Effects of Multiple Use on Water Quality of High-Mountain Watersheds: Bacteriological Investigations of Mountain Streams

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Bacteriological studies in 1968 and 1969 corroborated earlier findings that a municipal watershed which had been closed to public entry since 1917 yielded water with four to six times the coliform count found in an adjacent mountain watershed open to recreational activities. Similarly, chemical investigations showed higher concentrations of most ions in water from the closed area. Physiological differentiation of coliform and enterococcal bacteria revealed similar types of organisms in both animal droppings and stream water, with fecal coliforms accounting for as much as 70% of the coliform counts observed in the closed area in 1969. Opening of the closed drainage for limited recreation and expanded logging operations in the spring of 1970 coincided with an unexpected decrease in bacterial contamination of that stream. It is postulated that these human activities drove from the watershed a large wild animal population which had contributed substantially to the previous bacterial pollution. It would seem that the practice of closing high-mountain watersheds to public entry is questionable if governmental standards for water quality are to be met, and it also seems that the standards themselves should be reexamined.

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, the quality of the streams must receive prime consideration. However, as Teller (Ph.D. Thesis, Univ. of Washington, Seattle, 1963) indicates, there is insufficient information to determine what natural water quality is or to what extent bacterial numbers in a stream can be attributed to man-made or to natural causes. The fact that Fair and Morrison found bacterial pathogens in "clean" streams high in the Colorado Rockies substantiates this (7). The effect of clear-cut logging on the water temperature of mountain streams with small flow volumes may be extremely significant in the management of Pacific Northwest watersheds according to Brown and Krygier (5). The question of public use of reservoirs and municipal watersheds has been studied by Carswell et al. (6) and Van Nierop (Ph.D. Thesis, Cornell Univ., Ithaca, N.Y., 1963), resulting in essential agreement that recreation in and around water supplies results in little or no deterioration in bacteriological water quality. Presently, there are several satisfactory techniques for such investigations. Geldreich (8), Kunkel (11), and Schuettpeiz (13) agree that the fecal coliform test provides a good indication of fecal water pollution by warm-blooded animals, and several studies have demonstrated the value of coliform counts in evaluating mountain watersheds (3, 12, 14, 15). The practical use of certain streptococci as indicators of pollution has been described by Geldreich and Kenner (9).

Extensive high elevation bacteriological studies on an open and closed watershed within 20 miles of Bozeman, Mont., have been conducted since 1964 (4, 10, 15). The present study concerns bacteriological findings in 1968, 1969, and 1970 of a municipal water supply obtained from these two adjacent sources which have been termed "opened" and "closed" to general public use. In the open Hyalite watershed (Middle Creek) of approximately 30,080 acres, the public has had free access to the area for recreational purposes and commercial logging since an earth dam was constructed in 1939. The Mystic watershed (Bozeman Creek), including about 5,760 acres, has been closed to public entry, except for limited

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logging, since 1917. It was opened to foot and horseback travel in the spring of 1970 as well as for camping, fishing, and hunting. Vehicular travel is permitted only for logging, mainly in the South Fork of Bozeman Creek (Fig. 1).

Earlier results (15) revealed greater numbers of indicator organisms in the closed Mystic drainage than in the open Hyalite watershed. This report furnishes data to explain these unexpected findings.

**MATERIALS AND METHODS**

**Sampling.** Weekly samples were collected from different sites (Fig. 1) during the summer months as soon as roads were suitable for traveling the 70 miles involved. Routinely, the first sample was collected from the surface of Mystic Reservoir about 9 AM, and the others were collected subsequently at approximately the same time on each occasion. All samples were returned to the University laboratory by 1:00 PM. All containers were stored in a Coleman cooler until tested, usually within 4 hr. Water and air temperatures and pH were recorded for each site.

**Testing.** Procedures recommended in the 1965 edition of *Standard Methods for the Examination of Water and Wastewater* (1) were followed, except where noted. Coliform and enterococcal counts were obtained by the membrane filter technique with filter type HAWG 047 SO (Millipore Corp.) with a pore size of 0.45 μm. All plates were incubated for 40 hr at 35 C, so as to include attenuated organisms in this high-quality water environment.

For coliform counts, 1.0- and 10-ml samples were filtered, and the membranes were rolled onto pads (Millipore) saturated with 1.8 ml of m-endo broth (Difco) in disposable, sterile, plastic petri dishes (50 by 12 mm; Falcon Plastic, box type). For enterococcal counts, 100 to 250 ml of water was sampled, and the filter was placed on 10 ml of m-enterococcus agar (Difco) in disposable, sterile, plastic petri dishes (60 by 15 mm; Falcon Plastic, no. 1007). Standard plate counts (SPC) were obtained by plating 1.0- and 0.1-ml samples with tryptone glucose extract agar (Difco).

**Examination of animal droppings in the closed watershed.** Many of the animals in the Mystic drainage were known to be in the high country, hence trail bikes were used to gain access into areas where droppings of moose, elk, deer, and bear were easily located. Fecal samples were collected aseptically in 35-ml bottles containing four types of media, respectively: Brilliant Green-lactose-bile broth for detecting coliform bacteria; azide dextrose broth for enterococci; selenite broth for isolating salmonellae, shigellae, and other gram-negative enteric bacteria; and lactose broth for enrichment of entrobacteria prior to using selenite broth. The samples were shaken vigorously and then
used to determine nitrate and orthophosphate. Conductivity measurements were taken on a Lab Line Lectro MHO meter (model MC-1, Mark IV). Analyses of nutrients as well as anions and cations followed Standard Methods for the Examination of Water and Wastewater (1).

RESULTS

Quantitative bacteriological studies of water samples. "Bacteriological profiles" of geometric means for the Mystic and Hyalite watersheds in 1968, 1969, and 1970 are shown in Fig. 2 to 4. These profiles were based on 8, 9, and 13 weekly collections for the respective years. Geometric means were used to reduce variations in counts that occurred throughout the summer months caused mainly by fluctuations in water levels and flow rates.

During 1968 and 1969, no great difference was observed between the standard plate counts in the two watersheds. In 1970, a 5- to 10-fold increase in total organisms was obtained at each site. This increase can be attributed to incubating plates at

**Fig. 2. Bacteriological profile of Bozeman Creek (closed Mystic area) and Middle Creek (open Hyalite area) showing geometric means of organisms per 100 ml. SPC (■), coliform (●), and enterococcus (▲) counts geometrically averaged from 8 weekly collections during the summer of 1968.**

**Fig. 3. Bacteriological profile of Bozeman Creek (closed Mystic area) and Middle Creek (open Hyalite area) showing geometric means of organisms per 100 ml. SPC (■), coliform (●), and enterococcus (▲) counts geometrically averaged from 9 weekly collections during the summer of 1969.**

transported to a field laboratory near the Mystic reservoir. All cultures were incubated for 18 to 24 hr in a water bath at 35 C until taken to the University laboratory for subculturing in appropriate media. After 24 hr of incubation, cultures were streaked onto differential media, eosin methylene blue agar, Barn's (or m-enterococcus) medium, and MacConkey agar, respectively. Representative colonies were picked onto stock culture agar slants, incubated for 24 hr at 35 C, and held in the refrigerator for future testing.

**Differential tests.** Typical coliform bacteria were further differentiated as to fecal coliforms, *Escherichia coli*, *Enterobacter (Aerobacter) aerogenes*, and intermediates (1).

Gram-negative enteric bacteria other than the coliforms were differentiated with Kliger's iron agar, urea broth, lysine decarboxylase, dulcitol, citrate, and indole.

Determination of enterococcal species in 1970 was based on a scheme presented by Ayres et al. (2) utilizing production of ammonia from arginine, acid production from lactose and mannitol broth, growth on potassium tellurite agar, and growth on 5% horse blood-agar.

**Chemistry.** A filtered sample collected in glass was
and diversion dam (site 3) of Mystic gave higher counts. Comparison of the two curves (Fig. 2 and 3) for each watershed indicates excellent correlation for all stream sites.

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

Examination of the enterococcal counts in 1968 and 1969 reveals slightly greater contamination in the closed Mystic area. The contamination once again increased in both watersheds as the water flowed downstream. An adequate explanation of this increase has yet to be determined. The enterococcal counts in 1970 were nearly identical at all sites in both watersheds.

In 1969, a study of the South Fork (Fig. 1) was undertaken to determine the water quality of the major tributary of Bozeman Creek draining the upper basin of the Mystic watershed. The bacteriological profiles for the 2 years (Fig. 5

![Graph](http://aem.asm.org/)
and 6) reflect essentially straight lines for the SPC. The coliform profiles were, however, quite different. In 1969, the geometric means progressed from a low at A (62 per 100 ml) to a peak at E (219 per 100 ml) and subsequently decreased in numbers as the water flowed downstream to I (146 per 100 ml). In 1970, there was a definite decrease in coliform densities with a minimum of 22 per 100 ml at A and only 49 per 100 ml at E, representing a fourfold decrease at the latter site.

In 1970, samples were taken from two small tributaries (designated as sites D and F in Fig. 1) of the South Fork to determine what effect they might have on resulting bacterial densities farther downstream in the South Fork. The geometric mean at D was 20 coliforms per 100 ml, and at F it was 52 coliforms per 100 ml. A proper interpretation of these results is not yet possible.

The enterococcal profiles of the South Fork were essentially the same in 1969 and 1970. However, a slight increase was observed at B (36 per 100 ml) in 1969. No peaks were observed in 1970, the profile being basically a straight line.

Qualitative bacteriological studies of water samples: coliforms. A comparison of the differentiated coliform bacteria obtained from the two watersheds in 1968, 1969, and 1970 is shown in Table 1. The percentage of *Escherichia coli* was higher in Mystic than Hyalite for all 3 years. Also, the percentages of fecal coliforms were higher in the Mystic area. An even greater percentage of fecal coliforms was obtained from the South Fork area than from the lower Bozeman Creek in 1969 and 1970.

Qualitative bacteriological studies of water samples: enterococci. Differentiation of 448 enterococcal isolates to species resulted in the following: *Streptococcus faecium, S. faecium* var. *durans, S. faecalis, S. faecalis* var. *liquefaciens,* and *S. bovis.* Over 62% of the enterococci were found to be *S. faecalis* and *S. faecalis* var. *liquefaciens* occurring with nearly equal frequency.

### Table 1. Percentage distributions of *Escherichia coli, Enterobacter aerogenes, intermediates,* and *fecal coliforms* obtained at different sites

| Year        | Determination            | Mystic | Hyalite | Settling basin | South Fork | Animal droppings |
|-------------|--------------------------|--------|---------|----------------|------------|-----------------|
| 1968 (220   | *Escherichia coli*       | 33     | 25      | 46             | —          | —               |
| isolates)   | *Enterobacter aerogenes* | 23     | 43      | 0              | —          | —               |
|             | Intermediates            | 44     | 32      | 54             | —          | —               |
|             | Fecal coliforms          | 32     | 29      | 46             | —          | —               |
| 1969 (311   | *Escherichia coli*       | 47     | 38      | 31             | 41         | 80              |
| isolates)   | *Enterobacter aerogenes* | 42     | 41      | 46             | 46         | 0               |
|             | Intermediates            | 11     | 21      | 23             | 13         | 20              |
|             | Fecal coliforms          | 64     | 52      | 38             | 70         | 100b            |
| 1970 (535   | *Escherichia coli*       | 35     | 12      | 31             | 47         | 74              |
| isolates)   | *Enterobacter aerogenes* | 10     | 24      | 4              | 15         | 12              |
|             | Intermediates            | 55     | 64      | 65             | 38         | 14              |
|             | Fecal coliforms          | 42     | 29      | 48             | 54         | 100b            |

*Not determined.

*b Fresh droppings.*
TABLE 2. Comparison of mean water temperature, pH, and conductivity values at different sites in Mystic (closed) and Hyalite (open) watersheds

| Sitea | Year | Water temp (C) | pH  | Conductivity (micromhos) at 25°C |
|-------|------|----------------|-----|----------------------------------|
|       |      | Mystic | Hyalite | Mystic | Hyalite | Mystic | Hyalite |
| S     | 1968 | 16.4  | 15.3    | 7.8   | 7.6    | -      | -      |
| 1     |      | 10.7  | 9.1     | 7.3   | 7.3    | -      | -      |
| 2     |      | 8.8   | 10.1    | 8.0   | 7.4    | -      | -      |
| 3     |      | 9.2   | 10.3    | 8.0   | 7.5    | -      | -      |
| S     | 1969 | 18.6  | 17.6    | 8.4   | 9.0    | 175    | 75     |
| 1     |      | 10.3  | 9.7     | 7.8   | 7.8    | 205    | 73     |
| 2     |      | 8.7   | 10.7    | 8.2   | 8.1    | 215    | 117    |
| 3     |      | 9.3   | 11.1    | 8.3   | 8.1    | 221    | 120    |
| S     | 1970 | 16.1  | 14.4    | 7.8   | 7.6    | 149    | 58     |
| 1     |      | 9.0   | 8.5     | 7.2   | 6.9    | 168    | 60     |
| 2     |      | 7.5   | 10.1    | 7.4   | 7.0    | 166    | 93     |
| 3     |      | 7.9   | 10.2    | 7.6   | 7.1    | 179    | 96     |

* Surface of reservoir (S), reservoir outlet (1), halfway point (2), and diversion dam (3).

Bacteriology of animal droppings. Animal droppings from elk, moose, bear, deer, and horse were collected from the closed Mystic area, especially in the South Fork drainage area. Different genera of gram-negative organisms including Escherichia, Enterobacter, Proteus, Salmonella, and Shigella as well as various streptococcal species were isolated from these fecal droppings and differentiated with physiological tests. Serological studies by Bissonnette et al. (4) indicated that identical E. coli serotypes were isolated from both animal droppings and water samples during 1969.

Physical and chemical results of water analyses. Chemical analyses indicated greater concentrations of most ions in the Mystic water than in the open watershed, whereas the settling basin showed intermediate values reflecting the mixture of the two. The South Fork waters contained even lesser concentrations of most ions than the open Hyalite watershed.

Water temperatures for the summers of 1968, 1969, and 1970 shown in Table 2 revealed no appreciable differences between the Mystic and Hyalite watersheds. The pH of the two streams (Table 2) was similar in both 1968 and 1969; however, the pH values in 1970 showed a decrease at all sites in both watersheds. A reflection of the greater chemical concentrations in Mystic is indicated from conductivity measurements shown in Table 2, Mystic having the higher measurements at all sites when compared to Hyalite. The South Fork had the lowest measurements.

DISCUSSION

The results from 1968 and 1969 corroborate the findings reported earlier by Walter and Bottman (15) in that the numbers of coliform and enterococcal bacteria were observed to be higher in water from the closed area (Mystic) than in water from the area which is open to public use (Hyalite). This has been consistently true over 6 years of study. It is interesting to note that the numbers of coliform bacteria in the open watershed are only about twice the standard of 50 per 100 ml set for class A closed municipal water supplies by the Montana State Water Pollution Control Council, whereas the adjacent closed watershed exceeds this standard nearly sixfold.

Since Bozeman Creek (closed) was found to have higher concentrations of the various chemical constituents tested for, it would probably be capable of supporting larger bacterial populations than Middle Creek (open). However, relatively faster growth of bacteria in Bozeman Creek is questionable because of the low water temperature (4 to 10 C), as is borne out by the observation that the overall productivity of the two streams, as measured by the SPC, was nearly the same (Fig. 2 and 3). It was also found by Goodrich et al. (10) that some of the highest coliform counts occurred in the highest, coldest, and most primitive tributaries of Bozeman Creek, the South Fork. Hence, some other hypothesis is required to explain these data.

As can be seen in Table 1, similar indicator organisms were found in both water and animal droppings and fecal coliforms made up a larger percentage of the coliform population in Bozeman Creek (closed) water than in Middle Creek (open) water. Also, serological relationships between E. coli from Bozeman Creek water and from elk and bear droppings were demonstrated by Bissonnette et al. (4).

The results from 1970 indicate that a significant change occurred in the Mystic watershed during that year. Specifically, notable decreases in coliform densities were observed at sites 2 and 3 during 1970 than were obtained in previous years (Fig. 2 to 4). Additionally, the coliform concentrations decreased in the South Fork area during 1970. Further indication of decreasing pollution in the Mystic watershed was reflected in an approximate 20% decrease in fecal coliform isolates in 1970 from both the Bozeman Creek and the South Fork.

A possible explanation for the observance of higher indicator organism densities in the Mystic watershed before 1970 was that this area had
become a game reserve since it had been closed to public entry for 50 years. Specifically, a large concentration of about 300 to 500 elk and other big game animals was reported by Forest Service personnel in the South Fork drainage. The decrease in coliform densities in the Bozeman and South Fork drainages in 1970 is believed to be a result of a decreased wild animal population in the South Fork area. In the spring of 1970, the Mystic area was opened for limited public use. Possibly, this entrance of human activity forced the animals from their normal habitat in the South Fork area. Additionally, extensive logging practices had taken place in 1970 in the same vicinity. The combination of sudden human activity and logging in the natural wild animal habitat apparently caused an exodus of animals from this area. This is reflected by a decline in coliform densities in the closed area in 1970.

Probable fecal coliform densities, calculated from geometric mean values developed on total coliform counts and fecal coliform percentages (Table 2), also lend support to this interpretation. The highest fecal coliform density observed during the study (150 per ml) occurred in the most primitive area of the closed watershed, the South Fork of Bozeman Creek, in 1969. Probable fecal coliform densities at M1 were 130 per 100 ml in 1969, whereas the 1970 figure of 38 per 100 ml was similar to those of H3 in 1969 and 1970 (31 and 24 per 100 ml, respectively).

With the increasing concern for the preservation of our natural environment, it is quite unique that the results of this study have shown that human activity has coincided with lowered bacterial pollution. As a result, it seems that in the future careful consideration should be given to the advisability of closing high-mountain municipal watersheds to public entry if governmental water quality standards are to be met, and it may also be necessary to reexamine the standards themselves.

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