DISTRIBUTION AND CHARACTERIZATION OF *E. coli* WITHIN THE DUNES CREEK WATERSHED, INDIANA DUNES STATE PARK

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I. INTRODUCTION

Fecal coliforms, particularly *Escherichia coli* (*E. coli*), are considered a reliable indicator of recreational water quality (USEPA 1986). The United States Environmental Protection Agency recommends a maximum geometric mean of 126 *E. coli* colony-forming units (CFU)/100 ml water from five samples over a 30-day period for swimming waters; the single sample criteria is set at 235 CFU/100 ml. Regulatory and management agencies generally assume that *E. coli* is generated from warm-blooded animals with special emphasis on human sources. However, it has become increasingly clear that *E. coli* and other fecal indicators such as enterococci can persist and perhaps grow in soil, water, and plants/plant materials, under certain subtropical/tropical, and even temperate conditions (Anderson et al., 1997; Bermudez and Hazen, 1988; Carrillo et al., 1985; Fujioka et al., 1999; Hardina and Fujioka, 1991; Muller et al., 2001; Mundt, 1963; Ott et al., 2001; Solo-Gabriele, 2000; Whitman et al., 2001). Investigators generally ignore non-animal riparian sources generated within the watershed because there is a limited acceptance by the public heath community and regulators for the existence of significant background physical reservoirs of *E. coli*.

While in recent years the effects of animal input from domestic and non-domestic sources have become increasingly realized (e.g., dog, cattle, sheep, birds) (Anderson et al., 1997), the characteristics and distribution of fecal indicators derived from these non-human sources have not been well studied. Research has shown that *E. coli* and enterococci bacteria are ubiquitous and can persist for long periods of time in tropical and sub-tropical soils and water (Fujioka, 1983; Fujioka et al., 1988; Fujioka et al., 1999; Hardina and Fujioka, 1991; Solo-Gabriele, 2000). Circumstantial evidence supports regrowth under certain conditions (Anderson et al., 1997; Byappanahalli and Fujioka, 1998; Desmarais et al., 2002; Fujioka and Byappanahalli, 2001; Solo-Gabriele et al., 2000). Aside from Whitman et al. (2001), few comparable studies addressing the persistence of *E. coli* have been conducted in temperate or boreal lake watersheds, and even fewer for comparable riparian situations. Most *E. coli* source studies within streams focus on exogenous influences (e.g., sewage, feedlot operations, wildlife) since the assumption is that the stream is intrinsically clean.

Indiana Dunes State Park, located along the shore of southern Lake Michigan, is a popular recreational park that attracts thousands of visitors annually, with a significant number of these visits associated with beach and swimming activities. High *E. coli* levels and consequent swimming advisories have been a chronic problem at the park. Concerns over these closures have prompted land managers and regulatory officials to seek the sources of excessive *E. coli*. The immediate source of this bacterium is undoubtedly Dunes Creek, which empties directly onto the park's only swimming beach. Dunes Creek is one of three perennial streams draining and connecting the Great Marsh system and Lake Michigan. While there are no other stream systems within the state park, a significant portion of the subject branch of Dunes Creek arises from rural and suburban lands. Sister streams, Derby and Kintzele Ditches, have also been implicated in pollution of neighboring beaches. Earlier studies suggested similar *E. coli* longitudinal distribution, loadings, and seasonality in these streams (Whitman et al., 1995; Stewart et al., 1997).

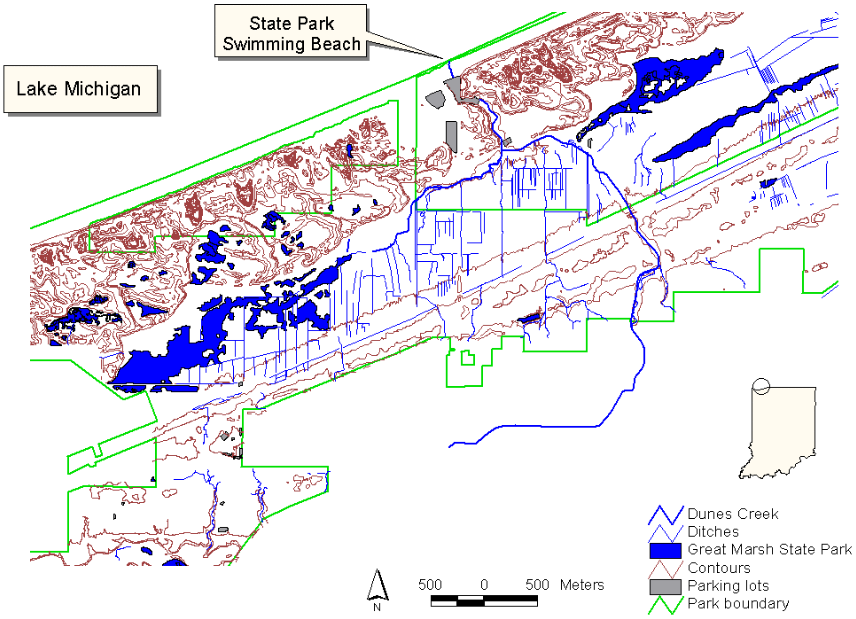
Although there is no direct evidence for point source contamination of these creeks, previous studies on Derby Ditch and Dunes Creek suggested that the bacterial loadings into these creeks were mostly derived from nonpoint source contamination (e.g., wild animals, birds), rather than any significant human fecal input (Whitman et al., 1995). Even though these earlier studies did not specifically address whether soil and sediments along the creeks were potential sources of *E. coli*, they provided significant circumstantial evidence that the persistent and increased concentrations of *E. coli* in the creeks were perhaps due to runoff from soil/sand adjacent to the creeks and/or resuspension of the sediment-borne bacteria. In this study, we characterize the persistence and distribution of *E.* coli within a small northern Indiana sandy stream. We explore the possibility that the ubiquitous occurrence of this bacterium within the subject stream serves as a continual source and reservoir, which compromises assumptions underlying its use as a human/animal waste indicator. We focus on riparian sediment and soil as potential sources of *E. coli* loading and discuss water quality remediation implications of our findings. The specific objectives of this study were to (1) confirm that Dunes Creek significantly increases *E. coli* concentrations in the State Park's beach water, (2) describe the spatial distribution of *E. coli* in Dunes Creek and related watersheds, and (3) determine the physical watershed inputs of *E. coli* into Dunes Creek.

II. MATERIALS AND METHODS

A. Site Description

Most studies were conducted within the watershed of Dunes Creek’s central branch, especially the portion within the park (**Fig. 1 A-C**). The creek is a natural stream that drains wetlands and seeps, but it has historically been ditched extensively to facilitate drainage and municipal development. Creek water is highly colored, circum-neutral, and moderately hard. The riparian zone around the sampling sites is dominated by white oak, although suburban developments, agricultural lands, and train and highway corridors occur upstream, especially along the eastern branch known as Kemper Ditch. Study area soils are mostly sandy with abundant deciduous litter and fine detritus. Riffles and margins are mostly clean to detritus-laden sands interspersed with slack areas and pools with silts grading to deeper sands. During the course of the riparian study, which occurred during the summer of 2001, the stream depth and width ranged from 3 to 10 cm and less than 1m for upstream and 10 to 20 cm and 2 to 3.5 m for downstream portions. Eastern and western branches of the creek converge with the central branch at the park’s campground, then freely flow for about 250 m before entering a 400 m culvert that delivers the waters to a swimming beach on Lake Michigan. The central branch is among the shortest and least disturbed tributaries of Dunes Creek.

(A)



(B)

Figure 1. Study site. (A) Map showing Dunes Creek and its major branches. The study was carried out along the central branch of the creek; the thin blue lines indicate extensive ditching of the wetlands in the watershed. (B) and (C) Pictures showing the downstream and upstream sections of the creek.

B. Annual Monitoring at Beach Outfall

During 1997 to 2001, *E. coli* concentrations were monitored weekly from just east and west of the creek's outfall into Lake Michigan. The samples were analyzed for *E. coli* by the membrane filtration (MF) technique (APHA, 1995; APHA, 1998). In this report, data from only 1999 and 2000 have been used. Sampling was conducted between April and November in 1999 and 2000. Sampling and testing at beach locations were repeated whenever *E. coli* counts exceeded EPA recommended criteria for swimming. Samples were collected in knee-deep (45-cm) water in the morning, placed in a cooler on ice, and analyzed within four hours of collection.

C. Previous Stream Monitoring

Weekly water samples were taken from 14 sites along Dunes Creek in 1999 and 2000 (**Fig. 2**). Sampling occurred from June to November 1999, and February to November 2000. These samples were analyzed for *E. coli* using the MF technique. Various parameters such as stream discharge, dissolved oxygen, and temperature were also measured.

Site 1 – Dunes Creek at lakefront outfall. Samples were collected where the creek exited the cement abutment.

Site 2 – Dunes Creek at parking lot, post- weir. Samples were collected on the downstream side of the weir, where the creek enters the culvert that passes under the parking lot.

Site 3 – Dunes Creek at parking lot, pre-weir. Samples were collected 15 m upstream of the weir over which the creek flows.

Site 4 – Dunes Creek at Trail 2. This low-flow site is located about 5 m from the campground.

Site 5 – Dunes Creek at confluence. Samples were collected 6 m downstream from the actual confluence of the east and west branches.

Site 6 – Dunes Creek west branch near confluence. Samples were collected from the south side of the bridge on the road, which leads towards the campground.

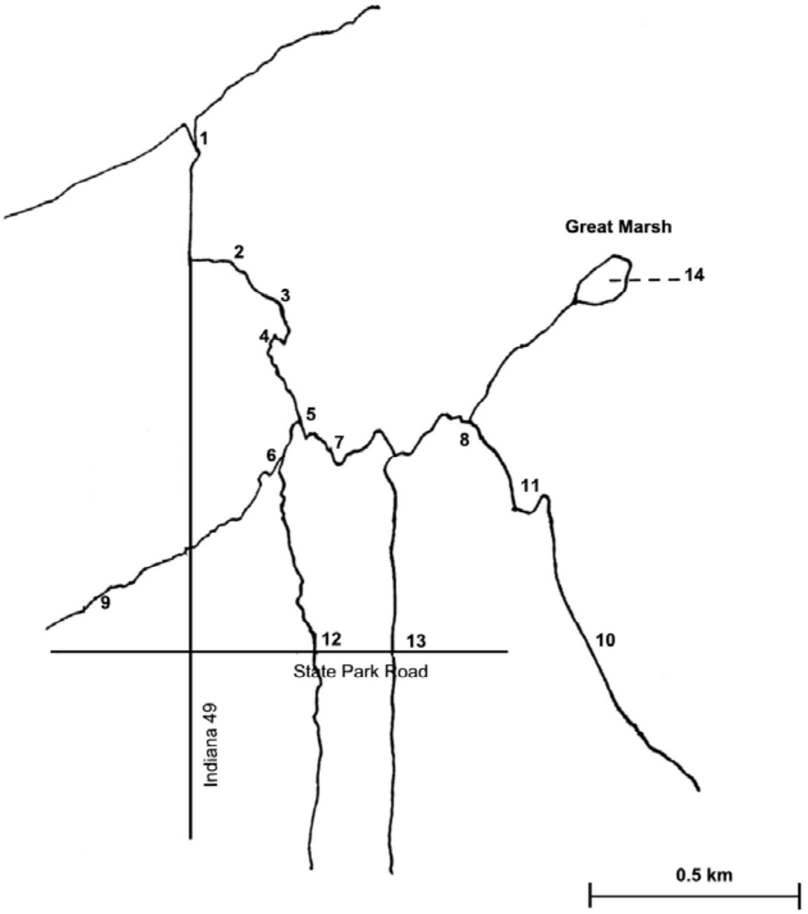


Figure 2. Map of 14 sampling sites chosen along the Dunes Creek watershed for monitoring weekly *E. coli* counts during 1999 and 2000. Sampling occurred from June to November (1999) and February to November (2000).

Site 7 – Dunes Creek east branch near confluence. As with site #6, the samples were collected from the south side of the bridge. Sediment was mostly silt and clay.

Site 8 – Dunes Creek east branch at the Great Marsh. This site was accessed from the road adjacent to the Wilson Shelter. Floating plants blanketed the sampling site.

Site 9 – Dunes Creek west branch at Waverly Road. Samples were collected from the eastern side of Waverly Road.

Site 10 – Dunes Creek east branch at Calumet Bike Trail. Samples were collected approximately 3 m downstream from the drainage pipes that direct the creek beneath the Calumet Bike Trail.

Site 11 – Dunes Creek east branch at Wilson Shelter. The sampling site was in close proximity to two pit toilets.

Site 12 – Dunes Creek west branch at State Park Road east of Route 49. Samples were collected on the north side of the road, approximately 6 m downstream from the bridge.

Site 13 – Dunes Creek east branch at State Park Road and western road. The creek was sampled on the north side of State Park Road approximately 3 m downstream from the bridge.

Site 14 – Occurs within the Great Marsh proper at Marsh Bridge along the park's Trail #8.

D. Lateral and Longitudinal Distribution

A series of sampling efforts that included both systematic and random sampling were used in this study to determine the potential sources of *E. coli* in the Dunes Creek watershed between June and August 2001. A preliminary, systematic sampling of sand and sediments was conducted along five pre-selected transects perpendicular to the creek, covering an area of 20 m by 32 m; samples were collected at 0 (creek center) and at 1, 2, 4, 8, 16 and 32 m from the creek's margin. Fifteen transects perpendicular to the stream were then randomly selected. At each transect, creek water, submerged sediment, margin sediment, and soil samples at 1 and 4 m were taken along the center branch of Dunes Creek.

1. Lateral studies

Five equally spaced transects were selected at a site approximately 750 m upstream from the Dunes Creek outfall to Lake Michigan, heretofore called the Nissaki 1 site. The selected location at the Nissaki 1 site measured 20 m (length) by 32 m (lateral distance that extended into the adjacent riparian forests). The initial transect was randomly selected, and the remaining four transects were equally spaced at 5 m intervals and were perpendicular to the stream and ran southward. Sediment, bank sand, and forest soil samples were taken from the middle of the stream, at the stream's margin and 1, 2, 4, 8, 16, and 32 m inland. Within 8 cm of each selected sampling point, 5 sub-samples (about 2 ml each) were taken using a sterile, liquid-medicine dispenser. The subsamples were mixed thoroughly, and a portion of the samples was later used for further analysis. Special care was taken to avoid collection of overlying stream water when submerged sediments were collected. Large litter and twigs were carefully removed before forest soils were sampled. Sampling points were carefully inspected for animal droppings and suspect areas were avoided. Sampling substrate depth did not exceed 6 cm. Three water samples, one each between transects 1 and 2, 2 and 3, and 4 and 5, were collected during this time. The samples were kept in a cooler on ice and analyzed within 4 hours of sampling. All samples were analyzed using the IDEXX Colilert-18 (Westbrook, Maine) system, which is based on a defined substrate technology (Edberg et al., 1990; Edberg et al., 1991). *E. coli* was elutriated from sediments and soils as detailed below. Air, sediment, and water temperature were determined in the field using a digital thermometer. Sediment moisture content was determined in the laboratory by gravimetric method.

2. Longitudinal studies

Fifteen randomly selected transects along the 800 m central tributary were selected using ArcView GIS program (version 3.2) (Environmental Systems Research Institute Inc., Redlands, CA). These points were located in the field using a Geographic Positioning System (GPS III Plus, Garmin International, Olathe, KS). The sample collection techniques were generally similar to the approach used in the lateral studies; however, there was slight difference in the approach in which the samples were collected within each transect. Lateral studies had demonstrated that soils beyond 4 m from shore were generally unpredictable in terms of *E. coli* distribution, as well as numbers. Consequently, in this phase of the study, soil samples were not collected beyond 4 m from the stream margin. Water and sediment/sand samples were collected from mid-stream, margin, 0.25, 1, and 4 m landward and perpendicular of the stream. Stream water, sediment, and air temperature were recorded at each location. Samples were analyzed for *E. coli* within 6 h after collection.

E. Reference Sources Surveys

We surveyed the following source waters and associated sediments of Dunes Creek: 1) seep water and sediments mostly above and below the park's campground and lateral ditch of the Nissaki 1 site; 2) artesian spring, marginal sediment, and streamlet in the Beverly Shores area between Beverly Drive and US 20; 3) artesian springs, surrounding sediments, and associated seeps along a secondary branch of Dunes Creek near Wilson Shelter. Also sampled during these surveys were 1) artesian well and associated sediments in Warren Woods State Park (Sawyer, Michigan) 100 meters from the margin and high above the floodplain of the St. Joseph River; 2) spring water about 500 meters from the St. Joseph River; and 3) submerged sediment, water, and marginal sands along Painterville Creek, a small coastal stream within Warren Dunes State Park, Michigan that was very similar to Dunes Creek in land use and limnology.

F. Laboratory Analyses

Water, sand, sediment, and soil samples were analyzed for *E. coli* using the Colilert-18 method. To analyze water samples, volumes ranging from 0.01 to 100 ml were used for the Colilert assay. For analyzing sand, sediment, and soil samples, an initial elutriation step was necessary to release the bound bacterial cells. Briefly, 5 g of the homogenized sample (sand, sediment, or soil) was weighed into a 50-ml centrifuge tube, to which 35 ml of sterile distilled water was added. The mixture was shaken on a vortex mixer for 2 minutes. After standing for 2 minutes, the suspension was then serially diluted. Sample volumes ranging from 0.001 to 10 ml were used for enumerating *E. coli* in the same approach as the water samples. For the determination of moisture content of sand and other sediment samples, about 10 g of each fresh sample was placed in a 100°C dry oven for 24 h, and the weight differential was recorded. The moisture content was calculated using the formula weight of fresh sample-weight of dry sample/weight of dry sample X 100.

The general protocol for *E. coli* identification in this study was as follows: (1) growth from wells that fluoresced (upon exposure to long-wavelength UV light) was initially swabbed on m TEC agar; plates were incubated at 44.5°C for 22 h, and yellow or yellow-brown colonies that developed on the membrane were confirmed for *E. coli* by the substrate test (urease activity) (APHA, 1998), (2) well-isolated colonies from the m TEC plates (at least two per plate) were further streaked for primary isolation on MacConkey agar, followed by a secondary isolation on the same medium, (3) at least two colonies from each MacConkey plate were again confirmed for their β-glucuronidase activity, which is a positive test for *E. coli* identification, by growth on nutrient agar with MUG, and subsequently these were stored in tryptic soy broth for later use, and (4) finally, the isolates that were presumed to be *E. coli* were speciated using the BBL crystal identification scheme (Becton Dickinson Microbiology Systems, Sparks, MD). For quality control and quality assurance (QA/QC), a standard isolate (e.g., *E. coli* ATCC 25922) was included in the identification protocol.

G. Antimicrobial Sensitivity Assay

The use of multiple antibiotic resistant (MAR) patterns has been shown to be a promising tool for identifying human and non-human sources of *E. coli*. The assay is based on the notion that *E. coli* isolates from human waste should show patterns of resistance to antibiotics that are normally used to fight infections in humans. Likewise, *E. coli* derived from domestic animals should possess resistance against antibiotics that are commonly used in animal husbandry. On the other hand, *E. coli* derived from ambient sources (soil, water) and wildlife are generally not exposed to antibiotics; consequently, such isolates are expected to be susceptible to most antimicrobial agents in contrast to human/domestic animal strains. Researchers working in this area have demonstrated that a large number of isolates from various sources (e.g., human, domesticated animals, wild animals) have to be analyzed to establish a database that consists of clear and distinguishable patterns to reflect sources (Hagedorn, et al., 1999;

Harwood et al., 2000). Once such a database is available, isolates from environmental waters can be analyzed to determine the source of fecal contamination. Nevertheless, such an approach is tedious, expensive, and requires a periodic update of the database for proper source identification.

Since the focus of this study did not relate directly to the above issues, a small study was undertaken to find out the relative abundance of susceptible/resistant strains of *E. coli* in Dunes Creek to certain selected antibiotics (that are commonly used in humans and animals for clinical and/or subtherapeutic purposes) using the disk diffusion method, which is also known as the Kirby-Bauer test (Bauer et al., 1966). In brief, the experimental protocol was as follows. Pure cultures of *E. coli*, originally isolated from the creek, were grown overnight on tryptic soy agar; three to four well-isolated colonies from each plate were aseptically transferred to a tube containing 2 ml of saline to prepare the cell suspension. Using a sterile cotton swab, a bacterial lawn was prepared on the surface of Mueller Hinton agar plate by gently rolling the swab three times, turning the plate 60° between swabbing to obtain even inoculation. Within 15 minutes after preparing the plates, selected antibiotic disks were applied on the agar surface using a disc dispenser, and the plates were incubated at 35°C. After 16-18 h of incubation, the plates were examined to record the zones of inhibition to determine whether the isolates were susceptible, intermediate, or resistant to the antibiotics used in this study.

H. Statistical Analyses

Data were maintained on Excel® spreadsheets and are attached in the Appendices and electronically on diskette. Statistical analysis and graphics preparation were performed using SPSS Version 10.01. Statistical significance level was set at $\alpha \leq 0.05$ unless otherwise stated.

III. RESULTS

A. *E. coli* Counts at Dunes Creek Outlet

Dunes Creek opens directly onto the State Park's only swimming beach. Weekly water samples from bathing areas immediately to the east and west of the creek outfall are generally monitored for *E. coli* from Memorial Day to Labor Day. Signs are posted when the counts exceeded 235 CFU/100 ml. The samples analyzed during 1999-2000 clearly demonstrated that *E. coli* concentrations in Dunes Creek were significantly correlated with that of the park's beach water (p<0.0001, r=0.520, n=139; **Fig. 3**). These results provided ample evidence that Dunes Creek was directly impacting the quality of bathing water at the State Park beach.

*Color due to tannin-rich wetland inputs

Correlation of *E. coli* Spearman's rho	r Significance
East Side vs. West Side	0.291 <0.0001
East Side vs. Dunes Creek	0.403 <0.0001
West Side vs. Dunes Creek	0.319 <0.0001

Figure 3. The effect of Dunes Creek on water quality at the Indiana Dunes State Park beach: correlation matrix of *E. coli* counts between the creek near Lake Michigan outfall and in east and west beach areas.

B. Annual Stream Monitoring

The mean *E. coli* concentrations in Dunes Creek tended to be higher during warmer months, peaking in late summer (**Fig. 4 A-B**). During cooler months, *E. coli* concentrations were lower; however, *E. coli* numbers did not fall below 1.5 log units, indicating that the creek was a perennial source of *E. coli*. These data further demonstrated that variations in *E. coli* numbers were substantially high both within and between years. For instance, the overall mean and standard error during 1999 was 1,061 CFU/100 ml ± 65 compared to 700/100 ml ± 63 for comparable periods during 2000. The Levene's test of equality of log-transformed variance between years failed to reject the assumption that the variances were equal ($p=0.884$) while Kolmogorov-Smirnov test showed that log-transformed *E. coli* data were normally distributed for 2000 ($p=0.163$) but not 1999 ($p=0.035$).

Over the course of the two-year monitoring study, only stream samples from station 8 were significantly lower in *E. coli* numbers. This location immediately drains a wetland area of the Great Marsh complex. Site 14, which is 200 m upgradient of Site 8 and located in the Great Marsh, had *E. coli* counts that were significantly lower than the creek ($p<0.05$) (Table 1). In general, *E. coli* counts tended to increase downstream. Mean *E. coli* at Sites 1-7 (downstream) was 921 CFU/100 ml compared to 486 CFU/100ml for Sites 8-14 (upstream).

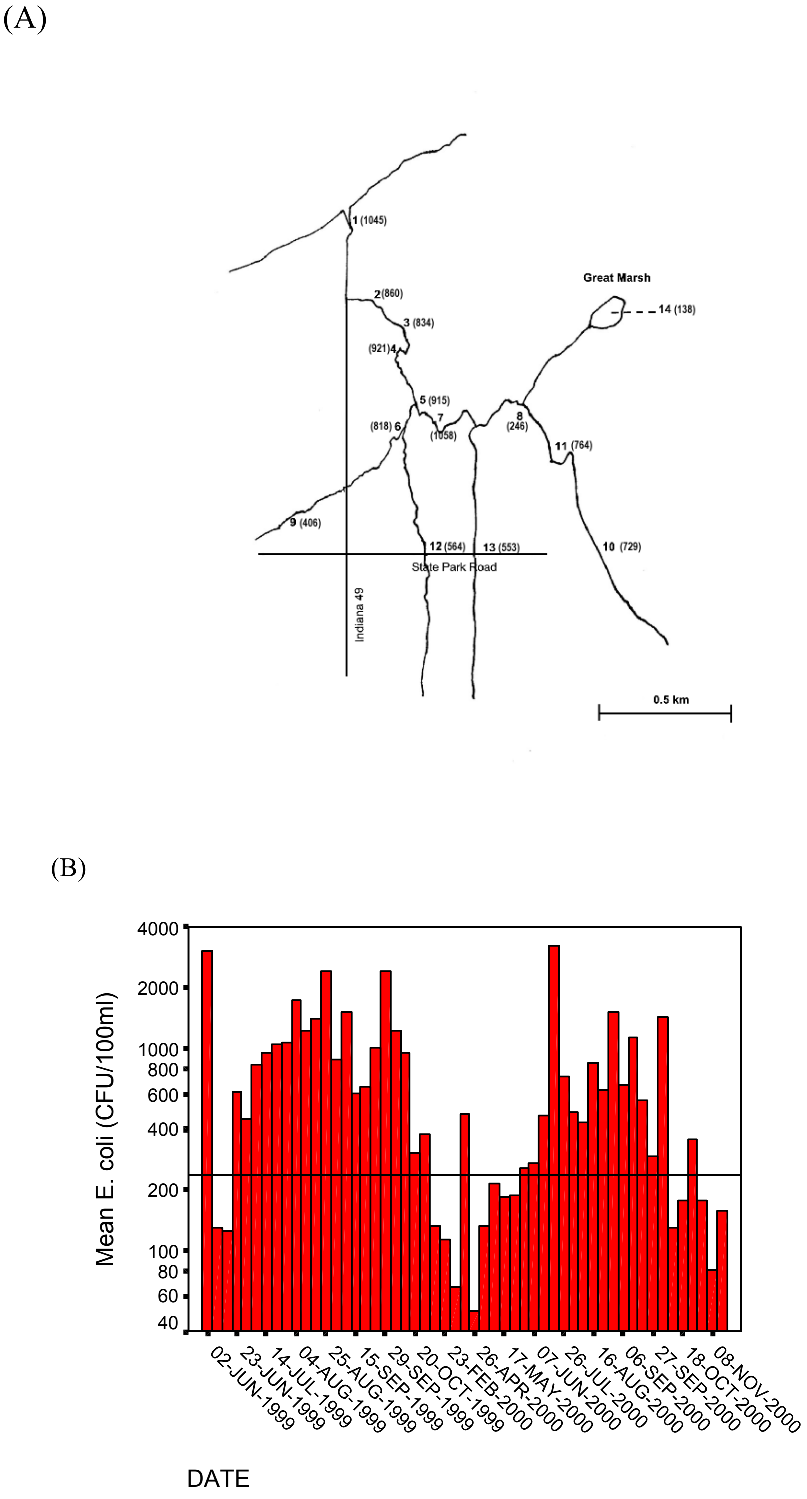


Figure 4. ***E. coli*** **distributions (CFU/100 ml) at the 14 locations monitored along the Dunes Creek during 1999 and 2000. (A) Overall mean densities (numbers in parentheses) at the 14 locations. (B) Mean** ***E. coli*** **counts for all stations on each date sampled.**

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	51.546	13	3.965	13.807	0.000
Within Groups	196.998	686	0.287		
Total	248.544	699			

Tukey HSD

	N	Subset for alpha = 0.05		
STATION		1	2	3
14	38	1.6597		
8	36	1.9514		
9	32		2.3454	
13	53		2.5141	2.5141
12	53		2.5284	2.5284
11	52		2.5363	2.5363
10	59		2.6169	2.6169
5	52		2.6335	2.6335
6	52		2.6643	2.6643
7	52		2.6687	2.6687
3	52		2.6770	2.6770
2	52		2.6892	2.6892
1	58		2.6916	2.6916
4	59			2.7446
Sig.		0.293	0.085	0.691

Table 1. ANOVA table of log-transformed mean *E. coli* counts for each of 14 sites sampled for two years. A post-hoc Tukey's test indicates that sites 8 and 14 are significantly different from the other sites sampled.

C. Lateral Riparian *E. coli* Distribution

A preliminary sampling of Dunes Creek water, underlying sediments, and exposed sands at 1, 2, 4, 8, 16, and 32 m from the stream margin was conducted to determine the relative distribution of *E. coli* within a small section (Nissaki 1) of the study area. The results indicated that there was a significant difference in sand *E. coli* numbers as a function of distance from the creek (**Fig. 5**). Counts were significantly higher in submerged sediments ($p \leq 0.05$), trending lower farther from the streambed. Variations in *E. coli* counts increased with distance from streambed. In general, *E. coli* counts decreased rapidly away from the stream. Although the sand moisture content accounted for only 25% ($r^2=0.25$) of *E. coli* variations, the data indicated that other factors, in addition to moisture, were probably controlling *E. coli* numbers.

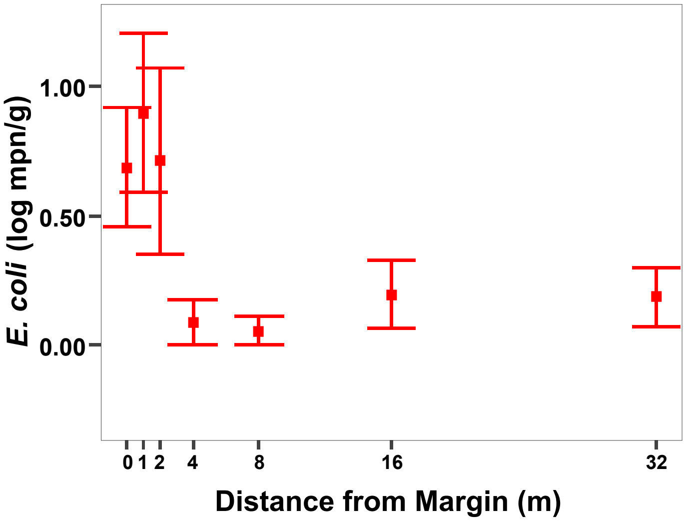
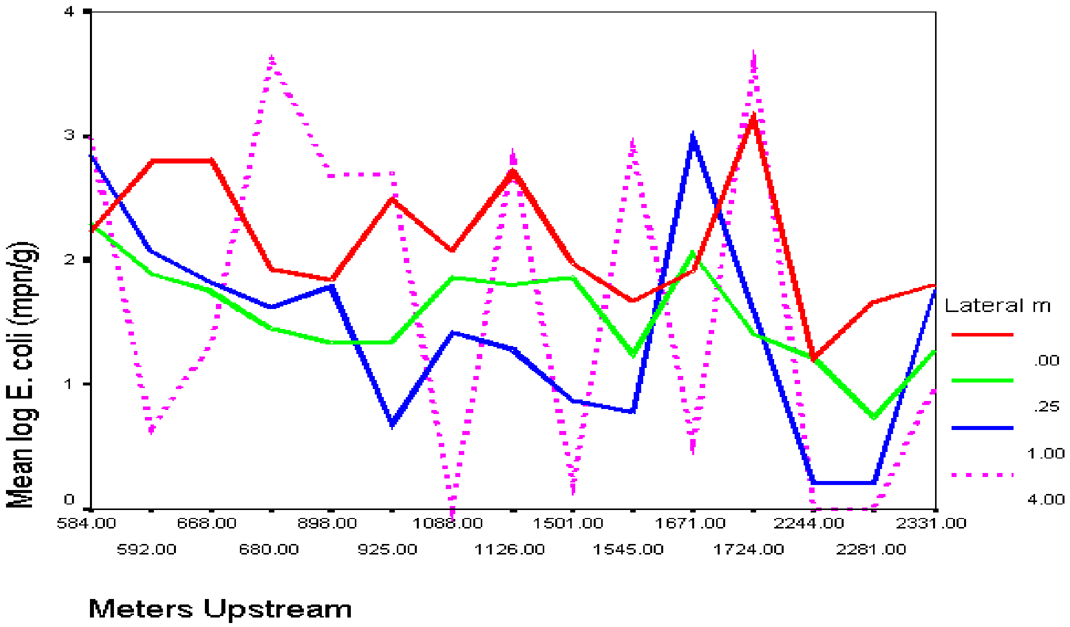


Figure 5. Lateral *E. coli* distributions (from five transects) in sediments as a function of distance from creek's margin.

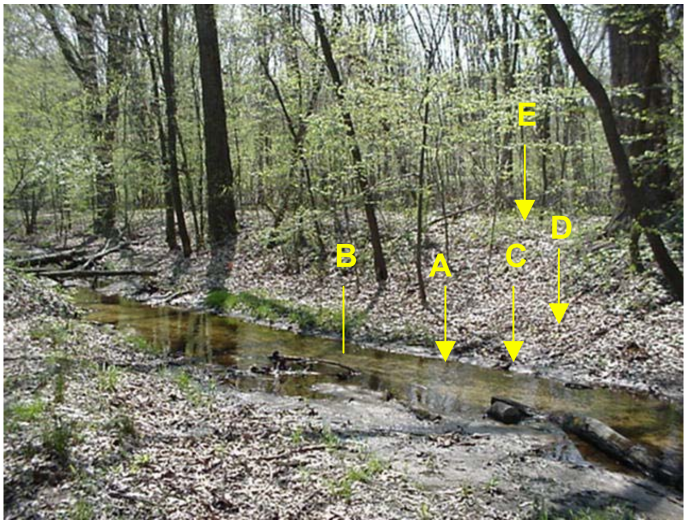
D. Lateral Basin *E. coli* Distribution

Having determined from a previous study that *E. coli* was consistently found in submerged sediments and exposed sand (up to 4 m from margin) within a small section of Dunes Creek, the next objective was to extend this study to the center branch of the creek. Samples of surface water, submerged sands, and exposed sands (at 0.25, 1 and 4 m landward) were collected from 15 randomly selected points along the branch and assayed for concentrations of *E. coli*. The median concentration of *E. coli* in water was 1,089 MPN/100 ml, whereas submerged and exposed sands at 0.25, 1, and 4 m from the margin contained 71, 28, 21, and 13 MPN/g. Stream sediments had higher concentrations than exposed sands ($p \leq 0.05$). *E. coli* counts in overlying water and submerged sands were correlated ($p \leq 0.05$), as were margin and sands 1 m inland ($p \leq 0.01$) (**Fig. 6 A-B**). These studies clearly indicated that *E. coli* could persist in stream waters and adjacent riparian sands, especially along wetted margins.

(A)



(B)



Stream Water-A
Stream Sand-B
Margin Sand-C
Sand @ 1 m from margin-D
Soil @ 4 m from margin-E

Figure 6. (A-B) Lateral *E. coli* distributions from 15 randomly selected transects along the central branch of Dunes Creek.

In studies extended to other habitats, particularly the sediments along seeps and springs, it was found that water samples issuing from artesian wells and springs were generally lower in *E. coli* concentrations than sediments surrounding seeps and spring/creek banks (**Fig. 7**). The mean and range of *E. coli* counts from these samples are summarized in Appendix 5. These results were consistent with other data that indicated persistence of *E. coli* in sediments, particularly along wetted margins of creeks in the riparian system.

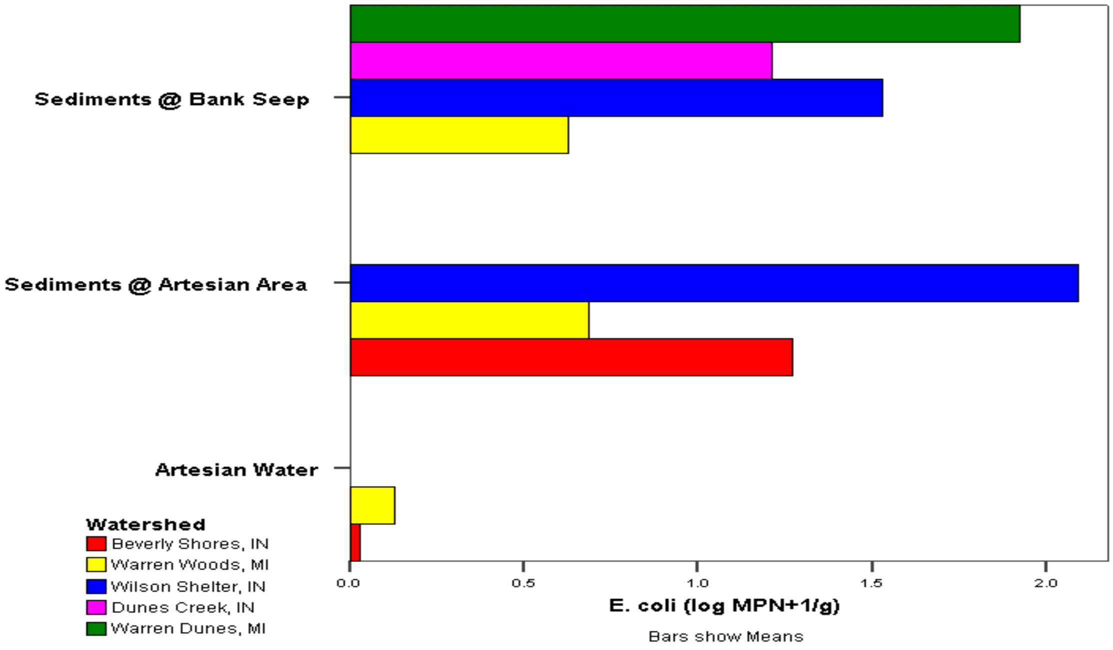


Figure 7. Concentrations of *E. coli* in water and sediment samples collected from springs and seeps of Dunes Creek (Indiana) and Berrien County (Michigan) watersheds.

E. *E. coli* Identification and Confirmation

The presumptive *E. coli* isolates collected from soil, sediment, sand, and water samples were identified and speciated using the BBL crystal identification scheme. A total of 405 isolates were collected over a period of six months. Of the 405 presumptive isolates, 182 were tested for positive identification using the BBL crystal identification scheme; 168 (92%) isolates were typed as *E. coli,* and the remaining 14 (8%) were not identified due to ambiguous test reactions (data not shown). These results clearly indicate that the riparian soil, creek water, and sediments, as well as sand in the Dunes Creek watershed harbor *E. coli,* and the persistently elevated counts in the water are perhaps due to the washings of the sediment-borne organisms to the water.

F. Antibiotic Resistance Testing of *E. coli*

Of the 444 *E. coli* antibiotic-isolate combinations examined, 317 (71.4%) were fully susceptible to the antibiotics examined, 80 (18%) isolates showed resistance (mainly to penicillin and erythromycin), and 47 (10.6%) isolates displayed intermediate resistance (mainly streptomycin) (Figure 8). Surprisingly, little resistance was found in antibiotics commonly used in human medicine and thus generally associated with domestic sewage (e.g., ampicillin, amoxicillin, tetracycline, and sulfamethoxazole). While penicillin and erythromycin are included in the antibiotic resistance surveys, they are generally ineffective at killing or inhibiting gram-negative bacteria such as *E. coli*. When these two antibiotics were excluded from consideration, the percentage of *E. coli* showing complete susceptibility rose to 85.7% compared to 2.2% of the isolates displaying resistance. Intermediate resistance remained stable at 12.2%.

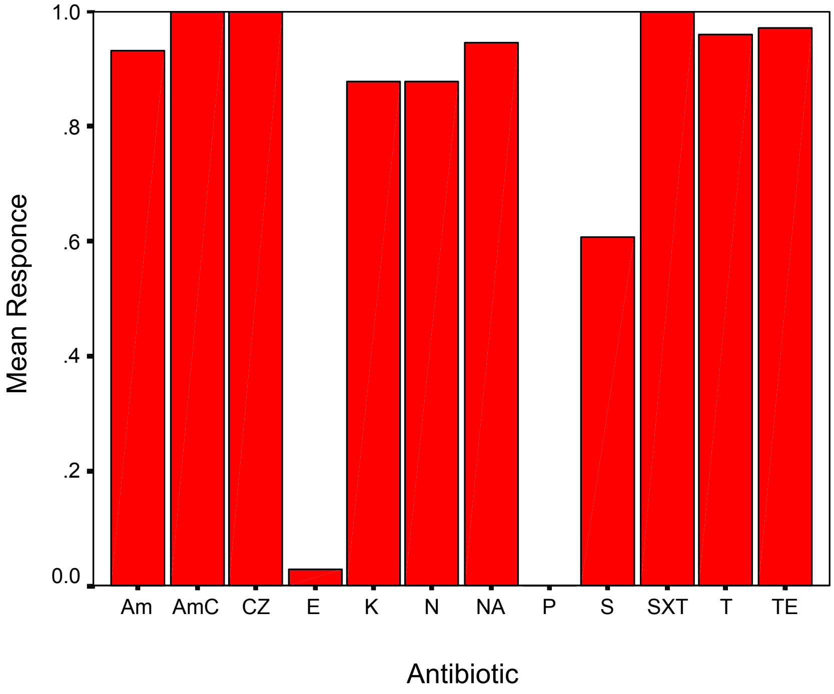


Figure 8. Response of *E. coli* isolates (37), obtained from Dunes creek, to various antimicrobial agents used in humans and animals.

IV. DISCUSSION

With existing evidence that most of the *E. coli* in the Dunes Creek watershed was from nonpoint sources (Whitman et al., 1995), we focused the study on other non-point sources contributing *E. coli* to the creek. An intensive survey of the main branch of Dunes Creek was undertaken to determine whether *E. coli* could occur in certain ambient environments. This was accomplished by analyzing samples—creek water, underlying sediments, exposed sands, and riparian soils—collected from 15 randomly selected locations along the creek's main branch. The results were consistent with our previous findings that indicated high concentrations of *E. coli* in stream sediments and exposed bank sands to 4 m landward. These results provided ample evidence that *E. coli* could persist in creek sediments and adjacent riparian sands, particularly along wetted margins.

Although *E. coli* counts in the riparian forest soils were highly variable (for instance, often less than one to several thousand organisms per g dry weight), the results were not surprising since microbial counts in terrestrial environments vary greatly between and within a sampling location. High population patchiness makes estimation of mean densities of *E. coli* difficult. Nonetheless, our findings suggested that increased *E. coli* counts in Dunes Creek are mostly attributable to contributions from nonpoint sources such as submerged sediments by re-suspension of bound bacterial cells. These results are consistent with those found by Gary and Adams (1985), Hardina and Fujioka (1991), and Solo-Gabriele et al (2000).

The current findings are very significant because, unlike studies from tropical areas that have reported the occurrence of *E. coli* and other indicator bacteria in natural habitats (Byappanahalli, 2000; Fujioka et al., 1988; Solo-Gabriele et al., 2000), there are few comparable studies addressing the persistence and possible growth of *E. coli* within temperate or boreal lake watersheds and even fewer for comparable riparian situations aside from Whitman et al. (2001). Further, most *E. coli* source studies within streams, lakes, and rivers generally focus on allochthonous influences (e.g., sewage, feedlot operations, wildlife) (Patrick et al., 1992) since the assumption is that these water bodies are intrinsically clean. Extensive studies conducted in Dunes Creek and related watersheds over the last 8-10 years have clearly shown that the elevated *E. coli* counts in the creek are due to nonpoint contamination rather than any sewage input (Whitman et al., 1995; 1999). The current study provides support to the previous observations.

Even though previous Dunes Creek studies (Whitman et al., 1995; 1999) suggested nonpoint source contamination (wild animals, stream water, sediments, and riparian soils) as the primary reason for increased *E. coli* levels in the Dunes Creek watershed, other indirect contributions have received little attention. One important factor that needs recognition is the human impact, more specifically, extensive manipulation through ditching of the Great Marsh system. Over the years, the Great Marsh has been ditched extensively to drain the wetlands and accommodate urban and municipal development. Draining the wetlands, which in turn feed the creeks that eventually empty into Lake Michigan, has modified the creeks in the watersheds extensively. For instance, Dunes Creek, which used to be a small, natural stream, now has extensive drainage connections as a result of the massive ditching of wetlands. This has led to an increase in complexity of the Dunes Creek stream order (a term used to define the hierarchy of streams and rivers; a higher order stream/river will have more confluences (e.g., primary, secondary, tertiary orders, **Fig. 9**). The relationship between sediment loading, salinity, and stream order is well known (Leopold et al., 1964). Extensive ditching of the wetlands has increased the stream order of Dunes Creek, which in turn has presumably increased the *E. coli* loadings to the creek (**Fig. 10 A-B**).

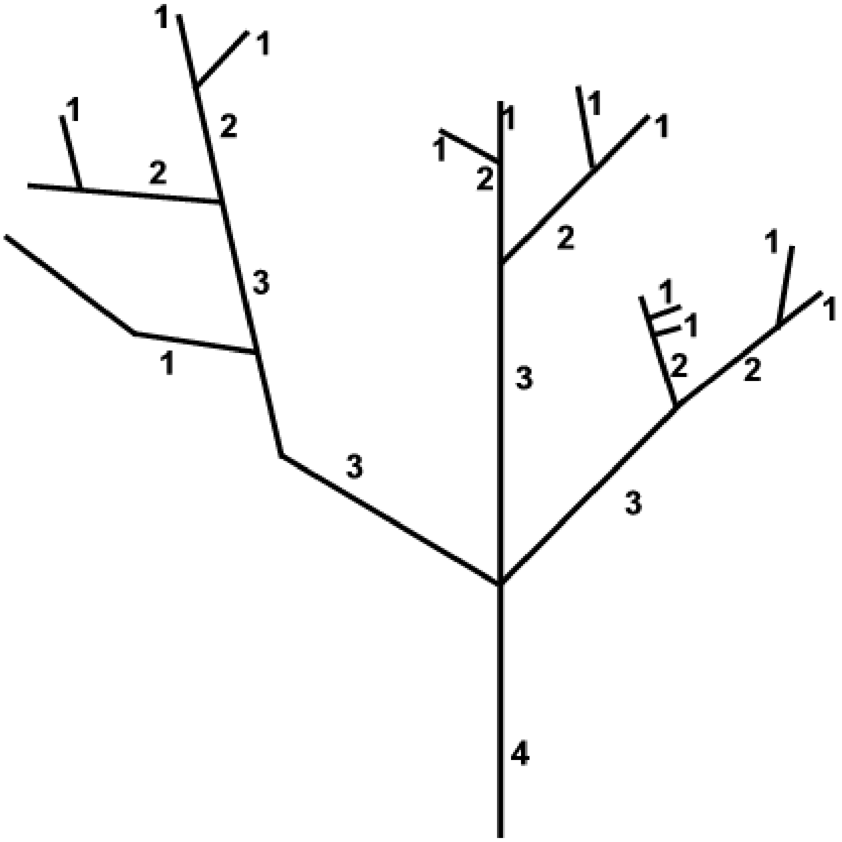


Figure 9. Illustration of stream order concept. Stream order increases only where equal or greater ordered branches meet.

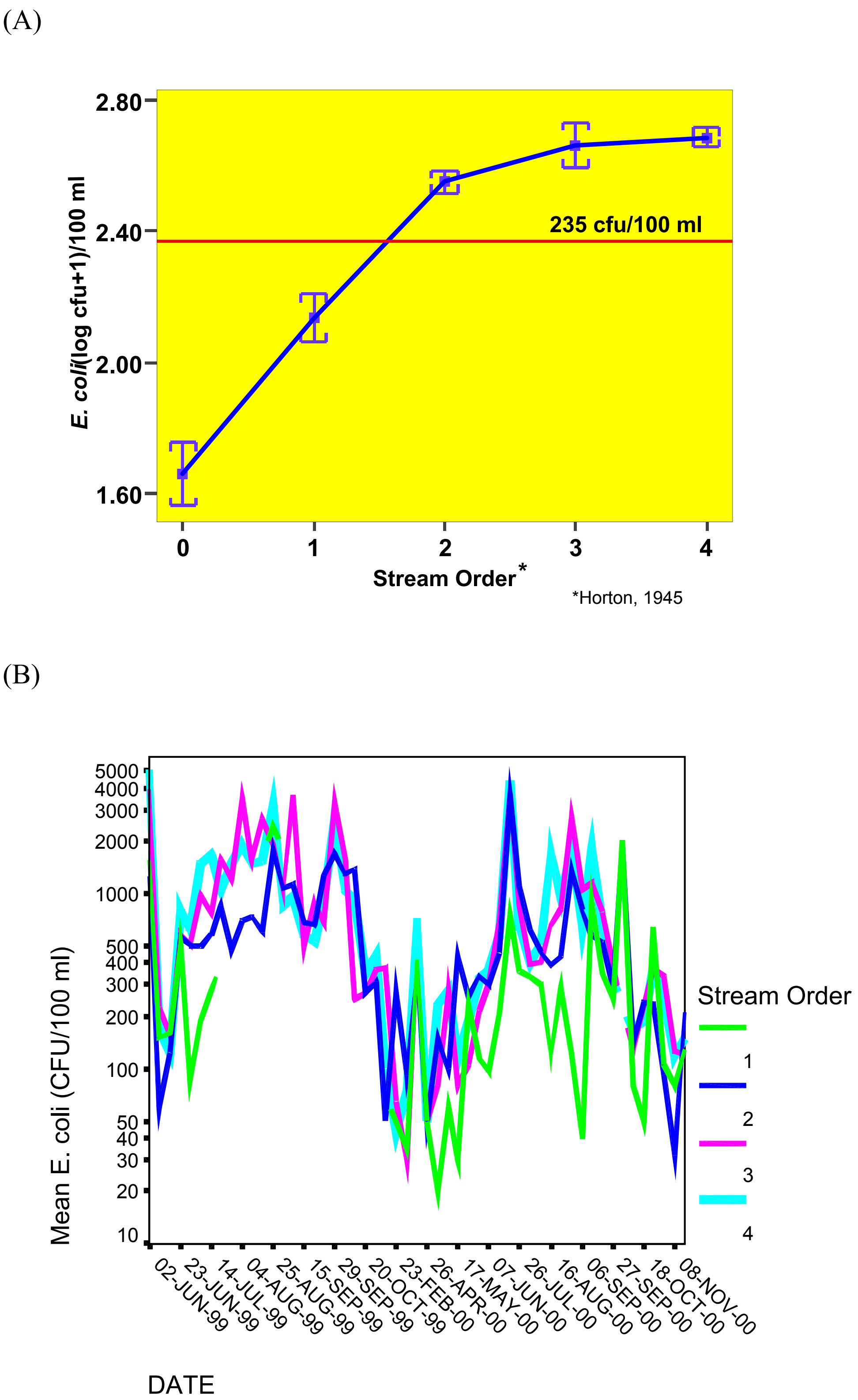


Figure 10. (A-B). Mean *E. coli* concentrations in Dunes Creek during 1999-2000 by stream order.

Most streams within the Midwest carry substantial quantities of *E. coli* relative to currently accepted federal guidelines for swimmable waters. The tendency to relate *E. coli* occurrences to these guidelines is unfortunate, since most of these streams, ditches, and creeks are not used for swimming and are influenced largely by non-point factors. Regulators and sanitarians most often look for point source and human-derived sources of fecal indicators when seeking the source of contamination. This bias can lead to frustration in conducting sanitary surveys because point sources may be elusive when *E. coli* is ubiquitous within a particular drainage. The integrated nature of streams and its associated drainage area is more complex than water flowing freely from source to destination. The contributors to *E. coli* loadings are complex; separating factors such as groundwater, land usage, runoff, riparian soils, microflora, nutrients, fauna, vegetation, and associated ecosystem processes, that are closely tied is a daunting task. While every stream system is unique, all share several characteristics. The tendency of organic material, salts, and microbial load to increase downstream is just one such generality. Past disturbance can cause environmental perturbations and exaggerations of these limnological processes. Over time, the stream will regain equilibrium, but often it is too slow for the beneficial uses sought. Such is the case for the extensive ditching that took place in the Dunes Creek watershed. Inadvertent increases in microbial input, more specifically *E. coli* loadings, via riparian birds and mammals, long-term bacteria-laden sediment loadings, loss of wetland functioning, and hydrological disturbance may need to be addressed most suitably by remediation. Even with complete remediation, there will always be a natural background load of *E. coli* in Dunes Creek.

V. SUMMARY

There is little doubt that Dunes Creek is the major source of *E. coli* at the State Park beach and consequently the main reason for chronic closures. The relationship is undoubtedly related to wind direction and stream discharge. Comparisons to Derby Ditch *E. coli* patterns show strong similarities. Both demonstrate a generalized occurrence of *E. coli* throughout the watershed and suggest non-point source origins. This is not surprising since the two creeks drain the same general watershed and have similar land use and hydrology. The similarity in *E. coli* distribution patterns is further evidence of the non-point, natural *E. coli* loading of the respective streams. Sampling of Painterville Creek, Warren Dunes State Park, Michigan suggests a patterns of bacteria distribution similar to Dunes Creek as well (Fig. 7).

A more complex and uncertain issue is the public health implication of the *E. coli* loadings onto the State Park beach. Point source pollution arising from sewage has been clearly linked to health effects, particularly gastrointestinal illness. There are few well-documented parallel data supporting non-point source health effects. That is not to say that the risks are minimal, but they are likely far less than those posed by human sewage. A good epidemiological study would help confirm this, and efforts to coordinate such a study with EPA and CDC would be useful.

This study investigated the distribution of *E. coli* at both a fine and coarse level within the Dunes Creek watershed in order to understand more fully the nature and source of the bacteria input and fate. The study revealed that the bacteria mostly occurred in submerged and marginal sediments and that these particles were likely the transport medium from source to sink. The likely source thus was forest sediments, whether they arose from ditches, depressions, or forest soils. There was a close association between the stream and bacteria occurrence, and suspected bacteria were linked to submerged sands, marginal sediments, and bank occurrence. The relationship between basin *E. coli* and forest soils was less statistically clear even though *E. coli* was often quite high in these soils. The unpredictable nature of soil *E. coli* made it difficult to relate the aquatic and terrestrial occurrence of the bacteria.

Seasonal distribution of *E. coli* was not as pronounced as was previously suspected. It was believed that *E. coli* levels in winter were universally low. There were many times in mid-winter when *E. coli* was abundant in the stream, even during periods of prolonged freezing. This phenomenon argues for septic field or groundwater input, but confirmatory tests (i.e., coliphage—male-specific F-RNA coliphage—occurrence) were not supportive. Nonetheless, the creek and margins remained ice-free and thus, bacteria-laden sediments were free to be suspended and transported along the stream. Interestingly, *E. coli* concentrations in wetlands were consistently low, suggesting that sources could be narrowed down to forest riparian associated inputs or direct runoff. In addition, wetlands seem to reduce overall *E. coli* inputs, perhaps through retention and deposition of particulate-bacteria complexes and inactivation of bacteria through light exposure. Unditched and originating tributaries generally had lower *E. coli* counts. In general, *E. coli* tends to decrease only moderately upstream, but lower reaches cannot be differentiated statistically from upstream stations.

There appears to be a strong positive relationship between the amount of ditching and the loading of *E. coli* occurring in Dunes Creek. This suggests not only that ditching is a major factor in excessive *E. coli* loading but also that the occurrence of the bacteria is from non-point origins. This is consistent with the finding that the major transport mechanism is via sediments washing into the creek or possibly natural populations of *E. coli* growing in wetted stream margins. Thus, while there is limited evidence of human-induced contamination from sewage inputs within the Dunes Creek watershed, there is strong support of anthropogenic influence through ditching, drainage of wetlands, changes in land-use patterns, and runoff. Restoration of wetlands and filling of ditches should have a positive effect in reducing *E. coli* concentrations. Nonetheless, the stream will never completely meet state *E. coli* criteria for swimmable waters regardless of the reasonable remediation of the stream hydrology. Key to the issue is the relative public health concerns presented by Dunes Creek water quality and the efficacy of using *E. coli* to track those risks.

VI. CONCLUSIONS

1. Dunes Creek adversely influences the water quality of Indiana Dunes State Park Beach.
2. *E. coli* within the Dunes Creek watershed is abundant and ubiquitous. It
    a. occurs primarily in submerged sediments and margin sands.
    b. occurs sporadically in forest soils.
    c. is found in seeps and along springs.
    d. is found in standing and running ditch water.
    e. is relatively high throughout the year.
    f. is relatively low in wetland waters and unditched drainage.
3. Similar distribution patterns were found in other stream systems (e.g., Derby Ditch, Indiana; Warren Dunes and Warren Woods, Michigan).
4. *E. coli* decreases rapidly as one moves away from the streambed and is less variable in stream sand and water than forest soil.
5. *E. coli* steadily increases downstream.
6. The relationship between *E. coli* concentration and stream order suggests that excessive ditching and, consequently, nonpoint source input is responsible for *E. coli* occurrence. The major transport of the bacteria is via sediments.

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REFERENCES

1. **American Public Health Association.** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Inc., Washington, D.C.
2. **American Public Health Association.** 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Inc., Washington, D.C.
3. **Anderson, S. A., S. J. Turner, and G. D. Lewis.** 1997. Enterococci in the New Zealand environment: Implications for water quality monitoring. Water Sci. Tech. **35:**325-331.
4. **Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by standardized single disk method. Am. J. Clin. Pathol. **45:**493-496.
5. **Bermudez, M., and T. C. Hazen.** 1988. Phenotypic and genotypic comparison of *Escherichia coli* from pristine tropical waters. Appl. Environ. Microbiol. **54:**979-983.
6. **Byappanahalli, M. N.** 2000. Ph.D. thesis. University of Hawaii at Manoa, Honolulu.
7. **Byappanahalli, M. N., and R. S. Fujioka.** 1998. Evidence that tropical soil environment can support the growth of *Escherichia coli*. Water Sci. Tech. **38:**171-174.
8. **Carrillo, M., E. Estrada, and T. C. Hazen.** 1985. Survival and enumeration of the fecal indicators *Bifidobacterium adolescentis* and *Escherichia coli* in a tropical rain forest watershed. Appl. Environ. Microbiol. **50:**468-476.
9. **Desmarais, T., H. M. Solo-Gabriele, and C. J. Palmer.** 2002. Influence of soil fecal indicator organisms in a tidally influenced subtropical environment. Appl. Environ. Microbiol. **68:**1165-1172.
10. **Dukta, B. J.** 1973. Coliforms are an inadequate index of water quality. J. Environ. Health **36:**39-45.
11. **Edberg, S. C., M. J. Allen, D. B. Smith, and N. J. Kriz.** 1990. Enumeration of coliforms and *Escherichia coli* from source water by the defined substrate technology. Appl. Environ. Microbiol. **56:**366-369.
12. **Edberg, S. C., M. J. Allen, and D. B. Smith.** 1991. Defined substrate technology method for rapid and specific simultaneous enumeration of total coliforms and *Escherichia coli* from water: collaborative study. J. Assoc. Off. Anal. Chem. **74:**526-529.
13. **Fujioka, R. S.** 1983. Stream water quality assessment based on fecal coliform and fecal streptococcus analysis. Water Resources Research Center, University of Hawaii, Technical Memorandum Report No. 70, pp.1-37.
14. **Fujioka, R. S., and M. N. Byappanahalli.** 2001. Microbial ecology controls the establishment of fecal bacteria in tropical soil environment, p. 273-283. In T. Matsuo, K. Hanaki, S. Takizawa and H. Satoh (eds.), Advances in water and wastewater treatment technology: Molecular technology, nutrient removal, sludge reduction and environmental health. Elsevier, Amsterdam, Netherlands.

15. **Fujioka, R. S., K. Tenno, and S. Kansako.** 1988. Naturally occurring fecal coliforms and fecal streptococci in Hawaii's freshwater streams. Toxicity Assess. **3:**613-630.
16. **Fujioka, R.**, **C. Sian-Denton, M. Borja, J. Castro, and K. Morphew.** 1999. Soil: the environmental source of *Escherichia coli* and enterococci in Guam's streams. J. Appl. Microbiol. **85:**83S-89S.
17. **Gary, H. L., and J. C. Adams.** 1985. Indicator bacteria in water and stream sediments near the snowy range in southern Wyoming. Water, Air, Soil Poll. **25:**133-144.
18. **Hagedorn, C., S. L. Robinson, J. R. Filtz, S. M. Grubbs, T. A. Angier, and R. B. Beneau.** 1999. Determining sources of fecal pollution in a rural Virginia watershed with antibiotic resistance patterns in fecal streptococci. Appl. Environ. Microbiol. **65:**5522-5531.
19. **Hardina, C. M., and R. S. Fujioka.** 1991. Soil: the environmental source of *E. coli* and enterococci in Hawaii's streams. Environ. Toxicol. Water Qual. **6:**185-195.
20. **Harwood, V. J., J. Whitlock, and V. Withington.** 2000. Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: Use in predicting the source of fecal contamination in subtropical waters. Appl. Environ. Microbiol. **66:**3698-3704.
21. **Leopold, L. B., M. G. Wolman, and J. P. Miller.** 1964. Fluvial processes in geomorphology. W. H. Freeman and Company, San Francisco, CA.
22. **Muller, T., A. Ulrich, E. M. Ott, and M. Muller.** 2001. Identification of plant-associated enterococci. J. Appl. Microbiol. **91:**268-278.
23. **Mundt, J. O.** 1963. Occurrence of enterococci in animals and wild environment. Appl. Microbiol. **11:**141-144.
24. **Ott, E. M., T. Muller, M. Muller, C. M. A. P. Franz, A. Ulrich, and M. Gabel.** 2001. Population dynamics and antagonistic potential of enterococci colonizing the phyllosphere of grasses. J. Appl. Microbiol. **91:**54-66.
25. **Patrick, R., F. Douglass, D. M. Palavage, and P. M. Stewart.** 1992. Surface water quality: Have the laws been successful? Princeton University Press, Princeton, New Jersey.
26. **Solo-Gabriele, H. M., M. A. Wolfert, T. R. Desmarias, and C. J. Palmer.** 2000. Sources of *Escherichia coli* in a coastal subtropical environment. Appl. Environ. Microbiol. **66:**230-237.
27. **Stewart, P. M., J. T. Butcher, and M. E. Becker.** 1997. Ecological assessment of the three creeks draining the Great Marsh at Indiana Dunes National Lakeshore. Report submitted to the National Park Service, Water Resources Division and the Indiana Dunes National Lakeshore, pp. 1-138.
28. **USEPA.** 1986. Bacteriological ambient water quality criteria; availability. Federal Register. **51:**8012-8016.
29. **Whitman, R. L., T.G. Horvath, M. L. Goodrich and M. B. Nevers, M. J. Wolcott, and S. K. Haack.** 2001. Characterization of *E. coli* levels at 63rd Street Beach. Project completion report submitted to City of Chicago, pp. 1-85.

30. **Whitman, R. L., A. V. Gochee, W. A. Dustman, and K. J. Kennedy.** 1995. Use of coliform bacteria in assessing human sewage contamination. Nat. Areas J. **15:**227-233.
31. **Whitman, R. L., M. B. Nevers, and P. J. Gerovac.** 1999. Interaction of ambient conditions and fecal coliform bacteria in southern Lake Michigan beach waters: Monitoring program implications. Nat. Areas J. 19:166-171.

APPENDIX 1

E. coli counts from the Dunes Creek outfall at Indiana Dunes State Park in 1999-2000. Sites include the creek, and Lake Michigan to the east and west of the outfall. All counts are in number of CFU/100 ml water.

Date	Dunes Creek	East	West
4/8/99	180	31	8
4/16/99	50	15	43
4/22/99	175	72	16
4/29/99	1090	66	384
5/7/99	970	48	219
5/13/99	380	27	182
5/20/99	470	91	10
5/28/99	240	20	1
6/2/99	6900	2000	5
6/3/99	6300	1160	
6/4/99	890	74	161
6/11/99	390	107	22
6/18/99	260	37	68
6/25/99	913	136	36
7/2/99	20130	12	2067
7/3/99			22
7/9/99	2160	221	37
7/16/99	1320	180	16
7/23/99		253	28
7/24/99		241	
7/29/99	3138	95	83
8/6/99	5755	82	105
8/13/99	703	219	405
8/20/99	11950	77	2346
8/21/99	2575		320
8/22/99	790		141
8/27/99	470	107	77
9/3/99	10400	18	32
9/10/99	397	5	12
9/17/99	260	112	250
9/24/99	325	27	24
10/1/99	650	65	44
10/8/99	435	35	80
10/15/99	1005	67	86
10/22/99	230	19	7
10/29/99	140	17	352
11/4/99	165	85	87
11/11/99	280	314	186
11/19/99	305	14	5
4/21/00	560	17	107

4/28/00	105	1	55
5/3/00	195	6	65
5/5/00	120	41	2
5/12/00	115	26	8
5/19/00	175	255	92
5/26/00	505	34	14
6/2/00	590	83	253
6/9/00	157	57	6
6/16/00	2800	541	0
6/23/00	423	137	96
6/30/00	377	60	96
7/7/00	250	186	111
7/14/00	117	52	26
7/21/00	1170	62	121
7/28/00	1793	29	583
8/4/00	977	279	335
8/11/00	487	101	101
8/18/00	10800	111	840
8/25/00	1433	11	101
9/1/00	223	15	23
9/8/00	203	2	60
9/15/00	1117	169	13
9/22/00	757	87	3
9/29/00	273	101	97
10/6/00	3260	100	52
10/13/00	120	9	15
10/20/00	160	26	44
10/27/00	183	21	171
11/3/00	177	468	99
11/9/00		31	28

APPENDIX 2

E. coli counts for 14 sites sampled along Dunes Creek in 1999-2000 (Site 14 was sampled but not analyzed in this report. It is located at Marsh Bridge near Wilson Shelter on Trail 8).

SITE	DATE	RESULT
1	6/2/99	5200
2	6/2/99	4500
3	6/2/99	3300
4	6/2/99	5200
5	6/2/99	7000
6	6/2/99	3800
7	6/2/99	4100
8	6/2/99	1700
9	6/2/99	1400
10	6/2/99	1600
11	6/2/99	1100
12	6/2/99	1200
13	6/2/99	1100
14	6/2/99	1200
1	6/9/99	120
2	6/9/99	220
3	6/9/99	270
4	6/9/99	140
5	6/9/99	90
6	6/9/99	320
7	6/9/99	130
8	6/9/99	100
9	6/9/99	200
10	6/9/99	45
11	6/9/99	70
12	6/9/99	85
13	6/9/99	40
14	6/9/99	10
1	6/16/99	80
2	6/16/99	140
3	6/16/99	120
4	6/16/99	130
5	6/16/99	150
6	6/16/99	190
7	6/16/99	120
8	6/16/99	160
9	6/16/99	160
10	6/16/99	220
11	6/16/99	64
12	6/16/99	140
13	6/16/99	70
14	6/16/99	10
1	6/23/99	450
2	6/23/99	800
3	6/23/99	670
4	6/23/99	840
5	6/23/99	1300
6	6/23/99	300
7	6/23/99	870
8	6/23/99	130
9	6/23/99	890
10	6/23/99	780
11	6/23/99	650
12	6/23/99	180
13	6/23/99	640
14	6/23/99	40
1	6/30/99	730
2	6/30/99	610
3	6/30/99	620
4	6/30/99	600
5	6/30/99	470
6	6/30/99	350
7	6/30/99	660
8	6/30/99	30
9	6/30/99	140
10	6/30/99	500
11	6/30/99	880
12	6/30/99	400
13	6/30/99	230
14	6/30/99	20
1	7/7/99	1800
2	7/7/99	1900
3	7/7/99	1300
4	7/7/99	820
5	7/7/99	1600
6	7/7/99	1100
7	7/7/99	840
8	7/7/99	200
9	7/7/99	180
10	7/7/99	380
11	7/7/99	510
12	7/7/99	170
13	7/7/99	920
14	7/7/99	15
1	7/14/99	1100
2	7/14/99	2700
3	7/14/99	2500
4	7/14/99	1400
5	7/14/99	560

6	7/14/99	960
7	7/14/99	600
8	7/14/99	280
9	7/14/99	320
11	7/14/99	280
12	7/14/99	1200
13	7/14/99	530
14	7/14/99	10
1	7/21/99	900
2	7/21/99	800
3	7/21/99	600
4	7/21/99	1600
5	7/21/99	1400
6	7/21/99	1300
7	7/21/99	1800
10	7/21/99	300
11	7/21/99	1600
12	7/21/99	680
13	7/21/99	800
14	7/21/99	800
1	7/28/99	4000
2	7/28/99	900
3	7/28/99	1300
4	7/28/99	660
5	7/28/99	540
6	7/28/99	620
7	7/28/99	1800
10	7/28/99	460
11	7/28/99	580
12	7/28/99	440
13	7/28/99	420
1	8/4/99	4000
2	8/4/99	920
3	8/4/99	540
4	8/4/99	1800
5	8/4/99	2200
6	8/4/99	2800
7	8/4/99	4100
10	8/4/99	980
11	8/4/99	800
12	8/4/99	340
13	8/4/99	680
1	8/11/99	480
2	8/11/99	1600
3	8/11/99	1500
4	8/11/99	1900
5	8/11/99	1900
6	8/11/99	1300
7	8/11/99	1800
10	8/11/99	660
11	8/11/99	1400
12	8/11/99	380
13	8/11/99	480
1	8/18/99	350
2	8/18/99	650
3	8/18/99	1500
4	8/18/99	1600
5	8/18/99	3600
6	8/18/99	4400
7	8/18/99	850
10	8/18/99	680
11	8/18/99	400
12	8/18/99	240
13	8/18/99	1100
1	8/25/99	4700
2	8/25/99	2400
3	8/25/99	3200
4	8/25/99	3600
5	8/25/99	1700
6	8/25/99	1000
7	8/25/99	2800
9	8/25/99	2400
10	8/25/99	1900
11	8/25/99	2000
12	8/25/99	1000
13	8/25/99	2100
2	9/1/99	650
3	9/1/99	750
4	9/1/99	1400
5	9/1/99	600
6	9/1/99	550
7	9/1/99	1500
10	9/1/99	2500
11	9/1/99	1200
12	9/1/99	250
13	9/1/99	350
14	9/1/99	<50
1	9/8/99	550
2	9/8/99	1200
3	9/8/99	1400
4	9/8/99	850
5	9/8/99	800
6	9/8/99	1000
7	9/8/99	6300
10	9/8/99	460
11	9/8/99	750
12	9/8/99	700
13	9/8/99	2600
1	9/15/99	700
2	9/15/99	900
3	9/15/99	150
4	9/15/99	400
5	9/15/99	750
6	9/15/99	300
7	9/15/99	700
10	9/15/99	1000
11	9/15/99	750
12	9/15/99	300

13	9/15/99	700
15	9/15/99	1
1	9/17/99	150
2	9/17/99	600
3	9/17/99	550
4	9/17/99	650
5	9/17/99	700
6	9/17/99	1300
7	9/17/99	500
10	9/17/99	1200
11	9/17/99	550
12	9/17/99	400
13	9/17/99	500
15	9/17/99	1
1	9/22/99	600
1	9/22/99	680
1	9/22/99	440
1	9/22/99	580
1	9/22/99	600
1	9/22/99	420
1	9/22/99	340
1	9/22/99	560
2	9/22/99	1700
3	9/22/99	2400
4	9/22/99	1100
4	9/22/99	880
4	9/22/99	1100
4	9/22/99	920
4	9/22/99	1100
4	9/22/99	800
4	9/22/99	1100
4	9/22/99	940
5	9/22/99	1200
6	9/22/99	1300
7	9/22/99	60
10	9/22/99	1600
10	9/22/99	1600
10	9/22/99	1300
10	9/22/99	1300
10	9/22/99	1500
10	9/22/99	1500
10	9/22/99	1400
10	9/22/99	1600
11	9/22/99	400
12	9/22/99	480
13	9/22/99	1200
1	9/29/99	4800
2	9/29/99	2400
3	9/29/99	2200
4	9/29/99	2000
5	9/29/99	1900
6	9/29/99	820
7	9/29/99	5700
10	9/29/99	860
11	9/29/99	4200
12	9/29/99	900
13	9/29/99	740
1	10/6/99	850
2	10/6/99	650
3	10/6/99	850
4	10/6/99	950
5	10/6/99	1900
6	10/6/99	1500
7	10/6/99	1600
10	10/6/99	1400
11	10/6/99	1500
12	10/6/99	1300
13	10/6/99	1000
1	10/13/99	1100
2	10/13/99	400
3	10/13/99	700
4	10/13/99	2100
5	10/13/99	200
6	10/13/99	400
7	10/13/99	100
10	10/13/99	500
11	10/13/99	200
12	10/13/99	3800
13	10/13/99	1000
1	10/20/99	320
2	10/20/99	60
3	10/20/99	220
4	10/20/99	1100
5	10/20/99	40
6	10/20/99	200
7	10/20/99	340
10	10/20/99	460
11	10/20/99	80
12	10/20/99	80
13	10/20/99	460
1	10/27/99	80
2	10/27/99	220
3	10/27/99	180
4	10/27/99	1300
5	10/27/99	400
6	10/27/99	420
7	10/27/99	320
10	10/27/99	220
11	10/27/99	<20
12	10/27/99	300
13	10/27/99	700
1	11/10/99	240
2	11/10/99	80
3	11/10/99	40
4	11/10/99	100
5	11/10/99	40
6	11/10/99	180
7	11/10/99	580

10	11/10/99	120
11	11/10/99	<20
12	11/10/99	40
13	11/10/99	20
1	2/23/00	60
2	2/23/00	50
3	2/23/00	60
4	2/23/00	40
5	2/23/00	10
6	2/23/00	30
7	2/23/00	100
8	2/23/00	<10
9	2/23/00	90
10	2/23/00	380
11	2/23/00	290
12	2/23/00	230
13	2/23/00	190
14	2/23/00	60
1	3/29/00	30
2	3/29/00	130
3	3/29/00	130
4	3/29/00	60
5	3/29/00	20
6	3/29/00	30
7	3/29/00	30
8	3/29/00	<10
9	3/29/00	60
10	3/29/00	10
11	3/29/00	<10
12	3/29/00	240
13	3/29/00	110
14	3/29/00	60
15	3/29/00	1
1	4/19/00	700
2	4/19/00	900
3	4/19/00	1000
4	4/19/00	400
5	4/19/00	600
6	4/19/00	400
7	4/19/00	400
8	4/19/00	330
9	4/19/00	500
10	4/19/00	600
11	4/19/00	400
12	4/19/00	300
13	4/19/00	100
14	4/19/00	<100
21	4/19/00	300
22	4/19/00	600
23	4/19/00	100
24	4/19/00	700
25	4/19/00	100
26	4/19/00	500
27	4/19/00	300
28	4/19/00	300
31	4/19/00	300
32	4/19/00	700
33	4/19/00	600
34	4/19/00	600
35	4/19/00	900
36	4/19/00	800
37	4/19/00	700
38	4/19/00	600
41	4/19/00	600
42	4/19/00	800
43	4/19/00	700
44	4/19/00	1200
45	4/19/00	700
46	4/19/00	900
47	4/19/00	500
48	4/19/00	1000
1	4/26/00	<50
2	4/26/00	50
3	4/26/00	50
4	4/26/00	<50
5	4/26/00	<50
6	4/26/00	<50
7	4/26/00	<50
8	4/26/00	<50
9	4/26/00	<50
10	4/26/00	50
11	4/26/00	<50
12	4/26/00	<50
13	4/26/00	<50
14	4/26/00	<50
1	5/3/00	180
2	5/3/00	300
3	5/3/00	340
4	5/3/00	180
5	5/3/00	140
6	5/3/00	140
7	5/3/00	20
8	5/3/00	<20
9	5/3/00	20
10	5/3/00	60
11	5/3/00	<20
12	5/3/00	120
13	5/3/00	160
14	5/3/00	<20
1	5/10/00	340
2	5/10/00	280
3	5/10/00	300
4	5/10/00	230
5	5/10/00	220
6	5/10/00	270
7	5/10/00	290
8	5/10/00	10
9	5/10/00	110

10	5/10/00	100
11	5/3/00	100
12	5/3/00	460
13	5/3/00	110
14	5/3/00	20
1	5/17/00	<20
2	5/17/00	240
3	5/17/00	100
4	5/17/00	130
5	5/17/00	80
6	5/17/00	110
7	5/17/00	50
8	5/17/00	30
9	5/17/00	30
10	5/17/00	60
11	5/17/00	1400
12	5/17/00	200
13	5/17/00	90
14	5/17/00	40
1	5/24/00	190
2	5/24/00	180
3	5/24/00	170
4	5/24/00	270
5	5/24/00	130
6	5/24/00	110
7	5/24/00	100
8	5/24/00	260
9	5/24/00	200
10	5/24/00	110
11	5/24/00	160
12	5/24/00	310
13	5/24/00	450
14	5/24/00	10
21	5/24/00	320
22	5/24/00	300
23	5/24/00	280
24	5/24/00	260
25	5/24/00	350
26	5/24/00	200
27	5/24/00	230
28	5/24/00	140
31	5/24/00	170
32	5/24/00	120
33	5/24/00	310
34	5/24/00	120
35	5/24/00	190
36	5/24/00	170
37	5/24/00	120
38	5/24/00	150
41	5/24/00	90
42	5/24/00	30
43	5/24/00	70
44	5/24/00	40
45	5/24/00	110

46	5/24/00	110
47	5/24/00	60
48	5/24/00	50
1	5/31/00	530
2	5/31/00	260
3	5/31/00	250
4	5/31/00	310
5	5/31/00	260
6	5/31/00	190
7	5/31/00	240
8	5/31/00	<10
9	5/31/00	220
10	5/31/00	690
11	5/31/00	320
12	5/31/00	170
13	5/31/00	160
14	5/31/00	<10
1	6/7/00	330
2	6/7/00	340
3	6/7/00	470
4	6/7/00	430
5	6/7/00	270
6	6/7/00	340
7	6/7/00	260
8	6/7/00	20
9	6/7/00	170
10	6/7/00	530
11	6/7/00	390
12	6/7/00	160
13	6/7/00	110
14	6/7/00	10
1	6/14/00	590
2	6/14/00	600
3	6/14/00	580
4	6/14/00	620
5	6/14/00	460
6	6/14/00	640
7	6/14/00	740
8	6/14/00	30
9	6/14/00	380
10	6/14/00	570
11	6/14/00	650
12	6/14/00	430
13	6/14/00	180
14	6/14/00	90
1	6/21/00	4700
2	6/21/00	6100
3	6/21/00	4500
4	6/21/00	4000
5	6/21/00	2700
6	6/21/00	3500
7	6/21/01	3900
8	6/21/00	100
9	6/21/00	1400

10	6/21/00	4400
11	6/21/00	6400
12	6/21/00	1900
13	6/21/00	1600
14	6/21/00	120
21	6/21/00	660
22	6/21/00	3500
23	6/21/00	3400
24	6/21/00	4500
25	6/21/00	4000
26	6/21/00	4000
27	6/21/00	3800
28	6/21/00	1900
31	6/21/00	3800
32	6/21/00	3100
33	6/21/00	3100
34	6/21/00	4600
35	6/21/00	2800
36	6/21/00	2800
37	6/21/00	4300
38	6/21/00	2900
41	6/21/00	4300
42	6/21/00	3800
43	6/21/00	5100
44	6/21/00	4700
45	6/21/00	5500
46	6/21/00	5900
47	6/21/00	3200
48	6/21/00	4300
1	6/28/00	120
2	6/28/00	120
3	6/28/00	260
4	6/28/00	150
5	6/28/00	170
6	6/28/00	110
7	6/28/00	130
8	6/28/00	30
9	6/28/00	160
10	6/28/00	280
11	6/28/00	170
12	6/28/00	110
13	6/28/00	130
14	6/28/00	10
1	7/5/00	380
2	7/5/00	390
3	7/5/00	260
4	7/5/00	310
5	7/5/00	240
6	7/5/00	310
7	7/5/00	210
8	7/5/00	50
9	7/5/00	220
10	7/5/00	700
11	7/5/00	740
12	7/5/00	750
13	7/5/00	440
14	7/5/00	40
1	7/12/00	220
2	7/12/00	380
3	7/12/00	240
4	7/12/00	190
5	7/12/00	210
6	7/12/00	270
7	7/12/00	180
8	7/12/00	20
9	7/12/00	130
10	7/12/00	1600
11	7/12/00	350
12	7/12/00	210
13	7/12/00	60
14	7/12/00	60
1	7/19/00	890
2	7/19/00	350
3	7/19/00	440
4	7/19/00	430
5	7/19/00	460
6	7/19/00	390
7	7/19/00	480
8	7/19/00	130
9	7/19/00	450
10	7/19/00	480
11	7/19/00	470
12	7/19/00	200
13	7/19/00	490
14	7/19/00	50
21	7/19/00	570
22	7/19/00	720
23	7/19/00	480
24	7/19/00	560
25	7/19/00	480
26	7/19/00	440
27	7/19/00	450
28	7/19/00	380
31	7/19/00	460
32	7/19/00	570
33	7/19/00	630
34	7/19/00	490
35	7/19/00	410
36	7/19/00	520
32	7/19/00	440
38	7/19/00	420
41	7/19/00	400
42	7/19/00	480
43	7/19/00	370
44	7/19/00	410
45	7/19/00	490
46	7/19/00	390
47	7/19/00	430

48	7/19/00	440
1	7/26/00	820
2	7/26/00	490
3	7/26/00	690
4	7/26/00	460
5	7/26/00	620
6	7/26/00	1300
7	7/26/00	410
8	7/26/00	360
10	7/26/00	430
11	7/26/00	2600
12	7/26/00	390
13	7/26/00	960
14	7/26/00	30
1	8/2/00	500
2	8/2/00	470
3	8/2/00	410
4	8/2/00	300
5	8/2/00	330
6	8/2/00	520
7	8/2/00	270
8	8/2/00	450
9	8/2/00	220
10	8/2/00	630
11	8/2/00	350
12	8/2/00	820
13	8/2/00	680
14	8/2/00	830
1	8/9/00	420
2	8/9/00	520
3	8/9/00	680
4	8/9/00	500
5	8/9/00	420
6	8/9/00	440
7	8/9/00	360
8	8/9/00	310
9	8/9/00	300
10	8/9/00	530
11	8/9/00	460
12	8/9/00	430
13	8/9/00	440
14	8/9/00	200
1	8/16/00	5400
2	8/16/00	700
3	8/16/00	720
4	8/16/00	1000
5	8/16/00	710
6	8/16/00	340
7	8/16/00	980
8	8/16/00	100
9	8/16/00	140
10	8/16/00	330
11	8/16/00	630
12	8/16/00	390
13	8/16/00	220
14	8/16/00	170
1	8/23/00	2100
2	8/23/00	600
3	8/23/00	720
4	8/23/00	500
5	8/23/00	820
6	8/23/00	800
7	8/23/00	820
8	8/23/00	160
9	8/23/00	420
10	8/23/00	120
11	8/23/00	580
12	8/23/00	960
13	8/23/00	100
14	8/23/00	<20
21	8/23/00	1800
22	8/23/00	1800
23	8/23/00	1600
24	8/23/00	1700
25	8/23/00	1800
26	8/23/00	1700
27	8/23/00	1800
28	8/23/00	1700
31	8/23/00	580
32	8/23/00	660
33	8/23/00	620
34	8/23/00	520
35	8/23/00	760
36	8/23/00	500
37	8/23/00	600
38	8/23/00	540
41	8/23/00	160
42	8/23/00	160
43	8/23/00	140
44	8/23/00	100
45	8/23/00	100
46	8/23/00	40
47	8/23/00	100
48	8/23/00	200
1	8/30/00	2200
2	8/30/00	700
3	8/30/00	660
4	8/30/00	1500
5	8/30/00	3800
6	8/30/00	2400
7	8/30/00	3000
8	8/30/00	120
10	8/30/00	920
11	8/30/00	980
12	8/30/00	3100
13	8/30/00	360
14	8/30/00	<20
1	9/6/00	760

2	9/6/00	660
3	9/6/00	500
4	9/6/00	700
5	9/6/00	580
6	9/6/00	1100
7	9/6/00	980
8	9/6/00	40
10	9/6/00	1200
11	9/6/00	1200
12	9/6/00	400
13	9/6/00	400
14	9/6/00	<20
1	9/13/00	1400
2	9/13/00	1900
3	9/13/00	1900
4	9/13/00	1700
5	9/13/00	1900
6	9/13/00	1200
7	9/13/00	1100
8	9/13/00	480
9	9/13/00	1300
10	9/13/00	580
11	9/13/00	920
12	9/13/00	240
13	9/13/00	540
14	9/13/00	700
15	9/13/00	1
1	9/20/00	820
2	9/20/00	660
3	9/20/00	740
4	9/20/00	500
5	9/20/00	620
6	9/20/00	460
7	9/20/00	1100
8	9/20/00	40
9	9/20/00	660
10	9/20/00	440
11	9/20/00	920
12	9/20/00	300
13	9/20/00	440
14	9/20/00	60
15	9/20/00	1
21	9/20/00	980
22	9/20/00	580
23	9/20/00	840
24	9/20/00	1000
25	9/20/00	820
26	9/20/00	600
27	9/20/00	960
28	9/20/00	860
31	9/20/00	460
32	9/20/00	600
33	9/20/00	600
34	9/20/00	360
35	9/20/00	480
36	9/20/00	480
37	9/20/00	700
38	9/20/00	540
41	9/20/00	860
42	9/20/00	580
43	9/20/00	420
44	9/20/00	660
45	9/20/00	820
46	9/20/00	600
47	9/20/00	480
48	9/20/00	760
1	9/27/00	320
2	9/27/00	380
3	9/27/00	440
4	9/27/00	240
5	9/27/00	240
6	9/27/00	320
7	9/27/00	460
8	9/27/00	80
9	9/27/00	420
10	9/27/00	380
11	9/27/00	200
12	9/27/00	320
13	9/27/00	260
14	9/27/00	20
1	10/4/00	TNTC
2	10/4/00	TNTC
3	10/4/00	TNTC
4	10/4/00	TNTC
5	10/4/00	TNTC
6	10/4/00	TNTC
7	10/4/00	TNTC
8	10/4/00	2000
9	10/4/00	TNTC
10	10/4/00	TNTC
11	10/4/00	TNTC
12	10/4/00	1800
13	10/4/00	1900
14	10/4/00	20
1	10/11/00	220
2	10/11/00	200
3	10/11/00	120
4	10/11/00	180
5	10/11/00	120
6	10/11/00	160
7	10/11/00	100
8	10/11/00	<20
9	10/11/00	140
10	10/11/00	120
11	10/11/00	200
12	10/11/00	120
13	10/11/00	120
14	10/11/00	<20

1	10/18/00	160
2	10/18/00	160
3	10/18/00	140
4	10/18/00	160
5	10/18/00	320
6	10/18/00	320
7	10/18/00	140
8	10/18/00	40
9	10/18/00	60
10	10/18/00	160
11	10/18/00	180
12	10/18/00	540
13	10/18/00	80
14	10/18/00	<20
1	10/25/00	360
2	10/25/00	360
3	10/25/00	400
4	10/25/00	380
5	10/25/00	340
6	10/25/00	460
7	10/25/00	280
8	10/25/00	1000
9	10/25/00	280
10	10/25/00	100
11	10/25/00	100
12	10/25/00	320
13	10/25/00	420
14	10/25/00	220
15	10/25/00	1
21	10/25/00	280
22	10/25/00	320
23	10/25/00	460
24	10/25/00	340
25	10/25/00	260
26	10/25/00	380
27	10/25/00	200
28	10/25/00	260
31	10/25/00	300
32	10/25/00	200
33	10/25/00	380
34	10/25/00	340
35	10/25/00	300
36	10/25/00	180
37	10/25/00	280
38	10/25/00	160
41	10/25/00	20
42	10/25/00	60
43	10/25/00	20
44	10/25/00	20
45	10/25/00	20
46	10/25/00	80
47	10/25/00	40
48	10/25/00	60
1	11/1/00	240
2	11/1/00	150
3	11/1/00	180
4	11/1/00	160
5	11/1/00	510
6	11/1/00	230
7	11/1/00	440
8	11/1/00	80
9	11/1/00	130
10	11/1/00	70
11	11/1/00	180
12	11/1/00	60
13	11/1/00	60
14	11/1/00	10
1	11/8/00	130
2	11/8/00	200
3	11/8/00	80
4	11/8/00	100
5	11/8/00	70
6	11/8/00	140
7	11/8/00	110
8	11/8/00	<10
9	11/8/00	150
10	11/8/00	30
11	11/8/00	30
12	11/8/00	50
13	11/8/00	20
14	11/8/00	<10
1	11/29/00	100
2	11/29/00	160
3	11/29/00	170
4	11/29/00	130
5	11/29/00	180
6	11/29/00	120
7	11/29/00	120
8	11/29/00	100
9	11/29/00	160
10	11/29/00	90
11	11/29/00	30
12	11/29/00	90
13	11/29/00	630
14	11/29/00	140

APPENDIX 3

Lateral Distribution of *E. coli* in Dunes Creek.

DISTANCE	TRANSECT	Water Content (%)	*E.coli* (MPN/gm d.w.)
.00	1.00	23.9	6.6
1.00	1.00	31.5	1.2
2.00	1.00	29.3	3.4
4.00	1.00	18.3	.0
8.00	1.00	14.0	.0
16.00	1.00	27.6	.0
32.00	1.00	21.1	.0
.00	2.00	20.4	.0
1.00	2.00	32.1	15.4
2.00	2.00	33.4	2.4
4.00	2.00	8.8	.0
8.00	2.00	11.8	.9
16.00	2.00	20.4	.0
32.00	2.00	16.7	.0
.00	3.00	25.7	1.1
1.00	3.00	40.5	32.3
2.00	3.00	39.7	118.6
4.00	3.00	9.8	1.8
8.00	3.00	8.4	.0
16.00	3.00	19.8	.0
32.00	3.00	22.4	2.1
.00	4.00	16.9	8.3
1.00	4.00	28.5	25.5
2.00	4.00	14.8	.0
4.00	4.00	5.6	.0
8.00	4.00	28.0	.0
16.00	4.00	8.4	3.6
32.00	4.00	18.9	.0
.00	5.00	28.1	17.8
1.00	5.00	42.8	.0
2.00	5.00	23.7	1.1
4.00	5.00	12.5	.0
8.00	5.00	10.7	.0
16.00	5.00	26.1	1.1
32.00	5.00	31.4	76.0

APPENDIX 4

Longitudinal Distribution of *E. coli* in Dunes Creek

Station*	Lateral distance	Dilution**	MPN/gm D.W.
1	0	80	176.88
1	0	160	162.54
1	0.25	80	347.42
1	0.25	160	111.17
1	1	80	617.84
1	1	160	807.98
1	4	80	1306.28
1	4	160	765.75
2	0	80	619.96
2	0	160	619.96
2	0.25	80	88.83
2	0.25	160	66.40
2	1	80	137.58
2	1	160	96.10
2	4	40	45.08
2	4	80	4.82
2	4	160	2.41
3	0	80	705.26
3	0	160	592.72
3	0.25	80	71.42
3	0.25	160	43.49
3	1	80	74.86
3	1	160	56.86
3	4	80	22.15
3	4	160	19.06
4	0	80	87.85
4	0	160	81.01
4	0.25	80	32.70
4	0.25	160	23.33
4	1	80	37.03
4	1	160	44.51
4	4	40	5295.31
4	4	80	5295.31
4	4	160	3094.18
5	0	80	95.40
5	0	160	49.89
5	0.25	80	29.61
5	0.25	160	14.55
5	1	80	79.73
5	1	160	45.44
5	4	80	660.49
5	4	160	345.06
6	0	80	333.71
6	0	160	295.51

6	0.25	80	49.50
6	0.25	160	8.86
6	1	80	6.46
6	1	160	2.08
6	4	80	646.38
6	4	160	380.88
7	0	80	855.25
7	0	160	315.81
7	0.25	80	73.81
7	0.25	160	50.40
7	1	80	35.07
7	1	160	8.98
7	4	80	987.01
7	4	160	393.86
8	0	80	182.61
8	0	160	77.62
8	0.25	80	87.27
8	0.25	160	59.12
8	1	80	30.44
8	1	160	21.47
8	4	80	0.00
8	4	160	0.00
9	0	80	123.87
9	0	160	71.82
9	0.25	80	129.83
9	0.25	160	39.68
9	1	80	11.22
9	1	160	3.56
9	4	40	3.11
9	4	80	0.00
9	4	160	1.55
10	0	80	68.60
10	0	160	31.45
10	0.25	80	26.18
10	0.25	160	10.16
10	1	80	7.05
10	1	160	3.44
10	4	80	949.11
10	4	160	671.22
11	0	80	109.51
11	0	160	59.55
11	0.25	80	162.98
11	0.25	160	79.61
11	1	80	1363.59
11	1	160	685.36
11	4	40	8.87
11	4	80	1.71
11	4	160	3.41
12	0	80	2266.71
12	0	160	868.59
12	0.25	80	30.80

12	0.25	160	19.41
12	1	80	56.64
12	1	160	26.24
12	4	80	3821.69
12	4	160	3821.69
12	4	320	3821.69
12	4	800	2233.11
13	0	80	31.07
13	0	160	7.36
13	0.25	80	15.71
13	0.25	160	15.16
13	1	80	1.53
13	1	160	0.00
13	4	40	1.61
13	4	80	0.00
13	4	160	0.00
14	0	80	75.87
14	0	160	27.15
14	0.25	80	9.45
14	0.25	160	1.82
14	1	80	0.00
14	1	160	1.53
14	4	40	3.10
14	4	80	0.00
14	4	160	0.00
15	0	80	97.42
15	0	160	39.90
15	0.25	80	27.72
15	0.25	160	11.42
15	1	80	61.76
15	1	160	51.10
15	4	80	21.34
15	4	160	3.16

*The stations represent fifteen randomly chosen sampling points along the central branch of Dunes Creek.

**The samples that were collected were analyzed for *E. coli* with COLILERT-18 and two different dilutions.

APPENDIX 5

Occurrence of *E. coli* in water and sediment collected from springs and seeps of Dunes Creek (IN) and Berrien County (MI) watersheds.

Habitat	Location	Mean *E.coli* Counts (CFU/ml or MPN/g)	Range
Artesian Springs	Beverly Shores	0.074	0 – 0.144
	Warren Woods	.8	0-1.6
	Wilson Spring	0	0-0
Spring Sediments	Beverly Shores	22.25	3.65 – 52.95
	Warren Woods	3.87	1.05 – 7.81
Seep Sediments	Dunes Creek	35.69	1.31 – 118.94
	Warren Dunes	83.5	68.25 – 101.10
	Wilson Shelter	85.62	1.56 – 169.68

APPENDIX 6

Antibiotic susceptibility patterns of *E.coli* isolates from Dunes Creek.

Isolate Number	AmC	Am	CZ	E	K	NA	N	T	P	S	SXT	TE
1A	S	S	S	R	S	S	S	S	R	R	S	S
1B	S	S	S	R	S	S	S	S	R	I	S	S
2A	S	S	S	R	I	S	S	S	R	S	S	S
2B	S	S	S	R	S	S	S	S	R	I	S	S
2C	S	S	S	R	I	S	S	S	R	S	S	S
3A	S	S	S	R	I	I	S	S	R	R	S	S
3B	S	S	S	R	S	S	S	S	R	R	S	S
3C	S	S	S	R	S	S	S	S	R	I	S	S
4A	S	S	S	R	S	S	S	S	R	I	S	S
4B	S	I	S	R	S	S	S	S	R	I	S	S
4C	S	S	S	R	S	I	S	S	R	S	S	S
5A	S	S	S	R	S	S	S	S	R	S	S	S
5B	S	S	S	R	S	S	S	S	R	I	S	S
5C	S	S	S	R*	S	S	S	S	R	I	S	S
6A	S	S	S	R	S	S	S	S	R	I	S	S
6B	S	S	S	R	I	S	S	S	R	I	S	S
6C	S	S	S	I	S	S	S	S	R	S	S	S
7A	S	S	S	R	I	S	I	S	R	R	S	S
7B	S	I	S	R	S	S	I	S	R	I	S	S
7C	S	S	S	I	S	S	S	S	R	I	S	S
8A	S	S	S	R	S	S	S	S	R	S	S	S
8B	S	S	S	R	I	S	I	S	R	I	S	S
8C	S	I	S	R	S	S	S	S	R	S	S	S
9A	S	I	S	R	S	R	S	S	R	I	S	S
9B	S	S	S	R	S	S	S	S	R	S	S	S
9C	S	S	S	R	S	S	S	S	R	S	S	S
10A	S	I	S	R	S	S	S	S	R	S	S	S
10B	S	S	S	R	S	S	S	S	R	S	S	S
10C	S	S	S	R	S	S	S	S	R	S	S	S
11A	S	S	S	R	S	S	I	S	R	I	S	S
11B	S	S	S	R	S	S	S	S	R	S	S	S
12A	S	S	S	R	S	S	I	S	R	I	S	S
12B	S	S	S	R	I	S	I	S	R	R	S	S
12C	S	S	S	R	I	S	I	R	R	I	S	R
13A	S	S	S	R	S	S	I	S	R	I	S	S
13B	S	S	S	R	S	S	S	S	R	I	S	S
13C	S	S	S	R	I	S	I	I	R	I	S	S

(sample 11A kanamycin: disk fell off agar plate, no reading taken)
Samples 1A-3C are from the wetland near Wilson shelter in the State Park
The remaining samples were taken along the main northern branch of Dunes Creek

AmC- Amoxicillin/clav.
AM- Ampicillin
CZ- Cefazolin
E- Erythromycin
K- Kanomycin
NA- Nalidixic acid

N- Neomycin
T- Oxytetracycline
P- Penicillin
S- Streptomycin
SXT- Sulfa/Trimeth
TE- Tetracycline

R= Resistant
I= Intermediate
S= Susceptible