# MANAGING NONPOINT FECAL COLIFORM SOURCES TO TIDAL INLETS

**George M. Simmons, Jr.**
**Alumni Distinguished Professor**
**Sue A. Herbein**
**Research Associate**
**and**
**Carey M. James**
**Undergraduate Research Assistant**
Biology Department
Virginia Polytechnic Institute and State University

## INTRODUCTION

The purpose of this research was to identify and explain nonpoint sources of fecal coliforms to tidal inlets on Virginia's Eastern Shore, particularly those inlets that were closed, or were threatened with closure. The goal of this research project was to demonstrate that nonpoint sources, once identified, could be managed in such a way that closed areas could be reopened for the economic benefit of the local community.

Fecal contamination from nonpoint sources has been recognized as a major threat to water quality for all types of surface water (Geldrich *et al.*, 1968; Faust, 1976; Gilliland and Baxter-Potter, 1987; Kay, *et al.*, 1994). In many cases, the level of contamination leads to closure of the water for recreational purposes or shellfish harvest. Closure of tidal inlets for shellfish harvest is more than an academic exercise related to water quality. In many cases, closure of an inlet has serious economic repercussions because of the burgeoning aquaculture industry. The number of acres condemned over the past decade for shellfish harvest in Virginia increased approximately 55% from 1970 levels (62,272 acres condemned in 1970 vs 96,826 acres in 1994; data kindly provided by Division of Shellfish Sanitation, Richmond, VA). Identifiable sources have not been evident for a large number of these condemnations (Schima *et al.*, 1994).

The major identifiable source generally regarded to be the cause of closure for shellfish waters is improperly functioning On-Site-Waste-Disposal-Systems (OSWDS) (Kator and Rhodes, 1993). While many studies have indicated that OSWDS can be a source of potential contamination (Reneau and Pettry, 1975; Hendry and Toth, 1982; Cogger, *et al.*, 1988; Chen, 1988; Reneau, *et al.*, 1989; Hayes, *et al.*, 1990, and others), many of the inlets in this study on the Eastern Shore had few or no homes associated with them. Other potential nonpoint sources have been identified as run-off from agricultural areas, from wild animals (Leonard, *et al.*, 1989), and from seagulls (Levesque, *et al.*, 1993).

## STUDY SITES

The geographic area for this study was in Northampton County, Virginia at the most southern tip of the Delmarva Penninsula (Fig. 1). The land use pattern in this area has been characterized as ~31% urban, ~31% forest, and ~48% agriculture. The upland soils are characterized as well drained. These sites were characteristic of many of the Bay-side tidal creeks that have narrow channels; maximum depths of 3 - 5 meters; broad, shallow shoal areas with irregularly spaced fringing marshes; forested buffer zones; and widely spaced private residences that are generally set back 50+ meters from the shoreline.

The Gulf is a 1.0 $km^2$ tidal inlet that drains westward into the Chesapeake Bay from Eastville, Virginia and has only one major tributary. The upper two-thirds of this inlet was closed to shellfish harvest during the study. Cherrystone Inlet has several major tributaries, is the larger of the two study sites, and has an area of approximately 6.0 $km^2$ (Reay, *et al.*, 1995). Two tributaries were closed and a second was threatened with closure during this study. Four ponds on Fisherman's Island (a barrier island at the tip of the Delmarva Penninsula, owned by the US Fish and Wildlife Service) were studied for comparative purposes because Fisherman's Island is uninhabited and lacked human fecal coliform input. The ponds ranged in size between approximately 500 and 5000 $m^2$.

### Water Samples

Water samples for fecal coliform determination followed Amer. Publ. Health Assoc., *et al.* (1992). Water was collected in 100 milliliter sterile bottles. Fecal coliform density was measured by the Fecal Coliform Direct Test using A-1 medium and the five tube, three dilution technique (Amer. Publ. Health Assoc., *et al.*, 1992). Density was recorded as a Most

Probable Number (MPN)/ 100 milliliters. In addition, the fluorescent dye, MUG (4-methylumbelliferyl-ß-D-glucuronide), was used to corroborate the presence of gas in the A-1 medium and also to test for the presence of false positives. Specific identification was made with the Analytab Products (API) 20E System.

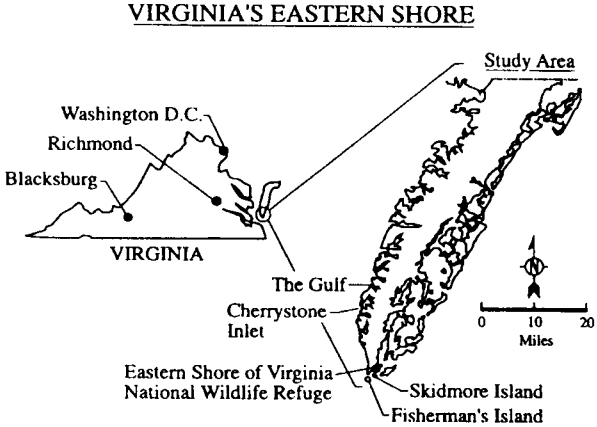



**Figure 1. Study site locations.**

All water samples for nutrient analyses were collected in Nalgene© bottles that had been acid-washed and rinsed 3X in distilled/deionized water. Samples for nutrient analyses were filtered through pre-rinsed 0.45 $\mu$m Nucleopore© filters and 2-3 replicates were analyzed for each nutrient in each water sample. Nutrient analyses, except ammonium, followed the methodology of the U.S. Environmental Protection Agency (U.S.E.P.A., 1983). Ammonium was determined according to Strickland and Parsons (1972). Salinity was measured in the laboratory with a Beckman Industrial Induction Salinometer (Rosemont Analytical, Model RS-10). Temperature was measured with a calibrated longstem thermometer, and oxygen was measured by a microWinkler Technique using the Azide Modification of the Iodometric Technique (American Public Health Assoc., *et al.*, 1992).

Genetic Fingerprinting

*Escherichia coli* from raccoon, goose, otter, and muskrat were characterized by contour-clamped homogenous electric field (CHEF) pulsed field gel electrophoresis (PFGE). PFGE has been used to resolve restriction fragments in bacterial genomes ranging from microorganisms responsible for nosocomial infections (Allardet - Servant, *et al.*, 1989) *Vibrio* species colonizing oysters (Buchrieser, *et al.*, 1995), *Listeria* species contaminating vegetables (Del Rosario, *et al.*, 1995), to coliforms isolated from water distribution systems (Edberg, *et al., 1994).* Fresh fecal samples were collected and stored in sealed sterile plastic bags at 4°C until analysis. A 0.5-1.0 g aliquot was placed in 20 ml sterile, buffered distilled water for inoculation into A-1 Broth. One tube of A-1 Broth (2X) was inoculated with 10 ml of a fecal/water sample and one tube A-1 Broth + MUG (1X) was inoculated with a 1 ml sample. Following 3 hr @ 37°C and 21 hr @ 44.5°C, a (+) A-1 broth sample was diluted $10^{-1}$ - $10^{-8}$ and spread-plated to Violet Red Bile Agar. The plate containing approximately 15-50 colonies was selected, each colony subcultured to Nutrient Agar, preserved in glycerol, identified by API 20E as *E. coli*, and prepared for PFGE according to the protocol of GenePath Group 2 Reagent Kit, Bio-Rad Laboratories (1994). The DNA from each strain was cut with the *Not*I restriction enzyme, the DNA fragments separated at 6V/cm (200v), 14°C, and ramped pulse 5.3-49.9 seconds for 20 hrs. The 1% agarose gel was stained with ethidium bromide, de-stained in distilled water, and photographed on a long-wave UV transilluminator with a Polaroid camera (aperture setting 7.5, shutter speed 1). The *Not*I fingerprint of each *E. coli* isolate could then be compared with the other isolates, both within each animal species and between animal species.

Tracking NonPoint Sources

Nonpoint fecal coliform sources can be tracked using shoreline survey techniques and discrete sampling methods over small areas (on a scale of centimeters or meters) at land/water interfaces. More specifically, samples should be collected by wading or walking, wearing Marsh Walkers© when necessary, using sterilized 60 milliliter hypodermic syringes for water collection (because the streams will be too small and/or shallow to immerse an entire bottle), and by sampling in a sequential fashion across marshes or up creeks, following the coliform signal (Fig. 2). Areas that consistently show few or no coliforms should be abandoned in favor of rivulets that show high coliform counts. Once the coliform source is identified, it should be monitored over an extended time period to verify its nature and degree of consistency (Simmons, 1994).

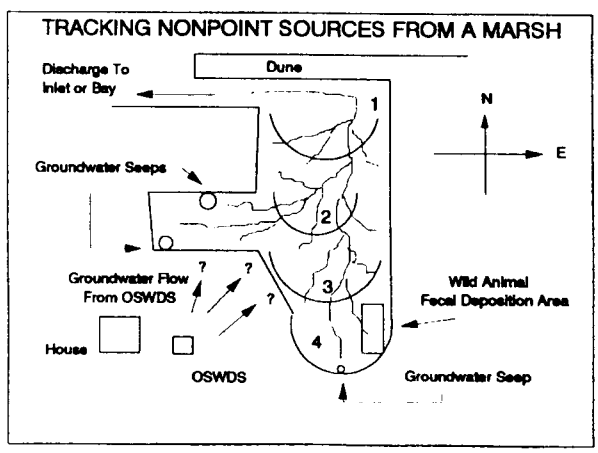



**Figure 2. Tracking nonpoint sources.**

## RESULTS

Developing the Insight to Manage Nonpoint Sources:

Simmons (1994) outlined the case history studies which led to the understanding that the majority of nonpoint fecal coliform sources are of nonhuman origin and associated with groundwater seeps at marsh/upland (woods) interfaces. To test the theory of animal origin, an enclosure pen was placed around two groundwater seeps at marsh/upland interfaces. Figure 3 describes the history of fecal coliform counts at a groundwater seep before and after enclosure and Figure 4 is a replicate of this exercise. As both data sets indicate, fecal coliform counts could be reduced by simply restricting animal access to the groundwater seep. Both experimental sites were located along the edge of Cherrystone Inlet.

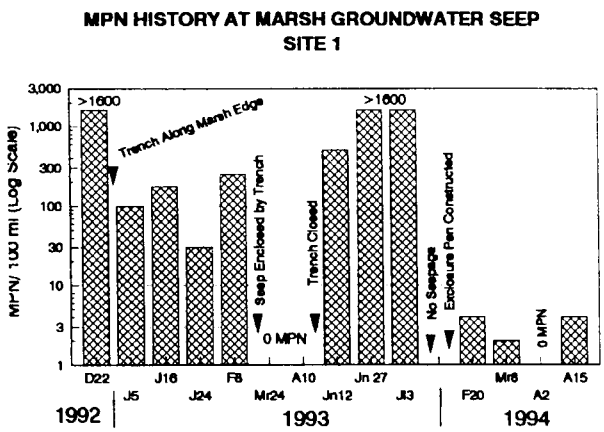



**Figure 3. Enclosure Effect at Site 1.**

Evidence of a "Marsh Effect" on Nonpoint Fecal Coliform Sources:

The significance of fecal coliform sources from natural marsh drainage could be demonstrated by measuring fecal coliform density in the headwaters of drainage basins and comparing this density with the density in tidal creek water below freshwater impoundments that interrupted the flow of these headwater streams to their tidal embayments. The impoundments served as a natural biological filter and the downstream areas below the impoundment were sampled before any possible anthropogenic inputs could occur. Table 1 shows the impoundments were quite effective in removing fecal coliforms, but the density quickly returned in the tidal portion of the creeks when the only source of water was 1) from the impoundments, and 2) from the marshes and their associated groundwater seeps. Direct observation of the marshes themselves indicated numerous trails across the marshs with frequent side "cul-de-sacs" which were used as defecation areas. The cul-de-sacs were ~10 X 10 $m^2$ in area and were covered at high tide. During the falling tide, drainage from these areas, as well as the groundwater seep areas, contributed to the elevated counts in tidal creeks below impoundments.

Table 1

Fecal Coliform Densities Suggesting a "Marsh Effect"

| Statistics | Creeks Above Ponds | Below Ponds | In Marshes |
|---|---|---|---|
| Means (MPN/100ml) | 584 | 29 | 796 |
| Range | 8 - 1600 | 0 - 50 | 8 - >1600 |
| N (Sample No.) | 13 | 11 | 14 |

(Based on February, April, and June, 1994 sampling)

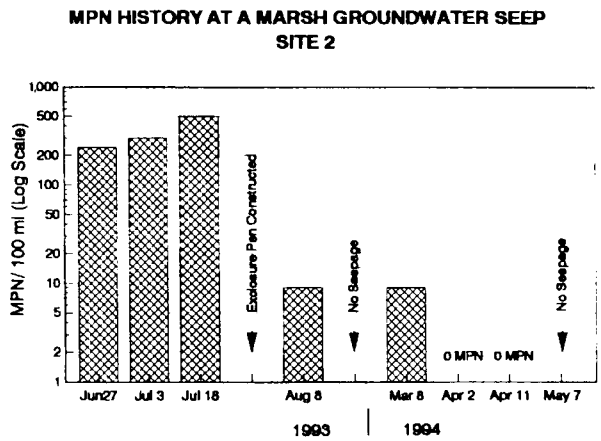



**Figure 4. Enclosure effect at Site 2.**

Managing the "Marsh Effect":

The fecal coliform burden coming off one marsh during a tidal cycle was measured in September 1993 (Fig. 5 - solid line). These data showed the fecal coliform density increased with tidal discharge - particularly when the water in association with the sediment/water interface began to drain off the marsh. Because the data suggested a wild animal source, particularly raccoons, the density of raccoons in the vicinity of this particular marsh on both sides of the creek was reduced. Reduction amounted to approximately 80 raccoons in a 50 acre area immediately behind this marsh and approximately 100 raccoons from the farm directly across the creek from this marsh.

The same study was repeated during September 1994 and the total fecal coliform burden was reduced by ~80% (Fig. 5 - dotted line). Furthermore, by summer's end, data collected by the Division of Shellfish Sanitation indicated this particular creek had moved away from the brink of closure (Division of Shellfish Sanitation, personal communication). Discharge volume from the marsh between the two years, based on stadia rod measurements of water depth in the marsh inlet, and all other major environmental variables between 1993 and 1994 (rainfall, hurricanes, tides, earthquakes, solar flux, etc.)were similar. The only majorchange was the density of raccoons.

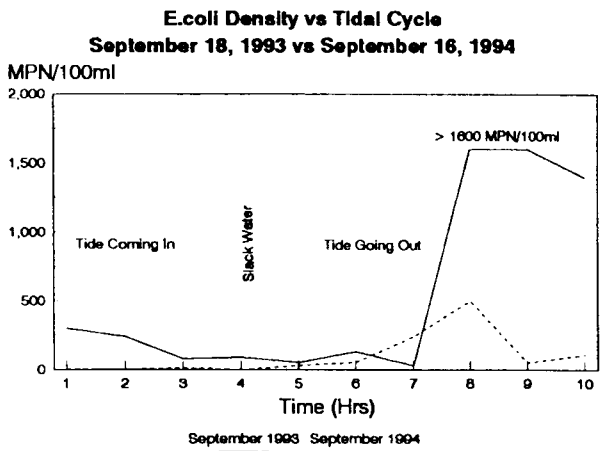



**Figure 5. Effect of raccoon removal.**

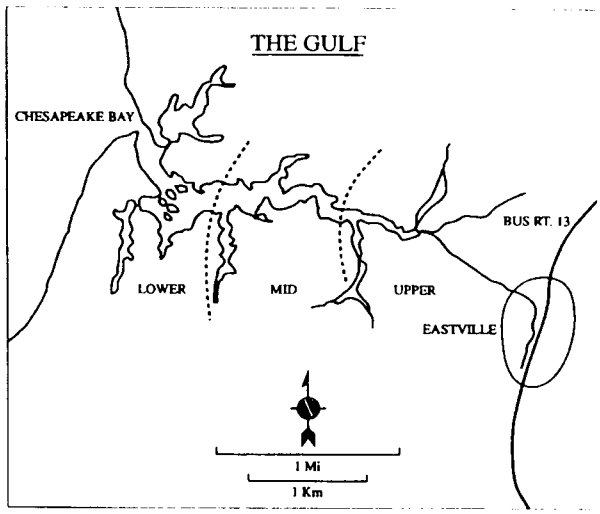



**Figure 6. Collecting zones in The Gulf.**

The Importance of Economic Incentive in Watershed Management of Nonpoint Fecal Coliform Sources:

Simultaneously with the field work and shoreline surveys on Cherrystone Inlet, similar studies began on the The Gulf which was the next tidal inlet to the north (Fig.1). It seemed that resolution of non-point sources on this inlet would be easier to achieve. On the lower end around the southern entrance to The Gulf was a small community, all homes of which used OSWDS. Another small community occupied the main headwater tributary of The Gulf and all homes, including the municipal building, utilized OSWDS. Historical data from the Shellfish Sanitation Office showed that elevated fecal coliform counts on The Gulf increased above the community at the mouth and increased going upstream. In fact, "closure" signs began immediately above the most upstream homes.

Additional sampling by our laboratory on outgoing tides indicated fecal coliform density was highest in the upstream areas, decreased in the mid-regions of the inlet and was lowest at the mouth (Fig. 6, Table 2). This trend remained even after the small tributaries draining the headwaters of the upstream community dried up during the summer. Fecal coliform densities reached their highest numbers (>1600 MPN/100 ml) in small rivulets draining fringing marshes along margins of The Gulf.

Unlike the marshes on Cherrystone Inlet, the closure area on The Gulf does not compromise economic activity, such as aquaculture potential. Therefore, the removal of offending species to enhance water quality becomes an exercise in environmental stewardship. Present plans by several citizen groups involve a remediation effort to reduce fecal coliform numbers. This study site will be an excellent case history study to evaluate the resolve of the citizens to improve water quality in their immediate area without economic incentives.

Table 2
Fecal Coliform Densities from the Upper and Mid-Reaches of The Gulf

| Statistics | Upper Reaches | Mid-Reach |
|---|---|---|
| Means (MPN/100ml) | 398 | 40 |
| Range | 4 - >1600 | 0 - 300 |
| N (Sample No.) | 37 | 40 |

March 1993 - June 1995

Genetic Fingerprinting

Before ascribing an *E. coli*, *Not*I DNA fingerprint to a particular animal, it was necessary to determine how many strains of the bacterium were present in a fecal sample. Table 3 lists the API 20E and PFGE profiles determined from each major marsh inhabitating animal studied thus far. The number of spread plate isolates per animal group was: Raccoon = 46; Goose = 34; Otter = 14; Muskrat = 29. The single fecal sample, per animal group, does not allow meaningful statistical comparisons among groups at this time. However, the presence/absence of certain API 20E proviles, and the number of isolates contained within each profile may prove to be a first level of discrimination among animals. For example, API 20E profile 5144572 is found within each of the four groups, but profile 5044572 is found only in raccoon and muskrat. In addition, profile 5144572 contains 64% of otter isolates, but only 20% goose, 9% raccoon, and 3% muskrat isolates, while profile 5044572 is the major isolate group (74% and 97% respectively) in raccoon and muskrat. The signifance of API 20E profile distribution may become more evident as replicate animals are studied.

Computer scans of all *Not*I DNA restriction fragment patterns in each animal group are shown in Figures 7-9. Lanes are labelled at the top of the gel while restriction

fringerprint patterns and molecular weight standards are labelled at the base of the gel. Figures 7A and 7B represent raccoon patterns, Figure 8 represents goose, and Figure 9 represents raccoon, otter, and muskrat. After a restriction fragment fingerprint was determined for each isolate within an animal group, each unique fingerprint pattern was assigned an arbitary number and the number of isolates exhibiting that particular fingerprint enumerated (see Table 3 for numbers per fingerprint).

Even though DNA restriction fragment technology is well developed for general use, a number of problems were encountered. For example, some *E. coli* strains isolated from raccoon grew as aggregates in Luria Broth, the growth medium used prior to preparation of DNA samples. The aggregates could not be separated physically and yielded restriction fingerprints that were difficult to characterize (Figure 9, Lanes 4 and 7). Considerable time was spent attempting to eliminate the in-lane, uneven, banding but clarification was not successful. Also noteworthy was that each animal group, except goose, contained isolates for which no banding pattern was discernible and only a diffused smear located near the bottom quarter of the agarose gel could be obtained (Figure 9, Lanes 2 and 3). Maslow, *et al.*, (1993) suggested smearing was due to the presence of endogenous nucleases possibly from bacteriophage infections of the *E. coli*. Subsequent PFGE runs on muskrat samples suggested that the effect of the nucleases may possibly be diluted out by increasing the concentration of plug DNA. The presence and number of isolates exhibiting smears was noted, but at this point the banding pattern contained within the smears has not been determined.

The restriction fragment fingerprint patterns are presently being compared both manually by marking and measuring migration distance distances in relation to a λ concatemer ladder and using NIH Image, gel documentation software down-loaded from Internet. Interpretation of the fingerprint patterns will be as follows: **Identical:** band patterns are the same; **Related:** may be substrains; differ by 1-2 genetic events, a 1-3 band difference; **Different:** considered different strains; differ by ≥3 genetic events, a > 4 band difference (Maslow, *et al.*, 1993; Goering, 1993).

It seems that genetic fingerprinting has considerable potential to corroborate field work, but further studies in our laboratory using molecular epidemiology techniques needs to examine variability between replicates and seasonal effects.

Table3
Summary of Restriction Fragment Isolates from Major Marsh Animals in Study Area

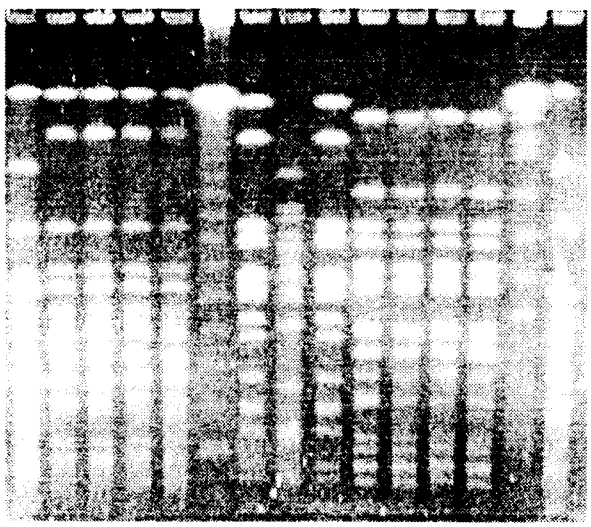
| Animal | API 20E Profile | #[1] | PFGE Pattern[2] | #[3] |
|---|---|---|---|---|
| Raccoon | 5044572 | 34 | R-4 | 14 |
| | | | R-Smear | 10 |
| | | | R-6 | 4 |
| | | | R-3 | 2 |
| | | | R-5 | 2 |
| | | | R-7 | 2 |
| | 5044552 | 8 | R-2 | 4 |
| | | | R-smear | 4 |
| | 5144572 | 4 | R-1 | 4 |
| Otter | 5144572 | 9 | O-2 | 6 |
| | | | O-3 | 1 |
| | | | O-4 | 1 |
| | | | O-smear | 1 |
| | 5044552 | 5 | O-1 | 5 |
| Goose | 5044552 | 20 | G-7 | 8 |
| | | | G-8 | 8 |
| | | | G-9 | 2 |
| | | | G-10 | 1 |
| | | | G-11 | 1 |
| | 5144572 | 7 | G-1 | 3 |
| | | | G-2 | 1 |
| | | | G-3 | 1 |
| | | | G-4 | 1 |
| | | | G-5 | 1 |
| | 1044572 | 3 | G-6 | 3 |
| | 5144573 | 1 | G-5 | 1 |
| | 5144552 | 1 | G-12 | 1 |
| | 4144572 | 1 | G-13 | 1 |
| | 5144512 | 1 | G-14 | 1 |
| Muskrat | 5044572 | 28 | M-1 | 22 |
| | | | M-2 | 1 |
| | | | M-smear | 5 |
| | 5144572 | 1 | M-3 | 1 |

[1] Number of spread plate isolates represented by preceding API 20E Profile.
[2] Arbitrary restriction fragment pattern number assigned **within** each animal group.
[3] Number of spread plate isolates represented by preceding PFGE pattern.

## Figure 7a

Composite PFGE gel of *E.coli* isolates from one raccoon fecal sample. Molecular weight standard is a lambda ladder (lane 6)

Lane: 6

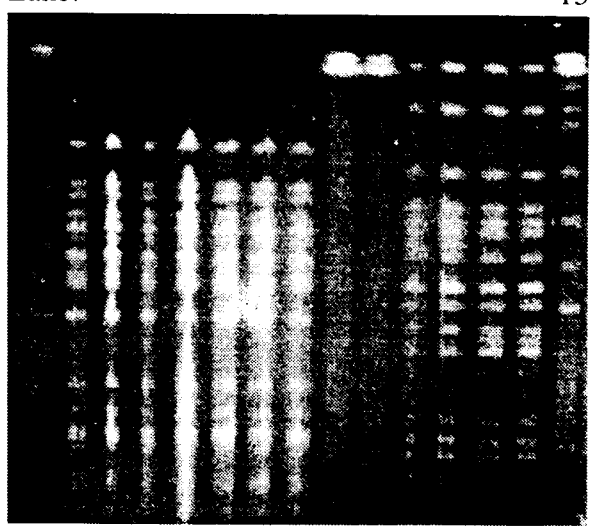


Pattern: R-1 R-2 R-3 R-4

## Figure 7b

Composite PFGE gel of *E.coli* isolates from one raccoon fecal sample. Molecular weight standard is a *S.cerevisiae* chorosome (SC)-(lane 15).

Lane: 15

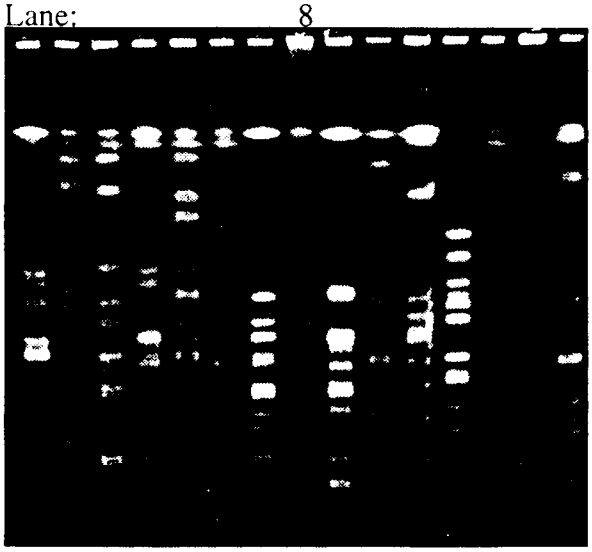


Pattern: R-5 R-6 R-7 SC

## Figure 8

PFGE gel of E.coli isolates from one goose fecal sample (lambda= lane 8).

Lane: 8



Goose Patterns (G-x)

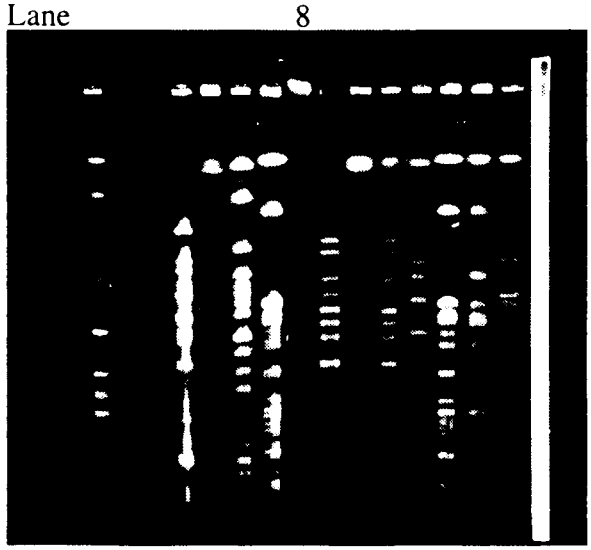
1 3 5 7 9 11 13

2 4 6 8 10 12 14

## Figure 9

PFGE gel of E.coli from one raccoon (1-7),one otter (9-12), and one muskrat (13-15).(lambda= lane 8)

Lane 8



Patterns:

1.....7 9.....12

13....15

## DISCUSSION

Nonpoint fecal coliform sources generally have been regarded to be too diffused to be amenable to resolution (Faust and Goff, 1977; Kator and Rhodes, 1993; and others). In many cases, this may be true. However, this report and others (Simmons, 1993; 1994) suggest that nonpoint sources can be identified by small-scale, discrete sampling at land/water interfaces. The effect of exclosures suggested that raccoons and other wild animal species use groundwater seeps at forest/marsh interfaces as sources of drinking water as the animals transit the marsh and forage for food. Personal observation indicated that such seeps are also used for defecation by raccoons. Furthermore, when wild animal densities were lowered, fecal coliform density in outgoing tidal water from marshes decreased dramatically.

Data accumulated from analysis of one fecal sample each from a raccoon, otter, goose, and muskrat suggest that API 20E profile patterns of *E. coli* isolates within these animal groups and between the animal groups may be useful as a screening test to eliminate a particular animal group, or groups, from consideration as a nonpoint source pollutant. Additional experimentation, now in progress, is required to determine if the DNA restriction fragment patterns of *E. coli* strains isolated from waters contaminated with fecal material may then represent a second, more discriminating, level of identification. The unique restriction fragment patterns from raccoon (N=7), otter (N=4), goose (N=14), and muskrat (N=3) are different based upon visual inspection, **both among *E. coli* strains within a given animal group and strains between animal groups.** The statistical significance of these fingerprints, and their use as an epidemiological tool, cannot be fully assessed until additional replicates of each animal species are analyzed.

The effect of reduction in fur bearing species by trapping on water quality does not appear to have been assessed in a quantitative manner. Data for trapping activities in Virginia was kindly provided by Mr. Dennis Martin (Virginia Department of Game and Inland Fisheries, (Fig. 10). Because these trapping data are based on a state-wide basis, it would be difficult to correlate these data with shellfish bed closure rates or fecal coliform density in any specific tidal inlet.

In addition, Siemer, *et al.* (1994) indicated that trapping activity declined significantly during the period 1980-1990 in New York State. Furthermore, enrollment in New York's mandatory trapper education courses declined by 90% over the same period. The decline in trapping activity was associated with the decline in pelt value of fur-bearing species. The decline in pelt value has been generally associated with animal rights/anti-

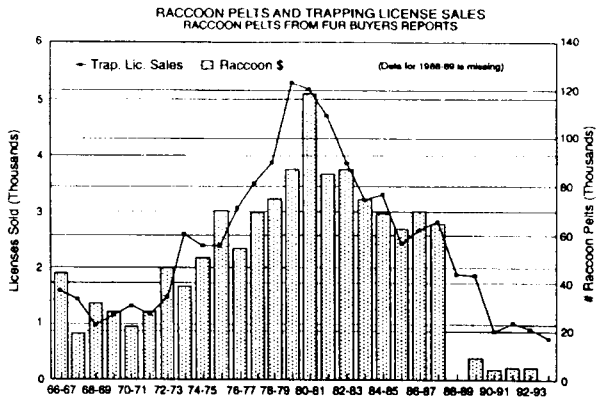



**Figure 10. Decline in trapping activity.**

trapping sentiment by the public at large (Siemer, *et al.*, 1994).

The data reported by Mr. Martin do corroborate the same trend reported by Seimer, et al. (1994) in that trapping activitity has declined significantly in Virginia over the last decade. On a local level, the same trend seems to have prevailed in our study area of the Chesapeake Bay (personal communication from several landowners and former trappers on Cherrystone Inlet).

Studies by Enzinger and Cooper (1976), McCambridge and McMeekin (1980), Anderson *et al.* (1983), and Rhodes and Kator (1988) suggested the microbiota (including plaque-forming microorganisms) in estuarine water were among the primary agents responsible for coliform removal. While sunlight is known to be an effective bacteriocide (Fujioka and Narikawa, 1982), there is evidence to show that *E. coli* can not only live for a long time, but may also reproduce under temperatures normally found in marshes during the summer period. For example, several deer pellets were placed in a shaded spot near a marsh/forest interface in the spring of 1993. Each time the site was visited, a pellet was removed, and a 0.1 gm mass was placed in 100 ml of autoclaved distilled water. For seven months, counts >1600 MPN/100 ml were recorded from those 0.1gm samples. Viability may be longer than seven months, but the supply of pellets was exhausted by then. Kator and Rhodes (1993) discussed the potential for *E. coli* to grow saprophytically in estuarine water. The degree to which *E. coli* density may be enhanced by natural growth in these tidal inlets was not investigated. However, it was determined that, under laboratory conditions*E. coli* can grow normally in the marsh water investigated in this study (James and Simmons, 1995).

Questions concerning the effect of waterfowl also have been rasied in conjunction with this study. Numerous attempts failed to measure a waterfowl signature in or around Cherrystone Inlet and The Gulf. The only positive signature that has been registered to date was in

a series of ponds on Fisherman's Island. Several ponds had been monitored during the summer because of their almost complete isolation from humans, their near isolation from mammals, but their total exposure to birds.

Sampling during the fall indicated a low fecal coliform density. However, the ponds were influenced during November, 1994 by Hurricane Gordon off the North Carolina coast. The Virginia coast was stormy with high winds and high tides, and on that occasion, the ponds were used quite heavily by waterfowl as a refuge. Bird feces were so abundant in the ponds that weekend, the mass was rolled into windrows at the windward ends. Sampling in those areas showed fecal coliform densities >1600 MPN/100ml. However, sampels taken four weeks later from the same ponds showed the fecal coliform density had decreased, and the counts continued to decline to a MPN of 5 in January, 1995. Further studies indicated the fecal coliforms were not "hidden" in the sediments. The data suggested that in order to see a bird signature, there must be a lot of birds crowded into a small area with no flushing or diluting effects due to tidal action.

Figure 11 represents a summary of the geometric means of fecal coliform densities in the four ponds on Fisherman's Island. However, Figure 12 shows that not all ponds have followed the same trend. There is, in general, a bimodal pattern of fecal coliform distribution. The fall pulse can be explained by the arrival of migratory waterfowl and the ponds serving as a refugium during bad weather. However, that doesn't explain why the fecal coliforms declined during the winter months when the bird fauna was as dense as ever. Moreover, as Figure 13 shows, fecal coliforms increased in three of the four ponds in the June, 1995 sampling. Interestingly, the smallest pond showed the largest increase. All ponds have experienced significant loss in volume due to evaporation. There is also much evidence of muskrat activity around the ponds, and the degree to which the mammals, rather than the waterfowl, may be contributing to the fecal coliform densities in the ponds will have to wait for further DNA analyses of pond *E.coli*.

A considerable number of water samples for nutrient chemistry were collected with water samples for fecal coliform determination. There was no correlation found between coliform density and any other water quality property. For example, elevated *E. coli* densities could be found in water that was warm or cold, high or low salinity, nutrient rich or nutrient poor, or oxygen rich or oxygen poor. Low *E. coli* densities could be found under the same range of environmental factors. The one environmental property which seemed to show a high correlation was habitat - proximity to a land/water

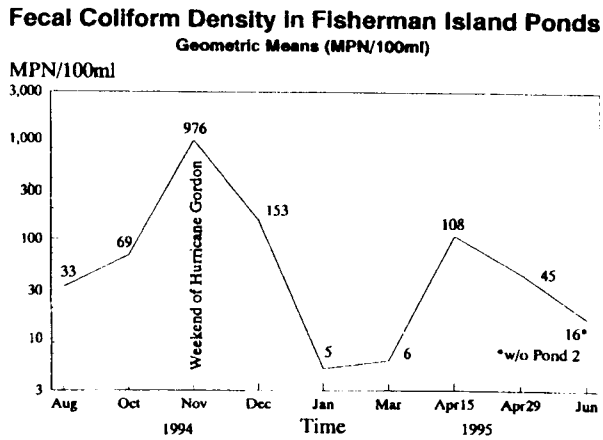



**Figure 11. Geometric mean of ponds.**

interface environment. In the absence of an OSWDS, the effect of wildlife interacting with the aquatic habitat and compromising its quality would have to be considered as a potential source.

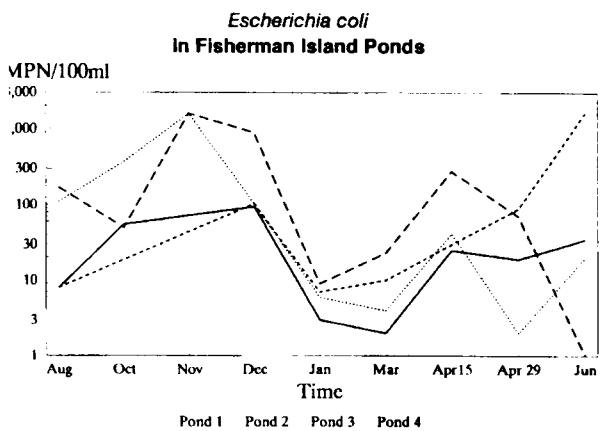



**Figure 12. *E.coli* trends in four ponds.**

Lastly, there is the issue of OSWDS, the original focus of nonpoint sources. Studies by Miles and Simmons (1994) and Touya (1995) showed that if an OSWDS is not overloaded, located a sufficient distance from the water table, and not broken, it will probably function as intended and remove all, or nearly all, of the *E. coli*. This work was conducted in sandy, beach sediments at Assateague National Seashore Park and included conventional, as well as a mounded OSWDS. While the freshwater plume, and sequence of nitrification could be followed in the groundwater, the movement of *E. coli* in groundwater from these systems could not be demonstrated.

## SUMMARY

1. The results obtained to date on Virginia's Eastern Shore suggest the nonpoint source problem of fecal contamination of tidal inlets, bays, and estuaries does have an explanation which, to a large extent, can be

attributed to nonhuman origin. Furthermore, this project demonstrates that techniques and technology are available to resolve and remediate "nonpoint" sources of water quality contamination by fecal coliform bacteria.

2. These nonhuman sources appear to be primarily furbearing animal species, such as raccoons, whose populations have increased over the past several decades due to the lack of predation, or trapping.

3. Reduction of specific wild animal populations resulted in remediation of the fecal coliform densities coming off marshes, protection of one major tidal creek, and the opening of a second.

4. The Eastern Shore provides an excellent habitat to resolve nonpoint source fecal coliform problems. Understanding and remediating such problems on a watershed basis on the Eastern Shore should provide valuable experience in resolving similar problems in areas of the Chesapeake Bay where humans and/or domestic animals may contribute more significantly to the fecal coliform problem.

5. Based on DNA restriction fragment profiles (i.e., genetic fingerprints), there appear to be different *E. coli* strains associated with different animal hosts. These differences provide the opportunity to develop a genetic library of strains to identify potential hosts, through their *E. coli* signature in the water, for purposes of effective wildlife management, domestic husbandry, or for OSWDS repair.

6. These studies suggest migratory waterfowl play an insignificant role in the *E. coli* problem in open, flushed aquatic environments.

7. This study and other studies suggest that On-Site Wastewater Disposal Systems are very effective at removing *E. coli*, provided the OSWDS is not mechanically damaged, or overloaded, and the system is sufficiently elevated above the water table.

## REFERENCES CITED


Allardt-Servent, A, N Bouziges, M-J Carles-Nurit, G Bourg, A Gouby, and M Ramuz. 1989. Use of low-frequency-cleavage restriction endonucleases on DNA analysis in epidemiological investigations of nosocomial bacterial infections. J. Clin. Microbiol. 27:2057-2061.

American Public Health Association, American Water Works Association, and Water Environment Federation. 1992. Standard Methods for the Examination of Water and Wastewater. Eighteenth Edition. American Public Health Association, 1015 Fifteenth Street, NW, Washington, DC 20005, 10 sections.

Anderson, IC, MW Rhodes, and HI Kator. 1983. Seasonal variation in survival of *Escherichia coli* exposed *in situ* in membrane diffusion chambers containing filtered and nonfiltered estuarine water. Appl. and Environ. Microbiol. 45:1877-1883.

Buchrieser, C, VV Gangar, RL Murphree, ML Tamplin, and CW Kaspar. 1995. Multiple *Vibrio vulnificus* strains in oysters as demonstrated by clamped homogenous electric field gel electrophoresis. Appl. and Environ. Microbiol. 61:1163-1167.

Bio-Rad Laboratories. 1994. GenePath® Group 2 Reagent Kit for use with *E. coli* . Instruction Manual. 17pp.

Chen, M. 1988. Pollution of ground water by nutrients and fecal coliforms from lakeshore-septic tank systems. Water, Air and Woil Pollut. 37:407-417.

Cogger, CG, LM Hajjar, CL Moe, and MD Sobsey. 1988. Septic system performance on a coastal barrier island. J. Environ. Qual. 17:401-408.

Del Rosario, L, SS Barth, M Rutter, B Ray. 1995. Molecular Analysis of *Listeria monocytogenes* isolates associated with the consumption of *Listeria* contaminated mixed vegetables. Abstract from 95th General Meeting of ASM, Washington, DC.

Edberg, SC, JE Patterson, and DB Smith. 1994. Differentiation of distribution systems, source water, and clinical coliforms by DNA analysis. J. Clin. Microbiol. 32:139-142.

Enzinger, RM and RC Cooper. 1976. Role of bacteria and protozoa in the removal of *Escherichia coli* from estuarine waters. Appl. and Environ. Microbiol. 31:758-763.

Faust, M. 1976. Coliform bacteria from diffuse sources as a factor in estuarine pollution. Water Research 10:619-627.

Faust, MA, and NM Goff. 1977. Basin size, water flow and land-use effects on fecal coliform pollution from a rural watershed. Watershed Res. II:611-634.

Fujioka, RS and OT Narikawa. 1982. Effect of sunlight on enumeration of indicator bacteria under field conditions. Appl. Environ. Microbiol. 44:395-401.

Geldreich, EE, LC Best, BA Kenner, and DJ vanDonsel. 1968. The bacteriological aspects of stormwater pollution. J. Water Pollut. Control Fed. 40:1861-1872.

Gilliand, MW and W Baxter-Potter. 1989. A geographic information system to predict non-point source pollution potential. Water Resources Bull. 23:281-291.

Goering, R. 1993. Molecular epidemiology of nosocomical infection: Analysis of chromosomal restriction fragment patterns by pulsed-field gel electrophoresis. Infect. Control Hosp. Epidemiol. 14:595-600.

Hayes, S, L Newland, K Morgan, and K Dean. 1990. Septic tank and agricultural non-point source pollution within a rural watershed. Toxicol. Environ. Chem. 26: 137-155.

Hendry, GS, and A Toth. 1982. Some effects of land use on bacteriological water quality in a recreational lake. Water Research 16:105-112.

James, C and GM Simmons, Jr. 1995. Saprophytic growth potential of *Escherichia coli* in estuarine water without nutrient amendments and grazers. Water Resources Research Conference, Richmond, VA. (Abstr.)

Kator, H and M Rhodes. 1993. Comprehensive Literature Review of Indicators in Molluscan Shellfish and Their Growing Waters. In M. Pierson and C. Hackney (editors), Indicators of Shellfish Safety. VanNostrand and Rheinhold, New York, NY (In Press).

Kay, D, JM Fleisher, RL Salmon, F Jones, MD Wyer, AF Godfree, Z Zelenauch-Jacquotte, R Shore. 1994. Predicting likelihood of gastroenteritis from sea bathing: results from randomised exposure. Lancet 334:905-909.

Leonard, DL, MA Broutman, and KE Harkness. 1989. The quality of shellfish growing waters on the east coast of the United States. National Oceanic and Atmospheric Administration. Ocean Assessments Division. Rockville, MD 45 pp.

Levesque, B, P Broussequ, P Simard, E Dewailly, M Meisels, D Ramsay, and J Joly. 1993. Impact of the Ring-Billed gull (*Larus delawarensis*) on the microbiological quality of recreational water. Appl. Environ. Microbiol. 59:1228-1230.

Maslow, JN, AM Slutsky, and RD Arbeit. 1993. Application of Pulsed-Field Gel Electrophoresis to Molecular Epidemiology, pp. 563-572. *In* DH Persing, TF Smith, FC Tenover, and TJ White (eds.), *Diagnostic Molecular Biology Principles and Appplications.* American Society of Microbiologists, Washington, D.C.

McCambridge, J and TA McMeekin. 1980. Relative effects of bacterial and protozoan predators on survival of *Escherichia coli* in estuarine water samples. Appl. and Environ. Microbiol. 40:907-911.

Miles, ET and GM Simmons, Jr. 1994. A comparative study between conventional and elevated sandmound on-site-waste-water-disposal systems in coastal plain sediments. National Park Service. Assateague National Seashore Park, Assateague, VA (Final report for Park Service and basis for Masters Thesis).

Reay, WG, DL Gallagher, and GM Simmons, Jr. 1995. Sediment-water column oxygen and nutrient fluxes in nearshore environments of the lower Delmarva Peninsula, USA. Mar. Ecol. Prog. Ser. 118:215-227.

Reneau, RB, Jr, and DE Pettry. 1975. Movement of coliform bacteria from septic tank effluent through selected coastal plain soils of Virginia. J. Environ. Qual. 4:41-44.

Reneau, RB, Jr, C Hagedorn, and MJ Degen. 1989. Fate and transport of biological and inorganic contaminants from on-site disposal of domestic wastewater. J. Environ. Qual. 18:135-144.

Rhodes MW and H Kator. 1988. Survival of *Escherichia coli* and *Salmonella* spp. in estuarine environments. Appl. Environ. Microbiol. 54:2902-2907.

Schima, FJ, WG Reay, DL Gallagher, GM Simmons, Jr., JL Waldon, and KK Reay. 1994. Groundwater transport of fecal coliform bacteria to open coastal waters of Virginia's coastal plain: a GIS approach. Unpublished final report. 100p.

Siemer, WF, GR Batcheller, RJ Glass, and TL Brown. 1994. Characteristics of trappers and trapping participation in New York. Wildl. Soc. Bull. 22:100-111.

Simmons, GM, Jr. 1993. Potential sources for fecal coliforms in tidal inlets. Interstate Seafood Conference, Proceedings. Ocean City, Maryland, pp 49-67.

Simmons, GM, Jr. 1994. Potential sources for nonpoint introduction of *Escherichia coli (E. coli)* to tidal inlets. Interstate Seafood Conference, Proceedings. Rehobeth Beach, Deleware. (In Press)

Strickland, JDH, and TR Parsons. 1972. A Practical Handbook of Seawater Analysis. Bull. Fish. Res. Bd. Canada. 310 pp.

Touya, EJM. 1995. A comparative study of a convential and a mound on-site wastewater disposal

system in a coastal environment. Master of Science Thesis, Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA. 187p.

U.S. Environmental Protection Agency. 1983. Methods for the chemical analysis of water and wastes. EPA-600/4-79-020.

## ACKNOWLEDGEMENTS

Many individuals contributed to the success of these research efforts either by providing access to their land and specific marshes, through financial contributions, or through the sharing of data. In particular, the following individuals should be noted: Roger Buyrn and The Cherrystone Inlet Preservation Group; Gordon Putnam and Citizens for a Better Eastern Shore; Sherman Stairs and Jim Wood and The Eastern Shore of Virginia National Wildlife Refuge; and, Paul Widgen and Dr. Bob Croonenberghs with the Virginia Department of Health, Division of Shellfish Sanitation. The Virginia Department of Conservation and Recreation, Division of Soil and Water Conservation provided valuable research support and Mr. Charles Lunsford was particularly important in supporting the outcomes of this study. The authors also appreciate the support of the Virginia Water Resources Research Center for its financial support in the development of the Pulsed-Field Gel Electrophoresis genetic library of *E. coli* strains.

Numerous landowners were generous with permission to allow us unlimited access across their property to marshes, inlets, and streams. The US Fish and Wildlife Service allowed access to barrier islands, normally closed to human visitation, for purposes of collecting water samples for comparative study that provided a water quality baseline without human presence or overpopulated animal groups.

Messers. Jeff Mecham and Eduardo Miles Touya were research assistants that contributed valuable field and laboratory assistance. I also benefited from the field assistance of Messers Jake Waller and Dave Loeks.

## BIBLIOGRAPHIES OF AUTHORS

George M. Simmons, Jr. is an Alumni Distinguished Professor and Associate Department Head in the Biology Department at Virginia Tech. His current research interests focus on the ecology of *E. coli* and remediation efforts to restore water quality in Chesapeake Bay tidal inlets.

Sue A. Herbein is the research associate in charge of microbial analyses and DNA restriction fragment studies. She was the Director of the microbiology laboratory program in the Biology Department at Virginia Tech for 11 years.

Carey M. James is completing an undergraduate degree and undergraduate research project in the Biology Department at Virginia Tech and will be pursuing graduate work in the area of molecular ecology after his senior year.