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Seasonal Occurrence and Distribution of Microbial Indicators and Pathogens in the Rhode River of Chesapeake Bay

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The seasonal incidence and occurrence of indicator organisms and pathogens were studied at four sites in the Rhode River, a subestuary of Chesapeake Bay. The highest frequency of occurrence of total and fecal coliforms and fecal streptococci was in Muddy Creek, a marsh area receiving pasture land runoff. Second highest frequency of occurrence of these bacteria was in Cadle Creek, a populated area. Lowest measurements of these parameters were obtained at stations in the central portion of the Rhode River. No *Salmonella* spp. were detected by the methods employed in this study. However, it is concluded that if these organisms are present, the concentrations are ≤1 organism per liter. The presence of *Clostridium botulinum* was detected in 12% of the samples tested.

For many years, coliforms and fecal streptococci have been employed as indicators of the presence of pathogenic bacteria associated with fecal contamination (1). In recent years, natural water systems have received increasingly heavier nutrient loads. Increased nutrients in an aquatic system can act to protect microorganisms from effects of salinity and temperature, with the net result being survival of bacterial strains that would otherwise die off. Indicator organisms themselves demonstrate variability. It has been discovered that there are non-lactose-fermenting or H_2S-producing *Escherichia coli* strains in natural bodies of water (16, 17). Such variability, in conjunction with the presence of damaged cells entering water from treatment plants (5), contributes to increasing doubts about the significance of indicator organisms. Such doubts, coupled with a strong awareness of the problem of human pathogenic viruses transmitted via water supplies, has led to reevaluations of the currently employed indicator organisms and to the search for more reliable indicators (7, 18). In addition, pathogenic bacteria and viruses often are enumerated directly (6).

The objective of this study was, therefore, to evaluate indicator organisms as a part of a larger study of the microbial ecology of a small subestuary area of the Chesapeake Bay.

MATERIALS AND METHODS

The relationships between indicator organisms, i.e., fecal coliforms and fecal streptococci, and pathogenic bacteria, such as *Salmonella* spp. and *Clostridium botulinum*, in the Rhode River were studied over an annual cycle. Four sites, or stations, in the Rhode River, a tributary embayment of the Chesapeake Bay, were examined (see Fig. 1).

Station numbers indicate the distance, in miles, from the mouth of the Rhode River, except for CC0.6, which refers to the distance from the mouth of Cadle Creek which empties into the Rhode River. Station 0.0 is located at the junction of the Rhode and West Rivers and was selected for study because it provides useful information about bacteria entering and leaving the Rhode River system. Station 3.38, located at a relatively deep-water site, is considered to be representative of the main body of water in the Rhode River area. Cadle Creek is lightly populated, and the development includes several gas stations and some small marinas. Station 5.4 is a site in a marsh area of Muddy Creek.

Collection of samples. Water samples were collected at a depth of 1 m using a Niskin water sampler. The water samples were immediately transferred to a sterile bottle. Sediment was collected using a grab sampler. The sediment samples were placed in individual sterile, closed beakers. The samples were stored in ice when collected during the winter and in an insulated cooler in the summer. Total elapsed time between sampling and plating was ≤1 to 3 h because the work was done at the Smithsonian Institution field station located at the Rhode River.

Conductivity, salinity, and temperature were measured using an electrodeless induction salinometer (Oceanography Unlimited, Hoboken, N.J.). Dissolved oxygen was measured with a model 51A Oxygen Meter (YSI Instruments, Inc., Yellow Spring, Ohio). A secchi disk was used for turbidity measurements.

Enumeration of aerobic heterotrophic bacteria. Water samples (0.1 ml) were plated in triplicate on estuarine salt-water-yeast extract agar. Colonies appearing on the spread plates were counted after

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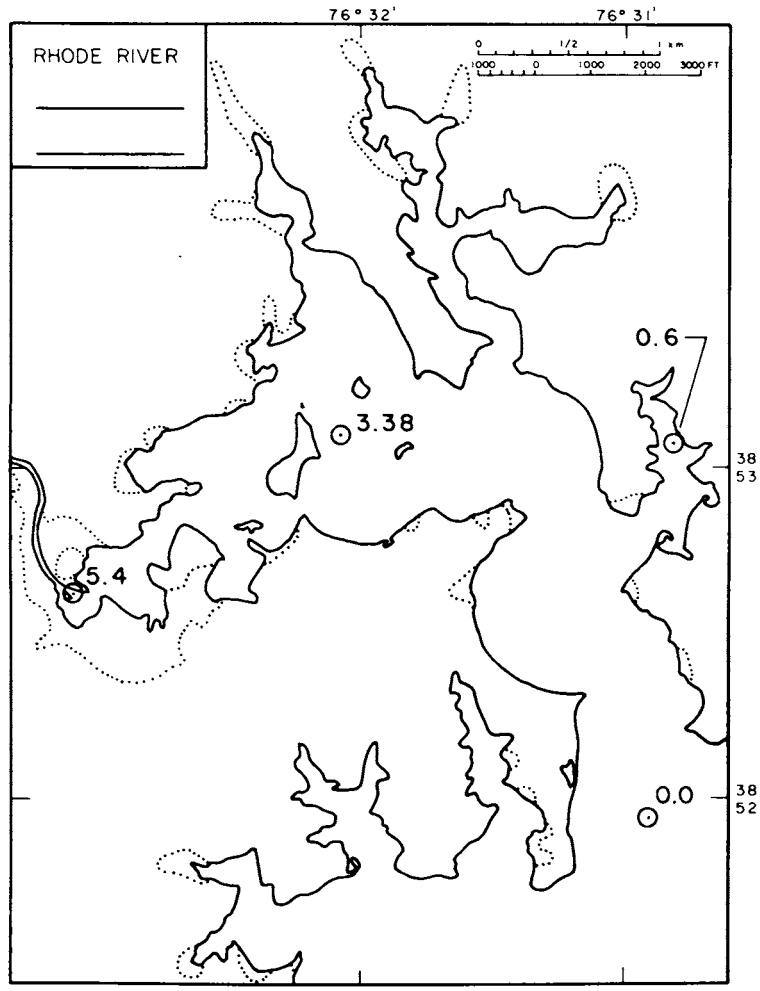

FIG. 1. *Map of the Rhode River subestuary showing the four sampling stations: 0.0, 3.38, 5.4, and CC 0.6.*

incubation at 15 C for 2 weeks. The estuarine salt-water-yeast extract medium consisted of 0.1% yeast extract, 0.1% proteose peptone, and 2.0% agar in a salts solution containing 0.23% magnesium sulfate heptahydrate, 0.025% potassium chloride, and 1.0% sodium chloride made up with distilled water, pH 7.2 to 7.4. Water samples were diluted 1:10 in sterile salts solution for enumeration. Sediment samples were prepared using volume displacement in 180 ml of sterile salts solution.

Enumeration of indicator organisms. Standard methods were employed for enumeration and isolation of indicator organisms (1). Three five-tube, 10-fold dilutions of lactose broth were used to determine most probable number (MPN) of coliforms. After incubation at 35 C for 24 to 48 h, positive tubes were confirmed by transfer to brilliant green lactose bile broth, or plated on eosin methylene blue agar. The inoculated brilliant green lactose bile tubes were incubated at 35 C for 48 h. A five-tube, three 10-fold dilution series of EC broth (Difco) was inoculated, and the tubes were incubated at 44.5 C (±0.5 C) for 24 h for fecal coliform determinations. Fecal streptococci MPN was determined using a five-tube, three 10-fold dilution sodium azide dextrose broth series inoculated and incubated at 35 C for 48 h. Confirmation of fecal streptococci was achieved by transfer of positive sodium azide dextrose broth cultures to ethyl violet azide broth tubes, which were incubated, after inoculation, at 35 C for 48 h. Cultures from ethyl violet azide-positive tubes were streaked on M-enterococcus medium and subsequently tested for catalase, oxidase, growth in 6.5% sodium chloride, and ability to hydrolyze starch and sodium hippurate (9). All media were obtained from Difco Laboratories, Detroit, Mich. Correlation coefficients were determined using the Stat 12 program. Calculations were done on the Control Data Corporation CDC 6600 computer, University of Maryland Computation Center.

Detection of C. botulinum. The presence of *C. botulinum* was determined indirectly by testing for the presence of specific neurotoxins (8). Sediment samples (ca. 1 g) were added to a sterile cooked meat medium which had been boiled for 10 min and cooled immediately prior to inoculation. The inoculated tubes were overlaid with mineral oil, incubated at 25 C for 5 days, and frozen at −70 C until examined further.

Polyvalent antiserum and specific anti-A, B, C, D, and E antisera were obtained through the courtesy of V. Dowell, Center for Disease Control, Atlanta, Ga.

Isolation of Salmonella spp. Water was examined for the presence of *Salmonella* by filtering 1 liter of a sample through membrane filters (Millipore Corp., 0.45 μm), after which the filters were folded and immersed in selenite-cysteine broth. The broth was overlaid with mineral oil and incubated at 43 C. After 24 h, the cultures were streaked on desoxycholate, bismuth sulfite, or *Salmonella-Shigella* agar. Cultures suspected to be *Salmonella* spp. were isolated and tested for catalase, oxidase, urease, and production of hydrogen sulfide. Presumptive *Salmonella* spp. were characterized using the API system (Analytab Products, Inc., New York, N.Y.), and identification was confirmed by serological methods.

Presence of VPLO. A five-tube, three 10-fold dilution MPN series employing a salt colistin broth medium was inoculated and incubated at 35 C for 12 to 24 h. Each tube showing turbidity was streaked on TCBS agar plates. The inoculated TCBS agar plates were incubated at 35 C for 24 h. A shortage of colistin necessitated a change in the method, and a direct enumeration by plating on TCBS plates was subsequently employed. Greenish, typical *V. parahaemolyticus* colonies were recorded as *V. parahaemolyticus*-like organisms (VPLO).

RESULTS

Enumeration of aerobic heterotrophic bacteria. A preliminary study was undertaken to compare the effect of incubation temperature on heterotrophic counts. A series of incubation temperatures was used, including 2, 5, 15, 25, 35, 41, and 55 C. The highest number of colonies was obtained when plates were incubated for 2 weeks at 15 C. The total viable population of aerobic, heterotrophic bacteria in the water column was found to vary between 1.9×10^2 bacteria/ml in December and 2.2×10^5 bacteria/ml in February (Fig. 2). The counts at stations 0.0, 3.38, and CC0.6 followed a similar general pattern, showing a rise in counts during the period January through March. Although the total numbers of aerobic, heterotrophic bacteria in the water column at Station 5.4 paralleled that of the other stations during the period January through April, sporadic increases were observed, particularly in November. Counts at the Muddy Creek Station were always at least 1.7×10^3 bacteria/ml, and counts at Cadle Creek were at least 1.3×10^3 bacteria/ml.

No readily discernible pattern was noted for numbers of aerobic, heterotrophic bacteria in the sediments (Fig. 3). Counts varied from 1.4×10^4 to 9.4×10^6 bacteria/g. Both the extremely low and the high total viable counts were recorded at Station 5.4 in October 1973 and June 1974, respectively. Oscillations in the total counts for the sediment were observed at all stations, without strong evidence of a correlation with given stations or for the time of sampling.

MPN of indicator organisms. Total coliforms were found in low numbers in the water at Stations 0.0 and 3.38, with both stations showing elevated coliform counts in June (Table 1). Coliform counts in the water at Station 5.4 were relatively high, falling below 100/100 ml only in November 1973. The total number of coliforms at Cadle Creek was higher than at Station 0.0 or 3.38 but, in general, was lower than counts obtained at Station 5.4. Total coliforms in the sediments, although present in higher numbers than in the water, did not parallel counts for the water column, nor were

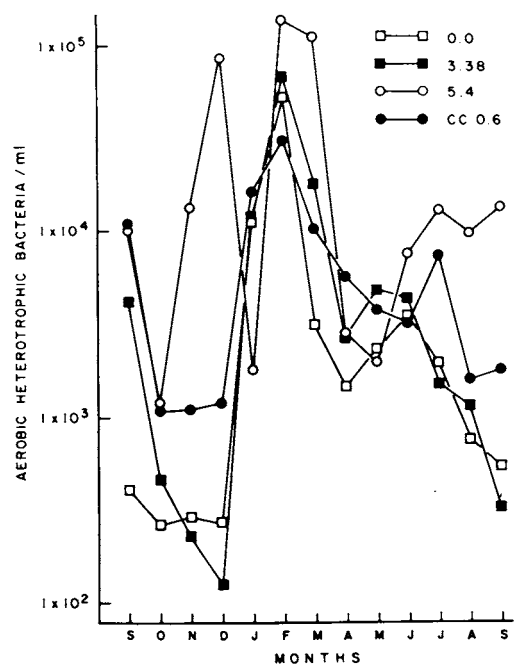

FIG. 2. *Total number of aerobic heterotrophic bacteria (per milliliter of water), September 1973 through September 1974.*

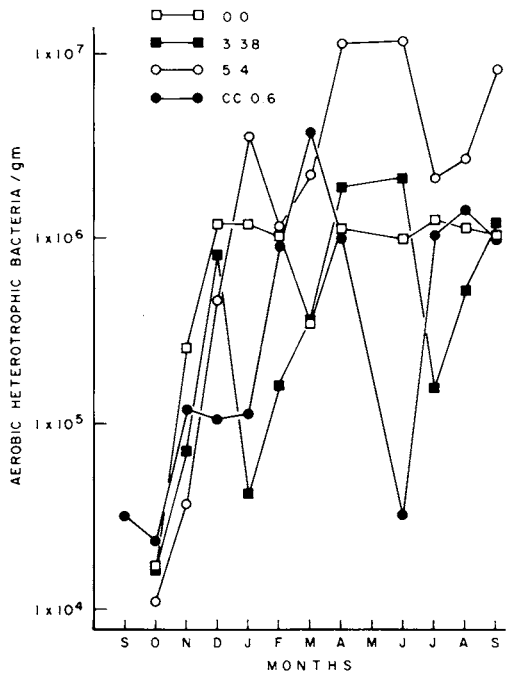

FIG. 3. *Total number of aerobic heterotrophic bacteria (per gram of sediment), September 1973 through September 1974.*

sediment counts higher after a peak was observed for the water column the previous month. The greatest numbers of total coliforms for all four stations, in both water and sediment, were observed in June 1974.

As expected, the numbers of fecal coliforms were much lower than the total coliform count (Table 2). Very high numbers of fecal coliforms were found in December 1973 and June 1974, but only at Station 5.4. The sediment counts were higher than the water counts, except at Station 5.4, but were not enormously high, with the exception of the May samples collected at Cadle Creek and in June at Stations 0.0 and 5.4. Frequently fecal coliforms could not be detected in the water and sediment samples. When the portion of the total coliform population represented by coliforms of fecal origin was determined, it became obvious that most of the total coliform population probably was derived only in small part from fecal wastes. Fecal contamination was more often responsible for coliforms in the water column, however, than in the sediment. In May almost all of the coliforms were probably derived from fecal contamination of the water.

When the variation in bacterial levels at the four stations was normalized by determining the number of fecal coliforms per 10^6 aerobic, heterotrophic bacteria, it was apparent that the coliforms formed a small but relatively consistent portion of the sediment population (Table 3). Only in June and August at Station 5.4, and in December at Station 3.38, did the coliforms in water show a significant increase. The coliforms varied in numbers from fewer than one fecal coliform organism per 10^6 to as many as 6,067/10^6 in the water at Muddy Creek. In every case, fecal coliforms comprised less than 1% of the total population. The ratio of fecal coliforms in the water to fecal coliforms in the sediments was observed to be between 0 and 1 for Stations

TABLE 1. *MPN of total coliforms for samples collected in the Rhode River, September 1973 through September 1974*

Month	MPN of total coliforms in:							
	Water (100 ml)				Sediment (10 g)			
	0.0[a]	3.38	5.4	CC0.6	0.0	3.38	5.4	CC0.6
Sept.	33	33	≧2,400	920				
Oct.	17	11	240	22				
Nov.	0	70	11	240				
Dec.	17	79	≧2,400	540	140	140	260	170
Jan.	130	33	130	240		350	1,600	350
Feb.	30	50	540	90	170	240	920	90
Mar.	10	20	350	90	280	920	≧2,400	350
Apr.	60	220	1,600	180	≧2,400	920	≧2,400	350
May	49	79	540	79	140	350	1,600	1,600
June	3,500	790	5,400	24,000	≧2,400	5,400	3,500	2,400
July	0	40	490	490	460	1,300	1,300	1,400
Aug.	20	80	28,000	2,400	60	1,100	1,700	3,300
Sept.	7	33	540	23	26	79	180	350

[a] Station number.

TABLE 2. *MPN of fecal coliforms for samples collected in the Rhode River, September 1973 through September 1974*

Month	MPN of fecal coliforms in:							
	Water (100 ml)				Sediment (10 g)			
	0.0[a]	3.38	5.4	CC0.6	0.0	3.38	5.4	CC0.6
Sept.	2	2	46	17				
Oct.	17	5	33	17				
Nov.								
Dec.	0	22	≧2,400	130	0	70	260	0
Jan.	5	0	49	70				
Feb.	20	0	20	10	0	0	20	10
Mar.	10	10	220	90	20	10	40	40
Apr.	20	90	60	40	10	10	60	10
May	49	79	220	79	140	240	17	1,600
June	170	0	5,400	110	330	0	490	0
July	0	0	80	17	50	20	20	50
Aug.	0	20	130	20	0	50	70	80
Sept.	0	17	49	5	0	0	8	2

[a] Station number.

TABLE 3. *Number of fecal coliforms/10^6 aerobic, heterotrophic bacteria observed in water and sediment samples collected in the Rhode River, September 1973 through September 1974*

Month	No. of fecal coliforms/10^6 bacteria							
	Water				Sediment			
	0.0[a]	3.38	5.4	CC0.6	0.0	3.38	5.4	CC0.6
Sept.	30	3.0	460	13				
Oct.	35	71	194	130				
Nov.	—[b]	—	—	—				
Dec.	0	1,157	255	76.0	0	7.6	37	0
Jan.	3.5	0	14.8	24	—	—	—	—
Feb.	2.6	0	0.9	1.8	0	0	1.3	1.0
Mar.	17.9	3.0	15.7	82	0.33	1.6	0.97	0.6
Apr.	80	183	117	50	0.66	2.9	0.35	1.0
May	13.9	109	594	127				
June	1.6	0	6,067	200	330	0	2.7	0
July	0	0	38	20	2.5	7.1	0.5	3.8
Aug.	0	125	1,300	68	0	6.6	1.4	3.2
Sept.	0	298	21.0	14	0	0	0.08	0.2

[a] Station number.
[b] —, Not determined.

0.0 and 3.38, with one exception. At Cadle Creek, ratios varied from 0.25 to 4, whereas at Station 5.4 they were always greater than 1.

Very low numbers of fecal streptococci were found in the water column, with the exception of samples collected at Muddy Creek in May 1974 (Table 4). With the single exception of samples collected in June, no more than two fecal streptococci were found in the water at Station 0.0 or 3.38; low numbers of fecal streptococci were found in the sediment at these two stations, with higher numbers obtained at Station 5.4 and Cadle Creek. Transfers were made from all tubes showing a precipitate, whether white or purple, onto M-enterococcus agar. More than 95% of these cultures were confirmed as fecal streptococci according to a set of selected tests suggested by Facklam et al. (9).

The fecal coliform-fecal streptococci index calculated from the data given in Tables 1 to 4 revealed that higher ratios were found more frequently in the water column than in the sediment, notably at Stations 0.0, 5.4 and CC0.6 (Table 5).

Correlation coefficients, given in Table 6, indicate that there were detectable differences between the two seemingly similar locations, Stations 0.0 and 3.38. Strong correlation was

TABLE 4. *MPN of confirmed fecal streptococci for samples collected in the Rhode River, September 1973 through September 1974*

Month	MPN of confirmed fecal streptococci							
	Water (100 ml)				Sediment (10 g)			
	0.0[a]	3.38	5.4	CC0.6	0.0	3.38	5.4	CC0.6
Jan.	2	0	23	5	23	0	≧2,400	6
Feb.	2	0	0	0	7	0	10	14
Mar.	2	0	17	17	9	17	17	33
Apr.	0	0	13	7	33	0	26	5
May	0	0	≧2,400	8	17	0	≧2,400	≧2,400
June	13	0	0	70	33	0	79	160
July	0	0	17	10	0	0	0	220

[a] Station number.

observed for indicator organisms at Station 0.0 in the water column and for the total coliforms and fecal streptococci in the sediments. No correlation was observed for the indicator organisms at Station 3.38 in either the water or sediment. Values for total coliforms and fecal coliforms showed a positive correlation for Station 5.4, and a low, but significant, correlation for Station CC0.6. Interestingly, all values for indicator organisms showed correlation for Cadle Creek, both for the water column and the sediment. Total counts, in general, did not show significant correlation with the occurrence of indicator organisms, except for the fecal streptococci in the sediment at Station 3.38 and Cadle Creek.

Presence of potentially pathogenic bacteria. Sediment samples collected during January, April, May, June, July, and August were examined for the presence of *C. botulinum* by testing for *C. botulinum* toxin. *C. botulinum* toxin was detected in April, June, and July. In June, *C. botulinum* toxin type E was identified. April and July samples indicated the presence of *C. botulinum* toxin types B or E. The amount of sample was insufficient for further discrimination.

No *Salmonella* spp. were detected at any of the four stations. *Salmonella*-like organisms were isolated on the various differential media employed, but these isolates commonly were found to be *Proteus* spp., *Citrobacter* spp., or other enteric organisms.

No VPLO were found in the water or sediment at Stations 3.38 and 5.4 during January. VPLO appeared in the water column in April; the numbers averaged 50/ml in the water and 100/g in the sediments. In June and July, the VPLO counts ranged from 0 to 700/ml in the water and from 0 to 44,000/g in the sediments.

Physical parameters. Turbidity was observed to increase in the winter months at Stations 0.0, 3.38 and CC0.6. Transparency of the water at Station 5.4 was rarely greater than 0.25 m. Correlation coefficients ranging from 0.75 to 0.88 were noted for turbidity and total viable counts at Stations 0.0, 3.38 and 5.4, suggesting that there was either increased runoff or resuspension at these stations. No correlation was noted for these parameters at Cadle Creek.

Salinity (5 to 9‰) was lowest in the winter and increasing in the spring to a maximum during September and October (10 to 18‰). Dissolved oxygen, measured at a depth of 1 m at all stations, varied from 7.4 μl/liter in July to 13.5 μl/liter in January. Lowest temperatures, 2 to 3 C, occurred in January-February, and the highest, 28 C, occurred in September.

DISCUSSION

An increase in total viable, aerobic, heterotrophic bacteria in the water column was observed at all stations examined in the Rhode River during the 3-month period January through March. Similar results have been recorded in studies of hydrocarbon-utilizing bacteria at several Chesapeake Bay Stations (7a). Since the Muddy Creek site in the Rhode River is shallow, with relatively turbid water, much of the sediment can be considered to be resuspended. It is a narrow creek, with significant input from terrestrial runoff. Either or both of these conditions account for the sporadic increases in bacterial counts which were observed in this study (Fig. 2). No seasonal distribution was found for the populations of bacteria in sediment. It should be pointed out that a vertical distribution of heterotrophs has been recorded by other investigators for sediment, with the greatest number of bacteria found in the upper 1 cm (2). It was very difficult to sample the upper 1 cm of sediment with the

grab samplers available for this study because of the sediment-water mixing that occurred at the interface upon closure of the grab. Such turbulence is much greater in grab samplers which require that the entire contents be removed to sample the surface portion. Consequently, although attempts were always made to achieve uniform sampling, a mixture of the first few centimeters very likely contributed to the differences in counts observed for sediment samples.

Fecal coliforms were found predominantly at the Muddy Creek Station. Higher fecal coliform counts were observed at Cadle Creek compared with Stations 0.0 and 3.38. Dilution occurring at the entrance of Muddy Creek to the Rhode River may account for the reduced coliform counts at Stations 3.38 and 0.0. Die-off may also be a factor. Resuspension of coliforms and heterotrophs can occur. However, the volume of water at Stations 0.0 and 3.38 is such that a very large number of microorganisms would have to occur in the sediment before resuspension could result in such an increase in the observed

TABLE 5. *Ratio of fecal coliforms to fecal streptococci (FC/FS) in water and sediment samples collected in the Rhode River, September 1973 through September 1974*

Month	Ratio of FC/FS							
	Water				Sediment			
	0.0[a]	3.38	5.4	CC0.6	0.0	3.38	5.4	CC0.6
Jan.	2.5	ND[b]	2.1	14				
Feb.	10.0	0	ND	ND	0	0	0.2	0.7
Mar.	5.0	ND	12.9	5.3	2.2	2.5	2.3	1.2
Apr.	ND	ND	4.6	5.7	0.3	ND	2.3	2.0
May	ND	ND	0.1	9.9	8.2	ND	0	0.5
June	13	0	ND	1.6	10	0	6.2	0
July	0	0	4.7	1.7	ND	ND	ND	0.2

[a] Station number.
[b] ND, Fecal coliforms not detected.

TABLE 6. *Two-way table of correlation coefficients for indicator organisms and total viable counts*[a]

Indication organism	Correlation coefficient					
	Water			Sediment		
	FC	FS	TVC	FC	FS	TVC
0.0[b]						
TC	0.96	0.98	−0.13	0.58	0.96	−0.54
FC		0.92	−0.09		0.54	−0.48
FS			−0.00			−0.44
3.38						
TC	−0.08	0.00	−0.14	−0.37	−0.23	0.09
FC		0.00	−0.03		0.13	0.06
FS			0.00			0.93
5.4						
TC	0.96	−0.18	−0.29	−0.54	0.46	−0.43
FC		−0.15	−0.25		0.36	0.58
FS			−0.28			−0.31
CC0.6						
TC	0.61	0.98	−0.30	0.81	0.88	0.82
FC		0.72	−0.63		0.97	0.70
FS			−0.44			0.79

[a] Values higher than 0.71 are significant at the 0.01 level. TC, Total coliforms; FC, fecal coliforms; FS, fecal streptococci; TVC, total viable count.
[b] Station number.

counts. In shallower areas, such as Muddy Creek or Cadle Creek, resuspension does occur.

It should be noted that fecal coliforms were found in ≥75% of the sediment samples examined. If the fecal coliform counts are normalized with respect to the aerobic, heterotrophic population, an increase in the entire population can thereby be distinguished from an increase in an indicator organism. By this computation, the increased counts at Station 3.38 in December and at Station 5.4 in June and August indicated a significant increase in the number of fecal coliforms. Rather than a seasonal trend, there appears to be a low background level of coliforms with sporadic increases occurring throughout the year.

Very few fecal streptococci were isolated from water samples collected at Stations 0.0 and 3.38 or in sediment samples collected at Station 3.38. In general, the fecal streptococci were present in much lower numbers than the fecal coliforms. However, very low but detectable numbers of fecal streptococci were found in sediment samples collected at Stations 0.0, 5.4, and Cadle Creek in ≥90% of the samples (Table 4).

In 1969, Geldreich and Kenner (11) suggested that the ratio of fecal coliforms to fecal streptococci could be used to determine the source of pollution, with ratios greater than 4.0 indicating sewage effluents and those lower than 0.7 suggesting nonhuman, warm-blooded animal sources. This was supported by Feachem, who suggested human and pig sources of pollution could be separated (10). The fecal coliforms-fecal streptococci ratios for Station 0.0, Muddy Creek, and Cadle Creek indicated that recent sources of pollution existed at these sites, particularly in the water column and during the spring months at Muddy Creek and Cadle Creek. It is necessary, in future research work, to determine whether this increase is a result of more rapid and widespread runoff during spring rains, from a defined point source, or from nonpoint sources of pollution.

Several methods for detection of indicator organisms were employed in these studies. The MPN tube technique was concluded to be preferable to filtration because of difficulty in enumerating sediment populations by the latter method. Whereas no difficulty was encountered with the methods employed for enumeration of total coliforms and fecal coliforms, the tests themselves cannot be considered accurate. Not all *E. coli* organisms are able to ferment lactose with consequent gas production. In this study, tubes showing turbidity without gas production were observed. Such cultures may have been coliforms, especially since effluents from sewage treatment plants can contain *E. coli* organisms injured by chlorination treatment and unable to grow or, if retaining the capability to grow, not producing gas in lactose broth (5). Studies on survival of *Salmonella* spp. and other pathogenic bacteria subjected to sewage treatment procedures, including chlorination, obviously are needed.

Methods employed in this study for the enumeration of fecal streptococci were found to be reliable. Results of selected biochemical tests revealed that ≥95% of the cultures giving a positive reaction contained organisms subsequently identified as enterococci.

Van Donsel and Geldreich (19) observed a correspondence between the presence of *Salmonella* and fecal coliforms in sediments. By extrapolating from their data for results obtained in this study, it was determined that in any given sediment sample there would have been only a 20% possibility of finding *Salmonella*, even with high coliform counts. Cohen and Shuval (7) concluded that fecal streptococci are more resistant to die-off in natural waters and suggested that a better correlation for measurement would be survival of fecal streptococci and presence of enteric viruses. Hoadley and Cheng (12) have shown that *Streptococcus faecalis* organisms are much more resistant than *E. coli* to stress, in particular when presented with selective media. Considering the erratic coliform counts observed in this study for Rhode River water and sediment samples and the problems encountered with the coliform procedures as cited above, greater reliance might better be placed on fecal streptococci as an indicator of fecal pollution and/or the presence of potentially pathogenic organisms. Enumeration of coliforms by coliphage counts (15) is another approach which has been considered.

Water and sediment samples were examined directly for pathogenic bacteria, including *Salmonella* and *C. botulinum* in this study. None of the cultures that were presumptive *Salmonella* spp. on selective media were confirmed as *Salmonella* spp. In general, very large volumes of water are required for the direct isolation and identification of *Salmonella*. Results, similar to those reported here, were obtained in our laboratory during the course of a study of water, sediment, and suspended sediments of the upper Chesapeake Bay (G. Sayler, R. R. Colwell, A. Hirsch, and J. Nelson, manuscript in preparation). Improved methods for direct isolation and identification of *Salmonella* spp. such as, for example, the fluorescent antibody technique (5), may be more efficient for detection of *Salmonella* spp. occurring at extremely low numbers.

Counts of *Clostridium perfringens* in sedi-

ments have been found to parallel areas of increased pollution (4). An indirect method, i.e., toxin production, was successful in detecting *C. botulinum* in 12% of the samples tested. The technique for detection of toxin is simple and reliable. Unfortunately, a rather long period of time is required for growth and testing.

VPLO were found in the warmer months in both water and sediment. These results corroborate those of Kaneko and Colwell (13), with the exception that VPLO were detected in the water column somewhat earlier than previously reported. Since a strong relationship of VPLO to *V. parahaemolyticus* has been suggested (13), direct enumeration of VPLO may be a useful indicator for the presence of *V. parahaemolyticus*.

The general conclusions to be drawn from the results of this study are as follows. In the Rhode River, a subestuary of the Chesapeake Bay, fecal coliforms, fecal streptococci, and the bacterium *C. botulinum* can be isolated despite the lack of obvious point sources of human fecal pollution. Entry of coliforms to the Chesapeake Bay via such subestuary routes can, considering the morphological structure of the Chesapeake Bay tributary system, account for significant enteric bacterial input in the aggregate. It should be emphasized that nonpoint sources of pollution, with ever-increasing development of Chesapeake Bay wetlands, become an important pollution problem.

Coliform, fecal coliform, and fecal streptococci counts, although helpful in assaying the general quality of the estuarine aquatic environment in gross terms, are insufficient indicators of the potential presence of enteric bacterial pathogens. Although no *Salmonella* spp. were isolated, the presence of *C. botulinum* was confirmed in 12% of the samples, and VPLO were also found in the warmer months of the year.

The effects of the estuarine environment on enteric pathogens such as *Salmonella* and *Shigella* remain to be determined. Such pathogens may not survive in estuaries or may become debilitated or altered by the low temperature, high salt concentration, and other environmental influences, so that selective media are, in essence, a "final blow." It may eventually be shown that more protective, less harsh media are required for recovery of *Salmonella* from estuarine and coastal waters, as well as sampling larger volumes of water. *V. parahaemolyticus*, native to the estuarine environment (13), may ultimately be a better indicator of water quality for estuarine and coastal waters.

The problem of human viruses and their transmission via water has only recently been recognized, and methods for isolation and enumeration of enteric viruses in natural waters are available but are not yet routinely applied to environmental monitoring. Clearly, there is a need for a reliable and more widely applicable indicator system than the coliform procedures currently in use.

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