1968, 1969, and 1970 shown in Tables 13 and 14 revealed no significant differences between the Mystic and Hyalite watersheds.

The pH of the two streams (Table 15) was very similar in both 1968 and 1969; however, the pH values in 1970 showed a decrease at all sites in both watersheds.

Chemical analyses indicated greater concentrations of most ions in the Mystic water than in the open watershed, while the settling

Table 8.* Reactions of Unheated E. coli Isolates With Individual A-Antisera

	0111:B4	055:B5	0127:B8	026:B6
0111:B4	NR	1520 (Elk)	NR	NR
055:B5		1516 (Elk)	1522 (Elk)	NR
		0127:B8	NR	NR
			026:B6	NR

47

Additional Cross Reactions

1271 (M_4) - all four antisera

1273 (M_4) - 0111:B4, 055:B5, 026:B6

Table 9.* - Reactions of Unheated E. coli Isolates with Individual B Antisera

	086:B7	0119:B14	0124:B17	0125:B15	0126:B16	0128:B12
086:B7	NR	1066(B) 1426(Bear)	NR	1522(Elk)	NR	1315 (SH)
0119:B14		985(H ₃) 1059(M ₄) 1061(M ₄) 1067(B) 1072(C)	1073(C) 1074(C) 1076(D) 1224(Elk)	1068(B) 1518(Elk) 1519(Elk)	1527(Elk)	937(Elk) 1370(A)
			0124:B17 1422(Bear) 1423(Bear)	NR	NR	NR
			0125:B15	NR	NR	NR
				0126:B16	NR	NR
					0128:B12	1275(A) 1318(H ₃)

Additional Cross Reactions

- 814(M₄) - 0119:B14, 0124:B17, 0128:B12
- 1222(Elk) - 0119:B14, 0126:B16, 0128:B12
- 1271(M₄) - all six antisera
- 1273(A) - all six antisera
- 1424(Bear) - 0119:B14, 0124:B17, 0125:B15
- 1427(Bear) - 0119:B14, 0124:B17, 0125:B15, 0126:B16
- 1428(Bear) - all six antisera
- 1521(Elk) - 0119:B14, 0124:B17, 0125:B15

Table 10. Reactions of Heated E. coli Isolates with Individual B Antisera

	086:B7	0119:B14	0124:B17	0125:B15	0126:B16	0128:B12
086:B7	NR	NR	NR	NR	NR	NR
0119:B14		1059(M ₄) 1066(B) 1067(B)	NR	NR	NR	NR
		0124:B17	814(M ₄) 1423(Bear) 1424(Bear)	NR	NR	NR
			0125:B15	NR	NR	NR
				0126:B16	NR	NR
					0128:B12	1318(H ₃)

Additional Cross Reactions

1521 (Elk) - 0125:B15, 0126:B16, 0128:B12

1525 (Elk) - 086:B7, 0119:B14, 0124:B17, 0128:B12

Table 11.* Reactions of Unheated E. coli Isolates With Individual C Antisera

	018:B21	020:B7	020:84B	028:B18	044:K74	0112:B11
018:B21	801(M ₁) · 1275(A) 805(M ₂) · 1279(B) 972(SB) · 1280(B) 1223(Elk) · 1429(Bear) 1274(A) · 1526(Elk)	NR	NR	1524(Elk)	NR	NR
	020:B7	NR	NR	NR	1297(M ₂)	NR
	020:84B		NR	NR	NR	NR
	028:B18			NR	NR	NR
	044:K74				NR	NR
					0112:B11	1428(Bear)

Additional Cross Reactions.

1271(M₄) - 020:B7, 020:84B, 028:B18, 0112:B11

1273(A) - 020:B7, 020:84B, 028:B18, 0112:B11

1527(Elk) - 018:B21, 020:84B, 028:B18

Table 12.* Reactions of Heated E. coli Isolates With Individual C Antisera

	018:B21	020:B7	020:84B	028:B18	044:K74	0112:B11
018:B21	801 (M ₁) 972 (SB) 1280 (B) 1527 (Elk)	NR	NR	NR	NR	NR
	020:B7	NR	NR	NR	NR	NR
		020:84B	NR	NR	NR	NR
			028:B18	NR	NR	NR
				044:K74	NR	NR
					0112:B11	1297 (M ₂)

Additional Cross Reactions

- 1125 (C) - all six antisera
- 1428 (Bear) - all six antisera
- 1429 (Bear) - all six antisera
- 1517 (Elk) - all six antisera

Table 13. Comparison of Water Temperatures (C) Obtained at Different Sites in Mystic (closed) and Hyalite (open) Watersheds

SITE*	MYSTIC		HYALITE	
	RANGE	ARITHMETIC MEAN	RANGE	ARITHMETIC MEAN
SUMMER 1968				
S	11.0-19.0	16.4	13.5-18.0	15.3
1	8.0-16.0	10.7	7.5-10.0	9.1
2	7.5-11.5	8.8	8.0-13.0	10.1
3	8.0-12.0	9.2	9.0-14.0	10.3
SB	9.0-12.0	10.6		
SUMMER 1969				
S	11.0-21.0	16.8	8.0-22.0	16.1
1	7.0-13.0	10.2	7.5-13.0	9.5
2	7.5-10.5	8.6	8.0-13.0	10.3
3	7.0-10.0	9.0	8.0-14.0	10.6
SB	8.0-13.0	10.6		
SUMMER 1970				
S	7.5-21.0	16.1	6.5-18.2	14.4
1	5.0-13.0	9.0	5.0-11.1	8.5
2	5.1-10.1	7.5	5.5-14.3	10.1
3	6.5-11.7	7.9	6.2-13.8	10.2
SB	6.2-11.2	8.6		

* S = surface of reservoir; 1 = reservoir outlet; 2 = halfway point;
3 = diversion dam

Table 14. Comparison of Air Temperatures (C) Obtained at Different Sites in Mystic (Closed) and Hyalite (Open) Watersheds

MYSTIC			HYALITE	
SITE*	RANGE	ARITHMETIC MEAN	RANGE	ARITHMETIC MEAN
SUMMER 1968				
S	8.5-23.0	14.3	10.0-27.0	12.5
1	8.0-25.0	13.9	11.0-25.0	17.1
2	6.0-22.0	14.6	10.0-24.0	17.4
3	11.0-28.5	17.3	11.5-26.0	19.0
SB	9.0-25.0	17.2		
SUMMER 1969				
S	6.0-23.0	16.7	11.0-23.0	18.3
1	8.0-19.0	15.2	10.5-21.5	16.1
2	8.5-19.5	15.7	10.5-23.5	16.4
3	8.0-20.5	16.3	10.0-26.0	17.5
SB	13.0-24.0	18.2		
SUMMER 1970				
S	9.5-26.5	17.5	9.6-28.7	20.5
1	10.0-27.8	15.2	9.9-24.3	18.4
2	11.4-30.2	17.5	11.5-27.7	18.9
3	13.5-32.6	19.1	11.5-28.6	20.9
SB	7.8-34.8	19.6		

* S = surface of reservoir; 1 = reservoir outlet; 2 = halfway point;
3 = diversion dam

Table 15. Comparison of pH Obtained at Different Sites in Mystic (Closed) and Hyalite (Open) Watersheds

SITE*	MYSTIC		HYALITE	
	RANGE	ARITHMETIC MEAN	RANGE	ARITHMETIC MEAN
SUMMER 1968				
S	7.4-8.6	7.8	7.0-9.9	7.6
1	6.5-8.2	7.3	6.7-8.1	7.3
2	7.6-8.3	8.0	6.9-8.1	7.4
3	7.6-8.3	8.0	7.0-8.2	7.5
SB	7.4-8.0	7.6		
SUMMER 1969				
S	7.9-9.2	8.5	8.2-10.0	9.0
1	7.2-8.5	7.8	7.4-8.5	7.9
2	7.9-8.5	8.2	7.8-8.6	8.0
3	8.0-8.6	8.3	7.8-8.5	8.1
SB	7.6-8.2	7.8		
SUMMER 1970				
S	6.8-9.9	7.8	7.1-9.1	7.6
1	6.6-8.6	7.2	6.3-8.7	6.9
2	6.9-9.0	7.4	6.5-8.3	7.0
3	7.3-8.2	7.6	6.6-8.0	7.1
SB	6.9-7.8	7.3		

* S = surface of reservoir; 1 = reservoir outlet; 2 = halfway point;
3 = diversion dam

basin showed intermediate values reflecting the mixture of the two (Tables 16, 17, 18, 19, and 20). Calcium was the dominant cation followed by magnesium, sodium, and potassium. The dominant anion was bicarbonate, followed by sulfate and chloride.

Chemical profiles of calcium, magnesium, bicarbonate, conductivity, nitrate, turbidity, and orthophosphate in the two watersheds are shown in Figures 10, 11, 12, and 13. Greater concentrations of calcium, magnesium, bicarbonate, and conductivity were found in the closed watershed when compared to the Hyalite area. Also, the concentrations of these constituents increased as the water flowed downstream. The nitrate profile was essentially the same in both areas. Turbidity and orthophosphate profiles reflect considerable variation. Orthophosphate was nearly identical in concentration at the spillways of both reservoirs; however, the concentration was greater at the halfway point in Mystic (0.33 mg/l) when compared to the halfway point in Hyalite (0.26 mg/l).

Chemical analyses were also performed on eight weekly water collections obtained from the South Fork drainage area in 1970. Only water temperature, air temperature, pH, and conductivity determinations were made in 1969. Ranges and means for these factors in 1969 and 1970 are shown in Tables 21 and 22. The water temperatures for the South Fork sites were quite cold with an approximate range of 4-8 C. The

Table 16. Comparison of Conductivity @ 25 C (Micromhos) Obtained at Different Sites in Mystic (Closed) and Hyalite (Open) Watersheds

MYSTIC			HYALITE	
SITE*	RANGE	ARITHMETIC MEANS	RANGE	ARITHMETIC MEANS
SUMMER 1969				
S	167-194	175	60-99	75
1	180-230	205	69-84	73
2	193-234	215	103-146	117
3	212-229	221	104-150	120
SB	118-215	146		
SUMMER 1970				
S	125-162	149	51-66	58
1	145-189	168	51-72	60
2	142-190	166	70-113	93
3	149-217	179	70-110	96
SB	103-189	141		

* S = surface of reservoir; 1 = reservoir outlet; 2 = halfway point;
3 = diversion dam

Table 17. Comparison of Calcium and Magnesium Concentrations Obtained at Different Sites in Mystic (Closed) and Hyalite (Open) Watersheds During the Summer of 1970 (9 Weekly Collections)

SITE*	MYSTIC		HYALITE	
	RANGE	ARITHMETIC MEAN	RANGE	ARITHMETIC MEAN
CALCIUM (mg/l)				
S	15.6-23.6	17.4	4.7-7.7	5.5
1	18.6-21.5	19.9	4.9-7.3	6.1
2	18.2-25.7	22.0	7.7-13.5	10.5
3	21.8-27.1	24.3	7.8-13.6	11.3
SB	13.4-20.0	16.4		
MAGNESIUM (mg/l)				
S	0.6-5.1	4.3	1.1-2.2	1.6
1	4.7-6.6	5.5	0.9-1.9	1.4
2	4.3-7.5	5.9	1.8-3.7	2.8
3	5.9-7.8	6.6	2.2-3.7	3.0
SB	3.3-5.4	4.3		

*S = surface of reservoir; 1 = reservoir outlet; 2 = halfway point;
3 = diversion dam

Table 18. Comparison of Sodium and Potassium Concentrations Obtained at Different Sites in Mystic (Closed) and Hyalite (Open) Watersheds During the Summer of 1970 (9 Weekly Collections)

MYSTIC		HYALITE		
SITE*	RANGE	ARITHMETIC MEAN	RANGE	ARITHMETIC MEAN
SODIUM (mg/l)				
S	3.7-4.8	4.1	0.6-3.1	1.3
1	2.8-4.8	3.8	0.6-1.3	0.9
2	1.6-2.7	2.1	0.7-2.4	1.4
3	1.4-3.1	2.3	1.1-2.4	1.6
SB	1.4-2.2	1.8		
POTASSIUM (mg/l)				
S	0.9-2.0	1.7	1.0-3.9	1.8
1	1.1-1.8	1.5	1.2-1.8	1.3
2	1.2-2.0	1.7	1.0-1.8	1.4
3	1.4-2.4	1.9	1.0-2.0	1.5
SB	1.1-2.0	1.5		

* S = surface of reservoir; 1 = reservoir outlet; 2 = halfway point;
3 = diversion dam

Table 19. Comparison of Major Anions Obtained at Different Sites in Mystic (Closed) and Hyalite (Open) Watersheds During the Summer of 1970 (9 Weekly Collections)

SITE*	MYSTIC		HYALITE	
	RANGE	ARITHMETIC MEAN	RANGE	ARITHMETIC MEAN
BICARBONATE (meq/l)				
S	1.23-1.40	1.29	0.47-0.62	0.54
1	1.37-1.49	1.43	0.45-0.68	0.55
2	1.56-1.78	1.68	0.66-1.11	0.90
3	1.79-1.97	1.88	0.70-1.00	0.90
SB	1.16-1.53	1.33		
SULFATE (mg/l)				
S	8.3-16.0	12.2	1.5-8.2	4.7
1	10.6-17.8	12.9	1.1-8.5	4.3
2	6.5-11.9	8.5	2.3-10.0	5.0
3	5.7-12.0	8.2	2.3-10.0	5.5
SB	3.9-12.0	6.8		
CHLORIDE (mg/l)				
S	0.03-0.73	0.30	0.01-0.25	0.12
1	0.09-0.40	0.19	0.01-0.30	0.11
2	0.10-0.40	0.22	0.00-0.21	0.10
3	0.03-0.40	0.17	0.05-1.00	0.23
SB	0.01-0.45	0.19		

* S = surface of reservoir; 1 = reservoir outlet; 2 = halfway point;
3 = diversion dam

Table 20. Comparison of Nitrate, Orthophosphate, and Turbidity Determinations Obtained at Different Sites in Mystic (Closed) and Hyalite (Open) Watersheds During the Summer of 1970 (9 Weekly Collections)

SITE*	MYSTIC		HYALITE	
	RANGE	ARITHMETIC MEAN	RANGE	ARITHMETIC MEAN
NITRATE (mg/1 N-NO ₃ ⁻)				
S	0.01-0.14	0.05	0.00-0.15	0.05
1	0.01-0.15	0.05	0.00-0.12	0.05
2	0.01-0.16	0.05	0.01-0.16	0.06
3	0.01-0.18	0.06	0.01-0.16	0.06
SB	0.00-0.15	0.06		
ORTHOPHOSPHATE (mg/1 PO ₄ ⁻³)				
S	0.11-0.56	0.29	0.10-0.23	0.15
1	0.21-0.32	0.27	0.22-0.35	0.28
2	0.27-0.45	0.33	0.22-0.35	0.26
3	0.20-0.40	0.27	0.17-0.35	0.26
SB	0.21-0.32	0.26		
TURBIDITY (Jackson Turbidity Units)				
S	0-41	15	0-60	16
1	0-35	13	0-27	6
2	0-53	12	0-29	9
3	0-33	11	0-29	12
SB	0-27	9		

* S = surface of the reservoir; 1 = reservoir outlet; 2 = halfway point;
3 = diversion dam

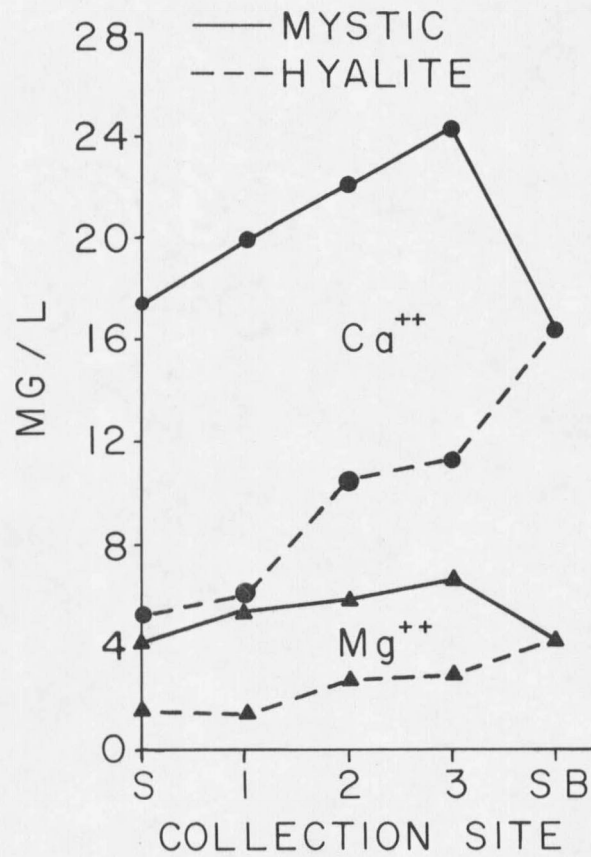


Figure 10. Chemical Profile of Average Calcium (●mg/l) and Magnesium (▲mg/l) Concentrations Obtained From Ten Weekly Collections at Different Sites on the Bozeman (Mystic) and Middle (Hyalite) Creeks During the Summer of 1970

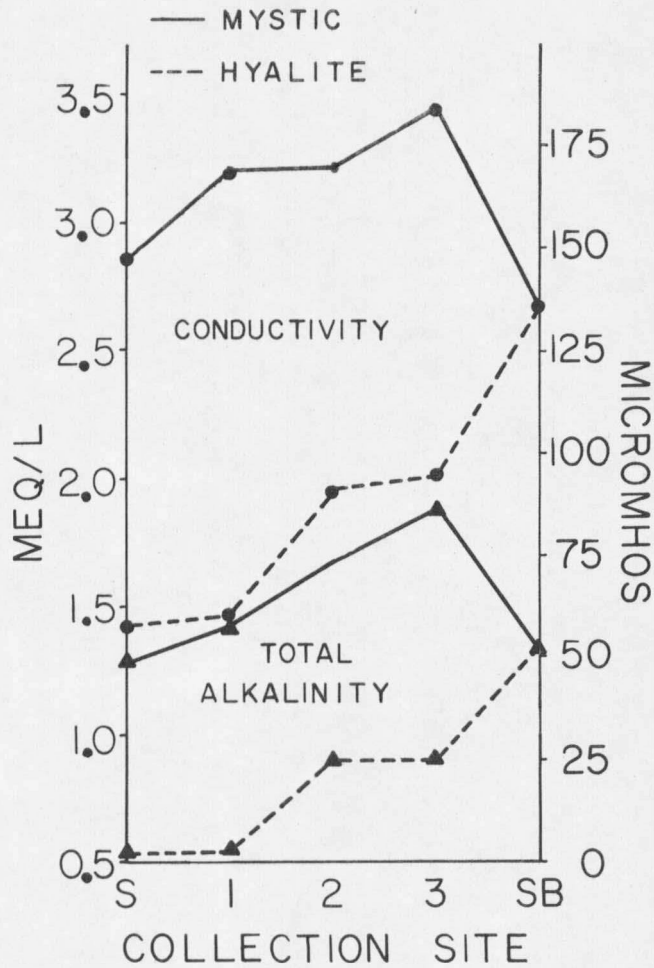


Figure 11. Chemical Profile of Average Conductivity (● micromhos) and Total Alkalinity (▲ meq/l) Concentrations Obtained From Ten Weekly Collections at Different Sites on the Bozeman (Mystic) and Middle (Hyalite) Creeks During the Summer of 1970

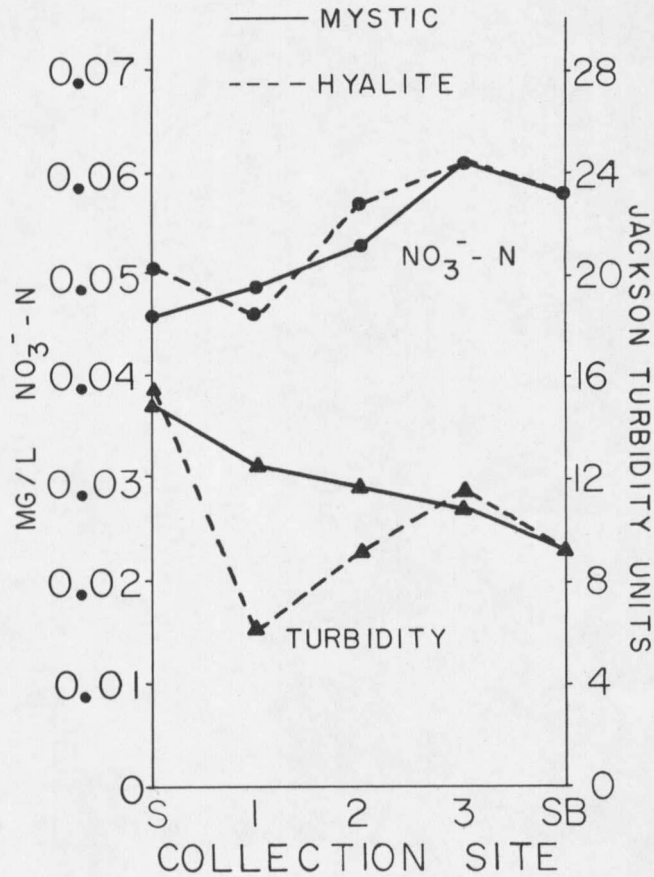


Figure 12. Chemical Profile of Average Turbidity (▲ Jackson Turbidity Units) and Nitrate (● mg/l NO₃⁻-N) Concentrations Obtained From Ten Weekly Collections at Different Sites on the Bozeman (Mystic) and Middle (Hyalite) Creeks During the Summer of 1970.

