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

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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Date 5/26/71

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Approved:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

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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, Teller [37] 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 Nierop[38] 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. Geldreich[13] 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. Schuettpelz[34] 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. Kunkle[26] 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 Burman[6] 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 Benson[4] 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. Mundt[32] 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 Morrison[9] 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 Spino[11] 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 Project[36] 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, Glantz[21] 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 preceeded 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 Kunkle[26] in a Vermont stream study. Schuettpelz[34] 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 Furfari[29] 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 Meiman[27] 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

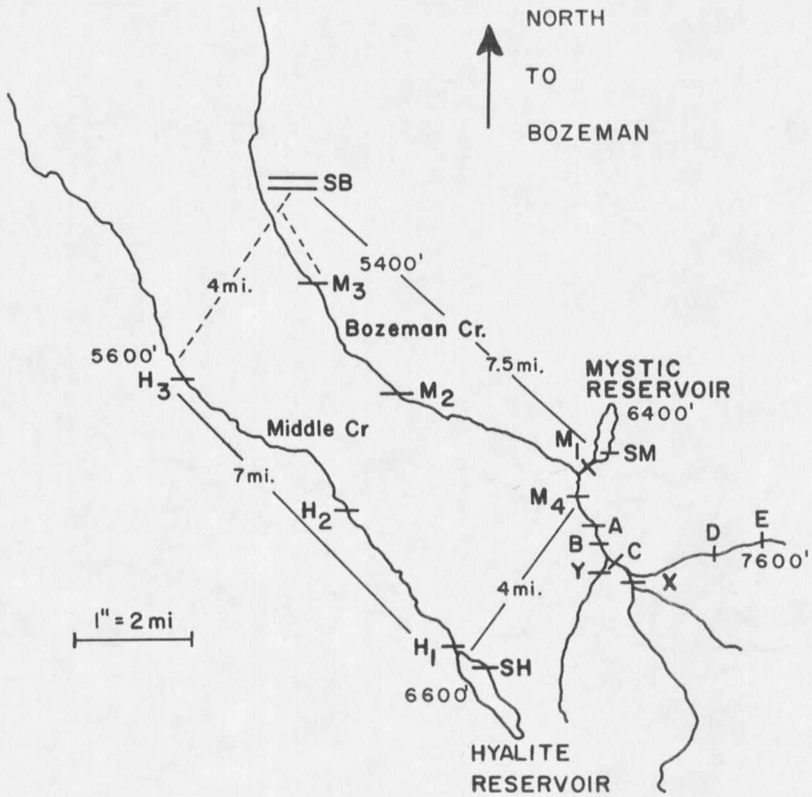

Figure 1. Elevations and Sampling Sites of Mystic (M) and Hyalite (H) Watersheds, Surface of Reservoir (S), Reservoir Outlet (1) Halfway Point (2), Diversion Dam (3), Settling Basin (SB), and the South Fork Sites: M_4, A, B, C, D, E, and South Fork Tributaries X and Y

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 M_1 - Mystic reservoir outlet

Site M_2 - Halfway point of Bozeman Creek

Site M_3 - Diversion dam of Bozeman Creek

Site SH - Surface of the Hyalite reservoir

Site H_1 - Hyalite reservoir outlet

Site H_2 - Halfway point of Middle Creek

Site H_3 - Diversion dam of Middle Creek

Site SB - Settling basin

Sites M_4, 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 M_4. 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 Wastewater[1] 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 Methods[1] 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 SO 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.