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Occurrence of indicators of fecal pollution in water and sediment of a subestuary impacted by non-point pollution

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Introduction

The overall sanitary microbiological quality of a subestuarine shellfish growing area is necessarily related to the types and magnitudes of fecal pollution in the watershed. The distribution of fecal pollution in the estuary can vary as a function of input characteristics, precipitation, the effects of site hydrography, physical/chemical processes such as adsorption/sedimentation, and importantly, the survival characteristics of enteric microorganisms. The latter is a complex function of microorganism physiology and as well as ecological interactions with various biological and physical factors such as temperature, sunlight, predation/antagonism, chemical inhibition and adsorption. The relative role of these factors will be a function of the unique characteristics of each environment, which can be expected to vary in both time and space.

Pollution sources may include direct human pollution, septic tank discharge or subsurface leaching and infiltration, discharge from an STP, transient discharge from vessels, and pollution from both domestic and wild animals. Pollution from animals presents a difficult and perplexing issue, one which has led to serious questioning of the applicability and public health significance of the current coliform or fecal coliform growing area standards in receiving waters where pollution sources other than wild or domestic animals are unknown or have been eliminated. The problem stems mainly from the recognition that although animals can contribute to fecal coliform loadings in receiving waters, it is generally agreed that animal fecal pollution is intuitively "less dangerous" than human pollution because animals are not sources of the more virulent shellfish-transmitted human enteric viral pathogens, i.e., hepatitis, Norwalk agent. Resolving the "animal issue" will require a number of basic research initiatives which include determining the health risk associated with consumption of shellfish from such areas and importantly, evaluation of alternate indicators and the development of methods to differentiate human from animal fecal pollution. It is the latter topic which is addressed in this report.

As stated there is direct and indirect evidence that populations of domestic and/or wild animals contribute to fecal pollution of estuarine receiving waters and thereby negatively impact the water quality of shellfish growing areas. Unfortunately, other than the sanitary survey, proven and reliable techniques are unavailable to differentiate human from animal fecal pollution. The overall objective of this study was to evaluate candidate indicators of fecal pollution (in conjunction with the standard APHA fecal coliform MPN method) which may provide this capability. In particular, we chose to evaluate the applicability of these methods in Timberneck Creek, Virginia, in order to examine the allegation that a local livestock farm ("the Catlett farm") is a major contributing factor to the deterioration of sanitary water quality in the creek. This subestuary of the York River is closed to direct harvesting of shellfish due to elevated levels of indicator bacteria. Finally, we also desired to evaluate the hypothesis that the use multiple indicators with contrasting source specificities and dieoff characteristics may provide a means to identify source and assess "age" of fecal contamination.

The indicators used in this study included the fecal coliform, Streptococcus bovis, a fecal streptococcus found in domestic farm animals; sorbitol-fermenting strains of Bifidobacterium spp. (sorbitol-fermenting bifids are considered relatively unique to human sources); and Rhodococcus coprophilus, an actinomycete which has been isolated exclusively from animal and not human feces. Finally, a new indicator, the so-called "male-specific" phage, i.e., RNA phages which infect only male strains of Escherichia coli, was added during the latter portion of this study. These phages are considered to be specific to sewage and the feces of warm blooded animals. Male strains of E. coli contain a small circular piece of DNA which is found in the cell cytoplasm and not part of the chromosome. This DNA is called the sex factor or F^+ (fertility factor) and cells containing it are called male in that they are donors in mating; F^- (female) cells lack this factor and are recipients. A method for the selective detection of these phages from environmental samples (Havelaar et al., 1984), was used in this study.

Materials and methods

Study site. Timberneck Creek (Figure 1) is comparatively small (ca. 7.2 x $10^{-1}km^2$) tidal creek located on the north shore of the York River in Virginia. This portion of the river is estuarine and creek salinities were comparatively homogeneous longitudinally with a mean value of 22 o/oo at the mouth. Feeder streams varied considerably in cross section and volume, these properties being related somewhat to precipitation or lack thereof. (Some of the following characteristics of the Timberneck Creek watershed were obtained from the Bureau of Shellfish Sanitation shoreline survey (dated 11-2-88), Virginia Department of Health.) Topographically, the area is characterized by low and marshy elevations of 1-2 m near the mouth and moderate elevations which can rise to 15-20m at the headwaters. Soil characteristics of the area are generally well drained to moderately well drained with dominantly loamy subsoil. The area is primarily residential but businesses are concentrated along Route 17. Development in the area has increased rapidly and there are fairly dense populations on the eastern side of the creek watershed adjacent to Route 17. Considerably fewer single family homes are sparsely distributed on the western shore. All homes surveyed were served by individual on-site sewage facilities and there are no septage disposal sites, animal wastes facilities or sewage treatment plants in the area. There is one comparatively large livestock farming operation ("the Catlett farm", see Figure 1)) and at least 9 other residents were identified as "hobby" farmers and classified by the Bureau of Shellfish Sanitation as "contributing animal pollution". Eight residencies were cited as "contributing pollution" owing to frank septic discharge or full privies according to the most recent sanitary survey. Timberneck Creek is currently classified as a Condemned Shellfish area.

Sampling, sample collection and transport. Timberneck Creek was initially sampled at approximately monthly intervals and after rain events. Following a number of preliminary but comprehensive surveys, we developed a sampling plan to focus on selected feeder streams and potential "hot spots". Water samples were collected in sterile glass bottles by grab sampling at 0.3 -1.0 m depth except at shallow sites where the bottles were immersed immediately beneath the surface. Care was taken to avoid disturbing the sediment when sampling.

Surface sediment (ca. top 0.5 cm) from feeder streams was collected with a clean spatula or with a small "Petersen-type" grab in the estuary. Samples were maintained at ambient temperature in insulated containers during transport to the laboratory. Total elapsed time from sampling to processing was 2 h or less. Ambient water and sediment temperatures were measured at the time of sampling; salinity and turbidity were determined in the laboratory using a temperature compensated refractometer (American Optical Corp.) and a turbidometer (Hach Co.).

Bacterial indicator enumeration. Water samples were diluted or membrane filtered as required. Sediment homogenates were prepared by mixing 20 g wet sediment in 180 ml sterile estuarine (mainstem stations) or phosphate buffer dilution water (APHA, 1985) (feeder stream stations) water for 60-90 seconds. Fecal coliform densities were determined by the five-tube MPN technique (APHA, 1985). Volumes of 10 ml or 1g portions of sediment and selected dilutions thereof were inoculated into lactose broth. Positive EC tubes from selected samples were examined for the presence of Escherichia coli by IMViC, lysine and ornithine decarboxylase tests (APHA, 1985).

S. bovis and sorbitol-fermenting bifidobacteria were enumerated by membrane filtration. A range of sample volumes was processed because the density of these organisms in feeder streams and estuarine waters was unknown. Quadruplicate 25 ml and triplicate 1 ml water volumes were filtered. Sediment homogenates were diluted 10x and 100x in phosphate buffer (APHA, 1985) or sterile estuarine water. Phosphate buffer (APHA, 1985) and gelatin diluent (NCDC, 1968), used for membrane filtration of S. bovis and the bifidobacteria, were added prior to filtration of 1.0 ml sample volumes to insure even distribution of cells and for rinsing filtration units. S. bovis was enumerated using modified membrane-Bovis agar (m-BA) (Oragui and Mara, 1981). Sorbitol-fermenting bifidobacteria were enumerated on human bifid sorbitol agar (HBSA) (Mara and Oragui, 1983). Plates were incubated anaerobically in Gas Pak systems (BBL Microbiology Systems, Cockeysville, Md.) systems for 4.0 h at room temperature and subsequently for 48-72 h at 39.5 (S. bovis) and 35 C (Bifidobacterium spp.). Selected typical colonies on m-BA were subcultured for purity and characterized according to recommended methods (Manual of Clinical Microbiology, 1985). Physiological tests included: growth in 6.5% NaCl and at

10 C, hydrolysis of starch, arginine and bile esculin; fermentation of lactose, sorbitol, raffinose, inulin and mannitol; and glucan production. Sorbitol-fermenting colonies were considered to be bifidobacteria if they were obligately anaerobic and displayed typical bifurcated "Y and V" morphological forms.

Rhodococcus coprophilus was enumerated by spread plating 0.1 ml of a heat treated sample (Rowbotham and Cross, 1977) in triplicate onto MM3 agar (Mara and Oragui, 1981). Plates were incubated at room temperature for about two weeks and subsequently exposed to sunlight in the laboratory for 4-7 days. Typical stellate colonies with bright orange central papillae were considered R. coprophilus if by microscopic observation they were asteroidal with substrate mycelia and were sensitive to 7% NaCl in Bennett's agar and did not produce a pellicle on the surface of Bennett's broth (Rowbotham and Cross, 1977).

F-specific phage. Phage assays were performed using as the single bacterial host strain, Salmonella WG49, which was designed for enumeration of male-specific coliphage (Havelaar et al., 1984). Tryptone-yeast extract-glucose medium (TYEG) was supplemented with nalidixic acid (100 mg/1) and kanamycin sulfate (20 mg/1) to prevent background microbial growth and to maintain selective pressure on the host strain. The host was grown overnight at 35°C in TYEG broth and diluted 1:100 into fresh broth several hours prior to sample processing.

Environmental samples were examined using a single-layer agar technique whereby the sample was mixed with TYEG agar and sufficient volume of host added to yield approximately 2.5 ml host culture per 100 ml sample-assay agar mixture. Water samples were processed by adding a 100 ml sample to 100 ml tempered molten TYEG (2% agar), followed by gentle shaking and distributing over 9-10 petri dishes (100 mm diameter). Sediment samples were initially processed using three procedures: (1) 10 ml sediment homogenate (ca. 1 g) was mixed with 90 ml TYEG (1% agar) and poured into five petri dishes (100 mm diameter); two 10 g quantities were shaken with either (2) 30 ml 3% beef extract, pH 9.0-9.5 or (3) 30 ml 0.1% peptone in 0.1% Tween 80 at 200 rpm for 15 minutes and centrifuged at 5000 RCF at 5 °C for 15 minutes. The sediment

was discarded and the supernatant (after adjustment to pH 7.0-7.2 for procedure (2)) was mixed with 30 ml TYEG (2% agar) and distributed over 3 petri dishes (100 mm diameter). If a site was suspected as heavily contaminated, then 6 ml of eluate, representing ca. 2 g sediment, was mixed with 90 ml TYEG (1% agar) and distributed over five petri dishes (100 mm diameter) and the remainder processed with 30 ml TYEG (2% agar). (Procedure (3) was discarded after the 6-27-88 survey due to low phage recovery.) After solidifying, plates were inverted and incubated overnight at 35°C. Plaques were counted using oblique light.

Selected plaques were subcultured onto TYEG double-layer agar plates (2% agar base and 1% top agar containing the host). The top agar layer on plates showing confluent lysis was harvested using TYEG broth, centrifuged and the supernatant filtered through 0.22 um membrane filters. Phage isolates were examined for plaque production on (1) Salmonella WG49 and WG45, the latter being the female parent strain of WG49, to check for the presence of somatic Salmonella spp. phages, and (2) Salmonella WG49 with and without RNAase in the top agar to distinguish male-specific RNA from DNA phages.

Results and discussion

Distribution of fecal coliforms in the water column. Fecal coliform densities for each sampling date for water column stations are shown in Table 1. Temperature, salinity and rainfall prior to sampling are included. The locations of all stations, mainstem and feeder streams, are shown in Figure 1. Feeder streams are identified by uppercase letters. Similar data for feeder streams are shown in Table 2. Mean values of fecal coliform densities and relevant physical data are shown for all stations in Table 3. Mean water temperatures during the study ranged from a minimum of 3°C (February) to a maximum of 30°C (July). In general, the longitudinal salinity gradient from mainstem headwaters to the creek mouth was comparatively small. Mean fecal coliform densities (Figure 2 and Table 3), displayed a somewhat typical pattern, with maximum densities occurring in the feeder streams and other sites of input, and decreasing densities moving from the headwaters, to the mainstem and mouth, respectively. Except at the immediate creek mouth, mean

fecal coliform densities from mainstem samples exceeded the approved shellfish area growing standard of 14 FC/100 ml. Correlation analysis (Table 4) showed that log FC was significantly and positively correlated ($p \leq 0.05$) with temperature, negatively correlated ($p \leq 0.05$) with salinity when all stations were combined, and positively correlated ($p \leq 0.05$) with rainfall and turbidity when either all stations or only mainstem stations were considered. Turbidity correlated significantly and positively ($p \leq 0.05$) with temperature and rainfall (3d prior). These correlations reflect the association of elevated fecal coliform densities in estuarine receiving waters with non-point source runoff.

Occurrence and distribution of alternate indicators in the water column at selected creek locations. Results from water samples processed for detection of candidate bacterial indicators previously discussed and for phage lysing the male specific assay strain WG49 are shown in Table 5. Densities of alternate indicators detected are in bold type simply as a visual aid. Representative sampling locations were chosen on the basis of proximity to the livestock farm (#7 and #10) or as indicative of headwater (#23), mouth (#2) and small cul-de-sac areas (#9). Compared to fecal coliforms, which were detected on every sampling date and at most stations (Table 4), alternate bacterial indicators were either undetected (sorbitol-fermenting bifidobacteria), or if detected (S. bovis, R. coprophilus), occurred infrequently at comparatively lower densities. Phage lysing WG49 were detected more frequently and at more locations than the bacterial indicators. The occurrences of alternate bacterial indicators were so low that any statistical analysis would be meaningless. Alternatively, the parameter "frequency of occurrence" (number of times detected/total number of surveys) was calculated for each indicator and the results summarized for each station (along with mean FC densities) in Figure 3. (Values in bold signify the site at which the maximum density of a given indicator occurred.) Systematic relationships between mean fecal coliform densities and the frequencies of occurrence of the alternate bacterial indicators were not evident. Although sorbitol-fermenting bifidobacteria were not detected, S. bovis and importantly, R. coprophilus, assumed indicators of animal fecal pollution, were detected at one or both of the stations (#7 and #10) adjacent to the "Catlett farm". Although S. bovis was recovered most frequently at the cul-de-sac station (#9) on the eastern

side of the creek, R. coprophilus was detected only at stations #7 and #10. R. coprophilus is considered an indicator of ruminant feces although it can be found in other animals (including birds) which may come in contact with these feces. The high incidence of S. bovis at station #9 remains enigmatic because neither S. bovis or other alternate indicators were detected in G, "its" feeder stream. Perhaps the hydrographic characteristics of the system favor concentration or lateral transport of fecal pollution to this part of the creek. Importantly, none of the alternate indicators were detected at the creek mouth (station #2).

Occurrence and distribution of fecal coliforms and alternate indicators in sediments at selected creek locations. Compared to water samples, the relative densities (expressed on a per unit volume basis) of indicators detected in sediments were generally much higher (Table 6). (Values of alternate bacterial indicators in bold to aid visualization.) This was evident in the densities of fecal coliforms, the 3-4 log increases in concentrations of phage lysing WG49 and R. coprophilus. Phage lysing WG49 and R. coprophilus were also detected at comparatively high sampling frequencies (Figure 3) at all stations except #2 located at the creek mouth. When detected, the highest levels of R. coprophilus occurred at station #7 adjacent to the farm. Maximum levels of phage lysing WG49 also occurred at this station on four of the seven sampling dates. S. bovis was detected once in sediment at station #7, this being the only location where it occurred at least once in both water and sediment. The absence of this indicator in the estuary proper, which (as will be seen) was in contrast to its widespread and more frequent occurrence in feeder streams, is consistent with high positive values of S. bovis dieoff constants observed at temperatures above 10°C (Kator and Rhodes, 1988). Log fecal coliforms in water and log fecal coliforms in sediment were significantly and positively correlated for all data groupings analyzed (Table 4), thereby supporting the notion that both water and sediment phases are "coupled" to fecal pollution carried by feeder streams.

Occurrence of fecal coliforms and alternate indicators in water and sediment of selected feeder streams. Results of sampling water and sediment in feeder streams to Timberneck Creek are shown in Table 7 and Figure 4 (values in bold signify the site at which the maximum density of a given indicator occurred).

Mean densities of fecal coliforms in feeder stream waters were comparatively larger than mean densities in mainstem creek waters (Table 3). As anticipated, levels of fecal coliforms in 100 g of sediment were generally much larger than corresponding densities in 100 ml samples from feeder streams. The concentration of fecal coliforms in the water was significantly ($p \leq 0.05$) correlated with temperature and inversely correlated with the occurrence of rain 7 days prior to sampling (Table 4). The latter observation would be consistent with gradual dilution by rainfall of comparatively small and intermittent sources. Fecal coliform densities in the water were positively correlated ($p \leq 0.05$) with levels in feeder stream sediments (Table 4), again implying coupling of both the sediments and bulk water layers to chronic addition of this indicator. In contrast to mainstem microbial indicator data, all alternate bacterial indicators were detected, albeit if only on a single occasion, in feeder streams. Phage lysing WG49 was detected in the water but occurred more frequently and at higher densities in sediments. Sorbitol-fermenting bifidobacteria were detected on two surveys, at two locations during a period of seasonally low water temperature and once in May. Importantly, feeder E was the site of maximum densities for both bifids and fecal coliforms. The former was never detected in sediment. R. coprophilus was found once in the water of a feeder stream during July and only five times in various feeder stream sediments. However, these feeder streams sediments (D, E, and F) were also the locations of the highest mean sediment fecal coliform densities. In contrast to the comparatively low frequencies of occurrence of the aforementioned bacterial indicators, S. bovis was frequently recovered in water from most feeder streams over the duration of this study. Its frequent occurrence and poor survival characteristics are not inconsistent with the hypothesis that its presence reflects a continual addition of recent animal fecal pollution. However, an unequivocal assessment of the ability of this indicator (and others) to reflect source "freshness" cannot be made until definitive studies describing its survival in feces, soils and fresh/estuarine receiving waters can be made. S. bovis was detected in feeder stream sediment on only one occasion. In contrast to feeder stream waters, its comparatively low occurrence in creek waters and sediments reflects its limited survival capacity in the estuary, distance from contamination and dilution. However, with the exception of feeder B, S. bovis was most likely to be detected in feeder stream waters with the highest mean fecal coliform densities.

Relationship of indicator occurrence to shoreline survey. A final step in this study was to analyze and relate the indicator data to real or potential sources of fecal pollution as identified by the shoreline survey or on the basis of recent field observations. An updated sanitary survey was performed during this study, in part because of previous sanitary deficiences or observations made during the course of this study. Potential or known pollution sources were identified as shown in Figure 5. With the exception of potential animal pollution associated with the "Catlett farm" (stations #7 and #10), most sanitary violations, both actual and potential, were on the developed eastern side of the creek. As measured by indicator microorganisms, this was reflected in detection of human bifids in feeder streams E and C-1; by the comparatively high levels or frequent detection of S. bovis at feeder locations D,B and F; by the presence of R. coprophilus in the water and sediment at D; and by the correspondingly elevated densities of fecal coliforms associated with the presence of alternate indicators. The high density of human development, the presence of known human violations, and the presence of numerous species of domestic farm animals correlated with maximum levels of S. bovis and fecal coliforms (Table 5). The presence of R. coprophilus at feeders D and E must be attributed to the comparatively high numbers of domestic animals other than pets. Detection of human bifids at feeder A suggests possible unreported sanitary violations. Detection of S. bovis at feeder F suggests unreported domestic animals. Finally, the absence of alternate bacterial indicators from feeder stream G, a stream which purportedly drains an area without known sanitary violations, corroborates the assumption that these indicators should not be found in the absence of fecal pollution.

The multiplicity of apparent pollution sources in the eastern creek drainage area and the corroborating alternate indicator data fail to support the hypothesis that the "Catlett farm" is significantly degrading creek water quality. However, our results suggest that the farm is not without some impact, as seen in the occurrence of bacterial indicators of fecal pollution proximate to stations #7 and #10. As previously revealed, the largest mean fecal coliform and R. coprophilus densities were detected in the sediment (#7) immediately adjacent to the farm (Figure 3). Furthermore, this was the only station where S. bovis was detected in both water and sediment, its presence

in the sediment being the one time it found in this milieu. R. coprophilus was also found at comparatively high frequency at #10. Although strongly suggestive of the farm as the source, in fairness it must be noted that interpretation of these results is not completely unequivocal, as wild bird populations were observed in the shallow waters adjacent to station #7.

"Mass balance" calculation: impact of feeder streams on creek. Another way to illustrate the comparative effect of the farm on creek water quality is to perform a relatively crude but revealing mass balance calculation based on the input of fecal coliforms to the creek in feeder streams. This input can be estimated using mean observed fecal coliform densities in feeder streams and representative feeder stream flow rates. The latter were calculated by measuring the cross sectional area of a given length of feeder stream and using a drouge to measure stream speed. Table 8 illustrates the use of these data to calculate total daily fecal coliform inputs to selected segments of the creek (Figure 6). Interestingly, daily inputs can be converted to theoretical estimates of the number of persons required to produce the observed fecal coliform loading by dividing the daily input by 2.0×10^9 (the estimated per capita contribution of fecal coliforms). The values obtained are comparatively small but not unrealistic in terms of actual numbers of sanitary violations. The second part of Table 8 predicts what the fecal coliform concentration would be based on daily loading from those feeder streams contributing to various segments of the creek and segment volume. Mean fecal coliform densities calculated for field data based on stations within each segment are compared to predicted values. Note that the theoretical fecal coliform density for segment I is remarkably close to the observed mean fecal coliform density from actual field data. Although this "back of the envelope" calculation requires simplistic assumptions and conservative estimates, the results suggest that the level of contamination introduced via the feeder streams impacting segment I is sufficient to account for the observed levels of fecal coliforms in the creek. Theoretical calculated values for segments II and III, although of similar magnitude were considerably lower than the mean field values. However, if the contribution of fecal coliforms from the segment immediately upstream (assuming only 10% was transported) is combined with the input from the feeder stream, the theoretical FC concentration value for segment II approaches the observed mean. The relatively small change in the

theoretical value for segment III may reflect the presence of unrecognized sources of contamination or removal by flushing or other mechanisms. Overall, these calculations serve to emphasize the dominance which feeder streams exert over fecal coliform concentrations in the creek per se and thus minimize the contribution of the "Catlett farm" as a major source of fecal pollution. The calculations also highlight the frequent realization that the levels of fecal coliforms in small subestuarine systems can be extremely sensitive to comparatively small amounts of contamination, especially when viewed in terms of per capita inputs.

Phage lysing Salmonella typhimurium assay strain WG49. During the course of this investigation comparatively large numbers of phage capable of lysing WG49, the male specific host strain, were observed in sediments (Tables 5 and 6) in a number of locations in the study area. Consequently, by way of confirmation phages plaquing on WG49 were also tested for RNAase resistance and ability to plaque on WG45. The ability to plaque on WG45, the "female" host strain, i.e., the strain not producing male pili, would suggest the presence of so-called "somatic" phages. Resistance to RNAase would mean the plaques formed would be due to DNA containing phages, since the RNAase present should effectively prevent replication of the RNA containing F-specific phages. On the basis of these tests, use of another male specific coliphage host, and subsequent resampling of Timberneck Creek in early 1989, we concluded that the WG49 parent strain had undoubtedly detected non-coliphage somatic phages which are apparently present in large numbers in creek sediments. Consequently, the sanitary significance of the results reported for the male specific phage remains undetermined and the data were not discussed in this report. However, it is interesting that a significant population of phage capable of lysing the assay strain, which is the enteric pathogen Salmonella typhimurium, does exist since this can affect use of this or similar virus indicators.

Conclusions

An important conclusion of this study is that the loading of fecal coliforms to Timberneck creek is derived from diverse and widespread sources and apparently introduced to the creek in feeder streams. Gross mass balance calculations based on feeder stream fecal coliform densities suggested that most if not all of the fecal coliform loading could be accounted for by these streams. Malfunctioning or poorly operating septic systems, multiple potential sources of domestic animal pollution, as well as unrecognized pollution sources are probable sources. Consequently, although alternate indicators provided evidence of animal fecal contamination probably originating from the "Catlett farm", it was not necessary to attribute significant fecal indicator loading to this source. The frequent detection of S. bovis in most feeder streams suggested animal pollution is continual and widespread. Finding R. coprophilus in sediments proximate to the "Catlett farm" is corroborative of the hypothesis that farm animals have been contributing fecal pollution to the creek. The very low but measurable occurrence of human sorbitol-fermenting bifidobacteria implies that human sanitary deficiences are present. Finally, this evaluation of alternate indicators supports their value as an investigative "tool", whose presence can be used to qualitatively identify sources and "age" of fecal pollution. Identification of contributing sources through use of these indicators may lead to ameliorative action designed to reduce or eliminate certain kinds of pollution sources.

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Fecal coliforms/100 ml

Station	Date								
	10-13-87	2-9-88	3-8-88	4-19-88	5-17-88	6-6-88	7-18-88	8-1-88	9-13-88
1	nd	2	2	2	2	nd	4	ID	nd
2	ID	4.5	4.5	4.5	ID	4.5	4.5	ID	14
3	33	4.5	33	22	1300	13	46	2	11
4	3.7	2	4.5	7.8	33	38	4.5	4.5	2
5	7.8	2	2	23	110	49	7.8	13	130
6	7.8	ID	13	6.8	49	46	17	11	330
7	17	7.8	7.8	230	1400	46	220	23	130
8	23	2	33	23	130	79	11	2	170
9	130	33	23	17	1300	490	230	79	700
10	13	4.5	17	17	130	79	49	4.5	460
11	490	ID	13	70	790	49	490	23	1100
12	23	ID	17	7.8	170	79	22	7.8	170
13	49	2	11	7.8	230	230	49	14	700
14	33	4.5	13	26	330	170	49	22	330
15	170	2	5	27	490	230	79	79	490
16	33	ID	22	46	230	70	110	31	790
17	70	49	33	49	330	79	49	49	700
18	49	ID	13	79	230	130	49	33	460
19	220	7.8	17	34	490	330	17	33	490
20	79	2	4.5	33	490	220	49	17	490
21	490	7.8	4.5	33	490	490	330	79	330
22	130	6.8	13	23	16000	nd	6.8	33	790
23	490	14	79	95	1700	nd	46	79	130
Ranges, physical data:									
Temp. (°C)	14-16	3-8	9-12	14-16	20-24	21-28	29-30	28-29	20-24
Sal. (o/oo)	20-22	10-18	16-19	15-18	12-18	8-16	19-20	19-21	20-23
Rainfall (") (3d prior to sampling)	0.0	0.0	0.48	0.04	0.69	1.04	0.00	0.04	0.22

nd= sample not collected owing to unfavorable weather conditions or extremely low water. ID = indeterminate value, $\leq$ 1.8 fecal coliforms/100 ml.

Table 2. Fecal coliform densities in water from major feeder streams to Timberneck Creek.

Station	Fecal coliforms/100 ml				
	5-31-88	6-27-88	7-11-88	8-08-88	9-19-88
A	220	1300	4900	230	140
B	70	3300	33	1400	490
C1	490	460	230	280	220
C2	nd	1700	33	280	7900
D	330	3300	1300	460	170
E	490	1300	1300	13000	24000
F	490	7900	2800	3500	1300
G	nd	70	330	280	23
Ranges, physical data:					
Temp. (^{o}C)	17-22	16-23	17-27	19-26	16-20
Rainfall (") (3d prior to sampling)	0.33	0.54	0.04	0.28	0.00

nd = not determined

coliform densities in creek and feeder streams.

Station	n	Temperature °C	Salinity o/oo	Turbidity NTU	Fecal coliforms /100 ml
Creek					
1	6	17	19	12	2.2
2	9	18	19	18	3.8
3	9	18	19	39	23
4	9	19	19	23	6.2
5	9	19	19	24	16
6	9	19	19	26	18
7	9	18	19	29	60
8	9	19	19	21	22
9	9	20	17	35	140
10	9	20	19	22	30
11	9	19	18	29	89
12	9	19	19	25	23
13	9	19	18	20	39
14	9	19	18	23	47
15	9	20	18	32	63
16	9	19	18	23	51
17	9	19	18	30	85
18	9	20	18	25	50
19	9	20	17	31	71
20	9	20	18	24	47
21	9	20	17	28	100
22	8	20	17	29	72
23	8	19	17	19	120
Feeder streams					
A	6	19	0	31	340
B	6	19	<1	16	280
C1	6	18	0	9	200
C2	4	23	14	17	590
D	6	19	0	30	360
E	6	19	0	16	1500
F	5	21	0	8	2200
G	4	18	0	5	110

Water samples:

Sample sites	n	Parameter	Temp.	Sal.	Rn	Rnt	Turb.
All	244-245	LogFCW	0.36	-0.42	0.26		0.16
Mainstem	205-206	LogFCW	0.39		0.32		0.31
Feeders	39	LogFCW	0.57			-0.46	
All	244-245	Turb.	0.44		0.38	0.13	
Mainstem	205-206	Turb.	0.49	-0.17	0.40		

Water and sediment sampled simultaneously:

Sample sites	n	Parameter	Temp. water	Temp. sed.	Sal.	Rn	Rnt	Turb.	LogFCW	LogFCS
All	54-68	LogFCW			-0.53					0.68
		LogFCS	-0.30	-0.28	-0.84		-0.41	-0.28	0.68	
Mainstem	24-34	LogFCW			-0.36					0.67
		LogFCS			-0.45				0.67	
Feeders	30-34	LogFCW								0.45
		LogFCS							0.45	

Definitions: RN, rainfall 3 days prior to sampling; RNT, rainfall 7 days prior to sampling; logFCW, log fecal coliforms/100 ml; logFCS, log fecal coliforms/100g dry sediment.

in Timberneck Creek.

Date	Station	Fecal coliforms[a]	Phage lysing WG49[b]	Sorbitol-fermenting bifidobacteria[b]	*S. bovis*[b]	*R. coprophilus*[c]
		/100 ml				/ml
10-13-87						
	2	ID	nd	ID	ID	ID
	7	17	nd	ID	ID	ID
	9	130	nd	nd	nd	nd
	10	13	nd	ID	ID	ID
	23	490	nd	ID	ID	ID
2-09-88						
	2	4.5	nd	ID	ID	ID
	7	7.8	nd	ID	ID	ID
	9	33	nd	nd	nd	nd
	10	4.5	nd	ID	ID	ID
	23	14	nd	ID	ID	ID
2-16-88						
	2	nd	nd	nd	nd	nd
	7	23	nd	ID	ID	3.3
	9	130	nd	ID	43	ID
	10	nd	nd	nd	nd	nd
	23	nd	nd	nd	nd	nd
3-08-88						
	2	4.5	1	ID	ID	ID
	7	7.8	ID	ID	ID	ID
	9	23	2	ID	ID	ID
	10	17	3	ID	ID	ID
	23	79	2	ID	ID	ID
4-19-88						
	2	4.5	6	ID	ID	ID
	7	230	ID	ID	ID	3.3
	9	17	ID	ID	1	ID
	10	17	ID	ID	ID	ID
	23	95	ID	ID	ID	ID

Table 3. Cont'd.

5-17-88						
	2	ID	ID	ID	ID	ID
	7	1400	5	ID	ID	ID
	9	1300	9	ID	250	ID
	10	130	1	ID	ID	ID
	23	1700	2	ID	ID	ID
6-06-88						
	2	4.5	ID	ID	ID	ID
	7	46	ID	ID	ID	ID
	9	490	5	ID	ID	ID
	10	79	2	ID	ID	ID
	21	490	4	ID	ID	ID
7-18-88						
	2	4.5	ID	ID	ID	ID
	7	220	130	ID	ID	ID
	9	230	81	ID	ID	ID
	10	49	61	ID	ID	3.3
	23	46	ID	ID	ID	ID
8-01-88						
	2	ID	ID	ID	ID	nd
	7	23	1	ID	ID	nd
	9	79	ID	ID	ID	nd
	10	4.5	ID	ID	ID	nd
	23	79	ID	ID	ID	nd
9-13-88						
	2	14	ID	ID	ID	ID
	7	130	ID	ID	4	ID
	9	700	ID	ID	ID	ID
	10	460	ID	ID	ID	ID
	23	130	ID	ID	ID	ID

---

[a]ID = $\leq$ 1.8/100 ml, [b]ID = $\leq$ 1.0/100 ml, [c]ID = $\leq$ 3.3/ml, nd = not determined.

Table 6. Occurrence of alternate indicators and fecal coliforms in estuarine sediments at selected sites in Timberneck Creek.

Date	Station	Fecal[a] coliforms /100 g dry sediment	Phage[b] lysing WG49 /100 g dry sediment	Sorbitol-[c] fermenting bifidobacteria /1 g dry sediment	S. bovis[c] /1 g dry sediment	R. coprophilus[c] /1 g dry sediment
4-19-88	2	40	ID	ID	ID	ID
	7	1100	35000	ID	ID	**35000**
	9	640	190	ID	ID	**190**
	10	130	96	ID	ID	**96**
	23	490	640	ID	ID	**630**
5-17-88	2	41	910	ID	ID	ID
	7	1600	1200	ID	ID	**210**
	9	660	16000	ID	ID	**98**
	10	2600	11000	ID	ID	**110**
	23	1700	3500	ID	ID	ID
6-06-88	2	270	310	ID	ID	ID
	7	840	790	ID	ID	**250**
	9	1500	11000	ID	ID	**98**
	10	950	2900	ID	ID	ID
	21	1900	6900	ID	ID	ID
7-18-88						
	2	ID	ID	ID	ID	ID
	7	55	63000	ID	ID	**2800**
	9	560	1700	ID	ID	ID
	10	210	4300	ID	ID	**210**
	23	95	4300	ID	ID	**1700**
8-01-88						
	2	ID	370	ID	ID	nd
	7	2200	42000	ID	ID	nd
	9	2700	2400	ID	ID	nd
	10	220	1300	ID	ID	nd
	23	620	1500	ID	ID	nd

Table 6. Cont'd.

9-13-88					
2	36	ID	ID	ID	ID
7	6800	9800	ID	**98**	ID
9	2700	1700	ID	ID	**110**
10	130	1900	ID	ID	ID
23	1000	930	ID	ID	ID

---

[a]ID = ≤18/100 g wet sediment, [b]ID = ≤10/100 g wet sediment, [c]ID = ≤33/g wet sediment for bifidobacteria, S. bovis, and R. coprophilus, nd = not determined

feeding Timberneck Creek.

Date Station	Water: Fecal coliforms[a]	Phage lysing WG49[b]	Bifids[b]	S. bovis[b]	R. coprophilus[c]	Sediment: Fecal coliforms[d]	Phage lysing WG49[e]	Bifids[f]	S. bovis[f]	R. coprophilus[f]
	/100ml				/ml	/100g dry sed		/g dry sediment		
2-16-88										
A	33	nd	ID	nd	nd	Sediments not sampled prior to 5-31-88				
B	95	nd	ID	nd	nd					
C1	21	nd	1	14	ID					
C2	nd	nd	nd	nd	nd					
D	22	nd	ID	6000	ID					
E	49	nd	4	110	ID					
F	nd	nd	nd	nd	nd					
G	nd	nd	nd	nd	nd					
5-31-88										
A	220	ID	2	ID	ID	4600	ID	ID	ID	ID
B	70	ID	ID	13	ID	88000	ID	ID	ID	ID
C1	490	ID	ID	ID	ID	70000	ID	ID	ID	ID
C2	nd					nd				
D	330	1	ID	ID	nd	110000	2000	ID	ID	540
E	490	4600	ID	57	ID	150000	120000	ID	ID	ID
F	490	1	ID	8	ID	80000	ID	ID	ID	49
G	nd					nd				
6-27-88										
A	1300	ID	ID	ID	ID	9700	ID	ID	ID	ID
B	3300	1	ID	350	ID	440000	19	ID	ID	ID
C1	460	ID	ID	ID	ID	330000	27	ID	ID	ID
C2	1700	1	ID	78	ID	59000	3500	ID	ID	ID
D	3300	ID	ID	ID	ID	490000	110	ID	ID	46
E	1300	16	ID	75	ID	770000	13000	ID	ID	73
F	7900	61	ID	730	ID	870000	320	ID	ID	54
G	70	ID	ID	ID	ID	67000	11000	ID	ID	ID

7-11-88										
A	4900	ID	ID	**70**	ID	39000	13	ID	ID	ID
B	33	ID	ID	**70**	ID	89000	18	ID	ID	ID
C1	230	ID	ID	**3**	ID	250000	100	ID	ID	ID
C2	33	2	ID	**12**	ID	5600	1700	ID	ID	ID
D	1300	2	ID	**3400**	**3.3**	650000	2100	ID	**34**	ID
E	1300	9	ID	**6**	ID	40000	8900	ID	ID	ID
F	2800	75	ID	**10**	ID	1200000	69	ID	ID	ID
G	330	2	ID	ID	ID	30000	1700	ID	ID	ID
8-08-88										
A	230	ID	ID	ID	ID	62000	13	ID	ID	ID
B	1400	ID	ID	**35**	ID	160000	57	ID	ID	ID
C1	280	ID	ID	ID	ID	310000	18	ID	ID	ID
C2	280	ID	ID	ID	ID	7800	680	ID	ID	ID
D	460	ID	ID	ID	ID	46000	28	ID	ID	ID
E	13000	9	ID	ID	ID	150000	1100	ID	ID	ID
F	3500	43	ID	ID	ID	91000	13	ID	ID	ID
G	280	ID	ID	ID	ID	1900	570	ID	ID	ID
9-19-88										
A	140	ID	ID	ID	ID	68000	14	ID	ID	ID
B	490	ID	ID	**18000**	ID	180000	26	ID	ID	ID
C1	220	ID	ID	ID	ID	270000	25	ID	ID	ID
C2	7900	ID	ID	**5**	ID	10000	890	ID	ID	ID
D	170	ID	ID	**1**	ID	790000	340	ID	ID	ID
E	24000	28	ID	**40**	ID	660000	7800	ID	ID	ID
F	1300	38	ID	**620**	ID	350000	440	ID	ID	ID
G	23	ID	ID	ID	ID	1100	1400	ID	ID	ID

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[a] ID = ≤ 1.8/100 ml, [b] ID = ≤ 1.0/100 ml, [c] ID = ≤ 3.3/ml, [d] ID = ≤ 18/100 g wet sediment, [e] ID = ≤ 10/100 g wet sediment, [f] ID = ≤ 33/g wet sediment, nd = not determined.

flow rates, mean fecal coliform densities calculated from field data and observed creek flow rates[1].

Feeder Stream	Flow rate, l/sec	$\overline{FC}$/100 ml	$\overline{FC}$ added/day	Segment impacted	Per capita equivalents[2]
A	6.2	340	1.8×10^9	I	0.9
B	124.0	280	3.0×10^{10}	I	15.0
C-1	6.6	200	1.1×10^9	I	0.6
D	5.4	360	1.7×10^9	II	0.6
E	16.3	1500	2.1×10^{10}	III	10.5

Theoretical mean FC concentrations calculated independently for each segment by dividing the total mean FC loading/day to each segment by the volume of that segment (Figure 6) at mean low water. These values are compared with mean FC from data. The latter are simple means of observed FC concentrations for the stations located in segments I, II or III, respectively.

Segment I (feeders A, B, C-1)	Mean FC, field data	Segment II (feeder D)	Mean FC, field data	Segment III (feeder E)	Mean FC, field data
107 FC/100 ml	97 FC/100 ml	10 FC/100 ml	56 FC/100 ml	12 FC/100 ml	53 FC/100 ml

Theoretical FC concentrations in segments downstream from segment I assuming contribution of FC from segment immediately upstream as well as feeder inputs. For the sake of argument only 10% of the upstream theoretical mean FC contribution is used to contaminate the downstream segment.

Segment I	Mean FC, field data	Segment II	Mean FC, field data	Segment III	Mean FC, field data
107 FC/100 ml	97 FC/100 ml	31 FC/100 ml	56 FC/100 ml	14 FC/100 ml	53 FC/100 ml

[1] "Streams" F and G were not considered as inputs in these calculations being characterized as very low or intermittent flow. [2] Daily per capita fecal coliform contribution = 2.0×10^9

Figure 1. Timberneck Creek sampling locations. Creek stations are identified by numbers; feeder streams by upper case letters. The dashed insert labeled "Livestock Pasture Land" is the "Catlett farm" grazing area.

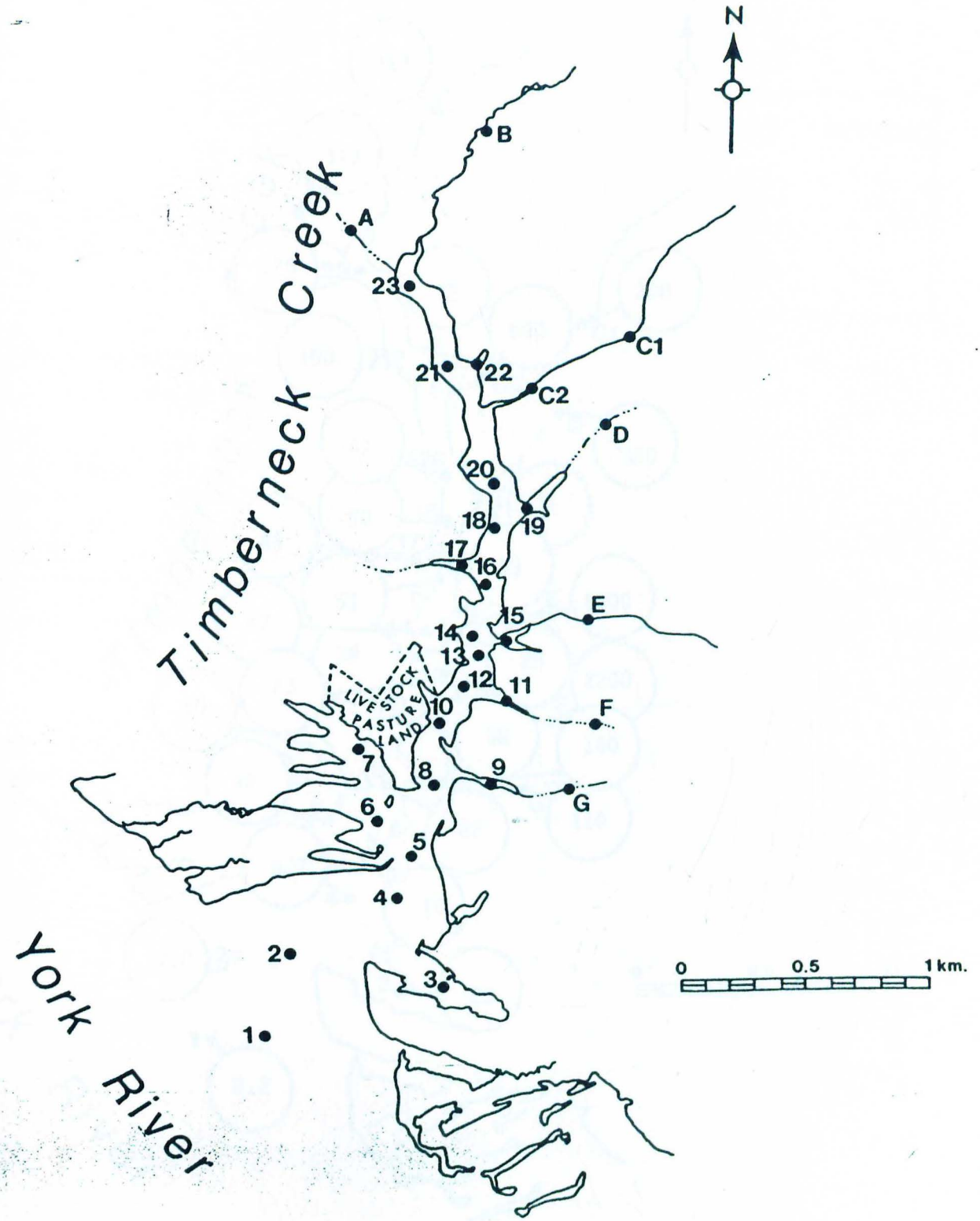


Figure 2. Mean fecal coliform densities (FC/100 ml) for all water samples.

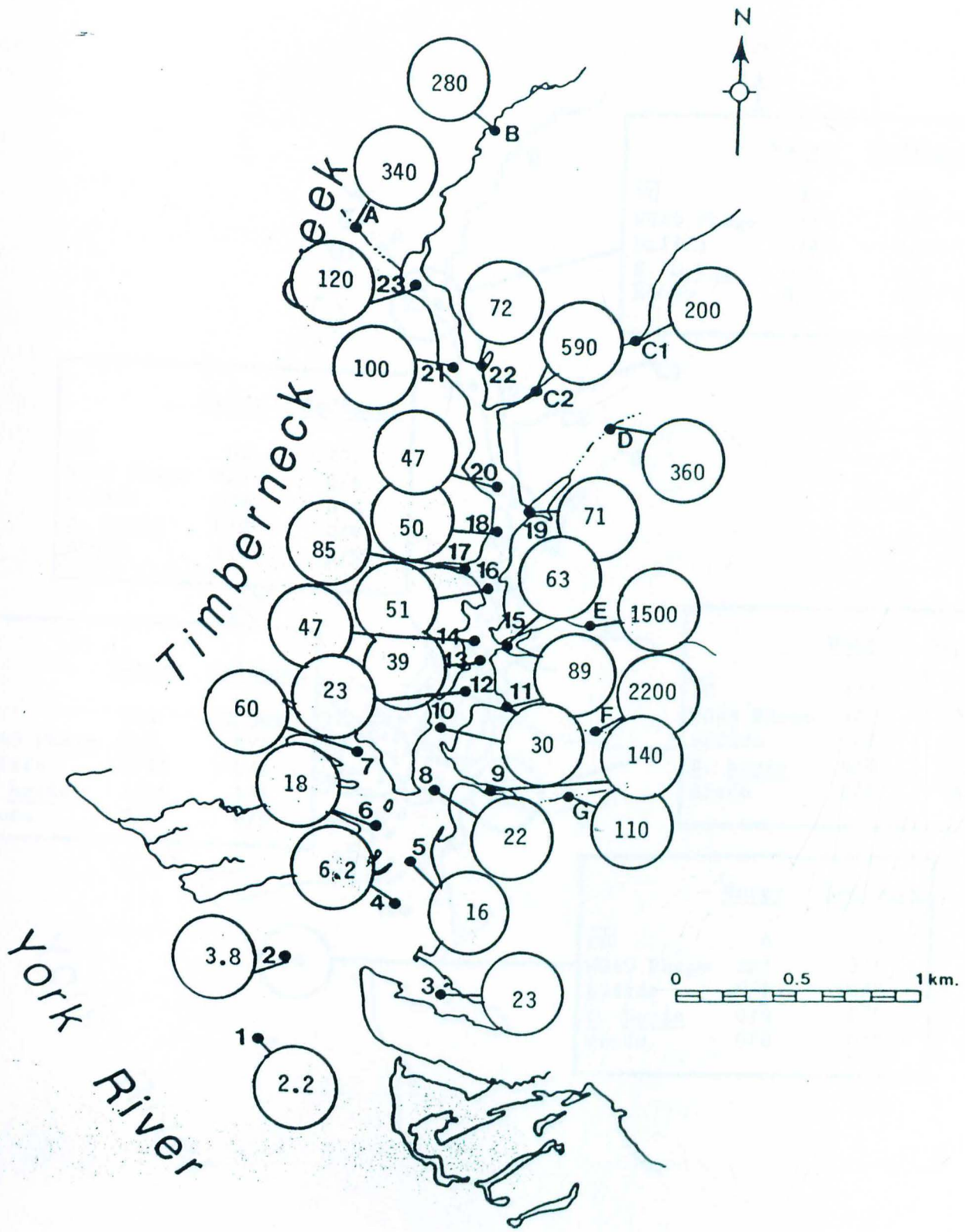


Figure 3. Incidence of alternate indicators and mean fecal coliform densities (FC/100 ml or 100 g) in water and sediment from selected locations in Timberneck Creek.

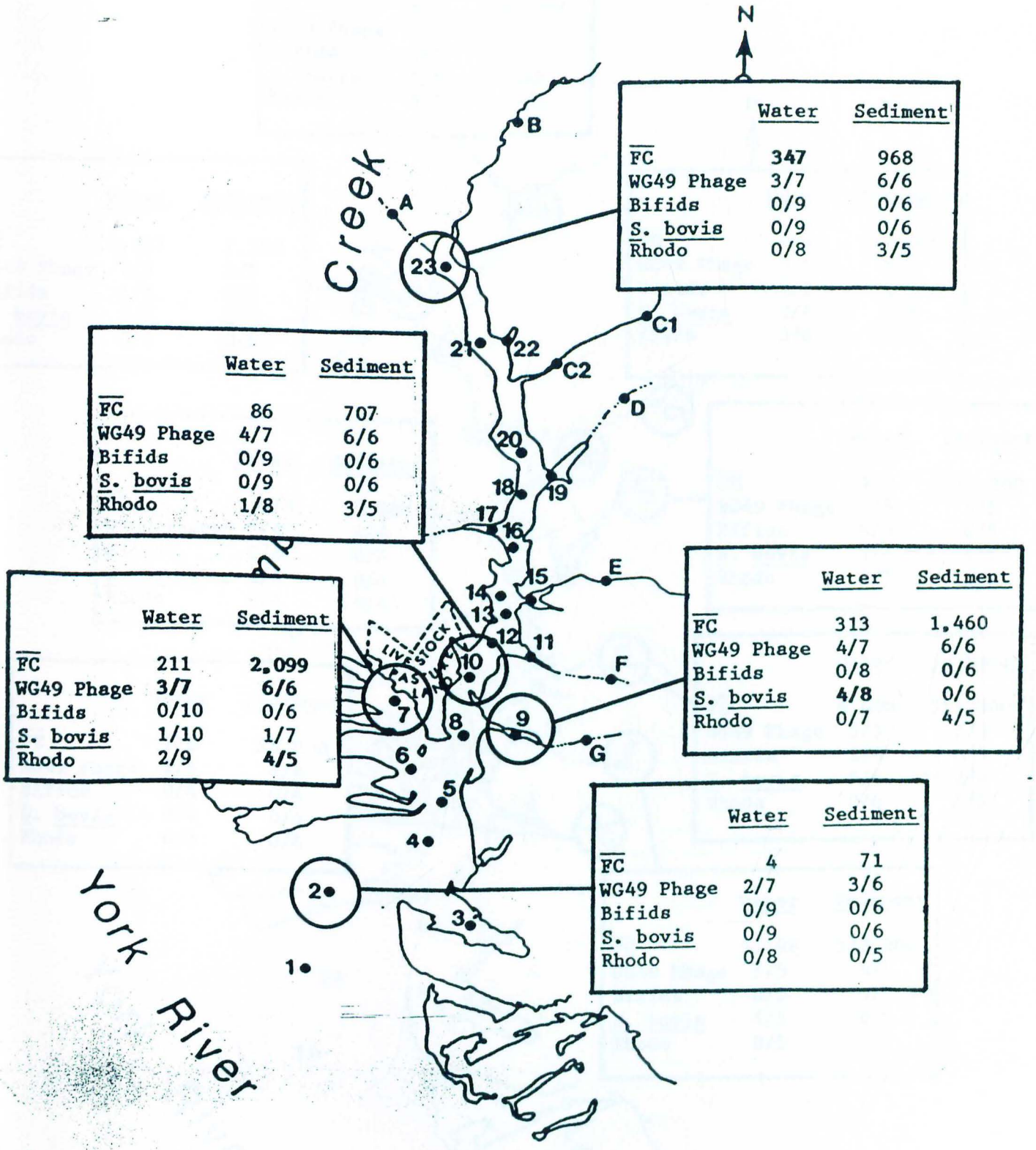


Figure 4. Incidence of alternate indicators and mean fecal coliform densities (FC/100 ml or 100 g) in water and sediment at selected feeder streams.

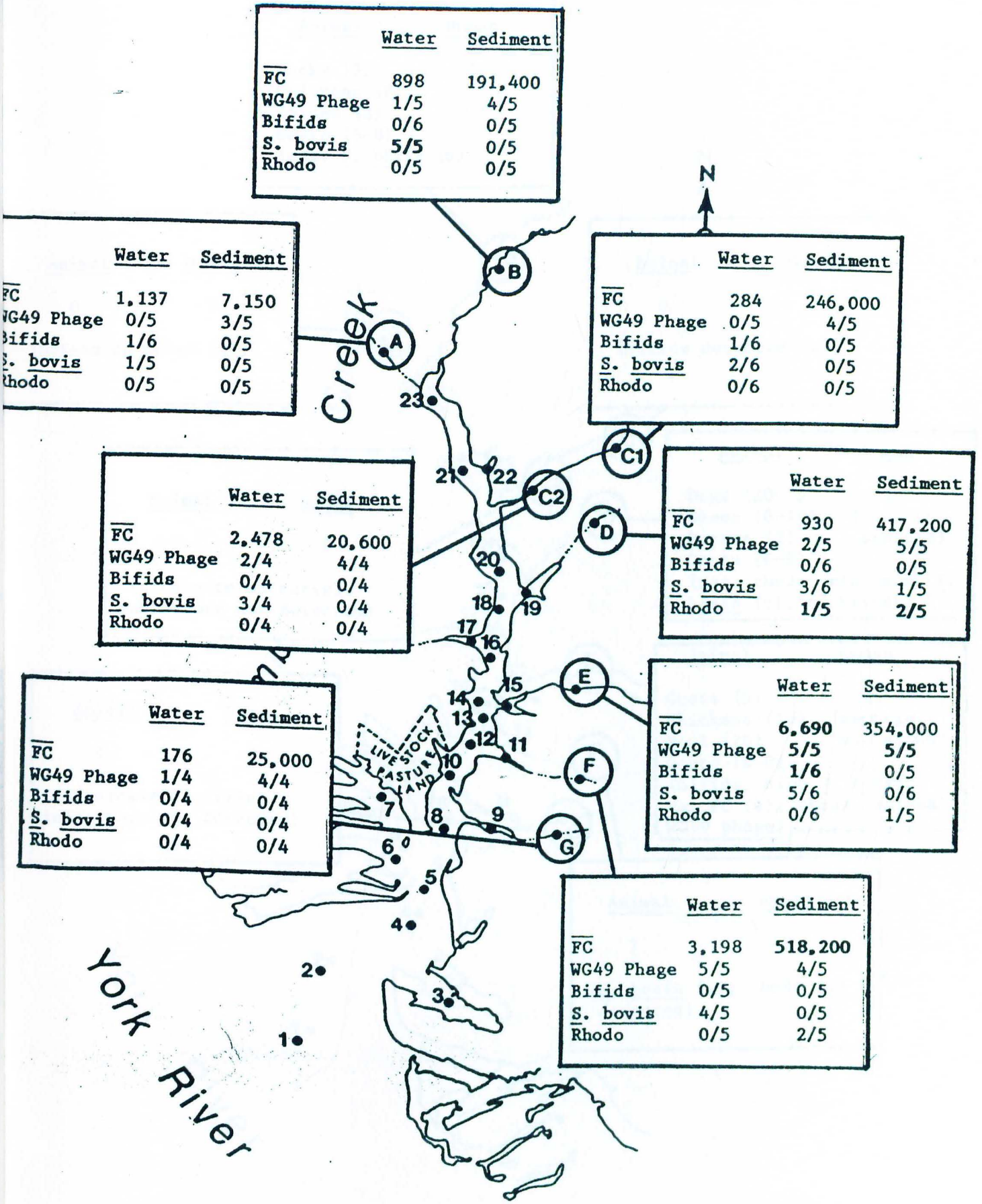


Figure 3. Potential and known contributions of fecal pollution to Timberneck Creek based on sanitary survey data and independent field observations.

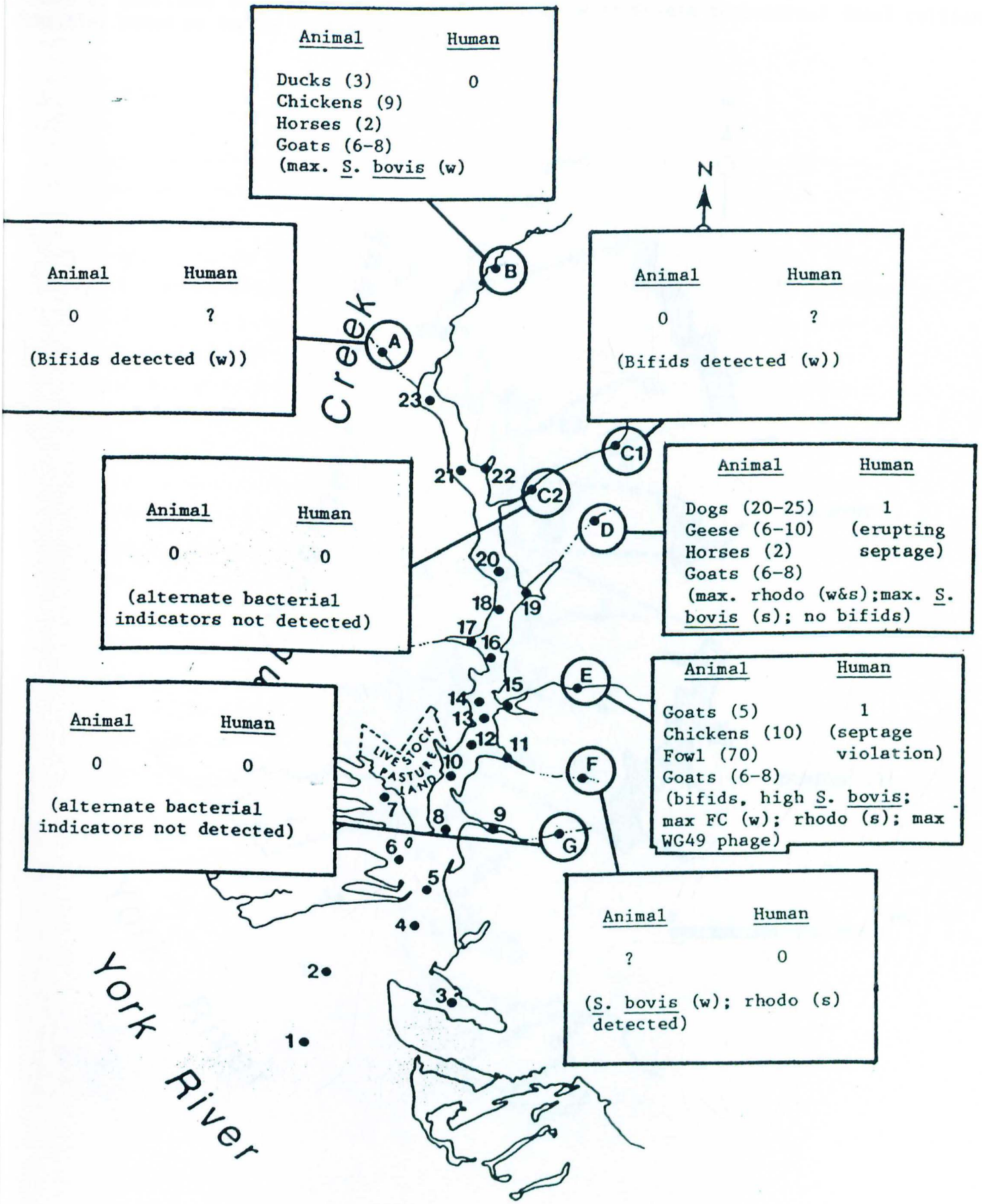
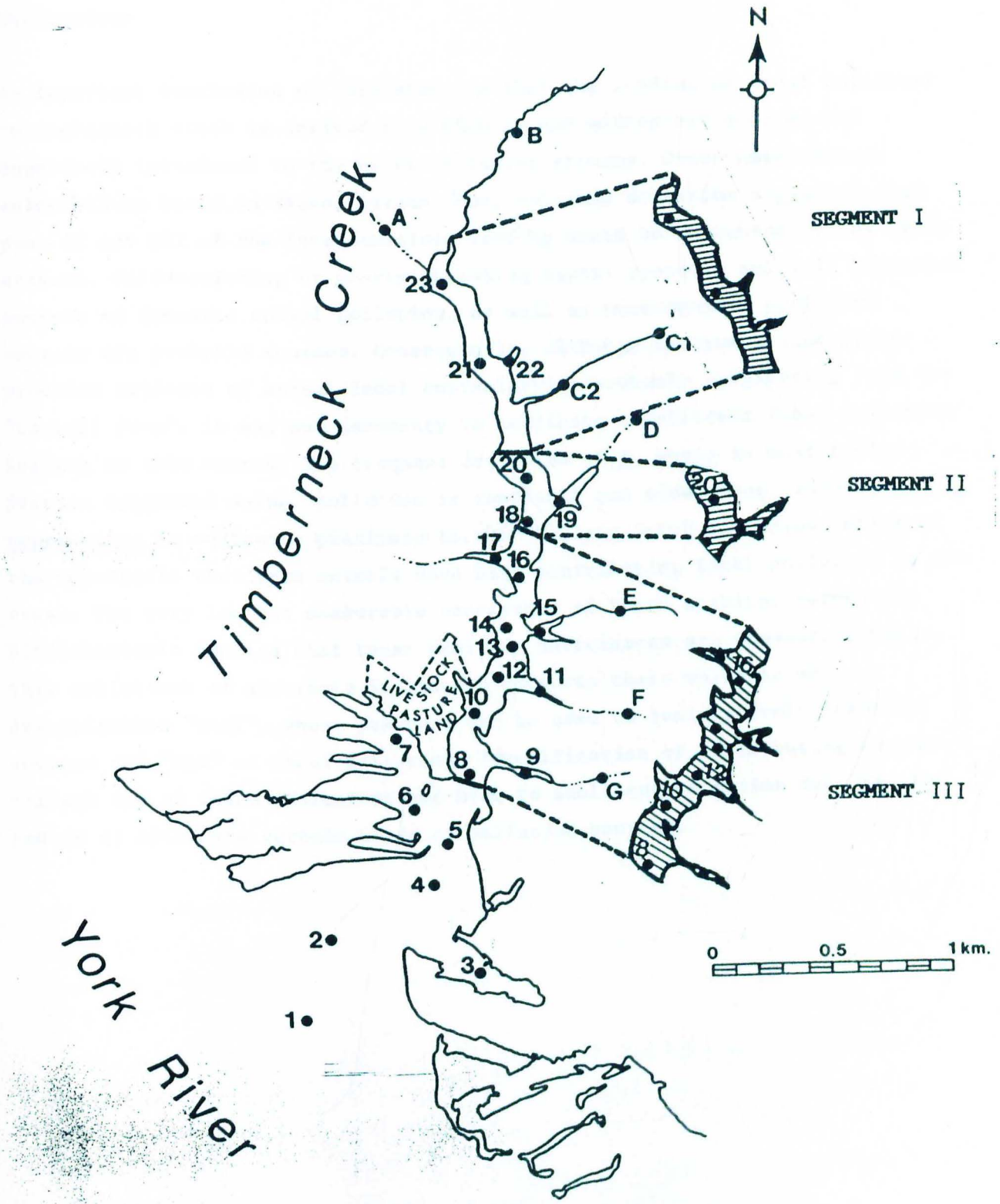


Figure 6. Locations of segments I, II, and III used to calculate theoretical fecal coliform densities based on feeder stream inputs.



Conclusions

An important conclusion of this study is that the loading of fecal coliforms to Timberneck creek is derived from diverse and widespread sources and apparently introduced to the creek in feeder streams. Gross mass balance calculations based on feeder stream fecal coliform densities suggested that most if not all of the fecal coliform loading could be accounted for by these streams. Malfunctioning or poorly operating septic systems, multiple potential sources of domestic animal pollution, as well as unrecognized pollution sources are probable sources. Consequently, although alternate indicators provided evidence of animal fecal contamination probably originating from the "Catlett farm", it was not necessary to attribute significant fecal indicator loading to this source. The frequent detection of S. bovis in most feeder streams suggested animal pollution is continual and widespread. Finding R. coprophilus in sediments proximate to the "Catlett farm" is corroborative of the hypothesis that farm animals have been contributing fecal pollution to the creek. The very low but measurable occurrence of human sorbitol-fermenting bifidobacteria implies that human sanitary deficiences are present. Finally, this evaluation of alternate indicators supports their value as an investigative "tool", whose presence can be used to qualitatively identify sources and "age" of fecal pollution. Identification of contributing sources through use of these indicators may lead to ameliorative action designed to reduce or eliminate certain kinds of pollution sources.

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