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Effects of Urban Stormwater Runoff on Fathead Minnows: Mitigating Potential of Best Management Practices

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Effects of Urban Stormwater Runoff on Fathead Minnows:

Mitigating Potential of Best Management Practices

by

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Abstract

Aquatic ecosystems located near urban landscapes are often contaminated by a complex mixture of contaminants of emerging concern (CECs). These landscapes are defined by an abundance of impervious surfaces that act as conduits during precipitation events moving contaminants into aquatic ecosystems. Prior research on the introduction of CECs into surface waters frequently focused on municipal wastewater treatment plants and agricultural runoff. This study investigates the effects of urban stormwater runoff on fathead minnows. In addition, I examined the mitigating potential of retention ponds and iron-enhanced sand filtration (IESF) as best management practices. I collected inflow and outflow water samples following precipitation events during snow melt, spring flush, and summer rains from seven stormwater ponds across the greater metropolitan area of St. Paul, MN, USA. CECs were commonly detected in stormwater runoff with greater concentrations in inflows when compared to pond outflows. In some instances, CEC concentrations rivaled those reported for treated wastewater effluent. Endpoints measured include survival, growth, foraging efficiency, and predator avoidance performance. Results indicated that seasonality had a significant effect on all biological outcomes ($p<0.01$) Moreover, stormwater from summer was the most detrimental to fathead minnows (declining survival and foraging efficiency,). Results for treatment were inconclusive with non-significant improvement for biological outcomes following exposure to stormwater treated with standard retention ponds, and the addition of IESF revealed varied and unexpected results. IESF treatment appeared to have an adverse effect on survival, with fathead minnows exposed to IESF treated stormwater surviving significantly less than those exposed to reference well water ($p<0.01$). IESF treatment also increased total escape response, with fathead minnows exposed to IESF treated stormwater having a quicker response than those exposed to untreated stormwater inflow ($p=0.03$). This study suggests that best management practices provide some benefit in reducing biological effects from exposure to urban stormwater although the biological benefits are seasonally limited. Furthermore, the addition of IESF has constrained and potentially adverse effects on biological outcomes.

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Chapter 1. Literature Review

1.1 Introduction

As the human population increases, the impacts of our existence on the environment are becoming progressively evident. Numerous anthropogenic chemicals routinely find their way into freshwater systems. Personal care and cleaning products are rinsed down the drain, pharmaceuticals are excreted into sewer systems, and lawn care treatments such as fertilizers and pesticides are washed away with stormwater runoff. Consequently, it is well established that freshwater systems around the globe contain a diverse and complex mixtures of contaminants (Kolpin et al. 2002; Ellis 2006; Zgheib et al. 2011; Meffe & de Bustamante 2014; Sorensen et al. 2015; Conley et al. 2017; Edwards et al. 2017; Jorgenson et al. 2018).

Urban development will increase in conjunction with the population, and this expansion will likely result in an increase of impervious surfaces such as roadways, parking lots, sidewalks, and rooftops (Shuster et al. 2005) impervious surfaces prevent precipitation from infiltrating into soil which increases the amount of runoff (Konrad 2003). It is to be expected that urban stormwater runoff will eventually in one form, or another become contaminated (Arnold & Gibbons 1996). Thus, stormwater management and treatment methods are of worldwide interest (Gromaire-Mertz et al.

1999; Lee & Bang 2000; Mireri et al. 2007; Erickson et al. 2012; Dodder et al. 2014; Hobbie et al. 2017).

1.2 Urban Stormwater Runoff

Urban stormwater runoff originates when precipitation does not infiltrate and therefore flows downgradient to a receiving water. Large volumes of stormwater runoff can be generated in a short period of time, predominantly due to the increase in impervious surfaces which are signature to urban areas (Leopold 1968). The primary means of controlling these large influxes of water is to discharge it, untreated, into streams, rivers, and lakes. Furthermore, when the infrastructure designed to manage stormwater runoff becomes overwhelmed, flash flooding can occur.

The results of flooding can be costly to a city not only from the damage to property but also from the resulting environmental harm. Flooding causes an increase in the erosion of soil and shorelines (Konrad 2003). This erosion also causes an increase in the amount of total suspended solids in the surface water. More suspended solids cause an increase in the turbidity of the water resulting in less available sunlight for aquatic plants. Solids will also settle out of suspension when the water slows down causing sedimentation of the waterways which decreases the available habitat for aquatic organisms (MN PCA). In addition to erosion and sedimentation, runoff from urban areas can also increase the temperature of receiving surface waters via conductive heat transfer from impervious surfaces and solar energy heating unshaded stormwater ponds and channels (Kieser & Spoelstra 2003).

Another complication with urban stormwater runoff lies not with the water itself, but with what is carried along with it. As stormwater crosses the impervious surfaces it combines with contaminants and transports them to surface waters.

1.3 Urban Stormwater Contamination

Traditionally, the contaminants of interest for stormwater have been excess nutrients (nitrogen and phosphorous) (Wang et al. 2001; Wendling et al. 2013), sediments (Sansalone et. al. 2004; Osouli et al. 2017), metals (Brown & Peake 2006), and polycyclic aromatic hydrocarbons (PAHs) (Brown & Peake 2006). However, stormwater runoff from urban areas is additionally often contaminated with complex mixtures of chemicals including pharmaceuticals and personal care products, industrial products, insecticides, and lawn / garden chemicals (Boyd et al. 2004; Ritchie et al. 2007; Zgheib et al. 2011; Page et al. 2014; Burant et al. 2018; Fairbairn et al. 2018). This can result in the contamination load of urban stormwater runoff being comparative to those found in the effluent of wastewater treatment plants (Buerge et al., 2006; Fairbairn et al., 2016; Vogel & Moore, 2016).

A study of stormwater pollution in Paris, France found 55 chemical substances including pesticides (diuron, isoproturon, metaldehyde, aminotriazole, glyphosate, AMPA), metals (Pb, Cu, Zn), PAHs, polychlorinated biphenyls, and alkylphenols (nonylphenol, para-tert-octylphenol, 4-ter-butylphenol) (Zgheib et al. 2011). Similarly, a Minnesota (USA) urban stormwater study (Fairbairn et al. 2018) detected 123 contaminants including industrial-commercial compounds, pharmaceuticals, pesticides, and lifestyle-personal care compounds. In addition to a complex mixture of contaminants, there is also a seasonality to the variation of contaminates found in urban stormwater runoff. Generally, runoff from fall and winter contains higher concentrations of flame retardants and alkylphenols, while spring and summer runoff shows higher concentrations of pesticides and industrial contaminates (Fairbairn et al. 2018, Westerhoff et al. 2018). Likewise, Anderson and colleagues (2016) also showed a variability in the concentration of contaminants with different rainfall events.

Many of the contaminants found in urban stormwater runoff are referred to as contaminants of emerging concern (CEC) (Ritchie et al. 2007; Dodder et al. 2014; Bhadra and Jhung 2017; Edwards et al. 2017). Contaminants of emerging concern include synthetic or naturally occurring chemicals that have not previously been detected in surface waters, or that are being detected at concentrations different than expected and may pose risks to humans and the environment (Ritchie et al. 2007).

Examples of chemical classes considered CECs include: antibiotics (tetracycline), solvents (ethanol, kerosene), flame retardants (polybrominated diphenyl ethers), pesticides and insecticides (permethrin, DEET), herbicides (atrazine, metolachlor), pharmaceuticals (opiates, antibiotics, contraceptives - ethinylestradiol and 17α-ethynylestradiol), personal care products (para-hydroxybenzoate), plasticizers (bisphenol A (BPA), phthalates, tributoxyethyl phosphate), lifestyle chemicals (caffeine, nicotine) and endogenous hormones (estrone, estradiol, and estriol).

1.4 Sources of Contamination

Stormwater contamination can originate from a variety of potential sources. Some pharmaceuticals are not entirely removed with standard wastewater treatment (Ternes et al. 1998). Pharmaceuticals are bioactive by design and intended to accomplish a biological effect on a target species such as humans or livestock (Henschel et al. 1997). These compounds are often not entirely eliminated by the body and can be excreted unchanged (Heberer 2002). Once in the environment pharmaceuticals are indifferent to their intended objective and can effect non-target organisms (Fent et al. 2006).

Additionally, sanitary sewer overflows and combined sewer overflows are potential sources for the introduction of untreated wastewater into stormwater and surface waters (Balmforth 1990; EPA 2004). Sewer system are commonly designed to transport and retain wastewater and stormwater through separate conveyers. However, wastewater and stormwater can be unintentionally combined during a sewer overflow which happens when untreated wastewater from a collection system is released before it reaches a treatment facility (Novotny 1996; EPA 2014).

Potential causes of sewer overflows include blocked pipes, line breaks, mechanical/power failures, and improper designs or other defects which allow excessive infiltration and inflow of stormwater (EPA 2014). The EPA estimated that the sanitary sewer overflows and combined sewer overflows release a collective total of around 850-860 billion gallons of untreated wastewater and stormwater yearly (EPA 2004). This release of untreated wastewater into surface waters has contributed to beach closures, shellfish bed closures, contaminated drinking water supplies, and other public health concerns (EPA 2004).

Additional sources of stormwater contamination include runoff from streets (Bannerman et al 1993); roofs, including those with artificial stormwater infiltration (Bucheli et al. 1998) residential, and commercial or park areas (Huang et al. 2007). Runoff from the aforementioned surfaces can be contaminated by a multitude of sources including pets and other animals (Ram et al. 2007), seal coating pavements (Watts et al. 2010), pesticides (Weston et al. 2009), and automotive sources including residue from tires and brakes (McKenzie et al 2009).

1.5 Effects of exposure to contaminates

The effects of exposure to contaminants commonly found in urban stormwater can be as diverse as the contaminants themselves. For instance, excess nutrients can cause toxic algal blooms, oxygen deficiency, shifts in the food web, loss of habitat, and decreased biodiversity (Rabalais 2002). Suspended sediment, while natural and to be expected in aquatic environments, can be problematic in higher concentration. Suspended solids in excess of 9000 mg/l have been shown to decrease the fertilization success of coho and sockeye salmon (Galbraith et al. 2006).

Metals and PAHs are the most frequently detected contaminant found in road runoff (Douben 2003). Metals have been found to affect the foraging efficiency of fathead minnows, with those exposed to lead acetate showing an increase in the time spent feeding and in the number of missed attempts (Weber et al. 1991). Biological effects of exposure to PAHs are well documented and include decreased growth, developmental disorders, cancer, and alterations in genetic - immune functions (Delistraty 1997; Grung et al. 2016).

In addition to the aforementioned contaminates, there is also a known presence of CECs in urban stormwater. Many of these CECs including BPA, dioxins, polybrominated diphenyl ethers, polychlorinated biphenyls, and estrogens have endocrine disrupting potential (Davies 2017). The endocrine system is a complex network of glands and organs that secrete and regulate hormones within the body. These hormones then regulate functions of the body including growth, metabolism, and reproduction. A challenge with endocrine disrupting chemicals is they are bioactive and can then interfere with the system by mimicking naturally occurring hormones (Tyler et al. 1998). This imitation can alter the natural hormonal system of an organism, hindering its responses to environmental changes (Diamanti-Kandarakis et al. 2009). Additionally, the interference from endocrine disrupting chemicals can alter the natural production of hormones or their receptors, negatively effecting growth and reproduction (Bergman et al. 2012). For instance, it is well established that 17α-ethynylestradiol and other estrogens can cause feminization of male fish in wild populations (Jobling et al. 1998; Thorpe et al. 2003; Matthiessen et al. 2018)

Exposure to CECs can have detrimental effects on the morphology and physiology of organisms. The degree to which an organism is affected is dependent upon various factors including the type of organism, the contaminant, and the exposure level. Recurrently exposure to CEC's has been shown to alter aspects of an organism's development (Henry et al 1997; Lefebvre et al. 2004; Zhang et al. 2017), survival (Rearick et al. 2014), and reproduction (Thrupp et al. 2018). Aquatic organisms can often be continuously exposed to contamination throughout their lifecycle. Continuous exposure to CECs from the point of fertilization can have detrimental teratogenic effects. When exposed to caffeine, ibuprofen, carbamazepine, and novobiocin the sea urchin *Paracentrotus lividus* showed a significant decrease in normally developed pluteus-larvae (Aguirre-Martínez et al. 2015).

An organism's ability to obtain food is critical to its survival. Piscivorous fish have the additional challenges of encountering, detecting, subduing, and consuming prey. These demands can be further taxed by the introduction of environmental stressors (Heugens et al. 2001; Sokolova et al. 2013). CECs have been shown to affect different aspects of fish foraging efficiency such as rate of consumption, feeding attempts, and capture success. Perch (*Perca fluviatilis)* exposed to a selective serotonin reuptake inhibitor showed a decrease in feeding (Hedgespeth et al. 2014). The overall reported effect is ultimately a reduction in the amount of food a fish is able to acquire (Brown et al. 1985; Beitinger 1990; Weber et al. 1991; Atchison et al. 1996). Exposure to CECs can also affect an organism's ability to avoid predation. The predator avoidance performance of Larval fathead minnows (*Pimephales promelas*) were adversely affected following exposure to the hormone estrone (McGee et al. 2009), and the antidepressants fluoxetine and venlafaxine (Painter et al. 2009).

Exposure to CECs results in a variety of reproductive effects. For instance, male fathead minnows showed less aggressive nest defense behavior following exposure to opioids (hydrocodone, methadone, oxycodone), antidepressants (fluoxetine, paroxetine, venlafaxine), and a sleep aid (temazepam) (Schoenfuss et al. 2016). Also, larval fathead minnows exposed to wastewater effluent which contained those same pharmaceuticals resulted in a significant decrease in body length (Schoenfuss et al. 2016). Furthermore, exposure to the anti-diabetic medication metformin was found to cause intersex of male fish as well as a reduction in size and fecundity (Niemuth & Klaper 2015). Additionally, female bluegill sunfish exposed to the estrogen 17β-estradiol produced significantly less eggs than control females (Elliott et al. 2014).

Adverse population level effects have also been attributed to CECs. Rearick et al. (2018) found that larval fathead minnows exposed to 17β-estradiol were more vulnerable to predation due to delayed response times and slower speeds. A population model established that an increase in predation mortality during early life stages could result in a decline of the population (Rearick et al. 2018). Similarly, a lake exposed to the synthetic estrogen 17α-ethynylestradiol experienced two years of consecutive reproduction failures for fathead minnows and ultimately the collapse of the entire population (Kidd et al. 2007). Additionally, male fathead minnows were found to have an increase in vitellogenin production (Kidd et al. 2007). Vitellogenin is a precursor protein for egg-yolk and is a sign of estrogenic exposure when expressed in males (Purdom et al. 1994; Harries et al. 1997)

1.6 Toxicological Stormwater Studies

Despite the documented presence of CECs in urban stormwater and their known biological effects, few toxicological experiments using stormwater runoff have been conducted. When studied, the demonstrated effects of urban stormwater runoff are varied. For example, Waara and Färm (2008) reported that highway runoff had no apparent toxicity. In contrast, Schiff and colleagues (2002) showed organism dependent toxicity with the purple sea urchin being sensitive to stormwater, while mysid shrimp were not at all sensitive. Similarly, urban stormwater runoff to a stream in Denmark were toxic to the algae *Pseudokirchneriella subcapitata*, but not toxic to *Daphnia magna* (Christensen et al. 2006). Furthermore, untreated stormwater samples from California (USA) were found to be toxic to amphipods and midges but not to daphnids or fathead minnows (Anderson et al. 2016).

Also demonstrating the variability in stormwater effects, Westerhoff et al. (2018) reported that the responses of *Daphnia magna* and fathead minnows were often subtle, inconsistent, or unexpected, and that larval fathead minnows exposed to both iron enhanced sand filter (IESF) un-treated and IESF treated stormwater fed less than fish in reference water. Additionally, zebrafish embryos exposed to urban stormwater runoff showed a range of developmental abnormalities such as delayed hatching, reduced growth, pericardial edema, reduced eye size, reduced swim bladder inflation, and acute lethality (McIntyre et al. 2014).

1.7 Stormwater Best Management Practices

The Clean Water Act 33 U.S.C. §1251 *et seq*. established the basic structure for regulating the discharging of pollutants, including stormwater runoff, into United States waters. Section 402 of the Clean Water Act 40 C.F.R. §122.1. authorized the creation of the National Pollutant Discharge Elimination System (NPDES). The NPDES permit program addresses water pollution by regulating three main potential point sources that discharge pollutants into waters of the United States. In 1990 the NPDES program required cities with populations of 100,000 or greater to obtain a permit and develop stormwater management programs in order to control pollution. Many of those programs incorporate best management practices (BMP) in order to limit or filter out pollutants.

Best management practices are the first line of defense between the contaminants of stormwater runoff and the aquatic organisms living downgradient. Current BMPs for stormwater include structures that are designed to store, treat, and infiltrate stormwater onsite before it is able to reach surface waters. These practices include flow-through dry ponds, wet retention ponds, constructed wetlands, buffer strips, rain gardens, and porous pavements (Anderson et al. 2016).

Biofiltration or bioretention is a BMP which promotes the filtering of stormwater runoff through plants and soil to reduce contaminants before they reach waterways, examples include raingardens and bioswales (Zhang et al. 2014). The addition of plants also makes the BMPs more aesthetically pleasing to inhabitants (Davis 2005). Most people living near these forms of green infrastructure cannot differentiate between a treatment area and a conventional garden (Suyeon & Kyungjin 2017). Yang and colleagues (2010) found that raingardens had exceptional removal efficiency for phosphate (89–100%) and atrazine (84–100%). Similarly, bioswales have been found to reduce the toxicity of stormwater and the concentrations of suspended solids (81%), metals (81%), hydrocarbons (82%), and pyrethroid pesticides (74%) (Anderson et al. 2016).

Treatment of stormwater is critical to aid in the reduction of adverse biological effects, and the potential removal of CECs. Most stormwater treatment methods rely on filtration or settling to remove solid particles, and few have the capability to capture pollutants once dissolved (Erickson et al. 2012). Sand filtration is a BMP used to treat suspended solids by filtering the stormwater using an aggregate media. Different types of sand filtration include the "Austin" sand filter which is similar to a dry retention basin but includes a sand filled area that filters water before releasing it. Additionally, the "Delaware" sand filter is designed to treat runoff underground in a concrete channel before discharging it (Weiss et. al. 2007). In an effort to improve the performance of sand filters, a recently developed BMP approach mixes iron (typically 5%) into the sand filtering portion of a stormwater pond creating an IESF (Erickson et al. 2012). The IESF filter can also be referred to as the "Minnesota" sand filter (MN PCA 2015). The main purpose of this addition is the ability of the IESF to mitigate excess dissolved phosphorous. Dissolved phosphorous in stormwater binds with the oxidized iron within the filter and is removed at an average of 80-90% (Erickson et al. 2012).

Apart from phosphorous, treatment of stormwater runoff with IESF can also reduce concentrations of CECs, metals, and nutrients (Westerhoff et al. 2018). Iron and manganese treatments have been shown to remove pharmaceuticals from water via physico-chemical, chemical, and biological process (Liu et al. 2016). Following IESF treatment, the concentrations of 17 organic contaminants were significantly reduced including PAHs and their derivates (89%-100%), lifestyle and pharmaceutical compounds such as caffeine (72%), nicotine, and acetaminophen (89%-100% removal), as well as commercial contaminates including BPA, phenol, and DEET (36%) (Fairbairn et al. 2018).

1.8 Fathead Minnow Uses in Aquatic Toxicology

The fathead minnow is a small-bodied, ray-finned, omnivorous fish with a full-grown weight between 2-5g, and a full-grown length ranging from 2.5-7.5 cm (Paetz, 1992). They live on average for 3-4 years, although environmental factors can limit this to approximately 2 years in wild populations (Kidd et al., 2007). Both males and females are deep bodied with a blunt rounded head, slightly forked caudal fin, and a single soft rayed dorsal fin (Paetz, 1992), however males are larger than females (Becker, 1983). They belong to the Cyprinidae family and have a broad distribution across most of North America (Ankley & Villeneuve 2006). In natural settings, fathead minnow reproduction begins May when waters reach 15°C and an appropriate 16:8 light:dark cycle is achieved (Prather, 1957; Duda, 1989; Danylchuk & Tom, 2001).

Fathead minnows were chosen for this experiment because they have historically been used as a model species for aquatic toxicology (Parrot & Wood 2002; Kidd et al. 2007; McGee et al. 2009; Anderson et al. 2016; Schoenfuss et al. 2016; Westerhoff et al. 2018), they are native to the area of study, and they are readily accessible. In addition, fathead minnows can continuously reproduce in a laboratory setting once they reach sexual maturity (Brungs 1971; Jensen et al. 2001), which makes them a useful species for laboratory studies which assess embryonic development. Fathead minnows are also a suitable choice for laboratory use because they have a well-defined reproductive and developmental cycle, as well as being tolerant to a broad range of water quality parameters including pH, alkalinity, hardness, turbidity, and temperature (Ankley & Villeneuve 2006).

The rapid increase of urbanization combined with the contemporary use and presence of CECs has resulted in the know contamination of stormwater. Additionally, these contaminates are also known to have adverse biological effects. What is unknown is the extent of the effects exposure to CECs may have on organisms or the mitigation potential of current BMPs. To answer those questions, the objective of this study is to determine if urban stormwater runoff has adverse biological effects on fathead minnows, and to assess the effectiveness of current BMP in mitigating those effects.

Chapter 2. Characterization and Biological Effects of Urban Stormwater Runoff from the Metropolitan area of Saint Paul, Minnesota

2.1 Introduction

Urbanization of the United States has been steadily increasing over the last 200 years with currently just over 80% of the United States population living in an urban area (US census 2010). Furthermore, this transition from a primarily rural to urban population in not unique to the United States. Currently 55% of the world population lives in an urban area, with an expected increase to 68% by 2050, and a majority of that growth occurring in Asia and Africa (UN 2018). This urbanization is not only characterized by an increase in population density, but also by an increase in the percentage of impervious surface cover. Impervious surfaces act as conduits during precipitation events to move contaminants into storm sewers and on to aquatic ecosystems. This allocates to the percentage of the impervious surfaces effectively indicating the impacts of urban development on aquatic ecosystems (Arnold et al. 1996; Finkenbine et al. 2000; Ladson et al. 2006). The threshold at which water quality begins to suffer from this urban input is debated, with most studies suggesting that adverse effects are detectable at an impervious surface percentage of 10 to 20 (Klein 1979; Schueler 1994; Holland et al. 2004; Kim 2016).

In addition to increased population density and impervious surfaces, urban areas are also characterized by the presence of CECs (Bai et al., 2018; Fairbairn et al. 2018). Traditionally, the contaminants of interest for stormwater have included superfluous nutrients (Wang et al. 2001; Wendling et al., 2013), suspended sediments (Sansalone el al. 2004; Osouli et al. 2017), metals (Brown & Peake 2006), and polycyclic aromatic hydrocarbons (PAHs) (Brown & Peake 2006). In addition, stormwater runoff from urban areas is often contaminated with complex mixtures of CECs (Boyd et al. 2004; Ritchie et al. 2007; Zgheib et al. 2012; Page et al. 2014; Burant et al. 2018; Fairbairn et al. 2018). In some instances, the contamination load of urban stormwater runoff can be comparable to those found in the effluent of wastewater treatment plants (Buerge et al., 2006; Fairbairn et al., 2016; Vogel & Moore, 2016). The combination of contamination present and impervious surfaces makes urban stormwater runoff a substantial contributor to water quality impairment (Li 2009, O'Driscoll 2010). In order to treat and regulate influxes of stormwater runoff during precipitation events, most cities install BMP infrastructure that includes retention ponds, constructed wetlands, buffer strips, porous pavements, and bioretention (Anderson et al. 2016; Zhang et al. 2014).

Unfortunately, little is known about CECs in stormwater and few toxicological studies have been conducted to assess their effect on biota in urban aquatic ecosystems. In the few available studies, the effects of exposure to CECs in urban stormwater runoff are often varied. Most research shows a level of organismal dependent toxicity and a range of developmental abnormalities (Schiff el al. 2002; Christensen et al. 2006; McIntyre et al. 2014; Anderson et al. 2016; Westerhoff et al. 2018). Fewer still are studies which examine the seasonality of CECs, or the potential of BMPs to mitigate effects or total numbers and/or concentrations of CECs.

Previous studies have demonstrated that treatment of stormwater runoff can reduce contaminant loads. Raingardens have been shown to remove phosphate and atrazine from urban stormwater (Yang et al. 2010). Additionally, bioswales reduce the toxicity of stormwater and the concentrations of suspended solids, metals, hydrocarbons, and pesticides (Anderson et al. 2016). Installation of IESF has been shown to diminish the concentrations of CECs, metals, and nutrients in the filtered runoff (Westerhoff et al. 2018). Despite these indicators of the positive impact of BMPs on CEC loads in stormwater, many questions remain regarding the biological effects of urban stormwater runoff and the mitigating potential of BMPs.

The question regarding CEC contamination of urban stormwater runoff is no longer of its existence. But rather one of concentrations of CECs, their biological effects, and the mitigating potential of BMPs. In an effort to answer these questions the objectives of this study are trifold: (i) to determine the seasonality of the biological effects to fathead minnows following exposure to urban stormwater runoff; (ii) to examine the effectiveness of stormwater BMP in mitigating biological effects of runoff on fathead minnows; and (iii) to assess the efficiency of the addition of IESF in mitigating adverse biologic effects. I hypothesize that (i) adverse biological effects will be greatest in summer and winter, with decreased adverse effects in the transition seasons. (ii) urban stormwater runoff treated with standard retention pond "outflow" will have fewer adverse biological effects than untreated stormwater "inflow"; (iii) Treatment of urban stormwater outflow with IESF will further reduce adverse biological effects over standard retention pond outflow and untreated inflow.

2.2 Materials and Methods

Over the course of two years (2018-19), I assessed the biologic effects of urban stormwater runoff and remedial potential of BMPs across the metropolitan area of St. Paul (Minnesota, USA). This was accomplished by the concurrent sampling of stormwater pond inflows and outflows, some of which included the addition of IESF filtration. In an attempt to demonstrate the variability of stormwater pollution, five seasonal sampling events took place (Table 1). Larval fathead minnows (1-22 days old) were then exposed to aide in understanding the biologic effects of exposure to urban stormwater.

Table 1. Seasonal stormwater collection attributes including date of collection, seasonal representation, geographic area (refer to figure 1 for location) and 24-hour precipitation amounts from https://www.ncdc.noaa.gov.

Sample Collection Date	Seasonal Representation	Area	24 Hour Precipitation (cm)
June 26, 2018	Summer	North	2.72
		Central	2.21
		South	2.79
September 4, 2018	Fall	North	1.73
		Central	4.09
		South	3.25
March 24, 2019	Winter	North	Snow Melt
		Central	Snow Melt
		South	Snow Melt
May 19, 2019	Spring	North	4.45
		Central	1.12
		South	1.12
August 14, 2019	Summer	North	0.13
		Central	0.00
		South	1.22

2.2.1 Study Sites

Potential sampling sites were reconnoitered across the metropolitan area of St. Paul, Minnesota (USA). Sites examined included stormwater ponds outfitted with IESF and standard stormwater retention ponds with no additional filtration. Site selection was based on land use, acres of watershed treated, accessibility, sample-ability (having both inflow and outflow), as well as proximity to other sites. After visiting and surveying the locations, seven stormwater ponds were found to be suitable for the current study (Table 2). Five ponds fitted with iron enhanced sand filters were chosen: Golden Lake Pond (GOL; Blain, MN), William Street Pond (WIL; Roseville, MN), Trout Brook Nature Sanctuary ponds-Maryland, Magnolia, and Jenks (MAR, MAG, JEN; St. Paul, MN). In addition to a pair of standard retention ponds: Southview Blvd (SOU; South St. Paul, MN), and Birchwood Acres (BIR; Lino Lakes, MN) (Figure 1).

Stormwater runoff from five seasonal precipitation events was collected. Sampling coincided with a summer rain (2018), a fall rain, snowmelt, a spring rain, and a second (2019) summer rain event. The snowmelt collection was timed with the thawing of the ground to allow for operation of the IESFs. Snow melt sampling took place during the first consecutive days with an air temperature above freezing. The spring, summer, and fall rainfall events were collected when there is a forecast of over 2.5 cm of rain. All collection bottles were cleansed using first Alconox® detergent (Alconox, Inc., White Plains, NY, USA) followed by rinsing with 99% isopropal alcohol. Thirty 1-L water samples for bioassays were collected from inflows and outflows of each site using established USGS protocols (Appendix A). Stormwater samples were collected from manholes, with catch poles, weighted buckets, or by hand and then stored at -20 °C before being used for bioassays. Water quality parameters of temperature, dissolved oxygen, conductivity, pH, total dissolved solids, and salinity were recorded at the time of sampling using a YSI model 556MPS (YSI Inc., Yellow Springs, OH, USA) (Table 3).

A consequence of the variability in hydrology of the IESF sites occasionally resulted in the inability to isolate the IESF treated outflow (Table 4). Increased amounts of precipitation would elevate the water level of the pond causing outflow to bypass the IESF in order to prevent flooding.

Table 2. Characteristics of stormwater field collection sites including both iron enhanced sand filtration sites and standard stormwater pond sites. Characteristics include site name with abbreviation, location, pond area, filter area, watershed area, land usage, and percent impervious surfaces.

Iron Enhanced Sand Filter Site	Location (latitude, longitude)	Pond Area (m^2)	Filter Area (m^2)	Watershed Area (km^2)	Land Use (%)		Impervious Surface (%)
					Residential	Industrial	
Golden Lake (GOL)	45° 9' 7.36" N, 93° 10' 4.31" W	7288	439	0.82	100	0	-
William Street (WIL)	45° 0' 7.20" N, 93° 6' 36.76" W	3196	46	0.68	-	-	-
Maryland (MAR)	44° 58' 34.37" N, 93° 5' 35.89" W	1885	418	0.14	-	-	47
Magnolia (MAG)	44° 58' 26.19" N, 93° 5' 35.30" W	610	130	0.17	-	-	24
Jenks (JEN)	44° 58' 15.55" N, 93° 5' 32.69" W	756	223	0.27	-	-	17
Standard Stormwater Pond Sites							
Southview Blvd (SOU)	44° 53' 13.78" N, 93° 3' 39.42" W	7689	n/a	0.68	-	-	-
Birchwood Acres (BIR)	45° 8' 13.81" N, 93° 6' 52.79" W	10165	n/a	0.15	100	0	-

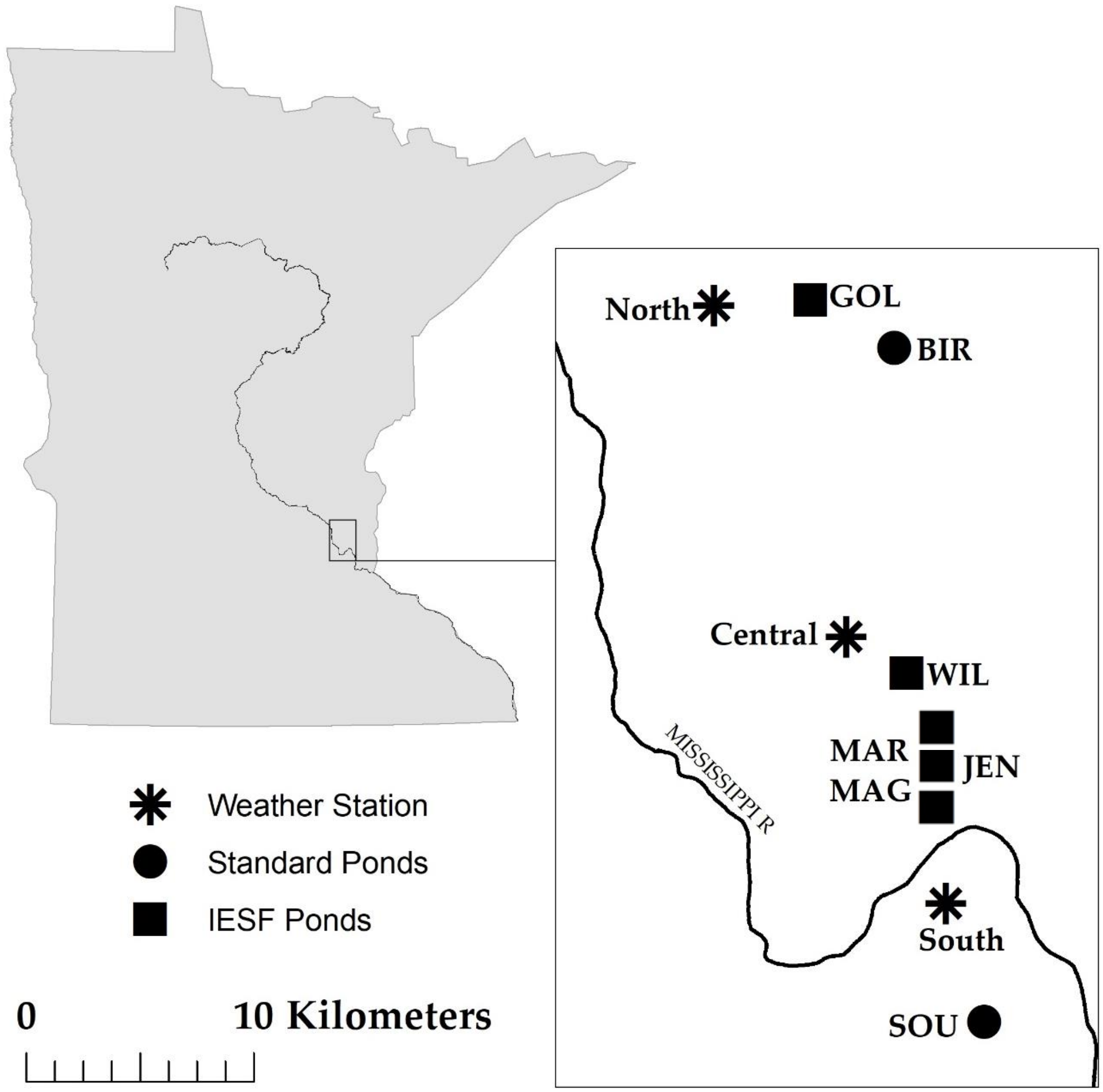

Figure 1. Map of sampling sites located around the greater St. Paul (Minnesota, USA) metropolitan area. Sites include five stormwater ponds fitted with iron enhanced sand filters: Golden Lake Pond (GOL) in Blain, William Street Pond (WIL) in Roseville, and Trout Brook Nature Sanctuary ponds: Maryland (MAR), Magnolia (MAG), and Jenks (JEN) in St. Paul. Also included are two standard retention ponds Southview Blvd/Anderson Pond (SOU) in South St. Paul, and Birchwood Acres (BIR) in Lino Lakes.

Table 3. Seasonal Water quality parameters of dissolved oxygen, conductivity, pH, total dissolved solids, salinity, and nitrate as measured at time of sampling for each site.

Site	Season	Dissolved O2 (mg/l)	Conductivity (µs/cm)	pH	Total Dissolved Solids	Salinity	Nitrate (mg/l)
GOL IN	Summer	8.12	95	8.76	0.061	0.04	0.9
	Fall	3.75	231	7.98	0.150	0.11	4.8
	winter	13.36	188	9.88	0.122	0.09	20.5
	Spring	12.10	174	7.59	0.110	0.08	35.2
	Summer	3.65	208	7.13	0.135	0.10	6.4
GOL OUT	Summer	3.09	396	7.11	0.257	0.19	0.7
	Fall	1.91	416	7.49	0.270	0.20	5.7
	winter	9.03	198	9.32	0.129	0.09	19.1
	Spring	7.83	730	7.55	0.475	0.30	12.3
	Summer	3.35	452	7.29	0.294	0.22	4.0
BIR IN	Summer	8.63	467	7.34	0.303	0.23	3.5
	Fall	4.88	94	8.06	0.061	0.04	11.9
	winter	13.36	240	9.03	0.156	0.11	61.1
	Spring	9.58	350	7.84	0.228	0.17	33.1
	Summer	4.18	714	7.26	0.464	0.35	8.7
BIR OUT	Summer	6.09	463	7.00	0.301	0.22	0.8
	Fall	4.37	159	7.88	0.103	0.07	7.0
	winter	9.07	169	8.83	0.110	0.08	25.2
	Spring	7.69	374	8.06	0.243	0.18	26.5
	Summer	4.41	398	7.36	0.259	0.19	8.2
WIL IN	Summer	3.40	354	7.08	0.230	0.17	1.0
	Fall	5.60	67	8.20	0.044	0.03	2.4
	winter	12.66	217	8.42	0.141	0.10	11.9
	Spring	3.73	534	7.93	0.347	0.26	15.1
	Summer	0.65	362	6.74	0.235	0.17	8.4
WIL OUT	Summer	1.80	458	7.06	0.298	0.22	0.8
	Fall	3.18	360	7.69	0.234	0.17	1.8
	winter	5.31	333	8.45	0.217	0.16	6.3
	Spring	3.70	554	7.75	0.360	0.27	24.2
	Summer	1.74	409	7.15	0.266	0.20	5.1
MAR IN	Summer	1.93	1074	6.87	0.698	0.53	1.6
	Fall	4.94	97	7.99	0.063	0.04	13.4
	winter	14.96	1006	8.26	0.653	0.50	26.1
	Spring	10.40	203	8.26	0.132	0.10	28.3
	Summer	4.52	206	7.04	0.134	0.10	9.9

Site	Season	Dissolved O2 (mg/l)	Conductivity (µs/cm)	pH	Total Dissolved Solids	Salinity	Nitrate (mg/l)
MAR OUT	Summer	2.56	1159	6.88	0.753	0.58	1.6
	Fall	3.01	406	7.32	0.264	0.19	2.1
	winter	8.01	799	8.46	0.518	0.39	20.1
	Spring	6.42	1042	7.86	0.678	0.52	38.9
	Summer	2.68	277	7.38	0.180	0.13	7.6
MAG IN	Summer	3.53	115	7.66	0.075	0.05	2.5
	Fall	5.31	34	7.66	0.022	0.01	2.8
	winter	12.98	236	8.55	0.153	0.11	14.4
	Spring	10.40	203	8.26	0.132	0.10	24.6
	Summer	n/a	n/a	n/a	n/a	n/a	n/a
MAG OUT	Summer	2.26	355	7.34	0.231	0.17	0.9
	Fall	4.14	195	7.26	0.127	0.09	9.0
	winter	7.12	302	8.21	0.196	0.14	13.8
	Spring	2.95	240	7.82	0.156	0.11	62.4
	Summer	n/a	n/a	n/a	n/a	n/a	n/a
JEN IN	Summer	3.19	174	7.29	0.113	0.08	1.1
	Fall	5.34	30	7.57	0.019	0.01	5.5
	winter	12.47	170	8.27	0.111	0.08	31.3
	Spring	8.20	13	7.99	0.086	0.06	21.6
	Summer	2.58	145	6.89	0.094	0.07	12.4
JEN OUT	Summer	1.92	283	7.27	0.184	0.13	0.8
	Fall	3.77	232	7.29	0.151	0.11	4.5
	winter	7.23	290	8.29	0.188	0.14	21.2
	Spring	7.61	415	7.81	0.270	0.20	36.8
	Summer	3.88	402	7.34	0.261	0.19	5.0
SOU IN	Summer	5.46	490	7.22	0.318	0.24	3.8
	Fall	5.83	435	7.27	0.283	0.21	3.8
	winter	15.10	774	8.09	0.503	0.38	12.5
	Spring	8.96	518	7.95	0.337	0.25	43.7
	Summer	6.14	724	7.94	0.471	0.35	20.6
SOU OUT	Summer	4.40	581	7.21	0.378	0.28	1.3
	Fall	5.39	180	7.49	0.117	0.08	5.9
	winter	10.70	890	8.11	0.579	0.44	12.3
	Spring	15.73	887	7.90	0.577	0.44	19.4
	Summer	4.40	807	7.67	0.525	0.40	5.7

Table 4. Differences in treatment level of stormwater runoff by season. All sites had representative inflows, with differences occurring at the outflow. Changes in treatment level of IESF ponds resulted from increased amounts of precipitation overwhelming and bypassing the filter. Refer to table 2 for site abbreviations.

	Summer 18	Fall 18	Winter 18	Spring 19	Summer 19
GOL	Standard outflow	IESF	Standard outflow	Standard outflow	IESF
WIL	IESF	Standard outflow	IESF	Standard outflow	IESF
MAR	IESF	IESF	IESF	IESF	IESF
MAG	IESF	IESF	IESF	IESF	Not Sampled
JEN	IESF	IESF	IESF	IESF	IESF
SOU	Standard outflow	Standard outflow	Standard outflow	Standard outflow	Standard outflow
BIR	Standard outflow	Standard outflow	Standard outflow	Standard outflow	Standard outflow

2.2.2 Juvenile Fathead Minnow Exposures

Juvenile fathead minnow exposures were conducted at the St. Cloud State University Aquatic Toxicology Laboratory (St. Cloud, MN, USA) using grab samples collected during precipitation events and stored at -20 °C until used. Post-hatch fathead minnow larvae (<24 hours old) were shipped from Environmental Consulting & Testing (Superior, WI, USA) overnight to St. Cloud. For each treatment, larvae were randomly separated into tanks (3 liter) each containing 5 replicates, each replicate contained 15 larvae and consisted of a 6.5 cm internal diameter x 10 cm tall glass tube with a mesh bottom secured to the glass with silicone. The fish were exposed for 21 days under a 50% daily static renewal protocol. A laboratory reference treatment used non-chlorinated water from a dedicated well. Environmental conditions were maintained at a temperature of 22.7 ± 1.5 °C and a photoperiod of 16:8 hour light:dark, within a laminar flow hood using HEPA filtration. Fish were fed twice daily *ad libitum* with freshly hatched brine shrimp (Brine Shrimp Direct, Ogden, UT, USA). Every third day water quality parameters of hardness and alkalinity were recorded using HACH PRO Aquachek test strips (Hach Company, Loveland, CO, USA), while temperature, pH, and dissolved oxygen where recorded using a YSI Pro 1020 water meter.

2.2.3 Biological Analysis

Following exposure, juvenile fathead minnows (21 days old) were analyzed for survival, growth, feeding efficiency, and predator avoidance performance. Survival was assessed as the percent remaining in each replicate following 21 days of exposure. Growth was assessed as the total body length in mm after 21 days of exposure. Subjects were measured for growth using a digital video recorded from a Redlake MotionScope high-speed camera (1000 frames per second; Tucson, AZ) positioned ~25 cm vertically above a test arena. The arena featured a 1 mm × 1mm grid background used in combination with ImageJ software to determine the overall length of the fish.

Feeding efficiency was assessed by transferring larvae to a 6-well plate (VWR International, Radnor, PA) containing 10 ml of conditioned well water and allowing them to acclimate for 12 hours. The food source consisted of 15±1 recently hatched brine shrimp that where counted using a microscope. The subject was then allowed to forage for 1 minute on an available brine shrimp until being euthanized using a lethal concentration of NaCO2-buffered MS -222 (Argent Chemical Laboratories, WA, USA). Following the assay, remaining brine shrimp were counted using a microscope to determine the percent reduction on shrimp. A more detailed description of the feeding assay can be found in (Appendix B)

Predator avoidance performance was assessed using four variables (latency, escape velocity, escape angle, and total escape response ((bodylength / distance traveled in 20msec)/ latency in msec)) following established protocols (McGee et al. 2009). At the start of each trial, a randomly selected subject was placed into a clear-bottomed testing arena (5-cm diameter) containing 10 mL of conditioned well water. The subject was allowed to acclimate for 1min before to the induction of the stimulus. The arena was positioned on a back-lit pad containing a speaker used to deliver a non-point source vibration lasting 0.6 seconds. A 1 mm × 1mm grid backdropped the arena to allow for quantification of the response. The entire testing area was illuminated via a Kessil A150 fiber optic light source (Richmond, CA, USA) angled above the arena. Each response was recorded at 1000 frames per second using a Redlake MotionScope high-speed camera (Tucson, AZ, USA) positioned ~25 cm vertically above the test arena. Recordings were then analyzed using ImageJ software (NIH). Trials in which the subject failed to respond to the stimulus after 3 attempts were deemed to have no response and excluded from the analysis. Additionally, trials in which the response latency was found to be less than six milliseconds after the induction of the stimulus were considered false-starts and eliminated from the analysis. After completion of the exposure period and bioassays, fish were euthanized following St. Cloud State University IACUC approved guidelines using a lethal concentration of $NaCO_2$-buffered MS-222 (Argent Chemical Laboratories, WA, USA). A more detailed description of the predator avoidance assay and the process for digitizing the data can be found in (Appendix C).

2.2.4 Statistical Analysis

Statistical analysis for the results of treatment effectiveness by site (inflow vs. outflow or IESF) was completed using a paired t-test. Statistical analysis for the results of seasonality (summer 2018, fall 2018, winter 2018, spring 2019, summer 2019) and treatment (inflow, outflow, IESF, reference) were analyzed with a two-way analysis of variance using a least square means model. A standard least square means model was used due to the variation in sample sizes. Fixed independent variables included season and treatment. Dependent variables included survival (%), body length (mm), reduction in shrimp (%), latency (ms), escape velocity (Bl/ms), escape angle, and and total escape response (Bl/ms). All models tested the effects of season, treatment, and the interaction between these two terms. All variables were analyzed using JMP pro 14 (SAS, Cary, NC, USA). Tukey HSD posttest was used for analysis of seasons, and the Dunnett's post-test was used for treatment level effects with reference well water as the control.

2.3 Results

Juvenile (21-day) fathead minnow exposure experiments were conducted for urban stormwater runoff samples coinciding with precipitation events representing summer (2018), fall, winter, spring and an additional summer (2019) (independent variable). Dependent variables assessed for juvenile fathead minnows included survival, growth, feeding efficiency, latency, escape velocity, escape angle, and total escape response. All dependent variables showed a significant difference between seasons ($p<0.01$) which demonstrates an inherent difference based on the variability of precipitation events.

2.3.1 Effects on Reference Fish

Biological effects of exposure to the well water “reference” treatment varied between exposures, with significant differences occurring for each dependent variable. An exposure to stormwater collected in winter yielded the best survival, followed by summer^{19}, spring, fall, and summer^{18} with the worst survival. To account for possible effects on survival resulting from laboratory practices, “relative survival” was used to relate the survival of reference fish to treatment fish for each seasonal exposure period. Relative survival was calculated as (100 / average survival of reference fish) * (average survival of treatment replicate).

Growth and feeding efficiency of reference fish varied less than survival between exposures. Fish grew to their largest size in stormwater collected in the winter, fall, and summer^{18}, while fish were smaller at the end of the 21-day exposure in stormwater collected in spring and summer^{19}. Feeding efficiency of reference fish varied more than growth, but not as much as survival. Fish exposed to stormwater collected in the fall season consumed the most shrimp, followed by winter, summer^{18}, summer^{19}, and the fewest shrimp were consumed in spring.

Furthermore, the predator avoidance end points of latency, escape velocity, escape angle, and total escape response had differing amounts of variability. There was negligible variability regarding the latency between exposures. Escape velocity showed a slight variation with the fastest velocity being observed in an exposure to stormwater collected in summer^{18} and fall, and the slowest found in summer^{19}, winter, and spring. Escape angle and total escape response showed increased variably over latency and escape velocity. The largest angle occurred in an exposure to stormwater collected in summer^{18}, followed by fall, summer^{19}, spring, and then winter with the smallest. The fastest total escape response occurred in an exposure to stormwater collected in summer^{18}, followed by fall, summer^{19}, winter, and then spring with the slowest.

2.3.2 Effects of Treatment by Site

Biological effects of exposure to urban stormwater runoff varied by site, when comparing mitigation potential of treatment. Reviewing first the effects on survival following treatment (Figure 2), as determined by relative surviving percentage (%) per replicate, sampling site GOL showed greater survival following treatment for fish exposed to summer^{18} non-IESF treated outflow when compared to the untreated inflow from summer^{18} ($p=0.02$).

In contrast exposure to stormwater collected in the fall resulted in decreased survival following treatment with IESF when compared to untreated inflow from fall ($p=0.03$). Survival then increased for fish exposed to non-IESF treated stormwater collected in spring when compared to the untreated inflow from spring ($p=0.05$). Following a similar pattern, survival decreased once more for fish exposed to IESF treated stormwater from summer[19] when compared to the untreated inflow from summer[19] ($p<0.01$).

A similar pattern was seen for the WIL site with decreased in survival of fish exposed to summer[18] runoff treated with IESF ($p<0.01$). Survival of fish then increased in an exposure to stormwater collected in spring which was not treated with IESF ($p<0.01$). The MAR and MAG sampling sites showed no significant difference between treatments. Additionally, the JEN sampling site indicated that fish exposed to IESF treated runoff from winter also had decreased survival when compared to the inflow ($p<0.01$). Non-IESF sites SOU and BIR also showed decreased survival of fathead minnows exposed to stormwater from treated outflow when compared to untreated inflow from summer[18] ($p<0.01$). Additionally, fish exposed to stormwater from the BIR site exhibited decreased survival following treatment for summer[18] ($p=0.03$), followed by increased survival for fall ($p=0.01$), and summer[19] ($p<0.01$).

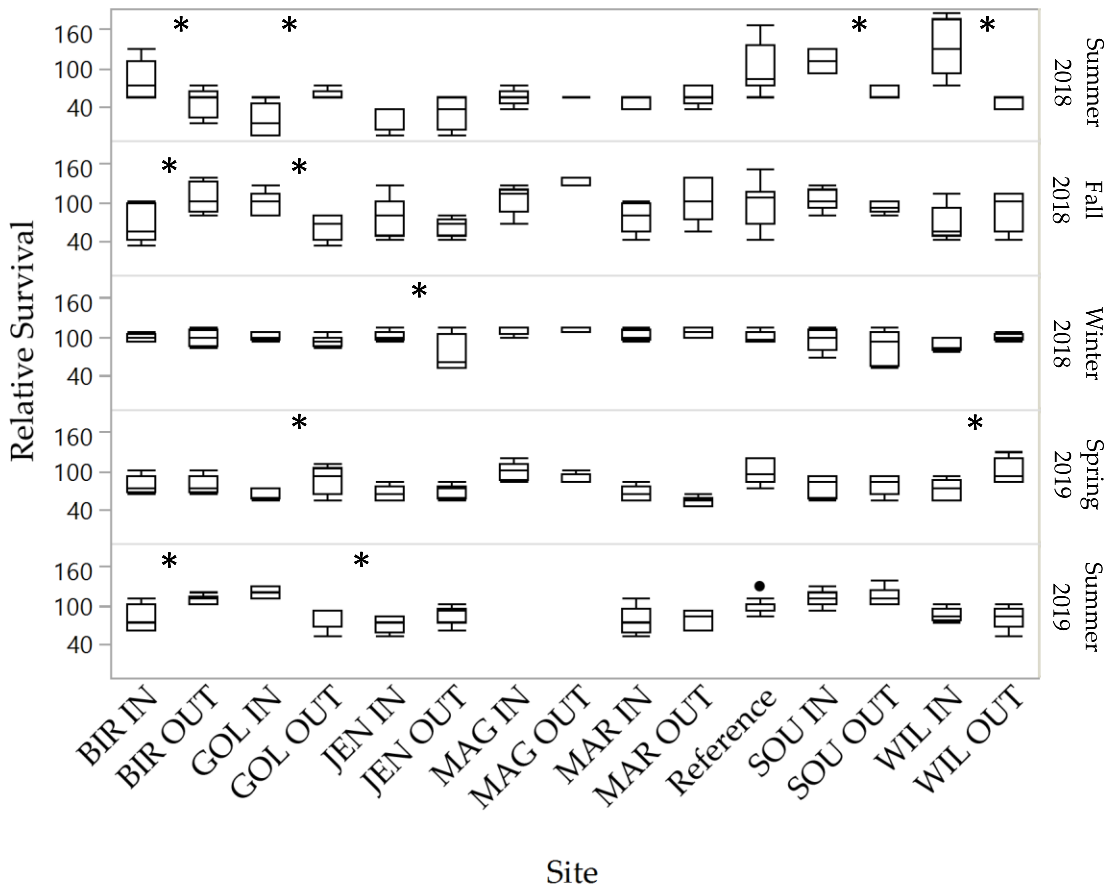

Figure 2. Relative Survival of fathead minnows exposed to stormwater runoff as compared to survival of reference fish. Inflow for each site is denoted as “in” and outflow is denoted as “out”. Refer to table 2 for site abbreviations, and table 4 for treatment level. An “*” indicates a significant difference of p≤0.05 for outflow when compared to inflow.

Effects on growth (Figure 3) of fathead minnows following 21-day exposure to pond inflows and outflows had varying results. When comparing inflow to outflow, exposure to stormwater collected from the GOL site caused a decrease in size following standard retention pond treatment for winter ($p<0.01$). Additionally, size was also decreased following exposure to IESF treated runoff from summer[19] when compared to untreated inflow ($p=0.01$). The WIL site showed no significant effect by treatment for any season. While exposure to IESF treated outflow from the MAR site in summer[18] resulted in an increase in size for fish when compared to untreated inflow ($p<0.01$). Similarly, the MAG site showed fish exposed to IESF treated runoff had an increase in size following treatment for winter ($p<0.01$). On the contrary the JEN site showed a decrease in size of fish following IESF treatment of stormwater collected from summer[19] ($p=0.03$). The standard retention pond site SOU had an increase in size following treatment for fish exposed to winter snowmelt ($p<0.01$). While BIR showed no significant effect based on treatment by season.

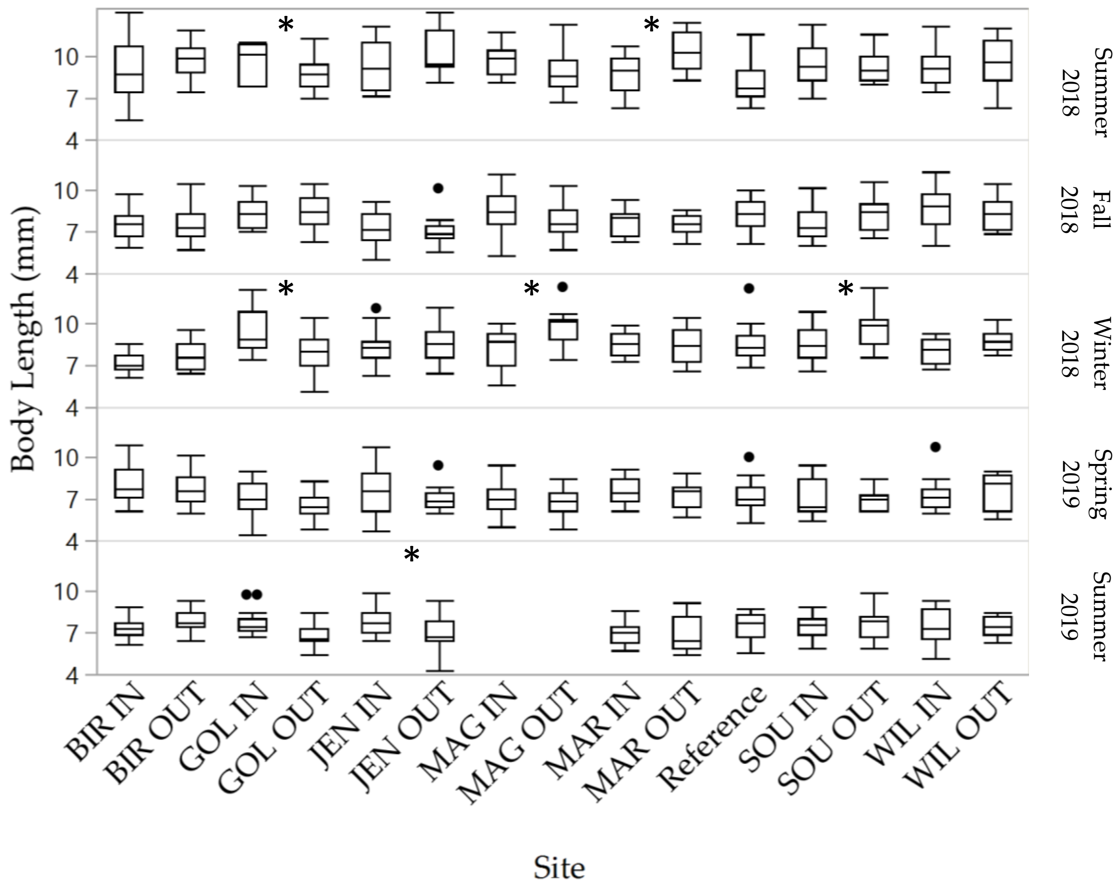

Figure 3. Total body length of fish exposed to urban stormwater runoff. Inflow for each site is denoted as "in" and outflows are denoted as "out". Refer to table 2 for site abbreviations, and table 4 for treatment level. An "*" indicates a significant difference of $p \leq 0.05$ for outflow when compared to inflow.

Effects of treatment on feeding efficiency (Figure 4) were measured as the percentage (%) of live brine shrimp consumed in a one-minute period for untreated inflow compared to treated outflow (standard or IESF). Results showed that fish exposed to IESF treated stormwater from the GOL site had a reduction in the percent shrimp consumed following exposure to stormwater collected in the fall ($p<0.01$). Likewise, fish exposed to IESF treated stormwater from the MAR site in summer[19] also had a decrease in the percent shrimp consumed ($p=0.03$). Additionally, an increase in the percent reduction in shrimp was shown for fish exposed to fall runoff from the standard retention pond sites SOU ($p=0.05$), and BIR ($p=0.04$). While the sampling sites WIL, MAG, and JEN showed no significant treatment level effects for any season.

Predator avoidance performance of fish (latency, escape velocity, escape angle, and total escape response) (Figure 5) showed no significant difference for any of the seasons based on treatment by site alone.

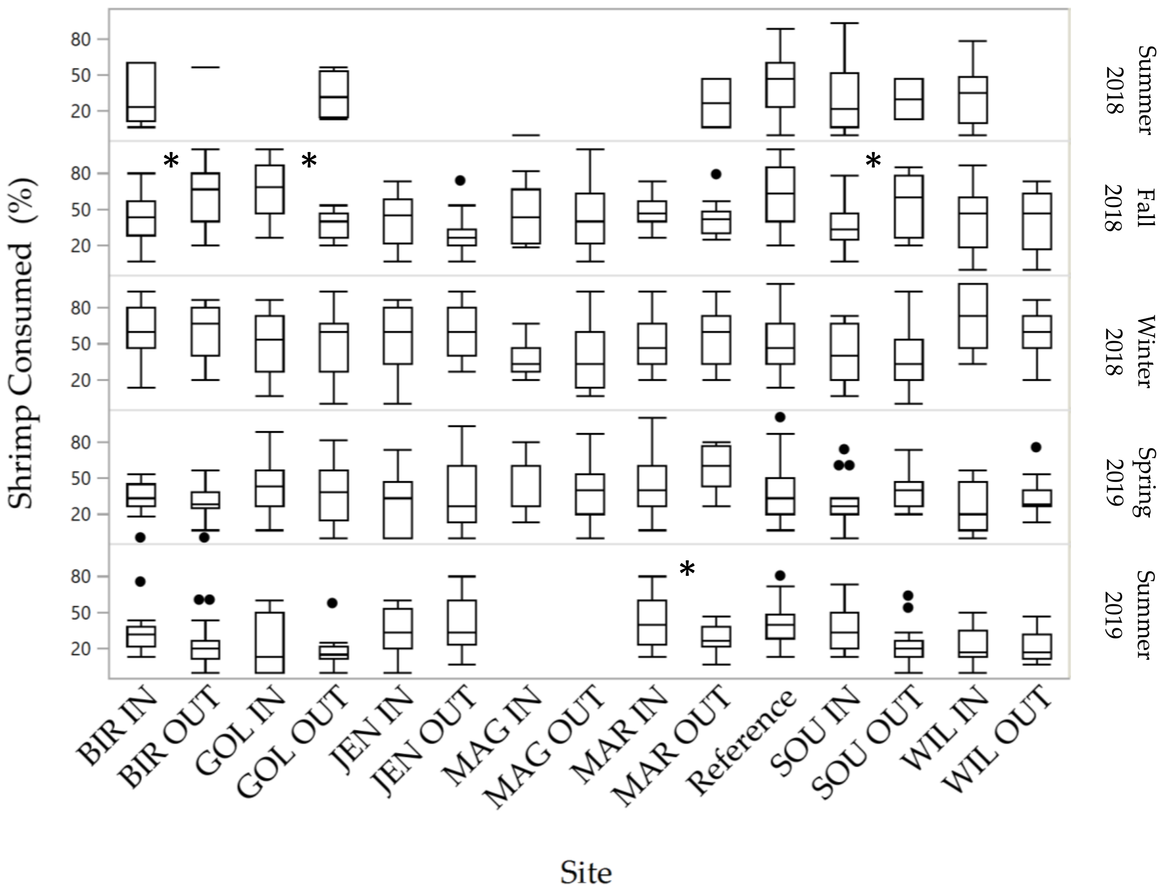

Figure 4. Percent reduction of shimp following exposure to urban stormwater runoff. Inflow for each site is denoted as "in" and outflow is denoted as "out". Refer to table 2 for site abbreviations, and table 4 for treatment level. An "*" indicates a significant difference of p≤0.05 for outflow when compared to inflow.

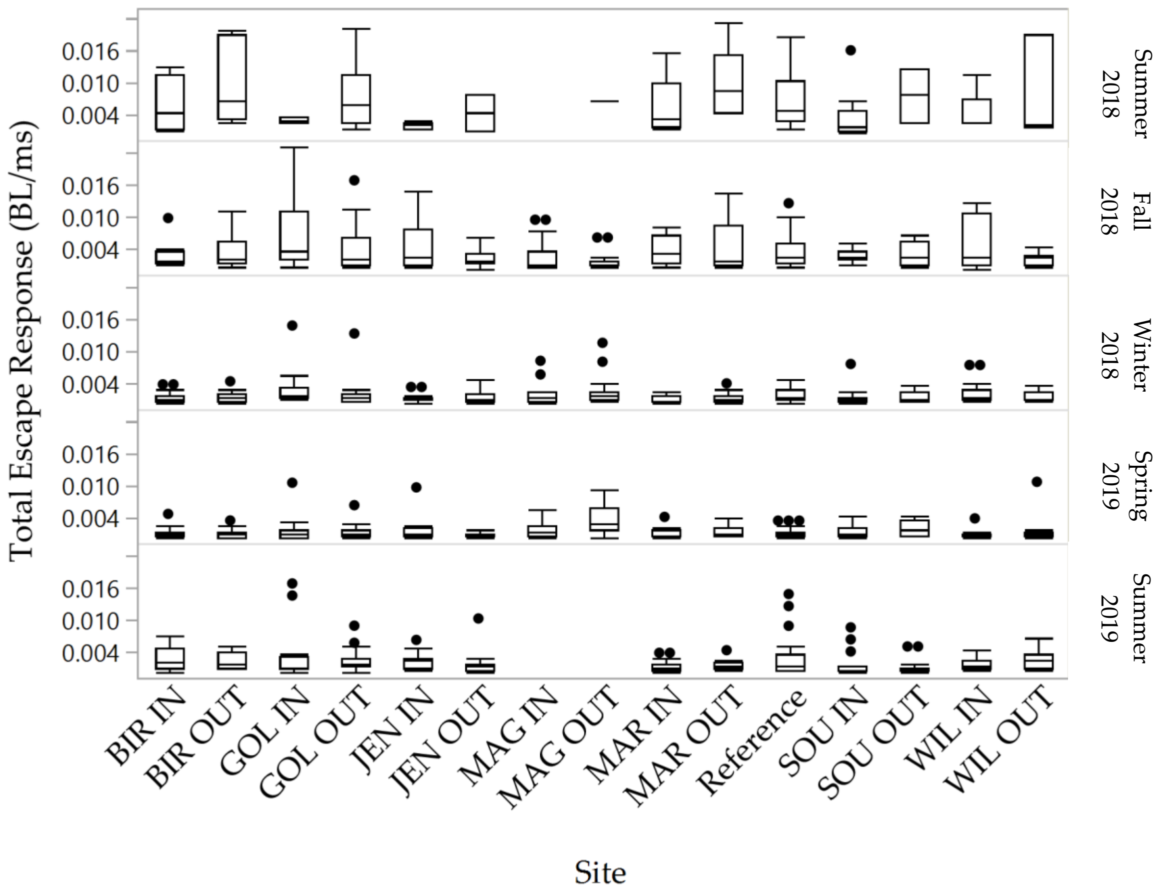

Figure 5. Total escape response calculated as ((bodylength / distance traveled in 20msec)/ latency in msec). Inflow for each site is denoted as "in" and outflow is denoted as "out". Refer to table 2 for site abbreviations, and table 4 for treatment level.

2.3.3 Season – Treatment Interactions

When independent variables of season (summer 2018, fall 2018, winter 2018, spring 2019, summer 2019) and treatment (inflow, outflow, IESF, and reference) are analyzed independent of site, survival differed between season and treatments (Figure 6). Survival showed significant ($p \leq 0.05$) seasonal effects with the greatest survival in winter, summer[19], and fall, followed by fall and spring, with fish exposed to summer[18] stormwater surviving the worst. Treatment level effects were noted with the Dunnett posttest showing a significant decrease in survival following exposure to untreated inflow ($p<0.01$), Standard outflow ($p=0.03$), and IESF treated outflow ($p<0.01$) as compared to reference well water. Additionally, the survival of fish exposed to standard outflow was significantly ($p\leq0.05$) greater than those exposed to IESF treated outflow. However, standard retention pond outflow did not show a significant improvement over untreated inflow. Survival was also influenced by the interaction between season and treatment ($p<0.01$) (Figure 7).

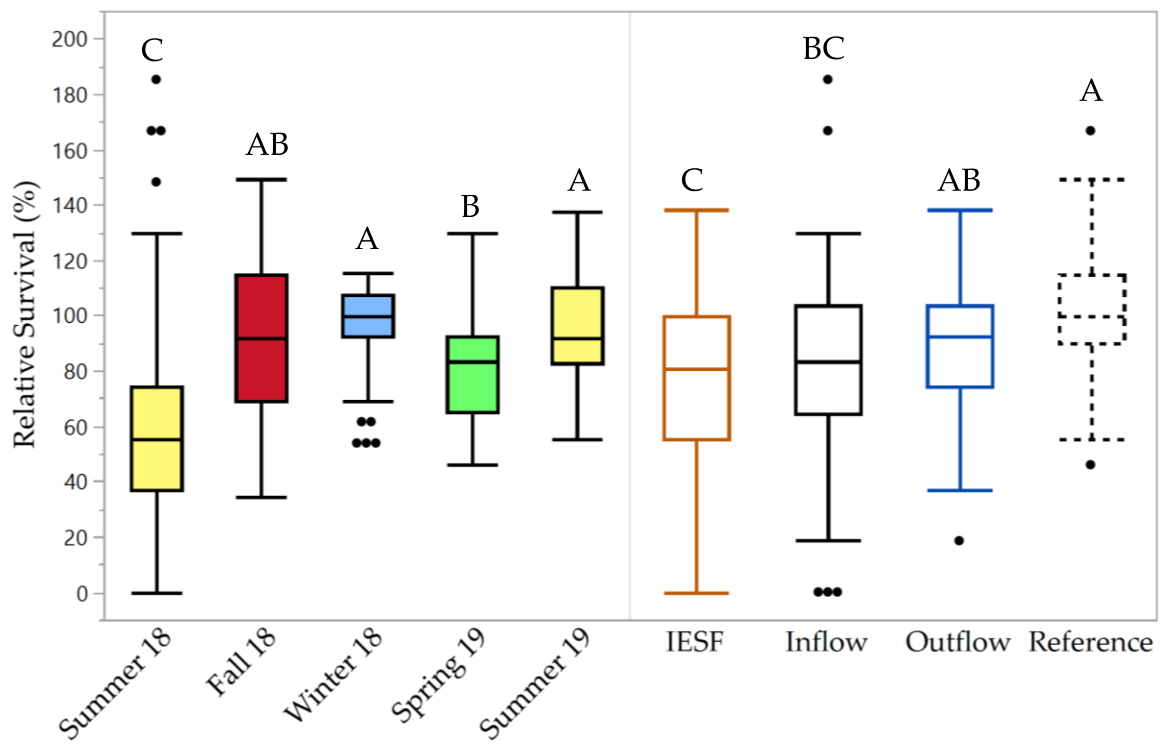

Figure 6. Percentage of fathead minnows that survived following 21 days of exposure to various seasonal representations (left) and treatment levels (right) of urban stormwater runoff. Ordered letter report is the result of a Tukey posttest with a significance of ($p\leq0.05$).

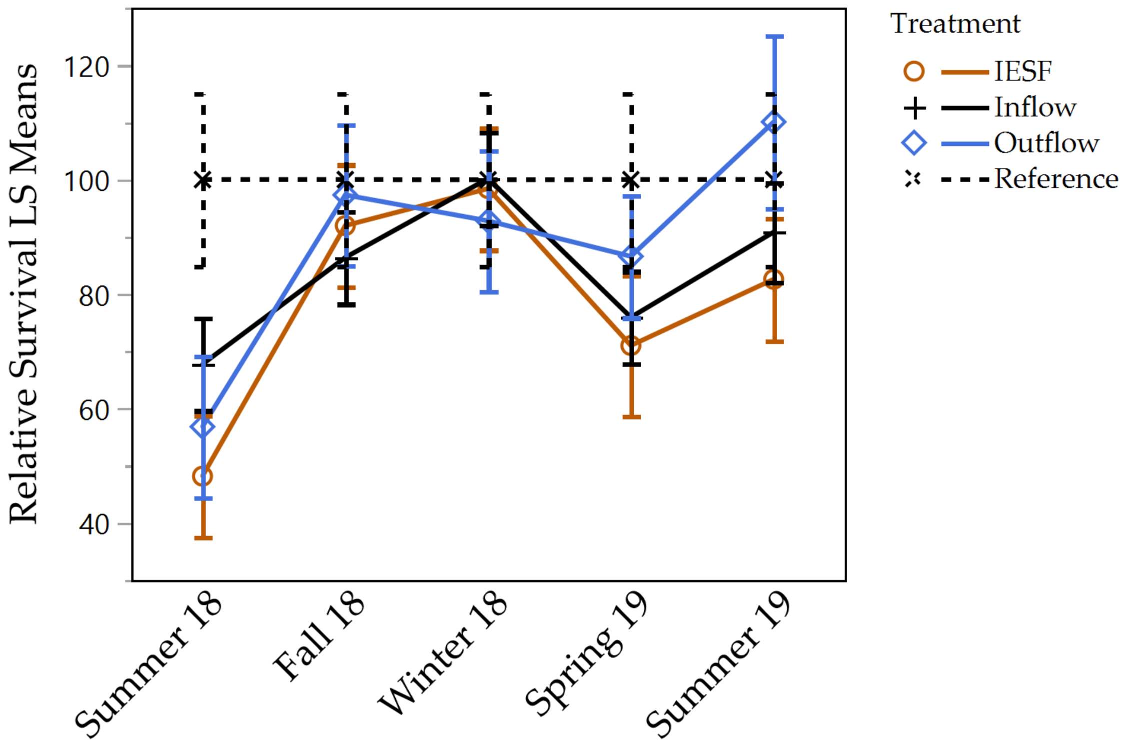

Figure 7. Plot showing the effect of interaction between season and treatment on survival of fathead minnows exposed to urban stormwater runoff.

The growth of larval fathead minnows differed significantly ($p \leq 0.05$) between seasons (Figure 8) with the largest fish in summer[18], followed by winter, then fall, and the smallest fish in spring and summer [19]. However, treatment at any level did not have a significant effect on growth. Additionally, growth was influenced by the interaction between season and treatment ($p < 0.01$) (Figure 9).

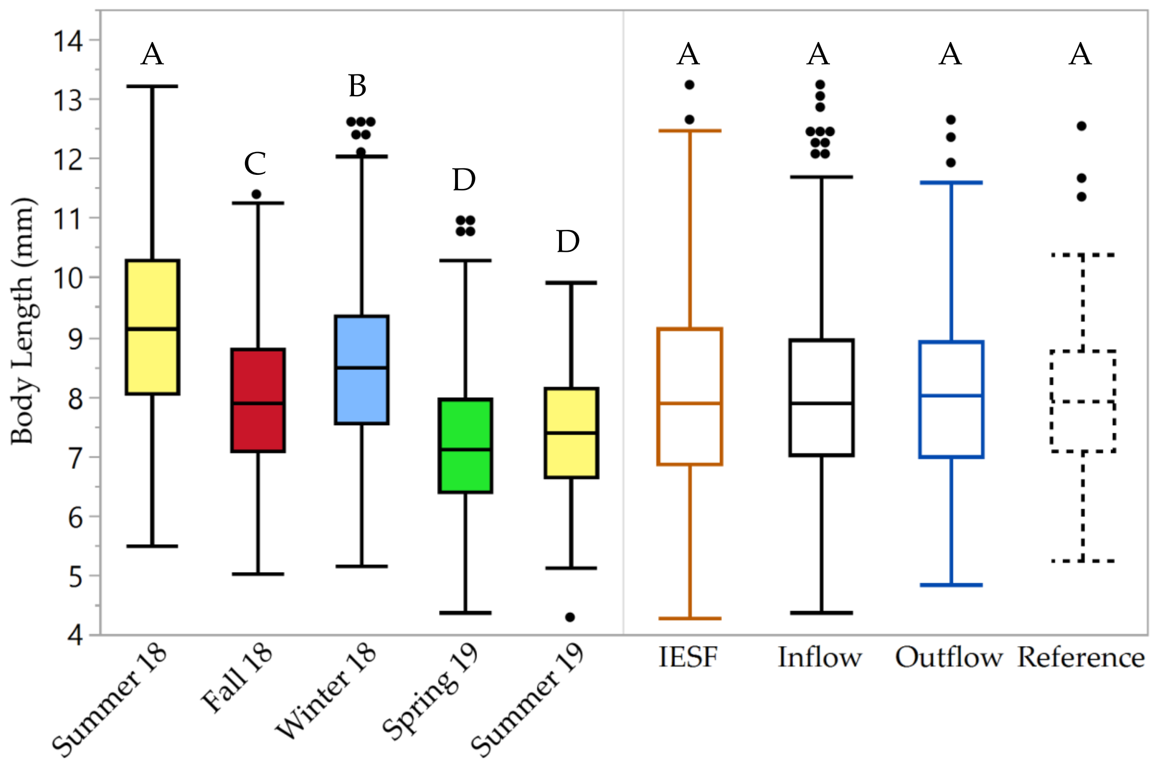

Figure 8. Body length in mm of fathead minnows following 21 days of exposure to various seasonal representations (left) and treatment levels (right) of urban stormwater runoff. Ordered letter report is the result of a Tukey posttest with a significance of ($p \leq 0.05$).

The feeding efficiency of juvenile fathead minnows was affected by both season and treatment (Figure 10). Shrimp consumption was significantly greater ($p \leq 0.05$) in fall and winter, followed by spring and summer[18], then summer[18] and summer[19]. The Dunnett's post-test indicated a significant decrease ($p<0.01$) in the amount of shrimp consumed by fish exposed to untreated inflow when compared to those exposed to reference well water. Furthermore, the feeding efficiency of fish did not differ significantly between those exposed to untreated inflow, standard retention pond outflow, or IESF treated outflow. Additionally, feeding efficiency was influenced by the interaction between season and treatment ($p<0.01$) (Figure 11).

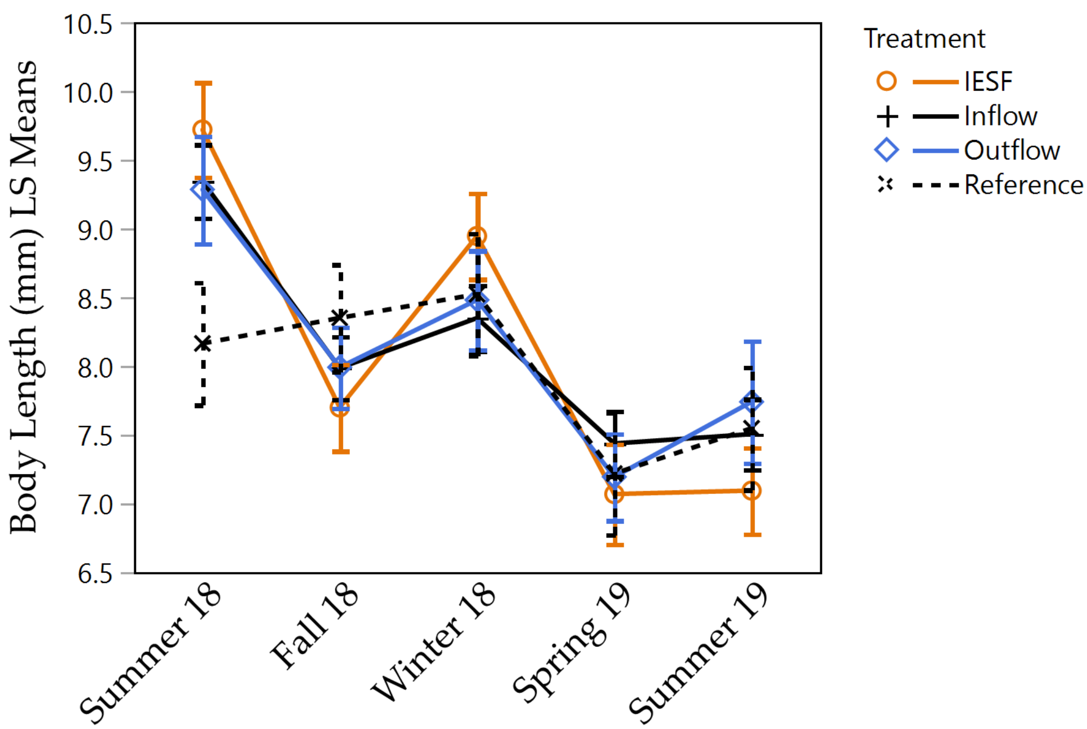

Figure 9. Plot showing the effect of interaction between season and treatment on growth of fathead minnows exposed to urban stormwater runoff

Predator avoidance performance was assessed using a combination of four variables (latency, escape velocity, escape angle, and total escape response) (Figure 12). Latency, the reaction time of a juvenile fathead minnow responding to a simulated predator stimulus, was significantly ($p\leq0.05$) affected by seasons. Fish reacted the fastest following exposure to stormwater collected from spring and winter, followed by winter and summer[19], then summer[19] and fall, with exposure to summer[18] runoff resulting in the slowest reaction time. However, treatment of stormwater did not have a significant effect on the latency of fathead minnows.

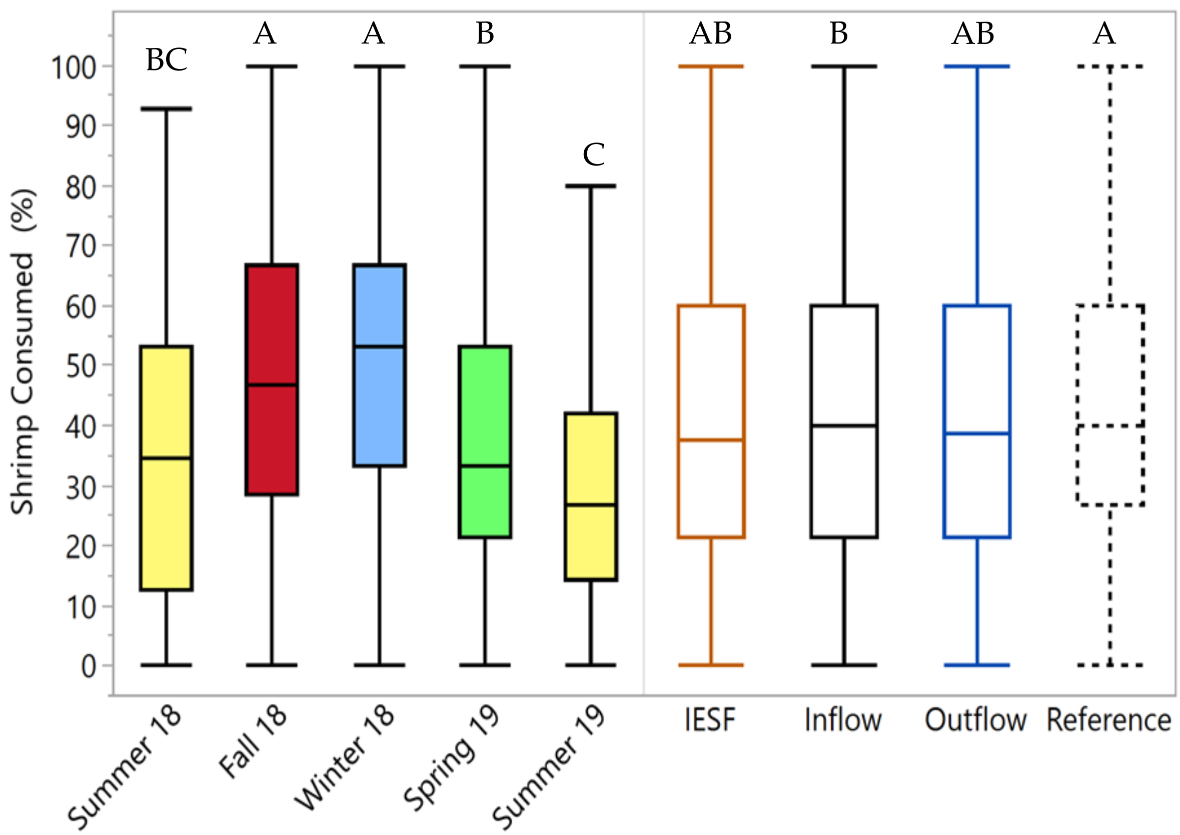

Figure 10. Percent reduction of shrimp for fathead minnows following 21 days of exposure to various seasonal representations (left) and treatment levels (right) of urban stormwater runoff. Ordered letter report is the result of a Tukey posttest with a significance of ($p \leq 0.05$).

Similarly, there was a significant ($p \leq 0.05$) seasonal effect for escape velocity (normalized to body lengths per millisecond) of juvenile fathead minnows. The greatest escape velocity was observed in summer[18], followed by fall, and then summer[19], winter, and spring. Again, there was no significant effect on escape velocity with any treatment level. However, there was a significant ($p= <0.01$) interaction between season and treatment (Figure 13).

The escape angle of juvenile fathead minnows was significantly affected by both season and treatment ($p \leq 0.05$). With the largest escape angle being shown in fish exposed to runoff from summer[18] and fall, followed by fall and summer[19], then summer[19] and spring, and finally spring and winter with the smallest. Dunnett's post-test did not indicated a significant difference when comparing stormwater exposed fish to reference fish. However, a Tukey posttest indicated there was an increased escape angle for fathead minnows exposed to IESF treated stormwater when compared to untreated inflow ($p= 0.03$).

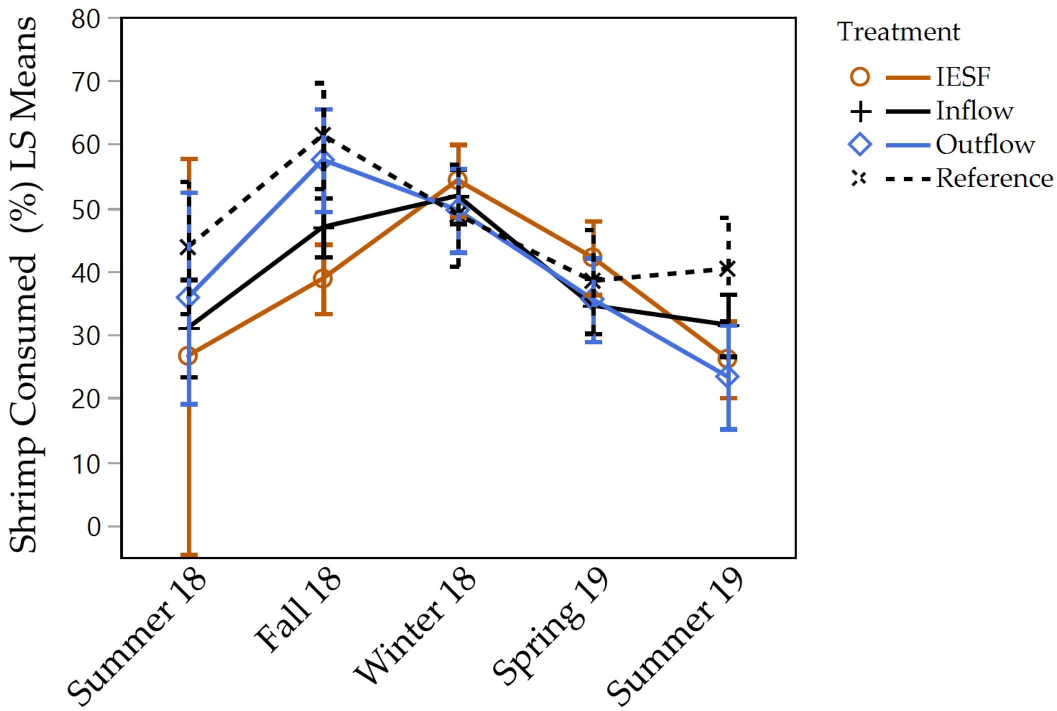

Figure 11. Plot showing the effect of interaction between season and treatment on feeding efficency of fathead minnows exposed to urban stormwater runoff.

Likewise, the total escape response, a combination of latency and escape velocity was also significantly altered by both season and treatment ($p \leq 0.05$). The greatest escape response was found in fish exposed to stormwater collected in summer[18], followed by fall, and then summer[19], winter, and spring with the slowest response. Again, the Dunnett's post-test did not indicated a significant difference when comparing stormwater exposed fish to reference fish. Although, the Tukey posttest showed a significant ($p \leq 0.05$) increased in escape response of fathead minnows exposed to IESF treated stormwater when compared to those exposed to untreated inflow. Additionally, there was a significant ($p<0.01$) interaction between season and treatment (Figure 14).

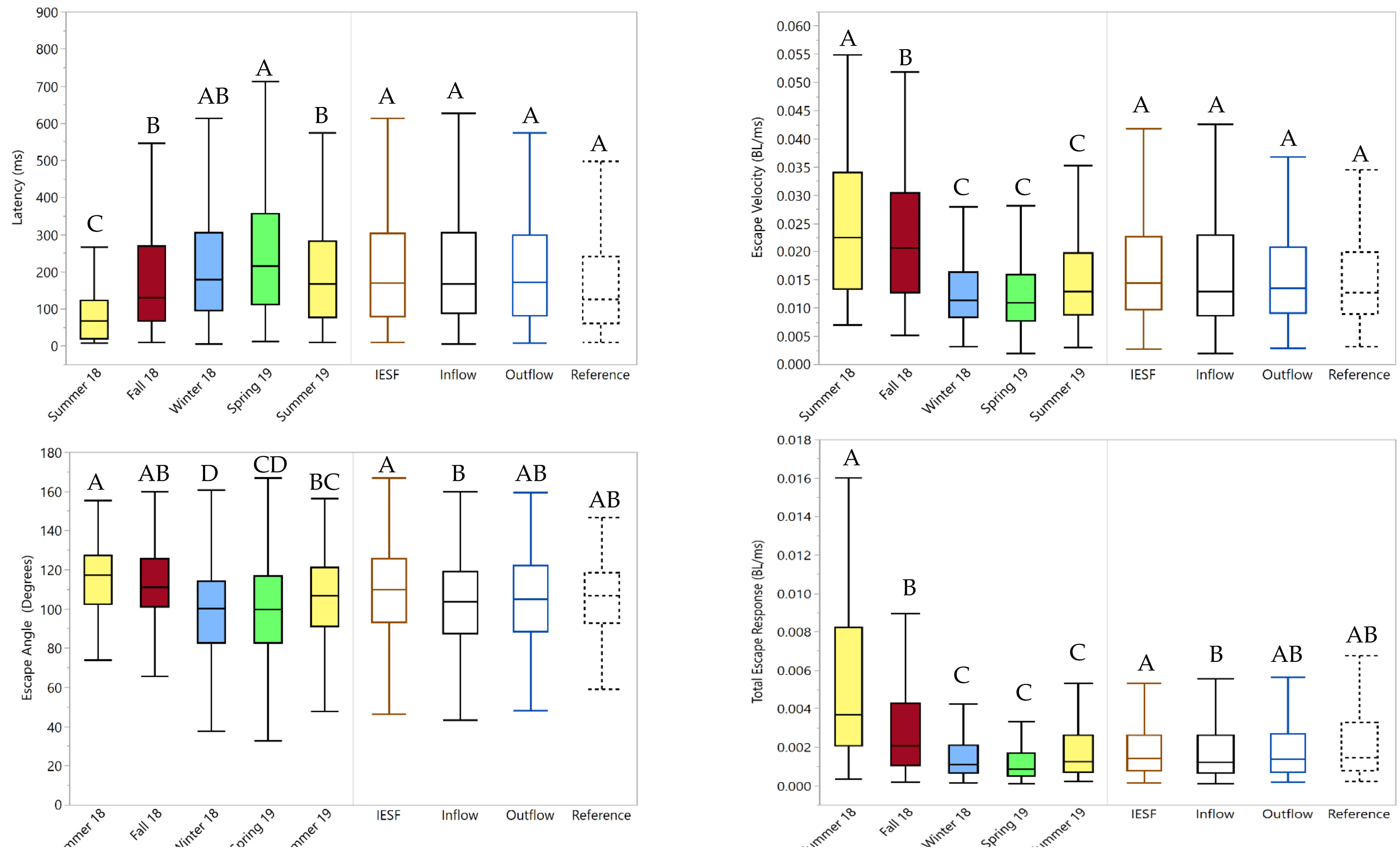

Figure 12. Predator avoidance variables of Latency, escape velocity, escape angle, total escape response of fathead minnows following 21 days of expsure to various sesonal representations (left) and treatment levels (right) of urban stormwater runoff. Ordered letter report is the result of a Tukey posttest with a significance of (p≤0.05).

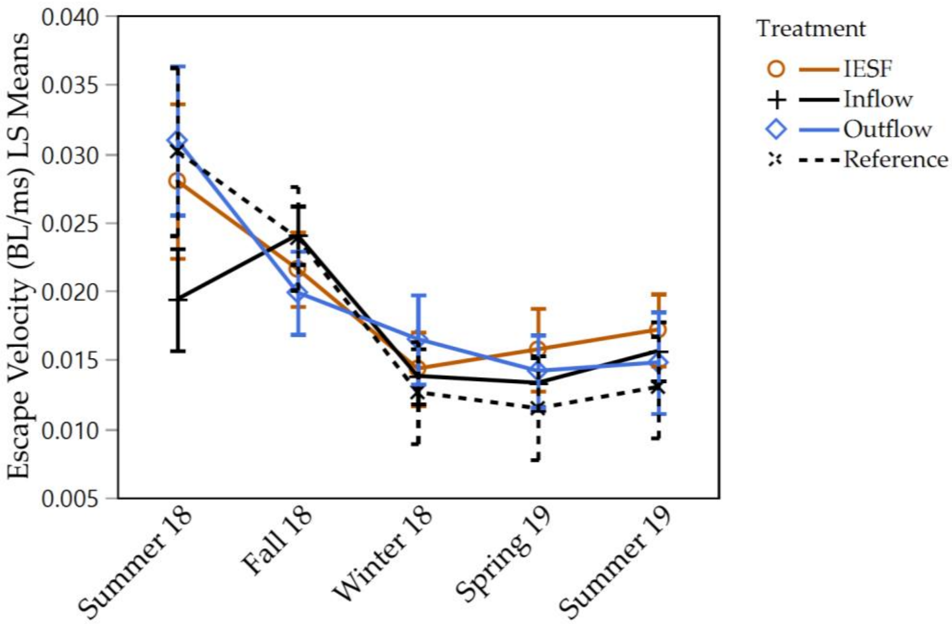

Figure 13. Plot showing the effect of interaction between season and treatment on the escape velocity of fathead minnows exposed to urban stormwater runoff.

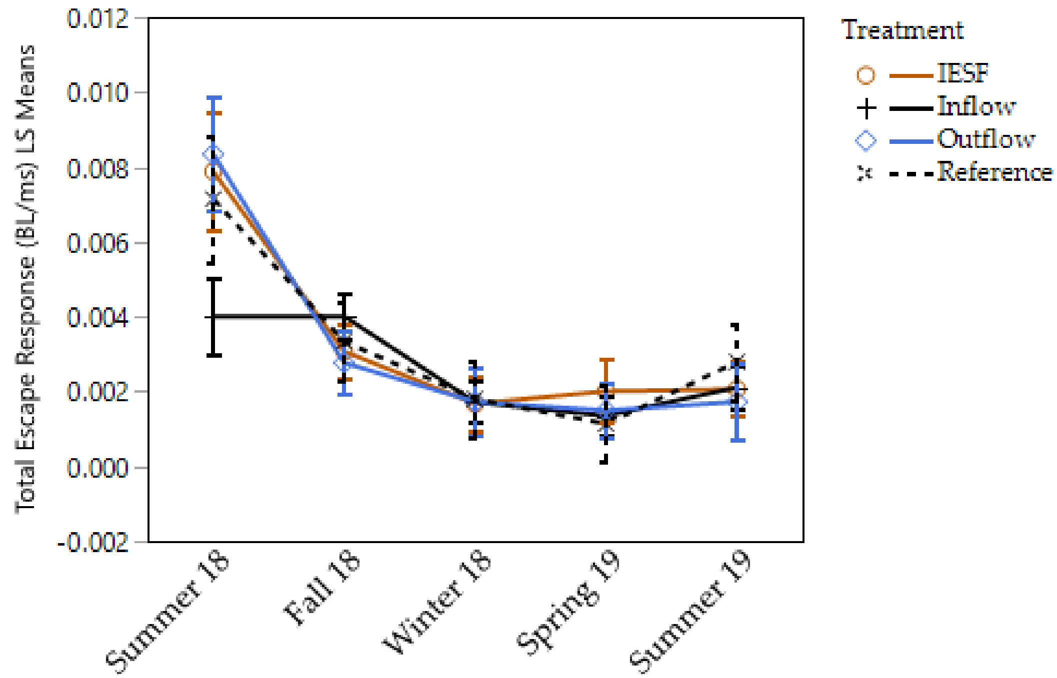

Figure 14. Plot showing the effect of interaction between season and treatment on the total escape response of fathead minnows exposed to urban stormwater runoff.

2.4 Discussion

I hypothesized that (i) biological effects would be greatest in summer and winter, with decreased effects in the transition seasons of fall and spring; (ii) urban stormwater runoff outflow treated with a standard retention pond will have fewer adverse biological effects than untreated stormwater inflow; (iii) treatment of urban stormwater runoff with IESF will further reduce adverse biological effects over standard retention pond outflow and untreated inflow.

Results of this experiment did not support the hypotheses. (i) Summer and winter did have the greatest biological effects, yet that varied from year to year, while the transitional seasons also showed adverse effects similar to summer and winter. (ii) Results for treatment were also inconclusive with non-significant improvement for biological outcomes following exposure to stormwater treated with standard retention ponds. (iii) The addition of IESF caused varied and unexpected results with fish exposed to IESF treated runoff showing decreased survival and an increased total escape response.

Despite indications of the positive impact BMPs could have on the CEC loads of stormwater, many questions remain regarding the seasonality of biological effects following exposure to urban stormwater runoff, and the mitigation potential of treatment using current BMPs.

Seasonality appeared to have the greatest effect on biological outcomes, with all dependent variables exhibiting a significant seasonal difference. This could be attributed to variations resulting from the inherent seasonality of storms. For instance, at the time of sampling for summer 2018 there was a combined total of 33 cm of precipitation from January – June, averaging to 0.18 cm/day. Whereas summer 2019 had considerably more precipitation with 79 cm from January – August, averaging 0.33 cm/day. Results of the experiment reflected this pattern of variability with fish exposed to runoff from summer 2018 exhibiting decreased survival when compared to runoff from summer 2019.

Previous stormwater studies have shown that variability and uncertainty in stormwater contamination is largely dependent on the amount and precipitation (Tiefenthaler et al. 2001). Likewise, a study by Launay et al. (2016) on the introduction of organic pollutants into surface waters found that personal care products and industrial chemical were diluted by rain events, while biocides and PAHs levels were increased indicating stormwater runoff as the likely source of pollution.

Results for growth were dependent on season, with the largest fish occurring in an exposure to stormwater collected in summer [18]. This effect could be attributed to density-dependent growth were fish in replicates with increased mortality have more available resources when compared to those in replicates with greater survival. This effect was also observed in a study that found body growth of laboratory reared zebra fish was largely density dependent (Hazlerigg et al. 2012).

Effects of stormwater exposure among seasons on shrimp consumption did not correlate with the precipitation pattern seen with survival. Fish consumed the most following exposure to runoff from fall (average 0.26 cm/day) and the least when exposed to runoff from summer[19] (average 0.33 cm/day). With no apparent effect from the amount of precipitation, it is likely that the effect is the result of the types or concentration of CECs present in rainfall at that time. Numerous studies have reported a “first flush” effect in which concentrations of contaminants are significantly higher during the first part of the wet season, or time of the year when most of the annual rainfall occurs (Lee et al. 2004; Soller et al. 2009; Schiff & Tiefenthaler 2011). In most cases this effect was greatest for metals (Soller et al. 2009; Schiff & Tiefenthaler 2011) as well as organics and minerals (Lee et al. 2004). Pollutant concentrations for initial storms were on average 1.2 to 20 times greater than concentrations toward the end of the season (Lee et al. 2004; Schiff & Tiefenthaler 2011).

Chemistry results for this experiment are not complete, however we can speculate on the types and concentrations of CECs based on previous studies. A pilot study (Westerhoff et al. 2018) found increased concentrations of CECs in summer and winter, with the least amount being found in fall. Furthermore, Westerhoff et al. (2018) also reported a distinct seasonality for the types of CECs present with increased sterols, flame retardants, and alkylphenols in fall and winter; and increased pesticide use in spring and summer. Likewise, a seasonal stormwater study found concentrations of suspended solids, lead, copper and cadmium were higher for snow melt, when compared to rain (Westerlund & Bäckström 2003). Similarly, Helmreich et al. (2010) found an increase in concentrations of Cu, organic carbon, suspended solids, pH values, and Zn during the cold season.

Treatment of stormwater by means of standard retention ponds did not significantly improve biological outcomes for juvenile fathead minnows over untreated inflow into the pond. However, although the effect was not significant this study showed there was a consistent improvement in biological outcomes following treatment of stormwater using standard retention ponds. This effect was expected with multiple stormwater studies reporting a decrease in contamination (suspended solids, nutrients, and heavy metals) following treatment with standard stormwater retention ponds (Van Buren et al. 1997; Pettersson et al. 1999). Removal of dissolved constituents, nutrients, suspended solids, metals, and organic contaminants occurred primarily during rain events indicating that removal appears to be influenced by sedimentation (Van Buren et al. 1997). Additionally, increased removal efficiency of suspended solids, nutrients, and metals was shown for ponds with greater surface areas (Pettersson et al. 1999).

The addition of plants has been shown to further aid in the removal of pollutants such as suspended solids, biological oxygen demand, total hydrocarbons, phosphorous, nitrogen, Cu, and PAHs (LaBarre et al. 2016; Leroy et al. 2016; Manka et al. 2016). Furthermore, stormwater ponds showed an increase in removal efficiency for metals, nutrients, and suspended solids following the establishment of aquatic vegetation (Kantrowitz & Woodham 1995). Additionally, constructed wetlands have been found to remove CECs including pesticides, pharmaceuticals, and estrogens (Gorito et al. 2017). However, removal efficiency varied depending on the compound with increased reduction for some pesticides (pentachlorophenol and endosulfan), and inefficient removal for others (atrazine) (Gorito et al. 2017). Similarly, removal of pharmaceuticals and estrogens varied with increased removal of estrogens, and negligible removal for the antibiotic azithromycin (Gorito et al. 2017).

Further treatment of runoff from retention ponds via IESF had conflicting and unexpected results. Survival appeared to be negatively affected with the worst outcomes following exposure to runoff treated with IESF. This effect could be attributed to iron leaching out of the filter. For instance, an iron concentration of above 1.5 mg/liter has been shown to cause a reduction in survival, growth, and hatchability in fathead minnows (Smith et al. 1973). Similarly, Dalzell & Macfarlane (1999) found that brown trout had physically clogged and damaged gills following exposure to iron sulfate used to control algae. Moreover, white suckers caged in a lake contaminated with iron ore tailings exhibited decreased growth when compared to those caged in a reference lake (Payne 2005).

In contrast, fish exposed to runoff treated with IESF were also shown to have an increased total escape response compared to those exposed to untreated inflow. This could be the result of the IESF reducing the number or concentration of CECs, which might suppress total escape response. The pilot study conducted by Westerhoff et al. (2018) showed treatment with IESF resulted in a decrease for both the number and concentration of CESs. However, in addition to a reduction of CECs, Westerhoff et al.

(2018) also reported a reduction in D. magna reproduction following exposure to runoff treated with IESF. Consequently, treatment of stormwater with IESF may result in negative outcomes for the survival, growth, and reproduction of aquatic organisms.

The addition of iron to IESF utilizes the process of sorption to improve the removal efficiency of dissolved phosphorus (Erickson et al. 2015). Phosphorus and metals may then have the opportunity to outcompete trace organics for IESF sorption sites based on electrochemical interactions (Reddy et al. 2014). Thus, the variability in biological outcomes following IESF treatment could also be attributed to the persistence of more bioavailable hydrophobic contaminants.

2.5 Conclusion

This study presented a unique set of challenges that need consideration. First sampling stormwater events from broadly distributed sites resulted in a variation in the amount of precipitation reported each site. Ideally the first portions of rain from the storm would be collected at the inflow, and then outflow would be collected following an appropriate retention time. However, due to the time required to collect water samples and the distance between sites, the collection for outflow had to occur just after the collection for inflow.

An analysis of fish exposed to urban stormwater runoff from differing seasons and treatment levels highlights the potential for current best management practices to diminish harmful biological consequences. This study represents a small fraction of current BMPs, including only standard retention ponds and retention ponds fitted with IESF installations. It is important to note that IESF relies on filtration and iron sorption more than on biological processes (which may take place within the pond and which are the focus of other BMPs including raingardens and bioswales). If IESFs are removing contamination as intended to improve the quality of aquatic ecosystems downstream, but are also causing adverse biological outcomes, then additional consideration is needed to determine how BMPs can be best utilized to improve the condition of aquatic environments.

Additionally, results of this study may also benefit those responsible for making decisions regarding the treatment of urban stormwater runoff. Often financial concerns are an important factor to consider when stormwater ponds are being constructed. Treatment of stormwater with IESF has been shown to be effective at removing dissolved phosphorous, while this study also demonstrated an IESF duality with decreased survival and increased in total escape response. As a result, those responsible for evaluating stormwater systems must consider the level of treatment required based on the needs of the watershed. If phosphorous fueled algal blooms are of principle concern, IESF may be a good choice. However, if the goal is preserving biodiversity, other treatments such as biofiltration may be a better option. This study highlighted the inherent variability of stormwater events, treatment outcomes of BMP, and the intricacies of using bioassays to assess effects of stormwater runoff which contains a complex mixture of pollutants.

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Appendix A

St. Cloud State University

Aquatic Toxicology Laboratory

Standard Operating Procedure - SOP

Water Sampling & Labeling for Analytical Chemistry

Introduction and aim of procedure

This SOP details the procedure to take and label water samples for analytical chemistry.

Supplies needed for assay:

- Sampling vials/ bottles/ containers (usually determined by analytical requirements)
- Labels
- Chain of Custody or Excel data sheet

Step 1 – General Considerations

- Water samples are taken for various reasons (embryo/ larval assay; extraction; analytical chemistry; etc.) which may require specific sampling techniques (i.e., larval assays)

- When water samples are taken for analytical chemistry, the overriding instructions are those provided by the analytical lab (for example: USGS Water Quality Lab Denver; Wooster College; AXYS Analytical, Canada) and should always be followed first. However, those instructions can be further augmented by the considerations below.

- The nature of the research of the Aquatic Toxicology Laboratory – using minute quantities of pollutants – requires extreme attention to cross-contamination hazards. Always (!) wear gloves; always start with control samples; always cap sampling containers quickly; always avoid any chance for cross-contamination!!!

Step 2 – Labeling

- Every sample needs to be labeled. Any label needs to include (i) identification of the Aquatic Tox Lab; (ii) a unique label code; (iii) date; and (iv) treatment code. Additional information may include: (a) approximate sample volume; (b) nominal concentration of chemical(s) in the sample; (c) duplicate sample; (d) other information either requested by the analytical chemistry lab or considered helpful for later purposes (i.e., weather conditions).

- Unique Label Code – this is NOT the same as the treatment name and date (Mix-Low 8.8.18 – is a treatment name that could be on the sampling container but is NOT a unique label). Here are the requirements for unique label codes:
    - Same number of characters for ALL samples (for example: three-digit number, dash, three letter code 111-aaa)
    - Brief to be easy to write, long enough to be fully unique for all samples
    - Code avoids any chance for confusion be separating letters from numbers and by underling the entire code
    - → MAKE SURE THE CODE IS DESCRIBED IN YOUR LAB NOTEBOOK!!!!

Step 3 – Sampling Considerations

- ALWAYS sample all treatments including carrier control and blank control
- ALWAYS sample in duplicate
- ALWAYS rinse bottle at least 3 times downstream before collection
- Store sample and duplicate sample in different places (i.e., different freezers) if possible.

- Apply label before you sample
- Always start with least contaminated sample and work up to highest concentrations (for example: blank > ethanol control > low > medium > high)
- Do everything to avoid cross-contamination – realize that your body is the most likely source for cross-contamination!
- Cap sample containers as soon as they are full
- Store sample containers appropriately (i.e., fridge/ freezer) as soon as possible
- Maintain a data sheet of all samples

Step 4 – Storage Considerations

- If possible, store duplicate samples away from main sample
- If cap was left loose for freezing, tighten as soon as sample is solidly frozen
- Ship samples to analytical lab as soon as possible (keep back duplicate sample)
- Make sure to fill out chain-of-custody forms and keep good records.

Appendix B

St. Cloud State University Aquatic Toxicology Laboratory

Fathead Minnow Larval Feeding Assay SOP

Introduction and goal of procedure:

The purpose of this SOP is to test the effects of any given water sample on the feeding efficiency of larval fathead minnows.

Necessary Supplies:

- Recently hatched live brine shrimp
- Larval (21 day old) fathead minnows exposed to sample water
- Dissecting microscope
- 6-well VWR sterile culture plate (~10mL volume wells)
- Pipette
- Microscope slides
- Stopwatch
- MS-222

Procedure

1. Two days before the assay

Start brine shrimp eggs (1 tsp salt, 1 tsp frozen eggs, 1 liter well water. Aerate in 1 lt Erlenmeyer flask)

2. The day before the assay

    1. Bring larvae to behavior analysis laboratory (ensure proper light cycle)
    2. Fill wells of VWR plate with 8ml of treatment water (3 wells per replicate)
    3. Carefully transfer one larva to each well (3 larvae per replicate)
    4. Allow time to acclimate before the assay (overnight)

3. The day of the assay

    1. Obtain live brine (approximately 150ml of shrimp from flask into separation funnel, strain/wash, and combine with ~50ml well water)
    2. Pipette single drops of shrimp mixture onto a microscope slide and count out **15±1** shrimp using a dissection microscope (record # on data sheet)
    3. Wash shrimp into well containing larva and start a **1-minute** timer
    4. After 1 minute immediately euthanize larva with ms-222
    5. Count remaining shrimp using a dissection microscope (record on data sheet)

Appendix C

St. Cloud State University Aquatic Toxicology Laboratory

C-start SOP

Introduction and goal of procedure:

The purpose of this SOP is to test the effects of exposure on the preditor avoidance performance of larval fathead minnows.

Necessary Supplies:

- High Speed Camera
- External Stimulus device
- Microsoft Excel
- Image J computer software
- Videos collected of C-start response
- MS-222
- Petri dish

Procedure

1.Bring larvae to behavior analysis laboratory the day before testing to acclimate. (ensure proper light cycle and air supply)

2.The day of testing position tanks and limit unnecessary light and movement to minimize disturbance of the fish

3.Very gently transfer the larvae to the testing arena (petri dish filled with aerated well water) under high speed camera

4.Give the fish approximately 1-2 minutes of acclimation time in the testing arena.

5.Arm the camera and stimulus device

6.Wait until the larvae is positioned in the center of the arena and staying still before delivering the stimulus.

7.If no C-start was observed try again up to 3 times before declaring it a "no response"

8.Save the video

9.Repeat the process until 3 larvae from each replicate have been tested

10.After testing euthanize larvae with MS-222

Digitizing C-Start Videos

1. Open the provided excel spread sheet titled "Template for C-Start Data"

2.Download ImageJ from http://rsbweb.nih.gov/ij/download.html

3. Open ImageJ

4. From ImageJ, open the video from the hard drive.

5. A window called "AVI Reader" will pop up- click OK

6. Video will load. If the video contains too many frames a new window will pop up saying "Out of memory." Click OK. (Only have one video open at a time- the AVI Reader can only read so many frames in total at a time; having another video open will grossly limit how many frames you'll be able to see in the next video.)

7. In the ImageJ menu window click on the box with the 5 yellow diamonds ("Point or multi-point selections"). Right click the red triangle and specify "point tool" That box should be highlighted while you work in ImageJ

8. Use "<" and ">" to move back and forth through time in the video window.

9. Scroll forward in time until the light in the corner comes on. Click on the center of the light the precise frame the light comes on. A yellow square should show up where you clicked.

10. Hit "M" to mark that point. A new window titles "Results" should show up. It will have an area, mean, min, max, x, y, and slice along the top. If you take a point and decide that it's wrong, highlight that row in this box and delete it. To delete the point from on the video push and hold Ctrl and click on point.

11. Push the magnifying glass button in the ImageJ menu window and put the cursor over the fish and hit the "+" sign to zoom in. After zooming, push the point selection button again.

12. Scroll forward in time (>) until the fish moves. This is usually best seen when the tip of the fish's nose moves. This decision is subjective- sometimes the fish jerks violently and it is easy determine when the fish moves. Other times the fish shows a weak reaction or no reaction at all to the stimulus. If there is no reaction, scroll to the end of the video and complete steps 1-14. If the reaction is weak, then scroll to when the fish first moves. If, at first, there is a weak reaction followed by a more prominent reaction, scroll to the more prominent reaction (when the fish jerks).

13. Measure 1mm: The fish is swimming on top of a grid. Place the cursor in a corner of a square near the fish (the refraction of light through water distorts the grid, so a measurement near the fish is better). Click on the corner and a yellow square should show up where you clicked. Then hold shift and click on a corner directly to the side of it. There should now be two yellow squares labeled 1 & 2 that mark two corners of a square. Click "m". These points should appear in the "Results" window at Points #2 and #3. In the X column, the numbers should be different. In the Y column, these two points should have the same number. If the numbers are different in the Y column, then your markers were not level. Delete these rows in the results and repeat the process.

14. Measure the length of the fish: Click on tip of the nose of the fish. Hit "m" to record the result. Click on the tail of the fish and then click on "m" to record the result. Be

careful not to click on the shadow of the fish- it's easier to scroll forward and back a few frames in order to see the tail move.

15. In the top left-hand corner of the video is the frame count. For instance, "257/391" means that you are on frame 257 out of 391. After taking the tail measurement, scroll forward 20 frames. Then click on the tip of the fish's nose. Click "m" to record the result.

16. Scroll forward another 20 frames. Click on the tip of the nose and click "m" to record the result.

17. Scroll back to just before fish reacts. Click on the Angle tool, then click on the tip of the tail and then click on the nose of the fish. A line should appear the length of the fish. Then scroll forward until the tail passes the across the and click on the nose again. Click "m" to record the result

18. In the results window, there should now be 8 points taken:

#1- when the light first comes on

#2 & #3- the length of 1mm based on the grid (when fish first moves)

#4- tip of the fish's nose when it first moves

#5- tip of the fish's tail taken at the same time as #4

#6- tip of the fish's nose after 20 frames

#7- tip of the fish's nose after another 20 frames

#8- angle of the fish (tail-nose-nose) (wait until the tail passes the line for second nose)

If the video is too short or there is no reaction, then complete points #1-5.

19. Select all the data from the Results window and copy.

20. Paste this data in the excel spreadsheet under the “original raw data” tab (make sure the fish ID matches the video). The data should begin in the “Point Number” column and should end in the column labeled “Count”.

21. Clear the contents out of the Results window.

22. Repeat for each video. It is advisable that you label each data set by the file name in the hard drive.

23. Mark any inconsistencies such as when the AVI Reader cannot read the file, the video is too short, etc.

24. Digitize Raw Data: In Excel, open the tab titled “Digitized Raw Data”.

From the original Excel page, copy everything from the Treatment Fish/Point number/X/Y/Angle/Slice columns and paste it in the Digitized Raw Data page under the same headings.

25. Analysis of Data: In Excel, open the third tab titled “Analysis.”

Copy everything (Treatment Fish/Point number/X/Y/Angle/Slice) from the "Digitized Raw Data" tab and paste it in column J-Q. the data will then be transferred to the appropriate columns A-H.

Make sure to enter the Treatment/Replicate (#)/Trial ID (A,B,C).

example (5.9.19_BIR_IN_1_A) Treatment=BIR_IN, Replicate=1, Trial=A

For the videos that had issues (i.e. no reactions, false starts, video could not be opened) list those in the appropriate rows.

Highlight videos that have a latency of less than 10. Those might be false starts and should be noted.