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Environmental assessment of stormwater pond impacts on a Rouge River tributary

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ENVIRONMENTAL ASSESSMENT OF STORMWATER POND IMPACTS ON A ROUGE RIVER TRIBUTARY

by

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Abstract

Environmental Assessment of Stormwater Pond Impacts on a Rouge River Tributary

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This study was conducted to evaluate impacts of stormwater ponds in the Municipality of the Town of Richmond Hill on select physical (temperature, dissolved oxygen, and pH) chemical (nutrients) and biological (macroinvertebrate community, macrophyte and experimental *Hyalella azteca* and *Daphnia magna* enclosures) aspects of a Rouge River tributary. Over a five-month period five sites along the tributary close to the outfall of the stormwater ponds were sampled to determine if there were any impacts, cumulatively (with increasing number of pond outfalls along the tributary) and locally (above and below an outfall). Physical and nutrient parameters showed no significant degradation in water quality, either cumulatively or locally. Macrophytic data showed some decrease in biomass at downstream sites, but no decrease in diversity or species richness. Survivorship in the enclosures containing *Hyalella azteca* and *Daphnia magna* showed no significant cumulative change. Analysis of the macroinvertebrate community showed no cumulative or local impact until the farthest downstream site.

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LIST OF ABBREVIATIONS

ANOVA – Analysis of Variance

APHA – American Public Health Association

BMP – Best Management Practices

CEES – Center for Earth and Environmental Science

CSO – Combined Sewer Overflow

DO – Dissolved Oxygen

EC – Environment Canada (when referring to an organization)

EC – *Escherichia coli* (when referring to biological contamination)

FC(U) – Fecal Coliform (Unit)

GIS – Geographical Information System

IC – Inorganic Carbon

LEL – Lower Effects Level

LSD – Least Significant Difference

MOEE – Ministry of Environment and Energy

OBBN – Ontario Benthos Biomonitoring Network

NPS – Non-Point-Source

NWC – National Water Council (Great Britain)

PAH – Polyaromatic (Polycyclic Aromatic) Hydrocarbon

PBDE – Polybrominated Diphenyl Ether

PCB – Polychlorinated Biphenyl

PSQG – Provincial Sediment Quality Guidelines

TC – Total Carbon

TOC – Total Organic Carbon

TSS – Total Suspended Solids

US EPA – US Environmental Protection Agency

INTRODUCTION

This project was conducted to find out if select stormwater ponds in the Municipality of the Town or Richmond Hill have an adverse impact on the receiving waters of a Rouge River tributary. To this end physical, chemical, and biological assessment of five sites in the study region was performed to determine if there is any cumulative (increasing number of pond outfalls) or local (above and below the outfall) impacts. In the course of conducting this assessment a protocol utilizing biological components was developed that may be used by municipalities in future assessments of potential stormwater pond impacts.

Stormwater, or, rather, anything that may be carried by runoff during a storm event, has been long considered non-point-source (NPS) pollution. In a situation where nothing has been done to redirect stormwater, it will follow the path of least resistance to the nearest water body, or infiltrate the groundwater.

The normal hydrological cycle depends on proper infiltration of water after a storm. Growing urbanization of the landscape in many countries is preventing this. As cities grow, the pavement and buildings cover more and more area, cutting down on the permeability and decreasing the amount of water that infiltrates to the groundwater. However, since the rainfall does not decrease simply because a city is being built, the water has to go somewhere. This water runs off the paved streets, roofs, driveways, parking lots, and lawns (the amount that cannot be absorbed by the lawn soil). Depending on the city design, it can either be discharged directly into the local waterways (receiving waters), it can be diverted to stormwater management/treatment facilities via dedicated storm sewers, or it can be directed to the combined sewer overflows (CSOs).

How is an increased urban surface runoff problematic? One of the major problems is the amount of anthropogenic contaminants that are carried with it to the receiving waters. These contaminants may present a danger to the wildlife in the surrounding ecosystem. Also, the sheer volume and force of water during a strong storm event, if directed to a receiving waterway, can easily lead to erosion of water channels. Lack of surface permeability and infiltration can cause excessive amounts of water to swell the receiving water ways, which can, in turn, cause flooding downstream. Surface water in the urban environment is also unwelcome, as it can cause temporary flooding of the streets as well as any part of a building below the ground level unless it is properly isolated. This problem can be somewhat remedied by incorporation of stormwater sewers into the urban infrastructure. Such sewers divert the water from the streets to underground channels and away from populated areas.

There are two different stormwater sewer systems – dedicated stormwater sewer systems and combined sewer overflows (CSOs). A dedicated stormwater sewer system is built separately from the municipal sewer that carries the wastewater generated by the population. In a CSO system, the storm sewer and the municipal sewer overlap. During dry weather all water in the sewer goes to the local wastewater treatment plant. During a storm event, up to a certain point all water, municipal wastewater and stormwater still go to the wastewater treatment plant; however, if the amount of rainfall exceeds the treatment plant capacity, the overflow from the combined sewers, including the municipal sewage, is released directly into the receiving waters, bypassing the treatment facilities. This presents obvious problems, as the sanitary sewers carry various pollutants (Gromaire et al. 2001), which can (and do) make their way to the surrounding ecosystem causing severe contamination of the receiving waters (Rochfort et al. 2000).

As mentioned previously, a major problem with large amount of surface runoff is the amount of anthropogenic pollutants that it carries. Contaminants that stormwater potentially carries to receiving waters can include sediment (Marsalek et al. 1997), metals (Mayer et al. 1996; Marsalek et al. 1997), organics (Murakami et al. 2004), nutrients (Mayer et al. 1996), and faecal coliforms (Marsalek and Rochfort 2004). Some studies have even investigated heat being transferred from large paved areas to stormwater during a rain event (e.g. Van Buren et al. 2000). Some of these contaminants, such as sediments and the metals and organics that are adsorbed to them, can be accumulated in the stormwater ponds (Marsalek et al. 1997). The following section will provide an overview of several studies that focused on specific anthropogenic substances that are present in, or can make their way into stormwater runoff.

Contaminants

Sediment

Erosion is a natural process, which takes place in any area – even those not developed by man. In an urbanized area sediment input into the local ecosystem through runoff can come from sanding in the winter, dust, construction, erosion of lawns, gardens, and parks. Particulate matter, which is light enough to be carried by the runoff is referred to, collectively, as suspended solids. It should be noted, however, that while all sediment that is carried by runoff (and is, therefore, "suspended") is classified as suspended solids, not all suspended solids are sediments. As mentioned above, a portion of the suspended matter is minute organic debris and is not considered sediment.

Depending on the type of the urban environment, suspended solids will have different fractions of sediment of different grain sizes. Grain size in sediment is very important, as it determines the sedimentation (settling) velocity in the water column. Settling velocity is the speed with which a particle moves down in the water column (Reynolds and Richards 1995). Settling velocity is determined by the mass of the particle and its surface area such that heavier particles with a smaller surface area settle out faster than lighter particles with a larger surface area (Reynolds and Richards 1995). This property of solids determines how far the particle will travel with the stormwater, and how fast it will settle out into the sediment. Based on the settling velocity, gravel and coarse sand will settle out fairly fast, fine sand will travel further, and dust and clay will travel furthest. This property is defined by the equation (1) (Hemond and Fechner-Levy 2000)

$$\omega_f = \frac{(2/9) \times g \times (\rho_s / \rho_f - 1) \times r^2}{\eta_f} \tag{1}$$

Where:

ω_f is the settling velocity

g is acceleration due to gravity

ρ_s is the density of the spherical particle

ρ_f is the density of the fluid, and

η_f is the kinematic viscosity of the fluid

The formula above is configured for spherical particles; however, it can be used for non-spherical particles if its ρ_s is determined (Hemond and Fechner-Levy 2000).

Another property that determines how fast a particle will settle out of the water column is the particle charge. Some very small particles are called colloids and have an electrostatic charge associated with them (Reynolds and Richards 1995). This charge makes the particles repel each other, causing them to remain in suspension longer or not settle at all (Reynolds and Richards 1995).

Suspended solids provide a sorbtion medium for various contaminants such as metals and organic compounds (Tessier et al. 1979; Hemond and Fechner-Levy 2000). This property of the suspended sediment imparts on it a toxicity that may not have been present originally. It also facilitates the aquatic transport of generally non-polar (hydrophobic) organic compounds (Hemond and Fechner-Levy 2000).

Besides the fact that the suspended sediment acts as a carrier for metals and organic compounds, the physical presence of suspended solids and turbidity associated with it has been shown to cause problems. Large amounts of suspended solids cause high turbidity, which decreases light penetration in water (Gliwicz 1986; Mackie 2001). This decreases primary productivity, which generates impacts up the food chain to the higher trophic levels. High levels of suspended solids can also impair the ability of zooplankton and benthos to filter-feed and graze by clogging the feeding apparatus (Arruda et al. 1983; Kirk 1991). It was also found that different organisms in the invertebrate population have different tolerances to suspended sediments, which allows for competitive exclusion of certain organisms in the presence of suspended solids (Kirk 1991). While, as discussed above, the speed with which suspended solids settle out depends on the surface area and weight of the particle, unless the particle is a colloid (or has colloidal properties) it will, eventually, settle out. This may present a problem to the benthic invertebrates. Bowlby et al. (1987) found that the abundance of benthic macroinvertebrates decreased downstream of a construction site and Gray and Ward (1982) found that the Chironomidae decreased in abundance 90% when sediment was introduced to the system. It is interesting, though, that they found that densities of Ephemeroptera, generally considered clean water species (Mackie 2001) increased below this construction site (Grey and Ward 1982).

Numerous studies have been conducted on stormwater and total suspended solids are usually measured as one of the parameters (e.g. Hoffman et al. 1984; Martin 1988; Oberts and Osgood 1991; Mayer et al. 1996; Mallin et al. 2002; Graves et al. 2004; Olding et al. 2004; Wood et al. 2004 and others). As mentioned before, the amount of suspended solids varies greatly with type of land use in the catchment. In Mayer et al. (1996) the data showed that in residential areas the total suspended solids varied from 5.0 mg/L to 267.0 mg/L, in industrial sector the range was between 8.8 and 119 mg/L, and open space concentrations varied from 0.5 to 66.8 mg/L. These readings were taken at the inlet of stormwater management facilities, providing an estimate of the suspended solids content before they had a chance to settle.

Nutrients

A major source of nutrients in stormwater runoff is fertilizer used on residential lawns, golf courses, and municipal recreation parks – basically, any large grass area which is habitually fertilized. Mayer et al. (1996) conducted a study on levels of various nutrients, metals, and suspended solids in several stormwater ponds. They found that, among other things, the total dissolved phosphorus at the inlet of the stormwater ponds (runoff that has gone through the catchment and through the storm sewers) ranges from 0.007 mg P/L to 0.480 mg P/L, nitrate (+ nitrite) nitrogen ranges from 0.203 mg N/L to 1.600 mg N/L, and ammonia nitrogen ranges from 0.013 mg N/L to 1.082 mg N/L. Other studies have found that at the inlet of the stormwater treatment facilities the concentrations could be 0.1 to 18.2 mg P/L (total phosphorus), 0.2 to 1.3 mg N/L (nitrate + nitrite), and 0.1 to 0.2 mg N/L as ammonium (Oberts and Osgood 1991;

Mallin et al. 1992; House et al, 1993; Stanley 1996). Environment Canada guidelines for drinking water state that the levels of nitrate nitrogen in surface waters should not exceed 10 mg/L and ammonia nitrogen should not exceed 0.5 mg/L (Environment Canada 1984). No guidelines are available for phosphate levels, however the Center of Earth and Environmental Science (CEES 2005) reports the levels of phosphate phosphorus for groundwater are considered excellent if they are below 0.1 mg/L and poor if they are above 3.0 mg/L.

To relate the nutrient input to urbanization, a GIS study published by Carle et al. (2005) showed that increased urban density and impervious area leads to an increase in total Kjeldahl nitrogen and total phosphorus. Interestingly, this same study suggested that the mean age of the buildings in an urban area is, actually, inversely correlated with the amount of nutrients in the runoff.

Nutrient input effect can be two fold. On one hand, nutrients are vital for the primary producers, which form a base of the food web in any ecosystem (Mackie 2001). As such, input of extra nutrients will lead to increase of primary producers (Schindler 1977; Pentecost 1984; Perrin et al. 1995; Gobler et al. 2007), which may be translated up the food chain to higher trophic levels, resulting in increased abundance of other organisms in the system (Krebs 2001). On the other hand, while moderate nutrient input into the aquatic ecosystem may stimulate the overall aquatic community growth, the increase in certain primary producers may also lead to adverse conditions in the ecosystem. Some algae (e.g. *Microcystis* and *Lyngbya majuscule*) produce toxins and their blooms can release enough to lead to impaired grazing behaviour in zooplankton (Gobler et al. 2007), fish die-offs (Reifel et al. 2002; Albay et al. 2005), and skin irritation in humans (WHO 1984). Nutrient influx can also cause blooms of benign algae which may lead to foul smells and reduction in dissolved oxygen through increased decomposition (Schindler 1977; Pentecost 1984).

It is interesting, however, that some results show that input of certain nutrients can actually inhibit the production of blooms. Dugdale et al. (2006) found that high concentrations of ammonia prevented the nitrate uptake in phytoplankton, reducing the formation of blooms. Unless there is a constant influx of ammonia, however, this effect would most likely be temporary as ammonia is oxidized to nitrate (Mackie 2001).

Metals

One category of the major chemical pollutants is heavy metals. Metals are naturally present in water and sediment. Some metals are essential micronutrients and are necessary for any biological community to thrive. However, excess inputs of heavy metals (for the rest of this paper "metals" refers to heavy metals) into an ecosystem can result in metal toxicity and detrimental effects on biota. Metals in urban pollution can come from numerous sources, but the major sources are roof (Athanasiadis et al. 2004; Chang et al. 2004) and road (Barrett et al. 1995; O'Reilly Brophy and Graney 2004) runoff.

A study by Chang et al. (2004) was conducted to study metal content in runoff from roofs made of common roofing materials, specifically, wood shingle, composition shingle, painted aluminum, and galvanized iron. The authors measured concentrations of Cu^{2+}, Mn^{2+}, Pb^{2+}, Zn^{2+}, Mg^{2+}, and Al^{3+}. They found that all metals with the exception of Mg^{2+} exceeded USEPA (1999) drinking water quality guidelines for at least one roof type. The exceedence was most severe for Cu^{2+} and Zn^{2+}, ranging from 59.6% on composition shingle to 77.9% on aluminum for Cu^{2+}, and from 99.5% on wood shingle and composition shingle to 100% on aluminum and galvanized iron for Zn^{2+}.

Another study, acknowledging that the presence of metals (specifically, zinc) poses a problem to the surrounding environment as non-point contaminants, experimented with ways to remove the metal from the roof runoff before it merges with main flows of the receiving waters. In their study Athanasiadis et al. (2004) looked at two different filters (geotextile and clinoptilolite) that may be used to achieve this. They found that, while rainwater that did not touch the roof had near zero concentrations of Zn^{2+} (suggesting little to no Zn^{2+} in the air being picked up by rainwater) Zn^{2+} content increased to as high as 25 mg/L after it hit the roof. They also found that Zn content in rainwater decreased dramatically after the first flush. Out of the two filters tested, a geotextile filter, while removing some zinc, did not perform well overall. On the other hand, a clinoptilolite filter reduced zinc content of the roof runoff to near zero. This study was conducted to examine the possibility of using filters to ameliorate roof runoff. Such filters are not in common use today and, from the results of this study, more work is still needed before technology is perfected.

The metals can adsorb to particulate matter that is carried by the stormwater. The fraction of the metals that are adsorbed to the particulate matter or dissolved in water depends on pH (Tessier et al. 1979) where acidification facilitates desorption of metals and dissolution in water. Higher pH leads to increased adsorption. A study by Sansalone (1999) was conducted to test the efficiency of two media (silica sand and iron oxide coated silica sand (OCS)) in adsorbing various metals. It was found that a) OCS, while having approximately the same mass per grain, has 300 to 500 times greater surface area and, as such, was much more efficient in providing surface for metal adsorption, and b) when the stormwater runoff was passed through a bed of pavement that raised the runoff pH from 6 to 8.5, the adsorption efficiency raised significantly.

It has been consistently shown that heavy metals in the aquatic environments have adverse effects on the local biota such as algae (Baptista and Vsconcelos 2006; Lin et al. 2007), macrophytes (Naqvi and Rizvi 2000; Kamal et al. 2004; Sanita di Toppi et al. 2007), invertebrates (Canli 2005; Ward et al. 2006; De Schamphelaere et al. 2007), and fish (Diamond et al. 2005; Brooks et al. 2006; Federici et al. 2007). Many plants and phytoplankton accumulate metals (Naqvi and Rizvi 2000; Kamal et al. 2004; Canli 2005; Sanita di Toppi et al. 2007) and if they are then consumed by organisms higher on the food chain, the metals are passed on to these organisms and may cause toxicity in them (Canli 2005; Sanita di Toppi et al. 2007). Dissolved metals can also cause adverse effects in aquatic organisms (Diamond et al. 2005; Brooks et al. 2006; Ward et al. 2006; Federici et al. 2007). One study also found that small zooplankton, such as *Daphnia* (especially *Daphnia* neonates) can act as carriers for metals, which adsorb to their carapaces and can contribute to dietary toxicity at higher trophic levels (Robninson et al. 2003).

Organic Contaminants

Various organic compounds such as polychlorinated byphenyls (PCBs), polyaromatic (polynuclear aromatic) hydrocarbons (PAHs), chlorinated benzenes, and organic pesticides and herbicides that are used (or were historically used) in everyday life can make their way into the stormwater runoff.

Marsalek and Schroeter (1988) calculated annual loadings of some of these contaminants in the Great Lakes basin. They looked at PCBs, organochlorine pesticides, PAHs, and chlorinated benzenes. They found that vast majority of these compounds were found in the stormwater runoff (i.e. they were above detection limits).

Soller et al. (2005) analyzed the fractions of the above mentioned organic compounds in the first flush of a rainstorm event. They found that organophosphorus pesticides were the most commonly detected of the compounds tested, and were present in 34% of first flush samples and 51% of baseline storms. The second most prevalent organics were PAHs, found in 10% of first flush samples and 9% of baseline storm samples.

From the literature it appears that PAHs and pesticides are most common organics of interest in the urban stormwater runoff. Pesticides are used on the lawns, residential gardens, golf courses, and in parks. While there is still no provincial legislation that prohibits, or limits pesticide use, some municipalities have, proactively, passed by-laws to ban pesticide use (e.g. Toronto, Guelph, Oakville, Hamilton, and others). Other municipalities are following suit (e.g. Burlington) following the Hudson P. Q. decision by the Supreme Court.

Polyaromatic hydrocarbons, while some are naturally occurring, are a common by-product of combustion. Studies have shown that a considerable amount of PAHs come from roads (Harrison et al. 2003; Murakami et al. 2004) and roofs (Murakami et al. 2004). Hoffman et al. (1984) looked at different sources of PAHs in urban runoff, such as residential, commercial, industrial and highway and the data showed that, on average, PAH contribution was slightly higher from industrial and highway sources, though this was not found to be statistically significant. Different urban sectors mentioned above – residential, commercial, industrial, and highway- will contribute different contaminants to the total pollution. Dickhut et al. (2000) showed that PAHs from different sources, such as coal and wood burning, smelting, and internal combustion engines mix in different ratios with each other and have different residence time in air, water, and sediment. This suggests that different media in the same location will have different effects on the local ecosystem, i.e. PAHs that stay longer in the aerosol phase will be transported further and have more wide-spread effects than the ones that readily mix with water and/or get bound up in the sediment.

While non-polar organics have different partitioning coefficients between air and water and water and sediment, the popular adage that "what goes up must come down" holds true for most organics and even ones that are most prevalent in atmosphere will eventually make their way to a waterway via rain. As such, in an urban environment there are some organic compounds present in each phase (Hoffman et al. 1984; Dickhut et al. 2000; Harrison et al. 2003). The mixtures they and the products of their degradation create can cause toxicity effects in local fauna (Boxall and Maltby 1997; Boxall and Maltby 1995)

Heat

While heat is not a contaminant per se, it is considered thermal pollution. Van Buren et al. (2000) showed that increased paved areas such as roadways and parking lots contribute a significant amount of heat, during the summer season, during the first flush of a rain event. This study showed that a large paved area (such as a parking lot) contributed, on average, 3.6°C to the runoff temperature. The same study also showed that if the runoff had a chance to go through the storm sewer or shaded areas, it lost, on average, 1.0°C. This shows, however, that the runoff that came from a catchment that included a large paved area was still almost 2°C warmer than normal.

An increase in temperature is known to increase metabolic processes according to Van't Hoff's principle (Randall 1997). It's been shown that higher temperature increases the rate of decomposition in aquatic environments (Costantini et al. 2004). Since decomposition is an aerobic process (Mackie 2001), it may lead to low oxygen concentrations in the water. This effect, coupled with the fact that oxygen solubility decreases with temperature (Mackie 2004) may lead to severe adverse effects on the biota. Even if the oxygen concentrations remain in the acceptable ranges, it has been shown that temperature may affect developmental processes of fish and aquatic invertebrates, causing them to emerge early (Markarian 1980; Beer and Andersen 2001; Cassie 2006). Pelagic zooplankton has also been shown to be sensitive to the temperature of the ambient waters (Nour El-Din and Al-Khayat 2001). Thermal pollution may also change the species distribution by driving out species that cannot survive in warmer waters, and attracting those that can (Zvyaginstev et al. 2004).

Biological

While physical and chemical contamination is most commonly considered in stormwater runoff, microorganisms can also enter stormwater ponds in runoff. Fecal coliforms (specifically *Escherichia coli*, which comprises 90-100% of all fecal coliforms) can cause mild to severe gastroenteritis (Health and Welfare Canada 1992). In 2004, Marsalek and Rochfort published a review paper on the presence of fecal coliforms in stormwater. They postulated that the coliform contamination can come not only from combined sewer overflows (as discussed above, when municipal sanitary sewers overflow during a storm even), but also from stormwater runoff that does not utilize that conveyance system. They found that fecal coliforms (FC) or *Escherichia coli* (EC) ranged in stormwater from 350 units/100 mL to 430,000 units/100 mL. To put this in perspective, Health and Welfare Canada's (1992) guidelines for recreational waters is 100-200 EC units/100 mL. As for drinking water, the USEPA (2006) standards are 0 EC per sample and only 5% of samples taken over a period of one month can test higher than this standard (i.e. any presence of *E. coli* at all). Olivieri et al. (1989) list the possible causes for presence of fecal coliforms in storm sewers as urban animal life (pets and wild) as well as land use, lack of sanitation, and bacterial growth in standing water in the sewers between rain events.

Residential and municipal lawns and parks are not, however, the sole source of fecal coliform contamination. A study in Austin, Texas found that runoff from highways contains on average from 13,000 to 116,000 FC units/100 mL (Barrett et al. 1995). Also, Carle et al. (2005) showed that with the increase in household density and imperviousness of the ground cover, the fecal coliform densities in the runoff increase, making it more likely that the standards listed earlier will be exceeded.

It has been known that stormwater presents a problem for quite some time. In the past two decades the municipalities began to design new developments so that some degree of stormwater management/treatment is incorporated. There is a wide variety of best management practices (BMPs) that have been designed to deal with the stormwater runoff and they must be chosen to fit a specific development plan. The following section describes some of these facilities.

Stormwater management facilities

Swales and miscellaneous grassed areas

Grassed areas can be places strategically around paved areas, such as parking lots, to slow down the runoff and detain any debris that is large enough to get caught in the grass (Debo and Reese 1995). Lawns and grass strips also provide infiltration area for precipitation, which in turn reduces the runoff volume. Swales channel the sheet water flow, directing it to stormwater management facilities. They are commonly vegetated and are placed alongside roads to direct the water flow away from the roadways during storm events (Davis and McCuen 2005). The advantages of the grassed areas are that they can double as parks and recreation areas and are very cost effective. Disadvantages include the fact that this type of stormwater control requires considerable land commitment from the municipality (Debo and Reese 1995).

Infiltration facilities

Infiltration facilities can range anywhere from a driveway covered with porous pavement or brick to full infiltration basins. As mentioned previously, as an area is urbanized, the ground surface is covered with pavement and houses, the area that previously allowed the water to filter down to the ground water table is no longer available. This may cause the water table in the area to drop causing problems for any facilities, homes, or ecosystems that depend on groundwater input. To deal with this problem, growing municipalities began to incorporate various porous areas, wherever possible, to supplement the groundwater input (Mays 2004). This also lessens the amount of surface water runoff into the surrounding ecosystems.

Creating artificial infiltration beds may seem a great solution to the urban stormwater problem. Infiltration of the urban runoff, however, brings its own problems. Simply allowing the urban stormwater to infiltrate the ground is not a very good idea since, as was discussed above, urban stormwater runoff carries with it a myriad of anthropogenic pollutants. Allowing these pollutants to reach the groundwater is undesirable as many municipalities use groundwater as a source of raw drinking water. Birch et al. (2005) published a study, which looked at the efficiency of an infiltration basin in removing various urban contaminants. They found that the infiltration basin under study was effective in removing Cu, Pb, and Zn, but it seemed to actually increase the concentrations of Cr, Fe, and Ni. As for other inorganic compounds – total phosphorus, total nitrogen, and total Kjeldahl nitrogen were decreased after passing through the infiltration basin, but nitrites and nitrates went up. The infiltration basin was also shown to reduce total suspended solids and fecal coliform content. A previously discussed study by Sansalone (1999) showed that if the filtration bed is lined with oxide covered silica sand, it retains metal contaminants much better than regular silica sand, and even more so if the pH is raised to 8.5 by having the water pass through porous pavement. A common problem with infiltration facilities is that they become clogged with the sediment that they filter out of the stormwater (Debo and Reese 1995). This means that these facilities require regular maintenance to remain functional.

Constructed wetlands

A distinction should be made between constructed wetlands and stormwater ponds. Stormwater ponds are used to manage peak water flows during storm events in urban areas (Wren et al. 1997). As natural area is urbanized, the ratio of impermeable to permeable area increases, decreasing water infiltration during storm events. This leads to higher surface runoff in the watershed (Wren et al 1997). Stormwater ponds have become very common in urban development to ameliorate this problem by retarding the water during storm events and then releasing it gradually into the receiving waters.

Constructed wetlands are used when the water input is much more stable, such as effluent from waste water treatment plants (Wren et al. 1997). Constructed wetlands are ill equipped to handle large variations in runoff, such as flooding during a storm event, or prolonged periods of very little input of water (Wren et al. 1997).

There is still some overlap between the two, as both are often colonized by local wildlife soon after construction. Also, it may happen that a stormwater detention pond, if left unattended and allowed to overgrow with aquatic vegetation, may transform into a wetland. Depending on the history of such a pond, this may be either a good or a bad development. If it is undesirable the stormwater pond becomes over-vegetated it may need to be cleared out to return to its original condition.

Although, as mentioned above, wetlands are ill suited to manage stormwater runoff, they have been shown consistently to be very efficient in retaining urban pollutants, as well as removing nutrients from the water and preventing eutrophication downstream (Johengen and LaRock 1993; Wren et al 1997; Bishop et al 2000a,b).

One of the drawbacks of the wetland system is that it comes with a significant land requirement (Debo and Reese 1995). Since such land requirements are not easily met by municipalities, if some processes (such as enhanced nutrient removal) that take place in the wetlands can be duplicated without having to reproduce the entire wetland, it could be used to improve other, simpler methods of stormwater/wastewater management. Davis et al (2001; 2006) have looked at creating bioretention media boxes, which, without taking up much space, provide excellent metal (>90%) and moderate nutrient (20-80%) removal. If, however, the land requirement is not a problem, the problem that wetlands cannot handle large inputs of water at some time and little to no water input at others can be remedied by building a detention pond (to regulate the water release) and a wetland (to improve water quality) in series. This has been done in several municipalities, and has shown promise by producing removal efficiencies of up to 83% of various contaminants, such as metals and nutrients (Martin 1988; Oberts and Osgood 1991; Johengen and LaRock 1993).

Detention and retention ponds

When speaking of stormwater ponds, detention and retention are often used interchangeably. This, however, is wrong. Retention ponds are built to "retain" runoff and, as such, do not have an outlet to receiving waters (Haestad and Durrans 2003). Such ponds are usually constructed to service facilities that cannot allow runoff into local ecosystems (Haestad and Durrans 2003). Detention ponds, on the other hand, have an outlet to release the water once it has been treated. For the purposes of this paper, "stormwater ponds" and "detention ponds" are used interchangeably.

The advantages of stormwater ponds include the fact that they can provide runoff control for large catchments, when properly constructed they can be aesthetically pleasing and can play a role in the urban environment (Debo and Reese 1995). They can also provide a habitat for urban wildlife (Debo and Reese 1995; Wren et al 1997; Bishop et al 2000), however it is debatable whether this is an advantage or a disadvantage, as will be discussed further. There are some drawbacks of having detention ponds as a stormwater control measure, such as the possibility of the detention ponds becoming eutrophic and providing a breeding area for pests (Debo and Reese 1995)

Construction of stormwater ponds is one of the more popular end-of-pipe methods to deal with the urban surface runoff. These ponds provide many of the same ecosystem services identified for constructed wetlands, including retention of nutrients, particulate matter, and contaminants. When constructed properly these ponds can be made to appear as part of urban landscape and can be integrated into community parks. As discussed above, the main purpose of the stormwater ponds is to retard water during peak flow times, and to allow the particulate matter to settle and temperature to stabilize before discharging it into the receiving waters. Depending on the area from which any particular pond receives the runoff, as well as the quality of design, the ponds may vary in their efficiency of contaminant removal.

The following section describes studies on the capacity of stormwater ponds to ameliorate water quality.

Contaminant Removal Efficiency

Sediment

Earlier it was discussed that the determinants of how fast particulate matter settles out of the water column are its shape, size, and mass. Based on these properties and the features of the detention ponds, the removal efficiency will vary for different types of suspended solids, as well as between different ponds.

Mayer et al. (1996) investigated the removal efficiency of several urban contaminants, including total suspended solids. They found that the stormwater ponds removed, on average, 24% TSS from the runoff from residential areas and 25% TSS from the industrial runoff. TSS from the open areas was not decreased. It should be noted that on several occasions the concentration of suspended sediments actually increased in the water after the trip through the ponds. This is due to sediment re-suspension during the heavy flows of a storm event.

The sediment removal function of the stormwater ponds has a two-fold benefit. First, it prevents the deposition of extra sediment in the receiving water way and second, the chemicals that sorb to the particulate matter that settles out in the ponds settle out with it. This helps reduce the overall contaminant load.

Again, not all suspended solids settle out in the ponds. The colloids and other particles that have colloidal properties remain in suspension and are carried on to the receiving waters when the stormwater ponds discharge. In wastewater treatment technology, a process called flocculation is used to remove such particles from the water. In this process, chemicals are added to the water which form a "fluffy" precipitate to which the colloidal suspended solids adhere (Reynolds and Richards 1995). Floc settles to the bottom, taking the colloids with it. This kind of procedure is tricky to implement in stormwater ponds, because leaking of these chemicals into the surrounding ecosystem must be considered, as well as the amounts of stormwater that would need to be treated. To this effect, Wood et al. (2004) published a study on the feasibility of stormwater treatment using flocculant addition. The authors did not add flocculant directly to a stormwater treatment facility, but, rather, set up a clarifier which was fed by the storm sewer. They found that a) polymer flocculant addition to the stormwater resulted in higher TSS removal efficiency (83%), b) more is not necessarily better and that the moderate addition of the polymer flocculant yielded best effects, and c) as the duration of a storm event increased, the TSS load decreased (which is consistent with the "first flush" phenomenon discussed above) and the amount of the polymer flocculant can be adjusted based on turbidity to conserve the flocculant and reduce the risk of inadvertent release into the surrounding ecosystem.

The stormwater ponds in the study area are designed to provide Level 1 quality control (80% suspended solids removal) (Olding et al. 2004). A study conducted on the Richmond Hill ponds in general showed that, while TSS can be fairly high (up to 908 mg/L), 75% of the stream samples had TSS concentrations below 60 mg/L (Olding et al. 2004).

Nutrients

It is difficult to estimate the efficiency of the nutrient removal in stormwater ponds since the ponds are often home to wildlife and vegetative community that may add, remove, or transform the nutrients that come into the system (Wren et al. 1997). Depending on the degree to which the stormwater pond is colonized with vegetation, it can aid in removal of nutrients by plant uptake and/or adding to the nutrient content by contributing decomposing matter.

Mayer et al. (1997) reported a limited ability for nutrient removal of the stormwater ponds investigated, which the authors attributed to poor removal of fine particulate matter to which nutrients are adsorbed. Other studies have found that presence of vegetation facilitates the removal of nutrients (Johengen and LaRock 1993; Groffman and Crawford 2003). It has been shown that constructed wetlands, which, by definition, have a much more extensive macrophyte component structure, provide 60 to 90 percent removal for such nutrients as nitrates, phosphates and ammonia (with varying efficiencies) (Johengen and LaRock 1993). It also has been shown that riparian zones around urban waterways, which are not, strictly speaking, part of a waterway, aid in denitrification (Grofman and Crawford, 2003). Martin (1988) found fairly high removal efficiencies for phosphorus and ammonia (72% and 55% respectively), but actually saw an increase in nitrates. He hypothesized that the increase in nitrate was due to oxidation of other nitrogen forms (including ammonia) to nitrate. Oberts and Osgood (1991) found the total nitrogen and total phosphorus removal to be at 76% and 79% respectively, and Stanley (1996) found that ammonia and nitrates increased (8% and 2% respectively) on average after going through a detention pond, but that phosphates decreased 19%. Both studies (Martin 1988; Oberts and Osgood 1991) were conducted on systems where there was a constructed wetland in series with the stormwater pond; however the removal efficiencies given here are for the pond portion of the series, which precedes the constructed wetland.

It is clear that the nutrient removal efficiencies vary greatly with the treatment facility being studied. As suggested previously, this is likely due to such differences as design and macrophyte community. In more heavily vegetated detention ponds, with greater water retention times, we may expect nutrient removal efficiency to approach that of constructed wetlands.

Metals

The ability of stormwater ponds to remove metals is, just as with nutrients, tied to a large extent to the pond's ability to retain suspended solids. It also depends on a variety of factors, such as pH of the incoming stormwater runoff and type of suspended solids that are carried in it. Mayer et al. (1997) found little change in metal concentration, which, as with nutrients, they attributed to poor suspended solids removal. Having said this, many studies have found that metals accumulate in the sediment in the stormwater ponds. For example, Campbell (1994) compared the Cd, Ni, Cu, Pb, and Zn content in the composite sediment from several stormwater ponds from Orlando, Florida to control sites and found that for all metals under study, except for Pb, the concentrations in the stormwater pond sediment were higher than at the control sites (statistical significance could not be established in this study due to small the sample size and the masking effects of the composite nature of the sediments). Another study of several stormwater ponds in the Guelph and Toronto areas showed that, while highly variable, the average levels of Cu, Pb, and Zn in the stormwater pond sediment exceeded the Ontario provincial sediment quality guideline (PSQG) LEL (Bishop et al 2000b).

Martin (1988) found that the removal efficiencies for Pb and Zn were 42% and 50% respectively. This removal was credited mainly to the settling of the suspended solids (Martin 1988). Stanley (1996), on the other hand, found lower average removal efficiencies for Cd (24%), Cr (42%), Cu (29%), Pb (44%), Ni (40%), and Zn (27%).

Organic Contaminants

While there are quite a few studies that look at the presence of organic contaminants in the environment (e.g. Hoffman et al. 1984; Marsalek and Schroeter 1988; Dickhut et al. 2000; Blanchard et al. 2002; Harrison et al. 2003; Murakami et al. 2004 and others) and their effects on aquatic organisms (e.g. McCloskey and Oris 1993; Boxall and Maltby 1995; Huang et al 1995; Weinstein et al 1997; McCarthy et al 2004; Xie et al 2005 and others) there are very few studies that focus on the stormwater management facilities' effects on organic contaminant retardation. One study on several Guelph and Toronto stormwater ponds and constructed wetlands showed that for 11 polyaromatic hydrocarbons (phenanthrene, fluoranthene, pyrene, beno[a]-anthracene, chrysene, benzo[b]-fluoranthene, benzo[k]-fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]-pyrene, dibenzo[a,h]-anthracene, and benzo[g,h,i]-perylene), if they were present (i.e. above method detection limit), for the most part they were also above the PSQG LEL (Bishop et al 2000b). Out of the 11 PAHs examined fluoranthene, pyrene, and chrysene were found in the highest concentrations in the sediments. The same study examined the presence of various organochlorine pesticides (alpha-bhc, gamma-bhc, gamma-chlordane, alpha-chlordane, p,p'DDE, and beta-endosulfan) and total PCBs in the sediment. Organochlorine pesticides were found to be mostly below the method detection limit and only gamma-bhc was found to exceed the PSQG LEL (Bishop et al 2000b), while total PCBs, although they were only detected in two out of 16 facilities under study, were found to exceed PSQG LEL by an order of magnitude both times (Bishop et al 2000b). The fact that the contaminants are found in the sediments in stormwater ponds suggests that some contaminant retention is taking place; however, no studies were found that compared the stormwater pond sediment content of organic contaminants to that of receiving waters.

One aspect that should not be ignored is that, while there are few specific studies as to the fate of organics in the stormwater management ponds, the normal processes of complex chemicals should be taken into account. The stormwater management facilities are usually built to be approximately 2 to 2.5 m deep (Debo and Reese 1995). Keeping in mind that stormwater management detention ponds are at their fullest during a storm event, and at other times the water is at lower levels. This exposes anything in the upper layer of water to solar radiation. Many complex chemical compounds undergo changes when exposed to UV radiation (Hemond and Flechner-Levy 2000), but it also seems that at times, the coupling of UV radiation with the presence of polyaromatic hydrocarbons increases their toxicity (Huang et al. 1995; Weinstein et al. 1997). Numerous studies have documented this effect on organics in the water (Huang et al. 1995; Ireland et al. 1996; Weinstein et al. 1997; McConkey et al. 2002). In some cases, the toxicity of modification products seems to increase relative to that of the original compound (Huang et al. 1995). Weinstein et al. (1997) looked at photo-induced toxicity of fluoranthene and its effect on the fathead minnow. Weinstein et al. (1997) found that simultaneous exposure of fish to fluoranthene and SUVR (solar UV radiation) caused edema, bleeding, and vasoconstriction in the gills of the fathead minnow. They hypothesized that ultraviolet radiation excited PAH molecules into releasing reactive oxygen species, which led to damage of the gill tissue and subsequent death of the test organisms (Weinstein et al. 1997). Huang et al. (1995)

investigated the effect of five different PAHs (anthracene, benzo[a]pyrene, fluoranthene, phenanthrene, and pyrene) on duckweed (*Lemna gibba*) and found that, when exposed to UV light, anthracene was most toxic, with complete death occurring within 3 days. The authors attributed the enhanced toxicity to the breakdown products of the original compound due to the fact that the half-life of the compounds measured in this experiment was minutes to hours, while the toxicity tests lasted for 8 days.

Wildlife in stormwater ponds and constructed wetlands

As mentioned previously, stormwater ponds can be made to look like a part of a park or other municipal recreation areas. These areas are often frequented by wildlife that is not easily scared away by the human population. Again, as discussed above, the stormwater ponds and constructed wetlands are designed to remove and retain anthropogenic contaminants from the storm water. It is worth investigating, then, what, if any, effect these contaminants have on the colonizing wildlife.

A study by Wren et al. (1997) focused on two types of stormwater management facilities (constructed wetlands and stormwater ponds), the input of various contaminants into these facilities, and the effects they have on the wildlife that uses these facilities as habitat. They found that most stormwater facilities provide a home for a wide variety of life including macrophytes, invertebrates, fish, amphibians, reptiles, birds, and mammals, more so in constructed wetlands than stormwater ponds due to more extensive vegetative coverage. They caution that, because the number of uncontaminated and natural wetlands is decreasing, more and more fauna find their home in wetlands constructed for remediation of anthropogenic impacts. This leads to wildlife exposure to the compounds these facilities are built to remove from the runoff/effluent.

As stormwater management facilities are created for the very purpose of reducing contaminant loads to the receiving waters, a portion of these contaminants is retained in the sediment and water of these structures. If and when wildlife colonizes these artificial water bodies, these contaminants may accumulate in animal tissues (Table 1).

Table 1: Concentrations of common urban runoff contaminants in organisms living in or around stormwater treatment facilities

	Largemouth bass (mg/kg)	Bluegill sunfish (mg/kg)	Freshwater mussel (*Ellipto complanata*) (mg/kg)	Resident vs. migratory mallard ducks (µg/kg)
Cd	3.16[1*]	0.006[1]	0.27[Δ]-0.53[2]	
Ni	2.46[1]	0.156[1]	9[Δ]-13[2]	
Cu	3.81[1]	2.08[1*]	9-12.5[2*]	
Pb	12.04[1*]	0.77[1]	0.7[*]-1.15[2*]	
Zn	29.99[1*]	36.61[1]		
Cr			0.95-1.95[2*]	
Octa-chlorostyrene				115 vs. 56[3]
Hexa-chlorobenzene				30 vs. 8.7[3]
Penta-chlorobenzene				1.5 vs. 0.4[3]

Grapentine et al. (2004) looked at the contaminant accumulation in the amphipods that were used for toxicity testing. They found that metals were not elevated between the upstream and downstream regions around the stormwater outfall, but that the PAH accumulation below the

[1] Campbell 1994
[2] Anderson et al. 2004
[Δ] Significantly lower than control
[*] Significantly higher than at control sites
[3] Hebert et al. 1990 (sample size was too small to determine significance)

outfall was more than twice that of above (Grapentine et al. 2004). The authors also examined the tissue contaminant content in the indigenous fauna and found that the PAH accumulation below the outfall exceeded accumulation above the outfall for hydrophsychids (caddisflies) and amphipods in Etobicoke region, and for mayflies in the East Humber region (Grapentine et al. 2004). Interestingly, they also found that the hydropsychids in the East Humber region did not differ in PAH accumulation above and below the outfall (Grapentine et al. 2004).

Bishop et al. (2000 a,b) investigated the contaminants in several stormwater ponds around Guelph and Toronto area, and found that the sediment and water in these facilities often exceeded the pertinent guidelines. They also conducted a comprehensive wildlife survey and found that many ponds were colonized by plants, invertebrates, fish, amphibians, reptiles, mammals, and birds (Bishop et al. 2000a). While they did not conduct any tissue testing to see if the contaminants that were found in the sediments and water of the stormwater ponds made their way into wildlife bodies, it is a fair assumption given the findings of the previous studies (Wren et al 1997; Anderson et al. 2004; Grapentine et al 2004).

Bioassessment methods

While it is important to know what kind of chemical pollutants make their way into (and, more importantly, out of) the stormwater treatment facilities, ultimately what we should be concerned with is the impact that the urban runoff (treated or untreated) has on the biological communities in the receiving water. Aquatic biological communities contain very complex relationships between primary producers (macrophytes and algae), microbes, benthic macroinvertebrates, fish, amphibians, reptiles, mammals, and birds. These organisms are listed by their increasing ability to move away from their place of habitation, should there be a disturbance that prevents them from living as they were. The latter three have the ability to migrate between watersheds. Both, fish and amphibians can migrate along the waterways. Macrophytes, attached algae (periphyton), and benthic invertebrates are very limited in their range of movement, while microbes are at the mercy of the currents. Based on these relative mobilities to avoid stressors, macrophytes, attached algae, and benthic invertebrates are good candidates for bioassessment in moving waters. Microbes and planktonic algae can also be used in lentic environments, such as lakes.

Macroinvertebrate community

There are many methods to determine the impact (or lack thereof) based on the biological community. The most popular target in such an assessment, by far, is the invertebrate community (e.g. Rochfort et al 2000; Grapentine et al. 2004; Vanden-Bossche and Usseglio-Polatera 2005; Liess and Von Der Ohe 2005; Chessman at al. 2006; Smith et al. 2007; Jackson et al. 2007) for several reasons: these organisms have a very limited range of movement (they are not as mobile as fishes), they are fairly easy to collect, and are abundant (Jones et al. 2007). Different tolerances of different species allow for extrapolation of the habitat conditions from the respective macroinvertebrate community structures (Jones et al. 2007).

There is a wide variety of methods for macroinvertebrate bioassessment that can be used, but there is one constant – the site set-up has to be chosen depending on the conditions, both spatial and temporal, of the possible impact. Figure 1 presents a decision tree, which can be used to select the appropriate method of bioassessment. The decision tree in Figure 1 was first described in Green (1979). The underlined decision branches were added by Bowen and Sommers (2005).

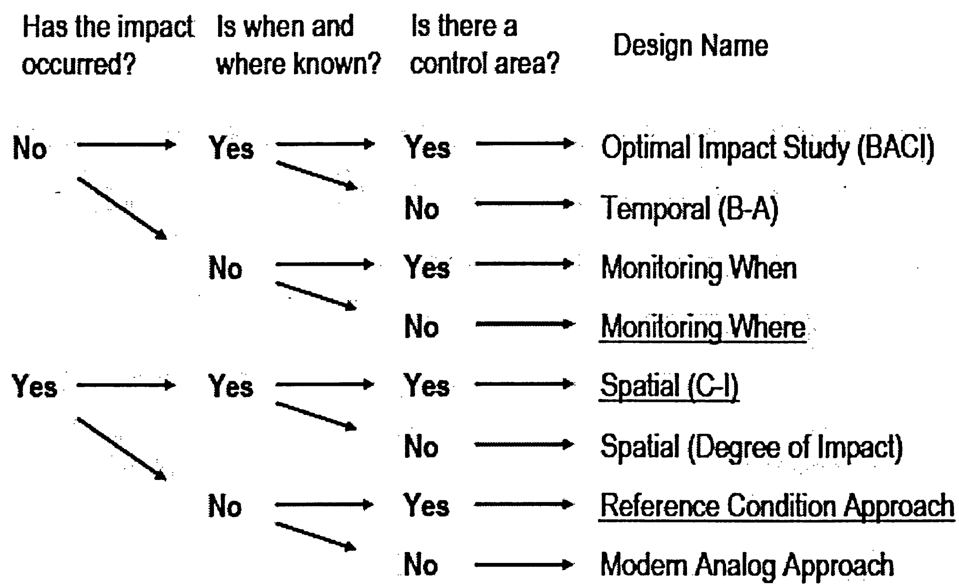


Figure 1: Bioassessment decision tree (Bowman and Somers 2005)

BACI – Before/After/Control/Impact – This study set-up is valid if the impact has not occurred yet, and it is known when and where the stressor is going to occur, AND there is a control area that is unaffected by the stressor (Green 1979). This sort of situation is fairly rare, but is considered the optimal design (Green 1979). In this case it is possible to sample the control area and the forecasted stressed area before the stressor has been introduced, and, again, after. Unless an environmental assessment is requested before urbanization or industrialization of an area, bioassessments are usually done to determine the damage after the stressor has already been introduced into the ecosystem.

B-A – This is a variation on the BACI, where the stressed site can be assessed before the stressor is introduced and, again, after; however in this set up there is no control site and the impact must be inferred from the temporal differences (Green 1979).

Monitoring When/Where – In the original Green (1979) referred to as "baseline or monitoring study", these two designs are information gathering methods, which are used when there has been no outside stress on the ecosystem (and none is coming in the foreseeable future), but the sites are sampled to determine the community composition in an non-stressed area (Green 1979).

C-I – This is, again, a variation on the BACI, however is not described in the original Green (1979). This set-up is used when the assessment is being done for the first time after the stressor has been introduced into the ecosystem, but there is a control area that is not influenced by the stressor (Jones et al. 2007).

Spatial (Degree of Impact) or Simple Gradient – This method is utilized when there is no control site. This method relies on the availability of several sites at varying distances from the stressor and makes inferences from spatial differences (Green 1979). An example of such a study would be a single discharge location and several sites downstream at ever increasing distance.

RCA (Reference Condition Approach) – There is some variability as to the use of this method. In the decision tree, it implies that there is a control site for comparison with the test site. This is an idealized control or reference site. In this method, several sites that have been sampled while they were not exposed to a stressor (e.g. during Monitoring When/Where) are grouped by conditions and are matched to the test site (Bailey et al. 2004). Statistical analysis is conducted to determine whether the test site falls into the "normal" range for the chosen reference group (Bailey et al. 2004).

Modern Analog Approach – referred to as "When and Where? Is the question" in the original Green (1979) text. The hardest design of all, since it is unclear where or when the impact occurred, therefore it is difficult (if not impossible) to decide what to sample and compare

(Green 1979). An example of such a predicament would be acid rain – the nature of the pollutant is known, but not the time and place of the release.

Since control sites are rarely available and it is hard to predict when and where a particular stressor will be introduced into an ecosystem, the RCA is growing in popularity (Jones et al 2007). Its drawback, however, is the fact that to compare the test sites to a cluster of reference sites with similar physical conditions, such reference sites must first be identified and sampled. The Ontario Benthos Biomonitoring Network as made this considerably easier by creating a database, which holds habitat and benthological data for numerous sites in Ontario. Not all of them are considered in reference condition, but those that are, are tagged as such. To use the data from this database, the samples at the test sites must be collected according to OBBN protocol.

Once it has been determined what the test site is being compared to (control, reference cluster, gradient), the indices based on which the test site will be compared to the control site(s) must be selected. Metcalfe (1989) summarized the various indices used in macroinvertebrate assessment. The following section summarizes the most prevalent indices along with advantages and disadvantages of each:

Diversity index

This index is most often used with macroinvertebrates; however, it can be calculated for any community. It is based on three criteria: species richness, species evenness, and species abundance. The most widely used diversity index is the Shannon-Weaver index (Shannon and Weaver 1963). It is calculated according to equation 2:

$$\bar{d} = -\sum \frac{Ni}{N} \log_2 \frac{Ni}{N} \qquad (2)$$

Where:

d – diversity
N – total number of individuals
Ni – number of individuals of a particular species

This approach is advantageous because it provides a good metric for statistical analysis (quantitative and dimensionless), it is independent of the sample size, no subjective tolerance values are used, and it is not very labour-intensive (Metcalfe 1989). There are some criticisms of the diversity index – it may provide false positives in the downstream communities due to changing physical conditions in moving to larger (higher order) streams/rivers (Metcalfe 1989). The fact that the tolerance values are not used is a double-edged sword, and all organisms are treated as having the same tolerance (Metcalfe 1989). The values may increase with mild biological (or nutrient) pollution, boosting abundance without eliminating species (Metcalfe 1989). Diversity can, theoretically, be calculated at any level; however, all taxa must be at the same level (e.g. species, genus, family, order), and diversity values calculated at different levels of taxonomic resolution cannot be compared (Jones unpublished). The diversity values also should not be compared for different dates, or significantly spatially separated habitats (Jones et al. 2002).

Trent Biotic Index (TBI)

The Trent score was developed by Woodiwiss (1964) while working for the Trent River Authority (Mackie 2001). This index uses macroinvertebrate orders, families, and species by assigning a score based on how many families are represented per order, how many species (1 or more than 1) are represented per family, and the tolerance of the organisms (Chandler 1970). The stream score ranges from 10-clean, to 0-polluted. It is simple to use, and the identification can be done to family once it has been established that there is more than one species present (Metcalf 1989). The drawbacks of this index are lack of sensitivity and the fact that it ignores abundance (Metcalfe 1989).

Chandler's Score

Chandler's Score is an extension of TBI and was developed by Chandler (1970). The improvement over the TBI is the inclusion of abundance rank (present, few, common, abundant, very abundant). This index is fairly comprehensive in taking into account both abundance and tolerance, but it is complicated to calculate (Metcalfe 1989).

NWC/BMWP/ASPT Index

The National Water Council/Biological Monitoring Working Party/Average Score Per Taxon index was developed in the United Kingdom and slightly modified by Armitage et al. (1983). The organisms are identified to family and assigned a score based on their tolerance (high for pollution intolerant, low for pollution tolerant). Scores for all families are then added up and divided by the total number of families. The score can range between 10-clean, or 0-polluted (Mackie 2001). This index is not affected by sample size, it is easy to calculate, and requires little expertise (Metcalfe 1989). This method requires a certain level of expertise in invertebrate identification to prevent mislabeling of the taxa tolerances.

Indice Biotique (IB)

Indice biotique is also derived from TBI and was developed by Tuffery and Verneaux (1968) specifically for use in France. There are some differences, such as a larger number of indicator taxa, exclusion of taxa represented by a single individual, necessity to sample both, lotic and lentic habitats, and exclusion of species with aberrant tolerances from the family score. The scoring is the same at TBI – 10-clean, 0-polluted (Metcalfe 1989).

Belgian Biotic Index Method (BBI)

This index combines the IB and TBI and modifications were added by De Pauw & Vanhooren (1983). The adjustments included exclusion of nematodes, division of Chironomidae into *thummi-plumosus* group and non- *thummi-plumosus*, and most ID was set to family level to avoid misidentification. Results produced using this metric were reproducible, so it proved successful.

% Relative Abundance

Some of the easiest biological indices are the % relative abundance of a certain taxa. The most commonly used ones are % EPT (Ephemeroptera+Plecoptera+Trichoptera), which represent the clean water organisms, and % Chironomidae (Mackie 2001). Organisms in the family Chironomidae are called "bloodworms" because they have hemoglobin in their hemolymph, which allows them to live in low-oxygen conditions and they are considered tolerant species (Mackie 2001).

Taxa Richness

Taxa richness is, simply put, the number of taxa collected in the sample (Mackie 2001). The taxa should all be enumerated at the same resolution level (e.g. species, genus, family, order). This index is, probably, the easiest to calculate.

Multivariate Indices

All indices described above are considered univariate (Jones et al. 2002). The data are condensed to a single value on the basis of which the test site is compared to a reference, or control site. Multivariate analysis uses all the variables (e.g. abundances of all taxa collected). An example of multivariate analysis is Correspondence Analysis (CA). CA is based on abundance and presence/absence data. A Correspondance Analysis chart provides a graphical representation of how alike/different the sites are. The closer the sites are together – the more similar their benthic invertebrate compositions. The taxa that are found predominantly only at one site will be closer (spatially) to that site. The taxa that are common to all sites will be located approximately in the middle of the cluster. This allows the investigator to see which taxa are responsible for the separation of the sites. This analysis can be done easily in MSExcel using a Biplot plug-in available freely on the internet (Jones et al. 2007). This analysis is usually part of RCA, but can be used on its own to summarize abundance/presence data of the community composition.

Although these indices have been used for several decades, studies do not necessarily use them to interpret the results. It is often the case that the statistical summaries of abundances of various species are presented and relationships are inferred on the basis of community make up as a whole (Rochfort et al. 2000). Also, over the years, different researches began to question the validity of some of the indices and introduced changes that they saw fit (Rabeni and Wang 2001). No metric presented above should be used on its own to conduct bioassessment. Several indices used together will provide a much fuller picture.

For this study, Diversity, Taxa Richness, % EPT, % Chironomidae, and Correspondence Analysis have been chosen. Diversity takes into consideration richness, evenness, and relative taxa abundance, giving a representative community composition score. Taxa Richness is very easy to understand, calculate, and interpret. % EPT and % Chironomidae represent the opposite ends of the tolerance spectrum; and Correspondence Analysis provides a multivariate statistical analysis of the sites.

Macrophyte community

Bioassessments using macrophytes is not very widespread and there are very few methods described in literature. One protocol for macrophyte-based assessment of rivers was developed in Germany (Meilinger et al. 2005). The rivers in the study were classified based on their current water hardness as follows:

- MRS – fast flowing, softwater
- MRK – fast flowing, hard water
- TR – fast flowing rivers and brooks
- TN – slow flowing, low land rivers

The method itself involves cataloguing emergent and submergent macrophytes using an underwater viewing aid. The plants observed are divided into 3 groups: Group A – taxa common at reference (control) sites, but not at test sites; Group B – taxa common at test sites, but not at reference sites; and Group C – taxa not showing any preference for either site type. The Reference Index (RI) was then calculated according to the following formula (3):

$$RI = \frac{\sum_{i=1}^{n_A} Q_{Ai} + \sum_{i=1}^{n_V} Q_{Vi} - \sum_{i=1}^{n_C} Q_{Ci}}{\sum_{i=1}^{n_g} Q_{gi}} \qquad (3)$$

Where:

- Q_{Ai} is plant quantity of the i-th taxon of species in group A
- Q_{Ci} is plant quantity of the i-th taxon of species in group C
- Q_{Vi} is plant quantity of the i-th taxon of species in group V (which exist only on MRS type rivers)
- Q_{gi} is plant quantity of the i-th taxon of all groups (A, B, C, V)
- $n_{A,C,V}$ is total number of taxa in group A, C, V
- n_g is total number of taxa in all groups

Diversity (according to Shannon-Weaver formula (2)) and Evenness (4) were also calculated.

$$E = \frac{d}{\ln s} \tag{4}$$

Where:

- E is Evenness index
- s is total taxon number of the biocoenosis
- d is Index of diversity (calculated using equation (2))

In-situ testing

While collecting indigenous macroinvertebrates can provide a reliable picture of ecosystem health, a more targeted analysis can be performed by exposing certain organisms with known tolerances to the environment under investigation. *Daphnia magna* and *Hyalella azteca* are two organisms that are widely used for such purposes (e.g. Kubitz et al. 1994; Hatch and Burton 1999; Rochfort et al. 2000; Grapentine et al. 2004; McCarthy et al. 2004). While invertebrates are a common choice for in-situ studies, fish have also been extensively used for this purpose (e.g. Hatch and Burton 1999; de la Torre et al. 2000; Jardine et al. 2005; Orrego et al. 2006; Todd et al. 2007).

STUDY RATIONALE

As is described in the previous section, the management of storm drainage is a problem in any urban environment. There have been many strategies to deal with stormwater over the years, including the most current process of directing tributary area storm drainage to a stormwater management (SWM) facility for treatment, prior to being discharged to a receiving watercourse or waterbody. This strategy is currently employed by the Town of Richmond Hill, utilizing source, conveyance, and SWM facilities (*i.e.* treatment train approach) (pers. comm. John Nemeth, Engineering and Public Works Department, Town of Richmond Hill 2005) to address both water quantity and quality issues.

It is unclear, however, the degree to which the outgoing water has an impact on any given receiving water body. This would depend on the particular land use around the stormwater treatment facilities (which would determine the kinds and amounts of contaminants that are carried by the stormwater) and the design of the stormwater facilities themselves (such as total volume, turnover time, and discharge channel design). To this end, this study endeavors to present a picture of the physical, nutrient, and biological impacts water discharged from stormwater ponds has on receiving waters at the Richmond Hill SWM facilities. Although physical parameters and nutrients were measured, heavy emphasis in this study has been placed on the biological component of the assessment, following the philosophy that, while there may be a contaminant input into an ecosystem, it does not necessarily have a negative impact on the biological community living in the ecosystem. Both indigenous community sampling, as well as *in situ* toxicity testing with organisms of known tolerances were performed.

Unfortunately, few municipalities have the staff or resources to assess the impacts on the environment of stormwater that has run through city streets collecting anthropogenic chemicals and particulate matter. While many municipalities conduct physical and chemical assessments, such as water temperature, pH, flow, nutrients, TSS, perhaps even major contaminants such as heavy metals and organics (Nemeth pers. Comm.), the biological component remains unexplored. Conducting a bioassessment requires resources, and, most importantly, expertise in invertebrate taxonomy and statistical analysis methods. Therefore, another objective of this study is to create a simplified bioassessment protocol that the municipalities can use in efficient and representative assessment of possible impacts of stormwater runoff on the receiving waters.

Study region

The Town of Richmond Hill is a fast-growing municipality located in the Rouge River watershed and the Oak Ridges Moraine. The stormwater ponds in the area drain into the main channel and tributaries of the Rouge River. This is a dedicated stormwater conveyance system which does not combine with the sewer system (as opposed to combined sewer overflow systems (CSOs)). The town is built in a very regular fashion and is divided into quadrants by major streets. The present study examined the impact the aforementioned facilities have on the receiving waters of the Rouge River tributary in Quadrant 19 cadastre division of the Municipality of the Town of Richmond Hill (Figure 2). For more detailed maps, see Appendix A.

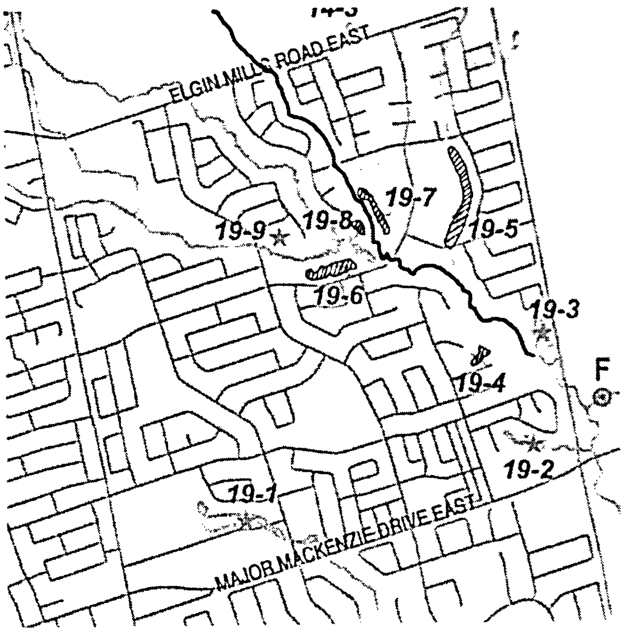


Figure 2: Region under study - Quadrant 19 of the Municipality of Town of Richmond Hill, Ontario (Water Resources Section 2005) (Bordered by Leslie St on the East Bayview Rd on the West)

When a specific effect on an ecosystem is unknown, it is important to look at a variety of indicators. In this study, to investigate the potential impact on the receiving waters of Richmond Hill's stormwater ponds, the following aspects have been analyzed: physical (temperature, pH, and dissolved oxygen), nutrients (NH_3, NO_3^-, PO_4^{3-}, and total organic carbon (TOC)), and biological (macrophyte and macroinvertebrate communities, as well as *in situ* impact testing). Due to the lack of documented bioassessment protocols for aquatic environments using macrophytes in the literature, a new protocol was developed for this study. The scientific objectives of the study were 1) to determine if there was an impact at the site of the pond outfall when comparing conditions immediately upstream and immediately downstream of the outfall, 2) to determine if there was a cumulative impact on the waterway due to the increasing number of stormwater ponds that empty into it, and 3) develop a protocol that municipalities could implement in the future to determine any effects a given disturbance may have on a receiving waterway. The emphasis was placed on environmental relevance, sensitivity, and simplicity of the assessment design.

MATERIALS AND METHODS

The following describes the methods used to collect and analyze the physical (temperature, pH, and dissolved oxygen), nutrient (NH_3, NO_3^-, PO_4^{3-}, and TOC) and biological (macrophytes, macroinvertebrates, caged organisms) data in the sampling region.

Sampling Protocol

Samples were collected over the course of the 2006 field season, which lasted from May 8th to October 18th. The stretch of the Rouge River studied here is fairly shallow, rarely more than 0.5 m in depth. Except for Site A, which is a first order stream with the width of approximately 0.5 meters, the channel width varies from approximately 3 to 6 meters.

At each site an OBBN data sheet was filled out which outlined the basic geography and physical conditions at each site (Appendix A).

Headwater site

The Headwater site is a reference site established for the current study where the tributary originates. It is located in an undeveloped field, more than 500 meters from the nearest buildings or roads. It is an ephemeral stream on the Oak Ridges Moraine and is overgrown with reed canary grass.

Site A

Site A is a first-order (no other tributaries) stream, according to the Strahler Index (Mackie 2001), running through a park with a fairly narrow (~0.5m) channel and silt and clay sediment (sediment characterized as per Jones et al. (2007)). Banks were heavily overgrown with herbaceous vegetation. This site receives water from the municipal stormwater pond 19-7 (Figure 2, Figure A-1).

Pond 19-7 was built in 1998 and is a level 1 quality control pond, meaning it is designed to provide 80% suspended solids removal (Olding et al. 2004). It provides drainage area for 27.5 ha and has a permanent pool volume of 2789 m^3 (Olding et al. 2004).

Site B

Before Site B, the tributary is a first-order stream. At Site B, this tributary merges with a third-order channel and, itself becomes third-order. Site B is located in the same park as Site A. It shows some bank erosion; however, this is probably due solely to the channel geography and not to any stormwater pond outfall. Bank vegetation is dominated by grasses and there are a few bushes overhanging the water, but no trees. In the sampling area, the current is fairly slow producing a hydraulic head of approx. 5 mm (as per Jones et al. 2007), and the sediment is mostly fine to coarse sand and gravel. Ponds 19-6 and 19-8 empty into the tributary above this site (Figure 2, Figure A-1).

Pond 19-6 was built in 1997 and provides Level 1 quality control (Olding et al. 2004). It provides drainage area for 50 ha and has a permanent pool volume of 6400 m^3 (Olding et al. 2004). Pond 19-8 was constructed in 2005 and the above information for this pond is unavailable.

Site C

Site C is a third-order channel in a small undeveloped area in the middle of urban development where the pond outfall is clearly visible. Bank vegetation is composed of a mix of grasses and horsetails (*Equisetum sp.*). Bushes and trees line the channel. In the sampling areas, both upstream and downstream of the outfall, the sediment is comprised of silt, gravel, and cobble/boulders and the current is fairly fast. Site C receives water from pond 19-5 (Figure 2, Figure A-1).

Pond 19-5 was built in 1998 and provides Level 1 quality control (Olding et al. 2004). It provides drainage area for 83.1 ha and has a permanent pool volume of 5765 m^3 (Olding et al. 2004).

Site D

Site D is a third-order channel approximately 0.5 m deep in a lightly wooded area behind a residential area. Besides trees, the bank vegetation consists of grasses and horsetails. Sediment is fine to coarse sand, small cobble, and few large boulders scattered through the channel. Pond 19-4 empties into the tributary above this site (Figure 2, Figure A-1).

Pond 19-4 was constructed in 1997 and provides Level 1 quality control (Olding et al. 2004). It provides drainage area for 38.5 ha and has a permanent pool volume of 2527 m^3 (Olding et al. 2004).

All assessment sites, with the exception of the Headwater site, were located in Quadrant 19 of the Municipality of the Town of Richmond Hill, Ontario (Figure 2). These sites represent a downstream progression with the Headwater site being the most upstream and D being the most downstream site of the set. For Sites A and C, the outfalls of their respective stormwater ponds were accessible and this made the establishment of immediate upstream (A-US and C-US of pond 19-7 and 19-5 respectively) and downstream (A-DS and C-DS of pond 19-7 and 19-5 respectively) sampling sites possible.

Physicochemical Measurements

Measurements of water temperature, dissolved oxygen and pH were taken on a weekly basis at every site during the entire field season using a Traceable® Digital Oxygen Meter (Control Company, Friendswood, Texas) and ColorpHast® (EMD Chemicals Inc., Gibbstown, NJ) Indicator Strips (pH 5-10), respectively.

Obtained values were analyzed using ANOVA blocking on the sampling date. This was to account for the variability in measurements, which is due to sampling date, as opposed to sampling site.

Water Analysis

Water samples were collected for nutrient analyses. For these analyses, the upstream and downstream regions at sites A and C were treated as separate sites. The collected samples were analyzed according to the following protocols.

Ammonia

Ammonia measurements were taken weekly. Two unfiltered 10 mL samples were collected in the field using acid-washed plastic 50 mL centrifuge tubes. A 400 µL aliquot of 10% phenol solution was added in the field to the 10 mL of samples to preserve them. Samples were transported to the laboratory on ice and analyzed using a modified phenate method for ammonia analysis (American Public Health Association (APHA) 1998) within 24 hours of collection. For the modified method refer to Appendix C.

Phosphate

Two 5 mL water samples for phosphate analysis were collected bi-weekly using acid-washed 50 mL plastic centrifuge tubes. Samples were filtered in the field using Whatman GF/F filters, transported to the laboratory on ice, and frozen for later analysis. A modified ascorbic acid method was used for phosphate analysis (American Public Health Association (APHA) 1998). For the modified method refer to Appendix C.

Nitrate

Two 5 mL water samples for nitrate analysis were collected bi-weekly using acid washed 50 mL plastic centrifuge tubes. Samples were filtered in the field (Whatman GF/F), transported to the laboratory on ice, and frozen for later analysis. A modified cadmium reduction method was used for analysis of nitrates and nitrites (American Public Health Association (APHA) 1998). For the modified method refer to Appendix C.

It should be noted that this method assumes negligible amounts of nitrite in the original sample.

The analytical methods for ammonium, nitrates, and phosphates are colorimetric, using different reagents to develop color in solution. The absorbance at a specific wavelength can be measured using a spectrophotometer and is proportional to the concentrations of the nutrients of interest.

Total Organic Carbon

Water samples for TOC analysis were collected bi-weekly. Samples were brought to the lab on ice and frozen for later analysis. The samples were analyzed using a Shimadzu® TOC-

VCS analyzer to determine the total carbon (TC) and the inorganic carbon (IC) in the samples. TOC was determined by subtracting the IC from the TC.

Macrophyte Community Analysis

Macrophytes were collected at each site bi-monthly from a 0.3 m^2 area of the bank and 0.2 m^2 area of the river bottom. If no emergent macrophytes were present, only the bank vegetation was collected. Plants were identified to the species, dried for 12 hours at 100°C, and weighed to obtain dry biomass. Richness (total number of groups at the same taxonomic level) and diversity were determined. Diversity takes into account the relative abundance of each taxon and was calculated using the Shannon-Weaver diversity index formula (2).

Species richness (total number of taxa at the same taxonomic level) was also determined. Sites were compared on the basis of these indices. For this analyses only the downstream regions (relative to the pond outfall) were sampled.

Macroinvertebrate Community Analysis

Benthic macroinvertebrates were collected at each site (and upstream and downstream regions of Sites A and C) monthly. The method described here, which was used for invertebrate collection, preservation, and identification, was adapted with slight changes from the Ontario Benthos Biomonitoring Network (OBBN) protocol for sampling streams (Jones et al. 2007), which is readily available online at the Ontario Benthos Biomonitoring Network website. See Figure 4 for sampling equipment. The sampling protocol used at each site (upstream and downstream regions at sites A and C are treated as separate sites for this assessment) is described in detail in Appendix C.

Taxa richness, diversity (Shannon-Weaver), percent composition of the taxonomic orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) relative to the total number of individuals (% EPT), and percent composition of the family Chironomidae relative to the total number of individuals (% Chironomidae) (Mackie 2001), were determined and sites were compared on the basis of these calculated indices. Diversity was calculated at the order level, which is provided, with some adjustments, by the OBBN level of identification. For organisms that were identified to family, a coarser level of identification was used for diversity calculations. Correspondence analysis was conducted on 3 dates: May 8 (Spring), July 20 (Summer), and September 7 (Fall) using MicroSoft Excel and the Biplot plug-in. The steps of Correspondence Analysis are described in Appendix C.

In-Stream Cages

This method was adapted from Grapentine et al. (2004). Cages were deployed at the Headwater site and at Site D in the spring, and at Sites B and D in the fall (Headwater site became dry in early June). Each 54cm x 42cm x 25cm cage divided into 15 cells of equal size made of 1 mm aluminum mesh, 5 of which (in a staggered pattern) were occupied by mini-cages with 0.25 mm nylon mesh (Figure 3). Each mini-cage housed 10 *Hyalella azteca* and 10 *Daphnia magna*. Survivorship was assessed each week for 4 weeks. Percent survivorship was determined by establishing the numbers of animals that survived after the first week as a baseline to account for any deaths due to transfer shock, and mating behaviour was assessed for *H. azteca* by counting the percent of individuals engaged in mating pairs.

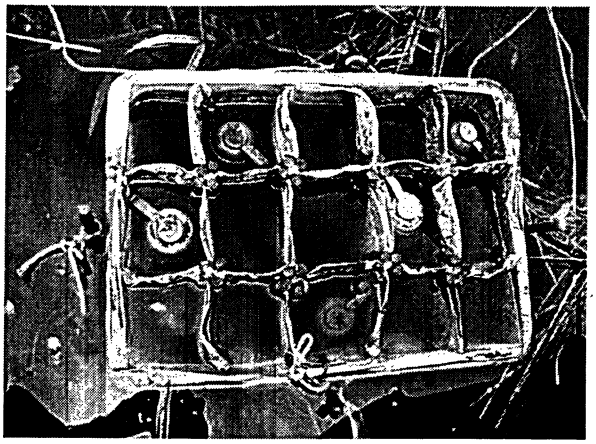


Figure 3: ***In-situ*** **invertebrate testing set-up**

Data Analysis

Data were compared among sites using ANOVA, blocking on the sampling date to account for temporal variation within sites. ANOVA with blocking was selected in favour of two-way ANOVA as the comparisons of interest were sites, and temporal differences within sites, or time-site interactions had to be isolated from the pertinent results. Where differences between sites were detected, a Fisher's Least Significant Difference (LSD) test was used to determine how sites differed. Fisher's LSD test is a commonly used post-hoc test following a global test of the null hypothesis that all treatment groups under study are equal (such as ANOVA). Where the results of the global test allow rejection of the null hypothesis, Fisher's LSD test can be used to perform all pair-wise comparisons of treatment groups at the same level of significance. Upstream versus downstream data analyses at Sites A and C were done using paired *t* tests, with each date representing a replicate. All statistical analyses were done using SYSTAT version 12 (Systat Software, Inc., Richmond, California).

RESULTS

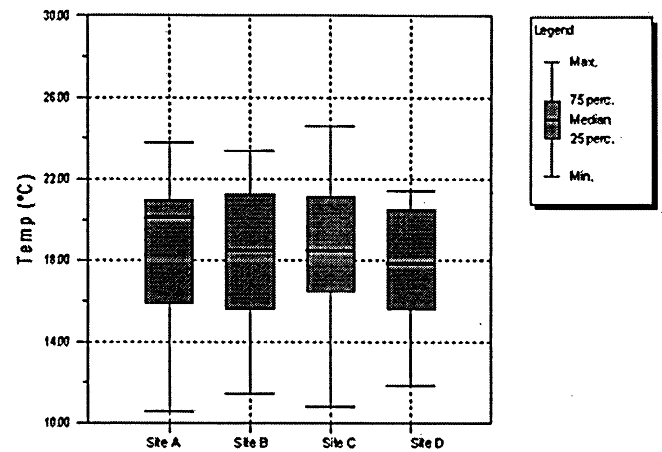
Due to the unforeseen and massive drought in the summer of 2006, the selected Headwater site dried up one month into the field season. However, while water was present, the results from this site were used in macrophyte community composition analyses, as well as in the spring caged experiments. Other results have been excluded from long-term analysis and the invertebrate indices calculation.

Physicochemical Conditions

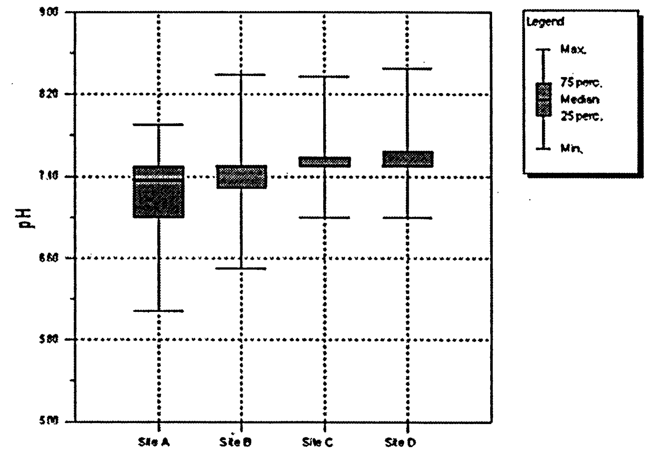
When temperature was analyzed, no significant differences were found among the sites ($p=0.28$), or between the upstream and the downstream locations (relative to pond inflows) for Sites A and C ($p=0.07$ and $p=0.6$, respectively). Figure 4(a) shows that the seasonal ranges of temperatures at all four sites are quite similar.

There was no significant difference in pH moving downstream from Site A to Site D ($p=0.342$; Figure 4(b)). No significant differences were found in the immediate upstream vs. downstream locations of Site A and Site C ($p=0.36$ and $p=0.39$ respectively).

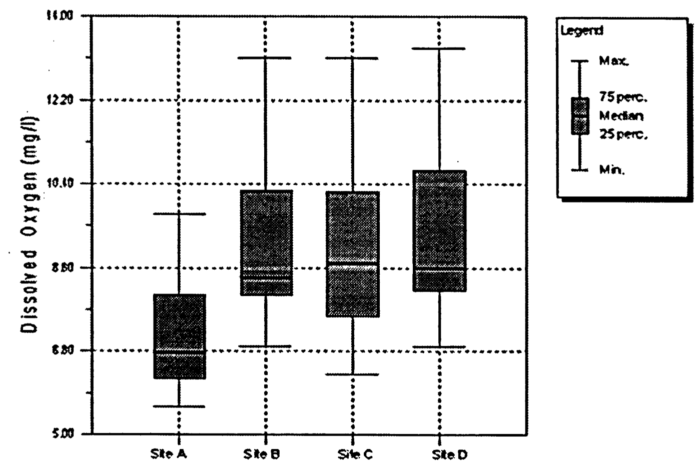
Dissolved oxygen was found to be significantly lower at Site A compared to the other sites ($p<0.001$; Figure 4(c)). No significant differences were found in the upstream and downstream region at Site A ($p=0.63$); however dissolved oxygen was marginally lower downstream relative to upstream at Site C ($p= 0.08$).



a)



b)



c)

Figure 4: Physicochemical properties of Sites A, B, C, and D over the sampling season; a) temperature, b) pH, c) Dissolved Oxygen

Water Analysis

Ammonium levels were found to be significantly higher at Site A compared to the other sites ($p=0.001$; Figure 5(a)). No significant differences were found in the immediate upstream and downstream regions of Site A and Site C ($p=0.25$ and $p=0.55$ respectively).

Nitrate concentrations were found to be significantly lower at Site A compared to the rest of the sites ($p=0.002$) (Figure 5(b)). No significant differences were found in the upstream versus downstream comparison at Sites A and C ($p=0.42$ and $p=0.41$ respectively).

Phosphate levels were not found to be significantly different moving downstream ($p=0.273$). Comparison of the regions immediately upstream and downstream at Sites A and C showed no significant differences ($p=0.47$ and $p=0.65$ respectively). Figure 5(c) shows a steady increase in phosphate from Site A to Site D; however, this is not statistically significant.

Total organic carbon was not found to be significantly different among sites ($p=0.221$), although concentrations were generally highest at Site A (Figure 5(d)). Upstream and downstream regions at Sites A and C were not significantly different either ($p=0.94$ and $p=0.13$ respectively).

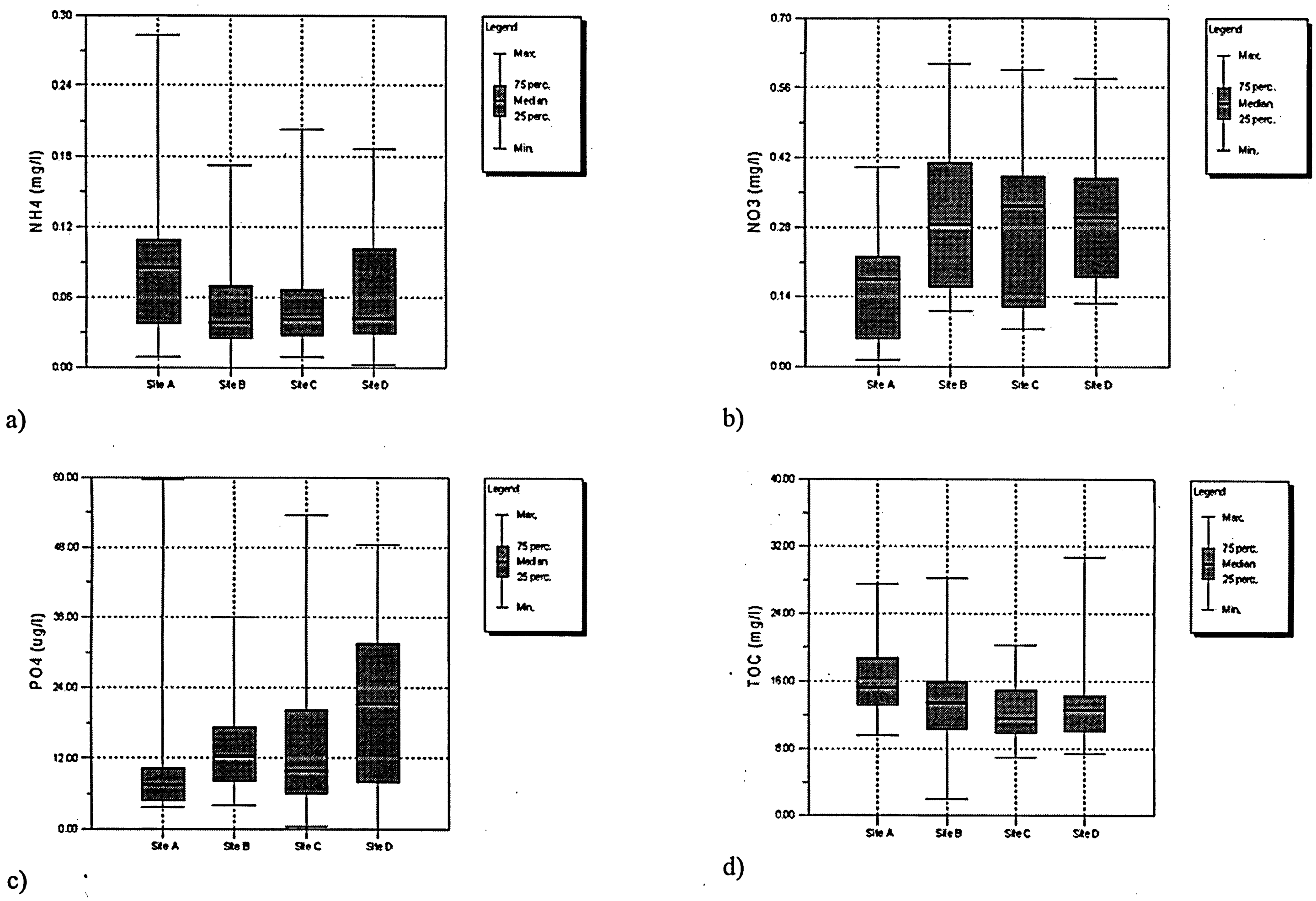


Figure 5: Nutrient levels at Sites A, B, C, and D over the sampling season; a) ammonium, b) nitrate, c) phosphate, d) TOC

Macrophyte Community Analysis

There was little emergent vegetation; therefore, the analyses are based primarily on the macrophytes collected from the adjacent bank area. Macrophyte diversity showed a marked increase moving downstream during the spring season (Figure 6(a)). However, diversity was similar among sites in the summer, with the exception of Site B, which showed an increase over the other sites. In the summer collection, only one species was collected from the Headwater site (reed canary grass), returning a Shannon-Weaver diversity index value of 0. Species richness presents no pattern moving downstream from the Headwater site to Site D either in spring or summer (Figure 6(b)). Dry biomass showed a general decrease moving downstream from the Headwater site to Site D (Figure 6(c)).

a) b) c)

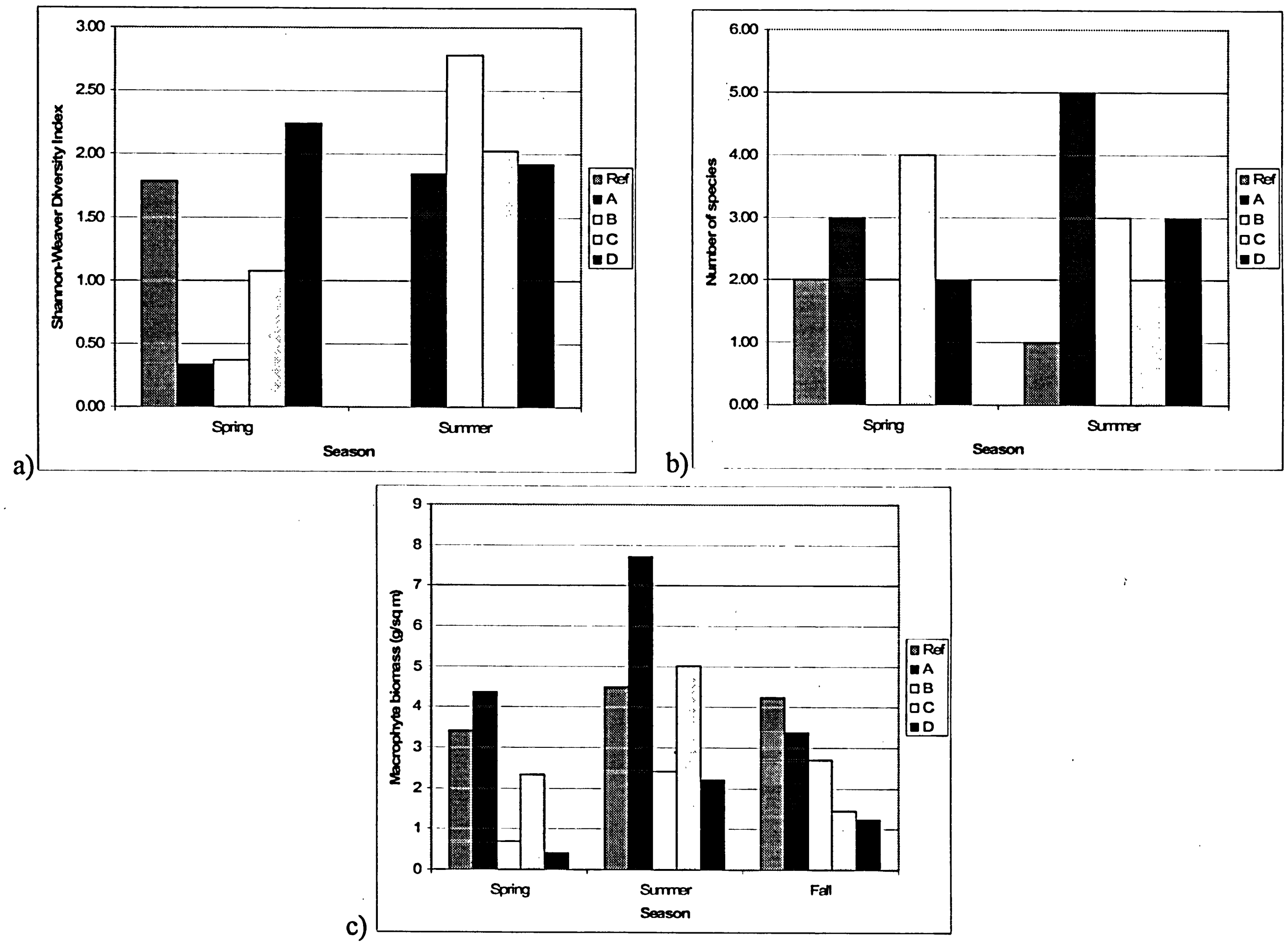


Figure 6: Seasonal macrophyte diversity (a), richness (b), and dry biomass (c)

Over the sampling season, the Headwater site yielded marsh marigold (*Caltha palustris*) and reed canary grass (*Phalaris arudinacea*). At Site A, meadow foxtail (*Alopecurus pratensis*), purple loosestrife (*Lythrum salicaria*), goldenrod (*Solidago canadensis*), wild mint (*Mentha avensis*), reed canary grass (*Phalaris arudinacea*), and field horsetails (*Equistum pratense*) were observed. Canary reed grass (*Phalaris arudinacea*), meadow foxtail (*Alopecurus pratensis*), goldenrod (*Solidago canadensis*), rice cut grass (*Leersia oryzoides*), and field horsetail (*Equistum pratense*) were observed at Site B. At Site C, reed canary grass (*Phalaris arudinacea*), meadow foxtail (*Alopecurus pratensis*), field horsetail (*Equistum pratense*), and goldenrod (*Solidago canadensis*) were collected. Site D yielded field horsetail (*Equistum pratense*), meadow foxtail (*Alopecurus pratensis*), and goldenrod (*Solidago canadensis*).

Macroinvertebrate Community Analysis

The Headwater site dried up one month into the field season, but macroinvertebrate analyses from the first month revealed that the benthic community was made up of gastropods and oligochaetes. The Headwater site was excluded from further analyses.

The following macroinvertebrates were found in the study region over the field season (Table 2). Note, the OBBN names do not always correspond to the names given in Table 2 – this is because Table 2 gives the basic Phylum/Class/Order/Family breakdown, whereas some of the OBBN names are Sub- and Super- divisions of the basic classification. Refer to Appendix B to find out the exact taxonomic level of the OBBN groups.

Table 2: Taxonomic inventory of the benthic invertebrates in the study region (organisms were grouped at the order level for subsequent analyses using indices)

Phylum	Class	Order	Family	Common name
Annelida	Hirudinea	Rhychnobdellida		Leeches
Annelida	Oligochaeta	Tubificida		Earthworms
Arthropoda	Arachnida	Actinedida		Mites
Arthropoda	Insecta	Coleoptera		Beetles
Arthropoda	Insecta	Diptera	Chironomidae	Non-biting midges
Arthropoda	Insecta	Diptera	Ceratopogonidae	Biting midges
Arthropoda	Insecta	Diptera	Tabanidae	Horse/deer flies
Arthropoda	Insecta	Diptera	Tipulidae	Crane flies
Arthropoda	Insecta	Diptera	Simuliidae	Blackflies
Arthropoda	Insecta	Ephemeroptera		Mayflies
Arthropoda	Insecta	Hemiptera		True bugs
Arthropoda	Insecta	Odonata		Dragonflies
Arthropoda	Insecta	Trichoptera		Caddisflies
Arthropoda	Malacostraca	Amphipoda	Gammaridae	Sow bugs
Arthropoda	Malacostraca	Dacapoda		Crayfish
Arthropoda	Malacostraca	Isopoda		Scuds
Cnidaria	Hydrazoa	Hydroida		Hydra
Mollusca	Gastropoda	Opisthobranchia		Aquatic snails
Nemata	Secernentea♦	Rhibditida♦		Nematodes
Platyhelmenthes	Turbellaria	Seriata	Planeriidae	Flatworms

Cumulative Analysis

Diversity generally increased for all sites (Figure 7 (a)) and Sites A and C scored consistently higher than Sites B and D. Richness presents no temporal pattern, but, as with diversity, Sites A and C score consistently higher than B and D (Figure 7 (b)). The % EPT increased over the course of the sampling season and was overwhelmingly higher at sites A and C than and B and D (Figure 7 (c)). The % Chinornomidae decreased at Sites A and C over the sampling period and at Sites B and D it decreased until August and increased again in September (Figure 7 (d)). Sites A and C scored consistently lower in % Chironomidae.

♦ Unconfirmed. All nematodes were treated as one group due to lack of sufficient identification expertise

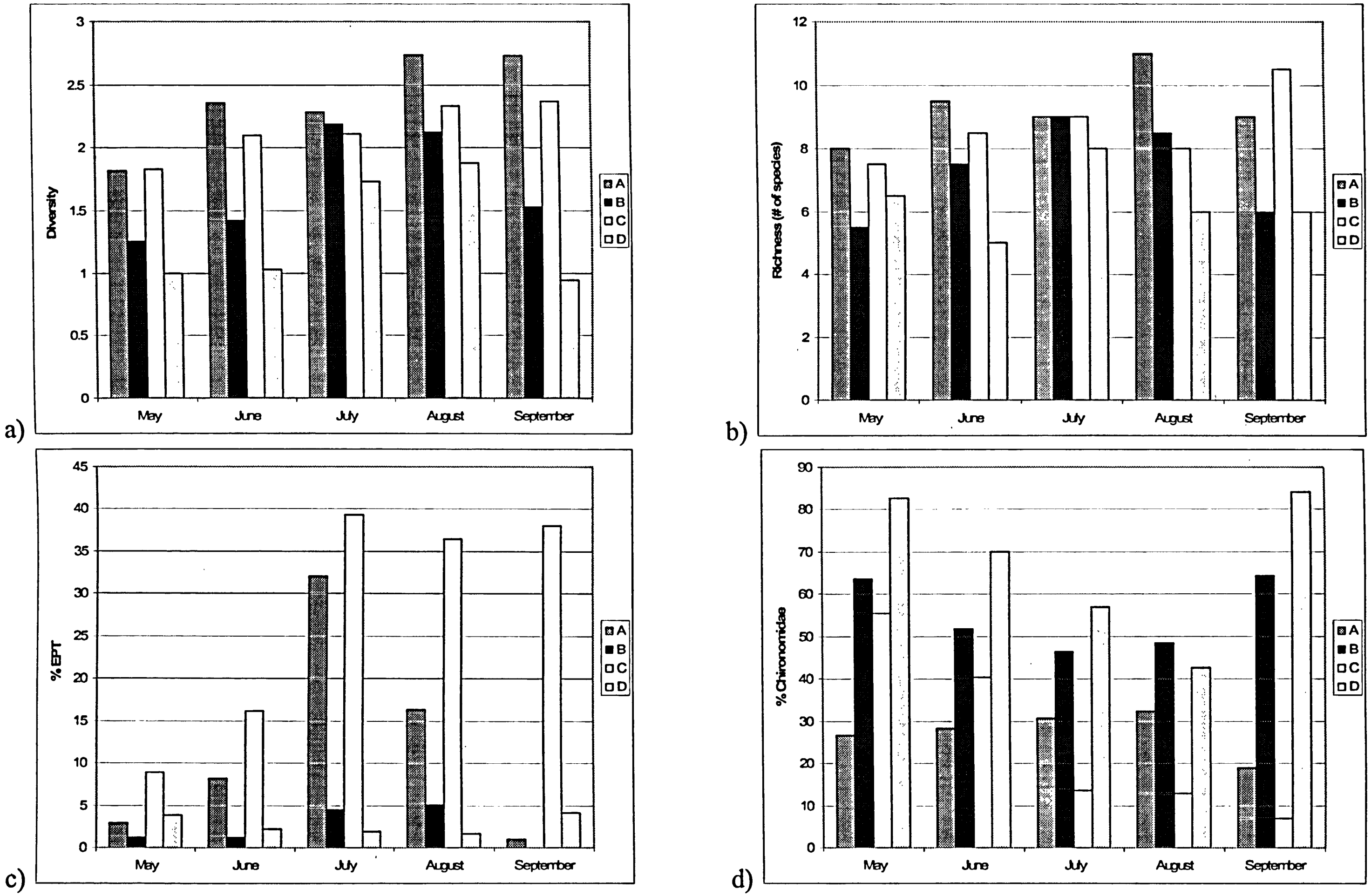


Figure 7: Monthly macroinvertebrate index scores for the study region

Local analysis

The macroinvertebrate community analysis for Sites A and C showed no pattern in upstream vs. downstream community composition. Richness both increased and decreased between the upstream and downstream regions of Site A over the three months and at the upstream and downstream regions of Site C, the species richness increased or stayed the same. Diversity increased for both sites at the downstream location for all months, except for Site C in September, when the diversity decreased from upstream to downstream locations. The % Chironomidae increased at the downstream regions of both sites in August and October, but decreased (again, at both sites) in September. The % EPT either decreased slightly, or stayed the same between the upstream and downstream regions of Site A, and at Site C % EPT decreased in the downstream region in August, but increased in September and October (Table 3).

Table 3: Macroinvertebrate community composition comparison between the upstream and downstream regions at Sites A and B (values averaged over 2 sub-samples at each site)

		Site A		Site C	
		Upstream	**Downstream**	**Upstream**	**Downstream**
Richness	*August*	8	11	8	8
	September	10.5	9	8	10.5
	October	9.5	7.5	7.5	8.5
Diversity	*August*	2.03	2.77	2.32	2.33
	September	2.41	2.73	2.60	2.37
	October	2.22	2.33	1.92	2.54
% Chironomidae	*August*	16.56	32.38	12.25	12.88
	September	45.04	19	20.5	7.04
	October	17.86	23.86	4.76	11.22
% EPT	*August*	17.38	16.38	41.08	36.43
	September	1	1	23.46	38.01
	October	0.97	0.48	46.93	48.29

Correspondence Analysis

The following three figures show the results of the Correspondence Analysis for Spring (Figure 8), Summer (Figure 9), and Fall (Figure 10). In the Spring, it appears that Site C and Site D are very close in community composition. Dragonflies, crayfish, caddisflies, and water mites are the taxa clustered around them, which results in their separation. Site A seems equidistant from Sites B and D, and slightly further yet from C.

In the Summer, Sites A, C, and D are most separated in terms of community composition. leeches, damselflies, dragonflies, and mayflies characterize Site A; aquatic moths, horseflies, and craneflies are responsible for separation of C; true bugs, crayfish, and flatworms were abundant at B and D, separating them from the rest.

In the Fall, Sites B and D are very similar based on community composition. Upstream and downstream regions of site C are also close to each other, although C-US is as close to B as it is to C-DS. Upstream and downstream regions of Site A show fairly large differences, with water mites, snails, and sowbugs influencing A-US, and damselflies and leeches influencing A-DS.

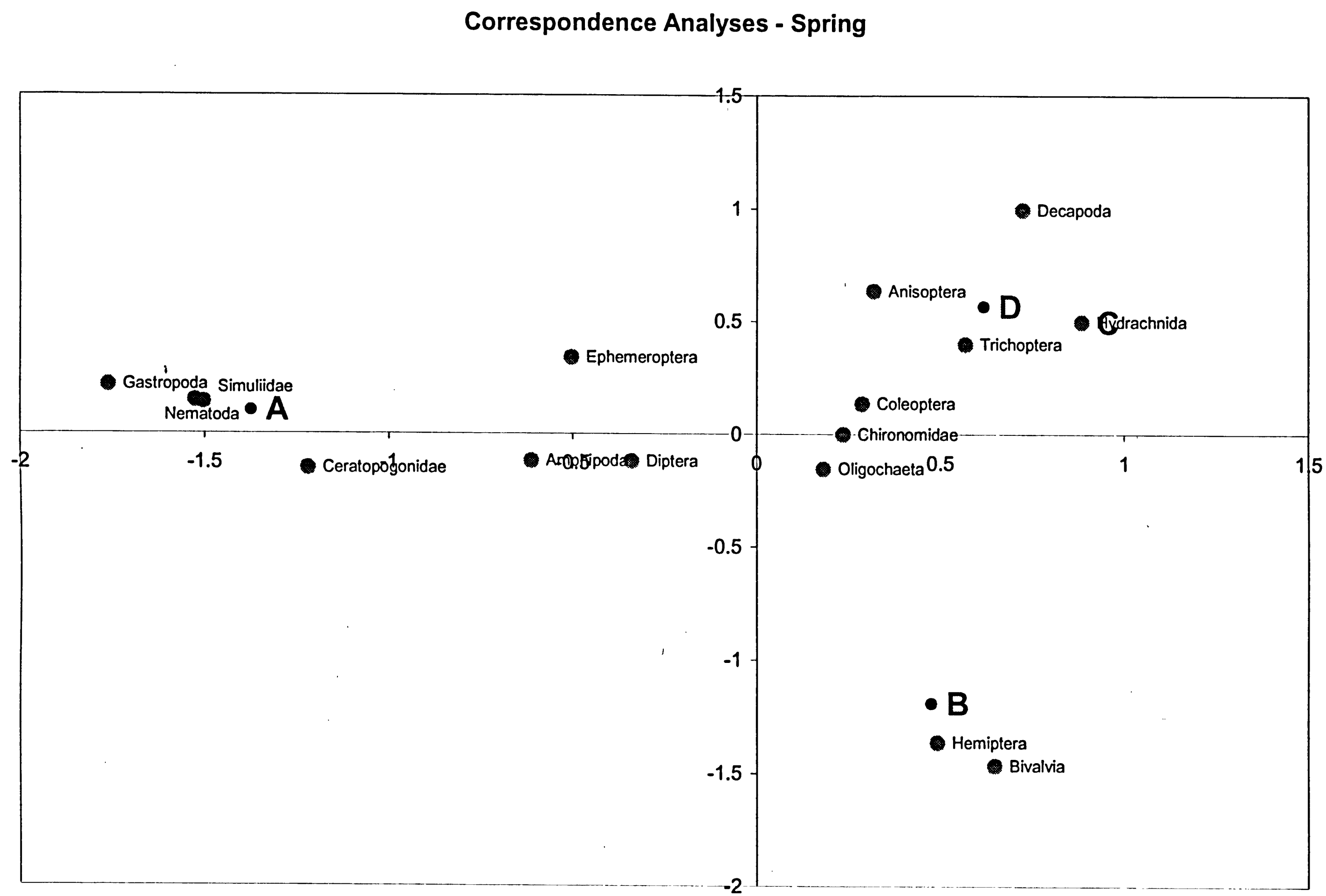


Figure 8: Spring Correspondence Analysis for macroinvertebrate community

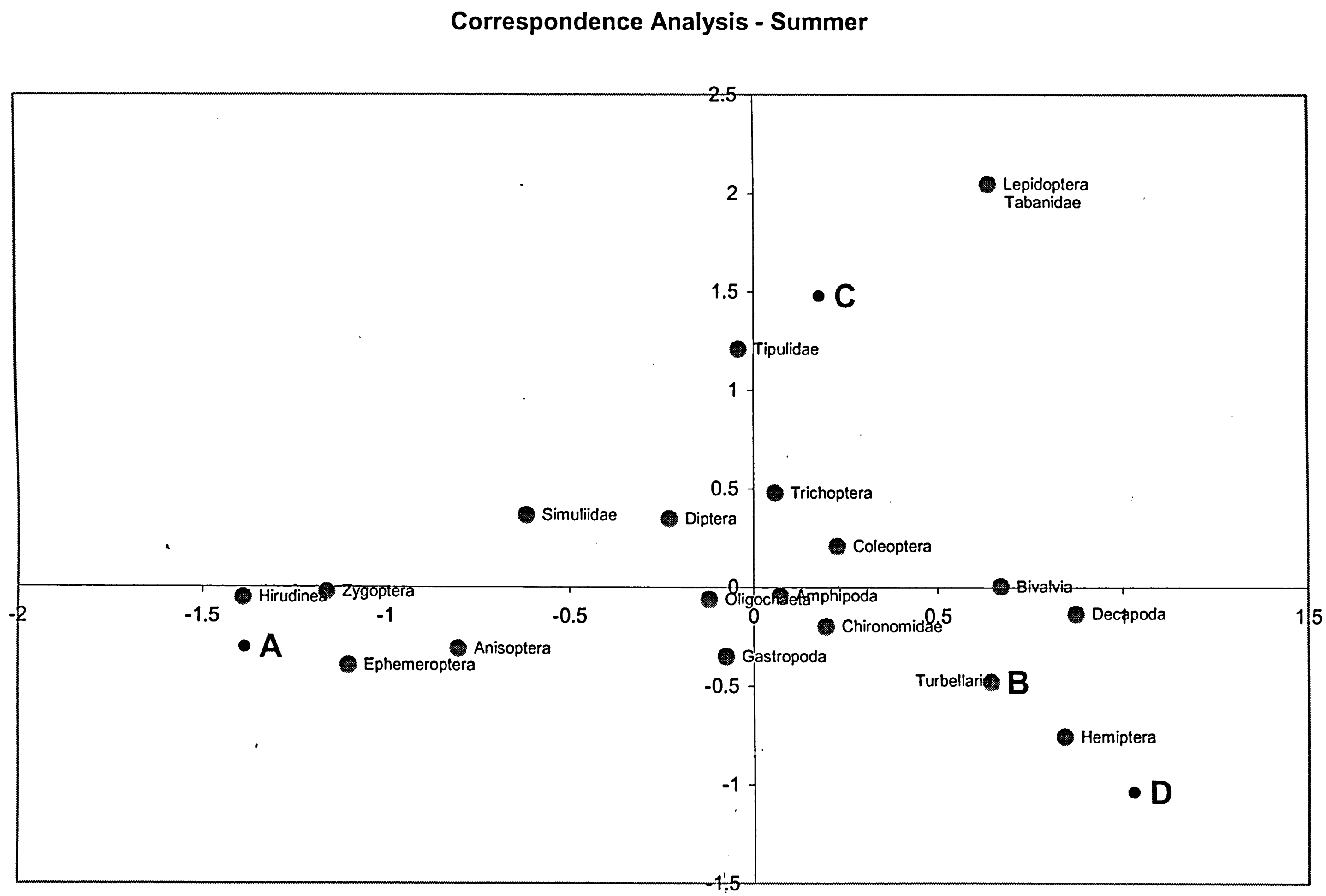


Figure 9: Summer Correspondence Analysis for macroinvertebrate community

Correspondence Analysis - Autumn

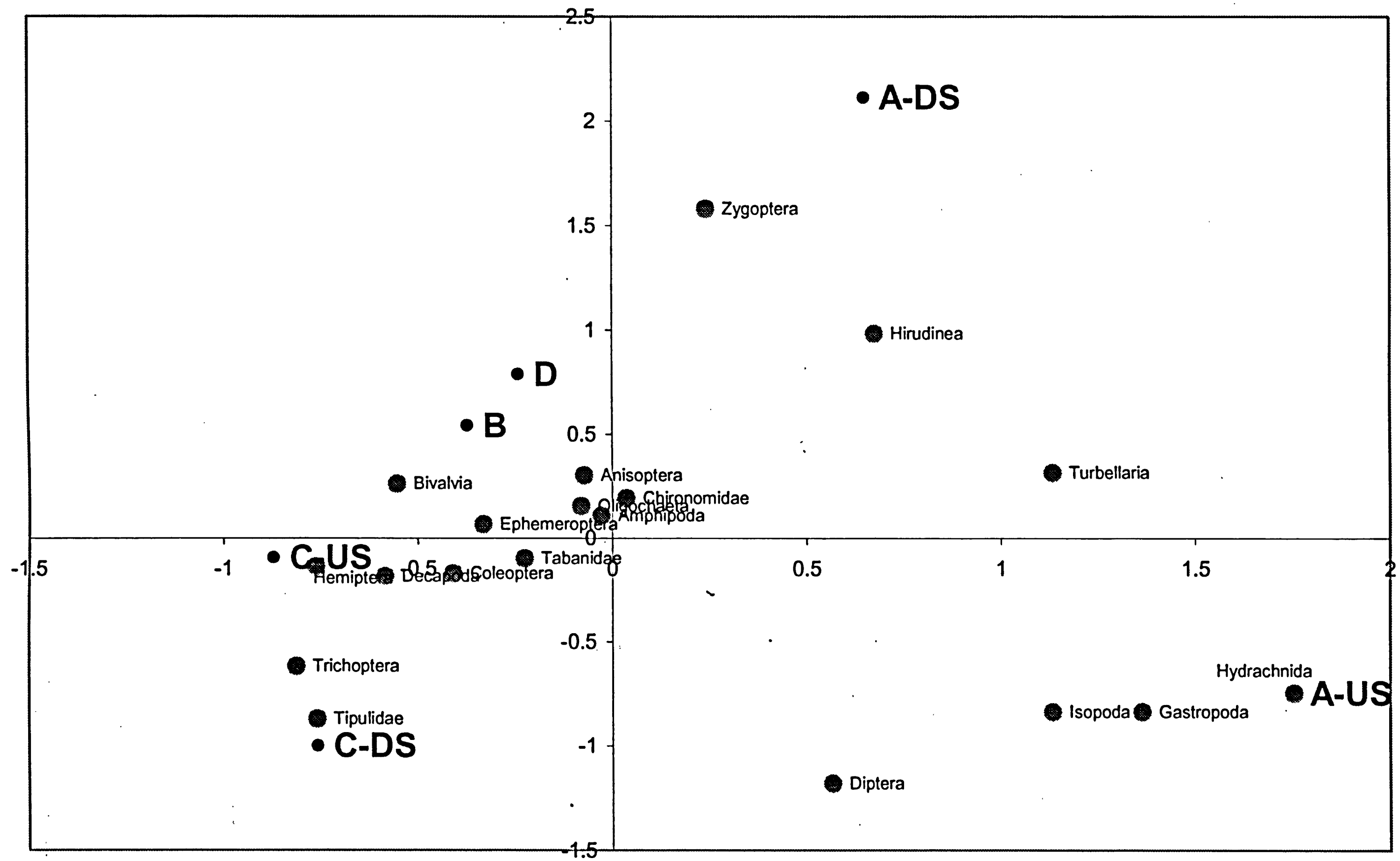


Figure 10: Autumn Correspondence Analysis for macroinvertebrate community

In-Stream Cages

No significant differences in *H. azteca* survival or mating were observed between Headwater and Site D in the spring or between Sites B and D in the fall (p=0.14 and p=0.85, respectively for spring; p=0.69 and p=0.43 for fall). In the first two weeks of spring deployment, the *D. magna* population increased beyond practical field enumeration at both sites. The population remained high for the duration of the spring deployment, suggesting little toxicity or reproductive impairment. In the fall, the population did not show any significant differences in survivorship between Sites B and D (p=0.21). These results suggest that there is little or no toxicity in the upstream sites, and this observation does not change moving downstream to Site D.

Table 4: Survivorship of *D. magna* and survivorship and mating behaviour of *H. azteca*

		D. magna	*H. azteca*	
		Survivorship (%)±SD	Survivorship (%)±SD	Change in Mating[⊗] ±SD
Spring	Headwater	100+	64.13±35.36	14.41±24.83
	D	100+	103.14±36.32	16.67±36.82
Fall	B	0±0	128.75±39.66	15.47±17.98
	D	27.23±82.79	147.02±83.24	36.25±35

[⊗] Calculated by subtracting % individuals in mating pairs on first week from % individuals in mating pairs on last week (positive numbers mean increase in mating behaviour)

DISCUSSION

The physicochemical results (Figure 6) indicate that there is no physical impact of the stormwater ponds, either locally or cumulatively, on the receiving waters. The only anomaly is the low dissolved oxygen values at Site A and that is likely due to a large quantity of decomposing plant matter. As stated in site descriptions earlier, Site A is heavily overgrown with herbaceous plants and the channel at this site is very narrow with silty sediment, creating conditions for high oxygen demand by microbial activity.

The nutrient levels (Figure 7) indicate that there is no impact, either cumulatively or locally, from stormwater ponds. While the ammonium levels at Site A are higher than the rest of the sites, other nutrients are low, suggesting that the influx of the stormwater from the ponds does not contribute to the eutrophication of this site. This is supported by the TOC values. The high levels of ammonium and TOC can be explained by decomposing detritus, which also agrees with the dissolved oxygen results discussed earlier. It should also be noted that, while ammonium values are significantly higher at Site A, ammonium and nitrate do not exceed the Environment Canada guidelines (0.5 mg/L for ammonium and 10 mg/L for nitrate nitrogen (Environment Canada 1984); no guidelines have been established for phosphorus in surface waters, but values below 0.10 mg/L are considered excellent for groundwater (Center for Earth and Environmental Science 2005).

It should be noted that since upstream and downstream regions did not show significant differences in nutrient concentration, stormwater ponds neither increased nutrient loading, nor had a discernable impact on nutrient retention. This, in turn, suggests that the nutrient concentration in the pond is similar to the ambient aquatic nutrient content. The next step in this investigation would be to sample the inlet and outlet of the stormwater pond to determine if the ponds are changing nutrient concentrations in a measurable way.

The macrophyte community analyses (Figure 6) do not indicate any cumulative impact from the stormwater ponds. While a decrease can be seen in the biomass, it can be explained by the change in macrophyte community composition. At the upstream sites, grasses dominate, while at downstream sites, horsetails are prevalent. Grasses are very fibrous, while horsetails have a more spongy cortex, which holds more water. The different tissue composition in the two types of plants may lead to a discrepancy in weight when they are dried.

The macroinvertebrate community analysis (Figure 7) shows that, there appears to be no consistent cumulative impact at Sites A, B, and C. Site D shows a consistent marked decrease in species richness and diversity, as well as a decrease in sensitive EPT and an increase in tolerant chironomids; however, so does Site B. Site C, which is between the two, shows high diversity and richness, as well as a healthy compliment of Trichoptera and Ephemeroptera coupled with low chironomid numbers. These results are inconsistent with cumulative impact, since then we would expect to see a steady degradation with increasing number of stormwater pond outfalls. These results do suggest an impact, but localized at Sites B and D, and, since the physical and nutrient analyses failed to provide an explanation, further chemical analyses of possible organic and inorganic contaminants are warranted in future studies.

Species richness showed both an increase and a decrease between the upstream/downstream locations, depending on the sampling date. This suggests that species richness at these sites is probably equal and the variations were incidental. Species diversity showed a consistent increase between upstream and downstream regions, except for September sampling at Site C. There is no clear adverse effect on species diversity due to stormwater input.

Percent Chironomidae (tolerant species) showed both, an increase and a decrease between the upstream and downstream sampling regions, depending on sampling date. There is an increase in percent Chironomidae in the downstream regions of both Sites A and C for the August and October sampling dates; however, there is a marked decrease at the same sites in September. This suggests that the conditions that may cause this effect are not constant. Further study is warranted to determine whether these fluctuations are random, or if they are in any way tied to the stormwater pond discharges. Percent EPT (sensitive species), as species richness and percent Chironomidae, showed both an increase and a decrease between the upstream and downstream locations, depending on the sampling date.

Correspondence analysis showed that on two out of three dates (summer and fall; Figures 9 and 10) Sites B and D were very close in close in community composition, and that at Site C, the upstream and downstream benthic communities are very similar. It also shows that the upstream and downstream communities at Site A are not very similar. Looking at the other indices for that date (Table 3; September) it can be seen that the richness decreases, diversity increases, % chironomids decreases and % EPT stays the same. This suggests that, while the community composition may have been different, it is still fairly diverse. It should be noted that correspondence analysis cannot be used to gauge impact unless there is a non-impacted site present with the same habitat characteristics. Correspondence analysis is used in this capacity when RCA is conducted. Then it is used to look at a test site relative to a cluster of reference sites. In lieu of a reference site, this metric should be looked at in conjunction with other indices.

The organisms in the *in-situ* experimental enclosures did not show any differences in survivorship between upstream and downstream deployment sites, either in the spring, or in the fall, suggesting no acute toxicity of the water or sediment at the study sites.

The changes (or lack thereof) in the indices discussed above do not suggest any adverse effect on the invertebrate community discharge from these stormwater ponds.

Summary

Previous studies have shown that stormwater can carry urban pollutants such as sediment, heavy metals, and organic contaminants (Mayer et al. 1996; Marsalek and Rochfort 2004). It has also been shown that some pollutants, such as polyaromatic hydrocarbons, are adsorbed to particulate matter (dust) and can be washed off from urban surfaces such as roofs and roads and add to wet weather pollution (Murakami et al. 2004). Stormwater ponds are designed to retard the water flow during (and after) a storm event to allow certain contaminants to be removed from the run-off. It has been shown that the stormwater ponds contain high amounts of nutrients and metals sometimes exceeding the pertinent MOE guidelines (Mayer et al. 2007), and allows for some reduction of total suspended solids (Mayer et al. 1996; Marsalek et al. 1997). However, the latter depends quite heavily on residence time.

In this study, knowing that such pollutants may be making their way into the stormwater ponds of a developing municipality, we wanted to elucidate whether outflow from these ponds had a measurable negative impact on the receiving waters.

The results indicate that none of the nutrient or physical parameters measured in the receiving waters exceeded Environment Canada guidelines (Environment Canada 1984), and while biological criteria revealed shifts in community composition for both macrophytes and macroinvertebrates, these shifts cannot be attributed to deleterious impacts of inflow from SWT facilities due to the lack of consistent patterns in these shifts. The invertebrate community shows some deterioration at Sites B and D; however, if this were due to the stormwater pond influence, it would be expected that Site C should fall somewhere between the two in the indices. That is not the case (Figure 7). It is probable that any impact due to the water from the stormwater ponds is overshadowed by local conditions such as channel geometry, substrate type, bank vegetation, etc. Similar findings have been reported previously by Grapentine et al. (2004). Also, macrophytes, while showing a marked decrease in biomass when moving downstream from the Headwater site, increase in species diversity (Figure 6). The decreasing biomass is likely due to the change in species composition of the macrophyte community. In this regard, diversity and richness present more reliable indicators of ecosystem health than biomass.

Protocol Development

While the first two objectives of this study were to a) whether there is a discernible cumulative impact and b) whether there is a discernible local impact of stormwater ponds on receiving bodies, the final objective was to develop a simple protocol that municipalities could utilize in long-term monitoring of the receiving waters in their jurisdictions and any impact that may arise from development. As such, several protocols have been adjusted and simplified in the current study, with additional protocols developed.

Physicochemical and nutrient measurements

The adjusted Standard Methods for Water and Wastewater analyses were used to determine the ammonia, nitrate, and phosphate concentrations in the water. The methods were adjusted to utilize smaller volumes to make sampling logistics more efficient. Smaller volumes, nonetheless, yielded consistent results. In a few cases, reagents were substituted (e.g. NaH_2PO_4

for KH_2PO_4); however, such substitutions do not change the reactive ingredient and, therefore, did not change the result of the tests.

Macrophyte community

No established protocol for waterway assessment using macrophytes has been reported in the literature, except for one developed in Germany which utilized surveys of 100 m stretches of the river (Meilinger et al. 2005). Studies that have looked at macrophytes usually conduct a census of the macrophytes in the area of interest (Bishop et al 2000a). This kind of assessment would not be feasible for a municipality's routine monitoring program; however, the level of effort and expertise required to collect and analyze macrophyte communities as presented in the current study could reasonably be implemented. Our protocol yielded results that agree with those of other methods of assessment utilized, showing that it perhaps can be used successfully in the future in this type of assessment.

Macroinvertebrate community

One of the most common ways to assess river ecosystem health is to look at the macroinvertebrate community (Rochfort et al. 2000; Grapentine et al. 2004). However, this assessment requires a certain level of expertise in identifying the organisms to provide a reliable result. The convention is to identify the organisms to family or even genus/species level (Metcalfe 1989). Implementation of routine macroinvertebrate community analysis by a municipality would, in most cases, be impractical. In comparison to macrophyte community analyses, macroinvertebrate community analysis is more time-consuming and requires greater expertise. Having samples analyzed by a third party means considerable expenditures whenever such an assessment is considered, leading to very few bioassessments using macroinvertebrate communities actually being conducted by the municipalities (pers. comm. Nemeth 2006).

This study looked at a simplified approach to macroinvertebrate bioassessment utilizing the order level of identification. Ontario Benthos Biomonitoring Network (OBBN) has developed a protocol in an attempt to standardize sampling techniques to allow data sharing between different organizations. While the OBBN encourages family or genus level of identification, the 27 group ID is the threshold for participation. The protocol used here calls for the order level of identification, which is acceptable under the OBBN guidelines (Jones et al. 2007). This approach can be considered a simplified alternative to the conventional approach, and may allow for macroinvertebrate community analysis to be implemented as part of a municipality's biomonitoring protocol. It is recommended that the municipalities create a library of the invertebrate specimens that are found in their area, which will allow for easier training.

The chosen indices are representative and simple to calculate. Having said this, they are not the only ones that can be used in an assessment based on the macroinvertebrate community. Individual taxon tolerance scores and multivariate analyses described in the introduction can also be used for this purpose.

In this study, due to some differences in the sample collection protocol, the RCA analysis could not be conducted. It is recommended, however, that the protocol be adjusted as follows:

- The mesh of the kick net should be 500 μm
- As much as possible the test sites should be far enough apart to include two riffles and a pool in the sampling design

If these changes are applied to the protocol presented in the Methods portion of this report, the RCA can be conducted on the subsequent studies in this region to determine if the fluctuations in the invertebrate community composition are due to the natural habitat conditions, or if it is being influenced by the discharge of the stormwater ponds.

In this study, a protocol of *in situ* assessment was adapted from Grapentine et al. (2004). *Daphnia magna* and *Hyalella azteca* were used as the test species. The lack of response indicates that, in this particular instance, the end-point measurements chosen for the assessment may not be sensitive enough (ie. lethality and reproductive behavior). This part of the protocol should be amended to include growth and developmental end-points.

While *D. magna* and *H. azteca* are well-recognized organisms used for toxicity testing (Grapentine et al. 2004; Hatch and Burton 1999; Kubitz et al. 1994; McCarthy et al. 2004; Rochfort et al. 2000), they are not the only ones. There are other organisms that can be used, such as *Ceriodaphia dubia* (Kubitz et al. 1994; Ireland et al. 1996), *Gammarus pulex* (Boxall and Maltby. 1995; Boxall and Maltby. 1997), *Chironomus riparius* (Rochfort et al. 2000), *Hexagenia spp.* (Burton et al. 2005; Riba et al. 2006), *Lumbriculus variegates* (Burton et al. 2005), and others. The organisms should be selected based on the local fauna as to not introduce any exotic species, and suspected toxicity, since all of these organisms have different tolerance levels.

It should be noted that when *H. azteca* and *D. magna* are used together for a toxicity test, it is preferable to house them in separate containers since it has been observed in this study (during transport of the organisms) that *H. azteca* may prey on *D. magna* when put in close proximity.

CONCLUSION

From the results discussed above it is unlikely that the stormwater ponds on the stretch of Rouge River in Quadrant 19 of the Municipality of the Town of Richmond Hill are having an adverse impact on the ecosystem of the receiving waters. All results for physicochemical parameters and nutrient levels can be explained by the biological activity in the region (Site A). No impact is seen in the macrophyte community or in the *in situ* experiments. While macroinvertebrate indices suggest impact at Site D and, to a lesser extent B, warranting further investigation, all sites taken together do not show consistent impact. No impact is observed locally.

From the nutrient results (showing no nutrient input or dilution effect) it is aparent that the stormwater ponds not having an effect on nutrient or organic carbon retention, nor are they acting as a source of nutrient pollution.

The protocol developed is suggested for use by the municipalities to assess the rudimentary biological impacts from any municipal stressors, not necessarily stormwater. This protocol was developed so that it requires little expertise with macrophytes, macroinvertebrates, or statistical manipulations with the numbers obtained in the field. It is suggested that the same team of people carry out the assessment from year to year to build expertise, as well as keep variation in sampling and processing technique to a minimum.

Since most municipalities already conduct physical and chemical (to various degrees) monitoring of the stormwater ponds on their property, the biological protocols presented in this report could be incorporated into the municipal monitoring protocols. It is highly recommended that the invertebrate community assessment be made an integral part of monitoring routine. Macrophytes are recommended as well; however if there are no observable emergent macrophytes in the study region, macrophytes should be paid less attention than the invertebrates.

If an impact is detected during an assessment, measures should be taken to remediate it and the assessment repeated following remediation efforts to make sure that the measures implemented served their purpose. If no impacts are found (such as in this case) the assessment should be repeated on a regular basis (every few years or so) to make sure that the situation remains acceptable.

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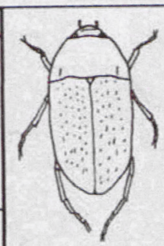
APPENDIX A
Maps of the Study Region and OBBN Data Sheets of the Study Sites

Figure A-1: Study region in the Municipality of the Town of Richmond Hill – Rouge River stretch and stormwater ponds under study are highlighted

Ontario Benthos Biomonitoring Network Field Sheet: STREAMS



Date: 14-Jun-07	Stream name: Rouge River
Time 11:15	Site #: Headwater 43°53'47"N
Agency: Ryerson University	Location: centroid of 3 replicates; Lat/Long or UTM 79°24'31"W
Investigators: Alexandra Chmakova	Elevation (m asl):
	Datum/zone:

Water Quality

Water Temperature (°C): n/a	Conductivity (uS/cm): n/a	pH: n/a	
DO (mg/l): n/a	Alkalinity (mg/l as $CaCO_3$ n/a		

Site Description and Map

Draw a map of the site (with landmarks) and indicate areas sampled. Attach photograph (optional)
Show north arrow.

Benthos Collection Method (circle one):
- Traveling Kick & Sweep
- Grab Sample
- Other (specify):

Gear Type (circle one)
- D-net
- Ponar
- Other (*specify*):
- Ekman
- Rock Baskets

Mesh Size: 500 micron (or specify)

Sub-samples	Sampling distance covered (m)	Time (min.)	Max. Depth (m)	Wetted Width (m)	Max. Hydraulic Head (mm)	# Grabs pooled per sample
Sample 1: Riffle (cross-over)	n/a	n/a	n/a	n/a	n/a	n/a
Sample 2: Pool	n/a	n/a	n/a	n/a	n/a	n/a
Sample 3: Riffle (cross-over)						

Substrate

Enter dominant substrate class and second dominant class for each sub-sample

	Sample 1	Sample 2	Sample 3
Dominant	2	2	
2nd Dominant	1	1	

Class	Description
1	Clay (hard pan)
2	Silt (gritty, < 0.06 mm particle diameter)
3	Sand (grainy, 0.06 - 2 mm)
4	Gravel (2 - 65 mm)
5	Cobble (65 - 250 mm)
6	Boulder (> 250 mm)
7	Bed Rock

Substrate Notes

The silt and clay dried into mud

Organic Matter-Areal Coverage

Use 1: Abundant, 2: Present, 3: Absent

	Sample 1	Sample 2	Sample 3
Woody Debris	3	3	
Detritus	1	1	

Riparian Vegetative Community

Use: 1 (None), 2 (cultivated), 3 (meadow), 4 (scrubland), 5 (forest, mainly coniferous), 6 (forest, mainly deciduous)

Zone (dist. From water's edge)	Left Bank	Right Bank (facing downstream)
1.5-10 m	3	3
10-30 m	3	3
30-100 m	3	3

% Canopy Cover (circle one)

0-24	25-49
50-74	75-100

If instrument used, record type:

Aquatic Macrophytes and Algae (Use: 1 (Abundant), 2 (Present), 3 (Absent). Circle dominant type.

Macrophytes	Sample 1	Sample 2	Sample 3
Emergent	1	1	
Rooted Floating	3	3	
Submergent	3	3	
Free Floating	3	3	

Algae	Sample 1	Sample 2	Sample 3
Floating Algae	1	1	
Filaments	1	1	
Attached Algae	2	2	
Slimes or Crusts	3	3	

Stream Size/Flow

Bank Full Width (m):

Discharge (m^3/s, optional, indicate method):

River Characterisation (circle one) Perennial Intermittent Unknown

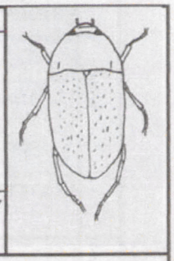
Notes (esp. related to land-use, habitat, obvious stressors)

Headwater stream, dried up at the end of May. Water returned mid-October

Candidate reference Site - Minimally Impacted? (circle one) Yes No

General Comments

Ontario Benthos Biomonitoring Network Field Sheet: STREAMS



Date:	14-Jun-07	Stream name:	Rouge River
Time	11:30	Site #:	A-US 43°53'20.9"N
Agency:	Ryerson Univeristy	Location: centroid of 3 replicates; Lat/Long or UTM	79°24'06.9"W
Investigators:	Alexandra Chmakova	Elevation (m asl):	
		Datum/zone:	

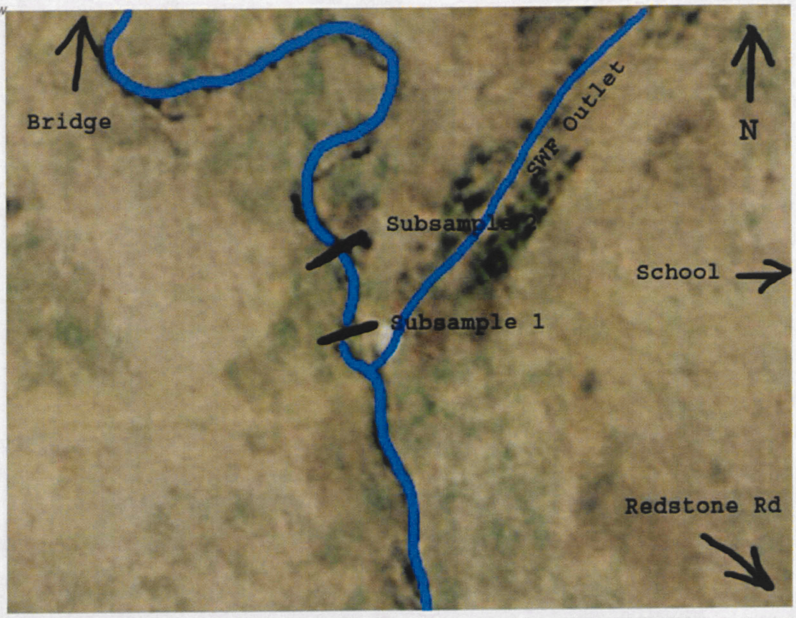
Water Quality

Water Temperature (°C):	19.7	Conductivity (uS/cm):	pH: 7
DO (mg/l):	8.4	Alkalinity (mg/l as $CaCO_3$):	

Site Description and Map

Draw a map of the site (with landmarks) and indicate areas sampled. Attach photograph (optional)

Show north arrow



Benthos Collection Method (circle one):
- Traveling Kick & Sweep
- Grab Sample
- Other (specify):

Gear Type (circle one)
- D-net
- Ponar
- Other (specify):
- Ekman
- Rock Baskets

Mesh Size: 500 micron (or specify) 1000 micron

Sub-samples	Sampling distance covered (m)	Time (min.)	Max. Depth (m)	Wetted Width (m)	Max. Hydraulic Head (mm)	# Grabs pooled per sample
Sample 1: Riffle (cross-over)	2	3	0.09	0.6	1.3	n/a
Sample 2: Pool	2	3	0.09	0.6	1.3	n/a
Sample 3: Riffle (cross-over)						

Substrate	Sample 1	Sample 2	Sample 3
Enter dominant substrate class and second dominant class for each sub-sample			
Dominant	2	2	
2nd Dominant	1	1	

Class	Description
1	Clay (hard pan)
2	Silt (gritty, < 0.06 mm particle diameter)
3	Sand (grainy, 0.06 - 2 mm)
4	Gravel (2 - 65 mm)
5	Cobble (65 - 250 mm)
6	Boulder (> 250 mm)
7	Bed Rock

Substrate Notes

Silt, mud, and clay

Organic Matter-Areal Coverage
Use 1: Abundant, 2: Present, 3: Absent

	Sample 1	Sample 2	Sample 3
Woody Debris	3	3	
Detritus	1	1	

Riparian Vegetative Community
Use: 1 (None), 2 (cultivated), 3 (meadow), 4 (scrubland), 5 (forest, mainly coniferous), 6 (forest, mainly deciduous)

Zone (dist. From water's edge)	Left Bank	Right Bank (facing downstream)
1.5-10 m	3	3
10-30 m	3	3
30-100 m	2	2

% Canopy Cover (circle one)

0-24	25-49
50-74	75-100

If instrument used, record type:

Aquatic Macrophytes and Algae (Use: 1 (Abundant), 2 (Present), 3 (Absent). Circle dominant type.

Macrophytes	Sample 1	Sample 2	Sample 3
Emergent	3	3	
Rooted Floating	2	2	
Submergent	3	3	
Free Floating	3	3	

Algae	Sample 1	Sample 2	Sample 3
Floating Algae	2	2	
Filaments	2	2	
Attached Algae	2	2	
Slimes or Crusts	3	3	

Stream Size/Flow

Bank Full Width (m): 1.2 Discharge (m^3/s, optional, indicate method):

River Characterisation (circle one) Perennial Intermittent Unknown

Notes (esp. related to land-use, habitat, obvious stressors)

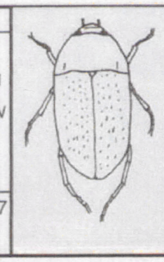
Site is located in a municipal park, well maintained, but not manicured

Candidate reference Site - Minimally Impacted? (circle one) Yes No

General Comments

Very narrow channel, dense herbaceous vegetation

Ontario Benthos Biomonitoring Network Field Sheet: STREAMS



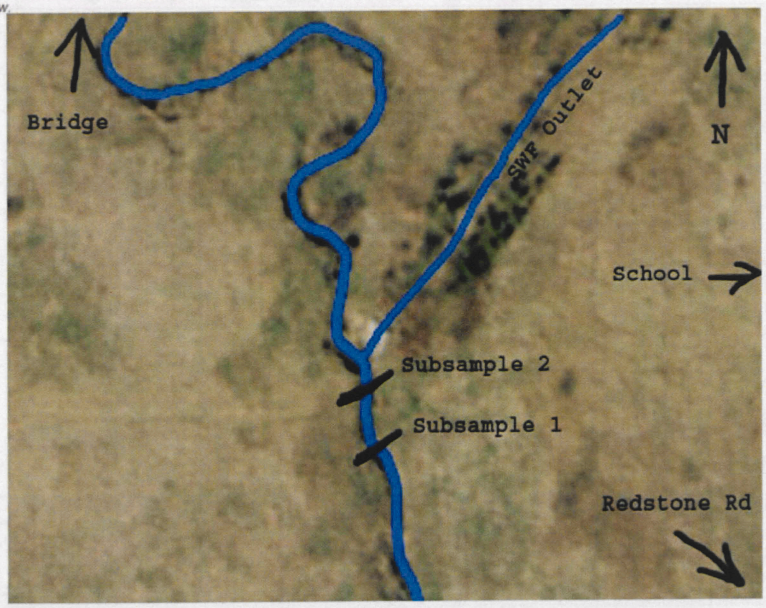
Date:	14-Jun-07	Stream name:	Rouge River
Time	11:00	Site #:	A-DS
Agency:	Ryerson University	Location: centroid of 3 replicates; Lat/Long or UTM	43°53'20.5"N 79°24'06.7"W
Investigators:	Alexandra Chmakova	Elevation (m asl):	
		Datum/zone:	

Water Quality

Water Temperature (°C):	19.8	Conductivity (uS/cm):	
		pH:	7
DO (mg/l):	8.8	Alkalinity (mg/l as $CaCO_3$):	

Site Description and Map

Draw a map of the site (with landmarks) and indicate areas sampled. Attach photograph (optional)
Show north arrow.



Benthos Collection Method (circle one):

- Traveling Kick & Sweep
- Grab Sample
- Other (specify):

Gear Type (circle one)

- D-net
- Ponar
- Other (*specify*):
- Ekman
- Rock Baskets

Mesh Size: 500 micron (or specify) 1000 micron

Sub-samples	Sampling distance covered (m)	Time (min.)	Max. Depth (m)	Wetted Width (m)	Max. Hydraulic Head (mm)	# Grabs pooled per sample
Sample 1: Riffle (cross-over)	2.5	3	0.15	0.6	15	n/a
Sample 2: Pool	3	3	0.15	0.6	15	n/a
Sample 3: Riffle (cross-over)						

Substrate

Enter dominant substrate class and second dominant class for each sub-sample

	Sample 1	Sample 2	Sample 3
Dominant	2	2	
2nd Dominant	1	1	

Class	Description
1	Clay (hard pan)
2	Silt (gritty, < 0.06 mm particle diameter)
3	Sand (grainy, 0.06 - 2 mm)
4	Gravel (2 - 65 mm)
5	Cobble (65 - 250 mm)
6	Boulder (> 250 mm)
7	Bed Rock

Substrate Notes

Silt, mud, and clay

Organic Matter-Areal Coverage

Use 1: Abundant, 2: Present, 3: Absent

	Sample 1	Sample 2	Sample 3
Woody Debris	3	3	
Detritus	2	2	

Riparian Vegetative Community

Use: 1 (None), 2 (cultivated), 3 (meadow), 4 (scrubland), 5 (forest, mainly coniferous), 6 (forest, mainly deciduous)

Zone (dist. From water's edge)	Left Bank	Right Bank (facing downstream)
1.5-10 m	3	3
10-30 m	3	3
30-100 m	2	2

% Canopy Cover (circle one)

0-24	25-49
50-74	75-100

If instrument used, record type:

Aquatic Macrophytes and Algae (Use: 1 (Abundant), 2 (Present), 3 (Absent). Circle dominant type.

Macrophytes	Sample 1	Sample 2	Sample 3
Emergent	3	3	
Rooted Floating	2	2	
Submergent	3	3	
Free Floating	3	3	

Algae	Sample 1	Sample 2	Sample 3
Floating Algae	2	2	
Filaments	2	2	
Attached Algae	2	2	
Slimes or Crusts	3	3	

Stream Size/Flow

Bank Full Width (m): 1.2 Discharge (m^3/s, optional, indicate method):

River Characterisation (circle one) Perennial Intermittent Unknown

Notes (esp. related to land-use, habitat, obvious stressors)

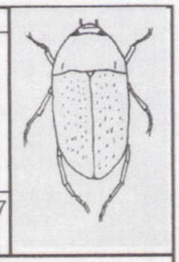
Site is located in a municipal park, well maintained, but not manicured

Candidate reference Site - Minimally Impacted? (circle one) Yes No

General Comments

Very narrow channel, dense herbaceous vegetation

Ontario Benthos Biomonitoring Network Field Sheet: STREAMS



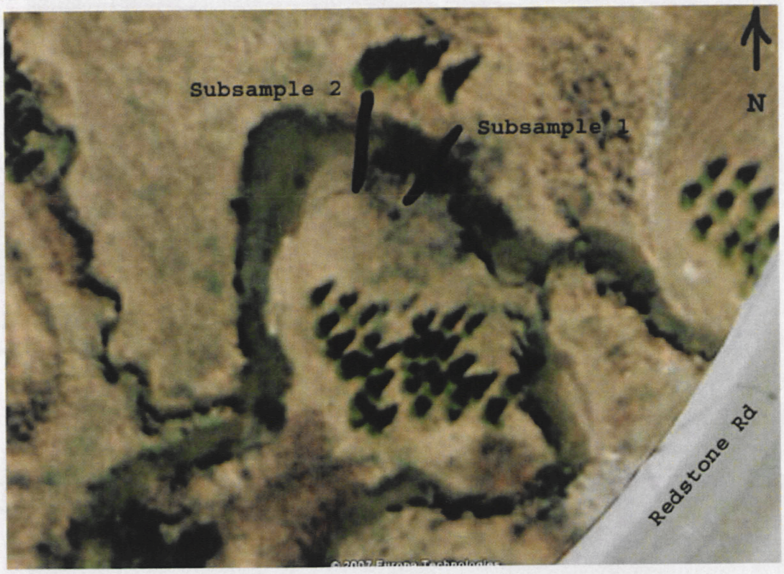
Date: 14-Jun-07	Stream name: Rouge River
Time 12:30	Site #: B 43°53'19.5"N
Agency: Ryerson University	Location: centroid of 3 replicates; Lat/Long or UTM 79°24'05"W
Investigators: Alexandra Chmakova	Elevation (m asl):
	Datum/zone:

Water Quality

Water Temperature (°C): 22.3	Conductivity (uS/cm):	pH: 7	
DO (mg/l): 7	Alkalinity (mg/l as $CaCO_3$):		

Site Description and Map

Draw a map of the site (with landmarks) and indicate areas sampled. Attach photograph (optional)
Show north arrow.



Benthos Collection Method (circle one):
- Traveling Kick & Sweep
- Grab Sample
- Other (specify):

Gear Type (circle one)
- D-net
- Ponar
- Other (*specify*):
- Ekman
- Rock Baskets

Mesh Size: 500 micron (or specify) 1000 micron

Sub-samples	Sampling distance covered (m)	Time (min.)	Max. Depth (m)	Wetted Width (m)	Max. Hydraulic Head (mm)	# Grabs pooled per sample
Sample 1: Riffle (cross-over)	4	3	0.27	4.2	5	n/a
Sample 2: Pool	4	3	0.3	4.2	5	n/a
Sample 3: Riffle (cross-over)						

Substrate

Enter dominant substrate class and second dominant class for each sub-sample

	Sample 1	Sample 2	Sample 3
Dominant	4	4	
2nd Dominant	5	5	

Class	Description
1	Clay (hard pan)
2	Silt (gritty, < 0.06 mm particle diameter)
3	Sand (grainy, 0.06 - 2 mm)
4	Gravel (2 - 65 mm)
5	Cobble (65 - 250 mm)
6	Boulder (> 250 mm)
7	Bed Rock

Substrate Notes

Fine to coarse sand, gravel, cobble, scattered boulders

Organic Matter-Areal Coverage

Use 1: Abundant, 2: Present, 3: Absent

	Sample 1	Sample 2	Sample 3
Woody Debris	2	2	
Detritus	2	2	

Riparian Vegetative Community

Use: 1 (None), 2 (cultivated), 3 (meadow), 4 (scrubland), 5 (forest, mainly coniferous), 6 (forest, mainly deciduous)

Zone (dist. From water's edge)	Left Bank	Right Bank (facing downstream)
1.5-10 m	3	2
10-30 m	2	2
30-100 m	2	2

% Canopy Cover (circle one)

0-24 25-49

50-74 75-100

If instrument used, record type:

Aquatic Macrophytes and Algae (Use: 1 (Abundant), 2 (Present), 3 (Absent). Circle dominant type.

Macrophytes	Sample 1	Sample 2	Sample 3
Emergent	3	3	
Rooted Floating	3	3	
Submergent	3	3	
Free Floating	3	3	

Algae	Sample 1	Sample 2	Sample 3
Floating Algae	2	2	
Filaments	2	2	
Attached Algae	2	2	
Slimes or Crusts	2	2	

Stream Size/Flow

Bank Full Width (m): 6 Discharge (m^3/s, optional, indicate method):

River Characterisation (circle one) Perennial Intermittent Unknown

Notes (esp. related to land-use, habitat, obvious stressors)

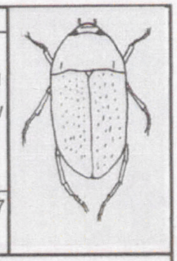
Site is located in a municipal park, well maintained, but not manicured

Candidate reference Site - Minimally Impacted? (circle one) Yes No

General Comments

Wide channel with a deep pool. Significant erosion noted on southeast bank, and deposition on the northwest bank.

Ontario Benthos Biomonitoring Network Field Sheet: STREAMS



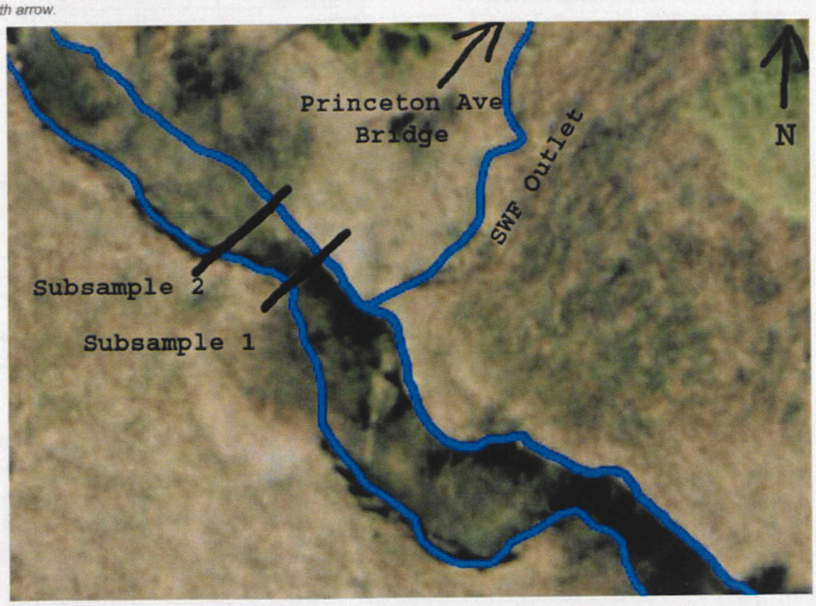
Date: 14-Jun-07	Stream name: Rouge River
Time 1:00	Site #: C-US 43°53"16.7"N
Agency: Ryerson University	Location: centroid of 3 replicates; Lat/Long or UTM 79°23'53.7"W
Investigators: Alexandra Chmakova	Elevation (m asl):
	Datum/zone:

Water Quality

Water Temperature (°C): 23.7	Conductivity (uS/cm): pH: 7
DO (mg/l): 6	Alkalinity (mg/l as $CaCO_3$):

Site Description and Map

Draw a map of the site (with landmarks) and indicate areas sampled. Attach photograph (optional)
Show north arrow.



Benthos Collection Method (circle one):
- Traveling Kick & Sweep
- Grab Sample
- Other (specify):

Gear Type (circle one)
- D-net
- Ponar
- Other (*specify*):
- Ekman
- Rock Baskets

Mesh Size: 500 micron (or specify) 1000 micron

Sub-samples	Sampling distance covered (m)	Time (min.)	Max. Depth (m)	Wetted Width (m)	Max. Hydraulic Head (mm)	# Grabs pooled per sample
Sample 1: Riffle (cross-over)	2	3	0.25	3.1	10	n/a
Sample 2: Pool	2.5	3	0.3	3.1	10	n/a
Sample 3: Riffle (cross-over)						

Substrate

Enter dominant substrate class and second dominant class for each sub-sample

	Sample 1	Sample 2	Sample 3
Dominant	5	5	
2nd Dominant	4	4	

Class	Description
1	Clay (hard pan)
2	Silt (gritty, < 0.06 mm particle diameter)
3	Sand (grainy, 0.06 - 2 mm)
4	Gravel (2 - 65 mm)
5	Cobble (65 - 250 mm)
6	Boulder (> 250 mm)
7	Bed Rock

Substrate Notes

gravel and cobble, scattered boulders

Organic Matter-Areal Coverage

Use 1: Abundant, 2: Present, 3: Absent

	Sample 1	Sample 2	Sample 3
Woody Debris	2	2	
Detritus	2	2	

Riparian Vegetative Community

Use: 1 (None), 2 (cultivated), 3 (meadow), 4 (scrubland), 5 (forest, mainly coniferous), 6 (forest, mainly deciduous)

Zone (dist. From water's edge)	Left Bank	Right Bank (facing downstream)
1.5-10 m	4	4
10-30 m	4	4
30-100 m	2	2

% Canopy Cover (circle one)

0-24	25-49
50-74	75-100

If instrument used, record type:

Aquatic Macrophytes and Algae (Use: 1 (Abundant), 2 (Present), 3 (Absent). Circle dominant type.

Macrophytes	Sample 1	Sample 2	Sample 3
Emergent	3	3	
Rooted Floating	3	3	
Submergent	3	3	
Free Floating	3	3	

Algae	Sample 1	Sample 2	Sample 3
Floating Algae	2	2	
Filaments	2	2	
Attached Algae	2	2	
Slimes or Crusts	2	2	

Stream Size/Flow

Bank Full Width (m): 3.7 Discharge (m^3/s, optional, indicate method):

River Characterisation (circle one) Perennial Intermittent Unknown

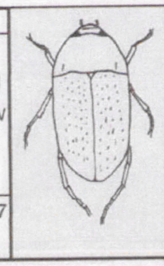
Notes (esp. related to land-use, habitat, obvious stressors)

Site located approximately 100 m away from the residential area. Fairly inaccessible.

Candidate reference Site - Minimally Impacted? (circle one) Yes No

General Comments

Ontario Benthos Biomonitoring Network Field Sheet: STREAMS



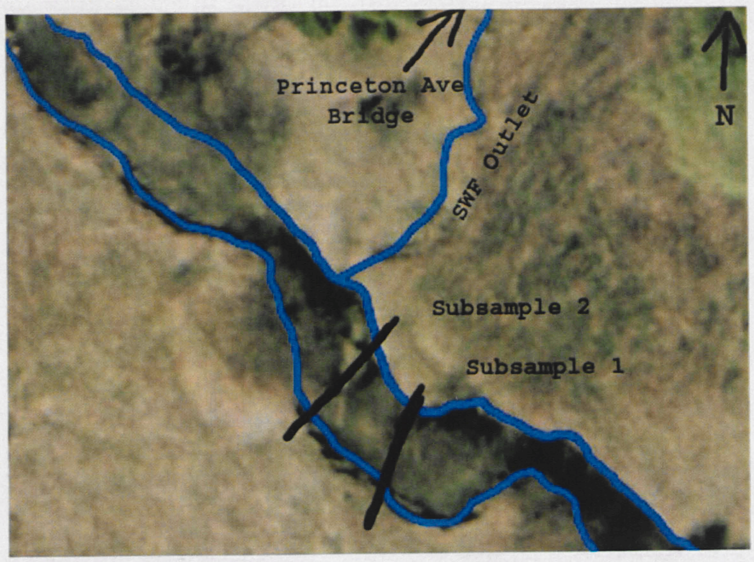
Date:	14-Jun-07	Stream name:	Rouge River
Time	1:15	Site #:	C-DS
Agency:	Ryerson University	Location: centroid of 3 replicates; Lat/Long or UTM	43°53'14.2"N 79°23'53.4"W
Investigators:	Alexandra Chmakova	Elevation (m asl):	
		Datum/zone:	

Water Quality

Water Temperature (°C):	23.6	Conductivity (uS/cm):	pH: 7
DO (mg/l):	6.1	Alkalinity (mg/l as $CaCO_3$):	

Site Description and Map

Draw a map of the site (with landmarks) and indicate areas sampled. Attach photograph (optional)
Show north arrow.



Benthos Collection Method (circle one):
- Traveling Kick & Sweep
- Grab Sample
- Other (specify):

Gear Type (circle one)
- D-net
- Ponar
- Other (*specify*):
- Ekman
- Rock Baskets

Mesh Size: 500 micron (or specify) 1000 micron

Sub-samples	Sampling distance covered (m)	Time (min.)	Max. Depth (m)	Wetted Width (m)	Max. Hydraulic Head (mm)	# Grabs pooled per sample
Sample 1: Riffle (cross-over)	3	3	0.35	3.6	10	n/a
Sample 2: Pool	2.5	3	0.35	3.6	10	n/a
Sample 3: Riffle (cross-over)						

Substrate				Class	Description
Enter dominant substrate class and second dominant class for each sub-sample				1	Clay (hard pan)
	Sample 1	Sample 2	Sample 3	2	Silt (gritty, < 0.06 mm particle diameter)
Dominant	5	5		3	Sand (grainy, 0.06 - 2 mm)
2nd Dominant	4	4		4	Gravel (2 - 65 mm)
				5	Cobble (65 - 250 mm)
				6	Boulder (> 250 mm)
				7	Bed Rock

Substrate Notes

gravel and cobble, scattered boulders

Organic Matter-Areal Coverage
Use 1: Abundant, 2: Present, 3: Absent

	Sample 1	Sample 2	Sample 3
Woody Debris	2	2	
Detritus	1	1	

Riparian Vegetative Community
Use: 1 (None), 2 (cultivated), 3 (meadow), 4 (scrubland), 5 (forest, mainly coniferous), 6 (forest, mainly deciduous)

Zone (dist. From water's edge)	Left Bank	Right Bank (facing downstream)
1.5-10 m	4	4
10-30 m	4	4
30-100 m	2	2

% Canopy Cover (circle one)

0-24	25-49
50-74	75-100

If instrument used, record type:

Aquatic Macrophytes and Algae (Use: 1 (Abundant), 2 (Present), 3 (Absent). Circle dominant type.

Macrophytes	Sample 1	Sample 2	Sample 3
Emergent	3	3	
Rooted Floating	3	3	
Submergent	3	3	
Free Floating	3	3	

Algae	Sample 1	Sample 2	Sample 3
Floating Algae	2	2	
Filaments	2	2	
Attached Algae	2	2	
Slimes or Crusts	2	2	

Stream Size/Flow

Bank Full Width (m): 3.9 Discharge (m^3/s, optional, indicate method):

River Characterisation (circle one) Perennial Intermittent Unknown

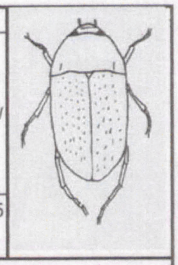
Notes (esp. related to land-use, habitat, obvious stressors)

Site located approximately 100 m away from the residential area. Fairly inaccessible.

Candidate reference Site - Minimally Impacted? (circle one) Yes No

General Comments

Ontario Benthos Biomonitoring Network Field Sheet: STREAMS



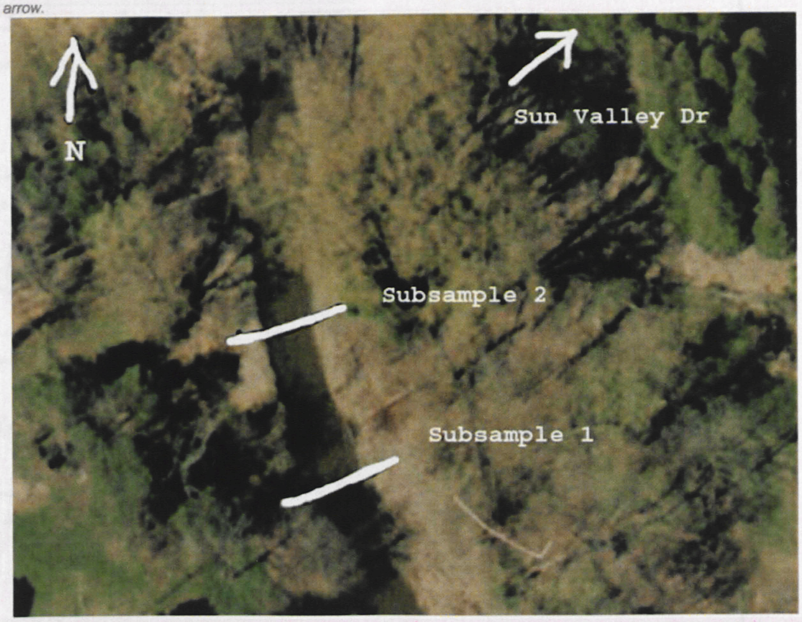
Date:	14-Jun-07	Stream name:	Rouge River
Time	1:45	Site #:	D
Agency:	Ryerson University	Location: centroid of 3 replicates; Lat/Long or UTM	43°53'08.2"N 79°23'40.6"W
Investigators:	Alexandra Chmakova	Elevation (m asl):	
		Datum/zone:	

Water Quality

Water Temperature (°C):	23.8	Conductivity (uS/cm):		pH:	7.5
DO (mg/l):	6.7	Alkalinity (mg/l as $CaCO_3$):			

Site Description and Map

Draw a map of the site (with landmarks) and indicate areas sampled. Attach photograph (optional)
Show north arrow.



Benthos Collection Method (circle one):

- Traveling Kick & Sweep
- Grab Sample
- Other (specify):

Gear Type (circle one)

- D-net
- Ponar
- Other (*specify*):
- Ekman
- Rock Baskets

Mesh Size: 500 micron (or specify) 1000 micron

Sub-samples	Sampling distance covered (m)	Time (min.)	Max. Depth (m)	Wetted Width (m)	Max. Hydraulic Head (mm)	# Grabs pooled per sample
Sample 1: Riffle (cross-over)	4	3	0.22	5.2	2	n/a
Sample 2: Pool	3	3	0.23	5.2	2	n/a
Sample 3: Riffle (cross-over)						

Substrate

Enter dominant substrate class and second dominant class for each sub-sample

	Sample 1	Sample 2	Sample 3
Dominant	4	4	
2nd Dominant	5	5	

Class	Description
1	Clay (hard pan)
2	Silt (gritty, < 0.06 mm particle diameter)
3	Sand (grainy, 0.06 - 2 mm)
4	Gravel (2 - 65 mm)
5	Cobble (65 - 250 mm)
6	Boulder (> 250 mm)
7	Bed Rock

Substrate Notes

gravel and cobble, scattered boulders

Organic Matter-Areal Coverage

Use 1: Abundant, 2: Present, 3: Absent

	Sample 1	Sample 2	Sample 3
Woody Debris	2	2	
Detritus	2	2	

Riparian Vegetative Community

Use: 1 (None), 2 (cultivated), 3 (meadow), 4 (scrubland), 5 (forest, mainly coniferous), 6 (forest, mainly deciduous)

Zone (dist. From water's edge)	Left Bank	Right Bank (facing downstream)
1.5-10 m	4	4
10-30 m	4	4
30-100 m	2	4

% Canopy Cover (circle one)

0-24	25-49
50-74	75-100

If instrument used, record type:

Aquatic Macrophytes and Algae (Use: 1 (Abundant), 2 (Present), 3 (Absent). Circle dominant type.

Macrophytes	Sample 1	Sample 2	Sample 3
Emergent	3	3	
Rooted Floating	3	3	
Submergent	3	3	
Free Floating	3	3	

Algae	Sample 1	Sample 2	Sample 3
Floating Algae	2	2	
Filaments	2	2	
Attached Algae	2	2	
Slimes or Crusts	2	2	

Stream Size/Flow

Bank Full Width (m): 5.3 Discharge (m^3/s, optional, indicate method):

River Characterisation (circle one) Perennial Intermittent Unknown

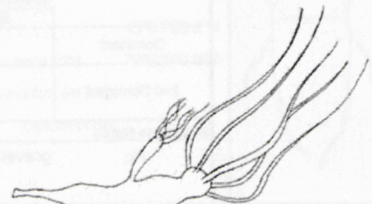
Notes (esp. related to land-use, habitat, obvious stressors)

Site located approximately 100 m away from residential area. Easily accessible.
Litter noted on several occasions.

Candidate reference Site - Minimally Impacted? (circle one) Yes No

General Comments

APPENDIX B: 27 OBBN Invertebrate Groups Illustrated

<table>
<tr><th>Group Name</th><th>Specimen Image[▽]</th><th>Schematic Representation[4]</th></tr>
<tr><td>Coelenterata (Hydras)</td><td></td><td></td></tr>
<tr><td>Turbellaria (Flatworms)</td><td>
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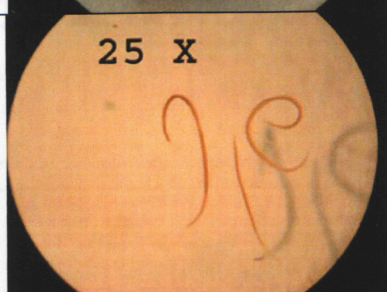
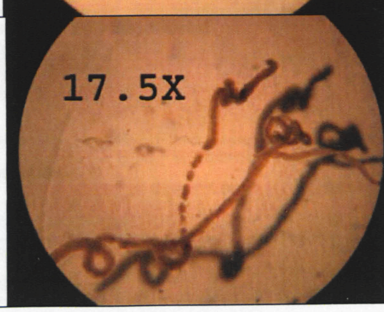
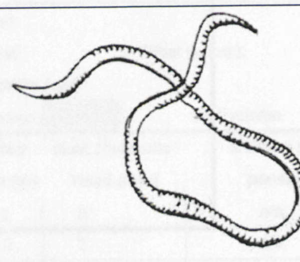
<tr><td>Nematoda (Roundworms)</td><td>
</td><td></td></tr>
<tr><td>Oligochaeta (Aquatic Earthworms)</td><td>
</td><td></td></tr>
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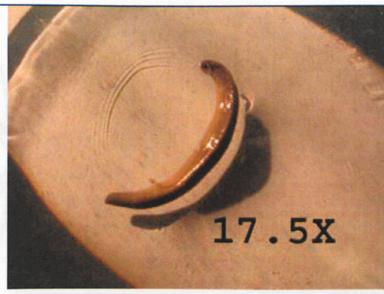
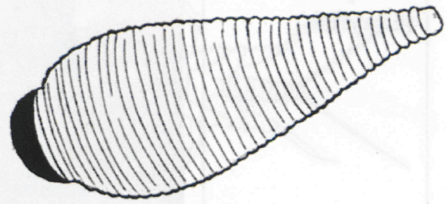
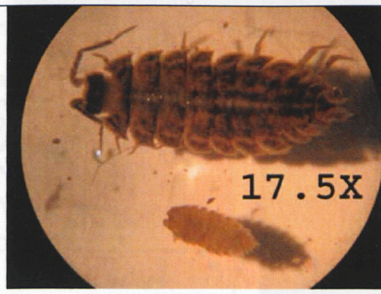
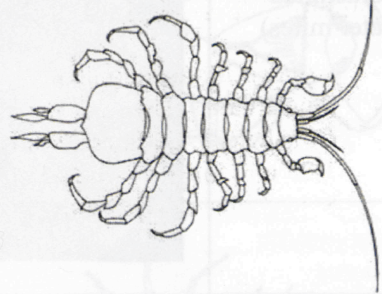
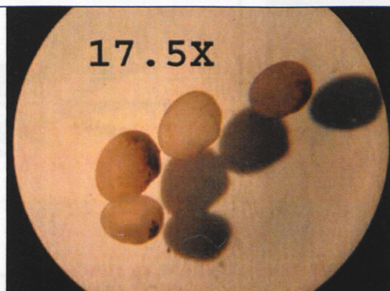
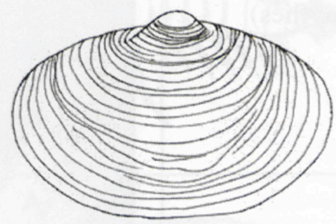
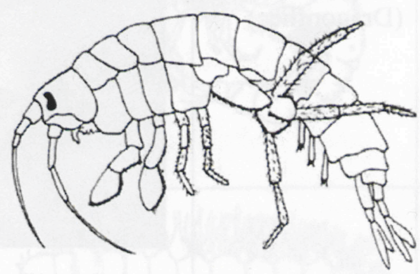
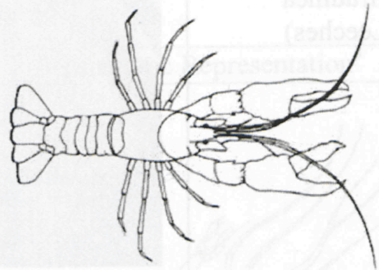
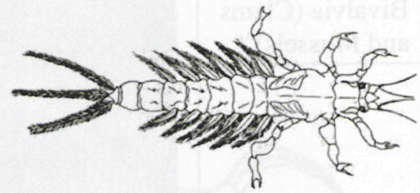
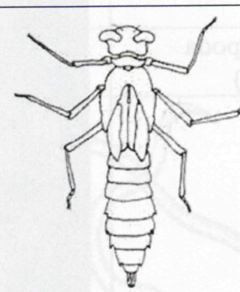
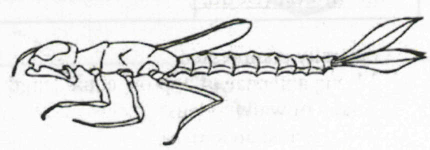
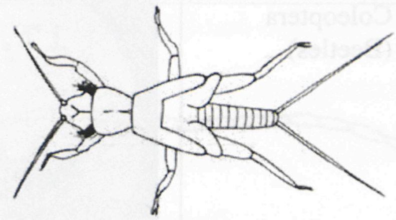
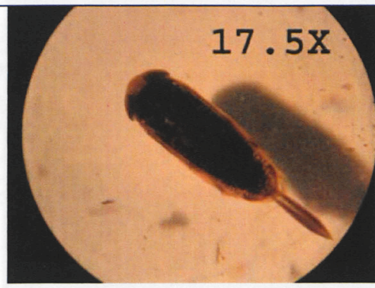
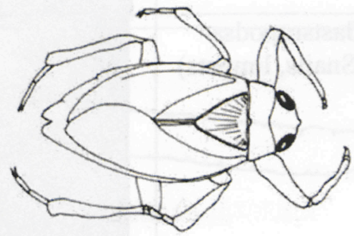
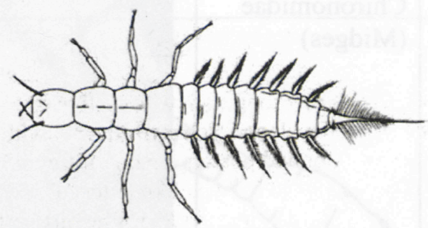
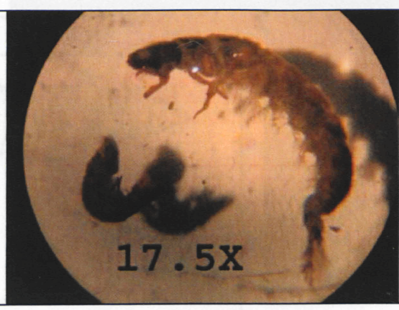
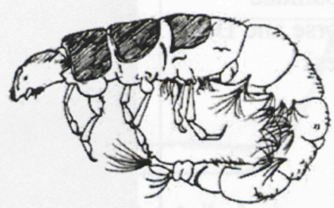
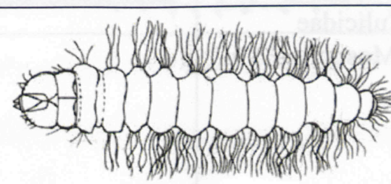
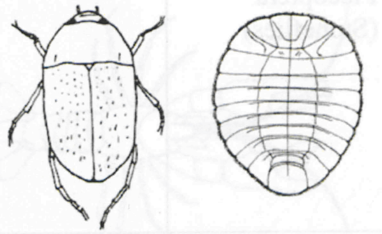
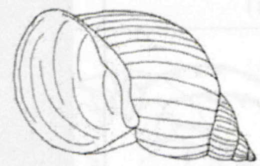
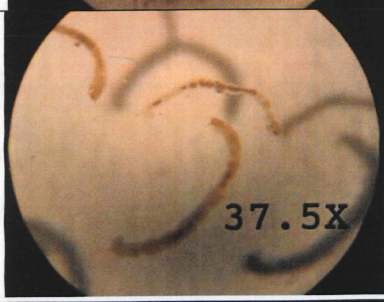
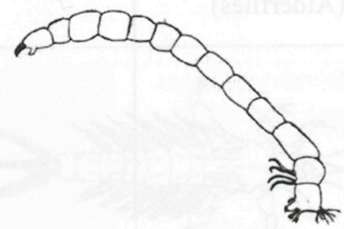
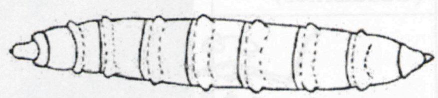
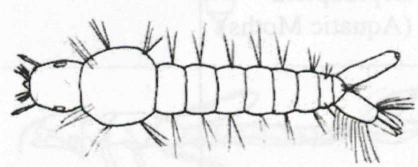
[▽] When no specimen image is provided, none of the group's representatives were encountered during the ennumeration

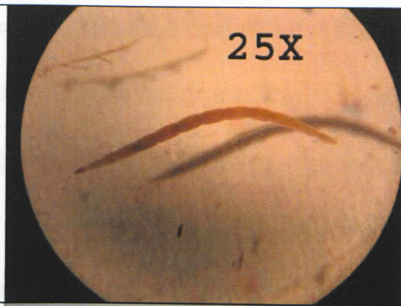
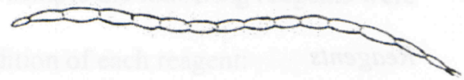
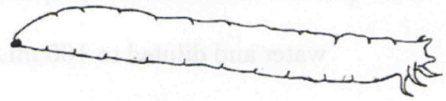
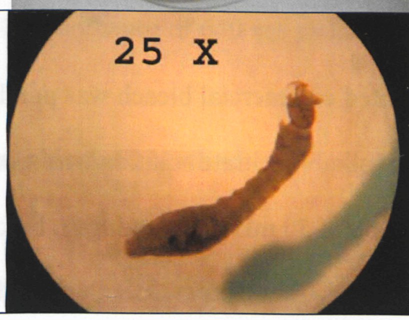
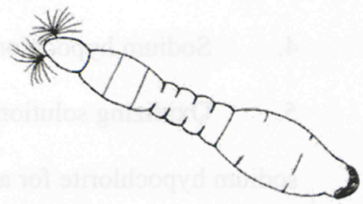
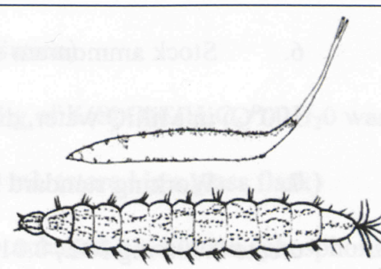
[4] Reprinted with permission from Jones et al 2007

Hirudinea (Leeches)		
Isopoda (Sow Bugs)		
Bivalvia (Clams and Mussels)		
Amphipoda (Scuds)		

Decapoda (Crayfish)		
Hydrachnida (Water mites)		
Ephemeroptera (Mayflies)		
Anisoptera (Dragonflies)		
Zygoptera (Damselflies)		

Plecoptera (Stoneflies)		
Hemiptera (True Bugs)		
Megaloptera (Alderflies)		
Trichoptera (Caddisflies)		
Lepidoptera (Aquatic Moths)		

Coleoptera (Beetles)		
Gastsropodsa (Snails, limpets)		
Chironomidae (Midges)		
Tabanidae (Horse and Deer Flies)		
Culicidae (Mosquitos)		

Ceratopogonidae (Biting midges)		
Tipulidae (Crane Flies)		
Simuliidae (Black Flies)		
Misc. Diptera (Misc. True Flies)		

APPENDIX C – Procedure Protocols

Ammonium Analysis – Phenate method

Reagents

1. Phenol solution: 4 mL liquefied phenol was diluted to a final volume of 40 mL with 95% v/v ethanol to produce 10% phenol solution (prepared weekly)
2. Sodium nitroprusside: 0.5 g sodium nitroprusside was dissolved in 100 MilliQ water and diluted to 100 mL (stored in foil-wrapped glass flask; prepared monthly)
3. Alkaline citrate: 200 g of trisodium citrate and 10 g sodium hydroxide was dissolved in MilliQ water and diluted to 1000 mL (stored in glass flask at room temperature)
4. Sodium hypochlorite: unscented commercial bleach was purchased every month
5. Oxidizing solution: 40 mL of alkaline citrate solution was mixed with 10 mL of sodium hypochlorite for a final volume of 50 mL (prepared fresh before each analysis run)
6. Stock ammonium solution – 1 g N/L: 3.819 g of anhydrous NH_4Cl (dried at 100°C) in MilliQ water, diluted to 1000 mL
7. Working standard for ammonium: the stock ammonium solution was used to create 0.005 mg N/L, 0.01 mg N/L, 0.05 mg N/L, and 0.1 mg N/L standard solutions and MilliQ water was used as a true zero (no ammonia present, but with all reagents added)

Procedure

1. Samples (to which 10% phenol solution was added in the field) were brought to room temperature

2. To each standard (including true zero) 400 µL of phenol solution was added

3. To each standard (including true zero) and sample the following reagents were added sequentially with thorough mixing after addition of each reagent:

   a. 400 µL sodium nitroprusside solution

   b. 1.0 mL oxidizing solution

4. The standards and samples were covered with parafilm and left to develop for 2 hours

5. After 2 hours the samples were read using Perkin Elmer® UV/VIS Spectrophotometer Model Lambda 20 and Perkin Elmer® UV WinLab V.2.85.04 software at absorbance of 640 nm and cuvette path length of 1 cm

Phosphate – Ascorbic Acid Method

Reagents

1. Phenolphthalein indicator

2. Sulfuric acid: 5N H_2SO_4 (as purchased, no dilution)

3. Potassium antimonyl tartrate solution: 1.3715 g of $K(SbO)C_4H_4O_6 \cdot 1/2H_20$ was dissolved in 400 mL MilliQ water and diluted to 500 mL (stored in a glass flask)

4. Ammonium molybdate solution: 4% w/v as purchased, no dilution (phosphorus free for phosphate analyses)

5. Ascorbic acid: 1.76 g L-ascorbic acid was dissolved in MilliQ water and diluted to 100 mL (prepared weekly, stored at 4°C)

6. Combined reagent: 50 mL 5N H_2SO_4, 5 mL potassium antimonyl tartrate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution were mixed to

yield 100 mL mixed reagent; the solution was mixed after addition of each reagent; if turbidity developed, the solution was allowed to stand until turbidity disappeared (prepared immediately before analysis)

7. Stock phosphate solution: 222.585 mg anhydrous NaH_2PO_4 was dissolved in MilliQ water and diluted to 1000 mL

8. Working standard for phosphate: 50.0 mL of stock phosphate solution was diluted to 1000 mL with MilliQ water; this solution was further diluted to create 0.001 mg P/L, 0.005 mg P/L, 0.01 mg P/L, and 0.05 mg P/L standards, as well as a true zero (MilliQ water with no phosphate, but all reagents added)

Procedure

1. To create a standard curve, the following reagents were added to the 5 mL aliquots of standard solutions (including true zero):

    a. 1 drop of phenolphthalein indicator; if solution turned pink, 5N H_2SO_4 was added dropwise until the color was discharged

    b. 0.8 mL of combined reagent

2. Standards were allowed to develop for 15 minutes, and then read using Perkin Elmer® UV/VIS Spectrophotometer Model Lambda 20 and Perkin Elmer® UV WinLab V.2.85.04 software to create a standard curve at absorbance of 880 nm and cuvette path length of 1 cm

3. The standard curve was saved and used for subsequent sample analyses

4. The following reagents were added to the samples:

a. 1 drop of phenolphthalein indicator ; if solution turned pink, 5N H_2SO_4 was added dropwise until the color was discharged

b. 0.8 mL of combined reagent

5. The samples were allowed to develop for 15 minutes and then read using Perkin Elmer® UV/VIS Spectrophotometer Model Lambda 20 and Perkin Elmer® UV WinLab V.2.85.04 software at an absorbance of 880 nm and cuvette path length of 1 cm

Nitrate – Cadmium Reduction Method

Reagents

1. Nitrite free water

2. Color reagent: to 80 mL MilliQ water add 10 mL 85% phosphoric acid and 1 g sulfanilamide. After dissolving sulfanilamide completely, add 0.1 g *N*-(1-naphthyl)-ethylendiamine dihydrochloride. Mix to dissolve, then dilute to 100 mL with MilliQ water. Stable for 1 month in dark bottle at 4°C

3. Stock nitrate solution: dissolve 0.7218 g KNO_3 (which was dried in an oven at 105°C for 24 h) in MilliQ water and dilute to 1000 mL; 1.00 mL = 100 µg NO_3^--N

4. Intermediate nitrate solution: dilute 100 mL stock nitrate solution to 1000 mL with MilliQ water; 1.00 mL = 10.0 µg NO_3^--N

5. Ammonium bromide-EDTA solution: dissolve 13 g NH_4Br and 1.7 g disodium ethylenediamine tetraacetate in 900 mL water. Adjust to pH 8.5 with concentrated NaOH and dilute to 1 L

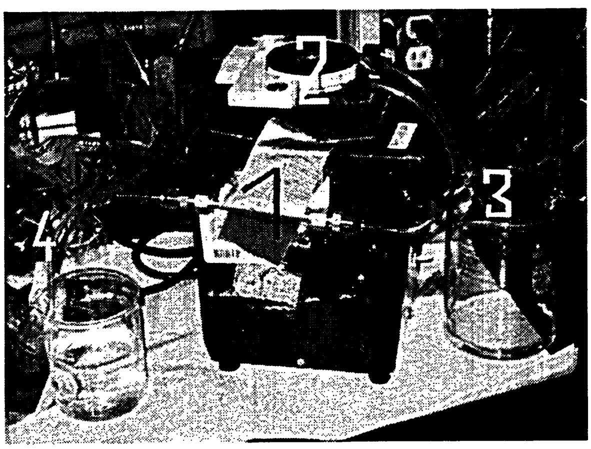
6. Dilute ammonium bromide-EDTA solution: Dilute 300 mL NH_4Br-EDTA solution to 500 mL with MilliQ water

7. Hydrochloric acid 10N

8. Copper sulfate solution, 2%: Dissolve 10 g $CuSO_4 \cdot 5H_2O$ in 500 mL water and dilute to 1 L

9. Standards 0 mg/L (MilliQ water) 0.1 mg N/L, 0.25 mg N/L, 0.5 mg N/L, and 1.0 mg N/L were prepared using sequential dilutions of intermediate nitrate solution with MilliQ water

Apparatus



1. Cu- Cd reduction column
2. Peristaltic pump
3. Original sample
4. Treated sample

Figure C-1: The cadmium reduction column and the peristaltic pump set-up

1. Column was constructed from 12 cm piece of stainless steel tube, 0.4 cm diameter, total volume <4.0 mL

2. Wash new or used 20- to 100-mesh Cd granules with 10N HCl and rinse with water. Swirl Cd with 100 mL 2% $CuSO_4$ solution 3 times until blue color partially fades. Decant and repeat with fresh $CuSO_4$ until a brown colloidal precipitate begins to develop. Gently flush with MilliQ water to remove all precipitated Cu.

3. Insert glass wool plug into bottom of reduction column and fill with water; pack the column with Cu-Cd granules – maintain water level above granules to prevent entrapment of air; wash column with 20 mL dilute NH_4Br-EDTA solution

4. Activate column by passing through it at max 5 mL/min at least 50 mL of solution composed of 25% 1.0 mg NO_3^--N/L standard and 75% NH_4Br-EDTA solution

5. Fill and store with dilute buffer. Never let the column run dry

6. Attach the column to a peristaltic pump

Procedure

1. To each 5 mL nitrate standard or sample, 10 mL of buffer was added

2. For each standard or sample 5 mL was run through the column to flush it and the next 5 mL were collected for analysis

3. 200 µL of color reagent was added immediately after collection to prevent oxidation

4. The standards and samples were allowed to develop for 15 minutes and were then read using Perkin Elmer® UV/VIS Spectrophotometer Model Lambda 20 and Perkin Elmer® UV WinLab V.2.85.04 software to establish a calibration curve at absorbance of 543 nm and cuvette path length of 1cm

Benthic Macroinvertebrate Sample Collection

1. A segment of waterway was identified for collection. Where possible, the segment included 2 riffles and a pool, however this was not possible at all sites.
2. The first sub-sample was taken at the downstream most section of the sampling segment as follows:

a. The person collecting the sample stood at the right bank facing downstream holding the D-net with the net touching the substrate and facing upstream

b. The substrate was kicked and the net was held downstream and swept side to side to catch any organisms that were dislodged and caught in the current

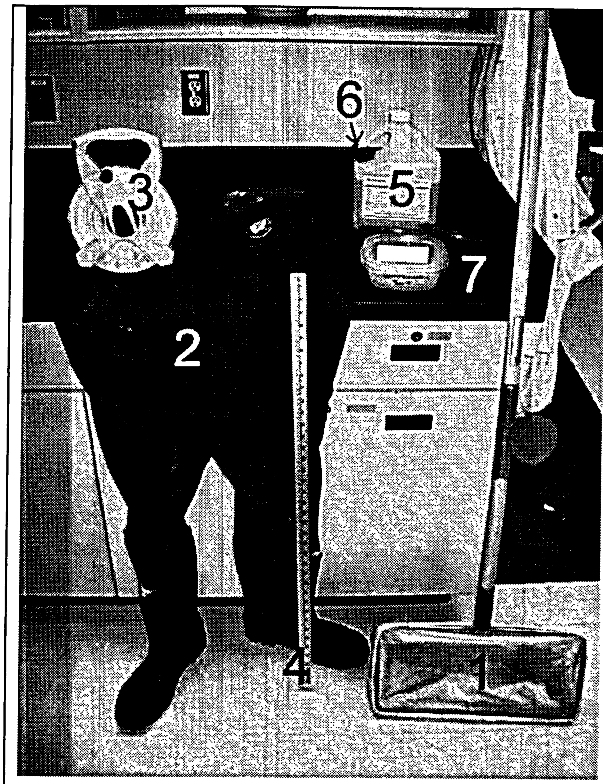
c. Once the spot was considered exhausted, the sampler moved one step to the left and repeated the procedure (if it is impossible to move to the left (i.e. very narrow stream), the sampling person took one step backwards)

d. Steps a – c were repeated for 3 minutes

e. After 3 minutes the collected sample was rinsed in the water to remove excess fine sediment and transferred to a plastic Ziploc container, which was labeled and contained a paper strip with the date, site, and sub-sample number written on it in pencil (pencil does not wash off in ethanol preservative)

f. Ethanol was added to preserve the samples in the field

3. At each site, a second sub-sample was collected as per steps a – f at the downstream most undisturbed point in the sampling segment



1) D-net (kick net)
2) Hip waders or chest waders
3) Tape measure
4) Meter stick
5) Ethanol
6) Stopwatch
7) Plastic container with wide opening, labeled with sample Site, Date, and Number/Designation (Riffle1/2, Pool)

Figure C-2: Benthic invertebrate sampling equipment

The OBBN protocol calls for three sub-samples, however, due to the fact that the sites were so close together, to prevent extensive habitat destruction, only two sub-samples were taken at each site.

Macroinvertebrate Sample Analysis

1. Samples were washed and sieved in the lab using 250 μm mesh to remove fine sediment such as silt and clay
2. Fresh ethanol was added to washed samples
3. At least 100 individual organisms were collected from each Ziploc container using the "teaspoon" sub-sampling technique (it is not necessary to use an actual

teaspoon – the sub-sample should simply be small, no much more than what a teaspoon can hold)

a. A small portion of the sample was removed and placed under a dissecting scope (OMANO, No: 0402572) – this is considered a "teaspoon" sub-sample
b. The "teaspoon" sub-sample was picked clean of all individual organisms
c. If less than 100 organisms were removed (cumulatively, from all "teaspoon" sub-samples from this particular Ziploc container), another "teaspoon" sub-sample was selected and picked
d. If 100 organisms has been reached, the picking of the "teaspoon" sub-sample under study had to be completed
e. The collective substrate from the "teaspoon" sub-samples was dried and weighed
f. The remaining sample in the Ziploc container was dried and weighed
g. By taking the ratio of the "teaspoon" sub-sample substrate weight to full Ziploc sample weight, the percent sampled was determined
h. This allowed for approximation of total abundance

4. The individual organisms were identified to the 27 OBBN groups (See Appendix B)
5. If the OBBN level only called for Class of Phylum identification, the identification was carried down to Order

The "teaspoon" sub-sampling technique is a simpler (and cheaper) alternative to the Marchant box sub-sampling technique often used in benthic invertebrate enumeration. Marchant box has 100 cells and the sample is randomly separated into these cells. Each cell is considered a sub-sample and is picked in the same manner as a teaspoon sub-sample. There is little difference in the sub-sampling technique; however, a Marchant box can cost up to several hundred dollars.

Correspondence Analysis using Microsoft Excel Biplot Plug-In

1. The data was arranged in a spreadsheet so that first row listed sites, and first column – the taxa observed on that date
2. After all data for that particular date was entered, all taxa that did not have a non-null value for at least one replicate were removed
3. The dataset was log-transformed to fit a standard distribution by using the "log(X+1)" formula, where X is the value of the original cell, and the "log(X+1)" is the value of the log-transformed value
4. BiPlot -> Singular Value Decomposition was selected
5. For "Data Range for Y's" the entire dataset, including the column and row headings was selected
6. In the **Method** frame, "Covariance Analysis" was selected
7. A cell for output range was selected at the bottom of the dataset
8. "Use First Column for Row Labels" and "Use First Row for Column Labels" were checked
9. "Chart Output" was un-checked

10. "Number of Components to Extract" was set to 2 and the first phase of Covariance Analysis was performed by clicking **[OK]**

The resultant dataset is displayed below the log-transformed table. There are two distinct data sets – Column Coordinates and Row Coordinates. Each data set has a column of labels, and 2 columns of numbers. These numbers are X and Y coordinates of the data point (taxa or site) calculated using the Covariance Analysis. The Column Coordinates are the site coordinates, but there are two replicates for each site. They have to be averaged – two X coordinates for two replicates are averaged to make one for the site, and two Y coordinates for two replicates are averaged to make one for the site. Once the coordinates are averaged, so that there is only one X and one Y for each site, the following steps were completed to create a Covariance Analysis graph:

1. The site names and coordinates were copied and pasted as values
2. The taxa coordinates were copied and pasted under the site coordinates
3. BiPlot -> BiPlot Chart was selected from the Main Menu
4. In the frame **Columns**
    a. X-coordinates for sites was selected for "Input X range"
    b. Y-coordinates for sites was selected for "Input Y range"
    c. Site names were selected for "Input labels' range"
5. In the frame **Rows**
    a. X-coordinates for taxa was selected for "Input X range"
    b. Y-coordinates for taxa was selected for "Input Y range"
    c. Taxa names were selected for "Input labels' range"

6. In the **Singular Values** frame, two cells under "Singular Values" at the very bottom of the original Correspondence Analysis output were selected for "Input range"

7. "Show labels for data points" and "Show axes" options were checked

8. "Row scaling (JK or RMP) was selected

The chart (phase 2 of covariance analysis) was created by clicking **[OK]**