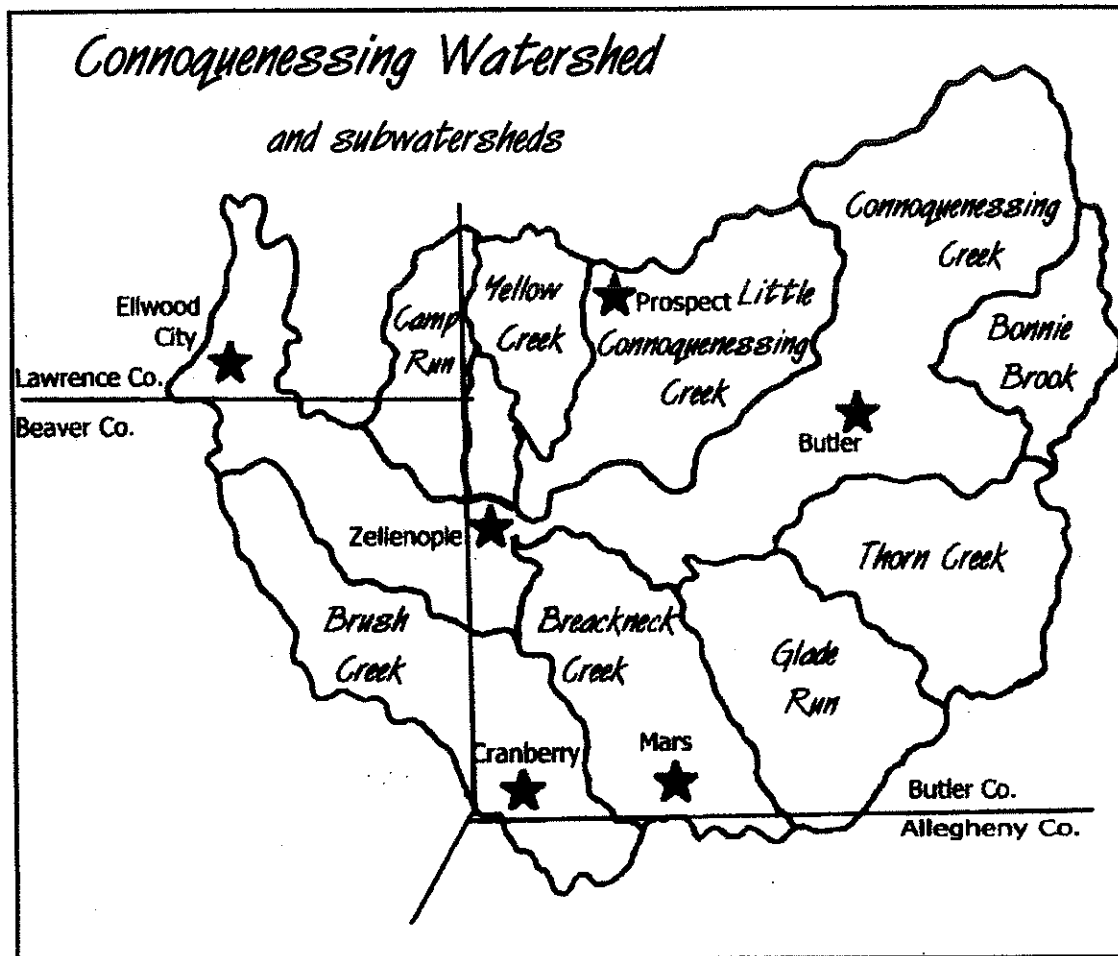


MONITORING BACTERIA IN THE STREAMS OF THE CONNOQUENESSING WATERSHED



**CONNOQUENESSING WATERSHED ALLIANCE
and
BUTLER COUNTY, PENNSYLVANIA**

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Monitoring Bacteria in the Streams of the Connoquenessing Watershed

INTRODUCTION

For the past four years, Connoquenessing Watershed Alliance, Inc. (CWA) has been conducting a volunteer water quality monitoring program in the Connoquenessing Watershed. The work force behind this monitoring consists of approximately thirty trained volunteers who have sampled and field tested stream water at 25 different sites, including 14 locations in Butler County. Using protocols established by the Environmental Alliance for Senior Involvement (EASI), the volunteers test for physical and chemical parameters and also periodically survey for benthic macro invertebrates. In 2002, CWA volunteers completed a scientifically designed freshwater mussel survey on twenty stream segments in the watershed. That survey has provided baseline data on past and present mussel populations that will be used to help gauge the long term health of the watershed ecosystem.

An assessment of Connoquenessing Watershed completed in 2002 by consultant Killam Associates, identified malfunctioning on-lot sewage systems as a potential problem in the watershed. CWA is investigating what role it can play as a non-profit, volunteer organization in addressing this issue. In order to help determine to what extent sewage systems may be creating a problem, CWA decided to add a bacteriological component to its existing monitoring program. In the past the presence of fecal coliform bacteria in natural waters was used as an indicator of the extent of fecal contamination. However, since the Beaches Environmental Assessment and Coastal Health Act of 2000 was passed, the U.S. Environmental Protection Agency has recommended testing for the bacteria *Escherichia coli* (*E. coli*) as the best indicator of health risk from water contact in recreational waters. (<http://www.epa.gov/waterscience/beaches/act.html>) This act also directed any states with coastal areas to adopt new standards equal or better than those of the EPA. As of 2003, several states, excluding Pennsylvania, have complied. (<http://www.epa.gov/volunteer/stream/vms511.html>) Various methods available to identify and quantify *E. coli* in samples of stream water were considered. CWA selected the Coliscan® Easygel® test kit, a product of Micrology Laboratories. These kits use Easygel®, a pre-measured, sterilized culture medium that requires no mixing, autoclaving, or

hot water baths as with other traditional media. A measured quantity of stream water is mixed with the Easygel® solution and then poured onto the pretreated petri dish that is provided with each kit. The petri dish is then incubated for 24 hours at 95 degrees F. If present, the colonies of *E. coli* are differentiated by color and are able to be counted. For more details regarding the Coliscan® Easygel® method, refer to (<http://www.micrologylabs.com>).

In 2004, CWA was awarded a Butler County Environmental Mini-grant that funded the purchase of equipment and supplies needed to field test for *E. coli* concentrations. Enough kits for three hundred tests were purchased for the project. A detailed review of potential sampling sites was conducted prior to starting this project. Samples were collected at intervals of three to four weeks over a three month period at various CWA monitoring sites. Based on the initial results of the testing, several stream segments were selected for more intensive sampling. Comparison of *E. coli* counts during wet weather and dry weather conditions was not possible because there was no dry weather during the project period. All labor and transportation costs were donated by CWA volunteers. As with all of CWA's monitoring work, the results of this testing are solely for educational and informational use, and will not be relied on for any legal purposes.

SAMPLING AND HANDLING PROTOCOL AND PROCEDURES

A - SITE SELECTION:

In any decision regarding where to conduct sampling, there must be consideration regarding the types of sites needed to answer the question at hand. This question needs to be framed as clearly as possible so it can be used as a guide to choosing relevant sites.

Sites were selected with three things in mind. First and foremost was their relevance to the assessment of the fecal contamination of the Connoquenessing and its tributaries. Access and accessibility also needed to be considered. Most of the sampling sites were within Butler County. However, since watersheds do not respect political boundaries, it was necessary to sample several tributaries at points downstream of the county to get a more complete picture of the overall condition of this watershed.

(1)Relevance: This study had two purposes in mind. First: to assess the suitability and usability of the Coliscan® Easygel® system for volunteer watershed groups, such as CWA, which may have no particular scientific background in this sort of activity; and to establish a set of protocols, working requirements which could then be made available to such groups which may then benefit from our experience. Second, inasmuch as this *E. coli* sampling would be the first systematic program of its kind in the watershed, as far as could be determined, the purpose in this first set of examinations was to gather some sense of how much fecal contamination the Connoquenessing and some of its major tributaries might be carrying - a survey, if you will, of present conditions. These were the "questions."

(2) Access: direct access to any creek will invariably involve traversing some land to get to the water. Good manners, if nothing else, require a respect for the ownership of that property. In some cases, the sites used were chosen such that access was available from public land (parks) or semi-public land (church or grocery store parking lots, for example). In other cases where adjoining land was privately owned, permission for access from the property owners was obtained beforehand. The majority of the sites ultimately used were in fact CWA water quality monitoring sites of long standing, where permission for access had long since been assured.

(3)Accessibility: permission aside, one must still be able to actually get to the water with relative ease and safety. Sites where the banks were very steep and slippery, or where debris presented unsafe obstacles, were considered unacceptable. Sites with reasonably gentle and unobstructed paths to the water were chosen. Also, since the ideal method for taking samples involves wading out into the middle of the stream, sites were chosen where the water was shallow enough to do that, and where the current was mild enough to not present a safety hazard.

For the initial sampling rounds, a total of 10 sites were selected. Three of these sites were on the main stem of the Connoquenessing (Connie), each site separated from the others by a number of miles, and were chosen in order to get a picture of what level of bacteriological contamination may be present at different locations along the length of the steam.

Additional sites were chosen on seven of the major tributaries (one site each) to give an idea of what those tributaries may contain, and what they may be therefore contributing to the main stem. An attempt was made to choose tributary sites that were within one mile from the confluence with the

Connie. However, due to access and accessibility requirements, three of these tributary sites were more than one mile upstream.

These first 10 sites (in Butler County unless otherwise indicated) were:

Connoquenessing Creek

- McCalmont Road crossing (Butler Twp.) - to represent the Connie as it left Butler.
- Ash Stop Road crossing (Forward Twp.) - to represent the possible contributions of Renfrew and the tributaries in between, particularly Thorn Creek and Glade Run.
- ECOZ Park (Zelienople Borough) - to represent the possible contributions of Breakneck Creek, Harmony and Zelienople, and the numerous "camps" along the creek in the general Evans City/Harmony area.

Thorn Creek

Along Renfrew Road, at Three Degree Rd. intersection (Penn Twp.)

Little Connoquenessing

Shannon Road crossing (Connoquenessing Twp.)

Glade Run

Brownsdale Road crossing (Forward Twp.)

Breakneck Creek

Textor School Road crossing (Jackson Twp.)

Brush Creek

Harper's Ferry Road at Chapel Road (North Sewickley Twp., Beaver Co.)

Muddy Creek

Currie Road crossing (Worth Twp.)

Slippery Rock Creek

Armstrong Bridge in McConnell's Mill State Park (Perry Twp., Lawrence Co.)

After the first couple of sampling rounds, the preliminary findings indicated elevated levels of *E. coli* in Brush Creek and Breakneck Creek in particular, and to some extent, Slippery Rock Creek and Glade Run, as well. These findings led to an expansion of the investigation by the addition of several more sites on those creeks in the hopes of trying to determine a more

specific geographic area within which the source of these elevated levels may lie. These additional sites were:

Breakneck Creek

Behind the Baptist Church, Washington and Jackson Sts. (Evans City)
End of Glenwood Avenue (Forward Twp.)
Kline Road crossing (Adams Twp., near Gallery)
Hook St. crossing (Mars)

Brush Creek

Brush Creek Park (Marion Twp., Beaver Co.)
Green Valley Park (New Sewickley Twp., Beaver Co.)

Slippery Rock Creek

West Park Road crossing (Worth Twp.)

Glade Run

Three Degree Road crossing (Adams Twp.)

B - ON-SITE COLLECTION OF WATER SAMPLES

Precautions - In the interest of safety, the sample collectors went on their sampling rounds in teams of at least two people.

For the collection of sample water to be tested, the Coliscan® Easygel® system provides sterile plastic bottles of ~30 ml. volume. It is most important that great care be taken in handling these bottles to avoid contamination of the interior of both the bottle and cap by anything other than the water being sampled. When opening the bottle, care must be taken to not touch the lip of the cap or of the bottle. If at any time during the collection process either of these areas is touched by the collector's hands, or any other object, the bottle should be discarded and a fresh bottle employed.

It is also most important that care be taken to label the bottles as to where the samples were taken to avoid confusion later on. Each bottle and cap were labeled with an indelible marker just prior to entering the stream, using a pre-arranged designation system that would eliminate any later uncertainty in identifying the location where that sample had been collected.

If it will be more than one hour before the samples collected are "plated" (see below), they should be stored in a small iced cooler during transport. If plating is to occur on a subsequent day, samples should be

refrigerated until use. In all cases for this study, coolers were used, and plating was done on the same day as collection.

Sample Collection - Ideally, the sample should be taken from the middle of the stream. If the stream is so broad, deep or swift at the center that this is not feasible or safe, the sample should at least be taken far enough out from shore so that the water comes from the main current, away from any eddys or slack water along the shore.

With the collector in position and facing upstream, the bottle, with cap still in place, was placed underwater in front the collector. While taking care to avoid touching the lip of both the cap and bottle, the cap was unscrewed underwater to open the bottle. The cap was kept underwater in one hand, and the open bottle in the other was tilted and faced into the current. The bottle was "swung", under water, to the right and left, in a "u" -shaped arc back and forth in front of the collector, keeping the mouth facing upstream, until all bubbles ceased escaping. The lid was replaced tightly, all still underwater.

There were times during this study when heavy rains caused periodic elevations in the water level which in some locations rendered in-stream sampling less safe than under more normal conditions. This was especially the case after hurricanes Frances and Ivan. To assist in sampling at such times, a sampling "tool" was devised from an extendable pole usually used for painting. This allowed the sample to be taken without the collector having to enter the stream. The sample bottle was attached to one end of the pole with rubber bands and the cap carefully removed. The lid was removed and held with the non-dominant hand while the bottle was plunged into the stream, tilted so that it faced into the current, and swung in an arc. The lid was immediately placed on the bottle once the pole was retracted from the stream.

C - PLATING - In this report, the word "plating" refers to that part of the process in which inoculum of specific concentrations of sample water were introduced onto petri dishes (the "plates") for incubation.

Precautions - In all phases of this operation, exceeding care was taken to avoid contamination of the sample water, to insure that it would not come into contact with anything other than itself, or the components of the Coliscan® Easygel® system. Care was also taken to avoid contamination of the work area. Inexpensive, disposable plastic tablecloths (such as are used for picnics) were placed over work tables. Latex or vinyl gloves were worn by

all those handling the samples and equipment. Purel® hand disinfectant was available and frequently used during the plating procedure.

Inoculum amounts - Lacking any previous data regarding *E. coli* concentrations in these streams, inoculum concentrations of 1, 2 and 3 mL (milliliter) were used the first time. According to Alabama Water Watch, a watershed group with extensive experience, a 1 mL sample will reveal contamination levels as low as 1 *E. coli*/mL. (<https://www.auburn.edu/>) Triplicate samples are also recommended, but it was decided to use three different volumes rather than just one. Otherwise, the chosen volume may not be large enough to grow any colonies or the colonies may be TNTC (Too Numerous To Count). In either case the result would be no valid data for that sampling date. Subsequent sampling event inoculum amounts were based on previous results in order to estimate the correct dilution needed to obtain "countable plates". In other words, if 1, 2 & 3 mL were used previously, and the result was one or less colonies on the 1 mL samples, sample sizes of 2, 3, 4, mL might be used the next time. Conversely, if CFUs were TNTC at a particular dilution, smaller sample sizes would be used the next time, and a fourth sample of less than one mL may be utilized.

Set-up - During each round of sampling, at least three different inoculum (see below) concentrations were plated for each site, sometimes 4, as described above. Each concentration for each site utilized one pre-packaged bottle of liquid growth medium and one plastic petri dish with lid.

The first step was to set out the total number of petri dishes and bottles of liquid media that would be required for that plating session. Each petri dish was first "hinged" by placing a small amount of transparent tape on one side which attached the lid to the dish. This was to help to prevent accidental dislodging of the lid (and therefore possible contamination) during the plating procedure and the period of solidification which occurs immediately afterwards, as well as during transport to and from the incubator. Each dish lid was then labeled, with indelible marker, with the site name and the inoculum concentration amount ("1" for a 1 mL. concentration, "3" for 3 mL., etc.). Dish labeling was done on the top of the lid, but at the edge, using the smallest legible writing, so that the labeling would not interfere with later colony counting. Each bottle of medium to be used was labeled correspondingly.

Inoculating the medium - A bottle of field sample water from a site was selected. The petri dishes for that site were arrayed in front of the plater.

Just in front of each petri dish the corresponding bottle of medium was placed.

The Easygel® kit contains individually pre-packaged sterile 1 ml and 3 ml. plastic pipettes, graduated by 0.5 ml. (Unless contaminated, one pipette was used for all inoculation samples for one site. A fresh pipette was used for the next site's samples). The pipette package was opened at the bulb end, and the pipette was aseptically removed from the packaging. Once removed, the shaft of the pipette was never allowed to touch anything other than sample water, and the insides of the bottles from which that sample was being withdrawn or into which it was being placed. Between inoculations it was held by the bulb between the index and middle fingers (as one might hold a cigarette).

Just before each inoculation sample was withdrawn, the sample bottle was gently agitated to encourage uniform distribution of any possible bacteria throughout. This was done by holding the sample bottle by the lid, and tapping the sides with the fingers for 10 to 15 seconds. The designated number of milliliters were withdrawn, and placed into the corresponding bottle of medium. The bottle of medium with its cap replaced was then gently agitated in the same way. At this point the combination of the medium and the sample water is referred to as the "inoculum".

Plating - In introducing the inoculum to the plates, care was taken that the inside of the petri dish and its lid were not exposed to the "outside" any more than was necessary. The lid of the corresponding petri dish was lifted, opposite the "hinge", just enough to allow the inoculum to be poured into the plate, and immediately closed. During the next 45 minutes, the combination of the inoculum and the coating of medium within the petri dish react in such a way that the solution becomes solidified. After solidification of the contents, the dishes may be moved to the incubator without danger of spillage.

Interim Precautions - After completing the inoculation and plating for one site, and before moving on to the next, gloved hands were dosed with Purel® to eliminate any possible contamination of the next site's field sample from any sample or inoculum from the site just completed. A fresh pipette was used for the next site's inocula.

Inoculated petri dishes were gently moved off to one side to allow solidification to occur undisturbed. Empty bottles of inoculum, and the completed field sample bottle, were removed to avoid any confusion.

Clean-up - Tablecloth, absorbent pads, and gloves were bundled together and burned. Sample and media bottles, and pipettes, were disinfected with bleach water and were either recycled or discarded. Pens, markers, and the work surface were disinfected with Lysol.

D - INCUBATION - A simple incubator was assembled using an 18 gallon Rubbermaid storage container and a standard light bulb as the heat source. An aluminum shielded "work light" assembly was suspended from a hole punched in the lid. A minimum-maximum thermometer was used to keep track of the temperature inside the incubator. After some experimenting with different set ups, it was determined that a 100 watt bulb controlled by a thermostat would provide the uniform 95 to 98 degree temperature range recommended by the Coliscan® Easygel® manufacturer. Plates were placed in the incubator within two hours of being inoculated and incubated for 20 to 22 hours prior to being counted.

E - COUNTING - in this report, "counting" refers to the counting of individual *E. coli* colonies after the incubation period as described above.

Precautions - As with the plating procedure described above, exceeding care must be taken in handling the petri dishes and avoiding contamination - in this phase, not so much contamination of the samples, but contamination by them. One must remember at all times that bacteria have been deliberately encouraged to grow within the dishes. At this stage they are still doing that actively, and one cannot be completely sure what may be thriving there. Latex or vinyl gloves were worn by all those handling the samples and equipment. Purel® hand disinfectant was available and frequently used during the counting procedure. Touching of any part of one's body with a gloved hand, or of any equipment or surface area outside of the work zone, was assiduously avoided.

Great care was also taken to avoid contamination of the work area. Inexpensive, disposable plastic tablecloths were again placed over work tables. Absorbent pads (or several layers of paper towels) were placed at each work station to collect any spilled liquid.

Set-up - To facilitate accuracy in counting the number of colonies, paper grids were prepared beforehand. These consisted of a square slightly larger than the diameter of the dish, filled with smaller squares about $\frac{3}{4}$ " on a side. When placed underneath a dish, the grid was clearly visible through the medium, and served to divide the counting area into smaller segments such

that it was easier to keep track of what colonies had or had not yet been counted.

Also prepared beforehand were "tally sheets". These sheets were constructed in tabular form, with space provided for CFU (colony forming units) and CFU per 100 ml. for each inoculum concentration for each site being counted. Space was also provided to record the date and time of both sampling and counting, and for any remarks regarding conditions at the sampling site. (See Table 1)

Counting for each round of sampling was done at a single session, always between 24 and 28 hours after initial plating, with at least three "counters" present.

Procedure - During incubation, it is not uncommon for some amount of condensation to form on the inside of the lid. This condensation can interfere with accurate counting "through" the lid. A better "view" was obtained by either (1) turning the whole plate upside down and reading from the back (the solidified medium is transparent); or (2) by placing the dish face up and tilting the lid open (using the tape hinge). Either method will result in the leakage of some of the condensate onto the work surface (hence the absorbent pads). For this study the latter method was most often employed.

Each counter assembled all the dishes for one site (three or four, as described above), and using the grid underneath, counted and recorded the colonies present in each dish. Colonies were counted that could be seen with the naked eye, though in some instances hand lenses were used to help determine the color of small colonies. Upon completion, the counted dishes were passed along to the next counter, and a new set of site dishes begun. Dishes were rotated between counters, such that in the end, all dishes were counted by all counters.

Reconciliation - upon the completion of all counting, results for each concentration for each site were compared. It was not uncommon for there to be some discrepancies in the number of colonies observed in individual dishes here and there caused by interruption during counting, a small colony sitting directly over a grid line and going unobserved, simple oversight, etc.. When such discrepancies were found, those dishes were checked again by all counters until agreement was reached.

Calculation - The results for each concentration were then presented as CFU per 100 mL. This is the unit of measure used formerly to count coliforms, and accepted currently by the EPA for enumerating *E. coli*. The three or four results from each site were then rendered as an average.

TABLE 1

Sample Date & time Analysis Date & Time

| Site Name | CFU | # mL | CFU/100 mL | CFU | # mL | CFU/100 mL | CFU | # mL | CFU/100 mL | CFU | # mL | CFU/100 mL | AVG | NOTES: |
|----------------------|-----|------|------------|-----|------|------------|-----|------|------------|-----|------|------------|-----|--------|
| Connie-McCalmont | | 4 | | | 6 | | | 8 | | | | | | |
| Thorn Creek | | 1 | | | 3 | | | 5 | | | | | | |
| Renfrew Rd. | | 3 | | | 5 | | | 7 | | | | | | |
| Glade-3 Degree Rd. | | 4 | | | 5 | | | 6 | | | | | | |
| Glade | | 3 | | | 4 | | | 5 | | | | | | |
| Brownsdale Rd. | | 3 | | | 5 | | | 7 | | | | | | |
| Connie-Ashstop Rd. | | 1 | | | 3 | | | 5 | | | | | | |
| BNC Mars | | 0.25 | | | 0.5 | | | 0.75 | | | | | 1 | |
| Hook St. | | 0.25 | | | 0.5 | | | 0.75 | | | | | 1 | |
| BNC Callery | | 0.25 | | | 0.5 | | | 0.75 | | | | | 1 | |
| Kline Rd. | | 4 | | | 5 | | | 6 | | | | | | |
| BNC Glenwood | | 2 | | | 3 | | | 4 | | | | | | |
| BNC Evans City | | 2 | | | 4 | | | 6 | | | | | | |
| Baptist Church | | 2 | | | 4 | | | 6 | | | | | | |
| BNC Textor School | | 2 | | | 3 | | | 4 | | | | | | |
| Little Connie | | 2 | | | 4 | | | 6 | | | | | | |
| Shannon Rd. | | 2 | | | 3 | | | 4 | | | | | | |
| Connie - ECOZ | | 2 | | | 4 | | | 6 | | | | | | |
| Brush-Gr. Valley | | 2 | | | 3 | | | 4 | | | | | | |
| Brush-B. C. Park | | 2 | | | 4 | | | 6 | | | | | | |
| Brush-Harper's Ferry | | 2 | | | 4 | | | 6 | | | | | | |
| Muddy-Curry Rd. | | 5 | | | 7 | | | 9 | | | | | | |
| SR-West Park Rd. | | 4 | | | 5 | | | 6 | | | | | | |
| SR-Armstrong Brdg. | | 2 | | | 3 | | | 4 | | | | | 5 | |

Clean-up - Tally sheets used at the counting table were invariably contaminated so each time the data was transcribed onto a fresh sheet away from the counting table. Great care was taken in bundling up the table covering, absorbent pads, gloves, grids, contaminated tally sheets and petri dishes into a biohazard bag. Then, the bag contents were either sterilized in an autoclave, immersed in concentrated bleach water, or burnt. All pens, lenses, work surfaces, work lights, and chairs, were disinfected with Lysol.

RESULTS

| TABLE 2: AVERAGE E. COLI LEVELS CFU/100 mL | | | | | | | | | | |
|---|--------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|------------|-------------|
| | DATES | | | | | | | | | |
| LOCATIONS | 83104 | 101604 | 103104 | 110604 | 112004 | 120504 | 121804 | 122904 | SUM | AVE |
| Connie. McCalmont Rd. | 228 | 19 | 106 | 0 | 152 | 0 | | 18 | 523 | 75 |
| Thorn Creek Renfrew Rd. | 17 | 70 | 57 | 302 | 61 | 458 | | 99 | 1064 | 152 |
| Glade Run Three Degree Rd. | | | | | | 24 | | 118 | 142 | 71 |
| Glade Run Brownsdale Rd. | 189 | 271 | 73 | 126 | 140 | 241 | | 54 | 1094 | 156 |
| Connie. Ashstop Rd. | 228 | 58 | 398 | 50 | 23 | 125 | | 33 | 915 | 131 |
| Breakneck Ck. Mars, Hook St. | | | | | | 87 | 75 | 228 | 390 | 130 |
| Breakneck Ck. Callery, Kline Rd. | | | | | | 111 | 106 | 147 | 364 | 121 |
| Breakneck Ck. Glenwood Dr. | | | | | | | 2108 | 83 | 2191 | 1096 |
| Breakneck-E.C. Baptist Church | | | | | | 5322 | 700 | 25 | 6047 | 2016 |
| Breakneck Creek Textor School Rd. | 1677 | 142 | | 331 | 900 | 1007 | 1800 | 50 | 5907 | 844 |
| Little Connie. Shannon Rd. | 22 | 64 | 23 | 6 | 70 | 135 | | 12 | 332 | 47 |
| Connie. ECOZ - Zelie. | 350 | 0 | | 204 | 104 | 233 | | 8 | 899 | 149 |
| Brush Creek. Green Valley Rd. | | | | | | 296 | | 172 | 468 | 234 |
| Brush Creek Brush Creek Pk. | | | | | | 69 | | 864 | 933 | 466 |
| Brush Creek Harper's Ferry Rd. | 1677 | 83 | | 178 | 838 | 25 | | 106 | 2907 | 485 |
| Muddy Creek Currie Rd. | 28 | 42 | | 0 | 28 | 6 | | 0 | 104 | 17 |
| Slippery Rock West Park Rd. | | | | | | 13 | | 22 | 35 | 18 |
| Slippery Rock Armstrong Bridge | 1938 | 0 | | 283 | 27 | 438 | | 11 | 2697 | 450 |

Figure 1
E. coli Levels in the Connoquenessing Creek Watershed
Connoquenessing Creek Main Stem
(Monitoring Locations in Upstream to Downstream Order)
Fall/Winter 2004

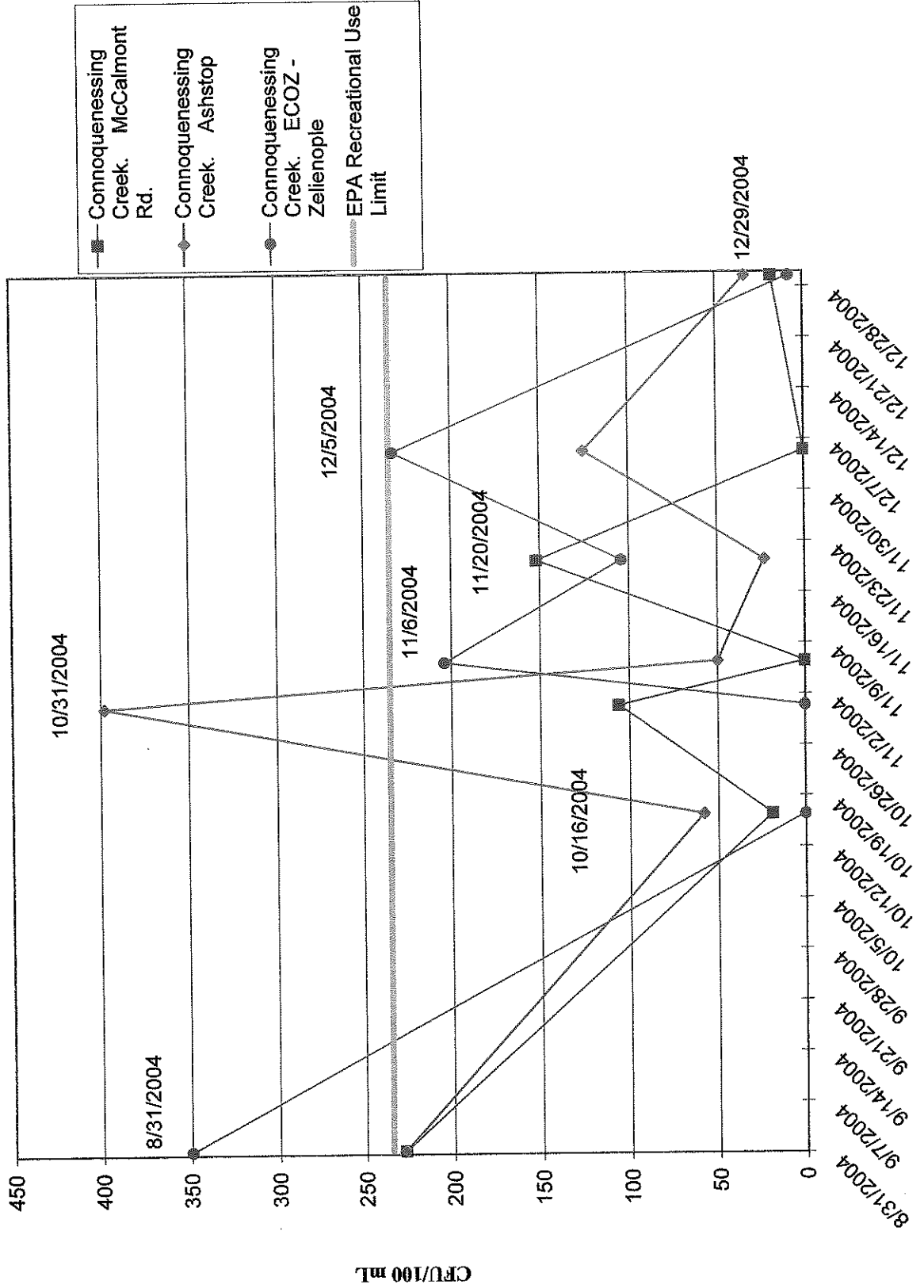


Figure 2
***E. coli* Levels in the Connoquessing Creek Watershed**
Thorn Creek
Fall/Winter 2004

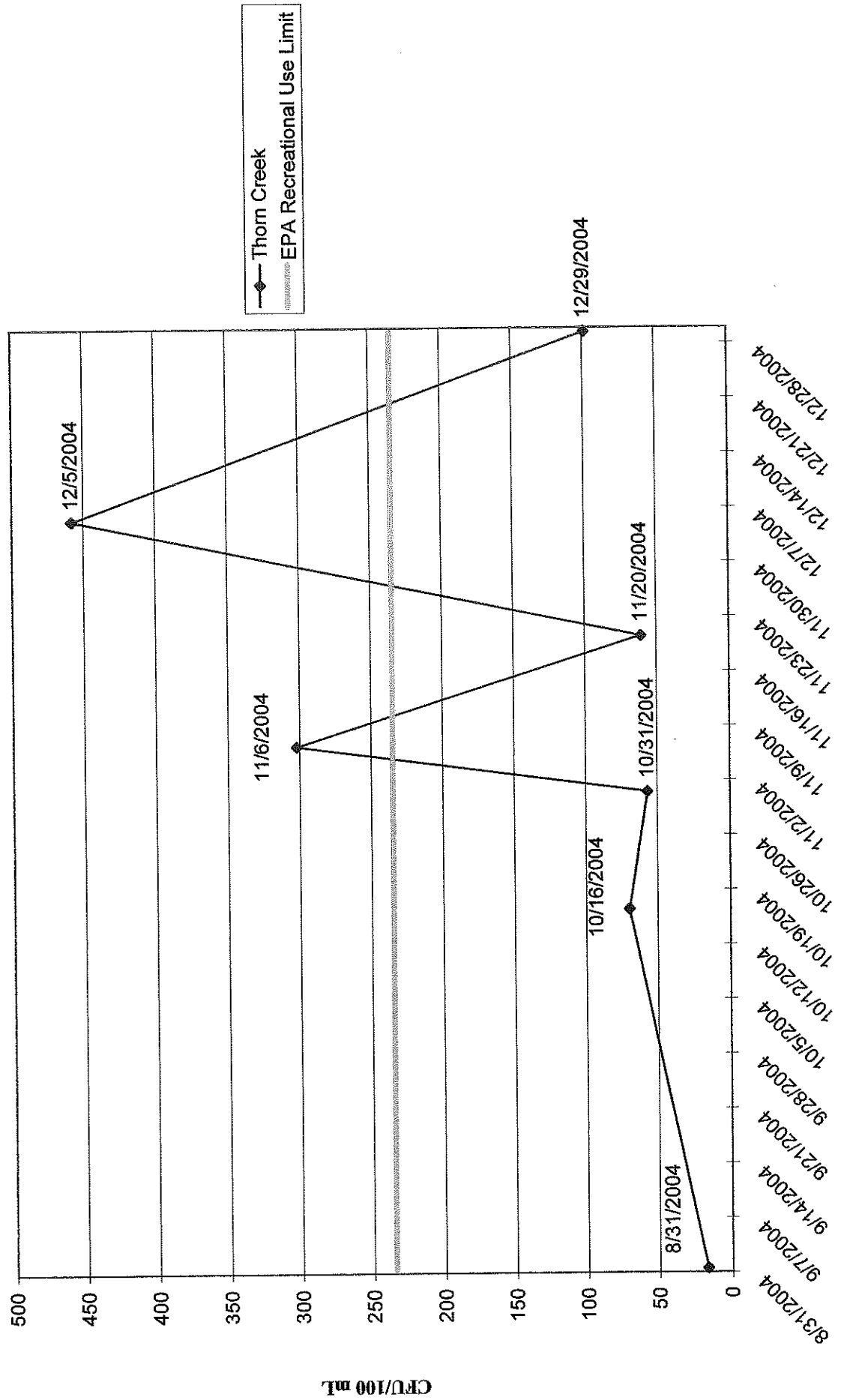


Figure 3
***E. coli* Levels in the Connoquessing Creek Watershed**
(Monitoring Locations in Upstream to Downstream Order)
Glade Run Locations
Fall/Winter 2004

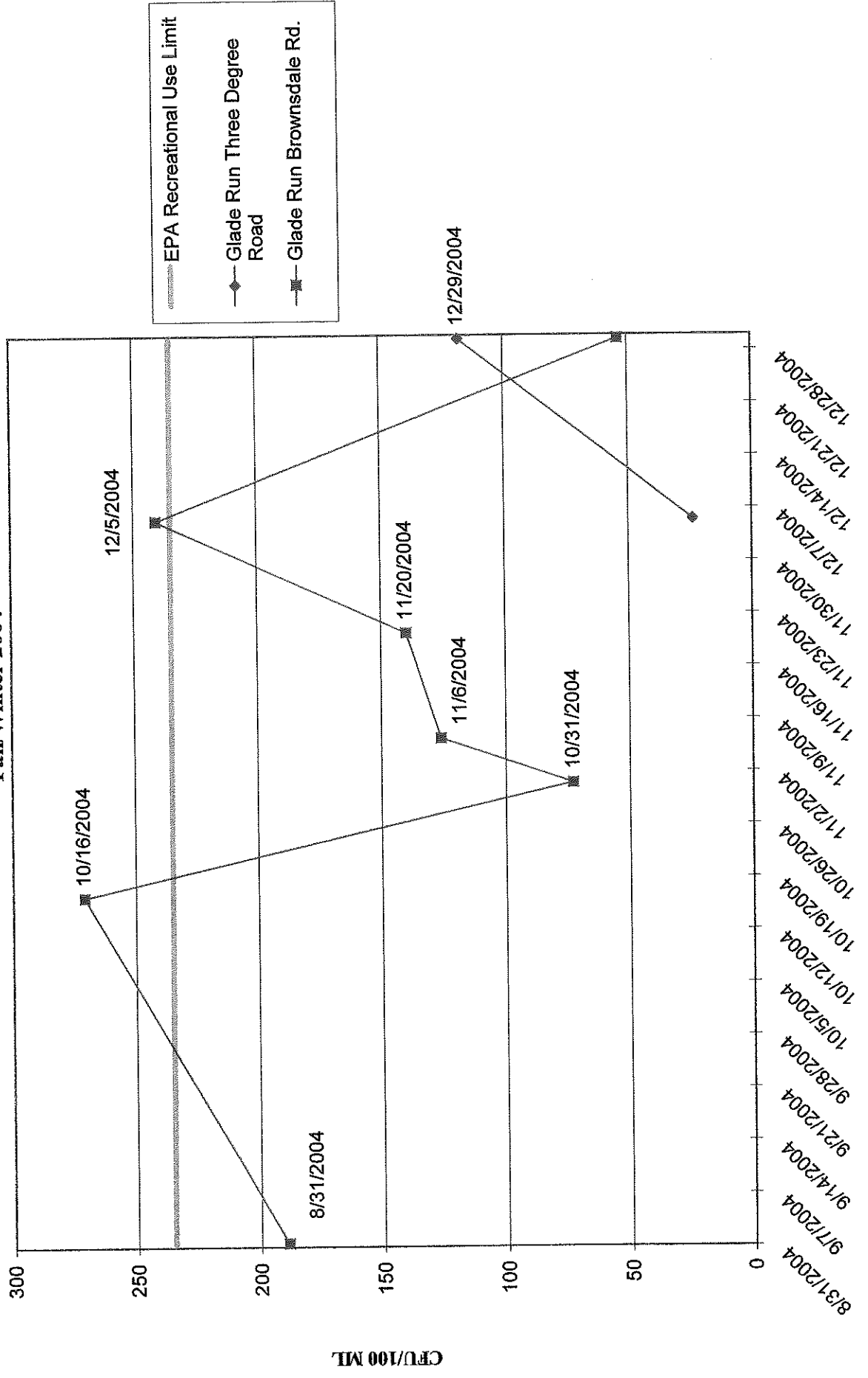


Figure 4
E. coli Levels in the Connoquenessing Creek Watershed
Breakneck Creek Locations
Fall/Winter 2004

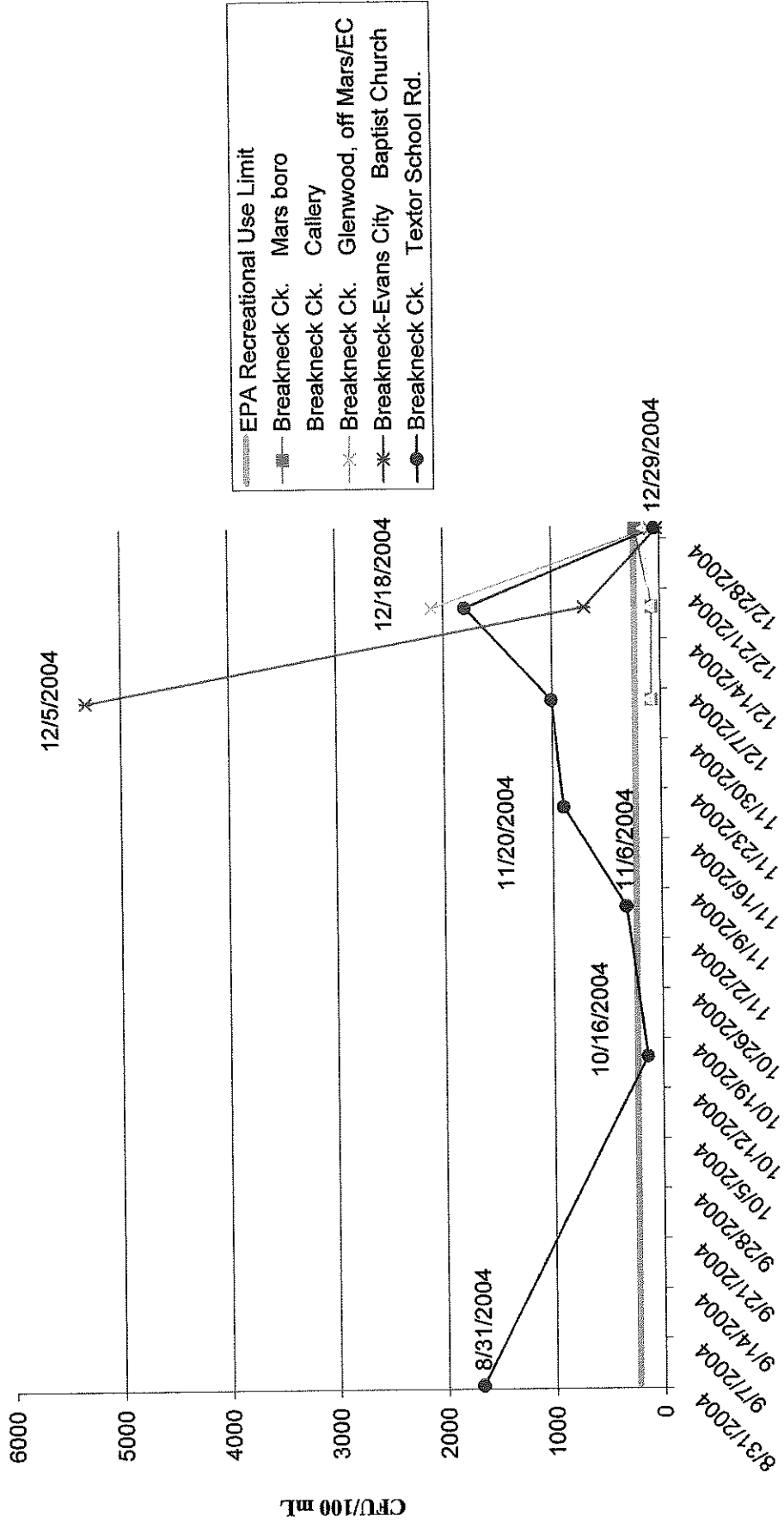


Figure 5
***E. coli* Levels in the Connoquenessing Creek Watershed**
Breakneck Creek Concentrations Vs. River Mile Index
December 5, 2004

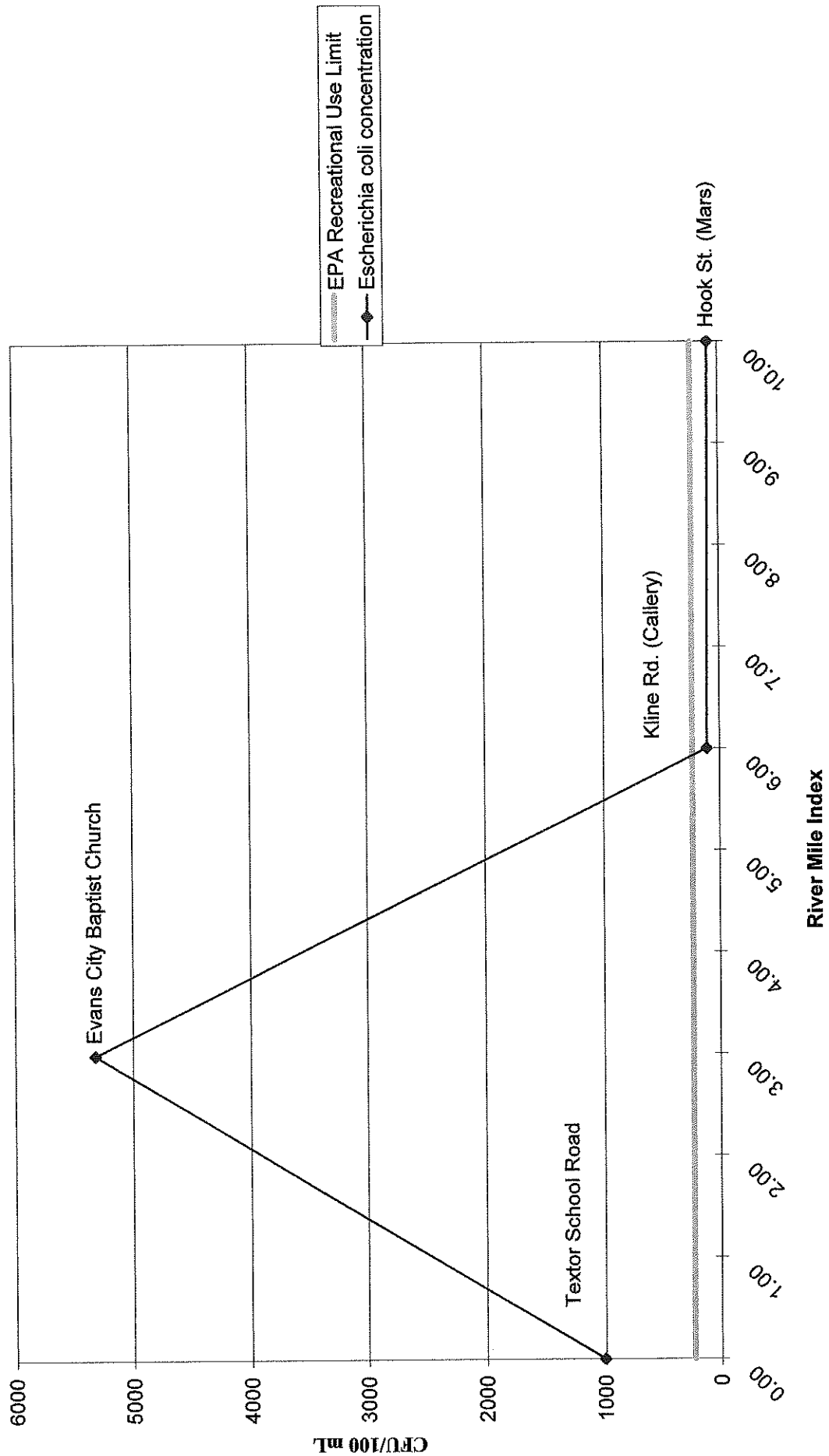


Figure 6
***E. coli* Levels in the Connoquenessing Creek Watershed**
Breakneck Creek Concentrations Vs. River Mile Index
December 18, 2004

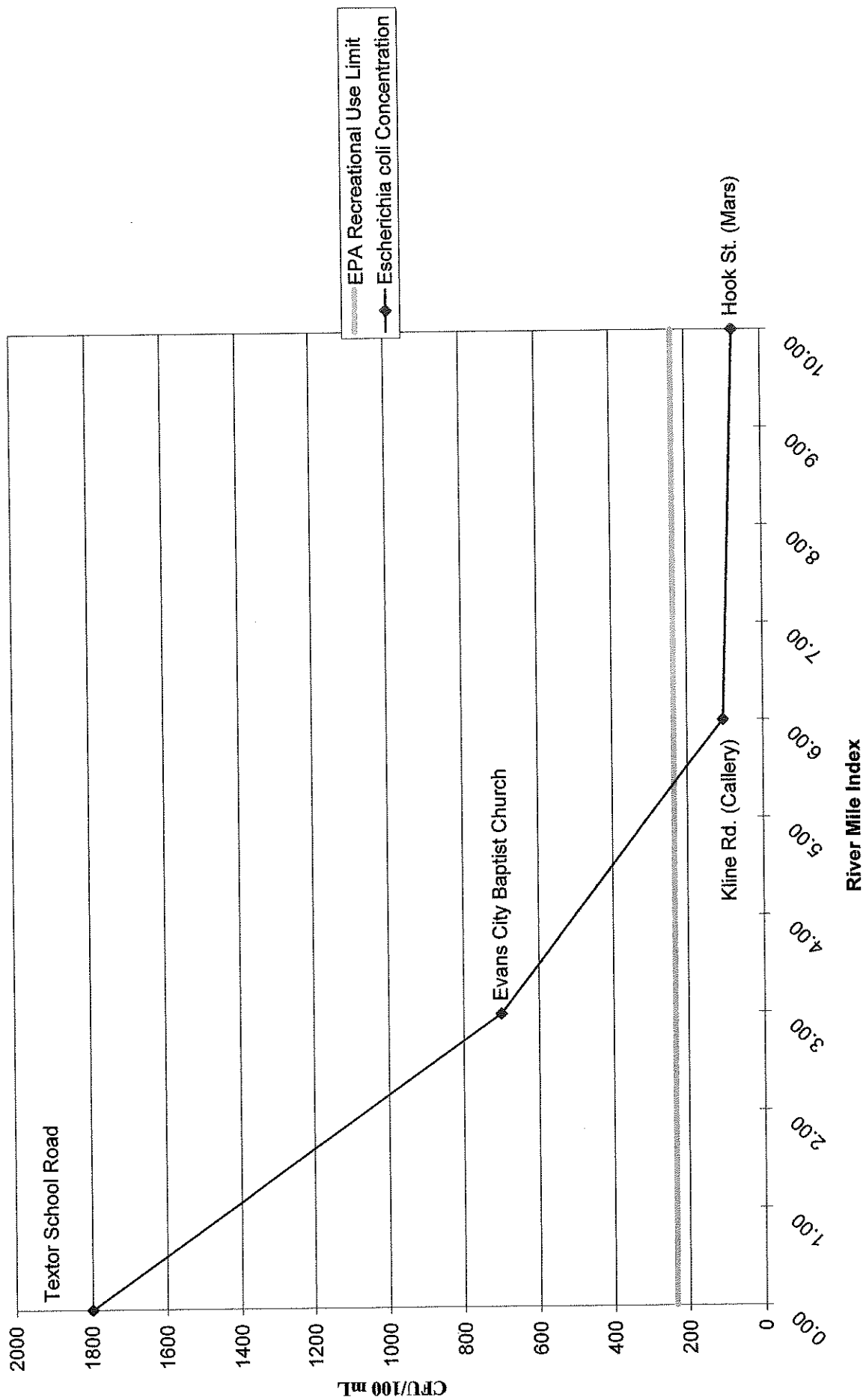


Figure 7
***E. coli* Levels in the Connoquenessing Creek Watershed**
Breakneck Creek Concentrations Vs. River Mile Index
December 29, 2004

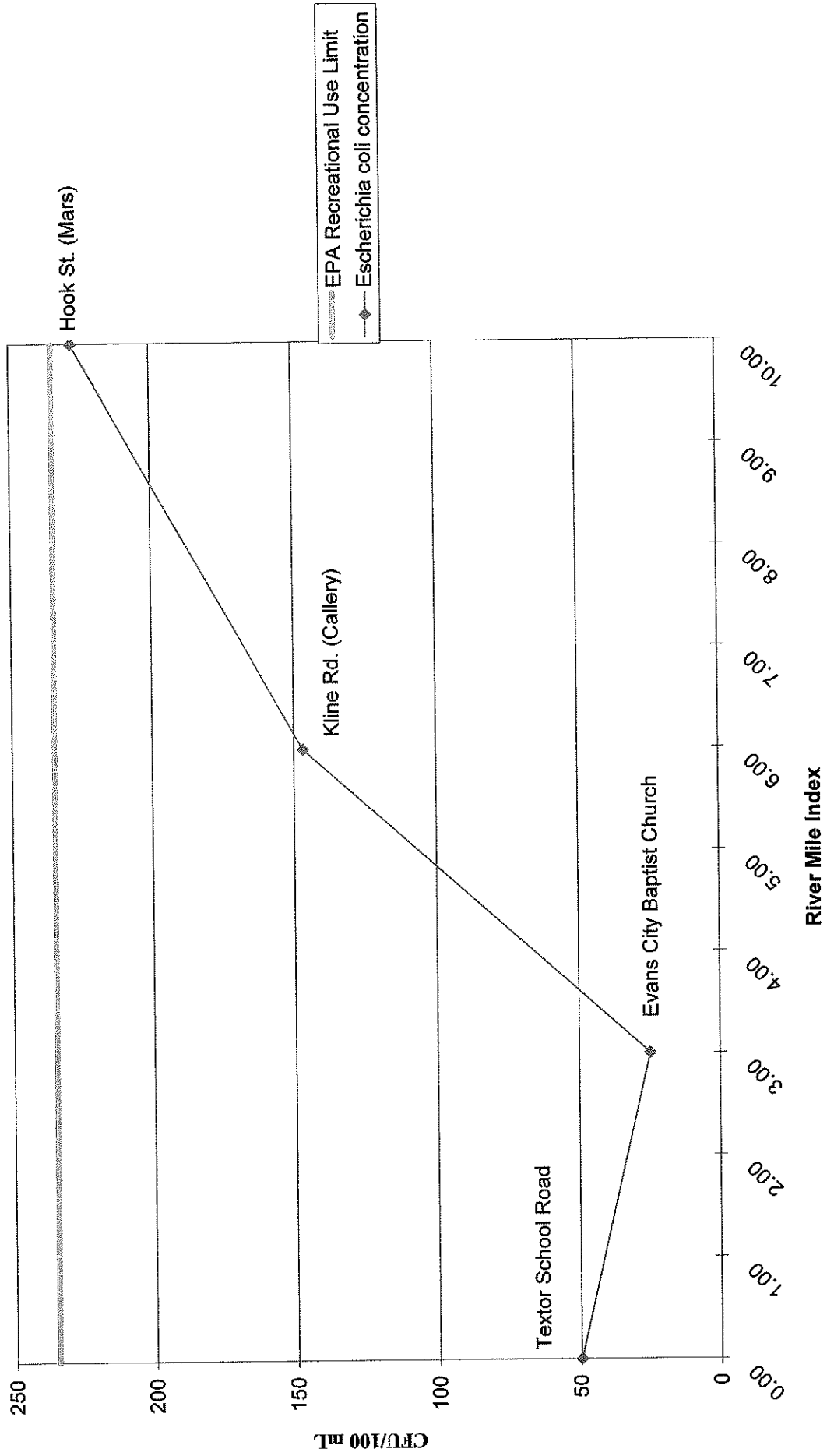


Figure 8
E. coli Levels in the Connoquenessing Creek Watershed
Little Connoquenessing Creek
Fall/Winter 2004

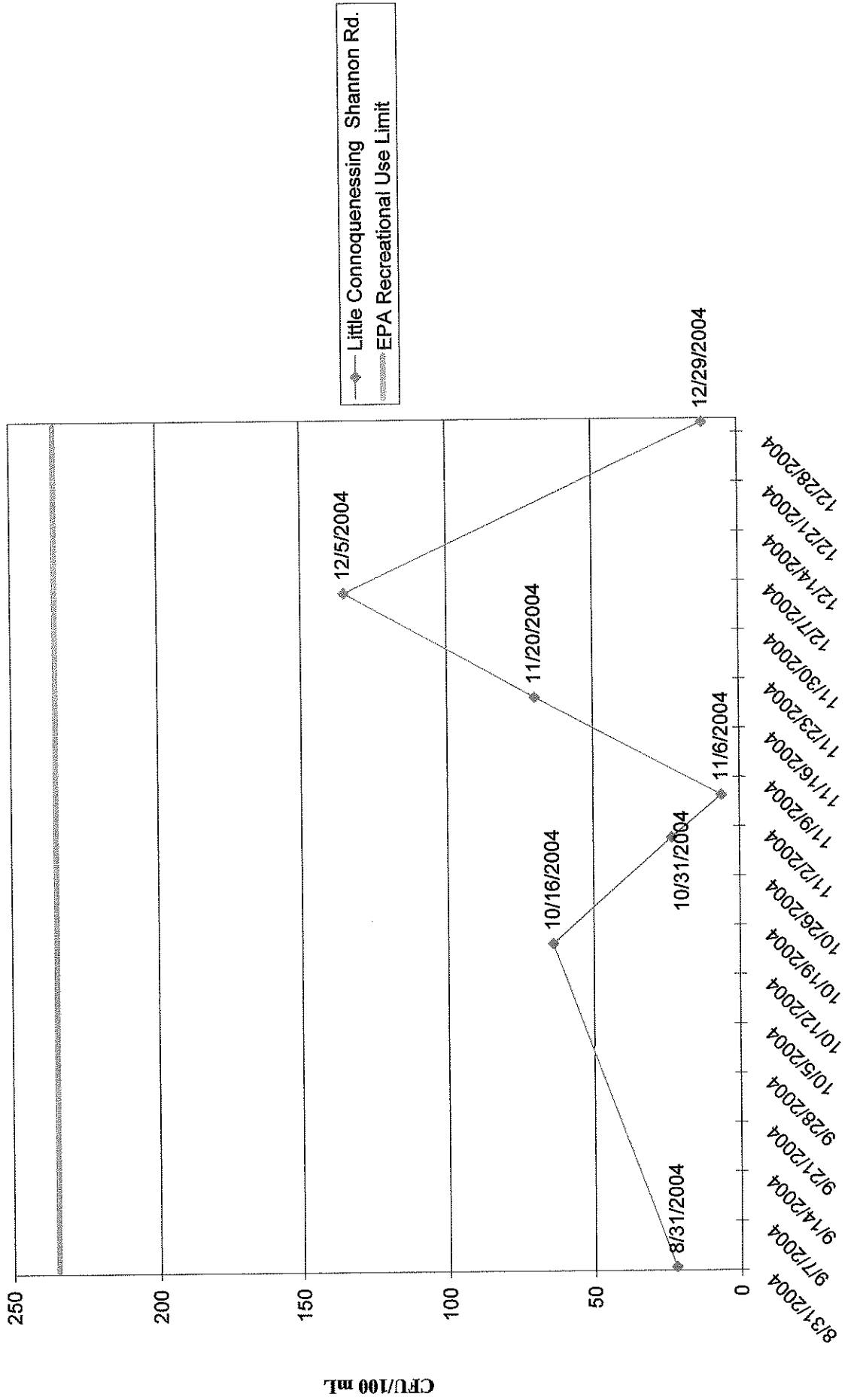


Figure 9
E. coli Levels in the Connoquenessing Creek Watershed
Brush Creek Locations
(Monitoring Locations in Upstream to Downstream Order)
Fall/Winter 2004

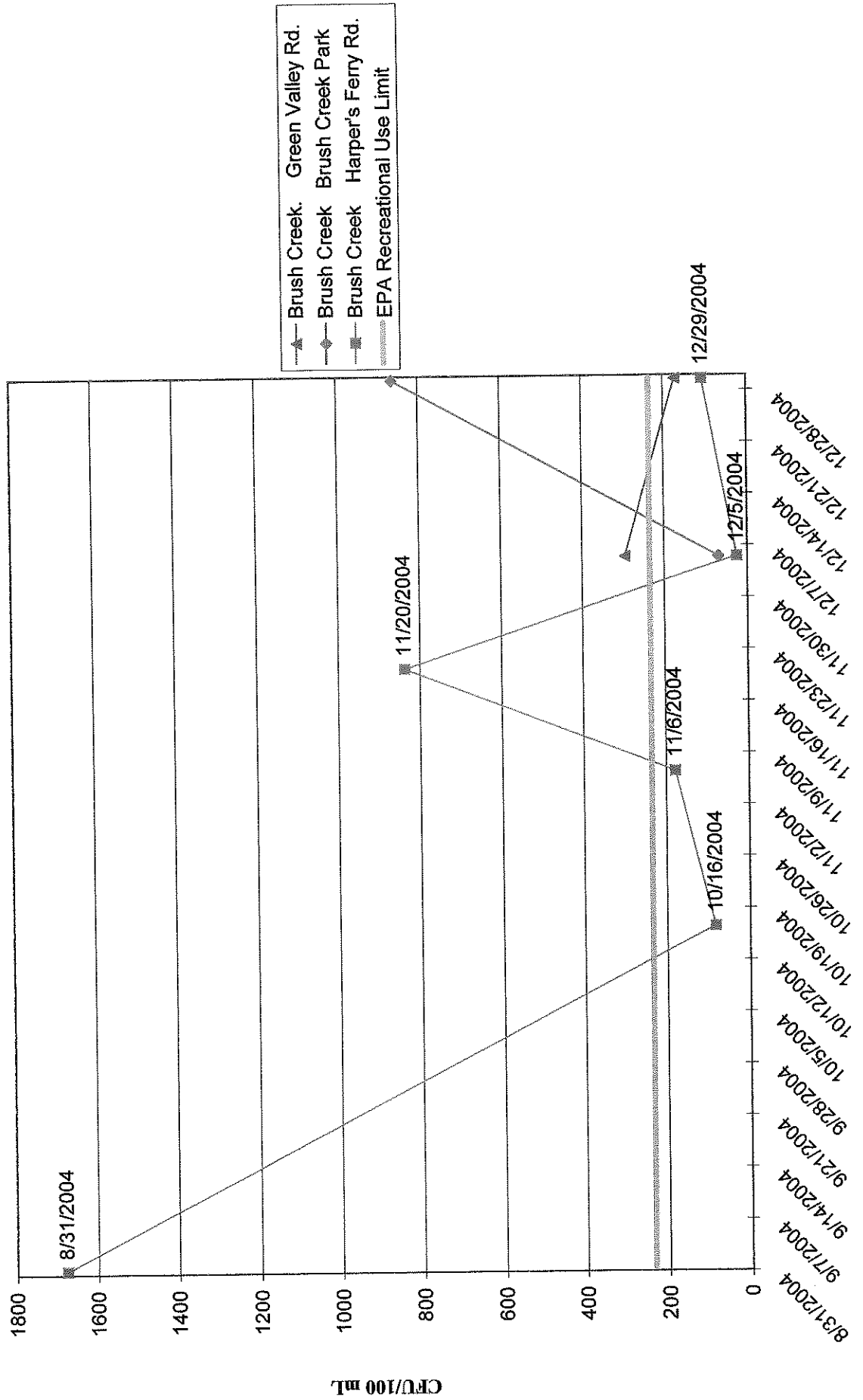


Figure 10
***E. coli* Levels in the Connoquenessing Creek Watershed**
Brush Creek Concentrations Vs. River Mile Index
December 5, 2004

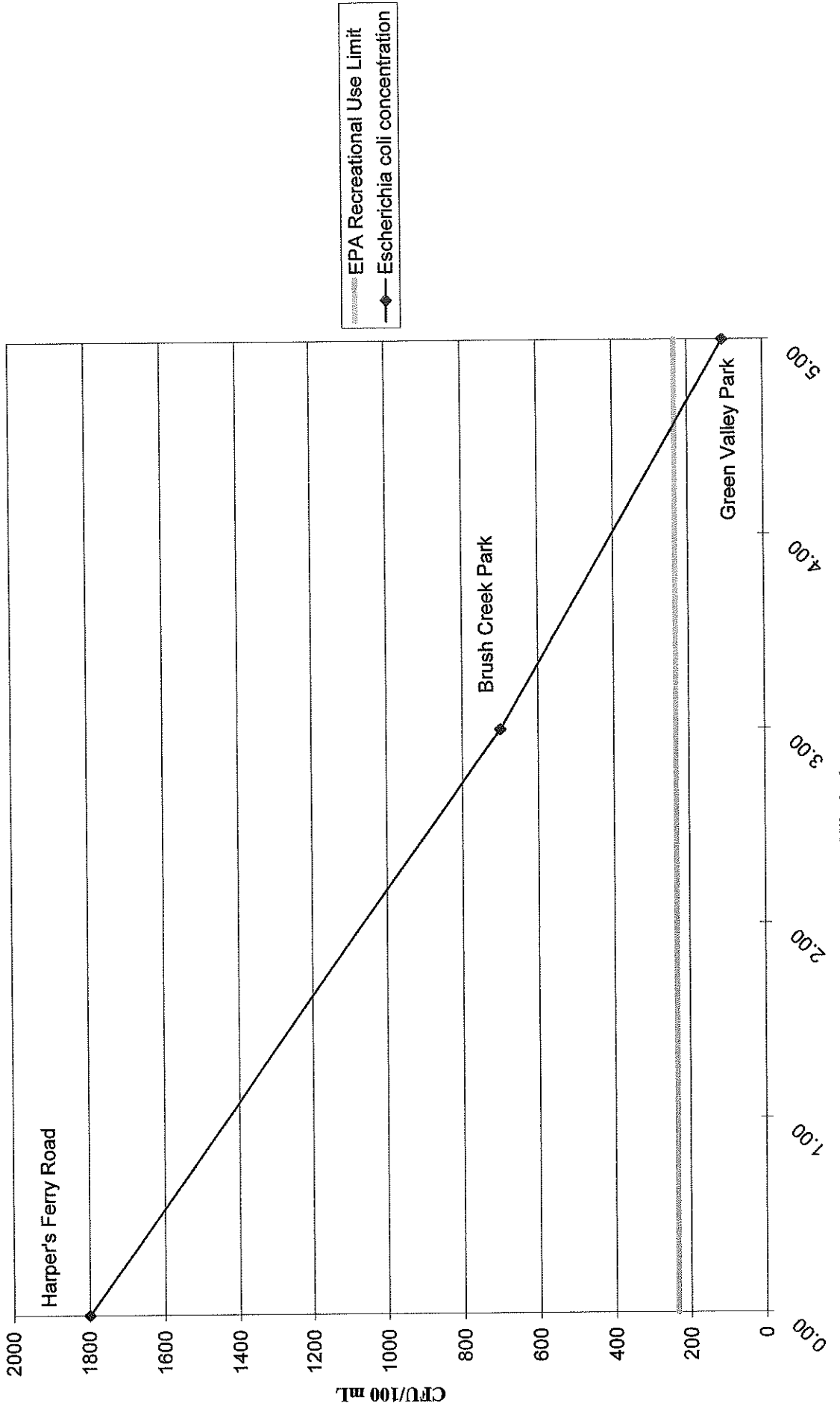


Figure 11
***E. coli* Levels in the Connoquenessing Creek Watershed**
Brush Creek Concentrations Vs. River Mile Index
December 29, 2004

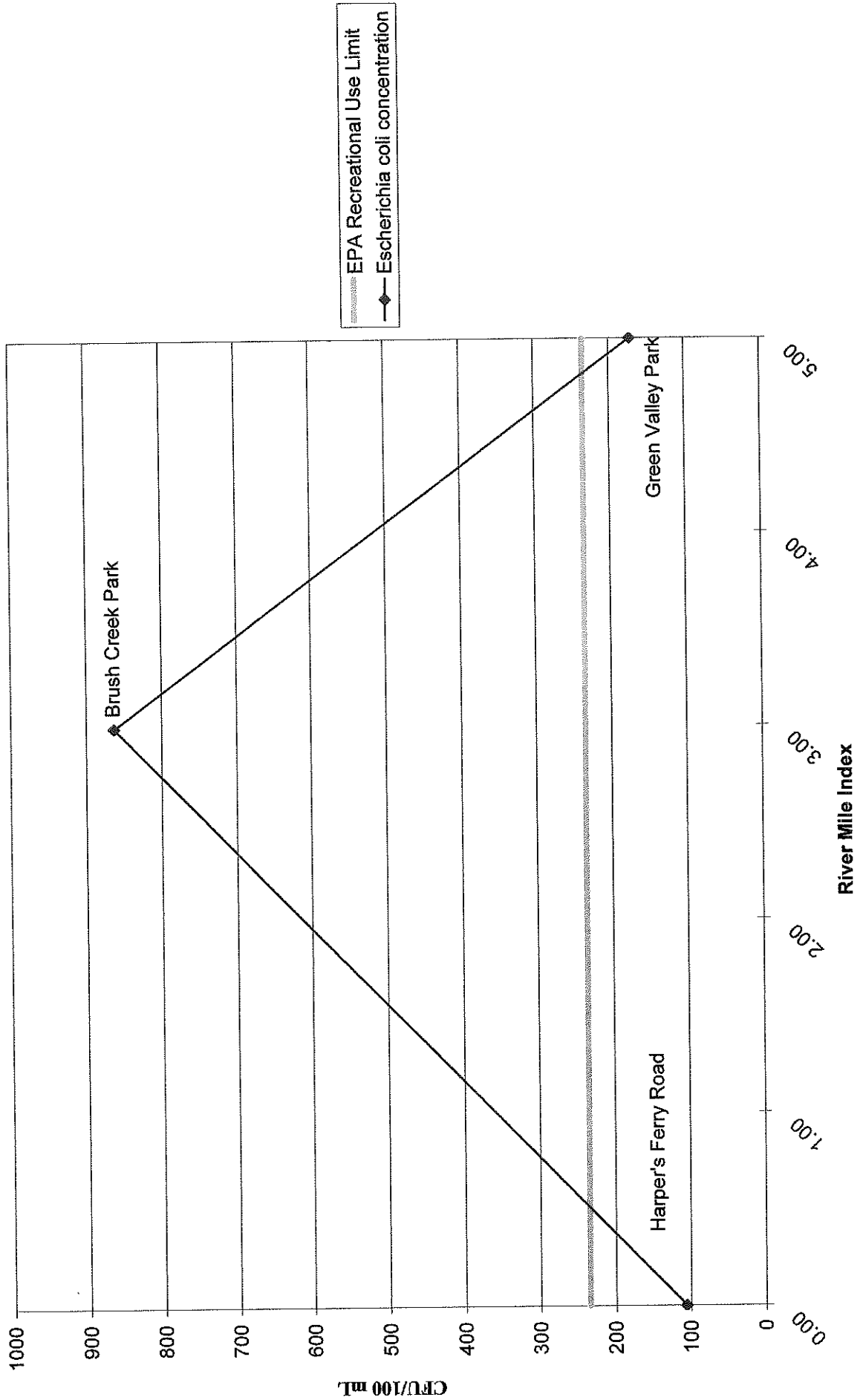


Figure 12
E. coli Levels in the Connoquenessing Creek Watershed
Muddy Creek
Fall/Winter 2004

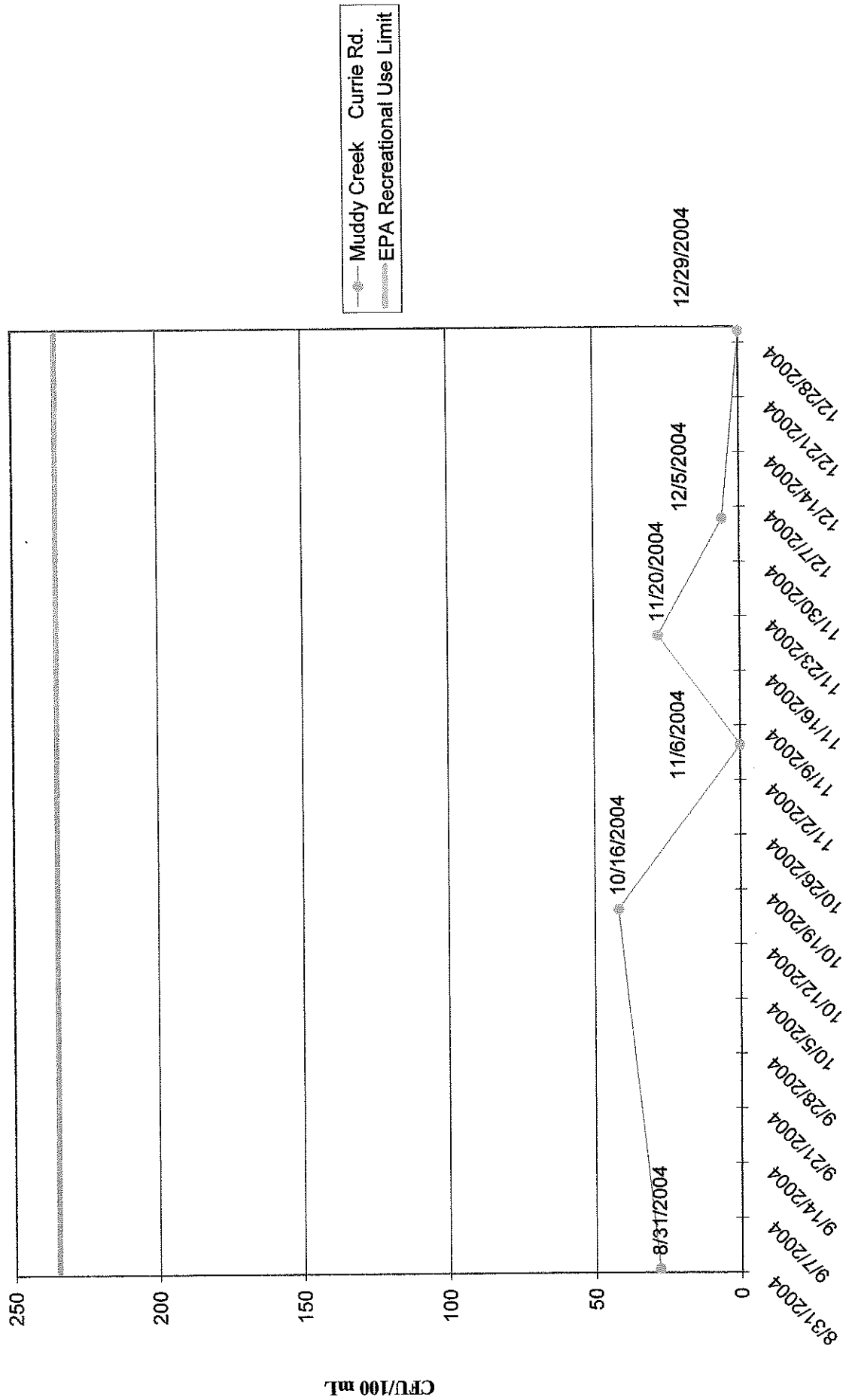


Figure 13
E. coli Levels in the Connoquenessing Creek Watershed
Slippery Rock Creek Locations
(Monitoring Locations in Upstream to Downstream Order)
Fall/Winter 2004

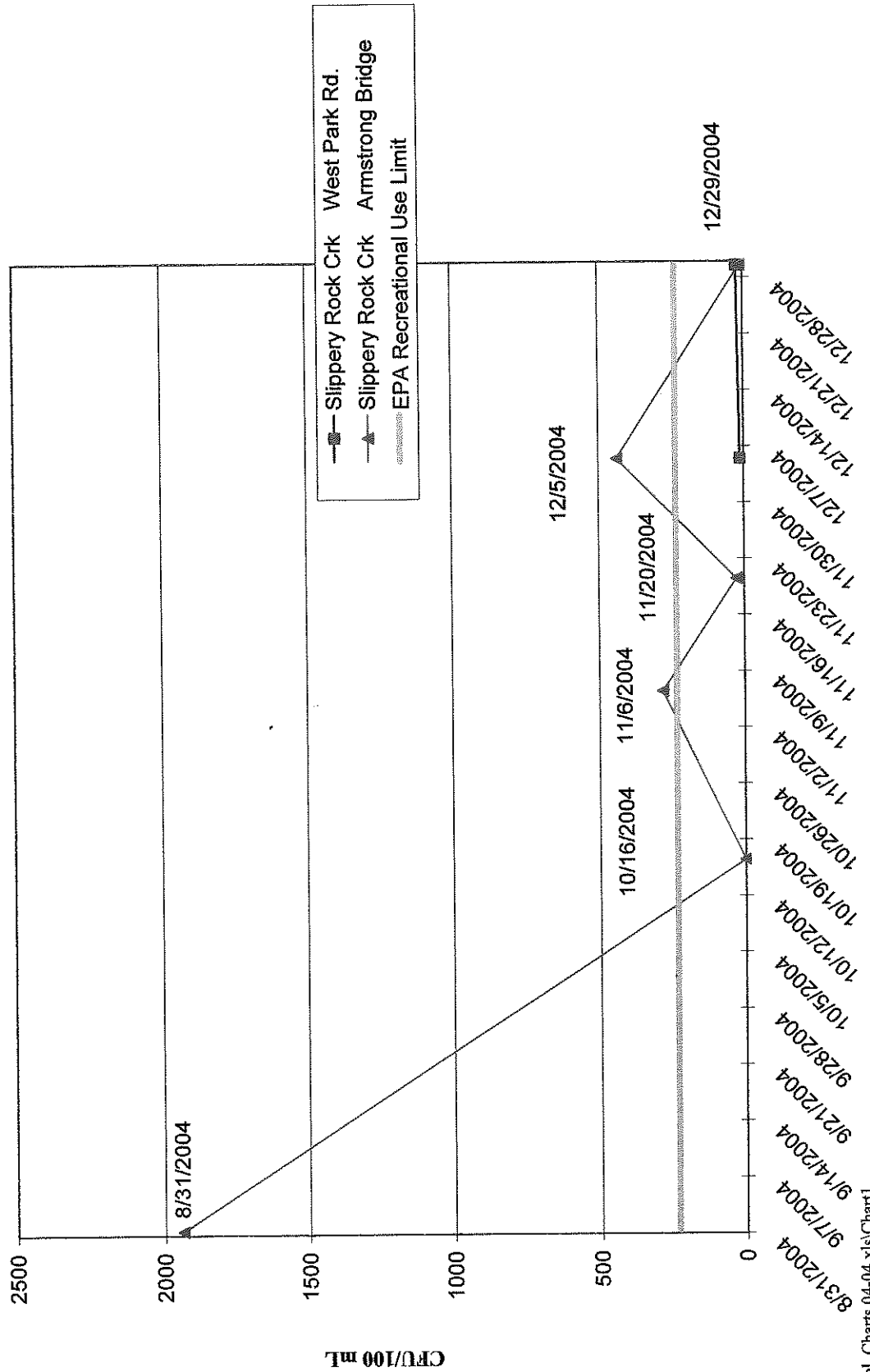
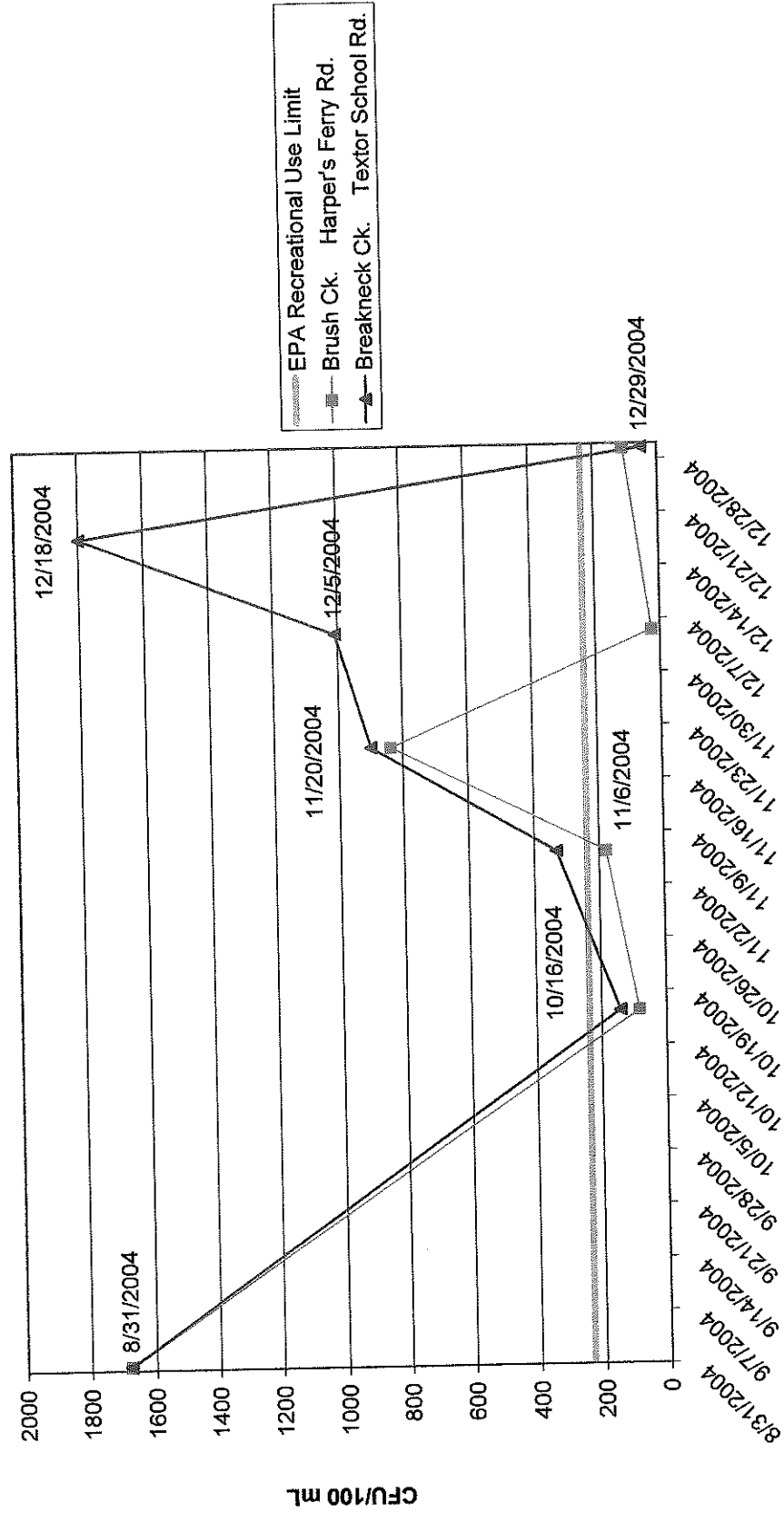


Figure 14
E. coli Levels in the Connoquenessing Creek Watershed
Breakneck and Brush Creek Locations
Fall/Winter 2004



DISCUSSION

A – FIELD SAMPLING RESULTS

The EPA recommends using a maximum geometric mean of 126 *E. coli* CFU/100 mL as a measure of fecal contamination. This is to be calculated from five samples taken over a 30 day period. This type of testing asks for a bigger commitment than the vast majority of volunteers can commit to since each testing requires two sequential days, for a total of 10 days per month. Consequently, the EPA (USEPA) single sample criterion of 235 *E. coli* CFU/100mL for recreational use was used as a benchmark, instead, to evaluate the fecal contamination of the streams. This benchmark is represented as a turquoise-colored line on all the figures.

It is relevant to note that local streams were significantly impacted by three hurricanes during the late summer and fall of 2004. Charlie occurred in August before our first sampling date which was on August 31, 2004. Frances passed through in early September, and the severe flooding of Ivan nine days later delayed a resumption of testing until October. The ground was saturated, not only from the hurricanes, but also from the higher than normal precipitation of the last two years. The combination of these factors appear to have led to a “flushing effect” in all of the tested streams which reduced *E. coli* counts for a period of time after the first testing date. After that, counts in most streams increased.

Most sites were sampled seven times spread over a four month period between Aug. 31, 2004 and Dec. 29, 2004. However, some sites were only sampled one or two times because they were added late in the study in an attempt to bracket problem areas. The data from all sites is recorded on **Table 2**.

The main stem of the Connoquenessing was sampled a total of twenty-one times, seven each at three different sites. It exceeded the recreational limit of 235 CFUs only two times (**See Figure 1**), once at Zelianople at the end of August, and again at Ashstop Rd. at the end of October. However, since the Connie is the largest stream in the watershed, dilution in this greater volume of water may present a picture of water quality which is not necessarily an accurate reflection of quality in the tributaries. Therefore, sites along the major tributaries were also selected for this study. These tributaries are discussed below in upstream to downstream order.

Thorn Creek was sampled seven times. The CFUs exceeded 235 two out of those seven times (**See Figure 2**). In fact, on 12/5/04 when the CFUs peaked at 458, there were also many pink (other coliform) colonies. Since the cause of these spikes is unknown, Thorn Creek is recommended for further study.

Glade Run also had two peaks somewhat above 235 CFUs (**See Figure 3**). They were on October 16 and Dec. 5th. On the last two sampling dates an additional site was added to try to determine where the problem was occurring. On Dec. 5th the number of CFUs increased by 217 between the Three Degree Road site and the Brownsdale Road site. The next time, however, CFUs at both sites were below the limit and Brownsdale Road was higher instead. There are many recreational camps along Glade Run. Malfunctioning septic systems in these camps may account for occasional spikes.

At first only one site on Breakneck Creek was sampled, on Textor School Rd., downstream of Evans City. The results of three out of four of these initial samplings yielded results over the recreational maximum. Consequently, three more sampling sites were added beginning on 12/5/2004 to begin to narrow down where the problem was occurring. These additional sites were at Mars, Callery, and Evans City. The *E. coli* count soared between Callery and Evans City from 111 CFU to 5322 (**See Figure 4**). While a specific cause for this high count was not confirmed, one speculation centered upon the possibility that Hurricane Ivan may have damaged the sewer line which runs along the creek from Callery to Evans City. (**See Figure 5**)

On 12/18/2004 one more site was added at Glenwood Dr. between Callery and Evans City. On this date (**See Figure 6**) the Mars and Callery sites were again below recreational limits, but the CFUs rose to 2108 at the new site, and then mysteriously dropped to 700 CFUs in Evans City just one mile downstream. On both of these dates the Textor School Rd. site also exceeded the recreational limit at 1007 and 1800 CFUs respectively.

Our last sampling date of 12/29/04 (**See Figure 7**) yielded much lower results, however; none of the five sites exceeded 235 CFUs. When CFUs at the Textor School site are lower than upstream, it may be caused by periodic releases of chlorinated effluent from the Evans City Sewage Treatment Plant which is upstream.

It remains uncertain what is causing the periodically high *E. coli* counts between Callery and Evans City. Consequently, this stream is also recommended for further study.

[Figures 4, 5 & 6, as well as Figures 10 & 11 which are discussed below, show the results obtained from all sampling sites on the stream in question on a given day. A particular site's "River mile index" refers to the distance in miles from that site to the mouth of the stream.]

The Little Connoquenessing Creek was also sampled seven times. It was a consistently "clean" stream with no *E. coli* readings even above 135 CFUs (See Figure 8). Like Thorn Creek, a small spike occurred on December 5th. Consequently, no further testing is recommended at this time.

A former sewage enforcement officer recommended Brush Creek for study. The initial sampling site was at Harper's Ferry Rd. The initial reading was very high at 1677 CFU/100 mL. Subsequent data from that site only exceeded the limit one additional time on 11/20/2004. (See Figure 9) Due to these very high counts, two additional sites were added, Brush Creek Park and Green Valley Park, beginning on 12/5/2004 (See Figure 10-11). The results were inconsistent and confusing. This stream is also recommended for additional testing.

Muddy Creek discharges from Lake Arthur in Moraine State Park. A site downstream from this outlet was chosen for sampling because in recent years there have been several sewage overflows into Lake Arthur from the collection lines that feed into a nearby sewage treatment plant. Even though this site was sampled six times, there were no readings above the recreational limit (See Figure 12). In fact, no counts exceeded 50 CFUs.

Slippery Rock Creek is a major tributary to the Connie. It is a heavily used recreational stream. Our first reading at Armstrong Bridge in August was 1938 CFUs (See Figure 13). The five subsequent readings oscillated from very low to slightly over the recreational limit. Consequently, in December we added another sampling site fifteen miles upstream to begin to get more data on this stream. Since a lot can happen in fifteen miles, Slippery Rock Creek is recommended for further study.

The results of this sampling series showed that Breakneck and Brush Creeks in particular seemed to be intermittently impacted to some extent by an unknown source(s) of fecal contamination. Figure 14 compares the fluctuations of these two streams. It is interesting to note that both streams

had similar low *E. coli* counts for the first five samplings before they began to rise significantly above the recreational limit. This may have been due to a "flushing" effect from Hurricane Ivan's flooding.

As has been indicated above, both Thorn Creek and Slippery Rock Creek also had occasional significant spikes of *E. coli*. Therefore, CWA intends to perform a second round of sampling in 2005 which will concentrate on these four streams. More sites on each stream will be sampled in an attempt to isolate ("bracket"), the sources of the fecal contamination.

B – COLISCAN® EASYGEL® SYSTEM EVALUATION

In order for coliforms to ferment lactose (i.e., have lunch), they must produce certain enzymes. Different types of coliforms produce different sets of enzymes. Identifying the presence of these enzymes can be used as an indicator for the presence of coliforms, and to some extent which types of coliforms are present. The Easygel® system used "chromogenic" substrates in the media which react to these enzymes by producing different color pigments which then color the colonies as they grow.

Four different colors of colonies are generally present in an incubated dish:

- Purple/blue – *E. coli*
- Pink/reddish – other coliform genera such as *Citrobacter*, *Enterobacter*, and *Klebsiella*, which are not necessarily fecal in origin
- Teal – non-coliform bacteria, possibly *Salmonella spp.* or *Shigella spp.*
- Pale gold/yellowish – non-coliform bacteria

The purple/blue colonies were counted since they are *E. coli*. However, it was noted if pink colonies were plentiful, or if any teal were present since such information provides supporting documentation of fecal contamination.

Some difficulty was occasionally experienced in differentiating purple/blue colonies from teal green, even though the manufacturer provided an actual comparison photograph that came with each kit. A few colonies appeared to be an intermediate color, rather than distinctly one or the other.

Early on in the study, as this difficulty was being noted, the lab technician in the group streak-plated several of these ambiguous colonies along with some pink colonies onto EMB (eosin methylene blue) agar. EMB is a differentially selective agar upon which *E. coli* colonies typically appear purple to blue/black with a green metallic sheen, while other colonies lack that green metallic sheen. For example, *Enterobacter aerogenes*, another

fecal coliform, will not have a green metallic sheen, and will characteristically be mucoid with a black center. The majority of the blue colonies selected from our plates were confirmed to be *E. coli* since they were dark with a green sheen on EMB; whereas, pink colonies streaked onto EMB did not exhibit this green sheen.

The use of hand lenses was sometimes useful in helping to differentiate these colonies one way or the other. In addition, experience and increasing familiarity appeared to improve the ability of the team members to determine whether or not a blue/green colony should be considered teal and therefore not to be included.

C – EXPERIENCES OF OTHER GROUPS WITH EASYGEL®

Since this study was completed in 2004, other groups have tested Coliscan® Easygel®. In the summer of 2005 the St. Louis River-River Watch program, along with Citizens Monitoring Bacteria, compared the reliability of Coliscan® Easygel® and 3M Petrifilm Kits with results obtained from an analytical lab. The *E. coli* counts obtained using Coliscan® Easygel® paralleled the analytical lab more closely than those from the 3M Petrifilm kits. (<http://www.uwex.edu/ces/csreesvolmon/EColi/FondduLacposter2005.pdf>)

According to Citizens Monitoring Bacteria a few other groups in the United States have used the Coliscan® Easygel® system to test local waters. These groups include the Alabama Water Watch in Alabama, Hoosier Riverwatch in Indiana, and IOWATER in Iowa. (<http://www.uwex.edu/ces/csreesvolmon/EColi/USVolunteerBactMonitor.htm>)

“In October 2003, six upper Midwestern states were awarded funding through the USDA Cooperative State Research Education, and Extension Service (CSREES) National Integrated Water Quality Program to assess the quality, reliability and usability of five different *E. coli* test kits with volunteer stream monitors.” During the summers of 2004 and 2005, Iowa’s IOWATER Program and Indiana’s Hoosier Riverwatch compared six methods of testing for fecal pollution using *E. coli*. They evaluated the accuracy, reliability, and usability of these kits by volunteers. The Coliscan® Easygel® was tested both with and without incubation. Incubated Coliscan® Easygel® was judged both the easiest to learn and the most reliable by the Indiana volunteers. However, they agreed that doing a good job distinguishing between teal and blue is challenging and is definitely a learned skill. (<http://www.usawaterquality.org/volunteer/EColi/ResultsProducts.htm>)

D – COLISCAN® EASYGEL® SUMMARY

Another problem noted was that sample sizes of 5 mL and above often did not set up as completely as lesser sample sizes in this study. The Midwestern study noted the same problem. Consequently, future studies will limit the maximum sample size to 5 mL.

The Coliscan® Easygel® System can be a useful tool for watershed groups as an adjunctive resource in helping to assess water quality. While for this study CWA had the participation of one of its members who is an experienced lab technician, it appears that this system may be reasonably employed by other adults who have no particular background in scientific and/or laboratory procedures as long as they take the recommended precautions. The components of the system are not difficult to use, and the instructions for use provided are not difficult to understand.

It is hoped that the *modus operandi* that has been developed here will be useful to other watershed groups who may contemplate adding a coliform study to their water quality assessment program. In the "Sampling and Handling Protocol and Procedures" section above, the methods employed were outlined in detail from start to finish. In particular, the great care and vigilance that must be constantly present when collecting and handling the samples was stressed in order to insure as much as possible that the results obtained are a reasonably accurate reflection of what's actually present in the sample, and that no one develops infections from any pathogens that are possibly present. This latter cannot be overstated.

The Easygel® system is not designed to be a "lab quality" test, therefore, "lab quality" results should not be expected. It is a field test, and as such the results obtained should be considered more as being approximate rather than precise. Still, assuming that the procedures are carefully followed, the results obtained can indicate whether or not enough *E. coli* bacteria, and therefore fecal contamination, are present to make such waters unsafe for recreational use. Plus it can give a pretty fair idea of the scope of that problem. Such results can be very useful in helping to determine if a more formal assessment, conducted under stricter laboratory protocols and conditions, is warranted.