APPENDIX B

Optical Brightener Sampling Report

RUTGERS
New Jersey Agricultural Experiment Station

Musquapsink Brook Watershed Restoration and Protection Plan
Optical Brightener Sampling Report

Developed by the Rutgers Cooperative Extension Water Resources Program

Funded by the New Jersey Department of Environmental Protection
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Background

Sources of pathogenic enteric bacteria in waterways include human, farm animal and/or wildlife excrement. Methods for detecting fecal coliform bacteria and identifying pathways from their sources are important in addressing point and nonpoint source pollution in watersheds (Tavares et al., 2008). Bacterial Source Tracking (BST) involves a series of microbiological and chemical analyses to determine sources of fecal bacteria in environmental water samples. One such source tracking method to identify *human* bacterial contamination in surface water is the fluorometric detection of optical brighteners. Optical brighteners are compounds added to laundry detergents and soaps, and have no natural sources. Because household plumbing systems combine effluent from washing machines and toilets, optical brighteners are associated with human sewage in sewer lines, septic systems and wastewater treatment plants (Hartel et al., 2007). Their presence in surface water, therefore, can be an indicator of an illicit connection, leaking pipes, or contamination from wastewater.

Data results obtained from surface water quality sampling in the Musquapsink Brook watershed show both wet and dry weather sources of *E. coli* and fecal coliform contamination. Microbial Source Tracking (MST) sampling using qPCR analysis has indicated the presence of human sources of bacterial loadings to the watershed. Potential human sources include leaking sewer lines and illicit connections. The project partners are required to identify and quantify sources of pollution in the watershed, as outlined by the tasks presented in the Quality Assurance Project Plan (QAPP) submitted for this project in January 2007 and as outlined by the objectives described in the original proposal for the Musquapsink Brook Watershed Restoration Plan, submitted in May 2006. These objectives and tasks were developed so that appropriate management practices are implemented and resources are allocated efficiently and economically throughout the watershed. Investigation beyond MST sampling is required to track down areas of detected human sources of pathogenic contamination so that point sources within the watershed can be adequately identified and addressed in the final Watershed Restoration and Protection Plan. Rutgers Cooperative Extension (RCE) Water Resources Program proposes to accomplish this using fluorometric analysis to detect the presence of optical brighteners in the stream.

Methods

Two rounds of optical brightener sampling and fluorometric analysis were completed between May and August 2010 during dry conditions (no recorded precipitation within 48 hours of sampling event). Initially, there were 16 sites sampled. Two additional sites were added for the August sampling event. See Figure 1 below for locations of sampling sites. Site M03 was sampled in May 2010 but data is not included since the site location lies just outside of the watershed boundary.

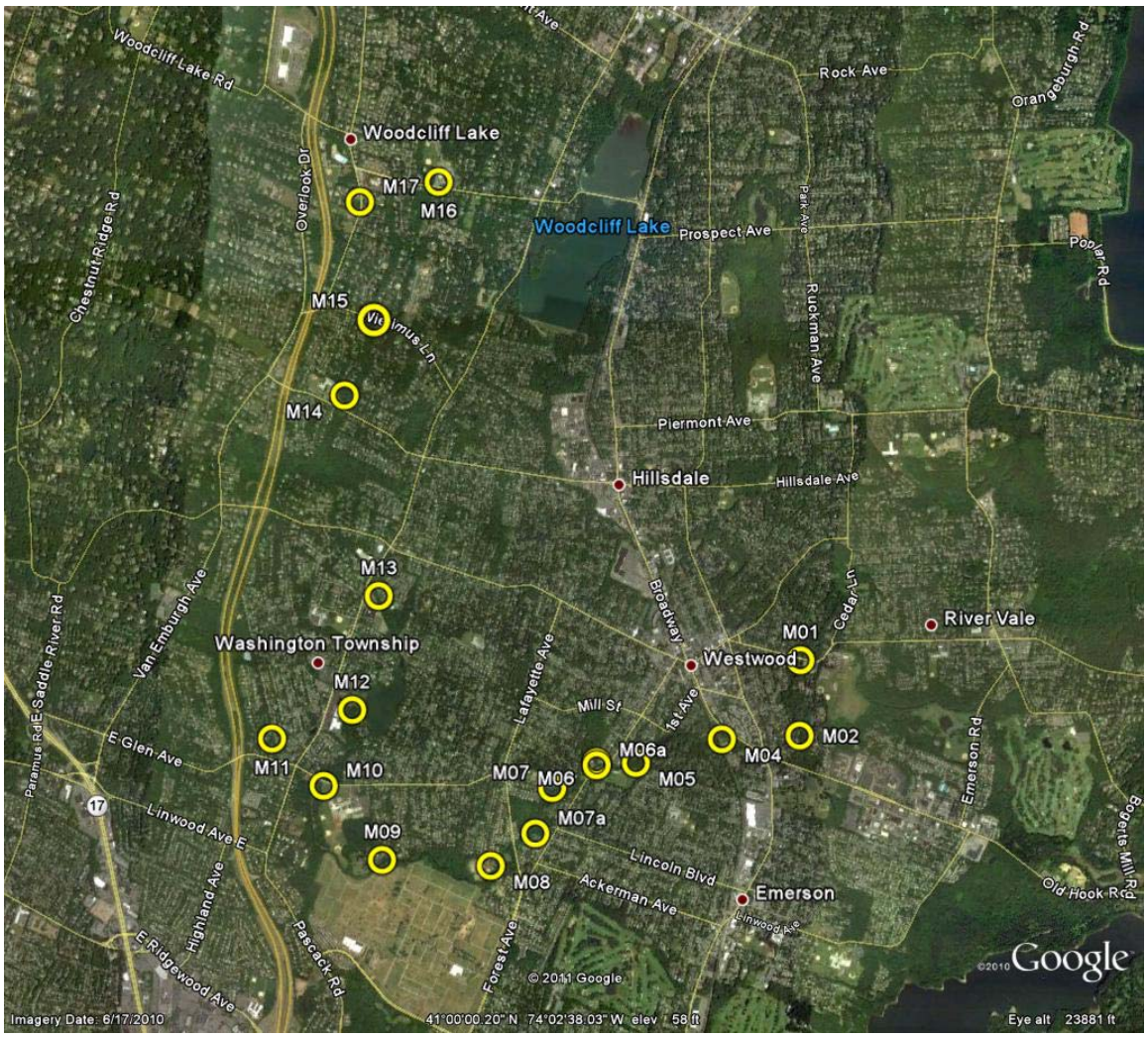

Figure 1 Optical Brightener Sampling Locations in the Musquapsink Brook Watershed

Data Summary

Fluorescence measurements were recorded from fluorometric analysis of the samples collected. The relative concentration of optical brighteners was measured in comparison to a blank solution with a known concentration of optical brighteners used in calibration. This data, as well as *in-situ* measurements of pH, dissolved oxygen (DO), and surface water temperature recorded during sampling, is provided in Appendix A. The average fluormetric reading for each sampling site is shown in Figure 2 below.

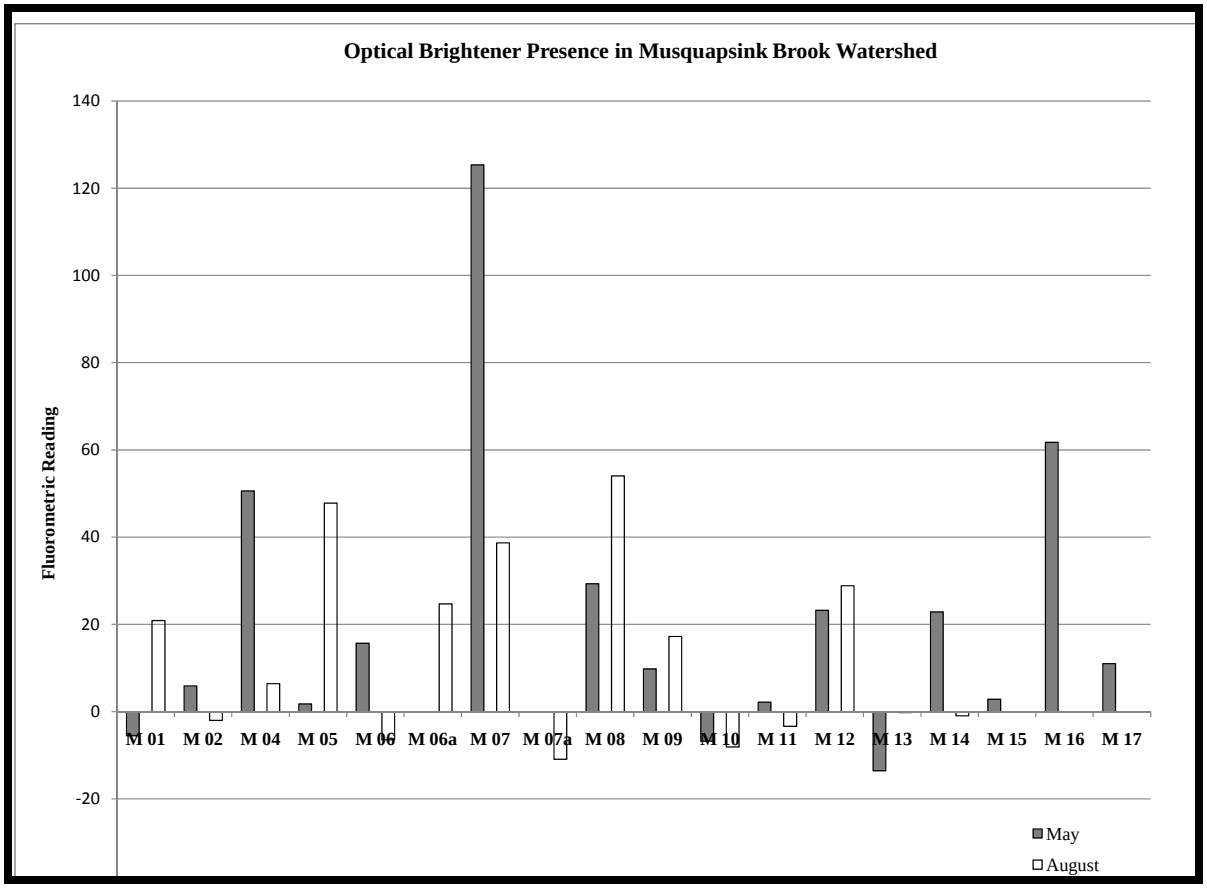

Figure 2 Average Fluorometric Readings for Samples Collected in May and August, 2010

The magnitude of the fluorescence reading indicates the relative strength of optical brightener in the sample. The highest fluorescence readings recorded were for samples collected from sites M04, M07, and M16. To further refine the trackdown of bacteria sources, fluorometric analysis results from the first round of sampling were used to adjust the location of sampling sites in the second round. Due to limited stream access and low-flow conditions, additional sampling locations could not be included in the regions of M04 and M16. M06a and M07a are located downstream and upstream, respectively, of

site M07. Values at or below zero indicate little to no presence of optical brightener in the sample.

Source tracking investigations completed by other research groups have reported positive correlations between fecal bacteria numbers and optical brightener levels, linking high levels of both indicators to human contamination. The RCE Water Resources Program study attempted to link physical surface water parameters (pH, DO, temperature) to optical brightener levels. The Pearson Product Moment is the ratio of covariance between the variables to the product of their standard deviations. The numerical value of the Pearson Product Moment ranges from -1.0 to +1.0. The closer the calculated coefficients are to +1.0 or –1.0, the greater the strength of the linear relationship between two independent variables. Correlations between *in-situ* physical surface water parameters and optical brightener levels were found to be, in general, weak and therefore no overlying conclusions could be drawn from this set of data. Further experimental design and laboratory research may provide further insight into the relationship between pH, DO, temperature and optical brightener presence in surface water.

APPENDICES

APPENDIX A
Tabulated Data

May 2010

Location ID	pH	DO	Temperature	Flourometric Units[1]		
		(mg/L)	(degree Celsius)	Reading 1	Reading 2	Reading 3
M01	7.11	5.93	20.4	-12	-8.9	4.22
M02	7.12	6.45	20.4	-13	16.1	14.5
M04	7.02	6.54	20.45	43.8	53.3	54.6
M05	6.79	3.81	19.5	-56	41.9	13
M05b*	6.79	3.81	19.5	22.1	-32	21.6
M06	6.74	3.56	19.15	36.3	12.4	-1.7
M07	6.84	4.19	19.45	122	118	136
M08	6.82	4.26	19.7	25.3	28	34.6
M09	6.83	3.83	20.15	-6.1	19.1	16.4
M10	7.08	6.41	21.4	2.82	-22	-1.1
M11	7.38	8.63	16.15	10.8	-5.8	1.5
M12	7.25	6.9	22.95	48	22	47.3
M12b*	7.25	6.9	22.95	4.3	22.1	-4.4
M13	7.42	8.84	17.55	-30	-1.8	-9
M14	7.47	8.6	18.55	24.9	27.1	16.5
M15	7.36	8.44	18.75	-22	-11	16.8
M15b*	7.36	8.44	18.75	29.1	3.4	0.56
M16	7.12	7.3	17.85	74.5	54.9	55.8
M17	7.28	8.11	19.1	12.2	9.6	11.1

*Duplicate samples were collected at this location
[1]Based on a scale of 0 to 100, with 100 indicating strong presence of optical brighteners in surface water sample

August 2010

Location ID	pH	DO	Temperature	Flourometric Units[1]		
		(mg/L)	(degree Celsius)	Reading 1	Reading 2	Reading 3
M 01	7.64	6.33	23.1	12.2	36.1	14.3
M 02	7.64	6.67	23.2	-3.1	10.2	-13.1
M 04	7.25	6.8	23.6	54	9.8	5.4
M04*	7.25	6.8	23.6	-32	-11	12.3
M 05	7	3.52	22.8	89.2	53.9	0.23
M 06a	7.22	4.21	23.1	24.1	37.8	12.2
M 06	7.23	3.35	22.2	5.4	-9.5	-15.3
M 07	7.32	5.11	22.6	45.2	54.2	16.7
M 07a	7.28	5.3	22.3	-5.6	1.2	-22.3
M07a*	7.28	5.3	22.3	5.4	-19.7	-24.7
M 08	7.18	5.29	22.6	15.3	67.9	78.9
M 09	7.18	5.49	23.2	34.2	15.4	2.1
M 10	6.87	5.64	21.6	-22	8.7	-11
M 11	7.94	8.74	23	3.9	-5	-14
M11*	7.94	8.74	23	-12	5.6	1.3
M 12	6.66	4.49	24.6	35.6	24.1	26.9
M 13	7.76	10.7	22.2	-23	10.1	12.3
M 14	7.6	7.54	24.4	17.3	-2.4	-17.8
M 15[2]	N/A	N/A	N/A	-	-	-
M 16[2]	N/A	N/A	N/A	-	-	-
M 17[2]	N/A	N/A	N/A	-	-	-

*Duplicate samples were collected at this location

[1]Based on a scale of 0 to 100, with 100 indicating strong presence of optical brighteners in surface water sample

[2]Sites with little to no flow. No samples collected.

APPENDIX B
Optical Brightener Standard Operational Procedure

RUTGERS
School of Environmental and Biological Sciences

STANDARD OPERATING PROCEDURE
OPTICAL BRIGHTENER ANALYSIS BY FLUOROMETRY

Authors: Jillian Thompson and Robert Miskewitz
Developed: January 2010

I. Background

Optical brighteners are compounds added to nearly all modern laundry detergents, which adhere to fabric and absorb and emit light, countering the yellowing appearance of whites and making other colors appear brighter. These compounds are excited by light in the near UV range (360-365nm) and emit light in the blue range (400-440 nm). After light absorption, fluorescence is given off during the second exited state and can be measured by a fluorometer (Tavares et al. 2008).

Because household plumbing systems combine effluent from washing machines and toilets, optical brighteners are associated with human sewage in septic systems, sanitary sewer systems, and wastewater treatment plants (Hartel et al., 2007). Their presence in surface water, therefore, indicates contamination from wastewater.

II. Materials

A. Fluorometer (Model 10-AU-000, Turner Designs, Sunnyvale, California).
B. Optical Brightener Optical Kit (Turner Designs, part number10-302R): lamp (10-049) emitting near UV light at 310-390nm; a filter (10-069R) for the 300-400 nm light range; a 436 nm filter to greater decrease background fluorescence
C. Tide® Powder Original Scent (no bleach)
D. Deionized water
E. Timer
F. Nalgene 250 mL opaque collection bottles
G. Transfer bottle
H. Refrigerator
I. Glass Cuvettes
J. Cooler
K. Scale (1.0 mg readability)

III. Sample Collection and Storage

A. Collect samples from the targeted waterbody in Nalgene 250 mL sampling bottles that have been acid cleaned and stored with 1% HCl (~5mL).
B. Transfer bottle is rinsed three (3) times with sample water before filling.
C. Sample water is collected with the transfer bottle placed 10cm below water surface facing upstream. Water is poured from the transfer bottle into a sample bottle.
D. Sample bottles are labeled and kept on ice and in a dark cooler after collection.
E. Upon arrival to the lab samples may be read after reaching room temperature or refrigerated at 4°C for up to five (5) days.

IV. Flourometric Calibration and Standard Curves

A. An optical brightener optical kit is installed in the fluorometer before any samples are read. This kit includes a lamp (10-049) emitting near UV light at 310-390 nm, a filter (10-069R) for the 300-400nm light range, and finally a 436 nm filter to greater decrease background fluorescence.
B. Make two-fold serial dilutions from a solution of 100mg powdered Tide in one liter deionized water (100 ppm).
    a. Mix 500 mL of the 100 ppm Tide solution with 500 mL deionized water to create the first dilution (50 ppm).
    b. Mix 500 mL of the 50 ppm solution with 500 mL deionized water to create the second dilution (25 ppm).
    c. Mix 500 mL of the 25 ppm solution with 500 mL deionized water to create the third dilution (12.5 ppm).
C. Create a standard curve using the serial dilutions from 100mg of Tide in one liter of deionized water (100 ppm).
    a. Adjust the fluorometer to a 80% sensitivity scale.
    b. The fluorometric value of 0 should be set equal to pure deionized water.
    c. The fluorometric value of 100 should be set equal to 100ppm of Tide in 1 liter of deionized water. This sets the equipment calibration.
    d. Record fluorometric readings of the solutions created from the serial dilution procedure.
    e. All results should be graphed (Fluormetric Reading vs. Concentration) to obtain a linear standard curve
D. Create a second standard curve using two-fold serial dilutions of 100mg Tide in one liter of ambient water. A standard curve created with ambient water will indicate the influence of background organic matter on fluorescence readings.
    a. Adjust the fluorometer to a 80% sensitivity scale.
    b. The fluorometric value of 0 should be set equal to deionized water.
    c. The fluorometric value of 100 should be set equal to 100ppm of Tide in 1 liter of deionized water. This sets the equipment calibration.

d. Once the two-point equipment calibration is established, create serial dilutions of Tide in ambient water.
e. Record fluorometric readings of the serial dilutions (100ppm, 50ppm, 25ppm, 12.5ppm)
f. All results will be graphed (Fluormetric Reading vs. Concentration) to obtain a second linear standard curve.

E. Compare the two standard curves. If organic matter in the ambient water is contributing to fluorescence readings, the ambient water solution readings will be higher than the deionized water solution readings.
a. The average difference between ambient water and deionized water fluorescence readings are calculated. This average represents a fluorescence reading due to background organic matter.
b. Any sample providing a reading at or below this calculated average will be considered to have only background sources of fluorescence.

V. Sample Analysis

A. Allow fluorometer and samples to warm up for 30 minutes
B. Shake each sample well before analysis.
C. Pour 9 mL sample water into cuvette (approximately 1/3 full). Place in fluorometer and start 10 second countdown.
D. Record reading.
E. Dispose of 9mL sample water and rinse cuvette with deionized water.
F. Repeat steps C through E three times for each sample.
G. Rinse the cuvette three times with deionized water before analyzing the next sample.

Sample analysis will provide qualitative results. Any fluorescence reading above the average difference between ambient water and deionized water fluorescence readings from the standard curves provide insight into the presence of optical brighteners in the sampled waterway. The magnitude of the fluorescence reading indicates the relative strength of optical brightener through multiple result and multiple site comparisons.

VI. Statistical Analysis

The three fluorometric readings recorded for each sample will be averaged and presented with the standard deviation. All data (both field and fluorometric) will be compiled to determine if significant relationships exist between optical brightener readings and other parameters. Data will be statistically analyzed using Microsoft Excel. A correlation analysis of the entire set of data will be completed to determine the relationship between optical brightener values and pH, dissolved oxygen, and water temperature measurements, respectively. The Pearson's Product Moment analysis will be used to determine correlation coefficients. Coefficients will be presented with p-values to demonstrate statistical significance.

VII. References

1. Hartel, Peter G., Jennifer McDonald, Lisa Gentit, Sarah Hemmings, Karen Rodgers, Katy Smith, Carolyn Belcher, Robin Kuntz, Yaritza Rivera-Torres, Ernesto Otero, and Eduardo Schroder. "Improving Fluorometry as a Source Tracking Method to Detect Human Fecal Contamination." Estuaries and Coasts 30 (2007): 551-61.

2. Tavares, Mary E., I. H. Spivey, Matthew McIver, and Michael A. Mallin. "Testing For Optical Brighteners and Fecal Bacteria To Detect Sewage Leaks in Tidal Creeks." University of North Carolina Wilmington Center for Marine Sciences (2008). http://people.uncw.edu/hillj/classes/EVS595/Optical%20brightener%20paper%20for%20NCAS.pdf

3. Leeds Point Chemistry Laboratory Standard Operating Procedures: Optical Brighteners. New Jersey Department of Environmental Protection. December 2006.