# The City and the Stream: Impacts of Municipal Wastewater Effluent on the Riffle Food Web in the Speed River, Ontario

by

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# 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.

I understand that my thesis may be made electronically available to the public.

# Abstract

Fast paced population growth in urban areas of southern Ontario is putting increased pressure on the surrounding aquatic environment. The City of Guelph uses the Speed River to assimilate its municipal wastewater effluent. With a projected 57% population increase in the watershed by 2031, the assimilative capacity of the river may be challenged in the coming years. The Guelph Wastewater Treatment Plant uses tertiary treatment methods greatly reducing ammonia, suspended solids and phosphate concentrations in the effluent. However there are still impacts detectable related to excessive nutrients released into this relatively small river ($6^{th}$ order) which promotes algae and aquatic macrophyte growth. There is also concern about a variety of emerging contaminants that may enter the river and impact the health of the ecosystem. The research in this thesis examined the seasonal and spatial variability and extent of the impacts of the wastewater effluent on the riffle fish communities in the Speed River. Stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) were used to understand the changes in the dominant benthic fish species, Rainbow Darters (*Etheostoma caeruleum*) and Greenside Darters (*E. blennioides*), relative to changes in invertebrate signatures and their abundance. Rainbow Darters were extremely abundant relative to Greenside Darters at the site immediately downstream of the effluent outfall, particularly in August. The benthic invertebrate community was distinctly different downstream of the effluent outfall, especially in the summer, with a reduced abundance of Elmidae beetle larvae and increased abundance of isopods (*Caecidotea intermedius*) compared to upstream. $\delta^{13}C$ and $\delta^{15}N$ of the two darters species were similar at all sites in May and July, but in August and October Rainbow Darter signatures were more enriched in the two heavier isotopes at sites downstream of the effluent outfall. The vast majority of invertebrate taxa sampled were also enriched at the downstream sites. An analysis of Rainbow and Greenside Darter stomach contents revealed that Rainbow Darters incorporated more isopods and other invertebrates in their diet, especially at the immediate downstream sites suggesting that they are more adaptable to the altered downstream environment. The feeding habits of Greenside Darters appear to change between July and August in response to changes in habitat and food availability. They are potentially consuming food organisms with less enriched isotopic signatures, which results in their isotopic signatures not rising during these months like most of the invertebrates and other fish. Alternatively, the Greenside Darters may move across the stream to feed on invertebrates that remain unexposed to the wastewater effluent. These impacts, although subtle, may be a reflection of the Speed River ecosystem being compromised by nutrient inputs from the wastewater effluent. With the impending increase in demand on the treatment plant (e.g., population growth), ongoing treatment and infrastructure improvements may be needed in the future to maintain the current ecosystem structure.

# Acknowledgements

This degree has been an extended ride that has somehow pushed me more than I thought it would. It's easy to be done at the end and think that nothing has changed other than the passing of time, but if I actually think about it, I've grown a lot as a person and as an academic. I've had the chance to meet a lot of great people that I would never have met if I had not chosen the grad school route.

The field of stream ecology is an area that is completely open to exploring and learning outside of school. By completing this degree, I believe that I have established a lifelong interest in the field of ecology. A quote that I once read, and now misquote, "Enjoyment comes from the understanding of what we see" sticks in my mind as being a very relevant statement on enjoying the world around us. I also believe that in order to create real and long-term environmental change, it is necessary for the majority of the population to understand and appreciate the organisms that live around us. I hope that my graduate work has, in some small way, contributed to promoting this concept. For these personal revelations, I have many people to thank.

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I hope you all enjoy the story of the city and the stream.

## Dedication

I dedicate this work to Mom, Dad, Ben and Jamie. This is my family.

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# Chapter 1:
# Introduction

The fast growing population of southern Ontario is placing many demands on water resources and current municipal infrastructure. The area just west of Toronto, surrounding the Grand River, is heavily influenced by this rapid growth. Intensive agriculture and many urban areas in the watershed rely greatly on the groundwater and surface water supplies. An issue of particular concern in the area is the unknown capacity of the river system to withstand increasing inputs of contaminants and nutrients from municipal wastewater effluents (MWWEs) and agricultural runoff. For this reason, municipalities contained within the watershed will face increased pressure to protect and conserve their water resources in the coming years. The projected population growth for the Grand River watershed is a 57% increase between 2001 and 2031, with the majority of the growth occurring in the five major urban centres, Kitchener, Waterloo, Cambridge, Guelph and Brantford (Grand River Conservation Authority 2005). The City of Guelph is built around the Speed River, a small 6$^{th}$ order tributary of the Grand River, which is used to assimilate their treated effluent. River discharge at the effluent outfall can sometimes reach a flow as low as 1.5 $m^3/s$ in the summer months (Grand River Conservation Authority, personal communication). These circumstances have caused the City of Guelph to invest large amounts of money in a long-term management plan, which includes continuous wastewater treatment plant (WWTP) upgrades to maintain a high effluent quality (CH2M Hill 2009). Beyond the conventional secondary biological treatment (activated sludge), the plant employs tertiary treatment, involving nitrifying biological contactors and sand filtration to reduce ammonia loads, suspended solids and particulate organic matter loads in the final effluent (City of Guelph 2007). A dam built upstream of the city in 1976 also helps to mitigate low flows during the dry summer months, improving the diluting power of the river. Although the state of the river is much improved since the 1970's when dissolved oxygen (DO) levels would sometimes drop to near zero (Gowda 1983), the role of assimilating increasing loads of sewage nutrients is an ongoing challenge for such a small river ecosystem.

Recent studies have shown that the two most abundant riffle-dwelling fish in this portion of the Speed River are the Rainbow Darter (*Etheostoma caeruleum*) and the Greenside Darter (*Etheostoma blennioides*) (Brown et al. 2011). Rainbow Darters are common in streams and rivers of southern Ontario while the Greenside Darter's range has only recently spread its range in southern Ontario and the Grand River (Bunt et al. 1998). Recent work by Brown et al. (2011) has shown a variety of changes in the relative abundances and condition of these fish downstream of the Guelph MWWE outfall. In addition, changes in the isotopic signatures ($\delta^{13}C$, $\delta^{15}N$) suggest a change in the nutrient cycling and diet of darters downstream of the outfall (Brown et al. 2011; Loomer 2008). Recent work has also reported changes in gene expression (in the endocrine system, metabolism, and stress response) in Rainbow Trout (*Onchorynchus mykiss*) exposed to this municipal wastewater outfall (Ings et al. 2011b). Exposure of Rainbow Trout to this effluent also caused a stress response and reduced the ability of fish to respond to secondary stressors (Ings et al. 2011a). A variety of PPCPs have been found in both the water and fish of the Speed River near the wastewater outfall (Wang 2010). Nutrient loads to this relatively small receiving environment also have the ability to alter the physical environment with heavy algae and macrophyte growth observed in the summer months.

## Effects of Sewage Effluent on Aquatic Ecosystems

Raw wastewater contains a mixture of residential wastewater, industrial wastewater, and storm water, which may contain a large variety of compounds. One of the major threats of sewage effluent to fish and aquatic life is eutrophication, which causes a reduction in DO and alters habitat. Often caused by high concentrations of nitrogen and phosphorus, eutrophication causes an increase in plant and algal growth (Environment Canada 2001) which eventually die and become consumed by bacteria which in turn use the oxygen in the aerobic respiration process (Chambers et al. 1997). Ammonia, chlorine and other chemicals in effluent have been known to cause acute toxicity effects in fish (Tsai 1975). Endocrine disrupting substances pose newer and more subtle threats that have the potential to cause changes to reproductive function in exposed organisms (Kidd et al. 2007). Low concentrations of pharmaceuticals and personal care products (PPCPs) have also been detected in wastewaters (Wang 2010), and can cause impacts on reproduction and development (Daughton and Ternes 1999). These chemicals might not produce acute responses, like those of low DO, ammonia or chlorine (compounds/conditions that fish may be able to avoid), so detecting the effects of these contaminants is a scientific challenge.

A typical cascade of effects occurs in the ecology of rivers exposed to the organic enrichment from sewage effluent (Hynes 1960). During the early days of wastewater treatment, it was observed that rivers receiving untreated wastewater experience distinct zones of pollution response (Tsai 1975). The breadth and extent of the downstream impacts depend on the type of ecosystem, but also on the combined discharges of the effluent and river, the amount of dilution, the amount of aeration at the outfall, and the quality of the effluent itself (Hynes 1960). Rivers receiving untreated sewage would often experience "sewage fungus", low DO, increased suspended solids, and an abundance of extremely tolerant benthic invertebrates such as Oligochaeta immediately downstream of the outfall (Hynes 1960). In the case of the

Guelph WWTP, the combined discharge and amount of natural aeration are low, but the high effluent quality should reduce the length of the impact zone. Since the majority of ammonia is removed from the effluent, the downstream effects should be drastically lessened and the severity of impact should be comparable to that seen far downstream of a raw sewage outfall as described in Hynes (1960). When oxygen depletion is not a factor, the increased nutrient load has the potential to cause an increase in production, as the effluent contains food for many organisms. In Europe, *Asellus sp.* (a genus of Isopoda) was shown to be a beneficiary of nutrients, as they were a common species in the oxygen recovery zone (Hynes 1960).

Recent studies on the impacts of wastewater in Canadian waters have shown effects in fish from nutrient and other contaminant input (Chambers et al. 1997). Wastewater effluent has been shown to alter condition factors, liver and gonad sizes (Brown et al. 2011), as well as inhibiting the production of sex steroids, particularly in male fish (Jeffries et al. 2008). Male Rainbow Darters and Greenside Darters in the Grand River were shown to have incidents of intersex (primary oocytes in the testes) downstream of major urban areas in the main branch of the watershed (Tetreault et al. 2011).

In 2009, the Canadian Council of Ministers of the Environment (CCME) developed a strategy to manage wastewater quality and to ensure standard guidelines for effluent quality across the country (Hovland 2009). New regulations proposed by the federal government for wastewater quality, aim to accomplish goals of the CCME (Government of Canada 2010), and are intended to initiate cleaner effluent from all discharges in Canada. The country's wastewater management strategy will be similar to those of other regulated effluents (e.g. pulp and paper mill effluent, mine effluent) specifying improvements to treatment processes and the policies that govern them to ensure human health and environmental protection.

## Fish Communities

Fish are key components in aquatic food webs. They occupy many feeding guilds, consuming primary and secondary consumers as well as primary producers. Like terrestrial food webs, the aquatic environment can contain intricate networks of trophic and spatial interactions among species. These interactions, combined with other biotic and abiotic factors, heavily influence which species of fish will be found in any particular waterbody. Impacts to the base of the food web may cause detectable changes in fish and other organisms at higher trophic levels. Based on a known regional pool of fish, the number and types of species present at a given site give insight as to the condition of the environment. This has been the rationale for many fish community studies, which have focused on lacustrine (Xi and Kitchell 1990), coastal (Azzurro et al. 2010) and riverine (Orrego et al. 2009) habitats. Rivers and streams represent a very unique subset of these aquatic environments because they are quite variable from the headwaters to the mouth (Vannote et al. 1980). Flowing waters produce areas of fast and slow, warm and cold, shallow and deep, and clear and turbid waters. As a result many habitat types are created for bacteria, algae, macrophytes, invertebrates and fish (Lobb III and Orth 1991; Cushing and Allan 2001). Competition and predation between these species will further affect which organisms will ultimately make up the riverine ecosystem. It is generally believed among ecologists that it is the combination of these biotic and abiotic factors that influence the assemblage of fish that will be found at any given site (Jackson et al. 2001). It is clear that the list of factors can be numerous, and when considering that they may work in tandem or at different times throughout the year, the study of fish assemblages becomes quite complex.

In some instances, it may appear that there is no predictable pattern as to what species one may encounter from year to year and from season to season (Strange et al. 1993; Grossman et al. 1982). This has spawned debate as to whether or not fish assemblages are even controlled deterministically or stochastically. With ongoing research, it has become more evident that there are indeed patterns and reasons for certain fish assemblages, but their causes are difficult to understand. It has been clearly shown that different habitats will harbour different fish species (Lobb III and Orth 1991), and so fish assemblages are most accurately identified and comparable to other assemblages if they are examined within one habitat guild. Factors such as temperature, dissolved oxygen (Jackson et al. 2001), discharge (Strange et al. 1993), stream size (Rahel and Hubert 1991), predation (MacRae and Jackson 2001), and food partitioning (van Snik Gray et al. 1997; Hlohowskyj and White 1983) can also play important assemblage determining roles. The practice of using fish communities, especially in streams, has been quite extensive for measuring the impacts of a pollution source or environmental change over time (Rahel and Hubert 1991; Sandström 1994; Azzurro et al. 2010). Although changes in individual fish at the molecular or physiological level are important, it is generally the effects at the population and community level that are the focus of environmental risk and protection work. Fish community studies will often compare the assemblage of fish species in two or more areas of the same waterbody, or similar waterbodies (Tonn et al. 2003; Schlosser 1982). Karr (1981) utilized different parameters of a fish assemblage to create a numerical score to determine the similarity amongst different sites, or to analyze change at a site over time or from a stressor. There are difficulties in sampling fish quantitatively, and it can be argued as to whether or not fish community assessment is a useful and reliable indicator for determining ecosystem health (Grossman et al. 1982; Yant, et al. 1984). However, comparisons done with appropriate control and design that consider natural variability (i.e. habitat, natural gradients, etc.) can provide insights into changes in fish communities associated with specific or cumulative stressors.

## Stable Isotopes

Naturally occurring stable isotopes of carbon and nitrogen are useful for tracing nutrient cycling in food webs, particularly those of aquatic environments. There are two naturally occurring isotopes of carbon and of nitrogen that are both common in living tissues of all organisms. Their quantification is measured relative to international standards and interpreted as an isotopic signature (δ) (Equation 1) (Peterson and Fry 1987).

$$\delta_{sample} = \left( {R_{sample}}/{R_{s\tan dard}} - 1 \right) \times 10^3 \qquad \text{Equation 1}$$

R= isotope ratio, either ${}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$

The isotope ratio (R) is the ratio of the heavy isotope to the light isotope found in the tissue, which is compared to the isotope ratio in an international standard. The standard used for carbon is Pee Dee Belemnite, and for nitrogen it is atmospheric $N_2$.

The alteration of isotopic signatures, called fractionation, occurs frequently in aquatic ecosystems through equilibrium and kinetic reactions (Peterson and Fry 1987). Equilibrium reactions occur when there are large quantities of a particular molecule changing from one state or form to another. In biological systems, and most aquatic chemistry, it is the kinetic reactions that control fractionation, as the reactants are not always infinitely abundant. In many of these types of reactions, the lighter isotope will go through with the reaction faster, leaving the original source more enriched with the heavier isotope. An example is the volatilization of $NH_3$ from water. ${}^{14}NH_3$ more readily volatizes into the atmosphere, so the remaining water is more enriched with ${}^{15}NH_3$. Kinetic fractionation by metabolic reactions in an organism can create different isotopic signatures between body tissues and different organisms (Peterson and Fry 1987). These reactions can produce products with depleted and enriched isotope signatures. The result is a mix of tissues with depleted and enriched signatures compared to the dietary signatures (Tieszen et al. 1983). The tissue turnover time also plays a role in determining tissue signatures. Very young fish and small fish have fast turnover rates, because large amounts of their energy are put into growth (Hesslein et al. 1993; Vander Zanden et al. 1998). This means that as these small fish grow, the isotopic signatures of their diets will be more quickly reflected in their body tissues. Fish that grow larger will have slower C and N turnover times as they age and their bodies lengthen (Maruyama et al. 2001). “Isotope routing” may also cause variation in the signature of a particular tissue. For example, if a high protein tissue uses dietary protein directly, that tissue will reflect the signature of the dietary protein more than the bulk diet (Gannes et al. 1997). This is a factor that could cause variation in $\delta^{13}C$ and $\delta^{15}N$, making it very important to choose the proper tissue to measure. Pinnegar and Polunin (1999) found the white muscle of fish to be the most reliable tissue for measuring $\delta^{13}C$ and $\delta^{15}N$, as it produces the least amount of variation.

Carbon and nitrogen based molecules moving through an aquatic food web face many potential sources of fractionation, and isotopic signatures can range widely. Fortunately, these fractionation processes create patterns which give indications as to what each organism is consuming. This information combined with other knowledge of the ecosystem such as stomach contents and abundances of fish can tell an ecological story. Nitrogen and carbon react differently in the food web and in the surrounding aquatic environment, so their signatures contain different information. Particulate and dissolved forms of $\delta^{13}C$ and $\delta^{15}N$ in sewage effluent are typically different from that of background levels in the environment, and organisms that incorporate these nutrients, usually as food, will in some way reflect these signatures (Rogers 1999; DeBruyn and Rasmussen 2002). Differential uptake and fractionation can alter the signatures of primary producers depending on the organisms and the availability of various forms of the nutrients (Loomer 2008). These changes in the isotopic signatures at the base of the food web can be strongly reflected in the higher trophic levels.

### *Nitrogen*

The nitrogen isotope signature of an organism can be indicative of its trophic level, because $\delta^{15}N$ typically increases from prey to predator (Peterson and Fry 1987). The nitrogen signatures of the tissues of carnivores have been shown to typically increase by 3.4‰ ± 1.1‰ from that of its food source (Minagawa and Wada 1984). Lower organisms that feed on organic particles or macrophytes tend to experience smaller and more variable increases in $\delta^{15}N$ from their food (Vander Zanden and Rasmussen 2001). The result however, is normally an increase in $\delta^{15}N$ as one moves up through the trophic levels of the food web.

Other factors that may influence $\delta^{15}N$ are the water temperature, and ration size. Barnes et al. (2007) and Power et al. (2003) found similar impacts on fish and *Daphnia magna* respectively, where the increasing temperature produced $\delta^{15}N$ that were more depleted. It was hypothesized that it was because of an increased investment in growth that the organisms excreted less waste $^{14}N$, thereby incorporating more of it into their body. In contrast, the effects of a reduced diet ration seemed to increase the $\delta^{15}N$ of *Daphnia sp.* (Adams and Sterner 2000). It was speculated that with a decrease in dietary nitrogen, the *Daphnia sp.* needed to use its body's nitrogen reserves, in which the process would favour the use and subsequent excretion of the lighter $^{14}N$ isotope. Vinson and Budy (2011) also found $\delta^{15}N$ to increase as fish length increased, further indicating that it is necessary to control for fish size when comparing $\delta^{15}N$.

### Carbon

$^{12}C$ and $^{13}C$ are the stable isotopes of carbon found in nature, with $^{12}C$ being the most common of the two. $^{13}C$ signatures indicate the signature of the primary source of carbon fixation. For example, plants that undergo $C_3$ and $C_4$ photosynthesis pathways have distinctly different signatures because the photosynthetic reactions fractionate atmospheric $CO_2$ very differently. $C_3$ plants have highly depleted $^{13}C$ signatures compared to atmospheric $CO_2$ (~ -28‰), while $C_4$ plants, the most common of which are grasses, have less depleted signatures (~ -13‰) (Peterson and Fry 1987). The fractionation of consumed carbon in organisms from one trophic level to the next is variable. Several studies have found a slight average enrichment in $^{13}C$ of less than one part per thousand (Vander Zanden and Rasmussen 2001; DeNiro and Epstein 1978). This small amount of fractionation means that an organism, regardless of its trophic level, will have a $\delta^{13}C$ similar to that of the source of primary production of its diet (DeNiro and Epstein 1978). Besides varying from tissue to tissue, $^{13}C$ signatures vary between molecules. Metabolic reactions produce lipids with $\delta^{13}C$ that are more depleted than other macromolecules (Post et al. 2007; DeNiro and Epstein 1977). As well, lipids contain a large amount of carbon atoms, so the % carbon to % nitrogen ratio (C:N) is indicative of the amount of lipid in a tissue.

In aquatic systems, a large source of carbon fractionation occurs at the base of the food web in the carbon fixation process of algae. Algal carbon uses dissolved $CO_2$ for photosynthesis, therefore the $\delta^{13}C$ of the $CO_2$ source along with the biomass, photosynthetic rate and water velocity can all impact the $\delta^{13}C$ of the algae (Finlay 2004). A greater biomass of algae acts by producing a high demand for $CO_2$ and causing the resulting fixed carbon to become more enriched in $^{13}C$ (Finlay et al. 2002). Dense algae populations will consume high amounts of $CO_2$, and as a result leave less of a $CO_2$ source to draw from for the remaining algal cells. Similarly, when in the presence of intense direct sunlight, the rate of algal photosynthesis will increase and enrich the resulting $\delta^{13}C$ of the algae (MacLeod and Barton 1998). This happens because the faster photosynthetic rate increases the amount of $CO_2$ use, and thus reduces the amount of fractionation during diffusion of $CO_2$ into the algal cells. Water velocity impacts algae $\delta^{13}C$ by controlling the speed at which dissolved $CO_2$ becomes available (Finlay 2001). In slower moving waters primary producer $\delta^{13}C$ become enriched, because the available $CO_2$ is not replaced very quickly, and the algae are forced to use the heavier $^{13}CO_2$ as molecules of $^{12}CO_2$ become less available. Inversely, $\delta^{13}C$ of algae in faster flowing waters are more depleted, because there is a greater supply of dissolved $CO_2$ to draw from.

Fractionation by primary and secondary consumers can also become impacted by environmental factors. Increased water temperatures, for example, tend to enrich the $\delta^{13}C$ of fish and *Daphnia magna* (Barnes et al. 2007; Power et al. 2003). It is expected that fish in warmer waters require less fat storage, and since lipids are more $^{13}C$ depleted this becomes reflected in enriched $\delta^{13}C$ compared to signatures at cooler temperatures. In *Daphnia* it was thought that greater respiration of depleted $CO_2$ associated with less food intake at higher temperatures, produced higher body $\delta^{13}C$. These two mechanisms may work in tandem, and likely resulted in increased $\delta^{13}C$ seen in fish (Barnes et al. 2007).

It is difficult to conclusively link an organism to a specific carbon source, because of the complex processes that can impact $\delta^{13}C$. At this time, it requires large $\delta^{13}C$ differences between possible sources to be able to determine any significant relationships (DeNiro and Epstein 1978; Finlay 2001). Fortunately, a combination of different food sources may combine to produce unique $\delta^{13}C$ among fish and invertebrates analyzed.

## Objectives

The rapid growth in the City of Guelph is putting an increasing amount of pressure on the aquatic environment to assimilate the treated wastewater effluent. Previous research in the Speed and Grand Rivers has found potential impacts caused by endocrine disrupting chemicals such as PPCPs (Tetreault et al. 2011; Ings et al. 2011a). $^{13}C$ and $^{15}N$ isotopic signatures in benthic fish and invertebrates were shown to reflect inputs of nutrients from MWWEs and other non-point sources (Brown et al. 2011; Loomer 2008). Brown et al. (2011) found impacts at the population level in the Speed River, where Rainbow Darters were more abundant at effluent exposed sites than Greenside Darters. Isotopic evidence suggested that effluent altered the diets of Rainbow Darters to give them a competitive advantage over Greenside Darters in the late summer. However, the seasonality of this occurrence and the relationship between isotopic signatures and the actual fish diets were not investigated.

The objectives of this research were to determine: (1) the seasonal and spatial changes to the species composition and abundances of riffle dwelling fish and benthic invertebrates exposed to tertiary treated wastewater effluent in the Speed River downstream of the City of Guelph WWTP; and (2) how stable isotopes ($\delta^{13}C$, $\delta^{15}N$) in Rainbow Darters and Greenside Darters vary in relationship to the effluent outfall, season and diet.

# Chapter 2:
# Spatial and temporal variation in fish and benthic invertebrates compositions in a mid-order stream food web impacted by a tertiary treated municipal wastewater effluent

The following people contributed to the results in this chapter:

C.S. Robinson was the principal investigator who designed the study, performed the collections, analysis and writing of the manuscript.

M.R. Servos served as supervisor of the work.

M.E. McMaster acted as an advisor on various aspects of the fish collections.

D.R. Barton contributed to the sampling and identification of invertebrates.

All authors helped in the preparation and review of the text.

# Introduction

The challenges of population growth are an issue that many municipalities in southern Ontario face. This area in particular is experiencing a large amount of urban growth, increasing the amount of residential and industrial wastewater being treated and released into the environment. The City of Guelph, west of Toronto, is a mid-sized Canadian city that is expected to have a population increase of nearly 65% between 2001 and 2031 (Grand River Conservation Authority 2005). With a current average of 450 L of wastewater produced per person per day, this growth will put increasing pressure on the Speed River especially during low flows of only 1.5 $m^3$/s in the dry summer months (Grand River Conservation Authority, personal communication). Historically, the Speed River downstream of Guelph has had very poor water quality, suffering from low minimum DO levels brought on by the high inputs of nutrients from the city's effluent. The river suffered from extreme eutrophication and low DO concentrations until 1976, when an upstream reservoir was installed to maintain river flows in the summer months, and a series of WWTP upgrades in the subsequent years lowered the nutrient load to the river (Grand River Conservation Authority 2008). The nitrogenous oxygen demand (NOD), from nitrifying bacteria converting the ammonia to nitrates, combined with bacterial and macrophyte respiration consumed the majority of DO in the water. Minimum DO levels of 1.5 mg/L were recorded six kilometres downstream of the outfall in September 1976 (Gowda 1983). The multiple WWTP improvements, including an upgrade from secondary treatment to tertiary treatment, played a major role in increasing the minimum DO levels above the target of 4 mg/L (Cooke 2006).

Largely because of the small receiving environment and historical issues, the City of Guelph now maintains high water quality standards for their effluent. The Guelph Wastewater Treatment Plant (WWTP) currently uses activated sludge and tertiary treatment processes, including sand filtration technology, on the raw wastewater before releasing the treated water into the river. Excess nutrients in a freshwater system, particularly phosphate, will cause eutrophication (Environment Canada 2001; Graham and Wilcox 2000) leading to increased plant and algal growth, changes to the physical habitat, and cause potential fluctuations in dissolved oxygen (DO). More recently, concerns have also been raised for the diversity of trace contaminants found in wastewater that may alter endocrine function and impact growth and reproduction in fish and other organisms (Kidd et al. 2007).

Brown et al. (2011) found the relative abundances of two particularly common riffle-dwelling fish, the Rainbow Darter (*Etheostoma caeruleum*) and the Greenside Darter (*Etheostoma blennioides*), were impacted immediately downstream of the Guelph WWTP. Rainbow Darters were found to be significantly more abundant immediately downstream of the effluent outfall, while Greenside Darters were less abundant. The altered relative abundance was hypothesized to be a response to altered food availability and utilization downstream of the effluent. However, these earlier studies looked at two species of darters, used a limited number of sites and focused only on the late summer period. The objective of the current study was to determine the seasonal and spatial changes to the species composition and abundances of riffle dwelling fish and benthic invertebrates exposed to tertiary wastewater effluent in the Speed River downstream of the City of Guelph WWTP. The potential for change in the river with increased development and the cost of infrastructure upgrades makes it important to understand the scope of the current impacts in the river.

# Methods

## Study Area

The Speed River is a major tributary of the Grand River, with roughly 50% of the land in the Speed River watershed used for agriculture and the other half consisting mainly of forest and urban cover (Grand River Conservation Authority 2005). Although the municipal wastewater effluent from the City of Guelph contributes heavily to the nutrient load in the lower portion of the river, the upper portion maintains relatively high water quality (Cooke 2006). The City of Guelph WWTP serves over 115,000 people, treating 55,896 $m^3$/day of residential and industrial water, with the capacity to treat 64,000 $m^3$/day (CH2M Hill 2009). The plant operates using tertiary treatment techniques with activated sludge aerobic digestion and chlorine disinfection. Soluble phosphorus is removed during secondary treatment using the flocculant ferrous chloride to produce a removable phosphorus precipitate. The tertiary treatment process uses Rotating Biological Contactors and sand filtration. The contactors contain nitrifying bacteria to convert the remaining ammonia to nitrite and nitrate. Sand filtration, with silica sand and anthracite, further removes the remaining suspended solids which helps to reduce the biochemical oxygen demand of the effluent (City of Guelph 2007). The final effluent still contains high levels of nitrate and phosphorus, and low levels of ammonia (Table 1). Chloride levels, from road salt, water softening salts and wastewater disinfection, are elevated compared to upstream levels (Cooke 2006).

At the outfall, the Speed is a relatively small $6^{th}$ order stream using the Strahler method, but using the Shreve method, it is a $549^{th}$ order stream (indicating that it consists of 549 first-order tributaries) (Grand River Conservation Authority 2011). Upstream and downstream of the city, the river follows a typical riffle-run-pool low gradient path, however, within the city the channel has been modified, and small weirs and dams interrupt the flow to create areas of slower deeper water, which tend to promote an increase in water temperatures as well as algae and bacteria growth.

Sampling was conducted at 14 sites (Figure 1; Table 2) during the four sampling periods of May 15-22, July 8-15, August 20-September 3, and October 26-November 11 2009. Not all sites were sampled at all times because circumstances in the field did not always allow it. Sites were wadeable reaches of shoreline riffle/run habitat 100 m long and 10 m wide, containing gravel, cobble, and boulder substrates. The sites were selected for their depth, ease of sampling, and their similarity to one another in an attempt to minimize variation in fish habitat (Brown 2010). Four sites were selected upstream of the effluent outfall and were used as reference sites. Six sites were selected downstream of the outfall on the west side of the river to show a gradient of effects going to ~10 km downstream. Three other sites were downstream of the outfall, but were on the east side of the river opposite to where the effluent is released.

Table 1: Summary of average monthly water quality parameters of the final effluent from the City of Guelph WWTP, during the period of May to November 2009. The average, minimum, and maximum values were calculated from monthly averages for each parameter from May to November. BOD – biological oxygen demand, TSS – total suspended solids, TKN – total Kjehldahl nitrogen. Data was collected and provided by the City of Guelph WWTP.

| | Monthly Averages | | |
|---|---|---|---|
| **Parameter** | **Avg.** | **Min.** | **Max.** |
| **Alkalinity (mgCaCO3/L)** | 216.60 | 193.15 | 236.04 |
| **Ammonia (mg/L)** | 0.99 | 0.14 | 4.88 |
| **Nitrate (mg/L)** | 22.39 | 20.11 | 27.53 |
| **Nitrite (mg/L)** | - | 0 | 1.4 |
| **TKN (mg/L)** | 1.93 | 1.07 | 6.02 |
| **pH** | 7.87 | 7.70 | 7.96 |
| **BOD (mg/L)** | 29.33 | 25.75 | 31.04 |
| **Total Phosphorus (mg/L)** | 0.12 | 0.10 | 0.14 |
| **TSS (mg/L)** | - | <1 | 7 |
| **E. Coli (CFU/100mL)** | - | <10 | 710 |
| **Discharge (m3/s)** | 4.4 | 2.1 | 10.4 |

### Site Characterization

Measurements of conductivity and temperature were taken 5 m from each shore along the river between sites U3 and D4 on November 4, 2009 to characterize the effluent plume. Stowaway Tidbit temperature loggers (Hosking Scientific, Burlington, ON) recorded hourly water temperatures at sites U2,

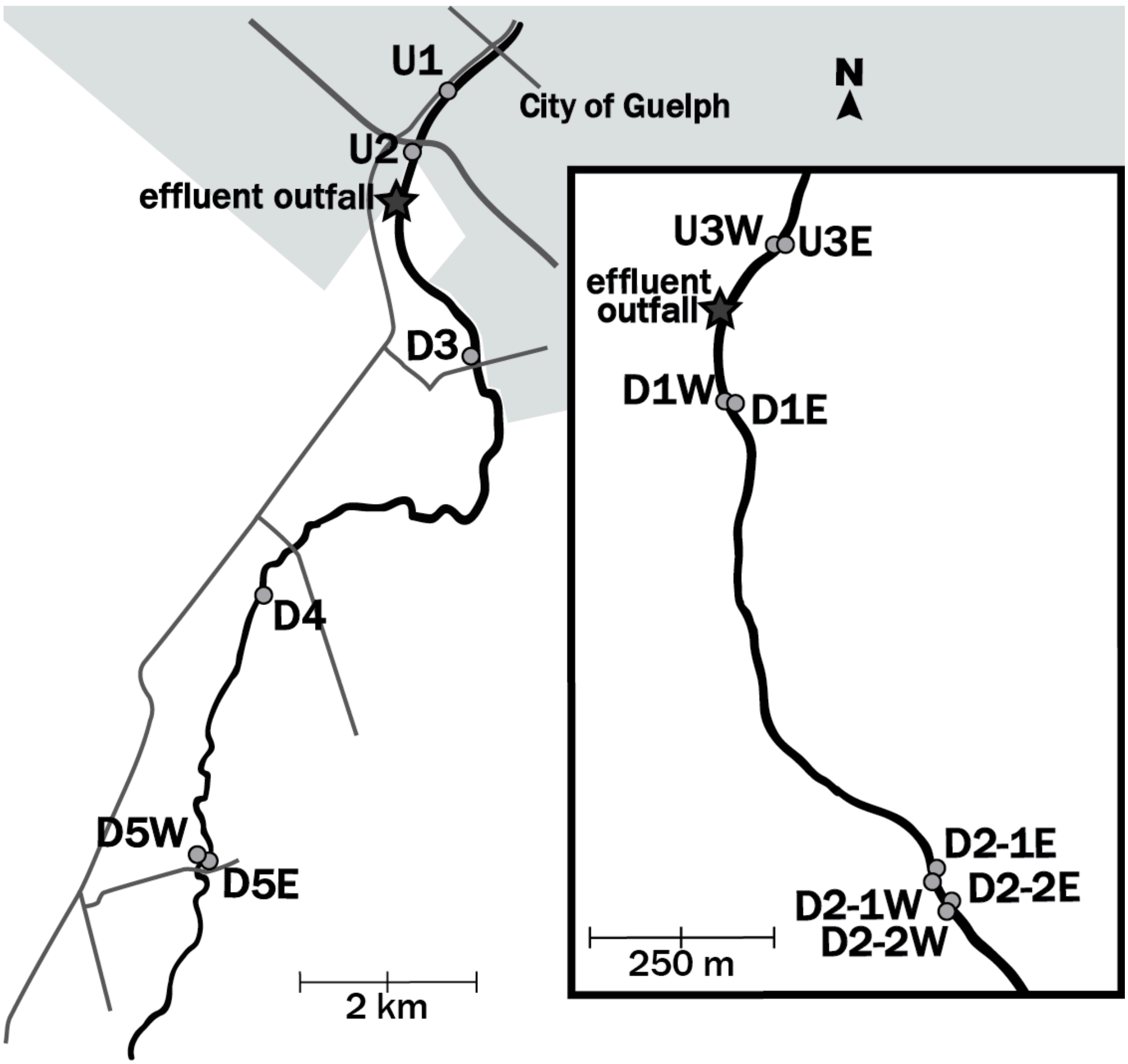


Figure 1: Map of Speed River sampling sites used for fish and invertebrate collections.

Table 2: Sampling sites on the Speed River. The effluent outfall is on the west side of the river. "F" indicates that fish were collected and "B" indicates that benthic invertebrates were collected.

| | Co-ordinates | | Fish Sampling Periods | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Site** | **N** | **W** | **May** | **July** | **August** | **October** | **Side of River** | **Km DS of outfall** |
| **U1** | 43.31'51.06" | 80.15'25.88" | F | F | F/B | | west | -1.22 |
| **U2** | 43.31'31.89" | 80.15'41.09" | F | F/B | F/B | F/B | west | -0.53 |
| **U3W** | 43.31'20.74" | 80.15'47.78" | F | F/B | F/B | F/B | west | -0.11 |
| **U3E** | 43.31'20.86" | 80.15'47.06" | | F | F/B | F | east | -0.12 |
| **D1W** | 43.31'12.70" | 80.15'51.17" | F | F/B | F/B | F/B | west | 0.13 |
| **D1E** | 43.31'13.00" | 80.15'50.73" | | | F/B | F | east | 0.12 |
| **D2-1E** | 43.30'51.42" | 80.15'39.86" | F | F | F/B | F | east | 0.95 |
| **D2-1W** | 43.30'47.84" | 80.15'38.72" | | F | F/B | F | west | 1.07 |
| **D2-2E** | 43.30'47.96" | 80.15'37.99" | | | F | | east | 1.08 |
| **D2-2W** | 43.30'45.67" | 80.15'36.36" | | | F | | west | 1.16 |
| **D3** | 43.30'08.95" | 80.15'14.93" | F | F | F/B | F | west | 2.43 |
| **D4** | 43.28'47.17" | 80.17'11.97" | | | F/B | F | east | 7.51 |
| **D5E** | 43.27'07.43" | 80.17'52.66" | | | F | | east | 10.92 |
| **D5W** | 43.27'06.27" | 80.17'51.06" | | | F | F | west | 10.92 |

U3W, D1E, D1W, D3 (east shore), D3 (west shore), and D4 from June 24 to December 7, 2009. As well, depth, flow (Stanfield 2007), and qualitative observations of algae and macrophyte growth were recorded at each site during the fish community sampling in July, August and October.

### Fish Community

Each site was divided into ten sub-sites measuring 10m by 10m, and six of these were chosen randomly to represent the site. Each sub-site was sampled in a zig-zag pattern covering the whole sub-site in 300 seconds, using a backpack electroshocker (Smith-Root Model 12, LR-24, Smith-Root Inc., Vancouver, WA). Due to wastewater effluent exposure, the sites varied in their conductivity, and the electroshocker settings (voltage and pulse) were adjusted at each site to maintain a consistent “stunning” effect on fish. Using a netter on each side of the electroshocker, as many fish as possible of all species were captured. It was determined that the time of day had little effect on the total number of fish caught (Brown 2010; Appendix A), so fish collections were typically performed between 7:00 and 11:00 am, usually beginning just after daybreak. All fish were identified to species, and measured for length (±1 mm) and weight (± 0.01g). All fish were handled according to protocols approved by the University of Waterloo Animal Care Committee (University of Waterloo AUP # 04-24 and 08-08).

### Benthic Invertebrate Community

The benthic invertebrate community was sampled at all sites during August and at two upstream sites and the immediate downstream site in July and October, using a modified version of the Canadian Aquatic Biomonitoring Network (CABIN) protocol. One five minute traveling kick and sweep collection was made at the centre of each site using a standard 500 μm mesh D-net. The area sampled covered the nearshore and mid-stream habitats. It began at the shore and the collector progressed perpendicularly toward the centre of the stream kicking to a depth of ~5 cm beneath the substrate surface (Reynoldson et al. 1999). Samples were preserved in 70% ethanol until processing. Sub-sampling was performed by weighing out small portions of the entire sample, and picking out invertebrates until at least 300 cumulative invertebrates were collected (at which point the remaining invertebrates were picked from the last sampled portion) (Sebastien et al. 1988). Invertebrates were identified to the family level using Mackie (2005). Isopoda, Gastropoda, Bivalvia, Megaloptera, Neuroptera, Plecoptera, Hydracarina, Hemiptera, Zygoptera, Anisoptera, and Lepidoptera were not identified any further than either class or order because these taxa were not common enough to cause differences in benthic invertebrate community interpretation.

### Statistical Analysis

Species richness, Shannon's diversity index, Evenness and Simpson's Index (Krebs 1972) were used to compare the diversity of species at each site. Differences in Rainbow Darter and Greenside Darter abundances between sites were tested using a Kruskal-Wallis and a Mann-Whitney U post-hoc test, as the data between sites failed the homogeneity of variance test. A Mann-Whitney U test was also used to compare the abundances of Rainbow Darters and Greenside Darters within each site. An alpha (α) value of 0.05 was used to determine significance. IBM SPSS Statistics 19.0 was used for all statistical analyses.

The Bray-Curtis index of dissimilarity (Equation 3) was used to compare the benthic invertebrate communities at sites sampled in August 2009. The taxonomical categories used were: Ephemeroptera, Trichoptera, Chironomidae, Coleoptera, Isopoda, Amphipoda, Mollusca and Other. Each taxon was expressed as a percentage of total invertebrates from that site. To compare downstream sites to upstream conditions, the percent compositions of each taxon were averaged amongst the reference sites (U1, U2, U3E and U3W), and these values were compared to those of each downstream site. This generated a dissimilarity value for each downstream site compared to the upstream mean. A mean Bray-Curtis dissimilarity value was calculated for the three upstream reference sites, by averaging the three dissimilarity values calculated between each pair of reference sites. If the downstream dissimilarity values fell outside of two standard deviations of the mean reference value, then the site was deemed significantly different from upstream. Unless otherwise stated, an alpha (α) value of 0.05 was used to test for significance.

$$BC = \frac{\sum_{k=1}^{n} \left| x_k - y_k \right|}{\sum_{k=1}^{n} \left( x_k + y_k \right)} \qquad \text{Equation 3}$$

## Results

### Site Characterization

The effluent is released on the west side of the river, and this side remains much more heavily exposed until the two sides of the river completely mix about six kilometers downstream (Figure 2). The temperature of the effluent impacts the temperature of the river immediately downstream, by keeping the water slightly cooler on the very warm days and warmer on the cooler days (Figure 3). Diel fluctuations in temperature at the exposed site were more stable in the warm months and less stable in the cooler months

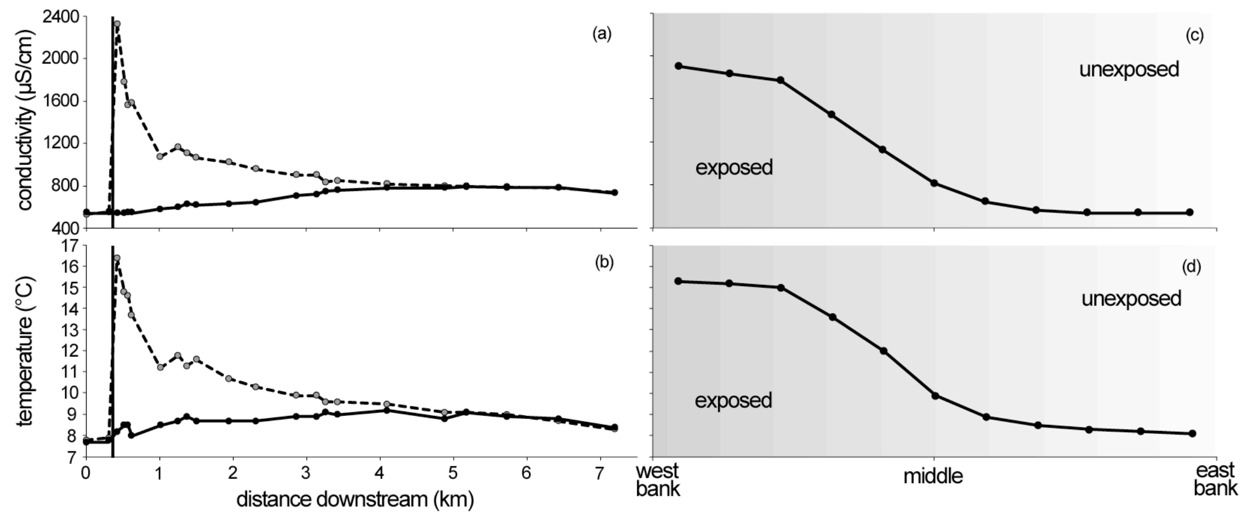


Figure 2: Conductivity (a) and temperature (b) measured five metres off of the west bank (dashed line) and the east banks (solid line). Conductivity (c) and temperature (d) measured on a transect from the west bank to the east bank of the river immediately downstream of the effluent outfall at site D1W.

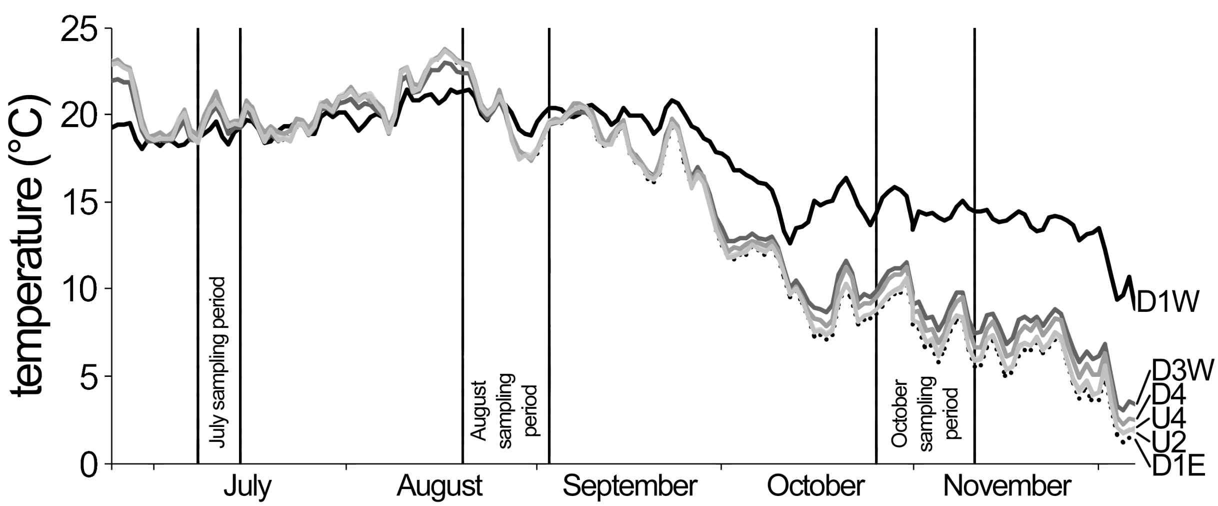


Figure 3: Average daily temperatures of selected sites in the Speed River 2009. Site D1W is immediately downstream of the effluent outfall.

than compared to the opposite side of the river (Figure 4). Some sites in the warmer months, particularly those exposed to the effluent, experienced macrophyte growth which altered the physical habitat (Table 3). The immediate downstream site (D1W), had heavy aquatic moss (Bryophyta) growth in both July and August. Thick filamentous *Cladophora sp.* also lined the water along the shore in July.

### Fish Community

Darters (Percidae) were the most abundant fish species at all sites with the exception of sites U1 in August and D2-1E and D4 in October (Table 4). Greenside Darters and Rainbow Darters were the most prominent darter species at all sites and at all times. In October, Percidae still dominated, but Catostomidae and Cyprinidae were more abundant at all sites than they had been in previous months. Species richness was the highest in August, and it was the most similar among sites in October (Table 4). Simpson's Index was above 0.5 (indicating any two randomly chosen fish have a 50% chance of being the same species) at site D1W in July and August (0.8 and 0.6), indicating the prevalence of only a few different species. Site U2 in May and July, site D2-1E in May, and site D2-2W in August also had Simpson's Index scores higher than 0.5.

At site D1W in August, catch-per-unit-effort (CPUE, measured as fish/300 shocking seconds) was 71.8 ± 11.8 for Rainbow Darters and only 4.7 ± 1.3 for Greenside Darters (Table 5). The largest abundances of total fish were also found in August (Figure 5). The CPUE for total fish (all species) peaked at 97.2 ± 16.2 immediately downstream (D1W), and then decreased until 1.16 km downstream (D2-2W). Below site D2-2W, the total number of fish increased until peaking 10.9 km downstream (D5E). In October, the total numbers of fish among sites were similar except for the furthest downstream sites (D4 and 5E), which increased similarly to August. At site D1W, there the CPUE for Rainbow Darters was 17.3 ± 4.5 and 1.5 ± 0.6 for Greenside Darters. The ratio of abundances between the two species (RD:GD) at site D1W was 15.2, 15.4 and 11.6 in July, August and October respectively, which were higher than any other sites except for U1 in July (16.0) (Figure 6). These numbers essentially indicate the number of Rainbow Darters for every one Greenside Darter.

At all sampling times, the immediate downstream site (D1W) had significantly more Rainbow Darters than Greenside Darters, although relative abundances of darters within sites showed many differences during the various sampling periods (Figure 7). In May, Rainbow Darters were more abundant than Greenside Darters at all sites except at D2-1E. Rainbow Darters were more abundant than Greenside Darters in July at sites U1, U2, U3W and D1W. Site D2-1E was heavily dominated by Greenside Darters, and the remaining sites contained similar amounts of both species. In August, Rainbow Darters were more

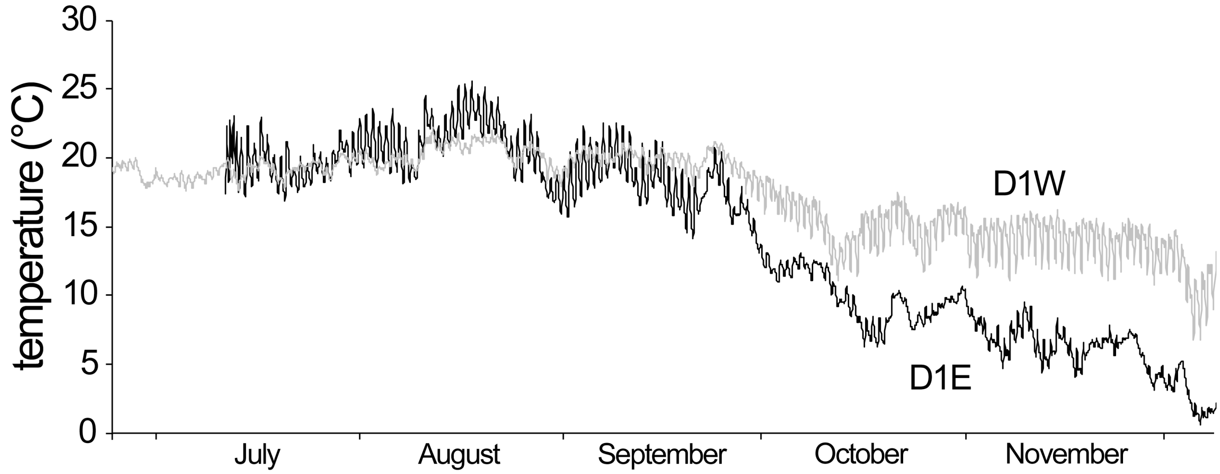


Figure 4: Average hourly temperatures at sites D1W and D1E in 2009. Both sites are immediately downstream of the effluent outfall, D1W is on the west bank completely exposed to the effluent, and D1E is on the opposite east bank.

Table 3: Average depth and flow (± SE) and habitat variables at sampling sites in July, August and October. The presence of aquatic moss (Bryophyta), filamentous algae (mainly *Cladophora sp.*) and other macrophytes (numerous species) were measured as the number of sub-sites out of ten that contained an obvious amount of growth. Dashes indicate no presence (0/10).

| | | | | Presence (# sub-sites/10) | | |
|---|---|---|---|---|---|---|
| | **Site** | **Avg. Depth (cm)** | **Avg. Flow (m/s)** | **Moss** | **Fil. Algae** | **Macro.** |
| **July** | **U1** | 29.4 ± 1.6 | 0.35 ± 0.01 | - | - | 4 |
| | **U2** | 36.8 ± 2.8 | 0.51 ± 0.06 | - | - | - |
| | **U3W** | 31.2 ± 6.2 | 0.34 ± 0.01 | - | - | - |
| | **D1W** | 47.3 ± 2.7 | 0.52 ± 0.09 | - | 10 | - |
| | **D2-1E** | 42.9 ± 4.8 | 0.43 ± 0.07 | - | - | - |
| | **D2-1W** | 37.9 ± 1.5 | 0.45 ± 0.04 | - | 10 | - |
| | **D3** | 36.0 ± 2.0 | 0.33 ± 0.02 | - | - | - |
| **August** | **U1** | 34.6 ± 1.2 | 0.34 ± 0.02 | - | - | 10 |
| | **U2** | 31.9 ± 1.9 | 0.43 ± 0.05 | - | 2 | - |
| | **U3W** | 28.7 ± 3.7 | 0.38 ± 0.04 | 5 | - | 3 |
| | **U3E** | 42.6 ± 2.9 | 0.32 ± 0.02 | 1.0 | - | - |
| | **D1W** | 31.1 ± 2.4 | 0.32 ± 0.01 | 6 | 9 | 3 |
| | **D1E** | 37.7 ± 3.8 | 0.52 ± 0.05 | 2 | - | - |
| | **D2-1E** | 45.1 ± 3.4 | 0.44 ± 0.09 | - | - | - |
| | **D2-1W** | 41.1 ± 1.7 | 0.35 ± 0.02 | 7 | 2 | 3 |
| | **D2-2E** | 34.2 ± 1.7 | 0.35 ± 0.03 | - | - | - |
| | **D2-2W** | 45.5 ± 2.2 | 0.31 ± 0.02 | - | - | - |
| | **D3** | 28.9 ± 2.5 | 0.33 ± 0.03 | - | - | - |
| | **D4** | 36.4 ± 1.4 | 0.38 ± 0.01 | - | 10 | - |
| | **D5W** | 33.7 ± 2.9 | 0.32 ± 0.03 | - | - | - |
| | **D5E** | 30.2 ± 4.1 | 0.29 ± 0.03 | - | 2 | - |
| **October** | **U2** | 39.2 ± 2.5 | 0.32 ± 0.03 | - | - | - |
| | **U3W** | 29.0 ± 5.1 | 0.43 ± 0.04 | - | - | - |
| | **U3E** | 43.9 ± 3.8 | 0.38 ± 0.06 | - | - | - |
| | **D1W** | 41.8 ± 2.9 | 0.52 ± 0.06 | 7 | 3 | 5 |
| | **D1E** | 48.7 ± 3.3 | 0.80 ± 0.04 | - | - | - |
| | **D2-1E** | 48.8 ± 4.5 | 0.42 ± 0.04 | - | - | - |
| | **D2-1W** | 45.1 ± 2.2 | 0.27 ± 0.02 | 3 | 8 | 1 |
| | **D3** | 33.5 ± 3.0 | 0.50 ± 0.20 | - | - | - |
| | **D4** | 36.2 ± 1.2 | 0.40 ± 0.03 | - | - | - |
| | **D5E** | 31.5 ± 3.0 | 0.31 ± 0.03 | - | - | - |

Table 4: Total abundances of all fish species at sites on the Speed River in 2009. Unidentified fish made up 0.28% of all fish, and were not included in diversity calculations. Site totals are a combination of fish from all six sampled sub-sites.

| | | U1 | | | U2 | | | | U3W | | | | U3E | | | D1W | | | | D1E | | D2-1E | | | | D2-1W | | | D2-2E | D2-2W | D3 | | | | D4 | | D5W | D5E | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Family | Species | May | Jul | Aug | May | Jul | Aug | Oct | May | Jul | Aug | Oct | Jul | Aug | Oct | May | Jul | Aug | Oct | Aug | Oct | May | Jul | Aug | Oct | Jul | Aug | Oct | Aug | Aug | May | Jul | Aug | Oct | Aug | Oct | Aug | Aug | Oct |
| Percidae | Rainbow Darter | 33 | 81 | 43 | 62 | 96 | 104 | 84 | 156 | 133 | 199 | 87 | 54 | 105 | 82 | 59 | 213 | 431 | 104 | 140 | 20 | 14 | 102 | 223 | 48 | 102 | 150 | 5 | 85 | 135 | 69 | 64 | 126 | 57 | 74 | 16 | 101 | 62 | 66 |
| | Greenside Darter | 8 | 9 | 17 | 12 | 6 | 18 | 48 | 30 | 36 | 100 | 65 | 74 | 130 | 88 | 23 | 14 | 28 | 9 | 43 | 14 | 76 | 138 | 156 | 90 | 72 | 68 | 14 | 13 | 12 | 9 | 38 | 61 | 58 | 195 | 83 | 171 | 228 | 186 |
| | Johnny Darter | 22 | 23 | 36 | 3 | 3 | 17 | 9 | 1 | 0 | 14 | 9 | 0 | 8 | 5 | 10 | 1 | 19 | 11 | 18 | 4 | 0 | 2 | 10 | 5 | 0 | 11 | 12 | 11 | 9 | 0 | 4 | 11 | 9 | 20 | 5 | 141 | 189 | 146 |
| | Fantail Darter | 8 | 12 | 9 | 2 | 10 | 8 | 1 | 2 | 11 | 15 | 4 | 26 | 13 | 2 | 0 | 2 | 13 | 5 | 6 | 9 | 11 | 15 | 15 | 5 | 3 | 10 | 0 | 8 | 10 | 4 | 2 | 6 | 3 | 32 | 18 | 8 | 0 | 0 |
| | Blackside Darter | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 6 | 0 |
| | Least Darter | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Yellow Perch | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Centrarchidae | Smallmouth Bass | 1 | 3 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 1 | 4 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 1 |
| | Largemouth Bass | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Bluegill | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Pumpkinseed | 18 | 5 | 7 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Rock Bass | 5 | 0 | 5 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 4 | 0 | 2 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 1 | 2 | 13 | 3 | 0 |
| Catastomidae | Northern Hog Sucker | 0 | 2 | 1 | 0 | 0 | 0 | 6 | 2 | 0 | 5 | 2 | 0 | 3 | 3 | 0 | 0 | 1 | 7 | 2 | 7 | 1 | 0 | 2 | 8 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 |
| | White Sucker | 11 | 26 | 65 | 0 | 3 | 5 | 15 | 4 | 22 | 29 | 10 | 5 | 2 | 3 | 8 | 8 | 34 | 12 | 2 | 2 | 0 | 0 | 2 | 7 | 10 | 9 | 27 | 1 | 1 | 9 | 1 | 11 | 3 | 30 | 19 | 15 | 33 | 38 |
| Cyprinidae | Blacknose Dace | 0 | 0 | 1 | 0 | 4 | 0 | 2 | 10 | 4 | 10 | 11 | 1 | 6 | 3 | 7 | 3 | 19 | 25 | 8 | 14 | 1 | 3 | 0 | 1 | 10 | 2 | 10 | 1 | 0 | 1 | 3 | 5 | 6 | 2 | 0 | 0 | 0 | 0 |
| | Longnose Dace | 0 | 1 | 5 | 2 | 0 | 4 | 5 | 42 | 28 | 34 | 23 | 2 | 6 | 6 | 0 | 0 | 24 | 8 | 9 | 4 | 1 | 3 | 1 | 1 | 7 | 15 | 1 | 9 | 0 | 1 | 1 | 7 | 1 | 20 | 30 | 16 | 3 | 1 |
| | Hornyhead Chub | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| | Creek Chub | 3 | 2 | 21 | 0 | 0 | 1 | 2 | 2 | 1 | 2 | 4 | 1 | 3 | 1 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| | Common Shiner | 0 | 0 | 69 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 7 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 13 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 4 |
| | Striped Shiner | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | Spotfin Shiner | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Mimic Shiner | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | Fathead Minnow | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 3 |
| | Bluntnose Minnow | 2 | 0 | 20 | 1 | 2 | 2 | 7 | 0 | 0 | 2 | 1 | 2 | 0 | 0 | 6 | 0 | 0 | 3 | 0 | 1 | 0 | 0 | 0 | 4 | 0 | 0 | 15 | 0 | 0 | 2 | 0 | 0 | 2 | 10 | 84 | 12 | 15 | 125 |
| | Central Stoneroller | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Common Carp | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | unknown | 2 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 1 |
| Esocidae | Northern Pike | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Umbridae | Central Mudminnow | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 4 | 3 | 1 | 2 | 0 |
| Gasterosteidae | Brook Stickleback | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ictaluridae | Brown Bullhead | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Cottidae | Mottled Sculpin | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 3 | 1 | 2 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 0 | 1 | 2 | 9 | 20 | 31 | 16 | 0 | 0 | 0 | 0 | 0 |
| | Total Fish | 134 | 169 | 315 | 87 | 124 | 164 | 187 | 251 | 237 | 419 | 218 | 173 | 281 | 194 | 120 | 243 | 583 | 186 | 238 | 82 | 109 | 266 | 416 | 179 | 205 | 271 | 105 | 134 | 172 | 106 | 135 | 259 | 166 | 393 | 263 | 480 | 544 | 572 |
| | Species Richness | 12 | 11 | 18 | 10 | 7 | 11 | 14 | 10 | 8 | 16 | 11 | 12 | 12 | 10 | 10 | 7 | 13 | 11 | 14 | 11 | 10 | 8 | 11 | 13 | 7 | 11 | 11 | 10 | 8 | 10 | 10 | 9 | 11 | 13 | 11 | 11 | 10 | 10 |
| | Shannon Index | 3.1 | 2.3 | 3.3 | 1.6 | 1.3 | 1.9 | 2.5 | 1.8 | 1.9 | 2.3 | 2.4 | 2.1 | 1.9 | 1.8 | 2.3 | 0.8 | 1.5 | 2.3 | 2.0 | 2.9 | 1.6 | 1.5 | 1.5 | 2.2 | 1.7 | 1.9 | 3.0 | 1.8 | 1.2 | 1.8 | 2.0 | 2.2 | 2.4 | 2.3 | 2.5 | 2.3 | 2.0 | 2.3 |
| | Evenness | 0.9 | 0.7 | 0.8 | 0.5 | 0.5 | 0.6 | 0.6 | 0.5 | 0.6 | 0.6 | 0.7 | 0.6 | 0.5 | 0.5 | 0.7 | 0.3 | 0.4 | 0.7 | 0.5 | 0.8 | 0.5 | 0.5 | 0.4 | 0.6 | 0.6 | 0.5 | 0.9 | 0.5 | 0.4 | 0.6 | 0.6 | 0.7 | 0.7 | 0.6 | 0.7 | 0.7 | 0.6 | 0.7 |
| | Simpson Index | 0.1 | 0.3 | 0.1 | 0.5 | 0.6 | 0.4 | 0.3 | 0.4 | 0.4 | 0.3 | 0.3 | 0.3 | 0.4 | 0.4 | 0.3 | 0.8 | 0.6 | 0.3 | 0.4 | 0.2 | 0.5 | 0.4 | 0.4 | 0.3 | 0.4 | 0.4 | 0.1 | 0.4 | 0.6 | 0.4 | 0.3 | 0.3 | 0.3 | 0.3 | 0.2 | 0.3 | 0.3 | 0.2 |

Table 5: Mean abundances (± SE) of Rainbow and Greenside Darters at sites from four sampling periods in the Speed River in 2009. Letters in brackets indicate the results of a Mann-Whitney U test within species and sampling times. Sites within the same column with the same letter are not significantly different. The shaded rows indicate sites exposed fully to wastewater effluent.

| | Rainbow Darters | | | | Greenside Darters | | | |
|---|---|---|---|---|---|---|---|---|
| **Site** | **May** | **Jul** | **Aug** | **Oct** | **May** | **Jul** | **Aug** | **Oct** |
| **U1** | 5.5 ±1.6 (ade) | 13.5 ±4.2 (ad) | 7.2 ±1.5 (a) | | 1.3 ±0.5 (ab) | 1.5 ±0.6 (ab) | 2.8 ±1.0 (a) | |
| **U2** | 10.3 ±1.1 (b) | 16 ±4.0 (abc) | 17.3 ±3.7 (bdfgjh) | 14 ±3.4 (a) | 2 ±0.4 (ab) | 1 ±0.5 (b) | 3 ±0.7 (a) | 8 ±0.6 (a) |
| **U3W** | 26 ±6.5 (e) | 22.2 ±5.7 (bcf) | 33.2 ±4.3 (c) | 14.5 ±2.7 (a) | 5 ±1.5 (c) | 6 ±2.1 (c) | 16.7 ±4.5 (bcfg) | 10.8 ±2.2 (ab) |
| **U3E** | | 9 ±1.4 (d) | 17.5 ±3.0 (dfhj) | 13.7 ±2.2 (a) | | 12.3 ±2.2 (d) | 21.7 ±1.9 (ce) | 14.7 ±2.0 (b) |
| **D1W** | 9.8 ±3.3 (abd) | 35.5 ±5.7 (e) | 71.8 ±11.8 (e) | 17.3 ± 4.5 (a) | 3.8 ±1.3 (abc) | 2.3 ±0.9 (a) | 4.7 ±1.3 (ad) | 1.5 ±0.6 (c) |
| **D1E** | | | 23.3 ±4.1 (cfg) | 3.3 ±1.3 (bc) | | | 7.2 ±1.3 (df) | 2.3 ±0.7 (c) |
| **D2-1E** | 2.3 ±0.8 (ae) | 17 ±6.2 (df) | 37.2 ±8.9 (cg) | 8 ±1.4 (a) | 12.7 ±3.4 (c) | 23 ±5.2 (e) | 26 ±5.8 (e) | 15 ±2.6 (b) |
| **D2-1W** | | 17 ±4.9 (bcf) | 25 ±5.8 (cbghi) | 0.8 ±0.5 (b) | | 12 ±1.7 (d) | 11.3 ±2.2 (f) | 2.3 ±0.7 (c) |
| **D2-2E** | | | 14.2 ±3.4 (abehi) | | | | 2.2 ±1.1 (a) | |
| **D2-2W** | | | 22.5 ±1.6 (begij) | | | | 2 ±0.6 (a) | |
| **D3** | 11.5 ±2.1 (ab) | 10.7 ±2.2 (d) | 21 ±6.4 (cghi) | 9.5 ±2.8 (ac) | 1.5 ±0.4 (ab) | 6.3 ±1.3 (c) | 10.2 ±1.9 (f) | 9.7 ±1.1 (ab) |
| **D4** | | | 12.3 ±3.1 (afh) | 2.7 ±0.8 (bc) | | | 32.5 ±4.1 (beg) | 13.8 ±1.6 (b) |
| **D5W** | | | 16.8 ±7.5 (acehj) | | | | 28.5 ±12.5 (ef) | |
| **D5E** | | | 10.3 ±4.2 (afh) | 11 ±8.0 (ac) | | | 38 ±7.1 (e) | 31 ±5.7 (d) |

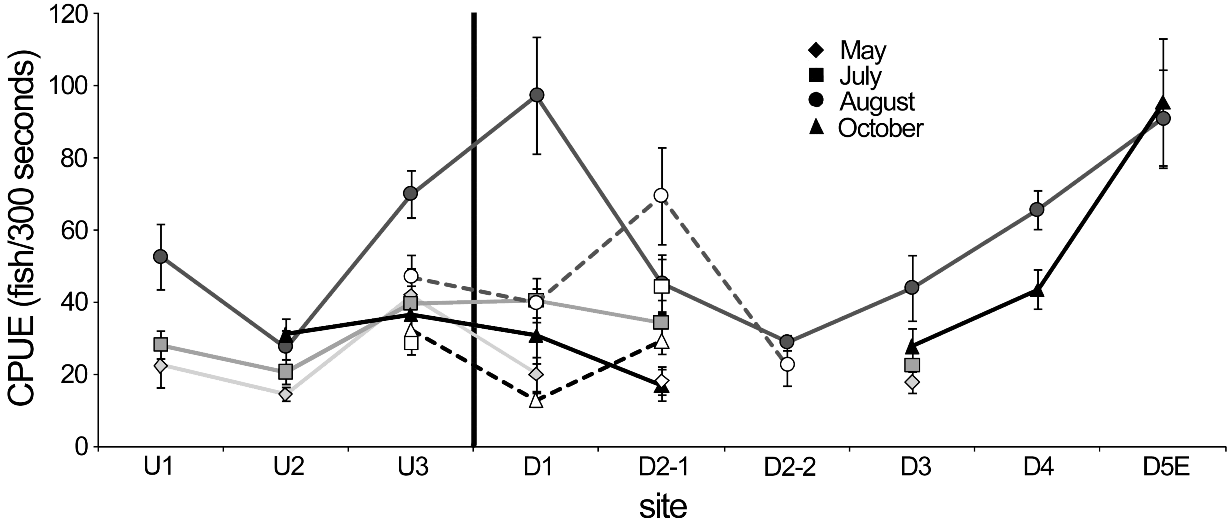


Figure 5: Mean abundances (± SE) measured as catch-per-unit-effort (CPUE) of total fish caught in 2009 at sites on the Speed River. Darkened shapes are sites that are exposed to the effluent and on the west side of the river, and hollow shapes with dotted lines are sites that are on the east (less exposed) side of the river.

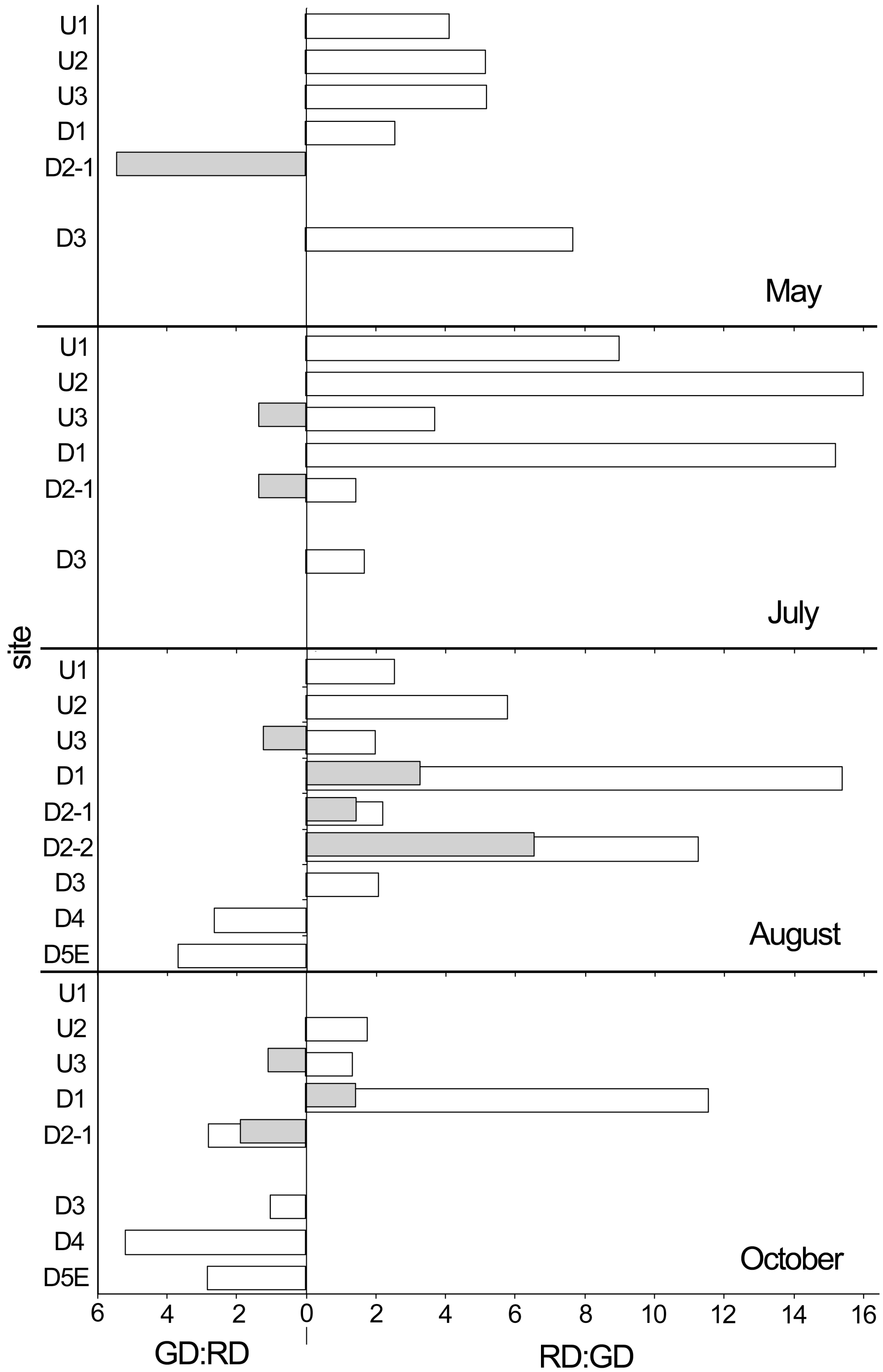


Figure 6: Ratios of total abundances of Rainbow Darters (RD) to Greenside Darters (GD) (and vice-versa depending on which species was dominant) at sites from May to October 2009. White bars indicate sites on the west bank exposed to effluent, and grey bars indicate sites on the east bank unexposed to effluent.

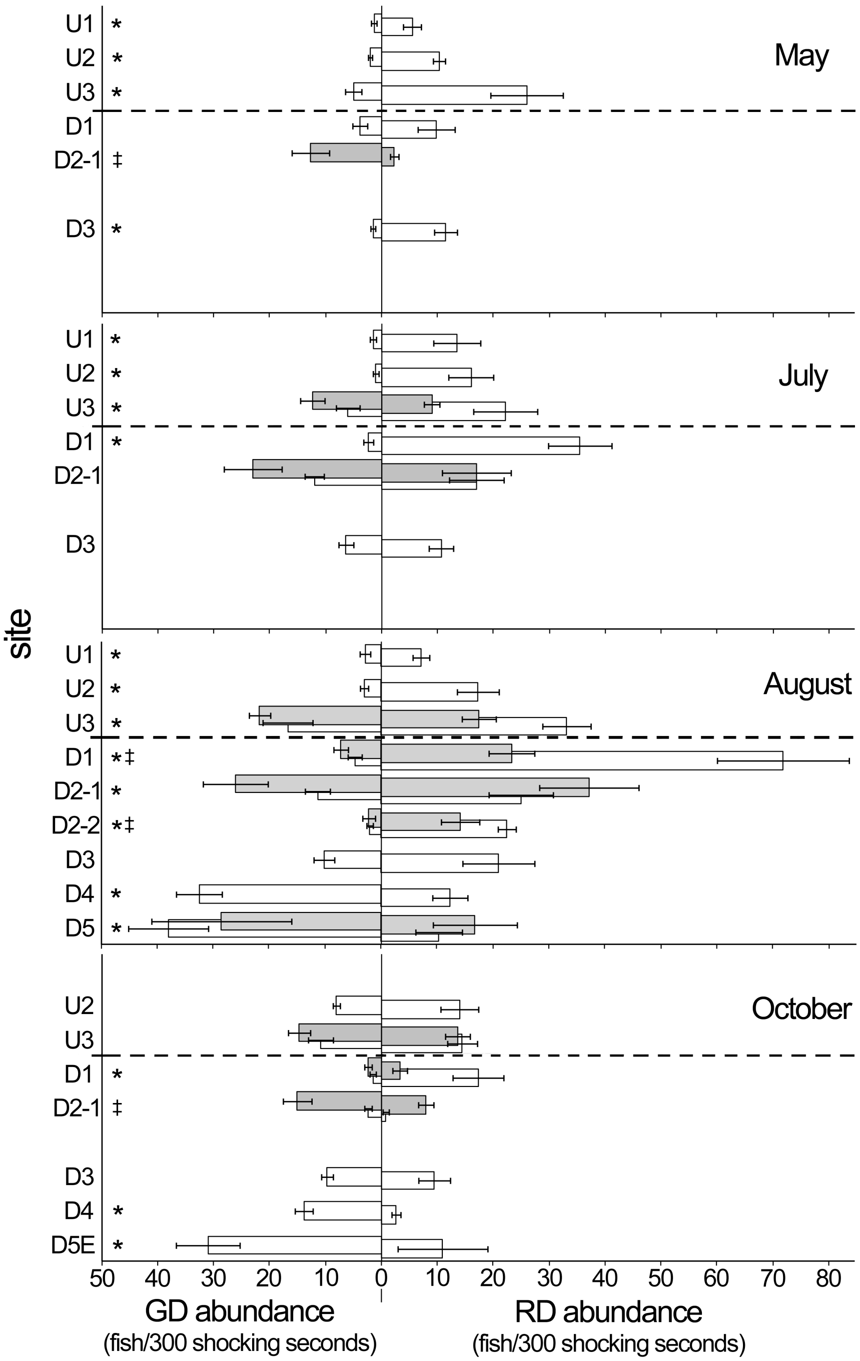


Figure 7: Mean abundances (± SE) of Rainbow and Greenside Darters at sites from four sampling periods in the Speed River in 2009. White bars indicate sites on the west bank, and grey bars indicate sites on the east bank. Asterisks (*) indicate a significant difference (α=0.05) between species abundances on the west bank, and double daggers (‡) on the east bank.

abundant than Greenside Darters at all sites except for U3E, D2-1E, D3, D4, D5W and 5E. At sites D4 and 5E, Greenside Darters were dominant. In October, Rainbow Darters were the dominant darter species at sites U1 and D1W, while Greenside Darters were the dominant darter species at sites D2-1E, D4 and 5E. The two species were similarly abundant at the remainder of the sites (Figure 7).

The length distributions of captured Rainbow and Greenside Darters in August were quite variable between sites (Figure 8). They indicated that both species contained a wider variety of fish lengths at the far downstream sites (D4 and D5E), which included many small fish. At site D5E the mean length of Rainbow Darters was 48.8 mm and the interquartile range (middle 50% of fish) (IQR) was 17 mm, and the Greenside Darters had a mean length of 60.3 mm surrounded by an IQR of 32 mm. The small darter sizes that were present farther downstream were rare at the further upstream sites. Despite the large number of Rainbow Darters caught in August at site D1W (n=431), there was much less variation in the size range. The mean length was 48.5 mm and the IQR was only 4 mm. This implies that there were no young-of-the-year fish, and that most of them were likely in the $2^{nd}$ to the $3^{rd}$ year age classes. The IQR of Greenside Darter lengths at site D1W was smaller too, spanning a range of 10 mm. A similar trend of small fish at far downstream sites also occurred in October.

### Benthic Invertebrate Community

The benthic invertebrate community changed immediately downstream (D1W) of the municipal wastewater effluent outfall in July, August and October 2009 (Table 6). Sites D1E, D2-1W, D3 and D4 also differed from the upstream reference sites in August. The substantial change experienced at the immediate downstream site (D1W) was highlighted by an increased proportion of the isopod *Caecidotea intermedius*, and a decreased proportion of beetle larvae (Elmidae) family, compared to upstream sites. Other sites on the west bank that were exposed to the effluent (D2-1W, D3W and D4) experienced shifts in *C. intermedius* and larval Elmidae compositions, but began to return to the upstream pattern further downstream (Table 7). By site D4, the number of *C. intermedius* returned to the upstream conditions, but the overall species compositions were different than upstream sites, highlighted by a large proportion of Ephemeroptera. The proportion of larval Elmidae did not return to those observed upstream.

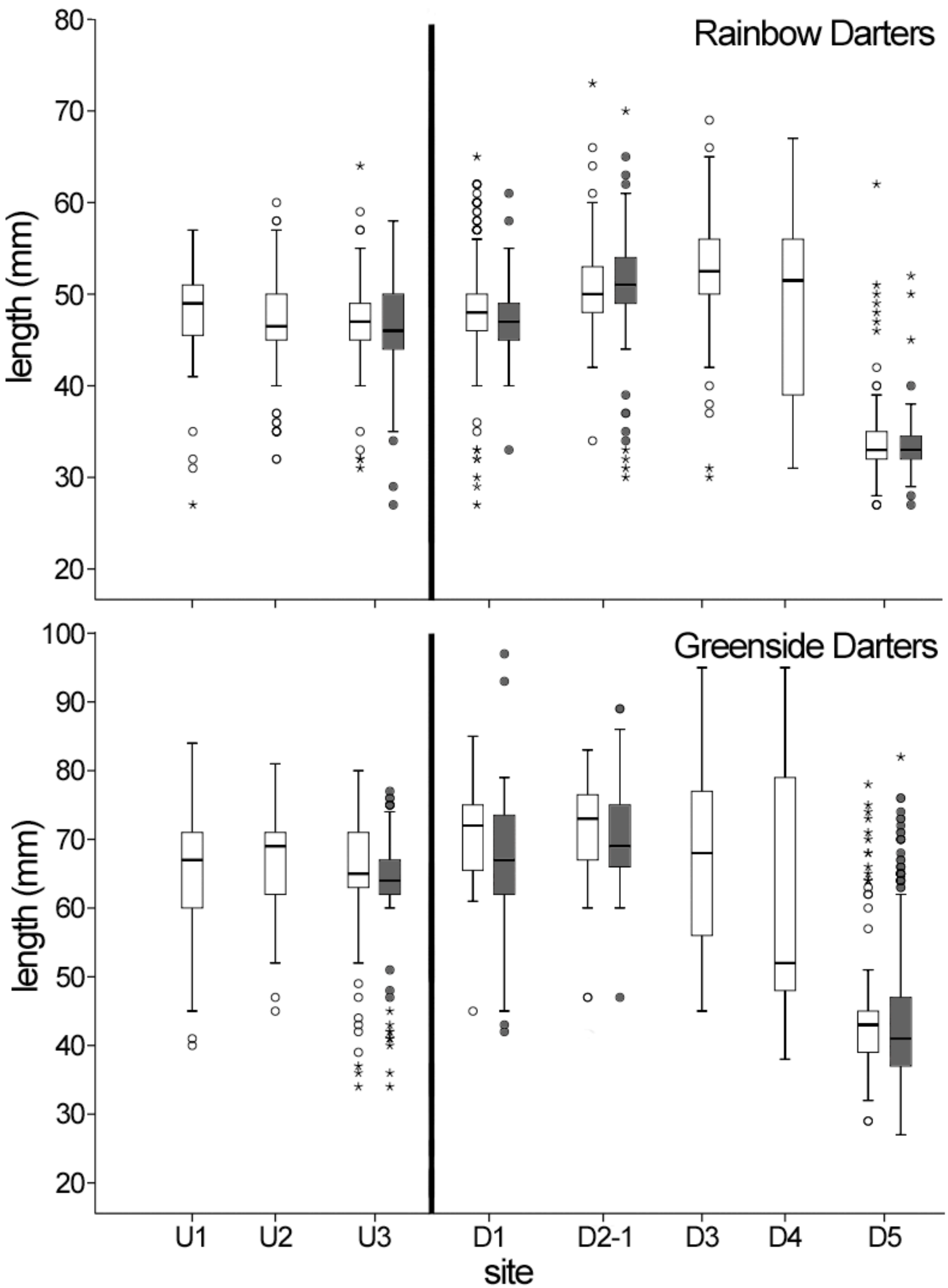


Figure 8: Mean lengths of Rainbow Darters in August 2009. White boxes are sites on the west side of the river, and grey boxes are on the east side. The boxed portion of each dataset is the interquartile range (IQR) (50% of fish from that site). The thick vertical bar indicates the point of the effluent outfall.

Table 6: Bray-Curtis indices of dissimilarity between downstream sites and an upstream reference average. Shaded columns are sites that are exposed fully to wastewater effluent on the west side. An asterisk (*) denotes no difference from the reference condition.

| | Site | | | | | |
|---|---|---|---|---|---|---|
| **Month** | **D1W** | **D1E** | **D2-1E** | **D2-1W** | **D3** | **D4** |
| **July** | 0.791 | - | - | - | - | - |
| **August** | 0.571 | 0.391 | 0.045* | 0.378 | 0.268 | 0.402 |
| **October** | 0.482 | - | - | - | - | - |

Table 7: Percent compositions (percentage of total invertebrates captured from the site) of benthic invertebrate taxa at sites in July, August and October 2009. Shaded columns are sites that are exposed fully to wastewater effluent.

| | | Site | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Month** | **Taxa** | **U1** | **U2** | **U3W** | **U3E** | **D1W** | **D1E** | **D2-1E** | **D2-1W** | **D3** | **D4** |
| **July** | **Ephemeroptera** | - | 5.98 | 32.00 | - | 10.84 | - | - | - | - | - |
| | **Trichoptera** | - | 10.30 | 8.31 | - | 13.25 | - | - | - | - | - |
| | **Chironomidae** | - | 10.96 | 7.38 | - | 7.53 | - | - | - | - | - |
| | **Coleoptera** | - | 17.61 | 17.23 | - | 3.61 | - | - | - | - | - |
| | **Isopoda** | - | 20.60 | 16.31 | - | 45.18 | - | - | - | - | - |
| | **Amphipoda** | - | 2.99 | 0.62 | - | 2.11 | - | - | - | - | - |
| | **Mollusca** | - | 27.24 | 12.00 | - | 15.66 | - | - | - | - | - |
| | **Other** | - | 3.99 | 6.15 | - | 1.81 | - | - | - | - | - |
| **August** | **Ephemeroptera** | 8.36 | 21.56 | 28.42 | 29.31 | 16.56 | 63.18 | 26.25 | 25.24 | 23.08 | 64.79 |
| | **Trichoptera** | 0.00 | 2.99 | 6.28 | 4.02 | 6.49 | 5.78 | 6.25 | 3.79 | 8.79 | 5.92 |
| | **Chironomidae** | 1.74 | 9.88 | 11.20 | 7.47 | 8.77 | 6.86 | 10.00 | 5.36 | 9.89 | 6.51 |
| | **Coleoptera** | 0.35 | 26.35 | 21.58 | 39.66 | 2.60 | 13.36 | 29.06 | 6.94 | 17.86 | 9.17 |
| | **Isopoda** | 10.80 | 11.38 | 10.11 | 5.17 | 63.96 | 1.44 | 4.38 | 46.69 | 25.82 | 7.69 |
| | **Amphipoda** | 64.81 | 2.69 | 9.29 | 2.87 | 0.97 | 2.89 | 5.00 | 3.47 | 9.89 | 0.89 |
| | **Mollusca** | 10.10 | 19.16 | 10.11 | 9.48 | 0.00 | 1.08 | 13.13 | 7.26 | 4.12 | 0.30 |
| | **Other** | 3.83 | 5.99 | 3.01 | 2.01 | 0.65 | 5.42 | 5.94 | 1.26 | 0.55 | 4.73 |
| **October** | **Ephemeroptera** | - | 31.55 | 33.22 | - | 8.46 | - | - | - | - | - |
| | **Trichoptera** | - | 5.65 | 10.30 | - | 4.78 | - | - | - | - | - |
| | **Chironomidae** | - | 14.88 | 8.31 | - | 19.49 | - | - | - | - | - |
| | **Coleoptera** | - | 13.69 | 16.28 | - | 2.57 | - | - | - | - | - |
| | **Isopoda** | - | 9.82 | 11.63 | - | 47.06 | - | - | - | - | - |
| | **Amphipoda** | - | 17.56 | 8.31 | - | 5.51 | - | - | - | - | - |
| | **Mollusca** | - | 2.08 | 6.98 | - | 8.46 | - | - | - | - | - |
| | **Other** | - | 4.76 | 4.98 | - | 3.68 | - | - | - | - | - |

# Discussion

The highly significant differences between Greenside Darter and Rainbow Darter abundances in August at the immediate downstream site (D1W) coincided with a large increase in the abundance of Rainbow Darters compared to any other site. This site was the most heavily exposed to the municipal wastewater effluent outfall, and it appears that the conditions created by the effluent were favourable for Rainbow Darters. When looking at the total fish community, Evenness and Simpson's indices in July and August, indicate the dominance of only a few species at site D1W. Increased nutrient levels from sewage effluent have been shown to alter downstream fish communities by increasing the total number of fish, and causing a decrease in intolerant species and an increase in tolerant species (Porter and Janz 2003). Rainbow Darters are not widely considered to be a tolerant species (Lyons 1992; Barbour et al. 1999) so the reason for their dominance at this site is not clear. This Rainbow Darter dominance trend was also observed in the Speed River in August and October of 2008 (Brown et al. 2011), and at sites downstream of other MWWEs in the Grand River in the same year (Brown 2010).

In August and October the total number of fish increased immediately downstream of the effluent outfall at site D1W relative to the reference sites and then decreased to a low 1.08 km downstream (D2-2W), with the total number of fish being heavily influenced by the prevalence of Rainbow Darters. Moving further downstream, the number of total fish at each site increased. A similar trend occurred in a similar study on the Grand River in 2008 whereby the total number of fish increased downstream of the sewage effluent outfall, but 4.6 km later, the total number of fish decreased to the lowest catch-per-unit-effort (CPUE) of any site (Brown 2010). Hypoxic conditions in the Speed River historically existed at a monitoring station 7 km downstream of the effluent outfall (Cooke 2006), however due to summer flow augmentation and improvements at the WWTP, the extent of oxygen depression has been minimized. Therefore low DO concentrations were not likely the cause of low fish abundance at site D2-2W.

Fish community data are known to be quite variable (Grossman et al. 1982), and biased by the sampling gear used to catch the fish (Curry and Munkittrick 2005). The variability in overall species distributions in the Speed River was also quite large, being complicated by the combination of the sampling method (backpack elecroshocking) and the complexity of sampling mobile fish species. Although the habitat was similar among most sites, in some cases, deviations in the fish community assemblages appear to be related to differences in the habitat at the site. For example, the habitat at site U1 in August had sand, cobble and gravel substrate with extensive aquatic grass growth, which likely contributed to 41% of the fish being Cyprinidae. The sampling method in the current study did however detect differences in the relative abundances of the less mobile benthic fish species, such as darters.

In the streams of North America, there are many similar darter species of the genus *Etheostoma* that live sympatrically, and likely coexist as a result of habitat partitioning (van Snik Gray and Stauffer 1999; Stauffer et al. 1996; Page and Swofford 1984). Carlson and Wainwright (2010) concluded that the mouths of species in the *Etheostoma* genus were very similar in gape size, and so it was likely that their selection of habitat drove the evolution of mouth morphology. In a study of minnows in a small Ozark watershed, Matthews (1982) found there to be no evidence for mutual exclusion of fish species based on similar morphological traits. This provides evidence that fish with similar morphologies can cohabitate with one another, and may not directly compete. We hypothesize that it was not competitive exclusion that allowed Rainbow Darters to dominate at site D1W over other darter species, but it was a combination of habitat variables that Rainbow Darters were more suited to exploit. The mouth morphologies of the two darters are very different, and the altered habitat could have given Greenside Darters less of an opportunity to feed, while at the same time creating a large amount of available food to Rainbow Darters (i.e. isopods). The conditions downstream of the sewage treatment plant effluent outfall varied from season to season, but there was a noticeable increase in macrophyte growth beginning in early June (Chapter 2) which continued as temperature, nutrients and other determining variables changed throughout the year (Cushing and Allan 2001; Mackie 1998). The downstream conditions also consisted of higher, more stable water temperatures (Figure 4), increased biological oxygen demand (Gowda 1983; Mackie 1998), and exposure to numerous chemicals including pharmaceuticals (Wang 2010). It may be possible that the two fish species respond differently to chemicals in the effluent, although the toxicological sensitivity of these two species has not been studied. The altered conditions may have supplied the Rainbow Darter with a more desirable habitat and food resource than its other benthic fish counterparts.

Macrophyte growth, particularly in the month of August, may have been partly responsible for the dominance of Rainbow Darters at the site immediately downstream (D1W). Increased nutrients and temperatures brought about an increase in the presence of aquatic moss (Bryophyta) in the mid-summer months, which attached to and covered the gravel and cobble substrate. That growth, combined with slightly elevated levels of silt and sand compared to other sites (Brown, personal communication), created a somewhat uniform environment that may have taken away potential preferred Greenside Darter habitat. They are one of the largest darters in the *Etheostoma* genus, and their habitat typically consists of unembedded cobble and boulder substrate (Bunt et al. 1998; Hlohowskyj and Wissing 1986; Chipps et al. 1994) in deep, fast water (Stauffer et al. 1996; Chipps et al. 1994; Greenberg 1991). Rainbow Darters tend to utilize slower and shallower areas of water (Harding et al. 1998), and also have more generic preferences for substrate size, often willingly occupying substrate of various sizes (Hlohowskyj and Wissing 1986; Wynes and Wissing 1982; Schlosser and Toth 1984). They are smaller, and so are found in between and under rocks (Schlosser and Toth 1984; Welsh and Perry 1998). Their mouths are terminal, which allows them to feed on top and in between rocks (Page and Swofford 1984; Schlosser and Toth 1984). Greenside Darters have sub-terminal mouths and have been shown to feed mainly on the tops of rocks (Page and Swofford 1984). Greenside Darters have often been associated with vegetation (Greenberg 1991; McCormick and Aspinwall 1983) and the filamentous green-algae genus *Cladophora* (Bunt et al. 1998). It has been hypothesized however, that it may just be that *Cladophora sp.* growth is often associated with larger cobble and boulders, creating an inaccurate correlation between the algae and Greenside Darter abundance (Hlohowskyj and Wissing 1986). There has been only a small connection made in the literature between aquatic moss and Greenside Darters (Winn 1958; Dalton 1991), the dominant macrophyte at site D1W in August, but the relationship is reported in regards to their breeding habits. The morphology of Rainbow Darters seems to be better suited for feeding amongst the moss and silty/sandy gravel. Wynes and Wissing (1982) also found that Rainbow Darters consumed food from a greater number of taxa and a greater size range than their counterparts. Since multiple studies report darter species to feed opportunistically on a variety of benthic invertebrates throughout the year (Hlohowskyj and White 1983; Wynes and Wissing 1982), our data implies that the Rainbow Darter behaviour may allow them access to more space and food. The distinct morphologies of the two species are very indicative of differential microhabitat use, and so combined with the more generalist behaviour of the Rainbow Darter, the small spaces amongst the moss and rocks were probably more suitable for this species of fish.

Hlohowskyj and Wissing (1985; 1987) also explored the maximum temperature and minimum DO tolerances of both species. Rainbow Darters were shown to have a greater ability to withstand an increase in temperature, particularly in the summer. Also in the summer, Rainbow Darters were found to be able to withstand low levels of oxygen down to 1.93 mg/L. The minimum summer DO level for Greenside Darters was only 3.39 mg/L. Despite DO levels in the Speed River staying above 4 mg/L in recent years, these findings still suggest that in a warmer, more oxygen demanding environment, similar to that of site D1W, Rainbow Darters are potnetially more suited to take advantage of the conditions.

Overall it seems as though Rainbow Darters are more of a generalist species than Greenside Darters and are more adaptable to a changing environment (Wynes and Wissing 1982).

The majority of these Rainbow Darters captured at site D1W in August were large and of the $2^{nd}$ to $3^{rd}$ year age class. Therefore the large abundance observed was not due to the presence of young-of-the-year fish that may have been too small to catch earlier in the year. This suggests that fish moved into this area between the July and August sampling periods to take advantage of the conditions. A similar trend occurred with darters in October 2008 (Brown 2010), in which few young-of-the-year were found at this site. A few potential causes may have accounted for these trends. Juvenile fish are easily affected by fluctuating river flows (Schlosser and Toth 1984; Schlosser 1985), which tend to be more variable in headwater streams. Juveniles can be swept downstream by high flows, and tend to fare better in areas of more stable flows, such as unregulated streams were unpredictable releases of water from dams will not be impactful (Freeman et al. 2001). This may have accounted for low abundances of young of the year fish at upstream sites and high abundances at the potentially more stable far downstream sites (D4 and D5E). Schlosser and Toth (1984) also found Rainbow Darters to move from riffles to raceway/pool habitats during the late summer and fall, as the flow in the river decreased. This may have been the reason for lower darter abundances in October than in August. At the very least, this indicates that Rainbow Darters may migrate back and forth from riffles to raceway/pools in the Speed River throughout the year. The habitat at the immediate downstream sites may also have been less suitable for the young-of-year darters.

The change in habitat at sites exposed to wastewater effluent, mainly from macrophyte growth, also caused the benthic invertebrate community to change. The major changes seen were a decrease in abundance of Elmidae larvae, particularly *Stenelmis* and *Optioservus*, and an increase in the isopod *C. intermedius*. These changes likely coincided with the change in habitat, highlighted by the aquatic moss and slightly sandier conditions compared to upstream sites. Members of the family Elmidae are scrapers (Mackie 2005) that are considered to be modestly tolerant of pollution and typically live in erosional habitats (Merritt and Cummins 1996). *C. intermedius* are collector-gatherers and are considered to be tolerant of adverse conditions (Mackie 1998), and could easily inhabit the downstream conditions that are created by exposure to wastewater effluent.

This study demonstrates the ability of a tertiary treated wastewater effluent to alter the fish and invertebrate communities in a small receiving environment. The wastewater increased the nutrient load and altered the physical habitat and thermal regime to create conditions that favoured Rainbow Darters and isopods (*C. intermedius*). Although the changes persisted for several kilometers downstream, the responses in the fish and invertebrate communities were highly variable through the seasons. The effluent does not fully mix for several kilometers and this has implications for future monitoring programs. The changes observed, although minor, may be an indication that without further wastewater management to address increases in nutrient and contaminant inputs resulting from population growth and other activities, the Speed River ecosystem could be altered to a greater extent.

# Chapter 3:
# The spatial and temporal effects of a municipal wastewater effluent on the carbon and nitrogen stable isotopic signatures of two darters (*Etheostoma blennioides* and *E. caeruleum*) in a small river

The following people contributed to the results in this chapter:

C.S. Robinson was the principal investigator who designed the study, performed the collections, analysis and writing of the manuscript.
M.R. Servos served as supervisor of the work.
M.E. McMaster acted as an advisor on various aspects of the fish collections.
D.R. Barton contributed to the sampling and identification of invertebrates.
All authors helped in the preparation and review of the text.

# Introduction

Southern Ontario is currently in the midst of heavy population growth that will be continuing into the foreseeable future. Parts of the Greater Toronto Area and an area to the west, including portions of the Grand River watershed, are expected to absorb steady influxes of new residents in the coming years. This will stress the current wastewater infrastructure of those affected municipalities. The City of Guelph and its surrounding area are expecting a population increase of 65% over the next 20 years (Grand River Conservation Authority 2005), which will undoubtedly place increased pressure on the Guelph Wastewater Treatment Plant. The plant releases its treated effluent into the nearby Speed River, a mid-sized tributary of the Grand River. Low flows in the summertime (which dipped to $1.5m^3/s$ in September 2009 (Grand River Conservation Authority, personal communication), are a major concern. The city's wastewater will likely contain additional chemical and nutrients in the future, and there is concern that the assimilative capacity of the river could be reached resulting in degradation of the aquatic environment. As recently as 1976, prior to the building of an upstream dam and WWTP upgrades, the river downstream suffered from extreme oxygen depression and overall poor environmental health (Grand River Conservation Authority 2008; Gowda 1983). The wastewater currently undergoes activated sludge secondary treatment and tertiary treatment that includes rotating biological contactors and slow sand filtration resulting in the effective removal of ammonia (0.99 mg/L average) and suspended solids (0-7mg/L). Nitrate is the dominant form of nitrogen released (22.39 mg/L average), and some soluble phosphorus is removed during early treatment, lessening the downstream nutrient exposure (City of Guelph, personal communication).

Stable isotope analysis of carbon and nitrogen is a useful tool for understanding aquatic systems (Peterson and Fry 1987). Sources of pollution, such as wastewater effluents and agriculture runoff, typically have different carbon and nitrogen isotopic signatures, which allow for the tracing of these anthropogenic inputs (DeBruyn and Rasmussen 2002; Loomer 2008). Wastewater effluents generally have more enriched carbon and nitrogen signatures, and organisms that directly or indirectly consume sewage derived nutrients often reflect, the enriched signature in their body tissues (DeBruyn and Rasmussen 2002;  Loomer 2008). However, isotope fractionation can be complex, and is reliant on the relative concentrations and forms of the nutrient compounds available for uptake in primary producers (Peterson and Fry 1987). Previous work in the Speed River in the summer of 2008 found that the two most abundant species of fish in the riffle habitats, Rainbow Darters (*Etheostoma caeruleum*) and Greenside Darters (*E. blennioides*), have similar $\delta^{13}C$ and $\delta^{15}N$ signatures at reference sites (Brown, et al.

2011). However, Rainbow Darters collected downstream of the City of Guelph municipal wastewater effluent outfall in August of 2008 had elevated isotopic signatures compared to Greenside Darter signatures, which remained similar to reference values. It was hypothesized by Brown et al. (2011) that, since the Rainbow Darter isotopic signatures increased by about one trophic level over the Greenside Darters at the downstream site, the Rainbow Darters were possibly able to exploit a higher trophic level food source in the altered downstream environment. This difference may give Rainbow Darters a competitive advantage resulting in an observed increased relative abundance at these effluent exposed sites (Brown et al. 2011). However, the variability of fish abundance (Chapter 2) and isotopic signatures in darters (Loomer 2008) in the Grand River have been shown to change seasonally in response to wastewater effluent outfalls.

The objective of this study was to identify the influence of Guelph WWTP effluent on the downstream food web, including two species of darters (Rainbow and Greenside Darters), across several seasons in the Speed River. The $\delta^{13}C$ and $\delta^{15}N$ of benthic invertebrates and fish were determined spanning five different sampling periods (May 2009 to March 2010), upstream and downstream of the municipal wastewater effluent outfall. Stomach contents were also determined to assess potential changes in diets of the two darter species that may influence the isotopic signatures.

## Methods

### Study Area

The study area was the Speed River which runs through the City of Guelph, Ontario (Figure 9). Fish and invertebrate sampling was performed at 11 sites over five sampling periods spanning 2009 and 2010: May (May 15-22, 2009), July (July 8-15, 2009), August (Aug. 20-Sept. 3, 2009), October (October 26-November 11, 2009) and March (March 2, 2010). Sites chosen were the same sites as used for fish community sampling outlined in Chapter 2. There were four reference sites upstream of the effluent outfall, and seven sites downstream (Figure 9). Three of the downstream sites were on the east bank, and therefore experienced only partial exposure to the sewage effluent.

### Fish and Benthic Invertebrate Collections

Six to fifteen Rainbow and Greenside Darters were collected from each site using backpack electroshocking (Chapter 2), spanning the five sampling periods. Rainbow Darters chosen for analysis

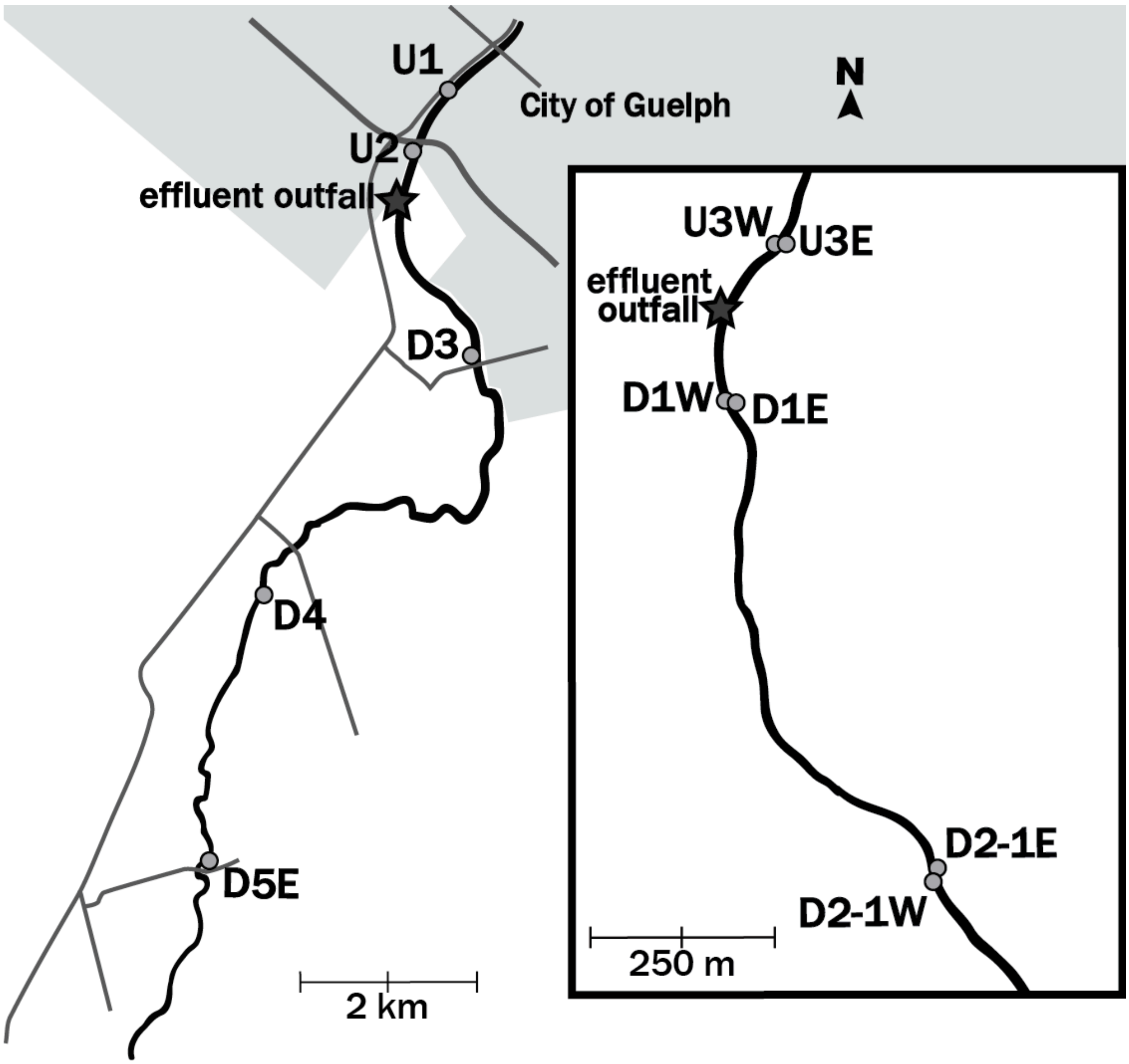


Figure 9: Map of Speed River fish and invertebrate sampling sites.

were in the size range of 39-61 mm and Greenside Darters in the range 48-82 mm. The species, sex, length and weight were recorded for each fish. Fish were collected according to protocols approved by the University of Waterloo Animal Care Committee (University of Waterloo AUP # 04-24; 08-08).

Benthic invertebrates were collected at all sites during the four sampling periods of 2009. A standard 500 μm mesh D-net was used to collect common invertebrates from each site. Organisms were collected live using tweezers and stored at -20°C until sorting could take place. For stable isotope analysis, invertebrates were sorted by trophic guild and taxon (order, family or sub-family) (Merritt and Cummins 1996).

### Stable Isotope Analysis

Both fish and invertebrate samples were prepared for instrumental analysis using methods similar to those applied by Loomer (2008) and Brown et al. (2011). Briefly, a small fillet of skinless dorsal epaxial white muscle, anterior and ventral to the dorsal fin, was removed from each fish for analysis (Pinnegar and Polunin 1999). Whole bodies of benthic invertebrates were cleaned with distilled water to remove any debris and grouped by organism type and trophic guild to create workable sample sizes. All samples were then dessicated in a 60°C oven for 24 h, and subsequently ground into a fine homogenous powder using a mortar and pestle. The samples were weighed out to 0.25-0.3 mg, surrounded in foil, and then submitted to the Environmental Isotope Lab at the University of Waterloo. A Delta Plus Continuous 26 Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan / Bremen-Germany) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108 - Italy) was used to measure $\delta^{15}N$ and $\delta^{13}C$, as well as % elemental carbon and nitrogen (Drimmie and Hemmskerk 2005).

### Stomach Content Analysis

Stomachs of Rainbow and Greenside Darters used for stable isotope analysis were removed and dissected. The invertebrate contents were identified to family level when possible (Mackie 2005). In cases of partial degradation, the items were identified to the lowest possible taxonomic level. Six to eleven fish were used to represent each site per season, which is considered to be sufficient to quantify the total number of families that comprise their diets (Alford and Beckett 2007). Sites immediately upstream (U3W) and downstream (D1W) of the treatment plant were sampled in all four collection periods. In July 2009, additional sites were sampled (U1, U2, D2-1E, D2-1W, and D3).

## Condition Factor

Condition factor was calculated using the lengths and weights from Greenside and Rainbow Darters caught during fish community sampling described in Chapter 2. Accurate determination of sexes was not posssible during field sampling, so for analysis, males and females of the same species were pooled together at each site. A length-weight plot was used to determine and remove obvious outliers that were likely the result of transcription errors in the field notes.

## Statistical Analysis

Isotope data did not meet the criteria for parametric testing, so a Kruskal-Wallis test and corresponding Mann-Whitney U tests were used to determine differences between isotope signatures. Comparisons were made between Rainbow Darters and Greenside Darters at each site, as well as between sites within each species. The total number of prey per fish was also compared between species using a Kruskal-Wallis test and corresponding Mann-Whitney U test. An alpha ($\alpha$) value of 0.05 was used to test for significance. A one-way ANOVA was used to compare condition factors among sites. IBM SPSS Statistics 19.0 was used for all statistical analyses.

# Results

## Stable Isotope Analysis

### *Nitrogen*

In July, the $\delta^{15}N$ of both Rainbow Darters and Greenside Darters increased gradually downstream with a large increase of ~1.5‰ at the site immediately downstream of the effluent outfall (D1W) (Figure 10; Table 8). $\delta^{15}N$ of most invertebrates also increased at this site (D1W) in May and July, with Tanypodinae containing the highest $\delta^{15}N$. In August, the $\delta^{15}N$ of Rainbow Darters increased at the immediate downstream site (D1W), by a slightly higher margin than in July, reaching a peak of 17.3‰. Downstream of site D1W, the signatures decreased gradually to values comparable to those of upstream sites (Figure 11). In contrast to the Rainbow Darter, the Greenside Darter $\delta^{15}N$ did not increase at the immediate downstream site (D1W). The $\delta^{15}N$ of Greenside Darters peaked farther downstream at site D2-1W at 16.0‰, and decreased at the further downstream sites. The signatures of the two darter species were different at all downstream sites in August, with Rainbow Darters maintaining a heavier signature. All $\delta^{15}N$ of invertebrate taxa, except Baetidae, peaked at the immediate downstream site (D1W), dipped down at D2-1W and then gradually increased at downstream sites (Figure 11). The

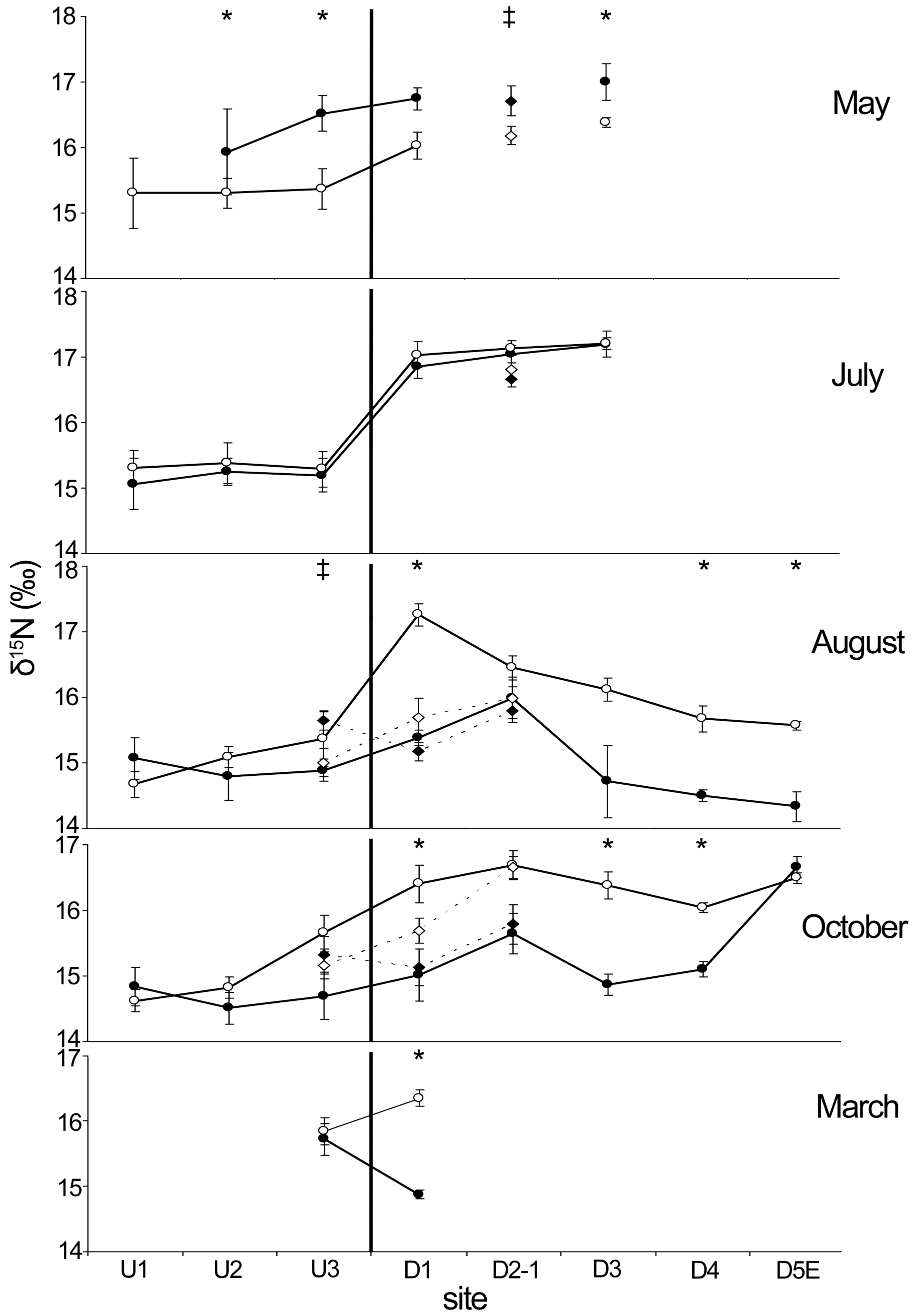


Figure 10: Mean (± SE) $\delta^{15}N$ of Rainbow Darters (○) and Greenside Darters (●) between May 2009 and March 2010. Diamonds (◊ and ♦) indicate unexposed/less exposed sites on the west bank of the river. Black asterisks (*) indicate a significant difference (α=0.05) between species at sites on the west bank, and double daggers (‡) at the sites on the east bank. Error bars represent the standard error.

Table 8: Site to site Mann-Whitney U comparisons (α=0.05) within species of $\delta^{13}C$ and $\delta^{15}N$ of Rainbow and Greenside Darter $^{13}C$ and $^{15}N$ signatures. Shaded rows indicate sites that were fully exposed to the effluent on the west bank of the river. Fish from site D5 were collected from both sides of the river (5E and 5W).

| | Carbon | | | | | | | | Nitrogen | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rainbow Darters | | | | Greenside Darters | | | | Rainbow Darters | | | | Greenside Darters | | | |
| **Site** | **May** | **Jul** | **Aug** | **Oct** | **May** | **Jul** | **Aug** | **Oct** | **May** | **Jul** | **Aug** | **Oct** | **May** | **Jul** | **Aug** | **Oct** |
| **U1** | abc | a | ac | a | | a | ae | acd | ab | a | a | a | | a | adfh | abc |
| **U2** | b | ab | ab | ab | ab | b | bd | ad | b | a | ab | a | a | a | efh | bdg |
| **U3W** | c | ab | abcd | bce | b | c | c | abde | bc | a | ach | bcd | a | a | ch | abc |
| **U3E** | | | bd | bdf | | | bcd | c | | | ad | acd | | | dg | ce |
| **D1W** | abc | c | e | c | c | a | bc | ade | acd | b | e | bef | a | bc | def | cde |
| **D1E** | | | abd | be | | | bc | ade | | | bcdh | df | | | cfi | abce |
| **D2-1E** | cb | ab | df | cde | a | b | bde | d | cde | b | bcfh | e | a | b | dg | ehf |
| **D2-1W** | | d | ef | cf | | ab | a | ade | de | b | fg | e | | bc | g | acf |
| **D3** | abc | bd | ef | cd | ac | a | f | be | | b | gh | eg | a | c | adghi | abcg |
| **D4** | | | abd | de | | | f | ab | | | bch | fg | | | ah | abce |
| **D5** | | | bd | de | | | a | d | | | c | beg | | | ahi | fh |

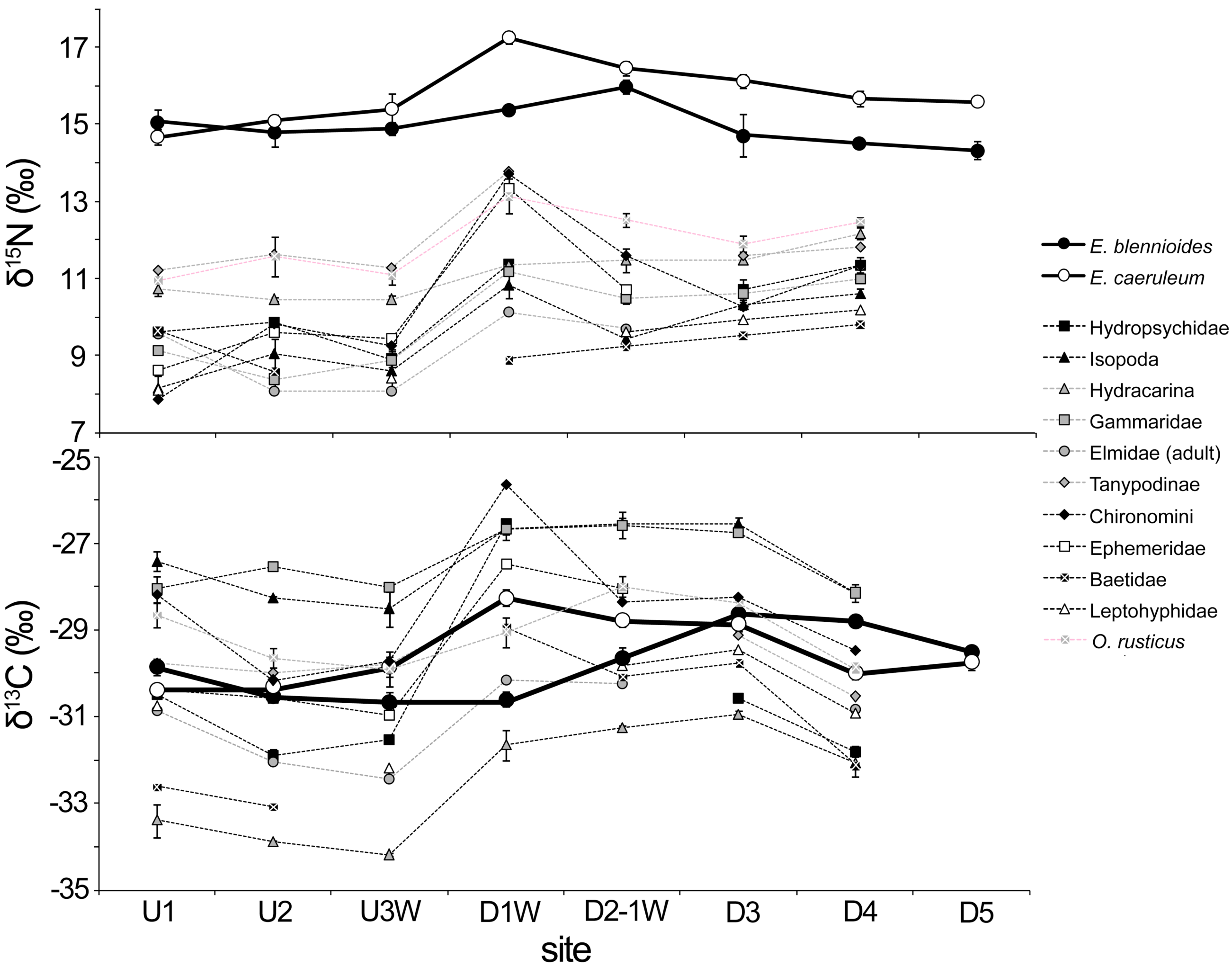


Figure 11: Mean (± SE) $\delta^{13}C$ and $\delta^{15}N$ of Rainbow Darters, Greenside Darter (muscle) and abundant invertebrates at sites on the west bank in August 2009. Data at site D5 is from both the east and west banks (5E and 5W). Error bars represent the standard error.

Baetidae $\delta^{15}N$ seemed relatively unaffected by the MWWE input.

In October, Rainbow Darter $\delta^{15}N$ began increasing at U3W while those of Greenside Darters did not. Both species' signatures peaked at D2-1W, but signatures between the two remained different at all sites. Rainbow Darters maintained a heavier signature at D5E, at which point Greenside Darters increased drastically while Rainbow Darters increased only slightly. The $\delta^{15}N$ of invertebrates showed no major trends amongst the sites sampled in October (Figure 12).

### *Carbon*

Fish carbon signatures were found in the middle of the range of invertebrate signatures at all sampling times. In July, $\delta^{13}C$ signatures in Rainbow Darters and Greenside Darters increased at the immediate downstream site (D1W) relative to upstream (Figure 13; Table 8). Invertebrate taxa also increased in $\delta^{13}C$ at D1W in July with Tanypodinae being the invertebrate with the most enriched signature. In August, the $\delta^{13}C$ of Rainbow Darters increased at the immediate downstream site (D1W), in contrast to the $\delta^{13}C$ in Greenside Darters' signature which did not increase. Rainbow Darter signatures peaked at -28.3‰ at D1W, while Greenside Darter signatures peaked farther downstream (D2-1W) at -28.6‰. Invertebrate signatures generally increased at the site immediately downstream (D1W) relative to upstream, and continued to gradually increase downstream until site D4, at which point they began to decrease.

In October, the $\delta^{13}C$ of Greenside Darters remained similar at all sites, while Rainbow Darter signatures significantly increased downstream of the outfall, and then gradually decreased further downstream to values similar to those upstream. Invertebrates slightly increased in $\delta^{13}C$ at the immediate downstream site (D1W). $\delta^{13}C$ in all invertebrate species decreased between sites D4 and D5E.

## Seasonal Trends

Throughout the year, the upstream carbon and nitrogen isotopic trends remained similar between fish species (Figure 14). $\delta^{15}N$ of Rainbow and Greenside Darters diverged downstream between July and August and stayed this way right through until the following March. Downstream $\delta^{13}C$ signatures of fish species were inconsistent with each other, and did not maintain similar trends. $\delta^{15}N$ of Hydropsychidae and Hydracarina at the immediate downstream site (D1W) were the most enriched in July and May respectively, while $\delta^{13}C$ trends increased, particularly in Hydracarina downstream of the effluent outfall (Figure 15).

## Stomach Content Analysis

The percent compositions of taxa in darter diets were similar among the two species in May and July at the immediate downstream site (D1W) (Table 9). In August and October the two species' diets diverged from each other. Greenside Darters consumed more trichopterans in August and more

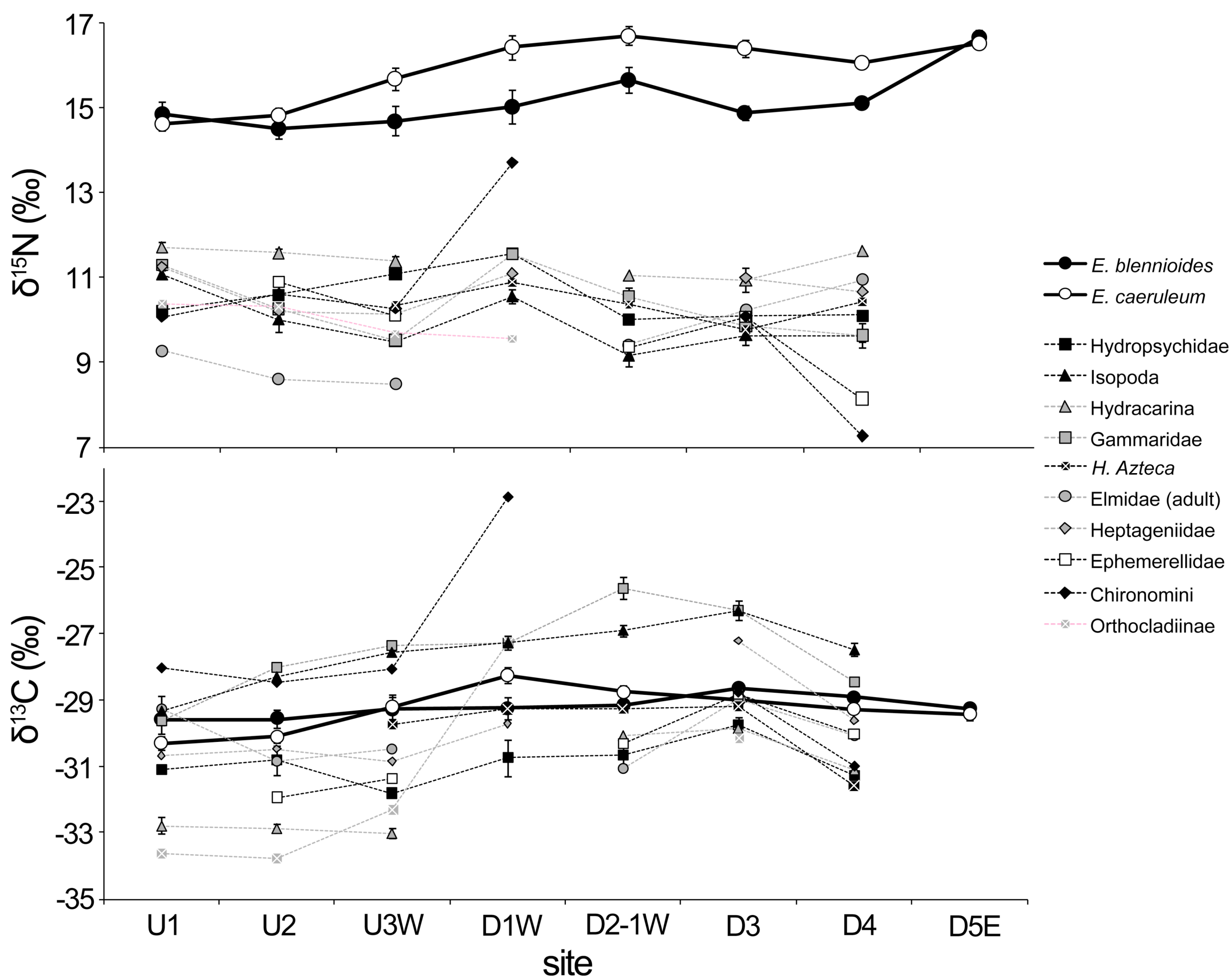


Figure 12: Mean (± SE) $\delta^{13}C$ and $\delta^{15}N$ of Rainbow Darters, Greenside Darter (muscle) and abundant invertebrates at sites on the west bank in October 2009. Data at site D5 is from both the east and west banks (5E and 5W).

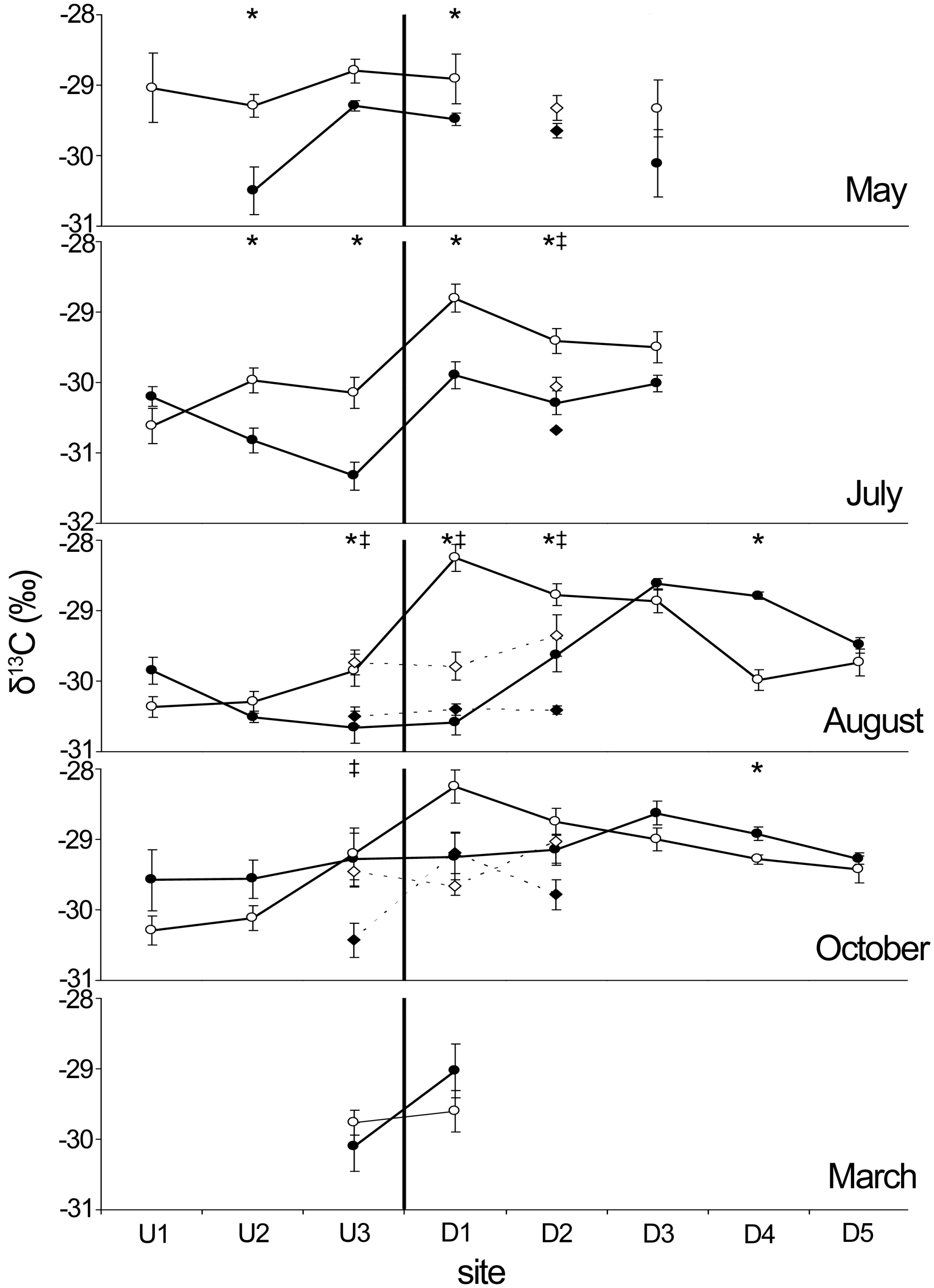


Figure 13: Mean (± SE) $\delta^{13}C$ of Rainbow Darters (○) and Greenside Darters (●) between May 2009 and March 2010. Diamonds (◊ and ♦) indicate unexposed/less exposed sites on the east bank of the river. Asterisks (*) indicate a significant difference (α=0.05) between sites on the west bank, and double daggers (‡) on the east bank.

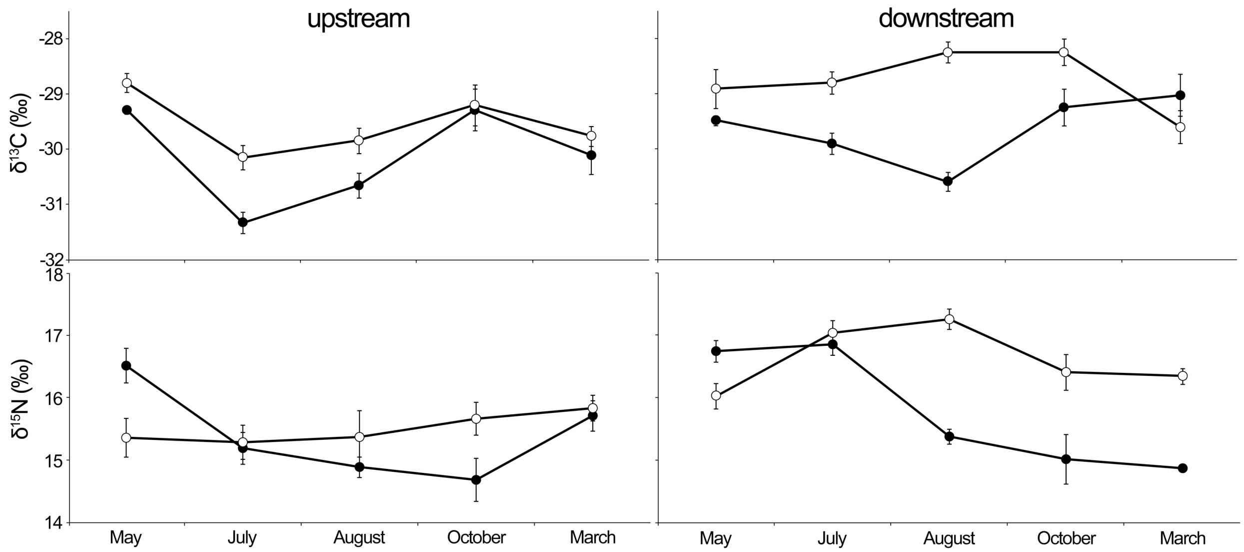


Figure 14: Mean (± SE) $\delta^{13}C$ and $\delta^{15}N$ of Rainbow Darters (○) and Greenside Darters (●) in all sampling months immediately upstream (site U3W) and immediately downstream (site D1W) of the Guelph WWTP.

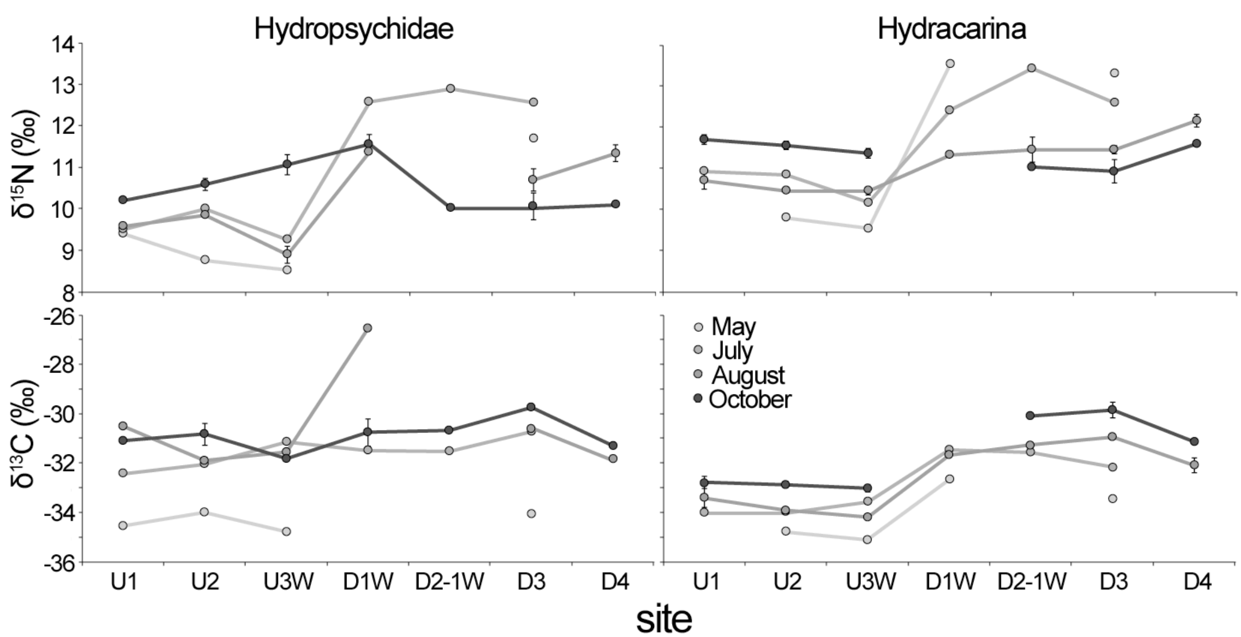


Figure 15: Mean (± SE) $\delta^{13}C$ and $\delta^{15}N$ of Hydropsychidae and Hydracarina for sampling periods May, July, August and October. Data points with no error bars have only one data point.

Table 9: Percent compositions of all identifiable prey in stomach contents of Rainbow (RD) and Greenside Darters (GD) in sampling periods in 2009 and 2010. Values were calculated based on the total prey from all fish sampled of one species at each site. Shaded columns represent sites fully exposed to wastewater effluent on the west bank.

| | | U1 | | U2 | | U3W | | D1W | | D2-1E | | D2-1W | | D3 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Month** | **Taxa** | **GD** | **RD** | **GD** | **RD** | **GD** | **RD** | **GD** | **RD** | **GD** | **RD** | **GD** | **RD** | **GD** | **RD** |
| **May** | **Chironomidae** | | | | | 97.1 | 93.1 | 91.3 | 91.2 | | | | | | |
| | **Ephemeroptera** | | | | | 0.0 | 1.3 | 2.2 | 0.0 | | | | | | |
| | **Trichoptera** | | | | | 2.6 | 2.6 | 6.5 | 2.6 | | | | | | |
| | **Isopoda** | | | | | 0.0 | 0.0 | 0.0 | 0.0 | | | | | | |
| | **Other** | | | | | 0.3 | 3.0 | 0.0 | 6.1 | | | | | | |
| **Jul** | **Chironomidae** | 16.7 | 39.1 | 71.5 | 33.3 | 67.6 | 30.5 | 33.3 | 20.0 | 45.5 | 34.8 | 19.6 | 30.8 | 56.0 | 26.3 |
| | **Ephemeroptera** | 0.0 | 4.3 | 1.3 | 0.0 | 0.0 | 1.2 | 1.1 | 5.0 | 3.6 | 2.9 | 0.0 | 23.1 | 9.9 | 47.5 |
| | **Trichoptera** | 16.7 | 17.4 | 27.2 | 63.9 | 32.4 | 61.0 | 65.6 | 63.3 | 49.1 | 36.2 | 79.5 | 28.2 | 33.3 | 23.2 |
| | **Isopoda** | 0.0 | 4.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 0.0 | 1.4 | 0.0 | 5.1 | 0.0 | 0.0 |
| | **Other** | 66.7 | 34.8 | 0.0 | 2.8 | 0.0 | 7.3 | 0.0 | 8.3 | 1.8 | 24.6 | 0.9 | 12.8 | 0.8 | 3.0 |
| **Aug** | **Chironomidae** | | | | | 82.4 | 48.4 | 35.4 | 39.5 | | | | | | |
| | **Ephemeroptera** | | | | | 2.0 | 3.2 | 21.7 | 7.9 | | | | | | |
| | **Trichoptera** | | | | | 14.2 | 22.6 | 42.3 | 10.5 | | | | | | |
| | **Isopoda** | | | | | 0.7 | 9.7 | 0.0 | 26.3 | | | | | | |
| | **Other** | | | | | 0.7 | 16.1 | 0.6 | 15.8 | | | | | | |
| **Oct** | **Chironomidae** | | | | | 95.2 | 85.0 | 68.9 | 35.3 | | | | | | |
| | **Ephemeroptera** | | | | | 0.6 | 2.8 | 2.8 | 20.6 | | | | | | |
| | **Trichoptera** | | | | | 3.2 | 4.7 | 17.9 | 11.8 | | | | | | |
| | **Isopoda** | | | | | 0.0 | 0.0 | 0.9 | 20.6 | | | | | | |
| | **Other** | | | | | 1.0 | 7.5 | 9.4 | 11.8 | | | | | | |
| **Mar** | **Chironomidae** | | | | | 95.7 | 91.4 | 75.0 | 92.5 | | | | | | |
| | **Simuliidae** | | | | | 2.9 | 7.5 | 21.9 | 2.5 | | | | | | |
| | **Other** | | | | | 1.4 | 1.1 | 3.1 | 5.0 | | | | | | |

chironomids in October than did Rainbow Darters. Isopods were a notable inclusion to the diets of Rainbow Darters in the months of July, August and October. They represented over 20% of identifiable prey in the stomachs of those fish downstream of the effluent outfall in August and October. Isopods were not as prevalent in Rainbow Darter diets upstream of the effluent outfall, or in Greenside Darters upstream or downstream of the outfall. In the months of July and August, Chironomidae became less dominant in both species. Trichoptera were the dominant prey in July in both species (except at one upstream site (U3W) for Rainbow Darters). The diets of Rainbow Darters diversified upstream (U3W) and downstream (D1W) in August. The diverse diet remained similar at the immediate downstream site D1W in October, but was dominated by chironomids upstream (U3W). Greenside Darters contained more total identifiable prey in their stomachs than did Rainbow Darters at all sites with the exception of site D1W in March 2010 (Table 10). At 6 of those 14 sites, Greenside Darters consumed significantly more identifiable prey than Rainbow Darters. Chironomidae was the most common taxon found in the stomachs of Greenside and Rainbow Darters During the months of May 2009, October 2009 and March 2010, both species of fish immediately upstream (U3W) and immediately downstream (D1W) contained mainly Orthocladiinae. The lone exception was Rainbow Darters at the immediate downstream site (D1W) in October 2009.

### Condition Factor

Condition factors of both species showed no obvious trends in relation to the effluent outfall (Figure 16). In August, Rainbow Darters at the immediate downstream site (D1W) had significantly greater condition factors than three of the unexposed sites (U3W, U3E, and D1E). Greenside Darters at site D1W had significantly greater condition factors than fish at site U3E in August.

## Discussion

Exposure to the wastewater effluents downstream of the City of Guelph outfall caused enrichment in $\delta^{13}C$ and $\delta^{15}N$ of most invertebrates and fish in late summer and fall. The majority of sewage effluents are enriched in both $^{13}C$ and $^{15}N$ compared to the unimpacted sites (Loomer 2008; Wayland and Hobson 2001; Lake et al. 2001; Cabana and Rasmussen 1996), and these signatures were reflected in the tissues of those organisms within the effluent plume (Rogers 1999; DeBruyn and Rasmussen 2002; Loomer 2008; Hansson et al. 1997; McClelland et al. 1997; DeBruyn et al. 2003). Nitrogen from human waste, which is already somewhat enriched from our mid to high trophic status (Cabana and Rasmussen 1996; Heaton 1986), is further enriched by the volatilization of depleted

Table 10: Total number of fish sampled, and mean number of identifiable prey contained in fish stomachs of each species (GD – Greenside Darter, RD – Rainbow Darter). Lightly shaded columns represent sites fully exposed to wastewater effluent on the west bank. Darker shaded areas represent sites with a significant difference between the number of identifiable prey in Rainbow and Greenside Darters (α=0.05).

| | | U1 | | U2 | | U3W | | D1W | | D2-1E | | D2-1W | | D3 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Month** | | **GD** | **RD** | **GD** | **RD** | **GD** | **RD** | **GD** | **RD** | **GD** | **RD** | **GD** | **RD** | **GD** | **RD** |
| **May** | **# fish** | | | | | 10 | 10 | 10 | 11 | | | | | | |
| | **# items** | | | | | 37.9 | 46.4 | 13.8 | 10.4 | | | | | | |
| **July** | **# fish** | 7 | 7 | 7 | 7 | 7 | 7 | 10 | 8 | 7 | 7 | 7 | 7 | 7 | 7 |
| | **# items** | 5.1 | 3.3 | 43.1 | 5.1 | 15.0 | 11.7 | 18.0 | 7.5 | 7.9 | 9.9 | 16.0 | 5.6 | 36.0 | 14.1 |
| **August** | **# fish** | | | | | 10 | 10 | 14 | 15 | | | | | | |
| | **# items** | | | | | 14.8 | 3.1 | 12.5 | 2.5 | | | | | | |
| **October** | **# fish** | | | | | 8 | 9 | 7 | 9 | | | | | | |
| | **# items** | | | | | 39.1 | 11.9 | 15.1 | 3.8 | | | | | | |
| **March** | **# fish** | | | | | 5 | 5 | 5 | 7 | | | | | | |
| | **# items** | | | | | 83.0 | 37.2 | 6.4 | 11.4 | | | | | | |

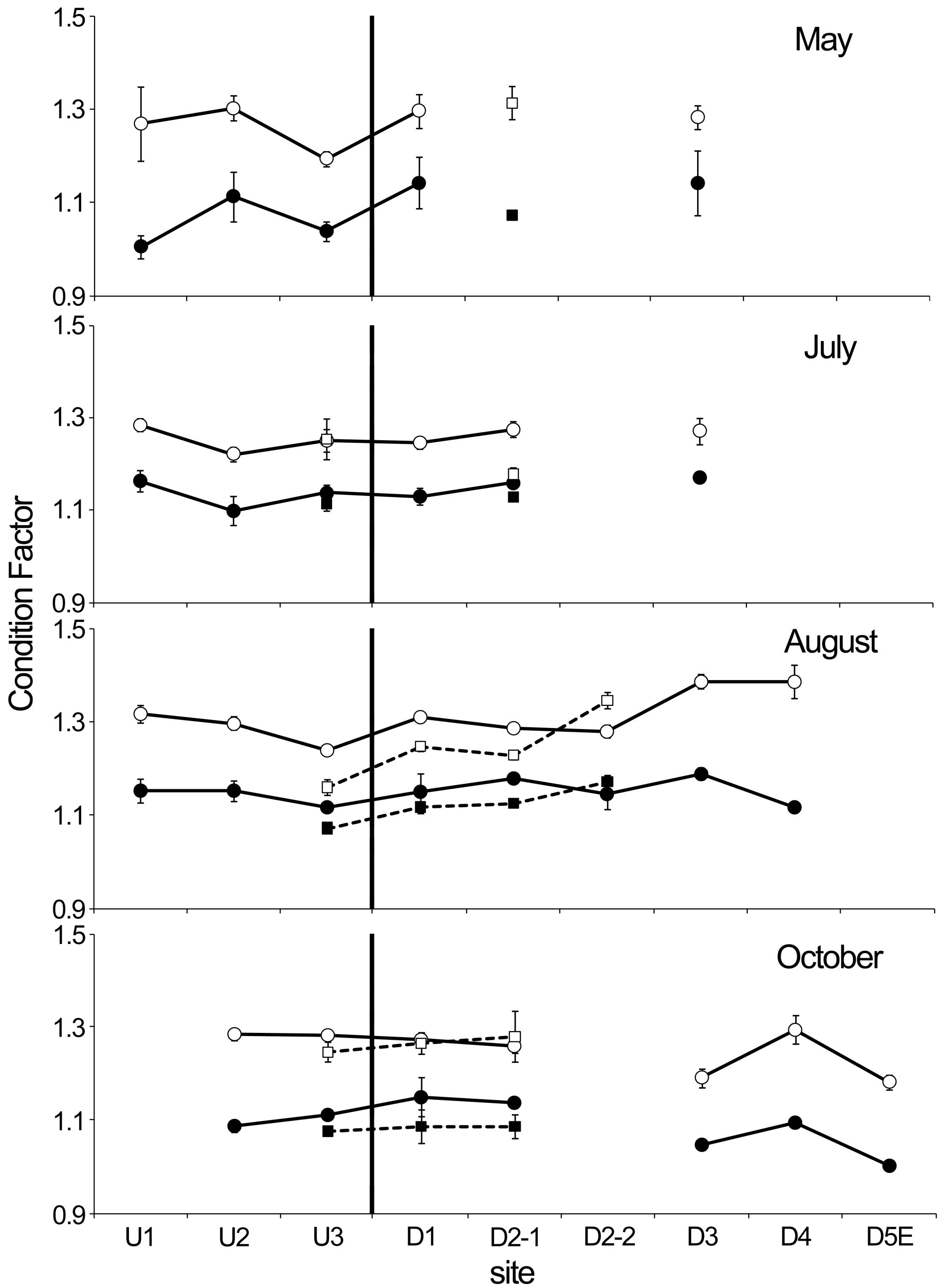


Figure 16: Condition factors of Rainbow (○) and Greenside Darters (●) between May 2009 and March 2010. Squares (□ and ■) indicate unexposed/less exposed sites on the east bank of the river. The vertical bar indicates the point of the WWTP effluent outfall. Error bars represent the standard error.

ammonia during mixing and settling treatment, and the subsequent nitrification of the remaining enriched ammonia (Heaton 1986). Rotating Biological Contactors (RBCs) in the Guelph WWTP tertiary treatment process contribute to the nitrification of ammonia to nitrate (City of Guelph 2007), likely contributing to enriched $\delta^{15}N$ in the WWTP effluent. The carbon enrichment process can be more complicated, but Loomer (2008) speculated that it was enriched $CO_2$ that was the source of enriched $^{13}C$ in the effluent. The cause of this may be from the uptake of dissolved $CO_2$ by algae in the clarifier ponds. This would occur when algae preferentially use the lighter $^{12}C$ atoms, thus enriching the remaining $CO_2$. Atmospheric $CO_2$ replacement is slow in stagnant water, but contains enriched carbon, which further leads to more enriched DIC in the clarifier ponds (Wayland and Hobson 2001).

$\delta^{13}C$ and $\delta^{15}N$ of Greenside Darters diverged from those of Rainbow Darters at the immediate downstream site (D1W) in August and October 2009, as seen similarly between the same fish species in October 2008 by Brown et al. (2011). The divergence in isotopic signatures in the two species of darters took place some time after July, as the $\delta^{13}C$ and $\delta^{15}N$ of both fish were very similar in July and followed similar trends in May. This occurrence coincided with a large increase in Rainbow Darter abundance compared to that of Greenside Darters at the immediate downstream site (D1W) (Chapter 2). Brown et al. (2011) hypothesized that a possible reason for the 2008 results was a shift of Rainbow Darters to a food source with a higher trophic level. This would imply that Greenside Darter diets would not change downstream as the fish continued to feed normally in the effluent plume on organisms of the same trophic level (and isotopic signature). The data from the present study do not support this hypothesis, but seem to indicate that the relative shift in isotopic signatures is a result of a change in diet of the Greenside Darter. Invertebrate taxa from the immediate downstream site in May, July, and August 2009 had more enriched $\delta^{13}C$ and $\delta^{15}N$ than invertebrates from the nearest upstream site, indicating that enriched nutrients from the WWTP effluent were indeed incorporated into the base of the food web. Grazer, collector and predator benthic invertebrate isotopic signatures shifted similarly downstream, and seemingly incorporated sewage derived nutrients similarly. Invertebrates (incorporating multiple different feeding guilds) showed a large range of $\delta^{13}C$ and $\delta^{15}N$, placing the $\delta^{15}N$ of fish about 1-2 trophic levels more enriched than that of the entire range of benthic invertebrates. Since carbon isotopes fractionate very little between trophic levels, the $\delta^{13}C$ of fish should have remained similar to their diet. However, from the data it was difficult to pinpoint any specific species of prey that contributed heavily to the $\delta^{13}C$ of either darter species. The $^{13}C$ signatures of fish are in the middle of the range of invertebrates, which is in support of the stomach content data suggesting that both darters eat a variety of different taxa. The increase of both isotope signatures in Rainbow Darters is consistent with a general increase in $\delta^{13}C$ and $\delta^{15}N$ in the food web. White Sucker (*Catostomus commersonii*) and Longnose Dace (*Rhinichthys cataractae*) collected in August also showed isotopic enrichment downstream of the outfall (Appendix B).

This new hypothesis is supported by the analysis of stomach contents of fish in 2009, which revealed variability in seasonal and spatial diet data between darter species. The immediate downstream diets of the two darter species differed from one another in August and October, supporting the notion that differences between diets resulted in a divergence in isotopic signatures. In these late summer and early fall months, which can have some of the most diverse feeding habits (Hansen et al. 1986), the diets of Rainbow Darters were more variable than Greenside Darters. The benthic invertebrate community downstream as a whole was very different from upstream, containing mainly isopods (*Caecidotea intermedius*) (Chapter 2) which were present in the stomachs of Rainbow Darters. The Rainbow Darters' ability to consume a larger variety of prey is consistent with previous findings (Hansen et al. 1986). Considering the wide range of $\delta^{13}C$ and $\delta^{15}N$ among invertebrate taxa, diet differences may have contributed to the diverging isotopic signatures of the two fish. In particular, the greater prevalence of Ephemeropterans in Greenside Darter stomachs in August may have consisted of Ephemeropterans that contained low $\delta^{13}C$ and $\delta^{15}N$. This could mean that, despite the more enriched invertebrate signatures at site D1W, Greenside Darters chose to eat food that, despite incorporating WWTP nutrients, had lower isotopic signatures than invertebrates in the upstream diet. For instance, Baetidae had relatively lower isotopic signatures in August than other Ephemeropterans and Trichopterans. If the Greenside Darters incorporated these into the diet it may have lead to a slightly more depleted diet that would be reflected in their muscle tissue. The Greenside Darters did show an increase in Ephemeroptera in gut contents in August relative to upstream and the Rainbow Darter. Unfortunately, degradation of body parts made them difficult to identify to the genus level. Alford and Beckett (2007) found some darter species to be very specific as to which species of chironomids they ate, regardless of their availability. This could indicate that the identification of stomach contents to the genus or species level is important to more accurately interpret isotopic signatures. Interestingly, orthocladiinae chironomids, the most common sub-family of chironomids found in darter stomachs, were not commonly caught in benthic community collections. It is not clear as to why this was, but it could be that darters were selectively choosing orthoclads as food, or they were too small to be collected by the 500 µm net used in benthic invertebrate collections.

Our hypothesis is further upheld by seasonal diet shifts that differ between the two darters, presumably in response to the seasonally changing benthic invertebrate community and isotopic signatures. Based on stomach contents, the diets shifted from Chironomidae in May to mainly Trichoptera in July, and were more diverse in August demonstrating that these darters exhibit the generalist and opportunistic behavior common to many stream fishes (Gerking 1994). Under typical conditions, both species have been found to eat mainly chironomids (Hlohowskyj and White 1983; Schlosser and Toth 1984; Hansen et al. 1986; Wehnes 1973), but some diversity has been seen in Rainbow Darters, which have fed on significant rations of Trichopterans and Ephemeropterans (Hlohowskyj and White 1983; Adamson and Wissing 1977). Small crayfish, snails, and minnow and lamprey eggs have also shown up in the stomachs of Rainbow Darters (Winn 1958; Turner 1921). The different mouth and body morphologies of the two species allow different feeding strategies (Page and Swofford 1984). Rainbow Darters have a terminal mouth and so may pick food off of the tops and sides of rocks or plants (Page and Swofford 1984; Schlosser and Toth 1984), while Greenside Darters have a subterminal mouth and feed off of the tops of rocks (Page and Swofford 1984). Wehnes (1973) found darters with subterminal mouths to be positively correlated with the amount of chironomids eaten, potentially demonstrating the limited feeding capabilities of Greenside Darters. In a direct comparison of the two diets, Wynes and Wissing (1982) found Rainbow Darters to consume a larger range of food sizes and a larger range of taxa. Hansen et al. (1986) found diet diversity peaked in both species in mid to late summer, while Hlohowskyj and White (1983) found their diets to overlap the least in the summer. Collectively, the literature supports our theory that changes in Rainbow and Greenside Darter diets occurring downstream of the WWTP in the summer, contributed to their diverging $\delta^{13}C$ and $\delta^{15}N$ signatures.

Another possible reason for the divergence of the $\delta^{13}C$ and $\delta^{15}N$, and stomach contents, of the two darters at the site immediately downstream of the outfall (D1W) in August and October is that Greenside Darters may have been consuming food that was not exposed to the effluent. The monthly fluctuation patterns of the two darter species' $\delta^{13}C$ and $\delta^{15}N$ at site D1W changed differently from one another. The $\delta^{13}C$ of fish at the immediate upstream site (U3W) maintained a very similar trend over time indicating similar influences on primary food sources at this reference site. This trend changed though in the presence of sewage effluent, only 240 m downstream (D1W), where the $\delta^{13}C$ of the two darters followed different trends, with the greatest divergence between the two in August. Downstream seasonal $\delta^{15}N$ trends of darters diverged the most in August as well, giving credence to this possibility that Greenside Darters captured within the effluent plume were feeding outside of the plume. If Greenside Darters were feeding outside of the plume, it would mean that they were either feeding upstream of the effluent outfall or towards the middle of the river and the opposite shore (D1E). Feeding upstream of the effluent outfall (more than 100 metres upstream) and living downstream does not seem reasonable for a small benthic fish, and it also seems unlikely that all of the Greenside Darters caught at D1W and other exposed sites were fish that had been living upstream and were migrating downstream. It is most plausible that

Greenside Darters were feeding near the middle or opposite shore. Very similar $\delta^{13}C$ and $\delta^{15}N$ of Greenside Darters from the opposite shore of the immediate downstream site (D1E) and site D1W support this hypothesis. Isotopic signatures at the next downstream site are also supportive, particularly the $\delta^{15}N$ of both species at site D2-1E (the opposite side of the river to site D2-1W). These signatures are both less than the signature of Rainbow Darters at D2-1W, and have become more enriched than at site D1E, reflecting the mixing of the plume with the rest of the river and the incorporation of enriched sewage-derived nitrogen into the food base.

It is difficult to understand why Greenside Darters would feed on one side of a river and take refuge on the other side of the river. One explanation may be that Greenside Darters fed nocturnally or early in the morning on the unimpacted side of the river, and then took cover on the effluent-exposed side when fish collections took place later in the morning. The effluent exposed side contained aquatic moss, which may not have been a desirable habitat for Greenside Darter feeding in mid-summer. Greenberg (1991) observed large specimens on the tops of rocks at night, which may be an indication of a nocturnal feeding habit. This contrasts however with the consensus that all darters are visual feeders (Greenberg 1991; Adamson and Wissing 1977). As well, stable isotope studies have revealed that benthic fish tend to inhabit small home ranges in a river during the summer, with little side to side (Brown 2010) and upstream-downstream movement (Gray et al. 2004; Cunjak et al. 2005). A more plausible second explanation may be that the Greenside Darters collected at site D1W were caught close to the middle of the river where there is less effluent exposure. Since our sampling method consisted of a 10 m wide reach that was measured perpendicularly from the shore, there was not enough spatial resolution to be able to distinguish if a fish was caught 1 m from shore or 10 m from shore. The Speed River is a small river, little more than 20 m wide at most sites; it is possible that Greenside Darters were caught at the very edge of our sampling reach, where the thalweg brought swifter flowing water and larger substrate. This habitat type is more appealing to Greenside Darters (Stauffer et al. 1996; Hlohowskyj and Wissing 1986; Chipps et al. 1994; Greenberg 1991). Low relative abundances of Greenside Darters and high abundances of Rainbow Darters at the immediate downstream site (D1W) were reported in Chapter 2, indicating that the immediate downstream environment was not very desirable for Greenside Darters. It is possible that Greenside Darters ceased to feed on invertebrates in the effluent plume, when conditions in the summer began to change. As mentioned, the aquatic moss and *Cladophora* sp. growth from nutrients in the effluent at D1W was substantial, and definitely contributed to an altered feeding environment. Since Rainbow Darters were so abundant at this downstream site, particularly in August, they may have also contributed to deterring Greenside Darters from feeding in this zone.

In this study, the highest $\delta^{15}N$ signatures of Hydracarina and Hydropsychidae at the immediate downstream site peaked in May and July respectively. This was observed differently by Loomer (2008) who found benthic invertebrate signatures at the same treatment plant to increase from May to September. It was thought that $^{15}N$ accumulated in the tissues of organisms as they grew throughout the summer, thus increasing their respective $\delta^{15}N$. The factors that affect the $\delta^{13}C$ and $\delta^{15}N$ in the muscle of fish are numerous. The turnover rate of fish muscle is highly dependent on fish size (Vander Zanden et al. 1998) and rate of growth (Hesslein et al. 1993; Maruyama et al. 2001). Perga and Gerdeaux (2005) found that in Whitefish (*Coregonus lavaretus*) muscle, there was a lag of 4-5 months between the consumption and incorporation of the $\delta^{13}C$ and $\delta^{15}N$ of its prey. Darters are much smaller fish and so the lag time may be close to a month. In the autumn and winter months, isotopic signatures of muscle did not reflect those of its food, because it was hypothesized that the consumed food was used directly for energy production and gonad growth, and not somatic growth. The incorporation of food into muscle tissue is likely seasonal in darters, which may make it difficult to directly link diet to changes in isotopic ratios. Both species spawn in early May in the Grand River and previous work has shown that both species grow rapidly during the early summer (Brown et al. 2011). Both darter species fed heavily on Orthocladiinae chironomids in the riffles in early March, and then changed to Trichoptera as they became more available. The diets diverged immediately downstream in August as the availability of prey changed (Chapter 2) and Bryophyte and *Cladophora sp*. growth dominated the habitat. It appears that the isotopic signature changes observed downstream in August were likely due to diet or food source shift of invertebrate prey that occurred in mid-summer. The signatures of fish tissues in October may be a result of continued changes in diet or a lag in the incorporation of food to the isotopic signatures.

Food consumption may have also impacted $^{15}N$ signatures of darters. Greenside Darters consumed significantly more prey than Rainbow Darters at many of the sites and at most times of the year. Since $\delta^{15}N$ have been shown to increase from limited food availability due to increased $^{14}N$ excretion (Adams and Sterner 2000), there is a chance that $\delta^{15}N$ of Rainbow Darters were increased at the immediate downstream site (D1W) as they appeared to consume less food in August. This may have resulted from increased competition for food as there were very high densities of Rainbow Darters at this site at the time (Chapter 2). However, from qualitative benthic invertebrate analysis there did not appear to be limiting food resources at any of the sites, particularly at site D1W. Greenside Darters are also a larger fish than Rainbow Darters that live in fast flowing water, and so likely have higher energy demands. This could easily explain their greater food consumption. It is also important to remember that stomach content analysis is only a snapshot of what fish were eating within a small timeframe, so the number of prey in their stomachs could change throughout the day as it has been shown to do in other different darter species (Adamson and Wissing 1977). The mass or size of each food item was not measured either, so Rainbow Darters may have consumed larger prey limiting their need to consume as much food. The difference seen in food consumption likely did not contribute to large changes in isotopic signatures, but it helps to further reveal differences in feeding behaviours between these two darter species.

Condition factors of the fish downstream were similar to those at upstream sites. Fish exposed to treated sewage effluent have been shown to experience an increase in condition (Porter and Janz 2003; Galloway et al. 2003), however in this study only a slight increase in Rainbow and Greenside Darters in August immediately downstream was observed, and no increase in the other months. The differences between the sexes may have contributed to obscuring any observable effects. The C:N ratios in August (Appendix C) were higher than any other months, indicating that the lipid stores were the highest during this time. This means that the slight increase in August condition factors were likely a result of increased energy storage due to an abundance of food during the summer.

In conclusion, all of the invertebrates exposed to the wastewater effluent utilized the sewage-derived nutrients enriched in $\delta^{13}C$ and $\delta^{15}N$. Rainbow Darters reflected these signatures, but Greenside Darters did not in August and October 2009. The results are similar to those reported by Brown et al. (2011) for fish collected in August 2008. Most invertebrates and fish, including Rainbow Darters, followed similar seasonal and spatial isotopic patterns in response to nutrient enrichment of the wastewater outfall. In contrast, the lack of a shift in isotopic signatures in late summer in Greenside Darters appears to be linked to a change in food selection habits or in the source of its food. They may be selecting different prey items that have lower isotopic signatures, or foraging outside of the effluent plume. The results suggest that there are subtle yet detectable changes in the behavior of fish in response to effluent discharges of a tertiary treated wastewater plant. Despite the high quality of the effluent, if the city's population grows at the projected rate, these subtle impacts could become more pronounced in the future as more effluent enters the river.

# Chapter 4:
# Conclusion

The tertiary effluent from the Guelph WWTP caused minor changes to the relative abundances of Rainbow Darters (*E. caeruleum*), Greenside Darters (*E. blennioides*), and benthic invertebrates in the downstream receiving environment of the Speed River. At the site with the greatest effluent exposure (D1W), Rainbow Darters were by far the most abundant species in the warmer months of the summer. This was particularly true in August, when the majority of fish were of the 2nd to 3rd year class. The effluent also caused a shift in the benthic invertebrate community from high proportions of the Elmidae family upstream at sites U2 and U3W to high proportions of the isopod *C. intermedius* downstream. The $\delta^{13}C$ and $\delta^{15}N$ of all invertebrates sampled in May, July, and August increased downstream of the effluent outfall, indicating the assimilation of sewage derived nutrients into the tissues of primary consumers in the food web. In May and July, these were reflected similarly in the signatures of the two fish species, however in August and October, Greenside Darters failed to show heavier $\delta^{13}C$ and $\delta^{15}N$ downstream. Both the $\delta^{13}C$ and $\delta^{15}N$ of the two fish stayed diverged at most exposed downstream sites during these months. The stomach contents of both darter species were variable, but they suggested that the Rainbow Darters were able to diversify their diets more so than the Greenside Darters.

Evidence from this research suggests that the altered downstream conditions, which consisted of heavy primary production, an altered temperature regime and an altered food base, were more suited for Rainbow Darters. The Rainbow Darter isotopic signatures increased in late summer similar to the majority of invertebrates. Greenside Darter isotopic signatures did not increase in a similar pattern to the Rainbow Darter or other fish. This divergence is possibly a result of altered feeding behaviours triggered by the altered downstream physical and ecological conditions. It is possible that the changes in habitat and food availability resulted in a change in diet that lowered the isotopic ratios in the diet relative to those upstream. Alternatively it is possible that the Greenside Darters fed more towards the middle or opposite side of the river where the effluent exposure was less, and the habitat more suitable for feeding. This is supported by the $\delta^{13}C$ and $\delta^{15}N$ of Greenside Darters captured on the east banks (unexposed shore) of the two most immediately downstream sites which were very similar to signatures of fish on the west bank (Figure 13).

Future studies should focus on identifying the microhabitat use of Rainbow and Greenside Darters in the Speed River. This could be done through direct observation (eg. mask and snorkel) or tagging. Utilizing a spot electroshocking technique, whereby a small space directly around the anode is sampled, in tandem with a detailed habitat survey may also be useful. A new technology to fisheries called side-scan sonar may accomplish this by assessing the physical habitat for the entire width of the river. In combination with electroshocking, this may allow for an accurate depiction of which habitats are used by which fish species.

The chemical habitat was only briefly assessed in these studies as part of a standard field protocol. Although low dissolved oxygen (DO) was never detected during morning field collections or at GRCA monitoring stations, a diel profile of the DO, especially at the immediate downstream site (D1W) would be useful in determining if oxygen depletion remains a contributing factor that can potentially affect the fish community in the Speed River. The heavy macrophyte growth at site D1W during July and August certainly must have caused a nightly DO demand, however it is unlikely that values ever dipped low enough to cause effects.

Stomach content analysis was another aspect of this research that could be enhanced. Fish diet data from this study was unable to show any conclusive evidence that diet was responsible for isotopic shifts seen in darters. Since food item identification was typically only taken to the family level, it left the possibility that the diets of Greenside Darters contained specific species of chironomids or other organisms that did not uptake sewage derived nutrients or had lower signatures. Since Alford and Beckett (2007) found four species of darter to only eat a few chironomid species, a more in-depth stomach content analysis to identify individual species could prove informative. The use of another method such as gastric lavage may be a viable option to not only sample more fish without sacrificing them, but may help to extract food items more quickly so as to avoid partial digestion, which may have been a problem with the method used in this study.

When measuring the isotopic signatures in fish, Perga and Gerdeaux (2005) reported that the time for the isotopic signature of food to be incorporated into the liver is about one month, and is much faster than the lag time for muscle. Since the fish is sacrificed anyhow, to supply the muscle sample, using fish livers for stable isotope analysis may give a more reliable indicator of when a change in diet occurs throughout the year. Jardine et al. (2011) found that fin clips are also reliable indicators of the $\delta^{13}C$ and $\delta^{15}N$ of fish muscle. Fin clips on small darters would likely compromise their swimming ability, but this technique could be useful in larger predatory fish to determine if sewage derived nutrients reach the predators in the river. Smallmouth Bass (*Micropterus dolomieu*) and Northern Pike (*Esox lucius*) are the two largest bodied predators in the river that could be analyzed using this method.

Of the invertebrate taxa analyzed for $^{13}C$ and $^{15}N$, all of them increased immediately downstream of the effluent outfall, making it unnecessary to collect them all. It may also be useful to collect those specific taxa that are present at all sites during all sampling seasons such as crayfish, Hydropsychidae, and individual genera of Chironomidae. This way, multiple samples could be collected for each taxon and the variability could be more accurately calculated. Since one of the inadequacies of this study was that the invertebrates used for isotope analysis were not exactly indicative of the darters' diets, in the future, focus may be better placed on collecting the actual invertebrates that the fish were eating by immediately analyzing the stomach contents of the fish and then targeting those invertebrates from isotope analysis. This could allow for more direct diet to fish isotope comparisons, and reduce speculation. To further characterize the impacts of the effluent on the isotopic signatures, it will be useful to measure the actual signatures of the nitrogen and carbon sources in the effluent. This could allow for estimates of the percentage of sewage derived nutrients used by these invertebrates (DeBruyn and Rasmussen 2002).

The findings of these studies show that with a high quality effluent, the impacts to river ecosystems can be limited even in a small receiving environment. The current effluent increased the total number of fish immediately downstream, but made it useful habitat only for certain adaptable species. An increase in wastewater load will likely mean that the Guelph WWTP will need to maintain high quality treatment and adapt to increasing wastewater loads to maintain the current condition of the aquatic environment and avoid any further impacts.

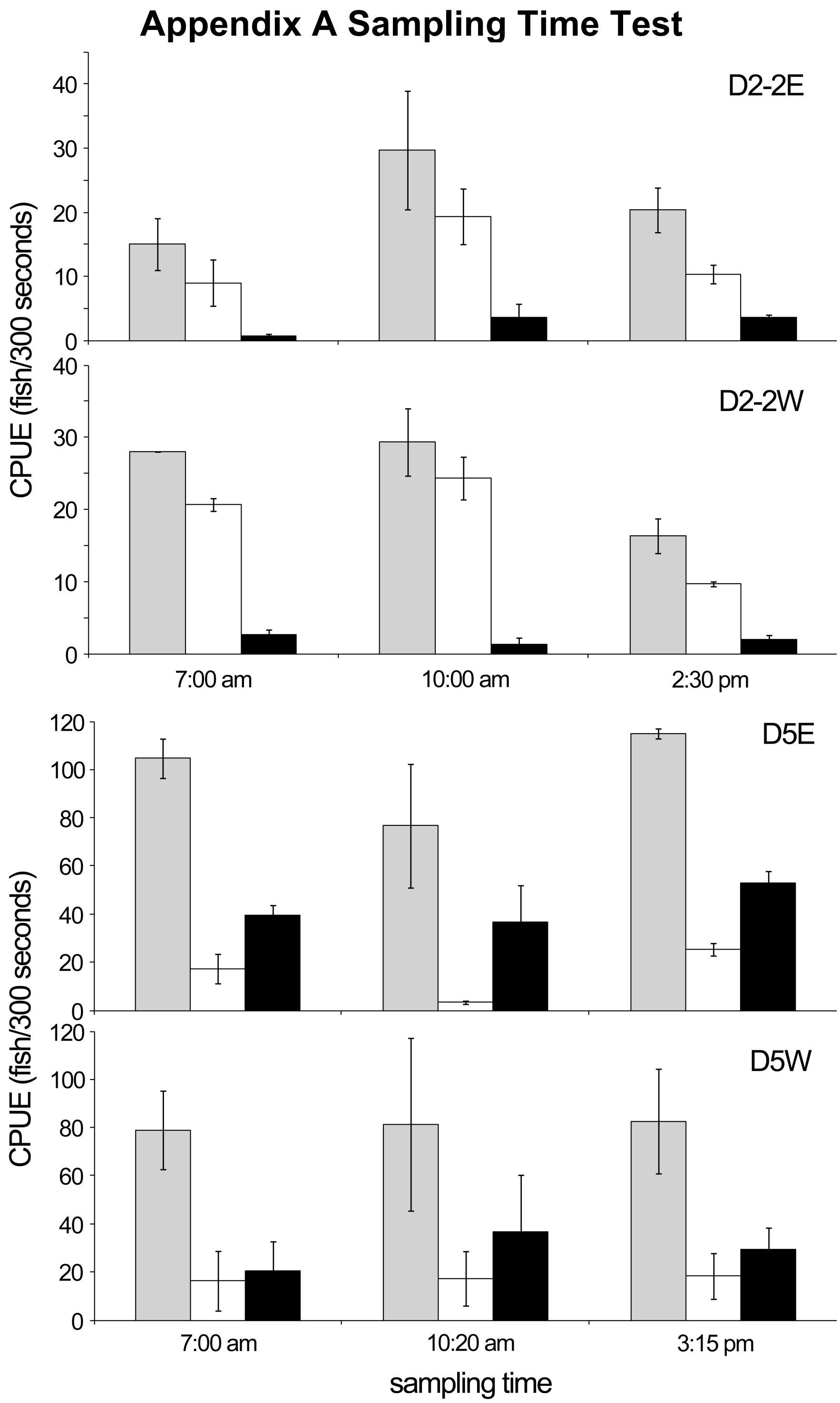


Figure 17: Mean abundances (± SE) of fish from four sites in August 2009 (n=6). Grey bars are total fish, white are Rainbow Darters, and black are Greenside Darters. The three sampling times chosen represent early morning, mid-morning, and afternoon.

## Appendix B $\delta^{13}C$ and $\delta^{15}N$ of White Sucker and Longnose Dace

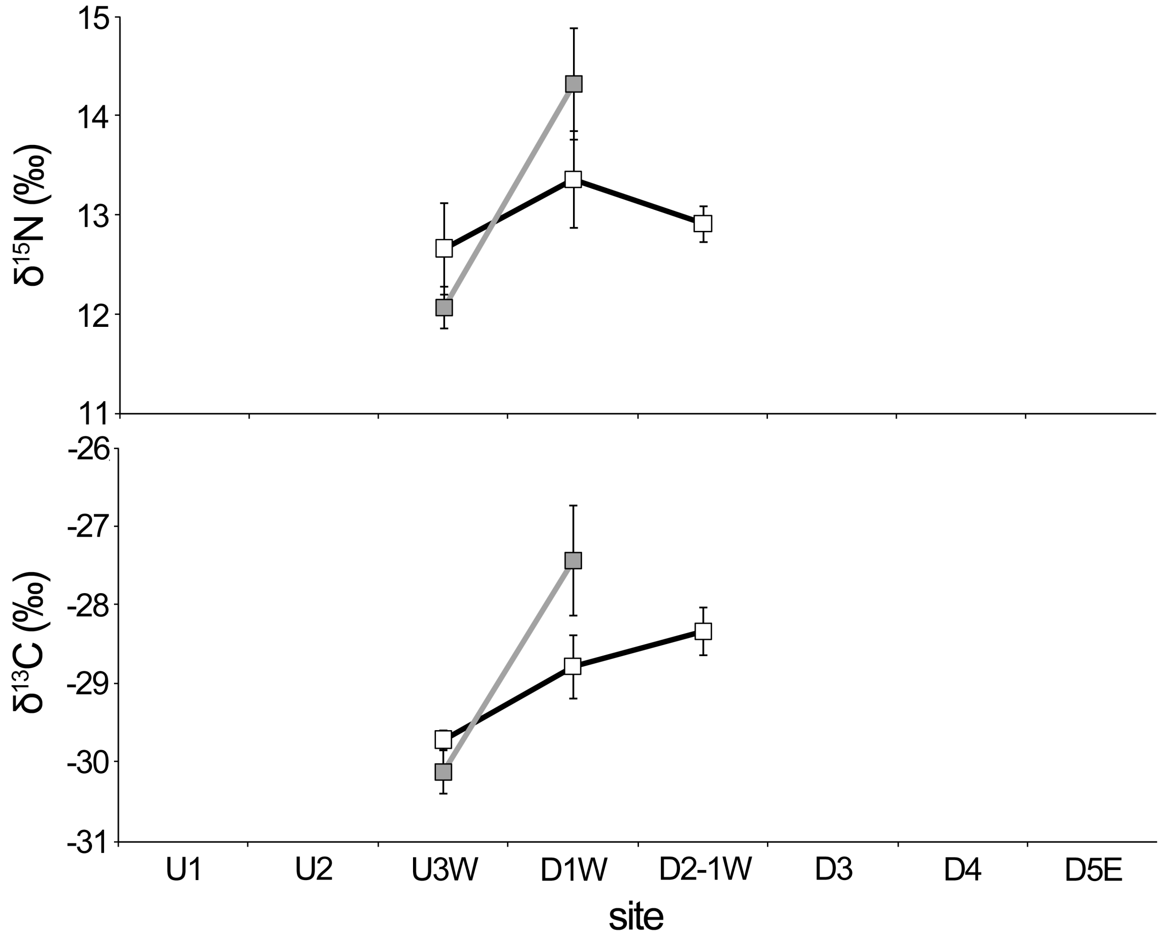


Figure 18: Mean $\delta^{13}C$ and $\delta^{15}N$ of Longnose Dace (*Rhinichthys cataractae*) (white squares) and White Suckers (*Catostomus commersonii*) (grey squares) immediately upstream and downstream of the Guelph WWTP effluent outfall in August 2009.

## Appendix C C:N Ratios

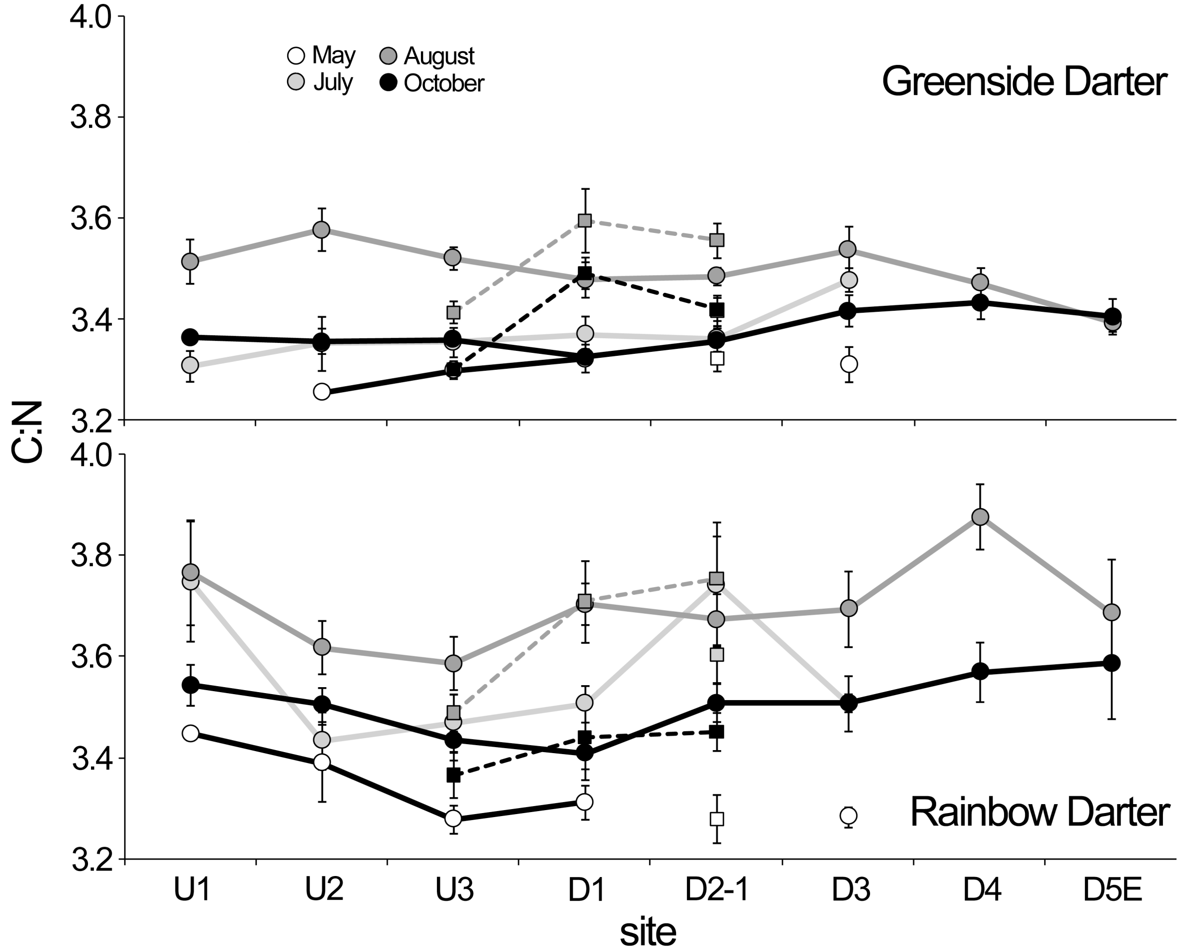


Figure 19: Mean C:N ratios (± SE) for Greenside and Rainbow Darters in 2009. Circles represent exposed sites on the west bank, and squares represent sites unexposed or partially unexposed on the east bank.

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