Microbial studies of a high alpine water supply used for recreation
by Sidney Arthur Stuart

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Microbiology
Montana State University
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Abstract:
Selected waters from the high alpine zone within Grand Teton National Park, Wyoming, were studied during the past four summers to determine the influence of various factors on the quality of these waters. The water samples collected were analyzed for populations of indicator bacteria. Water that originated in remote areas contained some indicator bacteria and these populations increased as the water flowed toward the valley. In general, the magnitude of this increase was not significantly influenced by the presence or absence of human visitors but, rather, by the nature of the biological community through which the streams flowed. It was determined that it is possible for coliforms of the non-fecal type to grow and multiply in alpine streams using extracellular products excreted by algae but it was not determined to what extent (if any) this occurs in Grand Teton National Park. Once in the valley lakes, the indicator bacteria declined to very low levels. A minority of the coliforms that were recovered from all of the sites proved to be fecal coliforms. The fecal streptococci isolated were identified as the species that were found primarily in the fecal material of the native rodent and moose populations. It is concluded that management questions that relate to the carrying capacity of alpine areas should be approached with the aid of other biological parameters along with levels of indicator bacteria in the streams.
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MICROBIAL STUDIES OF A HIGH ALPINE WATER SUPPLY USED FOR RECREATION

by

SIDNEY ARTHUR STUART

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Microbiology

Approved:

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Bozeman, Montana

December, 1975
ACKNOWLEDGMENTS

The author would like to thank Dr. Gordon A. McFeters for his guidance and assistance throughout the course of this study. Thanks are also due to Dr. David G. Stuart, Dr. John C. Wright and Mr. John E. Schillinger for their technical and editorial assistance. A sincere thanks to Susan Harrigan, Jim Hawkins and Sandra Dunkel for their field and laboratory assistance.

The help of Peter Hayden and Frank Betts, of the National Park Service, is gratefully acknowledged. The use of facilities at the Jackson Hole Biological Research Station under the direction of Dr. Oscar H. Paris is also appreciated.

This work was supported by the National Park Service (Contracts No. CX-12004B025, CX-6000-3-0087, 929-0-P20067 and PX-1200-50892), the United States Environmental Protection Agency (training fellowships No. U-910413-01 and U-910687-01-0) and the New York Zoological Society.
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ABSTRACT

Selected waters from the high alpine zone within Grand Teton National Park, Wyoming, were studied during the past four summers to determine the influence of various factors on the quality of these waters. The water samples collected were analyzed for populations of indicator bacteria. Water that originated in remote areas contained some indicator bacteria and these populations increased as the water flowed toward the valley. In general, the magnitude of this increase was not significantly influenced by the presence or absence of human visitors but, rather, by the nature of the biological community through which the streams flowed. It was determined that it is possible for coliforms of the non-fecal type to grow and multiply in alpine streams using extracellular products excreted by algae but it was not determined to what extent (if any) this occurs in Grand Teton National Park. Once in the valley lakes, the indicator bacteria declined to very low levels. A minority of the coliforms that were recovered from all of the sites proved to be fecal coliforms. The fecal streptococci isolated were identified as the species that were found primarily in the fecal material of the native rodent and moose populations. It is concluded that management questions that relate to the carrying capacity of alpine areas should be approached with the aid of other biological parameters along with levels of indicator bacteria in the streams.
INTRODUCTION

Water originating in the high alpine zone contributes significantly to the water supply for much of the western part of the nation. In the past, this alpine zone has been visited by relatively few people. However, the quality of water in high-elevation mountain areas is becoming an increasingly important consideration to agencies such as the National Park Service and the U.S. Forest Service who have expressed concern both for the possible public health consequences of drinking untreated "pure mountain stream" water, and for ecological consequences of nutrient additions. This increased concern over the water quality of high-elevation mountain areas is partly due to the increasing numbers of people who visit these areas each year.

Significant increases in wilderness-related outdoor recreation have taken place and continue to do so at a rapid rate. Barbaro et al. (4) report a seven percent increase per year over the last decade. In effect, the movement of large numbers of people each summer from metropolitan centers to remote regions in the western United States represents periodic, seasonal urbanization of many National Parks and forests. Grand Teton National Park is such an area where in 1975, 2,549,030 people visited the park with
approximately 32,045 of these people traveling to the alpine back-country within the park boundaries. With this substantial use of park facilities there is growing concern over the impact of visitor use on the aesthetic and wilderness values, campsite deterioration, and particularly the lowering of water quality. Any of these adverse impacts could irrevocably damage the unique qualities of such areas.

The drinking water used by these park visitors originates in the back-country where summer visitor use is heavy and sanitary facilities are limited or non-existent. Visitors also contribute to the natural nutrient budget of the lakes and streams by addition of sanitary wastes, outboard motor wastes, detergents and solid wastes. In 1969, for example, solid wastes left behind by recreational users of the Boundary Waters Canoe Area in Minnesota, totaled an estimated 163,440 Kg (360,000 lb) (44). According to Barton (5), this is equivalent to the addition of 1 ton of phosphates and 13 tons of nitrogen to these waters. In addition, heavy campsite use may accelerate soil erosion through deterioration of vegetation and compaction, which will further increase nutrient contributions to the waters. These observations point to the importance of understanding man's impact on the natural quality of waters within
wilderness areas and their safety for human use both in the remote back country and the more populated adjoining land.

Grand Teton National Park provides a unique opportunity to study the impact of various recreational uses on water quality, since the water from the high mountain elevations can be followed as it is exposed to a succession of recreational influences, as shown below.

1. **Mountain climbing.** Many of the highest and most remote alpine areas in the park are used primarily by mountain climbers for both day and overnight activities. This form of recreation influences the quality of the water as it originates as snowmelt in the uppermost areas of the alpine country.

2. **Backpacking and day hiking.** As the streams flow toward the valley they become more accessible by trails and as a consequence are more heavily used than the more remote streams. The use of the streams in these areas vary from consumption as drinking water to fishing and bathing by some hikers and backpackers. In the present investigation, the impact of these forms of recreation on water quality was studied.
3. **Horse-back riding.** Trails along the lower reaches of some streams within the park are heavily used by horses. Data obtained from streams above and below this activity will reflect the influence of this use on the quality of water.

4. **Swimming, fishing and boating.** The lakes and streams located in the valley floor of Grand Teton National Park, which collect water from the higher, more remote areas, are used for such activities as fishing, swimming and recreational boating including both motorized and non-motorized crafts. The valley floor is also the location of housing for seasonal and permanent park employees and concessionaires who operate climbing schools, boat tours, horse rides and general stores. Data obtained from waters in these areas will reflect the influence of such activities on the quality of the water within the valley floor.

Another desirable and unique quality provided by Grand Teton National Park is that the high alpine zone is a relatively simple ecosystem when compared with those of the lower wooded areas. Of particular importance in the high alpine zone is the lack of the topsoil component which
normally serves as a biological and physical filter to re­duce or eliminate the quantity of fecal pollutants which reach the water course. This observation suggests that the high alpine zone is more fragile and can, therefore, suc­cessfully withstand less human visitation before water quality deteriorates and become a public health hazard. This simplified ecosystem also makes it easier to study the effects of the surrounding ecosystem on the types and occurrence patterns of bacterial flora of sanitary impor­tance found in high alpine waters.

Because of the need to study the quality of water originating in the high alpine zone and because of the unique and desirable study situations provided by Grand Teton National Park the present study was carried out on streams and lakes within that particular park. Drainages were investigated that allowed the comparison of high al­pine areas exposed to intensive recreational use with those having little or no human activity. This study also pro­vided the opportunity to follow waterborne bacterial micro­flora from the source of the streams in the high alpine zone and observe changes in this population as the waters progressed toward the valley floor passing through biologi­cal communities of increasing complexity.
The objectives of this study were:

(1) To determine the types and occurrence patterns of the bacterial flora of sanitary importance in a high alpine water supply.

(2) To evaluate the impact of various recreational activities on the indicator bacterial flora of a high alpine water supply.

(3) To determine the effects of the surrounding ecosystem on the bacteriological water quality.

(4) To provide a basis relative to water quality and usefulness of indicator bacteria, for the management of various areas within Grand Teton National Park.
LITERATURE REVIEW

Effects of Recreational Use on Water Quality

In recent years a number of studies have been made to determine the nature and extent of water quality changes in mountain streams resulting from recreational use. While water quality deterioration has been observed, the results of most studies indicate that there is little correlation between the quantity of pollution and the extent of recreational use. Benedetti (10), in an investigation of the effects of recreational use on municipal watersheds in the Pacific Northwest, determined that there was very little change in the water quality with recreation. The most significant effect of recreation was found to be the disruption of soil and vegetation around the water causing heavier contamination during times of runoff. Carswell et al. (14) found that there was little or no deterioration of water quality in five separate watersheds as a result of recreation permitted on streams and reservoirs of municipal watersheds. It was concluded that the effects of recreation on aesthetics, damage to facilities, complications in administration of public property, and political and sociological impacts were of greater significance than the effects of recreation on water quality.
In an extensive study of the chemical and bacteriological analysis of three mountain watersheds in Oregon and Washington, by Lee et al. (48), it was shown that there was no significant differences between the effects of a heavily used recreational area on water quality as compared with the effects of lightly used and closed watersheds. It was concluded that recreational use of an area is not always followed by high coliform counts. Also, it was determined that wild animal populations were responsible for most of the indicator organism densities, particularly at low levels of stream flow (48, 61). In a similar study carried out in the Bozeman Creek Watershed, which was closed to public entry from 1917 to 1970, while the adjacent Hyalite Watershed was developed during this period by the Forest Service for public use, a significant difference in the quality of the water was found between the two areas (11, 60, 65). In this study, the open watershed consistently produced water of a higher quality than did the closed watershed over a six-year period. Even the highest, most undisturbed streams in the closed watershed had coliform counts exceeding state criteria for "A-Closed" municipal water supplies (30). It was also believed that the large population of big game animals in the closed area contributed
to this observed lower water quality (60). Van Nierop (64) has shown that, with proper practices being observed, public uses of reservoirs and municipal watersheds such as logging, fishing, boating, etc., can be permitted without detrimental effects on water quality.

In studying the Boundary Waters Canoe Area of Minnesota, Barton (5) observed that there were some serious water quality problems attributed to recreational use. It was estimated that recreational visitors contributed approximately 9 tons NaCl, 1 ton phosphorus and 13 tons nitrogen to the waters of this remote area in one year. King and Mace (44) also conducted investigations into the effects of recreation on water quality of lakes in the Boundary Waters Canoe Area. Of the parameters measured, total coliform populations and available phosphate concentrations seemed to be affected by recreational use of selected campsites. It was concluded that the leaching of effluent from pit privies near the campsites was probably responsible for water quality changes. Shoreline activities such as swimming, washing dishes, cleaning fish, and boat launching were also listed as probable causes of contaminations. Skinner et al. (57,58), in studies of alpine and non-alpine regions in Wyoming, noted that bacteriological water
quality appeared to be adversely influenced by recreational use of the land and stream flow. In a study done by the California Water Resources Center (17) on the Bishop Creek Wilderness Area high fecal coliform and high fecal streptococci counts were closely associated with human intervention. Studies done by Johnson and Middlebrooks (41) on the South Fork of the Ogden River indicate that there is insufficient contamination from present land uses along the river to exceed stream standards or to create a health hazard. However, within the recreational area, water quality data could be used to indicate differences in use at particular recreational sites.

Methods of Studying Water Quality in High Alpine Regions.

Probably the most useful water quality parameter in correlating recreational use with water pollutions is the indicator bacterial population. Strong technical foundations have been laid for this type of work. Both qualitative and quantitative standard methods have been developed for the determination of bacterial indicators of pollution (1,22). Fair and Morrison (50) and Kunkle and Meiman (46,47) have provided information on proper sampling and handling of data concerning high quality water from
mountainous regions. Studies on Flathead Lake in Montana demonstrated the effectiveness of using bacterial indicators of pollution to assess water quality in a relative pristine lake (6). Peterson and Boring (52) used coliform counts to assess the impacts from recreation and grazing on two isolated mountain streams. Although it was found that these uses had an influence on coliform counts, it was not determined how much of an influence came from specific land uses and how much was naturally occurring. Kabler and Clark (43) determined that fecal coliform counts are more reliable indicators of fecal contaminations than total coliform counts. Geldreich (26) states that the fecal coliform test is the most accurate determination for detecting water pollution by the feces of warm-blooded animals. In a study done on the Buffalo Lake Recreation Area, Geldreich (28) again offers a rationale for the fecal coliform concept with respect to: sanitary significance, density relationships with fecal streptococci and use of fecal coliforms as a bacteriological approach to a study of the recreational area. Geldreich has also suggested that fecal streptococci counts can be extremely useful in determining the source of pollution (29). Although fecal coliform counts have an excellent correlation with fecal
contamination from warm-blooded animals, they do not differentiate between contamination from humans and animals. Geldreich found that *Streptococcus bovis*, *S. equinus* and *S. faecium* are found in very large numbers in warm-blooded animals other than man. On the other hand *S. faecalis* is rather unique to the human intestinal tract. Geldreich and Kenner (27) used a ratio of fecal coliforms to fecal streptococci (FC/FS) to determine the exact origin of contamination. A high ratio (4:1 or greater) indicates human origin, while a low ratio (1:0.7 or less) indicates animal origin. A modification of the FC/FS ratio was proposed by the Ohio River Valley Sanitary Commission (51). This approach uses the fecal coliform total coliform ratio (FC/TC) to measure the effects of human contamination on a river or stream. However, use of the FC/TC ratio was suggested more as a measure of the seriousness and extent of human fecal contamination rather than to differentiate between sources of pollutions.

One of the purposes for measuring coliform densities is to get an indication of the probable presence of pathogens. Fair and Morrison (21), however, have isolated salmonellae from unpolluted Colorado streams containing only 30 coliforms per 100 ml. Gallagher and Spino (25) point
out that low total and fecal coliform counts do not, by themselves indicate the absence of pathogens. Dutka (19) attacks the use of the total coliform count as an indicator based on studies showing that coliforms do not fulfill any of the criteria for a true indicator organism. Geldreich (29) has suggested that fecal coliform counts are more sensitive as indicators of pathogens. It was found that salmonella species occurred 85 percent of the time when fecal coliform counts were between 200 and 2000 per 100 ml. Smith and Twedt (59) observed that no salmonellae were isolated at concentrations of less than 200 fecal coliforms per 100 ml, but that in almost every case for densities higher than this, these pathogens were found.

**Effect of Surrounding Ecosystem (other than man) on Water Quality**

In a study of mountain streams by Morrison and Fair (50), it was found that runoff was the most important factor in influencing bacteriological counts. Coliform counts, as well as chemical concentrations, were found to be the highest during times of overland flow. In a similar study by Kunkle and Meiman (46), it was found that the highest total and fecal coliform counts occurred during the spring runoff and the lowest counts occurred during the
winter. In a continuation of their mountain stream studies, Kunkle and Meiman (47) determined that total coliform counts varied on a diurnal basis as well as seasonally and with land uses.

Kittrell and Furfari (45) determined that coliform counts were not only related to levels of organic loading, but also determined by temperature, rainfall, runoff, stream characteristics, pH and turbidity. It was also observed that total coliform counts actually increased under conditions of suitable stream temperature and pH. Hendricks (36), using a chemostat, found maximal specific growth rates for various enteric bacteria including pathogenic strains occurred at 30°C in autoclaved river water taken 750 m below a wastewater outfall. Culture generation times ranged between 33.3 and 116 hr. Little or no growth occurred in the water at incubation temperatures of 20 and 5°C and neither the stock cultures nor the aquatic strains were capable of growth in autoclaved river water taken above the wastewater outfall at the three different temperatures tested. Survival of bacterial indicators in subarctic Alaskan river under total ice cover and water temperature at 0°C was studied by Gordon (31) who reported that after 7 days flow time, total coliforms were reduced to 3.2 to
6.5 percent of the initial count, fecal coliforms 2.1 to 4.2 percent and fecal streptococci 18.1 to 37.3 percent. Another study done by Hendricks (34) on growth of selected enteric bacteria in clear mountain stream water indicated that the aquatic environment associated with a clear mountain stream not only can maintain populations of enteric bacteria but also can supply sufficient nutrients to initiate multiplications and de novo protein synthesis. Jannasch (40) calculated generation times of 20 to 200 hr for aquatic bacteria in natural waters as a result of significant differences between dilution rates and washout rates in a chemostat. The measured growth rates were affected by the treatment of the water samples (type of sterilization) and by competition with the natural microflora for the unknown growth-limiting substrate. Bott (12) using direct measurement of bacterial growth rates in a natural stream showed that the unicellular and filamentous populations had doubling times of 42 to 51 hr when the water temperature was 0 to 5°C, 8.4 to 10.8 hr when the water temperature was predominantly 11 to 16°C, and 2.8 to 6.0 hr when the water temperature was 16.5 to 21.0°C. Hendricks (37) in studying sorption of heterotrophic and enteric bacteria to glass surfaces, showed an initial rate of attachment equivalent
to a doubling time of about 24 hr. After 24 hr both the sorbed and suspended populations stabilized with a mass doubling time approximating 100 hr. Hendricks (35) also demonstrated that bottom sediments of streams have a high adsorptive capacity for basic nutrients derived from the flowing water, retaining them in a form readily usable by various enteric bacteria including pathogenic strains. Sedimentation and adsorption of bacteria also occur in stream bottom sediments resulting in higher microbial populations than that observed in overlying waters.

The survival of intestinal bacteria in water may also be influenced by the antagonistic action of organisms in the indigenous flora, particularly by available predators. Johnstone and Kubinski (42) found that a few species of ciliated and flagellated protozoans were a major mechanism responsible for the removal of fecal bacteria in high quality waters. However, initial predatory responses required a lengthy lag period of 4 to 5 days.

Indicator bacteria must have certain quantities of organic carbon present for their survival and multiplication. Studies by Butterfield (13) and more recently by McGrew and Mallette (49), have shown that intestinal bacteria, including Escherichia coli can survive and later multiply in media.
that contained less than 5 µg/ml glucose. Hendricks and Morrison (34) also point out the fact that enteric bacteria can grow and reproduce in extremely dilute nutrient concentrations.

There is good evidence that healthy, actively growing phytoplankton species release a considerable proportion of their photoassimilated carbon into the aquatic environment (2,15,23,24,33,38,55,56,62). Hellebust (33) reported that some phytoplankton are capable of excreting up to 25 percent of their photoassimilated carbon during their log growth phase. Therefore, when large populations of algae are present, adequate supplies of carbon should be present for the survival of some heterotrophic water borne bacteria. Ward and Moyer (66) reported that organics excreted by algae during growth could serve as bacterial nutrient sources. Bell et al. (9) observed that dominant bacterial populations associated with algal blooms are a result of both stimulation and inhibition mediated by the release of extracellular products. Using C\(^14\)-labeled glycollate, Wright (69) measured uptake by the natural planktonic microorganisms in a lake in eastern Massachusetts. Bacteria able to grow on glycollate exhibited the same uptake pattern as seen in the lake. Bauld (7,8) in studying benthic
algal-bacterial mats in alkaline hot springs, demonstrated that C¹⁴-labeled organic compounds excreted during algal photosynthesis could be subsequently assimilated by natural populations of the bacteria present in the mat.
MATERIALS AND METHODS

The present research was carried out at the Jackson Hole Biological Research Station, Montana State University and at various sampling sites within Grand Teton National Park.

Travel to Sampling Sites

Travel to the sampling sites from the lab headquarters at the research station was by car and on foot. The trip to the trail heads by car was about 12 miles and the hike to the farthest sampling site was about 8 miles on foot with the other sites along the way.

Location of Study Sites

The main study areas, Figure 1, were the streams and lakes in Leigh and Cascade Canyons as well as the lakes in the valley into which these drainages empty (Leigh, String and Jenny Lakes) in addition to the streams in Glacier Gulch and Garnet Canyon. One phase of the overall study centered around a microbiological evaluation of the heavily used Cascade Canyon with Lake Solitude (elevation 9035 ft) at its upper end. Site C-1 (Lake Solitude) was located above timberline in a glaciated cirque. Sites C-2 through C-8 were located below timberline with Sites C-3 through C-8 being
Figure 1. Location of sample sites within Grand Teton National Park.
influenced by the south fork as well as the north fork of Cascade Creek. Control samples that were taken along Leigh Creek and at Mink Lake (elevation 8898 ft) gave an indication of the microflora of a similar alpine stream that was seldom visited by man. Mink Lake (Site L-1) was located above timberline with the remaining sites in Leigh Canyon below timberline.

The microbiological water quality of the valley lakes (Leigh, String and Jenny) was determined by obtaining samples from the inflow and outflow of each lake. These sites were useful in determining the impact of activities along these lakes. Jenny Lake, which served as the drinking water supply for a campground and visitor center, was used for motorized boating, canoeing and fishing. String and Leigh Lakes were used for fishing, swimming and canoeing. Samples were also taken at 1/8 mile intervals along Cottonwood Creek, which drains Jenny Lake, to determine the source of the contamination found in that stream.

Another phase of the study that was initiated in 1973 involved an examination of the waters in Garnet Canyon. Most of this drainage was located above timberline and was formed from small streams that originated as snowmelt (Site G-1) at elevations around 11,549 ft. The water
then flowed through a granite boulder field and emerged at Site G-2 at an elevation of 10,000 feet. The water proceeded through alpine meadows (Site G-3) and dropped below timberline at Site G-6 at an elevation of 3,793 ft. This drainage along with Surprise Lake outlet which drained into the same canyon was used to determine the effects of the surrounding ecosystem on the bacteriological water quality.

Testing

1. **Bacteriology.** Bi-weekly samples were collected from the sites during the summer months (from late June to early September). Water samples were collected aseptically, iced and carried directly (in less than 5 hours) to the field laboratory at Moran, Wyoming for microbiological evaluation. Samples were tested, according to Standard Methods (1), for standard plate count at 35°C, and for total coliform and fecal streptococci by membrane filtration techniques. Data obtained each year were processed to provide geometric means for each site.

   Representative colonies were picked from the coliform and streptococcus plates, put on enrichment media and transported to Montana State University water microbiology laboratory where they were characterized further:
I. Coliform bacteria were classified as INViC type and fecal origin (1).

II. Fecal streptococci were differentiated to species according to Facklam (20).

2. Animal dropping examination. Fresh dropping samples of moose, elk and marmot were aseptically collected and refrigerated. These samples were then transported to the Montana State University water microbiology laboratory where the numbers and types of fecal streptococci in the droppings were determined.

3. Visitor use data. In order to better correlate human use of the study area with microbiological data, the recreational uses made of the various areas were determined by trail counts and back country use data collected by the National Park Service.

4. Physical and chemical water analysis. Temperature, pH and conductivity were taken at all sites to provide a general physical and chemical evaluation of the waters under investigation. Weather conditions were noted before, during and after sampling periods and weather records were obtained from park headquarters at Moose in
order to correlate bacteriological results with rainfall and runoff.

5. Ecological testing. In order to correlate the growth of algae in the streams with the presence or growth (if any) of bacteria in the stream, several studies were conducted.

a. Determination of chlorophyll content of periphyton samples: The apparatus used to collect periphyton on artificial substrates was an adaptation from that used by Bahls (3). A single acrylic plastic plate with a collecting surface area on each side of 100 cm² was bolted to a 1 m concrete reinforcing rod which was secured by rocks so that it was positioned lengthwise across the stream. All plates were positioned uniformly so that the top surface of each plate was approximately 1 to 3 cm below the water surface and parallel to the direction of the current flow. Six plates were positioned in Surprise Lake outlet. The first plate was analyzed after two weeks and one plate was analyzed each following week for the next five weeks. The periphyton sample was scraped at the field site with a single-edge razor blade from the 100 cm² sampling area of each plate into a 250 ml wide mouth sampling
bottle containing 200 ml of sterile water. The sample was then covered, iced and transported to the laboratory where a 10 ml aliquot was taken from the bottle and tested for coliforms. The remaining sample was filtered onto a membrane filter (Millipore HAWP 04700), put into a dessicator that was sealed from light, and held at 4C until phytopigment analyses could be conducted at Montana State University. When this was done, the filter was placed in a centrifuge tube with 5 ml of 90 percent acetone solution and allowed to sit in the dark at 4C for 24 hr. After the initial extraction period, the samples were centrifuged for 10 min at 500x g and the absorbance of the supernatant was determined at 750, 665, 645 and 630 nm using a Varian Techtron (Model 635). The absorbance data were then used to determine the amount of chlorophyll a, b and c using formulas presented in Standard Methods (1). When the concentration of pigment in the extract was determined, the amount of pigment per unit surface area of sample was calculated as follows:

\[
\text{mg chlorophyll/m}^2 = \frac{\text{mg chlorophyll/l} \times \text{volume of extract (liters)}}{\text{area of substrate (m}^2)}
\]

b. Determining growth of coliform bacteria in presence of algae: Algae attached to the rocks within
Surprise Lake outlet were scraped into 250 ml sample bottles containing river water and were transported to Montana State University. To aid in isolation and identification, the algae samples were placed in Gorham's medium (39) and allowed to grow for several weeks under constant illumination by fluorescent light. The cultures were then streaked onto a solid Gorham's medium (32) (Gorham's medium and 1.5 percent agar) containing 30 μg/ml of tetracycline to prevent bacterial growth and allowed to grow for several more weeks under constant illumination at 10°C. Algal colonies were then picked from solid Gorham's into Gorham's medium and the growth was followed over a period of time using a G. K. Turner fluorometer to measure the chlorophyll a concentration of the growing culture. The algal cultures were under constant illumination by two G.E. Cool White fluorescent tubes with an approximate light intensity of 500 ft-candles during time of growth. Cultures were periodically checked to see if they had remained axenic by plating 1 ml aliquots with tryptone glucose yeast extract agar and checking for bacterial growth.

Selected bacteria isolated from Surprise Lake outlet waters were grown in tryptone glucose yeast extract (TGE) broth for 24 hr. After the 24 hr, 1 ml aliquots of
the selected bacterial suspensions were added to the growing algal cultures during various phases of its growth curve. The bacterial populations, which started out at approximately $1 \times 10^5$ organisms/ml, were then followed over a period of time by plating 1 ml samples of the algal-bacterial mixed cultures on TGE agar at one day intervals. Controls used were bacteria in Gorham's medium alone and algae in Gorham's alone. Growth of the algae was also followed at the same time using a G. K. Turner fluorometer.

Algal excretions were tested for their ability to stimulate bacterial growth by growing large axenic batch cultures of the selected algae. The batch cultures were then centrifuged using a continuous flow centrifuge and the supernatant minus the algae was used for testing. The supernatant was treated in various ways (autoclaving, filter sterilizing and flash evaporating) then again selected 24 hr cultures of bacterial isolates from Surprise Lake outlet were added to the supernatant and their growth followed over a period of time by plating out on TGE agar at one day intervals.
RESULTS

In general, the waters in the study area were found to be pristine in nature exhibiting low bacterial counts. Specific conductance for all sites ranged from 9.8 to 24.2 micromhos and bacterial counts were usually less than 50 organisms per ml for total coliforms and fecal streptococci and less than 50 organisms per ml for standard plate count (Figs. 2-4). The temperature increased throughout the summer, but ranged from 0 to 3°C in the upper canyons to the low teens in the lower canyons and valley sites. The pH values of these waters were 6.5 to 7.1.

Quantitative Bacteriological Studies of Water Samples

Counts of indicator bacteria in Leigh Canyon waters were consistently less than 30 per 100 ml at Mink Lake (Site L-1, Figs. 1 and 2). As this water flowed down Leigh Canyon through brush then below timberline, the bacterial populations increased (Site L-2, Figs. 1 and 2). The water flowing into Leigh Lake at the lower end of this canyon and Leigh Lake itself showed a decrease in bacterial numbers (Sites L-3 and V-3; Figs. 1 and 2). Bacterial numbers decreased even more in the valley lakes until they approached the lowest numbers at the outflow of Jenny Lake (Site V-6; Figs. 1 and 3).
Figure 2. Bacteriological profile of Leigh and Cascade Canyons showing geometric means of bacterial populations. Standard plate count, total coliform and fecal streptococcus counts are for data collected in 1972, 1973, 1974, 1975.
Figure 3. Bacteriological profile of valley lakes and Cottonwood Creek showing geometric means of bacterial populations. Standard plate count, total coliform and fecal streptococcus counts are for data collected in 1972, 1973, 1974, 1975.
The bacterial counts in the upper waters of Cascade Canyon were also low at Lake Solitude (Site C-1; Figs. 1 and 2) and increased as the water flowed down canyon below timberline (Sites C-2 and C-3). Again, bacterial populations decreased as the water flowed through the lower portions of the canyon, meandering through a nearly level wooded section of the canyon (Site C-5), and then cascading into Jenny Lake passing heavily used parts of the canyon trail (Site C-8). In general, the counts in Cascade and Leigh Canyons did not differ greatly; however, the values from Leigh Canyon were slightly higher (Fig. 2).

In Garnet Canyon a similar pattern was seen as in Cascade and Leigh Canyons. Indicator bacterial counts were low near the origin of the stream (Sites G-1 and G-2), increased as the water flowed through more complex biological communities (Site G-3), and decreased as the water slowed as it passed through the more level sections of the canyon (Sites G-4 and G-6; Figs. 1 and 4). The high populations of fecal streptococci and standard plate counts at Site G-1 were a reflection of difficulties in collecting water samples from snowmelt without obtaining some dirt and detritus from the area surrounding the snowfield. These high counts were not seen in years where the snowmelt was
Figure 4. Bacteriological profile of Garnet Canyon showing geometric means of bacterial populations. Standard plate count, total coliform and fecal streptococcus counts are for data collected in 1973, 1974, 1975.
ORGANISMS/100mL (SPC/ml)

TOTAL COLIFORMS
FECAL STREPTOCOCCI
STANDARD PLATE COUNT

COLLECTION SITES

ORGANISMS/100mL (SPC/ml)

TOTAL COLIFORMS
FECAL STREPTOCOCCI
STANDARD PLATE COUNT

COLLECTION SITES

G-1
G-2
G-3
G-4
G-6

36
sufficient to allow collection from a small drainage without obtaining dirt in the sample.

The microbiological quality of upper Cottonwood Creek was studied more extensively in 1974 and 1975 than previously. Figure 3 shows the occurrence of high coliform counts at Sites V-7 (Lupine Meadow Bridge) and V-8 while the counts at V-6 (Jenny Lake Boat Dock) and other valley sites remained low. The other bacterial categories tested showed similar results (Fig. 3). A more detailed examination for indicator bacteria was conducted at approximately 100 yard intervals between the Jenny Lake Boat Dock (Site V-6) and the Lupine Meadows Bridge (Site V-7). This study indicated that there was at least one location where the coliform counts made significant increases (Fig. 5). This site on Cottonwood Creek (Site 6) was 20 yards from the south end of the corral, 100 yards below where drainage was seen entering the main creek. Fecal coliform, fecal streptococci ratios (FC/FS) were calculated on data from all 8 sites for a series of 10 sampling runs in 1975 and a series of three runs in 1974. In every case the FC/FS ratio was 0.7 or less.

Several other sites within the park also exhibited high coliform counts. These were the outflow of Surprise
Figure 5. Mean and highest recorded coliform bacterial populations at eight sites in upper Cottonwood Creek between Sites V-6 and V-8 for 1975. The number represents FC/FS ratios for sampling cycle where highest count was obtained.
1975

GEOMETRIC MEANS FOR 1975
HIGHEST COUNT SEEN IN 1975
NUMBERS REPRESENT FC/FS

COLIFORM BACTERIA/100 ml

COLLECTION SITES

1  .06
2  .04
3  .03
4  .01
5  .03
6  .005
7  .006
8  .004
Lake (Site G-5) which had a geometric mean of 122 coliforms per 100 ml in 1975 and lower Glacier Gulch (Site G-7) which had a geometric mean of 115 coliforms per 100 ml in 1975. In order to examine this unexpected result, samples were taken at weekly intervals from 6 different sites along the creek below the outlet of Surprise Lake. The coliform counts were low just below Surprise Lake (Site 1) and increased as the stream progressed down the steep narrow canyon to Site G-5 (Fig. 6). The relatively low counts at Site G-5 reflect the observation that this site dried up each year before the bacterial populations in the stream started to show an increase later in the summer.

In studying Glacier Gulch more closely, samples were taken at two sites above G-7 (bridge across Glacier Gulch on Amphitheater Lake trail). Both sites, which were close to Delta Lake, showed significant lower coliform counts (geometric means of 6.5 and 15 coliforms per 100 ml) when compared with Site G-7.

Qualitative Bacteriological Studies of Water Samples: Coliforms

Of the bacterial colonies counted as total coliforms on the original millipore plate, only a fraction were confirmed as being fecal coliforms using standard
Figure 6. Geometric mean of coliform bacterial populations at six sites in Surprise Lake outlet between outlet of lake and Site G-5 for 1975.
microbiological procedures. The results from 1972 through 1975 are seen in Table 1. Of 1459 coliform colonies picked from original plates, 17.6 percent were confirmed as being fecal coliforms.

**Qualitative Bacteriological Studies of Water Samples: Fecal Streptococci**

Differentiation of fecal streptococci to species yielded the results seen in Table 2. The percentage of *Streptococcus faecium* was higher in all canyons than the percentage of *S. faecalis*. *S. equinis* was isolated from Cascade Canyon (8.4 percent of colonies picked) and the valley lakes and streams (8.6 percent of colonies picked).

**Bacteriology of Animal Droppings**

Droppings from the major animals in the study area were collected and the fecal streptococci differentiated and counted in 1972. Marmot droppings were taken to represent the large rodents and the moose as the primary ungulate within the study area. The results of the analysis are also seen in Table 2.

**Human Use Patterns in the Study Area**

In order to relate the microbiological data to human use within the study area, trail count information...
Table 1. Percent of coliform organisms isolated that are characterized as fecal

<table>
<thead>
<tr>
<th>Year</th>
<th>Leigh Canyon</th>
<th>Cascade Canyon</th>
<th>Valley Sites</th>
<th>Garnet Canyon</th>
<th>Glacier Gulch</th>
<th>Surprise Lake</th>
</tr>
</thead>
<tbody>
<tr>
<td>1972</td>
<td>42</td>
<td>23</td>
<td>4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1973</td>
<td>18</td>
<td>10</td>
<td>18</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1974</td>
<td>42</td>
<td>19</td>
<td>35</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>1975</td>
<td>54</td>
<td>18</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2. Percentage distribution of fecal streptococcus species obtained at different sites and in animal droppings. These are geometric means of data collected in 1972, 1973, 1974, 1975

<table>
<thead>
<tr>
<th>Species</th>
<th>Cascade Canyon (309)a</th>
<th>Leigh Canyon (175)a</th>
<th>Valley Lakes (215)a</th>
<th>Garnet Canyon (221)a</th>
<th>Moose (35)b</th>
<th>Marmot (8)b</th>
<th>Human (20)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus bovis</td>
<td>4.1</td>
<td>6.7</td>
<td>2.0</td>
<td>4.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus faecium</td>
<td>8.9</td>
<td>10.9</td>
<td>27.7</td>
<td>12.1</td>
<td>55</td>
<td>89</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>3.1</td>
<td>3.5</td>
<td>1.9</td>
<td>2.8</td>
<td>0</td>
<td>0</td>
<td>75</td>
</tr>
</tbody>
</table>

a Total number of colonies picked

b The number of samples analyzed
was collected. The upper reaches of Leigh Canyon were virtually untraveled by humans, however, the lower mile of the canyon was visited, with 196 backpacker nights being recorded for 1974 and 629 backpacker nights being recorded for 1975. Cascade Canyon, on the other hand, was heavily used (4557 backpacker nights in 1974 and 4753 backpacker nights for 1975). On an average mid-summer day, approximately 300 people reached the trail head at the bottom of Cascade Canyon (Site C-8). Of these about half were scenic hikers and went no farther than Hidden Falls (1/2 mile) or Inspiration Point (1 mile). Of those who went beyond Inspiration Point, there were day hikers (84 percent), backpackers (14 percent) and climbers (less than 5 percent). About 30 people reached Lake Solitude (Site C-1) each day in August; about eight by horseback. In the valley, hikers and backpackers used the edges of Leigh, String and Jenny Lakes, and there was some boating in each lake. During late July and in August there was considerable swimming activity in String Lake within the 1/2 mile above Site V-4. Garnet Canyon also received intense use (3115 backpacker nights in 1974, 3056 in 1975) with climbers comprising more than 75 percent of these visitors.
Bacterial Correlations With Rainfall and Runoff

When bacterial counts were plotted for various sites over the sampling season, a correlation was seen between periods of heavy rainfall and high counts (Fig. 7). In 1974, afternoon rains and thundershowers were noted on the week of July 17, increasing with frequency and length through the week of August 10, then decreasing until the end of the study period on the week of September 5. Other years did not show as good a correlation as did 1974 because rainstorms occurred intermittently throughout the entire summer rather than being concentrated into one period during the summer.

Bacterial Correlations With Algae

The benthos of Surprise Lake outlet consisted of complex communities containing many types of protozoa and algae of which the genera *Gloeocapsa*, *Stigonema* and *Chlorella* have been tentatively identified. The communities appeared as thin green films that covered the rocks which were under 1 to 3 cm of water. Chlorophyll a accrual (determined between Sites 4 and 5 in Surprise Lake outlet) was low at the start of the summer increased about mid August then decreased near the end of the summer (Fig. 8). The water
Figure 7. Total coliform bacterial populations at Sites V-7 and C-2 with dates of sample collection during summer of 1974.
Figure 8. Chlorophyll $a$ accrual for one site and total coliform bacterial populations for three sites in Surprise Lake outlet with dates of sample collection during the summer of 1975.
1975

CHLOROPHYLL

BACTERIA

COLIFORM BACTERIA / 100 ml

CHLOROPHYLL a ACCRUAL (mg/m² / 2 weeks)

JULY

AUGUST

SEPT

G-5

S-4

S-5
temperature of Surprise Lake outlet was 0C at the start of the summer, increased gradually to 10C by July 22, peaked out at 13C around August 26, then started to decrease until it was 7C by September 2. Bacterial counts in Surprise Lake outlet followed a pattern similar to the algae.

Of the algae isolated from Surprise Lake outlet *Chlorella* was chosen as the primary experimental algae for laboratory studies because it was the easiest of those observed to isolate and cultivate. The experiments done with *Chlorella* in the laboratory by no means represent all of the possible interactions that could have taken place within the complex communities in Surprise Lake outlet. Rather, the experiments were designed to see if extracellular products excreted by algae could support the growth of selected bacteria, of sanitary importance, from that stream.

Figure 9 shows the growth curve of *Chlorella* in Gorham's medium, growth of *Chlorella* in Gorham's with a selected bacterium from Surprise Lake outlet present, the population changes of the bacterium in Gorham's medium alone and the population changes of the bacterium with *Chlorella* present. In three trials (two with isolates picked from coliform plates done at Surprise Lake outlet and one laboratory EC+ culture isolated from an East
Figure 9. Growth rates at 10°C of algae in Gorham's medium, algae in Gorham's medium with bacteria present, bacteria in Gorham's medium and bacteria in Gorham's medium with algae present.
Gallatin sample) all bacterial populations dropped off dramatically after a few days when in the presence of algae. Mixed bacterial populations obtained from filtering one liter of water from Surprise Lake outlet onto a membrane filter were tested in algal supernatant. The algal supernatant was obtained from centrifuging a batch culture of *Chlorella* that was in the stationary growth phase. When the filters containing the mixed bacterial populations were placed into the algal supernatant, dramatic increases in bacterial numbers were seen after 5 days at both 4 and 35°C (Fig. 10). Mixed bacterial populations added to algal supernatant that had been concentrated by flash evaporation also showed marked increases in numbers after 5 days. Selected pure cultures (two isolates from Surprise Lake outlet and an EC+ culture isolated from the East Gallatin River) showed increases in 5 days when tested in algal supernatant that had been treated in several ways (boiling for 15 min, filter sterilized and autoclaved). Total carbon analysis done on growing algal cultures in lag phase of growth showed that the total carbon content of algal supernatant was approximately 10 to 20 mg C/l which is essentially what is obtained when Gorham's medium is tested.
However, the total carbon content from the supernatant of algae in stationary phase of growth have total carbon contents in excess of 50 mg C/l.
Figure 10. Growth rates at 4C and 35C of mixed bacterial populations in supernatant obtained from centrifuging a 10 batch culture of algae in the stationary phase of growth.
BACTERIA IN SUPERNATANT AT 35°C

BACTERIA IN SUPERNATANT AT 4°C

LOG NUMBER OF BACTERIA/ml

DAYS

10^4

10^5

10^6

10^7
DISCUSSION

The quantitative bacteriological results seen in Figures 2 through 4 show that the three types of bacteriological populations enumerated in this study (standard plate count, total coliforms and fecal streptococci) are not excessive when compared with other high elevation mountain areas (11,18,57,60,65) and are below 200 fecal coliforms per 100 ml which has been recommended as the limit for primary contact recreational waters (67). Water from the alpine region of Grand Teton National Park consistently contained some indicator bacteria and an increase in this population was demonstrated as the water flowed toward the valley (Figs. 2, 3 and 4). As the flow rate slowed in the lower canyons and the valley lakes, the bacterial populations were reduced probably by a variety of factors such as die-off, settling and predation (Figs. 2, 3 and 4).

As the water that originated in the high alpine country flowed toward the valley, no gross evidence of adverse bacteriological impact resulting from human activities was found. This can be seen in the data comparing Leigh Canyon, which receives little human use with Cascade Canyon which receives extensive visitor use (Fig. 2). This
is further supported by the bacteriological identification that revealed the fecal streptococci were generally of the types found in the feces of the native moose and rodent populations (Table 2). These results from the high alpine zone compare in some respects with observations made by others in lower watersheds. In those studies comparing the aquatic microflora of municipal watersheds that were either open or closed to human activities, the numbers of indicator bacteria were consistently higher in the areas where human use was excluded (60, 65). Our data (Fig. 2) support that conclusion. Other researchers have also determined that there has been very little change in the water quality with recreation (10, 14, 48). However, Skinner et al. (58) reported that the numbers of selected bacterial populations appeared to increase from recreational use for short periods in some cases. In comparing Leigh and Cascade Canyons, the conclusion may be reached that man has not contributed to any large scale contamination as far as the bacterial quality of the water is concerned. This can also be supported by the studies done in Garnet Canyon which receives extensive overnight use with limited sanitary facilities. Most sites in that canyon that were commonly used for camping were in close proximity to the
stream. There were, however, low numbers of indicator bacteria present in the water. It should also be noted that the populations of indicator organisms were higher in regions where human use was absent (Fig. 2). This observation suggests that these bacteria may be considered "natural" in some pristine waters.

From the information gained over the past four summers, it appears that waters originating in the high alpine zone contain only a few indicator bacteria. As these waters merge and flow toward the sub alpine valley, they pass through a succession of biological communities that increase in complexity and density. The flora and fauna of this continually changing series of biological communities contribute bacteria to the water in proportion to the biological density and diversity such that the numbers of indicator bacteria increase. This can be observed from the data that were obtained in Garnet Canyon (Fig. 4) as the water flowed from the steep rocky slopes (Site G-1 and 2) through alpine meadows (Site G-3) and then meandered through a lightly wooded alpine canyon (Sites G-4 and 6). Data obtained from water in the lower more heavily wooded canyons, such as upper Leigh Canyon (Site L-2), also support this idea of a higher "natural" population of
indicator bacteria because of the more complex biome surrounding the stream. In Cascade Canyon there was a similar microbiological pattern (Fig. 2).

Once the rivers emptied into the valley lakes and the flow rate was slowed, the bacterial populations were reduced, probably by a variety of effects such as die-off and settling. This reduction was seen in all four years (Fig. 3) as the water from both Leigh and Cascade Canyons slowly passed through the valley lakes. Because of this occurrence, the water at the Jenny Lake east boat dock (Site V-6) consistently had the lowest indicator bacterial populations observed in the study area. However, one-quarter mile below the boat dock on Cottonwood Creek, the numbers of indicator bacteria rose dramatically (Figs. 3 and 5) indicating a potential problem. Possible sources might have been the horse corral on the west bank of Cottonwood Creek or the dwellings used by the boat concessionaires, guide service and horse concessionaires. When studied more closely the FC/FS ratios and the correlations of high counts with rainfall tended to implicate surface contamination originating from the horse corral. The low fecal coliform counts (Table 1) and the low percentage of *Streptococcus equinis* and other streptococci that originate
in the intestine of the horse (Table 2), suggest that the increased counts are partly due to "natural" flora of the stream that would appear there even if the horse corral were absent. Figure 5, however, does show at least one input source probably coming from the horse corral.

Several other sites within the park showed elevated counts of indicator bacteria; specifically total coliforms. Surprise Lake outlet was one of these areas containing a geometric mean of 122 coliforms per 100 ml at site G-5. On the basis of observations in 1974 that bacterial populations appeared just after the establishment of algal communities in the stream a hypothesis was put forth that algal communities provided a suitable environment for the growth of some types of non-fecal coliform bacteria. Several authors have indicated that the aquatic environment associated with a clear mountain stream not only can maintain populations of enteric bacteria but also can supply sufficient nutrients to initiate multiplication and de novo protein synthesis (12,34,35,40). However, generation times ranged between 20 and 200 hr depending on temperature. It has been shown by several authors that organic chemicals excreted by algae during growth could serve as bacterial nutrient sources (7,8,9,33,66,69).
Figure 8 shows a correlation between chlorophyll a and bacterial populations in Surprise Lake outlet. This is not to say that the bacterial populations necessarily increased because of the algae since both could have been increasing because of some other unidentified variable such as temperature or nutrient loading.

Hellebust (33) reported that some phytoplankton are capable of excreting up to 25 percent of their photosynthesized carbon during their log growth phase. Based on the amount of chlorophyll a in Surprise Lake outlet (Fig. 3) and an assimilation number of 3.7 mgC/mg chla/hr as given by Ryther and Yentsch (54) and again later by Wright (68), one would expect 1.98 mgC being photoassimilated per m² of surface area per hr. Based on McGrew and Mallette's study, this would be sufficient to maintain any bacteria that are present with the algae. However, one must take into consideration such things as inhibitory substances excreted by algae. Figure 9 shows a rapid decline in the numbers of bacteria when exposed to a growing culture of algae. The same bacterium shows no decline when put into the medium without any algae. One must also take into consideration temperature. Bott (12) using direct measurement of bacterial growth rates in a natural
stream showed that selected bacterial populations had generation times of 2.8 to 6.0 hr when the temperature was 16.5 to 21.0°C. However, generation times increased to 42 to 51 hr when the temperature was lowered to 0 to 5°C. Hendricks (36) observed little or no growth of various enteric bacteria in water with incubation temperatures of 20 and 5°C. This is also seen in our data where bacterial counts only started going up after the stream temperature reached approximately 10°C. Figure 10 shows the growth curves of a mixed bacterial culture in algal supernatant at 35 and 4°C. Although the total carbon content was high (approximately 50 mgC/ℓ), substantial growth was noted at 4°C.

At this point it is difficult to say with certainty that bacteria were actually growing in the stream. The present studies have shown that it is possible but further work is needed to determine how many of these bacteria are being washed into the stream and how many are actually growing there.

Another area that exhibited high coliform counts was Glacier Gulch. Based on indicator bacterial counts taken at various locations in the drainage, the high counts at G-7 were probably of animal origin. In addition,
coliforms might also have been introduced from the swamp located above G-7.

An important source of variation in the bacterial numbers found in these waters was rainstorms which caused some overland flow (Fig. 7). This observation was also reported by Morrison and Fair (50) and by Kunkel and Meiman (46).

Table 2 shows the qualitative distribution of fecal streptococci in four portions of the study area within Grand Teton National Park observed in 1972, 1973, 1974 and 1975. These data indicate that *Streptococcus bovis* and *S. faecium* made up a high percentage of the fecal streptococcus population in the study areas. From the data relating the characteristic fecal streptococcus species found in representative animals that were within the study area, it may be seen that non-human sources were possibly responsible for the fecal contamination that was indicated by the coliform bacteria and the fecal streptococci found in the water. This assumption is based on the observation that human fecal material contains a substantial portion of *S. faecalis* and further, that this organism is seldom found in animal droppings.
Management Implications

From the results of this research it may be tentatively concluded that man was not responsible for any consistent, large scale contamination of back country waters within the study area in 1972, 1973, 1974 and 1975. This says little, however, about the safety of drinking untreated water within this area since the actions of a single careless individual could create a health hazard. In this connection, Fair and Morrison (21) isolated salmonellae from unpolluted Colorado streams containing only 30 coliforms per 100 ml. It was concluded that unpolluted, potable surface water sources do not exist. This brings out the question of usefulness of indicator bacteria for detecting sources of contamination in high alpine lakes and streams and points out some of the difficulties in establishing meaningful water quality criteria for these waters.

It should also be noted that man's absence does not imply that the water is safe to drink. Several bacteria known to cause disease in man have been isolated from wild animals. Of these, salmonellae appear to be ubiquitous and are shed in the feces of infected animals (16,53). Recently Thomason et al. reported that salmonellae may
survive harsh environments such as weather pools (63). Leptospirosis is another disease transmissible from animals to humans and is generally transmitted by contact with water contaminated by urine from infected animals. Leptospiral antibodies have been found in many types of wild animals as reported in a review by Reasoner (53), indicating widespread occurrence of this bacterium in nature. Of protozoan diseases Giardiasis has been noted in the Rocky Mountain States (16). Out of 54 people who had been camping in the Unitah Mountains in Utah 28 were positive for *Giardia lamblia* and all had obtained their drinking water from a stream at an altitude of 8,000 ft. *Giardia* sp. have been found in many wild and domestic animals.

The management question of the maximal carrying capacity of alpine area such as Grand Teton National Park is complex but may be approached from the standpoint of aquatic microbiology as well as from other environmental considerations. At present the back country areas of Grand Teton National Park have not yet reached the maximum carrying capacity as far as the water quality is concerned and could withstand increased visitor use. However, it is relatively easy to state that a high level of indicator bacteria shows water to be unsafe, but it is extremely
difficult to indicate exactly when water is safe for human consumption and activity because of the diversity of potential infectious agents and their sources. It would probably be wise at present to make decisions relating to carrying capacity with the aid of macroscopic biological parameters such as the numbers and variety of flora observed within areas of concern.

The present study indicates that the horses in the upper Cottonwood Creek area are likely the primary contributors of fecal contamination of the river. In terms of the number of coliform bacteria that were detected in upper Cottonwood Creek, the water is "contaminated" but not heavily so in the area below the horse corral. A federal standard that would apply to this situation is the U.S. Public Health Service Drinking Water Standards for Group 2 water if it is to be used for human consumption. This category of water requires only that disinfection be performed prior to use as drinking water. In order for water to be classified as Group 2 water it "should not average more than 50 coliform bacteria per 100 ml in any month." The water in the rest of the park that we have tested (except for Glacier Gulch and Surprise Lake outlet) could be classified as Group 2 water, however, the water from
the upper area of Cottonwood Creek exceeded this standard in July and August in the years under this study. From this standpoint a comprehensive examination of the use policy in this area of the park is in order. This might include the elimination of the horses from their present location and the reduction of the human habitation in the area or the installation of more vault type human sanitary facilities that are pumped on a regular basis and the elimination of the older out-houses.
SUMMARY

Selected waters from the high alpine zone within Grand Teton National Park, Wyoming, were studied during the past four summers to determine, 1) the types and occurrence patterns of the bacterial flora of sanitary importance, 2) to evaluate the impact of various recreational activities on the indicator bacterial flora, 3) to determine the effects of the surrounding ecosystem on bacteriological water quality and 4) to provide a basis relative to water quality and usefulness of indicator bacteria, for the management of various areas within Grand Teton National Park.

The water samples collected were analyzed for total coliforms, fecal coliforms, fecal streptococci and standard plate count at 35°C. Drainages were investigated that allowed the comparison of high alpine areas exposed to intensive recreational use with those having little or no human activity. In order to correlate the growth of algae in the streams with the presence or growth (if any) of bacteria in the stream several studies were conducted.

In general, the three types of bacteriological populations enumerated were not excessive when compared with other high elevation mountain areas. Water from the alpine
region consistently contained some indicator bacteria and an increase in this population was seen as the water flowed toward the valley. When this flow rate slowed in the lower canyons and valley lakes, the bacterial populations were probably reduced by a variety of factors such as die-off, settling and predation.

From the results of this research it may be tentatively concluded that man was not responsible for any consistent large scale contamination of back country waters within the study area. Within the valley, however, it was shown that horses are likely the primary contributors of fecal contaminations of upper Cottonwood Creek.

Correlations were made between chlorophyll a concentrations and total coliform populations as well as growth responses of mixed bacterial populations in algal supernatant at 4C. It was concluded that non-fecal type coliforms may grow and multiply in alpine streams using extracellular products excreted by algae. It was not determined, however, to what extent (if any) this occurs in the alpine streams with Grand Teton National Park.

Other areas exhibiting high indicator bacterial counts were influenced by high animal concentrations and swamp-lands located above the sampling sites. Another
important cause of variation in bacterial numbers appeared to be rainstorms which caused some overland flow.

Questions were brought out concerning the usefulness of indicator bacteria for detecting sources of contamination in high alpine lakes and streams and the difficulties in establishing meaningful water quality criteria in these areas. It was concluded that management questions relating to the carrying capacity of alpine areas should be approached with the aid of other biological parameters along with levels of indicator bacteria in the streams.


Microbial studies of a high alpine water supply