BACKGROUND

Recreational water quality is based primarily on the presence and pervasiveness of pathogens in the water that can pose risks to human health through body contact or ingestion. Since it is not practical to analyze for every possible pathogen found in human waste, indicator bacteria typically are used. Concentrations of these bacteria are relatively easy and cost effective to analyze and are good indicators of fecal contamination. Indicator bacteria results provide regulators with a means to determine the likelihood that human pathogens may be present in recreational waters. Historically, many states have used total fecal coliform as the indicator bacteria for determining the sanitary condition of recreational waters to protect human health. Fecal coliform primarily are found in the waste of humans or other warm-blooded animals; however, at least one type has non-fecal sources, including the effluent of paper mills, textile processing plants, and cotton mills (Wilhelm and Maluk, 1998).

In 1986, the U.S. Environmental Protection Agency (USEPA) published updated recommendations for states based on better knowledge of which indicator bacteria best correlated with gastrointestinal illness in humans. The USEPA recommends that states use either *Escherichia coli (E. coli)* or enterococci as indicators in freshwater and enterococci for saltwater (USEPA, 1986; USEPA, 2002). The presence of *E. coli* and enterococci in recreational waters is direct evidence that fecal contamination from humans or other warm-blooded animals has occurred (USGS, 2006).

The USEPA-recommended criteria are intended to control pathogens by keeping concentrations of indicator organisms at a level that corresponds with acceptable risks of acute gastrointestinal illness to recreational water users (USEPA, 2002). Gastroenteritis is a term for a variety of diseases that affect the gastrointestinal tract and are rarely life-threatening. Symptoms include vomiting, diarrhea, stomach ache, nausea, headache, and fever. Most people affected by gastroenteritis will experience these flu-like symptoms several days after exposure but rarely associate their illness with the ingestion of pathogen contaminated water. Other illnesses or conditions affecting the eyes, ears, skin, and upper respiratory tract can be contracted from contaminated water as well. Although people are affected differently, certain subgroups, such as children and the elderly, are more susceptible to contracting waterborne illnesses. In some studies, gastroenteritis was linked more closely to enterococci exposure, while skin rashes and ear ailments were linked to fecal and total coliform (Noble et al., 2000).

Ongoing research on which types of indicator bacteria are correlated most closely with outbreaks of gastroenteritis in humans continues to show that *E. Coli* and enterococci are both better indicators than fecal coliform (USEPA, 2002). Enterococci typically are used as the indicator bacteria in marine systems because they have a longer life in saltwater then do *E. coli*. However, some studies show that enterococci are a more sensitive indicator in freshwater, resulting in many more recreational closings due to high levels of bacteria (Kinzelman et al., 2003; John and Rose, 2005). In a California study, researchers found that one out of every three indicator bacteria violations was for enterococci alone and that fewer than half of the enterococci violations were paired with an exceedance of another indicator bacteria type. This suggests enterococci are a more sensitive indicator of bacteriological water quality than either total or fecal coliform (Noble et al., 2000). In another study, children who drank from private wells that tested positive for coliform were not at risk for diarrheal disease. However, children who drank from private wells that contained enterococci were six times as likely to become ill with diarrhea (Borchardt et al., 2003).

Some states have replaced their fecal coliform criteria with water quality criteria for *E. coli* and/or enterococci; however, many states, including Pennsylvania, have not yet made the transition (USEPA, 2002). In this study of the Yellow Breeches Creek Watershed, all three of the indicator bacteria (*E. coli*, enterococci, and fecal coliform) were sampled and the results were compared.

METHODS

DATA COLLECTION

SRBC staff collected bacteriological samples using standard PADEP protocol (PADEP, 2006). Four 30-day periods were sampled during the 2006 calendar year: February and early March, May, August, and November. Bacteria samples were collected by hand at eleven sites in 125-ml screw-capped polypropylene wide-mouth bottles that had been pre-sterilized and contained sodium thiosulfate. Samples were collected from the middle of the channel, and any sediment disturbed by the collector was allowed to settle before the sample was collected. Bottles were submersed approximately eight inches under the surface of the water, facing upstream, and filled with water. Bottles were immediately capped, put into a plastic zip-sealed bag, and placed on ice. Duplicate bacteria samples were collected at a rate of at least one per day and were taken once at each site during the 30-day sampling period. A field blank also was taken at least once per day to test for any kind of field contamination. Samples were delivered to the PADEP laboratory within 24 hours of collection.

The sampling sites (Appendix A) were selected so that data collected during this survey can be utilized as background information by PADEP and other interested parties, including water suppliers in the Yellow Breeches Creek Watershed. Additional sites have been added on tributary streams to provide better coverage of the watershed. The locations for sites were chosen to evaluate the pervasiveness of bacteria pollution along the mainstem and contamination in and from the various tributaries.

In addition to bacteria sampling, during each sampling visit, staff measured stream discharge and completed field

chemistry measurements at each site. Stream flow was measured at each site using a Scientific Instruments pygmy or AA meter according to U.S. Geological Survey (USGS) methods (Buchanan and Somers, 1969). The only exception was the site at the mouth of the Yellow Breeches Creek where flow conditions did not allow SRBC staff to take a wading discharge measurement. At this site, stream discharge was estimated using the USGS gage located three miles upstream combined with water withdrawal information from the water supplier directly upstream of the site.

Staff collected water for field chemistry using a hand-held, depth integrated sampler at six verticals across the stream channel. The water was put into a churn splitter, mixed thoroughly, and used to determine temperature, dissolved oxygen, conductivity, pH, turbidity, field acidity, and field alkalinity. Temperature was measured in degrees Celsius with a field thermometer. A Cole-Parmer Model 5996 meter was used to measure pH. Conductivity was measured with a Cole-Parmer 1481 meter and dissolved oxygen was measured with a YSI 55 meter. Turbidity was measured using a Hach 2100P portable turbidimeter. Alkalinity and acidity were determined using field titrations. Alkalinity was measured in the field by titrating a known volume of sample water to pH 4.5 with 0.02N H2SO4. Acidity was measured in the field by titrating a known volume of sample water to pH 8.3 with 0.02N NaOH.

DATA ANALYSIS

The 1986 USEPA-recommended criteria were used to determine violations for enterococci and *E. coli*. The single sample maximum standards for the USEPA-recommended criteria are based on level of human body contact. The most stringent criteria are for designated beach areas, followed by "moderate use full body contact" recreation, "light use full body contact" recreation, and the least stringent standards are in areas of "infrequent use full body contact" recreation. For this analysis, the single sample maximum standards for "moderate use full body contact" recreation were used for *E. coli* and enterococci. This criteria level was chosen because the Yellow Breeches Creek is used heavily throughout the year for a variety of recreational activities, such as swimming, tubing, fishing, kayaking, and canoeing.

Table 1. *Laboratory Methods for Bacteria Enumeration*

Bacteria Type	Description	Method
Fecal coliform	Fecal coliform membrane filter procedure	Standard Method 9222D
E. Coli	Modified mTEC agar with membrane filtration	USEPA Method 821/R-97/004
Enterococci	mEI agar with membrane filtration	USEPA Method 1600

The current recreational water quality criterion in the Commonwealth of Pennsylvania is based on fecal coliform as the indicator bacteria, and there are different standards during and outside of the recreation season. Indicator bacteria concentrations generally are reported as colony forming units per 100 milliliters of sample (cfu/100 ml). The recreation season is from May 1-September 30, and during this time the geometric mean limit is 200 cfu/100 ml, or no greater than 10 percent of the samples may exceed 400 cfu/100 ml. During the remainder of the year, the geometric mean standard is 2,000 cfu/100 ml, and there are no single sample maximum criteria during this time (Table 2).

Currently, there are no USEPA-recommended criteria for *E. coli* or enterococci that differentiate between limits based on a recreational season; USEPA leaves that up to each individual state's discretion. Therefore, only data for the recreational season, from May to September, were used in this analysis to compare the fecal coliform results to those of enterococci and *E. coli*. The enterococci and *E. coli* data for February and November are summarized according to the USEPA-recommended standards.

Steady state value is used synonymously with geometric mean throughout the report and refers to the calculated geometric mean of the six samples (five different days and one duplicate sample) taken throughout the 30-day sampling period. There are three geometric means, one for each type of indicator bacteria, calculated for each of the 11 sampling locations for all four sampling periods. Single sample maximum refers to the concentration of bacteria that cannot be exceeded by more than 10 percent of the samples. This number varies with indicator bacteria type and the designated water use. The geometric mean is used instead of the arithmetic mean, because it reduces the effect of very high or very low values. This is helpful when analyzing bacteria concentrations because levels may vary widely over a given period.

When bacteria results were reported at lower than the detection limit (PBQ), one-half of the detection limit was used in the geometric mean calculation. For a majority of the samples, the detection limit was 20 cfu/100 ml; thus, 10 cfu/100 ml was used in the calculations. For a few samples taken between February 28, 2006, and March 6, 2006, the detection limit was 10 cfu/100 ml, and in these cases 5 cfu/100 ml was used in the calculations. In all cases, a PBQ was listed in the results when the reported value was below the detection limit, and in no case did using half the detection

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Bacteria sampling in Yellow Breeches Creek.

limit cause a site to be in violation. Field blanks were taken at least once per day to determine any source of bacterial contamination coming from field sampling protocol. All of the blanks came back below the detection limit, showing no bacterial contamination in the field sampling procedure.

Precipitation data were obtained from three National Oceanic and Atmospheric Administration rain gages located in Pine Grove Furnace State Park, in the southwest portion of the watershed; in Shippensburg, just outside the northwestern watershed boundary; and in Harrisburg, just outside the eastern watershed boundary. These three rain gages recorded daily rainfall totals and were the closest available sites to the Yellow Breeches Creek Watershed that had a continuous rainfall record for all of the sampling periods. The data from the three locations were averaged together to get an estimated daily rainfall value for the watershed.

Table 2. *Water Quality Standards and Aquatic Life Tolerances*

Parameter	Limit	Reference Code
Temperature	> 25 degrees	a,d
Dissolved oxygen	< 4 mg/l	a,e
Conductivity	> 800 mmhos/cm	c
pH	< 5	b,d
Alkalinity	< 20 mg/l	a,e
Total fecal coliform	Geometric mean of 200 CFUs/100ml during recreation season or a single sample result of 400 CFUs/100 ml; 2000 CFUs/100 ml during non-recreation season	a
E. coli	Geometric mean of 126 CFUs/100 ml or a single sample maximum of 298 CFUs/100 ml for moderate full body contact recreation	f
Enterococci	Geometric mean of 33 CFUs/100 ml or a single sample maximum of 78 CFUs/100 ml for moderate full body contact	f

Reference Code & References

a. http://www.pacode.com/secure/data/025/chapter93/s93.7.html
b. Gagen and Sharpe (1987) and Baker and Schofield (1982)
c. http://www.uky.edu/WaterResources/Watershed/KRB_AR/wq_standards.htm
d. http://www.hach.com/h2ou/h2wtrqual.htm
e. http://sites.state.pa.us/PA_Exec/Fish_Boat/education/catalog/pondstream.pdf
f. EPA recommended criteria, Ambient Water Quality Criteria for Bacteria – 1986

RESULTS

Of the 11 sampling sites, 6 were on the mainstem Yellow Breeches Creek, which included sites from the headwaters to the mouth (Figure 4). Results are organized from upstream to downstream with the tributaries discussed in the order in which they enter the mainstem. Tributaries sampled included Mountain Creek, Dogwood Run, Trout Run, Stony Run, and Cedar Run. Numbers following the stream abbreviation denote river mile distance from mouth to sampling site.

BY SAMPLING LOCATION

Yellow Breeches Creek 51.6

The most upstream sampling point was YLBR 51.6, located along Rehoboth Road near New Lancaster, Cumberland County. The majority of the 12-square-mile drainage area to this site is forested; however, the adjacent land use is agricultural crop land. Bacteria levels exceeded the geometric mean for each of the three indicator bacteria in August, but there were no other steady state violations at this site (Table 3).

Table 3. *Steady State Violations at YLBR 51.6*

Month	*E. coli*		Enterococci		Fecal coliform	
	Geometric mean standard (cfu/100ml)	Calculated geometric mean (cfu/100 ml)	*Geometric mean standard (cfu/100ml)*	Calculated geometric mean (cfu/100 ml)	*Geometric mean standard (cfu/100ml)*	Calculated geometric mean (cfu/100 ml)
August 2006	*126*	305	*33*	337	*200*	462

A majority of the single sample exceedances were in August at this site for all three indicator bacteria types, with the exception of one enterococci violation in November. Overall, 25 percent of the fecal coliform samples and 21 percent of the *E. coli* and enterococci samples exceeded their single sample maximum limits at YLBR 51.6.

Stream flow at YLBR 51.6 was quite variable with very low flows of only 0.2 cubic feet per second (cfs) in the summer and up to 40 cfs after rain events. There was a general trend of decreasing levels of bacteria with increasing stream flow. This suggests that there is some relatively constant source of bacteria contamination that becomes more concentrated at low flow and is diluted at higher flows. Possible sources of contamination for this site could be improperly functioning septic systems, as it is a very rural area with no public sewer service, and cattle access to the stream, which was observed several times during sampling. The data for this site did not show any increase in bacteria levels after a rainfall event.

Yellow Breeches Creek 40.7

The next downstream mainstem site was YLBR 40.7, located along West Yellow Breeches Creek Road east of Montsera, Cumberland County. This site drains 46.8 square miles of primarily agricultural land, with some low density residential areas. YLBR 40.7 is located within a stream reach that is impaired