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BY

JOE OLIVER GRAVES, JR.

B.S., The University of Mississippi, 1964

A Thesis
Submitted to the Faculty of
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in Partial Fulfillment of the Requirements
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JOE OLIVER GRAVES, JR.

Lyman A. Magee
Professor of Biology and
Chairman of the Department of Biology
(Director of the Thesis)

Julia M. Cruse
Professor of Biology and
Research Professor of Immunology

M. E. Morrison
Professor of Biology

Dean of the Graduate School

ABSTRACT

ENTERIC BACTERIOPHAGES IN SAINT LOUIS BAY, MISSISSIPPI

JOE OLIVER GRAVES, JR. B.S., The University of Mississippi, 1964. M.S., The University of Mississippi, 1972. Thesis directed by Professor Lyman A. Magee.

Samples from four stations in Saint Louis Bay, Mississippi were examined monthly for the presence of enteric bacteriophages over the period from July 1971 to March 1972. The samples were of surface and bottom water, bottom sediment, and surface and gut of fish and oysters. Host bacteria tested were Escherichia coli, Enterobacter aerogenes, Salmonella schottmuelleri and Shigella alkalescens. No bacteriophages for Salmonella schottmuelleri or Shigella alkalescens were detected. Bacteriophages of Escherichia coli and Enterobacter aerogenes were isolated regularly, most commonly from surface water samples.

MPN estimations of E. coli bacteriophage concentrations in surface water samples were determined over the period from December to March. Wide variations in E. coli bacteriophage concentrations were encountered. The data indicated a pattern of seasonal variation. This method provided a rapid and simple means for estimation of coliphage concentrations in water.

Enteric bacteriophages could possibly be used as indicators of viral pathogens of fecal origin in water, in a manner similar to the present use of coliforms. Comparisons of E. coli bacteriophage and enteric virus survival in water are necessary to determine the usefulness of these bacteriophages as indicators in routine water quality tests.

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LIST OF FIGURES AND TABLES

INTRODUCTION

This study was part of an environmental survey of Saint Louis Bay, Mississippi. The main objectives of testing were to conduct a baseline survey for the presence of various enteric bacteriophages, and simultaneously to determine the feasibility of present techniques for phage detection, and to adapt these techniques to this specific sampling program.

Enteric bacteriophages occur in large numbers in feces and sewage. The possibility of their use as indicators of viral contamination in natural waters is suggested. This study points out advantages and necessary precautions in applying this concept.

LITERATURE REVIEW

Bacteriophages were first noted by Twort in 1915 and D'Herrelle in 1917. Early work with bacteriophages centered on disease therapy. However, bacteriophages have not proved effective in the treatment of bacterial infections. Current research has involved use of bacteriophages in bacterial genetics and also as a model for study of viral replication. For a comprehensive treatment of all phases of bacteriophage research, the work of Adams (1959) should be consulted.

Bacteriophages have been demonstrated for many species of bacteria. Enteric bacteriophages may inhabit the intestinal tracts of humans and animals, and are excreted in large numbers in feces. Phages can be easily isolated from feces and sewage by incubation in broth seeded with the proper host bacterium, and can be enumerated by a direct overlay inoculation onto a host-seeded nutrient agar (Adams, 1959). Thus, it would appear that enteric bacteriophages could be used as indicators of fecal pollution in natural waters.

Emphasis in this area, however, has been centered on indicator bacteria: coliforms, fecal coliforms, and fecal streptococci. Quantitative tests follow either a multiple tube Most Probable Number procedure or a membrane filter direct enumeration. Possible presence of pathogenic microorganisms and degree of hazard are indicated by arbitrary limits according to the number of indicator bacteria present (American Public Health Association, 1971).

Indicator bacteria are of questionable value in demonstrating viral contamination in water. Often waste treatment processes that reduce indicator bacteria to legally acceptable limits may fail to reduce significantly the levels of enteric viruses, allowing them to be released in large numbers into the aquatic environment. In addition many of these viruses have radically different die-off rates than indicator bacteria, and may persist in raw waters for long periods of time under certain conditions (Berg, 1967).

Groups of potentially hazardous viruses in water include the virus of infectious hepatitis, and the enterovirus, adenovirus, and reovirus groups. All of these groups may be excreted in great numbers in the feces of infected or carrier individuals. Present methods for indicating the presence of these organisms in natural waters are based on two types of assay: the routine indicator bacteria methods previously mentioned, or detection of the actual viruses. Neither of these methods is completely satisfactory. Under certain circumstances samples assayed by indicator methods may show low populations or absence of indicator bacteria, but may actually contain infectious levels of enteric viruses (Mack, Lu, and Coohon, 1972). Virus isolation methods require extremely tedious sampling, concentration, and cultivation techniques; and, in addition, quantitative techniques have not been perfected. A general summary of both indicator methods and virus isolation techniques are given in Standard Methods for the Examination of Water and Wastewater, 13th edition (American Public Health Association, 1971).

Viruses may be adsorbed to gauze pads suspended in water, or large volume grab samples may be taken (Melnick et al, 1954). The

viruses are then concentrated and isolated, usually in cell culture. Concentration may be accomplished by any of several different methods, the most promising of which is the membrane filter procedure (Cliver, 1967; Wallis and Melnick, 1967; Berg, Dean, and Dahling, 1968). This is a quantitative method, and in theory large volumes of water may be examined. Certain shortcomings are apparent in this method. In actual practice, unless samples are unusually free of particulate material, the sample volume is limited by filter clogging. Also, the collection ability is due to adsorption of the virus to the membrane surface, since the pores of the filter are much larger than the actual virions. If the membrane becomes coated by certain substances that may be present in the sample, the adsorption may be interrupted, and the virions may pass through the filter and into the filtrate (Wallis and Melnick, 1967). These difficulties in the indication of viral contamination by water suggest a need for the development of indicator methods using enteric bacteriophages.

Bacteriophages could possibly be used as indicators of contamination of natural waters by enteric viruses. Yet, little information has been published on isolation and enumeration of bacteriophages from natural waters. Problems arise when low phage concentrations are encountered, since the large sample volumes required are not compatible with direct plating techniques.

Adams (1959) and Eisenstark (1967) gave methods for isolation of phages from water. Large sample volumes were used, resulting in recovery of viruses present in low concentrations. These isolation procedures gave only rough quantitative estimates of phage concentrations; however, quantitative assays could be adapted from them.

Phages usually occur in sewage in greater numbers than enteric viruses, but in slightly lower concentration than coliforms (Kott, 1966b). Thus, in waters containing fecal contamination, phage enumeration methods similar to those for coliforms would seem applicable.

Loehr and Schwegler (1965) proposed a method for enumeration of Escherichia coli bacteriophages from water using a membrane filter procedure. Samples were collected, seeded with E. coli strain B, mixed to allow adsorption of phage, and then filtered through filters of 0.45 micron average pore diameter. The filters were placed on pads saturated with m-Endo Broth in petri plates and incubated 24 hours at 35° C. Plaques appeared as clear areas in the confluent growth of the E. coli cells. An inadequacy of this test was the poor recovery of phages. Comparative counts with the direct overlay method showed a recovery of only about 5% of the phages present when using the filter method. Another disadvantage was the length of time required for filtration.

A method for the estimation of E. coli bacteriophages in water has been suggested that involves a modified multiple tube Most Probable Number procedure (Kott, 1966a). This method proved adequate for detection of E. coli phages in concentrations as low as 2 per 100 ml of sample.

In oceans and estuaries coliform die-off is rapid (Carlucci and Parmer, 1960; Cook and Childers, 1970). Concentrations of E. coli phages have been compared to coliform concentrations in these waters (Kott, 1966b). Survival studies of E. coli phages in the marine environment have been conducted (Mitchell and Jannasch, 1969; Acton and Evans, 1968; Kott, 1966b; Kott and Ari, 1968). E. coli phages

generally have a longer survival time than coliforms under these conditions; therefore, the phage MPN is recommended in preference to the coliform test in the examination of marine waters (Kott, 1966b).

Kott's MPN method and the detection method of Eisenstark were chosen for this study and have been adapted to meet specific sampling requirements.

MATERIALS AND METHODS

I. Sampling

Monthly samples were taken from each of four locations in Saint Louis Bay, Mississippi. These stations are shown in Figure 1, page 8. Four types of samples were taken at these stations: surface water, bottom water, bottom sediment, and bottom-feeding fish (croaker or flounder species). The surface samples were collected in one gallon sterile jars, using a grab method. Bottom samples were collected in sterile 8 oz. prescription bottles by means of a modified ZoBell Sampler. Bottom sediment was collected with a Phleger-type core sampler. Fish were taken by trawling and placed in sterile bottles, with as little handling as possible. An oyster sample was obtained by dredging from the oyster bed shown in Figure 1, page 8. All samples were placed on ice immediately and remained there until assay.

Samples were taken on the following dates: July 6, August 17, September 28, October 26, and December 7, 1971; January 11, February 18, and March 29, 1972. No samples were taken in November, 1971.

II. Media

Media are listed in Table 1, page 9. The MPN broth was a cultivation broth with added inorganic salts. A similar medium is commercially available (Phage Isolation Broth, Fisher Scientific Company). The plaque agar consisted of the MPN broth plus 2% agar. The overlay agar is 0.7% agar with no added nutrients. All media were sterilized at 121° C for 15 minutes.

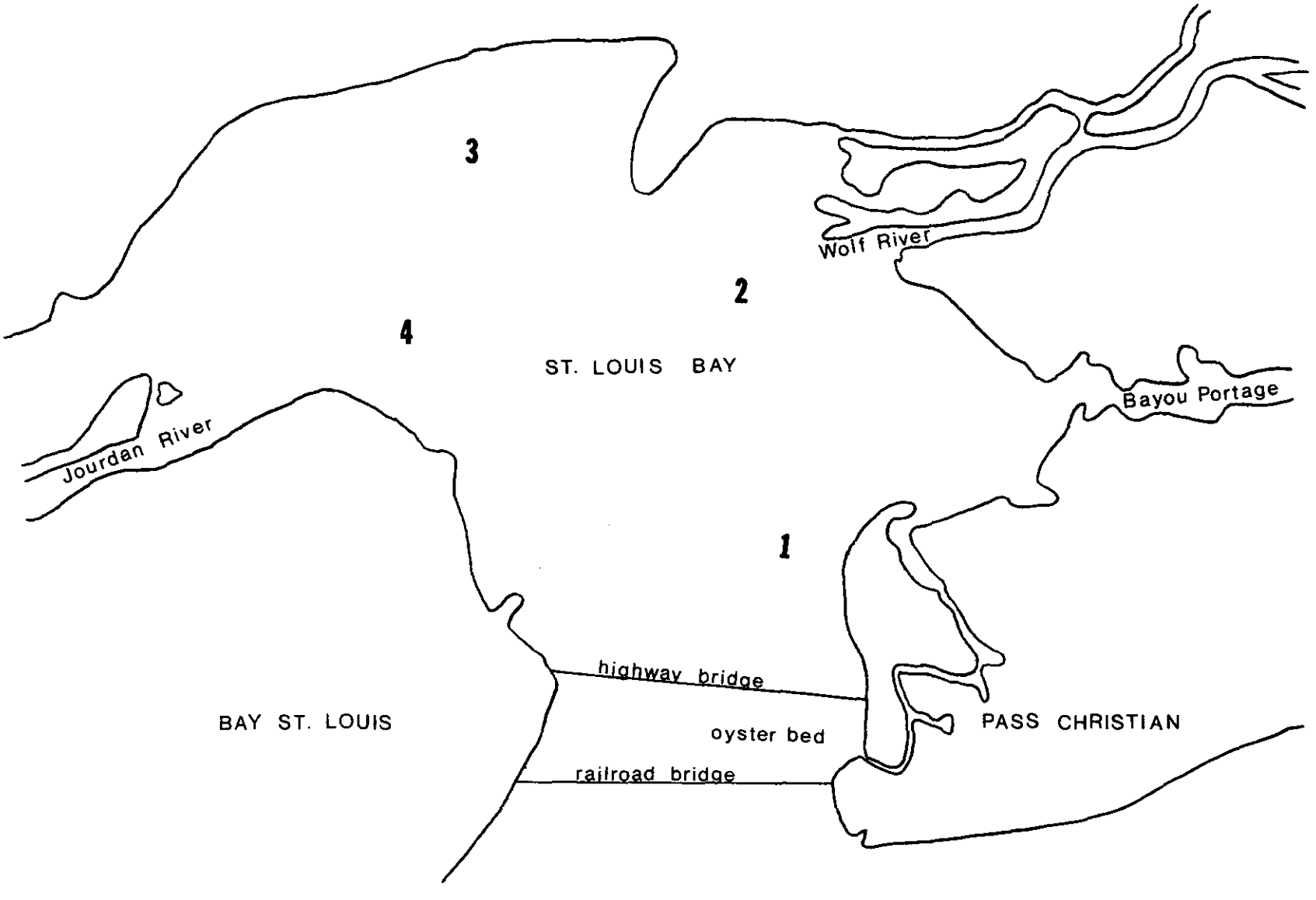

FIGURE 1. Sample locations

TABLE 1. Assay media

I. MPN broth

Beef extract	3 g
Sodium chloride	5 g
Tryptone	5 g
Magnesium sulfate	0.2 g
Manganese sulfate	0.05 g
Distilled water	1000 ml
Calcium chloride (added as a sterile solution after autoclaving)	0.15 g/l

II. Plaque agar

Isolation broth and 2% agar

III. Overlay agar

0.7% agar (no added nutrients)

III. Host bacteria

The host organisms chosen for this study were Escherichia coli, Enterobacter aerogenes, Shigella alkalescens, and Salmonella schottmuelleri (paratyphi B). Characterizations and phylogenetic relationships of these bacteria may be found in Bergey's Manual of Determinative Bacteriology, 7th edition (Breed, Murray, and Smith, 1957). E. coli ATCC No. 4157 was used in assay of the July and August samples. E. coli strain B was used for all following samples. The other host bacteria were cultures from the Microbiology stock collection at the University of Mississippi. Phage-free stocks of these organisms were carried in the MPN broth, with periodic checks for contamination using the plaque agar. Stocks were stored at 4° C between transfers. Log phase cultures were used in all assay procedures.

IV. Assay techniques

Three different types of assays were performed. The first type was designated as "Standard Samples." This assay was done on samples from each collection date. Surface and bottom water inocula were approximately 5 ml. (Double strength broth was used for these samples.) Bottom sediment, fish surface, fish gut, oyster shell liquor and oyster gut inoculations were made with sterile cotton applicators. Phage detection was accomplished by adaptations of the method of Eisenstark (1967). Four 5 ml broth tubes were inoculated from each sample. This resulted in four sets of sample tubes, one set for each of the host bacteria to be tested. Each set was then inoculated with 0.1 ml of a log phase culture of the respective host organism and incubated at 35° C for 16 to 24 hours. A 2 ml sample from each tube was then

centrifuged in a sterile 10 ml tube for 15 minutes. Test plates, prepared at least 24 hours before use, were made by pouring a bottom layer of the plaque agar. These plates were seeded immediately before use with 0.2 ml of a log phase culture of the host organism. This inoculum was distributed over the agar surface with a sterile glass spreader, and allowed to dry. Then an inoculation loop of broth from each of the centrifuge tubes was placed on a labeled area of the seeded agar plate. The number of areas were limited to five or less per plate. These drop areas were allowed to dry, and the plates were covered with a thin layer (about 5 ml) of seeded overlay agar held at 45° C in a water bath. The plates were incubated at 35° C. Plaque zones could usually be observed at 6 to 18 hours. The positive plaque zones appeared as clear areas, or as halos around any bacterial growth present from the sample drops. A positive plaque area indicated the presence of phages specific for the host organism in that particular sample.

The second type of assay ("Large Volume Samples") was done on surface water samples only. Sample volumes were 250 ml. Each sample was placed in 250 ml of double-strength broth and inoculated with 1 ml of a log phase culture of the proper host organism. Further assay of these samples was identical to that of the Standard Samples. This type of assay was done on samples collected from September through March.

The third type of assay was on surface water samples, using a multiple tube Most Probable Number quantitative procedure adapted from Kott (1966a). Only the E. coli strain B host was used in these assays. Many steps in this procedure are similar to the coliform MPN techniques. Sample volumes were usually 10 ml, 1 ml, and 0.1 ml. Five

tubes containing 10 ml of broth were inoculated with each volume. (The tubes for 10 ml sample volumes contained double-strength broth.) Each tube was then inoculated with 0.1 ml of a log phase culture of E. coli strain B (equivalent to about 10^8 cells/ml). Further procedures were identical to those for Standard Samples. Each tube of a sample was assayed for phages. The number of positive tubes for each volume of the sample was recorded, and the MPN value of the sample was obtained using standard MPN tables (American Public Health Association, 1970).

For all three types of assay, if the presence of large numbers of bacteria from the sample resulted in questionable plaque areas, broth from each of the sample tubes was retested after filtration through a sterile membrane filter (0.22 micron pore diameter) instead of centrifugation.

RESULTS AND DISCUSSION

I. Standard samples

Bacteriophages for Escherichia coli strain B were detected, but no phages were found for Shigella alkalescens, Salmonella schottmuelleri, Enterobacter aerogenes, and E. coli ATCC No. 4157 in these samples. These results are summarized in Table 2, page 14. Most of the positive samples were from surface water samples. E. coli bacteriophages were also isolated from one bottom water and one fish gut sample. No phages were isolated from bottom sediment, fish surface, or oyster samples. December and January surface water samples were positive at all four stations.

Station 4 had the most positive isolations: four surface water samples, one bottom water sample, and one fish gut sample. Stations 1, 2, and 3 had three positive samples each, all surface water.

II. Large volume samples

These samples were 250 ml surface samples. Bacteriophages for E. coli strain B and Enterobacter aerogenes were isolated from these samples. No phages were found for Shigella alkalescens, Salmonella schottmuelleri, and E. coli ATCC No. 4157. These results are summarized in Table 3, page 15.

E. coli bacteriophages were detected in the sample from Station 1 in September, at Stations 2, 3, and 4 in October, and at Stations 1, 2, 3, and 4 in December, January, February, and March.

TABLE 2. Bacteriophages from Saint Louis Bay, Mississippi: standard samples[1]

Sample type	Station	Sampling dates[2] Oct. 26	Dec. 7	Jan. 11	Feb. 18
Surface water	1		+	+	+
	2		+	+	+
	3		+	+	+
	4	+	+	+	+
Bottom water	1				
	2				
	3				
	4		+		
Bottom sediment	1				
	2				
	3				
	4				
Fish surface	1				
	2				
	3				
	4				
Fish gut	1				
	2				
	3				
	4		+		
Oyster (surface and gut)	1				
	2				
	3				
	4				

+ indicates presence of phage for Escherichia coli strain B

[1]No phages for Enterobacter aerogenes, Salmonella schottmuelleri, or Shigella alkalescens were detected by this procedure.

[2]Standard samples for July 6, Aug. 17, Sept. 28, and Mar. 29 were negative for all hosts.

TABLE 3. Bacteriophages from Saint Louis Bay, Mississippi: 250 ml surface water samples

Sampling dates	Sta.	Escherichia coli[1]	Enterobacter aerogenes	Salmonella schottmuelleri	Shigella alkalescens
July 6	1				
	2				
	3				
	4				
Aug. 17	1				
	2				
	3				
	4				
Sept. 28	1	+			
	2				
	3				
	4				
Oct. 26	1				
	2	+			
	3	+			
	4	+			
Dec. 7	1	+	+		
	2	+	+		
	3	+	+		
	4	+	+		
Jan. 11	1	+	+		
	2	+	+		
	3	+	+		
	4	+	+		
Feb. 18	1	+	+		
	2	+	+		
	3	+	+		
	4	+	+		
March 29	1	+			
	2	+	+		
	3	+	+		
	4	+			

+ indicates presence of phages

[1]ATCC No. 4157, July-Aug.; strain B, Sept.-Jan.

TABLE 4. *Escherichia coli* bacteriophage concentrations from Saint Louis Bay, Mississippi

Sampling dates	Phage MPN/100 ml			
	Station 1	Station 2	Station 3	Station 4
July - Nov.[1]	-	-	-	-
Dec. 7	110	49	94	130
Jan. 11	2400	790	1300	940
Feb. 18	12	14	9	27
March 29	10	6	1	2

[1]Enumeration not attempted.

TABLE 5. Bacteriophages from Saint Louis Bay, Mississippi: combined results

Sampling dates[1]	Station	Escherichia coli[2] 5 ml	Escherichia coli[2] 250 ml	Escherichia coli[2] MPN/100 ml[3]	Enterobacter aerogenes 5 ml	Enterobacter aerogenes 250 ml
Sept. 28	1		+			
	2					
	3					
	4					
Oct. 26	1					
	2		+			
	3		+			
	4	+	+			
Dec. 7	1	+	+	110		+
	2	+	+	49		+
	3	+	+	94		+
	4	+	+	130		+
Jan. 11	1	+	+	2400		+
	2	+	+	790		+
	3	+	+	1300		+
	4	+	+	940		+
Feb. 18	1	+	+	12		+
	2	+	+	14		+
	3	+	+	9		+
	4	+	+	27		+
March 29	1		+	10		
	2		+	6		+
	3		+	1		+
	4		+	2		

+ indicates presence of phages from surface water samples

[1]No phages were found in July and Aug. samples; no phages for Salmonella schottmuelleri or Shigella alkalescens were found in any samples.

[2]Station 4 bottom water and fish gut samples were positive for E. coli phage on Dec. 7.

[3]E. coli phage MPN's were assayed Dec. - March. Phage MPN's were not made for Enterobacter aerogenes.

the presence of phages by plaque areas from enrichment broth plated on host-seeded agar. Several factors in this assay were found to be critical to detection of bacteriophages:

1. Indigenous bacteria multiplied in the broth tubes, especially in low salinity samples. These bacteria at times were present in large enough numbers to mask plaque formation when the broth was transferred to agar plates. This necessitated centrifuging or filtering the broth from each tube to reduce the bacteria to a level that did not mask plaque formation. Tubes with high bacterial concentrations were filtered through a sterile 0.22 micron pore size membrane filter before being placed on seeded agar. For most samples centrifugation proved adequate. However, this step added time to the assay procedure. In field applications of bacteriophage detection, in which large numbers of samples must be assayed; it would be advantageous to run preliminary tests to determine if centrifugation is necessary.

2. Occasionally questionable plaque areas were encountered. In this case, presence of bacteriophages was confirmed by inoculation needle transfer from the questionable area to another seeded agar plate.

3. The host bacteria used in seeding procedures were from cultures in the logarithmic phase of growth. In tests of this type it is important that the phages attach to viable and actively multiplying host cells. Otherwise, in samples containing low numbers of phages, a large portion of the phages may attach to non-viable bacteria (Adams, 1959). Thus, phage replication may be greatly reduced or not occur at all; resulting in negative reports for phages

in single tube samples and lower estimates of phage concentration in MPN samples.

4. Periodic checks were made to assure that the host bacteria were phage-sensitive. Control tubes and plates were assayed in parallel with the samples to assure proper handling technique and absence of contaminating phages. No laboratory contamination by bacteriophages was observed. Cultures, except as noted, remained phage-sensitive over the test period. E. coli ATCC No. 4157 proved very insensitive to phages from these waters. This strain was used as an indicator in assay of the July and August samples, but no phages that would lyse this strain were found. E. coli strain B was substituted for all subsequent samples, and showed good sensitivity to phages. The Enterobacter aerogenes strain possessed low sensitivity to phages, resulting in poor plaque formation.

No phages for Shigella alkalescens or Salmonella schottmuelleri were available, so sensitivity tests were not performed on these cultures.

5. The media were prepared and used as indicated for optimum bacteriophage recovery. Inclusion of the inorganic salts assured proper attachment to the host (Adams, 1959). The agar plates were dried overnight before use to prevent running and confluence of plaque areas. The use of 2% agar also helped reduce this problem. Sample drops were not crowded on the agar plates. Although Kott (1966a) placed as many as 15 drops per plate, a more reliable procedure was to limit the drops to no more than 5 per plate.

VI. Discussion and conclusions

When assays were made using *E. coli* strain B, detectable numbers of E. coli phages were found in surface water samples, but were seldom encountered in other samples. No phages were isolated using *E. coli* ATCC No. 4157 (July and August samples). *Enterobacter aerogenes* phages formed poorly visible plaques on the host strain, and could be demonstrated only when present in high titer, using large sample volumes and enrichment techniques.

The *E. coli* bacteriophage MPN test was the most useful of the assay procedures. Estimations of actual phage concentrations could be obtained using this method. Using the data from this assay, factors influencing phage survival in natural waters could be studied.

Variation in phage concentration was compared to coliform variation. Coliform and fecal coliform MPN estimates obtained from parallel samples were provided by Dr. David Cook of the Gulf Coast Research Laboratory. The results of phage assay, along with these counts, are shown in Table 6, page 22. Some rough correlation was apparent, but no set ratio of coliforms to phages was found. Several factors influencing phage survival seem to be dissimilar to those for coliform survival. Kott (1966b) has shown similar results in comparing phage and coliform survival at varying distances from a sewage outfall in a marine environment.

As previously stated, coliform levels have been shown to be reduced drastically in estuarine waters. Salinity does not necessarily have a direct effect. Mitchell and Jannasch (1969) related this die-

TABLE 6. Concentrations of *Escherichia coli* bacteriophages, coliforms, and fecal coliforms from Saint Louis Bay, Mississippi

Sampling dates	Test	MPN per 100 ml Sta. 1	Sta. 2	Sta. 3	Sta. 4
Dec. 7	Phage MPN	110	49	94	130
	Coliform MPN	930	930	430	2400
	Fecal coliform MPN	930	240	93	240
Jan. 11	Phage MPN	2400	790	1300	940
	Coliform MPN	1500	1500	2400	930
	Fecal coliform MPN	430	430	2400	210
Feb. 18	Phage MPN	12	14	9	27
	Coliform MPN	460	4600	240	4300
	Fecal coliform MPN	43	930	93	390
March 29	Phage MPN	10	6	1	2
	Coliform MPN	33	170	40	170
	Fecal coliform MPN[1]	-	-	-	-

[1]Fecal coliform MPN estimates for March 29 not determined.

Coliform and fecal coliform MPN estimates for Dec. 7, Jan. 11, and Feb. 18 courtesy Dr. David W. Cook, Gulf Coast Research Laboratory.

off to the presence of large numbers of certain microorganisms (Bdellovibrio and the marine amoeba, Vexillifera telmathalassa). Carlucci and Parmer (1960) found that salinity, indigenous microorganisms, and lack of nutrients were factors detrimental to E. coli survival in this environment.

Variation in phage concentrations were compared to salinities of samples. No definite effects could be observed. Salinities for December 7 samples ranged from 2 parts per thousand to 10.5 ppt between stations, but phage concentrations were very similar at all stations. Salinities for January 11 and February 18 samples were less than 1 ppt for all samples; yet there was considerable variation in phage densities between these two dates, although there was not a wide range in densities between samples on the same date.

Concentrations of phages were generally higher at Stations 1 and 4. This indicated that a high proportion of the phages probably resulted from drainage from the local population centers adjacent to Stations 1 and 4. Lower concentrations were found at Stations 2 and 3, which are distant from inhabited areas. This in turn suggested that relatively low proportions of the phage concentrations were added by the river systems flowing into Saint Louis Bay.

A pattern of seasonal variation is suggested by the data. Phages appear to occur in higher concentrations in the winter months, indicating a temperature-related survival factor. Further testing will be necessary to determine the effects of these and other factors on survival of bacteriophages in these types of waters.

SUMMARY

Monthly samples from Saint Louis Bay, Mississippi were examined for the presence of bacteriophages for various enteric organisms. Phages specific for E. coli strain B and Enterobacter aerogenes were isolated. The great majority of positive samples came from surface water. The E. coli phage concentrations varied from 1 to 2400/100 ml in these samples. No phages for Shigella alkalescens or Salmonella schottmuelleri were found.

Salinity apparently had no effect on phage survival. A seasonal variation in phage concentration was indicated. Coliphage die-off occurred at different rates than coliform die-off. Thus, there is the possibility of detectable enteric phages in waters that are free of viable coliforms. As stated previously, enteric viruses can also be present in coliform-free waters. This reinforces the concept of the use of E. coli phages as indicators of contamination of water by enteric viruses.

There is great diversity among the groups of enteric viruses, shown by variations in size, morphology, and persistance in the aquatic environment (Berg, 1967; Goodheart, 1969). Nevertheless, E. coli bacteriophages are more closely related to the enteric viruses in these respects than are coliform bacteria and would logically be more representative indicators of enteric viruses in water, dependent on the development of suitable and adequately sensitive quantitative phage enumeration procedures.

Kott's MPN method of E. coli bacteriophage enumeration makes possible quantitative research in this field. This test is presently suggested for use in preference to coliform tests in marine waters (Kott, 1966b; Kott and Ari, 1968), and should prove equally useful in evaluation of fresh water sources. With the adaptations and precautions previously noted, the E. coli phage MPN gave excellent quantitative results in this study. The test was simple, inexpensive, and rapid; results could be obtained in 24 to 30 hours after sampling.

Further studies of the E. coli bacteriophage enumeration procedure, in parallel with animal virus detection studies, are indicated in order to obtain comparative survival data. If similar results are noted, the phage MPN should provide a useful additional method in routine water quality testing.

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