



# **Fecal-Indicator Bacteria and *Escherichia coli* Pathogen Data Collected Near a Novel Sub-Irrigation Water-Treatment System in Lenawee County, Michigan, June–November 2007**

By Joseph W. Duris and Stephanie Beeler

In cooperation with Lenawee County Conservation District

Open File Report 2008–1025

**U.S. Department of the Interior  
U.S. Geological Survey**

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Suggested citation: Duris, J.W., and Beeler, Stephanie, 2008 fecal-indicator bacteria and *Escherichia coli* pathogen data collected near a novel sub-irrigation water-treatment system in Lenawee County, Michigan, June–November 2007: U.S. Geological Survey Open-File Report 2008–1025, 13 p.

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## Conversion Factors

| <b>Multiply</b> | <b>By</b> | <b>To obtain</b> |
|-----------------|-----------|------------------|
| Length          |           |                  |
| inch (in.)      | 2.54      | centimeter (cm)  |
| foot (ft)       | 0.3048    | meter (m)        |
| meter (m)       | 3.281     | foot (ft)        |
| yard (yd)       | 0.9144    | meter (m)        |
| Volume          |           |                  |
| gallon (gal)    | 3.785     | liter (L)        |

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$^{\circ}\text{F}=(1.8\times^{\circ}\text{C})+32$$

Small sample volumes are reported in milliliters (mL) and microliters ( $\mu\text{L}$ ); very small masses are given in nanograms (ng).

Bacteria concentrations are given in colony-forming units per 100 milliliters (CFU/100 mL).

# **Fecal-Indicator Bacteria and *Escherichia coli* Pathogen Data Collected Near a Novel Sub-Irrigation Water-Treatment System in Lenawee County, Michigan, June–November 2007**

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## **Abstract**

The U.S. Geological Survey, in cooperation with the Lenawee County Conservation District in Lenawee County, Mich., conducted a sampling effort over a single growing season (June to November 2007) to evaluate the microbiological water quality around a novel livestock reservoir wetland sub-irrigation system. Samples were collected and analyzed for fecal coliform bacteria, *Escherichia coli* (*E. coli*) bacteria, and six genes from pathogenic strains of *E. coli*.

A total of 73 water-quality samples were collected on nine occasions from June to November 2007. These samples were collected within the surface water, shallow ground water, and the manure-treatment system near Bakerlads Farm near Clayton in Lenawee County, Mich. Fecal coliform bacteria concentrations ranged from 10 to 1.26 million colony forming units per 100 milliliters (CFU/100 mL). *E. coli* bacteria concentrations ranged from 8 to 540,000 CFU/100 mL. Data from the *E. coli* pathogen analysis showed that 73 percent of samples contained the *eaeA* gene, 1 percent of samples contained the *stx2* gene, 37 percent of samples contained the *stx1* gene, 21 percent of samples contained the *rfbO157* gene, and 64 percent of samples contained the LTIIIa gene.

## **Introduction**

The management of animal wastes on dairy farms is commonly driven by the need of farmers to effectively manage nutrient content in soils where crops are grown, as well as to control and manage manure in a way that avoids negative effects on local surface water and ground water. Typically, the management of manure in and around a farm is locally controlled by use of comprehensive nutrient-management plans that define how manure will be handled on the farm under various conditions. To meet the guidelines of these management practices, the Bakerlads Farm, working with the U.S. Department of Agriculture and the Lenawee County Conservation District, in 2005 installed novel manure-management system called a Livestock Reservoir Wetland Sub-Irrigation System (LRWSIS) on the farmstead, which is near Clayton, Mich (fig. 1; all figures and tables are at back of report).

The U.S. Geological Survey (USGS), in cooperation with the Lenawee County Conservation District (LCCD) in Lenawee County, Mich., sampled nine sites (surface water and ground water) over a single growing season, from June to November 2007. The purpose of this study was to evaluate the microbiological water quality around the LRWSIS. By studying the populations of fecal coliform and *E. coli* bacteria and by characterizing the types of *E. coli* pathogens and other bacterial markers in manure sources, we can assess how manure-management practices may contribute to indicator and pathogen loading in environmental waters. This could lead to a better understanding of how and why bacterial indicators and pathogens might be found in other environmental settings affected by agriculturally influenced waters. In addition, this work builds on a growing body of work by the USGS Michigan Water Science Center related to understanding how manure management affects the transport of fecal indicator and pathogenic bacteria. Samples were collected and analyzed for fecal coliform bacteria, *Escherichia coli* (*E. coli*), and six genes from pathogenic strains of *E. coli*, including *E. coli* O157:H7. This report summarizes the results of this sampling.

## Methods

### Site Information

The LRWSIS directs dairy barn wash water and runoff from the entire facility into a primary settling reservoir. Once settled, the liquid waste is then transferred into an 80,000 gal constructed wetland via a 3-in. subsurface PVC pipe that bridges a small unnamed tributary to the South Branch of the River Raisin. The liquid waste from the influent pond of the constructed wetland is allowed to move via subsurface flow through a packed layer of sand and pea gravel, planted with wetland vegetation, into the subsurface irrigation holding pond. This water is then pumped directly into a closed subsurface tile irrigation system below a planted cornfield, where it supplies water and nutrients to the crop that is planted above.

Samples for this study were collected from nine locations in and around the Bakerlads Farm (table 1 and fig. 1). For flowing water, the tributary to the South Branch of the River Raisin was sampled at three locations: upstream from the sub-irrigated field and the LRWSIS, downstream from the sub-irrigated field, and downstream from the LRWSIS. For ground water, samples were collected from two piezometers within the cropped area of the sub-irrigated field and one piezometer 10 m from the bank of the tributary to the South Branch of the River Raisin. For ponded water, samples were collected from the primary settling pond, the influent pond of the wetland system, and the sub-irrigation holding pond of the wetland system to evaluate potential inputs to the ambient water. Samples were collected on nine dates over the 2007 growing season, from June 25 through November 27, 2007 (table 2).

### Sample Collection

Samples were collected by the LCCD staff using standard USGS sampling procedures documented by the USGS National Field Manual (Myers, 2003). Each sample was evaluated for fecal coliform bacteria and *E. coli* and for six genes from pathogenic *E. coli*.

## Bacteriological Indicator Organisms

Samples were examined for the presence of fecal-indicator bacteria by means of standard membrane filtration and serial dilution methods as indicated in Britton and Greeson (1989), whereas *E. coli* analysis followed protocols of the American Public Health Association and others (1998), method 9222D/9222G. Fecal coliform bacteria from the membrane filter with the most growth for each site and date were collected and suspended in 1 mL of phosphate buffered saline (PBS) with a final concentration of 15 percent glycerol. This fecal coliform stock (FC) was used for all subsequent analysis for pathogenic *E. coli*.

## *E. coli* Gene Analysis

The FC stocks were analyzed by use of three polymerase chain reaction (PCR) methods. Deoxyribonucleic acid (DNA) was extracted from one-tenth of the volume of the FC stock, using alkaline polyethylene glycol (APEG) DNA extraction (Chomczynski and Rymaszewski, 2006). A volume of 100  $\mu$ L of FC stock was transferred to a volume of 1,000  $\mu$ L of APEG reagent and incubated at 55°C for 30 minutes, at which point it was stored at 4°C until PCR analysis for *E. coli* pathogen genes.

A volume of 1  $\mu$ L of DNA representing 1–100 ng of DNA was used to conduct all PCR analysis. Samples were analyzed for the gene responsible for the O157 type (*rfbO157*) as indicated by Osek (2003). The gene targeted by this assay is the gene responsible for the production of the specific antigen found on the surface of an *E. coli* O157 cell.

The samples were also analyzed for the pathogenic *E. coli* toxin genes *eaeA*, *stx2*, and *stx1* (Fagan and others, 1999; Sabat and others, 2000). The genes targeted by this assay are those required to confirm the presence of enterohemorrhagic *E. coli* in clinical samples (Nataro and Kaper, 1998; Fagan and others 1999). The method was modified to include analysis for a gene common to all *E. coli*, both pathogenic and nonpathogenic, which was used to confirm the presence of *E. coli* (Duris and others, 2003; Fogarty and others, 2005). The LTIIa gene, carried by some pathogenic *E. coli* strains common to bovine sources, was analyzed according to Khatib and others (2002) and Chern and others (2004). A summary of all genes and their common functions and associations can be found in table 3. All gene assays followed standard protocols (U.S. Environmental Protection Agency, 2004). All methods included positive controls and method blanks.

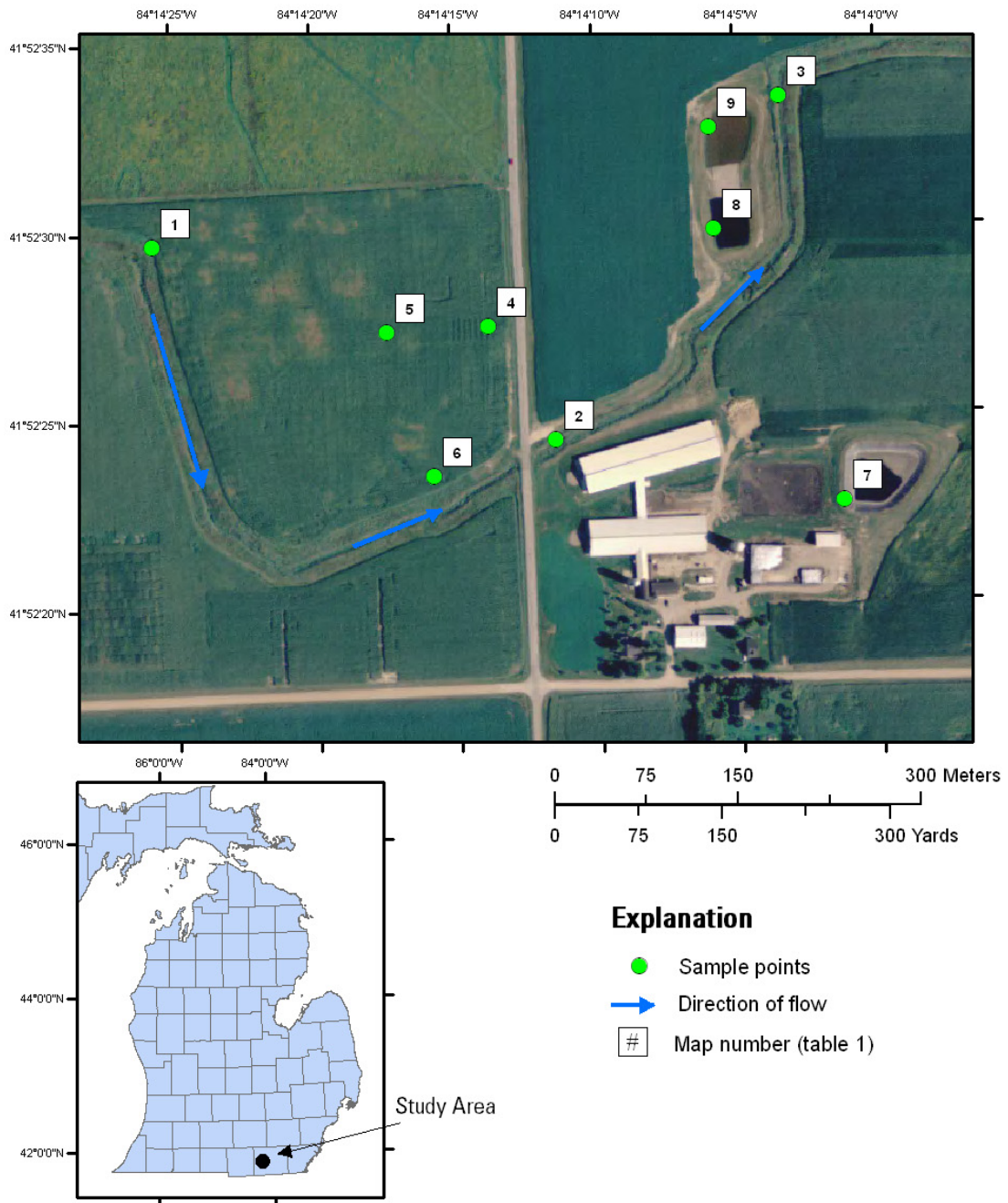
## Results

Water-quality samples were collected on nine occasions from June to November 2007. A total of 73 water samples were collected within the surface water, shallow ground water, and manure-treatment system near Bakerlads Farm, in Lenawee County, Mich. Fecal coliform bacteria concentrations ranged from 10 to 1.26 million CFU/100 mL. *E. coli* bacteria concentrations ranged from 8 to 540,000 CFU/100 mL. Data from the *E. coli* pathogen analysis showed that 73 percent of samples contained the *eaeA* gene, 1 percent of samples contained the *stx2* gene, 37 percent of samples contained the *stx1* gene, 21 percent of samples contained the *rfbO157* gene, and 64 percent of samples contained the LTIIa gene. The EC gene that is common to all *E. coli* was present in 97 percent of samples. The data from the fecal-indicator bacteria testing at each site are presented in table 4 and figures 2–10. Data from the *E. coli* pathogen analysis are presented in table 4. Those samples that had countable *E. coli* but no detectable EC gene were considered not amplifiable by PCR and are noted in table 4.

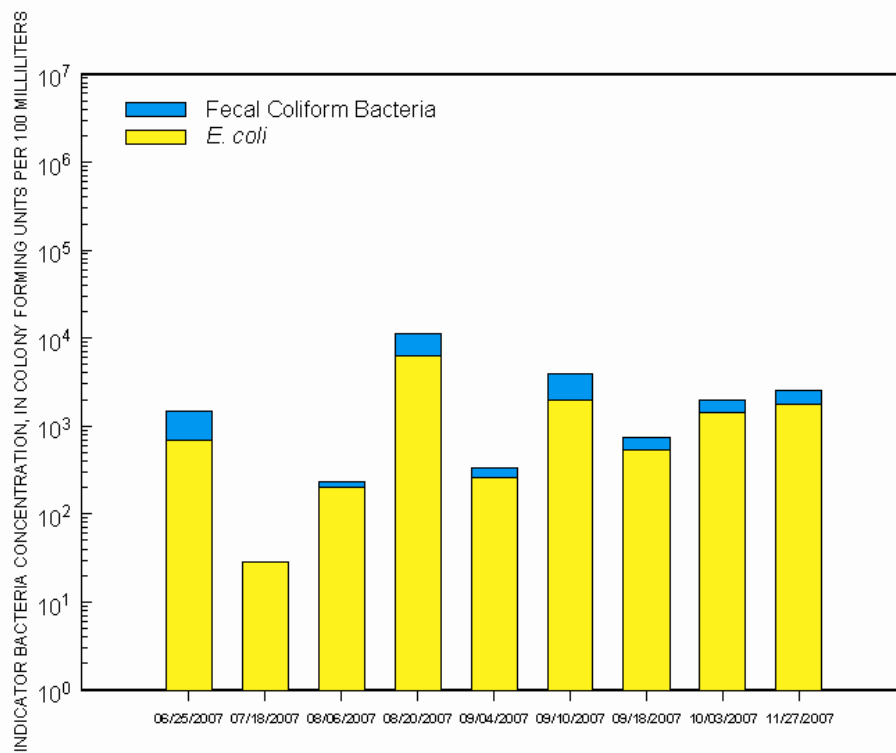
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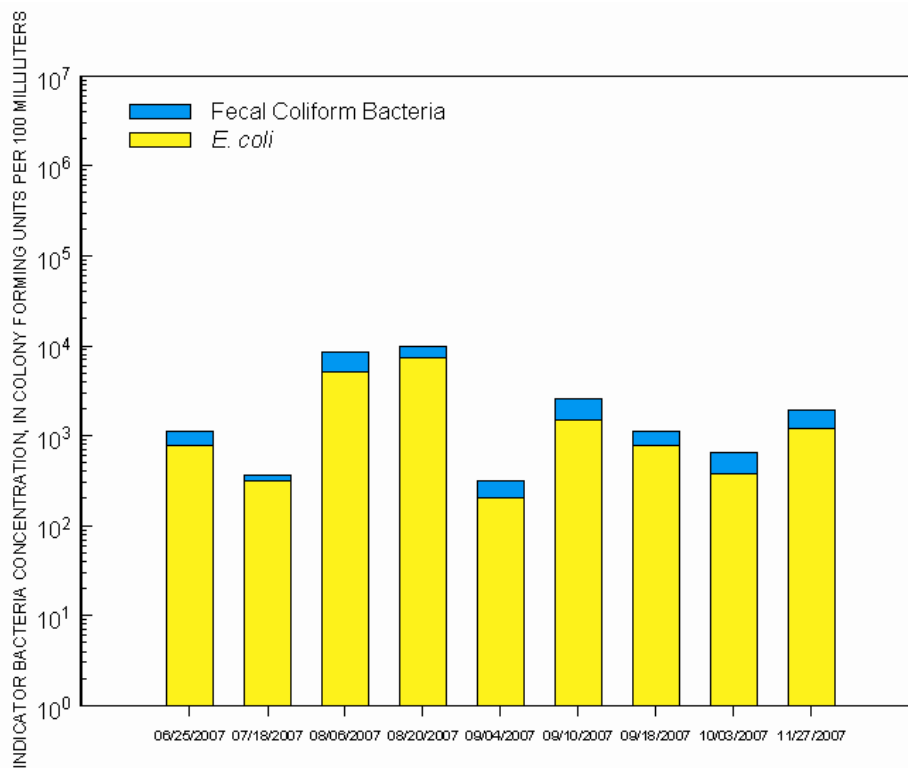




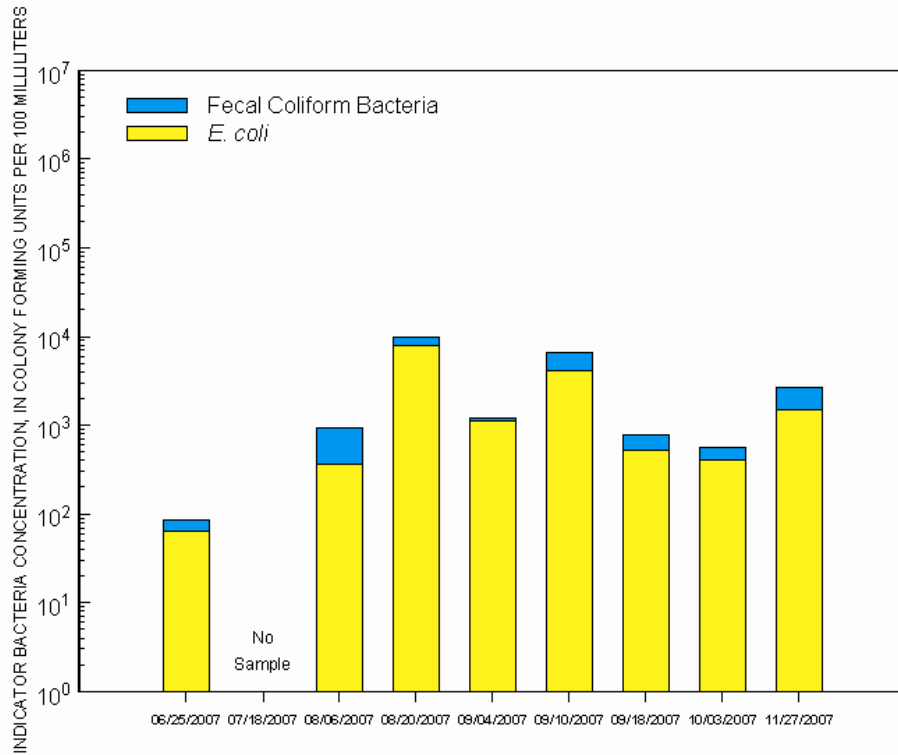
**Figure 1.** Sampling locations for fecal indicator bacteria and *E. coli* pathogen study, Lenawee County, Mich., 2007.



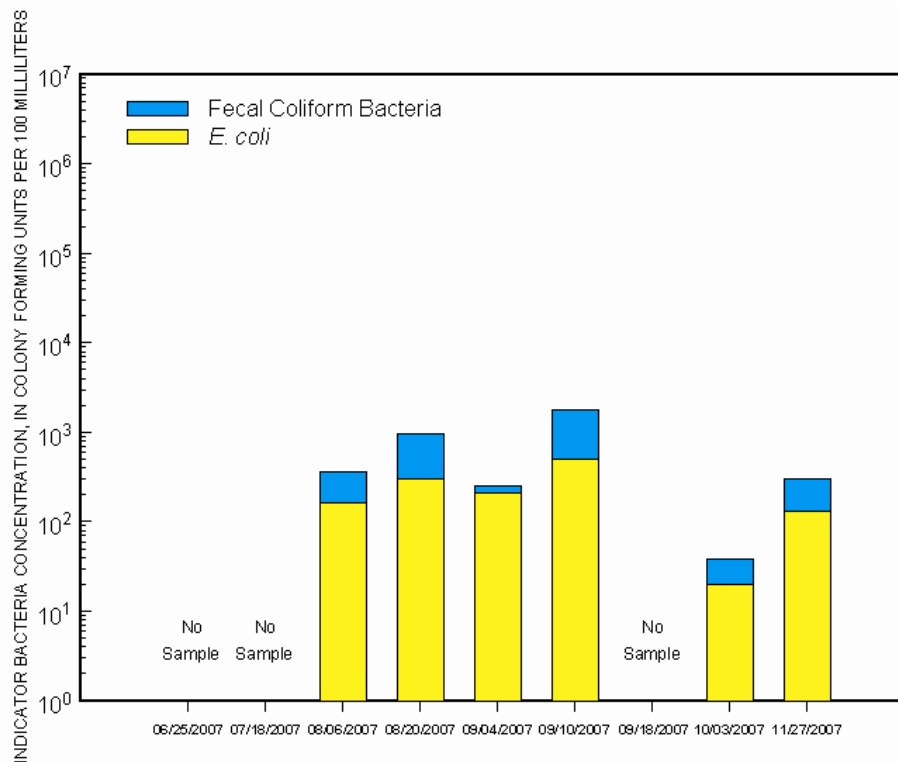
**Figure 2.** Fecal coliform bacteria and *Escherichia coli* concentrations by date for station 415233084140309 (D1), in Lenawee County, Mich., 2007.



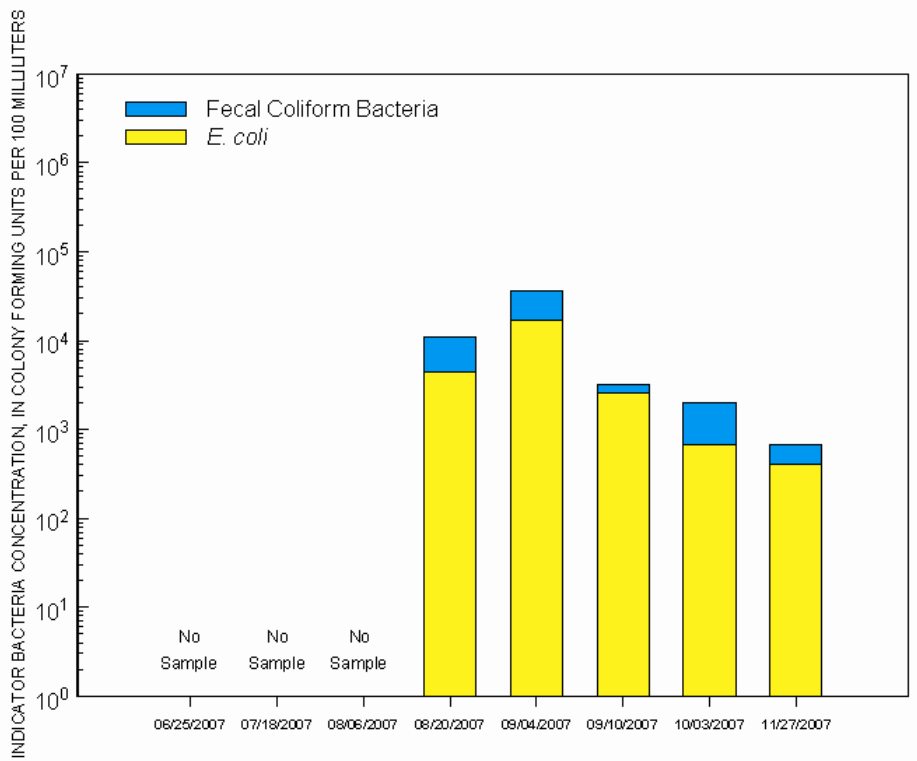
**Figure 3.** Fecal coliform bacteria and *Escherichia coli* concentrations by date for station 415224084141108 (D2), in Lenawee County, Mich., 2007.



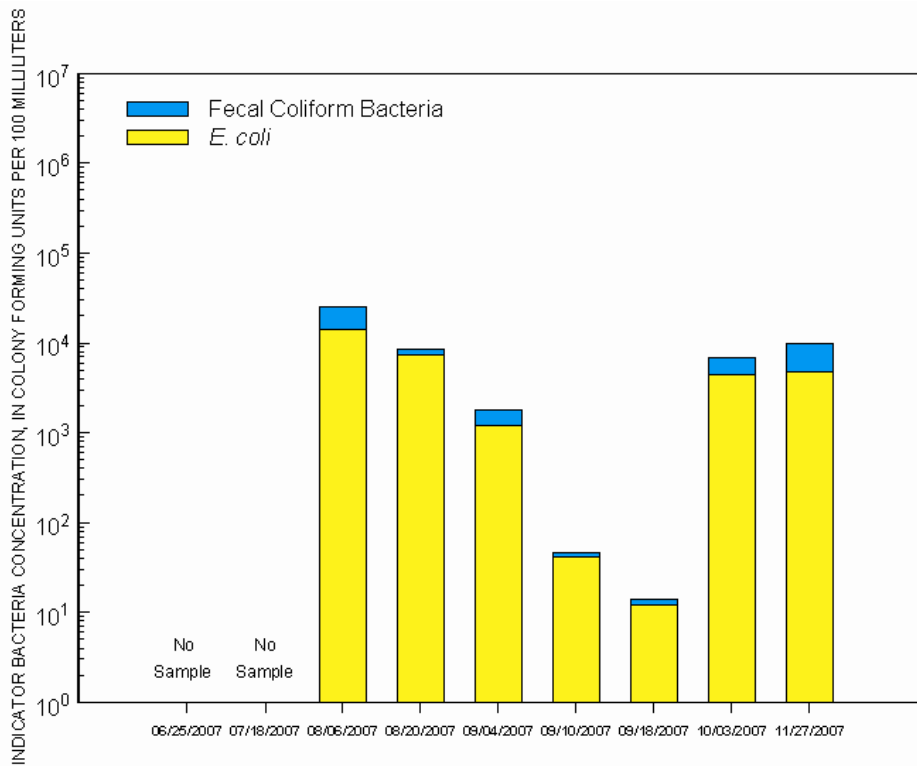
**Figure 4.** Fecal coliform bacteria and *Escherichia coli* concentrations by date for station 415230084142607 (D4), in Lenawee County, Mich., 2007.



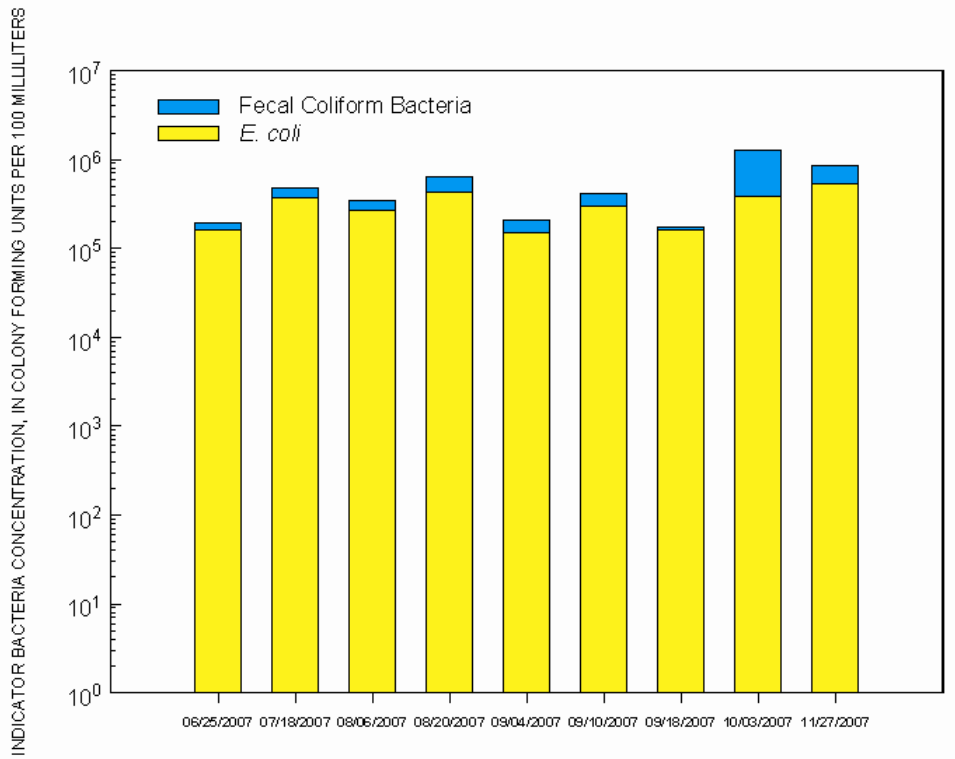
**Figure 5.** Fecal coliform bacteria and *Escherichia coli* concentrations by date for station 415224084141604 (W1), in Lenawee County, Mich., 2007.



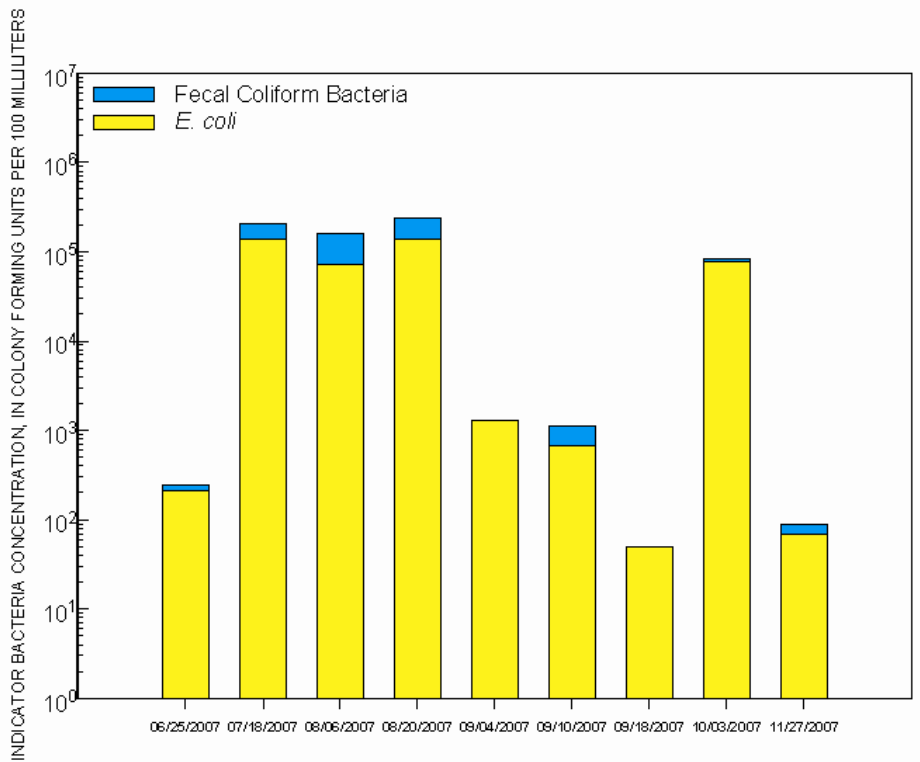
**Figure 6.** Fecal coliform bacteria and *Escherichia coli* concentrations by date for station 415227084141405 (W2), in Lenawee County, Mich., 2007.



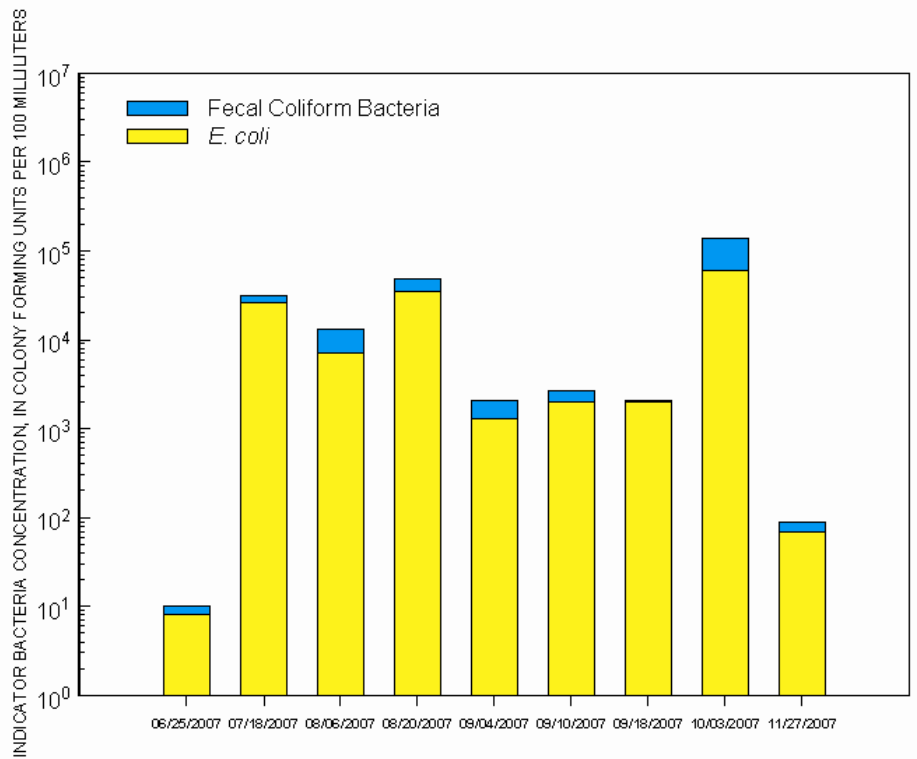
**Figure 7.** Fecal coliform bacteria and *Escherichia coli* concentrations by date for station 415227084141806 (W3), in Lenawee County, Mich., 2007.



**Figure 8.** Fecal coliform bacteria and *Escherichia coli* concentrations by date for station 415223084140101 (P2), in Lenawee County, Mich., 2007.



**Figure 9.** Fecal coliform bacteria and *Escherichia coli* concentrations by date for station 415230084140602 (P3), in Lenawee County, Mich., 2007.



**Figure 10.** Fecal coliform bacteria and *Escherichia coli* concentrations by date for station 415233084140603 (P4), in Lenawee County, Mich., 2007.

**Table 1. U.S. Geological Survey stations sampled in Lenawee County, Michigan.**  
 [USGS, U.S. Geological Survey; ID, Identification]

| USGS station ID | USGS field ID | Map number | USGS station name   | Station type  |
|-----------------|---------------|------------|---|---------------|
| 415230084142607 | D4            | 1          | Tributary to South Branch River Raisin (site D4) near Clayton, MI | Surface water |
| 415224084141108 | D2            | 2          | Tributary to South Branch River Raisin (site D2) near Clayton, MI | Surface water |
| 415233084140309 | D1            | 3          | Tributary to South Branch River Raisin (site D1) near Clayton, MI | Surface water |
| 415227084141806 | W3            | 4          | Unnamed Well W3 near Clayton, MI                                  | Ground water  |
| 415227084141405 | W2            | 5          | Unnamed Well W2 near Clayton, MI                                  | Ground water  |
| 415224084141604 | W1            | 6          | Unnamed Well W1 near Clayton, MI                                  | Ground water  |
| 415223084140101 | P2            | 7          | Unnamed Storage Lagoon P2 near Clayton, MI                        | Place of use  |
| 415230084140602 | P3            | 8          | Unnamed Wetland Pond P3 near Clayton, MI                          | Place of use  |
| 415233084140603 | P4            | 9          | Unnamed Wetland Pond P4 near Clayton, MI                          | Place of use  |

**Table 2. Sampling dates and conditions.**

| Sample number | Sampling date | Condition                              |
|---------------|---------------|--|
| 1             | 6/25/2007     | 4 days prior to treatment system start |
| 2             | 7/18/2007     | Day 20 of system operation             |
| 3             | 8/6/2007      | Day 39 of system operation             |
| 4             | 8/20/2007     | Day 53 of system operation             |
| 5             | 9/4/2007      | Day 68 of system operation             |
| 6             | 9/10/2007     | Day 74 of system operation             |
| 7             | 9/18/2007     | Day 82 of system operation             |
| 8             | 10/3/2007     | 10 days post system shutdown           |
| 9             | 11/27/2007    | 37 days after field drained            |

**Table 3. Microbiological analytes and supplemental information for fecal-indicator-bacteria analysis.**  
 [*E. coli*, *Escherichia coli*; NA, Not Applicable]

| Analyte        | Category        | Gene product         | Importance   |
|----------------|-----------------|----------------------|--|
| Fecal coliform | Indicator group | NA                   | Commonly used group of fecal indicator bacteria.   |
| <i>E. coli</i> | Indicator group | NA                   | Commonly used species of fecal indicator bacteria.   |
| <i>eaeA</i>    | Gene            | Intimin              | Product causes tight binding of pathogenic <i>E. coli</i> to intestinal cells (Nataro and Kaper, 1998; Fagan and others, 1999).                      |
| <i>stx1</i>    | Gene            | Shiga toxin 1        | Product causes intestinal cell death. <i>stx1</i> is associated with bovine sources (Nataro and Kaper, 1998; Fagan and others, 1999).                |
| <i>stx2</i>    | Gene            | Shiga toxin 2        | Product causes intestinal cell death. When found with <i>eaeA</i> is associated with human disease (Nataro and Kaper, 1998; Fagan and others, 1999). |
| EC             | Gene            | 16s rRNA             | Common to all <i>E. coli</i> , pathogenic and nonpathogenic (Fogarty and others, 2005).  |
| <i>rfbO157</i> | Gene            | O157 surface protein | Gene is marker for <i>E. coli</i> O157:H7 (Osek, 2003).  |
| LTIa           | Gene            | Heat labile toxin    | Product causes diarrheal disease in calves, commonly associated with bovine sources (Khatib and others, 2002; Chern and others, 2004).               |

**Table 4. Microbiological data collected in Lenawee County, Michigan.**

[USGS, U.S. Geological Survey; *E. coli*, *Escherichia coli*; EC, *E. coli* 16S ribosomal DNA gene that is common to all *E. coli*; +, gene present in sample; -, gene absent in sample; ns, no sample; na, not amplifiable with polymerase chain reaction]

| USGS station ID | Field ID | Date      | Colony forming units per<br>100 milliliters water |                | <i>Escherichia coli</i> gene data |             |             |    |            |       |
|-----------------|----------|-----------|---|----------------|-----------------------------------|-------------|-------------|----|------------|-------|
|                 |          |           | Fecal<br>coliforms                                | <i>E. coli</i> | <i>eaeA</i>                       | <i>stx2</i> | <i>stx1</i> | EC | <i>rfb</i> | LTIIa |
| 415233084140309 | D1       | 6/25/2007 | 1,500   | 700            | +                                 | -           | -           | +  | -          | +     |
| 415224084141108 | D2       | 6/25/2007 | 1,100   | 780            | +                                 | -           | -           | +  | -          | -     |
| 415230084142607 | D4       | 6/25/2007 | 84  | 64             | +                                 | -           | -           | +  | -          | -     |
| 415223084140101 | P2       | 6/25/2007 | 190,000   | 160,000        | +                                 | -           | +           | +  | -          | +     |
| 415230084140602 | P3       | 6/25/2007 | 240   | 210            | +                                 | -           | -           | +  | -          | -     |
| 415233084140603 | P4       | 6/25/2007 | 10  | 8              | -                                 | -           | -           | +  | -          | +     |
| 415224084141604 | W1       | 6/25/2007 | ns  | ns             | ns                                | ns          | ns          | ns | ns         | ns    |
| 415227084141405 | W2       | 6/25/2007 | ns  | ns             | ns                                | ns          | ns          | ns | ns         | ns    |
| 415227084141806 | W3       | 6/25/2007 | ns  | ns             | ns                                | ns          | ns          | ns | ns         | ns    |
| 415233084140309 | D1       | 7/18/2007 | 28  | 28             | +                                 | -           | -           | +  | -          | -     |
| 415224084141108 | D2       | 7/18/2007 | 370   | 310            | +                                 | -           | -           | +  | -          | +     |
| 415230084142607 | D4       | 7/18/2007 | ns  | ns             | ns                                | ns          | ns          | ns | ns         | ns    |
| 415223084140101 | P2       | 7/18/2007 | 480,000   | 370,000        | +                                 | -           | +           | +  | -          | +     |
| 415230084140602 | P3       | 7/18/2007 | 210,000   | 140,000        | +                                 | -           | +           | +  | -          | +     |
| 415233084140603 | P4       | 7/18/2007 | 31,000  | 26,000         | +                                 | -           | +           | +  | +          | +     |
| 415224084141604 | W1       | 7/18/2007 | ns  | ns             | ns                                | ns          | ns          | ns | ns         | ns    |
| 415227084141405 | W2       | 7/18/2007 | ns  | ns             | ns                                | ns          | ns          | ns | ns         | ns    |
| 415227084141806 | W3       | 7/18/2007 | ns  | ns             | ns                                | ns          | ns          | ns | ns         | ns    |
| 415233084140309 | D1       | 8/6/2007  | 230   | 200            | -                                 | -           | -           | +  | -          | -     |
| 415224084141108 | D2       | 8/6/2007  | 8,600   | 5,100          | +                                 | -           | +           | +  | -          | +     |
| 415230084142607 | D4       | 8/6/2007  | 930   | 370            | +                                 | -           | -           | +  | -          | +     |
| 415223084140101 | P2       | 8/6/2007  | 340,000   | 270,000        | +                                 | -           | +           | +  | -          | +     |
| 415230084140602 | P3       | 8/6/2007  | 160,000   | 73,000         | +                                 | -           | +           | +  | -          | -     |
| 415233084140603 | P4       | 8/6/2007  | 13,000  | 7,000          | +                                 | -           | -           | +  | -          | -     |
| 415224084141604 | W1       | 8/6/2007  | 360   | 160            | +                                 | -           | -           | +  | -          | -     |
| 415227084141405 | W2       | 8/6/2007  | ns  | ns             | ns                                | ns          | ns          | ns | ns         | ns    |
| 415227084141806 | W3       | 8/6/2007  | 25,000  | 14,000         | na                                | na          | na          | na | na         | na    |
| 415233084140309 | D1       | 8/20/2007 | 11,000  | 6,300          | +                                 | -           | +           | +  | -          | +     |
| 415224084141108 | D2       | 8/20/2007 | 10,000  | 7,400          | +                                 | -           | +           | +  | +          | +     |
| 415230084142607 | D4       | 8/20/2007 | 10,000  | 8,000          | +                                 | -           | +           | +  | -          | +     |
| 415223084140101 | P2       | 8/20/2007 | 630,000   | 430,000        | +                                 | -           | +           | +  | +          | +     |
| 415230084140602 | P3       | 8/20/2007 | 240,000   | 140,000        | +                                 | -           | +           | +  | +          | +     |
| 415233084140603 | P4       | 8/20/2007 | 49,000  | 35,000         | +                                 | -           | -           | +  | -          | +     |
| 415224084141604 | W1       | 8/20/2007 | 970   | 300            | na                                | na          | na          | na | na         | na    |
| 415227084141405 | W2       | 8/20/2007 | 11,000  | 4,500          | +                                 | -           | -           | +  | -          | +     |
| 415227084141806 | W3       | 8/20/2007 | 8,400   | 7,400          | -                                 | -           | -           | +  | -          | -     |
| 415233084140309 | D1       | 9/4/2007  | 330   | 260            | +                                 | -           | -           | +  | -          | +     |
| 415224084141108 | D2       | 9/4/2007  | 320   | 200            | +                                 | -           | -           | +  | -          | +     |
| 415230084142607 | D4       | 9/4/2007  | 1200  | 1,100          | +                                 | -           | -           | +  | +          | +     |
| 415223084140101 | P2       | 9/4/2007  | 210,000   | 150,000        | +                                 | -           | +           | +  | +          | +     |
| 415230084140602 | P3       | 9/4/2007  | 1,300   | 1,300          | -                                 | -           | -           | +  | -          | +     |



**Table 4. Microbiological data collected in Lenawee County, Michigan.—Continued**

[USGS, U.S. Geological Survey; *E. coli*, *Escherichia coli*; EC, *E. coli* 16S ribosomal DNA gene that is common to all *E. coli*; +, gene present in sample; -, gene absent in sample; ns, no sample; na, not amplifiable with polymerase chain reaction]

| USGS station ID | Field ID | Date       | Colony forming units per<br>100 milliliters water |                | <i>Escherichia coli</i> gene data |             |             |    |            |              |
|-----------------|----------|------------|---|----------------|-----------------------------------|-------------|-------------|----|------------|--------------|
|                 |          |            | Fecal<br>coliforms                                | <i>E. coli</i> | <i>eaeA</i>                       | <i>stx2</i> | <i>stx1</i> | EC | <i>rfb</i> | <i>LT11a</i> |
| 415233084140603 | P4       | 9/4/2007   | 2,100   | 1,300          | -                                 | -           | -           | +  | -          | -            |
| 415224084141604 | W1       | 9/4/2007   | 250   | 210            | +                                 | -           | -           | +  | -          | +            |
| 415227084141405 | W2       | 9/4/2007   | 36,000  | 17,000         | +                                 | -           | -           | +  | -          | -            |
| 415227084141806 | W3       | 9/4/2007   | 1,800   | 1,200          | +                                 | -           | -           | +  | -          | +            |
| 415233084140309 | D1       | 9/10/2007  | 3,900   | 2,000          | +                                 | -           | +           | +  | -          | +            |
| 415224084141108 | D2       | 9/10/2007  | 2,600   | 1,500          | +                                 | -           | +           | +  | +          | +            |
| 415230084142607 | D4       | 9/10/2007  | 6,500   | 4,100          | +                                 | -           | -           | +  | -          | +            |
| 415223084140101 | P2       | 9/10/2007  | 410,000   | 300,000        | +                                 | -           | +           | +  | +          | +            |
| 415230084140602 | P3       | 9/10/2007  | 1,100   | 670            | +                                 | -           | -           | +  | -          | -            |
| 415233084140603 | P4       | 9/10/2007  | 2,700   | 2,000          | -                                 | -           | +           | +  | -          | +            |
| 415224084141604 | W1       | 9/10/2007  | 1,800   | 500            | +                                 | -           | -           | +  | -          | -            |
| 415227084141405 | W2       | 9/10/2007  | 3,200   | 2,600          | -                                 | -           | -           | +  | -          | -            |
| 415227084141806 | W3       | 9/10/2007  | 46  | 42             | -                                 | -           | -           | +  | -          | +            |
| 415233084140309 | D1       | 9/18/2007  | 730   | 530            | -                                 | -           | -           | +  | -          | -            |
| 415224084141108 | D2       | 9/18/2007  | 1100  | 770            | +                                 | -           | -           | +  | -          | -            |
| 415230084142607 | D4       | 9/18/2007  | 770   | 530            | +                                 | -           | +           | +  | -          | +            |
| 415223084140101 | P2       | 9/18/2007  | 170,000   | 160,000        | +                                 | -           | +           | +  | +          | +            |
| 415230084140602 | P3       | 9/18/2007  | 50  | 50             | -                                 | -           | -           | +  | -          | -            |
| 415233084140603 | P4       | 9/18/2007  | 2,100   | 2,000          | -                                 | -           | -           | +  | -          | +            |
| 415224084141604 | W1       | 9/18/2007  | ns  | ns             | ns                                | ns          | ns          | ns | ns         | ns           |
| 415227084141405 | W2       | 9/18/2007  | ns  | ns             | ns                                | ns          | ns          | ns | ns         | ns           |
| 415227084141806 | W3       | 9/18/2007  | 14  | 12             | -                                 | -           | -           | +  | -          | +            |
| 415233084140309 | D1       | 10/3/2007  | 2,000   | 1,400          | +                                 | -           | -           | +  | -          | -            |
| 415224084141108 | D2       | 10/3/2007  | 660   | 380            | +                                 | -           | -           | +  | -          | -            |
| 415230084142607 | D4       | 10/3/2007  | 570   | 410            | +                                 | -           | +           | +  | -          | +            |
| 415223084140101 | P2       | 10/3/2007  | 1,260,000   | 390,000        | +                                 | -           | +           | +  | +          | +            |
| 415230084140602 | P3       | 10/3/2007  | 83,000  | 76,000         | +                                 | -           | +           | +  | -          | +            |
| 415233084140603 | P4       | 10/3/2007  | 140,000   | 60,000         | +                                 | -           | -           | +  | -          | +            |
| 415224084141604 | W1       | 10/3/2007  | 38  | 20             | -                                 | -           | -           | +  | -          | -            |
| 415227084141405 | W2       | 10/3/2007  | 2,000   | 670            | +                                 | -           | -           | +  | +          | -            |
| 415227084141806 | W3       | 10/3/2007  | 6,800   | 4,400          | +                                 | -           | -           | +  | +          | +            |
| 415233084140309 | D1       | 11/27/2007 | 2,500   | 1,800          | +                                 | +           | +           | +  | -          | +            |
| 415224084141108 | D2       | 11/27/2007 | 1,900   | 1,200          | +                                 | -           | +           | +  | +          | +            |
| 415230084142607 | D4       | 11/27/2007 | 2,700   | 1,500          | +                                 | -           | +           | +  | +          | +            |
| 415223084140101 | P2       | 11/27/2007 | 860,000   | 540,000        | +                                 | -           | +           | +  | +          | +            |
| 415230084140602 | P3       | 11/27/2007 | 90  | 70             | +                                 | -           | -           | +  | -          | +            |
| 415233084140603 | P4       | 11/27/2007 | 90  | 70             | -                                 | -           | -           | +  | -          | -            |
| 415224084141604 | W1       | 11/27/2007 | 300   | 130            | -                                 | -           | -           | +  | -          | +            |
| 415227084141405 | W2       | 11/27/2007 | 670   | 400            | -                                 | -           | -           | +  | -          | +            |
| 415227084141806 | W3       | 11/27/2007 | 9,900   | 4,700          | -                                 | -           | -           | +  | -          | -            |
| 415233084140309 | D1 Rep   | 8/6/2007   | 460   | 300            | -                                 | -           | -           | +  | -          | -            |
| 415223084140101 | P2 Rep   | 8/6/2007   | 390,000   | 310,000        | +                                 | -           | +           | +  | -          | +            |