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RESEARCH PROJECT SUMMARY

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Microbial Indicators of Human and Non-human Fecal Contamination of Coastal Waters and Shellfish

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ABSTRACT

The sanitary quality of New Jersey coastal waters is determined in part by measuring the levels of certain intestinally-derived (enteric) bacteria. Although such tests are very useful in assessing water quality, they are limited in that they cannot distinguish between animal- and human-derived pollution and they may not accurately predict the presence of pathogenic viruses. The objective of this study was to examine whether or not the presence of F+ RNA coliphage (FRNA phage), viruses which infect enteric bacteria, more accurately predict the presence of pathogenic human enteric viruses (HEV) in coastal waters and shellfish impacted by defined types of fecal pollution, including point and non-point, human and non-human fecal wastes. HEV cannot be monitored directly as they occur sporadically in the environment, usually at low concentrations, and laboratory detection methods are difficult, expensive, and time-consuming. Coliphage assays are as easy, inexpensive, and rapid to conduct as bacterial indicator assays and, if validated, could easily be incorporated into routine water quality

monitoring programs.

New methods were successfully applied to the detection of F+ RNA coliphages. FRNA phage were detected in 76% and 86% of water and shellfish samples, respectively, which were impacted by animal or human fecal pollution. The levels of phage were about ten-fold lower than bacterial indicator organism levels unless the sample was from a site impacted by a chlorinated sewage effluent. In such cases, phage levels were similar to or exceeded indicator bacteria levels. This finding is significant because fecal bacteria are more sensitive to the lethal effect of chlorine than are HEV. Epidemiological studies over the past several decades have documented the emergence of viruses as the microbial agents most commonly associated with swimming and shellfish consumption-associated diseases. Therefore, FRNA phage may be better predictors of the potential presence of HEV in polluted waters and shellfish than the presently used bacteria indicator tests.

HEV were detected in only 1 of 19 water samples from locations containing human-derived pollution. The HEV-positive sample was one of only 4 collected at Site E, the human, point source site and the site most likely to contain detectable HEV. Because there was only 1 HEV-positive sample, correlations could not be made between the presence and levels of HEV with that of FRNA phage or bacterial indicator organisms. Such correlations await ongoing and perhaps future studies.

FRNA phage and indicator bacteria levels were higher in oyster samples found to contain HEV compared to samples in which HEV were not detected. FRNA phage and HEV levels need to be studied in additional bivalve species of commercial interest to NJ and collected from conditionally approved and condemned as well as approved shellfish harvest waters.

Serotyping of FRNA phage may result in the ability to distinguish human from non-human pollution sources. FRNA phage serotyping was successfully performed and confirmed whether or not a sample was impacted by human, non-human, or a combination of these pollution sources. This finding has important estuary management implications since it is generally believed that HEV are derived solely from human pollution sources and that human-derived pollution poses a greater health threat than animal-derived pollution.

INTRODUCTION

Pathogens, microorganisms that cause disease, are found in several major groups; bacteria, viruses, and other parasites such as protozoa, intestinal worms and the like. The sanitary quality of bathing and shellfish harvest waters in New Jersey, as elsewhere, is assured in part through routine monitoring for fecal-derived, "indicator" bacteria. Indicator bacteria, like most enteric pathogens, inhabit the intestines of warm-blooded animals but for the most part are not pathogenic, (some pathogenic members exist) and are easy to measure compared to pathogens. When present in water above a specific concentration, they indicate the potential presence of pathogens.

Currently, New Jersey monitors bathing and shellfish harvest waters for the presence and amounts of fecal coliform (FC) bacteria, which include *Escherichia coli* and related organisms, and enterococci, a subgroup of fecal streptococci (FS) bacteria, consisting of *Streptococcus faecalis* and *S. faecium*. These waters are regulated based on fecal coliform counts [1]. Fecal coliform and fecal streptococci bacteria predict reasonably well the possible presence of bacterial pathogens including those responsible for typhoid fever (*Salmonella*), bacillary dysentery (*Shigella*), and cholera (*Vibrio*). However, some members of the FC and FS groups have non-fecal sources such as soil, vegetation, and certain industrial wastes [2]. Therefore, the presence of FC and FS in a water sample may not in all instances indicate the presence of fecal material.

Bacterial coliform standards were developed before the risks of viral illness from bathing or eating shellfish were adequately recognized. FC and FS bacteria are less reliable indicators of the potential presence of pathogenic human enteric viruses (HEV). Pathogenic HEV can cause diseases such as poliomyelitis, meningitis, infectious hepatitis, gastroenteritis, and respiratory infections. Some HEV persist longer in the environment and are more resistant to disinfection procedures such as chlorination than coliform bacteria [3]. Cases have been documented where HEV have been detected in coastal waters and shellfish meeting fecal or total coliform regulatory limits [4] and epidemiological studies over the past several decades have documented the emergence of viruses as the microbial agents most commonly associated with swimming and shellfish consumption-associated diseases [5].

Bacterial indicator organisms and pathogens are derived from animal as well as human waste. Pathogenic viruses are believed to be derived solely from human waste. It is not possible to distinguish human from animal pollution with the bacterial indicator organism tests and thus it is not possible to identify pollution sources that may contain pathogenic viruses. Furthermore, most scientists believe that the level of human health risk due to exposure to human-derived pollution is greater than that due to exposure to animal-derived pollution [6], although at present it is not possible to quantify this difference.

Because of the limitations of the currently used bacterial indicator organism tests, some bathing waters, shellfish, and shellfish waters may be improperly classified with respect to their sanitary quality. New Jersey and other governing bodies have begun to look for a test system which better predicts the potential presence of human pollution and viral pathogens. In this project, studies were initiated to detect and quantify *E. coli* (EC) and *Clostridium perfringens* (CP) bacteria in addition to FC and enterococci. EC and CP are fecal, but not human specific indicator organisms. *E. coli* is the fecal-specific component of the FC indicator group. *C. perfringens* forms an environmentally-stable spore and thus might be a good indicator for environmentally persistent pathogens (*eg* HEV) and of aged as well as recent pollution. In addition, F+ RNA coliphage were measured using an assay system developed by A.H. Havelaar and colleagues [7]. F+ RNA coliphage (FRNA phage) are viruses which infect "male" or F+ coliform bacteria by injecting their ribonucleic acid (RNA) through sexual pili; hair-like projections on the bacterial surface.

FRNA phage are consistently found in domestic, hospital and slaughterhouse wastewaters at levels (10^4 - 10^6 per 100 ml) outnumbering HEV by about 3 orders of magnitude [8]. The range of FRNA phage in the post-chlorinated effluents of 9 coastal NJ sewage treatment plants (STP) in 1987 ranged from 10^2 - 10^4 per 100 ml [9]. However, FRNA phage are present in only a small percentage of feces from humans (≈2%) or animals and hence these phage may not be suitable HEV indicators in all environmental situations (*eg* septic tank leachate; boat wastes). Therefore, FRNA phage monitoring data must be interpreted on the basis of site-specific information and under some circumstances, HEV may be present in the absence of FRNA phage.

Some studies have shown that FRNA phage have disinfection and environmental transport and survival characteristics more similar to that of HEV than FC or enterococci bacteria [10]. Thus, the F+ RNA coliphage assay offers the potential to predict the presence of viral pathogens more reliably than the currently used bacterial indicator tests.

F+ RNA coliphage can be divided into 4 groups (I,II,III, and IV) based on serotyping analysis. Limited studies have shown that type IV is found primarily and type I exclusively in animal feces, while type II is found primarily and type III exclusively in human feces [11]. Therefore, this assay appears to have the ability to distinguish between human and non-human sources of fecal contamination. Fecal coliform and enterococcal tests cannot make such a distinction (see above). HEV are generally believed to be derived solely from human pollution. Human exposure to low levels of fecal pollution from indigenous animal populations in coastal waters may not be totally preventable and the resultant health risk from this source may not be significant. Therefore, if confirmed, the ability of the coliphage assay to reliably distinguish animal and human fecal pollution has important coastal management and regulatory implications. Despite their limitations, the fecal coliform and enterococci indicator tests continue to serve a valuable role in water quality monitoring particularly with regard to bacterial pathogens and it is anticipated that, if the F+ RNA coliphage assay proves useful, this test would compliment, not replace bacterial indicator tests.

OBJECTIVES

1. Statistically compare male-specific (F+) coliphage and conventional bacterial indicators (fecal coliform, *E. coli*, enterococci and *C. perfringens*) to the levels of human enteric viruses in coastal waters and molluscan shellfish impacted by defined types of fecal pollution, including point and non-point, human and non-human fecal wastes.

2. Evaluate the ability of the F+ RNA coliphage assay to distinguish human and non-human fecal contamination in coastal waters and shellfish.

STUDY DESIGN AND METHODS

Site Selection

Four sites in New Jersey (Table 1) were selected for the collection of water samples based on whether the site was impacted by a point or a non-point contamination source and whether the predominant type of contamination was human or non-human. One site in coastal North Carolina was selected for analyses of oysters. The site, closed to shellfish harvesting, was impacted by chlorinated effluent from a secondary wastewater treatment facility.

Sampling

Subsurface grab samples of water were collected for bacterial and coliphage assays at 4 stations at each of four sites and, for HEV, at a portion of these stations at selected times over a 2 year period (N = 94). The stations were located at increasing distances from the suspected dominant pollution source. Oysters (1-2 dozen per sample) were collected at 2 stations at the North Carolina site twice a month for one year (N = 42).

Analytical Methods

Details of the microbial analytical procedures used can be found in the Final Reports. FC and EC in up to 100 ml volumes of water and FC and EC in 50 gram oyster tissue samples were enumerated in water using standardized methods [12]. Other, published methods were used for enterococci and CF in water and oyster tissues. Oysters were shucked and the tissue homogenized prior to analyses. Coliphage and bacteria data were reported as the number of organisms per 100 ml water or per 100 gram tissue. Lowest bacteria detection limit in water was 1 organism per 100 ml.

F+ RNA coliphages were enumerated in volumes from 1 to 2000 ml (3 different protocols used depending on sample) on *Salmonella* WG49, a host bacterium developed by A.H. Havelaar and colleagues [7]. The bacterium contains an *E. coli* plasmid responsible for sex pili production. Oyster tissue homogenate supernatants were analyzed following centrifugation to remove solids. Selected phage (in agar plaques) were shown to contain either RNA or DNA using enzyme treatments and to be either F+ or somatic Salmonella phage or coliphage using appropriate host organisms. Serotyping was conducted on 4 agar media each containing rabbit

antisera to one of the four serotypes. Lowest coliphage detection limits in water were 0.05-2 plaque forming units (PFU) per 100 ml, depending on method (detection limits are sample-dependent).

Enteric viruses were filtered from 100 liter volumes of water using 0.45 micron cartridge filters following addition of $AlCl_3$ and pH adjustment to 3.5. Viruses were eluted from the filters with beef extract/glycine fluid (pH 9.5) at the NJDEPE Marine Water Classification Laboratory, Leeds Point, NJ and, after adjustment to pH 7, overnight mailed to the University of North Carolina analytical lab where the eluate was precipitated with $FeCl_3$ at pH 3.5, centrifuged, resuspended in antibiotic-containing buffer at pH 7.0, and frozen at -70°C until assay. 50 gram homogenized, centrifuged oyster samples were analyzed for virus following precipitation, resuspension and purification procedures. Viruses were assayed by culture and subculture on African green monkey kidney (AGMK) and other cell lines and detected by observing cytopathic effects (CPE). CPE-negative cultures were further analyzed, following fixation, by enzyme immunoassay, and by transcription of RNA to DNA and amplification of the DNA by the polymerase chain reaction technique using pan-enterovirus primers followed by hybridization with specific oligonucleotide probes (gene probing) and visualization on stained electrophoretic gels. Virus concentrations were reported as most probable number of infectious units per unit weight or volume of sample. Detection limit for HEV in oysters was 2.3 infectious units per 100 grams.

Statistical analyses were done using ABSTAT software (Anderson Bell, Arvada, CO).

RESULTS & DISCUSSION

♦ F+ RNA coliphage (FRNA phage) were detected in 76% of the NJ water samples (Table 2). Most coliphage-negative samples were from stations distant from pollution sources. These samples had lower concentrations of bacterial indicator organisms (36-82% lower, depending on indicator) than did coliphage-positive samples. Significant positive correlations were observed between concentrations of FRNA phage and all four indicator bacteria. FRNA phage and the indicator bacteria were found at all times of the year.

♦ FRNA phage concentrations were approximately one order of magnitude lower than the concentrations of indicator bacteria (Table 2) unless the site was impacted by a chlorinated sewage treatment plant effluent. In such cases, phage concentrations were equal to or greater than the bacterial indicator concentrations (Table 3, Site E; see also Table 5), probably due to the fact that coliphage (and HEV) are known to be more resistant to chlorine than all indicator bacteria except *C. perfringens*. At Site E, FRNA phage concentrations were most strongly correlated with CP concentrations. The levels of FRNA phage found at 3 of the 4 sites in this study (Table 3) were similar to levels (measured using a different *E. coli* host organism) in ocean samples from 9 NJ beaches during the summer of 1988 (N = 212 [composite samples]; geometric mean = 1.0 PFU per 100 ml; range = 0.5 - 24 per 100 ml) [9].

♦ In 19 water samples containing human-derived pollution that were analyzed for human enteric viruses (HEV), only one was found to contain detectable HEV at an estimated concentration of 4.5 infectious units per 100 liters. 100 liters is a typical volume processed for HEV and other low-density pathogens such as protozoan parasites. This sample was taken from the station closest to the chlorinated sewage effluent at Site E, the human, point source and the site most likely to contain HEV. Only 4 samples were collected from site E. Therefore, correlations between HEV and F+ RNA coliphage and bacterial indicator organisms could not be determined.

Little or no HEV were expected to be found in the samples from sites A (N = 12) and B (N = 9) and HEV levels at site C (N = 8) were expected to be low or non-existent due to the limited population source (septic tank sources) and due to extensive dilution in the receiving water. No HEV were found at site D (N = 7), the site initially selected as the human, point source site but later found to contain considerable animal pollution.

Because FRNA phage are always found in wastewaters, in relatively constant and abundant levels, and because HEV are found in such waters only sporadically and in low numbers, correlations between FRNA phage and HEV concentrations can only be reliably determined following the accumulation of an extensive database that includes data from both groups of microorganisms.

♦ 16 of 31 oyster samples successfully analyzed

for viruses were found to contain HEV (52%). In samples from the site closest to the sewage effluent (1.1 km) the percentage was 63 and in the samples from the site 2.1 km from the effluent the percentage was 33. Most HEV-positive samples were detected by the standard cell culture method, but HEV were detected in three of the cell culture-negative samples (19% of all positive samples) using recently developed molecular biological techniques (reverse transcriptase [RT]/polymerase chain reaction [PCR]). Microbial indicator levels, including FRNA phage, were higher in HEV-positive samples (regardless of site) than in HEV-negative samples (Table 5).

♦ 36 of 42 oyster samples were successfully analyzed for coliphages and 86% contained coliphage. As expected, the levels of all indicators were higher at station 1 than station 2. In these samples, coliphage levels were similar to CP levels and about one order of magnitude higher than EC or FC levels (Table 5). Indicator and coliphage levels in the overlying waters were not measured. There were significant positive correlations between FRNA phage and some of the bacterial indicator organisms (*eg*, EC, r=0.67 [p <0.0001]; CP, r=0.71 [p <0.0001]). Correlations between FRNA phage and enterococci were poor and was unexpected since enterococci are considered to be relatively persistent in the environment and in shellfish. Regan *et al* also found low levels of enterococci in hardshell clams from Narragansett Bay [13]. They concluded that enterococci would be no better than FC in ensuring the sanitary quality of shellfish.

♦ Of the 61 FRNA phage serotyped in the water samples from the four NJ sites (Table 4), serotypes of human origin (Types II and III) were the only types found at Site E which was directly impacted by chlorinated sewage effluent. These serotypes were the most frequently found types (9 of 12) at Site C impacted by human, non-point source pollution. FRNA phage serotypes of animal origin (Types I and IV) were the only types found at the wildlife refuge (Site B) and were the predominant serotypes found (6 of 9) at Site A impacted mostly by non-human, non-point pollution. Examination of the quantitative bacterial indicator data in Table 3 shows that it is not possible to distinguish human from animal pollution using FC or the more pollution-specific EC indicator bacteria (compare sites B and E). Of the approximately 200 serotyped FRNA phage from oysters at 2 stations impacted by a chlorinated sewage effluent, $\geq$ 89.8% were human serotypes II and III

(mostly type II; data not shown). This percentage was higher (99.3%) at the station closest to the pollution source, the wastewater treatment plant outfall. Kator and Rhodes were able to distinguish marine waters impacted by a STP effluent and by wastewaters from a hog processing plant using the same FRNA phage typing antisera used in this study [14].

CONCLUSIONS

♦ Using newly developed methods, F+ RNA coliphage were successfully detected in coastal waters and shellfish from well characterized sites receiving defined sources of fecal contamination (human or non-human). The technology to perform F+ RNA coliphage analysis has been transferred to NJDEPE's Marine Water Classification Laboratory, Leeds Point, NJ. This provides NJ with an additional tool to evaluate the sanitary quality of bathing and shellfish harvest waters.

♦ The levels of the FRNA phage and the various bacterial indicator groups were, for the most part, positively correlated with each other. FRNA phage levels were about an order of magnitude less than the bacteria indicators unless the pollution source was a sewage treatment effluent. In this case, FRNA phage levels were equal to or greater than the bacterial indicator levels. The reason for this was probably the known greater resistance of FRNA phage to chlorine disinfection compared to that of the bacteria.

♦ An important finding was that serotyping of the isolated F+ RNA coliphage correctly identified that these indicators were derived from human or animal sources. Depending upon the outcome of additional studies, FRNA phage enumeration and serotyping could be used to identify if pollution is of human or non-human origin. FRNA phage serotyping may help assess the human health risk posed by HEV in coastal waters following storm events that cause elevations in bacterial indicator levels.

♦ Human enteric virus was detected in only 1 of 19 water samples (15 to 77 liters) containing human pollution. This sample was one of only 4 samples collected at the human, point source site; the site most likely to contain HEV. Therefore, the ability of the various bacterial or coliphage indicator organisms to predict the presence of HEV in water could not be determined in this study.

♦ HEV were detected in 16 of 31 samples of oysters (50 grams of meat) collected in a tidal river in North Carolina impacted by a sewage treatment effluent and closed to harvest. Levels of both bacterial indicators and of coliphage were higher in oysters in which HEV were detected compared to samples in which HEV were not detected.

RECOMMENDATIONS

♦ Various regulatory agencies are considering the use of coliphage as indicators of fecal and/or viral pollution [15]. The results of this study support the ability of F+ RNA coliphage to identify human pollution and the potential presence of HEV. The ability of the F+ RNA coliphage assay to indicate the presence of human-derived pollution in the New York Harbor is currently being investigated in studies sponsored by the New York/New Jersey Harbor Estuary Program [16]. Additional studies of this kind are needed so that the ability of the coliphage assay to determine the sanitary quality of bathing and shellfish harvest waters and to distinguish human from non-human pollution can be more thoroughly evaluated.

♦ Antisera to the four F+ RNA coliphage serotypes must become more readily available or alternative coliphage genotyping methods must be developed before the coliphage assay can be comprehensively confirmed as a valid human, viral pollution indicator by the scientific community.

♦ The project objective of correlating the presence and levels of coliphage and bacterial indicator organisms to HEV was not met for water samples as HEV were found in only 1 of 19 samples from human-impacted pollution sources. In future studies, greater emphasis should be placed on the examination of samples most directly and strongly impacted by human fecal waste sources, where HEV are most likely to be found, and using HEV isolation methods that sample greater volumes of water. One such study is underway [16].

FUNDING SOURCE

This work was funded by general research funds of the Division of Science and Research (DSR), the Division of Water Resources [since reorganized], and the Coastal Sewage Treatment Enforcement Act under Contracts P31040 and P32156.

ADDITIONAL INFORMATION

Copies of the Final Reports for this project are available from DSR (609-984-2212). A fee to cover the cost of reproduction may be charged. For general information about environmental research conducted and supported by DSR, call 609-984-6071, or write to the first page address. DSR Reference No. ____.

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TABLE 1. DESCRIPTION OF WATER SAMPLING SITES

Site	Category	Location	Pollution Source(s)
A	Non-point source, non-human	Navesink River; 4 locations downstream from horse farm area	Horses & livestock; some human possible (boats, marinas)
B	Point source, non-human	Forsythe Wildlife Refuge; drain pipes from 2 ponds & receiving bay waters	Waterfowl; wildlife
C	Non-point, human	Somers Point, Great Egg Harbor; 2 bay canal locations & 2 adjoining ship channel locations	Septic tank leachate; wildlife
E[a]	Point, human	Egg Harbor City, Landing Creek;[b] 4 locations downstream from STP outfall	STP effluent

a This site replaced Site D (Cox Hall Creek on the Delaware Bay) originally selected as the human, point site but later found to contain considerable non-human as well as human input.

b STP effluent = chlorinated effluent from a sewage treatment plant performing primary treatment.

TABLE 2. F+ RNA COLIPHAGE AND INDICATOR BACTERIA IN WATER SAMPLES FROM ALL SITES COMBINED (N = 94)

Indicator Organism[a]	Organism Concentrations per 100 ml[b]			
	N	Gm[c]	Median	Range
F+ Coliphage-SAL	92	3.0	3	<0.1-12,000
F+ Coliphage-MF	62	0.6	0.5	<0.05-200
Fecal coliforms	47	47.4	38	<1-4,780
E. coli	94	37.5	39.5	<1-4,300
Enterococci	94	17.4	19	<1-2,000
C. perfringens	92	15.8	10.5	<1-3,000

a SAL = single agar layer detection method; MF = membrane filter detection method.
b Coliphage units are plaque forming units per 100 ml. Bacteria units are colonies per 100 ml.
c Gm = Geometric mean.

TABLE 3. F+ RNA COLIPHAGES AND INDICATOR BACTERIA IN WATER SAMPLES FROM EACH STUDY AREA, 1989-1992

Indicator Organism[a]	Organism Concentrations per 100 ml			
	N	Gm	Median	Range
Site A: Non-human, non-point				
Coliphage	21	1.1	1.0	<0.1-25
FC	12	21.6	36	<1-290
EC	21	59.6	87	<1-1,960
Enterococci	21	17.6	18	<1-360
CP	21	17.9	17	3-200
Site B: Non-human, point				
Coliphage	20	0.5	1.0	<0.1-11
FC	7	96.1	63	<1.5-1,800
EC	20	18.4	34	1-2,200
Enterococci	20	4.9	2.5	<1-110
CP	20	2.0	2	<1-55
Site C: Human, non-point				
Coliphage	17	1.0	1.0	<0.1-26
FC	4	4.9	6.5	<1.5-10
EC	17	9.1	10	2-49
Enterococci	17	4.8	5	1-58
CP	17	5.1	7.5	1-25
Site E: Human, point[b]				
Coliphage	18	131	136	<0.1-12,000
FC	18	90.2	37.5	<1.5-4,780
EC	18	73.1	36.5	<1.5-4,300
Enterococci	18	71.7	57.5	<1.5-2,000
CP	18	216	165	25-3,000

a Coliphage = F+ RNA coliphage from the single agar layer (SAL) method; FC = fecal coliforms; EC = *E. coli*; CP = *Clostridium perfringens*.

b This site replaced Site D originally selected as the human, point site but later found to contain considerable non-human as well as human input.

TABLE 4. SEROTYPING OF F+ RNA COLIPHAGE ISOLATES

Site	Poll. Source	No. Isol.[a]	No. F+ RNA[b]	No. F+ RNA Coliphages in Serogroup[c]				
				I	II	III	IV	Other[d]
A	Non-hum. Non-pt.	77	9	4	1	0	2	2
B	Non-hum. Point	27	15	13	0	0	0	2
C	Human Non-pt.	40	12	3	9	0	0	0
E[e]	Human Point	28	25	0	9	2	0	14

a Total number of somatic and F+ phages isolated.

b Number that were F+ RNA coliphages.

c I = found only in animal waste; II = found primarily in human waste; III = found only in human waste; IV = found primarily in animal waste [9].

d F+ RNA coliphages that were not neutralized by any of the serogroup-specific antisera. They are considered genotypic and phenotypic intermediates [9].

e This site replaced Site D which was found to contain considerable non-human input.

TABLE 5. F+ RNA COLIPHAGES AND INDICATOR BACTERIA IN OYSTERS IMPACTED BY A CHLORINATED SEWAGE EFFLUENT

Indicator Organism[a]	Organism concentrations per 100 g		
	Gm	Median	Range
Station 1 (n = 21)[b]			
FRNA Phage	6,028	19,904	<3.2-188,800
FC	153	231	<4.5-9,200
EC	46	22	<2.8-1,120
Enterococci	350	210	50-14,250
CP	2,118	2,686	57-14,500
Station 2 (n = 21)[b]			
FRNA Phage	167	256	<3.2-10,740
FC	6	8	<0.2-2,450
EC	2	2	<0.2-55
Enterococci	68	58	<2.5-1,746
CP	339	476	29-3,429
HEV-Positive Samples, Stations 1 and 2 (n = 16)[c]			
FRNA Phage	3,372	2,928	<3.2-188,800[1]
FC	117	170	<4.5-2,450[2]
EC	37	15	<2.8-946[3]
Enterococci	244	173	38-2,900[4]
CP	1,578	2,686	57-14,500[5]
HEV-Negative Samples, Stations 1 and 2 (n = 15)[c]			
FRNA Phage	408	256	<3.2-122,240[1]
FC	21	12	<0.2-9,200[2]
EC	5	10	<0.2-295[3]
Enterococci	154	138	<2.5-14,250[4]
CP	603	610	29-6,137[5]

a FRNA Phage = F+ RNA coliphage; FC = fecal coliforms; EC = *E. coli*; CP = *C. perfringens*.
b Station 1 is 1.1 km from the sewage effluent and station 2 is 2.1 km from the effluent.
c Samples from either station where human enteric viruses (HEV) were or were not detected.

Wilcoxon Rank Sum 2-sample test p < :
1 = 0.046; 2 = 0.125; 3 = 0.093; 4 = 0.502; 5 = 0.059