



## Impacts of manure management practices on stream microbial loading into Conesus Lake, NY

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### ABSTRACT

The microbiology of stream water has a seasonal component that results from both biogeochemical and anthropogenic processes. Analysis of nonevent conditions in streams entering Conesus Lake, NY (USA), indicated that total coliform, *Escherichia coli*, and *Enterococcus* spp. levels peak in the summer in all streams, independent of the agricultural use in the stream sub-watershed. Prior to implementation of management practices, *E. coli* in water draining Graywood Gully, a sub-watershed with 74% of the land in agriculture, reached as high as 2806 CFU/100 mL, exceeding the 235 CFU/100 mL EPA Designated Bathing Beach Standard (EPA-DBBS). In contrast, North McMillan Creek, a sub-watershed with <13% of its land in agriculture, had *E. coli* maxima generally near or below the EPA-DBBS. Graywood Gully at times had a higher microbial loading than North McMillan Creek, a sub-watershed 48 times larger in surface area. Over a 5-year study period, there was a major decrease in bacterial loading during nonevent conditions at Graywood Gully, especially after manure management practices were implemented, while bacterial loading was constant or increased in streams draining three other sub-watersheds. *E. coli* levels dropped more than 10 fold to levels well below the EPA-DBBS while the yearly maximum for *Enterococcus* dropped by a factor 2.5. Similarly, exceedency curves for both *E. coli* and *Enterococcus* also showed improvement since there were fewer days during which minimum standards were exceeded. Even so, Graywood Gully at times continued to be a major contributor of *E. coli* to Conesus Lake. If wildlife represents a significant source of indicator bacteria to Graywood Gully as has been reported, stream remediation, management efforts and compliance criteria will need to be adjusted accordingly.

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### Introduction

Bacterial levels exceeding Federal, State and Provincial standards occur at beaches throughout the Great Lakes basin (Great Lakes Commission, 2005). Nearshore, river, and embayment recreational water quality is often impaired because of microbiological contamination, and beaches are closed out of concern for public health. Sources of microbiological contamination to the Great Lakes and lakes in general are many, including combined or sanitary sewer overflows, unsewered residential and commercial areas, failing private household and commercial septic systems, fecal coliforms from animal/pet fecal waste, and wildlife waste (Great Lakes Commission, 2005). Another source is agricultural runoff, especially manure. Manure is an agricultural by-product that is usually returned to the land to enhance soil productivity, increase soil organic matter, and increase infiltration rates (Gilly and Risse, 2000; McDowell et al., 2004; Smith et al., 2007). However, if improperly applied or applied in excess, manure conta-

minants can pollute adjacent waterways and infiltrate into groundwater (Zebarth et al., 1996).

Conesus Lake, one of the Finger Lakes of New York State, has microbial problems similar in many ways to the Great Lakes. Levels of indicators of microbial pollution are at times well above the levels (SOCL, 2001) recommended by the EPA for bathing or even casual contact with the water (USEPA, 2000). Besides microbial problems, this eutrophic lake (Forest et al., 1978; Makarewicz, 2009) has nuisance algae, invasive aquatic weeds, and large populations of zebra mussels. Lake stakeholders have concerns about the water quality at local beaches and at the shoreline cottages where residents swim and children play in the shallows (SOCL, 2001). In addition, Conesus Lake has a New York State Department of Environmental Conservation (DEC) Classification of AA, serves about 20,000 local residents both as a recreational resource and as a source of drinking water, and is a focal point for regional tourism (NYSDEC, 2006).

Water enters the lake from the surrounding sub-watersheds throughout the year as nonevent (baseline) flow and during ~13 to 15 annual events that are caused by significant rainfall or snowmelt conditions. During events, massive amounts of water and materials, including fecal pollution, are transferred to the lake in a short period of time (Simon and Makarewicz, 2009). Fecal pollution enters the lake

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from several sources, including surrounding farms, some of which house large numbers of cattle. A perimeter sewer system collects waste from homes surrounding the lake, and leaks from this system are a possible source of pollution (SOCL, 2001). Nearly one-thousand homes are set back from the main road and from the perimeter sewer system and have free-standing septic systems that with age and improper management can also act as a microbial source. Finally, there is a large wildlife population in the area ranging from deer to birds such as ducks and geese. Each of these species introduces fecal material that can make its way into streams draining sub-watersheds (Somarelli et al., 2007).

In an effort to improve the quality of the water entering the lake from the sub-watersheds, we have worked closely with farmers on nutrient and animal waste management (Herendeen and Glazier, 2009) to reduce the levels of fecal contaminants leaving farms and being transported to the lake. We view the Conesus Lake catchment system as an excellent surrogate system for a Great Lakes watershed. The lake's catchment has multiple small sub-watersheds within a few kilometers of each other for convenient sampling, are primarily in agriculture, and are owned or operated by one or two farms that allow some control of land use (Makarewicz et al., 2009). In addition, the large number of small watersheds allowed evaluation of the effects of different agricultural management systems on the loads of nutrients and fecal pollution in the streams that drain into the lake. Because of the steep-sided slopes of the sub-watersheds, water transit times were short, and changes in conditions are rapidly reflected in the water quality draining the sub-watershed.

Here we evaluate the seasonal and spatial abundance of microbial populations during hydrometeorological nonevent periods in four streams draining sub-watersheds mostly in agriculture. Nonevent periods are characterized by hydrologic and material export conditions that differ significantly from that of storm flows (Pionke et al., 1999). From a potential pathogen perspective, nonevent flow represents the conditions in the stream (probably more than 300 days a year) to which humans are actually exposed and for which there are State and National exposure limits for "indicator bacteria" (USEPA, 1986; NYSDEC, 2006). Our goal was to test the hypothesis that elevated levels of bacteria during nonevent flows were due to poor manure practices, and finally to determine the extent to which manure management could reduce microbial loading into downstream aquatic systems.

## Methods

### Implementation of BMPs related to microbiology

Six sites were chosen as study sub-watersheds based on several criteria (Makarewicz et al., 2009). Here we focus not only on the Graywood Gully sub-watershed but also provide comparative data on three other sub-watersheds: Long Point Gully, Sutton Point Gully, and North McMillan Creek (Fig. 1). The Graywood and Long Point sub-watersheds had resident populations of dairy cows, while North McMillan Creek is primarily a forested watershed and portions of Sutton Point Gully are in row crops. Graywood Gully is one of the smallest catchments (38 ha) in the Conesus Lake watershed. Land use is mostly in agriculture (74%), consisting of a single dairy-farm operation with approximately 100 head of cattle and row crops including corn and beans. Starting in the fall of 2003, "Whole Farm Planning" has been instituted at Graywood Gully, and a myriad of structural and cultural Best Management Practices (BMPs) aimed at controlling nutrient and animal waste pollution were implemented based on soil testing, evaluation of the P index, and common agricultural management practices (Herendeen and Glazier, 2009). Changes implemented were designed to improve both the nutrient and microbial characteristics of the runoff from the dairy farm to Conesus Lake, the ultimate recipient of the runoff. At Graywood Gully, many of the BMPs controlled water movement from the farm, kept

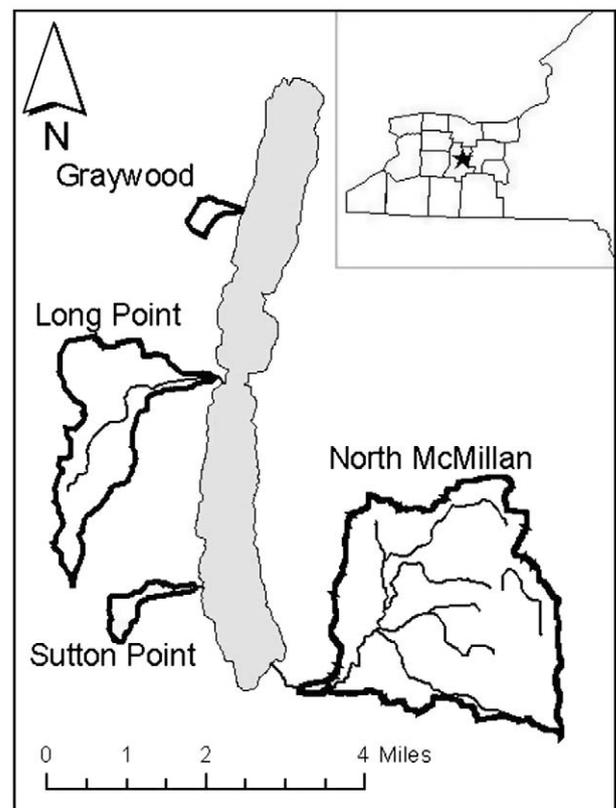


Fig. 1. Conesus Lake sub-watersheds used in this study and their location in Western New York.

cows out of streams, and limited the spreading of manure. The BMPs included: the installation of 20,000 subsurface drainage construction tiles (6250 m); the addition of a standpipe and watering source for a heifer pasture area; the fencing of cattle to prevent them from entering the stream; roof water separation allowing for the clean water to stay clean and be safely discharged away from the contaminated barnyard areas; and finally, the elimination of winter but not spring, summer and early fall spreading of manure (Herendeen and Glazier, 2009).

At Long Point Gully, the one dairy in this sub-watershed ceased operations and dairy cattle were removed from the sub-watershed in 2003. Additionally, a 37% reduction (76.7 ha) in crop acreage occurred by 2004, although manure spreading continued on the land through 2007. No physical infrastructure improvements were implemented in this watershed until 2007 when gully plugs were added at the end of the project. At Sutton Point Gully a significant and increasing portion of the sub-watershed has been in alfalfa/grass production since 2002 (37% in 2003 to 60.3% in 2006). This indicated that manure slurry was not added to the sub-watershed during the study period. At North McMillan Creek only 12% of the sub-watershed was in agriculture and over 77% was in vacant land, in abandoned land that included agricultural parcels in early forest succession, or in single family use (SOCL, 2001). No management practices were implemented in this sub-watershed in this study.

### Stream sampling

All streams were monitored continuously for five annual cycles with a differential pressure transducer (ISCO 720) attached to an ISCO continuously recording flow meter (Model 6700) equipped with an automatic sampler from 1 Sep 2002 to 31 Aug 2007 (Makarewicz et al., 2009). As defined in an accompanying study by Makarewicz et al. 2009, a Water Year (WY) is the period from 1 Sep to 31 Aug of the

following year. For example, WY 1 extended from 1 Sep 2002 to 31 Aug 2003, WY 2 extended from 1 Sep 2003 to 31 Aug 2004, etc. Water samples were taken using two different methodologies: weekly manual grab samples and automated hydrometeorological event samples (Makarewicz et al., 2009). A total of 5 water years of daily discharge data was collected on all creeks starting on 1 Sep 2002 and completed on 31 Aug 2007 (Makarewicz et al., 2009). Most of the time there was low flow in the stream – defined as a nonevent period. Hydrometeorological events were associated with dramatic changes in stream water level and were defined as a rise in the creek level of 2.54 cm in 30 min. After reaching a discharge peak, the end of an event was defined by a leveling off of the descending limb of the stream hydrograph (Makarewicz et al., 2009).

Stream temperature was measured *in situ* (Fisher Traceable Thermometer) weekly during nonevent conditions. Simultaneously, water samples were taken for both turbidity and microbial analysis within an hour of each other. Water was transported to the laboratory at SUNY Geneseo and processed for microbial measurement within 6 h. Turbidity was determined using a portable turbidimeter (Orbeco-Hellig Model 966).

The microbial quality of water was measured using surrogates for fecal pollution. New York State uses fecal coliform count levels as the standard for recreational waters and bathing beaches (Public Health Law §225, Chapter 1 State Sanitary Code Subpart 6-2). However, the EPA guidelines for water quality recommend the measurement of *Escherichia coli* and *Enterococcus* levels since they provide the best correlation to the presence of water-related human gastrointestinal disease (USEPA, 1999, 2000). In this study, both *E. coli* and *Enterococcus* levels were routinely measured in all samples. Additionally, the general microbial composition of stream waters was measured by total coliform and total heterotrophic counts (APHA, 1999).

Since most stream water flowing into Conesus Lake have low turbidity, samples were analyzed for total coliform and *E. coli* (CFU/100 mL) using a membrane filter (MF) method employing m-ColiBlue24 (Millipore®) medium (Grant, 1997). *Enterococcus* levels (CFU/100 mL) were determined using a MF method by placing filtered dilutions of water samples on m-Enterococcus Agar (Difco 0746) (Kaneko et al., 1989). Total heterotrophic bacteria were measured following growth on R2A medium (Difco 1826) at 25 °C for 48 h. A method of “spot plating” was used to facilitate these measurements, as multiple 20- $\mu$ L samples were spotted on R2A agar plates, and micro-colonies were observed under a dissecting microscope after 48 h. This method allows for quantitation of bacterial numbers with many fewer plates and in a shorter time than when sample dilutions are merely spread on plates of growth medium.

#### Data analysis

Comparison of the temperature differentials between streams assumed that the differences in temperature on any given day were random allowing Chi-square analysis. Only nonevent microbial data are presented here. Event data are presented in Simon and Makarewicz (2009). Microbial data were analyzed directly without transformation. To dampen the effect of extremes typically associated with intra-sample and analytical variability of microbial populations, comparisons were made using monthly geometric means as recommended by the EPA (USEPA, 1999). Most microbial counts showed seasonal periodicity and peaked between July and September or October (see below). Therefore, microbial analysis was done on a calendar year basis because a water year basis would combine numbers from half a seasonal peak in 1 year with numbers from half a seasonal peak of the following year. Linear regression analyses were carried out to examine the trends in bacterial loading over time and to determine relationship between loading of suspended solids and bacteria. (SigmaStat 3.5, SYSTAT Software Inc.).

Microbial “exceedency” curves were constructed for *E. coli* and *Enterococcus*. Exceedency curves allow for the evaluation of the percentage of time in any given period that microbial levels exceed a particular value. In such a curve, the comparison is as a percentage of time, and each microbial measurement is assumed to be held for a period of time between pre- and post sample, in this study usually 7 days. Because events may only last for a short period of time, only nonevent data were employed in determining an exceedency curves. To develop such a curve, the bacterial levels were arranged from highest to lowest, and then each value was multiplied by the time period in days that it represented. Time weighted averages were not necessary because the microbial sampling was done at regular weekly intervals. A calculation was done to determine what fraction of the total time (1 year) each level represented, and then *E. coli* and *Enterococcus* levels (CFU/100 mL) were plotted against the percent of the time that any particular microbial level was exceeded. For a complete discussion of exceedency curves, see the National Center for Water Quality Research (NCWQR, Richards and Baker, 1993).

## Results

### Stream hydrology

Seasonal weather in western New York has large fluctuations in temperature and rainfall (snow) that dominate stream hydrology. Nonevent flow was generally lower in the summer and higher in the spring (Fig. 2a). Streams have liquid water even during the coldest winter months with flow occurring under the ice. Fig. 2b gives the monthly nonevent flow in Graywood Gully as a fraction of the total flow and shows that the 4-year average of nonevent flow provided 77% of the total yearly water load. This was quite variable over time, reflecting wet years versus dry years. In some months 90% of the water

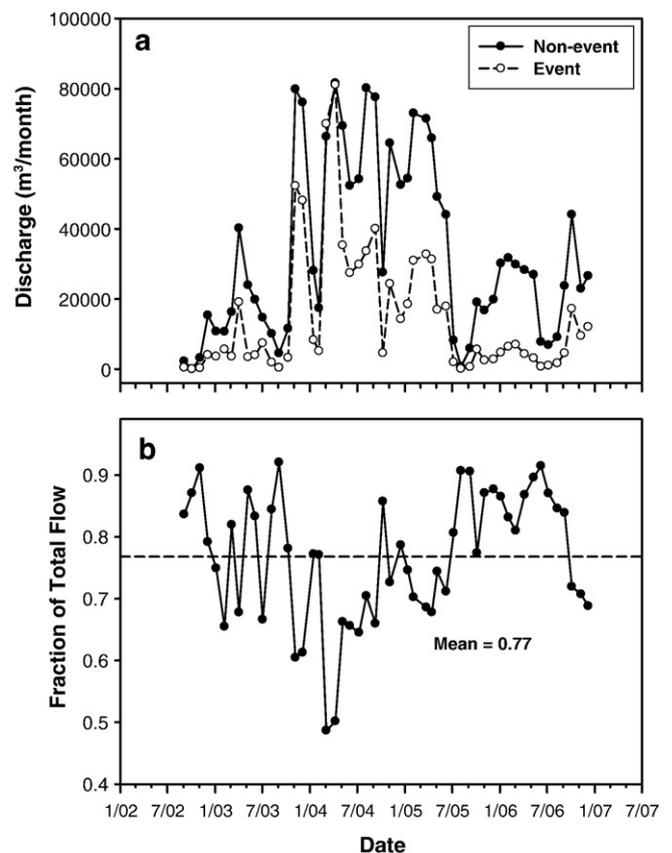


Fig. 2. Graywood Gully stream flow showing (a) nonevent and event discharge in m<sup>3</sup> per month and (b) monthly nonevent flow as a fraction of total flow.

flow was nonevent flow. In a small sub-watershed like Graywood Gully, stream flow may cease in years during limited rainfall as in 2002. However, 2004 was a very wet year and event discharges were significant relative to nonevent flow.

High variability in event versus nonevent discharge within a sub-watershed and between sub-watersheds was evident. In Graywood Gully, areal weighted nonevent discharge ( $\text{m}^3/\text{ha}$ ) runoff was greater in all 5 water years than event discharge. In Sutton Point Gully, areal event runoff was higher in 1 of 5 years; in both Long Point Gully and North McMillan Creek it was higher in 2 of 5 years (Table 1). Even when and where event flow was the dominant yearly stream contribution to Conesus Lake, it was no more than 1.7 times as much water as the nonevent flow during the rest of the year. As Makarewicz et al. (2009) have suggested, areal weighted discharge can be much higher in Graywood Gully than in other study streams. For example, in WY 2 Graywood Gully had almost 3.6 times the discharge per ha than did North McMillan Creek. Especially in wet years, external sources outside of the traditional topographical definition of sub-watershed were likely impacting this measurement (Noll and Magee, 2009).

As expected, the seasonal variability in stream temperatures was typical of temperate regions with maxima in the summer and minima in the winter (Fig. 3a). However, a Chi-square test of the temperature differentials between various sub-watersheds indicated that Graywood Gully was significantly warmer than both Sutton Point Gully ( $P < 0.001$ ,  $df = 164$ ) and Long Point Gully ( $P = 0.013$ ,  $df = 131$ ) but not North McMillan Creek ( $P = 0.755$ ,  $df = 164$ ) (Figs. 3b–d).

### Microbiology

The *E. coli*, *Enterococcus*, and total coliform levels as indicated by the geometric monthly mean concentrations in the nonevent flow of all streams were seasonal; bacteria were generally present in highest numbers from June to September, with peak amounts in the month of August (Fig. 4). At the beginning of this study prior to the initiation of BMPs, Graywood Gully was a major source of microbial pollution. Abundances of *E. coli* in stream water during nonevents reached as high as 2806 CFU/100 mL during 2003 when management practices concerning the application of manure on snow were not in place. No

other sub-watersheds experienced such high *E. coli* levels during 2003 (Table 2, Fig. 5). Long Point Gully, which still received manure application on fields after the closing of a dairy operation, had high and variable *E. coli* levels throughout the study period. North McMillan Creek, the reference sub-watershed with the lowest amount of land in agriculture (<13%), had maximum *E. coli* levels generally near or below the EPA's Designated Bathing Beach Standard (Table 2), while Sutton Point Gully, a sub-watershed in agriculture (76%) but with no dairy farms or manure application activities, had maximum *E. coli* levels only slightly over the EPA Beach criteria.

A small winter peak in Long Point Gully in 2003–2004 was also observed. No such peaks were found in Sutton Point Gully, and a small peak was found in North McMillan Creek. The large July 2005 peaks were associated with significantly elevated discharge rates at this time. With a few exceptions, heterotrophic bacterial levels did not change during the year and were often higher in the winter than they were in the summer (Fig. 4).

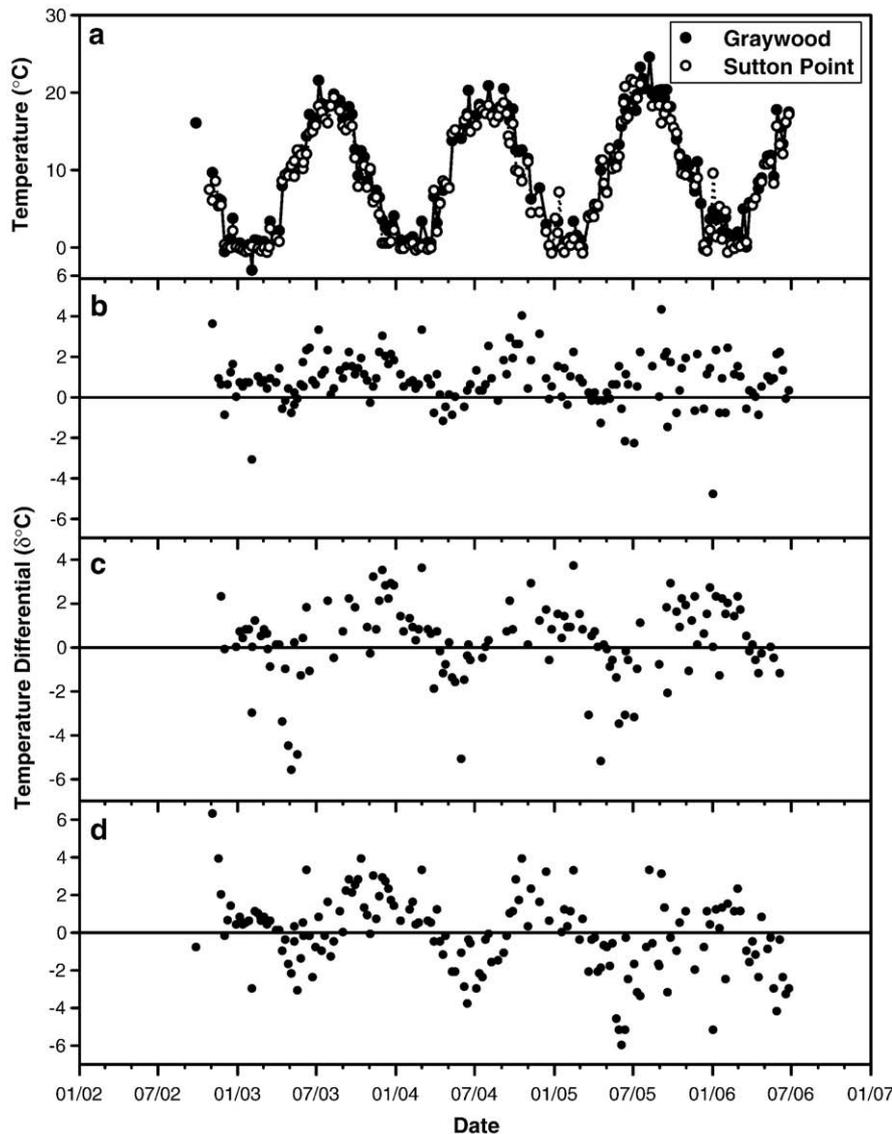
Over a 4-calendar year period, a major decrease in bacterial levels in nonevent Graywood Gully stream water was observed. *E. coli* levels in Graywood Gully dropped more than 10 fold to levels significantly below the 235 CFU/100 mL EPA Bathing Beach Standard (Table 2) while the yearly maximum for *Enterococcus* dropped by a factor 2.5. The box plot of monthly (June to September) range of values for *E. coli* and *Enterococcus* decreased over time (Fig. 5a). The decreases in the median values of *E. coli* ( $r^2 = 0.823$ ) and *Enterococcus* ( $r^2 = 0.546$ ) (Fig. 5b) in Graywood Gully contrasted with little to no change over the same time period for Long Point Gully, Sutton Point Gully, and North McMillan Creek. Graywood Gully was the only stream where the peak median for total coliforms dropped ( $r^2 = 0.982$ ), whereas all other streams had increases in total coliform levels. These trends persisted in 2007.

Exceedency curves (Fig. 6a) also demonstrated a decrease in the levels of *E. coli* in Graywood Gully over the study period. In 2003, 33% of the samples taken were above the EPA Standard for infrequent contact, whereas by 2006 that number dropped to 20%. Additionally, in 2003 only 43% of the yearly samples met the EPA Beach Standard for *E. coli* (USEPA, 1999); this increased to 62% by 2006. The improvement in Graywood Gully, which began in 2003, was also seen in the decrease in samples that had the highest levels of *E. coli*. In 2003, 12% of the time the samples were above 10,000 CFU/100 mL, but by 2006

**Table 1**  
Yearly nonevent and event discharge from Graywood Gully, Sutton Point Gully, Long Point Gully, and North McMillan Creek.

Year	Area (ha)	Watershed	Nonevent discharge ( $\text{m}^3$ )	Event discharge ( $\text{m}^3$ )	Ratio event/base	Nonevent discharge ( $\text{m}^3/\text{ha}$ )	Event discharge ( $\text{m}^3/\text{ha}$ )
WY 1	38	Graywood Gully	121,998	107,685	0.88	3210	2834
	68	Sutton Point Gully	148,360	58,188	0.39	2182	856
	588	Long Point Gully	477,296	1,228,372	2.57	812	2089
	1778	N. McMillan Creek	3,701,677	6,806,584	1.84	2082	3828
WY 2		Graywood Gully	616,429	436,795	0.71	16,222	11,495
		Sutton Point Gully	505,322	543,390	1.08	7431	7991
		Long Point Gully	2,919,546	239,498	0.08	4965	407
		N. McMillan Creek	6,605,063	7,594,317	1.15	3715	4271
WY 3		Graywood Gully	668,751	176,740	0.26	17,599	4651
		Sutton Point Gully	652,338	242,108	0.37	9593	3560
		Long Point Gully	2,133,648	2,504,593	1.17	3629	4260
		N. McMillan Creek	6,324,154	6,206,475	0.98	3557	3491
WY 4		Graywood Gully	214,823	65,058	0.30	5653	1712
		Sutton Point Gully	386,652	35,829	0.09	5686	527
		Long Point Gully	1,348,078	1,202,166	0.89	2293	2045
		N. McMillan Creek	4,068,563	2,294,813	0.56	2288	1291
WY 5		Graywood Gully	336,007	124,205	0.37	8842	3269
		Sutton Point Gully	553,187	85,312	0.15	8135	1255
		Long Point Gully	1,659,679	419,169	0.25	2823	713
		N. McMillan Creek	7,657,713	3,463,875	0.45	4307	1948

A water year (WY) was defined as the period from 1 September to 31 August of the following year.



**Fig. 3.** Stream temperatures ( $^{\circ}\text{C}$ ) and temperature differentials ( $\delta^{\circ}\text{C}$ ) in Graywood Gully and other sub-watersheds. (a) Weekly stream temperature in Graywood Gully and Sutton Point Gully temperature. Temperature differences between individual weekly samples in Graywood Gully and (b) Sutton Point Gully, (c) Long Point Gully, and (d) North McMillan Creek.

values this high occurred <4% of the time. *E. coli* in 2005 was an anomaly in that a few very high levels occurred. The majority of *Enterococcus* samples (<60%) remained at levels above the EPA recommendation for infrequent contact of 576 CFU/100 mL (USEPA, 1999) (Fig. 6b).

Microbial loading is similar in concept to nutrient loading into a lake. Rather than simply considering the microbial abundance per unit volume, we multiplied discharge of stream water times microbial concentration to obtain loading. Since WY trends in flow were used in these calculations, total microbial loading trends are presented as a function of water years. The loading of *E. coli*, *Enterococcus*, and total coliforms varied over the year because of the seasonality of microbial growth and stream flow. Higher stream flows sometimes occurred in late fall and early spring, times when indicator bacterial levels were low; but during wet years, summer rains often coincided with peak microbial numbers. On an areal (number per hectare) basis, Graywood Gully at times delivered *E. coli* to Conesus Lake that were more than an order of magnitude higher than the other three study streams (Table 3).

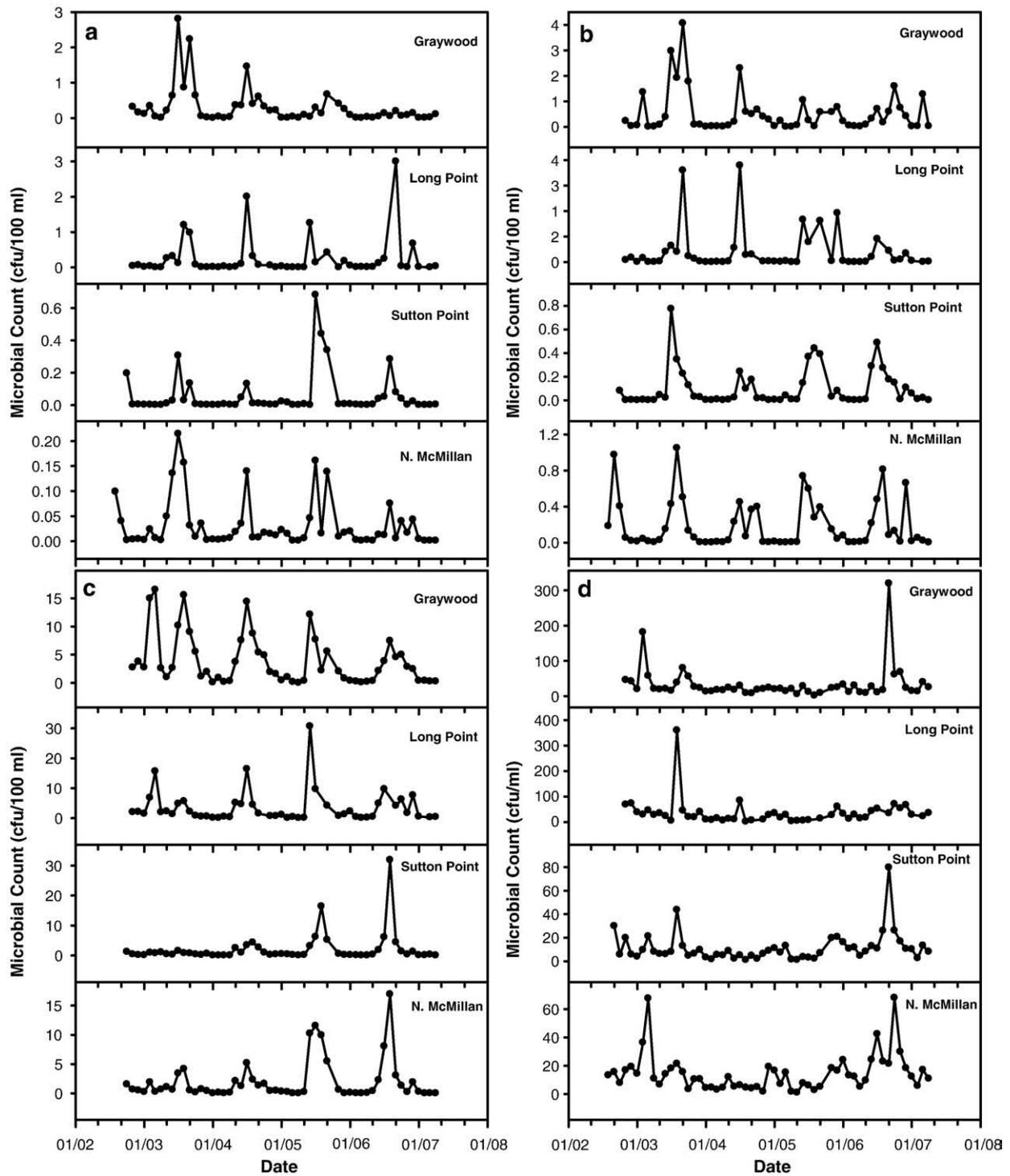
The relationship between stream microbiology from June to September (the time of peak indicator microbial levels) and turbidity

was examined. In all cases, as turbidity increased, stream microbial numbers (*E. coli*, *Enterococcus*, total coliforms, and total heterotrophic bacteria) increased ( $r^2$  from 0.262 to 0.576, Fig. 7).

## Discussion

Both nonevent and event discharge contributed to water entering the lake. While it is impressive that up to 40% of the total yearly volume of water discharged into Conesus Lake originated from ~13 to 15 events (Makarewicz et al., 2009), nonevent flow did represent a substantial amount of water to the lake. In Graywood Gully, nonevent runoff exceeded event runoff in all years between WY 1 and WY 5 and accounted for 53% to 79% of the total discharge.

Analysis of the microbial characteristics of Conesus Lake sub-watersheds indicated that for nonevent conditions, total coliform, *E. coli*, and *Enterococcus* levels peaked in the summer in all streams, whether or not livestock were present; that is, the periodicity observed was independent of the particular agricultural use in the stream sub-watershed. For example, North McMillan Creek had only 10.2% agricultural land use and no livestock but had a similar



**Fig. 4.** The geometric mean of monthly microbial counts in Graywood Gully, Long Point Gully, Sutton Point Gully, and North McMillan Creek. (a) *Escherichia coli*, (b) *Enterococcus*, and (c) total coliforms are given in units of CFU/100 mL. Total heterotrophic bacteria (d) are in CFU/mL.

seasonal periodicity for *E. coli* and *Enterococcus* as Graywood Gully, a sub-watershed with 100 head of dairy cattle. *E. coli* derived from wildlife may be a major contributor to the normal stream microbial components and may help define this periodicity. Microbial source typing using Rep-PCR indicated that as many as half of the *E. coli* found in Conesus Lake streams were from wildlife sources (Somarelli et al., 2007). However, autochthonous soil microbial communities may include a component of *E. coli*, which is distinguishable genetically from the communities found in the gut of most common wild animals such as geese and deer (Byappanahalli et al., 2006; Ishii

et al., 2006). These naturalized *E. coli* from Great Lakes watersheds also showed seasonal variability (Ishii et al., 2006) and thus may contribute to the overall *E. coli* levels found in streams.

Particulate material (total suspended solids, TSS) and microbial numbers rose dramatically during stream events (Richards et al., 2001; Makarewicz et al., 2009; Simon and Makarewicz, 2009). This was expected, as large volumes of moving water often transfer solids from land to stream as well as resuspending stream sediments, a known source of *E. coli* (Stephenson and Rychert, 1982; Jamieson et al., 2003, 2005). However, during nonevents there were both particulate matter and

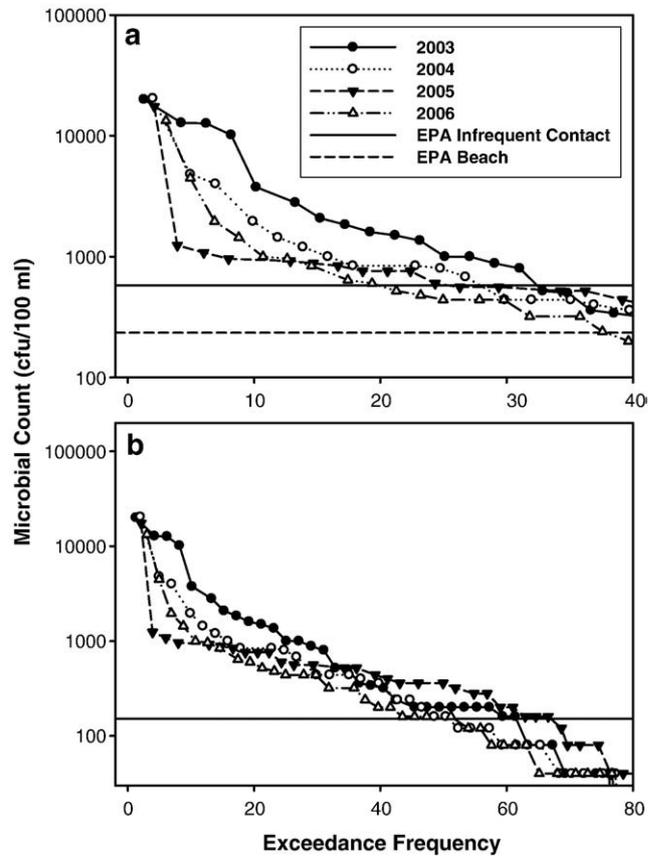
**Table 2**  
Yearly calendar maxima (CFU/100 mL) of *Escherichia coli* and *Enterococcus* (*Enter.*) in Graywood Gully, Long Point Gully, Sutton Point Gully, and North McMillan Creek.

Year	Graywood Gully		Long Point Gully		Sutton Point Gully		N. McMillan Creek	
	<i>E. coli</i>	<i>Enter.</i>	<i>E. coli</i>	<i>Enter.</i>	<i>E. coli</i>	<i>Enter.</i>	<i>E. coli</i>	<i>Enter.</i>
Y 1 – 2003	2806	4059	1200	3589	306	775	214	1048
Y 2 – 2004	1454	2298	2002	3788	131	234	140	449
Y 3 – 2005	668	1046	1257	1661	680	440	161	738
Y 4 – 2006	196	1592	3000	908	283	488	75	801

EPA standards for “Recreational Fresh Water”, “Designated Bathing Beach”, and “Infrequent Body Contact” are as follows: *E. coli*: 126, 235, and 576 CFU/100 mL, respectively; *Enterococcus*: 33, 62, and 151 CFU/100 mL, respectively (USEPA, 1999).

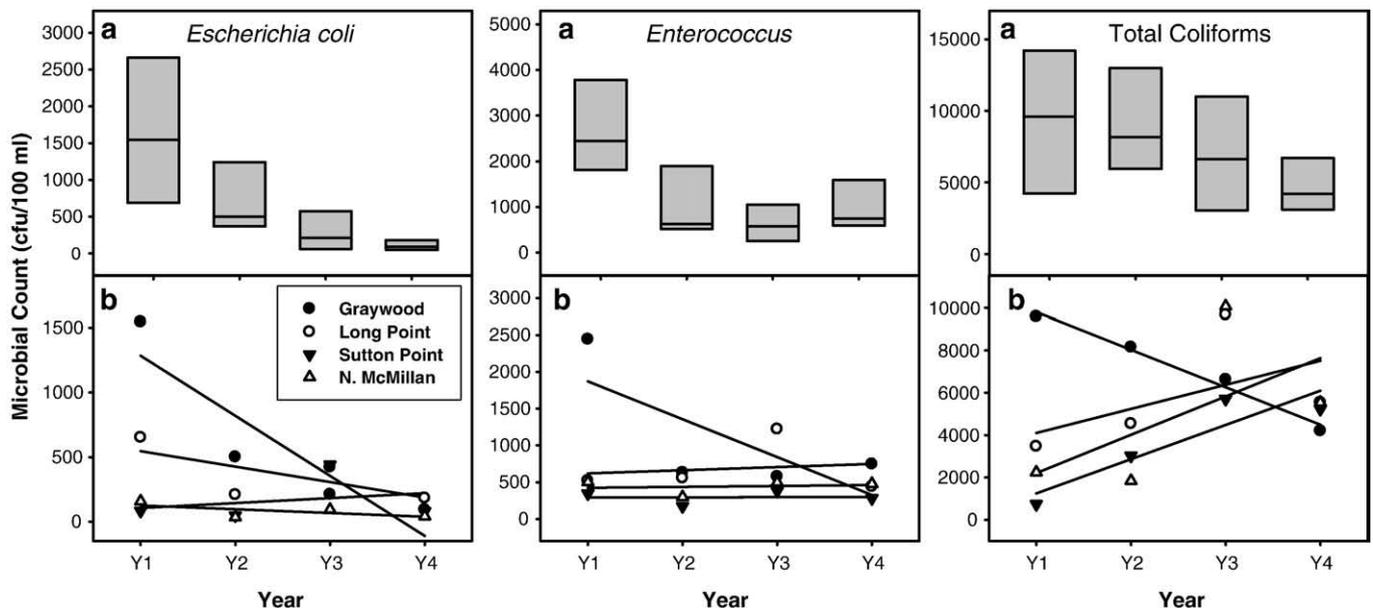
bacteria in Conesus Lake streams, and bacterial levels were positively correlated with water turbidity (Fig. 7). Meals (1989) also reported a significant correlation for bacteria and TSS in the LaPlatte Reservoir, Vermont. While the relationship between bacteria and particulates could be due to similar mechanisms being responsible for bringing them into suspension, it may simply be a reflection of the observation that bacteria in nature are generally bound to particulate matter and have higher metabolic rates when in this condition (Crump et al., 1999; Luef et al., 2007).

There can be little doubt that improvements in the microbial quality of the water in Graywood Gully occurred following the application of BMPs for manure management in the sub-watershed. The geometric mean monthly levels of *E. coli* and *Enterococcus* decreased over the study. The biggest drops occurred in the first years of the study, perhaps not unexpectedly, coming after changes in the patterns of manure spreading. By the end of the study, *E. coli* levels in Graywood Gully were below the “Designated Beach Area” standard of 235 CFU/100 mL set by the EPA. Although *Enterococcus* levels have decreased, they were still much higher than recommended (Table 2) (USEPA, 1999). Meals (1989) observed a significant decrease in fecal coliforms and *Streptococcus* in Vermont agricultural watersheds after changes in management practices, but to a lesser degree than observed here. Inamdar et al. (2002) observed a slight decrease in fecal coliforms and a larger decrease in fecal *Streptococcus* during a 10-year study on BMPs for manure management in the Piedmont region of Virginia.



**Fig. 6.** Graywood Gully exceedency curves for (a) *Escherichia coli* and (b) *Enterococcus* for 2003 to 2006. EPA *E. coli* standards for “Infrequent Body Contact” and “Designated Bathing Beach” are 576 and 235 CFU/100 mL, while *Enterococcus* “Infrequent Body Contact” is 151 CFU/100 mL (USEPA, 1999).

Total coliform levels, a broader measure of the presence of Gram-negative microbial communities, have decreased in Graywood Gully waters but increased in the Long Point, Sutton Point, and North McMillan streams. There was no clear reason why the total coliform



**Fig. 5.** Graywood Gully *Escherichia coli*, *Enterococcus*, and total coliform levels 2003 to 2006. (a) Range and median for the peak months of bacterial abundance, June to September. (b) Annual median values for Graywood Gully, Long Point Gully, Sutton Point Gully, and North McMillan Creek.

**Table 3**

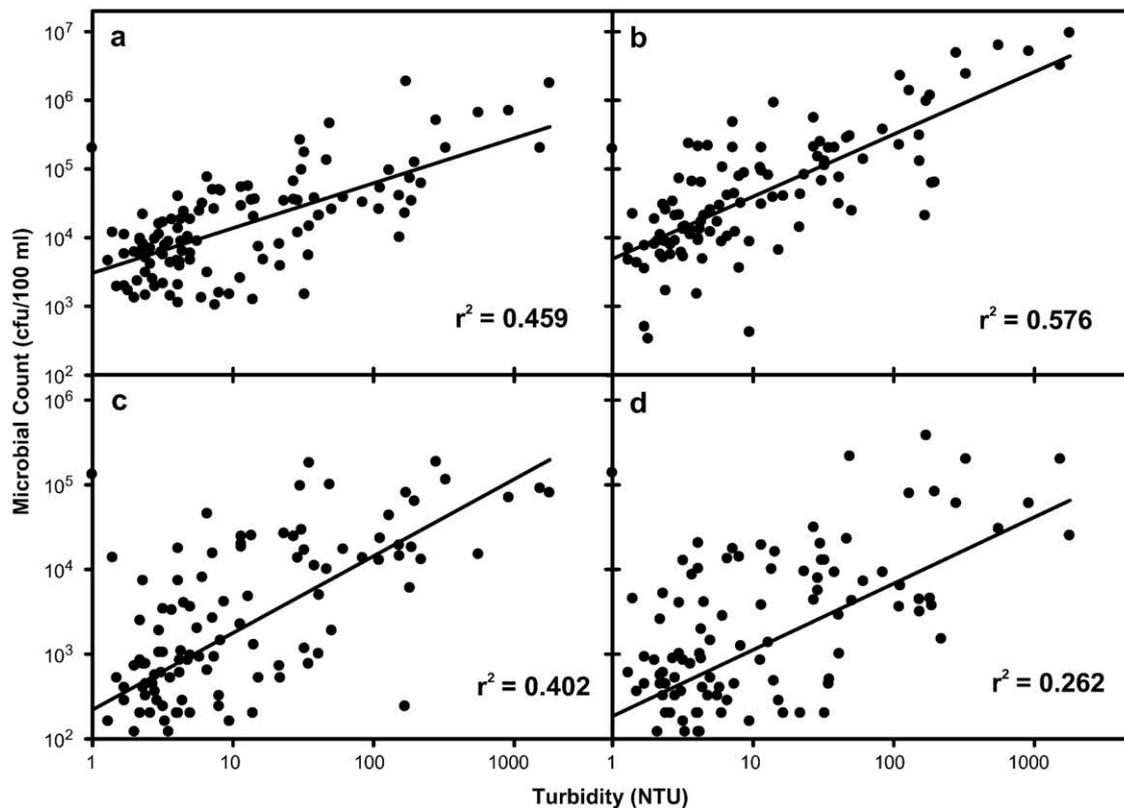
Average *Escherichia coli* loading (CFU/month) and areal weighted *Escherichia coli* loading (CFU/ha/month) from Graywood Gully, Long Point Gully, Sutton Point Gully, and North McMillan Creek.

		Graywood Gully	Long Point Gully	Sutton Point Gully	North McMillan Creek
WY1	Average CFU loading/mo	4.35E + 10	1.10E + 11	1.33E + 09	1.47E + 11
	Loading ratio to Graywood	1.00	2.52	0.03	3.37
	Average CFU/mo/ha	1.14E + 09	1.87E + 08	1.96E + 07	8.25E + 07
	Areal load ratio to Graywood	1.00	0.16	0.02	0.07
WY2	Average CFU loading/mo	1.30E + 11	6.73E + 10	6.79E + 09	9.14E + 10
	Loading ratio to Graywood	1.00	0.52	0.05	0.70
	Average CFU/mo/ha	3.44E + 09	1.14E + 08	9.99E + 07	5.14E + 07
	Areal load ratio to Graywood	1.00	0.03	0.03	0.01
WY3	Average CFU loading/mo	8.03E + 10	5.32E + 10	1.15E + 10	8.27E + 10
	Loading ratio to Graywood	1.00	0.66	0.14	1.03
	Average CFU/mo/ha	2.11E + 09	9.05E + 07	1.70E + 08	4.65E + 07
	Areal load ratio to Graywood	1.00	0.04	0.08	0.02
WY4	Average CFU loading/mo	1.67E + 10	4.13E + 10	8.05E + 09	4.20E + 10
	Loading ratio to Graywood	1.00	2.47	0.48	2.51
	Average CFU/mo/ha	4.41E + 08	7.02E + 07	4.34E + 09	2.36E + 07
	Areal load ratio to Graywood	1.00	0.16	9.85	0.05
WY5	Average CFU loading/mo	1.83E + 10	4.76E + 10	1.18E + 08	5.83E + 10
	Loading ratio to Graywood	1.00	2.60	0.01	3.18
	Average CFU/mo/ha	4.82E + 08	8.09E + 08	6.38E + 07	3.28E + 07
	Areal load ratio to Graywood	1.00	1.68	0.13	0.07

The average values have been used to compare monthly loadings and monthly areal loadings between Graywood Gully and the other streams. mo = month. ha = hectare.

level should rise over the 4-year period of monitoring. Levels of heterotrophic bacteria remained unchanged in all sub-watersheds. This might be expected because the numbers of total bacteria in stream water could be 3 to 4 orders of magnitude greater than the numbers of those bacterial species that were measured to assess water quality. Also, soil and plant detritus were present all year and may be a large and variable natural source of heterotrophic bacteria (Fierer and Jackson, 2006; Fierer et al., 2007).

Exceedency curves provide the opportunity to examine all stream water samples in a given year and to detect trends, especially in those samples that are at the extremes of water quality. Such curves for both *E. coli* and *Enterococcus* (Fig. 6) showed that the water in Graywood Gully had improved. While there were times during a year when water quality was above that set by the EPA for infrequent human contact, the numbers of those times have decreased steadily as the effects of implemented BMPs in the Graywood Gully sub-



**Fig. 7.** Microbial levels versus water turbidity in Graywood Gully. (a) Total coliforms, (b) heterotrophic bacteria, (c) *Enterococcus*, and (d) *Escherichia coli*. Because of the seasonality of bacterial abundance, only water samples from June to September were used in this analysis.

watershed became evident. The distribution of the elevated indicator levels during any given year was scattered, so that monthly geometric averaging corrects for these high levels (USEPA, 1999). The source of these elevated levels was not immediately obvious, but it is known that there were variations in stream flow and elevated stream flows, just below those that might define an event. Also, it was not possible to rule out the role of wildlife just upstream prior to the time of sampling. In spite of extraordinary efforts to prevent fecal pollution on a stream with a single farm at Graywood Gully, the exceedency curves demonstrated that EPA infrequent contact standards in nonevent flow were exceeded 20% of the year for *E. coli* and 50% of the year for *Enterococcus*. To meet the EPA standards, either non-agricultural sources of fecal pollution, such as that from wildlife (Somarelli et al., 2007), will have to be addressed or that there will need to be a change in expectations about the acceptable level of stream microbiology standards at bathing beaches. Similar conclusions regarding compliance with current water quality standards have been raised previously (Inamdar et al., 2002; Jamieson et al., 2003). If wildlife represents a major source of indicator bacteria, stream remediation and management efforts and compliance criteria should be adjusted accordingly. Dealing with wildlife contributions will require new approaches and considerations.

Graywood Gully at times had higher total monthly microbial loading than North McMillan Creek, a sub-watershed that is 48 times larger in surface area (Fig. 3). This is a remarkable result and hints at how land use does impact microbial populations that are leaving a sub-watershed. Long Point Gully, a sub-watershed 15.5 times the area of Graywood Gully, had a higher *E. coli* total loading in WY 1 than Graywood Gully. By WY 2 and WY 3, Long Point Gully loading per hectare was >20 fold less than Graywood Gully due to the closing of the dairy operation in that watershed. Loading of *E. coli* from Graywood Gully was 50–100 times greater than from Sutton Point Gully, an agricultural sub-watershed that did not house animals. Changes in farming practice were most likely the cause of the increase in Long Point Gully *E. coli* output in WY 5 and demonstrated the value of utilizing stream bacterial abundance and bacterial loading as a tool to evaluate farm practices (Kay et al., 2007).

There is ample evidence that livestock operations and manure application can elevate fecal coliform and fecal *Streptococcus* abundance in runoff from agricultural lands (Kunkle, 1970; Doran et al., 1981; Baxter-Potter and Gilliland, 1988; Niemi and Niemi, 1991). In this study we applied BMPs to a small sub-watershed in Conesus Lake and asked whether the manipulations reduced microbial loading in comparison to similar small agricultural watersheds as well as to a larger heavily forested sub-watershed. Is this experimental approach reasonable for microbial studies? At first glance the answer would appear to be no because there may be major differences in the physical and biological conditions in the sub-watersheds. For example, fluctuations in physical conditions such as temperature, light, rainfall, etc. may be different due to microclimates. Seasonal variations in critical nutrients that also serve as substrates for microbes (i.e., nitrate, total phosphorous, suspended solids) differ between sub-watersheds (Makarewicz et al., 2009). Sodium levels may be different (Makarewicz, Personal Communication, The College at Brockport) as a result of local differences in deicing salt usage during the winter (Kaushal et al., 2005; Kelly et al., 2009). Even adjacent sub-watersheds may have significantly different water temperatures and discharge when corrected for area, which indicated there were additional sources of groundwater input not present in the other sub-watersheds (Table 1). These may result from differences in rainfall patterns as well as variations in aspect (topography), soil, vegetation, land use, cultivation pattern, etc. There were also seasonal land use differences between sub-watersheds, as farmers rotated crops, changed locations for manure spreading, and varied chemical application of fertilizer and pesticides in their fields (Herendeen and Glazier, 2009). These differences must translate into differences in both the chemical and

microbial processes that take place in the sub-watershed and in the stream itself and likely account for some of the variability seen in the bacterial levels between sub-watersheds (Fig. 5). Even in a single, relatively small lake, there is complexity and variability among its sub-watersheds. This requires that the efficacy of BMPs on microbial quality be evaluated at the sub-watershed level (Makarewicz, 2009; Inamdar et al., 2002; Jamieson et al., 2003). In this study we used this particular approach and demonstrated that the application of BMPs in the Graywood Gully sub-watershed led to major reductions in the delivery of microbial populations to downstream aquatic systems in contrast to trends in three other sub-watersheds.

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