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Sanitary Sewer Overflows in Columbia, South Carolina and their Impact on Mercury and Metal Cycling

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SANITARY SEWER OVERFLOWS IN COLUMBIA, SOUTH CAROLINA AND THEIR
IMPACT ON MERCURY AND METAL CYCLING

by

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ABSTRACT

Objectives. Microbial pathogens and their threat to human health have been the primary concern of sanitary sewer overflows (SSO); however, SSOs may also be a source of metals, including methylmercury, to local waterways. We hypothesized that SSOs had elevated concentrations of metals compared to nearby reference creeks.

Methods. Unfiltered and filtered (0.2 μm) surface water samples were collected monthly between November 2015 and March 2016 from three sewage-impacted creeks in Columbia, South Carolina. During this period, three sewage events were captured including an active SSO, a ruptured force main sewer pipe, and one site downstream from SSOs. In October 2016 and January 2017, three additional SSOs were sampled during two heavy rainfall events. Total mercury and methylmercury concentrations were quantified in unfiltered and filtered surface water samples ($n=56$), and 34 other metals were determined in filtered samples ($n=51$). Dissolved organic carbon concentrations (DOC) ($n=41$) were measured, and we investigated whether the stable carbon isotopic composition of DOC differed for sewage events and creek reference sites.

Results. Compared to creek reference sites, unfiltered methylmercury concentrations were enriched by factors of 1.7 to 3.3 during three of the six sewage events while during all six events, total mercury was enriched by factors of 2.0 to 9.8 compared to the reference sites. In addition, several metal concentrations had elevated concentrations in the sewage events above the average reference sites concentrations. DOC concentrations were elevated in the sewage events, and the $\delta^{13}\text{C}$ values of DOC were more positive for

the SSO and ruptured sewer main compared to the creek reference sites, suggesting that the DOC source between the sewage events and the creek reference sites differed.

Conclusions. Results indicate that SSOs are a potential source of metals, including methylmercury, that in combination with DOC may have an impact on receiving waterbodies. With thousands of SSOs occurring annually in the United States, it is important to further investigate how these events may influence the biogeochemical cycling and bioavailability of metals in aquatic environments.

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LIST OF ABBREVIATIONS

EPA.....	United States Environmental Protection Agency
Hg.....	Mercury
MeHg	Methylmercury
PCA.....	Principal Component Analysis
SSO	Sanitary Sewer Overflow
THg	Total Mercury
WWTP	Wastewater Treatment Plant

CHAPTER 1

INTRODUCTION

Across the United States, municipalities are serviced by approximately 20,000 separate sanitary sewer systems that, under normal conditions, collect and transport municipal sewage and industrial wastewaters to wastewater treatment plants (WWTP) to be properly treated and disposed of (United States Environmental Protection Agency 2004a). Separate sanitary sewer systems differ from combined sewer systems which also carry storm-water runoff within shared piping systems with wastewaters. Excess volumes of storm-water runoff are known to cause combined sewer overflows in which WWTPs must bypass a portion of their untreated influents into local waterbodies when capacities are exceeded (USEPA 2011). Although separate sanitary sewer systems are not designed to convey storm-water runoff, these systems can also experience overflows. Blockages, line breaks, power failures, insufficient system capacity and inflow/infiltration can all lead to untreated sewage being discharged from separate sanitary sewer systems into the environment prior to reaching wastewater treatment facilities, a condition known as a sanitary sewer overflow (SSO) (USEPA 2004a).

One of the biggest challenges that cities face regarding their sewer systems is aging infrastructure. Corrosion, cracks, poor pipe connections and unsealed manholes allow excess storm-water and groundwater to infiltrate into separate sanitary sewer systems. When this occurs, these systems function more like combined sewers with excess flows, particularly during heavy rainfall events, overwhelming a system's capacity

and causing wet-weather SSOs. While wet-weather conditions are not the leading cause of SSOs, it has been reported that they account for nearly 75% of the total volume discharged across the nation (USEPA 2004a).

Within Columbia, South Carolina, SSOs have been a chronic water quality-related problem due to Columbia's deteriorating separate sanitary sewer systems. In 2013 and 2014, 203 and 185 SSOs were reported to have occurred within the Lower Broad and Lower Saluda watersheds near Columbia, releasing approximately 1.8 and 1.9 million gallons of untreated wastewater, respectively (South Carolina Department of Health and Environmental Control 2017). Under a signed consent decree with the United States Justice Department, United States Environmental Protection Agency (USEPA) and South Carolina Department of Health and Environmental Control, the City of Columbia, the City's largest sewer provider, agreed to implement extensive capital improvement projects to bring the Columbia Metro WWTP and sanitary sewer collection system up-to-date with the goal of minimizing SSOs (USEPA 2013). Upgrades to infrastructure include rehabilitation and capacity enhancements to pump stations and gravity and main sewer line improvements (City of Columbia 2015). In October 2015, however, historical rainfall and widespread flooding damaged significant sections of the sewer system and set progress back on many improvement projects (Trainor 2016). Other extreme rain events including Tropical Storm Hermine (September 2016) and Hurricane Matthew (October 2016), have since triggered SSO events. During 2015 and 2016, 205 and 184 SSOs were reported near Columbia (not including the October 2015 flooding) releasing an estimated 5.6 and 2.4 million gallons of untreated sewage into the environment, respectively, more than what had been spilled in previous years (SCDHEC 2017).

SSOs have the potential to threaten public health and cause environmental degradation. The primary concern of sewer overflows has been exposure to harmful microbial pathogens, including bacteria, viruses, and parasites, due to fecal contamination (McLellan, et al. 2007; Donovan, et al. 2008; Fong, et al. 2010). Exposure to untreated sewage by those recreating, drinking, and/or eating fish and shellfish from SSO contaminated waters can cause illnesses (USEPA 2004a). Studies have shown a positive association between the release of untreated or partially treated sewage into sources of drinking water and the number of reported cases of gastrointestinal illnesses (Redman, et al. 2007; Jagai, et al., 2015). Other pollutants that have been reported in sewer overflows include suspended solids, nutrients, oxygen depleting substances, organic compounds and toxic pollutants (USEPA 2004a). In addition to human health, these contaminants can have devastating environmental impacts including hypoxia, algal blooms, and aquatic habitat degradation (USEPA 2015).

The USEPA (2004) has reported that approximately 70% of SSOs reach surface waters. One potential contaminant in SSOs that is a concern for public and environment health are metals. Metals, such as mercury (Hg), can bioaccumulate through aquatic food webs and concentrate in the bodies of those that consume contaminated fish and shellfish (Chen et al, 2000; Clarkson and Magos 2006). Elevated metal concentrations within the human body can lead to adverse health effects. For instance, Hg is a well-known, potent neurotoxin (Clarkson 1997), lead can affect cognitive and behavioral development (Goldstein 1990), cadmium can cause renal and bone disorders (Jarup and Akesson 2009) and arsenic is a carcinogen that can affect multiple organs (Smith et al, 1992). Metals are persistent in the environment which increases the chance for long term exposure. Urban

waterways have been found to be particularly enriched in trace metals due anthropogenic sources including dry and wet deposition of industrial metal particulates, leaching from metal infrastructure, vehicle travel and wear, and urban runoff (Timperly, et al. 2005, Dean, et al. 2005, Rose and Shea, 2007). Studies have shown that combined sewer overflow effluents during wet-weather events often contain measurable metal concentrations including Hg, copper, lead and zinc, primarily due to large inputs of storm-water runoff (Mullis, Revitt and Shutes 1997; Gasperi, Garnaud and Vincent 2008). Within sewer systems, metals may also be sourced from industrial and/or municipal wastewaters. Hg, for example, is excreted from the body through both urine and feces in the form of inorganic Hg (II) (Clarkson and Magos 2006; Rothenberg, et al 2016) and could be introduced into sewer system along with human wastes. SSOs, which contain raw municipal sewage, other wastewaters, and in some cases, storm-water and/or groundwater, may also contain metals including Hg, and could be an additional contributing factor to elevated metal concentrations in urban waterbodies.

The unprecedented rainfall and flooding of October 2015 in Columbia, SC caused massive sewage spills across the city (Trainor 2016). Motivated by the events of the October 2015 floods, SSOs in Columbia were investigated with the objective to determine whether sewage inputs were a source of metals. It was hypothesized that metal concentrations in the SSOs would be elevated above concentrations in nearby creek reference sites that received the SSO effluents as well as in the treated effluents from a WWTP outfall.

CHAPTER 2

METHODS

2.1 Approach

Over a 5-month sampling period following the October 2015 flooding in Columbia, South Carolina (November 2015-March 2016), surface water samples from three sewage-impacted creeks, Crane Creek, Stoop Creek and Gills Creek, were collected monthly and analyzed for total mercury (THg), methylmercury (MeHg), and 34 other metals (Table 2.1). During this period, three sewage events that impacted the creeks were captured including a SSO at Crane Creek (December 10, 2015), a ruptured force main sewer pipe at Stoop Creek, and one site downstream from SSOs in Gills Creek. Two additional SSOs at Crane Creek during Hurricane Matthew (October 9, 2016) and one SSO during a heavy rain event (January 3, 2017) were captured following initial sampling. At Crane Creek and Stoop Creek, surface water samples from downstream and upstream reference sites were collected while only one site in Gills Creek was sampled. For Crane Creek and Stoop Creek, average metal concentrations in the sewage events were compared to the mean of the nearby creek reference sites; in Gills Creek, metal concentrations downstream from SSOs were compared to the average metal concentrations measured in Gills Creek on other sampling days when SSOs were not occurring. Metal concentrations in sewage events were also compared to average metal concentrations from the WWTP outfall (i.e. treated sewage) that discharges effluents into Stoop Creek.

In addition to metal concentrations, dissolved organic carbon (DOC) ($n=41$) and the $\delta^{13}\text{C}$ values of DOC were measured for a subset of the samples ($n=34$). Relationships between metals and DOC in the sewage events and the creek reference sites were examined to look at the potential impacts of elevated DOC concentrations from sewage events on metal cycling. The $\delta^{13}\text{C}$ values of DOC were determined to indicate possible differences in the source of DOC between the SSO events and the creek reference sites. Additionally, amino acid concentrations for a select subset of samples ($n=7$) were measured to help identify the degree to which microbial activity had influenced DOC.

2.2 Study Locations

Sampling locations were situated in three creeks that drain into the Lower Broad, Lower Saluda and Congaree Rivers (Figure 2.1). All three rivers are used for primary (swimming) and secondary (boating and fishing) recreation (SCDHEC 2007; SCDHEC 2011). In addition, the Lower Broad is a source for Columbia's drinking water supply (approximately half the City's 375,000 customers), via the Broad River Diversion Canal (www.columbiasc.net/drinking-water/educational-programs/facts). As of 2016, five sites within the Lower Broad River, Lower Saluda River and Congaree River were listed as impaired on South Carolina's 303(d) list for high metal concentrations, including Hg and copper, as well as high *E. coli* levels (Table 2.2) (SCDHEC 2016a). Due to Hg impairments, fish consumption advisories have been issued by the South Carolina Department of Health and Environmental Control for both the Lower Saluda River and the entire length of the Congaree River (from the City of Columbia to the Santee River) (SCDHEC 2016b). Three tributaries of these major river systems, Crane Creek, Stoop Creek, and Gills Creek, were selected for this study because they have a history of being

impacted by SSOs and/or had experienced sewage-related issues during the October 2015 flooding.

2.2.1 Crane Creek.

SSOs have been a chronic problem at Crane Creek, a tributary to the Lower Broad River, due to the aging infrastructure of the sanitary sewer system within the Crane Creek Basin (USEPA 2013). This sanitary sewer system is capacity limited, meaning it cannot handle additional flows of groundwater and storm-water that infiltrate the leaky system during heavy precipitation events. In 2014 and 2015 (excluding the 2015 flood), approximately 1.0 and 4.5 million gallons of sewage was released near Crane Creek, respectively (SCDHEC 2017a). To eliminate future wet weather SSOs, capacity enhancement projects are planned and/or are under construction to increase the size of the gravity sewer line (City of Columbia 2017a) that runs along a portion of Crane Creek as well as replace a sewer main to expand capacity and increase overall operation efficiency (City of Columbia 2017b).

Sampling in Crane Creek included downstream and upstream reference sites as well as from SSOs and a ditch which funneled untreated sewage from the SSOs directly into Crane Creek (Figure 2.2). Samples were collected directly from an overflowing manhole adjacent to the creek during three separate SSOs. An additional sample was collected from an overflowing manhole within the same sanitary sewer system, located upstream, where effluents also flowed into Crane Creek. Additional SSOs occurred during our sampling period that were not captured. On multiple occasions, routine monthly sampling occurred several days or weeks following a SSO event (Figure 2.3). Two reference sites were established and sampled from within the creek, one located

upstream and across the creek (approximately 25 meters) from the ditch (approximately 25 meters) and one located downstream from the ditch (approximately 50 meters). Metals concentrations were comparable in both reference sites, and therefore, only the downstream site was sampled routinely.

2.2.2 Stoop Creek

Located at Stoop Creek, a tributary to the Lower Saluda River, is a WWTP that treats minor domestic wastes and discharges treated wastewater directly into Stoop Creek (USEPA 2017a). In the past, this WWTP has been cited for being out of compliance due to high levels of fecal coliform, nitrogen and chlorine (USEPA 2017b). Samples were collected from the WWTP outfall as well as from five reference sites within the creek: one upstream (approximately 100 m), two near the WWTP (directly above and directly below the WWTP), and two sites further downstream (approximately 75m and 90m) (Figure 2.4).

In February 2016, during our monthly sampling, a ruptured force main sewer pipe owned by the City of Columbia was discovered within the creek further downstream (approximately 125 m). Following discovery of the broken sewer pipe, repairs were conducted within 48 hours which included the replacement of the broken pipe segment followed by encapsulating the new pipe with cement to prevent future external damage (Bill Stangler, Congaree Riverkeeper, personal communication). The South Carolina Department of Health and Environmental Control reported that approximately half a million gallons was spilled (SCDHEC 2017a); however, without knowing the duration of the spill, the actual amount of untreated sewage released into Stoop Creek remains

unknown. Samples were collected twice at the site of the broken sewer pipe in the subsequent month following repairs (Figure 2.5).

2.2.3 Gills Creek

Gills Creek flows through the northeastern section of Columbia through a series of lakes before draining into the Congaree River south of the city (SCDHEC 2011). Sampling in Gills Creek occurred downstream from Lake Katherine (Figures 2.6), an area which is routinely impacted by wet-weather SSOs (Figure 2.7), as follows. In 2015 (excluding the 2015 flood) and 2016 approximately 81,000 and 90,000 gallons of untreated sewage was spilled near Lake Katherine (SCDHEC 2017a). In addition, the October 2015 flooding greatly impacted the Gills Creek watershed. Like at Crane Creek, the City of Columbia has capacity enhancement projects planned for the Gills Creek Basin to expand and upgrade the sanitary sewer system in this area, including the Lake Katherine Sewer Capacity Enhancement Project which will replace the outdated gravity sewer line which experiences wet-weather SSOs near Lake Katherine (City of Columbia 2017).

2.3 Equipment and Sample Preparation

2.3.1 Hg

Boston round narrow-mouth amber glass bottles (125-mL) with Teflon-lined lids (Thermo Scientific, #149-0125) were used to collect and store samples for THg and MeHg analyses to reduce the potential of photochemical degradation of MeHg. Prior to use, the bottles were acid-cleaned using 1.2N hydrochloric acid (HCl): filled for ≥ 24 hours and then tripled-rinsed with ultrapure water ($\geq 18 \text{ M}\Omega \text{ cm}^{-1}$). Samples were filtered and preserved on the same day of collection. One duplicate bottle for each sample was

filtered using a 30-mL sterile syringe (BD Luer-Lok, #302832) and a mixed cellulose esters syringe filter (0.22 μm) (Fisherbrand, #09-720-004). Unfiltered and filtered samples were stored at 4° C in double-bagged, acid-cleaned amber glass bottles for THg analysis while unfiltered and filtered samples for MeHg analysis were stored at -80° C in double-bagged, 50 mL Falcon conical centrifuge tubes. All samples were preserved using 0.5% (v/v) ultrapure HCl (OmniTrace).

2.3.2 *Other Metals*

Acid-cleaned wide-mouth polyethylene bottles were used to collect samples for the other metal analyses. Prior to use, bottles were soaked in 10% nitric acid (≥ 24 hours) and then triple rinsed with ultrapure water ($\geq 18 \text{ M}\Omega \text{ cm}^{-1}$). Samples were filtered and preserved on the same day of collection. Approximately 8 mL of each sample was filtered using a 12-mL sterile syringe and hydrophobic filter (0.22 μm) into 15 mL acid-cleaned Falcon conical centrifuge tubes. Filtered samples were preserved using 0.5 mL concentrated nitric acid. To each filtered sample, 80 μL of 1 ppb indium was added as an internal standard to monitor drift.

2.3.3 *DOC, $\delta^{13}\text{C}$ Values of DOC, and Amino Acids*

Samples were collected in acid-washed (10% HCl overnight, followed by 100% Milli-Q water overnight), low density polyethylene cubitainers and stored in the dark until processed in the laboratory, within 24 hours. Samples for DOC, the isotopic composition of DOC, and amino acids were filtered through 0.2 μm polyethersulfone syringe filters into acid-washed, high density polyethylene bottles and stored frozen until analysis.

2.3.4 pH and Temperature

The pH and temperature was measured using a pH/mV/°C meter (Oakton pH 11 Series) with a temperature probe attachment. The meter was calibrated for pH in the laboratory at 25 °C within one day prior to sampling using 3 pH standards. For pH measurements collected without the temperature probe, a temperature correction factor was applied using the Nernst equation (i.e., for every 10°C change in temperature between the sample and the calibration standards, pH is expected to change by 0.03 units per log unit).

2.4 Laboratory Methods

2.4.1 THg and MeHg Analysis.

Filtered and unfiltered HCl-preserved water samples were analyzed for MeHg concentrations within six months of sample collection following the U.S. EPA Method 1630 (EPA 2001). Briefly, samples (45 mL) were aliquoted into 60 mL acid-washed Teflon distillation vials along with 200 µL of 1% ammonium pyrrolidine dithiocarbamate solution used to improve MeHg recovery. Samples (35 mL) were distilled using nitrogen gas(N²) into 60 mL Teflon receiving vials (Brooks Rand, Seattle, WA). Sample pH was adjusted to 4.9 using 2M acetate buffer, and 1% sodium tetraethyl borate dissolved in 2% potassium hydroxide was used as a derivatizing agent. Samples were purged with N² gas onto Tenax columns, thermally desorbed and MeHg was quantified using gas chromatography and cold vapor atomic fluorescence spectrometry (CVAFS) (Brooks Rand, Seattle, WA).

Filtered and unfiltered HCl-preserved water samples were analyzed for THg concentrations following U.S. EPA Method 1631, Revision E (EPA 2002). Briefly,

samples were digested with 0.5% (v/v) 0.2 N bromine monochloride (BrCl) for a minimum of 12 hours. Digested samples (5-10 ml) was aliquoted into 40 mL borosilicate vials with Teflon-line caps (Brooks Rand, Seattle, WA). To neutralize BrCl, 0.3% hydroxylamine hydrochloride was added, and then the samples were reduced using tin (II) chloride to convert inorganic mercury to elemental mercury. Reduced samples were purged with N² gas, and elemental mercury was captured and then thermally desorbed from gold traps (Brooks Rand, MERX-T). THg was quantified using CVAFS (Brooks Rand Model III, Seattle, WA).

2.4.2 Other Metal Analysis

Metal analysis were performed using trace metal clean techniques. Samples were analyzed for 34 elements using high resolution inductively coupled plasma mass spectrometry (HR-MS-ICP) (ThermoFisher ELEMENT II). Prior to analysis the instrument was configured for operations and tuned for the metals of interest at low, medium, and high resolutions.

2.4.3 DOC, $\delta^{13}\text{C}$ of DOC and Amino Acid Analysis

DOC concentrations were analyzed by high temperature combustion using a Shimadzu total organic carbon and total nitrogen analyzer. Deep seawater reference standards were injected every 6th sample and were within the range of reported values (41-44 $\mu\text{mol/L}$).

The $\delta^{13}\text{C}$ of DOC was analyzed by the method of Lang et al. (2012). In brief, 4 mL of sample was transferred to a pre-combusted (500°C, 5 hrs) 12 mL borosilicate Exetainer® vial (Labco, High Wycombe, UK) and acidified to a pH <3 with phosphoric acid (H₃PO₄). 1 mL of sodium persulfate oxidizing solution (100 mL H₂O + 4 g Na₂S₂O₈

+ 200 μL H_3PO_4) was added, the vial sealed, and the samples were flushed with high purity helium (Grade 5.0, 99.999% He) for 5 minutes at 100 mL/min. The samples were heated at 100°C for 1 hour to convert organic carbon to carbon dioxide (CO_2). The isotopic signature of the resulting CO_2 was analyzed using a GasBench II preparation device connected to a ConFlo IV interface and a Delta V Plus mass spectrometer (Thermo Fisher Scientific). Values were determined using standards prepared over a concentration range that bracketed the samples and had been previously calibrated to IAEA standards (Sucrose, -12.4 ‰; Phthalic Acid, -33.6 ‰) and are reported versus Vienna Pee Dee Belemnite (VPDB).

Amino acid concentrations were determined using a method adapted from Kaiser and Benner (2005). Sample aliquots (400 μL) were subjected to vapor phase hydrolysis with a CEM Discover Microwave system. The Teflon PFA hydrolysis vessel contained 10 mL of 6N HCl and was set to the following conditions: Power = 150 W, ramp to 165°C in 5 min, hold for 15 minutes, cool to <30°C before ending. After hydrolysis, 100 μL of MilliQ-H₂O was added to each and dried under a N₂ stream to neutralize the sample pH. Once dried, 400 μL of MilliQ-H₂O was added to each and the pH of the samples was determined. Neutral samples were either analyzed by HPLC immediately, or dried and stored at -20°C.

The concentrations of L- and D- enantiomers of amino acids contained in the hydrolyzed samples were determined with a Thermo UltiMate 3000 UHPLC. Derivatization of the samples with o-Phtaldialdehyde (OPA) + N-isobutyryl-L-cysteine (IBLC) and o-Phtaldialdehyde (OPA) + N-isobutyryl-D-cysteine (IBDC) was completed in-line immediately prior to injection. 150 μL of sample was combined with 10 μL of

either OPA/IBLC or OPA/IBDC and reacted for 10 min. 5 μ L of sample was injected and separation was completed on an Accucore XL C18 column (100x3 mm, 4 μ m particle size). A gradient mobile phase was used beginning with 95% (A) 25 mM Sodium Acetate (pH = 5.3) and 5% (B) Methanol, reaching 20% A: 80% B at 24 min before returning to the initial conditions. The HPLC flow was 0.8 mL min⁻¹ and the run time was 32 min. The system equilibrated for 10 min while the next sample was being derivatized. Fluorescence detection was utilized with excitation at 350 nm and emission recorded at 420 nm. Standards for each amino acid analyzed were prepared ranging in concentration from 5 to 250 nM. The limit of detection was 5 nM.

2.5 Quality Control and Quality Assurance (QA/QC)

2.5.1 THg and MeHg

QA/QC parameters for THg and MeHg are summarized in Table 2.3. For THg, mean recoveries for matrix spikes ($n=15$) and standard reference materials ($n=20$) ranged from 93 to 105%. Water spikes ($n=21$) and standard reference materials ($n=24$) for MeHg, resulted in mean recoveries ranging from 69-108%. For THg field duplicates ($n=3$), RSDs (100 x standard deviation/mean) averaged 25% (range: 15-36%). For MeHg field duplicates ($n=4$), RSDs averaged 6.9% (range: 3.4-8.8%). Additional field duplicates had been collected; however, values were below the method detection level (MDL), so RSDs were not calculated.

THg and MeHg concentrations were calculated using a daily calibration curve with a minimum of five standard points and a regression coefficient (R^2) ≥ 0.99 . The MDL was estimated from the region of the calibration curve where there was a significant change in sensitivity (for THg = 5 pg, for MeHg = 0.5 pg) (40 CFR 136,

Appendix B). For THg and MeHg, the MDL was 0.5 ng/L and 0.01 ng/L, respectively. Measurements that were <MDL were computed as half the MDL for statistical computation. All unfiltered THg were >MDL while 12 filtered THg samples were <MDL. For unfiltered and filtered MeHg, 4 and 7 samples were <MDL, respectively.

2.5.2 Other Metal QA/QC

QA/QC parameters for the other metals are summarized in Table 2.4. RSDs for field duplicates ($n=12$) ranged from 1.5% to 24% for 34 metals. Sample concentrations were determined using a 1 ppb standard that was verified using an Icelandic Basalt, BIR-1 rock external standard (U.S. Geological Survey). For the major cations (magnesium, calcium and iron), the BIR-1 rock standard was used to calculate concentrations in the samples.

2.6 Data Analysis

Statistical calculations were conducted using R Version 3.3.2 software. Bivariate relationships were explored using box-and-whisker plots (for categorical variables) and two-way scatterplots (for continuous variables). Correlations between metals were investigated using Spearman's correlation. Metal correlations were also examined using principle component analysis (PCA) which was run using 36 metals as variables. Prior to running PCA, variables were \log_{10} -transformed to correct skewness, centered and scaled. Wilcoxon rank sum test was used to compare difference between two groups while Kruskal-Wallis test was used for groups ≥ 3 . An alpha-level of 0.05 was used as a guide for significance for all analyses.

Table 2.1 Locations and dates of sampling sites for creek reference sites and sewage events.

Creek	Depth/Width	Location	Longitude	Latitude	Dates
1. Stoop	0.37m/4.85 m	a. Upstream Reference Site	-81.11456	34.029496	11/18/15, 12/10/15, 01/18/16, 02/17/16, 03/31/16
	0.21m/4.13m	b. Above WWTP Outfall Reference Site	-81.113761	34.028806	11/18/15
	0.38m/6.38m	c. Below WWTP Outfall Reference Site	-81.113822	34.028647	11/18/15, 12/10/15, 01/18/16, 02/17/16, 03/31/16
	0.32m/4.79m	d. Downstream Reference Site 1	-81.113292	34.028089	11/18/2015, 12/10/16
	0.18m/5.06m	e. Downstream Reference Site 2	-81.113592	34.02801	11/18/15, 12/10/15, 01/18/16, 02/17/16, 03/25/16, 03/31/16
	0.34m/7.50m	f. Downstream Reference Site 3	-81.113571	34.027825	03/25/16, 03/31/16
		g. Alpine Utilities WWTP Outfall	-81.113858	34.028732	11/18/15, 12/10/15, 01/18/16, 02/17/16, 03/31/16
		h. Ruptured Force Main	-81.113571	34.027825	02/17/16
2. Crane	1.4 m/16.5 m ^a	a. Upstream Reference Site	-81.06001	34.054043	12/10/15, 01/03/17 12/10/15, 01/16/16, 01/18/16, 01/22/16, 02/17/16, 03/31/16, 10/09/16
		b. Downstream Reference Site	-81.059926	34.054030	12/10/15, 12/31/15, 01/16/16, 01/18/16, 01/22/16, 02/04/16, 02/17/16, 03/31/16, 10/09/16
		c. Ditch	-81.060273	34.054030	12/31/15, 10/09/16, 01/03/17
		d. SSO next to Creek	-81.060470	34.054030	10/09/16
		e. SSO Upstream	-81.058228	34.054030	11/18/15, 11/19/15, 12/10/15, 01/18/16 02/05/16, 02/17/16, 03/31/16
3. Gills	0.43m/19.2 m ^b	a. Downstream from Lake Katherine (Downstream from SSOs)	-80.974488	34.054030	

^aDepth and width measurements taken from road bridge adjacent to upstream reference site during low flow.

^bDepth and width measurements taken from road bridge just upstream from sampling location during low flow.

Table 2.2 South Carolina Section 303(d) list of impaired waters within study area.

<u>Watershed</u>	<u>Waterbody</u>	<u>Designated Use</u>	<u>Impairment</u>
1. Broad	a. Broad River	Aquatic Life	Copper
	b. Crane Creek	Aquatic Life	Dissolve Oxygen
		Aquatic Life	Macroinverbrates
2. Saluda	a. Saluda River	Recreation	E. coli
		Fish	Mercury
		Aquatic Life	Turbidity
	b. Congaree River	Recreation	E. coli
		Fish	Mercury
		Aquatic Life	Copper
	c. Broad River Diversion Canal	Recreation	E. coli
	d. Stoop Creek	Aquatic Life	Macroinverbrates

Table 2.3 Quality assurance/quality control for THg and MeHg analysis including mean percent recoveries for standard reference materials, matrix spikes and aqueous spikes and the relative standard deviations for the field duplicates.

Analyte	Standard Reference Material				Matrix Spike % (n)	Aqueous Spike % (n)	Field Duplicate % (n)
	NIST-955c % (n)	NIST-1568b % (n)	TORT-2 % (n)	ERM-CC58 % (n)			
THg	105 (4)	97 (14)	N/A	93 (2)	98 (15)	N/A	26 (3)
MeHg	88 (8)	N/A	108 (16)	N/A	N/A	69 (21)	6.2 (4)

Table 2.4 Quality assurance/quality control for metal analysis including mean percent recoveries for standard reference materials and relative standard deviations for field and analytical duplicates.

Metal	Standard Reference Material		Field Duplicates % (n=12)	Analytic Duplicates % (n=5)
	1 ppb % (n=4)	BIR-1 % (n=2)		
Rb	102	113	2.0	2.4
Sr	104	103	1.8	2.5
Cd	106	128	4.4	4.9
Cs	104	80	2.8	4.5
Ba	107	97	2.1	1.8
Tl	107	81	9.2	3.3
Pb	109	106	4.7	3.1
U	104	103	3.9	3.6
Mg	n/a ^a	106	1.5	2.4
Al	78	108	5.1	4.6
Ca	n/a ^a	103	1.6	6.2
V	107	115	2.8	3.5
Cr	108	112	7.1	2.5
Mn	107	105	1.5	2.3
Fe	n/a ^a	103	5.3	3.8
Co	106	105	1.8	2.4
Ni	108	111	2.0	3.1
Cu	108	107	2.0	2.8
Zn	110	107	4.8	4.8
Ga	106	104	7.4	5.6
As	95	N/A	7.1	7.5
La	98	93	4.3	4.0
Ce	98	94	4.1	3.9
Pr	101	100	4.6	2.9
Nd	104	100	4.6	3.5
Sm	105	103	3.8	2.7
Eu	105	103	5.1	3.8
Gd	111	60 ^b	2.9	2.5
Dy	106	110	5.4	2.7
Ho	106	107	4.3	2.3
Er	105	111	3.4	4.0
Tm	106	107	5.3	3.9
Yb	107	108	4.9	3.4
Lu	104	117	24	5.7

^aConcentrations for major cations (magnesium, calcium and iron) too low in 1 ppb standard so Icelandic Basalt, BIR-1 rock external standard used to calculate major cation concentrations in samples.

^bLow percent recovery for gadolinium in Icelandic Basalt, BIR-1 rock standard attributed to oxidation interference.

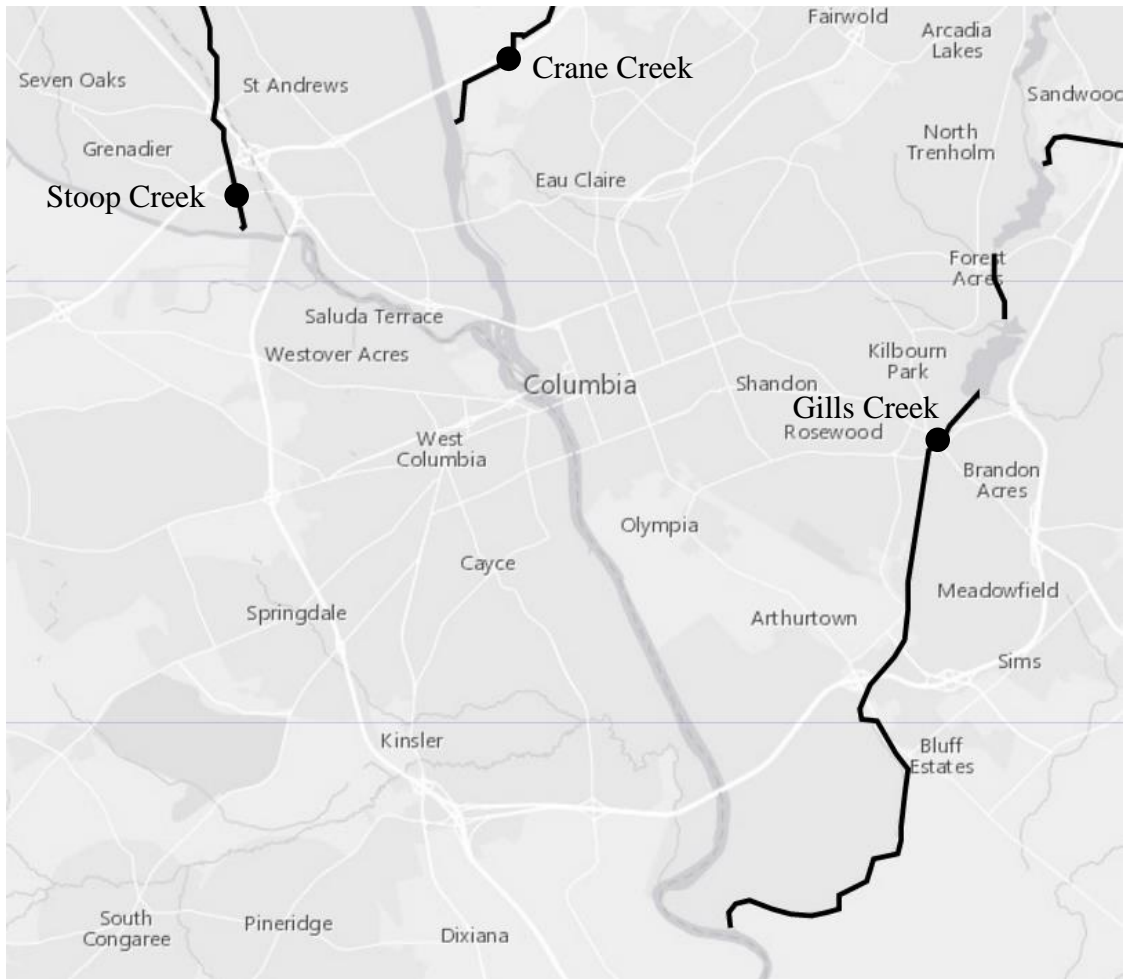


Figure 2.1 Location of creeks and sampling locations.

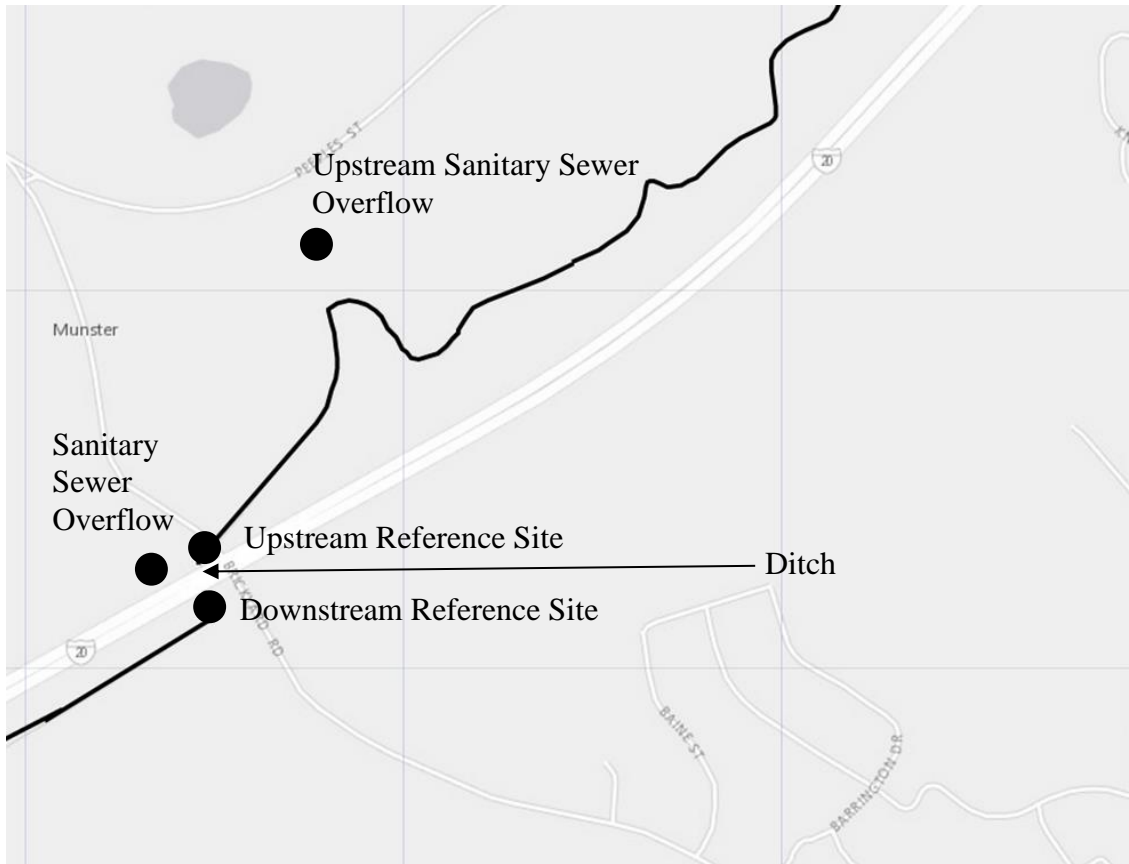


Figure 2.2 Crane Creek reference sites, sanitary sewer overflows and ditch.

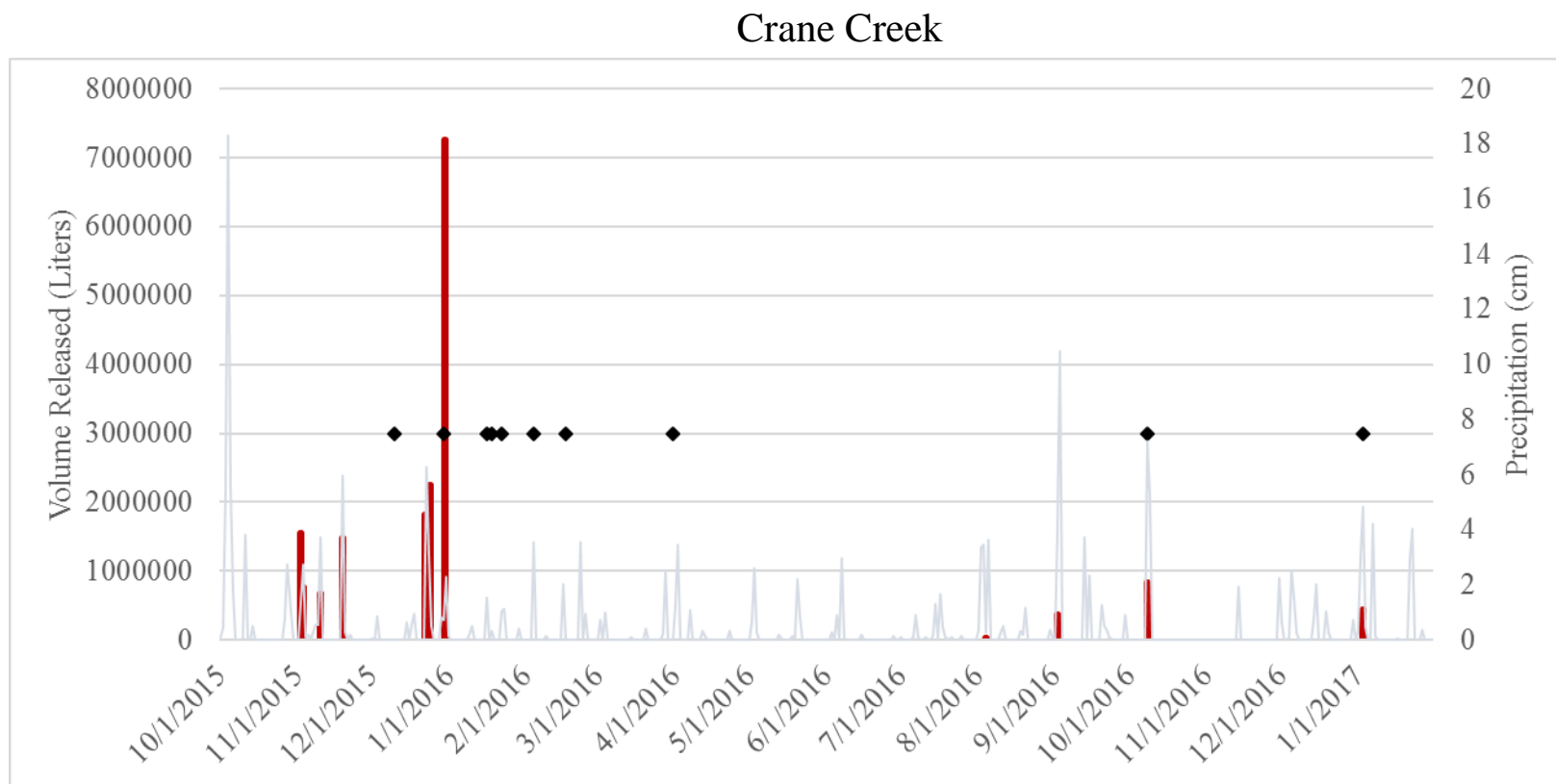


Figure 2.3 Sampling dates (black diamonds) compared to volumes released during sanitary sewer overflows (red bars) at Crane Creek and precipitation amounts (light gray line).

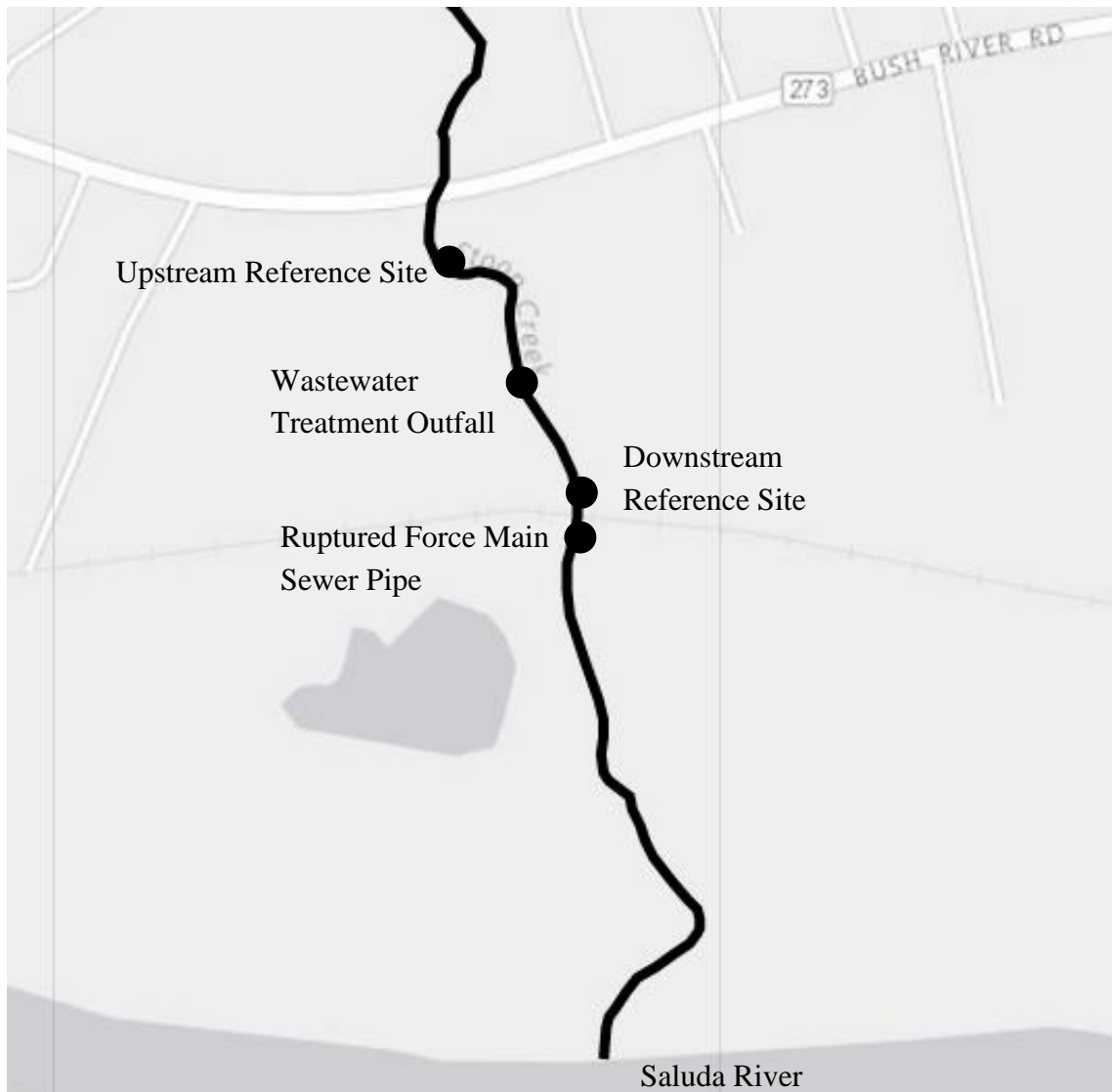


Figure 2.4 Stoop Creek upstream and downstream reference sites, wastewater treatment plant outfall, and ruptured force main sewer pipe.

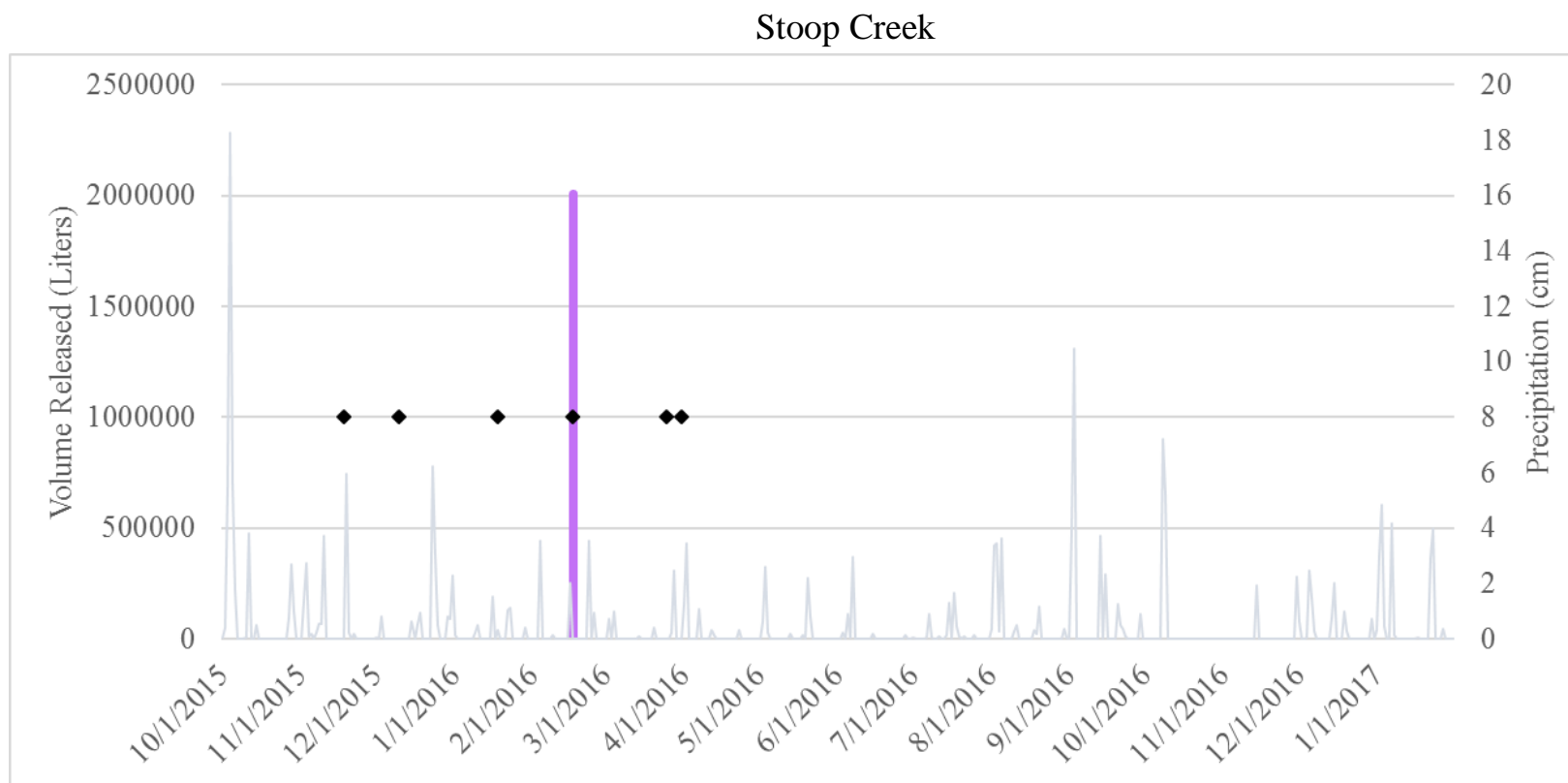


Figure 2.5 Sampling dates (black diamonds) compared to volume released from ruptured force main sewer pipe (purple bar) at Stoop Creek and precipitation amounts (light gray line).

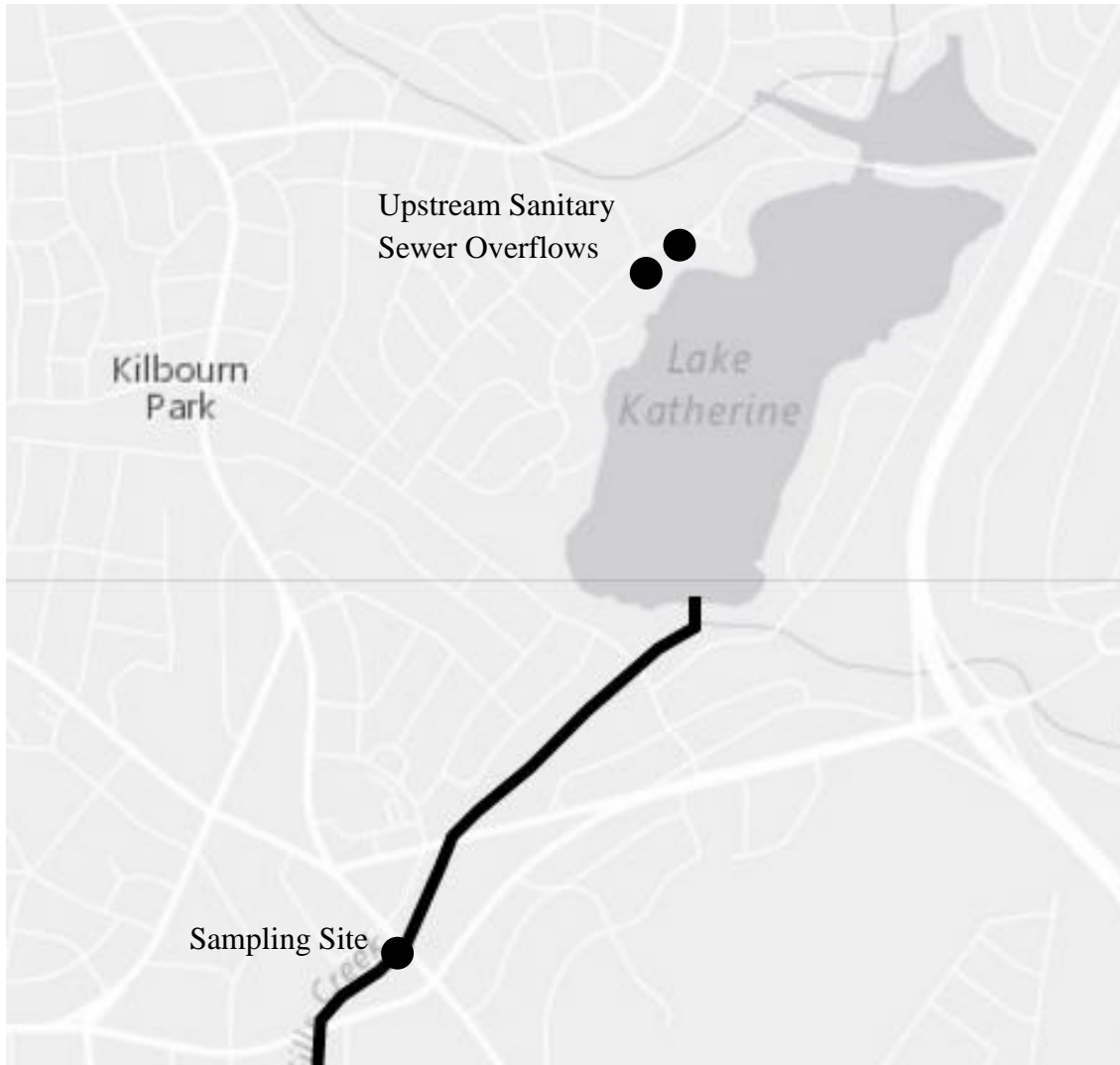


Figure 2.6 Gills Creek sampling site and upstream sanitary sewer overflow.

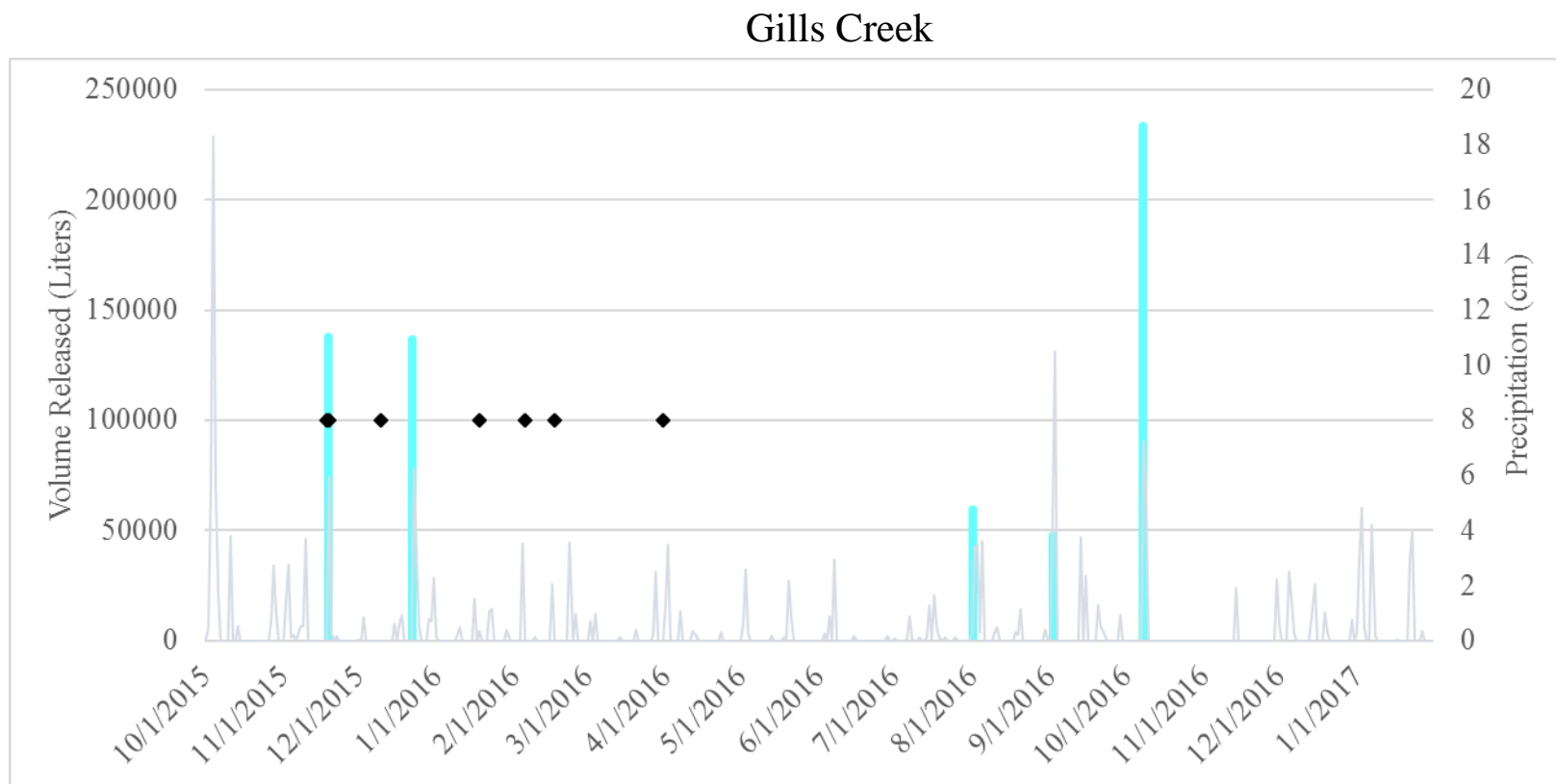


Figure 2.7 Sampling dates (black diamonds) compared to volume released from upstream sanitary sewer overflows (light blue bars) at Gills Creek and precipitation amounts (light gray line).

CHAPTER 3

RESULTS

3.1 THg and MeHg concentrations in the reference sites

Unfiltered and filtered THg and MeHg concentrations are summarized in Tables 3.1 and 3.2, respectively. As noted above (Section 2.2), Stoop Creek and Crane Creek included upstream and/or downstream reference sites while the mean concentrations observed in Gills Creek during sampling dates when SSOs were not occurring were used as references for comparisons. Spatial differences were observed in the unfiltered and filtered THg and MeHg concentrations between the creek reference sites (Figure 3.1 and 3.2). In the three creeks, the trend for average and median THg concentrations (unfiltered and filtered) was Crane Creek > Gills Creek > Stoop Creek (Kruskal-Wallis, $p < 0.01$). This same trend was observed for average unfiltered MeHg concentrations and median MeHg concentrations (unfiltered and filtered), while the trend for the average filtered MeHg varied slightly: Crane Creek > Stoop Creek > Gills Creek (Kruskal-Wallis, $p < 0.01$, for all). On average, reference sites in Crane Creek had the highest unfiltered and filtered THg and MeHg concentrations while Stoop Creek had the lowest unfiltered and filtered THg and unfiltered MeHg.

Unfiltered %MeHg (of THg) ($100 \times C_{\text{MeHg}}/C_{\text{THg}}$) varied between 0.59% to 8.9% while filtered %MeHg (of THg) had a wider range of 0.17% to 38% (Table 3.3). The trend observed in average unfiltered and unfiltered %MeHg (of THg) was Stoop Creek > Crane Creek > Gills Creek. While Stoop Creek had the highest %MeHg (of THg) and

Gills Creek had the lowest %MeHg (of THg) for both the unfiltered and filtered fractions, there was no significant difference in %MeHg (of THg) between the creek reference sites (Kruskal-Wallis, $p > 0.20$).

THg and MeHg %particulate fractions ($100 \times (C_{\text{unfiltered}} - C_{\text{filtered}}) / C_{\text{unfiltered}}$) are shown in Tables 3.1 and 3.2, respectively. In the reference sites, THg concentrations were, on average, higher in the particulate fraction compared to the filtered fraction (mean %particulate: 73%, range: 47-92%). The trend for %particulate THg was Gills Creek > Crane Creek > Stoop Creek; however, there was no significant difference for %particulate THg between the three creeks (Kruskal-Wallis, $p = 0.33$). Conversely, differences in the %particulate MeHg concentrations between the three creeks was significant (Kruskal-Wallis, $p < 0.01$). The trend in the average %particulate MeHg was Gills Creek > Crane Creek > Stoop Creek. MeHg concentrations were higher in the filtered fraction, on average, compared to the particulate fraction in both Stoop Creek (mean %particulate: 29%, range: 0-71%) and Crane Creek (mean %particulate: 47%, range: 18-92%) while in Gills Creek, particulate-bound MeHg concentrations were, on average higher than filtered MeHg (mean %particulate=59%, range: 46-75%). Results suggest that MeHg in Gills Creek was more particulate-bound compared to the other two sites.

3.2 THg and MeHg concentrations during sewage events

As stated above (Section 2.2), the 5-month sampling campaign included the following sewage events: a SSO at Crane Creek (December 31, 2015), a ruptured force main in Stoop Creek (February 17, 2016), and downstream from two active SSOs (November 19, 2015) in Gills Creek. Two additional events were sampled at Crane

Creek: two SSOs during Hurricane Matthew (October 9, 2016) and one SSO during a heavy rain event (January 3, 2017). Samples were also frequently collected from the ditch at Crane Creek that conveyed sewage runoff into the creek ($n=10$, December 10-March 30, October 9).

Compared to the average concentrations observed in associated creek reference sites, sewage events had elevated THg and MeHg concentrations (Table 3.4). Unfiltered THg concentrations were elevated in all SSOs at Crane Creek (Figure 3.3); unfiltered and filtered THg concentrations in the SSO discharges at Crane Creek ($n=4$) were, 4.1 and 1.4 times higher, on average, than the mean of the Crane Creek reference sites. On average, unfiltered and filtered MeHg concentrations were comparable to the Crane Creek reference sites; however, during the December 31 SSO, unfiltered and filtered MeHg concentrations were both elevated by factors of 2.3 and 1.8, respectively, compared to the mean of the Crane Creek reference sites. Elevated unfiltered and filtered MeHg concentrations (2.9 and 3.3 times higher, on average) were also observed in the ditch at Crane Creek while unfiltered and filtered THg were, on average, comparable or lower than the mean of the Crane Creek reference sites. In Stoop Creek (Figure 3.4), the ruptured force main sewer pipe had unfiltered and filtered THg concentrations that were 3.3 and 2.0 times higher, respectively, than the average of the Stoop Creek reference sites while only unfiltered MeHg was elevated by a factor of 3.8. Following repairs, concentrations of THg and MeHg at the site of the previously broken sewer pipe declined and were comparable to the average of the Stoop Creek reference sites. In Gills Creek (Figure 3.5) while SSOs were occurring upstream (November 19, 2015), unfiltered and filtered THg concentrations were, on average, 3.5 and 1.8 times higher, than the mean

concentration observed at Gills Creek on other dates, respectively, and only unfiltered MeHg was elevated by a factor 1.7.

While THg and MeHg concentrations were, on average, higher in the sewage events compared to the reference sites, mean unfiltered and filtered %MeHg (of THg) (1.4% and 2.9%, respectively) was significantly lower than the creek reference sites (mean: 3.2% and 11%, respectively) (Wilcoxon rank sum test, $p < 0.02$, for both). This result makes sense, however, because THg concentrations were significantly elevated in the sewage events compared to the creek reference sites (Wilcoxon rank sum test: $p < 0.01$). The %MeHg (of THg) is dependent on THg concentrations, therefore, significantly higher THg concentrations will result in lower %MeHg (of THg).

In the ditch at Crane Creek that conveyed SSO effluents ($n=10$), average unfiltered and filtered %MeHg (of THg) were 3.0 and 2.5 times higher than the mean of the Crane Creek sites, respectively. On December 10, both %MeHg (of THg) and MeHg concentrations were elevated (Figure 3.3). Compared to the mean of the Crane Creek reference sites, unfiltered and filtered MeHg concentrations were 12 and 17 times higher, respectively, while unfiltered and filtered %MeHg (of THg) were 10 and 7.5 times higher. No active SSOs were occurring during this date; however, prior to sampling in the ditch on December 10, three separate SSOs (November 2nd, 10th and 19th) had in combination, released more than 1 million gallons of untreated sewage near Crane Creek (SCDHEC 2017a). Elevated MeHg concentrations following SSOs suggest that repeated inflows of sewage and Hg may have contributed to elevated MeHg concentrations at Crane Creek.

THg concentrations in the sewage events were, on average, higher in the particulate fraction compared to the filtered fraction (mean %particulate-bound: 86%, range: 79-98%). MeHg concentrations were also higher in the particulate fraction, on average, compared to the filtered fraction (mean %particulate-bound=66%, range: 0-97%). One SSO had a %particulate-bound value of 0 because the unfiltered and filtered MeHg concentrations were both below the MDL. When excluded, the mean %particulate-bound of the remaining sewage events increases to 76%. MeHg partitioning in the sewage events differed from what was observed in the Crane Creek and Stoop Creek reference sites (Section 3.1) which had higher MeHg concentrations in the filtered fraction than the particulate fraction, on average.

3.3 Other metal concentrations in reference sites

Spatial variation was found for filtered metal concentrations in the three reference creeks. Eight metals had higher, average filtered concentrations in the Stoop Creek reference sites, including cadmium, cobalt, nickel, zinc, rubidium, strontium, magnesium and calcium compared to Crane Creek and Gills Creek reference sites (Figures 3.6-3.13). Filtered zinc concentrations were, on average, 4.4 and 5.3 times higher compared to Gills and Crane Creek, respectively (Kruskal-Wallis, $p < 0.01$, for all). Other metals, listed above, that had higher filtered concentrations in Stoop Creek reference sites were, on average, between 1.3 and 4.0 times higher compared to Crane Creek and Gills Creek average concentrations (Kruskal-Wallis, $p < 0.05$, for all). Of the eight metals that had higher concentrations in Stoop Creek, four (including zinc, rubidium, strontium and calcium) were elevated in the WWTP at Stoop Creek compared to the average Stoop Creek reference sites (Figures 3.14-3.17). Upstream reference sites, above the WWTP,

had lower concentrations of these four metals compared to reference sites below the WWTP, indicating that one source of these four metals was most likely the WWTP.

In Gills Creeks (excluding the November 19 sewage event), filtered lead and uranium concentrations were higher compared to the reference sites in the other two creeks (Figures 3.18 and 3.19). Average filtered lead concentrations were 3.4 and 5.4 times higher than that of the Crane Creek and Stoop Creek reference sites, respectively (Kruskal-Wallis, $p=0.02$). Filtered uranium concentrations were, on average, 1.5 and 2.6 times higher compared to Crane Creek and Stoop Creek reference sites, respectively (Kruskal-Wallis, $p<0.01$).

At Crane Creek, filtered aluminum concentrations were comparable to Gills Creeks (only 1.2 times higher) but 3.0 times higher, on average, than Stoop Creek (Kruskal-Wallis, $p<0.01$) (Figure 3.20). Additionally, filtered concentrations of light rare earth elements (REE) and europium were highest in Crane Creek, averaging 1.5-2.5 times higher than the other two creeks (Kruskal-Wallis, $p<0.05$, for all) (Figure 3.21). The filtered concentrations of the heavy REEs (except gadolinium) were lower in Stoop Creek, but comparable between Gills and Crane Creek (Kruskal-Wallis, $p<0.01$, for all). All other measured metals had, on average, similar filtered concentrations in the reference sites of the three creeks (Kruskal-Wallis, $p>0.1$, for all).

3.4 Other metal concentrations in sewage events

Sewage events had elevated concentrations of some metals when compared to the reference sites (Table 3.4). In the Crane Creek SSOs, 26 metals as well as the REEs had higher concentrations than the Crane Creek reference sites ranging from 1.5 to 7.0 times higher, on average (Figure 3.22). The ditch at Crane Creek that was impacted by the

SSOs had 8 metals with higher concentrations compared to the reference sites, ranging from 1.5 to 7.0 times higher, on average (Figure 3.23). At Stoop Creek, the ruptured force main had 5 metals with higher concentrations, compared to associated reference sites, ranging from 1.5 to 2.7 times higher (Figure 3.24). Following repairs to the broken sewer pipe, three of these metals (cobalt, manganese and vanadium) had concentrations that were still higher than the mean of the Stoop Creek reference sites (1.5-2.7 times higher) while the other metals that had been elevated in the broken sewer pipe were lower or comparable (Figure 3.25). In the Gills Creek sewage event (November 19, 2015), 12 metals as well as the REEs had higher concentrations than the Gills Creek average, ranging from 1.5 to 3.8 times higher (Figure 3.26).

3.5 Comparing metal concentrations in sewage events to wastewater treatment outfall

When metal concentrations in the sewage events were compared to the metals measured in the outfall effluent (i.e, treated sewage) at the Stoop Creek WWTP (Table 3.5), the average concentrations in the sewage events had elevated levels of THg and MeHg, as well as an additional 16 other metals and the REEs. Unfiltered and filtered THg concentrations in the sewage events were 15 and 4.9 times higher, on average, than the WWTP effluents, respectively, while average unfiltered and filtered MeHg concentrations in the sewage events were 11 and 2.5 times higher, respectively. In addition, 24 other metals had elevated concentrations that were between 1.5 to 10 times higher in the sewage events, on average, compared to the WWTP outfall.

While the sewage events had elevated metal concentrations compared to the reference sites and WWTP outfall, the concentrations for eight metals that are regulated by the USEPA in drinking water (including arsenic, barium, cadmium, chromium,

copper, inorganic mercury, lead and thulium) did not exceed the maximum contaminant or action levels (USEPA 2009). Metal concentrations for these eight metals in the sewage events, were, on average, 3.4-470 times lower than the maximum contaminant and action levels.

3.6 Associations between metals in creek reference sites and sewage events

REEs were used in this study as a proxy for continental crust derived material to infer the background contribution to the dissolved metal load. Within the environment, REEs typically occur together due to similar chemical properties (Gaillardet et al. 2003). In the reference sites, REEs were significantly, positively correlated to one another (Spearman's rho: 0.68-0.98, $p < 0.01$). One exception was gadolinium which was not correlated with the other REEs (Spearman's rho: -0.12-0.11, $p > 0.5$, for all); however, several studies have reported positive gadolinium anomalies in the dissolved fraction of natural waters in densely populated areas and in waterbodies receiving sewage treatment plant effluents, which has been attributed to the use of gadopentetic acid in magnetic resonance imaging (Bau and Duluski 1996; Moller et al. 2000; Verplanck et al. 2005). Aluminum, iron, lead, THg (filtered and unfiltered), chromium and uranium, had significant, positive relationships with all the REEs (excluding gadolinium) (Spearman's rho: 0.69-0.96, $p < 0.01$). Conversely, zinc, rubidium and strontium had significant, moderate inverse relationships with most of the REEs (Spearman's rho: -0.40 to -0.62, $p < 0.05$).

In the sewage events, all REEs including gadolinium were also significantly positively correlated (Spearman's rho: 0.61-1.0, $p < 0.03$, for all). Similar to creek reference sites, filtered THg, aluminum, lead, chromium, and uranium had significant,

strong positive relationships with the REEs, (Spearman's rho: 0.67-0.92, $p < 0.05$); however, only aluminum had a significant, positive correlation with europium (Spearman's rho: 0.72, $p < 0.01$). Iron was not significantly correlated with any of the REEs (Spearman's rho=0.06-0.15, $p > 0.1$, for all), but did show a moderate, non-significant, positive trend with europium (Spearman's rho: 0.42, $p = 0.2$). In addition, vanadium and cesium were also significantly, positively correlated with the REEs (Spearman's rho > 0.75 , $p < 0.05$), except for europium. Significant, inverse relationships were observed between the REEs and manganese (Spearman's rho: -0.82 to -0.74, $p < 0.01$), except for europium.

Principal component analysis (PCA) was used to examine the differences in filtered metal concentrations in the sewage events and reference sites. The first three principal components explained 80% of the variance between samples. In Figure 3.27, the three sampled sites have some overlap within PCA space; however, Stoop Creek falls primarily on the negative end of Component 1 while Crane Creek falls mostly on the positive end of Component 1. Gills Creek lays between Stoop Creek and Crane Creek along Component 1. All three creeks fall in both sides of Component 2 and 3; however, Gills Creek is mostly in the positive end of Component 3. While differences were observed in the metal concentrations between the three creeks, overlap indicates some commonality between the three sites. In Figure 3.28, the WWTP outfall at Stoops Creek fall together at the negative end of Component 1 and the positive end of Components 2 and 3 except for one sample that falls on the negative end of Component 2 and 3 with samples from the Crane Creek ditch. Crane Creek SSOs and the sewage event at Gills Creek (November 19, 2015) fall together on the positive end of Components 1 and 2. The

SSO and ditch at Crane Creek on December 31 as well as the Gills Creek sewage event discriminated from the reference sites while the other sewage events were not as distinctly separated. The broken sewer pipe at Stoop Creek fell between the Crane Creek SSOs and the WWTP outfall on Component 1. This was not surprising because samples obtained from this spill were a mixture of both sewer discharge and creek water below the WWTP effluent outfall. The Crane Creek ditch fell both near the SSOS as well as with the reference sites. Ditch samples that clustered with the Crane Creek SSOs were collected when the SSOs were occurring and thus had similar metal concentrations to the SSO effluents. Results from the PCA support that the concentrations of some metals in the SSOs at Crane Creek as well as at Gills Creek downstream from SSOs were different from the creek reference sites.

3.7 Associations between DOC and metals in the reference sites and sewage events

DOC is known to bind with metals affecting their speciation, solubility and mobility in aquatic environments (Ravichandran 2003; Tang and Johannesson 2003; Aiken et al 2011). In the creek reference sites (Figures 3.29 and 3.30), unfiltered and filtered THg and MeHg had significant, positive relationships with DOC (Spearman's ρ : 0.42-0.58, $p < 0.05$, for all, $n=28$). In addition, there were significant, positive trends between DOC and nine of 13 REEs (including lanthanum, cerium, praseodymium, neodymium, samarium, gadolinium, dysprosium, holmium, and erbium) as well as aluminum (Spearman's ρ : 0.40-0.54, $p < 0.05$, for all, $n=28$) (Figures 3.31 and 3.32). A significant inverse relationship was also observed between DOC and barium (Spearman's ρ : -0.38, $p=0.048$). No significant relationships were observed between DOC and the

other 23 metals (Spearman's rho: -0.29 to 0.36, p:0.06-0.98, for all, n=28) in the reference sites.

We also investigated associations between metals and DOC using all data, including the sewage events, the ditch and the treated sewage (n=41). When all data is included, most relationships with DOC did not change. Exceptions include filtered MeHg which was borderline significantly, positive (Spearman's rho: 0.30, p=0.06) and barium which had marginally significant inverse relationship with DOC (Spearman's rho: -0.28, p=0.08). Relationships between DOC and THg (unfiltered and filtered), MeHg (unfiltered), and aluminum remained significantly, positive (Spearman's rho 0.43-0.54, p<0.05, n=41). In addition, 12 of the 13 REEs had observed significantly, positive relationships with DOC (Spearman's rho: 0.37-0.49, p<0.05) while europium was borderline significantly positive (Spearman's rho: 0.31, p=0.55).

3.8 Isotope composition of DOC of sewage events and reference sites

Stable carbon isotope values of DOC ($\delta^{13}\text{C}$) can provide insight into the source of DOC inputs in aquatic environments (Finley and Kendall 2007). The $\delta^{13}\text{C}$ values were measured on a subset of samples (n=30) (Table 3.6). The $\delta^{13}\text{C}$ values of DOC were relatively constant in the creek reference sites, ranging from -26.1 to -28.6‰ (Kruskal-Wallis, p=0.09). Three sewage events had measured $\delta^{13}\text{C}$ values of DOC: Crane Creek SSO (December 2015), the broken sewer pipe at Stoop Creek, and Gills Creek (November 19, 2015). Both the Crane Creek SSO and broken sewer pipe had elevated DOC concentrations (Table 3.7) and more positive $\delta^{13}\text{C}$ values than the reference creeks, -25.6‰ and -25‰, respectively. While the DOC concentrations were elevated in Gills Creek (November 19) compared to the average DOC concentrations observed during

other sampling dates when SSOs were not occurring, the $\delta^{13}\text{C}$ value of DOC (-27.7‰) was in the range of the reference sites. Similarly, within the ditch at Crane Creek, the $\delta^{13}\text{C}$ values of DOC were comparable to the reference sites, averaging -27.6‰. In Figure 3.33, the $\delta^{13}\text{C}$ value of DOC are plotted against DOC concentrations ($\mu\text{mol/L}$). Two distinct fields are apparent corresponding to the reference sites from the three creeks (as well as the November 19 Gills Creek sample) and the two sewage events with the more positive $\delta^{13}\text{C}$ values, as well as the ditch at Crane Creek that carried SSO effluent elevated in DOC during the December 31 SSO.

3.9 Total hydrolyzable amino acids

Concentrations of total hydrolyzable amino acids (THAA) and the relative molar distributions of individual amino acids were determined for an additional subset of the samples collected ($n=7$), including the ditch at Crane Creek ($n=5$), a Crane Creek reference site ($n=1$), and the ruptured sewer pipe at Stoop Creek ($n=1$), to investigate the extent of decomposition of DOC and the potential role of microbial activity. Previous studies have found that THAAs are enriched in less degraded organic matter and are preferentially used during microbial decomposition (Cowie and Hedges 1994; Davis et al. 2009). Higher THAA concentrations were observed in the broken sewer pipe (12 $\mu\text{mol/L}$) as well as from the ditch during the December SSO event (9.3 $\mu\text{mol/L}$) compared to the ditch samples without SSO effluents and the reference site (0.94-2.1 $\mu\text{mol/L}$). THAAs accounted for a higher percentage of DOC in the December ditch sample (4.6%) and the ruptured sewer pipe (6.3%) compared to the other samples (1.4-2.1%). Among the amino acids present were glycine and non-protein amino acids, γ -aminobutyric acid (GABA) and β -alanine (BALA), whose mol% have been found to

have increased during DOC decomposition in riverine samples (Benner and Kaiser 2011). The mol% of GABA and glycine in the SSO effluents in the ditch (1.9% and 7.5%, respectively) and the broken sewer pipe (1.3% and 5.6%, respectively) were lower than those of the other ditch samples and reference site (GABA: 3.3-5.6%, glycine: 8.6-13%); the mol% of BALA in the SSO effluents in the ditch and broken sewer pipe (2.6% and 4.8%, respectively) were comparable to the other samples (3.6-5.8%). Higher %THAA of DOC and lower mol% of GABA and glycine in the ruptured sewer pipe and the ditch during an SSO suggests that the DOC present in these sewage events were less degraded. As potential sources of less degraded DOC, SSOs may provide more labile DOC that is more readily utilized by heterotrophic bacteria within these impacted areas (Cowie and Hedges 1994).

Table 3.1 Summary statistics for total mercury (THg) concentrations (ng L^{-1}) for unfiltered and filtered fractions as well as percent distributed in the particulate fraction (%particulate) for reference sites and sewage events.

<i>Location</i>	<i>Unfiltered</i>		<i>Filtered</i>		<i>% Particulate</i>	
	Mean \pm 1 SD	Median (Range)	Mean \pm 1 SD	Median (Range)	Mean \pm 1 SD	Median (Range)
Reference Sites (n=37)	4.6 \pm 2.9	3.7 (1.2-11)	1.3 \pm 1.0	0.95 (BDL-4.3)	73 \pm 13	75 (47-93)
Stoop Creek Reference Sites (n=21)	2.7 \pm 0.10	2.6 (1.2-5.2)	0.87 \pm 0.74	0.73 (BDL-2.8)	71 \pm 16	75 (47-92)
Crane Creek Reference Sites (n=10)	8.3 \pm 2.7	8.2 (5.2-11)	2.2 \pm 1.1	1.9 (0.83-4.2)	73 \pm 8.3	72 (60-87)
Gills Creek Reference Sites (n=6)	4.6 \pm 1.3	4.0 (3.5-6.8)	1.0 \pm 0.80	0.73 (BDL-2.5)	81 \pm 10	81 (64-93)
Wastewater Treatment Plant Outfall (n=5) ^a	1.9 \pm 0.68	1.6 (1.2-2.9)	0.54 \pm 0.47	0.25 (BDL-1.3)	73 \pm 15	79 (55-88)
Sewage Events (n=6)	28 \pm 22	16 (9.0-72)	2.7 \pm 1.2	2.3 (1.7-5.1)	86 \pm 7.6	81 (79-98)
Broken Force Main (n=1) ^b	9.0		1.8		80	
Crane Creek SSOs (n=4) ^c	34 \pm 24	26 (15-72)	3.0 \pm 1.3	2.9 (1.7-5.1)	87 \pm 8.7	81 (79-98)
Gills Creek Downstream from SSOs (n=1) ^d	16		1.8		89	
Crane Creek Ditch (n=10)	9.8 \pm 8.4	6.5 (4.1-30)	2.0 \pm 1.2	1.7 (0.53-3.9)	74 \pm 17	76 (39-87)
All Locations (n=56)	8.0 \pm 11	4.2 (1.2-72)	1.5 \pm 1.2	1.3 (BDL-5.1)	75 \pm 14	78 (39-98)

^aWastewater treatment plant effluent outfall at Stoop Creek.

^bRuptured force main sewer pipe at Stoop Creek (February 17, 2016).

^cSanitary sewer overflow events (SSO) at Crane Creek.

^dGills Creek site downstream (approximately 2000m) from two SSOs (November 19, 2015).

Table 3.2 Summary statistics for methylmercury (MeHg) concentrations (ng L⁻¹) for unfiltered and filtered fractions as well as percent distributed in the particulate fraction (%particulate) for reference sites and sewage events.

<i>Location</i>	<i>Unfiltered</i>		<i>Filtered</i>		<i>% Particulate</i>	
	Mean \pm 1 SD	Median (Range)	Mean \pm 1 SD	Median (Range)	Mean \pm 1 SD	Median (Range)
Reference Sites (n=37)	0.13 \pm 0.083	0.093 (0.020-0.34)	0.077 \pm 0.055	0.056 (BDL-0.28)	40 \pm 23	39 (0-92)
Stoop Creek Reference Sites (n=21)	0.085 \pm 0.042	0.085 (0.020-0.23)	0.063 \pm 0.031	0.057 (0.021-0.13)	29 \pm 22	28 (0-71)
Crane Creek Reference Sites (n=10)	0.22 \pm 0.091	0.23 (0.066-0.34)	0.13 \pm 0.075	0.14 (BDL-0.28)	47 \pm 21	44 (18-92)
Gills Creek Reference Sites (n=6)	0.095 \pm 0.017	0.097 (0.073-0.12)	0.039 \pm 0.011	0.036 (0.027-0.055)	59 \pm 10	58 (46-75)
Wastewater Treatment Plant Outfall (n=5) ^a	0.022 \pm 0.024	0.015 (BDL-0.063)	0.027 \pm 0.025	0.016 (BDL-0.063)	3.1 \pm 5.5	0 (0-13)
Sewage Events (n=6)	0.23 \pm 0.16	0.18 (BDL-0.52)	0.068 \pm 0.087	0.027 (BDL-0.23)	66 \pm 34	79 (0-97)
Broken Force Main (n=1) ^b	0.32		0.049		85	
Crane Creek SSO (n=4) ^c	0.23 \pm 0.19	0.18 (BDL-0.52)	0.083 \pm 0.10	0.027 (BDL-0.23)	57 \pm 37	56 (0-97)
Gills Creek Downstream from SSOs (n=1) ^d	0.17		0.012		93	
Crane Creek Ditch (n=10)	0.63 \pm 0.76	0.40 (0.13-2.7)	0.41 \pm 0.31	0.26 (BDL-2.1)	43 \pm 26	41 (0-96)
All Locations (n=58)	0.22 \pm 0.37	0.11 (BDL-2.7)	0.13 \pm 0.28	0.061 (BDL-2.1)	40 \pm 28	40 (0-97)

^aWastewater treatment plant effluent outfall at Stoop Creek.

^bRuptured force main sewer pipe at Stoop Creek (February 17, 2016).

^cSanitary sewer overflow events (SSO) at Crane Creek.

^dGills Creek site downstream (approximately 2000m) from two SSOs (November 19, 2015).

Table 3.3 Summary statistics for the percent methylmercury of total mercury (%MeHg of THg) for unfiltered and filtered fractions for reference sites and sewage events.

<i>Location</i>	<i>Unfiltered</i>		<i>Filtered</i>	
	Mean \pm 1 SD	Median (Range)	Mean \pm 1 SD	Median (Range)
Reference Sites (n=37)	3.2 \pm 1.9	2.9 (0.59-8.5)	11 \pm 9.6	8.5 (0.17-38)
Stoop Creek Reference Sites (n=21)	3.5 \pm 2.0	3.1 (1.3-8.5)	14 \pm 11	9.9 (2.0-38)
Crane Creek Reference Sites (n=10)	3.2 \pm 2.0	2.8 (0.59-6.1)	7.9 \pm 5.9	7.8 (0.18-17)
Gills Creek Reference Site (n=6)	2.0 \pm 0.72	2.0 (1.0-3.0)	5.1 \pm 4.0	3.5 (0.69-11)
Wastewater Treatment Plant Outfall (n=5) ^a	1.5 \pm 2.1	0.51 (0.35-5.2)	7.5 \pm 9.8	3.5 (0.86-25)
Sewage Events (n=6)	1.4 \pm 1.5	0.84 (0.021-3.6)	2.9 \pm 3.5	0.94 (0.11-8.6)
Broken Force Main (n=1) ^b	3.6		2.8	
Crane Creek SSO (n=4) ^c	1.3 \pm 1.5	0.43 (0.021-3.4)	4.0 \pm 4.5	3.7 (0.11-8.6)
Gills Creek Downstream from SSOs (n=1) ^d	1.0		0.69	
Crane Creek Ditch (n=10)	9.9 \pm 9.8	6.5 (0.42-33)	27 \pm 28	14 (0.11-82)
All Locations (n=56)	4.1 \pm 5.1	2.9 (0.021-33)	13 \pm 16	8.3 (0.11-89)

^aWastewater treatment plant effluent outfall at Stoop Creek.

^bRuptured force main sewer pipe at Stoops Creek (February 17, 2016).

^cSanitary sewer overflow events (SSO) at Crane Creek.

^dGills Creek site downstream (approximately 2000m) from two SSOs (November 19, 2015).

Table 3.4 Ratios between metal concentrations in sewage events and associated creek reference sites.

Metals	<i>Stoop Creek</i>	<i>Gills Creek</i>	<i>Crane Creek</i>	
	Ruptured Sewer Pipe	Downstream from SSOs	SSOs	Ditch
Unfiltered THg	3.8	3.5	4.1	1.2
Filtered THg	0.8	1.8	1.4	0.90
Unfiltered MeHg	3.3	1.7	1.0	2.9
Filtered MeHg	2.0	0.32	0.65	3.3
Rb	1.2	1.5	2.4	1.4
Sr	1.2	0.97	1.6	1.6
Cd	0.90	1.6	1.8	1.2
Cs	1.1	1.1	7.0	1.3
Ba	0.75	0.91	1.1	2.5
Tl	0.71	1.4	0.94	1.0
Pb	0.83	1.5	2.4	0.75
U	1.2	2.6	1.5	0.59
Mg	1.3	0.95	1.2	1.1
Al	1.0	3.5	1.7	0.73
Ca	1.1	1.0	1.7	1.9
V	1.6	3.0	2.9	1.0
Cr	0.8	1.8	1.5	0.86
Mn	2.7	0.4	1.3	7.0
Fe	1.0	1.1	1.4	2.4
Co	1.6	0.6	2.4	4.6
Ni	1.1	1.1	2.2	1.2
Cu	1.9	1.5	2.2	1.0
Zn	0.8	1.5	3.5	1.8
Ga	2.1	2.5	2.6	0.64
As	n/a	1.6	2.2	1.5
La	0.5	3.8	1.7	0.32
Ce	0.58	3.6	1.7	0.43
Pr	0.57	3.6	1.7	0.36
Nd	0.56	3.5	1.8	0.40
Sm	0.65	3.5	1.8	1.5
Eu	0.53	2.4	1.6	1.0
Gd	0.65	3.8	1.8	0.43
Dy	0.74	3.4	1.6	0.46
Ho	0.78	3.1	1.6	0.49
Er	0.84	2.9	1.6	0.51
Tm	0.87	2.6	1.6	0.54
Yb	0.78	2.6	1.7	0.55
Lu	0.92	2.2	1.6	0.59

Note: Bolding indicates higher metal concentration in sewage event compared to associated creek reference sites.

Table 3.5 Ratios between metal concentrations in sewage events and the wastewater treatment plant effluent outfall at Stoop Creek.

Metals	<i>Stoop Creek</i>	<i>Gills Creek</i>	<i>Crane Creek</i>		<i>All Events</i>
	Ruptured Sewer Pipe	Downstream from SSOs	SSOs	Ditch	
Unfiltered THg	4.8	8.5	18	2.9	15
Filtered THg	3.2	3.3	5.6	3.1	4.9
Unfiltered MeHg	15	7.7	10	37	11
Filtered MeHg	1.8	0.46	3	21	2.5
Rb	0.86	0.56	0.77	0.39	0.74
Sr	1.1	0.43	0.74	0.73	0.73
Cd	1.7	2.0	1.5	1.1	1.5
Cs	0.79	0.59	1.2	0.16	1.1
Ba	0.94	1.2	1.1	2.6	1.1
Tl	1.4	4.5	1.5	1.4	1.9
Pb	1.1	11	4.9	1.2	4.9
U	5.6	31	12	4.6	14
Mg	1.4	0.42	0.72	0.69	0.75
Al	0.70	5.7	3.4	1.5	3.2
Ca	0.9	0.37	0.52	0.63	0.53
V	1.3	2.7	2.5	0.66	2.3
Cr	1.2	3.5	2.8	1.6	2.6
Mn	1.6	0.40	0.94	4.8	0.90
Fe	2.2	3.1	4.2	7.7	3.4
Co	1.3	0.39	1.3	2.5	1.1
Ni	1.4	0.65	1.8	0.94	1.5
Cu	1.7	1.4	1.3	0.54	1.3
Zn	0.5	0.24	0.46	0.21	0.41
Ga	1.4	1.5	1.6	0.34	1.5
As	n/a	2.4	2.7	1.7	2.7
La	2.4	16	16	2.8	13
Ce	1.6	13	12	2.7	10
Pr	2.0	14	14	2.7	12
Nd	1.8	13	13	2.8	11
Sm	1.8	13	12	9.6	10
Eu	1.2	6.2	6.0	3.5	5.0
Gd	0.56	2.1	1.8	0.39	1.6
Dy	1.6	13	8.7	2.3	8.0
Ho	1.6	11	7.7	2.2	7.1
Er	1.7	10	6.7	2.1	6.3
Tm	1.6	9.2	5.9	1.9	5.6
Yb	0.95	5.3	3.3	1.1	3.1
Lu	1.5	7.6	4.7	1.8	4.5

Note: Bolding indicates higher metal concentration in sewage event compared to wastewater treatment effluent from the outfall.

Table 3.6 Summary statistics for $\delta^{13}\text{C}$ (‰) for reference sites and sewage events.

<i>Location</i>	$\delta^{13}\text{C}$ of <i>DOC</i> (‰)	
	Mean \pm SD	Median (Range)
Reference Sites (n=20)	-27.3 \pm 0.58	-27.4 (-28.6,-26.1)
Stoop Creek Reference Sites (n=12)	-27.2 \pm 0.63	-27.1 (-28.6,-26.1)
Crane Creek Reference Sites (n=3)	-27.5 \pm 0.21	-27.6 (-27.7,-27.3)
Gills Creek Reference Sites (n=5)	-27.7 \pm 0.73	-27.8 (-28.6,-26.7)
Wastewater Treatment Plant Outfall (n=3) ^a	-26.3 \pm 0.13	-26.1 (-27.7,-25.2)
Sewage Events (n=3)	-26.1 \pm 1.41	-25.6 (-27.7,-25.0)
Broken Sewer Main (n=1) ^b	-25.0	
Crane Creek SSO (n=1) ^c	-25.6	
Downstream from SSOs (n=1) ^d	-27.7	
Crane Creek Ditch (n=5)	-27.3 \pm 1.04	-27.9 (-28.3,-26.1)
All Locations (n=30)	-27.1 \pm 0.90	-27.4 (-28.6,-25.0)

^aWastewater treatment plant effluent outfall at Stoop Creek.

^bRuptured force main sewer pipe at Stoops Creek (February 17, 2016).

^cSanitary sewer overflow events (SSO) at Crane Creek (December 31, 2015).

^dGills Creek site downstream (approximately 2000m) from two SSOs (November 19, 2015).

Table 3.7 Summary statistics for dissolved organic carbon ($\mu\text{mol/L}$) for reference sites and sewage events.

<i>Location</i>	<i>Dissolved Organic Carbon ($\mu\text{mol/L}$)</i>	
	Mean \pm SD	Median (Range)
Reference Sites (n=28)	430 \pm 77	440 (280-560)
Stoop Creek Reference Sites (n=16)	410 \pm 65	440 (280-520)
Crane Creek Reference Sites (n=5)	510 \pm 52	510 (460-560)
Gills Creek Reference Sites (n=6)	390 \pm 79	380 (300-510)
Wastewater Treatment Plant Outfall (n=4) ^a	390 \pm 79	380 (300-510)
Sewage Events (n=3)	630 \pm 290	530 (330-1100)
Broken Sewer Main (n=1) ^b	920	
Crane Creek SSO (n=1) ^c	1100	
Downstream from SSOs (n=1) ^d	600	
Crane Creek Ditch (n=7)	530 \pm 240	452 (330-990)
All Locations (n=41)	470 \pm 170	440 (280-1100)

^aWastewater treatment plant effluent outfall at Stoop Creek.

^bRuptured force main sewer pipe at Stoops Creek (February 17, 2016)

^cSanitary sewer overflow events (SSO) at Crane Creek (December 31, 2015).

^dGills Creek site downstream (approximately 2000m) from two SSOs (November 19, 2015)

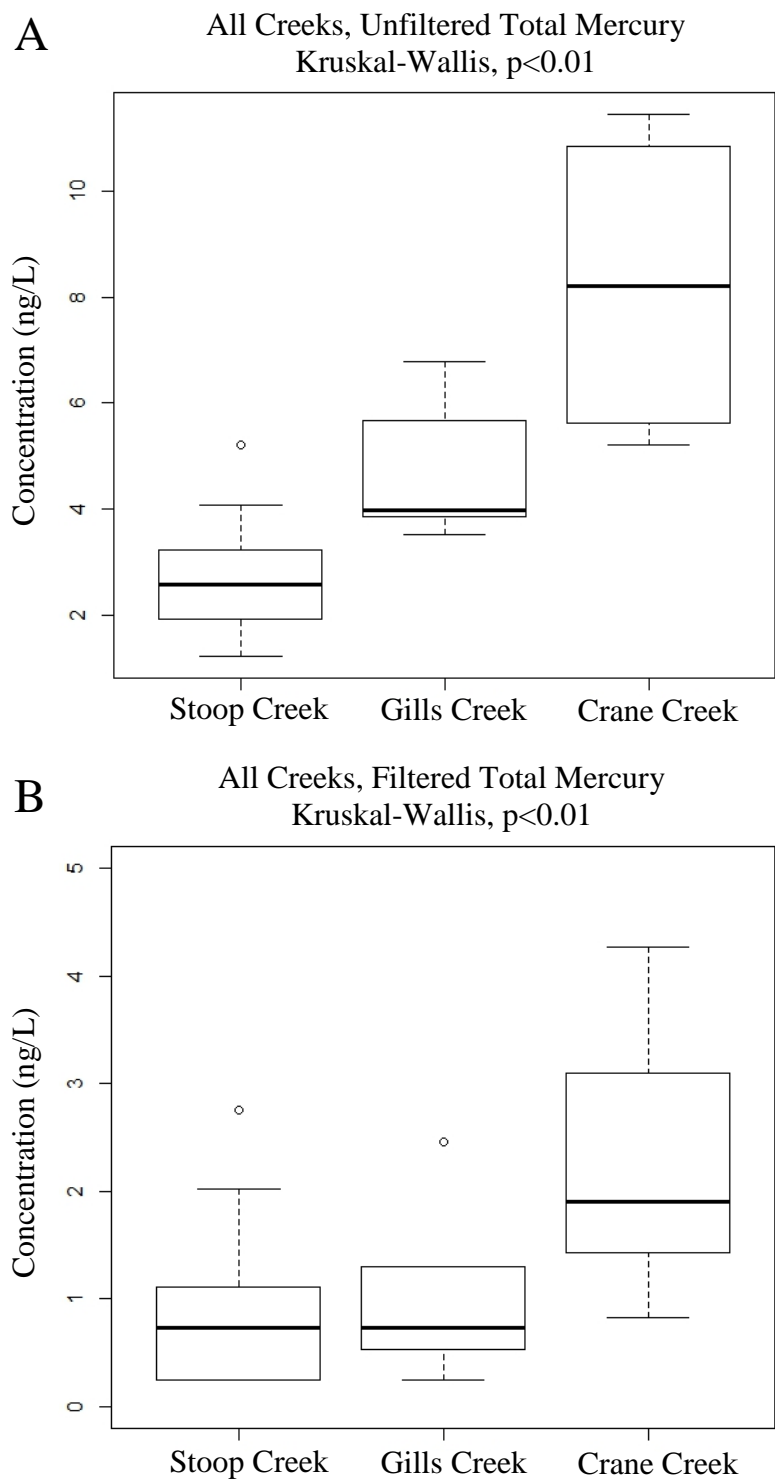


Figure 3.1 Boxplots for (A) unfiltered and (B) filtered total mercury concentrations (ng/L) vs. reference creek locations (n=37).

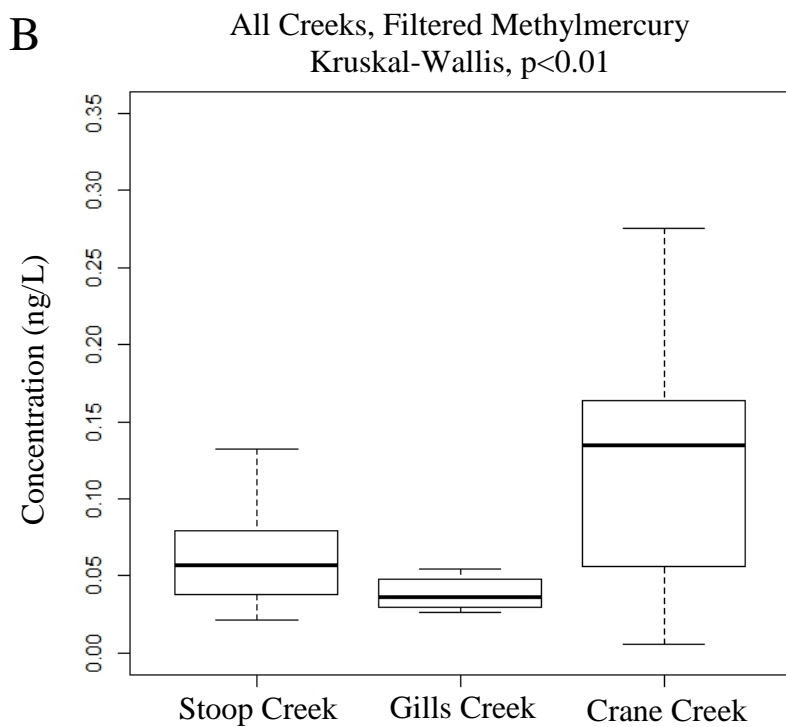
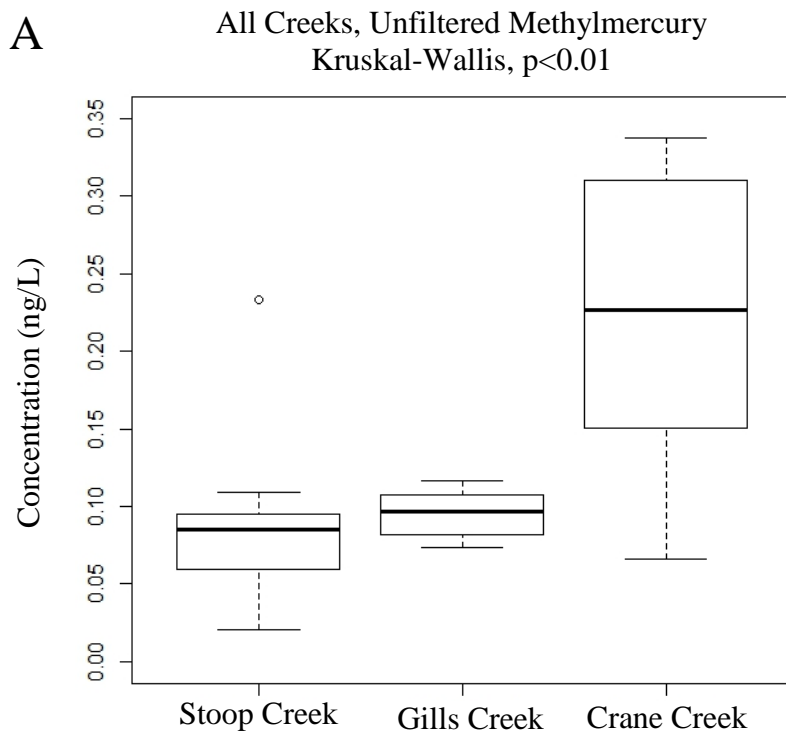
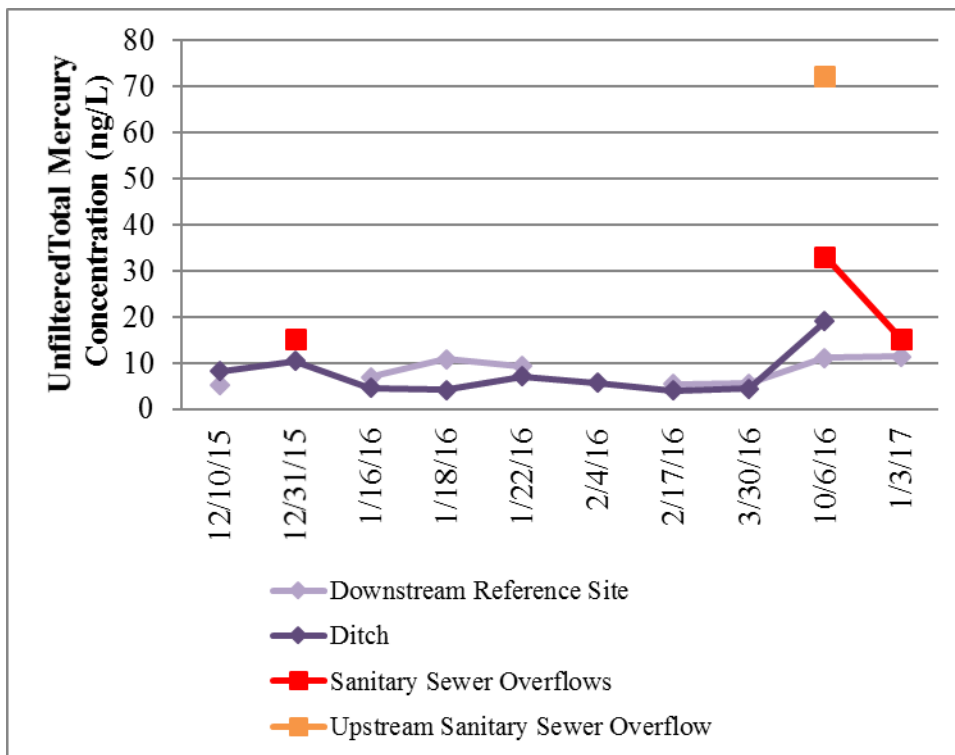


Figure 3.2 Boxplots for (A) unfiltered and (B) filtered methylmercury concentrations (ng/L) vs. reference creek locations (n=37).

A Crane Creek



B

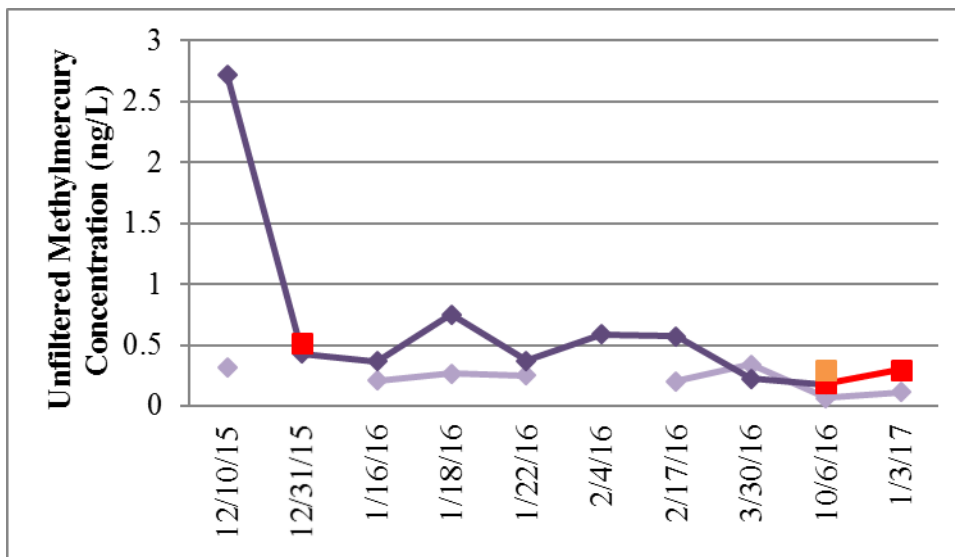
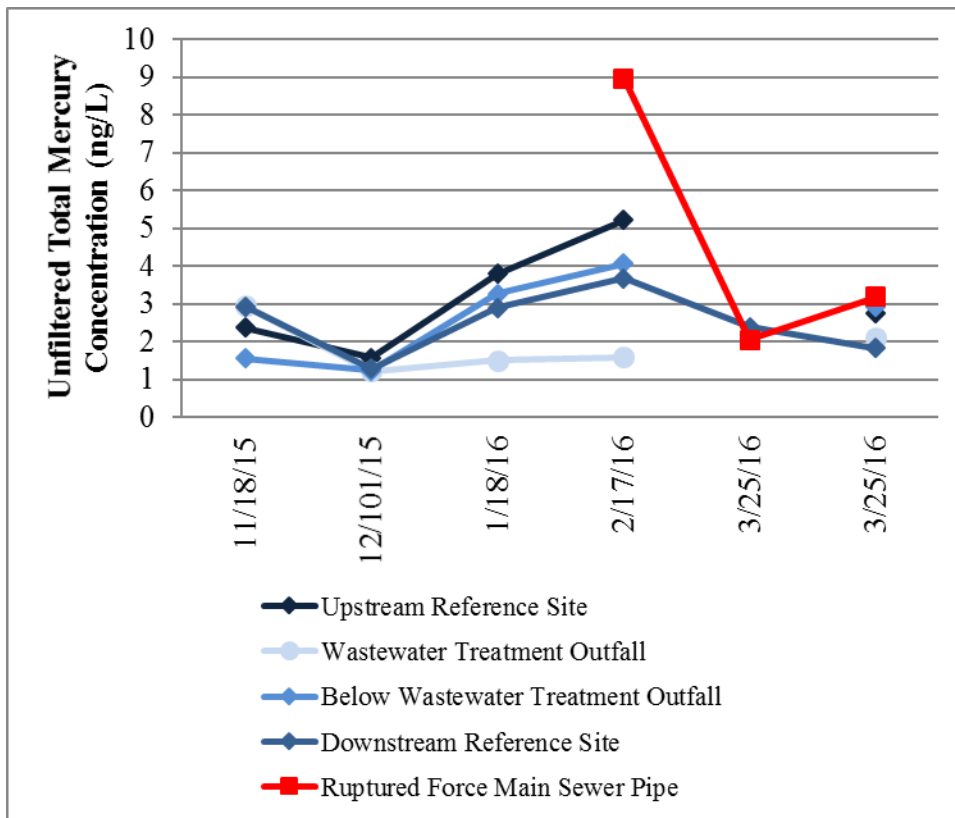


Figure 3.3 Temporal trends for (A) unfiltered total mercury and (B) unfiltered methylmercury at Crane Creek.

A Stoop Creek



B

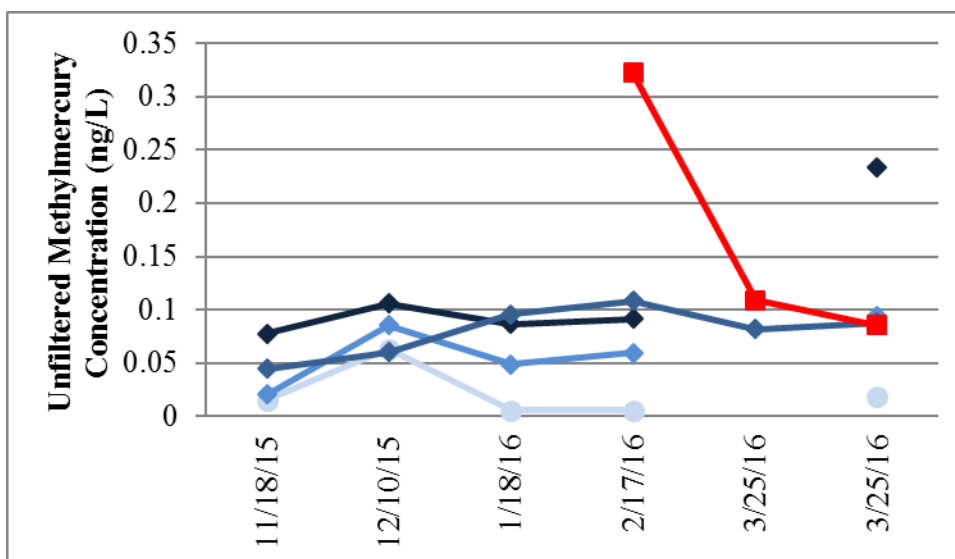
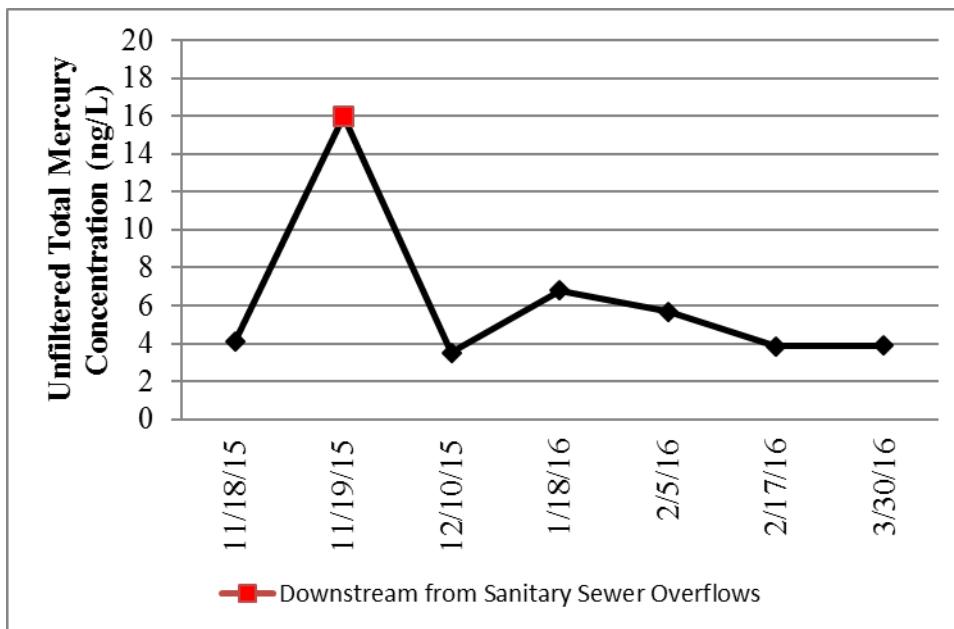


Figure 3.4 Temporal trends for (A) unfiltered total mercury and (B) unfiltered methylmercury at Stoop Creek.

A Gills Creek



B

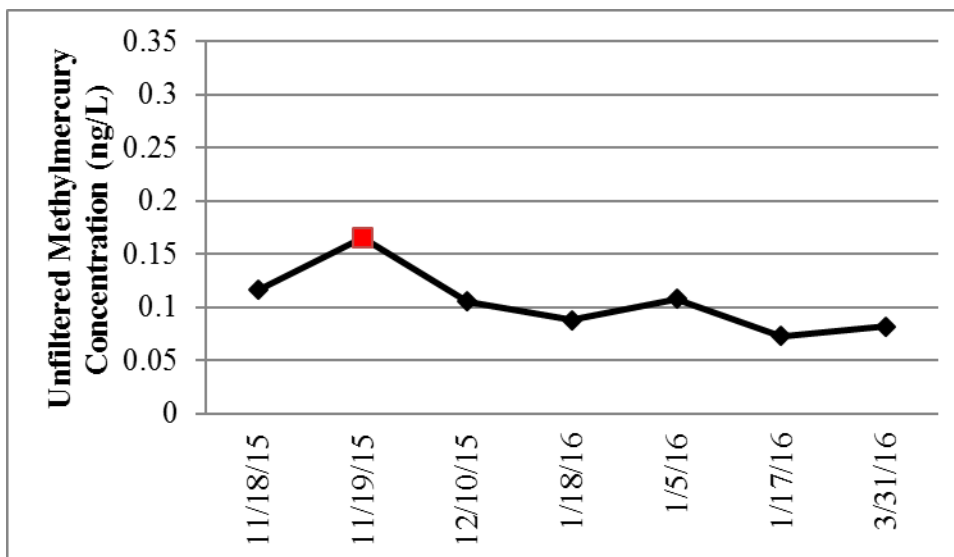


Figure 3.5 Temporal trends for (A) unfiltered total mercury and (B) unfiltered methylmercury at Gills Creek.

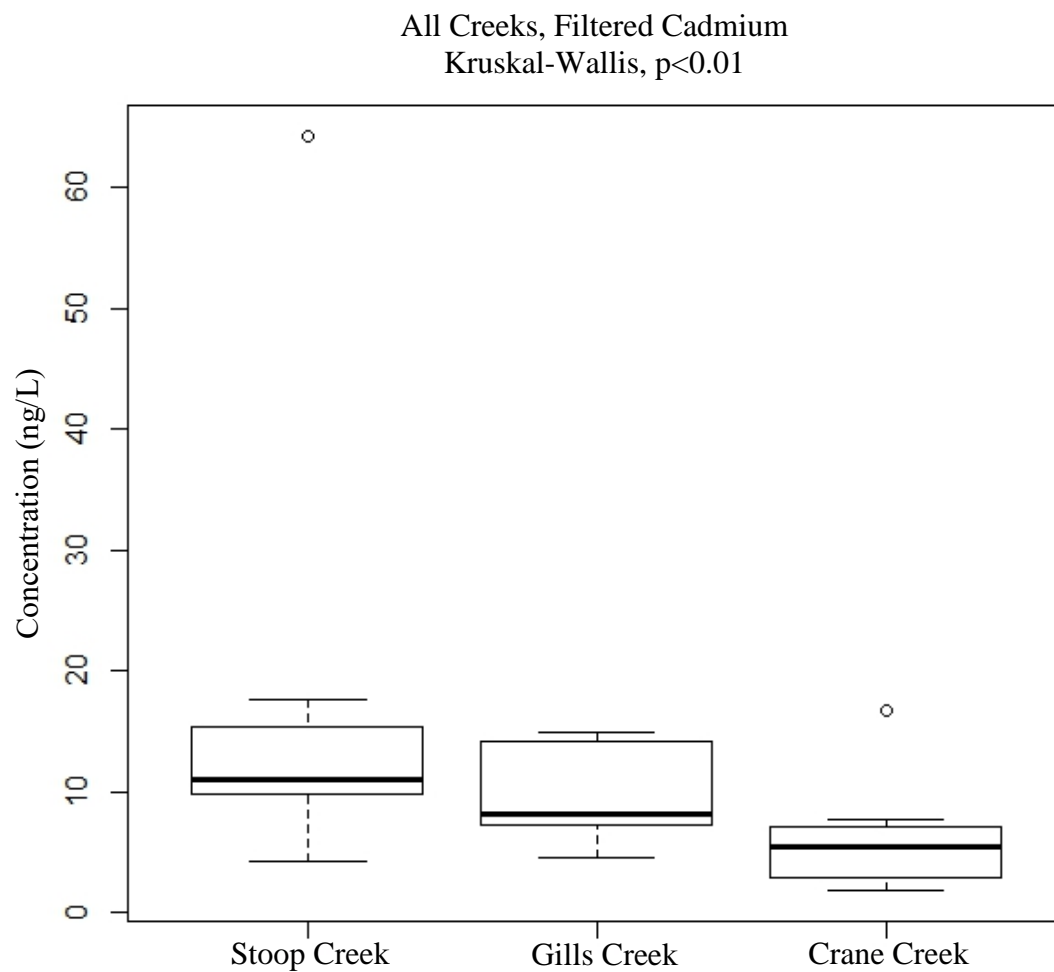


Figure 3.6 Boxplots of filtered cadmium concentration (ng/L) vs. creek reference locations (n=33).

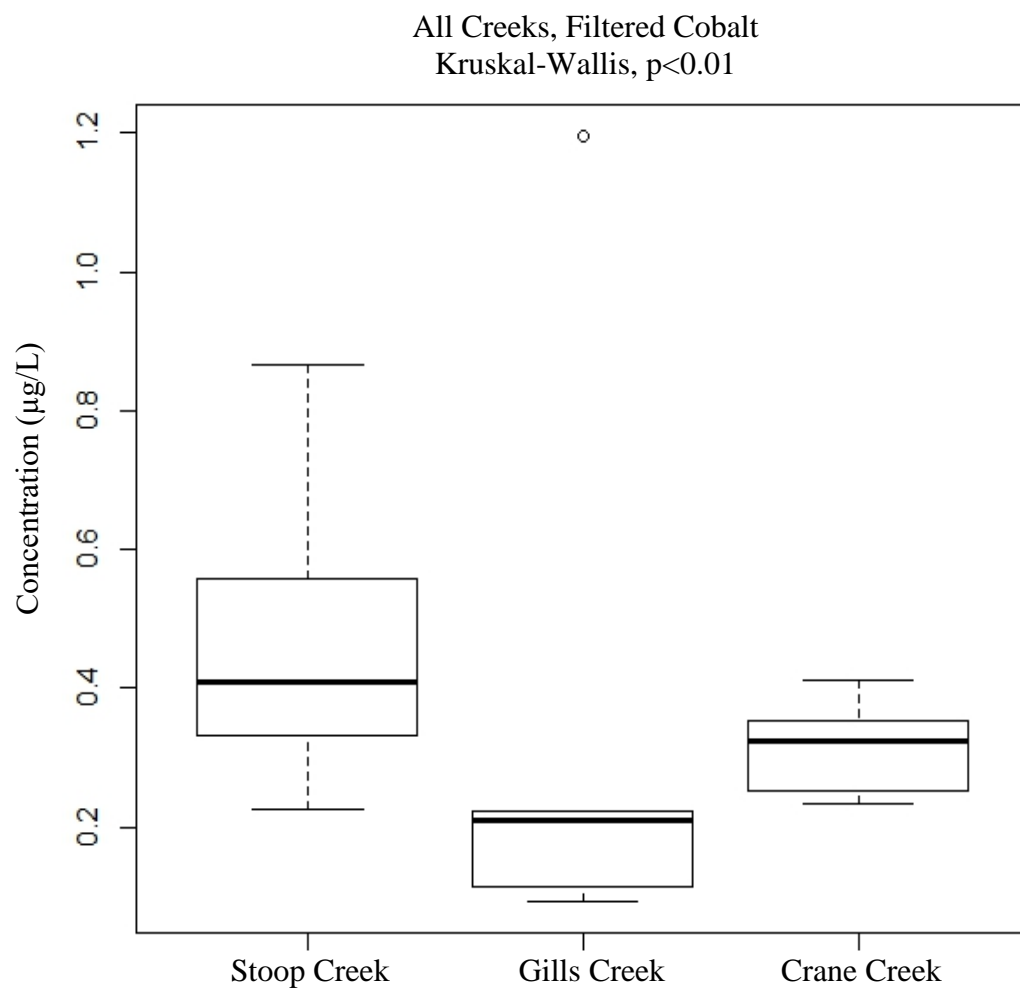


Figure 3.7 Boxplots of filtered cobalt concentration ($\mu\text{g/L}$) vs. creek reference locations ($n=33$).

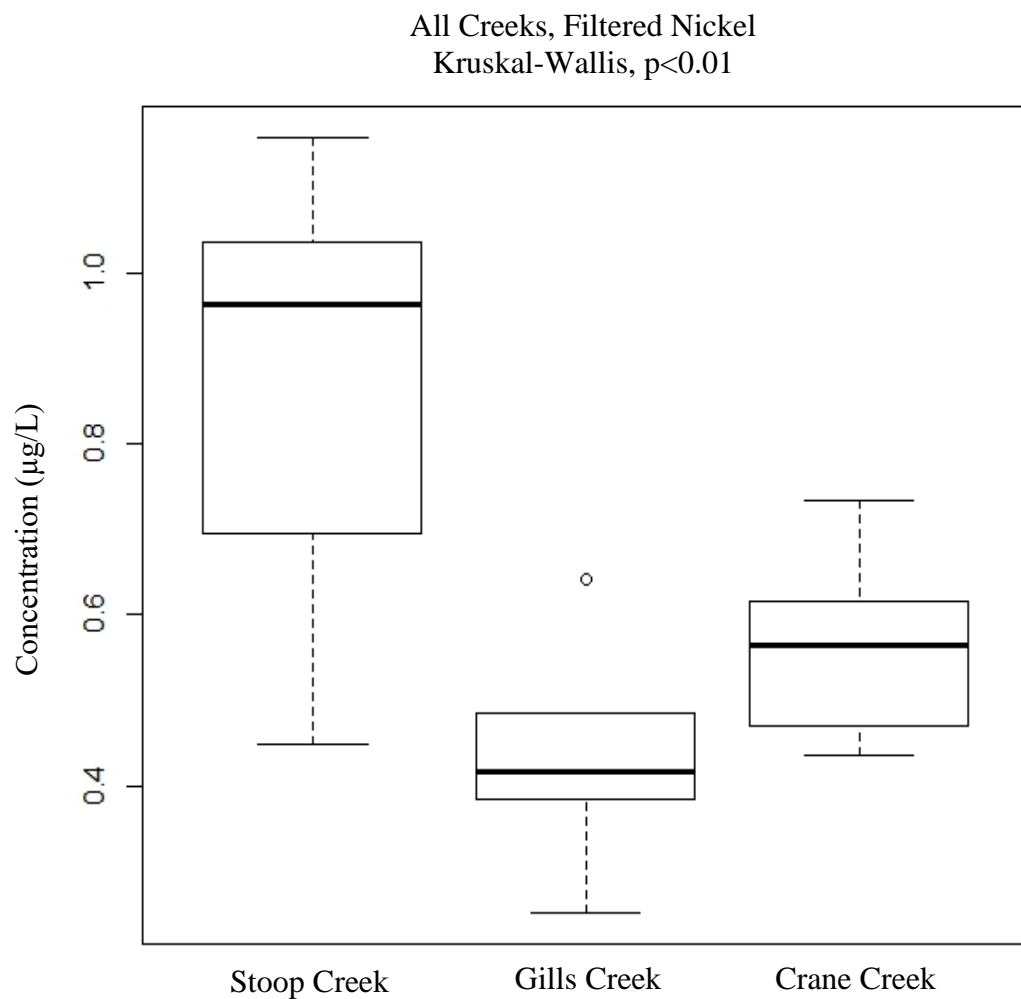


Figure 3.8 Boxplots of filtered nickel concentration ($\mu\text{g/L}$) vs. creek reference locations ($n=33$).

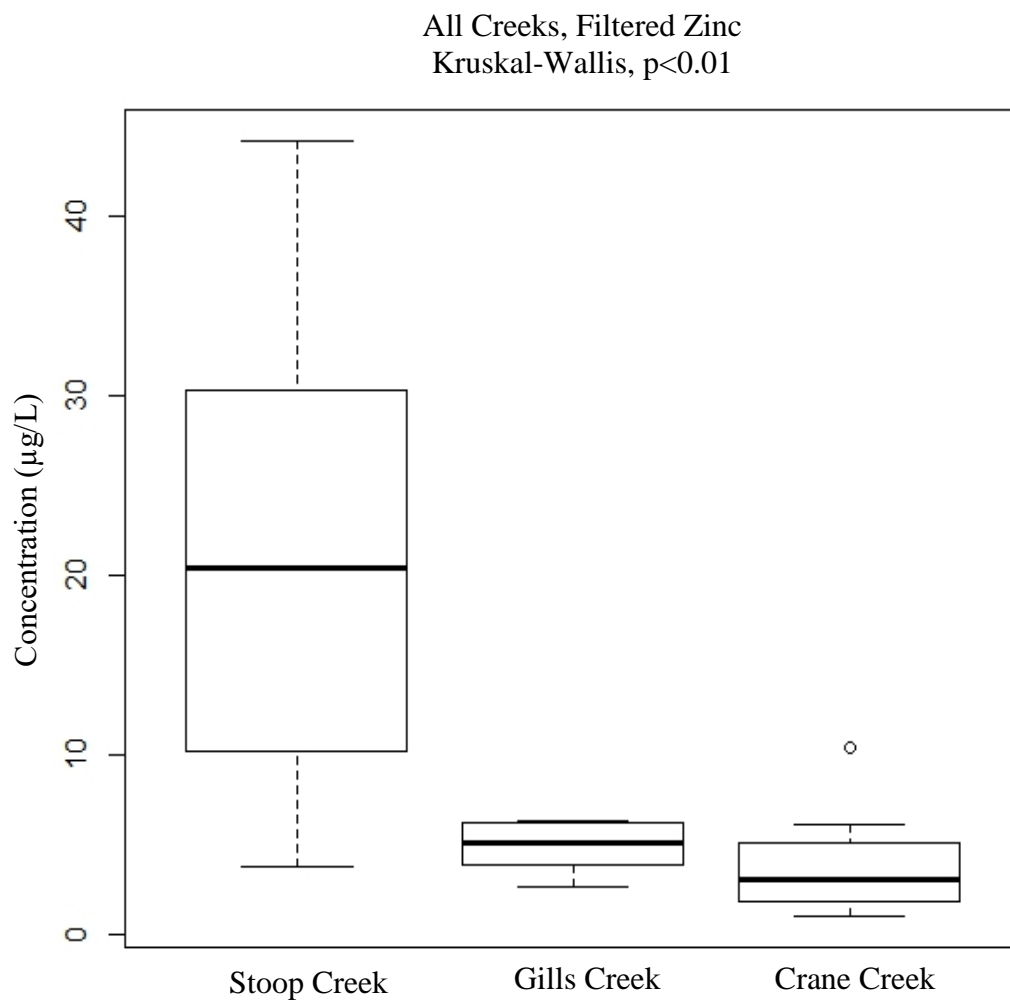


Figure 3.9 Boxplots of filtered zinc concentration ($\mu\text{g/L}$) vs. creek reference locations ($n=33$).

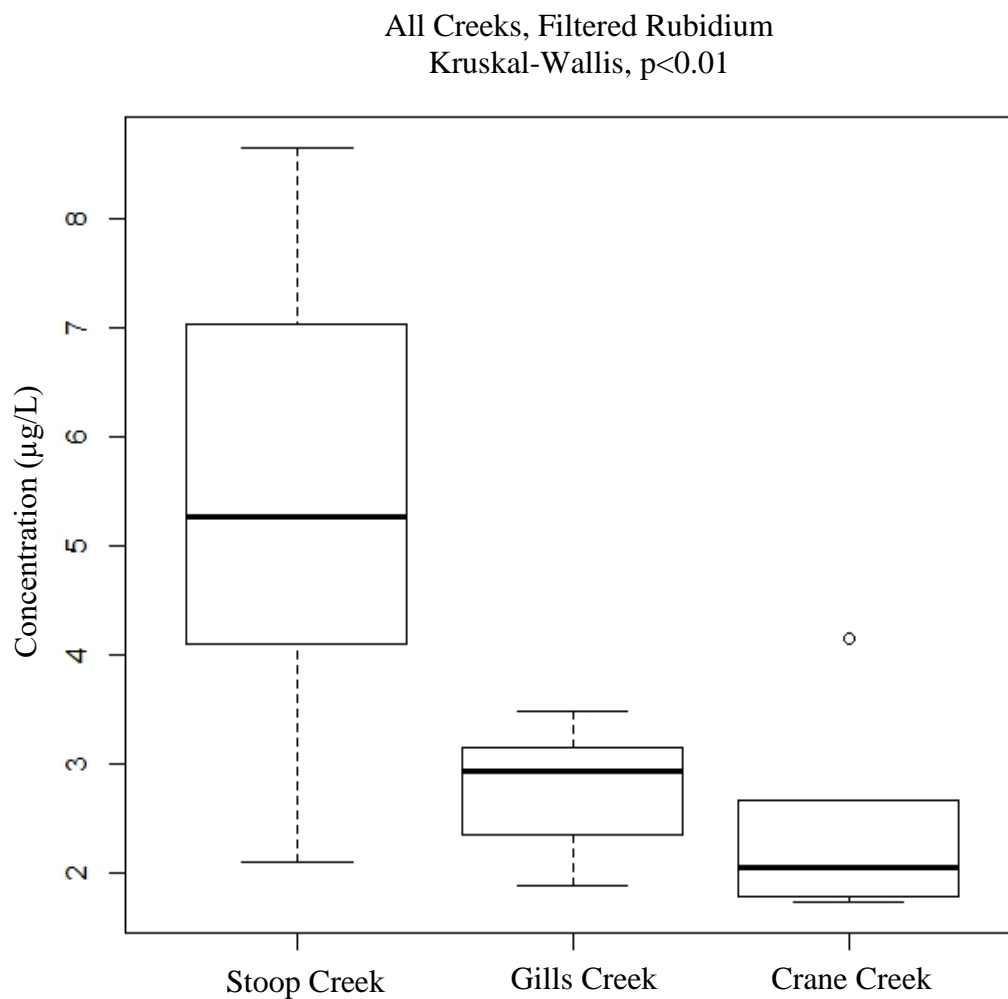


Figure 3.10 Boxplots of filtered rubidium concentration ($\mu\text{g/L}$) vs. creek reference locations ($n=33$).

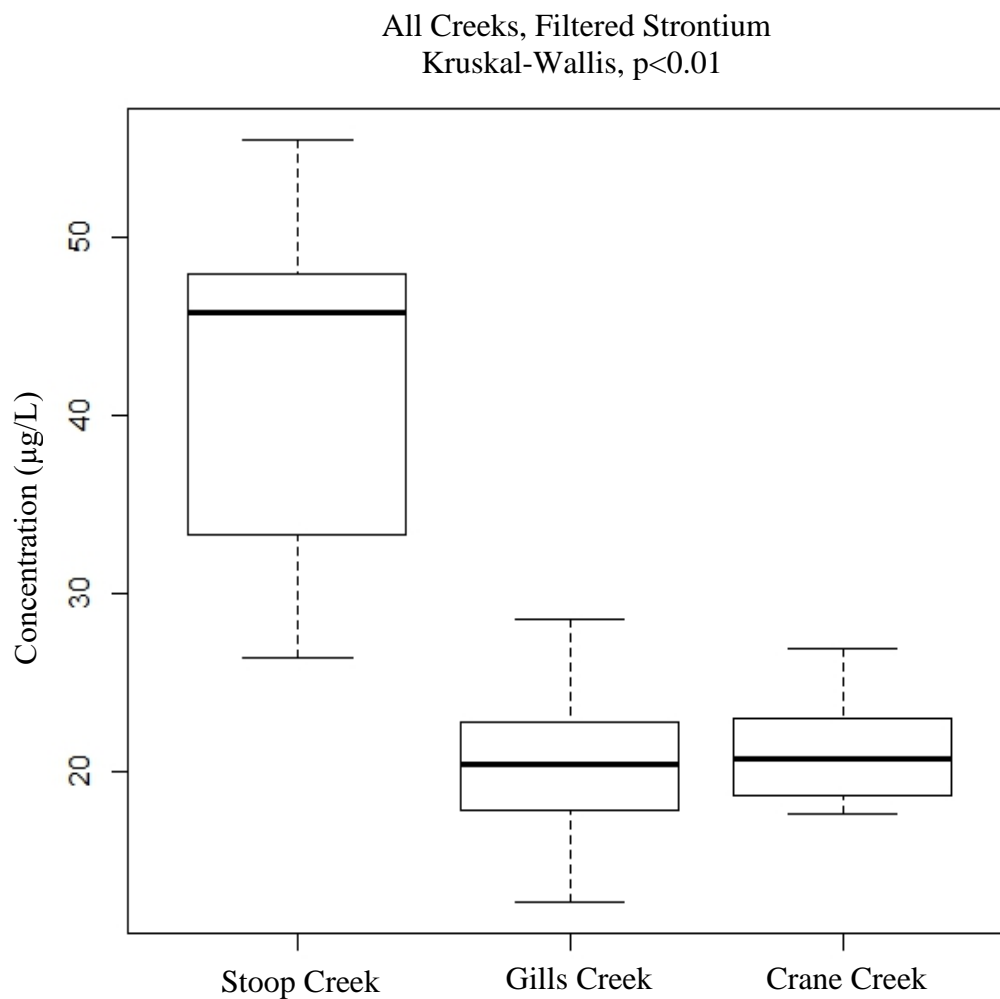


Figure 3.11 Boxplots of filtered strontium concentration (µg/L) vs. creek reference locations (n=33).

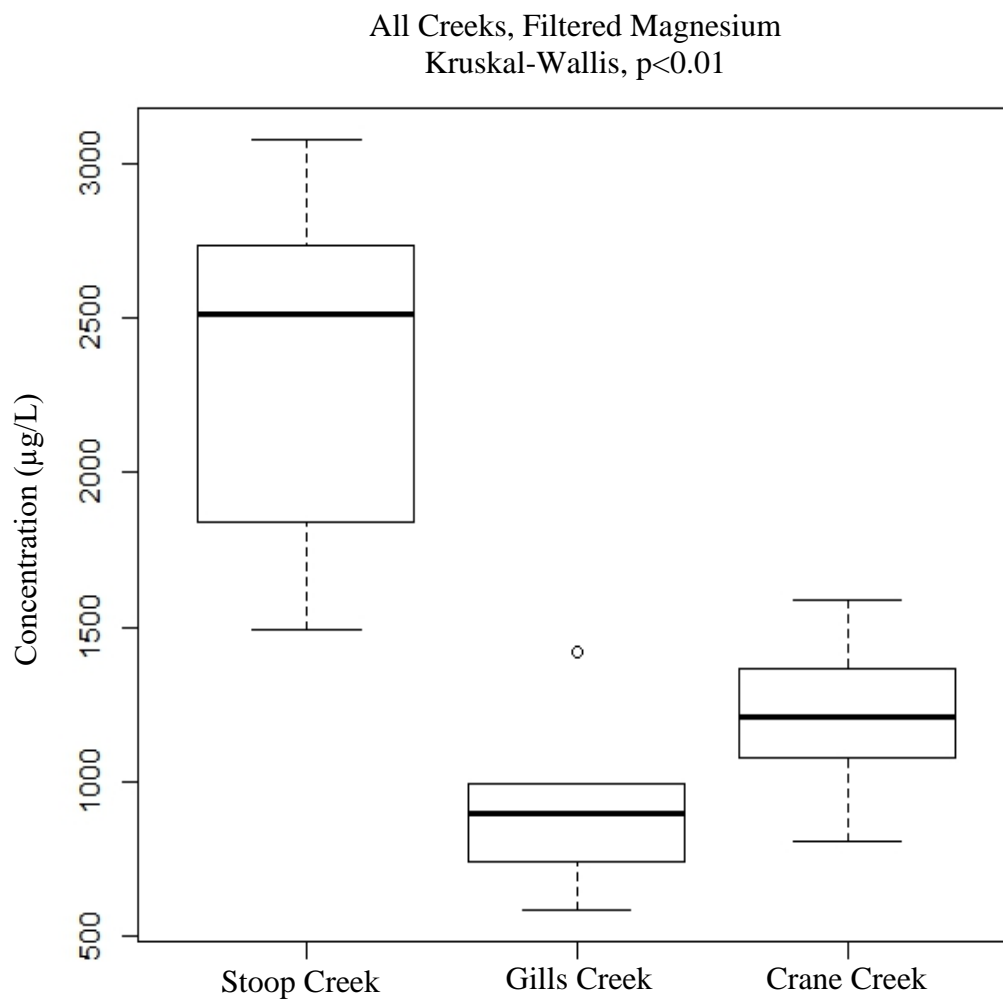


Figure 3.12 Boxplots of filtered magnesium concentration ($\mu\text{g/L}$) vs. creek reference locations ($n=33$).

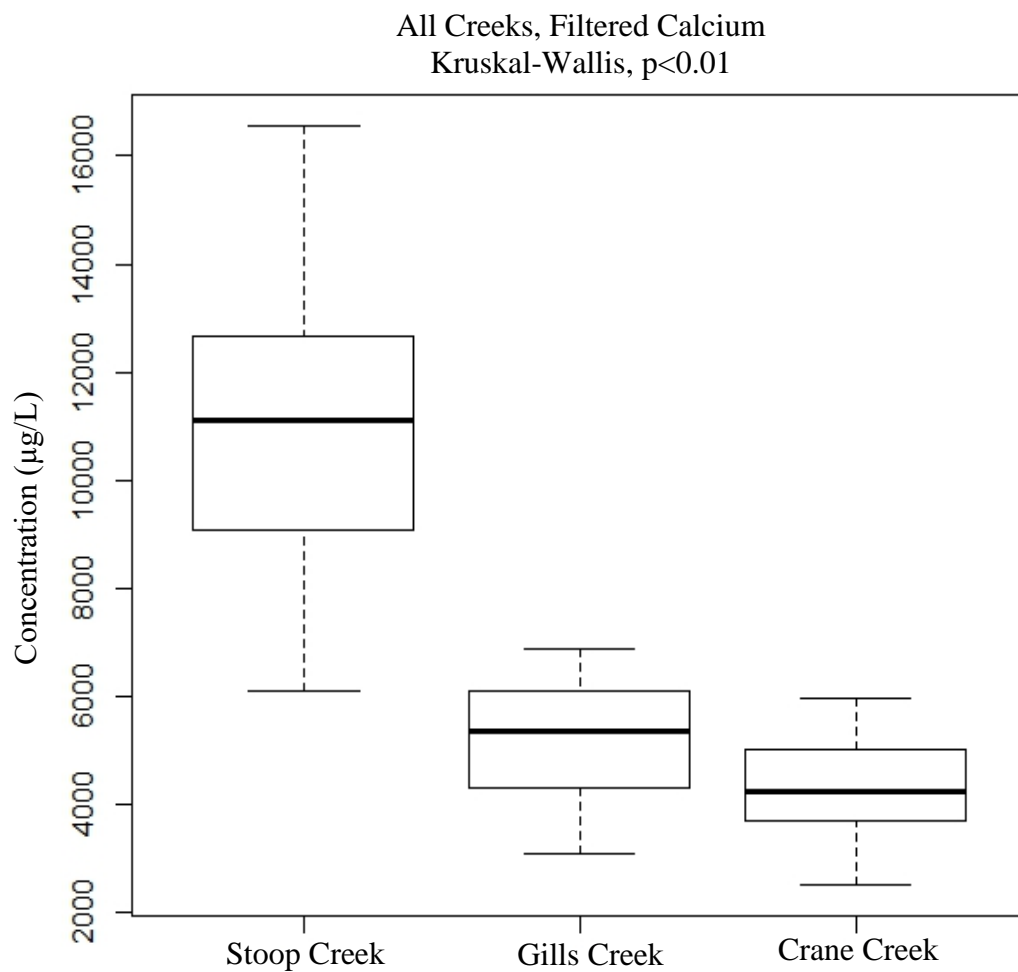


Figure 3.13 Boxplots of filtered calcium concentration ($\mu\text{g/L}$) vs. creek reference locations ($n=33$).

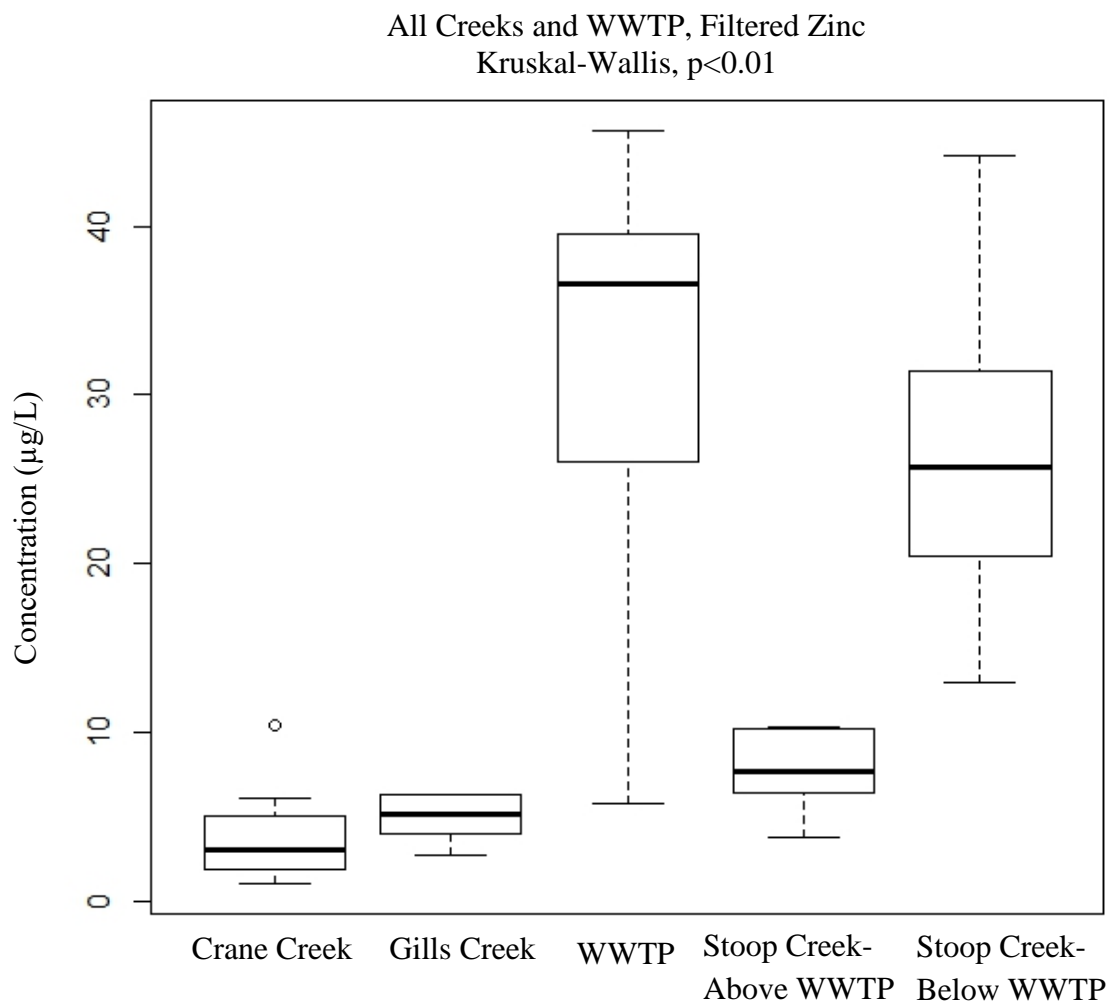


Figure 3.14 Boxplots for filtered zinc concentration ($\mu\text{g/L}$) vs. reference sites in Crane Creek ($n=8$), Gills Creek ($n=6$), and Stoop Creek both upstream ($n=6$) and downstream ($n=10$) reference as well as the wastewater treatment plant outfall (WWTP) ($n=5$).

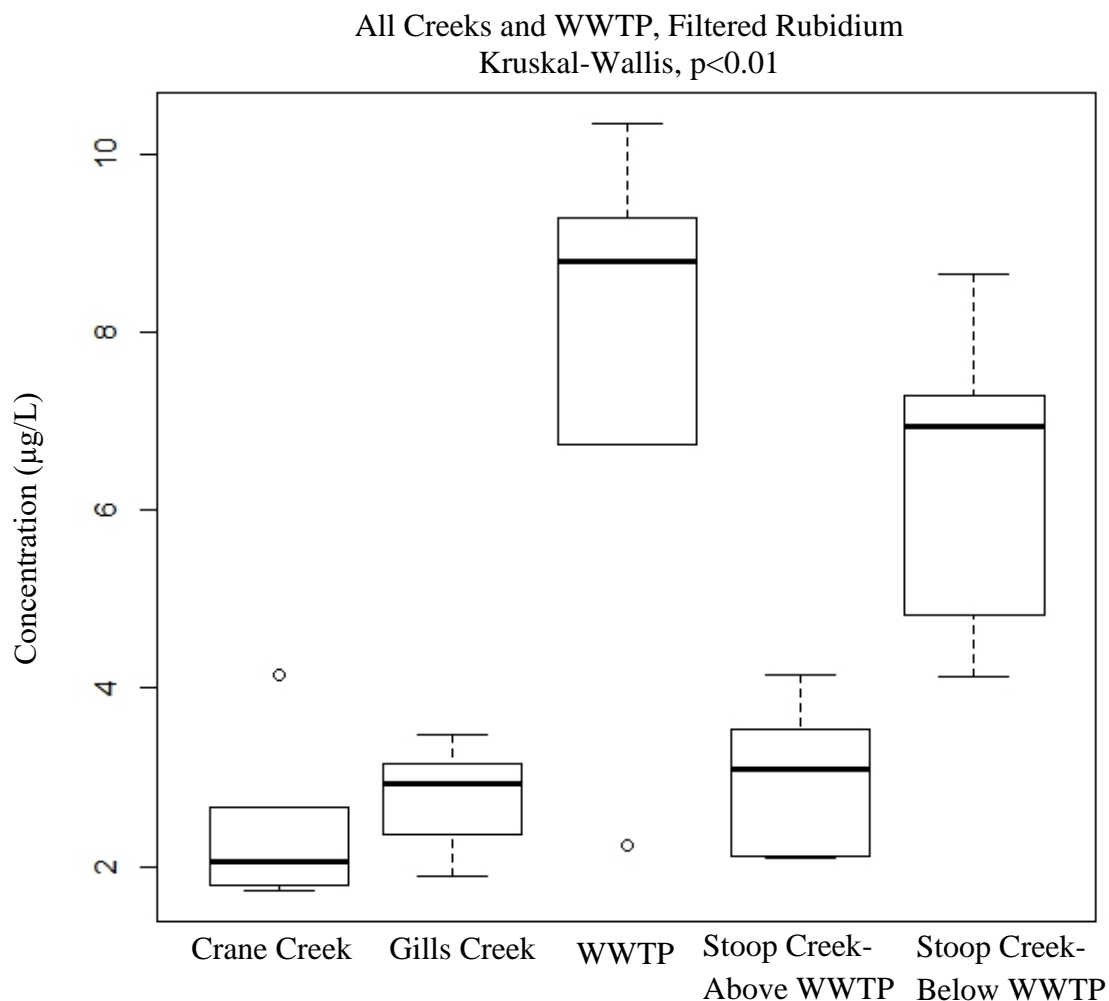


Figure 3.15 Boxplots for filtered rubidium concentration ($\mu\text{g/L}$) vs. reference sites in Crane Creek ($n=8$), Gills Creek ($n=6$), and Stoop Creek both upstream ($n=6$) and downstream ($n=10$) reference as well as the wastewater treatment plant outfall (WWTP) ($n=5$).

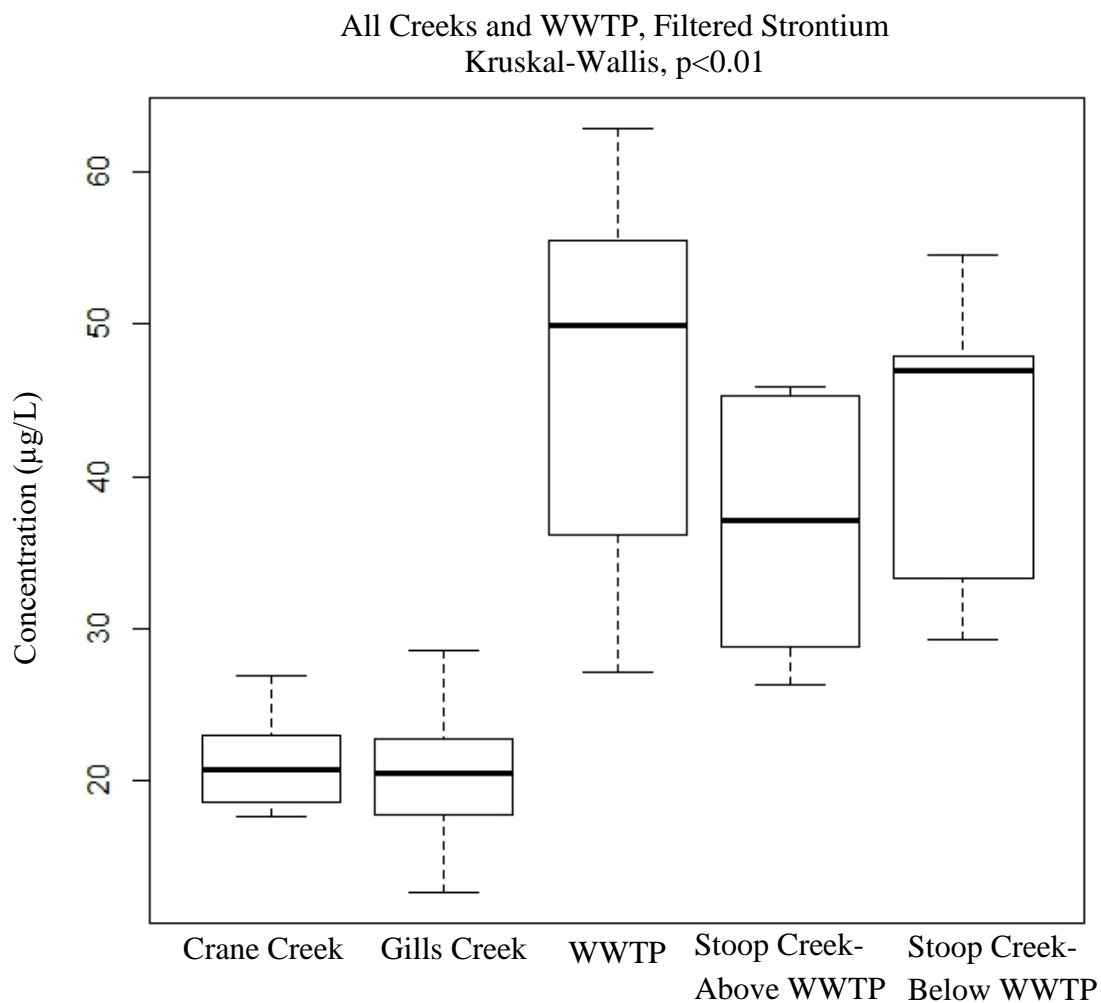


Figure 3.16 Boxplots for filtered strontium concentration ($\mu\text{g/L}$) vs. reference sites in Crane Creek ($n=8$), Gills Creek ($n=6$), and Stoop Creek both upstream ($n=6$) and downstream ($n=10$) reference as well as the wastewater treatment plant outfall (WWTP) ($n=5$).

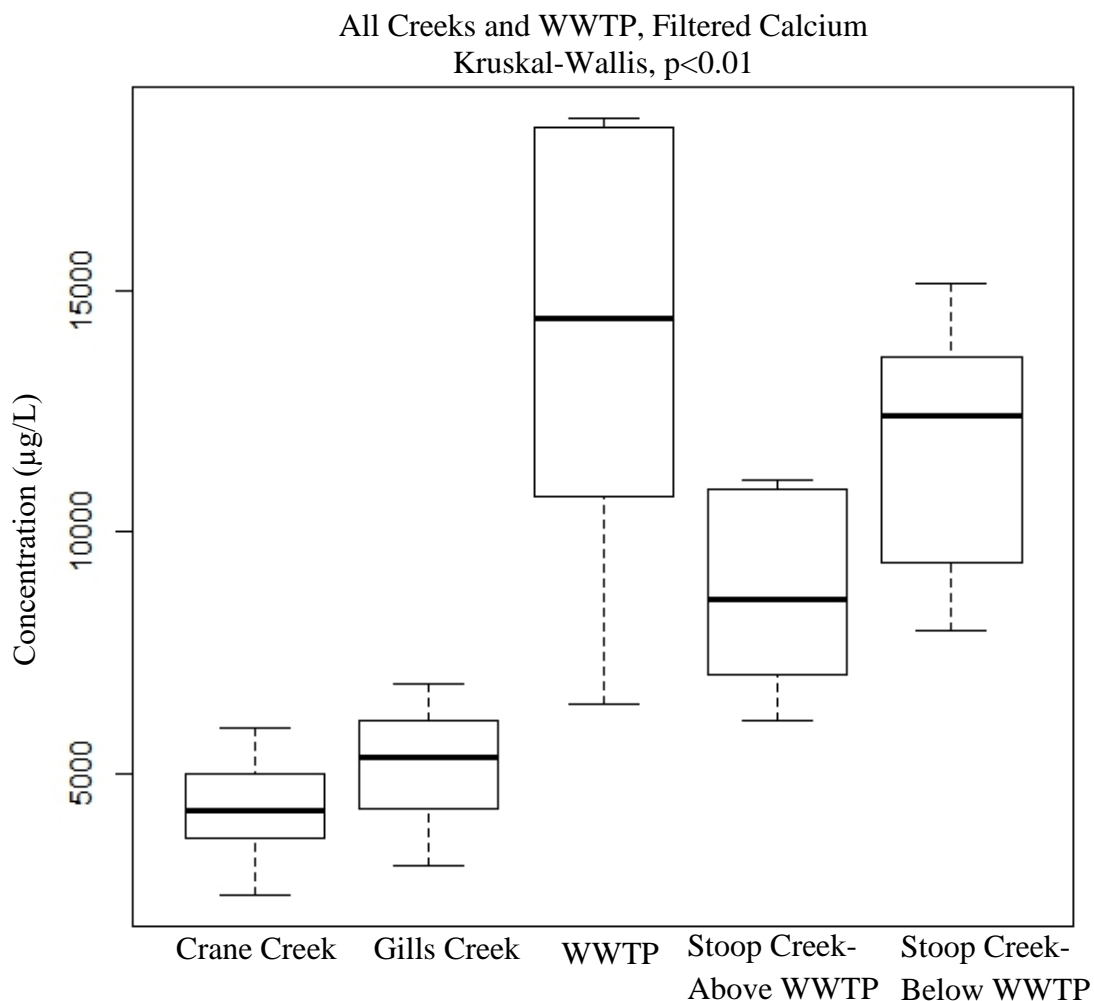


Figure 3.17 Boxplots for filtered calcium concentration ($\mu\text{g/L}$) vs. reference sites in Crane Creek ($n=8$), Gills Creek ($n=6$), and Stoop Creek both upstream ($n=6$) and downstream ($n=10$) reference as well as the wastewater treatment plant outfall (WWTP) ($n=5$).

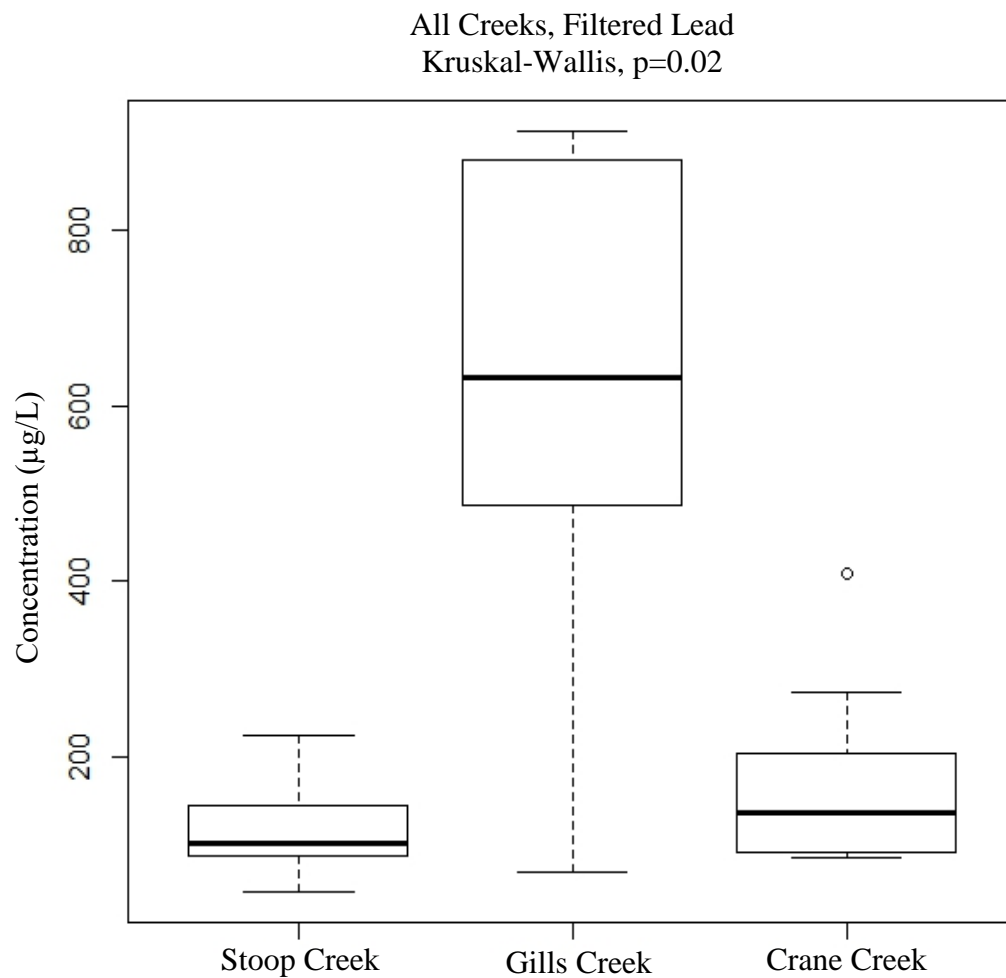


Figure 3.18 Boxplots of filtered calcium concentration ($\mu\text{g/L}$) vs. creek reference locations ($n=33$).

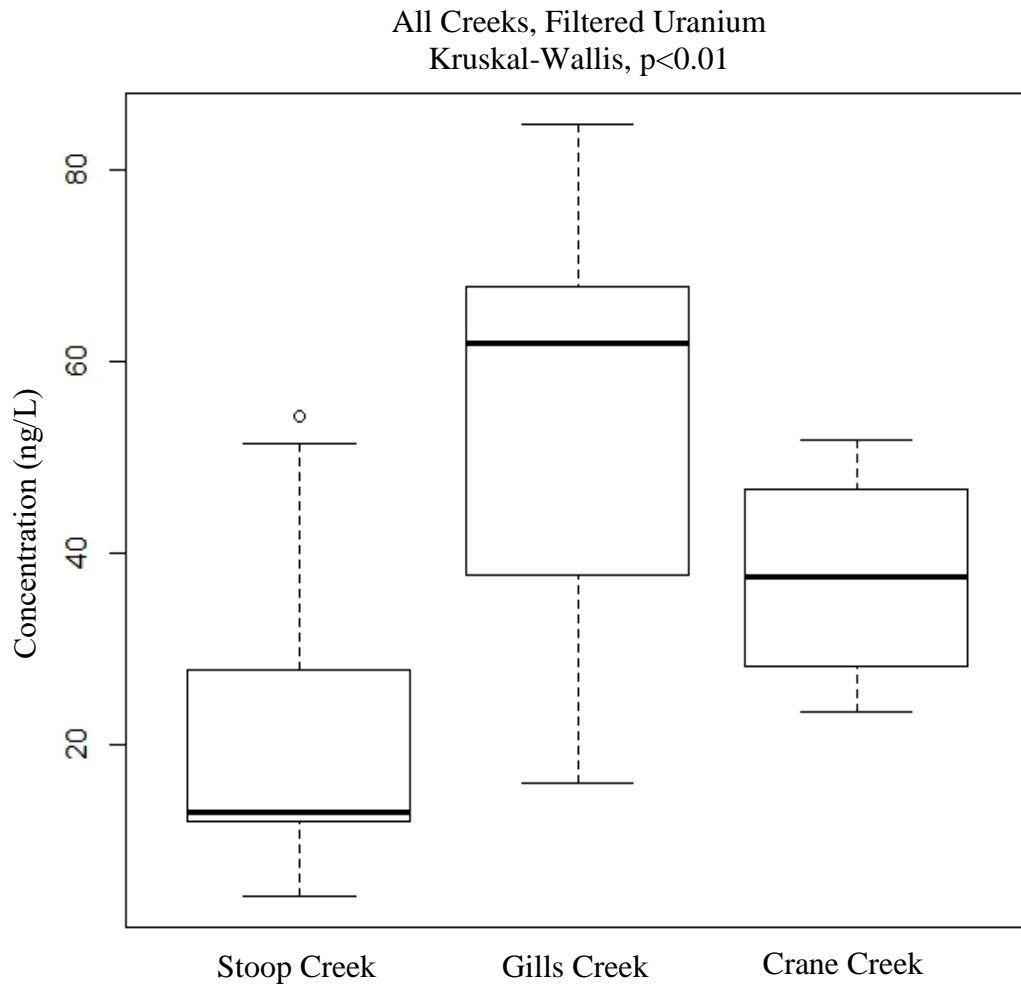


Figure 3.19 Boxplots of filtered uranium concentration (ng/L) vs. creek reference locations (n=33).

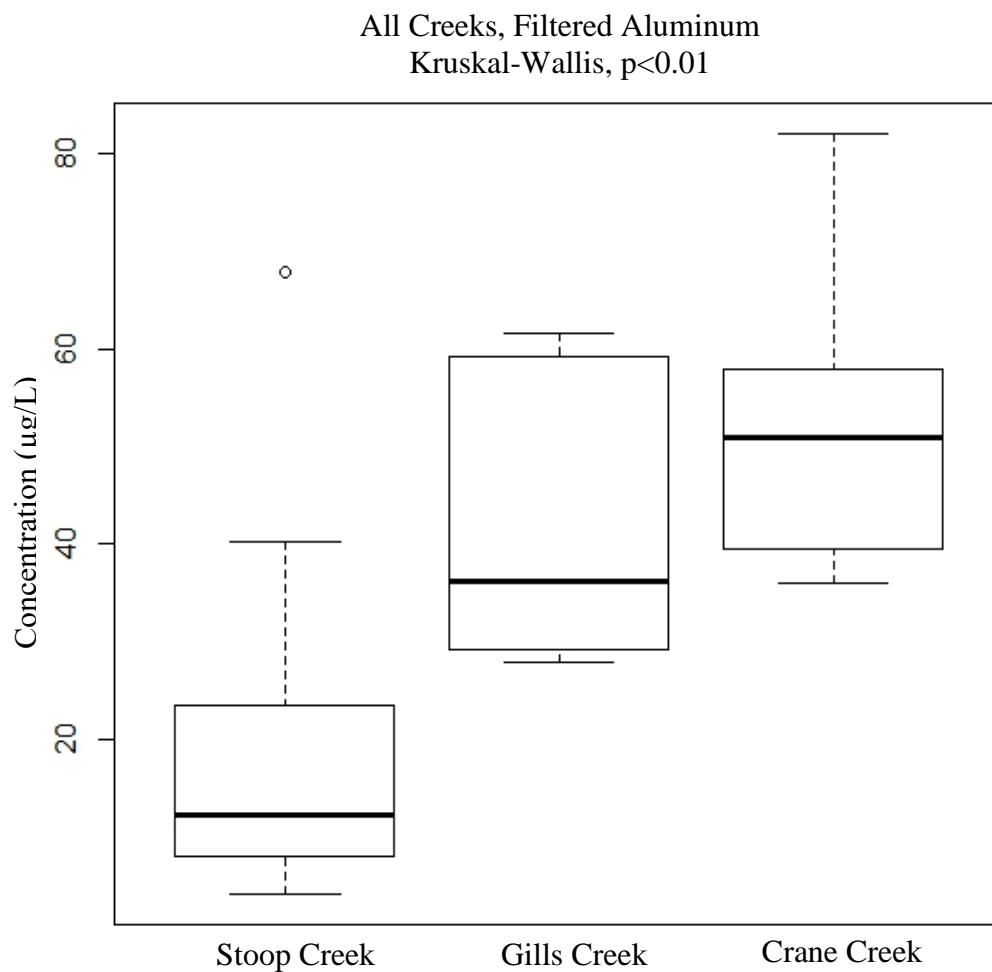


Figure 3.20 Boxplots of filtered aluminum concentration ($\mu\text{g/L}$) vs. creek reference locations ($n=33$).

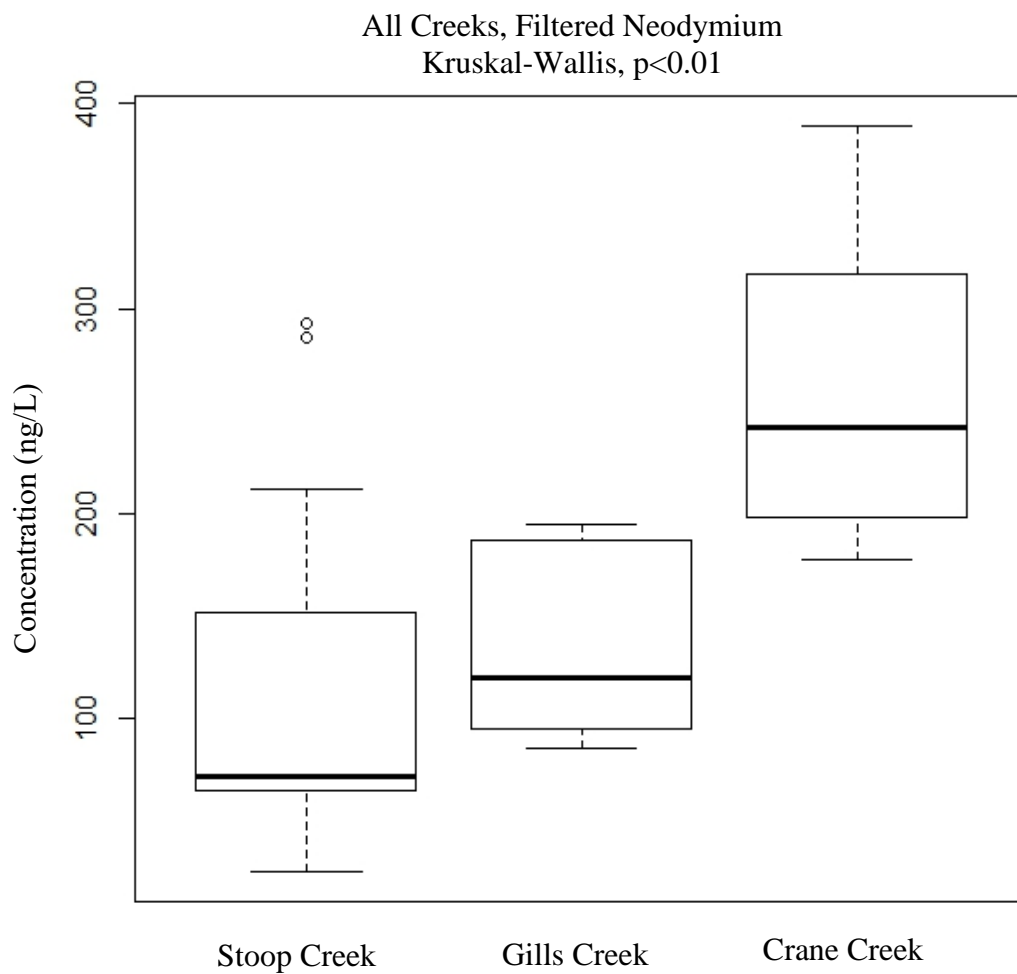


Figure 3.21 Boxplots of filtered neodymium concentration (ng/L) vs. creek reference locations (n=33) (representative of trend observed in all light rare earth elements).

Crane Creek Sanitary Sewer Overflows

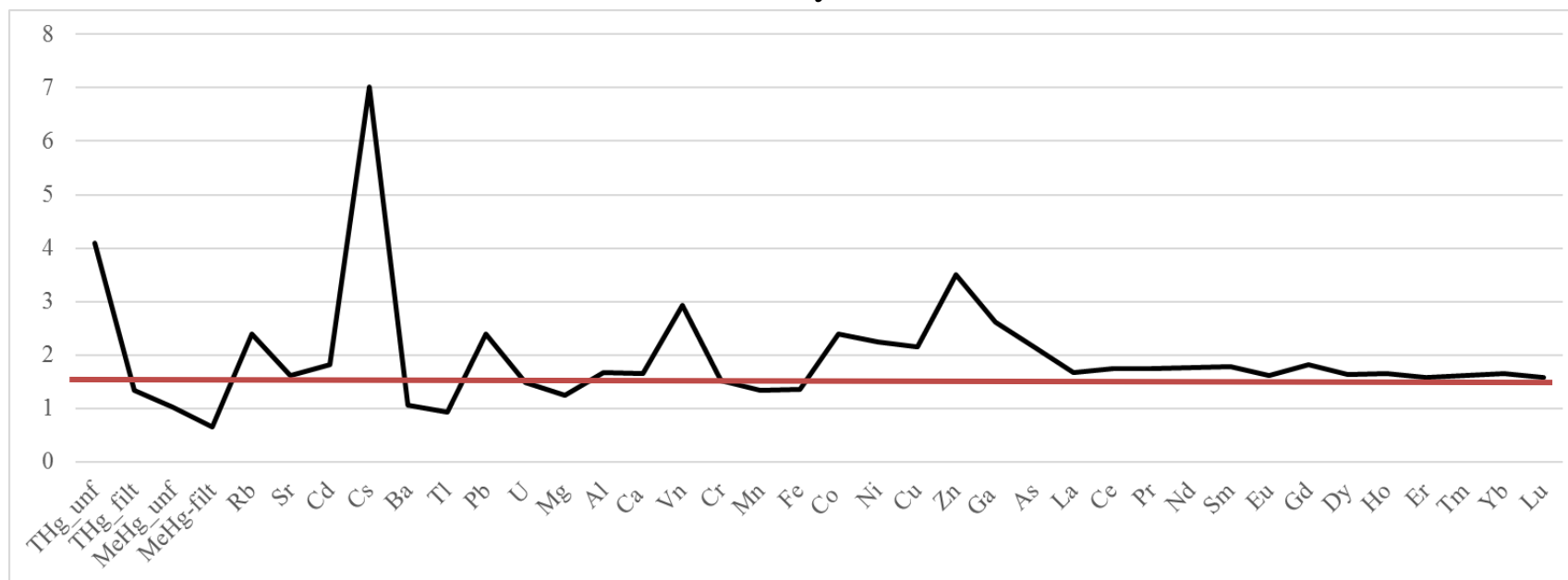


Figure 3.22 Ratio between average metal concentrations in Crane Creek sanitary sewage overflows (for total mercury and methylmercury n=5; for all metals n=4) compared to the Crane Creek reference sites (for total mercury and methylmercury n=10; for other metals n=7) (above red line indicates average concentrations are ≥ 1.5 than reference sites).

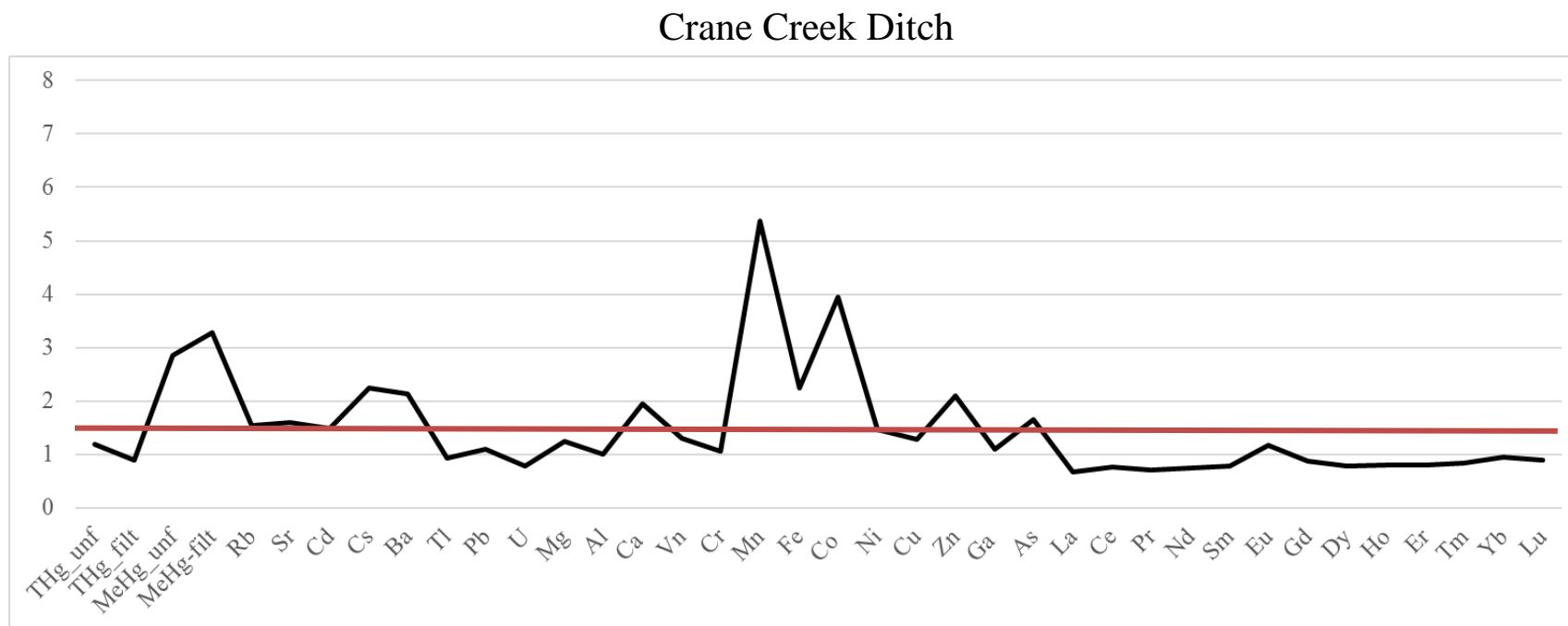


Figure 3.23 Ratio between average metal concentrations in Crane Creek ditch (for total mercury and methylmercury n=10; for other metals n=7) compared to the Crane Creek reference sites (for total mercury and methylmercury n=10; for other metals n=7) (above red line indicates average concentrations are ≥ 1.5 than reference sites).

Stoop Creek Ruptured Force Main Sewer Pipe

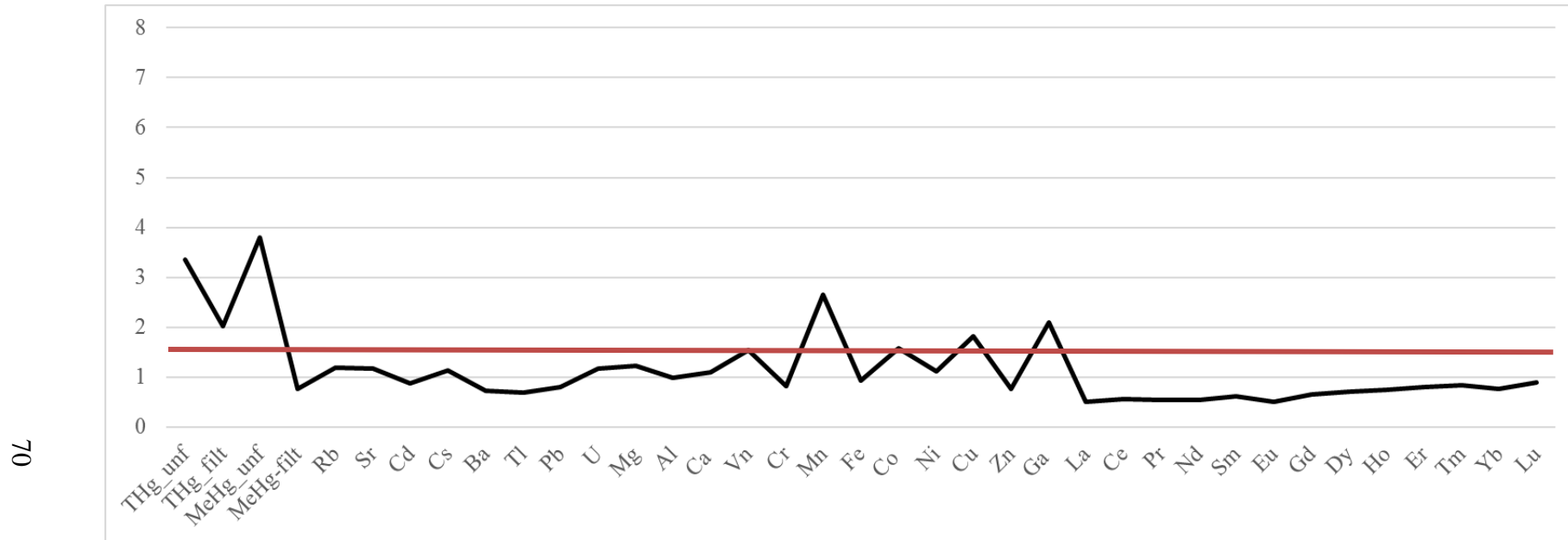


Figure 3.24 Ratio between average metal concentrations in Stoop Creek ruptured force main sewer pipe (n=1) compared to the Stoop Creek reference sites (n=20 for all metals) (above red line indicates concentrations are ≥ 1.5 than reference sites).

Stoop Creek Following Repairs to Ruptured Force Main Sewer Pipe

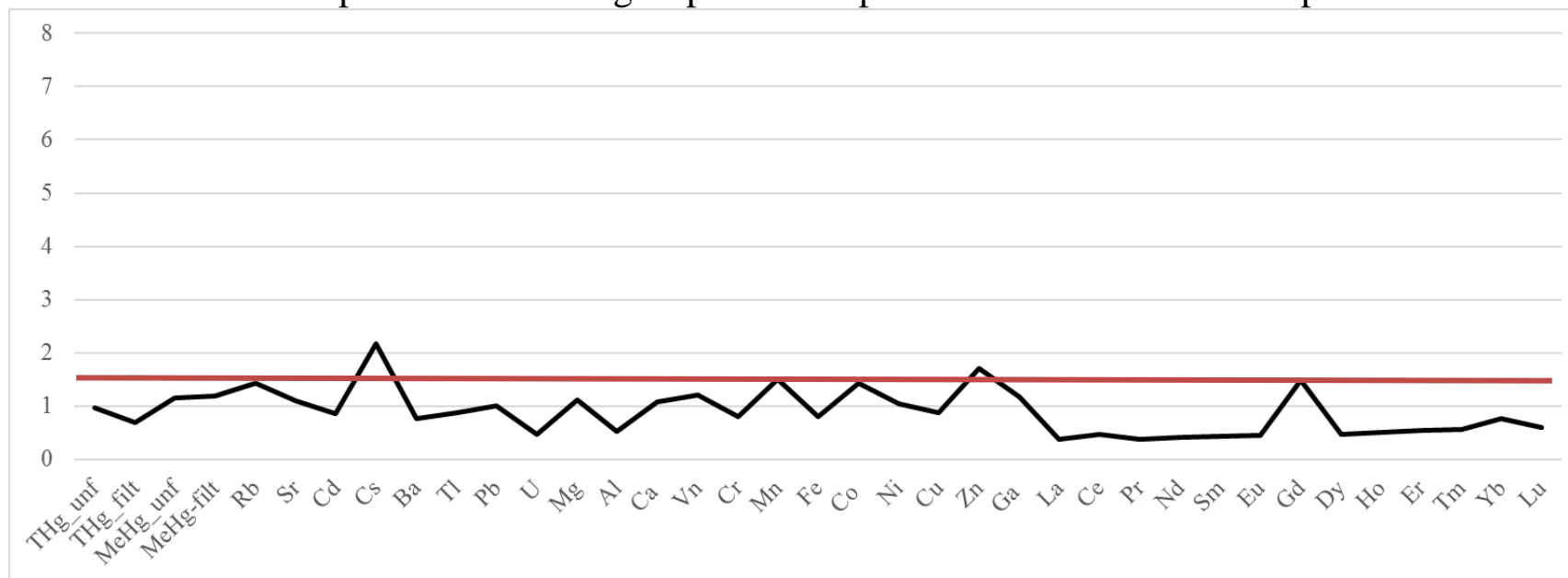


Figure 3.25 Ratio between average metal concentrations in Stoop Creek following repairs to the ruptured force main sewer pipe (n=2) compared to the Stoop Creek reference sites (n=20 for all metals) (above red line indicates average concentrations are ≥ 1.5 than reference sites).

Gills Creek Downstream from Sanitary Sewer Overflows

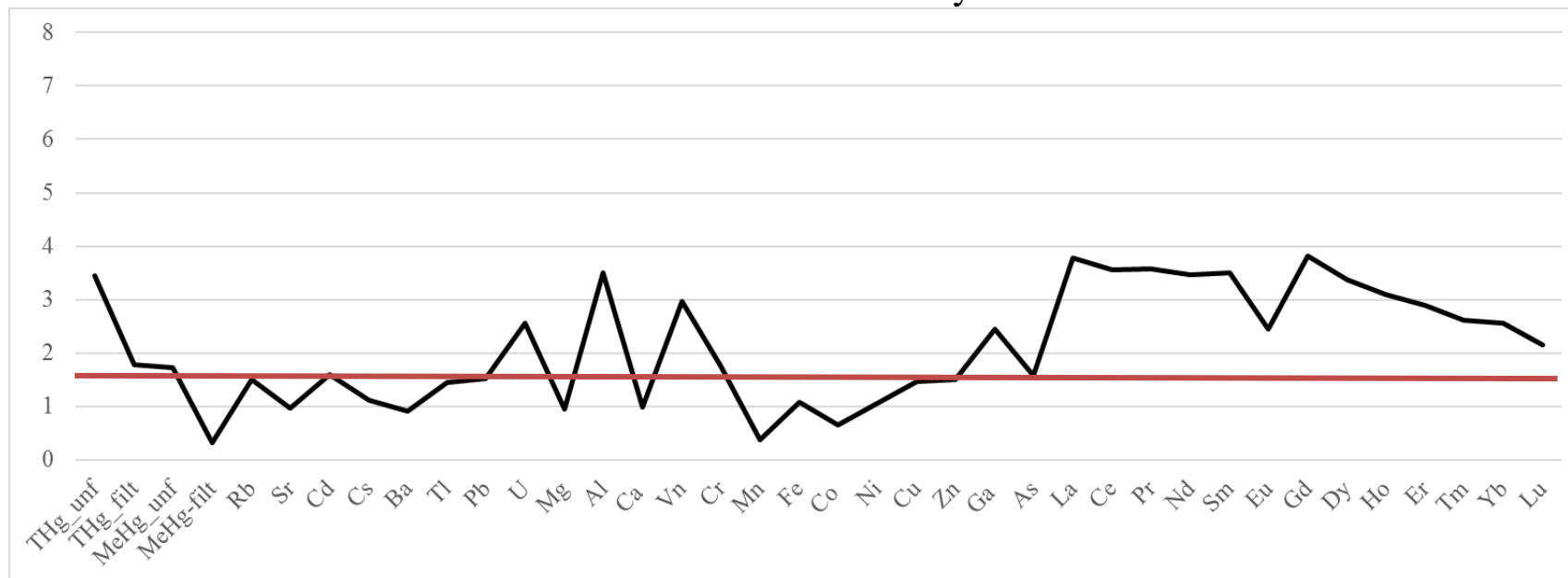
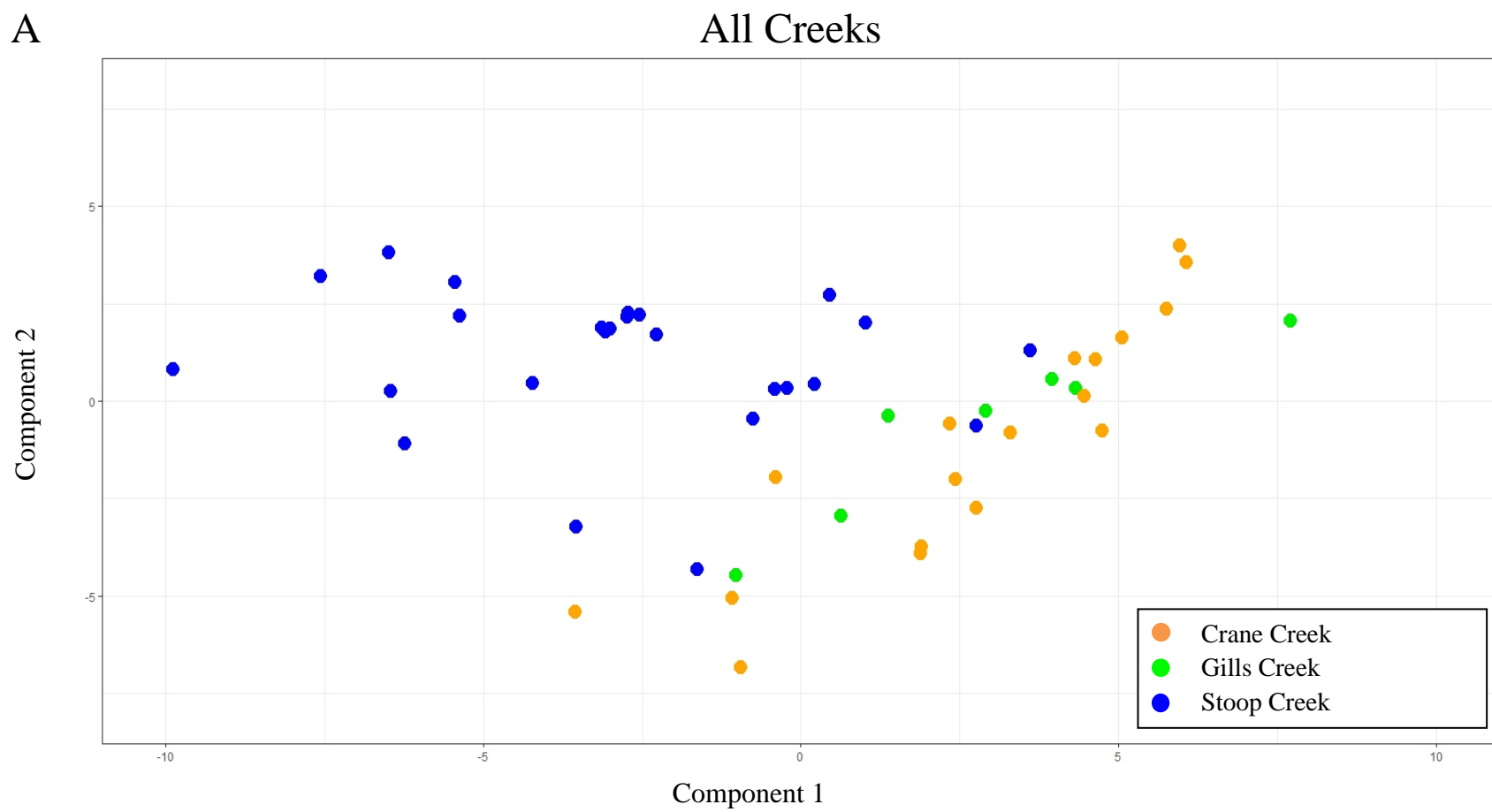


Figure 3.26 Ratio between average metal concentrations in Gills Creek downstream from two active sanitary sewer overflows (n=1) compared to the average metal concentrations at Gills Creek when no sanitary sewer overflows were occurring (n=6 for all metals) (above red line indicates average concentrations are ≥ 1.5 than reference sites).

A



B

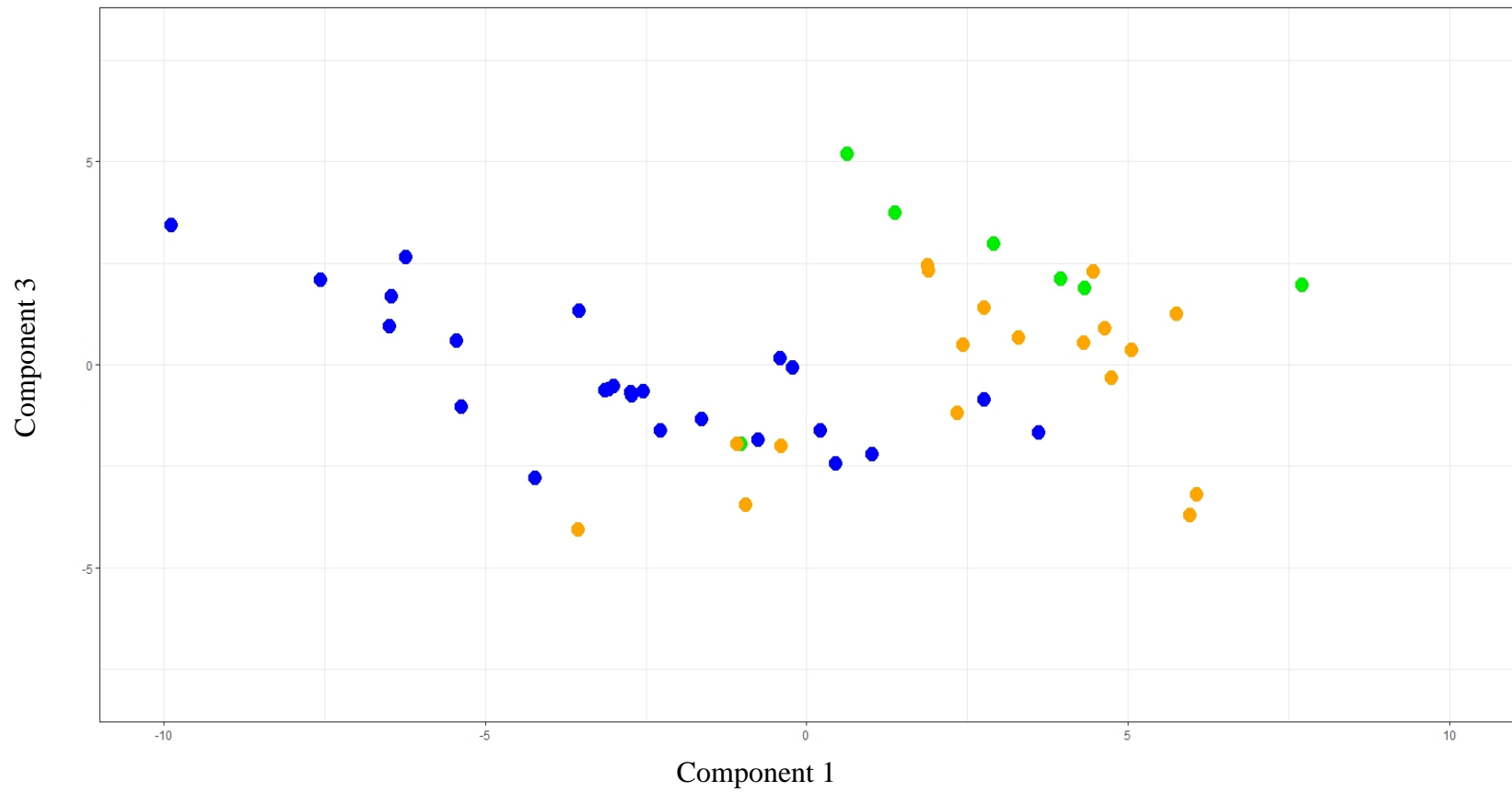
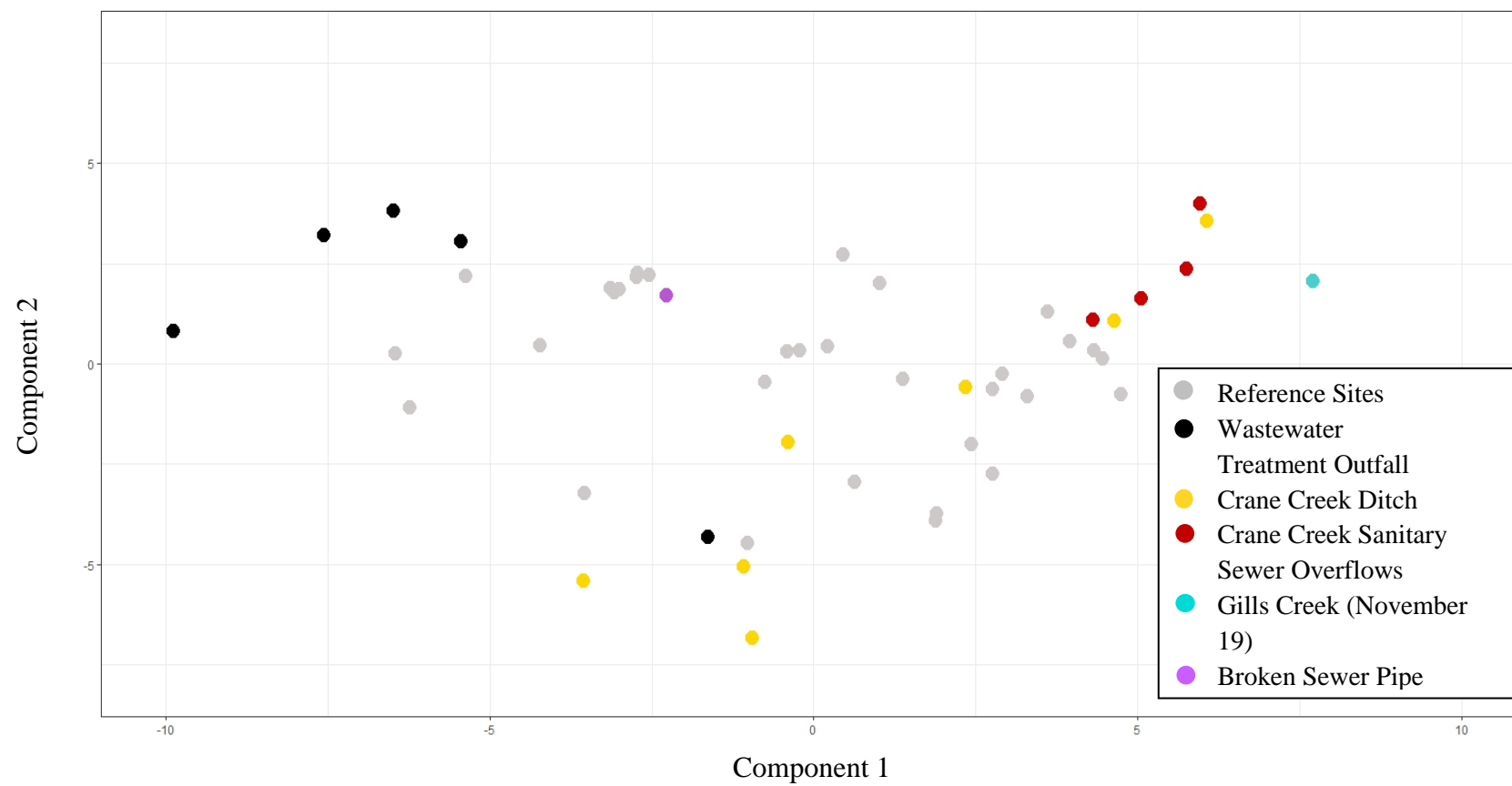


Figure 3.27 Principal component analysis results for (A) Components 1 and 2 and (B) Components 1 and 3 with events separated by creek.

A

All Creeks



B

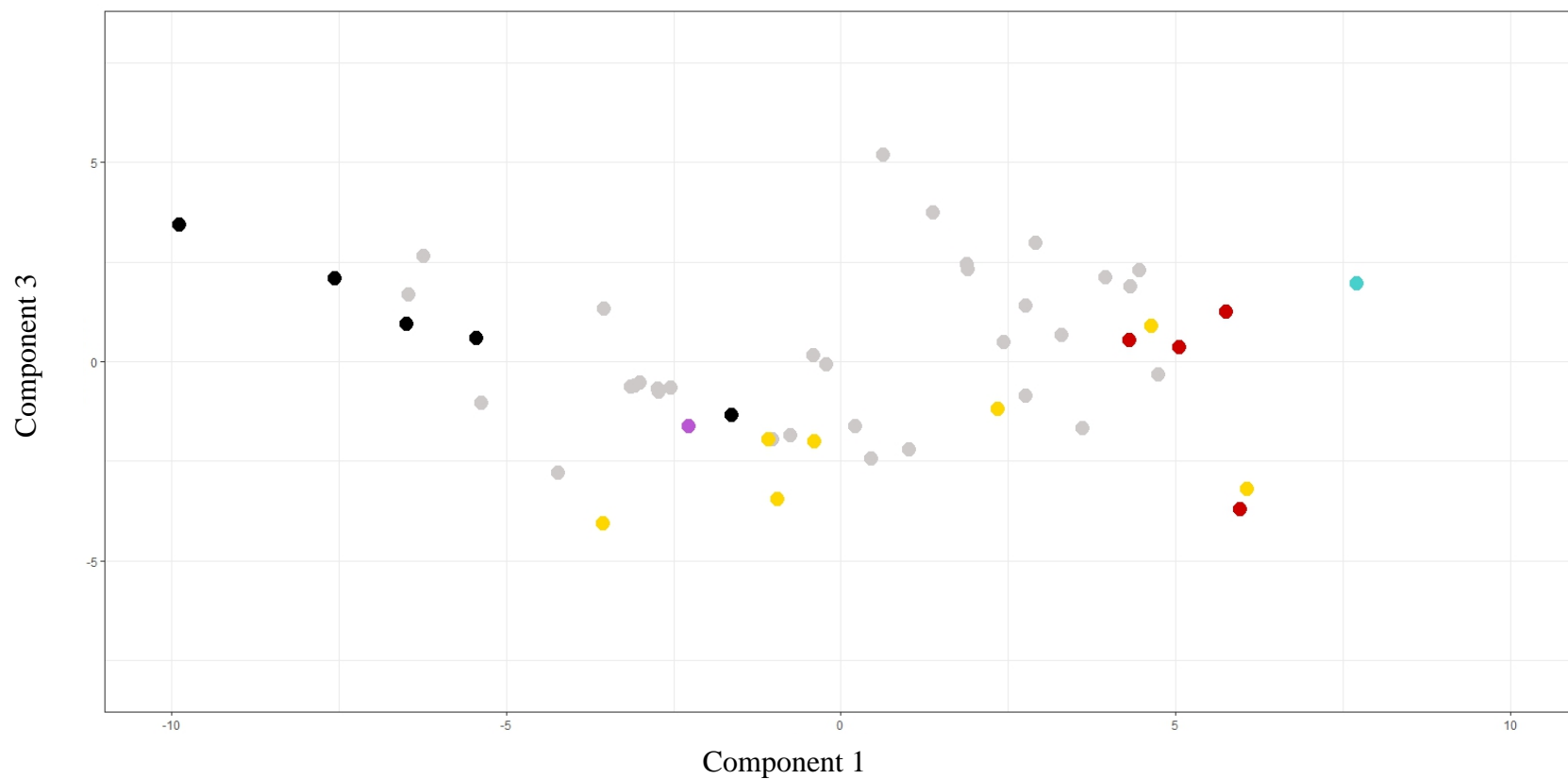
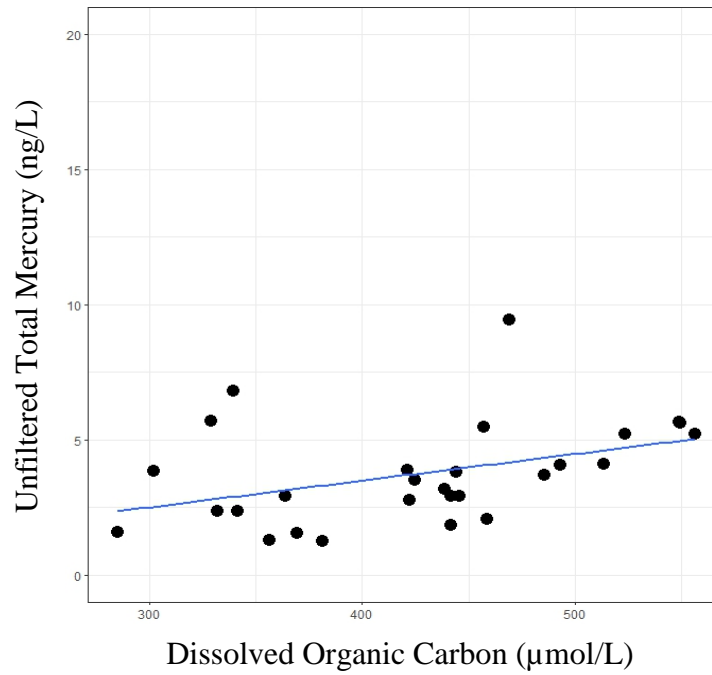


Figure 3.28 Principal component analysis results for (A) Components 1 and 2 and (B) Components 1 and 3 with events separated by sewage events and reference sites.

A

All Creeks, Spearman's rho: 0.43, p=0.02



B

All Creeks, Spearman's rho: 0.42, p=0.03

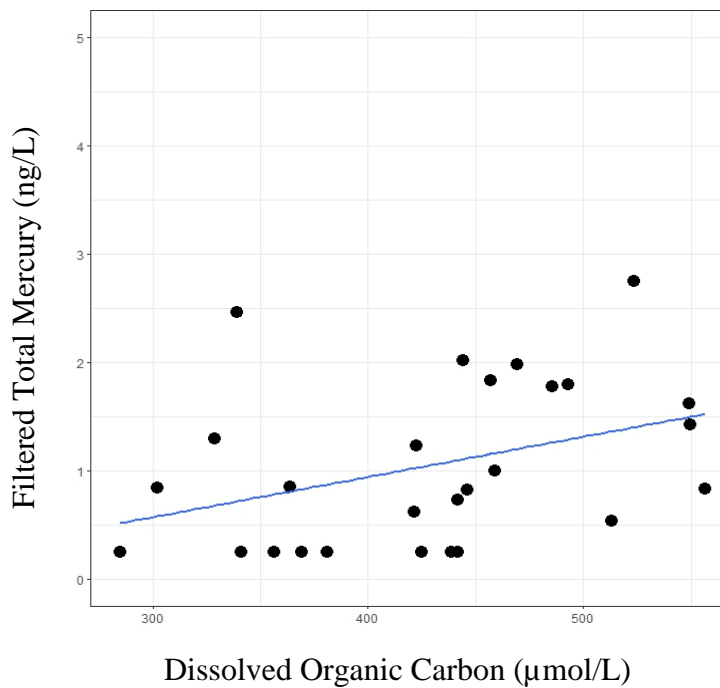


Figure 3.29 (A) Unfiltered and (B) filtered total mercury (ng/L) vs. dissolved organic carbon (μmol/L) concentrations for creek reference sites (n=20).

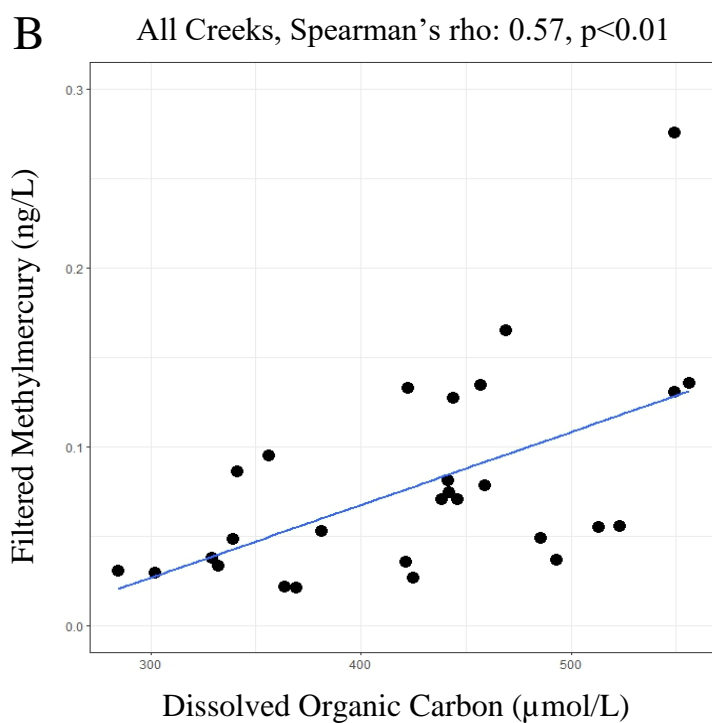
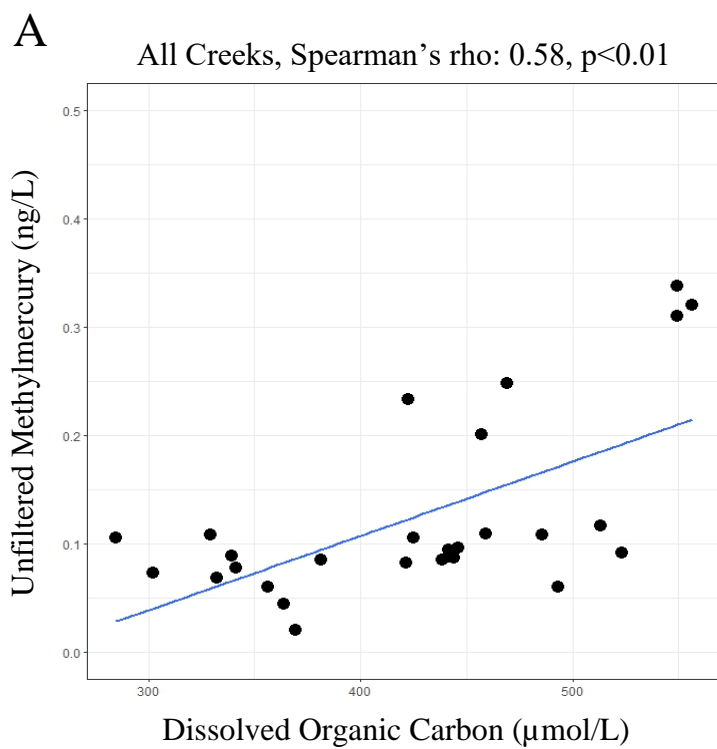
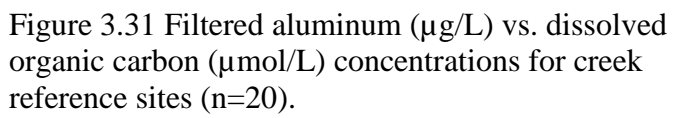


Figure 3.30 (A) Unfiltered and (B) filtered methylmercury (ng/L) vs. dissolved organic carbon ($\mu\text{mol/L}$) concentrations for creek reference sites ($n=20$).



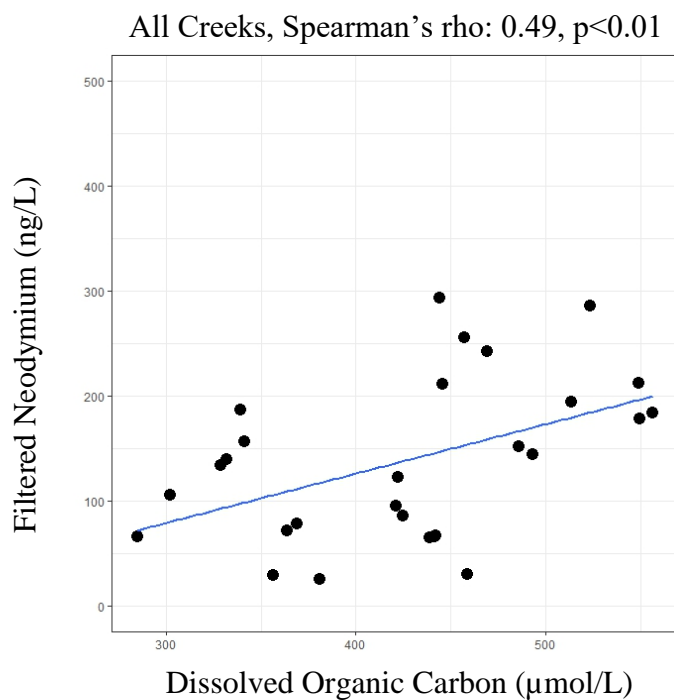


Figure 3.32 Filtered neodymium (ng/L) vs. dissolved organic carbon ($\mu\text{mol/L}$) concentrations for creek reference sites ($n=20$) representative of the nine rare earth elements that exhibited significantly, positive relationships with dissolve organic carbon (Spearman's rho: 0.40-0.54, $p < 0.05$, for all).

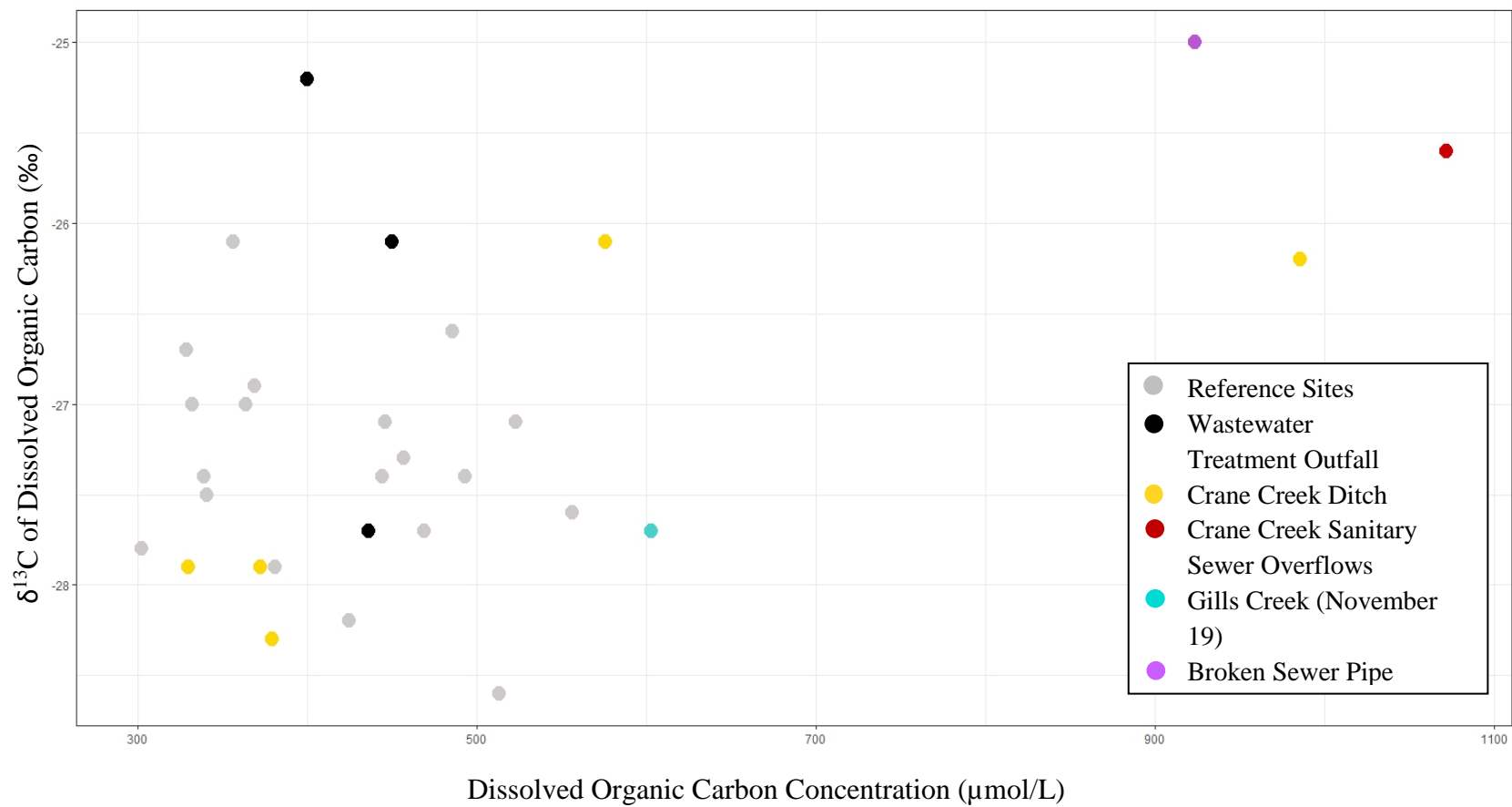


Figure 3.33 $\delta^{13}\text{C}$ values of dissolved organic carbon vs. dissolved organic carbon for sewage events and reference sites.

CHAPTER 4

DISCUSSION/CONCLUSION

Between 2015-2016, the concentrations of 36 metals, including THg and MeHg, were quantified in creek reference sites and during sewage events, including four SSOs, a ruptured force main sewer pipe and at one site downstream from SSOs. DOC concentrations and their corresponding $\delta^{13}\text{C}$ values were also measured. Sewage events were found to have higher DOC concentrations with more positive $\delta^{13}\text{C}$ values than those observed in the creek reference sites, which suggests the SSO effluents and the source of DOC in the SSOs differed from the creeks. Sewage events had greater concentrations of several metals compared to the associated creeks that received the SSO effluents. Compared to nearby reference sites, unfiltered THg concentrations in the sewage events were, on average, elevated by factors of 1.9-8.7, while unfiltered MeHg levels were enriched by factors of 1.7-3.8 within three of the six sewage events. A total of 5-16 other filtered metal concentrations were, on average, 1.5-7.0 times higher than the reference sites. Compared to the treated wastewater effluent, collected directly from the WWTP outfall, 26 metals, including THg and MeHg, were 1.5-15 times higher, on average, in the sewage events.

While the primary concerns of sewer overflows have focused on exposure to microbial pathogens (McLellan et al. 2007 Donovan et al. 2008; Fong et al. 2010) results suggest that SSOs are also a potential source of metals, including MeHg. Most sewage treated by the Columbia Metro WWTP comes from municipal sources while

approximately 5% originates from industries (<http://www.columbiasc.net/wastewater>). Prior to discharging their wastes into public sanitary sewage systems, industries are often required to remove toxic chemicals, including metals, through pretreatment processes (USEPA 2011). Although not all municipal WWTPs are required to implement specific treatment technologies that target metals, metal removal often occurs during primary and secondary treatments that are mandatory for all WWTPs that discharge into the nation's waters. During primary treatment, which is designed to remove settleable solids (USEPA 2004b), metals with a higher affinity for particulates, such as cadmium and lead, have been found to partially settle out into primary sludges (Buzier, et al. 2006). Secondary treatment, a process involving the removal of organic contaminants by microorganisms (USEPA 2011), can also lead to the further metal removal by biosorption (Chipasa 2003). Due to these treatment processes, a decrease in metal concentrations from the influent to the effluent of WWTPs has been observed for many metals including Hg, chromium, lead, nickel, cadmium and zinc (Karvelas, et al. 2003; Balogh and Nollet 2008; Gbondo-Tugbawa, et al 2010). Metals are often present in municipal wastewater; therefore, it is not surprising that sewage from the SSOs were more enriched in some metals.

One unexpected result, however, was that MeHg concentrations were higher in the effluent of one of the four SSOs at Crane Creek (2.3 times higher) and the broken sewer pipe (3.8 times higher) compared to nearby creek reference sites. Unlike inorganic Hg(II), MeHg is formed within the environment primarily through microbial-mediated processes (Benoit, et al. 2003; Parks, et al. 2013). One explanation for higher MeHg levels in the sewage events is that MeHg was introduced into the sewer system with other wastes or wastewaters. While most Hg emitted by the human body is inorganic Hg(II),

MeHg can also be excreted through feces (Clarkson and Magos 2006; Rothenberg, et al 2016), so it is possible that MeHg was sourced from raw sewage. Alternatively, higher MeHg concentration could be a result of in situ Hg methylation within the sewer system. In the environment, Hg methylation typically occurs in anoxic zones by anaerobic microorganisms, predominately sulfate-reducing bacteria, when inorganic Hg(II) is available (Benoit et al 2003; Hsu-Kim, et al 2013). Similar dark, anoxic conditions may be found within sewer collection systems which are known to contain microbes, including sulfate-reducing bacteria, within anaerobic biofilms on pipe walls (EPA 1992). Both inorganic Hg(II) and dissolved organic carbon which can fuel bacteria activity were present in the SSOS, therefore sewer systems could potentially be a suitable environment for Hg methylation. However, while THg concentrations were elevated in all six sewage events (2.0 to 9.8 times higher than reference sites), MeHg concentrations were enriched in only one of the four SSOs and the ruptured sewer pipe suggesting that SSOs are a potential source of inorganic Hg (II), but not a consistent source of MeHg.

In addition, SSOs may be a contributing factor to Hg methylation within environments that have been repeatedly impacted by SSO events due to enrichments of organic matter and inorganic Hg (II). Of the three sampled creeks, Crane Creek has experienced the greatest number of SSOs with just over 60% of the sewage spilled in Columbia during 2015 and 2016 occurring at Crane Creek (SCDHEC 2017a). Crane Creek reference sites had, on average, elevated THg and MeHg concentrations compared to the reference sites at Gills Creek and Stoop Creek. Unfiltered THg concentrations in the Crane Creek reference sites were 3.1 and 1.8 times higher, on average, compared to the Stoop and Gills Creek reference sites, respectively while unfiltered MeHg

concentrations were, on average, 2.6 and 2.3 times higher, respectively. SSOs at Crane Creek occurred near the creek's bank as well as at an upstream location in an area that is often inundated following rain events, two areas that may be ideal for Hg methylation. Higher methylation rates are often observed in saturated, surface sediments with higher microbial activity where there are constant inputs of fresh organic matter (Benoit, et al. 2003). Riparian zones and wetlands are known to be hotspots for Hg methylation (Driscoll, et al 1998; Rothenberg et al 2008; Vidon et al 2010). While SSO effluents flowed into Crane Creek, it is likely that inorganic Hg(II) and organic matter were deposited within the riparian zone of the creek, including the ditch that conveyed the SSO effluents, potentially providing anaerobes with the inorganic Hg(II) and labile carbon energy sources that support microbial methylation. These favorable conditions, however, were not observed at Stoop Creek and Gills Creek, so environmental factors and repeated SSOs could be contributing factors to the observed higher MeHg concentrations in the reference sites at Crane Creek compared to the other two creeks.

Unlike the SSOs and ruptured sewer pipe, the sewage event in Gills Creek was collected downstream from two active SSOs (approximately 2000m) during a heavy rain event rather than directly from or near the SSOs. Several metal concentrations were elevated (1.5-3.8 times higher) at this site compared to the average concentrations observed in the same creek during other sampling dates when SSOs were not occurring upstream. Concentrations of unfiltered THg and MeHg were 3.5 and 1.7 times higher than the creek average, respectively. These elevated concentrations were coupled with higher percentages of THg and MeHg that were particulate-bound (89% and 93%, respectively) indicating that Hg was potentially being transported with sediments from

upstream. This aligns with the results of other studies which have observed higher THg concentrations during storm flows due to high particulate loading and the tendency for Hg to bind strongly to particle matter (Mason and Sullivan 1998; Lawson, et al. 2001). DOC concentrations were also elevated; however, the corresponding $\delta^{13}\text{C}$ values did not differ from the reference sites, so it is unknown if the higher metal concentrations observed on this date were being sourced from the SSOs upstream. Coupled with higher rainfall amounts on this sampling date, other likely sources of the elevated metal concentrations could include metal-laden sediments entrained during higher flow rates, as indicated by the higher %particulate-bound THg and MeHg and/or urban runoff which has been found to carry higher concentrations of both particulate-bound and dissolved metals (Sansalone and Buchberger 1997; Dean, et al. 2005).

In conclusion, an estimated 23,000-75,000 SSOs release between 3 and 10 billion gallons of untreated sewage each year in the United States (EPA 2004a); therefore, the impact that SSOs have on the nation's waters could be substantial. Within the past four years, Columbia, SC alone has experienced just over 750 individual SSOs that have amounted to approximately 12 million gallons (not including during the October 2015 flooding event) of untreated, spilled wastewater (SCDHEC 2017). The entire 50-mile length of the Congaree River and Lower Saluda River are listed as impaired for Hg due to elevated fish tissue Hg concentrations (SCDHEC 2016b). It is possible that SSOs have contributed to these impairments. To minimize SSOs, replacements and capacity upgrades to the City of Columbia's separate sanitary sewer system began in 2013; however, conditions under the consent decree are not required to be fulfilled until 2025 (City of Columbia 2013). Until replacements and capacity upgrades in the City's sewer

lines are complete, SSOs will continue to occur, particularly during heavy precipitation events. Although SSO events are intermittent, the resulting impacts may not be temporary as metals are persistent contaminants that are not as easily removed from the environment. With SSOs likely continuing to be one potential source of metals to nearby waterbodies across the nation, it is important to further investigate how they influence the biogeochemical cycling and bioavailability of metals in impacted aquatic environments.

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