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Non-point Sources and Point Sources for Nutrient and Fecal Microbe Contamination in a Typical Upland Stream: Tates Creek, Madison County, Kentucky

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ABSTRACT

Tates Creek (Madison County, Kentucky) is characterized by an oversupply of nutrients and fecal microbe contamination. Its watershed is dominated by pastureland and immature woodlands with scattered settlements served by septic systems, whereas ~5% of the watershed drains urban areas of Richmond, Kentucky. Creek waters are eutrophic and commonly display levels of *Escherichia coli* deemed unfit for human contact by United States Environmental Protection Agency standards. Both point and non-point sources existed for stream contaminants. A secondary sewage treatment plant (STP) discharged effluent into the creek until mid-2011 and was a point source for nitrate and phosphate. Pastureland likely contributes dissolved nutrients as well. High ammonium levels occurred sporadically, only sometimes related to plant discharge. After plant shutdown, nutrient levels downstream of the former STP decreased markedly for nitrate and phosphate, but phosphate then became the principal nutrient contaminant, suggesting that nitrogen may be the limiting nutrient within the stream ecosystem. Fecal microbes enter the stream from the sewer system serving the town of Richmond, from cattle farming, and from large developments served by septic systems. We cannot demonstrate any fecal contamination from single-residence septic systems.

KEY WORDS: stream, water quality, nutrient, ammonium, nitrate, phosphorus, *E. coli*

INTRODUCTION

Tates Creek is a secondary stream located in Madison County, Kentucky (Figure 1). The stream is listed as impaired by the United States Environmental Protection Agency (US EPA 2010) as reported by the Kentucky Division of Water (KDW 2006, 2008, 2010, 2012). Causes for degradation of water quality include nutrient loading, biological indicators of eutrophication, organic enrichment, and low dissolved oxygen (USEPA 2010). The nature and extent of pollution within this local watershed mirrors that seen nationally, dependent on similar land use. We seek to assess the water quality of Tates Creek in more detail and to identify plausible sources of contamination. A more detailed understanding of contaminant sources can be used to plan measures to mitigate entry of pollutants into the watershed.

Point Sources Versus Non-Point Sources

The nature of pollution within American waterways has shifted greatly since the mid-1900's. Industrial pollution, emanating from easily-recognizable single sources or point sources, was the principal cause of degraded water quality (e.g., Loehr 1974). The environmental movement of the 1970's spurred government to pass laws requiring industry to discharge only treated, cleaner water from facilities. Subsequent years have seen vast improvements in most waterways so that industrial, point-source pollution is no longer the primary concern in terms of water quality for most regions within the United States. Now non-point sources, that is contamination from diffuse sources, are the greatest threat to water quality in most cases. Degradation of water quality arises when common, widespread, anthropogenic activities introduce contaminants in the form of nutrients, fecal microbes, or sediment into freshwater ecosystems from

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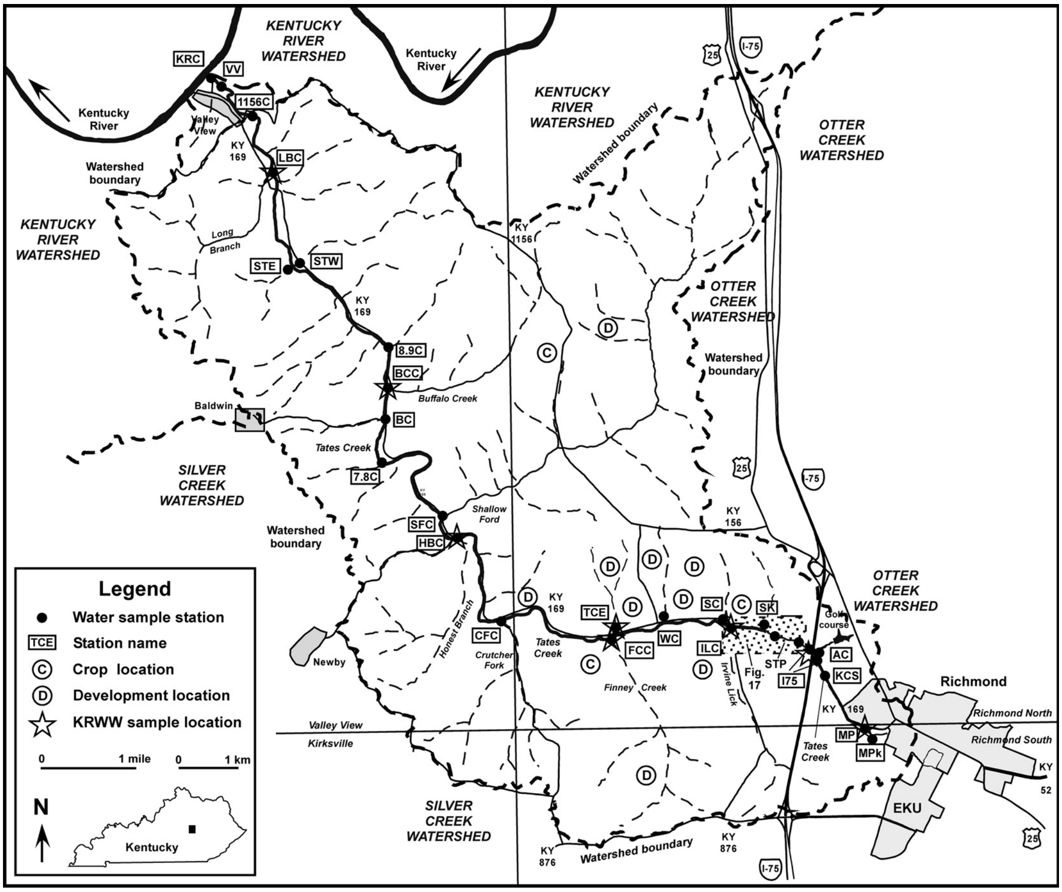

Figure 1. Map of the Tates Creek watershed and location of sampling stations (filled circles). Station codes are shown within boxes and are keyed to Table 1. Stars show sampling sites of the Kentucky River Watershed Watch (KRWW). The location of significant crops are shown with open circles enclosing a "C"; circled "D's" refer to the location of housing developments on septic systems. Footprints of Richmond (including Eastern Kentucky University, EKU) and selected towns are shown with gray polygons. Stippled rectangle near the sewage treatment plant (STP) is the location of Figure 17. Map base is compiled from the United States Geological Survey, 7.5-minute quadrangle topographic map series (Kirksville, Richmond North, Richmond South, and Valley View quadrangles).

agricultural, suburban, and urban settings (e.g., Myers et al. 1985; Baker 1992).

Nutrient Pollution

Surface waters of the United States have seen increases in dissolved nutrients that produce chronic and widespread contamination (e.g., Smith 2003, 2006). Eutrophication, or the oversupply of nutrients, causes a cascade of problems in ecosystems. Deleterious effects include rampant growth of cyanobacteria and algae, loss of biodiversity, and low dissolved oxygen concentration (e.g., Carpenter et al. 1998; Correll 1998; Parr and Mason 2004). Compounds of nitrogen and phosphorus are key nutrients and mainly exist in nature as ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-}). Land use by humans typically determines the severity of nutrient pollution by adding nutrients from sources such as fertilizers, farm animal manure, atmospheric deposition, and human sewage (e.g., Dubrovsky et al. 2010).

Few firm standards for dissolved nutrient levels for surface waters exist, largely because of huge ecosystem differences, variable land use, disparate rainfall and runoff conditions, and wide tolerance differences of aquatic biota (USEPA 2000). For example, the United States Environmental Protection Agency (USEPA) has not determined maximum contaminant

Table 1. National data showing nutrient concentration levels in pristine streams and in streams impacted by anthropogenic agricultural activity. Data are from Dubrovsky et al. (2010).

Condition	Nutrient		
	$N\text{-}NH_4$ (mg/L)	$N\text{-}NO_3$ (mg/L)	$P\text{-}PO_4$ (mg/L)
Pristine[a]	0.025	0.24	0.010
Median impacted[b]	0.095	2.8	0.095

[a] Pristine levels represent 75th percentile values (Table 4–1), Dubrovsky et al. (2010).

[b] Data from agricultural lands, estimated from graphs (Figure 4–4), Dubrovsky et al. (2010).

levels (MCLs) for ammonium, nitrate, or phosphate analogous to those for metals and pesticides (USEPA 2014). Because varied, multiple factors control dissolved nutrient values and because wide variations in nutrient measurements occur even within similar drainage systems, any guidelines or reference points are determined statistically and are somewhat arbitrary. The USEPA has determined typical contamination levels for nutrients within drainage basins with similar climate, land use, bedrock, etc., termed "ecoregions"; Madison County, Kentucky is within Ecoregion IX (USEPA 2000). "Reference conditions" are defined as the 25th percentile of sample values taken from streams in a particular ecoregion that display a spectrum of water quality. Thus, the reference condition is used as a realistic target for nutrient concentrations based on cleaner streams within a particular ecoregion. An analogous statistical method used by Dubrovsky et al. (2010) defines background nutrient concentrations as the 75th percentile of values measured from samples taken from pristine streams.

The USEPA has established standards for Ecoregion IX for total nitrogen and phosphorus (USEPA 2000), but not for specific nitrogen or phosphorus species as do Dubrovsky et al. (2010). Reference concentration values for total N and P from these two different sources are within about 16% and 8% of one another, respectively. Because Dubrovsky et al. (2010) specifically determine standards for specific dissolved nutrient species for both pristine and impacted streams, we will use their values (Table 1) as comparators when analyzing our data. Moreover, Dubrovsky et al. (2010) have an extensive statistical database covering the United States that ties dissolved nutrient values to land use.

Fecal Microbe Pollution

Fecal material from humans and agricultural animals contributes nutrients to freshwaters and also directly affects water quality by introducing fecal microbes that are a health threat to humans. Sewage treatment systems are typically effective in preventing both nutrients and microbes from entering streams. However, because of infrastructure costs, sewage systems typically serve only urban communities, whereas suburban and rural areas usually rely on septic systems. Septic systems that function inadequately, leak, or serve a high density of residences may release nutrients and microbes to surface and ground water (e.g., Steffy and Kilham 2004). Soil types and bedrock characteristics also impact the proper functioning of septic systems. Clay soils generally have poor permeability so that septic tank effluent may come to the surface and run-off into natural waters, especially during periods of prolonged precipitation (McKinney et al. 2007). Septic systems improperly situated in karst regions may channel effluent directly into surface or ground water. Parts of Kentucky are notorious for the occurrence of "straight pipes" – that is, discharge of human waste directly into natural waterways. Fecal material from grazing animals like cattle can also directly enter streams from runoff (Howell et al. 1995; Weiskel et al. 1996; Crowther et al. 2002). Most gut microbes are benign, but some are pathogenic and can cause severe health problems in humans.

The USEPA has established single-sample standards for *Escherichia coli* counts (USEPA 1986, 2004; for background, see also Borowski et al. 2012); these standards are also accepted by Kentucky in the form of Kentucky Administrative Regulations (KAR 401 5:031). *E coli* microbes themselves are generally not harmful, but *E. coli* counts serve as a reliable proxy for the quantity of fecal material and fecal microbes, some of which may be pathogenic, within natural waters. Overall standards for fecal microbe concentrations are: *bathing acceptable* (≤235 colony-forming units per 100 milliliters, or cfu/100 mL), *recreation only* (236–574 cfu/100 mL), and *human contact not recommended* (≥575 cfu/100 mL).

Study Site

Tates Creek is one of northern Madison County's principal watersheds, arising in Richmond and coursing about 21 km (13 miles) to the Kentucky River in draining approximately 95.8 km^2 ($\sim$37 mi^2) of land (Figure 1). No detailed picture of the water quality of the stream exists, although work by the KDW and some serial measurements by the Kentucky River Watershed Watch (KRWW) have identified contamination problems. The creek drains some of Richmond's urban setting at its headwaters ($\sim$5% of watershed area), but mainly is affected by suburban housing developments on septic systems ($\sim$5%), by small settlements and single residences in its valley ($\sim$5%), and by agricultural uses ($\sim$85%) mainly in the form of cattle farming, although some crop farming does occur (Figure 1). Principal threats to water quality are therefore expected to be contamination by nutrients and fecal microbes donated by both human and cattle waste, but fertilizer use on residential areas and croplands are also plausible as nutrient sources.

The watershed is further characterized by shallow, clay-rich soils developed atop bedrock. The underlying rocks are Middle and mostly Upper Ordovician limestone and shaly limestone (Greene 1966; Simmons 1967) with the potential for karst development in the subsurface. We did not investigate karst and other groundwater contributions to the watershed, although these subsurface conduits have the potential to quickly deliver contaminants to surface waters (e.g., Howell et al. 1995). Watershed streams generally flow on top of, and through alluvium, although both Tates Creek and its tributaries commonly flow over broad reaches of horizontal to shallow-dipping bedrock as well.

A sewage treatment plant (STP) existed on Tates Creek on the outskirts of Richmond (Figure 1). As a secondary plant, it was designed to remove solid waste from sewage (primary treatment) and to treat waste water to remove fecal microbes (secondary treatment) within regulated limits (monthly average, 130 colony forming units per 100 mL). The plant had operated since the 1960's but closed its operation on 19 Jul 2011 when all sewage from the city of Richmond was routed to a new tertiary treatment plant located within the Otter Creek watershed, $\sim$9.5 km (6 miles) to the northeast. The site on Tates Creek remains as a pumping station and storm water repository, but its wastewater is eventually treated at the Otter Creek plant. Our first year of sampling thus documents the effects of both plant operation and closure, whereas our sampling in 2012 characterizes stream waters without any STP discharge. The situation allows us to evaluate the STP as a potential point source for contaminants. We continue to sample the stream and will be able to document longer term responses of the stream to STP closure in the future.

Previous Work

Sporadic dissolved nutrient and fecal microbe measurements from Tates Creek were taken by the Kentucky River Watershed Watch (KRWW). The KRWW is a network of citizens led by professionals who sample and analyze surface waters of the Kentucky River watershed on a volunteer basis. The KRWW has sampled since about 1997 and has utilized six sites along Tates Creek for occasional sampling (Table 2, asterisks; Figure 1, starred locations). Their efforts identify Tates Creek as a "historically-troubled stream" (McAlister and Ormsbee 2007) with high nutrient concentrations and elevated fecal microbe counts. For example, Tates Creek displayed the highest total nitrogen and phosphate concentrations in the Kentucky River watershed in 2005 and 2007 (Ormsbee and McAlister 2005; McAlister and Ormsbee 2007) with the second and fifth highest concentrations, respectively, in 2006 (McAlister and Ormsbee 2006). Moreover, Tates Creek commonly had *E. coli* counts in excess of the USEPA standard for human contact (575 cfu/100 mL) or that for bathing (235 cfu/100 mL) (USEPA 1986, 2004). Our study includes all the KRWW sites and comprehensively expands the sampling of Tates Creek watershed waters (Table 1).

MATERIALS AND METHODS

Water Sampling

We established 28 sampling stations in the Tates Creek watershed along the entire length of the stream (Table 2; Figure 1), and sampled

Table 2. List of sampling stations, Tates Creek, Madison County, Kentucky. Sample codes are keyed to Figure 1. Sites sampled by the Kentucky River Watershed Watch (KRWW) from 2000 through 2012 are noted with an asterisk (*) with station names in parentheses; site designations before 2010 contain a "K" whereas new identification numbers adopted in 2010 appear thereafter. At tributary confluences with Tates Creek, we typically sampled within the tributary itself (inflow) and in the trunk stream above (up) and below (down) the confluence.

Sample code	Sampling site	Latitude	Longitude	Sampling	Number of samples
MPk-E	Million Park - east fork	37° 44′ 56.3040"	84° 18′ 11.2980"	Creek only	1
MPk-W	Million Park - west fork	37° 44′ 53.8080"	84° 18′ 17.2080"	Creek only	1
MP	McCready pond (2924)	37° 44′ 59.5680"	84° 18′ 31.9140"	Inflow, outflow	2
KCS	Opposite golf course	37° 45′ 24.8520"	84° 15′ 56.3280"	Drainage only	1
AC	Arlington golf course	37° 45′ 39.9000"	84° 19′ 4.3500"	Drainage only	1
I75*	Interstate I-75 overpass (K407, 1076)	37° 45′ 40.2960"	84° 19′ 6.0720"	Upstream, down	2
SP-u	Sewage plant - upstream	37° 45′ 46.6020"	84° 19′ 16.6140"	Upstream	1
SKC	South Keeneland drainage	37° 45′ 52.6980"	84° 19′ 31.0860"	Drainage only	1
SP-d	Sewage plant - downstream	37° 45′ 48.5040"	84° 19′ 30.1380"	Downstream	1
ILC*	Irvine Lick confluence (K209, 939)	37° 45′ 53.6100"	84° 20′ 1.7460"	Inflow, up, down, bridge	4
SC	Substation drainage	37° 45′ 59.2200"	84° 20′ 4.8840"	Drainage only	1
WC	Wellington development	37° 45′ 58.3320"	84° 20′ 46.4520"	Drainage only	1
TCE*	Tates Creek Estates dev. (K514, 1181)	37° 45′ 56.5140"	84° 20′ 57.2760"	Drainage only	1
FCC	Finney Creek confluence (3172)	37° 45′ 49.2720"	84° 21′ 22.4040"	Inflow, up, down	3
CFC	Crutcher Fork confluence	37° 45′ 59.2200"	84° 22′ 39.2820"	Inflow, up, down	3
HBC*	Honest Branch confluence (K515, 1182)	37° 46′ 44.9640"	84° 23′ 11.2980"	Inflow, up, down, big	4
SFC	Shallow Ford confluence	37° 46′57.2760"	84° 23′ 16.4820"	Inflow, up, down	3
7.8C	Stream at 7.8 mi. confluence	37° 47′ 27.2040"	84° 23′ 59.6400"	Inflow, up, down	3
BC	Baldwin confluence	37° 47′ 50.2140"	84° 23′ 56.9100"	Inflow, up, down	3
BCC*	Buffalo Creek confluence (K530, 1197)	37° 48′ 7.8480"	84° 23′ 53.7360"	Inflow, up, down	3
8.9C	Stream at 8.9 mi. confluence	37° 48′ 29.9160"	84° 23′ 52.1100"	Inflow, up, down	4
STC	Stringtown confluence	37° 49′ 15.2160"	84° 24′ 57.8880"	Up, down	2
STE, STW	Stringtown east, west tributary	37° 49′ 15.0660"	84° 24′ 59.4060"	Inflow, road	3
LBC*	Long Branch confluence (K59, 797)	37° 50′ 5.6880"	84° 25′ 12.3120"	Inflow, up, down	3
1156	KY 1156, tributary	37° 50′ 37.2180"	84° 25′ 28.3320"	Inflow, down	2
VV	Valley View	37° 50′ 47.9160"	84° 25′ 41.8560"	Creek only	1
KRC	Kentucky River confluence	37° 50′ 56.6700"	84° 25′ 51.3660"	Creek only	1
				Total samples	**57**

KEY:
u, up = upstream
d, down = downstream
*KRWW location

during the summer months of 2011 and in May, June, and July in 2012. Stream conditions and sewage plant discharge varied widely on different sampling days (Table 3; Figure 2). In 2011, sewage plant discharge exhibited a background outflow of ~1.5 million gallons per day (mgd) but peaked during and immediately after rainfall events. Rainfall controls flow within streams, particularly within smaller tributaries that were often dry or ponded with no flow on some sampling days (Table 3). However, STP discharge also contributed water to Tates Creek, which maintained a higher degree of flow than would otherwise be seen during times of little or no rainfall.

Sampling stations were established throughout the watershed where significant tributaries entered the trunk stream of Tates Creek and near suspected contaminant sources. At confluences, samples were taken within tributaries well above the influence of the trunk stream and also in the trunk stream upstream and downstream of confluences within riffles, where stream waters were well mixed.

Table 3. Sampling conditions during each sampling day in 2011 and 2012.

Sampling date	Rainfall history / stream conditions	STP operating?
31 May 2011	Last rain 26 May; all tributaries flowing.	Yes
20 Jun 2011	Rain last 3 days including hard rain 19 June; most streams flowing.	Yes
7 Jul 2011	No rain since last sampling; no flow in small streams.	Yes
4 Aug 2011	Little rain, shower day before; small streams with no flow, pooled water.	No
15 May 2012	All day rain on 13 May; all streams flowing.	No
12 Jun 2012	Small showers on 11 June; no flow in small tributaries.	No
11 Jul 2012	No rain since 8 July; no flow in small tributaries, weak flow in upper trunk.	No
16 Jul 2012	Substantial rain 13 and 14 July; no flow in small tributaries.	No

Samples targeted for nutrient analyses were filtered on location through nylon, 0.45 µm syringe filters and split into subsamples for analysis of ammonium, nitrate, and phosphate. These subsamples were acidified with H_2SO_4 to bring the sample to pH of ≤ 2 in order to prevent sample degradation (Eaton et al. 2005a). Samples were placed on ice in the field, transported to the laboratory, and then refrigerated. Nutrient measurements generally occurred within 1 to 3 days of sampling, well within the target interval of 28 days (Eaton et al. 2005a).

Water samples for microbe analyses were collected at the same time as nutrient samples but were not filtered, nor acidified. Samples

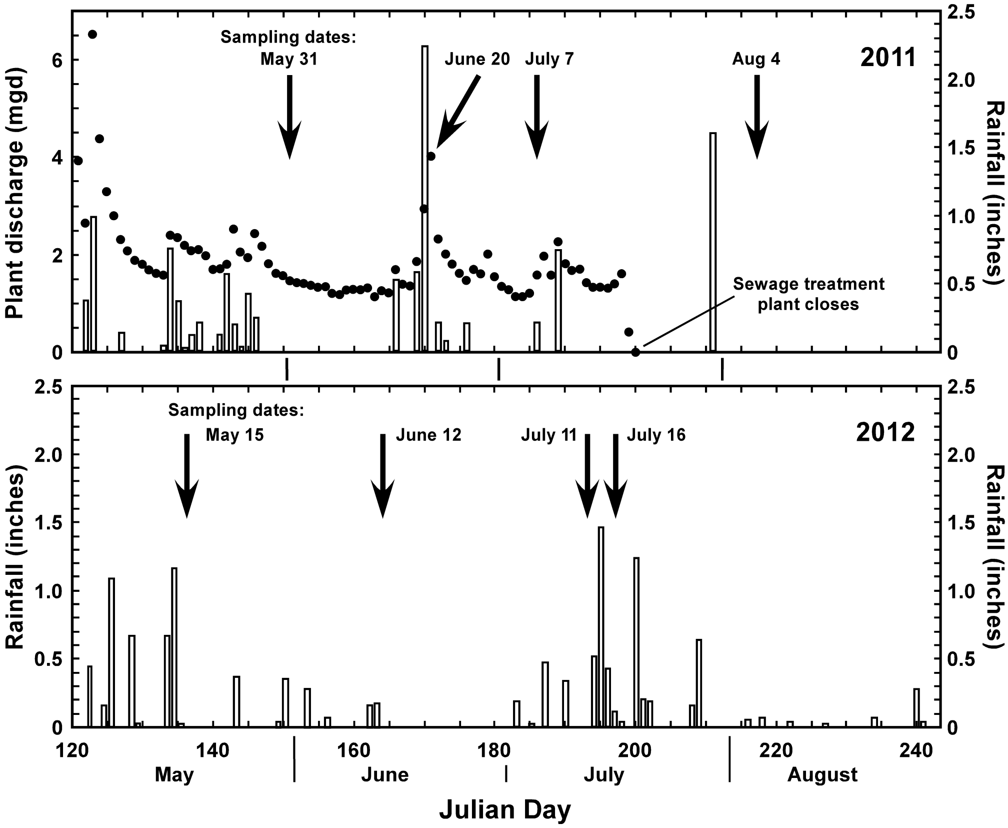

Figure 2. Graphs showing rainfall data (columns) in inches, sewage treatment plant outflow (points) in millions of gallons per day (mgd), and sampling dates for the field seasons of 2011 and 2012 (arrows). Data are courtesy of Richmond Utilities. The sewage treatment plant permanently shut down on 19 Jul 2011, consequently there are no outflow data thereafter. Rainfall data was collected from rain gauges either at the Tates Creek or Otter Creek (~9.5 km, or 6 miles, to the northeast) sewage treatment plants dependent on whether the Tates Creek plant was operating.

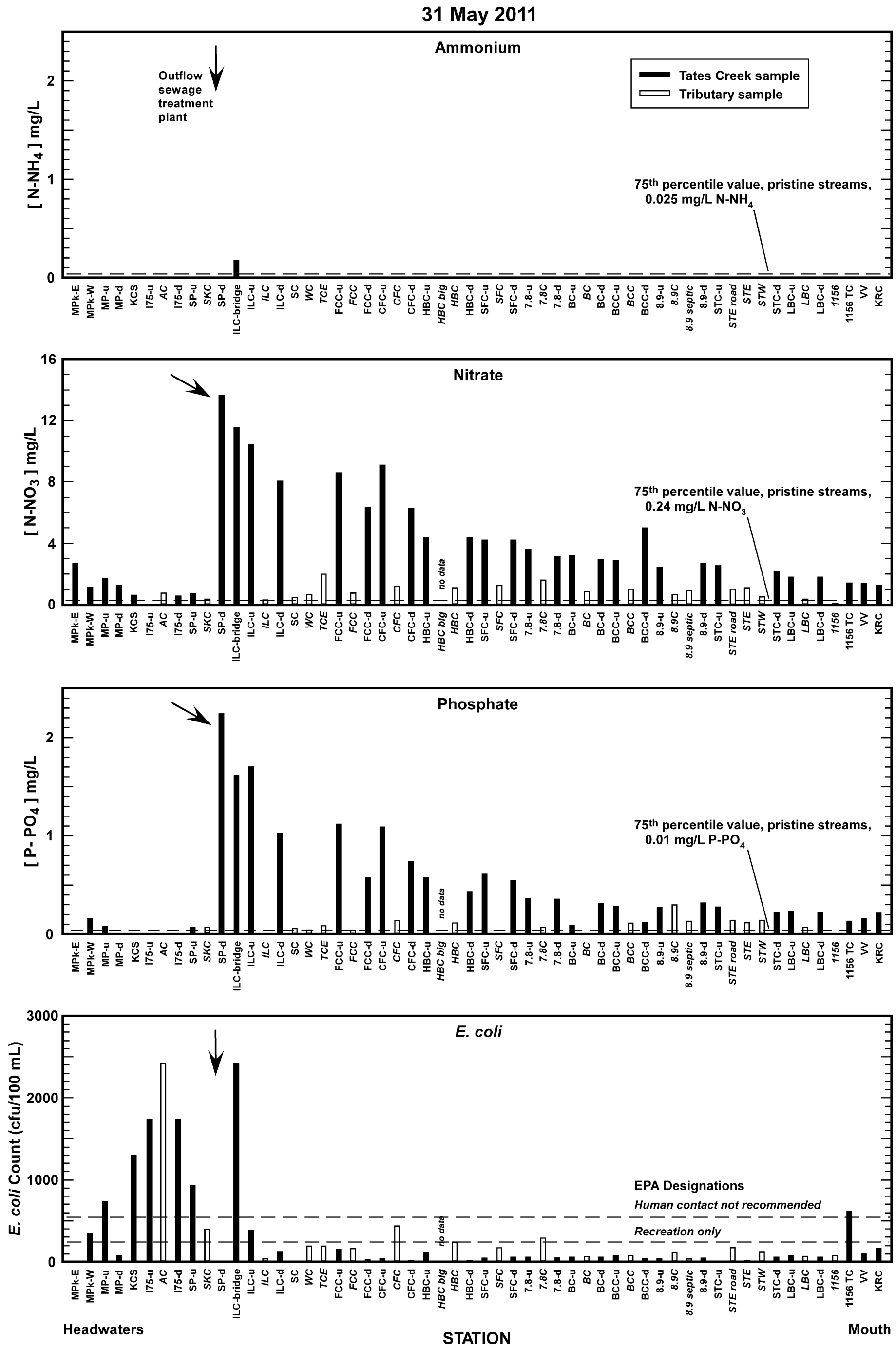

Figure 3. Graphs showing concentration of nitrogen as ammonium ($N\text{-}NH_4$), nitrogen as nitrate ($N\text{-}NO_3$), phosphorus as phosphate ($P\text{-}PO_4$), and *E. coli* counts for samples taken within the Tates Creek watershed for the sampling day of 31 May 2011. Nutrient concentrations are in units of milligrams per L (mg/L; equivalent to parts per million, ppm) in terms of the amount of nitrogen (N) within ammonium ($N\text{-}NH_4$) and nitrate ($N\text{-}NO_3$), and phosphorus (P) within orthophosphate ($P\text{-}PO_4$). Bacterial counts are in colony forming units per 100 milliliters (cfu/100 mL). From left to right, stations progress downstream from the headwaters to the confluence of Tates Creek with the Kentucky River. See

were collected in 100-mL sterile containers, stored on ice in the field, and incubated overnight (Dichter 2011; IDEXX 2006, 2010).

Nutrient Measurements

Ammonium. We used the Berthelot reaction (phenol hypochlorite method) to measure ammonium concentration as described by Solorzano (1969) (see also Gieskes et al. 1991; Eaton et al. 2005b) using colorimetry and a UV-VIS spectrophotometer (Thermo Scientific, Evolution 201). The method collectively measures ammonium (NH_4^+) and ammonia (NH_3) (Eaton et al. 2005b), but ammonium is the predominant species *in situ* because of the pH range (6.61–8.38) of Tates Creek waters. Standard solutions (0.0 to ~5.0 mg/L N-NH_4) were used to create a linear standard curve. For all nutrient analyses, two spiked samples (0.5, 1.0 mg/L) were prepared for quality control; both standards and samples were treated identically. Detection limits are <0.1 mg/L (Eaton et al. 2005b); we report values to the nearest 0.1 mg/L.

Nitrate. Nitrate concentration was measured using cadmium reduction (Eaton et al. 2005c), using Hach NitraVer 5 reagent packets (Hach 2009). Because we acidified our samples (Eaton et al. 2005a), the method measures combined nitrite (NO_2^-) and nitrate (NO_3^-) (Eaton et al. 2005c), but we report the values as N-NO_3, because nitrate is the predominant species in nature. Our range of standard solutions (0.0 to12.6 mg/L N-NO_3) encompassed any concentrations in stream waters and produced linear standard curves. Using this method, the detection limit for nitrate is theoretically 10 µg/L (Eaton et al. 2005c) 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.

Phosphate. Phosphate (PO_4^{3-}) concentration was measured using the ascorbic acid, colorimetric method (Strickland and Parsons 1972; Gieskes et al. 1991; Eaton et al. 2005d). Because we filtered our samples, the method measures only dissolved orthophosphate. The range of standard solutions was 0.0 to 2.5 mg/L P-PO_4. The detection limit is theoretically 10 µg/L (Eaton et al. 2005d) 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.

Fecal Microbe Measurements

We determined the distribution and abundance of *Escherichia coli* and total coliform bacteria using the rapid assay method developed by IDEXX (IDEXX 2006; Dichter 2011). The IDEXX method uses *Colisure-18*® materials and is accepted by the USEPA, 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 (e.g., IDEXX 2010; Eaton et al. 2005e; Edberg et al. 1990). The method gives an approximate count of microbe abundance in terms of colony-forming units per 100 mL (cfu/100 mL), as estimated from statistical methods that determine the most probable number (MPN) of colonies. See the procedure outlined in Borowski et al. (2012) for details. Without sample dilutions, the IDEXX method can only quantify up to 2419.6 cfu/100 mL, so that counts reaching this level may or may not contain appreciably larger numbers of *E. coli*.

The IDEXX method (IDEXX 2006) quantifies both total coliform and *E. coli*. However, workers have found that total coliform bacteria are not necessarily sourced only from feces so that *E. coli* is the best, rapid-assay indicator of fecal contamination of

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Table 1 for station designations and see Figure 1 for station locations. Samples from the trunk stream are shown with filled columns; open columns designate samples from tributaries entering Tates Creek. The station (SP-d) nearest the outflow of the sewage treatment plant is shown with an arrow. Dashed lines show reference concentrations for nutrients and *E. coli*. Dubrovsky et al. 2010 report these typical levels of nutrient concentrations in pristine streams: 0.025 mg/L N-NH_4, 0.24 mg/L N-NO_3, and 0.010 mg/L P-PO_4 (Table 1). The USEPA (1986, 2004) classifies use of natural waters into the following designations dependent on *E. coli* concentration: *bathing acceptable* (≤235 cfu/100 mL), *recreation only* (236–574 cfu/100/mL), and *human contact not recommended* (≥575 cfu/100 mL).

stream waters (e.g., Edberg et al. 2000). Thus, we report and discuss only *E. coli* counts.

RESULTS

Nutrient (ammonium, NH_4^+; nitrate, NO_3^-; and orthophosphate, PO_4^{-3}) concentrations in units of dissolved nitrogen (N) and phosphorus (P), and *Escherichia coli* counts are graphed in Figures 3 through 10 for our sampling days. The sewage treatment plant (STP) on Tates Creek was operating during the first 3 sampling days in 2011, then shut down on 19 Jul 2011 so that the sampling day of 4 Aug 2011 and all sampling days in 2012 occurred when no direct STP discharge entered Tates Creek.

Nutrient Concentration

Ammonium levels in the watershed varied considerably both temporally and spatially. During STP operation, samples taken on 31 May 2011 contained no or very low levels (station ILC-bridge) of ammonium, even when nitrate and/or phosphate concentrations were considerable (Figure 3). Otherwise ammonium was at highest concentration immediately downstream of STP discharge (station SP-d) and then dissipated downstream to sporadic values generally between 0.2 to 0.4 mg/L N-NH_4 on 20 June 2011 (Figure 4) or to values around 1.0 mg/L on 7 Jul 2011 (Figure 5). Once the STP stopped operating, ammonium levels were generally 0 mg/L or very low (4 Aug 2011, 15 May 2012, 12 June 2012; Figures 6 through 8) with the exception occurring on 11 Jul 2012 (Figure 9) when concentrations rose to 0.2 to over 0.5 mg/L N-NH_4 along the length of Tates Creek. Otherwise higher ammonium levels occurred sporadically (12 Jun 2012, Figure 8) or spiked upward in excess of 2 mg/L N-NH_4 at specific stations (AC, TCE; 16 Jul 2012, Figure 10).

Patterns of nitrate concentration were very different during and after STP operations. During STP operation on two of three sampling dates (31 May 2011, 7 Jul 2011), nitrate values within the trunk stream were generally 1 to 2 mg/L N-NO_3 upstream of the STP, increased to high concentration (10–15.5 mg/L N-NO_3) immediately downstream of the STP (stations SP-d; ILC-bridge, ILC-u), and generally showed progressive decrease downstream to 5–6 mg/L N-NO_3, although in some instances nitrate values approached those near the STP (Figures 3, 5). On 20 Jun 2011, N-NO_3 values were between 3 and 7 mg/L along the length of Tates Creek without showing a dominant peak immediately downstream of STP discharge (Figure 4). After STP shut down, nitrate values were typically below 0.5 mg/L on 4 Aug 2011 and on all sampling dates in 2012 (Figures 6–10).

Phosphate values generally mirrored those of nitrate but some variance did occur. Before the STP ceased operation, phosphate concentrations were also low upstream of the STP, hovering around 0.1 mg/L P-PO_4; were highest just downstream of STP discharge, ranging from 1.0 to 2.2 mg/L P-PO_4; and then dissipated downstream to values between 0.2 and 0.5 mg/L P-PO_4 (Figures 3–5). Unlike the case for nitrate on 4 Aug 2011, enriched phosphate values ranging from 0.2 to 0.6 mg/L P-PO_4 occurred along the length of Tates Creek from station SP-u to station LBC) (Figure 6). Then in 2012, phosphate concentration was very low, generally at 0 to 0.2 mg/L P-PO_4 (Figures 7–10).

Fecal Microbe Counts

The concentration of fecal microbes, expressed as the number of colony-forming units per 100 mL (cfu/100 mL), gives information about the amount of fecal material from animals and humans entering stream systems. Here we refer only to *E. coli* counts because they are a reliable indicator of fecal contributions (e.g., Edberg et al. 2000).

E. coli counts varied greatly, especially between the field seasons of 2011 and 2012. In 2011, bacterial counts were generally high with many stations reaching our maximum count level of 2419.6 cfu/100 mL, and many more with counts in excess of 575 cfu/100 mL, exceeding the USEPA standard of *no human contact recommended* (USEPA 1986, 2004) (Figures 3 to 6). For 31 May 2011, *E. coli* counts were highest upstream of the STP (except for station ILC-bridge) and fell to levels generally below 235 cfu/100 mL (*bathing acceptable*, USEPA 1986, 2004) (Figure 3). On 20 Jun and 7 Jul 2011, *E. coli* counts were high above the STP and remained high at many stations downstream and in several

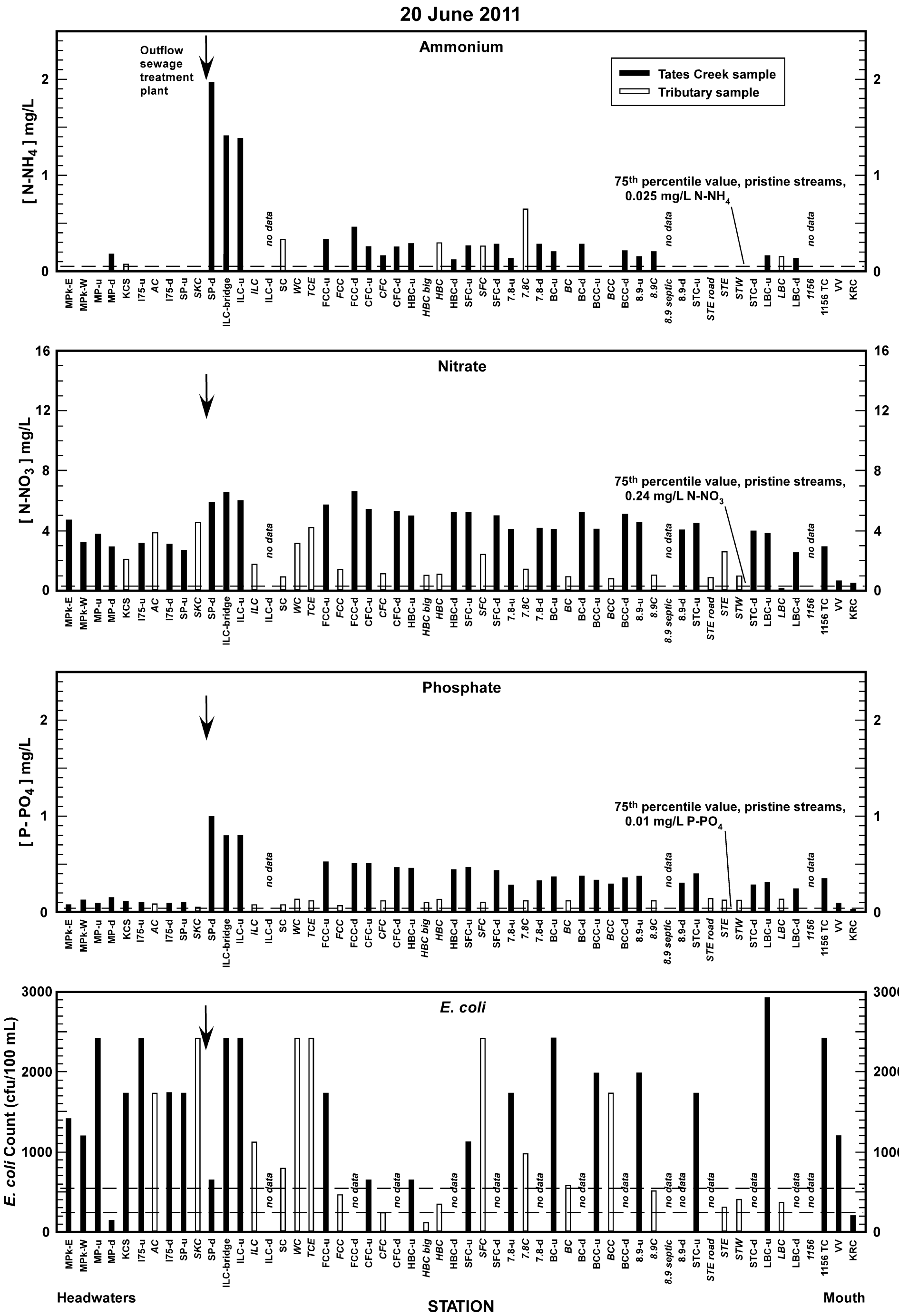

Figure 4. Graphs showing concentration of nitrogen as ammonium (N-NH_4), nitrogen as nitrate (N-NO_3), phosphorus as phosphate (P-PO_4), and *E. coli* counts for samples taken within the Tates Creek watershed for the sampling day of 20 Jun 2011. See caption of Figure 3 for further explanation.

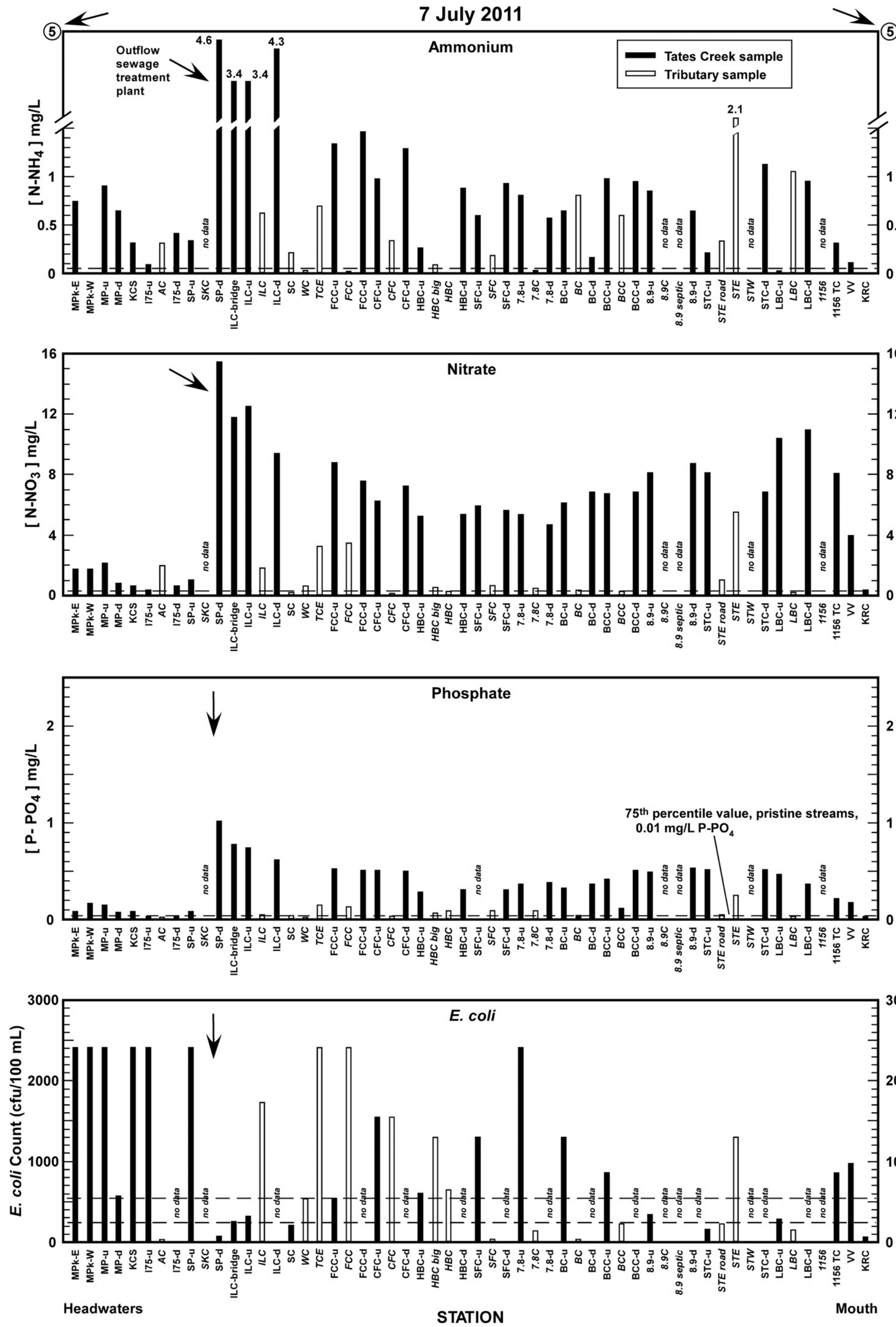

Figure 5. Graphs showing concentration of nitrogen as ammonium (N-NH_4), nitrogen as nitrate (N-NO_3), phosphorus as phosphate (P-PO_4), and *E. coli* counts for samples taken within the Tates Creek watershed for the sampling day of 7 Jul 2011. Note the break in the y-axis scale for ammonium values. See caption of Figure 3 for further explanation.

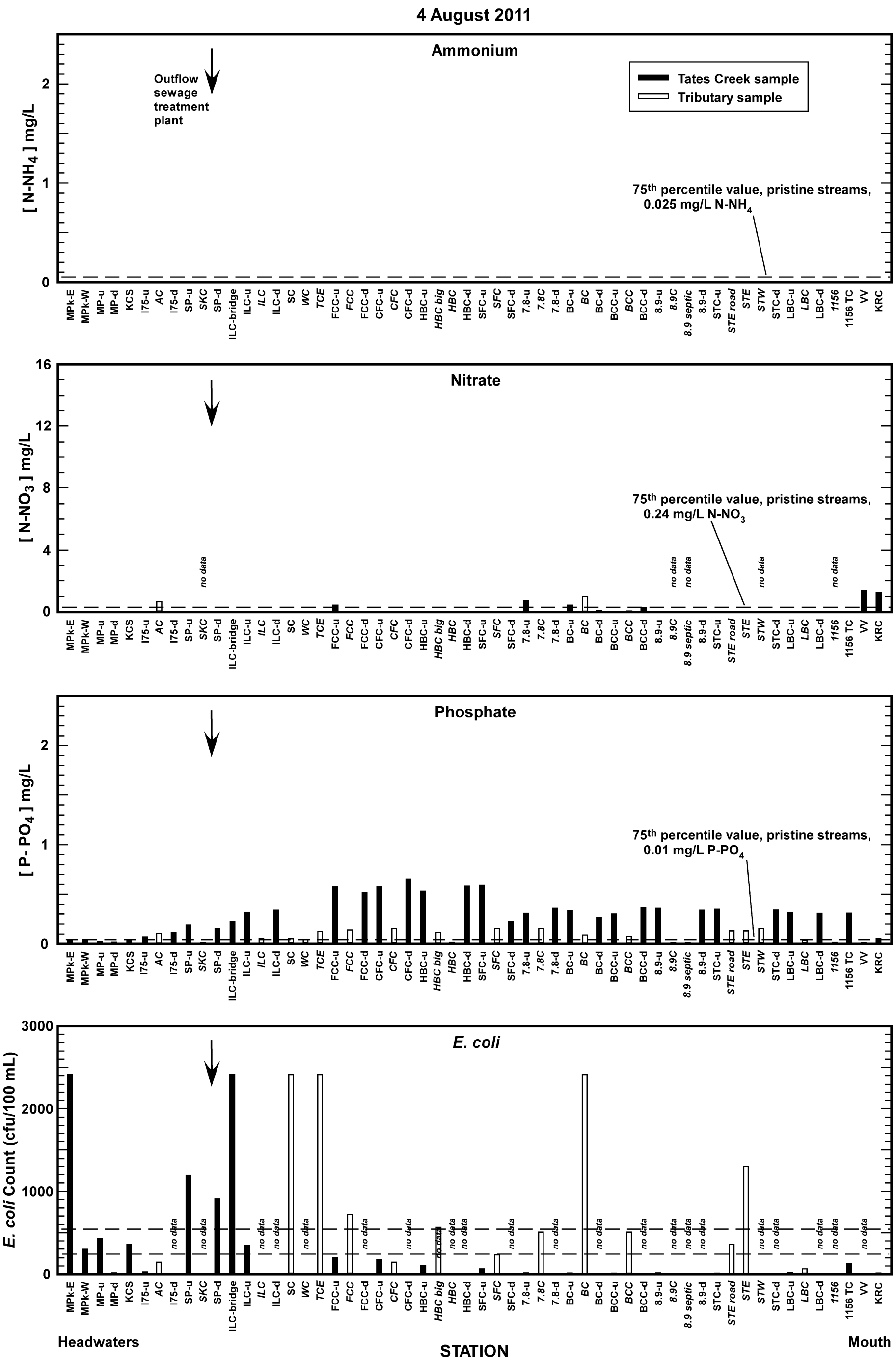

Figure 6. Graphs showing concentration of nitrogen as ammonium ($N\text{-}NH_4$), nitrogen as nitrate ($N\text{-}NO_3$), phosphorus as phosphate ($P\text{-}PO_4$), and *E. coli* counts for samples taken within the Tates Creek watershed for the sampling day of 4 Aug 2011. See caption of Figure 3 for further explanation.

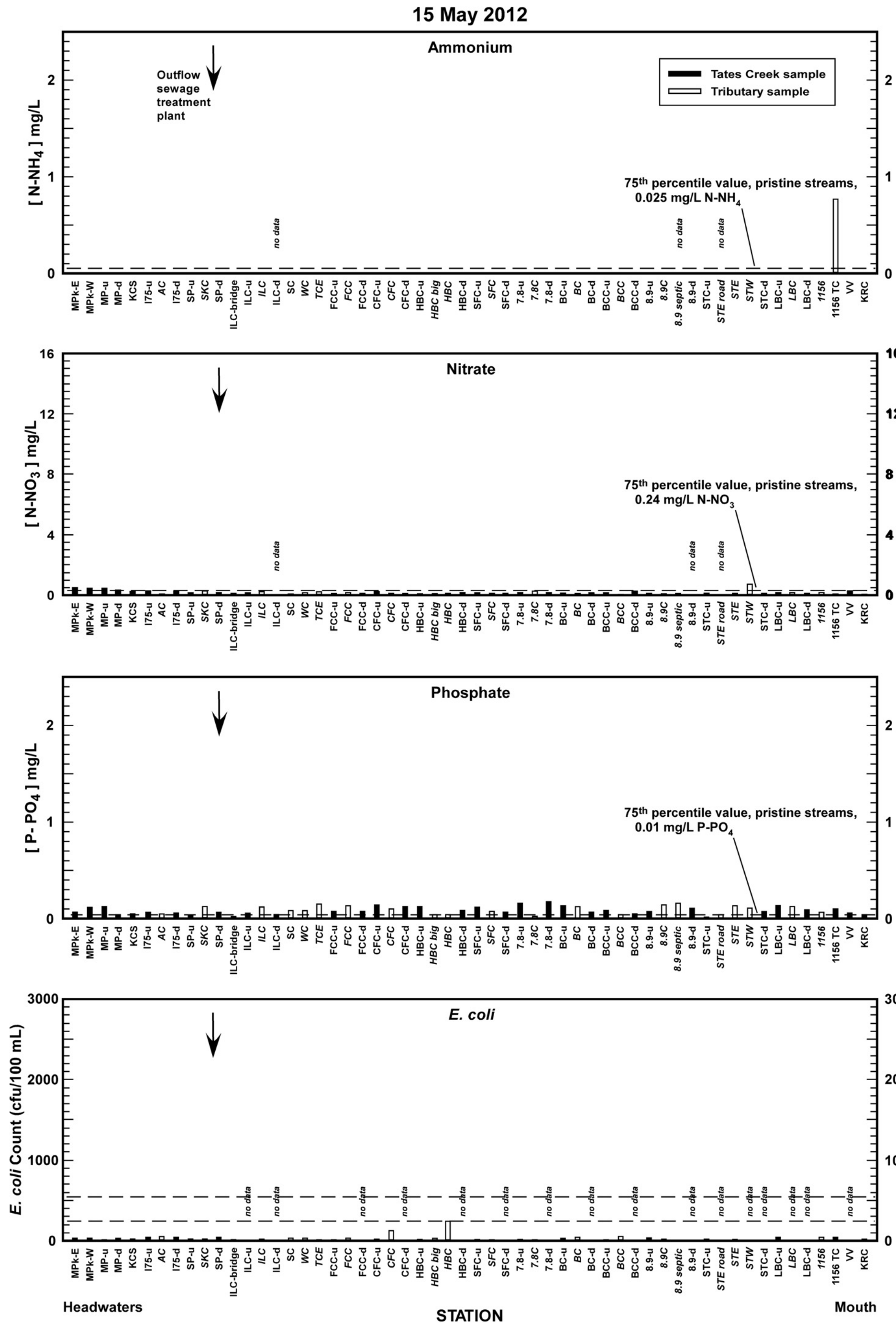

Figure 7. Graphs showing concentration of nitrogen as ammonium (N-NH_4), nitrogen as nitrate (N-NO_3), phosphorus as phosphate (P-PO_4), and *E. coli* counts for samples taken within the Tates Creek watershed for the sampling day of 15 May 2012. See caption of Figure 3 for further explanation.

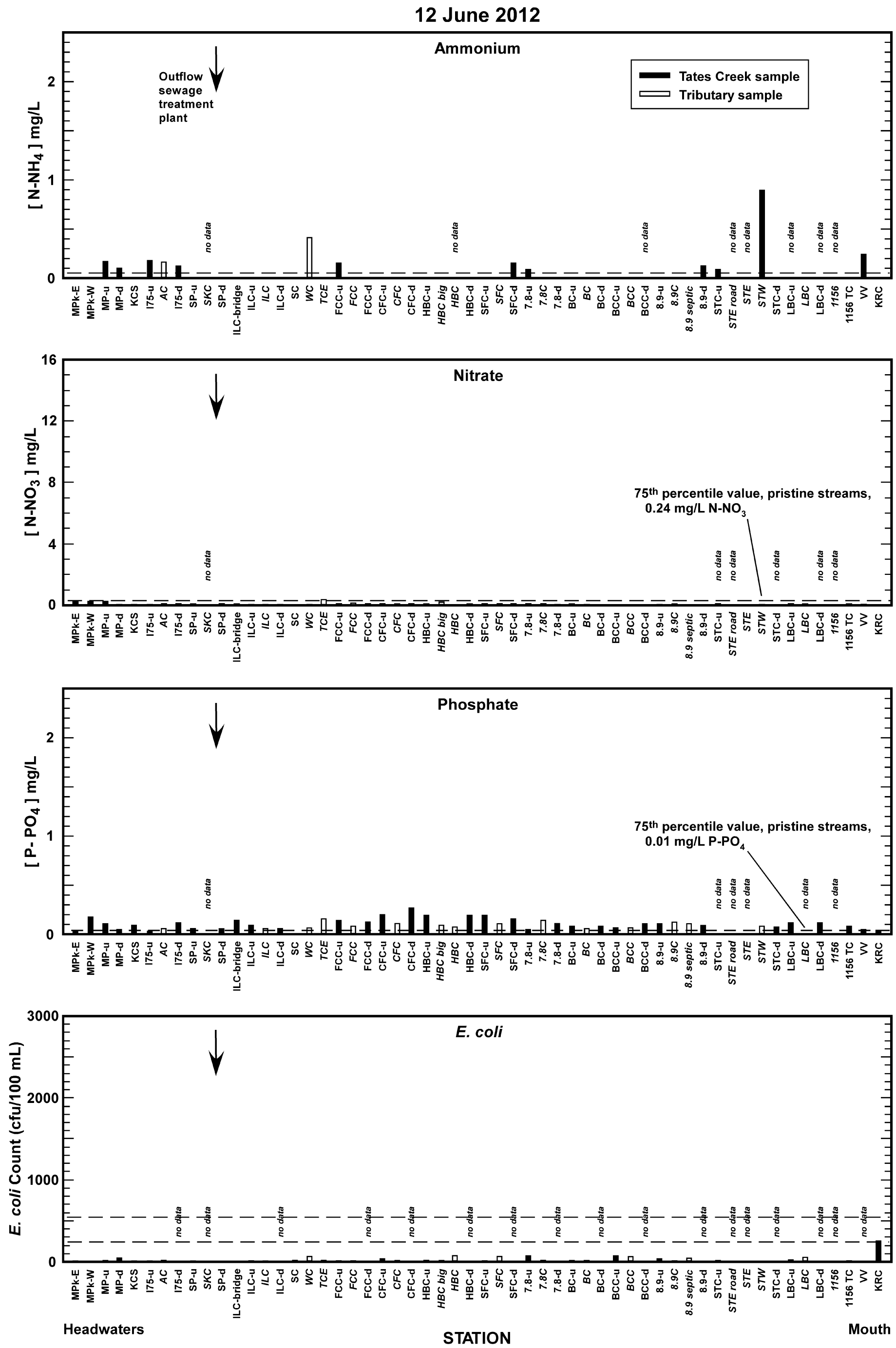

Figure 8. Graphs showing concentration of nitrogen as ammonium (N-NH$_4$), nitrogen as nitrate (N-NO$_3$), phosphorus as phosphate (P-PO$_4$), and *E. coli* counts for samples taken within the Tates Creek watershed for the sampling day of 12 Jun 2012. See caption of Figure 3 for further explanation.

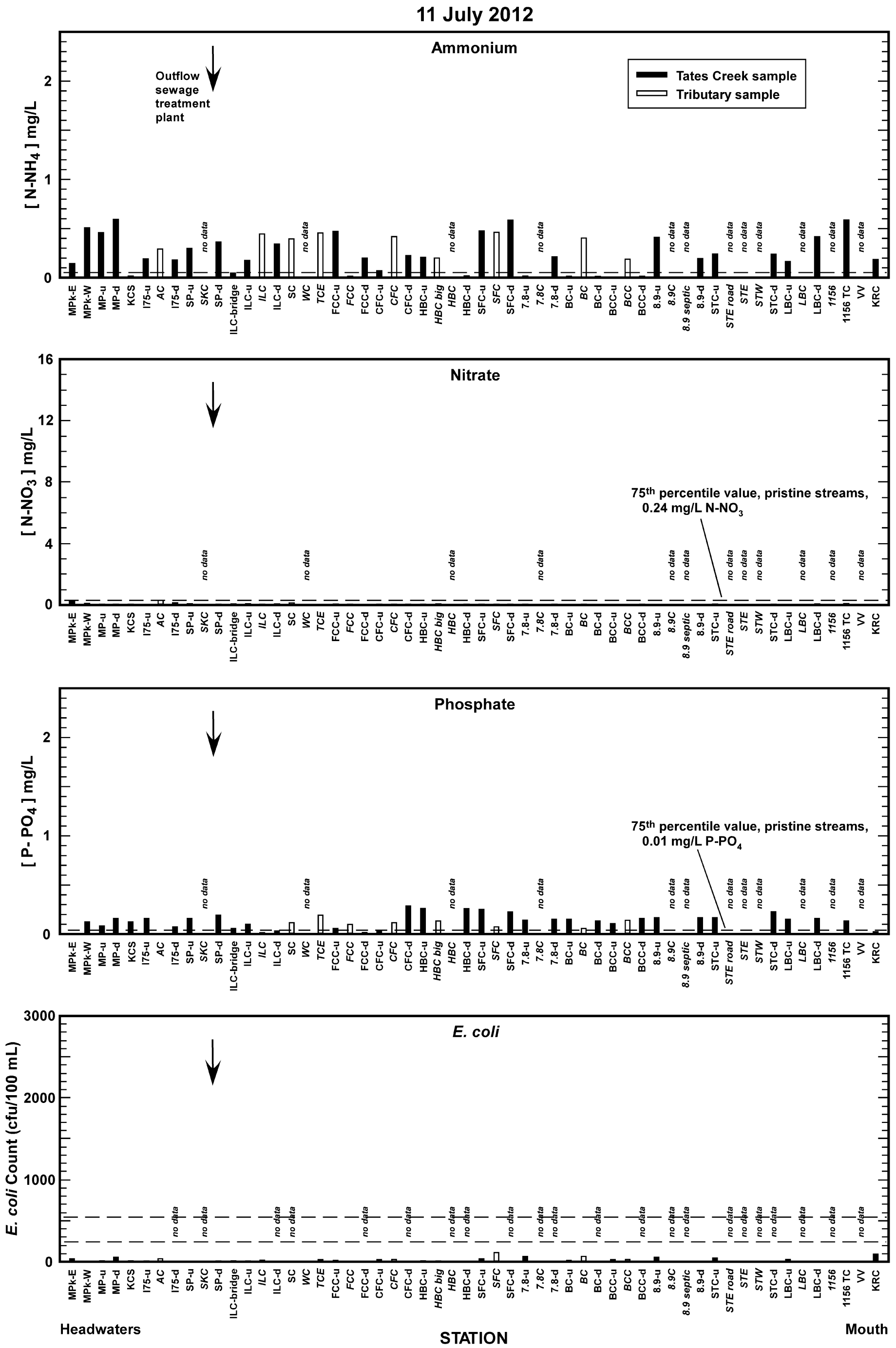

Figure 9. Graphs showing concentration of nitrogen as ammonium ($N\text{-}NH_4$), nitrogen as nitrate ($N\text{-}NO_3$), phosphorus as phosphate ($P\text{-}PO_4$), and *E. coli* counts for samples taken within the Tates Creek watershed for the sampling day of 11 Jul 2012. See caption of Figure 3 for further explanation.

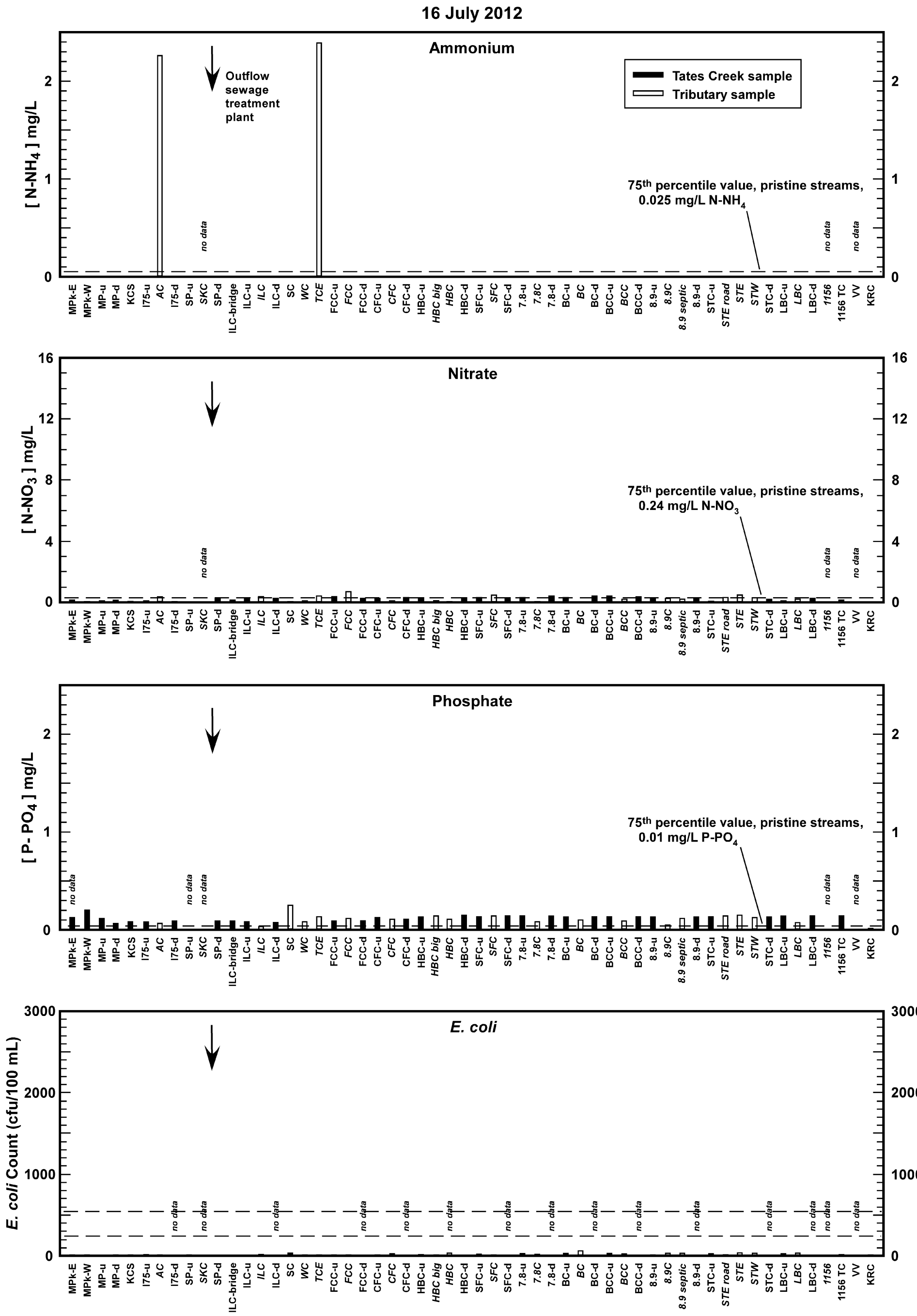

Figure 10. Graphs showing concentration of nitrogen as ammonium (N-NH_4), nitrogen as nitrate (N-NO_3), phosphorus as phosphate (P-PO_4), and *E. coli* counts for samples taken within the Tates Creek watershed for the sampling day of 16 Jul 2012. See caption of Figure 3 for further explanation.

Table 4. Nutrient concentrations immediately upstream (station SP-u) and downstream (station SP-d) of discharge from the sewage treatment plant (STP) situated on Tates Creek (Figure 1). Nutrient concentrations are expressed in milligrams per liter (mg/L; equivalent to parts per million, ppm) of nitrogen (N) in ammonium ($N\text{-}NH_4$) and nitrate ($N\text{-}NO_3$), and of phosphorus (P) as phosphate ($P\text{-}PO_4$). Note dates in 2011 when the STP was operating (31 May, 20 Jun, 7 Jul 2011) versus those dates after shutdown (Aug 2011; all of 2012).

		Nutrient concentration (mg/L)					
		$N\text{-}NH_4$		$N\text{-}NO_3$		$P\text{-}PO_4$	
Sampling date	Plant operation	SP-u	SP-d	SP-u	SP-d	SP-u	SP-d
31 May 2011	on	0.0	0.0	0.8	13.6	0.1	2.2
20 Jun 2011	on	0.0	2.0	2.7	5.9	0.1	1.0
7 Jul 2011	on	0.3	4.6	1.0	10.6	0.1	1.0
4 Aug 2011	off	0.0	0.0	0.0	0.0	0.2	0.2
15 May 2012	off	0.0	0.0	0.2	0.2	0.0	0.1
12 Jun 2012	off	0.0	0.0	0.1	0.1	0.1	0.1
11 Jul 2012	off	0.3	0.4	0.1	0.1	0.1	0.2
16 Jul 2012	off	0.0	0.0	0.0	0.3	–	0.1

tributaries (Figures 4 and 5). On 4 Aug 2011, after shutdown of the STP, *E. coli* levels were high upstream but then reached minimal levels downstream of the STP; several downstream tributaries also reached maximal *E. coli* counts (Figure 6). In 2012, *E. coli* counts were uniformly low on all sample dates (Figures 7 to 10) with every sample (except station KRC, 12 Jun 2012) with values below 235 cfu/100 mL (*bathing acceptable*, USEPA 1986, 2004).

DISCUSSION

Both point and non-point sources for contaminants exist within the Tates Creek watershed. Our data suggest that dissolved nutrients enter the stream from both source types whereas fecal microbes enter from non-point sources only. The operation of the sewage treatment plant acted as a strong point source and greatly impacted dissolved nutrient levels within trunk stream waters.

Nutrient Point Source

During its operation, the sewage treatment plant (STP) on Tates Creek was a point source for dissolved nutrients, mostly for nitrate and phosphate, and in some cases ammonium. We were unable to directly sample the STP effluent, but sampled stream waters approximately 100 m upstream (station SP-u) and downstream (station SP-d) from the plant discharge. For the first three sampling dates in 2011, samples downstream of plant discharge generally showed sizeable enrichments in nutrients relative to upstream sites, especially for nitrate and phosphate (Table 4; Figure 11). STP effluent showed enrichment of ammonium in two of three cases (20 Jun, 7 Jul 2011). Note the very high values at four sites downstream of the STP on 7 Jul 2011 with the highest concentration of 4.6 mg/L $N\text{-}NH_4$ at station SP-d, which was about 15 times higher than upstream values of $N\text{-}NH_4$. Nitrate concentrations were elevated by an average factor of ~10 but reached as high as 17 times the upstream values. Phosphate values were elevated by a factor of 10 to 22 times. Moreover, enrichments in nitrate and phosphate progressively decreased downstream from the STP (Figures 3, 5, and 11), indicative of both dispersal and dilution due to entry of additional water from tributary streams and presumably groundwater. Figures 3 through 5 show that maximum nutrient concentrations at and near station SP-d typically fell from station ILC through stations FCC, CFU, and HBC, and then reached background levels near station SFC (~7.3 km, 4.6 downstream of SP-d). In some cases, nutrient concentrations don't change much within the trunk stream (nitrate and phosphate, 20 Jun 2011, Figure 4) or spike upward in the lower reaches of Tates Creek (nitrate, 7 Jul 2011, Figure 5).

Overall nutrient concentration in Tates Creek waters decreased markedly after plant closure. After shutdown on 19 Jul 2011, there was no significant difference in values of dissolved nutrients at stations upstream and downstream of the STP (Table 4; Figure 11). Average ammonium, nitrate, and phosphate concentrations along the length of Tates Creek

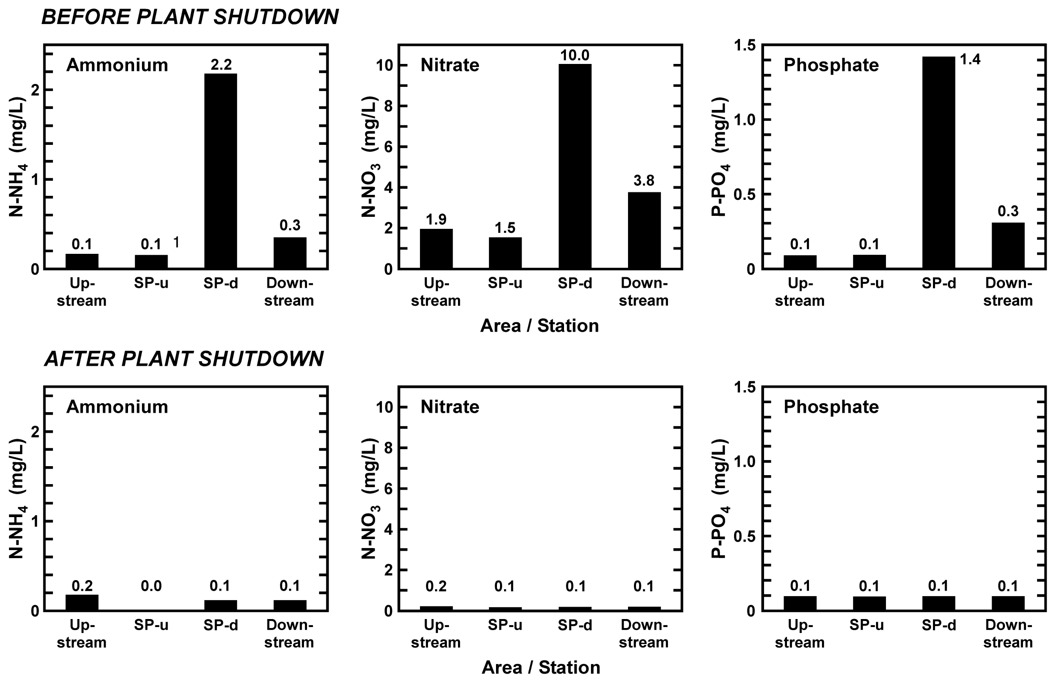

Figure 11. Graphs showing average nutrient concentrations before and after shut down of the sewage treatment plant (STP) situated on Tates Creek. Aggregate data for locations upstream of the STP include the six stations on the trunk stream of Tates Creek, whereas aggregate data for locations downstream of the STP include 14 stations on Tates Creek. Stations SP-u and SP-d are located on Tates Creek immediately upstream and downstream of STP discharge, respectively (Figure1; Table 1).

decreased by factors of 10, 56, and 5, respectively, after STP closure. The mean concentration of ammonium changed from ~0.5 to <0.1 mg/L; that of nitrate from ~5.6 to 0.1 mg/L; and that of phosphate from ~0.5 to 0.1 mg/L (Figures 3 through 10), establishing the STP as a point source for nutrients in the Tates Creek watershed.

Interestingly, dissolved nutrient concentrations upstream of the STP also decreased dramatically after plant closure. The decrease in nutrient levels above the STP after closure suggests that there was an additional upstream source (point or non-point) of nutrients. The upstream decrease in nutrients is coincident with, but not necessarily caused by, STP shut down. We have no explanation for this co-occurrence.

Comparison of Nutrient Levels to National Data

Contamination levels of dissolved nutrients within Tates Creek waters must be put into context by comparison with other watersheds, both contaminated and pristine. Dubrovsky et al. (2010) present nutrient concentration data from a host of impacted and pristine streams within the United States (Table 1, Figure 12). Moreover, they also tie land use to contamination levels by providing concentration statistics for agricultural, urban, mixed use, and undeveloped lands. To correctly compare Tates Creek water quality to that of the nation, we look at different segments of the trunk stream. The upstream segment (station SP-u and above) mostly receives urban drainage whereas the downstream segment (station SP-d and below) drains suburban and agricultural lands and was affected by STP effluent discharge until 19 Jul 2011.

Our data indicate that the STP was a point source for nutrient contaminants and that contamination levels were significantly above values for streams across the United States (Figure 12). Median ammonium levels in Tates Creek were highest downstream of the STP when it was in operation and were higher than the corresponding median, and 75th and

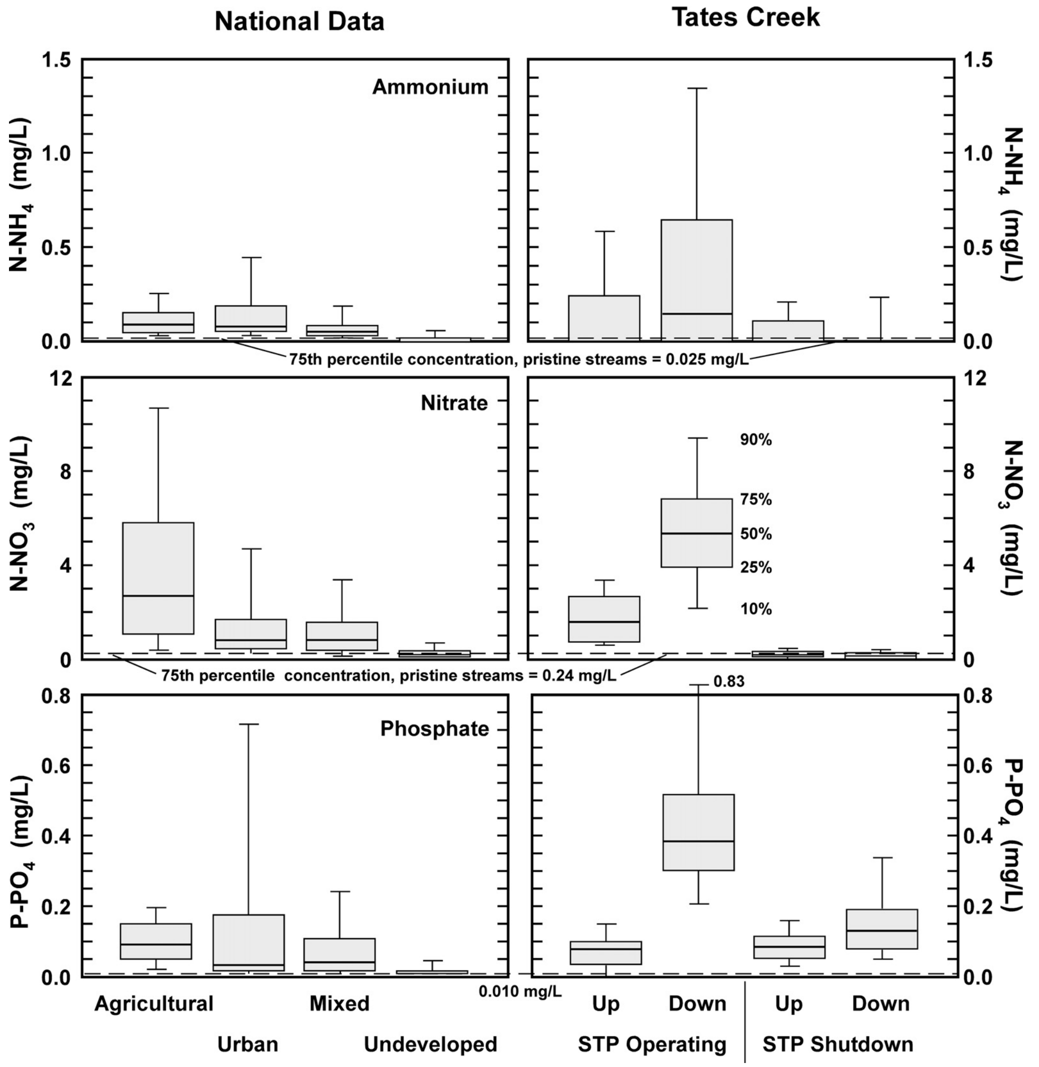

Figure 12. Box and whisker diagrams comparing surface-water nutrient concentrations in the United States (Dubrovsky et al. 2010) versus those in Tates Creek. Concentrations are in milligrams per liter (mg/L, or ppm) of nitrogen in ammonium ($N\text{-}NH_4$) and nitrate ($N\text{-}NO_3$), and of phosphorus in orthophosphate ($P\text{-}PO_4$). Solid horizontal bars represent median values, box limits represent values at the 25th and 75th percentiles, and whisker bars represent values at the 10th and 90th percentiles. Dashed horizontal lines for each dissolved nutrient show the 75th percentile concentration value for a national set of streams with minimal human impacts (from Dubrovsky et al. 2010, noted as "pristine" in Table 1). For the national nutrient concentration data, statistics are given on the basis of the dominant land use with mixed use referring to streams draining a mixture of agricultural and urban areas. The Tates Creek data have been separated into sets representing samples from the trunk stream upstream (up) and downstream (down) of the sewage treatment plant both during its operation (31 May, 20 Jun, 7 Jul 2011) and after it was shut down (4 Aug 2011 and thereafter in 2012).

90th percentile values of impacted streams in the nation by factors of 1.5, 3.5, and 3 respectively, regardless of land use (Figure 12). Once the STP ceased operations, ammonium concentrations in Tates Creek fell significantly below the median values for impacted streams (Figure 12), although sporadic high values did still occur (May through July 2012, Figures 7–10). Nitrate values were also highest downstream of the STP, and were generally higher at the corresponding 10th through 75th percentiles than national data; only the 90th percentile

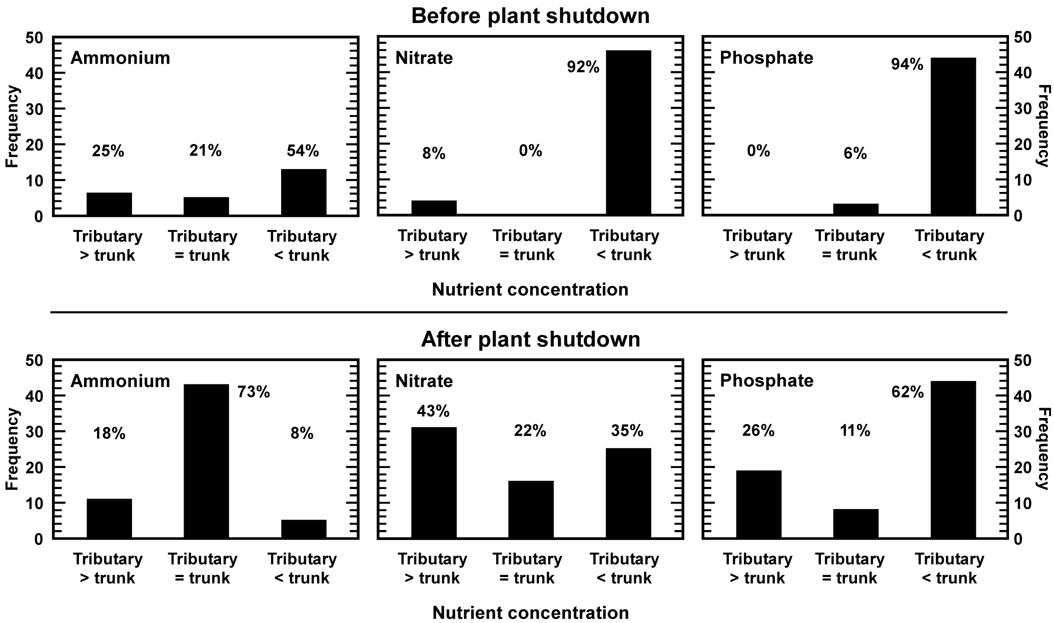

Figure 13. Graphs showing the frequency of cases in which stations along tributary streams display nutrient values greater than those of stations along the trunk stream of Tates Creek, where values of tributary streams equal those of the trunk stream, and cases where tributary stream values are lower than those of the trunk stream before and after the sewage treatment plant ceased operations.

value of national streams (agricultural lands) exceeded the value within Tates Creek (Figure 12). Before plant shutdown, the median value of nitrate contamination in Tates Creek was higher than that nationally by a factor of ~2; after plant shutdown, nitrate concentrations fell precipitously to approach those of undeveloped drainage areas in the nation (Figure 12). Phosphate contamination generally showed much higher contamination levels than those in the US database by factors of 1.1 to 6 during STP operation. Once the STP shut down, phosphate contamination markedly improved, but remained chronically higher than median values for impacted streams nationally.

To summarize, after STP shutdown, median values of ammonium and nitrate are near those of pristine streams nationally (Dubrovsky et al. 2010) in most reaches of Tates Creek, although ammonium values sporadically spike upward into the range of impacted streams. There are no longer any observed injections of nutrients from point sources following closure of the STP, but elevated background levels for phosphate are presumably from non-point-sources within the watershed.

Nutrient Non-Point Sources

Non-point sources occurred along the trunk stream of Tates Creek and along its tributary streams. Because nutrient discharge from the STP markedly increased downstream nutrient concentrations in the trunk stream, we must compare nutrient levels in tributary streams with those upstream of the STP, and then examine values at all stations after STP shutdown to identify additional, possible sources of nutrient input into the stream.

We compare nutrient concentrations in the trunk stream of Tates Creek versus its tributaries both before and after STP shutdown (Figure 13) to see that point-source contributions of elevated nutrient levels from the STP usually eclipse any contributions from tributaries. Before plant shutdown few tributary streams had nutrient concentrations higher than those of the trunk stream, especially with regard to nitrate and phosphate (Figures 3–5). Tributary streams exceeded trunk stream concentration in only 8% and 0% of cases for nitrate and phosphate, respectively, on three sampling dates before STP shut down (Figure 13). After the STP shut down, nutrient

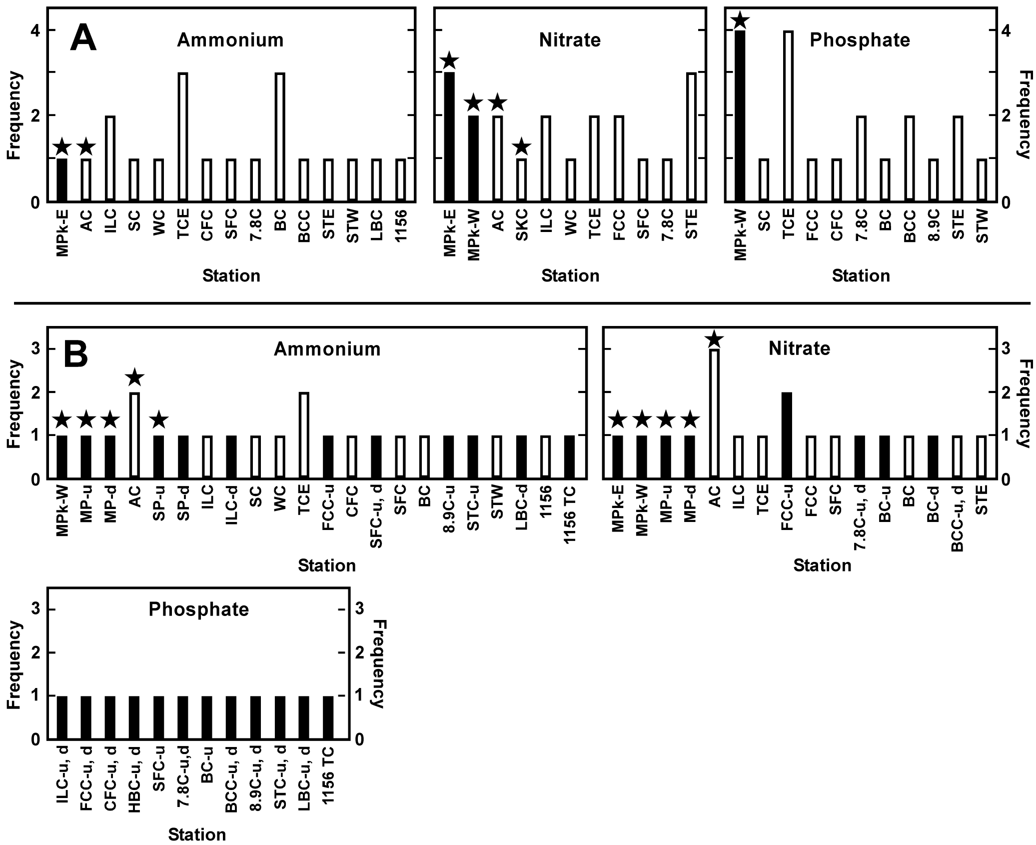

Figure 14. A. Graphs showing the frequency of sampling stations occurring in the top-20 measured concentrations for each dissolved nutrient (ammonium, nitrate, and phosphate) at trunk stations (filled columns) upstream of the sewage treatment plant (STP), and for all tributaries (open columns) on all sampling days. Stations are ordered from upstream to downstream (left to right). Stars refer to stations that sample waters that drain urban Richmond and do not have pastureland or residences on septic systems. B. Graphs showing frequency of all sampling stations occurring in the top-20 to 25 measured concentrations for dissolved nutrients (ammonium, nitrate, and phosphate) after STP shutdown.

concentrations fell sharply in Tates Creek at downstream stations so that more tributary streams had nutrient concentrations that were higher than those of the trunk stream, rising to 43% and 26% of cases for nitrate and phosphate, respectively, on the remaining 5 sample dates (Figure 13). For ammonium, there were more tributary streams with higher concentration before plant shutdown, and the percentage fell from 25% to 18% when the STP ceased operations. The percentage of cases where tributary concentration was lower than trunk streams decreased markedly from 54% to 8%, and many more stations showed equivalent ammonium concentration in both stream types.

Based on the information above, tributaries do play a role in adding nutrients to Tates Creek, especially after STP shut down. We can identify sites that were most influential in their nutrient contributions to perhaps identify nutrient sources. Stations with the highest 20 concentration measurements from tributaries and the upper watershed (upstream of the STP, which approximates the boundary of Richmond and is not influenced by STP discharge) for all sampling days are shown in Figure 14A. Fifteen stations produced the 20 highest ammonium concentrations with three stations (ILC, BC, TCE) producing multiple high cases. Nitrate and phosphate both had 11 stations that produced the top 20 concentrations with 7 and 5 stations, respectively, showing multiple high values. High-value stations are not identical between these two nutrients.

Locations of high nutrient concentration after STP shutdown (stations with the highest 20–25 concentration measurements after 19 Jul 2011), are charted in Figure 14B. Most stations showed high concentrations only once; the three stations with multiple high readings also had high readings before STP closure. Both the trunk stream and its tributaries had high levels of ammonium. Four of 12 cases in the trunk stream occurred at stations within the Richmond footprint, whereas 10 tributary streams had elevated ammonium concentrations. Nitrate values were elevated at 16 stations with a similar pattern of enrichment at stations in and near Richmond (4 of 8 cases in the trunk stream). For phosphate, all stations with elevated levels occur within the trunk stream of Tates Creek; no station was in or near Richmond, unlike the pattern observed for dissolved nitrogen nutrients.

Stations in and near Richmond (generally upstream of the STP) consistently showed high values of ammonium and nitrate both before (Figure 14A) and after (Figure 14B) STP closure. Station MPk-W was perhaps most prominent before STP closure with two instances of high nitrate values and three occurrences of high phosphate values; sister station MPk-E showed three cases of high nitrate values. High nutrient values in waters draining Richmond have two plausible sources: from fertilizer use and/or from contributions from leaky sewage/storm sewer pipes. Fertilizer application occurs mostly in spring whereas any sewer inputs are likely more constant, therefore we infer sewer input as most likely because of persistently high nutrient values observed throughout the sampling season.

Several stations are in the top 20 before STP shutdown for all nutrients: TCE, 7.8C, and STE (Figure 14). Station TCE is down-gradient of a large development served by septic systems (Figure 1). We did not observe a spring pulse of nutrients at station TCE, but did observe elevated *E. coli* counts at this site in 2011 (see *Fecal Microbes* below). Thus, septic systems are likely the most significant source for nutrients at TCE. Note also that TCE contributions are most notable for phosphate. Stations 7.8C and STE sample tributaries that drain woodland and pastureland in the upper reaches of their drainages, and residences on septic systems within the Tates Creek valley. We specifically tested site STE for septic contributions but found no differences in nutrient values up-gradient versus down-gradient of the septic system (see *Effluent from Septic Systems* below). Thus, runoff from pastureland seems the most likely source of nutrient enrichment at these sites.

Stations AC, ILC, and FCC are other notable nutrient contributors (Figure 14A). Station AC samples water emanating from two ponds that collect runoff from a golf course (Figure 1). Golf courses are notorious users of fertilizer, so high levels of dissolved nutrients are no surprise but it is interesting that AC waters do not contribute higher amounts of phosphate. Station ILC captures water from Irvine Lick, which has elevated ammonium and nitrate, but not phosphate levels. The creek is a major tributary to Tates Creek that drains urban Richmond, including a major shopping center and a series of cattle pastures (Figure 1). The tributary is also down-gradient of a major housing development served by septic systems and a chain of single residences are strung along the stream near its entry point into Tates Creek. Any and all of these sources could be delivering dissolved nutrients into Irvine Lick. Station FCC receives waters from Finney Creek which drains pastureland and has several single-family residences within its watershed (Figure 1). It showed high levels of nitrate and phosphate on multiple occasions.

The dominant pattern of nutrient enrichment in Tates Creek is one of non-point source contamination after STP shutdown. The majority of stations do not stand out, but a few stations had consistently high nutrient concentrations. Even stations that had multiple instances of high readings showed variability across the sampling period. This is not surprising because most of the watershed drains rural land, which is affected by season, rainfall, and other environmental factors. Tates Creek should be expected to receive its excess nutrients from pastureland and scattered septic systems serving single residences (and perhaps from fertilizer use at residences and croplands, which are rare) that occur throughout the expanse of the watershed.

Nutrient Loading

Nutrient contamination in the Tates Creek watershed is now dominated by phosphorus in the form of dissolved orthophosphate. Nitrogen loading from ammonium was observed throughout the watershed on two sampling dates before STP shutdown (20 Jun, 7 July 2011) but then only once after STP shutdown (11 Jul 2012). Otherwise observed high concentrations were sporadic. Nitrogen in the form of nitrate dropped precipitously in 2012 to levels of pristine streams in the United States (Dubrovsky et al. 2010). However, although mean phosphate levels fell by a factor of ~2.8 after STP shutdown, relative phosphate values remained much higher than those for nitrate and are on par with values seen nationally for streams impacted by agricultural activity (Dubrovsky et al. 2010). These data suggest but do not prove that nitrogen is now the limiting nutrient within the Tates Creek watershed.

There are several plausible reasons for continued phosphorus loading within the watershed. Phosphate-containing rocks do occur within the limestone bedrock of the watershed and may donate dissolved phosphate to stream waters with weathering and runoff. Also, nitrogen may be lost to the watershed system through volatilization of ammonia (NH_3) and denitrification of nitrate to nitrogen gas (N_2), thereby leaving the system relatively enriched in phosphate.

Fecal Microbe Contamination

Fecal microbe contamination, as recognized by *E. coli* counts, is significant within the Tates Creek watershed. Possible fecal microbe sources include runoff from pastureland supporting livestock, effluent from septic systems in suburban developments and individual residences of rural areas, and effluent from leaky sewage pipes routed to the STP.

Unlike the case for nutrient contamination, the sewage treatment plant was not a point source for fecal microbes because it should have removed fecal microbes, including possible pathogens, from water discharged into Tates Creek. *E. coli* counts were almost always significantly higher upstream versus downstream of STP discharge (Table 5). We infer that higher microbial concentrations observed within upstream waters (e.g., station SP-u) were diluted by STP effluent containing few or no microbes, which acted to decrease *E. coli* counts downstream (station SP-d).

Table 5. Fecal microbe concentration of *Escherichia coli* (*E. coli*) expressed as colony forming units per 100 mL (cfu/100 mL) in samples immediately upstream (station SP-u) and downstream (SP-d) of discharge from the sewage treatment plant (STP) situated on Tates Creek (Figure 1). Note dates in 2011 when the STP was operating (31 May, 20 Jun, 7 Jul 2011) versus those dates after shutdown (Aug 2011; all of 2012).

	E. coli count (cfu/100 mL)	
Date	SP-u	SP-d
31 May 2011	920.8	<1
20 Jun 2011	1732.9	648.8
7 Jul 2011	>2419.6	74.8
4 Aug 2011	1203.3	920.8
15 May 2012	19.1	37.5
12 Jun 2012	4	9.2
11 Jul 2012	0	1
16 Jul 2012	1	0

We have no national data base of fecal microbe contamination for comparison, but USEPA designations are useful to assess the level of fecal microbe contamination within the Tates Creek watershed. Most samples (74%) had *E. coli* counts below 235 cfu/100 mL (Figure 15), indicating their waters as *bathing acceptable* (USEPA 1986, 2004). Only 9% of samples rated a *recreation only* designation, whereas 16% of samples showed microbe counts at *human contact not recommended* levels. Fecal microbe contamination was much worse in 2011 (Figures 3–6) as compared to 2012 (Figures 7–10) with 15% and 44% of samples in 2011 rated as *recreation only* and *human contact not recommended*, respectively. In 2012, only one sample tested in the *recreation only* designation and no sample rated *human contact not recommended*.

High counts of fecal microbes occurred within both the trunk stream and tributaries (Figures 2–10) throughout the Tates Creek watershed, illustrating non-point source contamination. Stations along the trunk stream and along tributaries both show instances of high *E. coli* counts with some differences (Figure 15). More tributary stations than trunk stream stations had counts within the *recreation only* designation (13% vs. 7%), but 21% of trunk stream samples reached *human contact not recommended* levels whereas only

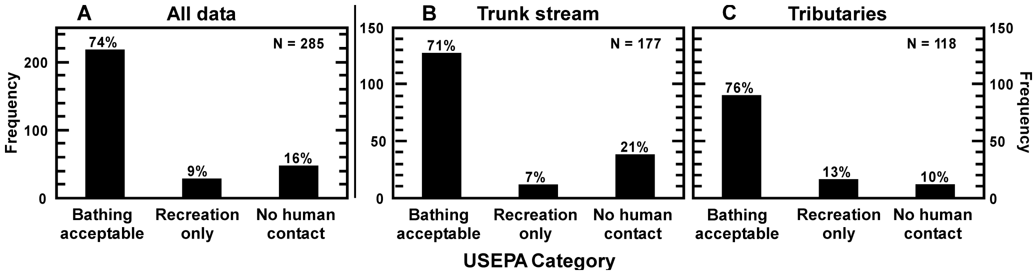

Figure 15. Graphs showing the frequency of samples for all sampling dates categorized into USEPA designations for human use of natural waters based on *E. coli* counts. Graph A shows all data (trunk stream and tributaries), Graph B shows data for the trunk stream only (Tates Creek), and Graph C displays data for tributaries only. USEPA designations are *bathing acceptable* (<235 colony forming units/100 mL), *recreation only* (236–574 cfu/100 mL), and *human contact not recommended* (>575 cfu/100 mL) (USEPA 1986, 2004).

10% of tributary waters showed such high microbe concentrations.

Although there is no point source for fecal microbe contamination, several patterns do emerge. First, higher *E. coli* counts occur with greater frequency at stations where stream waters drain urban areas, when looking at counts >575 cfu/100 mL (*human contact not recommended*) (Figure 16). Station SP-u, immediately upstream of the STP, shows the highest frequency (4) of *E. coli* contamination followed by 4 stations that also drain the Richmond area that each have 3 occurrences (MPk-E, MP-u, KCS, I75-u). Other stations that are within the general footprint of Richmond include MPk-W and AC (2 instances each); and MP-d, I75-d, and SKC (1 case each).

We attribute higher microbe counts in the upper portion of the Tates Creek watershed to leaky sewage distribution pipes. No cattle farming takes place up-gradient of station SP-u, eliminating bovine fecal matter as a potential source. Fecal matter from pets in this residential area is a possible source because these wastes occur outside of the sewage treatment system (USEPA 2001), but given that humans far outnumber pets we infer that human waste is the most likely source of fecal microbes. Because this area is served by city sewer, leaky or broken sewage delivery pipes are most consistent with the data, although we did not directly observe compromised pipes at any particular location.

Other stations with high frequency of high *E. coli* counts likely have different sources of fecal contamination. Two other stations with high microbe counts were 1156TC (3 cases) and VV (2 cases) (Figure 16). These sites occur near the confluence of the Kentucky River where stream waters are ponded during low flow and where the community of Valley View is situated. Residences here are on septic systems that may contribute fecal microbes. Station KRC, which lies closest to the confluence

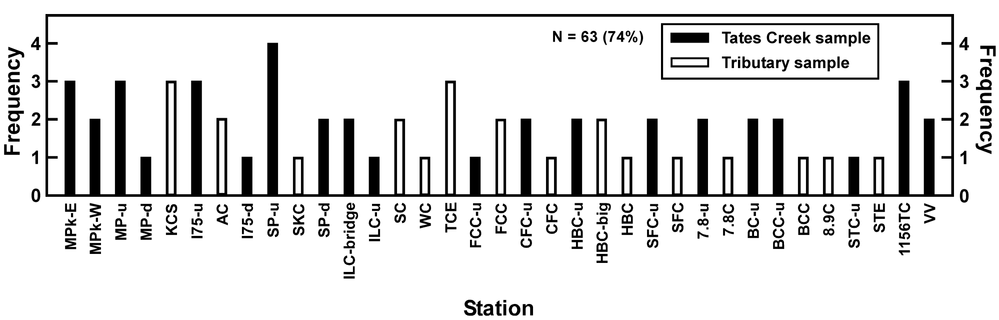

Figure 16. Graph showing stations that reached *E. coli* counts equivalent to an USEPA designation of *human contact not recommended* (>575 cfu/100 mL; USEPA 2004) one or more times for all sampling dates. Data from trunk streams are shown by solid columns, whereas data from tributaries are shown by open columns. Stations are organized from upstream to downstream (left to right). Stations MPk-E through SKC lie within or proximal to the town of Richmond.

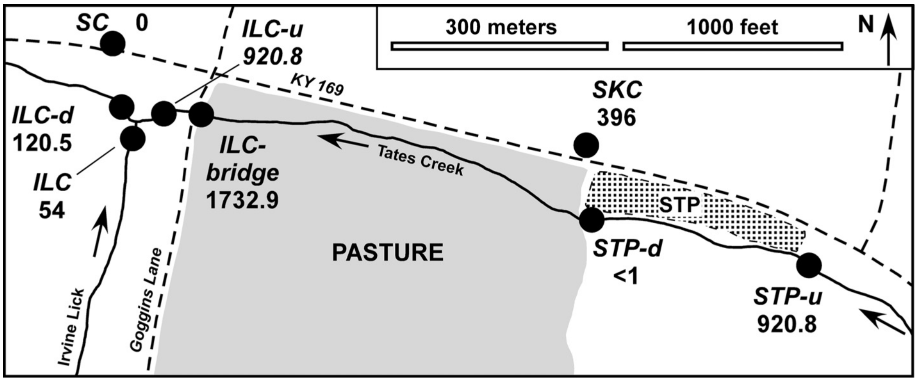

Figure 17. Map showing key features (streams in solid lines, roads in dashed lines) and sample locations (filled circles) with regard to fecal microbe contamination. Station codes are from Table 1; see Figure 1 for location of this map. Numbers refer to *E. coli* counts in colony-forming units per 100 ml (cfu/100 mL) for stations sampled on 31 May 2011. Note the location of the sewage treatment plant (STP) and key pastureland as stippled and shaded polygons, respectively.

and is just downstream of Valley View, does not show high fecal microbe counts, perhaps because the station is strongly influenced by waters of the Kentucky River. Lastly, station AC shows 3 cases of high *E. coli* counts. It lies downstream of 2 ponds that receive drainage from a golf course. The ponds are frequented by waterfowl that may be a source of fecal microbes.

The remaining stations with *E. coli* concentration designated as *human contact not recommended* (Figure 16) occur both within the trunk and tributary streams. There are no easily identifiable sources of fecal microbes at these sites. These stations, as well as others with the designation *recreation only*, likely receive contributions of fecal microbes from cattle feces associated with pastureland (see below).

Fecal Microbes from Cattle

Data from the sampling day of 31 May 2011 definitively shows that fecal material from cattle can elevate *E. coli* counts in Tates Creek. Figure 17 shows the vicinity of the sewage treatment plant and locations of key sampling stations with regard to land use proximal to the stream. *E. coli* counts upstream of the plant are generally high, including that of station SP-u (920.8 cfu/100 mL). The land surrounding the STP is fenced to exclude cattle and our STP sampling sites (SP-u and -d) are generally not impacted by cattle grazing. Although we did not directly measure microbial counts in STP effluent, the value seen within Tates Creek downstream of the discharge is <1 cfu/100 mL. We infer that discharge from the plant, which is about 70% by volume of the stream discharge, is substantially microbe-free and greatly dilutes microbial counts within stream waters measured at the upstream location (SP-u). Once the stream flows through the heavily-used pastureland just downstream of SP-d, *E. coli* counts at the next station (ILC-bridge) increased dramatically to 1732.9 cfu/100 mL. Some fecal microbes enter Tates Creek from station SKC (396 cfu/100 mL), but the flow from this drainage is at least an order of magnitude below that of Tates Creek and of STP discharge thereby producing a negligent to small influence on microbial counts at ILC-bridge. Thus, we attribute the great increase in *E. coli* counts to fecal matter from cattle. Cattle in this heavily-used pasture directly wade into the creek, and runoff from pasture certainly entrains fecal microbes as well, carrying them into stream waters. Many workers have documented similar fecal microbe contributions from grazing cattle (e.g., Crowther et al. 2002; Hubbard et al. 2004) and have shown that increased grazing intensity leads to higher fecal microbe counts in stream waters (e.g., Gary et al. 1983).

Effluent from Septic Systems

Septic systems within the watershed serve housing developments, communities, and single residences and have the capacity to contribute both nutrients and fecal microbes to the watershed. Station TCE is a top contributor of ammonium both before (3 cases, Figure 14) and after (2 cases) STP shutdown, and of nitrate (2 cases before, 1 case after). Station TCE also showed three instances of *E. coli* counts in the *human contact not recommended* category (Figure 16). This station captures waters that drain a large development served by septic systems, thus, we infer that these septic system clusters are a source for nutrient and fecal microbe contamination. Station WC also receives waters from a similar development and showed two cases of high nutrient and one case of high *E. coli* contributions (Figures 14 and 16). It is also possible that septic systems of the community of Valley View contribute fecal material to Tates Creek.

We did not document contributions of nutrients and fecal microbes from single-residence septic systems. At several locations within the watershed, we established ancillary stations both upstream and downstream from single-family residences or churches served by septic systems (stations ILC, SFC, BCC, 8.9C, STE, 1156C; see Table 1 and Figure 1). Measurements from these stations showed little or no difference in nutrient or *E. coli* levels downgradient of septic systems. Sonzogni et al. (1980) report similar results in watershed studies, and many studies suggest that clustered single-residence septic systems are more likely to contaminate surface waters with fecal microbes (e.g., Lipp et al. 2001; Borowski et al. 2012).

Effects of Rainfall

Rainfall frequency, duration, and intensity should affect the concentration of contaminants through the competing effects of flushing contaminants into watershed streams and by diluting stream-borne contaminants during higher discharge. Conversely, lack of rainfall minimizes overland entry of contaminants, but may concentrate them within stream waters because of decreased water volume. To completely assess these scenarios, both the concentration of contaminants and the volume of stream water must be known over time. Unfortunately, we have no stream discharge data, so can only incompletely appraise the effects of rainfall on contaminants within Tates Creek waters.

We chose sampling days to occur throughout the summer of 2011 and 2012, but also to test the effects of rainfall by deliberately sampling on 20 Jun 2011 and 16 Jul 2012, just after significant rainfall events of 2.80 and 2.02 inches, respectively (Figure 2; Table 3). Extended periods of little or no rainfall occurred before our sampling days of 4 Aug 2011 and 12 Jun 2012 (Figure 2; Table 3). 2012 was significantly drier and hotter than 2011.

Nutrient levels on 20 Jun 2011 (Figure 4), after substantial rain, showed little difference as compared to those on 31 May and 7 Jul (Figures 3 and 5). Stations upstream of the STP showed some higher values and discharge of nitrate and phosphate was lower at station SP-d. Nutrient values downstream of the STP were comparable. The exception was ammonium, which is measurable at only one station (ILC-bridge) on 31 May 2011. In 2011, *E. coli* counts are highest on Jun 20 2011 (Figure 4) following the rainfall event on the preceding day (Figure 2).

Nutrient levels were much lower throughout the 2012 sampling season than in 2011. Ammonium levels were higher across the watershed on 11 Jul 2012, a sample period that was not associated with either a prolonged dry spell or significant rainfall. The highest levels of ammonium in 2012 occurred in two tributaries (AC and TCE) during the post-rainfall sampling of 16 Jul 2012 (Figure 10). Nitrate levels were low throughout 2012, but rose to detectable levels in the post-rainfall, 16 Jul 2012 sample, although they generally remained below the level for pristine streams (Figure 10). Phosphate concentrations remained low on sampling dates before (11 Jul) and after (16 Jul) a rainfall event (Figures 9, 10). *E. coli* counts were uniformly low throughout 2012, independent of rainfall.

The effects of rainfall with respect to contaminants are equivocal. In 2011 nutrient values seemed unaffected by rainfall, but *E. coli* counts on 20 Jun 2011 were generally highest, suggesting contributions from runoff. In 2012, nitrate did increase after a rainfall event, but ammonium concentrations returned to low levels. Phosphate values remained steady across all sample dates. *E. coli* counts apparently did not vary with rainfall. With the exception of a clearly increased contribution of fecal microbes on 20 Jun 2011, rainfall events do not seem to significantly affect contaminant levels in Tates Creek.

SUMMARY

Tates Creek is a typical watershed of the Blue Grass region of Kentucky with anthropogenic contaminants, whose entry into stream waters is dependent on land use. Nutrients from both point and non-point sources, and fecal microbes from non-point sources are the major contaminants that impact water quality. A secondary sewage treatment plant (STP) was a dominant source of the dissolved nutrients nitrate and phosphate, and sometimes ammonium, which placed Tates Creek in the group of waterways with highest levels of nutrient loading nationally. After plant closure in July 2012, nutrient loading decreased markedly so that ammonium and nitrate concentrations approached those of undeveloped, pristine streams. Phosphate loading continued as a chronic problem from non-point sources that we infer include leaky sewage delivery pipes in Richmond, runoff from cattle pastureland, and effluent from sizeable developments on septic systems. This suggests but does not indicate that nitrogen may be the limiting nutrient within the disturbed Tates Creek ecosystem.

Fecal microbe contamination, as recognized by *E. coli* counts, also degrades water quality. The sewage treatment plant on Tates Creek did not add microbes to the stream, thus its closure did not affect fecal microbe contamination. Using EPA categories of fecal microbe contamination, 74% of all samples show conditions as *suitable for bathing*, 9% *for recreation only*, and 16% as *human contact not recommended*. Fecal microbe counts were appreciably worse in 2011 with 15% and 44% of cases in the *for recreation only* and *human contact not recommended* categories, respectively. As in the case of nutrients, leaky sewage distribution pipes within Richmond seem to be a source of fecal microbes in watershed headwaters. Farther downstream within rural areas, runoff from cattle pastureland is a non-point source of fecal microbes throughout the watershed. There is also evidence that large developments served by septic systems are a contributory source. We could not demonstrate single septic systems as significant sources of fecal microbes.

A large difference in contaminant levels occurred between 2011 and 2012 independent of STP operation. In 2012, nutrient concentrations upstream of the former STP were significantly lower than they were at those sites in 2011, and *E. coli* counts were also lower throughout the watershed. We attribute this difference to less rainfall occurring in 2012 which limited contaminant entry into stream waters. Sampling after rain events in 2012 demonstrated little or no effect on stream-borne contaminants, perhaps because more infiltration into soil and less runoff into streams occurred because of persistent drought conditions.

Water quality in terms of nutrient concentration has greatly improved within Tates Creek waters since cessation of sewage treatment at its STP. We suspect that fecal microbe contamination remains a problem in the Tates Creek watershed and that lower counts of *E. coli* in 2012 were likely anomalous because of low summer rainfall that year.

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