Aquatic Macroinvertebrate Data Mining Report
Hinkson Creek

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SECTION 1

PROJECT INTRODUCTION

1.1 Site Setting

Hinkson Creek lies within a 90 square mile mixed-land-use watershed and flows approximately 26 miles through Boone County, Missouri from northeast of Hallsville to its confluence with Perche Creek on the western edge of the City of Columbia. Hinkson Creek is considered a Missouri Ozark border and riffle/pool complex stream, located within a transitional zone between the Glaciated Plains and Ozark Natural Divisions (Thom and Wilson 1980). From the mouth upstream to approximately Providence Road (~ 8 miles) Hinkson Creek (Waterbody Identification #1007) is classified as a Class P stream, meaning that even in periods of drought the stream maintains permanent flow. From approximately Providence Road upstream (~ 19 miles) to its source Hinkson Creek (Waterbody Identification #1008) is classified as a Class C stream, meaning that it may cease flow during dry conditions but maintains permanent pools capable of supporting aquatic life. Beneficial uses of both sections include the protection of warm water aquatic life (Ashcroft 2020). Hinkson Creek is within the Ozark/Moreau/Loutre ecological drainage unit (EDU) of the state's aquatic ecological system (**Figure 1**). An EDU is a region in which the aquatic biological communities and habitat conditions are expected to be similar. Streams within an EDU that represent the least impacted and best attainable biological communities are considered Reference Streams. Reference Streams in the Ozark/Moreau/Loutre EDU include Boeuf Creek, Burris Fork Creek, Moniteau Creek, and the Loutre River. Bonne Femme Creek was added as an additional category of stream (Control), due to its similar size to rural Hinkson Creek, its proximity to the City of Columbia, and reduced urbanization in the watershed.

1.2 Background

Hinkson Creek's proximity to federal and state agencies as well as the University of Missouri makes it one of the most investigated streams in the state. Since the 1960's numerous stream studies have been performed by various entities investigating water quality, aquatic life, habitat, hydrology and other aspects of the creek. Since the 1970's, the City of Columbia and Boone County have worked to eliminate wastewater inputs to Hinkson Creek and nearby streams and directed them to a centralized sewer, significantly reducing inputs of domestic organic wastes. In 1982, the Columbia Regional Wastewater Treatment Plant was initially constructed. Since then more than 100 small wastewater treatment systems have been eliminated in Columbia (IMP 2018).

Hinkson Creek was originally placed on the 1998 303(d) list for "unspecified pollution due to urban nonpoint runoff." The United States Environmental Protection Agency (USEPA, 1994) stated that nonpoint source pollution was the greatest cause of water quality impairment in the

United States. Because no known pollutant was specified, in 2002 the impairment was revised to "unknown pollutant" and no source was listed. In order to determine the specific pollutant (s) and source (s) of the impairment, the Missouri Department of Natural Resources (MDNR) initiated a series of studies to determine whether the aquatic life in Hinkson Creek were impaired and, if so, determine the cause and sources of impairment. Using the Missouri bioassessment protocol that allows for a comparison of aquatic macroinvertebrate communities in streams within a given EDU to the appropriate reference streams (MDNR 2002), the MDNR conducted bioassessment surveys in 2001 and 2002. These macroinvertebrate bioassessments used the four primary metrics of Total Taxa richness , Ephemeroptera, Plecoptera, and Trichoptera taxa richness (EPT), Missouri Biotic Index and Shannon's Diversity Index to derive a Missouri Stream Condition Index (MSCI) which confirmed sections of Hinkson Creek downstream of the I-70 interchange, encompassing portions of WBID 1007 and WBID 1008 were not fully meeting aquatic life thresholds. However, bioassessments performed by MDNR in subsequent years have not consistently found an impaired aquatic community within both segments of Hinkson Creek. In some instances, and at some sites, MSCI scores have ranged from non-supporting to fully supporting from one sampling season to the next. The upstream rural segment of Hinkson Creek achieves fully supporting status more often than the urban portion of Hinkson Creek (64 percent rural vs 23 percent urban, MDNR 303d listing for 2018).

1.3 Collaborative Adaptive Management

In 2012, Boone County, the City of Columbia, the University of Missouri, the USEPA and the MDNR jointly agreed to use a Collaborative Adaptive Management (CAM) approach to address water quality concerns in Hinkson Creek. CAM is a stakeholder-based adaptive management process for decision-making, dealing with scientific and socio-economic complexities and uncertainties inherent in many ecosystems. CAM utilizes an iterative process to make changes and determine the effect of those changes on water quality. CAM is also a method for taking management actions and mapping their influence on the health of the stream ecosystem (CAM 2012).

The fundamental goals and objectives of CAM are to implement the TMDL and improve Hinkson Creek by:

- Identifying primary pollutants of concern, if possible;
- Improving diversity of key indicator macroinvertebrate species;
- Improving stream ecosystem health and general water quality in Hinkson Creek;
- Establish a meaningful stakeholder process that ensures appropriate actions are taken within reasonable timeframes; and
- Achieve the goal of Hinkson Creek meeting applicable water quality standards, as developed by MDNR, and approved by the Missouri Clean Water Commission and the USEPA.

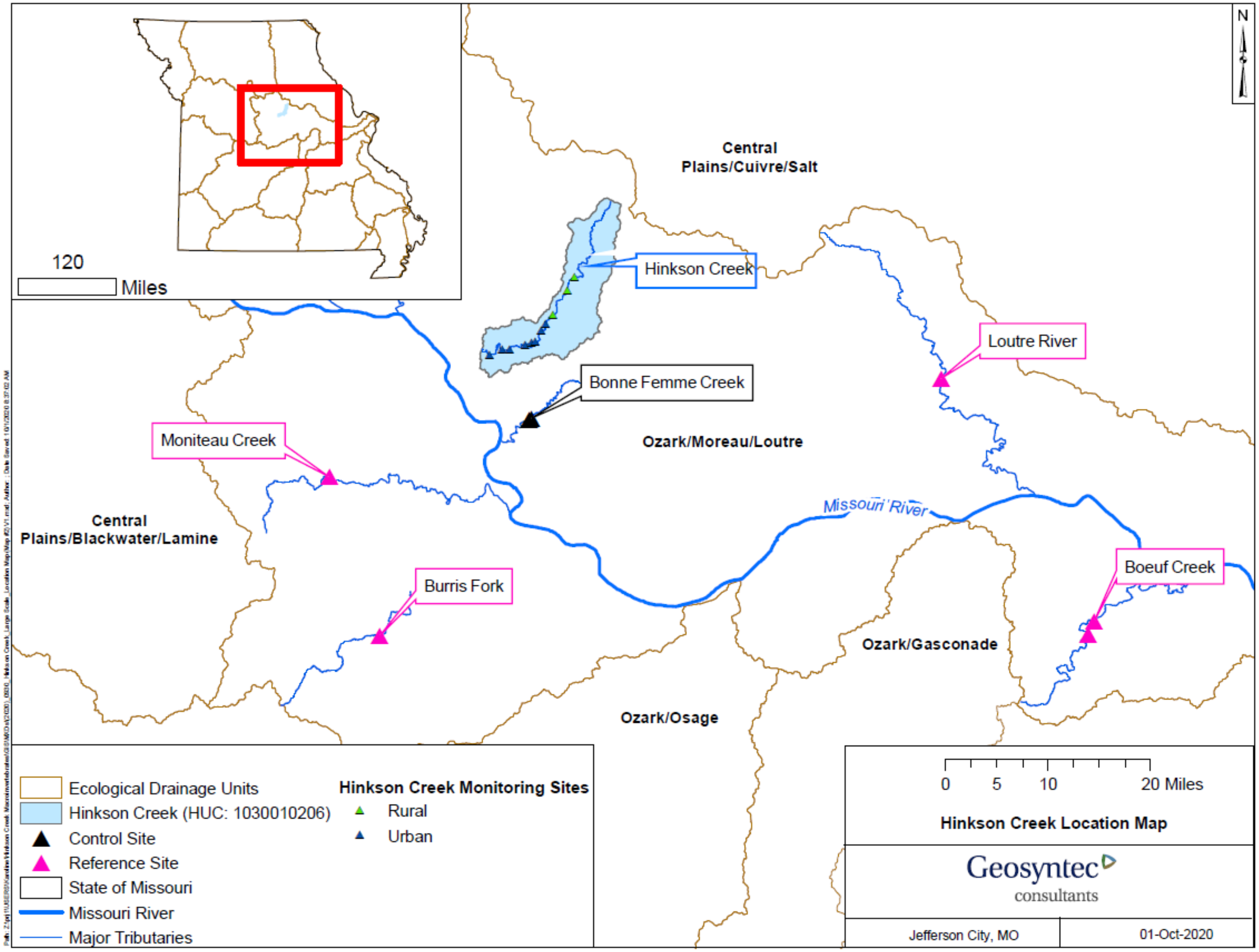

FIGURE 1. Ozark/Moreau/Loutre EDU Reference Streams, Control Stream and Hinkson Creek.

1.4 Project Goals and Objectives

The overall goal of the Hinkson Creek Aquatic Macroinvertebrate Data Mining project is to assist the CAM in the analyses and interpretation of existing macroinvertebrate and water quality and physical data from Hinkson Creek and relevant EDU Reference and Control streams to diagnose stressors causing the Hinkson Creek aquatic life impairment. An additional objective of the project is to determine "best" indicator metrics for stressor identification and for assembling multi-metric indices for diagnosing causes for aquatic life impairment in Hinkson Creek.

SECTION 2

LITERATURE REVIEW

Concurrently with the calculation, analyses and interpretation of macroinvertebrate metrics, a review of literature pertaining to urban effects on stream quality was performed to examine dynamics found in other urban settings and/or previous investigations of Hinkson Creek.

2.1 Potential Stressors in Urban Streams

Increased urbanization of lands surrounding streams increases stress on aquatic communities within those streams. Many studies assessing these streams have documented increasing degradation of physical, chemical and biological attributes of streams passing through urban areas (Poulton et al. 2007, Morley et al. 2002, Cuffney, et al. 2010). Within the Hinkson Creek watershed numerous studies have been performed that document stream degradation within the watershed, (MDNR 2002, 2004, 2005, 2006, 2012, 2013, 2014, 2015, and others) but identifying specific stressor(s) has been difficult. As stated in the US Environmental Protection Agency Guidance Document for Stressor Identification (USEPA 2000) linking biological effects to their causes is complex and is best accomplished using integrated information from a variety of sources. Ultimately through a strength of evidence approach the probable cause(s) may be identified giving managers the tools to eliminate, control and monitor identified stressors. According to the Stressor Identification Guidance Document, the core of the process consists of three main steps:

1) Listing candidate causes of impairment;
2) Analyzing new and previously existing data to generate evidence for each candidate cause; and
3) Producing a causal characterization using the evidence generated in Step 2 to draw conclusions about the stressors that are most likely to have caused the impairment.

A summary of some potential candidate stressors within urban streams in general and Hinkson Creek are provided below.

2.1.1 Hydrology

Increased urbanization in a watershed is nearly always associated with a decrease in pervious land cover. This limits the potential infiltration and results in increased runoff during rain events (Paul and Meyer 2001). This is frequently reflected by a more flashy hydrograph due to the generally increased runoff from impervious surfaces, as well as greater efficiency in runoff transport by stormwater drainage systems (Walsh et al. 2005). The altered hydrological regime is strongly related to a variety of water quality and habitat degradations as discussed below. Coleman et al. (2011) found that the magnitudes and frequencies of hydrological disturbances adversely affected stream communities.

2.1.2 Temperature

Temperature is a critical component to stream macroinvertebrate assemblages, influencing metabolism, growth, development and reproduction (Ward 1992). Increased stream temperatures are strongly related to factors such as the removal of riparian vegetation and the "heat island" effect of the urban area (Paul and Meyer 2001; Rutherford et al. 2004; Somers et al. 2012) while lower temperatures can mitigate the effects of some chemical constituents on aquatic life (Jackson et al. 2019). Generally, water quality investigations include water temperature measurements but only occasionally are these measurements collected in a manner where accurate comparison can be made between study areas, i.e., data are obtained from multiple locations simultaneously.

As part of a series of water quality investigations that coincided with macroinvertebrate sampling (MDNR 2006), dataloggers were placed at Hinkson Creek rural (HCr) and Hinkson Creek urban (HCu) locations to continuously record temperature and dissolved oxygen concentrations over an eight-week period extending from spring into summer of 2006. During this time temperature fluctuations occurred in a normal diurnal pattern at both monitoring sites. In addition, no evidence of significant temperature increases were noted at any monitoring site following runoff events.

Zeiger et. al. 2015 collected continuous instream temperature data in Hinkson Creek at multiple stations (both rural and urban) for four water years between 2009 and 2013. While their primary goals were to quantify water temperature and test model predictions in a mixed-use urbanized watershed, the intensive effort of data collection could be useful in evaluating differences in instream temperatures of rural versus urban Hinkson Creek stream segments. The maximums, minimums, and means of water temperatures during the four water years for each of the five monitoring sites were presented in the Zeiger report. Maximum temperature values of all sites ranged from 32.1 degrees Celsius (^{0}C) to 36.1^{0}C during the summer and mean temperature values ranged from 13.7^{0} to 14.4^{0}C. These data did not reveal large deviations (increases) in water temperature in the urban portions of Hinkson Creek as compared to the rural portion.

2.1.3 Dissolved Oxygen

As part of the state's water quality investigation that coincided with macroinvertebrate sampling (MDNR 2006) data loggers collected temperature and dissolved oxygen at HCr and HCu locations for an eight-week period during the spring and summer of 2006. Dissolved oxygen concentrations generally followed a typical diurnal pattern. Lower dissolved oxygen concentrations coincided with pool stagnation at low flows from extended dry periods (week or more) than with increased flows from storm events. Dissolved oxygen conditions were noted to typically improve following rainfall events. During an extended dry period, dissolved oxygen concentrations fell below the 5.0 mg/L water quality criterion 10 to 15 percent of the time at the HCr site (Hwy. 63) and 44 to 62 percent of the time at the HCu site (Broadway Blvd.).

2.1.4 Multiple Chemical Stressors

Multiple chemical stressors are frequently implicated in impaired urban stream settings (MDNR 2004, Nichols et al 2016, and others). Near stream activities and runoff from stormwater and snowmelt that drain a variety of urban uses such as commercial, industrial, and residential can contribute to instream pollutants through intentional or unintentional releases. Impacts from runoff can occur acutely (immediate) or chronically (long-term) through periodic inputs from commercial and industrial activities, parking lot runoff, road maintenance, etc. A wide range of contaminants such as metals, organic compounds (i.e. petroleum products, pesticides), fertilizers, dissolved chemicals, surfactants, etc. all can potentially contribute to stream inputs that can affect the quality of the water in a stream (MDNR 2004).

The MDNR Environmental Emergency Response Tracking System (MEERTS) and Missouri Department of Conservation Pollution and Fish Kill Investigation Reports document adverse ecological events that have occurred throughout Missouri. A map compiling reported adverse ecological events in the Hinkson Creek watershed from 2001-2017 is presented in **Figure 2**. Adverse ecological events are more frequently documented along the interstate and highway corridors, as well as within HCu locations. Significantly fewer adverse ecological events are documents in HCr locations. Sources of MEERTS and Fish Kill events include;

- Vehicular accident (petroleum leaked into waterway);
- Fire or explosion (Brookside Apartments and O’Reilly Auto Parts);
- Improper swimming pool water disposal; and
- Equipment malfunction of potable water or sewer systems.

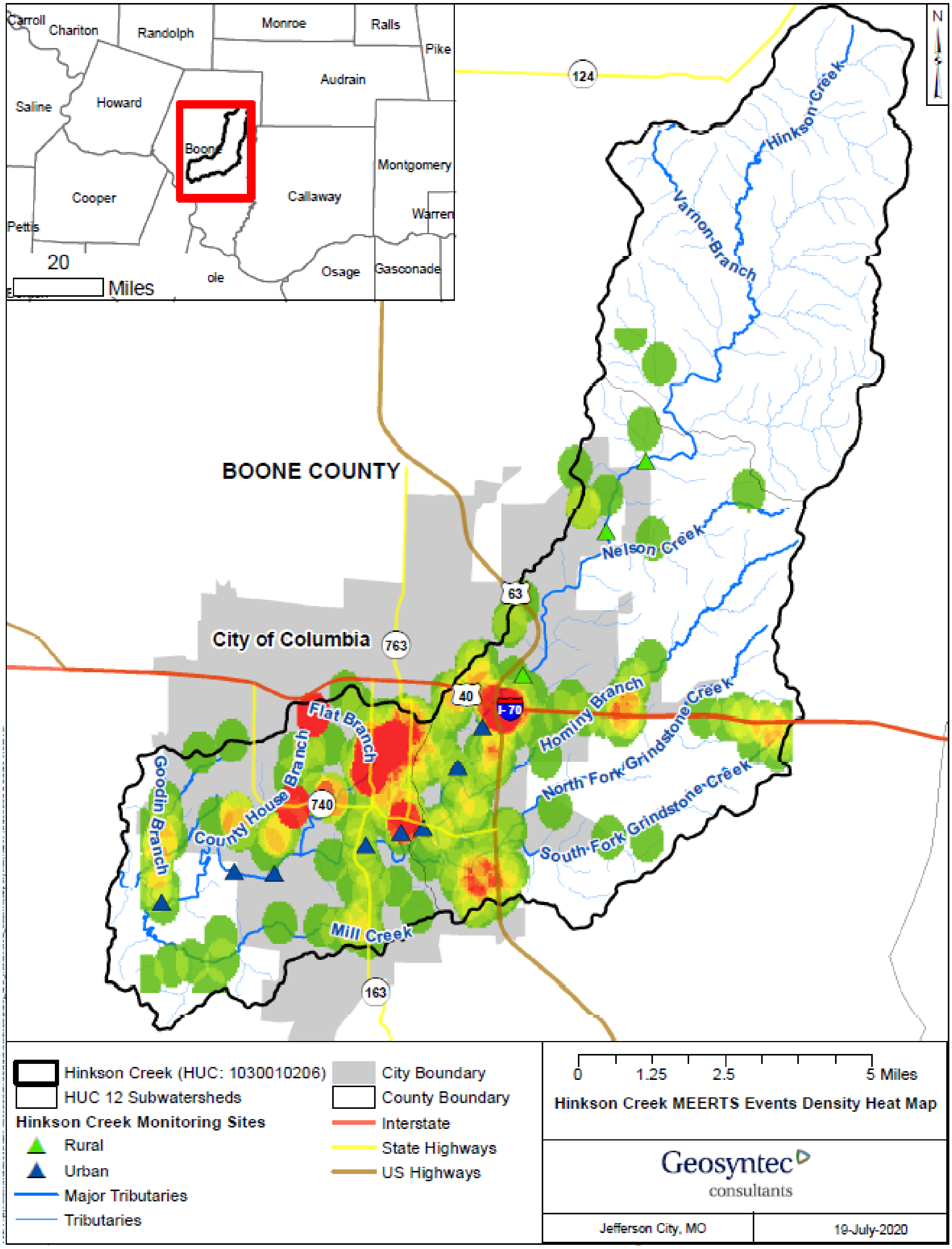

FIGURE 2. Hinkson Creek MEERTS Reported Events Heat Map.

2.1.4.1 Chloride

One of those stressors frequently cited in urban settings is chloride, typically sodium or calcium chloride associated with winter treatment of roads, sidewalks, and parking lots (Corsi et al. 2014, Gillis 2011, MDNR 2004). In addition, brine from domestic and industrial use of water softening agents can contribute to chloride inputs (e.g. lawn watering with potable water), especially in urban settings. The current USEPA ambient water quality criteria for chloride (USEPA 1988) is 860 mg/L (acute) and 230 mg/L (chronic). While the validity of these criteria has been questioned in recent years due to additional data demonstrating how important factors such as water hardness and temperature are to chloride toxicity (Iowa DNR, Soucek et al 2005, Elphick et al 2010, Geosyntec 2012), they are still widely used for regulatory purposes and are in effect in Missouri's Water Quality Standards.

Various Hinkson Creek investigators have reported acute and chronic chloride criteria to be exceeded on occasion in Hinkson Creek or its tributaries (MDNR 2002, 2004, 2006, Allert et al 2012, Nichols et al 2016, Hubbart et al 2017). MDNR 2002 observed high chloride levels and toxicity to daphnids (standard freshwater invertebrate toxicity test organism known to be pollutant intolerant) from direct snowmelt and rainfall events during winter and spring months in Hinkson Creek tributaries. In addition, toxicity attributed to factors other than chloride (i.e. petroleum compounds, pesticides, metals) were also detected. Allert et al 2012 attributed acute and chronic toxicity in daphnids to elevated chloride concentrations found in Hinkson Creek water samples during winter low-flow conditions and Nichols et al 2016 found that chloride concentrations were on average 126 percent higher in urbanized reaches in the spring. Hubbart et al 2017 performed an intensive chloride investigation of Hinkson Creek and determined that chloride loading increased from the rural headwaters to the primarily urbanized portion of Hinkson Creek and that the greatest frequency of elevated chloride conditions occurred in the mid-watershed portion during late winter/early spring melting periods, implicating road salt applications.

The effects of chloride on the aquatic life in Hinkson Creek have been documented but resolutions to mitigate these effects while maintaining and prioritizing safety considerations during winter snow/ice conditions remain elusive. In addition, a lack of information exists that looks at the long-term effects of chloride concentrations that are below chronic criteria but exceed natural background levels.

2.1.4.2 Organics and Metals

In addition to regular macroinvertebrate bioassessment monitoring, the MDNR performed a series of stream studies that included collection of baseflow samples throughout the watershed and stormwater and snowmelt runoff samples within the impaired section of Hinkson Creek. Monitoring was performed between the summer of 2003 and the summer of 2006 (MDNR 2004, MDNR 2005, MDNR 2006). Primarily concentrating within the impaired section of Hinkson

Creek, these studies focused on periods of snowmelt and stormwater runoff to identify chemical constituents present in runoff that may contribute to a depressed aquatic community. Both stormwater and baseflow samples were subjected to standard acute toxicity tests using Microtox (a bacterial bioluminescence test), and a daphnid, *Ceriodaphnia dubia*. Samples that exhibited toxicity were further subjected to Toxicity Identification Evaluation (TIE) manipulations (USEPA 1991) and follow up testing to determine the constituents or broad class of compounds that are most likely causing the observed toxicity.

Results of these studies found no toxicity in samples of Hinkson Creek collected upstream from the 63-Connector interchange. However, toxicity was detected in snowmelt/stormwater runoff samples collected near Hinkson Creek Stations 5.5 and 6 (between the 63-Connector Interchange and Broadway Blvd.) and instream at Hinkson Creek Station 5.5 (Broadway Blvd.). TIE results and follow-up chemical analyses suggested that at various times, in addition to chloride, non-polar organics (i.e. waste oil, herbicides) and metals may contribute to the observed toxicity although none of the sampling sites were consistently toxic. While chemical analyses associated with these tests rarely found individual chemical constituents that exceeded water quality criteria, mixtures of various constituents (i.e. metals) may exhibit toxicity at levels lower than that of the individual metal's criterion (Spehar and Fiandt 1986). Toxicity testing results of samples collected downstream of Hinkson Creek Station 5.5 were even more sporadic, implicating metals and organic constituents as likely contributors to observed toxicity. While these constituents are commonly implicated in the degradation of urban streams, mitigating their effects is challenging and no single management technique has proven successful in improving water quality and biological communities within urban watersheds.

2.1.4.3 Nutrients

The effects of nutrients (total phosphorus, total nitrogen, nitrate + nitrite nitrogen, and ammonia nitrogen) on water quality are documented (Vannote et al. 1980; Dodds et al. 1988) and have become standard parameters of most water quality investigations. However, as wastewater conveyance and treatment has improved, degradation associated with nutrients is less a problem in urban settings than in agricultural settings (USGS 1999). While most previous Hinkson Creek water quality studies have included nutrient evaluations, all reported values have generally been at or near detection limits for analytical testing, below values that would be deemed harmful to aquatic life, and similar to Reference stream concentrations.

2.1.5 Habitat

Stream habitat assessments were performed by MDNR during their initial investigation (MDNR 2002 and 2004) of Hinkson Creek and again in 2016 (MDNR 2016). According to MDNR habitat assessment protocols, for a study site to fully support a biological community the habitat scores per the stream habitat assessment protocol (SHAP 2016) should be 75 percent to 100 percent similar to the mean of the Reference streams site scores. All Hinkson Creek sites in both early and recent assessments have been within this range of habitat similarity and several of the sites in the urban portion score more favorably than habitat in the rural portion. Only the most downstream Hinkson Creek station (Station #1) scored below 90 percent of the reference mean and its score of 88 percent was within the 75 percent minimum habitat similarity score required by MDNR to be sufficient to fully support biological communities.

2.1.5.1 Land Use and Land Cover

Urbanization generally involves a replacement of vegetated or otherwise pervious surfaces with impervious surfaces, primarily in the forms of rooftops and transportation-related surfaces (Schueler 1994). Case studies have reported the impacts of numerous variables on stream quality that were related to changes in land use and land cover (LULC). These include hydrological alterations (Booth et al. 2004), water quality variables (Roy et al. 2003b; Collier and Clements 2011), and substrate alterations (Sponseller et al. 2001). The effect of increased developed land is not linear linear (i.e., the relationship between the amount of impervious surface and the level of environmental disturbance is not a one to one ratio) but is greater at the low end of the gradient – generally considered 10 percent of watershed area or less (Stepenuck et al. 2002; Allan 2004; Schueler et al. 2009). Moreover, historic land use likely has a long-term negative effect on stream quality ((Harding et al. 1998; Maloney et al. 2008). In the case of Hinkson Creek, the earlier agricultural land use and mining were probable environmental disturbance that preceded urbanization.

Cuffney, et al. 2010, investigating nine metropolitan areas found that where urbanization consisted of agricultural lands being converted to urban lands, invertebrate assemblages showed weaker or nonsignificant relations with urban development because they were already degraded by conversion to agriculture. In the Hinkson Creek study area, this may account for the similarity of habitat scores throughout the watershed as well as the inconsistent presence of clear differences in many of the environmental variables and biological metrics between the urban and rural sections of Hinkson Creek.

2.1.5.2 Riparian Corridor

The riparian borders on either side of a stream are important in terms of shading, filtration of potential pollutants, and as a food source (Groffman et al. 2003). It is assumed that reduction or removal of these areas will have profound influences on the quality of stream ecosystems (Naiman and Decamps 1997). However, Walsh et al. (2007) reported that overall watershed urbanization was often a more important factor than riparian disturbance because modern stormwater transport technology reduces disturbance of riparian zones.

2.1.5.3 Sedimentation

Sedimentation or sediment deposition is a stressor often associated with impaired aquatic communities in urban environments (USEPA 2006, Owens et al, 2005). It has been associated with many TMDLs throughout the country and has been implicated as a primary source of impairment in streams of the United States (USEPA 2000). Its effects on aquatic communities have been well documented (Rabeni et al. 2005, Ciao, E., and Wallace, B. 2003, and others). Various investigations into physical habitat and sediment have been conducted within the Hinkson Creek watershed (MDNR 2004, Hooper et al., 2016, Zeiger et al., 2016, Kellner et al. 2019, Nichols et al., 2016, Hubbart et al., 2019, and others) but relatively few have been tied directly with aquatic community assessments.

In 2003-2004 MDNR conducted visual fine sediment estimates in conjunction with macroinvertebrate sampling at three locations on Hinkson Creek and one on Bonne Femme Creek (MDNR 2004). The Hinkson Creek Broadway and 63-Connector (urban) sites averaged 96 percent and 79 percent fine sediment coverage, the Hinkson Creek Road site (rural) was 64 percent covered with fine sediment. The Bonne Femme Creek site averaged 28 percent fine sediment coverage, which is representative of what could be considered relatively unimpacted. This limited investigation suggests that both urban and rural portions of Hinkson Creek suffer from fine sediment impacts, but more work would be needed to tie instream sediment with aquatic life impairment.

As part of a multiple stressor study of Hinkson Creek, Nichols, et al 2016 investigated the effects of substrate composition on macroinvertebrate communities. In conjunction with macroinvertebrate sampling they evaluated core samples using gravimetric methods to estimate the amount of fine sediment in each sample. Increases in fine sediment in the lower reaches were noted and suggested to influence macroinvertebrate assemblages but were not significantly related through regression analyses.

Previous studies that characterized bottom substrates of Hinkson Creek (Hooper 2015, Hubbart 2015) used a pebble count method, identifying 15 substrate particles in 100-meter length stream segments. This is a standard procedure, but it could be vulnerable to skewed results if the relative presences of the sampled particles are not truly representative of the segment and/or not performed consistently over time to capture stream morphology changes. In the above referenced studies, that appeared to be the case. Sampled segments corresponding to the most downstream Hinkson Creek macroinvertebrate sites were documented to have extensive gravel and rock, but visual inspection by the study team performed on September 19, 2019 indicated that the stream bottom was silt and sand dominated. Additionally, Hinkson Creek segments corresponding to macroinvertebrate Site 4 and Site 5 did not report the extensive bedrock areas that the study team visual inspection revealed.

The above studies of fine sediment in Hinkson Creek are too limited in scope to sufficiently characterize sediment, sediment transport, and its effects on the aquatic communities within the watershed. More emphasis on a comprehensive investigation into sediment within Hinkson Creek and sediment studies in conjunction with future macroinvertebrate sampling should be undertaken to determine its importance as a stressor to the macroinvertebrate community.

SECTION 3

METHODS

The following section outlines the methods and processes for accomplishing the project's goals and objectives.

Publicly available macroinvertebrate and chemical/physical data from Hinkson Creek, Bonne Femme Creek, and EDU Reference Streams collected between 2001 and 2017 were selected for analyses and interpretation. These data were obtained from MDNR's aquatic macroinvertebrate (AQUID) and surface water quality databases.

For the purposes of this project, the portion of Hinkson Creek upstream of the I70 and the Highway 63 connector are considered HCr and the portion downstream of the connector are considered HCu (**Figure 3** and **Table 1**). As previously mentioned, Bonne Femme Creek sites are considered the Control stream and Burris Fork, Moniteau Creek, Bouef Creek and the Loutre River are the EDU Reference Streams. Collectively, these sites derive the four treatment groups used for data analyses; HCr, HCu, Control, and Reference (**Table 1**). Site numbers provided in **Table 1** are consistent with MDNR bioassessment studies of Hinkson Creek.

TABLE 1. Project Macroinvertebrate Sample Locations.

Treatment Group	Stream Name	Site #	Location Description	Watershed Area*
Hinkson Creek Urban (HCu)	Hinkson Creek	1	Scott Blvd.	80
	Hinkson Creek	2	Twin Lakes	76
	Hinkson Creek	3	Forum Blvd.	76
	Hinkson Creek	3.5	Recreation Dr.	71
	Hinkson Creek	4	Rock Quarry Rd.	68
	Hinkson Creek	5	Capen Park	53
	Hinkson Creek	5.5	Green Valley Dr.	45
	Hinkson Creek	6	Walnut St.	43
Hinkson Creek Rural (HCr)	Hinkson Creek	6.5	Upstream Connector	41
	Hinkson Creek	7	Hinkson Creek Rd.	34
	Hinkson Creek	8	Rogers Rd.	30
Control	Bonne Femme Creek	1	Downstream Nashville Church Rd.	39
	Bonne Femme Creek	2	Upstream Nashville Church Rd.	39
Reference	Boeuf Creek	1	Hoeman Rd.	62
	Boeuf Creek	2	Stone Church Rd.	64
	Burris Fork	1	Union Ford Rd.	63
	Loutre River	1	Lick Access	211
	Moniteau Creek	1	Dicks Mill Dr.	59

Notes: "*" = square miles.

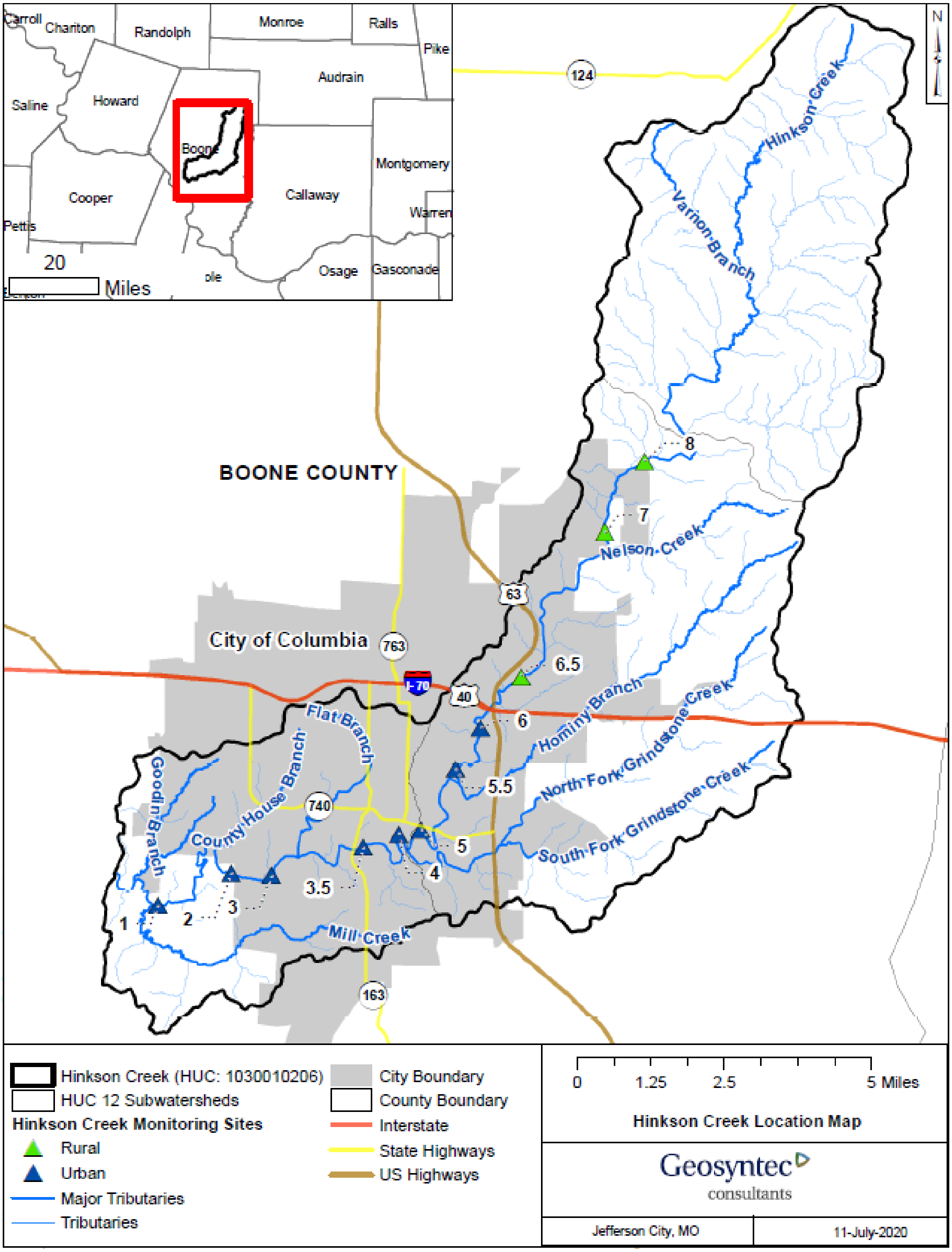

FIGURE 3. Hinkson Creek Watershed Macroinvertebrate Monitoring Locations.

3.1 Macroinvertebrate Data and Metric Calculation

From the 2001- 2017 period, only valid macroinvertebrate community samples collected in accordance with the MDNR Semi-Quantitative Macroinvertebrate Stream Bioassessment Project Procedure (MDNR 2015) were included for further analyses. Invalid macroinvertebrate samples were removed for analyses for the following reasons; catastrophic drought conditions (fall 2012), missing habitat (root-mat, fall 2001, spring 2002, fall 2005), and absence of flowing water (fall 2001). **Appendix A** provides a timeline of valid and invalid macroinvertebrate community samples collected from 2001-2017 from Hinkson Creek, Control stream and Reference streams. Upon removing invalid macroinvertebrate samples, 172 valid, riffle/pool complex, macroinvertebrate samples were included for analyses (**Table 2**).

TABLE 2. Valid Macroinvertebrate Samples Per Treatment Group.

Treatment Group	Count
Hinkson Creek Urban	96
Hinkson Creek Rural	32
Control Stream	29
Reference Streams	15

MDNR biologists periodically collected duplicate samples for quality assurance purposes and identify these samples as "A" and "B". For consistency purposes, the sample denoted with an "A" was considered for evaluation.

Forty-four (44) indicator metrics (**Table 3**) values were calculated for each of the 172 valid macroinvertebrate samples from Hinkson Creek, Control and Reference streams. Indicator metric categories include richness (#8), composition (#12), habitat (#5), pollution tolerance (#4), dominance/diversity (#4), ratio (#2) and trait states (#9) and are provided in **Table 3**. Taxa that were encountered in large/rare searches but not in the formal subsample (MDNR 2015) were only counted in richness metrics. For example, if the large caddisfly taxon *Pycnopsyche* was not found in the subsample but was found in the subsequent search for large/rare taxa, it was only included in the appropriate richness metrics but not for any metrics that included relative abundance calculations. Higher-level taxa (e.g., family) were not counted in richness metrics if it was possible that members of that taxon were also encountered at a lower-level (e.g. genus) entry. For example, the taxon "Chironomidae" was not counted if there were any members of the family identified to lower levels.

Categorization provided in Poff et al. (2006) were utilized for the nine metrics summarizing life history characteristics relating to reproductive strategies (fast or slow seasonal development), mobility (ability to exit [the stream] as adults, rare or abundant in the drift), morphological features (streamlined body shape) and habit (non-swimmers or strong swimmers, sprawlers). Since that guidance was limited to insect taxa only, non-insect taxa were not included in the calculations of these metrics.

Four of the invertebrate community metrics were indices (of pollution tolerance) calculated using relative proportions of taxa in a sample. Values provided by the MDNR database were used for Shannon Diversity Index and Missouri Biotic Index, and values provided in Davenport and Kelly (1983) were used for Macroinvertebrate Biotic Index. Tolerance values from Zweig and Rabeni (2001) were used to calculate deposited sediment tolerance index. For this study, deposited sediment tolerance values were only available for 27 taxa. Therefore, the total number of organisms used for this equation included only those that had tolerance values.

TABLE 3. Macroinvertebrate Indicator Metric/Categories and Expected Response to Increased Environmental Disturbance.

Metric	Category	Expected Response	Metric	Category	Expected Response
Total Taxa	Richness	Decrease	% Clingers + Climbers	Habitat	Decrease
Diptera	Richness	Decrease	% Filterers	Habitat	Variable
Chironomidae	Richness	Decrease	% Predators	Habitat	Variable
EPT	Richness	Decrease	% Scrapers	Habitat	Decrease
Ephemeroptera	Richness	Decrease	% Shredders	Habitat	Decrease
Plecoptera	Richness	Decrease	% Dominant Taxon	Dominance/Diversity	Increase
Trichoptera	Richness	Decrease	% Dominant 2 Taxa	Dominance/Diversity	Increase
Clinger + Climber	Richness	Decrease	% Dominant 5 Taxa	Dominance/Diversity	Increase
% Diptera	Composition	Increase	% Intolerant (≤ 4) Taxa	Dominance/Diversity	Decrease
% Chironomidae	Composition	Increase	Missouri Biotic Index	Tolerance	Increase
% Tanytarsini	Composition	Decrease	Macroinvertebrate Biotic Index	Tolerance	Increase
% Oligochaeta	Composition	Variable	Shannon Diversity Index	Tolerance	Decrease
% Corbicula	Composition	Increase	Deposited Sediment Tolerance Index	Tolerance	Increase
% Other Diptera + Non-Insects	Composition	Increase	% Fast Seasonal Development	Trait State	Increase
% EPT	Composition	Decrease	% Slow Seasonal Development	Trait State	Decrease
% Ephemeroptera	Composition	Decrease	% Ability to Exit as Adults	Trait State	Increase
% Plecoptera	Composition	Decrease	% Rare in Drift	Trait State	Decrease
% Trichoptera	Composition	Decrease	% Abundant in Drift	Trait State	Increase
% Ephemeroptera, Plecoptera	Composition	Decrease	% No Swimming Ability	Trait State	Increase
% Hydropsychidae	Composition	Variable	% Strong Swimming Ability	Trait State	Decrease
EPT/Chironomidae	Ratio	Decrease	% Streamlined Body Shape	Trait State	Decrease
Scraper/Filterer	Ratio	Decrease	% Sprawlers	Trait State	Decrease

Notes: "EPT" = Ephemeroptera, Plecoptera, Trichoptera.

Total Taxa richness metric measures the overall variety (i.e., the number of separate taxa in the sample) of the macroinvertebrate community (Barbour et al. 1999) and is used in the condition indices of numerous states, including Missouri. It is expected to decrease with increasing environmental disturbance.

Diptera richness/Chironomidae richness metrics represent the variety of the taxon (chironomids) that typically dominates macroinvertebrate samples. Although these groups are generally considered tolerant of pollution and may be the last to disappear under environmental degradation (DeShon 1995), their richness levels are expected to decrease with increasing environmental disturbance.

EPT richness metric represents the variety of the mayfly, stonefly, and caddisfly orders, and is a component of the multi-metric indices of many states, including Missouri. It is expected to decrease with increasing environmental disturbance.

Ephemeroptera richness/Plecoptera richness/Trichoptera richness are separate metrics which are collectively the constituents of the EPT richness metric. Ephemeroptera richness and Trichoptera richness are used in Ohio's multi-metric index of macroinvertebrate community condition (DeShon 1995). They are all expected to decrease with increasing environmental disturbance.

Clingers + Climbers richness metric is a measure of the variety of invertebrate groups that are adapted for attachment to surfaces of rocks or wood (clingers) or living on vascular hydrophytes (aquatic plants) or woody debris (climbers) in flowing water (Cummins et al. 2008). They are expected to decrease with increasing environmental disturbance.

Percent Diptera/Percent Chironomidae represent the relative abundance of these groups in the samples and are expected to increase with increasing environmental disturbance.

Percent Tanytarsini metric is considered indicative of good water quality in Ohio (DeShon 1995). However, most members of this group in this present study were relatively pollution tolerant. Therefore, their response to environmental disturbance is variable.

Percent Oligochaeta metric was reported to have a variable response to increasing environmental disturbance (Kerans and Karr 1994). However, in this present study their increased relative abundance was indicative of reduced habitat quality.

Percent Corbicula metric is expected to increase with increasing environmental disturbance (Kerans and Karr 1994).

Percent Other Diptera and Non-Insects metric is expected to increase with increasing environmental disturbance (DeShon 1995). It was modified from the Ohio EPA calculation be excluding all chironomids instead of only those in the tribe Tanytarsini (OEPA 1987).

Percent EPT/Percent Ephemeroptera/Percent Plecoptera/Percent Ephemeroptera + Plecoptera/Percent Trichoptera represent the relative abundance of each of these groups in relation to the total sample and are all expected to decrease with increasing environmental disturbance. Percent Ephemeroptera and percent Trichoptera are included in Ohio's multi-metric index of macroinvertebrate community condition (DeShon 1995).

Percent Hydropsychidae metric represents a filter-feeding trichopteran family, whose distribution dynamics have been reported to be sensitive to environmental disturbance (Camargo 1992; Garcia and Ferreras-Romero 2008). In this study, the group largely consisted of the genus *Cheumatopsyche*, with occasional *Hydropsyche* individuals, neither is particularly pollution intolerant. Therefore, their response to environmental disturbance was considered variable.

Percent Clingers + Climbers metric is a measure of the relative abundance of invertebrate groups that are adapted for attachment to surfaces of rocks or wood (clingers) or living on vascular hydrophytes or woody debris (climbers) in flowing water (Cummins et al. 2008). They are expected to decrease with increasing environmental disturbance.

Percent Filterers metric represents a subgroup of collectors, which primarily feed on decomposing fine particulate organic matter (FPOM) (Cummins et al. 2008). Their response to environmental disturbance is considered variable (Barbour et al. 1999).

Percent Predators metric consists of invertebrates that feed on living animals but includes omnivores. Their response to environmental disturbance is considered variable (Barbour et al. 1999), but the expected ratio of predators to the remainder of the assemblage is 10 – 20 percent (Cummins et al. 2008).

Percent Scrapers metric represents invertebrates that graze on periphyton (algae attached to substrates) and associated material on mineral and organic surfaces. This group is expected to decrease in abundance with a reduction of rock (cobble and larger) surface habitat (Cummins et al. 2008). Additionally, urbanization effects of increased amounts of sediment (Garie and McIntosh 1986) or chemical contamination (Quinn and Hickey 1993) may reduce the palatability of the periphyton and further inhibit scraper abundance.

Percent Shredders metric consists of taxa that are adapted to chew decomposing coarse particulate organic matter (CPOM) (Cummins et al. 2008). In stream segments where less allochthonous (tree and shrub leaves and branches) material reaches the stream, e.g., because riparian zones have been cleared, this group is expected to decrease.

Percent Dominant Taxon/Percent Dominant 2 Taxa/Percent Dominant 5 Taxa metrics generally correspond to reduced diversity in the invertebrate assemblage. They are expected to increase with increasing environmental disturbance (Barbour et al. 1999).

Percent Intolerant (≤ 4) Taxa metric consists of taxa considered intolerant to organic pollution. In this study, it included taxa with pollution tolerance values less than or equal to 4 (MDNR 2010). It is expected to decrease with increasing environmental disturbance.

EPT/Chironomidae Ratio metric accentuates the relationship between the generally pollution-sensitive EPT taxa and the generally pollution-tolerant chironomids. It is expected to decrease with increasing environmental disturbance.

Scrapers/Filterers Ratio – this metric is expected to reflect the availability of rock substrate. It is thus expected to decrease with increasing environmental disturbance, particularly habitat loss.

Percent Fast Seasonal Development metric is expected to increase with increasing environmental disturbance. Zuellig and Schmidt (2012) found that it was increased in urbanized sites as compared to least disturbed sites in the southern Appalachians and the temperate plains of the continental U.S.

Percent Slow Seasonal Development in contrast to the metric above, this metric is expected to decrease with increasing environmental disturbance.

Percent Ability to Exit as Adults metric is expected to increase with increasing environmental disturbance. Zuellig and Schmidt (2012) found that it was increased in urbanized sites as compared to least disturbed sites in the southern Appalachians and the temperate plains of the continental U.S.

Percent Abundant in Drift metric is expected to increase with increasing environmental disturbance.

Percent Rare in Drift in contrast to the metric above, this metric is expected to decrease with increasing environmental disturbance.

Percent No Swimming Ability metric, similarly to percent Chironomidae and the other associated metrics, it is expected to increase with increasing environmental disturbance.

Percent Strong Swimming Ability metric is expected to decrease with increasing environmental disturbance. In the current study, the only common or abundant representatives of this metric were the baetid mayflies *Acentrella, Acerpenna, Baetis*, and *Procloeon*.

Percent Streamlined Body Shape metric refers to invertebrates with a flat, fusiform body type (Poff et al. 2006). Taxa in this metric that were common or abundant in the present study included the coenagrionid damselflies *Argia* and *Enallagma*, the baetid mayflies, the heptageniid mayflies *Stenacron* and *Stenonema femoratum*, and the perlid stonefly *Perlesta*. Therefore, this metric would be expected to decrease with increasing environmental disturbance.

Percent Sprawlers metric represents invertebrate groups that move across the soft substrates of depositional habitats (Cummins et al. 2008). Taxa that were included in this metric and were common or abundant in this study included the dipterans Ceratopogoninae, *Hemerodromia*, and *Hexatoma*, the tanypod midges *Ablabesmyia* and *Thienemannimyia* gp., and the mayflies *Caenis* *latipennis* and *Tricorythodes*. None of these taxa are considered intolerant of disturbance; therefore, this metric's response to environmental disturbance should be considered variable.

Missouri Biotic Index/Macroinvertebrate Biotic Index metrics reflect the average pollution-tolerance of the sampled assemblage, although the tolerance values of the particular taxa differ moderately. The former index is a component of MSCI and both biotic indices are expected to increase with increasing environmental disturbance.

Shannon Diversity Index metric represents a combination of the taxa richness and the "evenness" of numbers among taxa. It is a component of MSCI and is expected to increase with increasing environmental disturbance.

Deposited Sediment Tolerance Index metric reflects the average sedimentation tolerance of the community, based on the values of 30 taxa that are often commonly collected (Zweig and Rabeni, 2001). It is expected to increase with increasing environmental disturbance in the form of sedimentation.

3.2 Chemical and Physical Data

Publicly available chemical and physical data from 2001-2017 were compiled for comparisons to macroinvertebrate community metrics. Chemical (water quality) data were available in two (2) distinct data sets; 1) data collected simultaneously with macroinvertebrate community samples, hereinafter referred to as "Paired" and 2) an "Inclusive" data set of year-round intermittent samples, which includes more parameters and frequency of collection as well as Paired data. Available chemical parameters were most often nutrients, instantaneous discharge, dissolved oxygen, temperature, pH, conductance (also referred to as specific conductance, which is a measure of how well water can conduct electricity, which increases with increasing amounts of ions), total suspended solids (TSS), turbidity, hardness, chloride, and sulfate.

Physical data include information regarding LULC, general stormwater outfalls, national pollution discharge elimination (NPDES) site-specific outfalls, continuous flow volume, and in-stream habitat data collected from Hinkson Creek.

3.3 Data Analyses

Upon data compilation of environmental (chemical and physical) data and macroinvertebrate metric calculations, a stepwise data analyses process was performed to assess potential relationships between macroinvertebrate metrics of sites and treatment groups to environmental (chemical and physical) variables for stressor identification. The stepwise data analyses process included:

- Analyses of Variance;
- Spatial and Temporal Trends Analyses;

- Correlation Analyses;
- Ordination Analyses; and
- Indicator Taxa Analyses.

Analyses of variance was used to assess whether metrics/communities differed between season, treatment group, and to assess differences in water chemistry results. One-way analysis of variance (ANOVA) tests were performed on macroinvertebrate metrics using Minitab 14 statistical software. The factors were season (spring and fall) and site type (Control, HCr, and HCu), using a p-value of less than 0.05 to consider treatment groups significantly different. If the normality assumption of a test was violated, the data were transformed using a Box-Cox recommendation. If the normality assumption was still violated after transformation, and/or if the equal variance assumption was violated, a non-parametric Kruskal-Wallis (KW) test was performed as a substitute for the ANOVA. If the ANOVA indicated significant difference, a Tukey's test for multiple comparisons was performed for the site type factor (3 treatment groups). The correction applied by this test increased the 95 percent confidence intervals to 98.09 percent. The KW test was followed by the Mann-Whitney rank sum test to determine differences between treatment groups. Non-parametric KW tests were used to evaluate water quality differences between treatment groups in R statistical analyses software. Reference streams data were not included as an additional treatment group in the ANOVA and KW analyses of variance tests as these macroinvertebrate community samples were collected from multiple streams and generally in different years compared to the Control stream and Hinkson Creek samples.

Even though numerous metrics were identified to auto correlate, it was the opinion of the CAM project committee to continue spatial/temporal and correlation analyses for all macroinvertebrate metrics. Autocorrelation refers to the same response of one metric as another due to taxa similarity or overlap and abundance.

Spatial and temporal trend analyses were performed to identify potential spatial (location) and temporal (time) trends of individual sites and treatment groups. Tests of statistically significant temporal trends may assist in understanding natural variability of reference streams and the variability exuded by Hinkson Creek and the Control stream. Analyses were performed using Mann Kendall trend test using R statistical software for each site and treatment group. Due to the analyses of variance demonstrating a seasonal influence on macroinvertebrate metrics, spatial and temporal trends were also evaluated seasonally. Sites and/or treatment groups with 3 or fewer samples could not be evaluated.

Relationships between macroinvertebrate metric values and environmental variables were examined using correlation analyses. Correlation analyses were performed using R statistical software and the non-parametric Spearman Rho rank-based correlation coefficient test. Correlation coefficients greater than zero indicate a positive relationship while values less than zero indicate a negative relationship. Treatment groups were selected for correlation analyses to provide a broader data set for comparison versus a more reduced site-specific data set. Correlation analyses could not be performed for static data such as one-time habitat assessment scores, stream gradient, and substrate characterization, or data sets of sample size less than four. Significant correlations were evaluated based on ecological significance. Ecological significance refers to whether a correlation made sense based on best professional judgement. For example, if a macroinvertebrate metric that is known to decrease with increasing disturbance (e.g., EPT richness) was positively correlated with an environmental metric that indicates greater disturbance (e.g., chloride concentration), it was interpreted as ecologically insignificant.

Ordination analyses was performed based on macroinvertebrate community composition using non-metric multidimensional scaling (NMDS) in R statistical analyses software. NMDS is used to collapse multiple variables (taxa) into fewer dimensions so that it can be interpreted and visualized in a 2-dimensional scatter plot. The absolute position of a sample to the center of a scatter plot is arbitrary; however, it is the distance between different samples that are meaningful (those that plot close together are more similar than those that plot farther away). To accommodate the NMDS analyses in the R program, a reduction in distinct taxa was performed by removing taxa that appeared infrequently (in total less than or equal to 14 occurrences). This community adjustment reduced the total # of distinct taxa in the overall macroinvertebrate data set from 386 to 195 taxa. Thereby, reducing the total number of organisms from the 172 valid macroinvertebrate community samples from 215,233 to 214,442 (791 removed) organisms.

An analyses of good water/habitat quality indicator taxa was performed for Hinkson Creek. Indicator taxa indicative of good water/habitat quality were evaluated based on the following attributes:

- Taxon had to be common enough in Hinkson Creek to be found with cost-effective (e.g., rapid bioassessment protocols) effort;
- Mature larvae needed to be large enough to be visible and identifiable in the field;
- Taxon must be pollution-sensitive, as indicated by a low (<3) tolerance value;
- Taxon must be sensitive to habitat sedimentation (clinger/climber habit category); and
- Taxon should be a member of two or more life history trait groups (Poff et al. 2006) that decrease in abundance with increasing environmental disturbance.

An additional indicator taxa analyses was performed on the macroinvertebrate community data matrix for each treatment group using R statistical software (Caceres 2020). Indicator taxa were determined using the relationship between occurrence and abundance in each treatment group. Consideration to taxa whose abundances were strongly associated with a treatment group or subset thereof, as indicated by correlation values greater than 0.7, were reported.

Indicator metrics analyses was performed for Hinkson Creek macroinvertebrate metrics based on the following response attributes:

- Exhibits a consistent difference between disturbed (HCu) and less disturbed (HCr, Control stream, Reference streams) locations;
- Associated with either pollution or habitat related stream degradation; and
- Varies over large gradients, facilitating the ability to perceive differences.

SECTION 4

RESULTS

Results discussed below were separated into two distinct seasons (spring and fall) as macroinvertebrate metrics exhibited seasonal variation. Seasonal variation is discussed in more detail in **Sections 4.2.2** and **4.2.5**.

4.1 Environmental Data

Environmental data consist of water chemistry and physical (LULC, outfalls, et.) data. As previously mentioned, chemical water quality data were available in two distinct data sets (Paired and Inclusive). For presentation purposes the Inclusive water chemistry statistics are provided below. However, Paired water chemistry data were used for specific metric/stressor analyses and evaluation, as these data were collected simultaneously with macroinvertebrate data.

4.1.1 Water Chemistry Data

The MDNR Inclusive water quality database includes approximately 98 water chemistry parameters and more than 9,000 results collected from the Control stream, Reference streams, and Hinkson Creek and its tributaries. These water quality parameters consist of nutrients, general water chemistry (i.e. dissolved oxygen, pH, temperature and conductance), and TSS. Specific water quality studies also included polycyclic aromatic hydrocarbons (PAH), and volatile/semi-volatile organic compounds (VOC/SVOC) and metals; however, they were not analyzed on a regular basis. Within mainstem Hinkson Creek, the only water quality parameters detected above laboratory quantification limits were general water chemistry parameters, nutrients and suspended sediment. Within the tributaries and stormwater conveyances of Hinkson Creek PAH, VOC/SVOC, and metals were present above laboratory quantification limits but were below laboratory quantification limits in mainstem Hinkson Creek.

Of the 172 valid macroinvertebrate samples, 153 include Paired water chemistry data with more than 2,400 results collected from the Control stream, Reference stream, and Hinkson Creek. Paired water quality data results include general water chemistry parameters, nutrients and suspended sediment.

Table 4 through **Table 16** below provide statistics of the Inclusive water chemistry data that were above laboratory quantification limits in the designated treatment groups which include; dissolved oxygen, water temperature, conductance, pH, instantaneous flow, hardness, ammonia, total nitrogen, total phosphorus, chloride, sulfate, TSS and turbidity. Spring season water chemistry data include the months of November through April and fall season months include May through October. **Appendix B** presents box whisker plots of the Inclusive water chemistry data set.

TABLE 4. Seasonal Dissolved Oxygen Values in Milligrams per Liter.

Treatment Group	Spring					Fall				
	Count	Min	Max	Avg.	Median	Count	Min	Max	Avg.	Median
Control Stream	32	8.9	13.8	10.6	10.3	55	2.5	11.2	6.7	6.7
Hinkson Creek Rural	41	7.8	16.4	11.3	11.2	33	3.8	13.0	8.3	8.0
Hinkson Creek Urban	128	6.4	17.6	11.2	10.7	188	3.5	12.3	7.7	7.6
Reference Streams	41	5.7	16.2	11.1	11.5	47	1.6	12.4	7.1	7.3

TABLE 5. Seasonal Water Temperature Values in Degrees Celsius.

Treatment Group	Spring					Fall				
	Count	Min	Max	Avg.	Median	Count	Min	Max	Avg.	Median
Control Stream	32	1.6	19.4	11.2	11.1	55	10.0	30.7	19.79	20.3
Hinkson Creek Rural	50	0.0	21.0	10.0	9.0	33	9.0	30.0	19.94	19.2
Hinkson Creek Urban	138	0	21.4	10.3	10.8	193	9.6	29.8	20.81	20.7
Reference Streams	41	0.6	22.2	10.6	9.0	46	11.8	33.4	22.39	22.15

TABLE 6. Seasonal Chloride Values in Milligrams per Liter.

Treatment Group	Spring					Fall				
	Count	Min	Max	Avg.	Median	Count	Min	Max	Avg.	Median
Control Stream	17	10	37.4	19.8	19.0	18	5.0	14.1	10.1	10.0
Hinkson Creek Rural	43	9.1	116.0	26.0	20.0	32	7.4	217.0	30.5	19.8
Hinkson Creek Urban	103	12.0	333.0	49.0	43.3	108	10.0	93.0	37.3	36.0
Reference Streams	41	4.4	48.1	13.5	12.5	36	2.3	36.1	11.0	10.0

TABLE 7. Seasonal Instantaneous Flow Values in Cubic Feet Per Second.

Treatment Group	Spring					Fall				
	Count	Min	Max	Avg.	Median	Count	Min	Max	Avg.	Median
Control Stream	28	0.4	14.5	5.2	3.6	56	0.1	13.4	2.6	0.9
Hinkson Creek Rural	37	0.2	28.4	6.5	4.3	27	0.0	9.2	1.8	0.5
Hinkson Creek Urban	96	0.9	275.0	24.9	10.7	162	0.1	134.0	9.1	2.1
Reference Streams	34	0.5	124.0	21.3	12.7	32	0.0	73.7	7.0	1.4

TABLE 8. Seasonal Hardness Values in Milligrams per Liter.

Treatment Group	Spring					Fall				
	Count	Min	Max	Avg.	Median	Count	Min	Max	Avg.	Median
Control Stream	8	179	219	200	200	8	211	298	240	239
Hinkson Creek Rural	15	137	247	196	199	10	169	375	256	240
Hinkson Creek Urban	44	184	348	253	255	41	205	327	260	250
Reference Streams	30	68	275	187	193	26	83	235	173	172

TABLE 9. Seasonal Ammonia Values in Milligrams per Liter.

Treatment Group	Spring					Fall				
	Count	Min	Max	Avg.	Median	Count	Min	Max	Avg.	Median
Control Stream	16	0.03	0.07	0.04	0.03	37	0.02	0.08	0.04	0.03
Hinkson Creek Rural	37	0.03	0.13	0.04	0.03	28	0.03	0.12	0.04	0.03
Hinkson Creek Urban	97	0.03	0.64	0.05	0.03	98	0.02	0.28	0.04	0.03
Reference Streams	32	0.03	0.16	0.05	0.03	38	0.00	0.24	0.07	0.05

TABLE 10. Seasonal Total Nitrogen Values in Milligrams per Liter.

Treatment Group	Spring					Fall				
	Count	Min	Max	Avg.	Median	Count	Min	Max	Avg.	Median
Control Stream	16	0.12	0.78	0.35	0.30	15	0.26	0.80	0.42	0.36
Hinkson Creek Rural	37	0.18	1.09	0.60	0.56	26	0.29	2.51	0.80	0.56
Hinkson Creek Urban	95	0.18	3.26	0.51	0.41	94	0.05	2.49	0.60	0.42
Reference Streams	41	0.15	3.73	0.85	0.64	38	0.14	2.15	0.64	0.41

TABLE 11. Seasonal Total Phosphorus Values in Milligrams per Liter.

Treatment Group	Spring					Fall				
	Count	Min	Max	Avg.	Median	Count	Min	Max	Avg.	Median
Control Stream	16	0.02	0.11	0.05	0.05	15	0.01	0.13	0.06	0.06
Hinkson Creek Rural	37	0.01	0.48	0.06	0.05	26	0.01	0.42	0.08	0.04
Hinkson Creek Urban	97	0.01	0.10	0.04	0.04	94	0.01	0.37	0.07	0.04
Reference Streams	41	0.01	0.19	0.06	0.04	47	0.02	1.30	0.14	0.09

TABLE 12. Seasonal Specific Conductance Values in Microsiemens Per Centimeter.

Treatment Group	Spring					Fall				
	Count	Min	Max	Avg.	Median	Count	Min	Max	Avg.	Median
Control Stream	32	125.3	533.0	395.3	411.5	55	292.0	543.0	406.4	408.0
Hinkson Creek Rural	45	299.0	1,180.0	583.9	532.0	33	183.0	1,540.0	594.0	563.0
Hinkson Creek Urban	133	301.0	1,330.0	671.0	626.0	190	212.0	914.0	542.7	552.5
Reference Streams	41	128.0	554.0	366.1	371.0	45	188.0	724.0	381.0	384.0

TABLE 13. Seasonal Total Suspended Solids Values in Milligrams per Liter.

Treatment Group	Spring					Fall				
	Count	Min	Max	Avg.	Median	Count	Min	Max	Avg.	Median
Control Stream	14	5.0	3,800.0	276.4	5.0	15	5.0	3,770.0	258.5	6.0
Hinkson Creek Rural	38	5.0	7,150.0	196.2	6.5	38	5.0	3,870.0	124.8	8.5
Hinkson Creek Urban	108	5.0	23,400.0	380.3	5.0	111	5.0	4,560.0	149.3	7.0
Reference Streams	7	5.0	7,180.0	1033.0	9.0	6	5.0	4,160.0	699.3	5.5

TABLE 14. Seasonal Turbidity Values in Nephelometric Turbidity Units.

Treatment Group	Spring					Fall				
	Count	Min	Max	Avg.	Median	Count	Min	Max	Avg.	Median
Control Stream	19	1.9	8.6	3.6	2.6	21	2.3	15.1	5.4	4.1
Hinkson Creek Rural	35	1.4	22.0	11.1	12.1	41	1.9	467.0	35.8	8.4
Hinkson Creek Urban	103	1.9	242.0	12.0	7.8	139	1.2	649.0	36.6	7.0
Reference Streams	25	1.2	111	17.5	8.3	23	1.1	50.2	9.0	6.5

TABLE 15. Seasonal Sulfate Values in Milligrams per Liter.

Treatment Group	Spring					Fall				
	Count	Min	Max	Avg.	Median	Count	Min	Max	Avg.	Median
Control Stream	8	10.9	16.7	14.2	15.7	8	5.21	10.4	8.3	9.0
Hinkson Creek Rural	15	64.2	114.0	85.5	84.6	10	44.3	202.0	110.3	93.1
Hinkson Creek Urban	43	78.6	180.0	101.5	95.3	41	47.8	179.0	89.4	77.4
Reference Streams	29	10.3	38	25.3	26	27	4.4	29.2	16.5	15

TABLE 16. Seasonal pH Values in Milligrams per Liter.

Treatment Group	Spring					Fall				
	Count	Min	Max	Avg.	Median	Count	Min	Max	Avg.	Median
Control Stream	29	7.3	8.7	7.9	7.8	55	7.0	8.2	7.6	7.6
Hinkson Creek Rural	43	6.9	8.5	7.9	8.0	33	7.3	8.6	8.0	8.0
Hinkson Creek Urban	127	5.2	8.8	7.8	8.0	187	6.3	8.6	7.8	7.8
Reference Streams	40	6.8	8.7	7.9	7.9	47	6.6	8.7	7.9	7.8

4.1.1.1 Winter 2019/2020 Continuous Conductance Monitoring

Observing elevated chloride levels in Hinkson Creek and its tributaries compared to the reference and control stream, and the strong linear relationship of chloride and conductance (**Figure 4**) in both the Paired and Inclusive water quality data sets led Geosyntec to investigate Hinkson Creek water quality conditions during the critical winter season. Prior to winter related events, YSI 600 XLM water quality instrumentation were deployed at five Hinkson Creek sites consistent with historic macroinvertebrate monitoring locations (**Table 17**). Hinkson Creek water temperature and conductance data were collected continuously every 30-minutes from December 12, 2019 to May 12, 2020.

TABLE 17. Hinkson Creek Winter 2019/2020 Monitoring Locations.

Treatment Group	Site #	Location Description
Hinkson Creek Urban	1	Scott Blvd.
	3.5	Recreation Dr.
	5.5	Green Valley Dr.
Hinkson Creek Rural	7	Hinkson Creek Rd.
	8	Rogers Rd.

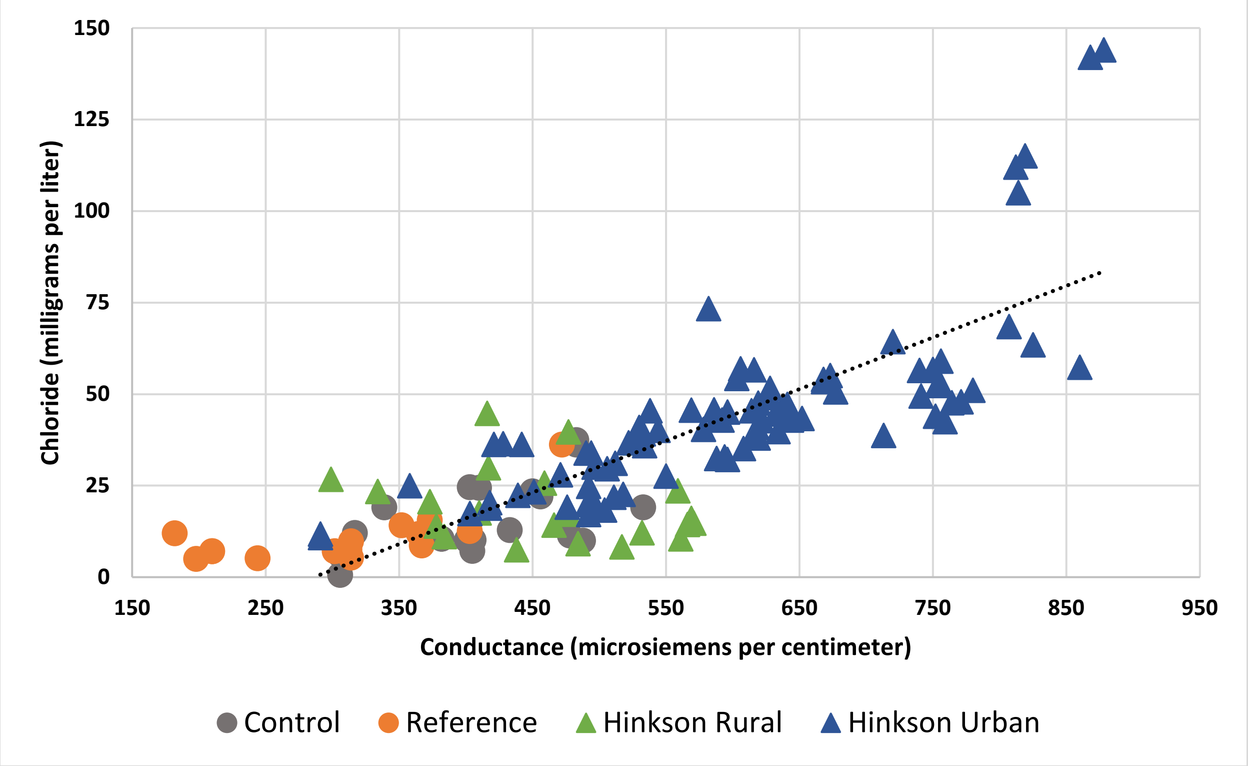

FIGURE 4. Paired Water Quality Data Chloride and Conductance Linear Relationship.

A summary of conductance data collected from Hinkson Creek during the winter of 2019/2020 is provided in **Table 18**. More than 30,000 conductance values were collected during the monitoring period. Conductance values increased in Hinkson Creek from upstream to downstream. All urban Hinkson Creek monitoring locations exhibited significantly higher conductance values compared to rural monitoring locations. The maximum conductance value was measured at urban Hinkson Creek site 5.5 just downstream of Broadway Blvd. However, the upper percentile (90th) conductance values consistently increased from upstream to downstream.

FIGURE 5. Hinkson Creek Site 7 Continuous Conductance Monitoring Instrumentation.

TABLE 18. Winter 2019/2020 Hinkson Creek Specific Conductance Monitoring Results.

Specific Conductance (µS/cm)	**Hinkson Creek Rural**		**Hinkson Creek Urban**		
	Site 8	**Site 7**	**Site 5.5**	**Site 3.5**	**Site 1**
Min	100	92	122	134	146
Max	623	685	3,323	2,149	2,604
Mean	301	391	604	562	598
Median	299	393	567	531	558
25th Percentile	208	321	381	359	357
75th Percentile	371	482	680	669	711
90th Percentile	448	528	857	876	996
Count (# records)	6,187	5,445	5,484	7,252	6,659

The first winter precipitation (snow/ice) event of the 2019/2020 season occurred on December 16 and 17, 2019, resulting in the highest conductance values measured at all urban Hinkson Creek locations during the monitoring period. Elevated conductance conditions persisted for approximately one week in the urban portions of Hinkson Creek. A timeseries of Hinkson Creek conductance response to the December 16 and 17, 2019 winter event is presented in **Figure 6**. Conductance values dramatically decreased at all monitoring locations on December 29, 2019 in response to a rainfall event that dramatically increased Hinkson Creek flow conditions.

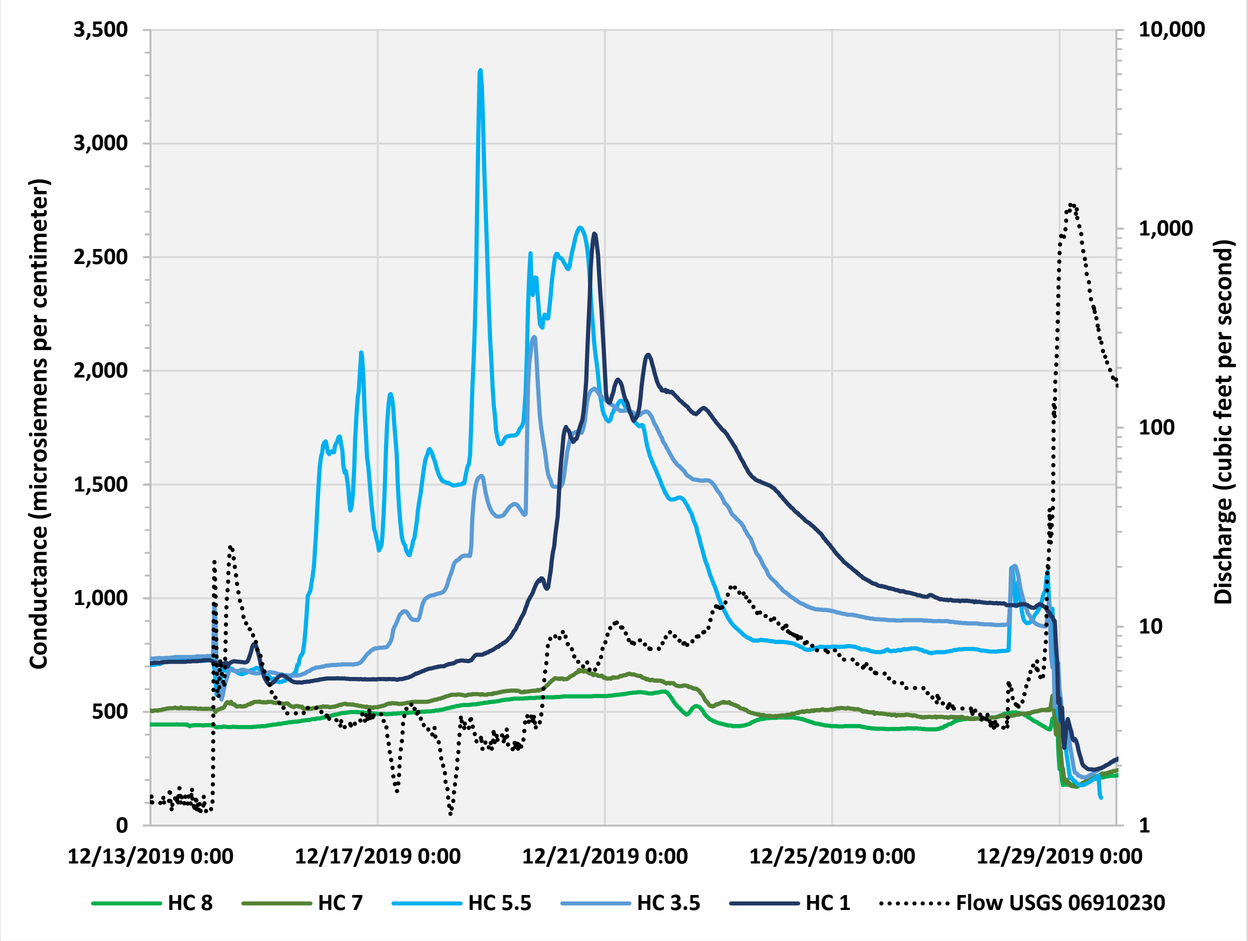

FIGURE 6. Hinkson Creek Conductance and Flow Data Timeseries. Flow data were obtained from Hinkson Creek at Columbia, Missouri USGS Station 06910230.

4.1.2 Physical Data

LULC data were evaluated from the 2001 and 2016 national land cover database (NLCD) and characterized as developed, forested, grassland, and cropland (**Table 19**). HCr locations have a similar percentage of developed land compared to Control and Reference streams. Generally, Reference streams have a higher composition of forest and grassland than HCu sites. From 2001 to 2016, HCu locations 1, 2, 3, 3.5 and 4 have experienced an increase in developed landscape with a corresponding decrease in forested and grasslands (**Table 20**). **Appendix C** provides maps comparing the 2001 and 2016 LULC data sets for all Hinkson Creek sites.

TABLE 19. Watershed Percent Land Use Land Cover Data.

Treatment Group	Site #	2001 NLCD (%)				2016 NLCD (%)			
		Dev.	Forest	Grass	Crop	Dev.	Forest	Grass	Crop
Hinkson Creek Urban (HCu)	1	21.9	36.6	32.0	7.7	26.4	35.2	28.3	8.5
	2	20.0	37.2	33.2	8.1	24.4	35.8	29.4	9.0
	3	19.7	37.2	33.5	8.2	24.2	35.7	29.6	9.1
	3.5	15.0	38.6	36.0	8.8	19.8	37.1	31.9	9.7
	4	14.8	38.3	36.4	9.0	19.3	37.1	32.3	10.0
	5	13.5	38.1	37.1	9.7	16.0	37.3	34.1	11.1
	5.5	10.2	38.9	39.7	9.8	12.0	38.6	36.9	11.2
	6	9.0	39.2	40.4	10.0	10.8	38.9	37.5	11.4
Hinkson Creek Rural (HCr)	6.5	5.8	40.1	42.2	10.5	7.5	39.9	39.2	12.0
	7	4.5	38.8	43.9	11.4	4.7	38.9	41.6	13.3
	8	4.1	39.7	44.0	11.2	4.2	39.9	41.6	13.4
Bonne Femme Creek (Control)	1	6.1	31.3	38.2	23.4	6.9	31.3	36.0	24.8
Boeuf Creek (Reference)	1	NC	NC	NC	NC	3.7	63.5	28.2	3.6
Burris Fork (Reference)	1	NC	NC	NC	NC	4.2	16.4	67.1	11.7
Loutre River (Referecne)	1	NC	NC	NC	NC	4.6	40.8	26.8	26.2
Moniteau Creek (Reference)	1	NC	NC	NC	NC	3.7	20.8	34.3	40.6

Notes: NLCD = National Land Cover Database; NC = not calculated: Dev. = Developed; Grass = Grassland; Cont. = Control.

TABLE 20. Hinkson Creek Watershed LULC Percent Change from 2001 to 2016.

Treatment Group	Site #	Percent (%) Change			
		Developed	Forest	Grassland	Crop
Hinkson Creek Urban (HCu)	1	20	-4	-12	10
	2	23	-4	-12	11
	3	23	-4	-12	11
	3.5	32	-4	-12	11
	4	30	-3	-11	11
	5	19	-2	-8	13
	5.5	18	-1	-7	14
	6	20	-1	-7	14
Hinkson Creek Rural (HCr)	6.5	29	0	-7	14
	7	4	0	-5	16
	8	0	1	-5	18

Site-specific and general stormwater NPDES permitted outfalls were tabulated for each Hinkson Creek locations from the most recent MDNR NPDES database. The most upstream Hinkson Creek location includes 5 site-specific and 3 general NPDES outfalls while the most downstream site includes 46 and 112, respectively (**Table 21**). A map of site-specific and general stormwater NPDES permits, along with design flow volumes of site-specific NPDES permits is provided in **Appendix D**.

TABLE 21. Hinkson Creek NPDES Outfall Summary.

Treatment Group	Site #	Site-Specific	General Stormwater*
Hinkson Creek Urban (HCu)	1	46	112
	2	46	108
	3	46	104
	3.5	40	91
	4	39	90
	5	21	50
	5.5	18	37
	6	18	33
Hinkson Creek Rural (HCr)	6.5	18	24
	7	17	8
	8	5	3

Continuous flow data were available from the USGS station 06910230 Hinkson Creek at Columbia, Missouri from 2007 to 2017, which corresponds with Hinkson Creek Site 3.5 macroinvertebrate monitoring station. Continuous flow data prior to 2007 were unavailable as the station was out of service. Therefore, continuous flow data that correspond with macroinvertebrate samples were from spring and fall of 2012 to 2017. A period of five weeks prior to macroinvertebrate sampling was chosen to assess hydrology characteristics and potential macroinvertebrate community responses. Approximately five weeks is often considered a standard period for macroinvertebrate colonization to artificial habitats (OEPA 1987). Hydrology characteristics included minimum flow, maximum flow, median flow, 90th percentile flow, flow variability (standard deviation), flow duration exceedance frequency condition (0.0 to 1.0 scale, 0.0 indicates highest condition and 1.0 indicates lowest condition), and flow disturbance frequency (count of flow pulses over long-term 90th percentile flow or 106 cubic feet per second, **Table 22**).

TABLE 22. Hinkson Creek Site 3.5 Hydrology Characteristics.

Year/Season	Minimum	Maximum	Median	90th Percentile	Flow Variability	Exceedance Frequency	Flow Disturbances
2012 Spring	4.0	927	19.2	147.6	450.2	0.679	2
2013 Spring	18.7	6,400	67.1	587.0	796.3	0.533	6
2014 Spring	1.7	6,200	8.1	103.0	553.7	0.263	2
2014 Fall	0.4	7,520	8.5	141.3	809.7	0.402	4
2015 Spring	2.4	565	37.3	115.0	69.7	0.663	2
2015 Fall	0.9	26	3.2	8.5	3.4	0.636	0
2016 Spring	5.0	90	11.3	34.3	11.9	0.591	0
2016 Fall	1.8	2,030	9.8	105.4	192.9	0.987	4
2017 Spring	1.5	1,690	21.1	127.6	153.7	0.454	4
2017 Fall	0.1	134	0.5	4.8	10.3	0.971	1

4.2 Data Analyses and Stressor Evaluation

Hinkson Creek, Control stream and Reference streams macroinvertebrate community data analyses included the calculation of macroinvertebrate metrics, analyses of variance, temporal/spatial trend analyses, correlation analyses of chemical/physical data with macroinvertebrate metrics, ordination analyses, and identification of indicator taxa and metrics. Environmental data/variables collected once or those that remained static (consistent) during the study period were omitted from statistical analyses.

4.2.1 Macroinvertebrate Metrics

The 172 valid macroinvertebrate samples consist of 386 distinct macroinvertebrate taxa ranging in abundance of 1 to 19,911. Forty-four (44) macroinvertebrate metrics were calculated for 172 valid macroinvertebrate samples from Hinkson Creek, Control stream and Reference streams. A summary of macroinvertebrate metrics per treatment group are provided for the fall and spring season in **Appendix E**.

4.2.2 Analyses of Variance

Analyses of variance were performed to assess seasonal, metric and water quality differences of the treatment groups.

4.2.2.1 Seasonal Variance

Thirty-eight of the 44 metrics differed between spring and fall seasons, reflecting a substantially different macroinvertebrate community composition between seasons (**Table 23**). In the Control and Hinkson Creek treatment groups, the seasonal variation in richness and relative abundance metrics were driven by the greater presence of chironomids in the spring season. The only metrics unrelated to chironomids that were significantly greater in the spring were Plecoptera richness, percent Plecoptera, and percent Intolerant Taxa. Metrics that reflect the presence of EPT and other major taxa groups were routinely greater in the fall.

TABLE 23. Hinkson Creek and Control Stream Results of Seasonal Analyses of Variance. Symbols in parentheses after metric names indicate transformations to normalize data. EDU Reference stream means are provided for comparison but were not included in the tests.

Metric (Transformation)	HCr, HCu and Control Means		Test Type	Result	Reference Streams Means	
	Spring	Fall			Spring	Fall
Total Taxa Richness	71.7	69.8	A	ns	77.2	78.5
Diptera Richness	36.6	33.4	A	**sig**	32.8	34.2
Chironomidae Richness (Y^2)	30.6	26.7	A	**sig**	27.0	27.8
EPT Richness ($\sqrt{Y}$)	10.9	12.7	A	**sig**	17.2	15.2
Ephemeroptera Richness	5.0	7.5	A	**sig**	6.6	8.5
Plecoptera Richness	1.6	0.1	KW	**sig**	4.2	0.5

Metric (Transformation)	**HCr, HCu and Control Means**		**Test Type**	**Result**	**Reference Streams Means**	
	Spring	**Fall**			**Spring**	**Fall**
Trichoptera Richness (√Y)	4.3	5.0	A	**sig**	6.4	6.2
Clinger/Climber Richness	27.3	31.4	A	**sig**	33.0	36.2
% Diptera	65.0	31.8	KW	**sig**	33.0	29.1
% Chironomidae	60.7	29.2	KW	**sig**	28.2	26.9
% Tanytarsini	6.7	4.8	KW	**sig**	3.8	6.9
% Oligochaeta	6.0	7.6	KW	ns	4.1	4.5
% Corbicula	0.4	2.3	KW	**sig**	0.1	0.0
% Other Diptera and Non-Insects (√Y)	15.5	20.6	A	**sig**	17.3	17.2
% EPT	13.3	31.1	KW	**sig**	37.5	36.2
% Ephemeroptera (√Y)	10.6	20.3	A	**sig**	27.3	29.2
% Plecoptera	1.6	<0.1	KW	**sig**	8.1	0.2
% EP	12.3	20.3	KW	**sig**	35.4	29.4
% Trichoptera	1.1	10.7	KW	**sig**	2.1	6.9
% Hydropsychidae	0.3	7.4	KW	**sig**	0.3	3.3
% Clinger/Climber	59.9	66.5	A	**sig**	70.2	69.5
% Filterers	8.8	19.5	KW	**sig**	6.9	11.5
% Predators (√Y)	10.8	12.4	A	**sig**	14.4	16.4
% Scrapers (√Y)	15.3	20.7	A	**sig**	19.1	16.6
% Shredders	29.0	10.5	KW	**sig**	11.6	9.9
% Dominant Taxon	20.9	17.5	KW	**sig**	25.9	20.7
% Dominant 2 Taxa (√Y)	33.0	28.0	A	**sig**	36.7	30.1
% Dominant 5 Taxa (√Y)	53.7	47.6	A	**sig**	57.8	47.2
% Intolerant (≤ 4) Taxa	8.8	5.7	KW	**sig**	20.0	5.0
EPT/Chironomidae Ratio	0.31	1.21	KW	**sig**	1.51	1.48
Scrapers/Filterers Ratio	3.12	1.48	KW	**sig**	6.68	1.54
% Fast Seasonal Development	66.8	41.9	KW	**sig**	44.4	47.0
% Slow Seasonal Development (√Y)	12.4	25.4	A	**sig**	28.2	25.4
% Able to Exit as Adults	61.1	30.1	KW	**sig**	28.6	27.8
% Rare in Drift	12.2	19.3	KW	**sig**	24.3	23.0
% Abundant in Drift	61.0	34.3	KW	**sig**	30.9	42.0
% No Swimming Ability	74.3	55.1	KW	**sig**	48.5	46.2
% Strong Swimming Ability	1.0	4.7	KW	**sig**	6.6	2.4

Metric (Transformation)	HCr, HCu and Control Means		Test Type	Result	Reference Streams Means	
	Spring	Fall			Spring	Fall
% Streamlined Body Shape (√Y)	5.0	13.4	A	**sig**	14.5	12.3
% Sprawlers	14.8	16.9	KW	ns	23.5	29.6
Missouri Biotic Index	6.71	6.77	A	ns	6.01	6.70
Macroinvertebrate Biotic Index	5.83	5.43	A	**sig**	4.91	5.34
Shannon Diversity Index (Y^5)	3.08	3.23	A	**sig**	2.96	3.30
Deposited Sediment Tolerance (log Y)	1.58	1.58	KW	ns	1.53	1.43

Notes: "A" indicates statistical test ($p < 0.05$) performed using ANOVA; " KW " indicates statistical test ($p < 0.05$) performed using Kruskall-Wallis; sig = significantly different; ns = not significantly different. Data Transformation: "Y2" squared; "√Y" square root ; "Y5" fifth power ; "log Y" base 10 log .

Many metrics exhibited clear seasonal patterns at the Control stream, HCr and HCu treatment groups that were not evident at the Reference streams. There were five general categories of similarity or contrast:

- The seasonal patterns and their magnitudes were essentially the same;
- The patterns were the same, but the magnitudes of the seasonal trends were different;
- There was not much difference for one, but a moderate to large difference for the other;
- The patterns were opposite, but the differences were small; and
- The seasonal patterns were clearly opposite

The metrics for which the seasonal patterns were approximately the same include: Ephemeroptera richness, Plecoptera richness, clinger/climber richness, percent Oligochaeta, percent Plecoptera, percent Trichoptera, percent Hydropsychidae, percent filterers, percent predators, all 3 dominant taxa metrics, scrapers/filterers ratio, percent sprawlers, and Shannon Diversity Index.

Several metrics exhibited a slight seasonal pattern in the Reference streams, but a large seasonal difference in the same direction was observed in the Control stream and Hinkson Creek samples. Most of these were directly associated with the greater abundance of chironomids in the spring. These included: percent Diptera, percent Chironomidae, percent Ephemeroptera, percent shredders, percent able to exit as adults, and percent non-swimmers. In contrast, percent Intolerant Taxa were moderately greater in the spring in the Control stream and Hinkson Creek samples but distinctly greater in the spring at the Reference streams.

Metrics that were similar between seasons at Reference streams but exhibited a strong seasonal difference in the Control stream and Hinkson Creek samples were: Trichoptera richness, percent Corbicula, percent other Diptera and non-insects, percent EPT, percent clinger/climber, EPT/Chironomidae ratio, percent fast seasonal development, percent slow seasonal development, percent rare in the drift, and percent streamlined body shape. In contrast, means of Missouri Biotic Index and Deposited Sediment Tolerance Index were similar between seasons in the Control stream and Hinkson Creek, but differed somewhat at the Reference streams (Missouri Biotic Index greater in the fall and Deposited Sediment Tolerance Index greater in the spring).

For ten metrics, the seasonal patterns were opposite in Reference streams as opposed to the Control stream and Hinkson Creek metrics. Metrics for which the differences were not large included: Total Taxa richness, Diptera richness, Chironomidae richness, and EPT richness. The opposite seasonal patterns were more discernable for percent Tanytarsini, percent EP, percent scrapers, percent abundant in the drift, percent strong swimmers, and Macroinvertebrate Biotic Index. Specific seasonal and treatment group patterns of each macroinvertebrate metric are discussed in detail below.

<u>Total Taxa richness</u> was lower in the HCu compared to HCr in both spring and fall (**Table 24** and **Table 25**) but never by a large margin. It was also greater in Control than HCr samples in both seasons, but by a significant margin only in the fall. Total Taxa richness was generally greater in Reference streams than HCr or HCu or Control stream. In the fall, HCr samples were similar to the Reference streams. This metric did not consistently differ between seasons. It was greater in the fall at HCr but similar between seasons at the other treatment groups.

<u>Diptera richness</u> was very strongly associated with Chironomidae richness but was generally 5 to 7 taxa greater. Diptera richness did not differ between Control stream, HCr, and HCu samples in either spring or fall (**Table 24** and **Table 25**).

<u>Chironomidae richness</u> was strongly correlated with Diptera richness ($r = 0.85$ [spring] and $r = 0.84$ [fall]). Chironomidae richness did not differ between Control, HCr, and HCu samples in either spring or fall (**Table 24** and **Table 25**). It was 4 – 5 taxa greater in the spring at Control and HCu, but similar between seasons at HCr and Reference streams.

<u>EPT richness</u> was lower in HCu samples than in those of HCr in both spring and fall (**Table 24** and **Table 25**). It was also lower at HCu than at the Control stream in the spring. EPT richness was generally greater in Reference streams than at the other treatment groups. In the fall; however, HCr samples were similar to the Reference streams. This metric did not consistently differ between seasons; it was greater in the spring at Control and Reference streams, but greater in the fall in both HCr and HCu.

<u>Ephemeroptera richness</u> was strongly related to EPT richness, and generally contributed 40 to 60 percent of its total. Ephemeroptera richness did not differ between Control, HCr, and HCu samples in either spring or fall (**Table 24** and **Table 25**). It was generally 1 to 2 taxa greater in Reference streams than HCr, HCu, or Control stream. In the fall; however, HCr samples were similar to the Reference streams. Ephemeroptera richness was greater in the fall than the spring at all treatment groups.

Plecoptera richness is comprised much less of the total of EPT richness, particularly in the fall. In the spring, the level at Control stream was similar to that of Reference streams, and was greater than HCr, which in turn was greater than HCu (**Table 25**). In the fall, no statistical differences in the levels of this metric were noted between treatment groups (**Table 24**). Plecoptera richness was markedly greater in the spring at all sites.

Trichoptera richness was positively related to EPT richness, and contributed 34 to 44 percent of its total, on average. This metric was greater in Reference streams than at Control stream or HCu in both seasons (**Table 24** and **Table 25**). Trichoptera richness was more similar between HCr and the Reference streams. It was significantly greater in HCr samples compared to HCu in spring and fall; in the fall HCr was greater than Control stream.

Clinger/Climber richness metric contains taxa from all major aquatic insect orders, it was strongly correlated ($r = 0.78$ in spring and $r = 0.81$ in fall) with EPT richness. It was significantly greater in HCr than HCu in both seasons (**Table 24** and **Table 25**). In the fall, Control stream was greater than HCu. Clinger/Climber richness levels were moderately higher in Reference streams in spring and fall. This metric was 2 to 5 taxa higher in the fall at all treatment groups.

Percent Diptera was very strongly associated with percent Chironomidae, particularly in the spring ($r = 0.97$). It was significantly greater at HCu than HCr and Control stream in the spring (**Table 25**). In the fall, it was intermediate between the two, but the differences were not significant (**Table 24**). At Control stream, HCr, and HCu, percent Diptera was much greater in the spring, reflecting the large seasonal difference in chironomid abundance. At the Reference streams, the seasonal difference was the same, but of much smaller magnitude.

Percent Chironomidae site and seasonal differences examined by analysis of variance were the same for percent Diptera. Chironomids contributed approximately 90 percent of the dipterans collected, and so this metric was highly correlated with Percent Diptera.

Percent Tanytarsini was greatest at HCu in both seasons, but the trend was significant only in the spring. This metric was greater in the spring at HCr and HCu, but greater in the fall at Control stream and in the Reference streams (**Table 24** and **Table 25**).

Percent Oligochaeta was consistently greater in samples from HCu and Control stream than from HCr and Reference streams, but never by a significant margin. It was greater in the fall at all treatment groups, but by moderate margins only at HCu and Control (**Table 24** and **Table 25**).

Percent Corbicula was generally found in low numbers but was significantly greater in samples from HCu than HCr and Control stream in both seasons (**Table 24** and **Table 25**). Corbicula specimens were very rarely collected at Reference streams. At HCu, they were much more abundant in the fall.

<u>Percent Other Diptera and Non-Insects</u> did not differ significantly among site groups in either season (**Table 24** and **Table 25**) and was slightly to moderately greater in fall samples from Control stream, HCr, and HCu. It was similar between seasons in Reference stream samples.

<u>Percent EPT</u> was significantly greater at HCr than at HCu, with Control intermediate between them in the spring (**Table 25**). No statistical difference was evident for this metric in the fall (**Table 24**). Percent EPT was much higher in Reference stream samples in the spring but only slightly higher in the fall. It was 2 to 3 times greater in the fall than in the spring at HCr, HCu, and Control stream, but similar between seasons in the Reference streams.

<u>Percent Ephemeroptera</u> were consistently the major contributors to the Percent EPT metric, accounting for 60 to 85 percent of the total, on average. In spring samples, it was significantly greater at HCr than at Control stream, with HCu intermediate between them (**Table 25**). No statistical difference was evident for this metric in the fall (**Table 24**). This metric was markedly higher in Reference stream samples than in the other treatment groups in both seasons. At the Control stream and HCu, percent Ephemeroptera levels in the fall were approximately double those of spring. At HCr, fall levels were moderately greater than spring levels, and in the Reference streams the levels were similar between seasons.

<u>Percent Plecoptera</u> are important contributors to the totals of EPT organisms at Control stream (mean = 38.6 percent) and Reference streams (21.6), in the spring. They were less numerous at HCr (10.6) and HCu (3.5) in the spring (**Table 25**). In the fall, stoneflies were found in low numbers at all treatment groups (**Table 24**). These patterns were further reflected by significantly greater spring levels of percent Plecoptera at the Control stream than at HCr, which was also significantly higher than HCu. Spring levels of this metric were even higher in samples from the Reference streams. In the fall, no statistical differences were noted.

<u>Percent Ephemeroptera + Plecoptera (EP)</u> was significantly greater at HCr than at HCu in the spring, with Control stream intermediately between them. No statistical differences among treatment groups were evident for this metric in the fall. In Reference streams, percent EP was much greater than HCu in the spring and moderately greater in the fall (**Table 24** and **Table 25**).

<u>Percent Trichoptera</u> contributed approximately 6 to 10 percent, on average, to the total number of EPT organisms in the spring, and 20 to 40 percent in the fall. It did not significantly differ among HCr, HCu, and Control stream samples in either season (**Table 24** and **Table 25**). It was slightly higher in the Reference streams than the other treatment groups in the spring, but lower than all of them in the fall.

<u>Percent Hydropsychidae</u> was greater in HCu samples than those of HCr or Control stream in both seasons, but by a significant margin only in the spring (**Table 24** and **Table 25**). It was found in similar levels in Reference streams to the other treatment groups in the spring but was markedly lower than them in the fall. It was much greater in fall samples, particularly at HCu.

Percent Clingers + Climbers did not significantly differ among treatment groups in either spring or fall (**Table 24** and **Table 25**). It was moderately greater in Reference streams than the other treatment groups in the spring, but only slightly greater in the fall. At HCr, HCu, and the Control stream, percent clingers plus climbers was greater in the fall than in the spring but was similar between seasons at the Reference streams.

Percent Filterers was greater in the fall than in the spring at all treatment groups (**Table 24** and **Table 25**). In the spring, it was significantly greater at HCu than at the Control stream, with HCr intermediate between them. No difference between sites was evident in the fall. Percent filterers at Reference streams were lower than both HCr and HCu, but not Control in spring samples and lower than all other sites in the fall.

Percent Predators did not differ among treatment groups in either spring or fall (**Table 24** and **Table 25**). It was moderately greater in Reference stream samples in both seasons. It was slightly greater in spring samples at the Control stream, and slightly greater in fall samples at HCr, HCu, and Reference streams.

Percent Scrapers was slightly to moderately greater in the fall than in the spring at HCr, HCu, and Control stream, but slightly higher in the spring at the Reference streams (**Table 24** and **Table 25**). It was significantly greater in samples from the Control stream and HCr than at HCu in the spring. In the fall, no differences among treatment groups were noted.

Percent Shredders was markedly greater in the spring than in the fall at the Control stream, HCr, and HCu, but was only slightly higher in the spring at Reference streams (**Table 24** and **Table 25**). It was significantly greater at Control stream and HCu than at HCr in the spring, but no difference between sites was noted in the fall.

Percent Dominant Taxon in the spring was significantly greater at the Control stream than at HCu, with HCr intermediate between them (**Table 25**). No difference among treatment groups was evident in the fall (**Table 24**).

Percent Dominant 2 Taxa was highly correlated (r = 0.93 in spring and 0.90 in fall) with percent dominant taxa. It was moderately greater in spring samples than in those from the fall at all treatment groups (**Table 24** and **Table 25**). It was significantly greater at the Control stream than at HCu in the spring, with HCr between them; but it was similar among treatment groups in the fall.

Percent Dominant 5 Taxa was strongly correlated (r = 0.91 in spring and 0.86 in the fall) with percent dominant 2 taxa. It comprised 52 to 58 percent of the total sample in the spring and 47 to 49 percent in the fall (**Table 24** and **Table 25**). It did not statistically differ between treatment groups in either season.

Percent Intolerant (≤ 4) Taxa was slightly greater in the spring than the fall at the Control stream and HCr, moderately greater in the spring at HCu, and markedly greater in the spring at the Reference streams (**Table 24** and **Table 25**). In the fall, it was significantly greater at control stream and HCr than at HCu, but no differences among treatment groups were evident in the spring.

EPT/Chironomidae Ratio was significantly greater at HCr than HCu in the spring, with Control stream intermediate between them. It did not differ among treatment groups in the fall. It was 2 to 5 times greater in the fall at HCr, HCu, and Control stream, reflecting the high numbers of chironomids in the spring samples of those locations (**Table 24** and **Table 25**). It was moderately (fall) to considerably (spring) greater in Reference streams than at the other treatment groups.

Scrapers/Filterers Ratio was 2 to 3 times greater in the spring than in the fall at HCr, Control stream, and the Reference streams; it was also slightly higher at HCu in the spring (**Table 24** and **Table 25**). Treatment group differences were only noted in the spring, when scrapers/filterers ratio was significantly greater at Control stream and HCr than at HCu.

Percent Fast Seasonal Development was highly correlated ($r = 0.94$) with percent Chironomidae in the spring but slightly less so ($r = 0.84$) in the fall. It was much greater in the spring than in the fall at HCr, HCu, and Control stream, but similar between seasons at Reference streams (**Table 24** and **Table 25**). In the spring, it was significantly greater at HCu than at HCr, with Control stream intermediate between them, but did not differ among treatment groups in the fall.

Percent Slow Seasonal Development was significantly greater at HCr than at HCu in the spring, with Control stream intermediate between them (**Table 25**). In the fall, it did not differ among treatment groups (**Table 24**). It was considerably greater in Reference streams as compared to HCr, HCu, and Control stream in the spring, but was similar among all treatment groups in the fall (**Table 24** and **Table 25**).

Percent Ability to Exit as Adults was completely correlated ($r = 1.00$) with percent Chironomidae in both seasons. Site and seasonal differences examined by analysis of variance were the same for both metrics.

Percent Rare Drift was significantly greater at HCr than at HCu and Control stream in the spring. In the fall, it was greater at HCr than Control stream, and HCu was intermediate between them (**Table 24** and **Table 25**). It was clearly greater in Reference streams as compared to HCr, HCu, and Control stream in the spring. In the fall, HCr was similar but still somewhat greater than HCu, and less at control stream.

Percent Abundant in Drift was highly ($r = 0.87$ in the fall) to completely ($r = 1.00$ in the spring) correlated to percent Chironomidae. Like that metric, percent abundant in drift was much greater in the spring than in the fall at HCr, HCu, and Control stream, but was only moderately greater in the fall at Reference streams (**Table 24** and **Table 25**). In the spring, it was significantly greater at HCu than at HCr, with Control stream intermediate between them, but did not differ among treatment groups in the fall.

Percent No Swimming Ability was highly correlated (r = 0.91) with percent Chironomidae in the spring, and less so (r = 0.59) in the fall. It was somewhat greater in the spring than in the fall at HCr, HCu, and Control stream, but similar between seasons at Reference streams (**Table 24** and **Table 25**). It did not significantly differ among treatment groups in either the spring or the fall.

Percent Strong Swimming Ability was significantly greater at HCr than at HCu and Control stream in the spring. In the fall, it was greater at HCu than the Control stream, and HCr was intermediate between them (**Table 24** and **Table 25**). It was markedly greater in Reference streams compared to HCr, HCu, and Control stream in the spring. In the fall; however, it was lowest in the Reference streams. Fall levels of percent strong swimming ability were greater at HCr, HCu, and Control stream, whereas this metric was greater in the spring in Reference streams.

Percent Streamlined Body Shape did not differ among treatment groups in either season (**Table 24** and **Table 25**). It was 2 to 3 times greater in the fall than in the spring at HCr, HCu, and Control stream but was similar between seasons at the Reference streams.

Percent Sprawlers was significantly greater at HCr and HCu than at Control stream in the spring. In the fall, it did not differ among treatment groups. It was moderately greater in the Reference streams than at HCr, HCu, and Control stream in both seasons (**Table 24** and **Table 25**).

Missouri Biotic Index did not differ among treatment groups in either spring or fall (**Table 24** and **Table 25**). At the Reference streams, the Missouri Biotic Index averaged 6.01 in the spring, which was lower than the HCr, HCu, and Control stream treatment groups. Otherwise, it varied over a narrow range (6.59 to 6.83, on average), and was slightly but consistently greater in fall samples at HCr, HCu, and the Control stream.

Macroinvertebrate Biotic Index was significantly greater at HCu than at HCr or Control stream in the spring, but in the fall the treatment groups did not differ (**Table 24** and **Table 25**). It was considerably lower at the Reference streams in the spring, but similar to the other treatment groups in the fall. Macroinvertebrate Biotic Index scores were lower in the fall at HCr, HCu, and Control stream, but lower in the spring at Reference streams.

Shannon Diversity Index varied over a narrow range (2.96 to 3.30, on average). It did not differ significantly among treatment groups in either season (**Table 24** and **Table 25**). It was slightly (HCr) to moderately (Control stream, HCu, and Reference streams) greater in the fall than in the spring.

Deposited Sediment Tolerance Index was significantly greater at HCr than at Control stream in the spring, with HCu intermediate between them (**Table 25**). In the fall, no significant differences among treatment groups were noted (**Table 24**). Deposited Sediment Tolerance Index levels at Reference streams were similar to or slightly lower than the other treatment groups in both seasons.

4.2.2.2 Metric Variance

Differences in metrics between treatment groups are more likely to reflect disturbance rather than natural variability. Due to the potential obscuring effect of the prevalent seasonal differences, separate tests were conducted for spring and fall data.

In the fall, 7 of 44 metrics differed among treatment groups (**Table 24**), and only 5 indicated lower community quality at HCu. These included significantly lower levels of EPT richness, Trichoptera richness, clinger/climber richness, and percent Intolerant Taxa, and a greater level of percent Corbicula.

In the spring, 30 of the 44 metrics differed among treatment groups (**Table 25**). Many of these likely reflected an impacted macroinvertebrate community at HCu as compared to HCr and/or the Control Stream. These included significantly lower levels of Total Taxa richness, EPT richness, Plecoptera richness, Trichoptera richness, clinger/climber richness, percent EPT, percent Plecoptera, percent EP, percent scrapers, EPT/Chironomidae ratio, scraper/filterer ratio, percent slow seasonal development, percent rare in the drift, and percent strong swimmers. Metrics that were significantly greater at HCu were percent Diptera, percent Chironomidae, percent Corbicula, percent Hydropsychidae, percent filterers, percent fast seasonal development, percent able to exit as adults, percent abundant in the drift, and Macroinvertebrate Biotic Index.

In general, levels of metrics in EDU Reference stream samples reflected macroinvertebrate communities better than those of the Control stream and HCr, and of a much higher quality than HCu. This was primarily evident in the spring, when 19 “positive” metrics (e.g., EPT richness) were greatest and nine “negative” metrics (e.g., Missouri Biotic Index) were lowest in reference stream samples (**Table 25**), possibly indicating a more pronounced effect of urbanization in this season. In the fall, the differences between macroinvertebrate communities at Reference streams and the other treatment groups were much less pronounced, particularly with respect to HCr. Even so, seven positive metrics were greatest and five negative metrics were lowest in Reference stream samples (**Table 24**).

TABLE 24. Fall Macroinvertebrate Metrics Analyses of Variance Results. Statistical differences ($p < 0.05$) are indicated by superscript letters. Means without superscript letters are not significantly different. EDU Reference streams means are provided for comparison purposes only. Symbols in parentheses after metric names indicate transformations to normalize data.

Metric (Transformation)	Test Type	Control	HCr	HCu	Reference
Total Taxa Richness	A	72.7	77.2	70.1	78.5
Diptera Richness	A	34.9	35.7	32.4	34.2
Chironomidae Richness	A	27.5	28.6	26.1	27.8
EPT Richness	A	11.7^{b}	15.3^{a}	12.4^{b}	15.2
Ephemeroptera Richness	KW	7.2	8.4	7.5	8.5
Plecoptera Richness	KW	0.2	0.2	0.1	0.5
Trichoptera Richness	A	4.3^{b}	6.7^{a}	4.9^{b}	6.2
Clinger/Climber Richness	A	31.3^{ab}	34.6^{a}	30.8^{b}	36.2
% Diptera	A	34.0	28.6	31.8	29.1
% Chironomidae	A	31.2	25.7	29.4	26.9
% Tanytarsini	KW	6.9	4.8	7.9	6.9
% Oligochaeta	KW	9.6	5.1	7.5	4.5
% Corbicula	KW	0.1^{b}	0.1^{b}	3.5^{a}	0.0
% Other Diptera and Non-Insects	A	22.6	19.9	20.1	17.2
% EPT	A	27.9	32.0	31.8	36.2
% Ephemeroptera	A	16.7	19.5	21.6	29.2
% Plecoptera	KW	0.1	<0.1	<0.1	0.2
% EP	A	16.8	19.5	21.6	29.4
% Trichoptera	A	11.2	12.5	10.2	6.9
% Hydropsychidae ($\sqrt{Y}$)	A	6.2	6.1	8.1	3.3
% Clinger/Climber	A	64.2	65.5	67.4	69.5
% Filterers (log Y)	A	18.4	15.9	20.6	11.5
% Predators	A	10.6	13.2	12.7	16.4
% Scrapers	A	23.1	24.1	19.2	16.6
% Shredders	KW	11.2	8.3	10.7	9.9
% Dominant Taxon	A	18.5	20.0	16.7	20.7
% Dominant 2 Taxa (log Y)	A	29.3	28.9	27.4	30.1
% Dominant 5 Taxa	A	48.8	47.6	47.2	47.1
% Intolerant (≤ 4) Taxa	A	7.5^{a}	7.8^{a}	4.7^{b}	5.0
EPT/Chironomidae Ratio	KW	0.98	1.24	1.28	1.48
Scrapers/Filterers Ratio	KW	1.88	2.07	1.23	1.54
% Fast Seasonal Development	A	41.7	34.8	43.5	47.0
% Slow Seasonal Development	A	22.9	29.5	25.3	25.4
% Able to Exit as Adults	A	31.7	26.4	30.4	27.8
% Rare in Drift	A	17.0^{b}	25.0^{a}	18.7^{ab}	23.0
% Abundant in Drift	A	33.7	27.6	36.1	42.0
% No Swimming Ability	A	59.2	56.3	53.6	46.2

Metric (Transformation)	Test Type	Control	HCr	HCu	Reference
% Strong Swimming Ability (log Y+1)	A	2.9^{b}	3.1^{ab}	5.5^{a}	2.4
% Streamlined Body Shape	A	11.3	11.4	14.4	12.3
% Sprawlers	KW	13.6	17.8	17.7	29.6
Missouri Biotic Index	A	6.69	6.63	6.83	6.70
Macroinvertebrate Biotic Index	KW	5.44	5.21	5.47	5.34
Shannon Diversity Index (Y^3)	A	3.20	3.14	3.25	3.30
Deposited Sediment Tolerance (log Y)	A	1.70	1.57	1.55	1.43

Notes: "A" indicates statistical test ($p < 0.05$) performed using ANOVA; " KW " indicates statistical test ($p < 0.05$) performed using Kruskall-Wallis.

TABLE 25. Spring Macroinvertebrate Metrics Analyses of Variance Results. Statistical differences ($p < 0.05$) are indicated by superscript letters. Means without superscript letters are not significantly different. EDU Reference streams means are provided for comparison purposes only. Symbols in parentheses after metric names indicate transformations to normalize data.

Metric (Transformation)	Test	Control	HCr	HCu	Reference
Total Taxa Richness	A	73.6^{a}	71.2^{ab}	68.1^{b}	77.2
Diptera Richness	A	38.4	36.4	36.1	32.8
Chironomidae Richness	A	31.4	28.9	31.0	27.0
EPT Richness	A	13.3^{a}	12.9^{a}	9.3^{b}	17.2
Ephemeroptera Richness	A	4.6	5.5	4.9	6.6
Plecoptera Richness	KW	4.2^{a}	2.1^{b}	0.6^{c}	4.2
Trichoptera Richness	KW	4.5^{ab}	5.3^{a}	3.8^{b}	6.4
Clinger/Climber Richness	A	29.2^{a}	29.0^{a}	26.0^{b}	33.0
% Diptera	KW	59.3^{ab}	59.2^{b}	69.1^{a}	33.0
% Chironomidae	A	57.1^{ab}	51.9^{b}	65.5^{a}	28.2
% Tanytarsini	KW	5.9^{b}	8.9^{b}	13.2^{a}	3.8
% Oligochaeta ($\sqrt{Y}$)	A	5.9	4.8	6.5	4.1
% Corbicula	KW	0.0^{b}	0.1^{b}	0.7^{a}	0.1
% Other Diptera and Non-Insects	KW	17.9	17.5	14.0	17.3
% EPT ($\sqrt{Y}$)	A	14.0^{ab}	17.8^{a}	11.3^{b}	37.5
% Ephemeroptera ($\sqrt{Y}$)	A	7.8^{b}	14.6^{a}	9.8^{ab}	27.3
% Plecoptera	KW	5.4^{a}	1.9^{b}	0.4^{c}	8.1
% EP ($\sqrt{Y}$)	A	13.1^{ab}	16.6^{a}	10.2^{b}	35.4
% Trichoptera	KW	0.9	1.2	1.0	2.1
% Hydropsychidae ($\sqrt{Y}$)	KW	0.1^{b}	0.2^{b}	0.4^{a}	0.3
% Clinger/Climber	A	59.9	59.9	59.9	70.2
% Filterers	KW	4.8^{b}	8.4^{ab}	10.2^{a}	6.9
% Predators	A	11.7	9.8	11.0	14.4
% Scrapers (log Y)	A	20.8^{a}	19.4^{a}	11.9^{b}	19.1
% Shredders	A	31.3^{a}	22.5^{b}	31.0^{a}	11.6
% Dominant Taxon	KW	25.4^{a}	21.2^{ab}	19.3^{b}	25.9

Metric (Transformation)	Test	Control	HCr	HCu	Reference
% Dominant 2 Taxa	A	39.0^{a}	33.5^{ab}	31.0^{b}	36.7
% Dominant 5 Taxa	A	57.2	54.9	52.2	57.8
% Intolerant (≤ 4) Taxa	KW	9.5	10.8	7.9	20.0
EPT/Chironomidae Ratio	KW	0.36^{ab}	0.48^{a}	0.23^{b}	1.51
Scrapers/Filterers Ratio	KW	5.98^{a}	4.41^{a}	1.72^{b}	6.68
% Fast Seasonal Development	KW	62.6^{ab}	60.5^{b}	70.7^{a}	44.4
% Slow Seasonal Development	KW	11.1^{ab}	16.7^{a}	11.0^{b}	28.2
% Able to Exit as Adults	A	57.4^{ab}	52.7^{b}	65.7^{a}	28.6
% Rare in Drift	KW	8.6^{b}	17.4^{a}	11.1^{b}	24.3
% Abundant in Drift	A	57.3^{ab}	52.3^{b}	65.7^{a}	30.9
% No Swimming Ability (Y^3)	A	70.0	70.7	77.0	48.5
% Strong Swimmers	KW	0.7^{b}	2.0^{a}	0.7^{b}	6.6
% Streamlined Body Shape (log Y+1)	A	6.2	5.9	4.2	14.5
% Sprawlers	KW	9.4^{b}	17.9^{a}	15.2^{a}	23.5
Missouri Biotic Index	A	6.59	6.60	6.79	6.01
Macroinvertebrate Biotic Index	A	5.56^{b}	5.67^{b}	5.98^{a}	4.91
Shannon Diversity Index (Y^3)	A	2.98	3.11	3.09	2.96
Deposited Sediment Tolerance (1/Y)	A	1.50^{b}	1.65^{a}	1.57^{ab}	1.53

Notes: "A" indicates statistical test ($p < 0.05$) performed using ANOVA; " KW " indicates statistical test ($p < 0.05$) performed using Kruskall-Wallis.

4.2.2.3 Water Quality Variance

Water quality variances between treatment groups were evaluated for the Paired water chemistry data. Water quality differences during the spring season were not evident for ammonia, dissolved oxygen, and TSS (**Table 26**). Notable spring differences include higher chloride in HCu compared to HCr and Control, higher temperature in both HCr and HCu than Control, higher sulfate in both HCr and HCu than Control, higher total nitrogen and total phosphorus in HCr than HCu, and high turbidity in HCr than HCu and Control. Notable fall water chemistry variances include higher chloride in HCu than HCr and Control, higher dissolved oxygen and temperature in HCr and HCu than Control. Variance during the fall were not evident for ammonia, total nitrogen, total phosphorus, turbidity, and TSS.

TABLE 26. Paired Water Chemistry Data Seasonal Analyses of Variance. Statistically different ($p < 0.05$) median values are indicated by superscript letters. Reference stream median values are provided for comparison purposes and were not included in statistical tests.

WQ Parameters (units)	Fall				Spring			
	Control	HCr	HCu	Ref.	Control	HCr	HCu	Ref.
Ammonia (mg/L)	0.036	0.036	0.050	0.050	0.035	0.040	0.047	0.044
Chloride (mg/L)	9.9^{b}	14.3^{b}	41.7^{a}	8.02	22^{b}	17.4^{b}	42.9^{a}	11.7
Dissolved Oxygen (mg/L)	7.0^{b}	8.5^{a}	7.9^{a}	7.6	10	10.7	10.7	9.9

WQ Parameters (units)	Fall				Spring			
	Control	HCr	HCu	Ref.	Control	HCr	HCu	Ref.
Flow (cfs)	1.18	2.71	2.10	0.40	4.05^{b}	7.60^{b}	15.45^{a}	26.40
pH (SU)	7.7^{b}	8.0^{a}	7.8^{ab}	7.7	7.9^{b}	8.3^{a}	8.2^{a}	8.1
Conductivity (µS/cm)	406^{b}	451^{ab}	585^{a}	314	410^{b}	472^{b}	605^{a}	244
Temperature (C)	17.7^{b}	19.7^{ab}	19.9^{a}	17.5	9.0^{b}	15.1^{a}	13.9^{a}	20.0
Hardness (mg/L)	235	224	249	NA	200^{b}	199^{b}	254^{a}	NA
Nitrate plus Nitrite (mg/L)	0.22	0.11	0.04	0.05	0.09^{a}	0.05^{ab}	0.01^{b}	0.41
Sulfate (mg/L)	9.6^{b}	68.2^{a}	76.0^{a}	NA	13.5^{c}	84.6^{b}	95.1^{a}	NA
Total Nitrogen (mg/L)	0.42	0.51	0.43	0.31	0.33^{b}	0.55^{a}	0.42^{b}	0.73
Total Phosphorus (mg/L)	0.066	0.072	0.051	0.050	0.050^{ab}	0.053^{a}	0.042^{b}	0.038
TSS (mg/L)	5.0	5.0	5.0	5.0	5.0	6.5	5.0	5.0
Turbidity (NTU)	5.2	7.1	6.1	7.0	3.2^{c}	12.2^{a}	6.0^{b}	4.0

Notes: Ref = Reference; mg/L = milligrams per liter; cfs = cubic feet per second; SU = standard units; uS/cm = microsiemens per centimeter; C = Celsius; NTU = nephelometric turbidity units.

4.2.3 Temporal Trend Analyses

Of a possible 1,419 trends analyses performed, 86 of statistical significance were evident. No statistically significant trends were evident in the Reference streams. Of the 44-macroinvertebrate metrics, 32 expressed statistically significant trends in either HCr, HCu, or Control stream during the spring or fall season. Those metrics that were not statistically significant in either season include: trait states (ability to exit as adults, abundant in drift, fast seasonal development, rare in drift, streamlined body shape, and strong swimming ability) composition (percent Chironomidae, percent Diptera, percent Ephemeroptera, and percent EP), richness (Ephemeroptera), and habitat (percent shredders).

More statistically significant trends were evident during the spring season; however, historically more macroinvertebrate community samples have been collected during the spring season. More statistically significant trends were evident in HCu than HCr; however, historically more macroinvertebrate community samples have been collected from HCu. Statistically significant temporal trend results of treatment groups are provided in **Table 27** and **Table 28**. Of statistically significant trends, percent Plecoptera and percent Predators were the most common descending metrics, and percent Intolerant Taxa and percent Trichoptera were the most common ascending metrics. Spring macroinvertebrate metrics of the Control stream are all descending except Missouri Biotic Index which is ascending.

TABLE 27. 2001 to 2017 Fall Season Macroinvertebrate Metrics Spatial and Temporal Trends of Significance.

Treatment Group	Metric	Significant Trend
HCr	% Intolerant (≤ 4) Taxa	Ascending
HCr	% Trichoptera	Ascending
HCu	% Corbicula	Ascending
HCu	% Dominant 2 Taxa	Descending
HCu	% EPT	Ascending
HCu	% Intolerant (≤ 4) Taxa	Ascending
HCu	% Predators	Descending
HCu	% Slow Seasonal Development	Ascending
HCu	Chironomidae Richness	Descending
HCu	Diptera Richness	Descending
HCu	Deposited Sediment Tolerance Index	Descending
HCu	Total Taxa Richness	Descending
HCu	Trichoptera Richness	Ascending
Control	% Clingers + Climbers	Ascending
Control	% EPT	Ascending
Control	% Filterers	Ascending
Control	% Hydropsychidae	Ascending
Control	% Intolerant (≤ 4) Taxa	Ascending
Control	% No Swimming Ability	Ascending
Control	% Oligochaeta	Descending
Control	% Predators	Descending
Control	% Slow Seasonal Development	Ascending
Control	% Trichoptera	Ascending
Control	Missouri Biotic Index	Descending
Control	Scraper/Filterer Ratio	Descending
Control	Deposited Sediment Tolerance Index	Descending

TABLE 28. 2002 to 2017 Spring Season Macroinvertebrate Metrics Spatial and Temporal Trends of Significance.

Treatment Group	Metric	Significant Trend
HCr	% Corbicula	Ascending
HCr	% Plecoptera	Descending
HCr	% Scrapers	Descending
HCr	% Tanytarsini	Ascending
HCr	% Trichoptera	Ascending
HCr	Clinger + Climber Richness	Descending
HCr	EPT Richness	Descending

Treatment Group	Metric	Significant Trend
HCr	Plecoptera Richness	Descending
HCr	Total Taxa Richness	Descending
HCu	% Dominant 2 Taxa	Descending
HCu	% Dominant 5 Taxa	Descending
HCu	% Dominant Taxon	Descending
HCu	% Intolerant (≤ 4) Taxa	Ascending
HCu	% Oligochaeta	Descending
HCu	% Other Diptera + Non-Insects	Descending
HCu	% Predators	Ascending
HCu	% Sprawlers	Ascending
HCu	% Tanytarsini	Ascending
HCu	% Trichoptera	Ascending
HCu	Chironomidae Richness	Ascending
HCu	Diptera Richness	Ascending
HCu	Macroinvertebrate Biotic Index	Descending
HCu	Missouri Biotic Index	Descending
HCu	Shannon Diversity Index	Ascending
Control	% Intolerant (≤ 4) Taxa	Descending
Control	% Plecoptera	Descending
Control	% Predators	Descending
Control	% Slow Seasonal Development	Descending
Control	% Trichoptera	Descending
Control	EPT/Chironomidae Ratio	Descending
Control	Missouri Biotic Index	Ascending
Control	Total Taxa Richness	Descending
Control	Trichoptera Richness	Descending

4.2.4 Correlation Analyses

Correlation analyses were performed for 14 water chemistry parameters collected from HCr, HCu, Control and Reference streams for the following parameters; temperature, dissolved oxygen, conductance, pH, field flow, total nitrogen, total phosphorus, ammonia, nitrate plus nitrite, chloride, sulfate, TSS, turbidity and hardness. Physical parameters correlation analysis of the HCu treatment group include 2016 NLCD LULC and site-specific and general stormwater NPDES outfalls. HCr correlation analyses of macroinvertebrate metrics and physical parameters were unable to be performed due to insufficient sample size (less than four).

Correlation analyses of Hinkson Creek Site 3.5 macroinvertebrate metrics to long-term Hinkson Creek USGS Station 06910230 flow statistics were performed for the period of 2012 to 2017 for each season. A period of five weeks prior to macroinvertebrate sampling was chosen to assess flow statistics. Flow statistics included minimum flow, maximum flow, median flow, 90th percentile flow, flow variability (standard deviation), flow duration exceedance frequency condition (0.0 to 1.0 scale, 0.0 indicates highest condition and 1.0 indicates lowest condition), and flow disturbance frequency (count of flow pulses over long-term 90th percentile flow or 106 cubic feet per second).

Approximately 4,530 water chemistry bivariate correlations were performed for the treatment groups, of which, 934 of statistical significance were evident (**Table 29** through **Table 32**). HCu had the most correlations of significance with 444. More correlations of significance were evident in HCu during the spring (count = 274) season than the fall (Count = 170) season; although, historically more data have been collected during the spring season.

Approximately 608 correlation analyses were performed for Hinkson Creek Site 3.5 hydrology and macroinvertebrate metrics. Correlations were evident during the spring season (Count = 21) but not during the fall season. A summary of statistically significant correlations is presented in **Table 33.** Median flow was the most common significant hydrology characteristic, positively correlating with percent EPT, percent Ephemeroptera, EPT/Chironomidae ratio, percent slow seasonal development, percent rare in drift, percent sprawlers and percent EP. Flow disturbance frequency was not significantly correlated with any macroinvertebrate metric.

Approximately 170 outfall correlation analyses were performed for HCu macroinvertebrate metrics. Correlations were evident during both seasons. A summary of statistically significant correlations is presented in **Table 34.**

Approximately 508 LULC correlation analyses were performed for HCu macroinvertebrate metrics. Correlations were evident during both seasons. A summary of statistically significant correlations is presented in **Table 35.**

TABLE 29. Count of Fall Treatment Group Macroinvertebrate Metric Significant Correlations to Water Chemistry Parameters.

Metric	Fall Season			
	HCr	HCu	Control	Reference
Total Taxa Richness*	7	9	2	3
Diptera Richness*	0	8	1	1
Chironomidae Richness*	0	5	1	2
EPT Richness	**6**	**0**	**2**	**0**
Ephemeroptera Richness*	0	1	0	0
Plecoptera Richness*	2	0	0	0
Trichoptera Richness	**3**	**3**	**1**	**1**
Clinger + Climber Richness	**3**	**2**	**2**	**3**
% Diptera*	8	2	1	1
% Chironomidae*	8	3	1	0
% Tanytarsini*	3	2	3	0

Metric	**Fall Season**			
	HCr	**HCu**	**Control**	**Reference**
% Oligochaeta*	4	10	5	1
% Corbicula	**0**	**10**	**1**	NA
% Other Diptera + Non-Insects*	6	5	3	0
% EPT*	3	10	3	5
% Ephemeroptera*	1	4	2	4
% Plecoptera*	NA	NA	NA	NA
% Trichoptera*	3	8	3	1
% EP*	1	4	2	4
% Hydropsychidae*	0	3	3	1
% Clingers + Climbers*	0	3	2	0
% Filterers*	8	9	2	2
% Predators*	0	2	3	2
% Scrapers*	10	1	5	1
% Shredders*	0	3	1	0
% Dominant Taxon*	0	0	1	0
% Dominant 2 Taxa*	1	1	1	0
% Dominant 5 Taxa*	7	4	2	0
% Intolerant (≤ 4) Taxa	**0**	**8**	**2**	**0**
EPT/Chironomidae Ratio*	0	4	2	4
Scraper/Filterer Ratio*	8	3	4	0
% Fast Seasonal Development*	1	2	4	0
% Slow Seasonal Development*	9	9	3	0
% Ability to Exit as Adults*	8	3	1	0
% Rare in Drift	**2**	**2**	**2**	**0**
% Abundant in Drift*	7	3	3	1
% No Swimming Ability*	2	2	3	3
% Strong Swimming Ability	**4**	**2**	**2**	**0**
% Streamlined Body Shape*	0	1	1	0
% Sprawlers*	2	1	1	0
Missouri Biotic Index*	1	4	3	0
Macroinvertebrate Biotic Index*	0	1	2	0
Shannon Diversity Index*	0	1	2	0
Deposited Sediment Tolerance Index*	4	12	4	1

Note: NA = not assessed due to 0 values and/or insufficient samples; "*" = differences of statistical significance were not evident in analyses of variance between HCr, HCu, and Control stream; **BOLD** values indicate differences of statistical significance were evident in analyses of variance between HCr, HCu, and Control stream.

TABLE 30. Count of Spring Treatment Group Macroinvertebrate Metric Significant Correlations to Water Chemistry Parameters.

Metric	Spring Season			
	HCr	HCu	Control	Reference
Total Taxa Richness	**1**	**4**	**0**	**0**
Diptera Richness*	1	6	1	1
Chironomidae Richness*	4	8	1	2
EPT Richness	**1**	**1**	**2**	**1**
Ephemeroptera Richness*	6	1	2	2
Plecoptera Richness	**1**	**3**	**0**	**0**
Trichoptera Richness	**2**	**3**	**0**	**1**
Clinger + Climber Richness	**3**	**2**	**1**	**0**
% Diptera	**3**	**7**	**3**	**0**
% Chironomidae	**3**	**11**	**3**	**0**
% Tanytarsini	**2**	**8**	**3**	**0**
% Oligochaeta*	9	9	3	1
% Corbicula	**5**	**4**	NA	**0**
% Other Diptera + Non-Insects*	5	8	4	1
% EPT	**1**	**9**	**2**	**4**
% Ephemeroptera	**2**	**10**	**2**	**4**
% Plecoptera	**1**	**7**	**1**	**0**
% Trichoptera*	0	10	2	0
% EP	**0**	**10**	**2**	**4**
% Hydropsychidae	**5**	**4**	NA	**0**
% Clingers + Climbers*	2	1	2	4
% Filterers	**5**	**9**	**0**	**3**
% Predators*	0	8	1	0
% Scrapers	**4**	**2**	**0**	**2**
% Shredders	**2**	**2**	**1**	**0**
% Dominant Taxon	**0**	**8**	**2**	**0**
% Dominant 2 Taxa	**1**	**8**	**2**	**0**
% Dominant 5 Taxa*	1	8	1	0
% Intolerant (≤ 4) Taxa*	0	2	0	2
EPT/Chironomidae Ratio	**2**	**10**	**3**	**3**
Scraper/Filterer Ratio	**6**	**6**	**1**	**4**
% Fast Seasonal Development	**4**	**10**	**2**	**4**
% Slow Seasonal Development	**2**	**9**	**2**	**1**
% Ability to Exit as Adults	**3**	**11**	**3**	**0**
% Rare in Drift	**2**	**11**	**4**	**0**
% Abundant in Drift	**3**	**9**	**3**	**0**
% No Swimming Ability*	2	10	1	2
% Strong Swimming Ability	**1**	**0**	**1**	**2**
% Streamlined Body Shape*	0	2	2	1

Metric	Spring Season			
	HCr	HCu	Control	Reference
% Sprawlers	**2**	**6**	**4**	**1**
Missouri Biotic Index*	2	8	0	0
Macroinvertebrate Biotic Index	**0**	**2**	**1**	**0**
Shannon Diversity Index*	0	6	1	0
Deposited Sediment Tolerance Index	**3**	**1**	**2**	**2**

Note: NA = not assessed due to 0 values and/or insufficient samples; "*" indicates differences of statistical significance were not evident in analyses of variance between HCr, HCu, and Control stream; **BOLD** values indicate differences of statistical significance were evident in analyses of variance between HCr, HCu, and Control stream.

TABLE 31. Count of Fall Water Chemistry Significant Correlations per Treatment Group to Macroinvertebrate Metrics.

WQ Parameters (units)	Fall Season			
	HCr	HCu	Control	Reference
Ammonia (mg/L)*	8	22	1	4
Chloride (mg/L)	**2**	**16**	**1**	**1**
Dissolved Oxygen (mg/L)	**16**	**0**	**10**	**7**
Flow (cfs)*	13	12	1	6
pH (SU)	**0**	**6**	**16**	**7**
Conductivity (µS/cm)	**10**	**16**	**7**	**1**
Temperature (C)	**15**	**11**	**1**	**2**
Hardness (mg/L)*	8	17	12	NA
Nitrate/Nitrite (mg/L)*	13	14	4	0
Sulfate (mg/L)	**9**	**12**	**20**	NA
Total Nitrogen (mg/L)*	13	17	5	6
Total Phosphorus (mg/L)*	12	11	2	5
TSS (mg/L)*	0	3	7	NA
Turbidity (NTU)*	13	13	5	2

Note: NA = not assessed due to 0 values and/or insufficient samples; "*" indicates differences of statistical significance were not evident in analyses of variance between HCr, HCu, and Control stream; **BOLD** values indicate differences of statistical significance were evident in analyses of variance between HCr, HCu, and Control stream.

TABLE 32. Count of Spring Water Chemistry Significant Correlations per Treatment Group to Macroinvertebrate Metrics.

WQ Parameters (units)	Spring Season			
	HCr	HCu	Control	Reference
Ammonia (mg/L)*	14	26	4	0
Chloride (mg/L)	**5**	**23**	**11**	**15**
Dissolved Oxygen (mg/L)*	2	11	1	5
Flow (cfs)	**6**	**12**	**3**	**9**
pH (SU)	**4**	**6**	**1**	**2**
Conductivity (µS/cm)	**8**	**18**	**0**	**5**
Temperature (C)	**11**	**16**	**1**	**1**

WQ Parameters (units)	**Spring Season**			
	HCr	**HCu**	**Control**	**Reference**
Hardness (mg/L)	**8**	**22**	**5**	NA
Nitrate/Nitrite (mg/L)	**9**	**27**	**10**	**1**
Sulfate (mg/L)	**5**	**23**	**13**	NA
Total Nitrogen (mg/L)	**13**	**27**	**4**	**5**
Total Phosphorus (mg/L)	**3**	**25**	**17**	**5**
TSS (mg/L)*	8	26	NA	NA
Turbidity (NTU)	**6**	**12**	**1**	**4**

Note: NA = not assessed due to 0 values and/or insufficient samples; "*" indicates differences of statistical significance were not evident in analyses of variance between HCr, HCu, and Control stream; **BOLD** values indicate differences of statistical significance were evident in analyses of variance between HCr, HCu, and Control stream.

TABLE 33. Significant Correlations Between Macroinvertebrate Metrics and Hydrological Metric at the USGS Gage on Hinkson Creek (Station 3.5).

Metric	Season	Hydrology	Coefficient
Chironomidae Richness	Spring	90th percentile flow	negative
EPT Richness	Spring	Minimum flow	positive
Ephemeroptera Richness	Spring	Minimum flow	positive
Clinger + Climber Richness	Spring	Minimum flow	positive
% Tanytarsini	Spring	Flow exceedance frequency	negative
% Oligochaeta	Spring	90th percentile flow	positive
% EPT	Spring	Median flow	positive
% Ephemeroptera	Spring	Median flow	positive
% Trichoptera	Spring	90th percentile flow	negative
% Hydropsychidae	Spring	Median flow	negative
% Clingers + Climbers	Spring	Minimum flow	negative
% Clingers + Climbers	Spring	Flow exceedance frequency	negative
% Filterers	Spring	Median flow	negative
EPT/Chironomidae Ratio	Spring	Median flow	positive
% Slow Seasonal Development	Spring	Median flow	positive
% Rare in Drift	Spring	Median flow	positive
% Strong Swimming Ability	Spring	Maximum flow	negative
% Strong Swimming Ability	Spring	Flow variability	negative
% Sprawlers	Spring	Median flow	positive
Missouri Biotic Index	Spring	Flow exceedance frequency	positive
% EP	Spring	Median flow	positive

TABLE 34. Results of Urban Hinkson Creek Site-Specific and General Stormwater NPDES Outfalls Significant Correlations.

Metric	Season	NPDES Type	Coefficient
Chironomidae Richness	Fall	Site-specific	positive
Chironomidae Richness	Fall	General stormwater	positive
%Scrapers	Fall	Site-specific	negative
%Scrapers	Fall	General stormwater	negative
% Intolerant (≤ 4) Taxa	Fall	Site-specific	positive
Scraper/Filterer Ratio	Fall	Site-specific	negative
Scraper/Filterer Ratio	Fall	General stormwater	negative
% Rare in Drift	Fall	Site-specific	negative
% Rare in Drift	Fall	General stormwater	negative
Missouri Biotic Index	Fall	Site-specific	negative
Missouri Biotic Index	Fall	General stormwater	negative
EPT Richness	Spring	General stormwater	negative
Trichoptera Richness	Spring	Site-specific	negative
Trichoptera Richness	Spring	General stormwater	negative
X.Ephemeroptera	Spring	Site-specific	positive
% Trichoptera	Spring	Site-specific	negative
% Trichoptera	Spring	General stormwater	negative
% Hydropsychidae	Spring	Site-specific	negative
% Hydropsychidae	Spring	General stormwater	negative
% Clingers + Climbers	Spring	Site-specific	negative
% Clingers + Climbers	Spring	General stormwater	negative
% Shredders	Spring	Site-specific	negative
% Shredders	Spring	General stormwater	negative
% Dominant Taxon	Spring	General stormwater	negative
% Dominant 2 Taxa	Spring	Site-specific	negative
% Dominant 2 Taxa	Spring	General stormwater	negative
% Dominant 5 Taxa	Spring	Site-specific	negative
% Dominant 5 Taxa	Spring	General stormwater	negative
% Intolerant (≤ 4) Taxa	Spring	General stormwater	positive
% No Swimming Ability	Spring	Site-specific	negative
% No Swimming Ability	Spring	General stormwater	negative
Missouri Biotic Index	Spring	General stormwater	positive
Shannon Diversity Index	Spring	Site-specific	positive
Shannon Diversity Index	Spring	General stormwater	positive
Sediment Tolerance Index	Spring	Site-specific	positive
Sediment Tolerance Index	Spring	General stormwater	positive
% EP	Spring	Site-specific	positive

TABLE 35. Results of Urban Hinkson Creek 2016 Land Use/Land Cover Significant Correlations.

Metric	Season	LU/LC Type	Coefficient
% Corbicula	Fall	Forest	positive
% Other Diptera + Non-Insects	Fall	Developed	negative
% Other Diptera + Non-Insects	Fall	Forest	positive
% Other Diptera + Non-Insects	Fall	Grassland	positive
% Other Diptera + Non-Insects	Fall	Cropland	positive
% No Swimming Ability	Fall	Developed	positive
% No Swimming Ability	Fall	Forest	negative
% No Swimming Ability	Fall	Grassland	negative
% No Swimming Ability	Fall	Cropland	negative
% Strong Swimming Ability	Fall	Forest	negative
Sediment Tolerance Index	Fall	Developed	positive
Sediment Tolerance Index	Fall	Forest	negative
Sediment Tolerance Index	Fall	Grassland	negative
Sediment Tolerance Index	Fall	Cropland	negative
% Oligochaeta	Spring	Developed	positive
% Oligochaeta	Spring	Forest	negative
% Oligochaeta	Spring	Grassland	negative
% Oligochaeta	Spring	Cropland	negative
% Strong Swimming Ability	Spring	Developed	positive
% Strong Swimming Ability	Spring	Forest	negative
% Strong Swimming Ability	Spring	Grassland	negative
% Strong Swimming Ability	Spring	Cropland	negative

Correlation analyses of each macroinvertebrate metrics and environmental variables are discussed in detail below.

Total Taxa richness

Total Taxa richness was negatively related to temperature at HCr and HCu and was positively associated with dissolved oxygen at HCr and Control stream in the fall. At HCu, this metric was negatively related to specific conductance (both seasons), chloride (spring only) and sulfate (fall only).

No significant correlations were found between Total Taxa richness and any of the hydrology variables tested, or with site-specific NPDES outfall or general stormwater outfalls. Total Taxa richness was not significantly correlated with any of the land use variables tested.

Diptera richness

Diptera richness was negatively correlated with chloride in the fall at HCr and HCu. It was also negatively associated with turbidity, TSS, nitrate plus nitrite, TN and TP at HCu in the spring. This metric was negatively related to sulfate concentration at HCu and Control stream in the fall. It was also negatively related to temperature at HCu in the fall.

No significant correlations were found between Diptera richness and any of the hydrology variables tested, or with site-specific NPDES outfall or general stormwater outfalls. Diptera richness was not significantly correlated with any of the land use variables tested.

Chironomidae richness

In spring samples from HCu, Chironomidae richness was negatively correlated with all nutrient variables, flow, turbidity, and TSS. This metric was negatively related to sulfate concentration at HCu and Control stream in the fall. It was also negatively related to temperature at HCu in the fall.

Chironomidae richness in the spring decreased with increasing 90th percentile flow volume during the 5-week period prior to sampling increased, but it was not associated to any of the other hydrology variables tested. In fall samples from HCu, this metric was positively related to site-specific NPDES outfall and general stormwater outfalls. Chironomidae richness was not significantly correlated with any of the land use variables tested.

EPT richness

EPT richness was negatively correlated with chloride levels in the spring at HCu and Reference streams. EPT richness was positively related to dissolved oxygen and flow, but negatively related to temperature, at HCr in the fall.

In the spring, EPT richness was positively related to minimum flow and negatively related to the number of general stormwater outfalls. This metric was not significantly correlated with any of the land use variables tested.

Ephemeroptera richness

Ephemeroptera richness was negatively correlated with chloride and TN at Reference streams in the spring. Ephemeroptera richness was also negatively associated with nitrate plus nitrite, TN, and TP at HCr in the spring. It was positively related to temperature at HCu and Control stream in the spring.

In spring samples, this metric increased when the minimum flow during the 5-week period prior to sampling increased. This metric was not significantly correlated with site-specific NPDES outfall or general stormwater outfalls, or with any of the land use variables tested.

Plecoptera richness

This metric was negatively correlated to specific conductance and chloride levels at HCu in the spring. It was negatively related to temperature and positively related to dissolved oxygen at HCr in the fall.

No significant correlations were found between Plecoptera richness in HCu and any of the hydrology variables tested, or with site-specific NPDES outfall or general stormwater outfalls.

Trichoptera richness

This metric was positively correlated to pH at HCu in both spring and fall samples. With other water quality variables, it was either not correlated or correlated inconsistently among treatment groups and/or between seasons.

No significant correlations were found between Trichoptera richness in HCu and any of the hydrology variables tested; however, in spring HCu samples, this metric was positively related to site-specific NPDES outfalls and general stormwater outfalls. It was not significantly correlated with any of the land use variables tested.

Clinger/Climber richness

This metric was positively correlated with dissolved oxygen at HCr, Control stream, and Reference streams in the fall. At HCr, it was positively correlated to flow in both seasons. Clinger/Climber richness was negatively related to temperature at HCr, and TN at Reference streams, in the fall.

In spring samples, this metric increased when minimum flow in the 5-week period prior to sampling increased. No significant correlations were evident between this metric and site-specific NPDES outfalls, general stormwater outfalls, or with any of the land use variables tested.

Percent Diptera

Percent Diptera is expected to increase with disturbances such as declining water quality conditions. Associations with water quality variables that indicated this trend included positive correlations with specific conductance (HCr in the fall), chloride (HCu in the spring), sulfate (HCu in the spring), turbidity (Reference streams in the fall), and ammonia (HCr and HCu in the spring).

No significant correlations were found between percent Diptera and any of the hydrology variables, site-specific NPDES outfalls, general stormwater outfalls, or with any of the land use variables tested.

Percent Chironomidae

Similarly, correlations with water quality variables were nearly identical to those noted for percent Diptera, with the exception of increasing percent Chironomidae with increasing specific conductance at HCu in both seasons.

No significant correlations were found between percent Chironomidae in HCu and any of the hydrology variables, site-specific NPDES outfalls, general stormwater outfalls, or with any of the land use variables tested.

Percent Tanytarsini

It was negatively correlated to all four nutrient variables at HCu in the spring. Percent Tanytarsini was negatively related to ammonia and TP at HCr in the spring, and negatively correlated with TN at Control stream in the spring. However, it was positively correlated with SC at HCu in the spring and Control in the fall, with chloride at HCu and Control in the spring and HCr in the fall, and with sulfate at HCu in the spring and HCr in the fall. These relationships were more indicative of a metric indicating poor water quality.

In spring samples, percent Tanytarsini decreased when disturbance frequency during the 5-week period prior to sampling increased. No significant correlations were evident between this metric and site-specific NPDES outfalls, general stormwater outfalls, or with any of the land use variables tested.

Percent Oligochaeta

This metric was significantly and consistently correlated with several water quality variables. In both spring and fall at HCu, percent Oligochaeta increased with increasing flow, turbidity, nitrate plus nitrite, TN, and TP. It also increased with increasing ammonia in the spring only. At HCr, it increased with increasing flow and nitrate plus nitrite in both seasons, and with TSS, turbidity, and TN in the spring only. At Control stream, it was positively related to nitrate plus nitrite (both seasons) and TN in the spring. Contrary to ecological expectations, percent Oligochaeta at HCu was inversely related to specific conductance and chloride (spring only) and to sulfate (both seasons). At HCr, it was also inversely related to specific conductance and chloride in the spring.

In spring samples, this metric increased as the 90th percentile flow volume increased in the 5-week period prior to sampling. No significant correlations were noted between this metric and site-specific NPDES outfalls or general stormwater outfalls. In spring samples from HCu, this metric was positively related to the percentage of developed land cover, and negatively related to the percentages of forested, grassland, and cropland cover (NLCD 2016).

Percent Corbicula

At HCu, this metric was positively correlated to temperature and negatively related to turbidity in both spring and fall, and positively correlated to specific conductance, chloride, and sulfate in the fall only. It was negatively correlated to ammonia, nitrate plus nitrite, and TN at HCu in the fall. At HCr, it was positively related to specific conductance and inversely related to TSS, nitrate plus nitrite, and TN in the spring.

No significant correlations were found between this metric and any of the hydrology variables tested, site-specific NPDES outfalls, or general stormwater outfalls. Percent Corbicula in fall samples at HCu was positively correlated with the amount of forested land cover (NLCD 2016).

Percent Other Diptera and Non-Insects

At HCr and HCu, percent other Diptera and non-insects was inversely correlated with specific conductance in both spring and fall. It was positively related to turbidity in Reference stream samples. At HCr and HCu, this metric was positively associated with TN and TP in both seasons. It was also positively correlated with ammonia at HCu in the spring, and with nitrate plus nitrite at Control stream in the spring.

No significant correlations were found between percent other Diptera and non-insects and any of the hydrology variables tested, site-specific NPDES outfalls, or general stormwater outfalls. In spring samples from HCu, this metric was negatively related to the percentage of developed land cover, and positively related to the percentages of forested, grassland, and cropland cover (NLCD 2016).

Percent EPT

Percent EPT trends were not consistently related to water quality variables among sites and between seasons. At HCu, it was negatively associated with specific conductance, chloride, and sulfate in the spring whereas in the fall it was positively correlated with these variables. In addition, this metric was positively correlated with ammonia, nitrate plus nitrite, and TN in the spring but negatively correlated with them in the fall at HCu. In Reference streams, percent EPT was inversely related to specific conductance and chloride in the spring, and TP in both seasons. At HCr and HCu, it was negatively correlated with turbidity in the fall.

In spring samples, this metric increased when the median flow during the 5-week period prior to sampling increase. No significant correlations were evident between this metric and site-specific NPDES outfalls, general stormwater outfalls, or with any of the land use variables tested.

Percent Ephemeroptera

At HCu, it was negatively correlated with sulfate in the spring, but positively correlated in the fall. The opposite trend was noted at this location for ammonia. However, chloride was negatively associated with percent Ephemeroptera at HCu, Control stream, and Reference streams in the spring. In both seasons, this metric was inversely related to TP at the Reference streams.

In spring samples, this metric increased when the median flow during the 5-week period prior to sampling was higher. It was also positively correlated to the number of site-specific NPDES outfalls in the watershed for spring samples from HCu, contrary to expectation. No significant correlations were evident between this metric and any of the land use variables tested.

Percent Plecoptera

At HCr and HCu, this metric was negatively correlated with specific conductance in the spring. It was also negatively related to chloride and sulfate at HCu in the spring. Stoneflies were collected too infrequently in the fall season to perform correlation tests.

No significant correlations were noted between this metric and any of the hydrology variables tested, site-specific NPDES outfalls, general stormwater outfalls, or with any of the land use variables tested.

Percent Ephemeroptera + Plecoptera

At HCu in the spring, this metric was positively correlated with temperature, TSS, ammonia, nitrate plus nitrite, and TN, and negatively correlated with specific conductance, chloride, sulfate, and dissolved oxygen. In the fall, percent EP was positively related to sulfate and negatively associated with ammonia. At HCr, percent EP was negatively correlated with ammonia in the fall. At the Control stream, this metric was positively correlated with TP in the spring and pH in the fall, but negatively correlated with chloride in the spring. At the Reference streams, percent EP was positively associated with dissolved oxygen and pH in the fall, and with flow in the spring. It was negatively correlated with specific conductance and chloride in the spring, with TN in the fall, and with TP in both seasons at the Reference streams.

In spring samples, this metric increased when the median flow during the 5-week period prior to sampling was higher. It was also positively correlated to the number of site-specific NPDES outfalls in the watershed for spring samples from HCu. No significant correlations were evident between this metric and any of the land use variables tested.

Percent Trichoptera

In both seasons at HCu, this metric was negatively associated with flow, turbidity, nitrate plus nitrite, TN, and TP. It was positively related to temperature, and negatively related to TSS and ammonia in the spring at HCu. At HCr, it was negatively correlated with TP in the fall. At Control stream, it was positively related to dissolved oxygen but negatively related to sulfate, in the spring, and positively related to pH in the fall.

In spring samples, percent Trichoptera decreased with increased 90th percentile streamflow volume in the 5-week period prior to sampling. It also decreased as the number site-specific NPDES and general stormwater outfalls increased in the watershed. No significant correlations were evident between this metric and any of the land use variables tested.

Percent Hydropsychidae

At HCr and HCu in the spring, this metric was positively correlated with dissolved oxygen and negatively correlated with ammonia. It was also negatively correlated with TN at HCu in the fall.

In spring samples, percent Hydropsychidae decreased as the median flow during the 5-week period prior to sampling increased. It also decreased as the number of site-specific NPDES and general stormwater outfalls increased in the watershed. No significant correlations were evident between this metric and any of the land use variables tested.

Percent Clingers + Climbers

At HCu, percent clingers plus climbers was negatively correlated with TN and TP in the fall. In the spring, it was also negatively correlated with TN at Control and Reference streams, and with chloride at Reference streams.

In spring samples, percent clingers plus climbers decreased as minimum flow and the frequency of high flow events increased during the 5-week period prior to sampling. It also decreased as the number site-specific NPDES and general stormwater outfalls increased in the watershed. No significant correlations were evident between this metric and any of the land use variables tested.

Percent Filterers
At HCu, this metric was positively correlated with specific conductance in the fall, chloride in both seasons, and sulfate in the spring. At HCr, it was positively correlated to specific conductance and sulfate in the fall. At HCu, percent Filterers was negatively associated with flow, turbidity, nitrate plus nitrite, TN, and TP in both spring and fall. Similarly, it was negatively related to flow (spring only), TP (fall only), and with turbidity, nitrate plus nitrite, and TN in both seasons at HCr.

In spring samples from HCu, this metric decreased with decreased median flow during the 5-week period prior to sampling. No significant correlations were found between this metric and site-specific NPDES outfalls, general stormwater outfalls, or with any of the land use variables tested.

Percent Predators
This metric was not consistently correlated to the water quality variables tested among treatment group or between seasons. It was negatively related to specific conductance (Control stream in the fall), temperature (Reference streams in the fall), flow and TSS (HCu in the spring), and TP (HCu in the spring and Reference streams in the fall). It was positively correlated to dissolved oxygen at HCu in the spring and Control stream in the fall, and with pH at HCu in the spring.

No significant correlations were found between this metric and any of the hydrology variables tested, site-specific NPDES outfalls, general stormwater outfalls, or with any of the land use variables tested.

Percent Scrapers
At HCr, percent Scrapers was negatively correlated with specific conductance, chloride, and temperature, and positively correlated with dissolved oxygen in the fall. At HCu, it was negatively correlated with sulfate in the fall. At Control stream, it was positively related to temperature, but negatively related to specific conductance, in the fall. This metric was positively related to turbidity, nitrate plus nitrite, and TN at HCr in both seasons, and to TP in the fall only. At HCu, it was positively correlated to ammonia and TP in the spring.

No significant correlations were found between percent Scrapers and any of the hydrology variables tested. At HCu in the fall; however, it decreased as the number of site-specific NPDES outfalls and general stormwater outfalls increased in the watershed. No significant correlations were evident between this metric and any of the land use variables tested.

Percent Shredders

Relatively few correlations of water quality variables and this metric were evident. At HCu, it was positively related to specific conductance and pH in the fall, and negatively associated with TSS and nitrate plus nitrite in the spring. Percent Shredders was negatively correlated with temperature and ammonia in the spring at HCr, and with turbidity at Control stream in the fall.

No significant correlations were found between percent Shredders and any of the hydrology variables tested. At HCu in the spring, it decreased as the number of site-specific NPDES outfalls and general stormwater outfalls increased in the watershed. No significant correlations were evident between this metric and any of the land use variables tested.

Percent Dominant Taxon

At HCu in the spring, percent dominant taxon was negatively correlated with temperature and positively correlated with flow, TSS, turbidity, nitrate plus nitrite, TN and TP. At the Control stream it was positively associated with pH in the fall and negatively related to TP in the spring.

No significant correlations were found between this metric and any of the hydrology variables tested. At HCu in the spring, it decreased as the number of general stormwater outfalls increased in the watershed. No significant correlations were evident between this metric and any of the land use variables tested.

Percent Dominant 2 Taxa

At HCu in the spring, percent dominant 2 taxa was negatively correlated with temperature and positively correlated with flow, TSS, turbidity, nitrate plus nitrite, TN and TP. It was negatively correlated with chloride at HCu in the fall. At HCr, it was positively related to pH in the spring and negatively related to temperature in the fall. At the Control stream, it was negatively associated with TP in the spring.

No significant correlations were found between this metric and any of the hydrology variables tested. At HCu in the spring, it decreased as the number of general stormwater outfalls and site-specific NPDES outfalls increased in the watershed. No significant correlations were evident between this metric and any of the land use variables tested.

Percent Dominant 5 Taxa

At HCu, percent dominant 5 taxa was negatively correlated with chloride in both seasons, negatively related to temperature in the spring, and to specific conductance and ammonia in the fall. In spring at HCu, it was positively correlated with TSS, turbidity, nitrate plus nitrite, TN, and TP. It was also positively correlated with TP in the fall at HCu. At HCr, percent dominant 5 taxa was positively associated with pH in the spring, and with flow, turbidity, nitrate plus nitrite, TN and TP in the fall. It was negatively correlated with specific conductance and sulfate at this site in the fall. At the Control stream, this metric was positively correlated with pH in the spring and negatively correlated with TP in the fall.

No significant correlations were found between this metric and any of the hydrology variables tested. At HCu in the spring, this metric decreased as the number of general stormwater outfalls and site-specific NPDES outfalls increased in the watershed. No significant correlations were evident between this metric and any of the land use variables tested.

Percent Intolerant (≤ 4) Taxa

At HCu, percent Intolerant Taxa was negatively correlated with TP in both seasons, and negatively correlated with flow, turbidity, nitrate plus nitrite, and TN in the fall. In the spring, it was negatively correlated with ammonia at HCu treatment group. It was positively correlated with temperature and specific conductance in the fall at HCu. At the Reference streams, this metric was negatively related to chloride and turbidity in the spring.

No significant correlations were found between this metric and any of the hydrology variables tested. Unexpectedly, it was positively correlated with the numbers of general stormwater outfalls (spring only) and site-specific NPDES outfalls (fall only) at HCu. No significant correlations were evident between this metric and any of the land use variables tested.

EPT/Chironomidae Ratio

Correlations with this metric and water quality variables rarely exhibited consistent patterns between seasons or among treatment groups. The exceptions were: (a) it was negatively related to chloride at HCu, Control stream, and Reference streams in the spring; (b) it was positively associated with temperature at HCu in both seasons; and (c) it was negatively correlated with TP at Reference streams in both seasons.

In spring samples, EPT/Chironomidae_ratio increased with median flow during the 5-week period prior to sampling. No significant correlations were found between this metric and general stormwater outfalls, site-specific NPDES outfalls, or with any of the land use variables tested.

Scrapers/Filterers Ratio

Scrapers/filterers ratio was negatively correlated with specific conductance at HCr, HCu, and the Control stream in the fall, with chloride at HCr in the spring and at HCu in both seasons, and with sulfate at HCr in the fall. It was positively associated with dissolved oxygen and pH at Reference streams in the spring. At HCr, it was positively related to flow and TSS in the spring, and with turbidity in both seasons. It was positively correlated with all nutrient-related variables at one or more of the treatment groups in both seasons.

No significant correlations were found between the scrapers/filterers ratio and any of the hydrology variables tested. At HCu in the fall this metric decreased as the number of general stormwater and site-specific NPDES outfalls increased. No significant correlations were evident between this metric and any of the land use variables tested.

Percent Fast Seasonal Development
Correlations with water quality variables demonstrated the mixed nature of this metric, which is comprised of all the chironomid taxa, but also several common or abundant mayflies in the treatment groups. At HCu, percent fast seasonal development was positively correlated with specific conductance, chloride, and sulfate, but negatively correlated with TSS, ammonia, nitrate plus nitrite, TN, and TP, in the spring. At HCr, it was negatively associated with ammonia, nitrate plus nitrite, and TN in the spring. At the Control stream, it was positively related to specific conductance (fall) and sulfate (spring), and negatively related to nitrate plus nitrite (spring) and TP (fall). At Reference streams, it was negatively correlated with chloride and TN in the spring.

No significant correlations were found between percent fast seasonal development and any of the hydrology variables, site-specific NPDES outfalls, general stormwater outfalls, or with any of the land use variables tested.

Percent Slow Seasonal Development
At HCu in the spring, percent slow seasonal development was negatively correlated with specific conductance, chloride, and dissolved oxygen, but positively correlated with temperature. In the fall, it was negatively correlated with specific conductance, flow, and turbidity, but positively correlated with chloride, sulfate, and temperature at HCu. At HCu, it was also positively related to ammonia, nitrate plus nitrite, and TN in the spring, but negatively associated with these variables in the fall. At HCr in the fall, percent slow seasonal development was positively correlated to temperature, but negatively correlated to specific conductance, dissolved oxygen, turbidity, ammonia, TN, and TP. At the Control stream, this metric was positively related to TP in the spring, and negatively related to pH in the fall. At the Reference streams, it was negatively correlated with chloride in the spring.

In spring samples, this metric increased with median flow during the 5-week period prior to sampling. No significant correlations were found between this metric and site-specific NPDES outfalls, general stormwater outfalls, or with any of the land use variables tested.

Percent Ability to Exit as Adults
All correlations with water quality and other environmental variables were as described for percent Chironomidae.

Percent Rare Drift
At HCu in the spring, percent rare drift was negatively correlated with specific conductance, chloride, and dissolved oxygen, and positively correlated with temperature, TSS, ammonia, nitrate plus nitrite, TN, and TP. In the fall, it was positively correlated to sulfate and negatively related to ammonia at HCu. At HCr, it was positively correlated with temperature and ammonia in the spring and negatively correlated with dissolved oxygen and ammonia in the fall. At the Control stream, percent rare drift was positively associated with nitrate plus nitrite, TN, and TP in the spring.

In spring samples, this metric increased with median flow during the 5-week period prior to sampling. At HCu in the fall, it decreased as the number of general stormwater and site-specific NPDES outfalls increased. No significant correlations were evident between this metric and any of the land use variables tested.

Percent Abundant in Drift

Nearly all correlations with water quality and other environmental variables were as described for percent Chironomidae. Two exceptions were noted: a positive correlation with percent abundant in drift and chloride at HCu in the fall, and a negative correlation with this metric and TP at Control stream in the fall.

Percent No Swimming Ability

At HCu in the spring, percent no swimming ability was positively correlated with specific conductance, chloride, sulfate, and negatively correlated with flow, TSS, ammonia, nitrate plus nitrite, TN, and TP. At HCr, it was positively associated with dissolved oxygen and ammonia in the fall, and negatively related to ammonia and TN in the spring. At the Reference streams, percent no swimming ability was positively correlated with specific conductance (spring), chloride (spring), and turbidity (fall), and negatively correlated with dissolved oxygen and pH in the fall. At Control stream, it was positively correlated with specific conductance in the fall and chloride in the spring.

No significant correlations were found between this metric and any of the hydrology variables tested. In the spring, it was negatively correlated with both general stormwater and site-specific NPDES outfalls. In fall samples from HCu, percent no swimming ability was positively related to the percentage of developed land and negatively related to the percentages of forested, grassland, and cropland (NLCD 2016).

Percent Strong Swimming Ability

This metric was not consistently correlated to the water quality variables tested among treatment groups or between seasons. At HCu, percent strong swimming ability was positively correlated with chloride in the fall. At HCr, it was positively related to specific conductance and sulfate, but negatively associated with TP, in the fall. It was negatively correlated to dissolved oxygen in the spring at HCr. At Control stream, this metric was positively related to pH (fall) and ammonia (spring), but negatively correlated with dissolved oxygen (fall). At Reference streams, percent strong swimming ability was negatively associated with chloride and turbidity in the spring.

In spring samples, this metric decreased as the maximum flow and flow variability increased during the 5-week period prior to sampling. No significant correlations were noted between this metric at HCu and the numbers of site-specific NPDES or general stormwater outfalls. In spring samples from HCu, percent strong swimming ability was positively related to the percentage of developed land, and negatively related to the percentages of forested, grassland, and cropland

(NLCD 2016). In fall samples from HCu, it was negatively correlated with the percentage of forested land (NLCD 2016).

Percent Streamlined Body Shape

This metric was rarely correlated with water quality or other environmental variables. At HCu in the spring, percent streamlined body shape was positively associated with pH, but negatively related to chloride. At Control stream, it was positively correlated with TP in the spring and pH in the fall, and negatively correlated with chloride in the spring. It was negatively correlated with turbidity at the Reference streams in the spring.

No significant correlations were found between percent streamlined body shape and any of the hydrology variables tested, site-specific NPDES outfalls, general stormwater outfalls, or with any of the land use variables tested.

Percent Sprawlers

At HCu in the spring, percent Sprawlers was positively correlated with temperature, ammonia, nitrate plus nitrite, and TN, and negatively correlated with chloride. In the fall, it was negatively correlated with ammonia at HCu. At HCr, this metric was positively correlated with temperature and ammonia in the spring, and negatively correlated with dissolved oxygen and ammonia in the fall. It was positively associated with turbidity and TP, but negatively related to sulfate, at Control stream in the spring. It was positively correlated with flow at Reference streams in the spring.

In spring samples from Hinkson Creek Site 3.5, this metric increased as the median flow increased during the 5-week period prior to sampling. No significant correlations were found between percent Sprawlers and site-specific NPDES outfalls, general stormwater outfalls, or with any of the land use variables tested.

Missouri Biotic Index

At HCu, Missouri Biotic Index was positively correlated with TSS, turbidity, and ammonia in the spring, and with nitrate plus nitrite, TN, and TP in both seasons. At HCr, it was negatively correlated with temperature and positively correlated with flow in the spring. At Control stream, it was positively associated with dissolved oxygen and negatively related to pH in the fall.

In spring samples from Hinkson Creek Site 3.5, Missouri Biotic Index increased as the frequency of high flow events increased during the 5-week period prior to sampling. At HCu, Missouri Biotic Index increased as the number of general stormwater outfalls increased. In the fall, Missouri Biotic Index was negatively correlated with both general stormwater and site-specific NPDES outfalls at HCu. No significant correlations were evident between this metric and any of the land use variables tested.

Macroinvertebrate Biotic Index

Macroinvertebrate Biotic Index patterns were rarely correlated with water quality or other environmental variables. At HCu in the spring, this metric was positively correlated with chloride and negatively correlated with pH. It was also negatively correlated with pH at Control stream in the fall.

No significant correlations were found between this metric and any of the hydrology variables tested, site-specific NPDES outfalls, general stormwater outfalls, or with any of the land use variables tested.

Shannon Diversity Index

At HCu in the spring, Shannon Diversity Index was negatively correlated with TSS, turbidity, nitrate plus nitrite, TN, and TP. It was negatively related to sulfate and pH at Control stream in the fall.

No significant correlations were found between this metric and any of the hydrology variables tested. Contrary to expectations, it was positively correlated with the numbers of general stormwater and site-specific NPDES outfalls in the spring at HCu. No significant correlations were evident between this metric and any of the land use variables tested.

Deposited Sediment Tolerance Index

At HCu, Deposited Sediment Tolerance Index was positively correlated with turbidity in both seasons. It was also positively associated with ammonia, nitrate plus nitrite, TN, and TP, and negatively related to temperature, in the fall at this treatment group. At HCr, it was positively correlated with flow and nitrate plus nitrite, and negatively related to temperature, in the fall. At Control stream, it was positively correlated with nitrate plus nitrite in both seasons and with TN in the fall. It was positively related to chloride at Reference streams in the spring.

No significant correlations were found between this and any of the hydrology variables tested. Deposited Sediment Tolerance Index was positively correlated with the numbers of general stormwater and site-specific NPDES outfalls in the spring at HCu. In fall samples from HCu, Deposited Sediment Tolerance Index was positively related to the percentage of developed land, and negatively related to the percentages of forested, grassland, and cropland (NLCD 2016).

4.2.5 Ordination Analyses

Aquatic ecological investigations are interested in comparing macroinvertebrate community descriptors such as diversity but also the composition of one macroinvertebrate community to the next, such as a Reference (best condition) stream. Ordination analyses (NMDS) was utilized to detect macroinvertebrate community patterns between season, treatment group and environmental variables.

4.2.5.1 Season

NMDS was utilized to evaluate and interpret macroinvertebrate community composition and potential seasonal differences among treatment groups. The position of a sample is arbitrary; however, the distance between samples is meaningful. NMDS clearly indicates a distinct macroinvertebrate community composition difference between the fall and spring season in each treatment group (**Figure** 7).

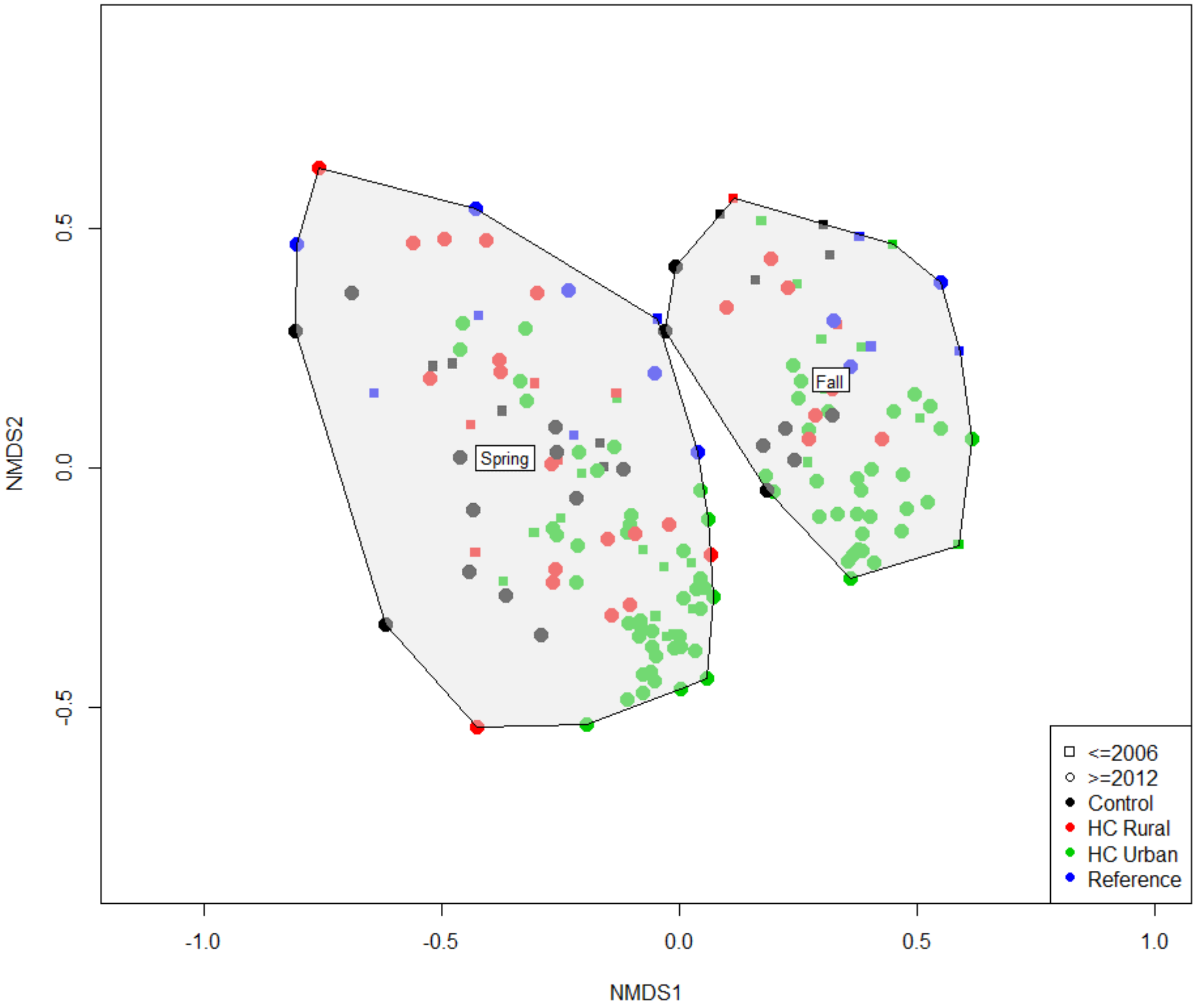

FIGURE 7. NMDS Seasonal Macroinvertebrate Community Composition Results. Abbreviations: "HC" = Hinkson Creek.

4.2.5.2 Treatment Group

NMDS was utilized to illuminate community composition differences in both the fall and spring season (**Figure 8** and **Figure 9**). During the fall season HCu community composition overlaps all other treatment groups. However, during the spring season HCu community composition only overlaps with HCr and Control Stream, and not the Reference Streams. In addition, HCu community composition during the spring season shifts further away from the Reference streams, HCr, and Control stream than the fall season.

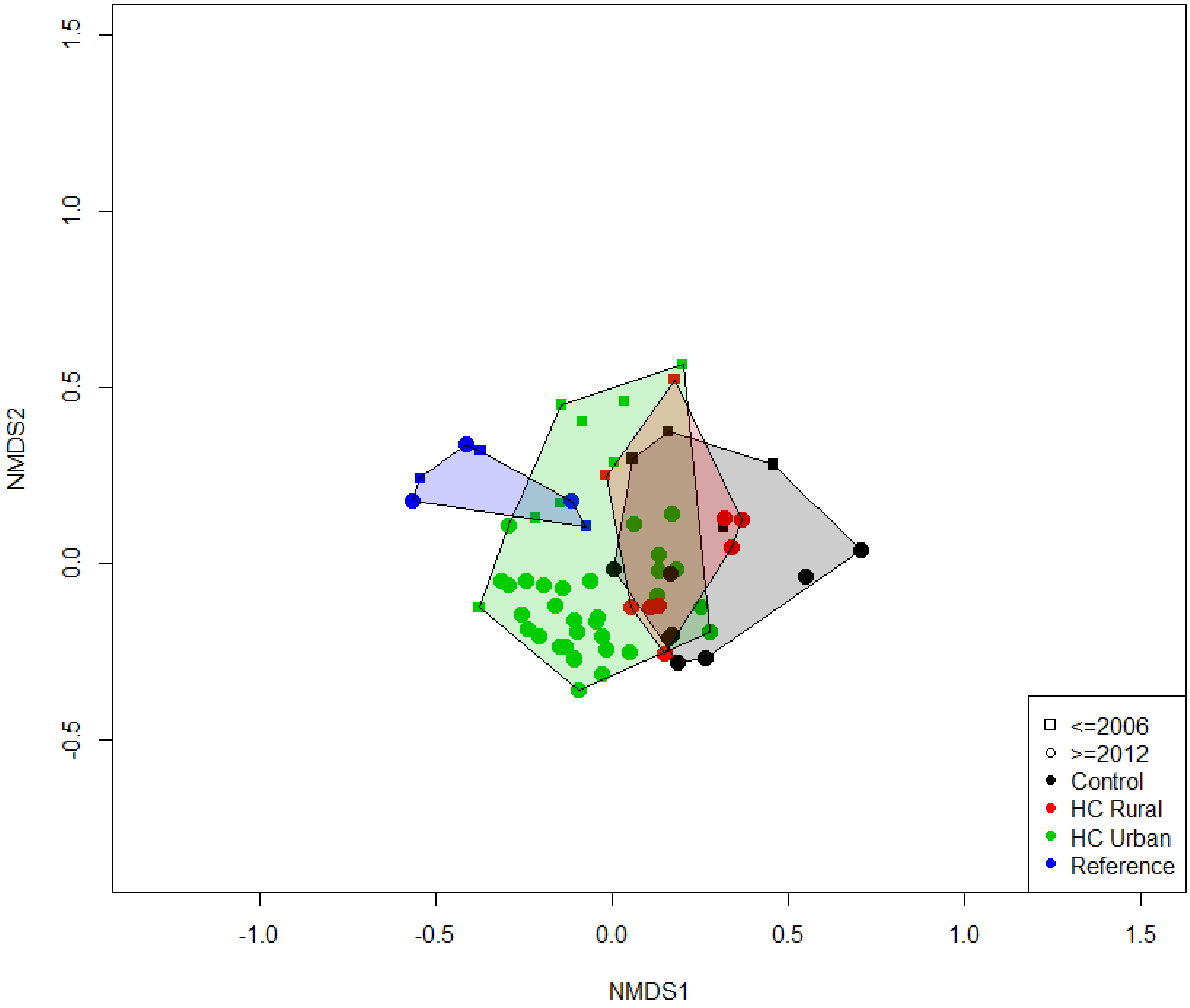

FIGURE 8. Fall Macroinvertebrate NMDS Community Composition Results. Abbreviations: “HC” = Hinkson Creek.

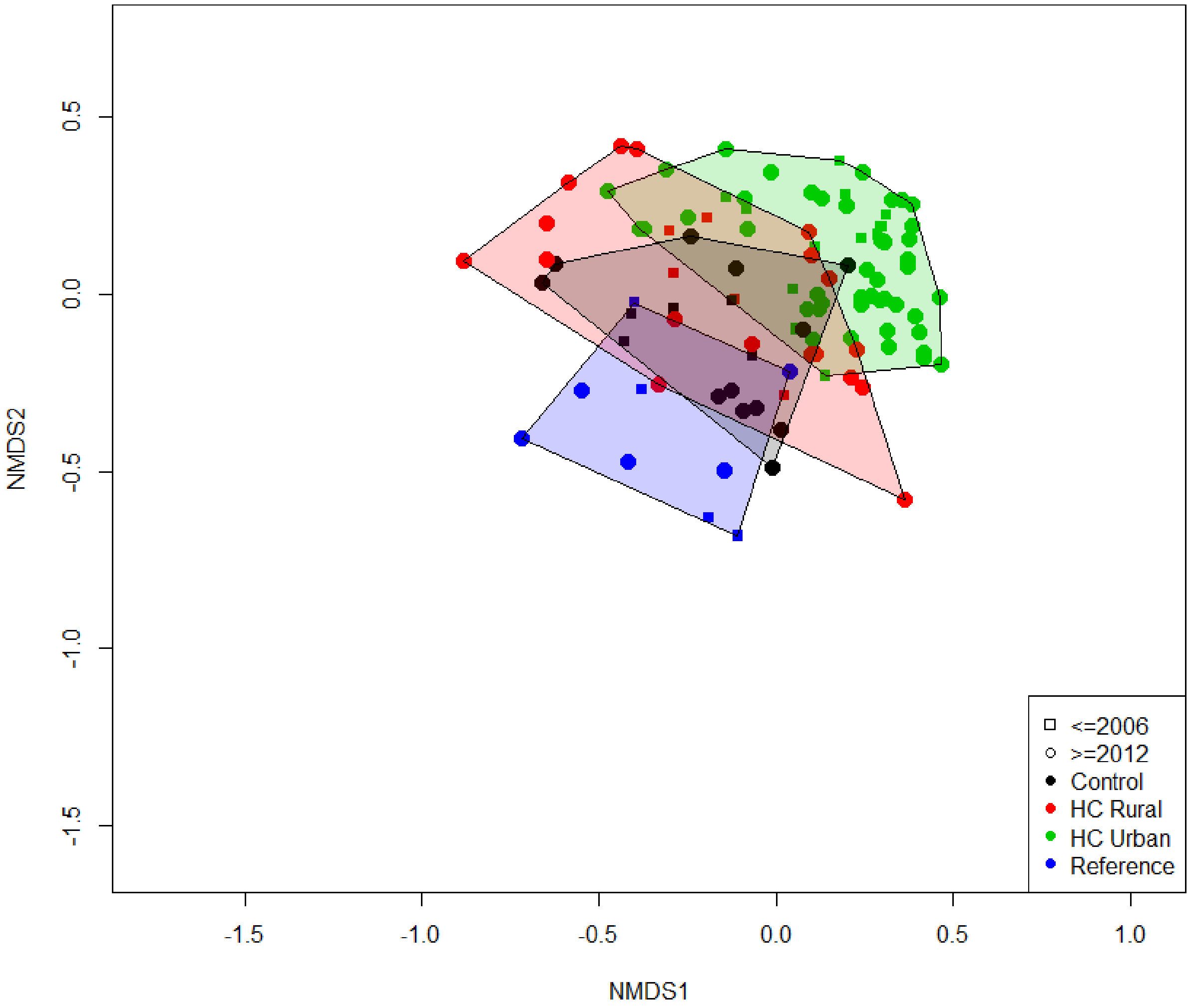

FIGURE 9. Spring Macroinvertebrate NMDS Community Composition Results. Abbreviations: "HC" = Hinkson Creek.

4.2.5.3 Water Chemistry

NMDS was utilized to illuminate community composition and underlying water chemistry differences of the macroinvertebrate matrix in each season (**Figure 10** and **Figure 11**). Association of the macroinvertebrate matrix to chemical variables is represented by the direction and length of the vector (arrows). The stronger the association increases the vector length and the direction of the vector indicates an association with a community matrix. Paired chemical data were utilized for this analysis as theses data were most concurrent to macroinvertebrate samples. Sulfate water chemistry data were omitted from this analysis as Paired sulfate were not collected during macroinvertebrate sampling at the Reference streams. TSS data were also omitted from this analysis as concentrations were static and primarily at or below laboratory quantification limits.

However, TSS data are represented in turbidity data. Nitrate plus nitrite data were omitted from the presentation due to consistent overlap with total nitrogen results. Macroinvertebrate community composition was also compared from samples collect prior to 2007 and samples collected after 2011.

During the fall season, increased conductivity, chloride and temperature are associated with HCu. Increased turbidity, total nitrogen, total phosphorus is associated with HCr, Control and Reference Streams. Flow, ammonia, dissolved oxygen and pH are less associated and therefore exhibit shorter vectors. Macroinvertebrate community composition during the fall season is less similar from the early (2001 to 2007) sampling period compared to the more recent (2012-2017) sampling periods for the treatment groups.

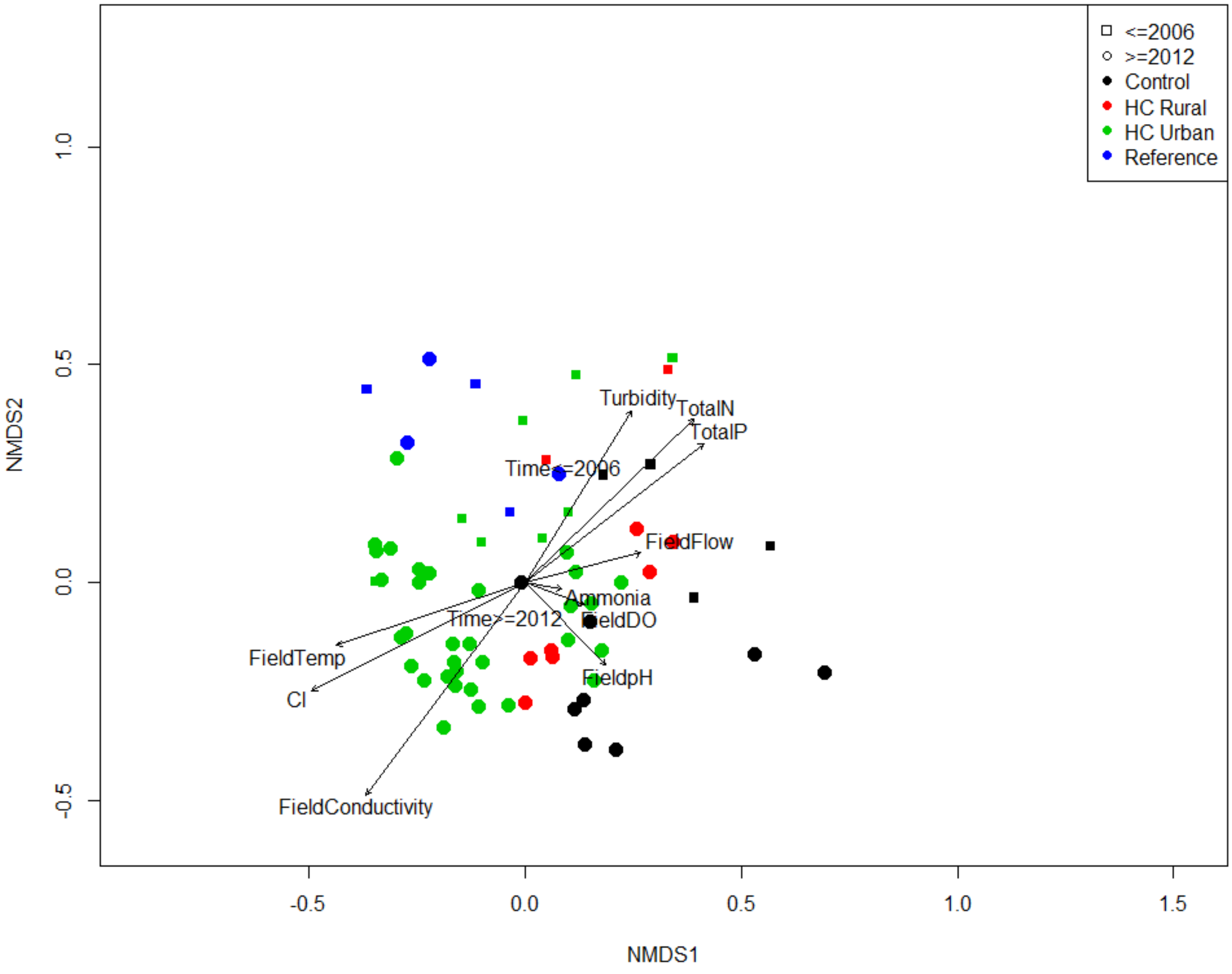

FIGURE 10. Fall Macroinvertebrate NMDS Community Composition and Water Chemistry Association. Abbreviations: “Cl” = chloride; “FieldDO” = dissolved oxygen; “FieldTemp” = temperature; “TotalP” = total phosphorus; “TotalN” = total nitrogen.

Conductance and chloride are very similar and strongly associated with HCu during the spring season. Increased turbidity is associated with HCu and HCr. Increased temperature is associated with the Control and Reference streams. Increased total nitrogen is associated with HCr, Control and Reference Streams. Flow, dissolved oxygen, total phosphorus, ammonia and pH vectors exhibit weak association. Macroinvertebrate community composition during the spring season is similar during early (2001 to 2007) and recent (2012-2017) sampling periods for the treatment groups.

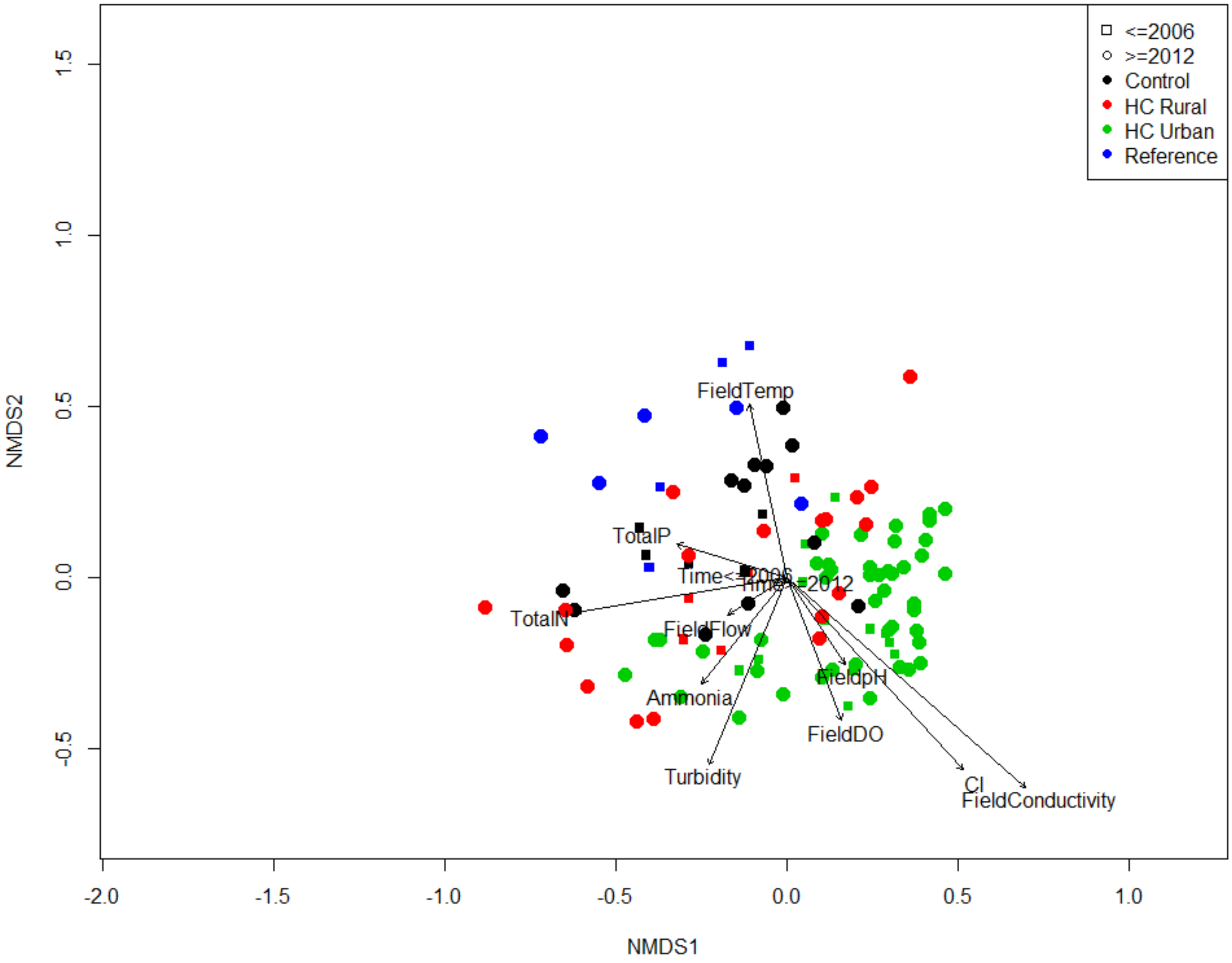

FIGURE 11. Spring Macroinvertebrate NMDS Community Composition and Water Chemistry Association. Abbreviations: “Cl” = chloride; “FieldDO” = dissolved oxygen; “FieldTemp” = temperature; “TotalP” = total phosphorus; “TotalN” = total nitrogen.

Increased chloride and conductivity were associated with HCu in both the fall and spring season, however, the association (strength and direction) of these two constituents were more similar during the spring season than the fall season. Nutrients were consistently associated with HCr, Control or Reference streams treatment groups in both seasons.

4.2.6 Indicator Taxa and Metric Analyses

Determining the occurrence or abundance of a small set of macroinvertebrate indicator species as an alternative to sampling the entire macroinvertebrate community may be useful in long-term environmental monitoring. Indicator taxa and metrics analyses were performed to identify taxa indicative of good water quality or an improved macroinvertebrate community, and taxa that are unique to a treatment group based on occurrence and abundance, which may also represent differences in habitat, community type or environmental disturbance.

4.2.6.1 Indicators of Good Quality

A goal of the study was to identify Hinkson Creek indicator taxa to be sought in future rapid bioassessment efforts. Based on the attributes of frequency (common), size (visible), sensitivity (low <3 tolerance), and life history (more than one trait) the following taxa were selected:

- *Perlesta* is a perlid stonefly. Its tolerance value for the Missouri Biotic Index calculation is 0. It was collected at all Hinkson Creek sites and was common in HCr but rare in HCu. It is a member of the clinger/climber, slow seasonal development, rare in the drift, and streamlined body shape trait groups.
- *Helicopsyche* is a caddisfly with a 0.0 tolerance value. It was present at all but the two most downstream Hinkson Creek sites and was relatively common in the middle and upper Hinkson Creek stations. It is a member of the clinger/climber, slow seasonal development, and rare in the drift trait groups.
- *Chimarra* is a philopotamid caddisfly with a tolerance value of 2.8. It was collected at every Hinkson Creek station and was relatively common in the middle and upper Hinkson Creek sites. It is a member of the clinger/climber, slow seasonal development, and rare in the drift trait groups.

4.2.6.2 Indicator Taxa Statistical Analyses

Indicator taxa analyses were performed for each season to identify taxa unique to a treatment group and subset thereof. Results of fall and spring season indicator taxa analyses are provided in **Appendix F**.

In the fall, taxa associated with the EDU Reference streams samples were *Choroterpes* and *Nectopsyche*. *Caecidotea, Crangonyx*, and *Parametriocnemus* were correlated with Control stream samples. HCu samples were strongly associated with *Corbicula*. *Triaenodes* was (as in the spring) associated with the three less disturbed treatment groups but not with HCu. *Centroptilum* was associated with the HCr and HCu treatment groups, but neither the Control stream nor Reference streams.

In spring samples, taxa that were strongly associated with a particular treatment group included *Acentrella* (Reference streams), *Caecidotea* (Control stream), and *Corbicula* (HCu). No taxon exceeded the 0.7 threshold for HCr. With regard to taxa that distinguished between disturbed and less disturbed treatment groups, *Hexatoma* was related to both Control stream and HCr samples, *Isoperla* and Chloroperlidae were associated with Control stream and Reference stream samples, and *Amphinemura* and *Triaenodes* were related to all three less disturbed treatment groups. None of these taxa were common at HCu. *Ormosia* was associated with the HCr and HCu treatment groups, but neither the Control stream nor Reference streams. *Tricorythodes* was associated with the HCu and Reference streams, but not the HCr or Control stream samples.

4.2.6.3 Indicator Metrics

Another goal of the study was to identify macroinvertebrate metrics that would be effective in distinguishing between disturbed communities and those indicating undisturbed or less disturbed conditions. Indicator metrics were derived based on the following attributes:

- Exhibits a consistent difference between disturbed (HCu) and less disturbed (HCr, Control stream, Reference streams) treatment groups;
- Associated with either pollution or habitat related stream degradation; and
- Varies over large gradients, facilitating the ability to perceive differences.

No individual metric met all conditions; however, five metrics were identified to have potential for use in future bioassessments of Hinkson Creek.

Total Taxa richness varied over a large range (54 – 94) and was strongly correlated with MSCI score ($r = 0.69$, $n = 105$ in the spring; $r = 0.58$, $n = 67$ in the fall). Total Taxa richness clearly higher in Reference streams than at HCu in both seasons.

EPT richness varied over a moderate range (4 – 21) and was strongly correlated with MSCI score ($r = 0.74$ in the spring; $r = 0.71$ in the fall). EPT richness was clearly higher at HCr, Control stream, and Reference streams in the spring and at HCr and Reference streams in the fall compared to HCu.

Clinger + Climber richness varied over a moderate range (19 – 42) and was strongly correlated with MSCI score ($r = 0.72$ in the spring; $r = 0.69$ in the fall). Clinger + climber richness was clearly higher at HCr, Control stream, and EDU reference streams in the spring and at HCr and EDU reference streams in the fall compared to HCu.

Percent Intolerant Taxa varied over a large range (0.3 – 58.6) and was positively correlated with MSCI score ($r = 0.28$ in the spring; $r = 0.32$ in the fall). Percent Intolerant Taxa consistently exhibited differences along a gradient, being significantly greater at the Control stream and HCr than at HCu in the fall. Percent Intolerant Taxa did not statistically differ among treatment groups in the spring but was considerably higher in Reference streams.

Scraper/filterer ratio varied over a large range (0.15 – 30.15) and was very weakly correlated with MSCI score (r = 0.03 in the spring; r = -0.01 in the fall). In the spring, scraper/filterer ratio exhibited significant differences among treatment groups following the expected pattern, i.e., greater at the Control stream and HCr than at HCu and greatest at the Reference streams. In the fall, the differences were not significant, but it was still greater at less disturbed treatment groups than at HCu.

SECTION 5

SUMMARY AND DISCUSSION

The goal of the project is to assist the CAM process in the computation and interpretation of aquatic macroinvertebrate community indicators for Hinkson Creek. Using publicly available data compiled from MDNR databases, a comprehensive review and analyses of the macroinvertebrate and environmental data was performed on rural and urban Hinkson Creek, Control stream and Reference streams to assist in the evaluation and diagnoses of potential stressors causing the aquatic life threshold impairment in urban Hinkson Creek.

Forty-four macroinvertebrate metrics were calculated for 172 valid macroinvertebrate community samples and evaluated for applicability in diagnosing potential stressors typically found in urbans stream systems. All metrics were evaluated for variance, temporal/spatial trends, and correlation to potential environmental stressors. Environmental stressors include water chemistry, LULC, NPDES outfalls, and hydrology, which was a very limited data set. One-time (i.e., habitat) or static environmental data (outfalls) are not applicable for stressor analyses. Ordination analyses were performed to illuminate community assemblage differences in sampling season, treatment group and water chemistry variables.

The analysis of variance and ordination analyses determined substantial macroinvertebrate metric and community assemblage differences between seasons (fall/spring). Therefore, temporal trends and correlation analyses of macroinvertebrate metrics and environmental stressors were considered seasonally. Of the 44 metrics, several metrics were less useful as they exhibited autocorrelations to one or more other metrics. Diptera richness was strongly autocorrelated to Chironomidae richness. Percent Chironomidae was strongly autocorrelated with percent Diptera, percent Fast Seasonal Development, percent Abundant in Drift, percent Ability to Exit as Adults, and percent No Swimming Ability. Additional autocorrelated metrics were the three metrics associated with Dominant Taxa, and percent Rare in the Drift which was strongly autocorrelated with percent Slow Seasonal Development.

Although urban and agricultural landscapes have expanded in the Hinkson Creek watershed, no consistent ecologically significant (improvement or degradation) trends were observed in Hinkson Creek in either season. However, more temporal trends of variability were evident in Hinkson Creek during the spring season. No significant temporal trends were observed in the Reference streams; however, the Control stream exhibited consistent ecologically significant (degradation) temporal trends in the spring season.

In general, macroinvertebrate community metrics did not exhibit constant relationships with environmental variables consistently indicating urbanization disturbances. This was likely due to the limited capacity of the invertebrate metrics to distinguish between relatively small environmental gradients and/or inadequate timing of environmental data collection. However, metrics that were considered advantageous in estimating community quality revealed useful patterns, as evidenced by significant correlations with water quality variables and significant differences among treatment groups in respective seasons. For example, Total Taxa richness, EPT richness, and scrapers/filterers ratio were inversely correlated to levels of specific conductance, chloride, and sulfate at multiple treatment groups and in both seasons. These patterns were also observed for metrics (e.g., percent EPT, percent Plecoptera, percent slow seasonal development) that were effective in illustrating community differences in the spring season.

With regard to nutrient concentrations, the only invertebrate metrics that exhibited consistent differences were percent Intolerant Taxa (moderately) and percent Oligochaeta (strongly). The influences of other environmental variables such as point and non-point source outfalls, and proportions of LULC were likely underestimated because of the difficulty of establishing a clear gradient between the treatment groups.

For future bioassessments, five (5) metrics were found to be most applicable to Hinkson Creek based on the following attributes: varied over a moderate to large range, exhibited a consistent difference between treatment groups (best in Reference and Control streams, and worst in urban Hinkson Creek), and may be associated with either a pollution or habitat related degradation. Metrics that had all or most of these attributes were Total Taxa richness, EPT richness, clinger/climber richness, percent Intolerant Taxa, and scraper/filterer ratio. The majority of metrics had some of the attributes listed above but were less effective in distinguishing community differences.

For future rapid bioassessments of Hinkson Creek, indicator taxa that exhibit characteristics of frequency (common), size (visible), sensitivity (low <3 tolerance), and life history (more than one trait) were selected. These taxa include *Perlesta*, a perlid stonefly, *Helicophysche*, a caddis fly, and *Chimarra*, a philopotamid caddisfly. These taxa were commonly found in the Reference streams, Control stream and rural Hinkson Creek but were uncommon in urban Hinkson Creek.

In addition to the outlined scope, Geosyntec deployed continuous conductance monitors at locations consistent with historic macroinvertebrate monitoring stations in rural and urban Hinkson Creek for the winter of 2019/2020. Notable gradient increases were observed from upstream (rural) to downstream (urban) and in direct response to winter snow/ice events.

Therefore, multiple stressors are likely causes for the aquatic life threshold impairment of urban Hinkson Creek. Of the potential stressors examined, analysis of hydrology and instream habitat (sediment) were limited due to the absence or lack of available data. Water quality variables such as temperature, dissolved oxygen, and nutrients that were available were either not significantly different between urban and rural segments of Hinkson Creek or do not appear to be correlated with macroinvertebrate metrics. Of data that were available, chloride concentrations (also represented by conductance) during the winter and spring seasons were noted to be near water quality thresholds. Chloride was also implicated in studies that specifically evaluated the toxicity of Hinkson Creek and its tributaries samples to standard bioassay organisms (MDNR 2002 and

Allert et al 2012). In addition, sporadic toxicity attributed to metals and organic constituents may also be affecting the urban portion of Hinkson Creek (MDNR 2002) but their episodic nature and lack of consistent monitoring made it difficult to document.

SECTION 6

RECOMMENDATIONS

As in most urban streams, multiple stressors are likely affecting the biota in Hinkson Creek. Over the years, many studies have been conducted and many steps have been recommended by various stakeholders. As a result, considerable effort to implement these recommendations have been performed to improve conditions within Hinkson Creek. Many of these projects (construction of retention basins, movement of a Missouri Department of Transportation storage facility, improved parking lot management, stormwater master planning, etc.) were designed to reduce sediment and pollutant (chloride, heavy metals, and organics) loading from nonpoint sources. It is vitally important to continue implementation and expansion of these efforts as the urban landscape continues to grow. Therefore, recommendations to preserve and improve the aquatic life in Hinkson Creek revolve around holistic watershed management, planning, best management practices (BMP), and monitoring tools to assess performance. A list of recommendations specific to the results of this study are provided below. Realizing resources are likely not available to perform the entire list of recommendations, prioritized recommendations are italicized.

- ***Watershed-Based Management Plan*** *for Hinkson Creek would guide appropriate urban expansion, identify critical areas for restoration or protection, and bolster the resources and implementation of non-point source BMP projects.*
- **Outreach and Education**
    - Enhance private landowners/businesses knowledge of appropriate impervious surface management, with specific focus on winter product application.
    - Promote cleanup, monitoring and educational efforts (Stream Teams, K-12 schools, University, etc.) of Hinkson Creek.
- **Non-Structural BMPs for Chlorides**
    - Winter road treatment product application equipment and decisions.
    - Investigate alternative winter road treatment products.
    - Perform street sweeping in late winter or after winter events.
    - Continue and enhance operator training.
    - Stockpile snow/ice away from sensitive areas.
    - Preserve and enhance riparian corridors along Hinkson Creek and its tributaries, with specific focus on the Hwy. 63 connector to Broadway Blvd. and upper portions of the Hinkson Creek watershed.
- **Structural BMPs**
    - Appropriate salt storage facilities (public and private).
    - Continue to design and construct stormwater control structures to reduce inputs of sediment and other pollutants.

      - Concentrate efforts on priority areas of Hinkson Creek (downstream of Hwy. 63 connector and downstream of Flat Branch Creek) which have shown to be of specific concern for chlorides.
    - Implement and enforce construction and/or land disturbance BMPs within the watershed to minimize soil erosion and pollutant transport into the stream system.
    - Incorporate permeable surfaces for parking lots that slow runoff and increase the infiltration of surface runoff.
  - **Performance Monitoring and Evaluation**
    - *Additional upstream flow monitoring station in the rural portion of Hinkson Creek (upstream of Hwy. 63 connector) would enhance the characterization of watershed hydrology and understanding of potential flow stressors by future investigations.*
    - *Flow monitoring stations should incorporate year-round temperature and specific conductance monitoring to assess long-term BMP efficacy and identify appropriate aquatic life thresholds while maintaining public safety.*
      - Future development of specific conductance and chloride regressions specifically for Hinkson Creek.
      - Future chloride data collection should be accompanied with hardness and sulfate data.
    - Comprehensive land cover analysis to determine total versus effective impervious surface in effort identify critical areas for BMPs.
    - *Perform high resolution winter season specific conductance monitoring (continuous) at numerous Hinkson Creek and tributary locations in effort to identify potential significant chloride sources and guide future BMP prioritization.*
      - *In conjunction with specific conductance monitoring, perform a macroinvertebrate drift survey to further evaluate the role chloride may influence macroinvertebrate community assemblage.*
    - *Sediment characterization and habitat assessments should be standardized to accompany future bioassessment.*
    - Future bioassessments of Hinkson Creek should consider Cedar Creek (Waterbody Identification 0737) as a Control stream for the following reasons:
      - Historic land (mining) uses resemble that of Hinkson Creek.
      - Proximity to Hinkson Creek (groundwater recharge). Bonne Femme Creek likely has higher groundwater recharge, as demonstrated by cooler fall and spring temperatures and lower dissolved oxygen concentration.

  - Increased urban expansion in Bonne Femme Creek watershed and decreasing spring macroinvertebrate community metric trends.
- Limited availability of data sets hindered the assessment of potential Hinkson Creek stressors (sediment, hydrology, temperature). It's our opinion that CAM should be the clearing house of data collection activities and future expenditures related to monitoring and evaluation and should have readily access to data funded by stakeholders.
- Future rapid bioassessments of Hinkson Creek should include use of identified indicator taxa.
- Future bioassessments of Hinkson Creek should include indicator metrics (Total Taxa richness, EPT Taxa richness, Clinger + Climber richness, percent Intolerant Taxa, and Scraper/Filterer ratio) for condition assessment. However, other monitoring components related to hydrology, specific conductance and habitat must be in place to better identify other potential stressors.

SECTION 7

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APPENDICES

APPENDIX A

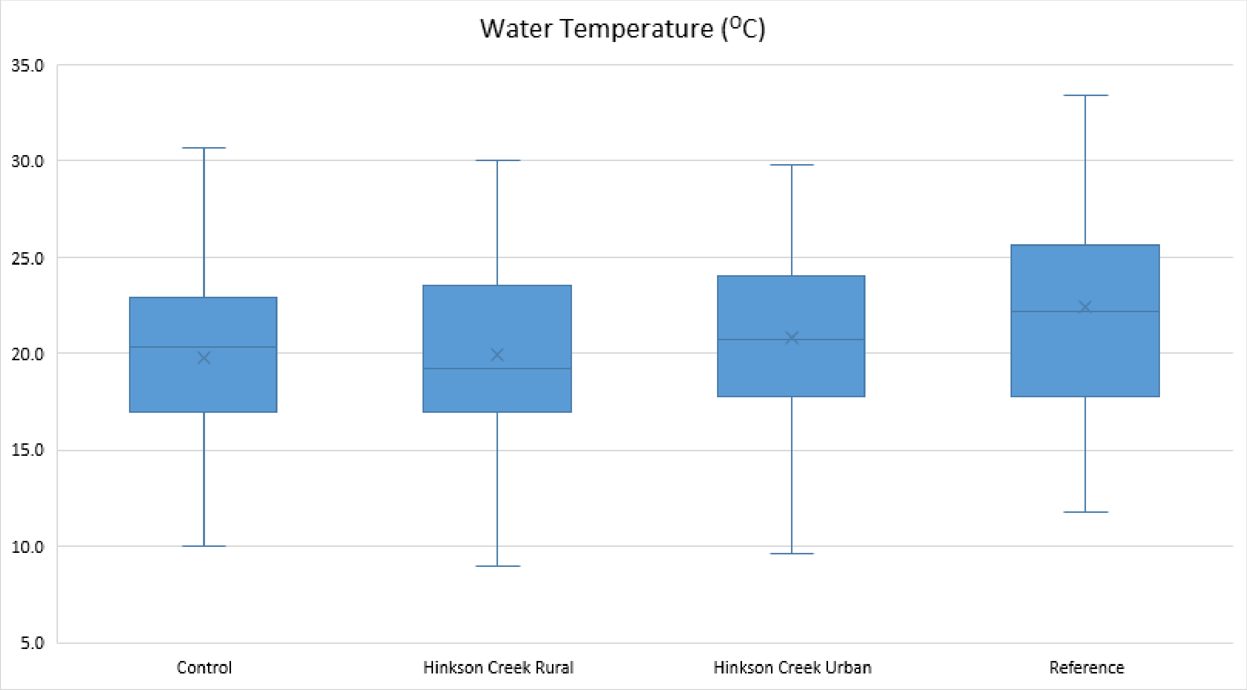
MDNR Riffle Pool Complex Ozark Moreau Loutre EDU Macroinvertebrate Samples

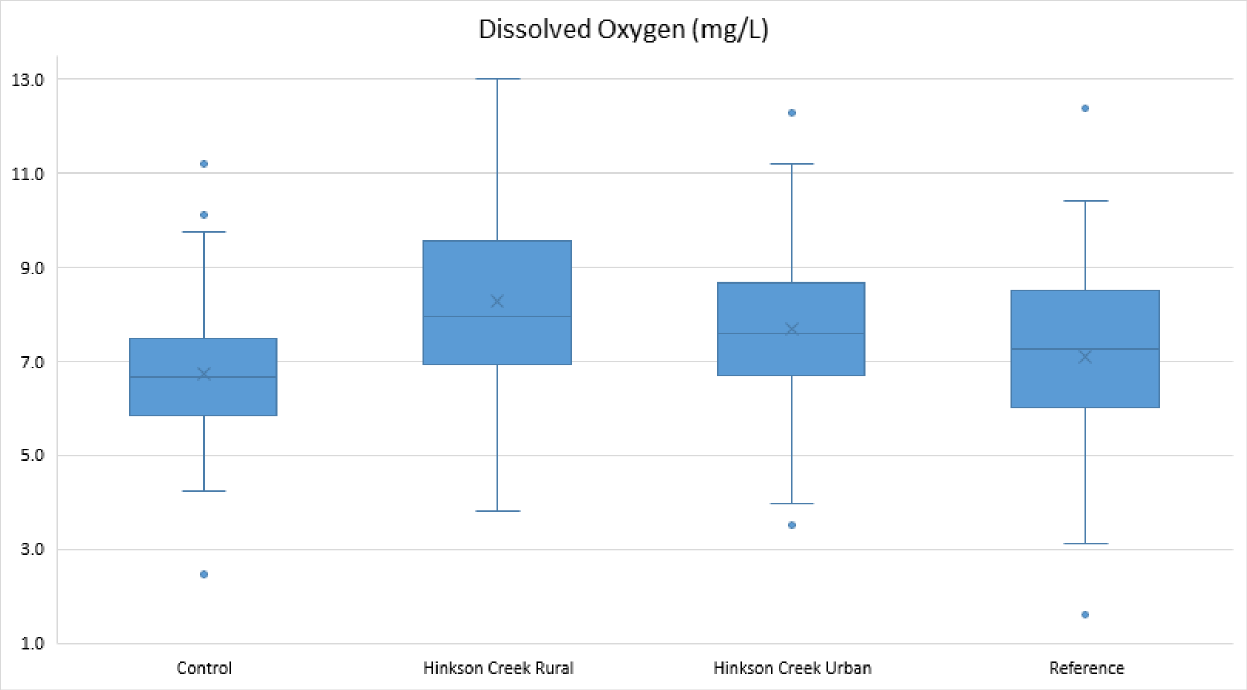
Treatment Group	Stream	Site #	WBID	Stream Class	Spring	Fall	Spring	Fall	Spring	Spring	Fall	Spring	Spring	Fall	Spring	Fall	Spring	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
					2001	2001	2002	2003	2004	2005	2005	2006	2008	2011	2012	2012	2013	2014	2014	2015	2015	2016	2016	2017	2017
Urban	Hinkson Creek	1	1007	P		✓	✓					✓				⦸	✓		✓	✓	✓	✓	✓	✓	✓
Urban	Hinkson Creek	2	1007	P		✓	✓					✓				⦸	✓	✓	✓	✓	✓	✓	✓	✓	✓
Urban	Hinkson Creek	3	1007	P		✓	✓					✓				⦸	✓	✓	✓	✓	✓	✓	✓	✓	✓
Urban	Hinkson Creek	3.5	1008	C						✓	✓				✓	⦸	✓	✓	✓	✓	✓	✓	✓	✓	✓
Urban	Hinkson Creek	4	1008	C		⦸	✓								✓	⦸	✓	✓	✓	✓	✓	✓	✓	✓	✓
Urban	Hinkson Creek	5	1008	C		⦸	⦸								✓	⦸	✓	✓	✓	✓	✓	✓	✓	✓	✓
Urban	Hinkson Creek	5.5	1008	C				✓	✓	✓	⦸				✓	⦸	✓	✓	✓	✓	✓	✓	✓	✓	✓
Urban	Hinkson Creek	6	1008	C		✓	⦸	✓	✓	✓	✓				✓	⦸	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rural	Hinkson Creek	6.5	1008	C					✓						✓		✓	✓	✓	✓	✓	✓	✓	✓	
Rural	Hinkson Creek	7	1008	C		⦸	✓	✓	✓	✓	✓				✓		✓	✓	✓	✓		✓	✓	✓	
Rural	Hinkson Creek	8	1008	C		⦸	✓								✓		✓	✓	✓	✓		✓	✓	✓	
Control	Bonne Femme Creek	1	0750	P		✓	✓	✓	✓			✓			✓		✓	✓	✓	✓	✓	✓	✓	✓	✓
Control	Bonne Femme Creek	2	0750	P		✓	✓	✓	✓						✓		✓	✓	✓	✓	✓	✓	✓	✓	✓
Reference	Boeuf Creek	1	1661	P	✓	✓																			
Reference	Boeuf Creek	2	1661	P	✓	✓							✓	✓	✓										
Reference	Burris Fork	1	0968	P	✓								✓	✓	✓										
Reference	Loutre River	1	1624	P										✓	✓										
Reference	Moniteau Creek	1	0809	C	✓	✓																			

Notes: WBID = waterbody identificaiton; ✓ = valid sample; ⦸ = invalid sample.

APPENDIX B

Appendix B: Fall Season Inclusive Water Quality Data Box-Whisker Plots





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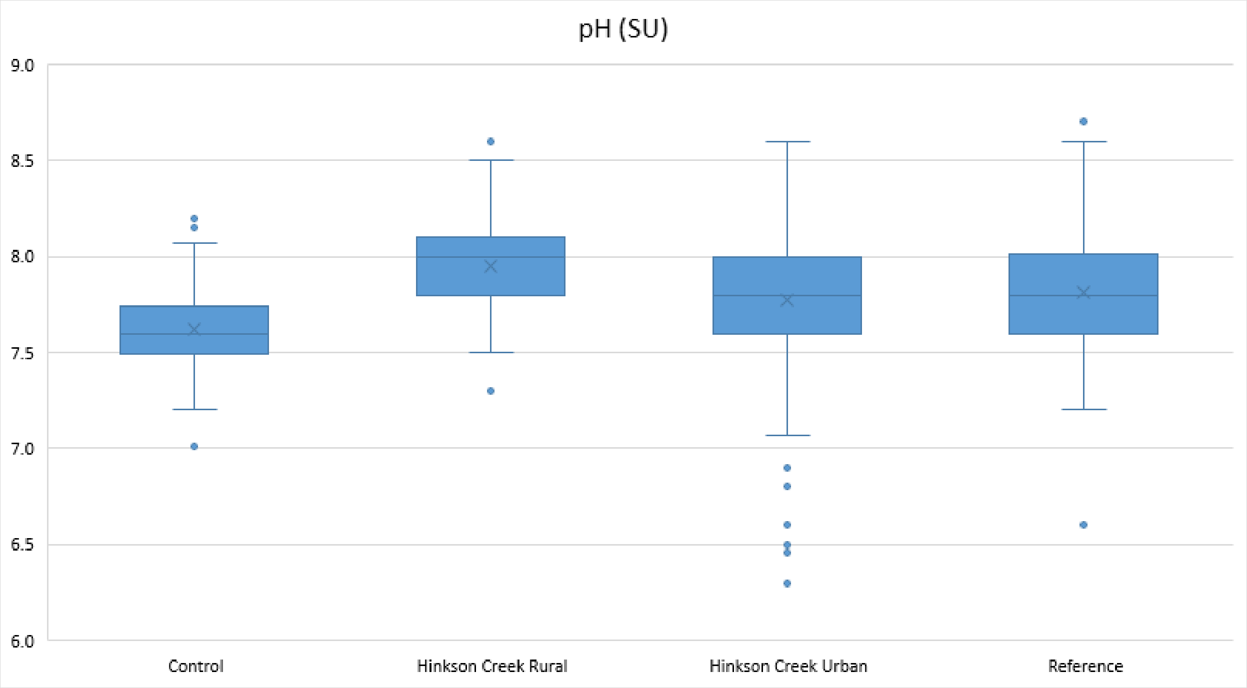

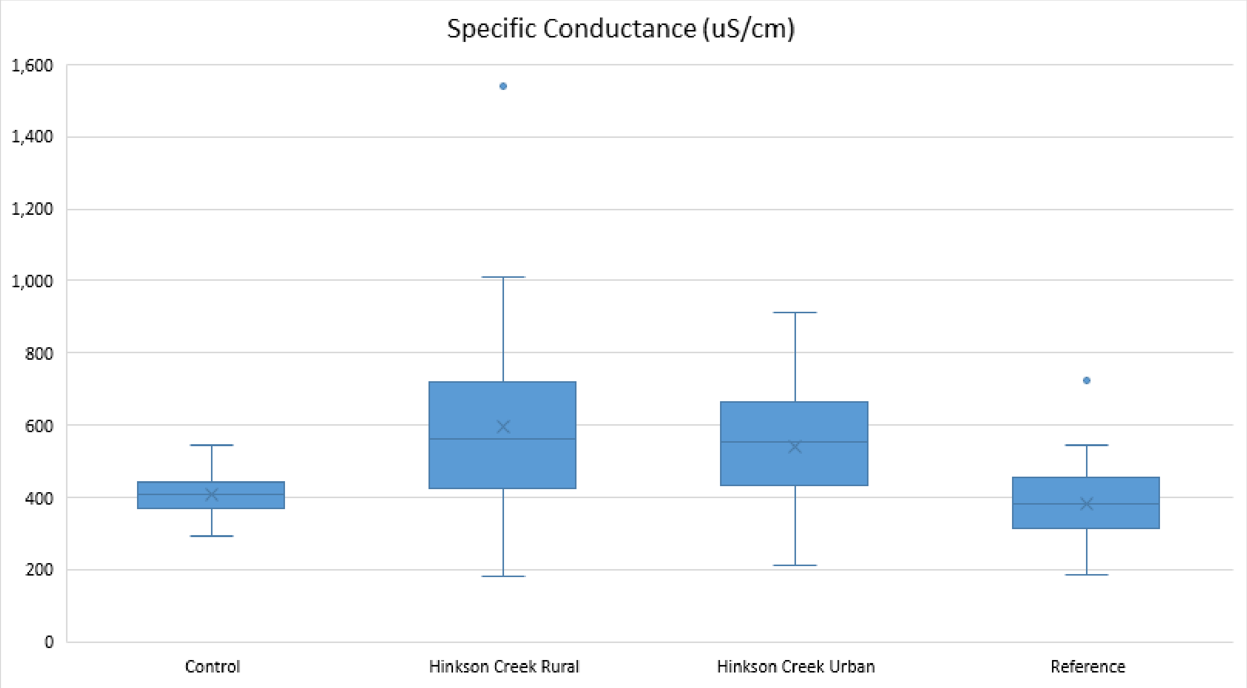

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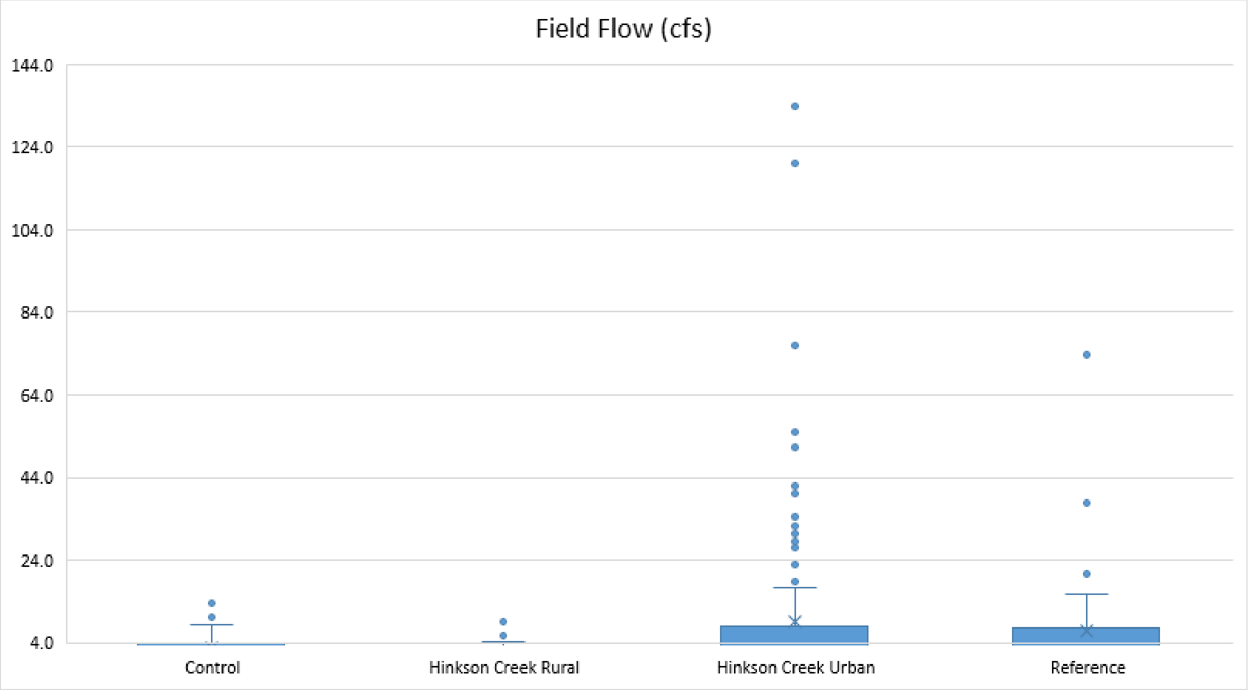

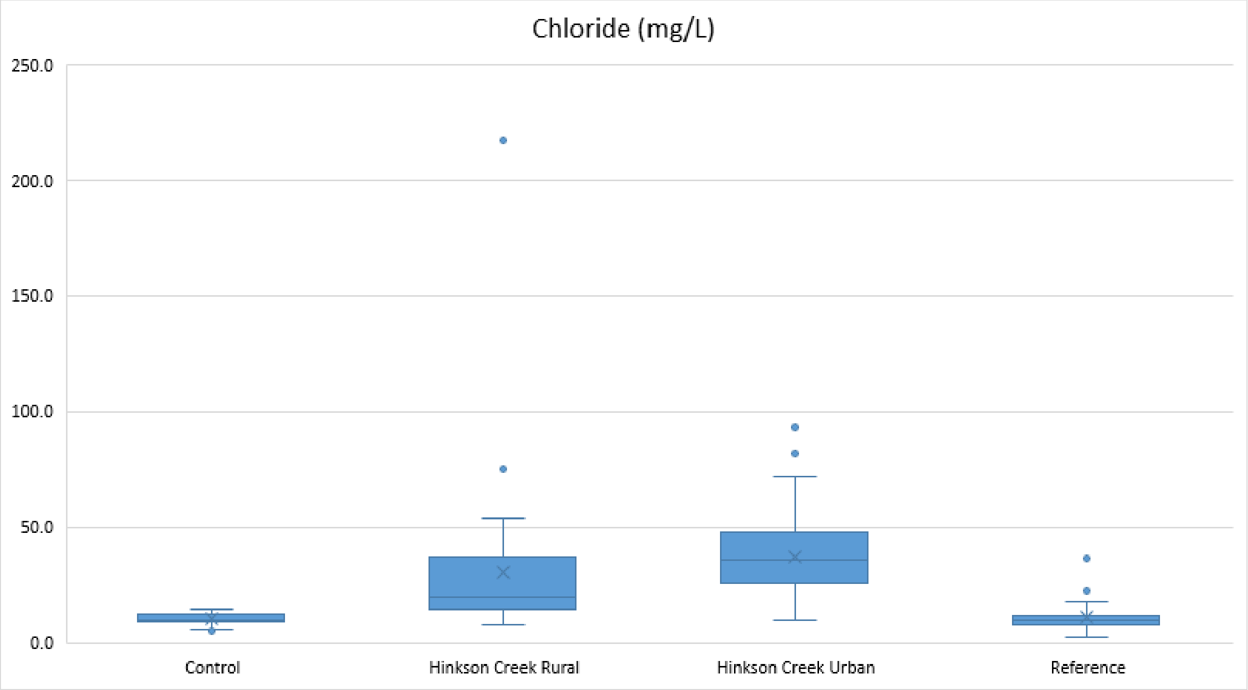

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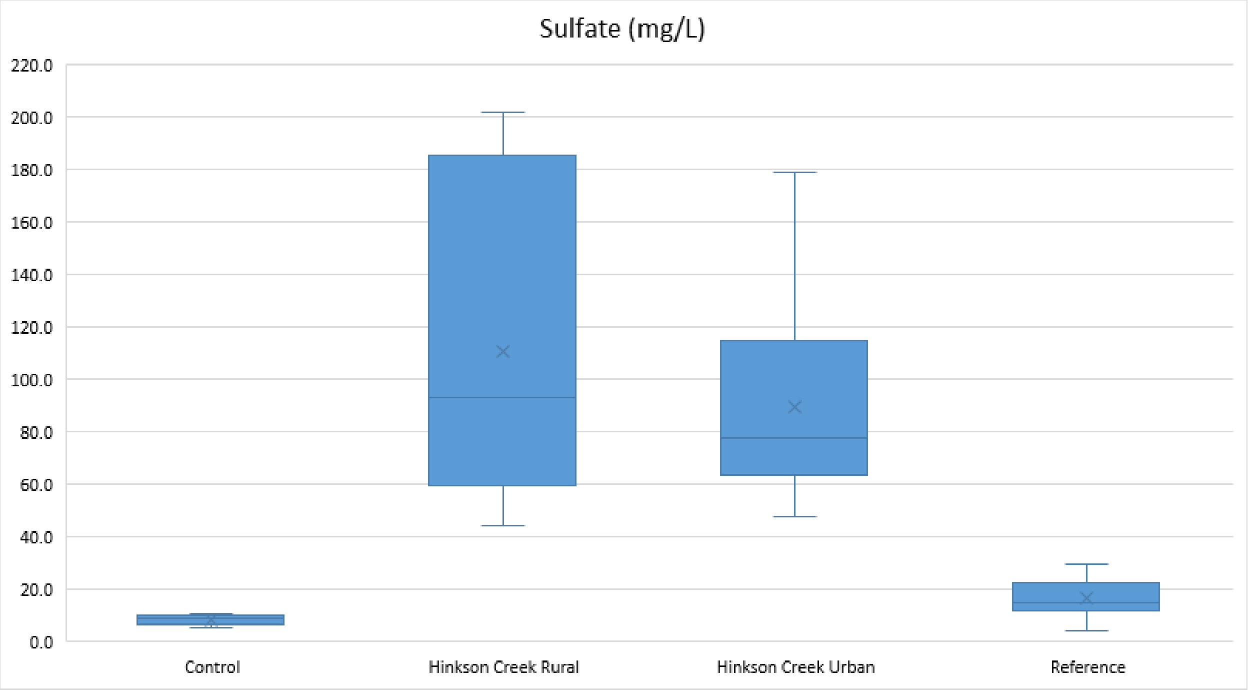

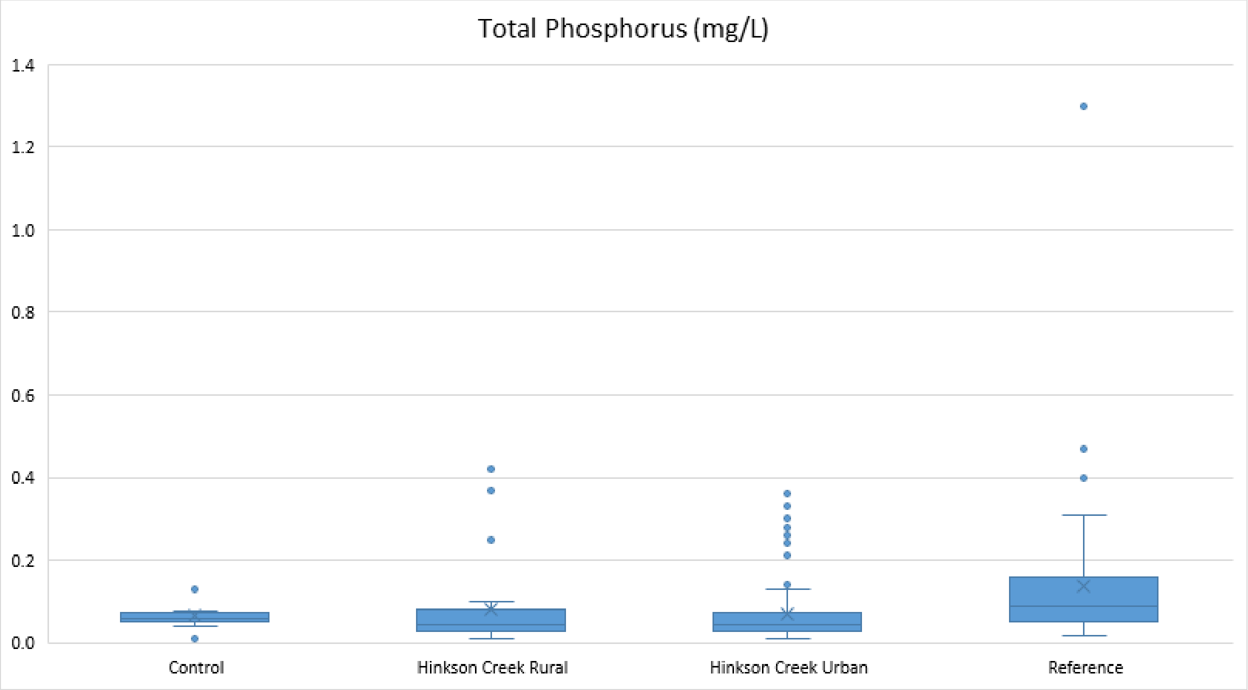

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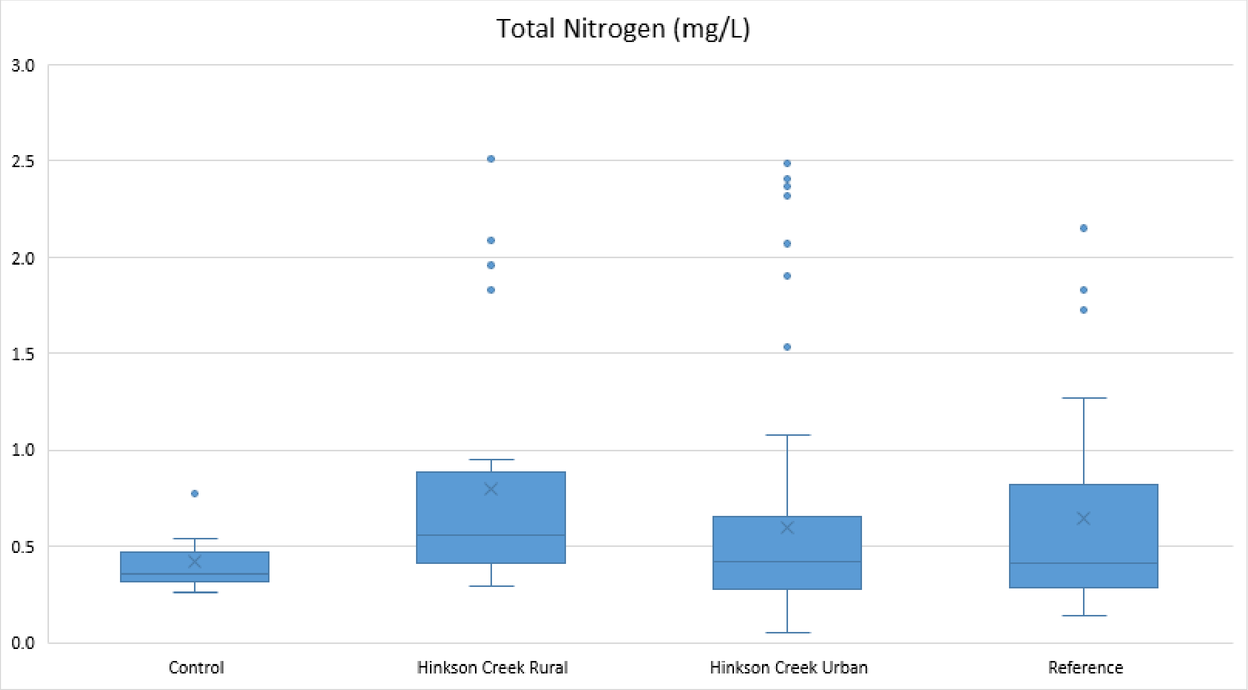

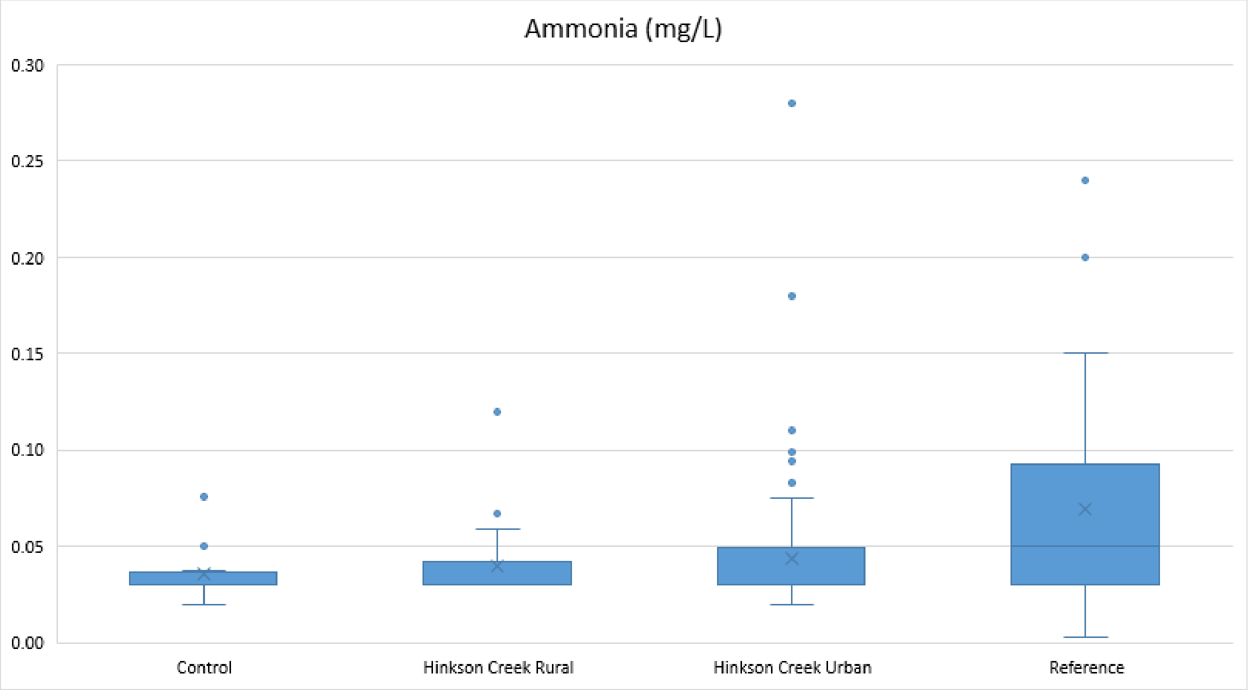

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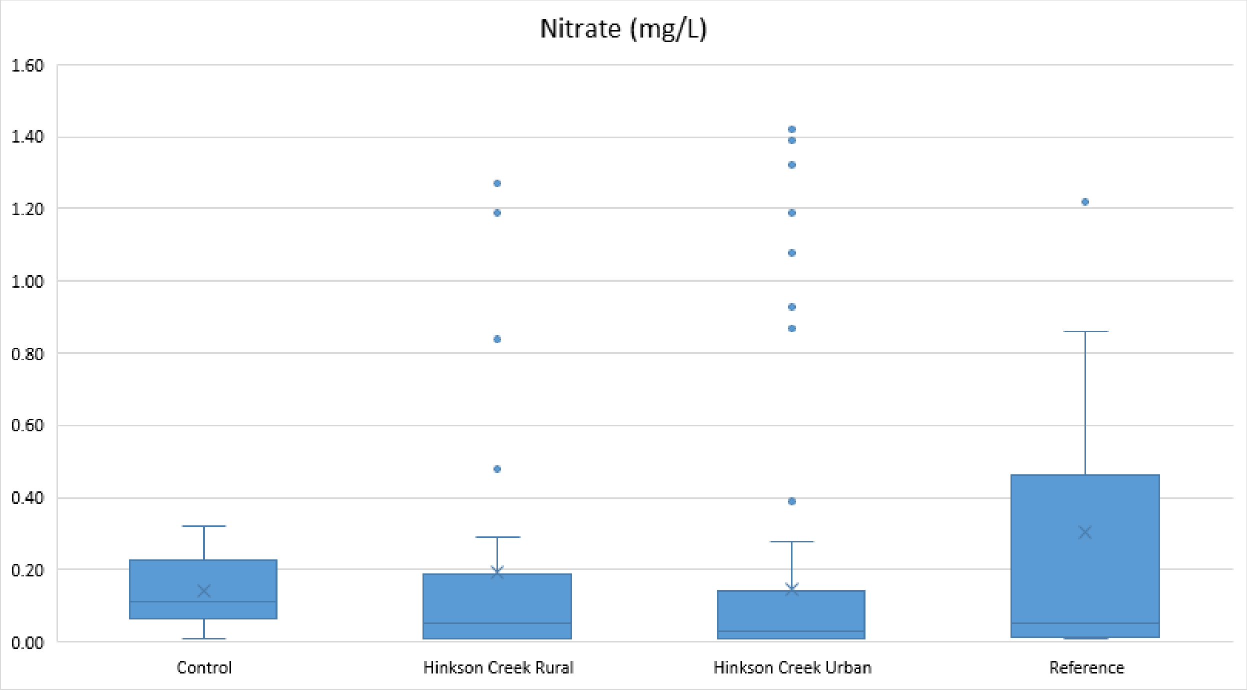

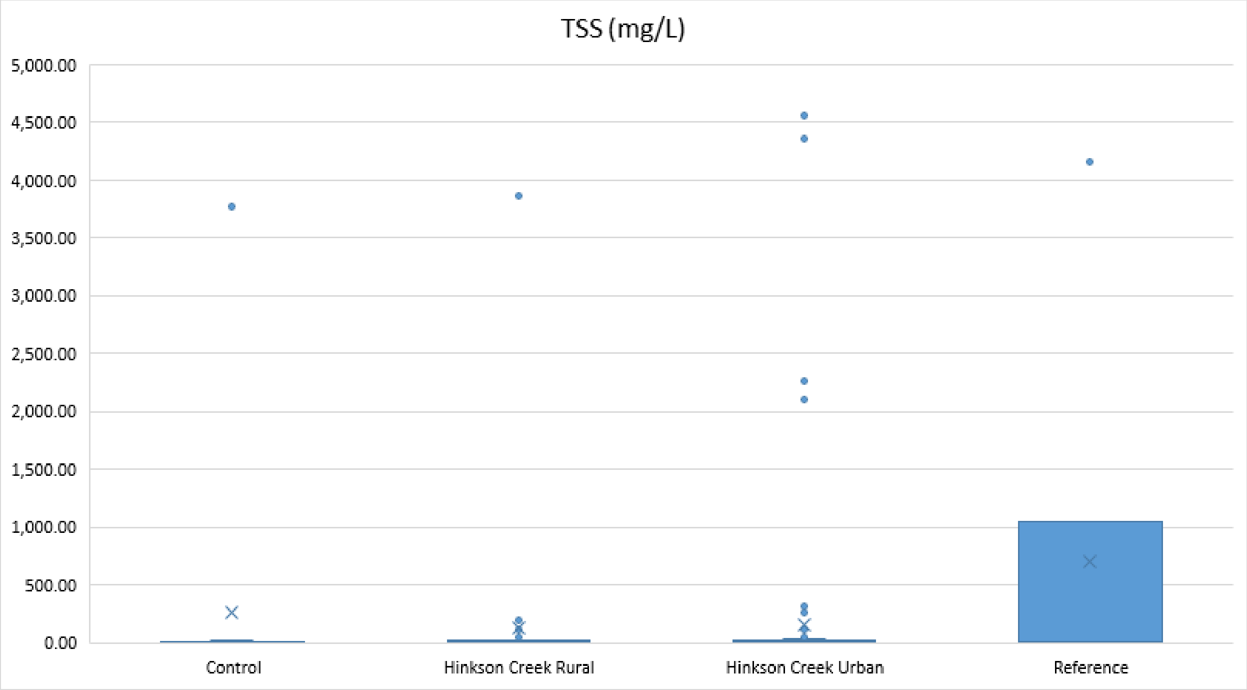

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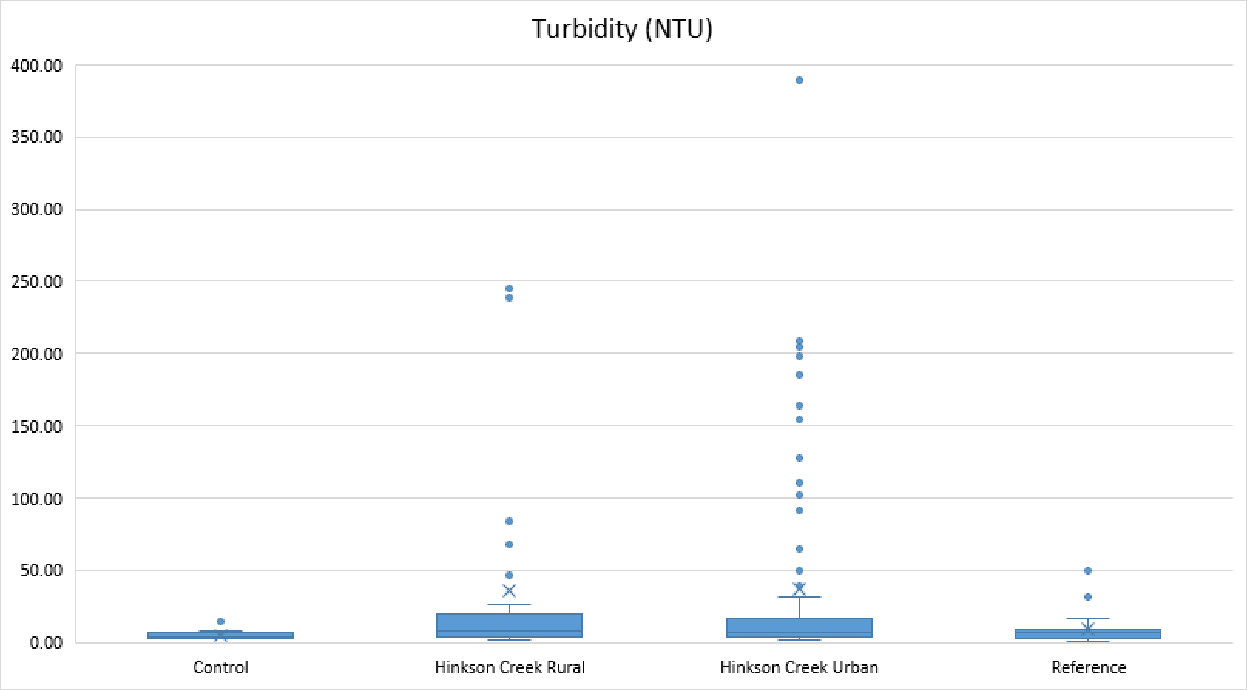

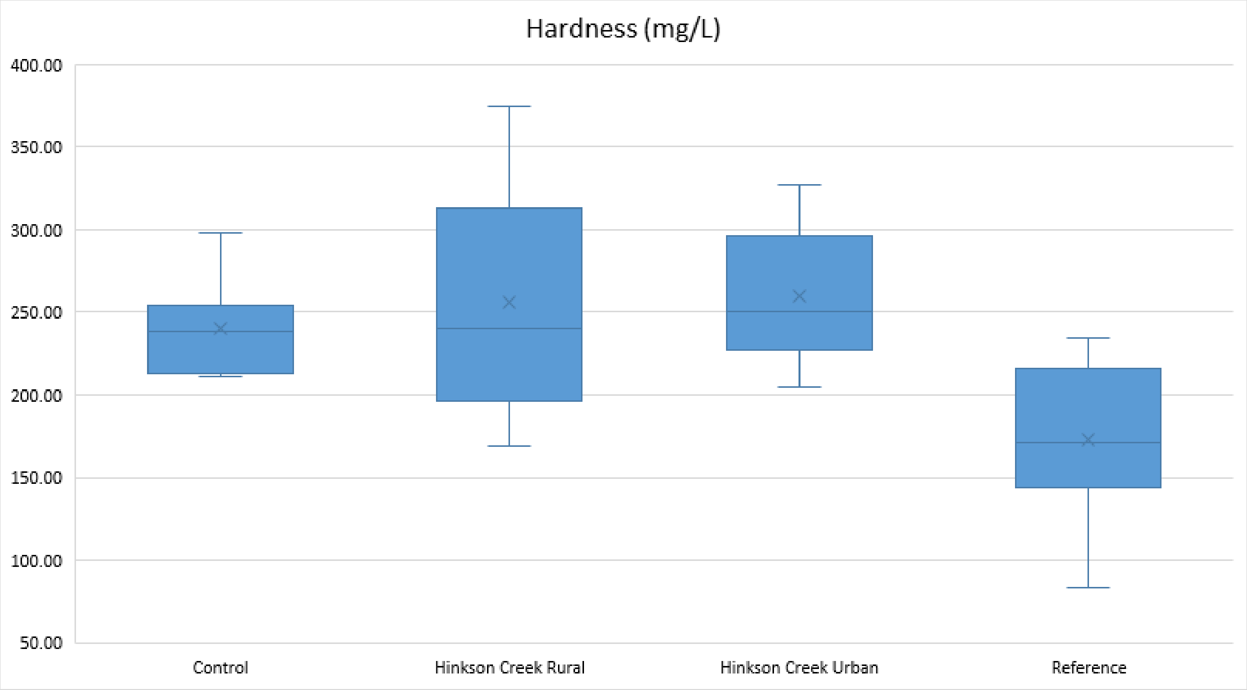

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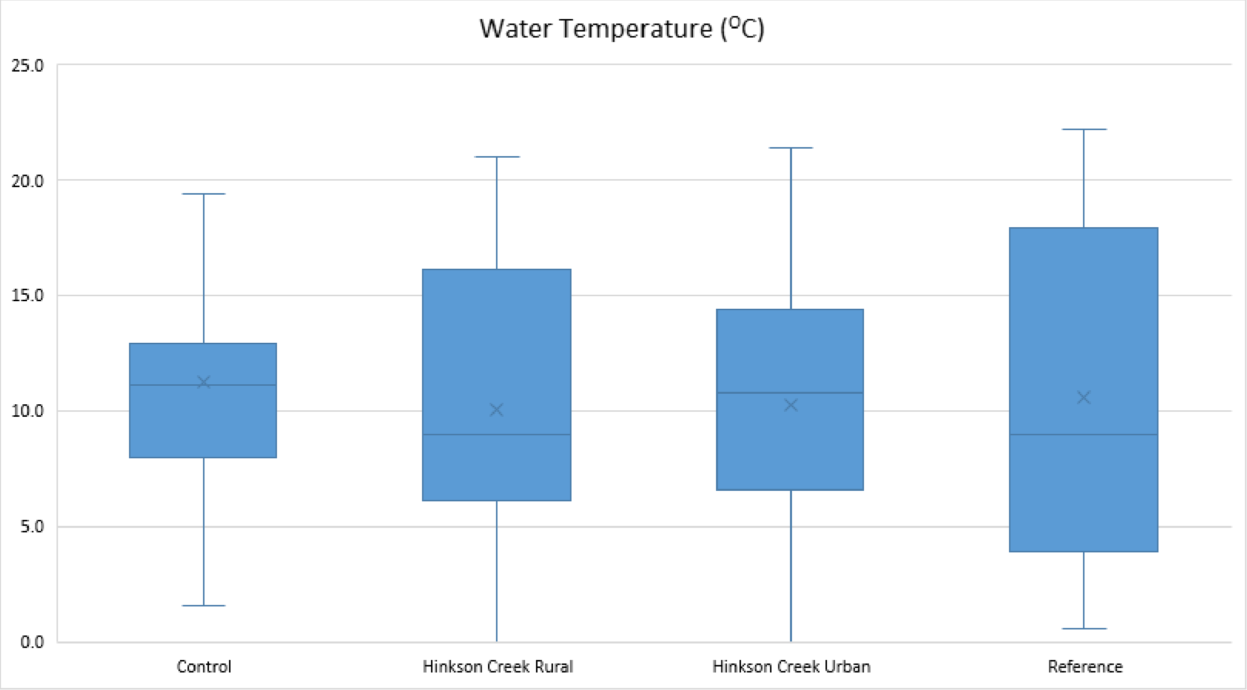

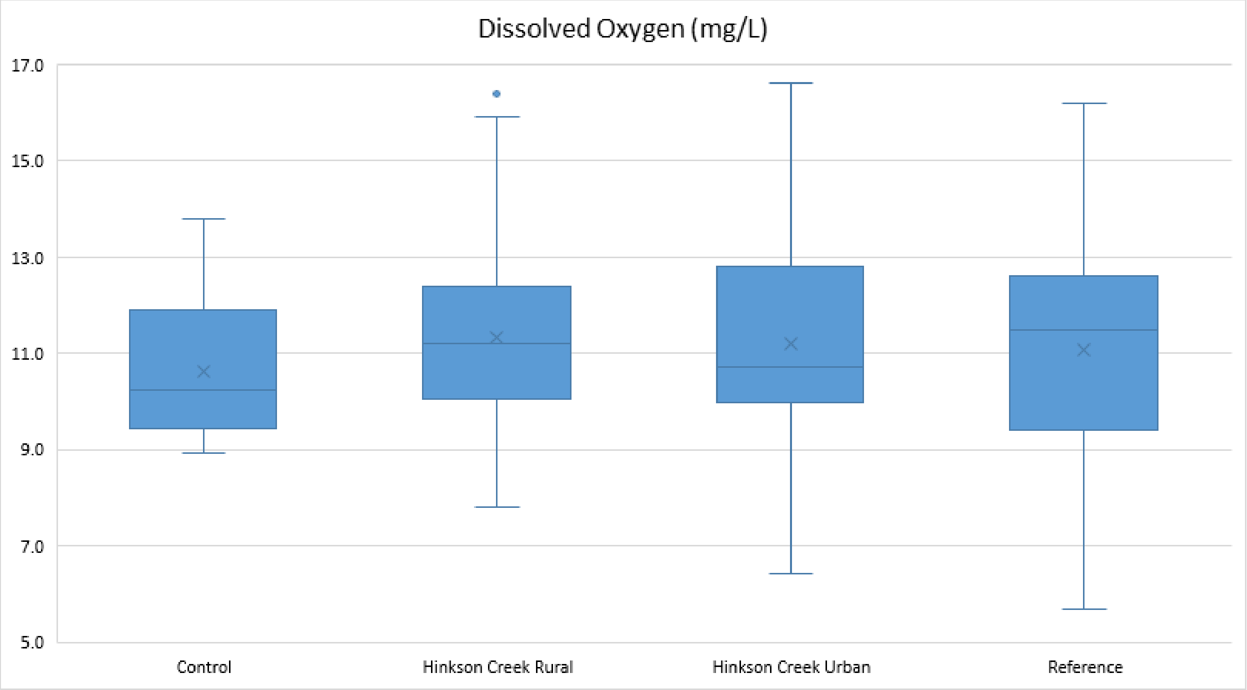

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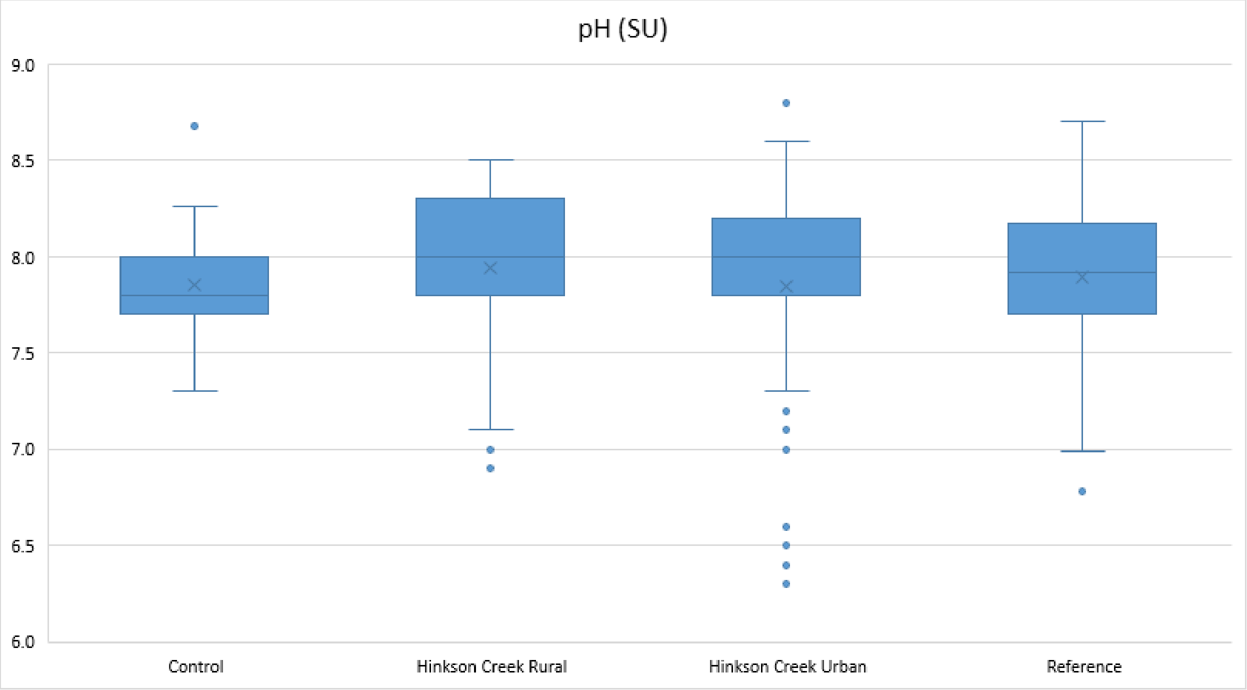

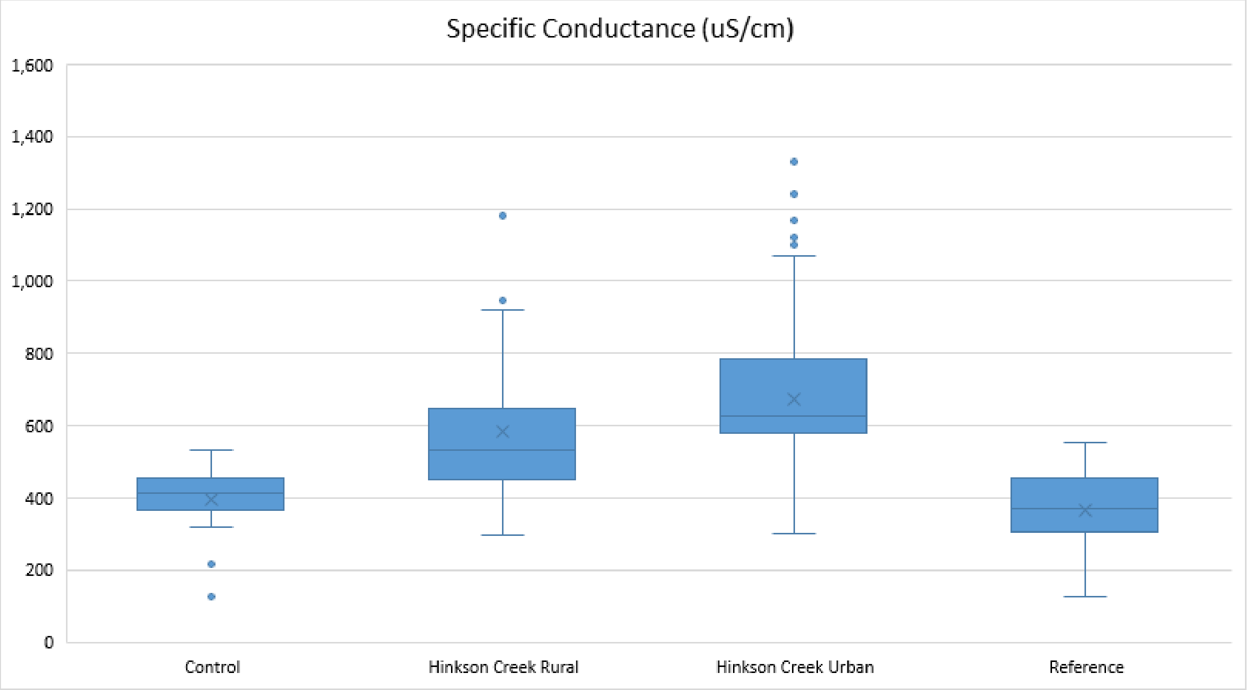

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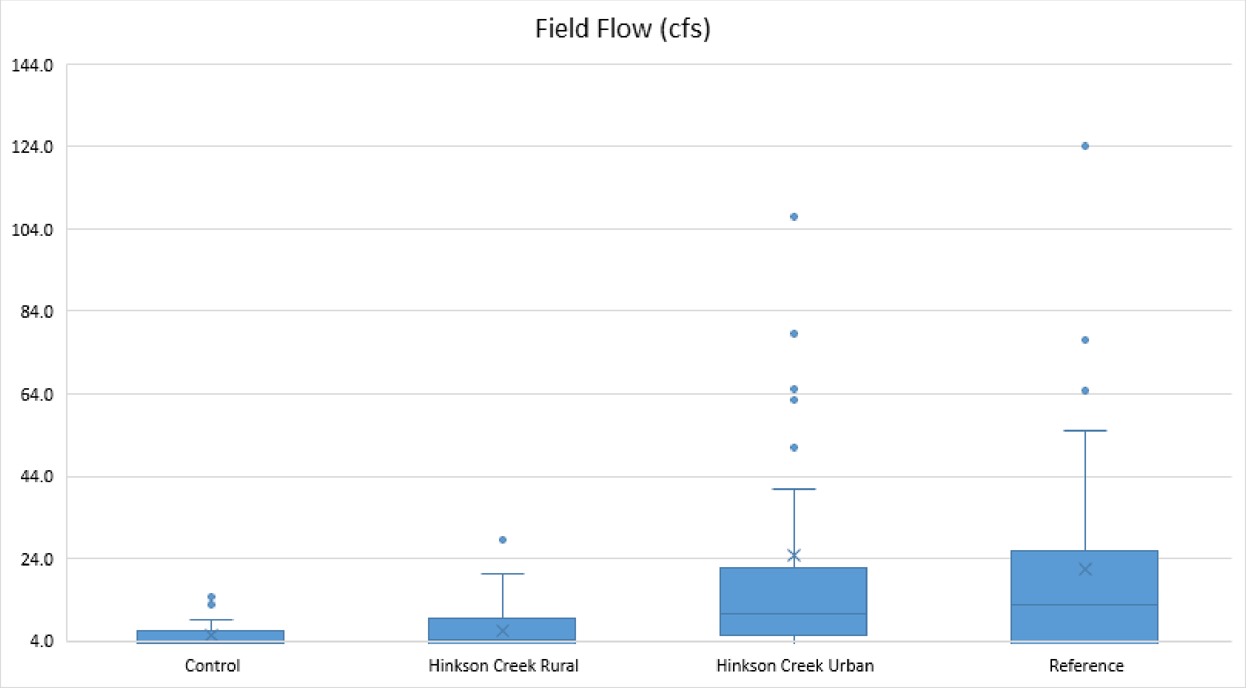

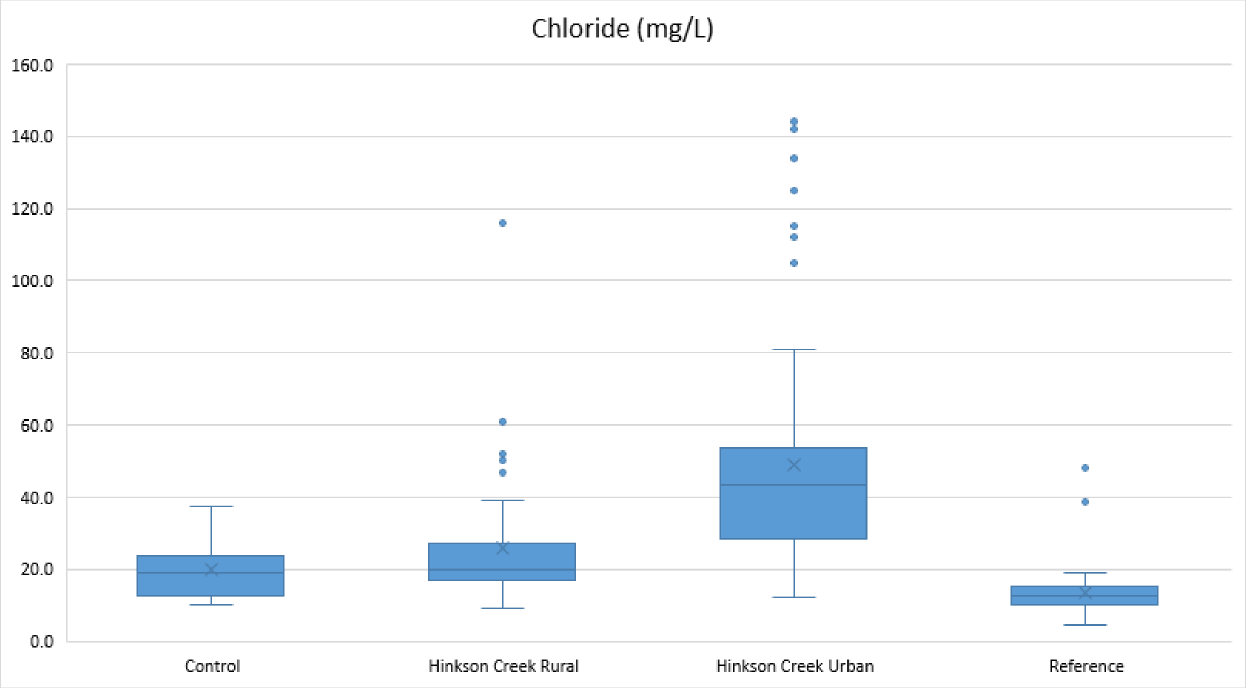

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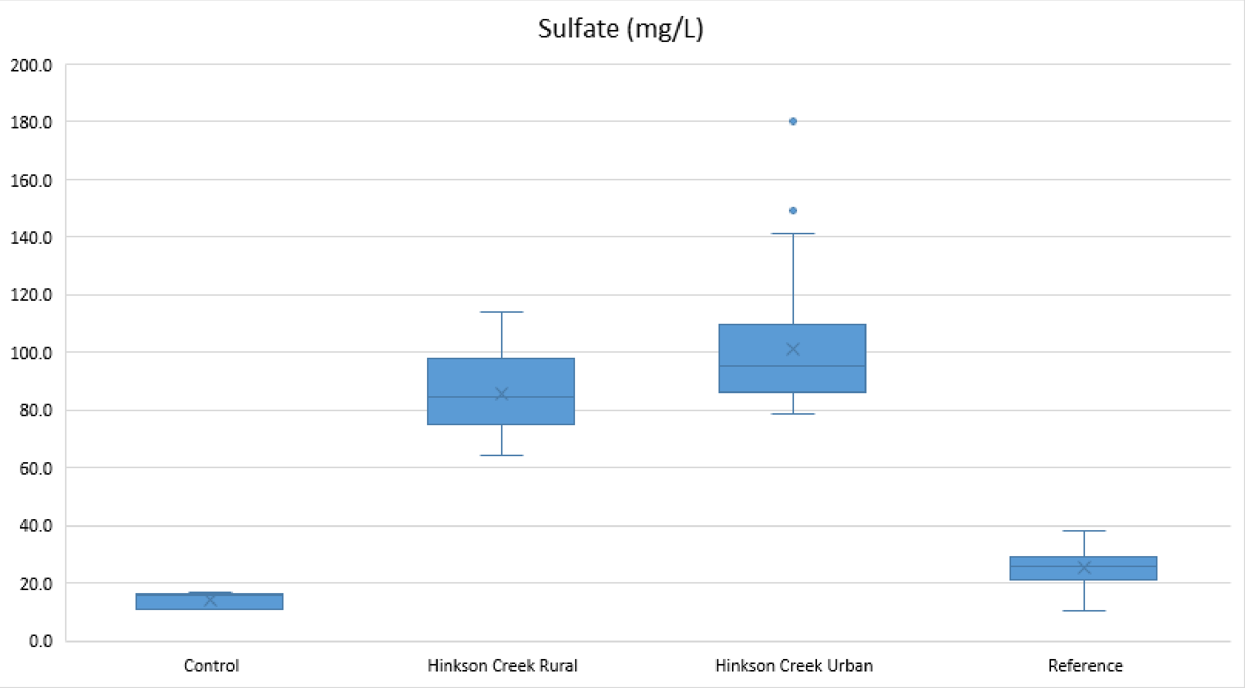

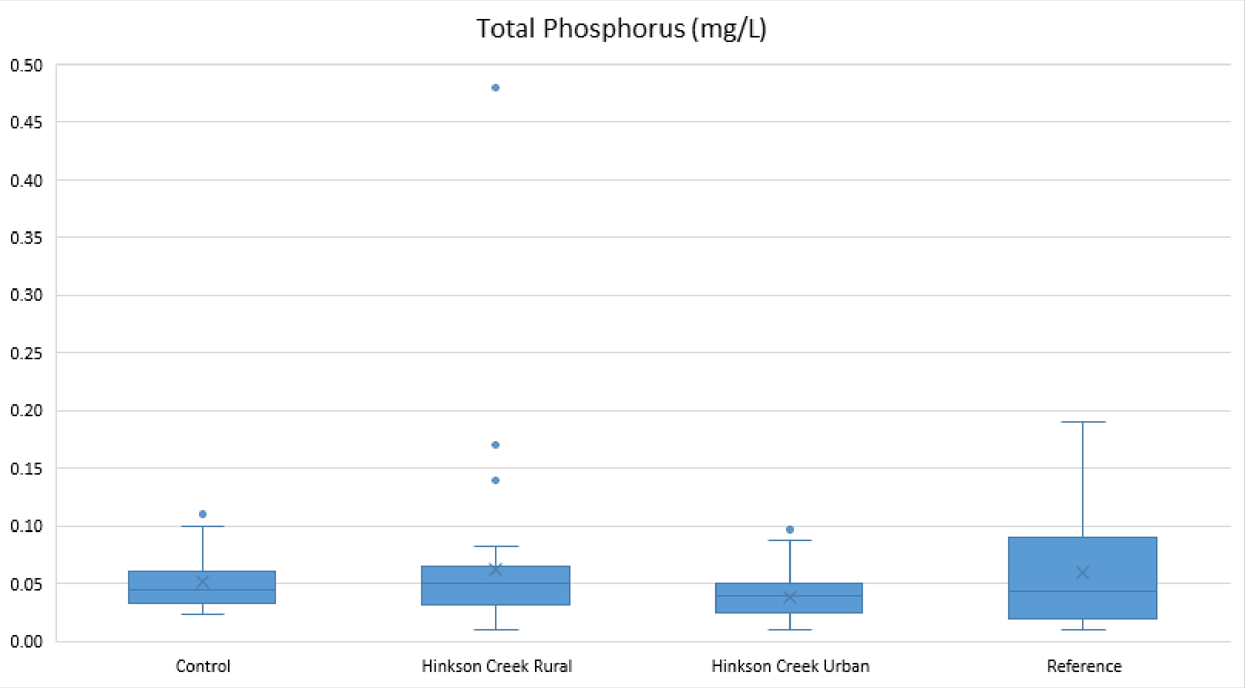

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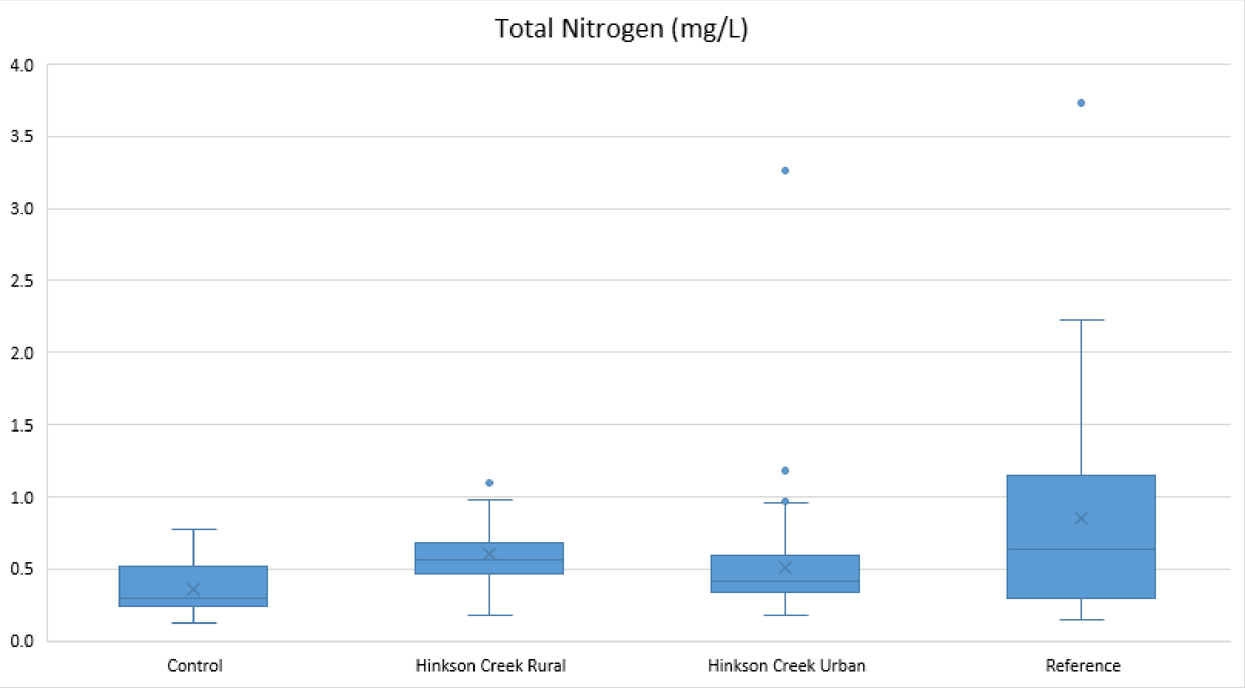

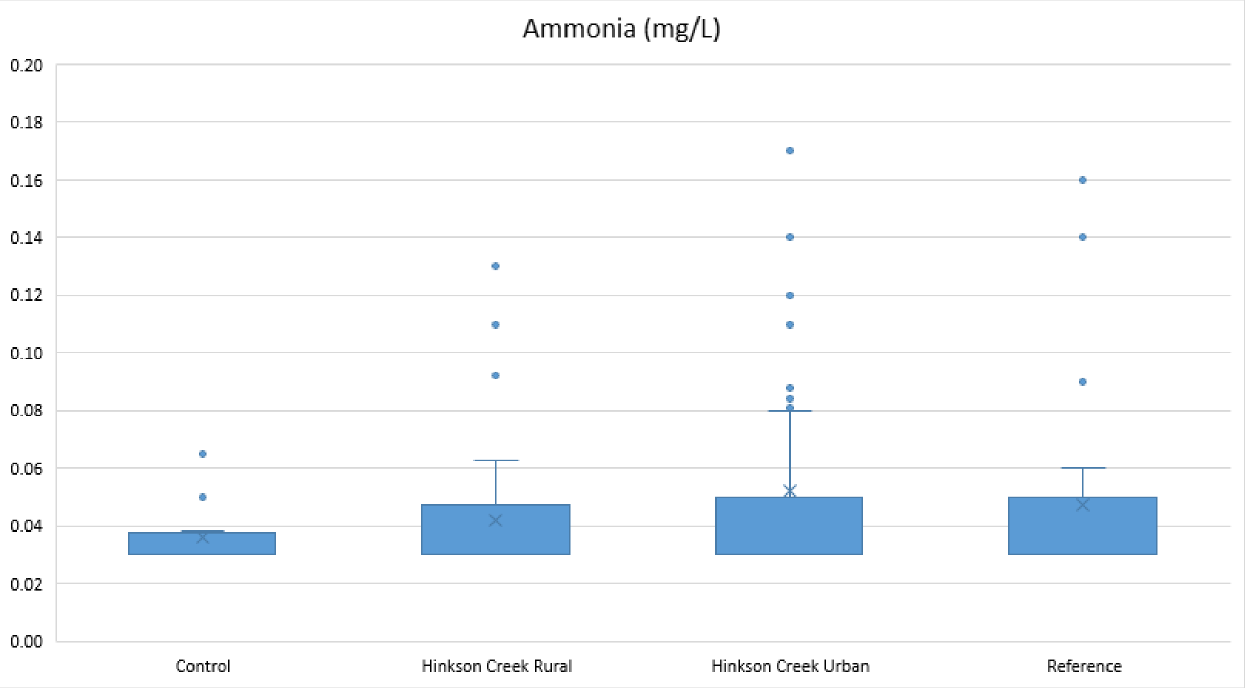

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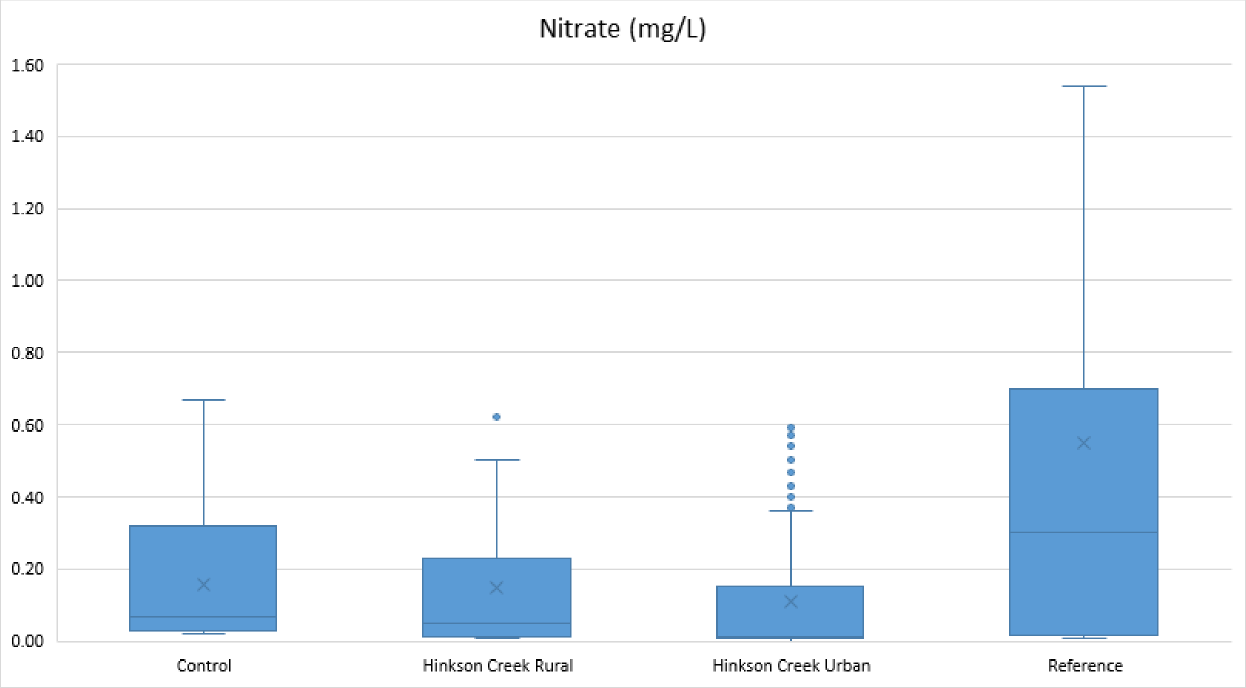

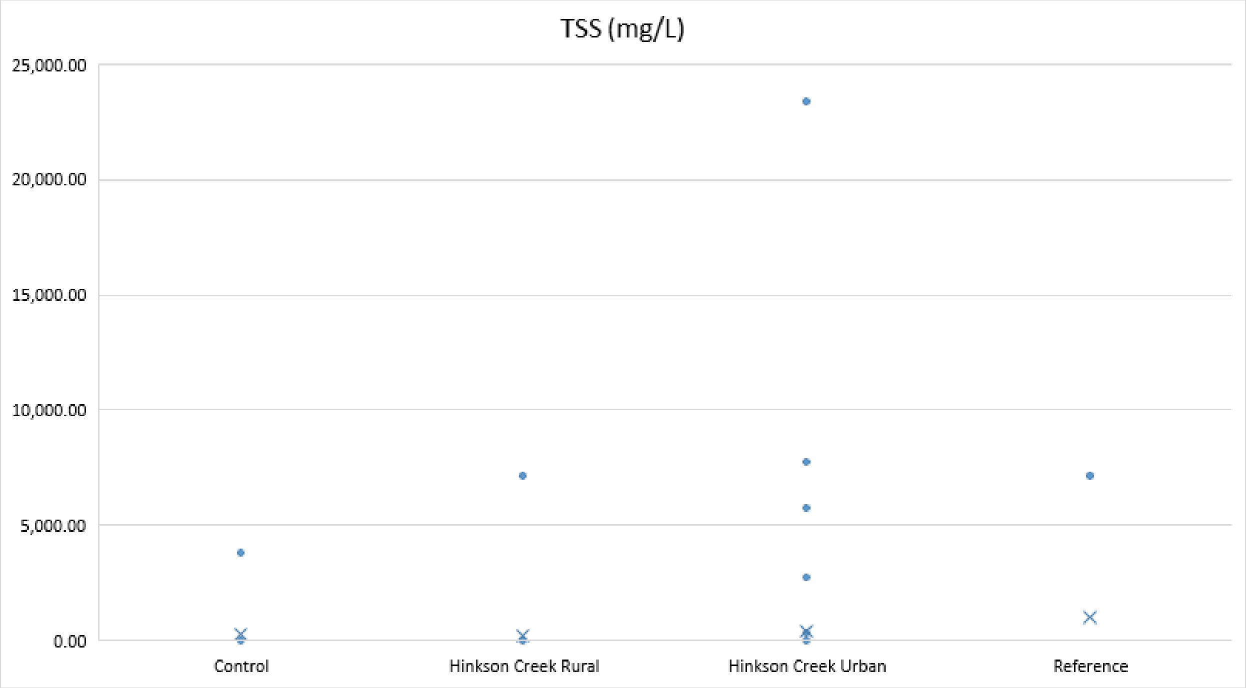

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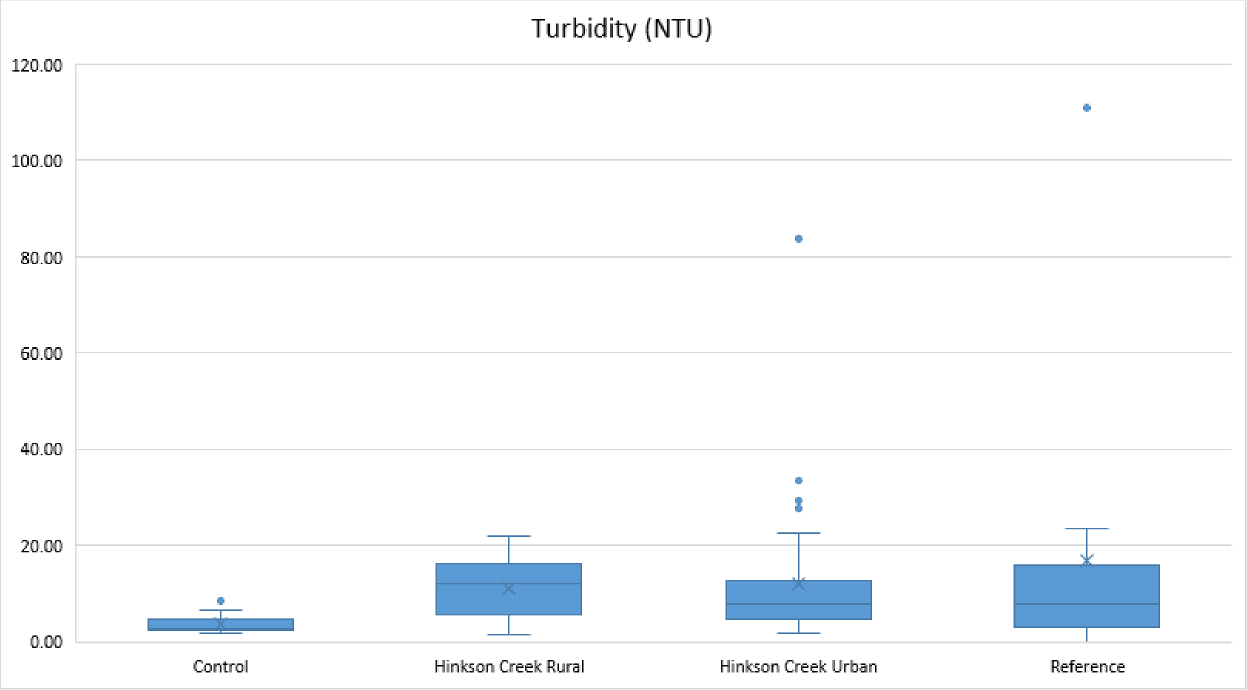
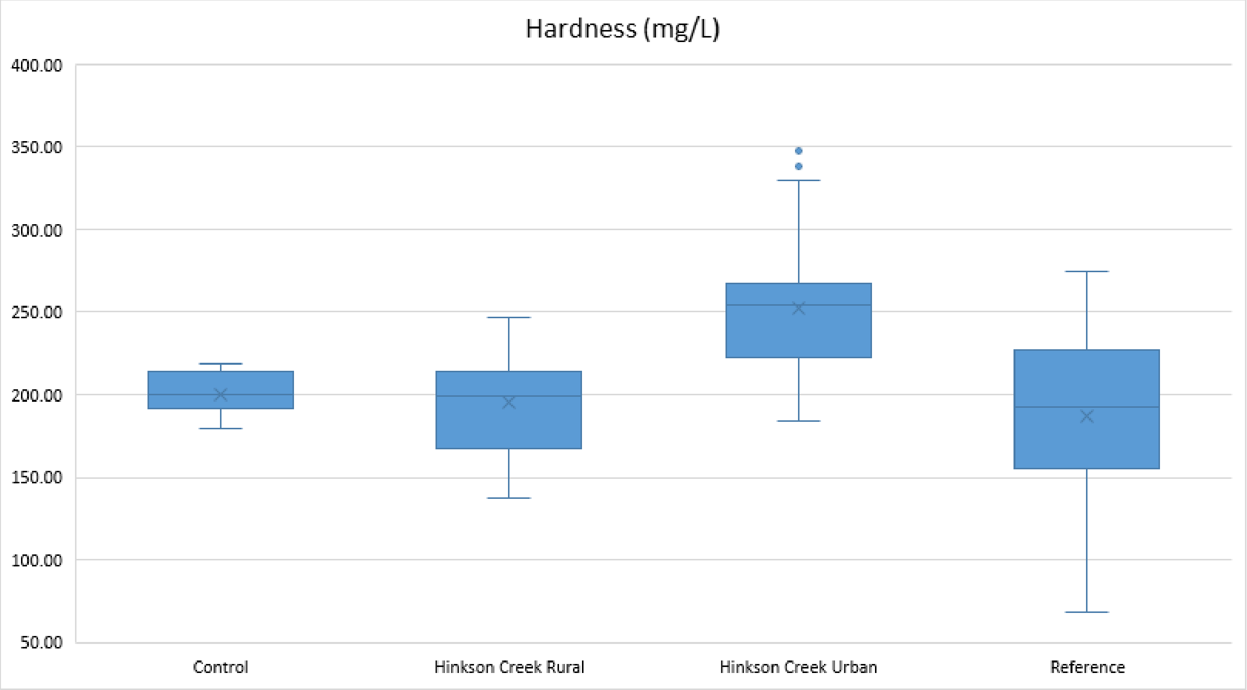
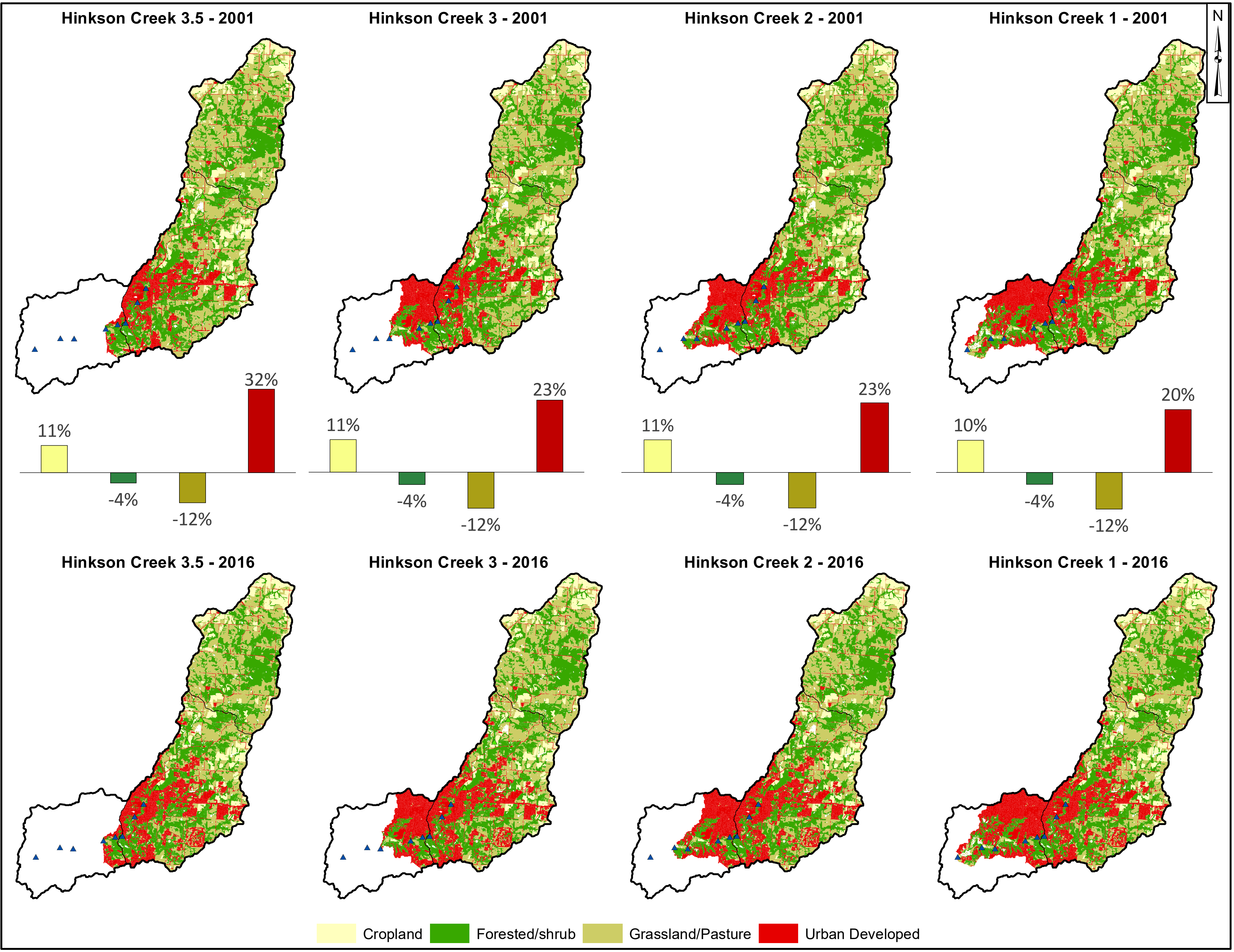




APPENDIX C



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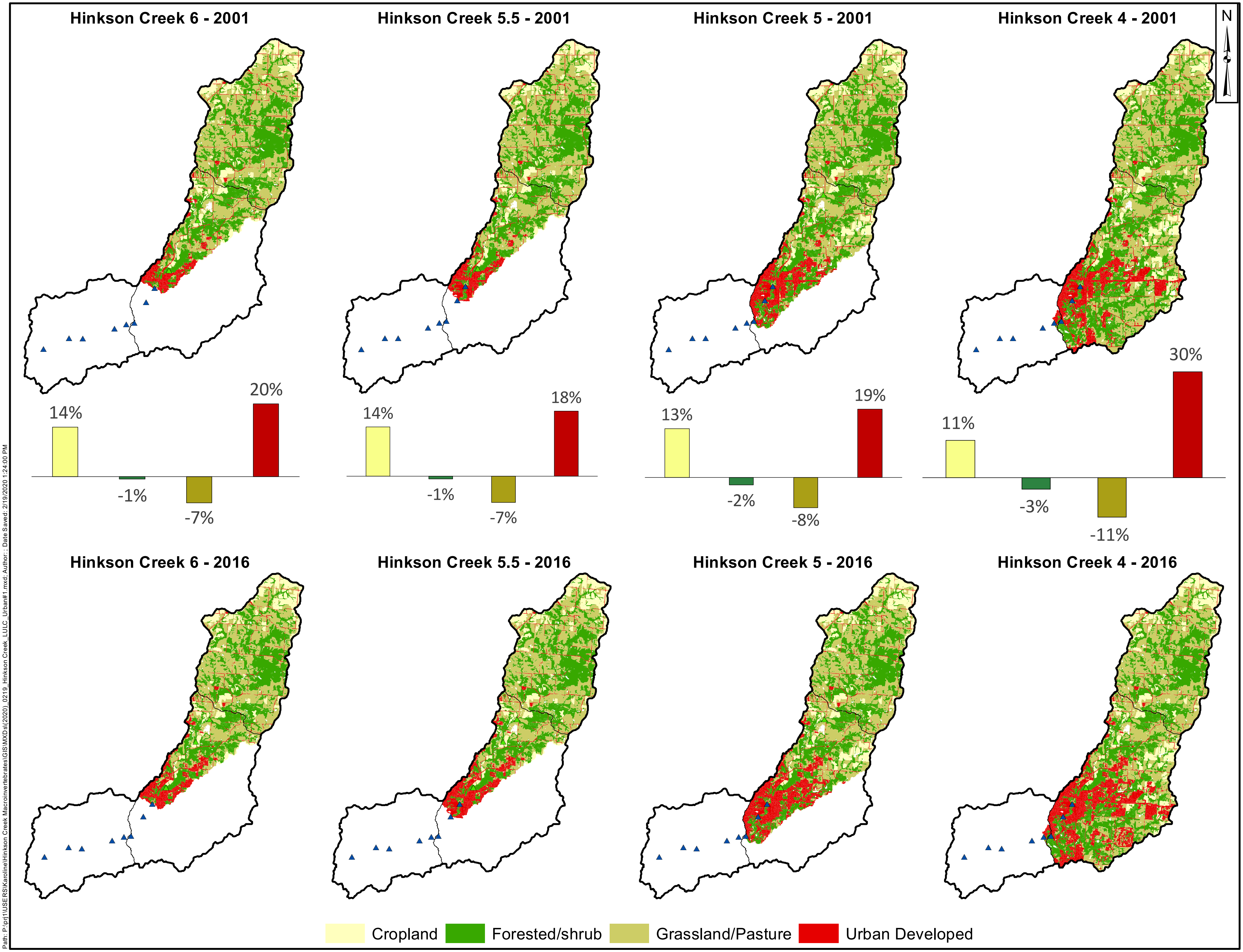

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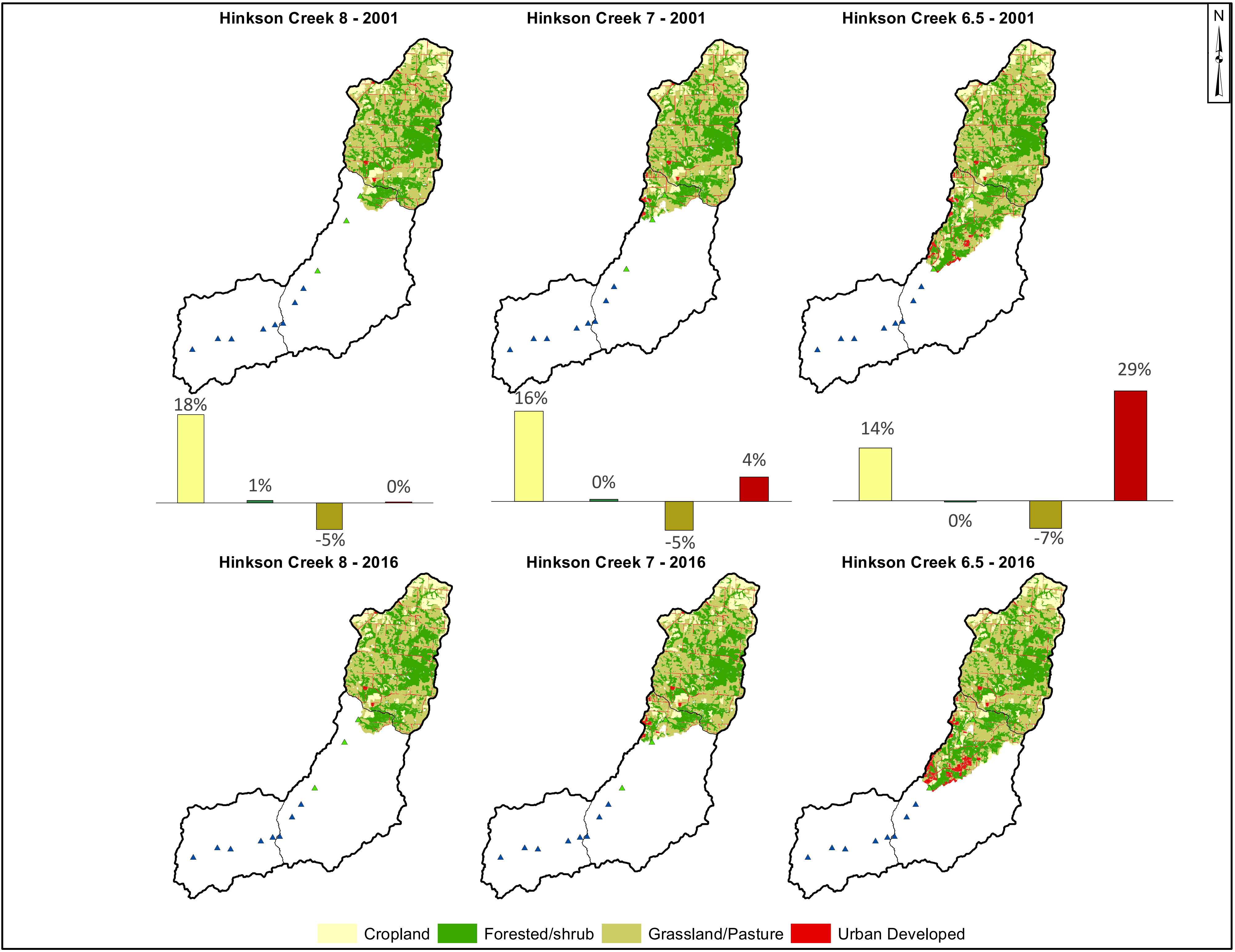

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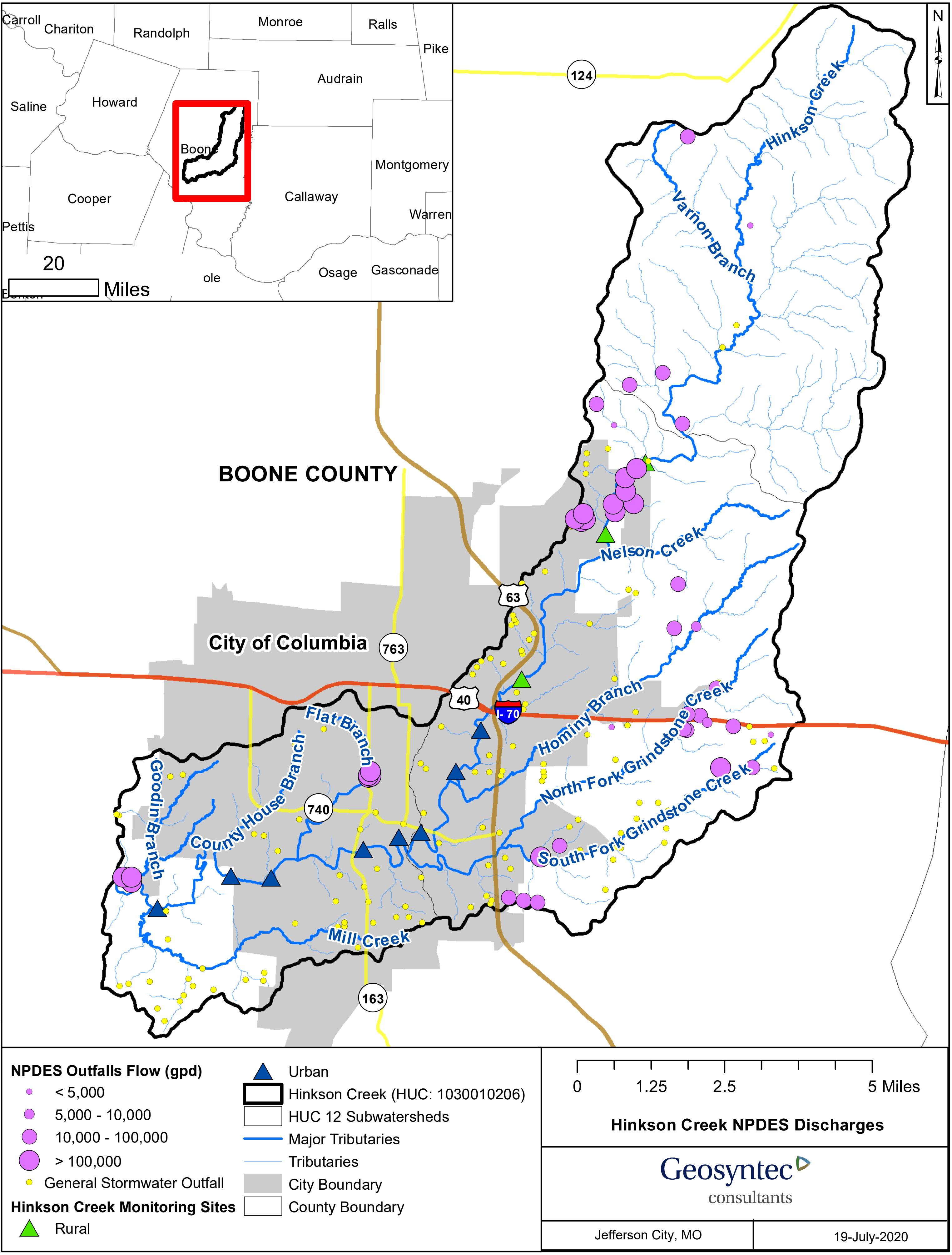
APPENDIX D



Hinkson Creek NPDES Discharges

Geosyntec
consultants

Jefferson City, MO

19-July-2020

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APPENDIX E

Appendix E: Fall Season Macroinvertebrate Metric Statistics per Treatment Group.

Treatment Group (# Samples)	**Control (#12)**				**Hinkson Creek Rural (#9)**				**Hinkson Creek Urban (#40)**				**Reference (#6)**			
Data Range	**2001 – 2017**				**2003 – 2016**				**2001 – Fall 2017**				**2001 - 2011**			
Metric Statistics	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**
Total Taxa Richness	60.0	83.0	72.7	72.5	63.0	94.0	77.2	75.0	55.0	87.0	70.1	70.0	75.0	83.0	78.5	78.0
Diptera Richness	28.0	40.0	34.9	34.5	33.0	39.0	35.7	36.0	21.0	42.0	32.4	33.0	30.0	37.0	34.2	34.0
Chironomidae Richness	21.0	32.0	27.5	27.0	27.0	33.0	28.6	28.0	14.0	33.0	26.1	27.0	25.0	31.0	27.8	27.5
EPT Richness	9.0	15.0	11.7	11.0	9.0	19.0	15.3	16.0	8.0	18.0	12.4	12.0	13.0	17.0	15.2	15.0
Ephemeroptera Richness	5.0	9.0	7.2	7.5	6.0	10.0	8.4	9.0	6.0	11.0	7.5	7.0	6.0	11.0	8.5	8.5
Plecoptera Richness	0.0	1.0	0.2	0.0	0.0	1.0	0.2	0.0	0.0	1.0	0.1	0.0	0.0	1.0	0.5	0.5
Trichoptera Richness	2.0	6.0	4.3	4.0	3.0	9.0	6.7	7.0	1.0	11.0	4.9	4.0	4.0	8.0	6.2	6.5
Clinger + Climber Richness	23.0	37.0	31.3	30.5	26.0	40.0	34.6	35.0	24.0	40.0	30.8	31.0	30.0	40.0	36.2	37.5
% Diptera	17.0	54.1	34.0	33.9	23.5	37.5	28.6	27.6	11.5	52.4	31.8	32.7	17.5	36.4	29.1	30.1
% Chironomidae	14.1	52.2	31.2	30.9	20.3	35.0	25.7	25.8	10.9	51.4	29.4	29.7	16.0	34.8	26.9	27.5
% Tanytarsini	2.2	14.2	6.9	6.5	1.8	8.7	4.8	4.6	1.7	22.1	7.9	6.2	5.5	8.8	6.9	6.7
% Oligochaeta	2.6	17.6	9.6	9.4	1.5	10.7	5.0	3.9	1.2	37.8	7.5	5.2	1.5	9.5	4.5	4.1
% Corbicula	0.0	0.3	0.1	0.0	0.0	1.0	0.1	0.0	0.0	12.4	3.5	2.5	0.0	0.0	0.0	0.0
% Other Diptera + Non-Insects	8.3	31.1	22.6	26.7	12.8	26.0	19.9	20.6	6.5	43.7	20.1	18.2	11.9	23.4	17.2	17.4
% EPT	13.5	48.1	27.9	28.5	20.7	43.7	32.0	34.6	10.5	52.1	31.8	31.2	20.7	43.9	36.2	41.3
% Ephemeroptera	9.2	28.0	16.7	14.9	8.4	33.1	19.5	20.6	9.9	36.6	21.6	21.2	14.9	36.9	29.2	32.8
% Plecoptera	0.0	1.1	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.4	0.2	0.0
% Trichoptera	1.9	27.6	11.1	7.4	1.6	19.8	12.5	12.8	0.5	22.5	10.2	9.4	4.9	12.6	6.9	6.0
% EP	9.3	28.0	16.8	15.0	8.4	33.1	19.5	20.6	9.9	36.6	21.6	21.2	15.4	37.3	29.3	32.8
% Hydropsychidae	0.9	22.2	6.2	3.7	0.1	13.9	6.1	5.8	0.3	21.8	8.1	7.2	0.6	7.3	3.3	3.3
% Clingers + Climbers	46.0	79.0	64.2	65.8	57.2	75.3	65.5	64.6	30.2	82.1	67.4	68.7	63.3	75.4	69.5	70.4
% Filterers	4.6	36.2	18.4	13.7	7.3	30.3	15.9	13.9	6.8	43.9	20.6	18.7	7.4	16.7	11.5	11.7
% Predators	5.2	21.0	10.6	10.4	8.6	16.5	13.2	14.2	7.1	21.0	12.7	11.7	9.6	22.6	16.4	16.2
% Scrapers	12.4	38.0	23.1	19.0	9.2	38.2	24.1	26.3	5.3	33.9	19.2	18.8	3.8	32.4	16.6	13.9

Treatment Group (# Samples)	Control (#12)				Hinkson Creek Rural (#9)				Hinkson Creek Urban (#40)				Reference (#6)			
Data Range	**2001 – 2017**				**2003 – 2016**				**2001 – Fall 2017**				**2001 - 2011**			
Metric Statistics	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**
% Shredders	4.3	32.3	11.2	8.5	2.6	13.6	8.3	8.8	3.6	17.4	10.7	10.9	3.8	19.4	9.9	8.3
% Dominant Taxon	9.6	33.1	18.5	17.2	10.6	27.8	20.0	21.6	10.0	35.2	16.7	15.9	14.4	26.1	20.7	20.6
% Dominant 2 Taxa	18.7	41.1	29.3	29.4	20.3	34.3	28.9	30.1	18.7	42.2	27.4	25.5	21.4	35.3	30.1	31.6
% Dominant 5 Taxa	37.3	56.5	48.8	50.8	37.9	53.1	47.6	48.5	36.4	60.8	47.2	46.2	38.8	52.9	47.2	47.6
% Intolerant (≤ 4) Taxa	1.4	17.0	7.5	7.5	2.2	13.0	7.8	7.9	0.4	11.4	4.7	4.3	2.5	7.6	5.0	4.8
EPT/Chironomidae Ratio	0.4	1.9	1.0	0.9	0.9	1.7	1.2	1.1	0.3	4.8	1.3	1.1	0.6	2.6	1.5	1.3
Scraper/Filterer Ratio	0.4	3.9	1.9	1.6	0.3	4.3	2.1	1.9	0.1	3.3	1.2	1.2	0.4	2.9	1.5	1.5
% Fast Seasonal Development	24.3	69.4	41.7	39.9	28.1	46.2	34.8	34.9	18.6	66.5	43.5	43.8	37.4	61.6	47.0	45.4
% Slow Seasonal Development	11.5	37.1	22.9	23.3	20.2	41.7	29.5	27.5	7.6	40.2	25.3	25.0	14.1	32.0	25.4	27.1
% Ability to Exit as Adults	14.6	52.4	31.7	31.6	20.4	35.1	26.4	26.4	11.8	51.5	30.4	30.2	16.3	35.4	27.8	28.6
% Rare in Drift	9.6	26.3	17.0	16.0	13.7	37.0	24.9	23.8	6.1	40.2	18.7	17.3	14.8	29.6	23.0	22.5
% Abundant in Drift	16.0	57.0	33.7	32.1	20.9	36.9	27.6	26.7	14.9	61.7	36.1	36.2	29.6	57.0	42.0	40.5
% No Swimming Ability	49.2	74.2	59.2	56.3	38.5	68.7	56.3	55.0	30.4	72.4	53.6	56.1	29.6	65.6	46.2	47.2
% Strong Swimming Ability	0.4	9.0	2.9	1.9	0.9	10.0	3.1	2.3	0.9	13.0	5.5	4.6	1.1	5.8	2.4	2.0
% Streamlined Body Shape	5.3	20.1	11.3	10.5	6.8	16.5	11.4	11.4	5.9	26.2	14.4	14.4	5.3	21.6	12.3	9.4
% Sprawlers	7.6	19.6	13.6	13.2	4.7	28.1	17.8	18.5	6.9	36.3	17.7	15.4	13.7	42.1	29.6	35.0
Missouri Biotic Index	6.3	7.2	6.7	6.7	6.1	7.2	6.6	6.6	6.2	7.7	6.8	6.8	6.4	7.0	6.7	6.7
Macroinvertebrate Biotic Index	5.1	6.1	5.4	5.4	5.0	5.7	5.2	5.2	4.8	7.4	5.5	5.4	5.2	5.7	5.3	5.3
Shannon Diversity Index	2.9	3.5	3.2	3.2	2.5	3.5	3.1	3.2	2.9	3.5	3.3	3.3	3.2	3.4	3.3	3.3
Deposited Sediment Tolerance Index	1.2	2.0	1.7	1.8	1.2	2.0	1.6	1.5	1.2	2.4	1.5	1.5	1.2	1.7	1.4	1.3

Appendix E: Spring Season Macroinvertebrate Metric Statistics per Treatment Group.

Treatment Group (# Samples)	Control (#17)				Hinkson Creek Rural (#23)				Hinkson Creek Urban (#56)				Reference (#10)			
Data Range	**2002 – 2017**				**2002 – 2017**				**2002 – 2017**				**2001 – 2012**			
Metric Statistics	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**
Total Taxa Richness	59.0	86.0	73.6	75.0	54.0	81.0	71.2	72.0	59.0	83.0	68.1	67.0	60.0	92.0	75.7	76.5
Diptera Richness	32.0	44.0	38.4	39.0	29.0	44.0	36.4	36.0	29.0	44.0	36.1	36.0	20.0	39.0	32.6	34.0
Chironomidae Richness	26.0	36.0	31.4	32.0	18.0	37.0	28.9	30.0	23.0	38.0	31.0	31.5	15.0	34.0	27.0	27.5
EPT Richness	8.0	19.0	13.3	13.0	9.0	17.0	12.9	13.0	4.0	17.0	9.3	9.0	10.0	21.0	16.5	18.0
Ephemeroptera Richness	2.0	8.0	4.6	4.0	3.0	8.0	5.5	5.0	1.0	7.0	4.9	5.0	4.0	10.0	6.5	7.0
Plecoptera Richness	3.0	5.0	4.2	4.0	0.0	5.0	2.1	2.0	0.0	2.0	0.6	1.0	0.0	6.0	3.8	4.5
Trichoptera Richness	1.0	10.0	4.5	5.0	1.0	8.0	5.3	5.0	1.0	9.0	3.8	4.0	3.0	11.0	6.2	6.0
Clinger + Climber Richness	21.0	38.0	29.2	28.0	19.0	35.0	29.0	29.0	19.0	34.0	26.0	26.0	22.0	42.0	32.3	32.0
% Diptera	21.2	79.6	59.3	61.7	28.0	89.0	59.2	67.5	28.4	89.2	69.1	72.2	22.0	84.9	38.2	35.9
% Chironomidae	18.9	78.4	57.0	59.3	14.1	86.9	51.9	51.5	25.2	86.8	65.5	67.5	15.4	83.8	33.7	30.9
% Tanytarsini	2.2	10.9	5.9	5.6	0.3	18.5	8.9	8.0	2.2	34.4	13.2	11.7	1.0	10.1	4.4	3.4
% Oligochaeta	0.6	20.0	5.9	5.5	1.0	16.1	4.8	3.2	0.3	32.9	6.5	5.1	0.8	13.9	4.2	3.2
% Corbicula	0.0	0.0	0.0	0.0	0.0	1.3	0.1	0.0	0.0	4.2	0.7	0.2	0.0	0.6	0.1	0.0
% Other Diptera + Non-Insects	6.5	37.1	17.9	16.8	3.4	38.7	17.5	13.7	3.1	40.7	14.0	11.7	6.4	24.8	16.2	15.8
% EPT	1.5	36.7	14.0	12.1	3.2	32.3	17.8	17.1	0.6	40.2	11.3	8.4	4.6	64.5	34.2	30.3
% Ephemeroptera	1.0	19.4	7.8	6.9	1.4	30.6	14.6	15.3	0.1	38.7	9.8	6.7	3.8	50.7	25.0	22.2
% Plecoptera	0.2	16.8	5.4	4.6	0.0	11.4	2.0	1.1	0.0	5.3	0.4	0.1	0.0	15.1	7.3	8.1
% Trichoptera	0.0	1.9	0.8	0.8	0.1	2.2	1.2	1.3	0.1	5.8	1.0	0.9	0.4	4.7	1.9	1.1
% EP	1.4	36.2	13.1	11.0	1.9	30.8	16.6	16.3	0.1	39.4	10.2	7.3	3.8	63.4	32.3	27.8
% Hydropsychidae	0.0	0.4	0.1	0.1	0.0	0.8	0.2	0.0	0.0	2.7	0.4	0.2	0.0	0.8	0.3	0.2
% Clingers + Climbers	34.2	70.6	59.9	63.4	39.5	74.4	59.9	58.4	40.5	76.2	59.9	60.9	56.5	80.6	68.8	67.7
% Filterers	2.1	8.3	4.8	4.8	2.1	25.9	8.4	5.2	2.2	29.5	10.2	8.9	1.6	20.4	6.9	4.6
% Predators	4.5	19.5	11.7	9.7	4.7	19.1	9.8	8.8	4.2	19.8	11.0	10.4	7.0	19.3	14.4	14.7
% Scrapers	10.0	39.6	20.8	20.0	5.1	34.0	19.4	15.6	2.8	31.1	11.9	10.8	5.0	47.3	18.7	14.3
% Shredders	8.1	50.1	31.3	31.5	6.7	42.8	22.5	22.5	5.5	54.3	31.0	31.5	3.8	39.7	14.5	10.2
% Dominant Taxon	12.1	37.0	25.4	28.4	12.0	33.0	21.2	20.1	10.2	37.8	19.3	17.5	8.6	36.2	26.2	27.5

Treatment Group (# Samples)	**Control (#17)**				**Hinkson Creek Rural (#23)**				**Hinkson Creek Urban (#56)**				**Reference (#10)**			
Data Range	**2002 – 2017**				**2002 – 2017**				**2002 – 2017**				**2001 – 2012**			
Metric Statistics	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**
% Dominant 2 Taxa	22.9	53.9	39.0	40.9	22.7	51.3	33.5	32.2	17.9	51.6	31.0	30.2	16.5	44.7	36.8	38.0
% Dominant 5 Taxa	47.5	71.7	57.2	56.1	44.5	69.3	54.9	54.1	36.9	67.4	52.2	51.7	36.5	66.9	57.6	57.7
% Intolerant (≤ 4) Taxa	5.0	18.2	9.5	9.5	4.4	36.6	10.8	8.5	0.3	31.2	7.9	5.8	1.8	58.6	18.2	14.7
EPT/Chironomidae Ratio	0.0	1.9	0.4	0.2	0.0	2.2	0.5	0.3	0.0	1.2	0.2	0.1	0.1	4.2	1.4	1.0
Scraper/Filterer Ratio	1.3	18.0	6.0	4.5	0.5	13.5	4.4	2.4	0.3	7.1	1.7	1.2	0.7	30.2	6.2	1.8
% Fast Seasonal Development	31.7	83.3	62.6	62.5	22.8	92.4	60.5	68.5	29.8	91.1	70.7	73.4	28.5	86.2	48.5	46.5
% Slow Seasonal Development	1.1	26.4	11.1	9.6	3.2	36.4	16.7	16.5	1.3	36.5	11.0	8.3	3.9	45.1	25.8	27.0
% Ability to Exit as Adults	18.9	79.0	57.4	59.7	18.2	87.2	52.7	51.7	25.4	87.1	65.7	67.7	15.8	84.0	34.1	31.5
% Rare in Drift	1.4	15.7	8.6	9.1	3.1	32.4	17.4	16.7	2.0	36.7	11.1	8.0	3.6	38.0	22.2	22.7
% Abundant in Drift	18.9	78.4	57.3	59.3	14.1	87.0	52.3	54.1	25.3	88.0	65.7	67.7	15.4	84.5	36.3	33.8
% No Swimming Ability	28.4	90.1	70.0	73.9	42.5	90.1	70.7	71.2	41.8	93.1	77.0	80.6	28.8	89.5	52.6	52.4
% Strong Swimming Ability	0.0	2.8	0.7	0.3	0.0	12.4	2.0	1.6	0.0	4.0	0.7	0.5	0.0	33.3	6.1	1.6
% Streamlined Body Shape	1.2	16.4	6.2	5.9	1.5	15.1	5.9	5.0	0.6	14.6	4.2	3.7	2.9	46.3	13.3	11.5
% Sprawlers	3.0	17.5	9.4	8.8	5.0	33.2	17.9	16.2	4.6	37.7	15.2	12.8	10.4	48.6	22.6	16.5
Missouri Biotic Index	6.2	7.5	6.6	6.5	5.8	7.3	6.6	6.6	5.9	8.0	6.8	6.9	4.8	7.1	6.1	6.2
Macroinvertebrate Biotic Index	4.6	6.4	5.6	5.6	5.0	6.4	5.7	5.7	5.4	7.2	6.0	6.0	4.5	6.1	5.0	4.9
Shannon Diversity Index	2.5	3.4	3.0	3.0	2.7	3.4	3.1	3.1	2.6	3.4	3.1	3.1	2.6	3.5	3.0	3.0
Deposited Sediment Tolerance Index	1.3	1.8	1.5	1.5	1.3	2.0	1.7	1.7	1.2	2.1	1.6	1.5	1.2	2.0	1.5	1.5

APPENDIX F

Appendix F: Fall Season Treatment Groupings Unique Indicator Taxa.

Treatment Groupings	Taxon	Correlation Value	Control		HCr		HCu		Reference		Tolerance Value
			Total #	Freq.	Total #	Freq.	Total #	Freq.	Total #	Freq.	
Control	*Caecidotea*	0.858	165	0.83	5	0.11	11	0.13	6	0.17	8.0
Control	*Crangonyx*	0.855	113	0.83	0	--	10	0.08	0	--	8.0
Control	*Parametriocnemus*	0.793	35	0.67	0	--	7	0.15	0	--	3.7
HCr	*Helicopsyche*	0.821	17	0.25	245	0.89	292	0.50	0	--	0.0
HCr	Leptophlebiidae	0.731	1	0.08	17	0.67	2	0.05	2	0.17	2.0
HCu	*Corbicula*	0.939	8	0.33	13	0.33	1730	0.95	0	0.50	6.3
Reference	*Tricorythodes*	0.924	5	0.33	3	0.33	1217	0.83	1091	1.00	5.4
Reference	*Macromia*	0.858	2	0.33	0	0.22	3	0.15	11	1.00	6.7
Reference	*Choroterpes*	0.856	1	0.08	7	0.11	0	--	38	0.83	6.0
Reference	*Caenis hilaris*	0.816	0	--	0	--	0	--	504	0.67	7.6
Reference	Heptageniidae	0.8	0	--	0	--	5	0.05	18	0.67	4.0
Reference	*Nectopsyche*	0.781	0	--	4	0.22	2	0.05	32	0.67	4.1
Reference	*Macronychus glabratus*	0.745	2	0.08	1	0.11	56	0.45	20	0.83	4.7
Reference	*Helichus lithophilus*	0.743	12	0.42	2	0.22	23	0.35	52	0.67	5.5
Reference	*Ancyronyx variegatus*	0.707	0	--	0	--	0	0.03	10	0.50	6.9
Control, HCr	*Microtendipes*	0.948	274	1.00	34	1.00	73	0.48	7	0.50	6.2
Control, HCr	*Paratendipes*	0.883	303	0.92	26	0.78	44	0.50	10	0.50	5.3
Control, Reference	*Hexatoma*	0.873	95	1.00	12	0.44	7	0.18	7	0.67	4.7
HCr, HCu	*Centroptilum*	0.737	7	0.33	34	0.78	106	0.55	0	--	6.3
HCr, Reference	Pisidiidae	0.873	23	0.58	174	1.00	247	0.63	40	1.00	7.3
HCr, Reference	*Pseudochironomus*	0.787	3	0.25	41	0.78	59	0.48	8	0.83	4.2
HCr, Control, Reference	*Chimarra*	0.916	666	1.00	380	0.78	510	0.73	145	1.00	2.8
HCr, Control, Reference	*Glyptotendipes*	0.898	127	0.92	71	0.78	70	0.30	77	0.83	8.5
HCr, Control, Reference	*Hyalella azteca*	0.895	429	0.92	349	0.89	472	0.68	194	0.83	7.9
HCr, Control, Reference	Scirtidae	0.881	95	0.83	22	0.89	57	0.48	25	0.83	5.0
HCr, Control, Reference	*Triaenodes*	0.881	35	0.75	73	0.89	34	0.38	35	0.83	3.7
HCr, Control, Reference	*Hemerodromia*	0.867	30	0.75	30	1.00	68	0.55	41	0.83	6.0

Treatment Groupings	**Taxon**	**Correlation Value**	**Control**		**HCr**		**HCu**		**Reference**		**Tolerance Value**
			Total #	**Freq.**	**Total #**	**Freq.**	**Total #**	**Freq.**	**Total #**	**Freq.**	
HCr, Control, Reference	Planariidae	0.839	125	0.83	41	0.56	104	0.58	58	1.00	7.5
HCr, Control, Reference	*Tabanus*	0.736	9	0.92	24	0.78	30	0.60	7	0.83	9.7
HCr, HCu, Reference	*Polypedilum halterale*	0.941	33	0.67	152	0.89	853	0.95	125	0.83	7.2
HCr, HCu, Reference	*Berosus*	0.889	3	0.25	54	0.56	254	0.83	60	1.00	8.6
HCr, HCu, Reference	*Hydroptila*	0.759	3	0.17	6	0.33	128	0.63	12	0.83	6.2

Note: Freq. = Frequency of occurrence in samples.

Appendix F: Spring Season Treatment Groupings Unique Indicator Taxa.

Treatment Groupings	Taxon	Correlation Value	Control		HCr		HCu		Reference		Tolerance Value
			Total #	Freq.	Total #	Freq.	Total #	Freq.	Total #	Freq.	
Control	*Caecidotea*	0.923	706	0.94	16	0.17	114	0.54	15	0.44	8.0
HCu	*Corbicula*	0.78	0	--	21	0.13	496	0.79	8	0.11	6.3
Reference	*Acentrella*	0.805	63	0.24	270	0.35	39	0.21	721	0.78	4.0
Reference	*Macromia*	0.753	1	0.12	1	0.17	3	0.14	8	0.67	6.7
Reference	*Cladopelma*	0.705	0	--	2	0.09	4	0.07	12	0.56	2.5
Control, HCr	*Hexatoma*	0.752	70	1.00	46	0.57	2	0.07	3	0.33	4.7
Control, HCr	*Tabanus*	0.736	24	1.00	47	0.83	20	0.54	3	0.56	9.7
Control, Reference	*Crangonyx*	0.897	842	1.00	122	0.48	202	0.59	100	0.78	8.0
Control, Reference	*Isoperla*	0.884	627	0.88	49	0.30	7	0.05	279	0.67	2.0
Control, Reference	*Chimarra*	0.799	50	0.76	43	0.35	57	0.32	71	0.89	2.8
Control, Reference	Chloroperlidae	0.76	43	0.65	0	--	0	--	8	0.44	1.0
Control, Reference	*Larsia*	0.708	48	0.53	30	0.26	20	0.14	24	0.89	8.3
HCr, Hcu	*Ormosia*	0.716	8	0.24	153	0.52	154	0.57	3	0.11	4.6
HCr, Reference	*Peltodytes*	0.711	11	0.29	40	0.70	50	0.39	23	0.67	8.5
Hcu, Reference	*Argia*	0.88	6	0.35	20	0.57	264	0.95	40	0.56	8.7
Hcu, Reference	*Tricorythodes*	0.721	4	0.12	0	--	121	0.52	297	0.56	5.4
Control, HCr, Hcu	*Phaenopsectra*	0.793	34	0.53	54	0.57	199	0.71	2	0.22	6.2
Control, HCr, Hcu	*Acerpenna*	0.741	38	0.47	127	0.52	323	0.61	3	0.22	3.7
Control, HCr, Hcu	*Nilotanypus*	0.729	32	0.59	58	0.30	181	0.61	0	--	6.0
Control, HCr, Reference	*Hyalella azteca*	0.882	162	0.71	517	0.96	275	0.43	306	0.89	7.9
Control, HCr, Reference	*Perlesta*	0.868	342	0.88	295	0.74	233	0.41	355	0.78	0.0
Control, HCr, Reference	*Amphinemura*	0.843	78	0.82	77	0.57	4	0.07	66	0.89	3.4
Control, HCr, Reference	*Triaenodes*	0.725	23	0.41	50	0.65	13	0.16	12	0.56	3.7
Control, HCr, Reference	*Glyptotendipes*	0.718	71	0.59	26	0.52	60	0.30	17	0.78	8.5
Control, HCr, Reference	*Hemerodromia*	0.71	30	0.53	45	0.52	35	0.34	26	0.67	6.0
HCr, HCu, Reference	*Hydroptila*	0.819	2	0.06	25	0.39	211	0.79	22	0.78	6.2
HCr, HCu, Reference	*Berosus*	0.805	2	0.12	23	0.35	186	0.75	22	1.00	8.6

Note: Freq. = Frequency of occurrence in samples.