2012

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Recommended Citation

Borowski, W.S., T.A. Aguiar, E.C. Jolly, J. Hunter, R.D. Stockwell, M.S. Albright, S.E. Godbey, B.E. West. 2012. Characteristics and environmental problems of a eutrophic, seasonally-stratified lake, Wilgreen Lake, Madison County, Kentucky. Journal of the Kentucky Academy Sciences, 73(1):41-69.

JOURNAL OF THE KENTUCKY ACADEMY OF SCIENCE

Official Publication of the Academy

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J. Ky. Acad. Sci. 73(1):41–69. 2012.

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ABSTRACT

Wilgreen Lake (Madison County, Kentucky) is listed as "nutrient impaired" by the United States Environmental Protection Agency and Commonwealth of Kentucky, and it also experiences high fecal microbe counts that restricts its use. The lake is a typical eutrophic lake, experiencing anoxia and dysoxia in its waters during summer stratification. Human activities in the watershed contribute additional nutrients to the lake that may exacerbate periods of anoxia, so knowing the sources of anthropogenic nutrient inputs to the lake would aid in developing best practices for development of lake shore areas and the watershed. Possible sources include residential fertilizers, cattle waste, and human sewage. High nutrient concentrations within surface waters generally occur only proximal to septic system clusters in the upper reaches of Taylor Fork. Bovine and human fecal microbes enter the lake causing periodic high fecal microbe counts, and are likewise restricted to shallow water areas especially after rain events. The areal distribution of high nutrient and fecal microbe values implicate septic systems as the most likely source of these pollutants, but runoff from pastureland must also contribute nutrients and fecal material. We plan to use additional tracing methods in the future to determine the main sources of nutrients and fecal microbes.

KEY WORDS: Eutrophification, nutrient, ammonium, phosphorus, fecal microbe, source tracking

INTRODUCTION

Wilgreen Lake is a eutrophic lake in Madison County formed by damming Taylor Fork (Figure 1), which ultimately feeds Silver Creek and the Kentucky River. The lake's watershed is relatively small (~41 km^2, ~16 mi^2) but is characterized by a variety of land uses that affect the lake ecosystem and impact its water quality. Wilgreen Lake is nutrient "impaired" according to the Environmental Protection Agency (2012) and by the 303(d) listing by Kentucky. The lake provides only non-swimming, recreational use because of episodic, high fecal microbe counts (U.S. EPA 2004a); it is not a source of drinking water. Wilgreen is subject to nutrient loading by livestock production, runoff from developed areas, and septic systems (U.S. EPA 2010). There is no large-scale farming in the watershed. New housing developments around the lake shore and in proximal areas of the drainage basin have added additional septic systems that ultimately drain into the lake. There is concern that continued and increased delivery of nutrients to the lake may degrade its water quality as eutrophication continues and perhaps accelerates.

We have studied the lake over a period of three years (2006, 2007, and 2008) to make a general assessment of water quality, and ultimately to identify the major nutrient sources that cause eutrophication of Wilgreen Lake. To do so we have monitored the physical and chemical characteristics of the lake (Jolly and Borowski 2007; Hunter and Borowski 2008; Aguiar 2009; Borowski et al. 2009; Stockwell and Borowski 2008), tested for pesticide pollution, and measured fecal microbe concentrations (Borowski and Albright 2007; Aguiar 2009). An advantage to such a concerted effort is to provide a baseline for recognizing changes in eutrophication as the watershed develops and as remediation steps are implemented.

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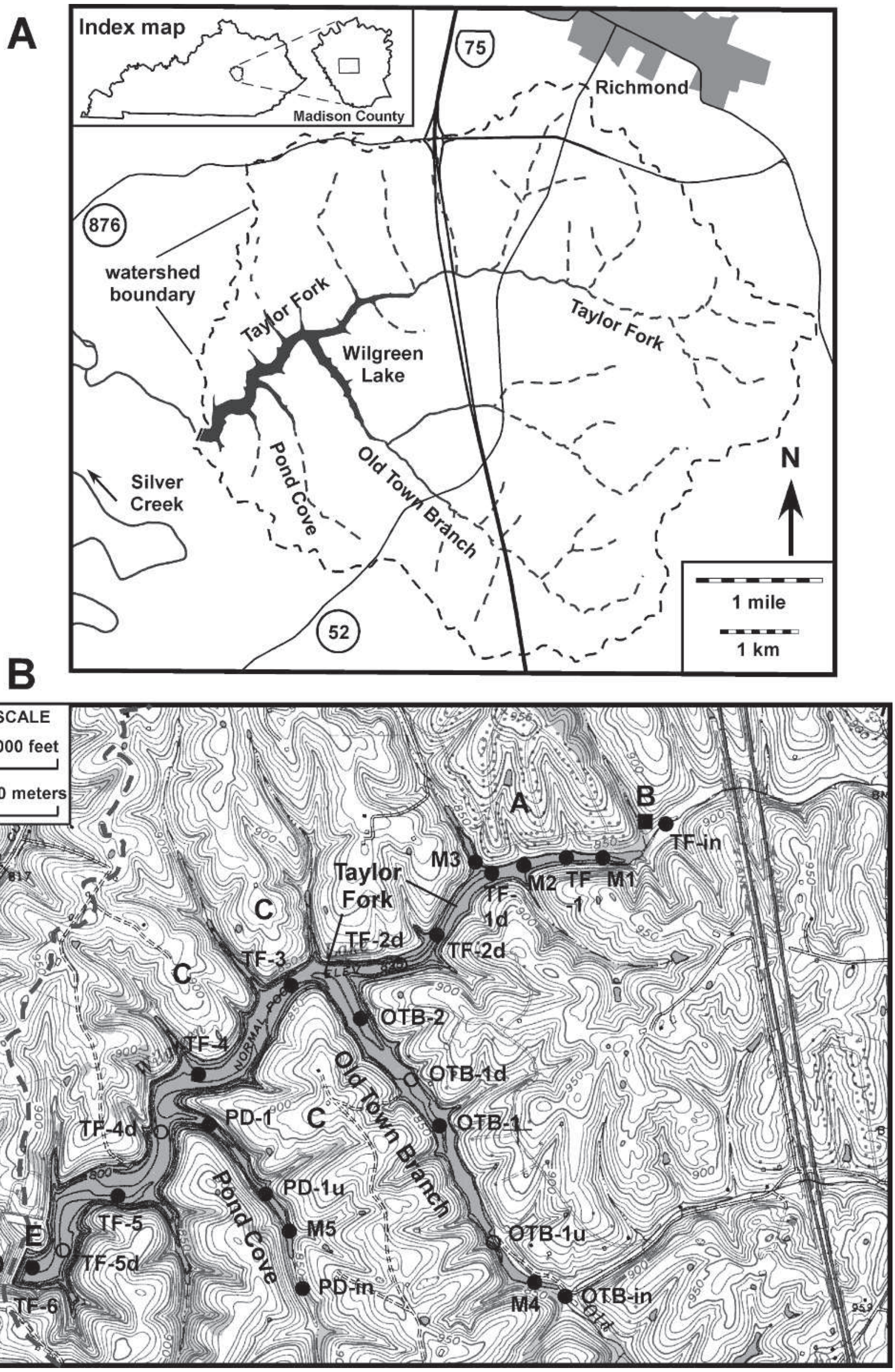

Figure 1. (*A*) Map of the Wilgreen Lake watershed, Madison County, Kentucky. Note the location of the main streams entering the lake: Taylor Fork, Old Town Branch, and Pond Cove. Also note downtown Richmond, which also extends to the south, east, and west of gray footprint. (*B*) Topographic map of Wilgreen Lake with stations and surrounding features. Water samples were taken regularly at stations denoted by filled circles; temperature, oxygen concentration, pH, and conductivity were measured at all stations but those with open circles were not regularly

Nature of the Drainage Basin

The watershed is developed within Upper Ordovician limestones, formally designated as the Garrard Siltstone, Calloway Creek Formation, and the Ashlock, Drakes, and Crab Orchard formations (Green 1966; Cressman and Peterson 1986). These units contain interlayered limestone muds, fossiliferous limestone, and siltstone. Numerous sinkholes occur in the drainage basin within the Crab Orchard Formation (Green 1966), and are especially evident in pastures adjacent to the lake (see map in Aguiar 2009) where they are not covered by forest or disguised by human activity. Thus, they likely also occur under developments adjacent to the lake, and at least one sinkhole occurs in Deacon Hills subdivision, about 135 meters from Wilgreen and it is also adjacent to an overland drainage runnel. Although most of our discussions will focus on overland runoff, we realize that groundwater also enters the lake; moreover, groundwater may flow quickly through subsurface karst conduits into the lake, perhaps even from outside the overland drainage basin. Karst conduits may therefore funnel nutrient- and microbe-rich effluent from pastureland, septic systems, and other sources directly into Wilgreen Lake. Unfortunately, it is beyond the scope and abilities of this study to assess the role and nature of these plausible karstic inputs.

Wilgreen Lake is fed by two major streams, Taylor Fork and Old Town Branch, and by several intermittent minor streams (Figure 1). Both of the principal tributaries experience seasonally low flow and during droughts like that of 2007 can fail to flow into the lake. These two watersheds drain land with very different uses. Taylor Fork begins in the urban and industrial areas of southeast Richmond, then winds its way through neighborhoods served by the Richmond sewage system. Only near its inflow to the lake does the drainage basin recover runoff from pastureland and sparse developments on septic systems. A sewage pumping station occurs approximately 80 meters from the inflow point, and this facility can release raw sewage into the shallow portions of the lake during flooding episodes. We will see that the existence of this pumping station complicates source interpretations for fecal microbes. In the shallowest portion of the lake formed by Taylor Fork, minor runoff occurs from the north and south. The southerly drainage comes from pastureland and wooded bottomland; the northerly drainage comes from the neighborhood of Deacon Hills and Idylwild. This development is characterized by ~240 households utilizing septic systems with as little as half-acre spacing. During the spring and summer, fetid odors near the lake are common in the subdivision indicating leakage of septic systems above ground with the potential of direct runoff into Wilgreen. We suspect this development of being a significant and chronic source for nutrients and fecal microbes to the lake from overland runoff and groundwater seepage; hence, the reason for closely-spaced sampling stations in the upper reaches of lake at Taylor Fork (Figure 1).

Old Town Branch is a very different drainage system passing through mostly widely-spaced households on septic systems and pastureland (Figure 1). Upstream, the source area of the northerly fork of Old Town Branch drains a small industrial area and a high-traffic, two-lane highway (52) before entering a mixture of suburbia and pastureland. The southerly fork covers a larger drainage area covered by widely-spaced dwellings and wooded bottomland. The confluence of the forks occurs only 80 meters from the lake waters. The east shore of lake portion of Old Town Branch is lined by widely-spaced households on septic systems that occur upgradient on relatively steep slopes that lead directly to the lake; the west side of the flooded stream channel is characterized by mostly pastureland at present.

Once the two major streams join, they form the trunk of Wilgreen Lake, which receives water from direct runoff, groundwater, and

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sampled for water. Letters on the map show pertinent information: (*A*) developments of Deacon Hills and Idylwild that are served by septic systems; (*B*) sewage pumping station on Taylor Fork; (*C*) areas of new developments along the lake served by septic systems; (*D*) dam; (*E*) boat dock. Map is from the 7.5 minute quadrangle series (1:24,000), Richmond South quadrangle (photo-revised in 1987), United States Geological Survey.

the small creek of Pond Cove (Figure 1; designated PD). The north side of the lake contains pastureland near the confluence with Old Town Branch, but downstream ~160 new homes (built after our base map, Figure 1B) have been constructed on the ridgeline and slopes overlooking Wilgreen. These homes also have septic systems with ~half-acre spacing. The south side of the lake trunk is occupied mostly by pastureland although an unoccupied development, cleared before 2006, occurs on the divide between Old Town Branch and Pond Cove. The development contains ~200 lots that will be served by septic systems. Near the dam, a floating boat dock provides recreational access to the lake.

Pesticide Pollution

To assess pesticide pollution within Wilgreen we assay for 2,4-dichlorophenoxyacetic acid (subsequently referred to 2,4-D), s-metolachlor, atrazine, and alachlor. 2,4-D is a chlorinated phenoxy compound that is used to control many types of broadleaf weeds; the U.S. EPA has determined that concentrations greater than 290 mg/L (290,000 µg/L) are potentially harmful to aquatic organisms U.S. EPA 2005). s-metolachlor is used to control certain broadleaf and annual grassy weeds; the U.S. EPA has determined that concentrations greater than 0.78 mg/L (780 µg/L) are potentially toxic to aquatic animals (U.S. EPA 1995). Atrazine is widely used to control broadleaf weeds and some grassy weeds; the U.S. EPA has determined that concentrations greater than 37 µg/L are potentially toxic to aquatic organisms (U.S. EPA 2006). Alachlor is used to control broadleaf and grassy weeds on a number of crops; the U.S. EPA has determined that concentrations greater than 0.1 to 0.2 mg/L (100 to 200 µg/L) are potentially toxic to aquatic animals (U.S. EPA 1998).

Nutrient Pollution

Increased nutrient (ammonia, nitrate, phosphate) levels within natural waters have become an acute and widespread problem in the natural waters of the United States (Dubrovsky et al. 2010). Although eutrophification does occur naturally, most nutrient pollution arises because of use of fertilizers, manure produced by farm animals, atmospheric deposition, and human sewage. Localities unaffected by human activities are few, but Dubravsky et al. (2010) have determined background, non-anthropogenic concentrations for ammonia, nitrate, and phosphate of 0.025, 0.24, and 0.01 mg/L, respectively, in streams.

The U.S. EPA and other governmental agencies have also determined acute and chronic criteria for nutrient levels within natural waters. Acute instances occur when concentrations of contaminants spike over a period of days; chronic conditions occur when concentrations are not as high but occur for longer time periods. The rationale for this rating system is that organisms have different tolerances for short-term versus long-term exposure. In the case of ammonia, acute and chronic criteria are dependent on pH, temperature, and whether salmonid fish and/or fish in early life stages are present in the aquatic system. For freshwater with pH of 8.0, the threshold values for acute criteria are 5.62 and 8.40 mg/L nitrogen, respectively, or 7.2 and 10.8 mg/L NH_4; chronic criteria for a pH of 8.0 at 16°C are identical for cases with and without fish in early life stages at 2.21 mg/L nitrogen, or 2.8 mg/L NH_4 (U.S. EPA 2009). The Minnesota Pollution Control Agency, using methods outlined by the U.S. EPA (1985), determined that nitrate standards for cold freshwater with and without lake trout should be 3.1 and 4.9 mg/L N, respectively, or 13.7 and 21.7 mg/L NO_3. Total phosphate in lakes should not exceed 0.05 mg/L whereas stream levels should be below 0.1 mg/L (MPCA 2010).

Nutrients in lake systems provide for increased growth of phytoplankton and macroalgae because nitrogen and/or phosphorus can be limiting factors in plankton growth. Eutrophification can be a natural process because lakes tend to accumulate nutrients, but it is usually intensified by anthropogenic addition of nutrients (Wetzel 1975). Added primary production can affect water quality by increasing the amount of biomass available for decomposition. Decomposition in deeper lake waters and sediments consumes oxygen leading to anoxia in bottom waters. Anoxia decreases living space for oxygen-utilizing organisms within the lake ecosystems. In extreme cases, eutrophification can lead to "dead" lakes, dominated by anaerobic microbes and excluding oxygen-utilizing animals. A host of other problems like harmful algal blooms, foul-smelling and tasting water, decrease in water clarity, and fish kills are also attributes of excessive eutrophification (Dubrovsky et al. 2010).

Possible Nutrient Sources

We suspect that leachate from the septic systems of Deacon Hills and Idylwild housing developments (Figure 1*B*) is a major contributor to eutrophication in Wilgreen Lake. Newer developments with approximately 160 collective households have been built in the watershed adjacent to Wilgreen Lake, and as their septic systems age there is possibility of additional septic effluent entering the lake through groundwater. Indeed, if situated on karstic conduits septic fluids may be already entering the lake from these newer developments. In the future, these new developments and new building on undeveloped lands may increase the nutrient load to the lake.

Other possible nutrient sources include stream input draining pastureland as well as direct runoff from pastureland into the lake, fertilizer run-off, and sewage from other sources entering the lake. Septic systems are used in developments ringing the lake and it can also contribute leachate to either surface or subsurface waters. Knowledge of the principal source or sources is essential in creating policies and strategies to abate further nutrient loading. For example, Madison County is encouraging the installation of fences along the lake border that prevent cattle from entering lake waters and directly depositing body wastes into the lake. If septic systems are the major source of nutrients and fecal matter, these proposed remediation steps will not improve the lake's water quality. Targeted remediation should be much more effective.

Fecal Microbe Pollution

Wilgreen Lake is designated as a recreational lake, suitable only for secondary human contact (non-swimming or recreational contact) because of high fecal microbe counts (U.S. EPA 2004a). The U.S. EPA first set standards for coastal recreational waters in 1986, using multiple water samples (a calculated geometric mean) in order to set safe levels for a variety of recreational uses (U.S. EPA 1986). Because of the difficulty and expense in procuring and processing multiple samples, the U.S. EPA sought to create standards based on single samples for state waters (EPA 2004a). Thus, standards for single-sample analyses for *Escherichia coli* have been established by the EPA (2004b) and are also accepted by Kentucky state government in the form of Kentucky Administration Regulations (KAR 401 5:031). The U.S. EPA designations for surface, recreational waters for use by humans are: *bathing acceptable* (<235 cfu/100 mL), *recreational use only* (236–574 cfu/100 mL), and *no human contact recommended* (>575 cfu/100 mL). There are also sub-designations for recreational use that are largely dependent on systematic, multiple samples (EPA 2004b), but we analyze our data in the light of the broader designations. Standards for total coliform counts have been established by Kentucky (KAR 401 5:031); the U.S. EPA has not established values for total coliform.

Total coliform and *E. coli* counts have been used to access water quality, but experience has shown that *E. coli* counts are more reliable as indicators of fecal contamination, whereas total coliform counts are have multiple sources other than fecal material (U.S. EPA 1986; Dick and Field 2004). Fecal material entering the lake is also a source for nutrients so that *E. coli* counts also represent addition of nutrients.

METHODS

Water Sampling

Twenty-four sampling stations have been established on Wilgreen to document the areal and vertical variability of physical and chemical parameters (Figure 1). Water samples were collected at 1-meter depth increments and were used to measure nutrient concentration; samples at depth were collected with a Van Dorn sampler. Each sample was filtered on location through a 0.45 micron (µm) syringe filter and split into three subsamples for analysis of ammonium, nitrate, and phosphate (Figure 2). These sub-samples were acidified with H_2SO_4 to bring the sample

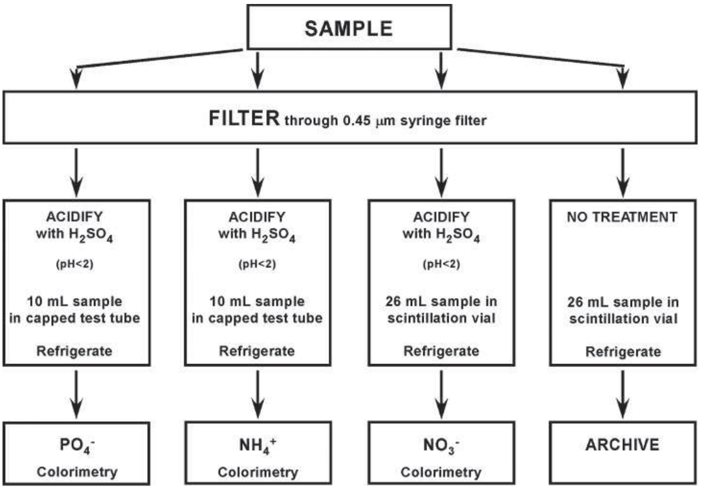


Figure 2. Diagram showing the treatment and use of water samples taken from Wilgreen Lake.

to pH of ≤2 in order to prevent sample degradation (Clesceri et al. 1998). Another 26-ml subsample was untreated and used as an archive sample. Samples were then placed in a cooler on ice, and ultimately transported to the laboratory, where they were refrigerated. Nutrient measurements were conducted within 30 days as specified by Clesceri et al., 1998.

Water samples for pesticide analyses were treated differently. At shallow stations, we collected only a near-surface sample whereas at deeper stations we also collected a sample from deeper, anoxic waters using a Van Dorn sampler. Samples were unfiltered, not acidified, and collected in pre-washed, amber bottles. Analyses occurred immediately after sampling.

Physical Properties

We use an YSI multi-parameter probe (model 556 MPS) to simultaneously measure temperature, conductivity, pH, and oxygen concentration. Probe calibration was completed daily with appropriate reference solutions (YSI User Handbook 2002); pH calibration used the three point method. Probe measurements of oxygen at extremely low levels (<0.09 mg/L) seem less reliable; we report oxygen concentration as zero (anoxic) for water samples containing hydrogen sulfide (H_2S) as identified by smell.

Pesticides

For measurement of 2,4-D, s-metolachlor, atrazine, and alachlor we used immunoassay kits supplied by Beacon Analytical Systems, Inc. We tested for 2,4-D and S-metolachlor using tube versions of the kits in the first round of testing in October of 2007. The kits supplied test tubes that were coated with polyclonal antibodies that bind the pesticide, an enzyme conjugate of the pesticide that will also bind to the antibodies coated on the tubes, substrate to react with the enzyme conjugate to produce a colored solution that absorbs at 450 nm, and pesticide standards needed in the analysis. Water samples, spikes, and standards were added to the tubes, followed by the addition of the enzyme conjugate. The pesticide in the samples will compete with the enzyme conjugate for binding sites on the antibodies. After a prescribed incubation period, unbound molecules were washed away with distilled water. A colorless substrate solution was then added to each test tube. The enzyme conjugate molecules that are bound to the antibodies convert the substrate to a blue compound. A darker solution indicated a lower concentration of pesticide because more antibodies are bound with the enzyme conjugate, which reacts with the substrate to produce the colored solution; the intensity of the color developed in the solutions during the analysis is inversely proportional to the pesticide concentration. Kit instructions were followed for each pesticide and the absorbances of the solutions in the tubes were measured using a spectrometer.

Table 1. Parameters for pesticide analysis.

Pesticide method	Standards range (µg/L)	Quality control spike levels (µg/L - % recovery)
2,4-D tube kit	2–100	10–150%
2,4-D plate kit	2–200	2–65%
		20–101%
s-melolachlor tube kit	0.05–3.0	0.6–98%
		0.6–83%
		0.6–118%
s-melolachlor plate kit	0.05–4.0	0.05–53%
		1.0–120%
atrazine plate kit	0.05–5.0	0.3–93%
alachlor plate kit	0.10–0.75	0.1–99%

More extensive testing for 2,4-D, S-metolachlor, atrazine, and alachlor was accomplished in May of 2008 using plate immunoassay kits (Beacon Analytical Systems, Inc.) The kits were analogous to the tube kits described above except that 96-well plates coated with the appropriate antibodies were supplied instead of coated test tubes. The reading of the absorbances in the 96-well plates was accomplished using a Biotek Synergy II plate reader.

The concentration range of the standards used for each immunoassay along with the spike recoveries appear in Table 1. Samples whose concentrations fall below the lowest standard are be reported as less than that standard concentration. Thus, the analyte can be either absent or either present at immeasurable levels if its concentration is less than the lowest standard concentration.

Ammonium Concentration

To measure ammonium concentration, we used the Berthelot reaction (phenol hypochlorite method) as described by Solorzano (1969) (see also Gieskes et al. 1991; Eaton et al. 2005, Method 4500-NH_3 F) using colorimetry and a spectrophotometer. The method is sensitive and specific to ammonium (NH_4^+) and ammonia (NH_3) (Eaton et al. 2005). Standards [0.0, 0.5, 1.0. 2.5, 5.0, and 12.5 mg/L NH_4] were used to create a linear standard curve, and two spiked samples [0.5, 1.0 mg/L] were prepared and measured with each batch of lake samples; standards and samples were treated identically. Standard curves have r^2 values (correlation coefficient) greater than 0.994. Detection limits are <0.1 mg/L (Method 4500-NH_3 F, Eaton et al. 1995); we report values to the nearest 0.1 mg/L and values <0.1 mg/L are reported as zero.

Nitrate Concentration

We used cadmium reduction, another colorimetric method (Eaton et al. 2005, Method 4500-NO_3 E), to measure nitrate using Hach NitraVer 5 reagent packets (Hach 1986). Because we acidified our samples, we actually measured nitrite (NO_2^-) and nitrate (NO_3^-) but report the values as NO_3. Nitrate data occur only for waters sampled during the 2008 field season. Standards [0.0, 0.5, 1.0. 2.5, 5.0, and 12.5 mg/L N-NO_3] yielded standard curves with r^2 values greater than 0.994. The detection limit for nitrate is theoretically 10 µg/L (~44 µg/L N-NO_3, Method 4500-NO_3 E, Eaton et al. 1995) but our experience shows reduced confidence in concentrations <0.1 mg/L (100 µg/L). Consequently, we report values to the nearest 0.1 mg/L and values <0.1 mg/L are reported as zero.

Phosphate Concentration

Phosphate (PO_4^{3-}) concentration of the lake water was measured using the ascorbic acid, colorimetric method (Strickland and Parsons 1968; Gieskes et al. 1991; see also Eaton et al. 2005, Method 4500-P E). Because we filtered our samples, the method measures only dissolved orthophosphate. Standards [0.0, 0.25, 0.5, 1.0, and 2.5 mg/L PO_4] were prepared with each batch of lake samples to establish a linear standard curve; typical r^2 values were greater than 0.996. Samples outside the linear portion of the curve (>2.5 mg/L) were re-analyzed using a dilution factor of 2 and in one case (station M1 – September 2007) it was necessary to use a dilution factor of 4. The detection limit is theoretically 10 µg/L (Method 4500-P E, Eaton et al. 1995) but our experience shows reduced confidence in concentrations <0.1 mg/

L (100 μg/L). Consequently, we report values to the nearest 0.1 mg/L and values <0.1 mg/L are reported as zero.

Fecal Microbes

We sampled the surface waters at selected stations across the lake to assess bacterial abundance on two instances in 2006 and 4 times in 2007, sampling during normal and dry conditions during the summer field season. Sampling was concentrated in the proximal, shallow-water areas of Old Town Branch, Taylor Fork, and Pond Cove but we also sampled at the deeper-water stations along the trunk of Taylor Fork.

We determined the distribution and abundance of *Escherichia coli* and total coliform bacteria within Wilgreen Lake using the rapid assay method developed by IDEXX (2006). Unfortunately, cost and equipment limitations prevented using other methods involving determination of atypical colonies (e.g., Brion and Mao 2000) and other potential indicators such as human epicoprostanol and fecal load indicators (Black et al. 2007). The IDEXX method uses *Colisure*® materials now accepted by the EPA, American Water Works Association, American Public Health Association, and Water Environment Federation as an established method in quantifying the occurrence and abundance of these microbes (Method 9223, Eaton et al. 2005). The method gives an approximate count of microbe abundance in terms of colony-forming units (cfu) per 100 mL, as estimated from statistical methods that determines the most probable number (MPN) of colonies (IDEXX 2006).

Samples were taken and analyzed over a period of two days. The procedure requires the collection of 100-mL water samples in sterile bottles according to standard collection methods (Method 9060, Eaton et al. 2005). Water samples were stored on ice in the field and transported to the laboratory within six hours (IDEXX 2006; Method 9060, Eaton et al. 2005). At the lab, processing began immediately when prepackaged *Colisure*® indicator reagent was added to samples and thoroughly mixed. Samples are then poured into a IDEXX Quanti-Tray®, sealed using a Quanti-Tray Sealer®, and incubated for 24 hours at 35°C. After incubation, total coliform was measured by counting the number of large and small wells that turned red/magenta. *E. coli* abundance was determined by counting the number of wells that fluoresce purple under UV light. The raw total coliform and the *E. coli* counts are then converted to the most probable number (MPN) of colonies per 100 ml using an IDEXX table. We present and analyze only *E. coli* data because these counts directly indicate fecal contamination.

The IDEXX method can only register a maximum microbial count of 2419 cfu/100 mL without sample dilution, so in some of these cases we diluted samples to obtain absolute counts, especially those samples from 30 June 2007. Subsequent to 30 June, some stations have replicate samples that were prepared using a 1:4 dilution as described by Brazos River Authority (2003), and we also tested a dilution factor of 1:10 to compare counts between undiluted and diluted samples. In most cases, only a slight discrepancy (<10% for *E. coli*) was noted (Aguiar 2009).

RESULTS

Physical and Chemical Properties

Wilgreen Lake is a typical eutrophic lake showing thermal stratification, which affects other physical and chemical parameters (Figure 3). Lake waters behave uniformly throughout the field season at the deep water stations along the trunk of Taylor Fork (stations TF-6 to TF-2), and extending to stations OTB-2 and PD-1 within the tributaries (Figure 1). Shallow-water stations near stream entries do not stratify and tend to exhibit more variability, responding to inflow from normal runoff and to rain events within the watershed. Wide variations in temperature and conductivity (0.307 to 0.900 mS/cm^2) occur dependent on inflow conditions with oxygen generally high (up to 17.72 mg/L) and pH ranging from 7.24 to 8.85.

The following description of lake properties relates mostly to deeper water stations of the lake that stratify and that contain the preponderance of the lake's volume. The upper layer is warm (typically over 20°C during the summer and Fall), the lower layer is cool (about 8 to 10°C) with a transitional zone

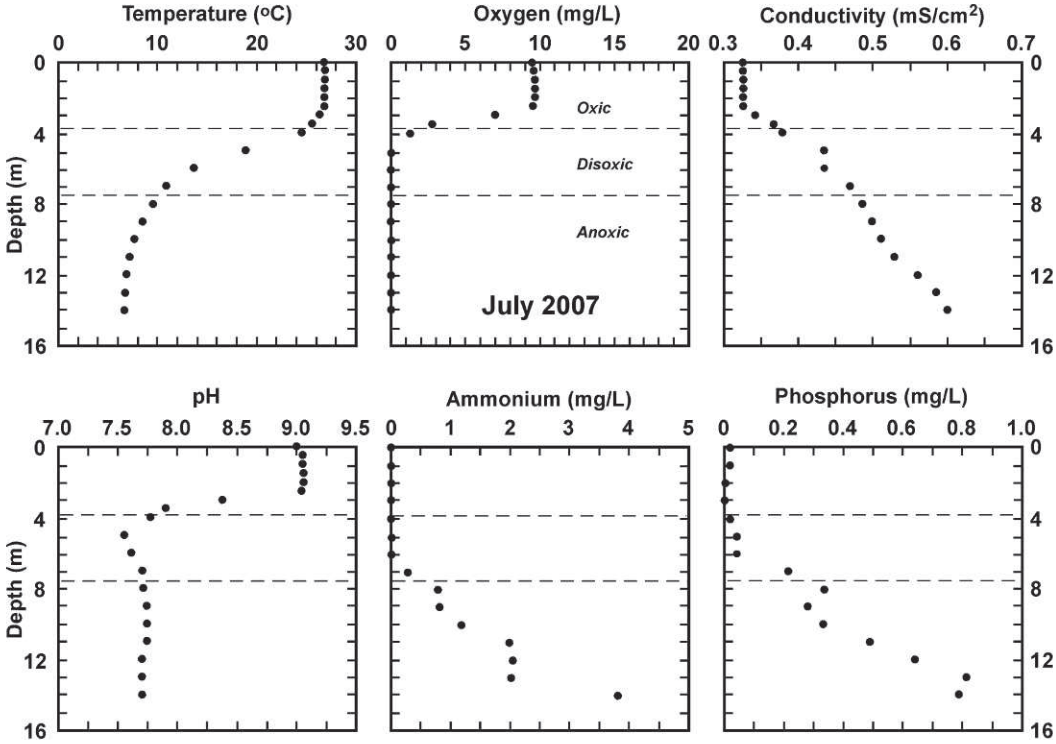


Figure 3. Typical profiles of selected parameters that occur during stratification in the summer. Measurements and samples were taken on 23 July 2007 at Station TF-6, the deepest sampling station (see Figure 1). Shown are profiles of temperature (degrees Centigrade, °C), oxygen concentration (milligrams/Liter, mg/L), conductivity (milliSeimens/square centimeter, mS/cm^2), pH, nitrogen concentration as ammonium (NH_4), and phosphorus concentration as phosphate (PO_4). Dashed horizontal lines refer to oxygen concentrations at either oxic (>2 mg/L), dysoxic (>0 to 2 mg/L), or anoxic levels (0 mg/L) within the water column. Data from Hunter and Borowski (2008); Aguiar (2009).

between containing the thermocline. During stratification, oxygen, conductivity, pH, and nutrient concentrations are very different in the upper versus lower layers (Figure 3). Oxygen concentration responds to stratification, water temperature, the balance between photosynthesis and respiration in the upper layer, and the rate of decomposition in deeper waters of the lake (e.g., Wetzel 1975). Oxygen levels are higher in the upper lake layer and are maintained by addition of oxygen from photosynthesis and by addition from the atmosphere, whereas deeper waters beneath quickly lose oxygen via microbial decomposition of organic matter to become dysoxic (O_2 between 0 and 2 mg/L) or anoxic (0 mg/L) (e.g., Wetzel 1975); anoxic waters smell strongly of hydrogen sulfide (H_2S). Conductivity is typically lower in the upper layer but increases markedly below the thermocline as dissolved ions are added by decomposition reactions. The upper layer generally has more alkaline pH (8.2–9.1), likely because slightly acidic rainwater is buffered by the limestone bedrock of the drainage basin, but deeper waters become more acidic concomitant with net decomposition of organic matter, which adds increasing amounts of dissolved carbon dioxide (CO_2) and organic acids (e.g., Wetzel 1975). Nutrient concentrations (nitrogen as ammonium, NH_4, and as nitrate, NO_3; phosphorus as phosphate, PO_4) are generally lowest in the upper layer where uptake by phytoplankton and algae occurs, and are highest in the lower layer as nitrogen and phosphorus are liberated by organic matter decomposition in the water column and sediments (Figure 3). All nutrients follow this same basic pattern with depth at the trunk stations. Anthropogenic sources of nutrients to Wilgreen Lake affect its water quality and we will focus on their sources below.

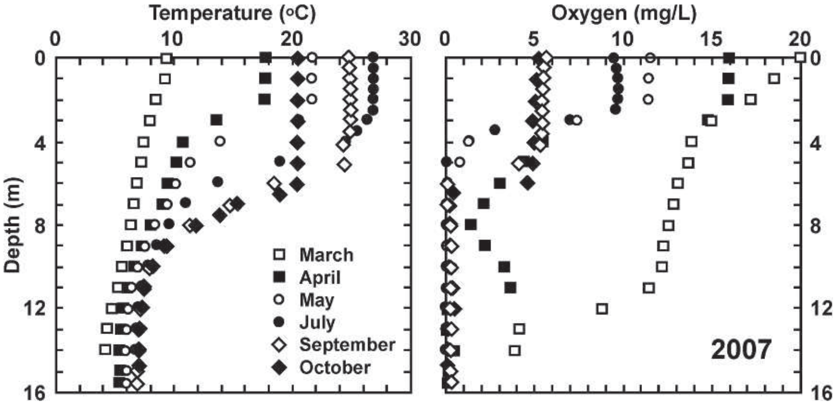


Figure 4. Seasonal variation in temperature and oxygen concentration as a function of water depth. Data represent profiles at Station TF-6 (see Figure 1) during the field season of 2007. Symbols are keyed to sampling on 13 March, 27 April, 17 May, 23 July, 12 September, and 12 October. Data from Hunter and Borowski (2008); Aguiar (2009).

Wilgreen Lake goes through a typical seasonal cycle for a eutrophic lake, showing stratification in the spring and summer with fall turnover occurring sometime in October or shortly thereafter, with stratification developing again in March (Figure 4). Deep waters approach 4°C at their coolest observed temperature whereas surface water fluctuates from freezing to slightly over 30°C; the highest observed temperature was 32.9°C occurring at Station TF-4 in August 2006 and 2007 (Aguiar 2009; Jolly and Borowski 2007; Hunter and Borowski 2008). Temperature data from 2007 shows that surface waters begin warming in March with stratification established by late April with the upper layer being about 2 meters thick (Figure 4). The lake remains stratified through the summer and into the fall, but the theromocline deepens from 2 meters in April, to 3 m in May, remaining there with some fluctuation through June, July and August with temperature of the upper mixed layer increasing from about 22 to 26, 27, and 30°C, respectively. Surface lake waters then begin to cool from 25° to 20°C in September and October as the thermocline deepens to 5 and 6 m respectively. Temperature and corresponding density differences between the upper and lower layers decline further and the mixed layer deepens until Fall turnover occurs; our observations do not witness the final collapse of summer stratification within the lake.

Dissolved oxygen concentrations also change seasonally (Figure 4) responding to water temperature, stratification, the balance between photosynthesis and respiration in the upper layer, and the rate of decomposition in deeper waters of the lake. In surface waters, oxygen concentration is at its maximum in the early spring (~20 mg/L in March 2007) and declines progressively into the summer with minimum values of ~5 mg/L occurring in September and October. Stratification profoundly influences oxygen distribution with depth as water remains well oxygenated in the upper layer and but is oxygen-poor below the thermocline. In 2007, March is the only observed month when deep waters are well-oxygenated, otherwise oxygen concentration below the thermocline is generally <1 mg/L. Cross sections of the lake (Figures 5, 6) show that the preponderant volume of Wilgreen is either dysoxic or anoxic during stratification. The thickness of the dysoxic layer is generally about 2 to 4 meters whereas the bottom-most 6 to 10 meters of the lake are anoxic through summer stratification at the deepest stations (TF 6 though TF-4), thinning upstream toward shallower stations.

Lake Bathymetry

Wilgreen lake is a dammed stream and a cross section of the lake shows the nature of the former stream topography (Figure 5). The lake is deepest at Station TF-6 just upstream from the earthen dam. The lake progressively shallows upstream along the trunk of the lake with a marked decrease in depth in the transition from stations TF-3 to TF-2d.

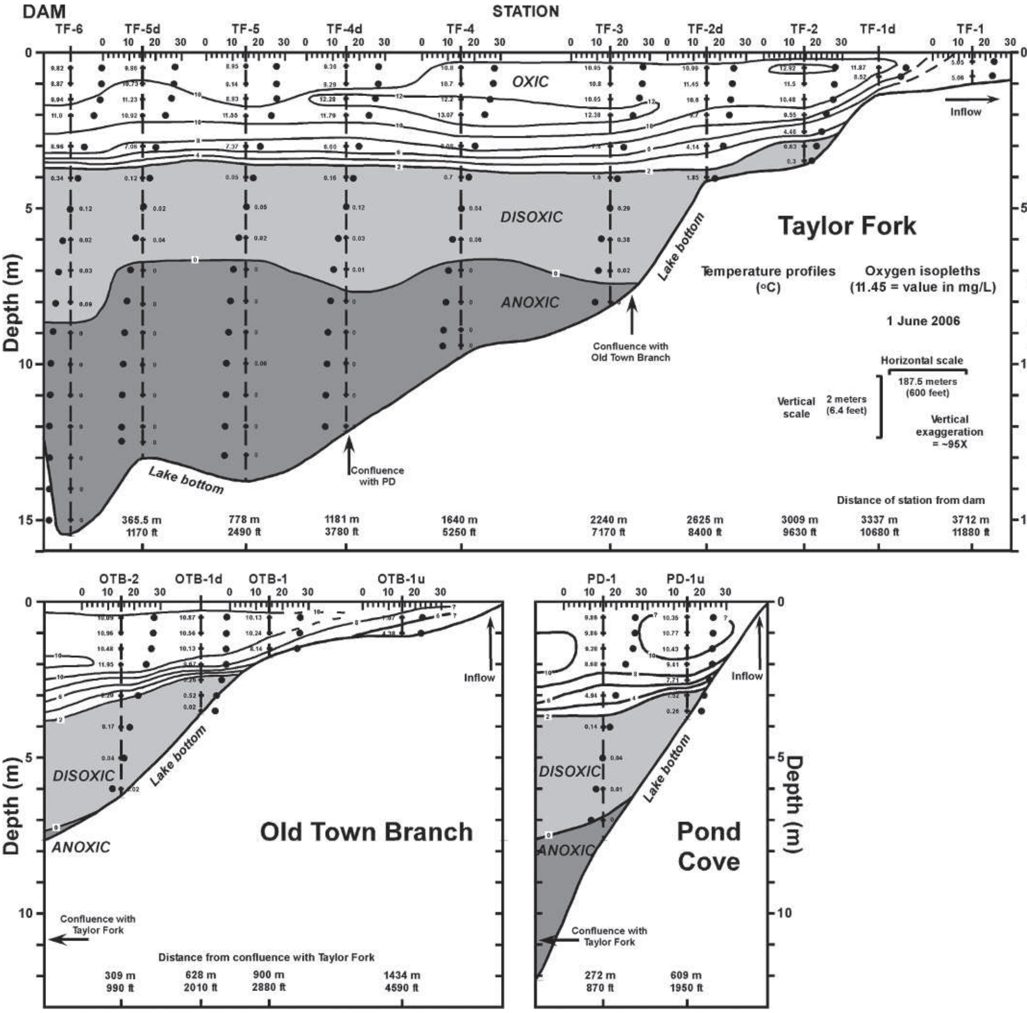


Figure 5. Cross section of Wilgreen lake along the main trunk of the lake (Taylor Fork) and its tributaries, Old Town Branch and Pond Cove (see Figure 1), showing graphed temperature data (°C, filled circles) and oxygen concentration isopleths for data measured on 1 June 2006. Oxygen values in units of milligrams per liter (mg/L) for each station are shown next to the corresponding depth; the contour interval for oxygen isopleths is 2 mg/L. Shading refers to oxygen concentrations at either oxic (>2 mg/L; no shading), dysoxic (>0 to 2 mg/L; light gray), or anoxic levels (0 mg/L; darker gray) within the water column. The entire lake segment is shown in the cross sections of Old Town Branch and Pond Cove, however a small portion of shallowest portion of Taylor Fork is not shown. Note the horizontal and vertical scales and the vertical exaggeration of ~95 times. Note also the locations of the confluences on each cross section, and the distances from either the dam (Taylor Fork) or a confluence (Old Town Branch, Pond Cove) shown at the bottom of each cross section panel. Data from Jolly and Borowski (2007).

Moving up Old Town Branch, the lake becomes noticeably shallower leaving the trunk of lake (station OTB-2) moving upstream to stations OTB-1 through M4. Within Pond Cove, Station PD-1 is deep but stations PD-1u and M5 are considerably shallower. At and upstream of stations TF-1d, OTB-1, and PD-1u lake waters are strongly influenced by inflow from streams and by other local conditions.

Homogeneity of Deep-Water Stations

Wilgreen Lake tends to be physically and chemically homogeneous over most of its areal extent, especially at deep-water locations, which contain the preponderance of its

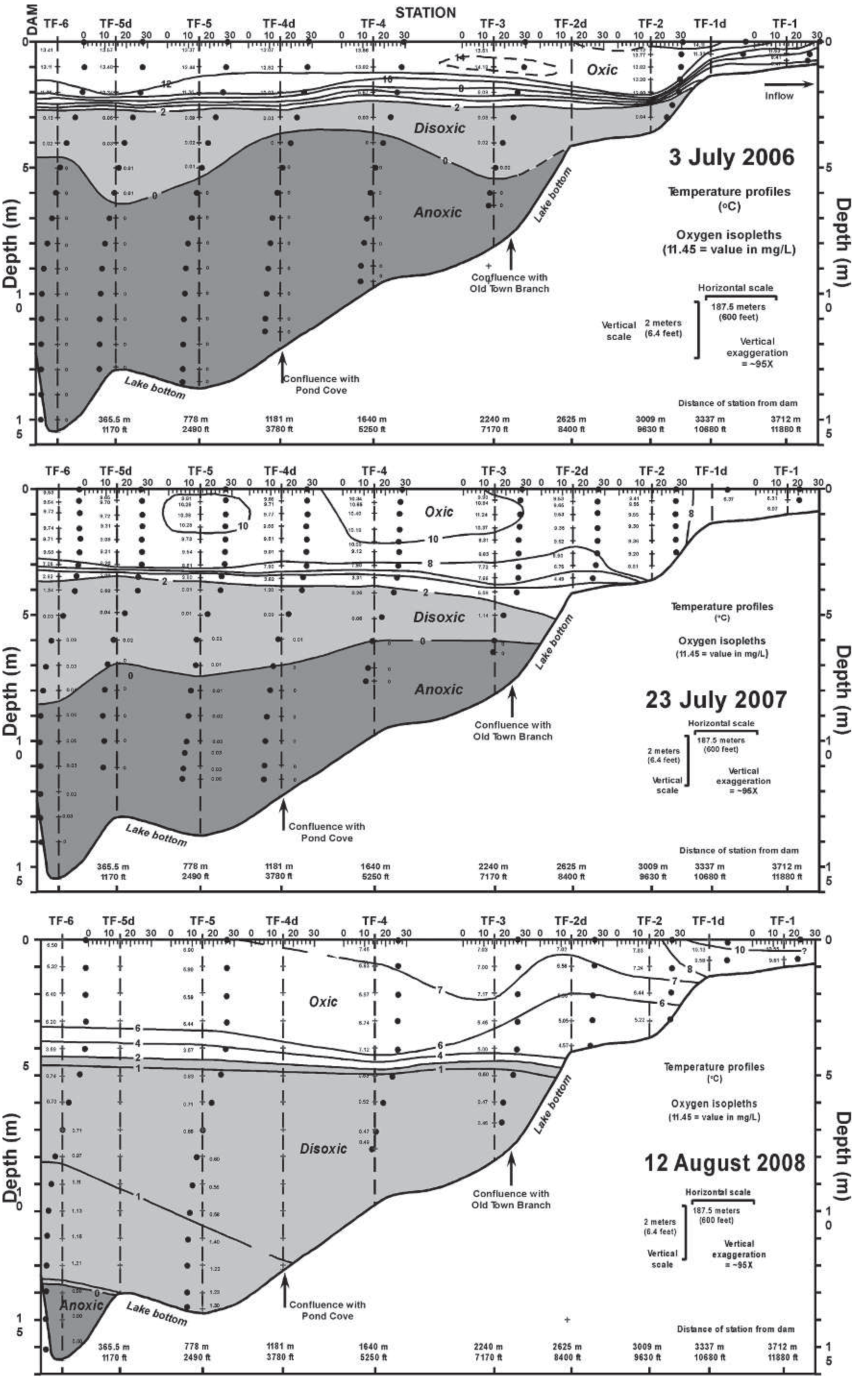


Figure 6. Cross sections of Taylor Fork during maximum stratification during the three successive years of 2006, 2007, and 2008. See caption of Figure 5 for explanations. Data from Jolly and Borowski (2007), Hunter and Borowski (2008), Stockwell and Borowski (2008), and Aguiar (2009).

volume. Figure 5 shows lake cross sections with temperature profiles and oxygen isopleths of the lake along the trunk of Taylor Fork and its tributaries, Old Town Branch and Pond Cove. Temperature data show a thermocline about 3 meters in depth that covers most of the deeper-water stations of the lake. Stratification becomes less pronounced at upstream stations (shallower than TF-2d, OTB-1d, and PD-1u), and where inflowing stream waters have more effect on lake properties. Oxygen concentrations are characteristically also similar across the lake. The cross sections clearly show that oxic waters occur in the upper, warmer layer of the lake and that oxygen decreases with depth to dysoxic and finally anoxic levels. Moreover, oxygen isopleths roughly parallel the lake surface across the deeper waters of the lake highlighting the strong correlation between physical and chemical stratification. Although not shown with any cross sections, nutrient concentrations are also homogeneous across the deeper portions of Wilgreen Lake with generally very low concentration in the uppermost, sunlit layer of the lake and progressively increasing concentration into colder, dysoxic to anoxic waters (Figure 3). Homogeneous nutrient concentrations observed at the deep-water stations of the lake allow us to recognize anomalously high nutrient concentrations in shallow-water portions of the lake for source tracking.

Annual Variation

Although lake parameters are generally uniform across the deep-water portions of the Wilgreen Lake, the lake displays marked differences in its properties from year to year. The lake always stratifies but temperature and oxygen properties change annually in response to solar heating and rainfall. Over the course of this study, the summer months of 2006 and 2007 were very dry but much wetter in 2008; 2006 was the hotter year. Figure 6 shows cross sections along the trunk of the lake during summer stratification from 2006 to 2008 during the height of stratification. The position of the thermocline is similar during each year but the strongest variation is seen in oxygen content of the lake. Oxygen isopleths defining the upper and lower layers of the lake are much closer in 2006 indicating a sharper oxygen gradient positioned at 2.5 m. In 2007, the oxygen gradient is not as sharp (depth a bit deeper than 3 m), and in 2008 oxygen gradients are diffuser still with the sharpest gradient slightly deeper at 4.5 m. Overall oxygen content mirrors this trend with the collective volume of dysoxic and anoxic water decreasing as the summer season becomes less hot and wetter from 2006 to 2008. The volume of anoxic water is also highest during 2006, overall the hottest and driest summer. Summer 2008 is most atypical with a very small amount of anoxic water hugging the bottom. These significant annual variations will make it difficult to recognize larger trends of nutrient loading that may occur within Wilgreen Lake in the future.

Pesticides

Our pesticide assay results are tabulated in Table 2. Potentially toxic levels of s-metolachlor (780 μg/L), atrazine (37 μg/L), and alachlor (100–200 μg/L) are not observed in any of our samples. All samples have <2 μg/L of 2,4-D, whereas the EPA level for potential toxicity for aquatic organisms is 0.29 μg/L. Thus, we cannot determine if Wilgreen waters contain potentially harmful amounts of 2,4-D. The lack of pesticide residues within our samples is not surprising as little or no commercial agriculture takes place within the Wilgreen drainage basin. In the absence of other evidence, we infer than 2,4-D behaves like the other measured pesticides and conclude that pesticide pollution is apparently not a problem in Wilgreen Lake, so will not discuss these findings further.

Outward Evidence of Eutrophification

Wilgreen Lake is listed as nutrient impaired and there are obvious signs of eutrophification. Large mats of algae exist particularly in the shallow reaches of Taylor Fork and Old Town Branch proximal to suspected nutrient sources. These mats consist of green algae (Chlorophyta); the dominant genera are *Oedogonium, Desmidium,* and *Microspora*; *Cosmarium*, and *Mougeotia* also occur (R. Creek pers. comm., 13 June 2009).

Overall Nutrient Concentration

Most samples contain nutrient concentrations higher than systems without human

Table 2. Pesticide measurements of 2,4-dichlorophenoxyacetic acid (2,4-D), s-metolachlor, atrazine, and alachlor during two different field seasons at Wilgreen Lake.

	October 2007				May 2008				
Station	Depth (m)	2,4-D (μg/L)[2]	s-Metolachlor (μg/L)[2]	Station	Depth (m)	2,4-D (μg/L)[2]	s-Metolachlor (μg/L)[2]	Atrazine (μg/L)[2]	Alachlor (μg/L)[2]
TF in[1]	—	<2	<0.05	TF in[1]	—	<2	<0.05	—	—
M1	0	<2	<0.05	M1	0	<2	<0.05	<0.1	<0.1
TF-1	2	<2	<0.05	TF-1	0	<2	<0.05	<0.1	<0.1
	2	<2	<0.05		—	—	—	—	—
M2	—	—	—	M2	0	<2	<0.05	<0.1	<0.1
TF-1d	—	—	—	TF-1d	0	<2	<0.05	<0.1	<0.1
M3	—	—	—	M3	0	<2	<0.05	<0.1	<0.1
TF-2	—	—	—	TF-2	—	—	—	—	—
TF-2d	—	—	—	TF-2d	—	—	—	—	—
TF-3	0	<2	<0.05	TF-3	0	<2	<0.05	<0.1	<0.1
	4	<2	<0.05		—	—	—	—	—
	4	<2	<0.05		—	—	—	—	—
TF-4	0	<2	<0.05	TF-4	0	<2	<0.05	<0.1	<0.1
	0	<2	<0.05		8	<2	<0.05	<0.1	<0.1
	6	<2	<0.05		—	—	—	—	—
TF-4d	—	—	—	TF-4d	—	—	—	—	—
TF-5	0	<2	<0.05	TF-5	0	<2	<0.05	<0.1	<0.1
	11	<2	<0.05		—	—	—	—	—
TF-5d	—	—	—	TF-5d	—	—	—	—	—
TF-6	0	<2	<0.05	TF-6	0	<2	<0.05	<0.1	<0.1
	13	<2	<0.05		8	<2	<0.05	<0.1	<0.1
OTB in[1]	—	<2	<0.05	OTB in[1]	—	<2	<0.05	<0.1	<0.1
M4	0	<2	<0.05	M4	0	<2	<0.05	<0.1	<0.1
OTB-1u	0	<2	<0.05	OTB-1u	0	<2	<0.05	<0.1	<0.1
OTB-1	1	<2	<0.05	OTB-1	0	<2	<0.05	<0.1	<0.1
OTB-1d	—	—	—	OTB-1d	—	—		—	—
OTB-2	0	<2	<0.05	OTB-2	0	<2	<0.05	<0.1	<0.1
	0	<2	<0.05		6	<2	<0.05	<0.1	<0.1
	4	<2	<0.05		—	—	—	—	—
	4	<2	<0.05		—	—	—	—	—
PD-in[1]	—	—	—	PD-in[1]	—	<2	<0.05	<0.1	<0.1
M5	0	<2	<0.05	M5	0	<2	<0.05	<0.1	<0.1
PD-1u	1	<2	<0.05	PD-1u	0	<2	<0.05	<0.1	<0.1
PD-1	0	<2	<0.05	PD-1	0	<2	<0.05	<0.1	<0.1
	6	<2	<0.05		7	<2	<0.05	<0.1	<0.1

[1] Stream samples taken without regard for depth.
[2] Analyte concentrations are reported as below the lowest standard concentration (see Table 1).

influence. The percentage of samples over the natural, background levels for ammonium (0.025 mg/L), nitrate (0.24 mg/L), and phosphate (0.01 mg/L) (Dubravsky et al. 2010) are 39%, 52%, and 68%, respectively. A much smaller percentage of samples shows nutrient values exceeding acute and chronic values for aquatic systems. For ammonium, none and 5.2% of samples exceed the threshold values of 10.8 mg/L and 2.8 mg/L for acute and chronic exposure, respectively (EPA 2009). For nitrate, only 0.4% of all samples exceed the threshold of 13.7 mg/L NO_3 (3.1 mg/L N-NO_3; MPCA 2010), assuming average pH of 8.0 and temperature of 16°C. For phosphate, 36% of samples exceed the threshold value of 0.05 mg/L (MPCA 2010). The location of higher nutrient concentrations, especially those in excess of accepted threshold values, should suggest major nutrient sources.

Nutrient Content of Deeper Waters

Once stratification occurs nutrient concentrations (ammonium, nitrate, phosphate) are almost always higher in lake waters below the thermocline relative to surface water values (Figure 3) (Aguiar 2009). Nutrients are at low concentration in the upper several meters of the lake at deep-water stations until Fall turnover occurs when dissolved nutrients in the deeper layers are injected toward the surface (Aguiar 2009). Average ammonium and phosphate concentration within the lower layer of the lake during stratification are 1.6 and 0.9 mg/L respectively; measured maximum values were 6.8 mg/L (TF-5, 13 m, September 2006) and 3.9 mg/L (TF-6, 8 m, October 2007), respectively (Aguiar 2009). None of the ammonium values exceed the recommended threshold amount for acute criteria, whereas about 3.9% of deep-water samples (3.2% of all samples) exceed threshold amounts for chronic criteria. For phosphate, 61% of deep-water samples exceed the threshold value (0.05 mg/L PO_4).

Nutrient Concentrations in Surface Waters

For shallow (<2 m) and surface waters, consistently high nutrient concentrations occur only in the shallow portions of the lake, proximal to suspected anthropogenic nutrient sources. For example, Figure 7 shows phosphate concentration in surface waters for 2007. Note that phosphate levels are generally <0.1 mg/L at deep-water stations (with some exceptions), whereas shallow-water stations have generally higher values. The highest illustrated values are between 4.4 and 8.2 mg/L (stations M2 and M1, respectively, September 2007) with other concentrations commonly exceeding 0.2 mg/L. Stream values can also be high with values commonly exceeding 0.2 mg/L with a maximum value of 1.5 mg/L (PD-in, August 2007).

Abundance of Fecal Microbes

E. coli counts vary temporally and areally over Wilgreen Lake in 2006 and 2007 (Borowski and Albright 2007; Aguiar 2009). In the majority of cases (95 of 123, 77%), *E. coli* counts are below 235 cfu/100 mL (Figure 8) and are therefore *suitable for bathing* as determined by the EPA (2004b). In 12 cases (9.7%), *E. coli* counts are deemed suitable *for recreation only* (236 to 574 cfu/100 mL; EPA 2004b), and in 16 cases (13%) counts specify *no human contact recommended* (>575 cfu/100 mL, EPA 2004b). The highest *E. coli* counts were 5199, 4813, 3921, and 3683 cfu/100 mL at stations TF-1, M1, M2, and TF-in, respectively (Table 3). High fecal microbe counts are not distributed evenly across the lake (Figures 8 and 9) and the relatively low incidence of fecal microbe pollution suggests that contamination is episodic.

Timing of Fecal Microbe Outbreaks

Rainfall events should sweep fecal microbes into stream and lake waters (e.g., Geldreich 1972; Kleinheinz et al. 2009). We have daily rainfall information from the City of Richmond at its sewage treatment plants of Dreaming Creek and Tates Creek. These locations are outside of the lake's drainage basin, about 3.5 miles to the northeast and 2.5 miles to the north-northeast of the lake, respectively, so any recorded (or unrecorded) rainfall may not represent rain conditions within the Wilgreen watershed. Moreover, summer rainfall in the form of thundershowers is notoriously local and spotty so our characterization of rainfall is not ideal.

The highest *E. coli* counts (*no human contact recommended*) take place on 26 and 30 June 2007 in the shallow waters of Taylor Fork and within inflowing streams (Figure 9). No appreciable rain took place for two weeks prior to rainfall occurring on 25 June (0.35 in., Dreaming Creek; 0.15 in., Tates Creek; Aguiar 2009) and this circumstance prompted us to sample on 26 June. Taylor Fork was flowing, Old Town Branch was trickling, and Pond Cove did not flow with its water only occurring in isolated pools. *E. coli* counts at all of the shallow-water stations of Taylor Fork (Figure 9C) exceeded 2419 cfu/100 mL so for complete quantification we also sampled on 30 June and counted diluted samples. These samples showed counts of up to 5199 cfu/100 mL (Figure 9D), and rain fell on 28 June (0.50 in., Tates Creek) and 29 June (0.75 in., Dreaming Creek; 0.15 in., Tates Creek) before this sample date.

Three to four rainfall events took place between our 30 June and 17 July samplings recording up to 1.35 in. (9 July, Dreaming Creek) but rainfall was insufficient to cause flow in the streams of Pond Cove and Old Town Branch on 17 July. We infer that any rainfall during this time interval did not sweep microbes into the lake to affect microbial counts. *E. coli* counts on 17 July (Figure 9E) exceed the designation of *bathing acceptable*.

Significant rainfall took place on 28–29 July (0.75 in., Dreaming Creek; 2.26 in., Tates

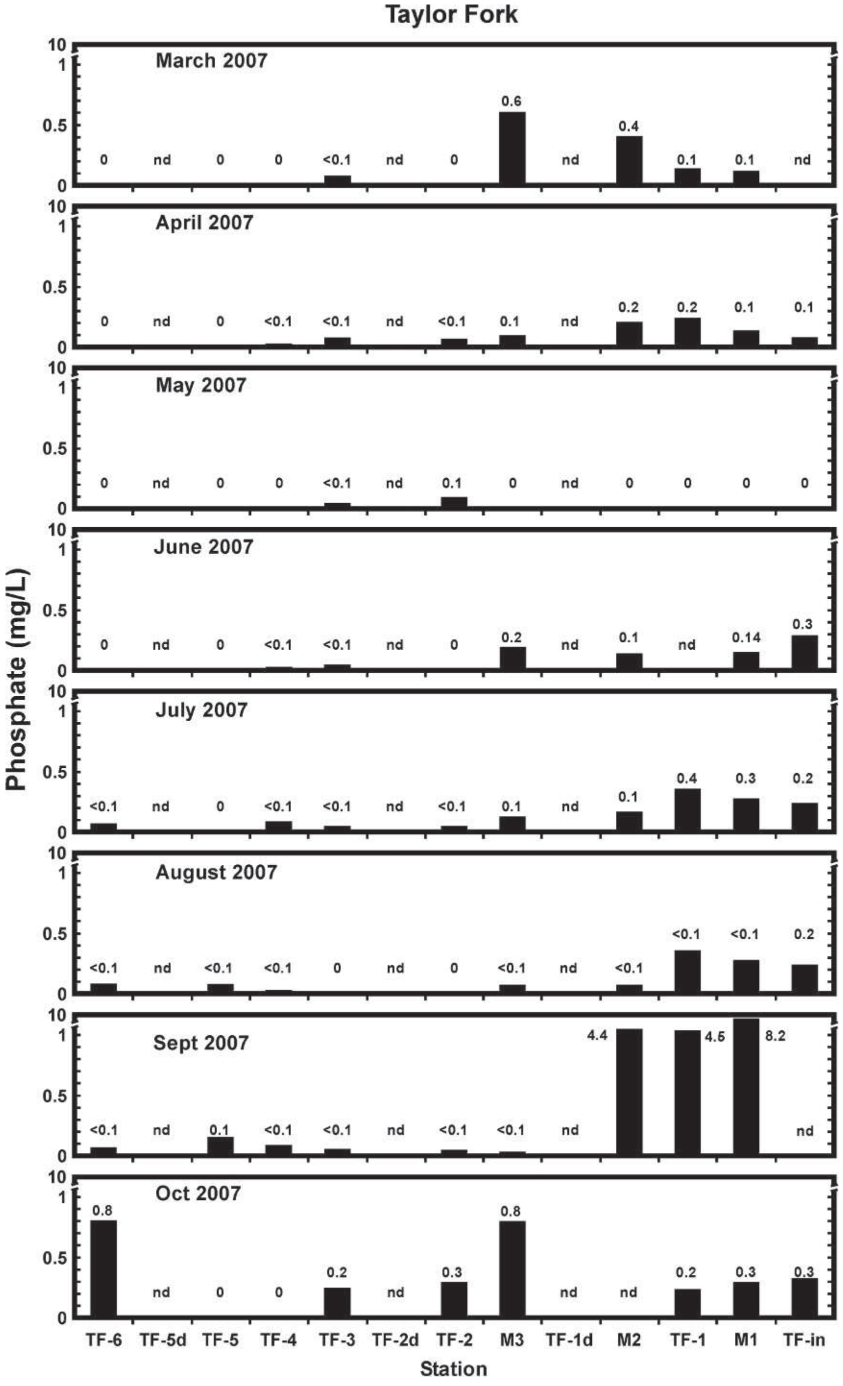
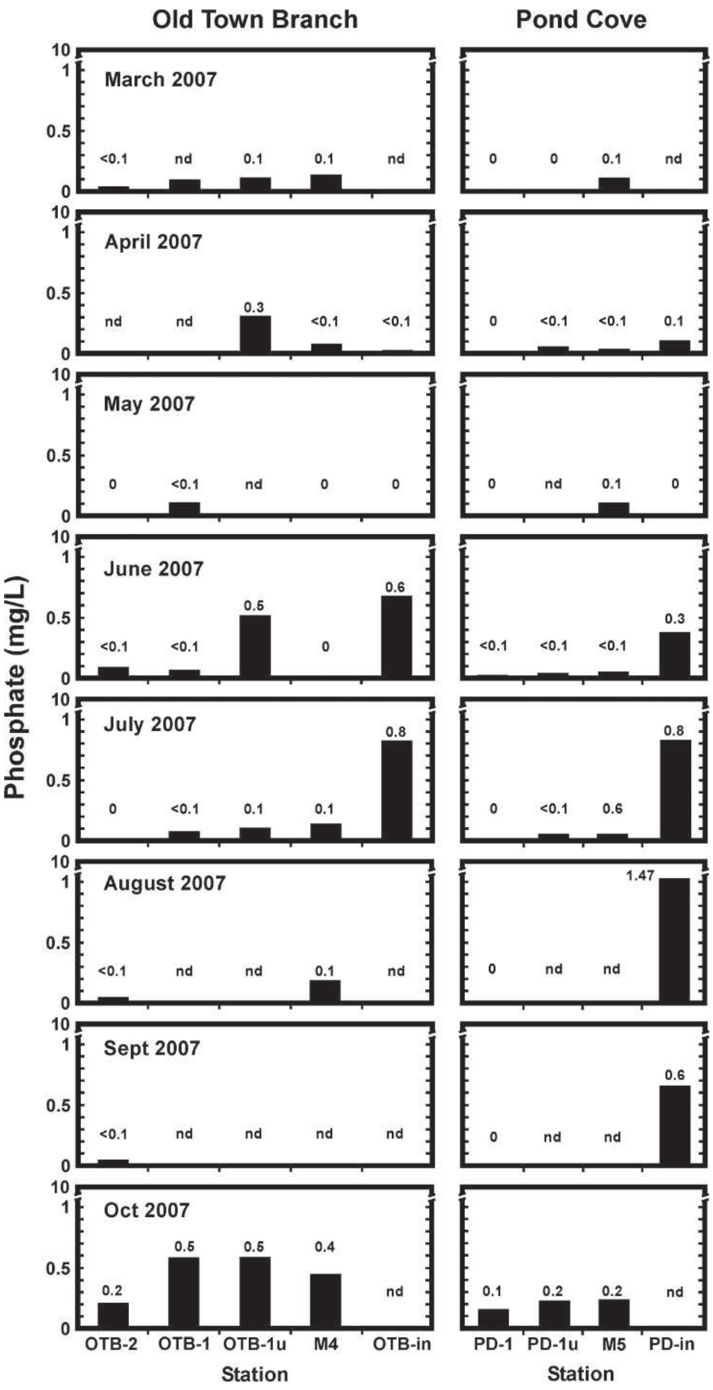


Figure 7. Graphs of phosphate concentration within surface waters through 2007 for Taylor Fork (TF), Old Town Branch (OTB) and Pond Cove (PD) areas of Wilgreen Lake. Water depth decreases from left to right on each panel as stations become more proximal to lake inputs; see Figure 1B for station locations. Note that the concentration scale breaks between 1 and 10 mg/L to clearly show the lower concentrations, and that concentrations are often in excess of that recommended for natural waters (0.05 mg/L PO_4; MPCA 2010). The numbers with the bars indicate phosphate concentration (milligrams/liter, mg/L); nd equals no data. Data from Hunter and Borowski (2008); Aguiar (2009).



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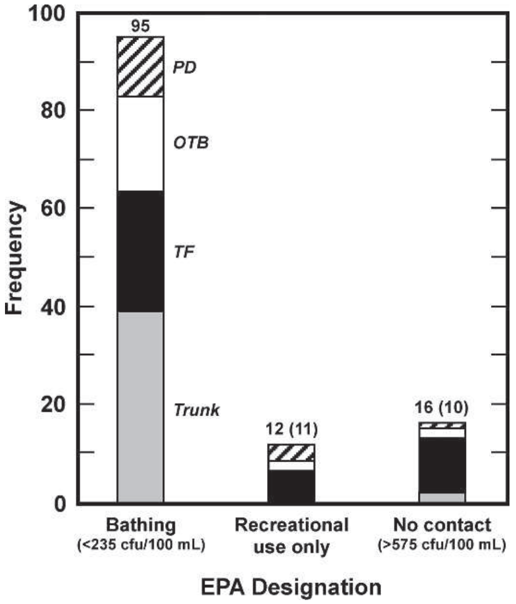

Figure 8. Histogram showing the frequency of *E. coli* counts related to EPA designations for human use: *bathing acceptable* (<235 cfu/100 mL), *recreational use only* (236–574 cfu/100 mL), and *no human contact recommended* (>575 cfu/100 mL) and), which are also related station location (trunk, gray; TF = Taylor Fork, black; OTB = Old Town Branch, white; PD = Pond Cove, striped pattern). See the text for definition of trunk versus shallow-water stations. Some counts exceeding the limit of the IDEXX methods (>2419.6 cfu/100 mL) were resampled and diluted to achieve fully quantitative values; these were samples first taken on 27 July 2007 with re-sampling on 30 July 2007. Numbers in parentheses refer to total number of samples in the class that were not replicated. See Table 3 for a listing of samples falling in the latter two categories. Data from Borowski and Albright (2007); Aguiar (2009).

Creek) between our microbial sampling dates of 17 July and 1 August. Several stations in the shallow-water portion of Taylor Fork had *E. coli* counts above the *for recreation only* designation; station PD-in had the highest count, exceeding *human contact not recommended* (Figure 9F).

Rainfall occurring between the sampling dates of 1 and 15 August was sporadic in the area. No significant rainfall occurred at Dreaming Creek but 0.9 in. was recorded at Tates Creek on 5 August. All *E. coli* counts excepting that of station OTB-in are below the *for recreation only* designation.

The relationship between rainfall and microbial counts is equivocal. We cannot demonstrate that high microbial counts within Wilgreen Lake are preceded by rainfall and runoff events. Our highest occurrences of *E. coli* counts exceeding the *human contact not recommended* designation did indeed occur after a rainfall event the preceding day. Apparently, significant rainfall (25, 28, and 29 June) also preceded very high counts recorded on 30 June but water levels within inflowing streams did not rise, suggesting that either appreciable rainfall did not occur within these watersheds and/or rain was insufficient to raise stream levels, perhaps because rain was immediately absorbed by dry soil preventing runoff. One could interpret that this rainfall did not affect microbial counts on 30 June, or alternatively that these highest counts occurred because of the rainfall. On other occasions, rainfall did occur prior to sampling but far enough in advance so that that *E. coli* counts could have spiked immediately after rainfall only to decline to levels documented by measurements. This uncertainty as to the timing of high microbe counts is also pertinent to the question of persistence of *E. coli* within lake waters.

Persistence of *E. coli*

Our data from the muddy environs of Wilgreen Lake suggest that *E. coli* can persist at moderate to high concentrations for 5 to 18 days. In the shallow waters of Taylor Fork, extremely high counts (>2419 cfu/100 mL) can continue in lake waters for at least 5 days (26 to 30 June 2007; Figure 9, panels C and D). Note that counts from Taylor Fork stream (TF-in) are also high, although lower than the highest counts at several other stations (M1, TF-1, M2). From these peak microbe counts on 30 June, *E. coli* counts decreased by a factor of 15 to 164 in the shallow waters and stream of Taylor Fork over an 18-day period to 17 July. The next sampling period (17 July to 1 August 2007; Figure 9, panels E and F) show apparent persistence of moderately high counts over 16 days, but then the subsequent period (1 to 15 August 2007; Figure 9, panels E and F) saw declines by a factor of 1.4 to 8 times over 15 days. These estimates assume that no new injection of fecal microbes from rain events took place between samplings. As we saw in the section above, periods between microbial sampling dates often experienced rainfall so that *E. coli* may have declined over

Table 3. Highest recorded *E. coli* counts in Wilgreen Lake segregated by EPA designation: *recreational use only* and *no human contact recommended* (EPA 2004b). Two sampling sessions occurred in 2006 (24 July, 6 August) and 4 sampling sessions took place in 2007 (27 June, 17 July, 1 August, 15 August). Note that some samples taken on 27 June 2007 exceeded the ability of the IDEXX method in obtaining absolute counts; these stations were re-sampled on 30 June 2007 and diluted to obtain the tabulated absolute counts. Data from Borowski and Albright (2007); Aguiar (2009).

	Recreational use only [1]			No contact human recommended [1]		
Sample date	Sample	*E. coli* count (cfu/100 mL)	Sample date	Sample	*E. coli* count (cfu/100 mL)	Re-sampled [2] *E. coli* (cfu/100 mL)
6 August 2006	M2	235	27 June 2007	M3	687	—
17 July 2007	TF-in	243	27 June 2007	TF-2d	816	—
1 August 2007	TF-1	243	1 August 2007	PD-in *	1259	—
1 August 2007	M1	243	27 June 2007	TF-2	1299	—
17 July 2007	TF-1	279	27 June 2007	TF-1d	>2419	1642
6 August 2006	TF-1	290	27 June 2007	OTB-in ^	>2419	2068
1 August 2007	TF-in ^	299	27 June 2007	TF-in ^	>2419	3683
15 August 2007	OTB-in ^	389	27 June 2007	M2	>2419	3921
17 July 2007	PD-in *	399	27 June 2007	M1	>2419	4813
27 June 2007	M4	410	27 June 2007	TF-1	>2419	5199
27 June 2007	PD-in *	488				

[1] Designations from EPA (2004b).
[2] High counts from 27 June 2007 were re-sampled on 30 June and diluted to produce absolute counts.
* No flow in stream.
^ Low flow in stream.

the entire interval between sampling, or declined then rose again after rainfall only to decline before the next sampling date.

High *E. coli* counts within Taylor Fork proximal to potential septic sources and stream inflow can persist whereas other localities show steeper decreases in microbe counts. Stations TF-2 and TF-2d show declines of 95% and 94% between 26 to 30 June over five days. Likewise over the same period, counts at station M3 decrease 90% from moderate (686 cfu/100 mL) to low levels (70 cfu/100 mL). These stations are distal to stream and potential septic input at the upper reaches of Taylor Fork. Counts seem to remain high at locations close to potential sources but decline more rapidly at stations away from these sources.

Our data show that high *E. coli* counts may persist for as long as 16 days but can also show marked declines over the same period. This suggests that new or continued input of fecal microbes is necessary for any persistence of moderate to high counts. Unfortunately, we cannot demonstrate this supposition because we cannot conclusively document the relationship of high *E. coli* counts to the timing and amounts of rainfall in the watershed. Nevertheless, distal stations (TF-2 and TF-2d, 26–30 June) can show large declines while no decreases are observed proximal to potential fecal sources. In addition, because steep declines in *E. coli* counts do occur over the period of 30 June to 17 July, we infer that *E. coli* can persist in the natural waters of Wilgreen Lake only for about 3 weeks, assuming no new addition of microbes. Similar persistence times of about 30 days have documented by Wcislo and Chrost (2000) in lake water, and about 28 days in sandy, marine sediment by Craig et al. (2004).

Distribution of High Fecal Microbe Counts

High *E. coli* counts most frequently occur in the shallow waters of Taylor Fork (stations TF-1d, -1, -in, M1, M2, and M3) followed by the shallow waters of Old Town Branch (OTB-1u, -in, and M4) and Pond Cove (PD-in) (Figure 9) (Table 3). Of the 10 highest counts above the EPA *no human contact* standard (not counting replicates), six cases occur at Taylor Fork stations (Table 3), proximal to the clustered septic systems of Deacon Hills and Idylwild (letter A, Figure 1B). Other stations from trunk sites, Old Town Branch, and Pond Cove have 2, 1, and 1 occurrences, respectively. Of the 21 cases exceeding the EPA bathing standard, 13 (62%), 3(14%), 3 (14%), and 2 (9%) cases occur at stations of Taylor Fork, Old Town Branch, Pond Cove, and trunk locations, respectively (Table 3). Stations with these high microbial counts include incoming streams in 38% of the cases (eight of 21), but seven of

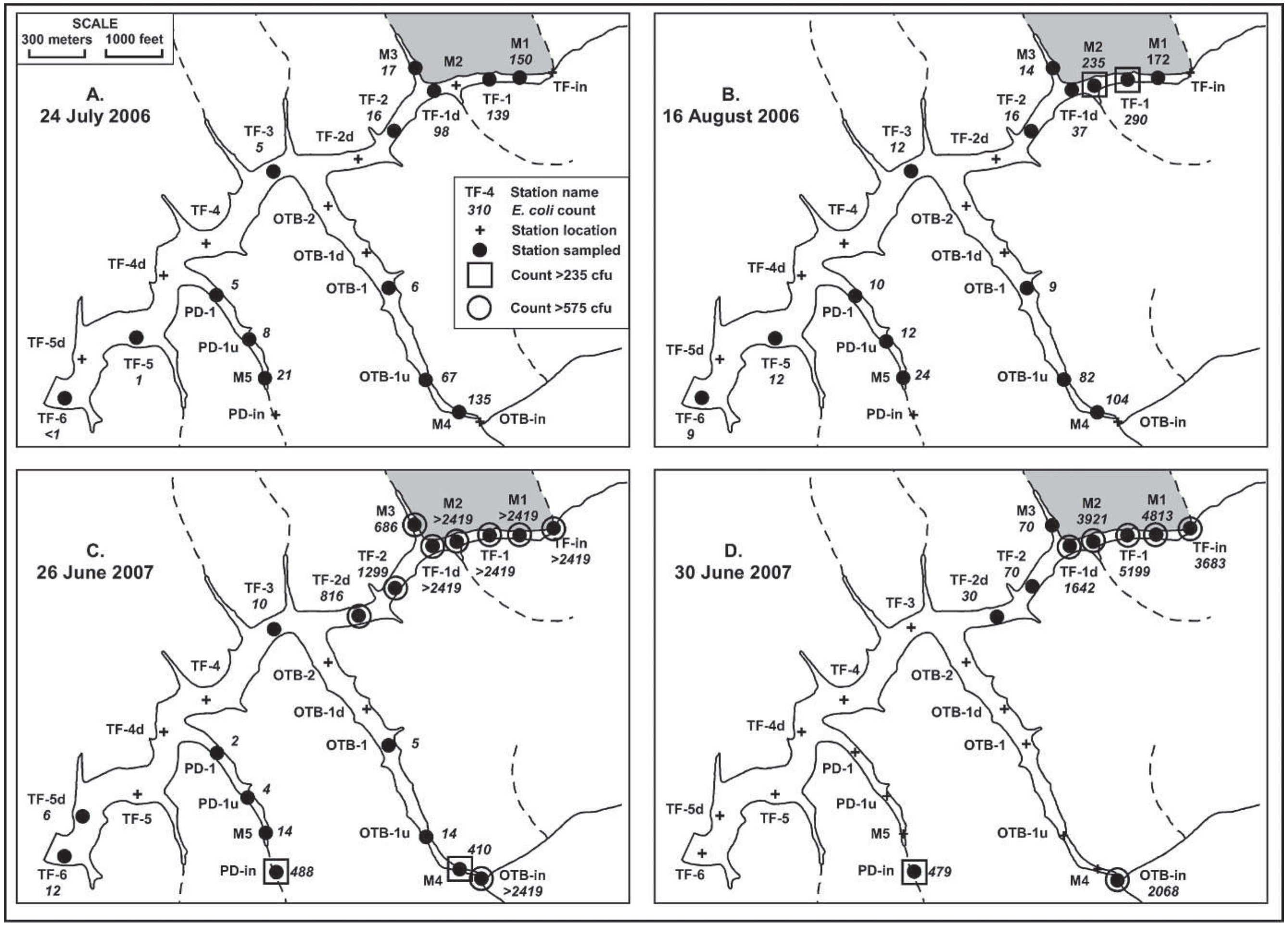

Figure 9. Maps showing the spatial occurrence of *E. coli* counts across Wilgreen Lake for two sampling sessions in 2006 (24 July, 16 August) and five sampling episodes in 2007 (26 June, 30 June, 17 July, 1 August, 15 August). Stations where microbial sampling took place are represented by filled circles and numbers are counts in colony forming units per 100 milliliters (cfu/100 mL). Stations with *E. coli* counts exceeding the EPA recommendation for *no human contact* (>575 cfu/100 mL; EPA 2004b) are highlighted with large, open circles; stations showing *E. coli* counts designated as *for recreation only* are shown with large, open squares. The shaded, gray area in each map shows the location of clustered septic systems within the Deacon Hills/Idylwild development. Data from Borowski and Albright (2007); Aguiar (2009).

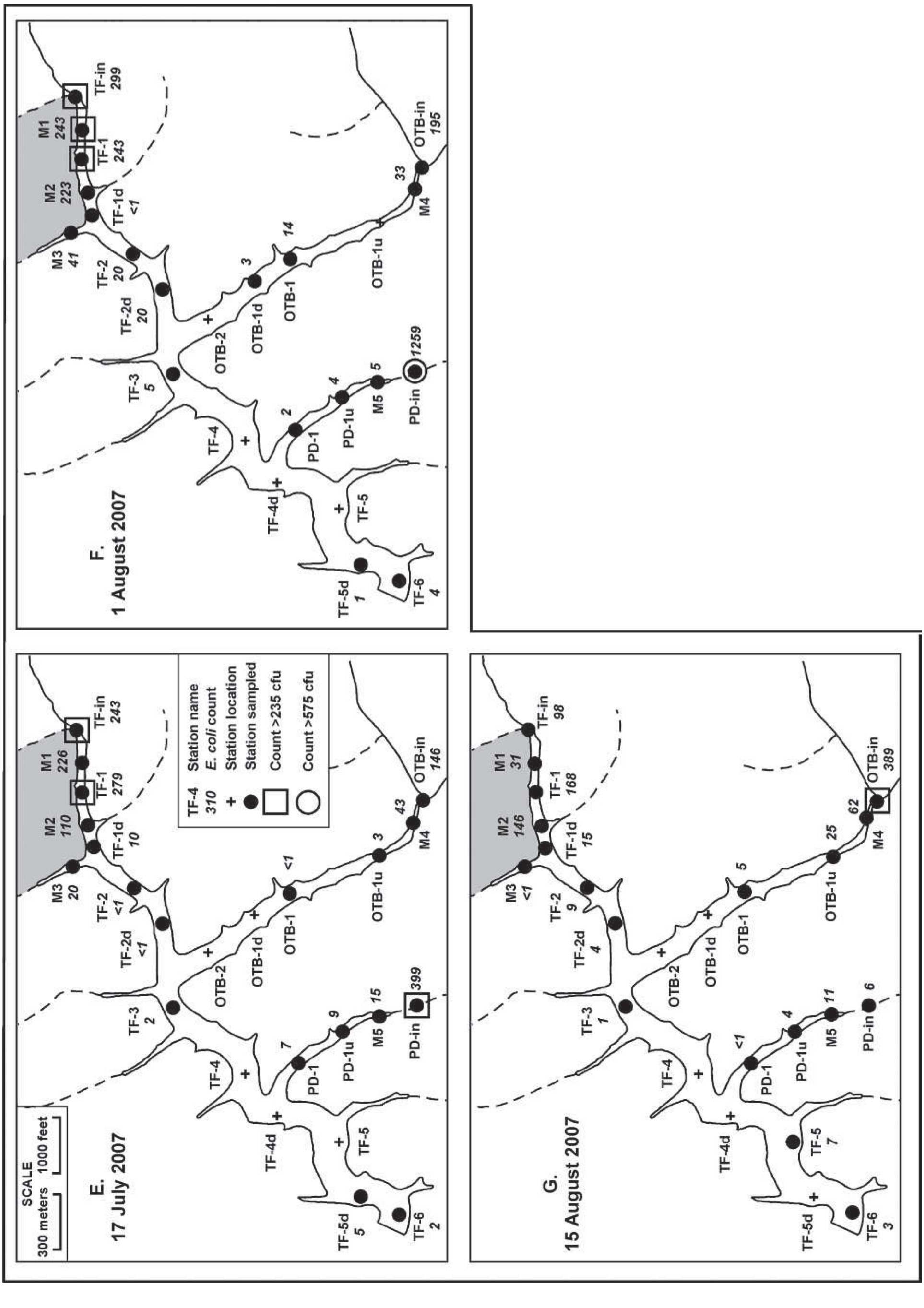

Figure 9. Continued.

eight of these occurrences happened when the streams were stagnant and drying up due to no rainfall.

Most of the lake is unaffected by high fecal microbial counts most of time, so fecal microbial contamination occurs only at discrete times. In 2006, none of the sampling stations showed *E. coli* counts higher than 575 cfu/100 mL, and only 2 stations (16 August 2006; TF-1 and M2) had counts within the *recreation only* designation by the EPA (EPA 2004b) (Figure 8; Table 3). In 2007, only samples taken on 26 and 30 June had samples exceeding the *no human contact* designation (Figure 8; Table 3). On 26 June all the shallow-water, Taylor Fork stations had the highest counts as did 2 of the trunk stations (TF-2, -2d) and OTB-in (Figure 9). We replicated sampling on 30 June to fully quantify the *E. coli* counts and to see how microbial patterns of distribution and abundance may have changed after these several days. High microbial counts still exceeded the *no human contact* designation at Taylor Fork and OTB-in, but *E. coli* counts decreased markedly to 70, 70, and 30 cfu/100 mL at M3, TF-2d, and TF-2, respectively (Figure 9). Thereafter, only PD-in (1 August) had high counts, and only stations in the upper reaches of Taylor Fork, PD-in, and OTB-in had *E. coli* counts at the *recreation only* standard (Figure 9).

DISCUSSION

Use of Nutrient Concentrations and Fecal Microbes as Source Indicators

Patterns of the distribution and abundance of high nutrient concentrations and *E. coli* counts should suggest sources for these contaminants. Dissolved nutrients are produced by decomposition of organic matter and can originate within lake waters or within its drainage basin. Any natural terrestrial habitat (woodlands, fields, etc.) can provide nutrients to natural waters as they become dissolved within rainwater and enter lakes from runoff and groundwater, but anthropogenic sources typically are responsible for severe eutrophication (e.g., Dubrovsky et al. 2010). Because Wilgreen Lake is homogeneous in terms of its physical and chemical properties, we use anomalous concentrations of dissolved nutrients as a source tracking parameter. Higher nutrient concentrations over the propensity of the lake are segregated within deep-waters trapped below surface and near-surface waters by density differences across the thermocline. Surface waters over most of the lake contain few nutrients because of uptake by photosynthesizers so only where nutrient supply exceeds uptake will nutrients accumulate in surface waters. Recognizing increased delivery of nutrients may identify plausible sources. Still, using anomalous nutrient concentrations to identify specific sources can be problematical because such sources may be numerous, because nutrient reservoirs are replete in natural systems (e.g., soils and sediments) especially those affected by humans, and because dissolved nutrients are mobile chemical species.

An additional tracer is fecal microbe occurrence within Wilgreen Lake. We link the occurrence of high nutrient concentrations and high fecal microbe counts because fecal matter contains enteric microbes and organic matter in varying stages of decomposition including nutrients that immediately or ultimately become dissolved in natural waters.

Use of *E. coli* as an indicator for sources of fecal and nutrient pollution depends on its reliability as a tracer. *E. coli* are enteric, facultative anaerobes (EPA 1986) that can persist in natural aquatic environments to some degree although their abundance typically declines with time (e.g., Crane and Moore 1986). Evidence suggests that predation by protozoans (Korhonen and Martikalnon 1991; Hartz et al. 2008) and sunlight (Chandran and Hatha 2005) are significant factors in the removal of *E. coli* from the environment; however, other studies have shown that these gut microbes can persist in refugia of sandy sediments (Craig et al. 2004) and can even grow within natural waters under favorable natural conditions (Carrillo et al. 1985). Thus, whereas governmental authorities like the EPA use *E. coli* counts as indicators of water quality (EPA 1986), the persistence of these microbes in natural waters complicates source tracking. Nevertheless we use the areal distribution of anomalously high nutrient concentrations in surface waters and *E .coli* counts to suggest plausible nutrient sources responsible for the eutrophication of Wilgreen Lake. Using anomalous nutrient concentrations and fecal microbe as tracers potentially allows identification of the two major sources of pollution in Wilgreen Lake (EPA 2010, 2012), so that results should also suggest remediation steps in improving the lake's water quality.

Areal Pattern of High Nutrient Concentrations in Surface Waters

Deep lake waters generally have higher nutrient concentrations because any nutrients released by decomposition are not utilized by photosynthesizers below the photic zone (e.g., Wetzel 1975). Shallow waters with high nutrient concentrations indicate that nutrient production or delivery is greater than uptake and may point to anthropogenic nutrient input. Excepting deep-waters (depth >2 m), the highest levels of ammonium and phosphate are consistently found at the shallow-water stations of Wilgreen Lake. Of the top 25 measurements of ammonium concentration, 72% are found at either shallow-water stations or incoming streams (Figure 10); of the top 50 measurements, 62% are found at either shallow-water stations or incoming streams. Of the top 25 measurements of phosphate concentration, 80% are found at either shallow-water stations or incoming streams (Figure 10); of the top 50 measurements, 70% are found at either shallow-water stations or incoming streams.

Of the shallow-water stations, the highest nutrient concentrations occur at Taylor Fork (Figure 10). Regarding ammonium and phosphate, 36% of the top-25 nutrient measurements occur in Taylor Fork samples. The next highest group of samples occurs at trunk stations, but these instances generally occur in deeper rather than surface samples. Old Town Branch samples correspond to 20% and 28% of the highest nutrient measurements for ammonium and phosphate, whereas Pond Cove samples for both nutrients represent 16% of the occurrences. High concentrations also occur in incoming streams indicating that the watershed does contribute significant amounts of nutrients to the lake. Stations OTB-in, PD-in, and TF-in have 2, 4, and 1 occurrences of high nutrient levels, respectively (Figure 10). Figure 7 also shows that stream waters contain appreciable amounts of nutrients.

These findings suggest high nutrient concentrations are sourced from either septic effluent entering through groundwater and/or direct runoff into the lake; and/or from streams draining their watersheds. We next examine *E. coli* data for added clues concerning nutrient sources.

Fecal Microbe and Nutrient Sources

High fecal microbe counts are generally restricted to the shallow waters of Taylor Fork, Old Town Branch, and Pond Cove and to their entry streams (Table 3, Figure 9). Moreover the clearest problem with fecal microbe pollution occurs at Taylor Fork, proximal to the concentrated septic systems in housing developments there (Figure 1B, letter A; Figure 9). The close spacing of residences with septic systems is a salient difference between settings of the 3 tributaries entering Wilgreen Lake.

Old Town Branch/Pond Cove. Shallow-water stations and streams of Old Town Branch and Pond Cove commonly contain high (*human contact not recommended*) and moderate (*for recreation only*) *E. coli* counts (Table 3, Figure 9), as well as high nutrient concentrations (Figures 7, 10). Stream waters are mostly responsible for these higher counts when water flow dwindled and the streams eventually ran dry in 2007. Of the lake stations, only station M4 shows moderate counts during these dry conditions (26 June 2007, Figure 9C).

When rainfall occurs and streams regain flow, they must contribute their load of fecal microbes to the lake; however, lake waters within these tributaries have low counts. We posit that once microbes are swept out into the lake, their numbers are diluted by lake waters because additional fecal sources from lake margins are lacking unlike the situation at Taylor Fork.

The drainage basin of Pond Cove is dominated by cattle pastureland, although some dwellings occur within the watershed directly positioned on the stream (outside the map area shown in Figure 1B). The drainage basin of Old Town Branch likewise is dominated by pastureland but comparatively more residences occur, albeit they tend to be widely spaced unlike those within the developments adjacent to Taylor Fork. Some housing

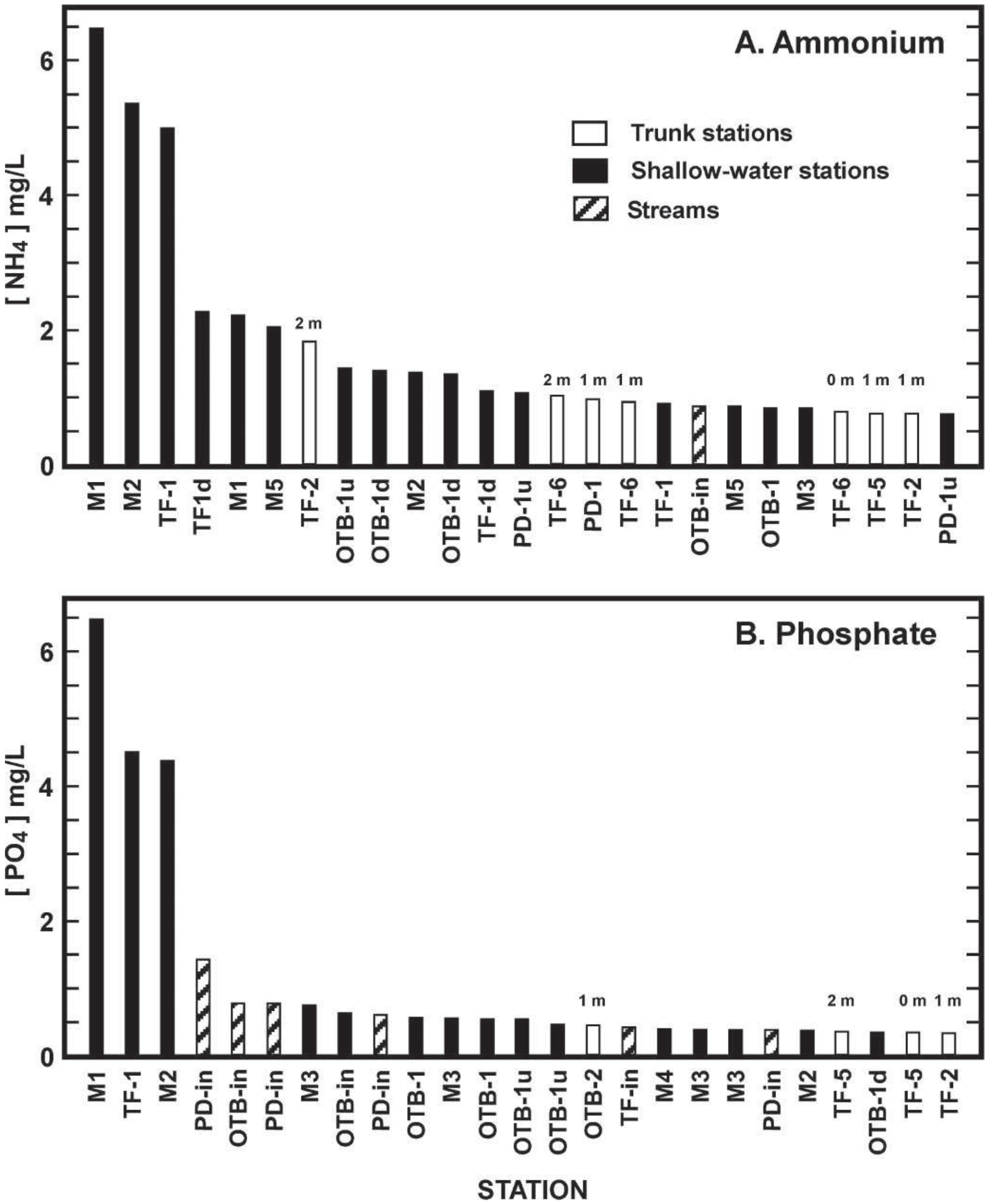

Figure 10. Graphs showing the highest 25 values of dissolved ammonium (A) and phosphate (B) in surface and near-surface (≤2 m) waters of Wilgreen Lake. Bars are coded to correspond to trunk stations (white), shallow-water stations (black), and inflowing streams (striped) of the Taylor Fork (TF), Old Town Branch (OTB), and Pond Cove (PD) portions of the lake. Labels with columns refer to the water depth of samples (e.g., 1 m); all other samples are surface samples. See Figure 1B for station locations and the text for definition of trunk versus shallow-water stations. Data from Jolly and Borowski (2007), Hunter and Borowski (2008), Stockwell and Borowski (2008), and Aguiar (2009).

developments on septic systems do occur in the drainage basin (see southeast portion, Figure 1B). Nutrients and fecal microbes from cattle manure may enter the lake through overland runoff, through groundwater especially in karstic conduits, and through direct deposits into the lake (cattle do enter the lake to drink).

Because pastureland is much more common and because dwellings on septic systems are so widely spaced, we infer that fecal sources in these watersheds most likely emanate from cattle, although human sources are still a possibility. Because fecal microbe counts of stations more distal from stream inputs (stations M5, PD-1u, OTB-1u, -1, -1d) tend to have very low counts, we also infer that direct runoff from pastureland and dwellings ringing lake margins is less significant as source of nutrients and microbes.

Taylor Fork stream waters. Microbes and nutrients carried by stream water entering Wilgreen Lake through Taylor Fork can come from several possible sources. The drainage basin of Taylor Fork is dominated by industrial, urban, and limited residential use without agricultural inputs (Figure 1). Only residential uses in the form of septic tanks or leaking and broken sewage lines could introduce fecal microbes into Taylor Fork stream, assuming that pet feces are not a significant source. Cattle manure or fertilizer use likewise should not contribute nutrients, also residential fertilizer use remains a possible source. The residences upgradient of the lake are generally on city sewer although some small developments are on septic systems. Thus, high fecal microbe counts within the stream (station TF-in) are consistent with either septic leachate or with sewage entering the stream from leaky sewer lines.

Another potential fecal microbe and nutrient source is from the sewage pumping station, located on Taylor Fork immediately adjacent to the lake (Figure 1B, letter B). At times of high water due to heavy rains, the sewage at the pump station may overflow into the stream and enter Wilgreen Lake. The City of Richmond operates the pump station and must report sewage releases; however, these episodes are the exception rather than the rule. For example, sewage overflows occurred on 14 April, 26 November, and 10 December during 2007 but not during the summer sampling season. No such overflows took place during our sampling times in 2006 either, so we may eliminate this potential fecal microbe source as affecting our data, especially because *E. coli* seem to persist in the lake for about 30 days or less. Although fecal microbes and human sewage certainly enters the lake via this mechanism, it is likely a small and episodic contributor to the lake system as a whole.

Taylor Fork lake waters. Nutrients and fecal microbes may also be sourced from pastureland and dwellings served by septic systems ringing Wilgreen Lake. Pastureland is limited along the shallow portions of Taylor Fork and cattle are prevented from entering lake waters upstream of station TF-1d by fencing, so we infer that cattle manure is not a significant source of nutrients and fecal microbes here.

Septic systems serving the residential area of Deacon Hills and Idylwild (letter A, Figure 1B) are another possible source of nutrients and fecal microbes. The septic systems are deployed in limestones that produce clayey soils and subsurface karst, and are thus prone to poor functioning. Evidence of septic malfunction includes fetid odors within the neighborhood that also occur right at the lake's edge indicating that sewage is leaking to the surface. A number of small runnels dissect the neighborhood adjacent to Taylor Fork and flow downhill into the lake and thus could directly inject nutrients and fecal microbes into the lake from surface flow. Contribution through the subsurface is also possible by conventional groundwater flow and by flow through karst conduits. Thus it is plausible that sewage containing nutrients and microbes enters lake waters of Taylor Fork upstream of and including station TF-1d.

A Natural Experiment

A drought occurring in summer 2007 provides a natural experiment in ascertaining nutrient and fecal microbe sources. Little rain fell during the summer months and entry streams ran dry (Aguiar 2009). In addition, many of the shallow-water stations of Wilgreen Lake were either dry or inaccessible by boat because of low water levels. Sampling at this time (12 September) established that phosphate (Figure 11) and ammonium levels were elevated only in the shallow-water stations of Taylor Fork. Dry stream beds and lack of rainfall eliminated two possible sources of nutrient delivery to the lake: that from direct runoff from lake margins or contributions from streams. The only other

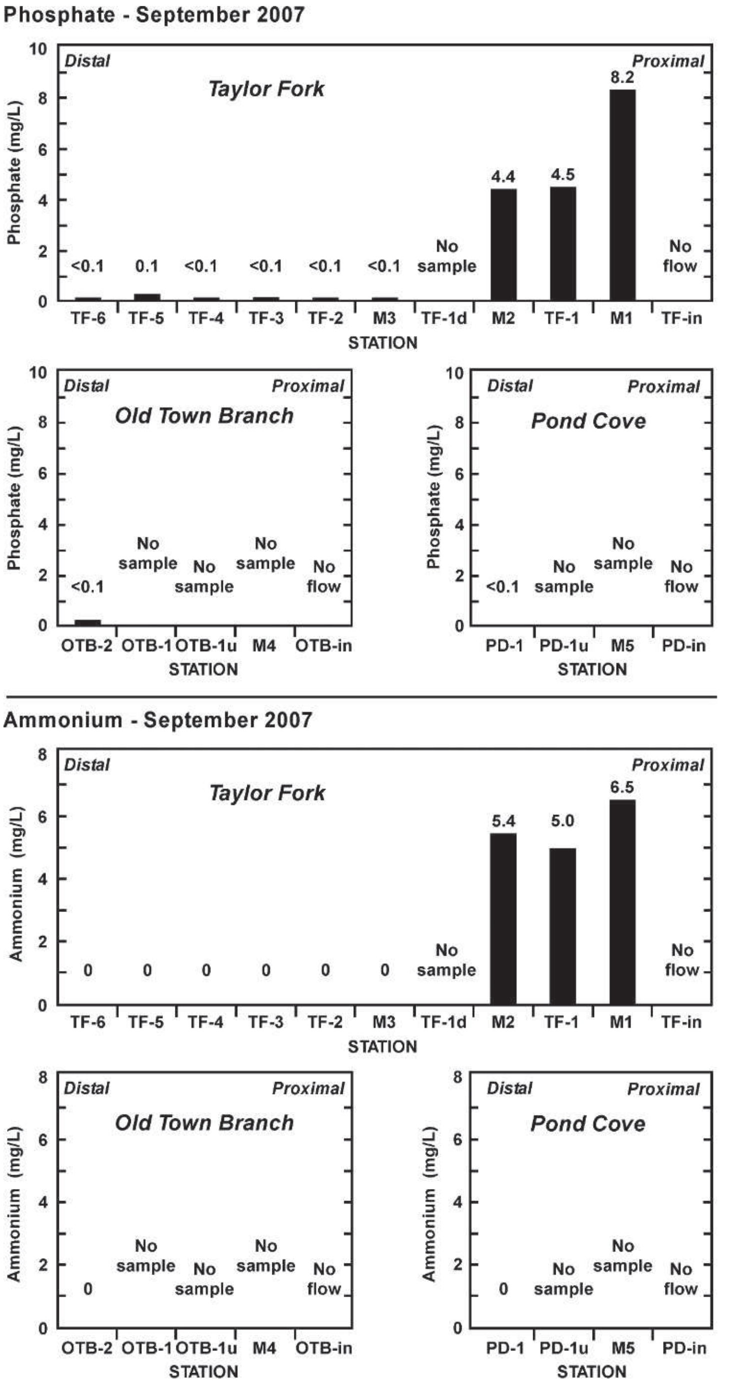

Figure 11. Graphs of phosphate and ammonium concentration within surface waters in September 2007 for the Taylor Fork (TF), Old Town Branch (OTB) and Pond Cove (PD) areas of Wilgreen Lake. Due to lack of rain, the lake had a very low water level and entry steams ceased to flow. Note that the highest dissolved nutrient concentrations are located at the shallowest portions of Taylor Fork, proximal to the developments (see Figure 1B, letter A) that possess clustered septic systems. See caption of Figure 7 for further explanation. Data from Hunter and Borowski (2008); Aguiar (2009).

sources for dissolved nutrients are from *in situ* decomposition of organic matter or from entry of groundwater. Sediment analyses from shallow-water sediments typically show low organic matter content (Aguiar 2009), leaving groundwater influx from the septic fields as the most likely source of nutrients during the drought. During normal periods, nutrient sources must also include run-off from streams and lake margins, but our natural experiment demonstrates that nutrients do enter the lake through groundwater. The source of nutrients within groundwater may not be solely from septic drainage, but effluent is a potential concentrated source for nutrients and consistent with our findings; however, we are unable to quantify the proportion of nutrient contribution from each plausible source.

Further Work

Circumstantial evidence above suggests that nutrient and fecal microbe pollution is associated with septic systems around the lake, especially those in the Deacon Hills/ Idylwild development (Letter A, Figure 1B) in the shallow reaches of Taylor Fork. Pastureland containing cattle is the other likely source. Nutrients occur concomitantly with fecal sources and will enter the lake along with fecal material. Although human sewage is clearly a source for nutrients and fecal microbes, we cannot quantify the proportion of human versus cattle contributions to lake waters. High nutrient levels, and microbial counts of total coliform and *E. coli*, although indicators of fecal sources (EPA 1986), do not specify host sources (Layton et al. 2006). Remediation efforts designed to control nutrient and fecal pollution must be targeted to specific sources to be effective. For example, if cattle manure is the dominate source for nutrients and fecal microbes within Wilgreen Lake, installation of costly sewage lines within Deacon Hills and Idylwild (estimated at $15K to $20K per household, 2005), although environmentally desirable, may be unnecessary to halt or decrease eutrophication. To effectively remediate nutrient and fecal microbe pollution effectively in Wilgreen Lake, the relative contributions from humans versus cattle must be determined. We plan to use nitrogen isotopes as tracer for nutrients (e.g., Silva et al. 2001) and PCR techniques utilizing the enteric obligate anaerobe, *Bacteroides* (e.g., Bernhard and Field 2000; Layton et al. 2006), to quantify these two most likely pollution sources.

SUMMARY

Circumstantial evidence in the form of increased nutrient concentrations and *E. coli* counts proximal to clustered septic systems in Wilgreen Lake suggest that septic systems are a significant source of nutrients and fecal microbes. However, these contaminants enter the lake through different mechanisms. Nutrients associated with the clustered septic system apparently enter the lake through groundwater, whereas fecal microbes enter the lake through runoff. Other sources for both contaminants are stream inputs and runoff form pastureland surrounding the lake. We cannot yet quantitatively access the individual contribution of these nutrient sources to Wilgreen Lake. We plan to use nitrogen isotopes and PCR assay techniques to distinguish the main sources of nutrients and fecal microbes.

ACKNOWLEDGEMENTS

Many people and organizations made this study possible. Mr. Randy Nunley actively supported our activities on the lake by offering the services of Wilgreen Boat Dock. We thank Mr. Steve Winkler (Richmond Utilities) for rainfall and sewage release data. Dr. Lori Wilson, Dr. Danita LaSage, Dr. Robert Creek, and Dr. Ron Jones also contributed to the project. Finally, anonymous reviews also improved the manuscript. Funding for the lake study came from Madison County Fiscal Court (with support from Dr. William Tudor, Dr. Paula Maionchi, and Judge Kent Clark); the Eastern Kentucky Environmental Research Institute (Dr. Alice Jones, Director); and with the support and collaboration of the Department of the Interior, U.S. Geological Survey and the University of Kentucky Research Foundation (grant number 06HQGR0087, 104B) funded through the Kentucky Water Resources Research Institute (with the support of Mr. James Kipp, Associate Director).

NOTE

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