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Microbial Indicator Levels in the Providence River and Upper Narragansett Bay 80 pp

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Narragansett Bay Estuary Program

MICROBIAL INDICATOR LEVELS IN THE PROVIDENCE RIVER AND UPPER NARRAGANSETT BAY

FINAL REPORT

February 1990

Study Conducted as Part of the Narragansett Bay Project

NBP-90-33

Principal Investigator

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FOREWORD

The United States Congress created the National Estuary Program in 1984, citing its concern for the "health and ecological integrity" of the nation's estuaries and estuarine resources. Narragansett Bay was selected for inclusion in the National Estuary Program in 1984 and designated an "estuary of national significance" in 1988. The Narragansett Bay Project (NBP) was established in 1985. Under the joint sponsorship of the U.S. Environmental Protection Agency and the Rhode Island Department of Environmental Management, the NBP's mandate is to direct a five-year program of research and planning focussed on managing Narragansett Bay and its resources for future generations. The NBP will develop a comprehensive management plan by December, 1990, which will recommend actions to improve and protect the Bay and its natural resources.

The NBP has established the following seven priority issues for Narragansett Bay:

* management of fisheries
* nutrients and potential for eutrophication
* impacts of toxic contaminants
* health and abundance of living resources
* health risk to consumers of contaminated seafood
* land-based impacts on water quality
* recreational uses

The NBP is taking an ecosystem approach to address these problems and has funded research that will help to improve our understanding of various aspects of these priority problems. The Project is also working to expand and coordinate existing programs among state agencies, governmental institutions, and academic researchers in order to apply research findings to the practical needs of managing the Bay and improving the environmental quality of its watershed.

This report represents the technical results of an investigation performed for the Narragansett Bay Project. The information in this document has been funded wholly or in part by the United States Environmental Protection Agency under assistance agreement #CX812768 to the Rhode Island Department of Environmental Management. It has been subject to the Agency's and the Narragansett Bay Project's peer and administrative review and has been accepted for publication by the Management Committee of the Narragansett Bay Project. The results and conclusions contained herein are those of the author(s), and do not necessarily represent the views or recommendations of the NBP. Final recommendations for management actions will be based upon the results of this and other investigations.

Executive Summary

Three, three-day sampling tours were conducted in the Providence River and upper bay of the Narragansett Bay Estuary during relatively dry weather. Surface water samples were collected during all three tours, and bottom water samples during the first two. The first tour was conducted during the winter and the last two during successive summers. In most instances, the samples were collected during a falling tide. During the first tour 20 stations were visited each day. Subsequently, the number of stations was increased to 24-26 in an attempt to better define the die-off characteristics of the microbial indicators.

About half the stations were located within the channel passing down through the Providence River and upper bay. The remaining half were sited at the mouths of sewage-receiving rivers which empty into the area, the "boils" for the Providence and East Providence sewage treatment plants (STPs), and the shellfish-growing area in the upper bay. In addition, pre- and postchlorination effluent samples were collected during each sampling day from the Providence and East Providence plants and twice from most of the other sewage treatment plants in the state.

Assays were performed for five microbial indicator systems. Fecal coliforms were examined because of existing standards; Escherichia coli was assayed as the most fecal-specific component of the coliform group; and enterococcus levels were determined because of their better correlation to swimming-associated illness in marine waters. F male-specific bacteriophages were assayed as a viral simulant for the environmental behavior, at least with regard to chlorination, of the Norwalk virus; and Clostridium perfringens was examined as a conservative tracer because of the environmental resistance of the endospores it produces. The E. coli and fecal coliform levels in the samples were very similar. Because of this, data on the E. coli levels were omitted and the fecal coliform levels were retained for comparison with historical data in the body of the report.

The fecal coliform levels in the prechlorinated effluents were about six times greater than those of the enterococci and about 46 times greater than those of the F phage. The coliform and enterococcus levels in the effluents were markedly reduced by chlorination (100-10,000 times) while those of the F phages, like those of C. perfringens, were reduced by less than a factor of ten. The differences were seen in the levels of the indicators in the postchlorinated effluents and reflected in part by the levels in the samples collected from the boils of the Providence and East Providence STPs.

As seen from the levels in the transect stations and their effect the levels in the Providence River, there were major inputs of all the indicators during the winter from the riverine sources to the north of the study area and, to a lesser extent, from the Pawtuxet River. In general, the indicator levels in these inputs during the summer were much less than those during the winter. Inputs of the enterococci were negligible during the summer. During the summer tours, the levels of the F phages, like those of the fecal coliforms, decreased rapidly with downstream travel, presumably due to die-off. This did not occur with the F phages during the winter tour. The levels of all the indicators were elevated in

the vicinity of Conimicut Point during the winter, as were those of C. perfringens during the summer. One explanation is that some of the contaminated water from the Pawtuxet River does not reach the channel until this point.

The indicator levels in the surface water generally were higher than those in the bottom water. The differences, however, became less with travel down the bay, especially during the summer tour of 1986.

Because of indicator inputs which reached the channel along the course of the estuary, biological decay coefficients were also calculated for segments of the channel sampling networks. Biological decay of the indicators generally was higher in the surface than bottom waters, and higher during the summer than the winter. Biological decay of the F phages in surface and bottom waters was minimal and appreciably less than that of the fecal coliforms or enterococci during the winter. During the summer, however, there was a marked increase in the biological decay of the F phages. The combination of the decreased inputs and high biological decay rates during the summer tours resulted in minimal levels of the F phages, enterococci and fecal coliforms south of Conimicut Point during the summer.

Any conclusions drawn from the study are limited to dry weather conditions and by the small number of sampling tours. In addition, they assume that the adequacy of the F phages as a simulant for the environmental behavior of the Norwalk virus, the most frequently identified agent of the most prevalent swimming- and shellfish-associated illness, an acute gastroenteritis.

Both the coliform and enterococcus indicator systems probably are defective in indexing the adequacy of wastewater chlorination in reducing the input levels of Norwalk virus and possibly other viral pathogens in the sewage effluents. Moreover, there probably has been too much reliance on wastewater chlorination as a means of control technology.

The above notwithstanding, since swimming occurs only during the summer, there should be a minimal and certainly acceptable risk of swimming-associated illness at beaches south of Gaspee Point from the discharges to its north, except following rainfall events or STP malfunctions. The risk of illness from the consumption of raw shellfish harvested from the conditional growing area should also be minimal during the summer.

The ratio of F phages to fecal coliforms in the water samples collected from the conditional shellfish growing area during the winter relative to the ratio in prechlorinated sewage suggests the possibility of measurable health effects from shellfish harvested during that time. The absolute levels of F phage in the shellfish themselves also indicates a potential health problem. Because of this, a major focus of future investigations should be the management of the shellfish resources in the conditional shellfish growing area during the winter and possibly the late the fall and early spring.

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Figures

Tables

INTRODUCTION

Narragansett Bay receives a number of municipal wastewater discharges directly or indirectly via rivers which empty into it. The major discharges, in terms of the volume of the sewage, enter the bay north of Gaspee Point (Figure 1). The dry weather flow from these discharges is in excess of 110 million gallons per day (MGD) or about 80 percent of the total sewage effluent discharged into the bay. About 73 MGD is discharged directly from the Providence and East Providence sewage treatment plants (STPs); about 23 MGD reaches the bay via the Seekonk River; and about 16 MGD of wastewater effluent is discharged into the Pawtuxet River.

Two types of resources can be adversely affected by the municipal wastewater discharges with regard to the potential for infectious disease among their users, e.g. bathing beaches and harvest areas for molluscan shellfish. Most of the heavily used bathing beaches in Narragansett Bay are in its southern part; and the quality of their waters as measured by the traditional fecal indicators is minimally affected by the effluent discharges north of Gaspee Point, except under most unusual circumstances. Although there are some bathing beaches in the Upper Bay, the major concern with regard to the potential for water-related infectious disease is a major shellfish harvesting area in the upper bay.

The area north of Conimicut Point has been closed to the harvesting of molluscan shellfish for direct consumption for many years. In addition, the area between Conimicut Point on the north and the northern tip of Prudence Island to the south has been managed as a "conditional harvest area." Because of the effect of rainfall on combined sewer overflows (CSOs), this conditional growing area is closed to the harvesting of shellfish for at least seven days following a rainfall in excess of 0.5 inches in a 24 hour period.

Total coliforms and fecal coliforms (a more but not completely fecal-specific component of the coliform group) have been used to assess the quality of both recreational and shellfish-growing waters with regard to the potential for waterborne infectious disease. The assumption has been that there is some quantifiable relationship of the levels of these fecal indicators in the water to the levels of pathogenic microorganisms in sewage and, hence, the risk of sewage pollution-related, infectious disease. It was further assumed that the decreases in the levels of the indicators during wastewater treatment and disinfection and transport between their sources (primarily sewage effluents) and the resources is similar to those for the enteric pathogens. This was probably is true of the bacterial pathogens such as Salmonella typhosa and other salmonellae but is questionable with regard to the viral agents such as the Norwalk virus.

Epidemiological studies conducted by the United States Environmental Protection Agency (USEPA) in the 1970s (Cabelli et al., 1982) showed that an acute gastroenteritis is the most common swimming-associated, pollution-related illness. They also showed that the levels in the bathing water of the enterococci, another fecal indicator which survives better than do the coliforms in marine waters (Fattal et. al. 1983), were best correlated to the risk of illness (Cabelli et. al., 1983; Cabelli, 1983). Because of this, the USEPA recently recommended a marine recreational water quality criterion and guideline based on the enterococcus levels in the water (USEPA, 1986). The findings from

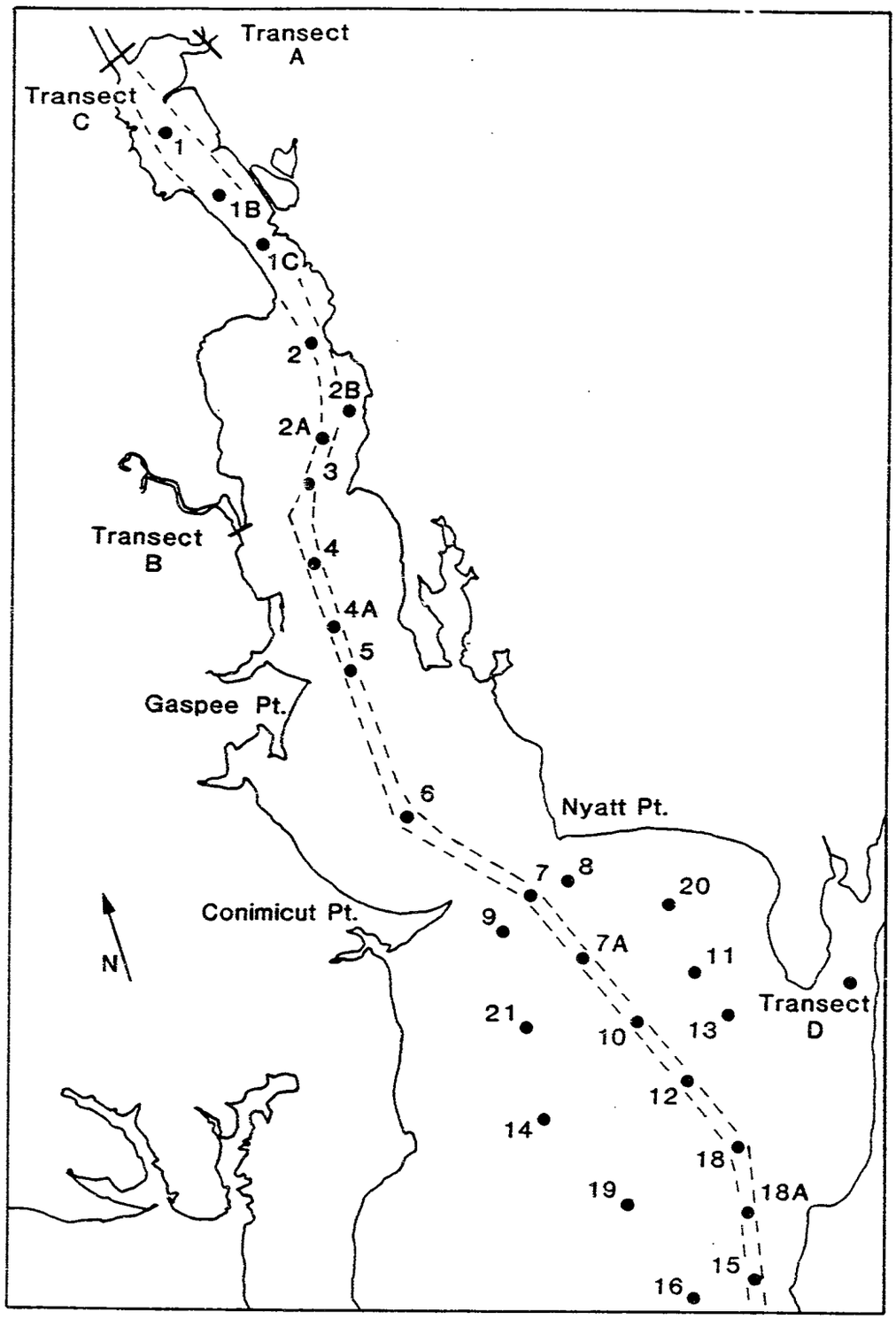

Figure 1. Location of sampling stations. ● sampling stations

the epidemiological studies are in agreement with the analysis of outbrezk reports (see Cabelli, 1982). Acute gastroenteritis is the most prevalent waterborne infectious disease (this includes shellfish and drinking water related outbreaks, as well); and the most frequently identified etiological agents for such outbreaks are the Norwalk-like viruses (Centers for Disease Control, 1987).

Three factors contribute to reduce the levels of the indicators and presumably the pathogens between the source, a sewage outfall, and the potentially affected resources. The first two are physical, dilution and sedimentation of particle-associated microorganisms. The third is biological, ie, die-off of the organisms due to a combination of factors such as salinity, temperature and sunlight. Theoretically, the reduction in the level of a given indicator in a wastewater effluent needed to achieve a given standard for this indicator at a resource could be extrapolated using a physical transport model into which a die-off or biological decay coefficient is included as an input parameter. Thus, one of the objectives of the study was to obtain such die-off or decay coefficients for the water quality indicators being measured.

The transport models for Narragansett Bay have been developed using salinity as the input parameter; therefore, the effect of sedimentation is not considered. Because of their origin, the fecal indicators and, presumably, the enteric pathogens tend to be particle-associated and, hence, are subject to sedimentation as well as dilution. This could preclude the use of a saline-driven transport model to predict microbial fecal indicator levels in the bay from those at the inputs or sources unless sedimentation as well as biological decay are included as input parameters to the model.

There also are a number of other factors, such as environmental repair of chloramine-damaged cells (so called regrowth), which could confound the predictions of coliform and enterococcus levels in the water, and there would be no way of knowing whether the weakness was in the transport model itself or in biological factors including the die-off coefficients introduced into the model. Thus, the evaluation of the use of the transport model for predicting indicator levels should be approached in a stepwise fashion. Ideally, the first step should employ measurements of a conservative microbial indicator (one which is minimally affected by die-off during chlorination and transport). In addition, the source of the indicator should be human fecal wastes so that sedimentation would affect it in a manner similar to that for the fecal indicators and pathogens. Clostridium perfringens, because it produces an environmentally resistant endospore and is found in human fecal wastes and sewage (about 10^4 CFU/100 ml), appears to satisfy the requirements and should be included in such studies.

A number of other factors can confound the predictions including the precision of the microbiological assays, tidal conditions, resuspension of the organisms from the sediments into the water column, and day to day variability in the levels of the organisms in the inputs and in the bay. To the extent feasible, these should be considered in the design of a study.

It is clear from the effect of by-passing wastewater treatment and disinfection due to rainfall-mediated combined sewer overflows (CSOs) that there is a heavy dependency on chlorination of the effluents and die-off during

transport to achieve the shellfish growing area coliform standards in the upper bay. It has been the contention of many environmental virologists that viruses survive die-off during wastewater chlorination and transport markedly better than do the coliform indicators (Scarpino et. al., 1972; Snead et. al., 1980; Berg, 1983). Moreover, a recent paper by Keswick et. al. (1985) reports that the Norwalk virus was the most chlorine-resistant of the other animal viruses studied and that only the f-2 male-specific bacteriophage was comparably resistant to the cidal effect of chlorine. These observations raise the possibility that, with regard to Norwalk virus acute gastroenteritis, coliform levels do not adequately index the potential for swimming- and shellfish associated illness in situations where there is a great dependency on die-off during wastewater chlorination to reduce the coliform levels.

The f-2 bacteriophage is one of the two groups of F male-specific bacteriophages, the other being Fd or f-1. Although these viruses, which attack specific bacterial cells are infrequently found in human feces (Havelaar and Hogeboom, 1986), they can be recovered from municipal sewage at levels of about 10^4 PFU/100 ml (data from our laboratory). The F male-specific bacteriophages can be assayed specifically by a technique developed in our laboratory (Debartolomeis, 1988). Thus, a second major objective of the project was to examine the die-off of F male specific bacteriophages during chlorination and transport and to compare it to that of the coliforms, enterococci and C. perfringens spores.

OBJECTIVES

The objectives of this project were:

1. to produce data sets on microbial indicator levels in the Providence River and Upper Narragansett Bay and in the major input sources of these indicators. These are for later use in the calibration of models to predict indicator levels in the bay.
2. to obtain biological decay coefficients for the indicators and to compare them with each other.
3. to compare the survival of bacterial (coliform and enterococcus) indicators, the simulant for the Norwalk virus (F male-specific bacteriophages), and the conservative tracer (C. perfringens) during wastewater chlorination at various STPs.
4. to compare the levels of the indicators to each other in the Upper Narragansett Bay.

MATERIALS AND METHODS

Study Area and Sampling Sites

The study area consisted of that part of the Narragansett Bay estuary referred to as the Providence River and Upper Narragansett Bay. The area extends south from the mouth of the Seekonk River to about the northern tip of Prudence Island (Figure 1).

A sampling tour consisted of the collection of water samples from each station on each of three successive days. Three such tours were conducted; the first from Dec. 9-11, 1985; the second from July 22-24, 1986; and the third from

July 28-30, 1987. During the first tour, samples were collected from a network of 20 stations. Ten of them were spaced within the portion of the ship channel extending from the head of Providence River through the upper bay. Another six stations were sited on both sides of the channel within the conditional shellfish growing area of the upper bay. Samples also were collected from the "boils" for the Providence and East Providence STP outfalls (stations B1 and B2, respectively) and from single stations at the mouths of the Seekonk and Pawtuxet Rivers (stations TnA and TnB, respectively). Pre- and postchlorinated grab samples were collected at the times of the sampling tours from the Providence, East Providence, Warren and Narragansett Village sewage treatment plants (STPs). Samples also were collected from several other STPs during November of 1985; these were assayed only for C. perfringens and F male-specific bacteriophages.

Following the examination of the data from December, 1985 sampling tour, the total number of samples collected from the bay was increased to 24-26 and some changes were made in the sampling locations. These will be described in the Results section.

Collection of Samples

Water samples were collected from the surface and bottom water for three consecutive days during each of the three sampling tours with the exception of the third tour when only surface water samples were taken. The surface samples were obtained from just below the surface (about 15 cm) of the water in sterile polypropylene bottles. The bottom water samples were collected from about one meter above the bottom sediments using a Kemmerer sampler, and the water was transferred to sterile polypropylene bottles. The bottles were iced upon collection, and the samples were assayed within eight hours of their collection.

The intent was to collect the samples on a falling tide insofar as possible. This was not achieved in all cases because of the number of samples to be collected with the single boat available to us. During the July, 1986 three-day sampling tour, the samples were not collected on a falling tide on the last two days.

The sewage samples were collected before and after chlorination. The postchlorination samples were taken just distal to the contact chamber so that they reflected residence (contact) time in the "chamber." The samples were collected in sterile polypropylene bottles which were iced after collection. They were assayed within 6-8 hours of collection. An appropriate quantity of sodium thiosulfate was added to the bottles used for the postchlorination samples.

Assay Parameters and Methods

Five microbiological parameters were measured. They were Escherichia coli and fecal coliforms by the mTEC method (Dufour et al., 1981; Cabelli et al., 1982), enterococci by the modified mE method (Levin et al., 1975; Dufour, 1980), Clostridium perfringens by the mCP method (Bisson and Cabelli, 1979), and F male-specific bacteriophages by a method developed in our laboratory (Debartolomeis, 1988) (see Appendix B). Fecal coliforms were measured because of the existing standards. Enterococci were enumerated because of epidemiological data showing that its levels were best correlated to swimming-associated illness

at marine bathing beaches and in anticipation of the recent USEPA marine recreational water quality criterion and guideline (USEPA, 1986). C. perfringens was measured as a conservative tracer because of the resistance of its endospores to environmental damage. The F male-specific bacteriophages were enumerated as the best available simulant for the environmental behavior of Norwalk virus (Keswick et. al., 1985).

The mTEC, mE and mCP methods employ membrane filtration. In general, up to 100 ml quantities of environmental water were assayed in duplicate. Thus the sensitivities of the assays were 0.5 colony-forming units (CFU) per 100 ml. With sewage, however, quantities in excess of 10-30 ml generally could not be passed through the membranes. In general, half-log quantities or dilutions of the samples were assayed so that the counting limits of methods would not be exceeded except near the sensitivity limits of the assays.

The sensitivity limit of the phage assay varied. If the concentration method was not used, it was 10 plaque-forming units (PFU)/100 ml. When the concentration method was used, it was reduced to 0.5-2.0 PFU/100 ml depending on the volume of sample water concentrated (Debartolomeis, 1988).

Salinity and temperature were measured on the samples except during the July, 1987 sampling tour. Where available, information on the total chlorine residuals and at times suspended solids was obtained from the sewage treatment plant operators.

RESULTS

Indicator levels at sewage treatment plants

The data obtained from the examination of the pre- and postchlorination effluent samples collected during those periods when the three-day sampling tours of the Providence River and Upper Narragansett Bay were conducted are given in Tables A1-A3. The means (Xs) and standard deviations (SDs) of the $\log_{10}$ transformed indicator recoveries from the prechlorination samples and the $\log_{10}$ reductions following chlorination for each sewage treatment plant (STP) during each sampling tour (12/7-11/1985, 7/21-24/1986 and 7/28-30/1987) are summarized in Tables 1-4. Initially, four STPs (Providence, East Providence, Warren, and a small "package" plant at Narragansett Village) were examined. The collection of samples from the Narragansett Village plant was abandoned in 1986 because of logistic problems. Samples were not collected from the Warren STP in 1987 because a transect sampling station had been established at the mouth of the Warren River.

Overall, the fecal coliform, enterococcus and C. perfringens levels in 100 ml quantities of the prechlorinated effluents from all plants were about 1.4×10^5, 2.3×10^4 and 9.1×10^3, respectively (Table 1). These levels are similar to those reported for secondary-treated effluents in earlier studies (Miescier and Cabelli, 1982; Bisson and Cabelli, 1980). The overall mean fecal coliform density was about six times that of the enterococci which, in turn, was about three times that of C. perfringens. The mean F male-specific bacteriophage (F phage) level was about 3.1×10^3/100 ml, a density which was about 45 times less than that of the fecal coliforms (FC).

Table 1. Summary of means (x) of $\log_{10}$ prechlorination indicator levels. Data from Tables A1-A3.

Sewage treatment plant	Mo-Yr[a]	N[b]	x for $\log^{10}$ indicator level/100 ml effluent			
			C. perfringens	F phage	Enterococci	FC
Providence	12-85	5	4.623	4.277	4.775[c]	5.519[c]
	07-86	4	4.353	4.212	3.949*	4.892*
	07-87	3	3.461*	3.605	~3.087*	~4.252*
East Providence	12-85	5	4.310[c]	3.746[c]	4.940[c]	5.688
	07-86	4	3.765*	3.106*	4.482*	5.490
	07-87	3	3.871*	3.252	4.451*	5.627
Warren	12-85	5	3.488	2.847	4.020	4.673
	07-86	4	3.087*	2.255*	4.314	4.996*
Narragansett Village	12-85	5	4.678	4.104	5.291	5.210
Mean		9	3.960	3.489	4.368	5.149

[a] Month and year samples collected.

[b] Number of samples collection during each period.

* Significantly different by Student's "t" tests than corresponding mean indicator level for 12-85 at $P<0.05$.

[c] December vs. July, $P<0.05$.

~ Approximation because indeterminate value included.

Since samples were collected and assayed only for 3-5 days during a single week in each "season" ... the examination of indicator levels at the plants and seasonal variations thereof was not an objective of this study..., the generalizations we make must be taken with caution. There was an indication, however, which was reinforced because the observation occurred at all three STP sampled in 12-85 and 7-86, that the indicator levels were generally higher during the winter than during the summer. In about half the instances, the differences between the 12-85 and 7-86 indicator levels were statistically significant (Table 1). The mean prechlorination indicator levels at the Providence STP, but not the East Providence STP, were lower during the week in July 1987 than in the one in 1986. The differences in the means, although appreciable, were not statistically significant because of the greater variances in the 1987 data (Table 2). The standard deviations for the mean prechlorination indicator levels (Table 2), but not the mean levels themselves (Table 1), at the Narragansett Village STP during the week in December generally were higher than the corresponding ones at the other three treatment plants.

The most important finding, which was a confirmation of some results obtained in an earlier study (Keswick et. al., 1986; Saad, unpublished data), was the marked resistance of the F phages to chlorination (Table 3). These viruses, like C. perfringens, were very resistant to the chlorination regimens which markedly reduced both the coliform and enterococcus levels. We suspect, however, that the fully formed spores are more resistant to chlorination than are the viruses and that the reductions in the C. perfringens levels were due to spores which were not fully formed (Bisson and Cabelli, 1979). The geometric mean F phage level for the postchlorinated effluents from the four STPs was about 1.3×10^3/100 ml. This density was approximately 50 and 30 times greater than those of the enterococci and fecal coliforms respectively (data from Tables A1-A3). The reductions in both the F phages and C. perfringens levels following chlorination at the Narragansett Village STP were appreciably and significantly greater than those at the other three plants during that week in December. The standard deviations for the differences in the $\log_{10}$ indicator levels following chlorination are given in Table 4. As with the prechlorination data, the greatest variability in the indicator levels was obtained with the July 1987 samples from the Providence STP and the December 1985 samples from the Narragansett Village STP.

Samples of the pre-and postchlorinated effluents were collected from several other STPs as well during November of 1985. These were assayed for C. perfringens spores and F phages only. The pre- and postchlorination levels for the two indicators are presented in Table A5. These data along with those obtained from the treatment plants sampled in December of 1985 were used to compare the levels of the two indicators in the prechlorinated effluents and the $\log_{10}$ reductions following chlorination at the eleven STPs (Table 5). In general, F phage levels were less than those of C. perfringens in the prechlorinated effluent, but the mean difference across plants was not significant. The mean F phage levels were higher than those for C. perfringens at only three STPs, and all three were primary plants. When these were eliminated from the statistical analysis (n = 8), the difference was highly

Table 2. Summary of standard deviations (SD) around the means of the $\log_{10}$ prechlorination indicator levels. Data from Tables A1-A3.

Sewage treatment plant	Mo-Yr[a]	N[b]	SD of $\log^{10}$ indicator level/100 ml effluent				
			C. perfringens	F phage	Enterococci	FC	Ave
Providence	12-85	5	0.253	0.159	0.214	0.274	0.225
	07-86	4	0.167	0.304	0.310	0.314	0.273
	07-87	3	0.939	0.609	0.755	1.074	0.844
East Providence	12-85	5	0.044	0.343	0.272	0.132	0.198
	07-86	4	0.114	0.144	0.093	0.216	0.142
	07-87	3	0.181	0.406	0.123	0.113	0.205
Warren	12-85	5	0.095	0.179	0.450	0.233	0.239
	07-86	4	0.340	0.400	0.651	0.134	0.381
Narragansett Village	12-85	5	0.521	0.377	0.579	1.174	0.662

[a]Month and year samples collected.

[b]Number of samples collection during each period.

Table 3. Summary of means (x) of $\log_{10}$ reductions in indicator levels following chlorination of sewage effluents. Data from Tables A1-A3.

Sewage Treatment Plant	Mo-Yr[a]	N[b]	x for $\log_{10}$ reduction after chlorination: *C. perfringens*	F phage	Enterococci	FC	Cl_2 resid[c] (mg/L)	SS[d]
Providence	12-85	5	0.527	0.440	~3.523*[e]	~3.205*	3.6	48
	07-86	4	0.351	0.263	NC[f]	2.653*	3.8	61.4
	07-87	3	-0.461	-0.023	~3.023*	~3.036*	ND[g]	ND
East Providence	12-85	5	0.672	0.406	~4.042*	4.165*	1.1	6.5
	07-86	4	1.123	0.331*	~4.241*	~4.435*	0.8	5.8
	07-87	3	0.404	0.170	4.259*	3.613*	ND	ND
Warren	12-85	5	0.734	0.479	~2.686*	~2.600*[g]	2.0	7.2
	07-86	4	~1.076	~0.437*	~3.454*	~3.160*	2.5	6.0
Narragansett	12-85	5	2.035[+]	0.959*[+]	~3.675*	~3.497*	3.9	190

[a] Month and year samples collected.

[b] Number of samples collected during each period.

[c] Average total chlorine residual in mg/liter.

[d] Average suspended solids in mg/100 ml.

[e] ~ approximation because of indeterminate values, usually at sensitivity of assay

[f] Could not be calculated because of indeterminate values.

[g] No data for two samples.

[h] No data

* Significantly different from *C. perfringens* at $P<0.05$.

[+] Significantly different from other STPs for 12-85 period at $P<0.05$.

Table 4. Summary of standard deviations (SD) around the means of $\log_{10}$ reductions in indicator levels following chlorination of sewage effluents. Data from Tables A1-A3.

Sewage treatment plant	Mo-Yr[a]	N[b]	SD of $\log_{10}$ reduction after chlorination C. perfringens	F phage	Enterococci	FC	Ave
Providence	12-85	5	0.270	0.245	0.706	0.748	0.492
	07-86	4	0.166	0.113	ind	0.186	0.155
	07-87	3	0.880	0.624	0.760	1.430	0.924
East Providence	12-85	5	0.187	0.346	1.037	0.248	0.455
	07-86	4	0.823	0.220	0.238	0.681	0.491
	07-87	3	0.380	0.570	0.378	0.181	0.377
Warren	12-85	5	0.575	0.197	0.575	0.376	0.431
	07-86	4	0.395	0.326	0.830	0.497	0.512
Narragansett Village	12-85	5	0.788	0.461	1.053	0.982	0.821

[a] Month and year samples collected.

[b] Number of samples collected during each period.

Table 5. Comparison of prechlorination levels and reductions following chlorination of F male-specific bacteriophages and C. perfringens spores. 1985 data.

Sewage treatment plant Location	Type[a]	N[b]	Cl_2 resid.[c] (mg/L)	Log_{10} pre-chlorination levels per 100 ml C. perfrin.	F phage[d]	Log_{10} reduction following chlorination C. perfrin.	F phage[d]
Narragansett Village	Pri,EA	5	3.9	4.678	4.104	2.035	0.959*
Providence	Sec,AS	7	3.6	4.597	4.463	0.461	0.407
Blackstone Valley	Sec,AS	2	3.1	4.247	4.133	0.491	0.611
Newport	Pri	2	2.9	4.258	4.874	-0.025	0.273
Quonset Pt.	Pri	1	2.9	4.230	4.322	1.026	1.860
Bristol	Pri	2	2.4	5.213	5.376	0.286	0.466
Warren	Sec,AS	7	2.0	3.495	2.874**	0.609	0.528
Jamestown	Sec,AS	1	2.0	3.663	2.663	0.171	1.265
E. Greenwich	Sec,TF	2	1.8	4.448	0.859	0.859	0.931
Fall River, MA	Sec,AS	2	1.4	4.190	3.898	0.717	0.750
E. Providence	Sec,AS	7	1.1	4.340	3.368*	0.770	0.334*
x[d]				4.305	4.039	0.673	0.762
Correlation coefficient (r) for C. perfringens vs F phage			n = 38[f]	0.67		0.32	
			n = 11[g]	0.84		0.38	

[a]Sec - secondary treatment; Pri - primary; AS - activated sludge; TF - trickling filter, EA - extended aeration

[b]Number of paired samples per plant

[c]Total chlorine residual in mg/L

[d]Mean for sewage treatment plants (n = 11)

*,**

Significantly different than C. perfringens at $P<0.05$ and 0.01, respectively.

[f]By sample

[g]By STP

significant by a paired "t" test ($P<0.02$). Another interesting relationship of the two indicators in the prechlorinated effluents was suggested from the regression analyses of the indicator levels against each other across both samples and plants. Inspection of the data suggested and the regression analyses confirmed that there was a good association of the levels of the two indicators with each other (correlation coefficients (r) of 0.67 and 0.84 for samples and plants, respectively).

Overall, the reductions in F phage and C. perfringens levels following chlorination were similar and not significantly different. There were, however, some marked differences in the reductions of the two indicators at some of the plants. At two of the plants, Narragansett Village and East Providence, the reductions in the phage levels following chlorination were appreciably and significantly lower then those of C. perfringens. Alternatively, the phage reductions were appreciably higher than those for C. perfringens at two other STPs, Quonset Point and Jamestown. Since these latter two STPs were sampled only once, the statistical significance of the differences could not be determined. There was not a good association of the log reductions of the two indicator levels to each other or to the total chlorine residual. This latter observation was understandable since other factors are equally important, eg contact time, suspended solids, chlorine demand, etc., in determining the effectiveness of chlorination.

Indicator levels in the Providence River and Upper Bay

The data from the three-day sampling tour of the Providence River - Upper Narragansett Bay sampling network in December, 1985 are shown in Tables A6 (surface water) and A7 (bottom water), along with the geometric means (GMs) and their upper and lower standard deviations (SDs). The means of the indicator levels in the surface and bottom water samples along with the distances of each station from Transect A are summarized in Table 6, and those for the surface water samples are shown graphically in Figure 2. The E. coli and fecal coliform levels in the samples were very similar. Because of this, data on the E. coli levels were omitted and the fecal coliform levels were retained for comparison with historical data in the body of the report.

The fecal coliform and enterococcus levels in the surface water within the channel north of Gaspee Point appeared to be dominated by the riverine inputs north of station 1 (Table 6, Figure 2). This was not so of the C. perfringens and F phage levels, and this observation is consistent with the markedly greater effect of chlorination on the coliforms and enterococci than the other two indicators (Table 3). There is an input of fecal contamination to the channel water in the vicinity of stations 6 and 7 as seen from the levels of all four indicators (Figure 2). This is probably due to inputs from the Pawtuxet River and the Narragansett Village STP which do not mix into the channel water until some distance south of the mouth of the river. The salinity data for both the surface and bottom water samples are consistent with this explanation. There appears to be yet another input which reaches the channel in the vicinity of station 12, and it was not reflected in the salinity data. It was seen from the levels of all four indicators in the surface water but not as clearly from those in the bottom water, suggesting that it does not come from resuspension of bottom sediments. Spurious contamina- tion from boats is one possibility, as is contamination from the Warren River into which the Warren STP discharges about 2.0 MGD of chlorinated effluent. Another possibility is a non-municipal discharge (eg. stormwater, fish processing plant etc.), and this would explain the smaller peak in the F phage levels (Figure 2).

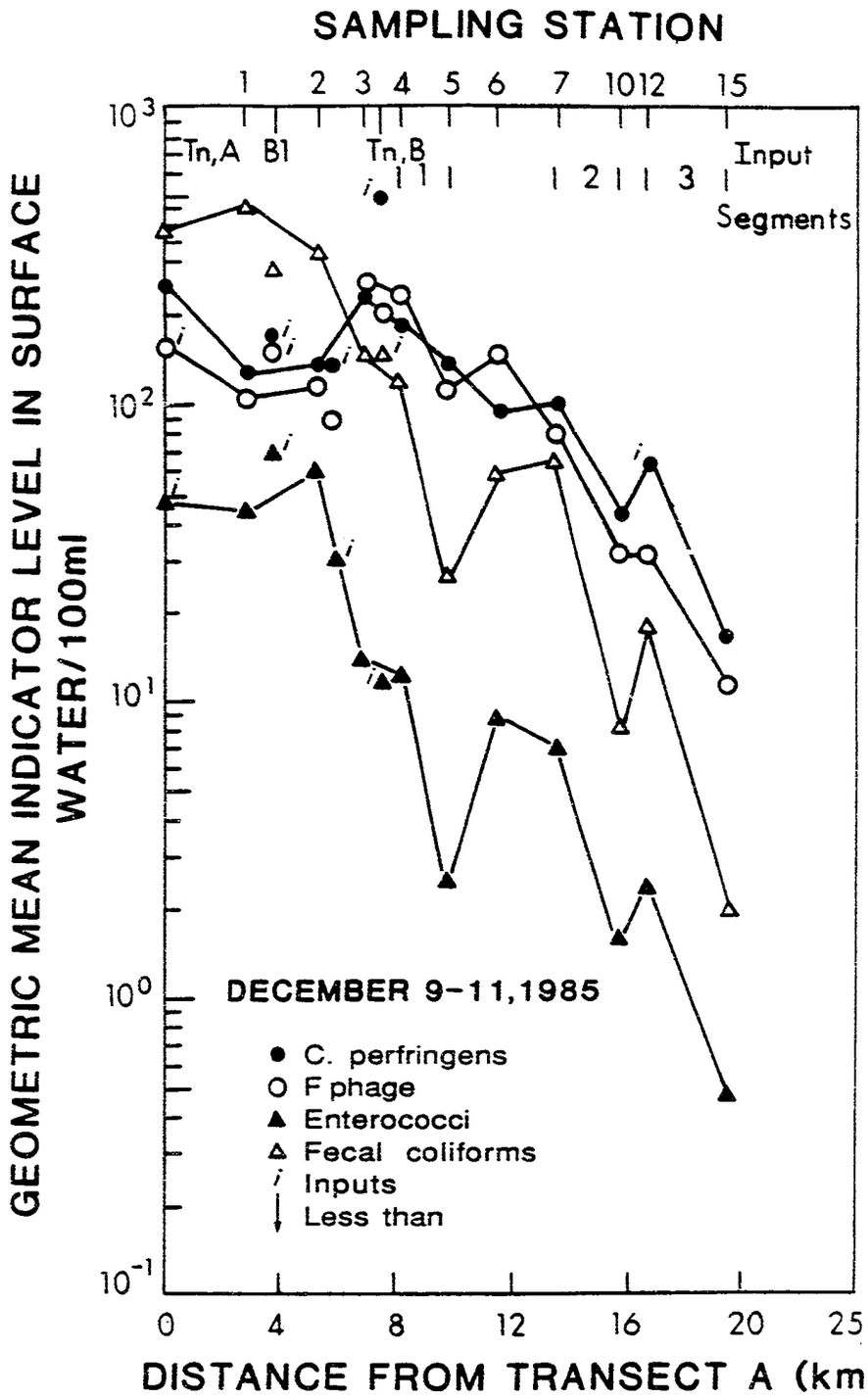

Figure 2. Geometric means indicator levels in surface water at channel stations by distance from head of Providence River for sampling tour conducted December 9-11, 1985. "i" denotes input levels at "boils" for discharges and at transect stations. Segments of network used to obtain decay coefficients are shown.

Table 6. Summary of geometric mean indicator levels at channel stations for sampling tour conducted December 9-11, 1985. Data from Tables 11 and 12.

Station	Distance[a] (km)	Geometric mean indicator level per 100 ml surface or bottom water							
		C. perfringens		F phage		Enterococci		Fecal coliforms	
		Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
TnA	0.00	265	274	163	76.1	47.1	47.8	392	244
1	2.83	132	66.5	110	-23.3[b]	44.7	2.5	474	22.9
2	5.38	142	5?.6	120	-13.7	60.6	3.5	339	14.0
3	6.92	240[i]	69.0	280[i]	-7.8	14.3	2.7	153	11.6
4	8.21	202	34.9	254	6.0	12.8	3.0	123	13.6
5	9.79	148	86.7	121	8.4	2.6	0.72	28.1	4.6
6	11.50	99.2	36.8	165[i]	-12.6	9.0[i]	-0.72	60.9[i]	2.2
7	13.48	109	53.8	84.9	-24.1	7.6	1.4	68.2	10.0
10	15.67	46.3	39.9	-34.8	-9.3	1.7	-0.63	8.9	3.8
12	16.68	69.5[i]	39.9	-34.2	-7.1	2.5[i]	1.1	19.0[i]	4.3
15	19.47	18.0	24.0	-12.6	10.0	-0.5	-0.5	2.1	1.5
Stations[c]		3-15	1-15	3-15	1-15	1-15	2-15	1-15	1-15
n		8	10	8	10	10	9	10	10
r		0.95	0.66	0.97	0.26	0.91	0.79	0.93	0.83
slope	$K_{t(d)}$	-0.079	-0.021	-0.106	-0.010	-0.110	-0.053	-0.127	-0.06

[a] Distance from Transect A.

[b] Approximation because of indeterminate values used in calcuating the means. These were usually due to values at the sensitivity limits of the assay. In such cases, the sensitivity limit was used in calculating the mean.

[i] Instances in which the lower SD of the mean was greater than the mean in the preceding station.

[c] Stations from which data was used in the regression of $\log_{10}$ indicator level (Y) against distance; n - number of values; r - correlation coefficient; $K_{t(d)}$ - total decay coefficient (slope of equation).

The indicator inputs along the course of the channel precluded obtaining releastic estimates values of biological decay coefficients against distance, much less time, from the entire data set for later use in water quality models. Nevertheless, the total decay coefficients against distance ($K_{t(d)}$) were calculated by regression analyses of the log mean indicator densities against distance from a major input at the head of the Providence River. They are included in Table 6 only for comparison among indicators. Certain of the increases in indicator levels at given stations relative to ones just to its north were great enough that the lower one SD from its GM was greater than the geometric mean level at the preceding station. These were probably due to inputs as noted above and are identified with the superscript "i" in Table 6. When this occurred within the first three stations, that station rather than station 1 was used as the first one in calculating the $K_t(d)$ values. In the surface water, the C. perfringens spore levels decreased the least, followed by F phages, enterococci and fecal coliforms in that order. This also was true for the bottom water data set with one exception, the decay for the phage in the bottom water was less than that for the spores. Total decay estimates as determined from the bottom water samples were consistently and appreciably less than those obtained from the surface water data.

An attempt was made to obtain more realistic total and even biological decay coefficients from the data for segments of the channel sampling network following a presumed indicator input and preceding a subsequent one. Three such segments were identified (Fig. 2). Unfortunately, there were only two stations in each segment; and this markedly limited the reliability of the estimate. The $\log_{10}$ indicator levels were regressed against the distances of the stations from Transect A to yield the $K_{t(d)}$ values for each indicator for each segment. The biological decay coefficients ($K_{b(d)}$ values) for the F phages, enterococci and fecal coliforms were obtained by subtracting the $K_{t(d)}$ values for C perfringens (used as a conservative tracer) from those for the other indicators. D_{90} values and T_{90} values were obtained, the former as $1/k_{b(d)}$ and the latter from the best available information we could obtain on the water velocity down the channel (3.5 cm/sec). Clearly, a measure of the flow in both the surface and bottom water in the channel at the time samples were collected would have provided a better estimate.

The $K_{t(d)}$ and $K_{b(d)}$ values from the surface water data for each indicator for each segment are given in Table 7. The individual $K_b(d)$ values, along with the $K_t(d)$ (total decay coefficients) for C. perfringens, are plotted against the average distance of the segment from Transect A in Figure 3. The coefficients for the phage were less than (more positive) those for the coliforms or the enterococci for each segment. Those for the enterococci were greater than those for the coliforms at the head of the estuary but less than those for the coliforms in the upper bay. The average $K_{b(d)}$ was then used to obtain the D_{90} and T_{90} estimates. The F phages had the least biological decay and the longest T_{90} estimate, and the fecal coliforms had the most biological decay and the shortest T_{90} estimates.

The data from the bottom water samples were not as amenable to obtaining absolute decay coefficients than those from the surface water samples for a

number of reasons. These relate to the source of the organisms and the movement of the water mass from which the organisms were recovered. Nevertheless, $K_{t(d)}$, $K_{b(d)}$, D_{90} and T_{90} values were calculated for comparison among the indicators and to those obtained from the surface water data. These are also given in Table 7. Although possibly spurious, the results of the comparisons are consistent with those from surface water data and understandable. They suggest less decay of the F phages than that of the enterococci or coliforms, whose die-off rates are comparable to each other. In general, the biological decay coefficients for the surface and bottom waters were not appreciably different during this winter sampling tour.

Three stations (7,8, and 9) from which water samples were collected in the December three-day sampling tour were close to the permanent closure line north of the conditional shellfish growing area. Another seven stations, 10-16, were well within the conditional area. Shellfish samples were collected twice in November 1985 from two stations near the closure line and from four stations well within the conditional area. The geometric means and 90 percentile values were calculated for each indicator from each of the four data sets for the water samples, and these were examined against the geometric means of the indicator levels in the shellfish. These comparisons are presented in Table 8 along with the geometric mean indicator levels in the prechlorinated effluents from the Providence and East Providence sewage treatment plants.

It can be seen from Table 8 that the fecal coliform levels in the conditional area (stations 10-16) did not exceed the standards for the geometric mean and 90th percentile (14 and 49 per 100 ml, respectively) (National Shellfish Sanitation Program, 1988) whether the surface water or the bottom water samples were used in the evaluation. Moreover, the fecal coliform levels in the shellfish themselves were well below the market standard. The enterococcus levels were less than those of the fecal coliforms in both the bottom and surface waters. The levels of enterococci in the shellfish exceeded those of the fecal coliforms, an observation consistent with reports of other investigators (Plusquellec et. al., 1986). The much higher C. perfringens than FC levels in both the bottom and surface waters was not unexpected because of the marked resistance of the spores to chlorination and die-off of the fecal coliforms in the water and in the bottom sediments. The markedly higher C. perfringens levels in the shellfish could have been due to accumulation of the spores from the sediments and/or differential die-off of the indicators in the shellfish. Some laboratory studies, in which the former possibility was eliminated, clearly showed that differential die-off in the shellfish was a major factor (Cabelli, 1988).

The most important finding, we believe, was the levels of the F phages in the water and especially the shellfish (This aspect of the work will be considered in a companion report). These viruses were being examined as a simulant for the environmental behavior of the Norwalk virus whose levels in the water can not be determined. The F phage levels were about 40 times less than those of the fecal coliforms in the prechlorinated effluents; but they were 1.8-3.1 times greater than the FC levels in the water and 20-160 times greater than the FC levels in the shellfish.

Table 7. Decay coefficients and D_{90} and T_{90} estimates for segments of the channel sampling network. December, 1985.

Parameter[a]	Segment[b]	C. perfringens SW[c]	C. perfringens BW[d]	F phage SW	F phage BW	Enterococci SW	Enterococci BW	Fecal coliforms SW	Fecal coliforms BW
$K_{t(d)}$	1	-0.086	-0.230	-0.204	0.093	-0.438	-0.392	-0.406	-0.298
	2	-0.170	-0.059	-0.177	-0.189	-0.297	-0.158	-0.406	-0.192
	3	-0.210	-0.079	-0.157	0.053	-0.251	-0.123	-0.343	-0.164
$K_{b(d)}$	1			-0.118	-0.157	-0.353	-0.642	-0.320	-0.548
	2			-0.007	-0.130	-0.127	-0.099	-0.236	-0.133
	3			-0.053	0.132	-0.041	-0.044	-0.133	-0.085
	x			-0.059	-0.052	-0.173*	-0.262	-0.230**	-0.255
	SD			-0.056	-0.159	0.161	0.065	0.094	0.029
D_{90} (Km)				16.9	19.2	5.78	4.31	4.35	3.92
T_{90} (hr)				134	152	45.9	34.2	34.5	31.1

[a] $K_{t(d)}$ - total decay coefficient, obtained as the slope of the line from the regression of the $\log_{10}$ indicator level (Y) against the distance from Transect A (x). $K_{b(d)}$ - biological decay coefficient for distance, obtained by subtracting $K_{t(d)}$ for C. perfringens from $K_{t(d)}$ for the indicator. $D_{90} = 1/K_{b(d)}$ - distance for 90 percent reduction in the indicator level. T_{90} - time for a 90 percent reduction in the indicator level, assumes an average flow of 3.5 cm/sec.

[b] Segments of the network: 1 - stns 4-5; 2 - stns 7-10; 3 stns 12-15.

[c] Data from surface water samples

[d] Data from bottom water samples

*, ** Differs significantly from that for F phage at $P<0.1$ and $P<0.01$; respectively by a pairing design "t" test.

Table 8. Comparison of mean indicator levels in water and shellfish samples collected near the permanent closure line and within the conditional shellfish growing area. November-December, 1985.

Indicator	Stat[a]	Indicator level per 100 ml water or per 100 gm shellfish[b]						
		Stations 7-9			Stations 10-16			Pre-Cl$_2$
		SW[c] (9)	BW[d] (9)	Shell (4)	SW (21)	BW (21)	Shell[d] (8)	Sewage x10^4
Fecal colif	GM	32.6	8.4	24.9	9.0	3.9	37.5	40.1
	90%	130	41		43	10		
Enterococci	GM	3.4	1.8	292	1.0	0.86	41.1	7.2
	90%	12	5.5		4.4	2.0		
F phage	GM	62.3	26.3	4040	26.0	7.3	734	1.0
	90%	140	100		89	22		
C. perfringens	GM	84	51.6	937	43.8	41.7	1050	2.9
	90%	130	76		73	80		

[a] GM - geometric mean; 90% - 90th percentile value, obtained graphically.

[b] In those cases where the indicator levels in at least half the samples were less than the sensitivity of the assay, the median was used.

[c] Surface water samples; ( ) number of samples.

[d] Bottom water samples.

[d] Shellfish samples collected in November, 1985.

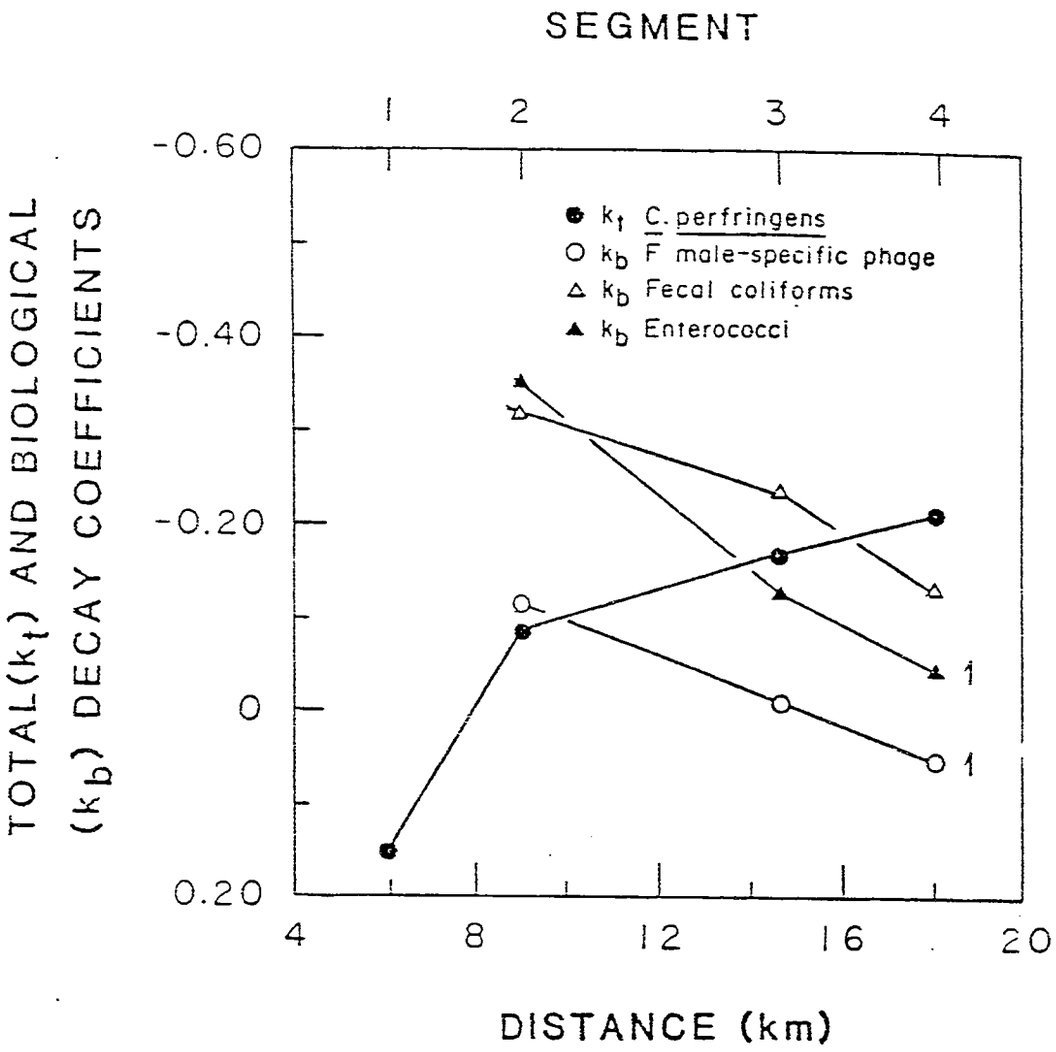

Figure 3. Biological decay (die-off) coefficients for fecal coliforms, enterococci and F male specific bacteriophages and total decay for C. perfringens as a function of distance from Transect A. K_t -total decay; K_b - biological decay; 1 - indicates decay greater than shown because of limits in assay sensitivity. Data from December 9-11 sampling tour.

A second three-day sampling tour was conducted July 22-24 of 1986. There were two problems with this tour. The first was that the samples were not collected on an outgoing tide on the second and third days, and this can be seen from the salinity data for the surface water samples shown in Figure 4. This required the segregation of the data from many of these stations into two groups, thereby decreasing the precision of the indicator levels obtained. Moreover, the means for days 2 and 3 could only be considered to reflect the conditions during a tidal change. The second problem was that in many of the assays the phage in water samples were not concentrated to provide a sensitivity limit of ≤ 2 PFU/100 ml. The need to do so was extremely critical, as will be shown, because of the low phage levels in the water and in the inputs to it during the summer. We have no explanation for the unusually high F phages and enterococcus levels which were found in the surface water samples collected on day 1 from stations 5 and 6 and 7, without corresponding increases in any of the other indicators (Table 9). A number of additional stations were added to those segments of the network used during the previous winter to obtain better decay coefficients. This was done in the hope of better defining total and biological decay within those segments.

The data obtained from the surface and bottom water samples for July 1986 tour are presented in Tables A8 and A9, respectively. Because of the problems noted above a somewhat different approach towards the analyses of the data from the channel stations. For those stations where the salinity for the first day was within one SD around the mean salinity for days 2 and 3, the indicator levels for all three days were used to obtain the geometric mean. When the salinity level for day 1 was not within one SD of the mean for days 2 and 3, the GM was obtained from the data for days 2 and 3. The means, and where appropriate, single values for the indicator levels in the channel stations are summarized for day 1 and days 2-3 in Table 9. The data for days 2-3 are shown graphically in Figure 4.

No estimate of the decay of the enterococci could be obtained either from the data for the entire network or for segments thereof due to the low input levels of this indicator. Additionally, the precision of decay estimates would have been poor because the numbers of colonies counted were near the lower sensitivity limit of the assay method and because data from three days were not always available in calculating the mean level for each station. The inputs for the F phages and C. perfringens were also markedly less than those observed in the winter sampling tour. Because of this and because, as noted earlier, the concentration procedure was not used in many of the phage assays, the precision of the estimates of the F phages in the water and, hence, the decay coefficient obtained were questionable and are not included in Table 10.

Low levels of enterococci and phage persisted in the surface water samples from the lower reaches of the sampling network during the previous winter. This phenomenon was not observed with the fecal coliforms during this tour and did not occur with the fecal coliforms, enterococci or F phages during the sampling tour conducted the following summer. Our best explanation for this observation is that, with the reversal of the tide, bottom water containing enterococci and phage, which remained at low levels because they were not exposed to sunlight and possibly because of the somewhat lower bottom water temperatures, were mixed with the surface water. This was less true of the coliforms, presumably because they do

Table 9. Summary of indicator levels for day 1 and the geometric means for days 2 and 3 channel sampling stations. July 22-24, 1986. Data from Table A8.

Station	Distance[a] (km)	Indicator level or geometric mean level/100 surface water for days							
		C. perfringens		F phage		Enterococci		FC	
		1	2-3	1	2-3	1	2-3	1	2-3
TnA[d]	0.00	26.1	26.1	46.6	46.6	4.9	4.9	183	183
1	2.83	38.0	12.8	<10	~10.3	1.6	4.8	582	170
2	5.38	130	8.1	14	19.6	3.3	1.7	566	34.6
2A[d]	6.23	28.4	23.4	~15.1	~15.1	0.93	0.93	38.2	38.2
3	6.92	30.0	6.4	23	5.0	1.7	~1.1	158	10.0
4[d]	8.21	26.6	26.6	17	17	2.2	2.2	23.2	23.2
4A	9.05	51.0	15.5	23	4.0	18.5	~1.1	47.5	7.4
5	9.79	24.7	2.9	114[o]	<10[o]	2.0	~1.8	ND	1.7
6	11.50	6.6	5.7	5	<10[o]	34.0	1.6	38.5	~5.7
7	13.48	8.2	2.4	~8	8	58.0	<0.5	16.0	3.3
7A	14.64	14.8	2.4	12	3	1.5	~0.71	14.5	<3.3[o]
10	15.76	9.9	1.7	12	<10[o]	3.0	~1.1	1.5	<0.5
12	16.68	10.0	1.4	6	<10[o]	7.0	~0.87	0.5	<0.5
18[d]	17.76	1.7	1.7	2	2	1.4	1.4	~0.5	~0.5
18A[d]	18.69	2.1	2.1	4.2	4.2	1.3	1.3	~0.5	~0.5
15[d]	19.47	0.87	0.87	<2	<2	<0.9	<0.9	0.71	0.71
Stations[c]		1-15	1-15	1-18	1-18			1-12	1-10
n		15	15	12	9			11	11
r		0.87	0.85	0.62	0.74			0.90	0.90
slope	$K_{t(d)}$	-0.098	-0.078	-0.038	-0.054			-0.184	-0.157

See Table 6 for footnotes

[d]Geometric mean from days 1-3

[o]Data omitted from regression analysis

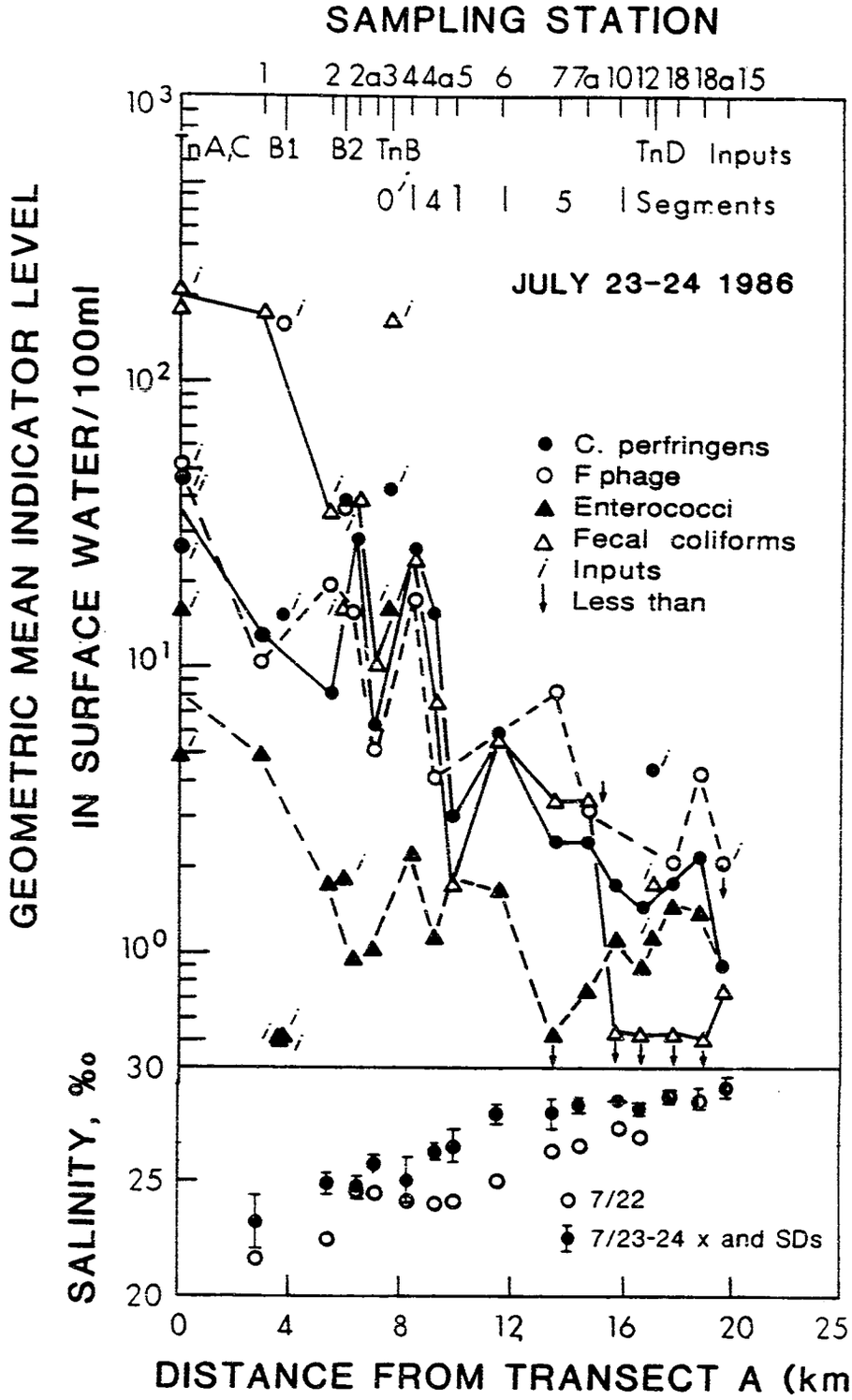

Figure 4. Geometric mean indicator levels in surface water at channel stations by distance from the head of the Providence River for sampling tour conducted July 22-24, 1986. Levels given are generally from 7/23 - 7/24 data because of differences in the salinities are shown. Inputs and segments indicated as in Figure 2.

not survive as well in saline waters (Fattal et. al., 1983). The data on the levels of the indicators in the bottom water (Table A9) was consistent with this explanation.

Two segments, of the sampling network (4 and 5) were used to derive decay coefficients. They were comprised of stations 4, 4A and 5 and 6,7, 7A and 10, respectively (Table 10). As can be seen from this table, neither F phages nor enterococcus decay coefficients were calculated for the reasons stated earlier. We have no explanation as to why the biological decay coefficient for the fecal coliforms was less than that obtained for the surface water during the previous December. There was less biological decay than during the following summer, suggesting that the decay coefficient may have also reflected bottom as well as surface water conditions.

The means of the indicator levels in the bottom water samples are given in Table A9 and summarized in Table 11. The C. perfringens and FC levels decreased in the upper part of the network. This could not be seen with the enterococci or F phages because of the low input levels (the exception was the boil for the Providence STP (Table A9). Very low levels of the indicators were found in the water samples from the stations south of station 7A. A single segment (6) of the sampling network comprised of stations 3-6 was used to obtain a biological decay coefficient for fecal coliforms. For the reasons noted above, meaningful decay coefficients for the enterococci and the F phages could not be determined. The one obtained for fecal coliforms (Table 10) was less than the one calculated for the surface water and much less than the one for the bottom water obtained the previous December.

The data from the sampling tour conducted July 28-30 1987 are given in Table A10, summarized in Table 12 and shown graphically in Figure 5. The indicator levels during the third day were appreciably lower at several of the stations. As in July of 1986, the levels of the indicators, including C. perfringens, were generally much lower than they were during the December tour. The major difference from the July 1986 data set was that the levels of all the indicators but C. perfringens were below the sensitivity levels of the assays (0.5 CFU/100 ml) at all the stations south of Conimicut Point. Detectable enterococcus levels were not found south of station 1 except for those in the samples from the two boils and Transect B.

In an attempt to better understand the differences in the indicator levels between the December 1985 and July 1987 data sets, the differences in the known inputs for which there were comparable data were examined. These included the data from Transects A and B, boil stations B1 and B2 and the pre- and postchlorinated effluents from the Providence and East Providence STPs. These comparisons are presented in Table 13. It is clear that, for the two periods in question, the lower indicator levels in July, 1987 can be ascribed largely to the smaller riverine inputs to the Providence River entering above station 1 during the week in July, 1987. Since this was also true of the C. perfringens spores, we find it hard to attribute the differences to die-off of the indicators in the river water. Differences between the inputs during the two periods were also found with all but the fecal coliforms from the comparisons of the data from the Pawtuxet River (TnB) station, although only the one for C. perfringens was statistically significant. The enterococcus and FC but not the C. perfringens

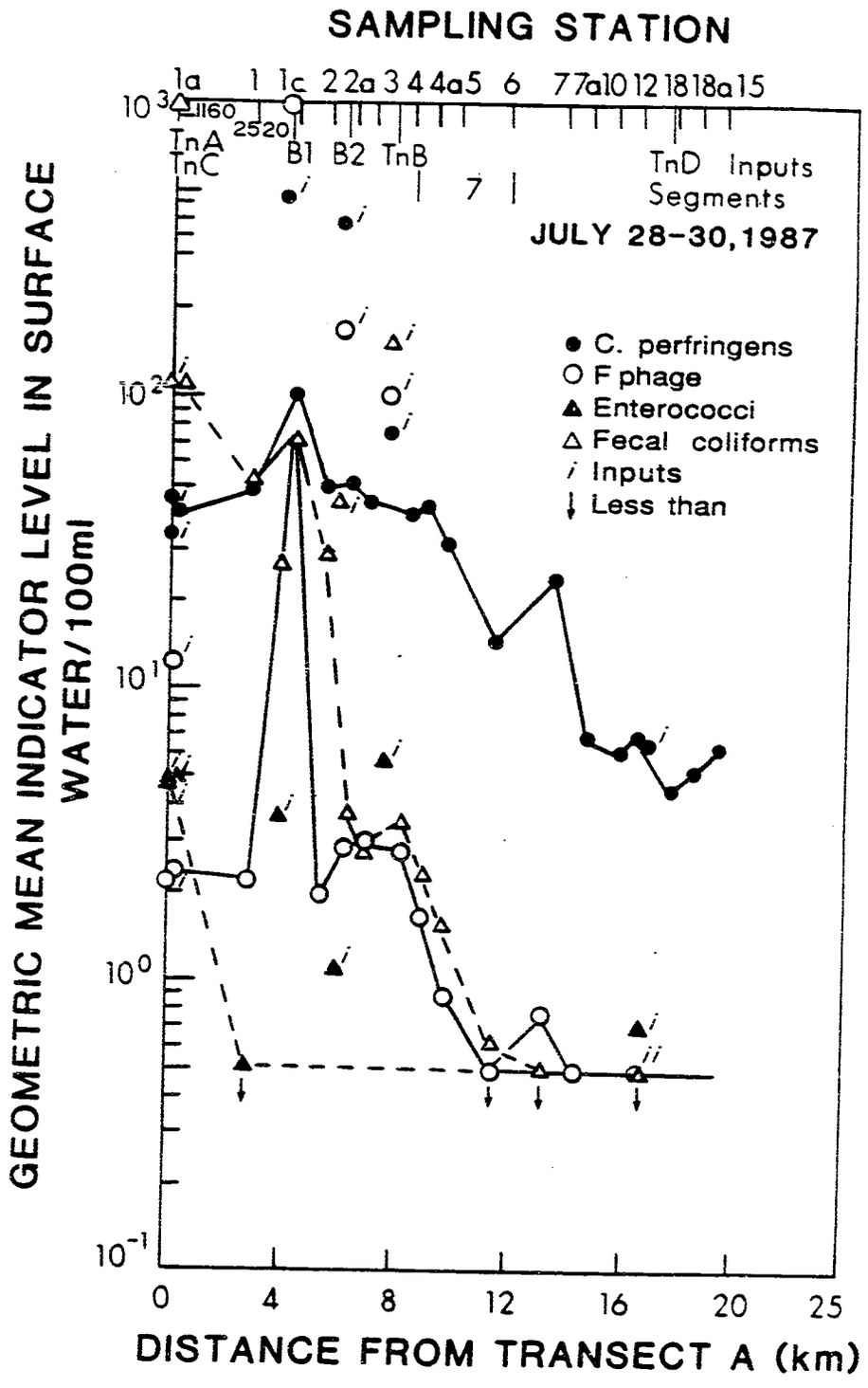

Figure 5. Geometric mean indicator levels in surface water at channel stations by distance from the head of the Providence River for sampling tour conducted July 28-30, 1987. Inputs and segments indicated as in Figure 2.

Table 10. Decay coefficients and D_{90} estimates for segments of the channel sampling network. July, 1986.

Parameter[a]	Segment[b]	Surface Water *C. perfringens*	FC	Segment[b]	Bottom Water *C. perfringens*	FC
$K_{t(d)}$	4	-0.601 (0.95)[c]	-0.716	6	-0.096 (0.97)	-0.168 (0.95)
	5	-0.118 (0.95)	-0.251 (0.96)			
$K_{b(d)}$	4		-0.115	6		-0.072
	5		-0.133			
	x		-0.124			-0.072
D_{90} (km)			8.06			13.9
T_{90} (hr)			64.0			110

[a]See Table 7

[b]Segments of the network: 5 - stns 4A-6; 6 - stns 7-10; 7 - stns 3-6

[c]Correlation coefficient, r.

Table 11. Summary of geometric mean indicator levels in bottom water from channel stations. July 22-24, 1986. Data from Table A9.

Station	Distance (km)	Geometric mean indicator level/100 ml bottom water			
		C. perfringens	F phage	Enterococci	FC
TnA	0.00	32.8	<10	3.4	116
1	2.83	<0.5	<10	2.3	89.7
2	5.38	19.8	~9	2.1	22.3
2A	6.23	13.3	~14	0.9	5.6
3	6.92	21.5	<10	1.0	11.3
4	8.21	13.0	~5.3	~0.8	5.2
4A	9.05	13.5	<10	1.0	6.2
5	9.79	10.4	~5.8	2.4	~4.1
6	11.50	7.4	<10	0.7	~1.7
7	13.48	3.3	~4	0.9	~2.3
7A	14.64	9.1	~8	~2.0	~1.6
10	15.76	2.4	~2.8	2.5	<1.0
12	16.68	2.5	<10	3.5	~1.0
18	17.76	4.3	~4	<0.6	<0.6
18A	18.69	4.2	<10	~0.96	~0.6
15	19.42	1.7	<2	1.0	0.8
Stations		2-7			1.6
n		8			8
slope	0.92 $K_{t(d)}$	0.84 -0.088			0.94 -0.179

See Table 6 for footnotes

Table 12. Geometric mean indicator levels in surface water samples from channel stations in Narragansett Bay. July 28-30, 1987.

Station	Distance[a] (km)	GM indicator level in water per 100 ml			
		C. perfringens	F phage	Enterococci	FC
TnA	0.00	45.9	2.2	4.8	112
1A	0.51	40.2	2.4	4.8	111
1	2.83	48.8	<2.2	<0.5	50.4
1C	4.28	100	78.1	<0.5	73.4
2	5.38	49.1	2.0	<0.5	30.2
2A	6.23	50.9	~2.9	<0.5	3.7
3	6.92	44.9	3.0	<0.5	~2.9
4	8.21	40.2	2.8	<0.5	~4.6
4A	9.05	43.3	~1.7	<0.5	~2.4
5	9.79	32.1	~0.9	~0.63	1.6
6	11.50	14.9	<0.5	<0.5	~0.63
7	13.48	24.4	0.79	<0.5	<0.5
7A	14.64	6.9	<0.5	<0.5	<0.5
10	15.76	6.3	<0.5	<0.5	<0.5
12	16.68	7.1	<0.5	<0.5	<0.5
18	17.76	4.6	<0.5	<0.5	<0.5
18A	18.69	5.4	<0.5	<0.5	<0.5
15	19.47	6.5	~0.5	<0.5	<0.5
Stations		1C-6	1C-6		1C-6
n		8	8		8
r		0.91	0.80		0.91
slope	$K_{t(d)}$	-0.087	-0.217		-0.254

[a] Distance from Transect A

[b] Approximate value because the level for at least one of the values was less than the sensitivity of the assay (0.5 CFU/100 ml).

[c] <at least two of the values less than the sensitivity of the assay.

Table 13. Comparison of indicator inputs to the Providence River and Upper Narragansett Bay during the December, 1985, and July, 1987, sampling tours.

Location	Station	Month/yr	Indicator level per 100 ml of sample			
			C. perfringens	F phage	Enterococci	FC
Seekonk R.	TnA	Dec/85	265***	163***	47.1***	392*
		July/87	50.5	1.7	5.0	83.2
		D/J[a]	5.2	96	9.4	4.7
Pawtuxet R.	TnB	Dec/85	515*	215	12.1	150
		July/87	87.3	103	4.0	160
		D/J	5.9	2.1	3.0	0.94
Prov. STP boil	B1	Dec/85	172	155***	69.8*	298*
		July/87	539	2740	3.3	37.9
		D/J	0.32	0.06	21	7.9
E. Prov. STP boil	B2	Dec/85	138***	92.1	30.8**	141
		July/87	379	180	1.0	48.1
		D/J	0.36	0.52	31	2.9
Providence STP	Post-Cl_2 effluent	Dec/85	12500	6890	27.2**	206
		July/87	4900	4260	1.2	16.4
		D/J	2.6	1.6	23	12.6
E. Providence STP	Post-Cl_2 effluent	Dec/85	4820	2180	7.9	33.4
		July/87	2930	1210	1.5	89.7
		D/J	1.6	1.8	5.1	0.37

[a]Mean level in Dec 1985/Mean level in July 1987

*,**,***

Significantly different from July/87 level at $P<0.05$, 0.01 and 0.001, respectively.

and F phage levels in the boils for the Providence and East Providence discharges were also less in July, 1987, than in December, 1985. However, the C. perfringens and F phage levels in the postchlorinated effluents were higher in December than July (Table 13). The comparison of the $\log_{10}$ reductions showed that, if anything, chlorination was less, not more, effective in the week of July, 1987, than in the week of December, 1985 (Table 3).

We question whether the B1 and B2 samples were collected from their respective boils during the December tour. However, they definitely were taken from the boils during the July, 1987, tour; and, because of this, we were able to estimate the initial dilution at the outfall from the comparison of the GM C. perfringens levels in the boils to those in the postchlorination effluents at the STPs. They were 9.1 and 7.7, respectively for the Providence and East Providence STPs. This type of estimate for initial dilution has the advantage over the use of a dye in that it considers initial sedimentation as well as dilution and does not require the addition of the dye to the water.

It can be seen from Table 13 that, in most cases, the coliform and enterococcus but not the F phages and C. perfringens levels in the samples from the boils for the discharges from both STPs were higher than those in the postchlorinated effluents. This was true for the discharges from both plants in the December, 1985 sampling tour and in that from the Providence STP during the July, 1987 tour. The simplest explanation for this observation is that the enterococcus and coliform levels in the waters which dilute the effluents are greater than those in the effluents themselves. An alternative explanation is so-called "regrowth", more probably environmental repair of chloramine-damaged cells. Regrowth will confound both the determination of die-off coefficients and the development and evaluation of models for predicting enterococcus or coliform levels in the bay.

The lower indicator levels found in the July, 1987 than the December, 1985 sampling tour can also be attributed to increased die-off from increased solar radiation and higher ambient water temperatures. There are considerable data showing that solar radiation is a major factor responsible for the biological decay of microorganisms in environmental waters (Fujioka et al. 1981, 1982). Biological decay coefficients for the F phages and fecal coliforms were obtained for a single, eight-station segment (stations 1C-6, Figure 5) of the channel network. No coefficient could be obtained for the enterococci since they were recovered only from station 1A samples, and then at very low levels. The coefficients are given in Table 14; and all the biological decay coefficients that could be obtained during the study are compared in Table 15.

The die-off of the F phages was higher in the surface water in July 1987 than in December 1985. Die-off of the fecal coliforms, however, was, if anything, less in July of 1987 than in December 1985. If this is a reality, there is an interesting explanation, better "regrowth" of the fecal coliforms during transport downstream during the summer. This notwithstanding, in July 1987, biological decay of the fecal coliforms was greater than that of the F phages (Table 14). Moreover, when decay coefficients for the F phages could be obtained, they were less than those for the fecal coliforms (Table 15); and,

during the winter tour in December, 1985, the difference was statistically significant (Table 7).

The D_{90} and T_{90} estimates for the bottom water along with the coefficients from which they were derived were included with marked reservations for the reasons given earlier; and even those for the surface waters have some limitations. Nevertheless, they are generally understandable.

The July 1986 and July 1987 data from the stations near and within the conditional shellfish growing area were examined as they were for the December, 1985 sampling tour (Table 8). The results are presented in Table 16 along with those for some assays conducted on shellfish harvested in July of 1986. As in the 1985 tour, there had not been a half inch of rain during a given 24 hour period in the week prior to collection of the water samples in 1986 or 1987. It is clear that the fecal coliform levels at the "closure line" and in the conditional area were well within the prescribed limits, although the 90 percentile values could not be estimated precisely from the only six data points. The F phage (virus) and C. perfringens (spore) levels in the water were higher than those of the fecal coliforms even though their numbers were markedly less in prechlorinated sewage. In general, the levels of the indicators both in the water and in the shellfish were appreciably less than they were in November-December 1985 (Table 8). This was especially true of the F phage levels in the shellfish. The exception was the C. perfringens levels in the shellfish, and we speculate that this may be due to high C. perfringens levels in the sediments during the summer and winter.

DISCUSSION

As noted earlier in this report, the sampling schedule at the sewage treatment plants precluded meaningful generalizations concerning plant operation. Since they occurred with C. perfringens spores as well as enterococci and coliforms, the higher indicator levels in December, 1985 may represent a seasonal effect on the indicator levels that derives from their better physical removal of the indicators More data are needed. The lower indicator levels at the Providence STP in July, 1987, as compared to those in July, 1986, suggest an improvement in secondary treatment since this did not occur at the East Providence STP.

Another interesting finding was the marked reduction in the C. perfringens and F phage levels at the Narragansett Village, extended aeration STP. The reductions could be explained by the higher total chlorine residuals, but the reductions in the enterococcus and coliform levels are not consistent with this explanation. An alternative explanation is that some aspect of the treatment train sensitized the spores and viruses specifically to the effect of subsequent chlorination.

The foregoing speculations are not important in themselves since more data are needed to examine them. They do, however, illustrate the range of questions that can be addressed by using microbial indicators in an instructive as well as regulatory mode. Moreover, the choice of the five easily measured indicators

Table 14. Decay coefficients and D_{90} and T_{90} estimate for segment of channel network. Surface water, July, 1987.

Parameter	Segment	C. perfringens	F phage	FC
$K_{t(d)}$	7	-0.087 (0.91)	-0.217 (0.80)	-0.254 (0.91)
$K_{b(d)}$	8		-0.130	-0.167
D_{90} (km)			7.69	5.98
T_{90} (hr)			61.1	47.5

[a]See Table 7 for relavent footnotes.

[b]Segment: 7 - stns 1C-6

[c]Correlation coefficient, r.

Table 15. Summary of biological decay coefficients for all sampling tours.[a]

Parameter	Month/Yr	Surface Water			Bottom Water		
		F phage	Enterococci	FC	F phage	Enterococci	FC
$K_{b(d)}$	Dec/85	-0.059	-0.173	-0.230	-0.052	-0.262	-0.255
	July/86			-0.124			-0.072
	July/87	-0.130		-0.167			
D_{90} (km)	Dec/85	16.9	5.78	4.35	19.2	4.31	3.92
	July/86			8.06			13.9
	July/87	7.69		5.98			
T_{90} (hr)	Dec/85	134	45.9	34.5	152	34.2	31.1
	July/86			64.0			110
	July/87	61.1		47.5			

[a]Values from Tables 7, 10 and 14.

Table 16. Comparison of mean indicator levels in the water and shellfish samples collected near the permanent closure line and within the conditional shellfish growing area. July 1986, July 1987.

Indicator	Stat	Yr	Indicator level/100 ml water or/100 g shellfish						
			Stations 6-7A			Stations 10-21			Pre-Cl$_2$
			SW (6)	BW (6)	Shell (3)	SW (24)	BW (24)	Shell (6)	sewage x10^4
Fecal colif.	GM	86	3.3	1.9	1.9	0.5	0.5	2.9	15.5
		87	<0.5			<0.5			8.7
	90%	86	20	34		1.5	2.5		
		87	1			0.5			
Enterococci	GM	86	0.5	1.3	17.4	1.0	1.0	7.8	1.64
		87	<0.5			<0.5			0.59
	90%	86	8			6.2	4.2		
		87	1			0.5			
F phage	GM	86	6.9	4.0	33.8	4.2	2.7	10.6	0.46
		87	0.5			<0.5			0.27
	90%	86	21	15		11	6		
		87	5			0.5			
C. perfringens	GM	86	3.9	5.9	1280	2.3	2.7	530	1.02
		87	12.9			6.0			0.46
	90%	86	20	18		9.0	8.6		
		87	41			10.0			

See Table 9 for appropriate footnotes.

allows the examination of different aspects of the treatment process, especially if measurements are made on the influent and primary settled effluent as well.

The good correlation between the prechlorination levels of C. perfringens and the F phages across STPs is somewhat confusing since the source of C. perfringens in sewage is human feces and the F phages are infrequently found therein and, when present, are there at very low levels. The source of the F phages found in domestic sewage needs to be determined. One possibility is that there is replication of the phages in the sewerage lines.

It is clear that, although the levels of the F male-specific bacteriophages in prechlorinated sewage are about 1.5 orders of magnitude less than those of the coliforms, their levels in the Providence River and Upper Narragansett Bay generally exceed those of the fecal coliforms, at times by as much as a factor of three. Moreover, the differences in the levels of the two indicators are even greater in shellfish and during the winter.

Clearly, the F phages (viruses) survived both wastewater chlorination and transport in marine waters much better than the fecal coliforms, and this confirms observations made elsewhere with other viruses (Scarpino et. al., 1972; Fattal et. al., 1983). These two factors can readily account for the differences in their environmental levels in the bay relative to those in the prechlorinated effluent.

The most critical issue raised by these findings is the risk shellfish-associated infectious disease deriving from the discharge of municipal wastewaters into marine waters during the winter, even when these waters meet the existing coliform standards. It is particularly important in situations, such as that in Narragansett Bay, where there is a critical need for disinfection (which, for the present, means chlorination) to achieve existing total coliform, fecal coliform or even enterococcus standards for major shellfish growing areas. The corollary to this issue is the adequacy of the coliform and enterococcus indicators in these specific situations. There is no question that, in the final analysis, both of these questions must be, and hopefully will be, resolved by the results from prospective epidemiological studies. Another corollary is the adequacy of wastewater strategies which depend heavily on chlorination to meet the existing microbial indicator standards.

A major concern, the management of the conditional shellfish growing area during the late fall, winter and early spring will be considered in the report on water/shellfish indicator relationships in the conditional shellfish growing area.

Bathing beaches, unless they are near an outfall for a wastewater discharge, should not pose a problem similar to that for shellfish growing areas. The swimming season in Narragansett Bay occurs in July and August; and there is appreciable die-off of the F male-specific bacteriophages and, hopefully, the Norwalk viruses, during transport in the water at this time.

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APPENDIX A

Table A1. Indicator levels in pre- and postchlorinated effluent samples from sewage treatment plants during December, 1985 sampling tour.

Sewage treatment[a] plant	Sample[b] type	Date	Log10 indicator level/100ml effluent C. perfringens	F phage	Enterococci	FC
Providence (Field's Point)	Pre-Cl_2	12/07	4.580	4.491	4.756	5.279
		12/08	4.602	4.114	4.708	5.362
		12/09	4.887	4.255	4.954	5.544
		12/10	4.236	4.146	4.996	5.431
		12/11	4.813	4.380	4.462	5.978
		x	4.623	4.277	4.775	5.519
		SD	0.253	0.159	0.214	0.274
	Post-Cl_2	12/07	3.630	3.869	1.176	1.672
		12/08	3.978	3.863	1.903	2.732
		12/09	4.447	3.929	1.690	3.230
		12/10	3.898	3.924	<0.519	1.255
		12/11	4.532	3.602	1.887	2.681
		x	4.097	3.838	~1.435	~2.314[c]
		SD	0.382	0.135	0.591	0.819
	log. red.	12/07	0.950	0.622	3.580	3.607
		12/08	0.624	0.251	2.806	2.630
		12/09	0.440	0.326	3.264	~2.314
		12/10	0.338	0.222	>4.477	4.176
		12/11	0.281	0.778	2.575	3.297
		x	0.527	0.440	~3.532	~3.205
		SD	0.270	0.245	0.706	0.748
East Providence	Pre-Cl_2	12/07	4.260	3.898	4.785	5.663
		12/08	4.362	4.255	4.591	5.477
		12/09	4.281	3.708	4.903	5.760
		12/10	4.295	3.591	5.164	5.826
		12/11	4.350	3.279	5.255	5.716
		x	4.310	3.746	4.940	5.688
		SD	0.044	0.363	0.272	0.132
	Post-Cl_2	12/07	3.560	3.903	1.813	1.568
		12/08	3.398	3.301	1.724	1.477
		12/09	3.618	3.362	<0.230	1.736
		12/10	3.732	3.255	0.519	1.724
		12/11	3.881	2.875	0.204	1.114
		x	3.638	3.339	~0.898	1.524
		SD	0.182	0.368	0.805	0.254
	log. red.	12/07	0.700	-0.005	2.972	4.095
		12/08	0.964	0.954	2.867	4.000
		12/09	0.663	0.343	>4.673	4.024
		12/10	0.563	0.336	4.645	4.102
		12/11	0.469	0.404	5.051	4.602
		x	0.672	0.406	~4.042	4.165
		SD	0.187	0.346	1.037	0.248

Warren	Pre-Cl_2	12/07	3.579	3.041	3.544	4.978
		12/08	3.398	3.000	3.699	4.653
		12/09	3.423	2.740	3.875	4.332
		12/10	3.439	2.839	4.394	4.643
		12/11	3.602	2.613	4.585	4.756
		x	3.488	2.847	4.020	4.673
		SD	0.095	0.179	0.450	0.233
	Post-Cl_2	12/07	3.100	2.681	1.124	2.176
		12/08	1.699	2.613	0.919	1.820
		12/09	2.602	2.204	<0.230	ND
		12/10	3.114	2.041	2.176	2.477
		12/11	3.255	2.301	2.220	ND[d]
		x	2.754	2.368	-1.334	2.158
		SD	0.640	0.272	0.856	0.329
	log. red.	12/07	0.479	0.360	2.420	2.802
		12/08	1.699	0.387	2.780	2.833
		12/09	0.821	0.536	>3.645	ND
		12/10	0.325	0.798	2.218	2.166
		12/11	0.347	0.312	2.365	ND
		x	0.734	0.479	-2.686	2.600
		SD	0.574	0.197	0.575	0.376
Narragansett Village	Pre-Cl_2	12/07	4.672	3.643	4.820	5.041
		12/08	4.686	4.613	5.799	6.568
		12/09	3.845	4.342	4.699	3.477
		12/10	5.248	3.944	5.146	4.987
		12/11	4.940	3.978	5.991	5.978
		x	4.678	4.104	5.291	5.210
		SD	0.521	0.377	0.579	1.174
	Post-Cl_2	12/07	2.806	3.447	0.519	0.398
		12/08	2.602	3.255	3.255	3.732
		12/09	2.875	3.079	1.398	<0.230
		12/10	2.064	3.041	<0.000	0.602
		12/11	2.869	2.903	>2.996	3.602
		x	2.643	3.145	-1.634	-1.713
		SD	0.342	0.210	1.454	1.789
	log. red.	12/07	1.866	0.196	4.301	4.643
		12/08	2.084	1.358	2.544	2.836
		12/09	0.970	1.263	3.301	>3.247
		12/10	3.184	0.903	>5.146	4.385
		12/11	2.071	1.075	<2.995	2.376
		x	2.026	0.959	-3.657	-3.497
		SD	0.788	0.461	1.053	0.982

[a]Type of treatment given in Table 5.

[b]Pre-Cl_2 - prechlorination; Post-Cl_2 - postchlorination; data from single grab sample; log. red. - (pre-chlorination $\log_{10}$ level) - (postchlorination $\log_{10}$ level).

Table A2. Indicator levels in sewage treatment plant pre- and post-chlorinated effluent samples during July, 1986 sampling tour.

Sewage treatment[a] plant	Sample[b] type	Date	Log10 indicator level/100ml effluent			
			C. perfringens	F phage	Enterococci	FC
Providence	Pre-Cl_2	07/21	4.531	4.365	4.398	5.290
		07/22	4.431	4.352	3.699	4.954
		07/23	4.301	4.375	3.898	4.778
		07/24	4.146	3.756	3.799	4.544
		x	4.353	4.212	3.949	4.892
		SD	0.167	0.304	0.310	0.314
	Post-Cl_2	07/21	4.398	4.260	2.996	2.778
		07/22	4.041	4.057	<2.000	2.477
		07/23	3.954	4.033	<2.000	2.000
		07/24	3.613	3.477	<2.000	1.699
		x	4.002	3.957	<2.249	2.239
		SD	0.323	0.336		0.482
	log. red.	07/21	0.133	0.105	1.402	2.512
		07/22	0.390	0.295	>1.699	2.477
		07/23	0.347	0.372	>1.898	2.778
		07/24	0.533	0.279	>1.799	2.845
		x	0.351	0.263	>1.700	2.653
		SD	0.166	0.113		0.186
East Providence	Pre-Cl_2	07/21	3.886	2.903	4.407	5.455
		07/22	3.799	3.196	4.544	5.199
		07/23	3.613	3.130	4.580	5.633
		07/24	3.763	3.243	4.398	5.672
		x	3.765	3.106	4.482	5.490
		SD	0.114	0.144	0.093	0.216
	Post-Cl_2	07/21	2.500	2.653	<0.230	1.000
		07/22	3.699	2.544	<0.519	1.699
		07/23	2.672	2.740	0.000	<0.519
		07/24	1.699	3.161	0.217	1.000
		x	2.642	2.775	~0.242	1.054
		SD	0.822	0.270	0.213	~0.486
	log. red.	07/21	1.386	0.250	>4.177	4.455
		07/22	0.100	0.602	>4.025	3.500
		07/23	0.941	0.390	>4.580	>5.114
		07/24	2.064	0.082	4.181	4.672
		x	1.123	0.331	~4.241	~4.435
		SD	0.823	0.220	1.238	0.681
Warren	Pre-Cl_2	07/21	3.580	2.477	4.352	5.097
		07/22	2.968	2.602	3.699	4.978
		07/23	3.000	1.699	5.204	5.097
		07/24	2.799	2.243	4.000	4.813
		x	3.087	2.255	4.314	4.996
		SD	0.340	0.400	0.651	0.134

Post-Cl_2	07/21	2.602	<1.699	1.000	2.021
	07/22	2.398	2.176	1.220	2.371
	07/23	<1.522	1.699	0.699	1.954
	07/24	<1.522	<1.699	<0.519	<1.000
	x	~2.011	~1.818	~0.860	~1.837
	SD	0.571	0.239	0.312	0.587
log. red.	07/21	0.978	>0.778	3.352	3.076
	07/22	1.570	0.426	2.479	2.607
	07/23	>1.478	0.000	4.505	3.143
	07/24	>1.277	>0.544	>3.481	>3.813
	x	~1.076	~0.437	~3.454	~3.160
	SD	0.395	0.326	0.830	0.497

[a] Type of treatment given in Table 5.

[b] $Pre\text{-}Cl_2$ - prechlorination; $Post\text{-}Cl_2$ - postchlorination; data from single grab sample; log. red. - (pre-chlorination $\log_{10}$ level) - (postchlorination $\log_{10}$ level).

[c] - approximation because an indeterminate value included in the calculation.

Table A3. Indicator levels in pre- and post-chlorinated effluents from Providence and East Providence sewage treatment plants during July, 1987 sampling tour.

Sewage treatment[a] plant	Sample[b] type	Date	Log10 indicator level/100ml effluent			
			C. perfringens	F phage	Enterococci	FC
Providence (Field's Point)	Pre-Cl_2	07/28	3.924	3.923	3.653	5.322
		07/29	2.380	2.892	<2.230	<3.230
		07/30	4.079	4.000	3.380	4.204
		x	3.461	3.605	~3.807	~4.252
		SD	0.939	0.619	0.755	1.047
	Post-Cl_2	07/28	3.644	3.792	<0.114	0.996
		07/29	3.813	3.602	0.079	1.732
		07/30	3.613	3.491	0.000	0.919
		x	3.690	3.629	~0.064	1.216
		SD	0.108	0.152	0.058	0.449
	log. red. (Pre-Post)	07/28	0.280	0.131	>3.539	4.326
		07/29	-1.433	-0.710	<2.150	<1.498
		07/30	-0.229	0.509	3.380	3.285
		x	-0.461	-0.023	~3.023	~3.036
		SD	0.880	0.624	0.760	1.430
East Providence	Pre-Cl_2	07/28	3.756	2.690	4.568	5.756
		07/29	4.079	3.431	4.462	5.580
		07/30	3.778	3.634	4.322	5.544
		x	3.871	3.252	4.451	5.627
		SD	0.181	0.406	0.123	0.113
	Post-Cl_2	07/28	3.644	3.104	0.699	1.851
		07/29	3.813	3.230	0.176	2.176
		07/30	2.945	2.910	-0.301	1.833
		x	3.467	3.081	0.191	1.953
		SD	0.460	0.162	0.500	0.193
	log. red. (Pre-Post)	07/28	0.112	-0.414	3.869	3.725
		07/29	0.266	0.210	4.286	3.404
		07/30	0.833	0.724	4.623	3.711
		x	0.404	0.170	4.259	3.613
		SD	0.380	0.570	0.378	0.181

[a] Type of treatment given in Table 9.

[b] Pre-Cl_2 - prechlorination; Post-Cl_2 - postchlorination; data from single grab sample; log. red. - (pre-chlorination $\log_{10}$ level) - (postchlorination $\log_{10}$ level).

[c] approximation because an indeterminate value included in the calculation.

Table A4. Flow rates, suspended solids and chlorine residuals for sewage samples collected. December, 1985, and July, 1986.

Date	Providence			East Providence			Warren			Narragansett Village		
	Flow[a]	SS[b]	Cl_2[c]	Flow	SS	Cl^2	Flow	SS	Cl^2	Flow	SS	Cl^2
12/07/85	42.3	43	3.8	6.5	ND	1.3	2.0	ND	2.0			3.5
12/08	40.8	53	ND	6.5	ND	1.1	2.0	1.0	1.8			4.0
12/09	47.3	39	3.3	6.3	13	1.0	1.9	1.0	2.1			4.0
12/10	48.7	41	3.8	6.5	15	1.1	2.0	6.1	3.1			4.0
12/11	62.3	50	3.4	6.5	16	1.0	2.1	20.	1.0			4.0
x	48.3	45	3.6	6.5	15	1.1	2.0	7.2	2.0	0.2[d]	190[d]	3.9
07/21/86	74.4	40	ND	5.3	ND	ND	1.0	14	ND			
07/22	52.6	18	3.3	5.2	5.4	1.4	1.3	5.0	2.0			
07/23	65.4	21	4.4	6.1	7.4	0.3	1.1	3.0	3.0			
07/24	53.3	21	3.6	6.7	29	0.6	1.2	2.0	2.5			
x	61.4	25	3.8	5.8	13.9	0.8	1.2	6.0	2.5			

[a]Flow in MGD (million gallons/day)

[b]Suspended solids in mg/100 ml

[c]Total chlorine in mg/liter

[d]Questionable value

Table A5. C perfringens and F phage levels in pre- and postchlorinated effluent samples collected November, 1985.

Sewage treatment plant	Date (1985)	Cl_2 resid.[a] (mg/L)	Indicator level/100 ml in effluent			
			Prechlorination		Postchlorination	
			C. perfringens	F phage	C. perfringens	F phage
Blackstone Valley	11/06	2.0	4.215	3.644	3.822	2.663
	11/21	4.1	4.279	4.623	3.690	4.380
Bristol	11/05	2.4	5.279[h]	5.671[h]	4.978[h]	5.079[h]
	11/21	2.4	5.146[h]	5.079[h]	4.875[h]	4.740[h]
East Greenwich	11/05	2.0	4.602	4.602	3.431	3.041
	11/21	1.5	4.295	4.114	3.748	3.813
East Providence	11/06	1.0	3.862	3.079	3.230	2.839
	11/21	1.1	4.964[h]	2.969	3.568	2.903
Fall River, MA	11/05	1.5	4.308	4.398	3.799	3.663
	11/21	1.3	4.000	3.398	3.146[l]	2.634
Jamestown	11/21	2.0	3.663[l]	2.663[l]	3.491	1.398[l]
Newport	11/06	3.0	4.214	5.000	4.176	4.724[h]
	11/21	2.8	4.301	4.748	4.380	4.477
Providence	11/05	3.8	4.663	4.887	4.380	4.681[h]
	11/21	3.7	4.398	4.964	4.079	4.519
Quonset Point	11/21	2.9	4.230	4.322	3.204[l]	2.462
Warren	11/06	1.9	3.391[l]	3.278[l]	2.906[l]	2.301[l]
	11/21	2.0	3.634[l]	2.602[l]	3.532	2.279[l]
x			4.302	4.112	3.802	3.478
SD			0.504	0.932	0.580	1.103

[a]Total chlorine residual

[h]Value greater than mean (x) by more than one standard deviation (SD)

[l]Value less than mean (x) by more than one standard deviation (SD)

Table A6. Indicator levels in surface water samples from Narragansett Bay. December 9, 10, 11, 1985.

Station	Day[a]	Indicator level per 100 ml in surface water					Salinity
		C. perfringens	F phage	Enterococci	FC	E. coli	o/ooo
TnA[b]	1	193	170	56.3	470	320	17.0
	2	310	160	39.6	533	400	17.5
	3	310	160	47.0	240	210	17.0
	GM	265	163	47.1	392	300	17.2
	SD	200-350	160-170	40-56	260-600	220-420	16.9-17.5
1	1	199	210	185	743	693	23.0
	2	105	80	11.0	435	400	21.0
	3	110	80	44.0	330	280	22.0
	GM	132	110	44.7	474	427	22.0
	SD	92-190	63-190	11-180	310-720	270-670	21.0-23.0
B1[c]	1	356	330	182	769	705	24.0
	2	90	140	45.5	250	206	21.2
	3	160	80	41.0	138	130	22.0
	GM	172	155	69.8	298	266	22.4
	SD	86-340	76-320	30-160	120-710	110-640	21.0-23.8
	1	112	180	127	640	615	26.0
	2	185	120	46.0	470	415	25.0
	3	137	80	38.0	130	110	22.5
	GM	142	120	60.6	339	304	24.5
	SD	110-180	80-180	32-120	150-790	120-750	22.7-27.5
B2[c]	1	131	163	70.8	300	292	28.0
	2	135	80	27.5	178	153	28.0
	3	150	60	15.0	53.0	52.8	25.2
	GM	138	92.1	30.8	141	133	27.1
	SD	130-150	55-150	14-67	58-340	56-320	25.5-28.7
3	1	174	270	58.0	355	333	30.0
	2	285	250	17.0	290	260	25.5
	3	280	340	3.0	35	25	24.0
	GM	240	280	14.3	153	129	26.5
	SD	180-320	240-330	3.2-64	42-550	31-540	23.3-29.6
TnB	1	195	210	19.6	43.5	37.0	15.0
	2	855	340	10.0	700	495	6.0
	3	820	140	9.0	110	85.0	10.0
	GM	515	215	12.1	150	116	10.3
	SD	220-1200	140-340	7.9-18	36-610	31-440	5.8-14.8
4	1	271	220	36.4	245	210	30.5
	2	168	440	10.5	153	142	25.5
	3	180	170	5.5	50	42	25.0
	GM	202	254	12.8	123	108	27.0
	SD	160-260	160-420	4.9-33	55-280	47-250	24.0-30.0

5	1	183	330	1.5	25.5	16.5	27.8
	2	160	90.0	2.0	31.3	ND	25.0
	3	110	60.0	6.0	28.0	28.0	21.0
	GM	148	121	2.6	28.2	21.5	24.6
	SD	110-190	50-300	1.3-5.4	25-31	15-31	21.2-28.0
6	1	77.1	200	12.5	101	89.1	27.8
	2	115	150	9.0	52.0	49.0	25.0
	3	110	160	6.5	43.0	38.0	25.0
	GM	99.2	169	9.0	60.9	54.9	25.9
	SD	80-120	150-200	6.5-12	39-95	35-85	24.3-27.6
7	1	104	170	9.9	112	89.1	30.0
	2	97.0	90.0	11.0	105	95.0	25.0
	3	130	40.0	4.0	27.0	23.0	24.9
	GM	109	84.9	7.6	68.2	60.0	26.6
	SD	94-130	41-180	4.3-13	31-150	26-130	23.7-29.5
10	1	19.9	<10	0.4	2.5	2.5	29.0
	2	69.0	60.0	4.0	70.0	48.0	26.9
	3	72.0	70.0	3.0	4.0	3.5	27.0
	GM	46.3	34.8	1.7	8.9	7.5	27.6
	SD	22-96	12-100	0.5-5.9	1.5-54	1.5-38	26.4-28.8
12	1	68.7	50.0	3.0	15.0	13.0	34.0
	2	77.5	80.0	2.5	40.0	33.0	26.5
	3	63.0	<10.0	2.0	11.5	10.0	27.0
	GM	69.5	34.2	2.5	19.0	16.2	29.2
	SD	63-77	11-100	2.0-3.0	9.9-37	8.7-30	25.0-33.4
15	1	28.0	20.0	0.5	1.5	1.0	33.5
	2	17.5	10.0	<0.5	4.0	4.0	27.8
	3	12.0	<10.0	0.5	1.5	1.0	28.2
	GM	18.0	12.6	0.5	2.1	1.6	29.8
	SD	12-28	8.4-19		1.2-3.7	0.7-3.5	26.7-33.0
8	1	60.2	80.0	3.5	33.0	25.5	29.0
	2	93.0	60.0	5.0	75.0	17.0	26.0
	3	77.0	40.0	0.5	22.0	17.0	26.0
	GM	75.5	57.7	2.1	37.9	19.5	27.0
	SD	61-94	41-82	0.6-7.1	20-71	15-25	25.3-28.7
9	1	74.4	100	8.5	26.5	17.5	31.0
	2	51.0	30.0	2.0	11.5	7.5	26.5
	3	100	40.0	1.0	8.0	7.0	26.0
	GM	72.4	49.3	2.6	13.5	9.7	27.8
	SD	52-100	26-90	0.9-7.7	7.3-25	5.8-16	25.1-30.6
11	1	45.0	<10	0.5	5.0	4.5	33.1
	2	63.0	100	1.5	25.0	20.0	27.0
	3	71.0	20.0	1.0	13.0	11.0	27.0
	GM	58.6	=27.1	0.91	11.8	10.0	29.0
	SD	46-74	8.3-88	0.5-1.6	5.2-25	4.7-21	25.5-32.6

13	1	42.0	20.0	0.5	2.5	2.0	33.5
	2	59.0	110	0.5	28.0	25.0	26.0
	3	72.0	40.0	1.5	9.5	8.5	27.0
	GM	56.2	44.5	0.72	8.7	7.5	28.8
	SD	43-74	19-100	0.4-1.4	2.6-29	2.1-27	24.8-32.9
14	1	59.7	70.0	4.0	22.0	17.5	32.0
	2	65.0	50.0	0.5	28.0	18.0	27.0
	3	48.0	20.0	1.0	8.5	7.0	26.7
	GM	57.1	41.2	1.3	17.4	13.0	28.6
	SD	49-67	22-79	0.4-3.6	9.2-33	7.6-22	25.6-31.5
16	1	29.0	50.0	<0.5	13.0	8.5	34.0
	2	25.5	40.0	0.5	5.0	4.5	28.1
	3	30.0	10.0	1.0	6.5	5.5	27.5
	GM	28.1	27.1	0.63	7.3	5.9	29.9
	SD	26-31	11-65	0.4-0.9	4.4-12	4.3-8.2	26.3-33.5

[a]Sample collection times and ambient water temperatures: 12/9 (Day 1) 1100-1415 hrs, 4.0-4.9°C; 12/10 (Day 2) 1040-1415 hrs, 3.7-4.9°C; 12/11 (Day 3) 1100-1435 hrs, 3.0-6.1°C.

[b]Single station transects near the mouths of the rivers. TnA - Seekonk River; TnB - Pawtuxet River.

[c]Stations located near the outfalls (boils) for the sewage treatment plants. B1, Providence STP; B2, East Providence STP.

[d]Approximation because of indeterminate values, usually due to values beyond the sensitivity limit of the assay. In such cases, the sensitivity limit was used in calculating the means.

Table A7. Indicator levels in bottom water samples from Narragansett Bay. December 9, 10, 11, 1985.

Station	Day[a]	Indicator level per 100 ml in bottom water					Salinity
		C. perfringens	F phage	Enterococci	FC	E. coli	o/oo
TnA[b]	1	163[e]	100	37.5	296	189	
	2	740[e]	100	69.3	266	233	21.9
	3	170	44.0	42.0	185	165	21.5
	GM	274	76.1	47.8	244	194	21.7
	SD	120-650	47-120	34-66	190-310	163-230	21.4-22.0
1	1	132	25	6.2	15.0	10.0	
	2	72	<10	1.0	66.6	16.6	25.5
	3	31	50	2.5	12.0	11.0	24.5
	GM	66.5	~23.3	2.5	22.9	12.2	25.0
	SD	32-140	10-52	1.0-6.2	9.0-58	9.3-16	24.3-25.7
B1[c]	1	53.0	7.0	10.4	23.0	20.0	
	2	415[e]	10.0	6.0	23.0	18.0	27.0
	3	43.0	38.0	3.0	1.0	1.0	28.0
	GM	98.1	13.9	5.7	8.1	7.1	27.5
	SD	28-340	5.7-34	3.1-10.7	1.4-49	1.3-39	26.9-28.2
2	1	28.5[e]	37	2.5	6.6	5.0	
	2	180[e]	<10	6.6	65	50	30.2
	3	30.0	7	2.5	6.6	3.3	28.1
	GM	53.6	~13.7	3.5	14.0	9.4	29.2
	SD	19-150	5.7-33	2.0-6.1	3.8-53	2.2-41	28.5-29.8
B2[c]	1	635[e]	<10	6.9	15.5	12.0	
	2	900[e]	30	9.9	79.0	68.0	30.0
	3	180	36	11.0	44.0	38.0	29.2
	GM	469	~22.1	9.1	37.8	31.4	29.6
	SD	200-1100	11-44	7.1-12	17-86	13-76	29.0-30.2
3	1	74.2	6.0	2.3	18.5	16.5	
	2	87.0	<10	1.5	6.0	5.0	28.0
	3	51.0	8.0	5.5	14.0	11.0	29.0
	GM	69.0	~7.8	2.7	11.6	9.7	28.5
	SD	52-91	6.1-10	1.4-5.2	6.4-21	5.3-18	27.8-29.2
TnB[b]	1	383[e]	140	20.4	38.5	30.0	
	2	820[e]	210	9.0	245	185	24.0
	3	820[e]	140	9.0	110	85.0	10.0
	GM	636	160	11.8	101	77.8	17.0
	SD	410-990	130-200	7.4-19	40-260	31-190	7.2-26.9
4	1	36.1	4.0[f]	1.5	8.5	6.5[f]	
	2	31.0	270[f]	3.5	16.5	<3[f]	30.0
	3	38.0	9.0	5.0	18.0	16.0	29.5
	GM	34.9	6.0	3.0	13.6	10.2	29.8
	SD	31-39	3.4-11	1.6-5.5	9.0-21	5.4-19	29.4-30.1

5	1	149	6.0	0.5	4.0	2.5	
	2	115	10.0	0.5	7.0	6.0	28.0
	3	38.0	10.0	1.5	3.5	3.5	28.2
	GM	86.7	8.4	0.72	4.6	3.7	28.1
	SD	42-180	6.3-11	0.4-1.4	3.1-6.7	2.4-5.8	28.0-28.2
6	1	20.0	40	<0.5	0.5	0.5	
	2	25.0	<10[c]	<0.5	3.5	<0.5	26.0
	3	100	40	1.5	6.5	5.5	28.5
	GM	36.8	12.6	~0.72	2.2	1.1	27.3
	SD	15-88	2.5-64	0.4-8.6	0.6-8.6	0.3-4.4	25.5-29.0
7	1	67.9	70	1.9	21.9	16.9	
	2	41.0	<10	1.0	3.5	3.5	27.5
	3	156	20	1.5	13.0	12.0	29.0
	GM	53.8	24.1	1.4	10.0	8.9	28.3
	SD	42-70	9.0-65	1.0-2.0	3.8-26	3.9-20	27.2-29.3
10	1	19.9	<10	0.5	2.5	2.5	
	2	37.5	10	1.0	9.0	7.0	30.0
	3	85.0	8.0	<0.5	2.5	2.5	29.5
	GM	39.9	9.3	0.13	3.8	3.5	29.3
	SD	19-83	8.2-11	0.4-0.9	1.8-8.0	1.9-6.4	29.4-30.1
12	1	42.0	2.0	1.0	3.0	2.5	
	2	33.0	<10	1.0	11.0	9.5	28.5
	3	46.0	8.0	1.5	2.5	1.5	28.5
	GM	39.9	7.1	1.1	4.3	3.3	28.5
	SD	34-47	3.8-13	0.9-1.4	1.9-9.8	1.3-8.5	
15	1	28.0	20.0	0.5	1.5	1.0	
	2	19.0	ND[g]	<0.5	1.5	1.0	29.0
	3	26.0	5.0	0.5	1.5	1.0	29.0
	GM	24.0	10.0	~0.5	1.5	1.0	29.0
	SD	20-29	3.8-27				
8	1	28.0	60	6.5	25.5	22.0	
	2	148.0	120	9.0	35.0	32.5	30.0
	3	64.0	30	1.0	8.5	7.5	28.2
	GM	64.2	60	3.9	19.6	17.5	29.1
	SD	27-150	30-120	1.2-13	9.3-41	8.2-37	27.8-30.4
9	1	47.5	5.0	0.5	0.5	0.5	
	2	141	<10	1.0	7.5	5.5	29.5
	3	74.0	40.0	2.0	7.0	7.0	29.0
	GM	79.1	12.5	1.0	3.0	2.7	29.3
	SD	46-140	4.4-36	0.5-2.0	0.6-14	0.6-12	28.9-29.6
11	1	86.4	2.0	0.5	4.0	3.5	
	2	64.0	ND	2.0	7.5	7.5	29.9
	3	41.0	17.0	0.5	5.0	4.5	29.0
	GM	61.0	5.8	0.80	5.3	4.9	29.5
	SD	42-89	1.3-26	0.4-1.8	3.9-7.3	3.3-7.2	28.9-30.1

13	1	64.0	<2.0	<0.5	3.0	2.0	
	2	20.5	ND	<0.5	9.0	7.5	29.0
	3	87.0	8.0	2.0	5.0	4.0	29.2
	GM	48.5	4.0	0.8	5.1	3.9	29.1
	SD	23-100	1.5-10.7	0.4-1.8	3.0-8.9	2.0-7.6	29.0-29.2
14	1	35.0	20	0.5	2.5	2.0	
	2	51.0	30	5.0	10.0	8.0	29.0
	3	48.0	20	1.0	8.5	7.0	26.7
	GM	44.1	22.9	1.4	6.0	4.8	27.9
	SD	36-54	19-28	1.1-1.7	2.8-12.7	2.2-10	26.2-29.5
16	1	29.0	<2.0	1.0	1.5	1.0	
	2	39.6	ND	1.5	5.0	5.0	28.7
	3	76.0	3.0	1.0	4.0	3.0	29.0
	GM	44.4	2.4	1.1	3.1	2.5	28.9
	SD	27-73	1.8-3.3	0.9-1.4	2.5-3.9	1.1-5.6	28.6-29.1

[a]Sample collection times and ambient water temperatures: 12/9 (Day 1) 1100-1415 hrs, 4.0-4.9°C; 12/10 (Day 2) 1040-1415 hrs, 3.7-4.9°C; 12/11 (Day 3) 1100-1435 hrs, 3.0-6.1°C.

[b]Single station transects near the mouths of the rivers. TnA - Seekonk River; TnB - Pawtuxet River.

[c]Stations located near the outfalls (boils) for the sewage treatment plants. B1, Providence STP; B2, East Providence STP.

[d]Approximation because of indeterminate values, usually due to values beyond the sensitivity limit of the assay. In such cases, the sensitivity limit was used in calculating the means.

[e]Suspect values probably due to sediment in bottom water samples. Values included in calculation of means.

[f]Data eliminated from calculation of means as being spurious.

Table A8. Indicator levels in surface water samples from Narragansett Bay. July 22, 23, 24, 1986.

Station	Day[a]	Indicator level per 100 ml in surface water					Salinity (o/oo)
		C. perfringens	F phage	Enterococci	FC	E. coli	
TnA[c]	1	18.1	37	10.0	~200	143	26.2
	2	46.0	88	2.0	85	30	22.0
	3	21.4	31	6.0	360	65	22.4
	GM(1-3)[e]	26.1	46.6	4.9	183	65.3	22.2
	SD	16-43	27-81	2.1-11	89-380	30-140	21.9-22.5
TnC[c]	1	77.5	52	15.0	400	245	25.5
	2	56.0	64	14.0	45	45	25.8
	3	25.0	41	19.5	500	285	24.1
	GM(1-3)	47.7	51.5	16.0	208	146	25.0
	SD	27-85	41-64	13-19	55-790	53-410	23.7-26.2
1	1	38.0	<10	1.6	582	377	21.7
	2	16.5	4.0	3.3	145	90.0	24.1
	3	9.9	27	7.0	200	150	22.7
	GM(2-3)M	12.8	~10.4	4.8	170	116	23.3
	SD	8.9-18	2.7-40	2.8-8.2	136-210	81-170	22.0-24.5
B1[d]	1	60.0	2620	10.0	2.5	2.5	19.7
	2	40.0	2670	<0.5	<0.5	<0.5	20.3
	3	15.0	158	0.5	<0.5	<0.5	23.8
	GM(1-2)	49.0	2640	~2.2	<1.1	<1.1	20.0
	SD	37-65	2600-2700	0.3-1.9	0.4-3.5	0.4-3.5	19.6-20.4
2	1	130	14	3.3	566	379	22.5
	2	20	64	1.0	40	30	24.8
	3	3.3	6	3.0	30	25	25.4
	GM(2-3)	8.1	19.6	1.7	34.6	27.3	25.1
	SD	2.3-29	3.7-100	0.8-3.8	28-42	24-31	24.7-25.5
B2[d]	1	5.0	17	1.6	294	99	24.6
	2	39.6	33	1.0	35.0	10.0	25.4
	3	36.3	38	3.3	6.6	6.6	26.7
	GM(2-3)	37.9	35.4	1.8	15.2	18.1	26.1
	SD	36-40	32.39	0.8-4.2	4.7-49	6.1-11	25.1-27.0
2A	1	31.0	19	3.3	94	48	24.5
	2	21.4	12	0.5	45	45	24.8
	3	34.6	<10	0.5	13.2	13.2	24.7
	GM(1-3)	28.4	~15.1	0.93	38.2	30.6	24.7
	SD	22-37	11-21	0.3-2.8	14-100	15-63	24.5-24.8
3	1	30.0	23.0	1.7	158.0	99	24.5
	2	8.2	5.0	<0.5	10.0	10.0	26.0
	3	5.0	<10	2.5	10.0	5.0	25.6
	GM(2-3)	6.4	~5.0	~1.1	10.0	7.1	25.8
	SD	4.5-9.1	ind	0.4-3.5	ind	4.3-12	25.5-26.1

TnB[C]	1	82.5	2260	66.0	400	375	23.4
	2	43.0	150	5.0	160	130	24.3
	3	20.0	260	11.5	65	30	23.0
	GM(1-3)	41.4	445	15.6	161	114	23.7
	SD	20-84	110-1900	4.2-58	65-400	32-400	22.7-24.6
4*	1	51.0	<10	5.0	42.0	23.5	24.2
	2	28.0	<10	1.0	59.4	25.0	25.8
	3	13.2	17	2.0	5.0	5.0	24.5
	GM(1-3)	26.6	~17	2.2	23.2	14.3	25.2
	SD	14-52		1.0-4.8	6.1-89	5.8-36	24.2-26.1
4A	1	51.0	23	18.5	47.5	30.5	24.0
	2	18.1	4	<0.5	16.5	5.0	26.4
	3	13.2	<10	2.5	3.3	3.3	26.2
	GM(2-3)	15.5	~4.0	~1.1	7.4	4.1	26.3
	SD	12-9		0.4-3.5	2.3-23	3.0-5.5	26.2-26.4
5	1	24.7	114	2.0	ND	ND	24.2
	2	5.0	<10	<0.5	1.7	1.7	27.1
	3	1.7	<10	6.5	1.7	1.7	26.1
	GM(2-3)	2.9	<10	1.8	1.7	1.7	26.6
	SD	1.4-6.3		0.3-11	ind	ind	25.9-27.3
6	1	6.6	5	34.0	38.5	31.5	25.0
	2	5.0	<10	0.5	10.0	6.6	28.2
	3	6.6	<10	5.0	<3.3	<3.3	27.7
	GM(2-3)	5.7	<10	1.6	~5.7	4.7	28.0
	SD	4.7-7.0		0.3-8.1	2.6-13	2.9-7.6	27.6-28.3
7	1	8.2	<10	58.0	16.0	15.5	26.3
	2	3.3	<10	<0.5	3.3	3.3	28.4
	3	1.7	8	<0.5	3.3	1.6	27.5
	GM(2-3)	2.4	~8	<0.5	3.3	2.3	28.0
	SD	1.5-3.8	ind	ind	ind	1.4-3.8	27.3-28.6
7A	1	14.8	12	1.5	14.5	13.0	26.6
	2	3.3	3	<0.5	<3.3	<3.3	28.2
	3	1.7	<10	1.0	<3.3	<3.3	28.5
	GM(2-3)	2.4	3	~0.71	<3.3	<3.3	28.4
	SD	1.5-3.8	ind	0.4-1.6	ind	ind	28.1-28.6
10	1	9.9	12	3.0	1.5	1.5	27.3
	2	1.7	<10	<0.5	0.5	<0.5	28.0
	3	1.7	<10	2.5	<0.5	<0.5	28.0
	GM(2-3)	1.7	<10	~1.1	<0.5	<0.5	28.0
	SD			0.4-3.5			
12	1	10.0	6	7.0	0.5	0.5	26.8
	2	4.0	<10	<0.5	<0.5	<0.5	28.3
	3	<0.5	<10	1.5	<0.5	<0.5	28.0
	GM(2-3)	1.4	<10	~0.87	<0.5	<0.5	28.2
	SD	0.3-6.2	1	0.4-1.9	ind	ind	27.9-28.4

TnD[c]	1	1.6	<10	18.1	12.5	8.5	28.8
	2	7.5	<10	0.5	144	138	28.3
	3	2.5	<10	2.5	1.7	1.7	28.2
	GM(1-3)	4.3	<10	1.1	1.7	1.7	28.3
	SD	2.0-9.4		0.4-3.5			28.2-28.3
18	1	1.0	<10	5.5	0.5	0.5	28.8
	2	3.0	2	<0.5	<0.5	<0.5	28.9
	3	1.0	<10	1.0	0.5	0.5	28.7
	GM(1-3)	1.7	~2.0	1.4	~0.5	~0.5	28.8
	SD	0.9-3.8	ind	0.4-4.8	ind	ind	28.7-28.9
18A	1	1.0	6	8.5	0.5	0.5	28.6
	2	2.5	3	<0.5	0.5	0.5	29.0
	3	1.7	<10	0.5	<0.5	<0.5	28.3
	GM(1-3)	2.1	<4.2	~1.3	0.5	~0.5	28.7
	SD	1.6-2.7	2.5-6.9	0.3-6.6	ind	ind	28.2-29.1
15	1	2.0	<2	3.0	1.5	1.5	29.0
	2	1.5	<10	<0.5	1.0	0.5	28.8
	3	<0.5	<10	<0.5	0.5	0.5	29.3
	GM(1-3)	~0.87	<2	<0.9	~0.71	0.5	29.1
	SD	0.4-1.9		0.3-2.6	0.4-1.6	ind	28.7-29.4
19	1	4.0	4.0	3.5	28.5	28.0	28.1
	2	1.0	<10	<0.5	<0.5	<0.5	28.8
	3	<0.5	<10	<0.5	1.0	1.0	28.6
	GM(2-3)	<0.71	~4	<0.5	0.71	~0.71	28.7
	SD	0.4-1.6		ind	0.4-1.6	0.4-1.6	28.6-28.8
20	1	6.5	<2	6.0	1.5	1.5	28.1
	2	5.0	<10	<0.5	1.5	0.5	28.2
	3	1.0	<10	1.0	<0.5	<0.5	27.8
	GM(1-3)	3.2	<2	~1.4	1.4	0.72	28.0
	SD	1.2-8.8		0.4-15.2	0.6-2.0	0.4-1.4	27.7-28.3
21	1	15.0	<10	3.5	1.5	1.5	29.5
	2	2.5	<10	0.5	2.0	2.0	28.0
	3	<0.5	<10	2.0	<0.5	<0.5	28.2
	GM(2-3)	~1.1	<10	1.0	~1.0	~1.0	28.1
	SD	0.4-3.5		0.4-2.7	0.4-2.7	04-2.7	27.8-28.4

[a]Sample collection times and ambient water temperatures: 7/22 (day 1) - 1030-1500 h, 21.2-23.2°C; 7/23 (day 2) - 1015-1430 h, 22.4-24.6°C; 7/24 (day 3) - 0945-1345 h, 21.8-23.5°C.

[b]Arithmatic mean and standard deviation for days 2 and 3. If the salinity for day 1 was outside the SD for days 2-3, the GMs for the indicator levels were calculated for days 2-3 as shown in parentheses under Day. Exception was station B1 where the mean salinity was for days 1-2.

[c]Single station transects near mouths of rivers. TnA - Seekonk R.; TnB - Pawtuxet R.; TnC - Moshassuck R.; TnD - Warren R.

[d] See "c" Table 11.

[e] ( ) days used to calculate geometric means (GM) and standard deviations (SD).

[f] See "d" Table 11.

Table A9. Indicator levels in bottom water samples from Narragansett Bay. July 23, 24, 25, 1986.

Station	Day[a]	Indicator level per 100 ml in bottom water					Salinity
		C. perfringens	F phage	Enterococci	FC	*E. coli*	o/ooo
TnA	1	15.0	<10	8.0	150	121	27.0
	2	235[e]	<10	2.0	75.0	55.0	28.2
	3	10.0	<10	2.5	140	60	25.0
	GM	32.8	<10	3.4	116	73.6	26.7
	SD	5.9-180		1.6-7.2	79-170	48-110	25.1-28.3
TnC	1	9.9	13	3.3	200	40.0	27.9
	2	31.3[e]	11	4.0	175	80.0	28.8
	3	5.0	<10	6.0	90.0	25.0	28.9
	GM	11.6	~11.9	4.3	147	43.1	28.5
	SD	4.6-29		3.2-5.9	96-230	24-77	28.0-29.1
1	1	0.5	<10	7.0	300	200	29.1
	2	<0.5	<10	3.5	43.0	23.0	28.1
	3	<0.5	<10	<0.5	56.0	46.0	24.6
	GM	<0.5	<10	~2.3	89.7	59.6	27.3
	SD			0.6-9.1	31-260	20-180	24.9-29.6
B1	1	135	360	46.0	700	550	28.5
	2	13.2	150	19.5	500	250	28.6
	3	5.0	55.0	4.0	25.0	20.0	28.2
	GM	20.7	144	15.3	206	140	28.4
	SD	3.8-110	56-370	4.4-53	33-1300	25-790	28.2-28.6
2	1	20.0	<10	4.0	55.0	48.0	17.0
	2	20.0	9	1.5	17.5	13.5	23.0
	3	19.5	<10	1.5	11.5	3.3	29.1
	GM	19.8	~9	2.1	22.3	12.9	23.0
	SD	20-20		1.2-3.7	10-50	3.4-49	17.0-29.0
B2	1	34.5	<10	2.0	32.5	22.5	28.3
	2	229[e]	4	1.0	6.6	5.0	26.2
	3	90.7	<10	2.0	15.0	11.5	26.0
	GM	89.5	< 4	1.6	14.8	10.9	26.8
	SD	35-230		1.1-2.4	6.7-33	5.1-23	25.6-28.1
2A	1	23.0	14	1.5	15.5	8.0	29.3
	2	13.5	<10	0.5	6.6	6.6	29.3
	3	7.5	<10	1.0	1.7	1.7	29.3
	GM	13.3	~14	0.9	5.6	4.5	29.3
	SD	7.6-23		0.9-0.9	5.6-5.6	1.9-10	29.3-29.3
3	1	54.0	<10	2.5	137	86.5	29.4
	2	30.5	<10	1.0	1.6	1.6	29.1
	3	6.0	<10	0.5	6.6	3.3	28.8
	GM	21.5	<10	1.0	11.3	7.7	29.1
	SD	6.9-67		0.5-2.4	1.2-110	0.9-65	28.8-29.4

TnB	1	445	53.0	16.5	200	145	27.4
	2	125	<10	28.0	400	395	24.2
	3	365	<10	6.5	15	5.0	26.1
	GM	273	<53	14.4	106	65.9	25.9
	SD	170-440		6.9-30	19-600	6.7-650	24.3-27.5
4	1	14.5	7.0	0.5	17.5	9.0	29.5
	2	16.0	4.0	<0.5	8.0	6.5	29.5
	3	9.5	<10	2.0	1.0	0.5	28.9
	GM	13.0	~5.3	0.8	5.2	3.1	29.3
	SD	9.9-17	3.6-7.9	0.4-1.8	1.2-23	0.6-15	29.0-29.6
4A	1	21.0	<10	2.0	14.5	8.5	29.2
	2	15.5	<10	1.0	5.5	5.0	29.4
	3	7.5	<10	0.5	3.0	1.0	27.2
	GM	13.5	<10	1.0	6.2	3.5	28.6
	SD	7.9-23		0.5-2.0	2.8-13.7	1.1-10.6	27.4-29.8
5	1	15.5	17	4.5	ND	ND	29.6
	2	8.0	<2	1.0	5.0	1.7	29.3
	3	9.0	<10	3.0	<3.3	<3.3	26.6
	GM	10.4	~5.8	2.4	~4.1	~2.4	28.5
	SD	7.3-14.8		1.7-3.4	3.0-5.4	1.5-3.8	26.8-30.2
6	1	12.0	<10	1.5	9.0	7.0	29.4
	2	8.5	<10	0.5	<0.5	<0.5	29.8
	3	4.0	<10	0.5	1.0	1.0	29.3
	GM	7.4	<10	0.7	~1.7	~1.5	29.5
	SD	4.2-13		0.4-1.4	0.4-7.5	0.3-6.9	29.2-29.8
7	1	2.5	<10	1.5	9.5	8.5	29.7
	2	3.5	4	1.0	2.5	2.5	29.8
	3	4.0	<10	0.5	<0.5	<0.5	28.0
	GM	3.3	~4	0.9	~2.3	~2.2	29.2
	SD	0.7-15		0.5-1.6	0.5-10	0.5-9.1	28.2-30.2
7A	1	13.5	8.0	10.5	16.0	16.0	29.8
	2	10.0	<10	<0.5	<0.5	<0.5	28.7
	3	5.5	<10	1.5	0.5	<0.5	28.2
	GM	9.1	~8	2.0	~1.6	<1.6	28.9
	SD	2.2-37.5		0.5-8.2	0.4-6.6	0.4-6.6	28.1-29.7
10	1	4.0	4.0	21.0	4.0	4.0	30.2
	2	1.0	2.0	0.5	<0.5	<0.5	29.3
	3	3.5	<10	1.5	<0.5	<0.5	28.2
	GM	2.4	~2.8	2.5	<1.0	<1.0	29.2
	SD	1.1-5.2	1.7-4.6	0.4-17.1	0.1-6.8	0.1-6.8	28.2-30.2
12	1	1.5	<10	21.0	2.5	2.5	30.2
	2	5.0	<10	1.0	1.0	<0.5	30.0
	3	2.0	<10	2.0	<0.5	<0.5	30.1
	GM	2.5	<10	3.5	~1.0	<0.9	30.1
	SD	1.3-4.6		0.7-17	1.0-1.0	0.9-0.9	30.0-30.2

TnD	1	38.0	3	13.2	0.5	0.5	28.4
	2	11.0	<10	1.5	16.0	0.5	22.8
	3	2.5	<10	1.5	<0.5	<0.5	25.2
	GM	10.1	~3	3.1	~1.6	~0.5	25.5
	SD	10-10		0.9-11	1.6-1.6	0.5-0.5	22.7-28.3
18	1	6.5	4	1.0	<0.5	<0.5	27.5
	2	5.0	<10	0.5	1.0	1.0	29.7
	3	2.5	<10	0.5	<0.5	<0.5	30.3
	GM	4.3	~4	0.6	<0.6	<0.6	29.2
	SD	4.3-4.3		0.6-0.6	0.6-0.6	0.6-0.6	27.7-30.6
18A	1	2.5	<10	3.5	<0.5	<0.5	30.0
	2	6.0	<10	<0.5	0.5	0.5	30.3
	3	5.0	<10	0.5	1.0	0.5	30.4
	GM	4.2	<10	~0.96	~0.6	~0.5	30.2
	SD	2.7-6.7		1.0-1.0	0.6-0.6		30.0-30.4
15	1	1.0	<2	1.0	0.5	0.5	26.5
	2	2.5	<10	1.0	0.5	0.5	29.9
	3	2.0	<10	1.0	2.0	<0.5	26.6
	GM	1.7	<2	1.0	0.8	~0.5	27.7
	SD	1.7-1.7		1.0-1.0	0.8-0.8		25.7-29.6
19	1	4.0	<2	1.5	2.5	2.5	28.7
	2	3.5	<10	<0.5	1.0	1.0	29.3
	3	0.5	<10	2.5	<0.5	<0.5	29.8
	GM	1.9	<2	~1.2	~1.1	~1.1	29.3
	SD	1.9-1.9		1.2-1.2	0.5-2.4	0.5-2.4	28.7-29.8
20	1	2.0	<10	1.5	1.5	1.5	29.3
	2	3.0	<10	<0.5	0.5	0.5	29.5
	3	2.0	<10	3.0	0.5	0.5	28.0
	GM	2.3	<10	~1.3	0.7	0.7	28.9
	SD	2.3-2.3		0.5-3.2	0.7-0.7	0.7-0.7	28.1-29.7
21	1	35.5	3	2.0	0.5	0.5	29.0
	2	7.5	<10	0.5	0.5	0.5	29.5
	3	1.5	<10	<0.5	<0.5	<0.5	29.7
	GM	7.4	~3	~0.8	~0.5	~0.5	29.4
	SD	1.5-36		0.8-0.8			29.0-29.8

[a]See Table 16 for sampling times. Ambient water temperatures and differential (surface-bottom): Day 1 - 17.5-21.2°C, 2.6±0.9°C; Day 2 - 18.9-22.7°C, 3.2±0.9°C; Day 3 - 19.0-22.1°C, 2.6±0.9°C.

[b]Arithmatic mean and standard deviation for the three days.

[c,d]See Table 16.

[e]Suspected spurious values due to sediment in bottom water samples; values included in calculating means.

Table A10. Indicator levels in surface water samples from Narragansett Bay. July 28, 29, 30, 1987[a].

Station	Day[a]	Indicator level per 100 ml in surface water				
		C. perfringens	F phage	Enterococci	FC	E. coli
TnA[b]	1	47.9	3.0	4.5	71.0	54.4
	2	53.0	1.0	5.5	97.4	56.1
	3	38.0	3.5	4.5	205	60.0
	GM	45.9	2.2	4.8	112	56.8
	SD	39-54	1.1-4.3	4.3-5.4	65-193	54-60
TnC[b]	1	41.3	35.0	11.1	>1390	>1360
	2	28.0	5.5	2.7	610	325
	3	33.0	10.5	3.3	1860	616
	GM	33.7	12.6	4.6	1160	648
	SD	28-41	4.9-32	2.2-9.9	650-2100	310-1300
1A	1	66.0	1.0	2.5	90.8	64.4
	2	24.5	6.0[e]	9.2	135	102
	3	ND	ND[e]	ND	ND	ND
	GM	40.2	2.4	4.8	111	81.0
	SD	20-81	0.7-8.7	1.9-12	84-150	59-110
1	1	ND	ND	ND	ND	ND
	2	57.5	<0.5	<0.5	18.2	3.3
	3	41.5	10.0	<0.5	140	67.7
	GM	48.8	<2.2	<0.5	50.4	14.9
	SD	39-62			12-210	1.7-130
B1[d]	1	>500	2640	3.1	26.4	13.2
	2	580	2840	3.5	54.5	52.8
	3	360	2150	4.5	15.0	14.0
	GM	470	2520	3.7	27.8	21.4
	SD	370-600	2200-2900	3.0-4.4	15-53	9.8-47
1C	1	277	94.5	<0.5	75.9	56.1
	2	47.9	1.0[o]	<0.5	71.0	31.4
	3	75.7	64.5	<0.5	0.5[o]	0.5[o]
	GM	100	78.1	<0.5	73.4	41.9
	SD	40-250	60-100		70-77	28-63
2	1	61.0	1.0	<0.5	23.1	8.3
	2	48.0	1.0	<0.5	39.6	28.0
	3	40.5	8.0	<0.5	3.0[o]	2.5[o]
	GM	49.1	2.0	<0.5	30.2	15.2
	SD	40-60	0.6-6.6		21-44	6.4-36
B2[d]	1	360	130	2.0	45.0	25.5
	2	400	251	<0.5	51.5	27.0
	3	410	158	1.5[f]	42.9	16.5
	GM	389	172	1.1[f]	46.3	22.4
	SD	360-420	120-240	0.6-2.4	42-51	17-29

2A	1	64.3	8.0	<0.5	5.0	3.3
	2	79.0	6.0	<0.5	2.5	0.5
	3	26.0	<0.5	<0.5	4.0	3.5
	GM	50.9	~2.9	<0.5	3.7	1.8
	SD	28-92	0.6-13		2.6-5.2	0.6-5.4
3	1	59.4	7.5	<0.5	8.3	3.3
	2	45.5	3.5	<0.5	6.0	3.0
	3	33.5	1.0	<0.5	<0.5	<0.5
	GM	44.9	3.0	<0.5	~2.9	~1.7
	SD	34-60	1.1-8.2		0.6-14	0.6-4.9
TnB[b]	1	140	60.0	1.9	180	120
	2	54.5	177	8.5	142	142
	3	57.8	ND	12.0	139	129
	GM	76.1	103	5.7	153	130
	SD	45-130	48-220	2.1-16	130-180	120-141
4	1	73.0	7.5	<0.5	13.5	8.5
	2	48.0	3.0	<0.5	14.0	7.5
	3	18.5	1.0	<0.5	<0.5	<0.5
	GM	40.2	2.8	<0.5	~4.6	~3.2
	SD	20-81	1.0-7.7		0.7-31	0.6-16
4A	1	74.0	4.0	<0.5	6.5	3.5
	2	61.0	2.5	<0.5	4.5	2.5
	3	18.0	<0.5	<0.5	<0.5	<0.5
	GM	43.3	~1.7	<0.5	2.4	~1.6
	SD	20-93	0.6-5.1		0.6-9.8	0.6-4.6
5	1	27.0	1.0	0.5	2.0	1.0
	2	47.0	1.5	1.0	3.5	3.0
	3	26.0	<0.5	<0.5	0.5	<0.5
	GM	32.1	~0.91	~0.63	1.6	~1.1
	SD	23-45	0.5-1.6	0.4-0.9	0.6-4.6	0.5-2.8
6	1	10.0	<0.5	<0.5	<0.5	<0.5
	2	28.5	<0.5	<0.5	1.0	0.5
	3	11.5	<0.5	<0.5	<0.5	<0.5
	GM	14.9	<0.5	<0.5	~0.63[f]	<0.5
	SD	8.4-26			0.4-0.9	
7	1	28.0	1.0	0.5	0.5	<0.5
	2	33.5	1.0	<0.5	<0.5	<0.5
	3	15.5	0.5	<0.5	<0.5	<0.5
	GM	24.4	0.79	<0.5	<0.5	<0.5
	SD	16-37	0.5-1.2			
7A	1	6.0	<0.5	<0.5	<0.5	<0.5
	2	12.0	<0.5	<0.5	<0.5	<0.5
	3	4.5	ND	<0.5	<0.5	<0.5
	GM	6.9	<0.5	<0.5	<0.5	<0.5
	SD	4.1-11				

10	1	7.5	<0.5	<0.5	<0.5	<0.5
	2	5.5	0.5	<0.5	<0.5	<0.5
	3	6.0	<0.5	<0.5	<0.5	<0.5
	GM	6.3	<0.5	<0.5	<0.5	<0.5
	SD	5.4-7.4				
12	1	6.5	<0.5	<0.5	<0.5	<0.5
	2	8.0	<0.5	<0.5	<0.5	<0.5
	3	7.0	<0.5	<0.5	0.5	<0.5
	GM	7.1	<0.5	<0.5	<0.5	<0.5
	SD	6.4-7.9				
TnD[C]	1	8.0	<0.5	<0.5	0.5	0.5
	2	10.5	<0.5	0.5	<0.5	<0.5
	3	3.5	<0.5	1.5	0.5	0.5
	GM	6.6	<0.5	0.72	<0.5	<0.5
	SD	3.8-12		0.4 - 1.4		
18	1	3.5	<0.5	<0.5	<0.5	0.5
	2	2.5	<0.5	<0.5	<0.5	<0.5
	3	11.0	<0.5	<0.5	<0.5	<0.5
	GM	4.6	<0.5	<0.5	<0.5	<0.5
	SD	2.1-10				
18a	1	4.5	<0.5	<0.5	<0.5	<0.5
	2	5.0	<0.5	<0.5	<0.5	<0.5
	3	7.0	<0.5	<0.5	<0.5	<0.5
	GM	5.4	<0.5	<0.5	<0.5	<0.5
	SD	4.3-6.8				
15	1	6.0	0.5	0.5	<0.5	<0.5
	2	5.0	0.5	<0.5	<0.5	<0.5
	3	9.0	<0.5	<0.5	0.5	0.5
	GM	6.5	0.5	<0.5	<0.5	<0.5
	SD	4.8-8.7				
19	1	3.5	<0.5	<0.5	<1.0	<1.0
	2	4.0	<0.5	<0.5	<0.5	<0.5
	3	11.5	<0.5	<0.5	<0.5	<0.5
	GM	5.4	<0.5	<0.5	<0.5	<0.5
	SD	2.8-10				
20	1	2.0	<0.5	0.5	<1.7	<1.7
	2	8.5	<0.5	<0.5	<0.5	<0.5
	3	10.0	<0.5	<0.5	<0.5	<0.5
	GM	5.5	<0.5	<0.5	<0.5	<0.5
	SD	2.3-13				
21	1	6.5	<0.5	<0.5	<0.5	<0.5
	2	7.0	<0.5	<0.5	<0.5	<0.5
	3	5.5	ND	<0.5	<0.5	<0.5
	GM	6.3	<0.5	<0.5	<0.5	<0.5
	SD	5.6-7.1				

[a]Sample collection: 7/28, 1030-1430 h; 7/29, 1130-1400 h 7/30, 1200-1500 h. Ambient water temperature.

[b]Transects: A - Seekonk R; B - Pawtuxet R; C - Moshassuck R.; D - Warren R.

[d]"Boils": B1 - Providence (Field's Point) Sewage Treatment Plant; B2 East Providence Sewage Treatment Plant

[e]ND - no data

[f]Approximation because less than half the value were less than the sensitivity of the assay or exceeded the upper counting limit of the method.

[o]Value omitted in calculating mean

APPENDIX B

ASSAY METHODS FOR F MALE-SPECIFIC BACTERIOPHAGES

Media

1. Tryptone Broth (per liter)

Bacto Tryptone	10.0 g
Dextrose	1.0 g
NaCl	5.0 g
Distilled water	1000 ml

Dissolve the ingredients and dispense in 100 ml quantities to 250 ml Erlenmeyer flasks and in 10 ml quantities to tubes as required. Autoclave at 121°C for 15 min and store in the refrigerator.

2. Antibiotic solution

Ampicillin	150 mg
Streptomycin sulfate	150 mg
Distilled water	100 ml

Filter sterilize the solution and store in the refrigerator. Prepare fresh every week.

3. Bottom Agar (Tryptone Agar)

Add 15.0 gm of agar to the ingredients in Tryptone broth (for 1 liter of medium). Autoclave at 121°C for 15 min. Cool to 50°C; add 20 ml of the antibiotic solution, mix, and dispense in about 38 ml quantities to sterile 150 x 15 mm petri plates.

4. Double strength (2X) Tryptone Soft Agar

Tryptone	20.0 g
Dextrose	2.0 g
NaCl	1.0 g
Agar	14.0 g
Distilled water	1000 ml

Heat to dissolve the ingredients. Add 1.0 of a 1 M $CaCl_2$ solution and dispense in 4 ml quantities to 16 x 150 mm tubes. Cap the tubes and store them in the freezer.

5. Single strength (1X) Tryptone Soft Agar

Reduce all the ingredients in the 2X Tryptone Agar by a factor of two.

Stock Cultures of Host Strain - Maintain the strain on slants of the bottom agar held in the refrigerator.

Concentration of Water Samples

C-1. During the late afternoon of the day before the assays are to be performed, aseptically add the antibiotic solution in 0.3 ml quantities to the number of tubes (10 ml) and in 3.0 ml quantities to the number of flasks (100 ml) of Tryptone broth needed. Inoculate them from the stock of the host strain, and incubate the cultures overnight at 37°C.

C-2. The following morning, inoculate flasks containing 100 ml amounts of Tryptone Broth (no antibiotics) with 10 ml quantities of the overnight culture of the host strain. Incubate the flasks for 3.5 ± 0.5 hours at 37°C to obtain log phase cells.

C-3. For each sample to be concentrated, add 1 g of Tryptone and 1 g of powdered beef extract to a sterile, capped, 250 ml centrifuge bottle.

C-4. For each sample add 100-200 ml of the water and 10 ml of log phase culture to one of the centrifuge bottles. Incubate the mixture in a water bath for 30 min at 30.5 ± 0.5 C with very gentle shaking.

C-5. Centrifuge the bottles at 9000 x g for 10 min in a refrigerated centrifuge at 4°C.

C-6. Decant the supernatant, leaving 7-8 ml behind in which to resuspend the pellet. Place bottle in the 50°C water bath for about one min and proceed to assay.

Assay of Concentrate

A-1. Remove the needed number of plates containing the bottom agar from the refrigerator the day before they are to be used. Remove an equal number of tubes of 2X soft agar from the freezer the morning of the day they are to be used.

A-2. Autoclave the tubes of soft agar at 121°C for 15 min. Place them in a water bath at 50°C.

A-3. Remove 4 ml of the resuspended pellet (step C-6) to a tube of 2X soft Tryptone Agar. Gently mix the contents of the tube by rolling it in the palms of your hands. Do this rapidly so that the agar will not harden, and avoid bubbles. Immediately pour the contents over a hard agar plate. Gently swirl the plate to distribute the soft agar over the surface of the hard agar. Similarly prepare a second plate from the remainder of the resuspended pellet. Allow the agar to harden; invert and incubate the plates at 37°C for at least 9 hours and no more than 18 hours.

A-4. Count the plaques and record the data.

Direct Assay of Water Samples or Sewage Effluents (no concentration)

a) Direct assay of duplicate 4 ml samples can be done with water samples collected near the sewage outfalls.

DA-1. Same as A-1

DA-2. Same as A-2

DA-3. Add 4 ml of the warmed (equilibrated to room temperature or 1 min at 50°C) water sample and 1 ml of the 3 hour culture of the host strain to a tube of 2X soft agar. Mix and pour the contents of the tube over a hard agar plate. Assay a second 4 ml portion of the sample in the same manner. Incubate as in A-3.

DA-4. Same as A-4

b) Direct assay of 1 ml (duplicate 0.5 ml quantities) or decimal dilutions thereof usually is required only with sewage effluents since samples with phage levels up to 1.0×10^4/100 ml can be assayed by direct plating of duplicate 4 ml portions.

DDA-1. 100 x 15mm plates containing about 17 ml of bottom agar are prepared as described in the Media Section.

DDA-2. 13 x 100 mm tubes containing 4.0 ml of single strength soft agar are prepared as described in the Media Section.

DDA-3. Same as A-2

DDA-4. Add 0.5 ml of the sample and 0.02 ml of the 3 hour culture of the host strain to a tube of single strength soft agar. Mix and pour the contents of the tube over a 100 x 15 mm bottom agar plate. Assay a second 0.5 ml portion. Incubate as in A-3.

DDA-5. Same as A-4

sampled during any given tour, and 5-7 such tours should be conducted during the six month period. The sampling frequencies are given in Table A-1.

C. Microbial Assays

1. Water and effluents: C. perfringens (mCP); enterococci (mE as modified by use of indoxyl β D glucoside); E. coli and fecal coliforms (mTEC); F male-specific phages (Cabelli method).
2. Sediments: Assays for C. perfringens by the "sonicate and settle" extraction method followed by membrane filtration by mCP. If the metaphosphate extraction method is used, MF assays can be done for enterococci (mE) and E. coli/fecal coliforms (mTEC) as well.
3. Shellfish: Clean, shuck and homogenize 9-12 animals as described in "Standard Methods for the Examination of Shellfish and Shellfish-Growing Waters." Then perform 5 tube, 3 dilution (1.0, 0.1 and 0.01 ml) MPN assays by the appropriate methods for C. perfringens, enterococci and fecal coliforms/E. coli. The MPNs/100 ml can be converted to MPN/100 g by weighing a given volume of the homogenate and using the values obtained for the conversion.
   a. E. coli/fecal coliforms: Inoculate tubes of LST broth as described in "Standard Methods." Then transfer to EC as they specify only use EC MUG (Difco) instead of EC. Read the EC tubes for fecal coliforms as they specify. Then place the tubes under "black light", long wave UV, and record fluorescence. Those that fluoresce contain E. coli. MUG is methyl umbelliferyl glucuronide.
   b. Enterococci: Inoculate tubes of Azide Dextrose Broth as given in "Standard Methods for the Examination of Water and Wastewater." After incubation, streak from the turbid tubes on the surface of a membrane filter placed on a MF plate of modified mE medium. Use half or a third of a plate per tube. Record blue growth on the membranes after incubation at 41°C for 24-36 hours as positive.
   c. C. perfringens: Inoculate tubes of Iron-Milk medium, incubate and read as described by Matches et al.
   d. F male-specific bacteriophages: Weigh 100 g of the homogenate into a sterile, screw-cap centrifuge tube, and centrifuge at 13000 x g for 15 min. Decant the supernatant to a sterile tube and record the volume. Let the fluid warm to room temperature. Add 5 ml

to 5 ml of 2 x soft agar; add 1 ml of the host cells and pour over the large plates. Incubate and count as given in the appended method for the phage assay.

MICROBIOLOGY SEMINAR SCHEDULE
(12:00 noon - Morrill 215)

Jan. 22 Mannitol Utilization in Clostridium difficile. Mike Kazamias, Department of Microbiology, URI.

Jan. 29 Characterization of the Ciliary Proteins of Wild-type Tetrahymena thermophila and a Mating Mutant. Lee Ju Cheng, Department of Microbiology, URI.

Feb. 5 No seminar

Feb. 12 To be announced.

Feb. 26 A Mouse Model for E. coli 0157:H7 Intestinal Pathogenesis. Dr. Elizabeth Wadolkowski, Dept. of Microbiology, Uniformed Services University Medical School.

Feb. 28 Transformation of Corn Using Microprojectile Bombardment. Dr. William Gordon-Kahn, Pfizer Central Research, Groton, Connecticut. (RANGER HALL, 4:00, ROOM 103)

Mar. 5 The Effect of Heat-Shock on Developmentally Regulated Genes in Myxococcus xanthus. Deborah Britt, Department of Microbiology, URI.

Mar. 19 Elimination of Microbial Indicators of Sanitary Quality by Bivalve Molluscs. Bill Burkhardt, Department of Microbiology, URI.

Mar. 26 Regulatory Molecules Involved is Signal Transduction and Secretion. Dr. Brigit Satir, Department of Anatomy and Cell Biology, Albert Einstein School of Medicine.

Mar. 27 Mitochondrial DNA and evolutionary genetics. Dr. Thomas Kocher, University of New Hampshire. (ZOOLOGY; BIO. SCI. BLDG.; 4:00 room A105).

Apr. 2 Mutational Analysis of the vir Region of an LHR Agrobacterium tumefaciens Ti Plasmid, Mike Zapor, Department of Microbiology, URI.

Apr. 9 The Expression of E. coli F 18 Type 1 Pili in the Ability to Colonize the Streptomycin-Treated Mouse Colon. Beth M. McCormick, Department of Microbiology, URI.

Apr. 16 A Simple Fluorescent Method to Assess Phagocytosis and Bactericidal Efficiency in Phagocytes from Marine Organisms. Thomas G. Daniels, Environmental Protection Agency, Narragansett, RI.

Apr. 25 Physiologic and Biochemical Responses to Stress by Cyanobacteria. Dr. Elliot Schubert, Dept. of Biology, University of North Dakota (RANGER HALL, room 103, 4:00).

Apr. 30 Elucidation of the Pathways Involved in the Suicide Response of Aeromonas caviae. Joe Newman, Department of Microbiology, URI.