Investigating the Effect of Septic Systems on Surface Water Quality in the Cayuga Lake and Hudson River Watersheds

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Abstract

The purpose of this study was to explore the effect of septic systems on surface water quality by comparing concentrations of faecal indicator bacteria data to the spatial distribution of septic systems and land use practices. The initial hypothesis was that a greater septic system presence in similar upstream segments would lead to greater bacterial concentrations, and additionally that agricultural land use would be more prevalent where this were true.

T-tests and Wilcoxon Rank-Sum tests were used to determine the difference in bacterial concentrations within each pair of upstream and downstream sampling points. In the Cayuga Lake Watershed the Brooktondale and Gasline points had the most significant difference (p value of 0.04). In the Hudson River Watershed Preston and Potter Hollow showed the greatest significant difference (p value of 0.13). Two way ANOVA was used for analyzing the seasonal effect on bacterial concentrations. The results varied by type of faecal bacteria. For E. coli all four seasons were tested for two downstream points with different septic system densities. There was a more significant difference in concentration due to season (p value of < 0.001) than to density (p value of 0.01), and the interaction of the variables was not significant (p value of 0.22). For enterococci the data and analysis is incomplete. The data was separated between base and storm flow but compared for different seasons. Spring and summer (stormflow) were tested along with septic system density and showed a p value of 0.3. The interaction had a p value of 0.35. The baseflow seasons of summer and fall had a p value of 0.70 for seasonal influence and 0.38 for the interaction of variables. Using regression, the average distance from septic system to stream, the number of septic systems between points, and the distance to agricultural land were the three most contributing factors in the Cayuga Lake Watershed. In the Hudson River Watershed these factors were the number of septic systems between the paired points, the percent agricultural land, and the distance to agricultural land. The data from the Cayuga Lake Watershed was revisited as the agricultural land use category could be further specified into a single category of cultivated land or land containing livestock. This variable had more of a significance in multiple regression models when combined with percent forested land, another reduced category, and distance to agricultural land from the sampling points (p values of 0.06, 0.04, and 0.48 respectively) (R^2 = 0.6). In the Hudson River Valley Watershed, it was only possible to divide the agricultural land category into a percent forested category. The regression model also improved with this and the distance to agricultural land (p values of 0.03, 0.04, and 0.06 for percent ag, percent forested, and distance to agricultural land respectively) (R^2 = 0.53).

These results demonstrate that it would be worthwhile to investigate land use further. Other methods that could offer greater insight are to keep a constant distance between the paired points and to find better suited control points. More sampling dates in general are also needed to more accurately evaluate seasonal variation and land use interactions. Finally, it would be vital to note that although septic systems may have an effect on water quality, perhaps the ones in the sites studied were not failing.

Introduction

With an adequate depth of soil (evaluated differently depending on hydrologic properties and slope) and surveillance, septic systems are efficient ways to treat waste water before it reaches surface or ground sources. However, a carelessly installed system can lead to pollution of water resources (Mass.Gov 2015). An improperly drained leach field, for instance, can cause
wastewater to mix with runoff and become a non point source pollution problem. It is important to know where and when this occurs to protect waters designated as recreational or fishing grounds, and to ensure drinking water reservoirs meet standards.

One way of analyzing the impact of septic systems is to look for bacteria in streams and lakes that are also present in faecal matter. These fecal indicator bacteria are commonly sampled for and reported by the NYSDEC and commonly include either E. coli or enterococci. High concentrations of either of these usually non harmful bacteria may indicate a risk of other pathogenic bacteria being present in the water. However, the source of the bacteria is not always the septic systems, as they may also come from manure from agricultural tracts close to the stream. Thus, controlling for different land uses and accounting for variables in the spatial distribution of septic systems, such as their density and the percent of agricultural land in the corresponding watershed, helps determine if septic systems are likely to be a source of pollution. If there is a clear correlation between the variables and the bacterial concentrations, then it is possible to determine whether they also play a role in increasing stream pollution.

Collecting water quality data (faecal indicator bacteria concentrations) from one single watershed, even if from different streams, also provides the possibility of standardizing against similar soils. Similar topography and climate allows for the elimination of more variables. In this study eight sampling points in the Cayuga Lake Watershed and eleven points in the Hudson River Valley Watershed were used to evaluate the effect of septic systems on surface water quality. The former involved an E. coli analysis and the latter enterococci.  

Note: The presence of E. coli and enterococci bacteria have shown to follow similar trends in this and other studies. They may be used together to support conclusions but they do not serve as direct substitutes or predictors of one another.

Methods

Two sources from New York that maintain easily accessible online public databases of water quality results are the Community Science Institute (CSI) and Riverkeeper. The latter has a water quality program that tests the Hudson River and its tributaries for enterococcus bacterial counts and physical/chemical data such as oxygen, temperature and salinity. The importance is given to enterococci because of their relation to faecal matter. These bacteria are present in human and animal intestines and are usually correlated with other pathogens originating from the same place but that may be more harmful. CSI also tests for faecal bacteria but includes many other analytes such as faecal coliform, total coliform, and E. coli; not enterococci. The extent to which the general results relating water quality to septic tanks can be compared between the two regions is dependent on the relationship between the faecal bacteria, as explained below.

Enterococci belong to a genus of bacteria that has been utilized as an indicator of contamination by faecal matter due to its relationship with gastrointestinal illnesses (Boehm & Sassoubre, 2014). However, it may also originate from some filamentous algae or, like E. coli, from animal feces (Kinzelman, Ng, Jackson, Gradus & Bagley, 2003). It would be of greater use in this project to differentiate between enterococcus species and E. coli strains that are more likely to only be present in human waste, but the tests done by the organizations from which the water quality data was acquired do not distinguish them. When E. coli and enterococci are looked at in terms of their faecal source, either animal waste or sewage from a primary municipal
wastewater outlet, both bacteria in animal waste are more likely to survive for longer than their counterparts in sewage. This may be due to the "greater particulate matter" that manure can offer as nutrients (Korajkic, McMinn, Harwood, Shanks, Fout & Ashbolt, 2013).

Additionally, E.coli, belonging to a different bacterial genus, behave differently in the environment than do enterococci. Enterococci are known to survive longer in salt water and are usually used to test the water quality of ocean beaches (United States Environmental, 2012). According to a study done in Lake Pontchartrain and its affluent, the correlation between E. coli, enterococci, and faecal coliform counts was more significant in the lake than in the drainage canal water where salinity and other extraneous variables could be different (Guang, Englande & Huei-Wang, 2004). Thus, this creates a difference in the relative concentrations of the bacteria wherever they are tested, as enterococci usually seem to be in greater abundance.

Apart from the Guang et al study, other research has also shown that E. coli are usually present in lower concentrations than enterococci. The general result is that they both follow the same distributions but their concentrations are not as highly correlated (Fisher & Dillard, 2003). This is seen in a study in a section of Lake Michigan that analyzed the concentrations of Enterococci versus E. coli and their impacts on beach closings. Consequentially, the beaches that are usually monitored using only faecal coliform indicators (E. coli), would exceed the 5 day geometric mean and single sample standards set by the EPA much more often than not. Thus, enterococci may not be used as a substitute for E. coli or vice versa. Each, with its own standards, can relate water quality to an acceptable or exceeded pollution level, and when tested for simultaneously they are even more beneficial. A study in Southeastern Australia used a biochemical fingerprinting method to show that E. coli and enterococci were better indicators when sampled for together, because in some cases one bacteria showed identical BPTs than those within the septic tanks (Ahmed, Neller & Katoili, 2004). Thus, if one indicated there was no pollution, the other could prove otherwise.

Once the bacterial relationship was established, the next step was to choose sampling points that controlled for other variables (land use) and that held varying spatial characteristics of septic systems. In each watershed there was a set of paired points (upstream and downstream) and a set of control points (one for ag and one pristine point with low ag and low septic system density). The following variables were measured for each location.

The variables related to the septic systems:
- the number of septic systems between paired points
- the number of septic systems within the overlap of a sampling point's 500 m buffer and its watershed
- the average density of septic systems within the overlap of the buffer and watershed
- the average distance from stream to septic system within the overlap of the buffer and watershed.

The variables related to land use:
- the shortest distance to agricultural land from each sampling point
- the percent agricultural land in the sampling point's watershed
- the percent forested land in the sampling point's watershed
In addition, CSI indicated which samples were taken during stormflow and which were baseflow. Riverkeeper did not provide this information but by using the data they had on rainfall over the previous few days, it was possible to manually determine which could be considered baseflow (those with no rain in the previous two days) (Brutsaert). This information was incorporated into the analysis as well.

**Statistical Analysis**

Paired t-tests were used to determine the significance in the difference between E. coli measurements in the upstream and downstream points for each of the paired sites. T-tests were also used to compare the control sites to each other and to all other sites. Wilcoxon Rank-Sum tests were used for comparison among some sites as well as further support of the results acquired from the t-tests. Regression analysis was used to gauge the influence of all the measured variables for each site on water quality (E. coli concentrations). Multiple regression served as a way to see the influence of various combined factors on water quality and the relative error they resolve. Finally, Two way ANOVA was used to simultaneously compare the effect of seasonal differences and that of low versus high septic density.

**Results**

**Downstream and upstream comparison**

In the paired points along a single stretch of stream, the downstream sites were hypothesized to have a larger concentration of E. coli or enterococci due to the density of septic systems below the upstream site. For the E. coli points in the Cayuga Lake Watershed the p values comparing upstream to downstream concentrations for each pair ranged from 0.04 (Brooktondale and Gasline) to 0.92. For enterococci in the Hudson River Watershed the p values for similar paired t tests ranged from 0.13 (Cauterskill and Leeds) to 0.77.

However, when the average E. coli concentrations are taken for each site (a synoptic analysis) the upstream points had mostly higher concentrations. The results for each site are seen in the chart below (Figure 1) and suggest that only those that are physically closer (Newfield sites) to each other support the hypothesis. However the difference between the upstream and downstream averages for this site is 1 cfu / 100 mL (p value of 0.92) and not considered significant.
Figure 1: The height of the bars represent the average E. coli concentration in each paired site from the Cayuga Lake Watershed. The dotted bars represent the percent of agricultural land in each site's watershed on a secondary vertical axis.

For enterococci in the Hudson River Watershed, the results are similar. Out of 5 pairs of points, 3 showed higher concentrations downstream. The other part of the hypothesis was that the downstream points that did have higher enterococci would also have higher percent agricultural land in their watershed. However this was not always the case as seen in Figure 2.

Figure 2: The height of the bars represent the average E. coli concentration in each paired site from the Hudson River Valley Watershed. The dotted bars represent the percent of agricultural land in each site's watershed on a secondary vertical axis.
The Wilcoxon Rank Sum tests for the Cayuga Lake Watershed points mostly matched the results given by the paired t-tests. This test was also used for comparing the control points to the other sampling sites. For example, as seen in Table 1 below, the downstream point D3 (Salmon Creek Mouth) had a p value of 0.25 when compared with the control site for agricultural land and a value of 0.06 when compared to the pristine site.

*Table 1*: The titles on the diagonal represent each upstream and downstream point in the Cayuga Lake Watershed as well as the two control points. The values to the left of the diagonal are the p values from the Wilcoxon - Rank Sum test for each combination of points tested, and the values to the left are the p values for the paired t-tests.

<table>
<thead>
<tr>
<th></th>
<th>U 1</th>
<th>0.04</th>
<th>0.02</th>
<th>0.41</th>
<th>0.39</th>
<th>0.46</th>
<th>&lt; 0.01</th>
<th>0.02</th>
</tr>
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<tr>
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<td>&lt; 0.1</td>
<td>D 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>0.54</td>
</tr>
<tr>
<td>U 3</td>
<td>0.92</td>
<td></td>
<td></td>
<td>0.21</td>
<td>0.35</td>
<td></td>
<td>0.89</td>
<td></td>
</tr>
<tr>
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<td>&lt; 0.9</td>
<td>D 2</td>
<td></td>
<td></td>
<td></td>
<td>0.39</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>0.21</td>
<td></td>
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<td>0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>U 3</td>
<td>0.25</td>
<td>0.16</td>
<td>0.51</td>
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<tr>
<td></td>
<td></td>
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<td>U 3</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>U 3</td>
<td>0.25</td>
<td>0.16</td>
<td>0.51</td>
<td></td>
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</tr>
</tbody>
</table>

Wilcoxon Rank-Sum tests

*All p values less than 0.1 are highlighted*
Table 2: The titles on the diagonal represent each upstream and downstream point in the Hudson River Valley Watershed as well as the two control points. The values to the left of the diagonal are the p values from the Wilcoxon - Rank Sum test for each combination of points tested, and the values to the left are the p values for the paired t-tests.

<table>
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<th></th>
<th>U1</th>
<th>0.33</th>
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<th>0.5</th>
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<th>U4</th>
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<th>U</th>
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<td>D2</td>
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<td>D3</td>
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<td>0.4</td>
<td>D4</td>
<td>0.67</td>
<td>0.67</td>
<td>0.67</td>
<td>0.4</td>
</tr>
<tr>
<td>D</td>
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<td>0.47</td>
<td>0.95</td>
<td>0.38</td>
<td>0.94</td>
<td>0.11</td>
<td>Pristine</td>
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<td>0.67</td>
<td>0.67</td>
<td>0.4</td>
<td>1</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Wilcoxon Rank-Sum tests

Seasonal variation

Seasonal variation was analyzed in two ways. One focused on just a single area of one watershed and the differences in E. coli averages at three points along the same stream between summer and winter. These E. coli concentrations were also compared to the control points' averages, shown in Figure 3.
Figure 3: Seasonal comparison of average E. coli concentrations in Brooktondale

The bar labeled 'Middle' is point between the upstream and downstream points chosen in the Brooktondale area.

For enterococci concentrations there were not enough data to compare summer and winter. The comparisons between summer and spring (Figure 4a) showed that there was no significant difference between them. In some sites the mean was larger for spring and in others it was larger for summer. However, this could be due to the dates being storm flow sampling days. In comparing summer and fall, and only base flow sampling days, the evidence was much clearer for summer's larger contribution to the enterococci counts (Figure 4b).
The second method was to use a two way ANOVA analysis, with replication, to compare the effect on E. coli concentrations of septic density and season. Two downstream points (from two different paired points) were chosen on the basis of having different septic system densities. The results showed that there is a significant difference (p value of < 0.001) between the concentrations associated with seasons (during summer, increasing E. coli) and a less significant difference when testing between septic system densities (p value of 0.01). This is supported by the average concentration at each site for each season shown in Table 3. This test also provides the significance of the interaction of the two variables on water quality and the result showed a p value of 0.22. The results for fall and spring are much less significant.
For enterococci the comparison between an upstream point and the pristine site was done for storm flow in the spring and summer and for base flow in the fall and summer (there were no sampling dates in the winter). The storm flow showed insignificant differences between the sites or within the season (p value of 0.39 and 0.30 respectively) and showed an insignificant interaction of the two variables (p value of 0.35). The baseflow analysis gave a slightly more significant value (p value of 0.25) for between sites and less significant values for seasonal difference (p value of 0.70) and the interaction of the two variables (p value of 0.38).

Table 3: Seasonal E. coli averages for a low density and a high density point chosen from the Cayuga Lake Watershed.

<table>
<thead>
<tr>
<th></th>
<th>Low Density (D2)</th>
<th>High Density (D1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>concentration (cfu / 100 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>107</td>
<td>213</td>
</tr>
<tr>
<td>Winter</td>
<td>13</td>
<td>60</td>
</tr>
<tr>
<td>Spring</td>
<td>110</td>
<td>45</td>
</tr>
<tr>
<td>Fall</td>
<td>90</td>
<td>90</td>
</tr>
</tbody>
</table>

Linear Regression

Linear regression was done as preliminary work, before multiple regression analysis, to compare the individual effect of the variables on E.coli and enterococci concentrations. Multiple regression on the Cayuga Lake Watershed data with all variables included had a R^2 value of 0.83 without including the pristine point and a value of 0.31 when it was included. The reason for excluding the pristine point is merely to demonstrate that all the variables tested for here might not be enough to declare what determines the indicator bacteria concentrations. Figure 5 includes the pristine point and shows an inverse relationship between agricultural land (the trend line is drawn on the plot) and E. coli concentrations, contrary to the original hypothesis. This is due to the pristine point having a relatively large concentration of E. coli even though its land use contributions would indicate otherwise; 0.73 percent is agricultural land and about 64 percent is forested land. Thus, the E. coli seen at that point may be due to an even more specific land use or another variable not tested for in this study. The same trend is seen in Figure 6 for enterococci.
Using multiple regression including the pristine point, the factors that emerged as the most significant contributors to E. coli concentrations in eight matched dates over 6 years were the number of septic systems within the 500 m radius (p value of 0.51) and the average density of septic systems within the radius (p value of 0.46). The average distance from septic system to stream (p value of 0.2 and R^2 of 0.22) was significant when regression was done for each variable independently. Without the pristine point included, percent agricultural land in the watershed replaced the number of septic systems as an important variable.

For the enterococci analysis, those with the most significant contribution to the model after multiple regression were the distance to agricultural land (p value of 0.70) from each sampling point and the percent agricultural land in the sampling points' watersheds (p value of 0.64). The number of septic systems between the paired points (p value of 0.48 and R^2 of 0.18) was at a similar contributing level as the percent agricultural land (p value of 0.25 and R^2 of 0.14) when regression was done for each variable independently.

Land use regression analysis

For the Cayuga Lake Watershed, regression analysis of the distance to agricultural land from each sampling point gave a p value of 0.98 and a R squared value of <0.01. Without including the pristine point, multiple regression analysis with percent agricultural land in the sampling point's watershed gave a p value of 0.56 for the distance variable and 0.07 for percent agricultural land (R^2 value of 0.51).

For the Hudson River Watershed the distance to agricultural land from each sampling point gave a p value of 0.97 and an R^2 of less than 0.01. When the percent agricultural land in the point's watershed was included the p value for distance was 0.65 and was 0.27 for percent agricultural land with an R^2 value of 0.15.

When more directed regression analysis was done on a fewer number of variables, the main contributing factors changed. More specific land use categories were chosen since percent agricultural land appeared as potentially important in both watersheds. The results also showed that seasonal variation was significant between the summer and winter, and thus that manure
spreading in the summer could be a cause of higher indicator bacteria. The agricultural land category was thus divided into forested land and that involving cultivation or livestock. The following variables were measured within each sampling point's watershed:

- the percent of agricultural (all types) land
- the percent forested land
- the percent of land designated as cultivated/ with livestock

Lower p values (0.93, 0.05, and 0.27 respectively) were extracted for the latter two factors than for the first. A second multiple regression with only percent forested and percent cultivated/ livestock land gave p values of 0.03 and 0.05 respectively. A third analysis included the variable of the average distance to agricultural land (of any category), since this factor proved to also be potentially contributing to the water quality. This regression gave p values of 0.04, 0.06 and 0.48 for percent forested, percent cultivated/ with livestock, and distance to agricultural land respectively, with an R^2 value of 0.60. Note that this was all done with the inclusion of the pristine point.

For enterococci in the Hudson River Watershed, multiple regression of percent agricultural land in the watershed, percent forested land in the watershed, and distance to agricultural land gave p values of 0.03, 0.05 and 0.06 respectively, with an R^2 value of 0.53.

The other variables originally measured for each sampling point did not show a significant correlation with the E. coli concentrations when analyzed individually. The available data was separated into the dates that matched among sites and each variable was graphed against water quality for that day. The plots are shown below for the five sites that had sampling dates in common.

Figure 7: The plot compares the E. coli concentrations to the average distance from septic system to stream for the five points in the Cayuga Lake Watershed that had sampling dates in common.

<table>
<thead>
<tr>
<th>Date</th>
<th>Data Points</th>
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<tr>
<td>July 30 2007</td>
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</tr>
<tr>
<td>July 28 2008</td>
<td></td>
</tr>
<tr>
<td>January 6 2009</td>
<td></td>
</tr>
<tr>
<td>June 1 2009</td>
<td></td>
</tr>
<tr>
<td>June 15 2010</td>
<td></td>
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<tr>
<td>June 21 2011</td>
<td></td>
</tr>
<tr>
<td>June 19 2013</td>
<td></td>
</tr>
<tr>
<td>December 1 2009</td>
<td></td>
</tr>
</tbody>
</table>
Figure 8: The plot compares the E. coli concentrations to the average septic system density for the five points in the Cayuga Lake Watershed that had sampling dates in common.

Figure 9: The plot compares the E. coli concentrations to the number of septic systems for the five points in the Cayuga Lake Watershed that had sampling dates in common.
For enterococci there were only two dates for which all the sites had baseflow data.

Figure 10: The plot compares the enterococci concentrations to the average distance from septic system to stream for the two points in the Hudson River Valley Watershed that had sampling dates in common.

Figure 11: The plot compares the enterococci concentrations to the average septic system density for the two points in the Hudson River Valley Watershed that had sampling dates in common.
Figure 12: The plot compares the E. coli concentrations to the number of septic systems for the five points in the Hudson River Valley Watershed that had sampling dates in common.

Stormflow and Baseflow

As both water quality sets collected from Riverkeeper and CSI included stormflow, or rainfall data to determine which dates recorded stormflow, the statistical tests were done separately from baseflow. In both bacteria's analysis, the results showed that stormflow diminished the significance in the differences between paired points concentrations. Table 4 below shows how the p values for the paired t-tests increased for E. coli.

Table 4: Change in stormflow t-test values for E. coli

<table>
<thead>
<tr>
<th></th>
<th>U 1</th>
<th>0.04</th>
<th>0.77</th>
</tr>
</thead>
<tbody>
<tr>
<td>D 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U 2</td>
<td>0.92</td>
<td>0.99</td>
</tr>
<tr>
<td>D 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U 3</td>
<td>0.25</td>
<td>0.37</td>
</tr>
<tr>
<td>D 3</td>
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<td>Pristine</td>
<td>0.25</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Ag</td>
<td></td>
<td></td>
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</tbody>
</table>

U = upstream  
D = downstream  
1 = Brooktondale area  
2 = Newfield Area  
3 = Salmon Creek
Discussion

The main purpose of determining the effect of septic systems on water quality, and the hypothesis that greater bacterial concentrations would be found in areas with more septic systems closer to streams was not secured with these results.

The difference between sites with varying spatial distributions of septic tanks was measured using distributional statistical tests. Thus, if a large load of faecal indicator bacteria were being added between an upstream and downstream point the latter would show a significant difference and a $p$ value of less than 0.05. This was seen in two of the paired points in the Cayuga Lake Watershed. In the Hudson River Watershed the most significant difference was seen in the Preston Hollow and Potter Hollow points.

To start defining the causes of the test results, seasonal variation was analysed. The difficulty was finding a large number of same day data points for each season shared by more than one point. For E. coli, one more point was added to the paired set in Brooktondale and all were compared along with the control points. For enterococci the data was even more scarce and no sampling was done in the winter. Thus, the summer averages were compared to spring and fall. Both bacteria showed high summer values when compared to the fall and winter, and a small difference when comparing summer and spring. Further studies expanding on this work may show whether this is due to high rainfall events in the summer and the effect of the thaw in the spring. The summer is also primetime for manure spreading and may cause a greater bacterial load.

When agricultural land in the Cayuga Lake Watershed was divided into that which was specifically designated as cultivated or with livestock, excluding areas such as orchards, the significance of the variable within the model increased. This could indicate that this is indeed a possible source of the E. coli. In the Hudson River Watershed land use/land cover layers specific to the state might offer more specific categories of agricultural land and insight on direct effects.

Enterococci measurements were all done in the same creek, Catskill Creek, except for the pristine point. This might also have decreased the differences in the bacterial concentrations or made them less apparent with the variables studied. Still, the seasonal trends in indicator bacteria in both watersheds are similar in addition to the significance of the land use percentages, as shown by multiple regression analysis.

Future work may analyze whether the lack of certainty of faecal indicator bacteria sources is due to differences in distances between the paired points. Also, many of the septic system variables were based on a 500 m buffer which might not be the adequate radius to capture everything affecting a sampling point.
References


