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Winter Survival of Fecal Indicator Bacteria in a Subarctic Alaskan River

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WINTER SURVIVAL OF FECAL INDICATOR BACTERIA IN A SUBARCTIC ALASKAN RIVER

By

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ABSTRACT

Survival of fecal indicator bacteria in a subarctic Alaskan river was studied during the winter of 1969-70 when there was total ice cover and the water temperature was 0°C. Most of the domestic pollution entered the river from one source. Since no additional pollution entered downstream from this source, an uninterrupted study covering seven days of flow time (210 river miles) was possible. Nine sample stations were established to obtain total coliform, fecal coliform, enterococcus and water chemistry data. Samples were collected four to eight times from each station during the two week period of data collection, and a discharge measurement was made at each station during the same period. Bacteria survival was examined with and without consideration for the effect of dilution. After seven days flow time, total coliforms were reduced to 3.2-6.5 percent of the initial count, fecal coliforms to 2.1-4.2 percent, and the enterococci to 18.1-37.3 percent depending on dilution consideration.

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SECTION I

SUMMARY AND CONCLUSIONS

A winter survival study of fecal indicator bacteria (total and fecal coliforms, and enterococci) was conducted on the 210-mile reach of the Tanana River between its confluence with the Chena and Yukon Rivers. A large volume of domestic sewage effluent from the Fairbanks area enters the Tanana at its confluence with the Chena. Since the Tanana receives no additional sewage effluent downstream from the Chena, it was possible to conduct an uninterrupted survival study for 7 days of flow time. This study was conducted during February and March of 1970 when there was total ice cover on the river and the water temperature was 0°C. Several conclusions can be drawn:

1. The numbers of fecal indicator bacteria per 100 ml of sample decreased progressively downstream. However, they survived in significant numbers throughout the 7 days of flow time with 380 total coliforms, 88 fecal coliforms and 15 enterococci remaining per 100 ml of sample at the end of the time period.

2. The greatest percentage reduction in total and fecal coliform numbers occurred during the first 1.2 days of flow time since they decreased to 26.7-35.0 and 20.2-25.6 percent of the original numbers respectively, depending on the method of handling dilution.

3. When the bacteria counts were adjusted for river flow in cubic feet per second, the percent survival decreased progressively downstream, and after 7 days of flow time, 3.26.5 percent of the total coliforms, 2.1-4.2 percent of the fecal coliforms, and 18.1-37.3 percent of the enterococci remained viable, depending on the method of handling dilution.

4. Fecal coliform survival was 2.7-5.4 times greater than indicated by winter survival data from more temperate climates, depending on the method of handling dilution.

5. Enterococci had a much higher survival rate than either total or fecal coliforms after 7 days of flow time.

6. Water was apparently lost from the river channel by infiltration into groundwater reservoirs.

7. The groundwater associated with the Tanana River may have been somewhat contaminated if infiltration from the river channel carried bacteria with it.

8. No satisfactory correlation was established between the loss of viability of bacteria and the observed changes in chemical parameters.

9. Additional studies need to be conducted on several other aspects of fecal microorganisms in arctic and subarctic rivers:

A. Summer data similar to the winter data is needed to ascertain if a seasonal difference in indicator bacteria survival exists.

B. The survival of enteropathogenic bacteria must be studied under the same conditions to obtain a correlation between pathogens and indicators.

C. Other potential bacteriological water quality indicators should be studied to determine their relationship to enteric pathogens at low water temperatures.

D. The extent of fecal bacteria penetration into the groundwater reservoirs should be determined.

E. An adequate disinfection method for sewage plant effluents must be developed for use in the extreme environmental conditions of Alaska.

F. Enteric virus survival at 0°C water temperature must be examined.

SECTION II

INTRODUCTION

Bacteria of the coliform and fecal streptococcus groups have been used to indicate the sanitary bacteriological quality of water for many years. The development and present usage of these bacteria to indicate the potential presence of enteric pathogens have been reviewed recently (7, 16). Organic substrate concentration, pH, dissolved oxygen concentration and water temperature are among the more obvious factors affecting fecal bacteria survival and growth (2, 6). In view of these and other factors, the traditional concepts concerning the quantitative relationship between fecal indicator and enteric pathogenic bacteria have come under scrutiny as Geldreich (17) pointed out in his recent report.

The temperature of the suspending medium appears to have a significant effect on the growth and survival of fecal bacteria. Several low temperature growth studies have been conducted with pure cultures of Escherichia coli. In 1934, Haines (20) found that a strain of E. coli produced visable growth at 0°C after 29 days of incubation. This early report of growth at 0°C has not been substantiated. However, Das and Goldstein (9) showed that during the first few hours of incubation, E. coli had limited capacity to synthesize protein at 0°C. Ingraham (24) found 8°C to be the minimum growth temperature and, more recent work has shown the minimum growth temperature to be between 7.5 and 7.8°C (31). Shaw (30) found filaments produced at 6°C, and stated that this was below the minimum growth temperature. In their review, Ballentine and Kittrell (5) stated that data from stream studies did not show aftergrowth of coliforms at low temperatures.

Coliform and enterococcus survival studies have been conducted under controlled laboratory conditions at temperatures between 0°C and 10°C in various suspending media (19, 21, 22). In all cases, the survival rate at the low temperatures was higher than found in parallel studies at 20° to 30°C. Evidence from field studies is, perhaps, not as clear cut as laboratory studies. Nevertheless, there is evidence that fecal bacteria survived longer in streams when the water temperature was low (winter conditions) than when the temperature was warm (summer conditions) (5, 14, 25). In the reviews by Kittrell and Furfari (25) and Ballentine and Kittrell (5), summer and winter water temperatures were considered to be above and below 15°C respectively, and their interpretation of temperature effect was based on this arbitrary dividing line.

Water temperature of the arctic and subarctic rivers in Alaska is 0°C for about 6 months of the year, with total ice cover during most of this period. The temperatures are below 5°C for an additional

4 months, and may exceed 15°C for about 2 weeks during the summer. As a result of low year-around water temperatures, fecal bacteria survival may be higher throughout the year than temperate climate data indicate. This may be more pronounced when the water temperature is at or near 0°C.

This study was conducted to gain some insight into fecal indicator bacteria survival during winter with total ice cover and 0°C water temperature because: [1] the probability of a higher survival rate makes extrapolation from temperate climate data questionable; [2] Ballentine and Kittrell (5) pointed out that few streams have sufficient water travel time between sewage outfalls in which to obtain survival data; and [3] Berg et al. (6) stated that there is a practical need for survival time parameters.

SECTION III

MATERIALS AND METHODS

Selection of River to be Studied

The Tanana River, one of the major rivers draining the interior of Alaska, rises near the Canadian border and flows generally northwest for several hundred miles to its confluence with the Yukon River. The Tanana carries a heavy silt load during the summer, but clears up in the fall and remains clear throughout the winter. The Chena River, which flows through Fairbanks, is a small tributary of the Tanana River. Raw domestic sewage and effluents from primary treatment plants enter the Chena in the Fairbanks area causing a high pollution level in the lower portion of the river. The Chena enters the Tanana a few miles below Fairbanks and is the major source of pollution in the Tanana. From the confluence of the Tanana and Chena Rivers, the Tanana flows 210 miles to the Yukon (Figure 1). Since none of and villages on the Tanana below the Chena have sewage collection systems, there are no significant additional sources of fecal material. The reach below the Chena confluence represents a little more than 7 days flow time and provides an excellent opportunity to study fecal indicator bacteria survival.

Sample Station Selection and Sampling Schedule

Nine sample stations were selected and their locations are shown in Figure 1. Seven stations (T-100 to T-700) divide the Tanana River into approximately equal reaches below its confluence with the Chena River. One station (T-800) above the Chena monitored the fecal bacteria from upstream. The Chena River station (C-100) was downstream from all domestic sewage sources in the Fairbanks area.

Field data was collected during the last week of February and the first week of March 1970 because U.S. Geological Survey measurements indicated that discharge is stable during this part of the winter. Also, it was early enough in the year to ensure total ice cover, and late enough to provide adequate daylight in which to sample all stations each day. Only two stations (C-100 and T-600) were accessible by road so it was necessary to use an aircraft equipped with skis for sampling. Stations T-100 through T-800 were reached by aircraft and C-100 by ground transportation.

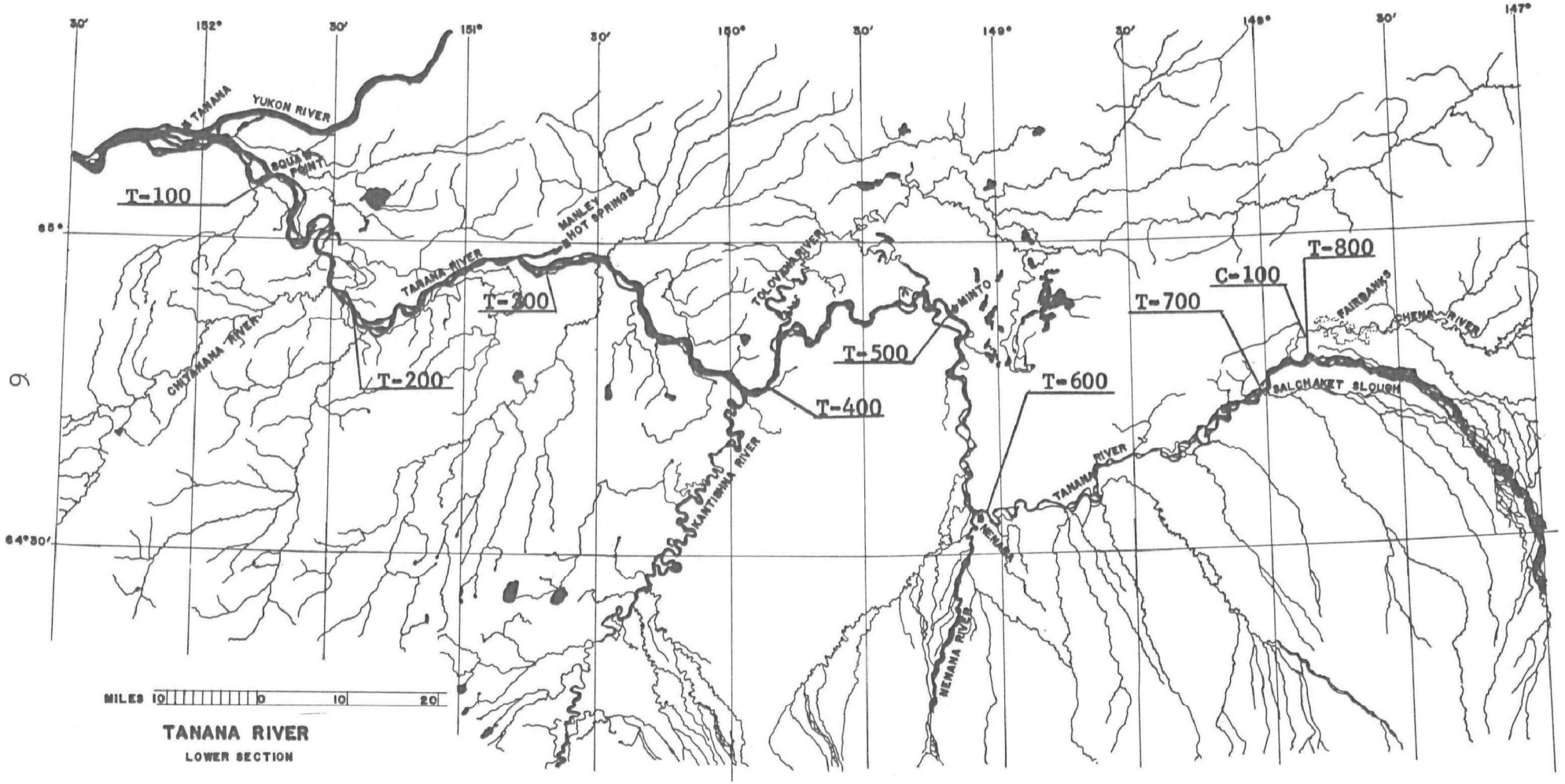

Figure 1. Map of the Lower Tanana River Showing the Location of Sample Stations

Eight sampling trips were scheduled and accomplished, at least in part, during the 2-week study period. However, weather-related problems encountered in the field prevented some sample stations from being reached each day. Samples were obtained as many as eight times, but no fewer than four times from all stations.

Sampling Techniques

A gasoline powered ice auger was used to cut through the 3 to 6 feet of ice encountered at each sample station. There were only a few occasions when it was not necessary to re-cut the hole each day.

A rod sampler (Figures 2, 3, and 4) was developed at the Alaska Water Laboratory for obtaining bacteriological and dissolved oxygen samples from below the ice. Two models of this sampler were made from aluminum tubing. Both models were capable of extension to 12 feet, one telescoping and the other in 3-foot breakdown sections. The upper end of each was equipped with a T-bar as a handle and the bottom with a support and clamp to hold the sample bottle. A rubber stopper attached to a nylon line was placed in the top of the sample bottle. When the bottle had been lowered to the desired depth, the stopper was pulled and the bottle allowed to fill completely with water.

Samples for bacteriological analysis were taken in sterile, 1-liter glass bottles attached to the rod sampler. As soon as a sample was brought to the surface, 50-100 ml of the water was poured from the bottle and a glass stopper inserted. Dissolved oyxgen samples were taken in 300 ml BOD bottles. The samples were fixed and the bottles stoppered as soon as they were brought to the surface. Samples for chemical analysis were taken just under the water surface in hand-held 500 ml polyethylene bottles. All samples were transported to the laboratory in ice chests for analysis.

Enumeration of Fecal Indicator Bacteria

Total coliforms (3), fecal coliforms (16) and enterococci (3) were enumerated by the membrane filter method. Total coliforms were grown on m-Coliform Broth (BBL) at 35°C for 24 hours, fecal coliforms on m-FC Broth Base (Difco) for 24 hours in a 44.5°C water bath, and enterococci on m-Enterococcus Agar (Difco) for 48 hours at 35°C. Three volumes of sample were used for each analysis and plates were made in triplicate at each volume. The three plates from the volume giving the best count were evaluated by the Q Test (32) to reject questionable results. All results from each sample station were treated mathmetically (28) to determine the arithmetic mean, standard deviation and 95 percent confidence limits.

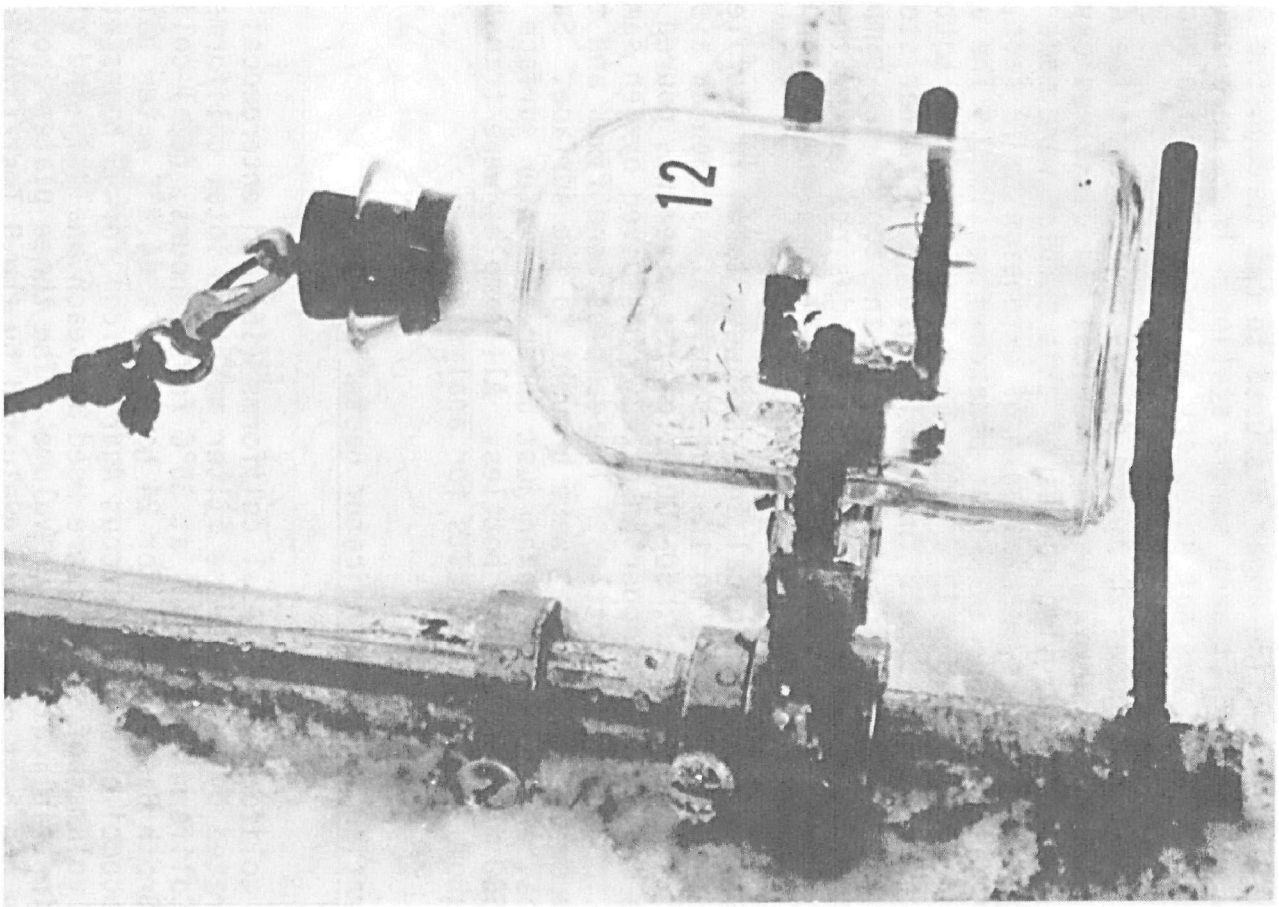

Figure 2. Rod Sampler with Attached BOD Bottle

Figure 3. Attaching Bacteriological Sample Bottle to Rod Sampler

Figure 4. Obtaining Sample from Beneath Ice with Rod Sampler

Chemical Analyses

Dissolved oxygen was determined by the azide modification of the idometric method as described in APHA Standard Methods (3).

Alkalinity, pH and conductivity were determined by the instrumental methods described in FWPCA Methods for Chemical Analysis of Water and Wastes (10).

A Technicon Auto Analyzer was used to determine ammonia nitrogen by the sodium phenolate method (10), and nitrate nitrogen by hydrazine reduction (10).

Total phosphorus was determined by the persulfate digestion method (10).

Hydrology

Discharge was measured at each sample station by U.S. Geological Survey personnel using the current-meter measurement procedure described by Buchanan and Somers (8), and a typical measurement is shown in Figure 5.

A dye study was conducted by U.S. Geological Survey personnel between stations T-600 and T-500 to aid in determining the time required for a water mass to move between stations. Rhodamine B dye was injected at T-600, and the passage of the dye was measured at T-500 using a Turner Model 111 Fluorometer with a high sensitivity door.

Figure 5. Discharge Measurements

SECTION IV

RESULTS

The travel time between stations and the water volume at each station was needed to interpret fecal indicator survival data. The most accurate time-of-travel information would have been obtained by a dye study between each pair of stations, but such an extensive dye study was impossible. However, it was feasible to conduct a dye study between one pair of stations. The reach between the T-600 and T-500 stations was selected because the gradient was the approximate average of all reaches.

Before the dye study was conducted, the velocity estimated for this reach was 1 mile per hour (mph) based on gradient. Figure 6 shows that the dye peak reached T-500 in 24 hours, which indicates the water moved at 1.25 mph. Since the Tanana is braided for most of its length below Fairbanks, the smaller and slower channels considered in the original estimate may have been frozen dry. This would mean that most of the water moved in the deeper and faster main channel. The cubic feet per second (cfs) discharge at each sample station, and water velocity estimates between stations, are presented in Table 1. These velocity estimates were the basis for determining the flow time between sample stations.

Since discharge usually increases as the water moves downstream, there are some apparent anomalies in the data presented in Table 1. The discharge increased 2490 cfs between T-600 and T-500 followed by a decrease of 2780 cfs between T-500 and T-400. The discharge again increased 980 and 760 cfs respectively at T-300 and T-200. This was followed by a decrease of 1350 cfs at T-100. These data indicate that far more water enters the Tanana than shown by the overall increase of 100 cfs between T-600 and T-100. The U.S. Geological Survey maintains a permanent gaging station at the T-600 station. Since discharge is measured regularly at this station, it is probably the most reliable measurement obtained during the study. On the first day of the study, discharge at T-100 and T-600 was nearly the same. Because of this, discharge at T-100 was measured again on the last day of the study. The two results differed by only 2 percent which indicated an acceptable degree of measurement reliability. Thus, only a small portion of the discharge anomaly can be attributed to measurement error.

The reason for the discharge anomalies evident in Table 1 has not been established. However, the most probable explanation is loss of water from the channel to adjacent groundwater reservoirs.

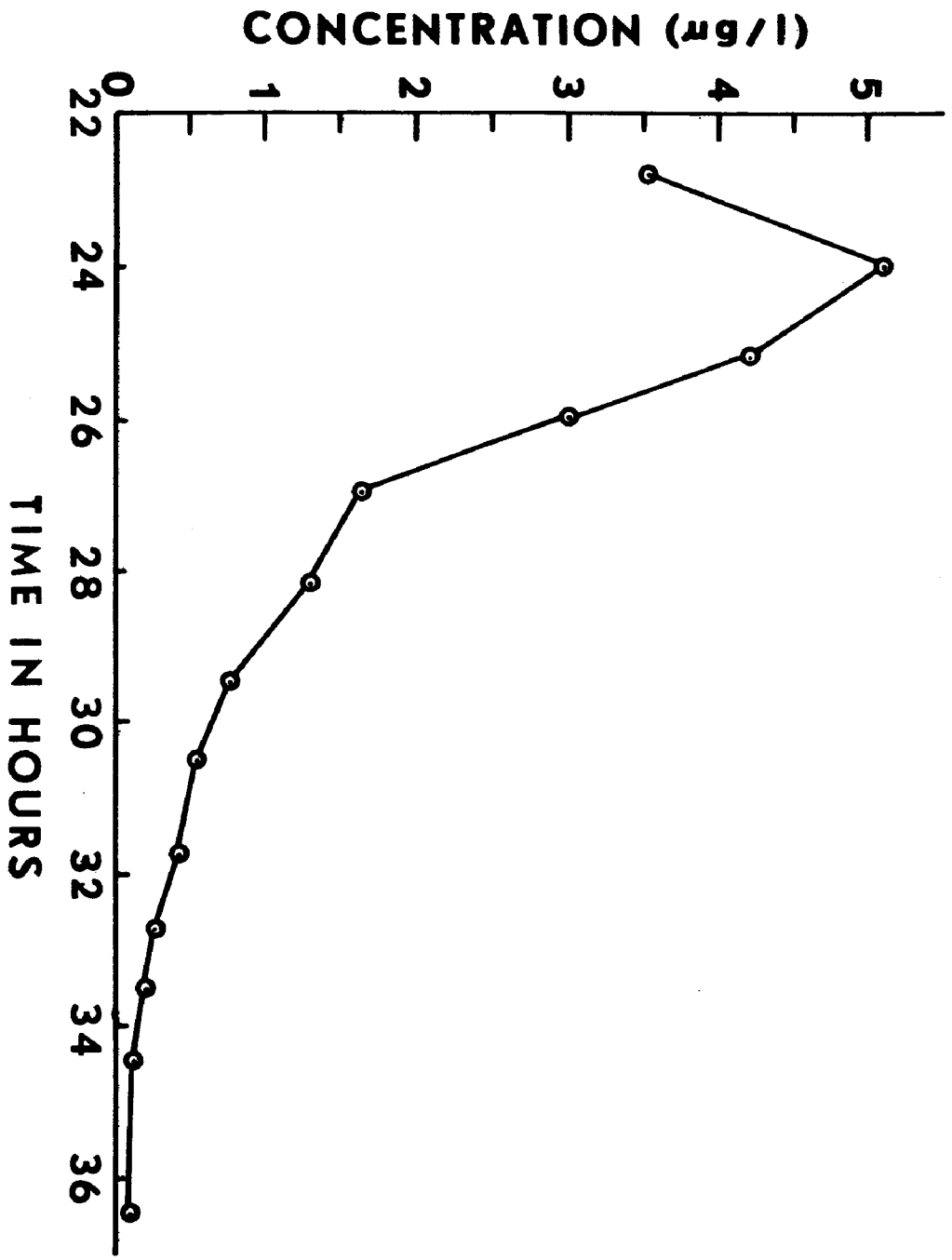

Figure 6. Time of Flow Dye Study from Station T-600 to Station T-500

TABLE 1

Discharge at Each Sample Station

and

Velocity Estimates Between Stations

Station	Discharge Cu.Ft./Sec.	Velocity Estimate Between Stations MPH*	Reliability of Measurement**
C-100	182		± 5%
		0.93	
Mouth of Chena			
		1.50	
T-700	5630		± 5%
		1.50	
T-600	7280		± 8%
		1.25***	
T-500	9770		± 8%
		1.25	
T-400	6990		± 5%
		1.00	
T-300	7970		± 8%
		1.00	
T-200	8730		± 5%
		1.00	
T-100	7380		± 5-8%

* Based on the gradient between stations and the dye study results.

** Judgement of Hydrologist at time of discharge measurement.

*** Determined from dye study conducted between stations T-600 and T-500.

Anderson (4) reported that the Tanana River is in a large alluvial flood plain consisting of well-stratified to lenticular silt, sand and gravel with a low ice content. He also stated that infiltration and permeability are moderate to good, and that groundwater availability is good because of extensive saturated thickness and abundant recharge. Abnormally dry conditions existed in the entire Tanana valley for the 2 years prior to this study so the groundwater supply may have been low. In view of the area geology and the dry conditions, the water lost from the Tanana River may have percolated into groundwater reservoirs.

The arithmetic mean, standard deviation and 95 percent confidence limits of the actual counts per 100 ml of river water at each sample station are presented in Tables 2, 3 and 4 for total coliforms, fecal coliforms and enterococci respectively. The high counts at the C-100 and T-700 stations indicated that the Chena was the major fecal bacteria source for the Tanana, while the numbers at T-800 represented background counts in the Tanana from one or more sources above the Chena. These data also show that fecal bacteria numbers decrease progressively between T-700 and T-100, and that a significant number of these bacteria remained at the T-100 station.

Since fecal indicator bacteria came from both the Chena and upstream on the Tanana, the survival study was started at T-700. In order to determine survival, it was necessary to adjust the bacteria counts for dilution at each sample station downstream from T-700. The discharge anomolies (Table 1) created some problems so two methods of dilution adjustment were employed. The resulting numbers of bacteria were considered to represent survival at each station. Sample calculations for these two methods are as follows:

Method I - Count/100 ml Adjusted for Dilution With Water Loss

The dilution adjustment factors were calculated for each station using the discharge measurements shown in Table 1. The following sample calculation is for station T-400:

$$\frac{\text{6990 cfs at T-400}}{\text{5630 cfs at T-700}} = 1.24 \text{ dilution factor}$$

The actual counts per 100 ml obtained each day were multiplied by the dilution factor to obtain the number of fecal bacteria present at each station. From these results, the data presented in the appropriate portions of Tables 2, 3 and 4 were calculated.

TABLE 2

Total Coliform Bacteria at Each Sample Station Showing Actual Count per 100 ml and Count Adjusted for Dilution

Station	Actual Count/100 ml			Count/100 ml Adjusted for Dilution with Water Loss			Count/100 ml Adjusted for Dilution Without Water Loss		
	Arithmetic Mean	Standard Deviation	95% Confidence Limits (Mean + or -)	Arithmetic Mean	Standard Deviation	95% Confidence Limits (Mean + or -)	Arithmetic Mean	Standard Deviation	95% Confidence Limits (Mean + or -)
C-100	400,000	133,000	89,000	---	---	---	---	---	---
T-800	740	160	130	---	---	---	---	---	---
T-700	12,000	6,100	4,000	---	---	---	---	---	---
T-600	3,200	680	800	4,200	880	1,000	---	---	---
T-500	2,200	990	660	3,900	1,700	1,200	---	---	---
T-400	1,100	290	240	1,300	360	300	1,900	510	420
T-300	590	130	98	840	190	140	1,100	250	190
T-200	460	99	73	710	150	110	940	200	150
T-100	380	65	48	500	86	63	780	130	98

TABLE 3

Fecal Coliform Bacteria at Each Sample Station Showing Actual Count per 100 ml and Count Adjusted for Dilution

Station	Actual Count/100 ml			Count 100/ml Adjusted For Dilution with Water Loss			Count 100/ml Adjusted For Dilution Without Water Loss		
	Arithmetic Mean	Standard Deviation	95% Confidence Limits (Mean + or -)	Arithmetic Mean	Standard Deviation	95% Confidence Limits (Mean + or -)	Arithmetic Mean	Standard Deviation	95% Confidence Limits (Mean + or -)
C-100	120,000	80,000	66,000	---	---	---	---	---	---
T-800	230	120	120	---	---	---	---	---	---
T-700	4,300	1,100	880	---	---	---	---	---	---
T-600	870	230	270	1,100	290	340	---	---	---
T-500	550	170	160	960	300	280	---	---	---
T-400	380	190	180	480	230	220	670	320	310
T-300	170	64	61	240	91	87	330	120	120
T-200	120	55	53	190	86	82	250	110	110
T-100	88	37	36	120	49	47	180	76	72

TABLE 4

Enterococcus Bacteria at Each Sample Station Showing Actual Count per 100 ml and Count Adjusted for Dilution

Station	Actual Count/100 ml			Count/100 ml Adjusted for Dilution with Water Loss			Count/100 ml Adjusted for Dilution Without Water Loss		
	Arithmetic Mean	Standard Deviation	95% Confidence Limits (Mean + or -)	Arithmetic Mean	Standard Deviation	95% Confidence Limits (Mean + or -)	Arithmetic Mean	Standard Deviation	95% Confidence Limits (Mean + or -)
C-100	2,400	530	440	--	--	--	--	--	--
T-800	11	1.7	1.6	--	--	--	--	--	--
T-700	83	19	18	--	--	--	--	--	--
T-600	39	4.7	8.0	50	6.1	10	--	--	--
T-500	40	14	11	69	23	19	--	--	--
T-400	29	6.7	6.4	36	8.3	7.9	51	12	11
T-300	20	2.7	2.6	28	3.9	3.7	37	5.2	5.0
T-200	19	6.4	6.1	29	10	9.5	38	13	13
T-100	15	4.4	4.2	20	5.8	5.5	31	9.0	8.6

Method II - Count/100 ml Adjusted for Dilution Without Water Loss

With this method, it was assumed for dilution purposes that no water was lost from the river channel. Thus, the discharge either increased (as indicated by actual measurement) or remained constant (where actual discharge decreased) at each station proceeding downstream. This resulted in stations T-700 through T-100 having discharges of 5630, 7280, 9770, 9770, 10750, 11510 and 11510 cfs respectively. The discharges presented here for stations T-400 through T-100 differ from the actual discharge measurements shown in Table 1. Therefore, the dilution adjustment factors for these stations differ from those used in Method I and the calculation for T-400 is as follows:

$$\frac{\text{9770 cfs at T-400}}{\text{5630 cfs at T-700}} = 1.74 \text{ dilution factor}$$

Again, as with Method I, the actual counts obtained each day were adjusted with the dilution factor to obtain the results in the appropriate sections of Tables 2, 3 and 4.

The total coliform, fecal coliform and enterococci counts at T-700 were considered to be 100 percent survival because this was the survival study starting point. Proceeding downstream (stations T-600 through T-100), a percent survival range was established from the arithmetic means in Tables 2, 3 and 4. Sample calculations showing the possible range of total coliform survival at station T-400 are as follows:

(1) Based on actual count/100 ml:

$$\frac{\text{1,100/100 ml at T-400}}{\text{12,000/100 ml at T-700}} \times 100 = 9.2 \text{ percent survival}$$

(2) Based on count/100 ml adjusted for dilution with water loss:

$$\frac{\text{1,300/100 ml at T-400}}{\text{12,000/100 ml at T-700}} \times 100 = 10.8 \text{ percent survival}$$

(3) Based on count/100 ml adjusted for dilution without water loss:

$$\frac{\text{1,900/100 ml at T-400}}{\text{12,000/100 ml at T-700}} \times 100 = 15.8 \text{ percent survival}$$

The total coliform percent survival range at each station from T-700 to T-100 is shown in Figure 7. These data indicate that the most rapid reduction in numbers occurred during the first 1.2 days of flow time (T-700 to T-600) with 26.7-35.0 percent remaining viable. The percent survival continued to decrease at each of the remaining stations with 18.3-32.5 percent remaining viable at T-500 (2.2 days of flow time), 9.2-15.8 percent at T-400 (3.4 days), 4.9-9.2 percent (4.7 days), 3.8-7.8 percent (5.8 days), and 3.2-6.5 percent (7.0 days) at T-300, T-200 and T-100 respectively.

The percent survival range for fecal coliforms at each station is presented in Figure 8, with composite winter data from Ballentine and Kittrell (5) shown for comparison. The most rapid reduction in numbers again occurred during the first 1.2 days of flow time with 20.2-25.6 percent remaining at T-600. The percent survival continued to decrease at a slower rate downstream from T-600 with 12.8-22.3 percent, 8.8-15.6 percent, 4.0-7.7 percent, 2.8-5.8 percent and 2.1-4.2 percent remaining viable respectively at T-500 through T-100. After the first 1.2 days of flow time, the fecal coliform decrease in the Tanana River proceeded at a much slower rate than suggested by the composite data from Ballentine and Kittrell (5). They indicated that 0.78 percent of these bacteria remained viable after 6 days of flow time, while the Tanana data showed that 2.1-4.2 percent remained viable after 7 days.

The enterococcus data presented in Figure 9 indicate that reduction in numbers of these bacteria proceeds at a much slower rate than observed with total or fecal coliforms (Figures 7 and 8). The 47.0-60.2 percent survival at T-600 after 1.2 days of flow time contrasts to 26.7-35.0 and 20.2-25.6 percent for total and fecal coliforms. Because the numbers of enterococci per 100 ml did not change between T-600 and T-500 or between T-300 and T-200 (Table 4), the percent survival either remained essentially constant or showed an increase depending on the method of handling discharge. In spite of the possible discrepancies caused by the discharge measurements, the enterococci decreased at a much slower rate than either coliform group. This is manifested by the much higher percent survival at all stations, 47.0-60.2, 48.2-83.1, 34.9-61.5, 24.1-44.6, 22.9-45.8 and 18.1-37.3 percent survival at stations T-600 to T-100 respectively.

The data from figures 7, 8 and 9, in which survival with dilution and water loss was considered, are compared in Figure 10 to show the relative rates of total coliform, fecal coliform and enterococcus decrease throughout the 7 days of flow time. In general, fecal coliforms in the Tanana appear to decrease somewhat more rapidly than total coliforms with the difference in rate becoming more accentuated during the third through the seventh day of flow time. Winter data from Ballentine and Kittrell (5) is also shown.

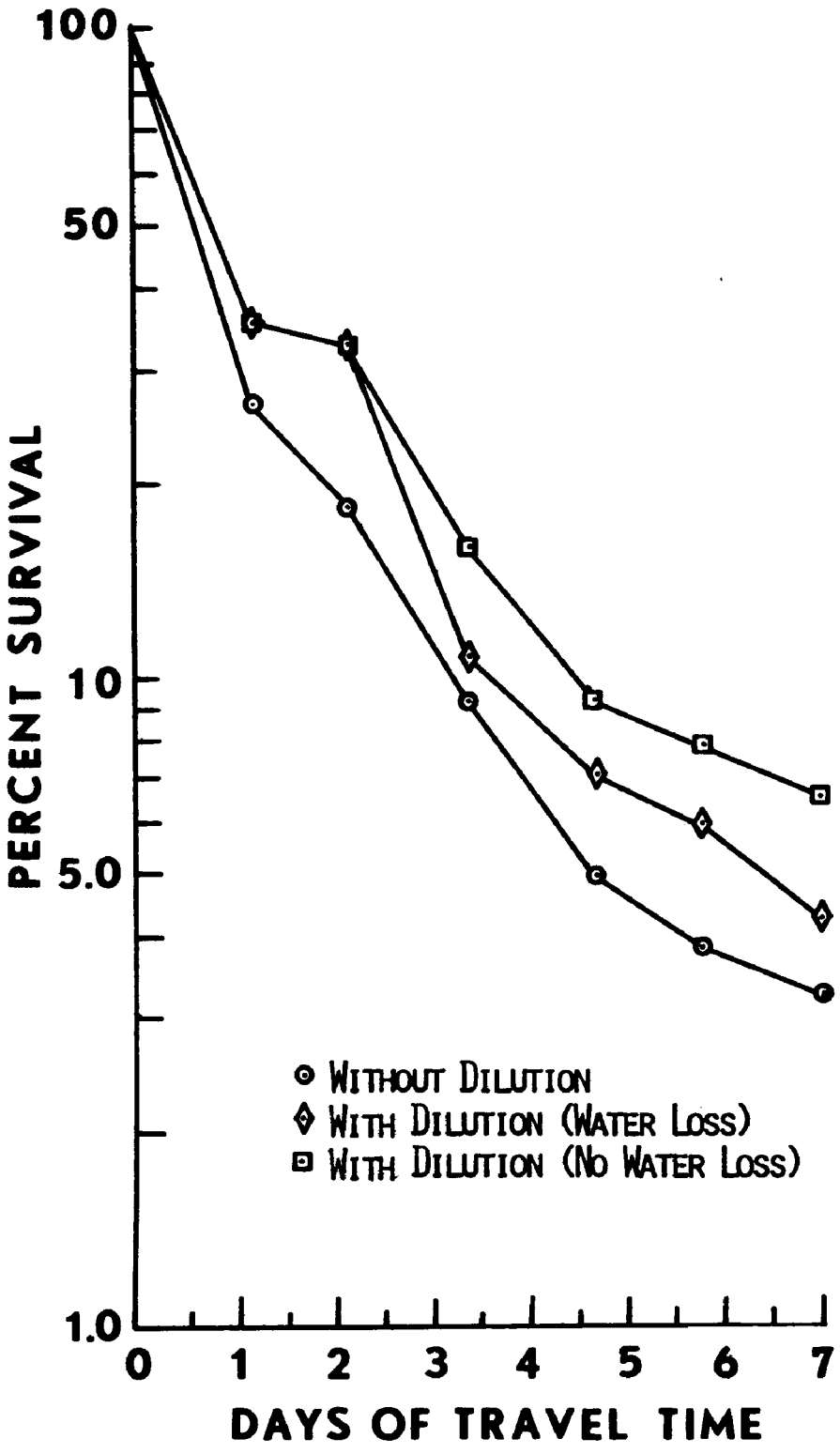

Figure 7. Percent Survival of Total Coliform Bacteria With and Without Discharge Consideration

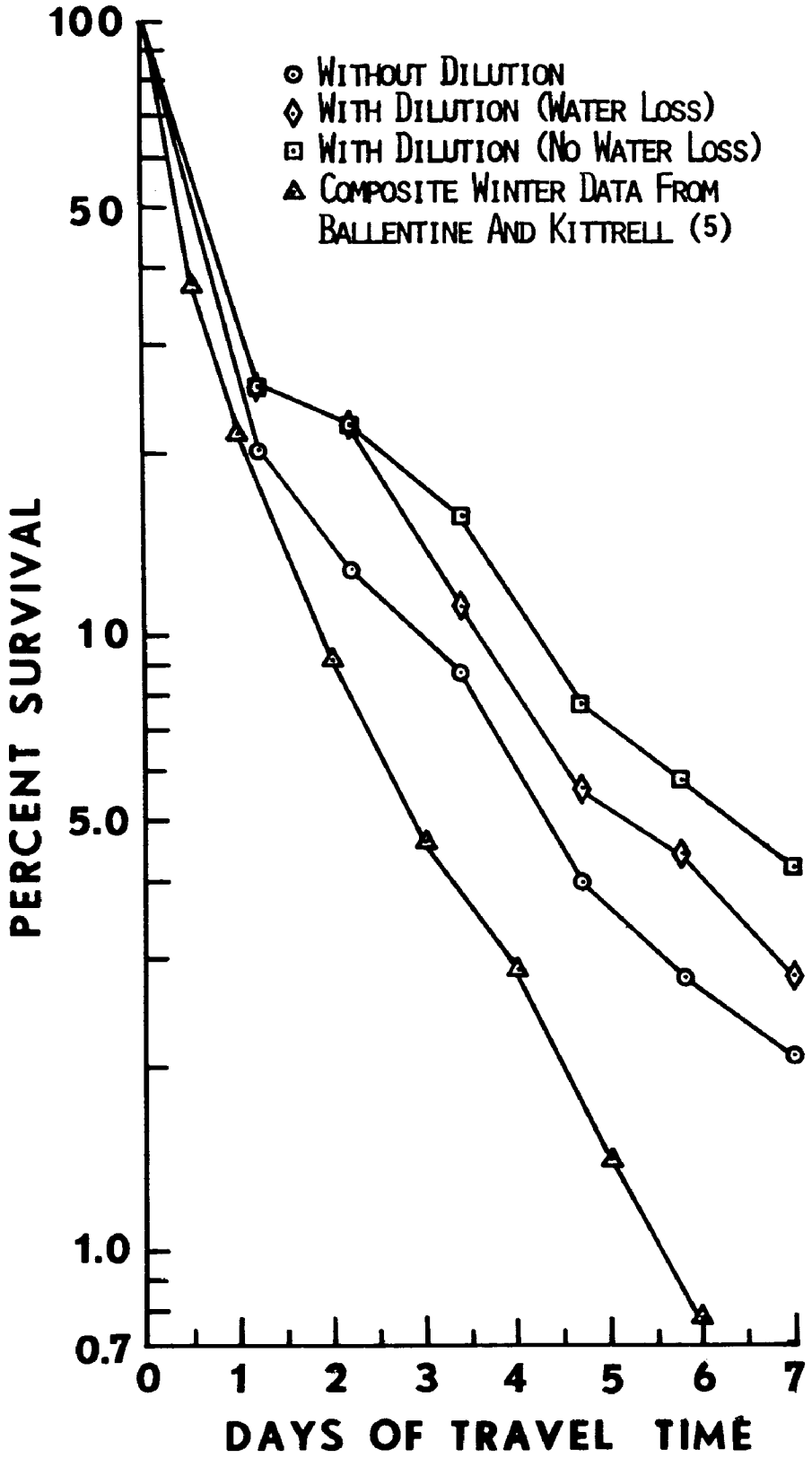

Figure 8. Percent Survival of Fecal Coliform Bacteria With and Without Discharge Consideration

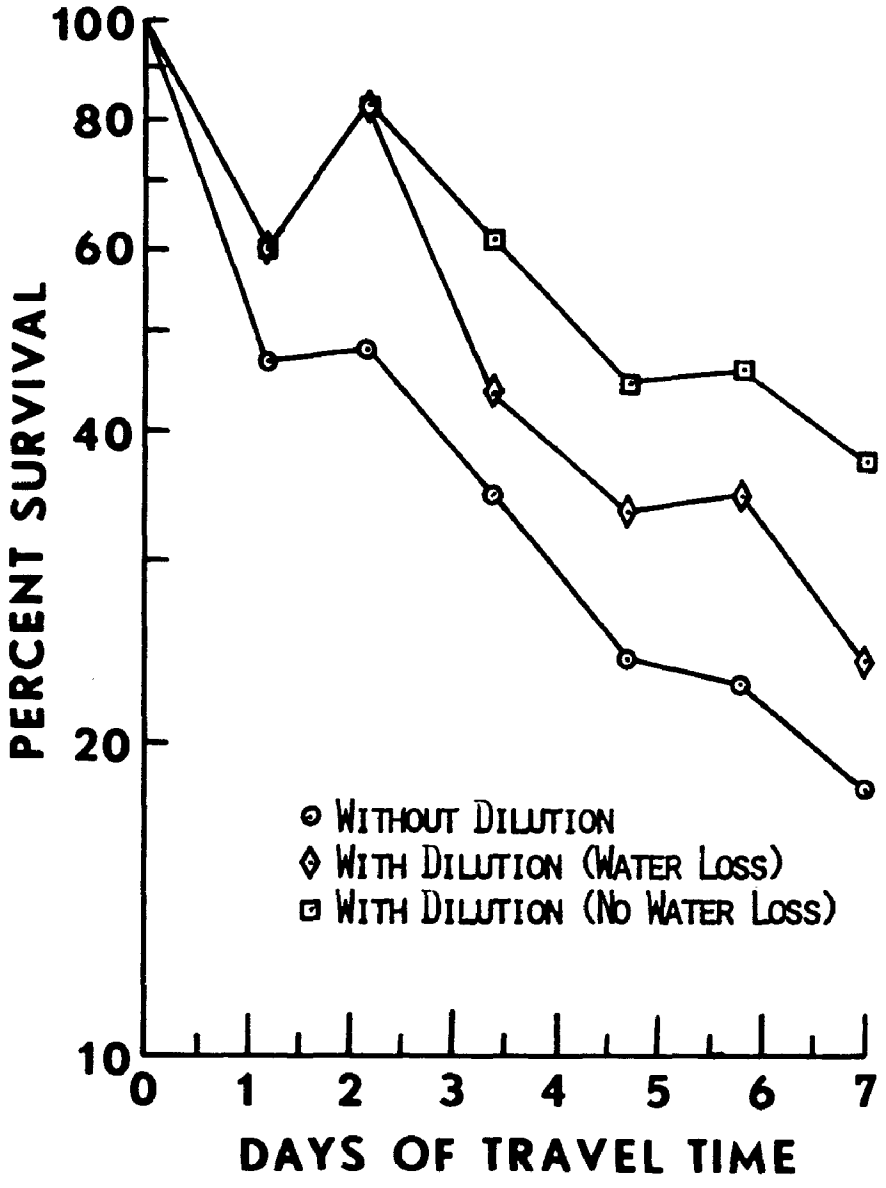

Figure 9. Percent Survival of Enterococcus Bacteria With and Without Discharge Consideration

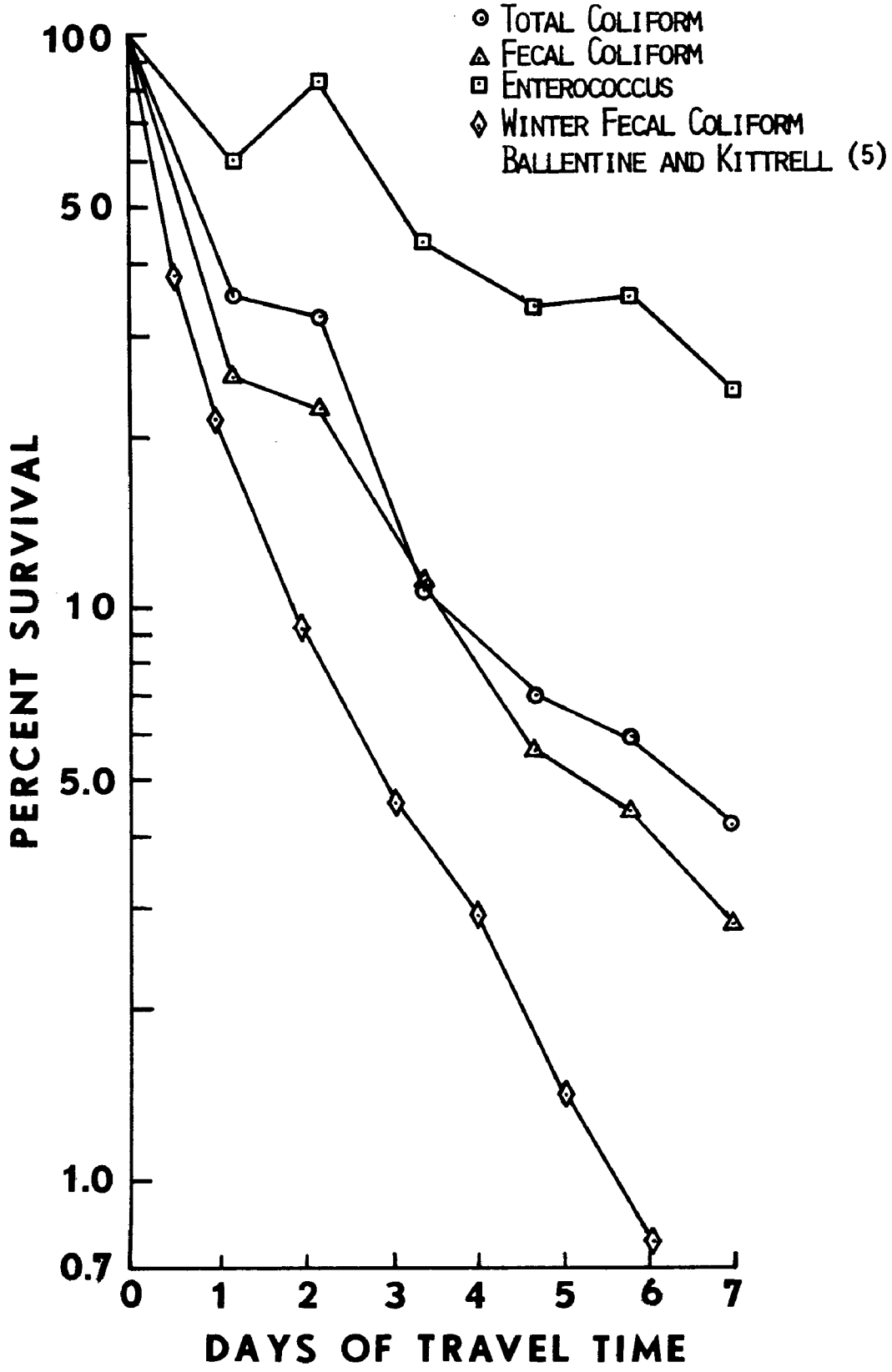

Figure 10. Comparison of Total Coliform, Fecal Coliform and Enterococcus Survival

Their composite data indicate that warmer temperatures (up to 15°C) result in a much more rapid reduction of fecal coliform numbers than observed in 0°C water under total ice cover. It is obvious from this data that enterococci have a much greater capability to survive than either total or fecal coliforms.

Several chemical parameters were measured during this study. The results indicated that the concentration of these components in the Chena River differed considerably from the Tanana River in nearly all cases as shown in Table 5. Perhaps the most significant differences were the pH and the concentrations of dissolved oxygen and ammonia nitrogen at C-100 and T-700. As the water moved downstream from T-700, a continuous change took place in all chemical parameters, except for the total phosphorus concentration which remained constant. The magnitude of these changes was small and no correlation with bacteria survival was established.

TABLE 5

Arithmetic Mean of the Conductivity and pH, and Concentration of Dissolved Oxygen, Alkalinity and Nutrients at Each Sample Station

Station	Dissolved Oxygen mg/l	Conductivity μ mhos/cm	Alkalinity mg/l as $CaCO_3$	pH	Nitrate Nitrogen mg/l	Ammonia Nitrogen mg/l	Total Phosphorus mg/l
C-100	4.68	277	123	6.72	0.02	0.79	0.05
T-700	10.04	304	122	7.32	0.05	0.07	0.01
T-600	9.14	314	126	7.19	0.06	0.08	0.01
T-500	8.98	323	131	7.18	0.05	0.08	0.01
T-400	8.19	333	133	7.08	0.07	0.09	0.01
T-300	6.67	333	138	7.07	0.05	0.11	0.01
T-200	6.70	338	139	7.04	0.08	0.11	0.01
T-100	6.10	345	143	6.96	0.07	0.11	0.01

SECTION V

DISCUSSION

No doubt, the discharge measurements (Table 1) accurately reflect the water in the river channel. However, these measurements did indicate that the river suffered a net flow reduction in two reaches. In view of this information, it is suggested that some volume of water may also have been gained in these two reaches and that water may well have been lost as well as gained in others. It was not possible to determine the volume of water contributed by each tributary and groundwater source or the volume of water lost to groundwater reservoirs, so the actual exchange of water must remain in the realm of speculation.

It was assumed that the fecal indicator bacteria were uniformly distributed throughout the water column. Hence, water leaving the channel would carry bacteria with it and the effect of water entering would be to dilute the numbers of bacteria per unit volume. Therefore, any loss or gain in discharge would affect the apparent bacterial survival. Because of the discharge indeterminates, the real discharge effect on bacterial numbers could not be assessed. However, discharge adjustment did permit a range of possible effects to be established. Minimum survival was observed when the number of bacteria counted per 100 ml of sample were examined without regard for discharge. Thus, any dilution effect would tend to increase survival and the actual percent survival probably lies somewhere between the minima and maxima shown in Figures 7, 8 and 9. A preliminary survey, conducted on the Tanana under similar conditions in 1968 (18) suggested that survival of fecal indicator bacteria was closer to the maxima shown here.

These data (Figures 7, 8 and 9) show that significant numbers of fecal indicator bacteria survive for an extended period in 0°C river water under total ice cover. This is in agreement with previous laboratory and field studies which indicated that survival rates at temperatures below 15°C were greater than above 15°C (5, 9, 14, 19, 20, 21, 22, 24, 25, 30). However, the extent of survival demonstrated in this study (Figure 10) appeared to exceed that reported in previous field work (5, 14, 25). This higher survival rate may have been caused by one or more factors such as lower water temperature, total ice cover, physical or chemical characteristics of the water, or a longer time in which to conduct the study without interruption by sewage from additional sources.

The actual disposition of the bacteria in water leaving the river channel is unknown, but there are at least two possibilities which are dependent on the porosity of the river bed. If the water passed through silt, the bacteria would probably have been filtered out in the silt. If sand or gravel were exposed, the bacteria would have been carried some unspecified distance as the water percolated down. The bacteria trapped in the silt constitute a potential source of increased numbers in the river channel under flow conditions which would resuspend the silt, and those carried through sand or gravel represent a source of contamination for groundwater reservoirs.

The role of fecal indicator bacteria is to indicate the possible presence of enteric pathogenic bacteria. Traditional concepts of the indicator-pathogen relationships are being questioned in the more temperate portions of the United States where this water quality parameter was developed (6, 15). Thus, it is imperative that similar questions be asked here in Alaska where lower water temperatures occur for longer periods. Most of the available data concerning survival of enteric pathogenic bacteria in water deal with Salmonellae. This group of bacteria are the only enteric pathogens which can be isolated from water with any degree of reliability; even so, satisfactory quantitative methods for their isolation have not been developed (15). Laboratory studies have shown that Salmonellae are capable of growth at 5°C (23, 26). These organisms were isolated from the Red River of the North during the winter (14), and there are indications that they persist in streams for at least as long as the fecal coliforms or possibly even longer (5). Because of the much larger numbers of coliforms than pathogens initially present, the longer survival of coliforms relative to pathogens may be more apparent than real (6). The presence of enteric infections in Alaska has been well documented (11, 12, 27, 29, 35), and Salmonellae have been isolated from the Chena River (27, 33). Since fecal indicator bacteria survive in large numbers for extended periods in at least one subarctic river, it is probable that pathogens can also be isolated at the same sample stations. However, when Gallagher and Spino (15) summarized the data from several field studies, they found little correlation between quantities of total or fecal coliforms and the probable isolation of Salmonellae. Thus, the question: Is there any number of fecal indicator bacteria below which raw water can be considered safe for human consumption? People in many villages along the rivers in Alaska still use raw water for drinking purposes without benefit of any form of treatment. Water consumed in some villages probably contains enteric pathogens, and may be a source of enteric infection.

The Water Quality Standards for Alaska (1) do not cover raw water used for drinking purposes. The minimum treatment specified is disinfection, and the raw water for this purpose must average less

than 50 total coliforms per 100 ml in any month. Since total coliforms survive for an extended period, numbers may be far in excess of 50 per 100 ml. Therefore, it is necessary to increase awareness of the problem, provide proper drinking water treatment facilities and adequately disinfect effluents from sewage treatment plants.

SECTION VI

ACKNOWLEDGEMENTS

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SECTION VII

REFERENCES

1. Alaska Department of Health and Welfare, Water Quality Standards for Interstate Waters within the State of Alaska and a Plan for Implementation and Enforcement of the Criteria (1967).

2. Allen, L. A., Pasley, S. M., and Pierce, M. A. F., "Some Factors Affecting the Viability of Faecal Bacteria in Water," Journal General Microbiology, 7, pp. 36-43 (1952).

3. American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 2nd Edition, American Public Health Association, Inc., New York (1965).

4. Anderson, G. S., "Hydrologic Reconnaissance of the Tanana Basin, Central Alaska," U. S. Dept. of the Interior, U. S. Geological Survey, Hydrolic Investigations Atlas, HA 319, Sheet 2 (1970).

5. Ballentine, R. K., and Kittrell, F. W., "Observation of Fecal Coliforms in Several Recent Stream Pollution Studies," Proceedings of the Symposium on Fecal Coliform Bacteria in Water and Wastewater, Bureau of Sanitary Engineering, California State Department of Public Health (1968).

6. Berg, G., Scarpino, P. V., and Bergman, D., "Survival of Bacteria and Viruses in Natural Waters," Dept. of the Interior, Federal Water Pollution Control Administration (1965).

7. Brezenski, F. T., "State of the Art, Microbiological Pollution Indicators," Dept. of the Interior, Federal Water Pollution Control Administration, pp. 1-72 (1968).

8. Buchanan, T. J., and Somers, W. P., "Discharge Measurements at Gaging Stations," Techniques of Water-Resources Investigations of the United States Geological Survey, Book 3, Chapter A8, Dept. of the Interior, U. S. Geological Survey (1969).

9. Das, H. D., and Goldstein, A., "Limited Capacity for Protein Synthesis at Zero Degrees Centigrade in Escherichia Coli," Journal of Molecular Biology, 31, pp. 209-226 (1968).

10. Federal Water Quality Administration, FWPCA Methods for Chemical Analysis of Water and Wastes, Dept. of the Interior, (1969).

11. Fournelle, J. H., Rader, V., and Allen, C., "A Survey of Enteric Infections Among Alaskan Indians," Public Health Reports, 81, pp. 797-803 (1966).

12. Fournelle, J. H., Wallace, I. L., and Rader, V., "A Bacteriological and Parasitological Survey of Enteric Infection in an Alaskan Eskimo Area," American Journal of Public Health, 48, pp. 1489-1497 (1958).

13. Freeman, H. M. "Current Practices in Water Microbiology," U. S. Dept. of the Interior, Federal Water Pollution Control Administration (1970).

14. Gallagher, T. P., Hagan, J. E., Thomas, N. A., and Spino, D. F., "Report on Pollution of the Interstate Waters of the Red River of the North (Minnesota-North Dakota)," U. S. Dept. of Health, Education and Welfare, Public Health Service (1965).

15. Gallagher, T. P., and Spino, D. F., "The Significance of Numbers of Coliform Bacteria as an Indicator of Enteric Pathogens," Water Research, Pergamon Press, 2, pp. 169-175 (1968).

16. Geldreich, E. E., "Sanitary Significance of Fecal Coliforms in the Environment," U. S. Dept. of the Interior, Federal Water Pollution Control Administration, WP-20-3 (1966).

17. Geldreich, E. E., "Applying Bacteriological Parameters to Recreational Water Quality," Journal American Water Works Association, 62, No. 2, pp. 113-120 (1970).

18. Gordon, R. C., Unpublished Data, Environmental Protection Agency, Alaska Water Laboratory (1968).

19. Gyllenberg, H., Niemela, S., and Sormunen, T., "Survival of Bifid Bacteria in Water as Compared with that of Coliform Bacteria and Enterococci," Applied Microbiology, 8, No. 1, pp. 20-22 (1960).

20. Haines, R. B., "The Minimum Temperatures of Growth of Some Bacteria," Journal Hygiene, 34, pp. 277-282 (1934).

21. Halton, J. E., and Nehlsen, W. R., "Survival of Escherichia Coli in Zero-Degree Centigrade Sea Water," Journal Water Pollution Control Federation, 40, No. 5, Part 1, pp. 865-868 (1968).

22. Hanes, N. B. Rohlich, G. A., and Sarles, W. B., "Effect of Temperature on the Survival of Indicator Bacteria in Water," Eutrophication Symposium, University of Wisconsin (1966).

23. Hendricks, C. W., and Morrison, S. M., "Multiplication and Growth of Selected Enteric Bacteria in Clear Mountain Stream Water," Water Research, Pergamon Press, 1, pp. 567-576 (1967).

24. Ingraham, J. L., "Growth of Psychrophillic Bacteria," Journal of Bacteriology, 76, No. 1, pp. 75-80 (1958).

25. Kittrell, F. W., and Furfari, S. A., "Observations of Coliform Bacteria in Streams," Journal Water Pollution Control Federation, 35, No. 11, pp. 1361-1385 (1963).

26. Matches, J. R., and Liston, J., "Low Temperature Growth of Salmonella," Journal of Food Science, 33, pp. 641-645 (1968).

27. Miller, L., Personal communication, U. S. Public Health Service, Arctic Health Research Center (1970).

28. Natrella, M. G., Experimental Statistics, National Bureau of Standards Handbood 91, U. S. Dept. of Commerce, National Bureau of Standards, pp. 2-2 and 2-3 (1963).

29. Pauls, F. P., "Enteric Disease in Alaska," Arctic, 6, pp. 205-212 (1953).

30. Shaw, M. K., "Formation of Filaments and Synthesis of Macromolecules at Temperatures Below the Minimum for Growth of Escherichia coli," Journal of Bacteriology, 95, pp. 221-230 (1968).

31. Shaw, M. K., Marr, A. C., and Ingraham, J. L., "Determination of the Minimal Temperature for Growth of Escherichia coli," Journal of Bacteriology, 105, pp. 683-684 (1971).

32. Skoog, D. A., and West, D. M., Fundamentals of Analytical Chemistry, Holt, Rinehart and Winston, New York, pp. 58-60 (1963).

33. Van Donsel, D., Personal communication, U. S. Public Health Service, Arctic Health Research Center, College, Alaska (1970).

34. Weiss, C. M., "Adsorption of E. coli in River and Estuarine Silts," Sewage and Industrial Wastes, 23, No. 2, pp. 227-237 (1951).

35. Williams, R. B., and Dodson, M. W., "Salmonella in Alaska," Public Health Reports, 75, pp. 913-916 (1960).

SECTION VIII

GLOSSARY OF TERMS

aftergrowth - increase of coliform bacteria numbers in the receiving water after effluent discharge from a waste treatment plant.

arctic - area north of the 10°C isotherm for the warmest month and the -10°C isotherm for the coldest month of the year.

background count - the number of coliform and enterococcus bacteria in the water upstream from the major source of these organisms.

BOD bottle - a bottle designed for biochemical oxygen demand (BOD) determinations and to contain samples for dissolved oxygen determination by the Winkler method.

braided - a stream flowing in several dividing and reuniting channels resembling the strains of a braid.

count per 100 ml - standard method for reporting the numbers of bacteria of sanitary significance in water.

discharge - volume of water passing a given point in a stream per unit time.

dissolved oxygen (DO) - elemental oxygen in solution.

effluent - flowing out; i.e., a sewer outfall into a stream.

enteric - pertaining to the lower intestinal tract.

enterococcus - a bacteria commonly found in significant numbers in feces of human or other warm-blooded animals.

enteropathogen - microorganism which causes diseases in the intestinal tract of humans or other warm-blooded animals.

fecal coliform - a total coliform bacteria subgroup which is specifically found in the feces of humans and other warm-blooded animals.

filament - a continuous protoplasm filled tube produced by some bacteria when cross walls, which produce normal cells, are not formed during cell division.

gaging station - location at which the discharge of a stream is measured.

groundwater - all water found beneath the surface of the ground.

indicator - bacteria generally found in large numbers in feces of humans and other warm-blooded animals and when found in water indicate the probable presence of enteropathogenic bacteria.

infiltration - movement of surface water into the ground.

isolation - separation of a species or strain of bacteria from other bacteria which may be present as contaminants.

membrane filter method - a standard method for obtaining bacterial cells from large volumes of water for enumeration of the number present.

parameter - any one chemical or biological determination which defines the condition of the system relative to that determination.

pathogen - an organism which causes a disease.

percolate - movement of water through the ground.

permeability - measure of the capacity of a material to transmit water through its interstices.

pollution - addition to any material which tends to degrade water quality with respect to a particular use.

primary treatment - a waste treatment process designed to remove floating and settleable solids, and removes 30-40 percent of the oxidizable organic material in solution, before discharge.

pure culture - a single strain or species of bacteria free from other bacteria.

raw domestic sewage - the water carried wastes from households before it has received any form of treatment.

raw water - fresh water which is potentially useful for drinking purposes but has received no treatment to remove foreign substances which may be present.

Salmonellae - pathogenic bacteria which belongs in the genus Salmonella.

sterile - free from any form of life.

subarctic - areas where the mean temperature is higher than 10°C for less than four months of the year and the mean temperature for the coldest month is less than 0°C.

suspending medium - any liquid in which particles are suspended; e.g., bacteria in water.

temperate climate - any area north of the Tropic of Cancer not previously defined as arctic or subarctic.

total coliform - heterogeneous group of bacteria which meet certain morphological and biochemical criteria, and are found in feces of human and other warm-blooded animals, as well as in other environmental situations.

viable - bacterial cells capable of growth and reproduction if appropriate conditions are present.

water column - a volume of water extending from the surface to the bottom of a water body.

water mass - a unit volume of water traveling more or less as a discrete unit.

SELECTED WATER RESOURCES ABSTRACTS

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4. Title: WINTER SURVIVAL OF FECAL INDICATOR BACTERIA IN A SUBARCTIC ALASKAN RIVER

5. Report Date

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7. Author(s): RONALD C. GORDON

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16. Abstract: Survival of fecal indicator bacteria in a subarctic Alaskan river was studied during the winter of 1969-70 when there was total ice cover and the water temperature was 0°C. Most of the domestic pollution entered downstream from this source. Since no additional pollution entered downstream from this source, an uninterrupted study covering 7 days of flow time (210 river miles) was possible. Nine sample stations were established to obtain total coliform, fecal coliform, enterococcus and water chemistry data. Samples were collected four to eight times from each station during the 2-week period of data collection, and a discharge measurement was made at each station during the same period. Bacteria survival was examined with and without consideration for the effect of dilution. After 7 days flow time, total coliforms were reduced to 3.2-6.5 percent of the initial count, fecal coliforms to 2.1-4.2 percent, and the enterococci to 18.1-37.3 percent depending on dilution consideration.

17a. Descriptors: *Rivers, *Enteric Bacteria, *Winter, *Coliforms, *Bioindicators, *Streptococcus, *Bacteria, Alaska, Water Pollution, Water Temperature, Discharge Measurement, Dissolved Oxygen, Conductivity, Alkalinity, Nitrogen Compounds, Hydrogen Ion Concentration, Phosphorus Compounds

17b. Identifiers: *Subarctic, *Total Coliforms, *Fecal Coliforms, *Enterococcus, *Survival

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