GROWTH, SURVIVAL AND EVALUATION OF TOXICANT EXPOSURE IN RAINBOW TROUT REARED IN URBAN STORMWATER PONDS

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Abstract

To create additional fishing opportunities in Alberta, Canada it has been suggested that urban stormwater ponds be stocked with rainbow trout. Thirty-one stormwater ponds were assessed for suitability to support put-and-take recreational fisheries. Six ponds met the physical, biological and water quality parameters necessary for trout survival and were each stocked with a minimum of four hundred rainbow trout per pond. To determine stocking success and if fish were exposed to urban contaminants, i) fish survival, ii) fish growth, and iii) several indicators of toxicant exposure (AChE, Hg, cortisol and vitellogenin) were evaluated. Fish survival was low in all six ponds. Poor water quality, predation (including angler induced mortalities) and interspecific competition were responsible for the poor survival in all ponds and complete failure of two ponds. Rainbow trout specific growth rates ranged from 0.3% to 2.9% body weight gain per day. Brain acetylcholinesterase values declined in fish from three ponds and may be indicative of acute pesticide exposure. Mercury concentrations in fish muscle tissues were below CCME guidelines in all ponds. Cortisol concentrations, as an indicator of stress, were similar in fish from all ponds and were comparable to hatchery fish. Two distinct vitellogenin groupings were identified that correspond to values reported for unpolluted male and female fish from other studies, suggesting that fish were not exposed to estrogens or estrogen mimicking compounds. Despite the low proportion of suitable stocking locations, stormwater ponds are very abundant on the urban landscape and recreational stormwater fisheries could be feasible if specific physical and chemical criteria are met.

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Dedication

To Lenore Stone: Thank-you for your support, patience and love.

Table of Contents

List of Tables

List of Figures and Illustrations

List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
ACA	Alberta Conservation Association
ACh	Acetylcholine
AChE	Acetylcholinesterase
ANOVA	analysis of variance
ESRD	Alberta Environment and Sustainable Resource Development
BChE	Butyrylcholinesterase
BIA	Bioelectrical Impedance Analysis
BMP	best management practice
CCME	Canadian Council of Ministers of the Environment
Cond	Conductivity
DO	dissolved oxygen
EDC	endocrine disruptive chemicals
Hg	Mercury
HPI	hypothalamo-pituitary-interrenal
ICP-MS	inductively coupled plasma mass spectrometry
IQR	inter quartile range
K	condition factor
LOEC	lowest observed effect concentration
MeHg	methyl mercury
NTU	nephelometric turbidity units
NURP	Nationwide Urban Runoff Program
PAH	polyaromatic hydrocarbon
PCB	polycyclic aromatic hydrocarbon
SD	standard deviation
SE	standard error
Se	Selenium
THg	total mercury
TN	total nitrogen
TP	total phosphorus

Epigraph

"Many men go fishing their entire lives without knowing it is not fish they are after."

- contributed to Henry David Thoreau

Chapter One: Stormwater Ponds as Potential Recreational Fisheries

1.1 Introduction

The population of Alberta, Canada, has increased by 63.5% in the last 30 years and 82% of Albertans now reside in urban centers (Statistics Canada 2012). Concurrently, increases in urban sprawl has displaced aquatic habitats and degraded urban waterbodies. The rapid population expansion and growth of urban sprawl is increasing pressure on water resources within Alberta. There are approximately 300,000 recreational anglers and only 1100 lakes and reservoirs in Alberta (Zwickel 2012), demonstrating that Alberta has insufficient water resources to support the current demand for recreational fisheries. With few fishing opportunities in Alberta, anglers have limited options of where to fish and what to fish for and consequently anglers accept lower catch rates if there are easily accessible waters (Patterson and Sullivan 2013). Ease of access can result in high fishing pressure which can quickly diminish fish stocks (Gunn and Sein 2000). Fisheries close to large urban centers attract 2.5 times more anglers per unit fish density than more remote lakes (Post et al. 2002). Thus, it is not surprising that in easily accessible areas with short travels times, there are low catch rates and in areas with longer travel times, there are higher catches rates (Post et al. 2002).

The creation of additional fisheries close to urban centers, such as stormwater fisheries, may offer anglers additional, highly valued, easily accessible, alternate fishing venues. Stormwater fisheries may help to alleviate fishing pressure on existing fisheries similar to how the Alberta Conservation Association's (ACA) stocked trout ponds have functioned to create highly valued, alternate fishing venues (Patterson and Sullivan 2013). However, many municipalities in Canada have adopted a 'non-contact' policy that discourages residents from participating in activities such as swimming or boating that may put them in direct contact with stormwater since stormwater and stormwater pond sediments may be polluted with urban contaminants (Karlsson et al. 2010). In addition to human contamination from direct contact activities with stormwater, consumption of fish from stormwater ponds could also pose a risk to human health. Thus, to evaluate the recreational fisheries potential of stormwater ponds, the present study was designed to 1) Determine the proportion of stormwater ponds suitable for stocking with rainbow trout (*Oncorhynchus mykiss*). 2) Evaluate toxicant exposure in rainbow trout stocked in stormwater ponds.

1.2 The Current Status of Stormwater Ponds

The influence of urbanization on aquatic ecosystem health is becoming an increasingly scrutinised issue. Several studies have identified urban runoff, precipitation falling on impervious surfaces where infiltration potential is low, as a major contributor to the degradation of urban streams and rivers (Pitt et al. 1995, Walsh et al. 2005). When uncontrolled, urban runoff negatively impacts ecosystem functioning and the health of aquatic organisms (Eckley and Branfireun 2009) through the introduction of high concentrations of numerous pollutants including heavy metals, suspended solids, oil and grease, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), pesticides, nutrients and bacteria (Bishop et al. 2000a, Bishop et al. 2000b, Karouna-Renier and Sparling 2001). Despite the abundance of information on urban runoff and the water quality within and downstream of urban development, few studies have quantified the negative impact urban runoff has on the health of aquatic organisms.

Urban runoff is a by-product of urban development since urban development can profoundly alter watershed basins through the degradation of the landscape and functioning of the hydrologic system (Booth and Jackson 1997). Urbanization alters the lands surface through the creation of impervious surfaces and the displacement of aquatic habitats (Paul and Meyer 2001). The manipulation of the land surface changes the hydrology of the watershed by reducing infiltration potential, leading to a reduction in groundwater recharge (Pitt et al. 1999). The acts of compacting soil, ditching, removing vegetation and paving over surfaces prevent water from entering the natural hydrologic cycle. Thus, for any given intensity and duration of precipitation, the peak discharge is greater, the water velocity higher and the volume of particulate and effluent release greater than in an undisturbed system (Sartor et al. 1974). Average runoff from developed subdivisions is reported to be as much as 10 times higher than in non-developed agricultural areas (Madison et al. 1979). Consequently, runoff in urban centers resulting from hydrologic disruption is no longer considered rainwater because of its intensity and toxicant load and is instead considered stormwater (US Environmental Protection Agency, 1983).

Stormwater is considered nonpoint source, urban runoff generated from rain and snowmelt occurring on anthropogenically altered surfaces such as impervious parking lots, streets, sidewalks and rooftops, and on other somewhat pervious urban land features such as lawns and construction sites where soil compaction is high (Pitt et al. 1995). Activities such as overwatering lawns and gardens, and washing vehicles in driveways also contribute to stormwater runoff. Worldwide trends in increased urbanization and increased urban sprawl have made impervious surfaces more prevalent (Alberta

Environmental Protection 1999, Paul and Meyer 2001). Due to the increase in impervious surfaces in recent decades, retention basins commonly referred to as stormwater ponds have been designed and implemented into urban landscapes to receive stormwater runoff.

Stormwater ponds are artificial catchment basins designed to collect and retain urban runoff. Originally, the primary purpose of stormwater ponds was to provide flood protection to surrounding infrastructure through the diversion and collection of urban runoff (Thomas 1998). However, in the last 30 years it has been recognized that design improvements to stormwater ponds were required to mitigate some of the negative consequences of stormwater runoff (Brown 2005). Documented effects of unmitigated stormwater to downstream aquatic ecosystems include increased water temperatures, increased sedimentation, increased stream bed and bank erosion, increased nutrient loading and increased toxicant accumulation in aquatic organisms (US Environmental Protection Agency 1983, Walsh et al. 2005). Stormwater ponds are now specifically designed to temporarily retain runoff so that fine sediments, contaminants and nutrients can settle out in ponds, and water exiting ponds can be released at a controlled rate, thereby reducing stream erosion and ameliorating the quality of water entering nearby rivers and streams (Bishop et al. 1999b).

In addition to the functional purpose of stormwater ponds, they also provide green space in urban areas, recreational opportunities, improved perceived quality of life for residents, and increased property values (Baxter et al. 1985, Ministry of the Environment 2003). Because of the inherent and extrinsic value of stormwater ponds, they are becoming a common feature in urban landscapes, often being required by city by-laws in Canada.

Stormwater ponds are small, shallow, saucer shaped, productive, unstratified, highly eutrophic lakes that commonly winterkill if fish are present. The morphometric attributes of stormwater ponds are similar to glacially formed pothole lakes of the Canadian prairies (Sunde and Barica1975). Stormwater ponds exhibit developed aquatic communities comparable to those in natural wetlands and pothole lakes, and stormwater ponds provide similar habitat for aquatic insects, birds, reptiles, amphibians and fish (Fritioff and Greger 2003, Sparling et al. 2004). The habitat provided by stormwater ponds acts as a final refuge for aquatic organisms in urban environments where natural aquatic habitats have all but been displaced (Le Viol et al. 2009). In some instances, stormwater ponds are being used as conservation sites for regionally endangered fishes (Schaeffer et al. 2012) and are recognized as creating biodiversity islands within urban landscapes (Scher et al. 2004).

Despite stormwater ponds having similar habitat features to pothole lakes, stormwater ponds can have substantially different water quality due to anthropogenic inputs and the fact that stormwater ponds are designed to retain pollutants (US Environmental Protection Agency 1983). However, stormwater ponds may have the potential to supplement pothole lakes as venues for recreational rainbow trout fishing depending on water quality and severity of toxicant loading.

Rainbow trout are useful indicator organisms because they readily accumulate many toxicants from food sources and water, and have a longer life span than invertebrates (Wren et al. 1997). There is more toxicological data for fish than other vertebrates because fish are important food sources for both humans and wildlife and rainbow trout are often used as model organisms in toxicological studies. Stocked rainbow trout are reliable test organisms because baseline toxicant levels are easily measured in fish tissues prior to fish being stocked and known stocking dates help define exposure duration. Thus, stocked rainbow trout could be used to assess the feasibility of exploiting stormwater ponds for the purpose of recreational fishing.

Rainbow trout are the most extensively stocked salmonid species in the world (Haddix and Budy 2005) and the most frequently farmed freshwater salmonid in both North America and Europe (Ellis et al. 2002). Approximately 3.7 million trout are stocked annually in Alberta (Park 2007). Rainbow trout are highly rated as sport fish and sought after by a wide arrary of anglers because of the acrobatics rainbow trout perform when hooked and the wide variety of fishing methods with which rainbow trout can be captured (Nelson 1991). Rainbow trout fill a broader ecological niche than other members of the salmonid family which enables rainbow trout to tolerate a wider temperature range and greater water chemistry variability (Kerr and Lasenby 2000). Thus, rainbow trout are more suited for stocking in highly variable waterbodies, such as stormwater ponds, than other members of the salmonid family.

Rainbow trout were first stocked in Alberta in 1919 within a few lakes in Banff and Jasper and later, in the 1950's, into many pothole lakes throughout the Canadian prairies and foothills (MacCrimmon 1971). A small, but successful commercial rainbow trout farming industry was established in the pothole lakes of the Canadian prairies by the 70's, but fish recoveries were highly variable from lake to lake (Lawler et al. 1974). Alberta's rainbow trout fisheries are now being stocked with triploid rainbow trout in an attempt to lessen the risk of hybridization with native or established fish stocks.

Precautions to mitigate stocking non-native fish species in stormwater ponds need to be considered to prevent fish escapement and hybridization with wild fishes. Alberta Environment and Sustainable Resource Development (ESRD) recommends that connected waterbodies, such as stormwater ponds, have screens installed on inflows and outflows in addition to being stocked with triploid fish since triploid induction is not always 100% effective (Solar et al. 1984). Triploid rainbow trout can normally be contained by fish screens since the trout are stocked at a predetermined size (usually 15 to 20 cm) that is too large to fit through the fish barriers. However, fish screens are ineffective at containing diploid species that are capable of spawning in lentic systems, such as northern pike (*Esox lucius)*, since newly hatched young are able to fit through the mesh of fish screens. Therefore, the availability and popularity of triploid rainbow trout, the successful introduction of rainbow trout into pothole lakes, and the lower risk of hybridization with wild fishes make triploid rainbow trout a suitable candidate for stocking within stormwater ponds.

Stormwater ponds are not built for the purpose of recreational fisheries and are therefore likely to have less-than-desirable water quality and habitat characteristics for rainbow trout fisheries. Since stormwater ponds are not considered traditional fisheries and little is known about the suitability of stormwater to support rainbow trout, it was necessary to develop a two tiered approach for this study. In the first part of this study, a preliminary investigation based on water quality and habitat characteristics was used to determine if stormwater ponds could support recreational rainbow trout fisheries. In the second part of this study, stormwater ponds were stocked with rainbow trout to monitor fish survival, growth and exposure to toxicants.

To determine the proportion of stormwater ponds suitable for recreational fisheries, a literature survey was conducted to identify key water quality thresholds and habitat characteristics critical for rainbow trout survival. Studies such as Ayles et al. (1976) that discuss the high variability of rainbow trout stocking success in shallow, eutrophic pothole lakes, the review on rainbow trout welfare by MacIntyre et al. (2008) and Canadian Council of Ministers of the Environment (CCME) guidelines were used to develop criteria for selecting stormwater ponds that would have the highest likelihood of fish survival.

Nine parameters were recognized as critical for rainbow trout survival and a guideline for assessing stormwater pond suitability for rainbow trout welfare was established: dissolved oxygen (DO), water temperature, pH, ammonia, secchi depth (as proximate of total suspended solids), water depth, water depth fluctuation, accessibility and density of macrophyte growth were all used to assess pond suitability.

Of the parameters necessary for trout survival, DO is the primary water quality measurement influencing rainbow trout health (Ayles et al. 1976, MacIntyre et al. 2008) and is also a leading contributor to poor stocking success in pothole lakes where summerkill is prevalent (Lawler et al. 1974). Dissolved oxygen concentrations depend on a number of variables including temperature, salinity, wave action and altitude (MacIntyre et al. 2008). If DO concentrations fall below optimal levels, a rainbow trout will experience stunted growth, reduced food conversion efficiency and a reduction in swimming ability (MacIntyre et al. 2008). At critically low DO levels, fish will stop feeding, eventually go unconscious and die (Davis 1975). CCME guidelines suggest a minimum concentration of 6.5 mg/L DO (1999) for the protection of aquatic life in cold water ecosystems. A critical DO requirement of 5-6 mg/L is frequently recommended for rainbow trout welfare in aquacultures (MacIntyre et al. 2008). We relaxed our guideline value to a minimum DO concentration of 4 mg/L to ensure that enough stormwater ponds could pass the initial screening process.

Water temperature is also a major factor and can affect fish growth, swimming capacity and oxygen consumption (Hokanson et al. 1977). The importance of cool water refugia in conserving salmonids during high summer water temperatures is well documented (Matthews and Berg 1997). Lethal water temperatures for diploid rainbow trout are > 25°C (Hokanson et al. 1977) while triploid rainbow trout have reduced survival in >22°C over an extended period of time (Ojolick et al. 1995, Hyndman et al. 2003). We set the maximum, one time, surface water temperature at 24°C for this study.

Rainbow trout are sensitive to pH conditions since unfavourable pH can reduce the swimming capability of rainbow trout, interfere with a fish's ability to excrete ammonia and reduce a fish's ability to transport oxygen (MacIntyre et al. 2008). Aside from the direct effects of acidity on fish welfare, pH affects other water quality parameters such as unionised ammonia concentrations and the solubility of heavy metals (Wren et al. 1997). The range of acceptable pH values for the protection of aquatic life is between 6.5 and 9.0 (CCME 1987) and were adopted as a criterion in this study as well.

Ammonia concentrations are a concern in pothole lakes and stormwater ponds where dense aquatic plant growth, common in eutrophic systems, releases nitrogen when decaying (Nichols and Keeney 1973). Nitrogen can be converted to ammonia at pH > 9 (Farnsworth-Lee and Baker 2000). Fish exposed to high ammonia concentrations may experience a loss in equilibrium, increased respiratory activity, impaired oxygen uptake and death (MacIntyre et al. 2008). Ammonia concentrations are difficult to predict owing to the complex interaction ammonia has with other water quality variables such as pH and the diurnal cycle ammonia follows (Lawler et al. 1974). The toxicity of ammonia to fish depends on DO concentration, pH, temperature, fish life-cycle stage and fish stress level (Thurston et al. 1984). A maximum ammonia concentration of 0.02 mg/L is recommended for the protection of aquatic life including rainbow trout (CCME 2001, Wedemeyer 1996) and was used in this study.

Suspended sediment is another factor that can be deleterious to fish; it can impair fish feeding, modify natural movements, damage gill mechanisms and impair predator avoidance (Wren et al. 1997, Henley et al. 2000). High levels of suspended sediments may indicate high levels of runoff and associated pollution and, in other instances, high turbidity levels may be associated with re-suspension of pollutants trapped in sediments. High turbidity can also influence surface water temperature since suspended particles absorb the sun's energy. At the same time, sediments, such as fine clay particles, can aid in the removal of heavy metals by adsorption (Hart 1982). Wedemeyer (1996) suggests 80-100 mg/L total suspended solids (TSS) as a guideline for maximum chronic exposure for rainbow trout. A minimum secchi depth of 0.5 m (as a proximate for TSS >70) was set as a selection criterion in this study.

In addition to water quality parameters, physical habitat features such as water depth can also affect fish welfare. Shallow water promotes dense growths of aquatic vegetation, high water temperatures and subsequent low DO levels. Low water volume ponds may also have insufficient DO under ice cover, leading to fish winterkill (Lawler et al. 1974). Shallow water can increase exposure to avian predators feeding on rainbow trout (Ayles et al. 1976). Shallow ponds also have less volume, and therefore frequently have short water retention times compared to deeper ponds with similar surface areas, and short retention time can limit zooplankton biomass, a key food resource for rainbow trout (Obertegger et al. 2007). Thus, a minimum mean water depth of 1.5 m and a maximum water depth fluctuation of 2.0 m were set as selection criteria for this study.

Aquatic vegetation can also influence fish survival and the public perception of a quality fishery. Dense blooms of cyanobacteria and dense growth of submerged aquatic macrophytes can contribute to anoxic water conditions and increase ammonia concentrations (Lawler et al. 1974). Dense aquatic plant growth is often correlated with pond features that may influence fish health including shallow water, high water temperature and high nutrient loading. Dense plant growth also snags lures and restricts shoreline angling which can diminish the angling experience. Therefore, ponds with dense growths of aquatic macrophytes and/or algae blooms were dismissed as possible stocking candidates in this study.

While all criteria were determined on an individual basis, we are fully aware that most of the parameters used to assess stormwater suitability are tightly linked and interactive effects might influence stocking success.

In addition to assessing stormwater ponds for water quality and habitat metrics, stormwater ponds also commonly contain toxicants that must be assessed to determine the suitability of stormwater ponds as recreational fishing venues. It was therefore necessary to develop criteria for evaluating toxicant exposure in rainbow trout reared in stormwater ponds. Stormwater ponds may exceed guidelines for heavy metals, suspended solids, oil and grease, PCBs, PAHs, pesticides, nutrients and bacteria (Bishop et al.

2000a, Bishop et al. 2000b, Karouna-Renier and Sparling 2001). Some stormwater contaminants biodegrade while others contaminants are more persistent and accumulate in sediments, adsorbing to fine clay particles (Bishop et al. 1999b). Stormwater pollutants have the potential to bioaccumulate in benthic organisms and, to some extent, in vertebrate consumers of benthic organisms (Karouna-Renier and Sparling 2001). Consequently, stormwater ponds might negatively influence the health of organisms inhabiting them in the process of protecting downstream ecosystems from toxicants.

Despite the abundance of information on stormwater ponds, a major unresolved issue is how much of a toxicological threat stormwater poses to wildlife (Campbell 1994, Walsh et al. 2005, Le Viol et al. 2009). Stormwater pollution can be traced to several major sources and is directly influenced by the intensity of land use and percent impervious land cover (Booth and Jackson 1997). Street pavement, motor vehicles, atmospheric fallout, anti-skid compounds, pet faeces, litter, illegal disposal of household hazard wastes and sewer overflows are all common sources of stormwater pollutants (US Environmental Protection Agency 1983). The impact of stormwater on downstream aquatic ecosystems varies depending upon the quality and quantity of wastewater entering the ecosystem and the ecosystem's ability to assimilate degrading substances: there is also considerable variability inherent to stormwater quality and detention basin morphometry. Thus, stormwater quality and stormwater fish toxicant burden must be assessed on a case by case basis to determine if there are potential risks to human health. There are several commonly observed stormwater contaminants that Wren et al. (1997) and Bishop et al. (2000b) recommend testing for that were used to assess the health of wildlife inhabiting stormwater ponds.

One common class of contaminant detected in stormwater ponds are pesticides, which are leached from surrounding agricultural areas and from lawns and gardens (Schiff and Sutula 2004). Organophosphate and carbamate pesticides used to control insects are toxic to a wide range of non-target organisms including fish, birds, reptiles and mammals due to the ability of these pesticides to inactivate the enzyme acetylcholinesterase (AChE) (Fulton and Key 2001). Inactivation of AChE can lead to overstimulation of the nervous system, resulting in continuous involuntary contraction of muscles, causing exhaustion and paralysis of muscles necessary for breathing, leading to death. In humans, organophosphate exposure may affect neurodevelopment and growth (Eskenazi et al. 1999). Since organophosphate and carbamate pesticides inhibit AChE activity, decreased AChE activity in rainbow trout brain tissue can be used as a biomarker for pesticide exposure. It is therefore of interest to determine AChE activity in rainbow trout brain tissues as a proxy for pesticides exposure in stormwater to evaluate fish toxicant exposure in stormwater ponds.

Several studies have detected elevated metal concentrations in the water and sediment of stormwater ponds that can lead to the impairment of aquatic health. Copper, lead and zinc are the most prevalent pollutants detected in urban runoff samples (US Environmental Protection Agency 1983, Cole et al. 1984, Ellis et al. 1987, Campbell 1994, Marsalek and Marsalek 1997). Although heavy metals are present in stormwater ponds (Marsalek and Marsalek 1997, Karlsson et al. 2010), few studies have investigated the influence of metals on fish health in stormwater ponds (Campbell 1994, Campbell 1995) even though metals can negatively influence the health of aquatic organisms (CCME1987). Chemical speciation and interaction with other inorganic and organic molecules influence the toxicity of metals and the toxicological impact of metals is further complicated by the behaviour and biology of the target organism (Deheyn et al. 2004). As such, it remains difficult to predict the toxic effect of metals in stormwater ponds on fish health. Few studies have focused on mercury (Hg) in fish taken from stormwater even though elevated Hg concentrations have been reported in urban waterways and Hg can biomagnify (Mason and Sullivan 1998, Lawson et al. 2001).

Atmospheric deposition is the dominant source of Hg and, during stormflow periods, elevated Hg concentrations have been observed in urban waterways (Eckley and Branfireun 2009). Mercury in rainbow trout muscle tissue is of concern because of Hg's persistence in the environment and the cells of organisms, and the ability of Hg to biomagnify. Fish absorb Hg directly through their gills and through the consumption of contaminated food sources (Phillips and Buhler 1978). Methyl mercury (MeHg) is the most toxic form of Hg with the consumption of fish and shellfish being the main source of MeHg exposure for humans (World Health Organization (WHO) 2007). Fish consumption guidelines exist for several drainages in Alberta because of Hg contamination (Alberta Environmental Protection 2013) and it is likely that elevated levels will be present in stormwater ponds (Mason and Sullivan 1998). Rainbow trout are expected to be the apex consumers in stormwater ponds and are therefore likely to have elevated levels of Hg within their tissues which could influence the health of fish consumers.

Pollutant mobilization into stormwater ponds is often dependent upon precipitation events (Eckley and Branfireun 2009). Due to temporal variation associated with precipitation events, aquatic organisms inhabiting stormwater ponds are exposed to large variations in water quality and chemistry, which may influence an organism's stress levels. Chronic stress, manifested as prolonged elevation of cortisol, can be detrimental to the immune status and growth of fish (Maule et al. 1989, Andersen et al. 1991). It is therefore useful to measure plasma cortisol concentrations in rainbow trout stocked in stormwater ponds to determine if they are physiologically competent and able to mount a normal stress response (Hontela 1998). The physiological competency of rainbow trout reared in stormwater can be used as a proxy for urban contaminants and health of aquatic organisms inhabiting stormwater.

Stormwater ponds receive a flux of synthetic chemicals from surrounding land use activities (Boyd et al. 2004). Some urban contaminants are known to disrupt the endocrine system, influencing the reproductive function of humans and wildlife (Thorpe et al. 2001). Organochlorine insecticides, nonylphenol, bisphenol, constituents of contraceptive pills and PCB's are a few of the chemical classes known to mimic estrogenic action. In male fish, environmental estrogens can influence testicular development and can lead to physiological and anatomical feminization (Cardinali et al. 2004). In the presence of estrogen or estrogenic endocrine disruptive chemicals (EDC's) male fish can express elevated levels of the egg yolk precursor protein, vitellogenin. Vitellogenin, which is synthesized in the liver, is not normally made nor secreted into the blood of male fish at appreciable levels, unless in the presence of estrogens (Thorpe et al. 2001). Therefore, high plasma vitellogenin concentrations in male fish can be used as a proxy for estrogenic pollution (Kime et al. 1999). Ploidy can also influence vitellogenin levels and triploid female fish have been shown to have lower vitellogenin levels than diploid female fish due to lower estrogen levels (Benfey 1999, Schafhauser-Smith and

Benfey 2001). Thus, ploidy should be taken into consideration when examining vitellogenin concentrations as an indicator of exposure to EDC's. Using vitellogenin concentrations as an indicator of EDC's is worthwhile since there is a growing body of evidence suggesting that feminization of male fish could have severe implications for fish populations (Kidd et al. 2007).

Physical, chemical and biological characterization of stormwater ponds are essential to understanding and addressing human health risks associated with activities that put humans in direct or indirect contact with stormwater such as fishing or consumption of fish from stormwater ponds. Monitoring stocked trout for bioaccumulation of toxicants and water quality for contaminants is necessary if stormwater ponds are to be used as venues for recreational fishing.

From the water quality and habitat literature survey, guidelines were established to select the most suitable stormwater ponds available for rainbow trout survival. If fish were able to survive in stormwater ponds, it would allow for the evaluation of rainbow trout exposure to toxicants such as those identified in the toxicant literature survey.

1.3 Stormwater Pond Preliminary Field Survey

Using the criteria selected by the literature analysis, thirty-one candidate ponds from seven different communities were investigated between July and August of 2011 to assess recreational fisheries potential of stormwater ponds. Ponds were characterized by physical, biological and chemical characteristics to identify what proportion of stormwater ponds would be suitable for stocking with rainbow trout. The initial screening process selected six suitable candidate ponds from the original 31 ponds investigated. Ponds not passing the initial screening process failed to meet at least one of the preliminary criteria that were established as necessary for either rainbow trout survival or as a location for a recreational fishery (Table 1).

Table 1: Water quality thresholds for rainbow trout survival used for selecting candidate ponds

Parameter	Threshold	Number of Ponds Failing Criteria (n=31)
Minimum Dissolved Oxygen	4 mg/L	8
Maximum Temperature	24°C	0
Minimum and Maximum pH	6.5 and 9.0	6
Maximum Ammonia	0.02 mg/L	9
Minimum Mean Water Depth	1.5 m	18
Minimum Secchi Depth	0.5 m	2
Maximum Water Depth Fluctuation	2.0 m	3
Excessive Macrophytes	-	4
Inaccessibility	-	7

Of the six ponds selected, three were located in the city of Lethbridge, one in the town of High River and two in the city of Lacombe (Table 2) (Figure 1). Lethbridge 1 and Lacombe 1 were aerated with subsurface oxygen diffusors while Lethbridge 2 and Lethbridge 3 had surface water fountains. Lethbridge 1, Lethbridge 2, Lethbridge 3 and High River were all connected to irrigation canals and supplemented with irrigation water to maintain aesthetically pleasing water levels. Lacombe 2 was the only pond that did not have some form of artificial water quality enhancement such as aeration or water supplementation. All ponds were at least five years old allowing for sufficient time to elapse for sediment and toxicant deposition from the surrounding watershed. Furthermore, vegetation cover and pond hydrology could have likely reached a steady state by the time ponds were at least five years old.

Table 2: Physical characteristics of stormwater ponds passing the initial screening process

Pond	Location (UTM)	Area (ha)	Max Depth (m)	Volume (m^3)
High River	12U 298120E 5606680N	4.3	8.1	297750
Lacombe 1	12U 315780E 5817520N	1.7	4.3	48190
Lacombe 2	12U 315940E 5816690N	0.5	6.7	18420
Lethbridge 1	12U 370190E 5502000N	1.2	3.0	28000
Lethbridge 2	12 U 370960E 5502840N	4.3	4.2	139280
Lethbridge 3	12U 362960E 5506240N	1.2	3.0	27690

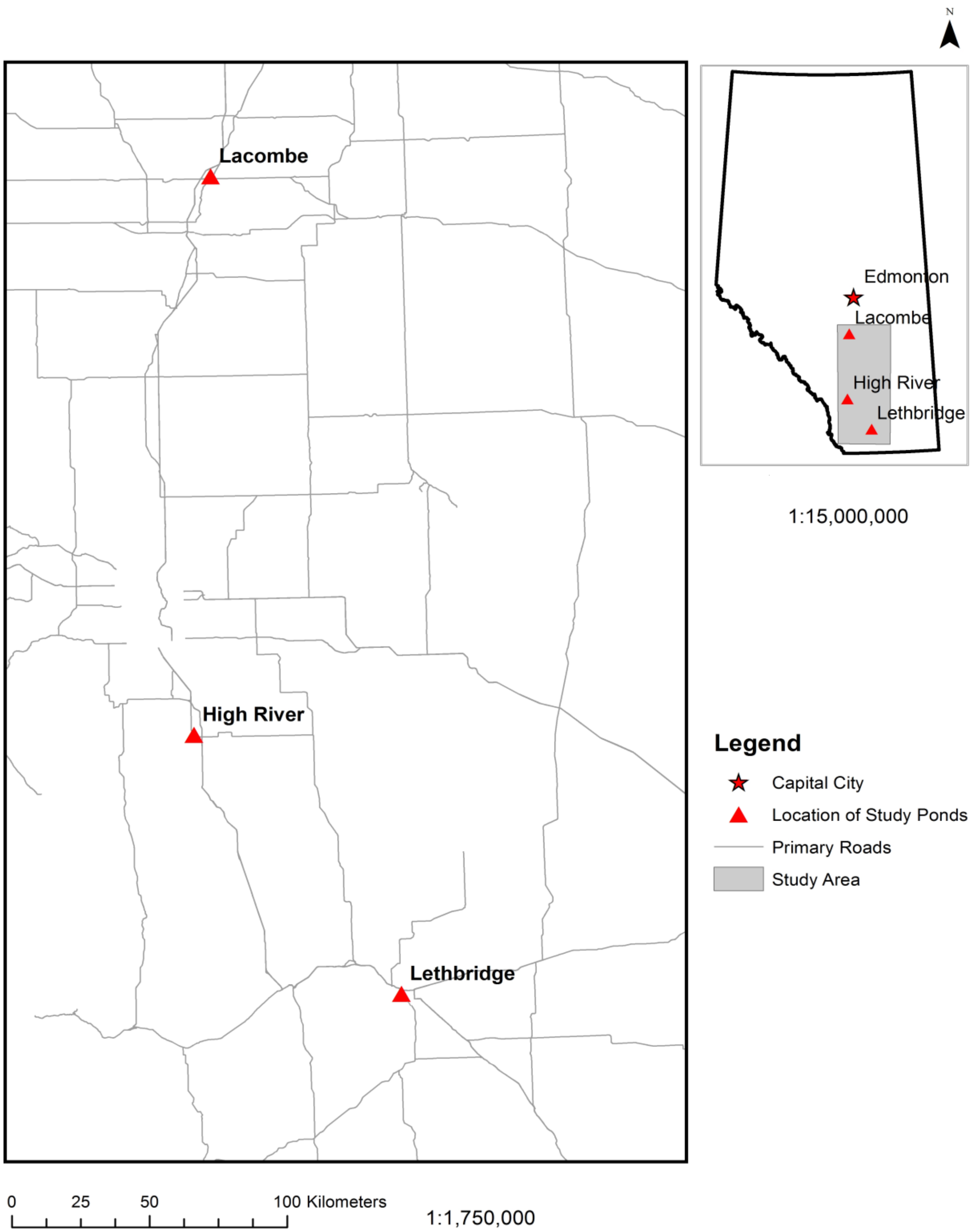

Figure 1: Location of selected study sites within Alberta passing preliminary criteria necessary for rainbow trout survival

Of the stormwater ponds surveyed, 25 out of 31 ponds (81%) did not meet water quality or habitat characteristics necessary for a rainbow trout fishery. There were high ammonia concentrations in 9 (29%) of the ponds and low DO levels in 8 (26%) of the ponds. In addition to poor water quality, there was insufficient water depth in 18 (58%) of the ponds and 7 (23%) of the ponds were inaccessible or had steep banks that would be public safety hazards. Thus, only a small percentage of stormwater ponds investigated had the appropriate physical, chemical and biological attributes for recreational fisheries.

It is not surprising that the majority of the ponds investigated for recreational fisheries potential failed the initial water quality and habitat assessment since stormwater ponds are not designed for fisheries. Despite only a small portion of stormwater ponds meeting rainbow trout water quality and habitat requirements, urban stormwater ponds are very abundant; in fact, several hundred are likely present in Alberta if ponds from both the cities of Edmonton and Calgary are included. The initial survey suggests that around (20%) of stormwater ponds might be appropriate for fish stocking, and therefore, at least 30-40 urban storm ponds suitable for summer rearing of put-and-take recreational fisheries likely exist within the province of Alberta.

The reviewed data and studies on stormwater ponds clearly outline the need for further investigation on the suitability of stormwater ponds as recreational fishing venues. The concerns identified in the literature, as well as through our own synoptic survey of ponds, indicated that few stormwater ponds would be suitable for stocking with rainbow trout. Furthermore, stormwater ponds have a high likelihood of containing harmful contaminants and are therefore commonly dismissed as locations for fisheries (Wren et al. 1997). The risks stormwater presents to wildlife health have not been studied extensively and several studies have identified the need to quantify these risks (Kadlec 1985, Helfield and Diamond 1997, Bishop et al 2000b). Thus, measuring growth, survival and toxicant exposure (chapter 2), in rainbow trout stocked into the stormwater ponds selected from the preliminary screening process is necessary to determine the suitability of stormwater ponds as alternative fishing venues to pothole lakes.

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Chapter Two: Growth, Survival and Indicators of Toxicant Exposure in Rainbow Trout Stocked in Stormwater Ponds

2.1 Introduction

Worldwide trends in increased urbanization and urban sprawl have created large tracts of impervious land surfaces and consequently, urban runoff (Paul and Meyer 2001). Urban runoff negatively influences downstream aquatic ecosystems by increasing water temperatures, sedimentation, stream bed and bank erosion, nutrient loading and toxicant deposition (US Environmental Protection Agency 1983, Walsh et al. 2005). Engineered structures known as stormwater ponds are now specifically designed to temporarily retain urban runoff so that fine sediments, contaminants and nutrients can settle out in the ponds, and water can be released at a controlled rate (Wren et al. 1997). The controlled release of stormwater reduces downstream erosion and ameliorates the quality of water entering nearby rivers and streams (Bishop et al. 1999b) and, as such, stormwater ponds are becoming common urban land features (Alberta Environmental Protection 1999, Paul and Meyer 2001). With limited water supplies and the subsequent lack of fisheries (Zwickel 2012) in central and southern Alberta, Canada, stormwater ponds represent an extensive, under-used water resource already present within the urban landscape that could be exploited to create additional fishing opportunities.

Stormwater ponds are similar in shape, size, flora and fauna to prairie pothole lakes of the Canadian prairies (Wren et al. 1997, Bishop et al. 2000a, Sparling et al. 2004, Casey et al. 2007) where a moderately successful commercial rainbow trout fishery has been developed over the past four decades (Lawler et al. 1974, Ayles et al. 1976). Consequently, the successful development of pothole lake fisheries might indicate that stormwater ponds have fisheries potential. However, numerous studies indicate that stormwater ponds contain elevated levels of pollutants associated with urbanization such as heavy metals, suspended solids, oil and grease, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), pesticides, nutrients and bacteria (Bishop et al. 2000a, Bishop et al. 2000b, Karouna-Renier and Sparling 2001). These urban pollutants are potentially harmful to aquatic organisms and are not commonly detected in pothole lakes. Thus, there are human health concerns associated with supplementing existing recreational pothole lakes fisheries with stormwater pond fisheries.

To date, the few studies that have examined toxicant accumulation in fish taken directly from stormwater ponds detected elevated levels of heavy metals in the muscle tissues of various warm water fish species (Campbell 1994, Campbell 1995). Species-specific feeding behaviours, fish exposure duration and individual pond characteristics have influenced fish toxicant burdens (Campbell 1994). No study has investigated the potential for stormwater ponds to be used as put and take recreational rainbow trout fisheries even though rainbow trout are the most extensively stocked species in the world (Haddix and Budy 2005).

To determine the recreational fisheries potential of stormwater ponds, rainbow trout growth, survival and toxicant accumulation in urban stormwater ponds will be characterized. Rainbow trout were chosen for stocking in stormwater ponds since rainbow trout are useful indicator organisms that readily accumulate many toxicants from their food sources and water and are often used as model organisms in toxicological studies (Wren et al. 1997). Furthermore, rainbow trout are a popular sport fish that have been stocked in Alberta for almost a century and are easy to obtain from hatcheries

(MacCrimmon 1971). This study will allow us to assess rainbow trout survival and growth in stormwater ponds and allow us to evaluate toxicant exposure in rainbow trout reared in urban stormwater ponds.

To estimate rainbow trout growth and survival, we proposed to measure catch per unit of gill netting effort (CPUE) and indicators of rainbow trout body condition such as weight, length, condition factor (K) and bioelectrical impedance analysis (BIA) in monthly intervals.

To test fish for exposure to urban contaminants, we propose to measure several common indicators of toxicant exposure including acetylcholinesterase (AChE) activity, muscle mercury (Hg) concentration, cortisol concentration and vitellogenin concentration. Reduced acetylcholinesterase activity is indicative of exposure to organophosphates and carbamate pesticides (Fulton and Key 2001). A reduction in AChE activity leads to an over stimulation of the nervous system which causes exhaustion and paralysis of muscles necessary for breathing, resulting in death. Mercury was a concern in this study because of Hg's persistence in the environment and the ability of Hg to biomagnify (Trudel and Rasmussen 2006). Fish consumption is the primary source of Hg exposure to humans (World Health Organization (WHO) 2007). Elevated Hg concentrations have been detected in urban waterways (Mason and Sullivan 1998, Lawson et al. 2001) and several watersheds in Alberta have fish consumption guidelines in place to prevent Hg exposure to consumers (Alberta Environmental Protection 2013). Cortisol concentrations in fish can be measured as a proxy for stress with fish stress levels being influenced by, but not limited to, exposure to metals, organic contaminants, water quality conditions and capture (Hontela et al. 1992). High cortisol concentrations indicate a fish under stress while low cortisol levels indicate no stress, exhaustion of the hypothalamo-pituitary-interrenal (HPI) stress axis, or impairment of the HPI axis (Hontela 1998, Miller et al. 2009). High plasma vitellogenin concentrations in male fish can be used as a proxy for estrogenic pollution since vitellogenin is normally not detectable in male fish (Kime et al. 1999). However, in the presence of estrogen or estrogenic endocrine disruptive chemicals (EDC's) male fish can express elevated levels of the egg yolk precursor protein, vitellogenin. In male fish, environmental estrogens can influence testicular development and can lead to physiological and anatomical feminization (Cardinali et al. 2004).

We expect to detect increased toxicant tissue burdens in fish exposed to stormwater. Fish exposure time and the ecology of the stormwater environment will likely play a significant role in the suitability of these ponds as trout fisheries. This study will be the first to give empirical data on toxicant exposure in fish reared in stormwater ponds for a known time period.

2.2 Materials and Methods

2.2.1 Study Sites

A subset of six stormwater ponds in central and southern Alberta, meeting specific physical, chemical and biological characteristics necessary for rainbow trout welfare were selected from a set of 31 candidate ponds (Chapter 1) (Table 1). Three selected ponds were located in the city of Lethbridge, one in the town of High River and two in the city of Lacombe (Figure 1). All selected ponds were less than 5 hectares and had maximum water depths ≥ 3.0 m (Table 2). Lethbridge 2 and Lethbridge 3 were aerated by water fountains, Lethbridge 1 and Lacombe 1 by oxygen diffusors and High

River and Lacombe 2 had no form of mechanical aeration. Lethbridge 1, Lethbridge 2, Lethbridge 3 and High River were connected to irrigation canals and could be supplemented with irrigation water to maintain aesthetically pleasing water levels. All ponds were at least five years old, allowing for sufficient time to elapse for sediment and toxicant deposition from the surrounding watershed. Vegetation cover and pond hydrology could have also likely reached a steady state within five years of being built.

2.2.2 Water Quality

Water temperature, oxygen, conductivity (subsurface water depth of 1.0 – 2.0 m) (YSI, model 85), secchi depth and water depth were measured once a month (May to October) in each pond from five locations corresponding with pond inlet, pond centre, pond outlet and two other locations to ensure all habitat types were sampled. Composite water samples combined from the five previously mentioned sampling locations within each pond (0.5 – 1.0 m subsurface water depth) were collected each month (May to September) for analysis of nutrient concentrations. Additional composite water samples were collected in May and July for analysis of trace metal concentrations (36 elements, ICP-MS) and in August for analysis of pesticide concentrations (69 target compounds) (see Appendix I, Table 8 for a list of pesticide compounds). All water samples were analyzed by Alberta Innovates and Technologies Futures (AITF) in Vegreville, Alberta. To capture diurnal fluctuations, hourly measurements of temperature, conductivity, dissolved oxygen (DO) and pH were recorded with data loggers (Manta Water Quality Multiprobe; 1 – 2 m subsurface water depth) that were alternated between ponds. Biofouling of oxygen sensor membranes was a common problem with the data loggers and therefore only the first two weeks from any collection interval were used to determine DO levels.

2.2.3 Invertebrates

Five Ekman dredge ($225 cm^2$) samples were collected in each pond monthly (May to September) to examine benthic invertebrates. Sampling locations were chosen to cover the range of habitat types and water depths present in each pond. Two sweep net samples were also taken from the shallow littoral zones and around macrophytes. Two zooplankton samples were collected from each pond at each sampling time using a vertically towed zooplankton net (110 µm mesh size) from a depth of 2 m to the surface. All invertebrate samples were frozen. In the lab, invertebrates were identified to order, dried (60°C for 72 hours) and dry biomass was estimated for each monthly sample.

2.2.4 Fish

Animal-use protocols were approved by the Animal Care Committee of the University of Lethbridge in accordance with national guidelines. Juvenile, triploid, rainbow trout from the Ackenberry Trout Farm, Camrose, Alberta (99.3 ± 45.7g) were stocked in High River while juvenile, triploid, rainbow trout from the Sam Livingston Fish Hatchery,Calgary, Alberta, (58.9 ± 22.1g) were stocked in the remaining five stormwater ponds. Prior to stocking, a subsample of fish from each hatchery were measured to establish baseline fish fork length (cm), weight (g), BIA and indicators of toxicant exposure (AChE, Hg, cortisol, vitellogenin). Fish were stocked in the six stormwater ponds in early May of 2012 at a rate of 400 fish per pond with the exception of High River which received 2500 as part of the Alberta Conservation Association’s (ACA) stocking program. Stocking densities were kept low to minimize fish stress and health issues related to overcrowding such as high cortisol concentrations and reduced growth rates caused by insufficient fish food sources (Lawler 1974, Ellis et al. 2002).

Subsamples of stocked trout were collected in July, August, September and October from each stormwater pond. Fish were captured using ≤ 2 hour gill net sets between 5 AM and 12 PM. Fish were euthanized with clove oil (160 ppm, in ethanol) immediately after removal from gill nets. Fish were kept on ice during transportation to the laboratory. Gill net set times were limited to morning hours to standardize for diurnal fluctuations in hormone levels, particularly cortisol. Multimesh gill nets ranging from 25mm – 120mm were used in the first round of sampling (July) but since all fish were captured in the 51-57 mm mesh sizes during the first sampling event, in subsequent sampling events only 51-57 mm mesh sizes were used. No fish were captured in June using a boat electrofisher.

Fork length, weight and BIA were recorded in the field immediately after fish had been captured and euthanized. Bioelectrical impedance analysis procedures were conducted according to Cox and Hartman (2005). Resistance and reactance were measured in series, which were then used to calculate phase angle φ ($\tan \varphi = \chi c R^{-1}$), as a reliable indicator of acute nutritional stress and malnourishment (Rasmussen et al. 2012). Condition factor, K = (weight (g) x 100)/ fork length^3 (cm) as an indicator of overall fish body condition, and specific growth rate, SGR = 100 x ($\log_e$ final weight (g) – $\log_e$ initial weight (g))/days after stocking, as an indication of weight gain per a day, were calculated. Blood samples were taken from the caudal vasculature with 1 mL heparinized needles following BIA measurements. Plasma was separated at 13 000 rpm for five minutes and then frozen in liquid nitrogen. Muscle, brain and liver samples were frozen at -80ºC ≃23 hours after netting for later toxicant analysis.

2.2.4.1 Acetylcholinesterase (AChE)

There are two main types of cholinesterase enzymes: AChE and butyrylcholinesterase (BChE). Of the two enzymes, AChE is the predominant enzyme in the brain of teleost fish (Sturm et al. 2000). AChE activity was measured using a kinetic spectrophotometric assay (Quinn et al. 2010) with some modifications. Briefly, 16 µL of supernatant containing of 1 g of brain tissue to 3 g of phosphate buffer was diluted with 160 µL of phosphate buffer to create a brain homogenate solution. The brain homogenate solution (5µL) and 120 µL of Tris Buffer (total cholinesterase: Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE), Tris Buffered Saline Tablets, Sigma T-5030) were pipetted into a microplate. The samples were gently agitated and incubated at room temperature for 10 minutes. Twelve µL of both DTNB (Sigma 5,5' Dithiobis (2-nitro-benzoic acid), D8130) and Acethylthiochlorine Iodide (AChI, Sigma minimum 98% TLC, A5751) were added. This was followed by a second agitation and incubation period of 10 minutes at room temperature. Microplate absorbance was read every 2 minutes for 10 minutes at 405 nm. AChE activity for each sample was determined by comparison with the slope of the standard curve, with the intensity of the product color being proportionate to the enzyme activity in the sample. Reference material (in house rainbow trout reference material), sample triplicates and controls (0.5 U/mL and 1.0 U/mL of eel acetylcholinesterase, Sigma C3389) were used to ensure the accuracy and precision of the assay. Protein concentrations in brain samples were measured with the Bradford method

(Bradford 1976) to correct AChE measurements for variations in brain homogenate protein concentrations.

2.2.4.2 Mercury (Hg)

Oven dried (60°C for 72 hours) fish muscle tissue was analyzed for total mercury (THg) as a reliable indicator of Methyl mercury (MeHg) (Harris et al. 2003). Muscle samples (0.100g ± 0.005) were digested in 2 mL of concentrated trace metals grade HNO_3 inside a capped 50 mL borosilicate tube placed in a dry aluminum hot block at 75ºC for 16 hrs. After digestion, the samples were cooled and a 300 µL aliquot was pipetted into a 15 mL polypropylene centrifuge tube. A 10 mL, 200 ppb gold-chloride solution was added to the sample to stabilize the Hg in solution and to achieve the appropriate dilution for analysis by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) on an Elan DRC-E. The detection limit for this method was 0.009 ppm Hg in muscle tissue and was assured through duplicates, blanks and TORT-2 standards (National Research Council Canada). Measured THg concentrations in TORT-2 averaged 0.023 ± 0.04 SD mg/kg (n = 17) with 87 % ± 13 % SD of recovery. Duplicate samples differed by an average of 8% (n = 12).

2.2.4.3 Cortisol

Cortisol concentrations in plasma were determined with radioimmunoassay diagnostic kits (MP Biomedicals Diagnostics Division 07-221102) as described by Hontela et al. (1995). Plasma samples were collected from hatchery fish in May of 2012 prior to stocking and from rainbow trout captured in gill nets in Lethbridge 1, High River, Lacombe 1 and Lacombe 2 four months after stocking (August 2012).

2.2.4.4 Vitellogenin

Vitellogenin concentrations in plasma were determined with an enzyme-linked immunosorbent assay kit (Biosense Laboratories V01004402). Assay procedures were conducted according to the manufacturer's directions. Briefly, a blocking buffer provided by the manufacturer was used to create a 2 fold dilution series of vitellogenin standards (10 µg) from 0.390 ng/mL to 200 ng/mL. Blocking buffer was also added to samples for 50x and 100x dilutions. Samples and standards were plated in duplicate on 96 well microtiter plates precoated with a vitellogenin primary antibody. Plates were sealed and incubated at 4°C overnight. Following the initial incubation, plates were rinsed three times using 200 µL of washing buffer solution. Then, 100 µL of detecting antibody was added to each well prior to the plates being resealed and incubated on an orbital plate shaker at room temperature (20-25°C) for 1 hour. Plates were re-rinsed five times using 200 µL of washing buffer solution after which 100 µL of substrate solution was added to each well. Plates were resealed and incubated on an orbital plate shaker at room temperature for 1 hour in the dark. Absorbance values were read on a microtiter plate reader at 405 nm in the same order as the substrate solution was added. Vitellogenin concentrations in plasma samples were calculated based on the standard curve obtained from the rainbow trout vitellogenin standard provided by the manufacturer.

2.2.5 Statistical Analysis

All statistical analyses were performed using JMP (Version 10.0 Software Package, SAS Institute Inc 2012) and all graphs produced using R (R Development Core Team, 2012), Rstudio (Version 2.15.2) and the library ggplot2 (Wickham 2009). Benthic invertebrate biomass, zooplankton biomass, fish weights, phase angles, specific growth rates, condition factors (K), muscle Hg concentrations, brain AChE activities, plasma cortisol concentrations and plasma vitellogenin concentrations were compared between ponds using one-way analysis of variance (ANOVA) for single factor comparisons and a post-hoc Tukey-Kramer HSD to test for significant differences between the means of ponds. Regression analyses were used to investigate the relationships between AChE activity and time, muscle Hg concentration and time, and muscle Hg concentration and weight. All analyses used $\alpha = 0.05$, and data were log transformed or box cox transformed to achieve normality.

2.3 Results

2.3.1 Water Quality

Dissolved oxygen, temperature, pH and ammonia were the four parameters most often reaching critical levels for rainbow trout welfare in the six ponds (Table 3). Minimum DO concentration reached critical levels (<5.5 mg/L) in Lacombe 1, Lacombe 2, Lethbridge 1, and Lethbridge 3. Water temperature reached critical levels (>22°C) in High River, Lacombe 1, Lethbridge 1, Lethbridge 2 and Lethbridge 3. Maximum water pH reached critical levels (>9.0) in Lacombe 2, Lethbridge 1, Lethbridge 2 and Lethbridge 3. Maximum ammonia concentration reached critical levels (>0.02 mg/L) in Lacombe 1, Lacombe 2, Lethbridge 1, Lethbridge 2 and Lethbridge 3 (See Appendix I, Table 7 for monthly means).

Table 3: Water quality characteristics of stormwater ponds (May – Sept).[1]

		Temp (°C)	DO (mg/L)	Cond (µS/cm)	pH	NH_3 (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)	TN (mg/L)	TP (mg/L)	Secchi (m)
High River	**Mean**	18.0	8.3	324	8.5	0.012	0.008	0.003	0.33	0.02	3.7
	Min	13.9	6.7	287	8.4	0.005	0.003	0.003	0.28	0.01	2.3
	Max	**22.4**	10.6	374	8.7	0.020	0.023	0.003	0.39	0.02	5.1
	Std Dev	3.2	1.1	27	0.1	0.006	0.009	0.000	0.05	0.00	0.7
Lacombe 1	**Mean**	17.1	8.4	1286	8.8	**0.207**	0.030	0.001	0.89	0.05	0.9
	Min	10.6	**3.9**	944	8.5	0.013	0.003	0.001	0.30	0.01	**0.4**
	Max	**22.3**	12.6	1537	9.0	**0.960**	0.125	0.002	1.23	0.08	2.0
	Std Dev	4.5	3.0	211	0.2	0.421	0.053	0.001	0.37	0.03	0.5
Lacombe 2	**Mean**	18.8	7.9	880	8.8	**0.133**	0.063	0.005	1.43	0.08	0.9
	Min	12.0	**3.4**	716	8.5	0.027	0.003	0.001	1.16	0.04	**0.3**
	Max	21.7	14.4	953	**9.1**	**0.510**	0.260	0.011	1.63	0.10	1.8
	Std Dev	3.1	3.0	67	0.2	0.211	0.111	0.004	0.19	0.02	0.6
Lethbridge1	**Mean**	17.7	7.3	991	9.2	**0.230**	0.275	0.042	1.43	0.06	2.0
	Min	13.1	**4.4**	663	8.9	0.020	0.198	0.020	1.24	0.04	**0.5**
	Max	**23.2**	14.5	1251	**9.5**	**0.430**	0.463	0.092	1.94	0.08	2.9
	Std Dev	4.0	3.0	230	0.2	0.172	0.126	0.034	0.34	0.01	0.8
Lethbridge 2	**Mean**	17.9	8.9	1330	9.2	0.018	0.078	0.008	1.33	0.05	0.8
	Min	12.0	5.9	1188	8.7	0.015	0.003	0.001	1.06	0.04	**0.5**
	Max	**23.5**	13.8	1576	**9.7**	**0.023**	0.256	0.018	1.44	0.06	1.7
	Std Dev	4.1	2.2	113	0.3	0.004	0.121	0.008	0.18	0.01	0.4
Lethbridge 3	**Mean**	17.3	8.1	494	**9.1**	**0.062**	0.179	0.008	1.16	0.06	1.1
	Min	11.6	**4.3**	382	**9.0**	0.017	0.003	0.002	0.86	0.04	**0.4**
	Max	**23.3**	11.9	576	**9.3**	**0.222**	0.498	0.015	1.54	0.08	2.5
	Std Dev	4.4	2.0	60	0.1	0.090	0.211	0.005	0.25	0.02	0.7
	Threshold	<22.0	> 5.5	-	6.0<<9.0	< 0.020	-	< 0.100	-	-	0.5

[1] Water quality measurements (n=5) were collected from 1.0 m water depth from five locations within each pond. Spatially composite nutrient samples (n=5) were collected from 0.5 m to 1.0 m water depth. Bolded values indicate water quality measurements falling outside selection criteria guidelines. Cond – conductivity, TN – total nitrogen, TP – total phosphorus.

Water samples contained traces of several metals above Canadian Council of Ministers of the Environment (CCME) guidelines for the protection of aquatic life (Table 4). Mean concentrations of aluminum in water were above the CCME (1987) 100 µg/L guideline in Lacombe 1. Mean copper concentrations in water were above the CCME (1987) 2 µg/L guideline in Lethbridge 1 and Lethbridge 3. Mean (Hg) concentrations were above the CCME (2003) 0.026 µg/L guideline in High River and Lacombe 2. Mean selenium (Se) concentrations in water were above the CCME (1987) 1 µg/L guideline in Lethbridge 1, Lethbridge 2, and Lethbridge 3.

Table 4: Mean metal concentrations (µg/L ± SD).[2]

Metal (µg/L)	High River	Lacombe 1	Lacombe 2	Lethbridge 1	Lethbridge 2	Lethbridge 3	CCME
Aluminum	54 ± 70	**149 ± 228**	70 ± 75	19 ± 22	28 ± 39	80 ± 65	100
Arsenic	0.75 ± .06	1.09 ± 0.29	2.23 ± .75	1.19 ± 0.48	2.93 ± 1.53	2.89 ± 2.00	5
Cadmium	0.008 ± 0.005	0.017 ± 0.018	0.020 ± 0.016	0.007 ± 0.006	0.024 ± 0.028	0.010 ± 0.004	0.09
Copper	0.85 ± 0.29	1.21 ± 0.24	1.65 ± 0.44	**2.50 ± 0.58**	1.76 ± 0.71	**2.29 ± 0.63**	2
Iron	21.41 ± 13.07	126.88 ± 62.36	90.85 ± 34.29	138.65 ± 25.24	91.15 ± 122.70	139.67 ± 101.36	300
Lead	0.070 ± 0.055	0.169 ± 0.180	0.147 ± 0.094	0.088 ± 0.014	0.025 ± 0.032	0.097 ± 0.100	1
Mercury	**0.031 ± 0.037**	0.015 ± 0.005	**0.041 ± 0.051**	0.010 ± 0.00	0.010 ± 0.00	0.008 ± 0.003	0.026
Molybdenum	0.85 ± .06	1.23 ± 0.16	1.91 ± 0.39	1.17 ± 0.30	1.34 ± 0.06	1.34 ± 0.07	73
Nickel	0.26 ± 0.22	0.88 ± 0.66	2.55 ± 0.64	0.33 ± 0.43	0.72 ± 0.82	1.48 ± 0.57	25
Selenium	0.29 ± 0.16	0.57 ± 0.29	0.63 ± 0.26	**2.91 ± 0.93**	**9.85 ± 0.50**	**3.37 ± 0.95**	1
Silver	0.001 ± 0.001	0.003 ± 0.002	0.002 ± 0.001	0.003 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.1
Thallium	0.0044 ± 0.0052	0.0112 ± 0.0160	0.0057 ± 0.0065	0.0045 ± 0.0001	0.0045 ± 0.0001	0.0067 ± 0.0034	0.8
Zinc	2.80 ± 2.71	1.89 ± 0.47	5.60 ± 2.74	3.85 ± 1.20	2.00 ± 0.56	1.92 ± 1.05	30

[2] Spatially composite water samples (n=2) collected in May and July of 2012 from 0.5 to 1.0 m water depth. Bolded values indicate metal concentrations above CCME guidelines for the protection of aquatic life.

In all ponds, pesticide concentrations in water were below CCME (1999) guidelines for the protection of aquatic life, or below the lowest observed effect concentration (LOEC) for Clopyralid since CCME guidelines were not available for Clopyralid (Fairchild et al. 2009) (Table 5). CCME and LOEC values were not available for Mecoprop (MCPP). The phenoxy herbicides, 2,4-D and MCPP were detected in all of the study ponds while MCPA was detected in Lacombe 1 and Lacombe 2. The benzoic acid herbicide, Dicamba, was detected in all of the study ponds with the exception of High River. The picolinic acid herbicide, Clopyralid, was detected in Lacombe 2 and Lethbridge 3. Lacombe 2 contained the largest variety of pesticides while High River had the fewest. Lethbridge 1 had the highest concentration of 2,4 D. Lacombe 2 had the highest concentrations of Dicamba and MCPP. Lethbridge 3 had the highest concentration of Clopyralid while Lacombe 1 had the highest concentration of MCPA.

Table 5: Pesticide concentrations (µg/L).[3]

Pond	2,4D (µg/L)	Dicamba (µg/L)	Clopyralid (µg/L)	MCPA (µg/L)	MCPP (µg/L)
High River	0.037	0	0	0	0.013
Lacombe 1	0.086	0.01	0	0.01	0.056
Lacombe 2	0.135	0.081	0.042	0.009	0.195
Lethbridge 1	0.404	0.033	0	0	0.067
Lethbridge 2	0.238	0.04	0	0.005	0.067
Lethbridge 3	0.029	0.202	0.057	0	0.136
CCME	4	10	-	2.6	-
LOEC	-	-	18000	-	-

[3] Spatially composite water samples collected from inlet and outlet of stormwater ponds in August 2012 from 0.5 m water depth. Lowest observed effect concentration (LOEC) from Fairchild et al. (2009). Zero (0) indicates that the analyte was not detected. A total of 69 pesticides were tested for including but not limited to: Aldicarb, Chlorpyrifus(Dursban), Diazinon, Dimethoate (Cygon), Disulfoton (Di-Syston), Ethion,

Malathion, Methomyl, Parathion, Phorate (Thimet), Terbufos and Triallate (Avadex BW).

2.3.2 Invertebrates

Mean benthic invertebrate biomass ranged from 0.1 g/m^2 in Lethbridge 2 to 6.5 g/m^2 in Lacombe 1. Benthic biomass was highest in Lacombe 1 and Lethbridge 1, but not significantly different from High River since Lacombe 1 and Lethbridge 1 biomass were highly variable between months (ANOVA, $F_{4, 20} = 7.82$, $p < 0.001$). Lacombe 2 benthic biomass was intermediate and not significantly different from any of the other ponds while Lethbridge 2 had the lowest biomass, but was not significantly different from Lacombe 2.

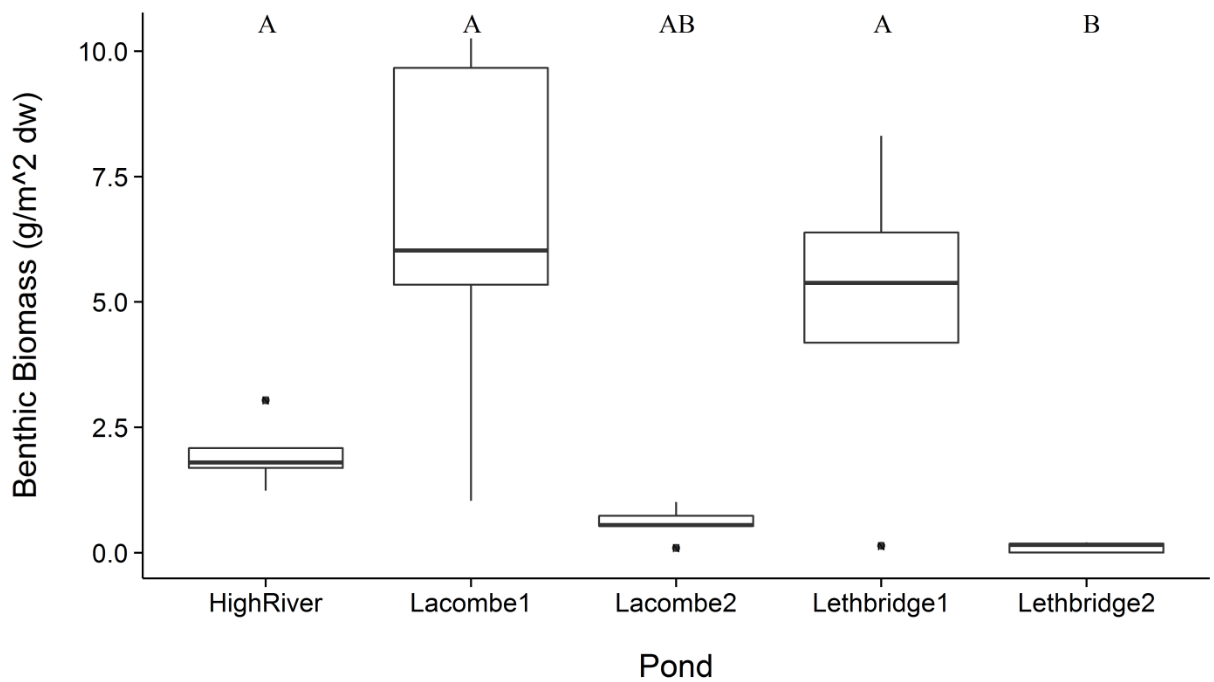

Figure 2: Mean benthic biomass (g/m²) collected from five locations within each stormwater pond (n_{lake}=5) (ANOVA, $F_{4, 20} = 7.82$, $p < 0.001$ and Tukey-Kramer HSD $\alpha = 0.5$). Capital letters represent significant differences between ponds. Whiskers represent 1.5 IQR of the upper and lower quartile, points represent outliers.

Mean zooplankton biomass ranged from 0.03 g/m^3 in Lethbridge 2 to 0.23 g/m^3 in Lacombe 2. Zooplankton biomass was significantly higher in Lacombe 2 and Lethbridge 1 than Lethbridge 2 and High River (ANOVA, $F_{4, 20} = 7.26$, $p < 0.001$). Lacombe 1 zooplankton biomass was intermediate, the most variable, and did not different from any of the ponds (Figure 3).

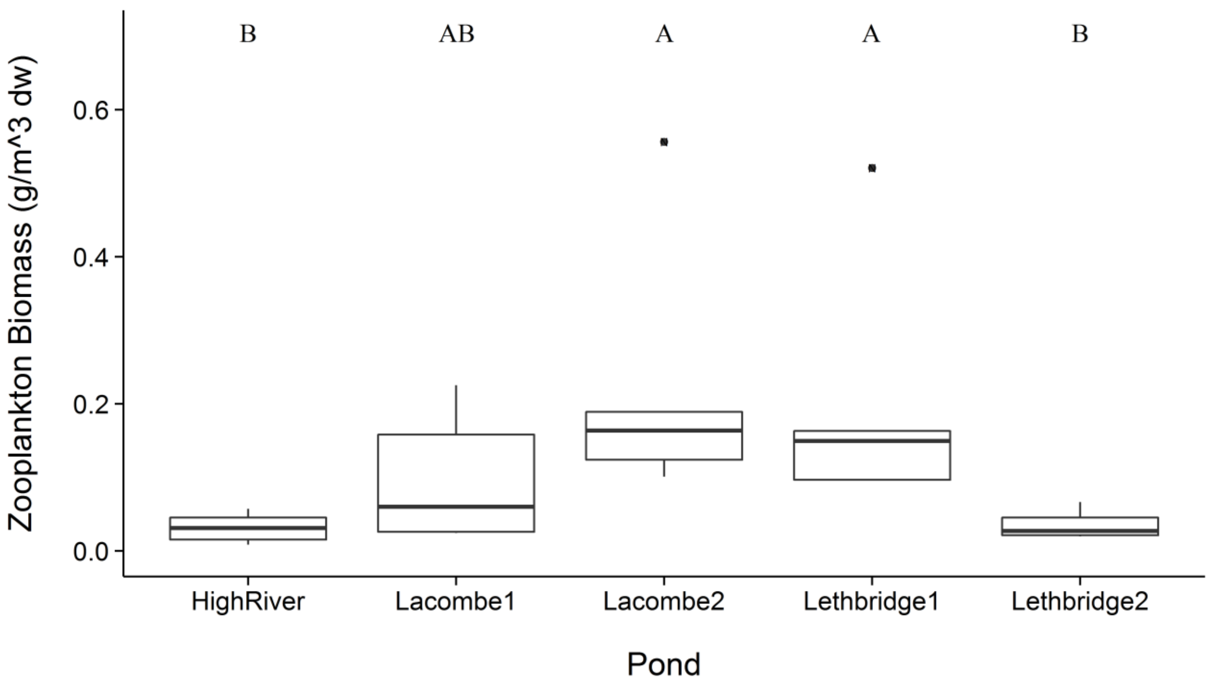

Figure 3: Mean zooplankton biomass (g/m^3) collected monthly from two locations within each stormwater pond (n_{lake}=5) (ANOVA, $F_{4, 20} = 7.26$, $p < 0.001$ and Tukey-Kramer HSD $\alpha = 0.5$). Capital letters represent significant differences between ponds. Whiskers represent 1.5 IQR of the upper and lower quartile, points represent outliers.

2.3.3 Fish

2.3.3.1 Growth and Condition

Fish weights were compared from the first round of sampling (July) since no rainbow trout were captured in Lethbridge 2 in subsequent sampling events. No rainbow trout were captured from Lethbridge 3 (Table 6) during any sampling events, but several large (>400g) northern pike (*Esox lucius)* were captured, thus Lethbridge 3 was excluded from the analysis. High River was also not included in the analysis since significantly heavier fish from a different hatchery were stocked in High River (t-test$_{30, 1}$ = 8.37, $p < 0.05$). Lethbridge 2 (111.1 ± 13.2 g) fish weighed less than Lethbridge 1(194.7 ± 12.6 g), Lacombe 1 (189.7 ± 16.5 g) and Lacombe 2 (173.3 ± 13.8 g) (ANOVA, $F_{36, 3} = 10.35$, $p < 0.001$) (Figure 4).

Table 6: Number of fish captured monthly (2012) from six stormwater ponds using gill nets.

Pond	July	August	September	October	Total
High River	11	10	3	2	26
Lacombe 1	7	8	2	1	18
Lacombe 2	10	14	1	3	28
Lethbridge 1	25	10	8	10	53
Lethbridge 2	12	0	0	0	12
Lethbridge3	0	0	0	0	0

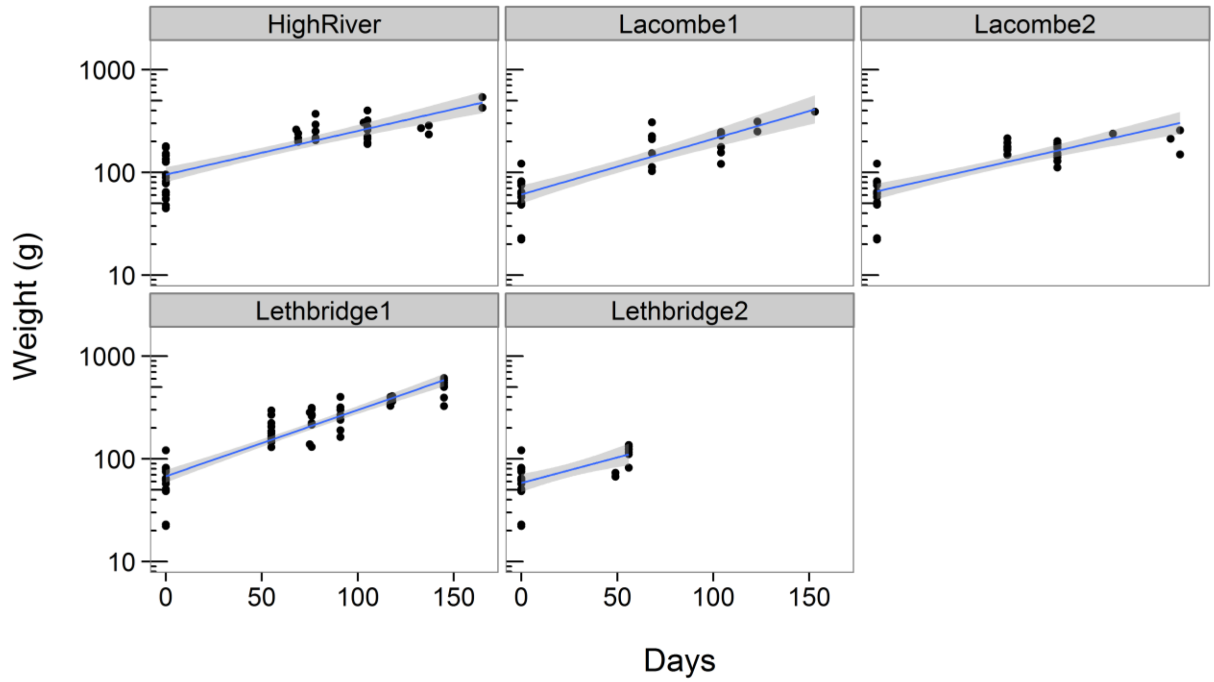

Figure 4: Weight (g) of rainbow trout reared in stormwater ponds from May to October, 2012. Each data point represents an individual fish and includes the line of best fit and 95% confidence intervals.

Mean specific growth rates of rainbow trout were lowest in High River (1.0 g/g*day) and highest in Lethbridge 1 (1.8 g/g*day). Fish from Lethbridge 1 had significantly higher specific growth rates than fish from any other pond. Fish in Lacombe 1, Lacombe 2 and Lethbridge 2 had similar growth rates while Lacombe 2, Lethbridge 2 and High River fish had similar, low, growth rates (ANOVA, $F_{131, 3} = 23.52$, $p < 0.001$) (Figure 5).

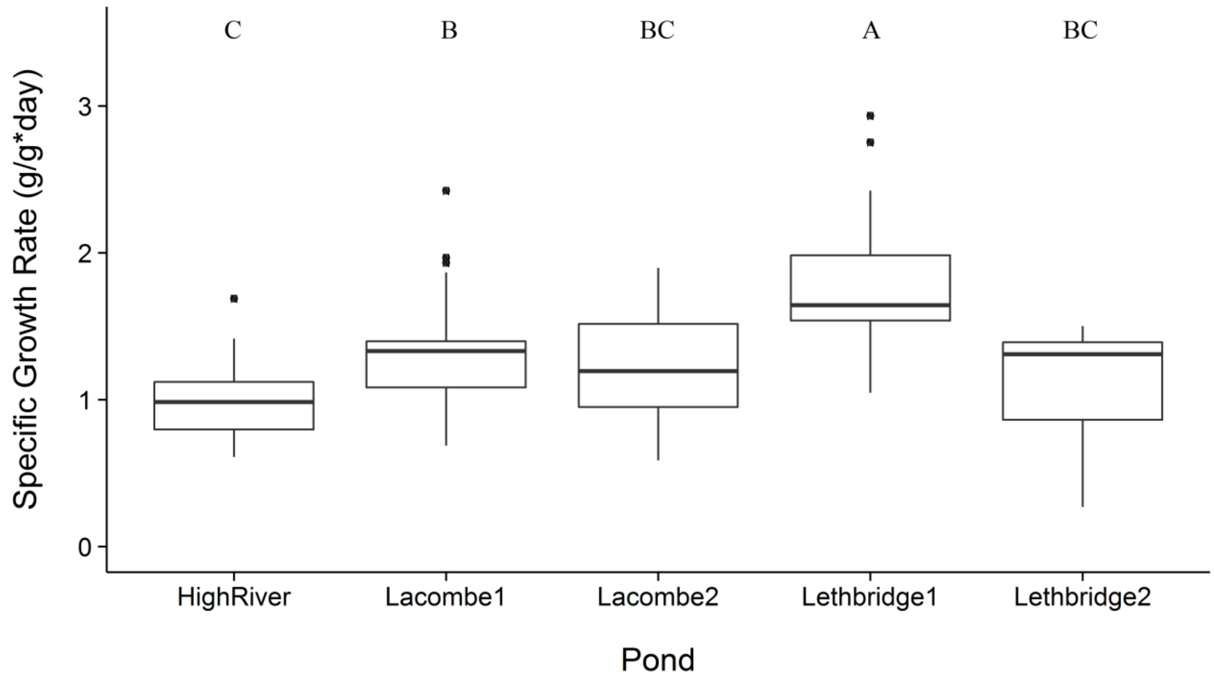

Figure 5: Specific growth rate (g/g*day) of rainbow trout reared in stormwater from May to October. Capital letters represent significant differences between ponds (ANOVA, $F_{131, 3} = 23.52$, $p < 0.001$ and Tukey-Kramer HSD $\alpha = 0.5$) (SGR = 100 x ($\log_e$ final weight – $\log_e$ mean initial weight)/days after stocking). Whiskers represent 1.5 IQR of the upper and lower quartile, points represent outliers. ($n_{High\ River} = 26$, $n_{Lacombe\ 1} = 18$, $n_{Lacombe\ 2} = 28$, $n_{Lethbridge\ 1} = 53$, $n_{Lethbridge\ 2} = 11$)

Lethbridge 1 fish had a significantly higher mean condition factor than all other fish and was also the most variable. Lacombe 1 and High River fish has similar condition factor while High River fish had similar condition factor as baseline fish. Baseline fish had similar condition factor as Lacombe 2 and Lethbridge 2 fish (ANOVA, $F_{161, 5} = 37.62$, $p < 0.001$) (Figure 6).

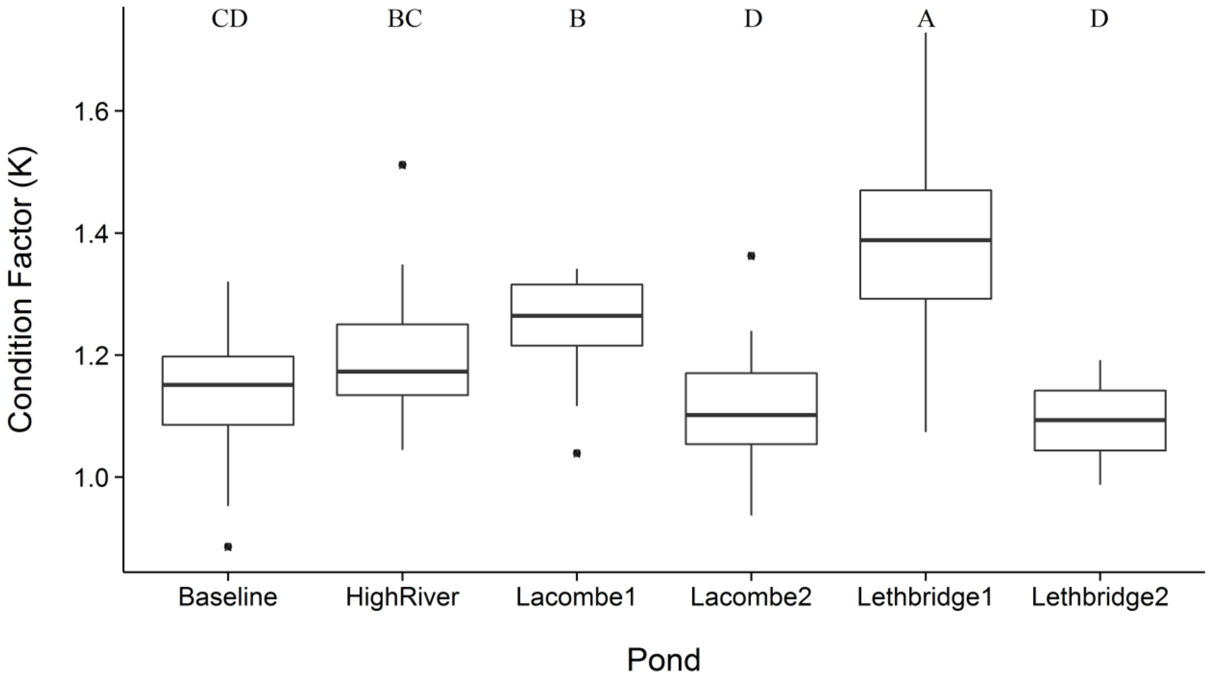

Figure 6: Condition factor of rainbow trout reared in stormwater from May to October, 2012. Capital letters represent significant differences between ponds (ANOVA, $F_{161, 5}$ = 37.62, $p < 0.001$ and Tukey-Kramer HSD $\alpha = 0.5$) (K = (weight x 100)/length^3). Whiskers represent 1.5 IQR of the upper and lower quartile, points represent outliers. ($n_{baseline} = 31$, $n_{High\ River} = 26$, $n_{Lacombe\ 1} = 18$, $n_{Lacombe\ 2} = 28$, $n_{Lethbridge\ 1} = 53$, $n_{Lethbridge\ 2} = 11$)

Bioelectrical Impedance Analysis was used to determine phase angle (tan φ) which was the highest in fish from High River, Lethbridge 1, and Lacombe 2. Lacombe 2 and Lacombe 1 fish showed no difference from each other. Lethbridge 2 fish had similar phase angle as Lacombe 1 fish, while baseline fish were lower than all other fish from other ponds (ANOVA, $F_{157, 5} = 76.06$, $p < 0.001$) (Figure 7).

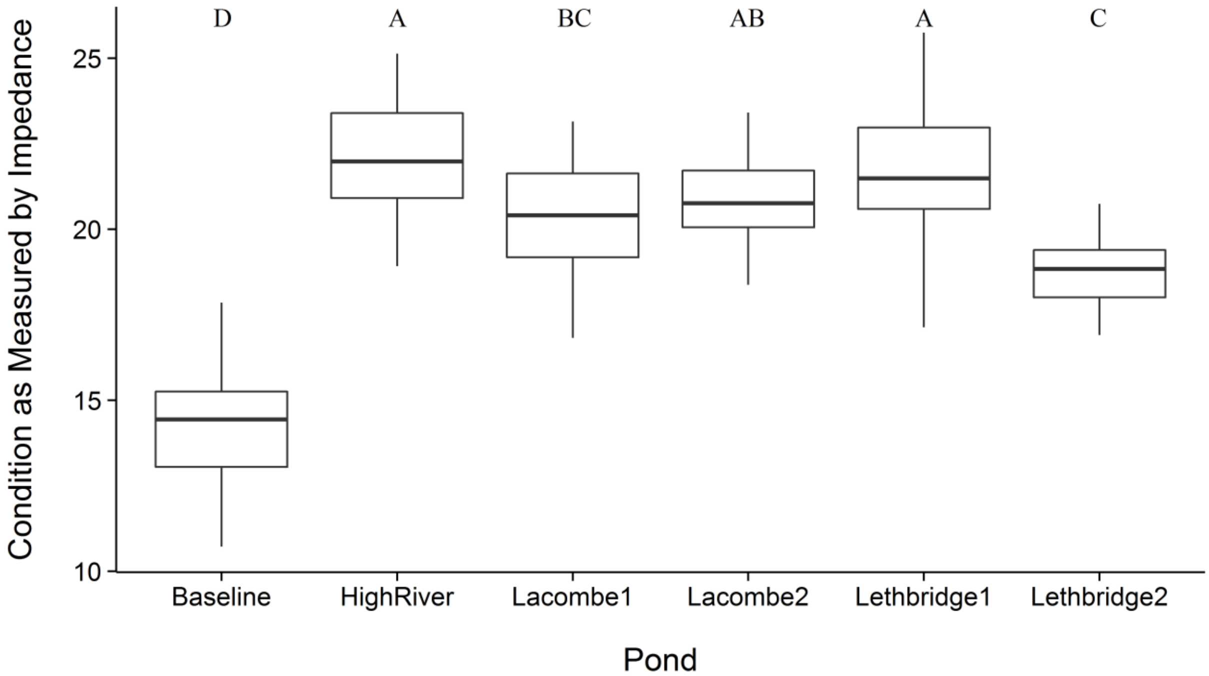

Figure 7: Condition as measured by impedance, (Phase Angle ϕ (arctan reactance / resistance * 180° / π)) of rainbow trout reared in stormwater from May to October, 2012. Capital letters represent significant differences between ponds (ANOVA, $F_{157, 5} = 76.06$, $p < 0.001$ and Tukey-Kramer HSD $\alpha = 0.5$) Whiskers represent 1.5 IQR of the upper and lower quartile, points represent outliers. ($n_{baseline} = 29$, $n_{High\ River} = 26$, $n_{Lacombe\ 1} = 18$, $n_{Lacombe\ 2} = 28$, $n_{Lethbridge\ 1} = 52$, $n_{Lethbridge\ 2} = 10$)

2.3.3.2 Fish population

In all stormwater ponds, with the exception of Lacombe 1, rainbow trout catch dropped to less than one fish per hour of gill-netting effort approximately 160 days after stocking. In Lacombe 1, rainbow catch per one hour of gill netting effort dropped to 2 fish in the same time frame (Figure 8). High River catch per unit effort decreased by 56% from August to October, while Lacombe 1 decreased by 98%, Lacombe 2 decreased by 99% and Lethbridge 1 decreased by 17%. No rainbow trout were captured in Lethbridge 2 after the first round of sampling (approximately 60 days after stocking) and no rainbow trout were ever captured in Lethbridge 3.

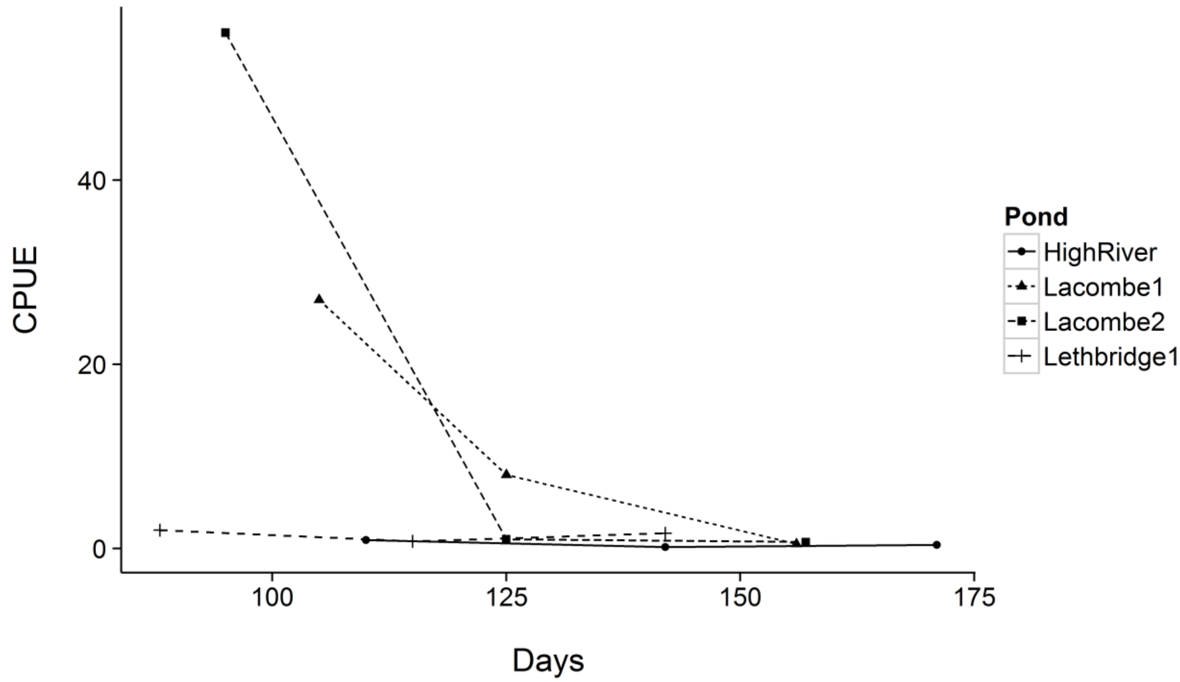

Figure 8: Rainbow trout catch per hour of gill netting effort in ponds stocked in May and sampled monthly from August to October 2012. Four hundred fish were stocked in Lacombe 1, Lacombe 2 and Lethbridge 1 and 2500 fish in High River.

2.3.3.3 Acetylcholinesterase

Mean brain AChE activity was similar in fish from Lacombe 1 (AChE = 776.3 ± 54.5 nmol/min/mg protein), baseline fish (AChE = 758.1 ± 37.9 nmol/min/mg protein), and Lethbridge 2 fish (AChE = 555.9 ± 46.9 nmol/min/mg protein). Fish from Lacombe 1, Lacombe 2 (AChE = 599.1 ± 52.5 nmol/min/mg protein) and Lethbrige 2 also had similar AChE activity. Lacombe 2, Lethbridge 2 and High River fish (AChE = 555.5 ± 47.2 nmol/min/mg protein) all had similar activity. Fish from Lethbridge 1 (AChE = 396.5 ± 22.0 nmol/min/mg protein) had lower AChE activity than fish from all other ponds. Baseline fish had higher AChE activity than Lacombe 1, Lacombe 2 and Lethbridge 2 fish (ANOVA, $F_{155, 5} = 16.74$, $p < 0.001$) (Figure 9). There was a significant

(Linear Regression $p < 0.05$) decrease in AChE activity over time in Lethbridge 1, Lethbridge 2 and Lacombe 2 fish, but not in High River and Lacombe 1 fish (see Appendix I, Table 9 for regression parameters).

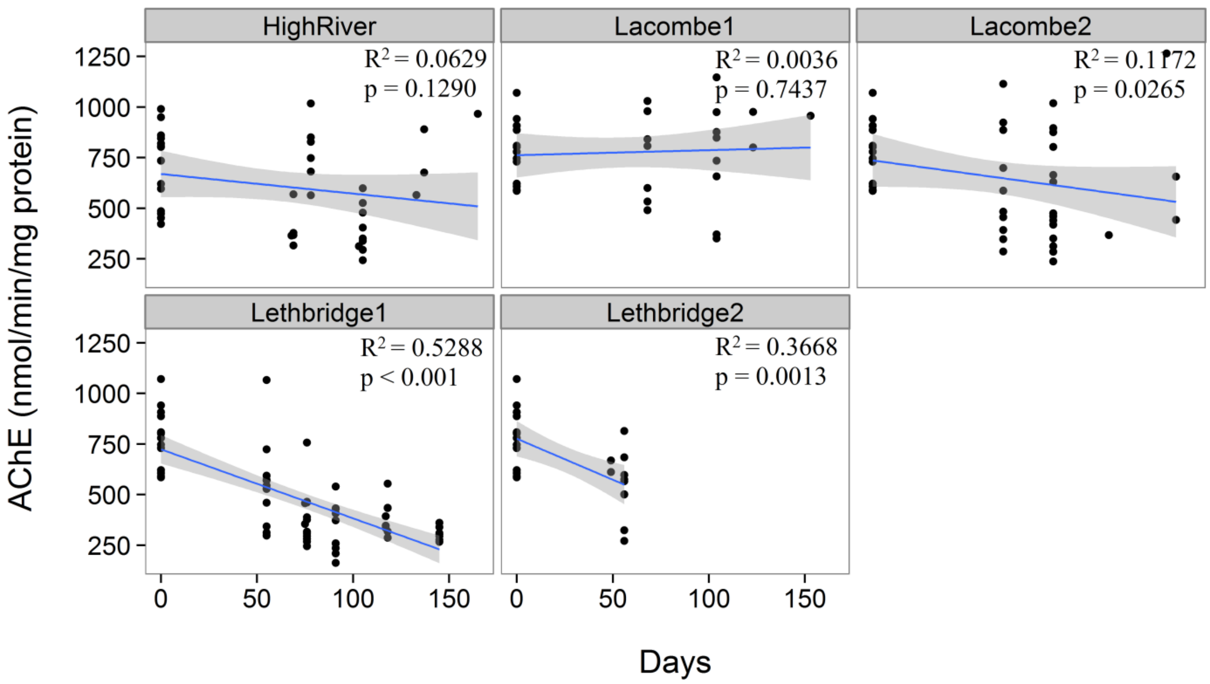

Figure 9: Brain acetylcholinesterase (nmol/min/mg protein) activity of rainbow trout reared in stormwater ponds from May to October, 2012. Each data point represents an individual fish and includes the line of best fit and 95% confidence intervals.

2.3.3.4 Mercury

Mean fish muscle Hg concentrations were highest in Lacombe 1 (Hg = 0.081 ± 0.009 mg/kg) and High River (Hg = 0.0615 ± 0.005 mg/kg) (ANOVA, $F_{161,\,5} = 57.29$, $p < 0.001$). High River and Lacombe 2 (Hg = 0.0493 ± 0.005 mg/kg) fish had similar Hg concentrations while Lacombe 1 fish had higher Hg concentrations than Lacombe 2 fish. Lethbridge 1 fish (Hg = 0.0270 ± 0.001 mg/kg) had lower Hg concentrations than

Lacombe 2 fish, but Lethbridge 1 fish had higher Hg concentrations than baseline fish (Hg = 0.0223 ± 0.004 mg/kg). Lethbridge 2 fish were exposed to stormwater for a shorter duration of time (Hg = 0.008 ± 0.001 mg/kg) and had lower Hg concentrations than baseline fish and all other ponds. There were significant positive relationships (Linear Regression, $p < 0.01$) between rainbow trout muscle Hg concentration and rainbow trout body weight, and rainbow trout muscle Hg concentration and exposure duration in all ponds except Lethbridge 2 (Figure 10 and Figure 11) (see Appendix I, Table 9 for regression parameters). When Hg concentration was standardized for a targetable sized rainbow trout weight (250g), Lacombe 1, Lacombe 2, baseline and High River fish had significantly more Hg (mg/kg) than Lethbridge 1 and Lethbridge 2 (ANOVA, $F_{160, 5} = 32.68$, $p < 0.001$) (Figure 12).

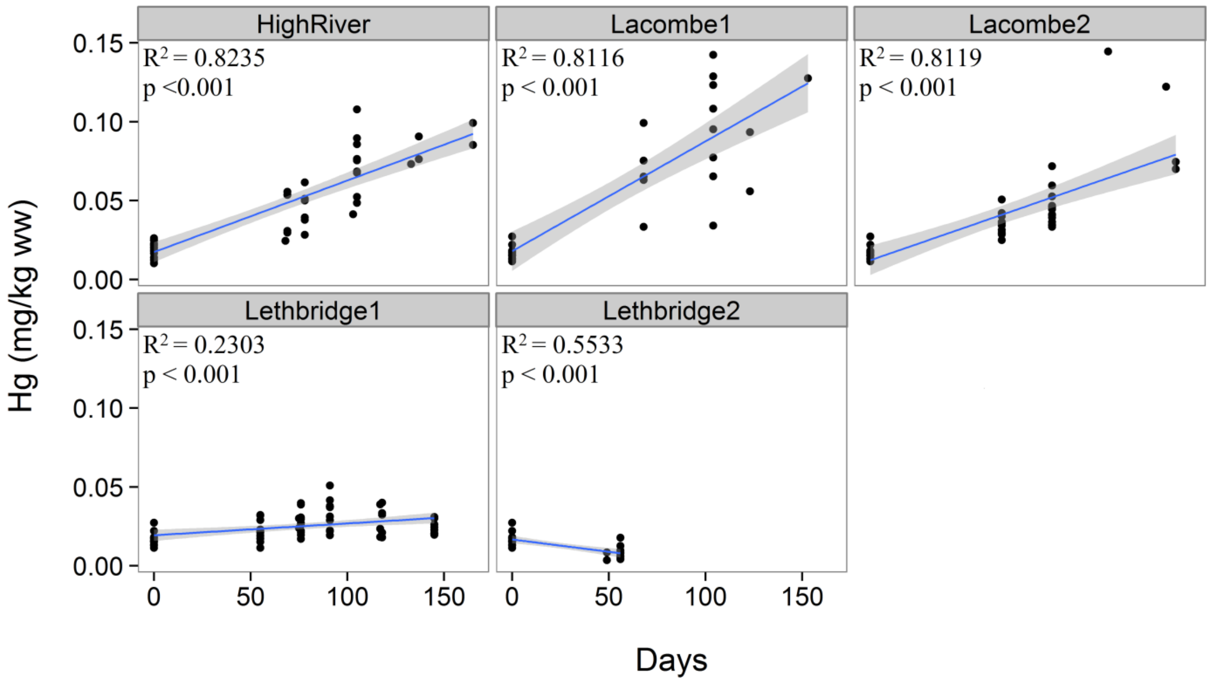

Figure 10: Muscle mercury concentrations (mg/kg wet weight) of rainbow trout stocked in early May and sampled from July to October, 2012. Each data point represents an individual fish and includes the line of best fit and 95% confidence intervals.

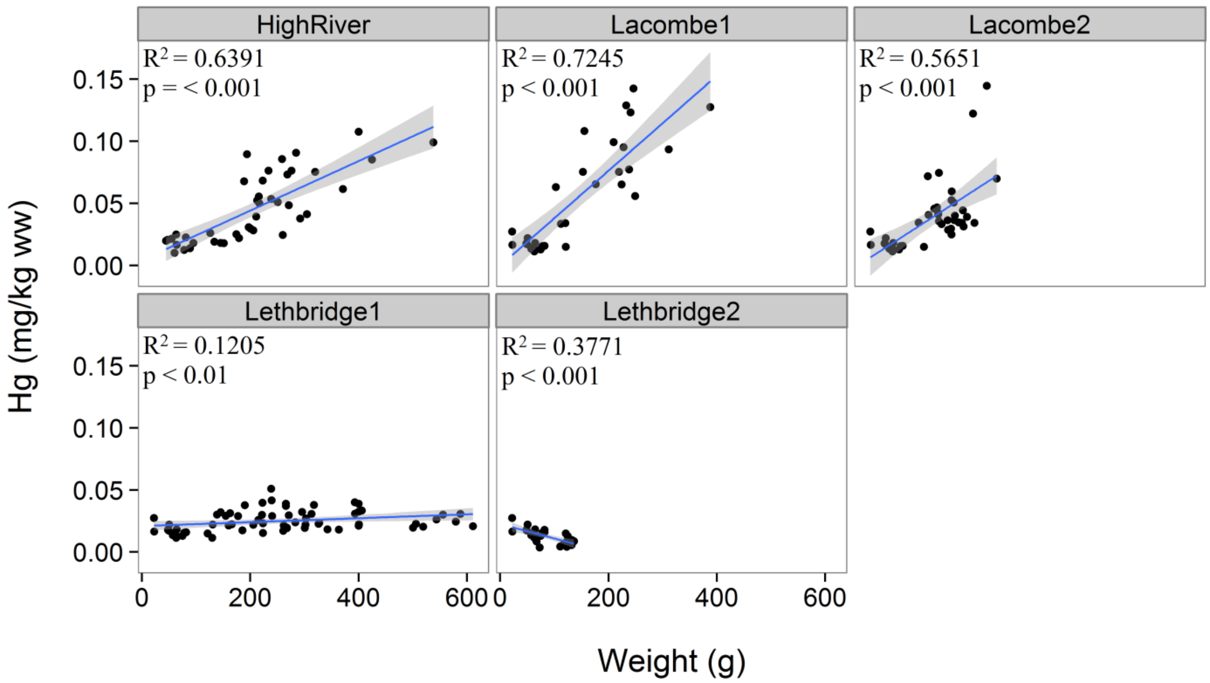

Figure 11: Muscle mercury concentrations (mg/kg wet weight) of rainbow trout in relation to body weight (g). Each data point represents an individual fish and includes the line of best fit and 95% confidence intervals.

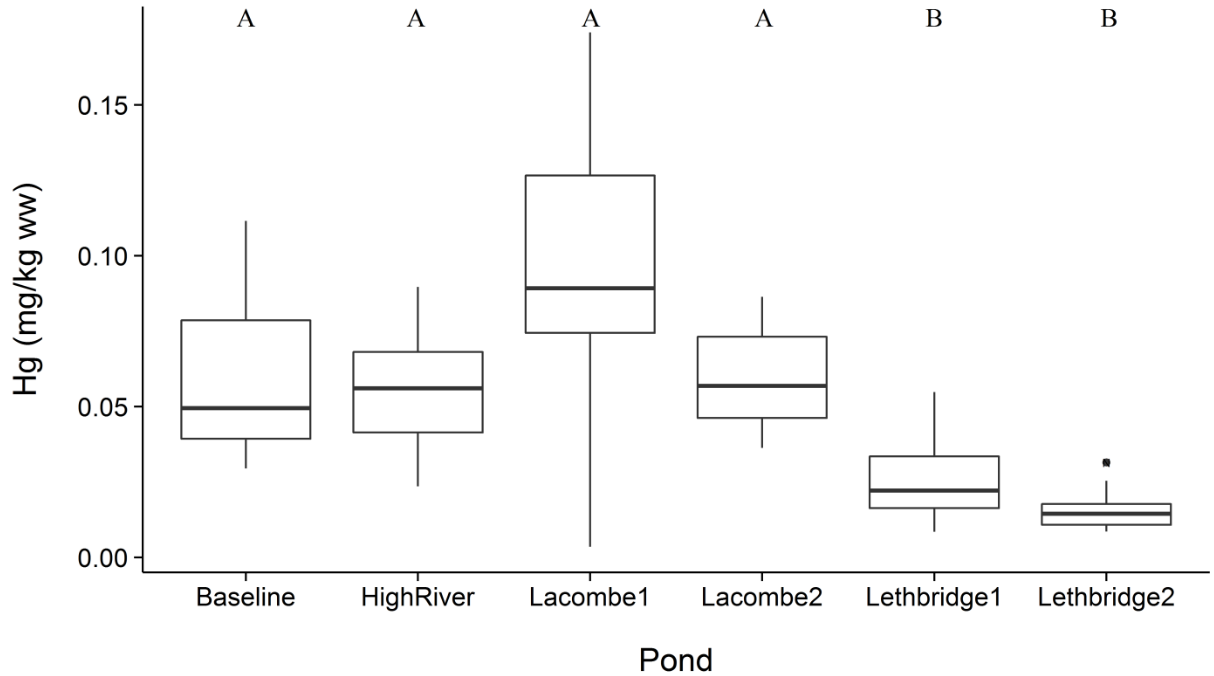

Figure 12: Muscle mercury concentrations (mg/kg wet weight) of rainbow trout standardized for 250g body weight. Capital letters represent significant differences between ponds (ANOVA, $F_{160, 5} = 32.68$, $p < 0.001$). Whiskers represent 1.5 IQR of the upper and lower quartile, points represent outliers. ($n_{baseline} = 30$, $n_{High\ River} = 26$, $n_{Lacombe\ 1} = 18$, $n_{Lacombe\ 2} = 28$, $n_{Lethbridge\ 1} = 53$, $n_{Lethbridge\ 2} = 11$)

2.3.3.5 Cortisol

Mean plasma cortisol concentrations were not significantly different between baseline, High River, Lacombe 1, Lacombe 2 and Lethbridge 1 fish (ANOVA, $F_{57, 4} = 39.15$, $p = 0.37$) (Figure 13). Mean cortisol concentration of all ponds was 83 ±11 S.D. ng/ml plasma.

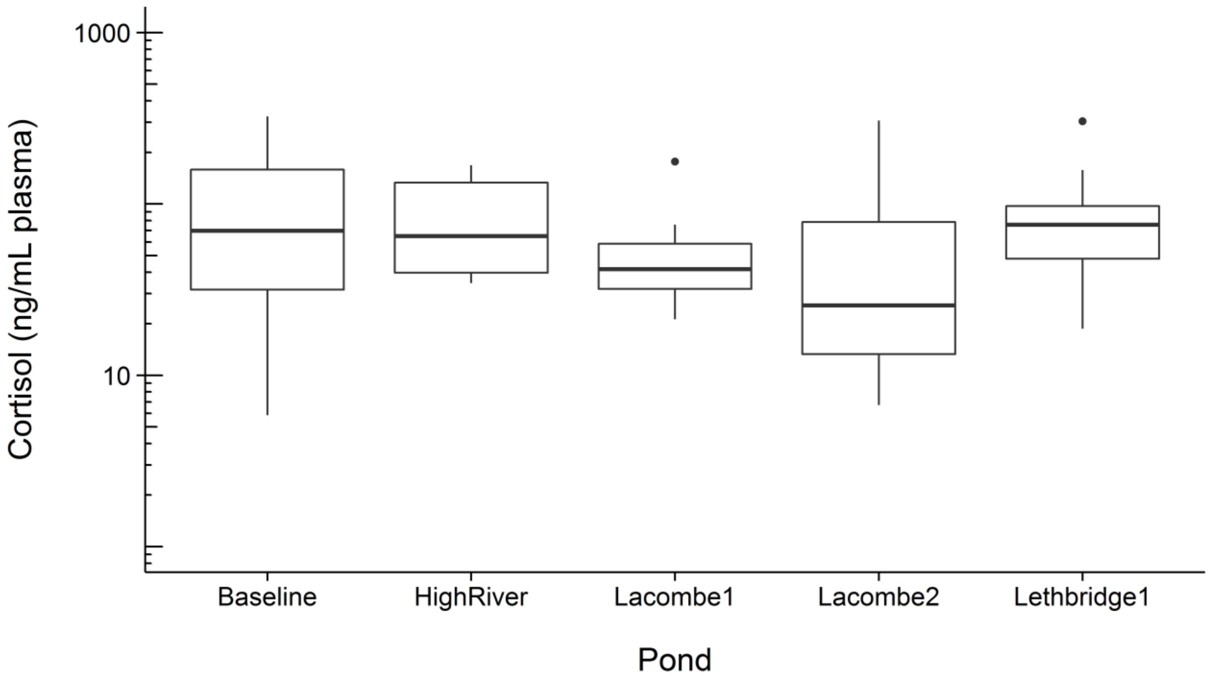

Figure 13: Plasma cortisol concentrations (ng/mL plasma) of rainbow trout stocked in stormwater ponds in May and netted in August, 2012 between 6 am and 12 pm; (ANOVA, $F_{57,4} = 39.15$, $p = 0.37$). Whiskers represent 1.5 IQR of the upper and lower quartile, points represent outliers. ($n_{baseline} = 24$, $n_{High\ River} = 8$, $n_{Lacombe\ 1} = 8$, $n_{Lacombe\ 2} = 13$, $n_{Lethbridge\ 1} = 9$)

2.3.3.6 Vitellogenin

Two distinct, separate, groups of plasma vitellogenin concentrations were detected in fish from each pond, with the exceptions of Lacombe 1 and baseline fish, that correspond to high vitellogenin concentrations (>15 µg/mL plasma) and low vitellogenin concentrations (<10 µg/mL plasma) (Figure 14). In both the Lacombe 1 fish and baseline fish, only the low vitellogenin concentration group was detected.

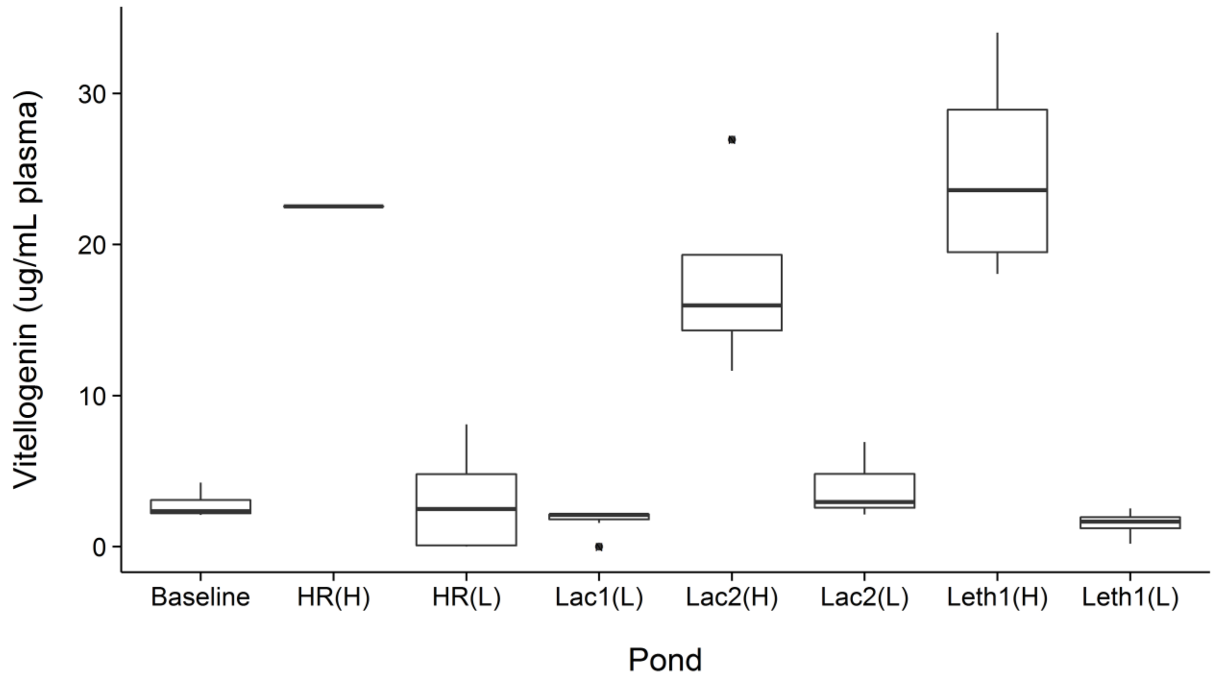

Figure 14: Plasma vitellogenin concentrations (µg/mL) of rainbow trout captured in August, 2012 and separated into high (H) and low (L) vitellogenin concentration groups. Whiskers represent 1.5 IQR of the upper and lower quartile, points represent outliers. ($n_{baseline}$ = 29, $n_{High\ River}$ = 8, $n_{Lacombe\ 1}$ = 7, $n_{Lacombe\ 2}$ = 12, $n_{Lethbridge\ 1}$ = 8)

2.4 Discussion

Rainbow trout stocked in stormwater ponds had low survival, despite our selection of ponds with the most suitable water quality and habitat for rainbow trout welfare. Low catch returns were recorded in all six stormwater ponds with no fish captured in 2/6 of the ponds by midsummer (Table 6). Thus, only 4 of the original 31 stormwater ponds surveyed (13%) had suitable water quality and habitat for rainbow trout survival over one open water season. Low fish survival can be attributed primarily to poor water quality but may have also been influenced by predation (including angler induced mortality) and interspecific competition.

Poor water quality resulted in one complete and three partial summer kills in the stocked stormwater ponds. High water temperatures, low DO concentrations, high pHs and high un-ionised ammonia concentrations were the primary water quality parameters reaching critical levels. The water quality of the stormwater ponds was similar to the water quality of stocked prairie pothole lakes of central Canada where summerkills are common (Lawler et al. 1974, Barica 1975). Consistent with Ayles et al. (1976), we detected water temperatures, DOs, pHs, and ammonia concentrations that would not be lethal on an individual basis, but together, would significantly reduce fish survival. Periods of poor water quality conditions occurred in July and August and resulted in fish death and subsequently low catch returns in September and October. Surviving fish exhibited compensatory growth (Quinton and Blake 1990) and grew to a targetable size of > 250g over the course of one open water season. Lethbridge 1 fish showed exceptional growth and grew to > 500 g over a six month period.

Selenium contamination was another water quality parameter that negatively impacted fish health in this study. The mean Se concentration detected in Lethbridge 2 water samples was almost ten times the CCME (1987) guideline for the protection of aquatic life. High selenium concentrations in water impair zooplankton and invertebrate communities (Swift 2002) which in turn influences fish growth and body condition. In Lethbridge 2, fish food sources were scarce and this could be due to selenium contamination (deBruyn and Chapman 2007). Fish catch per unit of gill netting effort in Lethbridge 2, during the first month of sampling, was low and fish exhibited poor body condition. Furthermore, no fish were captured in subsequent sampling events. Selenium is likely being leached from rocks used to armor the pond’s banks and littoral zone.

Shoreline armament is also discouraging macrophyte growth and limiting invertebrate habitat, further reducing fish food sources. Malnourished fish are less suited to withstand environmental stress (Koehn and Bayne 1989) and fish in Lethbridge 2 likely died from not being able to withstand poor water quality conditions due to being underfed.

Predation on rainbow trout by northern reduced stocked rainbow trout populations in two ponds and resulted in low rainbow trout catch returns. No trout were captured in Lethbridge 3 and can be attributed almost exclusively to predation by pike, of which, more than ten (>350 mm/~400g) were caught. Similarly, in High River, rainbow trout returns were low and several of the trout captured in gill nets had large lacerations that were likely caused by pike. Three larger pike were caught in that lake (>550 mm/>600g). Predation is one of the greatest sources of mortality to stocked salmonids (Kerr and Lasenby 2000) and pike are common predators that have the potential to negatively inpact stocking success in small, shallow lakes.

In addition to predation by pike, angler induced mortality of rainbow trout could explain the subsequent reduction in gill net catches of rainbow trout. Frequent angler presence on all of the stormwater ponds indicates high fishing pressure and, even if the fish were released by anglers, post hooking mortalities would likely be high (Schisler and Bergersen 1996). Where anglers have easy access to fishable water, they can greatly contribute to fish mortality (Gunn and Sein 2000, Kaufman et al. 2009) and access to stocked stormwater ponds was unlimited.

Interspecific competition with suckers and minnows for resources can impair rainbow trout growth (Barton and Bidgood 1979) and stocking success is negatively correlated with sucker presence (Kerr and Lasenby 2000). Minnows and suckers reduce the size and quantity of food sources present in aquatic systems (Tremblay and Magnan 1991, Hanson and Riggs 1995). Long nose suckers, white suckers and minnows were abundant in 3 of the 6 stocked stormwater ponds. The lowest combined fish food biomass (zooplankton and benthic invertebrates) and mean rainbow trout standard growth rates were recorded in the three ponds where suckers were present. Furthermore, with a higher number of fish per system, rainbow trout would have to expend more energy to acquire the same amount of food sources, which would reduce growth potential. In addition to competition for resources with stocked species, suckers are bottom feeders that disturb sediments and consequently resuspend potentially harmful pollutants, such as metals, previously buried. The resuspension of pollutants can subsequently increase the risk of contaminant exposure for both rainbow trout and invertebrate food sources. Thus, the presence of fish species other than rainbow trout in stormwater ponds will likely reduced rainbow trout growth and survival.

Our growth and survival results indicate that few stormwater ponds of form viable recreational fisheries since the majority of stormwater ponds have a high potential to summerkill. However, in ponds where fish can survive, rainbow trout can be grown to a targetable size in one open water season. Pond selection criteria should be further restricted and additional measures added to the selection process (Chapter 3) in order to reduce fish mortalities. Increasing minimum water depth requirements would be the most beneficial improvement for selecting successful stormwater fisheries. Increased water depth would help improve water temperatures and ammonia concentrations. Furthermore, including a gill netting component to the initial pond selection process would help ensure no other fish species are present to compete with or prey on stocked rainbow trout. In addition to amending pond selection criteria, installing oxygen diffusors into ponds that previously had no aeration would improve DO levels and replacing existing water fountains with oxygen diffusors would reduce water temperatures.

Analysis of water quality and fish tissues indicated that fish from this study had minimal exposure to pesticides, Hg, general pollutants and estrogen mimicking compounds. Acetylcholinesterase inhibiting pesticides were not detected in any of the water samples. However, AChE levels decreased in fish from three ponds and were likely from exposure to pesticides. Given that carbamate and organophosphate pesticides are rapidly broken down in freshwater compared to other forms of pesticides (Bondarenko et al. 2004) it is likely that carbamates and organophosphates were not detected by the one-time water sampling event. Other studies have reported that AChE activity decreases allometrically with fish size (Zinkl et al. 1987, Sturm et al. 1999, Beauvais et al. 2002), however, decreases in AChE acitivty did not appear to be a simple function of growth in this study and are more likely related to exposure to pesticides.

In all six stormwater ponds, Hg concentrations in fish muscle tissue were below Health Canada guidelines (0.50 mg/kg) despite Hg concentrations in water being above CCME guidelines in High River and Lacombe 2. High growth efficiency can dilute Hg contamination (Trudel and Rasmussen 2006) and, in nutrient rich systems such as stormwater ponds, high growth dilution is possible. Lethbridge 1 fish exhibited the fastest growth and the second lowest Hg concentrations in muscle tissue. However, it was fish from Lethbridge 2 that had the lowest Hg concentrations despite fish having only low to moderate growth rates. Fish from both Lethbridge 1 and Lethbridge 2 likely had some protection from Hg contamination through exposure to high concentrations of Se (Cuvin-

Aralar and Furness 1991). Regardless of mechanism, Hg concentrations in fish muscle tissues were well below (approximately 2.5x below) consumption guidelines.

High plasma cortisol concentrations indicated high stress levels in fish from all ponds, including hatchery fish. Detection of high cortisol concentrations in fish were to be expected since all fish were captured using gill nets except for baseline fish, which were corralled and then captured with dip nets. Observed cortisol concentrations were similar to other studies in which fish were experiencing handling stress (Barton and Peter 1982). The high cortisol concentrations in rainbow trout suggest that fish still had functioning stress axes that were capable of mounting a stress response and that their stress axes were not compromised from chronic exposure to environmental stressors (Hontela et al. 1992,Wagner et al. 1997, Miller et al. 2007).

Two distinct groups of plasma vitellogenin values were observed and placed into two categories: high fish (>15000 ng/ml plasma) and low fish (<10000 ng/ml plasma) and are considered putative female and male fish in this study. We were unable to validate fish sex using gonadal histology since all fish were triploid and immature. However, the high and low groups had similar values to baseline triploid female and triploid male rainbow trout, from other studies (Schafhauser-Smith and Benfey 2001). In each pond, with the exception of Lacombe 1, both the high and low vitellogenin concentration groups were observed. In Lacombe 1, only the low vitellogenin concentration group was observed, but a plausible explanation for this is the low sample size (n~8). The low vitellogenin groups did not differ from sexually immature hatchery trout (mean weight = 58.9 ± 22.1 g) where females would not be expected to be producing sex hormones. There is considerable variability in vitellogenin concentrations in the literature since water temperature and sexual maturity of fish influences vitellogenesis, making it difficult to draw strong conclusions (Copeland et al. 1986, Purdom et al. 1994, Jobling et al. 1996), but there was no evidence of feminiziation of male fish in this study.

Analysis of rainbow trout toxicant exposure suggests that rainbow trout were exposed to very low levels of toxicants. Our results contrast with previous studies that found fish in stormwater ponds in Orlando, Florida had elevated concentrations of metals in their muscle tissues (Campbell 1994). The contrasting results of our study and Campbell (1994) suggest that toxicant loading is variable between ponds and geographic location. Thus, caution should be used when applying overarching themes to these unique stormwater pond systems in which watershed pollutant loading can vary.

Our study did not find anything to suggest that stormwater ponds should not be used as recreational fisheries. However, the sustainability of such fisheries can be compromised by summerkill. Furthermore, other toxicants not analysed in this study such as PAH's which are known carcinogens need to investigated (Hwang and Foster 2006). Based on our findings, harmful exposure to contaminants from stormwater fisheries would be low for humans, especially since very few, if any, fish would survive more than one year. Slight alterations to existing and future stormwater ponds would make them better suited for rainbow trout stocking. At the present time, existing stormwater ponds may be better suited for other types of fish species or strains of rainbow trout that are more tolerant to low DO levels and warm water temperatures. If summerkill in stormwater ponds could be removed, stormwater fisheries could be highly successful in areas with limited fishing venues.

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Chapter Three: Recommendations for Future Stormwater Stocking Programs

3.1 Construction Considerations

Several of the water quality and fish habitat issues that were identified during our field study could be proactively addressed during stormwater pond construction. If biologist were consulted by developers and municipalities during the initial stormwater pond design, construction goals could be amended so that stormwater ponds could be built to better suit the water quality and habitat needs of desired fish species (rainbow trout in this case) while maintaining the principal function of ameliorating the quality of water entering nearby rivers and streams.

During stormwater pond design, primary consideration should be given to creating ponds with greater water depth since most of the ponds surveyed in this study failed as viable fisheries due to insufficient water depth. By building deeper ponds, cold-water trout refuges would be created and macrophyte growth reduced. Even though building deeper ponds would increase development costs, deeper ponds are more likely to support recreational fishing and are more aesthetically pleasing (less odour and visible 'green scum'), which may improve adjacent property values and help offset development costs. Existing stormwater ponds could be dug deeper when sediments are dredged to remove potential pollutants. Increasing water depth in stormwater ponds is an easy, attainable and relatively cheap solution that would increase the number of stormwater ponds capable of rearing rainbow trout.

Some stormwater ponds designed to have greater water depth would have to be supplemented with additional water to maintain water levels. Irrigation water could be used to maintain water levels in stormwater ponds, but there is some uncertainty associated with the availability of irrigation water in dry years. In some municipalities, tap water is being used to maintain water levels, which is an impractical, costly option. In our study, northern pike (*Esox lucius)* and suckers entered stormwater ponds through irrigation canals. The pike and suckers found in the stormwater ponds were likely able to fit through the fish screens installed on the stormwater pond inlets and outlets as juvenile fish. Reducing fish screen mesh sizes on stormwater ponds was not an option in this study since large mesh sizes must be used to ensure water can flow in and out of stormwater ponds unimpeded. One solution is to reduce the water depth in stormwater ponds prior to freeze-up to ensure all unwanted fish species and stocked species winterkill. Winterkill would reduce interspecific competition and predation on rainbow trout and also limit the duration rainbow trout are exposed to stormwater.

Aeration systems are often already part of the initial pond design since aeration discourages algal growth and foul odours. In most municipalities, water fountains are the primary form of aeration used in stormwater ponds. However, fountain aeration systems are likely detrimental to fish health. Fountain aeration can increase water temperatures by exposing cool water to warmer air temperatures, decreasing the DO capacity of water. In stormwater ponds where rainbow trout are stocked, fountain aeration systems should be used with caution since the tolerance of salmonids to low DO levels is greatly reduced by high water temperatures and the presence of ammonia in small concentrations (Gibson and Fry 1954, Merkens and Downing 1957). Thus, to avoid increasing water temperatures, water fountains should only be used in stormwater ponds at night when air temperatures are coolest. Alternatively, to increase DO levels, oxygen diffusers that continuously bubble oxygen through the water column could be used in stormwater ponds.

In addition to pond design considerations, the materials used for stormwater pond construction should be tested for pollutants prior to their implementation. Some stormwater ponds were contaminated with selenium (Se) which likely came from construction materials or supplemental water sources. Although it was not confirmed, it is probable that some of the ponds in this study had bank armor made of rock from the Crowsnest Pass/ Elk Valley region where coal mining is prevalent and high concentrations of Se are common (Lussier et al. 2003). Another potential source of the high Se concentrations detected in stormwater ponds is from agricultural drain water (Miller et al. 2009). Supplemental water sources and construction materials used in stormwater ponds should be given careful consideration to ensure they are free of potentially harmful pollutants.

3.2 Management Considerations

In addition to building “fish friendly” stormwater ponds, changes to the pond selection process and stocking program would improve fish survival. Several stormwater selection criteria were revised to reduce the risk of summerkill in stormwater ponds including increased water depth, increased DO concentration and reduced water temperature. Furthermore, additional requirements were added to the pond selection process, including gill netting, to identify ponds with fish species that would compete with and prey on rainbow trout (Figure 15). In addition to stricter selection criteria, data loggers should be installed into each stormwater pond for the entire open water season to measure hourly DO, water temperature, pH and, if possible, ammonia since these water quality parameters can fluctuate over a short period of time, can have an interactive effect and can severely influence fish health. For example, high temperature, low DO and high pH will all increase ammonia toxicity (Thurston et al. 1981a, Thurston et al. 1981b). Peaks in ammonia levels may be missed if water sampling does not occur frequently since ammonia toxicity can be temporally separated from macrophyte decomposition and last for as little as a few days, (Farnsworth-Lee and Baker 2000). Similarly, critical DO levels may last for as little as a week and be missed if water quality measurements are not recorded frequently (Burton and McGinn 2008). Several water quality measurements were suboptimal, but each water quality parameter was assessed individually and therefore not initially considered lethal to fish. However, when the water quality parameters were evaluated collectively, they indicated low fish survival and should therefore be monitored more closely.

Physical Characteristics

- Minimum Mean Water Depth = 2.5 m
- Minimum Dissolved Oxygen = 6.5 mg/l
- Maximum Water Temperature = 22°C

Biological Parameters

- Absence of Predators (Northern Pike) and Competitive Species (Suckers)
- Absence of Excessive Macrophyte Growth (Choked Ponds)
- Presence of Sufficient Zooplankton and Benthic Invertebrates

Chemical Characteristics

- Maximum Ammonia Concentration = 0.020 mg/L
- Maximum pH = 9.0
- Mean Metals and Metalloids Concentrations = Below CCME Guidelines
- Carbamate and Organophosphate Pesticides = Below CCME Guidelines

Pond Design Considerations

- Presence of Gently Sloped Banks
- Presence of Fish Escapement Barriers
- Presence of Public Access

Figure 15: Stormwater selection criteria for rainbow trout stocking. Candidate ponds must meet all of the above criteria to be considered a potential stocking location

Stocking different species of fish and strains of rainbow trout into stormwater ponds should also be investigated to further evaluate the potential of stormwater ponds as recreational fisheries. Species such as northern pike and yellow perch (*Perca flavescens*) would be better adapted to withstand the high summer water temperatures and low DO concentrations of stormwater ponds (Casselman and Lewis 1996). However, pike and perch are able to spawn in lentic systems and would likely invade receiving waters even if fish screens were installed on stormwater pond inflows and outflows for reasons previously mentioned. Ensuring that fish stocked in stormwater ponds are incapable of invading downstream systems should be considered when evaluating stormwater fisheries since introductions of non-native fish species can cause a decline in native fish species (Arismendi et al. 2009) and influence ecosystem functioning (Dunham et al. 2004). Since rainbow trout are not native to the majority of Alberta's rivers and streams, only triploid trout should be stocked in stormwater ponds even though Alberta has a long history of stocking diploid rainbow trout in various waterbodies (Nelson 1991). In addition to stocking triploid fishes, different strains of rainbow trout should be investigated since pothole lake studies reported significantly different growth and survival rates of several strains of rainbow trout (Ayles 1975, Ayles and Baker 1983).

In addition to stocking alternative species or strains of fish in stormwater ponds, stormwater ponds from larger city centers (Calgary and Edmonton) should also be incorporated into future studies to investigate the influence of traffic density and land use intensity on water quality. Likewise, concentrations of other urban pollutants such as hydrocarbons should be investigated since hydrocarbons are often detected in stormwater ponds and are known carcinogens (Hwang and Foster 2006).

Stormwater pond fisheries can provide recreational opportunities and, in the process, be used by managers to actively engage and educate the general public about water resources (particularly watershed protection) and the fate of household contaminants. Findings from the Oldman Watershed Council's 2004 Lethbridge Stormwater Education Program Design and Evaluation Report, "The River Starts Here", suggested that 91% of participating residents would like to increase their own knowledge of the influence residential yard practices have on water quality. Similarly, 92% of people polled felt that the community as a whole should increase their knowledge of urban runoff. Programs like the "Yellow Fish Road" by Trout Unlimited Canada, attempt to increase community awareness of stormwater drains and the Oldman Watershed Council's, "Urban Education Program" aids urban residents in implementing xeriscape and prairie urban gardens on their properties to promote water conscious living. Healthy watersheds are natural assets of great value and Alberta's growing urban population is increasing pressure on scarce water resources (Schindler and Donahue 2006). Creating additional fishing opportunities from stormwater ponds will aid in the promotion of alternative water use programs and increase public awareness of stormwater, water pollution and water preservation.

3.3 Closing Remarks

Even though the rainbow trout stocked in stormwater ponds had low levels of toxicant accumulation in this study, very few stormwater ponds could form viable recreational fisheries since the majority of stormwater ponds have a high potential to summerkill. The risk of summerkill in stormwater ponds is comparable to that in prairie pothole fisheries where there are well documented low fish survival rates in many pothole lakes and mixed reviews on the feasibility of pothole fisheries (Lawler et al. 1974, Barica 1975, Papst et al. 1980, Burton and McGinn 2008). The low proportion of suitable stormwater stocking locations makes a large scale rainbow trout stormwater fishery an impractical venture until the risk of summerkill can be reduced through stricter selection criteria (Figure 15) or improved stormwater design. However, stormwater ponds have the potential to be inexpensive and practical fishing locations that are already present within the urban landscape. Slight alterations to existing and future stormwater ponds would make them better suited for rainbow trout stocking. At the present time, existing stormwater ponds may be better suited for other types of fish species or strains of rainbow trout that are more tolerant to low DO levels and warm water temperatures. If fish summerkill in stormwater ponds could be removed, stormwater fisheries could be highly successful in urban areas with limited fishing venues.

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Appendix I

Table 7: Mean monthly water quality characteristics of stormwater ponds stocked with rainbow trout in 2012.[4]

High River

Date	Temp (°C)	pH	Cond (µS/cm)	DO (mg/L)	NH_3 (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)	TN (mg/L)	TP (mg/L)	Secchi (m)
May	14.0 ± 0.1	-	283 ± 103	10.2 ± 0.4	0.009	0.010	0.003	0.28	0.01	4.2 ± 0.2
June	18.3 ± 0.9	8.6	365 ± 9	8.4 ± 0.3	0.020	0.003	0.003	0.31	0.02	3.1 ± 0.5
July	21.8 ± 0.4	8.6	326 ± 3	8.5 ± 0.3	0.017	0.003	0.003	0.29	0.01	3.0 ± 0.1
August	21.1 ± 0.1	8.6	314 ± 0.4	7.6 ± 0.1	-	-	-	-	-	4.6 ± 0.6
September	14.9 ± 0.0	8.7	287 ± 0.4	6.9 ± 0.1	0.005	0.003	0.003	0.39	0.02	3.7 ± 0.3

Lacombe 1

Date	Temp (°C)	pH	Cond (µS/cm)	DO (mg/L)	NH_3 (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)	TN (mg/L)	TP (mg/L)	Secchi (m)
May	10.6 ± 0.1	-	947 ± 2	11.5 ± 0.3	0.013	0.018	0.001	1.18	0.08	0.6 ± 0.0
June	17.4 ± 2.2	8.8	1174 ± 70	11.9 ± 0.8	0.018	0.003	0.001	1.23	0.07	0.5 ± 0.0
July	21.2 ± 0.3	8.9	1419 ± 8	7.2 ± 0.3	0.021	0.003	0.001	0.89	0.03	1.2 ± 0.2
August	22.2 ± 0.2	8.9	1529 ± 6	7.1 ± 0.3	-	-	-	-	-	1.7 ± 0.2
September	14.1 ± 0.2	8.6	1362 ± 7	4.2 ± 0.3	0.024	0.003	0.002	0.86	0.04	0.4 ± 0.0

Lacombe 2

Date	Temp (°C)	pH	Cond (µS/cm)	DO (mg/L)	NH_3 (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)	TN (mg/L)	TP (mg/L)	Secchi (m)
May	12.0 ± 0.1	-	716 ± 3	14.5 ± 0.3	0.027	0.009	0.001	1.44	0.08	0.6 ± 0.1
June	18.5 ± 0.5	8.9	896 ± 15	7.5 ± 0.5	0.033	0.260	0.011	1.32	0.07	1.8 ± 0.0
July	21.3 ± 0.1	9.0	950 ± 2	9.3 ± 0.6	0.036	0.003	0.002	1.16	0.04	1.2 ± 0.1
August	21.6 ± 0.1	8.7	907 ± 4	10.1 ± 0.4	-	-	-	-	-	0.4 ± 0.0
September	15.1 ± 0.0	8.8	800 ± 2	3.5 ± 0.2	0.057	0.003	0.002	1.59	0.10	0.4 ± 0.0

Lethbridge1

Date	Temp (°C)	pH	Cond (µS/cm)	DO (mg/L)	NH_3 (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)	TN (mg/L)	TP (mg/L)	Secchi (m)
May	13.4 ± 0.2	-	789 ± 12	12.9 ± 0.9	0.020	0.198	0.020	1.25	0.06	0.6 ± 0.0
June	15.8 ± 0.4	9.2	666 ± 4	6.8 ± .1	0.282	0.230	0.020	1.24	0.07	2.4 ± 0.3
July	23.0 ± 0.1	9.2	1070 ± 17	5.7 ± 0.4	0.187	0.210	0.034	1.29	0.04	2.4 ± 0.3
August	21.6 ± 0.1	9.3	1241 ± 13	4.7 ± 0.3	-	-	-	-	-	2.5 ± 0.3
September	14.6 ± 0.1	9.2	1190 ± 7	6.3 ± 0.2	0.430	0.463	0.092	1.94	0.08	2.3 ± 0.2

Lethbridge 2

Date	Temp (°C)	pH	Cond (µS/cm)	DO (mg/L)	NH_3 (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)	TN (mg/L)	TP (mg/L)	Secchi (m)
May	12.9 ± 1.0	-	1286 ± 72	12.3 ± 0.9	0.023	0.256	0.018	1.37	0.04	0.5 ± 0.1
June	16.8 ± 0.1	9.2	1353 ± 39	9.5 ± 0.4	0.018	0.051	0.012	1.44	0.05	0.5 ± 0.0
July	23 ± 0.4	9.3	1494 ± 71	8.0 ± 1.5	0.015	0.003	0.002	1.06	0.04	0.5 ± 0.0
August	21.9 ± 0.2	9.3	1318 ± 80	6.4 ± 0.5	-	-	-	-	-	1.5 ± 0.1
September	14.7 ± 0.2	9.1	1201 ± 9	8.4 ± 0.8	0.016	0.003	0.001	1.44	0.06	0.7 ± 0.0

Lethbridge 3

Date	Temp (°C)	pH	Cond (µS/cm)	DO (mg/L)	NH_3 (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)	TN (mg/L)	TP (mg/L)	Secchi (m)
May	11.8 ± 0.2	-	478 ± 8	10.0 ± 1.4	0.222	0.108	0.007	1.08	0.06	2.0 ± 0.4
June	16.4 ± 0.4	9.1	407 ± 14	9.7 ± 0.7	0.029	0.278	0.015	1.10	0.04	1.5 ± 0.0
July	23.1 ± 0.2	9.1	468 ± 6	8.5 ± 0.5	0.022	0.006	0.004	0.86	0.06	0.4 ± 0.0
August	21.4 ± 0.2	9.2	565 ± 7	4.8 ± 0.4	-	-	-	-	-	1.0 ± 0.5
September	13.8 ± 0.3	9.1	553 ± 3	7.5 ± 0.3	0.020	0.003	0.002	1.24	0.08	0.5 ± 0.0

[4] Water quality measurements collected from 1.0 m water depth from five locations within each pond. Spatially composite nutrient samples collected from 0.5 m to 1.0 m water depth.

Table 8: Pesticides tested for in water samples collected in August 2012.[5]

	Pesticide	
2,4-D	Diclofop-methyl (Hoe Grass)	Methomyl
2,4-DB	Dieldrin	Metolachlor
2,4-DP	Dimethoate (Cygon)	Metribuzin
2,4-dichlorophenol	Disulfoton (Di-Syston)	Napropamide
4-chloro-2-methylphenol	Diuron	Oxycarboxin
Aldicarb	Ethalfluralin (Edge)	Parathion
Aldrin	Ethion	Phorate (Thimet)
Aminopyralid	Ethofumesate	Picloram (Tordon)
Atrazine	Fenoxaprop-P-ethyl	Propiconazole
Bentazon	Fluazifop	Pyridaben
Bromacil	Fluroxypyr	Quinclorac
Bromoxynil	Guthion	Quizalofop
Carbathiin (Carboxin)	Hexaconazole	Simazine
Chlorothalonil	Imazamethabenz-methyl(Assert)	Terbufos
Chlorpyrifos (Dursban)	Imazamox	Thiamethoxam
Clodinafop acid metabolite	Imazethapyr	Triallate (Avadex BW)
Clodinafop-propargyl	Iprodione	Triclopyr
Clopyralid (Lontrel)	Linuron	Trifluralin (Treflan)
Cyanazine	MCPA	Vinclozolin
Desethyl atrazine	MCPB	α-BHC
Desisopropyl atrazine	MCPP (Mecoprop)	α-Endosulfan
Diazinon	Malathion	γ-BHC (Lindane)
Dicamba (Banvel)	Metalaxyl-M	p,p-Methoxychlor

[5] Pesticide analyses were conducted by Alberta Innovates - Technology Futures (AITF), Vegreville, Alberta.

Table 9: Linear regression parameters by pond for AChE activity over time, Hg concentration over time, and Hg concentration by fish weight.

Interaction	Pond	R^2	R^2 Adjusted	P Value	Y-Intercept	Slope
(log10 AChE) x (Time)	**High River**	0.0629	0.0368	0.1290	2.8051	-0.0008
	Lacombe 1	0.0036	-0.0296	0.7437	2.8893	-0.0002
	Lacombe 2	0.1172	0.0951	0.0265	2.8693	-0.0013
	Lethbridge 1	0.5288	0.5215	0.0001	2.8603	-0.0030
	Lethbridge 2	0.3668	0.3392	0.0013	2.8993	-0.0032

Interaction	Pond	R^2	R^2 Adjusted	P Value	Y-Intercept	Slope
(log Hg) x (Time)	**High River**	0.8235	0.8191	0.0001	-3.9714	0.0110
	Lacombe 1	0.8163	0.8098	0.0001	-4.0439	0.0151
	Lacombe 2	0.8119	0.8071	0.0001	-4.1218	0.0110
	Lethbridge 1	0.2303	0.2183	0.0001	-4.0023	0.0034
	Lethbridge 2	0.5533	0.5338	0.0001	-4.1210	-0.0147

Interaction	Pond	R^2	R^2 Adjusted	P Value	Y-Intercept	Slope
(log Hg) x (Weight)	**High River**	0.6391	0.6301	0.0001	-4.2694	0.0048
	Lacombe 1	0.7246	0.7147	0.0001	-4.3674	0.0078
	Lacombe 2	0.5651	0.5540	0.0001	-4.4419	0.0074
	Lethbridge 1	0.1206	0.1068	0.0043	-3.9435	0.0008
	Lethbridge 2	0.3771	0.3488	0.0014	-3.6825	-0.0096