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CLOSTRIDIUM PERFRINGENS: AN ADJUNCTIVE INDICATOR
IN NONPOINT POLLUTION

by
Steven G. Eberl

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

of

MASTER OF SCIENCE

in

Civil and Environmental Engineering

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Logan, Utah
1986

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Steven G. Eberl

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	iv
LIST OF FIGURES	v
ABSTRACT	vi
INTRODUCTION	1
STUDY AREA	4
MATERIALS AND METHODS	7
RESULTS AND DISCUSSION	10
REFERENCES	22

LIST OF TABLES

Table	Page
1. Indicator bacteria (No./100) mL in monthly UBWPC river samples from the study area	11
2. Indicator bacteria in various point wastewater discharges .	12
3. Indicator bacteria in Worm Creek and the Cub River on 4-12-86	14
4. Indicator bacteria in various nonpoint pollutant sources	17
5. Indicator bacteria in various grazing animal fecal samples	20

LIST OF FIGURES

Figure	Page
1. The Cache Valley study area with location of [STORET] sampling stations	5
2. Decrease in CP-S concentrations in Worm Creek	15

ABSTRACT

Clostridium Perfringens: An Adjunctive Indicator
in Nonpoint Source Pollution

by

Steven G. Eberl, Master of Science

Utah State University, 1986

Major Professor: Dr. Darwin L. Sorensen
Department: Civil and Environmental Engineering

Clostridium perfringens (CP) was evaluated as an additional indicator in assessing impacts and sources of microbial pollution in the Idaho-Utah Cache Valley. Point, nonpoint, river water, and animal fecal samples were analyzed for CP, total coliforms, fecal coliforms, and fecal streptococci.

Monthly river samples consistently contained <20 CP/100 mL, but concentrations of the other indicators varied significantly by location and date. Two sample stations consistently had CP concentrations greater than 20/100 mL. One of these stations was influenced by an upstream wastewater discharge. Chlorinated effluent from this trickling filter plant contained greater than 10^3 CP/100 mL, but met a 400 FC/100 mL discharge standard. A consistent decrease in CP concentrations in samples taken downstream from this wastewater source were found, despite significant impact from adjacent nonpoint pollution. Lagoon and oxidation ditch wastewater effluents sampled contained <20 CP/100 mL.

Nonpoint sources sampled (e.g., cattle feedlot runoff) contained <20 CP/100 mL and 10^2 - 10^4 /100 mL coliforms and fecal streptococcus. Cattle, horse, and sheep feces analyzed contained 10^4 - 10^7 /g coliforms and fecal streptococcus, but less than 10^2 CP/g. Nonpoint pollution from

such animals may contribute significant coliforms and streptococci but not CP. Wastewater treatment effluents may or may not contain elevated levels of CP depending on factors such as wastewater residence time and particular treatment process employed. The occurrence of relatively high, i.e., $>10^2$ CP/100 mL, in areas impacted by nonpoint sources may suggest a municipal wastewater input. Coliform and streptococci indicators may not be able to distinguish municipal or domestic microbial loading in the presence of nonpoint source interferences in many circumstances.

INTRODUCTION

Various plans for municipal and industrial development of Bear River basin (Utah, Wyoming, and Idaho, U.S.A.) water have been proposed by the State of Utah (DWR, 1982, 1983). The feasibility of operating proposed Bear River reservoirs as municipal and industrial water supply sources will depend on expected water quality. Historical data from water quality monitoring efforts indicates that coliform concentrations have frequently exceeded standards for the use of Bear River water as a raw municipal and industrial supply source. This study evaluated the bacterial indicator Clostridium perfringens, in conjunction with coliforms and fecal streptococci as tools to differentiate sources of fecal contamination in the Bear River system. Fecal concentrations of C. perfringens (CP) vary between different animal species as do concentrations of the fecal streptococci (Pipes, 1982; Barnes and Mead, 1986). Fujioka and Shizumura (1985) found 10^3 - 10^4 CP/100 mL in chlorinated wastewater effluents, but nearly undetectable CP concentrations in waters impacted by only nonpoint sources of pollution. Bisson and Cabelli (1980) found CP spore concentrations to decrease by only about 1 Log unit through chlorination disinfection processes in typical wastewater treatment plants studied. The concentrations of CP in nonpoint sources such as cattle feedlot runoff have not been described in the literature. Determining CP concentrations in streams where non-point pollution is important may aid in the identification of specific sources of pollution when the coliform and streptococcal indicators are inadequate.

C. perfringens has been suggested as an alternative bacterial indicator for a variety of reasons. C. perfringens is consistently

associated with human wastes (Bisson and Cabelli, 1980; Pipes, 1982) and is entirely of fecal origin (Mara, 1974). C. perfringens is widely distributed in feces, sewage, and polluted waters (Bonde, 1977). It was suggested by Bisson and Cabelli (1980) that the ratio of vegetative cells to endospores could be used to indicate the recentness of pollution. Despite the advantages of CP, it has not been widely used in the U.S. due to difficulties in specific analysis for the organism. The development of a rapid membrane filter technique (mCP), combining high incubation temperature, biochemical characterization, and antibiotics, enables the recovery of CP vegetative cells or CP spores with minimum interference (Bisson and Cabelli, 1979).

An estimated one third of the pollutants entering United States waters come from nonpoint sources (Doran and Linn, 1979). Pollution in the Bear River basin and Cache Valley area from diffuse, nonpoint agricultural inputs appears to influence Bear River water quality much more than the point sources present (UWRL, 1974; Wieneke et al., 1980). Nonpoint microbial pollution is a concern to municipal and industrial water resources development because human pathogens, such as Salmonella and Campylobacter fetus subspecies jejuni, may be recoverable in animal excrement (Clinton et al., 1979; Ullman, 1979).

Fecal indicator organisms frequently employed in nonpoint source pollution studies include the total coliform (TC), fecal coliform (FC), and fecal streptococcus (FS) groups. Tests for indicators such as TC, FC, and FS are usually more convenient than direct testing for pathogens, due to the difficulties, time, and expense involved in analyses for the latter (Dept. of Health, 1970; APHA, 1985). Unfortu-

nately, no single indicator will provide all the information needed to evaluate a sample for various water uses (Cabelli, 1978).

The predominant indicator bacteria in cattle feces has been shown to be starch hydrolyzing Streptococcus bovis (Doran and Linn, 1979). The source of cattle grazing runoff, soil type, vegetation, and management practices have all been shown to effect bacterial counts obtained from grazing land runoff (Stephenson and Street, 1978). Jawson et al. (1982) found the occurrence of TC and FC groups in runoff from cattle grazing watersheds did not relate well to the recentness of grazing. Thelin and Gifford (1983) found FC concentrations of $4 \times 10^4 / 100$ mL to be released from 30 day old cattle feces exposed to laboratory simulated rainfall.

Seasonal variations in indicator bacteria concentrations are common. Hollon et al. (1982) found fecal coliform concentrations to reach a maximum during low streamflow, summer months in a study of dairy runoff. Potential regrowth of fecal coliforms and fecal streptococci in overland flow wastewater treatment resulting from warmer temperatures and increased availability of nutrients during the summer decreases the value of these indicators in nonpoint studies (Hunt et al., 1979). Alternative indicators such as CP may provide additional information when coliform and fecal streptococci results are ambiguous.

STUDY AREA

The Cache Valley (Figure 1) was chosen as the primary study area within the Bear River basin because of the intensive agricultural activities which take place within the valley. The Bear River enters the Idaho-Utah Cache Valley from the north. The Cub River is a tributary entering the Bear as it flows south to Cutler reservoir and the Little Bear and Blacksmith Fork Rivers join the Logan River which enters Cutler reservoir from the south. After passing through Cutler dam, the Bear flows south into the Great Salt Lake.

Cattle grazing, feedlots, dairy operations, manure spreading practices, and other animal grazing all may contribute substantial fecal indicator bacteria to the Bear River. Pasture and grazing land accounts for roughly 53 percent of the total land use in the Bear River basin (UWRL, 1974). At least 220 dairy and beef cattle feeding operations with the potential to discharge wastes into the Bear River in Cache Valley were identified by Wieneke et al. (1980). Most dairy and beef cattle livestock operations in Cache Valley contain between 30-150 head, many being located immediately adjacent to rivers. Over 10 percent of the cattle operations consist of over 150 head (Wieneke et al., 1980).

There are relatively few municipal and industrial point sources of wastewater in the Cache Valley. Major point sources include the Preston and Hyrum City wastewater treatment plants and the Logan and Wellsville City wastewater treatment lagoons (Figure 1).

At the present time, water from the Bear River and its tributaries is used for irrigation withdrawal and hydroelectric power generation only. Irrigation water use normally peaks during the late summer when flows in the Bear and its tributaries reach their minimum. Maximum

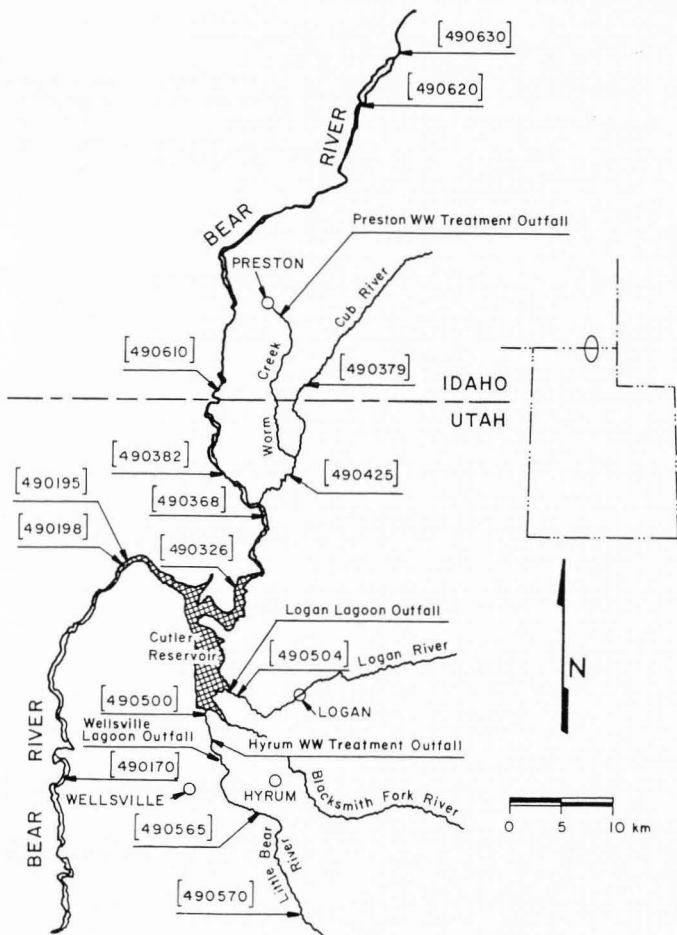


Figure 1. The Cache Valley study area with location of [STORET] sampling stations.

flows are normally reached in the late spring due largely to snowmelt runoff from upper mountain drainages. The mean streamflow for the Bear River below Cutler reservoir for the 1984 water year was 124 m³/s (U.S.G.S., 1985). Maximum and minimum flows at this location of 317 and 14 m³/s respectively indicate the wide variations resulting from spring snowmelt, irrigation water demand, and hydroelectric power generation.

MATERIALS AND METHODS

Project objectives called for water quality sampling of the Cache Valley area. In agreement with the Utah Bureau of Water Pollution Control (UBWPC), routine monthly samples were collected from 15 stations in the Cache Valley. These six digit U.S.E.P.A. STORET stations are designated by their numbers in Figure 1. In addition to this monitoring, a special series of field studies was instituted to examine the concentrations of indicator bacteria in various pollutant sources. Oxbow lake outflows, farmlot runoff, and other diffuse nonpoint sources were sampled. Determinations of indicator bacteria concentrations were made on dairy cattle, horse, and sheep fecal samples. These animals are major contributors of nonpoint microbial pollution in the Cache Valley.

The effects of point wastewater discharges were studied. The Hyrum City oxidation ditch wastewater treatment plant which uses chlorine disinfection and final rapid sand filtration was designed to treat 3785 m³/d. Flows exceeding twice the design rate for this plant have resulted from groundwater infiltration. The Preston City trickling filter wastewater treatment plant with chlorine disinfection has a design capacity of 3785 m³/d although groundwater infiltration frequently causes hydraulic overloads. Both these plants were functioning at design capacity when sampled. The decrease in indicator concentrations in Worm Creek downstream from the Preston wastewater outfall was investigated.

Logan City wastewater treatment lagoon effluent flow averaged 18,925 m³/d during the sampling period. Wastewater entering the lagoon had an average residence time of 45 days during the summer and 90 days over the

winter. Wellsville City wastewater lagoons were designed to be total containment facilities, but groundwater infiltration has necessitated a small, $<100 \text{ m}^3/\text{d}$ discharge to the Little Bear River since 1978.

The UBWPC determined TC, FC, and FS concentrations in monthly STORET station samples by the membrane filter method (APHA, 1985). Duplicate UBWPC samples were collected using sterile sample bags (Whirlpak, Nasco) and transported on ice to the Utah Water Research Lab (UWRL) for C. perfringens determinations within 12 h. All other sampling was done by the UWRL and analyses were undertaken within 6 h (APHA, 1985).

The concentrations of CP were determined using the mCP method of Bisson and Cabelli (1979). C. perfringens vegetative cells (CP-V) and C. perfringens spores (CP-S) were differentiated by the ability of CP-S to survive heat treatment of the sample for 15 minutes at 60°C (Bisson and Cabelli, 1979). Volumes of water exceeding 100 mL were normally examined due to the low concentrations of CP found in most samples. Animal fecal samples were examined by dispersing 1.0 g of feces in buffered dilution water and making appropriate dilutions until acceptable membrane filter counts could be obtained.

A culture of C. perfringens was obtained from the Utah State University Biology department and was used as a positive control. Confirmatory biochemical tests were performed on CP samples early in the study to ascertain reliability of the mCP procedure (Bisson and Cabelli, 1979). The sensitivity of the mCP media was compared by plating pure cultures of CP on both mCP and differential reinforced clostridial medium (DRCM), (Dept. of Health and Social Security, 1970). Counts of CP on the mCP medium were equal to those obtained on the less selective DRCM medium. TC, FC, and FS determinations were made using the membrane

filter technique (APHA, 1985). Duplicate analyses were run for a minimum of 10% of the samples collected and results were found to be within acceptable ranges suggested by APHA (1985). Analysis of indicator data was undertaken on a DEC VAX-11/780 computer, using the Pearson Correlation routine within the SPSS-X statistical package. Positive and negative controls for all the indicators were routinely used to assure media quality and sterility.

RESULTS AND DISCUSSION

The concentrations of TC, FC, FS, and CP-S indicators are summarized in Table 1 for five monthly UBWPC sampling dates. Indicator concentrations varied considerably between different locations and sampling dates. Regression analysis performed on Table 1 data showed the lack of any meaningful correlations between these indicators. C. perfringens spores in these river samples were frequently recovered, but at concentrations normally less than 20/100 mL. Only two of the fifteen stations sampled, [490425] on the Cub River and [490500] on the Little Bear River, showed CP-S concentrations consistently higher than 20/100 mL.

Station [490425] is located downstream from station [490379] on the Cub River. Worm Creek flows into the Cub River between these two Stations (Figure 1). CP-S concentrations always increased markedly between these two stations, while corresponding concentrations of TC, FC, and FS frequently remained constant or decreased over this same section of the Cub River (Table 1). Worm Creek receives effluent from the Preston Wastewater treatment plant 8, km upstream from it's confluence with the Cub River (Figure 1).

Various point wastewater effluents were analyzed for indicators and these data are shown in Table 2. Preston wastewater effluent contained 1900 and 2400 CP-S/100 mL on the two dates sampled. The Preston effluent contained only 5 FC/100 mL on 4-12-86 and routinely meets the FC discharge standard of 400/100 mL established for this plant. These data suggest that the Preston effluent routinely contributes more than 10^3 CP-S/100 mL, but adds only negligible concentrations of coliforms to Worm Creek.

Table 1. Indicator bacteria (No./100 mL) in monthly UBWPC river samples from the study area.

Sample Location	Sampling Date																			
	10 July 1984				7 August 1984				5 September 1984				2 October 1984				23 October 1984			
	TC	FC	FS	CP-S	TC	FC	FS	CP-S	TC	FC	FS	CP-S	TC	FC	FS	CP-S	TC	FC	FS	CP-S
[490630]	<100	8	800	10	--	100	<1	5	300	230	200	1	NA*	NA	NA	NA	NA	NA	NA	NA
[490620]	200	280	200	2	--	<1	3000	3	100	28	200	1	NA	NA	NA	NA	NA	NA	NA	NA
[490610]	100	96	2100	3	--	2200	500	3	300	240	1000	3	100	80	480	5	400	12	150	5
[490382]	100	84	300	10	--	5000	700	3	1500	1080	2100	7	250	40	1500	94	50	8	600	4
[490368]	<100	64	1500	5	--	4500	2800	5	100	170	1100	4	NA	NA	NA	NA	20	20	160	NA
[490326]	100	72	200	2	--	2000	300	7	300	370	300	6	210	120	330	4	200	48	300	2
[490198]	100	160	600	17	--	100	600	16	800	1300	1100	4	700	116	800	3	500	24	300	3
[490170]	<100	16	500	7	--	120	1700	10	200	160	500	9	100	60	600	7	NA	NA	NA	<1
[490195]	200	220	100	14	--	2000	6500	8	1800	260	800	7	300	90	3200	13	600	50	350	11
[490375]	<100	60	400	2	--	5300	100	4	800	180	1200	7	180	100	360	14	650	130	200	10
[490425]	100	56	900	47	--	3000	100	43	1700	280	700	73	--	160	2200	94	200	8	650	21
[490504]	<100	40	100	4	--	2000	800	14	--	--	--	3	170	48	240	6	550	8	100	5
[490570]	100	100	500	NA	--	2400	400	--	400	84	1900	3	140	20	140	NA	300	12	50	NA
[490565]	<100	52	1300	1	--	1500	900	1	200	26	1600	<1	70	8	340	<1	150	8	600	2
[490500]	200	80	800	84	--	3500	3500	410	7300	900	1000	125	--	330	1800	76	2000	70	150	39

*NA = not analyzed

Table 2. Indicator bacteria in various point wastewater discharges.

Sample source	Date	(No./100 mL)			
		TC	FC	FS	CP-S
Preston WW effluent	3-29-86	NA*	NA	NA	1900
Preston WW effluent	4-12-86	340	5	82	2400
Welsville lagoon effluent	4-12-86	6300	<20	540	<10
Hyrum WW effluent	3-29-86	NA	NA	NA	<1
Logan lagoon effluent	8-21-84	2000	240	1000	13

NA* = Not analyzed

The concentrations of indicators in Worm Creek as it flows to the Cub River were determined. Cattle grazing and other nonpoint sources of indicator bacteria are abundant along Worm Creek throughout this area. The reach of Worm Creek sampled has approximately 0.46% average grade, uniform stream velocity and flowrate. Results of the decay study are given in Table 3 and Figure 2. A decrease in CP-S concentrations downstream from the outfall is evident, but an appreciable concentration remains at the confluence with the Cub River (Figure 2). Concentrations of CP-S in the Cub River increased from 5/100 mL at Station [490379], above Worm Creek, to 100/100 mL at Station [490425], below Worm Creek. Concentrations of TC and FS increased substantially over the section of Worm Creek sampled, indicating input of nonpoint indicators had occurred downstream from the outfall (Table 3). In this instance, CP-S served as a conservative, non-ubiquitous tracer of Preston wastewater in the presence of interfering nonpoint source pollution. The CP-S concentrations were plotted versus downstream distance using a linear first order decay equation (not shown). Results gave a reasonably good fit of the data, but lacked sufficient sample points to fully substantiate this model of CP-S decrease. The source of elevated CP-S in the Cub River between stations [490379] and [490425] is undoubtedly Worm Creek, which receives these organisms in the effluent from the Preston Wastewater treatment plant. Coliform and streptococci data were unable to be applied in distinguishing between the point and nonpoint sources of pollution in this case.

Station [490500] on the Little Bear River also consistently contained more than 20 CP-S/100 mL during the UBWPC monthly sampling (Table 1). Concentrations of CP-S consistently increased between

Table 3. Indicator bacteria in Worm Creek and the Cub River on
4-12-86.

Sample source	(No./100 mL)			
	CP-S	TC	FC	FS
Worm Ck. 0.10 km above Preston outfall	85 (72-98)*	730	240	300
Worm Ck. 0.16 km below Preston outfall	>2400	-**	-	-
Worm Ck. 0.75 km below Preston outfall	2400	-	-	-
Worm Ck. 2.94 km below Preston outfall	1800	-	-	-
Worm Ck. 4.22 km below Preston outfall	740	-	-	-
Worm Ck. 5.54 km below Preston outfall	620	-	-	-
Worm Ck. 7.79 km below Preston outfall	640	2500	260	1200
[490379] Cub River above Worm Ck.	5 (4-6)	-	-	-
[490425] Cub River below Worm Ck	100 (94-106)	-	-	-

* - Values in parentheses indicate the range in duplicate samples.

** - Not analyzed.

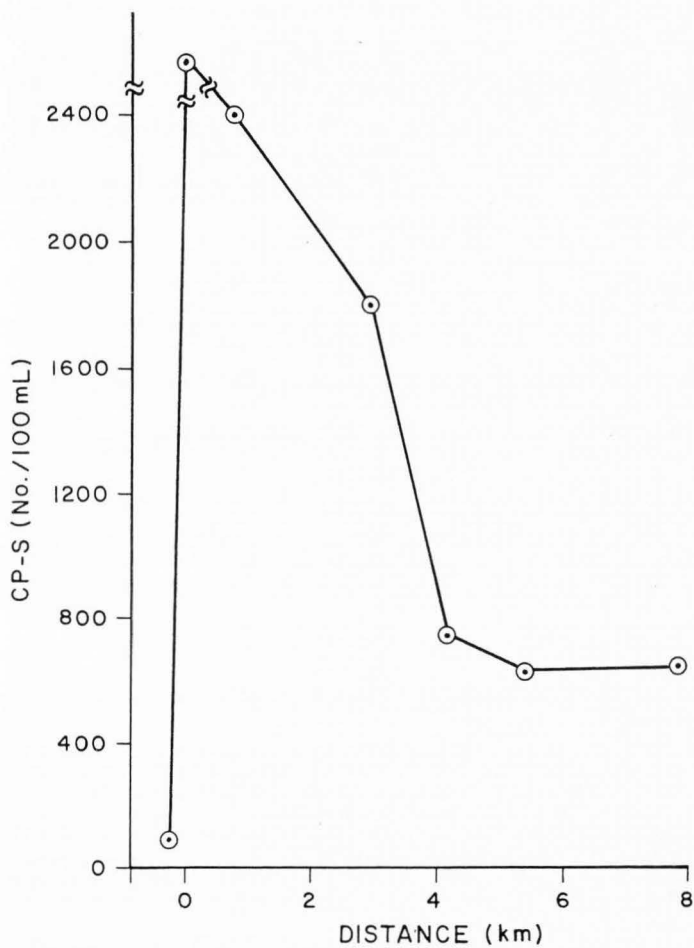


Figure 2. Decrease in CP-S concentrations in Worm Creek.

Stations [490565] and [490500], but corresponding TC, FC, and FS concentrations sometimes decreased. Wellsville wastewater lagoon effluent and Hyrum wastewater treatment plant effluent enter the Little Bear River between these two sample stations (Figure 1).

The Hyrum effluent sample collected 3-29-86 contained no detectable CP-S (Table 2). The effluent from the Hyrum plant routinely meets the Utah discharge standards of 200 TC/100 mL and 20 FC/100 mL established for this plant. Apparently, the rapid sand filtration process at Hyrum helps remove any CP-S not eliminated by either sedimentation in the treatment process or chlorination.

Wellsville lagoon effluent contained 6300 TC, <20 FC, and 540 FS/100 mL respectively (Table 2). The concentration of CP-S was <10/100 mL. Sedimentation of CP-S through the four cell lagoon and appreciable bird life near the effluent weir structure may help explain these data.

Neither the Hyrum nor the Wellsville wastewater effluents entering the Little Bear River contained measurable concentrations of CP-S. It is unlikely that they would have accounted for the elevated CP-S levels found at Station [490500], since the respective treatment processes had not changed since 1984.

Table 4 shows the concentrations of indicators in various nonpoint sources of microbial pollution to the Bear River and Cache Valley. Shallow oxbow lakes are immediately adjacent to the Bear River throughout most of the Cache Valley. These oxbow lakes were at their maximum capacity during the study due to above normal snowmelt runoff. River levels were high enough so that most of these oxbows and adjacent inundated farmlands were in hydraulic connection with mainstream flow. The oxbow and farmlot runoff samples analyzed contained 10^2 - 10^4 TC,

Table 4. Indicator bacteria in various nonpoint pollutant sources.

Sample source	Date	(No./100 mL)			
		TC	FC	FS	CP-S
Oxbow Lake at Smithfield, UT	8-21-84	500	360	9000	1
Oxbow Lake at Trenton, UT	8-21-84	3000	760	280	4
Oxbow Lake at Benson, UT	8-21-84	13000	380	500	8
Oxbow Lake at Cornish, UT	8-21-84	1000	400	520	1
Hopkins Slough	8-21-84	9000	1000	1100	12
Farmlot runoff to Hopkins S.	8-21-84	15000	2800	7500	4
Farm runoff at Collinston, UT	8-31-84	3000	500	2000	19
Farmlot runoff to L. Bear R.	9-12-84	5000	450	3000	1

10^2 - 10^3 FC, and 10^2 - 10^3 FS/100 mL respectively, but concentrations of CP-S never exceeded 20/100 mL. The unknown residence time of water within the oxbows makes application of the FC/FS ratio questionable for these samples (APHA, 1985). The FC/FS ratios for all farmplot runoff samples were below 0.7, suggesting pollution from animal sources.

The relative lack of CP-S in the river and nonpoint sources studied prompted analysis of animal fecal samples to determine CP-S, TC, FC, and FS concentrations. The results are shown in Table 5. Fresh composite fecal samples from cattle, horse, and sheep holding facilities were collected. Fecal coliform and FS concentrations of 10^4 - 10^7 /g feces were found to be in general agreement with typical values reported in the literature (Pipes, 1982). C. perfringens spore and vegetative cell concentrations were from 3 to 5 orders of magnitude below the coliform and streptococcal indicator concentrations. Human feces contain approximately 10^7 - 10^9 TC, 10^7 - 10^9 FC, 10^5 - 10^6 FS, and 10^6 - 10^7 CP/g feces respectively (Pipes, 1982). This indicates the relative similarity in these indicator concentrations that would be expected in typical municipal wastewaters. The animal feces analyzed help explain the abundance of TC, FC, and FS and the relative lack of CP-S in the nonpoint sources sampled. Microbial pollution originating from cattle, horses, or sheep, as in the Cache Valley study area, should therefore not be expected to contain elevated CP, but probably will contain very high concentrations of TC, FC, and FS indicators.

An attempt was made to identify any overlooked point sources of wastewater to the Little Bear River. Phone conversations with various regulatory agencies failed to ascertain any additional known point wastewater sources. Five samples of the Little Bear River were

Table 5. Indicator bacteria in various grazing animal fecal samples.

Indicator bacteria	(No./g feces)		
	Cattle	Sheep	Horse
TC	4.0×10^6	NA*	NA*
FC	4.75×10^6	1.6×10^7 **	1.26×10^4 **
FS	8.25×10^6	3.8×10^7 **	6.3×10^6 **
CP-S	60	85	<1
CP-V	150	<1	<1

NA* = Not analyzed.

** = From Pipes, Bacterial indicators of pollution, CRC press, 1982.

collected between Stations [490565] and [490500] on 4-12-86. Concentrations of CP-S increased from 4 to 14/100 mL along this river section, indicating that CP-S levels were no longer as elevated as during the 1984 UBWPC sampling. Larger streamflows during the spring runoff account for some dilution, but the Cub River Station [490425] also influenced by these high flows, still exhibited much higher relative CP-S levels on this same date (Table 3). The source of CP-S to the Little Bear River during the 1984 sampling was either relatively small in volume and obscured by dilution during high flows, or could no longer be detected during the spring of 1986. It is also possible that an intermittent source of wastewater discharges into the Little Bear River. Bird life in wetlands adjacent to the Little Bear River were not thought to be responsible for the elevated CP-S concentrations. The lack of CP-S in Wellsville lagoon effluent known to be heavily influenced by bird life does not suggest that birds are a significant source of CP to the Little Bear River.

Logan lagoon effluent was analyzed to complete the study of all wastewater sources within the Cache Valley. Logan lagoon effluent contained 2000 TC, 240 FC, and 1000 FS/100 mL respectively (Table 2). C. perfringens spores were recoverable, but present at a concentration of only 13/100 mL. The long residence time of wastewater in the lagoon probably helps in achieving removal of CP-S through sedimentation. Elevated levels of TC and FS may be partially attributable to the abundant bird life observed at the lagoon.

Typical wastewaters entering municipal treatment facilities contain 10^4 - 10^7 /100 mL, of TC, FC, FS, and CP (Bisson and Cabelli, 1980). The concentrations of these indicators in wastewater treatment effluents may

vary according to the type of treatment process used and other influencing factors. The data of Table 2 show that the concentrations of these indicators in wastewater effluents vary significantly in the different plants sampled.

Results of the study indicate that CP can provide additional valuable information in certain circumstances. C. perfringens was shown to be capable of differentiating wastewater effluents from nonpoint sources where other indicators were ambiguous. When CP is found in relatively high, i.e. approaching $10^2/100$ mL concentrations, a strong possibility exists that a municipal wastewater effluent or some other domestic point source of pollution is discharging to the stream. Wastewater effluents were shown to contain CP in both undetectable and $10^3/100$ mL concentrations, necessitating individual sampling of such suspected sources. The low concentrations of CP-S relative to other indicator bacteria in the majority of the Bear River and its tributaries are further evidence of the importance of nonpoint source pollution to this river system.

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