January 2016

Biotic Assessment Of Two Central Kentucky Streams: Examining The Effects Of Wastewater Treatment And Anthropogenic Disturbance

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BIOTIC ASSESSMENT OF TWO CENTRAL KENTUCKY STREAMS:

EXAMINING THE EFFECTS OF WASTEWATER TREATMENT AND ANTHROPOGENIC DISTURBANCE

By

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Submitted to the Faculty of the Graduate School of

Eastern Kentucky University

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

May, 2016

DEDICATION

My loving family, past and present, has made this possible.

I thank them for their support.

I am truly blessed.

ACKNOWLEDGEMENTS

I wish to thank the following individuals, without whom I could not have completed this thesis:

Steve Winkler, Sanitary Sewer Superintendent, Richmond Utilities

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Dr. Sherry Harrel, Eastern Kentucky University Department of Biological Sciences

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ABSTRACT

Globally, anthropogenic disturbance has altered many aquatic habitats, including lotic waters. Flowing, fresh water sustains life on Earth yet suffers the resulting waste products. Native, locally adapted ecosystems integrate or eliminate the byproducts of life. However an increase of human population, poor agricultural practices, accelerated overland runoff, a non-point source of pollution, and wastewater treatment plants (WTP), a point source of pollution, have all placed a strain on the world's flowing, fresh, waters. The de-commissioning of two WTPs in the Kentucky River basin, and the commissioning of a new WTP in an adjacent watershed, provided an opportunity to examine the effects of WTPs and land-use for potentially influencing stream degradation. Using multi-metric bioassessments for habitat, fishes, and macroinvertebrates this study sought to evaluate the relative health of both streams and establish a reference survey of the habitat and biota of these two streams, relative to the presence of a wastewater treatment facility. Although WTP activity has impacted both streams it is apparent that it is only one component responsible for the overall impairment of these streams.

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CHAPTER I

INTRODUCTION

Wastewater treatment plants (WTPs) serve as central collection points for untreated influent, and as discharge points of treated effluent. Through a region-wide system of sanitary sewers, modern and efficient WTPs collect and treat human biological waste, and are an important component of overall environmental health. Although treated wastewater may still contain antibiotics, pharmaceuticals, hormones, metals, surfactants, pesticides, and elevated levels of nitrates and PCBs (Allan, 2008), WTPs are the best solution for preventing untreated wastewater from entering surface and groundwater systems (Miller, 1977). Effluents can be very complex and variable mixtures, making their adverse effects on wildlife extremely difficult to predict.

Lotic waters are traditionally assessed according to the degree of functionality of the aquatic ecosystem, as evidenced by the efficiency of microbial decomposition of organic matter, elimination of organic carbon (OC), and release of plant nutrients in an organically polluted stream (Gucker, 2006). Modern WTPs efficiently remove OC, but removal of inorganic nutrients, primarily nitrogen and phosphorus, is economically and technologically limited (Gucker, 2006). Reducing the amount of phosphorus and nitrogen in WTP discharge water is a complex and dynamic process. Nutrient uptake efficiencies of streams are diminished by high nutrient inputs from the WTPs, retention by algae and vascular plants, and by surrounding land uses; thus, nutrient concentrations can remain high for long distances of the receiving stream (Gucker, 2006). These high nutrient concentrations may result in eutrophication of the stream, causing an increase in both autotrophic and heterotrophic processes, shifting the species composition and ultimately reducing biodiversity (Campbell, 2005); (Gucker, 2006). In addition, wastewater from sewage treatment plants often contains organic materials still being decomposed by microorganisms. The oxygen requirements of these microbes can dramatically lower the available oxygen in the receiving waters.

Urban streams often have higher algal biomass relative to less disturbed streams, attributed to the resulting increased nutrients, and increased light exposure to the stream (Wenger, 2009). Increased levels of nutrient and OC concentrations may cause the stream to become highly productive, as evidenced by the amount of fish and macroinvertebrate biomass (Goldstein, 1999), and can stimulate enough periphyton growth to alter the benthic habitat, leading to a cascade of higher trophic effects (Wenger, 2009). Dissolved Oxygen (DO) is a major limiting factor in the distribution of aquatic organisms, and in eutrophic waters much of the available DO is consumed during the high decomposition and respiration rates by algae, especially during the warm growing season. Streams can become hypoxic, exceeding the lower limits of biological oxygen demand (BOD), causing changes in activity, alternate habitat choice , facultative air breathing, and/or increased use of aquatic surface respiration, stressing organisms to the point of die-offs (Kramer, 1987).

A bioassessment of living organisms can be used as a measure of water quality or habitat (Rosenberg & Resh, 1993). Biological monitoring with multimetric indices relies on freshwater biota to assess uses the best indicator of human disturbance, the aquatic biota, to assess the health of a stream ecosystem (Karr J. R., 2000). Populations of differing species occupying the same ecosystem, collectively known as a community, are evaluated for their function or attribute. The use of multiple community attributes, such as abundance, distribution, and trophic makeup, has become widely accepted for assessing in-stream biological impairment (KDOW, 2002). Macroinvertebrates, in addition to fish and habitat, are most frequently used for this determination. The equally weighted metrics of Indices of Biotic Integrity are widely used for fish and macroinvertebrate communities to assess trends in species richness, species composition, trophic composition, and abundance over time at an individual site (Jones D. , 2001). Advantages of using fish as bioindicators include their ubiquitous distribution in all but the most impaired waters, multiple trophic levels, and extensive life-history information (Karr J. F., 1986). Disadvantages include gear bias related to water body size and fish size, and temporal-spatial movements. Additionally, stream size and zoogeography can mask water quality effects of land use on species composition and relative abundance (Messinger, 2001). Macroinvertebrates are ubiquitous in distribution, have relatively long life cycles, are sedentary, and many species exhibit a range of responses to disturbances and environmental stressors (Rosenberg & Resh, 1993). The combination of habitat assessment, fish surveys, and macroinvertebrate surveys yields a more complete determination of stream health.

Aquatic communities reflect existing and previous watershed conditions because they are sensitive to changes from many environmental factors (Karr J. F., 1986). Halting disturbance and other degradation does not ensure that a stream has regained biotic integrity, thus, the stream can only be considered restored if it can support a healthy, natural, biological community (Thomas J. , 2000). Streams that do not experience excess nutrient conditions have fish assemblages that tend to include herbivores while eutrophic streams, with large amounts of decaying biomass and heavy nutrient loading via WTP activity, are dominated by detritivores (Miranda, 2010). Macroinvertebrate communities are an important part of the aquatic food chain, typically made up of several functional feeding groups that break down organic material into nutrients for lower organisms, and provide forage for higher organisms (Miranda, 2010). Yet species composition in eutrophic conditions experiences an increase in scrapers, collector gatherers, and collector-filterers, and a decline in shredders, piercing herbivores, and predators. Areas of moderate to heavy siltation experience increased burrowing organisms such as Chironomids and Oligochaetes, and a decrease of riffle species such as Hydropsychids, Elmids, and Psephenids (Griswold, 1978).

Distributions of aquatic life may be changing rapidly due to environmental alteration yet baseline survey data is lacking for most stream systems (Ray, 1999). The continuous flow of effluent to a stream makes it an ideal ecosystem for studying the responses of aquatic organisms (Brooks, 2006). Biomonitoring has shown to be a reliable means of estimating the chronic biological effects of complex effluents on aquatic biota (Birge, 1989). The decommissioning of the outmoded Tates Creek WTP and the recent startup of the Otter Creek WTP on two streams in the Kentucky River system provided a unique opportunity to create an inventory of the aquatic organisms in these two streams, establish the degree of health of both streams, and examine plausible reasons for stream impairment. Using Kentucky Division of Water (KDOW) Rapid Biologic habitat assessment Protocol (RBP), physicochemical parameters, and Indices of Biotic Integrity (IBI), the relative health of these streams was determined. Otter Creek WTP began service in September 2010, replacing Tates Creek WTP and Dreaming Creek WTP (Winkler . pers.comm., 2013). The former Tates Creek WTP was originally operated as a secondary treatment plant (Borowski, 2016). The plant now operates as a collection point, pumping station, and storage facility with some primary treatment. Dreaming Creek WTP serves only as a collection point and pumping station. Otter Creek WTP is a tertiary treatment plant with an average daily flow of 8 million gallons and a peak hydraulic flow of 24 million gallons.

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CHAPTER II

METHODS AND MATERIALS

STUDY AREA

The Tates and Otter Creek watersheds are located in the Interior Plateau Geographic Province, Bluegrass Bioregion, of Central Kentucky and form high gradient, wadeable tributaries of the Kentucky River (Figure 1; Woods, 2002)[1]. The Tates Creek watershed drains 36.6 mi^2 (94.7 km^2) of northwest Madison County beginning in Richmond, KY near the former Tates Creek WTP (Figure 2). Along with its tributaries Tates Creek comprises a total of 28.6 stream miles (46.0 km), flowing mostly west through rural, rolling countryside, and emptying as a second order tributary into the Kentucky River at Valley View, KY (Kentucky Watershed Viewer, 2012). The upper reaches of Tates Creek are in the outer sub-region of the Bluegrass physiographic region, composed of undulating terrain, with moderate to rapid surface runoff, and moderate groundwater drainage rates (KY Water Research Institute, 2000). The lower reaches of Tates Creek are in the in the bluegrass sub-region of the Bluegrass physiographic region, composed of hilly terrain, with very rapid surface runoff, and slow groundwater drainage rates. Land use is primarily agricultural accounting for 85%, or 31.2 mi^2 (80.8 km^2). The remainder is a mix of residential and commercial uses. Five businesses hold Kentucky Pollutant Discharge Elimination System (KPDES) permits including the Tates Creek WTP, a hotel, a campground, and two gasoline stations. Arlington Golf course is permitted to withdraw water from Tates Creek, and has impounded tributaries to the upper reaches for irrigation (KY Water Research Institute, 2000).

The Otter Creek watershed drains 65.4 mi^2 (169.5 km^2) of north-central Madison County beginning south of Richmond, KY (Figure 3). Along with its tributaries Otter

[1] Figures located in Appendix B.

Creek comprises a total of 46.6 stream miles (75.0 km), flowing mostly north through eastern Richmond, and emptying as a third order tributary into the Kentucky River near Fort Boonesboro State Park (Kentucky Watershed Viewer, 2012). The upper reach, of the main stem, is impounded forming Lake Reba. Otter Creek is in the outer sub-region of the Bluegrass physiographic region, composed of undulating terrain, with moderate to rapid surface runoff, and moderate groundwater drainage rates (KY Water Research Institute, 2000). Land use is primarily agricultural accounting for 85%, or 55.7 mi^2 (144.3 km^2). The remainder is a mix of residential and commercial uses. Six businesses hold Kentucky Pollutant Discharge Elimination System (KPDES) permits including the Otter Creek WTP, a metal fabricator, a bus maintenance facility, a residential subdivision, a gasoline station, and a battery manufacturing and storage facility. No water withdrawal permits are known for Otter Creek (KY Water Research Institute, 2000).

SITE SELECTION

Habitat, fish, and macroinvertebrates were sampled at four sites along Tates and Otter Creeks. Fish communities were sampled during May and June, 2012 and again during October and November, 2012. Habitat assessment, using the Kentucky Division of Water (KDOW) Rapid Bioassessment Protocol (RBP), was performed concurrently with macroinvertebrate sampling during the summer index period for wadeable, high gradient streams, July and August, 2012. All sampling periods were consistent with KDOW guidelines (KDOW, 2011). At Tates Creek one sample reach was located approximately 17 km, or about 2/3 the distance downstream from the discharge of the WTP to the mouth of Tates Creek (TC1, distal to WTP discharge; access, bridge at Perkins-Ascraft Road, N37° 49’ 55.761” W84° 25’ 9.699”). A second site was located approximately 8 km, or about 1/3 the distance downstream from the discharge of the WTP to the mouth of Tates Creek (TC2, proximal to WTP discharge; access at Million Bible Church, N37° 46’ 46.186” W84° 23’ 9.898”). The third and fourth sites were located immediately below the WTP discharge (TC3), and immediately above the WTP discharge (TC4) (access at former WTP site, junction of TC3 & TC4, N37° 45' 47.400", W84° 19' 24.422").

At Otter Creek one sample reach was located approximately 12 km, or about 2/3 the distance downstream from the discharge of the WTP to the mouth of Otter Creek (OC1, distal to WTP discharge; access, pull-off along Red House Road (KY388), N37° 52' 48.922" W84° 16' 46.239"). A second site was located approximately 5 km, or about 1/3 the distance downstream from the discharge of the WTP to the mouth of Otter Creek (OC2, proximal to WTP discharge; access, bridge at Lost Fork Road, N37° 50' 5.839" W84 16' 22.248"). The third and fourth sites were located immediately below the WTP discharge (OC3), and immediately above the WTP discharge (OC4) (access at WTP, discharge, junction of OC3 & OC4, N37° 48' 5.984" W84°15' 38.975"). Sites were referenced using KDOW Watershed Viewer (Kentucky Watershed Viewer, 2012).

The arrangement of sampling sites above and below the former discharge at Tates Creek WTP, and above and below the discharge at the Otter Creek WTP, allowed for comparisons between an upstream reference site and the area downstream of the effluent discharge. Although Tates Creek WTP no longer discharges into Tates Creek the impairment of the area directly downstream of the WTP is expected to still persist, having had little time to recover, the expectation for both WTPs being that water quality, and biotic integrity (taxa richness, diversity, dominance), will be lower below the discharges than above. The decision to add two additional sites for each stream, one 1/3 and the other 2/3 the distance from each WTP discharge to the terminus of each receiving stream, is to be able to quantify and qualify the downstream persistence or dilution of effluent effects (Birge, 1989). Water quality and biological integrity is expected to improve moving downstream from each WTP. Findings similar to the WTP discharge site, however, would indicate effluent persistence although adjacent land uses may impact these downstream sites, as well.

To gain a better understanding of the potential effects of the WTP's on the receiving streams, an overview of the three main stages of the wastewater treatment process is described. For the primary stage of treatment, incoming effluent first goes through a screening machine that removes trash from the waste water and fecal matter (Dickenson, 2011). The resulting trash-free effluent is piped into a settling basin where solids, flotsam, and waste water stratify. The solids are removed from the bottom of the tank and either converted into an activated sludge for use in further treatment or placed in a solid waste facility. The flotsam, containing oils and fats, is skimmed from the top and separately processed. The resulting liquor is pumped into a lagoon for secondary treatment.

A secondary treatment disinfects and clarifies the water prior to discharge. Chlorine, ozone, and sometimes ultraviolet irradiation (used for secondary treatment at Otter Creek WTP) kill off excess microbes used in the treatment process, pathogenic bacteria associated with fecal matter, and indicator microbes such as benign strains of *Escherichia coli*. Using ultraviolet irradiation produces a less toxic discharge versus chlorine but it is less efficient in that layered microbes can effectively shield other microbes from irradiation. The water is oxygenated and released into a nearby water body. Tates Creek WTP operated as a secondary treatment facility prior to decommission. Currently the facility functions as a collection point, and pumping station, sending wastewater to the Otter Creek WTP for processing.

The Otter Creek Wastewater Treatment Plant goes through a tertiary treatment, prior to disinfection. Tertiary treatment seeks to reduce the level of nutrients available in the effluent liquor, mainly ammonia, nitrate, and phosphate, (Dickenson, 2011). The process relies on bacteria and protozoa converting the nutrients by feeding on the effluent. The addition of activated sludge also aids in the denitrifying process. Return and recycling flows can contain large amounts of nitrogen and phosphorus that organically overload the removal process, potentially exceeding the plant's discharge permit limits (Kang, 2008). Because of this possibility a portion of the microbe-rich water is returned to fortify activated sludge, providing microbes for the tertiary treatment cycle.

If the receiving water body is a well-planted, shallow, constructed wetland the effluent goes through an extended-tertiary (sometimes referred to as quaternary) treatment process. Rooted emergent plants (e.g. bulrush *Scirpus* spp., and cattail *Typha* spp.) uptake, utilize, and store much of the nutrients and contaminants while providing substrate for both aerobic and anaerobic microbial communities that assimilate constituents in the wastewater (Water Environment Foundation, 2011). Shallow, standing water allows sediment to settle and is further broken down by anaerobic microbes below the sediment, the standing water being further clarified by aerobic microbes, before the effluent enters the receiving stream. In the United States an average of 20% of directly released secondary and tertiary treated effluents receive less than 10-fold instream dilution; during low flow conditions this average rises to 60% (Brooks, 2006).

Three water quality studies, one in Tates Creek (Borowski, 2016), and two in Otter creek (Crockett, 2015) and (Wolfe, 2016), measured nutrient and fecal microbe impacts in the streams. The data from Borowski, et al., is summarized in Table 1[2] and the data from Crockett & Borowski is summarized in Table 2. *Escherichia coli* are measured as a proxy or indicator of the presence of pathogenic bacteria.

SAMPLING – HABITAT AND PHYSICOCHEMISTRY

Relative stream habitat health was assessed at each site using a combination physicochemical parameters and Rapid Bioassessment Protocol (RBP) (KDOW, 2011). Physicochemical measurements were taken at four transects per site; upper reach limit, lower reach limit, and at two riffle-run-pool combinations between each limit. These measurements concurred with spring and fall electrofishing. Targeted parameters water temperature (˚C), dissolved oxygen (DO; mg/l), pH, and conductivity (µmhos/cm^2) were measured in the thalweg of the sampling site using an YSI Professional Series multi-meter (Yellow Springs Instruments, Yellow Springs, OH.). Flow and channel profile

[2] Tables located in Appendix B.

were determined using a Marsh-McBirney model 2000 portable flow meter (Marsh-McBirney, Inc., Frederick, MD.), a top-set wading rod, and a meter stick (Central Scientific, Chicago, IL). Stream width (m) was measured and water flow velocity and depth were recorded at five equidistant points along each of the four perpendicular transects (Figure 4). Global positioning system (GPS) coordinates of all sample sites and transects were recorded using a DeLorme Earthmate PN-40 hand-held GPS unit (DeLorme, Inc.).

Rapid Habitat Assessment Protocol (RBP) examines the quality of the habitat that directly influences the biotic integrity of the stream, and should accompany any biological sampling (KDOW, 2011). An additional benefit of the RBP is the temporal documentation of physical changes to a stream sampling reach. Procedures outlined by KDOW (2011) were used to evaluate the biological quality of the stream and riparian habitat. High-gradient Bioassessment Stream Visit sheets were filled out streamside while wading each of the sampling site reaches. Land uses adjacent to each stream reach were recorded on the RBP stream visit sheet. Concurrently canopy cover was assessed for each sampling site using a GRS Densitometer (Geographic Resource Solutions, Arcata, CA.). The GRS densitometer is used to determine canopy presence or absence. Measurements were taken at ten transects, perpendicular to the stream, and readings (0% or 100%, absence or presence) of canopy cover were taken at one meter intervals across the width of the stream (Adikari, 2015). Transect results were used to determine the average canopy cover for each site. Canopy cover is an important factor in limiting light, limiting heating, and providing habitat. Partially shaded streams generally have the highest species diversity, for example, wadeable streams with 50% to 75% have sufficient shade to support indigenous organisms (KDOW, 2011).

SAMPLING - FISH

Recommended sampling protocol for fishes indicates a minimum distance of 100 meters from bridge crossings, unless the purpose of obtaining the fish community data is related to these influences (KDOW, 2010). In this study, where land use is a potential impact, these sampling sites were appropriate. Bridge crossings provided access for three sites. Care was taken to assure that these three bridge-associated sites were consistently upstream, lessening potential impacts.

Each sample reach consisted minimally of two riffles, two runs, and two pools. Fishes were sampled using a Smith-Root LR-24 backpack electro-fisher (Smith-Root, Inc., Vancouver, WA) during summer and fall sampling events. Sampling was performed in a downstream to upstream direction, sweeping from bank to bank, engaging the shocker near substrate, undercuts, and pools in order to sample all available habitats. Each site was electro-fished for approximately 2,000 seconds, over 200 stream-meters. One pass was made over the entire stream reach, taking care to budget the allotted 2,000 seconds evenly. Fishes were collected with dip nets and placed in aerated buckets until revived. They were then identified, counted, recorded, and released. A comprehensive measure of abundance and species richness was determined for each site.

SAMPLING - MACROINVERTEBRATES

Sampling for macroinvertebrates consisted of a composited, semi-quantitative, riffle sample and a composited, multi-habitat sample. For semi-quantitative sampling a 600µm mesh kick net, was used to collect benthic macroinvertebrates. Four $0.25m^2$ kick net samples, one in each of four riffles, were taken within the thalweg (KDOW, 2011). All four riffle samples were composited and combined, field-elutriated using a 600µm mesh wash bucket, transferred to a three-gallon plastic bag, labeled, and preserved with 95% ethanol. For qualitative sampling, benthic macroinvertebrates were collected from four separate rifle/run/pool complexes using an 800µm x 900µm D-frame dip net. Targeted habitat included undercut banks/root mats, sticks/wood, leaf packs, silt/sand/gravel, *Aufwuchs*, marginal and instream vegetation, and bed/slab rock. Where available five pieces of coarse woody debris, ranging in length from 3 to 6 m and 5 to 15 cm in diameter, were picked and rinsed into the wash bucket. In addition five large cobbles from each riffle, run, and pool, were picked and also rinsed into the wash bucket (KDOW, 2011). All multi-habitat samples were composited and field-elutriated using a 600µm mesh wash bucket, transferred to a three-gallon plastic bag, labeled, preserved with 95% ethanol, and sealed (Braccia . pers.comm., 2012).

Macroinvertebrates collected via semi-quantitative sampling were sub-sampled in the laboratory according to KDOW guidelines (KDOW, 2011). A segmented tray, and random number generator were used to sub-sample each site. Additional tray segments were randomly chosen, as necessary, to achieve the minimum of 300 specimens. Large, rare organisms from the entire semi-quantitative material were added to the qualitative sample for each site, and then coarsely picked for taxa absent in semi-quantitative sub-samples (Braccia . pers.comm., 2012). All specimens were identified to the lowest practical level, using the most current KDOW Master Taxa list as a taxonomic reference (KDOW, 2011).

DATA ANALYSIS

RBP metric scoring consists of ten visual evaluations for each sampling site ranking in-stream habitat, channel morphology, bank stability, and riparian vegetation on a scale from 0 (lowest) to 20 (highest). Condition categories are qualified as Poor (0-5), Marginal (6-10), Suboptimal (11-15), and Optimal (16-20) (KDOW, 2011). Documentation of physicochemical conditions, RBP score, and canopy cover provides an opportunity to monitor physical changes of the stream sampling reach.

The Kentucky Index of Biotic Integrity (KIBI) for fish was used to score the condition of the streams (KDOW, 2003). Core metrics included Native Richness (NAT), Darter, Madtom, and Sculpin Richness (DMS), Intolerant Richness (INT), Simple Lithophilic Spawners (SL), Relative Abundance of Insectivorous Individuals, excluding

Tolerant Individuals (%INSCT), Relative Abundance of Tolerant Individuals (%TOL), and Relative Abundance of Facultative Headwater Individuals (%FHW). These metrics are considered to be sensitive to different levels, types, and combinations of environmental stressors providing data on the abundance and diversity of tolerant species, intolerant species, indicator species, and trophic composition (Allan, 2008). Calculation of the KIBI converts these quantitative results to a qualitative biotic score, indicative of the condition and water quality of the stream. Richness and biodiversity of the fish community was assessed using the Shannon-Wiener Diversity Index (Krebs, 1999). In addition, the Jaccard's Similarity Index, ranging from completely dissimilar (0), to identical (1), was used to compare fish assemblages between sites, and between streams (Allan, 2008). For the spring and fall sampling periods Jaccard's was calculated comparing the above and below discharge sites of Tates Creek WTP (TC4 and TC3), the above and below discharge sites of Otter Creek WTP (OC4 and OC3), and the below discharge site of Tates Creek WTP (TC3) with the below discharge site of Otter Creek WTP (OC3).

Originally developed for fishes the adaptable Index of Biotic Integrity (IBI), gave rise to the Macroinvertebrate Bioassessment Index (MBI) (Karr J. R., 2000). For this study seven core metrics of Taxa Richness (TR), Ephemeroptera-Plecoptera-Trichoptera Richness (EPT), Modified Hilsenhoff Biotic Index (mHBI), Modified percent EPT abundance (m%EPT), Percent Ephemeroptera (%Ephem), Percent Chironomidae+Oligochaeta (%Chir+%Olig), and Percent Primary Clingers (%Clingers) were used to calculate MBI scores (KDOW, 2011). These metrics are considered to be sensitive to different levels, types, and combinations of environmental stressors providing data on the abundance and diversity of tolerant species, intolerant species, indicator species, and trophic composition (Allan, 2008). Calculation of the MBI converts these quantitative results to a qualitative biotic score, indicative of the condition and water quality of the stream.

CHAPTER III

RESULTS

HABITAT AND PHYSICOCHEMISTRY

Primary land use in the Tates Creek and Otter Creek watersheds is a mix of urban development and agriculture, including pasture, livestock, crops, manicured parks, golf courses, the city of Richmond, Kentucky and associated development (Table 3) (Table 4). RBP habitat scoring ranks Tates Creek as poor-to-fair, and Otter Creek as fair-to-good (Figure 5). Mean canopy cover, assessed in conjunction with macroinvertebrate sampling, ranged from 43% at TC2 to 95% at TC4 (Figure 6) and from 32% at OC2 to 61% at OC3 (Figure 7). Bedrock was the dominate substrate at all Tates Creek sampling sites (Figure 8) and at OC3 and OC2 (Figure 9). Cobble was dominant at OC4 and at OC1. Siltation was heavy to very heavy, and algal cover on substrate was light, at all Tates Creek sampling sites Siltation was moderate while algal cover on substrate was heavy in Otter Creek.

Physicochemical measurements are summarized Table 5 and Table 6. Spring DO levels ranged from 5.06 mg/l at TC3 to 10.20 mg/l at TC2 and from 5.59 mg/l at OC4 to 9.22 mg/l at OC1 and OC2. Fall DO levels ranged from 11.00 mg/l at TC3and TC4 to 12.30 mg/l at TC1 and from 9.40 mg/l at OC3 to 14.21 mg/l at OC2. Spring pH levels ranged from 8.49 at TC1 to 8.65 at TC2 and from 8.41 at OC3 to 9.83 at OC2. Fall pH levels ranged from 8.58 at the TC3 to 8.90 at TC2 and from 8.37 at OC3 to 9.55 at OC2. Spring conductivity values ranged from 431 µmhos/cm^2 at TC2 to 737 µmhos/cm^2 TC3 and from 502 µmhos/cm^2 OC4 to 1039 µmhos/cm^2 at OC2. Fall conductivity values ranged from 374 µmhos/cm^2 at TC1 to 492 µmhos/cm^2 at TC3 and from 468 µmhos/cm^2 at OC2 to 732 µmhos/cm^2 at OC3. Average water depth for spring sampling ranged from 102mm at TC4 to 285mm at TC1 and 115mm at OC4 and OC3 to 281mm at OC1.

Average velocity for spring sampling was 0.00 m/s at all Tates Creek sampling sites and ranged from 0.04 m/s at OC1 to 0.34 m\s at OC3. Average water depth for fall sampling ranged from 111mm at TC 4 to 263mm at TC1 and from 99mm at OC4 to 178 at OC1. Average velocity for fall sampling from 0.01 m/s at TC3 to 0.06 m/s at TC4 and from 0.20 m/s at OC4 to 0.36 m/s at OC2.

FISH

Spring electro-fishing for Tates Creek sampling sites yielded a total of 2,662 fishes consisting of 19 species, representing 6 families (Table 7). Fall electro-fishing yielded a total of 3,848 fishes consisting of 15 species, representing 5 families (Table 8). Total species identified from both spring and fall sampling was 20. Spring taxa richness was lowest at TC4 with 7 species, and highest at TC2 with 15 species (Figure 10). Fall taxa richness was lowest at TC3 with 6 species, and highest at TC1 with 13 species (Figure 11). Shannon-Wiener index values for the spring sampling event ranged from a low of 1.35 at TC4 to a high of 2.13 at TC2. Shannon-Wiener index values for the fall sampling event ranged from a low of 1.21 at TC2 to a high of 1.75 at TC1. KIBI results for the spring sampling event ranged from 37 (Fair) at TC1 to 60 (Excellent) at TC3 (Figure 12). KIBI results for the fall sampling event ranged from 40 (Fair) at TC1 to 59 (Excellent) at TC4 (Figure 13). Six species accounted for 93% of individuals identified during spring electrofishing at Tates Creek (Figure 14). In order of total abundance across all sampling reaches of Tates Creek are the creek chub (*Semotilus atromaculatus*), fantail darter (*Etheostoma flabellare*), bluntnose minnow (*Pimephales notatus*), central stoneroller (*Campostoma anomalum*), rainbow darter (*E. caeruleum*), and Western mosquitofish (*Gambusia affinis*). Six species accounted for 90% of individuals identified during fall electrofishing at Tates Creek (Figure 15). In order of total abundance across all sampling reaches are the bluntnose minnow, central stoneroller, striped shiner (*Luxilus chrysocephalus*), rainbow darter, scarlet shiner (*Lythrurus fasciolaris*), and western mosquitofish.

Spring electro-fishing for Otter Creek yielded 2,683 fishes consisting of 23 species, representing 6 families (Table 9). Fall electro-fishing yielded a sample size of 5,643 individuals consisting of 18 species, representing 7 families (Table 10). Total species identified from both spring and fall sampling was 24. Spring taxa richness was lowest at OC3 with 11 species, and highest at OC1 with 19 species. Fall taxa richness was lowest at OC3 with 12 species, and highest at OC1 with 15 species. Shannon-Wiener index values for the spring sampling event ranged from a low of 1.60 at OC3 to a high of 2.05 at OC1. Shannon-Wiener index values for the fall sampling event ranged from a low of 1.46 at OC4 to a high of 1.89 at OC1. KIBI results for the spring sampling event ranged from a low of 34 (Fair) at OC2 to high of 43 (Fair) at OC1. KIBI results for the fall sampling event ranged from a low of 36 (Fair) at OC2 to a high of 42 (Fair) at OC4. Six species accounted for 86% of individuals identified during spring electrofishing at the Otter Creek sampling sites (Figure 16). In order of total abundance across all sites are the central stoneroller, rainbow darter, western mosquitofish, fantail darter, bluntnose minnow, and creek chub. Six species accounted for 91% of individuals identified during fall electrofishing at the Otter Creek sampling sites (Figure 17). In order of total abundance across all sites are the central stoneroller, bluntnose minnow, rainbow darter, fantail darter, western mosquitofish, and the striped shiner.

During spring sampling the above discharge and proximal downstream sites of Otter Creek WTP (OC4 and OC2), scored a 1.0 indicating complete similarity for fish communities at both sites (Figure 18). Comparison of the above and below discharge sites of Tates Creek WTP (TC4 and TC3) produced a coefficient of 0.875, and the above and below discharge sites of Otter Creek WTP (OC4 and OC3) produced a coefficient of 0.769. Comparing the below discharge site of Tates Creek WTP (TC3) with the below discharge site of Otter Creek WTP (OC3) rendered a value of 0.727. During the fall sampling the comparison of the above and below discharge sites of Tates Creek WTP (TC4 and TC3) yielded a value of 0.857. The above discharge and proximal downstream sites of Otter Creek WTP (OC4 and OC2) have a similarity of 0.800. Comparison of the above and below discharge sites of Otter Creek WTP (OC4 and OC3) produced a coefficient of 0.733. Comparing the below discharge site of Tates Creek WTP (TC3) with the below discharge site of Otter Creek WTP (OC3) rendered a value of 0.500.

Fish community makeup, divided by predatory and generalist functional feeding groups, is shown for Tates Creek in Figure 19 and Figure 20. Spring sampling at all of the Tates Creek sites were dominated by predators while fall sampling at all of the Tates Creek sites were dominated by generalists. Spring sampling (Figure 21) at OC4, OC3, and OC1 was dominated by predators while fall sampling (Figure 22) at OC4, OC3, and OC2 was dominated by generalists.

MACROINVERTEBRATES

Laboratory identification following macroinvertebrate sampling at the Tates Creek sites yielded 1,340 individuals, representing 19 orders, 44 families, and 62 taxa (Table 11). Taxa richness ranged from a low of 30 at TC3 to a high of 37 at TC2. Ephemeroptera-Plecoptera-Trichoptera (EPT) richness ranged from a low of 3 at TC3 to a high of 9 at TC2. Only one Plecopteran individual was collected, Perlidae (*Acroneuria* sp.), at TC1. Modified Hilsenhoff Biotic Index ranged from a low of 5.58 at TC2 to a high of 7.44 at TC3 (Figure 23). Percent of Chironomidae-Oligochaeta ranged from a low of 9% at TC4 to a high of 16% at TC3. Percent of primary clingers ranged from a low of 10% at TC3 to a high of 76% at TC1. MBI results ranged from a low of 10.45 at TC3 to a high of 32.80 at TC2 (Figure 24). Five taxa (four species) of macroinvertebrates accounted for 57% of individuals collected and identified at the Tates Creek sampling sites (Figure 25). In order of total abundance of the combined Tates Creek sampling reaches are the Elmidae-beetle larvae (*Stenelmis* sp.), amphipod (*Crangonyx* sp.), caddisfly (*Cheumatopsyche* sp.), mayfly (*Caenis* sp.), and Elmidae beetle adult (*Stenelmis* sp.). Other select macroinvertebrate species are illustrated in Figure 26. Note that larvae and adults are only separated to illustrate the dominant taxa for Tates Creek. When calculating biotic indices larvae and adults of the same species were combined and considered as one taxon.

Laboratory identification following macroinvertebrate sampling at the Otter Creek sites yielded 1,801 individuals representing 20 orders, 32 families, and 53 taxa (Table 12). Taxa richness ranged from a low of 29 at OC2 to a high of 37 at OC4. Ephemeroptera-Plecoptera-Trichoptera (EPT) richness ranged from a low of 6 at OC1 to a high of 9 at OC4. Modified Hilsenhoff Biotic Index ranged from a low of 5.48 at OC1 to a high of 6.18 at OC4. Percent of Chironomidae-Oligochaeta ranged from a low of 19% at OC4 to a high of 34% at OC3. Percent of primary clingers ranged from a low of 53% at OC3 to a high of 69% at OC1. MBI results ranged from a low of 28.29 at OC3 to a high of 29.51 at OC2. Five species of macroinvertebrates accounted for 76% of individuals collected and identified at the Otter Creek sampling sites (Figure 27). In order of total abundance of the combined Otter Creek sampling reaches are the Elmidae-beetle larvae (*Stenelmis* sp.), midge (Chironomidae), and three caddisfly larvae (*Cheumatopsyche* sp., *Hydropsyche* sp., and *Hydroptila* sp). Other select macroinvertebrate species are illustrated in Figure 28.

CHAPTER IV

DISCUSSION

HABITAT AND PHYSICOCHEMISTRY

Agriculture, development, and erosion have reduced and even eliminated the riparian vegetation and over-story of both streams. A desirable range for canopy cover is 50% to 75% (KDOW, 2011). Above and below the discharge sites of the Tates Creek WTP (TC4 and TC3) canopy coverage was 95% and 86%, respectively (Figure 6). Canopy cover for TC4 was 100% Amur honeysuckle (*Lonicera spp.)* and for TC3 was 75% Amur honeysuckle. Most likely as a result of shading, these stream reaches experienced low productivity (light amounts of algae and moss), and lower biomass (lower fish and macroinvertebrate abundance). The above and below discharge sites of Otter Creek WTP (OC4 and OC3) (Figure 7), and the most downstream site of Tates Creek WTP (TC1), scored at 59%, 61% and 70%, respectively, falling within the desired canopy cover range. Canopy composition at these three sites was also primarily Amur honeysuckle. These five sites, exhibiting better than 50% shading, were sparsely represented by native trees such as box elder (*Acer negundo*), willow (*Salix* sp.), green ash (*Fraxinus pennsylvanica*), and sycamore (*Platanus occidentalis*) (Jones R. , 2005). The proximal and distal sites of Otter Creek WTP (OC2 and OC1), and the proximal downstream site of Tates Creek WTP (TC2), scored at 32%, 45%, and 43%, respectively, falling below the desired canopy range. These three sites had the widest channels, and the least amount of riparian vegetation, of all eight sites. However the presence of western or Nutall's waterweed (*Elodea nuttallii)*, at the above and below discharge sites of Tates Creek WTP (TC4 and TC3), was a positive and unexpected discovery as it is listed as "Threatened" by the Kentucky State Nature Preserve Commission (Jones R. , 2005).

PH is one of the most important environmental factors limiting the distribution of species in aquatic habitats. Although different species flourish within different ranges of pH, optimal range for most aquatic organisms falls between pH 6.5-8.0. U.S. E.P.A. water quality criteria for pH in freshwater suggest a range of 6.5 to 9.0. Fluctuating pH or sustained pH outside this range reduces biological diversity in streams as it physiologically stresses many species and can result in decreased reproduction, decreased growth, disease, or death (EPA CADDIS, 2013). The pH measurements for both streams range from 8.4 to 9.8, comparable to baking soda or sea water. A pH > 9.0 magnifies the effects of ammonia, a byproduct of excessive nutrient input, and can also damage the gills and the slime coat of fish. A pH > 10.0 is possibly fatal to fish and other aquatic organisms. Potential sources of elevated pH, per U.S. E.P.A., include inputs that exist within both watersheds. Agriculture, urbanization, and industry waste enters streams by leaching into groundwater or via storm-water runoff (EPA CADDIS, 2013). Another source of alkalinity, limestone, is common throughout both watersheds and forms the beds of both streams. The proximal downstream site of Otter Creek WTP (OC2) had the highest pH measurements for spring and fall at 9.83 and 9.55, respectively. OC2 also had, by far, the highest conductivity reading overall at 1039 µmhos/cm^2. The left hand bank at OC2 is a crumbling, 50 m, limestone cliff.

Below normal precipitation during 2012 made measuring flow and depth somewhat difficult. Sampling events had to be scheduled following precipitation events, after the initial flooding returned to within-bank levels. In Tates Creek during both the spring and fall sampling events flow ranged from -0.02 m/s to 0.06 m/s; this represents essentially no flow. Otter Creek flow measurements ranged from 0.04 m/s to a maximum flow for the entire study of 0.36 m/s. Aside from the weather this difference in flow can be explained two ways. First of all Tates Creek is a smaller, second order stream, draining an area of 94.7 km^2, versus Otter Creek, a third order stream, which drains and area of 169.5 km^2. Secondly the majority of all potable water used by residential, business, and public customers, connected to the Otter Creek WTP sanitary sewer system, ends up passing through the WTP and is discharged into Otter Creek. It is to be expected that this constant discharge keeps the water flowing in Otter Creek.

Seasonal DO levels fluctuate with water temperature. Cold water holds more oxygen than warm water making aquatic animals most vulnerable to lowered DO levels when stream flows are low, water temperatures are high, and aquatic plants have not been producing oxygen. DO concentrations also can determine whether excess nitrogen, from animal sources, forms ammonia, nitrate, or nitrite (Hynes, 1970). Nitrates are most common but the compounds change with relative ease with DO concentrations being a major factor. DO concentrations and iron (Fe) availability are the primary parameters affecting the release of phosphorous and its ability to bind and form soluble reactive phosphorous (SRP), the most biologically available form of phosphorous (Miranda, 2010). Dissolved oxygen levels below 3.0 mg/l are too low for fish population survival (Montana Science Partnership, 2013). Between 3.0 mg/l and 5.0 mg/l, conditions are stressing, tolerable for only twelve to twenty-four hours. Spawning can occur as levels rise above 6.0 mg/l, and those over 7.0 mg/l promote growth and activity. Dissolved oxygen levels greater than 9.0 mg/l can provide for abundant fish populations.

At 5.06 mg/l the below discharge site of the Tates Creek WTP (TC3) qualified as stressful. Sites in both streams were bare of vegetation, relying on algae as the source of photosynthetic oxygen; Otter Creek consistently exhibited high levels of algal cover. And although Otter Creek WTP oxygenates the discharge plume 200 meters from its confluence with Otter Creek (Winkler . pers.comm., 2013), levels of DO were consistently lower at the below discharge site of Otter Creek WTP (OC3), although not low enough to constitute stress. Similarly the below discharge site of Tates Creek WTP (TC3), consistently had the lowest readings for Tates Creek. Much cooler instream temperatures lead to the measurement of higher concentrations of dissolved oxygen during the fall 2012 sampling period.

Temperature readings were unremarkable for both streams during both the spring and fall sampling events with the exception of fall sampling above and below the discharge sites of Otter Creek WTP (OC4 and OC3). Immediately above the discharge (OC4) the water temperature was 9.9°C, consistent with temperatures at the proximal

(OC2, 9.0°C) and distal (OC1, 7.3°C) sites of Otter Creek. Directly below the discharge (OC3) the water temperature was 16.0°C, a difference of +6.1°C. No such spike was noted regarding spring temperature measurements, inferring that when the receiving waters are seasonably cooler the treated water, exiting the treatment buildings and culvert, adds relative warmth to the stream at the discharge.

Conductivity is a measure of the ability of water to pass an electrical current via ionized inorganic dissolved solids (EPA Water: Conductivity, 2013). Discharges to streams can change the conductivity depending on their make-up. A failing sewage system, direct and indirect inputs from agricultural, or runoff from urban environments, would raise the conductivity because of the presence of chloride, phosphate, and nitrate. In addition warmer water has higher conductivity. Stream conductivity is also affected by the geology of the area through which the water flows. Streams that run through areas with limestone bedrock tend to have higher conductivity because of the continual dissolution of the rock. In addition, streams that run through areas with clay soils tend to have higher conductivity because of the presence of materials that ionize when washed into the water. Indications are that streams supporting good mixed fisheries have a range between 150 and 500 µmhos/cm^2.; ≤ 750 µmhos/cm^2 is the desired range. Conductivity outside this range indicates unsuitable habitat for certain species of fish and macroinvertebrates. Rivers in the United States generally range from 50 to 1500 µmhos/cm^2 and Industrial waters can range as high as 10,000 µmhos/cm^2.Conductivity measures for Tates and Otter Creeks indicate a seasonal variation. Spring conductivity measurements for both streams ranged from a low of 431 µmhos/cm^2 to a somewhat-unhealthy 1039 µmhos/cm^2 at the proximal downstream site of the Otter Creek WTP (OC2). As mentioned before the left-hand bank of OC2 is a crumbling, 50 m, limestone cliff. Spring levels at both below discharge sites (TC3 and OC3) were within the desired range, ≤ 750 µmhos/cm^2. However spring conductance levels were so high in parts of some sampling reaches that the electro-fishing equipment shut down with an “Inverter Overload Error”, requiring reduction of shocking voltage. This includes part of the aforementioned site OC2 as-well-as part of the distal downstream site of the Otter Creek WTP (OC1) which produced a measurement of 806 µmhos/cm^2. Electro-fishing problems also occurred at the discharge, and for first 20 m downstream, of the below discharge site of Otter Creek WTP (OC3). Although OC2 averaged only 656 µmhos/cm^2 over the 200 m stream reach measurements, at the discharge, were around 1000 µmhos/cm^2. Fall conductivity measurements ranged from 374 µmhos/cm^2 to 732 µmhos/cm^2, well within the range for a healthy stream. Another difference may be linked to agricultural, lawn, golf course and park maintenance as these activities are typically concentrated in the spring of the year, and discontinued by fall. The only observed correlation between WTP activity and elevated conductivity is that the below discharge site of Otter Creek WTP (OC3), had the highest conductivity measurement for the fall at 732 µmhos/cm^2, well within range. A second Otter Creek site, the distal downstream site of the Otter Creek WTP (OC1), produced a fall reading of 654 µmhos/cm^2. The remaining 6 sites, in both streams, produced fall conductivity levels ≤ 500 µmhos/cm^2.

Silt coverage was heavy in Tates Creek during both spring and fall sampling. The above and below discharge sites of Tates Creek WTP (TC4 and TC3) were very heavy with silt primarily a result of unrestricted access to both reaches by cattle. At the distal downstream site of Tates Creek WTP (TC1) the siltation cover was heavy. The left-hand bank was unstable for the entire stream reach and past the upstream limit of this site. Low flow was also a contributing factor allowing sediment to settle and accumulate. Silt coverage was light to medium in Otter Creek during both spring and fall sampling. Although no livestock had access at any of the 4 sites on Otter Creek I believe the lower levels of silt coverage are attributable to the higher overall flow. More flow keeps sediments suspended in the water column and moving towards the Kentucky River. The constant throughput of water at Otter Creek WTP aids in maintaining flow and, possibly, flushing siltation from the stream.

Algal cover was light in Tates Creek during both spring and fall sampling. However Otter Creek consistently exhibited high levels of algal cover including many areas of eutrophication. It would be easy to say that nutrient loading is higher in Otter

Creek because of the WTP. But sewage was still leaching into Tates Creek during my sampling from the old lagoons at the WTP. And cattle freely and frequently relieved themselves in the stream. The difference was light. Canopy cover at the above discharge, below discharge, and distal downstream sites of Tates Creek WTP (TC4, TC3, and TC1) was much higher than any site at Otter Creek. The combination of high nutrient loading, and a more open canopy, could explain the high production of algae in Otter Creek.

RBP for habitat score ratings are described in and illustrated in Figure 5. RBP scores were “poor” above and below the Tates Creek WTP discharge (TC4 and TC3), “fair” above and below the Otter Creek WTP discharge (OC4 and OC3), improving to “fair” for the downstream sites of Tates Creek (TC2 and TC1), and improving to “good” for the downstream sites of Otter Creek (OC2 and OC1). Little or no riparian buffer zones, lack of substrate types, unstable banks, hydrogeology, and missing flow regime components kept the scores low.

FISH

The Commonwealth of Kentucky contains habitat that is utilized by 244 native, and 19 introduced, species of fish (Thomas M. , 2011). Yet even though all species are not suited to the same habitat-type, many species have been excluded from once-suitable habitat due to environmental and anthropogenic disturbance. Streams with impaired water quality, and high nutrient content, can exhibit high productivity and biomass, and low species richness. Total fish sampled and identified fell just short of 15,000 (14,936), 5,345 (36%) sampled during the spring, and 9591 (64%) sampled during the fall. A total of 25 taxa were identified for both sampling events. It is possible that cooler water temperatures, higher DO concentrations, higher water levels, and recruitment could explain the disparity in seasonal abundance. Also spatial, temporal and seasonal movements along the continuum of the stream are other possibilities. This study did not examine this parameter; however, a tracking study could shed some light on this possible factor. The spring sampling yielded 2,662 individuals from Tates Creek, and 2,683 individuals from Otter Creek, a difference of only 21 individuals between the two streams. However fall sampling was much different yielding 3,948 individuals from Tates Creek and 5,643 from Otter Creek, a difference of 1,695. The explosive amount of primary production is likely another factor in explaining both the seasonal differences and stream-to-stream differences. The eutrophic and near-eutrophic conditions that existed from mid- summer through late fall in Otter Creek certainly provided abundant resources for those individuals that can take advantage. Both streams were essentially void of macrophytes. But while Tates Creek had some benthic algal growth Otter Creek had many areas where the bottom was covered in long mats of filamentous algae.

Taxa richness for both spring and fall sampling was lowest at the above and below discharge sites of the Tates Creek WTP (TC4 and TC3) and was highest at the distal downstream site of the Otter Creek WTP (OC1). The consistently low richness at TC4 and TC3 was due to low flow (even in the fall), low amounts of substrate, and the unrestricted access of livestock. .The difference in taxa richness, comparing site-to-site, is much more pronounced for fish (Figure 10) and (Figure 11) than macroinvertebrates (Table 11) and (Table 12). An additional consideration is the size of the Tates Creek watershed at the WTP. Although the Tates Creek watershed drains 36.6 mi^2 (94.7 km^2) the Tates Creek WTP is near the top of the watershed, 2.3 mi (3.7 km) from the source, the stream only receiving input from 3 mi^2 (7.8 km^2) of the watershed. As a result the above and below discharge sites of the Tates Creek WTP (TC4 and TC3) intermittently go dry, especially in dry years like 2012, certainly acting as a barrier to colonization of these stream reaches. In contrast Otter Creek WTP is located 8.9 mi (14.3 km) from the source, Otter Creek receiving input from 20.8 mi^2 (53.9 km^2) of the watershed. However, even the best sites had very low species richness??

The Shannon-Wiener Diversity Index (D) was used to explore species richness and diversity at each site. The range for D is from 0.0 to ~4.6, an index approaching 0.0 indicating little or no diversity in the population sampled. Looking at both spring and fall sampling results the range for Tates Creek was from 1.21 to 2.13, and the range for Otter Creek was from 1.46 to 1.89. These were consistent results for both streams but much lower than desired. Two results stand out. The proximal downstream site of Tates Creek WTP (TC2) had both the highest overall score during spring sampling at 2.13, and the lowest overall score during fall sampling at 1.21. This site has very little canopy cover (43%), and became very shallow between spring and fall sampling events, during the summer of 2012. The distal downstream site of Otter Creek WTP (OC1) had the second highest overall score during spring sampling at 2.05, and the highest overall score during fall sampling at 1.89. Water levels remained higher at this site during the summer of 2012 due to the proximity of OC1 to the confluence of Otter Creek with the Kentucky River. Additionally four species, discussed below, were unique to OC1 and may have influenced the consistently higher relative scores.

The KIBI was used to calculate a quality-indicating score reflective of the fish population structure of each site. Five sites, the distal downstream site of Tates Creeks WTP (TC1), and all four sites on Otter Creek, are rated as “Fair” by their KIBI. As a comparison RBP habitat scores for these sites were also “Fair”, except the proximal and distal downstream sites of Otter Creek WTP (OC2 and OC1), which were scored as “Good” habitat. The proximal downstream site of Tates Creek WTP (TC2) had a KIBI scored as “Good” and the above and below discharge sites of Tates Creek WTP (TC4 and TC3) were scored as “Excellent” by their KIBI. A RBP habitat ranking of “Fair” and a KIBI ranking of “Good” for TC2 are comparable. However RBP habitat rankings of “Poor”, combined with KIBI rankings “Excellent”, make the results for TC4 and TC3 somewhat puzzling. One possibility is the high proportion of darters at these two sites (39% of TC4 individuals and 17% of TC3 individuals). Another possibility is linked to limitations of the KIBI. The reliability and consistency of the KIBI is more uncertain when assessing sites that are approaching the extremes of the recommended drainage areas (2.0-300.0 mi^2) (KDOW, 2003). Tates Creek, at the upper reaches that make up the above and below discharge sites of Tates Creek WTP (TC4 and TC3), receives input from only 3.0 mi^2 (7.8 km^2). Streams with drainage areas <3.0 mi^2 tend to have fish communities dominated by tolerant species, naturally low abundances, and naturally low diversity. Most importantly these communities may show little discrimination between high and low quality streams. The result may be related to watershed area instead of anthropogenic factors. For this reason I believe that the KIBI scores for TC4 and TC3 are anomalies and are not reliable results.

The Jaccard's Coefficient of Community Similarity Index was used to calculate the degree of taxonomic similarity between two sites in terms of species presence or absence. Values range from 0.000 to 1.000 increasing as similarity increases. Each site was compared to one of the 7 other sites, included between streams, for both the spring and fall sampling events. During spring sampling the above discharge and proximal downstream sites of Otter Creek WTP (OC4 and OC2), scored a 1.000 indicating complete similarity for fish communities at both sites. This was the highest value for spring sampling. Comparison of the above and below discharge sites of Tates Creek WTP (TC4 and TC3) produced a coefficient of 0.875, and the above and below discharge sites of Otter Creek WTP (OC4 and OC3) produced a coefficient of 0.769. Comparing the below discharge site of Tates Creek WTP (TC3) with the below discharge site of Otter Creek WTP (OC3) rendered a value of 0.727. During the fall sampling the comparison of the above and below discharge sites of Tates Creek WTP (TC4 and TC3) yielded a value of 0.857. The above discharge and proximal downstream sites of Otter Creek WTP (OC4 and OC2) have a similarity of 0.800. Comparison of the above and below discharge sites of Otter Creek WTP (OC4 and OC3) produced a coefficient of 0.733. Comparing the below discharge site of Tates Creek WTP (TC3) with the below discharge site of Otter Creek WTP (OC3) rendered a value of 0.500.

All species identified are considered native (KDOW, 2002) although there has been some debate over the historical range of the western mosquito fish (*Gambusia affinis*) in Kentucky. This species has been introduced for the control of mosquito larva in lentic water bodies and, although native to some Kentucky streams, frequent escapes have made it extremely difficult to determine their original range (Harrel . pers.comm., 2012). Mosquito fish give live birth to between 2 and 6 broods of 60 young each per year. This is a potential problem as *G. affinis* is an indiscriminate insectivore, mosquito larva being only part of its diet. If introduced it is in direct competition with insectivores already present.

At the distal downstream site of Otter Creek WTP (OC1) a spotted bass (*Micropterus punctulatus*), a channel catfish (*Ictalurus punctatus*), and a stonecat (*Noturus flavus*) were identified during spring sampling. These three species were part of the 19 different species identified from OC1 during spring sampling. Given that spring and fall sampling yielded a total of 25 species from all 8 sites it is no surprise that at 19 OC1 was, by far, the overall richest site for both periods. Fall sampling at OC1 produced 32 gizzard shad (*Dorosoma cepedianum*). None of these species were seen at the other 7 sites. As mentioned above these, unique to site OC1, may have something to do with this site's relatively higher diversity values. The proximity of OC1 to the confluence of Otter Creek with the Kentucky River, and the interaction with this higher order stream, would explain the presence of fish species typically considered common in larger water bodies.

Six species accounted for 81% of all fish sampled; the central stoneroller Cyprinidae (*Campostoma anomalum*), the fantail darter Percidae (*Etheostoma flabellare*), the rainbow darter Percidae (*E. caeruleum*), the creek chub Cyprinidae (*Semotilus atromaculatus*), the bluntnose minnow Cyprinidae (*Pimephales notatus*), and the striped shiner Cyprinidae (*Luxilus chrysocephalus*). *C. anomalum* and *P. notatus* consume detritus, filamentous algae, and insects, especially midge larva (chironomids) (Etnier, 2001). *L. chrysocephalus* feeds on filamentous algae and insects.

E. flabellare, *E. caeruleum*, and are primarily insectivores specifically of midge larva (chironomids), caddisfly larva (Hydropsychidae), amphipods, and isopods. *S. atromaculatus* feeds on large insects and small fish as does the green sunfish (*Lepomis cyanellus*). The only truly piscivorous fish, the rock bass (*Ambloplites rupestris*), the smallmouth bass (*Micropterus dolomieu*), and the Kentucky bass (*M. punctulatus*) were only found during spring sampling and only aggregated 14 individuals. The channel catfish (*Ictalurus punctatus*), is piscivorous but also will forage on large insects and algae. The one individual, sampled during the spring at the distal downstream site of Otter Creek WTP (OC1), was likely a transient from the nearby Kentucky River. Also straying from their home waters were the gizzard shad (*Dorosoma cepedianum*), planktonic feeders, that were counted during the fall sampling at OC1. The emerald shiner (*Notropis atherinoides*) feed on insects and algae, as does the yellow bullhead catfish (*Ameiurus natalis*), that also feeds on sewerage. The remaining species, the northern hogsucker (*Hypentelium nigricans*), the bluegill (*L. macrochirus*), the longear sunfish (*L. megalotis*), the spot-fin shiner (*Cyprinella spiloptera*), the silver-jaw minnow (*Ericymba buccata*), the scarlet shiner (*Lythrurus fasciolaris*), the big-eye shiner (*N. boops*), the stonecat (*Noturus flavus*), the greenside darter (*E. blennoides*), the logperch (*Percina caprodes*), and the western mosquito fish (*Gambusia affinis*) are all insectivorous. Most of these are indiscriminate insectivores while a few specialize on midge (chironomids), caddisfly (Hydropsychidae), and riffle beetle (Elmidae) larva. All these macroinvertebrates were found in large abundance.

Streams with high amounts of primary production classically have high abundances of detritivores and herbivores. In Tates and Otter Creeks the primary producer is algae, especially filamentous algae in Otter Creek. And although many of the fish identified in this study consume algae, none of the fish were said to forage on macrophytes, something lacking in both streams (Etnier, 2001). This narrow diversity of forage may exclude some herbivores, favoring only those herbivores that can utilize algae. Herbivores forage on the algae, detritivores feed on dead algae (and other matter), and small fish and macroinvertebrates use the algae for shelter. While this was true in both streams there really was a tri-dominance of trophic feeding groups. Insectivores, like *E. caeruleum* and *E. flabellare* for example, were also present in large numbers. This was due to the abundance of preferred prey such as caddisfly (Hydropsychidae) larva, midge (Chironomidae) larva, and isopods (Asellidae). Fish populations in both these streams were a mix of detritivores, herbivores, and insectivores. Generalist species like the "tolerant" *C. anomalum* that are able to forage as detritivores, herbivores, and insectivores were also a dominant part this ecosystem.

Seven species are simple lithophilic (SL) spawners, preferring to spawn over clean, gravel substrate (KDOW, 2002). The lack of suitable substrate indicates these are perhaps the most tolerant of the SL spawners. With resources and fish populations so abundant it may be sheer numbers that allow populations to overcome any impaired reproductive success. The northern hogsucker (*Hypentelium nigricans*), the striped shiner (*Luxilus chrysocephalus*), the emerald shiner (*Notropis atherinoides*), the big-eye shiner (*N. boops*), the greenside darter (*Etheostoma blennoides*), the rainbow darter (*E. caeruleum*), and the logperch (*Percina caprodes*) are all SL spawners.

Only 7 of the 25 overall species are considered "Tolerant" (KDOW, 2002). The green sunfish (*Lepomis cyanellus*), the bluegill (*L. macrochirus*), the striped shiner (*Luxilus chrysocephalus*), the big-eye shiner (*Notropis boops*), the creek chub (*Semotilus atromaculatus*), the yellow bullhead catfish (*Ameiurus natalis*), and the western mosquito fish (*Gambusia affinis*) are considered tolerant species. One metric of the KIBI considers the presence (and abundance?) of darters, madtoms, and sculpins (DMS). Present in large abundance was the rainbow darter (*Etheostoma caeruleum*) at 2,204 individuals, and the fantail darter (*E. flabellare*) at 1,533 individuals. Present in much smaller numbers were the greenside darter (*E. blennoides*) at 53 individuals, and the logperch (*Percina caprodes*) at 8 individuals. The one stonecat (*Noturus flavus*) was the only madtom, and the only "intolerant" species of the 14,936 individuals identified. No sculpins were encountered. The darters present in abundance, *E. caeruleum* and *E. flabellare*, exhibit higher tolerance than most darters and in fact forage on the insect larva, amphipods, and isopods abundant in impaired waters.

Areas within some sites were so impaired with deposited, sludge-like sedimentation that only Western mosquito fish (*Gambusia affinis*), facultative air breathers that can take advantage of the oxygen-rich surface film, existed as a monoculture. In spring sampling 37% of *G. affinis* came from the below discharge site of Tates Creek WTP (TC3) and 60% of *G. affinis* came from the below discharge site of Otter Creek WTP (OC3). During fall sampling 26% of *G. affinis* came from the below discharge site of Tates Creek WTP and 34% came from the proximal downstream site of

Otter Creek WTP (OC2). Two sites in the spring, and two sites in the fall, accounted for 97% and 60% of all *G. affinis* sampled, respectively. The below discharge site of Tates Creek (TC3) consisted, in part, of pasture with unlimited livestock access. Several pools, knee-deep in manure, yielded the majority of *G. affinis* collected during both spring and fall sampling events at this site. The *G. affinis* sampled at the below discharge site of Otter Creek WTP (OC3) came from two pools created by the uprooting of two large trees. These pools were opposite and 25 m downstream of the discharge. Although the low water during the spring provided only a small trickle of connection between these pools and the stream I don't feel that low DO was the reason for this concentration of *G. affinis*. It is possible that these pools were lower in DO concentration than the rest of the stream but measurements were not made to support this idea. Unlike the other three sites producing high concentrations of *G. affinis*, however, this site was not a monoculture. These pools also contained green sunfish (*Lepomis cyanellus*), striped shiners (*Luxilus chrysocephalus*), bluntnose minnows (*Pimephales notatus*), and creek chubs (*Semotilus atromaculatus*). The abundance of *G. affinis* in these two pools was likely a result of a preference by this species for lentic habitat. But, even though the proximal downstream site of Otter Creek WTP (OC2) showed no sign of livestock access, a condition similar to TC3 occurred. All *G. affinis* sampled at OC2 came from a pool, with a deep deposit of what appeared to be manure, which produced no other species.

MACROINVERTEBRATES

The Commonwealth of Kentucky has more lotic water than any other state other than Alaska (KY Film Office, 2014). Combined with several large impoundments, and countless ponds, Kentucky provides potential habitat for a wide variety of aquatic organisms. Macroinvertebrate species, like fish, are not all suited to the same habitat-type. Many species have been excluded from once-suitable habitat due to environmental and anthropogenic disturbance. Also like fish, macroinvertebrate communities in streams with impaired water quality, and high nutrient content, can exhibit high productivity and biomass, and low species richness. Collectively both streams yielded 3,141 individuals, identified as 73 different macroinvertebrate taxa. Sampling yielded 1,340 individuals from Tates Creek, and 1,801 individuals from Otter Creek, a difference of 461 (~15%) individuals. The explosive amount of primary production in Otter Creek is likely one of the factors in explaining the stream-to-stream difference. Otter Creek experienced eutrophic, and near-eutrophic conditions, from mid- summer through late fall. Tates Creek had some benthic algal growth but Otter Creek had many areas where the bottom was covered in long mats of filamentous algae. Both streams were essentially void of macrophytes. Otter Creek certainly provided abundant resources for those individuals equipped to use algae for forage and shelter. Another likely factor is the difference in the size of the streams. As previously mentioned Tates Creek, a second order stream, is supplied by 36.6 mi^2 (94.7 km^2) of watershed drained by a total of 28.6 stream miles (46.0 km). Otter Creek, a third order stream, is supplied by 65.4 mi^2 (169.5 km^2) of watershed drained by a total of 46.6 stream miles (75.0 km). Otter creek drains almost twice the area, and consists of nearly double the length of stream miles, of Tates Creek. Larger streams, with more primary production, provide more resources for more organisms.

Taxa richness was lowest at the proximal downstream site of Otter Creek WTP (OC2) at 29 individuals, and highest at the above discharge site of Otter Creek WTP (OC4) at 37 individuals (Table 11) and (Table 12). Richness numbers were enhanced by many specimens being collected only once, or in very small abundance, but from a variety of sites. Such a narrow range of results, only 8 individuals separate the most and least rich sites, indicates very little overall difference between sites. When comparing the above and below discharge sites of the Tates Creek WTP (TC4 and TC3) the results were 34 and 30, respectively, the difference attributable to several singular specimens. When comparing the above and below discharge of Otter Creek WTP (OC4 and OC3) the results were 37 and 31, respectively, a larger difference also attributable to several singular specimens. The difference in taxa richness, comparing site-to-site, is much less pronounced for macroinvertebrates than fish (Figure 10) and (Figure 11). Increasing taxa richness generally reflects increasing water quality, and increasing habitat diversity and/or suitability.

All macroinvertebrate taxa identified are considered native except for the Asian clam (*Corbicula fluminea*) (Cummings, 2010). At least one introduction came from the release of bilge water from Asian waters into the Great Lakes. Recreational boaters may have then transported live clams or glochidia from the Great Lakes to Kentucky waters. Although *C. fluminea* can outcompete native bivalves, especially fingernail clams (Sphaeriidae), their ubiquitous nature is owed to their high tolerance and high abundance in impaired waters. They thrive in nutrient rich streams and, due to their abundance, actually contribute to the filtering and cleaning the water. A large amount of *C.* fluminea, collected during qualitative sampling, was unaccounted for and discarded. Future metrics should account for this very large amount of discarded biomass.

Of the 3,141 individuals collected in both streams, five species accounted for 67% of the individuals sampled; the riffle beetle Elmidae (*Stenelmis* sp.) (larval and adult), the midge larva Chironomidae (Unidentified chironomid), the caddisfly larva Hydropsychidae (*Cheumatopsyche* sp.), the amphipod Crangonyctidae (*Crangonyx* sp.), and the caddisfly larva Hydropsychidae (*Hydropsyche* sp.). Both the larvae and adult Elmidae forage primarily by scraping algae from substrate, consuming the diatoms and bio-film associated with the algae (KDOW, 2002) (Allan, 2008). Chironomidae are burrowers and benthic collector-gatherers, generally increasing in abundance with increased siltation, consuming bits of fine particulate organic matter (FPOM) rich with protein from bio-film and microbes. Both Hydropsychidae genera identified for this study are collector-filterers. Ample algae provided these retreat-makers with plenty of raw material to build tent-like shelters. Hydropsychidae spin and attach a silk collection net to the shelter, filtering the water column for FPOM rich with protein from small autotrophs and microbes. The Crangonyctidae amphipods are swimmers and shredders. They break down coarse particulate organic matter, primarily leaves, from which they glean protein from fungus and microbes. The above and below discharge sites of Tates Creek WTP (TC4 and TC3) represented 28% of the macroinvertebrates collected from these two sites, and 97% of all the Crangonyctidae collected for this study. CPOM and potential predators are available at all eight sites. Their high abundance implies they could be foraging on the copious amounts of cow manure introduced into the stream at sites TC4 and TC3.

Ephemeroptera, Plecoptera, Trichoptera (EPT) are orders of generally pollution sensitive insects. Increasing EPT richness generally reflects increasing water quality, and increasing habitat diversity and/or suitability. EPT richness ranged from a low of 3 at the below discharge site of Tates Creek WTP (TC3), to 9 at the proximal downstream site of Tates Creek WTP (TC2) and the above discharge site of Otter Creek WTP (OC4). EPT taxa at TC3 was composed of 1 mayfly larva (Unidentified Baetidae), 1 caddisfly larva (*Hydroptila* sp.), and 9 caddisfly larva (*Cheumatopsyche* sp.) for a total abundance of 11 EPT individuals. In contrast EPT richness at TC2 included all 5 identified Ephemeropteran species, and 4 of the 5 identified Trichopteran species, for an EPT abundance of 160 individuals. EPT richness at OC4 included 4 of the 5 identified Ephemeropteran species, and all 5 of the identified Trichopteran species for an EPT abundance of 92 individuals. Plecoptera were virtually non-existent. Only 1specimen of this sensitive order, the stonefly Perlidae (*Acroneuria* sp.), was collected at the distal downstream site of Tates Creek WTP (TC1).

Modified Percent EPT Abundance (m%EPT) adjusts for the relatively tolerant and ubiquitous caddisfly genus *Cheumatopsyche* sp. by excluding this Trichopteran from the calculation. Removing the often abundant *Cheumatopsyche* sp. from the equation increases the sensitivity of this metric. *Cheumatopsyche* sp. accounted for 12% of all specimens collected for this study. Increasing m%EPT values indicate increasing water quality and/or habitat conditions. The below discharge site of Tates Creek WTP (TC3), with an EPT richness and EPT abundance of 2 after excluding *Cheumatopsyche* sp., had a dismal m%EPT of 1%. By comparison the below discharge site of Otter Creek WTP (OC3) had the second highest m%EPT at 24%. The site that co-ranked as highest in EPT richness at 9, the proximal downstream site of Tates Creek WTP (TC2), also had the highest m%EPT with 33%. At TC2, after excluding Cheumatopsyche sp., 94% of the EPT is represented by Ephemeroptera. TC2 was attractive to 73% of the mayfly larva Caenidae (*Caenis* sp.), and 57% of the mayfly larva Heptageniidae (*Maccaffertium* sp.), collected for the entire study. It was unexpected that Caenidae was only found in abundance at this one site. With operculate gills providing silt protection, and a relatively high tolerance value of 6.8, a wider distribution would have been expected. Something else is not to their liking at the other 7 sites. The above discharge sites of both Tates Creek WTP (TC4) and Otter Creek WTP (OC4) had the second lowest and lowest m%EPT at 4% and 14%, respectively, for each stream. Dewatering during the summer of 2012 likely explains the low m%EPT for TC4. The low m%EPT for OC4 is a bit puzzling. This site had a good mix of substrate, light siltation, and comparatively good hydrology. Algal cover, however, was heavy. At the upstream limit of OC4 the right-hand bank is the edge of a CSX railroad right-of-way. Five meters above the stream, and 10 meters away from the stream, CSX trains run several times a daily. This may have some acute influence to this stream reach but the overall implication is that unidentified anthropogenic inputs are coming from the 22.43 mi^2 (58.09 km^2) of the Otter Creek watershed upstream of this site.

Percent Ephemeroptera abundance (%Ephem) uses the relative abundance of mayflies to show impacts of metals and high conductivity. While generally associated with mining and oil well impacts it is also appropriate where urban inputs, such as lawn care and industrial runoff, may be influencing stream health. Decreasing %Ephem can be an indicator of the presence of brine and metal contamination. Seven of the sites scored ≤ 7% for %Ephem. The proximal downstream site of Tates Creek WTP (TC2), as with m%EPT, had the highest %Ephem at 31%. At TC2 Ephemeroptera represented 31% of the macroinvertebrates collected from this site, and 50% of all the Ephemeroptera collected for this study. With moderate siltation and algae cover TC2, the only site not to be rated “heavy” or greater in at least one of these parameters, may have provided the best Ephemeroptera habitat option of the 8 sites.

Percent Chironomidae+Oligochaeta (%Chir+%Olig) measures the relative abundance of these generally pollution tolerant organisms. Increasing abundance of these groups suggests decreasing water quality conditions. A total of 123 Chironomidae (including subfamily Tanypodinae), and 26 Oligochaeta, were collected from all four Tates Creek sites. The %Chir + %Olig proportions for Tates Creek ranged from 9.43% to 15.68%. In contrast a total of 152 Chironomidae (including subfamily Tanypodinae), and 3 Oligochaeta, were collected from one site, the below discharge site of Otter Creek WTP (OC3) alone. At 34% this was the highest %Chir + %Olig ratio in the entire study. The abundance of Chironomidae, and Oligochaeta was high at the other three Otter Creek sites as well. The above discharge site of Otter Creek WTP (OC4) produced 57 Chironomidae and 4 Oligochaeta for a %Chir + %Olig ratio of 19%. The proximal and distal downstream sites of Otter Creek WTP (OC2 and OC1) produced 107 and 116 Chironomidae and Tanypodinae, respectively, and 4 and 2 Oligochaeta, respectively. The %Chir + %Olig for OC2 and OC1 was 21% and 24%, respectively. Primary production likely explains the large abundance of this group. But the highest %Chir + %Olig ratio and highest abundance occurring at OC3 indicates a correlation with the WTP.

Percent Primary Clingers (%Clingers) measures the relative abundance of those organisms that need hard, silt-free substrates on which to "cling". Decreasing %Clingers is associated with increased levels of sedimentation and/or decreasing rock substrate. The above and below discharge sites of the Tates Creek WTP (TC4 and TC3) were composed of 89% and 84% hard substrate, respectively with very heavy siltation. However %Clingers for TC4 and TC3 were 35% and 10%, respectively (Figure 26).

Tates Creek WTP now functions as a storage and pumping facility. Very little is discharged from the plant into the stream. The relatively low %Clingers values for TC4 and TC3 were likely due to siltation, especially high due to livestock access, and below normal precipitation during the summer 0f 2012. TC4 and TC3 were the only sites judged to have very heavy siltation, in some cases approaching 50 cm in depth. The proximal and distal downstream sites of Tates Creek WTP (TC2 and TC1), were composed of 95% and 93% hard substrate, respectively, with moderate to heavy siltation. The %Clingers for TC2 and TC1 were 56% and 76%, respectively. The relatively high %Clingers value for TC1, the highest of all the sites in both streams, can be explained by one species. Larval and adult riffle beetles, Elmidae (*Stenelmis* sp.), comprised 54% (216 of 400) of the total organisms collected at TC1. Both the larva and the adults have operculate gills, and the ability to crawl, allowing them to protect their gills from sediment and move to clearer areas for respiration. The trend for Tates Creek indicates increased %Clingers, and less siltation, moving downstream.

The above and below discharge site of Otter Creek WTP (OC4 and OC3), were composed of 85% and 91% hard substrate, respectively , with light to moderate siltation. Sites OC4 and OC3, at 55% and 53% clingers, respectively, were the lowest %Clingers of the four Otter Creek sites (Figure 28). The proximal and distal downstream sites of Otter Creek WTP (OC2 and OC1), were composed of 95% and 88% hard substrate, respectively, with moderate siltation. The %Clingers for OC2 and OC1 were 65% and 69%, respectively. Although OC3 was the lowest %Clingers for all of Otter Creek but only 2% lower than OC4 upstream. This indicates no correlation with the WTP as both the above and below discharge sites essentially produced the same %Clingers. In addition OC3 was only 16% lower than the best Otter Creek site OC1 and 23% lower than the overall highest %Clingers at TC1 (76%). There is really no comparison between the below discharge sites, TC3 and OC3. The trend for Otter Creek indicates increased %Clingers, and less siltation, moving downstream.

The Modified Hilsenhoff Biotic Index (mHBI) summarizes the organic pollution tolerance of a benthic macroinvertebrate community. Tolerance values, having been regionally modified, are assigned to all macroinvertebrate species. The tolerance value for each species ranges from 0 to 10, with 10 being the most tolerant. The mHBI score, being an aggregate of species abundance and tolerance values, also ranges from 0 to 10. An increase in mHBI value indicates an increase in the relative abundance of pollution tolerant species in the macroinvertebrate community. Higher mHBI scores are indicative of decreasing water quality. Tates Creek, ranging from 5.6 to 7.4, indicates some variation between the four sites (Figure 23). However the below discharge site of

Tates Creek WTP (TC3) spiked at 7.4. Although some sewage leaching occurred during the demolition of Tates Creek WTP the problem here was the impairment to this site by years of livestock access; lots of standing water and manure-bottomed pools. The greatest abundance of amphipods (*Crangonyx* sp.), with a tolerance value of 7.2, were sampled at this site. Amphipods, the isopod (*Caecidotea* sp.) at 8.4, the midge larva (Tanypodinae) at 7.2, and the midge larva (Chironomidae) at 7.0, accounted for 65% of the individuals sampled from TC3. Otter Creek, ranging from 5.5 to 6.2, had no appreciable differences between any of the four sites. The below discharge site of Otter Creek WTP (OC3) scored a 6.0 indicating no correlation with WTP.

The MBI uses multiple community attributes, referred to as metrics, to assess instream biological impairment (KDOW, 2003). The metrics chosen are expected to contribute pertinent ecological information about the community under study. Metric combinations and scoring vary between ecoregions and stream sizes. This study used seven core metrics, as described in the methods section of this paper, recommended by the KDOW for high gradient, wadeable streams in the Bluegrass Bioregion. The MBI was used to calculate a score indicating the quality of the macroinvertebrate population structure of each site. The scoring criteria are represented in (

Figure 24). Two sites, the above and below discharge sites of Tates Creek WTP (TC4 and TC3), are rated “Very Poor” by their MBI score. Six sites, the proximal and distal downstream sites of Tates Creeks WTP (TC2 and TC1), and all four sites on Otter Creek (OC4, OC3, OC2 and OC1), are rated as “Poor” by their MBI score. As a comparison RBP habitat scores TC4 and TC3 were scored as “Poor” habitat. For sites TC2, TC1, and the above and below discharge sites of Otter Creek WTP (OC4 and OC3), habitat was scored as “Fair”. The proximal and distal downstream sites of Otter Creek WTP (OC2 and OC1) were scored as providing “Good” habitat.

Agricultural, municipal inputs, and excess nutrients may contribute to the impairment of both Tates and Otter Creeks (KY Water Research Institute, 2000). Both streams have been moved and heavily channelized for transportation and agricultural needs. A natural channel meanders in a helical, sinusoidal pattern migrating laterally as one bank is eroded, and the opposite bank receives sediment deposition (Schmal, 1978). Areas of localized heavy erosion are rare, and the cross section of the channel remains constant and stable even though the position of the channel does not. However levees, including hand-laid rock walls, rip-rap banks, concrete walls, and bulldozed mounds, have been constructed along both streams to reduce flooding. As a result all the streams' energy and water are contained in the channel. Without bends to slow the water and absorb the streams' energy, and because the streams cannot dissipate into their flood plains (Vannote, 1980), flooding events are more frequent and severe. Evidence of scouring events is common, substrate in both streams being composed of primarily bare bedrock. Heavy erosion is evident at all 8 sites. Stream banks are highly eroded, unstable and are a major source of sediment loading. Riparian vegetation, where present, clings to these unstable banks both holding them together and in imminent danger of being swept away in future flooding events. Channelization reduces habitat and substrate complexity, base flow, and biological diversity and favors highly tolerant species (Allan, 2008). Streams altered in this manor often drain their watersheds so efficiently, that the associated channels become dewatered during dry conditions (Griswold, 1978). Additional disturbance comes from livestock access above and below the discharge sites of Tates Creek WTP (TC4 and TC3). A large herd of cattle (>100) have unlimited access to 1300 meters of Tates Creek, adjacent to and downstream of the former WTP. Approximately 300 meters of streambed serves as a travel corridor between pastures. Pools in this stretch of Tates Creek have cattle manure deposits approaching 50 cm in depth.

Although much of the watershed is served by the sanitary sewer infrastructure of Otter Creek WTP, individual septic systems are still in use. Tates Creek has been relocated and heavily channelized to serve agricultural and transportation needs and is closely associated with a paved, two-lane, Kentucky state highway, with the shoulder of this roadway often serving as the stream bank. The land use adjacent to the Tates Creek WTP above discharge sampling site (TC4) and the below discharge sampling site

(TC3) appeared to be affecting the creek. Cattle had full access at these sites and were observed urinating and defecating directly into the creek. Additionally the razing and landfill of the Tates Creek WTP provided a non-point source of potential contaminants and sediment to the creek.

CHAPTER V

CONCLUSION

Having two physically defined point sources, Tates Creek WTP and Otter Creek WTP, provided a tremendous opportunity to evaluate, side by side, the effect of wastewater discharge on the receiving waters. While this effluent has effects on the stream, such as the nutrient loading determined in water chemistry studies of these two streams, it would appear that other anthropological disturbances also greatly affect the overall quality of the stream and the water. Alterations, levees, adjacent land use, and poor agricultural management practices also strain the biota and function of both streams.

Remediation, including the reduction of nutrient output from Otter Creek WTP, dilution and dissipation of the lingering effects from Tates Creek WTP, restoration of riparian buffers, and implementation of agricultural best management practices (BMP) could be effective in some reaches.

It is hoped that this study has produced a valuable inventory and evaluation of these two streams. It is also hoped that this data be periodically updated and be used as a tool for learning the temporal effects the former and current WTPs have had on their receiving streams.

CHAPTER VI

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APPENDIX A:

TABLES

Table 1 Tates Creek WTP data indicating levels of Ammonium, Nitrate, Phosphate, and Escherichia coli in relation to the Tates Creek WTP discharge (DC).

Date	**Ammonium (mg/l)**			**Nitrate (mg/l)**			**Phosphate (mg/l)**			***Escherichia coli* (cfu/100ml)**		
	↑DC	**DC**	**↓DC**	**↑DC**	**DC**	**↓DC**	**↑DC**	**DC**	**↓DC**	**↑DC**	**DC**	**↓DC**
31May11	0.0	0.0	-	3.3	51.1	-	0.2	7.1	-	>2420	1	-
20Jun11	0.0	2.5	-	12.1	26.1	-	0.1	1.0	-	1733	649	-
07Jul11	0.4	5.9	-	4.6	47.0	-	0.1	1.0	-	>2420	1533	-
05Aug11	0.0	0.0	-	0.0	0.0	-	0.2	0.2	-	>2420	>2420	-
15May12	0.0	0.0	-	0.7	0.6	-	0.1	0.2	-	36	36	-
13Jun12	0.0	0.0	-	0.3	0.2	-	0.2	0.2	-	n.a.	n.a.	-
11Jul12	0.3	0.4	-	0.4	0.3	-	0.5	0.6	-	n.a.	n.a.	-
16Jul12	0.0	0.0	-	0.0	0.8	-	n.a.	0.3	-	1	0	-
Discharge (DC) limits:	6.0 mg/l daily max			Report			Report			240 cfu daily max		

Table 2 Otter Creek WTP data indicating levels of Ammonium, Nitrate, Phosphate, and Escherichia coli in relation to the Otter Creek WTP discharge (DC) Discharge.

Date	**Ammonium (mg/l)**			**Nitrate (mg/l)**			**Phosphate (mg/l)**			***Escherichia coli* (cfu/100ml)**		
	↑DC	**DC**	**↓DC**	**↑DC**	**DC**	**↓DC**	**↑DC**	**DC**	**↓DC**	**↑DC**	**DC**	**↓DC**
2012	No data		-	-	-	-	-	-	-	-	-	-
21May13	0.0	0.0	0.0	0.5	26.7	13.4	0.1	3.0	1.4	68	43	35
17Jun13	0.3	0.0	0.0	1.6	20.6	13.0	0.2	1.8	1.0	1203	1	n.a.
08Jul13	0.0	0.0	0.0	5.5	19.2	9.1	0.1	1.6	0.5	866	2	649
05Aug13	0.0	0.0	0.0	0.0	33.7	32.1	0.1	1.6	1.3	142	6	87
Discharge (DC) limits:	6.0 mg/l daily max			Report			Report			240 cfu daily max		

Table 3 Observations of land use adjacent to the stream-sampling sites of Tates Creek.

Activity:	**Above DC (TC4)**	**Below DC (TC3)**	**Proximal Downstream (TC2)**	**Distal Downstream (TC1)**
Land disposal	√	√		
Pasture with livestock access	√	√		
Pasture without livestock access			√	√
Row crops				√
Residential or fallow land				√
Industrial	√	√		
Forested	√	√		√
Commercial	√	√	√	
Storm sewer/runoff	√	√		

Table 4 Observations of land use adjacent to the stream-sampling sites of Otter Creek.

Activity:	**Above DC (OC4)**	**Below DC (OC3)**	**Proximal Downstream (OC2)**	**Distal Downstream (OC1)**
Land disposal				
Pasture with livestock access				
Pasture without livestock access	√	√	√	√
Row crops				√
Residential or fallow land			√	
Industrial	√	√		
Forested	√	√		
Commercial	√	√		
Storm sewer/runoff	√	√		

Table 5 Physicochemical data, in relation to the Tates Creek WTP, as measured during spring and fall sampling periods.

Spring:	**Above DC (TC4)**	**Below DC (TC3)**	**Proximal Downstream (TC2)**	**Distal Downstream (TC1)**
pH	8.55	8.58	8.65	8.49
Flow (m/s)	-0.02	-0.01	0.00	0.00
Depth (mm)	102	105	123	285
Temperature (°C)	21.5	22.2	24.6	25.0
Dissolved Oxygen (mg/l)	9.35	5.06	10.20	6.25
Conductivity (µmhos/cm^2)	648	737	431	446
Siltation	Vheavy	Vheavy	Med	Heavy
Algal cover	Light	Vlight	Med	Light
RBP	101, Poor	112, Poor	122, Fair	120, Fair
Fall:				
pH	8.59	8.58	8.90	8.75
Flow (m/s)	0.06	0.01	0.04	0.02
Depth (mm)	111	150	127	263
Temperature (°C)	6.9	7.3	10.2	7.5
Dissolved Oxygen (mg/l)	11.00	11.00	11.60	12.30
Conductivity (µmhos/cm^2)	480	492	470	374
Siltation	Vheavy	Vheavy	Med	Heavy
Algal cover	Light	Vlight	Med	Light
RBP	101, Poor	112, Poor	122, Fair	120, Fair

Table 6 Physicochemical data, in relation to the Otter Creek WTP, as measured during spring and fall sampling periods.

Spring:	**Above DC (OC4)**	**Below DC (OC3)**	**Proximal Downstream (OC2)**	**Distal Downstream (OC1)**
pH	8.61	8.41	9.83	8.80
Flow (m/s)	0.20	0.34	0.14	0.04
Depth (mm)	115	115	137	281
Temperature (°C)	17.5	18.1	29.3	25.6
Dissolved Oxygen (mg/l)	5.59	6.70	9.22	9.22
Conductivity (µmhos/cm^2)	502	656	1039	806
Siltation	Light	Med	Med	Med
Algal cover	Heavy	Heavy	Heavy	Heavy
RBP	128, Fair	127, Fair	140, Good	147, Good
Fall:				
pH	8.51	8.37	9.55	9.53
Flow (m/s)	0.20	0.33	0.36	0.21
Depth (mm)	99	121	166	178
Temperature (°C)	9.9	16.0	9.0	7.3
Dissolved Oxygen (mg/l)	11.30	9.40	14.21	Meter Malfunction
Conductivity (µmhos/cm^2)	500	732	468	654
Siltation	Light	Med	Med	Med
Algal cover	Heavy	Heavy	Heavy	Heavy
RBP	128, Fair	127, Fair	140, Good	147, Good

Table 7 Totals and identification results for spring fish sampling, Tates Creek, sorted phylogenetically, and by sampling-site s in relation to Tates Creek WTP.

Fish Taxa			TC4	TC3	TC2	TC1	Total
Clupeiformes							
Clupeidae	*Dorosoma*	*cepedianum*					
Cypriniformes							
Cyprinidae	*Campostoma*	*anomalum*	16	1	143	185	345
Cyprinidae	*Cyprinella*	*spiloptera*					
Cyprinidae	*Ericymba*	*buccata*					
Cyprinidae	*Luxilus*	*chrysocephalus*			48	24	72
Cyprinidae	*Lythrurus*	*fasciolaris*			18	4	22
Cyprinidae	*Notropis*	*atherinoides*			19	4	23
Cyprinidae	*Notropis*	*boops*			3		3
Cyprinidae	*Pimephales*	*notatus*	70	191	131	56	448
Cyprinidae	*Semotilus*	*atromaculatus*	188	186	133	89	596
Catostomidae	*Hypentelium*	*nigricans*			7		7
Siluriformes							
Ictaluridae	*Ameiurus*	*natalis*			1	7	8
Ictaluridae	*Ictalurus*	*punctatus*					
Ictaluridae	*Noturus*	*flavus*					
Cyprinodontiformes							
Poeciliidae	*Gambusia*	*affinis*		227			227
Perciformes							
Centrarchidae	*Ambloplites*	*rupestris*	1	1			2
Centrarchidae	*Lepomis*	*cyanellus*	2	1	11	3	17
Centrarchidae	*Lepomis*	*macrochirus*				2	2
Centrarchidae	*Lepomis*	*megalotis*			26	5	31
Centrarchidae	*Micropterus*	*dolomieu*			1	1	2
Centrarchidae	*Micropterus*	*punctulatus*					

Table 7 (continued).

Fish Taxa			TC4	TC3	TC2	TC1	Totals
Percidae	*Etheostoma*	*blennoides*			8		8
Percidae	*Etheostoma*	*caeruleum*	26	69	63	162	320
Percidae	*Etheostoma*	*flabellare*	107	35	93	293	528
Percidae	*Percina*	*caprodes*				1	1
		Abundance	410	711	705	836	2662
		Taxa Richness	7	8	15	14	
		Shannon-Wiener	1.35	1.47	2.13	1.71	
		KIBI	59	60	51	37	

Table 8 Totals and identification results for fall fish sampling, Tates Creek, sorted phylogenetically, and by sampling-sites in relation to Tates Creek WTP.

Fish Taxa			TC4	TC3	TC2	TC1	Totals
Clupeiformes							
Clupeidae	*Dorosoma*	*cepedianum*					
Cypriniformes							
Cyprinidae	*Campostoma*	*anomalum*	96	48	333	308	785
Cyprinidae	*Cyprinella*	*spiloptera*					
Cyprinidae	*Ericymba*	*buccata*			73	17	90
Cyprinidae	*Luxilus*	*chrysocephalus*			467	87	554
Cyprinidae	*Lythrurus*	*fasciolaris*					
Cyprinidae	*Notropis*	*atherinoides*			108	177	285
Cyprinidae	*Notropis*	*boops*					
Cyprinidae	*Pimephales*	*notatus*	240	144	793	137	1314
Cyprinidae	*Semotilus*	*atromaculatus*	9	24	71	34	138
Catostomidae	*Hypentelium*	*nigricans*			2		2

Table 8 (continued).

Fish Taxa			TC4	TC3	TC2	TC1	Totals
Siluriformes							
Ictaluridae	*Ameiurus*	*natalis*					
Ictaluridae	*Ictalurus*	*punctatus*					
Ictaluridae	*Noturus*	*flavus*					
Cyprinodontiformes							
Poeciliidae	*Gambusia*	*affinis*	21	130			151
Perciformes							
Centrarchidae	*Ambloplites*	*rupestris*					
Centrarchidae	*Lepomis*	*cyanellus*	1		5	31	37
Centrarchidae	*Lepomis*	*macrochirus*				6	6
Centrarchidae	*Lepomis*	*megalotis*			7	1	8
Centrarchidae	*Micropterus*	*dolomieu*					
Centrarchidae	*Micropterus*	*punctulatus*					
Percidae	*Etheostoma*	*blennoides*			1	2	3
Percidae	*Etheostoma*	*caeruleum*	224	58	121	79	482
Percidae	*Etheostoma*	*flabellare*	50	33	2	7	92
Percidae	*Percina*	*caprodes*				1	1
		Abundance	641	437	1983	887	3948
		Taxa Richness	7	6	12	13	
		Shannon-Wiener	1.40	1.59	1.21	1.75	
		KIBI	59	58	48	40	

Table 9 Totals and identification results for spring fish sampling, Otter Creek, sorted phylogenetically, and by sampling-site.

Fish Taxa			OC4	OC3	OC2	OC1	Totals
Clupeiformes							
Clupeidae	*Dorosoma*	*cepedianum*					
Cypriniformes							
Cyprinidae	*Campostoma*	*anomalum*	39	34	422	139	634
Cyprinidae	*Cyprinella*	*spiloptera*				1	1
Cyprinidae	*Ericymba*	*buccata*	2	43	10		55
Cyprinidae	*Luxilus*	*chrysocephalus*	12	18	18	18	66
Cyprinidae	*Lythrurus*	*fasciolaris*				11	11
Cyprinidae	*Notropis*	*atherinoides*				11	11
Cyprinidae	*Notropis*	*boops*					
Cyprinidae	*Pimephales*	*notatus*	67	41	128	30	266
Cyprinidae	*Semotilus*	*atromaculatus*	7	42	81		130
Catostomidae	*Hypentelium*	*nigricans*				1	1
Siluriformes							
Ictaluridae	*Ameiurus*	*natalis*	2		14	3	19
Ictaluridae	*Ictalurus*	*punctatus*				1	1
Ictaluridae	*Noturus*	*flavus*				1	1
Cyprinodontiformes							
Poeciliidae	*Gambusia*	*affinis*	4	365	12		381
Perciformes							
Centrarchidae	*Ambloplites*	*rupestris*		1		1	2
Centrarchidae	*Lepomis*	*cyanellus*	14	3	13	42	72
Centrarchidae	*Lepomis*	*macrochirus*				4	4
Centrarchidae	*Lepomis*	*megalotis*	1		8	80	89
Centrarchidae	*Micropterus*	*dolomieu*				7	7

Table 9
(continued).

Fish Taxa			OC4	OC3	OC2	OC1	Totals
Centrarchidae	*Micropterus*	*punctulatus*				1	1
Percidae	*Etheostoma*	*blennoides*	7	1	1	16	25
Percidae	*Etheostoma*	*caeruleum*	110	155	121	158	544
Percidae	*Etheostoma*	*flabellare*	104	43	193	20	360
Percidae	*Percina*	*caprodes*				2	2
		Abundance	369	746	1021	547	2683
		Taxa Richness	12	11	12	19	
		Shannon-Wiener	1.77	1.60	1.72	2.05	
		KIBI	39	38	34	43	

Table 10 Totals and identification results for fall fish sampling, Otter Creek, sorted phylogenetically, and by sampling-site.

Fish Taxa			OC4	OC3	OC2	OC1	Totals
Clupeiformes							
Clupeidae	*Dorosoma*	*cepedianum*				32	32
Cypriniformes							
Cyprinidae	*Campostoma*	*anomalum*	898	687	456	63	2104
Cyprinidae	*Cyprinella*	*spiloptera*	1				1
Cyprinidae	*Ericymba*	*buccata*	52	6	23		81
Cyprinidae	*Luxilus*	*chrysocephalus*	1	55	10	126	192
Cyprinidae	*Lythrurus*	*fasciolaris*					
Cyprinidae	*Notropis*	*atherinoides*	6	2		118	126
Cyprinidae	*Notropis*	*boops*					
Cyprinidae	*Pimephales*	*notatus*	367	262	343	84	1056
Cyprinidae	*Semotilus*	*atromaculatus*		101	24		125
Catostomidae	*Hypentelium*	*nigricans*				3	3

Table 10 (continued).

Fish Taxa			OC4	OC3	OC2	OC1	Totals
<u>Siluriformes</u>							
Ictaluridae	*Ameiurus*	*natalis*	3		2	1	6
Ictaluridae	*Ictalurus*	*punctatus*					
Ictaluridae	*Noturus*	*flavus*					
<u>Cyprinodontiformes</u>							
Poeciliidae	*Gambusia*	*affinis*	94	69	170	16	349
<u>Perciformes</u>							
Centrarchidae	*Ambloplites*	*rupestris*					
Centrarchidae	*Lepomis*	*cyanellus*	11	3	9	10	33
Centrarchidae	*Lepomis*	*macrochirus*	1		3	44	48
Centrarchidae	*Lepomis*	*megalotis*	12	1	4	38	55
Centrarchidae	*Micropterus*	*dolomieu*					
Centrarchidae	*Micropterus*	*punctulatus*					
Percidae	*Etheostoma*	*blennoides*	2	5	1	9	17
Percidae	*Etheostoma*	*caeruleum*	145	195	162	356	858
Percidae	*Etheostoma*	*flabellare*	138	310	97	8	553
Percidae	*Percina*	*caprodes*				4	4
		Abundance	1731	1696	1304	912	5643
		Taxa Richness	14	12	13	15	
		Shannon-Wiener	1.46	1.62	1.69	1.89	
		KIBI	42	40	36	37	

Table 11 Totals and identification results for macroinvertebrate sampling sorted by order, family, genus (if possible), species (if possible), and by sampling-site in relation to Tates Creek WTP.

Macroinvertebrate Taxa		**TC4**	**TC3**	**TC2**	**TC1**	**Totals**
Amphipoda						
Crangonyctidae	*Crangonyx* sp.	80	133	3	1	217
Arhynchobdellida						
Haemopidae	*Haemophus* sp.	1				1
Basommatophora						
Physidae	*Physa* sp.	5	12	2	3	22
Coleoptera						
Elmidae	*Dubiraphia* sp.			1		1
Elmidae	*Stenelmis* sp.	64	19	77	216	376
Haliplidae	*Peltodytes* sp.	1	6			7
Hydrophilidae	*Berosus* sp.			1		1
Hydrophilidae	Un-id'd Hydrophilid	1	1			2
Hydrophilidae	*Tropisternus* sp.		1		1	2
Limnichidae	*Lutrochus* sp.			1		1
Psephenidae	*Ectopria* sp.	10		6	15	31
Psephenidae	*Psephnus* sp.	14		35	32	81
Chrysomelidae	*Un-id'd Chrysomelid*		1			1
Lampyridae	*Un-id'd Lampyridae*	1		1		2
Staphylinidae	*Un-id'd Staphylinidae*			1		1
Decapoda						
Cambaridae	*Orconectes juvenilis*			1		1
Cambaridae	*Orconectes rusticus*	1		4	3	8
Diptera						
Ceratopogonidae	*Probezzi* sp.				1	1
Chironomidae	*Pseudochironomus* sp.					
Chironomidae	Non Tanypodinae sp.	4	21	19	19	63

Table 11 (continued).

Macroinvertebrate Taxa		TC4	TC3	TC2	TC1	Totals
Empididae	*Hemerodromia* sp.					
Psychodidae	*Psychoda* sp.		1			1
Rhagionidae				1		
Stratiomyidae	*Stratiomys* sp.		1			1
Tanyderidae	*Protoplasa fitchii*				1	1
Tanypodinae	Un-id'd Tanypodinae	21	12	11	16	60
Tipulidae	*Hexatoma* sp.	1				1
Tipulidae	*Tipula* sp.	1	1	1		3
Ephemeroptera						
Baetidae	Un-id'd Baetid sp.		1	1	1	3
Baetidae	*Centroptilum* sp.	9		1		10
Caenidae	*Caenis* sp.			76	9	85
Heptageniidae	*Maccaffertium sp.*	1		30	13	44
Isonychiidae	*Isonychia* sp.			2		2
Haplotaxida						
Lumbricidae	Un-id'd Lumbricid	1	1	1	1	4
Tubificidae	*Branchiura sowerbyi*	1	4			5
Tubificidae	Un-id'd without cilia sp.	1	7	4	5	17
Hemiptera						
Corixidae	Un-id'd Corixid	1	7	1	1	10
Gerridae	*Aquarius* sp.		1			1
Gerridae	*Metrobates* sp.				2	2
Gerridae	*Trepobates* sp.			1		1
Gerridae	Un-id'd Gerrid					
Naucoridae						
Nepidae	*Ranatra* sp.					
Veliidae	*Microvelia* sp.	1	2			3

Table 11 (continued).

Macroinvertebrate Taxa		TC4	TC3	TC2	TC1	Totals
Heterodonta						
Sphaeridae	Un-id'd Spaehrid					
Isopoda						
Asellidae	*Caecidotea* sp.	24	21	1	5	51
Asellidae	*Lirceus* sp.	1	7	6	1	15
Lymnophila						
Ancylidae	*Ferrissia* sp.			8	12	20
Lymnaedae	*Galba* sp.	1	1		1	3
Lymnaedae	*Pseudosuccinea columella*	1	1	1		3
Planorbidae	*Heliosoma* sp.	1	1	3	1	6
Megaloptera						
Sialidae	*Sialis* sp.			1	1	2
Mesogastropoda						
Hydrobiidae	*Amnicola limosa*					
Hydrobiidae	Un-id'd Hydrobiid					
Pleuroceridae	*Elimia* sp.	1				1
Pomatiopsidae	*Pomatiopsis* sp.				1	1
Odonata						
Aeshnidae	*Boyeria* sp.					
Calopterygidae	*Calopteryx* sp.					
Coenagrionidae	*Argia* sp.	1		1	1	3
Gomphidae	*Dromogomphus* sp.				1	1
Libellulidae	*Ladona* sp.		1			1
Pelecypoda						
Corbiculidae	*Corbicula fluminea*	31	4	2	7	44
Plecoptera						
Perlidae	*Acroneuria* sp.				1	1

Table 11 (continued).

Macroinvertebrate Taxa		TC4	TC3	TC2	TC1	Totals
Rhynchobdellida						
Glossiphoniidae	*Helobdella stagnalis*	1	8			9
Glossiphonidae	*Placobdella* sp.					
Glossiphoniidae	Un-id'd Glossiphoniid	1	1			2
Piscicolidae	*Myzobdella lugubris*			1	1	2
Trichoptera						
Helicopsychidae	*Helicopsyche* sp.			1	1	2
Hydropsychidae	*Cheumatopsyche* sp.	13	9	43	22	87
Hydropsychidae	*Hydropsyche* sp.	1		1	1	3
Hydroptilidae	*Hydroptila* sp.		1			1
Philopotamidae	*Chimarra* sp.			5	3	8
Phylum: Nematoda						
■ = Reference Collection, 1x voucher specimen	Un-id'd Nematode	1			1	2
□ = Non-MBI Taxa	Semi-Quant Totals	298	288	355	401	1340
Taxa Richness-Overall		34	30	376	34	
Taxa Richness - MBI		32	28	34	32	
EPT Richness		4	3	9	8	
Percent Ephemeroptera		3%	0%	31%	6%	
Modified Percent EPT Abundance		4%	1%	33%	7%	
Percent Chironomidae		8%	11%	8%	9%	
Percent Chironomidae+Oligochaeta		9%	16%	10%	10%	
Percent Primary Clingers		35%	10%	56%	76%	
Modified Hilsenhoff Biotic Index		6.21	7.44	5.58	5.57	
MBI		18.23	10.45	32.8	29.92	

Table 12 Totals and identification results for macroinvertebrate sampling sorted by order, family, genus (if possible), species (if possible), and by sampling-site in relation to Otter Creek WTP.

Macroinvertebrate Taxa		**OC4**	**OC3**	**OC2**	**OC1**	**Totals**
Amphipoda						
Crangonyctidae	*Crangonyx* sp.	1	1			2
Arhynchobdellida						
Haemopidae	*Haemophus* sp.					
Basommatophora						
Physidae	*Physa* sp.	1	2	1	1	5
Coleoptera						
Elmidae	*Dubiraphia* sp.					
Elmidae	*Stenelmis* sp.	103	32	203	191	529
Haliplidae	*Peltodytes* sp.	1	1	1	1	4
Hydrophilidae	*Berosus* sp.	2	1	4	1	8
Hydrophilidae	Un-id'd Hydrophilid	1	1			2
Hydrophilidae	*Tropisternus* sp.	1		1	1	3
Limnichidae	*Lutrochus* sp.			1		1
Psephenidae	*Ectopria* sp.	2		1		3
Psephenidae	*Psephnus* sp.	4	1	1	16	22
Chrysomelidae	Un-id'd Chrysomelid					
Lampyridae	Un-id'd Lampyridae					
Staphylinidae	Un-id'd Staphylinidae					
Decapoda						
Cambaridae	*Orconectes juvenilis*	1	1			2
Cambaridae	*Orconectes rusticus*	1		1	1	3
Diptera						
Ceratopogonidae	*Probezzi* sp.					
Chironomidae	*Pseudochironomus sp.*		1			1
Chironomidae	Non-Tanypodinae sp.	46	147	96	99	388

Table 12 (continued).

Macroinvertebrate Taxa		OC4	OC3	OC2	OC1	Totals
Empididae	*Hemerodromia* sp.	1				1
Psychodidae	*Psychoda* sp.					
Rhagionidae						
Stratiomyidae	*Stratiomys* sp.			1		1
Tanyderidae	*Protoplasa fitchii*					
Tanypodinae	Un-id'd Tanypodinae	11	4	11	17	43
Tipulidae	*Hexatoma* sp.					
Tipulidae	*Tipula* sp.					
Ephemeroptera						
Baetidae	Un-id'd Baetid sp.	1		1		2
Baetidae	*Centroptilum* sp.	11	10	22		43
Caenidae	*Caenis* sp.	11	3	4	1	19
Heptageniidae	*Maccaffertium sp.*	1			8	9
Isonychiidae	*Isonychia* sp.		1			1
Haplotaxida						
Lumbricidae	Un-id'd Lumbricid	4	1		1	6
Tubificidae	*Branchiura sowerbyi*					
Tubificidae	UIW/OCS sp.		2	4	1	7
Hemiptera						
Corixidae	Un-id'd Corixid					
Gerridae	*Aquarius* sp.					
Gerridae	*Metrobates* sp.					
Gerridae	*Trepobates* sp.				1	1
Gerridae	Un-id'd Gerrid			1		1
Naucoridae				1		
Nepidae	*Ranatra* sp.				1	1
Veliidae	*Microvelia* sp.					

Table 12 (continued).

Macroinvertebrate Taxa		OC4	OC3	OC2	OC1	Totals
Heterodonta						
Sphaeridae	Un-id'd Spaehrid				1	1
Isopoda						
Asellidae	*Caecidotea* sp.	15	9	12	6	42
Asellidae	*Lirceus* sp.	14	16	11	5	46
Lymnophila						
Ancylidae	*Ferrissia* sp.	1	1		1	3
Lymnaedae	*Galba* sp.	1	1		2	4
Lymnaedae	*Pseudosuccinea columella*	1		1		2
Planorbidae	*Heliosoma* sp.	1	1		1	3
Megaloptera						
Sialidae	*Sialis* sp.				1	1
Mesogastropoda						
Hydrobiidae	*Amnicola limosa*				1	1
Hydrobiidae	Un-id'd Hydrobiid		1			1
Pleuroceridae	*Elimia* sp.					
Pomatiopsidae	*Pomatiopsis* sp.	1	1		1	3
Odonata						
Aeshnidae	*Boyeria* sp.				1	1
Calopterygidae	*Calopteryx* sp.		1			1
Coenagrionidae	*Argia* sp.	1	1	1	**2**	5
Gomphidae	*Dromogomphus* sp.	1				1
Libellulidae	*Ladona* sp.	1			1	2
Pelecypoda						
Corbiculidae	*Corbicula fluminea*	14	2	6	2	24
Plecoptera						
Perlidae	*Acroneuria* sp.					

Table 12 (continued).

Macroinvertebrate Taxa		OC4	OC3	OC2	OC1	Totals
Rhynchobdellida						
Glossiphoniidae	*Helobdella stagnalis*	1	1	1		3
Glossiphonidae	*Placobdella* sp.				1	1
Glossiphoniidae	Un-id'd Glossiphoniid					
Piscicolidae	*Myzobdella lugubris*	1		1		2
Trichoptera						
Helicopsychidae	*Helicopsyche* sp.	1				1
Hydropsychidae	*Cheumatopsyche* sp.	46	113	62	64	285
Hydropsychidae	*Hydropsyche* sp.	7	85	43	23	158
Hydroptilidae	*Hydroptila* sp.	7	7	33	5	52
Philopotamidae	*Chimarra* sp.	7	1	3	38	49
Phylum: Nematoda						
█ = Reference Collection, 1x voucher specimen	Un-id'd Nematode					
□ = Non-MBI Taxa	Semi-Quant Totals	325	450	529	497	1801
Taxa Richness - Overall		37	31	29	33	
Taxa Richness - MBI		37	31	27	32	
EPT Richness		9	7	7	6	
Percent Ephemeroptera		7%	3%	5%	2%	
Modified Percent EPT Abundance		14%	24%	20%	15%	
Percent Chironomidae		18%	34%	20%	23%	
Percent Chironomidae+Oligochaeta		19%	34%	21%	24%	
Percent Primary Clingers		55%	53%	65%	69%	
Modified Hilsenhoff Biotic Index		6.18	6.02	6.02	5.48	
MBI		28.92	28.29	29.18	29.51	

APPENDIX B:

FIGURES

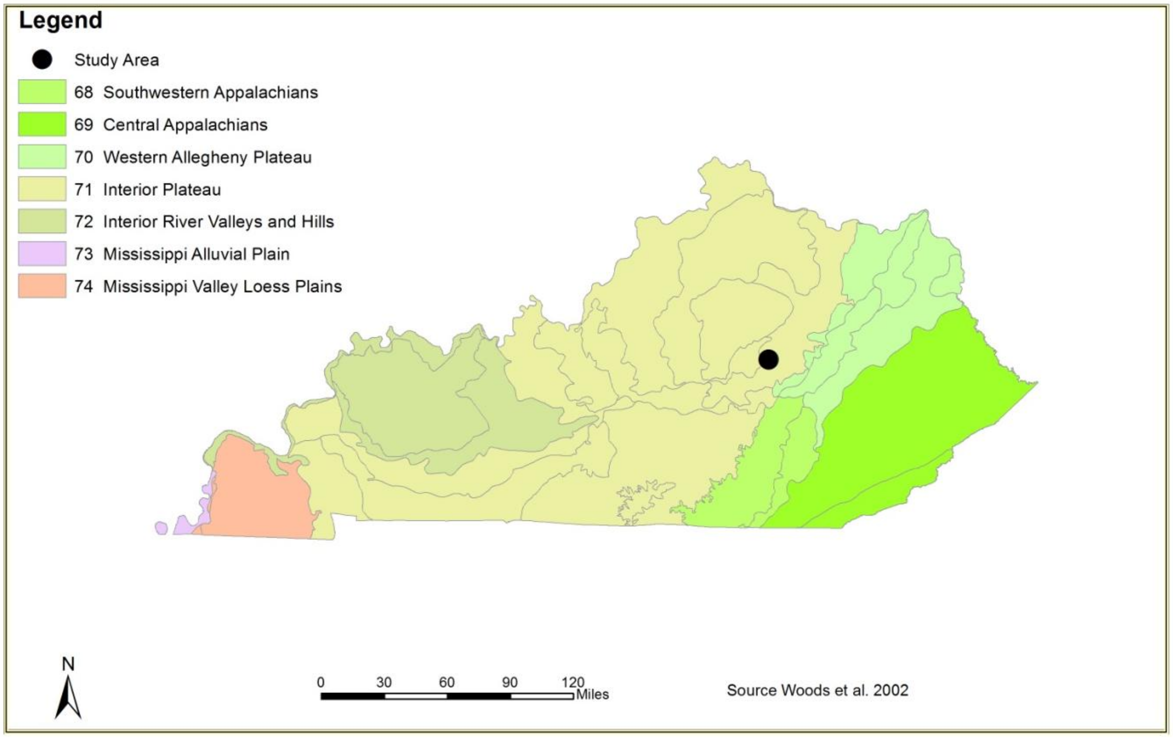

Figure 1 Map detailing the ecoregions of Kentucky indicating the relative position of the study area within the Interior Plateau Geographic Province, Bluegrass Bioregion, of Central Kentucky. (Woods, 2002).

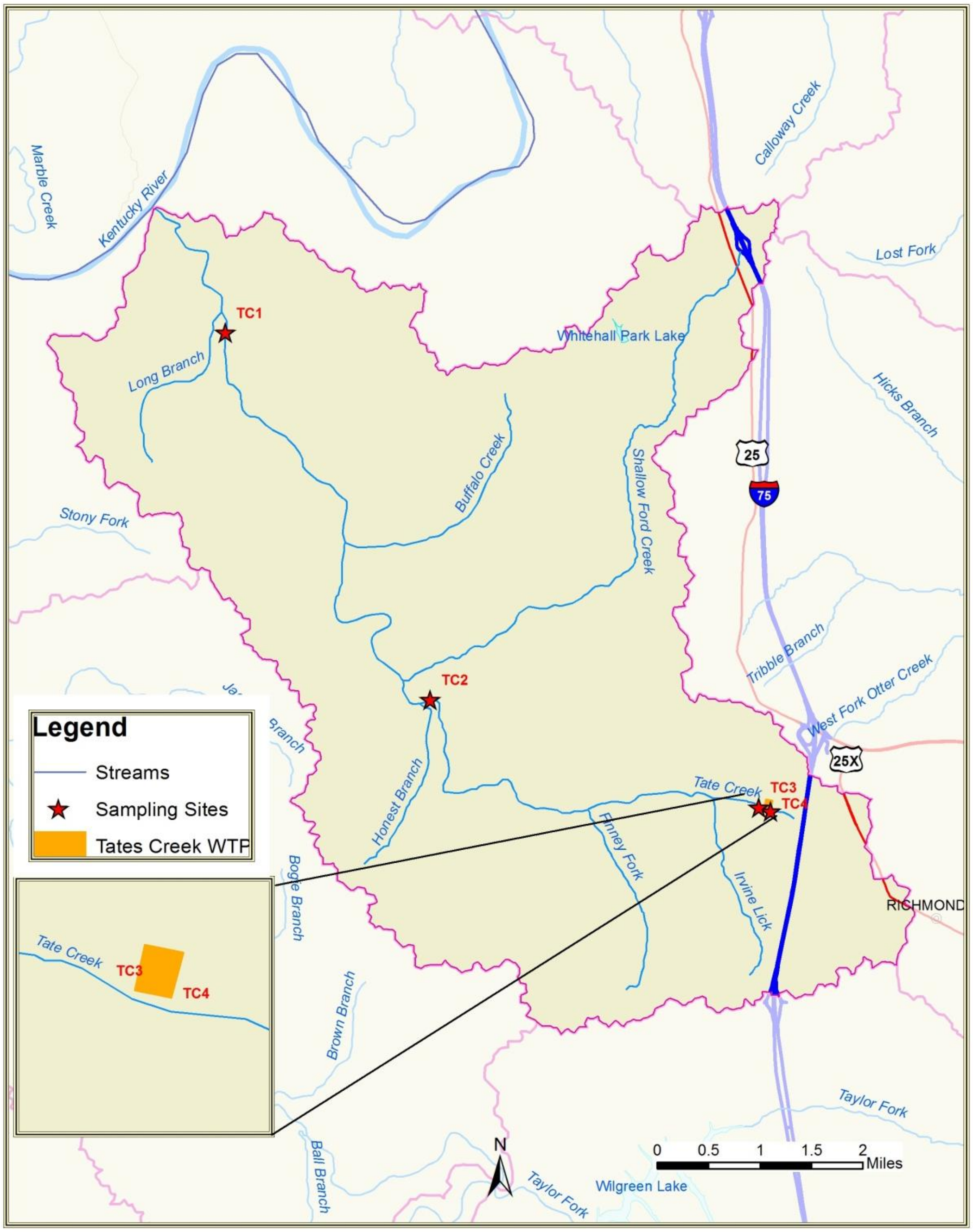

Figure 2 Tates Creek watershed with location of former Tates Creek WTP in relation to study-area sampling sites. Map courtesy of G. Sprandel, Kentucky Department of Fish and Wildlife Resources.

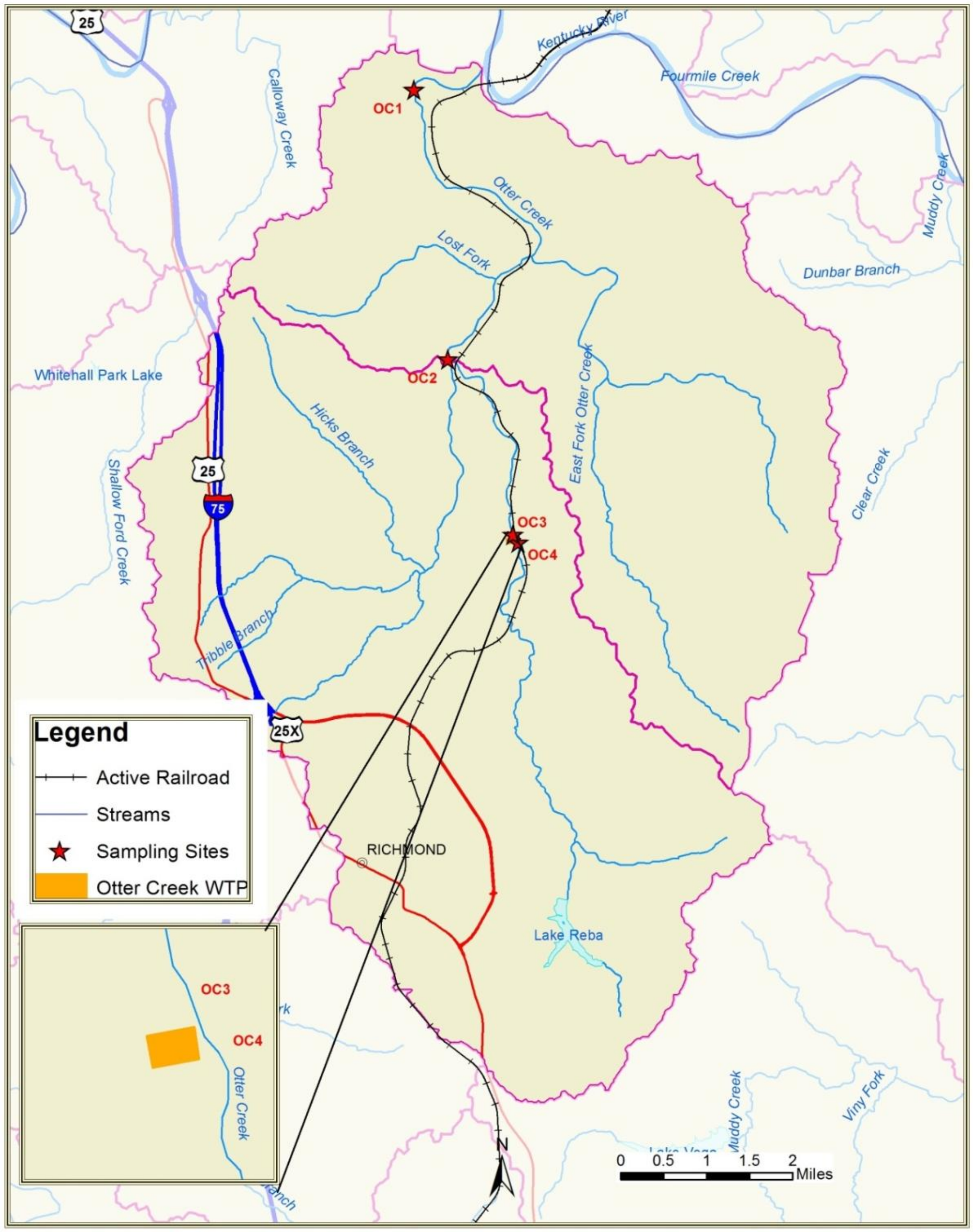

Figure 3 Otter Creek watershed with location of current Otter Creek WTP in relation to study-area sampling sites. Map courtesy of G. Sprandel, Kentucky Department of Fish and Wildlife Resources.

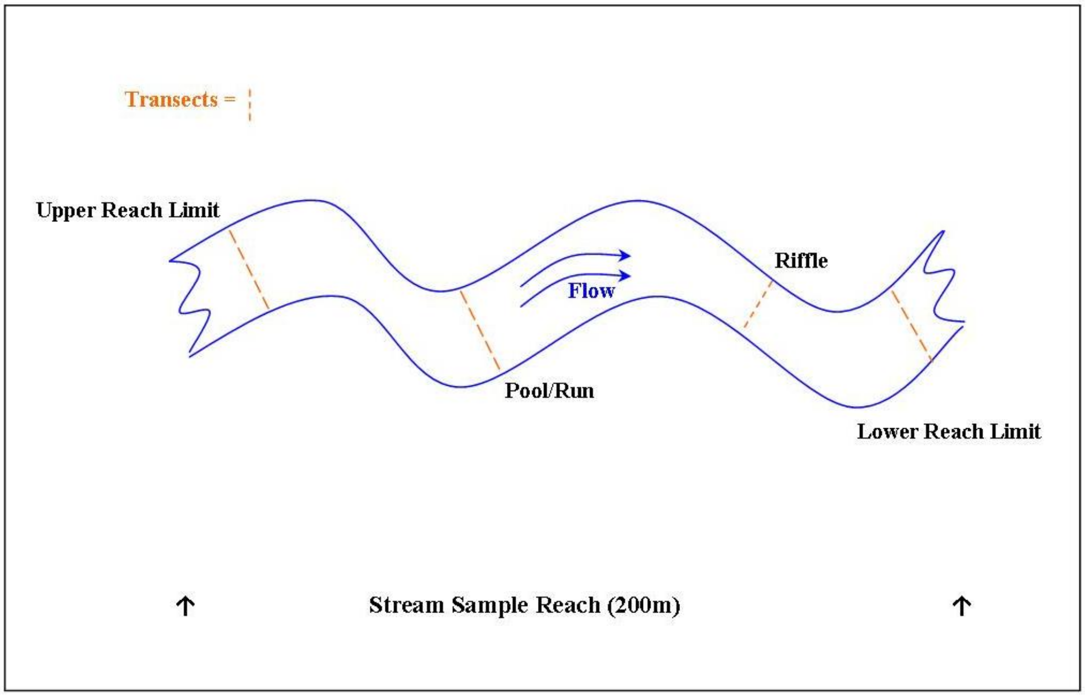

Figure 4 Idealized representation of typical stream sampling reach indicating upper and lower sampling reach limits, two flow regimes, and relative position of physicochemical measurement transects.

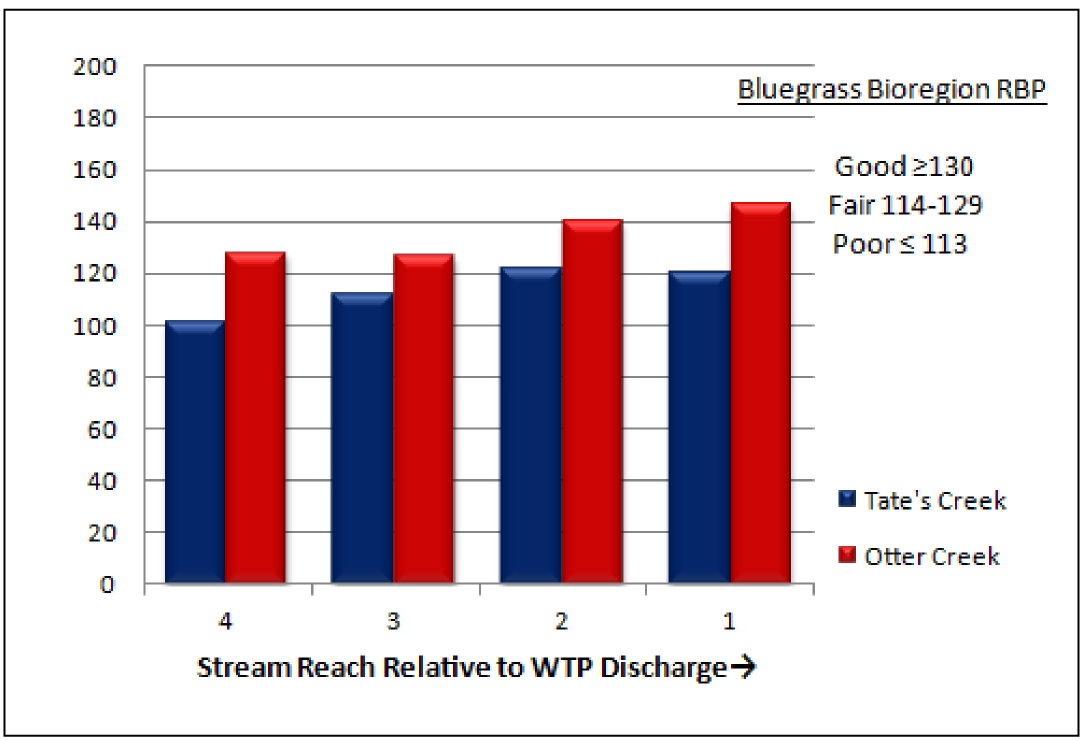

Figure 5 RBP habitat metric scores for Tates Creek, and Otter Creek, stream-sampling sites. An undisturbed, reference-quality stream could score the maximum of 200.

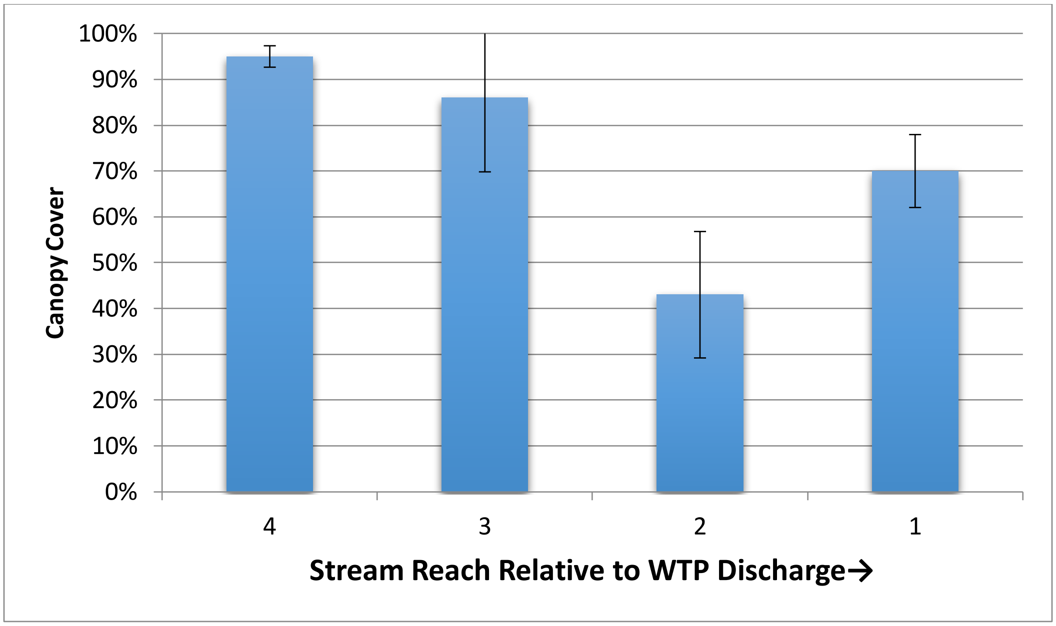

Figure 6 Mean canopy cover measured in relation to Tates Creek sampling-sites.

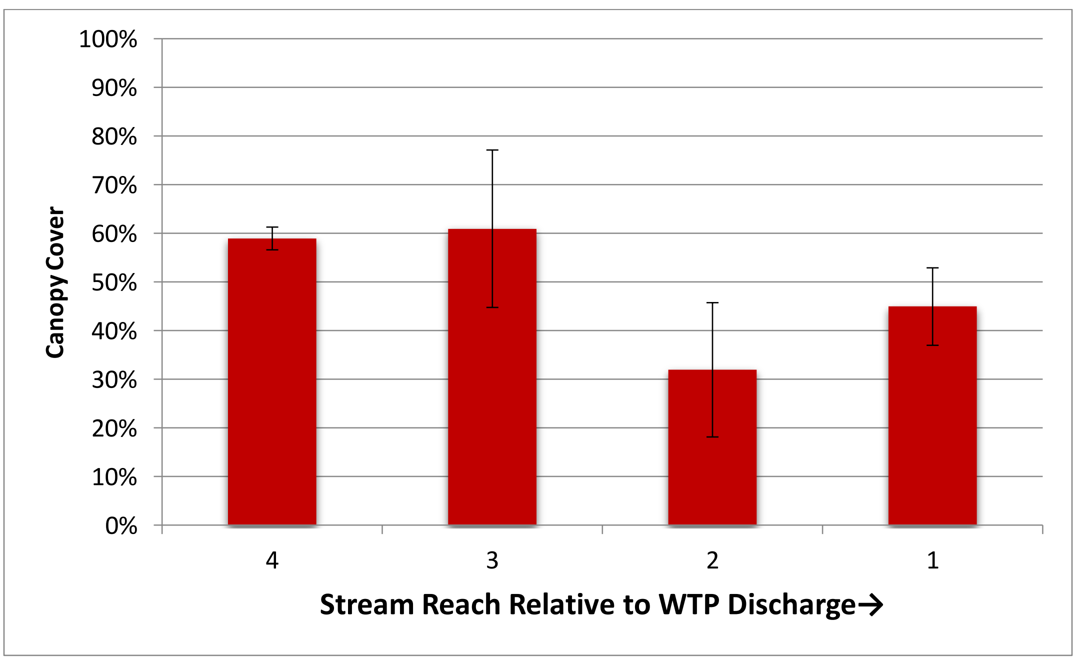

Figure 7 Mean canopy cover measured in relation to Otter Creek WTP sampling-sites.

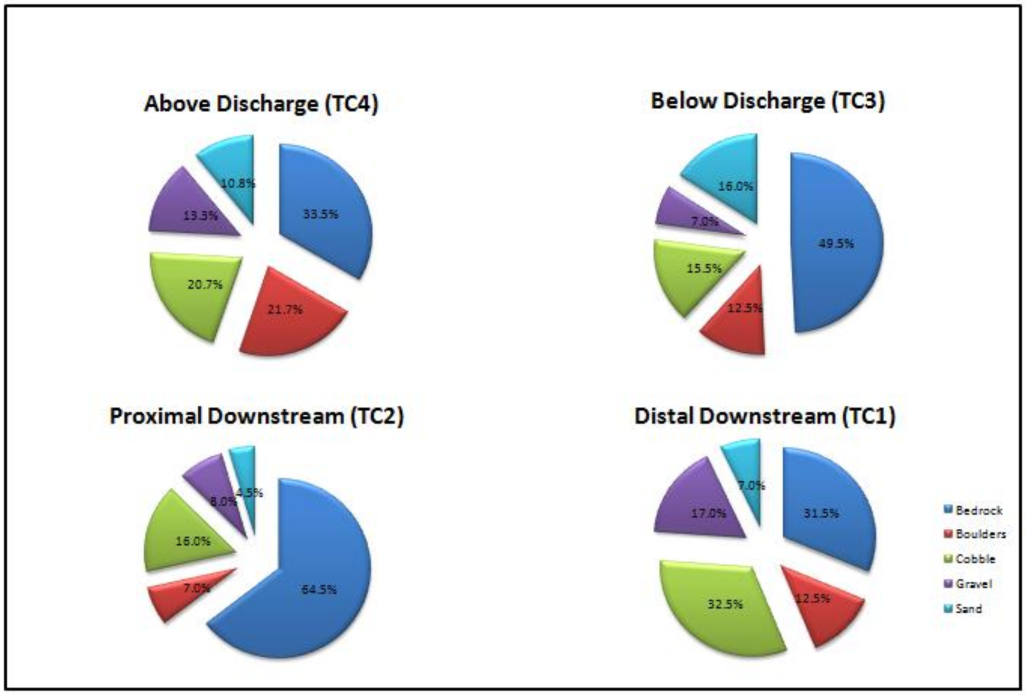

Figure 8 Generalized assessment of streambed substrate composition in relation to Tates Creek WTP sampling sites.

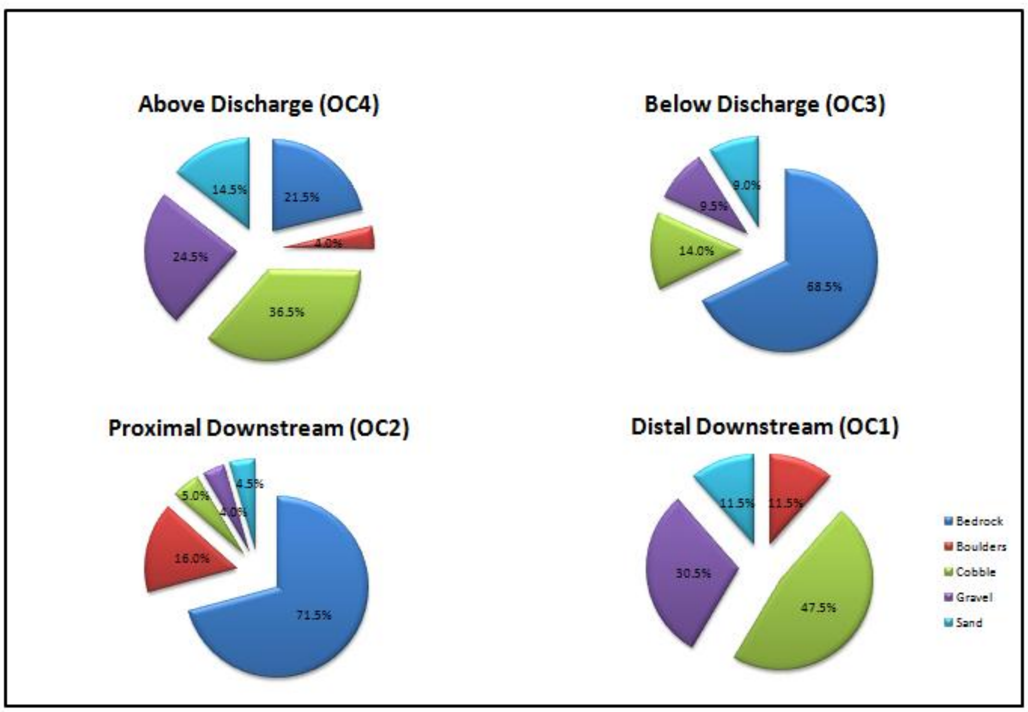

Figure 9 Generalized assessment of streambed substrate composition in relation to Otter Creek WTP sampling sites.

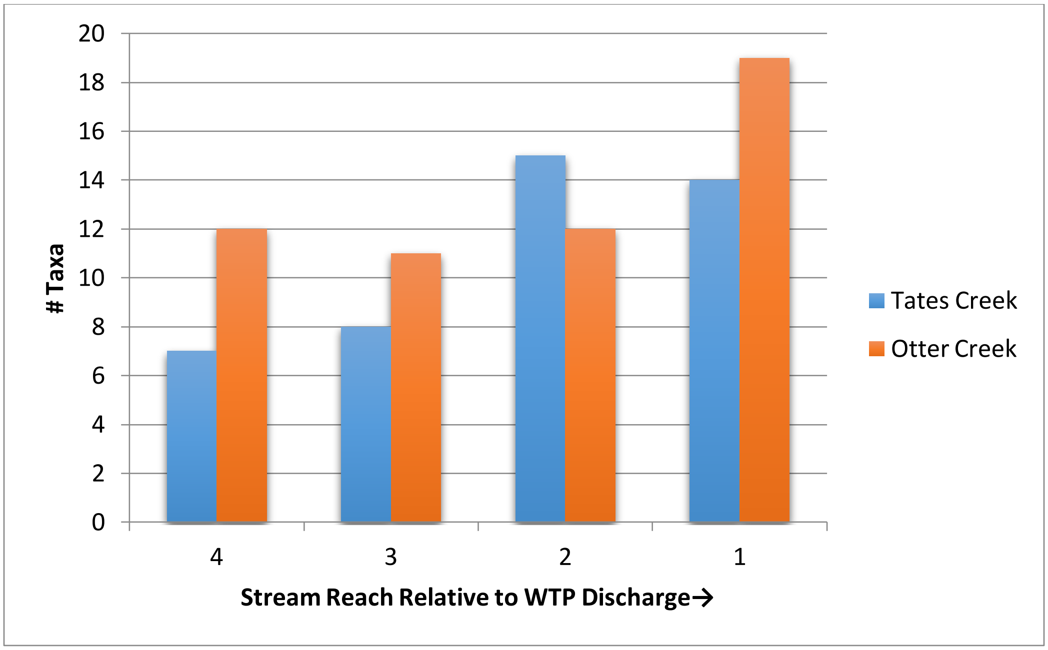

Figure 10 Fish taxa richness, spring, in relation to Tates Creek WTP and Otter Creek WTP stream-sampling sites.

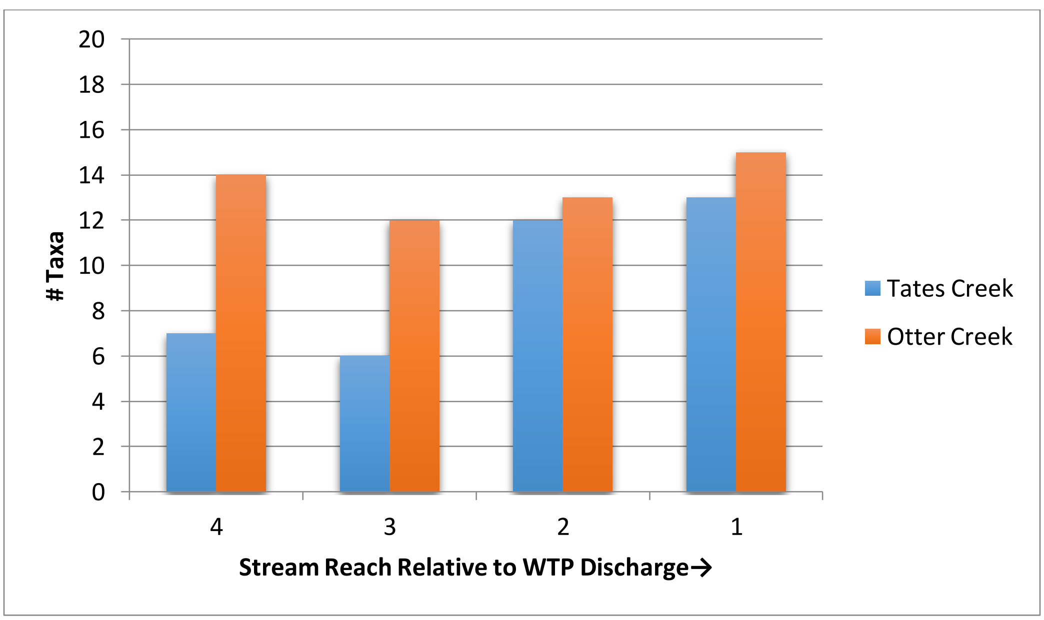

Figure 11 Fish taxa richness, fall, in relation to Tates Creek WTP and Otter Creek WTP stream-sampling sites.

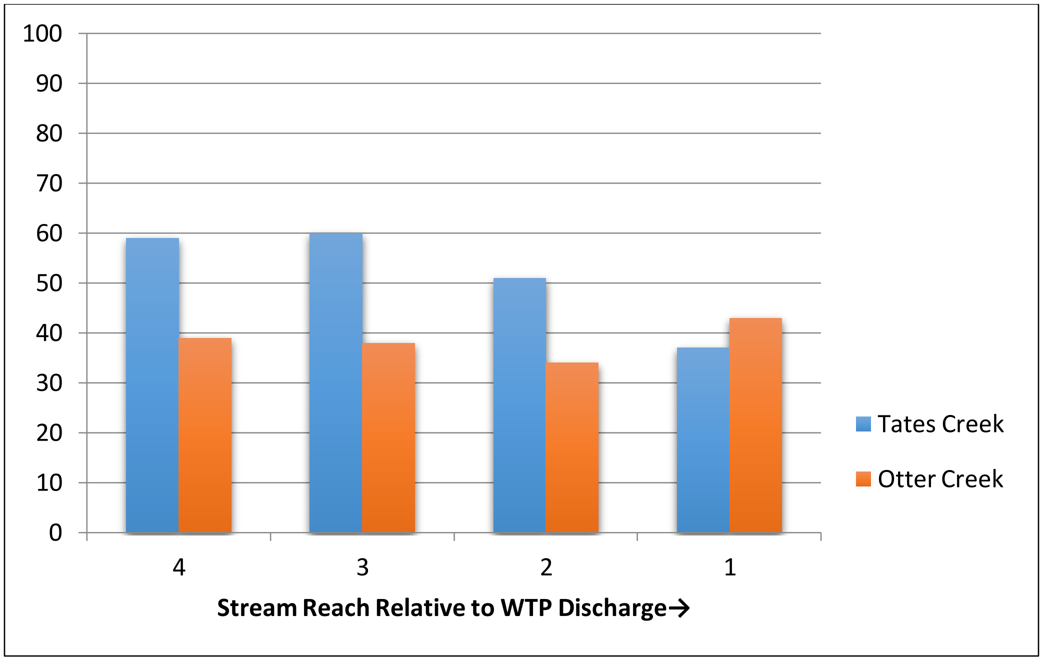

Figure 12 Kentucky Index of Biotic Integrity (KIBI), spring, in relation to Tates Creek WTP and Otter Creek WTP stream-sampling sites.

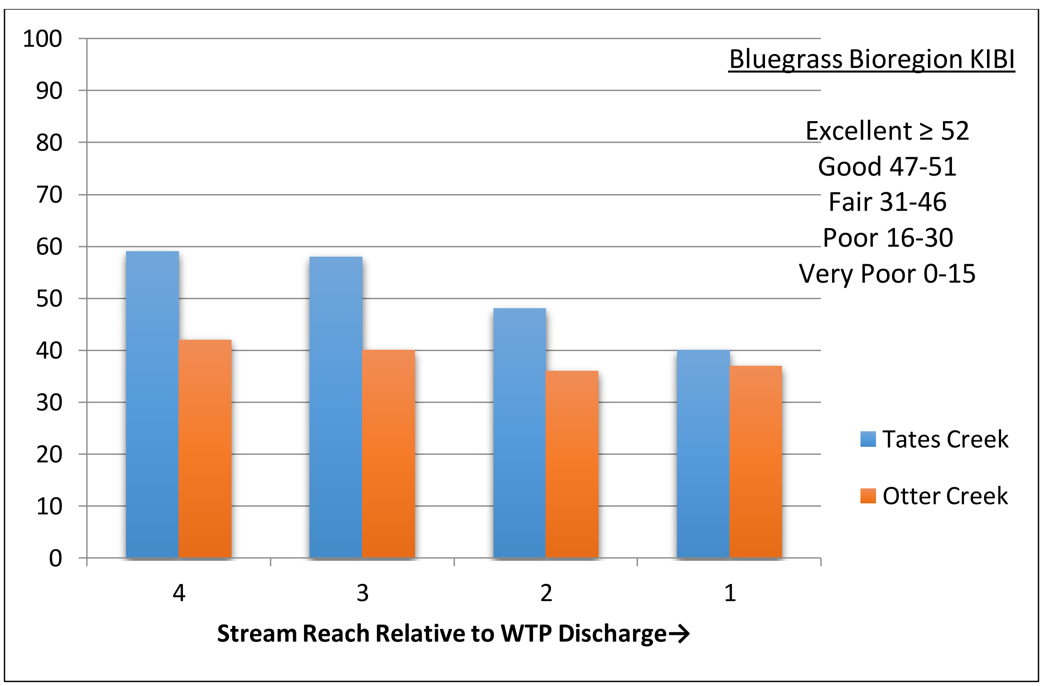

Figure 13 Kentucky Index of Biotic Integrity (KIBI), fall, in relation to Tates Creek WTP and Otter Creek WTP stream-sampling sites.

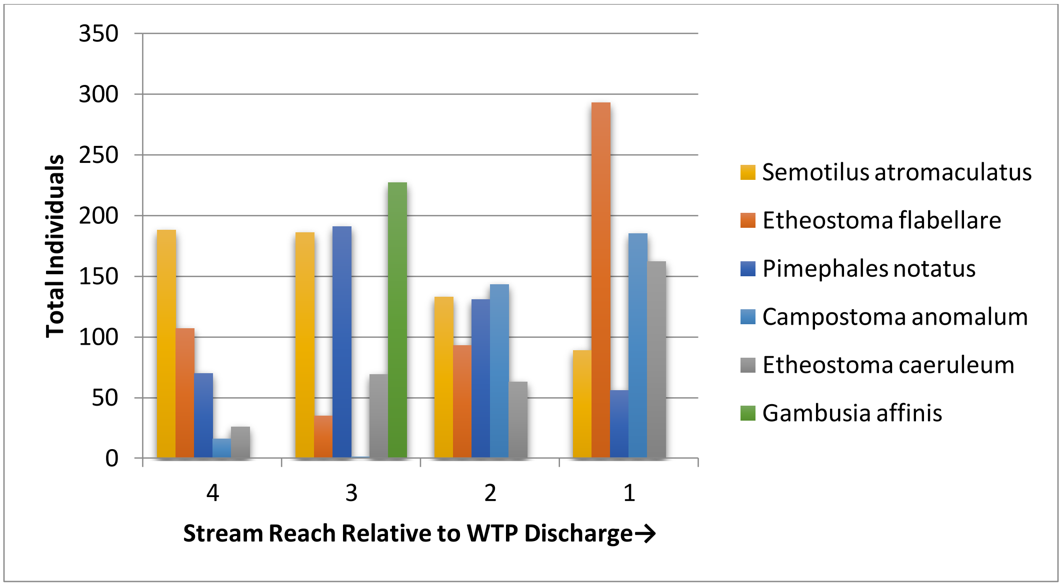

Figure 14 Dominant fish species identified in relation to spring electrofishing at Tates Creek WTP sampling sites.

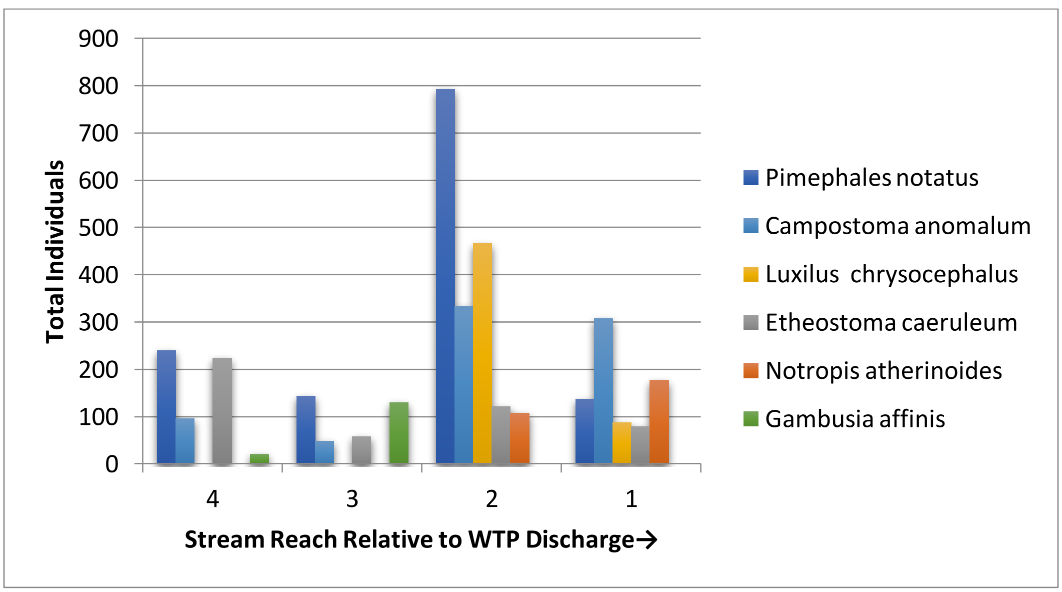

Figure 15 Dominant fish species identified in relation to fall electrofishing at Tates Creek WTP sampling sites.

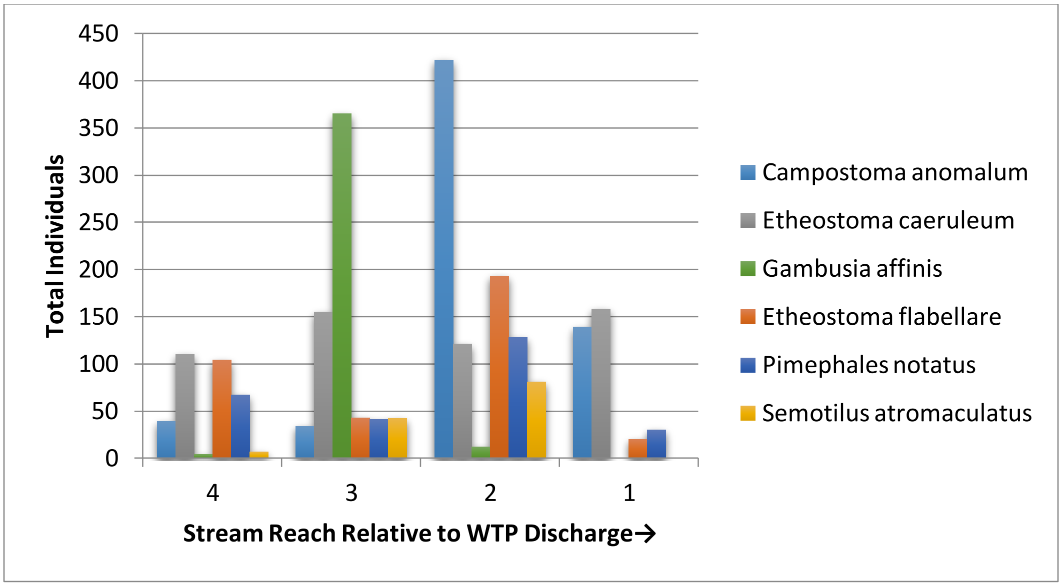

Figure 16 Dominant fish species identified in relation to spring electrofishing at Otter Creek WTP sampling sites.

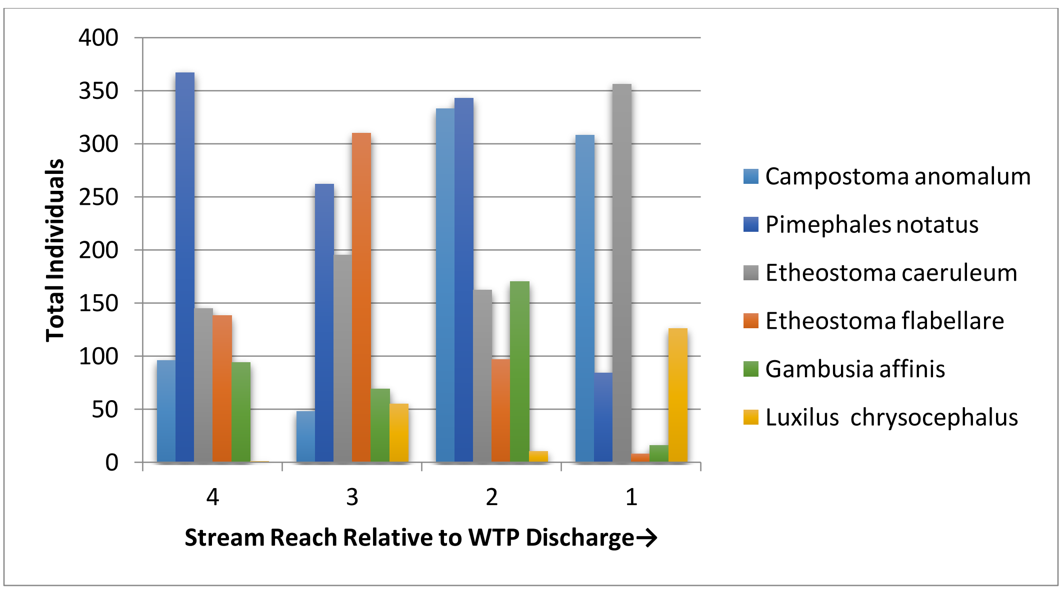

Figure 17 Dominant fish species identified in relation to fall electrofishing at Otter Creek WTP sampling sites.

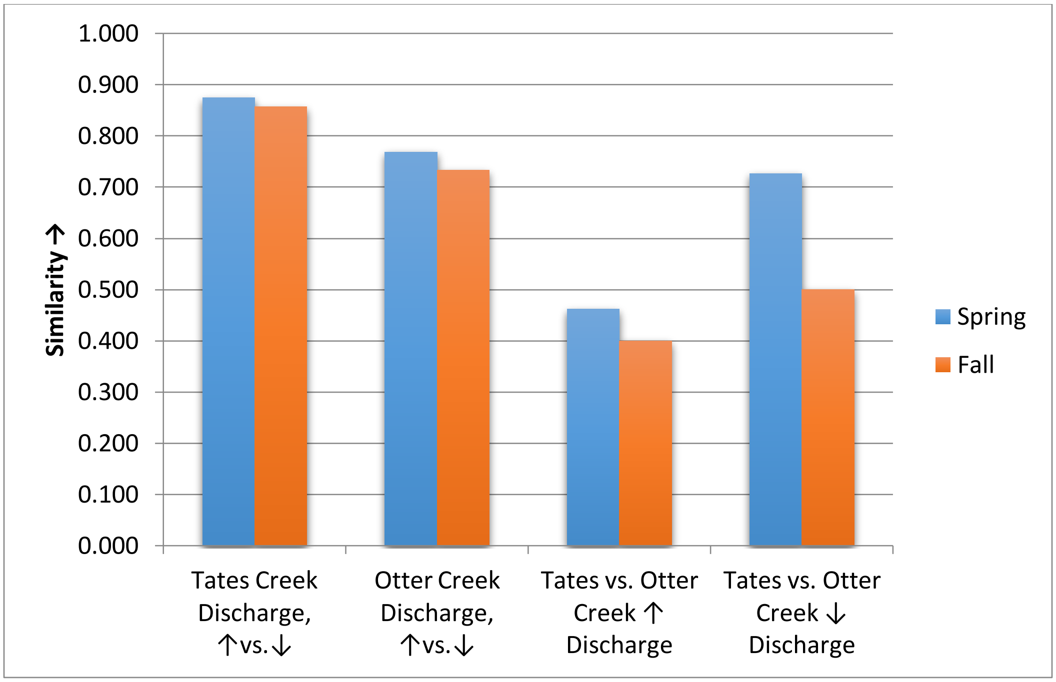

Figure 18 Similarity of fish communities above and below WTP discharges. Resulting metric scores calculated using Jaccard's Similarity Coefficient.

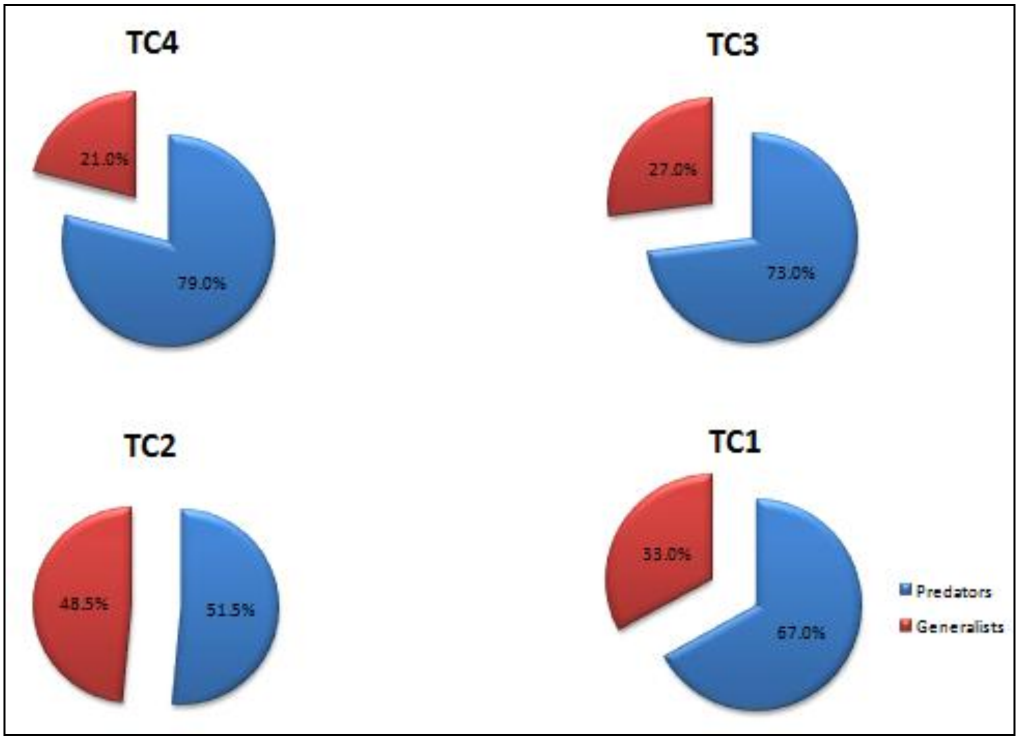

Figure 19 Fish functional feeding groups, spring, in relation to Tates Creek WTP discharge.

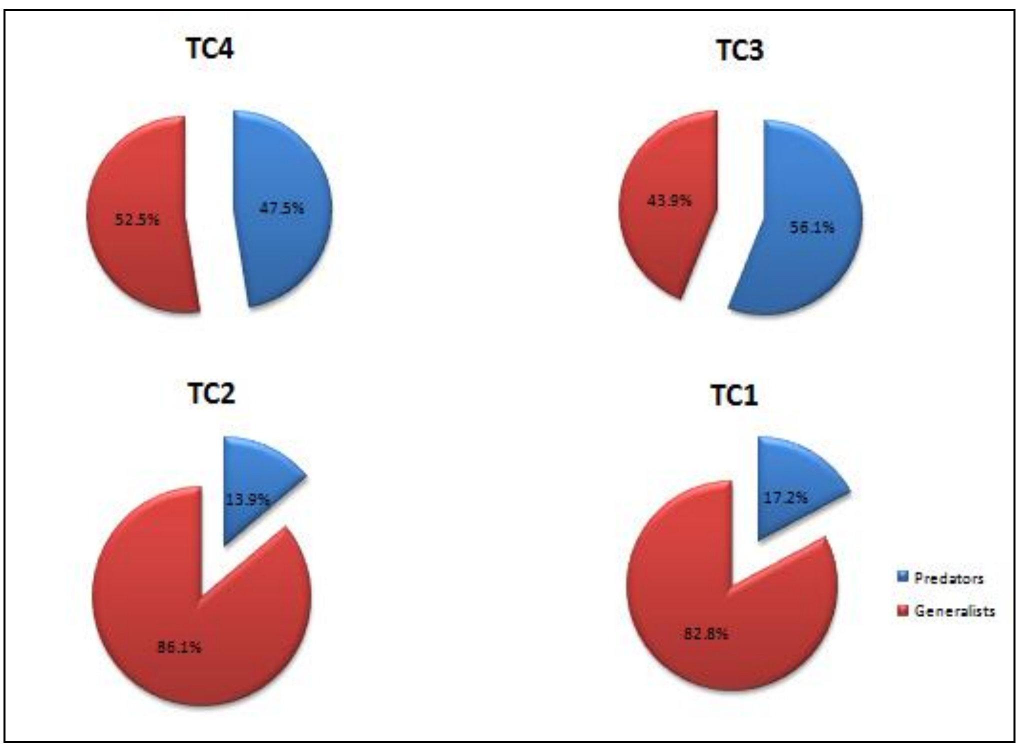

Figure 20 Fish functional feeding groups, fall, in relation to Tates Creek WTP Discharge.

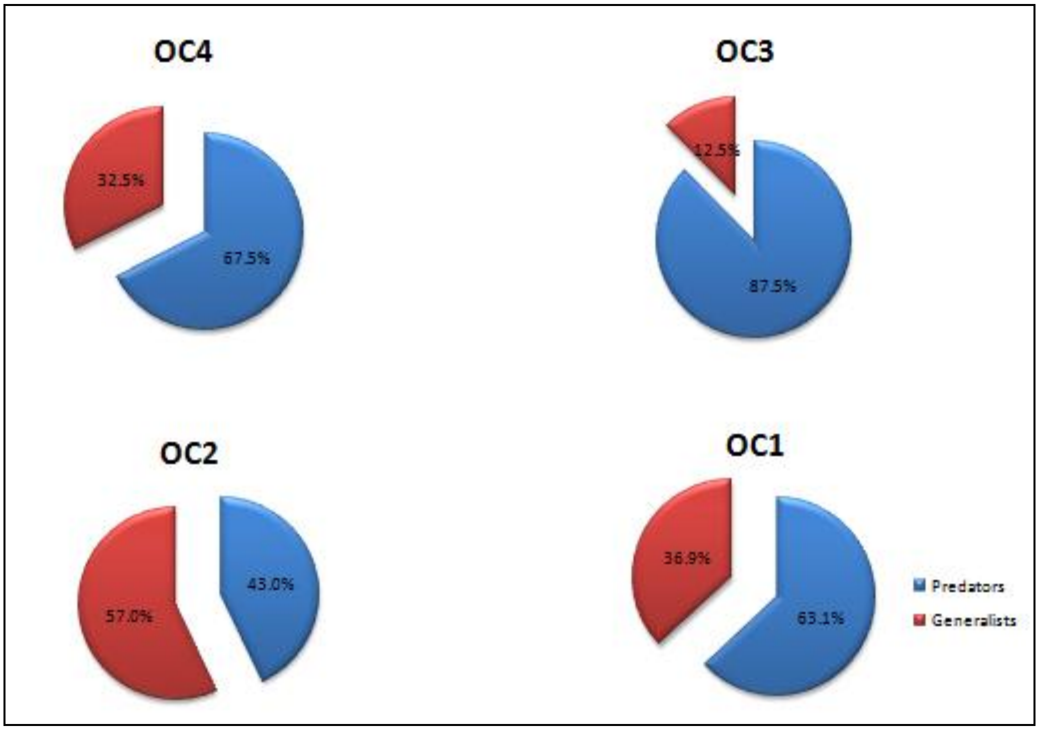

Figure 21 Fish functional feeding groups, spring, in relation to Otter Creek WTP discharge.

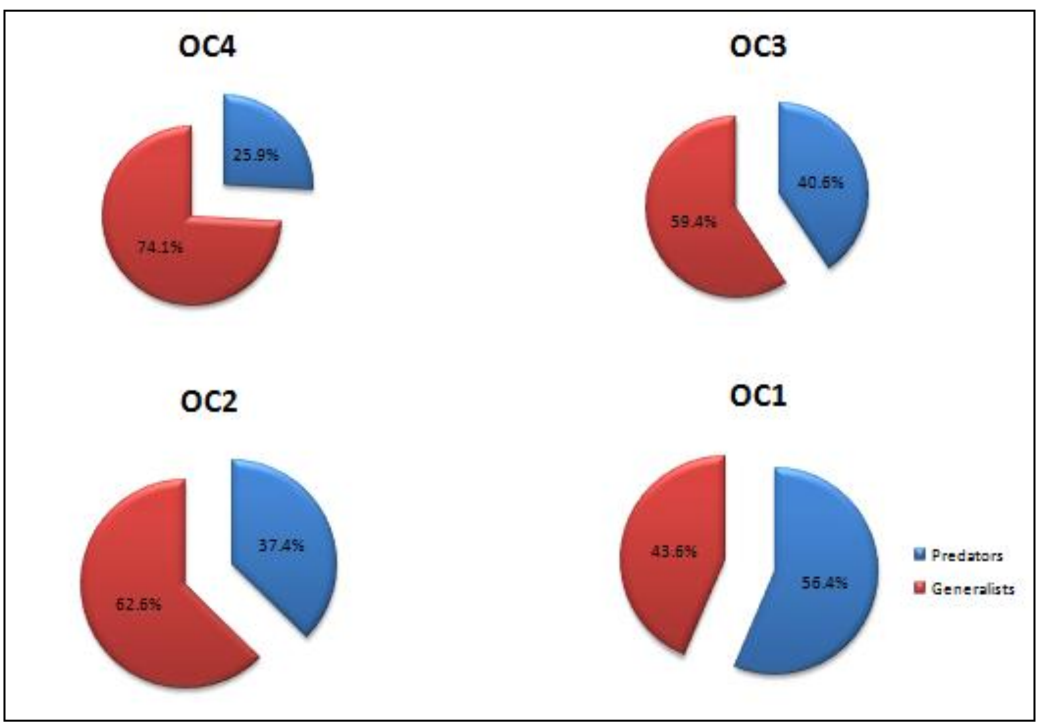

Figure 22 Fish functional feeding groups, fall, in relation to Otter Creek WTP discharge.

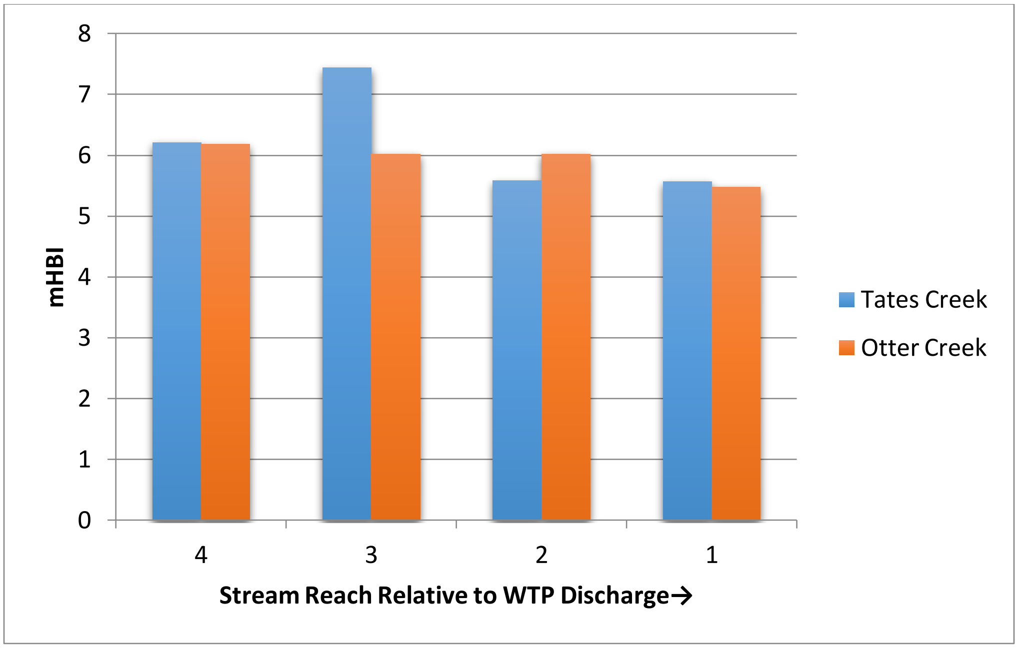

Figure 23 Comparison of Modified Hilsenhoff Biotic Index (mHBI) in relation to Tates Creek WTP and Otter Creek WTP, stream-sampling sites.

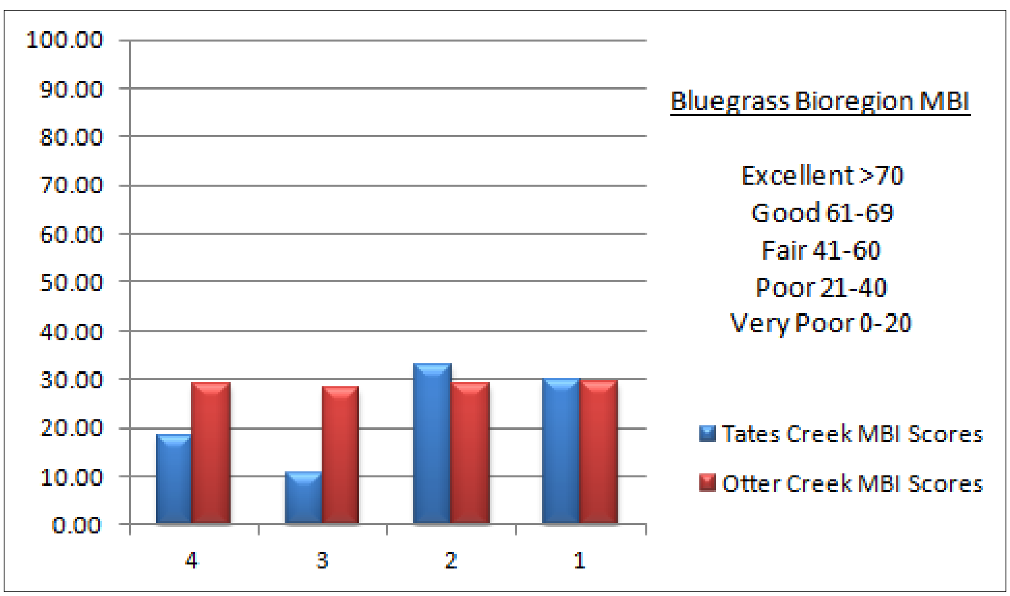

Figure 24 MBI scores in relation to Tates Creek WTP and Otter Creek WTP.

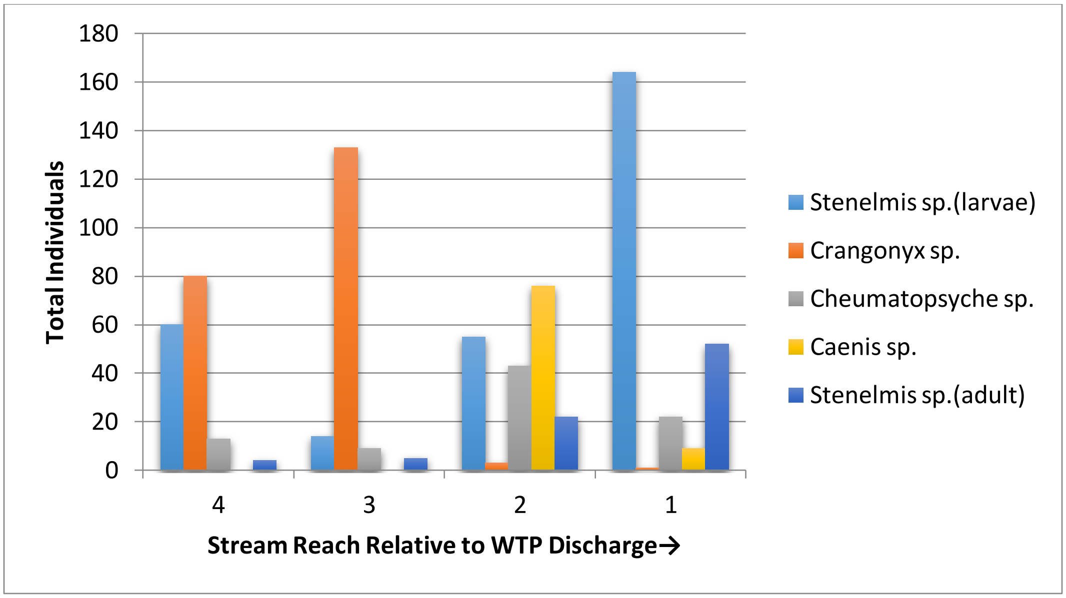

Figure 25 Dominant macroinvertebrate species collected, in relation to Tates Creek WTP stream-sampling sites.

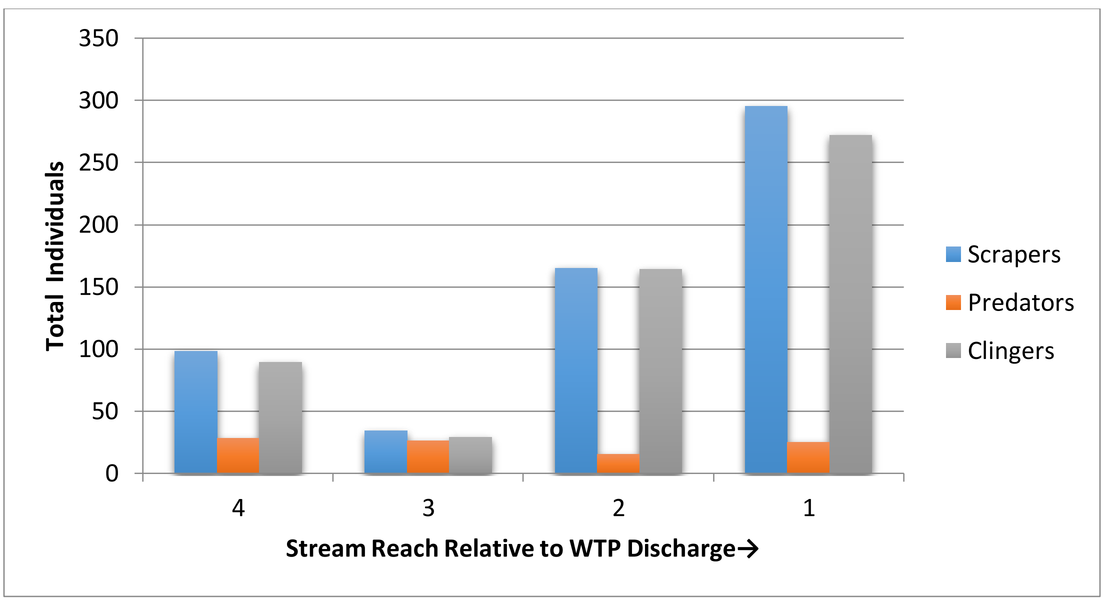

Figure 26 Selected macroinvertebrate functional group distributions in relation to Tates Creek WTP discharge.

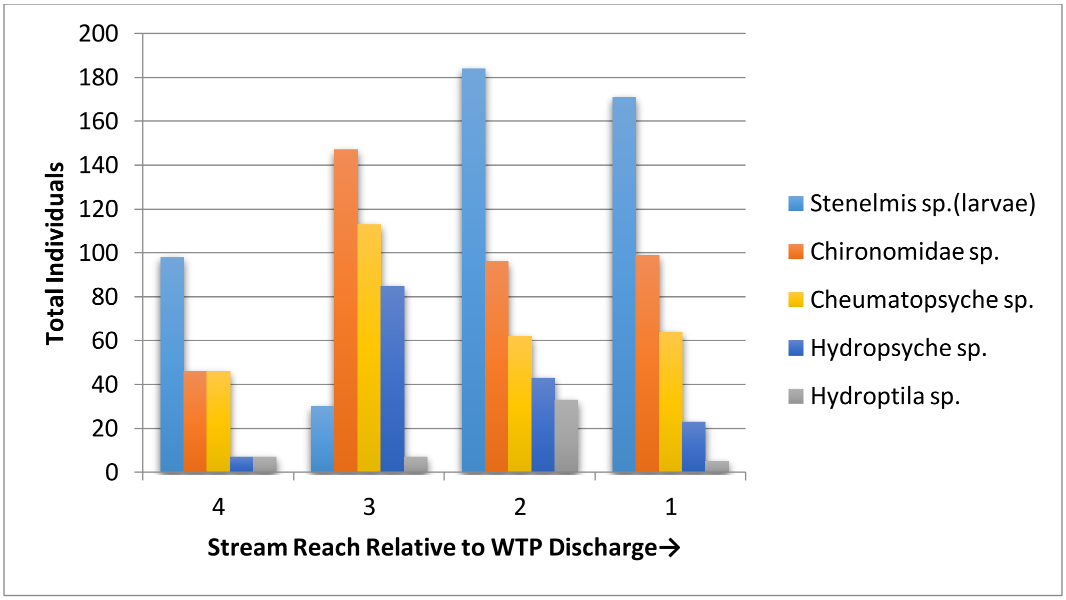

Figure 27 Dominant macroinvertebrate species collected, in relation to Otter Creek WTP stream sampling sites.

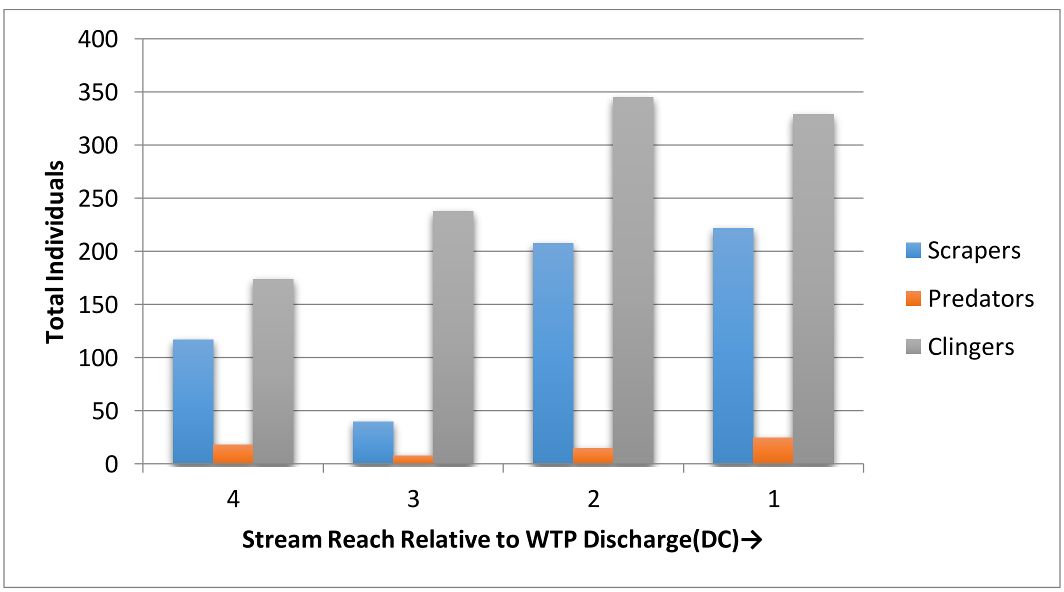

Figure 28 Selected macroinvertebrate functional group distributions in relation to Otter Creek WTP discharge.

VITA

Daniel John Ratterman was born November 18, 1962 in Oak Park, Illinois. He attended Jefferson County (Kentucky) primary schools and Trinity High School (Louisville, Kentucky) graduating May 1980. Daniel attended the University of Dayton (Dayton, Ohio) graduating May 1985 with a Bachelor of Science in Engineering Technology, major in Electrical Engineering, and minors in Biomedical and Mechanical Engineering. Following careers in the medical imaging field, agriculture and as a construction industry manager, he attended the University of Louisville completing post baccalaureate work and beginning graduate work in biology. Transferring to Eastern Kentucky University in January 2011, he earned a M.S. in aquatic and wildlife biology May 2016. Daniel has been passionate about the outdoors since childhood. Volunteerism includes water quality testing with Salt River Watershed Watch, interpreter at the Falls of the Ohio State Park, many projects at EKU's Taylor Fork Ecological Area, and as a conservation interpreter with Salato Wildlife Education Center, Kentucky Department of Fish and Wildlife Resources.