The **I**mpact of **S**eptic **T**ank **S**ystems on the **W**ater **Q**uality of **S**tream **R**eaches in the Oak Orchard **W**atershed

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Abstract

This study evaluated whether septic fields are a source of nonpoint source pollution in the Oak Orchard watershed. Accurate spatial information on septic fields was combined with hydrography and water quality sampling to identify stream reaches and parts of the shoreline that are most sensitive to septic systems. The regression analysis between septic field density and nitrate concentration shows a weak positive relationship. The regression analysis between number of fields within 200-meters and nitrate concentration also shows a weak positive relationship. The regression analysis between percent cropland and nitrate concentration, and percent cropland and SRP concentration, implies that agriculture does not have as large of an impact on nutrient pollution during the late summer. This study suggests that septic fields do have an impact on nitrate concentrations in the Oak Orchard watershed.

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Introduction

Eutrophication of inland waters is a problem caused by the increase of nitrogen and phosphorus cycling. Sources of nitrogen and phosphorus in a watershed include urbanization, deforestation, and agriculture (Pennington and Cech 2010). Nonpoint source pollution from septic fields has also been identified as a source of nitrogen and phosphorus in groundwater (Yates 1985), shorelines (Duda and Cromartie 1982; Reay 2004), streams (Hatt et al. 2004), and lakes (Hayes et al. 1990). Septic tanks can have a large impact on local stream chemistry by creating public health hazards and threatening surface and ground waters with chemical and biological pollutants (Alhajjar et al. 1990). As contributors of pollution, septic fields are considered to be a top 10 priority for management in the counties bordering Lake Ontario (Landre et al. 2004). Oak Orchard Creek, located in the Oak Orchard watershed is on New York State's "303(d)" list of impaired water bodies due to nutrient pollution (1972 Clean Water Act)(Makarewicz and Lewis 2009). Restoration and remediation of the Oak Orchard watershed has been a main goal of the Orleans County Soil and Water Conservation District for decades (Makarewicz and Lewis 2009). However, it is difficult to address issues caused by septic field pollution with a watershed policy; this is because the hydrologic connectivity between septic fields and water bodies is poorly understood, and the nutrient contributions septic fields make to water bodies have not been quantified.

The Septic System

Septic tank systems are used for onsite treatment and disposal of domestic wastewater. They are found in rural areas that do not have public sewer systems. Although most septic systems are capable of treating domestic wastewater, domestic wastewater contains a wide variety of potential pollutants including the following: pathogens, fecal bacteria, organic matter

(OM), phosphorus (P), nitrogen (N), ammonia (NH_4–N), biochemical oxygen demand (BOD), and suspended solids (SS), as well as pharmaceutical organic compounds and household detergents and chemicals (Gill et al. 2004; Wilhelm et al. 1994; Siegrist et al. 2012). All of the above pose a contamination risk to fresh waters.

Septic systems work by removing solid particles from the waste and treating the resulting liquid waste. Domestic waste first enters the septic tank. The waste settles into three layers: the sludge layer, the effluent, and the scum layer. The sludge layer consists of solid particles that sink. At the top of the tank is the scum layer containing solid particles that float. Between the scum and the sludge layers is the effluent. Anaerobic digestion of organic matter (largely fecal) occurs inside the tank, and the discharge from the tank travels to the septic field where it is treated.

The effluent entering the septic field is highly enriched in reduced inorganic nutrients such as methane (CH_4), ammonium (NH_4^+), soluble reactive phosphorus (SRP), sulfides (H_2S), manganese (Mn), and fecal indicator organisms (FIOs) (Withers et al. 2011). The effluent is released through holes in the pipes within the septic field. The effluent saturates the first 5-10cm of the surface soil, forming a biological mat (Wilhelm et al. 1994). As oxygen travels slowly through saturated soil, the mat is a site of further anaerobic digestion by microorganisms.

When the reduced nutrients and FIOs infiltrate into the unsaturated zone beneath the mat, the FIOs die off, and the nutrients are treated by oxidation and filtration. Microorganisms use the ample amount of O_2 in the unsaturated zone to oxidize organic carbon to carbon dioxide (CO_2) and NH_4^+ to NO_3, releasing H^+. Thus, treatment of wastewater in the drain field depends on the abundance of O_2. However, the abundance of O_2 makes the conversion of NH_4^+ to NO_3 unavoidable resulting in high concentrations of nitrate in the septic leachate. Phosphate is released in the septic field due to the oxidation of phosphorus. Sulfides are released as well due to the oxidation of H_2S. Chloride (Cl^-) is not effectively removed from wastewater by the septic system and is found to be mobile in water (Alhajjar et al. 1990). While many harmful pollutants, such as phosphorus and bacteria, are removed from the wastewater by filtration, other pollutants, such as nitrate and chloride, can move freely in the soil and reach the water table (Alhajjar et al. 1990).

Contamination of groundwater and surface water from septic leachate implies either direct discharge into a stream network; existence of a preferential flow pathway or shallow groundwater within the pathway; or a system failure due to poor design, soil type, location, age, or a lack of maintenance (Withers et al. 2011). Septic fields built in areas of shallow water table depths and/or permeable sandy substrates create a high risk environment for groundwater contamination (Reay 2004). Septic systems function best when the septic leachate has enough time to be filtered and oxidized before reaching a water body.

Optical Brighteners as Indicators for Septic Leachate

Optical brighteners, also known as fluorescent whitening agents, are whitening agents added to most laundry detergents to brighten the appearance of clothing (Cao et al. 2009). Optical brighteners adsorb ultraviolet light and fluorescence blue light (415-445nm) in the visible spectrum which gives fabric a white appearance (Boving et al. 2004). Optical brighteners can be detected by observing ultraviolet light adsorption. Since optical brighteners can pass through the septic system unscathed, they make an effective indicator of the presence of septic leachate (Alhajjar et al. 1990; Hartel et al. 2007;Cao et al. 2009). However, detection of optical brighteners can be difficult because of the presence of naturally occurring fluorescent compounds in the water. These compounds, such as humic acid, tannic acid, and other dissolved organic compounds, adsorb and emit light at wavelengths similar to optical brighteners. This causes difficulty in distinguishing fluorescence from just optical brighteners (Boving et al. 2004; Cao et al. 2009). Thus, optical brightener detection is only successful with water samples collected near to the groundwater-surface interface, making fluorometry not a recommended method. To resolve this problem, Hartel et al. (2007) suggested a method of optical brighter detection by combining fluorometry with exposure to ultra-violet light. Optical brighteners degrade when exposed to UV light, while the natural fluorescing compounds do not. Thus, optical brighteners can be detected in samples by measuring the change in fluorometric readings before and after UV exposure (Cao et al. 2009).

Previous Work

A conceptual model, proposed by Wilhelm et al. (1994), documents how biogeochemical processes alter domestic wastewater as it goes through a septic system and into groundwater. In the septic system treatment, two redox environments are formed. This first redox occurs in the tank. The tank is an anaerobic setting where the concentration of organic matter is high. Microorganisms oxidize the organic matter and produce CO_2, methane, H_2, and sulfide. The second redox occurs in an aerobic setting where the effluent flows into the drain field. Microorganisms use O_2 in the oxidation of C to CO_2 and NH_4^+ to NO_3^-. This causes high concentrations of NO_3^- in ground water. Thus, the product of NH_4^+ oxidation in a septic field will cause an increase in NO_3^- in drinking water, which can be 2 to 7 times the water drinking limit. The authors conclude that another form of wastewater disposal should be used or alternate systems that provide the proper sequence of redox zones and organic C supply to remove NO_3^- should be installed.

In another study, Reay (2004) evaluated shallow groundwater quality impacts from septic tanks and subsequent pollutant transport to estuarine surface waters. The U.S. Environmental Agency (1980) states that the volume of wastewater introduced into residential septic tanks systems is on the order of 160-200 L/day per capita, with concentrations of contaminants being 0.6-0.9mmol/L of total phosphorus, 2.5 -7.1mmol/L of total nitrogen, and $10^7$to 10^9 MPN/100mL of fecal coliforms. Since septic tank systems are not efficient in nutrient removal, estimates are that only 5-18% of nitrogen and 20-30% of phosphorus is removed (Hardisty 1974; Valiela et al. 1997). For two years, three residential sites that used septic tank systems were examined. It was found that shallow water table depths and permeable sandy substrates were a high risk setting for septic contamination. High concentrations of fecal coliforms, phosphorus, and nitrogen were measured. However, the mobility of phosphorus was low as concentrations measured were at or near background levels in groundwater adjacent to wastewater drain fields and along shorelines. Nitrogen had a high degree of mobility as concentrations were 50 to 100 times greater than adjacent water surface concentrations. Thus, as both the amount of people in a household and the amount of septic systems increase, an increased nitrogen loading should be expected.

In a study from Withers et al. (2011), data showed that the septic systems located near watercourses had direct discharge of effluent to adjacent streams. There were elevated levels of nutrients associated with anaerobic digestion of organic material and associated detergents including: SRP, NH_4^+, sodium (Na), potassium(K), Cl, boron (B), and Mn. Furthermore, discharge into headwaters was a concern due to lack of dilution from low flow volume. The authors also stated that under low flow conditions, septic tank leachate will lead to greater nutrient concentrations.

In a study regarding the fate of phosphorus in a drainfield, Sawhney and Starr (1977) used a 6-year-old septic system and measured the amount of phosphorus being released in the effluent. It was concluded that a soil with a deep water table below the drainfield should effectively treat wastewater effluent. Reay (2004) also found that phosphorus concentrations were high in the drainfield, but were limited to the drainfield. Another study reported that phosphorus was immobilized within meters of the distribution pipes, and concluded that significant movement of phosphorus is rare (Reneau et al. 1989). However, Sawhney and Starr (1977) stated that phosphorus can reach groundwater if there is a shallow soil with a high or perched water table.

Mallin and McIver (2012) conducted a study to investigate the impacts of nearby urbanization on public trust waters in the national park of Cape Hatteras National Seashore, USA. The authors found that at all sampling sites—exceptthe control—ammonium, phosphorus, and fecal bacteria concentrations were high, strongly seasonal, and significantly correlated with community water usage. These results indicate that increased septic tank usage, which occurred during tourist season, led to increased pollutant concentrations in area waterways. Furthermore, the authors suggested that in sensitive coastal areas with high water tables and sandy soils, alternatives to standard septic systems must be required to protect human health and the environment.

In conclusion, movement of nitrate, once released into the sediments of the septic field, is of significant concern for groundwater and surface water pollution. Many studies show an increase of nitrate concentration around septic fields. Thus, nitrate has the potential to reach surface water and groundwater, raising the concentration of nitrate in receiving water bodies.

Importance of Septic Field Locations

While watershed management policies are progressing with managing sources of nutrients from agricultural runoff, not many policies have been made in regards to septic field management. Septic field management has been non-existent due to the lack of precise and cost-effective septic field mapping. Current modeling of septic field fluxes uses a statistical approach based on population and household water use (Hayes et al. 1990). This approach does not include any information on location relative to nearby water bodies. However, several studies show that nutrient fluxes from septic systems are often influenced by the local geology, soil type, water table conditions, and distance from leach fields (Crosby et al. 1971; Waltz 1972; Childs et al. 1974; Reneau and Petri 1976). Thus, it is important to know the exact location of septic fields in order to correctly assess their influence. By identifying the location and number of septic systems in watersheds with greater precision, modeling of the septic contribution of nitrogen and phosphorus will be more accurate. The septic contribution to watershed total maximum daily load (TMDL) studies will be more defendable because the studies will be based on actual septic system density rather than demographics. Once this information is available, spatially-distributed models for predicting septic field fluxes can be developed. Mapping areas that are sensitive to septic tank inputs will allow watershed managers to direct policy changes towards problem areas rather than to all septic systems in the watershed. This will also give watershed managers an environmental and cost-effective way to determine whether septic systems or public sewer systems are better for an area.

Locating Septic Fields

Septic fields can be distinguished in aerial photography by 1) the hue of grass and 2) micro-relief. For example, Zhowet al. (2008) showed that color photography can be used to identify differences in grass color due to fertilizer. Septic leachate can have the same effect on grass as fertilizer because leachate adds water and nutrients to the area around the leach field. Imagery taken in the late summer, especially after a period of no rainfall, should show a difference between the color of the grass over a leach field and the color of the grass everywhere else (Figures 1A & 1B). Furthermore, another way to identify septic fields through aerial photography is by observing the micro-relief over the septic field. Burial of septic tanks and pipes during installation will disturb the soil, causing a different micro-relief than that of the undisturbed soil. Often, the disturbed soil in the leach field will sink between the pipes in the leach field, forming small trenches. In fall imagery, this micro-relief can be observed by leaves gathering in the trenches between pipes (Figure 1C).

Objectives

This study proposes to combine accurate spatial information on septic fields with hydrography and water quality sampling to identify stream reaches and parts of the shoreline that are most sensitive to septic systems. With this information, watershed managers will have the locations of sources of septic field pollution. It is hypothesized that stream segments with greater densities of septic fields, which are closer to the stream, will have greater nutrient concentrations and will be positive for optical brighteners. The objectives of this study are to 1) rank stream reaches in the watershed by their proximity to septic fields using spatial statistics, such as septic field density, and number within the 200-meter buffer and 2) physically measure water quality (including the presence or absence of optical brighteners) in the stream reaches to determine if septic leach fields are having a measurable impact on water quality. If the hypothesis is correct, there should be greater concentration of nitrate in stream reaches with catchments containing greater densities of septic fields. There may also be greater concentrations of nitrate in stream reaches that contain more septic fields within a 200-meter buffer.

Study Area

The study area for this research is the Oak Orchard watershed, located in Western New York in Genesee and Orleans counties (Figure 2). Eventually discharging into Lake Ontario, the Oak Orchard watershed is a contributor of nonpoint source pollution to the lake (Richards et al. 2010). The watershed area is approximately 1,173,794 acres, with the headwaters, about 14% of the watershed, located in Genesee County. The topography of the region is mostly flat with gently sloped small hills, a result of glacial and post-glacial erosion. The Oak Orchard watershed is made up of agricultural lands, forested and non-forested wetlands, and a few urbanized areas. The watershed is undergoing a TMDL for phosphorus and sediment, which makes it an ideal place for nutrient testing and analysis. Currently, the influence of septic field contributions on the load for this watershed is not well known.

Oak Orchard SWAT Model

In 2010, a soil water assessment tool (SWAT) was developed for the Oak Orchard watershed in order to evaluate sources and sinks of sediment and nutrients (Richards et al. 2010). The model included point sources for every subbasin and was calibrated for water flow and sediment using observed loading data collected by Makarewicz and Lewis (1999; 2009). The model used land cover and soil information to estimate nutrient loads, but did not explicitly include septic field inputs in its estimation of phosphorus and nitrogen loads. Results of the model had a Nash-Sutcliffe (NS) prediction efficiency of 0.81 for the calibration period (1997-1999). Calibration was excellent for flow and total phosphorus and fair for sediment flux. However, model performance in the validation period was weaker, which was attributed to climate and groundwater contribution differences in 2008. Thus, the model shows that there is a need for a better understanding of the timing and magnitude of groundwater inputs coming from the Onondaga Escarpment. The model results also suggest that the largest phosphorus contributions are from the mucklands and agricultural fields in the southern part of the watershed. This model helps us to understand some of the sources of nutrients in this watershed but omits septic field contribution.

Previous Septic Field Mapping in Oak Orchard

In a study of septic field distribution in the Oak Orchard watershed, septic fields were identified 70-75% of the time using Pictometry oblique imagery (Richards and David 2015). Canopy cover was the main problem with the technique, preventing the identification of 10% of the septic systems. Certain kinds of septic systems (e.g. cess pools) were not able to be mapped. However, these are older systems that are seldom used and not very common. Imagery taken in the early spring had the best results because the color differential of grass growing on septic fields was enhanced relative to grass that had not started growing from lack of spring nutrients and moisture. This work demonstrated that this technique can be used to map septic fields as well as septic systems with leaks.

A total of 1,277 septic fields were found in the watershed (Richards et al. 2016). Using the locations of the septic fields, a 200m buffer was created around all of the stream segments (Figure 3). Areas are considered to have a high density when greater amounts of septic fields arelocated within the buffer. Approximately 40% of the Oak Orchard septic fields are within the buffer. Location data on septic fields from the Oak Orchard study will be utilized in this thesis.

Methods

Water sample locations were determined by using a 200m buffer around all of the stream segments and identifying which areas had a high amount of septic tanks within the buffer. A total of 26 locations were chosen (Figure 4). Septic tank density was determined by defining the watershed subbasin based on each sample site, calculating the area, and then dividing the area by the number of septic fields contained within the watershed. Areas of high density were of greater interest because those areas pose the greatest amount of identifiable septic tank leachate.

Percentage of cropland was also evaluated for the catchment associated with each sample site. It has been assumed that the abundance of farming in the Oak Orchard watershed affects the water quality because of the addition of nitrate and phosphorus from fertilizer. The fertilizer used on the cropland travels as runoff from cropland to nearby streams. However, this study challenges that septic fields are the source of nitrate in the watershed. Cropland statistics were calculated using the 2010 NRCS Cropland raster layer, which was extracted from 2010 Landsat Imagery. Using GIS, the percentage of cropland was calculated for each subbasin by dividing the total area of the subbasin by the area of cropland within the subbasin.

Water quality parameters, such as temperature, electroconductivity, pH, and stream flow discharge, were collected in the field using a Marsh McBirnneyFloMate 2000 current meter, a YSI ECOSENSE 100 electroconductivity meter and a YSI pH meter. Water sampling consisted of grab samples that were collected in the middle of streams. Two grab samples were collected at each sample location. A sample was collected in an amber colored bottle for optical brightener (OB) analysis, and the other sample was collected in a white bottle for nutrient analysis. Both were put into a cooler on ice for further analysis in the lab.

Water samples were analyzed for the presence of optical brighteners using an *AquaFluor®* handheld fluorometer/turbidimeter and a 50ppm OB calibration solution with Tide 2X Original Scent as the OB agent. A modified Hartel et al. 2007 method was used for detection of optical brighteners, which suggested replacing the step requiring a 15% fluorescence reduction after five minutes of UV exposure with two steps (Cao et al. 2009). Three replicates for each sample were used. After five minutes of UV exposure, the first step required there to be a reduction of fluorescence of no less than 8%. A reduction less than 8% means the sample is negative for optical brighteners. A reduction no less than 30% means the sample is positive for optical brighteners. For a reduction of fluorescence between 8% and 30%, further exposure (five additional minutes, total of ten minutes exposure) to UV light was needed. A ratio of reduction after 10 minutes of UV exposure to reduction after 5 minutes of UV exposure must be less than 1.5 for the sample to be positive for optical brighteners.

Cao et al. (2009) used an initial fluorescence threshold of 5µL/L or 10 relative fluorescent units based on calculations of flow from their study site. They determined that an initial fluorescence less than 10 would indicate a negative reading for the presence of optical brighteners. This study used samples that were taken directly from sewage, or samples that were created by mixing detergent and stream water for known concentrations. Therefore, Cao et al. (2009) could calculate a known dilution threshold. To see if optical brightener analysis could still be conducted for samples with an initial fluorescence less than 10, a dilution curve was created using known concentrations of Tide 2X Original Scent detergent (Figure 5). For this study, an initial fluorescence threshold was not used; regardless of the initial fluorescence, decay ratios were calculated for all samples.

Testing for SRP, NO_3, SO_4, and Cl

Water samples were tested for SRP using the EPA 365.1/365.2 method. The EPA 353.2 method was used to test for NO_3. Testing for SO_4 used theEPA 375.4 method. Hach pillows were used as the reagent for each method. Known standards were made and a standard curve was created. For each nutrient concentration, 10mL of filtered sample was needed. Contents in the Hach pillow were emptied into a tube of 10mL of sample. A 5-minute reaction took place. The sample was then placed in a spectrometer, and the concentration was calculated by using the absorbance value in the standard curve.

Samples were analyzed for chloride using potentiometry using an ELIT Chloride electrode with a silver chloride reference electrode. A small amount of the concentrated unknown sample was added into a known largequantity of a lower concentration standard (10ppm). Measurements were taken using a chloride electrode which measures in voltage (millivolt). The subsequent change in electropotential between the known concentration sample and then the known sample with the added unknown sample were then used to determine the chloride concentration of the unknown (Rundle 2000).

Once samples were analyzed, a relationship between water quality and septic tank variables was determined by creating scatter plots between septic field statistics (septic field density and number of fields within 200-meters of sample site), nutrients (SRP and nitrate), and optical brighteners. For scatter plots that visually appear to have a relationship, a regression analysis was implemented and the adjusted R-squared was determined to assess the amount of variance controlled by each septic field's variable. P-values were determined for the slope of the least-squares fit. This value describes the fractional probability that the relationship is incorrect.

Low P-values indicate that the relationship between water quality parameters and septic field variables is accurate.

Results

Two rounds of low flow samples were collected in August 2016 (August 18) and in October 2016 (October 11 and 12). In August, eighteen sites representing first, second, and third order streams were visited. Only thirteen had any water present, and of those thirteen, only nine had flowing water. In October, eighteen sites were visited, but only ten had water present and only six had flowing water. One round of high flow samples were collected the following year in June 2017 (June 8). Twenty-two sites were visited, and all but one site had water present. Table 1 presents details on the site location, stream order, and septic field metrics (total number in the contributing area, contributing area, septic field density). Tables 2a, 2b, & 2c present the water quality data collected.

Based on the water quality data collected, sites A, E, G, H, J2, and N are the stream segments most impacted by nutrients. Two of these sites had low initial OB concentrations.However, their fluorescence ratio suggests that optical brighteners were present. Scatter plots revealed no obvious relationship between septic field density and SRP, sulfate, or chloride concentration.

In regression analyses, site N was considered an outlier and was excluded from the analysis. In August and October, site N had extremely high nitrate concentrations when compared to the concentrations from the other sample sites. Site N is located near a large farm and major road (Rt. 31), and it is assumed that there are additional sources of nitrate in this sample site such as fertilizer runoff into the stream.

A regressional relationship between septic field density and nitrate concentration shows a weak relationship between the two factors (r-sq=26.1%) (Figure 6). The p-value is 0.09 meaning that 91% of the time the septic field density will predict the nitrate concentration. A regressional relationship between the number of septic fields within 200m of the sample site and nitrate concentration for August suggests that the amount of fields within 200m of the sample site do not predict the nitrate concentration (r-sq = 12.5%) (Figure7). The p-value is 0.26 meaning that 74% of the time the number of fields within 200m will predict the nitrate concentration. Thus, the septic field density within the subbasin is a better predictor of nitrate concentration.

Optical Brighteners

In August, nine out of the thirteen sample sites were positive for optical brighteners. In October, sampled sites that were positive in August were still positive in October. However, in June, six sites that were positive during low flow showed negative readings for optical brighteners during high flow.

From the dilution curve created, it was determined that optical brighteners can still exist in a sample with an initial fluorescence value less than 10 (Table 3). As the standard was diluted, the ratio, which indicated the presence of optical brighteners (<1.5), remained constant while the initial fluorescence decreased. At initial fluorescence less than 10, the ratio still remained constant. Thus, a low initial fluorescence value that results in a ratio less than 1.5 can be considered positive for optical brighteners.

Optical brightener statistics consisting of either a “yes” or “no” for each sample site were used to assess the presence of OBs. The number of sites within 200m was compared to the percentage of yes’s and no’s. For sites with no septic fields within 200m of the sample site, 25% of the samples were positive for optical brighteners (Figure 8). For sites with one septic field within 200m of the sample site, 50% of the samples were positive for optical brighteners. For sites with two septic fields within 200m of the sample site, 67% of the samples were positive for optical brighteners. For sites with three septic fields within 200m of the sample site, 75% of the samples were positive for optical brighteners. A regression analysis of this data had an r-sq of 95% and a p-value of 0.02. This means that as the number of septic fields close to a stream segment increase, more samples are going to contain optical brighteners. Thus, there is a relationship between number of fields close to streams and the chance of leachate pollution.

In contrast to septic field statistics, relationships between nitrate and percentage of cropland were not as well-defined. The regression analysis of percent cropland and nitrate concentration did not yield a strong relationship (r-sq = 5.5%) (Figure 9). The slope had a p-value of 0.46. Since septic field density had a stronger relationship to nitrate concentration, septic fields have a greater influence than cropland on nitrate concentrations found in the streams of the watershed.

Discussion

Shown by this study, the stream segments that could be most impacted by septic fields are the reaches that have fields within 200-meters of the sample site. Results show that an increase in septic fields within 200-meters will increase the probability of a positive reading for optical brighteners. This means that there is a higher chance of septic leachate reaching stream segments and adding nutrient pollution to the streams. Septic field density within the watershed of the sample site did not have a strong correlation with nutrient pollution suggesting that while density may have an impact on nitrate concentration, density may not always be a reliable predictor of nitrate concentration. There are a variety of reasons why this could be the case. One possibility is the amount of groundwater inputs into the stream. Although the sampling strategy focused on low flow conditions, there are stream segments that receive a greater than normal quantity of groundwater flow by virtue of their geomorphic position. Higher groundwater input could dilute the septic field inputs and resultin lower nitrate concentrations. Such areas will be less susceptible to water quality changes related to septic field density. Water table conditions, slope, distance, and soil characteristics are known to impact nutrient fluxes from individual septic systems (Crosby et al. 1971**;** Waltz 1972**;** Childs et al. 1974**;** Reneau and Petri 1975**;**Reneau and Petri 1976;Reneau 1979; Rea and Upchurch 1980; Starr and Sawhney 1980**;**Gerrite et al 1995**;** Sherlock et al. 2002**;**Collick et al. 2006). An area with optimal geologic conditions for septic fields may have lower nutrient fluxes despite having a high density of systems. Similarly the age of the systems might be a factor in nutrient fluxes. Older developed portions of the watershed will have older systems than newer portions, making the age of development of the stream reach a factor. Related to this is the impact of the age of the fixtures that supply water to the septic field. New fixtures are much more water efficient and produce less waste water per person. A new (modern) subdivision will produce less wastewater than a subdivision that was constructed in the 1970'sfor the same density of septic fields.

Optical Brightener Analysis

The importance of measuring for the presence of optical brighteners is that their presence indicates that nutrient pollution is most likely coming from septic leachate. If the optical brighteners were negative for a site, then any nutrient pollution is assumed to be from other sources.

Most samples were below the initial fluorescence value of 5, which would indicate a negative reading for the presence of optical brighteners. However, this study shows that a low initial fluorescence could be from a lower concentration of detergent present in the sample; the sample can still be positive for optical brighteners with a low fluorescence. There is just a low concentration of optical brighteners in the sample. Thus, if detergents containing optical brighteners are diluted to such a point that the initial fluorescence is low, the leachate from the septic fields may also be diluted to a point where there is not an impact on stream chemistry. Low nutrient concentrations with low initial fluorescent values could mean that septic fields are not a problem for that area. Initial fluorescence values are thus useful for interpretation and should not be dismissed as they currently are using the "presence or absence" paradigm.

Another reason for low optical brightener values is that optical brighteners will decay out in the field under the UV light from the sun. Cao et al. (2009) found that their samples degraded rapidly later in the day when the UV index reached 2. Samples in the shade degraded more than 25% when the UV index reached 7. The first batch of samples was collected in August, potentially having UV index values higher than 7. The samples collected in October had UV index values between 3 and 7. Thus, optical brighteners may have already decayed in the stream water before being collected and measured. Collection of samples in tree canopied stream sites using opaque, UV resistant sample bottles is recommended.

Nitrate Analysis

While natural levels of nitrate in freshwater are 1ppm, the EPA drinking standard for nitrate is 10ppm (Behar 1996). Concentrations above 10ppm will cause blue baby syndrome. Since the sample sites (excluding Site N) had nitrate concentrations below 10mg/L, it could be said that septic fields are not producing enough nitrate pollution to cause health concerns in the watershed. However, there could still be palpable ecological impacts from excessive concentrations that are between 1 and 10ppm. Eutrophication at nitrate concentrations above 1ppm can occur. Several sample sites were above the 1ppm threshold suggesting that there is an anthropogenic source of nitrate in the watershed.

Nitrate is not the only pollutant that comes from septic fields. Recent studies show that a significant number of household pharmaceuticals can pass through septic systems intact (Carrara et al. 2008; Phillips et al. 2015). Hormones, antidepressants, and antimicrobials have serious ecologic impacts such as impairing the ability of native bacteria in groundwater discharge zones to reduce nitrogen concentrations (Underwood et al. 2011). Also, pharmaceuticals have had an impact on the physiological and behavioral aspects of fish (Hinck et al. 2009; Painter et al. 2009). Stream segments with high densities of septic fields may be the parts of the stream system that receive the highest concentrations of pharmaceuticals.

It is commonly assumed that the abundance of farming in the watershed adds additional nitrate into the watershed, but the regression analysis in this study shows no relationship between the amount of cropland and the concentration of nitrate (figure 6). However, site N was excluded from the analysis due to a possible source of nitrates from farmland. TMDL studies typically focus on agricultural sources of nutrient when they manage watersheds for nonpoint source pollution. This study suggests that septic fields may have more of an impact, than farmland, on nitrate concentrations in the watershed during the late summer (August). The TMDL study for Oak Orchard watershed, which is ongoing, may need to assess and consider mitigating septic field sources as well as agricultural sources.

SRP Analysis

In this study, several samples had concentrations of SRP over the EPA (1986) guideline of 0.05mg/l for streams discharging into a reservoir. However, a regression analysis shows that there is not a relationship between SRP and septic tank density. This is to be expected because SRP is retained in the sediments in and around the septic field. Areas that had high amounts of

SRP are receiving pollution from another source or a broken or poorly built septic field. Further investigation of high concentration sites would need to be done to determine the source. In a properly functioning septic tank, phosphorus should be retained within or around the septic field and should not cause a pollution problem in streams. This is shown by the lack of a relationship between SRP and septic field density. This suggests that sources of phosphorus are site specific. High concentrations of SRP cannot be predicted because the sources may be random in the watershed, such as a poorly functioning septic field or an overzealous farmer. SRP is also much more biologically available and subject to nutrient uptake and nutrient "spiraling" (Triska et al. 1989).

Future Work

This study could be expanded by adding more sample sites. Additional sample sites would make the regression analysis stronger. Further, groundwater samples could be taken at or near stream sample sites to determine if septic leachate is reaching the groundwater beforereaching the streams, or vice versa. In addition, this study could be applied to a different watershed where nitrate pollution is a problem and where septic systems are present.

Optical brightener analysis could be expanded by sampling in the same location at different times during the day. This would determine if and how much the optical brighteners are decaying before being analyzed. Groundwater could also be sampled and compared to stream water for optical brighteners to assess the loss of OB from the sun in streams and/or the effects of dilution from upstream baseflow.

Conclusions

This study evaluated whether septic fields are a source of nonpoint source pollution in the Oak Orchard watershed. Spatial information on septic fields was used to identify possible areas that could be affected by septic leachate due to high density of septic fields in the area. Water quality sampling was used to identify stream reaches where septic systems may have an influence on nutrient pollution. The regression analysis between septic field density and nitrate concentration shows a weak positive relationship. The regression analysis between number of fields within 200-meters and nitrate concentration also shows a weak positive relationship. The regression analysis between percent cropland and nitrate concentration, implies that agriculture does have a large of impact on nutrient pollution during the month of August. This study suggests that septic fields do have an impact on nitrate concentrations in the Oak Orchard watershed.

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Tables

Table 1. Details on the site location, stream order, and septic field metrics (total number in the contributing area, contributing area, septic field density, and number of fields within 200-meters of sample site).

Site ID	Stream Order	Watershed Area (sq km)	Septic Fields	Septic Field Density	Septic Fields within 200m of sample site
A	Second	15.1	52	3.5	1
B	First	0.4	2	5.6	3
C	First	0.8	7	9.2	1
D	First	2.4	12	5.1	2
E	First	3.2	7	2.2	1
F	First	1.8	21	11.4	3
G	Second	5.1	42	8.2	2
H	Third	47.0	96	2.0	2
J	Second	2.9	10	3.4	2
J2	First	0.8	5	6.0	0
K	First	3.7	7	1.9	2
L	First	0.9	24	27.5	1
M	Second	6.6	5	0.8	2
N	Second	7.0	29	4.1	1
N2	First	1.5	4	2.7	2
O	First	1.9	7	3.7	0
P2	Third	32.5	67	2.1	0
Q	First	1.6	15	9.4	1
Q2	Third	22.9	99	4.3	0
S1	Third	28.3	70	2.5	3
S2	Third	28.6	64	2.2	2
T	First	2.9	16	5.5	3

Table 2a. Nitrate, SRP, sulfate and chloride concentrations for samples taken in August 2016. A total of 18 sites were visited and samples were taken from 13 sites.

August				
Site ID	Nitrate (ppm)	SRP (ppm)	Sulfate (ppm)	Chloride (ppm)
A	1.17	9.48	60.16	283.32
B	2.17	0.04	71.20	479.47
C	*dry**	*dry*	*dry*	*dry*
D	*dry*	*dry*	*dry*	*dry*
E	2.63	0.88	107.80	244.32
F	8.28	0.07	131.46	73.05
G	1.26	0.02	675.30	231.98
H	4.90	1.77	944.68	153.32
J	0.00	0.24	1022.17	271.97
K	*dry*	*dry*	*dry*	*dry*
L	*dry*	*dry*	*dry*	*dry*
M	0.44	0.17	378.60	118.29
N	77.59	19.53	214.14	158.25
O	0.62	0.29	41.91	84.19
Q	1.26	0.12	44.04	39.45
S1	0.62	0.53	58.98	64.51
S2	0.44	0.54	57.58	77.78
T	*dry*	*dry*	*dry*	*dry*

*denotes sample sites where no water was present and no sample was collected

Table 2b. Nitrate, SRP, sulfate and chloride concentrations for samples taken in October 2016. A total of 17 sites were visited and samples were taken from 9 sites. Sample sites J2 and P2 were added.

October				
Site ID	Nitrate (ppm)	SRP (ppm)	Sulfate (ppm)	Chloride (ppm)
A	BD**	3.21	122.63	144.56
B	2.99	0.04	74.80	479.32
C	*dry*	*dry*	*dry*	*dry*
D	*dry*	*dry*	*dry*	*dry*
E	*dry*	*dry*	*dry*	*dry*
F	1.62	0.04	127.97	95.95
G	2.35	0.17	789.70	233.36
H	7.82	1.89	873.89	158.44
J	*dry*	*dry*	*dry*	*dry*
J2	BD	0.02	801.37	120.02
K	*dry*	*dry*	*dry*	*dry*
L	*dry*	*dry*	*dry*	*dry*
M	*dry*	*dry*	*dry*	*dry*
N	52.99	20.03	119.06	170.43
O	2.17	0.12	50.15	153.65
P2	BD	0.76	199.02	89.37
T	*dry*	*dry*	*dry*	*dry*

**denotes concentrations that were below detection (nitrate: absorbance of 0.26nm or less)

Table 2c. Nitrate, SRP, sulfate and chloride concentrations for samples taken in June 2017. A total of 22 sites were visited and samples were taken from 21 sites. Sample sites N2 and Q2 were added.

June				
Site ID	Nitrate (ppm)	SRP (ppm)	Sulfate (ppm)	Chloride (ppm)
A	13.34	0.03	93.47	140.47
B	2.70	BD**	130.18	333.25
C	0.98	BD	16.74	60.25
D	29.50	BD	101.09	147.40
E	19.16	0.14	56.56	125.94
F	12.40	BD	68.60	74.79
G	4.96	BD	801.98	121.98
H	5.72	0.08	1684.23	104.49
J	1.00	BD	678.42	72.50
J2	6.96	0.07	724.36	112.24
K	*dry*	*dry*	*dry*	*dry*
L	3.14	0.00	1351.23	350.00
M	1.78	0.09	48.54	38.00
N	5.77	0.05	92.27	98.00
N2	3.89	0.01	17.95	64.00
O	2.72	0.00	27.37	74.00
P2	3.24	0.00	111.93	66.00
Q	11.90	0.02	84.75	64.00
Q2	2.99	0.02	36.20	80.55
S1	3.20	0.03	35.80	162.99
S2	3.17	0.02	42.72	157.7
T	1.27	0.29	29.98	57.66

**denotes concentrations that were below detection (SRP: absorbance of 0.05nm or less)

Table 3. As the initial fluorescence decreases due to a decrease in detergent concentration, the ending ratio that determines the presence of optical brighteners remains relatively constant at value indicative for positive readings of optical brighteners. Below the red line is where, according to Cao et al. (2009), samples would be considered negative for optical brighteners based on initial fluorescence below 10 relative fluorescent unites. However, this table shows that even when initial fluorescence is below 5, there is still apresence of optical brighteners as indicated by the positive reading from the ending ratio.

Detergent Concentration (µl/L)	Initial Fluorescence (Tide Units)	Ratio
50	100.0	1.04
25	47.7	1.02
10	23.0	1.04
5	11.7	1.04
2.5	4.6	1.07
1	2.6	1.06
0.5	1.2	1.06

Figures

Figures 1A, 1B, and 1C. Images A and B show the darkening of grass where the septic field is. Image C shows the gathering of leaves over the septic field due to micro-relief.

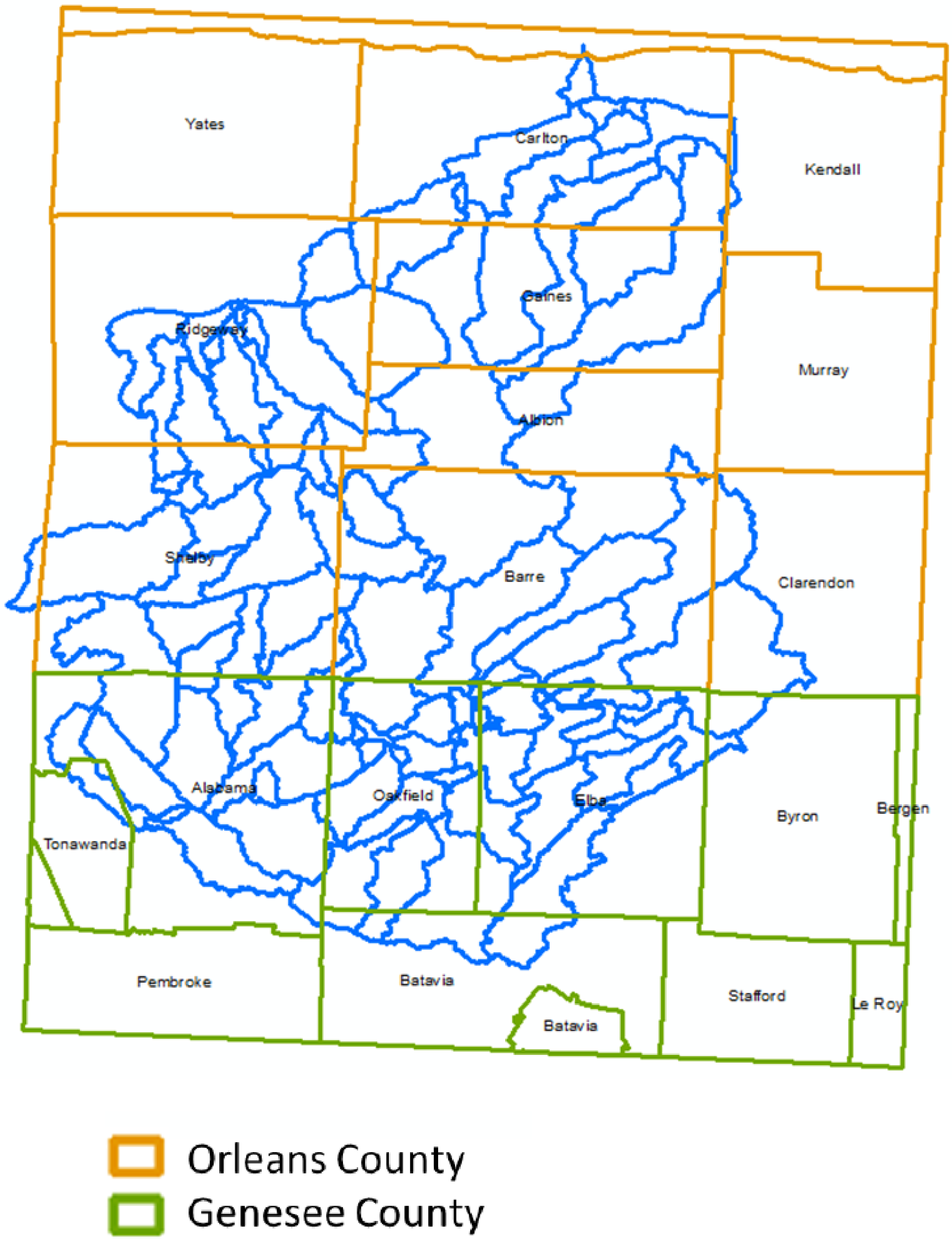


Figure 2. Map of study area showing Oak Orchard watershed and the two counties where the watershed is located.

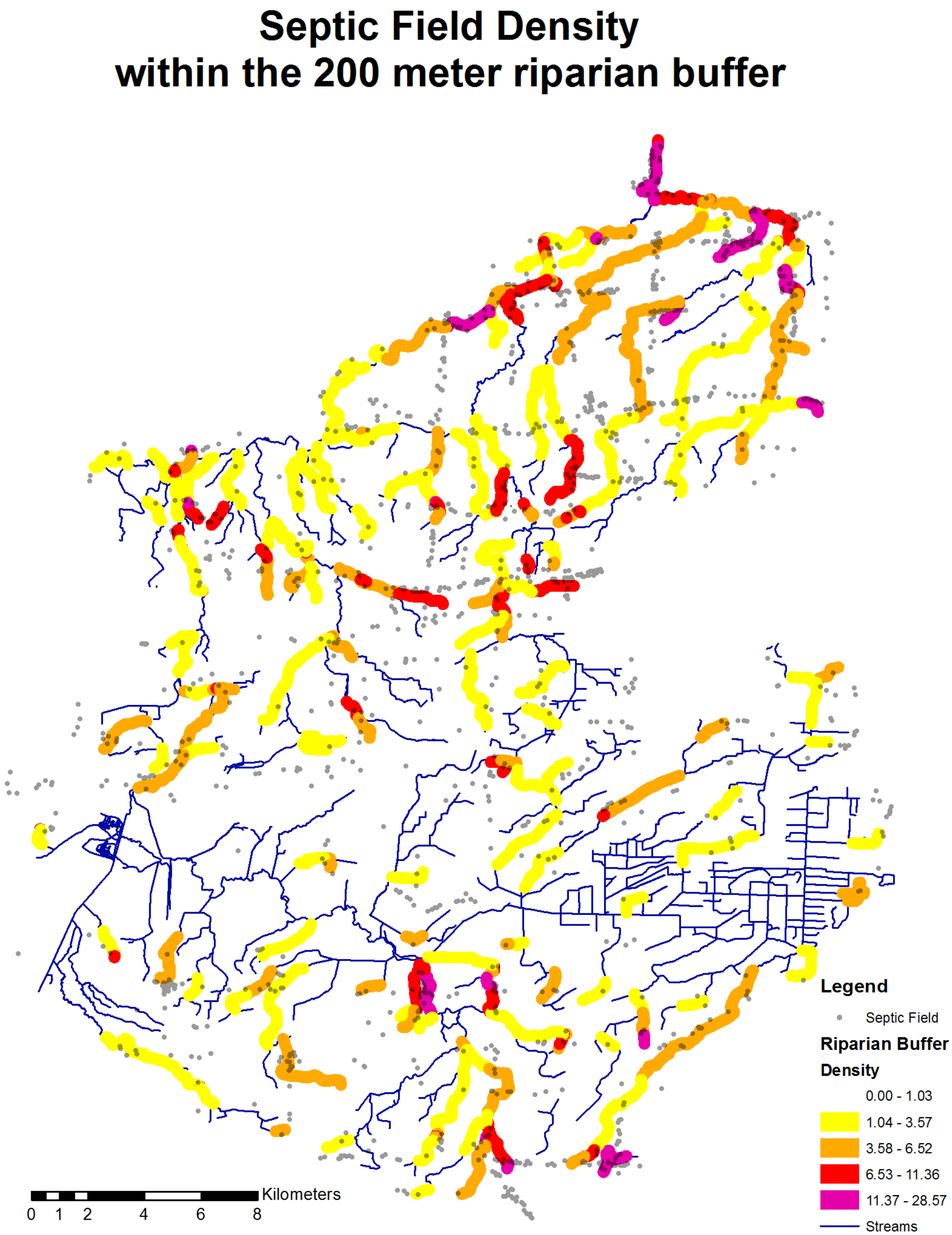


Figure 3. Septic field density within the 200m riparian corridor in the Oak Orchard watershed. Density is expressed as numbers of septic fields per square kilometer. Areas of stream segments with high densities are of interest because they may have greater septic inputs.

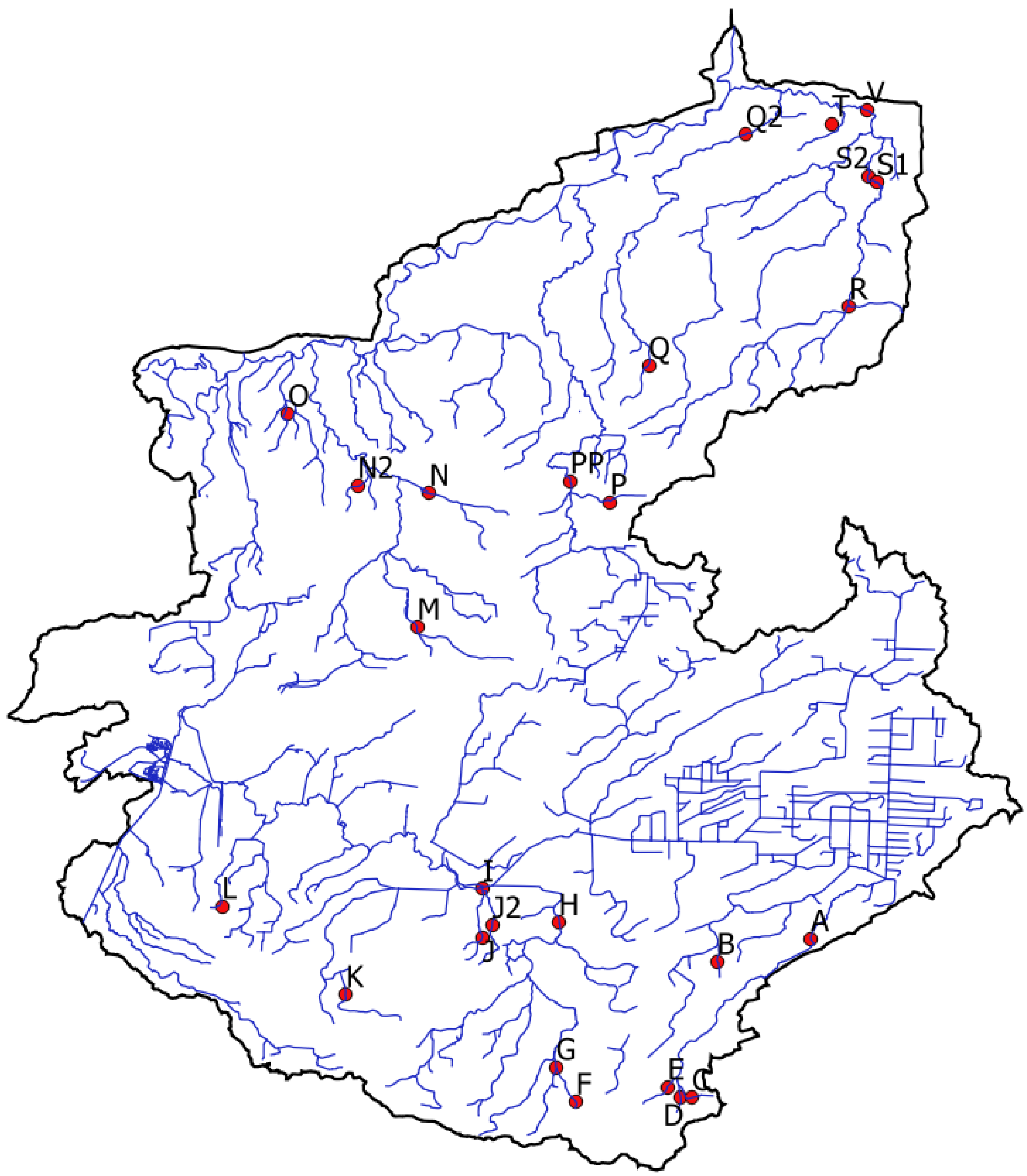


Figure 4. Locations and site names of sample sites in the Oak Orchard watershed.

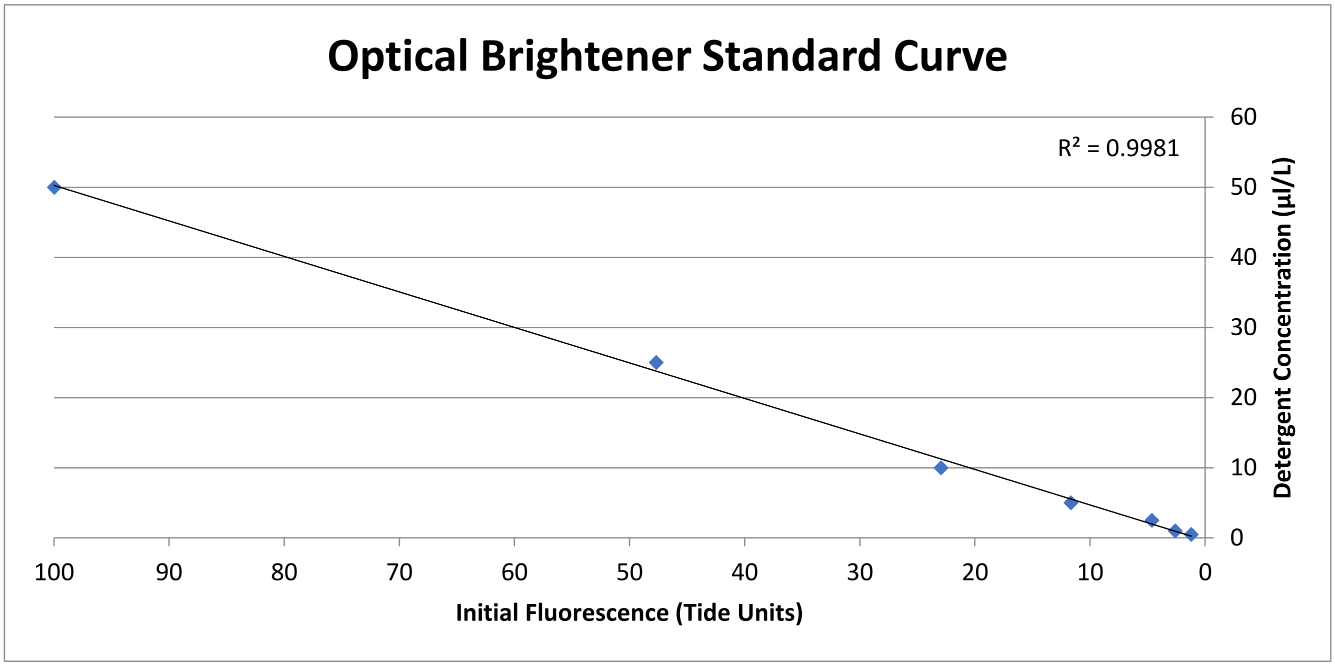


Figure 5. Initial fluorescence was measured for known concentrations of Tide 2X Original Scent detergent. From this graph, the amount of detergent in a water sample could be determined based on the initial fluorescence of a sample.

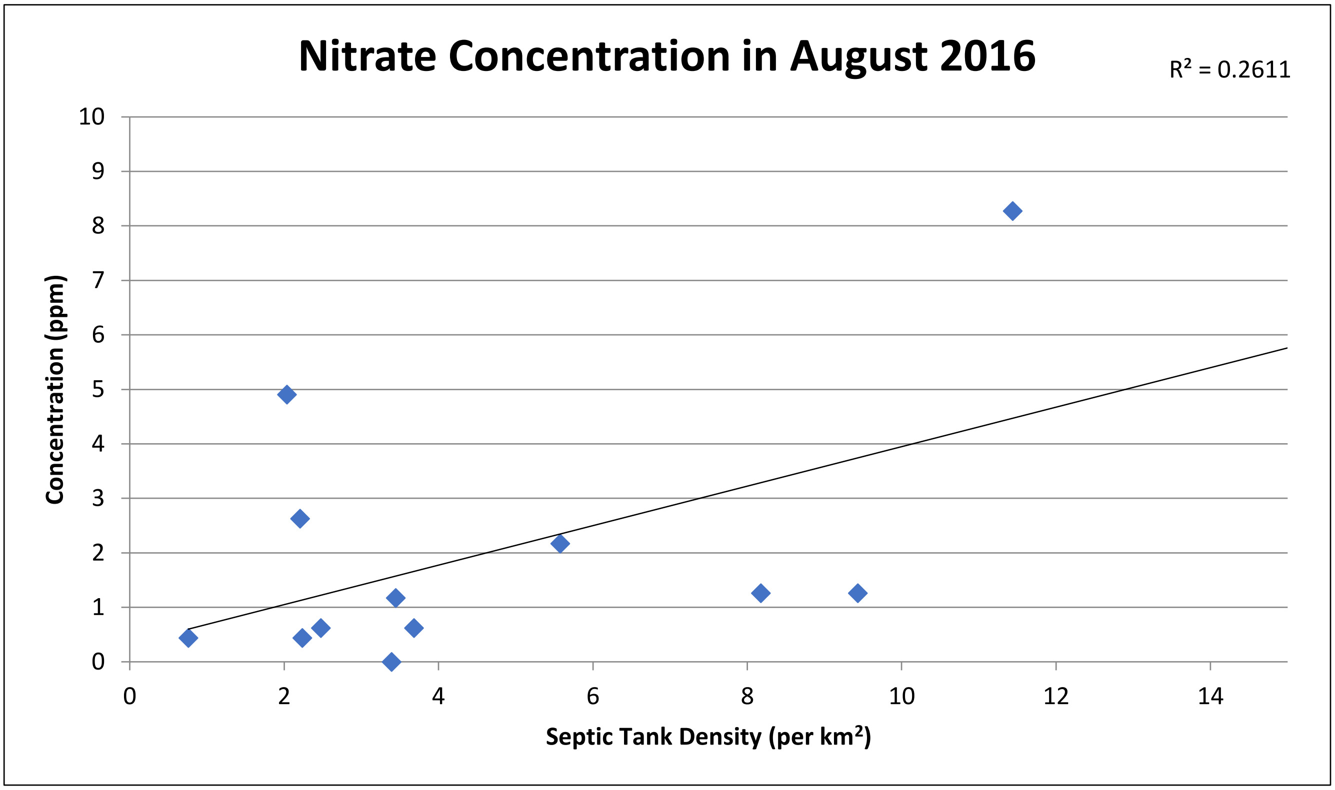


Figure 6. Nitrate concentrations were compared against the density of septic tanks within the watershed basin of the sample point. The R^2 is 26.1% and the p-value is 0.09.

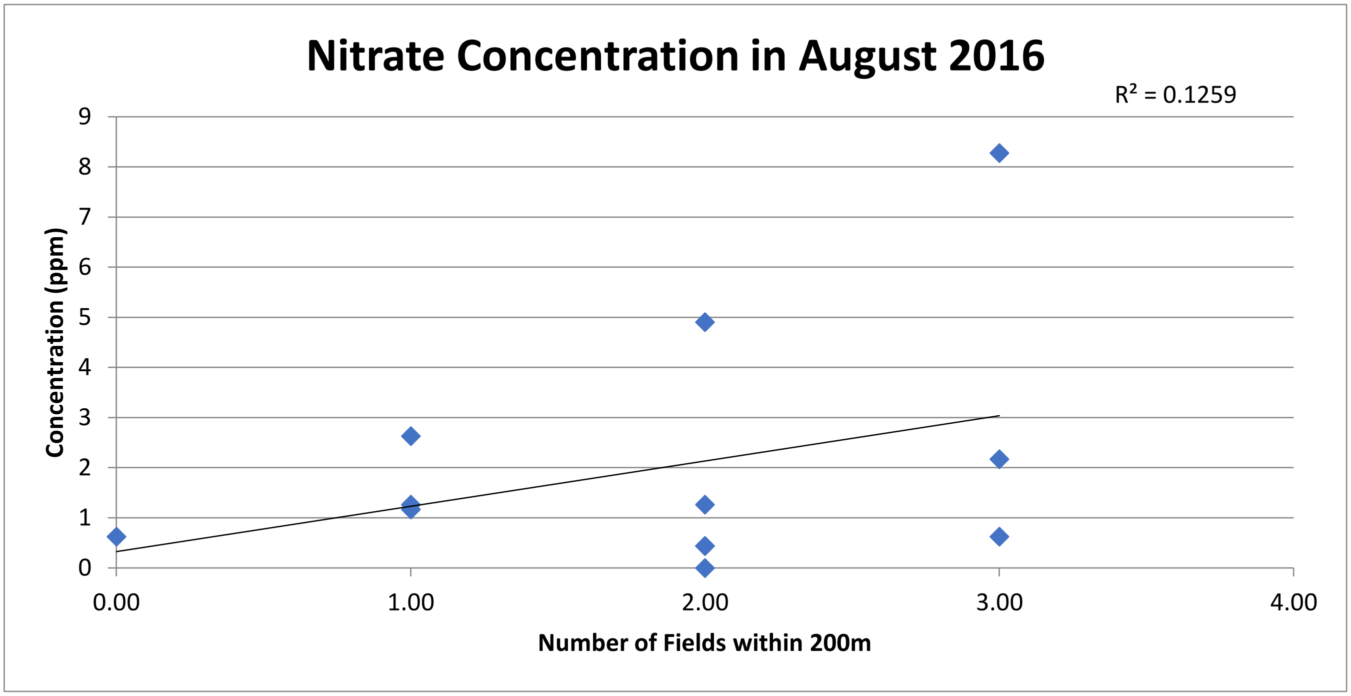


Figure 7. Number of septic fields within 200m of the sample site were compared to the concentration of nitrate in each sample site. The R^2 is 12.5% and the p-value is 0.26.

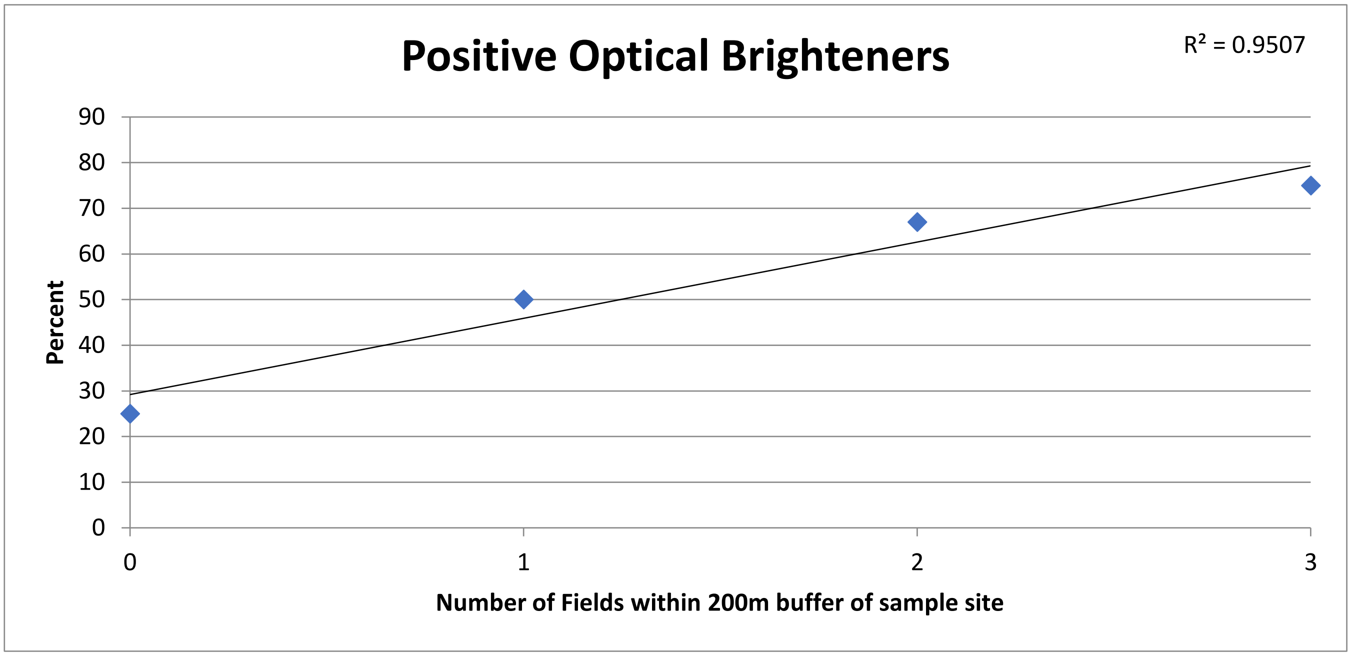


Figure 8. Number of septic fields within 200m of the sample site were compared to the percentage of samples that were positive for optical brighteners. The R^2 is 95% and the p-value is 0.02.

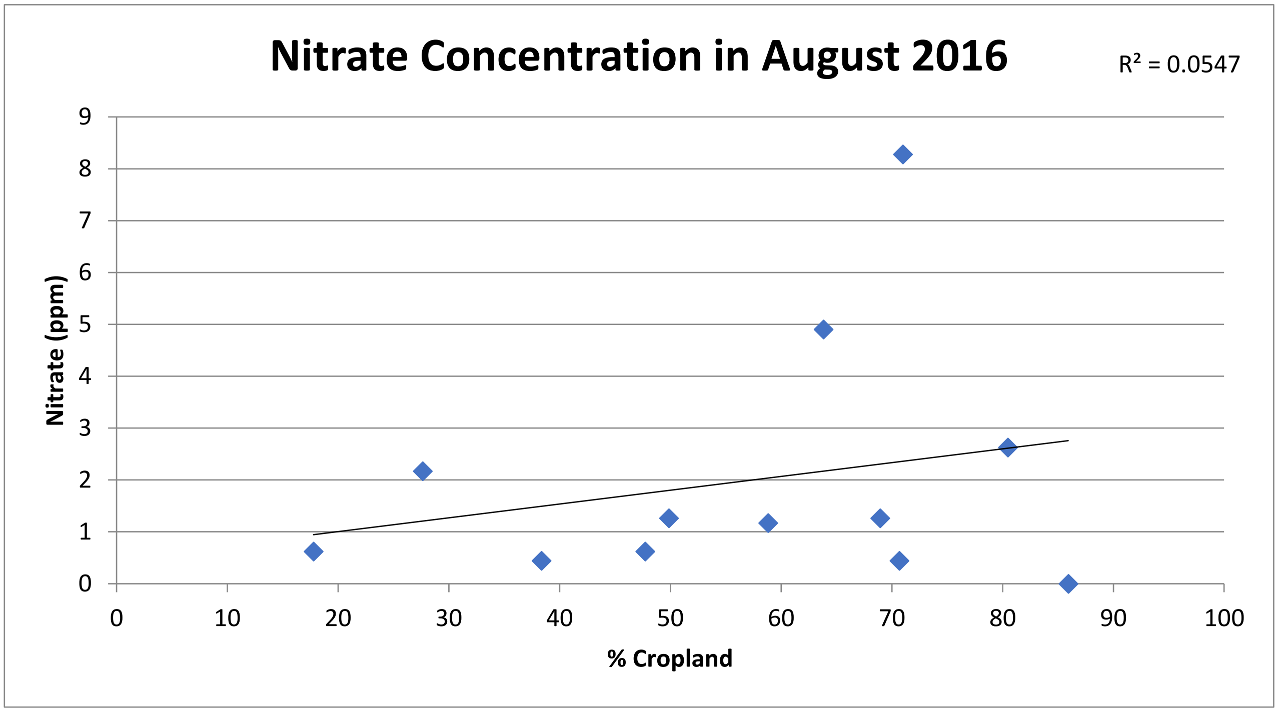


Figure 9. Nitrate concentrations were compared against the percentage of cropland within the watershed basin of the sample point. The R^2 is 5.4% and the p-value is 0.46. The weak relationship suggests that the amount of nitrate from agriculture is not the main contributor of nitrate to the surface water.

Appendix A

Preparing a 50ppm OB calibration solution:

A two step dilution process is used.

1. Prepare a 1 liter Erlenmeyer flask covered with aluminum foil to make it lightproof or a 1 liter amber bottle with 100 ml of DI water.
2. Using a piston style pipette, draw 0.5ml of OB agent (Tide 2X Original Scent is suggested). Wipe off excess OB agent that might have coated the pipette tip. Dispense the OB agent into the 1 liter vessel of DI water, cap and mix thoroughly. Allow foam to settle before next step*. This solution is 500ppm Tide 2X and can be reserved as stock for further use.
3. To then make the actual calibration solution (50.0ppm Tide 2X), add 5.0ml of the stock solution to 45ml of DI water in a foil wrapped Falcon tube. Cap the tube and mix thoroughly. Allow foam to settle before use*.

*It may take quite a long time for foam to settle

Calibration:

1. Turn the AquaFluor on by pressing <ON/OFF>. Wait 5 seconds for the AquaFluor to warm up.

2. Assign a Calibration Standard Value. This is the numeric value that you want the standard to read. Press the <STD VAL> button. Use the ↑ and ↓ arrow buttons to set the standard value to 100.

5. To perform a calibration, press the <CAL> button.

6. Press <ENT> to start the calibration.

7. Insert your blank sample and press <ENT> .AquaFluor will average the reading for 10 seconds and set the blanking zero point.

8. Insert the standard sample and press <ENT>. The reading is averaged for 10 seconds and the Standard Calibration value is set.

Reading the sample:

1. Insert your sample and close the black sample compartment cover.

2. The orientation and cleanliness of the cuvettes can have an impact on the accuracy of your results. Use clean cuvettes which are dry on the outside. Mark your cuvettes for consistent orientation.

3. Press the button. The instrument will measure and average the fluorescence signal for 10 seconds.

4. The reading result will be displayed on the top line of the Home screen.

5. The top left corner will then display “WAIT” for 3 seconds. Once “WAIT” disappears, another sample reading can be performed.

OB method:(Modified from Hartel et al. 2007 and Cao et al. 2009)

- Use three replicates for each sample.
- Read and record initial fluorescence of sample (prior to UV exposure).
- Place sample under UV light for 5 minutes.
- Read and record fluorescence of sample.
- Calculate the percent reduction:

$$\frac{Initial\ Fluorescence - Fluorescence\ after\ 5\ mintes\ of\ exposure}{Initial\ Fluorescence} * 100$$

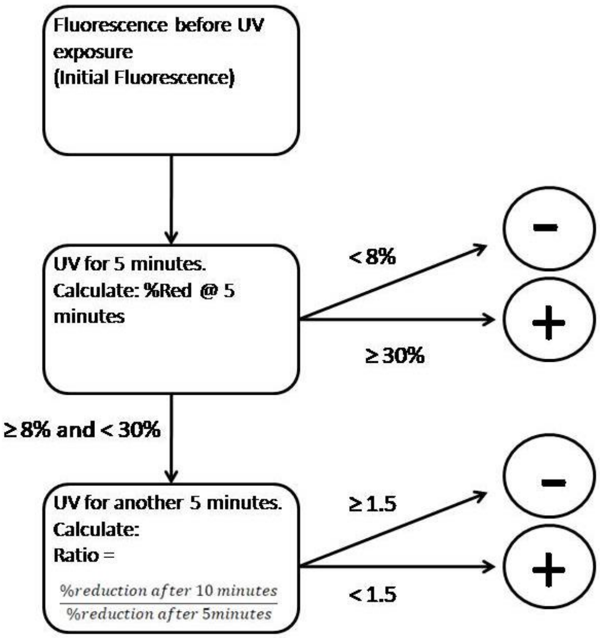
    - A reduction less than 8% means that the sample is negative for optical brighteners.
    - A reduction 30% or greater means the sample is positive for optical brighteners.
    - A reduction of at least 8% and below 30% means that the sample needs further exposure to determine the presence of optical brighteners.
- If needing further exposure, place sample back under UV light for 5 minutes.
- Read and record fluorescence of sample.
- Calculate the percent reduction:

$$\frac{Initial\ Fluorescence - Fluorescence\ after\ additional\ 5\ mintes\ (10\ total)\ of\ exposure}{Initial\ Fluorescence} * 100$$

- Calculate ratio of reduction:

$$\frac{\%reduction\ after\ 10\ minutes}{\%reduction\ after\ 5minutes}$$

    - A ratio of 1.5 or greater means that the sample is negative for optical brighteners.
    - A ratio less than 1.5 means that the sample is positive for optical brighteners.



- If two out of the three triplicate samples are positive for optical brighteners, then the optical brightener analysis is inconclusive.
- If all three triplicate samples are positive for optical brighteners, then the sample is positive for optical brighteners.