W&M ScholarWorks

Reports

10-15-1988

Evaluation of Alternate Microbial Indicators of Fecal Pollution in a Non-Point Source Impacted Shellfish Growing Area

Howard I. Kator
Virginia Institute of Marine Science

Martha Rhodes
Virginia Institute of Marine Science

Follow this and additional works at: https://scholarworks.wm.edu/reports

Part of the Marine Biology Commons

Recommended Citation
Kator, H. I., & Rhodes, M. (1988) Evaluation of Alternate Microbial Indicators of Fecal Pollution in a Non-Point Source Impacted Shellfish Growing Area. Special Reports in Applied Marine Science and Ocean Engineering (SRAMSOE) No. 297. Virginia Institute of Marine Science, College of William and Mary. http://dx.doi.org/doi:10.21220/m2-14rw-cy12

This Report is brought to you for free and open access by W&M ScholarWorks. It has been accepted for inclusion in Reports by an authorized administrator of W&M ScholarWorks. For more information, please contact scholarworks@wm.edu.

VIMS ARCHIVES

Archives
VIMS
GC
1
S67
no. 297

Evaluation of alternate microbial indicators of fecal pollution in a non-point source impacted shellfish growing area.

A final report submitted to the

Council on the Environment
Commonwealth of Virginia
903 Ninth Street Office Building
Richmond, Virginia 23219

by

Howard Kator
Associate Professor
of Marine Science

and

Martha Rhodes
Marine Scientist

of the

School of Marine Science
College of William and Mary
Virginia Institute of Marine Science
Gloucester Point, Virginia 23062

October 15, 1988

SRAMSOE No. 297

VIMS
GC
1
.S67
no.297

Title: Evaluation of alternate microbial indicators of fecal pollution in a non-point source impacted shellfish growing area.

Introduction

Non-point source pollution has been implicated as a major factor contributing to reduction of shellfish harvesting acreage in estuarine waters. As the classification of shellfish growing waters is based on the recognition of point sources as well as bacterial indicator densities, classification decisions may be based solely on microbial indicator data in the absence of known point sources. This has generated considerable discussion of the validity and relevance of the current indicator/standard when applied to growing waters located in rural or undisturbed areas where sources of fecal pollution may be restricted to domestic or wild animals. A number of alternate indicators have been proposed to differentiate human from animal sources of pollution. One of these, the ratio of fecal coliforms to fecal streptococci, has been widely discussed in the literature but its value appears somewhat uncertain, being affected by factors such as site, temperature, salinity, season, age of contaminant, source type, etc. Other alternate indicators proposed for this purpose have been evaluated primarily in freshwater. This report presents results of an evaluation of traditional and alternate fecal indicators (e.g., sorbitol-fermenting bifidobacteria, *Rhodococcus coprophilus*, and *Streptococcus bovis*) in an effort to identify sources of fecal pollution in a small productive subestuary closed to direct shellfish harvesting owing to elevated fecal coliform counts allegedly due to non-point source fecal pollution. Indicator densities are discussed as functions of temperature, runoff, presence in feeder streams and proximity

to potential sources of contamination. Also discussed are observations related to their survival and recovery methodology.

Methods

Study site. Bonum Creek is a small (ca.$5.4 \times 10^{-1} km^2$) tidal creek located on the south shore of the Potomac River in Virginia. This portion of the river is estuarine and creek salinities ranged from 14.0 o/oo at the mouth to 0.0 o/oo in marshy headwaters and feeder streams. Feeder streams varied considerably in cross section and volume, with a number disappearing during seasonally dry periods. (The following characteristics of the Bonum Creek watershed were obtained from the Bureau of Shellfish Sanitation Shoreline Surveys, Virginia Department of Health). Topographically, the area is characterized by elevations of 1-2 m near the mouth to 45 m bluffs near the headwaters. Soil characteristics of the upper elevations are generally well drained and sandy with deep water tables. Middle elevations share similar characteristics with those of the upper but show evidence of some high water tables. The lower elevations are variable and those with little relief, e.g., broad flat areas close to marshes and drainage paths, have very poor drainage. Standing water was observed in a number of areas with these characteristics. Development is sparse, with single dwelling homes distributed evenly around the creek although relatively greater concentrations of homes can be found in the vicinity of Stations #17 and #18. All homes are served by individual on-site sewage facilities and there are no septage disposal sites, animal wastes facilities or solid wastes sites in the area. The area is primarily agricultural and some residents indulge in "hobby" farming, keeping small numbers of domestic animals. A current shoreline survey was provided by the Bureau of Shellfish Sanitation,

Virginia Department of Health. This particular subestuary and its watershed were considered free of known point sources of pollution.

Sample collection and transport. 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. All samples were maintained at ambient temperature in insulated containers during transport to the laboratory. Total elapsed time from sampling to processing was 6 h or less.

Bacterial indicator enumeration. Fecal coliform densities were determined by the five-tube MPN technique (APHA, 1985). Volumes of 10 ml, 1 ml and dilutions thereof were inoculated into lactose broth. Positive EC tubes from selected samples were examined for the presence of *Escherichia coli* using IMViC tests (APHA, 1985) supplemented with API 20E test strips (Analytab Products, Inc., Plainview, New York).

Streptococcus 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. One and 10 ml volumes were membrane filtered in triplicate, 50 ml or 100 ml volumes were processed in triplicate in samples containing low suspended solids; if suspended solids were high, four 25 ml volumes were filtered. One ml samples were diluted prior to filtration to insure even distribution of cells on the filters and the filtration units rinsed with sterile phosphate buffer (APHA, 1985). Beginning with the November survey, this procedure was modified for bifidobacteria assays by substituting gelatin diluent (NCDC, 1968) for phosphate buffer. *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 (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, growth at 10 and 45 C, starch hydrolysis, arginine hydrolysis, fermentation of lactose, sorbitol, raffinose, inulin and mannitol, 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 either at room temperature (20 C) or 30 C 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 exhibited the physiological characteristics reported by Rowbotham and Cross (1977). Typical physiological tests included: growth in 7% NaCl, pellicle formation on the surface of Bennett's broth, thiamine requirement, and utilization of glucose, glycerol, mannitol, sorbitol, serine and alanine as sole sources of carbon and energy.

Enumeration efficiency of Bifidobacterium adolescentis. B. adolescentis (ATTC 15703) was cultured anaerobically in peptone-yeast extract glucose

broth (Manual of Clinical Microbiology, 1985) at 35 C for 48 h and enumerated by spread plating and membrane filtration onto non-selective reinforced clostridial agar and selective HBSA. Three replicates were provided for each dilution and treatment. Additionally, enumeration efficiency was compared using three different diluent solutions; phosphate dilution buffer (APHA, 1985), Ringer's solution (Microbiological Methods, 1984), and gelatin diluent (NCDC, 1968). All plates were incubated at 35 C for 72 h.

In vitro survival studies. The following organisms were grown overnight at 35 C on the indicated media: B. adolescentis (ATCC 15703) on sorbitol broth modified after HBSA and lacking antimicrobials or dye; S. bovis (ATCC 9809) on BHI; a human fecal isolate (experiment 1) or estuarine isolate (experiment 2) of E. coli on trypticase soy broth (TSB). R. coprophilus (ATCC 29080) was grown in Bennett's broth at 30 C for 3-5 days. All cultures were diluted 1:10,000 in double membrane filtered (0.45 and 0.2 um) freshly collected site water to final volumes of 500 ml (experiment 1) or 250 ml (experiment 2). Three sites of contrasting salinity were chosen and each of the four bottles from each site was inoculated with one of the test organisms. At selected intervals duplicate samples were removed from each bottle and enumerated for the test organisms by spread plating on RCA (B. adolescentis), TSA (E. coli), BHI (S. bovis), and Bennett's agar (B. coprophilus). With the exception of R. coprophilus (30 C for 7 days), all the remaining plates were incubated at 35 C for 72 h.

Enumeration of Salmonella spp. Salmonella spp. were enumerated using a three tube MPN procedure based on ambient-temperature primary nonselective enrichment in dulcitol broth, followed by selective enrichment in selenite cystine and isolated on xylose lysine desoxycholate agar (Kaper et al.,

1977). Presumptive colonies were screened on triple sugar iron agar and lysine iron agar and then characterized to the genus level using the API 20E system. Species identification was performed using serological methods by the Department of General Services, Richmond, VA.

Results

The distribution of fecal coliforms. Fecal coliform densities for all sample dates and stations are shown in Table 1. Figure 1 shows the locations of stations sampled. Mean fecal coliform densities and relevant physical data for the subestuary and major feeder streams are shown in Table 2. Mean water temperatures (Table 3) ranged from a seasonal low of 3.9 to a summer maximum of 28 ^{o}C. Samples were generally collected during slack before flood tidal conditions and rain occurred within 3 days preceeding sample collection on 8 of 11 occasions. Mean log fecal coliform densities (Figure 2) were maximal at feeder streams, headwaters and in small cul-de-sacs typified by station 6b. Mean densities decreased in the order feeder streams, headwaters and mainstem stations. Values exceeded the approved growing area standard of 14 fecal coliforms 100 ml^{-1} at mainstem stations upstream and including station #7 which overlies a productive shellfish bed. Correlation analysis (Table 4) showed that fecal coliform densities were significantly and positively correlated with rainfall, turbidity and temperature and negatively correlated with salinity.

Alternative indicators. The occurrence of alternate indicators was examined in feeder streams and three mainstem creek stations. Results for bifidobacteria, _S. bovis,_ and _R. coprophilus_ are shown in Tables 5-7. The

human specific sorbitol-fermenting bifidobacteria were detected only in feeder streams at generally very low densities and during those times of seasonally low water temperatures. However, bifidobacteria were detected at several feeder stream stations on more than one occasion. The occurrence of rain did not always correlate with detection of this or the other alternate indicators.

In contrast to the restricted seasonal occurrence of the bifidobacteria, S. bovis was detected during essentially all seasons and temperature conditions. Although maximum densities of the indicator were higher than those of the bifidobacteria (ca. 2 log units), it was similar in being more frequently detected in feeder streams as compared to the creek mainstem.

R. coprophilus was recovered on only four sampling dates and therefore was the least frequently detected alternate indicator. Consequently, it was not possible to discern a pattern of occurrence as a function of seasonal temperature. However, R. coprophilus was detected in the same feeder stream on two comparatively closely spaced sampling intervals and occurred once at a mainstem station.

Alternate indicators: in vitro survival studies. Significant differences in survival characteristics of the fecal coliform and alternate indicators were observed as a function of temperature (Table 8). Survival is expressed here using **k**, the exponential dieoff coeficient (where $-\mathbf{k} = \ln (C_2 - C_1) / t_2 - t_1$; and C = cell density at times t_2 and t_1). Because cells prepared under conditions to minimize stress will generally manifest aftergrowth for 1-3 days following exposure, values of **k** were calculated over intervals of 0-3 days and from 3 days to termination of the experiment. Overall, the results

for the fecal coliform, E. coli, confirmed its capacity to persist at low ambient temperatures, i.e., 6°C for a relatively prolonged time period despite a gradual decline (Figure 3) in cell density. Values of **k** for R. coprophilus and B. adolescentis revealed essentially similar survival patterns (Figures 4 and 5) and both showed greater dieoff than E. coli. In contrast, S. bovis (Figure 6) showed greater mortality than either of the alternate indicators or E. coli.

At an incubation temperature of 25°C, E. coli exhibited characteristic aftergrowth (large negative values of **k**) and persistence (Figure 7). Compared to their relative persistence at 6 °C, both B. adolescentis and S. bovis exhibited markedly rapid dieoff (Figures 8 and 9) at the higher temperature, the latter exhibiting the largest positive values of **k** measured. S. bovis was undetectable after 1 day at 25°C. R. coprophilus survival was similar to that of E. coli with apparent aftergrowth followed by a period of little change in **k** or density (Figure 10).

There was little evidence in both experiments of consistent patterns of persistence as a function of salinity, i.e., location from which the different exposure waters were collected. However, at 6°C survival of E. coli was favored in 0 o/oo and S. bovis survival was better at 17 o/oo.

Efficiency of recovery of alternate indicators. Results of an experiment to compare the effects of procedure on recovery of B. adolescentis are shown in Table 9. These results, where percent recovery was normalized to recovery on a non-selective medium with spread plating, revealed that although there were comparatively small differences in recovery with different diluents, use of the selective medium with spread plating resulted in a 75-85%

recovery loss and use of membrane filtration onto the selective medium could yield a 99% loss.

Discussion

The object of this study was to explain the origin of elevated fecal coliform counts which had resulted in closure of historically productive shellfish beds located near the mouth of Bonum Creek. Bonum Creek was characterized by several state regulatory groups as an area with "unsatisfactory bacteriological water quality with no known pollution sources". Our observations of the distributions of fecal coliform densities in the creek were not dissimilar to those in other small tidal creeks, i.e., high levels at the headwaters with values progressively diminishing toward the mouth. Intuitively, this pattern suggests inputs at the headwaters with reductions of indicator levels downstream due to a variety of factors which can include dilution, dieoff, removal owing to sedimentation, and net loss due to tidal exchange. Although, we had no access to flushing models or data, comparative measurements of tidal height changes immediately outside the mouth and within the creek suggested that the narrow mouth does restrict flushing. These measurements indicated tidal heights outside the creek were approximately 1/2 those in the creek at the beginning of flood tide. In hydrographic terms, the creek may act as a "sink" and therefore, indicators of fecal pollution with the capability to survive (such as the fecal coliform *E. coli*) may persist at elevated levels.

In an effort to understand the mass balance of the system in terms of the fecal coliform indicator, we measured the densities of this indicator in major feeder streams contributing to the creek. Basically, the question asked was whether the magnitude of these sources was sufficient to account

for the observed fecal coliform distribution? Furthermore, if the feeder streams were of importance to fecal coliform budget, could we employ a variety of candidate indicators of fecal pollution, including those allegedly specific to animals or humans, to identify sources of fecal contamination?

Results from correlation analysis of fecal coliform densities and physical factors such as turbidity suggested that non-point source runoff was a contributing factor to fecal coliform loading. Creek flow rates and indicator densities were measured concommitantly on several representative occasions. These data are summarized in Table 10. Knowing the instantaneous fecal coliform concentration and the stream flow allowed us to calculate an estimated amount of fecal coliforms added per day for each feeder stream. Interestingly, if this number is divided by the daily per capita contribution of 2.0×10^9 fecal coliforms/day, it is possible to estimate the number of humans theoretically required to yield the calculated input level and to compare this to the actual population density. Thus, in the case of station #21 on the SE leg, sanitary survey data indicated the residential population to be within the range required to provide the theoretically calculated level of contamination. To estimate the contributions of the feeder streams (in terms of fecal coliform concentrations) in the creek receiving waters, fecal coliform inputs to each leg were summed and divided by estimates of the volume of water in the SE leg or the smaller western leg. The latter values were calculated by planimetry for 3 depth scenarios (0.1, 0.5, and 1.0 m). These values were then compared to the mean fecal coliform densities for each leg calculated from actual field data. Considering the assumptions involved in these calculations it was surprising to note the close correspondence between

theoretically-calculated and actual concentrations in the large SE leg. These results support the hypothesis that the input of fecal coliforms from the feeder streams was adequate to account for the observed indicator levels in the creek proper.

A number of alternate indicators were used in the study in a preliminary effort to (1) corroborate their presence in estuarine waters and (2) to evaluate these indicators for their ability to separate and identify human from non-human sources of fecal pollution. The latter issue is one of great importance to management of the sanitary microbiology of shellfish growing areas. As previously noted, Bonum Creek was classified as an area closed despite the absence of known point sources of fecal pollution. At the inception of this study this classification was confirmed as well as the lack of known domestic animal pollution. For the purpose of this discussion these results are summarized in Figure 11 which shows recovery frequencies for various alternate indicators, Salmonella spp. and mean fecal coliform densities at feeder stations and selected mainstem creek locations. The frequency of occurrence is expressed using the convention "# times detected/ total # surveys". Sorbitol-fermenting bifidobacteria, allegedly specific to humans, were found in a number of feeder streams but not in the creek mainstem. This distribution was not unexpected considering the poor survival characteristics and low recoverability of the bifidobacteria, which if detected implies a very recent source. R. coprophilus, an indicator of ruminant (and perhaps other animal) feces, was detected on two occasions at the same feeder stream. S. bovis, presumably an indicator of animal feces, (although it may occur at a relatively low frequency in humans), was widely distributed with high occurrences in all feeder streams and much less frequently detected in mainstem stations. In view of the poor survival characteristics of this indicator (as well as the bifidobacteria at warm

temperatures), its widespread occurrence supports the existence of a significant number of comparatively fresh and continuous inputs. Interestingly, salmonellae were detected at all feeder streams at comparatively high frequencies (considering the # sampling events) except station #17, from which bifidobacteria were also absent.

Following an analysis of the aforementioned data, which suggested animal pollution was present as well as a widely distributed number of sources contributing human fecal pollution, we arranged a site visit with the Westmoreland county sanitarian. After a comprehensive site visit and review of updated sanitary survey data, a number of deficiences were identified and correlated with the indicator data. These deficiencies are shown in Figure 12. All feeder stream locations except #17 were impacted by residences with sanitary deficiences. These ranged from full privies, frank septic seepage to dwellings without facilities. Following the site visit, we were able to relate the occurrence of _R. coprophilus_ (twice in the same stream) to a proximate dwelling whose owner admitted she hobby-farmed cattle intermittently on a small scale for private use. The feeder stream with the highest frequency of _S. bovis_ correlated with the identification of a small hobby farm with at least 20 pigs. (The presence of this farm with its potential animal pollution was suspected on the basis of aerial recconaissance and had not been reported to the county sanitarian.) The highest frequencies of detection for human bifidobacteria correlated with high numbers of deficiencies (station #21) or raw sewage (stations #20, #19). It is significant that the feeder stream (station #17) from which no bifidobacteria or salmonellae were recovered drained a deficiency-free area. The presence of _S. bovis_ suggests that animals (wild/domestic?) impacted this station as well as others on a relatively continuous basis.

The question arises as to the mechanism(s) of transfer of feces to the feeder streams and ultimately the creek. The most obvious mechanism is through rain produced runoff and as we have seen there was a statistically significant correlation between rainfall, turbidity and fecal coliform densities. During our detailed sanitary survey we noted standing water in a number of roadside ditches and yards. These were especially evident in the soil drainage area impacting station #21. Subsequent conversations with the sanitarian and examination of soil survey data (Soil Survey of Westmoreland County, Virginia. 1981. USDA Soil Conservation Serivce) indicated soils in this area have very high seasonal water tables (6" to 1.5') and/or are comparatively poorly drained. It would appear that septic systems in such locations cannot operate properly and should be replaced with (ideally) central sewage systems or other means which do not rely on subsurface percolation of sewage. Similarly, the incidence of hobby-farming, which we suspected on the basis of an aircraft survey and the detection of an alternate indicator, must be known to area sanitarians. We recommend that residents must be encouraged, either through licensing and/or punitive action, to report hobby-farming and this information must be frequently updated within the current sanitary survey report. It is obvious that if these problems (which are evidently widespread) cannot be addressed, then the problem of non-point fecal pollution will never be eliminated. However, the fact that the problems identified can be remedied should be viewed as a positive result of this study. With new approaches it should be possible to devise a program to reduce or eliminate some sources of fecal pollution which can impact a shellfish growing area such as Bonum Creek. The efficacy of the program should be evaluated using methods which include the microbiological techniques used in this study.

Conclusions

The major conclusion from this study must be that the Bonum Creek area is closed to direct shellfish harvesting because of comparatively widespread and probably not atypical sources of "non-point source" fecal pollution. It would appear that the sources of fecal pollution from recognized sources, i.e., malfunctioning septic systems owing to poor soil characteristics, improper owner operation, and unreported hobby-farming, etc., are contributing factors to the deteriorated sanitary water quality. Consequently, although there was no need to invoke wild animal pollution as the unknown source of fecal pollution to explain the elevated levels of fecal coliforms, the widespread occurrence of *S. bovis*, an alleged indicator of animal pollution, suggests animals also contributed to pollution of the the watershed. More specific conclusions are:

1. Inputs of fecal pollution to feeder streams appeared to be adequate to account for the levels of fecal coliforms observed in impacted Bonum Creek headwaters.

2. Detection of human-specific bifidobacteria in feeder streams correlated with human sanitary deficiencies in the drainage area.

3. *S. bovis* was found in all feeder streams suggesting widespread sources of animal pollution.

4. *R. coprophilus*, an indicator of fecal pollution from ruminants (and some other animals), was detected in a feeder stream adjacent to a hobby farm whose owner periodically raised cows for slaughter.

5. Use of alternate indicators provided a "tool" which enabled identification and confirmation of sanitary deficiences, some of which can be corrected, thereby providing the potential for improved water quality. Although, the distribution of these indicators was restricted temporally and spatially owing to their differential survival characteristics, these properties were useful in assessing the "freshness" of the pollution and their probable sources.

6. Additional research is required to optimize the recovery of alternate indicators.

Literature cited.

1. American Public Health Association. 1985. Laboratory procedures for the examination of seawater and shellfish. Fifth edition. American Public Health Association, Washington, D.C.

2. American Society for Microbiology. 1985. Manual of clinical microbiology. Fourth edition. American Society for Microbiology, Washington, D.C. 20006

3. Kaper, J.B., G.S. Sayler, M.M. Baldini and R.R. Colwell. 1977. Ambient-temperature primary nonselective enrichment for isolation of _Salmonella_ spp. from an estuarine environment. Appl. Environ. Microbiol. 33: 829-835.

4. Mara, D.D. and J.I. Oragui. 1981. Occurrence of _Rhodococcus_ _coprophilus_ and associated actinomycetes in feces, sewage and freshwater. Appl. Environ. Microbiol. 42: 1037-1042.

5. Oragui, J.I. and D.D. Mara. 1981. A selective medium for the enumeration of _Streptococcus_ _bovis_ by membrane filtration. J. Appl. Bacteriol. 51: 85-94.

6. Mara, D.D. and J.I. Oragui. 1983. Sorbitol-fermenting bifidobacteria as specific indicators of human fecal pollution. J. Appl. Bacteriol. 55: 349-357.

7. National Communicable Disease Center. 1968. Laboratory methods in anaerobic bacteriology. Public Health Service Publication No. 1803, June 1968. U.S. Government Printing Office, Washington, D.C. 20402.

8. Rowbotham, T.J. and T. Cross. 1977. Rhodococcus coprophilus sp. nov.: an aerobic nocardioform actinomycete belonging to the 'rhodochrous' complex. J. Gen. Microbiol. 100: 123-138.

Table 1. Fecal coliform densities at selected sites in Bonum Creek and major feeder streams.

	Fecal Coliforms/100 ml										
STATION	2-3-87	2-23-28	3-17-87	4-14-87	5-12-87	6-23-87	7-27-87	9-21-87	11-16-87	12-7-87	2-2-88
1	2.0	23	ID	ID	2.0	4.5	6.8	17	2	7.8	2.0
2	ID	49	6.1	ID	23	7.8	17	17	4.5	11	2.0
3	ID	170	33	49	230	49	33	23	110	23	17
4	2.0	23	13	2.0	2.0	6.8	21	27	13	23	7.8
5	ID	7.8	2.0	4.5	4.5	23	700	130	4.5	7.8	7.8
6a	ID	27	11	23	46	17	33	84	13	4.5	22
6b	nd	170	110	110	330	33	130	330	49	79	22
7	ID	70	13	49	17	11	17	79	31	33	11
8	4.5	49	14	49	130	17	79	79	33	13	23
9	4.0	170	170	49	70	49	17	33	6.8	23	140
10	nd	11	1.8	110	46	49	70	130	31	2	2
11	nd	220	33	49	49	95	23	49	23	33	23
12	nd	49	23	110	46	33	79	11	79	79	11
13	nd	170	130	280	170	130	46	14	79	79	23
14	nd	1,600	230	330	170	130	110	13	49	23	46
15	nd	1,300	110	79	330	490	330	7.8	23	230	49
16	nd	nd	nd	33	310	220	790	33	170	nd	nd
17	79	350	49	350	230	170	490	140	230	220	490
18	79	49	49	79	490	350	1,400	330	70	6.1	20
19	nd	nd	nd	nd	460	700	490	79	330	430	3500
20	23	310	79	26	700	1,300	3,500	490	33	31	17
21	22	270	7.8	130	27	1,300	1,100	3,500	170	27	11
Rainfall - inches, previous 3d)	0.0	1.9	0.4	0.2	1.1	1.8	0.4	0.75	0.0	0.2	0.0

ID (Indeterminate) = <1.8 Fecal coliforms /100 ml, nd = Not Done. Note: On 9-21-87 there was an unusually large volume of water in the creek during low slack water.

Table 2. Mean values of log fecal coliform densities and selected physical variables for all dates sampled in Bonum Creek and feeder streams.

Station	n	Salinity o/oo	Turbidity NTU	Fecal coliforms /100 ml
1	11	12.1	9.9	0.61
2	11	11.4	11.0	0.88
3	11	10.9	14.6	1.57
4	11	11.5	10.9	0.94
5	11	11.6	12.7	1.05
6a	11	10.5	16.9	1.23
6b	10	10.2	16.7	1.99
7	11	10.7	23.5	1.31
8	11	10.5	17.1	1.48
9	11	9.9	12.4	1.58
10	10	10.8	17.7	1.27
11	10	9.6	12.7	1.65
12	10	9.4	12.5	1.61
13	10	8.5	18.7	1.91
14	10	8.4	17.6	2.03
15	10	7.3	17.2	2.11
16	6	8.0	12.5	2.17
17	11	0.0	7.4	2.32
18	11	0.1	11.5	1.91
19	7	0.0	14.1	2.68
20	11	0.0	16.9	2.15
21	11	0.2	23.0	2.06

Table 3. Average temperature, prior rainfall and tidal stage for each sampling date.

Sampling date	Mean water temperature (°C) all stations	Rainfall (") prior to sampling 3d	Rainfall (") prior to sampling 7d	Tidal Stage
2-03-87	4.0	0.0	0.0	SBF
2-23-87	3.9	1.9	1.9	SBE
3-17-87	7.6	0.4	0.4	SBF
4-14-87	15.3	0.2	0.2	SBF
5-12-87	20.4	1.1	1.1	SBF
6-23-87	26.0	1.8	1.8	SBF
7-27-87	28.0	0.4	0.4	SBF
9-21-87	22.4	0.8	0.8	SBF
11-16-87	11.2	0.0	1.2	SBF
12-07-87	6.5	0.2	0.4	SBF
2-02-88	10.5	0.0	0.0	SBF

Table 4. Pearson correlation coefficient for log fecal coliform MPN versus various physical measurements. Data are for all surveys and for station groups indicated.

Log FC vs.	Log FC	Temp	Salinity	Rainfall Previous 3d	Rainfall Previous Week	Turbidity
A. All stations:						
Coef	1.000	0.25	-0.55	0.28	0.27	0.32
p	0.000	0.0002	0.0001	0.0001	0.0001	0.0001
n	226	226	226	226	226	213
B. Main stem stations only:						
Coef	1.000	0.28	-0.48	0.30	0.29	0.29
p	0.000	0.0002	0.0001	0.0001	0.0001	0.0002
n	175	175	175	175	175	166
C. Feeder stream stations only:						
Coef	1.000	0.54	-	0.36	0.328	0.57
p	0.000	0.00001	-	0.011	0.019	0.0001
n	51	51	-	51	51	47

Table 5. Dectection of presumptive "human bifidobacteria" at selected sites in Bonum Creek and feeder streams.

Presumptive human bifids/100ml

	1987										1988	
Sta	Feb 2	Feb 23	Mar	Apr	May	Jun	Jul	Sept	Nov	Dec	Feb 2	Mar
1	-[a]	-	-	-	-	-	-	-	-	-	-	-
9	-	nd	-	-	-	-	-	-	-	-	-	-
15	nd[b]	-	-	-	-	-	-	-	-	-	-	-
17	nd	-	ns[c]	-	-	-	-	-	-	-	-	-
18	nd	-	ns	-	-	-	-	-	10	-	-	-
19	nd	nd	nd	nd	3	-	-	-	-	-	8	-
20	nd	87	ns	1.3	1.4	-	-	-	-	-	-	-
21	3.3	6.7	ns	-	-	-	-	-	-	-	-	-
		R	R	R	R	R	R	R		R		

[a]Indeterminate, ≤0.3 to ≤1.0 / 100ml depending on sample volume filtered.

[b]nd = not done, [c]ns = no sample results due to anaerobic system failure.

R = rain three days prior to sampling.

Table 6. Detection of confirmed Streptococcus bovis at selected sites and feeder streams.

Confirmed *S. bovis*/100ml

	1987									1988	
Sta	Feb 2	Feb 23	Mar	May	Jun	Jul	Sept	Nov	Dec	Feb	Mar
1	–[a]	10	–	–	–	–	–	–	–	–	–
9	0.3	nd	–	–	–	–	–	–	–	–	–
15	nd[b]	42	–	–	–	–	–	–	–	–	–
17	nd	850	72	–	–	–	13	3.4	5.8	–	–
18	nd	31	3.9	1.7	–	–	–	1,800	–	–	2.0
19	nd	nd	nd	–	–	–	–	41	–	–	–
20	nd	10	7.0	660	–	8.3	2.0	–	–	3.0	–
21	0.7	–	–	2.0	–	230	–	–	1.0	1.0	2.0
		R	R	R	R	R	R		R		

[a]Indeterminate, ≤0.3 to ≤1.0 / 100ml depending on sample volume filtered.

[b]nd = not done. R = rain three days prior to sampling.

Table 7. Detection of Rhodococcus coprophilus in Bonum Creek and feeder streams

Sta	Rhodococcus coprophilus/ml										
	1987									1988	
	Feb	Mar	Apr	May	Jun	Jul	Sept	Nov	Dec	Feb	Mar
1	–[a]	–	–	–	–	–	–	–	–	–	–
9	–	–	3.3	–	–	–	–	–	–	–	–
15	nd[b]	–	–	–	–	–	–	–	–	–	–
17	nd	–	–	–	–	–	–	–	–	–	–
18	nd	–	–	–	–	–	–	–	–	–	–
19	nd	nd	nd	–	–	–	–	–	–	–	–
20	nd	–	–	–	–	3.3	–	–	–	–	–
21	33[c]	–	3.3	–	–	–	–	–	–	–	–
	R	**R**	**R**	**R**	**R**	**R**	**R**		**R**		

[a]Indeterminate, <1 colony on each of three replicate plates (<3.3/ml).

[b]nd = not done. **R** = rain three days prior to sampling.

[c]Suspect R. coprophilus colonies were confirmed by additional testing for all surveys except 2-3-87.

Table 8. Survival[a] of selected bacterial indicators in filtered (0.2 um) water collected from three locations in Bonum Creek during two seasonal temperature regimes. Locations included the mouth (#1), an upstream location (#11 or #15), and a freshwater feeder stream (#17).

	E. coli			*R. coprophilus*			*B. adolescentis*			*S. bovis*		
	#1	#11	#17	#1	#11	#17	#1	#11	#17	#1	#11	#17
					Incubation temperature = 6 °C							
Day												
0-3	0.28[a]	0.31	0.63	0.01	0.27	0.15	0.20	0.18	0.21	0.42	0.39	0.66
3-42	0.11	0.15	0.25[d]	-0.15	0.04	0.04	0.23[c]	0.15	0.15	0.24[e]	0.40[d]	0.62[f]
					Incubation temperature = 25 °C							
Day												
0-3	-2.35	-2.88	-2.70	-0.48	-0.51	-1.88	1.03	3.68[b]	1.10	4.28[b]	1.44	4.12[b]
3-60	0.20	0.15	0.02	0.01[c]	-0.24	-0.02	--	--	--	--	--	--

[a]expressed as values of **k**, d^{-1}, the dieoff coefficient, where $-k = \ln (C_2/C_1)/T_2 - T_1$ and C equals cell density at time t; [b]0-1 day; [c]3-25 days; [d]3-22 days; [e]3-35 days; [f]3-14 days

Table 9. Percent recovery of *B. adolescentis* (grown in PYEG broth and diluted as indicated on nonselective reinforced clostridial agar (RCA) and selective human bifid sorbitol agar (HBSA) using spread plating and membrane filtration techniques.

Diluent	Percent recovery[a]			
	Spread plating		Membrane filtration	
	RCA	HBSA	RCA	HBSA
Phosphate buffer	100	24	26	1
Ringer	93	15	26	1
Gelatin	101	26	39	8

[a]Percent recovery relative to that obtained using phosphate buffer as the diluent and spread plating on RCA. Each value is the geometric mean of four replicate experiments.

Table 10. Input of fecal coliforms to Bonum Creek based on feeder stream flow rates and fecal coliform densities.

Feeder Stream Station	Flow rate, l/sec	FC/100 ml	FC added/day	Per capita equivalents
#17	120.0	140	1.7×10^{10}	8.5
#18	1.2	330	3.4×10^{8}	0.2
#19	1.3	79	8.6×10^{7}	0.04
#21	14.7	3500	4.4×10^{10}	22.0
#20	6.0	490	2.5×10^{9}	1.25

Theoretical FC concentration calculated using three depth values and dividing the FC added/day by the resultant volume of water in appropriate receiving legs.

Leg receiving #'s 17,18,19,21				Leg receiving #20			
Depth			Mean FC, field data	Depth			Mean FC, field data
0.25 m	0.50 m	1.0 m		0.25 m	0.50 m	1.0 m	
4.1×10^{2}	2.0×10^{2}	1.0×10^{2}	**1.3×10^{2}**	7.9×10^{2}	4.0×10^{2}	2.0×10^{2}	**8.1×10^{1}**

Figure 1. Station locations

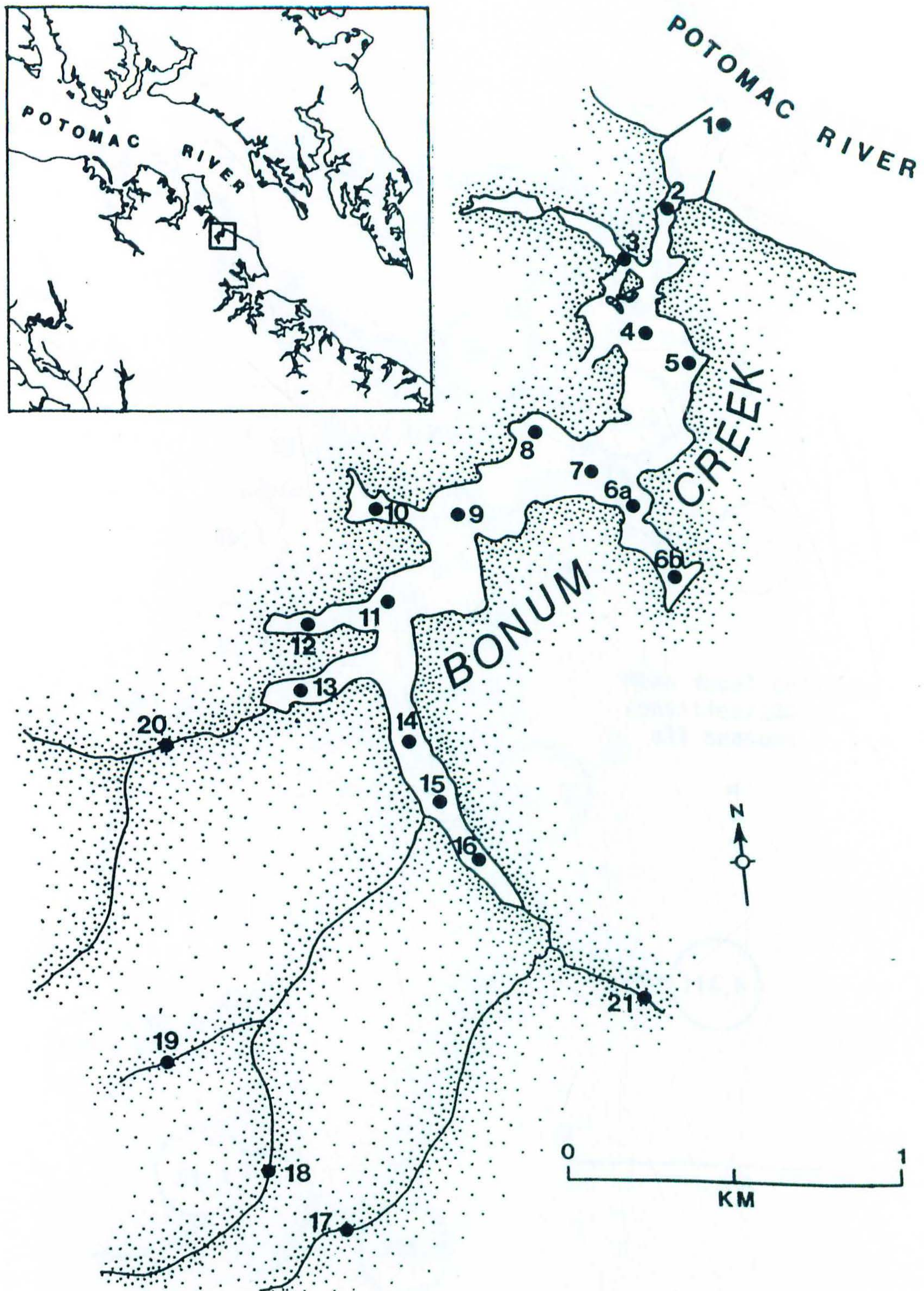


Figure 2. Mean fecal coliform densities, all stations, all dates

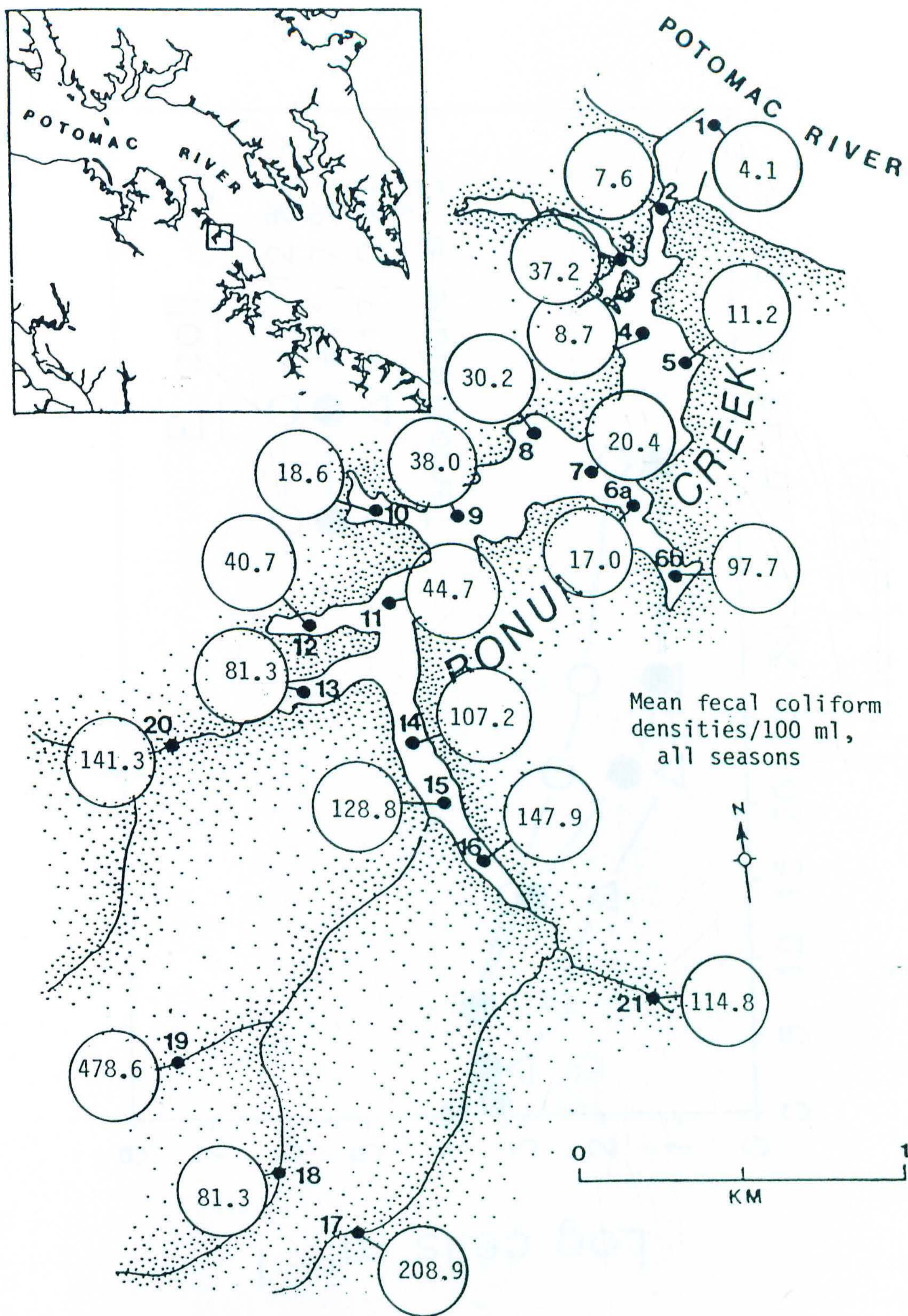


Figure 3. Survival of E. coli at 6°C in flasks containing filtered water from the indicated stations.

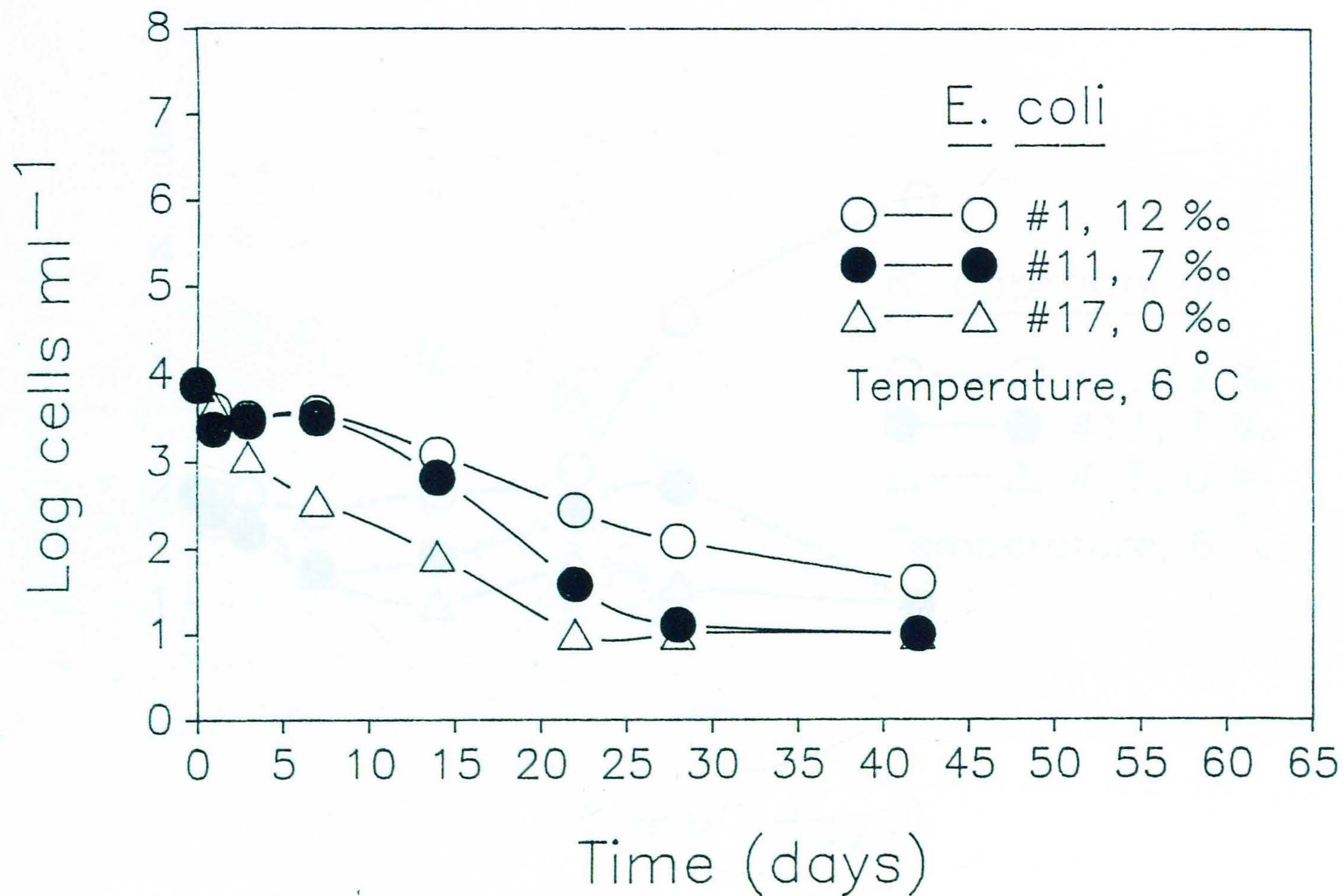


Figure 4. Survival of R. coprophilus at 6°C in flasks containing filtered water from the indicated stations.

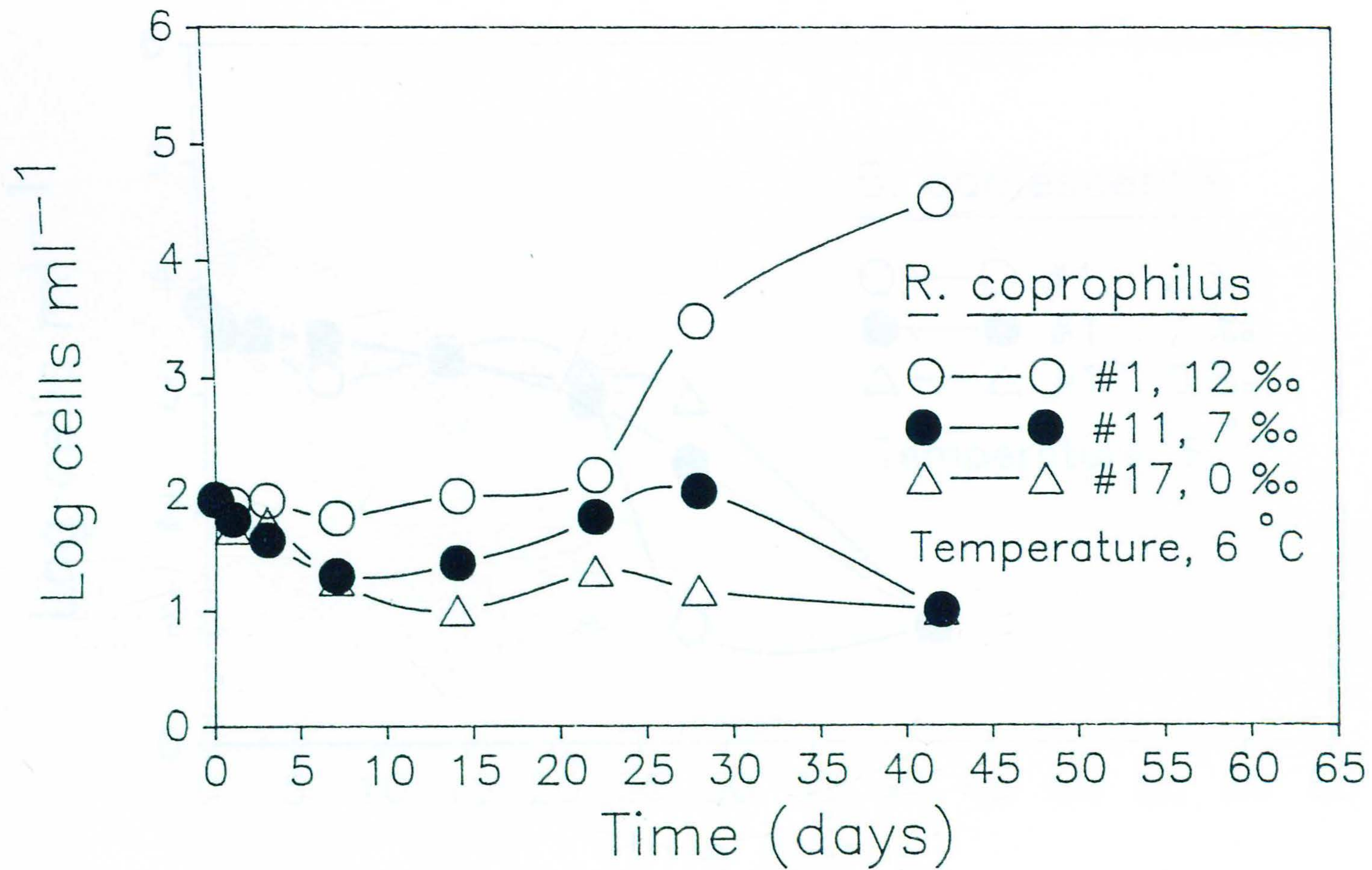


Figure 5. Survival of B. adolescentis at 6°C in flasks containing filtered water from the indicated stations.

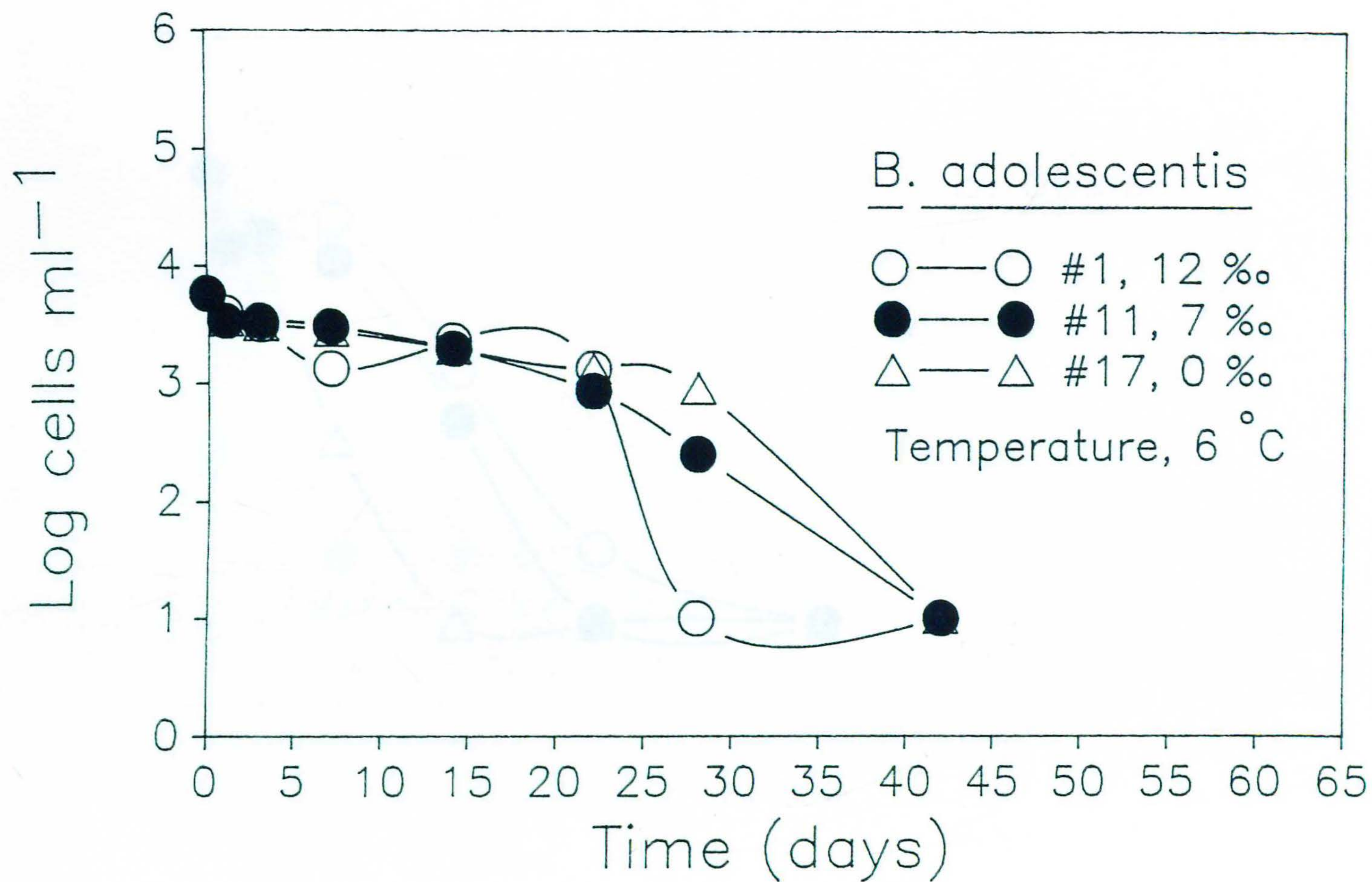


Figure 6. Survival of S. bovis at $6^{0}C$ in flasks containing filtered water from the stations indicated.

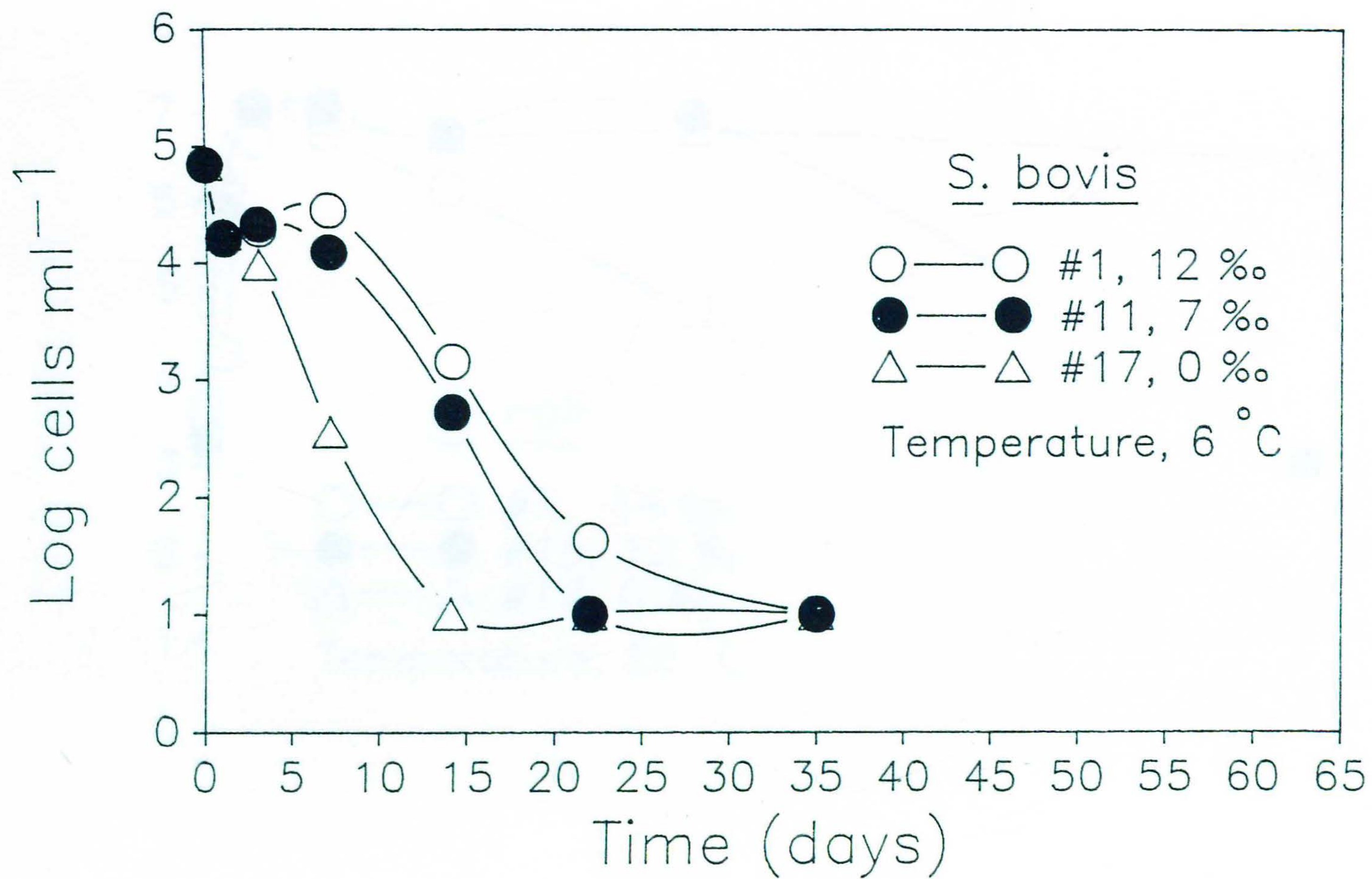


Figure 7. Survival of E. coli at 25°C in flasks containing filtered water from the stations indicated.

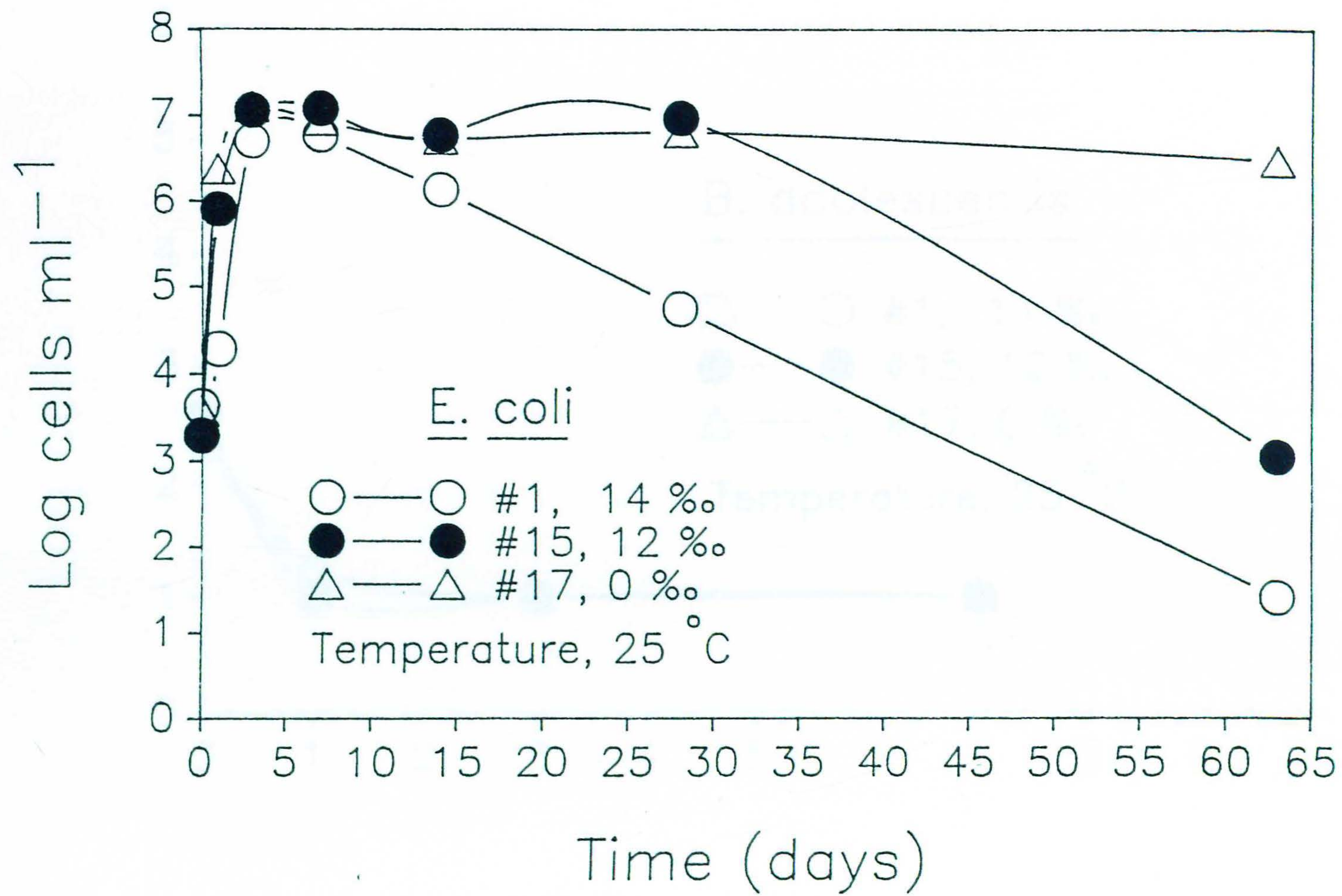


Figure 8. Survival of *B. adolescentis* at 25°C in flasks containing filtered water from the indicated stations.

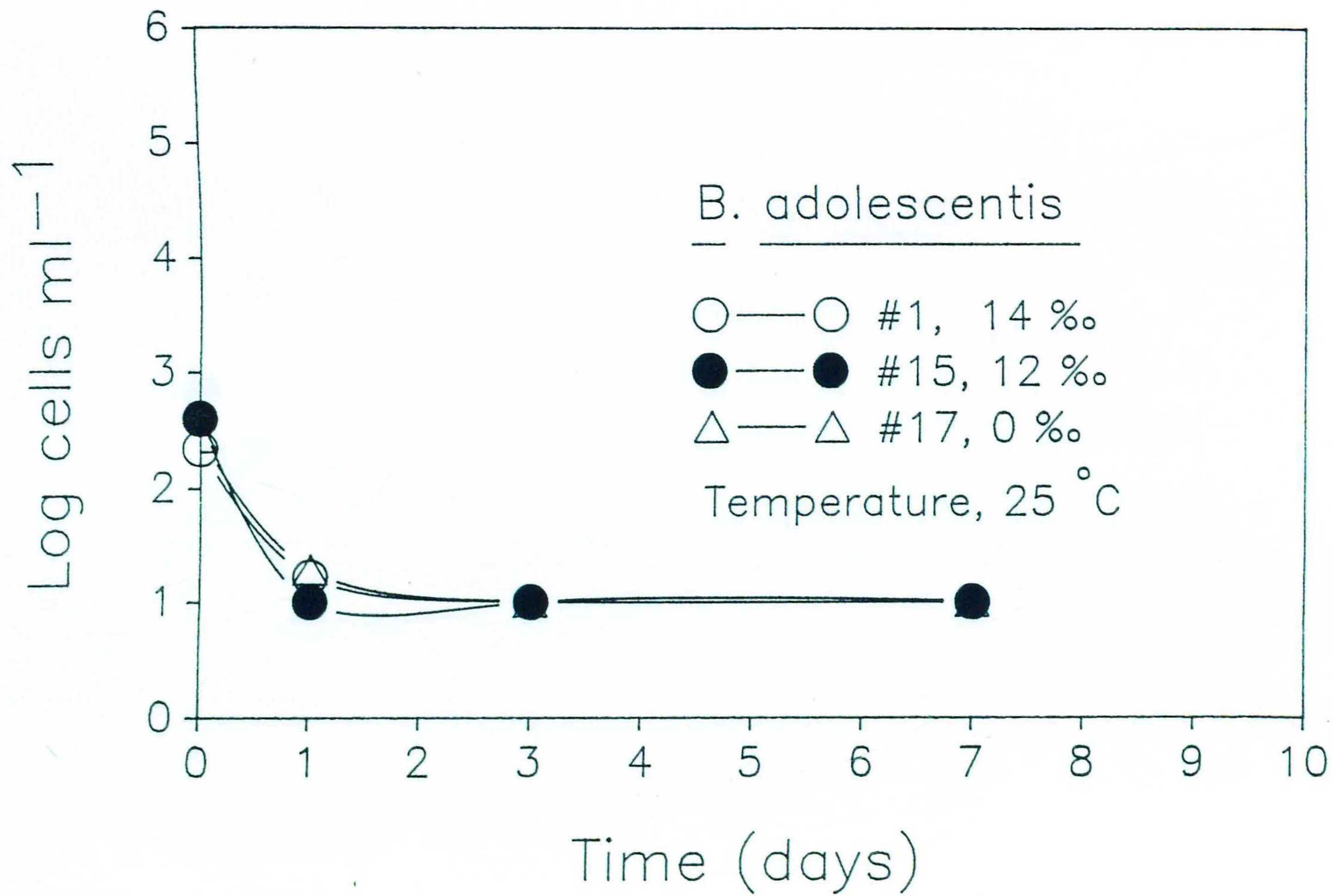


Figure 9. Survival of S. bovis at 25°C in flasks containing filtered water from the stations indicated.

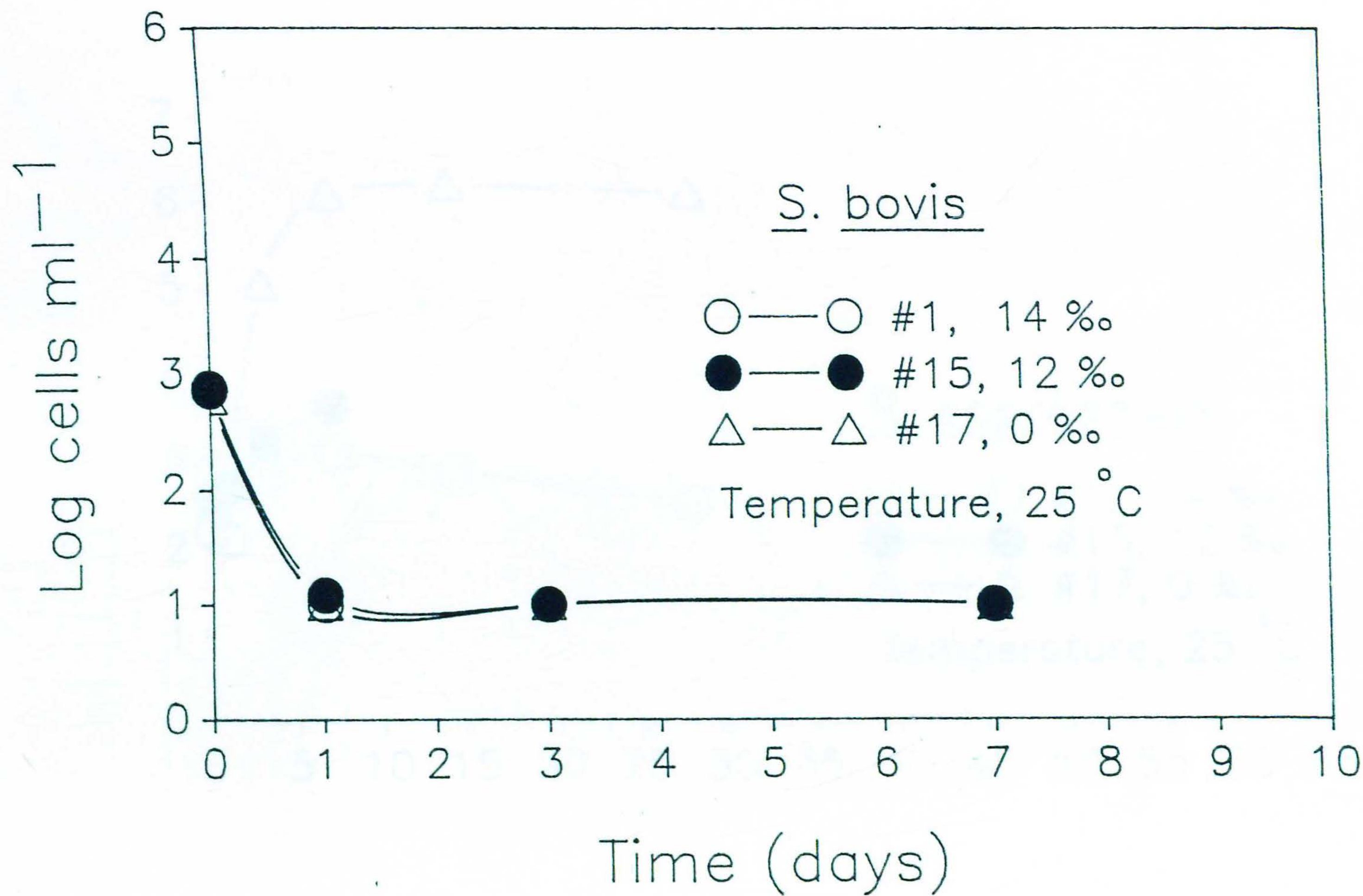


Figure 10. Survival of R. coprophilus at 25°C in flasks containing filtered water from the stations indicated.

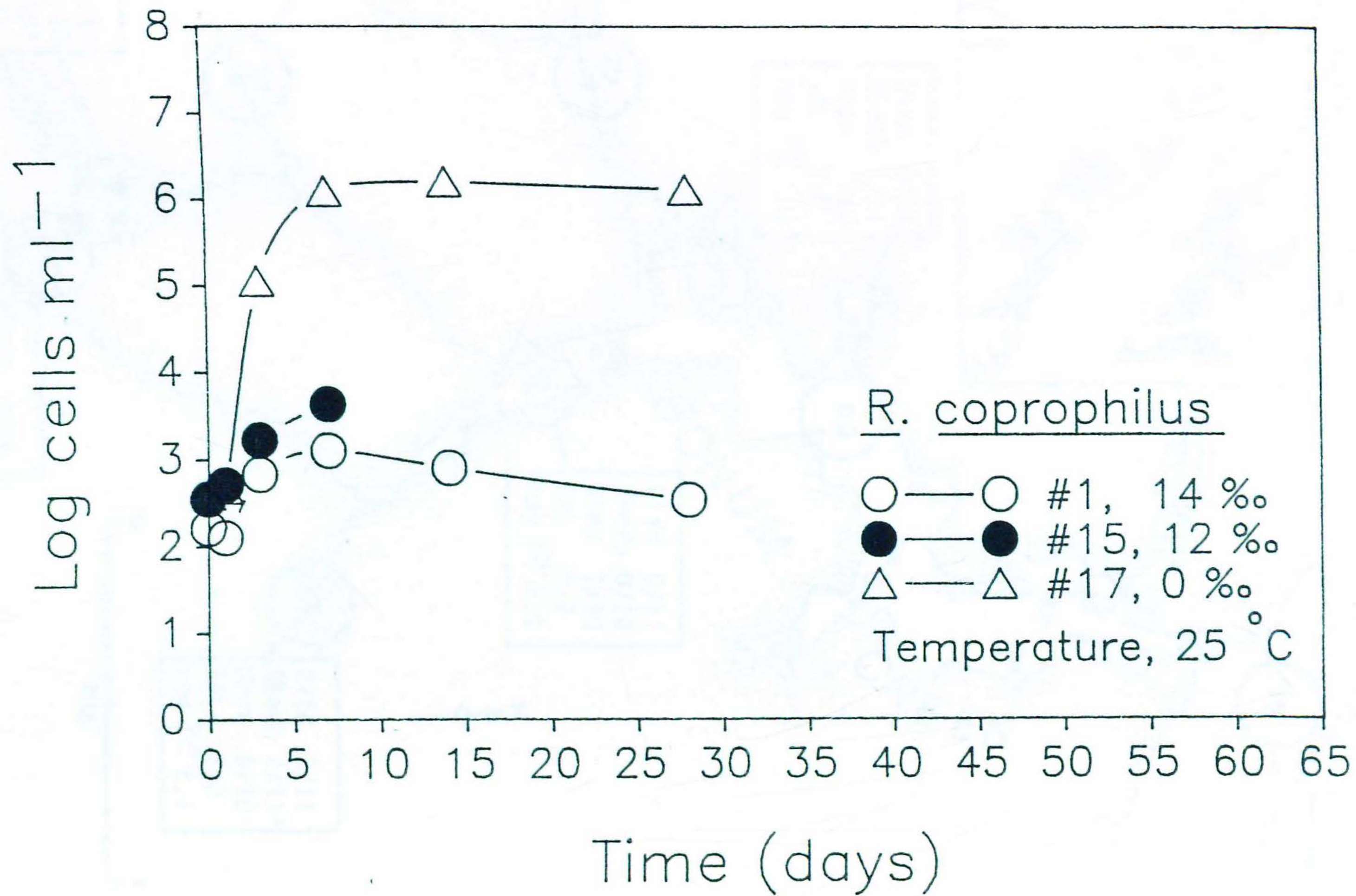


Figure 11. Summary of occurrence of alternate indicators, mean fecal coliform densities and incidence of salmonellae at selected stations in the Bonum Creek system.

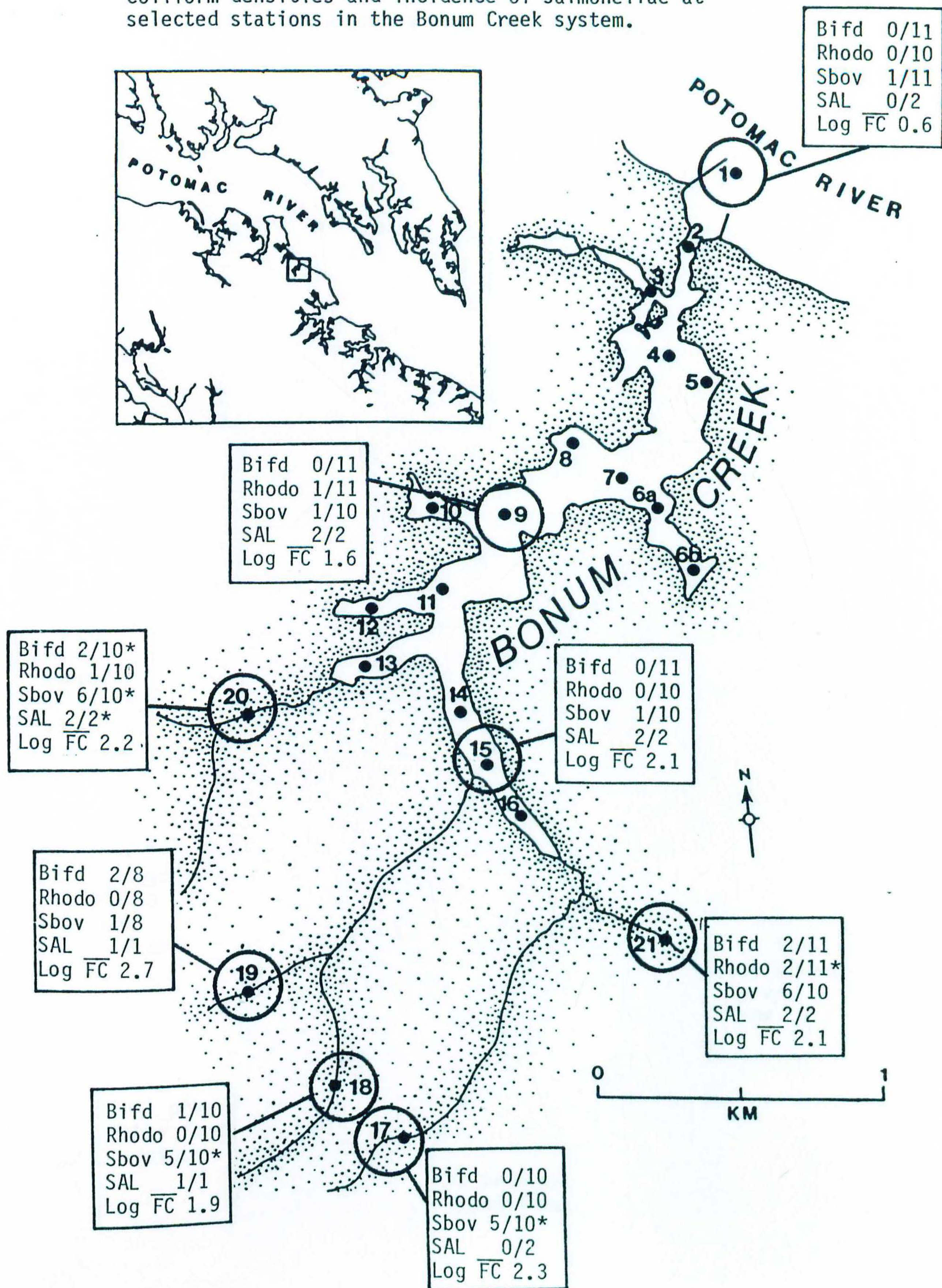
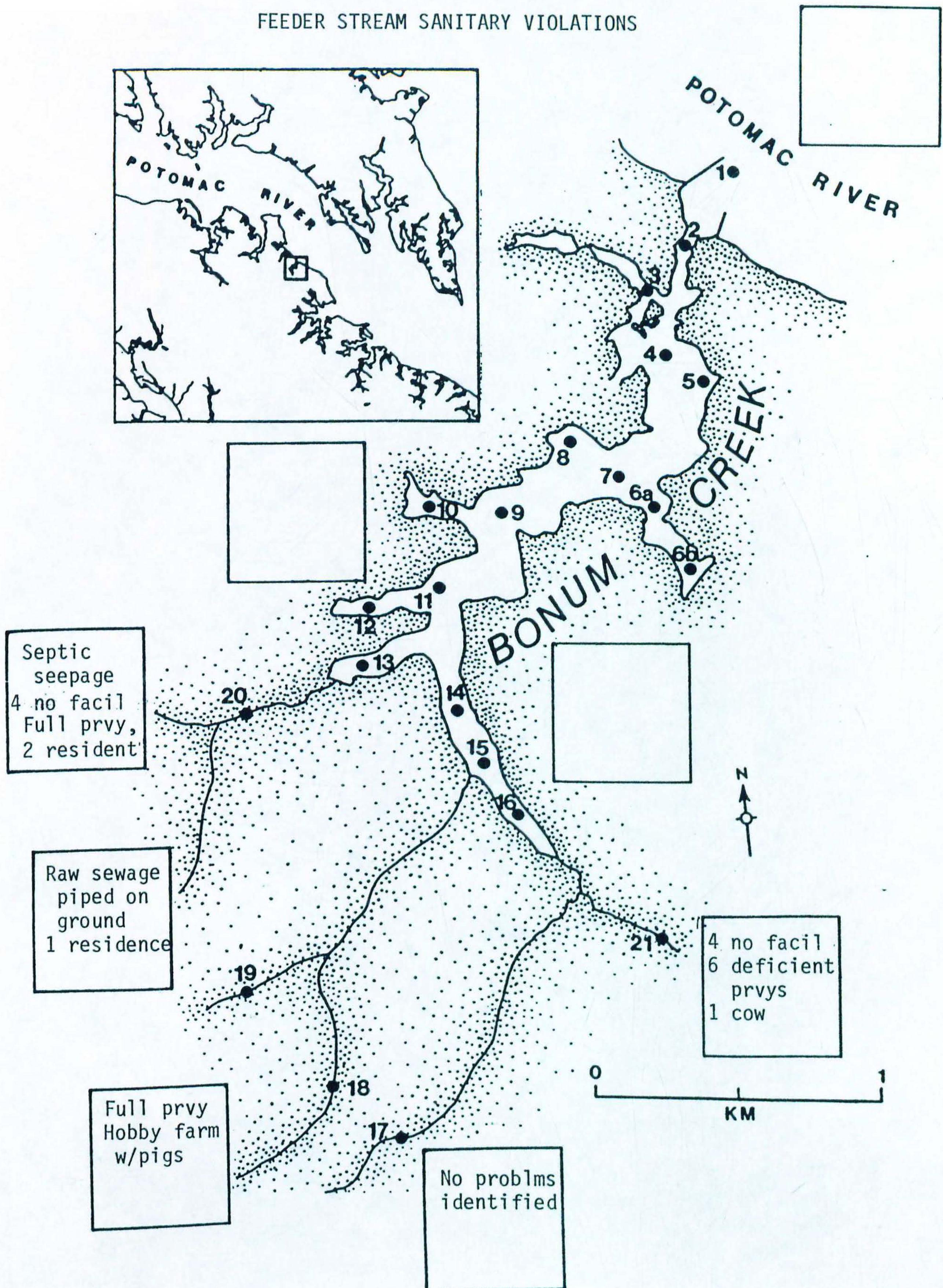


Figure 12. Sanitary violations on Bonum Creek watershed identified by detailed site visit and sanitary survey.



VIRGINIA INSTITUTE OF MARINE SCIENCE
1001181427