A Study of Surface Run-off from U.S. 31 and Seaway Drive on Little Black Creek, Muskegon County, MI

Final Report

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Executive Summary

Storm water runoff is a major contributor of pollutants to water bodies in the United States. In this study, we conducted a variety of field and laboratory assessments between 2008 and 2011 to determine the ecological impacts of storm water that enters Little Black Creek (LBC), a historically impaired water body in the Mona Lake Watershed in Muskegon County, MI. In particular, we examined storm water runoff from two major thoroughfares, U.S.31 and Seaway Drive (Business U.S. 31) into LBC. Our study examined water quality and quantity characterization, a geomorphic assessment, a toxicity assessment of the runoff water, an engineering assessment, and a suite of environmental analyses, consisting of a) laboratory algal bioassays, b) field surveys, c) mesocosm experiments, and d) laboratory fish experiments. In addition to collecting runoff during storm events, we also collected snow along the highways to examine the environmental impacts of snowmelt.

Water Quality and Quantity. The water quality of the storm water indicated that concentrations and loads of several key pollutants, including total phosphorus and several heavy metals, were elevated in storm water. Increases were more apparent at U.S. 31 than Seaway Drive; the difference in storm water effects can be attributed to the watershed position of the sites. The Seaway Drive site, positioned near the bottom of the watershed, receives storm water from the majority of the watershed, which overwhelms the influence of localized inputs from Seaway Drive. In contrast, U.S. 31 crosses LBC in the approximate middle of the watershed, upstream of the densely-populated urban areas of Muskegon and Muskegon Heights, and receives less storm runoff from upstream sources.

Although storm water from our study sites contained elevated concentrations of pollutants that are potentially harmful to aquatic life, it did not result in downstream concentrations that exceeded Michigan water quality standards. Oil and grease, and polycyclic aromatic hydrocarbons (PAHs), are contaminants of concern in road runoff, but our data suggest they are not a significant issue for LBC at our study sites. Average PAH concentrations in storm water were very low in our study (6-15 µg/L), and at times were below detection in storm water runoff. Depending on the duration and volume of snowmelt events, the potential exists for episodic stress to biota during these events. Snow collected from the roadside at our sites contained concentrations of chloride, copper, and zinc that exceeded state standards for acute effects to aquatic life. With concentrations 2-5X greater than the acute standard, chloride is the pollutant most likely to have negative effects on biota during snowmelt events.

Total phosphorus concentrations and loads were very high in both storm water and in LBC during storms. This elevated TP may have limited, episodic, effects on biota in LBC, but likely has greater consequences for Mona Lake, which is the receiving water body for LBC. TP concentrations exceeded the eutrophic threshold during base flow at the Seaway Drive site, and increased to hypereutrophic levels during storm events. Snowmelt TP concentrations were ~4X the hypereutrophic threshold, suggesting that melting events have the potential to deliver an intense pulse of TP to the system. This

phosphorus subsidy from snowmelt may be an important catalyst for spring phytoplankton growth in Mona Lake.

The amount of precipitation measured in sampled storm events ranged from small (0.07 in) to moderately large (1.04 in). As expected, total storm water volume, which includes storm water inputs from the study sites plus all upstream inputs over the entire duration of the storm, was directly related to rainfall amount. The percentage of flow composed of site-specific storm water was 3 to 34% at the Seaway Drive site and 13 to 50% at the US 31 site. Average storm flow discharge in LBC during the period of active road runoff (i.e., our sampling period) ranged from 0.01 to 0.26 m^3/s upstream and 0.02 to 0.38 m^3/s downstream at U.S. 31. Average storm flow discharge at the Seaway Drive site was greater, and ranged from 0.31 to 0.90 m^3/s, both upstream and downstream of the storm water outfall. Storm water contributed lower percentages at Seaway because of this site's location in the watershed; its placement near the bottom of the watershed leads to more flow coming from upstream. Of course, since some of the water reaching this site from upstream is also composed of storm water runoff, there is actually more storm water at Seaway than just the 3 to 34% contribution attributed strictly to runoff at the site. Unfortunately, we do not have the data to estimate with accuracy the degree of total storm water contribution (i.e., upstream and site-specific).

Storm flow duration in LBC was directly related to total storm water volume, with the longest storm pulses lasting over 50 hours. The extended period of storm flow during higher-rainfall events suggests that storm water detention may be occurring in the watershed, allowing for infiltration and helping to reduce extreme (i.e., "flashy") flows.

Geomorphic Assessment. Storm water from U.S. 31 resulted in increased suspended sediment concentrations (SSC) and loads in LBC; however, downstream concentrations remained below the 80 mg/L suspended sediment target for wet-weather events in LBC. Suspended sediment concentrations were extremely high in snowmelt, contributing to the aforementioned possibility of episodic stress to biota during snowmelt events. Our data show that bedload is the dominant form of sediment being transported in LBC. Substantial increases in bedload were measured downstream of the storm water outfalls at both locations. Storm water SSC did fall into the 'less than moderate' range for the protection of fish communities, suggesting the possibility of impairment.

Toxicity Assessment. Storm water runoff was toxic to *Ceriodaphnia dubia* during the winter and spring samples at the Seaway site but no toxicity was measured at the U.S. 31 site. This may be because runoff at this location is diluted by groundwater inputs. As a consequence, concentrations of metals and chloride were lower at U.S. 31 than at Seaway. In contrast to precipitation runoff, *snowmelt* from both locations was toxic to *C. dubia*.

Toxicity in our study was correlated with chloride, chromium, copper, nickel, and zinc. Additional testing involving the Toxicity Identification Evaluation (TIE) would be required to determine if chloride and/or metals were the toxic agent(s). Toxicity is usually strongest during the first flush, as runoff begins. In our study, we prepared just one flow-proportioned composite sample to represent the entire event; consequently, the toxicity of the discrete samples was not determined and we may have underestimated toxicity associated with first flush.

Engineering Assessment. Most of the toxicity associated with storm water and snowmelt from U.S. 31 and Seaway was associated with the fine particulate phase and attributed to heavy metals and, to a lesser extent, PAH compounds. Both of these materials have a high affinity for suspended solids. The results of the engineering assessment suggest that filtration and settling will remove the majority of toxic effects associated with storm water; however, storm water would need to be retained for at least 48 hr to be effective—this might require a settling lagoon or retention basin with a relatively large footprint. Given the magnitude of storm flows and the urban setting of the highways, the ability to locate a large settling pond in the vicinity of Little Black Creek may be limited. An alternative solution is baffled settling tank units, which provide a combination of screening and settling to remove pollutants associated with fine particulates. More detailed modeling, combined with a cost-benefit analysis that includes long-term maintenance costs and environmental benefits, is needed to determine the most appropriate BMPs and their locations.

Environmental Analyses. The environmental analyses consisted of laboratory algal bioassays, field surveys, mesocosm experiments, and laboratory fish experiments.

Lab algal bioassays revealed that snowmelt water was toxic to *Pseudokirchneriella subcapitatum;* heavy metals and chloride are the suspected causative agents. Since the snowmelt occurs during the winter when algal productivity is relatively low, the impact to stream autotrophs is likely limited, but there may be significant negative effects on invertebrates, which we did not measure.

The field surveys were conducted in 2008 and 2009 at both the U.S. 31 and Seaway Drive road-stream crossings. We sampled algae both upstream (control) and downstream (treatment) of the storm water outfall. Overall, storm water runoff did not have a strong effect on algal biomass or metabolic activity. The overall community composition was not significantly affected by location upstream or downstream of the storm water pipe, although some taxa were slightly affected by storm water, suggesting that community composition is a more sensitive measure of water quality than biomass. The design of our surveys did not allow us to separate the chemical effects of storm water from the hydrologic effects of increased flow, so it is difficult to know what influenced the algal samples the most. Either increases in current velocity or increases in metals concentrations downstream of the storm water pipe had a slight negative impact on the algae at these sites. Because of the variable nature of storm water runoff, this type of experiment is very context-specific, with the results at each study site heavily influenced by the composition of runoff water from a specific location.

The mesocosm experiments were conducted in indoor, replicated, 1300-liter fiberglass tanks (mesocosms). Experimental treatments consisted of 100%, 50%, and 0% (control) storm water, with control water coming from Muskegon Lake. Experiments were conducted in both 2008 and 2009 and lasted 31 and 28 days, respectively. Algae, snails, and fish (pumpkinseed sunfish) were exposed to the storm water treatments in each tank under a variety of nested treatments. Overall, we observed limited ecological effects of storm water. It is likely that the concentrations of metals in the storm water runoff were not high enough to negatively impact the biota. In 2008, only the concentration of Cu in the storm water pipe sample exceeded Michigan water quality standards for chronic exposure. In 2009, only the concentrations of Cu and Pb in one storm water pipe sample exceeded Michigan water quality standards for chronic exposure. Given that the storm runoff was collected from a major storm and during first flush, when concentrations should be relatively high, changes in hydrology, such as the increased frequency and magnitude of erosive flows, may have a greater impact on algal communities in this natural setting than the chemical composition of the storm water alone.

The laboratory fish study consisted of five experiments to determine impacts of storm water runoff on central mudminnow, one of the most abundant fishes in LBC. Storm water was collected from 3 rain events and roadside snow was collected in 2009 and 2011 to investigate the effects of snowmelt. Overall, storm water did not impact actual or instantaneous *growth* of central mudminnows in any of the experiments. However, storm water did impact *survival* of central mudminnows in some of the experiments. Both the summer 2008 and the 2009 snowmelt trials had significant mortality that we attributed to runoff source and concentration. In contrast, the 2011 snowmelt experiments did not affect growth or survival, but did show evidence of effects on condition of central mudminnows.

The lack of a strong effect of storm water on biota is likely because the concentrations of contaminants in the storm water runoff were not high enough to have negative impacts. There were occasional significant effects on some biotic responses, but they varied with time, space, and response variable, suggesting the ecological impacts of storm water runoff are very context-specific. Based on our field surveys, laboratory bioassays, and mesocosm experiments, the chemical concentration of storm water entering LBC from U.S. 31 or Seaway Drive has limited effects on stream biota impairment. However, altered hydrology associated with runoff, resulting in dislodgement of attached organisms, erosion of streambanks, and movement of sediment, all appear to be impacting the biota in Little Black Creek. As a consequence, we provide the following list of recommendations for consideration:

- Restore natural hydrology to the greatest extent practicable, by working with the Muskegon Area Municipal Storm Water Committee (MAMSWC) on identifying and implementing storm water retention best management practices (BMPs) in the watershed and determining if the discharge rates at the U.S. 31 pump station can be modified to reduce damaging storm water flows while still controlling groundwater discharge.

- Control the snowmelt to reduce toxic inputs by placing snow piles in locations where snow melt will flow on to pervious surfaces and not directly reach streams.
- Manage the wetland habitat to maintain a more natural flow regime and improve habitat by maintaining the existing wetlands and restoring or creating fringing wetlands throughout watershed
- Install cost-effective structural BMPs, such as hydrodynamic separators, to remove sediments at select locations

I. Introduction

Urban areas, and the human populations inhabiting them, are expanding around the world. The majority of humans now live in cities (UNPD 2005) and it is estimated that in the next 25 years, 1.7 billion people will move into urbanized regions (McDonald 2008). Activities associated with urban land use frequently involve polluting industries and dense road networks, as well as altered vegetative cover and hydrology (Booth et al. 2004). Although the total area of urban landscape is relatively small, its overall impact on stream health is disproportionately large (Paul and Meyer 2001). Urbanization creates large areas of impervious surface, such as parking lots, roofs, and roads that prevent the infiltration of water into the ground. Instead, water moves quickly off of these hardened surfaces, picking up pollutants along the way, and flows either directly into a stream, or into a storm drain and then into a stream. As a consequence, urban streams often exhibit symptoms indicative of impairment; collectively, these symptoms have been referred to as the "urban stream syndrome" (Walsh et al. 2005), which include changes to water chemistry, stream channel morphology, hydrology, and biotic richness (Paul and Meyer 2001; Walsh et al. 2005).

Storm water runoff from impervious surfaces is one of the major impacts on urban streams (Meyer et al. 2005). Urban snowmelt is another source of contaminated water affecting streams during winter and spring in temperate climates (Bennett et al. 1981, Brezonik and Stadelmann 2002). During storms and/or snowmelt, accumulated contaminants can wash off of impervious surfaces and into streams, thereby contributing nutrients, heavy metals (e.g., Pb, Zn, Cr, Cu, Mn, Ni, Cd), pesticides, and toxic organic compounds such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). Snowmelt has been shown to have higher (Brezonik and Stadelmann 2002) or lower (Bennett et al. 1981) pollutant concentrations than storm water. High concentrations of suspended solids, phosphorus, Pb, and Zn in urban snowmelt have been attributed to the use of sand and salt on roads in winter (Oberts 1986). These compounds potentially become major chemical stressors to the aquatic ecosystem and impact both water quality and stream ecosystem structure and function (Taylor et al. 2004, Walsh et al. 2005, Christensen et al. 2006).

Changes to stream hydrology also result from urbanization. Urban streams tend to have more frequent flow events in which peak discharge is larger and occurs more quickly (i.e., "flashier") than in non-urbanized watersheds (Walsh et al. 2005). This occurs because large areas of impervious surface and storm water pipes efficiently transport water into streams, much of which would naturally infiltrate the groundwater and slowly make its way to the stream (Paul and Meyer 2001). The magnitude and frequency of flow events regulate many ecological processes in streams, and alterations of these flows because of storm water run-off can lead to the scouring or wash-out of benthic biota, the loss of sensitive species, and the possible disruption of life cycles (Poff et al. 1997).

Stream degradation caused by urbanization has important consequences for both human and ecological health. Most stream ecosystems, including urban streams, provide ecosystem services such as primary and secondary production and leaf litter breakdown, carbon cycling, and removal of nutrients from the water column; these services allow waterways to be used by humans for municipal, industrial and agricultural purposes (Palmer et al. 2004, Meyer et al. 2005). Urban streams also provide opportunities for recreation and aesthetic enjoyment not always present in urban settings (Meyer et al. 2005). To continue providing ecosystem services in an urban environment, streams need to be protected or restored to a healthy state (Walsh et al. 2005).

Little Black Creek (LBC), an urban stream located in Muskegon County of west Michigan, served as the location for our study. This creek receives runoff from a federal highway (U.S. 31) and Business U.S. 31 (Seaway Drive). Because of prior industrial activities, the sediments in LBC are heavily contaminated (Steinman et al. 2006a), and represent both an ecological and human health risk to the region. In particular, LBC sediments are highly contaminated with cadmium, chromium, lead, PAH compounds, including benzo(a)pyrene, and PCBs. Most of the samples have contaminant concentrations that exceed the standards generally applied for the protection of aquatic life. In addition, concentrations of lead, benzo(a)pyrene, and cadmium are at levels that exceed human health criteria for long term direct contact.

Contaminated sediments can pose a limited risk to human and environmental health if they remain in place and are covered with stable layers of clean sediment. However, data collected over the past several years indicate that these toxic sediments are moving downstream and that the problem is not localized (Steinman et al. 2006a). One of the major reasons for sediment movement is erosion associated with water run-off from the highly concentrated road network in this region. Water moves quickly off these impervious surfaces and enters either directly from surface run-off or collects first through storm drains and then enters LBC. The erosive force of this run-off scours the sediments from the streambed, transports them in the water column, and redeposits them further downstream or to Mona Lake. Data collected in 2003 show that sediment contaminant concentrations in Mona Lake have increased dramatically since 1980, and to levels that have a high probability of causing ecological impairment (Steinman et al. 2006a); although that study did not examine the source of these contaminants, it is likely that LBC was a contributor given its past history and paucity of other contaminated tributaries.

Although we know that storm water run-off from the highly concentrated road network is resulting in environmental degradation in the LBC basin, it is not known which roads and associated storm drains are most responsible for these impacts. This study was designed to determine the extent of environmental impairment caused by road-induced runoff, and the associated industrial activities in the basin, so that the appropriate restoration and remediation activities can be implemented, if appropriate. The impacts of road runoff were studied at two locations where LBC crosses major roadways.

II. Methods

II.A. Site Description

LBC is one of the major tributaries in the Mona Lake Watershed, a small, impacted basin in west Michigan that connects directly to Lake Michigan (Fig. II.A.1). This second order stream flows through heavily urbanized areas, including portions of the cities of Muskegon and Muskegon Heights (Steinman et al. 2006a). LBC has an 18 km^2 catchment, of which 66.2% is developed; impervious surfaces cover 32.1% of this developed land area (Steinman et al. 2006a). Numerous industries are located adjacent to LBC and currently discharge storm water into the stream from 19 outfalls (Steinman et al. 2006a). The sediments in LBC are contaminated with a number of metals and organic chemicals that arose from a petroleum refinery, storm sewers draining foundry and metal finishing industries, a plating Superfund site, a municipal sanitary/industrial wastewater pump station, and a closed municipal landfill without a leachate collection system (MDEQ 2000, 2002). LBC is included on the Michigan Department of Environmental Quality's 303(d) list of impaired water bodies for a degraded benthic community (MDEQ 2003).

We had two study sites located at crossings of LBC and major roadways: Business U.S. 31 (Seaway Drive) and U.S. 31 (Fig. II.A.1). The study sites consisted of three sampling locations, one upstream of a storm water pipe, one downstream of the pipe, and one storm water location (see section II.B. for details) (Figs. II.A.2,3). The Seaway study site was a ~150 m section of stream that included a storm water outlet pipe, a sampling site ~ 97 m upstream of the pipe, and a sampling site ~53 m downstream of the pipe. Seaway Drive is a two-lane divided road with average daily traffic (ADT) of 25,300 vehicles per day (MDOT 2007). During storm events, water from an 8,094 m^2 area of Seaway Drive drains into LBC (Prein and Newhof 2007). The U.S. 31 study site was a ~40 m section of stream consisting of the storm water input pipe, a sampling site ~24 m upstream of the pipe, and a sampling site ~15 m downstream of the pipe. U.S. 31 is a four-lane divided highway and has an ADT of ~61,000 vehicles per day, which is near the top of the range for Muskegon County (range = 4,160-62,644 ADT; MDOT 2007). While the ADT on U.S. 31 is substantially lower than that of highways passing through larger urban areas such as Detroit, MI (~124,000 ADT) or Chicago, IL (~250,000 ADT), it is substantial for a moderate-sized metropolitan area such as Muskegon (IDOT 2003, MDOT 2007). During storm events, water from a 95,101 m^2 area of U.S. 31 drains into a pumping station (Prein and Newhof 2007) and is then discharged into LBC through a storm water outlet pipe.

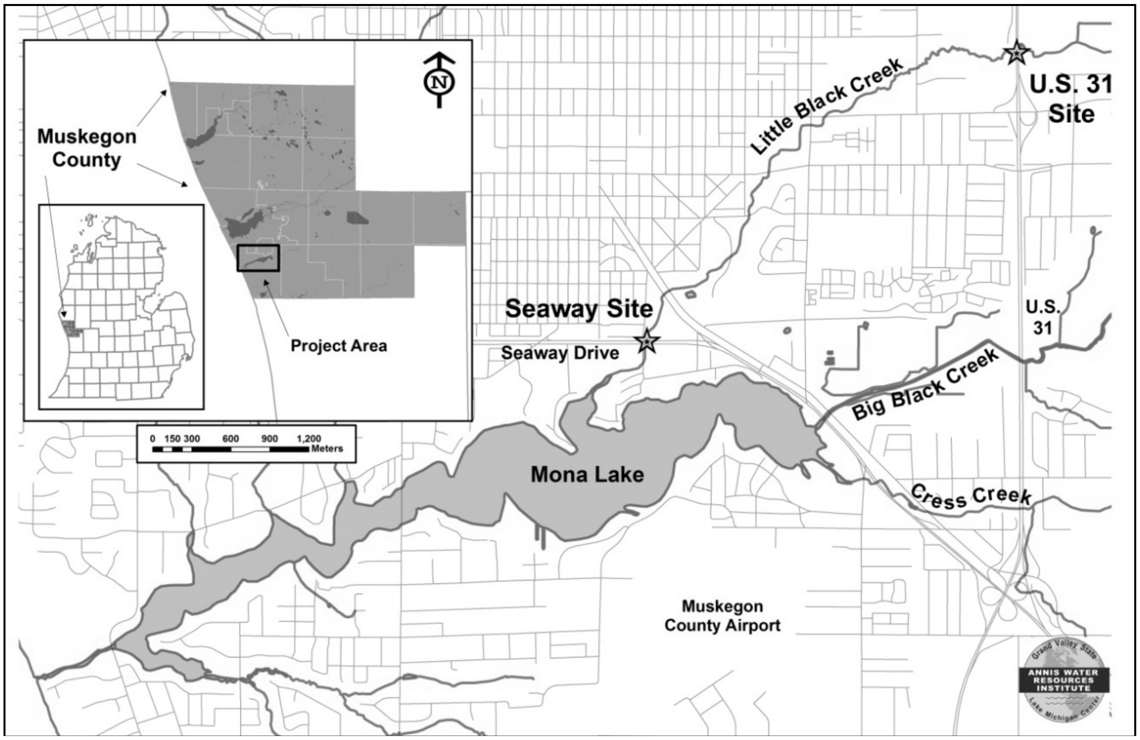


Fig. II.A.1. Mona Lake and three main tributaries: Little Black Creek, Big Black Creek, and Cress Creek. Study sites on Little Black Creek are shown by stars (Seaway and U.S. 31 sites). Small inset: Location of Muskegon County (shaded) in Michigan's lower peninsula. Large inset: Muskegon County, showing location of Mona Lake (enclosed in box).

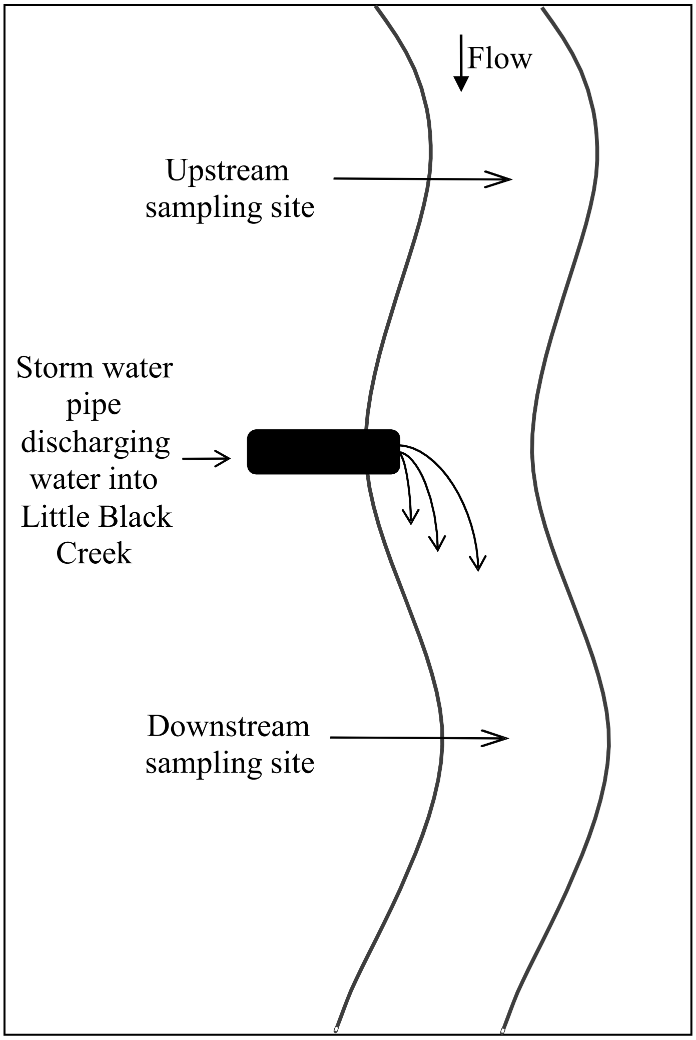


Fig. II.A.2. Schematic diagram of the basic layout of both the Seaway and U.S. 31 study sites. Both study sites consisted of a stream segment with sampling site upstream and downstream of a storm water pipe. The upstream and downstream sampling sites were the upper and lower bounds of the study site, respectively. See II.A.3 and text for details relating to each site. Diagram is not to scale.

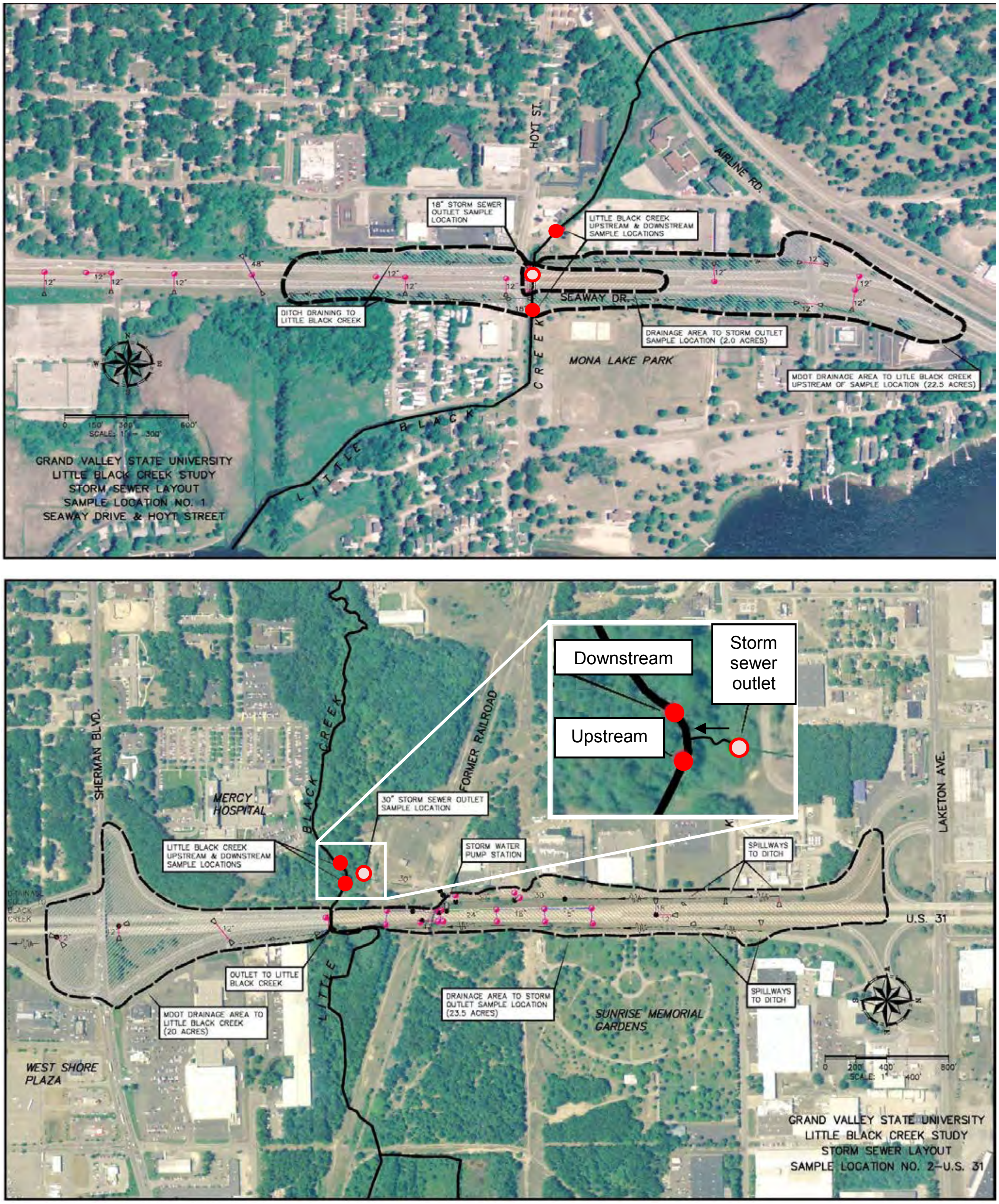


Fig. II.A.3. Satellite images of the two sampling sites, showing upstream and downstream locations (red dots),storm sewer pipes (red/white dots), and drainage areas (dashed lines). Top: Seaway Drive site; Bottom: U.S. 31 site. Note that north is on the right on the U.S. 31 map.

II.B. Water Quality and Quantity Characterization

Base flow sampling was conducted approximately monthly from April 30, 2008 – April 8, 2009, with a total of 11 base flow events. Grab samples for water quality analysis were collected from only the upstream location at Seaway Drive. Because the pump station at U.S. 31 continually discharges a small amount of groundwater, we sampled both the upstream and downstream locations at this site in order to characterize any differences in water quality caused by the groundwater discharge. During two base flow events (March 13 and April 8, 2009), water samples were also collected from the outfall pipe to characterize the groundwater discharge. Samples were collected in two 4 L glass jars and kept on ice until we returned to the laboratory for processing and analysis. A YSI 6600 sonde (Yellow Springs Instruments, Yellow Springs, OH) was used to measure dissolved oxygen (DO), temperature, pH, specific conductance, total dissolved solids (TDS), redox (ORP), turbidity, and chlorophyll *a* (Chl *a*).

In the lab, the two 4 L water samples from each site were poured into an 8 L Teflon churn splitter (Carnet Technology, Terry, MS), mixed thoroughly, and dispensed into sample bottles for water quality analysis. Alkalinity samples were titrated immediately. Samples for chloride (Cl), sulfate (SO_4), and nitrate-nitrogen (NO_3-N) were syringe filtered through a 0.45 μm nylon membrane and frozen until analysis by ion chromatography on a Dionex ICS 2100 (U.S. EPA 1993a). Ammonia-nitrogen (NH_3-N) analysis was conducted using the automated phenate method (U.S. EPA 1983) on a Braun+Luebbe AutoAnalyzer III. Total phosphorus (TP) and soluble reactive phosphorus (SRP; filtered and frozen, as above) was analyzed on a Bran+Luebbe AutoAnalyzer III (U.S. EPA 1983). Chemical analyses for metals (cadmium [Cd], chromium [Cr], copper [Cu], nickel [Ni], lead [Pb], zinc [Zn]), polycyclic aromatic hydrocarbon (PAH) compounds, and oil and grease were conducted using SW-846 (U.S. EPA 1994) methods. Water samples for total metals analysis were digested prior to analysis, conducted on a Perkin Elmer AAnalyst 800 Graphite Furnace. Suspended sediment concentration (SSC) samples were filtered using GF/A glass fiber filters and analyzed according to U.S. EPA (1983). Hardness was determined using the EDTA Titrimetric Method (U.S. EPA 1983).

Water quality was also sampled during 7 storm events over the study period. Water samples (two 4 L glass jars) were collected every 30 minutes from the upstream location, downstream location, and storm water outfall pipe. Upstream and downstream water was collected by grab sampling at both sites. Pipe water was collected by grab sampling at U.S. 31. At Seaway Drive, a weir was used to direct storm water runoff from the road into a 4 L glass jar that was submerged in the shoulder of the road. An automated sampler (Isco 6712, Teledyne Isco, Lincoln, NE) was used to collect water from the jar. At U.S. 31, sampling began when we observed an increase in water flow and turbidity in the storm water outfall and ended when water clarity improved and flow decreased to approximately base flow levels. At Seaway Drive, sampling began when water began flowing from the road into the glass collection jar and ended when runoff stopped. Storm samples were kept on ice until returned to the laboratory for processing and analysis.

General water quality parameters were measured with a YSI 6600 sonde, as described above, when water samples were collected.

In the lab, a composite water sample was created for each location (upstream, downstream, pipe) by flow proportioned-subsampling of half-hour samples, as follows. From our modeled discharge data (see below), we determined the percentage of total storm flow volume for each half-hour of the storm. Half-hour percentages were multiplied by 14 L (our target composite volume) to determine the appropriate flow-proportioned aliquots to collect from each half-hour sample. Half-hour water samples (two 4 L jars) were poured into an 8 L Teflon churn splitter, thoroughly mixed, and the flow-proportioned aliquots dispensed into a 14 L Teflon churn splitter. Once all half-hour samples were flow-proportioned, the composite storm sample was thoroughly mixed in the 14 L churn splitter and dispensed into sample bottles for water quality analysis, as described above.

Submersible pressure and temperature recording systems (Odyssey, Christchurch, New Zealand) were installed within PVC stilling wells at the upstream and downstream locations at both sites. Pressure was logged at 10-minute intervals throughout the study period and corrected for atmospheric pressure. Stream stage was measured manually during each visit using staff gauges attached directly to each stilling well. Atmospheric-corrected pressure readings were regressed against measured stage values and the resulting linear function was applied to the entire record of pressure readings to yield a high-frequency record of stream stage for the study period.

To determine stage-discharge relationships, manual flow measurements were taken at upstream and downstream locations over a range of stages from base flow to storm flow, with a total of 10-13 measurements per location. Water depth and velocity were measured at twelve equally-spaced points along permanent transects using a Marsh-McBirney Flow Mate 2000 flow meter attached to a top-setting wading rod, according to USGS protocols (Rantz et al. 1982). The Windows-based hydrologic software, HYDROL-INF (Chu 2006) was used to calculate stream discharge. Stream stage was converted to discharge by first calculating rating curves between stage and discharge and applying these functions to the high-frequency stage records. The result was a hydrograph for each location over the study period.

Loading rates for major nutrients, metals, PAHs, and oil and grease were calculated by multiplying constituent concentration by discharge at the time of sample collection. Discharge from the storm water pipes during storm events was calculated as the difference between downstream and upstream discharge at each site.

Storm water volume was calculated in two ways: site-specific and total storm event volume. Site-specific storm water volume was calculated using storm water pipe discharge for each 30-minute sampling interval and summed. This value represents the volume of road runoff from the storm water pipe during a given storm. Total storm event volume was calculated using the discharge data derived from the continuous pressure loggers at the downstream location at each site; water volume was calculated for each 10-minute time interval over the entire duration of the storm, beginning at the rise over base flow and continuing until the return to base flow, and summed. This value represents the total volume of storm water that entered LBC from all locations upstream of each study site.

In addition to base flow and storm sampling, snowmelt water quality was characterized in February 2009 (both sites) and February 2011 (U.S. 31 only). Snow was collected using snow shovels and was placed in multiple 50-gallon plastic tubs. Prior to snow collection the plastic tubs were washed and rinsed with tap water. At the Seaway site, snow was gathered from piles on the road shoulder near the crossing of LBC. At the U.S. 31 site, snow was gathered from piles on the shoulder of the highway on-ramp from Laketon Ave. The tubs were transported to AWRI and the snow from each site was placed indoors in a fiberglass mesocosm tank with a volumetric capacity of ~1,300 liters. Indoor temperature was held at 15.5º C and the snow was allowed to melt over several days. General water quality parameters were measured with a YSI 6600 sonde and water samples were collected in 4 L glass jars for analysis, as described above.

II.C. Geomorphic Assessment

Sediment dynamics were characterized by measuring both suspended and bedload sediment. During each base flow and storm event, samples for analysis of suspended sediment concentration (SSC) were dispensed from the churn splitter (see above). SSC samples were filtered using GF/A glass fiber filters and analyzed according to U.S. EPA (1983). Suspended sediment loads were calculated by multiplying SSC by discharge. Bedload was determined during each base flow and storm event. Bedload subsamples (1-minute duration) were collected using a 3x3" Helley-Smith sampler at five equally-spaced points across the stream at each site (5 minute total sampling time) and composited. During storm events, five bedload subsamples were collected hourly and composited to create one bedload sample for the entire event. Bedload sediment was dried at 105 °C for 24 hours and weighed. Sediment was then ashed in a muffle furnace at 550 °C for 24 hours to remove organic matter and re-weighed. Instantaneous bedload transport rate (Q_b) in kg/s was calculated as:

$$Q_b = \frac{M_b}{T} \times \frac{1}{N} \times \frac{W}{0.076\ \text{m}}$$

where M_b is the total mass of bedload sediment in kg; T, subsample duration in s (i.e., 60); N, number of subsamples; W, wetted width of the channel in m; 0.076 m represents the width of a 3x3" Helley-Smith sampler opening.

II.D. Toxicity Assessment of the Runoff Water

Water samples collected during base flow (n=11) and storm events (n=7) (see section II.B for details) were evaluated for toxicity. Toxicity tests using *Ceriodaphnia dubia* were performed according to USEPA (2002) guidelines. Test animals were obtained from Aquatic Biosystems of Fort Collins, Colorado and maintained in internal laboratory cultures. One week prior to test initiation, neonate (<24 hours old) water fleas were isolated from brood stock cultures and placed in individual holding cups containing clean culture water and food. Neonate selection for continuing culture is based on overall health and reproductive performance of the individuals in the current brood stock culture. Cups containing isolated females were placed in a polypropylene rack and the entire rack was placed in a temperature-controlled room maintained at 25 ± 1°C. Isolated females were transferred daily to cups containing fresh water and food. Neonates produced within the previous 24 hours were selected for testing if produced by individuals that had at least 3 broods of 8 or more neonates each over the course of the previous week. Organisms were fed a mixture of YCT (yeast-Cerophyll®-trout chow) and a suspension of *Pseudokirchneriella* algae daily according to EPA protocol guidance. The toxicity tests were carried out in 30 ml polystyrene cups with 15 ml of test solution. Each concentration had 4 replicate chambers. At test initiation, five neonates were transferred to each test chamber. Test chambers were then covered with clear Plexiglas™ covers and placed in a temperature-controlled room maintained at 25 ± 1 °C. Light was provided with cool-white fluorescent bulbs and maintained on a 16:8 hour light:dark cycle.. Control and dilution water consisted of Perrier®. The dilution series consisted of 6.25%, 12.5%, 25%, 50%, and 100% treatments. Water quality parameters of pH, DO, ammonia, conductivity, and temperature in addition to organism mortality were measured and recorded daily.

Toxicity data were evaluated for significant differences using Dunnett's multiple comparison test, provided that the data met criteria for homogeneity of variance and normal distribution. Data that did not meet these criteria were analyzed by the non-parametric Steel's Many-One Rank or Wilcoxon's tests. LC50s were calculated by Probit and Trimmed-Spearman Karber methods (ASTM 1995). Correlations between environmental variables and toxicity data were evaluated with Spearman's rho. Statistical analyses were conducted with SPSS 15.0 (SPSS Inc., Chicago, IL) and Toxstat 3.5 (Gully 1996)

In addition, toxicity data were assigned categories of Not Toxic, Potentially Toxic, and Significantly Toxic (Doherty et al. 1999). The Not Toxic category (0±10% response) was established on the basis of test acceptability for control treatments of standard biomonitoring tests. An acute *C. dubia* test is considered invalid if control organisms experience > 10% mortality by the conclusion of the test. The Potentially Toxic category (> 10% - < 25%) response spans the difference between the upper limit of the not toxic category and the lower limit of the significantly toxic category. The lower limit of the significantly toxic category (25-65% response) represents the most conservative percentage response at which there would be a statistically significant level of mortality in a sample treatment relative to the control as determined by Fishers exact test (assuming 0% mortality in the control treatment and a sample size of 20 individuals per treatment; Gulley 1996). The upper limit of the Significantly Toxic Category (>65%-100% response) represents the minimum level of response at which LC50s and EC50s can be reliably generated (ASTM 1995).

II.E. Engineering Assessment

Treatability Evaluation Methods

Treatability studies were performed on samples from 1 storm event and 1 snowmelt according to methods outlined by Pitt et al. (1995). The following treatability tests were performed:

1. Settling column (37 mm x 0.8 m Plexiglass® columns),
2. Filtration (series of II stainless steel sieves from 63 to 1,000 µm and a 0.45 µm membrane filter),
3. Photodegradation (2-L glass beaker with a 60-W broadband, incandescent light placed 25 em above the water, stirred with a magnetic stirrer with water temperature and evaporation rate also monitored),
4. Aeration (the same beaker arrangement as earlier, without the light, but with filtered compressed air keeping the test solution supersaturated and well mixed),
5. Undisturbed control sample (a sealed and covered glass jar at room temperature).

The bench-scale tests all were designed to use small sample volumes (approximately 1 L per test). Each test (except for filtration, which was an instantaneous test) was conducted over a 3-day period in triplicate. Subsamples (40 mL) were obtained for toxicity analyses at 0, 6, 12, 24, 48, and 72 hours.

Treatability was evaluated with respect to toxicity reduction using the Microtox™ 90% test. The photoluminescent bacteria, *Photobacterium phosphoreum,* were exposed to a concentration series of stormwater runoff for 15 min and toxic effects were expressed as a decrease in light output relative to controls. All reagents and the dehydrated bacteria were obtained from Azur (Carlsbad, CA). The modifications to the procedure involved using a more concentrated bacterial solution and buffer/salt solution so that more samples could be tested per batch of bacteria. Bacteria luminescence was measured using the photon sensing system of a Microtox™ Model 500 Analyzer Azur (Carlsbad, CA). The dilution series consisted of 6.25%, 12.5%, 25%, 50%, and 100% treatments using storm water and stream water. Samples were salinity adjusted prior to testing with a brine solution (22% NaCl) provided by Azur. Temperature, dissolved oxygen, pH, ammonia, and salinity were measured in all test samples prior to test initiation.

Toxicity identification evaluations

The nature of the toxicants present in the storm water was evaluated using Toxicity Evaluation Investigation (TIE) methods (USEPA 1993b). The following TIE treatments with targeted toxicants were performed for this study: (1) Baseline (none: unmanipulated sample); (2) EDTA addition (divalent cationic trace metals); (3) Sodium thiosulfate addition (oxidizable compounds, some trace metals); (4) C18 solid-phase extraction (non-polar organics); (5) C18 methanol elution (non-polar organic confirmation); and (6) Aeration (surfactants and volatile compounds). TIE methods were applied in a step-wise approach with identification and confirmation steps dependent upon results obtained during Microtox™ characterization of the raw storm water. The number and type of treatments applied for any given sample was dependent upon known water quality parameters of concern present in the sample. Treatments were performed on full-strength samples with appropriate method controls employed.

II.F. Environmental Analyses

II.F.1. Laboratory Algal Bioassays

The freshwater unicellular alga, *Pseudokirchneriella subcapitatum* (formerly known as *Selenastrum capricornutum*) 96-hour growth inhibition toxicity test was performed according to the U.S. EPA (2002) guidelines. The algal assays were performed on the storm event samples (n=5) and snowmelt samples (n=2). The stock culture used to inoculate each treatment was between 4 and 7 days old and in log-phase growth at the time of test initiation. The initial stock culture was purchased from Aquatic Biosystems of Fort Collins, Colorado and maintained in the laboratory using recommended culture media. Test chambers consisted of four replicate 125-ml Erlenmeyer flasks per sample. Test solutions were warmed to 25 ± 1 °C, and measurements of temperature, pH, DO, and conductivity were recorded. Fifty ml of prepared test solution was then distributed to each exposure chamber. The dilution series consisted of 6.25%, 12.5%, 25%, 50%, and 100% treatments using storm water and culture water. Each test chamber was aseptically inoculated with the algal stock solution to an initial concentration of ~10,000 cells per ml. Illumination was provided by a cool-white fluorescent light source suspended above the test vessels. Protocol-specific light levels (400 ± 40 foot-candles) were verified prior to test initiation. Test chambers were arranged randomly on shelves in the environmental chamber based on assigned numbers and covered with a clear Plexiglas™ sheet to prevent cross contamination.

For the duration of the test period, each test chamber was manually mixed twice each day and positions rotated under the light source (once in the morning and once in the evening). Temperature was monitored daily. At test termination, Chl *a* fluorescence was measured in an aliquot drawn from each test chamber using a Perkin Elmer fluorometer. An additional subsample of each replicate was counted for cell density using a Neubauer hemocytometer at 400× magnification.

II.F.2. Field Survey: Periphyton

Experimental Design

Two separate experiments were conducted upstream and downstream of the storm water pipes at two sites along LBC (Fig. II.A.2) in 2008 to examine the effect of storm water on algal biomass, metabolism, and community composition. The summer experiment lasted for 31 days, from June 9 to July 10, 2008 and the fall experiment lasted for 40 days, from September 25 to November 4, 2008.

Eight significant (>0.3 cm total rainfall) storm events occurred during the summer experiment. These ranged from a total of 0.30 cm to 2.4 cm of rainfall. Discharge declined in LBC during the course of the summer experiment, but spikes in discharge were seen after rain events (Fig. II.F.2.1). Six significant (>0.3 cm total rainfall) storm events occurred during the fall experiment. These ranged from a total of 0.3 cm to 1.7 cm of rainfall. Discharge generally correlated with these rain events (Fig. II.F.2.1).

Algae colonized 48 unglazed, 4-inch ceramic tiles, attached to six cement blocks, that were placed in LBC at the upstream sampling sites at both the Seaway and U.S. 31 study sites (Fig. II.A.3). Tiles were allowed to naturally colonize with algae for six weeks prior to the experiment.

At the beginning of the experiment (i.e. before tiles had been assigned to treatments), eight tiles were randomly selected from the Seaway and U.S. 31 study sites and analyzed for Chl *a* concentration and ash-free dry mass (AFDM) using the methods outlined in Steinman et al. (2006b). Then at each study site, half of the remaining tiles located at the upstream sampling location were moved downstream of the storm water pipe to the downstream sampling location; during the experiment, tiles were present both upstream and downstream of the pipe. In the process of moving tiles downstream they were removed from water for several minutes. To ensure that all tiles were exposed to similar disturbances, tiles remaining at the upstream sampling site were removed from the water for ~ 3 min and then returned to their original location. From the beginning of the tile incubation and throughout the experiment, a YSI 6600 sonde was used to measure temperature, specific conductivity, dissolved oxygen, turbidity, Chl *a*, and pH at both sampling sites. A Li-Cor quantum sensor was used to measure incident and underwater irradiance. Throughout the experiment, measurements were taken once on the same day of each week between 10 am and 12 pm.

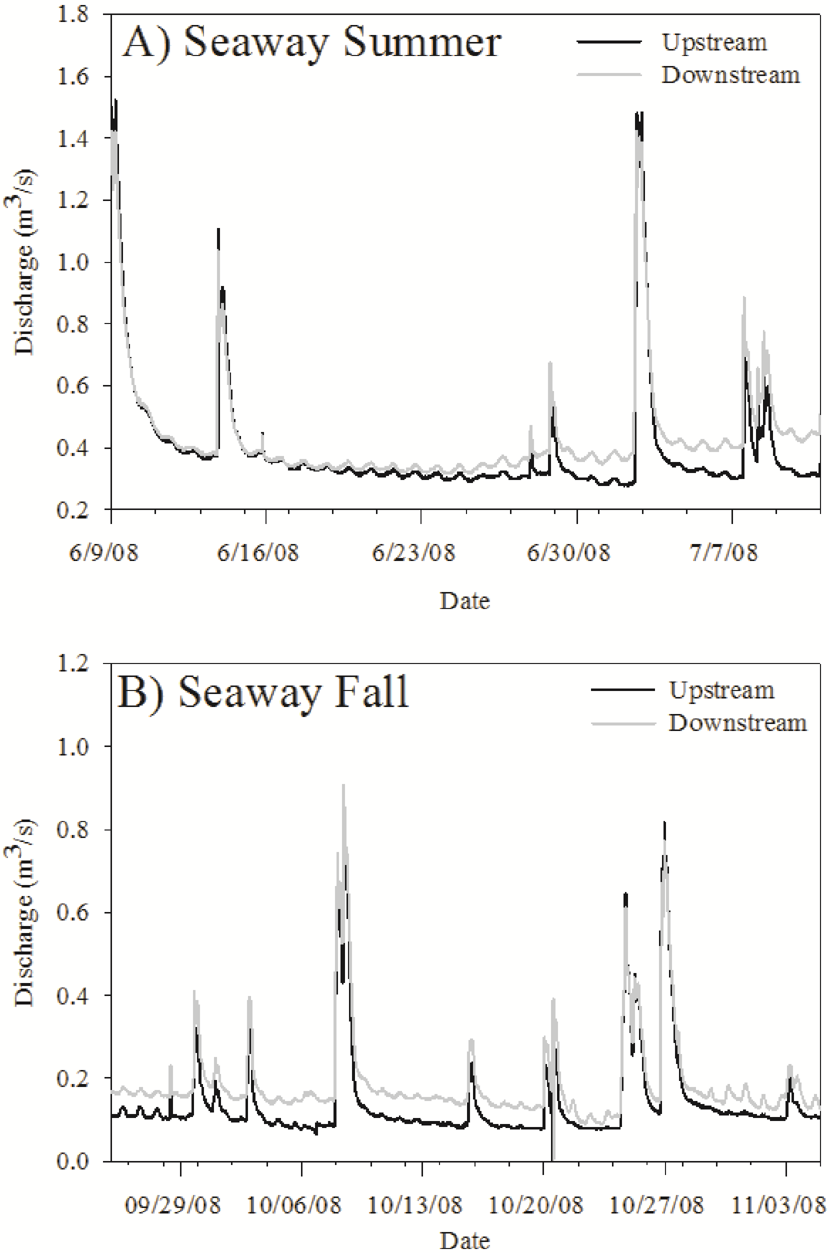


Fig. II.F.2.1. Discharge at the Seaway site during A) the summer experiment (6/9/08-7/10/08) and B) the fall experiment (9/25/08-11/4/08). Note the different scales on the discharge axes.

Sampling Methods

On day 31 or 40 of the summer and fall experiments, respectively, eight tiles from each treatment (upstream and downstream sampling sites) were removed from the stream at both the Seaway and U.S. 31 study sites (n = 16 tiles/study site). Five of the eight replicate tiles from a sampling site were analyzed for Chl *a*, AFDM, and community metabolism. Algal community composition was examined using the three remaining replicate tiles (see below). Community metabolism was estimated by measuring the rate of oxygen production from algal communities on each tile. All tiles were transported to the Annis Water Resources Institute in plastic containers filled with water and metabolism incubations began within two hours of the tiles being removed from the stream. Individual tiles were incubated in separate pint-size Ziploc® bags filled with ~ 0.5 L of water from the appropriate stream location (cf. Stewart 1987). Air was squeezed out of the Ziploc® bag so no bubbles were visible and the bag was then resealed with minimal disturbance to the water. This entire process took less than 10 seconds per bag.

Each bagged tile was placed in a 15 x 15 cm plastic container filled with water from the corresponding stream location, creating a water bath to help keep the temperature constant during the incubation. The temperature was similar to ambient conditions within LBC. During the summer experiment, containers holding the bags with tiles were then placed on the bottom of empty mesocosm tanks located in the field house at the Annis Water Resources Institute. For the fall experiment, the containers holding the metabolism bags were randomly assigned a location in a growth chamber (Thermo Fisher Scientific, Model RI-50-555-A). The chamber was set at 10°C, the ambient temperature in LBC. For both experiments, community respiration (R) was measured first by placing the containers in the dark for two hours. During the summer experiment, this treatment was created by turning off the overhead lights and completely covering the top of the mesocosm with black, opaque plastic to block out all light. During the fall experiment, the lights in the growth chamber were turned off. After measuring DO at the end of the dark treatment (see below), the black plastic was removed and lights were turned on, exposing the containers to the light for two hours to measure gross primary productivity (GPP). During the summer experiment, light was provided by metal halide lamps with a Sylvania 1000 Watt M47/S bulb suspended above each mesocosm, which provided full-spectrum photosynthetically active radiation. During the fall experiment, light was provided by GE plant and aquarium F20T12 fluorescent lights inside the growth chamber.

Metabolism was estimated by measuring changes in dissolved oxygen concentration from the beginning and end of each light treatment using Winkler titrations. To collect the water samples for analysis, one corner of the top of the bag was unzipped and a 10 ml water sample was collected using a syringe. Air was squeezed out of the Ziploc® bag and it was then resealed with minimal disturbance to the water. This entire water removal process took less than 10 seconds per bag. Light intensity was recorded at the beginning of the light treatment using a LiCor quantum sensor.

After the incubations, each tile was removed from its bag and remaining water was saved for biomass collection of any sloughed algae. Algae were scraped from the tiles using a razor blade and toothbrush. The algal slurry from the tile and the water from the metabolism bag were filtered through separate pre-ashed, pre-weighed Whatman GF/F glass fiber filters. AFDM and Chl *a* were measured for both the tile and water for each sample using the methods outlined in Steinman et al. (2006b). Analysis of pheophytin (a Chl *a* degradation product) was included in this method. Areal-specific metabolism measurements (GPP and community respiration) were calculated as the change in oxygen divided by the total area of the top surface of the tile. Chl *a*-specific metabolism measurements were calculated as the change in oxygen divided by the total biomass (Chl *a*) of the sample.

Algae from the three community composition analysis tiles were removed from the tiles in a similar manner as the algae removed from tiles for biomass analysis. An algal sub-sample was preserved in 1% Lugol's solution and stored in an opaque bottle for future microscopic determination of algal taxonomic composition. The community composition of the algal samples was determined using the inverted microscope method (Utermöhl 1958). Subsample volumes of 100-1500 μL were settled, and a minimum of 300 live (chloroplasts present) cells were counted at 400x with a Nikon Eclipse TE200 inverted microscope and identified to genus. Subsample volumes varied based on available biomass and the concentration of the sample. Mean biovolume of each algal taxon was determined from measurements of at least 15 cells per taxon using standard geometric formulae (Hillebrand et al. 1999).

Algal taxa were grouped into four categories based on their morphology and ability to attach to surfaces (Peterson 1996, Wehr and Sheath 2003, Hogsden and Vinebrooke 2006, Passy 2007). Growth form can be a predictor of algal response to grazing (Steinman 1996) and physical disturbance (Peterson 1996). The four categories used in this study were firm understory, loose understory, firm canopy, and loose canopy. Taxa possessing an attachment structure were placed in the firm category and taxa lacking the ability to tightly adhere to substrates were placed in the loose category (Hogsden and Vinebrooke 2006). Canopy taxa included those with filamentous and long-stalked growth forms, while understory taxa were prostrate or short-stalked (Hogsden and Vinebrooke 2006). During counting, no differentiation was made between filamentous cells and basal cells of the same taxon.

Statistical Analysis

The effect of location upstream or downstream of the storm water pipe on algal biomass (Chl *a* and AFDM), metabolic activity, and community composition was analyzed using two-way analysis of variance (ANOVA) tests. The factors in the tests were location (upstream or downstream of storm water pipe), season (summer or fall), and the interaction between location and season. Normality was tested using the Kolmogorov-Smirnov test, and equality of variance was tested using Levene's test. Seaway pheophytin, U.S. 31 pheophytin:Chl *a*, *Staurosirella*, and *Rhoicosphenia* values were $\log_{10}$ transformed, and U.S. 31 pheophytin values were square-root transformed prior to analysis to meet assumptions of normality and equal variance. All statistical analyses were conducted using Sigma Stat (version 3.1), and statistical significance was accepted at $p < 0.05$.

A multi-response permutation procedure (MRPP; Biondini et al. 1988, McCune and Grace 2002b) was used to test for differences in overall algal community structure among different treatments. MRPP is a non-parametric procedure for testing the hypothesis of no difference between two or more a priori defined groups. This test uses the statistic A to describe the degree of within-group homogeneity compared to that expected by chance. The MRPP tests in this experiment were based on relative number of algal cells and biovolume, and Bray-Curtis distance measures were used in the analysis. The NMDS ordinations, indicator species analysis, and MRPP were conducted using PC-ORD version 5.21 (McCune and Mefford 2006).

II.F.3 Environmental Analyses – Mesocosm Experiments

II.F.3.a. Mesocosm Design/Water Quality

2008 Mesocosm Design

To examine the effect of different concentrations of storm water runoff and grazing on algal biomass, metabolism, and community composition, experiments were conducted using mesocosms housed indoors at the Annis Water Resources Institute (AWRI) field station in Muskegon, MI. The experiment lasted 31 days, from June 6 to July 7, 2008. All storm water runoff used in the experiment was collected during a single rain event on June 5, 2008 from the storm water outlet pipe directed into LBC at the study site. A total of 2.79 cm of rainfall fell during this storm, with an average rainfall rate of 0.64 cm/hr. The most recent antecedent rainfalls had occurred on June 3 (0.31 cm) and May 30 (2.21 cm). The rain event from which storm runoff was collected was one of the largest in 2008 (Fig. II.F.3.a.1).

Water collection commenced just as storm water began to flow out of the outlet pipe. A 4-cycle Honda® GX25 pump, with a 1.5 horsepower engine, pumped water through a hose directly from the storm water outlet pipe to a ~1,900 L plastic tank positioned on a flatbed trailer. The pump and hose were rinsed with Muskegon Lake water and the plastic tank was pressure-washed with tap water prior to the water collection. The tank was towed directly to the AWRI field station after being filled. Water samples for chemical analysis were collected at the same time as water was collected for the experiment. These samples were collected in acid-washed glass jars directly from the storm water outlet pipe every half hour for three hours (six samples total) during the storm event and then transported back to the lab. The chemical analysis samples were flow composited into a single sample, meaning the proportion of each sample added to the composite was based on the proportion of total flow that occurred during the half hour the particular sample was collected. Continuous flow data were measured, both upstream and downstream of the storm water pipe, with pressure transducers installed at the sampling site prior to water collection.

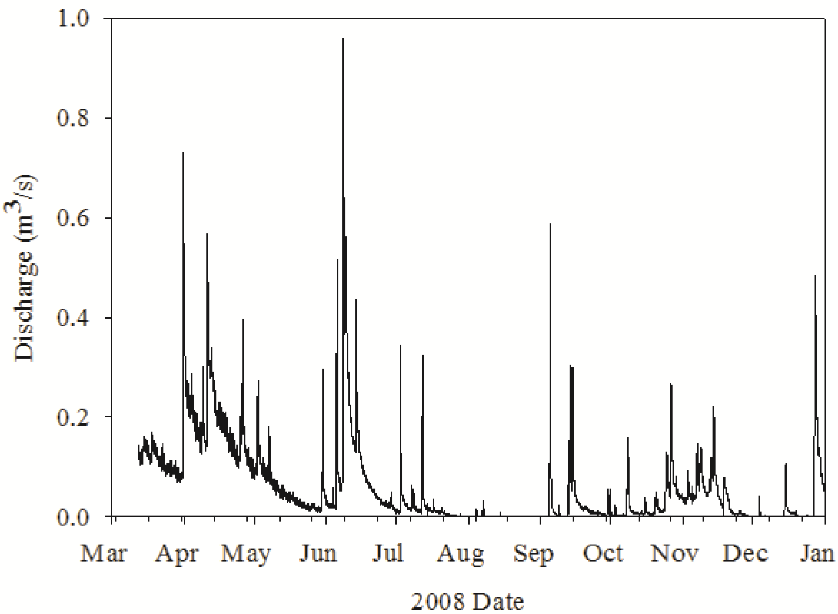


Fig. II.F.3.a.1. Hydrograph of LBC directly downstream of the storm water outlet pipe from which storm water was collected for the mesocosm experiment.

The experimental units were eleven fiberglass mesocosm tanks, each with a volumetric capacity of ~1,300 L. Three storm water treatments were created among these mesocosms: 100% storm water (n=3), 50% storm water (n=4), and 0% storm water (control; n=4). As a result of inadvertent loss of some storm water during transportation, a sufficient volume of runoff was not available to complete a fourth replicate for the 100% treatment. Each mesocosm was thoroughly cleaned with Muskegon Lake water prior to the experiment. Muskegon Lake water was used for the controls and dilutions. Given the relatively small influence of storm water on Muskegon Lake, it was believed the lake was a reasonable source for control water. This water was pumped directly from Muskegon Lake and filtered through a 300 μm filter to remove dreissenid mussels and other large particles. Approximately 220 L of the appropriate treatment water was pumped into each mesocosm. The storm water was transferred from the ~1,900 L tank to the appropriate mesocosms with the same pump and hose used for collection. In the 50% storm water treatments, ~110 L of storm water were pumped into the mesocosm, followed by ~110 L of Muskegon Lake water. Mesocosms were randomly assigned a storm water treatment and were filled in random order. Metal halide lamps with a Sylvania 1000 Watt M47/S bulb were suspended above each mesocosm and provided full-spectrum photosynthetically active radiation (~300 μmol m^{-2} s^{-1}). The photoperiod for the lamps was 4L:20D, and ambient light (~10-100 μmol m^{-2} s^{-1}) was present in the field station for a 14L:10D period. The photoperiod for the lamps was less than the ambient photoperiod to minimize evaporation, and keep water levels above the top of the exclosures (see below). A submerged Beckett Versa Gold 300 gph pump gently circulated water in each mesocosm.

Grab samples of water from each mesocosm were collected on the last day of the experiment (experiment day 31) in acid-washed 1 L glass jars and transported directly to the laboratory. All water samples were analyzed for major nutrients including total phosphorus (TP), soluble reactive phosphorus (SRP), nitrate (NO_3-N), ammonia (NH_3-N), chloride (Cl), and sulfate (SO_4), as well as total suspended solids (TSS), alkalinity, and potentially toxic compounds such as heavy metals and PAHs. Subsamples were filtered immediately in the laboratory for SRP, NO_3-N, Cl, and SO_4. Samples were frozen at -4°C until analysis. TP, SRP, and NH_3-N were analyzed colorimetrically on a Bran+Luebbe Autoanalyzer (U.S. EPA 1983). NO_3-N, Cl, and SO_4 were analyzed by ion chromatography on a Dionex DX500 (APHA 1999). Metal samples (Cr, Cu, Cd, Ni, Pb, Zn) were digested following Method 3010A from Standard Methods and run on a Perkin Elmer Analyst 800 THGA graphite furnace (U.S. EPA 1983). Working ranges were 1-25 ppb, depending on the element, and compounds with concentrations below detection limits were assigned a value of one-half the detection limit (Smith 1991). QA/QC procedures followed U.S. EPA method guidelines including 10% method blanks and 10% matrix spikes/matrix spike duplicates (± 15% limits for precision and accuracy; U.S. EPA 1983).

All treatments in this experiment contained an algal assemblage (see below); the presence of pumpkinseed sunfish (*Lepomis gibbosus)* and snails (*Physa* sp.) was manipulated within each mesocosm using a 2 x 2 factorial design. The four treatment combinations were algae only, algae + snails only, algae + fish only, and algae + snails + fish (Fig. II.F.3.a.2). Each mesocosm contained one each of these four treatment combinations, resulting in a total of 44 (11 mesocosms x 4 treatment combinations/mesocosm).

To obtain uniform algal samples to be used in the mesocosm experiment, 96 unglazed, 8-inch ceramic tiles, attached to 48 cement blocks, were placed in Cress Creek, a second order tributary in the Mona Lake watershed (Fig. II.A.1). Tiles were allowed to naturally colonize with algae for six weeks prior to the experiment. Tiles were incubated in Cress Creek instead of LBC because the catchment is not industrialized and does not have contaminated sediments (Steinman et al. 2006a). Thus, Cress Creek is relatively uncontaminated compared to LBC (Cooper et al. 2009), producing less-impacted algal communities for experimental use.

Pumpkinseed sunfish were collected by hook and line from Muskegon Lake and allowed to acclimate to Muskegon Lake water in the mesocosms (one fish per tank) for two weeks prior to the experiment. Black, opaque plastic was placed over the top of approximately one-third of each mesocosm for the entire experiment to provide a dark refuge for the fish while at the same time allowing algae exposure to the light (Fig. II.F.3.a.2). Prior to and throughout the experiment, snails were hand-collected from locations in Muskegon County including Cedar Creek near Holton and a pond in Veterans Memorial Park, North Muskegon. Approximately 7.5 grams of wet snails were added to each mesocosm tank every three days; 2.5 grams were scattered randomly within the tank, and the remaining 5 grams of snails were placed in or near the algae + snail only treatment exclosure (see below for exclosure description). To place the snails belonging in the algae + snail only exclosure, the screen blocking fish access was lifted as much as possible without disrupting the algal sample and the snails were placed inside the wooden exclosure; often, some of these snails floated out of the exclosure during the placement process and ended up outside of, but adjacent to the exclosure. The experimental design was intended to optimize fish feeding on the snails and snail grazing of the algae.

The snail and fish treatments were created within each mesocosm by placing four exclosure or enclosure structures on the bottom of the tanks (Fig. II.F.3.a.2). All of the treatment structures had wood frames, screen on four sides, pegboard on the bottom, and plexiglass covering the top. A single algal-colonized tile was positioned within each structure. Each enclosure or exclosure within a mesocosm differed in the presence and length of screen on its sides, thus determining the treatment it contained: algae only, algae + snails only, algae + fish only, or algae + snails + fish (Fig. II.F.3.a.2). Exclosures for the algae only treatment had all four sides entirely covered by screen, thus excluding fish and snails. The algae + snails only treatments had screen extended down just the upper two-thirds of each side of the structure; snails could freely enter and exit the structure, but fish were excluded. The algae + fish + snail treatments had no screen covering the sides, thus allowing both fish and snails free access to the structure. Lastly, algae + fish only exclosures had no screen covering the sides, allowing fish free access to the structure. Petroleum jelly was spread along the sides of the tile to exclude snails; petroleum jelly has been used on tiles as a barrier for invertebrates and has been especially effective in deterring *Glossosoma* caddisflies (McAuliffe 1984, Holomuzki and Biggs 1999). Researchers using petroleum jelly found that it was only moderately successful at inhibiting snail movement (Cooper and Dudley 1988, Dudley and D'Antonia 1991); however, snails in this experiment were never observed on the tiles coated with jelly. Each structure was ~23 cm x 23 cm with a height of 10 cm and each was randomly assigned one of four positions within the uncovered portion of each mesocosm (Fig. II.F.3.a.2).

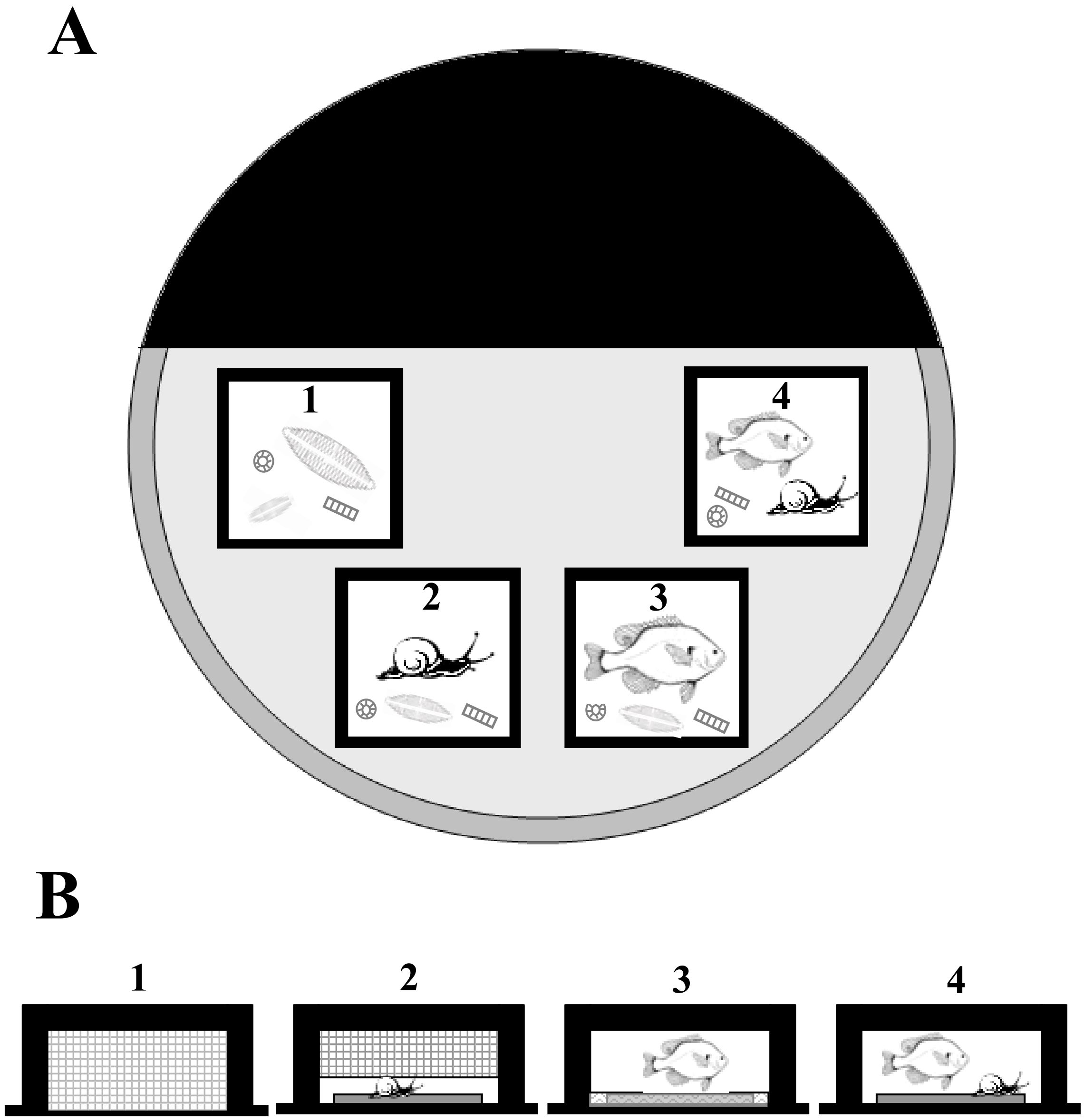


Fig. II.F.3.a.2. (A) plan view of a mesocosm containing the four exclosure treatments: 1) algae only, 2) algae + snails only, 3) algae + fish only, and 4) algae + snails + fish. The black portion of the mesocosm tank represents the plastic covering to create shade for the fish. (B) Side view of structures used in the fish and snail treatments: 1) algae only treatment (fish and snails were excluded), 2) algae + snails only treatment (snails had free access and fish were excluded), 3) algae + fish only treatment (fish had free access, but snails were excluded with petroleum jelly), and 4) algae + snails + fish treatment (both snails and fish had free access). This diagram is not to scale.

2009 Mesocosm Design

A second mesocosm experiment was conducted in 2009. The overall design was the same as in 2008, with the following differences:

1) The experiment lasted 28 days, from July 16 to August 13, 2009. All storm water runoff used in the experiment was collected during a single rain event on July 15, 2009 from the storm water outlet pipe directed into the LBC study site. A total 1.19 cm of rainfall fell during this storm, with an average rainfall rate of 0.2 cm/hr. The most recent antecedent rainfalls had occurred on July 11 (0.20 cm) and July 1 (0.10 cm). The median rainfall amount for all of the storm events in 2009 was 3 cm, with a range of 0.01-45.97 cm. The rain event from which storm runoff was collected for this experiment was in the top 25% of rainfall volumes for the year.
2) Water was collected via a pump with a 3.5 horsepower engine pumped water through a hose directly from the storm water outlet pipe to a ~1,900 L plastic tank positioned on a flatbed trailer. Water quality samples were collected in acid-washed glass jars directly from the storm water outlet pipe every half hour for one hour (two samples total) during the storm event and then transported back to the lab.
3) All biotic treatments were fully crossed, unlike 2008. As a consequence, all 12 mesocosms were used, with each mesocosm containing one each of the three treatment combinations, resulting in a total of 36 (12 mesocosms x 3 treatment combinations per mesocosm).
4) Pumpkinseed sunfish were collected via boat electrofishing (instead of angling) from Muskegon Lake and allowed to acclimate to Muskegon Lake water in the mesocosms (one fish per tank) for 98 days (instead of 14 days) prior to the experiment. Shelters for the fish measured 19 cm x 19 cm with a height of 25 cm and were constructed with six cement bricks (in lieu of black plastic). These shelters provided a shady refuge for the fish in the mesocosm tank (Fig. II.F.3.a.2). During the experiment, fish were fed night crawlers with a ration of approximately 2% of each fish's body weight per day. Prior to the experiment, snails were hand-collected from a pond in Veterans Memorial Park, North Muskegon. Approximately 1.1 grams of wet snails were placed on top of the algae sample and enclosed inside each algae + snail only enclosure (see below for enclosure description). The experimental design was intended to optimize snail grazing of the algae; in 2008, snails were not enclosed.

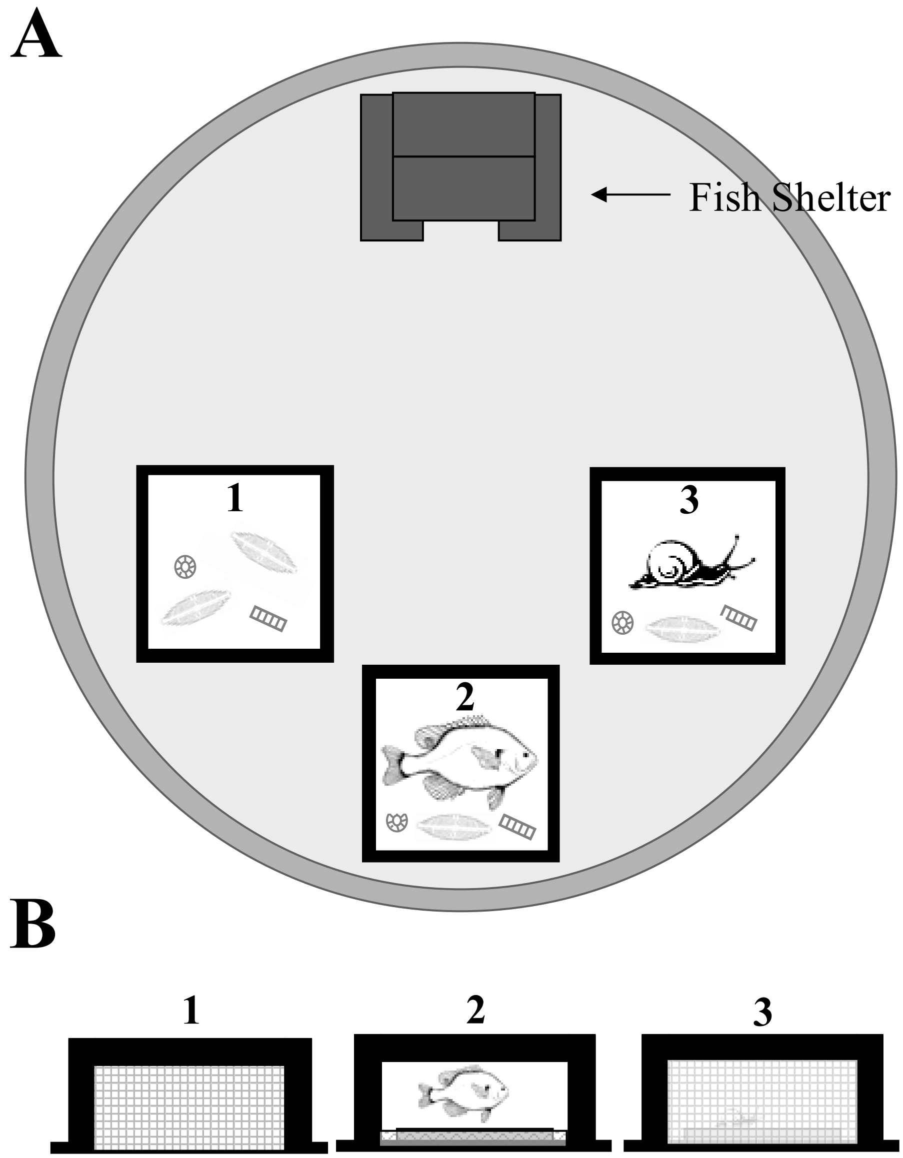


Fig. II.F.3.a.2. (A) plan view of a mesocosm containing three treatments: 1) algae only, 2) algae + fish only, and 3) algae + snails only. (B) Side view of structures used in the fish and snail treatments: 1) algae only treatment (fish and snails were excluded), 2) algae + fish only treatment (fish had free access, but snails were excluded), and 3) algae + snails only treatment (snails were enclosed and fish were excluded). This diagram is not to scale.

<u>II.F.3.b. Periphyton</u>

Sampling Methods (2008 and 2009)

At the beginning of the experiment (i.e., before tiles had been assigned to treatments), seven tiles (2008) or six tiles (2009) were randomly selected from Cress Creek and analyzed for Chl *a* concentration and AFDM using the methods outlined in Steinman et al. (2006b). Temperature, specific conductivity, dissolved oxygen, turbidity, Chl *a*, and pH were measured weekly throughout the experiment in the mesocosm tanks using a Yellow Springs Instruments (YSI) 6600 Multiprobe. A Li-Cor quantum sensor was used to measure incident irradiance at water level. Measurements were taken on the same day and within the same three hour time block each week.

On day 31 (2008) or day 28 (2009) of the experiment, community metabolism was estimated by measuring the rate of oxygen production from algal communities on each tile. Unlike 2008, tiles were acclimated in the appropriate treatment water for 15 min before the initial water samples were taken. Individual tiles (n = 4/tank) were incubated in separate gallon-size Ziploc® bags filled with ~ 2.5 L of water from the appropriate mesocosm; measurements for metabolism readings and biomass estimation followed the same procedure outlined in the Field Survey methods section (II.F.2) with the exception of the light and dark incubations lasting 4 hr instead of 2 hr.

The community composition of the algal samples was determined using the inverted microscope method (Utermöhl 1958), following the procedures in the Field Survey methods section (II.F.2).

Statistical Analysis

Differences in water chemistry parameters among base flow and day 31 (2008) or day 28 (2009) experiment values from the 100%, 50%, and 0% storm water treatments were examined using one-way analysis of variance (ANOVA) tests. Parameters were transformed, as necessary, prior to analysis to ensure the data were distributed normally. The Holm-Sidak multiple comparison test was used for mean separation.

The effects of storm water concentration and snail and fish treatments on algal biomass (Chl *a* and AFDM) and metabolic activity were analyzed using nested ANOVA. The main effect was storm water treatment, with 3 levels (100%, 50%, 0%), each of which was replicated 4x. The remaining factors were nested within the storm water treatments. Equal variance of the data was assessed by examining the residuals values plot. Data were transformed prior to analysis to ensure equal variance. The same nested ANOVA test used to identify treatment differences with the algal biomass data was used to identify differences in algal genera and growth form groups among treatments. The nested ANOVA analyses were conducted using SAS (version 9.1) and all other tests were conducted using SigmaStat (version 3.1). Statistical significance was accepted at $p < 0.05$.

Principal components analysis (PCA) was used to identify gradients in the environmental attributes of the samples (McCune and Grace 2002). Data from each of the water chemistry variables were correlated with NMDS axes 1 and 2 and significantly correlated environmental variables were used as factors in the PCA. This method was a way to determine which environmental variables fit with the gradients in community composition. When variables were auto-correlated, only the variable with the most significant correlation was used in the PCA.

Nonmetric multidimensional scaling (NMDS) ordinations (Kruskal 1964, Mather 1976), indicator species analysis (Dufrêne and Legrendre 1997), and multi-response permutation procedures (Biondini et al. 1988, McCune and Grace 2002) were used to identify overall gradients in algal community composition and growth form groups in the different storm water and fish and snail treatments. The NMDS ordinations were based on dissimilarity matrices calculated using relative abundance of cell number and biovolume for each sample and Bray-Curtis distance measures (Kruskal and Wish 1978). A Monte Carlo test was used to determine if a solution with comparable stress could be obtained by chance alone (i.e. to test whether the data had an extractable pattern). Separate ordinations were constructed for relative abundance of cell number and biovolume. The NMDS ordinations, indicator species analysis, and MRPP were conducted using PC-ORD version 5.21 (McCune and Mefford 2006).

II.F.3.c. Snails and Fish

Sampling Methods (2008 and 2009)

Mesocosm experimental design and biota collection methods were described in Section II.F.3.a.

In 2008, snail additions occurred every 3 days to provide a stable ration for pumpkinseeds and to ensure that grazers were present throughout the experiment. Snails were weighed, and 7.5 g (about 70 individuals) of snails were added to each mesocosm. During additions, most snails (5 g) were placed in the fish-excluded treatment combination with the remainder (2.5 g) scattered randomly throughout the mesocosm. This approach was used to decrease the rate at which snails were consumed by fish. In 2009, snails were added (4 individuals per exclosure) once at the beginning of the experiment to a cage that excluded fish so that snails were present for the duration of the experiment. Each snail was individually marked with paint. Length and mass of each snail was measured at the beginning and end of the experiment.

Pumpkinseeds were collected via angling and boat electrofishing from Muskegon Lake for both experiments. We collected fish of approximately the same size to minimize variation in individual growth rates, strengthening comparisons across storm water treatments. Each mesocosm contained one pumpkinseed. Pumpkinseeds were acclimated in the mesocosms 21 days in 2008 and 98 days in 2009 prior to storm water addition and fed snails (10 g) or one night crawler every 2-3 days during this period. During the experiment, a pumpkinseed was fed a restricted ration of approximately 2% of its body weight per day to maximize impacts of contaminants (Beyers et al. 1999). Mass (g) and total length (mm) of each fish were measured when it was first stocked in a mesocosm at the start of the acclimation (capture size) period and again immediately prior to the storm water addition (initial size). At the end of the 2008 experiment, numbers of snails remaining were counted and fish were measured for length and mass. At the end of the 2009 experiment, snail and fish were measured for length and mass. In both experiments, fish were not fed 2 days prior to the end of experiment to ensure complete digestion of food items.

Data Analysis

We used a one-way analysis of variance (ANOVA) to quantify effects of storm water concentration on absolute and instantaneous growth rates of fish and snails. Growth rates were calculated based on initial and final mass of individual fish and snails (see Van Den Avyle and Hayward 1999). The experimental unit for this analysis was each individual tank. To avoid pseudoreplication, mean growth rates of snails in each tank were used for ANOVA.

Differences in survival of fish and snails among storm water concentrations were evaluated using contingency tables (Agresti 1996). The experimental unit for this analysis was the individual fish or snail.

II. F.4. Environmental Analyses – Laboratory Fish Experiments

Experimental design: 2008-2009

Five experiments were conducted to determine impacts of storm water runoff on central mudminnow. We found that central mudminnow is one of the most abundant fishes in LBC through preliminary electro-fishing surveys. Storm water was collected from rain events on 6 June 2008, 9 May 2009, and 14 July 2009 (U.S. 31 only) for experiments. Additionally, roadside snow was collected on 3 February 2009, to investigate the effects of snowmelt (see Section II.B). Two experiments were conducted using the same snowmelt. In all experiments, we used a 5 × 2 × 2 factorial design to investigate the effects of storm water concentration (0, 25, 50, 75, and 100% storm water), runoff source (U.S. 31, Seaway Drive), and fish source (LBC, control) on the growth and mortality of central mudminnow. A concentration gradient of storm water was used to determine whether the effect of storm water on central mudminnows differed with concentration. Runoff from two sources was used to study variability in storm water among road-stream crossings. Fish from LBC and a control stream were used to control for the possibility that central mudminnows from LBC may be pollution tolerant or pollution stressed because LBC is heavily degraded. We selected control streams that lacked the legacy of pollution that LBC received. Cress Creek (Muskegon, Michigan), another tributary of the

Mona Lake watershed, was used as a collection site for central mudminnows for all experiments except the summer 2008 trial. Norris Creek (Fruitport, Michigan) was used as a collection site for the first experiment. Both streams are less impacted than LBC and support a diverse fish community (W. Keiper, personal observation).

Collection of storm water occurred simultaneously at both study sites during rain events. Runoff at the Seaway Drive site was collected using 10-L carboys positioned below the storm drain located above LBC. Runoff at the U.S. 31 storm drain was collected using 10-L carboys or a 1.5 hp Honda GX25 gas powered water pump (dependent on rain event). After collection, storm water was transported to Annis Water Resources Institute, where storm water from each site was kept in separate 114-L holding tanks in a refrigeration room (4 °C).

For each experiment, 60 central mudminnows were collected from two sites, LBC (30) and a control stream (30). Fish were collected via backpack electro-fishing and transported to AWRI in 45-L coolers. Before acclimating to laboratory conditions, fish were kept overnight in a 45-L cooler with aeration to allow recovery from stress associated with collection and transport. On day two, fish were individually weighed (g) and measured (mm) and then placed in aquaria with 2-L of dechlorinated tap water (de-ionized water was used in summer 2008 experiment). Each aquarium was aerated. To minimize stress to fish by people being nearby aquaria, the shelves holding aquaria were covered. Fish were held for 5 days and fed ad libitum amounts of amphipods (*Gammarus*) or freeze dried blood worms (Chironomidae) during the acclimation period, both of which are natural prey items of central mudminnow (Peckham and Dineen 1957). During the first experiment only *Gammarus* were used; ration consisted of freeze dried blood worms in subsequent experiments. After a 5-day acclimation period, individual fish were measured (mass and length) and then randomly assigned to experimental treatments.

Fish were fed a diet at approximately 2% their total body mass per day (40% of their maximum consumption) to maximize storm water effects (Beyer et al. 1999). Each fish was fed on Monday, Wednesday, and Friday. During the first experiment, feedings consisted of adding three *Gammarus* during each feeding until day 20 when rations were doubled to 6 *Gammarus* at each feeding for the remainder of the experiment. This was done to offset high amounts of mortality that were thought to be from the reduced ration. During subsequent experiments, rations consisted of freeze dried bloodworms fed ad libitum. Ammonia levels were monitored weekly (Quick Dip^{TM}, Jungle Laboratory Corp, Cibola, Texas) throughout the experiment to ensure nitrogenous wastes were not contributing to mortality. Fish mortalities were noted but not replaced once the experiment started. Each experiment was run for 28 days, after which final mass and final length of each fish was measured. All fish were euthanized with MS-222 at the end of the experiment.

Water samples were taken during each rain event and analyzed using methods previously described.

Experimental design: 2011

To assess the ecological effects of snowmelt to Little Black Creek, we collected roadside snow from U.S. 31 on 7 February 2011 and exposed central muddminnows *Umbra limi* to four concentrations (0%, 5%, 50%, and 100% snowmelt). There were 10 replicates of each snowmelt concentration (4 snowmelt concentrations × 10 replicates = 40 aquaria with on fish each). Central mudminnows were collected from Little Black Creek via backpack electrofishing and transported to Annis Water Resources Institute in a 45-L cooler. Fish were acclimated to laboratory conditions for 72 hours in a 45-L cooler with aeration in a REVCO incubator kept at 6°C. Fish were fed *ad libitum* (acclimation period only) a diet of freeze-dried blood worms (Chironomidae). After acclimation, fish were individually weighed (g) and measured for total length (TL; mm) and then randomly placed in 3-L aquaria containing one of the four snowmelt concentrations. Each aquarium was aerated and covered with mesh to prevent fish from escaping. All aquaria were held in a REVCO incubator at 6°C. Fish were again fed a diet of freeze-dried blood worms, but the ration was about 4% their total body mass per feeding. Fish were fed every other day for the entire experiment and checked every day for mortalities. A light regime (8 hours/day) was used to mimic natural conditions. Ammonia levels were monitored weekly (Quick Dip™, Jungle Laboratory Corp, Cibola, Texas) throughout the experiment to ensure nitrogenous wastes were not contributing to mortality. The experiment was run for 18 days, after which final mass and TL of each fish was measured. All individuals were euthanized at the conclusion of the experiment. The sex of each fish was determined by dissection.

Immediately after being euthanized, two pieces of white muscle tissue were taken from each fish (next to the back half of the dorsal fin on each side) to extract nucleic acids. Tissue samples were immediately flash frozen in liquid nitrogen and stored in a -80°C freezer until RNA/DNA extraction (Grant 1996; Imsland et al. 2002; Pilar Olivar et al. 2009). We used an extraction procedure developed and modified by Clemmesen (1988, 1993) to extract nucleic acids from 200-300 mg of tissue from each central mudminnow (Steinhart and Eckmann 1992; Imsland et al. 2002; Caldarone et al. 2006). Individual muscle tissue (4-24 mg wet mass) was placed in a vial containing 0.4 mL of Tris-NaCl buffer (0.05 M Tris, 0.1 M NaCl, 0.01 M EDTA and 2% SDS (sodium dodecyl sulphate) adjusted to pH 8.0 with HCl) and glass beads (diameter 0.2 and 2 mm). Samples were then mechanically homogenized for 20 seconds at max speed with a BioSpec Mini Beadbeater 115V and centrifuged for 5 minutes at max speed (140,000 rpm) in an Eppendorf 5424 centrifuge. The supernatant was then transferred to a new vial and 4 µL of Proteinase K was added and then the sample was incubated for 10 minutes at 55°C in a Boekel Scientific Heat bath. All steps were carried out on ice to minimize the effects of RNase and DNase.

Fluorescence was measured with a spectrofluorometer (Photon Technology International) at an excitation of 365 nm and an emission at 590 nm. A constant spectrofluorometer temperature was maintained at 25°C, along with constant room temperature (Clemmesen 1988, 1993; Steinhart and Eckmann 1992; Chicharo et al. 1998; Caldarone 2005; Caldarone et al. 2006). Standard curves were constructed with RNA from calf liver (16/23s) type IV (Sigma) and Lambda DNA. A master mix of sample was made with

250 µL of sample and 1250 µL of TEN buffer (pH = 8.0), then 500 µL were pipetted into two separate new vials. Ethidium bromide (EB) (50µL) was added to each vial. RNase was then added to one of the vials, which incubated for 1 hour at room temperate. While the RNase was incubating, the vial containing just EB and sample was read for total fluorescence. The sample containing RNase was then read and RNA was calculated by subtracting the total (1^{st} reading) from the RNase treated sample (2^{nd} reading). To measure residual fluorescence, samples were treated with both RNase and DNase. The addition of DNase did not alter the fluorescence. Also, endogenous fluorescence (raw sample before EB) was measured and proved to be negligible, so it was not considered in calculations. Therefore, for all samples, the entire fluorescence from the RNase treated samples was attributed to DNA (Clemmesen 1988, 1993; Steinhart and Eckmann 1992; Chicharo et al. 1998; Caldarone 2005; Caldarone et al. 2006).

Water samples were taken from each snowmelt concentration and analyzed using methods previously described.

Data analysis

For each experiment, we used a 5 × 2 × 2 factorial design to test the effects of runoff concentration (0%, 25%, 50%, 75%, and 100% runoff), runoff source (Seaway, U.S. 31), and fish source (LBC, control stream) on the growth and survival of central mudminnow. We quantified absolute and instantaneous growth rates of individual fish based on changes between initial and final mass (see Van Den Avyle and Hayward 1999). To examine the effect of treatment combinations on fish growth, we used a 3-way factorial analysis of variance (ANOVA). To examine the effects of treatment combinations on survival, we used logit models with likelihood ratio tests that were performed in the GENMOD procedures in SAS, version 9.2. Fish source was not included in logit models because it was not found to significantly affect fish survival using Fisher's exact test (Agresti 1996). Moreover, fish source did not significantly affect fish growth (see Results: Section III.E.4), which further justified not including it as a factor in logit models and simplified the analysis. We assessed overdispersion (i.e., greater variability than predicted by binomial model) for each logit model to guard against artificially underestimating standard error and inflating the probability of a type I error (Agresti 1996). Overdispersion was assessed based on whether the ratio of deviance to the degrees of freedom (df) was near one, and the data were considered overdispersed when the ratio of deviance to df was much greater than one (SAS Institute 1999). If overdispersion was detected, then we adjusted the scale of the dispersion parameter (i.e., scale=deviance) *so* that the dispersion parameter is estimated by the deviance divided by its degrees of freedom (SAS institute 1999). Only the summer 2008 logit model had overdispersion and was corrected. Fish in controls (i.e., 0% runoff) were not included in logit models because mortality in controls biased the effect of runoff location (i.e., control water was the same for each runoff location). Nevertheless, we report survival in controls for each experiment.

For the 2011 snowmelt experiment, we used a 4 × 2 factorial design to test the effects of snowmelt concentration (0%, 5%, 50%, 100% snowmelt), fish sex (male, female) on the survival, growth, and condition of central mudminnow. Our experiment had an unbalanced design (Table II.F.4.1) because we could not determine the sex of fish until after they were euthanized. We quantified absolute growth of individual fish based on changes between initial and final mass; condition was quantified as liver mass and nucleic acids (RNA:DNA ratio). In general, high liver mass and high RNA:DNA ratios are indicative of good condition (Busacker et al. 1990). To examine the effect of treatment combinations on fish growth and liver mass, we used an analysis of covariance (ANCOVA). The main effects were snowmelt concentration and fish sex, which were crossed in the ANCOVA. Initial fish size (measured as TL) was the covariate because we suspected that absolute growth and liver mass may vary according to fish size. To examine the effects of treatment combinations on the RNA:DNA ratio, we used a two-way analysis of variance (ANOVA). Both ANCOVA and ANOVA were performed in the GLM procedure in SAS, version 9.2. When a significant interaction was detected in ANCOVA or ANOVA, we used contrasts to make planned pairwise comparisons among all snowmelt concentrations within a sex. Otherwise, we used contrasts to make all pair-wise comparisons when we detected a significant main effect (e.g., snowmelt concentration). Based on residual plots, we determined that variance-stabilizing transformation was not necessary.

Table II.F.4.1. Sample size of central mudminnows used in 2011 snowmelt experiment.

	Snowmelt Concentration (%)			
Fish Sex	0	5	50	100
Male	6	7	7	8
Female	4	3	3	2

III. Results

III.A. Water Quality and Quantity Characterization

Water Quality

The water quality properties of storm water runoff were markedly different than in-stream conditions in LBC (Tables III.A.1 and 2). Parameters that elicited the greatest changes in downstream water quality during storms include total phosphorus (TP), ammonia (NH_3), chloride (Cl), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn). For all of these parameters, two generalizations can be made: 1) concentrations were higher in storm water runoff than in baseflow of LBC at both U.S. 31 and Seaway Drive, and 2) storm water inputs resulted in increased downstream concentrations and loads only at the U.S. 31 site (Figs. III.A.1-10). At Seaway Drive, the already-elevated constituent concentrations upstream of the storm water outfall, coupled with the high storm flow discharge rate of LBC (average ~0.5 m^3/s) at that site, minimized the influence of storm water on loads and concentrations. Additional details about each of these parameters are discussed in the paragraphs that follow.

Average TP concentrations were moderate at U.S. 31(0.010 mg/L upstream, 0.014 mg/L downstream) during base flow conditions (Fig. III.A.1). At Seaway Drive, average base flow TP concentration was high (0.035 mg/L) and exceeded the 0.03 mg/L eutrophic threshold. Storm water TP concentrations were very high (~0.2 mg/L), from 2X (Seaway Drive) to 10X (U.S. 31) greater than in-stream concentrations during storms (Fig. III.A.1). During storm flow, average in-stream TP concentrations downstream of the storm water outfalls exceeded the eutrophic threshold at U.S. 31 and the hypereutrophic threshold (0.1 mg/L) at Seaway Drive (Fig. III.A.1). Snowmelt TP concentrations were 2X higher than average storm water TP concentrations (Fig. III.A.1).

Average total ammonia concentrations were ~5X higher in storm water runoff than in LBC during storms (Fig. III.A.2). Mean snowmelt total ammonia concentrations were 1.2X (Seaway Drive) to 2X (U.S. 31) greater than storm runoff concentrations (Fig. III.A.2). Un-ionized ammonia concentrations did not exceed the State of Michigan's water quality criteria for chronic or acute NH_3 toxicity (MDEQ 2007).

Average chloride concentrations were ~2.5X higher in storm water runoff than in LBC during storms and at Seaway Drive exceeded U.S. EPA's (2009) maximum concentration for chronic effects to aquatic life (Fig. III.A.3). Chloride was extremely high in snowmelt and exceeded U.S. EPA's (2009) maximum concentration for acute effects to aquatic life by 2X (U.S. 31) to 5X (Seaway Drive) (Fig. III.A.3).

Average chromium concentrations were 7X (Seaway Drive) to 12X (U.S. 31) higher in storm water runoff than in LBC during storms, but did not exceed the State of Michigan's water quality criteria for chronic effects (MDEQ 2011) (Fig. III.A.4). Although snowmelt contained higher concentrations of chromium than were measured in storm water, they exceeded the chronic standard only during the 2009 snowmelt collection from U.S. 31 (Fig. III.A.4). The 2011 snowmelt collection from the same site had a concentration of chromium similar to the mean storm water concentration, yielding an average chromium concentration well below the chronic standard (Fig. III.A.4). Mean chromium loads were less than 0.1 g/d during base flow conditions and increased dramatically during storm events, with average loads of 300-400 g/d (Fig. III.A.4). At U.S. 31, in-stream loads of chromium averaged 25g/d upstream of the storm water outfall and were 10X higher downstream of the pipe (Fig. III.A.4).

Average copper concentrations in storm water were 3-4X greater than in LBC during storms, upstream of the storm water outfalls (Fig. III.A.5). Storm conditions resulted in elevated copper concentrations downstream of the storm water outfall at U.S. 31; already-elevated copper concentrations in LBC at Seaway Drive were not affected by storm water inputs from that site (Fig. III.A.5). Average copper concentrations in storm water exceeded the State of Michigan's standard for chronic effects at both sites; snowmelt exceeded the acute standard (MDEQ 2011) (Fig. III.A.5). Although average in-stream copper concentrations were below the chronic standard, this threshold was exceeded during 2 of 7 storm events downstream of the storm water outfall at U.S. 31 and at both locations at Seaway Drive. Similar to chromium, average copper loads were very low during base flow (<0.15 g/d) and increased dramatically during storm events, with average copper loads of 225 g/d at downstream U.S. 31 and 717 g/d at downstream Seaway Drive (Fig. III.A.5).

Although average nickel concentrations were greater in storm water and snowmelt than in LBC, all concentrations were well below the State of Michigan's standard for chronic effects (94 µg/L; MDEQ 2011) (Fig. III.A.6). Similar to chromium and copper, nickel loads were very low during base flow (<0.05 g/d) and increased dramatically during storm events. The increased nickel load during storms was especially pronounced at Seaway Drive, where in-stream loads were >400 g/d, but was not the result of runoff from that site given the already-elevated load upstream of the storm water pipe (Fig. III.A.6).

Average storm water concentrations of lead were 4X (Seaway Drive) to 14X (U.S. 31) greater than in-stream concentrations during storms, upstream of the storm water outfalls, and exceeded the State of Michigan's standard for chronic effects (Fig. III.A.7). Storm conditions resulted in elevated lead concentrations downstream of the storm water outfall at U.S. 31; already-elevated lead concentrations in LBC at Seaway Drive were not affected by storm water inputs from that site (Fig. III.A.7). Lead concentrations in snowmelt exceeded the chronic standard at U.S. 31 only during the 2009 collection. Average lead loads were similar to the metals previously discussed, with very low base flow loads (<0.05 g/d) increasing to 210 g/d at downstream U.S. 31 and 587 g/d at downstream Seaway Drive (Fig. III.A.7). Lead loads from U.S. 31 storm water resulted in elevated loads downstream, but already-elevated loads in LBC at Seaway Drive during storms were not influenced by storm water from that site (Fig. III.A.7).

Average zinc concentrations in storm water were 5X greater than in-stream storm flow concentrations upstream of the storm water outfalls, but translated to increased downstream concentrations only at U.S. 31 (Fig. III.A.8). Average zinc concentrations in storm water exceeded the State of Michigan's standard for chronic effects only at Seaway Drive (MDEQ 2011) (Fig. III.A.8). Zinc concentrations in snowmelt exceeded the acute standard at both sites during the 2009 collection, but were below the acute concentration at U.S. 31 in 2011 (Seaway Drive snowmelt was not sampled in 2011) (Fig. III.A.8). Characteristics of zinc load were similar to the previously-discussed metals. Average zinc load increased from <0.7 g/d during base flow to 1200 g/d at downstream U.S. 31 and 2600 g/d at downstream Seaway Dive (III.A.8).

Oil and grease and PAHs are contaminants of concern in storm water runoff from roads. Average concentrations of both pollutants were greater in storm water than in LBC, but storm events did not elicit increases in downstream concentrations (Figs. III.A.9-10). In-stream concentrations of oil and grease were similar under base flow and storm flow conditions. In-stream concentrations of PAHs were higher than base flow concentrations only at Seaway Drive (III.A.10). Concentrations of oil and grease were 2-4X greater and PAHs were 2X greater in snowmelt than in storm water (Figs. III.A.9-10).

Oil and grease loads were similar during storms and base flow at U.S. 31. At Seaway Drive, oil and grease loads were higher during storms than base flow, but were not influenced by storm water inputs from that site (Fig. III.A.9). Although average in-stream PAH loads during storms were greater than base flow loads, the increase was not the direct result of storm water inputs from our sampling sites (Fig. III.A.10).

Table III.A.1. Average values for physical and chemical water quality parameters measured during base flow, storm flow, and snowmelt conditions at Seaway Drive and U.S. 31. Sample number is indicated by n for each sample type. *n=2 for base flow samples from the storm water pipe at U.S. 31.

	Seaway Drive					U.S. 31						
	Base flow n=11	Storm flow n=7			Snow n=1	Base flow n=11*			Storm flow n=7			Snow n=2
		Up	Pipe	Down		Up	Pipe	Down	Up	Pipe	Down	
Temp, C	10.35	11.63	--	11.83	7.44	8.29	9.40	9.90	10.90	11.36	11.44	8.38
DO, mg/L	10.76	8.71	--	8.93	9.58	9.61	11.03	9.90	8.20	10.43	9.51	10.48
pH	8.00	7.77	--	7.76	7.21	7.70	7.89	7.83	7.75	7.90	7.80	7.99
SpCond, mS/cm	0.86	0.69	--	0.65	30	0.58	0.80	0.75	0.48	1.11	0.60	9.09
ORP, mV	322	356	--	357	393	316	99	295	299	343	366	419
TDS, g/L	0.56	0.43	--	0.40	19.36	0.38	0.52	0.49	0.30	0.68	0.32	5.74
Turbidity, NTU	3.3	19.6	--	27.4	496.1	2.9	2.3	5.7	7.4	125.4	56.5	508.1
Chl *a*, μg/L	3.02	8.98	--	10.57	23.8	4.17	3.60	5.33	5.32	7.25	5.55	20.65
Alk, mg/L	160	124	50	123	190	137	153	138	121	120	112	98
Cl, mg/L	157.9	135.7	316.9	126.7	4579	92.2	175.0	103.9	81.6	205.3	111.1	2016
SO_4, mg/L	34.45	26.67	14.50	24.29	49.00	26.13	34.00	27.71	22.06	22.57	20.29	52.5
NO_3-N, mg/L	0.77	0.71	0.64	0.66	0.16	0.34	0.81	0.40	0.33	0.56	0.43	0.43
NH_3-N, mg/L	0.08	0.13	0.73	0.15	0.94	0.05	0.10	0.06	0.05	0.23	0.12	0.47
SRP-P, mg/L	0.01	0.02	0.01	0.02	0.003	0.00	0.00	0.00	0.00	0.01	0.01	0.003
TP-P, mg/L	0.04	0.10	0.24	0.10	0.47	0.01	0.02	0.01	0.02	0.19	0.07	0.38

Table III.A.2. Average concentrations for heavy metals, PAHs, and oil and grease measured during base flow, storm flow, and snowmelt conditions at Seaway Drive and U.S. 31. Sample number is indicated by n for each sample type. *n=2 for base flow samples from the storm water pipe at U.S. 31.

	Seaway Drive					U.S. 31						
	Base flow n=11	Storm flow n=7			Snow n=1	Base flow n=11*			Storm flow n=7			Snow n=2
		Up	Pipe	Down		Up	Pipe	Down	Up	Pipe	Down	
Cd, μg/L	2.2	1.8	0.5	1.7	0.1	1.5	0.5	1.5	0.9	1.1	1.0	0.5
Cr, μg/L	2.45	7.51	54.03	6.82	91.05	1.07	0.50	1.35	2.15	26.12	12.09	95.10
Cu, μg/L	12.46	12.99	35.96	12.39	61.79	13.00	27.41	10.71	6.20	27.19	13.02	62.96
Ni, μg/L	11.57	9.09	23.20	8.02	30.12	11.28	6.91	9.69	6.43	12.42	8.59	30.23
Pb, μg/L	1.62	10.39	42.55	9.61	5.76	1.85	0.50	1.11	2.11	25.75	9.56	16.40
Zn, μg/L	25	54	287	47	561	25	25	25	25	141	58	402
Total PAHs, μg/L	0.5	5.8	16.4	9.8	40.5	0.5	0.5	0.5	0.8	6.9	0.9	13.8
Oil and Grease, mg/L	0.9	1.4	3.3	1.6	12.3	1.2	0.6	1.2	0.9	3.5	1.2	7.2

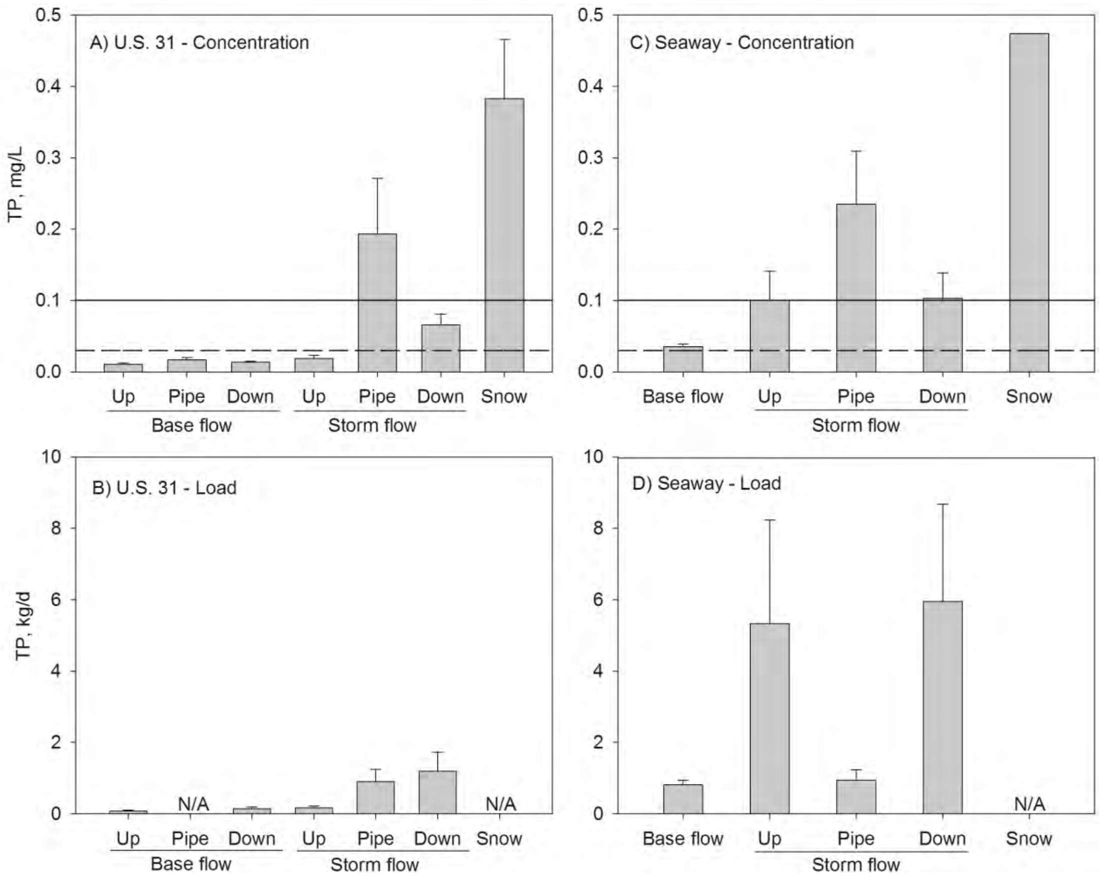


Figure III.A.1. Mean (±SE) total phosphorus concentrations (mg/L) and loads (kg/d) for U.S. 31 (A, B) and Seaway Drive (C, D). Dashed and solid lines on panels A and C indicate the 0.03 mg/L eutrophic and the 0.1 mg/L hypereutrophic thresholds for total phosphorus. N/A = not applicable due to lack of discharge data.

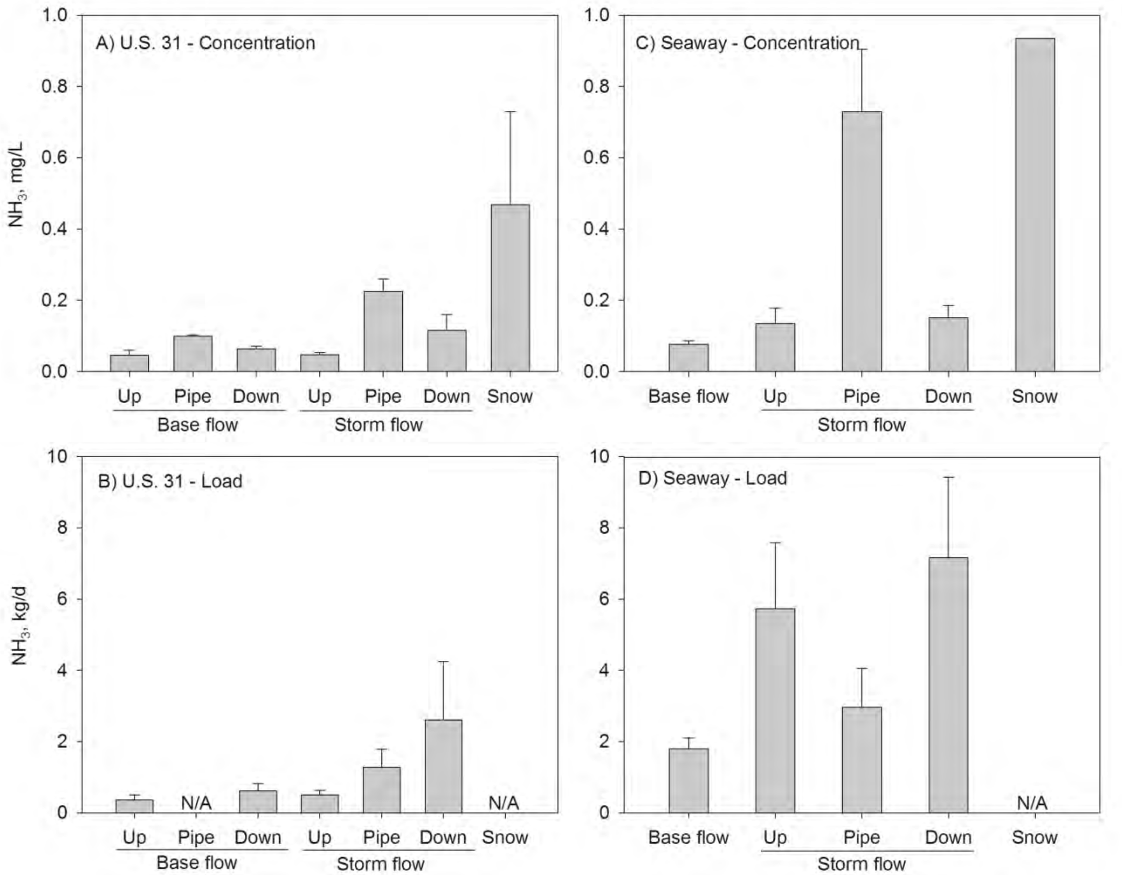


Figure III.A.2. Mean (±SE) total ammonia concentrations (mg/L) and loads (kg/d) for U.S. 31 (A, B) and Seaway Drive (C, D). N/A = not applicable due to lack of discharge data.

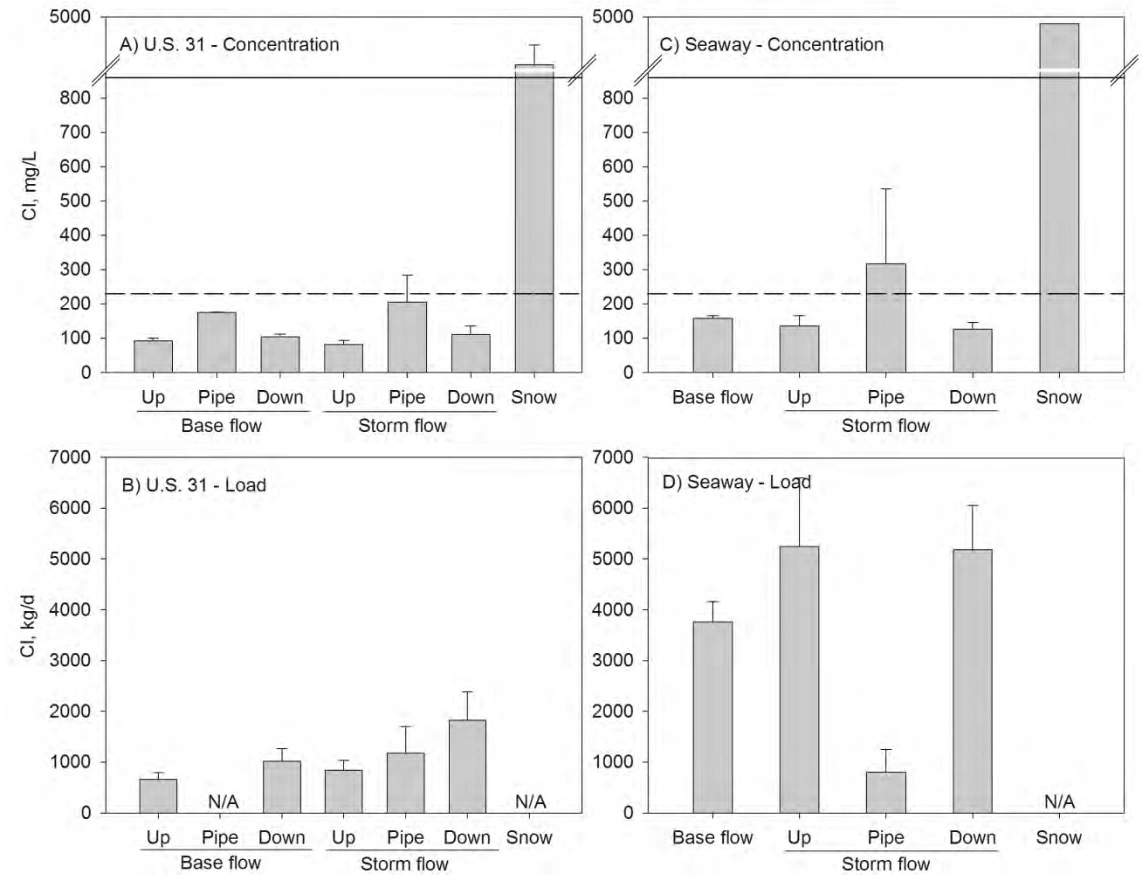


Figure III.A.3. Mean (±SE) chloride concentrations (mg/L) and loads (kg/d) for U.S. 31 (A, B) and Seaway Drive (C, D). Dashed and solid lines on panels A and C indicate U.S. EPA's 230 mg/L threshold for chronic effects and 860 mg/L threshold for acute effects to aquatic life (U.S. EPA 2009). N/A = not applicable due to lack of discharge data.

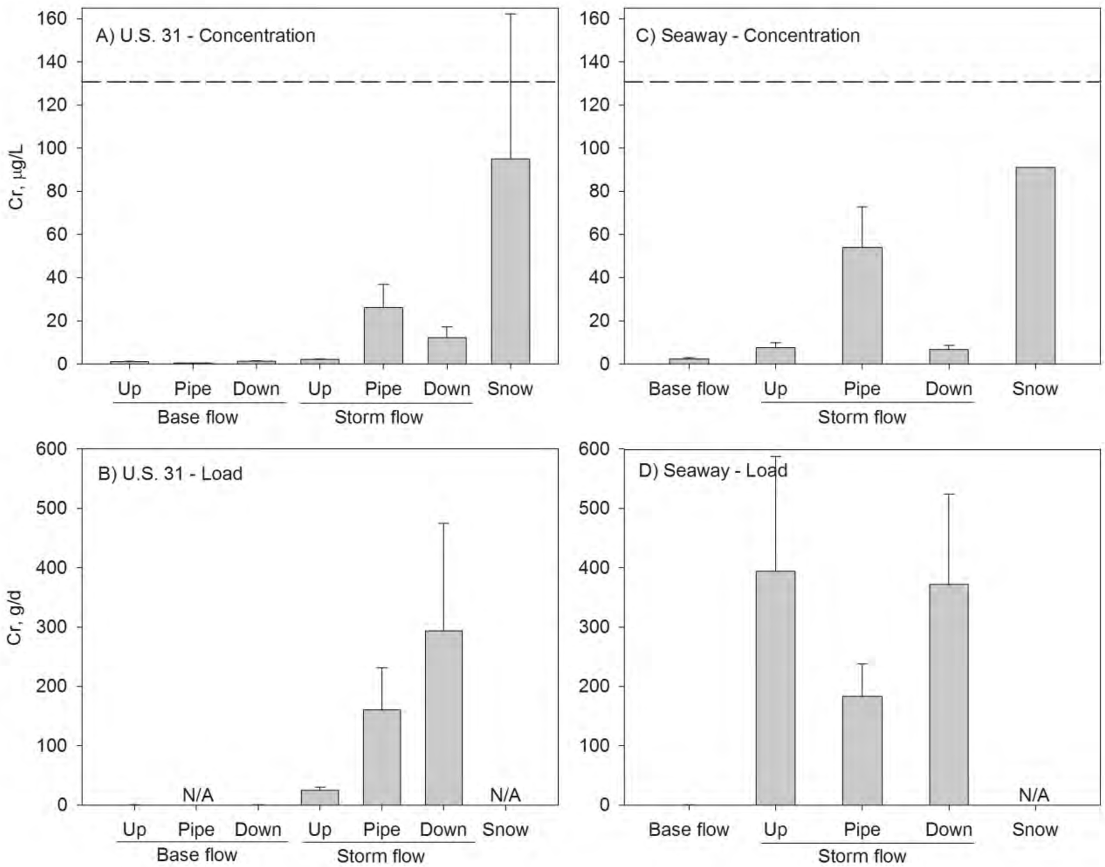


Figure III.A.4. Mean (±SE) chromium concentrations (μg/L) and loads (g/d) for U.S. 31 (A, B) and Seaway Drive (C, D). Dashed lines on panels A and C indicate the Michigan standard for chronic effects to aquatic life (MDEQ 2011). N/A = not applicable due to lack of discharge data.

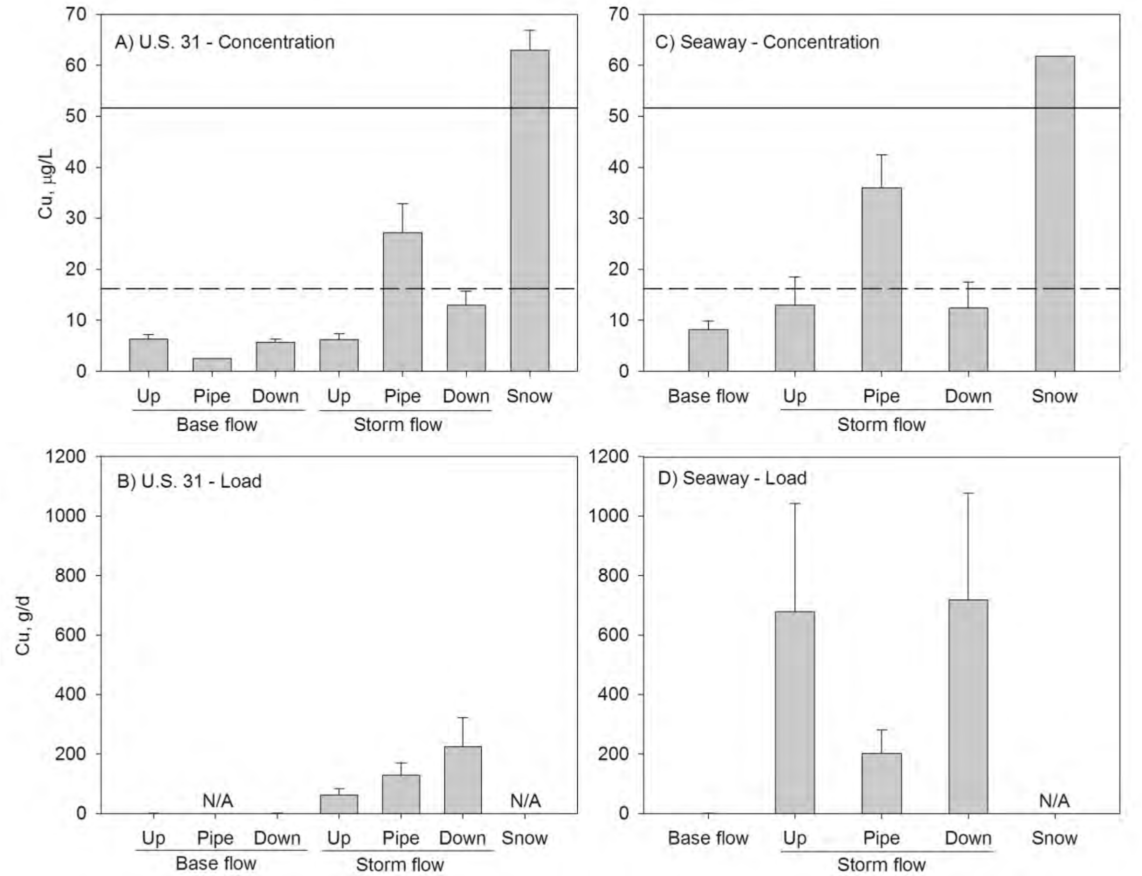


Figure III.A.5. Mean (±SE) copper concentrations (µg/L) and loads (g/d) for U.S. 31 (A, B) and Seaway Drive (C, D). Dashed and solid lines on panels A and C indicate the Michigan standards for chronic and acute effects to aquatic life (MDEQ 2011). N/A = not applicable due to lack of discharge data.

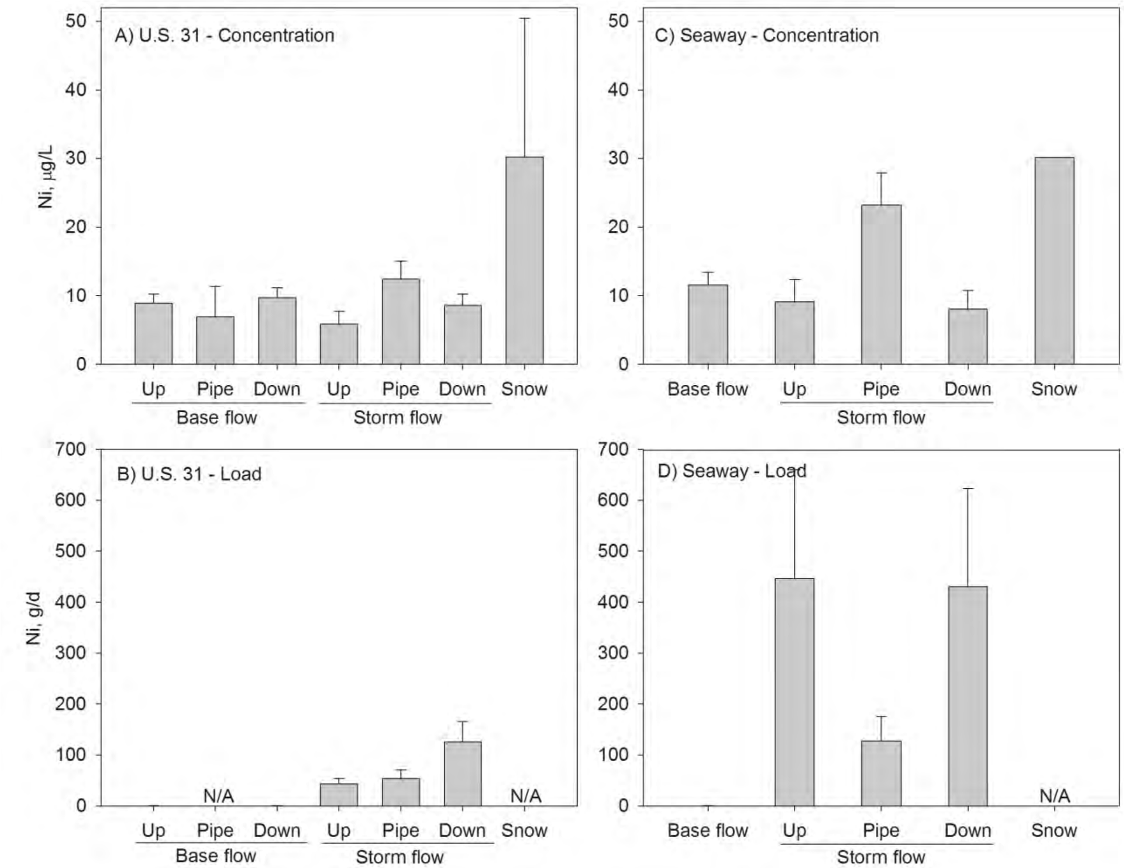


Figure III.A.6. Mean (±SE) nickel concentrations (µg/L) and loads (g/d) for U.S. 31 (A, B) and Seaway Drive (C, D). N/A = not applicable due to lack of discharge data.

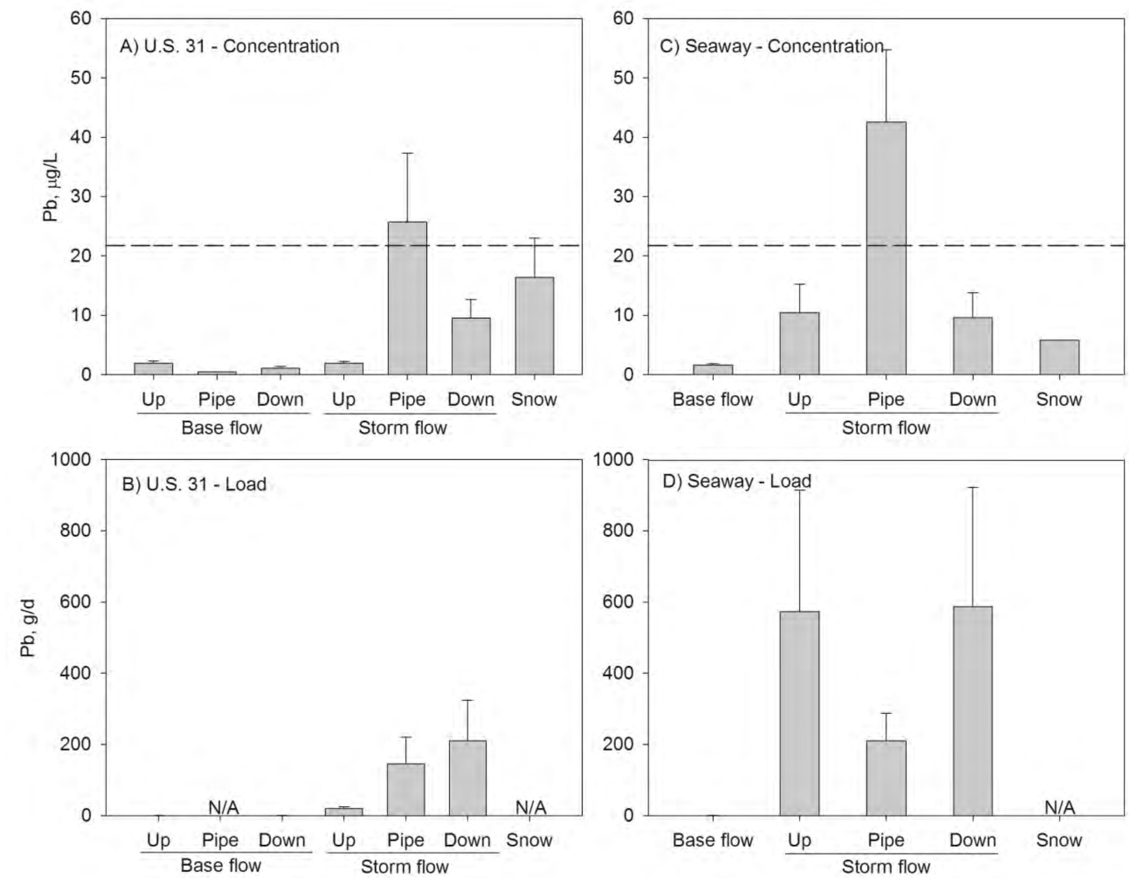


Figure III.A.7. Mean (±SE) lead concentrations (µg/L) and loads (g/d) for U.S. 31 (A, B) and Seaway Drive (C, D). Dashed lines on panels A and C indicate the Michigan standards for chronic effects to aquatic life (MDEQ 2011). N/A = not applicable due to lack of discharge data.

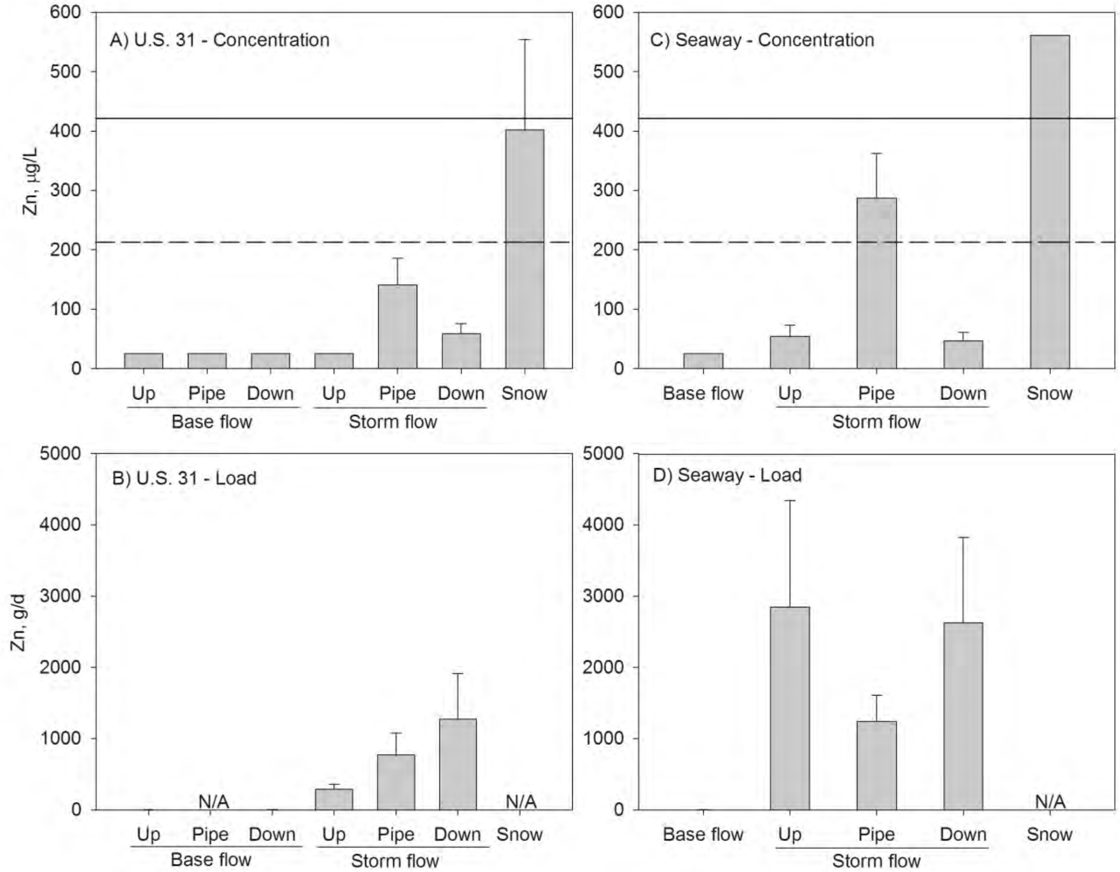


Figure III.A.8. Mean (±SE) zinc concentrations (µg/L) and loads (g/d) for U.S. 31 (A, B) and Seaway Drive (C, D). Dashed and solid lines on panels A and C indicate the Michigan standards for chronic and acute effects to aquatic life (MDEQ 2011). N/A = not applicable due to lack of discharge data.

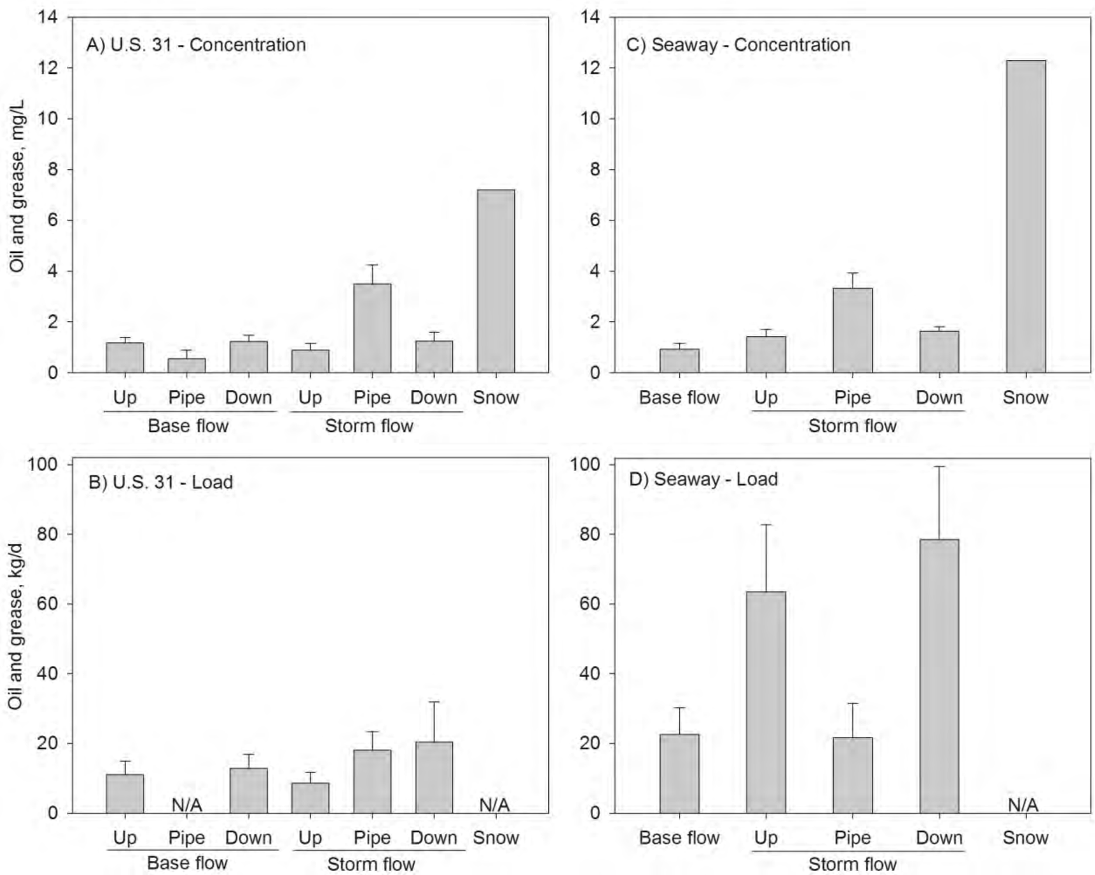


Figure III.A.9. Mean (±SE) oil and grease concentrations (mg/L) and loads (kg/d) for U.S. 31 (A, B) and Seaway Drive (C, D). N/A = not applicable due to lack of discharge data.

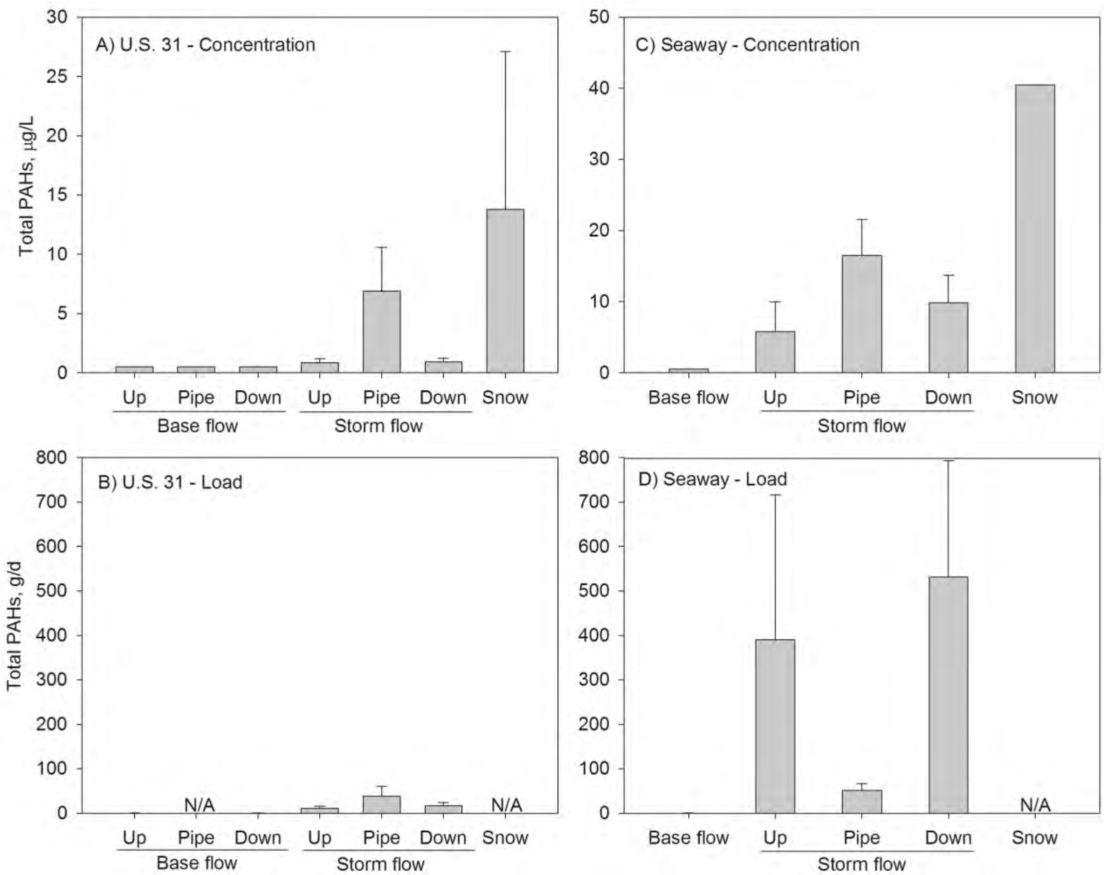


Figure III.A.10. Mean (±SE) total PAH concentrations (µg/L) and loads (g/d) for U.S. 31 (A, B) and Seaway Drive (C, D). N/A = not applicable due to lack of discharge data.

Water Quantity

Seven storms of varying rainfall amounts were sampled over the project period (Fig. III.A.11, Table III.A.3). Rainfall for the period of active road runoff, during which sampling took place, ranged from 0.07 in to 1.04 in (Table III.A.3). Despite the smaller storm water catchment area of the Seaway Drive site, a greater volume of storm water was generated there than at the U.S. 31 site during 2 of the 7 storms (Table III.A.3). However, the 1,216 m^3 volume calculated for Seaway Drive during the 8/4/08 event is likely an over-estimate. During base flow sampling on 7/28/08, stream stage downstream of Seaway Drive was 2 cm higher than upstream and greater discharge at the downstream site during the days preceding the storm is evident from the hydrograph (Fig. III.A.11). Because we estimated storm water discharge as the difference between downstream and upstream discharge, this pre-storm difference in discharge likely inflated the storm water discharge and volume estimates for this date. Excluding the 8/4/08 event at Seaway Drive, the storm with the greatest rainfall generated the greatest site-specific storm water volume at both sites (Table III.A.3). The relationship of site-specific storm water volume and rainfall amount was not perfect, however. Actual rainfall amounts at the study sites were likely different from the totals recorded the Muskegon County Airport, as the Seaway site was ~2 km and the US 31 site was ~ 5 km, from the airport. This may have affected this relationship, especially for storms with locally-intense cells rather than widespread showers. Rainfall rate also likely played a role in site-specific storm water volume. Ponding and/or lateral runoff (i.e., allowing infiltration) during a storm with a slower rate of rainfall would result in less runoff to the stream compared to a storm with a similar amount of rainfall falling at a faster rate.

Total storm water volume, which includes storm water inputs from the study sites plus all upstream inputs over the entire duration of the storm, was directly related to rainfall amount (Table III.A.3). Total storm water volumes estimated for the Seaway Drive site, which is located near the bottom of the watershed, integrate the majority of storm water entering LBC during storm events. Storm flow duration in LBC was directly related to total storm water volume, with the longest storm pulses lasting over 50 hours (Table III.A.3). The extended period of storm flow during higher-rainfall events suggests that storm water detention may be occurring in the watershed, allowing for infiltration and helping to reduce extreme (i.e., "flashy") flows.

Average storm flow discharge in LBC during the period of active road runoff (i.e., our sampling period) ranged from 0.01 to 0.26 m^3/s upstream and 0.02 to 0.38 m^3/s downstream at U.S. 31 (Table III.A.3). At Seaway Drive, average storm flow discharge in LBC ranged from 0.31 to 0.90 m^3/s, both upstream and downstream of the storm water outfall (Table III.A.3). In most cases, storm water was responsible for a greater percentage of downstream discharge in LBC at U.S. 31 than at Seaway Drive (Table III.A.3). The already-elevated storm flow discharge in LBC upstream of Seaway drive, resulting from upstream storm water inputs from the majority of the watershed, minimized the influence of storm water discharge at that site.

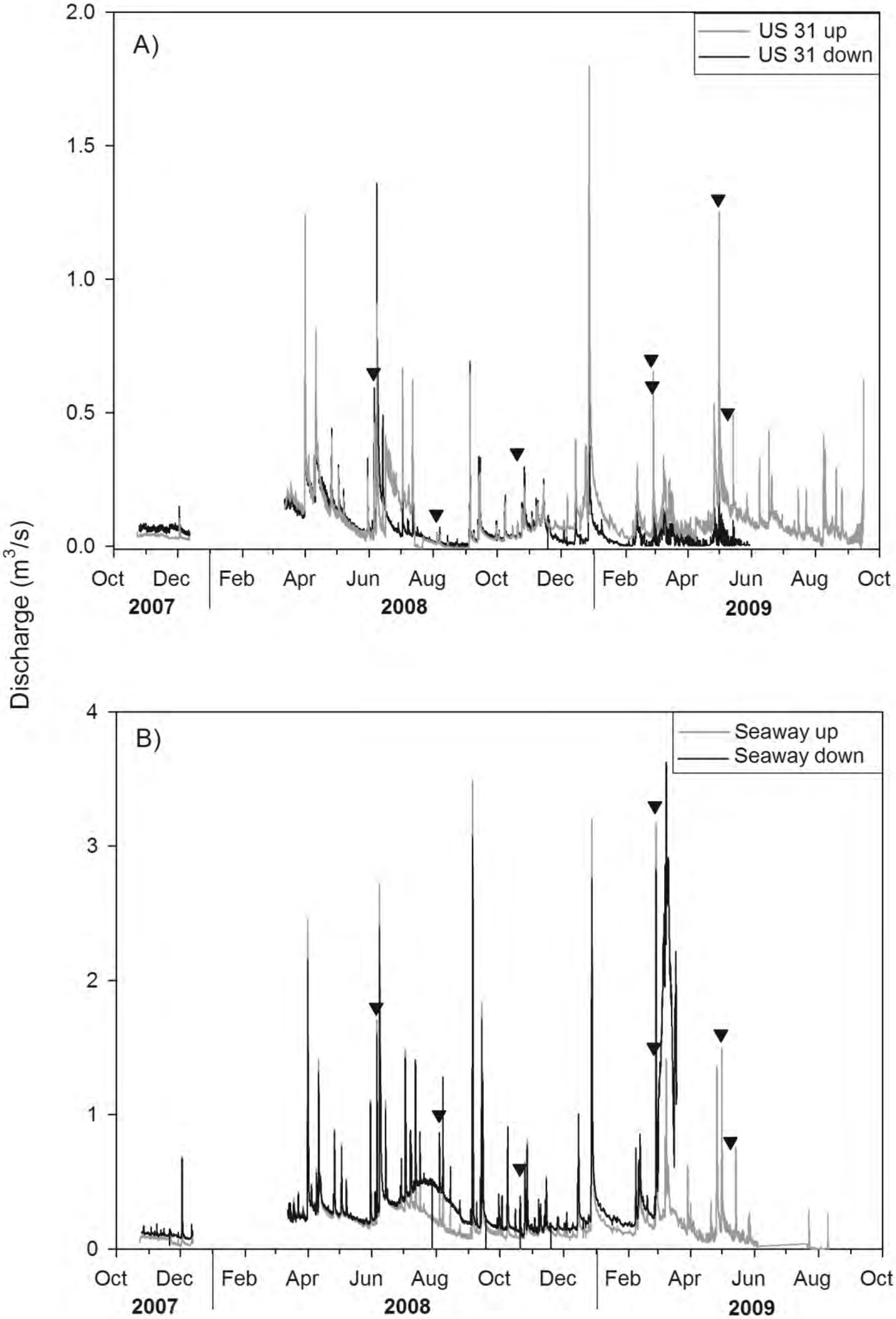


Figure III.A.11. Discharge (m^3/s), as calculated from stage-discharge models, for upstream and downstream locations at A) U.S. 31 and B) Seaway Drive over the study period. Inverted triangles indicate storm sampling events. Note different scales on y-axes.

Table III.A.3. Storm event characteristics for Seaway Drive and U.S. 31. Rainfall amounts were measured at the Muskegon County Airport (3°10'N, 86°14'W; ~ 2 km from the Seaway Drive site and ~5 km from the U.S. 31 site; see Fig. II.A.1) and obtained from the National Oceanic and Atmospheric Administration (NOAA) National Climatic Data Center (www.ncdc.noaa.gov). %SW is the percentage downstream discharge attributable to site-specific storm water. Dashes within cells indicate that data were not available due to technical issues with data loggers.

		Site-specific storm water volume, m^3		Total storm event volume, m^3		Storm flow duration, hr		Average storm flow discharge, m^3/s							
								Seaway				U.S. 31			
Date	Rainfall, in	Seaway	U.S. 31	Seaway	U.S. 31	Seaway	U.S. 31	Up	Pipe	Down	%SW	Up	Pipe	Down	%SW
6/5/2008	1.04	554	459	65,019	18,287	58	59	0.90	0.21	0.89	24%	0.16	0.04	0.21	19%
8/4/2008	0.37	1216	50	11,595	1,198	39	36	0.45	0.23	0.68	34%	0.01	0.01	0.02	50%
10/20/2008	0.09	12	44	5,156	507	19	13	0.34	0.01	0.32	3%	0.07	0.01	0.08	13%
2/25/2009	0.07	53	232	6,885	2,534	22	19	0.31	0.02	0.31	6%	0.11	0.06	0.18	33%
2/26/2009*	0.21	77	273	121,731	38,952	54	54	0.52	0.04	0.54	7%	0.16	0.08	0.23	35%
4/30/2009	0.23	38	206	--	--	--	--	0.45	0.02	0.47	4%	0.26	0.11	0.38	29%
5/8/2009	0.07	--	142	--	--	--	--	0.39	--	0.39	--	0.16	0.08	0.24	--

* Actual rainfall for entire event was 1.73 in, in multiple bands. We sampled the first band, which delivered 0.09 in of rain. The total storm event volumes for this date represent the entire storm, not just the band we sampled.

III.B. Geomorphic Assessment

Mean suspended sediment concentration (SSC) was low during base flow conditions at both sites, ranging from 1.4 (upstream U.S. 31) to 3.6 mg/L (downstream U.S. 31). Mean storm flow SSC was 10X greater than base flow concentrations at the downstream location of U.S. 31 and at both upstream and downstream locations at Seaway. At U.S. 31, storm water resulted in an increase in SSC, as evidenced by elevated SSC in the pipe samples and greater SSC downstream than upstream (Fig. III.B.1). Although Seaway storm water SSC was high, it did not result in elevated SSC at the downstream location. Snowmelt SSC was extremely high (>700 mg/L) at both sites during the 2009 collection and averaged 436 mg/L at U.S. 31 for the 2009 and 2011 collections (Fig. III.B.1).

In-stream SSC remained below the 80 mg/L suspended sediment target for wet-weather events, set forth in the LBC Total Maximum Daily Load (TMDL) for biota (MDEQ 2003). This limit was set based upon the upper limit for good to moderate conditions for the protection of fish communities, as described by Alabaster and Lloyd (1982). Storm water SSC fell into the less than moderate range for the protection of fish communities (Fig. III.B.1). Snowmelt SSC exceeded the less than moderate threshold, falling into the poor category. Base flow SSC was in the optimum range (<25 mg/L) for both sites.

During base flow conditions, the majority of the sediment load in LBC was in the form of bedload sediment (Fig. III.B.1). Bedload also dominated the total sediment load (i.e., suspended sediment + bedload) during storm events, except at the upstream location of Seaway Drive. Total sediment load was positively influenced by road runoff during storms at both sites. At U.S. 31, suspended sediment load was higher in the storm water pipe than upstream, and highest downstream of the storm water outfall. Bedload was also higher downstream than upstream of the storm water outfall at U.S. 31. At Seaway Drive, in-stream suspended sediment load was not influenced by storm water runoff, but bedload was greater downstream of the outfall than upstream (Fig. III.B.1).

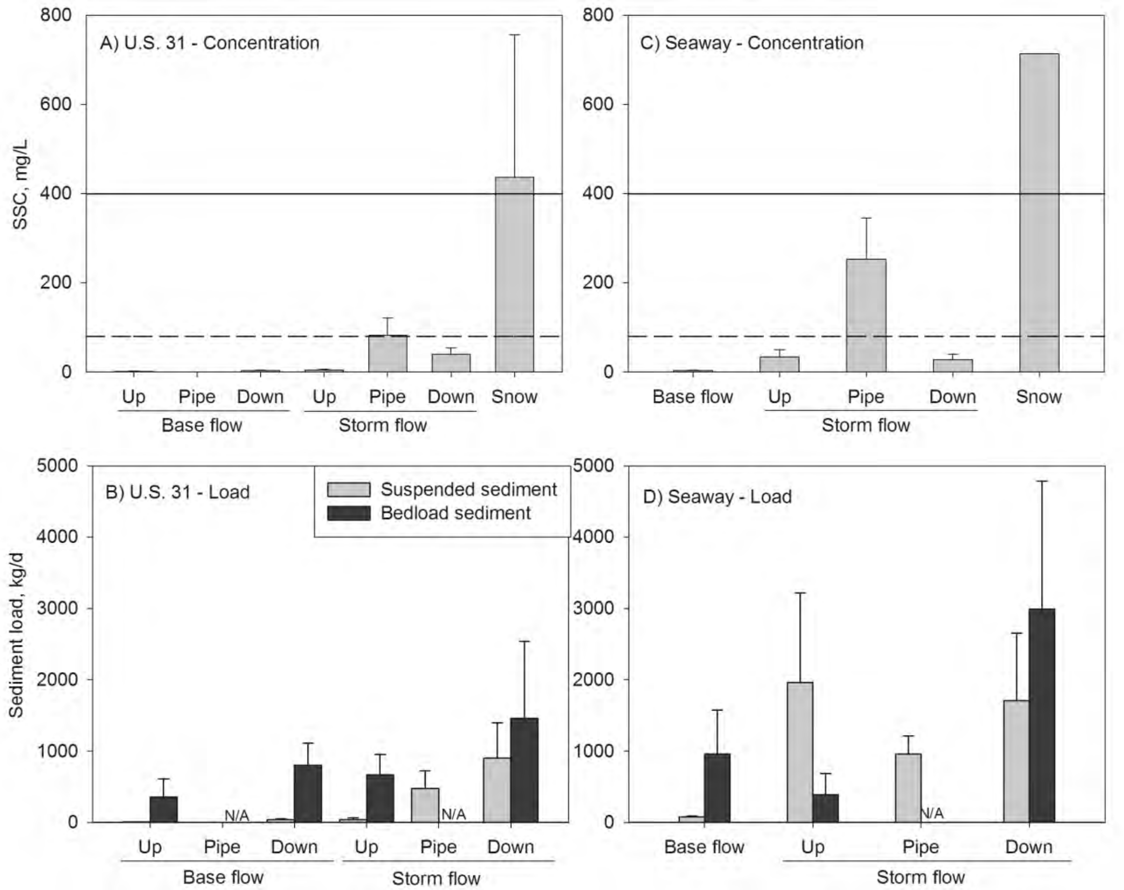


Figure III.B.1. Mean (±SE) suspended sediment concentration (mg/L) at A) U.S. 31 and C) Seaway Drive and mean (±SE) suspended sediment load and bedload (kg/d) at B) U.S. 31 and D) Seaway Drive. Bedload is not applicable (N/A) at storm water pipe outfalls. Dashed line on panels A and C represents the 80 mg/L upper limit for "good to moderate" conditions (Alabaster and Lloyd 1982), and the suspended sediment target for wet-weather events in LBC (MDEQ 2003); solid line represents the 400 mg/L upper limit for "less than moderate" conditions (Alabaster and Lloyd 1982).

III.C. Toxicity Assessment of the Runoff Water

The results of the 48 hr acute toxicity *Ceriodaphnia dubia* tests on the base flow samples are shown in Figure III.C.1. All controls were ≤10% mortality, indicating the tests were successful. The 100% dilutions were ≤10% mortality, signifying that no measurable toxicity was present in the streams. Some of the lower dilutions had mortalities of 15% and 20%. Since the 100% dilutions were ≤10% mortality, these data probably represent experimental variability.

The results of the acute toxicity *C. dubia* tests on the storm water samples are shown in Figure III.C.2. All storm water samples from U.S. 31 were not toxic (≤10% mortality). In contrast, toxicity at Seaway showed a seasonal pattern where winter and spring samples were significantly toxic on 2/25/09 and 4/30/09 (100% mortality) and potentially toxic on 2/26/09 (50% mortality). LC50s for 2/25/09, 2/26/09 and 4/30/09 were 66%, 100%, and 50%, respectively. Spearman's rho was calculated for metals and TPAH (Table III.C.1) and significant correlations were found for chloride ($r=0.80$; $p<0.001$), chromium ($r=0.71$; $p=0.003$), copper ($r=0.72$; $p=0.003$), nickel ($r=0.70$; $p=0.004$), and zinc ($r=0.74$; $p=0.001$). This set of three samples contained the highest concentrations observed during the study for chloride, chromium, copper, nickel, lead, and zinc. Fall and summer storm water samples from the Seaway pipe were not toxic on 6/8/08, 8/4/08, 10/20/08, and 5/9/09.

The results of the acute toxicity *C. dubia* tests on the snowmelt samples are shown in Figure III.C.2. All 2009 snowmelt samples from U.S. 31 were significantly toxic (100% mortality). LC50s for U.S. 31 and Seaway were 19% and 9%, respectively. Spearman's rho was calculated for metals and TPAH (Table III.C.1.) and significant correlations were found for chloride ($r=0.84$; $p<0.001$), chromium ($r=0.79$; $p<0.001$), copper ($r=0.75$; $p<0.001$), nickel ($r=0.84$; $p<0.001$), and zinc ($r=0.79$; $p<0.001$). Snowmelt samples from U.S. 31 were not toxic on in 2011.

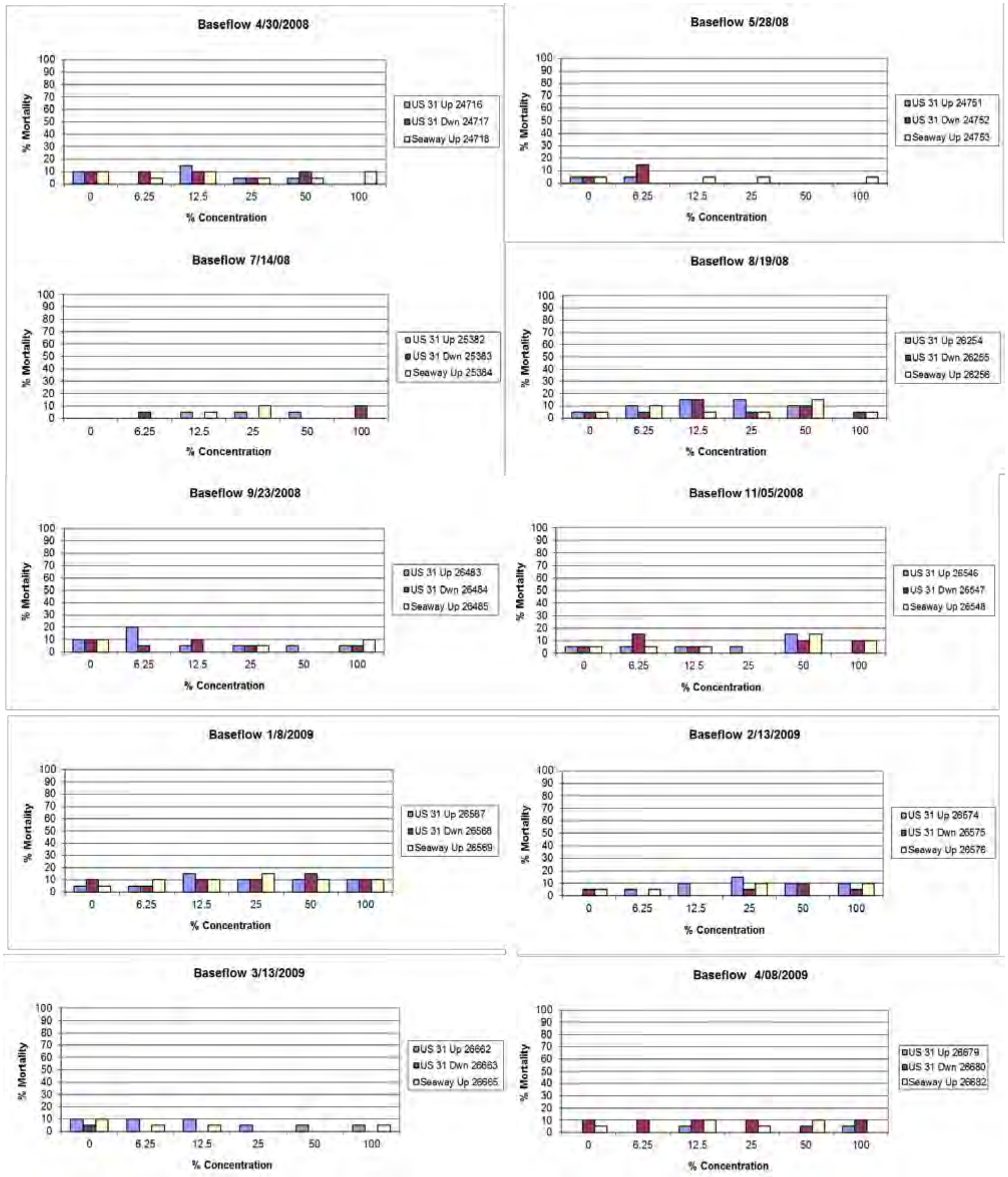


Figure III.C.1. The results of the 48 hr acute toxicity *C. dubia* tests on the base flow samples for U.S. 31 and Seaway 2008-2009.

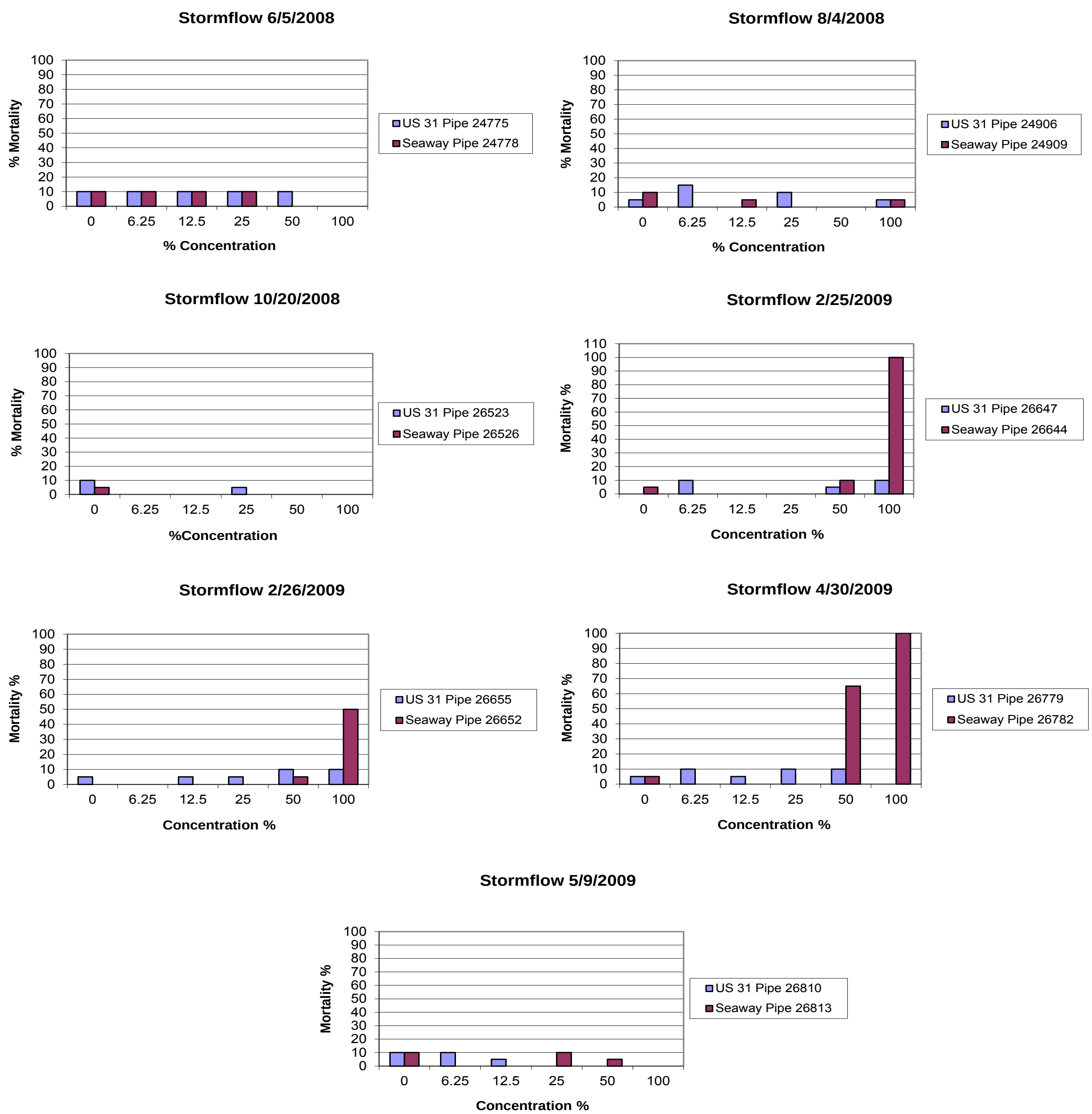
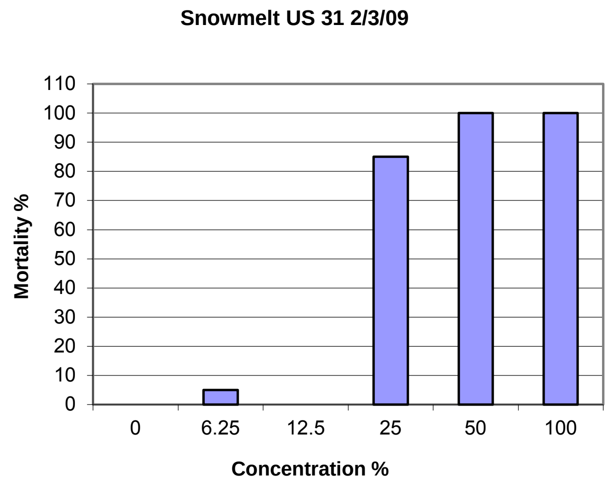
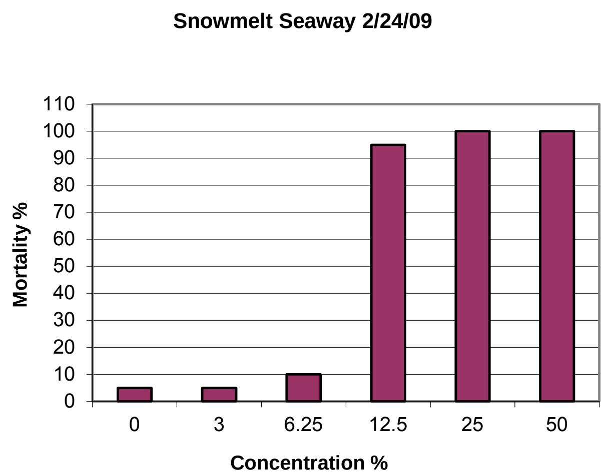


Figure III.C.2. The results of the 48 hr acute toxicity *C. dubia* tests on the storm flow samples from the U.S. 31 Pipe and Seaway Pipe, 2008-2009.

Table III.C.1. The results of the metals and TPH analyses and 48 hr acute toxicity *C. dubia* tests on the storm flow samples from the U.S. 31 Pipe, Seaway Pipe, and snowmelt samples from U.S. 31 and Seaway, 2008-2011.

Sample ID	Station	Date	Chloride mg/l	Cadmium ug/l	Chromium ug/l	Copper ug/l	Nickel ug/l	Lead ug/l	Zinc ug/l	TPAH ug/l	% Mortality
24775	US31 Pipe	6/5/08	85	<1.0	16	23	9.2	28	154	3.6	0
24906	US31 Pipe	8/4/08	94	<1.0	4.6	14	5.1	4.4	59	1.4	5
26523	US31 Pipe	10/20/08	78	<1.0	5.2	15	<5.0	6.2	63	1.3	0
26647	US 31 Pipe	2/25/09	515	<1.0	58	41	19	56	298	7.1	10
26655	US 31 Pipe	2/26/09	456	<1.0	68	41	16	73	283	3.5	10
26779	US 31 Pipe	4/30/09	143	<1.0	23	15	5.3	10	102	2.2	0
26810	US 31 Pipe	5/9/09	66	<1.0	7.6	8.4	<5.0	2.41	<50	5.1	0
24778	Seaway Pipe	6/5/08	5	<1.0	8.1	11	<0.5	10	65	0.5	0
24909	Seaway Pipe	8/4/08	15	<1.0	9.6	19	9.4	27	138	0.5	0
26526	Seaway Pipe	10/20/08	25	<1.0	7.4	18	6.66	13	110	29	0
26644	Seaway Pipe	2/25/09	1558	<1.0	83	37	30	53	431	23	100
26652	Seaway Pipe	2/26/09	535	<1.0	111	63	38	88	490	28	50
26782	Seaway Pipe	4/30/09	123	<1.0	120	54	39	82	550	0.5	100
26813	Seaway Pipe	5/9/09	35	<1.0	39	28	16	26	223	2.2	0
26648	Seaway snowmelt	2/24/09	3247	<1.0	180	96	42	14	616	27	100
26582	US 31 snowmelt	2/3/09	4062	<1.0	162	67	50	9.8	554	27	100
31148	US 31 Snowmelt	2/14/11	487	<1.0	0.06	11	21	44	470	<0.5	10





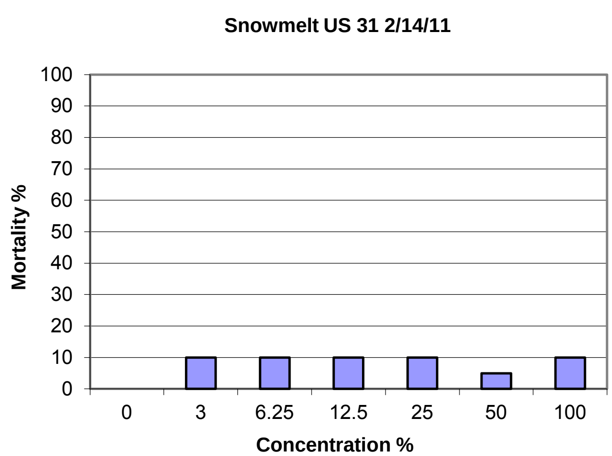


Figure III.C.3. The results of the 48 hr acute toxicity *C. dubia* tests on the snowmelt samples from U.S. 31 and Seaway, 2009 and 2011.

III. D. Engineering Assessment

Treatability evaluations were performed on the February 2009 storm water and snowmelt samples from U.S. 31 and Seaway. The U.S. 31 and Seaway snowmelt samples and Seaway storm water sample were toxic to *C. dubia*. The U.S. 31 storm water sample was not toxic to *C. dubia* but contained elevated levels of heavy metals. The following TIE treatments with targeted toxicants were performed for this study: (1) Baseline (none: unmanipulated sample); (2) EDTA addition (divalent cationic trace metals); (3) Sodium thiosulfate addition (oxidizable compounds, some trace metals); (4) C18 solid-phase extraction (non-polar organics); (5) C18 methanol elution (non-polar organic confirmation); and (6) Aeration (surfactants and volatile compounds). The results of the baseline toxicity studies are shown in Table III.D.1.

Table III.D.1. The results of *Photobacterium phosphoreum* light inhibition assays of storm water and snowmelt samples using the Microtox™ 15 minute assay (n=4 replicates).

Sample	Date	Mean % Light Inhibition Relative to the control (±SE)
U.S. 31 Snowmelt	2/3/2009	33% (3)
Seaway Snowmelt	2/24/2009	60% (6)
U.S. 31 Storm water	2/25/2009	35% (5)
Seaway Storm water	2/25/2009	48% (8)

Seaway snowmelt had the greatest toxic response in the Microtox™ 15 minute assay (60%). The results of the TIE manipulations are shown in Figure III.D.1. The addition of EDTA reduced light inhibition to the greatest extent indicating that heavy metals were the primary source of toxicity. C18 reduced the % light inhibition to a lesser extent indicating that PAH compounds may be responsible for some of the toxic response. Toxicity reductions were significant for all samples except the U.S. 31 storm water (Dunnett's test α=0.05). No change was observed for aeration and sodium thiosulfate, suggesting that the toxicants were not oxidizable or volatile compounds.

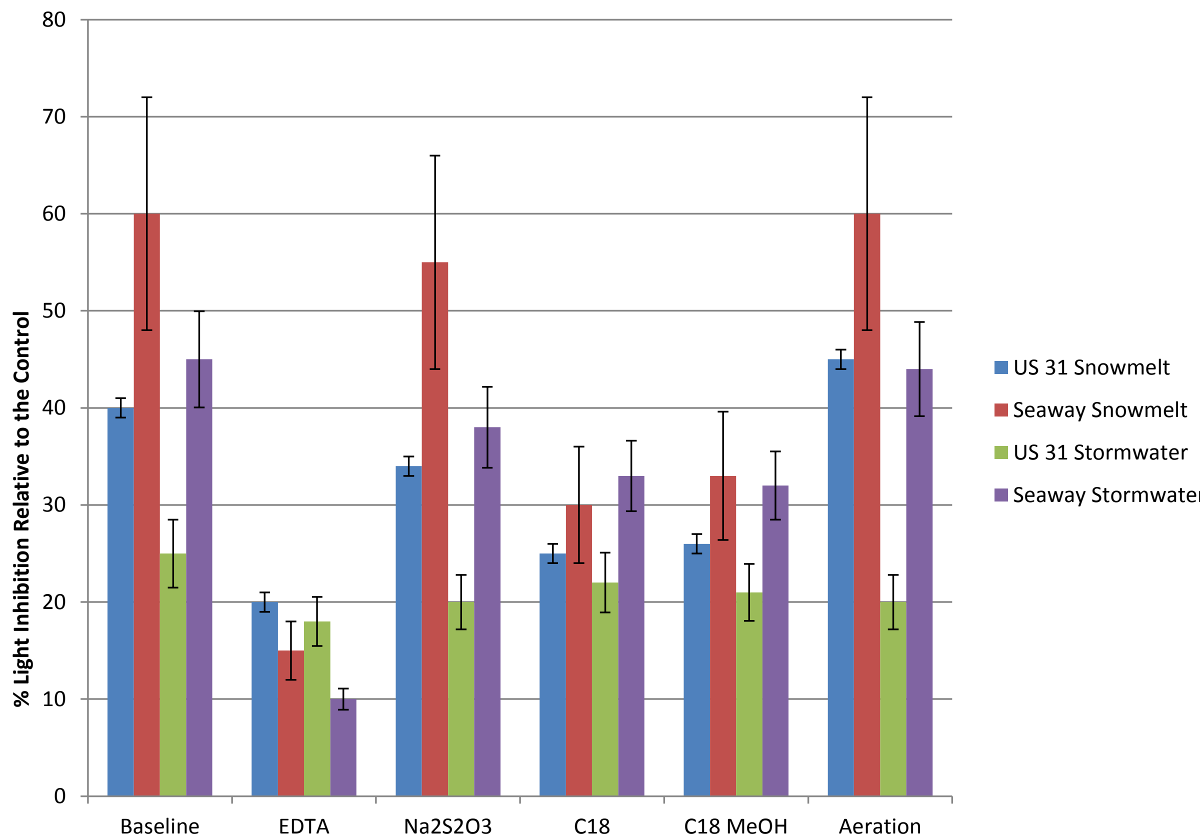


Figure III.D.1. Results of TIE manipulation experiments on storm water and snowmelt samples using the Microtox™ 15 minute assay.

The results of the filtration experiments are shown in Figure III.D.2. A majority of the toxicity was in the particulate phase as >80% of the light inhibition response was removed by filtration with the 0.45 µm membrane filter. Only 15-20% of the light inhibition was removed by the 63 µm filter, indicating the most of the toxicity was associated with fine clay and silt size particles. Settling experiments showed that toxicity reduction began to be observed after 12 hrs and was complete by 72 hrs (Figure III.D.3). Only 30-50% of the toxicity was removed after 4 hrs.

The results of aeration and photodegradation experiments are shown in Figures III.D.4 and III.D.5, respectively. Both of these treatments showed limited effectiveness, with aeration and photodegradation removing only 20-25% of the toxicity.

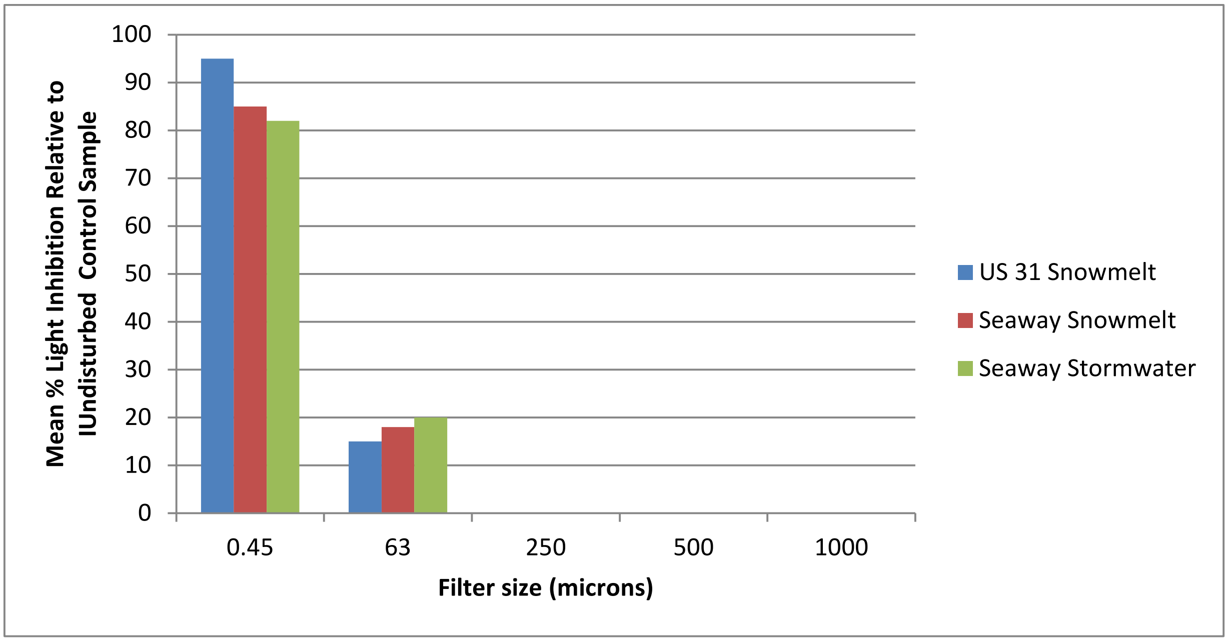


Figure III.D.2. Results of filtration experiments on stormwater and snowmelt samples using the Microtox™ 15 minute assay.

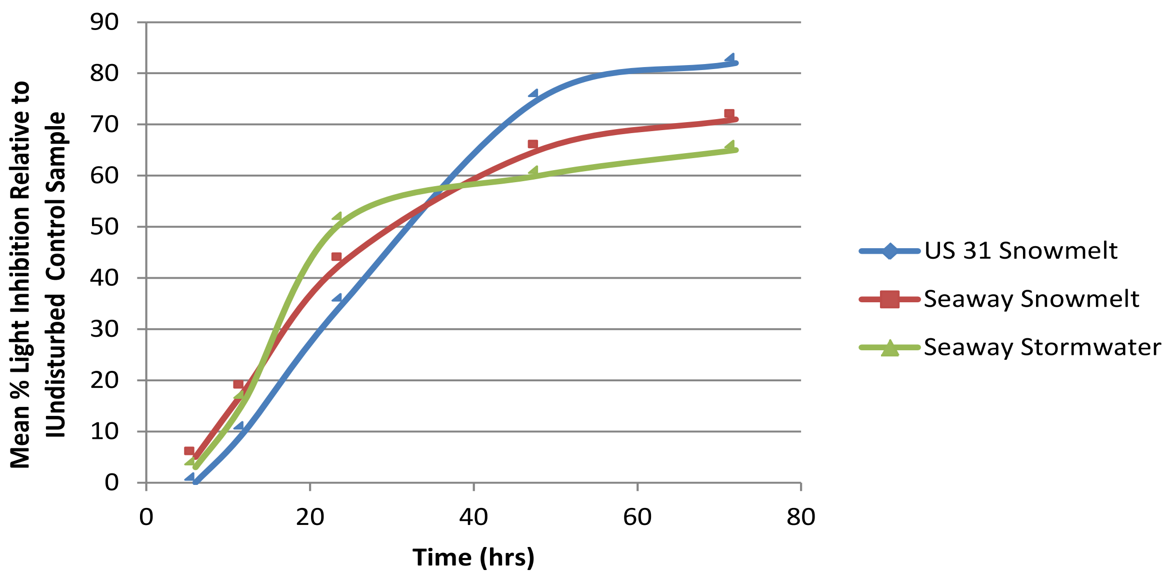


Figure III.D.3. Results of settling experiments on stormwater and snowmelt samples using the Microtox™ 15 minute assay.

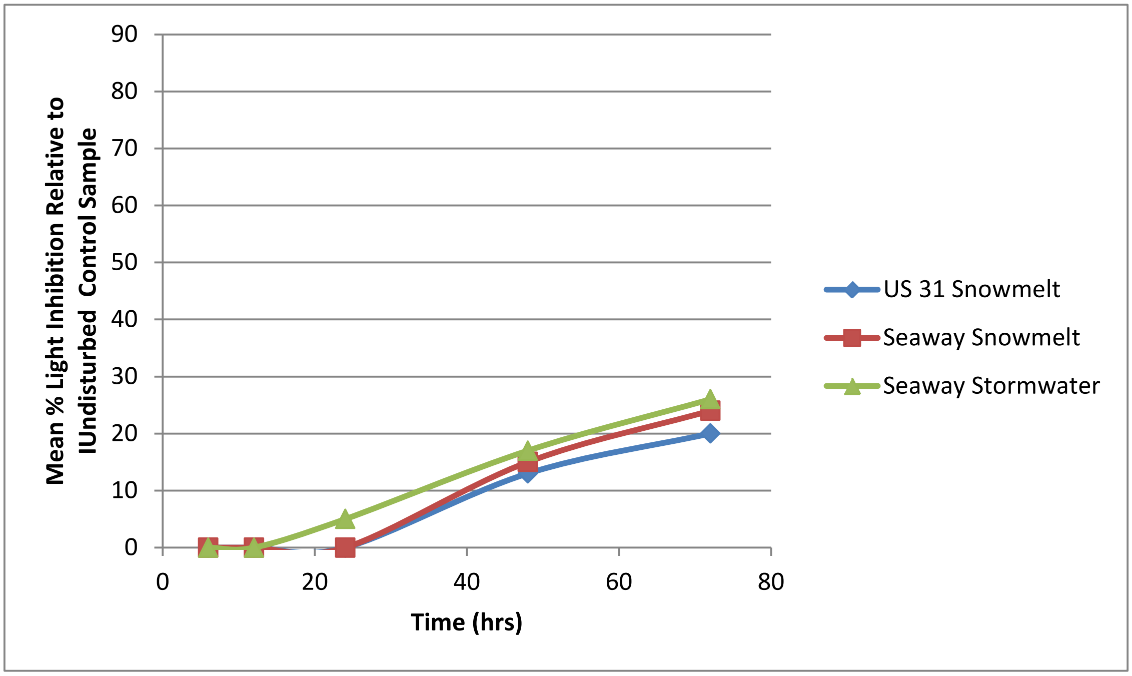


Figure III.D.4. Results of aeration experiments on stormwater and snowmelt samples using the Microtox™ 15 minute assay.

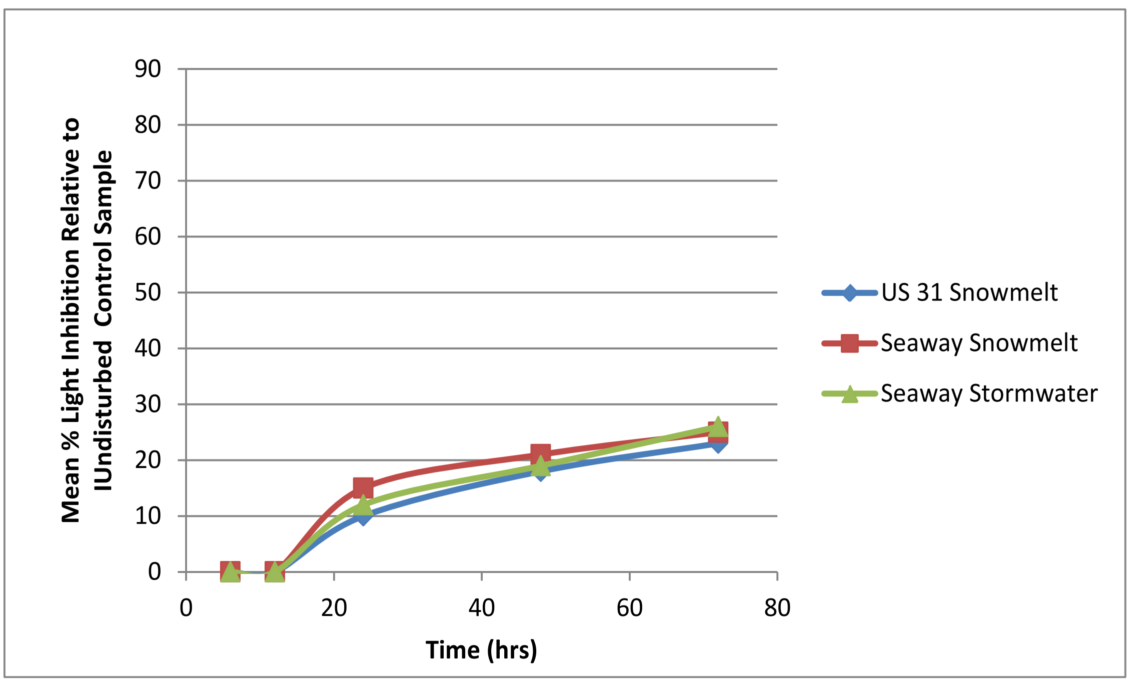


Figure III.D.5. Results of photodegradation experiments on stormwater and snowmelt samples using the Microtox™ 15 minute assay.

III. E. Environmental Analyses

III.E.1. Laboratory Algal Bioassays

The results of the 96 hr growth inhibition tests with *Pseudokirchneriella subcapitatum* on the storm water samples from U.S. 31 and Seaway are shown in Figures III.E.1.1 and III.E.1.2, respectively. *P. subcapitatum* grown in storm water from U.S. 31 had greater cell densities than the culture water control and similar growth as the control water from Little Black Creek (Figure III.E.1.1). The increased growth in U.S. 31 storm water vs the culture water control was statistically significant on 8/4/2008 and 5/9/2009 (Dunnett's Test α=0.050). There was no significant difference between algal growth in the LBC control and the storm water samples. Similar results were observed for the Seaway storm water on 6/5/2008, 8/4/2008, and 4/30/2011 with algal growth in the treatments being significantly greater than the culture water control (Dunnett's Test α=0.050) and no statistical difference with respect to the LBC control (Figure III.E.1.2). The sample from 10/25/2008 showed algal growth rates significantly greater than both the culture water and LBC controls for the 100%, 50%, and 25% treatments. The 12.5% and 6.25% dilutions were not significantly different than either of the two controls. The algal growth results for the storm water collected on 5/9/2009 were similar with the 100% and 50% dilutions having significantly greater cell density than both controls (Dunnett's Test α=0.050).

Snowmelt samples from U.S. 31 and Seaway collected on 2/3/2009 and 2/24/2009, respectively, were inhibitory to the growth of *P. subcapitatum* (Figure III.E.1.3). The 100% and 50% treatments had significantly less growth than both controls (Dunnett's Test α=0.010). Algal growth was reduced by a factor of 7 at 100%. Snowmelt from U.S. 31 also inhibited algal growth, but to a lesser extent. The 100% treatment for U.S. 31 had cell densities that were 2.5 times less than the control after 96 hrs. Both the 100% and 50% dilutions had significantly lower cell densities than the two controls (Dunnett's Test α=0.050). The Seaway snowmelt was higher in heavy metals than the U.S. 31 sample (Table III.C.1). No inhibition or stimulation was observed for the snowmelt sample collected on 2/14/2011 from U.S. 31 as all treatment cell densities were similar to the controls. This sample had heavy metal concentrations similar to several of the storm events.

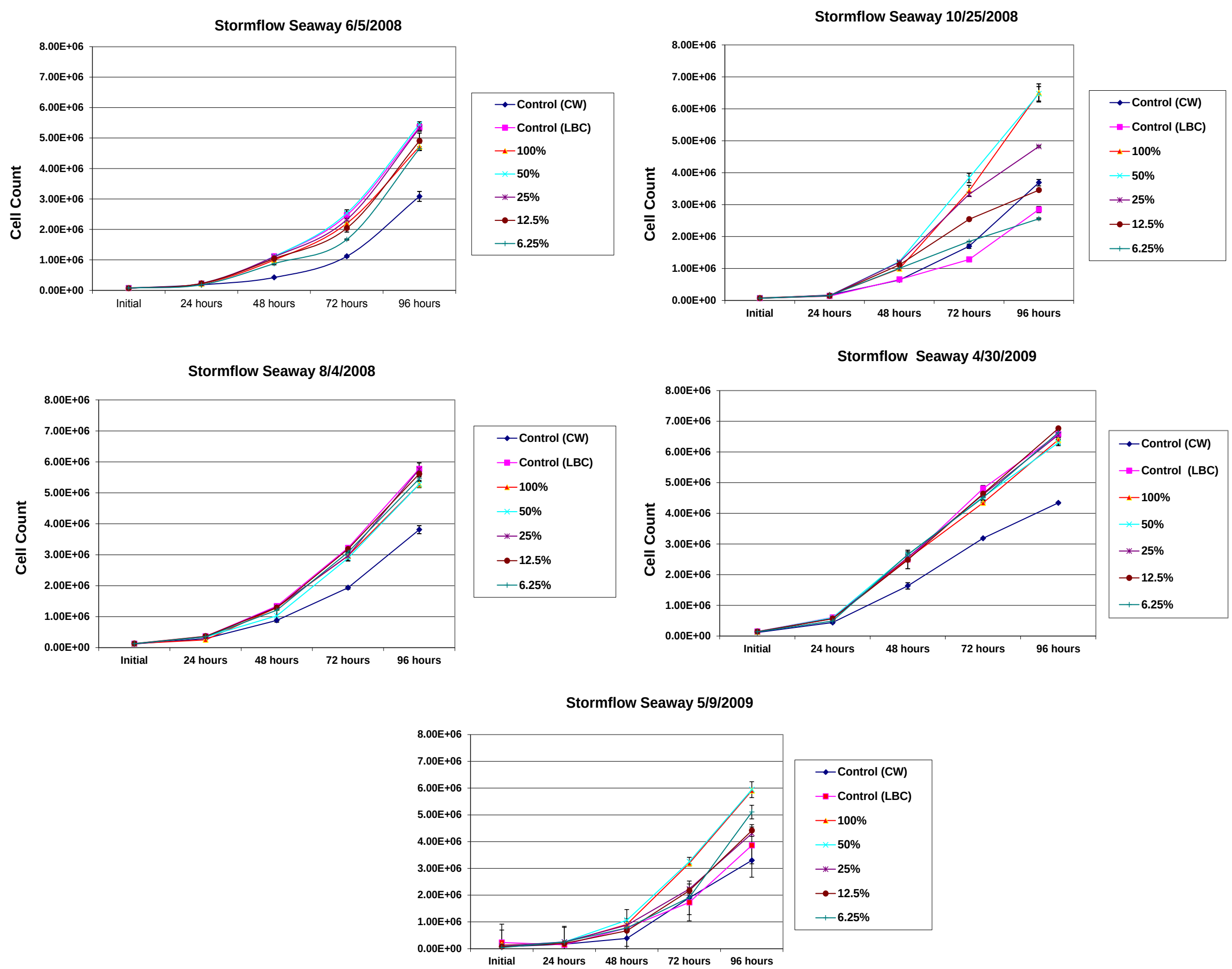


Figure III.E.1.1. The results of the 96 hr growth inhibition tests (mean ±SE) with *Pseudokirchneriella subcapitatum* tests on the storm water samples from U.S. 31, 2008-2009.

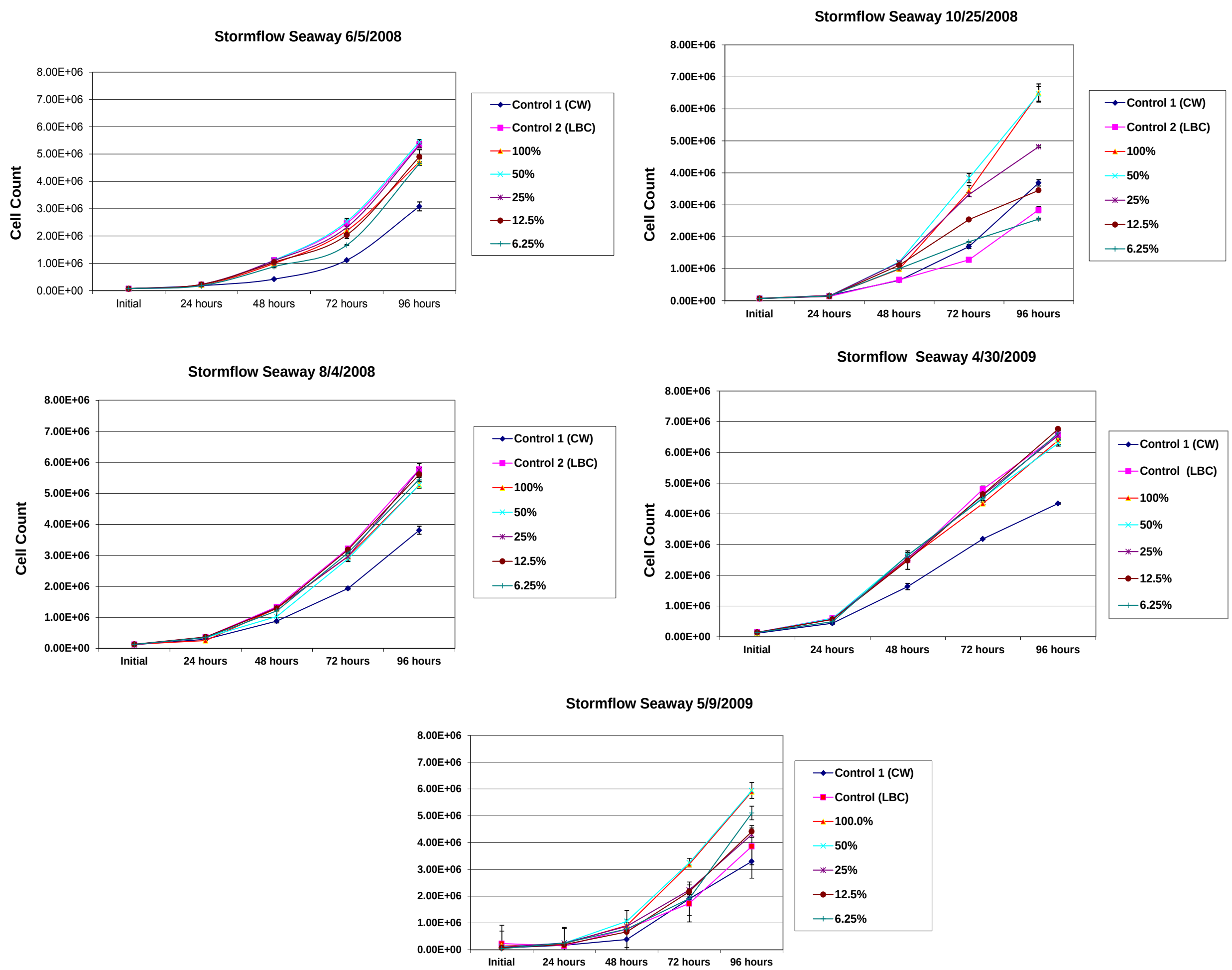


Figure III.E.1.2. The results of the 96 hr growth inhibition tests (mean ±SE) with *Pseudokirchneriella subcapitatum* tests on the storm water samples from Seaway, 2008-2009.

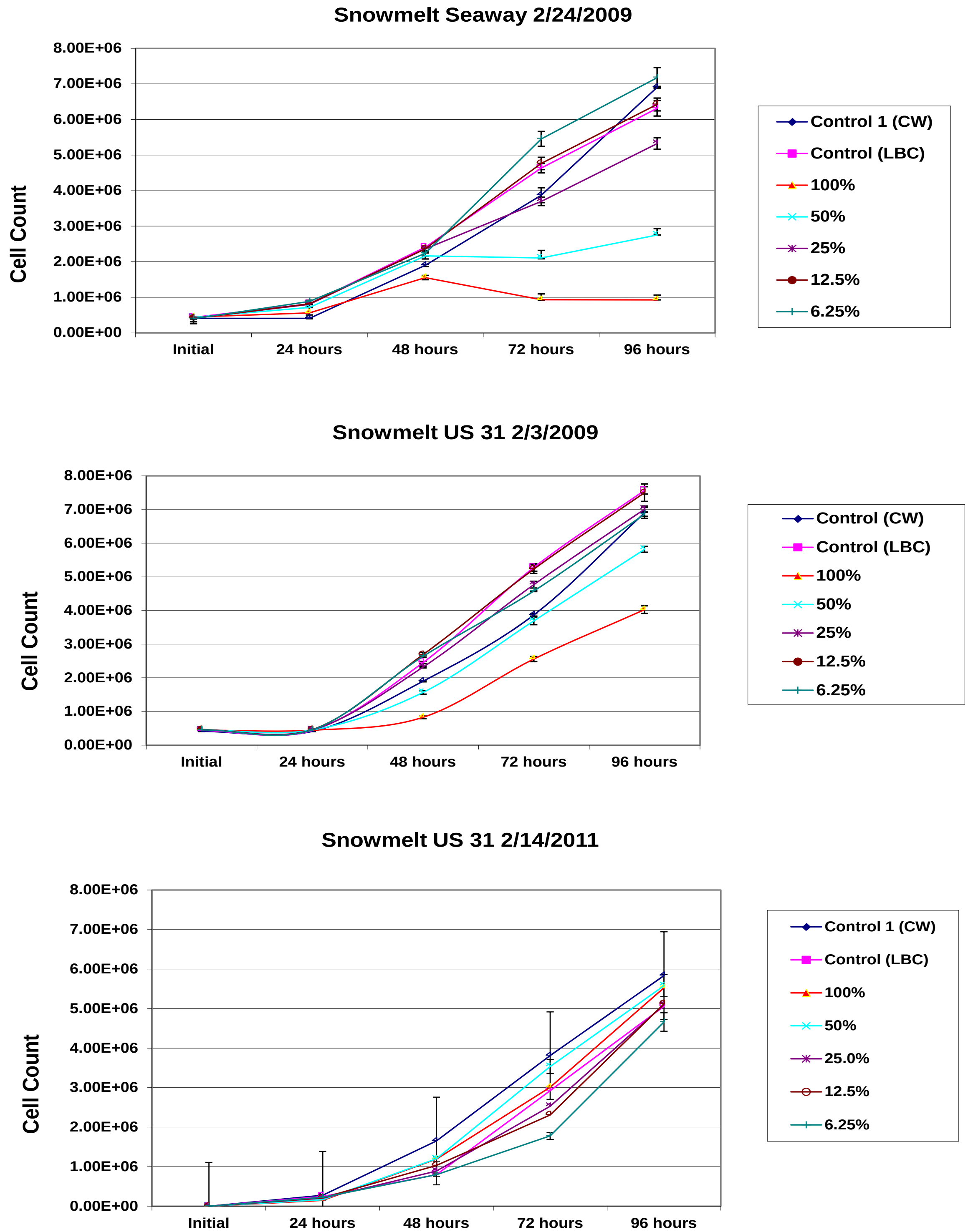


Figure III.E.1.3. The results of the 96 hr growth inhibition tests (mean ±SE) with *Pseudokirchneriella subcapitatum* tests on the snowmelt samples from U.S. 31 and Seaway, 2009 and 2011.

III.E.2. Field Survey: Periphyton

At the Seaway study site, location upstream or downstream of the storm water pipe did not have a statistically significant impact on algal biomass as measured by Chl *a* or AFDM (Table III.E.1.1, Fig. III.E.1.1), although mean values were lower at the downstream than at the upstream site in fall. AFDM:Chl *a* was significantly lower downstream of the storm water pipe compared to upstream (Table 1). Neither the concentration of pheophytin nor pheophytin:Chl *a* were significantly affected by location relative to the storm water pipe (Table III.E.1.1). Biomass as measured by Chl *a* and AFDM was not significantly different between the summer and fall experiments (Table III.E.1.1, Fig. III.E.1.1). Pheophytin was significantly lower in the fall experiment than in the summer experiment, and AFDM:Chl *a* and pheophytin:Chl *a* were both significantly higher in the fall experiment compared to the summer experiment (Table 1).

At the U.S. 31 site, location upstream or downstream of the storm water pipe had a significant influence on AFDM; values were lower in the downstream location compared to the upstream location (Table III.E.1.1, Fig. III.E.1.2). Other measurements related to biomass did not differ significantly based on location relative to the storm water pipe: Chl *a*, AFDM:Chl, pheophytin, or pheophytin:Chl *a* (Table III.E.1.1). Chl *a* values were significantly lower during the fall experiment than during the summer experiment, and AFDM values were significantly higher during the fall experiment compared to the summer experiment (Table III.E.1.1, Fig. III.E.1.2). AFDM:Chl, pheophytin, and pheophytin:Chl *a* values were all significantly higher during the fall experiment compared to the summer experiment (Table III.E.1.1).

Table III.E.1.1. Two-way ANOVA analysis of the final Chl *a*, ash-free dry mass (AFDM), AFDM:Chl a, pheophytin, and pheophytin: Chl *a* values at both the Seaway and U.S. 31 study sites. Storm water (SW) location refers to the sample's location upstream or downstream of storm water pipe, and season refers to differences between the summer and fall experiments. Bold values are significant ($p < 0.05$). Arrows represent values that are significantly higher (↑) or lower (↓) at the downstream site or during the fall experiment.

Seaway Site						U.S. 31 Site					
Source of Variation	**df**	**SS**	**MS**	**F**	**P**	**Source of Variation**	**df**	**SS**	**MS**	**F**	**P**
SW Location						**SW Location**					
Chl *a*	1	88.143	88.143	1.245	0.281	Chl *a*	1	0.817	0.817	3.597	0.076
AFDM	1	5.154	5.154	1.968	0.180	AFDM	1	0.156	0.156	7.470	**0.015↓**
AFDM: Chl *a*	1	0.015	0.015	6.756	**0.019↓**	AFDM: Chl *a*	1	0.001	0.001	0.171	0.685
Pheophytin	1	0.001	0.001	0.021	0.887	Pheophytin	1	0.096	0.096	1.894	0.188
Pheophytin:Chl *a*	1	0.031	0.031	0.550	0.469	Pheophytin:Chl *a*	1	0.029	0.029	0.235	0.634
Season						**Season**					
Chl *a*	1	211.095	211.095	2.982	0.103	Chl *a*	1	7.507	7.507	33.066	**<0.001↓**
AFDM	1	0.345	0.345	0.132	0.721	AFDM	1	0.255	0.255	12.245	**0.003↑**
AFDM: Chl *a*	1	0.042	0.042	18.325	**<0.001↑**	AFDM: Chl *a*	1	0.340	0.340	70.793	**<0.001↑**
Pheophytin	1	1.518	1.518	25.152	**<0.001↓**	Pheophytin	1	1.365	1.365	27.044	**<0.001↑**
Pheophytin:Chl *a*	1	0.706	0.706	12.438	**0.003↑**	Pheophytin:Chl *a*	1	4.157	4.157	33.970	**<0.001↑**
SW Location x Season						**SW Location x Season**					
Chl *a*	1	72.872	72.872	1.029	0.325	Chl *a*	1	0.272	0.272	1.198	0.290
AFDM	1	1.526	1.526	0.583	0.456	AFDM	1	0.012	0.012	0.577	0.459
AFDM: Chl *a*	1	0.000	0.000	0.088	0.771	AFDM: Chl *a*	1	0.000	0.000	0.092	0.766
Pheophytin	1	0.002	0.002	0.040	0.844	Pheophytin	1	0.003	0.003	0.056	0.815
Pheophytin:Chl *a*	1	0.121	0.121	2.137	0.163	Pheophytin:Chl *a*	1	0.259	0.259	2.119	0.165

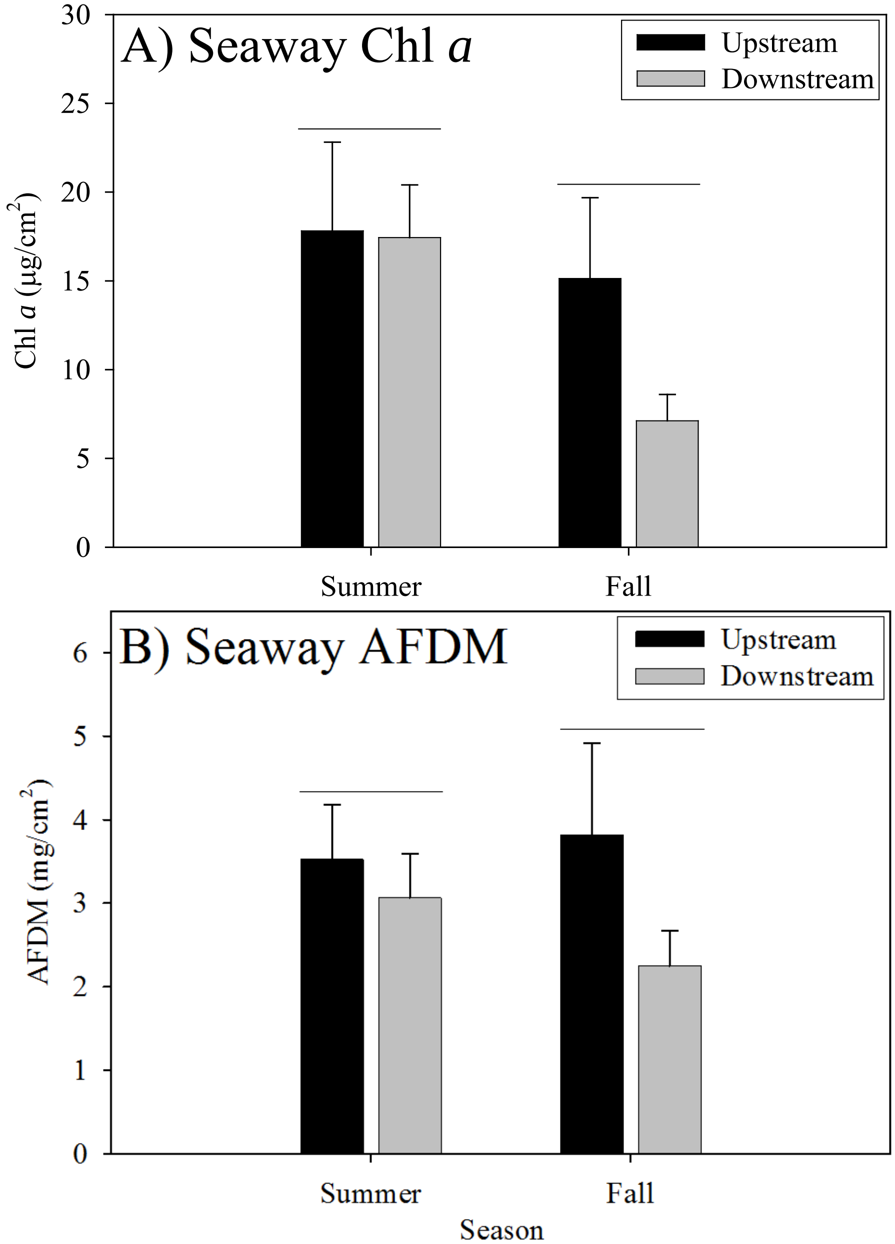


Fig. III.E.1.1. Seaway site biomass in terms of A) Chl *a* concentrations and B) ash-free dry mass (AFDM) values of algal samples upstream and downstream of a storm water outlet pipe in Little Black Creek during both the summer and fall experiments. Chl *a* or AFDM values connected by a bar are not significantly different from each other, and separate bars represent significant differences among values within a particular experiment only. Error bars represent standard error.

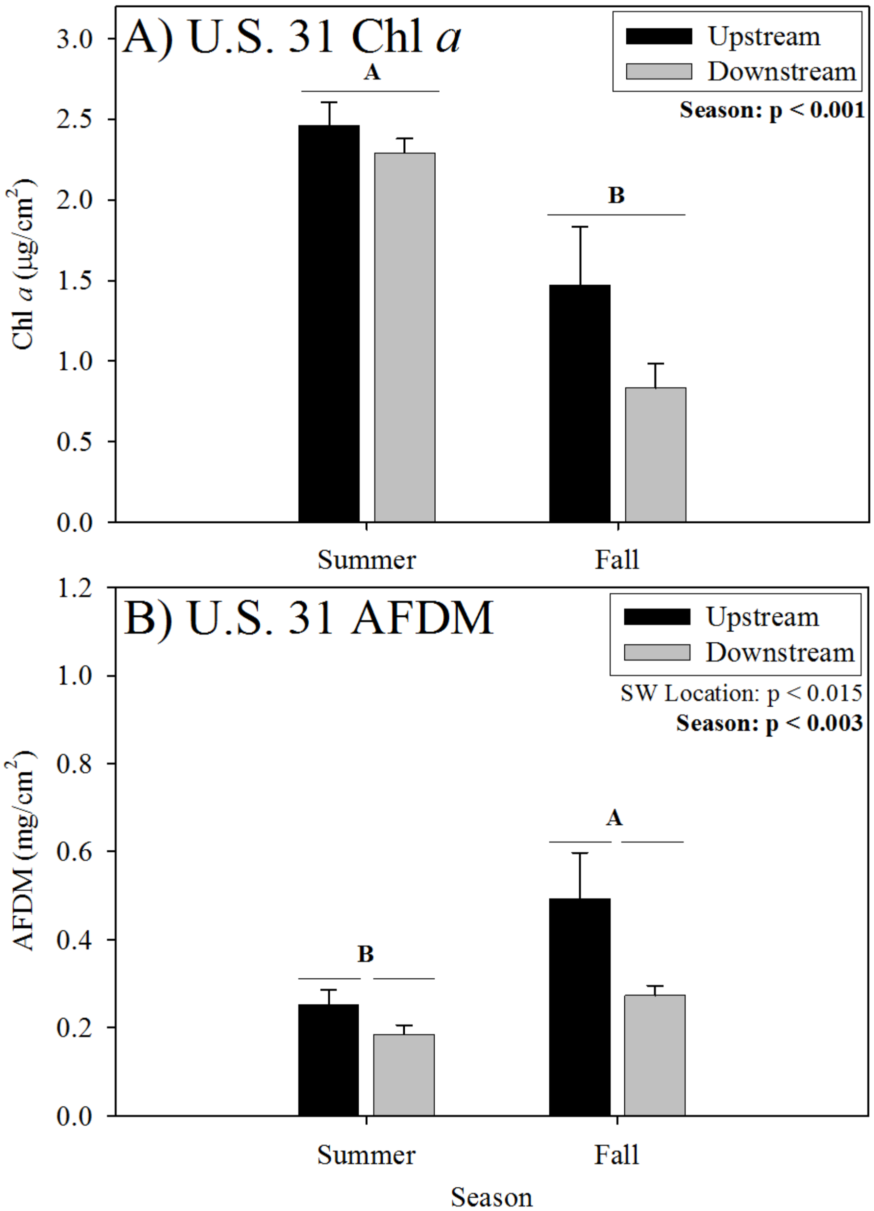


Fig. III.E.1.2. U.S. 31 site biomass in terms of A) Chl *a* concentrations and B) ash-free dry mass (AFDM) values of algal samples upstream and downstream of a storm water outlet pipe in Little Black Creek during both the summer and fall experiments. Chl *a* or AFDM values from upstream and downstream locations connected by a bar are not significantly different from each other, and separate bars represent significant differences among values within a particular experiment only. Bold letters represent significant differences between the summer and fall experiments. Error bars represent standard error.

Algal Metabolism

At the Seaway location, areal-specific GPP was significantly lower downstream of the storm water pipe compared to upstream of the pipe (Table III.E.1.2, Fig. III.E.1.3). Neither areal-specific community respiration (Fig. III.E.1.3) nor GPP:R values at this site were significantly affected by location relative to the storm water pipe (Table III.E.1.2). Respiration and GPP were significantly lower during the fall experiment compared to the summer experiment (Table III.E.1.2, Fig. III.E.1.3).

At the U.S. 31 study site, areal-specific respiration was significantly lower downstream compared to upstream of the storm water pipe, but only during the summer experiment (Table III.E.1.2, Fig. III.E.1.4). During the fall experiment, respiration was significantly higher downstream compared to upstream (Table III.E.1.2, Fig. III.E.1.4). Although, GPP values were not statistically different between the upstream and downstream sampling locations, GPP tended to be higher at the upstream location compared to the downstream location (Fig. III.E.1.4). GPP:R values did not differ based on location at the U.S. 31 site. GPP was significantly lower in the fall experiment than in the summer (Fig. III.E.1.4, Table III.E.1.2). A similar trend was observed both for Chl *a*-specific metabolism and areal-specific metabolism at both study sites.

Table III.E.1.2. Two-way ANOVA analysis of the final community respiration and gross primary production (GPP) values for both the Seaway and U.S. 31 study sites. Storm water (SW) location refers to the sample's location upstream or downstream of storm water outlet and season refers to differences between the summer and fall experiments. Bold values are significant (p < 0.05). Arrows represent values that are significantly higher (↑) or lower (↓) at the downstream site or during the fall experiment.

Seaway Site						U.S. 31 Site					
Source of Variation	**df**	**SS**	**MS**	**F**	**P**	**Source of Variation**	**df**	**SS**	**MS**	**F**	**P**
SW Location						**SW Location**					
Respiration	1	9.5E-08	9.5E-08	0.0195	0.891	GPP	1	6.28E-06	6.28E-06	0.866	0.367
GPP	1	0.0000355	0.0000355	17.903	**<0.001↓**	Respiration	1	2.72E-07	2.72E-07	0.358	0.558
Season						**Season**					
Respiration	1	0.0000241	0.0000241	4.931	**0.041↓**	Respiration	1	1.04E-06	1.04E-06	1.373	0.258
GPP	1	0.0000285	0.0000285	14.389	**0.002↓**	GPP	1	0.0000824	0.0000824	11.371	**0.004↓**
SW Location x Season						**SW Location x Season**					
Respiration	1	2.83E-07	2.83E-07	0.058	0.813	Respiration	1	0.0000045	0.0000045	5.931	**0.027***
GPP	1	2.15E-06	2.15E-06	1.084	0.313	GPP	1	0.0000197	0.0000197	2.713	0.12

* significantly lower downstream in summer experiment and significantly higher downstream in fall experiment

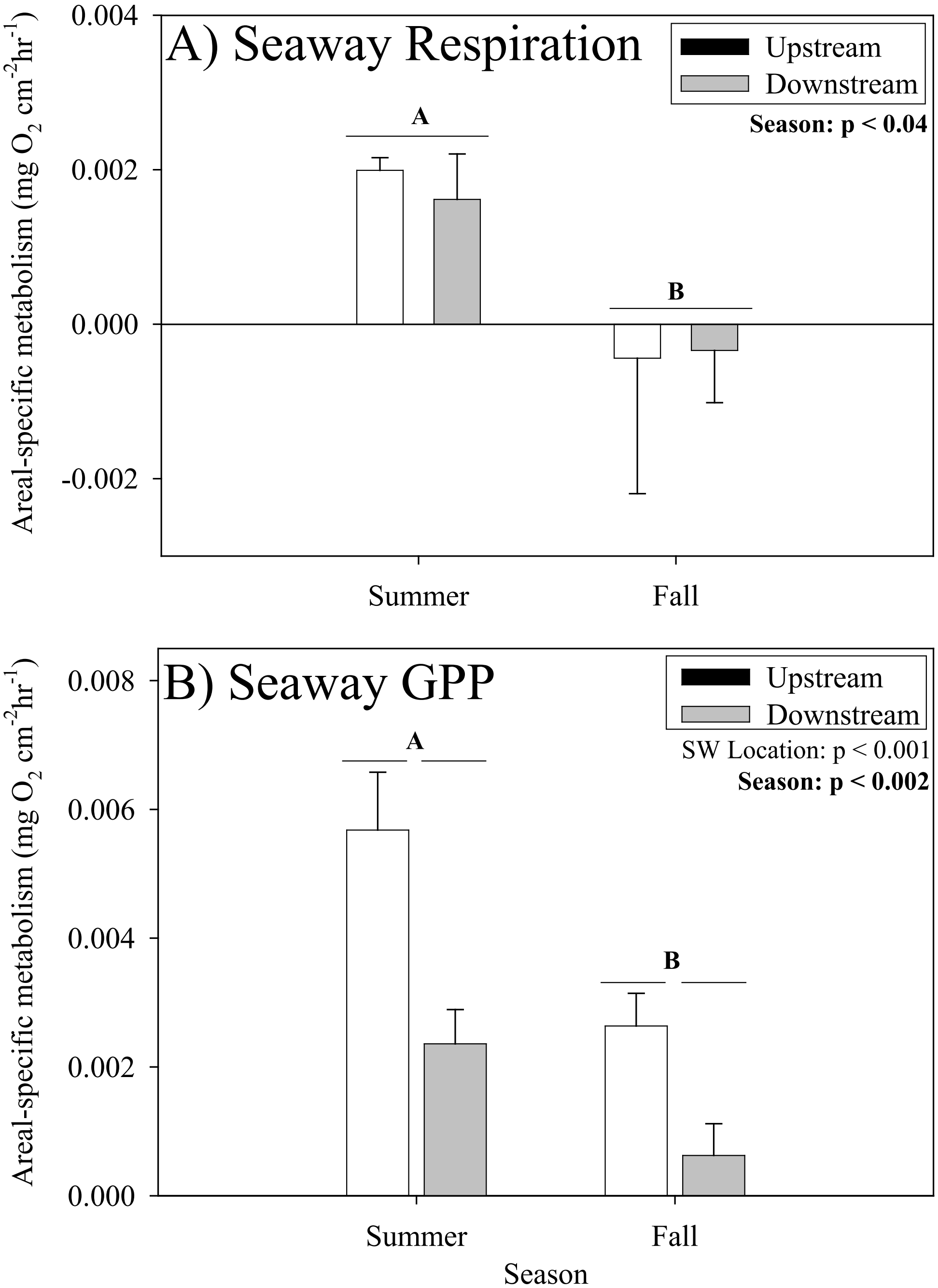


Fig. III.E.1.3. Seaway site areal-specific A) respiration and B) gross primary production (GPP) of algal samples upstream and downstream of a storm water input pipe in Little Black Creek during both the summer and fall experiments. Respiration and GPP values from upstream and downstream locations connected by a bar are not significantly different from each other, and separate bars represent significant differences among values within a particular experiment only. Bold letters represent significant differences between the summer and fall experiments. Error bars represent standard error.

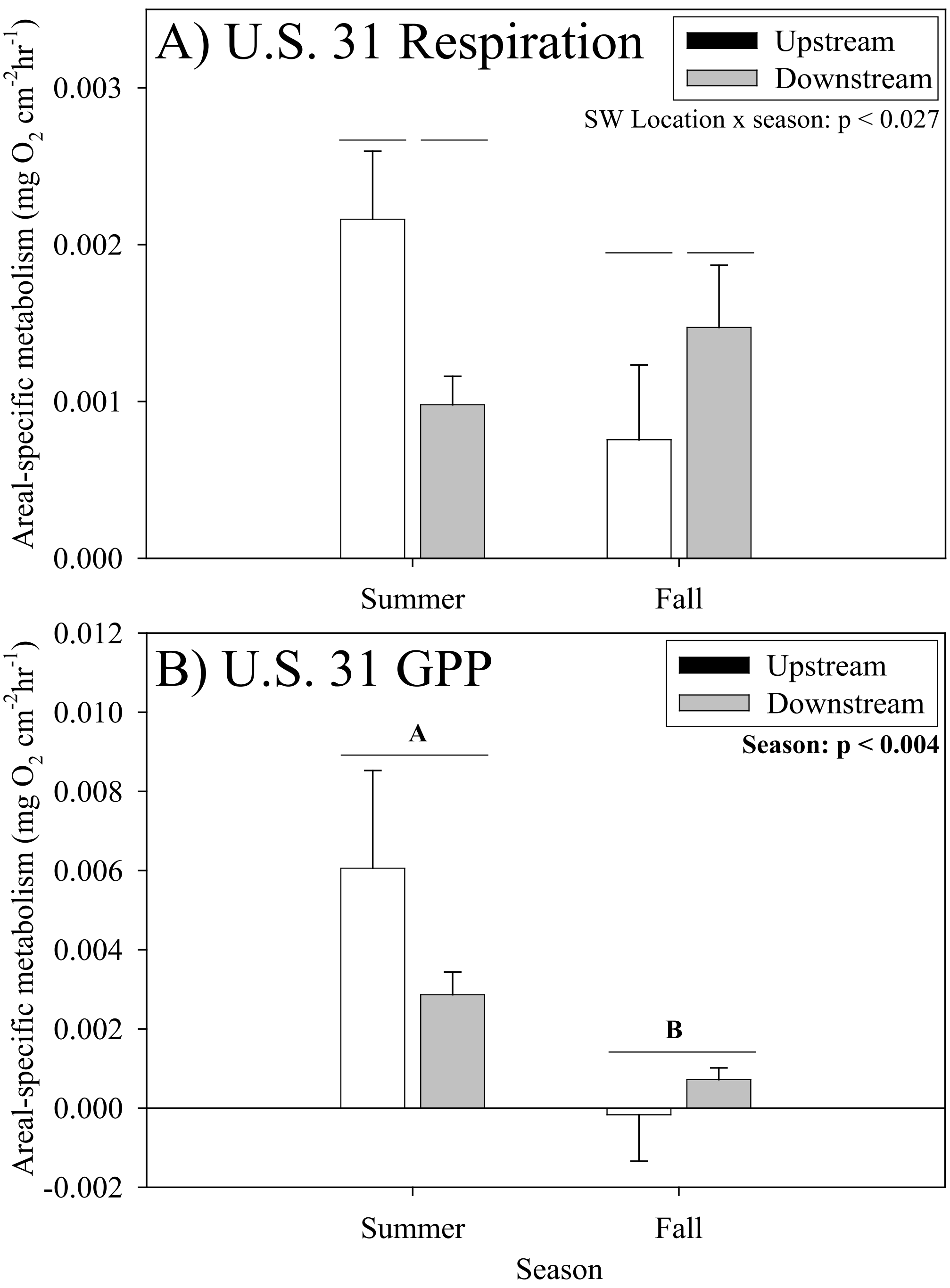


Fig. III.E.1.4. U.S. 31 site areal-specific A) respiration and B) gross primary production (GPP) of values of algal samples upstream and downstream of a storm water input pipe in Little Black Creek during both the summer and fall experiments. Respiration and GPP values from upstream and downstream locations connected by a bar are not significantly different from each other, and separate bars represent significant differences among values within a particular experiment only. Bold letters represent significant differences between the summer and fall experiments. Error bars represent standard error.

Algal Community Composition

Seaway site community composition

The algal communities at the end of these experiments were almost entirely dominated by diatoms (Bacillariophyceae) and green algae (Chlorophyceae). The eight most abundant genera (or taxonomic units) in terms of relative abundance of total cell numbers were *Achnanthes,* small naviculoid*, Navicula, Cocconeis, Staurosirella, Rhoicosphenia, Diatoma,* and *Cladophora* (Table III.E.1.3, Fig. III.E.1.5). Diatoms made up ~99% of total cell numbers with green algae contributing the remaining ~1%.

In terms of relative total biovolume, the eight most abundant taxa were *Navicula, Cocconeis,* small naviculoid, *Staurosirella, Diatoma, Achnanthes, Rhoicosphenia,* and *Cladophora* (Table III.E.1.4, Fig. III.E.1.6). Diatoms made up ~66% of total biovolume with green algae contributing the remaining ~34%. The discrepancy of green algal taxa abundance in terms of cell numbers and biovolume was because *Cladophora* cells were very large in size, but relatively few in number.

Location upstream or downstream of the storm water pipe had a small influence on community composition during both the summer and fall experiments in terms of relative abundance of total cell numbers, although the overall community composition was not significantly different between the two locations ($p > 0.367$). During summer, the relative abundances of total cell numbers of *Cocconeis* and *Rhoicosphenia* were most abundant upstream, while *Rhoicosphenia* and small naviculoid were most abundant downstream (Table III.E.1.4). During the fall experiment, *Navicula* was the most abundant taxon both upstream and downstream of the storm water pipe. Relative abundance of total cell numbers of *Cocconeis* was significantly higher upstream of the pipe compared to downstream, but only during the summer experiment (Table III.E.1.5). Relative abundance of total cell numbers of *Navicula* was significantly higher downstream of the storm water pipe compared to upstream, but only during the fall experiment (Table III.E.1.5).

Although several taxa had significant responses to location upstream or downstream of the storm water pipe, the overall communities between the locations were not significantly different in terms of relative total biovolume ($p > 0.308$). *Cladophora* made up the largest proportion of relative total biovolume both upstream and downstream of the storm water pipe in the summer experiment, and *Navicula* had the largest relative total biovolume in both storm water pipe locations during the fall experiment (Table III.E.1.4). *Navicula* and small naviculoid taxa had a significantly greater relative total biovolume downstream of the pipe compared to upstream, and *Cladophora* biovolume was significantly higher upstream compared to downstream of the pipe, although these differences were significant only during summer experiment (Table III.E.1.5). No taxa were significantly affected by location during the fall experiment (Table III.E.1.5).

The relative abundance of total cell numbers of numerous taxa was significantly affected by season. The overall communities in the summer and fall experiments were significantly different from one another ($p < 0.001$). *Navicula*, *Cocconeis*, and *Diatoma* all had a significantly higher relative abundance total cell numbers in the fall experiment compared to the summer (Table III.E.1.5), while *Staurosirella*, *Rhoicosphenia*, *Cladophora* and *Stephanocyclus* had significantly higher relative total cell numbers in the summer experiment compared to the fall (Table III.E.1.5).

The relative total biovolume of numerous taxa was also affected by season, and the overall community biovolume during the two seasons were significantly different from one another ($p < 0.001$). *Achnanthes*, small naviculoid, *Navicula,* and *Diatoma* all had a significantly higher relative total biovolume in the fall experiment compared to the summer (Table III.E.1.5), while *Cocconeis, Rhoicosphenia,* and *Cladophora* had a significantly higher relative total biovolume in the summer experiment compared to the fall (Table III.E.1.5).

The distribution of different algal physiognomies differed between upstream and downstream locations and between seasons in terms of relative abundance of total cell numbers. During summer, the community upstream of the storm water pipe was dominated by firm understory species. The community downstream during summer was not dominated by a single growth form, but loose understory taxa (34%) and firm canopy taxa (31%) made up the largest proportions of the community (Fig. III.E.1.7A). During the fall experiment, loose understory taxa were the predominant physiognomic group both upstream and downstream (Fig. III.E.1.7A). In both experiments, firm understory taxa had a significantly higher relative number of total cells upstream compared to downstream of the storm water pipe ($p < 0.003$), and loose understory taxa had a significantly higher relative number of total cells downstream compared to upstream ($p < 0.004$; Fig. III.E.1.7A). Firm canopy taxa and loose canopy taxa both had a significantly higher relative number of total cells in the summer experiment than in the fall ($p < 0.001$ and $p < 0.014$, respectively), and loose understory taxa had significantly more relative total cells in the fall experiment compared to the summer ($p <0.001$; Fig. III.E.1.7A).

Table III.E.1.3. Seaway site median relative abundance of total cell numbers of algal taxa from upstream (UPST) and downstream (DNST) of the storm water pipe in both seasons (summer and fall). Replicate samples (n=3) were combined to generate median values.

	Seaway relative abundance of total cell numbers (%)				
Taxon	Summer		Fall		
	UPST	DNST	UPST	DNST	Overall
Achnanthes	11.18	3.05	23.55	10.89	20.32
Small naviculoid	11.28	22.19	20.86	15.77	19.19
Navicula	9.87	9.69	50.36	66.01	11.23
Cocconeis	30.25	10.67	1.44	1.43	5.47
Staurosirella	9.24	11.88	0.55	1.63	5.35
Rhoicosphenia	24.33	24.39	0.00	0.00	0.81
Diatoma	0.00	0.00	1.39	0.95	0.44
Cladophora	1.78	0.31	0.00	0.00	0.16
All other algal taxa	2.06	17.83	1.85	3.32	37.03
Total	100.00	100.00	100.00	100.00	100.00

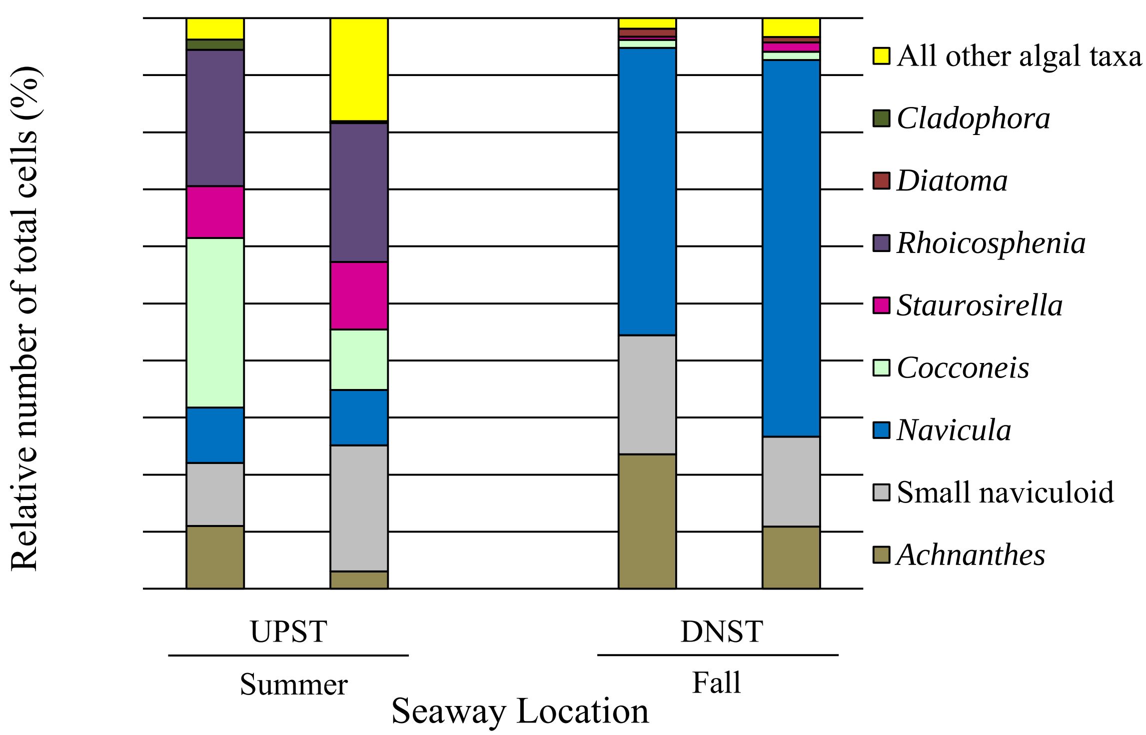


Fig. III.E.1.5. Seaway site median relative abundance of total cell numbers of algal taxa from upstream (UPST) and downstream (DNST) of the storm water pipe in both seasons (summer and fall). Replicate samples (n=3) were combined to generate median values.

Table III.E.1.4. Seaway site median relative biovolume of algal taxa from upstream (UPST) and downstream (DNST) of the storm water pipe in both seasons (summer and fall). Replicate samples (n=3) were combined to generate median values. Taxa are listed in the same order as in Table 3 for comparison purposes.

	Seaway relative total biovolume (%)				
Taxon	Summer		Fall		
	UPST	DNST	UPST	DNST	Overall
Achnanthes	0.08	0.16	1.67	0.64	0.83
Small naviculoid	0.23	3.89	4.16	2.67	3.09
Navicula	1.99	14.70	88.64	92.59	28.33
Cocconeis	3.82	5.13	1.55	1.08	4.47
Staurosirella	0.48	4.60	0.24	0.50	1.25
Rhoicosphenia	1.71	21.77	0.00	0.00	0.51
Diatoma	0.00	0.00	2.80	1.65	0.97
Cladophora	91.00	48.89	0.00	0.00	0.07
All other algal taxa	1.01	4.90	6.76	4.17	64.40
Total	100.00	100.00	100.00	100.00	100.0

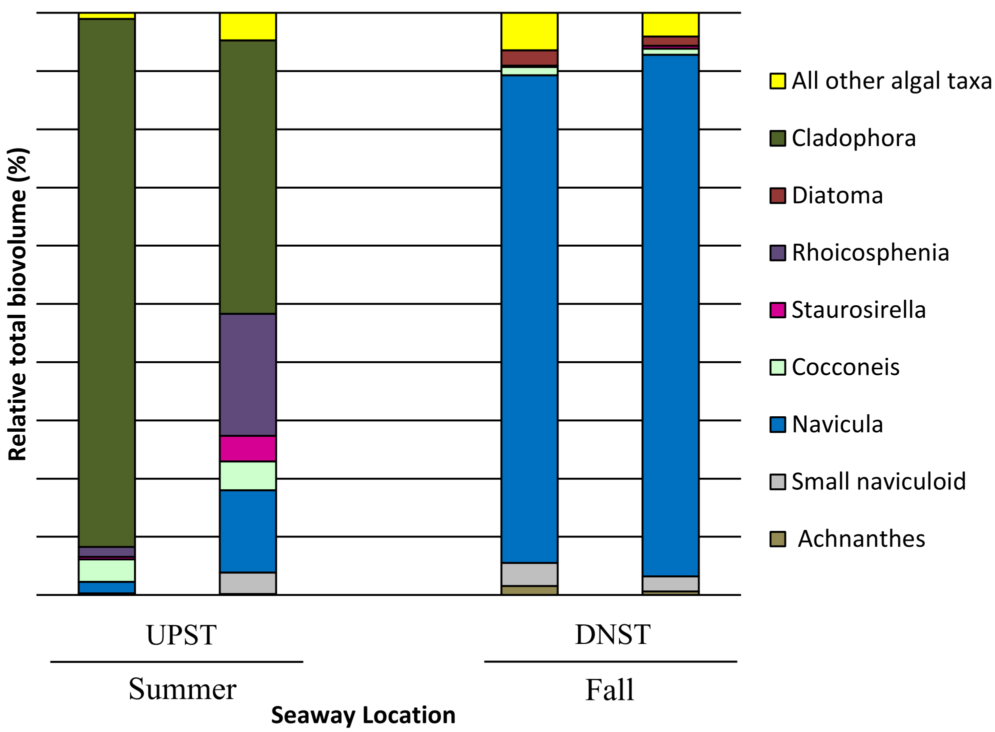


Fig. III.E.1.6. Seaway site median relative total biovolume of algal taxa from upstream (UPST) and downstream (DNST) of the storm water pipe in both seasons (summer and fall). Replicate samples (n=3) were combined to generate median values.

Table III.E.1.5. Seaway site ANOVA analysis of the relative abundance of total cells numbers and relative total biovolume data for each algal taxon (and physiognomic group) at the end of the experiments. Location upstream or downstream of the storm water pipe, season (summer or fall experiment), and location x season treatments were the factors. Bold values are significant (p < 0.05). Arrows represent values that are significantly higher (↑) or lower (↓) at the downstream site or during the fall experiment.

		Relative number of total cells				Relative total biovolume			
Source of Variation	**df**	**SS**	**MS**	**F**	**P**	**SS**	**MS**	**F**	**P**
***Achnanthes* (FU)**									
Location	1	768.82	384.41	2.28	0.15	0.02	0.02	0.24	0.64
Season	1	53.79	53.79	0.32	0.58	2.17	2.17	24.46	**<0.001**↑
Location x Season	1	265.09	132.55	0.79	0.48	0.62	0.62	6.94	**0.03***
Small naviculoid (LU)									
Location	1	41.70	41.70	0.58	0.47	0.61	0.61	6.91	**0.03**↑
Season	1	28.32	28.32	0.39	0.55	1.08	1.08	12.28	**0.01**↑
Location x Season	1	207.73	207.73	2.86	0.13	2.46	2.46	27.94	**<0.001**†
***Navicula* (LU)**									
Location	1	213.49	213.49	10.32	**0.01**↑	0.45	0.45	13.78	**0.006**↑
Season	1	8285.79	8285.79	400.55	**<0.001**↑	4.69	4.69	143.70	**<0.001**↑
Location x Season	1	397.52	397.52	19.22	**0.002**‡	0.39	0.39	12.05	**0.01**
***Cocconeis* (FU)**									
Location	1	0.41	0.41	10.61	**0.01**↓	0.01	0.01	0.16	0.70
Season	1	2.98	2.98	76.41	**<0.001**↓	1.20	1.20	17.55	**<0.001**↓
Location x Season	1	0.28	0.28	7.12	**0.03**†	0.17	0.17	2.50	0.15
***Staurosirella* (LC)**									
Location	1	0.20	0.20	1.47	0.26	78.12	78.12	1.91	0.21
Season	1	2.83	2.83	21.07	**0.00**↓	71.66	71.66	1.75	0.22
Location x Season	1	0.12	0.12	0.88	0.37	71.65	71.65	1.75	0.22
***Rhoicosphenia* (FC)**									
Location	1	0.09	0.09	0.43	0.53	159.50	159.50	4.64	0.06
Season	1	4.79	4.79	23.48	**<0.001**↓	226.84	226.84	6.60	**0.03**↓
Location x Season	1	0.02	0.02	0.11	0.75	163.08	163.08	4.74	0.06

Abbreviations: firm understory (FU), loose understory (LU), loose canopy (LC), and firm canopy (FC)

* increased only at the upstream location

† only during the summer experiment

‡ increased only during the fall experiment

Table continued below

Table III.E.1.5. Continued

		Relative number of total cells				Relative total biovolume			
Source of Variation	**df**	**SS**	**MS**	**F**	**P**	**SS**	**MS**	**F**	**P**
***Diatoma* (LC)**									
Location	1	0.04	0.04	0.37	0.56	1.29	1.29	1.24	0.30
Season	1	1.99	1.99	17.55	**<0.001**↑	14.78	14.78	14.24	**0.01**↑
Location x Season	1	0.74	0.74	6.53	**0.03**	4.47	4.47	4.30	0.07
***Cladophora* (FC)**									
Location	1	1.34	1.34	4.16	0.07	4255.07	4255.07	22.91	**<0.001**↓
Season	1	1.80	1.80	5.61	**0.04**↓	8908.46	8908.46	47.97	**<0.001**↓
Location x Season	1	1.34	1.34	4.16	0.07	4255.07	4255.07	22.91	**<0.001**†
***Stephanocyclus* (LU)**									
Location	1	0.09	0.09	1.22	0.30	0.03	0.03	3.87	0.09
Season	1	0.61	0.61	8.26	**0.02**↓	0.02	0.02	2.19	0.18
Location x Season	1	0.01	0.01	0.09	0.77	0.01	0.01	1.75	0.22

Abbreviations: firm understory (FU), loose understory (LU), loose canopy (LC), and firm canopy (FC)

† only during the summer experiment

In terms of relative biovolume, algal physiognomies differed slightly among location and season, but with less variation than was present in terms of relative total cell numbers. Communities both upstream and downstream of the storm water pipe during the summer experiment were dominated, in terms of relative biovolume, by firm canopy taxa (Fig. III.E.1.7). During the fall experiment, communities in both sampling locations were dominated by loose understory taxa (Fig. III.E.1.7). In terms of relative biovolume, loose understory was the only group with a significant response to location, having a higher relative biovolume in the downstream location compared to the upstream location, but this was only significant during the summer experiment ($p < 0.003$). A significantly higher relative biovolume of firm canopy taxa was present in the summer compared to the fall ($p < 0.001$; Fig. III.E.1.7). Firm understory and loose understory taxa had significantly higher relative biovolume in the summer compared to the fall ($p < 0.032$ and $p < 0.001$, respectively; Fig. III.E.1.7).

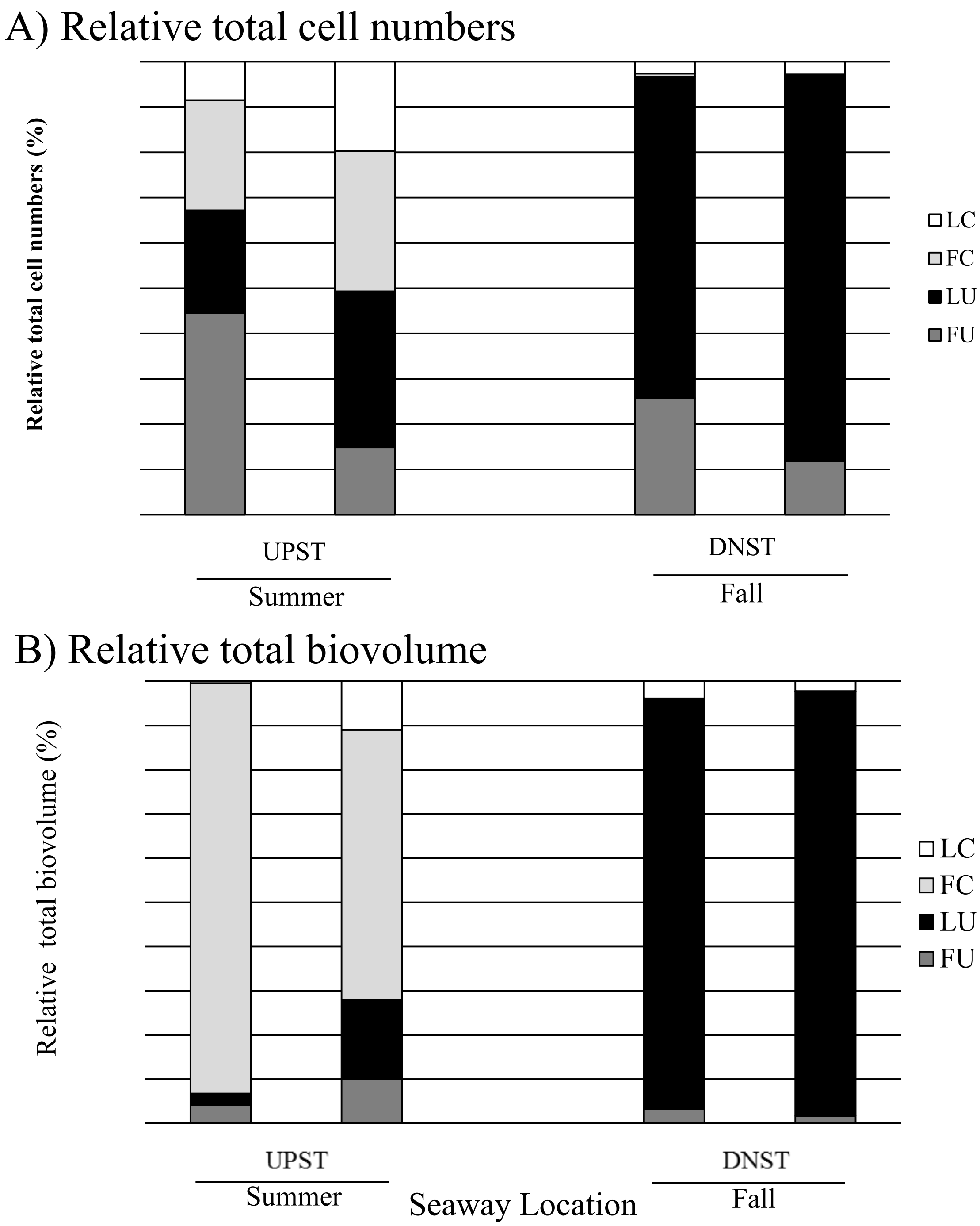


Fig. III.E.1.7A. Distribution of different algal physiognomic groups at the Seaway site in terms of relative A) abundance of total cell numbers and B) total biovolume. Abbreviations: upstream sampling site (UPST), downstream sampling site (DNST), loose canopy (LC), firm canopy (FC), loose understory (LU), and firm understory (FU). Samples were collected at the end of the experiments.

U.S. 31 site community composition

The algal communities at the end of these experiments were almost entirely dominated by diatoms and green algae. The eight most abundant genera (or taxonomic units) in terms of relative abundance of total cell numbers were *Cocconeis,* small naviculoid, *Achnanthes, Cosmarium, Rhoicosphenia, Staurosirella, Coleochaete* and *Navicula* (Table III.E.1.6, Fig. III.E.1.8). Diatoms made up ~80% of total cell numbers with green algae contributing the remaining ~20%.

In terms of biovolume, the eight most abundant taxa were again *Cocconeis,* small naviculoid, *Achnanthes, Cosmarium, Rhoicosphenia, Staurosirella, Coleochaete,* and *Navicula* (Table III.E.1.7, Fig. III.E.1.9). Diatoms made up ~74% of total biovolume with green algae contributing the remaining ~26%.

Location upstream or downstream of the storm water pipe had a small influence on community composition during both the summer and fall experiments, in terms of relative numbers of total cells, although the overall community composition was not significantly different between the two locations ($p > 0.498$). During summer, in terms of relative abundance of total cell numbers, *Cocconeis* was most abundant both upstream and downstream of the storm water pipe (Table III.E.1.6). During the fall experiment, *Cosmarium* was most abundant upstream and *Rhoicosphenia* was most abundant downstream of the pipe (Table III.E.1.6). *Rhoicosphenia* had significantly higher relative total cell numbers downstream of the storm water pipe, but only during the fall experiment (Table III.E.1.8). *Cosmarium* had a significantly higher relative total numbers of cells upstream of the pipe compared to downstream, also only during the fall experiment (Table III.E.1.8). No taxa were significantly affected by location during the summer experiment.

The two taxa significantly influenced by location upstream or downstream of the storm water pipe in terms of relative number of total cells were also affected in terms of relative total biovolume, although the overall communities in the different locations were not significantly different ($p > 0.489$). During summer, in terms of relative biovolume, *Cocconeis* was most abundant both upstream and downstream of the storm water pipe (Table III.E.1.7). During the fall experiment, *Cosmarium* was most abundant upstream and *Rhoicosphenia* was most abundant downstream of the pipe (Table III.E.1.7). *Rhoicosphenia* had a significantly higher relative biovolume downstream of the storm water pipe compared to upstream, and *Cosmarium* had a significantly higher relative biovolume upstream of the pipe compared to downstream, both only during the fall experiment (Table III.E.1.8).

The relative abundance of total cell numbers of numerous taxa was significantly affected by season. The overall communities in the summer and fall experiments were significantly different from one another ($p < 0.001$). *Cocconeis* and *Coleochaete* had a significantly higher relative number of total cells during the summer experiment compared to the fall experiment (Table III.E.1.8). *Achnanthes* also had a significantly higher relative number of total cells during the summer experiment compared to the fall, but only in the downstream locations (Table III.E.1.8). *Cosmarium* and *Rhoicosphenia* had a significantly higher relative number of total cells in the fall experiment compared to the summer experiment (Table III.E.1.8).

The relative biovolume of numerous taxa was also affected by season and the overall communities during the two seasons were significantly different from one another ($p < 0.001$). *Cocconeis* relative biovolume was significantly higher during summer compared to the fall (Table III.E.1.8). *Cosmarium* biovolume was significantly higher during fall compared to the summer (Table III.E.1.8).

The distribution of algal physiognomies differed between upstream and downstream locations and between seasons in terms of relative abundance of total cell numbers. In the summer experiment, the samples both upstream and downstream of the storm water pipe were dominated by firm understory taxa (Fig. III.E.1.10). During fall, upstream communities were dominated by loose understory taxa and downstream communities were dominated by firm canopy taxa (Fig. III.E.1.10). Loose understory taxa were significantly more abundant upstream compared to downstream, but only during the summer experiment ($p < 0.01$). The relative total cell numbers of taxa in the firm canopy group were significantly higher downstream of the pipe compared to upstream, but only during the fall experiment ($p < 0.001$). Firm canopy taxa had significantly higher relative total cell numbers in fall, but only at the downstream location, and loose understory taxa had significantly higher relative cell numbers in fall in both upstream and downstream locations ($p < 0.001$ and $p < 0.002$, respectively). Firm understory taxa had significantly more relative total cells during the summer experiment than during the fall experiment ($p < 0.001$).

Table III.E.1.6. U.S. 31 site median relative abundance of total cell numbers of algal taxa from upstream (UPST) and downstream (DNST) of the storm water pipe in both seasons (summer and fall). Replicate samples (n=3) were combined to generate median values.

	U.S. 31 relative abundance of total cell numbers (%)				
Taxon	Summer		Fall		
	UPST	DNST	UPST	DNST	Overall
Cocconeis	60.01	68.69	8.52	11.23	15.87
Small naviculoid	7.29	7.03	11.01	4.37	9.47
Achnanthes	6.84	9.27	7.55	3.62	7.28
Cosmarium	0.00	0.00	46.31	14.14	6.82
Rhoicosphenia	5.46	6.21	24.60	59.02	6.74
Staurosirella	1.37	1.78	1.42	2.41	1.85
Coleochaete	7.26	4.14	0.00	0.27	1.40
Navicula	0.61	0.00	0.58	0.53	0.59
All other algal taxa	11.16	2.89	0.01	4.41	49.99
Total	100.00	100.00	100.00	100.00	100.0

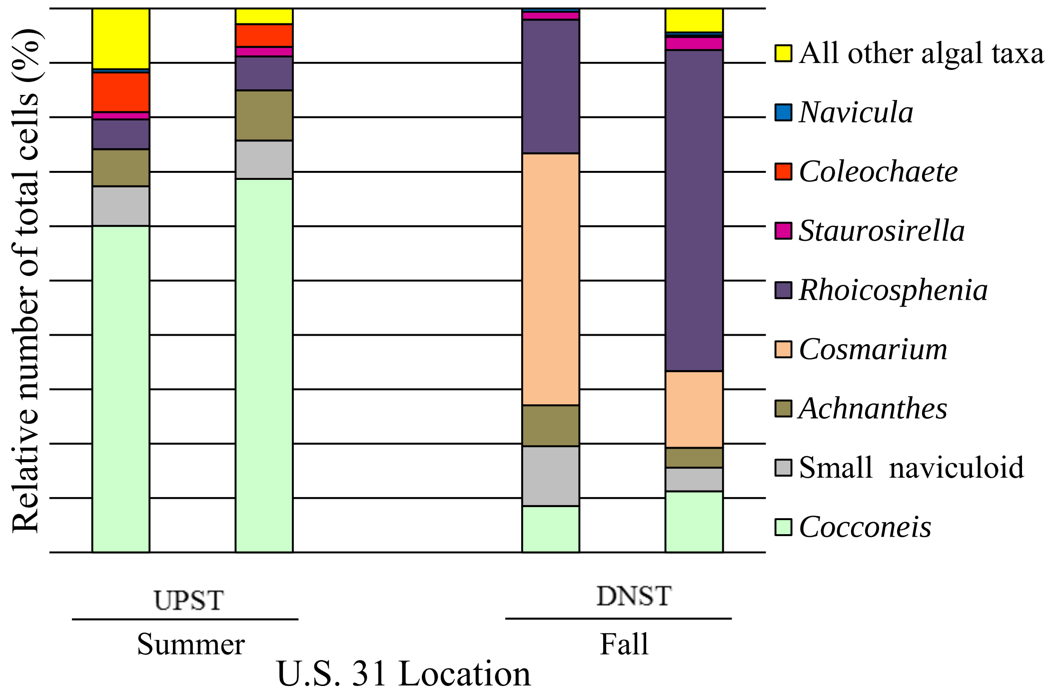


Fig. III.E.1.8. U.S. 31 site median relative abundance of total cell numbers of algal taxa from upstream (UPST) and downstream (DNST) of the storm water pipe in both seasons (summer and fall). Replicate samples (n=3) were combined to generate median values.

Table III.E.1.7. U.S. 31 site median relative biovolume of algal taxa from upstream (UPST) and downstream (DNST) of the storm water pipe in both seasons (summer and fall). Replicate samples (n=3) were combined to generate median values. Taxa are listed in the same order as in Table 3 for comparison purposes.

	U.S. 31 relative total biovolume (%)				
Taxon	Summer		Fall		
	UPST	DNST	UPST	DNST	Overall
Cocconeis	81.94	92.37	21.94	21.40	32.73
Small naviculoid	0.00	0.00	51.09	12.51	5.86
Achnanthes	1.85	1.67	5.22	1.53	2.56
Cosmarium	2.00	1.29	15.09	56.80	2.02
Rhoicosphenia	1.33	0.00	1.98	1.63	1.50
Staurosirella	0.63	0.80	1.30	0.42	0.92
Coleochaete	5.41	2.75	0.00	0.27	0.93
Navicula	0.75	0.93	1.49	1.87	1.31
All other algal taxa	6.10	0.19	1.90	3.56	52.17
Total	100.00	100.00	100.00	100.00	100.00

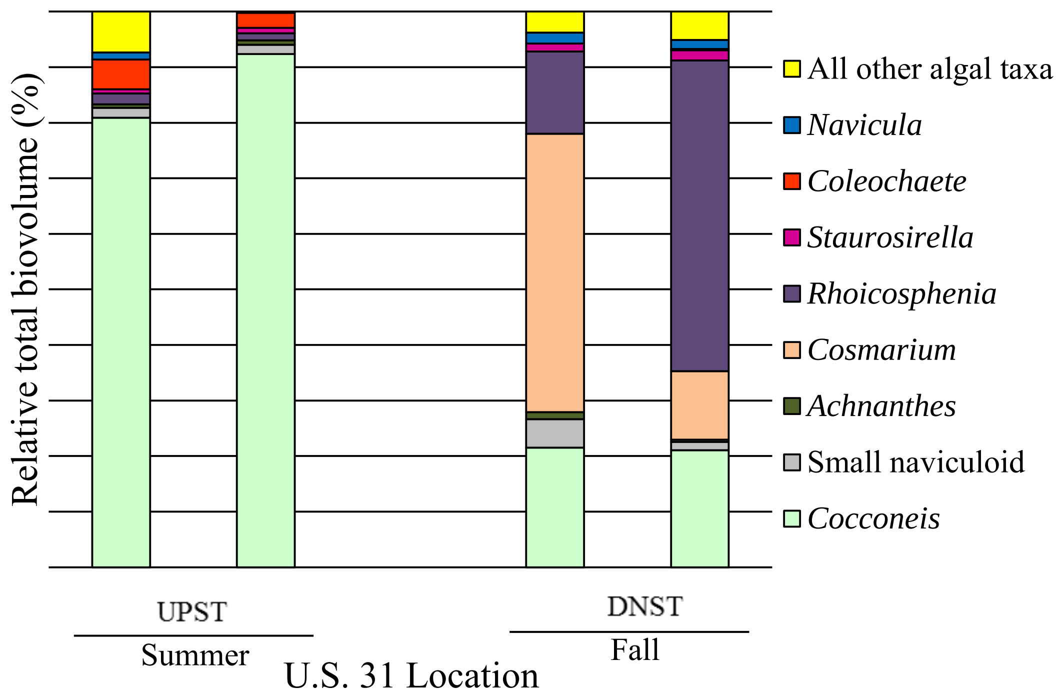


Fig. III.E.1.9. U.S. 31 site median relative total biovolume of algal taxa from upstream (UPST) and downstream (DNST) of the storm water pipe in both seasons (summer and fall). Replicate samples (n=3) were combined to generate median values.

Table III.E.1.8. U.S. 31 site ANOVA analysis of the relative abundance of total cell numbers and relative total biovolume data for each algal taxon (and physiognomic group) at the end of the experiments. Location upstream or downstream of the storm water pipe, season (summer or fall experiment), and location x season treatments were the factors. Bold values are significant ($p < 0.05$). Arrows represent values that are significantly higher (↑) or lower (↓) at the downstream site or during the fall experiment.

		Relative number of total cells				Relative total biovolume			
Source of Variation	**df**	**SS**	**MS**	**F**	**P**	**SS**	**MS**	**F**	**P**
***Cocconeis* (FU)**									
Location	1	0.17	0.17	3.17	0.11	0.00	0.00	2.32	0.162
Season	1	1.65	1.65	31.82	**<0.001**↓	0.00	0.00	27.51	**<0.001**↓
Location x Season	1	0.00	0.00	0.01	0.91	0.00	0.00	0.50	0.499
***Cosmarium* (LU)**									
Location	1	12.25	12.25	8.58	**0.02**↓	1632.60	1632.60	34.57	**<0.001**↑
Season	1	73.80	73.80	51.70	**<0.001**↑	2948.02	2948.02	62.42	**<0.001**↑
Location x Season	1	2.93	2.93	2.05	0.19	945.15	945.15	20.01	**0.002**‡
Small naviculoid (LU)									
Location	1	0.11	0.11	2.64	0.14	0.26	0.26	2.94	0.12
Season	1	0.03	0.03	0.69	0.43	0.01	0.01	0.12	0.74
Location x Season	1	0.03	0.03	0.63	0.45	0.04	0.04	0.45	0.52
***Rhoicosphenia* (FC)**									
Location	1	738.78	738.78	21.41	**0.001**↑	0.11	0.11	3.15	**0.011**↑
Season	1	3578.60	3578.60	103.69	**<0.001**↑	5.33	5.33	160.12	**<0.001**↑
Location x Season	1	1029.77	1029.77	29.84	**<0.001**‡	0.33	0.33	9.88	**0.012**‡
***Navicula* (LU)**									
Location	1	0.17	0.17	1.29	0.29	3.14	3.14	1.37	0.27
Season	1	0.01	0.01	0.11	0.75	0.31	0.31	0.13	0.72
Location x Season	1	0.36	0.36	2.69	0.14	2.69	2.69	1.17	0.31
***Staurosirella* (LC)**									
Location	1	0.26	0.26	0.30	0.60	0.00	0.00	0.02	0.89
Season	1	0.97	0.97	1.15	0.31	0.46	0.46	3.30	0.10
Location x Season	1	0.53	0.53	0.63	0.45	0.00	0.00	0.01	0.91

Abbreviations: firm understory (FU), loose understory (LU), firm canopy (FC), and loose canopy

‡ only during the fall experiment

Table continued below

Table III.E.1.8. Continued

		Relative number of total cells				Relative total biovolume			
Source of Variation	**df**	**SS**	**MS**	**F**	**P**	**SS**	**MS**	**F**	**P**
***Achnanthes* (FU)**									
Location	1	0.00	0.00	0.13	0.72	0.19	0.19	0.81	0.39
Season	1	0.22	0.22	7.98	**0.02↓**	0.06	0.06	0.24	0.64
Location x Season	1	0.14	0.14	5.13	**0.05****	0.71	0.71	3.04	0.12
***Coleochaete* (FC)**									
Location	1	0.02	0.02	0.01	0.91	0.01	0.01	0.01	0.94
Season	1	13.02	13.02	11.06	**0.01↓**	9.05	9.05	9.19	**0.01↓**
Location x Season	1	0.44	0.44	0.37	0.56	0.49	0.49	0.50	0.50
***Cymbella* (FU)**									
Location	1	1.19	1.19	1.15	0.31	1.06	1.06	1.16	0.31
Season	1	0.17	0.17	0.16	0.70	0.13	0.13	0.15	0.71
Location x Season	1	0.16	0.16	0.15	0.71	0.16	0.16	0.17	0.69

Abbreviations: firm understory (FU), loose understory (LU), firm canopy (FC), and loose canopy
**only downstream

Changes in physiognomy in terms of relative biovolume were similar to those in terms of number of total cells. In the summer experiment, firm understory taxa dominated the communities both upstream and downstream of the storm water pipe (Fig. III.E.1.10). During the fall experiment, loose understory taxa were dominant upstream and firm canopy taxa were dominant downstream. Firm canopy taxa had a higher relative biovolume downstream of the pipe compared to upstream, but this was only significant during the fall experiment ($p < 0.001$). The relative biovolume of firm understory taxa was significantly higher in the summer experiment compared to the fall ($p < 0.001$). Firm canopy taxa had significantly higher relative biovolume in the fall experiment compared to the summer, but only at the downstream site ($p < 0.001$).

Comparison of Seaway and U.S. 31 community composition

The algal communities at the Seaway and U.S. 31 study sites were fairly similar. Six of the eight most common taxa were present at both sites: *Achnanthes,* small naviculoid, *Navicula, Cocconeis, Staurosirella,* and *Rhoicosphenia.* Of all the taxa that were similar among study sites and significantly impacted by location relative to the storm water pipe, none were affected in the same way at both sites. In terms of relative number of total cells and relative biovolume, *Navicula* was significantly more abundant downstream of the storm water pipe at the Seaway site, but was not significantly affected by storm water pipe location at the U.S. 31 site. *Rhoicosphenia* was significantly more abundant downstream of the storm water pipe at the U.S. site, but was only marginally significantly affected in terms of biovolume at the Seaway site. At both the Seaway and U.S. 31 sites, *Cocconeis* was significantly more abundant during the summer experiment compared to the fall both in terms of relative total cell numbers and relative biovolume. *Rhoicosphenia* abundance (total cells and biovolume) was significantly higher during summer compared to fall at the Seaway site and was significantly higher during fall at the U.S. 31 site.

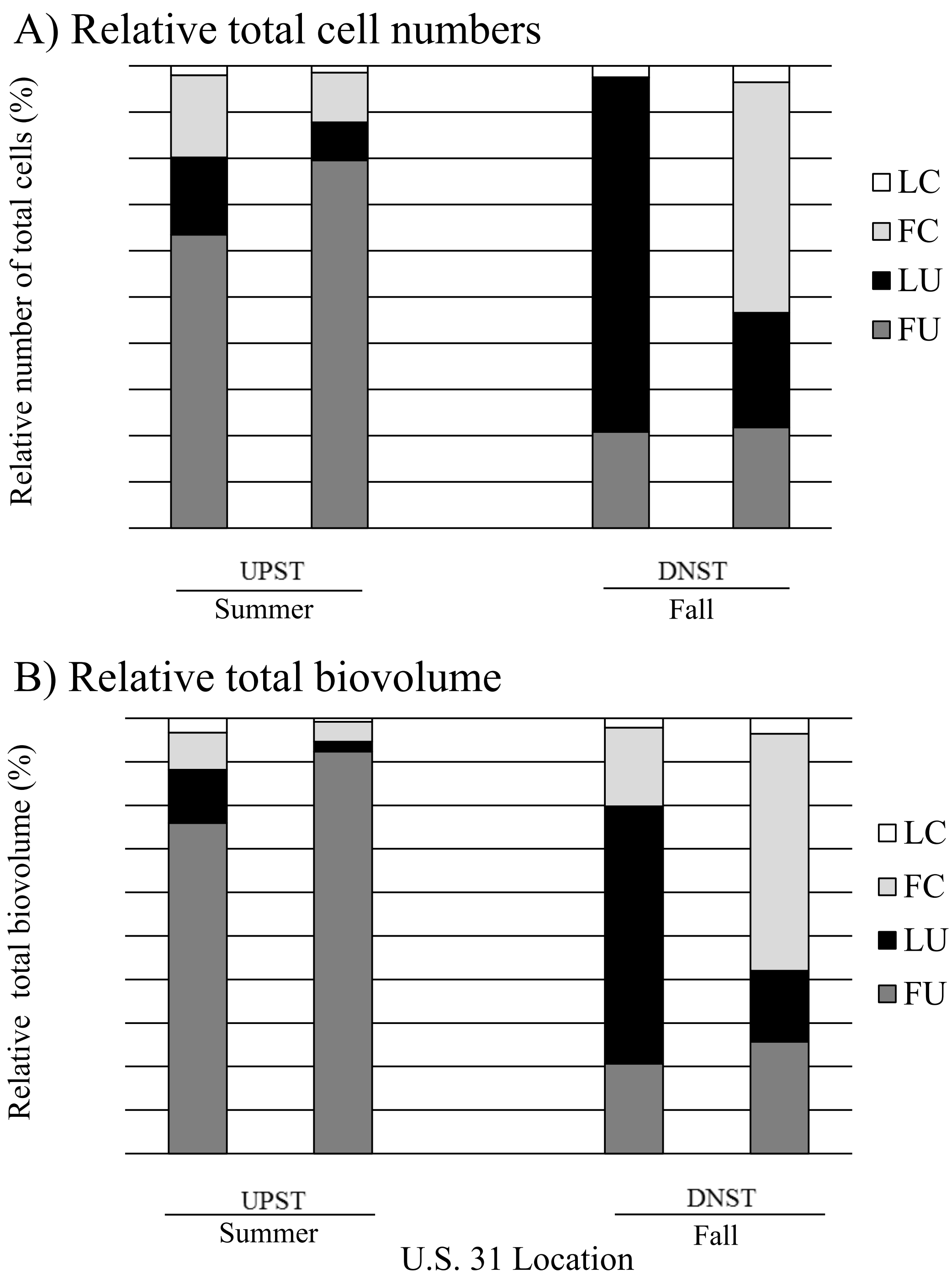


Fig. III.E.1.10. Distribution of different algal physiognomic groups at the U.S. 31 site in terms of relative A) relative number of total cells and B) relative total biovolume. Abbreviations: upstream

sampling site (UPST), downstream sampling site (DNST), loose canopy (LC), firm canopy (FC), loose understory (LU), and firm understory (FU). Samples were collected at the end of the experiments.

III.E.3. Mesocosm Experiments

III.E.3.a. Water Quality

2008

Comparison of LBC base flow and storm flow

In general, quality of the base flow in LBC differed from (1) storm flow upstream of the storm water pipe, (2) storm flow downstream of the storm water pipe, and (3) storm water directly from the pipe. For both TP and SRP, values upstream and downstream of the storm water pipe were similar to base flow and much lower than the values from the pipe (Table III.E.3.a.1, Figs. III.E.3.a.1). Concentrations of NO_3-N (Fig. III.E.3.a.2), NH_3 (Fig. III.E.3.a.2), and Cl, pH, and specific conductivity from base flow were similar to those from upstream and downstream of the pipe and the pipe storm water (Table III.E.3.a.1). SO_4 and alkalinity concentrations were higher in base flow than from any of the storm flow locations (Table III.E.3.a.1). All values were within the range found in other studies (Table III.E.3.a.2).

Organics and metals concentrations also differed between the storm water pipe and base flow. Zn, Cr, Pb, and Cu concentrations were much higher in the storm water than in base flow water, whereas levels of Ni and Cd were not as elevated in storm water compared to base flow (Table 3.E.e.a.3, Fig. III.E.2.3). In general, upstream storm flow values were similar to base flow, and downstream storm flow values were slightly less than pipe storm water concentrations for Zn, Pb (Fig. III.E.2.4), Cu (Fig. III.E.2.4), and Cr (Fig. III.E.3.a.5, Table III.E.3.a.3). Base flow levels of Ni were similar to upstream storm flow, and concentrations in downstream storm flow and pipe storm water were not as elevated above base flow as with Zn, Pb, Cu, and Cr (Table III.E.3.a.3). Cd levels were below detection limit at all sampling locations (Table III.E.3.a.3). The elevated concentrations of Cu and Pb in the storm water exceeded Michigan water quality standards for chronic, but not acute exposure (MDEQ 2011; Table III.E.3.a.4). All of the metal concentrations were within the range found in other studies (Table III.E.3.a.4). PAH levels were very low in this study (Table III.E.3.a.4).

Experimental storm water treatments

Several chemical parameters differed among (1) the three storm water treatments measured on day 31 of the experiment, (2) the storm water from the pipe and (3) base flow in LBC (Table III.E.3.a.1). TP concentrations were significantly higher in the 0% storm water treatment than in the 50% and 100% treatments, and base flow values were significantly less than the 0%, 50% and 100% treatments (Table III.E.3.a.1, Fig. III.E.3.a.1). TP concentrations were significantly lower in all storm water treatments than in the water from the storm water pipe, although the absolute TP concentration was still relatively high (Table III.E.3.a.1, Fig. III.E.3.a.1). NO_3-N concentration did not differ between storm water treatments and base flow and these values were not substantially less than values from the pipe storm water (Table III.E.3.a.1, Fig. III.E.3.a.2). On day 31 of the experiment, alkalinity was significantly different among all three storm water treatments, values from the storm water pipe, and base flow. The 0% storm water treatment had the highest alkalinity; the lowest alkalinity was found in the pipe (Table III.E.3.a.1). Cl levels and specific conductivity varied among different storm water treatments and base flow (Table III.E.3.a.1). Concentrations of SRP (Fig. III.E.3.a.1), NH_3-N (Fig. III.E.3.a.2), and SO_4 did not differ among storm water treatments or stream samples (Table III.E.3.a.1).

Table III.E.3.a.1. Median (and range) of select water chemistry variables from base flow in Little Black Creek (LBC), MI, water collected from LBC during a storm in June 2008: upstream of the storm water (SW) pipe, downstream of the SW pipe, and road runoff collected from a pipe directed into LBC, and the three SW treatments (100%, 50%, and 0%) from day 31 the experiment. Replicate samples (n = 4) were combined to generate median values. Lowercase letters represent significant differences among base flow and after experiment values in each SW treatment. Only one water sample was collected from the upstream and downstream storm flow and pipe SW, so a statistical comparison with other samples was not possible.

	Parameters								
Study	**TP-P**	**SRP-P**	**NO_3-N**	**NH_3-N**	**Cl**	**SO_4**	**pH**	**SpCond**	**Alkalinity**
	mg/L	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**		**µS/cm**	**mg/L**
Base flow[1]	0.013[c] (BD-0.024)	0.003 (BD-0.008)	0.36[a] (0.2-0.69)	0.05 (BD-0.13)	98.5[a] (64-139)	27[a] (23-35)	7.79 (7.29-8.06)	593[a] (457-1057)	137[c] (126-150)
Upstream of pipe (storm flow)	0.030	0.003	0.32	0.08	61	16.00	7.93 (7.82-8.08)	356 (284-545)	84
Downstream of pipe (storm flow)	0.070	0.005	0.24	0.09	50	11.00	7.94 (7.81-8.21)	318 (136-391)	78
Pipe SW	0.58	0.11	0.41	0.11	85	19	8.14 (7.87-8.68)	474 (104-537)	82
100% SW (experiment day 31)	0.04[b] (0.03-0.04)	0.003 (BD-0.006)	0.32[ab] (0.14-0.34)	0.03	73[ab] (51-83)	18[b] (14-22)	8.17 (8.09-8.23)	533[ab] (510-540)	124[d] (114-126)
50% SW (experiment day 31)	0.03[b] (0.03-0.06)	BD	0.23[b] (0.07-0.28)	0.04 (0.03-0.1)	53[bc] (35-67)	19[b] (15-24)	8.28 (8.26-8.32)	488[ab] (460-500)	144[b] (142-152)
0% SW (experiment day 31)	0.06[a] (0.05-0.07)	0.006 (BD-0.10)	0.27[ab] (0.18-0.28)	0.05	40[c] (39-44)	23[ab] (20-25)	8.24 (8.14-8.35)	408[b] (340-450)	162[a] (156-166)

Abbreviations: total phosphorus (TP), soluble reactive phosphorus (SRP), nitrate (NO_3-N), ammonia (NH_3-N), chloride (Cl), sulfate (SO_4), specific conductivity (SpCond), and below detection limit (BD)

Detection limits: TP = 0.01 mg/l, SRP = 0.005 mg/l, and NH_3-N = 0.01 mg/l

[1] Base flow measurements were collected on eleven dates between April 2008 and April 2009.

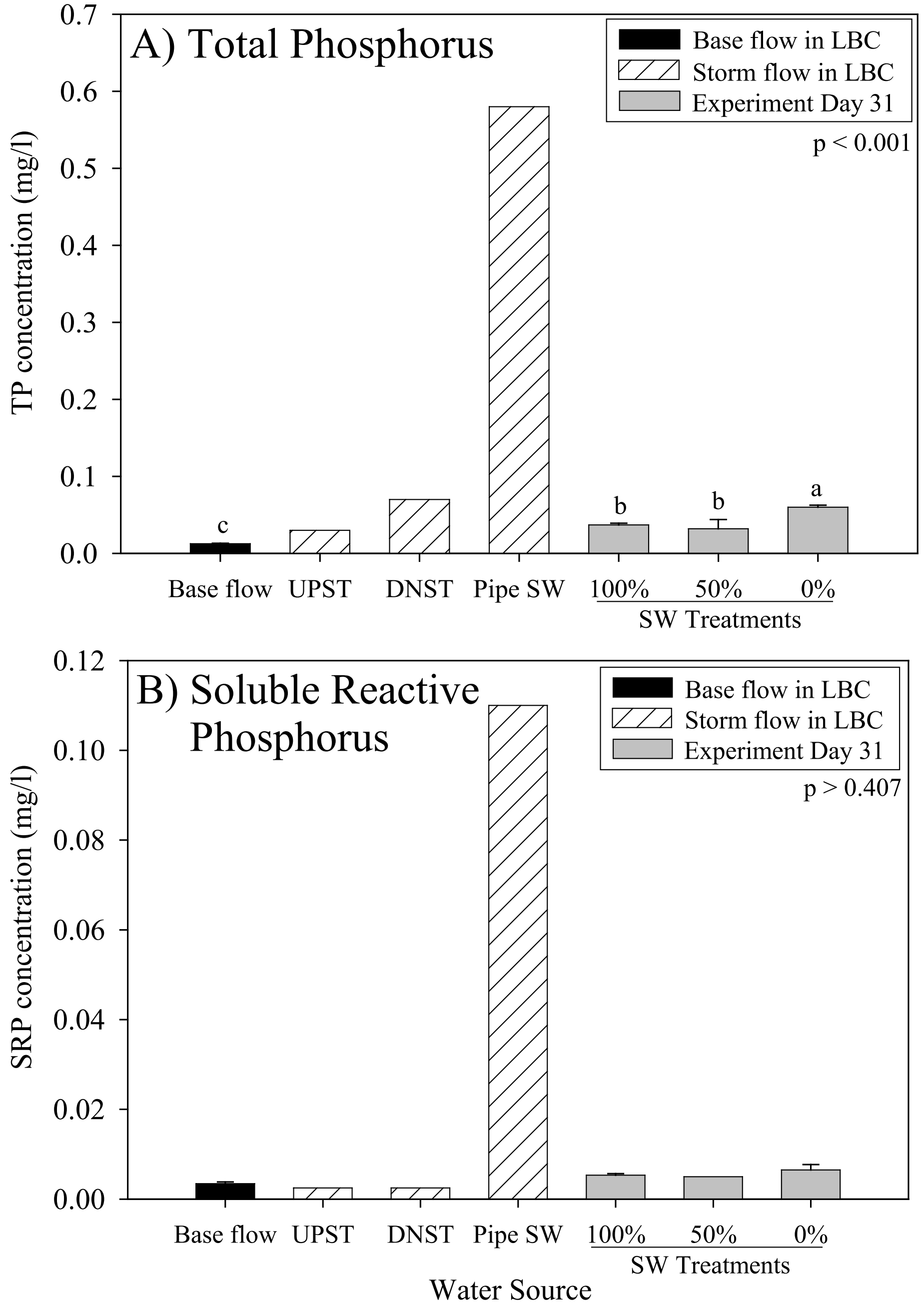


Fig. III.E.3.a.1. A) Total phosphorus (TP) and B) soluble reactive phosphorus (SRP) concentrations during base flow in LBC, water collected from LBC during a storm in June 2008: upstream of the storm water pipe (UPST), downstream of storm water pipe (DNST), and directly from the storm water (SW) pipe, and in the 100%, 50%, and 0% SW treatments on day 31 of the experiment. Lowercase letters represent significant differences among base flow and after experiment values only in each SW treatment. Error bars represent standard error. Only one sample was collected for UPST, DNST, and Pipe SW, so a statistical comparison with other samples was not possible.

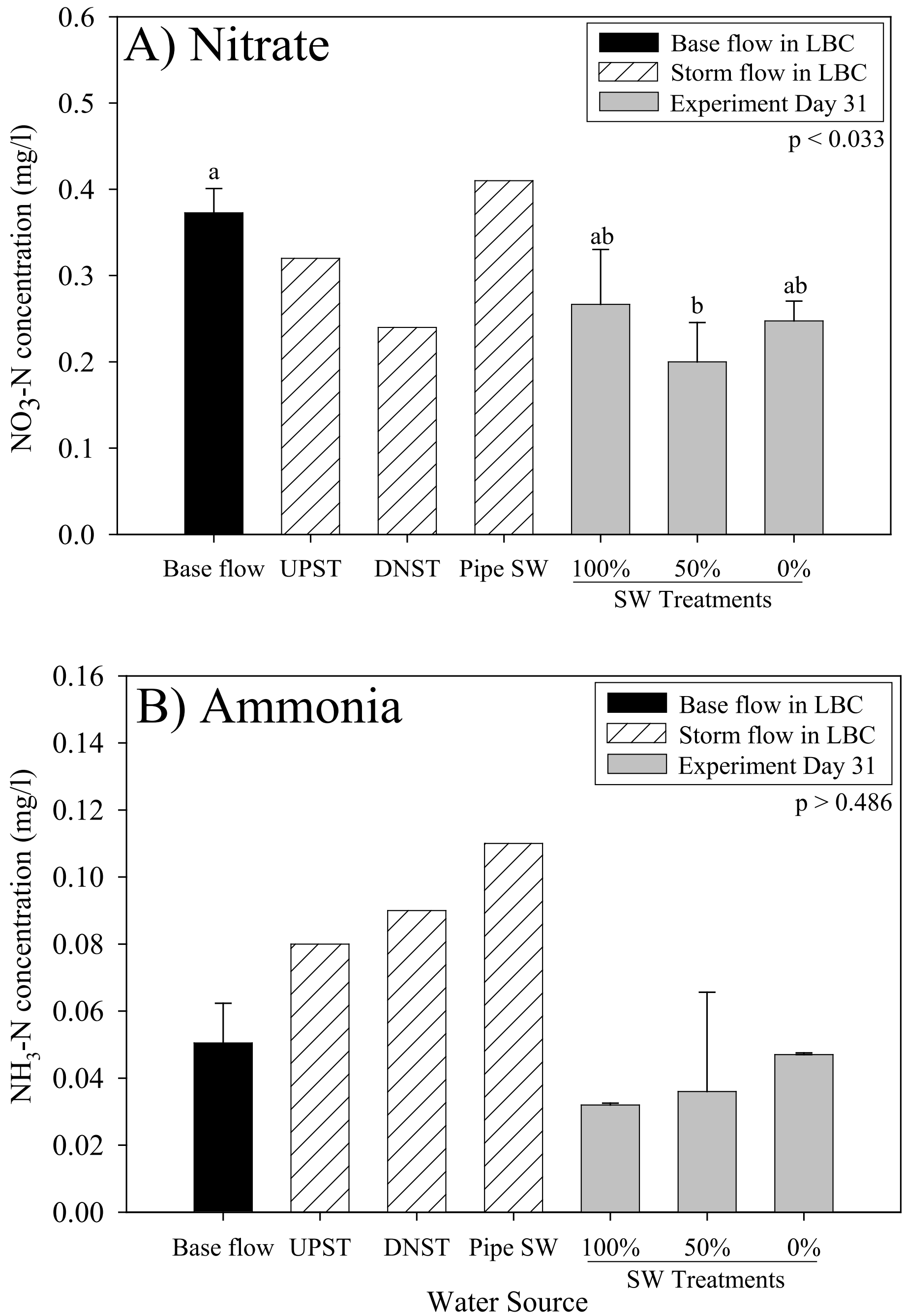


Fig. III.E.3.a.2. A) Nitrate (NO_3-N) and B) ammonia (NH_3-N) concentrations during base flow in LBC, water collected from LBC during a storm in June 2008: upstream of the storm water pipe (UPST), downstream of storm water pipe (DNST), and directly from the storm water (SW) pipe, and in the 100%, 50%, and 0% SW treatments on day 31 of the experiment. Lowercase letters represent significant differences among base flow and after experiment values only in each SW treatment. Error bars represent standard error. Only one sample was collected for UPST, DNST, and Pipe SW, so a statistical comparison with other samples was not possible.

Table III.E.3.a.2. Median (and range) of select water chemistry variables from road runoff collected in June 2008 from a pipe directed into Little Black Creek (LBC), MI, base flow in LBC, and other storm water studies from the literature (with study location in parentheses). Studies from the literature were chosen because they analyzed road run-off or sampled directly downstream of a road crossing and provided water chemistry data in the text.

	Parameters								
Study	**TP-P**	**SRP-P**	**NO_3-N**	**NH_3-N**	**Cl**	**SO_4**	**pH**	**SpCond**	**Alkalinity**
	mg/L	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**		**µS/cm**	**mg/L**
Little Black Creek (this study, pipe SW)	0.58	0.11	0.41	0.11	85	19	8.01 (7.87-8.68)	474 (104-537)	82
Little Black Creek (this study, base flow)[1]	0.013 (BD-0.024)	0.003 (BD-0.008)	0.36 (0.2-0.69)	0.05 (BD-0.13)	98.5 (64-139)	27 (21-35)	7.79 (7.29-8.06)	593 (457-1057)	137 (126-150)
Little Black Creek (Steinman et al., 2006)	0.045 (0.03-0.1)	0.005 (0.005-0.03)	0.9 (0.5-1.5)	—	125 (31-270)	—	—	747 (498-1042)	—
Boisson & Perrodin, 2006 (France)	—	—	5.05 (3.5-6.6)	—	16.5 (13-20)	32 (23-41)	6.35 (5.5-7.2)	217 (198-226)	—
Lee & Bang, 2000[2] (Korea)	5.86 (1.2-10.3)	—	1.2 (0.45-2.28)	—	—	—	—	—	—
Maltby et al.,1995 (Northern England)	—	—	44	—	112.1	111.6	7.69	—	—
Mangani et al., 2005 (Central Italy)	—	—	4.6 (3.0-8.0)	—	4.9 (3.8-6.5)	12.3 (0.0-14.4)	7.85 (7.1-8.0)	—	—
Taebi & Droste, 2004[2] (Iran)	0.274	—	—	—	—	—	7.3	507	—
Wu et al., 1998 (North Carolina, USA)	0.2 (0.04-1.54)	—	0.38 (0.08-13.4)	0.66 (0.50-1.74)	—	—	—	—	—

Abbreviations: storm water (SW), total phosphorus (TP), soluble reactive phosphorus (SRP), nitrate (NO_3-N), ammonia (NH_3-N), chloride (Cl), sulfate (SO_4), specific conductivity (SpCond), and below detection limit (BD)

Detection limits: TP = 0.01 mg/l, SRP = 0.005 mg/l, and NH_3-N = 0.01 mg/l

[1] Base flow measurements were collected on eleven dates between April 2008 and April 2009.

[2] Mean values are shown because median values were unavailable.

Table III.E.3.a.3. Median (and range) of select metals from base flow in Little Black Creek (LBC), MI, water collected from LBC during a storm in June 2008: upstream of the storm water (SW) pipe, downstream of the SW pipe, and road runoff collected from a pipe directed into LBC. Only one water sample was collected from the upstream and downstream storm flow and pipe SW, so a statistical comparison with other samples was not possible.

	Parameters					
Study	**Zn**	**Pb**	**Cu**	**Cr**	**Ni**	**Cd**
	µg/L	**µg/L**	**µg/L**	**µg/L**	**µg/L**	**µg/L**
Base flow[1]	BD	0.846 (BD-4.42)	2.5 (BD-60.51)	1.19 (BD-2.54)	BD	BD
Upstream of pipe (storm flow)	BD	3.28	BD	2.01	BD	BD
Downstream of pipe (storm flow)	53	10.16	10.46	7.19	5.52	BD
Pipe SW	153.52	28.41	22.84	16.42	9.21	BD

[1]Base flow measurements were collected on eleven dates between April 2008 and April 2009.
Abbreviation: below detection limit (BD)
Detection limits: Zn = 50 µg/l, Cr, Pb, & Cd = 1.0 µg/l, and Cu & Ni = 5.0 µg/l

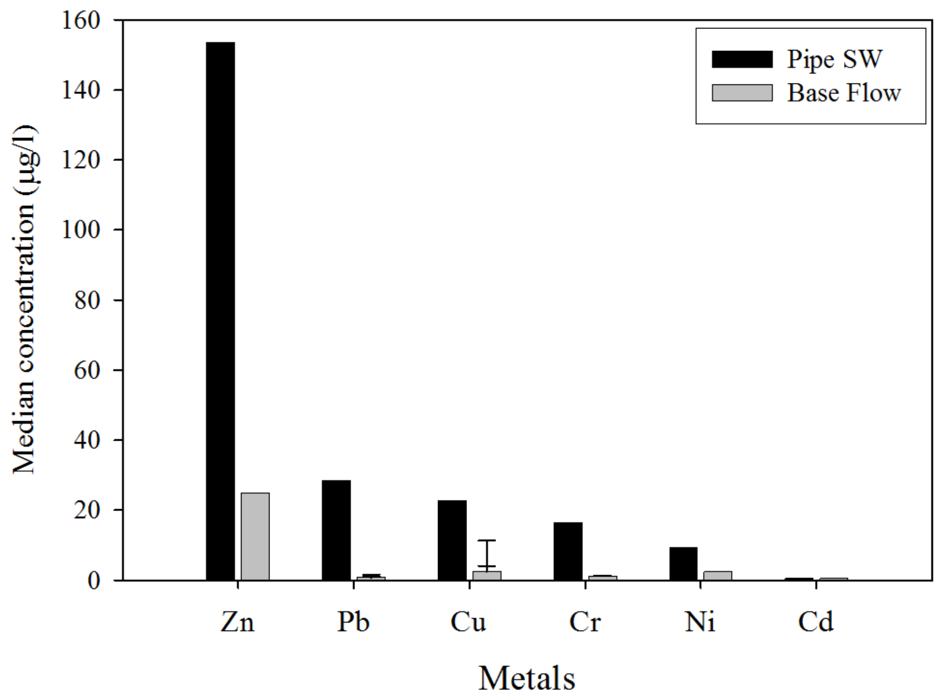


Fig. III.E.3.a.3. Median concentrations of various metals in the runoff from the storm water pipe (Pipe SW) and in base flow in LBC.

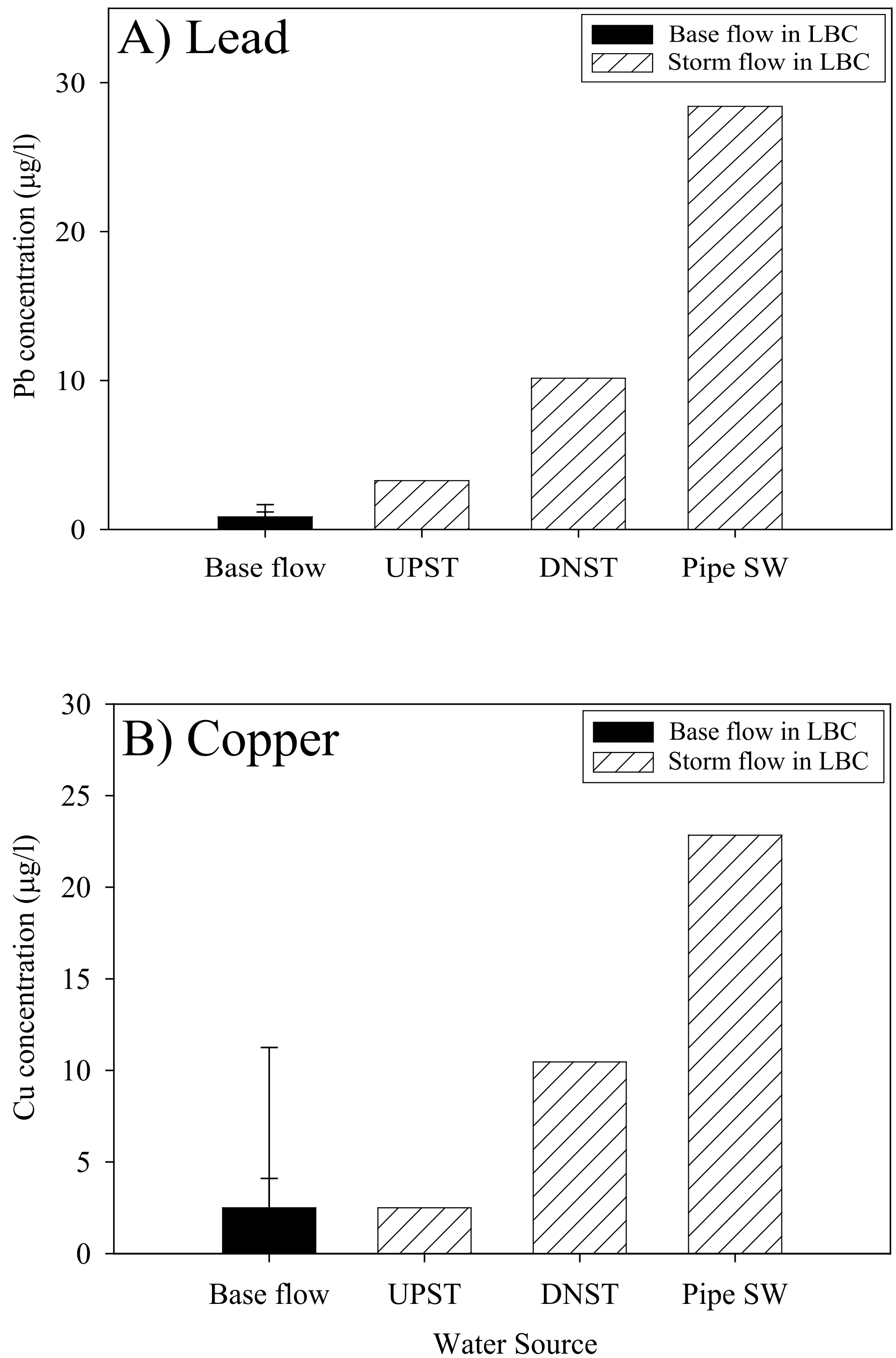


Fig. III.E.3.a.4. A) Lead and B) copper concentrations during base flow in LBC, water collected from LBC during a storm in June 2008: upstream of the storm water pipe (UPST), downstream of storm water pipe (DNST), and directly from the storm water (SW) pipe. Only one sample was collected for UPST, DNST, and Pipe SW, so a statistical comparison with other samples was not possible. Error bars represent standard error.

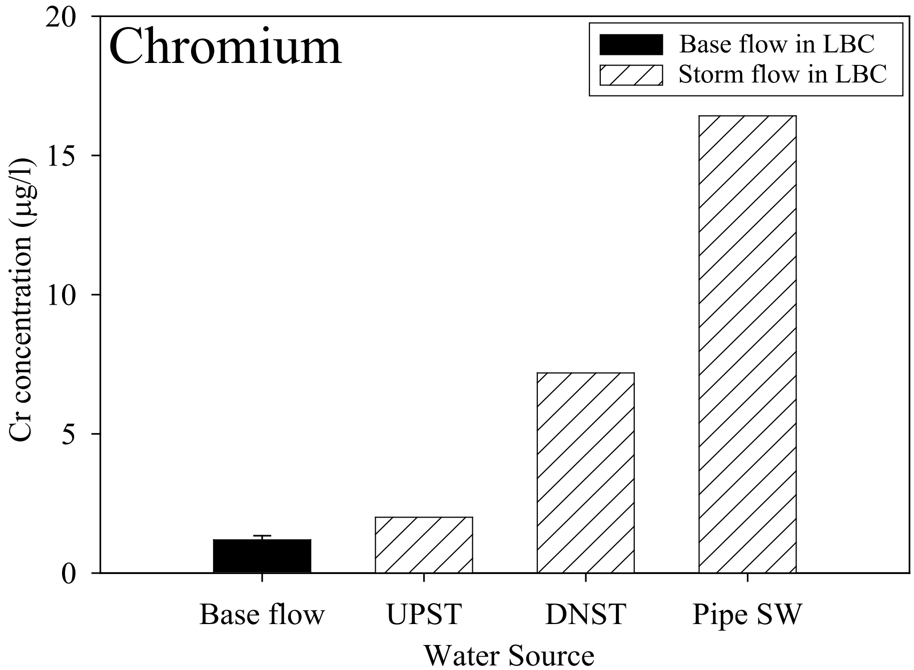


Fig. III.E.3.a.5. Chromium concentrations during base flow in LBC, water collected from LBC during a storm in June 2008: upstream of the storm water pipe (UPST), downstream of storm water pipe (DNST), and directly from the storm water (SW) pipe. Only one sample was collected for UPST, DNST, and Pipe SW, so a statistical comparison with other samples was not possible. Error bars represent standard error.

Table III.E.3.a.4. Median (and range) of selected metals and organics from road runoff collected in June 2008 from a pipe directed into Little Black Creek (LBC), MI, base flow in LBC, chronic and acute exposure water standards in Michigan, and other storm water studies from the literature (with study location in parentheses). Studies from the literature were chosen because they analyzed road run-off or sampled directly downstream of a road crossing and provided water chemistry data in the text.

	Parameters						
Study	Zn	Pb	Cu	Cr	Ni	Cd	PAHs
	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	mg/L
Little Black Creek (this study, pipe SW)	153.52	28.41	22.84	16.42	9.21	0.5	0.001
Little Black Creek (this study, base flow)[1]	25	0.846 (0.5-4.42)	2.5 (2.5-60.51)	1.19 (0.5-2.54)	2.5	0.5	0.001
Michigan Water Standards - Chronic	212.55	21.71	16.19	130.75	93.48	3.73	—
Michigan Water Standards - Acute	421.65	386.47	51.65	2010.33	1683.32	18.07	—
Boisson et al., 2006 (France)	—	7.25 (4.5-10.0)	83.3 (39.5-127)	—	430 (60-800)	—	—
Christensen et al., 2006 (Denmark)	16.0 (12-29)	2.9 (1.7-4.7)	3.55 (2.9-25.0)	0.50 (0.3-1.1)	—	—	—
Gan et al., 2008 [2] (Southern China)	1230 (700-1760)	105.3 (92.3-118.2)	140	40.4	18 (13.4-22.6)	1.55 (1.5-1.6)	—
Lee & Bang, 2000[2] (Korea)	—	133.5 (41-226)	—	—	—	—	165.4 (85.2-327.3)
Pitt et al., 1995 [2] (Alabama, USA)	58 (<1.0-130)	43 (1.5-150)	280 (<1.0-1250)	9.9 (<1.0-30)	17 (<1.0-70)	37 (<1.0-220)	—
Wu et al., 1998 (North Carolina, USA)	—	15 (<0.5-56)	15 (<0.5-52)	6.5 (<0.5-20)	9.0 (<0.5-17)	2.5 (<0.5-2.5)	—

Abbreviations: storm water (SW), polycyclic aromatic hydrocarbons (PAHs), and below detection limit (BD)

Detection limits: Zn = 50 µg/l, Cr, Pb, & Cd = 1.0 µg/l, Cu & Ni = 5.0 µg/l, and PAHs = 0.001 mg/l

[1] Base flow measurements were collected on eleven dates between April 2008 and April 2009.

[2] Mean values are shown because median values were unavailable.

2009 Data

Comparison of LBC base flow and storm flow

In general, quality of the base flow in LBC differed from (1) storm flow upstream of the storm water pipe, (2) storm flow downstream of the storm water pipe, and (3) storm water directly from the pipe. The TP values upstream and downstream of the storm water pipe and from the pipe itself were all slightly higher than in base flow (Table III.E.3.a.5, Fig. III.E.3.a.6). SRP, NO_3-N, and NH_3-N concentrations were elevated in storm water out of the pipe relative to base flow (Table III.E.3.a.5, Figs III.E.3.a.6, 7). Concentrations of both SRP and NH_3-N upstream of the pipe during storm flow were similar to base flow, while concentrations downstream of the pipe were more similar to the pipe storm water (Table III.E.3.a.5, Fig. III.E.3.a.6, 7). NO_3-N values both upstream and downstream of the pipe were similar to water directly from the storm water pipe (Table III.E.3.a.5, Fig. III.E.3.a.7). Cl, SO_4, specific conductivity, and alkalinity values were lower in the storm water than in base flow, and values upstream and downstream of the pipe were variable (Table III.E.3.a.5). pH was similar among all water sources (Table III.E.3.a.5). All water chemistry values were within the range found in other studies, although the TP concentration in this study was near the low end of the range found in other studies (Table III.E.3.a.6).

Metal concentrations also differed between base flow and storm flow. Zn, Cr, Pb, and Cu concentrations were much higher in the pipe storm water than in base flow water, whereas levels of Ni and Cd were not as elevated in pipe storm water compared to base flow (Table III.E.3.a.7, Fig. III.E.3.a.8). Zn and Ni were both present in storm water directly from the pipe, but were below detection limits in the upstream and downstream storm flows as well as in base flow (Table III.E.3.a.7). In general, Cr, Pb, and Cu concentrations increased incrementally from base flow (lowest value), to the upstream storm flow, to the downstream storm flow, and culminated with the pipe storm water having the highest concentration (Table III.E.3.a.7, Figs. III.E.3.a.9-10). Cd was below detection limit at all locations (Table III.E.3.a.7). The median concentration of metals collected from the storm water pipe did not exceed Michigan water quality standards for chronic or acute exposure (MDEQ 2011; Table III.E.3.a.8). However, one of the two samples from the storm water pipe had concentrations of Cu (19.56 µg/L) and Pb (24.04 µg/L) exceeding the Michigan water quality standards for chronic exposure (Table III.E.3.a.8). All the metal concentrations were within the range found in other studies from the literature (Table III.E.3.a.8). PAH levels were very low in this study (< 0.001 mg/L).

Comparison of the pipe storm water and day 0 experimental treatments

The three storm water treatments created from the pipe storm water for this experiment varied in their concentrations of different nutrients on day 0 of the experiment. Concentrations of TP in the 100% and 50% storm water treatments were not significantly different from one another, but were significantly greater than in the 0% treatment on day 1 (Table III.E.3.a.5, Fig. III.E.3.a.6A). The 0% storm water treatment had concentrations of TP similar to those in base flow (Table III.E.3.a.5, Fig. III.E.3.a.6). The concentrations of TP in the 100% and 50% treatments on day 0 of the experiment were substantially greater than the concentration in the pipe storm water; this phenomenon was not observed for any other chemical parameter and may be due to contamination of the storage tank. SRP, NO_3-N, and NH_3-N concentrations were not significantly different among the 100%, 50%, and 0% storm water treatments, but the values in those treatments were lower than the concentrations in the pipe storm water (Table III.E.3.a.5, Figs. III.E.3.a.6 and 7). Although the difference was not significant, the concentrations of NH_3-N in the 50% and 0% treatments were greater than in the 100% treatment (Table III.E.3.a.5, Fig. III.E.3.a.7). SO_4 concentrations, and Cl, pH, specific conductivity, and alkalinity also varied among different storm water treatments and the pipe storm water (Table III.E.3.a.5).

The concentrations of various metals also differed among the pipe storm water and the experimental treatments on day 0 of the study. The concentrations of Cr and Cu on day 1 were significantly different among the 100%, 50% and 0% storm water treatments; the 100% treatment had the highest concentration and the 0% treatment had the lowest (Table III.E.3.a.7, Figs. III.E.3.a.9 and 10). Storm water from the pipe had a higher concentration than the 100% treatment for both Cr and Cu (Table III.E.3.a.7, Figs. III.E.3.a.9 and 10). Pb concentrations were not statistically different between the 100% and 50% storm water treatments, but both were significantly higher than the 0% treatment (Table III.E.3.a.7, Fig. III.E.3.a.9). Similar to Cr and Cu, Pb values from the pipe storm water were higher than in the 100% treatment. Zn, Ni, and Cd were below detection limit in all of the experimental storm water treatments on day 0 of the study (Table III.E.3.a.7).

Table III.E.3.a.5. Median (and range) of select water chemistry variables from base flow in Little Black Creek (LBC), MI, water collected from LBC during a storm in July 2009: upstream of the storm water (SW) pipe, downstream of the SW pipe, and road runoff collected from a pipe directed into LBC, and the three SW treatments (100%, 50%, and 0%) from day 0 of the experiment. Replicate samples (n = 4) were combined to generate median values. Different letters indicate significant differences within a column. A statistical comparison with the upstream and downstream storm flow and pipe SW values and others was not possible because only one water sample was collected.

	Parameters								
Study	**TP-P**	**SRP-P**	**NO_3-N**	**NH_3-N**	**Cl**	**SO_4**	**pH**	**SpCond**	**Alkalinity**
	mg/L	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**		**µS/cm**	**mg/L**
Base flow[1]	0.013[c] (BD-0.024)	0.003 (BD-0.008)	0.36[a] (0.2-0.69)	0.05 (BD-0.13)	98.5[a] (64-139)	27[a] (21-35)	7.79[b] (7.29-8.06)	593[a] (457-1057)	137[ab] (126-150)
Upstream of pipe (storm flow)	0.03	BD	0.63	0.03	80	25	7.42	583	140
Downstream of pipe (storm flow)	0.05	0.007	0.62	0.15	59	16	7.6	422	82
Pipe SW	0.07 (0.04-0.09)	0.01 (0.009-0.011)	0.57 (0.55-0.58)	0.19 (0.16-0.22)	47.5 (42-53)	13 (12-14)	7.525 (7.42-7.63)	322 (270-373)	73.5 (68-79)
100% SW (experiment day 0)	0.21[a] (0.14-0.27)	BD	0.42 (0.36-0.51)	0.012 (0.009-0.014)	48.5[b] (43-57)	13.25[b] (12-15)	7.98[ab] (7.41-8.05)	343[b] (342-345)	72[c] (72-76)
50% SW (experiment day 0)	0.132[a] (0.12-0.15)	BD	0.41 (0.38-0.44)	0.07 (0.02-0.16)	43[b] (42-53)	16.25[b] (14-18)	8.00[ab] (7.85-8.14)	367[b] (365-380)	107[bc] (106-108)
0% SW (experiment day 0)	0.05[b] (0.03-0.07)	0.006 (BD-0.011)	0.24 (0.19-0.4)	0.08 (0.06-0.12)	39[b] (27-45)	18[b] (14-18)	8.24[a] (8.17-8.29)	389[ab] (387-391)	143[a] (136-146)

Abbreviations: total phosphorus (TP), soluble reactive phosphorus (SRP), nitrate (NO_3-N), ammonia (NH_3-N), chloride (Cl), sulfate (SO_4), specific conductivity (SpCond), and below detection limit (BD)

Detection limits: TP = 0.01 mg/l, SRP = 0.005 mg/l, and NH_3-N = 0.01 mg/l

[1] Base flow measurements were collected on eleven dates between April 2008 and April 2009.

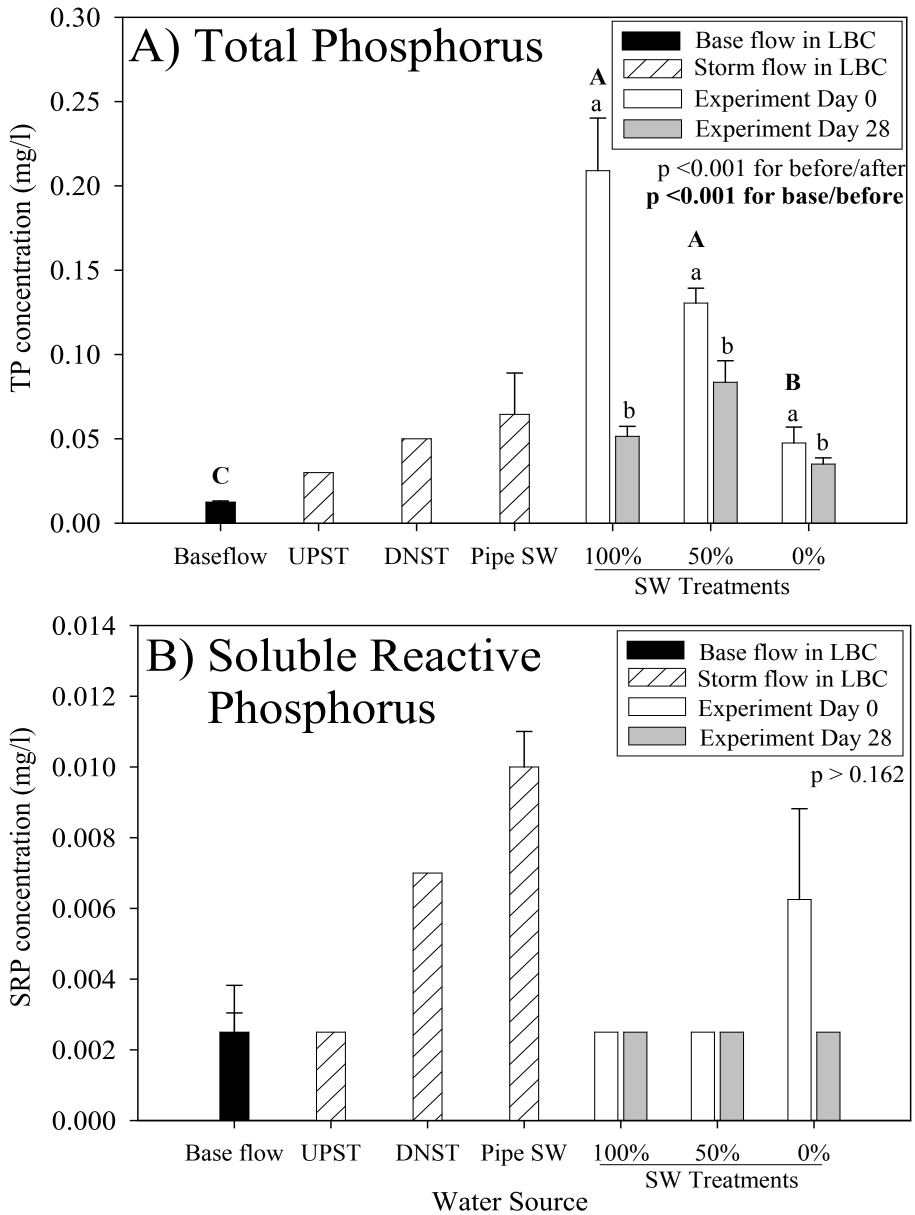
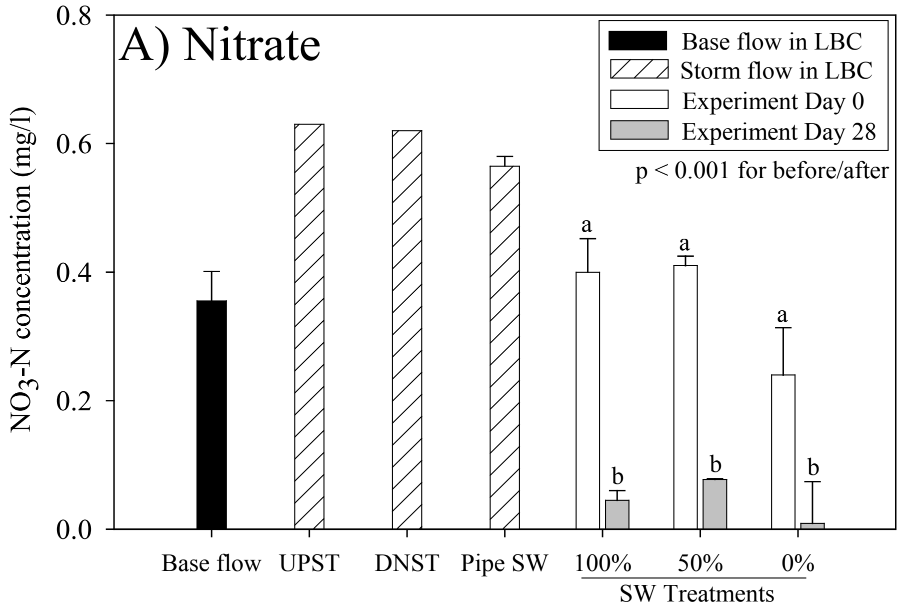


Fig. III.E.3.a.6. A) Total phosphorus (TP) and B) soluble reactive phosphorus (SRP) concentrations during base flow in LBC, water collected from LBC during a storm in July 2009: upstream of the storm water pipe (UPST), downstream of storm water pipe (DNST), and directly from the storm water (SW) pipe, and in the 100%, 50%, and 0% SW treatments on day 0 and day 28 the experiment. Bold, capitalized letters represent significant differences among base flow and day 0 values in each SW treatment. Lowercase letters represent significant differences between the day 0 and day 28 concentrations within each SW treatment. Error bars represent standard error. A statistical comparison with the UPST, DNST, and Pipe SW values with others was not possible because only one sample was collected.



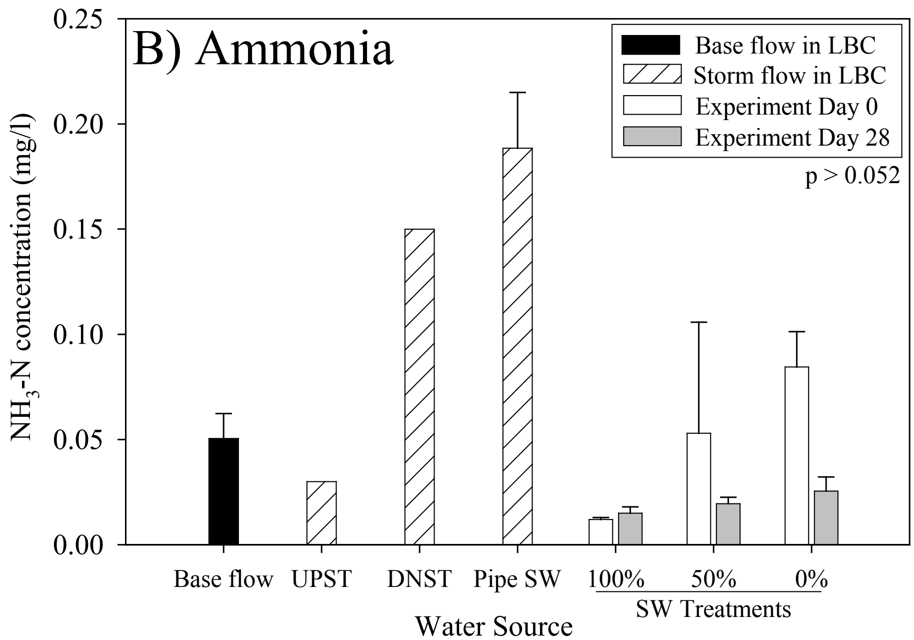


Fig. III.E.3.a.7. A) Nitrate (NO_3-N) and B) ammonia (NH_3-N) concentrations during base flow in LBC, water collected from LBC during a storm in July 2009: upstream of the storm water pipe (UPST), downstream of storm water pipe (DNST), and directly from the storm water (SW) pipe, and in the 100%, 50%, and 0% SW treatments on day 0 and day 28 the experiment. Bold, capitalized letters represent significant differences among base flow and day 0 experiment values in each SW treatment. Lowercase letters represent significant differences between the day 0 and day 28 concentrations within each SW treatment. Error bars represent standard error. A statistical comparison with the UPST, DNST, and Pipe SW values with others was not possible because only one sample was collected.

Table III.E.3.a.6. Median (and range) of select water chemistry variables from road runoff collected in July 2009 from a pipe directed into Little Black Creek (LBC), MI, base flow in LBC, and other storm water studies from the literature (with study location in parentheses). Studies from the literature were chosen because they analyzed road runoff or sampled directly downstream of a road crossing and provided water chemistry data in the text.

	Parameters									
Study	**TP**	**SRP**	**TSS**	**NO_3-N**	**NH_3-N**	**Cl**	**SO_4**	**pH**	**SpCond**	**Alkalinity**
	mg/L	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**		**µS/cm**	**mg/L**
Little Black Creek (this study, pipe SW)	0.07 (0.04-0.09)	0.01 (0.009-0.011)	48 (15-81)	0.57 (0.55-0.58)	0.19 (0.16-0.22)	47.5 (42-53)	13 (12-14)	7.525 (7.42-7.63)	322 (270-373)	73.5 (68-79)
Little Black Creek (this study, base flow)[1]	0.013 (BD-0.024)	0.003 (BD-0.008)	2 (0-10)	0.36 (0.2-0.69)	0.05 (BD-0.13)	98.5 (64-139)	27 (21-35)	7.79 (7.29-8.06)	593 (457-1057)	137 (126-150)
Little Black Creek (Steinman et al., 2006)	0.045 (0.03-0.1)	0.005 (0.005-0.03)	—	0.9 (0.5-1.5)	—	125 (31-270)	—	—	747 (498-1042)	—
Boisson & Perrodin, 2006 (France)	—	—	35 (20-50)	5.05 (3.5-6.6)	—	16.5 (13-20)	32 (23-41)	6.35 (5.5-7.2)	217 (198-226)	—
Lee & Bang, 2000[2] (Korea)	5.86 (1.2-10.3)	—	—	1.2 (0.45-2.28)	—	—	—	—	—	—
Maltby et al.,1995 (Northern England)	—	—	—	44	—	112.1	111.6	7.69	—	—
Mangani et al., 2005 (Central Italy)	—	—	—	4.6 (3.0-8.0)	—	4.9 (3.8-6.5)	12.3 (8.0-14.4)	7.85 (7.1-8.0)	—	—
Taebi & Droste, 2004[2] (Iran)	0.274	—	149	—	—	—	—	7.3	507	—
Wu et al., 1998 (North Carolina, USA)	0.2 (0.04-1.54)	—	215 (32-538)	0.38 (0.08-13.4)	0.66 (0.50-1.74)	—	—	—	—	—

Abbreviations: total phosphorus (TP), soluble reactive phosphorus (SRP), total suspended solids (TSS), nitrate (NO_3-N), ammonia (NH_3-N), chloride (Cl), sulfate (SO_4), specific conductivity (SpCond), and below detection limit (BD)

Detection limits: TP = 0.01 mg/l, SRP = 0.005 mg/l, and NH_3-N = 0.01 mg/l

[1] Base flow measurements were collected on 11 dates between April 2008 and April 2009

[2] Mean values are shown because median values were unavailable.

Table III.E.3.a.7. Median (and range) of select metals from base flow in Little Black Creek (LBC), MI, water collected from LBC during a storm in July 2009: upstream of the storm water (SW) pipe, downstream of the SW pipe, and road runoff collected from a pipe directed into LBC, and the three SW treatments from day 0 of the experiment. Replicate samples (n = 4) were combined to generate median and range values. Lowercase letters represent significant differences among base flow and before experiment values only in each SW treatment. A statistical comparison with the upstream and downstream storm flow and pipe SW values with others was not possible because only one water sample was collected.

	Parameters					
Study	Zn	Cr	Pb	Cu	Ni	Cd
	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Base flow[1]	BD	1.19[c] (BD-2.54)	0.846[b] (BD-4.42)	2.5[abc] (BD-60.51)	BD	BD
Upstream of pipe (storm flow)	BD	3.76	3.53	2.50	BD	BD
Downstream of pipe (storm flow)	BD	10.82	7.38	8.83	BD	BD
Pipe SW	85.93 (BD-146.86)	19.75 (11.18-28.33)	14.46 (4.89-24.04)	13.86 (8.16-19.56)	5.26 (BD-8.01)	BD
100% SW (experiment day 0)	BD	9.79[a] (9.27-15.95)	3.78[a] (3.65-4.38)	8.48[a] (7.14-10.69)	BD	BD
50% SW (experiment day 0)	BD	6.08[b] (5.85-8.18)	2.62[a] (2.3-3.0)	5.76[b] (5.0-6.49)	BD	BD
0% SW (experiment day 0)	BD	0.5[c] (BD-1.1)	BD[b]	BD[c]	BD	BD

Abbreviation: below detection limit (BD)

Detection limits: Zn = 50 µg/l, Cr, Pb, & Cd = 1.0 µg/l, and Cu & Ni = 5.0 µg/l

[1]Base flow measurements were collected on eleven dates between April 2008 and April 2009

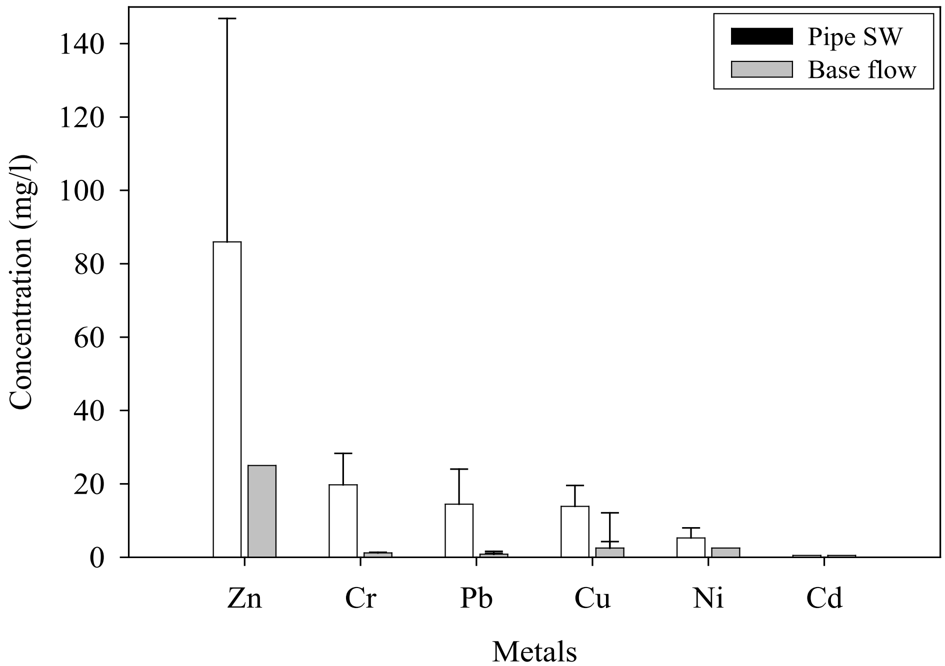


Fig. III.E.3.a.8. Median concentrations of various metals in the runoff from the storm water (SW) pipe collected in July 2009 and in base flow in LBC. Base flow measurements were collected on eleven dates between April 2008 and April 2009.

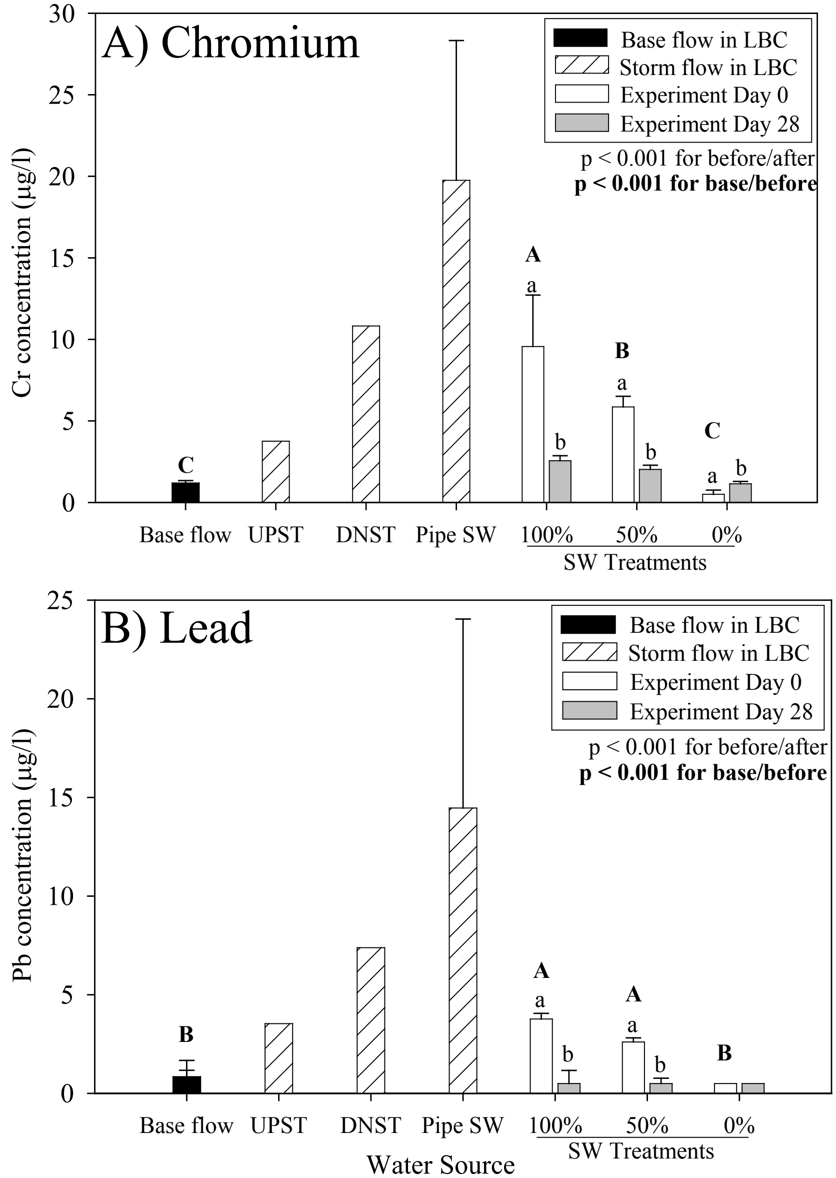


Fig. III.E.3.a.9. A) Chromium and B) lead concentrations during base flow in LBC, water collected from LBC during a storm in July 2009: upstream of the storm water pipe (UPST), downstream of storm water pipe (DNST), and directly from the storm water (SW) pipe, and in the 100%, 50%, and 0% SW treatments on day 0 and day 28 the experiment. Bold, capitalized letters represent significant differences among base flow and day 0 experiment values in each SW treatment. Lowercase letters represent significant differences between the day 0 and day 28 concentrations within each SW treatment. Error bars represent standard error. A statistical comparison with the UPST, DNST, and Pipe SW values with others was not possible because only one sample was collected.

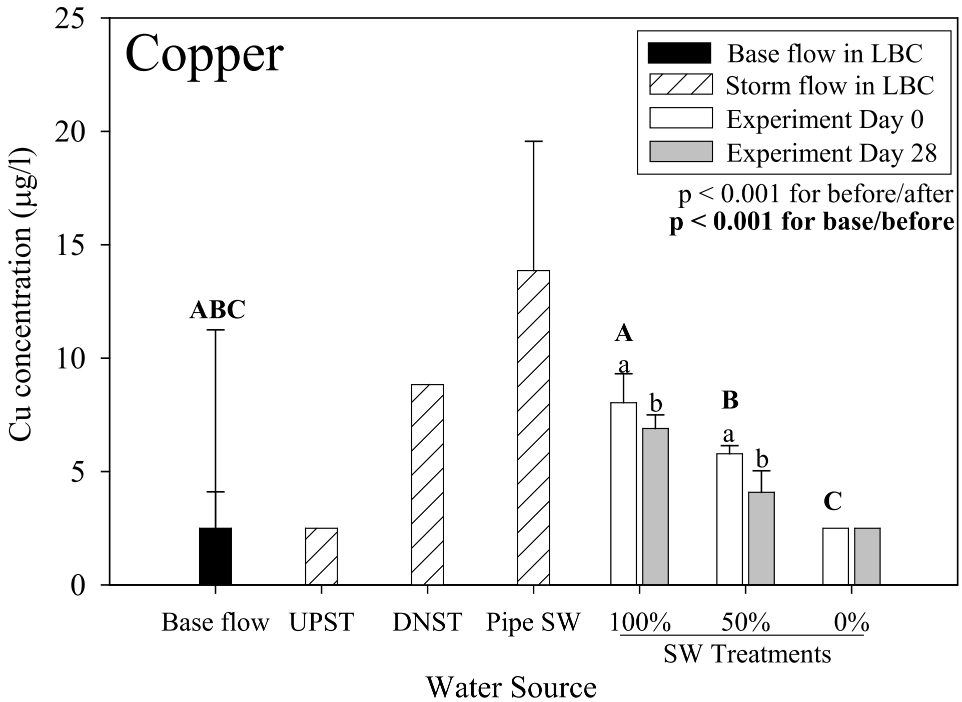


Fig. III.E.3.a.10. Copper concentrations during base flow in LBC, water collected from LBC during a storm in July 2009: upstream of the storm water pipe (UPST), downstream of storm water pipe (DNST), and directly from the storm water (SW) pipe, and in the 100%, 50%, and 0% SW treatments on day 0 and day 28 the experiment. Bold, capitalized letters represent significant differences among base flow and day 0 experiment values in each SW treatment. Lowercase letters represent significant differences between the day 0 and day 28 concentrations within each SW treatment. Error bars represent standard error. A statistical comparison with the UPST, DNST, and Pipe SW values with others was not possible because only one sample was collected.

Table III.E.3.a.8. Median (and range) of selected metals and organics from road runoff collected in July 2009 from a pipe directed into Little Black Creek (LBC), MI, base flow in LBC, chronic and acute exposure water standards in Michigan, and other storm water studies from the literature (with study location in parentheses). Studies from the literature were chosen because they analyzed road runoff or sampled directly downstream of a road crossing and provided water chemistry data in the text.

	Parameters						
Study	**Zn**	**Cr**	**Pb**	**Cu**	**Ni**	**Cd**	**PAHs**
	µg/L	**µg/L**	**µg/L**	**µg/L**	**µg/L**	**µg/L**	**mg/L**
Little Black Creek (this study, pipe SW)	85.93 (BD-146.86)	19.75 (11.18-28.33)	14.46 (4.89-24.04)	13.86 (8.16-19.56)	5.26 (BD-8.01)	BD	BD
Little Black Creek (this study, base flow)[1]	BD	1.19 (BD-2.54)	0.846 (BD-4.42)	2.5 (BD-60.51)	BD	BD	BD
Michigan water standards - Chronic	212.55	130.75	21.71	16.19	93.48	3.73	—
Michigan water standards - Acute	421.65	2010.33	386.47	51.65	1683.32	18.07	—
Boisson & Perrodin, 2006 (France)	—	—	7.25 (4.5-10.0)	83.3 (39.5-127)	430 (60-800)	—	—
Christensen et al., 2006 (Denmark)	16.0 (12-29)	0.50 (0.3-1.1)	2.9 (1.7-4.7)	3.55 (2.9-25.0)	—	—	—
Gan et al., 2008[2] (Southern China)	1230 (700-1760)	40.4	105.3 (92.3-118.2)	140	18 (13.4-22.6)	1.55 (1.5-1.6)	—
Lee & Bang, 2000[2] (Korea)	—	—	133.5 (41-226)	—	—	—	165.4 (85.2-327.3)
Pitt et al., 1995[2] (Alabama, USA)	58 (<1.0-130)	9.9 (<1.0-30)	43 (1.5-150)	280 (<1.0-1250)	17 (<1.0-70)	37 (<1.0-220)	—
Wu et al., 1998 (North Carolina, USA)	—	6.5 (<0.5-20)	15 (<0.5-56)	15 (<0.5-52)	9.0 (<0.5-17)	2.5 (<0.5-2.5)	—

Abbreviations: polycyclic aromatic hydrocarbons (PAHs), and below detection limit (BD)

Detection limits: Zn = 50 µg/l, Cr, Pb, & Cd = 1.0 µg/l, Cu & Ni = 5.0 µg/l, and PAHs = 0.001 mg/l

[1] Base flow measurements were collected on eleven dates between April 2008 and April 2009

[2] Mean values are shown because median values were unavailable.

Experimental storm water treatments

The concentrations of several nutrients in the mesocosms changed during the 28-day experiment. In all three storm water treatments, the concentrations of TP and NO_3-N declined significantly during the experiment (Table III.E.3.a.9, Figs. III.E.3.a.6, 7). SRP concentrations were below detection limit on both day 0 and day 28 of the experiment (Table III.E.3.a.9, Fig. III.E.3.a.6). NH_3-N concentrations were not significantly different on day 0 compared to day 28 of the experiment, but a larger decline occurred in the 50% and 0% treatments than in the 100% treatment (Table III.E.3.a.9, Fig. III.E.3.a.7). Cl, SO_4, pH, specific conductivity, and alkalinity concentrations also varied between day 1 and day 28 of the experiment (Table III.E.3.a.9).

The concentrations of certain metals also changed during the experiment. Cr, Pb, and Cu concentrations were significantly lower on day 28 than on day 0 in the 100% and 50% storm water treatments only (Table III.E.3.a.10, Figs. III.E.3.a.9, 10). Cr, Ni, and Cd were below detection limit both on both day 0 and day 28 of the experiment (Table III.E.3.a.10).

Table III.E.3.a.9. Median (and range) of select water chemistry variables from the 100%, 50%, and 0% storm water (SW) treatments created in the mesocosms. Samples were collected on day 0 and day 28 of the experiment. Lowercase letters represent significant differences between the day 0 and day 28 concentrations within each SW treatment.

	Parameters								
Treatment	**TP-P**	**SRP-P**	**NO_3-N**	**NH_3-N**	**Cl**	**SO_4**	**pH**	**SpCond**	**Alkalinity**
	mg/L	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**		**µS/cm**	**mg/L**
100% SW									
experiment day 0	0.21^{a} (0.14-0.27)	BD	0.42^{a} (0.36-0.51)	0.012 (0.009-0.014)	48.5^{b} (43-57)	13.25^{b} (12-15)	7.98 (7.41-8.05)	343^{b} (342-345)	72 (72-76)
experiment day 28	0.05^{b} (0.02-0.07)	BD	0.045^{b} (0.02-0.08)	0.02 (0.01-0.02)	90.5^{a} (70-113)	22.5^{a} (21-29)	8.01 (7.95-8.06)	449^{a} (448-458)	74 (66-76)
50% SW									
experiment day 0	0.132^{a} (0.12-0.15)	BD	0.41^{a} (0.38-0.44)	0.07^{a} (0.02-0.16)	43^{b} (42-53)	16.25^{b} (14-18)	8.00 (7.85-8.14)	367^{b} (365-380)	107^{a} (106-108)
experiment day 28	0.084^{b} (0.06-0.11)	BD	0.08^{b} (0.07-0.08)	0.02^{b} (0.02-0.03)	68^{a} (57-123)	28^{a} (22-39)	8.04 (7.99-8.11)	421^{a} (396-501)	85^{b} (74-100)
0% SW									
experiment day 0	0.05^{a} (0.03-0.07)	0.006 (BD-0.011)	0.24^{a} (0.19-0.4)	0.08^{a} (0.06-0.12)	39 (27-45)	18^{b} (14-18)	8.24 (8.17-8.29)	389 (387-391)	143^{a} (136-146)
experiment day 28	0.035^{b} (0.03-0.04)	BD	0.01^{b} (0.005-0.14)	0.03^{b} (0.02-0.04)	46 (45-59)	29.5^{a} (25-34)	8.06 (8.04-8.09)	389 (363-419)	110^{b} (102-124)

Abbreviations: total phosphorus (TP), soluble reactive phosphorus (SRP), nitrate (NO_3-N), ammonia (NH_3-N), chloride (Cl), sulfate (SO_4), specific conductivity (SpCond), and below detection limit (BD)

Detection limit of SRP = 0.005 mg/l

Table III.E.3.a.10. Median (and range) of select metals from the 100%, 50%, and 0% storm water (SW) treatments created in the mesocosms. Samples were collected on day 0 and day 28 of the experiment. Lowercase letters represent significant differences between the day 0 and day 28 concentrations within each SW treatment.

	Parameters					
Study	**Zn**	**Cr**	**Pb**	**Cu**	**Ni**	**Cd**
	µg/L	**µg/L**	**µg/L**	**µg/L**	**µg/L**	**µg/L**
100% SW						
experiment day 0	BD	9.79[a] (9.27-15.95)	3.78[a] (3.65-4.38)	8.48[a] (7.14-10.69)	BD	BD
experiment day 28	BD	2.56[b] (1.79-3.27)	0.5[b] (BD-1.83)	6.90[b] (5.85-8.18)	BD	BD
50% SW						
experiment day 0	BD	6.08[a] (5.85-8.18)	2.62[a] (2.3-3.00	5.76[a] (5.0-6.49)	BD	BD
experiment day 28	BD	2.03[b] (1.87-2.57)	0.5[b] (BD-1.03)	4.08[b] (BD-5.76)	BD	BD
0% SW						
experiment day 0	BD	0.5 (BD-1.1)	BD	BD	BD	BD
experiment day 28	BD	1.142 (BD-1.52)	BD	BD	BD	BD

Abbreviation: below detection limit (BD)
Detection limits: Zn = 50 µg/l, Cr, Pb, & Cd = 1.0 µg/l, and Cu & Ni = 5.0 µg/l

III.E.3.b. Periphyton

2008

Algal Biomass

Storm water concentration did not have a significant impact on algal biomass as measured by Chl *a* or AFDM (Table III.E.3.b.1, Fig. III.E.3.b.1). Other measurements related to biomass (AFDM:Chl *a*, pheophytin, or pheophytin:Chl *a*) also did not differ based on storm water treatment (Table III.E.3.b.1). In each storm water treatment, significantly less biomass was present on the tiles exposed to fish than on tiles not exposed to fish (Table III.E.3.b.1, Fig. III.E.3.b.1). This difference was significant for the algae + fish and algae + fish + snails treatments. Fish had no significant effect on either AFDM:Chl *a* or pheophytin:Chl *a*. Exposure to snails both in the algae + snails only treatment and algae + fish + snails treatment did not produce significant changes in algal biomass (Chl *a* or AFDM; Table III.E.3.b.1).

Algal Metabolism

Storm water concentration did not have a significant effect on either respiration or GPP (Fig. III.E.3.b.2). No differences in areal-specific or chl-specific metabolism (respiration and GPP*)* were detected between the fish or snail treatments. A similar lack of trend was observed both for Chl *a*-specific metabolism and areal-specific metabolism.

Table III.E.3.b.1. Nested ANOVA analysis of the final Chl *a*, AFDM, AFDM:Chl *a*, pheophytin, and pheophytin: Chl *a* values in the mesocosm experiment. Fish, snail, and fish x snail treatments are nested within the storm water treatments. Bold values are significant ($p < 0.05$).

Source of Variation	df	SS	MS	F	P
Storm water Treatment					
Chl *a*	2	0.073	0.037	1.09	0.348
AFDM	2	0.149	0.075	1.41	0.26
AFDM: Chl *a*	2	271.3	135.7	1.32	0.284
Pheophytin	2	0.039	0.02	0.72	0.493
Pheophytin:Chl *a*	2	0.053	0.027	2.54	0.096
Snail Treatments (Algae + Snails & Algae + Snails + Fish)					
Chl *a*	3	0.105	0.035	1.05	0.386
AFDM	3	0.246	0.082	1.55	0.222
AFDM: Chl *a*	3	149.3	49.76	0.48	0.696
Pheophytin	3	0.117	0.039	1.44	0.251
Pheophytin:Chl *a*	3	0.028	0.009	0.88	0.46
Fish Treatments (Algae + Fish & Algae + Snails + Fish)					
Chl *a*	3	2.795	0.931	27.8	**<0.001**
AFDM	3	2.058	0.686	12.98	**<0.001**
AFDM: Chl *a*	3	268.8	89.6	0.87	0.468
Pheophytin	3	1.88	0.628	23.03	**<0.001**
Pheophytin:Chl *a*	3	0.051	0.017	1.63	0.202
Algae + Snails + Fish Treatment					
Chl *a*	3	0.073	0.024	0.72	0.545
AFDM	3	0.205	0.068	1.29	0.296
AFDM: Chl *a*	3	67.84	22.61	0.22	0.882
Pheophytin	3	0.053	0.018	0.65	0.589
Pheophytin:Chl *a*	3	0.031	0.01	0.98	0.417

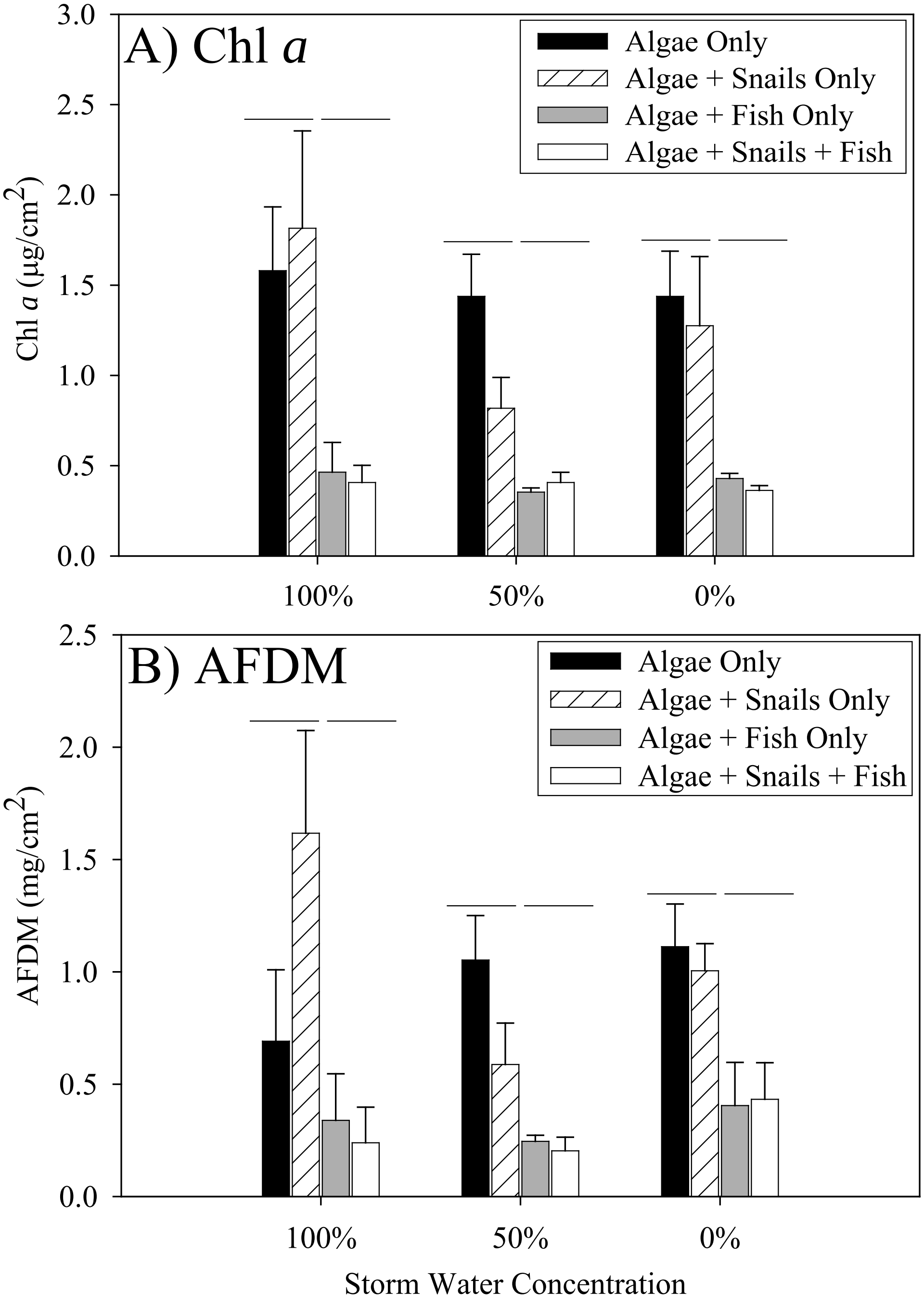


Fig. III.E.3.b.1. A) Chl *a* concentrations and B) AFDM after 31-day exposure to storm water treatments. Chl *a* and AFDM values connected by a bar are not significantly different from each other, and separate bars represent significant differences among AFDM values within a particular storm water treatment only. Error bars represent standard error.

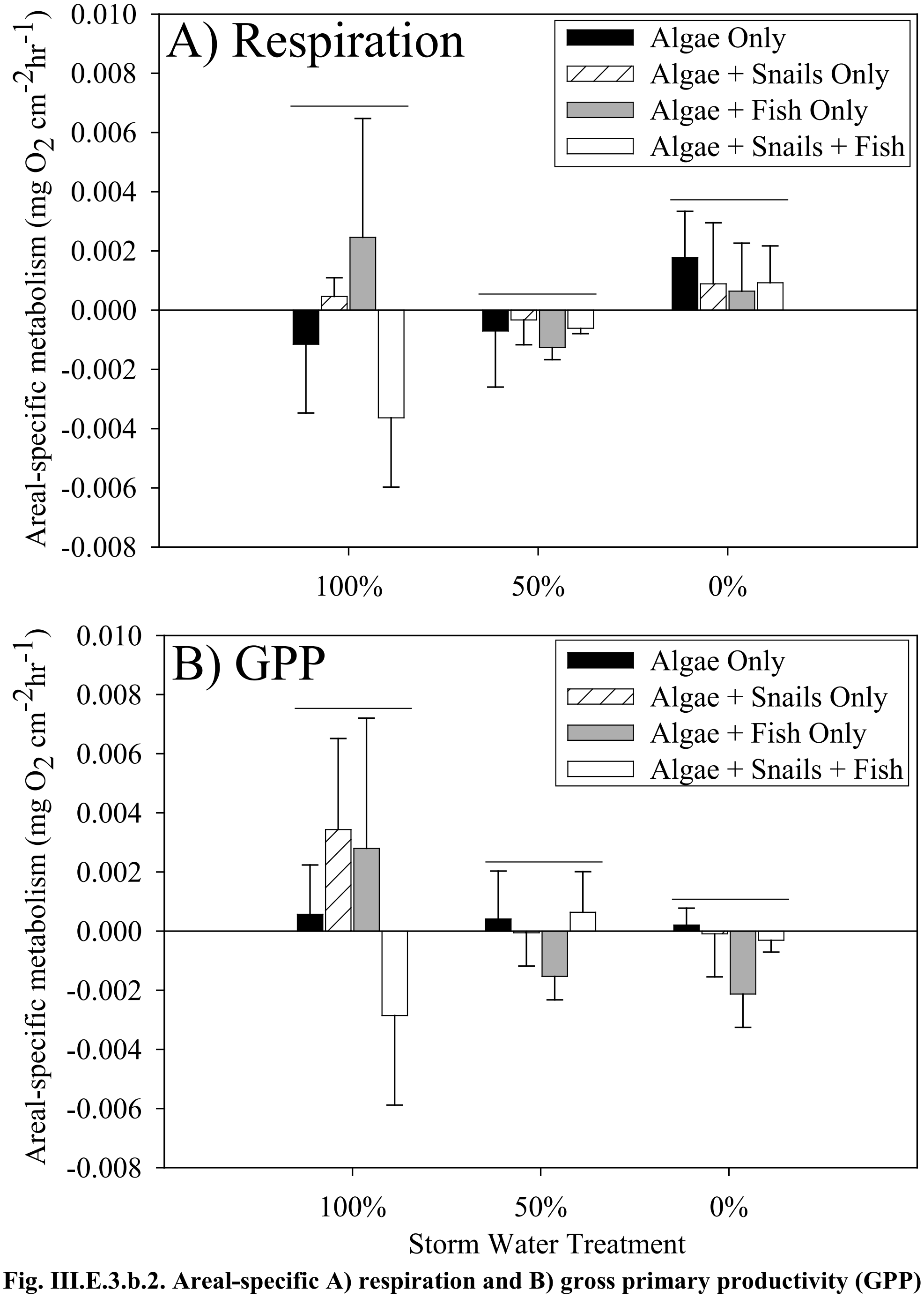


Fig. III.E.3.b.2. Areal-specific A) respiration and B) gross primary productivity (GPP) after 31- day exposure to different storm water treatments. Respiration and GPP values connected by a bar are not significantly different from each other, and separate bars represent significant differences among AFDM values within a particular storm water treatment only. Error bars represent standard error.

Storm water effects

The algal communities on day 31 of this experiment were almost entirely dominated by diatoms (Bacillariophyceae). The seven most abundant genera (or taxonomic units) in terms of relative abundance of total cells were *Navicula,* small naviculoid*, Melosira, Staurosirella, Nitzschia, Stephanocyclus,* and *Cocconeis* (Table III.E.3.b.2, Fig. III.E.3.b.3). Diatoms made up ~92% of total cell numbers with green algae (Chlorophyceae) contributing the remaining 8%. Green algal taxa (and their relative percentage of total cell numbers) included *Scenedesmus* (3.7%), *Ankistrodesmus* (2.7%), *Coelastrum* (0.7%), *Cosmarium* (0.5%), and *Pediastrum* (0.2%).

Diatoms contributed ~90.5% of total cell biovolume. The dominant diatom genera based on relative biovolume were similar to the dominant taxa in terms of relative abundance of total cells and included *Navicula,* small naviculoid*, Melosira, Staurosirella, Nitzschia, Stephanocyclus,* and *Cocconeis* (Table III.E.3.b.3, Fig. III.E.3.b.3). Green algal genera *Scenedesmus* (5.8%), *Pediastrum* (1.7%), and *Ankistrodesmus* (0.1%) made up most of the remaining total biovolume.

Table III.E.3.b.2. Median relative abundance of total cell numbers of algal taxa from each storm water and snail and fish treatment. Samples were collected on day 31 of the experiment and replicate treatments (n = 3 for 100% SW and n = 4 for 50% and 0% SW) were combined to generate median values.

	Relative abundance of total cell numbers (%)												
Taxon	100% Storm Water				50% Storm Water				0% Storm Water				
	A	A+S	A+F	A+S+F	A	A+S	A+F	A+S+F	A	A+S	A+F	A+S+F	Overall
Navicula	19.4	28.0	19.5	20.1	27.0	24.2	16.3	19.7	30.4	28.7	21.6	18.8	22.7
Small naviculoid	17.6	17.5	20.1	20.7	19.6	21.0	20.2	17.1	22.0	19.3	26.1	23.5	20.1
Melosira	21.2	17.0	11.0	7.2	19.5	14.7	9.9	9.8	14.1	14.0	6.6	7.2	11.2
Staurosirella	9.3	5.1	5.8	7.2	9.8	7.3	7.9	7.5	7.4	8.5	5.6	5.9	7.3
Nitzschia	8.0	6.3	7.3	5.2	1.2	4.5	7.8	7.8	2.2	4.2	8.4	9.4	6.4
Stephanocyclus	6.1	5.1	7.3	8.5	6.6	4.4	3.9	4.4	5.8	4.6	2.9	4.0	4.9
Cocconeis	4.5	1.6	5.4	7.2	4.0	2.6	7.8	5.9	3.1	3.4	7.3	6.3	4.7
Scenedesmus	0.6	1.8	3.7	3.9	2.0	1.7	7.4	7.2	2.5	1.2	4.1	3.6	2.7
All other algal taxa	13.20	17.45	19.94	19.89	10.34	19.54	18.88	20.63	12.53	16.17	17.32	21.41	20.05
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.0

Abbreviations: A = algae only treatment (fish & snails excluded), A+S = algae + snails only treatment (fish excluded), A+F = algae + fish only treatment (snails excluded), and A+S+F = algae + snails + fish treatment

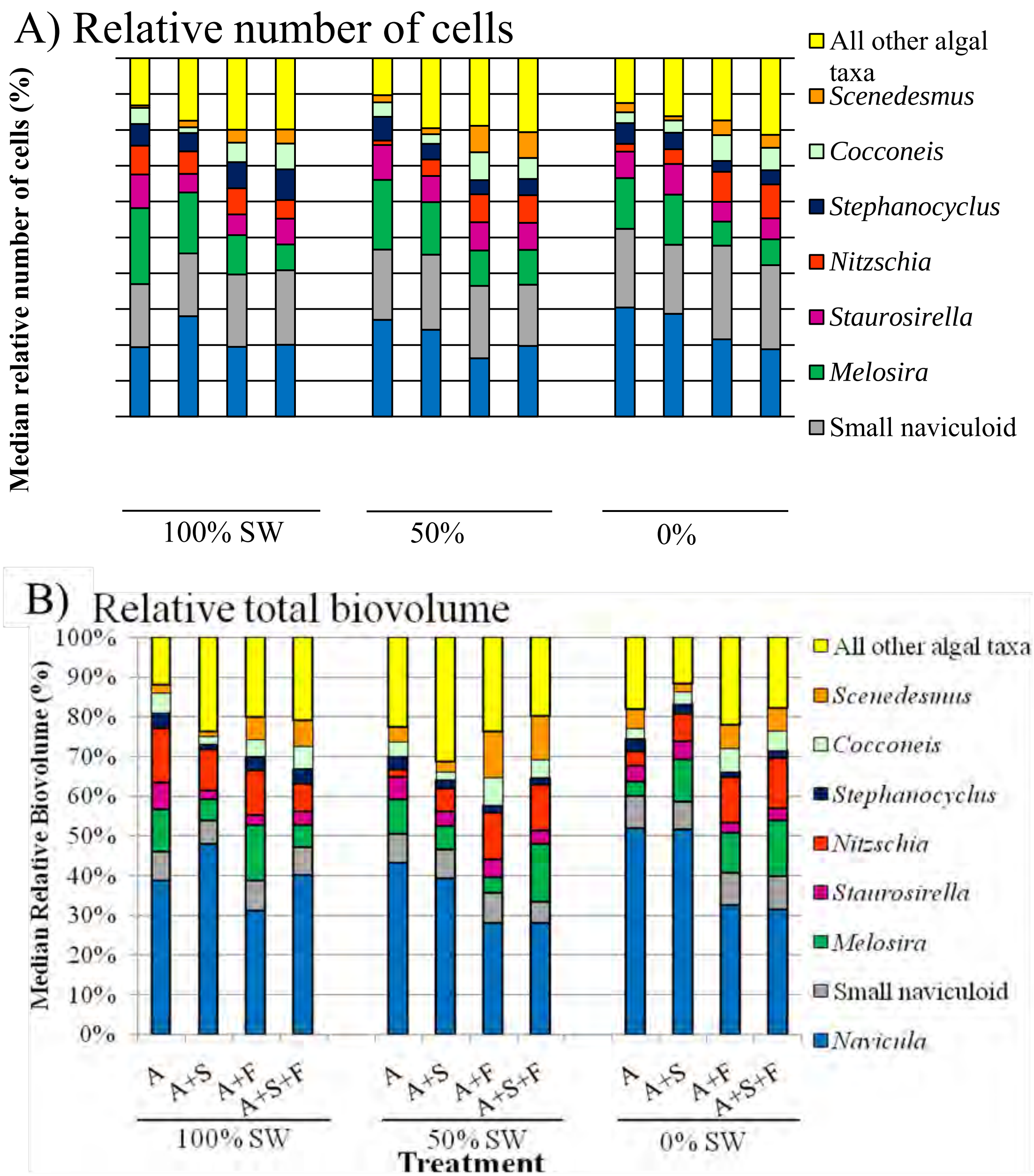


Fig. III.E.3.b.3. Median relative abundance of A) relative abundance of total cell numbers and B) relative total biovolume of algal taxa after exposure to different storm water and snail and fish treatments. Abbreviations: storm water (SW), algae only treatment (A), algae + snails only treatment (A+S), algae + fish only treatment (A+F), and algae + fish + snails treatment (A+F+S). Samples were collected on day 31 of the experiment and replicate treatments (n = 3 for 100% SW and n = 4 for 50% and 0% SW) were combined to generate median values.

The storm water treatments appeared to have a small influence on community composition during this experiment. *Navicula* and small naviculoid were the most abundant taxa, in terms of relative number of cells, in all three storm water treatments (Table III.E.3.b.2). The relative abundance of total cell numbers of small naviculoid and *Ankistrodesmus* taxa were significantly higher in the 0% storm water treatment than in the 100% storm water treatment, and the relative percentage of *Stephanocyclus* was significantly higher in the 100% storm water compared to the 0% treatment (Table III.E.3.b.4). The relative abundances of total cell numbers of the remaining algal taxa were not significantly affected by the storm water treatments.

Navicula and *Melosira* made up the largest percent of relative total biovolume in all storm water treatments, but were not significantly affected by storm water (Table III.E.3.b.3). In terms of biovolume, small *Achnanthes, Pediastrum,* and *Ankistrodesmus* were all significantly affected by storm water concentration (Table III.E.3.b.4). *Pediastrum* and *Ankistrodesmus* had a significantly higher relative biovolume in the 0% storm water treatment than in the other treatments while small *Achnanthes* had a significantly higher relative biovolume in the 100% treatment compared to the 50% and 0% treatments (Table III.E.3.b.4). The remaining algal taxa were not significantly affected by the storm water treatments.

The NMDS ordination of relative abundances of total cell numbers revealed some separation among storm water treatments (Fig. III.E.3.b.4). The algal community in the 0% storm water treatment was significantly ($p < 0.039$) different from the communities in the 50% and 100% treatments, which were not different from each other.

The NMDS ordination with relative total biovolume and storm water treatment showed considerable overlap in ordination space among the treatments (Fig. III.E.3.b.4). Unlike cell number, the communities in terms of biovolume were not significantly different among any storm water treatments. An outlier (100% SW) was present in the biovolume data and although this point condenses the ordination (Fig. III.E.3.b.4) it was not excluded from analyses because its exclusion did not alter the results of any statistical test.

In this analysis, indicator values never exceeded 45 (Table III.E.3.b.5), which indicates that algal taxa were not particularly restricted to certain treatments. For both the relative abundance of total cell numbers and relative biovolume, *Achnanthes* was the best indicator of the 100% storm water treatment and *Scenedesmus* was the best indicator of the 50% storm water treatment, although the indicator values were relatively weak overall. *Cymbella* was the best indicator for the 0% storm water treatment based on relative abundance of total cell numbers, but *Ankistrodesmus* was the best in terms of relative biovolume (Table III.E.3.b.5).

The algal communities in the different storm water treatments in this experiment did not differ significantly in the distribution of different physiognomies (Fig. III.E.3.b.5). Of the four groups defined in this experiment, only taxa in the loose understory, firm understory, and loose overstory groups were present. The two most abundant taxa in terms of relative abundances of total cell numbers in all three storm water treatments were both in the loose understory physiognomic group (*Navicula* and small naviculoid; Table III.E.3.b.4, Fig. III.E.3.b.5). In terms of relative biovolume, the loose understory (*Navicula*) and loose canopy (*Melosira*) groups were most abundant in all treatments. In terms of relative abundance of total cell numbers, loose understory taxa had significantly higher abundance in the 0% storm water treatment compared to the 100% treatment ($p < 0.001$).

PCA of environmental variables revealed a clear distinction between the storm water treatments in principal component (PC) 1, with all of the 100% storm water mesocosms receiving high scores and all of the 0% mesocosms receiving low scores (Fig. III.E.3.b.6). Specific conductivity and Cr correlated most strongly to PC 1 in the positive direction and SRP and alkalinity correlated most strongly with PC 1 in the negative direction.

Effects of snails and fish

Navicula and small naviculoid taxa had the highest relative number of total cells in the samples regardless of fish presence or absence (Table III.E.3.b.2). The relative number of total cells of many taxa was significantly affected by fish presence. *Navicula* and *Melosira* both had a significantly negative response to fish presence (Table III.E.3.b.4). *Nitzschia, Cocconeis,* small *Achnanthes, Scenedesmus, Pediastrum,* and *Ankistrodesmus* all had significantly higher relative abundances of total cell numbers when fish were present compared to when fish were excluded (Table III.E.3.b.4). Small *Naviculoid* taxon had a marginally significant increase in relative cell numbers when exposed to fish (Table III.E.3.b.4).

Navicula and *Melosira* had the highest relative biovolume in the samples regardless of fish presence or absence (Table III.E.3.b.3). The relative biovolume of several taxa was also significantly affected by fish presence. *Navicula* and *Stephanocyclus* were the only taxa that declined in biovolume when exposed to fish (Table III.E.3.b.4). *Nitzschia, Cocconeis, Scenedesmus, Pediastrum,* and *Ankistrodesmus* all increased in relative biovolume when exposed to fish (Table III.E.3.b.4).

Table III.E.3.b.3. Median relative total biovolume of algal taxa from each storm water and snails and fish treatment. Samples were collected on day 31 of the experiment and replicate treatments (n = 3 for 100% SW and n = 4 for 50% and 0% SW) were combined to generate median values. Taxa are listed in the same order as in Table 6 for comparison purposes.

	Relative total biovolume (%)												
Taxon	100% SW				50% SW				0% SW				
	A	A+S	A+F	A+S+F	A	A+S	A+F	A+S+F	A	A+S	A+F	A+S+F	Overall
Navicula	38.61	47.75	31.10	39.89	43.12	39.21	28.09	27.84	51.94	51.58	32.36	31.31	38.79
Small naviculoid	7.18	5.93	7.51	7.07	7.18	7.21	7.46	5.41	8.10	7.02	8.31	8.44	7.49
Melosira	10.75	5.29	14.08	5.65	8.81	6.05	3.92	14.68	3.61	10.55	9.93	13.97	9.76
Staurosirella	6.71	2.26	2.60	3.33	5.68	3.50	4.48	3.31	3.88	4.59	2.59	3.04	3.66
Nitzschia	13.91	10.44	11.09	6.97	1.83	5.81	11.65	11.38	3.68	6.91	11.57	12.76	9.87
Stephanocyclus	3.73	1.39	3.48	3.79	3.29	2.15	2.01	1.93	3.06	2.39	1.30	1.79	2.26
Cocconeis	4.86	1.76	4.13	5.69	3.49	1.93	7.09	4.45	2.76	3.19	5.67	4.89	4.02
Scenedesmus	2.29	1.32	5.79	6.72	3.87	2.86	11.54	11.03	4.68	2.10	6.01	5.85	4.27
All other algal taxa	11.97	23.86	20.22	20.90	22.73	31.28	23.77	19.98	18.30	11.66	22.27	17.96	19.87
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Abbreviations: A = algae only treatment (fish & snails excluded), A+S = algae + snails only treatment (fish excluded), A+F = algae + fish only treatment (snails excluded), and A+S+F = algae + snails + fish treatment

Table III.E.3.b.4. Nested ANOVA analysis of the relative abundance of total cell numbers and relative total biovolume data for each algal taxon (and physiognomic group) at the end of the 31-day experiment. Snail, fish, and snail x fish treatments are nested within the storm water (SW) treatments. Bold values are significant (p < 0.05). Arrows represent a significant increase (↑) or decrease (↓) relative to the control.

		Relative number of total cells				Relative total biovolume			
Source of Variation	**df**	**SS**	**MS**	**F**	**P**	**SS**	**MS**	**F**	**P**
***Navicula* (LU)**									
Storm Water	2	109.23	54.62	1.81	0.18	262.46	131.23	1.47	0.25
Snail (SW)	3	75.72	25.24	0.84	0.48	51.95	17.32	0.19	0.90
Fish (SW)	3	696.48	232.16	7.71	**<0.001** ↓	2359.63	786.54	8.80	**<0.001** ↓
Snail x Fish (SW)	3	3.90	1.30	0.04	0.99	20.93	6.98	0.08	0.97
Small naviculoid (LU)									
Storm Water	2	96.36	4.41	4.41	**0.02** ↓	8.90	4.45	1.53	0.23
Snail (SW)	3	19.80	0.60	0.60	0.62	6.84	2.28	0.78	0.51
Fish (SW)	3	104.06	3.17	3.17	0.05	2.39	0.80	0.27	0.84
Snail x Fish (SW)	3	18.53	0.57	0.57	0.64	4.54	1.51	0.52	0.67
***Melosira* (LC)**									
Storm Water	2	81.02	40.51	1.82	0.18	0.01	0.00	0.05	0.95
Snail (SW)	3	33.47	11.16	0.50	0.68	0.41	0.14	1.40	0.26
Fish (SW)	3	731.18	243.73	10.93	**<0.001** ↓	0.15	0.05	0.51	0.68
Snail x Fish (SW)	3	17.00	5.67	0.25	0.86	0.64	0.21	2.15	0.11
***Staurosirella* (LC)**									
Storm Water	2	21.25	10.62	1.46	0.25	3.37	1.69	0.79	0.46
Snail (SW)	3	23.38	7.79	1.07	0.38	18.85	6.27	2.94	**0.05** ↓*
Fish (SW)	3	37.83	12.66	1.73	0.18	15.48	5.16	2.42	0.09
Snail x Fish (SW)	3	27.49	9.16	1.26	0.31	19.43	6.48	3.03	**0.04**
***Nitzschia* (LU)**									
Storm Water	2	14.02	7.01	0.87	0.43	32.36	16.18	0.73	0.49
Snail (SW)	3	19.26	6.42	0.79	0.51	77.29	25.76	1.16	0.34
Fish (SW)	3	175.16	58.39	7.22	**<0.001** ↑	354.33	118.11	5.30	**0.005** ↑
Snail x Fish (SW)	3	5.12	1.71	0.21	0.89	22.78	7.59	0.34	0.80
***Stephanocyclus* (LU)**									
Storm Water	2	22.47	11.23	3.22	**0.05** ↑	4.56	2.28	1.92	0.16
Snail (SW)	3	1.07	0.36	0.10	0.96	2.26	0.75	0.63	0.60
Fish (SW)	3	34.09	11.37	3.25	0.07	12.76	4.25	3.58	**0.02** ↓
Snail x Fish (SW)	3	11.99	4.00	1.14	0.35	5.50	1.86	1.54	0.22

Abbreviations: loose understory (LU), firm canopy (FC), loose canopy (LC), and firm understory (FU)

* significant only in 100% and 50% SW treatments

Table continued below

Table III.E.3.b.4 Continued.

		Relative number of total cells				Relative total biovolume			
Source of Variation	**df**	**SS**	**MS**	**F**	**P**	**SS**	**MS**	**F**	**P**
***Cocconeis* (FU)**									
Storm Water	2	1.12	0.56	0.10	0.90	0.01	0.00	0.09	0.91
Snail (SW)	3	19.70	6.57	1.23	0.32	0.35	0.12	2.94	**0.05** ↓
Fish (SW)	3	112.82	37.61	7.02	**<0.001** ↑	0.88	0.29	7.31	**<0.001** ↑
Snail x Fish (SW)	3	10.04	3.35	0.63	0.60	0.31	0.10	2.56	0.07
***Achnanthes* (FU)**									
Storm Water	2	13.62	6.81	0.85	0.44	0.50	0.25	5.43	**0.01** ↑
Snail (SW)	3	23.22	7.74	0.96	0.42	0.11	0.04	0.79	0.51
Fish (SW)	3	174.04	58.01	7.20	**<0.001** ↑	0.17	0.06	1.25	0.31
Snail x Fish (SW)	3	7.44	2.48	0.31	0.82	0.17	0.06	1.21	0.32
***Scenedesmus* (LU)**									
Storm Water	2	16.26	8.13	1.07	0.36	45.79	22.90	1.25	0.30
Snail (SW)	3	13.79	4.60	0.60	0.62	27.65	9.22	0.50	0.68
Fish (SW)	3	164.78	54.93	7.20	**<0.001** ↑	386.17	128.72	7.02	**<0.001** ↑
Snail x Fish (SW)	3	2.78	0.93	0.12	0.95	3.10	1.03	0.06	0.98
***Pediastrum* (LU)**									
Storm Water	2	0.86	0.43	3.05	0.06	39.58	19.79	3.27	**0.05** ↓
Snail (SW)	3	0.29	0.10	0.68	0.57	13.27	4.42	0.73	0.54
Fish (SW)	3	1.82	0.61	4.29	**0.01** ↑	73.99	24.66	4.07	**0.015** ↑
Snail x Fish (SW)	3	0.06	0.02	0.15	0.93	2.12	0.71	0.12	0.95
***Ankistrodesmus* (LU)**									
Storm Water	2	9.31	4.65	3.73	**0.04** ↓	0.09	0.05	3.65	**0.04** ↓
Snail (SW)	3	0.37	0.12	0.10	0.96	0.00	0.00	0.01	1.00
Fish (SW)	3	16.76	5.58	4.48	**0.001** ↑	0.15	0.05	3.84	**0.02** ↑
Snail x Fish (SW)	3	0.42	0.14	0.11	0.95	0.00	0.00	0.09	0.96

Abbreviations: loose understory (LU), firm canopy (FC), loose canopy (LC), and firm understory (FU)

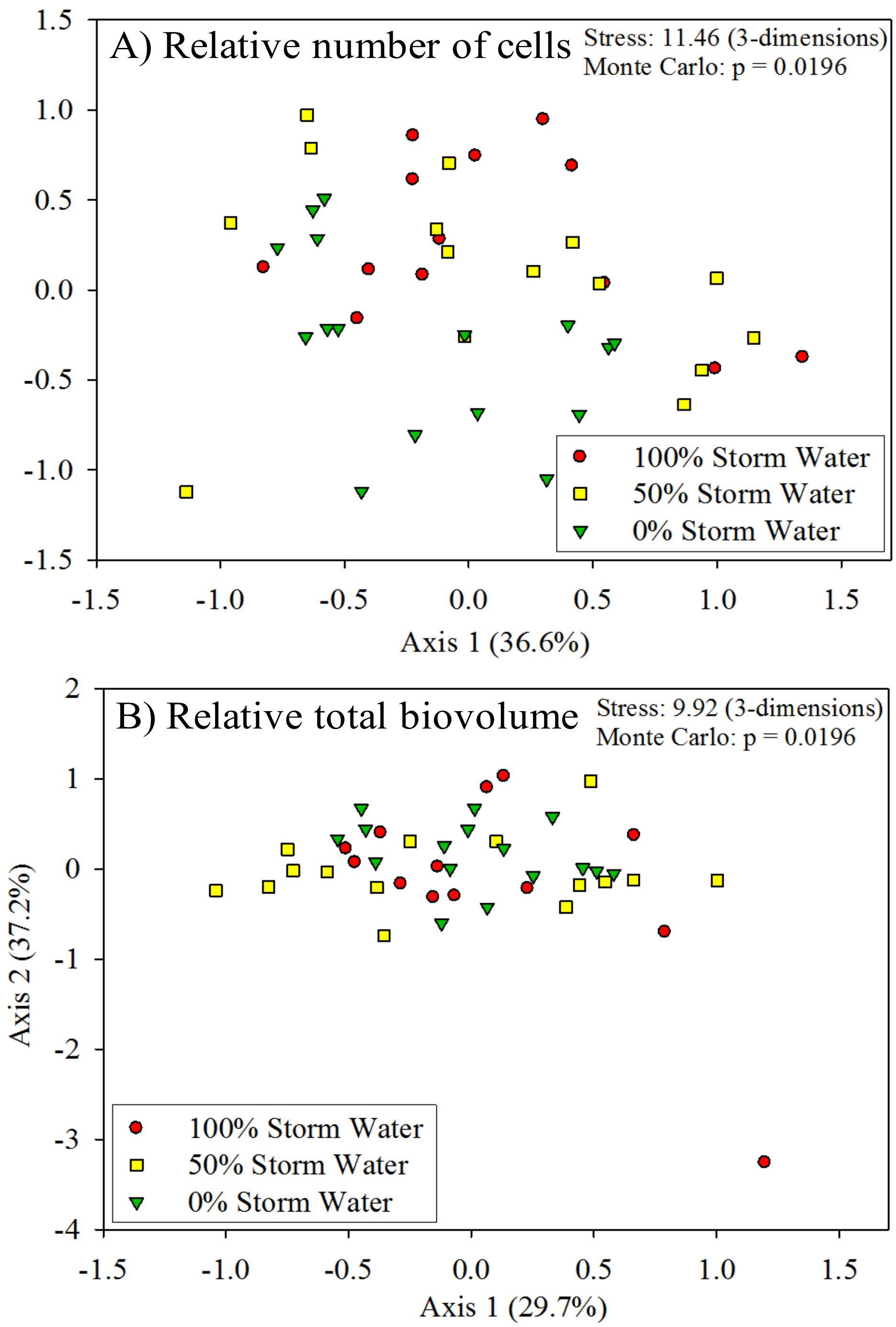


Fig. III.E.3.b.4. Nonmetric multidimensional scaling (NMDS) ordinations of the A) relative abundance of total cell numbers and B) relative total biovolume of the algal communities in each storm water treatment.

Table III.E.3.b.5. The taxon with the highest indicator value (in parentheses) for each of the three storm water (SW) treatments and four snail and fish treatments in terms of both relative number of total cells and relative total biovolume. Indicator values can range from 0 for a taxon that has the same occurrence and abundance in all groups of samples to 100 for a taxon that is restricted to a single group.

	Relative number of total cells	Relative total biovolume
SW Treatments		
100% SW	*Achnanthes* (42.1)	*Achnanthes* (42.7)
50% SW	*Scenedesmus* (40.8)	*Scenedesmus* (41.5)
0% SW	*Cymbella* (42.8)	*Ankistrodesmus* (43.4)
Fish/Snail Treatments		
Algae Only	*Melosira* (34.4)	*Staurosirella* (33.5)
Algae + Snails Only	*Melosira* (31)	*Navicula* (30)
Algae + Fish Only	*Scenedesmus* (38.5)	*Scenedesmus* (38.3)
Algae + Snails+ Fish	*Scenedesmus* (37)	*Scenedesmus* (36)

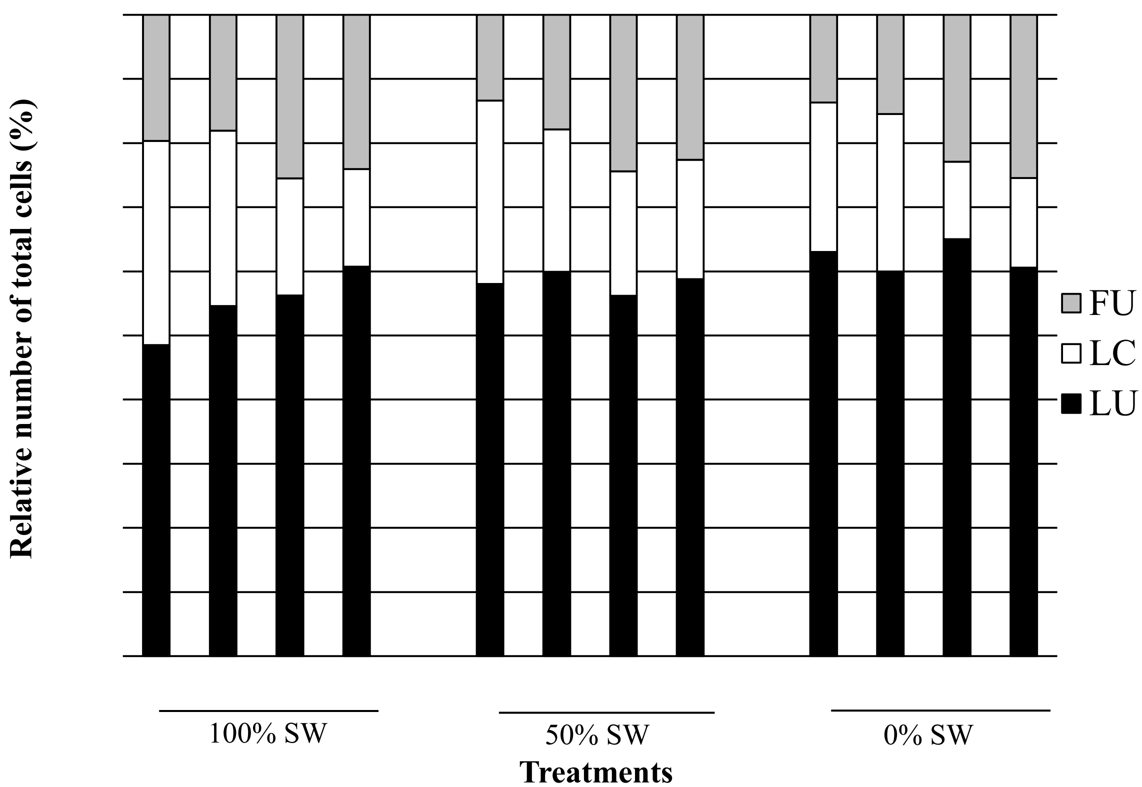


Fig. III.E.3.b.5. Physiognomy distributions in algal communities after exposure to different storm water and snail and fish treatments. Abbreviations: loose understory (LU), loose canopy (LC), firm understory (FU), storm water (SW), algae only treatment (A), algae + snails only treatment (A+S), algae + fish only treatment (A+F), algae + fish + snails treatment (A+F+S). Samples were collected on day 31 of the experiment.

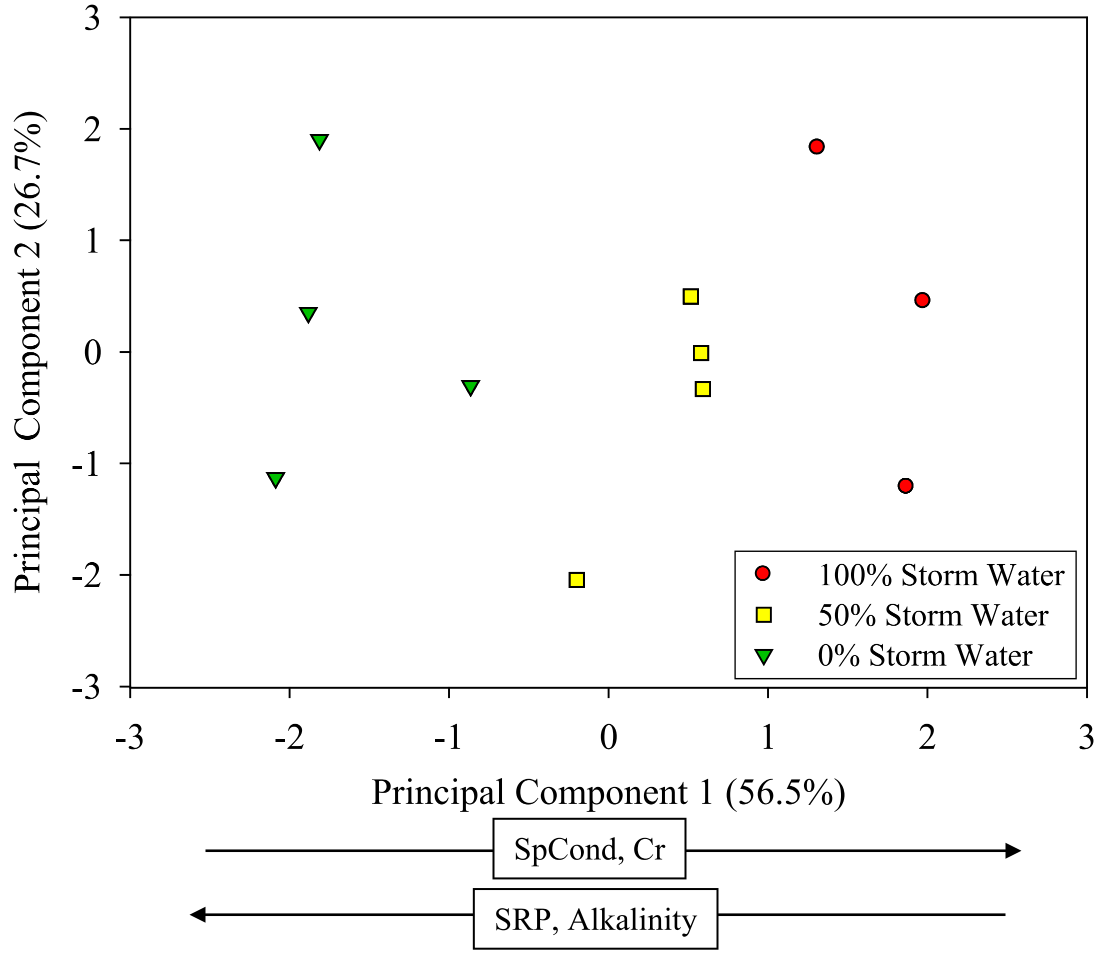


Fig. III.E.3.b.6. Principal components analysis (PCA) of four environmental variables collected from the end of the mesocosm experiment: specific conductivity, soluble reactive phosphorus (SRP), and alkalinity. Cr concentrations were available only from the pipe at the beginning of the experiment; values from the mesocosms were estimated (see text for details). Variables that loaded heavily into principal component 1 are listed.

The NMDS ordinations of both relative abundance of total cell numbers and relative total biovolume with snail and fish treatments revealed a tendency for the algae only and algae + snails only treatments to group together apart from the algae + fish only and algae + snails + fish treatments, which formed a second grouping in ordination space (Fig. III.E.3.b.7). This distinction is clearer in the relative number of cells ordination (Fig. III.E.3.b.7) than in the biovolume ordination because the latter is condensed due to the presence of an outlier (Fig. III.E.3.b.7). The algal communities present in the two treatments exposed to fish were significantly different than the communities in the treatments not exposed to fish ($p < 0.001$ for both number of cells and biovolume). For both relative abundance of total cell numbers and relative total biovolume, *Scenedesmus* was the best indicator for the algae + fish only and algae + snails + fish treatments (Table III.E.3.b.5). In terms of relative number of total cells, *Melosira* had the highest indicator values for both the algae only and algae + snails only treatments. In terms of relative biovolume, *Staurosirella* and *Navicula* had the highest indicator values for the algae only and algae + snails only treatments, respectively (Table III.E.3.b.5). As with the storm water treatments, the indicator values of the taxa in the fish and snail treatments were relatively low.

Algal physiognomy had a variable response to fish and snail presence (Fig. III.E.3.b.6). Overall, the relative abundances of total cell numbers in the firm understory group significantly ($p < 0.04$) declined with fish presence and the relative number of cells in the firm canopy group also significantly ($p < 0.003$) declined with fish presence. In terms of relative abundance of total cell numbers, the two taxa that responded negatively to fish presence were a loose understory taxon (*Navicula*) and a firm canopy taxon (*Melosira*; Table III.E.3.b.4). The taxa that were positively affected by fish presence were in the loose understory (*Nitzschia, Scenedesmus, Pediastrum,* and *Ankistrodesmus)* and firm understory groups (small *Achnanthes* and *Cocconeis*; Table III.E.3.b.4).

The relative biovolume of the firm understory group significantly ($p < 0.001$) increased with fish presence and loose understory taxa significantly ($p < 0.001$) declined with fish presence. In terms of relative biovolume, the only taxa that were negatively affected by fish presence were loose understory taxa (*Navicula* and *Stephanocyclus*; Table III.E.3.b.4). Taxa that responded positively to fish presence in terms of relative biovolume were the same as with relative number of total cells. Snail presence did not significantly affect the distribution of physiognomic groups.

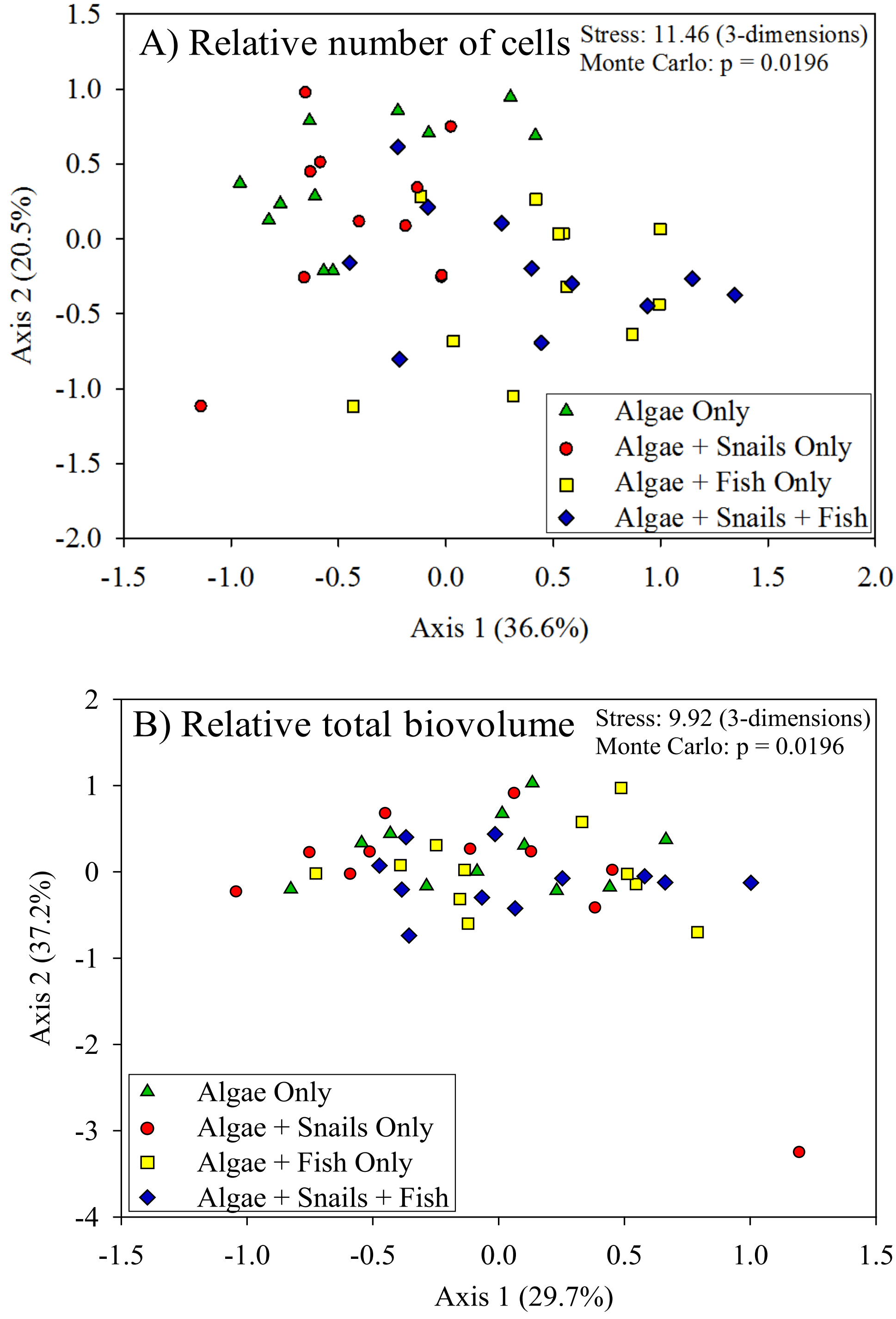


Fig. III.E.3.b.7. Nonmetric multidimensional scaling (NMDS) ordinations of the A) relative abundance of total cell numbers and B) relative total biovolume of the algal communities in each fish and snail treatment.

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Algal Biomass

Storm water concentration did not have a significant impact on algal biomass as measured by Chl *a* or AFDM (Fig. III.E.3.b.8, Table III.E.3.b.6). Other measurements related to biomass (AFDM:Chl, pheophytin, or pheophytin:Chl *a*) also did not differ based on storm water treatment (Table III.E.3.b.6). Fish presence significantly reduced the concentration of pheophytin, but only in the 50% storm water treatment (Table III.E.3.b.6). Fish significantly reduced pheophytin: Chl *a* in all storm water treatments and had a marginally significant negative effect on AFDM (Table III.E.3.b.6). Fish did not have a significant effect on Chl *a* or AFDM:Chl (Table III.E.3.b.6). Significantly less Chl *a* and AFDM were present in the 0% storm water treatment when snails were present (Table III.E.3.b.6). Mean values of both Chl *a* and AFDM were greater in the 50 and 100% storm water treatments than in the control treatment when snails were present, but this difference was statistically significant only in the 100% storm water treatment (Fig. III.E.3.b.8, Table III.E.3.b.6). This suggests that storm water did have an impact on snail behavior, perhaps resulting in reduced herbivory.

Algal Metabolism

Storm water concentration did not have a significant effect on either areal-specific respiration, GPP, or GPP:R (Fig. III.E.3.b.9). No differences in areal-specific or Chl *a*-specific metabolism (respiration and GPP) were detected between either the fish or snail treatments. A similar lack of trend was observed both for Chl *a*-specific metabolism and areal-specific metabolism.

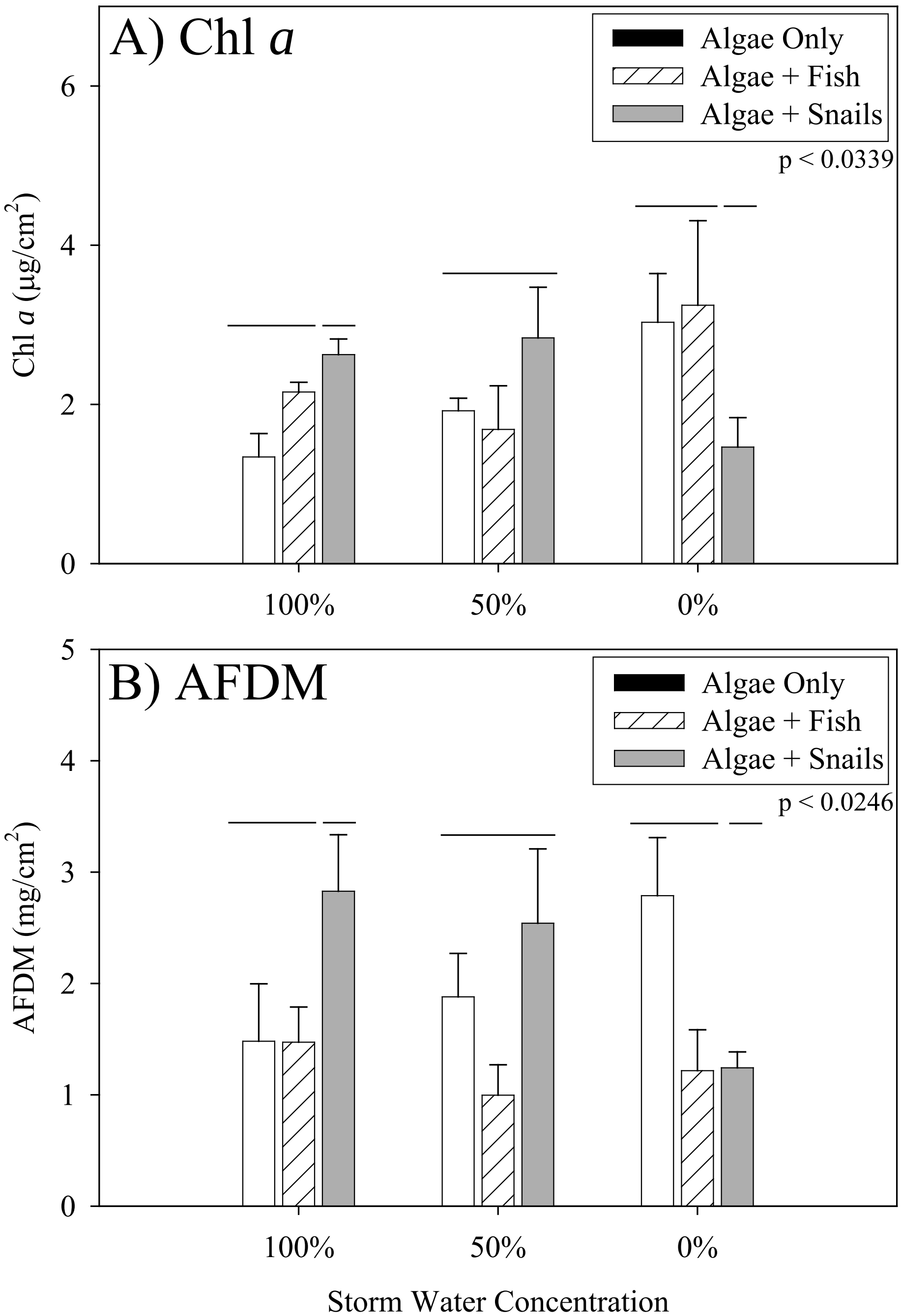
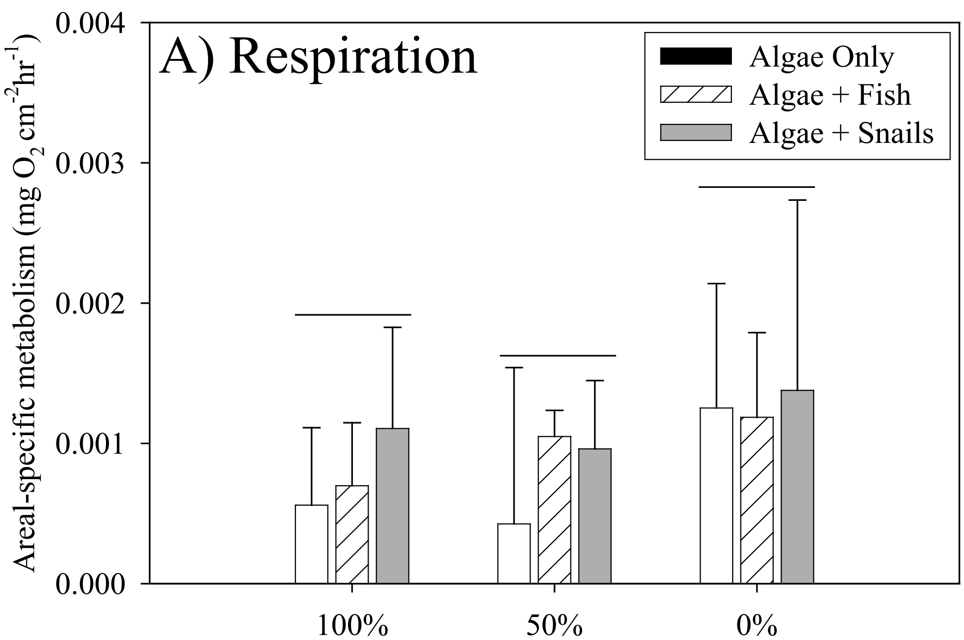


Fig. III.E.3.b.8. A) Chl *a* concentrations and B) AFDM collected on day 28 of the experiment. AFDM values connected by a bar are not significantly different from each other, and separate bars represent significant differences among AFDM values within a particular storm water treatment only. Error bars represent standard error.

Table III.E.3.b.6. Nested ANOVA analysis of the final Chl *a*, AFDM, AFDM:Chl *a*, pheophytin, and pheophytin: Chl *a* values in the mesocosm experiment. Bold values are significant (p < 0.05).

Source of Variation	df	SS	MS	F	P
Storm water Treatment					
Chl *a*	2	0.0527	0.0264	0.55	0.582
AFDM	2	0.1433	0.0716	0.6	0.555
AFDM: Chl *a*	2	0.0389	0.0195	0.4	0.676
Pheophytin	2	0.305	0.1525	1.51	0.240
Pheophytin:Chl *a*	2	0.243	0.1215	2.48	0.103
Algae + Fish Only Treatment (snails excluded)					
Chl *a*	3	0.1658	0.0553	1.16	0.343
AFDM	3	0.9915	0.3305	2.78	0.060
AFDM: Chl *a*	3	0.2282	0.0761	1.55	0.224
Pheophytin	3	1.5412	0.5137	5.08	**0.007**
Pheophytin:Chl *a*	3	1.4654	0.4885	9.97	< **0.001**
Algae + Snails Only Treatment (fish excluded)					
Chl *a*	3	0.4777	0.1592	3.34	**0.034**
AFDM	3	1.308	0.436	3.67	**0.025**
AFDM: Chl *a*	3	0.0288	0.0096	0.2	0.898
Pheophytin	3	0.6698	0.2233	2.21	0.110
Pheophytin:Chl *a*	3	0.0213	0.0071	0.15	0.932



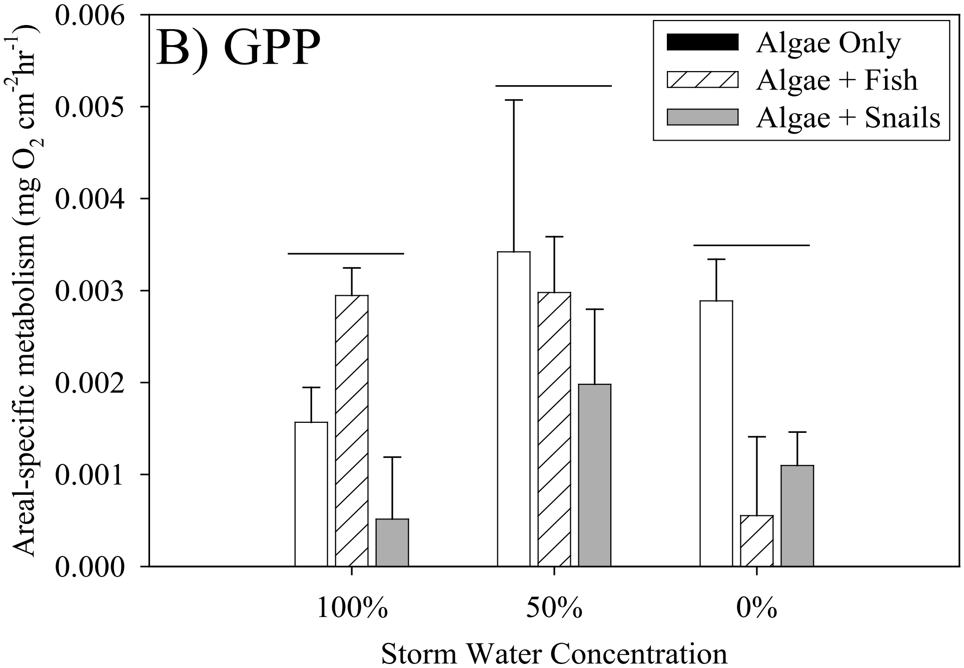


Fig. III.E.3.b.9. Areal-specific A) respiration and B) gross primary productivity (GPP) after 28-day exposure to different storm water treatments. Respiration and GPP values connected by a bar are not significantly different from each other, and separate bars represent significant differences among respiration and GPP values within a particular storm water treatment only. Error bars represent standard error.

Algal Community Composition

Day 0 experiment community composition

On day 0 of the experiment, the algal communities on the tiles removed from Cress Creek (which had been incubating in the stream for nine months) were almost entirely dominated by diatoms (Bacillariophyceae). The six most abundant genera (or taxonomic units) in terms of relative abundance of total cell numbers were *Navicula,* small naviculoid, *Achnanthes, Cocconeis, Rhoicosephenia,* and *Staurosirella* (Table III.E.3.b.7). The dominant diatom taxa based on relative biovolume were similar to the dominant taxa in terms of relative total cell numbers and included *Navicula,* small naviculoid, *Cocconeis, Rhoicosephenia* and *Staurosirella* (Table III.E.3.b.7). One difference between the algal communities in terms of relative total cell numbers and relative biovolume was that *Melosira* was the fifth most abundant taxon by biovolume, but was only the eight most abundant (0.57%) when assessed by cell number. Also, *Achnanthes* was the third most abundant taxa in terms of relative total cell numbers, but was only the ninth most abundant (0.3%) in terms of relative biovolume.

Table III.E.3.b.7. Median relative abundance of total cell numbers and total biovolume data for algal taxa from day 0 of the experiment (n = 6).

Relative number of total cells (%)		Relative total biovolume (%)	
Taxon	Experiment Day 0	Taxon	Experiment Day 0
Navicula	40.82	*Navicula*	76.68
Small naviculoid	34.18	Small naviculoid	6.98
Achnanthes	4.41	*Cocconeis*	5.03
Cocconeis	4.26	*Rhoicosphenia*	1.56
Rhoicosphenia	2.67	*Melosira*	1.38
Staurosirella	2.46	*Staurosirella*	1.18
All other algal taxa	11.20	All other algal taxa	7.20
Total	100	Total	100

Storm water effects

The algal community on day 28 of the experiment was composed of green algae (Chlorophyceae) and diatoms. The eight most abundant genera (or taxonomic units) in terms of relative number of total cells were *Mougeotia*, *Scenedesmus*, *Cocconeis*, small naviculoid, *Ankistrodesmus*, *Coelastrum*, *Synedra*, and *Pediastrum* (Table III.E.3.b.8, Fig. III.E.3.b.10A). Green algae made up ~82% of total cell numbers with diatoms contributing the remaining ~18%.

Green algae contributed ~84% and diatoms contributed the remaining ~16% of relative total algal biovolume at the end of the experiment. The eight most abundant taxa in terms of relative biovolume were *Mougeotia*, *Scenedesmus*, *Cocconeis*, *Pediastrum*, *Navicula*, *Synedra*, small naviculoid, and *Ankistrodesmus* (Table III.E.3.b.9, Fig. III.E.3.b.10B).

The storm water treatments appeared to have a small influence on community composition during this experiment. *Mougeotia*, *Scenedesmus*, and *Cocconeis* were the most abundant taxa, in terms of relative abundance of total cell numbers, in the 50% and 0% storm water treatments (Table III.E.3.b.8). In the 100% storm water treatment, *Mougeotia* and *Scenedesmus* were the two most abundant, but the third most abundant was *Ankistrodesmus* (Table III.E.3.b.8). The relative total cell numbers of *Synedra* and *Ankistrodesmus* were significantly higher in the 100% storm water treatment than in the 0% storm water treatment (Table III.E.3.b.10). The relative total cell numbers of *Mougeotia* were significantly higher in the 0% storm water compared to the 100% treatment (Table III.E.3.b.10). The relative abundances of total cell numbers of the remaining algal taxa were not significantly affected by the storm water treatments (Table III.E.3.b.10).

Mougeotia, *Scenedesmus*, and *Cocconeis* were the most abundant taxa, in terms of relative biovolume, in all storm water treatments (Table III.E.3.b.9). The relative biovolume of *Synedra, Stephanocyclus,* and *Staurosirella* were significantly greater in the 100% storm water treatment than the 0% treatment (Table III.E.3.b.10). *Mougeotia* and *Ankistrodesmus* relative biovolume was significantly higher in the 0% storm water compared to the 100% treatment (Table III.E.3.b.10). The relative biovolume of the remaining algal taxa was not significantly affected by the storm water treatments (Table III.E.3.b.10).

The NMDS ordination of relative abundance of total cell numbers revealed some separation among the treatments (Fig. III.E.3.b.11). The day 0 experiment communities were clearly separated, and significantly different ($p<0.001$) from the day 28 communities in the ordination ($p < 0.001$). Because of the overlap in ordination space among the three storm water treatments, it is likely that natural change in the algal communities over the 28-day period was responsible for part of the difference between day 0 and day 28 communities. Despite the overlap in the ordination, the overall algal community in the 0% storm water treatment was significantly ($p < 0.001$) different from the community in the 100% treatment. This difference was significant when the day 0 experiment values were both included in and excluded from the analysis.

The NMDS ordination of relative biovolume was quite similar to the relative number of total cells ordination (Fig. III.E.3.b.11). In terms of relative biovolume, the day 0 experiment communities were significantly ($p < 0.001$) different from all three storm water treatments. Also, the 100% storm water treatment was significantly ($p < 0.001$) different from the 0% storm water treatment.

Indicator values for some taxa exceeded 80 in some treatments (Table III.E.3.b.11), which indicates that algal taxa were somewhat restricted to specific treatments. For both relative number of total cells and relative biovolume, *Ankistrodesmus* was the best indicator of the 100% storm water treatment, *Scenedesmus* was the best indicator of the 50% storm water treatment, *Coelastrum* was the best indicator for the 0% storm water treatment, and *Navicula* was the best indicator for the day 0 experiment community (Table III.E.3.b.11). The indicator values for the taxa in the 100% storm water treatment and the taxa in the day 0 experiment community were relatively high, while those for the 50% and 0% storm water treatments were relatively low.

The algal communities in the different storm water treatments in this experiment differed significantly in the distribution of different physiognomies (Fig. III.E.3.b.12). Both relative abundance of total cell numbers and relative total biovolume of loose understory taxa were significantly greater in the 100% storm water treatment than in the 0% treatment ($p < 0.03$ for cell number and $p < 0.02$ for biovolume). Also in terms of both relative abundance of total cell numbers and relative biovolume, loose canopy taxa were significantly more abundant in the 0% storm water treatment than in the 50% and 100% treatments ($p < 0.04$ for cell numbers and $p < 0.05$ for biovolume). In terms of relative number of total cells, the 50% storm water treatment had significantly less ($p < 0.05$) firm understory taxa than the 100% and 0% storm water treatments

PCA of environmental variables did not reveal a clear distinction between the storm water treatments in principal components (PC) 1 and 2, although the 50% storm water treatments tended to be located in the negative end of PC 1 and the positive end of PC 2 (Fig. III.E.3.b.13). The positive end of PC 1 correlated most strongly with alkalinity and the negative end of PC 1 correlated most strongly with TP, NO_3-N, and specific conductivity (Fig. III.E.3.b.13). The positive end of PC 2 correlated most strongly with Cr and Cu concentrations (Fig. III.E.3.b.13).

Table III.E.3.b.8. Median relative abundance of total cell numbers of algal taxa from each storm water and the fish and snail treatment. Samples were collected on day 28 of the experiment and replicate treatments (n = 4) were combined to generate median values.

	Relative abundance of total cell numbers (%)									
Taxon	100% Storm Water			50% Storm Water			0% Storm Water			
	A	A+F	A+S	A	A+F	A+S	A	A+F	A+S	Overall
Mougeotia	18.23	35.25	19.19	46.65	54.34	33.80	41.60	60.84	53.03	38.49
Scenedesmus	34.61	27.09	39.22	31.46	22.65	37.12	16.27	7.88	17.94	18.76
Cocconeis	9.48	4.29	6.36	3.95	4.22	3.13	6.18	6.92	4.47	5.93
Small naviculoid	4.30	2.04	8.10	4.47	2.68	3.94	7.28	4.73	1.99	3.68
Ankistrodesmus	15.79	20.85	5.61	1.18	4.05	2.15	1.83	3.21	2.46	2.68
Coelastrum	3.16	1.94	1.00	1.56	0.49	0.68	6.42	4.55	4.82	1.56
Synedra	1.54	2.47	1.81	0.87	0.49	1.02	1.62	1.18	0.77	1.15
Pediastrum	0.91	0.67	1.82	1.01	0.16	1.65	1.27	1.27	1.10	1.10
All other algal taxa	11.97	5.38	16.88	8.85	10.91	16.51	17.53	9.42	13.40	26.66
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.0

Abbreviations: A = algae only treatment (fish & snails excluded), A+F = algae + fish only treatment (snails excluded), and A+S = algae + snails only treatment (fish excluded)

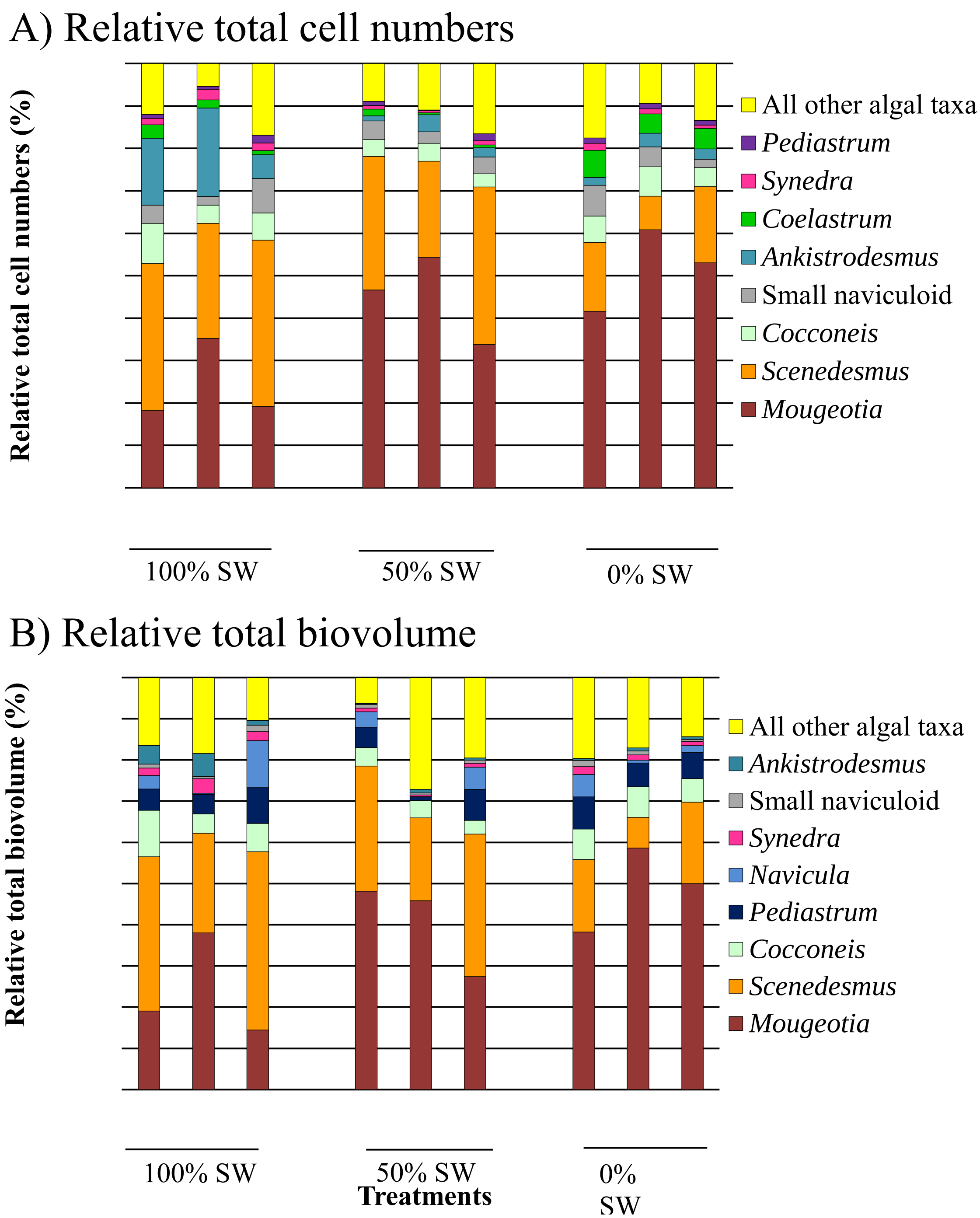


Fig. III.E.3.b.10. Median relative abundance of A) relative total cell numbers and B) relative biovolume of algal taxa exposed to different storm water and fish and snail treatments. Samples were collected on day 28 of the experiment and replicate treatments (n = 4) were combined to generate median values. Abbreviations: storm water (SW), algae only treatment (A), algae + fish only treatment (A+F), algae + snails only treatment (A+S).

Table III.E.3.b.9. Median relative total biovolume of algal taxa from each storm water and fish and snail treatment. Samples were collected on day 28 of the experiment and replicate treatments (n = 4) were combined to generate median values.

	Relative total biovolume (%)									
Taxon	100% Storm Water			50% Storm Water			0% Storm Water			
	A	A+F	A+S	A	A+F	A+S	A	A+F	A+S	Overall
Mougeotia	19.08	38.03	14.43	48.14	45.83	27.42	38.23	58.59	49.99	40.10
Scenedesmus	37.45	24.21	43.27	30.35	20.14	34.59	17.57	7.52	19.78	21.95
Cocconeis	11.24	4.63	6.90	4.49	4.20	3.35	7.46	7.35	5.71	6.52
Small naviculoid	0.98	0.52	1.53	0.84	0.46	0.78	1.47	0.95	0.50	0.68
Ankistrodesmus	4.54	5.63	1.19	0.29	0.85	0.50	0.49	0.70	0.69	0.67
Synedra	1.85	3.53	2.15	0.89	0.46	0.97	1.93	1.30	1.00	1.30
Pediastrum	5.16	4.77	8.68	5.00	0.91	7.53	7.76	5.80	6.32	6.21
Navicula	3.24	0.28	11.44	3.72	0.00	5.32	5.43	0.70	1.66	1.91
All other algal taxa	16.44	18.40	10.41	6.28	27.15	19.55	19.67	17.10	14.33	20.67
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.0

Abbreviations: A = algae only treatment (fish and snails excluded), A+F = algae + fish only treatment (snails excluded), and A+S = algae + snails only treatment (fish excluded)

Table III.E.3.b.10. Nested ANOVA analysis of the relative abundance of total cell numbers and relative total biovolume data for each algal taxon (and physiognomic group) on day 28 of the experiment. Fish, snail, and fish x snail treatments are nested within the storm water (SW) treatments. Bold values are significant ($p < 0.05$). Arrows represent a significant increase (↑) or decrease (↓) relative to the control.

		Relative total cell numbers				Relative total biovolume			
Source of Variation	**df**	**SS**	**MS**	**F**	**P**	**SS**	**MS**	**F**	**P**
***Mougeotia* (LC)**									
Storm Water	2	3558.92	1779.5	4.46	**0.02↓**	2830.36	1415.18	3.68	**0.04↓**
Fish (SW)	3	1076.9	358.98	0.9	0.45	762.4	762.4	1.98	0.17
Snail (SW)	3	876.77	292.26	0.73	0.54	151.9	151.9	0.39	0.54
***Scenedesmus* (LU)**									
Storm Water	2	1938.98	969.49	1.89	0.17	1799.73	899.87	1.73	0.2
Fish (SW)	3	245.78	81.92	0.16	0.92	663.36	663.36	1.27	0.27
Snail (SW)	3	161.86	53.95	0.11	0.96	325.2	325.2	0.61	0.44
***Cocconeis* (FU)**									
Storm Water	2	21.56	10.78	2.1	0.14	62.12	31.06	4.05	**0.03↑**‡
Fish (SW)	3	47.49	15.83	3.08	**0.04↓***	9.26	9.26	1.21	0.28
Snail (SW)	3	19.98	6.66	1.3	0.3	3.51	3.51	0.41	0.53
***Small naviculoid* (LU)**									
Storm Water	2	9.69	4.84	0.74	0.48	0.18	0.09	1.32	0.28
Fish (SW)	3	19.98	6.66	1.02	0.4	0.27	0.27	4.12	**0.05↓**
Snail (SW)	3	68.25	22.75	3.49	**0.03↑*↓**†	0.05	0.05	0.91	0.35
***Ankistrodesmus* (LU)**									
Storm Water	2	510.43	255.21	6.26	**0.01↑**	94.27	47.14	11.39	**0.001↓**
Fish (SW)	3	23.16	7.12	0.19	0.9	6.57	6.57	1.59	0.22
Snail (SW)	3	138.87	46.29	1.14	0.35	7.19	7.19	1.87	0.18
***Coelastrum* (LU)**									
Storm Water	2	61.77	30.89	1.59	0.22	1.26	0.63	2.75	0.08
Fish (SW)	3	51.27	17.09	0.88	0.46	0.74	0.74	3.21	0.08
Snail (SW)	3	31.24	10.41	0.54	0.66	0.11	0.11	0.45	0.51

Abbreviations: loose canopy (LC), loose understory (LU), and firm understory (FU)

* significant only in 100% SW treatment

† significant only in 0% SW treatment

‡ significant only in 100% and 0% SW treatments

Table continued below

Table III.E.3.b.10 Continued.

		Relative total cell numbers				Relative total biovolume			
Source of Variation	**df**	**SS**	**MS**	**F**	**P**	**SS**	**MS**	**F**	**P**
***Synedra* (FU)**									
Storm Water	2	7.36	3.68	4.04	**0.03↑**	16.33	8.17	6.82	**0.004↑**
Fish (SW)	3	1.34	0.45	0.49	0.69	0.28	0.28	0.23	0.63
Snail (SW)	3	1.44	0.48	0.53	0.67	0.45	0.45	0.37	0.55
***Pediastrum* (LU)**									
Storm Water	2	0.55	0.27	0.23	0.79	59.41	29.71	0.98	0.39
Fish (SW)	3	0.67	0.22	0.19	0.9	27.26	27.26	0.89	0.35
Snail (SW)	3	2.16	0.72	0.62	0.61	34.76	1.79	1.79	0.29
***Navicula* (LU)**									
Storm Water	2	39.82	19.9	1.66	0.21	0.09	0.04	0.3	0.75
Fish (SW)	3	34.08	11.36	0.95	0.43	3.63	3.63	25.02	**0.001↓**
Snail (SW)	3	97.77	32.59	2.71	0.06	1.27	1.27	6.12	**0.02↑**
***Stephanocyclus* (LU)**									
Storm Water	2	3.03	1.52	2.02	0.15	0.85	0.43	4.13	**0.03↑**
Fish (SW)	3	0.35	0.12	0.16	0.92	0.05	0.05	0.46	0.5
Snail (SW)	3	1.46	0.49	0.65	0.59	0.04	0.04	0.36	0.55
***Staurosirella* (LC)**									
Storm Water	2	4.26	2.13	1.81	0.18	1.86	0.93	3.85	**0.03↑**
Fish (SW)	3	4.72	1.58	1.34	0.28	0.35	0.35	1.46	0.24
Snail (SW)	3	3.6	1.2	1.02	0.4	0.23	0.23	0.9	0.35

Abbreviations: loose canopy (LC), loose understory (LU), and firm understory (FU)

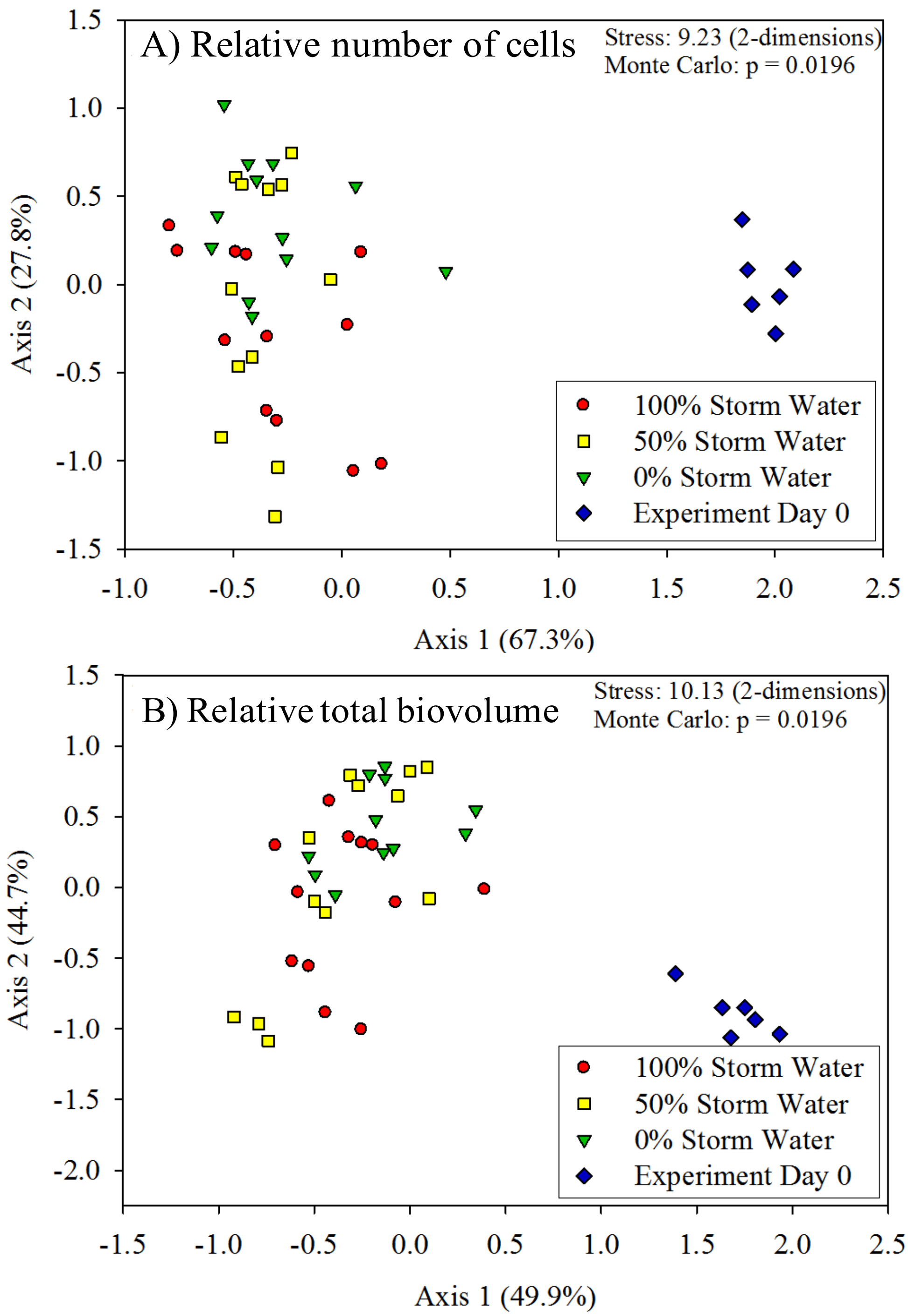


Fig. III.E.3.b.11. Nonmetric multidimensional scaling (NMDS) ordinations of the A) relative abundance of total cell numbers and B) relative total biovolume of the algal communities in each storm water treatment on day 28 of the experiment and the communities on day 1 of the experiment.

Table III.E.3.b.11. The taxon with highest indicator value (in parentheses) for each of the three storm water (SW) treatments, day 0 experiment communities, and three fish and snail treatments in terms of both relative abundance of total cell numbers and total biovolume. Indicator values can range from 0 for a taxon that has the same occurrence and abundance in all groups of samples to 100 for a taxon that is restricted to a single group.

	Realtive total cell numbers	Relative total biovolume
SW Treatments		
100% SW	*Ankistrodesmus* (71)	*Ankistrodesmus* (75)
50% SW	*Scenedesmus* (42)	*Scenedesmus* (39)
0% SW	*Coelastrum* (58)	*Coelastrum* (56)
Experiment day 0	*Navicula* (85)	*Navicula* (85)
Fish/Snail Treatments		
Algae Only	*Coelastrum* (39)	*Coelastrum* (37)
Algae + Fish Only	*Closterium* (57)	*Closterium* (54)
Algae + Snails Only	*Scenedesmus* (38)	*Scenedesmus* (37)

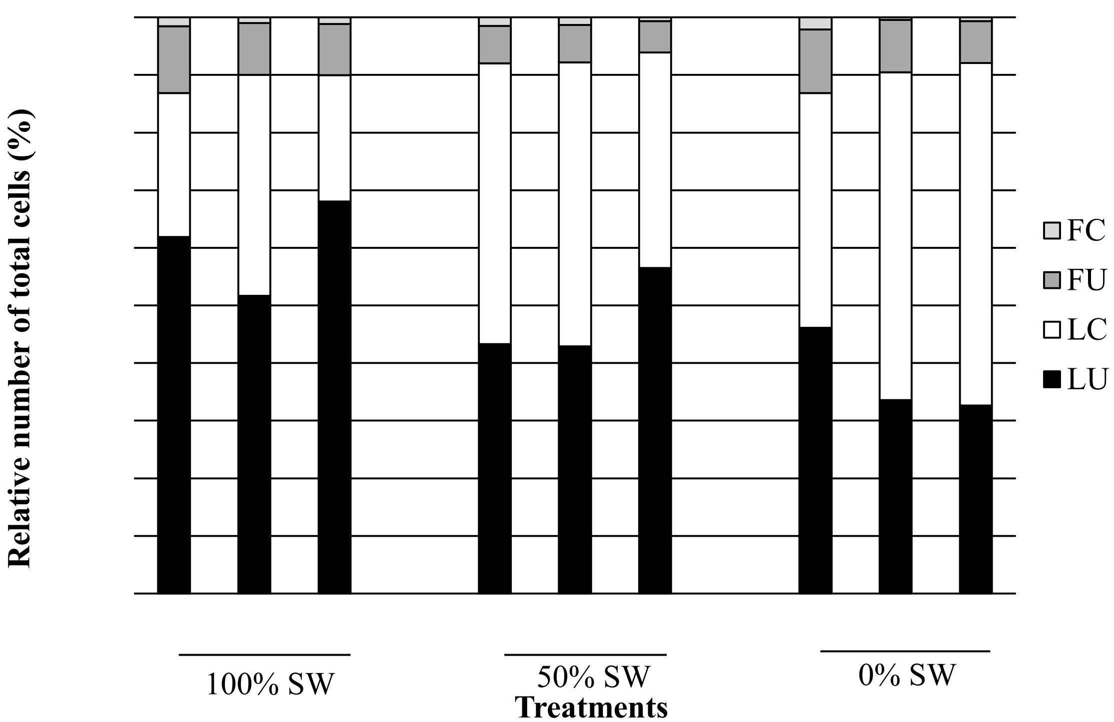


Fig. III.E.3.b.12. Relative abundance of total cell numbers of algal taxa growth forms after exposure to different storm water and fish and snail treatments. Samples were collected on day 28 of the experiment. Abbreviations: storm water (SW), firm canopy (FC), firm understory (FU), loose canopy (LC), and loose understory (LU).

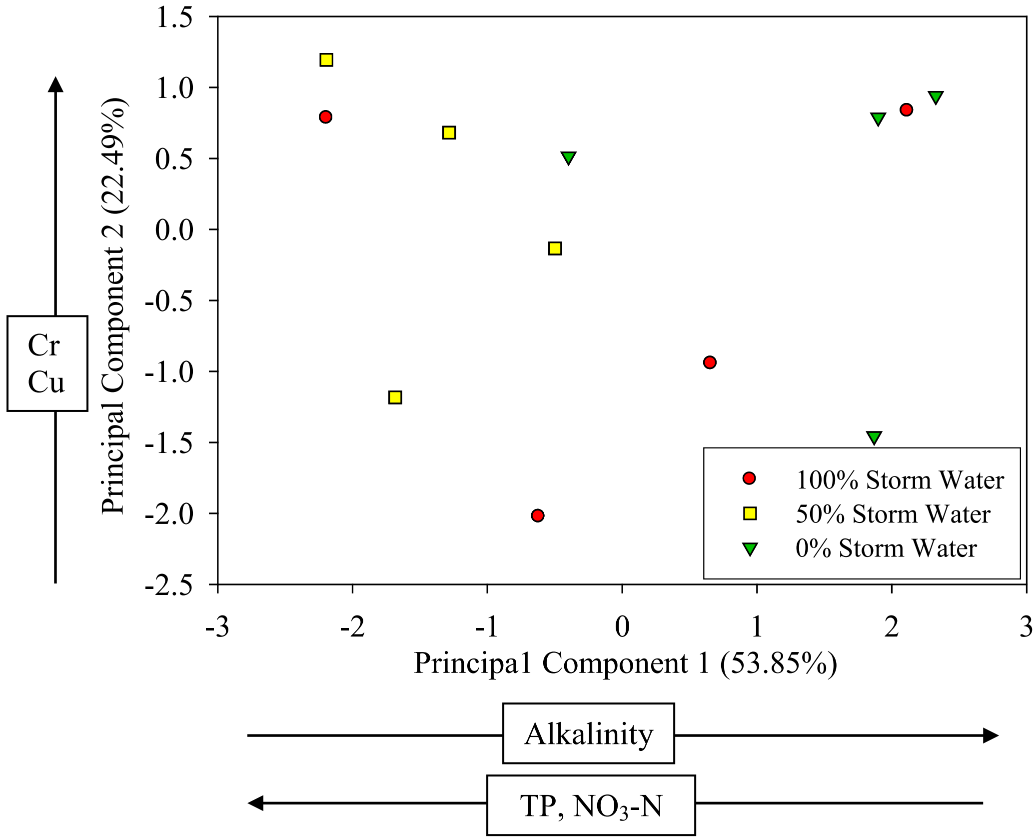


Fig. III.E.3.b.13. Principal components analysis (PCA) of six environmental variables collected on day 28 of the experiment. Alkalinity was positively loaded on principal component (PC) 1, total phosphorus (TP), nitrate (NO_3-N), and specific conductivity (SpCond) were negatively loaded on PC 1, and Cr and Cu were positively loaded on PC 2.

Effects of fish and snails

Mougeotia and *Scenedesmus* had the highest relative abundances of total cell numbers in the samples regardless of fish presence or absence (Table III.E.3.b.8). In terms of relative abundance of total cell numbers, *Cocconeis* declined in the presence of fish, but only in the 100% storm water treatment (Table III.E.3.b.10). In the algae + snails treatments, the small naviculoid taxon increased in relative abundance of total cell numbers in the 100% storm water treatment and declined in abundance in the 0% storm water treatment (Table III.E.3.b.10). The relative abundance of total cell numbers of the remaining taxa was not affected by the presence of fish or snails (Table III.E.3.b.10).

In terms of relative biovolume, only small naviculoid and *Navicula* were significantly influenced by the presence of fish or snails. Both small naviculoid and *Navicula* declined in abundance in the algae + fish treatments (Table III.E.3.b.10). Also, *Navicula* relative biovolume was greater in the algae + snail treatments than the treatments without snails (Table III.E.3.b.10). The NMDS ordinations of both relative number of total cells and relative biovolume with fish and snail treatments revealed a tendency for the day 0 experiment communities to group together apart from the day 28 algae only, algae + fish only, and algae + snails only treatments, which showed considerable overlap in the ordination (Fig. III.E.3.b.14). All three day 28 communities were significantly different from the day 0 communities. In terms of relative number of total cells, the algal communities in the algae only, algae + fish only, and algae + snail only treatments were not significantly different ($p > 0.05$). In terms of relative biovolume, all three day 28 treatments were significantly different from the day 0 communities ($p < 0.001$). The algal community in the algae + fish only treatment was significantly different from the community in the algae + snails only treatment ($p < 0.038$), but neither were different from the algae only treatment.

For both relative abundance of total cell numbers and relative biovolume, *Coelastrum* was the best indicator for the algae only treatment (Table III.E.3.b.11). *Closterium* had the highest indicator values for the algae + fish only treatments and *Scenedesmus* had the highest indicator values for the algae + snails only treatment (Table III.E.3.b.11). As with the storm water treatments, the indicator values of the taxa in the fish and snail treatments were relatively low.

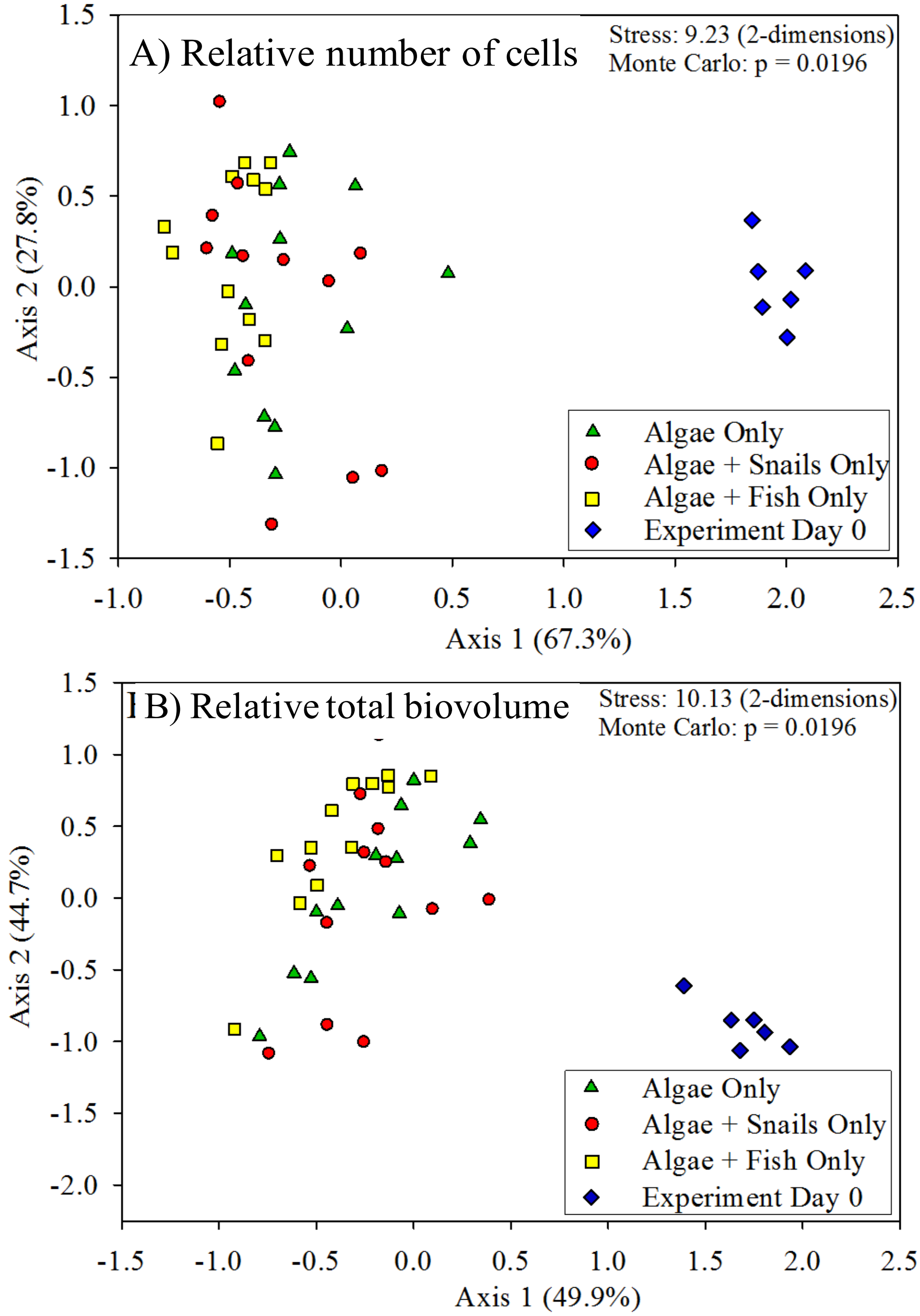


Fig. III.E.3.b.14. Nonmetric multidimensional scaling (NMDS) ordinations of the A) relative abundance of total cell numbers and B) relative total biovolume of the algal communities in each fish and snail treatment on day 28 of the experiment and the communities on day 0 of the experiment.

III.E.3.c. Fish

2008 Experiment

Storm water did not have a measurable effect on pumpkinseed growth or mortality during the 30-d experiment. No mortality occurred to pumpkinseeds exposed to storm water. Pumpkinseeds did lose mass during the experiment, which may have resulted from the restricted ration. However, neither actual ($F_{2,8}$ = 1.21, P = 0.346; Fig. III.E.3.c.1) nor instantaneous growth rates ($F_{2,8}$ = 1.08, P = 0.385; Fig. III.E.3.c.1) varied significantly across storm water concentrations.

We were unable to measure the response of snails to storm water in this experiment because pumpkinseeds consumed all snails present in mesocosms, often within a few minutes of being added. To reduce immediate consumption by pumpkinseeds, a portion of snails were placed in the snail only (fish excluded) exclosure. However, snails were not observed anywhere in the mesocosms 24 h after an addition.

2009 Experiment

Storm water did not have a measurable effect on pumpkinseeds growth or mortality during the 28-d experiment. No mortality occurred to pumpkinseeds exposed to storm water, and pumpkinseeds gained mass during the experiment. Storm water concentration did not significantly affect actual ($F_{2,9}$ = 0.16, P = 0.850; Fig. III.E.3.c.2) or instantaneous growth rates ($F_{2,9}$ = 0.01, P = 0.993; Fig. III.E.3.c.2).

Similar to the response of pumpkinseeds, storm water did not have a measurable effect on snail mortality or growth. Snail survival did not significantly differ across the storm water concentration gradient (χ^2_1 = 0.700, P = 0.850; Fig. III.E.3.c.3). Similarly, actual (mass: $F_{2,31}$ = 0.79, P = 0.464; total length: $F_{2,31}$ = 0.79, P = 0.199; Figs. III.E.3.c.4 and 4) and instantaneous growth rates (mass: $F_{2,1}$ = 0.73, P = 0.491; total length: $F_{2,31}$ = 0.73, P = 0.203; Figs. III.E.3.c.4 and III.E.3.c.4) of snails did not differ significantly across the storm water concentration gradient. Snail growth rates showed a pattern of lower growth in the 100% storm water treatment but differences were not significant (Fig. III.E.3.c).

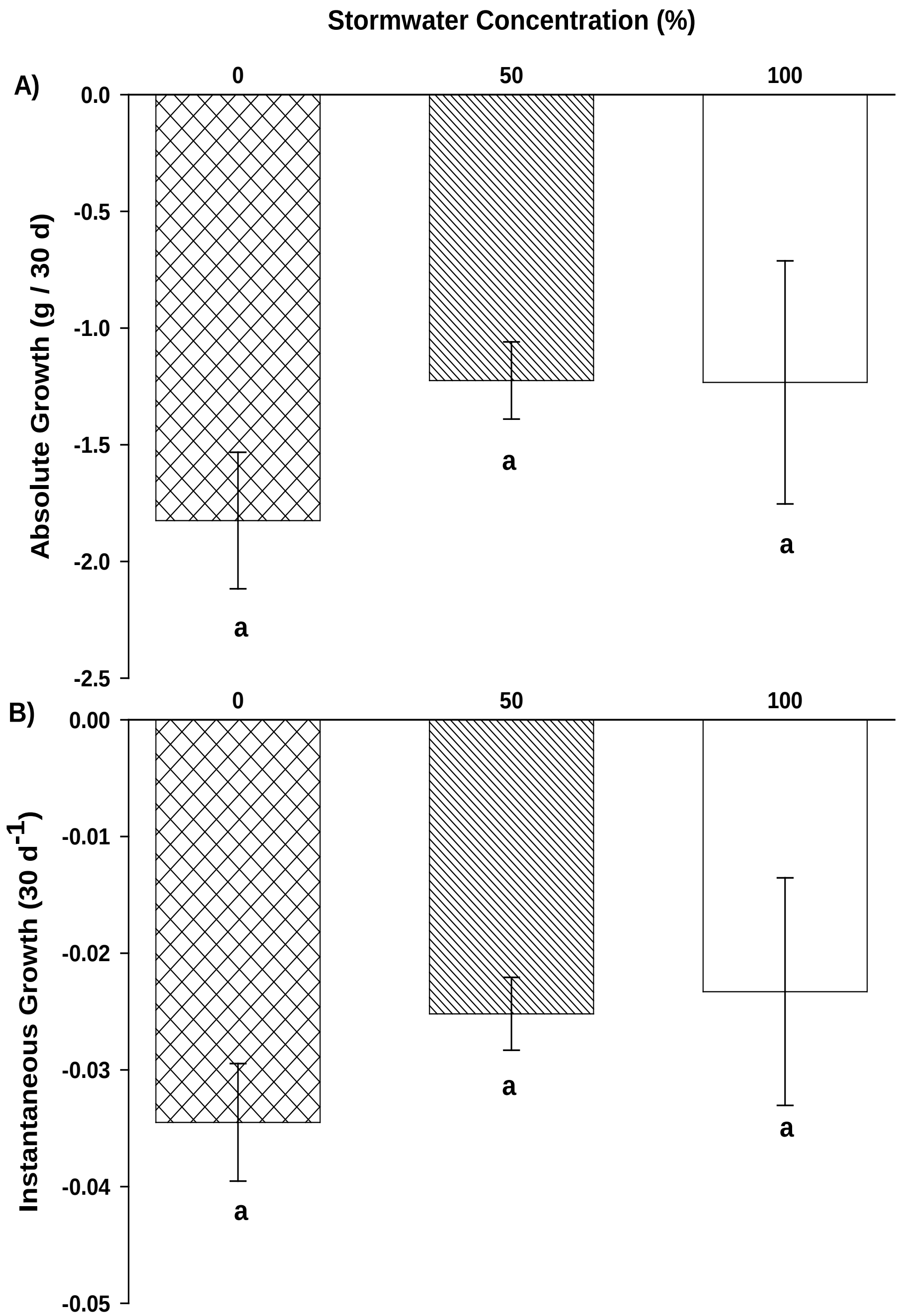


Fig. III.E.3.c.1. (A) Actual and (B) instantaneous growth rates of pumpkinseeds after 30-d exposure to storm water runoff collected during a rain event in June 2008 from a road- stream crossing on Little Black Creek (U.S. 31). Error bars represent ± 1 standard error. Means with similar lowercase letters were not significantly different (P > 0.05).

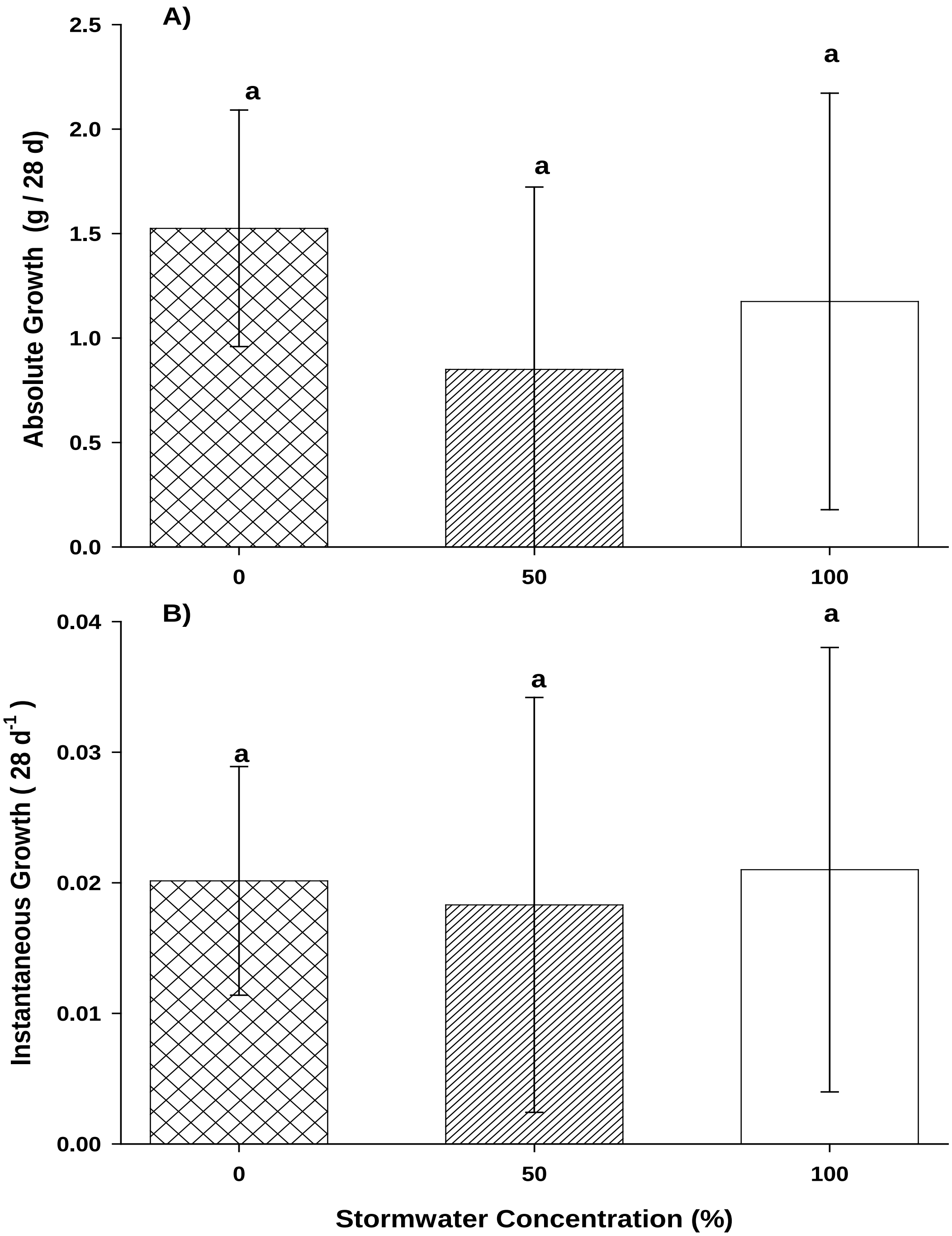


Fig. III.E.3.c.2. (A) Absolute and (B) instantaneous growth rates of pumpkinseeds after 30-d exposure to storm water runoff collected during a rain event in July 2009 from a road- stream crossing on Little Black Creek (U.S. 31). Error bars represent ± 1 standard error. Means with similar lowercase letters were not significantly different (P > 0.05).

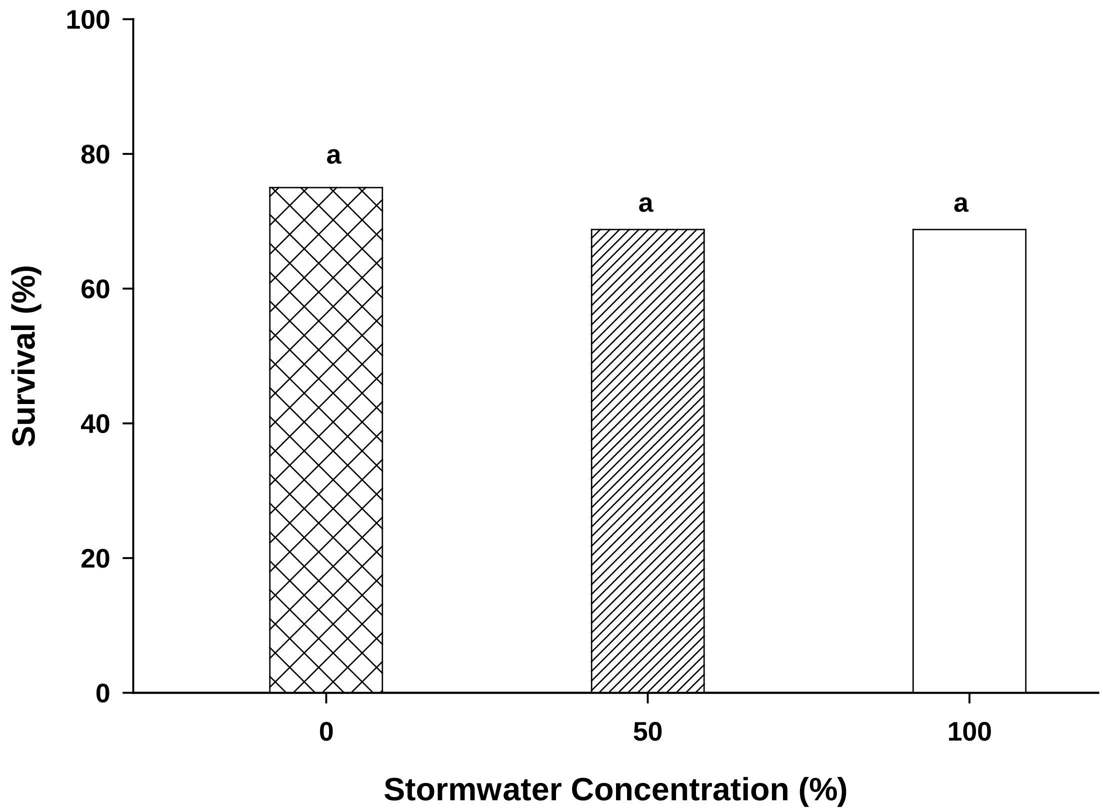


Fig. III.E.3.c.3. Snail survival rates (% survive) after 28-d exposure to storm water runoff collected during a rain event in July 2009 from a road-stream crossing on Little Black Creek (U.S. 31). Means with similar lowercase letters were not significantly different (P > 0.05).

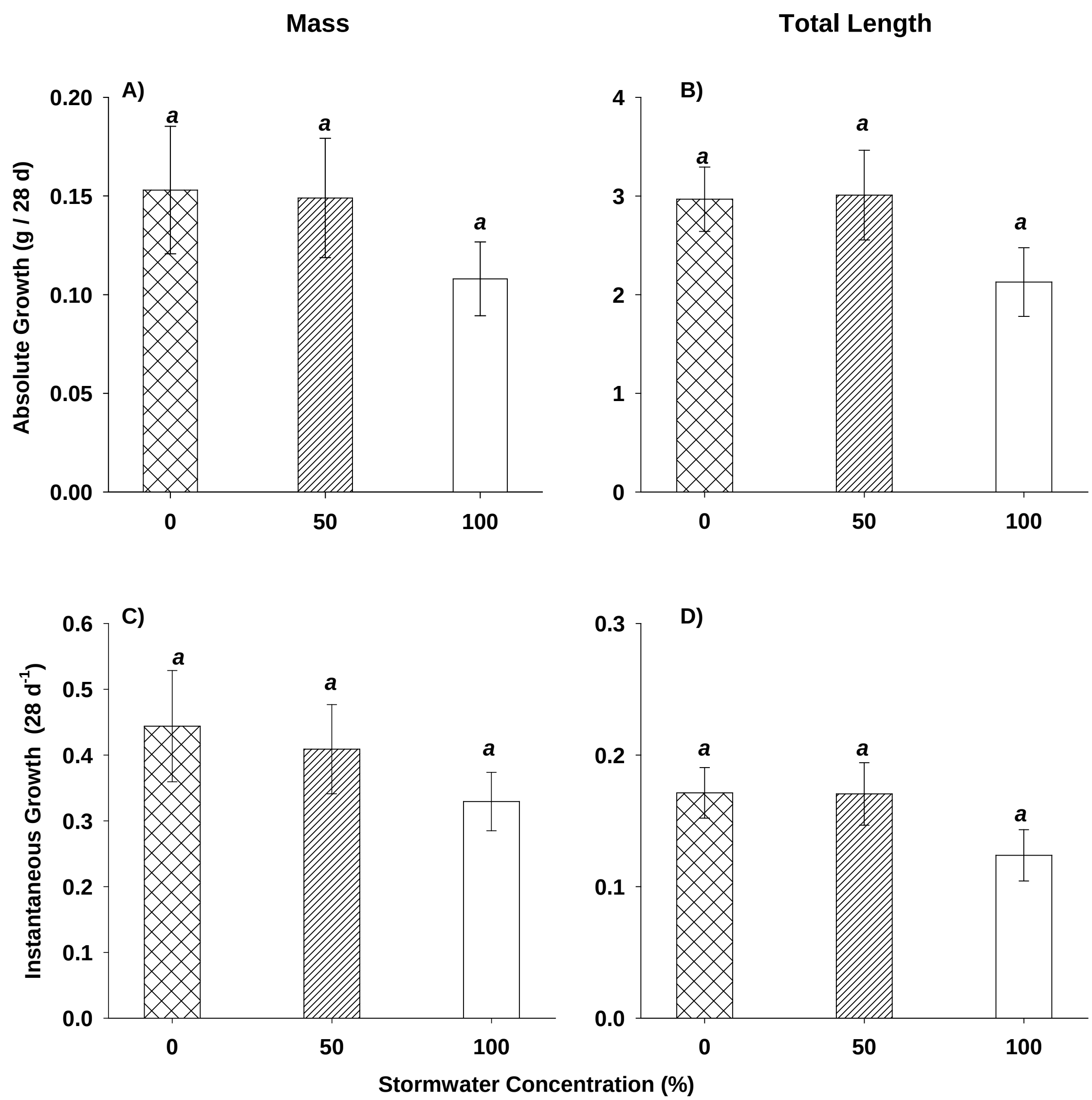


Fig. III.E.3.c.4. Absolute growth rates based on (A) mass and (B) total length, and instantaneous growth rates based on (C) mass and (D) total length of snails after 28-d exposure to storm water runoff collected during a rain event in July 2009 from a road-stream crossing on Little Black Creek (U.S. 31). Error bars represent ± 1 standard error. Means with similar lowercase letters were not significantly different (P > 0.05).

III.E.4. Environmental Analyses – Laboratory Fish Experiments

Summer 2008

Thirty-five of the initial 60 central mudminnows survived the 28-d exposure. Neither absolute nor instantaneous growth rates of central mudminnows significantly responded to storm water concentration, location where runoff was collected, source of fish, or the interaction of these factors (Table III.E.4.1; Fig. III.E.4.1). Survival in control was 55%. Survival of central mudminnow did not significantly differ with storm water concentration ($\chi^2_4 = 0.90$, $P = 0.342$), however, survival did significantly differ with runoff source ($\chi^2_4 = 7.30$, $P = 0.007$). Survival was significantly higher with central mudminnows exposed to U.S. 31 storm water than Seaway Drive storm water (Fig. III.E.4.2). The interaction of storm water concentration and runoff source was not significant ($\chi^2_4= 2.23$, $P = 0.135$).

Winter 2009

Results of the snowmelt effect on central mudminnows are based on two experiments using the same snowmelt collected from both study sites. Results of both experiments were pooled because outcomes were similar. Sixty-two of the initial 120 central mudminnows survived the 24-d exposure. Survival in controls was 53%. Neither absolute nor instantaneous growth rates of central mudminnows significantly differed with storm water concentration, location where runoff was collected, source of fish, or the interaction of these factors (Table III.E.4.2; Fig. III.E.4.3). Survival of central mudminnows did not significantly differ with fish source ($\chi^2_1 = 0.19$, $P = 0.658$). However, survival differed with storm water concentration ($\chi^2_1 = 23.15$, $P <0.001$), runoff source ($\chi^2_1 = 7.07$, $P = 0.008$), and the interaction of storm water concentration and runoff source ($\chi^2_1 = 18.82$, $P < 0.001$). Survival was significantly higher for central mudminnows exposed to lower concentrations of storm water and storm water from Seaway Drive (Fig. III.E.4.4). No survival of fish occurred above a 50% concentration of U.S. 31 storm water (Fig. III.E.4.4), and this mortality occurred within the first 4 days of exposure.

Spring 2009

Fifty-three central mudminnows survived the 24-d exposure. Survival of central mudminnows in control treatments was 45%. Neither absolute nor instantaneous growth significantly responded to storm water concentration, location where runoff was collected, or the interaction of these factors (Table III.E.4.3, Fig. III.E.4.5). However, central mudminnows from LBC had significantly lower absolute growth than control stream fish (Table III.E.4.3, Fig. III.E.4.5). Survival of central mudminnows did not differ significantly with storm water concentration ($\chi^2_1 = 0.09$, $P = 0.763$), runoff source ($\chi^2_1 = 0.46$, $P = 0.499$), or their interaction of storm water concentration and runoff source ($\chi^2_1 = 0.99$, $P = 0.320$; Fig. III.E.4.6).

Summer 2009

Only storm water from the U.S. 31 site was used for this experiment; therefore, the number of central mudminnows used during the experiment was reduced by half. Twenty-five of the original 30 fish survived the acclimation period. Survival of central mudminnows in control treatments was 80%. Neither absolute nor instantaneous growth rates of central mudminnows significantly responded to storm water concentration, fish source, or the interaction (Table III.E.4.4; Fig. III.E.4.7). Survival of central mudminnows did not significantly respond to storm water concentration ($\chi^2_1 = 0.96$, $P = 0.327$), fish source ($\chi^2_1 = 1.86$, $P = 0.173$), or the interaction of storm water concentration and fish source ($\chi^2_1 = 0.96$, $P = 0.327$; Fig. III.E.4.8).

Winter 2011

Snowmelt did not appear to affect the survival or growth of the central mudminnow in our laboratory experiment, although there was weak evidence that snowmelt may affect the condition of fish. All 40 of the central mudminnows survived the 18-d exposure to snowmelt, although they exhibited negative growth during the experiment (Fig. III.E.4.9). We found that initial size, sex, and the interaction between snowmelt concentration and sex had significant effects on the absolute growth of central mudminnows (Table III.E.4.6). There was a weak negative relationship between fish growth and initial total length, meaning that smaller fish lost less mass than larger fish. Males also tended to lose more mass than females for a given snowmelt concentration (Fig. III.E.4.9). More importantly, we found that the snowmelt effect in the experiment was dependent on sex. For male fish, snowmelt concentration had no significant effects on fish growth among pair-wise comparisons ($P > 0.223$; Fig. III.E.4.9). For female fish, we detected a significant difference in growth between 0% and 5% snowmelt ($P = 0.004$) and between 0% and 100% snowmelt ($P = 0.010$); however, we did not detect any significant differences among 0% vs. 50%, 5% vs. 50%, or 50% vs. 100% ($P > 0.116$; Fig. III.E.4.9). The incongruent response of female fish growth to the 5% and 50% snowmelt (i.e., if snowmelt negatively affect fish growth, then we expected negative growth to be lowest at 0% and 5% snowmelt and highest at 50% and 100% snowmelt, which is not what we observed for females; Fig. III.E.4.15) and the lack of a detectable effect on males suggest that there was no clear effect of snowmelt on the central muddminnow growth.

We found evidence of a weak effect of snowmelt on the condition of central mudminnows. The liver mass of fish significantly differed with snowmelt concentration (Table III.E.4.7). Liver mass tended to be higher for fish in the 0% and 5% snowmelt concentrations compared to the 50% and 100% (Fig. III.E.4.10). However, liver mass of fish in the 5% snowmelt was significantly greater than the 50% ($P = 0.001$) and 100% snowmelt ($P = 0.001$) as well as the 0% snowmelt ($P = 0.031$), and liver mass among the other snowmelt concentrations were not significantly different ($P > 0.130$; Fig. III.E.4.10). The effect of snowmelt on fish condition, as measured by liver mass, would have been more straightforward to interpret had liver mass in 0% snowmelt been more similar to what we measured in 5% snowmelt. We also found weak evidence that snowmelt negatively affected the condition of central mudminnows based on RNA:DNA ratios. We detected a significant interaction between snowmelt concentration and fish sex (Table III.E.4.8). For females, we saw a somewhat similar pattern to what we observed for liver mass in that the RNA:DNA ratio was significantly higher for fish in the 5% snowmelt than fish in the 50% ($P = 0.011$) and 100% snowmelt ($P = 0.019$) as well as the 0% snowmelt ($P = 0.001$), and the RNA:DNA ratio did not significantly differ among the other snowmelt concentrations ($P > 0.362$; Fig. III.E.4.11). For males, we found a more straightforward pattern where the RNA:DNA ratio of fish in the 0% snowmelt was significantly higher than fish in the 50% ($P = 0.014$) and 100% snowmelt ($P = 0.032$), whereas comparison of the RNA:DNA ratio of fish in the other snowmelt concentrations were not significantly different ($P = 0.060$ for 0% vs. 5%, $P > 0.495$ for other comparisons; Fig. III.E.4.11).

Temporal variation

Overall survival response of central mudminnows exposed to storm water from different storm events was mixed, likely due to constituents present in storm water. Central mudminnows responded differently to storm water for each storm event and among storm water concentrations (Figs. III.E.4.2, 4, 6, and 8). In all experiments except for summer 2008, a general trend existed where central mudminnow survival decreased with increasing storm water concentrations (Fig. III.E.4.12).

Variation in central mudminnow response to storm water among road stream crossings also varied among experiments (Fig. III.E.4.13). Central mudminnows clearly responded differently to storm water from different events across a temporal scale (Fig. III.E.4.13). A mixed response occurred where runoff from one site was not exclusively more toxic than the other (see Water Quality, below). These results only strengthen the fact that storm water is ever changing, thus impacting how biota respond.

Water quality

Concentrations of heavy metals varied greatly among storm events and road-stream crossings during single rain events (Tables III.E.4.5.9). Levels of heavy metals showed no consistent pattern where a single runoff source had higher or lower concentrations of metals. During many rain events, metal concentrations exceeded chronic water quality standards for aquatic life (MDEQ 2011; Tables III.E.4.5.9). Cadmium levels remained below chronic standard concentrations in all storm water collections. Chromium concentrations exceeded the chronic standard only at the U.S. 31 site for the 2009 snowmelt experiment (Tables III.E.4.5.9). Copper concentrations exceeded the chronic level during four of the five sampling events (Tables III.E.4.5.9). Nickel concentrations remained below chronic standard concentrations in all collected storm water (Tables III.E.4.5.9). Lead concentrations exceeded the chronic standards at the U.S. 31 site during one rain event and the 2011 snowmelt experiment, and at the Seaway Drive site during a one rain event (Table III.E.4.5.9). Zinc exceeded the chronic standard concentrations at both road-stream crossings in the 2009 and 2011 snowmelt and Seaway Drive storm water in the spring 2009 experiment (Table III.E.4.5.9). Storm water does appear to increase concentrations of metals in LBC during storm events since metal concentrations in runoff always exceeded average baseflow concentrations.

Concentrations of Cl remained low in most base flow and storm water samples, except in the snowmelt water (Fig. III.E.4.14, Table II.E.4.9). The high concentrations of Cl in the snowmelt are thought to be a result of additions of road salt to the highways during the winter months. Chloride concentrations in the snowmelt exceeded both the chronic and acute water quality standards in 2009, but exceeded only chronic standards in 2011 (U.S. EPA 1988; Fig. III.E.4.11, Table III.E.4.9). Thus, we were not surprised to find that snowmelt did not strongly affect the growth, condition, or survival of central muddminows during the 2011 snowmelt experiment.

Table III.E.4.1. Analysis of variance results for the summer 2008 experiment for the effects runoff source, fish source, storm water concentration (% sw) and all treatment interactions for both absolute and instantaneous growth rates (based on mass).

Source of variation	df	SS	F	P
Absolute growth				
runoff	1	0.376	0.56	0.463
fish	1	0.507	0.76	0.396
% sw	4	1.469	0.55	0.703
fish * % sw	4	0.158	0.06	0.993
runoff * % sw	3	0.235	0.12	0.949
runoff * fish	1	0.006	0.01	0.925
runoff * fish*% sw	2	0.196	0.15	0.865
Error	18	12.073		
Instantaneous growth				
runoff	1	0.017	0.82	0.378
fish	1	0.006	0.29	0.599
% sw	4	0.035	0.41	0.797
fish * % sw	4	0.002	0.03	0.998
runoff * % sw	3	0.030	0.47	0.709
runoff * fish	1	0.000	0.01	0.933
runoff * fish*% sw	2	0.008	0.18	0.834
Error	18	0.383		

Table III.E.4.2. Analysis of variance results for the winter 2009 snowmelt experiment for the effects runoff source, fish source, storm water concentration (% sw) and all treatment interactions for both absolute and instantaneous growth (based on mass).

Source of variation	df	ss	F	P
Absolute growth				
runoff	1	0.003	0.01	0.917
fish	1	0.618	2.31	0.136
% sw	4	1.605	1.50	0.218
fish * % sw	4	0.489	0.46	0.767
runoff * % sw	2	0.181	0.34	0.715
runoff * fish	1	0.010	0.04	0.849
runoff * fish* % sw	2	0.647	1.21	0.308
Error	15	12.046		
Instantaneous growth				
runoff	1	0.033	0.98	0.328
fish	1	0.001	0.00	0.953
% sw	4	0.172	1.26	0.301
fish * %sw	4	0.110	0.80	0.530
runoff * % sw	2	0.128	1.88	0.165
runoff * fish	1	0.005	0.16	0.694
runoff * fish* % sw	2	0.001	0.01	0.994
Error	15	1.538		

Table III.E.4.3. Analysis of variance results for the spring 2009 experiment for the effects runoff source, fish source, storm water concentration (% sw) and all treatment interactions for both absolute and instantaneous growth (based on mass). Bold values denote significant differences.

Source of variation	df	ss	F	P
Absolute growth				
runoff	1	0.006	0.05	0.835
fish	1	0.805	6.42	**0.022**
% sw	4	0.968	1.93	0.154
fish * % sw	4	0.454	0.91	0.484
runoff * % sw	4	0.321	0.64	0.641
runoff * fish	1	0.087	0.69	0.417
runoff * fish*% sw	3	0.211	0.56	0.649
Error	16	2.007		
Instantaneous growth				
runoff	1	0.000	0.00	0.945
fish	1	0.139	4.02	0.062
% sw	4	0.170	1.23	0.336
fish * % sw	4	0.073	0.53	0.717
runoff * % sw	4	0.098	0.71	0.596
runoff * fish	1	0.035	1.01	0.329
runoff * fish*% sw	3	0.052	0.51	0.682
Error	16	0.551		

Table III.E.4.4. Analysis of variance results for the summer 2009 experiment for the effects runoff source, fish source, storm water concentration (% sw) and all treatment interactions for both absolute and instantaneous growth rates.

Source of variation	df	Ss	F	P
Absolute growth				
fish	1	0.391	3.68	0.128
% sw	4	0.404	0.95	0.519
fish * % sw	2	0.186	0.88	0.484
Error	4	0.425		
Instantaneous growth				
fish	1	0.000	0.35	0.588
% sw	4	0.001	0.93	0.526
fish * % sw	2	0.001	0.86	0.490
Error	4	0.005		

Table III.E.4.5. Heavy metals concentrations for two road-stream crossings (U.S. 31 and Seaway Drive) on Little Black Creek, Muskegon, Michigan for four storm water collection periods between June 2008 and July 2009 compared to average base flow concentrations and MDEQ chronic value standards for aquatic life. Bold values denote concentrations above MDEQ chronic value standards where negative impacts to aquatic organisms should occur.

Experiment	Runoff source	Cd µg/L	Cr µg/L	Cu µg/L	Ni µg/L	Pb µg/L	Zn µg/L
Summer 08	U.S. 31	<1.0	16.42	**22.84**	9.21	**28.41**	153.52
	Seaway	<1.0	8.11	11.27	<5.0	9.50	65.25
Snowmelt	U.S. 31	<1.0	**162.20**	**66.91**	50.46	9.80	**554.00**
	Seaway	<1.0	91.05	**61.79**	30.12	5.76	**561.00**
Spring	U.S. 31	<1.0	7.63	8.38	<5.0	2.41	<50
	Seaway	<1.0	38.78	**28.20**	15.96	**25.56**	**223.03**
Summer 09	U.S. 31	<1.0	11.18	8.16	<5.0	4.89	<50
Base flow avg.	U.S. 31	<1.0	1.07	7.58	2.5	1.72	25.00
	Seaway	<1.0	2.45	8.10	2.50	1.73	25.00
Chronic values[a]		3.73	130.75	16.19	93.48	21.71	212.55

[a] Michigan Department of Environmental Quality Final Chronic Value (FCV) for aquatic life (MDEQ 2011)

Table III.E.4.6. Analysis of variance results for the 2011 experiment for the effects snowmelt concentration (0, 5, 50, 100%), initial total length of fish, and sex on the absolute growth (based on mass) of central muddminows.

Source of variation	df	SS	F	P
Initial size	1	0.1639	7.59	0.010
Sex	1	0.2793	12.94	0.001
Snowmelt	3	0.1094	1.69	0.190
Snowmelt×Sex	3	0.2748	4.24	0.013
Error	31	12.073		

Table III.E.4.7. Analysis of variance results for the 2011 experiment for the effects snowmelt concentration (0, 5, 50, 100%), initial total length of fish, and sex on the liver mass (an index of fish condition) of central muddminows.

Source of variation	df	SS	F	P
Initial size	1	0.00010	0.43	0.517
Sex	1	0.00028	1.13	0.296
Snowmelt	3	0.00470	6.42	0.002
Snowmelt×Sex	3	0.00054	0.74	0.538
Error	31	0.00757		

Table III.E.4.8. Analysis of variance results for the 2011 experiment for the effects snowmelt concentration (0, 5, 50, 100%) and sex on the RNA:DNA (an index of fish condition) of central muddminows.

Source of variation	df	SS	F	P
Sex	1	0.0184	2.20	0.148
Snowmelt	3	0.0713	2.83	0.054
Snowmelt×Sex	3	0.1519	6.03	0.002
Error	32	0.2686		

Table III.E.4.9. Water quality variables for four concentrations of snowmelt used to examine effects of fish survival and growth during the 2011 snowmelt experiment. Control water (0% snowmelt) was tap water. Values reported as '<' were below detection limits.

	Snowmelt Concentration (%)			
Variable (mg/L)	0	5	50	100
Cl	22	224	424	785
SO_4	33	37	27	37
NO_3	0.65	0.73	0.64	0.71
NH_3	0.03	0.04	0.13	0.21
SRP	< 0.005	0.005	< 0.005	< 0.005
TP	< 0.01	0.03	0.15	0.30
Cd	< 0.001	< 0.001	< 0.001	< 0.001
Cr	< 0.01	< 0.01	0.01	0.03
Cu	0.03	0.08	0.04	0.06
Ni	< 0.02	< 0.02	< 0.02	< 0.02
Pb	< 0.003	< 0.003	0.010	0.020
Zn	< 0.15	< 0.15	< 0.15	< 0.15

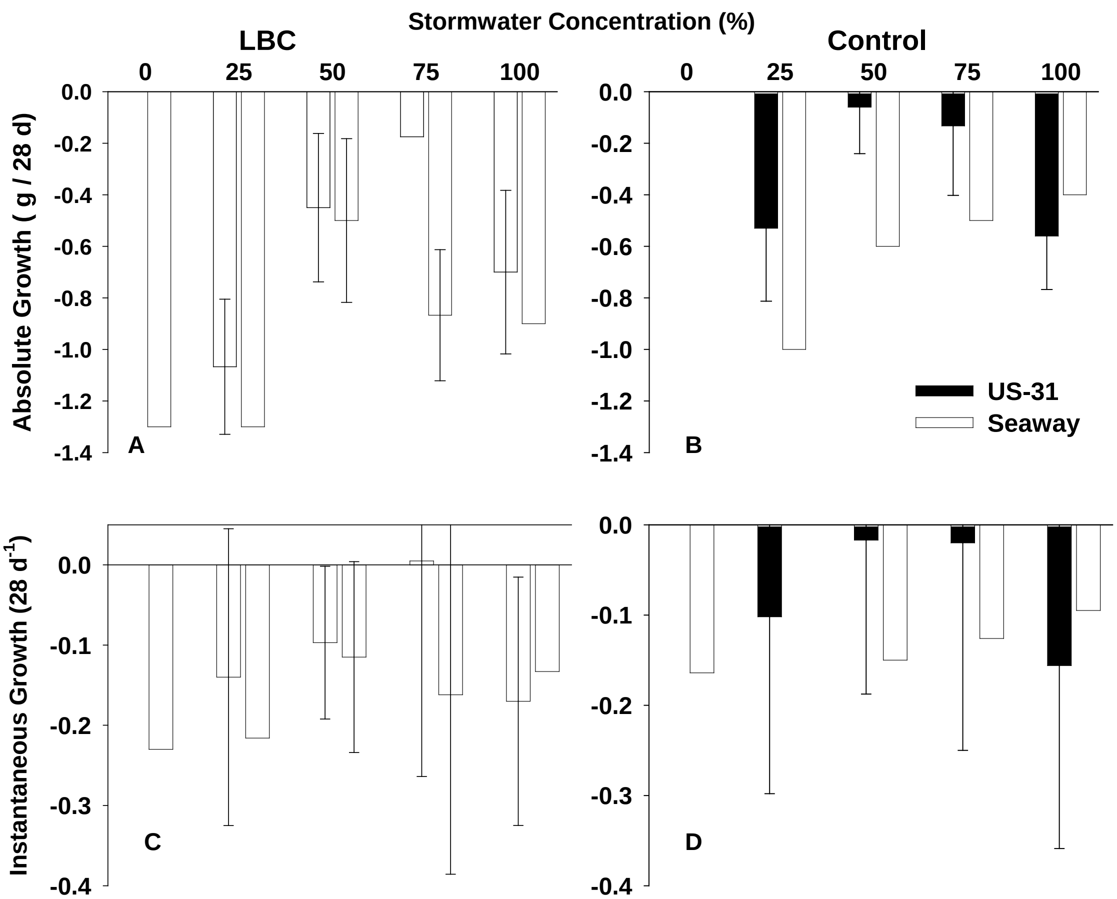


Fig. III.E.4.1. Absolute growth rates of central mudminnows from (A) Little Black Creek and the (B) control stream and instantaneous growth rates of central mudminnows from (C) LBC and (D) the control stream exposed to a concentration gradient of storm water runoff collected from two road-stream crossings (U.S. 31 and Seaway Drive) on 6 June 2008. Error bars represent ± 1 standard error.

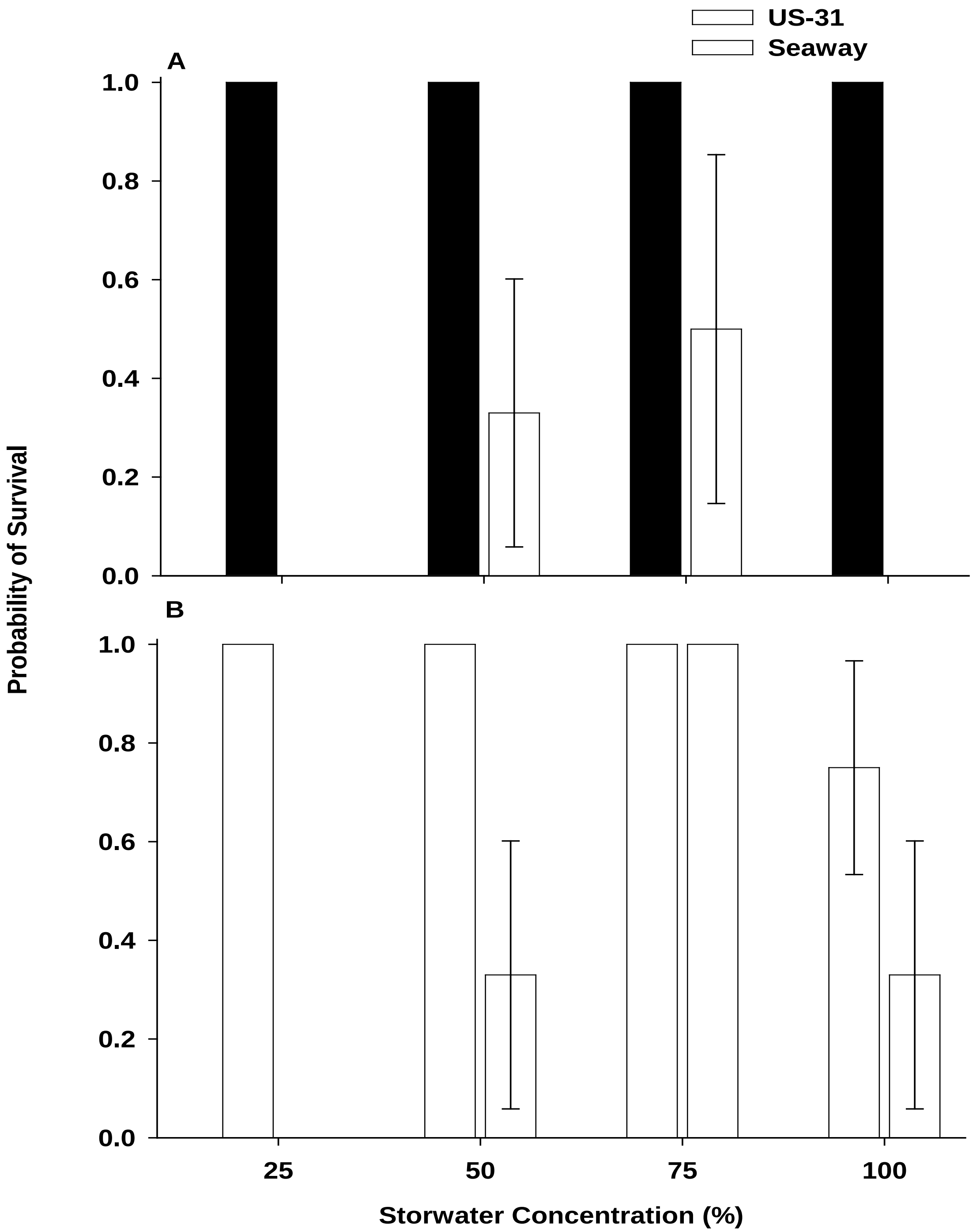


Fig. III.E.4.2. Survival (proportion alive after exposure) of central mudminnows from the (a) control stream and (b) Little Black Creek (LBC) after 28-day exposure to storm water runoff collected during a rain event on 6 June 2008 from two road stream crossing on LBC (U.S. 31 and Seaway Drive). Error bars represent ± 1 standard error.

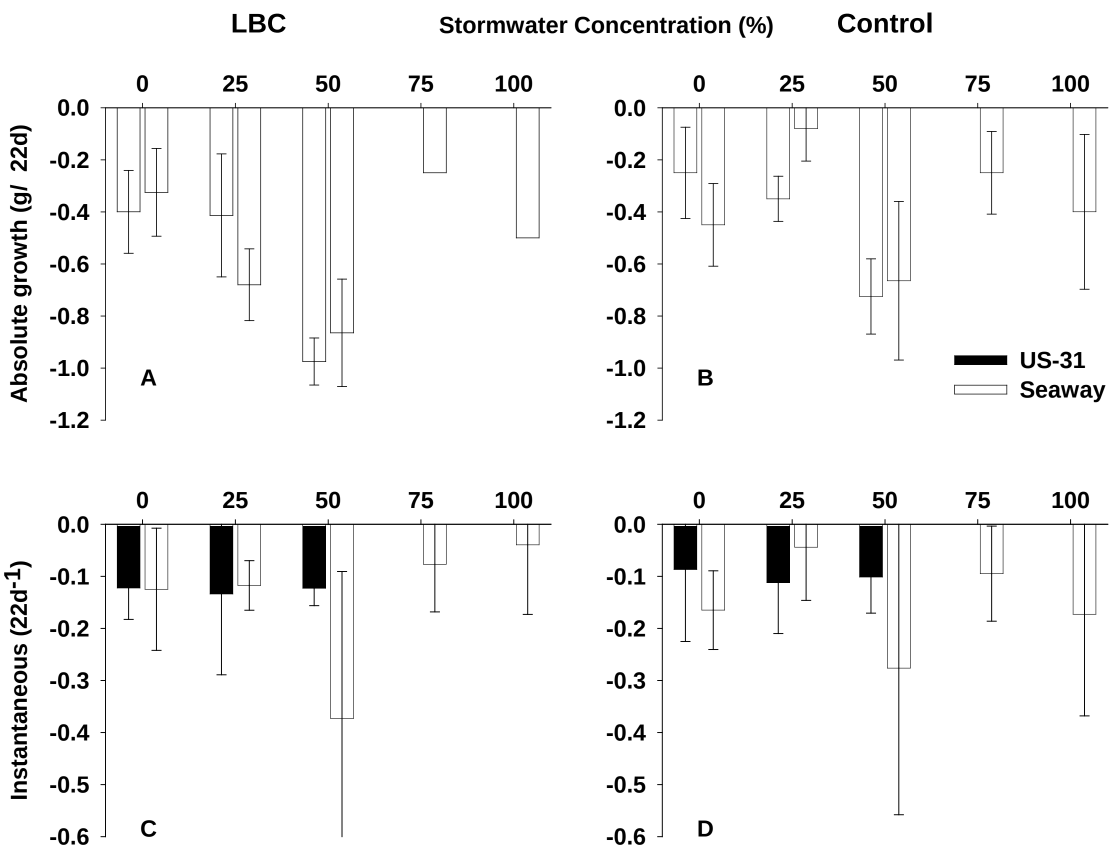


Fig. III.E.4.3. Absolute growth of central mudminnows from (A) Little Black Creek and the (B) control stream and instantaneous growth rates of central mudminnows from (C) LBC and (D) the control stream exposed to a concentration gradient of snowmelt collected from two road-stream crossings (U.S. 31 and Seaway Drive) in 3 February 2009. Error bars represent ± 1 standard error.

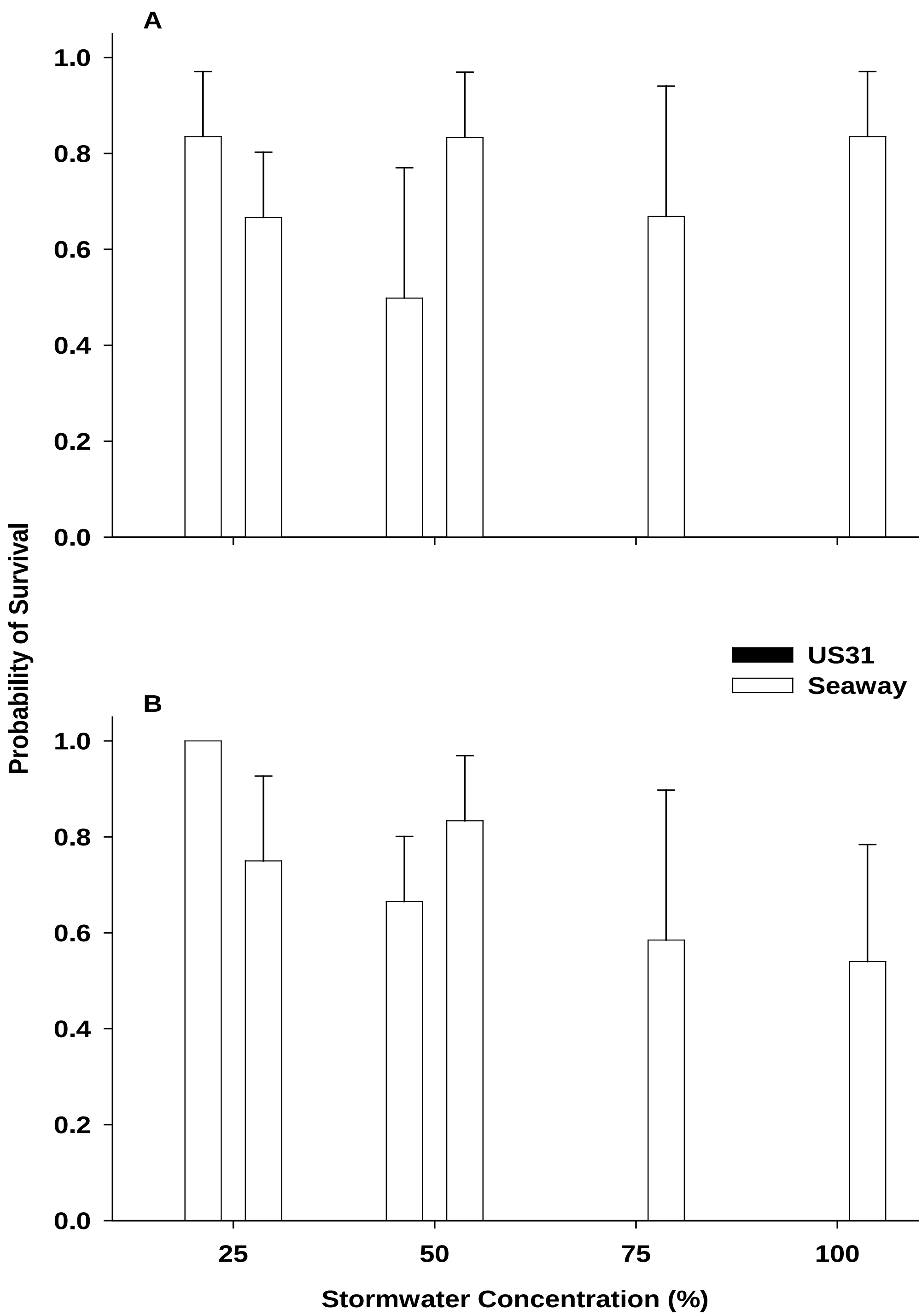


Fig. III.E.4.4. Survival (proportion alive after exposure) of central mudminnows from the (A) control stream and (B) Little Black Creek (LBC) after a 22 day exposure to roadside snowmelt collected on 3 February 2009 from two road stream crossing on LBC (U.S. 31 and Seaway Drive). Error bars represent ± 1 standard error.

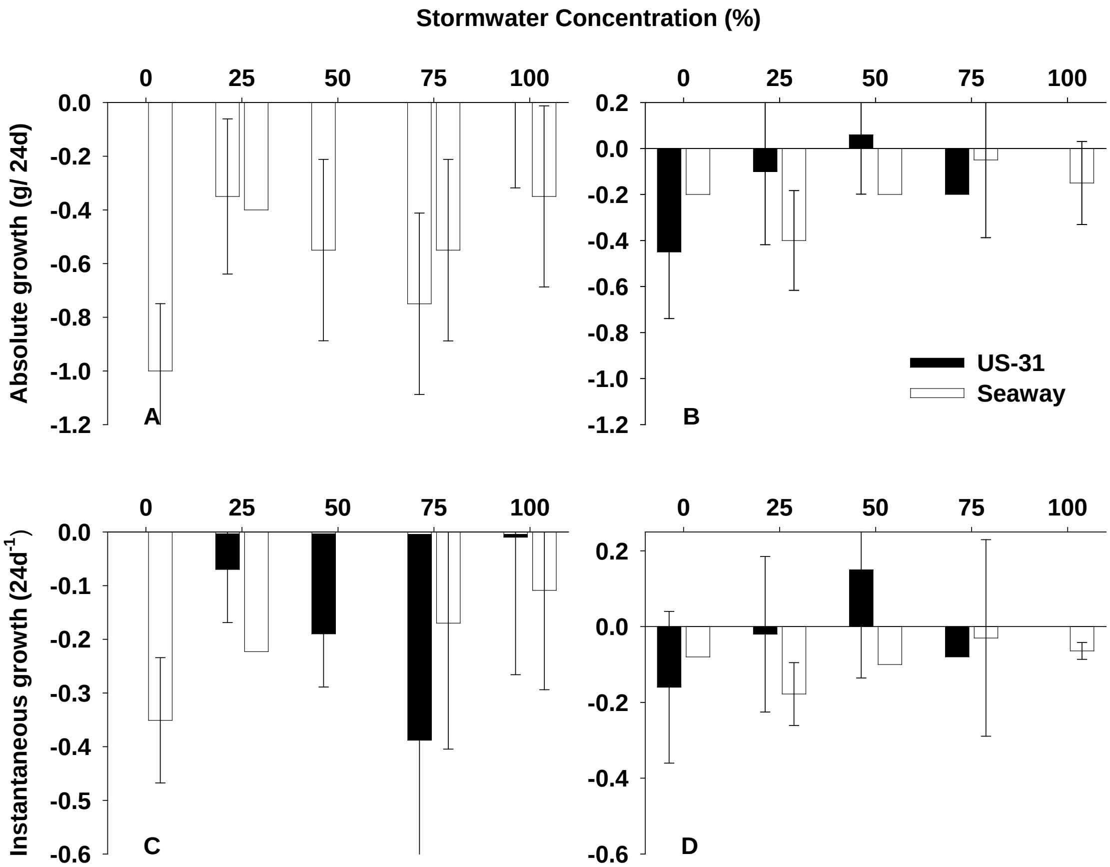


Fig. III.E.4.5. Absolute growth of central mudminnows from (A) Little Black Creek and the (B) control stream and instantaneous growth rates of central mudminnows from (C) LBC and (D) the control stream exposed to a concentration gradient of storm water runoff collected from two road-stream crossings (U.S. 31 and Seaway Drive) on 9 May 2009. Error bars represent ± 1 standard error.

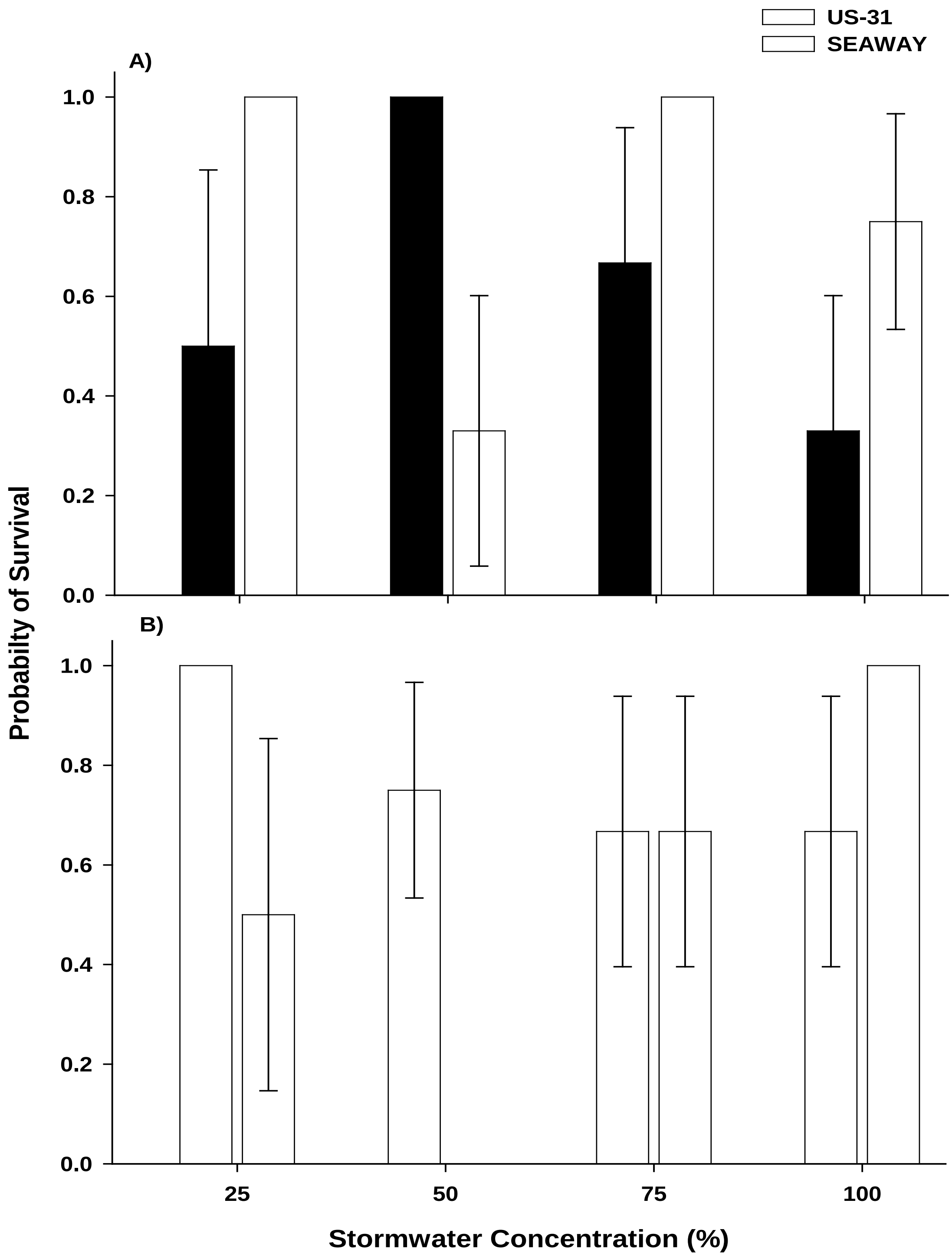


Fig. III.E.4.6. Survival (proportion alive after exposure) of central mudminnows from (A) the control stream and (B) Little Black Creek (LBC) after 24-day exposure to storm water runoff collected during a rain event on 9 May 2009 from two road stream crossings on LBC (U.S. 31 and Seaway Drive). Error bars represent ± 1 standard error.

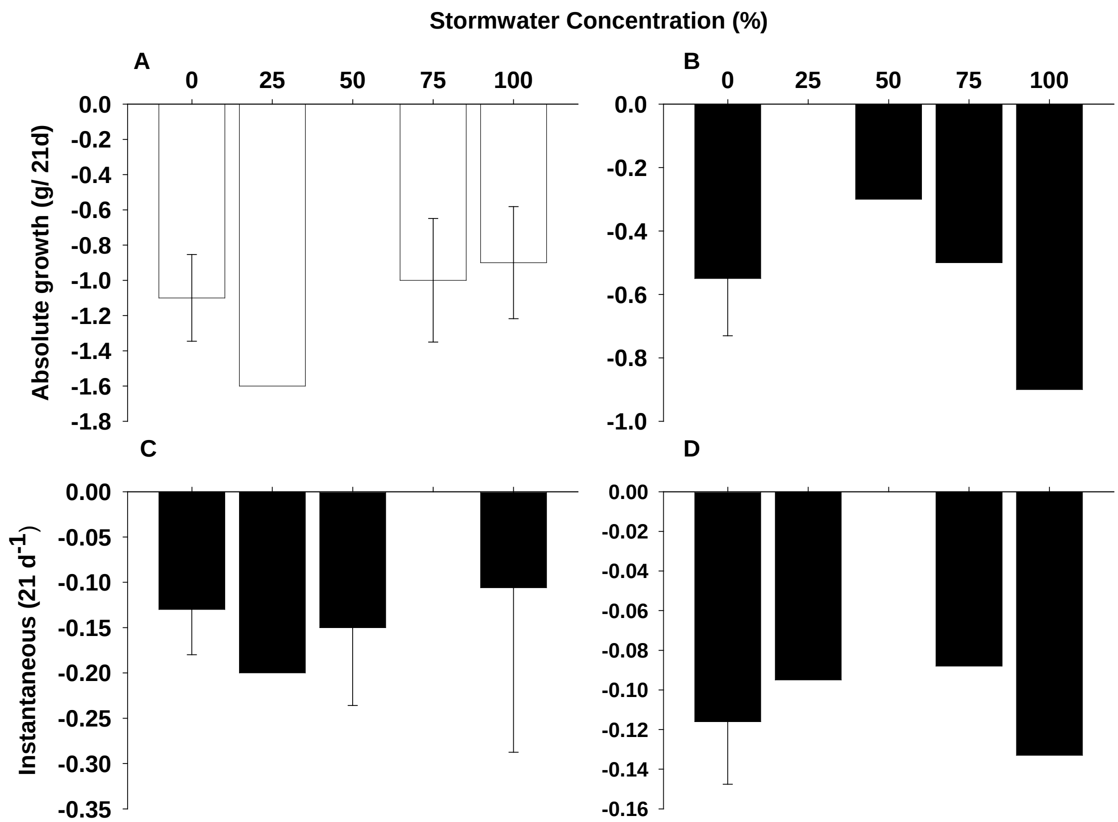


Fig. III.E.4.7. Absolute growth of central mudminnows from (A) Little Black Creek (LBC) and the (B) control stream and instantaneous growth rates of central mudminnows from (C) LBC and (D) the control stream exposed to a concentration gradient of storm water runoff collected from two road-stream crossings (U.S. 31 and Seaway Drive) on 14 July 2009. Error bars represent ± 1 standard error.

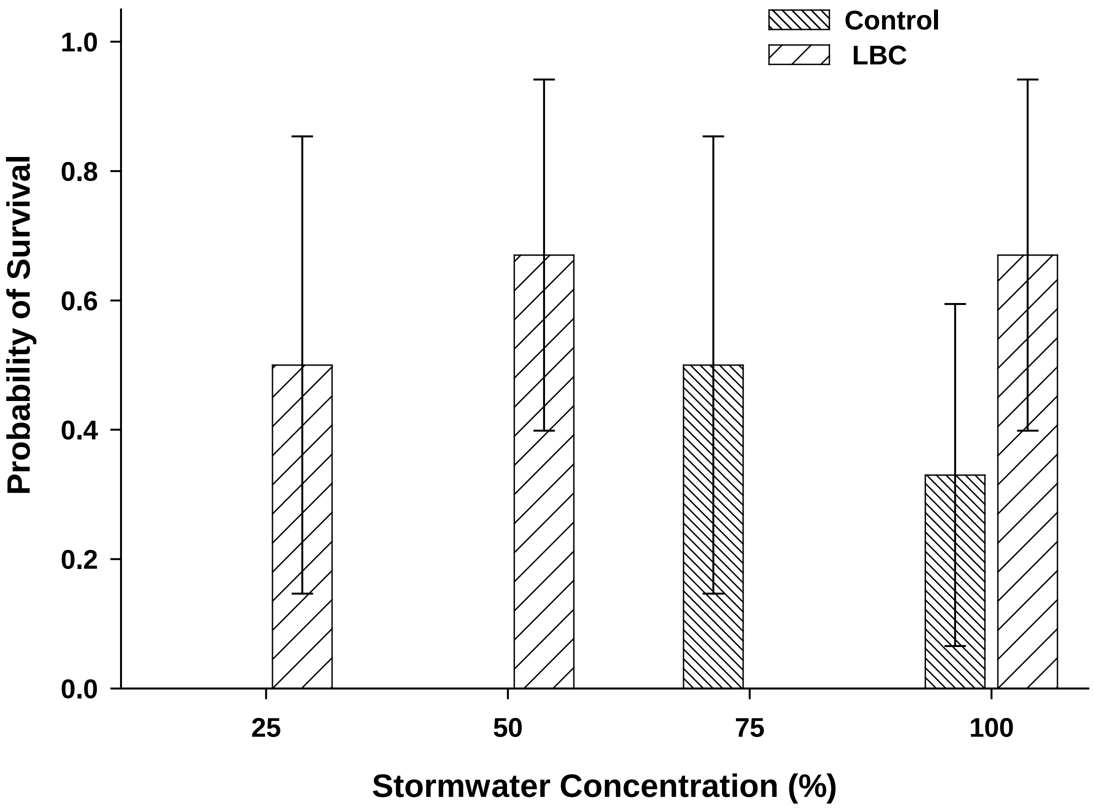


Fig. III.E.4.8. Survival rates (proportion alive after exposure) of central mudminnows from the control stream and Little Black Creek (LBC) after a 21-day exposure to storm water runoff collected during a rain event on 14 July 2009 from a single road stream crossing on LBC (U.S. 31). Error bars represent ± 1 standard error.

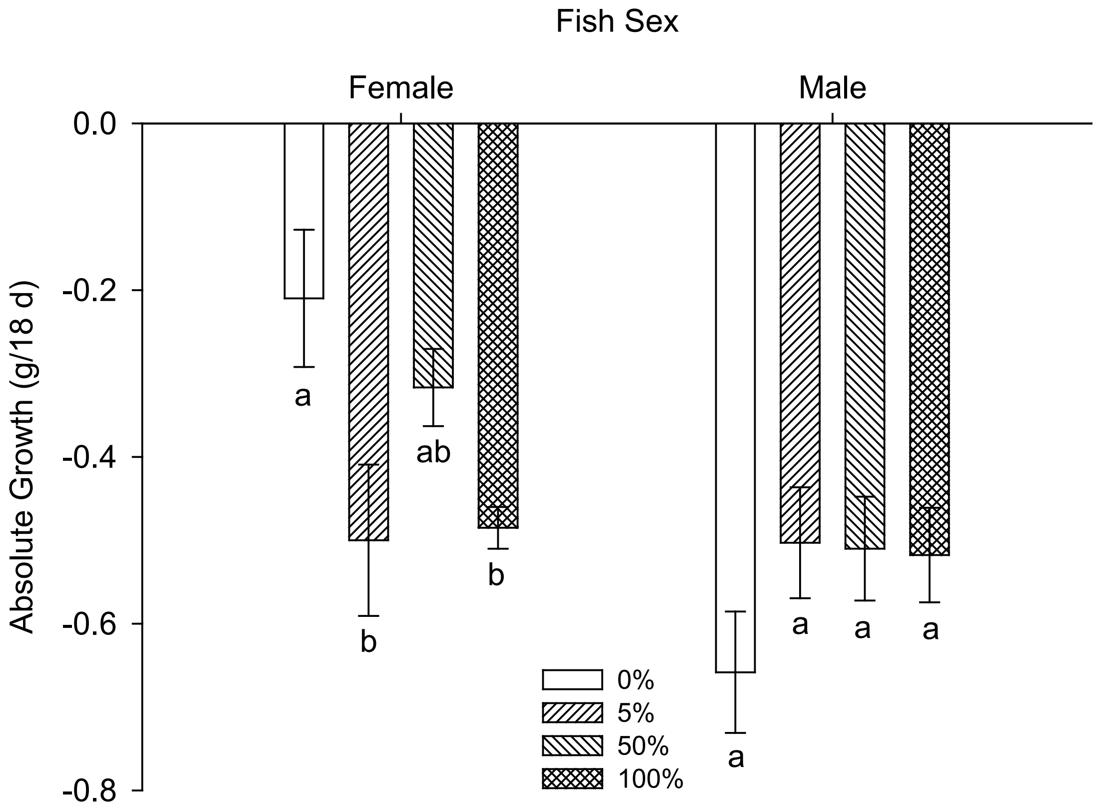


Fig. III.E.4.9. Absolute growth of female and male central mudminnows from Little Black Creek exposed to a concentration gradient of snowmelt (0%, 5%, 50%, and 100%) collected from U.S. 31 on 7 February 2011. Error bars represent ± 1 standard error. Lower case letters that differ indicate significant ($P < 0.05$) differences based on pair-wise comparisons among means; statistical comparisons were made for each sex (i.e., statistical comparisons were not made between males and females).

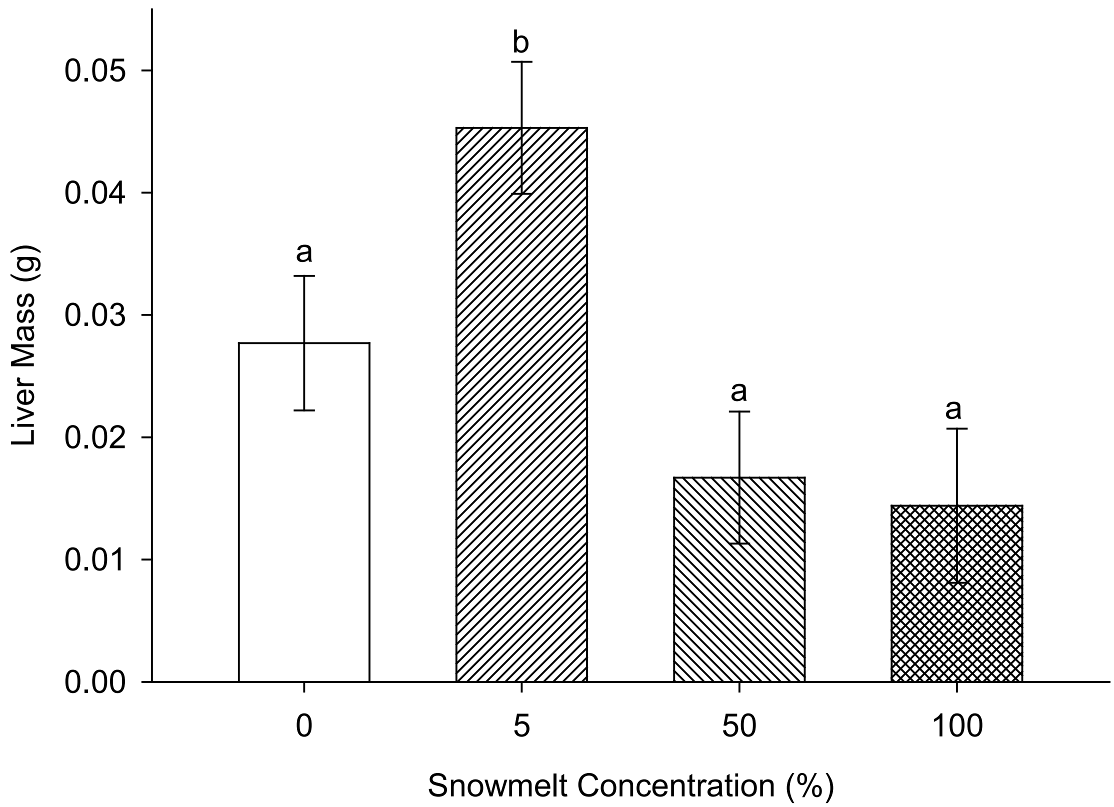


Fig. III.E.4.10. Condition (measured as liver mass) of central mudminnows from Little Black Creek exposed to a concentration gradient of snowmelt (0%, 5%, 50%, and 100%) collected from U.S. 31 on 7 February 2011. Error bars represent ± 1 standard error. Lower case letters that differ indicate significant ($P < 0.05$) differences based on pair-wise comparisons among means.

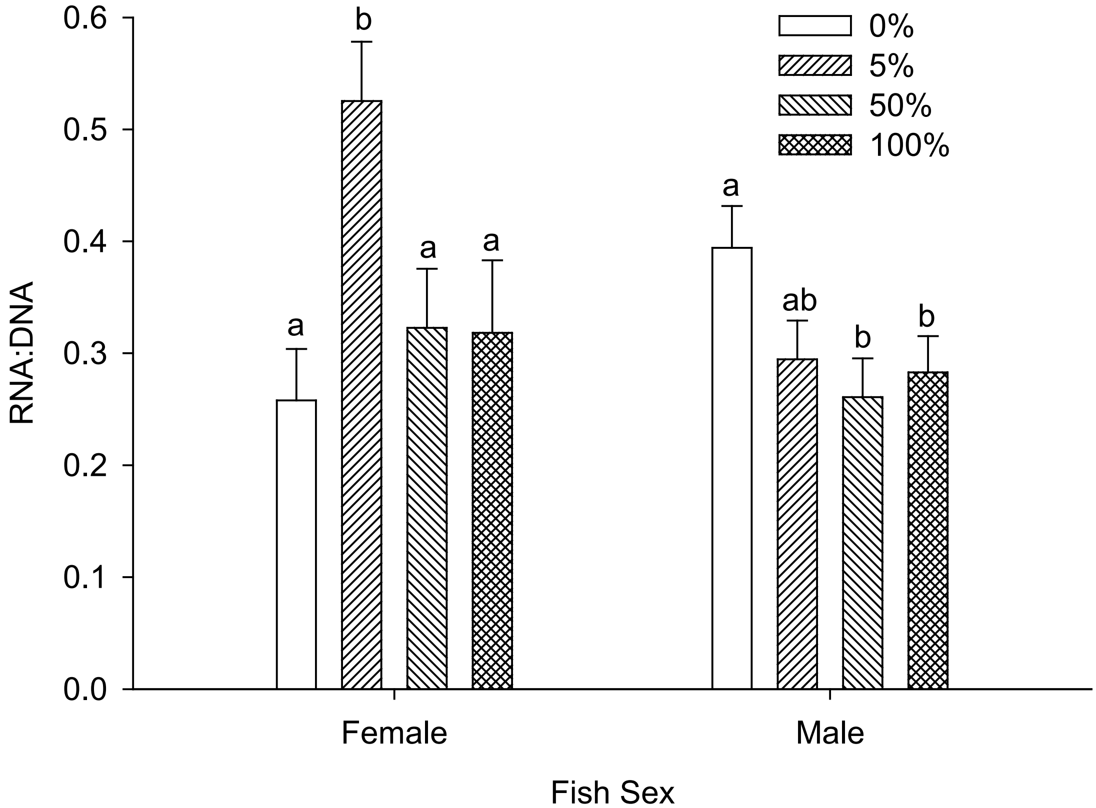


Fig. III.E.4.11. Condition (measured as RNA:DNA from fish tissue) of central mudminnows from Little Black Creek exposed to a concentration gradient of snowmelt (0%, 5%, 50%, and 100%) collected from U.S. 31 on 7 February 2011. Error bars represent ± 1 standard error. Lower case letters that differ indicate significant ($P < 0.05$) differences based on pair-wise comparisons among means; statistical comparisons were made for each sex (i.e., statistical comparisons were not made between males and females).

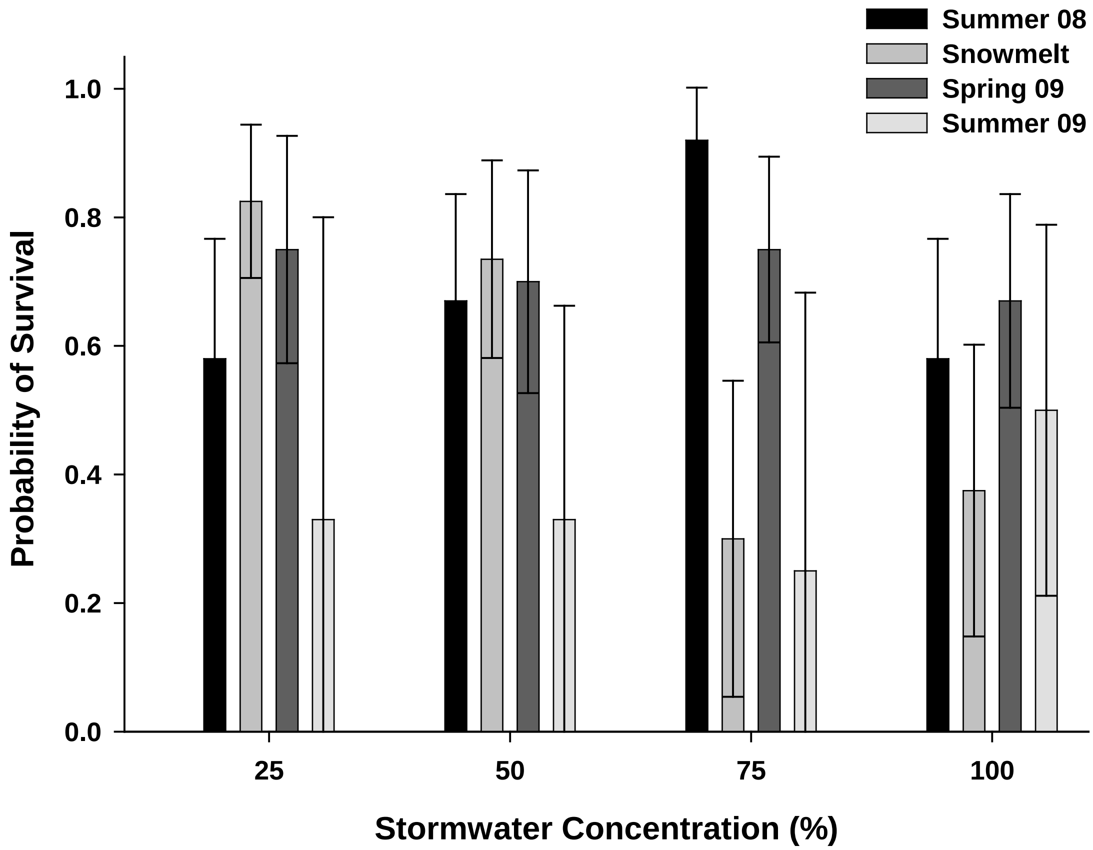


Fig. III.E.4.128. Survival rates (proportion alive after exposure) of central mudminnows exposed to storm water from five collection events between June 2008 and July 2009 across a concentration gradient of storm water (0, 25, 50, 75, and 100% storm water) collected in Little Black Creek (U.S. 31 and Seaway Drive). Error bars represent ± 1 standard error.

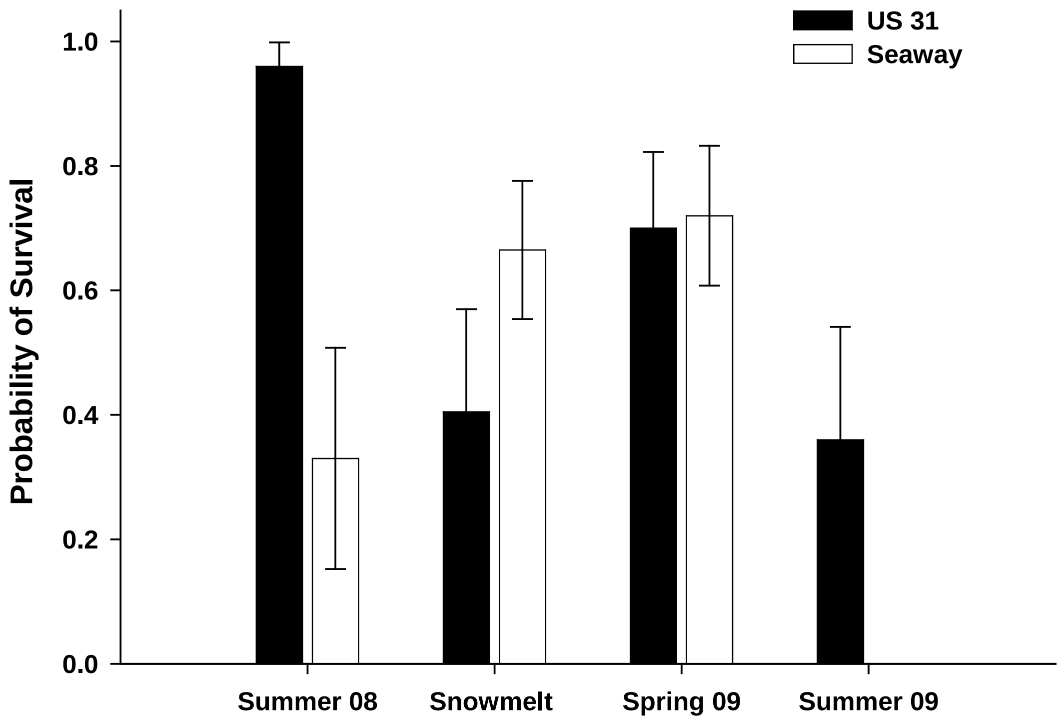


Fig. III.E.4.13. Survival rates (proportion alive after exposure) of central mudminnows exposed storm water from five collection events between June 2008 and July 2009 from two road stream crossing on Little Black Creek (U.S. 31 and Seaway Drive). Error bars represent ± 1 standard error.

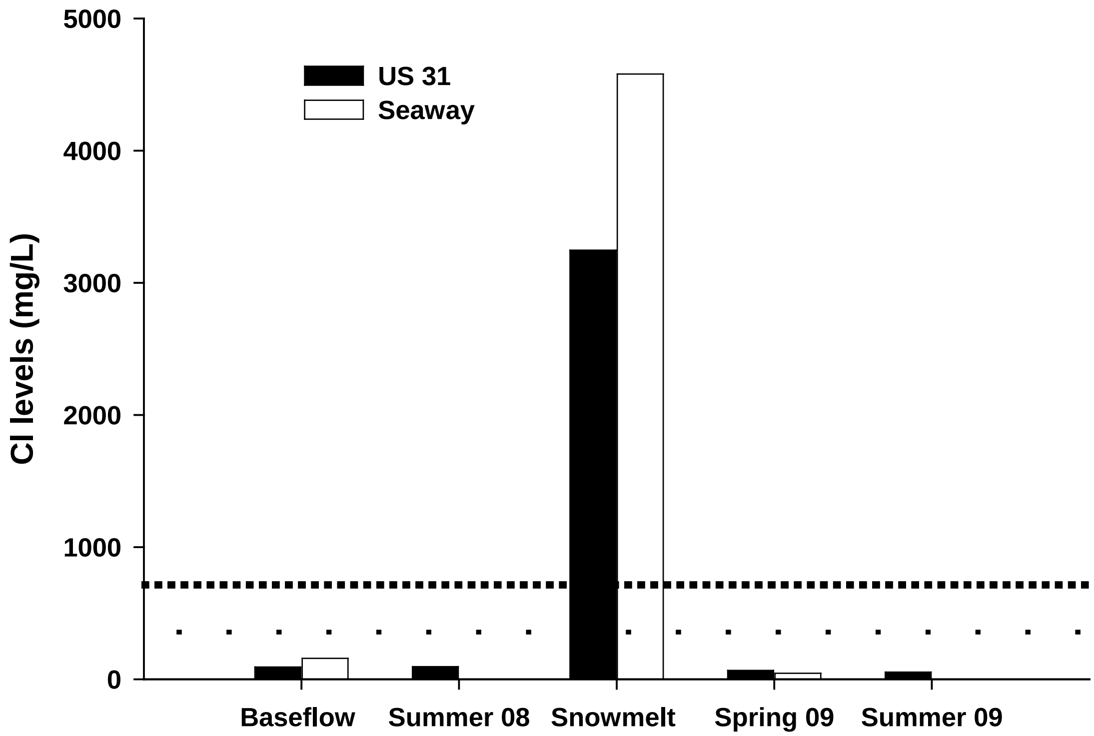


Fig. III.E.4.14. Concentrations of chloride (mg /L) for average base flow measurements and storm water collection events between June 2008 and July 2009 taken from two road/stream crossings (U.S. 31 and Seaway Drive) in LBC. Dashed line represents chronic water quality standard and dotted line represents acute water quality standard for freshwater organisms (U.S. EPA 1988).

IV. Discussion

IV.A. Water Quality, Quantity, and Geomorphology

Concentrations and loads of several key pollutants, including total phosphorus and several heavy metals, were elevated in storm water from U.S. 31 and Seaway Drive sites. Although storm water inputs resulted in increased concentrations and loads of these pollutants downstream of U.S. 31, storm water generated at Seaway Drive did not have a substantial effect on in-stream pollutants. This difference in storm water effects can be attributed to the watershed position of the sites. The Seaway Drive site, positioned near the bottom of the watershed, receives storm water from the majority of the watershed, which overwhelms the influence of localized inputs from Seaway Drive. In contrast, U.S. 31 crosses LBC in the approximate middle of the watershed, upstream of the densely-populated urban areas of Muskegon and Muskegon Heights, and receives less storm runoff from upstream sources. Thus, storm water inputs from U.S. 31 have a more direct impact on downstream pollutant concentrations and loads than those from Seaway Drive.

Although storm water from our study sites contained elevated concentrations of pollutants that are potentially harmful to aquatic life, it did not result in downstream concentrations that exceeded Michigan water quality standards. Depending on the duration and volume of snowmelt events, the potential exists for episodic stress to biota during these events. Snow collected from the roadside at our sites contained concentrations of chloride, copper, and zinc that exceeded state standards for acute effects to aquatic life. With concentrations 2-5X greater than the acute standard, chloride is the pollutant most likely to have negative effects on biota during snowmelt events (cf. Kaushel et al. 2005, Kelly et al. 2008, Gardner and Royer 2010).

Total phosphorus concentrations and loads were very high in both storm water and in LBC during storms. This elevated TP may have limited, episodic, effects on biota in LBC, but likely has greater consequences for Mona Lake, which is the receiving water body for LBC. TP concentrations exceeded the eutrophic threshold during base flow at the Seaway Drive site, and increased to hypereutrophic levels during storm events. Snowmelt TP concentrations were ~4X the hypereutrophic threshold, suggesting that melting events have the potential to deliver an intense pulse of TP to the system. This delivery of TP from snow melt often coincides with the onset of spring phytoplankton blooms in Mona Lake (Steinman et al. 2006a). Because internal P loading is not a significant P source to Mona Lake at this time of the year (Steinman et al. 2009), the external P subsidy from snow melt may be an important catalyst for spring phytoplankton growth in Mona Lake. The TP concentrations we measured in storm water were similar to those reported by Gan et al. (2008) for roads in rural areas of China and much lower than the 0.43-0.53 mg/L reported by Wu et al. (1998) for urban areas in North Carolina.

Oil and grease and PAHs are contaminants of concern in road runoff, but our data suggest they are not a significant issue for LBC at our study sites. Average PAH concentrations in storm water were very low in our study (6-15 μg/L) compared to those reported by Lee and Bang (2000) for urban areas in Korea (165 μg/L). Indeed, 1of 7 storm water samples from Seaway Drive and 3 of 7 from U.S. 31 had PAH concentrations below the detection limit (1 μg/L). Average oil and grease concentrations were similar to those in rural areas of China (Gan et al. 2008) and within the 1.3-4.4 mg/L range reported for urban areas in North Carolina (Wu et al. 1998).

Numerous other studies have documented the negative impacts that increased sediment can have on stream biota (Berkman and Rabeni 1987, Lemly 1982, Angradi 1999, Brim Box and Mossa 1999, Biggs et al. 1999, Schofield et al. 2004). Storm water from U.S. 31 resulted in increased suspended sediment concentrations and loads in LBC; however, downstream concentrations remained below the 80 mg/L suspended sediment target for wet-weather events in LBC (MDEQ 2003). Suspended sediment concentrations were extremely high in snowmelt, contributing to the aforementioned possibility of episodic stress to biota during snowmelt events. Our data show that bedload is the dominant form of sediment being transported in LBC. Substantial increases in bedload were measured downstream of the storm water outfalls at both locations. A study by Shofield et al. (2004) demonstrated that even small increases in bedload (60%) can result in negative effects to stream biota. In our study, storm water inputs resulted in average bedload increases of 117% at U.S. 31 and 663% at Seaway Drive. Potential negative effects of increased bedload sediment include habitat alteration, reduced densities of benthic biota, and altered food web interactions (Shofield et al. 2004).

The amount of precipitation measured in sampled storm events ranged from small (0.07 in) to moderately large (1.04 in). As expected, total storm water volume, which includes storm water inputs from the study sites plus all upstream inputs over the entire duration of the storm, was directly related to rainfall amount. The percentage of flow composed of site-specific storm water was 3 to 34% at the Seaway Drive site and 13 to 50% at the US 31 site. Storm water contributed lower percentages at Seaway because of this site's location in the watershed; its placement near the bottom of the watershed leads to more flow coming from upstream. Of course, since some of the upstream flow is also composed of runoff, which is not accounted for in the site-specific contribution, the 3 to 34% is an underestimate, but we cannot estimate with accuracy the degree of underestimation.

Storm flow duration in LBC was directly related to total storm water volume, with the longest storm pulses lasting over 50 hours. The extended period of storm flow during higher-rainfall events suggests that storm water detention may be occurring in the watershed, allowing for infiltration and helping to reduce extreme (i.e., "flashy") flows (cf. Chu and Steinman 2009).

Average storm flow discharge in LBC during the period of active road runoff (i.e., our sampling period) ranged from 0.01 to 0.26 m^3/s upstream and 0.02 to 0.38 m^3/s downstream at U.S. 31. Average storm flow discharge at the Seaway Drive site was greater, and ranged from 0.31 to 0.90 m^3/s, both upstream and downstream of the storm water outfall. This increased discharge resulted in elevated SSC at the downstream sampling locations at both sites, although concentrations remained below the 80 mg/L suspended sediment target for wet-weather events, set forth in the LBC Total Maximum

Daily Load (TMDL) for biota (MDEQ 2003). However, storm water SSC did fall into the less than moderate range for the protection of fish communities, suggesting the possibility of impairment.

IV.B. Toxicity Assessment

Storm water runoff generated from roadway and other land uses has been increasingly found to be the major source of non-point source pollution to receiving waters (Kayhanian et al. 2008). Only a few studies have been conducted on runoff that is predominantly or exclusively from roadways (Buckler and Granato 1999). Two toxicity studies conducted by Pitt et al. (1995) and Marsalek et al. (1999) found that roadway runoff had greater toxicity compared to the other land uses. In our study, storm water runoff was toxic to *C. dubia* during the winter and spring samples at the Seaway site but no toxicity was measured at the U.S. 31 site. This may be because runoff at this location contains both roadway runoff and groundwater. As a consequence, concentrations of metals and chloride were lower at U.S. 31 than at Seaway. In addition, snowmelt from both locations was toxic to *C. dubia*.

Toxicity in our study was correlated with chloride, chromium, copper, nickel, and zinc. Additional testing involving the Toxicity Identification Evaluation (TIE) would be required to determine if chloride and/or metals were the toxic agent(s). The cause of roadway runoff toxicity was hypothesized by Marsalek et al.(1999) to be partially due to road salts used for deicing while others have found heavy metals to responsible for the toxicity of roadway runoff to *Ceriodaphnia dubia* and *Daphnia magna* (Christensen et al. 2006; Kayhanian et al. 2008). In contrast, other investigators found no toxicity associated with roadway runoff to *Daphnia magna* (Waara and Färm 2008). Toxicity may be strongest during the first flush, as runoff begins. Indeed, Kayhanian et al. (2008) examined a series of 14 discrete samples collected during multiple storm events and found that 90% of the toxic samples were collected during the first 30% of storm duration, and that the first sample was the most toxic. In our study, we prepared only one flow-proportioned composite sample to represent the entire event; consequently, the toxicity of the discrete samples was not determined and we may have underestimated toxicity associated with first flush.

IV.C. Engineering Assessment

Treatability studies were similar to the results obtained by Pitt et al. (1995) where settling and filtration provided the greatest level of treatment for storm water while aeration and photodegradation were relatively ineffective. The storm water samples they evaluated contained a similar mixture of metals and PAH compounds, as was the case for our Seaway and U.S. 31 samples.

Storm water runoff generated from roadway and other land uses has been increasingly found to be the major source of non-point source pollution to receiving waters (Kayhanian et al., 2008). In order to achieve storm water-related water quality requirements, a wide range of best management practices (BMPs) are being implemented to remove toxic pollutants in order to protect aquatic life. The performance of these

BMPs is usually measured based on removal of pollutant concentrations or mass, and therefore, less attention has been made to evaluate toxicity (Kayhanian et al. 2008). Knowledge of the toxicity of roadway runoff toxicity is critical to accurately evaluate BMP effectiveness with respect to removal of the toxic fraction of pollutants, which is dependent on a variety of factors, including chemical speciation and interactions with other chemicals and physical water quality parameters such as suspended solids.

In the case of storm water and snowmelt from U.S. 31 and Seaway, most of the toxicity was found to be associated with the fine particulate phase and attributed to heavy metals and to a lesser extent, PAH compounds. Both of these materials have a high affinity for suspended solids. The results of the engineering assessment suggest that filtration and settling will remove the majority of toxic effects associated with storm water; however, storm water would need to be retained for at least 48 hr to be effective—this might require a settling lagoon or retention basin with a relatively large footprint. Given the magnitude of storm flows and the urban setting of the highways, the ability to locate a large settling pond in the vicinity of Little Black Creek may be limited. An alternative solution is baffled settling tank units similar to the Suntree Nutrient Separating Baffle Box$^{@}$, which provides a combination of screening and settling to remove pollutants associated with fine particulates (Charbeneau et al. 2004). More detailed modeling, combined with a cost-benefit analysis that includes long-term maintenance costs and environmental benefits, is needed to determine the most appropriate BMP and its siting.

IV.D. Laboratory Algal Bioassays

Storm water from roadway runoff can either stimulate, through nutrient addition (Kaczala et al. 2011), or inhibit, through toxic addition (Christensen et al. 2006, Kayhanian et al. 2008), the growth of algae. With respect to the U.S. 31 and Seaway storm water, nutrient levels were lower in the storm water (TP~0.05-0.1 mg/l) than in the culture medium (TP= 0.64 mg/l). The lack of elevated nutrient levels suggest that other factors such as trace metals and/or enhanced bioavailability may make the presence of storm water stimulate algal growth.

The snowmelt water was toxic to *P. subcapitatum* and the presence of elevated heavy metals supports the observations of other researchers as to the causative agent (Christensen et al. 2006, Kayhanian et al. 2008). Since the snowmelt occurs during the winter when algal productivity is low and dilution is high, the impact to stream autotrophs is limited, although there may be negative effects on invertebrates (cf. Gardner and Royer 2010) that we did not measure. The fact that spring, summer, and fall storm water was found to stimulate algal growth suggests that it may be factor in cultural eutrophication.

IV.E. Field Surveys

Aquatic ecosystems can become degraded when only 10-20% of the catchment area is covered with impervious surfaces (Arnold and Gibbons 1996). In LBC, impervious surfaces cover 32.1% of the developed land area (Steinman et al. 2006a), suggesting that the aquatic ecosystem is impacted. The hydrology in LBC is sensitive to rainfall events because of its small size and the high percentage of impervious surface in the catchment (Chu and Steinman 2009). In addition, the traffic volume at the U.S. 31 study site is high. This should result in high pollutant deposition, and runoff draining to the study site. Traffic volume is important because the number of vehicles on a road and the pollutant load in the runoff water are related (Barrett et al. 1998, Hallberg et al. 2007). Car parts such as brake linings, tires, and metal alloys in engine parts all contain heavy metals that can be released onto roads (Allan 2004). The traffic volume at the study site for this experiment is near the top of the range for Muskegon County (MDOT 2007), and is generally higher than volumes at sites examined in other road runoff studies: 5,500-25,000 ADT in Wu et al. (1998), >10,000 ADT in Boisson et al. (2005), 34,000-54,000 ADT in Boisson and Perrodin (2006), and 22,170 ADT in Gan et al. (2008). Boisson and Perrodin (2006) found that storm water runoff had a slightly positive effect on algal biomass. Runoff water from the Seaway study was not expected to have as large of a pollutant load as runoff from the U.S. 31 site because it has a much smaller traffic volume (~25,000 ADT).

Effects of Storm Water Chemistry

Effects on algal biomass and metabolism

Location downstream of the storm water pipe did not have a strong effect on algal biomass or metabolism. We suspect this was because the storm water was not potent enough and/or the increases in flow were not strong enough to see an algal effect. Toxins present in storm water runoff have the potential to decrease algal biomass and metabolism if their concentrations are high enough (Walsh et al. 2005), and nutrients generally increase algal biomass if the community is nutrient limited (Borchardt 1996).

It is unknown if the concentrations of metals in the storm water runoff were high enough to negatively impact algal biomass because water chemistry measurements were taken during only one storm event (fall experiment); no samples were collected during the summer experiment. Other storm water samples, however, were collected as part of this project (see Section III.A) throughout 2008 and 2009. These samples showed that the concentration of most metals did increase downstream of the storm water pipe, but no median metal concentration either upstream or downstream of the storm water pipe at either study site exceeded Michigan water quality standards for chronic or acute exposure (MDEQ 2011). In two of the storm events, the concentrations of Cu and Pb exceeded chronic, but not acute, water quality standards (MDEQ 2011). The exposure duration of storm water toxins in the stream was most likely an acute effect because the storm water is quickly diluted in the stream. Substantial reductions in concentrations of Zn, Cu, Pb, Cr, Ni, and Cd occurred at both study sites between the storm water pipe and downstream sampling location, a distance of ~53 m at the Seaway site and ~15 m at the U.S. 31 site.

The only significant periphyton biomass difference between upstream and downstream of the storm water pipe was AFDM at the U.S. 31 site. AFDM was significantly lower downstream of the pipe and although the difference was not statistically significant, the concentration of Chl *a* was also lower downstream. Also, at the Seaway site in the fall experiment only, both Chl *a* and AFDM tended to be lower downstream of the pipe compared to upstream in the fall experiment, but these differences were not statistically significant. The concentrations of all metals (except Cd) in storm water were higher at the downstream location at the U.S. 31 site than downstream at the Seaway site, perhaps accounting for the significant reductions at U.S. 31 but not at Seaway. Other studies that exposed algal communities to metal concentrations much higher than those in the current study, reported a slight increase in biomass (Boisson and Perrodin 2006), or no change in biomass (Maltby et al. 1995).

The levels of nutrients present in the storm water may not have been high enough to elicit a response (albeit positive) in algal biomass or the algae may not have been nutrient-limited. NO_3 concentration increased slightly downstream of a road-stream crossing at one site in a study by Maltby et al. (1995) and no change in algal biomass was detected; the NO_3 concentrations at their study site were much greater (66.6 – 74. 2 mg/L) than those generally found in base flow and even during storm flow in LBC. Boisson et al. (2005) also found that location upstream or downstream of a road runoff discharge pipe did not affect algal biomass when upstream water contained higher concentrations of NO_3-N (954 mg/L) and TP (37 mg/L) than were measured at either site in the present experiments. Nutrient concentrations in LBC during these experiments were suggestive of nutrient limited conditions, but the data were far from unequivocal. For example, SRP, the form of phosphorus readily available to the algae, was below 0.005 mg/L in most of the samples, so it was likely limiting unless nutrient demand was being met by P cycling within the periphyton matrix (cf. Steinman et al. 1995). NO_3-N has been shown to be limiting for algal communities at concentrations of 0.055 mg/L (Grimm and Fisher 1986) and 0.1 mg/L (Lohman et al. 1991). In the present experiment, NO_3-N concentrations were above 0.05 mg/L at both the upstream and downstream locations at both study sites. Separate nutrient limitation assays would have been necessary to definitively determine if the algal communities in this experiment were nutrient limited, and if so, by what nutrient.

The effects of location relative to the storm water pipe on algal metabolism were more complicated than the biomass results. At the U.S. 31 site in the summer experiment, a decline in biomass corresponded with a significant decline in algal respiration and a non-significant trend of less GPP downstream of the storm water pipe. A similar result was observed at the Seaway site; GPP and biomass were both lower downstream in the fall experiment. This decline in areal-specific metabolism is consistent with a decline in biomass because there would be less algal material to photosynthesize (Steinman 1996).

In the fall experiment at the Seaway site, however, areal-specific respiration and GPP were greater downstream of the pipe, despite the presence of less biomass. These results are inconsistent with the typical response of areal-specific metabolism to reductions in biomass. The results are more consistent with those of biomass-specific metabolism, which has been shown to increase as algal biomass declines because senescent cells, which contribute to biomass but not productivity, may have been removed (Lamberti et al. 1989, Steinman 1996). It is unlikely that a change in algal community structure was responsible for the increase in metabolic activity. Only one taxon increased in abundance downstream of the storm water pipe during the fall experiment at U.S. 31 and that was *Rhoicosphenia*, which was only the fifth most abundant taxon. It is possible that the samples that had greater metabolism downstream with lower biomass were affected by other impacts, such as light grazing pressure, which has been shown to positively affect areal-specific under some circumstances (Steinman 1996). Also, both respiration and GPP values were very low in these experiments, and this could have led to some measurement uncertainty, providing an explanation for the inconsistencies in the metabolism results.

Storm water and metal pollution have been shown to have variable effects on algal metabolism. In the present experiments, location relative to the storm water pipe had no effect, a positive effect, and a negative effect on algal metabolism depending on the study site and season. The increase in metabolism in the fall experiment at the Seaway site is consistent with studies by Boisson et al. (2005) and Boisson and Perrodin (2006), which found that production and respiration increased slightly with exposure to storm water. Also, GPP, respiration, and Chl *a* generally increased with increasing catchment area cleared and with increasing nutrient concentrations in a study by Fellows et al. (2006). In a study of benthic algal metabolism along a mine pollution gradient, both respiration and GPP were highest at the reference site and declined as the concentration of Zn, Mn, and Fe increased (Hill et al. 1997). The continuous exposure to high metal concentrations impaired the algal communities (Hill et al. 1997). Along with reductions in biomass, metal concentrations downstream of the storm water pipe may have contributed to the decline in metabolism in some treatments.

Effects of storm water chemistry on algal community composition

Small amounts of storm water runoff can affect algal community composition (Newell and Walsh 2005). Algal taxa vary in their tolerance to chemical stress due to differences in how these chemicals interact with intracellular and cell-surface binding sites in each particular taxon, and this can be reflected in differences in the relative abundances of algal taxa within a community (Genter 1996). The overall algal communities located upstream and downstream of the storm water pipe were not significantly different during either experiment, suggesting that the conditions downstream were not potent enough to cause large changes in algal taxa. A few individual taxa, however, were significantly affected by storm water location, but the results were not generally consistent among the Seaway and U.S. 31 sites or the summer and fall experiments.

Effects of Storm Water Flow

Effects on algal biomass and metabolism

Current velocity is one of the most important factors affecting benthic algal community variation among substrata in the same habitat (Stevenson 1996), and could have accounted for the slight decline in biomass downstream of the storm water pipe. The shear stress of increased current velocities has been shown to reduce algal biomass (Peterson 1987, Poff et al. 1990, Poff et al. 1997), although this depends on the intensity of the velocity. In Poff et al. (1990), biomass was on average 30-40x higher in treatments with slow current velocity (<1 cm/s and 17.1 cm/s) than treatments with high current velocity (41.6 cm/s and 29.2 cm/s). Taulbee et al. (2009) found that Chl *a* and AFDM concentrations were not significantly affected by exposure to velocity regimes of pre-development flow (base flow velocity: 26.1 cm/s) and post-development flow (base flow velocity: 16.7 m/s) vs. a storm flow current velocity of 40 cm/s. Average base flow velocities in LBC at the Seaway site were 33 cm/s upstream of the storm water pipe and 29 cm/s downstream of the pipe, and at the U.S. 31 site, base flow velocities were 21 cm/s upstream of the pipe and 33 cm/s downstream of the pipe. Unfortunately, storm flow current velocity is not available from the present study sites, but storm flow discharge was on average 1.43x higher downstream of the storm water pipe compared to upstream at the U.S. 31 site (upstream discharge = 0.129 m^3s^{-1} and downstream discharge = 0.184 m^3s^{-1}), suggesting that current flows may have increased enough in LBC during storms to have contributed to the decline in algal biomass downstream of the pipe in some treatments.

Modest increases in current velocity have also been shown to increase algal metabolism by increasing nutrient diffusion and the uptake of nutrients by the algae (Borchardt 1996). In a study of the effects of storm water runoff on benthic algae, production and respiration were significantly greater when exposed to storm water and high current velocities (~50 cm/s) than when exposed to storm water and slow current velocities (~12 cm/s; Boisson and Perrodin 2006). The high current velocities in the Boisson and Perrodin (2006) experiment are higher than base flow velocities in LBC, but may be comparable to current velocity during storm flow at the study sites. We did not mimic velocity differences in our incubations, so our results are not directly comparable to these studies.

Effects of storm water flow on algal community composition

Current velocity may have had some impact on algal taxa in these experiments. Poff et al. (1990) showed that slow current (~20 cm/s) environments were dominated by *Ulothrix zonata*, upright, filamentous taxa, and some diatoms, including *Cocconeis*, *Fragilaria*, and *Cymbella*; in contrast, fast current (~40 cm/s) environments were dominated by prostrate diatoms such as *Cocconeis*, *Nitzschia*, and *Cymbella*, as well as short filaments of *Ulothrix*. We did not see a similar response in our studies; algal taxa did not show clear patterns with respect to their location upstream or downstream of the storm water pipe, perhaps because of the relatively modest differences in current velocities. At the Seaway site, *Cocconeis*, a firm understory taxa, was significantly less abundant downstream of the pipe, which is contrary to expectations if increased current velocity was scouring the more loosely attached, upper canopy taxa.

Cladophora is a filamentous taxon that has been shown to have the greatest biomass in moderate to fast currents (Stevenson 1996). *Cladophora* made up a large part of the total algal biovolume at the Seaway site during the summer experiment, and in this experiment, *Cladophora* biovolume was significantly lower downstream compared to upstream of the storm water pipe. Any increases in current velocity downstream of the pipe may not have been large enough to have a positive effect on this taxon in this experiment.

Effects of Light and Grazing

Light is an important abiotic factor affecting benthic algal communities. In this experiment, light was not controlled for. The irradiance, however, did not differ substantially between the upstream and downstream sampling sites at a particular study site, which is consistent with the lack of strong effect of location relative to the storm water pipe. There was a large difference, however, between the irradiance at the Seaway and U.S. 31 study sites. The U.S. 31 site was predominantly shaded, with irradiance in the air directly above the tiles of ~30-100 $\mu mol\ m^{-2}\ s^{-1}$, depending on weather conditions, and the Seaway site had an open canopy, with an irradiance of ~1800-2100 $\mu mol\ m^{-2}\ s^{-1}$. Because photosynthesis of most benthic algal assemblages saturates above 100 $\mu mol\ m^{-2}\ s^{-}1$ (Hill 1996) algal communities at the U.S. 31 site may have been light limited. This may explain the discrepancy in algal biomass between the two sites; Chl *a* can be four to five time higher at open sites than at sites with full canopy cover (Hill 1996). The maximum Chl *a* at the Seaway site was ~6 times more than the maximum Chl *a* at U.S. 31.

Grazing is also an important biotic factor affecting benthic algae that was not controlled for this these experiments. Some algae were most likely removed from the sample tiles by grazers during the experiment, but this probably affected treatments upstream and downstream of the storm water pipe equally. Snails were occasionally observed on the tiles at the U.S. 31 site, but no grazers were observed on the tiles at the Seaway site; this, however, does not mean that no grazing occurred. Nutrients and grazing often have contrasting effects on algal communities; in a meta-analysis of studies examining the effects of grazers and nutrients, Hillebrand (2002) found that the effect of nutrients on algal biomass was stronger in ungrazed samples compared to grazed samples. If moderate grazing occurred during the present experiments, this may have reduced the positive effect nutrients from storm water had on the samples.

In conclusion, storm water runoff did not have a strong effect on algal biomass, metabolic activity, or taxonomic composition in these field-based experiments. There was a trend of lower biomass at the sampling sites downstream of the storm water pipe, although this was significant only for AFDM in one treatment and not significant for Chl *a* in any treatment. Metabolism tended to decline with algal biomass in most treatments. These declines in biomass and metabolism suggest that metal concentrations in the storm water or increases in current velocities may have negatively affected the algal communities. The overall community composition was not significantly affected by location upstream or downstream of the storm water pipe, although some taxa were slightly affected by storm water, suggesting that community composition is a more sensitive measure of water quality than biomass.

These field experiments did not separate the chemical effects of storm water from the hydrologic effects, so it is difficult to know what influenced the algal samples the most.

Either increases in current velocity or increases in metals concentrations downstream of the storm water pipe had a slight negative impact on the algae at these sites. Due to the variable nature of storm water runoff, this type of experiment is very context-specific, with the results at each study site heavily influenced by the composition of runoff water from one location.

IV.F. Mesocosm Experiments

Validity of Experimental Design

The lack of a strong storm water effect in this experiment was unexpected. It is believed that the experimental design was appropriate to elicit algal responses. For example, the storm water runoff used in this experiment was thought to be potent because of two reasons. First, the traffic volume at this site is high, which should result in high pollutant deposition, and runoff draining to the study site. Traffic volume is important because the number of vehicles on a road and the pollutant load in the runoff water are related (Barrett et al. 1998, Hallberg et al. 2007). Car parts such as brake linings, tires, and metal alloys in engine parts all contain heavy metals that can be released onto roads (Allan 2004). The traffic volume at the study site for this experiment (~61,000 ADT) is near the top of the range for Muskegon County (MDOT 2007), and is generally higher than volumes at sites examined in other road runoff studies: 5,500-25,000 ADT in Wu et al. (1998), >10,000 ADT in Boisson et al. (2005), 34,000-54,000 ADT in Boisson and Perrodin (2006), and 22,170 ADT in Gan et al. (2008). Boisson and Perrodin (2006) found that storm water runoff had a slightly positive effect on algal biomass.

The second reason that storm water collected for this experiment was expected to be potent was because water collection commenced just as storm water began to flow, thereby capturing the "first flush". First flush has been used to describe the disproportionately high concentration of constituents in runoff water during the initial portions of a runoff event (Sansalone and Cristina 2004). This occurs because many nutrients, metals, and other compounds are loosely attached to impervious surfaces and are washed off of these surfaces relatively quickly during a storm (Sansalone and Cristina 2004). On average, 90% of the toxicity of a storm water runoff sample is observed during the first 30% of the storm (Kayhanian et al. 2008).

2008 Experiments:

Effects of Storm Water on Algae

Effects on algal biomass

In this experiment, storm water did not have a significant effect on algal biomass or metabolism. This may be because the storm water was not potent enough to elicit a response from the algae and/or because the constituents in the storm water were not in a form that could impact the algae (also see section below: *Factors affecting storm water potency*). It is likely that the concentrations of metals in the storm water runoff were not high enough to negatively impact algal biomass. Of all the metals measured, only the concentration of Cu in the storm water pipe sample exceeded Michigan water quality standards for chronic exposure (16.19 µg/L; MDEQ 2011). In Boisson and Perrodin (2006) and Maltby et al. (1995), algal communities were exposed to levels of metals much higher than those in the present experiment. A slight increase in biomass was observed in Boisson and Perrodin (2006) and no change in biomass was observed in Maltby et al. (1995). Hence the lack of negative effects in the present study, where metal concentrations were much lower, is consistent with the findings of those studies. Numerous other studies have analyzed the chemical composition of storm water runoff (Wu et al. 1998, Lee and Bang 2000, Mangani et al. 2005, Christensen et al. 2006, Gan et al. 2008), but most do not examine the effects of this water on algal communities.

The levels of nutrients present in the storm water also may not have been high enough to elicit a response (albeit positive) in algal biomass. NO_3 concentration increased slightly downstream of a road-stream crossing at one site in a study by Maltby et al. (1995); the NO_3 concentrations at their study site were much greater (66.6 mg/L upstream and 74.2 mg/L) than in the present experiment and no change in algal biomass was detected. Boisson et al. (2005) also found that location upstream or downstream of a road runoff discharge pipe did not affect algal biomass when upstream water contained higher concentrations of NO_3-N (954 mg/L) and TP (37 mg/L) than were measured in the present experiment. Algal biomass was not affected in these studies, so it is consistent that the nutrient concentrations in the present experiment were too low to have an effect.

It is also possible that the algal communities were not nutrient limited. If the algal communities were not nutrient limited, then any increases in nitrogen or phosphorus would have limited effect on algal biomass. As noted in the previous section, nutrient concentrations in our systems were suggestive of nutrient limited conditions, but the data were far from unequivocal. Separate nutrient limitation assays would be necessary to definitively determine if the algal communities in this experiment were nutrient limited, and if so, by what nutrient.

Effects on Algal Community Composition

Small amounts of storm water runoff can affect algal community composition (Newell and Walsh 2005) without necessarily altering overall biomass, and this was observed in this experiment. Algal taxa vary in their tolerance to chemical stress due to differences in how these chemicals interact with intracellular and cell-surface binding sites in each particular taxon, and this can be reflected in differences in the relative abundances of algal taxa within a community (Genter 1996). The algal communities exposed to 100% and 50% storm water in this experiment were significantly different from communities not exposed to storm water, possibly because of differences in metals and nutrient concentrations.

Some taxa may have been influenced by increased metal concentrations in the 100% storm water treatment. Small members of the genus *Achnanthes* had the highest indicator values for, and increased in biovolume in, the 100% storm water treatment. This taxon possibly contributed to the significant difference among storm water treatments. Several authors have reported that small, adnately attaching species such as *Achnanthes minutissimum* are in highest abundance in metal-polluted environments (Medley and Clements 1998, Ivorra et al. 2000, Morin et al. 2008), which is consistent with the findings of the present study. Tightly attached species, such as *Achnanthes,* may be more likely to survive under metal stress because they are embedded in an organic matrix acting as a boundary towards metal toxicity (Burkholder et al. 1990). In a study by Sabater et al. (2002), *Achnanthes minutissima* and *A. lanceolata* significantly increased in abundance after seven days in an artificial channel with 15 µg/L of Cu added to the water. In the present study, however, *Achnanthes* was not identified to species, so it is impossible to know which species were part of the community.

Two of the three most abundant (in terms of biovolume) non-diatom taxa observed in the present experiment, the green algae *Ankistrodesmus* and *Pediastrum*, had a significantly greater abundance in the 0% storm water treatment compared to the 100% treatment. Although the data were variable, this suggests that these taxa were not as tolerant of, or successful in, the 100% storm water. Contrary to the findings in this experiment, a study by Serra et al. (2009) found that green algae and cyanobacteria were more tolerant than diatoms of continuous high concentrations of Cu (100 µg/L) for five weeks. Similarly, Genter et al. (1987) found that community composition shifted from diatoms to filamentous green algae to unicellular green algae as Zn concentrations increased. It is possible that the levels of metals in the 100% storm water treatment were not high enough to negatively impact the taxa and that the biovolume of these green algae was more influenced by increased nutrients in the 0% treatment than by metals.

In general, three taxa were most abundant at the end of all storm water treatment incubations (*Navicula*, small naviculoid, and *Melosira*), suggesting these taxa were more tolerant of the storm water constituents than the other taxa. Maltby et al. 1995 also found certain species of *Navicula* to be tolerant of storm water runoff. Because a large number of species exist in this genus, with varying tolerances to heavy metals, it is impossible to know which species were present in the different studies. *Melosira varians* has been shown to be sensitive to metal pollution (Medley and Clements 1998, Ivorra et al. 2000). In a study by Takamura et al. (1989), *Melosira varians* had a 50% inhibition of photosynthesis when exposed to 5.08 µg/L of Cu or 6670 µg/L of Zn for 24 hours. In the present experiment, however, *Melosira* did not respond negatively to the 100% or 50% storm water treatments, nor did it respond positively to the 0% treatment. This is consistent with a study by Rosemond et al. (1993) in which *Melosira varians* was also not affected by nutrients.

While the overall distribution of different physiognomies in the algal communities did not differ among storm water treatments, the relative number of loose understory taxa was significantly lower in the 100% storm water treatment than in the 0% treatment. Loose understory taxa, however, were dominant in every treatment in this experiment, as well as in other studies (Maltby et al. 1995, Newell and Walsh 2005). Understory taxa may have been most abundant in the 100% storm water treatment because they were less exposed to toxins in the water and therefore less vulnerable. Loose understory taxa may respond in a similar manner as firm understory taxa because of their location within the algal community matrix. The decline in relative abundance of loose understory taxa in the 100% treatment is inconsistent with the hypothesis that understory taxa are protected from toxins. These taxa simply could have been not affected by the levels of nutrients and metals in the experimental treatments.

Factors affecting storm water potency

The storm water collected for the mesocosm study may have had less potency than anticipated for 4 reasons:

First, high traffic volume on the road draining to the study site may not have translated to high concentrations of pollutants in the storm water if most of the constituents were not washed off the road during the storm event. The intensity of rainfall in this experiment was 6.43 cm/hr, which is significantly less than the 13.3 cm/hr required to wash off the maximum amount of pollutants (Egodawatta et al. 2007). Most common storm events are not capable of removing all of the built-up pollutants on the impervious surface and often remove only a fraction of the available pollutants (Vaze and Chiew 2002, Egodawatta et al. 2007).

Second, the concentration of metals and nutrients in the mesocosms may not have been as high as concentrations in the pipe storm water. Unfortunately the concentrations of metals in the mesocosms are not available for this experiment. However, storm water runoff was collected in July 2009 to perform another mesocosm experiment similar to the one described in this chapter; as described below in the 2009 mesocosm discussion, there was a ~40-70% reduction in concentration of metals from the pipe storm water to the water in the 100% storm water tanks at the beginning of the experiment. It is possible that the same phenomenon occurred in the 2008 mesocosm experiment. Third, metals present in the tanks may not have been available to the algae. And fourth, all the cells within the periphyton matrix may not have been exposed to toxins in the water to the same extent. Explanations for reasons 2-4 are discussed in the 2009 mesocosm section below.

Effects of Snails

There was no evidence in this experiment that storm water impacted snail grazing activity. Absence of a grazing effect may be a result of snails being consumed before they could cause significant reductions in algal biomass. Although gut analysis of the fish was not conducted, the fish were observed vigorously consuming snails during the experiment. The fish had full access to all snails inside the mesocosm except the ones inside the algae + snails only exclosure, which served as a refuge for the snails. Snails, however, were rarely observed in this exclosure except immediately after being placed in the mesocosm. Snails were free to enter and exit the algae + snails only exclosure, so it is possible that the snails left this exclosure and were consumed by fish, although this behavior seems counterintuitive. Turner et al. (1999) found that snails moved under covered habitats in the presence of pumpkinseed sunfish. The snails used in this experiment were most likely exposed to pumpkinseed sunfish or similar snail-consuming fish in their natural habitats, so it is probable that the snails in the exclosure recognized the fish as predators.

Effects of Fish

Because pumpkinseed sunfish are not known to consume algae (Becker 1983), the significantly lower biomass of algae on the tiles exposed to fish suggests that it was their movement inside the exclosures that disrupted and removed biomass. Even if they are not grazing, the movement of mobile grazers, including fish, through an algal assemblage can dislodge the loose, upper layer of algal taxa (Hill and Knight 1987, Lamberti et al. 1989, Cattaneo and Mousseau 1995), and this was observed in the present experiment. Fish swam around and entered the exclosures when feeding on snails, and despite being provided a shaded portion of the mesocosm, the fish were frequently observed hiding inside the exclosures (on top of the algal samples) regardless of whether overhead lights were on or off. The effect of this movement on algal biomass was not influenced by storm water concentration. In addition to reducing algal biomass on the tiles, fish presence was associated with significant increases in cell number and biovolume of *Achnanthes* and *Cocconeis*, firmly attached understory taxa. Also, in terms of biovolume, the amount of loose understory taxa declined significantly with fish. These changes suggest that the physical disruption caused by the fish movement resulted in a shift towards taxa more tightly adhered to the substrate and therefore less vulnerable to dislodgement, similar to the effects of grazing. Algal communities under grazing pressure are often dominated by taxa with prostrate growth forms (Steinman et al. 1987, Lowe and Hunter 1988, Lamberti et al. 1989, Rosemond et al. 1993).

Fish had no significant effect on the metabolic activity of the algal communities. This may be because the algal biomass on the tiles was too low to alter oxygen levels enough to detect an effect. The reduction in biomass in the fish-exposed treatments in this experiment may not have resulted in an increase in biomass-specific GPP because the algal mats may not have been thick enough to create a light and nutrient diffusion barrier (Lamberti et al. 1989, Steinman 1996).

2009 Experiments:

Effects of Storm Water on Algae

Effects on algal biomass

As was the case for the 2008 experiments, storm water did not have an overall effect on algal biomass or metabolism. However, storm water may have had an interaction effect with the fish and snail treatments (see section below: Effects of Snails). This may be because the storm water was not potent enough to elicit a response from the algae and/or because the constituents in the storm water were not in a form that could impact the algae (also see section below: *Factors affecting storm water potency*). Toxins present in storm water runoff have the potential to decrease algal biomass and metabolism if their concentrations are high enough (Walsh et al. 2005), while nutrients generally increase algal biomass if the community is nutrient limited (Borchardt 1996). The highest levels of both metals and nutrients were found in the 100% storm water treatments, so it is difficult to separate the effects of these two influences on the results of the experiment.

As noted in the previous section, it is likely that the concentrations of metals in the storm water runoff were not high enough to negatively impact algal biomass or metabolism. The concentrations of measured were below the water quality standards in the 100% storm water treatments on day 1 of the experiment. In addition, nutrient concentrations present in the storm water may not have been high enough to elicit a response (albeit positive) in algal biomass, or the algal communities may not have been nutrient limited. If the algal communities were not nutrient limited, then any increases in nitrogen or phosphorus would have limited effect on algal biomass. Some nutrient concentrations at the beginning of the experiment were suggestive of nutrient limited conditions, but the data were far from unequivocal. SRP, the form of phosphorus readily available to algae, was below 0.005 mg/L, and so was likely limiting, in all treatments at both the beginning and end of the experiment unless nutrient demand was being met by P cycling within the periphyton matrix (cf. Steinman et al. 1995). NO_3-N has been shown to be limiting for algal communities at concentrations of 0.055 mg/L (Grimm and Fisher 1986) and 0.1 mg/L (Lohman et al. 1991). In the present experiment, NO_3-N concentrations were above 0.05 mg/L in all treatments at the beginning of the experiment, but were below 0.05 mg/L at the end of the experiment in the 100% and 0% treatments. Separate nutrient limitation assays would have been necessary to definitively determine if the algal communities in this experiment were nutrient limited, and if so, by what nutrient.

Effects on Algal Community Composition

Small amounts of storm water runoff can affect algal community composition (Newell and Walsh 2005) without necessarily altering overall biomass, and this was observed in the present experiment. Algal taxa vary in their tolerance to chemical stress due to differences in how these chemicals interact with intracellular and cell-surface binding sites in each particular taxon, and this can be reflected in differences in the relative abundances of algal taxa within a community (Genter 1996). The algal communities exposed to 100% storm water in this experiment were significantly different from communities not exposed to storm water, possibly because of differences in metal and nutrient concentrations.

A shift from diatoms to green algae was observed for some taxa in the 100% treatment in present experiment. Two abundant taxa (*Mougeotia* and *Scenedesmus*) and the taxa most indicative of the 100% treatment (*Ankistrodesmus*) were all green algae, suggesting these taxa are tolerant of the higher metal conditions. *Mougeotia,* however, while being the most abundant taxa in all treatments, was actually significantly less abundant in the 100% storm water treatment than in the 0%. This decline in *Mougeotia* as well as the fact that several diatom taxa increased in abundance in the 100% storm water treatment (*Synedra, Stephanocyclus, Staurosirella*) potentially differs from the findings of Genter et al. (1987) and Serra et al. (2009). Genter et al. (1987) found that community composition shifted from diatoms to filamentous green algae to unicellular green algae as Zn concentrations increased. Similarly, Serra et al. (2009) found that green algae and cyanobacteria were more tolerant than diatoms of continuous high concentrations of Cu (100 μg/L) for five weeks. The results of the present experiment may reflect not only the effects of metals on the algal community, but also the effects of nutrients. Individual species may have responded more strongly to the nutrient influence than the metals, and this could explain some of the inconsistency with the general findings of Genter et al. (1987) and Serra et al. (2009). Also, diatom species vary greatly in their tolerance to pollutants; even species within the same genus can respond differently. In this experiment, diatoms were only identified to genus, so it is possible that the unknown species present were tolerant of the 100% treatment.

The algal community composition in this experiment did not appear to have been substantially influenced by the presence of nutrients in the water. In areas with increased nutrients, filamentous chlorophytes, mobile and stalked diatoms (DeNicola et al. 2006), as well as monofilaments (Hillebrand 2003) have been shown to increase in abundance. The only results from the present experiment that were consistent with these findings were the dominance of *Mougeotia*, a loose canopy taxa, in the 100% storm water treatment and the significantly greater biovolume of *Staurosirella*, also a loose canopy taxon, in the 100% treatment compared to the 0%. The rest of the results of the present experiment were not consistent with the findings of DeNicola et al. (2006) and Hillebrand (2003) because loose canopy taxa were also dominant in most treatments in the 50% and 0% storm water treatments. The abundance of loose canopy taxa was actually significantly less in the 100% treatment than in the 0%.

As noted in the 2008 mesocosm experiment, loose understory taxa were dominant in the 100% storm water treatment, as has been observed elsewhere (Maltby et al. 1995, Newell and Walsh 2005). Understory taxa may have been most abundant in the 100% storm water treatment because they were less exposed to toxins in the water and therefore less vulnerable. Loose understory taxa may respond in a similar manner as firm understory taxa because of their location within the algal community matrix. Tightly attached species, such as *Achnanthes,* have been shown to be more likely to survive under metal stress because they are embedded in an organic matrix acting as a boundary towards metal toxicity (Burkholder et al. 1990).

Factors Affecting Storm water potency

The storm water in this experiment was expected to be potent, in part due to the high traffic volume on the road draining to the study site, but this high volume may not have translated to high concentrations of pollutants in the storm water if most of the constituents were not washed off the road during the storm event. The intensity of rainfall in this experiment was 0.2 cm/hr, which is significantly less than the 13.3 cm/hr required to wash off the maximum amount of pollutants (Egodawatta et al. 2007). Most common storm events are not capable of removing all of the built-up pollutants on the impervious surface and often remove only a fraction of the available pollutants (Vaze and Chiew 2002, Egodawatta et al. 2007).

In addition to the lack of potential potency in the road runoff water, the (relatively) higher concentration of metals and nutrients (except TP) found in the pipe storm water during runoff was not observed in the 100% storm water mesocosms at the beginning of the experiment. Cr, Cu, Ni, Pb, and Zn were all present in the pipe storm water; however, Ni and Zn were below detection limit in all samples taken from the mesocosms on day 1 of the experiment. Even for the metals present in the mesocosm (Cr, Cu, Pb), there was a ~40-70% reduction in concentration of these metals from the pipe storm water to the water in the 100% storm water tanks at the beginning of the experiment. The pipe storm water samples may have overestimated the concentration of contaminants in the large volume of water (~ 1900 L) pumped into the tank for the experiment. Toxin concentrations during high flow may change dramatically in minutes, thus grab or composite flow-weighted samples may not replicate what organisms would realistically be exposed to (Crunkilton et al. 1996), nor accurately represent all of the water in the tank. Additionally, although the first flush water was collected, Barrett et al. (1998) suggested that the length of the first flush is variable and this effect may be limited to small volumes of runoff. The concentration of pollutants in the water generally decreases after the first flush because a greater intensity of rain is required to wash off more firmly attached constituents (Sansalone and Cristina 2004). Therefore, most of the toxin concentrations in the water collected for the mesocosms may have been dilute.

A third explanation for the lack of storm water effect is that the metals present in the tanks may not have been available to the algae. After the storm water was collected, it was stored in the ~1,900 L plastic tank for less than 24 hrs, so it is unlikely that adsorption of the metals on the container walls occurred during this time period (Struempler 1973). Once in the mesocosm tanks, however, many of the toxic compounds may have bound to the walls, settled out of the water, and/or became bound to settling particles during the experiment, which would decrease their bioavailability (McCarthy and Black 1988). The storm water runoff was very turbid when it was first pumped into the mesocosms, but within a few days most of the suspended solids had settled out and the water was fairly clear for the remainder of the experiment. In this experiment, turbidity declined from 10.3 NTU to 3.2 NTU during the first week. Unfortunately, the settling particles were not analyzed for metals, so it is not known if metals were settling out of the water column.

Finally, not all the cells within the periphyton matrix may have been exposed to toxins in the water to the same extent. Rose and Cushing (1970) found that after algal communities were exposed to zinc, the metal was found mainly on and within the upper layers of the community, indicating that a diffusion gradient existed within the algal mat. The transport rate of ions through algal mats has been shown to decrease with increased algal density (Stevenson and Glover 1993). Stevenson and Glover (1993) found that ion concentrations within the algal mat were significantly lower in samples with dense (AFDM of 8.9 mg cm^{-2}) compared to sparse (AFDM of 4.5 mg cm^{-2}) algal cover. Due to the very low algal biomass in our experiment (AFDM of 1.6 mg cm^{-2}), however, it is likely that a strong diffusion gradient did not exist to protect some cells from exposure to constituents in the water.

Effects of Snails

Snails appeared to have an effect on algal biomass in the 0% and 100% storm water treatments. The significantly less biomass present in the 0% storm water treatment presumably was attributed to snail grazing. This decline in biomass was not accompanied by a significant change in algal respiration or GPP. A difference in metabolic activity between the samples exposed to snails and those not exposed was expected because areal-specific GPP generally declines as biomass is removed due to grazing because less algal material is present to photosynthesize (Steinman 1996). Another possible result would have been for biomass-specific GPP to increase because senescent cells, which contribute to biomass but not productivity, are removed (Lamberti et al. 1989, Steinman 1996). Algal cells higher in the algal mat can block both light and nutrients from reaching cells lower in the mat, thus reducing those cells' ability to photosynthesize (Tuchman 1996). The reduction in biomass in the snail-exposed treatments in this experiment may not have resulted in an increase in biomass-specific GPP because the algal mats may not have been thick enough to create a light and nutrient diffusion barrier.

In the 100% storm water treatment, the concentrations of Chl *a* and AFDM were significantly greater in the presence of snails. The 100% treatment contained the highest concentrations of metals compared to the other treatments, and this may have negatively impacted the snails. Exposure to high levels of metals has been shown to negatively affect the consumption rates of land snails (Notten et al. 2006). Despite an increase in algal biomass in the 100% treatments, algal metabolism was not significantly affected in these treatments. Modest grazing pressure has been shown to increase primary productivity by increasing the availability of resources to the remaining cells (Lamberti et al. 1987, Abe et al. 2007). The results of our experiment, in which algal GPP, although not statistically significantly different, did tend to be lower in the presence of snails, suggest that at the time of sampling, the algal communities in the 100% may have been stressed.

It is also possible that the snails in the 100% treatment augmented the nutrients available to the algae via excretion. Herbivores can increase algal biomass by excreting nutrients (Liess and Hillebrand 2004) and freshwater snails are known to excrete ammonia and ammonium compounds (Friedl 1974). In general, the removal of biomass from grazing outweighs any positive effect of the additional nutrients (Mulholland et al. 1991). In this experiment, however, the nutrient excretion explanation may be valid if snail grazing pressure is reduced because of exposure to metals. Although the difference was not statistically significant, snail growth rates were lower in the 100% storm water treatment than in the 50% and 0% treatments both in terms of changes in mass and total length (Keiper unpubl. data); this adds support to the hypothesis that the snails were negatively impacted by the metals in the 100% treatment. Whether this affect is applicable to other members of the invertebrate community is unresolved at present.

Snails had a slight impact on the algal community composition. Significant grazing pressure results in a decline in the percentage of overstory taxa in the algal community (Steinman 1996), and grazed communities are often dominated by taxa with prostrate growth forms. Lowe and Hunter (1988) found that both low and high density of the grazer, *Physa integra*, significantly reduced the abundance of *Mougeotia,* a taxon with a loose canopy growth form. This is not consistent with the results of the present study because within the treatment containing snails, *Mougeotia* occupied a much higher relative percentage of total cells and biovolume in the 0% treatment compared to the 100% treatment. However, a study by Steinman et al. (1987) found that algal growth form can differ based on grazer density, especially when snails were the grazers. Steinman et al. (1987) reported that *Stigeoclonium tenue*, a taxon with a filamentous growth form, was present in samples exposed to low snail densities and because of spatial patchiness in the feeding behavior of the snails, displayed a patchy distribution of large and small individuals. In the present experiment, the distribution of *Mougeotia* consisted of many small filaments (1-2 cells) and some larger filaments (10-15 cells), so this is consistent with the results of Steinman et al. (1987).

The influences of nutrients and grazing are often strongly interdependent and depend on treatment intensities. Hillebrand (2002) conducted a meta-analysis of 85 experiments and found that nutrient enrichment and the removal of grazers had positive effects on algal biomass in all studies. Although algal biomass was strongly controlled by both nutrients and grazing, Hillebrand (2002) found that the absence of grazers had a stronger effect on algae than nutrients alone. This is supported Pan and Lowe (1994), who found that algal biomass increased when exposed only to phosphorus enrichment, but when exposed to phosphorus enrichment and grazers, the biomass was not significantly higher than communities without nutrient addition. Although our experiment did not examine nutrients directly, we did find that algal biomass increased when grazers were present in the 100% storm water treatment (which contained the highest concentrations of nutrients); this likely was because of the negative effect of metals on snails. Rosemond et al. (1993) found that nutrient and grazer effects were both important and neither had overwhelming control over the algae, although the herbivore effects seemed most important in determining algal community structure. In general, algal species were positively affected by nutrients were negatively affected by snail grazing (Rosemond et al. 1993). In our experiment, *Mougeotia* may have been positively affected by nutrients, but was not negatively affected by snail grazing.

Effects of Fish

Because pumpkinseed sunfish are not known to consume algae (Becker 1983), it is not surprising that their presence did not reduce algal biomass. The fish were rarely observed inside the exclosures containing the algal samples, but were often observed adjacent to the edge of the tiles containing the samples. Their movement did not remove biomass and only slightly affected community composition.

If the fish swam and moved inside the exclosures, however, their movement could have dislodged algal biomass and altered community composition (Hill and Knight 1987, Lamberti et al. 1989, Cattaneo and Mousseau 1995). This effect was not observed in the present experiment because *Mougeotia,* a loose canopy taxa, had the highest percentage of cells in the samples regardless of fish presence or absence, and did not decline in the presence of fish. The cell number and biovolume of small *Achnanthes* and *Cocconeis,* firm understory taxa, significantly increased when fish were present in the 2008 experiment. These changes suggest that the physical disruption caused by the fish movement in the 2008 experiment resulted in a shift towards taxa more tightly adhered to the substrate and therefore less vulnerable to dislodgement, similar to the effects of grazing or other types of disturbance (Steinman and McIntire 1990). This change was not seen in the 2009 experiment because the experimental design was altered slightly from the 2008 experiment. A brick shelter for the fish was placed in the mesocosms and this reduced the amount of time the fish spent inside the treatment exclosures and thus their impact on the algae.

In conclusion, storm water concentration did not have an overall effect on algal biomass or metabolic activity in this experiment; based on comparisons with other studies, it seems likely that relatively low concentrations of contaminants in the storm water were responsible for the absence of a strong effect on algal function. Algal community composition was slightly affected by storm water, suggesting that it is a more sensitive measure of water quality than biomass. The presence of snails caused a decline in algal biomass in the 0% storm water treatment only, and the presence of fish did not cause a decrease in algal biomass. Due to the variable nature of storm water runoff, this type of experiment is very context-specific, with the results heavily influenced by the composition of runoff water from one location and one storm event. Given that the storm runoff was collected from a major storm and during first flush, when concentrations should be relatively high, changes in hydrology, such as the increased frequency and magnitude of erosive flows, may have a greater impact on algal communities in this natural setting than the chemical composition of the storm water.

IV.G. Laboratory Fish Experiments

Overall, storm water did not impact actual or instantaneous growth of central mudminnows in any of the five experiments. However, storm water did impact survival of central mudminnows in some of the experiments. Both the summer 2008 and the 2009 snowmelt trials had significant mortality that we attributed to runoff source and concentration. In contrast, the 2011 snowmelt experiments did not affect growth or survival, but did show evidence of effects on condition of central mudminnows.

The lack of a strong effect of storm water on fish is consistent with the lack of a strong effect on algae. The same reasoning can be applied: the concentrations of metals in the storm water runoff likely were not high enough to negatively impact fish. There were occasional significant effects on some aspects of fish, but they varied with time, space, and response variable, suggesting again the ecological impacts of storm water runoff are very context-specific.

V. Synthesis and Recommendations

Urbanization is a pervasive issue that severely impacts stream ecosystems around the world. Many general effects of urbanization on streams are well known (Walsh et al. 2005), but two of the major effects are the input of storm water runoff from impervious surfaces and changes in stream hydrology. Both of these effects have the potential to impact biotic communities, which are sensitive to changes in water chemistry and flow.

Little Black Creek is highly impacted by urbanization and the biota are impacted as well. Years of urban expansion and industry along LBC have altered the stream, and these lingering effects may have a greater impact on biota than the inputs of storm water runoff. The sediments in LBC are contaminated with a number of metals and organic chemicals which arose from a petroleum refinery, storm sewers draining foundry and metal finishing industries, a plating Superfund site, a municipal sanitary/industrial wastewater pump station, and a closed municipal landfill without a leachate collection system (MDEQ 2000; MDEQ 2002). The substrate at the sampling sites in LBC used for the field experiments was almost entirely sand, and this is the case for many sites along LBC (Cooper et al. 2009).

Although storm water from our study sites contained elevated concentrations of pollutants that are potentially harmful to aquatic life, it did not result in downstream concentrations that exceeded Michigan water quality standards. Depending on the duration and volume of snowmelt events, the potential exists for episodic stress to biota during these events. Despite these changes in concentration, the storm water may not have been potent enough to elicit a response from the biota and/or the constituents in the storm water were not in a form that could impact the biota. Hence, it does not appear that the chemical concentration of storm water entering LBC from U.S. 31 or Seaway Drive is a major contributor to stream biota impairment.

The one aspect of storm water runoff that was potentially toxic according to our results was snowmelt. Snow collected from the roadside at our sites contained concentrations of chloride, copper, and zinc that exceeded state standards for acute effects to aquatic life. With concentrations 2-5X greater than the acute standard, chloride is the pollutant most likely to have negative effects on biota during snowmelt events.

In addition to the effects of constituents within storm water, the increased current velocity associated with storm water runoff can have negative impacts on stream biota due to sloughing of attached organisms, altering of habitat (e.g. sedimentation and erosion), and impaired food web interactions. Average discharge increased during storm events, and resulted in elevated sediment loads (suspended and bedload) at the downstream sampling locations at both sites, although SSC concentrations remained below the 80 mg/L suspended sediment target for wet-weather events. However, storm water SSC did fall into the less than moderate range for the protection of fish communities, suggesting the possibility of impairment.

Our findings result in a set of recommendations for MDOT regarding storm water runoff from U.S. 31 and Seaway Drive into Little Black Creek:

1) Hydrology control (reduce erosion and minimize sediment movement)
    - Work with the Muskegon Area Municipal Storm Water Committee (MAMSWC) on identifying and implementing storm water retention best management practices (BMPs) in the watershed.
    - Determine if the discharge rates at the U.S. 31 pump station can or need to be modified to reduce damaging storm water flows while still controlling groundwater discharge.
2) Snowmelt control (reduce toxic inputs)
    - Place snow piles in locations where snow melt will flow on to pervious surfaces and not directly reach streams.
    - Implement BMPs to handle snow melt runoff than cannot reach pervious surfaces.
3) Wetland management (maintain more natural flow regime and improve habitat)
    - Maintain wetlands already in place (e.g., LBC between Seaway and Summit)
    - Restore fringing wetlands throughout watershed
4) Structural BMPs
    - Evaluate the feasibility of installing hydrodynamic separators to remove sediments at select locations

Storm water is extremely variable, even among different storm events at the same location, so our experimental results are very context-specific. It is possible that road runoff from other areas draining into LBC may contain higher levels of pollutants than storm water from U.S. 31 and Seaway Drive, and have a greater effect on biota.

VI. References

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