Fish communities near municipal wastewater discharges in the Grand River watershed

by

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A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Master of Science
in
Biology

Waterloo, Ontario, Canada, 2010

Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Municipal wastewater effluent (MWWE) has the potential for aquatic degradation, as it is the largest, per volume, anthropogenic discharge in Canada and other areas in the world. With an increasing population in many areas, such as Southern Ontario, there is concern that infrastructure of wastewater treatment facilities will not be able to maintain adequate treatment and prevent further degradation of the environment. The Grand River watershed, in Southern Ontario, is predicted to have its population increase to 1.2 million people by 2031 (from 780,000 people in 2001). Although wastewater treatment has improved, concern remains for receiving environments due to inadequate treatment (i.e. Kitchener) and minimal dilution (i.e. Guelph). This research was conducted to understand current impacts of MWWE in the Grand River watershed on fish communities to support future management and protection. Study sites upstream and downstream were chosen for their proximity to the Guelph, Kitchener, and Waterloo MWWE outfalls, similarity in habitat, and wadeability. Habitat analysis indicated that there were no large physical differences among sites. Fish communities were collected in a standardized method with a backpack electroshocker at each site (six randomly selected 10 m by 10 m sub-sites for 5 min). Greenside Darter (*Etheostoma blennioides*) and Rainbow Darter (*E. caeruleum*), the most abundant species, were also analyzed for stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) at each site. Downstream of the Guelph outfall there were no changes in mean total catch per unit effort (CPUE) or mean total mass. Changes to diversity, resilience, and tolerance in the fish community were attributed to a decreased abundance of Greenside Darter and increased abundance of Rainbow Darter. Downstream of the Kitchener discharge, there was a trend towards decreasing mean total CPUE, especially for darter species, and an increase in mean total mass due to a community shift to larger species including Catostomids and Centrarchids. The changes in abundance of Rainbow Darter, Catostomids, and Centrarchids among reference and Kitchener MWWE exposed sites explained the pattern in resilience, tolerance, and diet classifications. Lower diversity downstream of all three MWWE outfalls can be attributed to the increase in Rainbow Darter abundance. Stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of Greenside Darter did not change downstream of the Guelph and Waterloo discharges, but signatures of Rainbow Darter increased immediately below the two outfalls. This shift may be due to the Rainbow Darter being able to take advantage of a change in the environment (i.e. food availability), resulting in its increased abundance and changes in isotopic signature. Directly downstream of the Kitchener outfall both darter species had an increase in $\delta^{13}C$ and a large decrease in $\delta^{15}N$, likely due to high nutrient inputs from the outfall. The Kitchener

wastewater discharge is also associated with a decrease in abundance of fish and a shift in community structure. MWWEs are currently affecting the aquatic environment, including fish communities in the Grand River watershed. Future investments in infrastructure and watershed management should be made to mitigate degradation of water quality in this watershed.

Acknowledgements

Originally I knew very little about what fish species there were and how to identify them. Ken Oakes and Gerald Tetreault were the first to sample with me and assist me in learning the roughly 33 species captured during this study. These two boys also helped a great deal in introducing me to the watershed and fish sampling practices. Thank you for all the time you spent in the hot July sun and cold November wind sampling with me.

Field work needs many hands and could not have been accomplished without the time of many members of the Mark Servos Lab from the University of Waterloo and Mark McMaster Lab from Environment Canada. I especially thank Brendan Smith who sampled with me almost everyday. You were instrumental to the collection of the habitat data. Additional thanks to Stephanie Dryden-Cripton, Ben DeJourdan, Natalie Feisthauer, and Stephanie Lyons, the non-lab associated field workers. Thank you to Brendan Knight for assisting in the field work and drying, grinding, and weighing all those samples for isotope analysis.

There were many organizations who assisted and I thank for their help: the Grand River Conservation Authority, notably Sandra Cooke and Mark Anderson for water quality information; Art Timmerman at the Guelph MNR for historic watershed conditions; Gord Gallant at the Peterborough MNR for river flow data; the City of Guelph Wastewater Services for treatment processes and river access; Doon Valley Golf Course for river access; and Joe Keene at Stantec, Guelph for assisting me with the crayfish identification. Robert Bailey at Ontario Western University also assisted by giving the equation for the Brenduler and Brewster Conant Jr. at the University of Waterloo provided a Swoffer.

My committee members were George Dixon and Kelly Munkittrick and gave a lot of time, assistance in analysis, and helpful suggestions. My supervisors Mark McMaster and Mark Servos were great help fishing, constructive criticism, and providing me with a trailer. I could not have accomplished this without your guidance and assistance.

Thanks go to my family and friends for their support and Linda Zepf in the Department of Biology for her “Sign Here” sticky notes. And a special thanks to Andy Chapman for his milkshakes.

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Introduction

The effects of municipal wastewater effluent (MWWE) on the environment and on human health are of increasing concern. The most common way to dispose of wastewater is by discharging into nearby waterways. Originally this waste was untreated and lead to serious problems such as odor, eutrophication, acute toxicity, and disease. Treatment of wastewater was developed to reduce these impacts, but some problems still persist in many Canadian locations (Chambers et al. 1997). Advances in chemical detection have found low concentrations of emerging contaminants of concern in wastewater such as solvents, pharmaceuticals, and personal care products. Many of these chemicals are endocrine disruptors and may alter growth and reproduction in fish at concentrations found in some effluents (Tyler et al. 1998).

MWWE is the largest anthropogenic discharge into the aquatic environment per volume in Canada (Kilgour et al. 2005), leading to a large potential for ecological impacts. Although wastewater treatment has improved dramatically in most jurisdictions, there are still concerns about degradation of ecosystem health in receiving waters. Chemical composition of final effluents are monitored regularly, but knowing the concentration of a limited number of analytes is not enough to protect aquatic ecosystems (Karr 1981, Birge et al. 1989). Wastewater effluent impacts on organisms in the environment need to be understood in order to define potential risks and formulate appropriate remedial actions on a site-specific basis.

Municipal wastewater includes liquid wastes from sanitary sewers (residential, industrial, and commercial) and stormwater (precipitation and runoff) that contains various microbes, metals, organic compounds, oils, and many other chemicals and solids. Municipalities vary in the level of treatment their sanitary sewers receive and also if stormwater is treated in a combined or separate system. In Canada, all levels of government have some responsibility for the regulation and collection, treatment, and release of effluents (Environment Canada 2001).

Wastewater treatment begins with primary treatment that removes large solids, such as plastics, gravel, and sand with a screen and settling tank. Secondary treatment removes the organic matter with bacteria and aerobic digestion followed by more settling tanks. Tertiary treatment removes additional dissolved or suspended substances, but processes are variable: some tertiary treatment is targeted at removal of phosphorous, ammonia, metals, specific organics, or colour. The final process is usually disinfection (usually only in summer months) using chlorine, UV radiation, and/or ozone (Kilgour et al. 2005).

In Ontario the level of treatment of the plant, the size of mixing zone, and the dilution power of the receiving environment determines allowable concentrations of chemicals and particulates in the final effluent (Ministry of Environment and Energy 1994a), with the Provincial Water Quality Objectives as a goal. Therefore, treatment is site-specific to the needs of each municipality and its receiving environment as determined by the provincial government. In Ontario, acceptable concentrations from continuous discharge into rivers are calculated from the low flow 7Q20 (over twenty year periods what is the average low flow for seven days). In other words, calculating the 5% chance that the flow will not give adequate dilution in any year (Ministry of Environment and Energy 1994a). In addition, whole effluent must not be acutely toxic (96 hr) to Rainbow Trout (*Oncorhynchus mykiss*) or *Daphnia magna* and water quality outside the mixing zone must not be degraded. In Ontario it is illegal to dump untreated sewage directly into the rivers and lakes and all treatment plants that discharge into waterways must have at least a secondary treatment system (Ministry of Environment and Energy 1994b).

Sometimes incoming volumes of wastewater are too much for the treatment plant to handle, leading to the wastewater bypassing treatment and the raw or partially treated sewage enters the environment. This happens more frequently with combined systems (i.e. stormwater) during or just after rainstorms (Canadian Council of Ministers of the Environment 2006). This wastewater can cause adverse effects in ecosystems and on human health downstream.

Despite treatment, municipal effluents contain a wide variety of oxygen depleting substances and contaminants that can impact the health of aquatic organisms and populations. When effluent has a high biological oxygen demand (BOD), the dissolved oxygen is consumed through organic processes, potentially suffocating or stressing aquatic organisms. High nutrient concentrations (i.e. nitrogens and/or phosphates) can lead to increased periphyton and macrophyte densities, leading to increased BOD and changes in habitat and species composition (Canadian Council of Ministers of the Environment 2006).

The mean LC_{50} (lethal concentration for 50% of the population) from 112 studies on ammonia (unionized form) toxicity in Rainbow Trout was reported as 0.481 mg/L (Environment Canada 2000), but lower chronic exposures may reduce fish growth and reproductive capacity. Ammonia is classified as toxic under the Canadian Environmental Protection Act, 1999 (Environment Canada 2000). The discharge of the City of Kitchener had a mean monthly total ammonia concentration of 18.05 mg/L in 2008 (Kitchener Wastewater Treatment Plant 2008). Based on a surface water temperature of 20^{o}C and pH of 7.90, the unionized ammonia concentration would be around 0.55 mg/L. It has also been suggested that various forms of nitrogen pollution, including nitrate, may act as an endocrine disruptor which could potentially alter the reproductive performance of aquatic animals (Guillette and Edwards 2005). Urbanized rivers have an increase in all forms of nitrogen, which often changes aquatic community structure (Ulseth and Hershey 2005).

Fish community effects from wastewater effluent have included increased abundance (Porter and Janz 2003, Winger et al. 2005, Yeom et al. 2007), decreased abundance (Dyer and Wang 2002, Ra et al. 2007), decreased diversity (Birge et al. 1989, Ra et al. 2007), increased diversity (Winger et al. 2005), increased tolerant species, omnivores, and deformities/lesions (Ra et al. 2007, Yeom et al. 2007), and generally an impacted ecosystem (Dyer and Wang 2002, Ra et al. 2007). Ecosystem performance can be confounded by watershed structure and habitat (Winger et al. 2005). Individual species downstream of MWWE have been found with increased vitellogenin (Tyler et al. 1998, Porter and Janz 2003), increased condition (Dyer and Wang 2002, Porter and Janz 2003, Winger et al. 2005, Yeom et al. 2007), and no young of the year present (Yeom et al. 2007). Effects of whole final MWWE in laboratory experiments have shown reduced growth in fathead minnows (Orr et al. 1992) and immonutoxicity, genotoxicity, changes in kidney structure, and feminization in roach (Liney et al. 2006). This demonstrates the potential for MWWE to have negative effects on the aquatic ecosystem.

Another concern in effluents is emerging contaminants (Tyler et al. 1998), in particular, endocrine disrupting chemicals that impact fish reproductive processes and may lead to loss of fecundity or recruitment. A variety of endocrine disrupting substances and pharmaceuticals have been found in Canadian waterways from MWWE (Metcalfe et al. 2003, Servos et al. 2007) including 17β-estradiol and estrone (Servos et al. 2005) and industrial compounds (Servos et al. 2003). Kidd et al. (2007) dosed Lake 260 in the experimental lakes area of North-Western Ontario to 17α-ethinylestradiol (approximately 5 ηg/L), the active ingredient in birth control pills. Within two years of exposure, recruitment of fathead minnows (*Pimephales promelas*) ceased, leading to a severe decrease in fathead minnow abundance. Exposed fish had an increased occurrence of intersex in males and increased blood vitellogenin concentrations in both sexes. A variety of impacts have been seen in environments receiving, including endocrine disruption, to fish exposed to municipal effluents. Feminized male wild fish in the United Kingdom and elsewhere have been found downstream of MWWE (Tyler et al. 1998). Endocrine disruption may be an important influence in the reproductive success of fish exposed to MWWE. However, there are many other trace contaminants in effluents that may have the potential to effect fish and other organisms through a wide variety of mechanisms.

Although contaminants in the effluent may be biologically active, the receiving environment may dilute and alter their bioavailabilty, decreasing or even resulting in no apparent toxicity. The interaction of the contaminant with the physical and biotic habitat of the organism influences toxicity (Emlen and Springman 2007). Chambers et al. (1997) found that few Canadian MWWEs lead to acute toxicity except where there is minimal treatment or dilution. However, the threat of long term impacts from exposure to low concentrations of chemicals in MWWE still needs to be considered.

Numerous studies have demonstrated the utility of using stable isotope signatures (stable isotope analysis) as a tool to understand food web structure and function in rivers. $\delta^{15}N$ increases 3-5‰ with each trophic level, allowing for estimation of trophic position of different species. $\delta^{13}C$ increases 0-1‰ with each trophic level, so is much more reliable as an indicator of carbon source (Ulseth and Hershey 2005).

For example, C_4 plants are heavier in the carbon stable isotope (~ -13.5‰) than C_3 plants (~ -28.1‰) (O'Leary 1981). Other factors, such as amount of dark respiration, carbon source, or CAM (crassulacean acid metabolism) processes will impact the carbon signature. Aquatic plants with similar photosynthetic processes have higher $\delta^{13}C$ values than terrestrial plants (O'Leary 1981), although phytoplankton has lower $\delta^{13}C$ than terrestrial litter in lake systems (France 1995). There is also an increase in $\delta^{13}C$ in autotrophs as CO_2 availability decreases (O'Leary 1981, Finlay 2004), which is likely the cause for the difference in aquatic and terrestrial species signature. Therefore, an organism's stable isotope signature will reflect its food and habitat (Peterson and Fry 1987).

Where an organism occurs in the watershed, headwaters or further downstream, can impact its isotopic signature. Finaly (2004) found an increase in $\delta^{13}C$ as watershed area grew, possibly due to the decrease in available CO_2, carbonate bedrock, and increasing anthropogenic influences. The river continuum concept supports the theory of a gradient from exogenous to endogenous energy sources within a river system (Vannote et al. 1980). This could lead to changes in CO_2 use, as carbon source shifts from external terrestrial sources to aquatic autotrophs in streams (France 1995). Loomer (2008) found no correlation of increasing $\delta^{13}C$ downstream to CO_2 concentrations in the Grand River. It was hypothesized that the high CO_2 concentrations were not a limiting factor in photosynthesis and variation in $\delta^{13}C$ was due to substrate source, which has an increased signature possibly from carbonate bedrock and anthropogenic inputs such as MWWE and dams. Dams have been shown to increase $\delta^{13}C$ in organism downstream (Loomer 2008), with a possible explanation being of increased CO_2 recycling within lake food webs (Lennon et al. 2006).

Food webs downstream of wastewater inputs in freshwater are enriched in stable isotopes of N and C (if food is an aquatic source) (Steffy and Kilham 2004, Ulseth and Hershey 2005, Northington and Hershey 2006). deBruyn and Rasmussen (2002) found in a river receiving secondary treated municipal effluents that > 60% of carbon and nitrogen incorporated in benthos near the outfall was of effluent origin. Sediment accumulation did not occur in this area and nutrients from other sources were more abundant. This suggested preferential uptake of wastewater derived particles. This preferential uptake in a watershed with many municipal outfalls could lead to a large disruption in energy cycling and further adverse effects.

The Grand River watershed (Figure 1) is the largest in Southern Ontario, Canada, 6,965km² draining into Lake Erie. In 2006, 76% of the surface area was agriculture, and 80% of the 900,000 residences were serviced by 26 municipal wastewater treatment plants (Cooke 2006). This population is expected to grow to 1.2 million by 2031, concentrating in the central urban area of the watershed (Ministry of Public Infrastructure Renewal 2006). This growth is due in part to the restriction of development in the Greenbelt which surrounds the Greater Toronto Area. With the watershed already impacted from agricultural inputs and existing MWWE, the Grand River may not have sufficient assimilative capacity for further large urban development. Continued growth in population in this region may be extremely detrimental to the watershed given these circumstances.

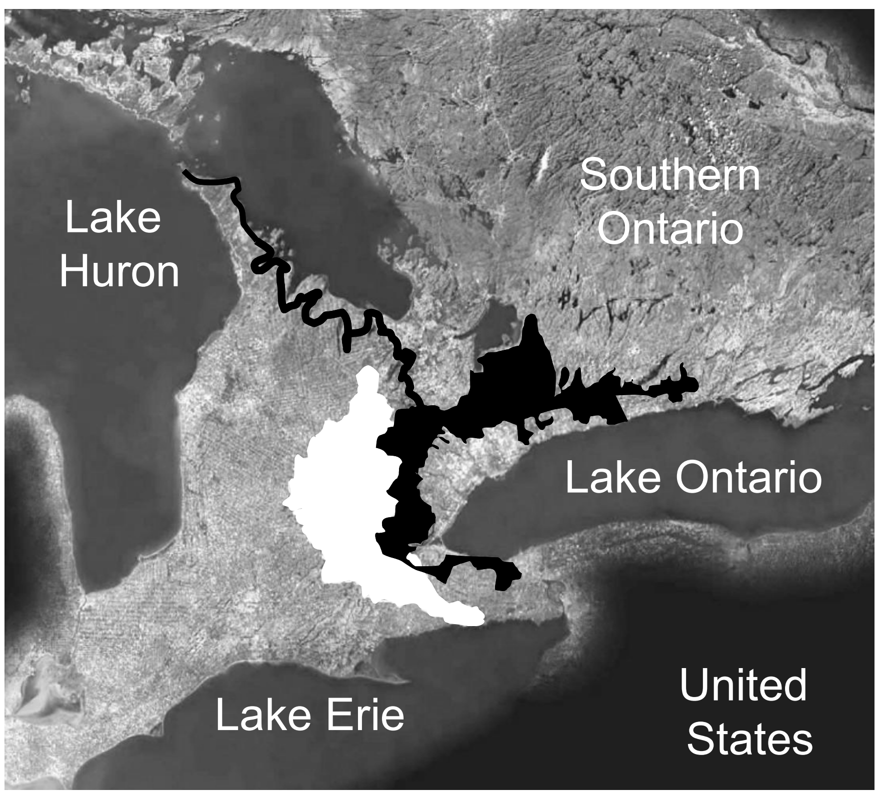

Figure 1: The Grand River watershed (white) in Southern Ontario, Canada with the Greenbelt (black), over lain (source: www.grandriver.ca).

The discharge of effluent from treatment plants in the Grand River watershed have historically had severe impacts on the aquatic receiving environments through eutrophication, oxygen depletion, and toxicity (Grand River Conservation Authority 2008). Although there have been considerable plant upgrades to address these issues, there is remaining concern for selected plants that have inadequate treatment (i.e. Kitchener) or discharge into small receiving environments (i.e. Guelph). Using ratios of stable isotopes of nitrogen and carbon, Loomer (2008) demonstrated that the municipal treatment plants in the watershed were altering the cycling of nutrients in the receiving environments. A variety of endocrine disrupting substances and pharmaceuticals have also been found in the Grand River (Metcalfe et al. 2003, Servos et al. 2005, Servos et al. 2007). Using an Environmental Effects Monitoring Program (EEM) protocol, Tetreault et al. (2008) found minimal changes in whole organism responses and indicators of reproductive impairment downstream of effluents from three plants. Although Tetreault et al. (2008) found a decrease in fish abundance downstream of the Kitchener outfall, there were no obvious effects on abundance downstream of the Waterloo or Guelph outfalls.

Fish communities are used to evaluate ecological health of waterways because they integrate conditions of other trophic levels (Kilgour et al. 2005). Species at certain sites can indicate a shift in food source at lower trophic levels by changes in the stable isotope signatures for both nitrogen and carbon (Jardine et al. 2006). Fish species are also fairly easy to process and are socioeconomically relevant (Simon 2006). They are responsive to endocrine and emergent chemicals found in MWWE, unlike most plant and invertebrate species (Tyler et al. 1998). Additionally, there is some evidence that fish communities are possibly impacted by MWWE in the Grand River watershed (Loomer 2008, Tetreault et al. 2008). A more complete understanding of the current status of fish communities and the factors that control them will be critical in supporting future management decisions related to wastewater treatment in this watershed.

Various protocols have been developed to assist in sampling fish communities. Existing protocols that use a backpack electroshocker are designed to sample all habitat (riffle, run, pool), bank to bank, in low order streams. Recommended site sizes are 20 x wetted width or between crossover points over 40 m for the US EPA, USGS, and OMNR protocols (Lazorchak et al. 1998, Moulton et al. 2002, Stanfield 2007). As the Speed River is a medium sized river (6^{th} order) with pools and shallow riffles, it would not be feasible to continuously measure 20 x wetted width with a backpack electroshocker or any other similar method.

Previously on the Grand River, backpack electroshockers used a random half of a 100 m site (Coleman 1992) to compare fish communities in urban and rural areas. Tetreault et al. (2008) developed an approach using six randomly selected 10 m by 10 m (from shore) sub-sites from a 100 m river section. This method was developed on the Grand River, using species richness as a guide for selecting how many sub-sites were needed to reduce variability in order to detect impacts. This standardized method was used in this study because of its feasibility and statistical power (replication).

Once the fish communities are sampled at each site, there are many ways to examine the community to determine if impacts exist. The Index of Biotic Integrity (IBI) is possibly the most well known, and was developed in warm water streams in central Illinois and Indiana (Karr 1981). This has been modified for other river types and sizes and is the primary method for fish community assessment by the US EPA. The IBI is composed of various matrixes that look at species richness and composition, trophic organization and function, indicator species, hybrids, and abnormalities of individuals (i.e. disease, deformities etc). Depending on reference conditions, a score of 1, 3, or 5 is given (with 5 being similar to reference), and each matrix is added together to give a final IBI score. This score can be compared to other sites in the area and to previous and future sampling at those sites. This approach can lead to ambiguity (Suter 1993). For example, two sites may be both given a score of 45. Some matrices could be very similar, but one matrix could be high while another low and the opposite for the other site, which means different fish communities exist at each site. Low abundance, or more omnivores, or more hybrids can indicate different types of change. Also, each of the matrices that make up the final IBI score have the same weight towards the final score, but may not be equal in terms of ecological function. Part of the IBI also includes maximum richness lines, which would be very similar for closely spaced sites such as the ones used in this study. Adaptation of the IBI for each specific watershed or region would also need to be developed. Additionally, it is not evident that an IBI could be applied to wadeable riffle communities as it is not including all fish within an area and biasing results towards benthic insectivores. For these reasons IBI was not considered appropriate for use in this study.

Many other studies examining fish communities have compared abundance, species richness, diversity, trophic level or tolerance composition, and condition of abundant species. Abundance is often standardized to catch per unit effort (CPUE), which takes abundance and divides it by the sampling time, area, sweeps, etc. that occurred. Simpson and Shannon-Wiener are popular diversity indices. The Simpson index doesn't weigh rare species as much as Shannon-Wiener index, so the selection of which index to use needs to be based on the study's objectives (Krebs 1999). Trophic level categories group species based on what they eat; omnivore, carnivore, invertivore etc. and is often reported as a percent composition of the fish community for each site. Similarly, species can be classified as tolerant, intermediate, or intolerant as percent site composition of tolerance of the fish community. Condition, also called condition factor or Fulton's condition factor, uses the equation $K = W/L^3$, where W is the weight and L the length. As the equation implies, a larger condition will result when the fish is heavier relative to its length. This value can be compared between reference and exposed sites to determine if there are differences on how populations grow and store energy (Nash et al. 2006).

Fish are sensitive to many pollutants in MWWE, and changes to community and nutrient cycling have been seen in the Grand River (Loomer 2008, Tetreault et al. 2008). The human population increase predicted for the watershed may degrade effluent quality, leading to further aquatic degradation. The current impacts of MWWE will assist in determining appropriate mitigation that would prevent further degradation or recovery.

This study has the following objectives:

1. To assess changes in riffle fish community structure associated with the wastewater treatment plant outfalls in the Grand River (Guelph, Waterloo, and Kitchener plants).

2. To assess changes in stable isotope signatures of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) in the dominant fish species associated with major municipal wastewater effluent outfalls in the Grand River (Guelph, Waterloo, and Kitchener plants).

The effects of tertiary treated municipal wastewater on fish communities in the Speed River, Ontario

This chapter will be submitted as a manuscript to Aquatic Toxicology.
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Overview

As in many parts of the world, urbanization is increasing in Southern Ontario. This will strain current infrastructure and possibly lead to environmental degradation unless changes are made. The City of Guelph is considering this problem and looking at their current impacts in the environment to assist with future management decisions. The city is located on the Speed River and has historically had problems with oxygen levels, which were below the provincial standard of 4 mg/L downstream of the wastewater outfall in the early 1970's. The receiving environment is relatively small and could not adequately dilute or assimilate the wastes. As a result steps were taken such as upgrading the wastewater treatment plant for the City of Guelph to tertiary treatment in 1979, which raised river oxygen levels above provincial standards. There is concern, however, that with rapid population growth and emerging contaminants, effluent quality may decrease and degrade aquatic ecosystems unless larger investments are made in additional advanced treatment. Fish communities were evaluated in two seasons of 2008 to assist in determining current conditions and to help support future decisions. Fish were collected with a backpack electroshocker at nine wadeable sites upstream and downstream of the outfall. There were no large habitat differences among the selected sites and mean total catch per unit effort and mean total mass of fish collected were not affected by the outfall. Sites upstream of the treatment plant were dominated by Greenside Darter (*Etheostoma blennioides*), but directly downstream of the discharge Rainbow Darter (*E. caeruleum*) became dominant and there were few young of the year Greenside Darter at downstream sites. Stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) increased in Rainbow Darter downstream of the outfall, but showed no change in Greenside Darter. At this downstream site Rainbow Darter may be exploiting a food source that is not present at upstream sites, leading to a higher isotope signature and giving it a competitive advantage over the Greenside Darter. MWWEs are complex and even tertiary treated effluent appears to have subtle effects on fish communities in small aquatic receiving environments. Larger investments may be necessary for this city and other parts of the world if degradation is to be avoided with the increase of urbanization.

Introduction

Urbanization is increasing in many parts of the world, with a prediction of an increase from 49% in 2005 to 60% of people living in cities by 2030, concentrating in areas like India, China, and the United States (United Nations 2006). This raises concern about many issues, including infrastructure to meet the needs of these people. In Southern Ontario urban areas are expected to grow by as much as 47% between 2001 and 2031. An Ontario government report, “Places to Grow” has designated many of the communities surrounding the Greenbelt of Greater Toronto as areas to accommodate this rapid growth (Ministry of Public Infrastructure Renewal 2006). The City of Guelph and surrounding County of Wellington is projected to increase in population by 67% within this same period of time. Of particular concern in this community are the potential impacts this projected population increase may have on the quality of municipal wastewater effluent (MWWE), and the impact on the Speed River, a relatively small tributary of the Grand River. Although the MWWE outfall has caused significant ecological change in the river in the past, investment in treatment and wastewater management has resulted in considerable improvements. For example, in the 1970s oxygen concentrations downstream of the outfall were frequently below provincial water quality guidelines of 4 mg/L (Cooke 2006). The construction of the Guelph Lake reservoir to allow for water flow regulation/augmentation and a series of upgrades of the wastewater plant, including tertiary treatment in 1979 (DenHoed and Robertson 2003), resulted in recovery of river oxygen levels to meet the provincial guidelines. This downstream reach is still an area of concern on the Speed River as it continues to have lower dissolved oxygen levels (Cooke 2006). With the projected increase in the population to be served by the plant, there is concern that without additional investment in treatment infrastructure, effluent quality may decrease and degrade the river ecosystem, reversing the impressive progress made over the past few decades. The impact cities have on the environment is a concern for many communities around the world as urbanization strains current resources. The City of Guelph is one example of the struggle urban areas face when considering the health of the environment.

Although water chemistry has been routinely monitored in the Speed River (Cooke 2006), it alone is not adequate to understand the effects of the discharge on aquatic life (Karr 1981). Organisms exposed to the effluent must be examined to understand the impacts from these chemical inputs. Fish are commonly used as bioindicators of river health and many protocols have been established (Barbour et al. 1999, Moulton et al. 2002, Stanfield 2007) because they are relatively easy to process, are of public concern (Simon 2006), integrate conditions in other trophic levels (Kilgour et al. 2005), and are responsive to endocrine and emergent chemicals found in MWWE (Tyler et al. 1998). The fish community present reflects those species and individuals that can survive the physiochemical and biological influences of that area in addition to the added stress of the effluent. This makes them one of the most direct ways to assess if there are impairments to ecological function for small systems receiving municipal effluents (Kilgour et al. 2005).

Previous studies have looked at fish communities in areas upstream and downstream of outfalls (Porter and Janz 2003, Yeom et al. 2007), between urban and rural areas receiving effluent (Dyer and Wang 2002), and comparing forested, restored, and unrestored urban areas receiving effluent (Northington and Hershey 2006). All of these studies have found changes downstream of the MWWE outfalls, although the changes seen are unique to each site. For example, some studies have found an increase in abundance (Porter and Janz 2003, Winger et al. 2005, Yeom et al. 2007), while others a decrease in abundance (Dyer and Wang 2002, Ra et al. 2007). Other common changes are decreased diversity (Birge et al. 1989, Ra et al. 2007), increased diversity (Winger et al. 2005), and increased tolerant species, omnivores, and deformities/lesions (Ra et al. 2007, Yeom et al. 2007). The size of municipality served, level of treatment, and the receiving environment are different for each outfall and effects seen at one outfall cannot be assumed to occur at another. Investigations must be site-specific if protection of each aquatic environment is to occur.

Other studies have used stable isotope analysis to look for site-specific changes because the stable isotope signature of each organism is unique to that individual based on its biological processes and its habitat (Peterson and Fry 1987). This has made stable isotope ratios of nitrogen and carbon useful tools to indicate shifts in energy flow through food webs (Jardine et al. 2006) and can be potentially applied to understand the effects of municipal effluent inputs. Previous work by Loomer (2008) on the Speed River has indicated an increase in isotopic signatures of carbon and nitrogen in Greenside Darter (*Etheostoma blenniodes*) and Rainbow Darter (*E. caeruleum*) and some benthic primary consumers downstream of the effluent. This shift in isotope ratios suggests that there are changes in how nutrients and energy are incorporated into food webs associated with the discharge of MWWE. Typically $\delta^{15}N$ can assist in determining position within a food chain and $\delta^{13}C$ is more useful to indicate the origin food because the carbon isotope signature depends on the photosynthetic processes that originated the food source (O'Leary 1981, Ulseth and Hershey 2005). Stable isotopes of carbon and nitrogen have been found to be different downstream of outfalls because the effluent's signature is different from the receiving environment or fractionation is occurring differently (Steffy and Kilham 2004, Ulseth and Hershey 2005, Northington and Hershey 2006).

The water and wastewater managers in the watershed are concerned about conditions of the aquatic environment downstream of the Guelph MWWE outfall. It is possible that with population growth additional investments will be needed to maintain effluent quality, but current conditions need to be understood before informed decisions can be made. The results from this study can be helpful information to other urban areas considering upgrades for their wastewater treatment and what environmental responses they may expect to see. The objectives of this study were to determine if there are changes on the Speed River associated with the MWWE in i) the fish community, and ii) stable isotope signatures of carbon and nitrogen in abundant fish species.

Methods

Study Area

The City of Guelph, Ontario, Canada discharges its municipal wastewater effluent into the relatively small tributary of the Grand River: the Speed River (Figure 2). The Speed River has a mean August discharge of 2.48 m^3/s (1950-2005; Water Survey of Canada 2007b), leading to the effluent consisting of an average 7.6% of river flow in 2008 (Atkinson 2010, Gallant 2010). Currently, the City of Guelph treatment plant has extended activated sludge with denitrification and rotating biological contactors with sand filtration to remove phosphorous (Table 1) with a retention time of 15 to 28 days (City of Guelph Wastewater Treatment Plant 2007), resulting in high effluent quality (Table 2). The There are no other major municipal effluent outfalls further upstream. However, there are other potentially confounding factors. Guelph Lake is an upstream reservoir, which was constructed to maintain river flow (and effluent dilution) in the drier summer months. The Speed River is channelized for much of its length within the city, and has many low-head impoundments, the last one being 1.4 km upstream of the effluent outfall. Across from the treatment plant there is an active gravel pit, which discharges groundwater 0.33 km downstream of the outfall. The City of Guelph piles winter snow beside and downstream of the treatment plant, providing a source of salt and sand during spring run-off. In addition, there are a number of creeks and municipal drains upstream and downstream of the outfall.

Table 1: Description of the City of Guelph wastewater treatment plant for 2008 (City of Guelph Wastewater Treatment Plant 2007, 2008).

Parameter	Description
Population Served	100,000
Capacity m^3/day	64,000
Discharge m^3/day	54,892
Retention Time	15 to 28 Days
Secondary Treatment	Conventional and Extended Activated Sludge
Tertiary Treatment	Rotating Biological Contactors and Sand Filtration
Combined Sewers	No
Disinfectant	Sodium Hyperchlorite
Dechlorination	Sodium Bisulphite
Current Upgrades	Various to reach effluent criteria for capacity of 73,330 m^3/day

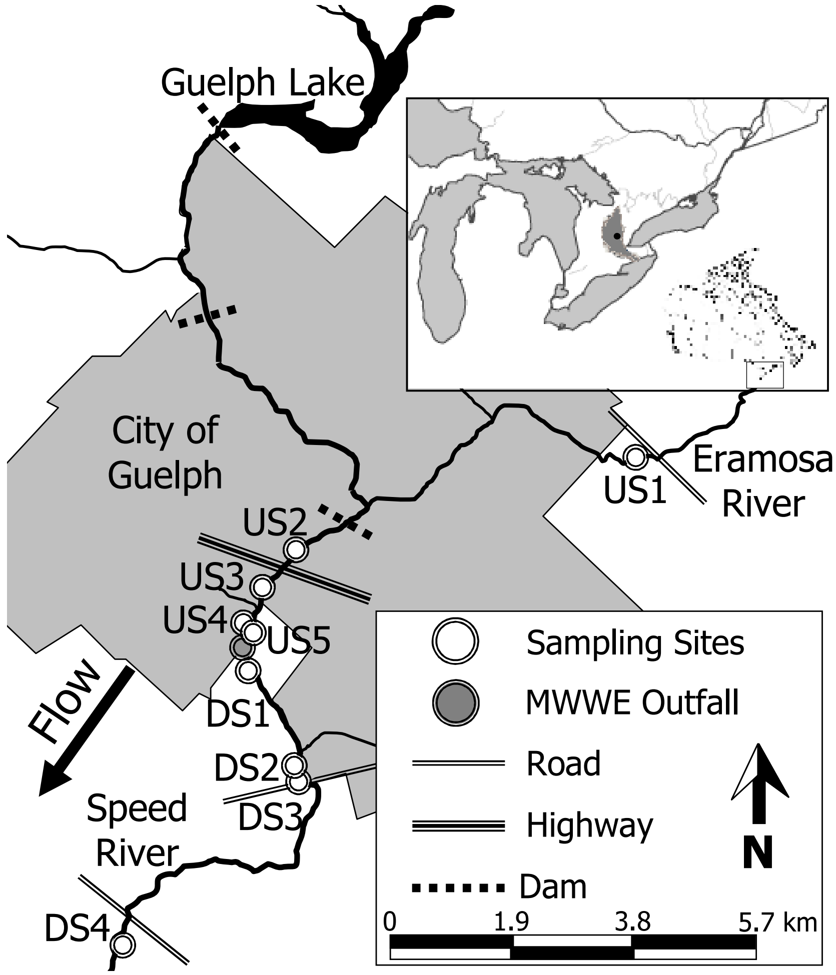

Figure 2: Map of the Speed River through Guelph, Ontario, Canada. Sampling sites for the 2008 study are labeled US1-US5 for upstream and DS1-DS4 for downstream of the Guelph municipal wastewater effluent outfall. Downstream of DS4 is the Hespeler Dam (6.4 km) and the Speed River enters the main branch of the Grand River (14.5 km) in Cambridge.

Table 2: Concentration of water quality parameters measured in the City of Guelph final municipal wastewater effluent, in 2008 (City of Guelph Wastewater Treatment Plant 2008) and dilution (Atkinson 2010, Gallant 2010). Biological oxygen demand (BOD) and suspended solids (SS) are a 24 hr composite sample (100 mL every 20 min). Final effluent BOD measured once per week and SS 6-7 times per week.

Parameter	Mean	Min (Month)	Max (Month)
BOD (mg/L)	2.90	2.0 (Aug/Nov)	4.8 (Dec)
SS (mg/L)	1.2	1 (all other)	3 (Dec)
P (mg/L)	0.10	0.08 (Apr)	0.25 (Dec)
$NH_3^-NH_4$ (mg/L)	0.8	0.11 (Jun)	1.58 (Dec)
NO_3^- (mg/L)	20.4	19.09 (Aug)	22.99 (Oct)
NO_2^- (mg/L)	0.5	0.2 (Jun/Aug)	1.01 (Oct)
Dilution (%)	7.6	3.2 (Apr)	12.1 (Jun)

Habitat and Water Quality

Habitat and water quality measures were collected at each site. Habitat information was collected in August, 2008 using methods adapted from the US EPA's Habitat Assessment in the Rapid Bioassessment Protocols for Wadeable Streams (RBA) (Barbour et al. 1999), and the Ontario Ministry of Natural Resources' Habitat Module in Ontario Stream Assessment Protocol (OSAP) (Stanfield 2007). At 1, 5, and 10 m from shore, estimates of percent of silt, sand, gravel (< 1", < 2"), cobble (< 4", < 8"), rock (> 8", > 12"), bed rock, and plant covering a 1 m by 1 m area was estimated. Rock shape, bank stability, bank vegetation, riparian width, canopy cover, river width, odor, and turbidity were also recorded. Water velocity was measured at 1, 5, and 10 m from shore using a Brenduler, a meter stick as described in Environment Canada's Canadian Aquatic Biomonitoring Network (CABIN) protocols (Environment Canada 2010). This method was validated with use of a Swoffer flow meter (Model 2100, $r^2 = 0.92$, $y = 0.79x - 0.034$, $n = 38$, depth = 0.14 – 0.72 m).

After habitat data was collected, the Ohio EPA Qualitative Habitat Evaluation Index (QHEI) (Rankin 2006) and the RBA were both used to convert habitat quality into a value for each site to determine if there were differences between sub-sites. No changes were made to the QHEI. In the RBA the “frequency of riffles” section was not applicable, so it was replaced with a percent of biofilm on rocks and turbidity of the water (which scored lower with higher percent/amount). Each section was also scored out of 10 instead of 20, resulting in a maximum habitat value of 100 per site.

Water quality information collected in August and October, 2008 included pH (Oakton pHTestr 2 Double Junction - Illinois), dissolved oxygen and temperature (YSI 55 Handheld Dissolved Oxygen – Yellow Springs). These endpoints were collected 5 m from shore between site 5 and 6 after electrofishing was complete.

Fish Community Sampling

Nine sites were sampled on the Speed River (Figure 2; Table 3) during August and October, 2008. These sites were chosen for their proximity to the MWWE outfall, wadeability, accessibility, habitat similarity, and safety.

Table 3: Co-ordinates for 2008 sampling sites on the Speed River.

Site	Latitude (N)	Longitude (W)
US1	43° 32' 54"	80° 10' 55"
US2	43° 31' 42"	80° 15' 36"
US3	43° 31' 32"	80° 15' 41"
US4	43° 31' 20"	80° 15' 49"
US5	43° 31' 19"	80° 15' 48"
GMWWE	43° 31' 18"	80° 15' 51"
DS1	43° 31' 15"	80° 15' 52"
DS2	43° 30' 25"	80° 15' 18"
DS3	43° 30' 9"	80° 15' 15"
DS4	43° 29' 4"	80° 17' 0"

The sampling approach used by Tetreault et al. (2008) was adapted for this study. This method was developed on the Grand River and used species richness to determine number of sub-sites necessary for variability reduction to detect impacts. Six randomly selected 10 m x 10 m sub-sites were selected from a 100 m river site. All possible fish were collected while electro-fishing (Smith-Root Model 12 with LR-24 and HT-2000 for back up) upstream in a zig-zag pattern (Moulton et al. 2002) with two netters for

5 min at each sub-site. Settings on the electroshocker were maintained between sites. Preliminary studies did not demonstrate a clear advantage to sampling at night so all sampling was completed between shortly after sunrise to late morning (Appendix A). Fish were identified to species (Scott and Crossman 1998), weighed (± 0.001 g), measured as fork length (mm) for forked tails or total length (mm) for other species, and any deformities were recorded for each individual. All fish were handled according to University of Waterloo's approved Animal Care Committee Protocols (AUP 02-24, 08-08). During the October sampling period an additional site, US4, was added upstream of the Guelph effluent outfall, while sites US1 and DS4 were not sampled.

Stable Isotopes

Greenside Darter, Rainbow Darter, and rusty crayfish (*Orconectes rusticus*) were collected from each site for stable isotope analysis of $\delta^{15}N$ and $\delta^{13}C$. Six fish from each site and species were euthanized by severance of the spinal cord, stored in labeled whirl pack bags on ice, and then frozen at $-20^{o}C$ in the lab. Fish were thawed, length and weight recorded, and the left dorsal muscle removed. Crayfish collected by electrofishing were identified to species (Crocker and Barr 1968, Karstad and the Project Crayfish Group 2008, Keene 2009) and tail muscle was sampled and processed in a similar manner (n = 2 to 8/site).

Tissue samples were cut into pieces and dried at $60^{o}C$ for 24 – 48 hr. The dried samples were ground and weighed (0.25 – 0.30 mg) in tin cups and submitted to the University of Waterloo Environmental Isotope Laboratory of Earth Science (Drimmie and Heemskerk 2005). Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan – Bremen, Germany) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108 - Italy) calculated % elemental composition. Results for crayfish were lipid corrected with the equation $\delta^{13}C_{corrected} = -3.32 + (0.99)\cdot(C:N)$ (Post et al. 2007). Lipid correcting for fish did not change statistical differences between sites and therefore fish were not corrected.

Analysis

Each species that had more than ten individuals captured had diagnostic analysis performed on the log length and log weight to identify outliers and transcription errors. Catch per unit effort (CPUE) was compared for individual species, families, and total catch between sites, calculated by averaging the abundance at sub-sites divided by shocking time (300 s). Simpson's diversity and evenness index were used to compare communities at each site (Krebs 1999). Species with high abundance at each site (10% or higher) had length frequency graphs produced to determine age structure (Gray et al. 2002). Condition factor (Nash et al. 2006) of the most abundant species and total mass were also examined.

For each species caught (Table 4), different characteristics were identified: tolerance—ability to adapt to disturbance and stress (Eakins 2009); resilience—ability to withstand exploitation (doubling time) (Froese and Pauly 2010); and vulnerability—catchability (Froese and Pauly 2010). The percent of individuals high, medium, or low for these characteristics was compared between sites. Similarly, diet classification (Scott and Crossman 1998, Eakins 2009, Froese and Pauly 2010) was also determined and percent of individuals grouped by piscivore, general carnivore, invertivore, benthic invertivore, omnivore, and benthic omnivore was compared between sites (Table 4).

Data collected was examined with Levene's test for non-homogeneity. If homogeneous, sites were compared using ANOVA followed by Tukey pair-wise comparisons when significant differences were found. For non-homogeneous data, a non-parametric Kruskal-Wallis test was performed, followed by Mann-Whitney-U tests if significant differences were found. Where there were only two means to compare, an independent t-test was performed. ANCOVA was performed when comparing condition factor between sites. All statistical analysis was completed with SAS 9.1.3 software © (2003) to $p< 0.05$ significance level. All statistical results are ANOVA followed by Tukey unless otherwise stated.

Table 4: List of species caught on the Speed River in 2008. Tolerance is the ability of a species to adapt to disturbance and stress (Eakins 2009), resilience is the species' ability to withstand exploitation (doubling time) (Froese and Pauly 2010), and vulnerability is related to catchability (Froese and Pauly 2010). Diet classification was determined from a combination of various sources (Scott and Crossman 1998, Eakins 2009, Froese and Pauly 2010). Rare species are found at two or fewer sites and are indicated by * found only upstream, † found only downstream, and ‡ found both upstream and downstream.

Family	Common Name	Species	Tolerance	Resilience	Vulnerability	Diet Classification	Spawning
Catostomidae	Northern Hogsucker	*Hypentelium nigricans*	Intolerant	Low	High	Omnivore	Spring
	White Sucker	*Catostomus commersoni*	Tolerant	Low	High	Benthic Omnivore	Spring
Centrarchidae	Largemouth Bass*	*Micropterus salmoides*	Intermediate	Low	Moderate	General Carnivore	Spring
	Pumpkinseed†	*Lepomis gibbosus*	Intermediate	Medium	Moderate	Omnivore	Spring-Summer
	Rock Bass	*Ambloplites rupestris*	Intermediate	Medium	Low	Piscivore	Spring
	Smallmouth Bass	*Micropterus dolomieui*	Intermediate	Medium	Moderate	General Carnivore	Spring
Cottidae	Mottled Sculpin	*Cottus bairdii*	Intermediate	High	Low	Benthic Invertivore	Spring
Cyprinidae	Blackchin Shiner	*Notropis heterodon*	Intolerant	High	Low	Invertivore	Summer
	Blacknose Dace	*Rhinichthys obtusus*	Tolerant	High	Low	Invertivore	Spring
	Bluntnose Minnow	*Pimephales notatus*	Tolerant	Medium	Low	Omnivore	Summer
	Common Shiner	*Luxilus cornutus*	Intermediate	Medium	Low	Invertivore	Spring
	Creek Chub	*Semotilus atromaculatus*	Tolerant	Medium	Moderate	Omnivore	Spring
	Fathead Minnow‡	*Pimephales promelas*	Tolerant	High	Low	Benthic Omnivore	Spring-Summer
	Hornyhead Chub*	*Nocomis biguttatus*	Intermediate	Medium	High	Omnivore	Spring-Summer
	Longnose Dace	*Rhinichthys cataractae*	Intermediate	Medium	Moderate	Benthic Invertivore	Spring-Summer
	Striped Shiner*	*Luxilus chrysocephalus*	Tolerant	Medium	Low	Invertivore	Spring-Summer
Esocidae	Northern Pike‡	*Esox lucius*	Intermediate	Low	High	Piscivore	Spring
Ictaluridae	Brown Bullhead*	*Ameiurus nebulosus*	Tolerant	Medium	Low	Benthic Omnivore	Spring
Percidae	Blackside Darter*	*Percina maculata*	Intermediate	Medium	Low	Benthic Invertivore	Spring
	Fantail Darter	*Etheostoma flabellare*	Intermediate	Medium	Low	Benthic Invertivore	Spring
	Greenside Darter	*Etheostoma blennioides*	Intermediate	Medium	Low	Benthic Invertivore	Spring
	Johnny Darter	*Etheostoma nigrum*	Intermediate	Medium	Low	Benthic Invertivore	Spring
	Rainbow Darter	*Etheostoma caeruleum*	Intolerant	High	Low	Benthic Invertivore	Spring
	Yellow Perch*	*Perca flavescens*	Intermediate	Medium	Low	General Carnivore	Spring

Results

Habitat and Water Quality

Habitat results from the Qualitative Habitat Evaluation Index (QHEI) (Rankin 2006) resulted in most scores ranging from 59 to 66, except for US2 (50). QHEI scores are ranked excellent (> 75), good (60 - 74), fair (46 - 59), poor (30 - 45), or very poor (< 30) (Rankin 2006), so that most sites are "good". The US EPA Rapid Bioassessment (RBA) (Barbour et al. 1999) were similar, with scores ranging from 64 to 77, except for US2 (56) and US5 (81) (Table 5). RBA follows a similar principal of classifying results, but does not give specific scores for where divisions occur (Barbour et al. 1999). Both methods rated US2 the lowest, likely a result of the anthropogenic influences of the low head impoundment directly upstream and city parks on both banks of the river. As each site had similar values and there was no trend of lower values at all upstream or at all downstream sites, it was determined that habitat was similar between sites and changes in the fish community are likely a result of other influences.

Water quality was similar between sites. pH ranged from 7.2 to 8.2 in both season and water temperature (^{o}C) 17.3 to 21.8 in August and 5.1 to 10.1 in October. The highest temperatures in October occurred downstream of the effluent. Dissolved oxygen had a downstream decreasing trend in August, and sites ranged from 6.62 to 10.60 mg/L. In October dissolved oxygen ranged from 11.36 to 13.81 mg/L.

Table 5: Summary of habitat data collected on the Speed River, 2008. RBA refers to results from the modified US EPA Habitat Assessment in the Rapid Bioassessment Protocols for Wadeable Streams (Barbour et al. 1999), and QHEI results from the Ohio EPA Qualitative Habitat Evaluation Index (Rankin 2006), each scored out of 100. Substrate 1 and 2 are the most common substrate type, with their percent in brackets. Biotic values are A = algae, Aq = aquatic plants, E = emergent plants, TD = terrestrial leaves, bark, branch debris, and DF = dead falls, logs. Velocity reported as August/October. NE indicates a site not sampled and * are sites where data was miscommunicated.

Site	RBA	QHEI	Stream Width (m)	Velocity (cm/s)	Substrate 1 (%)	Substrate 2 (%)	Biotic	Description
US1	64	59	20	34.3 / NE	Bedrock (33)	Gravel (28)	A, TD	Tributary, cut grass to shore part of site, low habitat diversity
US2	56	50	37	46.4 / 34.3	Gravel (49)	Sand (28)	A, 27% Aq, 5% E	Downstream low-head impoundment, flat, shallow, city park either shore, channelization
US3	74	60	22	92.9 / 59.4	Cobble (33)	Gravel (26)	E, Aq, TD	Shrub on shore, bridge to gravel pit downstream
US4	NE	NE	16	NE / 78	Cobble (NE)	Gravel (NE)	DF	Trees fallen into river, shallow
US5	81	64	16	50.5 / 68.6	Cobble (40)	Gravel (29)	Aq, TD	Stream in one sub-site, treed shore
DS1	69	59	21	56 / 101	Cobble (29)	Gravel (28)	A, Aq, DF	Trees fallen into river
DS2	75	65	31	46.4 / *	Gravel (41)	Cobble (28)	3% TD, DF	Treed shore, some bedrock
DS3	77	65	34	46.4 / 76.7	Cobble (40)	Gravel (22)	A, TD	Treed shore, bridge divides site, some bedrock
DS4	71	59	26	54.2 / NE	Bedrock (38)	Gravel (17)	A, TD	Natural grass shore, low habitat diversity

Fish Community

Of the twenty-four species that were captured, darter species, particularity Rainbow Darter and Greenside Darter dominated the fish community numerically (Table 6). There was < 1% occurrence of deformities in total for all species and no upstream or downstream influence was present. Mean total catch per unit effort (CPUE) was not consistently high or low upstream (Table 6), but US2 was significantly higher than US3 and DS1 in August and US3 was lower than US2, DS2, and DS3 in October (Kruskal-Wallis with Mann-Whitney-U test). Mean total mass was similar with no distinct downstream pattern (Table 6), but in August US1 and DS1 were lower than US2, US5, DS2 and DS3 (Kruskal-Wallis with Mann-Whitney-U test) and there was no difference between sites in October.

When species are grouped based on resiliency (high, medium, and low) and compared between sites, there was an increase in the proportion of high resilient species downstream of the treatment plant. Rainbow Darter is considered highly resilient, as population doubling time is less than 15 months, whereas Greenside Darter have a medium resilience because they take 1.4 - 4.4 years (Froese and Pauly 2010). Rainbow Darter was about 31% and 28% of the community at US5 and about 71% and 69% at DS1 for August and October, which accounts for the increase in high resilient species. The pattern of high resilience and medium resilience follow the abundance of Rainbow Darter and Greenside Darter abundance, respectively. Similar results occur with tolerance, as the Rainbow Darter influenced the increase of intolerant species at the DS1. As most species caught have low vulnerability (Table 4), there was no pattern observed.

Species were classified by diet (Table 4) and compared among sites. Benthic invertivores dominated at each site. This was due to darters dominating the community at each site, and all darter species caught in this study are benthic invertivores. Percent diet types compared between sites show a decrease in the variety of other classifications at US5 and all downstream MWWE sites. This is due to an increase in darter species dominance at these sites.

Of the nine species that were considered rare (species found at two or less sites), six were found at upstream sites only. These six species were not unique from other species in terms of classification: they were tolerant or intermediate tolerance; medium or low resilience; all levels of vulnerability; and all diet classifications (Table 4). The reasons for these species being found only at upstream sites is not as easily determined from the biological information given. Four of these species were found at US2, which resulted in higher species diversity at this site (Table 6). Of the common species captured, Mottled Sculpin was found at downstream sites only.

Greenside Darter mean CPUE was lowest at DS1 in both seasons while Rainbow Darter increased only in October (Figure 3) (Kruskal-Wallis with Mann-Whitney-U test). Percent composition of Greenside Darter also decreased at DS1 and Rainbow Darter increased during both sampling periods (Figure 4) (October Kruskal-Wallis with Mann-Whitney-U test). Also at DS1 no Greenside Darter young of the year (YOY) were captured in August but they were captured at all other sites. In October, site US4 was added to characterize both sides of the river and extra effort was added to increase fish numbers. With an extra half hour of shocking at DS1 in October, total catch was still less than fifty Greenside Darters, but there were some YOY. Rainbow Darter and other species had YOY and were abundant downstream of the MWWE.

Table 6: Common species collected on the Speed River in 2008 are listed, grouped by family, with abundance for each site, reported as August/October. Below is other information about each site, incorporating all species captured. NE indicates sites not sampled that month. Mean CPUE, CPUE SE, and Mean Total Mass were calculated by averaging the respective totals from sub-sites at each site. All other parameters are with sub-sites combined.

Family	Species	US1	US2	US3	US4	US5	DS1	DS2	DS3	DS4
Catostomidea	Northern Hogsucker	- / NE	7 / 10	3 / 10	NE / 16	3 / 7	- / 2	5 / 4	2 / 2	1 / NE
	White Sucker	- / NE	2 / 11	1 / 5	NE / 2	- / 5	- / 12	- / 14	- / 10	- / NE
Centrarchidae	Rock Bass	- / NE	5 / 3	1 / -	NE / 3	- / 1	- / 2	2 / -	- / -	1 / NE
	Smallmouth Bass	- / NE	5 / 3	- / 4	NE / -	7 / 2	2 / 2	4 / -	2 / 4	1 / NE
Cottidae	Mottled Sculpin	- / NE	- / -	- / -	NE / -	- / -	1 / -	1 / 1	5 / 4	1 / NE
Cyprinidae	Blackchin Shiner	- / NE	- / -	6 / -	NE / -	1 / -	- / -	- / -	- / -	8 / NE
	Blacknose Dace	- / NE	1 / -	- / -	NE / -	- / -	- / 1	2 / 1	- / -	- / NE
	Bluntnose Minnow	- / NE	31 / 6	- / 7	NE / 9	- / -	- / 4	- / 6	1 / 4	- / NE
	Common Shiner	- / NE	- / -	- / 3	NE / 1	- / -	- / -	- / 2	- / -	- / NE
	Creek Chub	- / NE	- / -	- / 1	NE / 1	- / -	- / -	1 / 4	- / -	- / NE
	Longnose Dace	2 / NE	4 / -	3 / 1	NE / 7	- / 2	1 / 5	7 / 5	6 / 1	2 / NE
Percidae	Fantail Darter	4 / NE	11 / 4	3 / 1	NE / 1	9 / 2	4 / 9	2 / 2	2 / 3	6 / NE
	Greenside Darter	41 / NE	53 / 65	30 / 43	NE / 61	83 / 81	18 / 24	45 / 59	47 / 90	51 / NE
	Johnny Darter	7 / NE	19 / 53	6 / 9	NE / 15	- / 1	2 / 11	8 / 24	3 / 17	30 / NE
	Rainbow Darter	56 / NE	57 / 46	29 / 33	NE / 50	47 / 39	70 / 165	96 / 94	59 / 166	42 / NE
Mean Catch Per Unit Effort (/300 s)		3.8 / NE	6.9 / 6.9	2.8 / 3.9	NE / 5.6	5 / 4.7	3.3 / 7.9	5.8 / 7.3	4.2 / 10.1	4.8 / NE
Catch Per Unit Effort SE		0.52 / NE	1.05 / 0.35	0.75 / 0.89	NE / 0.91	0.81 / 0.49	0.59 / 0.99	0.61 / 0.59	0.79 / 1.71	0.96 / NE
Mean Total Mass (g)		34.8 / NE	94.4 / 69.1	39.2 / 65.3	NE / 74.9	69.9 / 100.1	32.3 / 86.4	101.6 / 104.1	62.5 / 89.5	47.6 / NE
Species Richness		6 / NE	14 / 14	12 / 12	NE / 12	6 / 9	7 / 12	12 / 14	9 / 11	11 / NE
Family Richness		2 / NE	5 / 5	4 / 4	NE / 5	4 / 4	4 / 5	5 / 5	5 / 5	6 / NE
Simpson's Diversity		0.63 / NE	0.82 / 0.78	0.74 / 0.77	NE / 0.75	0.59 / 0.58	0.45 / 0.5	0.62 / 0.72	0.64 / 0.6	0.74 / NE
Simpson's Evenness		0.45 / NE	0.37 / 0.33	0.33 / 0.36	NE / 0.34	0.41 / 0.27	0.26 / 0.17	0.22 / 0.26	0.31 / 0.23	0.35 / NE
% Invertivore		- / NE	0.5 / -	7.1 / 3.4	NE / 0.6	0.7 / -	- / 0.4	1.2 / 1.4	- / -	5.7 / NE
% Benthic Invertivore		95.6 / NE	69.9 / 81.2	84.7 / 73.7	NE / 80.2	92.7 / 89.3	96.9 / 89.9	90.8 / 84.0	92.1 / 91.7	91.0 / NE
% Omnivore		4.3 / NE	20.9 / 8.7	4.7 / 15.3	NE / 15.6	2.0 / 5.0	- / 2.5	3.4 / 6.9	2.4 / 2.0	0.7 / NE
% Benthic Omnivore		- / NE	3.4 / 5.3	1.2 / 4.2	NE / 1.2	- / 3.6	- / 5.0	- / 6.5	- / 3.6	- / NE
% General Carnivore		- / NE	2.4 / 2.4	- / 3.4	NE / -	4.7 / 1.4	3.1 / 0.8	2.9 / 0.5	5.5 / 2.7	1.4 / NE
% Carnivore		- / NE	2.4 / 2.4	1.2 / -	NE / 2.4	- / 0.7	- / 1.3	1.1 / -	- / -	1.4 / NE
% Darter		93.9 / NE	68.0 / 81.2	81.2 / 72.9	NE / 76.1	92.7 / 87.9	95.9 / 87.8	86.8 / 81.7	87.4 / 91.4	89.6 / NE

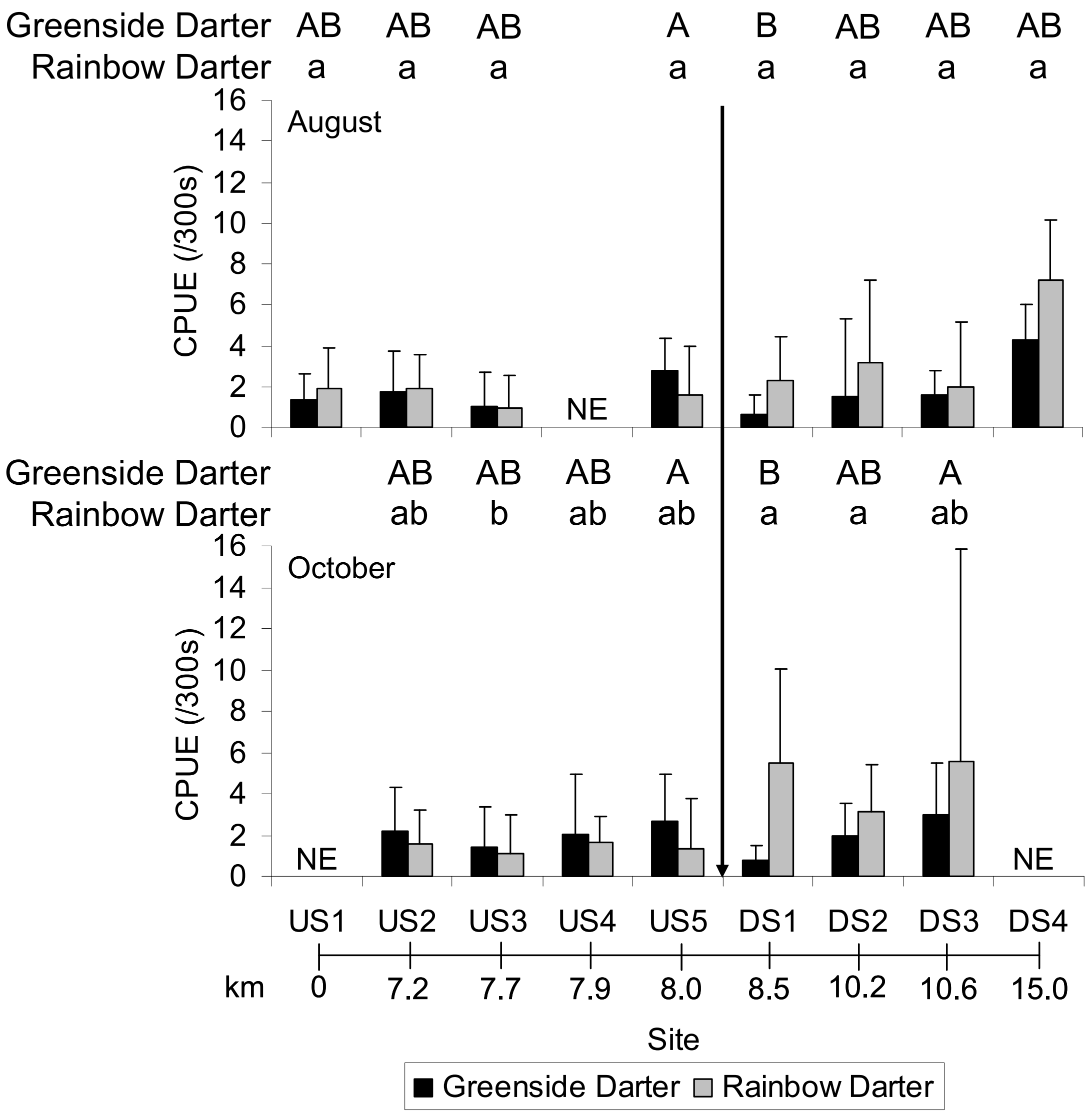

Figure 3: Mean catch per unit effort (CPUE +SE) in August and October for Greenside Darter and Rainbow Darter on the Speed River in 2008. Lettering indicates significant difference by ANOVA with Tukey, except for Rainbow Darter in October which is by Kruskal-Wallis with Mann-Whitney-U test. Arrow indicates the Guelph MWWE outfall. Kilometers show distance downstream from furthest upstream site. NE indicates not sampled.

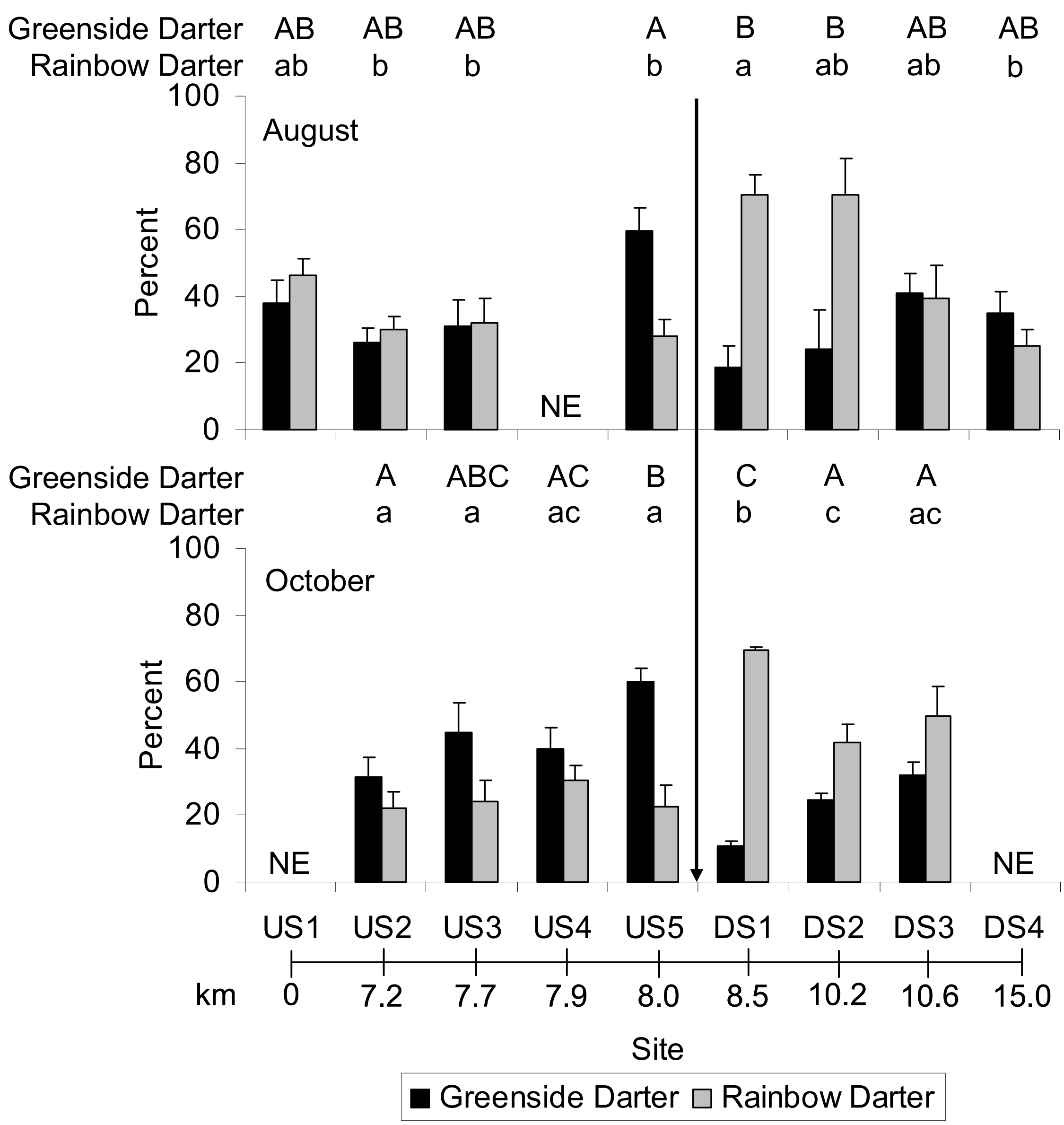

Figure 4: Mean percent site composition (+SE) in August and October on the Speed River, 2008, of Greenside Darter and Rainbow Darter. Lettering indicates significant differences by ANOVA with Tukey in August and Kruskal-Wallis with Mann-Whitney-U test in October for both species. Arrow indicates the Guelph MWWE outfall. Kilometers show distance downstream from furthest upstream site. NE indicates not sampled.

Sites were combined into upstream and downstream, then length frequency graphs were produced for Greenside Darter (Figure 5). Upstream Greenside Darter YOYs were considered to be less than 58 mm in August and 62 mm in October. Downstream they were less than 60 mm and 64 mm. There was an increase in the average length of YOYs and adults between August and October. Adults were longer downstream than upstream in both seasons. There was no difference between YOY in August upstream and downstream but YOY downstream were longer in October (Kruskal-Wallis test). Rainbow Darter did not show clear age divisions and were not examined further with this method.

When upstream and downstream sites were pooled separately and condition factor was compared (ANCOVA) for both Greenside Darter and Rainbow Darter, there was a significant increase in condition downstream for both species (Figure 6). When YOY and adults were analyzed separately, it was found that Greenside Darter YOY condition was higher downstream than upstream in October, but not in August, whereas adults were larger downstream in August, but not in October.

Using Simpson's Diversity Index, diversity was the lowest at DS1 in both seasons and evenness was lower downstream as well (Table 6). When Rainbow Darter and Greenside Darter are removed from these calculations, diversity and evenness are no longer different between sites. Lower diversity and evenness is a result of increased abundance of Rainbow Darter downstream in terms of site species composition. The lower evenness and diversity at US5 is a result of a high abundance of Greenside Darter. These two species dominate the community and the result of these indices is a reflection of their abundance.

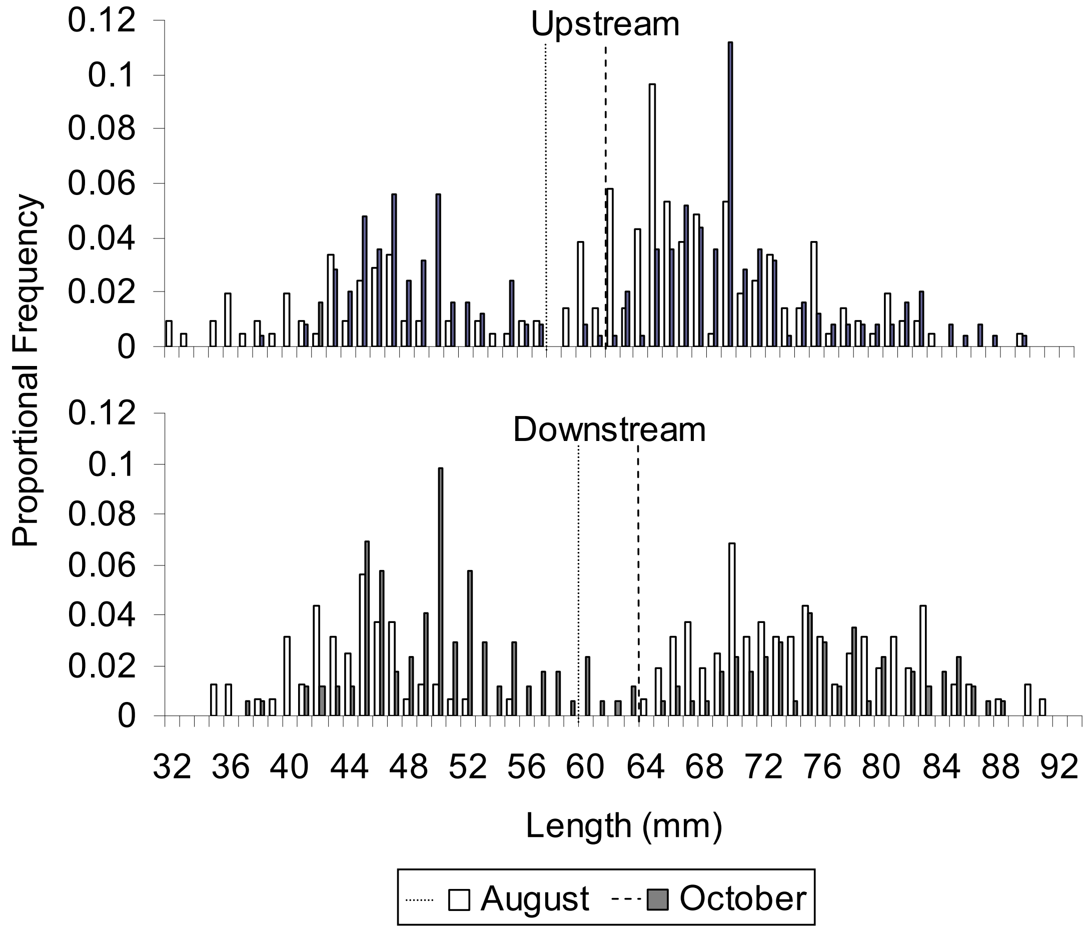

Figure 5: Greenside Darter length frequency, pooled sites upstream and downstream of the Guelph MWWE in August and October, 2008. The division between young of the year and adults is indicated by the dotted line for August and the dashed line for October.

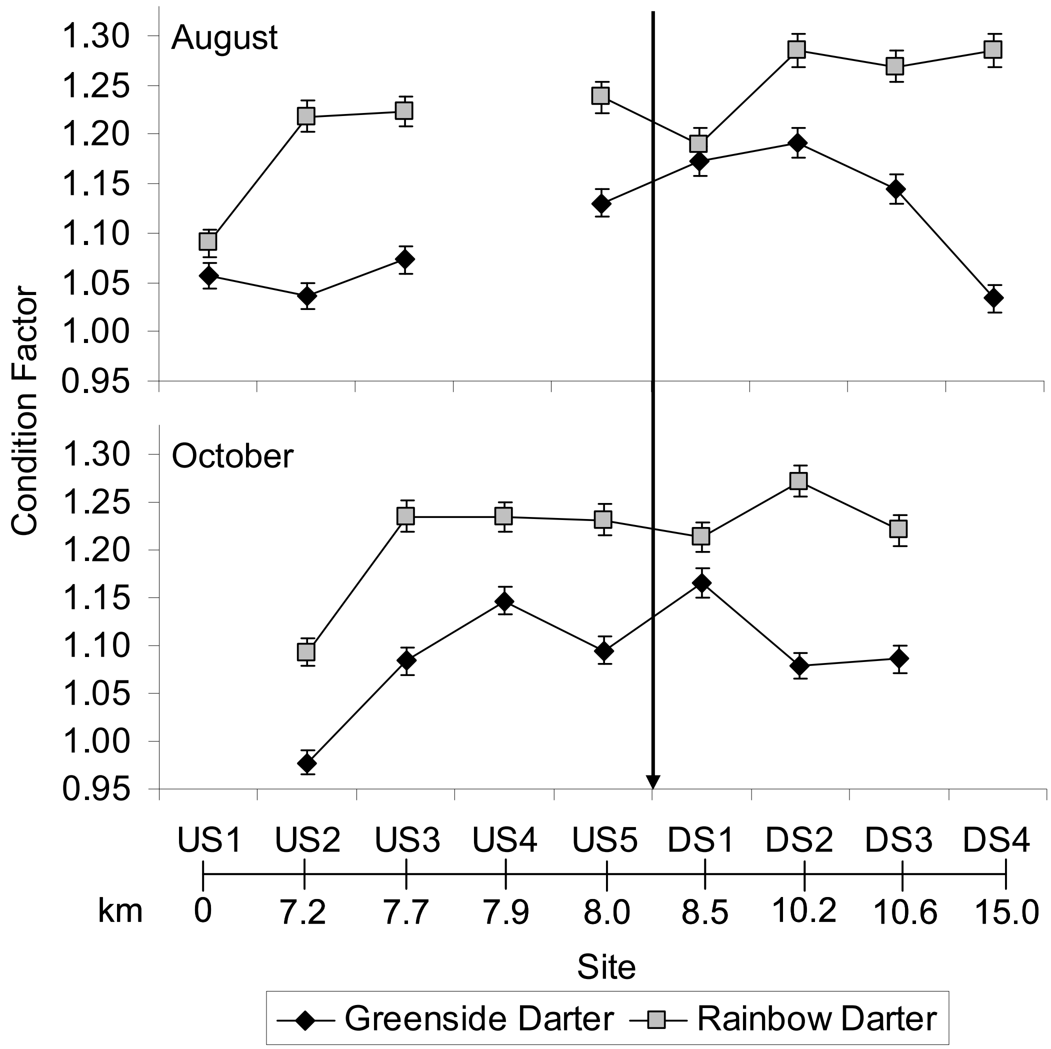

Figure 6: Condition factor (mean ± SE) for Greenside Darter and Rainbow Darter at each site in August and October, 2008. Arrow indicates the Guelph MWWE outfall. Pooled downstream sites were significantly higher than pooled upstream sites (ANCOVA) for both species.

Stable Isotopes

For all species, $\delta^{15}N$ and $\delta^{13}C$ had an increasing trend from upstream to downstream. Signatures of both stable isotopes in Rainbow Darter increased significantly downstream of the MWWE outfall in August but no differences were found for Greenside Darter (Rainbow Darter carbon Kruskal-Wallis with Mann-Whitney-U test) (Figure 7). Rainbow Darter's $\delta^{13}C$ signature was greater than Greenside Darter at DS1, and $\delta^{15}N$ was greater at US1, DS1, DS2, and DS3 (independent t-test). $\delta^{15}N$ was greater for Greenside Darter at US2 (independent t-test). Rusty crayfish showed a similar response as the Rainbow

Darter and was more enriched in $\delta^{13}C$, but less enriched in $\delta^{15}N$ compared to the darter species at all sites (Figure 8).

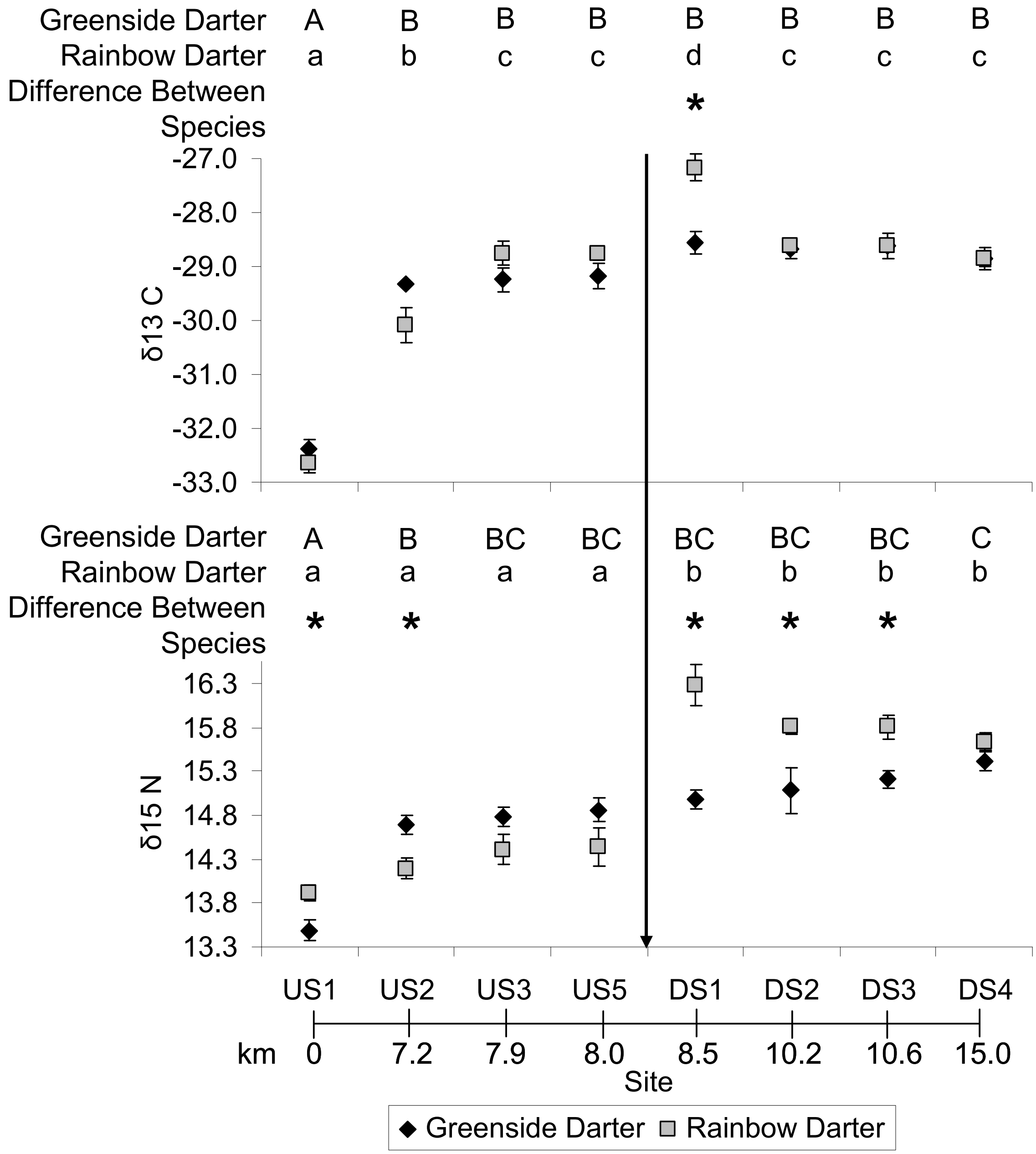

Figure 7: Stable isotope signature ($\delta^{15}N$ and $\delta^{13}C$, mean ± SE) for Greenside Darter and Rainbow Darter collected in August on the Speed River, 2008 (n = 6/site). Arrow indicates the Guelph MWWE outfall. Different letters correspond to significant site differences by ANOVA with Tukey, except carbon in Rainbow Darter by Kruskal-Wallis with Mann-Whitney-U test. * indicates significant difference between the two species as determined by independent t-test.

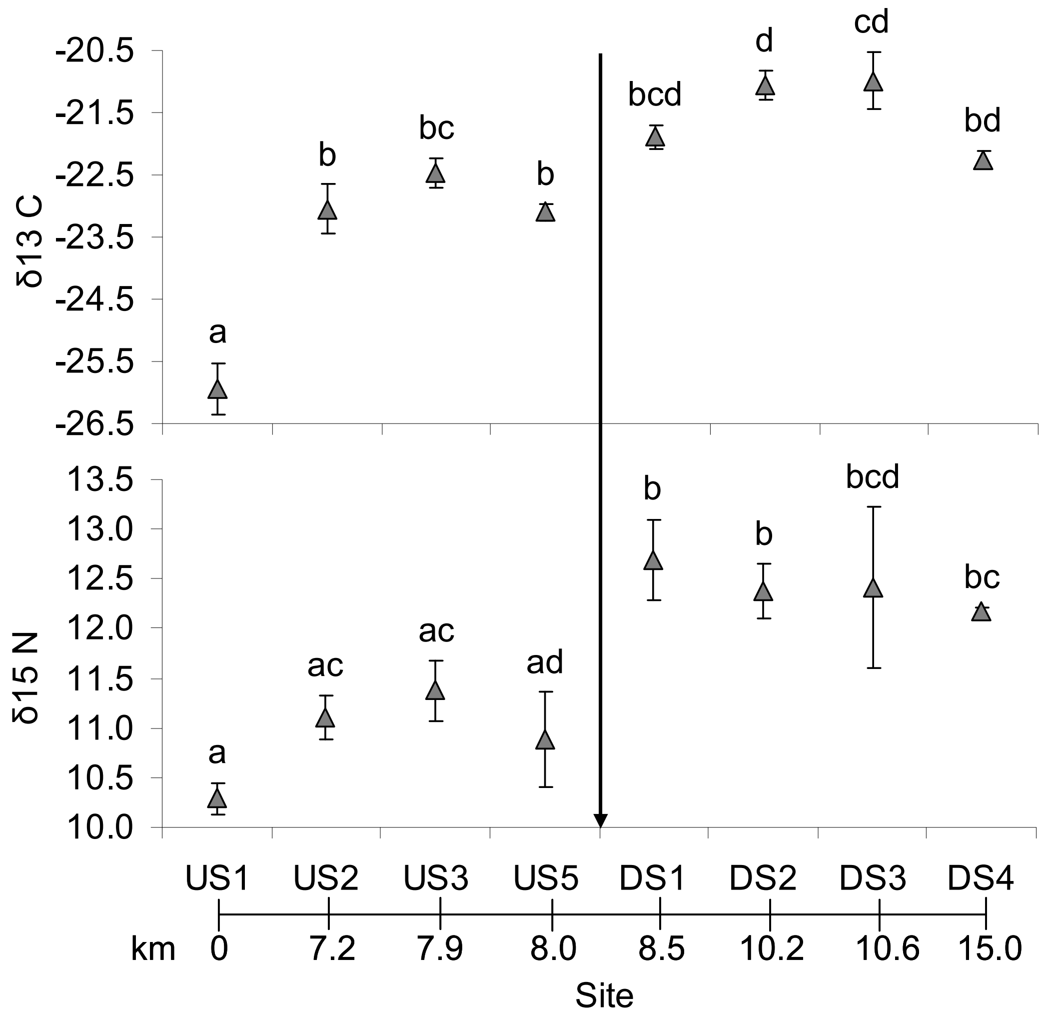

Figure 8: Stable isotope signature (δ^{15}N and δ^{13}C, mean ± SE) for rusty crayfish collected in August on the Speed River, 2008 (n = 2 to 8/site). Arrow indicates the Guelph MWWE outfall. Different letters indicate significant site differences by ANOVA with Tukey.

Discussion

Mean total CPUE and mean total mass were significantly different between some sites, but no trend for each individual season indicates a MWWE influence. Simpson's diversity, resilience, and tolerance showed a trend of possible MWWE influence on the fish community. The trend was related to the abundance of Greenside Darter and Rainbow Darter, which dominated the community. Greenside Darter abundance decreased at DS1 while Rainbow Darter increased. Also at this same site the Greenside Darter isotope signature did not change, while Rainbow Darter increased significantly. It is likely these species are not consuming the same prey and the changing environmental conditions downstream of the outfall are giving the Rainbow Darter a competitive advantage.

Mean total CPUE varied between some sites and was higher at downstream sites in October, possibly due to warmer temperatures and food availability as a result of the effluent discharge. CPUE in this study is proportional to abundance, as effort was the same at all sites. Porter and Janz (2003) sampled in September in Oklahoma and had an increase in abundance downstream of MWWE, but other studies in Ohio, Michigan, and Korea have found a decrease (Dyer and Wang 2002, Ra et al. 2007, Yeom et al. 2007). These studies were conducted over many months or years or were conducted in the spring. Many fish species are mobile and will travel large distances for better habitat or for spawning. In terms of the species in this study, the darters are unlikely to move between riffles, whereas Catostomids (Sweet 2007) and Centrarchids (Gatz and Adams 1994) might move between reference and exposed sites, especially during spawning season. As collections for this study were conducted outside of the spawning season, most fish should be fairly resident in the sites where they were collected, as indicated by their isotope signatures.

Another variable that would be influenced by fish movement is mean total mass. October sites varied in mass, because of the significant increase in mean total mass the presence of one Catostomid or Centrarchid can make. The most abundant species, darters, are small (2.03 ± 0.24 g August and 1.94 ± 0.27 g October), with about a thousand individuals in each season. Rock Bass (*Ambloplites rupestris)* are larger (17 g August, 20 g October, large error) with nine individuals across sites in each season. The addition of one large Rock Bass, or similar species, can increase the mean total mass at a site significantly. These larger species also have increased mobility, as previously discussed. Reporting mean total mass is also not as informative without total number of fish caught. Askey et al. (2007) reported biomass of three sport fish (*Oncorhynchus mykiss*, *Prosopium Williamsoni*, and *Salmo trutta*) on the Bow River influenced by Calgary's MWWE. The biomass of the fish species combined decreased downstream, but separating the species revealed that the Mountain Whitefish (*Prosopium williamsoni*) was decreasing while the two trout species (*Oncorhynchus mykiss* and *Salmo trutta*) were increasing. If they had reported total number of fish the analysis may have revealed whether biomass is changing due to abundance or individual's body mass, which can lead to different conclusions about the response to the effluent (Munkittrick et al. 2000). Mass appeared to be unaffected by the outfall, therefore we can conclude that the presence of larger species on the Speed River was not significantly influenced by the MWWE discharge.

Simpson's species diversity and evenness indices were compared among sites and there was a decrease in both indices downstream of the treatment plant due to the changes in Rainbow Darter and Greenside Darter abundance. Other studies have also seen a decrease in diversity downstream of other treatment plants (Birge et al. 1989, Ra et al. 2007), but did not indicate what were the dominating influences. In both studies there was a decrease in species richness at the lowest diversity sites.

Mottled Sculpin were only found at downstream sites in this study. This is a coldwater species (Eakins 2009) and downstream there is thought to be possible coldwater sources from groundwater upwellings. It is assumed the Mottled Sculpin is present in areas where the upwelling occurs and not connected to the presence of MWWE. Further investigation is required to determine why six species were found at upstream sites only.

Rainbow Darter and Greenside Darter were the most abundant species at sites sampled in 2008. The abundance of Greenside Darter decreased at DS1 while Rainbow Darter increased. Greenside Darters are less tolerant to higher temperatures than Rainbow Darters (COSEWIC 2006) and it is possible the higher temperature of the effluent is influencing abundance.

When all species are grouped by tolerance and resilience, there is an increase in intolerant and high resilient species directly downstream of the outfall. Rainbow Darter increases in abundance at DS1 and the pattern for intolerant and high resilient species follow the abundance of this species. Other researchers have found an increase in tolerant species downstream of other MWWE (Ra et al. 2007, Yeom et al. 2007). The process of determining tolerance is based on a subjective classification from studies on physical habitat and physiochemical water quality changes and is not specific to what is in the effluent. In many cases it has been found that there is different tolerances to different chemicals and having one classification to encompass all exposures may not be a helpful tool to determining impacts of human influences on fish communities (Meador and Carlisle 2007). Thus it is possible that the Rainbow Darter is not completely intolerant to chemicals in the Guelph MWWE. It is also possible the effluent is not toxic enough to be detrimental to this species. As this species increases in abundance at DS1, it would seem that it is benefiting from the MWWE.

Pooled upstream and downstream sites for condition factor demonstrated significantly higher condition downstream for both species. Other studies have found a general increase in condition or weight for individual species downstream of MWWE (Dyer and Wang 2002, Porter and Janz 2003, Yeom et al. 2007). Currently in Canada there is discussion of creating an Environmental Effects Monitoring Program for MWWE. Similar programs for pulp and paper and metal mining sectors already exist, and have influenced the development of a MWWE monitoring framework which suggests > 10% for condition or > 25% in weight at age as warning signs of impacts (Kilgour et al. 2005). Pooled downstream sites in this study for condition were 2.8% and 5.5% higher than pooled upstream sites for Greenside Darter and Rainbow Darter, respectively. This could mean that the increase downstream is not biologically significant or that > 10% is too large a factor for small bodied fish.

When Greenside Darter lengths were pooled into upstream and downstream sites, adults were longer downstream than upstream in both seasons. Greenside Darter grows to an average of 76 mm in Canada (age 3-4 years), achieving 60% of its length in its first year (COSEWIC 2006). There was no difference in YOY length in August, but there was in October, possibly a result of the river being warmer longer into the fall at the downstream site due to the inputs of warm effluent.

This study found upstream Greenside Darter YOYs were < 58 mm in August and < 62 mm in October while downstream they were < 60 mm and < 64 mm, respectively. As mentioned before, 60% of length is achieved in the first year, which would be an average of 45.6 mm from the Canadian length (COSEWIC 2006). Another source estimates total length to be 50-55 mm in the first year (Graham and Fink 2004). Fahy (1954) did an extensive investigation into biology and found males grow faster and are bigger. His YOY Greenside Darters were less than 43.5 mm for females and less than 47.5 mm for males (standard lengths) in January. The YOY in this study are longer than other studies. As this study is grouping upstream and downstream sites, growth rates may be different between individual sites. But the histogram gives a fairly clear division between YOY and adults, which has been found in other studies (Fahy 1954). It is assumed this gap exists because of the rapid growth in their first year of life, which makes distinguishing between YOY and adults relatively easy. Beyond the first year, growth rate decreases and it is difficult to tell the difference between age classes that are mature as the histograms overlap.

Other studies have found increased growth of fish downstream of MWWE because there are nutrients in the effluent that increase the amount of food (Chambers et al. 1997, McMaster et al. 2005). This is demonstrated by an increase in condition, liver size, or lipid storage which indicates that energy is being allocated to storage (Kilgour et al. 2005).

Length frequency graphs of Rainbow Darter did not show clear YOY and adult separation. Rainbow Darter life history studies have found first year lengths to be 34 mm standard length in Bayou Sara, LA and 43.3 mm total length in Wisconsin, while second year lengths are 40.5 mm standard length and 50.7 mm total length, respectively (Grady and Bart 1984). The same Wisconsin study found Rainbow Darters to reach 50% of the 1st year's length in the first 2 months. Eggs are lain between March and May (Grady and Bart 1984). If we use the Wisconsin study's total length in the 1st year and this growth rate, in late August Rainbow Darter YOY would be an estimated length of 22-30 mm. The mesh size of our nets was not adequate to capture all the YOY, which is likely the reason for the difficulty in determining age divisions for this species.

In 2008 Rainbow Darter and crayfish stable isotope signatures of carbon and nitrogen increased downstream of the MWWE outfall. Studies have found an enriching of carbon and nitrogen stable isotopes downstream of outfalls because i) the effluent has a higher signature than the environment, or ii) the effluent has more nutrients which leads to different uptake/fractionation (Ulseth and Hershey 2005, Northington and Hershey 2006). In a study looking at a small discharge of secondary treated effluent, it was found that > 60% carbon and nitrogen were of effluent origin, indicating a preferential uptake (deBruyn and Rasmussen 2002). Other studies on the Speed River have also found enrichment downstream, however sites are not all similar to this study (Tetreault 2010) or only included two upstream sites, one of which was on a tributary of the Speed River which resulted in a large difference in signature between sites (Loomer 2008). The only downstream site directly downstream of the outfall did increased in isotopic signature, but conclusions on this river were limited as there were only three sites. These studies support the idea that nutrients from the MWWE are being incorporated into the food chain or are supporting a primary producer that is fractionating the isotopes differently (which are consumed by the invertivores in the darter's diet).

In 2008 Greenside Darter signature did not change for either carbon or nitrogen. This indicates that nutrients from the MWWE are not incorporated in this species' food chain. An explanation may be that the Greenside Darter food chain is different than the Rainbow Darter, or that the Greenside Darter has greater movement, reducing exposure to the effluent.

COSEWIC (2006) reported findings in other studies, on different rivers, that Greenside Darter movement ranged from as far upstream as possible to no movement from a single riffle. Loomer's (2008) work on the Grand River suggests that darter species in this watershed do not move between sides of the river during the summer months (< 50 m), as stable isotope signatures were distinctly different. Crayfish also do not travel large distances, as PCB concentrations in crayfish on an exposed side of the Speed River were distinctly different from the unexposed opposite side (G. R. Craig & Associates 2006). This is also evident in the small standard errors on the isotope values between the individuals sampled at each site. It is unlikely that the Greenside Darter is not exposed to the effluent.

Stomach content analysis in Ohio (Turner 1921) showed both species' diets are similar, and consists mainly of mayfly, caddisfly, and midge larvae. Differences in content included fish parts, worms, and bottom debris in Greenside Darter, while Rainbow Darter had small crayfish and snails. A study in southern Indiana found mayfly, midge larvae, and caddisfly to be the main portion of stomach content of the Rainbow Darter, with fish eggs and black fly larvae occurring as well (Martin 1984). Other studies have also found major diet components to be black fly larvae in Greenside Darter and minnow and lamprey eggs in Rainbow Darter (Scott and Crossman 1998). The fish from these studies were in different watersheds than those of this study, so it is difficult to conclude whether these differences in diet would be the same on the Speed River.

MWWE has higher nutrients, leading to higher production downstream. It is possible these added nutrients can sustain organisms not present upstream, which would increase the availability of potential food items. Rainbow Darter $\delta^{15}N$ increased 1.84‰ between upstream and downstream sites. The difference between Rainbow Darter and Greenside Darter $\delta^{15}N$ at DS1 is 1.3‰. This indicates that the Rainbow Darter is not a trophic level higher than the Greenside Darter (which would be a 3-5‰ difference), but there is possibly a difference in food (Ulseth and Hershey 2005). The $\delta^{13}C$ supports this theory, as Rainbow Darter increases 1.6‰ between upstream and downstream and is 1.39‰ greater than the Greenside Darter. It is also possible that organism(s) in the Rainbow Darter food chain are preferentially uptaking nutrients from the MWWE and organisms in the Greenside Darter food chain are not. What ever the situation, the additional abundance of the Rainbow Darter at downstream sites also suggests that there are additional resources (i.e. food) for it to take advantage of and survive/outcompete the Greenside Darter.

When looking at the physiology of the two fish species, Greenside Darter has a subterminal mouth, a rounder body, and is larger. This can restrict its feeding to the top of large substrate. Rainbow Darter's narrow, smaller body and terminal mouth would allow access to the water column and to move between the rocks. Hlohowskyj and Wissing (1986) found that Greenside Darter preferred larger rocks, while the Rainbow Darter was less picky on substrate type. The Rainbow Darter also eats from substrate surfaces, putting it potentially in direct competition with the Greenside Darter, but has more ability to adapt to crevices. This gives the Rainbow Darter the ability to take advantage of a different food source which may be less accessible to the Greenside Darter.

Stable isotopes of carbon taken of Rainbow Darter and Greenside Darter in 2005 (Tetreault 2010) and 2007 (Loomer 2008) increased at downstream sites. Nitrogen stable isotope ratios in Greenside Darters did not change in 2005, but increased in 2007 at the site downstream of the MWWE. The years of 2005 and 2007 had lower precipitation, resulting in river levels below average in 2005 and below the 40^{th} percentile in 2007. In contrast, 2008 was a very wet year and river levels were above the 97^{th} percentile (August levels at Edinburgh Road from Water Survey of Canada 2007b, Gallant 2010). It is possible that the difference in isotope signature between the years is linked to water level. More water may increase habitat and the number of different organisms living downstream of the MWWE which the Rainbow Darter can consume. This would increase the difference in signature in 2008 as compared to 2007.

In these low flow years the effluent would contribute more to the flow of the river downstream, a significant influence to the volume of water in the river. It is possible that in 2005 and 2007 there was more habitat available downstream as compared to upstream because of this additional flow, which may result in more/different food items downstream. This would increase the stable isotope signature of carbon and nitrogen of the Greenside Darter by its predation of these additional food items only found downstream. It is possible that because the effluent was not a significant influence on river flow in 2008 that upstream and downstream habitat was not different, and food items were the same upstream and downstream, resulting in no change to stable isotope signature for the Greenside Darter. This is different than the Rainbow Darter, which signature is increased downstream of the MWWE in all years. The stable isotopic signature of the Rainbow Darter and Greenside Darter in 2008 indicates that there is a difference in diet between the species. The possible explanations are complex and will require further investigation.

Although habitat was similar between sites, there is still the possibility that subtle changes between sites are impacting the fish community. The City of Guelph has multiple low head impoundments, municipal drains, and channelization upstream of the outfall. There is also a transition from urban to natural shoreline near the treatment plant, which could decrease the effects of MWWE as compared to a more urban setting. There are multiple potential confounding influences that could not be controlled in this study, but should be kept in mind. In addition, our sampling method included only one gear type in wadeable areas and sampling with different equipment at a wider variety of habitats may give different results.

Urbanization is occurring in many places around the world, but there are impacts from this human activity on the environment that needs to be considered. The City of Guelph is one community that has concerns that current wastewater treatment infrastructure will need large investments to mitigate environmental degradation. This study looked at the condition of fish communities associated with the Guelph MWWE in August and October, 2008. There were differences between individual sites in terms of abundance and total mass, but there were no apparent MWWE effects with these parameters. Effects seen were isolated to Rainbow Darter increasing in abundance and isotopic signature, whereas Greenside Darter did not. Further study is needed to compare the diet and behavior of these two darter species and determine if the changes in nitrogen and carbon stable isotopic signatures are a result of a shift in food selection, resulting in a competitive advantage for the Rainbow Darter. From the isotope data of 2005, 2007, and 2008 there is a difference in the response of Greenside Darter, which may mean that different flows of the river will affect the species (or its food) differently. It is recommended that a long-term study be conducted to look at this possibility. Other areas receiving similarly treated effluent where these species are present should also be investigated for similar responses. These subtle impacts to fish communities from MWWE may change at this site if effluent quality decreases from an increase in human population growth. High quality treatment alone may not be enough to mitigate effects on aquatic ecosystems, and wastewater investments need to be considered carefully to ensure environmental protection in all communities.

Fish communities near secondary treated municipal wastewater outfalls in the Grand River, Ontario

This chapter will be submitted as a manuscript to Environmental Toxicology and Chemistry. The contributing authors are:

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Overview

Municipal wastewater effluent (MWWE) has the potential for widespread impacts on aquatic receiving environments in many parts of the world due to urbanization and discharges being one of the largest human influences, per volume, in the environment. Treatment of wastewater has improved to reduce many acute toxic effects, but concerns remain related to long-term chronic exposure to a variety of emerging chemicals. The Grand River is the largest watershed in Southern Ontario, Canada and receives the municipal effluents from 26 treatment systems. Fish communities were evaluated at thirteen sites upstream and downstream of two major effluent outfalls associated with large urban centers in the watershed in both September and November, 2008 using a backpack electroshocker. Rainbow Darter (*Etheostoma caeruleum*) and other darter species dominated the fish communities. At the farthest downstream sites sucker species, such as White Sucker (*Catostomus commersonii*) and Golden Redhorse (*Moxostoma erythrurum*), increased in abundance. Stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) in Rainbow Darter and Greenside Darter (*E. blenniodes*) differed directly downstream of the two outfalls. At the first MWWE outfall $\delta^{15}N$ increased in Rainbow Darter, but there was no change in Greenside Darter. In contrast, downstream of the second outfall for both species $\delta^{13}C$ increased, but $\delta^{15}N$ decreased dramatically, possibly as a result of high nutrient and ammonia inputs. These nutrients have lead to extreme diurnal variations in oxygen levels and in combination with enrichment and toxicity, may have altered energy flow in the ecosystem and influenced the change in fish community. Even with high dilution, MWWE can have effects on aquatic environments many kilometers downstream and other communities need to consider their impacts carefully with increasing urban populations.

Introduction

Municipal wastewater discharged into waterways cause many issues such as high oxygen demand, nutrient enrichment, and metal contamination (Chambers et al. 1997). More recently many industrial, pharmaceutical, and personal care products have been detected in low concentrations in receiving waters. Many of these emerging contaminants are endocrine disruptors and at environmentally relevant concentrations that can potentially interfere with fish growth and reproduction (Tyler et al. 1998). This is concerning for many communities around the world who have an increasing urban population demanding services (United Nations 2006). As the largest anthropogenic discharge by volume in Canada (Kilgour et al. 2005) there is concern over the potential impacts it may have on aquatic ecosystems. This has lead to government regulations on discharged wastewater quality (Ministry of Environment and Energy 1994a) and large investments in treatment to prevent environmental impacts.

Wastewater treatment was minimal in the early 1900s in Southern Ontario's Grand River watershed and substantial investment in treatment did not occur until the 1970s (Cooke 2006). With a continued increase in human population in urban areas, there is now concern that investments in wastewater infrastructure are not adequate to prevent degradation in receiving waters. Of particular concern is the area downstream of the Kitchener outfall, where there is significant nutrient enrichment and contaminant loads, and summer oxygen levels are often below the provincial water quality objective of 4 mg/L.

Fish are used as bioindicators of aquatic environmental degradation (Tsai 1975) because they are important socioeconomically (Simon 2006), respond to emerging contaminants (Tyler et al. 1998), and integrate conditions across lower trophic levels (Kilgour et al. 2005). This integration is expressed in parameters such as species composition, abundance, and health (growth and reproduction). In addition, stable isotope signatures of nitrogen and carbon in fish, can be used to detect subtle changes in nutrient and energy flow within the system (Jardine et al. 2006).

Previous studies looking at fish populations and communities downstream of municipal wastewater effluent (MWWE) discharges on other rivers have found a variety of effects including increased abundance (Porter and Janz 2003, Winger et al. 2005, Yeom et al. 2007), decreased abundance (Dyer and Wang 2002, Ra et al. 2007), decreased diversity (Birge et al. 1989, Ra et al. 2007), increased diversity (Winger et al. 2005), and increased deformities/lesions (Ra et al. 2007, Yeom et al. 2007). The difference in the fish community response between outfalls relates to differences in treatment process, effluent quality, receiving environment and species sensitivities. The interaction of contaminants and the physical and biotic habitat of the organism greatly influences exposure and toxicity (Emlen and Springman 2007).

The position and structure of organisms within food webs influences the stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of individuals (Peterson and Fry 1987). The differential metabolism and source (i.e. C_3 vs. C_4 plants) results in distinctly different signatures due to different processes in photosynthesis (O'Leary 1981). The heavier isotope of nitrogen is not excreted as readily, resulting in an increase in signature of ~3-5‰ with each trophic level (Ulseth and Hershey 2005). Anthropogenic sources of nitrogen and carbon, such as MWWE, can influence the signature of downstream organisms if nutrients from the outfall are incorporated into the food chain or alters the availability of the different forms of the elements (Jardine et al. 2006, Loomer 2008). Many studies have found an enrichment of stable isotopes in organisms associated with MWWE exposed ecosystems (Steffy and Kilham 2004, Ulseth and Hershey 2005, Northington and Hershey 2006). A change in stable isotope signature will indicate a change in nutrient source somewhere in the food chain, helping to understand the effects MWWE has on aquatic environments.

Tetreault et al. (2008), sampled fish communities at nine sites in the Grand River across 48 km through a major urbanized area that includes two secondary treated effluent outfalls. They found a decrease in fish abundance and the disappearance of some fish species and the appearance of larger mobile species at the furthest downstream sites. Loomer (2008) sampled fish and invertebrates at seven sites in this same area of the watershed and detected a dramatic change in nutrient cycling in the river using ratios of stable isotopes of carbon and nitrogen. These studies found changes likely caused by high nutrient output from MWWE, but it was suggested that more sites and combining the fish community and isotope signature methods in one study would give further insights into what impacts are occurring and how they might be linked.

The objective of this study was to determine if there were changes downstream of two municipal wastewater treatment outfalls (Waterloo and Kitchener) in fish community responses on the Grand River in Southern Ontario. In addition, stable isotope signatures of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) in two dominant fish species, Rainbow Darter (*Etheostoma caeruleum*) and Greenside Darter (*E. blenniodes*), were examined to determine changes in nutrient cycling. If effects of MWWE are better understood, than monitoring programs can be designed to help evaluate ecosystem health and management decisions may be made to prevent further degradation of receiving environments. The situation on the Grand River is not unique, and the knowledge gained from the effects of urbanization on this watershed can assist in many other communities.

Methods

Study Area

The Grand River watershed (Figure 9) is the largest in Southern Ontario, Canada (6,965 km^2), with 76% of its area dominated by agriculture (Cooke 2006). As a result the watershed is already impacted from nutrient runoff of these areas and has less of a capacity than it otherwise would to assimilate additional wastes from municipalities. The population within its boundaries is expected to increase 57% between 2001 and 2031 (Ministry of Public Infrastructure Renewal 2006), concentrating in urban cities such as Kitchener, Waterloo, Cambridge, Guelph, and Brantford in the central area of the watershed. In 2006 there were 26 municipal wastewater treatment facilities servicing 80% of the population in the watershed (Cooke 2006).

The Grand River flowing through the municipalities of Kitchener – Waterloo has two secondary wastewater treatment facilities, with discussion of a multi-million dollar investment for a new plant and/or upgrades (EarthTech 2007). Currently, both plants have conventional activated sludge as secondary treatment and chemical phosphorous removal (Table 7). In addition the Kitchener plant receives wastewater from other Regional plants which are stored in lagoons, resulting in high ammonia concentrations. The Certificate of Approval (provincial wastewater treatment permit) has no requirement for nitrification (EarthTech 2007) and as a result total ammonia effluent concentrations averaged 18.05 mg/L in 2008 (Table 8). When summer pH and temperature are taken into account the unionized concentration is about 0.55 mg/L, higher than the mean 0.48 mg/L LC_{50} for Rainbow Trout (*Oncorhynchus mykiss*) (Environment Canada 2000). The Grand River has a large dilution capacity with estimated August flows (1913-2007) near Kitchener – Waterloo around 11.5 m^3/s (Water Survey of Canada 2007a), which results in higher allowable concentrations in the effluent. These nutrients have lead to an extreme diurnal pattern in dissolved oxygen at downstream sites, possibly caused by the increase in primary production (Cooke 2006) and/or nitrification (Gujer 2010). There are many other inputs upstream, such as large tributaries, wastewater outfalls, dams, and agricultural runoff.

Table 7: Description of the City of Kitchener and the City of Waterloo wastewater treatment plants in 2008 (EarthTech 2007, Kitchener Wastewater Treatment Plant 2008, Waterloo Wastewater Treatment Plant 2008).

Parameters	Kitchener	Waterloo
Population Served	190,000	120,055
Capacity m^3/day	122,745	72,730
Discharge m^3/day	77,768	23,802
Secondary Treatment	Conventional Activated Sludge	Conventional Activated Sludge
Combined Sewers	Some Foundation Drains	No
Disinfectant	Sodium Hyperchlorite *	Sodium Hyperchlorite
Upgrades	Plans for disinfectant to UV and others	Currently disinfectant to UV and others
Other	Receives aerobic sludges in winter from other Regional plants, stored in lagoons	Increased regulations with discharge is over 54,600

* High ammonia from supernating lagoons interferes with disinfection

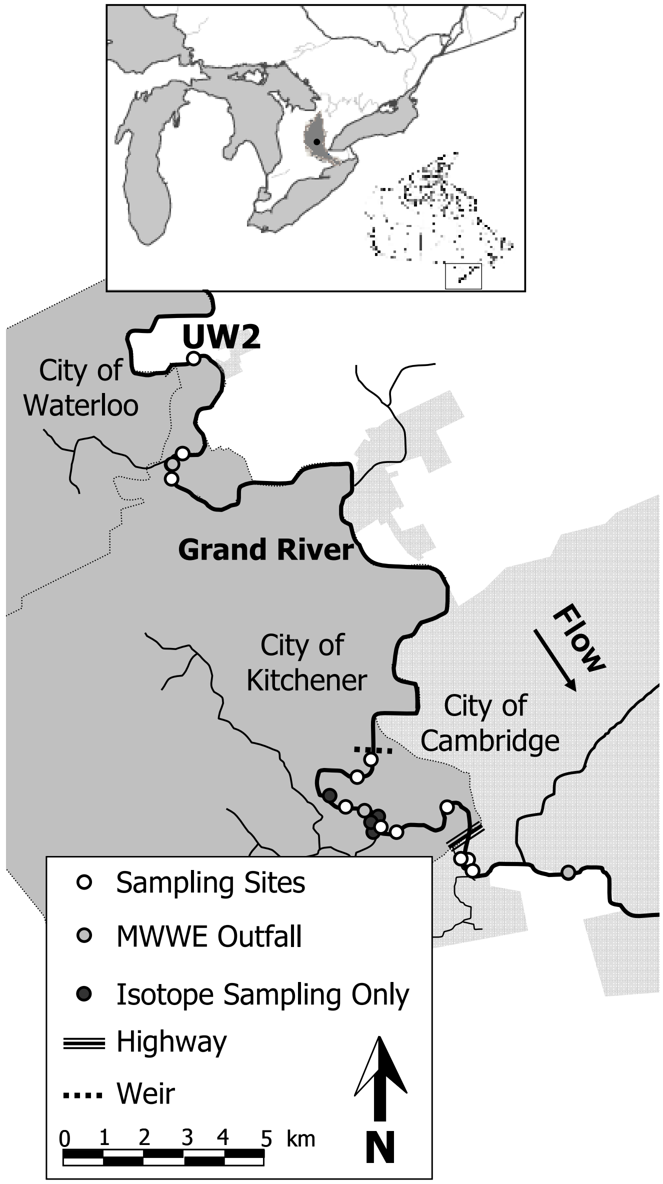

Figure 9: Map of Grand River through the City of Waterloo and City of Kitchener, Ontario, Canada. Sampling sites for 2008 in map start at UW2, with UW1 16.7 km and the Conestogo River 8.1 km upstream of UW2. Sites are in order downstream as listed in Table 9. The Speed River enters the Grand River 1.6 km downstream of DK6.

Table 8: Final effluent concentrations for various parameters in 2008 for the City of Kitchener and City of Waterloo MWWE (Kitchener Wastewater Treatment Plant 2008, Waterloo Wastewater Treatment Plant 2008).

		Waterloo		
		Average	Min (Month)	Max (Month)
Parameter (mg/L)	BOD	20.9	14.5 (Dec)	37.7 (Feb)
	SS	11.5	7.7 (Jan)	13.5 (Jul)
	P	0.53	0.23 (Nov)	0.87 (Apr)
	NH_3-NH_4	7.75	1.82 (Mar)	14.48 (Jan)
	NO_3^-	9.77	5.06 (Jun)	20.9 (Mar)
	NO_2^-	0.92	0.38 (Mar)	1.23 (Jan)
		Kitchener		
		Average	Min (Month)	Max (Month)
Parameter (mg/L)	BOD	5.22	2.95 (Sep)	8.46 (Jul)
	SS	9.84	6.75 (Nov)	14.00 (Mar)
	P	0.53	0.25 (Dec)	0.80 (Jan)
	NH_3-NH_4	18.05	13.15 (Dec)	24.26 (Feb)
	NO_3^-	2.7	1.65 (Aug)	4.31 (Nov)
	NO_2^-	0.86	0.02 (Jul)	1.94 (Oct)

Habitat and Water Quality

Habitat and water quality information on each site was compared to ensure that sites selected had similar characteristics. The US EPA Habitat Assessment in the Rapid Bioassessment Protocols for Wadeable Streams (RBA) (Barbour et al. 1999), and the Ontario Ministry of Natural Resources Habitat Module in the Ontario Stream Assessment Protocol (OSAP) (Stanfield 2007) were guides in determining which habitat data to collect. In September, percent of silt, sand, gravel (< 1", < 2"), cobble (< 4", < 8"), rock (> 8", > 12"), bed rock, and plant covering a 1 m by 1 m area were estimated at 1, 5, and 10 m from shore in each sub-site. Bank stability, bank vegetation, riparian width, canopy cover (% cover), river width, odor, biofilm (% cover), turbidity, and whether the rocks were round or flat was also recorded.

Canadian Aquatic Biomonitoring Network (CABIN) (Environment Canada 2010) method for water velocity measurement involves the Brenduler, a meter stick. This method was used at 1, 5, and 10 m from shore and validated with use of a Swoffer Flow Meter (Model 2100 - Washington, $r^2 = 0.9217$, $y = 0.7895x - 0.0343$, $n = 38$, depth = 0.14 – 0.72 m).

Data collected was input into the modified RBA and the Ohio EPA Qualitative Habitat Evaluation Index (QHEI) (Rankin 2006), both converting habitat quality into a numerical score that can be compared to determine if there are large differences between sites. Changes made in the RBA included counting each section in the RBA out of 10 instead of 20 and replacing the "frequency of riffles" section with one created on the amount of biofilm and turbidity, inversely proportional to the value. There were no alterations to the QHEI.

Water quality was measured with an YSI (6-Series Multiparameter - Ohio) in September and pH (Oakton pHTestr 2 Double Junction - Illinois), dissolved oxygen and temperature (YSI 55 Handheld Dissolved Oxygen – Yellow Springs) in November. These endpoints were collected 5 m from shore in the middle of the site.

Fish Community Sampling

Thirteen sites were sampled in September and November, 2008 (Table 9), with three additional sites sampled for isotopes only in September. Sites were chosen based on proximity to MWWE, wadability, similarity to areas downstream of the MWWE, and safety. UK1, DK5A and DK5B were not sampled in November.

Table 9: Co-ordinates for sampling sites on the Grand River 2008. River side is from perspective of looking upstream. DK5B shore was on an island.

Site	River Side	Longitude (W)	Latitude (N)
UW1	Right	80° 28' 58"	43° 35' 7"
UW2	Left	80° 28' 28"	43° 30' 19"
UW3	Right	80° 28' 27"	43° 29' 3"
WMWWE		80° 28' 55"	43° 28' 47"
DW1	Left	80° 28' 23"	43° 28' 26"
UK1	Left	80° 25' 1"	43° 24' 42"
UK2	Left	80° 25' 28"	43° 24' 31"
UK3	Right	80° 25' 59"	43° 24' 15"
UK4	Right	80° 25' 44"	43° 24' 7"
KMWWE		80° 25' 18"	43° 24' 5"
DK1A	Right	80° 24' 57"	43° 23' 55"
DK1B	Left	80° 24' 59"	43° 23' 54"
DK2A	Right	80° 24' 55"	43° 23' 51"
DK2B	Left	80° 24' 57"	43° 23' 48"
DK3	Right	80° 24' 40"	43° 23' 39"
DK4	Left	80° 23' 32"	43° 24' 7"
DK5A	Right	80° 23' 10"	43° 23' 24"
DK5B	Right (Island)	80° 23' 15"	43° 23' 23"
DK6	Right	80° 23' 8.0"	43° 23' 8"

The method of fish community sampling was originally developed by Tetreault et al. (2008) on the Grand River and used species richness to determine number of sub-sites to ensure sufficient statistical power by reducing variability. This method divided a wadeable 100 m site into ten equal sections. These sections extended 10 m into the river, creating ten 10 m x 10 m sub-sites. Six sub-sites were randomly selected, and each sampled for 300 seconds. Starting downstream, the person with the electroshocking backpack (HT-2000 for September and Smith-Root Model 12 in November with LR-24 for back up) and two netters moved upstream in a zig-zag pattern (Moulton et al. 2002) catching all possible fish. Electroshocker settings were maintained at each site. Sampling occurred in the morning, as preliminary studies were inconclusive as to the best time of day to sample (Appendix A). Fish species were identified (Scott and Crossman 1998), weight (± 0.001 g) and length (mm) measured (forked tails fork length, others total length), and any deformities recorded for each individual. All fish were handled according to the University of Waterloo's Animal Care Committee Protocols (AUP 02-24, 08-08).

Stable Isotopes

Six Rainbow Darter and four to six Greenside Darter were collected from each site, where possible, for stable isotope analysis of $\delta^{15}N$ and $\delta^{13}C$. Severance of the spinal cord euthanized fish according to a protocol approved by University of Waterloo's Animal Care Committee. These fish were stored in labeled whirl packs on ice, and then frozen at –20°C in the lab. Fish were thawed, their length and weight recorded, and the left dorsal muscle removed. Two crayfish species were also collected and shown in Appendix B.

Samples were cut into pieces and dried at 60°C for 24 – 48 hours. The dried samples were ground and weighed (0.25 – 0.30 mg) in tin cups and submitted to the University of Waterloo's Environmental Isotope Laboratory of Earth Science (Drimmie and Heemskerk 2005). Percent elemental composition was calculated with a Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan – Bremen, Germany) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108 - Italy).

Analysis

Diagnostic analysis was performed on the log length and log weight of species with ten or more individuals. Individual species, families, and mean total catch per unit effort (CPUE) were compared between sites, calculated by the mean abundance of sub-sites divided by shocking time (300 s). Community composition was compared between sites with Simpson's diversity and evenness index (Krebs 1999). Mean total mass and condition factor (Nash et al. 2006) of the most abundant species were also examined.

Characteristics such as tolerance (ability to adapt to disturbance and stress) (Eakins 2009), resilience (ability to withstand exploitation, doubling time) (Froese and Pauly 2010), and vulnerability (catchability) (Froese and Pauly 2010) where summarized from the literature for each species caught

(Table 10) and compared between sites. Diet classification (carnivore, general carnivore, invertivore, benthic invertivore, omnivore, benthic omnivore) was also determined from the literature (Scott and Crossman 1998, Eakins 2009, Froese and Pauly 2010) and percent site composition compared (Table 10).

Levene's test for non-homogeneity was used to determine normality of the data and if normal, ANOVA followed by Tukey (homogeneous) tests were conducted. If non-normal, non-parametric Kruskal-Wallis test was followed by a Mann-Whitney-U (non-homogeneous) tests were conducted instead. Independent t-tests were performed when only two means were compared and condition factor (length-weight relationship) was compared with ANCOVA. All statistical analysis was completed with SAS 9.1.3 software © (2003) with $p < 0.05$. All statistical results are ANOVA followed by Tukey unless otherwise stated.

Table 10: List of species caught in 2008 on the Grand River. Tolerance is the ability of a species to adapt to disturbance and stress (Eakins 2009), resilience is the species' ability to withstand exploitation (doubling time) (Froese and Pauly 2010), and vulnerability is related to catchability (Froese and Pauly 2010). Diet classification was determined from a combination of various sources (Scott and Crossman 1998, Eakins 2009, Froese and Pauly 2010). Rare species are found at three or fewer sites are indicated by * when found at upstream sites only, † downstream sites only, and ‡ for both up and downstream sites.

Family	Common Name	Species	Tolerance	Resilience	Vulnerability	Diet Classification	Spawning
Atherinidae	Brook Stickleback*	*Culaea inconstans*	Tolerant	High	Low	Invertivore	Spring-Summer
Catostomidae	Golden Redhorse	*Moxostoma erythrurum*	Intermediate	Low	Low	Benthic Invertivore	Spring
	Greater Redhorse‡	*Moxostoma valenciennesi*	Intermediate	Low	High	Benthic Invertivore	Spring
	Northern Hogsucker	*Hypentelium nigricans*	Intolerant	Low	Low	Omnivore	Spring
	White Sucker	*Catostomus commersonii*	Tolerant	Low	Low	Benthic Omnivore	Spring
Centrarchidae	Pumpkinseed	*Lepomis gibbosus*	Intermediate	Medium	Moderate	Omnivore	Spring-Summer
	Rock Bass	*Ambloplites rupestris*	Intermediate	Medium	Low	Piscivore	Spring
	Smallmouth Bass	*Micropterus dolomieu*	Intermediate	Medium	Moderate	General Carnivore	Spring
Cottidae	Mottled Sculpin†	*Cottus bairdii*	Intermediate	High	Moderate	Benthic Invertivore	Spring
Cyprinidae	Blacknose Dace*	*Rhinichthys obtusus*	Tolerant	High	Low	Invertivore	Spring
	Bluntnose Minnow	*Pimephales notatus*	Tolerant	Medium	Low	Omnivore	Summer
	Common Shiner	*Luxilus cornutus frontalis*	Intermediate	Medium	Low	Invertivore	Spring
	Creek Chub	*Semotilus atromaculatus*	Tolerant	Medium	Low	Omnivore	Spring
	Hornyhead Chub	*Nocomis biguttatus*	Intermediate	Medium	Low	Omnivore	Spring-Summer
	Longnose Dace	*Rhinichthys cataractae*	Intermediate	Medium	Low	Benthic Invertivore	Spring-Summer
	Rosyface Shiner†	*Notropis rubellus*	Intolerant	High	Low	Omnivore	Spring-Summer
	Striped Shiner	*Luxilus chrysocephalus*	Tolerant	Medium	Low	Invertivore	Spring-Summer
Ictaluridae	Brown Bullhead	*Ameiurus nebulosus*	Tolerant	Medium	Low	Benthic Omnivore	Spring
	Stonecat	*Noturus flavus*	Intermediate	Medium	Moderate	Benthic Omnivore	Summer
Percidae	Blackside Darter	*Percina maculata*	Intermediate	Medium	Low	Benthic Invertivore	Spring
	Fantail Darter	*Etheostoma flabellare*	Intermediate	Medium	Moderate	Benthic Invertivore	Spring
	Greenside Darter	*Etheostoma blennioides*	Intermediate	Medium	High	Benthic Invertivore	Spring
	Johnny Darter	*Etheostoma nigrum*	Intermediate	Medium	High	Benthic Invertivore	Spring
	Rainbow Darter	*Etheostoma caeruleum*	Intolerant	High	Low	Benthic Invertivore	Spring

Results

Habitat and Water Quality

The results of QHEI and RBA were similar between sites, with no trend upstream or downstream of the wastewater outfalls (Table 11). The QHEI had a score of 61-72 for most sites, except for UK1 (49) and DK3 (54) and the RBA scored 66-76. The QHEI scores are ranked as excellent (> 75), good (60 - 74), fair (46 - 59), poor (30 - 45), or very poor (< 30) (Rankin 2006). RBA follows a similar principals for defining ranges, although does not give specific values for what is excellent, good, fair or poor (Barbour et al. 1999). Most sites as evaluated by the QHEI and RBA are "good" and are within 15 points of each other. UK1 has the influence of a weir directly upstream and DK3 has a gravel bank for some of the sub-sites which are influencing the QHEI lower scores at these sites. Macrophyte growth increased at downstream sites, possibly from the nitrogen inputs from the Kitchener effluent.

Water quality was similar among sites. pH ranged from 7.0 to 8.28 in both seasons, water temperature (^{o}C) 15.1 to 26.5 in September and 4.3 to 10.0 in November, and dissolved oxygen (mg/L) 5.6 to 9.2 in September and 11.31 to 14.02 in November.

Table 11: Habitat summary for community sites sampled in 2008 on the Grand River. RBA represents habitat values from the modified US EPA's Habitat Assessment in the Rapid Bioassessment Protocol (Barbour et al. 1999) and QHEI is the habitat values from the Ohio EPA's Qualitative Habitat Evaluation Index (Rankin 2006), each scored out of 100. Velocity displayed as September/November. Substrate 1 and 2 are the most common substrate type, with their percent in brackets. Cover symbols are A = algae, Aq = aquatic plants, E = emergent plants, TD = terrestrial leaves, bark, branch debris. NE indicates sites not sampled.

Site	RBA	QHEI	Stream Width (m)	Velocity (cm/s)	Substrate 1 (%)	Substrate 2 (%)	Biotic	Description
UW1	68	68	40	19.8 / 50.5	Gravel (49)	Cobble (34)	A, 3% Aq, E	Cut grass to shore, upstream of major tributary confluence, low habitat diversity
UW2	72	61	50	42 / 57.7	Cobble (45)	Gravel (44)	Aq	Flat, shallow, natural grass shore
UW3	76	65	54	34.3 / 59.4	Cobble (58)	Rock (18)	A. Aq, E, TD	Bank stabilized by large rock
DW1	68	64	63	31.3 / 50.5	Gravel (43)	Cobble (30)	A, Aq	Flat, shallow, shoreline treed, low habitat diversity
UK1	67	49	82	14 / NE	Cobble (34)	Silt (33)	A, E	Downstream of weir, high sediment, flat, shallow
UK2	72	62	56	31.3 / 19.8	Gravel (45)	Cobble (41)	8% Aq	Flat, shallow, natural grass shore
UK4	69	65	43	77.9 / 65.7	Gravel (42)	Cobble (26)	Aq	Flat, shallow, natural grass shore, low habitat diversity
DK2A	76	70	54	39.6 / 67.1	Cobble (52)	Gravel (26)	A, Aq	Flat, shallow, natural grass shore
DK3	71	54	45	24.2 / 59.4	Gravel (47)	Cobble (28)	A	Flat, shallow, natural grass shore, gravel bar, low habitat diversity
DK4	66	67	58	57.7 / 75.4	Gravel (36)	Cobble (35)	TD	Shallow, shore has shrubs
DK5A	69	65	70	37 / NE	Cobble (47)	Gravel (28)	70% Aq	Flat, shallow, natural grass shore, high in stream cover
DK5B	76	72	21	39.6 / NE	Gravel (47)	Cobble (27)	8% Aq	Shallow, natural grass shore
DK6	70	71	94	37 / 0	Cobble (41)	Gravel (34)	38% Aq	Flat, shallow, shore treed, high instream cover

Fish Community

Twenty-four species of fish were captured in September and November 2008 sampling. The wadeable riffle communities sampled were dominated by Rainbow Darter and other darter species (Table 12). Five species were rare, caught at three or fewer sites. These rare species were not common to upstream, downstream, or any one site. Mean total CPUE was significantly different in both seasons, with a trend of decreasing abundance at downstream sites. DK5B was significantly lower than all sites upstream of DK2A, except UW1, in September, while DK3 was lower than all sites in November (both seasons Kruskal-Wallis with Mann-Whitney-U).

Mean total mass was significantly different between sites in both seasons (both seasons Kruskal-Wallis with Mann-Whitney-U) (Table 12). In September DK5A was higher than all other sites and DK3 was different than all other sites in November. DK5A has the highest mass and a low CPUE because a significant portion of the fish caught were larger species (Brown Bullhead (*Ameiurus nebulosus*), Golden Redhorse (*Moxostoma erythrurum*), and Rock Bass (*Ambloplites rupestris*)).

Rainbow Darter and other darter species decreased in abundance at the furthest downstream sites (Kruskal-Wallis with Mann-Whitney-U) (Figure 10). The highest abundance in September was between outfalls, whereas highest abundance in November was upstream of Waterloo. In November there is an increase in abundance of darters at DK2A.

Condition factors for Rainbow Darter and Greenside Darter were significantly different in both seasons between pooled UW, UK, and DK sites (ANCOVA). In both seasons Greenside Darter average condition was UW < DK < UK, while Rainbow Darter's condition was UW < UK < DK. Individual sites were plotted, but low abundance at the downstream sites prevented comparison between sites (Figure 11).

Species were grouped by resiliency (high, medium, low) and compared between sites. Below Waterloo and Kitchener treatment plants (DW1 and DK2A) and at UW2 there was an increase in highly resilient species. Species composition is dominated by Rainbow Darter throughout the river (Table 12), but increases at UW2, DW1, and DK2A. This species is classified as a highly resilient species (Table 10) and the pattern between sites of high resilience reflects its abundance. The pattern of medium resilience follows the abundance of other darter species, as they are all classified as medium resilience and are the next most abundant. There is an increase in low resilience at the furthest downstream sites because there is an increase in Catostomid, which take longer to reproduce. Tolerance showed similar results, with darters driving the results at all sites but the furthest downstream sites, which were influenced by Catostomid. Vulnerability did not show changes between sites as most individuals caught are low vulnerability.

Benthic invertivores made up the largest percentage at each site, but benthic omnivores and carnivores increased at the furthest downstream sites (Table 10; Table 12). This reflects the decrease in darters (benthic invertivores) and increase in Catostomid (benthic omnivores) and Rock Bass (carnivore) at these downstream sites.

Simpson's Diversity and Evenness Index show that UW2 and the sites directly downstream of the MWWE outfalls (DW1 and DK2A) have the lowest diversity and evenness (Table 12). When Rainbow Darter is removed from the diversity calculation there is no difference between sites. When Rainbow Darter is removed from evenness calculation, there is a dramatic increase in evenness at all sites, with UW2, DW1 and DK2A the highest. Rainbow Darter dominated at these sites, and its abundance has a large impact on the fish community of wadeable riffles on the Grand River.

Table 12: Total abundance of common species caught in 2008, presented as September/November. Below are summaries of the fish communities including all species caught. NE indicates sites not sampled. Catch Per Unit Effort (CPUE), CPUE SE, and Mean Total Mass were calculated by averaging the respective totals from sub-sites at each site. All other parameters are with sub-sites combined.

Family	Species	UW1	UW2	UW3	DW1	UK1	UK2	UK4	DK2A	DK3	DK4	DK5A	DK5B	DK6
Catostomidae	Golden Redhorse	- / 1	- / -	- / -	- / -	- / NE	- / -	- / -	- / -	- / -	2 / 1	38 / NE	15 / NE	- / 5
	Northern Hogsucker	- / 1	- / 12	1 / 8	1 / 1	- / NE	- / 2	1 / -	- / 3	- / 1	1 / 3	- / NE	- / NE	1 / 1
	White Sucker	2 / 6	2 / 5	3 / 6	6 / 15	- / NE	1 / 5	1 / 2	5 / 14	4 / 1	3 / 12	- / NE	4 / NE	26 / 36
Centrarchidae	Pumpkinseed	- / -	- / -	- / 3	- / 4	- / NE	- / -	- / -	1 / -	- / -	6 / 2	- / NE	- / NE	1 / -
	Rock Bass	1 / 3	- / -	9 / 20	1 / 6	10 / NE	2 / -	6 / -	- / -	- / -	8 / 1	15 / NE	9 / NE	16 / 1
	Smallmouth Bass	2 / -	- / -	3 / 36	1 / 5	- / NE	- / -	- / -	1 / 3	- / -	1 / -	- / NE	- / NE	1 / -
Cyprinidae	Bluntnose Minnow	- / 13	- / -	- / 3	2 / 1	- / NE	- / 3	- / 2	- / 5	- / -	2 / 4	- / NE	- / NE	1 / 1
	Common Shiner	- / -	- / -	- / 1	- / 3	- / NE	- / 9	2 / 2	- / 2	- / -	5 / 2	- / NE	- / NE	1 / 8
	Creek Chub	- / 2	- / 1	- / -	3 / -	- / NE	- / -	2 / -	- / -	- / -	- / -	- / NE	2 / NE	- / -
	Hornyhead Chub	- / 1	- / 1	- / 2	- / 3	- / NE	- / -	- / -	2 / 1	- / -	- / -	1 / NE	1 / NE	1 / -
	Longnose Dace	- / -	1 / 7	- / -	19 / -	- / NE	5 / 3	10 / 5	12 / 8	20 / 4	12 / 2	18 / NE	2 / NE	4 / 2
	Striped Shiner	28 / -	- / 1	1 / -	3 / 2	2 / NE	18 / 10	1 / 9	1 / 3	- / -	7 / 2	- / NE	- / NE	6 / 4
Ictaluridae	Stonecat	- / 1	2 / -	- / 2	- / 1	- / NE	2 / -	2 / 1	1 / -	- / -	- / -	- / NE	- / NE	- / -
Percidae	Blackside Darter	1 / -	- / -	- / 19	- / -	- / NE	- / -	7 / -	- / -	- / -	2 / -	- / NE	- / NE	- / -
	Fantail Darter	1 / 12	14 / 22	10 / 14	2 / 17	22 / NE	4 / 7	2 / 11	1 / 4	1 / -	2 / -	- / NE	- / NE	- / -
	Greenside Darter	11 / 31	3 / 16	23 / 45	7 / 9	19 / NE	14 / 17	35 / 14	7 / 19	7 / 3	21 / 10	10 / NE	1 / NE	12 / 6
	Johnny Darter	11 / 48	1 / 1	18 / 18	6 / 23	16 / NE	36 / 52	29 / 30	5 / 17	2 / 14	38 / 37	- / NE	7 / NE	11 / 17
	Rainbow Darter	39 / 119	119 / 226	64 / 111	131 / 134	116 / NE	105 / 63	107 / 41	108 / 163	78 / 5	30 / 18	16 / NE	30 / NE	59 / 11
Catch Per Unit Effort (/300 s)		3.2 / 8.03	4.8 / 9.8	4.4 / 9.6	6.1 / 7.5	6.2 / NE	6.2 / 7.7	6.8 / 3.9	4.8 / 8.1	3.7 / 0.9	4.7 / 3.2	3.7 / NE	2.4 / NE	4.7 / 3.2
Catch Per Unit Effort SE		2.8 / 4.7	3.1 / 3.8	3.1 / 3.8	6.3 / 3.1	2.9 / NE	2.2 / 2.6	5.4 / 1.5	3.0 / 4.5	3.5 / 1.5	2.8 / 3.1	2.3 / NE	3.4 / NE	3.1 / 4.8
Mean Total Mass (g)		15.7 / 83.3	32.4 / 90.6	55.1 / 188.4	47.3 / 158.5	36.2 / NE	63.0 / 46.6	61.2 / 25.2	43.6 / 86.7	22.8 / 9.5	43.7 / 58.7	364.2 / NE	32.9 / NE	94.2 / 82.1
Species Richness		9 / 13	8 / 12	9 / 15	12 / 14	6 / NE	9 / 11	13 / 10	11 / 13	6 / 6	17 / 14	8 / NE	10 / NE	14 / 13
Family Richness		4 / 6	4 / 3	4 / 5	4 / 5	3 / NE	5 / 3	5 / 4	5 / 4	3 / 3	4 / 5	5 / NE	5 / NE	5 / 4
Simpson's Diversity		0.72 / 0.69	0.30 / 0.40	0.70 / 0.80	0.47 / 0.62	0.57 / NE	0.63 / 0.76	0.67 / 0.78	0.43 / 0.53	0.48 / 0.68	0.85 / 0.79	0.79 / NE	0.78 / NE	0.77 / 0.79
Simpson's Evenness		0.40 / 0.25	0.18 / 0.15	0.38 / 0.33	0.16 / 0.19	0.39 / NE	0.30 / 0.41	0.24 / 0.45	0.16 / 0.18	0.32 / 0.53	0.38 / 0.36	0.67 / NE	0.40 / NE	0.28 / 0.40
% Invertivore		29.2 / 1.3	- / 0.7	0.76 / 0.35	1.6 / 2.2	1.1 / NE	9.6 / 10.9	1.5 / 9.4	0.7 / 2.1	- / -	8.5 / 4.1	- / NE	- / NE	5.0 / 12.6
% Benthic Invertivore		65.6 / 87.5	97.2 / 92.5	87.1 / 71.6	90.7 / 81.7	93.5 / NE	87.7 / 81.6	92.7 / 86.3	92.4 / 86.8	96.4 / 92.9	76.1 / 70.1	75.3 / NE	74.5 / NE	61.0 / 45.3
% Omnivore		- / 7.05	- / 4.8	0.8 / 5.5	3.3 / 4.0	- / NE	- / 2.9	1.5 / 1.7	2.1 / 3.7	- / 3.6	7.0 / 9.3	4.1 / NE	0.9 / NE	2.8 / 2.1
% Benthic Omnivore		2.1 / 2.9	2.8 / 1.7	2.3 / 2.8	3.3 / 7.1	- / NE	1.6 / 2.9	1.5 / 2.6	4.2 / 5.8	3.6 / 3.6	2.1 / 13.4	8.2 / NE	8.2 / NE	18.4 / 37.9
% General Carnivore		2.1 / -	- / -	2.3 / 12.5	0.6 / 2.2	- / NE	- / -	- / -	0.7 / 1.2	- / -	0.7 / -	- / NE	- / NE	1.4 / -
% Carnivore		1.0 / 1.2	- / -	6.8 / 6.9	0.6 / 2.7	5.4 / NE	1.1 / -	2.9 / -	- / -	- / -	5.6 / 1.0	12.3 / NE	13.6 / NE	11.3 / 1.0
% Darter		65.6 / 87.1	95.8 / 90.1	87.1 / 71.6	80.2 / 81.7	93.5 / NE	85.0 / 79.9	87.8 / 82.1	84.0 / 83.5	78.6 / 78.6	65.5 / 67.0	23.6 / NE	52.0 / NE	58.2 / 35.8

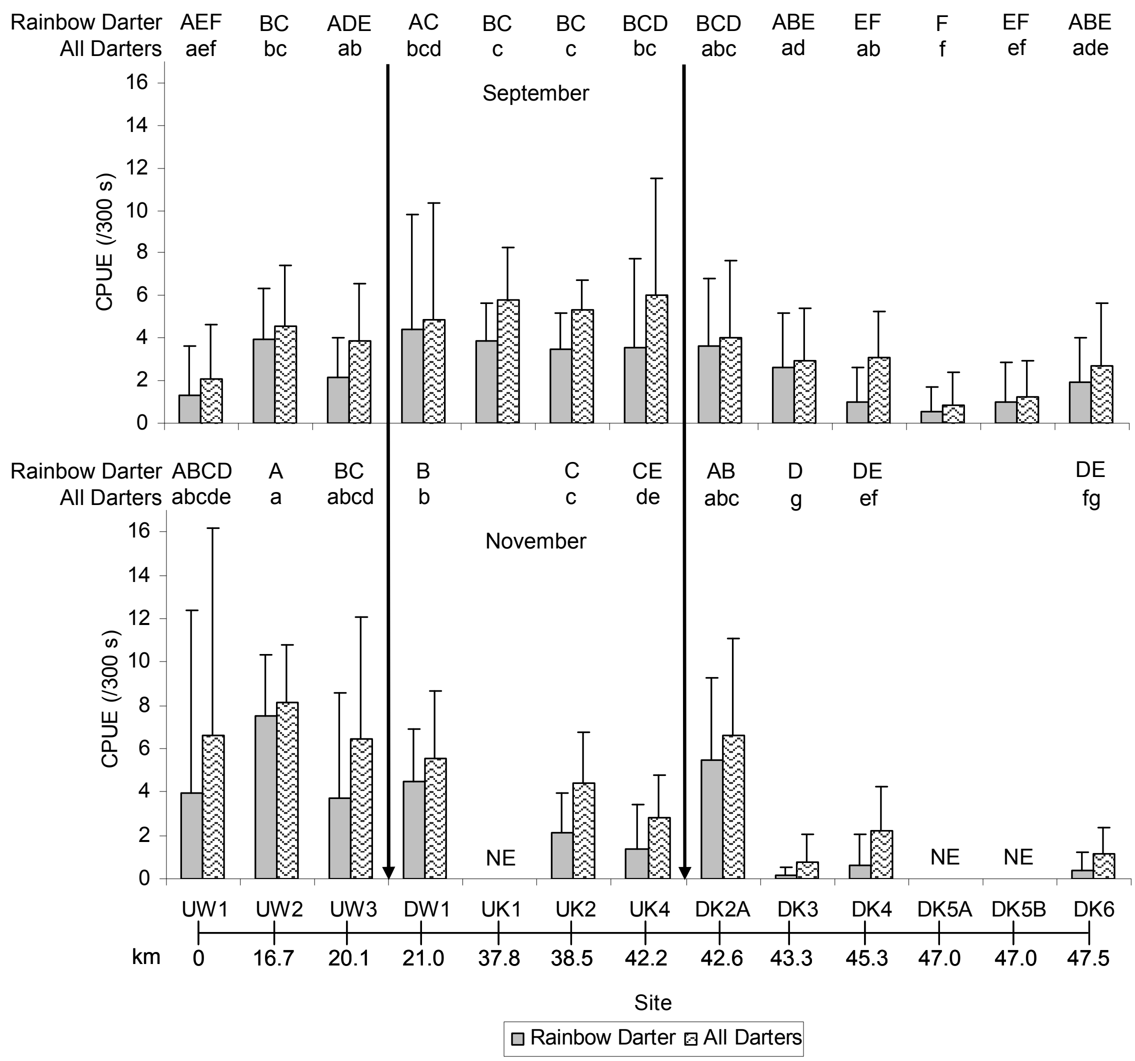

Figure 10: Mean catch per unit effort (CPUE, /300 seconds + SE) for Rainbow Darter and all darter species on the Grand River in September and November, 2008. Arrows indicate the Waterloo and Kitchener MWWE outfalls, respectively. Lettering indicates significant difference between sites by Mann-Whitney-U test. NE indicates sites not sampled. Distance from farthest upstream site indicated below sites.

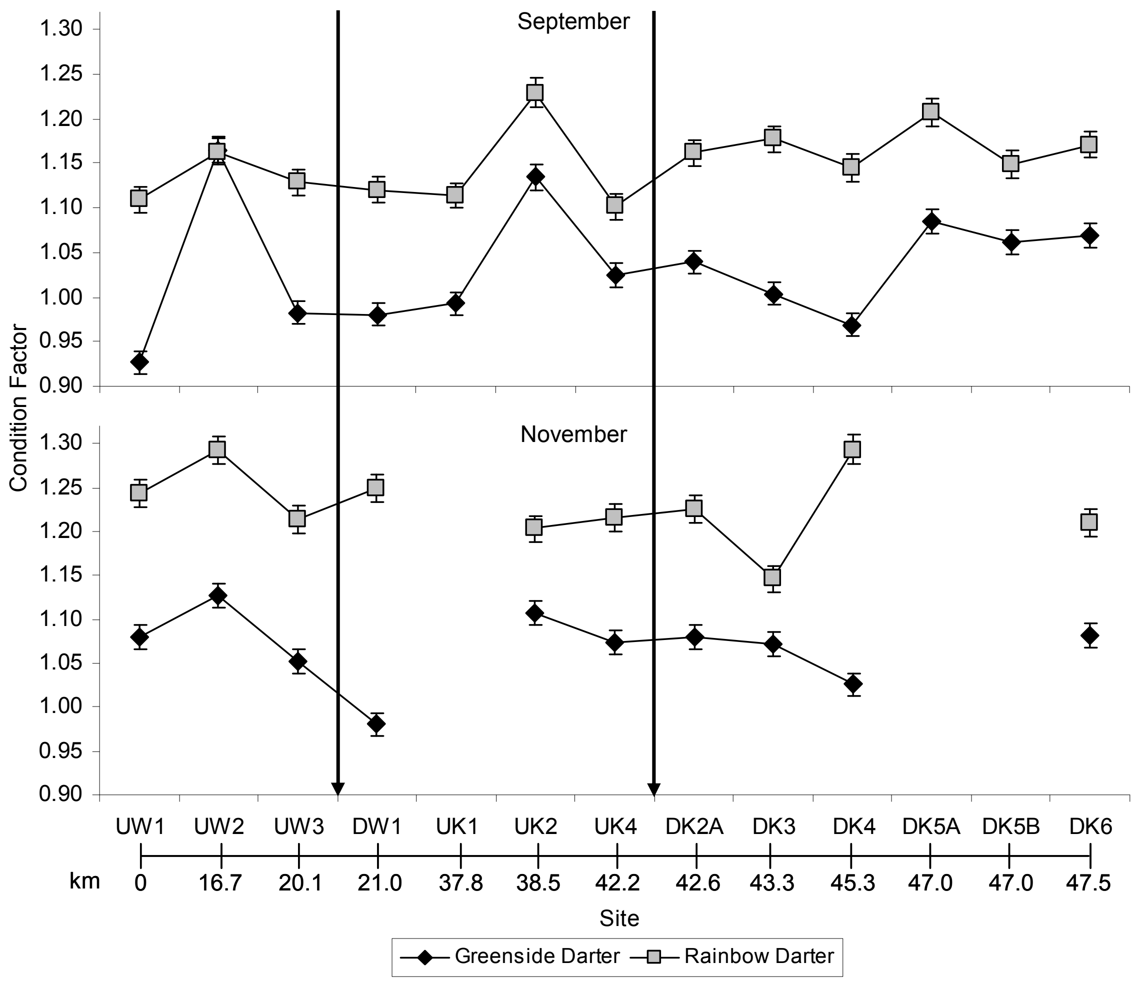

Figure 11: Condition factor (mean ± SE) for Greenside Darter and Rainbow Darter in September and November, 2008. Arrows indicate the Waterloo and Kitchener wastewater outfalls. Distance downstream from furthest upstream site shown below.

Stable Isotope

Rainbow Darter increased in stable isotope signature for nitrogen ($\delta^{15}N$) (Figure 12), but did not change significantly for carbon ($\delta^{13}C$) downstream of the Waterloo outfall. There was no change for Greenside Darter, $\delta^{13}C$ or $\delta^{15}N$, at this site (Figure 13). Fish of both species caught at downstream sites of the Kitchener outfall had an increase in $\delta^{13}C$ and a decrease in $\delta^{15}N$. Downstream of Kitchener both sides of the river were sampled, and the sites with higher effluent exposure (DK1A and DK2A) had a greater difference in $\delta^{13}C$ and $\delta^{15}N$ from upstream sites than the opposite side (DK1B and DK2B). Rainbow Darter signature ($\delta^{13}C$ or $\delta^{15}N$) was greater than Greenside Darter at many sites (Table 13), except at UW3 where Greenside Darter was greater for both $\delta^{13}C$ and $\delta^{15}N$. Nitrogen of both species were analyzed with Kruskal-Wallis followed by Mann-Whitney-U test.

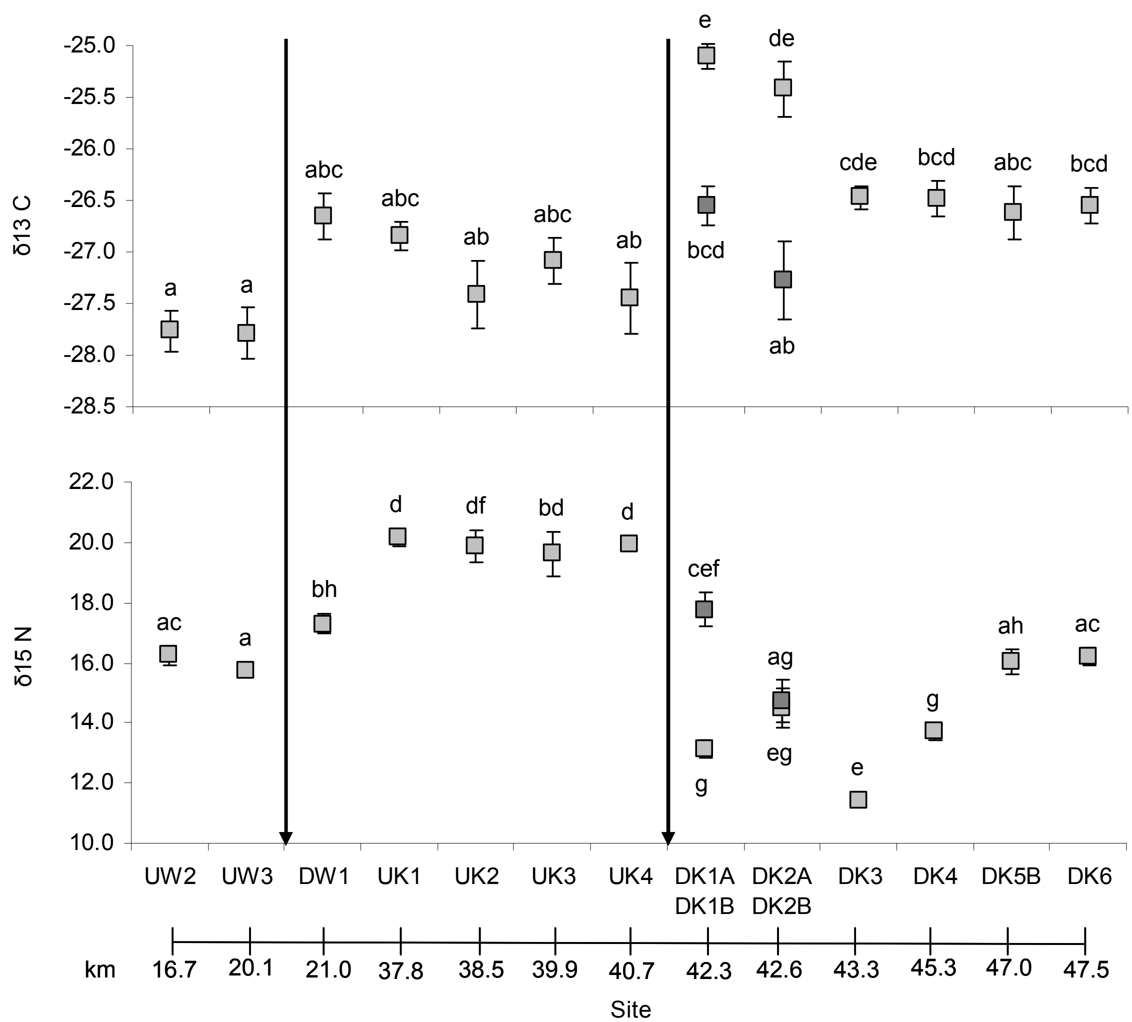

Figure 12: Stable isotope signatures ($\delta^{15}N$ and $\delta^{13}C$, mean ± SE) for Rainbow Darter (n = 6/site) collected September, 2008 on the Grand River. Lettering indicates significant differences between sites, carbon with Tukey and nitrogen with Mann-Whitney-U test. Kilometers downstream are from furthest upstream site. Arrows indicate the Waterloo and Kitchener MWWE outfalls. Darker shading indicates DK1B and DK2B.

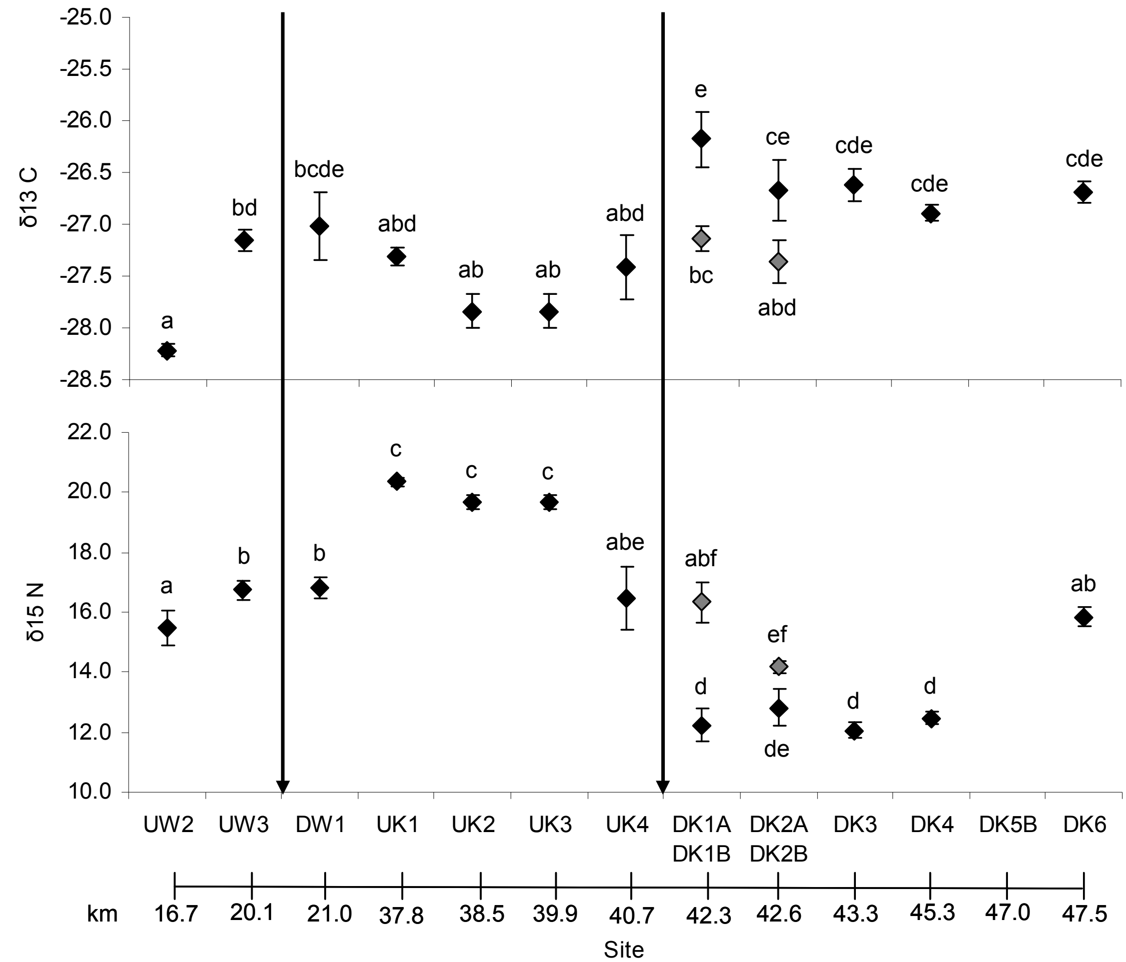

Figure 13: Stable isotope signatures ($\delta^{15}N$ and $\delta^{13}C$, mean ± SE) for Greenside Darter (n = 5 to 6/site) collected September, 2008 on the Grand River. Lettering indicates significant difference between sites of carbon with Tukey and nitrogen with Mann-Whitney-U test. Kilometers downstream are from furthest upstream site given. Arrows indicate the Waterloo and Kitchener MWWE outfalls. Lighter shaded diamonds indicate DK1B and DK2B.

Table 13: Statistical comparison between Rainbow Darter and Greenside Darter values for $\delta^{15}N$ and $\delta^{13}C$. 'Yes' indicates that there was a significant difference between the species at that site with an independent t-test.

	Site													
	UW2	**UW3**	**DW1**	**UK1**	**UK2**	**UK3**	**UK4**	**DK1A**	**DK1B**	**DK2A**	**DK2B**	**DK3**	**DK4**	**DK6**
C	No	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	No	Yes	Yes	No
N	No	Yes	No	No	No	No	Yes	No	No	No	No	No	Yes	No

Discussion

Downstream of the Kitchener MWWE changes were seen in species composition, darter abundance, and stable isotope signatures. This is potentially due to inadequate treatment of MWWE, which is leading to high nutrients, ammonia, and large diurnal patterns in dissolved oxygen concentrations (Cooke 2006). Low dissolved oxygen and toxic conditions can lead to a decrease and changes in species composition (Tsai 1975). MWWE has also lead to changes in stable isotopic signature in receiving environments, as nutrients provided are often preferentially incorporated (deBruyn and Rasmussen 2002).

MWWE was originally discharged into streams and rivers without treatment, leading to acute toxicity (Tsai 1975). Treatment has improved, but there are still areas where treatment is not adequate and effects are seen (Chambers et al. 1997). Studies in Michigan and Ohio looked at the effects of multiple wastewater outfalls within a watershed and found a trend of decreasing fish abundance downstream. In this study CPUE is proportional to abundance, as sampling time was the same at every site. Mean total abundance downstream of Kitchener had a decreasing trend, similar to studies previously mentioned. When groups of species were examined it was found the darter species abundance decreased as well. Other studies have found a decrease in Pale Chub (*Zacco platypus*) in Korea (Yeom et al. 2007) or an increase in total abundance in Oklahoma (Porter and Janz 2003). Both of these studies had only one downstream site. It is possible that if they included more sites there would be variation in abundance depending on how close they were to the outfalls (Tsai 1975).

Other effects of MWWE include changes in biomass. In this study mean total mass increased at downstream sites because of an increase in Centrarchid and Catostomid species. These species live longer and are larger than darter species (Scott and Crossman 1998). Another study downstream of Calgary's MWWE has also seen an increase in mass (Askey et al. 2007), but it is unclear if this is due to heavier fish or increased numbers of fish. The differentiation between the two is necessary to fully understand effects (Munkittrick et al. 2000).

Condition factor is a method to compare weight by standardizing it to length and indicates how fat the fish is. Larger values indicate a heavier individual relative to its length (Nash et al. 2006). Rainbow Darter's condition was larger at pooled sites downstream of Kitchener. Other studies have found enrichment from MWWE (Chambers et al. 1997, McMaster et al. 2005), and it is likely fish are heavier due to availability of food. Greenside Darter were heaviest between the Waterloo and Kitchener outfalls, which could be enriched in nutrients from the Waterloo outfall and other urban sources. The difference in the condition pattern between the species may be a difference in sensitivity to the effluent or other factors such as diet. MWWE may be elevated in temperature in certain seasons compared to the receiving environment. Greenside Darter is less tolerant to high temperatures than the Rainbow Darter (COSEWIC 2006) and may not be able to compete/survive as well in conditions downstream of the Kitchener outfall.

Diversity and evenness decreased at sites directly downstream of both effluent outfalls due to the increase in Rainbow Darter at these sites. There have been other studies that have found a decrease in diversity downstream of MWWE (Birge et al. 1989, Ra et al. 2007), but whether this is from an increase in a single species is not noted. It is unknown why UW2 had a large increase in Rainbow Darter as there are no obvious inputs or differences in habitat.

When diet, tolerance, and resilience are examined, the pattern seen can be attributed to the changes in abundance of darter, Centrarchid, and Catostomid species. Other studies have found an increase in tolerant species and omnivores downstream of MWWE to indicate impacts (Karr 1981, Ra et al. 2007, Yeom et al. 2007). The changes in community composition is reflective of those species that can compete/survive in the environment with the additional stress of the effluent, which may indicate impairment of ecological function (Kilgour et al. 2005). These changes are seen further downstream of the effluent, which may be due to mixing and different processes occurring at different distances from the outfall.

Often the areas around the outfall are grouped into zones (Tsai 1975). The number and names of zones varies, but they start where the wastewater enters the river. In this area DO is still high and fish abundance often greater than upstream as they take advantage of the increase in food. Further downstream the wastewater starts to decompose and lowers DO levels. Fish avoid or suffer in this area due to suffocation. Downstream of this area there is recovery where DO levels start to increase and so does fish abundance. Suckers and shiners are species often found in this area. The last zone is where the wastewater is no longer present, often termed “clean water”.

These zones are similar to what we have seen reflected in the fish community on the Grand River. There was high abundance of fish downstream of the outfall at DK2A, indicating non-toxic conditions with high food availability. The stable isotope signatures also changes in the fish at these sites, likely from a change in food source or nutrient availability from the effluent. Further downstream there was a decrease in abundance, an area of the river with documented low DO in summer (Cooke 2006). At DK6 we also see an increase in sucker species abundance, which may indicate that these sites are the start of recovery from the effects of the MWWE. Sites further downstream should be sampled if similar habitat is present, but the confluence of the Speed River and the influence of the Galt Dam affect responses in these areas.

Sampling in 2007 by Tetreault et al. (2008) was completed at six of the same sites to this study (UK1, UK2, DW1, UK1, DK2A, and DK6). Community composition was similar at sites between years except DK6, which had no darter species in 2007. The summer of 2007 was very dry, with water levels below the 10^{th} percentile. In contrast, 2008 was a very wet year, with water levels above the 97^{th} percentile (August levels at West Montrose from Water Survey of Canada 2007a, Gallant 2010). It is possible the lower flows in the summer of 2007 extended the decomposition zone, causing a more adverse environment at DK6. A long-term study to compare seasonal variations is recommended to understand the risk to fish communities from MWWE, especially with the predicted increase of water withdrawals and dry summers for this region (Colombo et al. 2007).

Stable isotope signature of nitrogen in Rainbow Darter on the Grand River in 2008 increased downstream of Waterloo. This is consistent with findings from the previous year in the same area (Loomer 2008) and many other locations downstream of other MWWE outfalls (Steffy and Kilham 2004, Ulseth and Hershey 2005, Northington and Hershey 2006). Greenside Darter had no change in signature downstream of the Waterloo outfall, which is contrary to the other studies, including the one conducted in 2007. As there were only three sites around the outfall sampled, it is possible that additional upstream and downstream sites would give a better understanding of the stable isotope signature pattern. The change in Rainbow Darter and no change in Greenside Darter was also seen on the Speed River downstream of the Guelph outfall in 2008 (Chapter 2). The pattern on the Speed River was hypothesized to be a result of a shift in diet between the species. It is possible that the signatures differ between the years because of the change in water flow from low in 2007 to high in 2008. Both Waterloo and Guelph effluents have lower concentrations of ammonia than Kitchener, which may be the reason for the difference in response downstream of these treatment plants. Both the Rainbow Darter and Greenside Darter are unlikely to be mobile (COSEWIC 2006, Loomer 2008), as is also indicated by isotope results for DK1 and DK2 A (effluent exposed) and B (not exposed). More sampling downstream of the Waterloo wastewater outfall may reveal a stronger connection of isotopes and fish community similar to what was seen on the Speed River.

Downstream of Kitchener there was an increase in $\delta^{13}C$ for both species, but $\delta^{15}N$ decreased dramatically. Loomer (2008) observed a similar trend in 2007 and hypothesized that the low signature represented consumption of autotrophs, who use lighter inorganic nitrogen from MWWE. Total ammonia, an inorganic form of nitrogen, was an average concentration of 18.05 mg/L in the

Kitchener effluent in 2008. The increase in fish abundance also indicates an increase in food, likely starting with an increase in primary production.

Habitat evaluation revealed an increase in macrophyte production at the furthest downstream sites, likely from nutrients provided by the MWWE. These obscured vision of the river bottom, and possibly fish, in September. Sampling in November, when the plants were dead, also resulted in low abundance, which indicates catchability in September was likely not a major factor. The macrophytes could influence the change in community by providing additional habitat. Both the QHEI and RBA scoring at sites where macrophytes were present were not significantly affected by their abundance. It is possible that this habitat feature is more important to fish community composition than these indices account for.

This study captured fish in wadeable areas with a backpack electroshocker. It is possible with different gear types and habitats that differences from this study would be seen. Also, only small bodied fish that are less mobile were used for stable isotope analysis. Other species that spend less time in the effluent may have different signatures, which is why the darters were chosen for the analysis. There are a number of tributaries, municipal runoff, low head impoundments and other factors that may confound results.

The Grand River has improved in water quality since wastewater treatment has been implemented. However, with increasing populations, effluent quality may degrade without further investments. The increase in dry years predicted for this area (Colombo et al. 2007) will increase years like 2007 where effluent dilution and oxygen levels were low. The difference between years of Tetreault et al. (2008) and this study indicate that further study between years will give insight into the effect of different flows on the toxicity of municipal wastewater effluent.

The fish community in 2008 was dominated by darter species, which decreased in abundance while Catostomids and Centrarchids increased in abundance at the furthest downstream sites. This is where low DO levels are known to occur, possibly from high primary productivity from high nutrient levels (Tsai 1975, Cooke 2006) and the conversion of ammonia to nitrate and nitrite (Gujer 2010). The decrease in the stable isotope signature of nitrogen downstream of Kitchener is likely from the large discharge of ammonia in the city's effluent being consumed by autotrophs. The inadequately treated effluent is changing the ecosystem of the receiving environment. Upgrades to treatment infrastructure are necessary, which the Region of Waterloo is planning (EarthTech 2007). The question remains what level of treatment is necessary to mitigate current and future degradation. Further sampling downstream of Waterloo and other treatment plants in the area may assist in these important management decisions. Impacts have occurred downstream of other outfalls, and the conditions found in the Grand River can assist in the investigation of other receiving environments to prevent aquatic degradation.

Conclusion

Municipal wastewater effluent is the largest anthropogenic discharge in Canada (Kilgour et al. 2005), that contains a variety of chemicals such as nutrients, metals, and pharmaceuticals. Although treatment has improved, there are still areas of concern related to current inadequate treatment and future population growth (Chambers et al. 1997).

The Grand River watershed is part of the provincial government's "Places to Grow" plan for population growth in Southern Ontario. The watershed is likely going to increase at least 50% from 2001 to 2031 (Ministry of Public Infrastructure Renewal 2006). This growth will mean an increase in demand for services, such as wastewater treatment. There is concern that effluent quality will decrease and lead to degradation of aquatic ecosystems. This study looked at current conditions associated with three central MWWE outfalls in the Grand River watershed to determine if there are impacts and to assist with future decisions related to infrastructure.

Fish communities were chosen to evaluate if there are current impacts to the aquatic ecosystem because they integrate conditions from all trophic levels (Kilgour et al. 2005) and have been seen as the most important water pollution indicator (Tsai 1975). Unfortunately there are no sampling protocols for the size of rivers sampled in this study. The rivers have a combination of shallow riffles and deep pools, meaning that multiple equipment types would have to be used to sample all habitats. Ensuring that habitat in pools are similar is more complicated than wadeable areas, so to reduce complexity only wadeable areas were sampled.

A method previously developed on the Grand River was adopted, which randomly sampled six of ten 10 m x 10 m sub-sites (Tetreault et al. 2008). Other protocols recommend sites 20 x wetted width or to sample between cross over points (Lazorchak et al. 1998, Moulton et al. 2002, Stanfield 2007). It is also often recommended that different gear types be used together to capture more of the community (Curry and Munkittrick 2005). This method of sampling should be tested and developed further. But, the sampling style used in this study did allow for a consistent, reproducible method that is not present in the literature for medium sized rivers.

The basic habitat analysis completed in this study was adopted from several published approaches. It demonstrated that there were no large differences among sites upstream or downstream of the effluent outfalls. These methods may not emphasize or evaluate habitat features that are important to fish or the fish species specific to this study. Further analysis, such as canonical correspondence analysis (D'Ambrosia and Williams 2009), may reveal habitat differences that explain the fish community seen. This will allow multiple species and multiple habitat variables to be compared simultaneously to determine if some species occur more or less frequently with a specific parameter.

Stable isotope signatures also assisted with the goals of this study by indicating food source of the Rainbow Darter and Greenside Darter. A change in signature of fish caught downstream of the outfall would indicate that the effluent has a different signature or the food chain is fractionating the high nutrients from the effluent differently, possibly changing energy cycling (Steffy and Kilham 2004, Ulseth and Hershey 2005, Northington and Hershey 2006, Loomer 2008). This assists in the investigation of current impacts on fish communities.

The City of Guelph discharges its wastewater into the Speed River, a major tributary of the Grand River. The Speed River is a relatively small receiving environment, and in the past has had problems downstream with low dissolved oxygen levels (Cooke 2006). The city upgraded the treatment plant to tertiary treatment in 1979 (DenHoed and Robertson 2003), and a further increase in population may require a much larger investment to maintain aquatic ecosystem quality.

Evaluation of wadeable fish communities on the Speed River revealed no impacts from the MWWE to mean total CPUE or mean total mass. The appearance of impacts to diversity, evenness, tolerance and resilience were all attributed to changes in Greenside Darter and Rainbow Darter abundance. The MWWE from Guelph seems to play a role in the interactions of these species, with the Rainbow Darter able to survive/outcompete the Greenside Darter downstream of the outfall. This is supported from the low abundance of Greenside Darter directly downstream of the outfall and the low number of YOY. Also, stable isotope signature of nitrogen and carbon did not change in the Greenside Darter, but increased in the Rainbow Darter. This suggests that there is a food source the Rainbow Darter is eating that the Greenside Darter is not. This altered food source availability may lead to the increase in Rainbow Darter abundance, condition, and isotope signature.

The Cities of Waterloo and Kitchener each have a secondary wastewater treatment plant that discharges into the main branch of the Grand River ~ 21 km apart. Downstream of Kitchener there are concerns of low oxygen, especially when river levels are low and temperatures are high in the summer months (Cooke 2006). In 2008 there was a decrease in fish abundance near the low oxygen area (~ 5.3 km downstream of outfall) and a decrease in $\delta^{15}N$ directly downstream of the outfall, both likely a result of Kitchener's high ammonia outputs. Previous studies have found similar results, with low fish abundance (Tetreault et al. 2008) and changes in stable isotope signatures (Loomer 2008). In addition, fish community studies in 2007 did not capture any darter species at the far field site, but they were present in 2008. This is likely due to the difference in water quality between the years as a result of low flow in 2007.

The difference in response to MWWE between Rainbow Darter and Greenside Darter in 2008 shows that these species may differ in their ability to respond to environmental change. Rainbow Darter increased in abundance (percent site composition) at all sites directly downstream of all outfalls. Wastewater adds nutrients to receiving environments that can increase growth of existing animals or allow additional organisms (numbers and/or species) to survive (Chambers et al. 1997, McMaster et al. 2005). These additional organisms may be providing food for the Rainbow Darter, which can account for the increase in abundance and stable isotope signatures at Waterloo and Guelph. The Greenside Darter had lower abundance downstream of the outfalls, and also did not change in isotopic signature downstream of Waterloo and Guelph. The interaction of these two darter species influences the wadeable fish community and it is important to understand the interaction with MWWE exposure to fully understand the impacts on the environment. Rainbow Darter and Greenside Darter's diet and biology should be further investigated.

A long term study would allow comparisons between wet and dry years. The river levels were below the 10^{th} and 40^{th} percentile quartile in August 2007, while in 2008 they were above the 97^{th} percentile (Water Survey of Canada 2007b, a, Gallant 2010). The change in water level may explain why there were no darters collected at DK6 in 2007, but there were in 2008, as there would be less dilution. There was also a difference in Greenside Darter isotopic response, with an increase downstream of Guelph and Waterloo in 2007, while there was no change downstream in 2008. The lower flows may lead to more toxic conditions, and regulations may need to change to accommodate the additional stress in these situations. With climate change, it is predicted that temperatures will increase and precipitation will decrease for the Grand River watershed (Colombo et al. 2007). In other words, conditions similar to 2007 will increase in frequency, which may increase the detrimental effects of MWWE to aquatic environments.

A popular method to evaluating impacts of anthropologic inputs in aquatic ecosystems is to evaluate a change in a sentinel species (Environment Canada 2003). The Rainbow Darter and Greenside Darter are abundant within the area of study, so it is possible that they could be chosen for such a task. If one species had been chosen without the other, different conclusions may have been drawn as they appear to be responding differently to the effluent. This emphasizes why initial studies should include a community survey (Kilgour et al. 2005), to determine species presence and abundance, but also so possible differences in response between species may be observed.

The population in Southern Ontario is growing and pressure on infrastructure to service these people will also increase. MWWE is currently affecting some aspects of the fish community in the Grand River watershed. It is possible that without additional investments, effluent quality will degrade and impact the receiving environment severely. The Region of Waterloo is planning upgrades and possibly a new treatment plant for Kitchener-Waterloo (EarthTech 2007) to try and mitigate the low oxygen, high nutrients, toxicity, and contaminants. What remains to be decided is what level a treatment will be necessary to prevent impacts. Additional study in this area, especially downstream of Waterloo, may aid in deciding what quality of effluent is necessary for the Grand River, especially with the increase in low flow years to come. Aquatic resources are important for recreation, irrigation, drinking water and more, and need to be protected before irreversible damage is done.

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Appendices

Appendix A Day versus Night Sampling

July 15-18, 2008 four sites were sampled in the morning and night to determine if there was a difference between the two time periods. Two of these sites were on the Speed River (US3 and DS3) and two were on the Grand River (DK2A and DK6). Mean total catch per unit effort (CPUE) was calculated by averaging the total CPUE of each sub-site and day and night compared at each site with an independent t-tests in SAS 9.1.3 software © (2003) with $p < 0.05$. All other parameters are with sub-sites combined. US3 had similar diversity between day and night (Table 14), but had an increase in CPUE, species richness, and decreases in diversity and evenness at night. DS3 did not change between day and night (CPUE). DK2A had a large increase in CPUE, richness, diversity and evenness at night. DK6 had similar results between sampling times for CPUE, richness, and diversity. Differences between sampling time were inconclusive, as there was difference between some sites and not others. Therefore, all sampling for this study was conducted in the morning to reduce potential variability and simplify logistics.

Table 14: Day and night sampling results for July, 2008. US3 and DS3 were sampled on the Speed River, While DK2A and DK6 were sampled on the Grand River. Mean catch per unit effort (CPUE) and CPUE Standard Error was calculated by averaging the total CPUE of each sub-site. CPUE is the abundance divided by the sampling time (300 s). All other parameters were calculated with sub-sites combined.

Parameter	US3 Day	US3 Night	DS3 Day	DS3 Night	DK2A Day	DK2A Night	DK6 Day	DK6 Night
Mean CPUE	1.3	4.1	4.5	5.6	6.8	13.2	4.4	2.9
CPUE Standard Error	0.94	0.92	0.42	0.73	0.53	0.69	1.02	1.26
Species Richness	9	12	9	9	13	17	10	11
Family Richness	4	4	5	5	5	7	4	4
Simpson's Diversity	0.82	0.76	0.7	0.7	0.76	0.96	0.66	0.79
Simpson's Evenness	0.61	0.35	0.37	0.37	0.32	1.5	0.29	0.43

Appendix B Grand River Crayfish

Crayfish collected by electrofishing on the Grand River in September, 2008, were identified to species (Crocker and Barr 1968, Karstad and the Project Crayfish Group 2008, Keene 2009) and prepared in the same manner to other isotope samples previously mentioned. The tail muscle was the tissue processed. Carbon was lipid corrected with the equation $\delta^{13}C_{corrected} = -3.32 + (0.99)\cdot(C:N)$ (Post et al. 2007).

Orconectes propquinus (northern clearwater crayfish) and *o. virilis* (virile crayfish) were the species most abundant with this sampling method. Waterloo treatment plant had no effect on the stable isotope signature of carbon or nitrogen in either species (Figure 14). Downstream of Kitchener (DK1A and DK2A) there is an increase in $\delta^{13}C$ and a decrease in $\delta^{15}N$. As with the Rainbow Darter and Greenside Darter at these same sites, this is likely due to a change in nutrient source for the food web. This is possibly from the consumption of autotrophs who may be increasing their uptake of inorganic nitrogen (ammonia) from the MWWE (Loomer 2008). The distinctive signature between river sides at DK1 and DK2 (A and B) further supports other findings that crayfish do not move between river sides (G. R. Craig & Associates 2006).

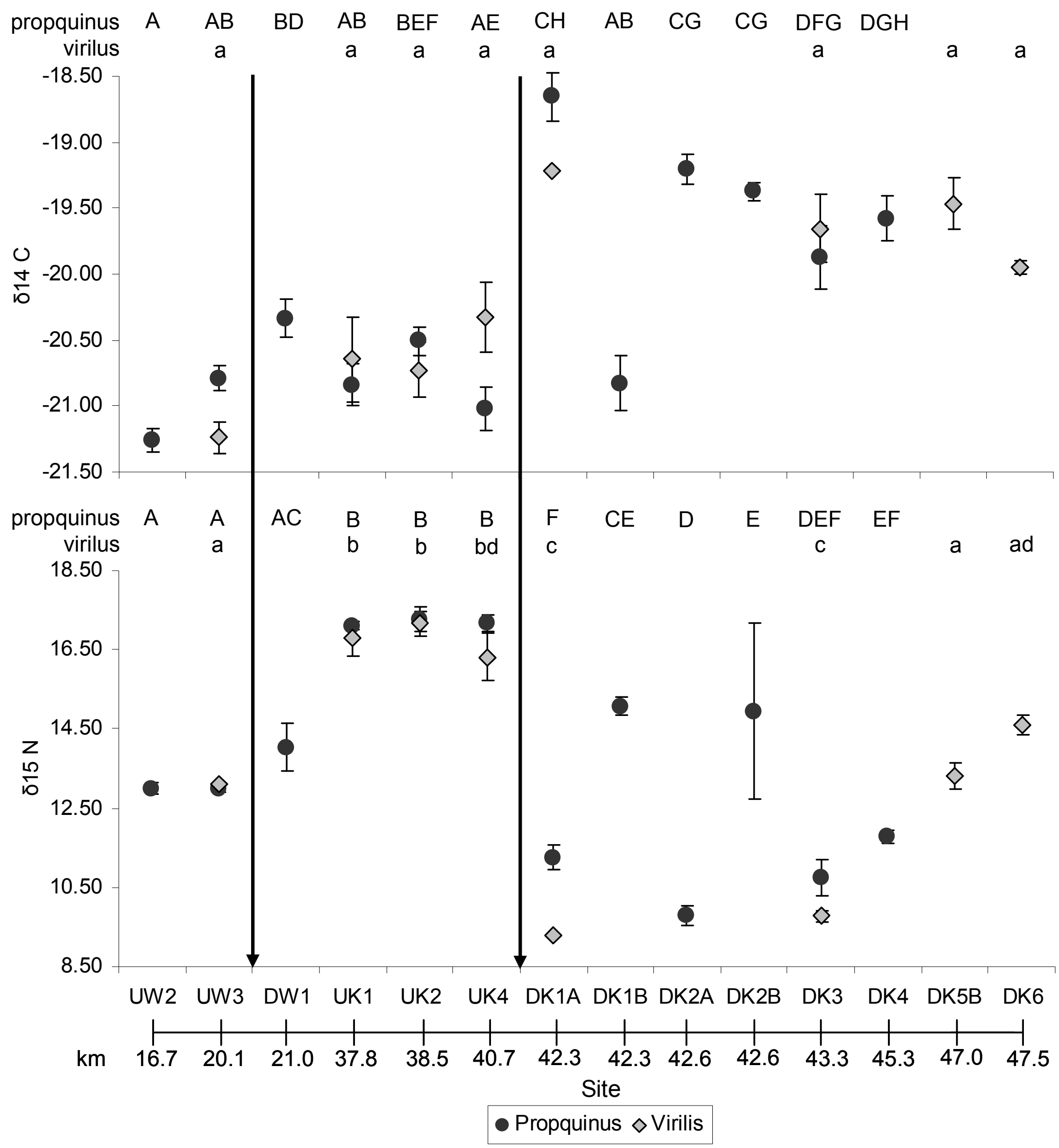

Figure 14: Stable isotope signatures (δ15N and δ13C, mean ± SE) for *Orconectes propquinus* (n = 2 to 6/site) and *O. virilis* (n = 1 to 4/site) collected in September on the Grand River, 2008. Arrows indicate the Waterloo and Kitchener MWWE outfalls. Different letters indicate significant site differences by ANOVA with Tukey, except for nitrogen *O. propquinus*, which is Kruskal-Wallis with Mann-Whitney-U test. Sites A are on the east/effluent side of the river, while sites B are on the opposite bank.