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Identification of pollutant sources contributing to degraded sanitary water quality inTaskinas Creek National Estuarine Research Reserve, Virginia

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Abstract

This study addressed a need to identify unknown sources of fecal pollution adversely impacting the sanitary water quality of the Taskinas Creek Reserve in Virginia. Taskinas Creek is a tidal creek marsh and a National Estuarine Research Reserve site located primarily within the York River State Park watershed but surrounded by areas ranging from undeveloped to low density urban development. The tidal portion of Taskinas Creek is closed to shellfish harvesting owing to high fecal coliform densities. Following a detailed sanitary survey to locate potential pollution sources, we applied a "suite" of approved and candidate indicators of fecal contamination to sample feeder streams in developed and Reserve areas of the watershed and tidal Taskinas Creek for one year. The absence of candidate human-specific indicators (sorbitol-positive bifidobacteria, fluorescent whitening agents) and the infrequent occurrence of FRNA coliphages in feeder streams in both reserve and developed areas did not implicate human contamination as responsible for elevated fecal coliform counts in shellfish harvesting waters of Taskinas Creek. Rather, the study revealed a widespread occurrence of the candidate animal fecal indicator, *Streptococcus bovis*, at all locations sampled within the watershed and in the Taskinas Creek tidal marsh. The occurrence of *S. bovis* was not correlated with seasonal temperature or salinity. Analysis of limited animal fecal samples corroborated dominant feral animals as sources of fecal coliforms or *S. bovis* to marsh waters and feeder streams. *S. bovis* has potential as a direct indicator of animal fecal contamination but its use requires additional validation and improved methods to confirm presumptive counts.

Keywords: animal fecal contamination, shellfish waters, *Streptococcus bovis*, candidate fecal indicators

Introduction

Taskinas Creek, a tidal creek with brackish and freshwater communities, is a NERRS site representative of the transition zone of the York River. Although reserve sites were established in part to provide long-term habitats that "will be protected as far as possible from immediate threats from development" (CBNERRS-VA 1991), increased residential development within the Taskinas Creek watershed is alleged to be compromising preservation of Taskinas Creek through degradation of water quality.

Crucial to evaluating the public health significance of nonpoint pollution and implementing management options and remediation strategies is (1) validation that the traditional coliform indicator is indeed "signaling" fecal pollution, and (2) determination of the source(s) of fecal contamination. It is generally accepted that human fecal contamination presents a greater health risk to shellfish consumers and bathers when compared to animal fecal contamination. However, approved coliform indicators are not source specific, i.e., do not discriminate human from animal sources. Furthermore, coliforms have come under considerable scrutiny and criticism for a variety of reasons. Although consistent and direct evidence is lacking, it is believed that conditions in estuaries can promote bacterial indicator survival (Erkenbrecker 1981; Kator and Rhodes 1991). Contributing factors include inorganic and organic nutrient loading, high suspended solids, elevated temperatures, the presence of fine grained organic rich sediment and poor tidal flushing, conditions characteristic of many tidal creeks. In response to these problems, we compared water quality in feeder streams located in developed and reserve sections of the Taskinas Creek watershed using a variety of candidate indicators to discriminate human from animal fecal contamination.

Major feeder streams located through aerial and onsite field surveys were identified in developed and Reserve portions of the watershed. Streams in the Reserve were chosen on the basis of isolation from residential development. Streams in the developed sections were chosen to integrate effects from residential single family developments or lower-density rural settings populated by livestock on hobby farms.

Indicators and methods evaluated included fecal coliforms and *Escherichia coli*, a suite of candidate microbiological indicators including *Streptococcus bovis*, human-specific sorbitol-fermenting bifidobacteria, and FRNA male-specific coliphage, and a chemical indicator based on whitening agents found in clothes detergents. During a one year period a total of five sampling surveys were conducted in Taskinas Creek under slack before flood tidal current conditions and eight surveys of feeder streams. In this report we present the results of this watershed study and compare the occurrences of different indicators.

Materials and methods

Site evaluation and station selection. Three reconnaissance surveys of Taskinas Creek and its watershed were conducted by land, boat and air. In addition, a detailed sanitary survey was conducted in conjunction with the Virginia Department of Health, Division of Shellfish Sanitation in accordance with National Shellfish Sanitation Program guidelines (FDA, 1993). Consequently, feeder stream and creek sampling sites could be selected proximate to potential inputs associated with development (i.e., the presence of domestic animals and homes with on-site domestic waste systems and in areas lacking development (Figure 1). Detection of nonpoint pollution was facilitated by sampling in shallow embayments and small streams and under contrasting surface runoff conditions.

Sample and physical data collection. Water samples were collected by grab sampling using sterile containers. Sampling bottles used for detection of bifidobacteria were filled to capacity to exclude air and sodium bisulfite and cysteine solutions added to final concentrations of 0.01% and 0.05%, respectively, as quenching agents (Cameron Hackney, VPI & SU, personal communication). Sediment samples were collected either using plastic core liners and extruding the upper 3 cm into sterile plastic bags or using a spatula to sample surficial sediment for transfer to sterile bags. Five independent sediment samples were pooled for each analysis. Animal scat deposits were collected with a clean spatula and transferred to sterile plastic bags. All samples were stored at ambient field temperatures in insulated containers during sampling and transport to the laboratory. Bacterial samples were generally processed within 6 hours after

sampling except for FRNA coliphage samples which were refrigerated overnight (4°C) and processed the next day.

Physical parameters were measured in Taskinas Creek using a calibrated Hydrolab H20/Surveyor system (Hydrolab Corporation, Austin, TX) and included temperature, pH, conductivity, sample depth, oxygen and sampling time. Temperature was measured in feeder streams with a calibrated thermometer.

Detection and enumeration of microbial indicators. Recently proposed media and methodology for enumerating bifidobacteria (Beerens, 1991) were evaluated prior to initiating field studies. Based on data presented in the results section, bifidobacteria were enumerated using two media: human bifid sorbitol agar (HBSA, Mara and Oragui, 1983) for detection of sorbitol-fermenting bifids indicative of human sources and HBSA lacking antimicrobial agents and modified to contain either 0.5% sodium propionate or propionic acid and adjusted to pH 5.0 (HBSAPA) (Beerens, 1991). Depending on bacterial densities and suspended solids either triplicate 1 and 10 ml volumes or quadruplicate 25 ml volumes were filtered through 0.45 μm membrane filters (GN-6, Gelman Sciences). Dilution and rinse water used were either gelatin diluent (NCDC, 1968) containing 0.05 % cysteine hydrochloride (VPI&SU Anaerobe Manual, 1977) or phosphate buffered saline prepared (PBS) using a stock buffer solution (APHA, 1992) and containing 0.85% NaCl amended to contain 0.01% sodium bisulfite and 0.05% cysteine hydrochloride. Filters were incubated on solid media using Oxoid Anaero*Gen* system (Unipath, Ogdensburg, NY), resuscitated for 4 h at 30°C, and subsequently incubated for 4-6 days at 37°C. HBSA plates were examined for yellow raised "domed" colonies and suspect colonies examined microscopically for characteristic bifid cellular morphology. Selected colony types appearing on HBSAPA were also examined microscopically as a preliminary evaluation step. Colonies showing "typical" bifid cellular morphology were transferred to agar plates and the requirement for anaerobic conditions evaluated by incubating in air.

Modified mBA medium (mmBA, Oragui and Mara, 1984) was used for detection of *Streptococcus bovis*. Depending on bacterial densities and suspended solids as described above, samples of appropriate volume were filtered (0.45 μm membrane filters, GN-6, Gelman Sciences) using PBS and

filters incubated anaerobically as described above, resuscitated for 4 h at 30°C, and subsequently incubated for 72 h at 39.5°C.

Selected typical yellow and atypical non-yellow colonies were subcultured for purity and key phenotypic characteristics determined (APHA, 1992; Knudtson and Hartman, 1992). Gram stain, catalase, and growth in brain heart infusion at 10°C, 45°C, and in the presence of 6.5 % NaCl were performed according to APHA (1992). Esculin hydrolysis and fermentation of arabinose, lactose, raffinose and ribose followed procedures described in Clinical Microbiology (ASM, 1991). Starch hydrolysis was determined using heart infusion agar containing 0.2% starch, a concentration shown to maximize detection of hydrolytic activity (Pavlova et al., 1971). Commercially available tests (Difco, Detroit, MI) were used for detection of pyrrolidonylase (PYR *Dry*Slide) and *beta*-galactosidase (ONPG differentiation discs).

The method presented for enumeration of FRNA coliphage is based on use of *Salmonella typhimurium* WG49 (Havelaar and Hogeboom, 1984). Selection of this assay method was based on a preliminary analysis of water and sediment samples comparing WG49 with *Escherichia coli* Famp (Debartolomeis and Cabelli, 1991) as hosts.

Male-specific FRNA coliphages were enumerated using the *S. typhimurium* WG49 (Havelaar and Hogeboom, 1984) host strain following published cultural protocols for plaque assays. Because the host assay system does not distinguish between FDNA and FRNA coliphages, all samples were parallel plated on appropriate media with and without RNase (Rhodes and Kator, 1991). The difference between plaque counts obtained without and with RNase is therefore generally equivalent to the density of target FRNA phage. However, when densities are low it is feasible to confirm the RNase sensitivity of individual plaques. Duplicate 1 ml samples of beef extract concentrates from water samples or dilutions thereof were enumerated by double agar overlayer for RNase-free and RNase-containing (1 ml of a 3 mg ml^{-1} solution of RNase added to 100 ml of DAL agar) media. Plates were incubated inverted overnight at 35°C and plaques then counted using a typical "colony counter" or oblique light. To confirm that phages recovered by *S. typhimurium* WG49 were FRNA male-specific phages, all plaques or a representative

fraction were transferred using sterile toothpicks to plates containing RNase (2 ml of a 4.8 mg/ml solution per 100 ml of DAL agar).

Detection of FRNA coliphage in environmental water samples was coupled with a concentration method to realize a detection limit of 3 pfu/100 ml. At each station replicate sample volumes of 250 ml were concentrated using a membrane filter adsorption-elution method modified after Farrah (1982). $MgSO_4$ was added to each water sample at a final concentration of 0.2 M, shaken to dissolve, and allowed to equilibrate to room temperature. Replicate 250 ml aliquots of the sample were filtered through Millipore HAWG nitrocellulose (0.45 μm) membrane filters at a vacuum no greater than 130 mm Hg. After filtration to dryness each filter was placed in a tube containing 15 ml cold sterile beef extract (3%, pH 9.0) and vortexed rapidly for 30 seconds followed by vigorous agitation on a rotary shaker (250 rpm) for 15 minutes. Aliquots of beef extract were analyzed using the double layer agar overlay method as described.

Enumeration of phages from sediment samples was by elution into 3% beef extract (pH 9). One part sediment and three parts beef extract were shaken vigorously for 15 minutes at 200 rpm and then centrifuged at 7000 rpm for 15 minutes at 4°C. The decanted supernatant was adjusted to pH 7.0-7.2 and phage enumerated by SAL and DAL.

Fecal coliforms (FC) were determined in environmental samples using a five-tube MPN procedure (APHA 1992) with lactose broth as the presumptive medium and EC medium as the confirmatory medium. Incorporation of a fluorogenic substrate, MUG (4-methylumbelliferyl-β-D-glucuronide) into EC medium provided an *E. coli* count (Feng and Hartman, 1982). Fecal coliforms and *E. coli* were enumerated from sediment by homogenization (90 seconds) of a 1:10 dilution of sediment (20 g of sediment in 180 ml of phosphate buffer (APHA, 1992)) followed by MPN analysis. Final MPN estimates were calculated as described in Standard Methods (APHA, 1992).

Animal fecal samples were weighed, and homogenized in chilled sterile blenders containing beef extract (3%, pH 9.0; 1:10 w/v) for 90 seconds. Stomaching of muskrat feces in beef extract was also found to be a clean, safe and effective method for homogenization. Dilutions were made as required in

phosphate buffer for enumeration of fecal coliforms and *E. coli*. in phosphate buffered saline for *S. bovis*, and in phage buffer (Tartera and Jofre (1987) for FRNA coliphages.

Detection of fluorescent whitening agents. The method used for detection of fluorescent whitening agents (FWAs) was adapted from Uchiyama (1979) and Close et al. (1989). Nonpolar organics were separated under high salt concentrations from water soluble FWAs by partitioning the nonpolars into dichloroethane. The dichloroethane was discarded, the FWAs complexed with an ion pairing agent, tetra-n-butyl ammonium iodide, and extracted into dichloroethane. The procedure we adopted used dichloromethane instead of dichloroethane because the former is less carcinogenic and disposal is less complicated.

Nonfiltered aliquots (100 ml) of samples collected from feeder streams or Taskinas Creek were added to solvent cleaned separatory funnels. Ten g NaCl was added and the funnel shaken to dissolve the salt. After dissolution 20 ml dichloromethane was added, the funnel shaken for not more than 5 minutes with careful venting and the layers allowed to separate. The lower layer containing nonpolar materials was carefully discarded into a waste container. Then 4 ml of the ion pairing agent tetra-n-butyl ammonium iodide (2.5% w/v solution) followed by 20 ml dichloromethane were added and the funnel shaken for about 5 minutes before allowing the layers to separate. An aliquot of the lower phase (containing any FWAs) was decanted into an acid and solvent cleaned cuvette and sealed to prevent solvent evaporation.

Aliquots of each extract were then analyzed by fluorometry using a filter set optimized for this purpose (excitation filter 10-069, emission filters 10-061 and 10-059, UV lamp 10-049; Turner Designs, Sunnyvale, CA). A Turner Model 10AU fluorometer (Turner Designs, Sunnyvale, CA) was used for the analysis with 13x100 mm cuvettes (Fisher Scientific). Standards (0.0, 0.01, 0.1, 1.0, 5.0 and 10 µg/l) based on a common FWA (Tinopal CBS-X, Ciba-Geigy) and procedural blanks were prepared and the instrument calibrated using the 1.0 µg/l standard. The lowest concentration of the standard FWA that could be detected was 0.1 µg FWA/l.

Design of fecal weathering experiment. The absence of frank human sources of fecal contamination and our consistent finding of muskrat fecal pellets on the intertidal margin of the marsh, precipitated analyses of pellet indicator composition as well as the design of preliminary experiments to measure: (1) the persistence of fecal coliforms in pellets incubated in vitro in estuarine water and (2) the physical pellet integrity under in situ marsh conditions. Accordingly, fresh muskrat pellets were collected and 5 g muskrat pellets sealed in small mesh (10 mesh Nytex) bags. Bags were placed on the intertidal marsh at low tide and covered with an inverted aluminum test tube basket to prevent them from being lost or affected by small animals or invertebrates (e. g. , *Uca*). At selected time intervals three replicate bags were removed, the residual material weighed and enumerated for fecal coliforms. A parallel experiment was conducted in the laboratory to evaluate the pellets as sources of fecal coliforms to water. Five grams of pellets were each carefully added to 495 ml of ambient creek water (13 psu) in three flasks and incubated statically at 15°C. At intervals water samples were removed and enumerated for fecal coliforms.

Analysis of data. Raw data were tabulated in a spread sheet program and geometric mean values for indicators calculated. Plotting and nonparametric data analysis were carried out using Statview 4.5 (Abacus Concepts, Inc.).

Results

Sanitary survey. Results of the sanitary survey are shown in Table 1 and detailed information provided in Appendix A. Significant numbers of farm animals were identified as having potential impact on feeder streams 3, 4, and 5. Feeders 5 and 6 are also surrounded by relatively recent high density residential development. Feeder streams 1, 2 and 7, located in the reserve portion of the watershed, were, judging by an abundance of tracks, frequented by resident populations of deer and raccoon. Personnel at the York River State Park facility had no animal census data available for these species.

Physical-chemical characteristics of Taskinas Creek and area rainfall. Physical and chemical characteristic of Taskinas Creek for the five surveys conducted are shown in Table 2. The salinity characteristics shown are not unexpected for a shallow tidal creek influenced by both tidal flow and freshwater input. Salinities ranged from a maximum of 12.5 psu at the mouth to a minimum of 0.1 psu as far upstream on one branch as was navigable. A typical range of seasonal water temperatures is evident as well as the interaction of temperature and biological activity on oxygen concentrations.

Rainfall is shown in Table 3. Precipitation prior to sampling can influence the densities of indicators in the creek proper and also the feeder streams. Data in Table 3 were obtained from a NOAA observation station proximate to Williamsburg, a location relatively close to the Taskinas watershed. Cumulative rainfall is listed on various days prior to sampling. Significant precipitation events occurred with 3 days prior to sampling on 10-17-94 and 6-26-95 for feeder streams and on 5-1-95 and 7-17-95 for tidal creek surveys.

Evaluation of microbiological methods prior to field surveys. Prior to initiating field studies, media and methodology for enumerating bifidobacteria were evaluated using sewage and pure cultures. Parallel bifid enumerations using HBSAPA prepared with sodium propionate and propionic acid showed that equivalent results were obtained using either component (Student's t test. $p < 0.05$. $n=7$). Subsequent enumerations were performed using sodium propionate since its liquid acid form is both toxic and corrosive.

The potential for improved recovery of bifids by resuscitation on a nonselective medium was examined by comparing counts on membrane filters which were incubated anaerobically on reinforced clostridial agar (RCA) for 4 h at 37°C prior to transfer to HBSAPA. with counts on filters which were immediately placed on the selective medium. When sewage was diluted into 0.22 μm filtered freshwater and incubated aerobically at 15°C. the two-step resuscitation approach did not appear to enhance recovery of bifids in the membrane filtered samples (Table 4). There was also no apparent difference in HBSAPA membrane filter counts of a pure culture suspension in filtered freshwater as a function of resuscitation

(Table 5). However, spread plate counts indicated that direct exposure to selective components in HBSAPA reduced recovery of *B. adolescentis*.

Selection of the bacterial host for enumeration of FRNA coliphage was partly based on replicate analyses of fourteen 250 ml water and three sediment samples. Samples were analyzed in parallel using *S. typhimurium* WG49 (Havelaar and Hogeboom, 1984) and *E. coli* Famp (Debartolomeis and Cabelli, 1991). All samples tested were negative for FRNA coliphage when analyzed using the latter host. Four isolates with partial sensitivity to RNase were obtained using the former host and subsequent serotyping indicated these isolates were neutralized by MS2 antiserum and belonged to FRNA coliphage group I (Furuse, 1987). Based on these observations, *S. typhimurium* WG49 was selected as the assay host for all subsequent FRNA phage analyses

Occurrence of indicators in feeder streams. Values for indicators found in feeder streams are presented in Table 6. Fecal coliforms and *E. coli* were found at every location and during all seasons in feeder streams located in both reserve and developed areas of the watershed (Figure 2). Similarly, *S. bovis* was also commonly detected throughout the watershed. As discussed below, presumptive *S. bovis* counts shown in this table should be adjusted by a factor of 0.47 to more accurately reflect the density based on the overall rate of isolate confirmation. Geometric mean values for these indicators are summarized in Table 7. A Friedman test for homogeneity of station means for these indicators was significant only for *S. bovis* ($p= 0.02$) apparently resulting from the low *S. bovis* densities at feeder station 2. Although Friedman test results for the coliform indicators revealed the data were homogeneous, feeder locations 3 and 6 were consistently highest in mean rank. Results of Wilcoxon signed rank tests for *S. bovis* were significant for feeder 2 versus feeder 3 ($p= 0.011$), and close to the $p= 0.05$ significance level for feeder 6 ($p= 0.07$) and feeder 5 ($p= 0.06$). Both bifidobacteria and FRNA coliphage were infrequently detected and when present generally occurred just above the level of detection.

Correlation and regression analyses were performed to establish if temperature or cumulative precipitation prior to sampling were significantly related to indicator densities. Figure 3 is a scatter plot of fecal coliform, *E. coli* and *S. bovis* densities against temperature. A direct association between levels

of log-transformed coliform densities with seasonal temperature was suggested but r^2 values by simple linear regression were < 0.3. Correlation analysis (Spearman rank) revealed a significant but modest degree of association between temperature and coliform indicator densities (r_S= 0.46 and 0.48) but not for *S. bovis*. (r_S= 0.15). Similar analyses did not reveal significant relationships between indicator densities and cumulative rainfall integrated over 1, 3 and 7 day periods prior to each sampling date (Table 3). Fecal coliform and *E. coli* densities were strongly correlated (r_S= 0.97, p<0.0001) with each other but not well correlated with *S. bovis* densities (r_S= 0. 34 and 0.35).

Occurrence of indicators in Taskinas Creek. Values for indicators detected in Taskinas Creek are presented in Table 8. Geometric mean values for these indicators are summarized in Table 7. Mean values of fecal coliforms exceeded the approved fecal coliform growing area standard at all locations sampled within Taskinas Creek. Mean values were as elevated in locations considered impacted by only reserve portions of the watershed (G or H) as those potentially affected by residential development (F or E). As with feeder stream samples, coliform indicators and *S. bovis* were generally detected at all stations and during all seasons. Detection of bifidobacteria and FRNA coliphage was generally as unremarkable as in feeder streams. Two exceptions were the surveys conducted on 1/23/95 and 7/17/95 when FRNA coliphage were detected at geographically separated sites. Elevated counts of FRNA coliphage on 7/17/95 were associated with the second largest cumulative rainfall amount recorded during creek surveys (Table 3). Regressions of indicator densities against cumulative rainfall at 1, 3 and 7 days were characterized by low r^2 values (<0.2). Correlation analyses also yielded very low and insignificant values of r_S for indicators and cumulative rainfall. Counts of coliform indicators were strongly correlated (r_S = 0.97, p= 0.001) with each other and in contrast to feeder streams, r_S values for *S. bovis* and fecal coliforms (0.68) and *S. bovis* and *E. coli* (0.66) were considerably larger and significant (p= 0.001).

Densities of indicators plotted as a function of salinity are shown in Figure 4. *S. bovis* was found at relatively elevated levels at all locations sampled, including the lower reaches and mouth of the creek. The association of decreasing densities of indicators with salinity suggested by this figure was not particularly strong as evidenced by simple linear regression analysis with r^2 values of 0.5 for coliforms.

0.53 for *E. coli.* and 0.23 for *S. bovis.* An inverse correlation of indicator densities with salinity was shown by Spearman rank values (r_S) of -0.67, -0.66, and -0.4, for fecal coliforms, *E. coli*, and *S. bovis*, respectively. Figure 5 shows indicator counts plotted versus temperature. No significant linear relationships were detected by regressing indicator counts on temperature (r^2 values for all indicators approximately 0.1 or less). Spearman rank r_S values for correlation of temperature with indicator densities were also small ($r_S = 0.32$ or less) and not significant.

Bacteriophage plaques showing partial or total sensitivity to RNase were more frequently observed in creek (53%; 24 plaques from 45 samples) than feeder stream samples (17%; 11 plaques from 65 samples). Feeder stream sites 1, 3, 6 (water and sediment), and 7 were each positive only on a single occasion whereas possible FRNA phage were recovered on three separate samplings at site 4 (upstream of creek site E). As noted incidence of coliphage isolates from creek samples was associated with rainfall with 23 of 24 phages recovered after rainfall events of ≥0.6 in. three days preceding sampling. The majority of plaques were detected at Taskinas Creek site E (10/24 plaques) after 1.1 in. rain the preceding day. The majority of purified phages from site D (2 isolates), E (8 isolates), and F (2 isolates) reacted with SP (FRNA serogroup IV) antiserum.

Methodological considerations affecting measurements of indicator densities. The specificity of modified mBA for *S. bovis* as determined by characterization of 310 target isolates was 47%. Based on gram stain, catalase, esculin and pyrrolidonylase reactions, 12% of target colonies were enterococci. Beta-galactosidase reactions were positive for 38% of isolates having gram stain, catalase, esculin and pyrrolidonylase reactions otherwise typical of *S. bovis*. Variable sugar fermentations and growth characteristics were observed for isolates in the latter group. The remainder of isolates had one or more of the following characteristics: esculin negative, catalase positive or noncoccal morphology. Examination of 20 nontarget colonies showed that 40% were false-negatives.

Occurrence of indicators in animal feces. During this study we paid particular attention to the hypothesis that uniform animal activity was responsible for elevated FC/*E. coli* counts in feeder streams

and estuarine waters. This perception was supported by the discovery of frequently occurring and widespread deposits of small fecal pellets on the intertidal marsh margin that were eventually attributed to muskrat, and less frequent but not uncommon raccoon scat found on fallen trees overlying the creek. One survey of a marsh area where raccoon tracks were plentiful failed to reveal scat deposits. Limited fecal samples from muskrat, raccoon, deer and an unknown animal were analyzed to characterize them as sources of indicator organisms. Results (Table 9) verified that feral animal feces contained fecal coliforms (ca. 10^2-10^9 MPN/g) with the highest densities from raccoon (ca. 10^9 MPN/g) and that deer and raccoon also contained high concentrations of *S. bovis* (ca. 10^6 cells/g). Furthermore, the feces from an unknown omnivore contained high levels of FRNA coliphage that belonged to serogroup SP. This was the first unequivocal observation of FRNA coliphages in scat from a feral animal. Unfortunately, we were unable to identify the animal source on the basis of its scat.

Persistence experiments demonstrated that although muskrat pellets weathered in situ in the intertidal marsh lost 60-80% of their initial weights over a seven day period, they remained a source of high numbers of fecal coliforms (Table 10). Similarly, muskrat pellets placed in estuarine water were sources of fecal coliforms for at least 7 days. Although these data are compelling in supporting the connection between animals and fecal contamination, our database is comparatively limited in number of samples and seasonal coverage.

Fluorescent whitening agents. Results from the analysis of 43 samples collected from Taskinas Creek and feeder streams in the watershed are shown in Table 11. As noted the limit of sensitivity for this analysis was 0.1 µg/l calibrated against the standard whitener manufactured by Ciba-Geigy and extracting 100 ml volumes. With one exception (feeder station 4, 10-17-94), the concentrations determined must represent baseline fluorescent values for the technique as these were similar for all stations, regardless of location. Most samples had varying loads of suspended material and colored humic materials although feeder station 4 was exceptionally turbid. Values obtained for various standards, procedural and solvent blanks are also shown.

Discussion

Major objectives of this research were to identify sources of fecal contamination reflected by the observed elevated fecal coliform densities in the Taskinas Creek watershed and to evaluate various candidate methods for detection of fecal contamination from animals. Our results, (unique in comparing approved fecal indicators with *S. bovis*) showed that animal fecal contamination was widespread within the watershed. The coincidence of comparatively high levels of *S. bovis* at feeder streams and in Taskinas Creek proper must reflect an active animal community within both reserve and developed portions of the watershed. This conclusion was reinforced by consistent field observations of animal tracks and fecal deposits at both feeder stream and creek sampling sites. Furthermore, limited fecal samples from dominant animals in the watershed, deer and raccoon, were found to be significant sources of *S. bovis*. Although *S. bovis* has been isolated from a very limited variety of birds, captive or exotic animals (e. g., Wheater et al., 1979; Rutkowski and Sjogren, 1986; Osawa, 1990), to the best of our knowledge these data are unique in confirming feral mammals typically resident in tidal and freshwater marsh habitats of the Chesapeake Bay as sources of *S. bovis*.

The absence of detectable fluorescent whitening agents, bifidobacteria indicative of human contamination, and the low occurrences of FRNA coliphage in feeder streams provides support for the notion that domestic farm and/or feral animals are the dominant sources of fecal coliforms to the Taskinas Creek watershed. Although human septage can contain high densities of FRNA phage (Kator and Rhodes, 1993a), the absence of failing septic systems identified during the comprehensive sanitary survey is consistent with nonhuman origins of the observed fecal pollution. That the majority of FRNA coliphage recovered from sites within Taskinas Creek were SP (group IV) suggests animal fecal contamination. Furuse (1987) reported that serologically-distinct groups of FRNA phage manifest different patterns of occurrence in animals and humans with serogroup I isolated from animals, serogroups II and III from humans. Serogroup IV phages were considered by Furuse (1987) to have a broad habitat, being isolated from human feces, feces from two tigers maintained in a zoo, and the gastrointestinal contents (not feces) of pigs. However, as the frequency of occurrence of these phages is the lowest of the four serogroups in domestic sewage, Osawa et al., (1963) concluded that it occurred

more frequently in animal feces. Of 1020 FRNA strains recovered from domestic sewage sources in Asian countries, only 3 belonged to group IV. These results are consistent with observations we have recorded for domestic sewage and septage (data not shown). Isolation of group IV FRNA coliphage has been limited to samples of animal feces from captive animals (skunk, young turkey, domestic goose), pig feces and rendering waste (Gwaltney Co.), and an unknown animal fecal sample in the Taskinas Creek watershed. Accordingly, we interpret the occurrence of group IV FRNA coliphage in Taskinas Creek as reflecting animal fecal contamination.

Close (1989) suggested that fluorescent whitening agents (FWAs) found in laundry detergents might be useful as tracers of septic tank leachate contamination of domestic wells and groundwater. Previously, Kerfoot and Skinner (1981) had reported measuring fluorescing organics in a freshwater lake as a method to identify underwater septic field plumes. Uchiyama (1979) detected fluorescent whitening agents in a small lake receiving sewage effluent at concentrations that ranged from undetected to 7 µg/l. Because baseline flow of feeder streams in developed areas of the Taskinas Creek watershed is groundwater driven, we proposed to measure FWAs as indicators of septic leachate contamination. Close et al. (1989) reported a mean concentration range of 0.1-0.5 µg/l FWAs in groundwater samples affected by septic effluents. A theoretical maximum FWA concentration of 300 µg/l in septic tank leachate based on an average washing rate and water usage was also calculated (Close et al. 1989). Obviously, estimates of this kind are sensitive to the unique characteristics of each source, the septic system, the geohydrology of each site, and temporal effects. Samples we analyzed from the developed areas in the Taskinas Creek watershed (sample locations 5 and 6) came from feeder streams under both base and wet flow conditions in ravines immediately adjacent to single family developments on the uplands. As the base flow to these streams is supplied by groundwater, the absence of detectable FWA signals either reflected the absence of defective septic systems, the limit of sensitivity of the method, or the effect of chance on FWA occurrence. Although failure to detect FWAs cannot "prove" the lack of human fecal contamination, their "absence" did not contradict occurrence data for the other human specific indicators of fecal contamination we analyzed. Concentration of larger volume (one liter) samples by rotary evaporation is a feasible way to improve the detection limit of the method but is time consuming and the gain factor return

is only about 10. A more efficient approach would be to develop a phase adsorption assay to increase the gain 100 to 1000x, provided that evidence supporting the use of FWAs to detect septic contamination is obtained by other investigators.

Feral mammals within the immediate vicinity of Taskinas Creek marshland include muskrats, raccoons, deer, opossum, squirrels, fox and nutria (CBNERRS, 1991). Because of physical evidence suggesting a significant muskrat population, densities of indicator organisms in scat droppings and its intimate association with creek waters, the potential of muskrat feces to impact receiving waters can be hypothesized by estimating their density. Although the presence of muskrats was evident by tracks and scat deposits, muskrat houses or lodges were not observed from the creek during sampling surveys. Houses may have been located along nonnavigable reaches, or around the landward edges as has been frequently observed for the Virginia muskrat (*Ondatra zibethica macrodon*) (Harris, 1950). Dozier (1953) noted that along the Atlantic coastal marshes, the tightly constructed hut is replaced by a flimsy feeding shelter which in southern areas may be used throughout the year. Mild winters experienced during the last several years may also have contributed to the absence of well defined dwellings. Since a ground effort to accurately count muskrat houses or feeding platforms throughout the marsh was beyond our capabilities, reported muskrat densities per acre of suitable habitat were used to estimate the muskrat population in Taskinas Creek. Using Dozier's (1953) density of 2.5 dwellings per acre as a threshold density, and assuming five muskrats per dwelling, a density of 12 muskrats per acre is assumed under ideal conditions. The inventory of Taskinas Creek vegetative communities shows that 7% and 53% of 83 acres of low tidal creek marsh are dominated by three square sedge and saltmarsh cordgrass, respectively (CBNERRS, 1991). The former is a preferred muskrat food source whereas the latter is not as desirable and supports lower muskrat populations (Harris, 1950). Arbitrarily assuming an ideal muskrat "yield" of 100% (sedge) and 25%(cordgrass), the acreage of these plant communities produces an estimate of 200 muskrats for Taskinas Creek marshes. These figures are probably underestimates because we routinely noted the presence of areas denuded of vegetation, "eat-outs", which result from high population densities (Dozier, 1953).

It is instructive to calculate a hypothetical fecal coliform loading to marsh waters from muskrats based on the observed fecal coliform burden in muskrat feces and an estimate of daily fecal production. Assuming a mean fecal coliform density of 3.4×10^5 FC g^{-1} muskrat feces, and a "typical" daily "conservative" dropping weight of about 100 g (according to data supplied by Kevin Campbell (Dept. of Zoology, University of Manitoba, Winnipeg, R3T 2N2), the mean dropping weight for eight feral animals studied in the months of May, Judy, and September was 190.7 g) , a muskrat population of consisting of two hundred animals would contribute at least 6.8×10^9 FC day^{-1} to the marsh for potential transport to the creek.

From a water quality view, the size of this FC input can be placed in perspective by calculating hypothetical volumes of creek water required to dilute the FC input to various concentrations. Thus, if we assume 200 animals impact the main stretch of creek containing stations A, B, C, and D, and we make various simplistic assumptions such as the mean creek depth of this segment is 1 m, and the pollutant is instantaneously dispersed throughout the volume, then the amount of diluting creek water required to yield an average FC density of 330 FC/100 ml (calculated from Table 7) in this creek segment is 2.1×10^6 liters (2.1×10^3 m^3) or about 6% of the water in this segment. To reduce the average FC/100 ml count to 58 FC/100 ml (the FC density at the creek mouth) would use 32% of the water or 1.2×10^7 liters (1.2×10^4 m^3). To achieve sanitary water quality equivalent to the fecal coliform growing area standard for direct harvesting (14 FC/100 ml) would require 4.9×10^7 liters (4.9×10^4 m^3) or 128% of the volume. This volume of creek water required would be roughly equivalent to an idealized segment of creek 2450 m long, 1 m deep , and 2 m wide. Although tidal flushing will mix and dilute this waste, flushing will only remove a portion of the waste, which is presumably "refreshed" on a daily basis, and the one preliminary water exposure experiment performed shows aftergrowth is also possible. However, fecal coliforms do not behave as conservative elements and other removal processes, e. g., light, predation, physiological stress (Kator and Rhodes, 1991, 1993b) can lead to reductions in numbers. This is of course a "back-of-the-envelope" calculation, but it does serve to illustrate how feral marsh animal fecal contamination can affect sanitary water quality and offers support for our observations of elevated indicator counts in creek water compared with feeder streams.

Methodological concerns associated with enumeration of various candidate indicators occurred and will influence recommendations for adoption. Using our method bifidobacteria were recovered from domestic sewage at densities equivalent to (Mara and Oragui, 1983, Munoa and Pares, 1988) or higher (Resnick and Levin, 1981) than reported values. However, analyses of watershed samples was problematic because of interference from high bacterial backgrounds in undiluted environmental samples. Samples that contained high densities of presumptive *S. bovis*, showed similar recoveries of colonies of nontarget coccoid bacteria on HBSA and HBSAPA. Six isolates representing deer feces processed on bifid media were characterized biochemically as *S. bovis*. In addition to problems associated with low densities of target bacteria and nonselectivity of the media, neither medium provided for differential enumeration. Examination of representative colony types for typical bifid morphology coupled with the requirement for anaerobic growth were the criteria used for verification. However, expression of pleomorphic "bifid" morphology is both species and culture condition dependent (Miller-Catchpole, 1989a), and confirmation based on microscopic examination could result in underestimation. More reliable verification requires either use of a commercial identification system, gas liquid chromatography or the fructose-6-phosphate phosphoketolase test (Miller-Catchpole, 1989b). Application of these tests for enumerative purposes is laborious and cost prohibitive when one considers that hundreds of isolates not showing bifid morphology could result from a single sample. Emergent methods involving nucleic acid probes could provide an alternative to conventional phenotypic approaches. Recently species-specific oligonucleotide probes have been developed for identification and detection of human intestinal bifidobacteria (Yamamoto et al., 1992).

The low specificity of the mm-BA method for enumerating *S. bovis*, 47%, was similar to that observed previously (Kator and Rhodes, 1991) and demonstrates the need for a more rapid and specific methodology. *S. bovis* confirmation by biochemical methods is tedious and expensive. Although miniaturized methods reduce labor and time, commercially available systems are expensive if large number of isolates are examined and more importantly, there are discrepancies between results of minaturized methods as well as with conventional tube results (Molitoris et al. 1985; Knudtson and Hartman, 1992)

In addition to problems associated with biochemical characterization of mmBA isolates, schemes employed to classify isolates may led to misidentification. For instance, in addition to *S. bovis*, *S. equinus* and *Enterococcus cecorum* (isolated from the caeca of chickens, Devriese et al., 1983; Williams et al., 1989), other gram-positive cocci which are catalase negative, esculin positive and pyrrolidonylase negative include *S. alactolyticus* isolated from cows and straw bedding (Farrow et al., 1984), *S. saccharolyticus* isolated from the intestines of pigs and feces of chickens (Farrow et al., 1984), the latter alternatively referred to as *E. saccharolyticus* (Rodrigues and Collins, 1990), and *E. columbae* isolated from pigeon intestines (Devriese et al., 1990). These enterococcal and streptococcal species were identified by other biochemical tests, DNA-DNA hybridization studies, and analysis of 16 S rRNA and would not have been identified using solely the scheme of Knudtson and Hartman (1992). The need for rapid and reliable identification has lead to the development of 16S and 23S RNA based oligonucleotide probes for selected *Lactococcus*, *Enterococcus* and *Streptococcus* species (Beimfohr et al., 1993). Whitehead and Cotta (1993) have described development of a DNA probe to an amylase gene of *S. bovis* and more recently a 16s rRNA probe has been used to differentiate ruminal and human strains of *S. bovis* (Nelms et al., 1995). The relatively low percentage of confirmation of mmBA isolates as *S. bovis*, and the possibility of misidentification using only biochemical testing underlines a need to evaluate improved methods for identifying *S. bovis*.

In summary, our observations suggest that the degraded sanitary water quality of shellfish growing waters in Taskinas Creek is not directly related to human development, but derived from feral and domestic animals based on the densities and distribution of the animal fecal indicator, *S. bovis*, the absence of human specific indicators, and the detection of FRNA coliphages belonging to a serogroup we associate with animal fecal contamination. These results continue to question the validity of the fecal coliform indicator to reflect human contamination and suggest that *S. bovis* may be a direct and specific indicator of animal fecal contamination. *S. bovis* thus has potential utility as an indicator to validate the fecal coliform indicator in nonpoint impacted shellfish waters through epidemiological studies, and may also provide an index of effort to manage animal populations within a watershed and to assess whether remediation efforts as now generally conceived, i. e., "cleaning up" human sites and septic systems, is an

effective management strategy. Impediments to use of *S. bovis* for these purposes relate to the time consuming activity of *S. bovis* isolate confirmation. As noted the recovery method lacks sufficient selectivity/specificity and requires a series of confirmatory tests. It is likely that use of DNA probes specific for *S. bovis* coupled with a colony lift-dot blot procedure and a nonradioactive labeling method would substantially shorten this process. Accordingly, future research should be directed toward adoption of existing probes or new probes for simultaneous testing against biochemically confirmed isolates of *S. bovis* from the Taskinas Creek watershed (water and fecal samples) and known *S. bovis* strains.

Figure 1. Taskinas Creek Research Reserve sampling sites

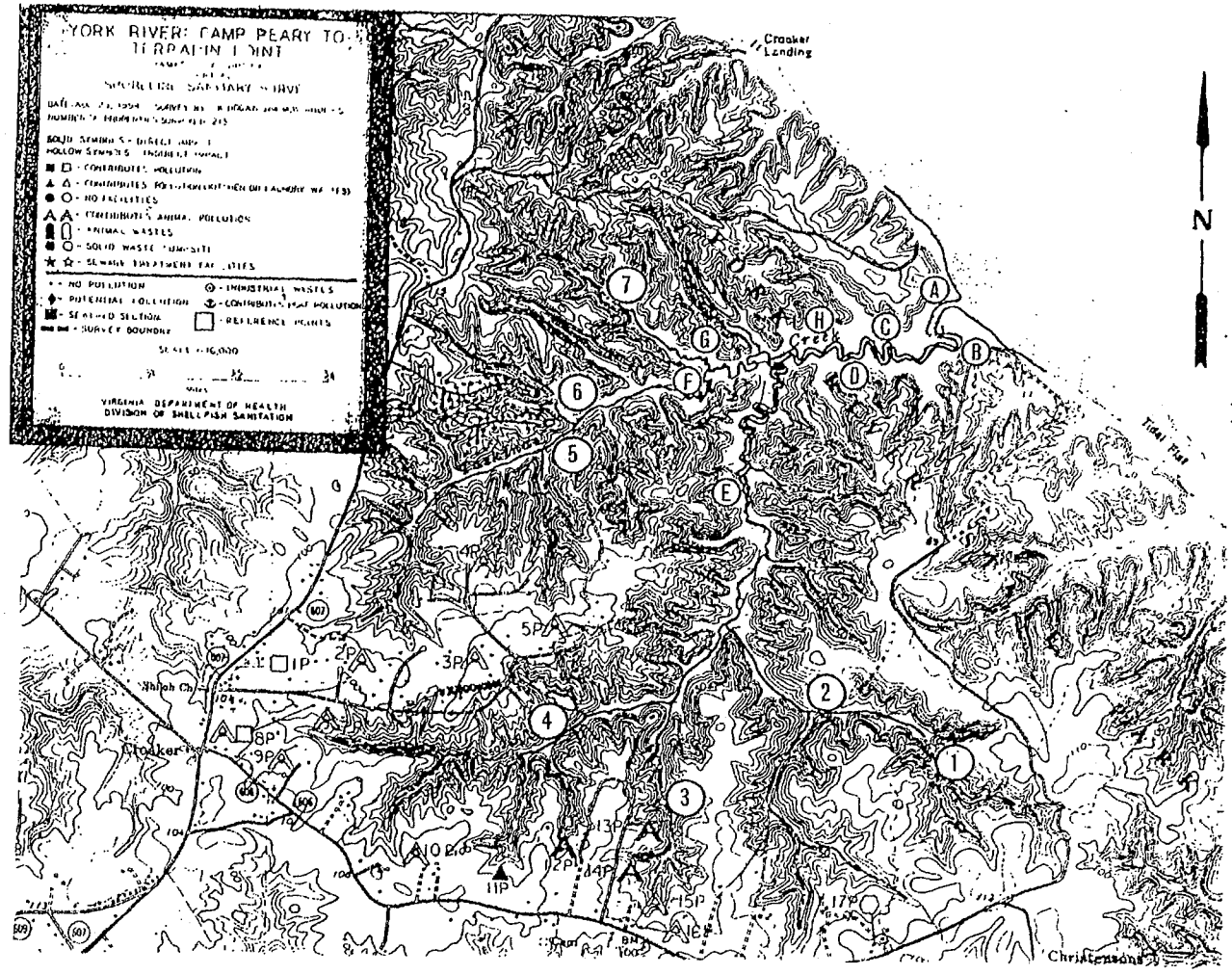

Figure 2. Indicator counts over all seasons for feeder streams in reference and developed areas of the Taskinas Creek watershed.

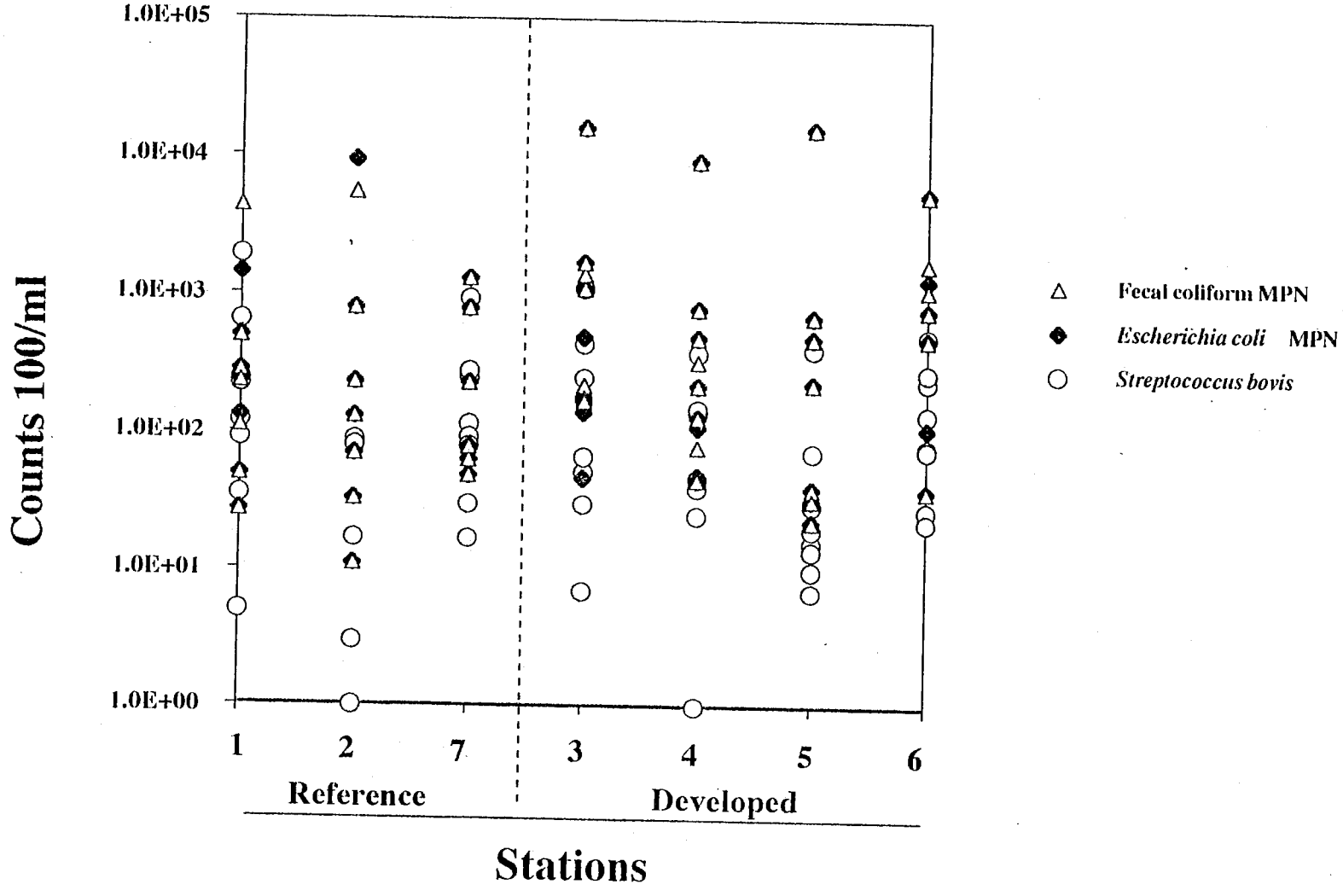

Figure 3. Indicator counts versus temperature: feeder streams.

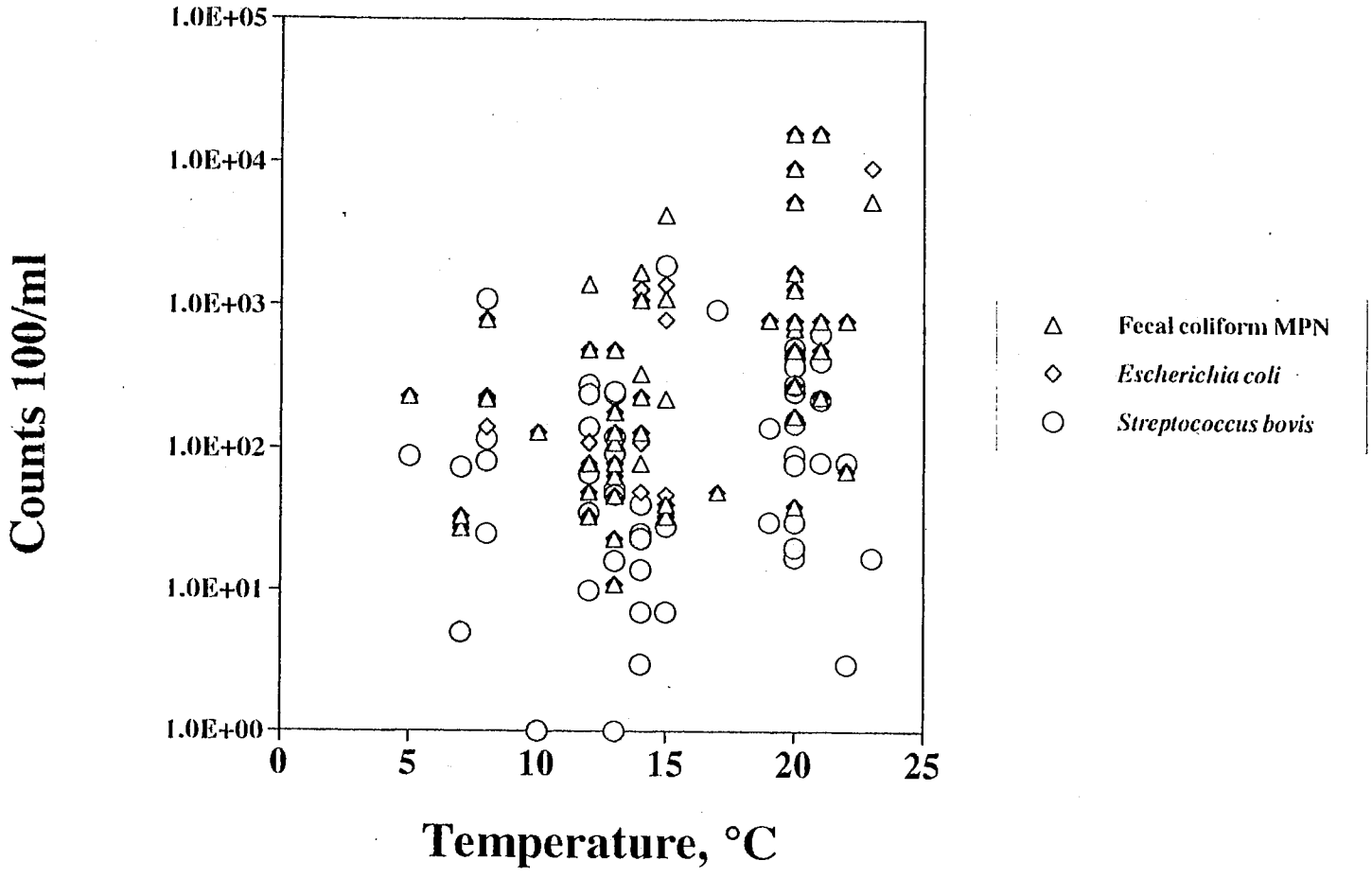

Figure 4. Indicator counts versus salinity: Taskinas Creek.

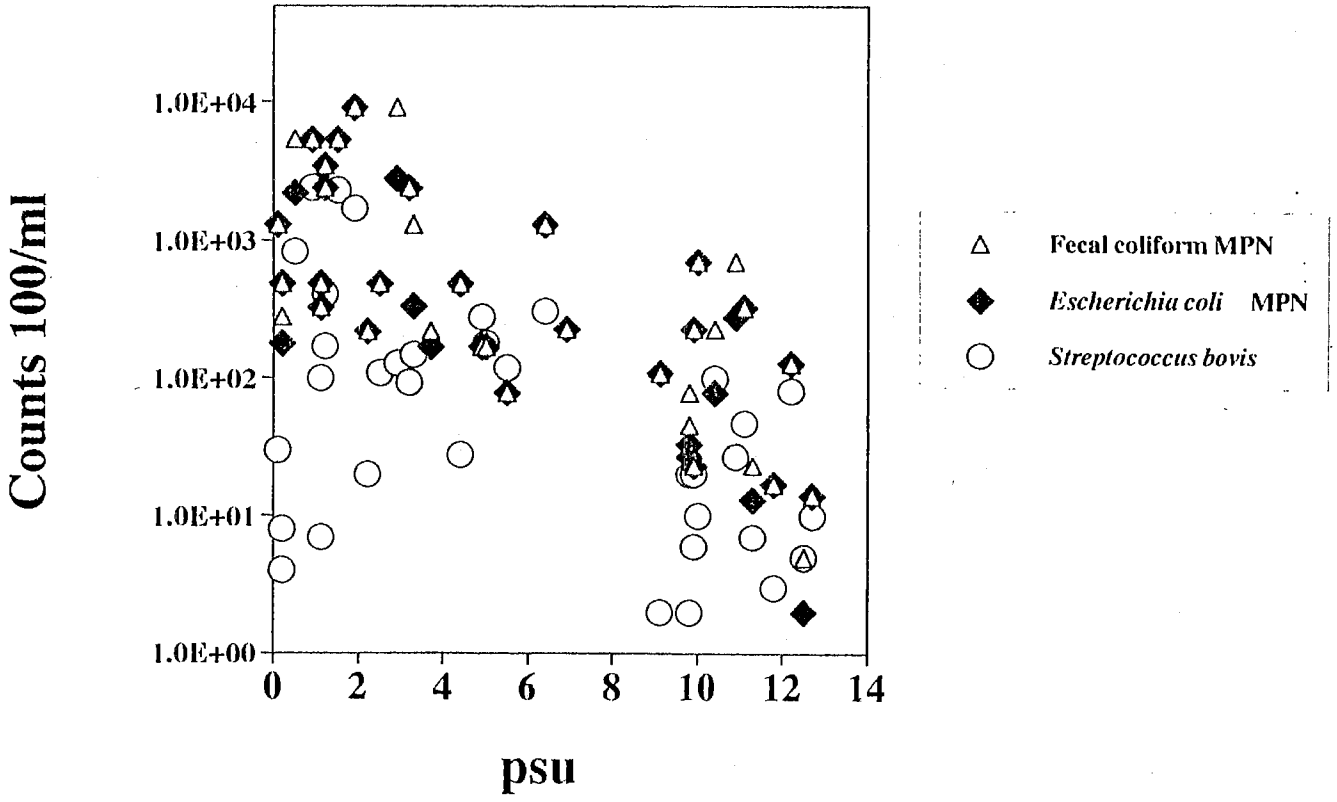

Figure 5. Indicator counts versus temperature: Taskinas Creek

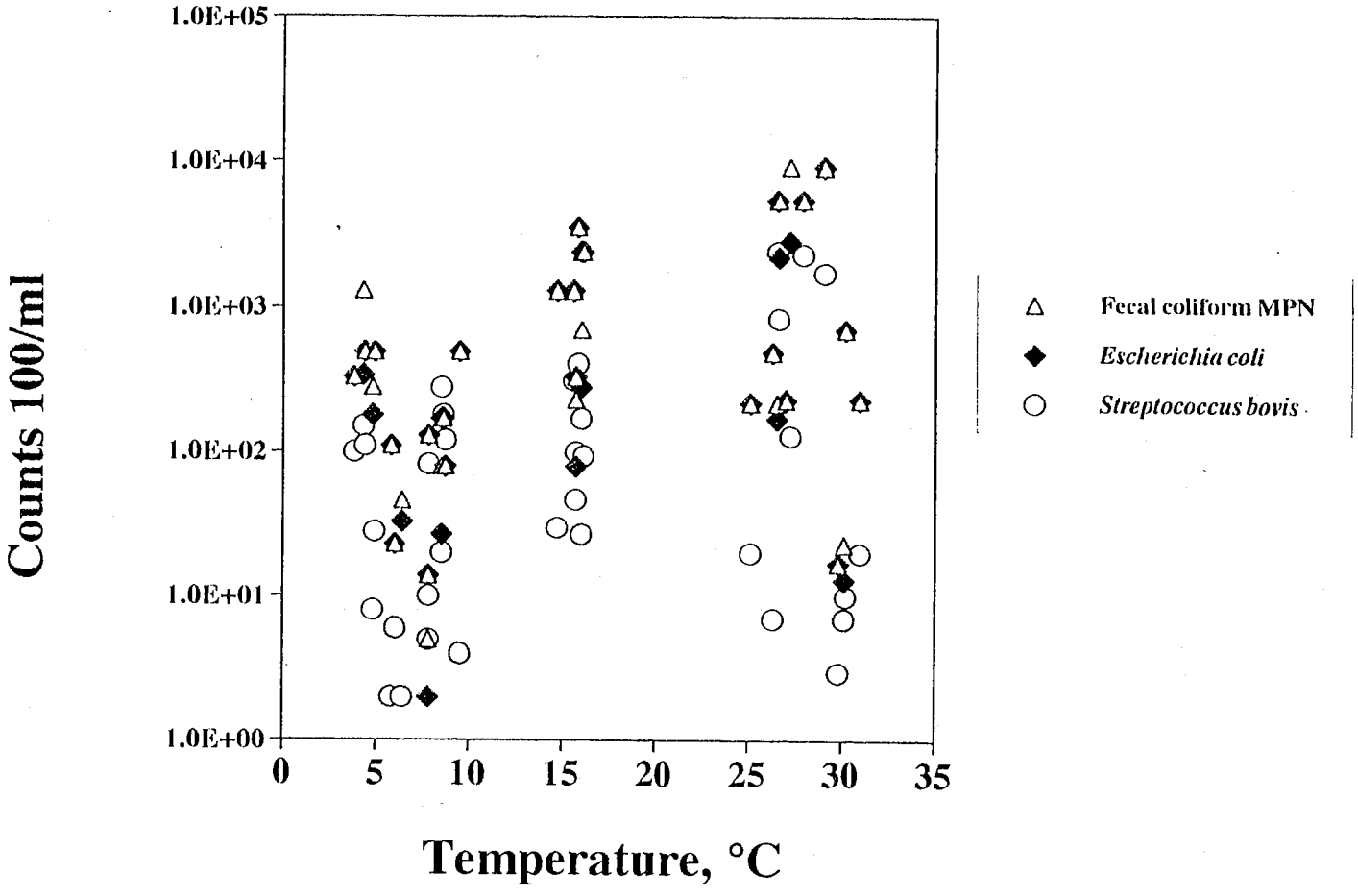

Table 1. Potential sources of fecal contamination to Taskinas Creek feeder streams identified by sanitary survey.

Feeder stream	Animal[a,b]						Septic as residential development intensity[c]		
	Bovine	Fowl	Goat	Equine	Pig	Rabbit	None	Moderate	High
1							X		
2							X		
3	4 (2)	178	10	8 (4)		10		X	
4	8 (8)	191	13	9 (4)	1	16		X	
5		29	2	20		4			X
6									X
7							X		

[a]Domestic pets (dogs, cats) not included. [b]Number of animals with access to creek in parentheses.
[c]Moderate, two or more acres per farm or residence; high, <1 acre per residence.

Table 2. Physical and chemical data from Taskinas Creek surveys.

Date	Station	Depth, meters	Temperature °C	Salinity, psu	pH	Dissolved oxygen, mg/l
1/23/95	A	0.33	6.4	9.8	7.7	10.4
	B	0.36	6.0	9.9	7.7	10.6
	C	0.29	5.8	9.1	7.6	10.5
	D	0.27	4.3	3.3	7.5	10.0
	E	0.15	4.8	0.2	7.7	11.4
	F	0.77	3.8	1.1	7.5	10.4
	G	0.04	4.4	2.5	7.5	10.2
	H	0.09	4.9	4.4	7.5	10.2
3/6/95	A	1.01	7.8	12.5	8.4	11.0
	B	1.01	7.8	12.7	8.4	11.0
	C	1.03	7.8	12.2	8.2	10.8
	D	1.02	8.5	4.9	7.7	10.0
	E	0.36	9.5	0.2	7.9	9.7
	F	0.91	8.6	5.0	7.7	10.2
	G	0.19	8.7	5.5	7.7	10.7
	H	0.35	8.5	9.8	8.1	11.3
5/1/95	A	0.99	16.0	10.4	7.6	7.1
	B	0.92	15.7	10.4	7.6	7.3
	C	0.97	15.7	11.1	7.3	7.4
	D	0.94	15.6	6.4	7.4	6.2
	E	0.43	14.7	0.1	7.6	5.3
	F	0.64	15.8	1.8	7.4	5.3
	G	0.18	16.0	1.2	7.5	5.4
	H	0.15	16.1	3.2	7.4	6.1
6/19/95	A	0.83	29.0	11.8	8.4	12.1
	B	0.25	30.1	11.3	8.4	13.1
	C	0.20	27.0	6.9	7.6	8.0
	D	0.23	26.5	3.7	7.5	7.5
	E	0.26	26.3	1.1	8.0	12.7
	F	0.23	25.1	2.2	8.3	14.1
	G	ns[a]	ns	ns	ns	ns
	H	ns	ns	ns	ns	ns
7/17/95	A	1.01	31.0	9.9	7.8	7.5
	B	0.81	30.2	10.0	7.7	6.9
	C	0.93	27.2	2.9	7.1	2.9
	D	0.18	27.9	1.5	7.9	8.8
	E	0.65	26.6	0.5	7.3	5.0
	F	0.27	26.5	0.9	7.2	5.4
	G	ns	ns	ns	ns	ns
	H	0.04	29.0	1.9	7.3	5.6

[a]ns-not sampled because of very low tides.

Table 3. Precipitation prior to field surveys.[a]

Location	Date	Cumulative rainfall prior to sampling (in.)[a]				
		1 day	2 days	3 days	5 days	7 days
Taskinas Creek	1-23-95	0.0	0.0	0.6	0.7	0.9
	3-6-95	0.2	0.2	0.2	0.2	1.4
	5-1-95	1.0	1.0	1.0	1.0	2.2
	6-19-95	0.0	0.0	0.0	0.0	0.1
	7-17-95	1.1	1.1	1.1	1.1	1.3
Feeder streams	10-17-94	0.0	0.1	1.2	1.2	1.2
	11-15-94	0.0	0.0	0.0	0.2	0.2
	1-30-95	0.2	0.4	0.4	0.4	0.4
	2-20-95	0.0	0.1	0.3	1.1	1.1
	5-8-95	0.0	0.0	0.1	0.2	1.5
	6-5-95	0.0	0.2	0.2	0.2	0.3
	6-26-95	0.0	0.8	0.8	1.1	1.1
	7-10-95	0.0	0.0	0.6	0.7	0.9

[a]Rainfall measurements made at NOAA National Weather Service observation station located 2 miles north of Williamsburg, VA.

Table 4. Comparison of recovery methods for sorbitol-fermenting bifidobacteria from membrane filtered (0.22 μm) freshwater containing raw sewage diluted 1:10 (v/v) and incubated in vitro at 15°C.[a]

Exposure time (days)	CFU/ml on HBSAPA[b]		
	Spread plating	Membrane filtration	
		Resuscitated	Not resuscitated
0	2.0E04	2.7E04	2.5E04
1	1.8E04	1.7E04	1.6E04
6	9.0E01	2.2E02	1.5E02
20	nd[c]	1.4E01	9.3E00
29	nd	1.1E01	8.3E00

[a]Enumerated on human bifid sorbitol agar with sodium propionate (pH 5) (HBSAPA) by direct spread plating or by membrane filtration with and without resuscitation on reinforced clostridium agar (RCA) for 4h at 37°C prior to transfer to HBSAPA. [b]Triplicate plate counts prepared for each dilution. [c]nd - not determined.

Table 5. Comparison of recovery methods for a *Bifidobacterium adolescentis* isolate from membrane filtered (0.22 μm) freshwater incubated in vitro at 15°C.[a]

Exposure time (days)	CFU/ml				
	Spread plating		Membrane filtration		
	RCA	HBSAPA	RCA	HBSAPA	
				Resuscitated	Not resuscitated
0	1.3E05	1.0E05	1.8E05	2.1E05	2.2E05
7	1.9E04	6.8E02	1.4E04	2.5E04	6.7E04
16	5.3E02	<1.0E01	5.7E01	1.0E01	2.8E02

[a]Bifidobacteria enumerated on RCA and HBSAPA by direct spread plating or by membrane filtration with and without resuscitation on RCA for 4h at 37°C prior to transfer to HBSAPA. [b]Triplicate plate counts prepared for each dilution.

Table 6. Microbiological and temperature data for Taskinas Creek watershed feeder streams collected on survey dates shown.

Date	Station	Temp. °C	Counts/100 ml or /100g sediment						
			Fecal coliform MPN	*Escherichia coli* MPN		Presumptive *Streptococcus bovis*[a]	Bifidobacteria		FRNA coliphage
				Water	Sediment		"Total"	Sorbitol+	Water[b]	Sediment
10/17/94	1	13	110	130		240	1	<3	<3	
	2	15	33	33		<1	<1	<3	<3	
	3	15	220	47		7	<1	<3	<3	<10
	4	14	330	110		25	<1	<3	<3	<10
	5	15	40	40		7	<1	<3	<3	<10
	6	15	1110	790		28	<1	<3	<3	
	7	17	49	49		930	3	<3	<3	
11/15/94	1	15	4300	1400		1900	<1	<1	<3/3	
	2	13	11	11		1	<1	<1	<3	
	3	14	1100	1100		7	<1	<1	<3/3	
	4	13	46	46		<1	<1	<1	<3/3	
	5	13	23	23		16	<1	<1	<3	
	6	14	1700	1300		23	<1	<1	<3	
	7	13	64	64		250	<1	<1	<3/3	
1/30/94	1	7	27	27		5	<1	<1	<3	
	2	5	230	230		88	<1	<1	<3	
	3	8	220	140		1100	<1	<1	<3	
	4	8	220	220		25	<1	<1	<3/3	
	5	7	33	33		73	<1	<1	<3	
	6	8	790	790		81	<1	<1	<3	
	7	8	230	230		115	<1	<1	<3	

Table 6 cont'd. Microbiological and temperature data for Taskinas Creek watershed feeder streams collected on survey dates shown.

Date	Station	Temp. °C	Counts/100 ml or /100g (sediment)							
			Fecal coliform MPN	*Escherichia coli* MPN		Presumptive *Streptococcus bovis*[a]	Bifidobacteria		FRNA coliphage	
				Water	Sediment		"Total"	Sorbitol+	Water[b]	Sediment
2/20/95	1	12	49	49		35	3	not done	<3	
	2	10	130	130		1	<1	not done	<3	
	3	13	180	180		52	<1	not done	<3	<10
	4	14	79	49		40	<1	<2	<3/<3	<10
	5	12	33	33		10	1	<2	<3	<10
	6	12	490	110		140	<1	<2	<3	<10/10
	7	12	79	79		280	<1	<2	<3	<10
5/8/95	1	13	490	490		120	<3	<3	<3	
	2	14	130	130	2300	<3	<3	<3	<3	<10
	3	12	1400	490	49000	67	<3	<3	<3	<10
	4	13	130	130	4900	47	<3	<3	<3	<10
	5	14	230	230		14	<3	<3	<3	
	6	12	490	490	4900	240	<3	<3	<3	<10
	7	13	79	79		93	<3	<3	<3	
6/5/95	1	20	280	280		90	<3	<3	<3/3	
	2	22	70	70		<3	<3	<3	<3	
	3	20	1700	1700		440	<3	<3	<3	
	4	19	790	790		140	<3	<3	<3	
	5	20	700	700		30	<3	<3	<3	
	6	20	39	39		510	<3	<3	<3	
	7	21	790	790		80	<3	<3	<3	

Table 6 cont'd. Microbiological and temperature data for Taskinas Creek watershed feeder streams collected on survey dates shown.

Date	Station	Temp. °C	Counts/100 ml or /100g (sediment)						
			Fecal coliform MPN	*Escherichia coli* MPN		Presumptive *Streptococcus bovis*[a]	Bifidobacteria		FRNA coliphage
				Water	Sediment		"Total"	Sorbitol+	Water[b]	Sediment
6/26/95	1	21	490	490		220	<3	<3	<3	
	2	23	5400	9200		17	<3	<3	<3	
	3	20	16000	16000		250	<3	<3	<3	
	4	20	9200	9200		150	<3	<3	<3/24	
	5	21	16000	16000		410	<3	<3	<3	
	6	20	5400	5400		77	<3	<3	<3	
	7	20	1300	1300		17	<3	<3	<3	
7/10/95	1	21	230	230		640	<3	<3	<3	
	2	22	790	790		80	<3	<3	<3	
	3	20	170	170		30	<3	<3	<3	
	4	20	490	490		380	<3	<3	<3	
	5	20	490	490		20	<3	<3	<3	
	6	20	790	790		280	<3	<3	<3	
	7	19	790	790		30	<3	<3	<3	

[a]All yellow colonies on mmBA were counted as presumptive *S. bovis*. Overall confirmation rate of presumptive *S. bovis* colonies was 47%.
[b]Unless phage were detected in at least one analytical replicate only a single value is shown

Table 7. Geometric means for indicators (counts/100 ml) shown for feeder stream and Taskinas Creek surveys.

Feeder stream stations

	Reserve			Developed areas			
	1	2	7	3	4	5	6
Fecal coliforms	240 (8)[a]	160 (8)	170 (8)	800 (8)	320 (8)	190 (8)	700 (8)
Escherichia coli	210 (8)	170 (8)	170 (8)	550 (8)	270 (8)	190 (8)	540 (8)
Streptococcus bovis[b]	140 (8)	10 (8)	110 (8)	97 (8)	47 (8)	38 (8)	82 (8)

Taskinas Creek stations

	A	B	C	D	E	F	G	H
Fecal coliforms	58 (5)	65 (5)	400 (5)	810 (5)	860 (5)	750 (5)	960 (3)	960 (4)
Escherichia coli	38 (5)	56 (5)	410 (5)	670 (5)	910 (5)	920 (5	440 (3)	840 (4)
Streptococcus bovis[b]	9.5 (5)	16 (5)	79 (5)	580 (5)	29 (5)	240 (5)	140 (3)	150 (4)

[a]Number of independent surveys. [b]All yellow colonies on mmBA were counted as presumptive *S. bovis*. The overall confirmation rate of presumptive isolates on this medium was 47%.

Table 8. Microbiological and hydrographic data for Taskinas Creek collected on survey dates shown.

Date	Station	Temp. °C	Salinity psu	Counts/100 ml or /100g							
				Fecal coliform MPN	*Escherichia coli* MPN		Presumptive *Streptococcus bovis*[a]	Bifidobacteria		FRNA coliphage	
					Water	Sediment		"Total"	Sorbitol+	Water[b]	Sediment
1/23/95	A	6	9.8	46	33		2	<1	nd	3	
	B	6	9.9	23	23		6	12	nd	6/<3	
	C	6	9.1	110	110		2	7	nd	<3/3	
	D	4	3.3	1300	340		150	<1	nd	<3/3	
	E	5	0.2	280	180		8	7	<1	<3	<10
	F	4	1.1	330	330		100	2	<1	<3	<10
	G	4	2.5	490	490		110	2	<1	<3	<10
	H	5	4.4	490	490		28	<1	<1	<3	<10
3/6/95	A	8	12.5	5	2		5	nd	<1	<3	
	B	8	12.7	14	14		10	nd	<1	<3	
	C	8	12.2	130	130		82	nd	<1	<3	
	D	9	4.9	170	170		280	nd	<1	<3	
	E	10	0.2	490	490	2300	4	nd	<1	<3	<10
	F	9	5.0	170	170	130000	180	nd	<1	<3	<10
	G	9	5.5	79	79	33000	120	nd	<1	<3	<10
	H	9	9.8	79	27	2200	20	nd	<1	<3	<10
5/1/95	A	16	10.9	700	280		27	<3	<3	<3	
	B	16	10.4	230	79		100	<3	<3	<3	
	C	16	11.1	330	330		47	<3	<3	<3	
	D	16	6.4	1300	1300		310	<3	<3	<3	
	E	15	0.1	1300	1300		30	<3	<3	<3	
	F	16	1.2	3500	3500		410	<3	<3	<3	
	G	16	1.2	2400	2400		170	<3	<3	<3	
	H	16	3.2	2400	2400		93	<3	<3	<3	

Table 8 cont'd. Microbiological and hydrographic data for Taskinas Creek collected on survey dates shown.

Date	Station	Temp. °C	Salinity psu	Counts/100 ml or /100g (sediment)						
				Fecal coliform MPN	*Escherichia coli* MPN		Presumptive *Streptococcus bovis*[a]	Bifidobacteria		FRNA coliphage	
					Water	Sediment		"Total"	Sorbitol+	Water[b]	Sediment
6/19/95	A	30	11.8	17	17		3	<3	<3	<3	
	B	30	11.3	23	13		7	<3	<3	<3	
	C	27	6.9	230	230		<3	<3	<3	<3	
	D	27	3.7	220	170		<3	<3	<3	<3/3	
	E	26	1.1	490	490		7	<3	<3	<3	
	F	25	2.2	220	220		20	<3	<3	<3	
	G	ns	ns	ns			ns	ns	ns	ns	
	H	ns	ns	ns	ns		ns	ns	ns	ns	
7/17/95	A	31	9.9	230	230		20	<3	<3	<3	
	B	30	10	700	700		10	<3	<3	<3	
	C	27	2.9	9200	2800		130	<3	<3	<3	
	D	28	1.5	5400	5400		2300	<3	<3	11/8	
	E	27	0.5	5400	2200		830	<3	<3	75/34	
	F	27	1	5400	5400		2400	<3	<3	10/11	
	G	ns	ns	ns	ns		ns	ns	ns	ns	
	H	29	1.9	9200	9200		1700	<3	<3	<3/8	

[a]All yellow colonies on mmBA were counted as presumptive *S. bovis*. Overall confirmation rate of presumptive *S. bovis* colonies was 47%.
[b]Unless phage were detected in at least one analytical replicate only a single value is shown. nd- Not done.
ns- Not possible to sample owing to very low tide.

Table 9. Densities[a] of fecal indicator bacteria in feral animal feces from the Taskinas Reserve watershed and tidal creek.

Animal	Mean indicator count/g feces				
	Fecal coliform/ *Escherichia coli* MPN	*Streptococcus bovis*, presumptive[b]	Bifidobacteria		Confirmed FRNA coliphage
			Total	Sorbitol +	
Deer	$\leq 10^2$	1×10^6	I[c]	I	<10
	($\leq 10^2$ - 2.4×10^2, n=2)	(10^4 - 10^7, n=10)	(n=10)	(n=10)	(n=10)
Muskrat	3.4×10^5	$<1 \times 10^3$	nd[d]	$<1 \times 10^4$[e]	<10
	(2.8×10^3 - $\geq 2.4 \times 10^4$, n=6)	(n=5)		(n=5)	(n=5)
Raccoon	1×10^9	$>1 \times 10^6$	I	I	<10
	(1×10^8 - $>1 \times 10^9$, n=3)	(1×10^5 - $>5 \times 10^6$, n=5	(n=5)	(n=5)	(n=5)
Unknown carnivore[f]	$>1 \times 10^6$	$<1 \times 10^3$ - $>5 \times 10^6$	I	I	1.1×10^4
	(n=1)	(n=2)	(n=2)	(n=2)	(3.6×10^3 - $>3.1 \times 10^4$, n=2)

[a]Values shown in parentheses are the range of values obtained and n, the number of fecal samples analyzed for a given indicator. [b]All yellow colonies on mmBA were counted as presumptive *S. bovis*. [c]I, interference from high densities of nontarget colonies prevented enumeration of bifidobacteria. Note that *S. bovis* will grow on both HBSA and HBSA base medium containing propionic acid, pH 5. [d]nd, not determined. [e]Mean of 10^4 nonsorbitol fermenting bifidobacteria were recovered on HBSA (human bifid sorbitol agar). [f]Based on material present in feces.

Table 10. Persistence of fecal coliforms/*Escherichia coli*[a] in (1) muskrat fecal pellets exposed in situ in mesh bags in a salt marsh (18 psu, 15°C), and (2) muskrat pellets added to estuarine water (1:100, w/v, 13 psu, 15°C) and incubated statically in vitro.

Days exposed		Weight change, pellets deployed in marsh (% of 0 day weight)	Fecal indicator MPN/g feces or ml water	
			Muskrat pellets, in situ marsh weathered	Muskrat pellets, in vitro estuarine water weathered
0		5	$\geq 2.4 \times 10^{6}$	2.0×10^{-2}
		5	$\geq 2.4 \times 10^{6}$	4.5×10^{-2}
		5	9.2×10^{5}	2.0×10^{-2}
	mean	5 (100)	$\geq 1.7 \times 10^{6}$	2.6×10^{-2}
1		1.9	1.4×10^{6}	3.3×10^{-2}
		1.3	1.3×10^{6}	7.9×10^{-2}/4.9×10^{-2}
		0.9	5.4×10^{6}	3.3×10^{-2}
	mean	1.4 (28)	2.1×10^{6}	4.4×10^{-2}/3.8×10^{-2}
2		0.7	3.5×10^{6}	1.1×10^{-2}
		1.2	5.4×10^{6}	4.6×10^{-2}
		1.1	$\geq 2.4 \times 10^{7}$	1.7×10^{1}
	mean	1.0 (20)	7.7×10^{6}	9.5×10^{1}
3		1.6	2.4×10^{6}	1.3×10^{1}
		1.0	3.5×10^{6}	3.3×10^{0}
		1.8	4.9×10^{5}	7.9×10^{0}
	mean	1.5 (30)	1.6×10^{6}	7.0×10^{0}
7		1.5	3.1×10^{3}	8.4×10^{0}
		1.6	1.7×10^{2}	4.6×10^{0}
		3.4	1.1×10^{6}[b]	2.2×10^{-1}
	mean	2.2 (44)	8.3×10^{3}	2.0×10^{0}

[a]Only one value shown when fecal coliform/*E. coli* MPN were the same. [b]This bag was found wedged between the other two bags on this date which may account for the high value.

Table 11. Analysis for fluorescent whitening agents in watershed samples.

Date	Sample type: Taskinas Creek station	Sample type: Watershed station	Fluorometer value, µg/l[a]	Date	Sample type: Taskinas Creek station	Sample type: Watershed station	Fluorometer value, µg/l
10/17/94		1	0.003	1/30/95		1	0.001
		2	0.006			2	0.003
		3	0.002			3	0.003
		4	0.023			4	0.000
		5	0.003			5	0.001
		6	0.000			6	0.000
		7	0.002			7	0.000
11/15/94		1	0.003	2/20/95		1	0.001
		2	0.006			2	0.002
		3	0.000			3	0.002
		4	0.002			4	0.003
		5	0.003			5	0.003
		6	0.002			6	0.000
		7	0.000			7	0.001
1/23/95	A		0.003	3/6/95	A		0.004
	B		0.004		B		0.003
	C		0.005		C		0.004
	D		0.004		D		0.005
	E		0.002		E		0.003
	F		0.005		F		0.004
	G		0.005		G		0.004
	H		0.005				
	10 µg/l standard		>0.99				
	5 µg/l standard		>0.99				
	1 µg/l standard		0.010				
	0.1 µg/l standard		0.001				
	0.01 µg/l standard		0.000				
	Procedural blank 1		0.000				
	Procedural blank 2		0.000				
	Solvent blank		0.000				

[a]Values shown are averages of two determinations.

Acknowledgment

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Appendix A. Detailed sanitary survey

YORK RIVER: CAMP PEARY TO TERRAPIN POINT

James City County

Partial Shoreline Sanitary Survey

Date: August 29, 1994

Survey Period: July 26 - August 8, 1994

Total Number of Properties Surveyed: 215

Surveyed by: Christine K. Hogan and Martha W. Rhodes

SECTION A: GENERAL

This survey area extends from Reference Point 50A at the end of the first road to the right off of Route 605, extended to the shoreline to Reference Point 50B at the end of the York River State Park entrance road, extended to the shoreline and includes the York River shoreline between these two points, Taskinas Creek and all of its tributaries.

Topography of the area is characterized by elevations that rise sharply near the shoreline to 50′ and on up to 100′ in the headwaters of Taskinas Creek. The population is concentrated in the subdivisions of Woodland Farms and Ware Manor Estates. The latter community has increased from 12 to 52 dwellings since the last survey in 1990. The economy of this survey area is based on recreation and commuters to nearby military installations and urban areas.

Meteorological data indicated that 2.01" of rain fell July 26-31 and 2.93" August 1-8 for a total rainfall of 4.94" for the survey period.

Sources of animal pollution have increased in this part of the survey area from 9 to 14 sources, of which 3 have a direct impact on shellfish waters. However, these all involve relatively small numbers of animals. There is one kennel facility (field #A204) with 18 runs. All wastes from kennel are washed down the floor drains and into a septic system separate from the owner's home.

The current restriction on shellfish harvesting is Condemned Shellfish Area No. 166, York River: Taskinas Creek, reissued April 27, 1989.

Information in this report is gathered by and primarily for use of the Division of Shellfish Sanitation, Virginia Department of Health, in order to fulfill its responsibilities of shellfish growing area supervision and classification. However, the data are made available to various agencies participating in shellfish program coordinated activities or other interested parties. The Engineering Appendix is available by request from the Richmond Office of the Division of Shellfish Sanitation.

Report copies are provided to the local health department for corrective action of deficiencies listed on the summary page in Sections B. II. and B. III. and the Department of Environmental Quality, Water Regional Office for classifications listed on the summary page in Section B. I. Local health departments are not responsible for correction of properties listed on the summary page in Sections A, B. I., C, D and E.

This report lists only those properties which have a sanitary deficiency or have other environmental significance. Individual field forms with full information on properties listed in this report are on file in the Richmond Office of the Division of Shellfish Sanitation and are available for reference until superseded by a subsequent resurvey of the area.

SECTION B: SEWAGE POLLUTION SOURCES

SEWAGE TREATMENT FACILITIES, DIRECT

-None-

SEWAGE TREATMENT FACILITIES, INDIRECT

-None-

ON-SITE DEFICIENCIES, DIRECT

11P. CONTRIBUTES POLLUTION (Kitchen or Laundry Wastes) - Archie C. Richardson, 5202 Riverview Road, Williamsburg 23185. Dwelling- white vinyl siding 1 story with blue shutters. 3 persons. The owner refused inspection. This property was previously listed as a Contributes Pollution (Kitchen or Laundry Wastes) on the last survey and has not been corrected. Listed on last survey as #881. No Sanitary Notice issued.

ON-SITE DEFICIENCIES, INDIRECT

1P. CONTRIBUTES POLLUTION - Owner: Robert P. Piggott, 8604 Croaker Road, Williamsburg 23185. Occupant: Linwood James, 8606 Croaker Road, Williamsburg. Dwelling- red and white housetrailer. 1 person. Pit to privy undermined, exposing contents of pit. Privy inaccessible due to door opening less than half way. Sanitary Notice issued 7-27-94 to field #A75.

8P. CONTRIBUTES POLLUTION - David A. Wolverton, 104 Woodland Road, Williamsburg 23185. Dwelling- white vinyl siding 1 story with green and black shutters and beige "Coachmen" camper trailer in side yard. No contact. Camper trailer sewer hose broken in two. No longer connected to septic system. No evidence of waste on ground at time of inspection. Sanitary Notice issued 8-4-94 to field #A153.

POTENTIAL POLLUTION

-None-

SECTION E: ANIMAL POLLUTION SOURCES

CONTRIBUTES ANIMAL POLLUTION, DIRECT

12P. CONTRIBUTES ANIMAL POLLUTION - Arthur Richardson, 5298 Riverview Road, Williamsburg 23185. Dwelling- white frame 2½ story with blue trim. 3 persons. Present at time of survey were approximately 70 assorted fowl, 8 cows, 11 goats, 1 pig and 11 dogs. Cattle have direct access to swamp leading to Taskinas Creek.

13P. CONTRIBUTES ANIMAL POLLUTION - Dawn Crump, 5330 Riverview Road, Williamsburg 23185. Dwelling- white house trailer with attached porch. 4 persons. Present at time of survey were 2 horses, 10 rabbits, and approximately 100 assorted fowl. Horses have direct access to a tributary of Taskinas Creek in pasture next to neighboring property.

14P. CONTRIBUTES ANIMAL POLLUTION - George Richardson, 5362 Riverview Road, Williamsburg 23185. Agricultural- private pasture. No contact. Present at time of survey were 2 cattle, 1 horse and 1 mule with direct access to pond in pasture and a tributary of Taskinas Creek.

CONTRIBUTES ANIMAL POLLUTION, INDIRECT

2P. CONTRIBUTES ANIMAL POLLUTION - Michael W. Kaspareck, 2 Croaker Circle, Williamsburg 23185. Dwelling- beige vinyl siding 1 story with white trim. No contact. Present at time of survey were 6 horses. There is a ravine in the pasture that drains to an intermittent stream of Taskinas Creek. Fence crosses ravine to keep horses out.

3P. CONTRIBUTES ANIMAL POLLUTION - Frederick W. Topke, 203 Stonehouse Road, Williamsburg 23185. Dwelling- brick and beige vinyl siding 1 story. 3 persons. Present at time of survey were approximately 25 assorted fowl in pens.

4P. CONTRIBUTES ANIMAL POLLUTION - Nelson L. St. Clair, Jr., 208 Stonehouse Road, Williamsburg 23185. Dwelling- brick 1 story with yellow trim and red shutters with red frame stable to left side of house. No contact. Present at time of survey were 9 horses, 2 goats and approximately 15 dogs, 9 of which were kenneled down the hill, behind the house.

5P. CONTRIBUTES ANIMAL POLLUTION - Marcel and Helen N. Walter, 307 Stonehouse Road, Williamsburg 23185. Dwelling- brick 1 story with white trim and black shutters. Present at time of survey were 4 goats, 1 horse and 31 geese. Cement drainage ditch located 5' from pasture.

6P. CONTRIBUTES ANIMAL POLLUTION - Charles N. and L.J. Hall, 124 Timberwood Drive, Williamsburg 23185. Dwelling- brown frame modern 2 story. 3 persons. Present at time of survey were 30 chickens and 1 goat. Chicken coop is located at edge of 50' bluff above Taskinas Creek tributary.

7P. CONTRIBUTES ANIMAL POLLUTION - Valda R. Anderson, 106 Woodland Road, Williamsburg 23185. Dwelling- brick 1 story with beige shutters and trim. No contact. Present at time of survey were 3 horses and 3 geese which had direct access to pond in pasture. Pond has overflow to intermittent stream of Taskinas Creek, but was no currently draining to stream.

8P. CONTRIBUTES ANIMAL POLLUTION - David A. Wolverton, 104 Woodland Road, Williamsburg 23185. Dwelling- white vinyl siding 1 story with black and green shutters. No contact. Present at time of survey was 1 horse with direct access to marsh that drains to an intermittent stream of Taskinas Creek.

9P. CONTRIBUTES ANIMAL POLLUTION - Minda F. Bishop, 4852 Riverview Road, Williamsburg 23185. Dwelling- white vinyl siding 1 story with blue shutters. 2 persons. Present at time of survey were approximately 50 chickens in pens.

10P. CONTRIBUTES ANIMAL POLLUTION - Harold M. Phegley, 5084 Riverview Road, Williamsburg 23185. Dwelling- green aluminum siding 1 story with white trim. 4 persons. Present at time of survey were 3 horses, 1 pony and approximately 40 assorted fowl. Pasture is located 15' from ditch.

15P. CONTRIBUTES ANIMAL POLLUTION - Ronald D. Richardson, 5390 Riverview Road, Williamsburg 23185. Dwelling- beige vinyl siding 1½ story with red shutters. 4 persons. Present at time of survey were 2 goats and 43 assorted fowl.

16P. CONTRIBUTES ANIMAL POLLUTION - Roby J. Nixon, Jr., 5396 Riverview Road, Williamsburg 23185. Dwelling- brick 1 story with black shutters and white trim. 3 persons. Present at time of survey were 31 assorted fowl, 2 cattle, 2 horses, 2 donkeys, 7 goats. In addition to the 31 fowl, there were also approximately 25 parakeets and cockatiels that are kept outside in two cages year round.

SECTION C: NONSEWAGE WASTE SITES

INDUSTRIAL WASTES, DIRECT

-None-

INDUSTRIAL WASTES, INDIRECT

-None-

SOLID WASTE SITES, DIRECT

-None-

SOLID WASTE SITES, INDIRECT

17P. Trina and Greg Mathews, 5624 Riverview Road, Williamsburg 23185. Dwelling- white frame 1 story with black trim. 4 persons. Domestic trash covered whole back yard and back of pickup truck filled with cans, bottles and other trash. Mrs. Mathews watches approximately 8 children and all were playing in and around garbage at time of inspection.

SECTION D: CONTRIBUTES BOAT POLLUTION

-None-

ANIMAL WASTES, DIRECT

-None-

ANIMAL WASTES, INDIRECT

-None-

SUMMARY

Area #50
York River: Camp Peary to Terrapin Point
August 29, 1994

SECTION B: SEWAGE POLLUTION SOURCES

I. SEWAGE TREATMENT FACILITIES

0 - DIRECT - None
0 - INDIRECT - None

0 - Section B. I. TOTAL

II. ON-SITE SEWAGE DEFICIENCIES

Correction of deficiencies in this section is the responsibility of the local health department.

0 - CONTRIBUTES POLLUTION, DIRECT - None
2 - CONTRIBUTES POLLUTION, INDIRECT - #1P, 8P
1 - CP (Kitchen or Laundry Wastes), DIRECT - #11P
0 - CP (Kitchen or Laundry Wastes), INDIRECT - None
0 - NO FACILITIES, DIRECT - None
0 - NO FACILITIES, INDIRECT - None

3 - Section B. II. TOTAL

III. POTENTIAL POLLUTION -

Periodic surveillance of these properties will be maintained to determine any status change.

0 - POTENTIAL POLLUTION - None

0 - Section B. III. TOTAL

SECTION C: NON-SEWAGE WASTE SITES

I. INDUSTRIAL WASTE SITES

0 - DIRECT - None
0 - INDIRECT - None

0 - Section C. I. TOTAL

II. SOLID WASTE SITES

0 - DIRECT - None
1 - INDIRECT - #17P

1 - Section C. II. TOTAL

SECTION D: CONTRIBUTES BOAT POLLUTION

0 - MARINAS - None
0 - OTHER PLACES WHERE BOATS ARE MOORED - None
0 - UNDER SURVEILLANCE - None

0 - Section D TOTAL

SECTION E: AGRICULTURAL POLLUTION SOURCES

I. CONTRIBUTES ANIMAL POLLUTION

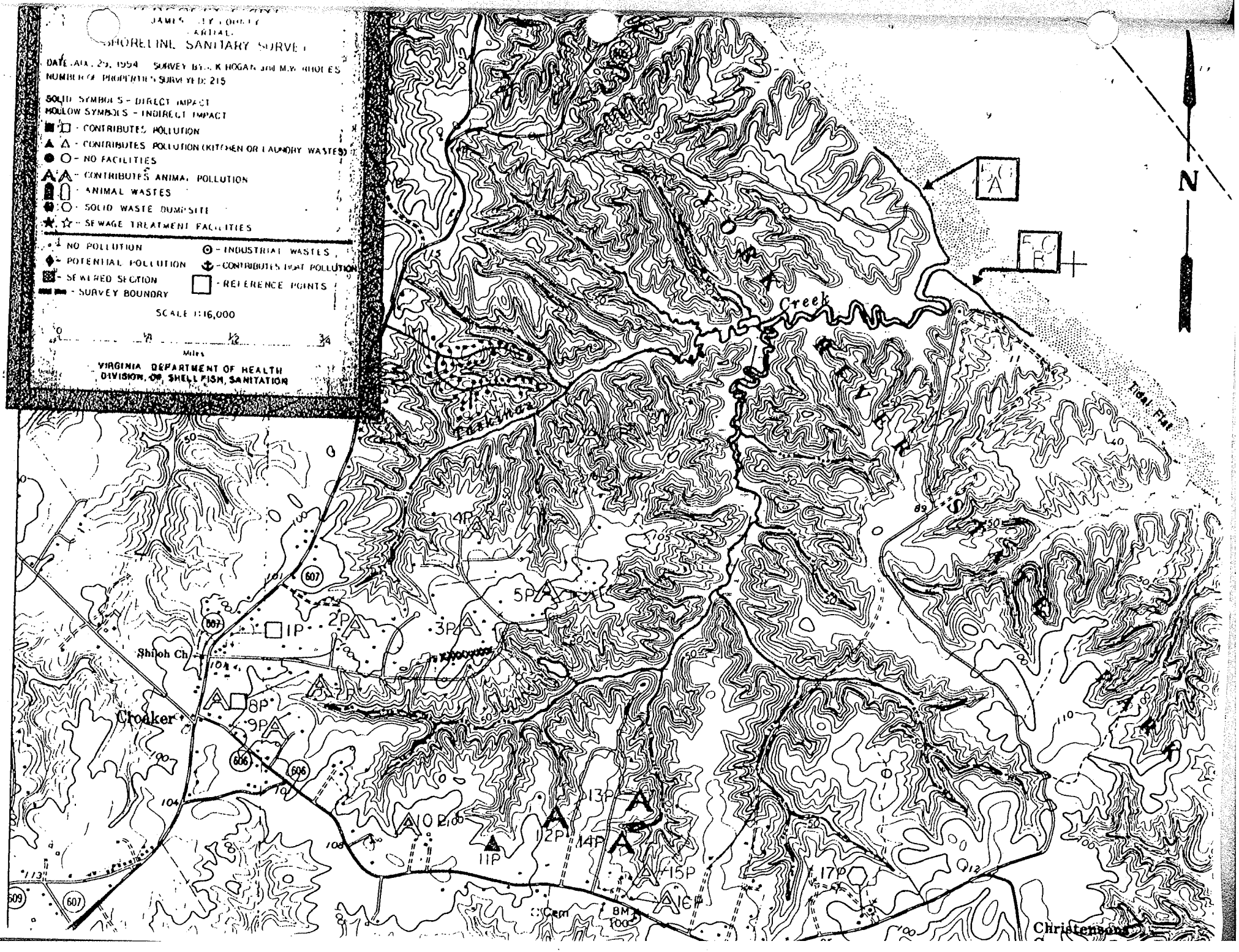
3 - DIRECT - #12P, 13P, 14P
11 - INDIRECT - #2P, 3P, 4P, 5P, 6P, 7P, 8P, 9P, 10P, 15P, 16P

14 - Section E. I. TOTAL

II. AGRICULTURAL POLLUTION

0 - DIRECT - None
0 - INDIRECT - None

0 - Section E. II. TOTAL


JAMES CITY COUNTY
AERIAL
SHORELINE SANITARY SURVEY
DATE AUG. 29, 1994 SURVEY BY: K HOGAN and M.W. WHOLES
NUMBER OF PROPERTIES SURVEYED: 215
SOLID SYMBOLS - DIRECT IMPACT
HOLLOW SYMBOLS - INDIRECT IMPACT
- CONTRIBUTES POLLUTION
- CONTRIBUTES POLLUTION (KITCHEN OR LAUNDRY WASTES)
- NO FACILITIES
- CONTRIBUTES ANIMAL POLLUTION
- ANIMAL WASTES
- SOLID WASTE DUMPSITE
- SEWAGE TREATMENT FACILITIES
- NO POLLUTION
- POTENTIAL POLLUTION
- SEWERED SECTION
- SURVEY BOUNDRY
- INDUSTRIAL WASTES
- CONTRIBUTES BOAT POLLUTION
- REFERENCE POINTS
SCALE 1:16,000
Miles
VIRGINIA DEPARTMENT OF HEALTH
DIVISION OF SHELLFISH SANITATION
N
Creek
Tidal Flat
Shiloh Ch
Croaker
Christenson
1P
2PA
3PA
4P
5PA
8P
9PA
10P
11P
12P
13P
14P
15P
17P
607
606
609