Eastern Kentucky University
Encompass

EKU Faculty and Staff Scholarship

5-2015

Analyses of nutrient and E. coli contamination within the Otter Creek watershed, Madison County, Kentucky

Nathaniel C. Crockett
Eastern Kentucky University

Walter S. Borowski
Eastern Kentucky University

ANALYSES OF NUTRIENT AND *E. COLI* CONTAMINATION WITHIN THE OTTER CREEK WATERSHED, MADISON COUNTY, KY

By

NATHANIEL C. CROCKETT

Senior Thesis

Bachelor of Science

Eastern Kentucky University

Richmond, KY

Submitted to Walter S. Borowski

Department of Geosciences

Eastern Kentucky University

May, 2015

ABSTRACT

The Otter Creek watershed exhibits dissolved nutrient (ammonium, NH_4^{1+}; nitrate, NO_3^{1-}; phosphate, PO_4^{3-}) and *Escherichia coli* contamination that compromises its water quality. The watershed covers a substantial portion of Madison County (~168 km^2) and consists of Lake Reba, Dreaming Creek, and east and west forks, all of which enter the trunk of Otter Creek before flowing into the Kentucky River. Suspected contaminate sources include leaky sewage system pipes, runoff from pasture land, and septic system leachate. We collected 330 water samples on three occasions during summer 2014 to determine the extent and sources of contamination, in hopes to mitigate contamination and improve water quality. Nutrients were measured using colorimetric methods, whereas *E. coli* counts were determined by using IDEXX materials.

We found highest nutrient concentrations immediately below discharge from the Otter Creek sewage treatment plant (STP), which is a point source for nitrate (3.5 – 4.4 mg/L N-NO_3) and phosphate (0.8 - 1.0 mg/L P-PO_3). Background levels were ~0.4 mg/L N-NO_3 and ~0.09 mg/L P-PO_4. Nitrate and phosphate values progressively decrease at stations downstream from the STP. Ammonium averages ~0.4 mg/L N-NH_4, ranging from 0 to 1.4 mg/L in May, but measurable ammonium occurs only sporadically in June and July. The highest observed value is 1.8 mg/L N-NH_4 (station CC, June) with the majority of stations having no measurable ammonium. 53% of samples exceeded EPA *E.coli* concentration standards for human contact (>575 cfu/100 mL) and are distributed throughout the watershed, displaying classic non-point-source pollution.

Phosphate and fecal microbes are the principal contaminants within the watershed. Compared to a national data set, phosphate contamination is most severe, often exceeding the 90th percentile value. Nitrate is generally below the 25th percentile level. Ammonium concentration is not related to STP discharge but exceeds the 90th percentile value in May; concentrations approach those of pristine streams in June and July. Non-point sources for nitrate, phosphate, and *E. coli* are likely due to leaky sewage pipes within the town of Richmond, and to pasture runoff in rural areas. Ammonium sources are more enigmatic, but seem associated with pasture land and septic systems. Sampling in June and July after rain events saw higher nitrate, phosphate, and *E. coli* concentrations, but lower ammonium levels relative to measurements in May.

INTRODUCTION

The Otter Creek watershed, located within Madison County, Kentucky has shown elevated dissolved nutrient and fecal microbe concentrations within its waters (Smith and Borowski, 2013; Kentucky River Watershed Watch, KRWW). Moderate amounts of nitrogen and phosphorus are essential for a healthy ecosystem, yet when nutrients are oversupplied degradation in the form of eutrophication can occur. Oversupply of nutrients acts as a catalyst for increased growth of algae, which can cause hypoxia (oxygen depletion) upon death and decomposition. During decay, microorganisms decompose organic matter, consuming considerable amounts of dissolved oxygen, to the point that dysoxic or anoxic conditions may result. When these conditions exist within a lake or stream, excess nutrients may concentrate within anoxic zones, which can then be resupplied as a limiting nutrient to primary producers, stimulating renewed eutrophication (Bartram et al., 1999). The primary limiting nutrient for eutrophication is usually phosphate, as it enhances phytoplankton and algae production while conversely decreasing the biodiversity of other photosynthesizers and consumers dependent upon the photosynthetic organisms. Thus, nutrient oversupply can alter the structure of ecosystems and its biotic components, which are often used as indicators of environmental health and water quality in surface waters (Khan and Muhammed, 2014).

Another potential threat to water quality are fecal microbes, such as *Escherichia coli,* which is commonly derived from the lower intestine of many endotherms (Singleton, 1999). *E. coli* is known to be hazardous to humans upon exposure or consumption. Consequences of consumption may include urinary tract infections, gastroenteritis, and neonatal meningitis. The EPA recognizes this threat and has established recommended designations for human contact in relation to *E. coli* concentrations (EPA, 2006). Most *E. coli* strains are harmless, although some serotypes can present the possibility of aforementioned medical hazards (CDC, 2012). But, *E. coli* is an excellent indicator organism, serving as a fecal contamination proxy for the existence of other disease-causing fecal microbes, and for monitoring water quality conditions within certain environments. This is due to the ability of the bacteria's cells to survive outside of the host organism upon excretion for a limited amount of time (Feng and Weagent, 2002).

Sources of contamination

Ammonium, nitrate, and phosphate are vital, naturally-occuring nutrients within waterways, but they are also byproducts of anthropogenic activity and related land uses. Sources of nutrient contamination may include input of detergents, industrial and domestic runoff, fertilizers, or other agriculture-related runoff (Werner, 2002). Nutrients are commonly contained in fertilizers for agricultural use due to their high solubility and biodegradibility. These factors allow for easy integration into surface water and groundwater. Groundwater generally flows more slowly than surface waters so that its dissolved nutrients may stimulate additional eutrophification at later times when surface stream flow is diminished. Nutrients are also generated by wastewater treatment and are usually discharged into surface streams.

Typical fecal microbe sources

Nutrient and fecal microbe contamination may be the result of both point and non-point sources. Point sources being those from discrete, easily identifiable locations, whereas non-point sources are those whose input comes from diffuse sources such as runoff from pastures, septic system leaching, and urban runoff.

Human activities and associated land use control the type and magnitude of stream contaminants, and this is also true of the Otter Creek watershed. Although the watershed does not provide Madison County's municipal water supply, its streams are located near both urban and rural residential areas that support recreational activities, so high water quality is desirable.

Description of Otter Creek watershed

The Otter Creek watershed covers an area of ~168 km^2 (>41,000 acres) within north-central Madison County (Fig. 1). Found within the Bluegrass physiographic region, the Otter Creek watershed is characterized by undulating terrain and moderate to rapid surface runoff and groundwater discharge (KRWW). Otter Creek is composed of four main segments, which collectively drain into the Kentucky River (Fig. 1): (1) A central trunk, whose headwaters begin in urban Richmond and upstream of Lake Reba, continuing downstream to the north; (2) Dreaming Creek, a tributary characterized by mainly urban-residential land-use within the city of Richmond in its upper reaches; (3) the east fork, consisting of pastureland and rural residences; and (4) the west fork, also dominated by fields and pasturelands.

Potential contaminants

Land-use in the watershed is predominantly rural (~85%) (Fig. 1), therefore potential nutrient and fecal microbe contamination most likely stems from pasture runoff, and perhaps from septic system leachate. These contributors are likely culprits in the east and west forks, and mid-to-southern portions of the central trunk. Fertilizer runoff from crops is likely negligible, as only a small portion of the watershed is farmland. In urban areas, contaminant input may be due to sewer system leakage, likely evident in the headwaters of the central trunk and the Dreaming Creek tributary. The Otter Creek wastewater treatment plant, operated by Richmond Utilities, discharges treated water into the central trunk of Otter Creek (Fig. 1). Tertiary wastewater treatment processes attempt to remove nitrogen from water through the process of nitrification, which converts ammonia to nitrate through biological oxidation. This is followed by denitrification processes which then turns nitrate into nitrogen gas, which is released from the water into the atmosphere (EPA, 2004). Phosphate mitigation is achieved through the process of enhanced biological phosphorus removal (EBPR), which enriches polyphosphate-accumulating organisms (PAO) within an anaerobic tank, thus enhancing the removal of phosphorus (Metcalf and Eddy, 2003). Despite these mitigation efforts, subsequent effluent from wastewater facilities may still contain high levels of dissolved nitrogen and phosphorus compounds, leading again to the problem of eutrophication and degradation of water quality.

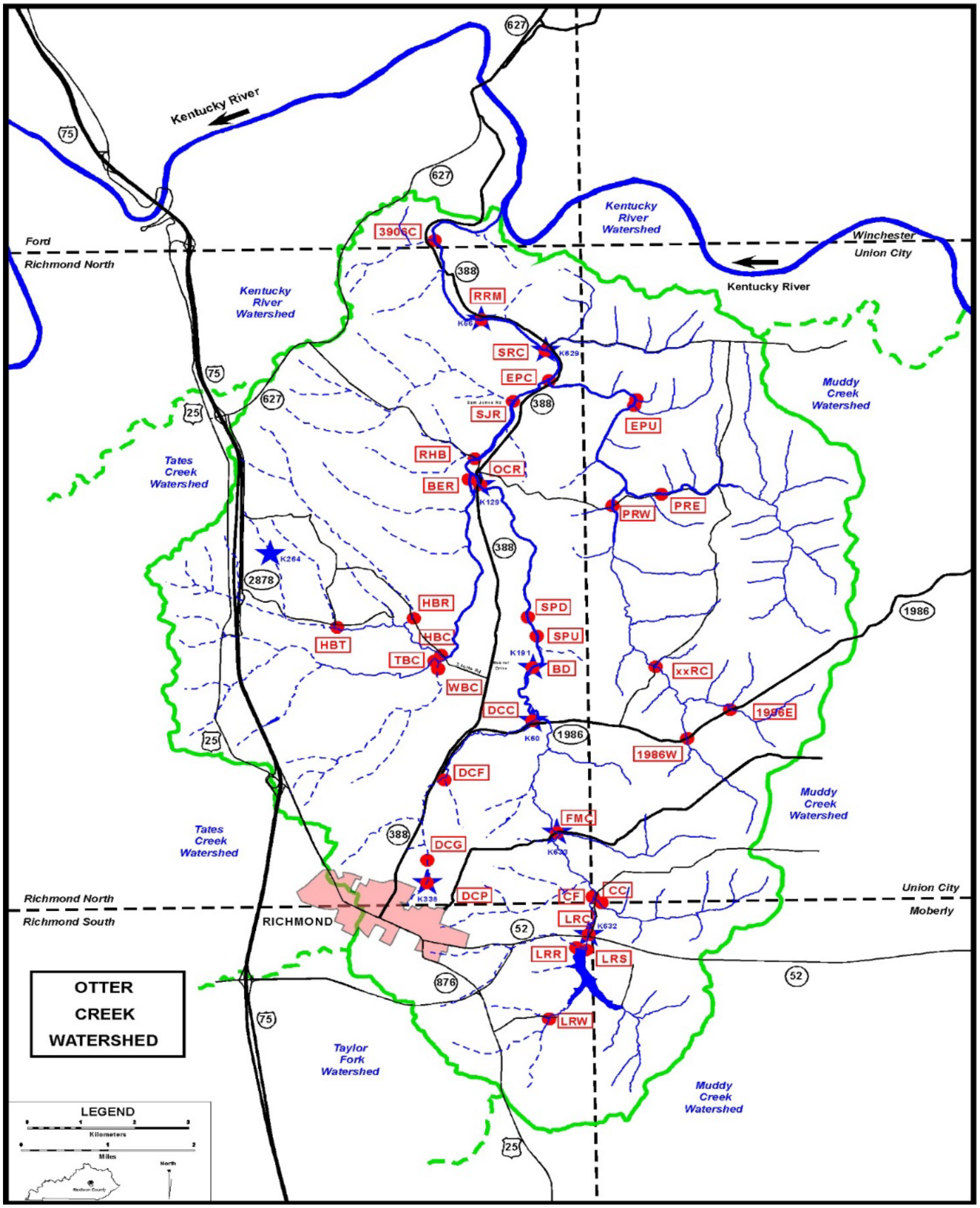

Figure 1. Map of Otter Creek watershed with labeled stations (Smith and Borowski, 2013). Stars show sampling sites of the Kentucky River Watershed Watch (KRWW) whereas closed circles shows sampling stations of this paper. Station codes are keyed to Table 1. United States Geological Survey (USGS) topographic maps (Richmond North, Richmond South, Union City, Moberly) served as bases.

Study objectives

Our research objectives are to: (1) assess levels of nutrient and *E. coli* contamination; (2) locate both point and non-point sources of pollution; and (3) ultimately identify steps to mitigate contamination in order to improve water quality within the watershed. We hypothesize that non-point source pollution is the primary source for nutrient and *E. coli* contamination, mainly in the form of pasture runoff and septic system leachate.

METHODS

Nutrient and fecal microbe concentration in stream waters can vary greatly within watershed locations varying upon land use, land cover, and contaminant sources. Sampling stations were selected to provide representative sampling of the entire watershed and its varied land use; accessibility was also a factor in selection. Water samples were taken at 40 stations (Table 1) that are distributed throughout the Otter Creek watershed and covered all four major stream segments, and some of their tributaries. At major stream confluences, we took samples upstream, downstream, and at the tributary within riffle areas. Sampling in riffles ensures that the waters are well mixed. Other sampling sites targeted possible contaminant sources such as pastureland and residential areas served by septic systems. There are also several stations that document contaminant levels where land use occurs with minimal human impact.

Field sampling

Field samples were taken three times May through July (Table 2). Water samples for nutrient measurements were collected in syringes, then filtered through 0.45 µm nylon filters and stored in 26-mL borosilicate scintillation vials which were pre-acidified at <2 pH according to Eaton et al. (2005). Filtration eliminates larger biota and detritus from the sample. Acidification keeps dissolved nutrients in solution and halts any microbial activity, thereby preserving the sample. Samples were placed on ice in a cooler in the field, then upon returning to the lab they were refrigerated, and measured one or two days after collection. Fecal microbe samples were collected in sterile 100-mL vials and then sealed, placed on ice, and processed on the day of collection.

Table 1. Table of sample locations with site description, runoff contribution, likely contaminants and number of samples per sampling day.

Fork	Sample Code	Sampling Site	Runoff Type	Likely Contaminants	Number of Samples
West	WFU	West Branch upstream of Three Forks road	Fields	Nutrients, microbes	1
	TBC-u	Tribble Branch, upstream West Fork confluence	Fields	Nutrients, microbes	1
	TRC	Tribble Branch, downstream West Fork confluence	Fields	Nutrients, microbes	1
	HBT	Hicks Branch tributary, upstream	Fields	Nutrients, microbes	3
	HBR	Hicks Branch road	Fields	Nutrients, microbes	1
	HBC	Hicks Branch confluence	Fields	Nutrients, microbes	1
	WFC	West Fork confluence, downstream Three Fork Rd	Fields	Nutrients, microbes	1
	BER	Bill Eades Road - upstream confluence	Fields, septic systems	Nutrients, microbes	1
East	1986E	Highway 1986, east	Fields, septic systems	Nutrients, microbes	1
	1986W	Highway 1986, west	Fields, septic systems	Nutrients, microbes	1
	BRC	Brookstown Road, confluence 3 streams	Fields, septic systems	Nutrients, microbes	3
	PRW	Peacock Road, west	Fields, septic systems	Nutrients, microbes	1
	PRE	Peacock Road, east	Fields, septic systems	Nutrients, microbes	1
	EPU	East Prong Road crosses stream	Fields, septic systems	Nutrients, microbes	2
	EPU-trib	Tributary near East Prong Road crossing	Fields, septic systems	Nutrients, microbes	1
	EPC	East Prong confluence	Fields, septic systems	Nutrients, microbes	3
Central	DCP*	Dreaming Creek, former ST plant (K338)	Urban, residential	Nutrients, microbes	1
	DCG	Dreaming Creek - downstream golf course	Recreational	Nutrients	1
	DCF	Dreaming Creek ford - intersection Hwy 388/1986	Urban, residential	Nutrients, microbes	1
	DCC*	Dreaming Creek confluence (K60)	Urban, residential	Nutrients, microbes	3
Central	LRW	West Lake Reba input	Urban, residential	Nutrients, microbes	1
	LRS	Lake Reba Spillway	Recreational	Nutrients, microbes	1
	LRR	Lake Reba spillway - road	Urban, residential	Nutrients, microbes	1
	LRC*	Downstream Lake Reba, Concord (K632)	Residential	Nutrients, microbes	1
	CC	Concord stream	Residential, Hwy	Nutrients, microbes	1
	CF	Concord ford	Residential, Hwy	Nutrients, microbes	1
	FMH*	Four Mile Road/Hunter Road confluence (K633)	Fields	Nutrients, microbes	1
	BD*	Beaver Drive (K191)	Fields, septic systems	Nutrients, microbes	1
	BD-trib	Drainage paralleling Beaver drive	Septic systems	Nutrients, microbes	1
	STP-u	Sewage Treatment plant - upstream	Fields, septic systems	Nutrients, microbes	1
	STP-dis	Sewage Treatment plant - effluent discharge	Plant operations	Nutrients, microbes	1
	STP-d	Sewage Treatment plant - downstream	Plant operations	Nutrients, microbes	1
	OCR*	Otter Creek Road, 388 bridge (K129)	Fields, septic systems	Nutrients, microbes	1
	BER	Bill Eades Road - upstream confluence	Fields, septic systems	Nutrients, microbes	1
	RHB	Ky 3377 crosses Otter Creek	Fields, septic systems	Nutrients, microbes	1
	RHB-trib	Tributary paralleling Lost Fork Road	Fields, septic systems	Nutrients, microbes	1
	SJR	Sam Jones Road, Otter Creek	Fields, septic systems	Nutrients, microbes	1
	SJR-trib	Tributary at bridge, Sam Jones Road	Fields, septic systems	Nutrients, microbes	1
	SRC*	Stony Run confluence (K29)	Fields, septic systems	Nutrients, microbes	3
	RRM*	Railroad crossing on Hwy 338 (K66)	Fields, septic systems	Nutrients, microbes	1
	3906 bridg	Road crossing at 3906	Upland, septic system	Nutrients, microbes	1
	3906C	3906 Redhouse Rd confluence	Upland, septic system	Nutrients, microbes	3
				TOTAL	55
		* Sampling site, Kentucky River Watershed Watch			

Table 2. Sampling dates with rainfall history and respective stream conditions.

Sampling Date	Rainfall History / Stream Conditions
27 May	Last rain 22 May; most streams flowing with exception of smallest courses
30 June	Soaking rain on 28 June; streams flowing and turbid
21 July	Rain event on 18, 19 July; most streams flowing with exception of smallest courses,
	main channels not filled with flow in east and west branches

Nutrient measurements

We measure three nutrients: (1) nitrate (NO_3^{-1}); (2) phosphate (PO_4^{-3}); and ammonium (NH_4^{+1}). Nutrient concentrations were all measured using established, colorimetric methods and an ultraviolet-visible spectrophotometer. Standards for each nutrient were prepared using conventional stoichiometric procedures and encompass the expected range of concentrations from the Otter Creek watershed.

Nitrate was measured using the cadmium reduction method and NitraVer5 assay packets (Hach, 1986). 20 mL of water sample is mixed with the prepared NitraVer5 packets, which is then ready for analysis after 2 minutes. Degree of nutrient concentration within a sample is made visible by increasing saturation of reddish hues within the vial. The range of standards was 0 mg/L to 56.2 mg/L N-NO_3. Standard curves had a mean r^2 value of 0.9925. Samples were ran through the UV-VIS spectrophotometer at a wavelength of 543 µm. The detection limit for nitrate is ~0.01 mg/L (Eaton et al.,1995), but we report concentrations to the nearest 0.1 mg/L N-NO_3 .

For ammonium measurements we followed the method of Gieskes et al. (1991), modified from Solarzano (1969), utilizing the sodium hypochlorite method. Reagents are placed within individual flasks and brought to full volume with nanopure H_2O and are used on day of mixing. The process requires 0.5 mL of phenol-alcohol solution, 0.5 mL sodium nitroprusside, 1 mL of sample, and 2 mL oxidizing solution (chlorox bleach) in a 100-mL alkaline solution. Once mixed, reagents were added to water samples and then developed for 3 hours. The range of standards was from 0 mg/L to 16.3 mg/L N-NH_4, with a mean r^2 value of 0.9988. Samples were measured at a wavelength of 640 μm. The detection limit for ammonium is <0.1 mg/L, but we report concentrations to the nearest 0.1 mg/L N-NH_4.

Phosphate measurements employed the ascorbic acid method as described by Gieskes et al. (1991), modified from Strickland and Parsons (1968). A mixed reagent is prepared with solutions of ammonium molybdate, sulfuric acid, ascorbic acid, and potassium antimonyl-tartrate. Standards ranged from 0 – 1.7 mg/L P-PO_4, with a mean r^2 value of 0.9785. For measurements, 1 mL of sample was combined with 1 mL nanopure water and 2 mL of mixed reagent. Vials were then placed in the dark for one hour until ready for measurement at a wavelength of 885 μm. The detection limit for phosphate is ~0.01 mg/L, but we report concentrations to the nearest 0.1 mg/L P-PO_4.

E. coli measurements

IDEXX rapid assay methods were utilized for measuring microbe concentrations as colony forming units per 100 mL (cfu/100-mL) of sample (IDEXX, 2006). Without dilutions, this method quantifies up to 2,419 (cfu/100 mL). Upon returning to the laboratory after field work, fecal microbe samples were spiked with *Colilert-18* media, poured into Quanti-trays, sealed, and then placed in an incubator at 35° C for 18 hours

(IDEXX, 2006). Quanti-trays contain 49 large cells and 48 smaller cells, with a total volume of 100 mL. Positive counts in each cell statistically yield the most probable number of microbe colonies (IDEXX, 2006). This procedure utilizes a nutrient indicator that produces a yellow color when metabolized by total fecal coliforms; *E. coli* are indicated by blue fluorescence under ultraviolet light. Although the method also enumerates total coliform bacteria, *E. coli* counts are more reliable indicators of fecal contamination (Edberg et al., 2007), therefore only *E. coli* counts will be addressed within this paper.

RESULTS

Results indicate that nutrient and *E. coli* concentrations remain fairly consistent for each stream segment across each sampling date, with the exception of varying ammonium concentrations. Typically, the two biggest factors we saw affecting concentrations were the sewage treatment plant (STP) and rain events.

Nutrients

Nitrate and phosphate behave similarly (Fig. 2, 3, 4; Tables A, B, C, Appendix). The east and west forks displayed low amounts for both nutrients: ~0.09 mg/L N-NO_3 and near-zero mg/L P-PO_4 were found in the east fork, whereas average concentrations for the west fork were ~0.21 mg/L N-NO_3 and ~0.04 mg/L P-PO_4. In the main trunk of Otter Creek, values are lower upstream of the sewage treatment plant (STP), but then spike to maximum levels at its discharge and progressively decrease downstream of the plant. Mean discharge concentrations from the STP were 3.9 mg/L N-NO_3 and 0.9 mg/L P-PO_4. Average concentrations at upstream locations were ~0.23 mg/L N-NO_3 and 0.04 mg/L P-PO_4, so that STP discharge is 16.9 and 22.5 times higher than average upstream concentrations. Concentrations downstream of the wastewater plant are elevated to 0.9 mg/L N-NO_3 and 0.26 mg/L P-PO_4. High values of both nitrate and phosphate were also found within Dreaming Creek, which are considerably higher than average levels; for each sampling date we saw average concentrations of ~1.4 mg/L N-NO_3 and ~0.11 mg/L P-PO_4.

Nitrate concentrations are collectively higher on 30 June relative to the other sampling dates. Values generally are 0.5 to 1.0 mg/L N-NO_3 with some concentrations

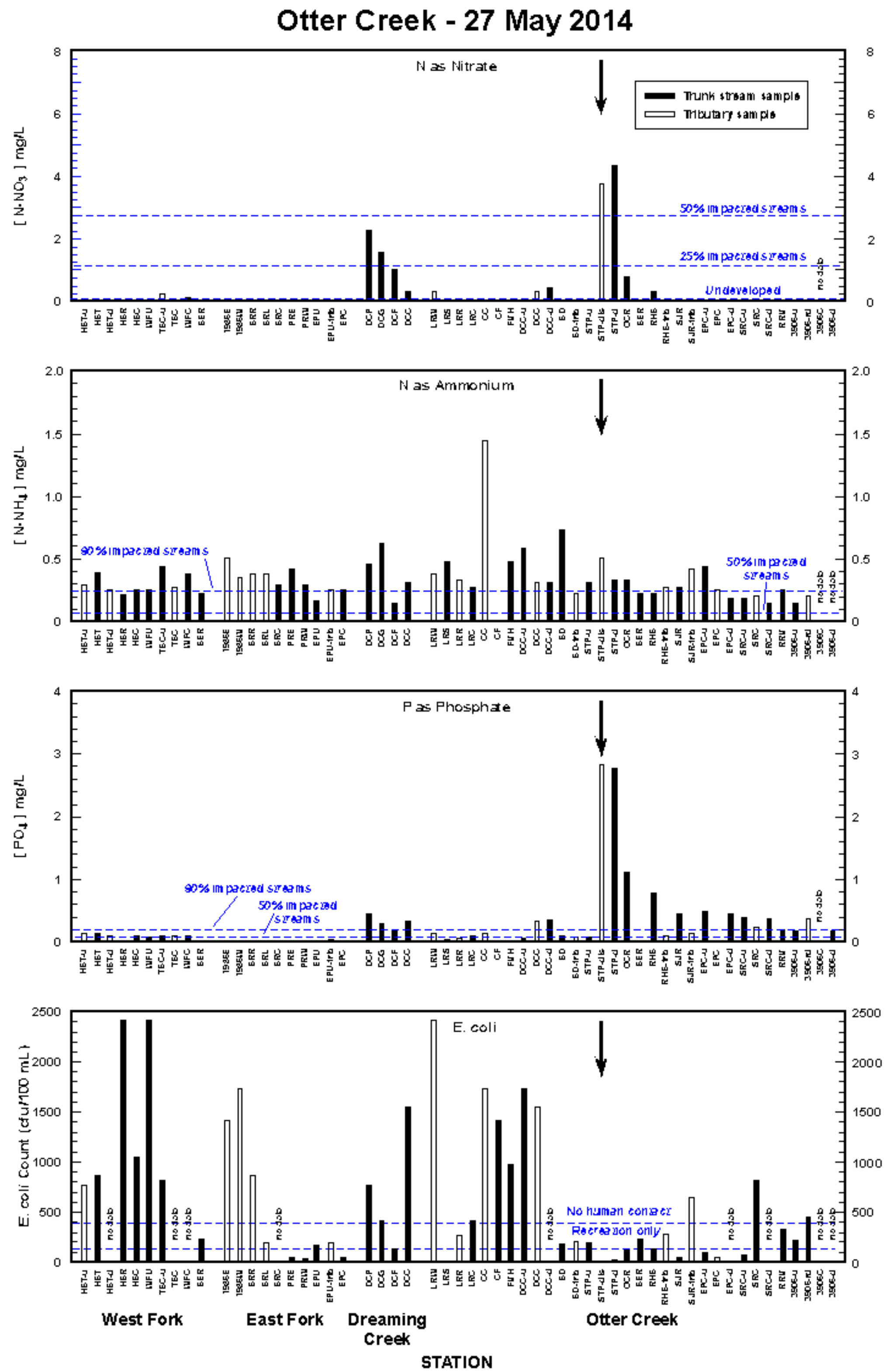

Figure 2. May sampling data for nutrients and *E. coli*. Stations are subdivided according to respective stream component, labeled on x-axis. Concentrations in mg/L lie on y-axis. Arrow indicates Otter Creek wastewater treatment plant discharge.

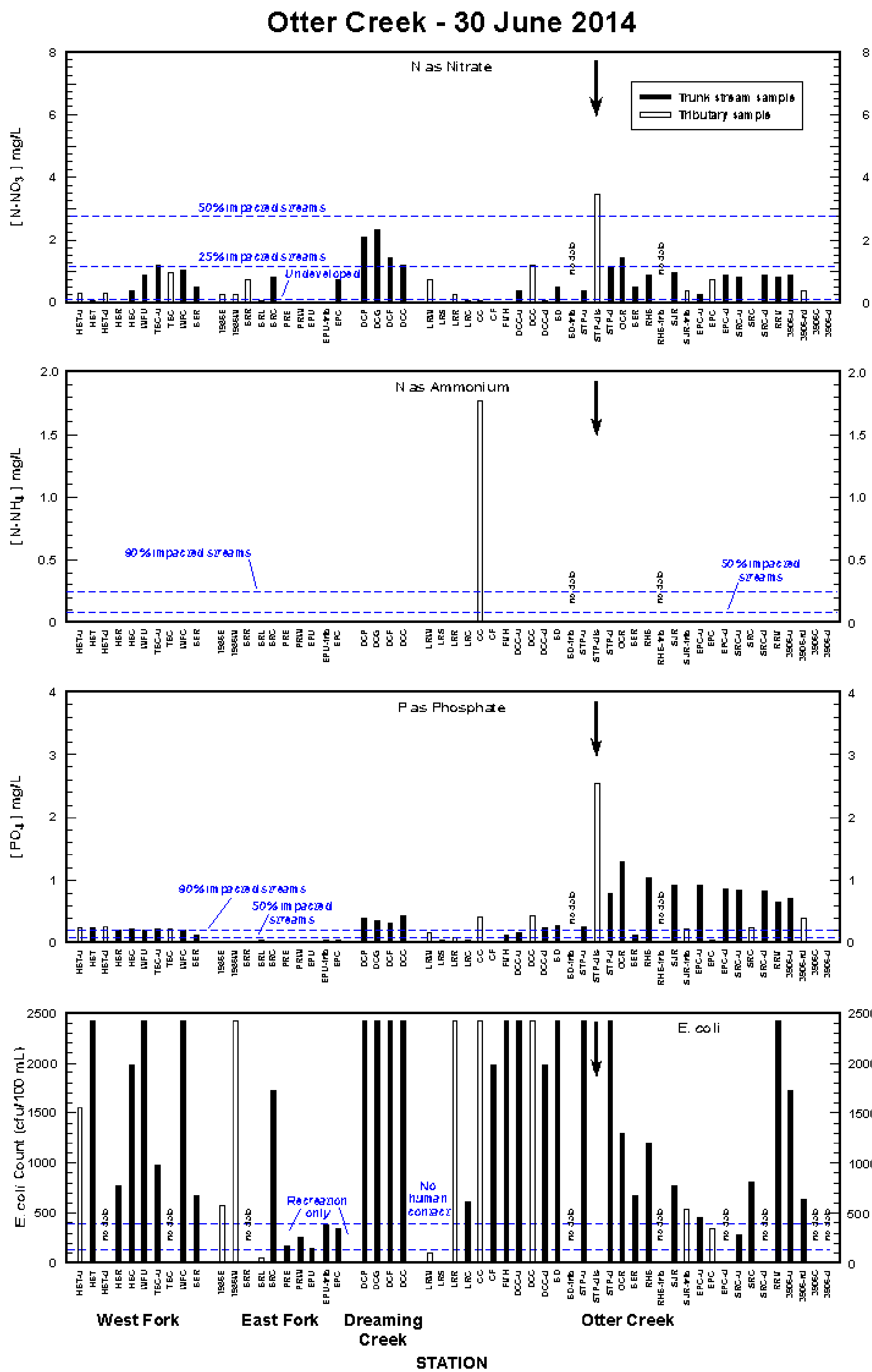

Figure 3. June sampling data for nutrients and *E. coli*.

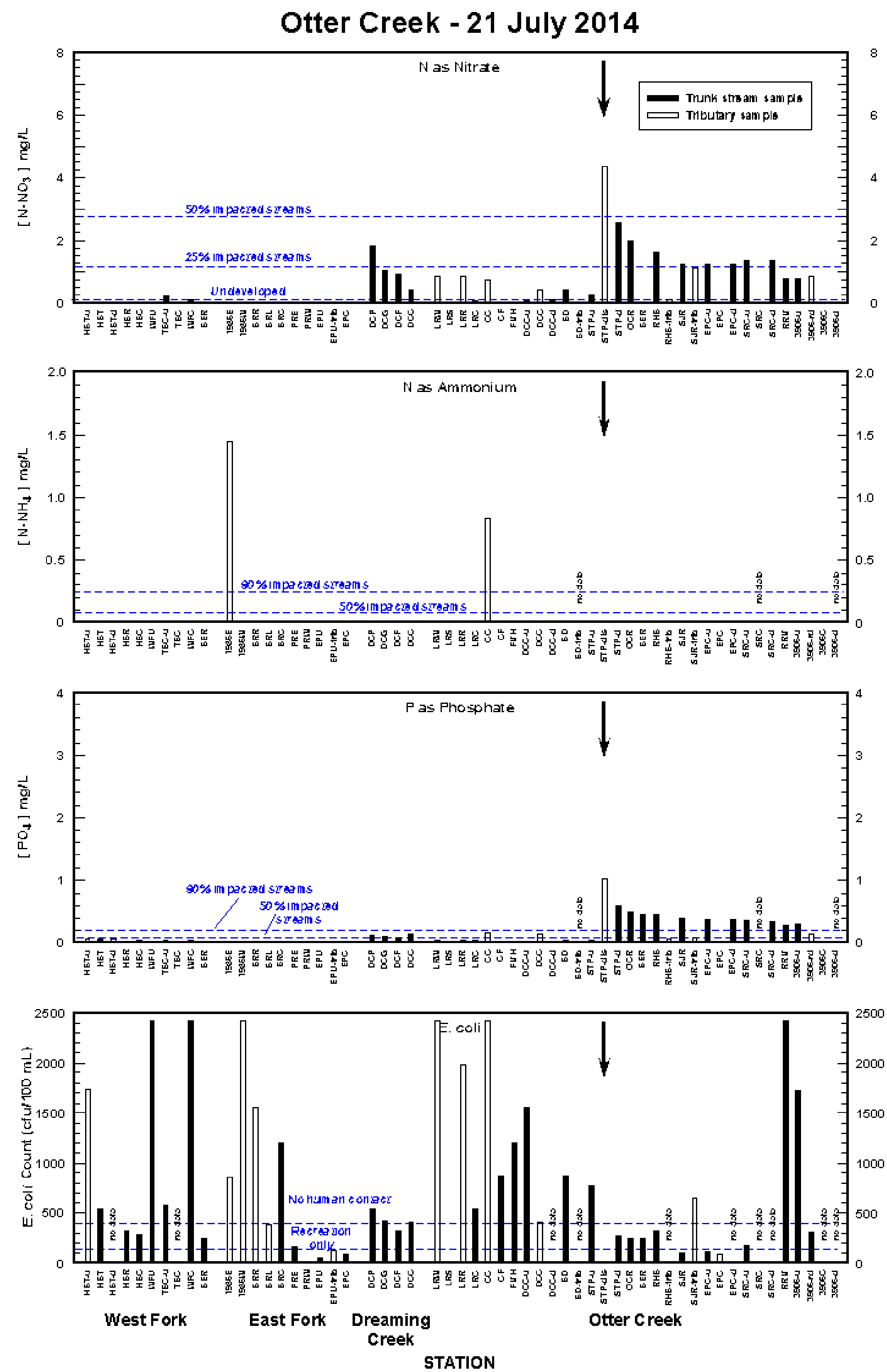

Figure 4. July sampling data for nutrients and *E. coli*.

rising above 2 mg/L at the Dreaming Creek stations and at the wastewater treatment plant's discharge (3.5 mg/L $N\text{-}NO_3$). A peak value of 4.4 mg/L (STP-discharge) was recorded in July, which was also reflected in high downstream concentrations at the central fork stations.

Phosphate concentrations were relatively consistent across all sampling dates, with highest values always occurring at and downstream of the sewage treatment plant, averaging ~0.9 mg/L $P\text{-}PO_4$. July sampling saw 1.0 mg/L $P\text{-}PO_4$ at station STP-discharge which was the highest concentration documented all season.

Results for ammonium were very different with respect to nitrate and phosphate, and are not tied to the sewage treatment plant. The highest background concentrations found were about 0.3 mg/L $N\text{-}NH_4$, during May sampling. Station CC recorded the highest value of 1.4 mg/L $N\text{-}NH_4$. Measurable ammonium occurs only sporadically with subsequent sampling dates. Only two stations (CC and 1986E) saw values exceeding 0 mg/L $N\text{-}NH_4$ for June and July, showing concentrations ranging from 1.4 to 1.8 mg/L. The wastewater treatment facility discharged significant levels of ammonium only on 27 May 2015, showing a value of 0.5 mg/L $N\text{-}NH_4$.

E. coli

High *E. coli* concentrations were seen among all stream segments on all sampling dates. The average of all samples taken on all three of the sampling dates was 955 cfu/100 mL *E. coli* (median, 630 cfu/100 mL). The United States Environmental Protection Agency (EPA) has developed standards for water quality using *E. coli* as an indicator for any fecal microbe contamination (Table 3). 53% of samples exceeded EPA standards for human contact (>575 cfu/100 mL). 30% of samples were deemed suitable for bathing with the remaining 17% of samples showing as suitable for only recreational purposes. *E. coli* counts diminished at the sewage treatment plant (average of ~7.37 cfu/100 mL) and at proximal downstream stations to the STP, with gradual increases moving further downstream. Mean upstream counts for all sampling dates was ~1,065.8 cfu/100 mL, compared to mean downstream counts of ~621.4 cfu/100 mL. Similar to nitrate and phosphate sampling, highest mean *E. coli* counts were seen in June, where 17 of 47 stations saw values that reached maximum counts (>2,419 cfu/100 mL).

Table 3. EPA designations for water quality according to *E. coli* counts (EPA, 2006).

Activity	**Count Threshold (cfu/100 mL)**
Suitable for bathing	<235
Recreation only	236 - 574
Not recommended for human contact	>575

DISCUSSION

Contaminant input into stream systems is dependent on anthropogenic activities such as land use, and natural events such as rainfall that have the ability to flush nutrients into stream systems at elevated levels. Moreover, contaminants from anthropogenic sources can be attributed to point sources, or from human activities that occur throughout a drainage basin (non-point sources).

Point source

We use our data to identify both point, and nonpoint-sources within the watershed. We also provide evidence that shows the Otter Creek sewage treatment plant as a point source for nitrate and phosphate contamination. Discharge from the STP contains up to 17 and 22.5 times more nitrate and phosphate, respectively, compared to nutrient values for streams with minimal human impacts (Dubrovsky et al., 2010) Dubrovsky et al. (2010) gathered data from streams across the United States comparing nutrient levels with four categories of land use by humans. Of particular interest are nutrient levels within streams with minimal human impacts, and Dubrovsky et al. (2010) use the 75^{th} percentile value as a national background level for each nutrient. Significant spikes of $N\text{-}NO_3$ and $P\text{-}PO_4$ occur at the STP discharge, and then progressively diminish downstream due to dilution and dispersion, and possibly because of uptake by primary producers. Concentrations for both nutrients are also comparably higher than those seen in the national data set (Fig. 5; Dubrovsky et al., 2010). Average levels of nitrate upstream of the STP fall among average background to 10^{th} percentile values, but increase to median ranges downstream of STP for urban and mixed land uses. Levels for phosphate were a little higher upstream of the facility, residing among median ranges for urban and mixed land uses, and around the 10th percentile for agricultural areas. Downstream of the STP, $P\text{-}PO_4$ concentrations elevate significantly to surpass the 90th percentile for agricultural and mixed land uses, while approaching the same percentile for urban areas as well.

Nitrate distribution was found to be highest within Dreaming Creek and within Otter Creek sewage treatment plant discharge and downstream locales showing median to

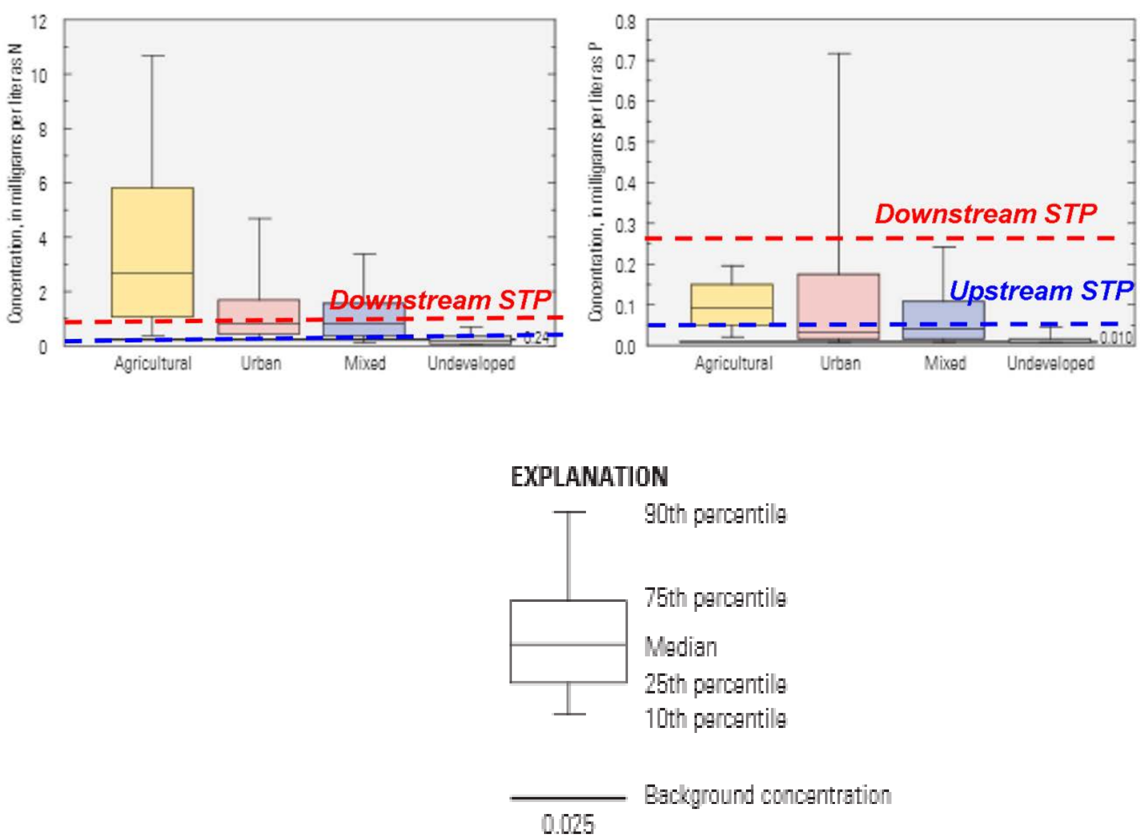

Figure 5. Data from Dubrovsky et al. (2010), showing levels of $N\text{-}NO_3$ (nitrate) and $P\text{-}PO_4$ (phosphate) within pristine stream waters throughout the United States. Note that data are categorized by land use, including nutrient levels in undeveloped watersheds. Average $N\text{-}NO_3$ and $P\text{-}PO_4$ concentrations from the Otter Creek watershed relative to STP are shown by dashed lines across the diagrams.

90th percentile ranges for urban, mixed, and undeveloped areas (Fig. 5). An exception occurred in the month of June, which presented a spike in N-NO_3^- concentrations that placed a majority of the stations within the 90th percentile range. N-NO_3^- concentrations in the sewage treatment plant's discharge and downstream location were shown to exceed those typically seen in agricultural areas.

Concentrations of phosphate were fairly consistent across all sampling days, with high concentrations recorded at the sewage treatment plant's discharge and downstream stations. These stations exceeded national percentile ranges for all land uses for each sampling event. We found an average of 0.04 mg/L P-PO_4 within the west fork, falling among median percentiles for urban and mixed land uses, and the 25th percentile for agricultural areas. The east fork never had values of P-PO_4 that exceeded 0 mg/L.

Overall, our findings show that the Otter Creek watershed is experiencing excessive N-NO_3^- and P-PO_4^- concentrations within its central fork, and particularly within the Otter Creek sewage treatment plant's discharge and downstream locales. The sewage treatment plant is a strong point source for nitrate and phosphate, but not for ammonium or fecal microbes (see below).

Non-point sources

Non-point sources of contamination within the watershed are more numerous and widespread. As most of Otter Creek is dominated by rural residences and pasture land, we infer that concentrations above national background levels (Dubrovsky et al., 2010) are the result of runoff from pasture and perhaps from septic system leachate.

Considerably high concentrations of nitrate and phosphate also occur in Dreaming Creek, among all of its stations, which is especially evident in the case of $N\text{-}NO_3$. Sampling sites located within Dreaming Creek provide a high average concentration of ~ 1.4 mg/L $N\text{-}NO_3$ as compared to national data for urban and residential land use (Dubrovsky et al., 2010). Because there should be limited overland sources of nutrients in a town setting, we hypothesize that elevated nutrient levels are the result of sewer system leaks. This inference is consistent with *E. coli* counts within Dreaming Creek, which are also elevated (see below).

Anomalous ammonium

$N\text{-}NH_4$ concentrations are very different than those of nitrate and phosphate for all sampling dates. June and July sampling identified only two stations with values exceeding 0 mg/L (Stations CC and 1986E), whereas May sampling shows widespread, elevated amounts of $N\text{-}NH_4^+$ that exceed most values from national data (Dubrovsky et al., 2010). $N\text{-}NH_4^+$ concentrations at stations CC and 1986E are well above the 90th percentile level of nutrient data (Dubrovsky et al., 2010), while $N\text{-}NH_4$ background concentrations of 0.025 mg/L or lower were usually found in all other stations (Fig. 6). Excessive $N\text{-}NH_4^+$ seen at 1986E is best attributed to runoff from its active pasture. The Concord station (CC) has multiple land uses upstream of its tributary with pasture in the upper reaches and several residences served by septic systems downstream and proximal to its confluence within Otter Creek. This, we are uncertain of the source of high ammonium at this station.

Appreciable levels of ammonium were only found in rural areas with pastureland and sparse residences on septic systems, therefore non-point sources of contamination are likely from pasture runoff and septic sources. Unlike the case for nitrate and phosphate, the sewage treatment plant lacks any influence on ammonium concentrations.

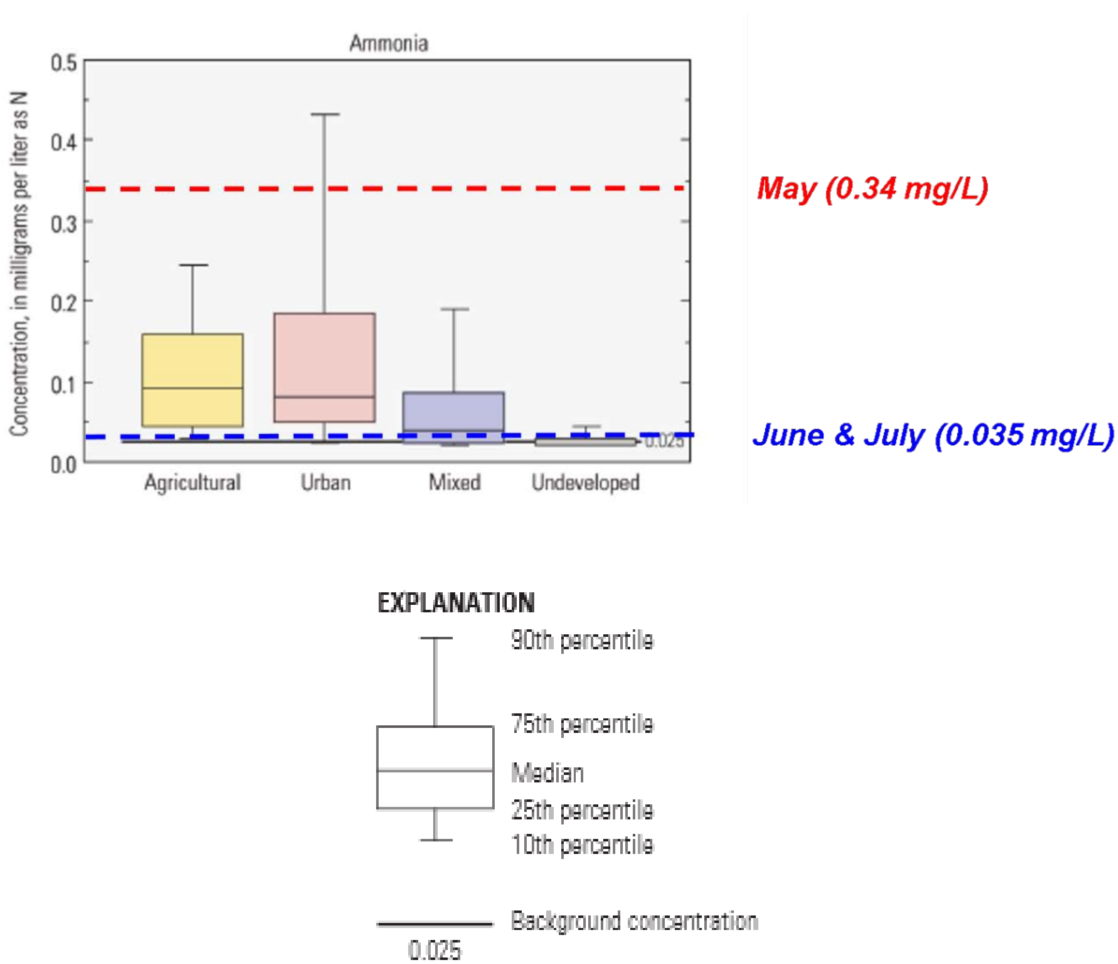

Figure 6. National data set for ammonium (N-NH_4) compared to our observations. Note that the data distinguish between sample dates of 27 May (drier) versus those in June and July (wetter).

E. coli

E. coli counts are consistently high within all stream segments of the watershed and degrade Otter Creek's water quality. Many sampling stations (53%) had *E.coli* counts deemed unsuitable for human contact (535 cfu/100 mL, EPA 1986) and 18.8% of all stations reached a maximum contamination count of >2419.6 cfu/100 mL (IDEXX, 2006). Sources for fecal microbe counts occur across all land uses, and illustrate classic non-point source contamination, as all stream segments are affected.

Consistently high counts were recorded at stations where pastureland is the dominant land use in both the west and east forks. For example, station 1986W, located within pastureland, was the primary culprit for high counts in the East Fork, as did its eastern counterpart (1986E). High counts within the central fork occurred at Lake Reba input (station LRW) and the road located next to its spillway (LRR), the Concord stream of Otter Creek proper (CC), and the railroad crossing on highway 388 (RRM). The LRW, CC, and RRM stations are associated with pastures, so we infer the cattle manure is the most likely source for fecal microbes. The source for *E. coli* at station LRR is problematical.

The upper portion of Dreaming Creek drains urban areas that are served by city sewer. Despite the lack of pastureland and septic systems, *E.coli* counts are almost always above that deemed unsuitable for human contact and often exceed maximum contamination count of >2419.6 cfu/100 mL (IDEXX, 2006). Due to the lack of other fecal sources, we infer that leaking city sewer pipes are contributing fecal microbes to Dreaming Creek.

As expected, the sewage treatment plan is not a source for fecal microbes. The average *E.coli* count in its discharge is ~7.37 cfu/100 mL as compared to the upstream average of 1065.8 cfu/100 mL. The STP is effectively removing fecal microbes from its waste stream.

Rain events

Contaminant concentrations within streams are impacted by rainfall and resulting runoff. Rain events seem to increase N-NO_3, P-PO_4, and *E. coli* concentrations within Otter Creek stream waters, whereas N-NH_4 concentrations decrease. Significant rain events occurred on days prior to both June and July sampling that led to increased runoff, coinciding with the higher values we see for nitrate and phosphate (Fig. 7). Conversely, ammonium values barely exceed 0 mg/L N-NH_4 for the same sampling dates. The presence of ammonium was much higher in the month of May, when five days had

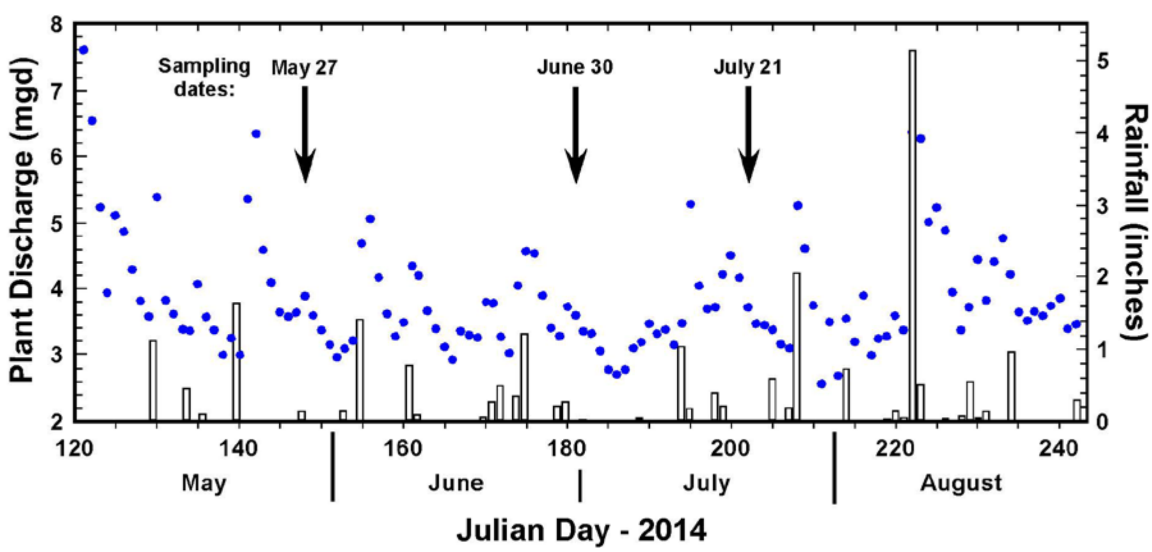

Figure 7. Rainfall and discharge data for the 2014 sampling season. Data courtesy of the Richmond Utilities Distract.

passed between a rain event and sampling.

The highest *E.coli* counts were recorded in the months of June and July after recent rain events. Maximum counts were seen at 17 stations in June, with overall higher counts documented in comparison to other sampling events. This could be in association with the significant rain event that occurred just two days prior which would have increased runoff substantially. Therefore, we infer that runoff from pasturelands are responsible for higher *E.coli* counts found in June and July. Runoff from rural residential areas may be an additional source for fecal microbes, but remains undocumented.

CONCLUSIONS

(1) A strong point-source for N-NO_3 and P-PO_4 contamination is the Otter Creek sewage treatment plant (STP), which discharges these nutrients into Otter Creek raising concentrations by a factor of ~17 times (N-NO_3) and ~22.5 times (P-PO_4) that of national data sets for pristine streams.

(2) Although the STP is a direct source for nitrate and phosphate, it does not seem to influence ammonium contamination, as N-NH_4 concentrations do not differ between stations upstream and downstream of the STP.

(3) Non-point sources for nutrients also occur in the Otter Creek watershed. The relationship between high concentrations of N-NO_3 and P-PO_4 to rural and pasture land uses indicates that non-point sources of contamination are likely pastureland runoff and leaking sewage systems. We attribute the consistently higher concentrations within Dreaming Creek tributary to leachate from residential sewer systems in the more urban areas of Richmond.

(4) The Otter Creek watershed contains only non-point sources for *E.coli* contamination in both rural and urban settings. The highest concentrations are associated with cattle pastureland. We also suspect that septic tanks of rural settlements and residences may contribute fecal microbes to the watershed, but have only circumstantial evidence for septic tank sources.

(5) In urban settings, elevated *E. coli* counts point to a leaky sewage distribution system as a likely source based on high microbe counts in the upper reaches of Dreaming Creek.

(6) The wastewater treatment facility is effective in removing microbes from the waste stream as evidenced by low *E. coli* counts in plant discharge (mean, ~ 7.37 cfu/100 mL).

REFERENCES

Bartram, J., Wayne W. Carmichael, Ingrid Chorus, Gary Jones, and Olav M. Skulberg (1999) Chapter 1. Introduction, in: *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. World Health Organization. URL: WHO document

Borowski WS, Aguiar TA, Hunter J, Jolly EC, Stockwell RD. 2009. Nutrient and fecal microbe sources for a eutrophic lake and recommended remediation steps, Wilgreen Lake, Madison County, Kentucky. Kentucky Water Resource Research Institute Symposium, March 2009.

Dubrovsky NM, Burow KR, Clark GM, Gronberg JOM, Hamilton PA, Hitt, KJ, Mueller DK, Munn MD, Nolan BT, Puckett LJ, Rupert MG, Short TM, Spahr NE, Sprague LA, Wilber WG. 2010. The quality of our nations's water – Nutrients in the nation's streams and groundwater, 1992-2004. USGS Circular 1350, 175pp.

Eaton AD, Clesceri LS, Rice EW, Greenberg AE (Eds.). 2005 (21st ed.). Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WEF. Baltimore: Port City Press.

Edberg, S.C., E.W. Rice, R.J. Karlin, M.J. Allen, 2000. Escherichia coli: the biological drinking water indicator for public health protection. Journal Applied Microbiology, 88:106S-116S.

Feng P, Weagant S, Grant, M (2002-09-01). "Enumeration of *Escherichia coli* and the Coliform Bacteria". *Bacteriological Analytical Manual (8th ed.)*. FDA/Center for Food Safety & Applied Nutrition. Retrieved 2007-01-25.

Gieskes JM, Gamo T, Brumsack H. 1991. Chemical methods for interstitial water analysis aboard the JOIDES Resolution. Ocean Drilling Program Technical Note 15, Ocean Drilling Program, Texas A&M University, 60 pp.

Hach. 1986. Nitrate, Instruction Manual, Pocket Colorimeter II. 73 pp.

IDEXX. 2006. Scientific basis of Quanti-Tray/2000. Available from the world-wide web, http://www.idexx.com/water/quantitray/science.jsp.

Khan, Nasir M., and F. Mohammad. 2014. "Eutrophication: Challenges and Solutions" in A. A. Ansari, S. S. Gill (eds.), Eutrophication: Causes, Consequences and Control, Springer Science and Business Media Dordrecht 2014; doi:10.1007/978-94-007-7814-6_5

Kentucky River Watershed Watch (KRWW), website: http://www.uky.edu/OtherOrgs/KRWW/index.html.

Metcalf & Eddy, (2003). *Wastewater engineering : treatment and reuse* (4 ed.). Boston: McGraw-Hill. p. 624. ISBN 0070418780.

Method 4500-NH3 F. Phenate method. IN Eaton, AD, Clesceri LS, Rice EW, Greenberg AE (Eds.), 2005 (21st ed.). Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WEF. Baltimore: Port City Press. p. 4-114.

Greenberg AE (Eds.), 2005 (21st ed.). Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WEF. Baltimore: Port City Press. pp. 4-123 to 4-125.

Method-P E. Ascorbic acid method. IN Eaton, AD, Clesceri LS, Rice EW, Greenberg AE (Eds.), 2005 (21st ed.). Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WEF. Baltimore: Port City Press. pp. 4-153 to 4-155.

Method 9223. Enzyme substrate coliform test. IN Eaton, AD, Clesceri LS, Rice EW, Greenberg AE (Eds.), 2005 (21st ed.). Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WEF. Baltimore: Port City Press. pp. 9-72 to 9-74.

Method 9060. Samples. IN Eaton, AD, Clesceri LS, Rice EW, Greenberg AE (Eds.), 2005 (21st ed.). Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WEF. Baltimore: Port City Press. pp. 9-20 to 9-22.

Richmond Utilities website, http://richmondutilities.com/pages/main/home.php.

Singleton P (1999). *Bacteria in Biology, Biotechnology and Medicine* (5th ed.). Wiley. pp. 444–454.

Smith KL, Borowski WS, 2013. Sources of nutrient and fecal microbe pollution in Otter Creek, Madison County, Kentucky. Meeting Kentucky Academy of Science, November 2013.

Solorzano L.1969. Determination of ammonia in natural waters by phenol-hypochlorite method. Limnology Oceanography. 14:799-801.

Strickland, JDH, Parsons TR. 1968. A manual for sea water analysis, Bull. Fisheries Research Board Canada, 167.

U.S. Center for Disease Control. “*Escherichia coli*”. CDC National Center for Emerging and Zoonotic Infectious Diseases.

U.S. Environmental Protection Agency. 1986. Water quality for bacteria – 1986. EPA Publication 440/5-84-002. Washington, D.C.: U.S. Government Printing Office. 24 pp.

Werner, Wilfried. "Fertilizers, 6. Environmental Aspects" Ullmann's Encyclopedia of Industrial Chemistry, 2002, Wiley-VCH,Weinheim. doi:10.1002/14356007.n10_n05.

APPENDIX

Appendix A. Data from sampling on 27 May 2014.

							TOTAL COLIFORM				*E. coli*	
							Number	Number		Number	Number	EC
Station	[NH4]	[N]	[NO3]	[N]	[PO4]	[P]	Large	Small	Count	Large	Small	Count
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	Wells	Wells	(cfc / 100 mL)	Wells	Wells	(cfc / 100 mL)
HBT-U	0.4	0.3	0.5	0.1	0.1	0.0	49	48	>2419.6	49	34	770.1
HBT	0.5	0.4	0.0	0.0	0.1	0.0	49	48	>2419.6	49	36	866.4
HBT-d	0.3	0.3	0.0	0.0	0.1	0.0	-	-	-	-	-	-
HBR	0.3	0.2	0.0	0.0	0.0	0.0	49	48	>2419.6	49	48	>2419.6
HBC	0.3	0.3	0.0	0.0	0.1	0.0	49	48	>2419.6	49	39	1046.2
TBC-up	0.3	0.3	0.0	0.0	0.1	0.0	49	48	>2419.6	49	48	>2419.6
TBC	0.6	0.4	1.0	0.2	0.1	0.0	49	48	>2419.6	49	35	816.4
WFU	0.4	0.3	0.0	0.0	0.1	0.0	-	-	-	-	-	-
WFC	0.5	0.4	0.5	0.1	0.1	0.0	-	-	-	-	-	-
BER	0.3	0.2	0.0	0.0	0.0	0.0	49	48	>2419.6	47	21	240
1986E	0.6	0.5	0.0	0.0	0.0	0.0	49	48	>2419.6	49	43	1413.6
1986W	0.5	0.4	0.0	0.0	0.0	0.0	49	48	>2419.6	49	45	1732.9
BRR	0.5	0.4	0.0	0.0	0.0	0.0	49	48	>2419.6	49	36	866.1
BRL	0.5	0.4	0.0	0.0	0.0	0.0	49	48	>2419.6	47	15	191.8
BRC	0.4	0.3	0.0	0.0	0.0	0.0	-	-	-	-	-	-
PRE	0.5	0.4	0.0	0.0	0.0	0.0	49	48	>2419.6	29	8	54.5
PRW	0.4	0.3	0.0	0.0	0.0	0.0	49	46	1986.3	27	3	42
EPU	0.2	0.2	0.0	0.0	0.0	0.0	49	17	290.9	49	6	172
EPU-trib	0.3	0.3	0.0	0.0	0.0	0.0	49	47	2419.6	48	12	193.5
EPC	0.3	0.3	0.0	0.0	0.0	0.0	40	41	207.1	32	6	59.1
DCP	0.6	0.5	10.1	2.3	0.5	0.2	49	48	>2419.6	49	34	770.1
DCG	0.8	0.6	7.1	1.6	0.3	0.1	49	48	>2419.6	49	23	410.6
DCF	0.2	0.1	4.5	1.0	0.2	0.1	49	48	>2419.6	46	8	137.6
DCC	0.4	0.3	1.5	0.3	0.3	0.1	49	48	>2419.6	49	44	1553.1
LRW	0.5	0.4	1.5	0.3	0.2	0.0	49	48	>2419.6	49	48	>2419.6
LRS	0.6	0.5	0.0	0.0	0.0	0.0	49	48	>2419.6	3	1	4.1
LRR	0.4	0.3	0.0	0.0	0.1	0.0	49	48	>2419.6	48	20	272.3
LRC	0.4	0.3	0.0	0.0	0.1	0.0	49	48	>2419.6	49	23	410.6
CC	1.9	1.4	0.0	0.0	0.1	0.0	49	48	>2419.6	49	45	1732.9
CF	0.0	0.0	0.0	0.0	0.0	0.0	49	48	>2419.6	49	43	1413.6
FMH	0.6	0.5	0.0	0.0	0.0	0.0	49	48	>2419.6	49	38	980.4
DCC-u	0.8	0.6	0.0	0.0	0.1	0.0	49	48	>2419.6	49	45	1732.9
DCC	0.4	0.3	1.5	0.3	0.3	0.1	49	48	>2419.6	49	44	1553.1
DCC-d	0.4	0.3	2.0	0.5	0.4	0.1	-	-	-	-	-	-
BD	0.9	0.7	0.0	0.0	0.1	0.0	49	48	>2419.6	47	14	185
BD-trib	0.3	0.2	0.0	0.0	0.1	0.0	49	48	>2419.6	48	14	209.8
STP-u	0.4	0.3	0.0	0.0	0.1	0.0	49	48	>2419.6	47	16	198.9
STP-dis	0.6	0.5	16.7	3.8	2.8	0.9	47	12	172.3	5	1	6
STP-d	0.4	0.3	19.2	4.3	2.8	0.9	49	48	>2419.6	20	3	28.8
OCR	0.4	0.3	3.5	0.8	1.1	0.4	49	48	>2419.6	42	16	130.1
BER	0.3	0.2	0.0	0.0	0.0	0.0	49	47	2419.6	46	10	146.7
RHB	0.3	0.2	1.5	0.3	0.8	0.3	49	48	>2419.6	49	17	290.9
RHB-trib	0.4	0.3	0.0	0.0	0.1	0.0	49	48	>2419.6	26	7	45.9
SJR	0.4	0.3	0.0	0.0	0.5	0.1	49	48	>2419.6	49	31	648.8
SJR-trib	0.5	0.4	0.0	0.0	0.2	0.0	49	48	>2419.6	40	7	90.8
EPC-u	0.6	0.4	0.0	0.0	0.5	0.2	40	41	207.1	32	6	59.1
EPC	0.3	0.3	0.0	0.0	0.0	0.0	-	-	-	-	-	-
EPC-d	0.2	0.2	0.0	0.0	0.5	0.1	49	48	>2419.6	34	9	70.8
SRC-u	0.2	0.2	0.0	0.0	0.4	0.1	49	48	>2419.6	49	35	816.4
SRC	0.3	0.2	0.0	0.0	0.2	0.1	-	-	-	-	-	-
SRC-d	0.2	0.1	0.0	0.0	0.4	0.1	49	48	>2419.6	49	19	325.5
RRM	0.3	0.3	0.0	0.0	0.2	0.1	49	48	>2419.6	49	12	224.7
3906-u	0.2	0.1	0.0	0.0	0.2	0.1	49	48	>2419.6	49	25	461.1
3906 road	0.3	0.2	0.0	0.0	0.4	0.1	-	-	-	-	-	-
3906C	-	-	-	-	-	-	-	-	-	-	-	-
3906-d	-	-	0.0	0.0	0.2	0.1	-	-	-	-	-	-

Appendix B. Data from sampling on 30 June 2014.

							TOTAL COLIFORM			*E. coli*	
						Number	Number		Number	Number	EC
Station	[NH4]	[N]	[NO3]	[N]	[PO4]	[P]	Large	Small	Count	Large	Small	Count
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	Wells	Wells	(cfc / 100 mL)	Wells	Wells	(cfc / 100 mL)
HBT-U	0.0	0.0	2.1	0.3	0.2	0.1	49	48	>2419.6	49	44	1553.1
HBT	0.0	0.0	1.0	0.1	0.2	0.1	49	48	>2419.6	49	48	>2419.6
HBT-d	0.0	0.0	2.1	0.3	0.3	0.1	-	-	-	-	-	-
HBR	0.0	0.0	0.0	0.0	0.2	0.1	49	48	>2419.6	49	34	770.1
HBC	0.0	0.0	2.4	0.4	0.2	0.1	49	48	>2419.6	49	46	1986.3
TBC-up	0.0	0.0	4.5	0.9	0.2	0.1	49	48	>2419.6	49	48	>2419.6
TBC	0.0	0.0	5.8	1.2	0.2	0.1	49	48	>2419.6	49	38	980.4
WFU	0.0	0.0	4.8	1.0	0.2	0.1	-	-	-	-	-	-
WFC	0.0	0.0	5.2	1.0	0.2	0.1	49	48	>2419.6	49	48	>2419.6
BER	0.0	0.0	2.8	0.5	0.1	0.0	49	48	>2419.6	49	32	686.7
1986E	0.0	0.0	1.7	0.2	0.0	0.0	49	48	>2419.6	49	29	579.4
1986W	0.0	0.0	1.7	0.2	0.0	0.0	49	48	>2419.6	49	47	2419.6
BRR	0.0	0.0	3.8	0.7	0.0	0.0	-	-	-	-	-	-
BRL	0.0	0.0	1.0	0.1	0.1	0.0	47	44	593.8	16	27	53.2
BRC	0.0	0.0	4.1	0.8	0.0	0.0	49	48	>2419.6	49	45	1732.9
PRE	0.0	0.0	0.4	0.0	0.0	0.0	49	48	>2419.6	48	10	178.9
PRW	0.0	0.0	0.0	0.0	0.0	0.0	49	48	>2419.6	49	15	261.3
EPU	0.0	0.0	0.4	0.0	0.0	0.0	49	47	2419.6	46	11	151.5
EPU-trib	0.0	0.0	0.0	0.0	0.1	0.0	49	48	>2419.6	49	22	387.3
EPC	0.0	0.0	3.8	0.7	0.1	0.0	49	48	>2419.6	49	20	344.8
DCP	0.0	0.0	9.6	2.1	0.4	0.1	49	48	>2419.6	49	48	>2419.6
DCG	0.0	0.0	10.6	2.3	0.4	0.1	49	48	>2419.6	49	48	>2419.6
DCF	0.0	0.0	6.9	1.4	0.3	0.1	49	48	>2419.6	49	48	>2419.6
DCC	0.0	0.0	5.8	1.2	0.4	0.1	49	48	>2419.6	49	48	>2419.6
LRW	0.0	0.0	3.8	0.7	0.2	0.1	45	47	424.5	24	47	106.1
LRS	0.0	0.0	0.7	0.0	0.1	0.0	38	48	207.7	9	0	9.8
LRR	0.0	0.0	1.7	0.2	0.1	0.0	49	48	>2419.6	49	47	2419.6
LRC	0.0	0.0	1.0	0.1	0.1	0.0	49	48	>2419.6	49	30	613.1
CC	2.3	1.8	1.0	0.1	0.4	0.1	49	48	>2419.6	49	48	>2419.6
CF	0.0	0.0	0.4	0.0	0.0	0.0	49	48	>2419.6	49	46	1986.3
FMH	0.0	0.0	0.0	0.0	0.1	0.0	49	48	>2419.6	49	48	>2419.6
DCC-u	0.0	0.0	2.4	0.4	0.2	0.1	49	48	>2419.6	49	48	>2419.6
DCC	0.0	0.0	5.8	1.2	0.4	0.1	49	48	>2419.6	49	48	>2419.6
DCC-d	0.0	0.0	1.0	0.1	0.2	0.1	49	48	>2419.6	49	46	1986.3
BD	0.0	0.0	2.8	0.5	0.3	0.1	49	48	>2419.6	49	48	>2419.6
BD-trib	-	-	-	-	-	-	-	-	-	-	-	-
STP-u	0.0	0.0	2.4	0.4	0.3	0.1	49	48	>2419.6	49	48	>2419.6
STP-dis	0.0	0.0	15.4	3.5	2.6	0.8	48	18	248.9	5	0	5.2
STP-d	0.0	0.0	5.5	1.1	0.8	0.3	49	48	>2419.6	49	48	>2419.6
OCR	0.0	0.0	6.9	1.4	1.3	0.4	49	48	>2419.6	49	42	1299.7
BER	0.0	0.0	2.8	0.5	0.1	0.0	49	48	>2419.6	49	32	686.7
RHB	0.0	0.0	4.5	0.9	1.0	0.3	49	48	>2419.6	49	41	1203.3
RHB-trib	0.0	0.0	-	-	-	-	-	-	-	-	-	-
SJR	0.0	0.0	4.8	1.0	0.9	0.3	49	48	>2419.6	49	34	770.1
SJR-trib	-	-	2.4	0.4	0.2	0.1	49	48	>2419.6	49	28	547.5
EPC-u	0.0	0.0	1.7	0.2	0.9	0.3	49	48	>2419.6	49	25	461.1
EPC	0.0	0.0	3.8	0.7	0.1	0.0	49	48	>2419.6	49	20	344.8
EPC-d	0.0	0.0	4.5	0.9	0.9	0.3	-	-	-	-	-	-
SRC-u	0.0	0.0	4.1	0.8	0.9	0.3	49	48	>2419.6	49	17	290.9
SRC	0.0	0.0	0.7	0.0	0.2	0.1	49	48	>2419.6	49	35	816.4
SRC-d	0.0	0.0	4.5	0.9	0.8	0.3	-	-	-	-	-	-
RRM	0.0	0.0	4.1	0.8	0.7	0.2	49	48	>2419.6	49	48	2419.6
3906-u	0.0	0.0	4.5	0.9	0.7	0.2	49	48	>2419.6	49	45	1732.9
3906 road	0.0	0.0	2.4	0.4	0.4	0.1	49	48	>2419.6	49	31	648.8
3906C	0.0	0.0	4.5	0.9	0.7	0.2	-	-	-	-	-	-
3906-d	-	-	-	-	-	-	-	-	-	-	-	-

Appendix C. Data from sampling on 21 July 2014.

												TOTAL COLIFORM				*E. coli*	
											Number	Number			Number	Number	EC
Station		**[NH4]**	**[N]**		**[NO3]**	**[N]**		**[PO4]**	**[P]**		**Large**	**Small**	**Count**		**Large**	**Small**	**Count**
		(mg/L)	**(mg/L)**		**(mg/L)**	**(mg/L)**		**(mg/L)**	**(mg/L)**		**Wells**	**Wells**	**:fc / 100 mL)**		**Wells**	**Wells**	**:fc / 100 ml**
HBT-U		0.0	0.0		0.7	0.0		0.2	0.1		49	48	>2419.6		49	45	1732.9
HBT		0.0	0.0		0.0	0.0		0.2	0.1		49	48	>2419.6		49	28	547.5
HBT-d		0.0	0.0		0.3	0.0		0.2	0.1		-	-	-		-	-	-
HBR		0.0	0.0		0.0	0.0		0.0	0.0		49	48	>2419.6		48	24	328.2
HBC		0.0	0.0		0.0	0.0		0.2	0.0		49	48	>2419.6		49	17	290.9
TBC-up		0.0	0.0		0.3	0.0		0.1	0.0		49	48	>2419.6		49	48	>2419.6
TBC		0.0	0.0		1.6	0.2		0.2	0.1		49	48	>2419.6		49	29	579.4
WFU		0.0	0.0		0.7	0.0		0.1	0.0		-	-	-		-	-	-
WFC		0.0	0.0		1.2	0.1		0.1	0.0		49	48	>2419.6		49	48	>2419.6
BER		0.0	0.0		0.0	0.0		0.0	0.0		49	48	>2419.6		49	14	248.1
															-	-	-
1986E		1.9	1.5		0.0	0.0		0.0	0.0		49	48	>2419.6		49	36	866.4
1986W		0.0	0.0		0.0	0.0		0.0	0.0		49	48	>2419.6		49	48	>2419.6
BRR		0.0	0.0		0.3	0.0		0.0	0.0		49	48	>2419.6		49	44	1553.1
BRL		0.0	0.0		0.3	0.0		0.1	0.0		49	48	>2419.6		49	22	387.3
BRC		0.0	0.0		0.3	0.0		0.0	0.0		49	48	>2419.6		49	41	1203.3
PRE		0.0	0.0		0.0	0.0		0.0	0.0		49	48	>2419.6		47	11	166.4
PRW		0.0	0.0		0.0	0.0		0.0	0.0		49	38	980.4		7	0	7.5
EPU		0.0	0.0		0.0	0.0		0.0	0.0		49	48	>2419.6		32	5	57.3
EPU-trib		0.0	0.0		0.0	0.0		0.1	0.0		49	48	>2419.6		43	13	128.1
EPC		0.0	0.0		0.0	0.0		0.1	0.0		49	48	>2419.6		37	13	91.1
DCP		0.0	0.0		8.7	1.9		0.4	0.1		49	48	>2419.6		49	28	547.5
DCG		0.0	0.0		5.1	1.1		0.4	0.1		49	48	>2419.6		46	10	146.7
DCF		0.0	0.0		4.7	1.0		0.3	0.1		49	48	>2419.6		49	19	325.5
DCC		0.0	0.0		2.5	0.4		0.5	0.1		49	48	>2419.6		49	23	410.6
LRW		0.0	0.0		4.3	0.9		0.2	0.1		49	48	>2419.6		49	47	2419.6
LRS		0.0	0.0		0.0	0.0		0.1	0.0		49	48	>2419.6		19	0	23.3
LRR		0.0	0.0		4.3	0.9		0.1	0.0		49	48	>2419.6		49	46	1986.3
LRC		0.0	0.0		0.7	0.0		0.1	0.0		49	48	>2419.6		49	28	547.5
CC		1.1	0.8		3.8	0.7		0.5	0.2		49	48	>2419.6		49	48	>2419.6
CF		0.0	0.0		0.0	0.0		0.1	0.0		49	48	>2419.6		49	36	866.4
FMH		0.0	0.0		0.0	0.0		0.1	0.0		49	48	>2419.6		49	41	1203.3
DCC-u		0.0	0.0		0.7	0.0		0.1	0.0		49	48	>2419.6		49	44	1553.1
DCC		0.0	0.0		2.5	0.4		0.5	0.1		49	48	>2419.6		49	23	410.6
DCC-d		0.0	0.0		1.2	0.1		0.1	0.0		-	-	-		-	-	-
BD		0.0	0.0		2.5	0.4		0.2	0.1		49	48	>2419.6		49	36	866.4
BD-trib		-	-		-	-		-	-		-	-	-		-	-	-
STP-u		0.0	0.0		1.6	0.2		0.2	0.1		49	48	>2419.6		49	34	770.1
STP-dis		0.0	0.0		19.7	4.4		3.2	1.0		49	22	387.3		9	1	10.9
STP-d		0.0	0.0		11.8	2.6		1.8	0.6		49	48	>2419.6		49	16	275.5
OCR		0.0	0.0		9.1	2.0		1.5	0.5		49	48	>2419.6		48	18	248.9
BER		0.0	0.0		0.0	0.0		0.0	0.0		49	48	>2419.6		49	14	248.1
RHB		0.0	0.0		7.8	1.7		1.4	0.5		49	48	>2419.6		49	19	325.5
RHB-trib		0.0	0.0		1.2	0.1		0.2	0.1		49	37	920.8		32	2	-
SJR		0.0	0.0		6.0	1.3		1.2	0.4		49	48	>2419.6		40	13	106.7
SJR-trib		0.0	0.0		5.6	1.2		0.2	0.1		49	48	>2419.6		49	31	648.8
EPC-u		0.0	0.0		6.0	1.3		1.2	0.4		49	48	>2419.6		43	10	117.8
EPC		0.0	0.0		0.0	0.0		0.1	0.0		49	48	>2419.6		37	13	91.1
EPC-d		0.0	0.0		6.0	1.3		1.2	0.4		-	-	-		-	-	-
SRC-u		0.0	0.0		6.5	1.4		1.1	0.4		49	48	>2419.6		47	12	172.3
SRC		-	-		-	-		-	-		-	-	-		-	-	-
SRC-d		0.0	0.0		6.5	1.4		1.1	0.4		-	-	-		-	-	-
RRM		0.0	0.0		3.8	0.7		0.9	0.3		49	48	>2419.6		49	47	2419.6
3906-u		0.0	0.0		3.8	0.7		0.9	0.3		49	48	>2419.6		49	45	1732.9
3906 road		0.0	0.0		4.3	0.9		0.459224	0.149726		49	48	>2419.6		49	18	307.6
3906C		-	-		-	-		-	-		-	-	-		-	-	-
3906-d		0.0	0.0		4.3	0.9		0.9	0.3		-	-	-		-	-	-