Effects of Natural and Anthropogenic Non-Point Source Disturbances on the Structure and Function of Tributary Ecosystems in the Athabasca Oil Sands Region

by

Christina Louise Suzanne
B.Sc., University of Calgary, 2009

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Supervisory Committee

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Abstract

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A multi-integrative approach was used to identify spatial and temporal relationships of natural and anthropogenic environmental variables affecting riverine ecosystem structure and function in the Athabasca Oil Sands Region (AOSR). A series of inter-related field studies were conducted to assess three key components of the freshwater food web (physico-chemical environment, basal productivity, benthic macroinvertebrates) utilizing an *a priori* environmental disturbance gradient experimental design. The gradient design was formulated to best discriminate the possible effects of natural and anthropogenic environmental variables on two river basins (Steepbank and Ells Rivers) each having different levels of oil sands (OS) land use disturbance. Findings from this study showed that natural variation explained most longitudinal and seasonal responses of physico-chemical environmental variables for both rivers, including possible mechanisms such as physical and chemical effects from the OS geological deposit and inputs from shallow groundwater upwelling. Basal productivity was likely controlled by natural variables within the Steepbank and Ells Rivers, such as potential OS deposit effects, nutrient availability and influences from turbidity and physical factors, with disturbance from OS development either negligible or not detected. Longitudinal and seasonal differences in benthic macroinvertebrate community composition were mostly related to natural variation, including possible mechanisms such as high discharge and sediment slump events on the Steepbank River, and community shifts from elevated metal concentrations from natural sources at upstream sites on the Ells River. This study demonstrated that developing baseline information on watersheds can be essential at discriminating sources of disturbance, with natural variation potentially confounding with anthropogenic factors. This study also highlights the need for further research to obtain an improved

understanding of mechanistic pathways to better determine natural and anthropogenic non-point source disturbances and cumulative effects on the structure and function of tributary ecosystems in the AOSR at relevant spatial and temporal scales.

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CHAPTER 1: INTRODUCTION

1.1 General Introduction

Large-scale anthropogenic land use perturbations on fluvial landscapes have been shown to impose ecological consequences on the structure and function of freshwater ecosystems (Carpenter et al. 1998; Dudgeon et al. 2006; Palmer et al. 2010). Human land use activities of this extent are readily observed in the watersheds of the Athabasca Oil Sands Region (AOSR) situated in northeastern Alberta, Canada. Since the late 1960s, landscapes surrounding the AOSR have been increasingly altered in relation to enhanced land-clearing, mining extraction operations and associated infrastructure development (Shiell and Loney 2007; Humphries 2008). Oil sands (OS) extraction along the Athabasca River has potential environmental effects on the surrounding ecosystem including air emissions, water use, wastewater production, potential surface and groundwater contamination, as well as land and habitat disturbances (Environment Canada 2011a).

The AOSR is located within the McMurray Formation (McMF), an early Cretaceous deposit of bitumen, sand, water and clay (Carrigy 1959). Many of the tributaries draining into the lower reaches of the Athabasca River are incised into the oil rich McMF, allowing potential for exposure of hydrocarbon-related non-point source contaminants into surrounding watersheds (Mossop and Flach 1983). Therefore, determining sources and pathways of disturbance is challenging due to co-variation from natural and anthropogenic non-point source perturbations (Allan 2004). This project focusses on two basins within the AOSR, the Steepbank and Ells River watersheds, due to their comparable catchment sizes, differences in land use disturbance, and situation within the OS geologic formation.

Numerous studies have been previously conducted in the AOSR, although, uncertainties still exist in assessing non-point source disturbances from OS development on surrounding riverine basins. These include: (1) determination of appropriate structural and functional endpoints for impact measurements; (2) extent of co-variation between anthropogenic and natural gradients; and (3) cumulative effects of multiple anthropogenic stressors on the ecosystem. Changes in benthic macroinvertebrate communities have been used as a structural and functional tool to assess the integrity of freshwater ecosystems in the AOSR, as they are sensitive to small physico-chemical and biological changes in lotic environments (Bonada et al. 2006).

1.2 Study Objectives

The purpose of this research is to use a multi-integrative approach to identify spatial and temporal relationships of natural and anthropogenic environmental variables on riverine ecosystem structure and function in the AOSR. A gradient sampling design of catchment-scale disturbance is implemented involving two Athabasca River tributaries (Steepbank and Ells Rivers), encompassing differing stages of OS land use activities. Specifically, three main objectives will be addressed separately in the next chapters (3, 4, and 5):

1) Examine the effects of natural and anthropogenic non-point source disturbances on physical and chemical environmental variables in Athabasca River tributaries. Investigate the within- and among-site and between-river basin physico-chemical spatial and temporal differences. (Chapter 3)

2) Examine the effects of natural and anthropogenic non-point source disturbances on the basal productivity of aquatic food webs in Athabasca River tributaries. Investigate the within- and among-site and between-river basin spatial and temporal differences in algal and biofilm biomass. (Chapter 4)

3) Examine the longitudinal and temporal differences in benthic macroinvertebrate community structure influenced by natural and anthropogenic non-point source disturbances on Athabasca River tributaries. This is assessed by:

- *Identifying which physico-chemical and basal production variables explain variation in benthic macroinvertebrate community composition.*
- *Determining whether elemental mercury can be identified as a contaminant at the base of the aquatic food web.* (Chapter 5)

Chapter 2 provides detailed information about the study area and experimental design. Chapter 6 contains general conclusions and recommendations on future research.

1.3 Background Literature

Anthropogenic catchment-scale disturbances have a measurable effect on lotic ecosystems (Resh and Grodhaus 1983; Petersen et al. 1987). Land use changes are considered the primary stressor on freshwater ecosystems, with watershed perturbations, and non-point source contamination being the greatest disturbances (Carpenter et al. 1992). Spatially, freshwater systems are impacted from local habitats to watershed ecosystems, as well as temporally at certain times of the year (Resh et al. 1988). Moreover, the local habitat and biological diversity of streams and rivers are largely influenced by both landform and land use within the surrounding landscape (Allan 2004).

A riverine ecosystem assessment can determine the condition of the watershed (Allan 2004); although, linking land use disturbance to measurable structural and functional endpoints in aquatic ecosystems is a key challenge because of the variety of biological, chemical, hydrological and geophysical components that must be incorporated (Gergel et al. 2002). Examining community structure of benthic macroinvertebrates has frequently been used in environmental monitoring and assessment of freshwater systems (Reynoldson and Metcalfe-Smith 1992). Patterns of species distribution and abundance are important elements of river health but often contribute little to an understanding of how a system works (Harris 1994). Therefore, ecosystem-level processes, such as benthic metabolism, are useful measures of freshwater integrity because they provide a holistic response to a wide-range of catchment-scale disturbances (Bunn et al. 1999).

Multi-integrative approaches investigating key components of the freshwater food web, including the physico-chemical environment, basal production and benthic macroinvertebrate communities can be implemented for riverine ecosystem assessments, through the measurement of a variety of structural and functional responses. Associating these responses to relevant environmental variables can assist in the discrimination of natural and anthropogenic disturbances (Barbour and Yoder 2000; Figure 1.1). Consequently, understanding the relationships between anthropogenic perturbations and lotic freshwater integrity is complex and many uncertainties and challenges still prevail, especially with regards to OS development (Shiell and Loney 2007).

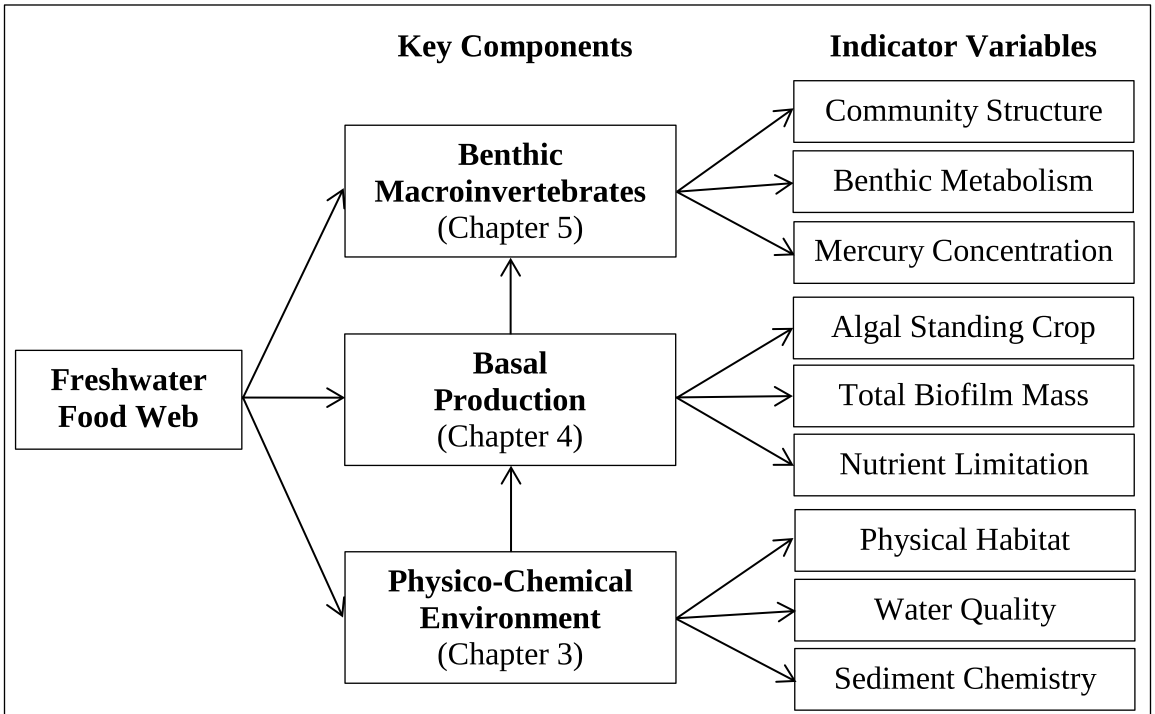

Figure 1.1. Conceptual diagram of the relationships among key components of the freshwater food web incorporated in this study and the relevant indicator variables which were quantified to assess river ecosystem health.

1.4 Current State of Knowledge on the Athabasca Oil Sands Region (AOSR)

One of the greatest landscape perturbations in Canada is the result of mining the AOSR. The geological deposit in the AOSR is one of four OS deposits in northern Alberta, Canada, containing an estimated 1.7 trillion barrels of bitumen. The deposit in the lower Athabasca River basin is the largest in North America and covers an area of 42,000 km^2 surrounding the town of Fort McMurray (Headley et al. 2005; Figure 1.2). The stratigraphy of the area is variable, creating complex river catchment geomorphology across the lower Athabasca River basin and associated tributaries. A significant portion of the bitumen in the AOSR is contained within fluvial deposits of the McMF that outcrop in the surrounding rivers catchments creating a natural oil seep into the watershed (Mossop and Flach 1983).

Figure 1.2. Map of the Athabasca Oil Sands Region (AOSR), Alberta, Canada.

Organisms living in these river systems have the potential for chronic exposure to low levels of naturally derived hydrocarbons in the water or on the substrate (Barton and Wallace 1980).

Bitumen trapped in the McMF in Alberta is one of the most important hydrocarbon sources in the world (Hubbard et al. 2011). Oil production from bitumen extraction in Alberta began with the Great Canadian Oil Sands Company in 1967 but started expanding rapidly around 2000 (CEMA 2003; Humphries 2008; Howell et al. 2014; Figure 1.3). The OS deposits in Alberta contain an overall estimated 170 billion recoverable barrels of bitumen, which make it second only to Saudi Arabia in proven oil reserves (Kraemer et al. 2009). About 80% of the reserves are considered recoverable by *in situ* methods (e.g., Steam Assisted Gravity Drainage; SAGD), and 20% by surface mining (Alberta Energy 2011). In 2012, bitumen production in Alberta exceeded 1.8 million barrels per day (b/d), and is expected to reach approximately 4.5 million b/d in 2025 following current trends (CAPP 2013).

A multitude of agencies have focused on the potential consequences of OS development on the surrounding landscape and river ecosystems in the AOSR, including: Alberta Environment (AENV), Environment Canada (EC), Regional Aquatics Monitoring Program (RAMP), and the Alberta Oil Sands Environmental Research Program (AOSERP; 1975-1985). However, several independent expert review panels have recently scrutinized the largest program, RAMP (industry-funded, multi-stakeholder environmental monitoring program), as their studies are likely insufficient to assess this landscape-scale disturbance with a lack of scientific oversight and inability to detect effects (Dillion et al. 2011).

In 2011, Environment Canada, in conjunction with the province of Alberta, implemented a two-phased monitoring plan (Canada-Alberta Joint Oil Sands Monitoring Program (JOSMP)) to quantitatively define any potential effects OS development may have on the regional air, land and water ecosystems (Environment Canada 2011b, c). With projected future development of the AOSR, further research is necessary to produce a precise representation of the consequences of disturbance on the surrounding region (Headley et al. 2001). Specifically, this thesis addresses prevalent uncertainties in assessing environmental effects of this rapidly-paced, large-scale perturbation on surrounding lotic ecosystems.

Figure 1.3. The first oil sands (OS) production facility, originally named The Great Canadian Oil Sands, now Suncor Energy Inc., adjacent to the Athabasca River (Production: 1967-Present).

1.5 Knowledge Gaps

To achieve a holistic characterization of the watersheds situated within the AOSR, information of both structural and functional components of the riverine ecosystems is required (Gessner and Chauvet 2002). Stressors from OS development can cause changes to structure but not function, function but not structure, or to both (Matthews et al. 1982). Structural and functional integrity are linked (Cummins 1973); however, functional responses, until recently, were often neglected from traditional ecosystem assessments (Bunn and Davies 2000). With the exclusion of functional components, an adequate ecosystem-scale assessment is limited, which can result in an inaccurate representation of potential OS developmental disturbance on surrounding freshwater environments.

Another challenge of assessing perturbations on fluvial ecosystems as a result of mining the AOSR is differentiating between variations caused by developmental disturbance and natural landscape features (e.g., natural oil seeps), also known as co-variation (Allan 2004). Patterns in landscape, created by geology and vegetation type, can account for variations in benthic macroinvertebrate community patterns at large spatial scales (Corkum 1999). Alternatively, the various OS development techniques (e.g., open-pit mining vs. *in situ* recovery (SAGD)) and development stages (e.g., land-clearing vs. mining extraction) among catchments can influence instream physico-chemical and biological variables, as well as riverine ecosystem structure and function (Sponseller et al. 2001).

Thus, separating the consequences of OS development from other catchment-scale features is complicated by the physical characteristics of rivers and streams being influenced by a variety of integrated landscape topographies (Richards et al. 1996). Understanding the hierarchical organization of fluvial habitats is necessary to comprehend how each scale is expected to incorporate factors that directly influence the biological assemblage (Frissell et al. 1986; Minshall 1988). Nevertheless, there are limited studies that assess the strength of the relationships between OS development and physico-chemical and biological processes occurring at different spatial and temporal scales.

An example of a poorly quantified stressor from OS development is pollutants from aerial deposition associated with bitumen mining and processing. Atmospheric deposition is a potentially important pathway of trace metals and polycyclic aromatic hydrocarbons (PAH) input to the landscapes surrounding the AOSR (Bari et al. 2014). PAHs are widely recognized as toxic, carcinogenic, or mutagenic and are relatively persistent in the environment (Headley et al. 2001; Yang et al. 2014). Kelly et al. (2009, 2010), and subsequently Kirk et al. (2014), documented higher than previously reported loadings of airborne particulates found in snowpack samples at sites located near upgrading facilities on the Athabasca River and tributaries. During spring melt, snow from the landscape, as well as on the frozen top of the river is deposited; however, there are no current studies on the effects of this contaminant-pulse on the *in situ* biota.

Mercury (Hg) is another contaminant of concern in the AOSR because of possible toxic effects from fish consumption, as well as the potential for Hg methylation (Kelly et al. 2010). Bioaccumulation of methylmercury (MeHg) in aquatic food chains needs to be investigated (Clarkson 2002), because fish form the major route of MeHg transfer to higher trophic levels (Langley 1973). Greater concentrations of elemental Hg particulates have been measured in snowpack samples on watersheds more disturbed by OS development compared to less developed landscapes, and Hg is found to be more common in highly developed areas (Kelly et al. 2010; Kirk et al. 2014).

Focusing on an individual stressor does not encompass the overall consequences of human modifications to freshwater ecosystems (Schindler 2001). Investigating perturbations from OS development on a landscape-scale allows for the incorporation of various anthropogenic non-point source perturbations. Measuring their cumulative effects is necessary for a comprehensive understanding of the river and its associated basin. Improved methods for cumulative effects assessment supported by integrated environmental effects monitoring are required to study local and landscape-scale perturbations from OS development (Dubé et al. 2006). Cumulative effects assessment assists in linking the different scales of environmental assessment (Therivel and Ross 2007), with understanding the consequences of incremental and accumulating perturbations of OS development on lotic ecosystems (Dubé et al. 2006).

1.6 Multi-Integrative Approach

An integrative analysis of physico-chemical, basal production and benthic macroinvertebrate variables was implemented to investigate the degree of ecosystem alteration from OS development on a landscape-scale. The diversity of habitats and stressors present in the AOSR suggests that a range of sampling methods of various parameters over several seasons is necessary to appropriately quantify the variation in key abiotic and biotic components of river environments. Below are the specific physico-chemical and basal production parameters (Table 1.1), as well as benthic macroinvertebrate community composition, benthic metabolism, and mercury concentration variables (Table 1.2) collected in this study, and a short explanation of the importance of each variable.

Table 1.1. Physico-chemical and basal production variables collected in this study and an explanation as to why they are important for riverine ecosystem assessments.

Indicator Variable	**Rational**
i) Physical Habitat Characteristics	The local physical environment directly influences the aquatic ecosystem assemblage (Maddock 1999). For example, flow velocity (i.e., fast or stagnant), water depth, and substrate composition (i.e., cobbles or sand) determine which organisms will inhabit a certain environment (Stark 1993). Additionally, discharge measurements can illustrate any hydrological extremes during the sampling period, which can ultimately influence the distribution of aquatic biota (Poff et al. 1997).
ii) Water Quality Parameters	Numerous land use patterns within a watershed can account for some of the variability in river water quality (Hunsaker and Levine 1995). Sampling water quality at a given location and time, as well as continuously over seasons can provide critical information in regards to the sampling environment. Spatial and temporal changes in water column chemistry, such as nutrients, metals and total dissolved solids (TDS) can indicate potential perturbations on the lotic ecosystem (Wetzel 1983).
iii) Sediment Chemistry	Metals and contaminants located in the river-bottom and fine sediments will directly influence aquatic biological communities, especially with the accumulation of sediment contaminants. Benthic macroinvertebrates reside in the river-bottom; therefore, assessing contaminant levels in this location provides insight into exposure levels of benthic biota over time (Reynoldson 1987).
iv) Algal and Biofilm Biomass/Nutrient Limitation	Benthic algae are critical primary producers in rivers, providing essential food sources for the biological community (Bott 2006). Moreover, periphyton is predominantly biofilm composed of microbial communities which contribute substantially to the ecosystems energy transfer (Lock et al. 1984). Nutrient limitation regulates algal primary and biofilm production in many rivers, and is an important component of stream ecosystem function (Wold and Hershey 1999). Nutrient availability varies both spatially and temporally in these systems because of seasonal and hydrological events (i.e., spring freshet).

Table 1.2. Benthic macroinvertebrate community composition, benthic metabolism and mercury concentration variables collected in this study and an explanation as to why they are important for riverine ecosystem assessments.

Indicator Variable	Rational
i) Community Composition	Benthic macroinvertebrates are sensitive to ecosystem changes. Alterations to the freshwater environment can influence the structure of the benthic community, with intolerant individuals being lost (e.g., EPT), and more tolerant taxa dominating (e.g., certain species of Chironomidae; Resh and Unzicker 1975). General declines in benthic macroinvertebrate community diversity and species abundance can result from many factors, including water quality degradation, chemical pollutants and streamflow alterations (Pesek and Hergenrader 1976; Rios and Bailey 2006; Dewson et al. 2007).
ii) Benthic Metabolism	The amount of organic carbon produced and consumed in rivers can be measured to determine longitudinal variations in carbon supply and demand in a river continuum (McTammany et al. 2003). Ecosystem respiration (ER) and net primary productivity (NPP) can be used to calculate gross primary productivity (GPP), and measurements of primary productivity, respiration, and factors that influence these rates provide information on energy relationships of aquatic communities (Osborne and Davies 1981). Understanding interactions among species that influence total productivity and energy mobilization, such as benthic macroinvertebrates, is fundamental in assessing ecological dynamics in aquatic ecosystems (Power 1984).
iii) Mercury Concentration	Total Hg (THg) and MeHg are contaminants of concern within benthic macroinvertebrates residing in river ecosystems of the AOSR, with the potential of bioaccumulation of MeHg to higher trophic levels (Clarkson 2002). Assessing Hg concentrations in benthic macroinvertebrates provides information on spatial and temporal movement of Hg in the river environment as well as a potential disturbance to invertebrate communities (Clements 1994).

1.6.1 Hypotheses

Numerous studies investigating the responses of catchment-scale disturbances on lotic ecosystems have shown that there will potentially be an identifiable signal of change in one or more measures of ecosystem structure and/or function along the environmental disturbance gradient (Palmer et al. 2010). Sites downstream of OS mining development could demonstrate altered physico-chemical conditions such as, low pH and high levels of TDS, sulfates, and nutrients, as well as increased sedimentation and physical perturbations of natural benthic substrates, which has been shown in other studies investigating the impacts of mining on river ecosystems, such as Bruns (2005). Sediment chemistry may exhibit elevated levels of metals and contaminants at sites downstream of mining activity, as a result of cumulative loadings from upstream disturbances (Axtmann and Luoma 1991).

Furthermore, sites within the OS geological deposit, specifically the McMF, would possibly contain heavy metals, PAHs and major ions that co-occur with elevated levels of naturally occurring petroleum hydrocarbons (Maclock et al. 1997). Additionally, large pieces of bitumen that erode from the river bank in the OS deposit are transported downstream, where they bind together to form a bedrock-like substrate. This substratum decreases surface area and sheltered areas for invertebrate colonization, potentially reducing invertebrate abundance (Barton and Wallace 1979).

Studies investigating the effects of crude oil on algal autotrophic production in freshwater ecosystems have demonstrated growth inhibition to occur in higher concentrations (Soto et al. 1975; Miller et al. 1978; Federle et al. 1979; Barsdate et al. 1980). Moreover, Bott et al. (1978) observed benthic algal communities with prolonged exposure to crude oil, shifted to a heterotrophic state as a result of deteriorated algae development, increased bacterial activity and reduced photosynthesis. Variations in nutrient availability in relation to algal standing crop have also been demonstrated among tributaries in the AOSR. Physical factors were found to be more important in controlling standing crop size than nutrient levels; however, nutrient limitation was an influential factor on epilithic algal standing crop and the individual species (Hickman et al. 1983). Non-point source nutrient inputs from catchment-scale disturbances could also potentially influence the nutrient availability in the downstream river ecosystems (Carpenter et al. 1998).

Longitudinal changes in benthic macroinvertebrate community structure from human land use activities could demonstrate signs of alteration through a decrease in taxonomic richness and diversity, uneven distributions and low numbers of sensitive taxa (Glozier et al. 2002). Previous studies on benthic macroinvertebrates in the AOSR determined less diversity in the community situated in the OS deposit, in comparison to sites outside of the deposit, even before large-scale OS development began (Barton and Wallace 1979). Furthermore, crude oil has been found to physically impede the gills of sensitive Plecoptera and Trichoptera species, affecting their respiration, and ultimately their abundance (Simpson 1980). Thus, sites within the OS deposit are expected to have lower Plecoptera and Trichoptera species abundances.

Based on the results of Kelly et al. (2009, 2010) and Kirk et al. (2014), sites closer to large-scale OS development or, "hot spots", are anticipated to contain aerial contaminants in water, sediment, and benthic macroinvertebrates, in greater concentrations than areas outside of these sites. Contaminants can alter ecosystem structure by reducing sensitive species which may initiate a trophic cascade or a release from competition that secondarily leads to responses in tolerant species. Furthermore, contaminant-induced changes in nutrient and oxygen dynamics may also alter ecosystem function (Fleeger et al. 2003).

Seasonal variations can influence lotic environmental variables through events such as increased discharge during spring freshet, summer rainfall events, and colder temperatures and freeze-up in fall (Bonsal et al. 2006; Ouyang et al. 2006). Impervious landscapes from human land use activities can also modify timing and quantities of catchment runoff during seasonal storms (Dunne and Leopold 1978). Therefore, comparing two basins with similar catchment geomorphology, but dissimilar land use disturbances can assist in determining both spatial and temporal consequences of non-point source perturbations.

Given the background information, changes in ecosystem structure and/or function along the environmental disturbance gradient are possible in relation to natural and anthropogenic non-point source perturbations, with greater evidence of ecosystem alteration from OS development on the Steepbank River. The implemented gradient design and multi-integrative approaches will ultimately facilitate the discrimination of natural versus anthropogenic environmental drivers on various components of tributary ecosystems in the AOSR. It is important to note that assessment of benthic metabolism as a functional endpoint will be exclusively addressed in subsequent papers to the thesis manuscript, utilizing *in situ* lotic respiration experiments similar to Osborne and Davies (1981).

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CHAPTER 2: STUDY DESIGN

To assess the effects of natural and anthropogenic non-point source disturbances on river ecosystems in the Athabasca Oil Sands Region (AOSR), an *in situ* based field experiment was conducted during the winter, spring, summer and fall seasons of 2012. An environmental disturbance gradient sampling design was implemented on two Athabasca River tributaries (Steepbank and Ells Rivers), which have comparable geomorphology but different levels of oil sands (OS) land use disturbance. A comparative analysis of the study systems investigated the potential consequences of different stages of the development process (i.e., land-clearing vs. open-pit mining) both spatially and temporally on the riverine basins.

The Steepbank River is an east-side tributary to the Athabasca River, with a heavily-developed watershed, and the Ells River is a west-side tributary with a catchment presently only disturbed by land-clearing for future development. Both rivers have similar watershed sizes, stream order, substrate composition, river gradients, and situation within the McMurray Formation (McMF; Headley et al. 2001; RAMP 2012). The gradient sampling design was intended to distinguish natural versus anthropogenic non-point source disturbances on the lotic ecosystems. Four sampling sites were located on the Steepbank River and three on the Ells River, from areas of low to high relative land use disturbance. Sites were also selected in “hot spot” areas of atmospheric deposition of contaminants from industrial upgraders, as identified by Kelly et al. (2009) and Kirk et al. (2014).

A multi-integrative approach was implemented during the 2012 field sampling campaign. Various sampling techniques were employed to collect physico-chemical and biological variables along the environmental disturbance gradient throughout all sampling seasons, which provided a comprehensive investigation of environmental stressors on these freshwater systems. Generalized linear parametric models and non-parametric, multivariate permutation tests were performed to assess within-, among-site and between-basin relationships for physico-chemical, basal production, and benthic macroinvertebrate variables. Ordination analyses were conducted to determine which environmental variables could explain variation in benthic macroinvertebrate community structure.

Studies to date examining the potential effects of OS development on surrounding river ecosystems in the AOSR have not routinely measured physico-chemical and biological variables with the experimental design implemented in this study for the Steepbank and Ells Rivers. Therefore, this chapter describes the study area, gradient sampling design, seasons sampled, and the statistical analysis, which were utilized to assess the effects of natural and anthropogenic non-point source disturbances in two tributaries of the AOSR.

2.1 Study Rivers and Area Description

The Steepbank and Ells River watersheds are situated within the Athabasca River basin in the expansive Canadian Boreal Plains (Schindler and Lee 2010). The Athabasca River watershed overlies the largest OS deposit in Alberta (Conly et al. 2002). Environmental concerns regarding OS development on river ecosystem health are concentrated in the lower Athabasca River basin, downstream of Fort McMurray (Figure 2.1).

Mining extraction for bitumen occurs extensively in the lower Athabasca River basin, with most of the bitumen presently extracted through surface mining processes, compared to *in situ* recovery (Steam Assisted Gravity Drainage; SAGD; Giesy et al. 2010). Surface mining in the AOSR is the second largest disturbance in the boreal region of Alberta, after naturally occurring fires (Allen 2008). The surface mineable area available in the AOSR is approximately 4,800 km^2, with currently 1,670 km^2 of the mineable surface either been mined or approved for development over the next decades (Government of Alberta 2014). The cleared area intensity for surface mining is 0.094 km^2/million barrels, with forests and wetlands cleared, dug up and drained before open-pit mining commences (Pembina Institute 2010). To date, 770 km^2 of boreal forest have been disturbed by this practice in northern Alberta (~0.2% of Alberta's boreal forest; Government of Alberta 2014).

For these reasons, this project focused exclusively on surface mining, compared to *in situ* recovery (SAGD), due to its extensive landscape-scale disturbance. Relevant open-pit mining project boundaries and statuses of development (as of 2012), including

2012 field sampling sites within the different bedrock formations of the AOSR are depicted in Figure 2.1.

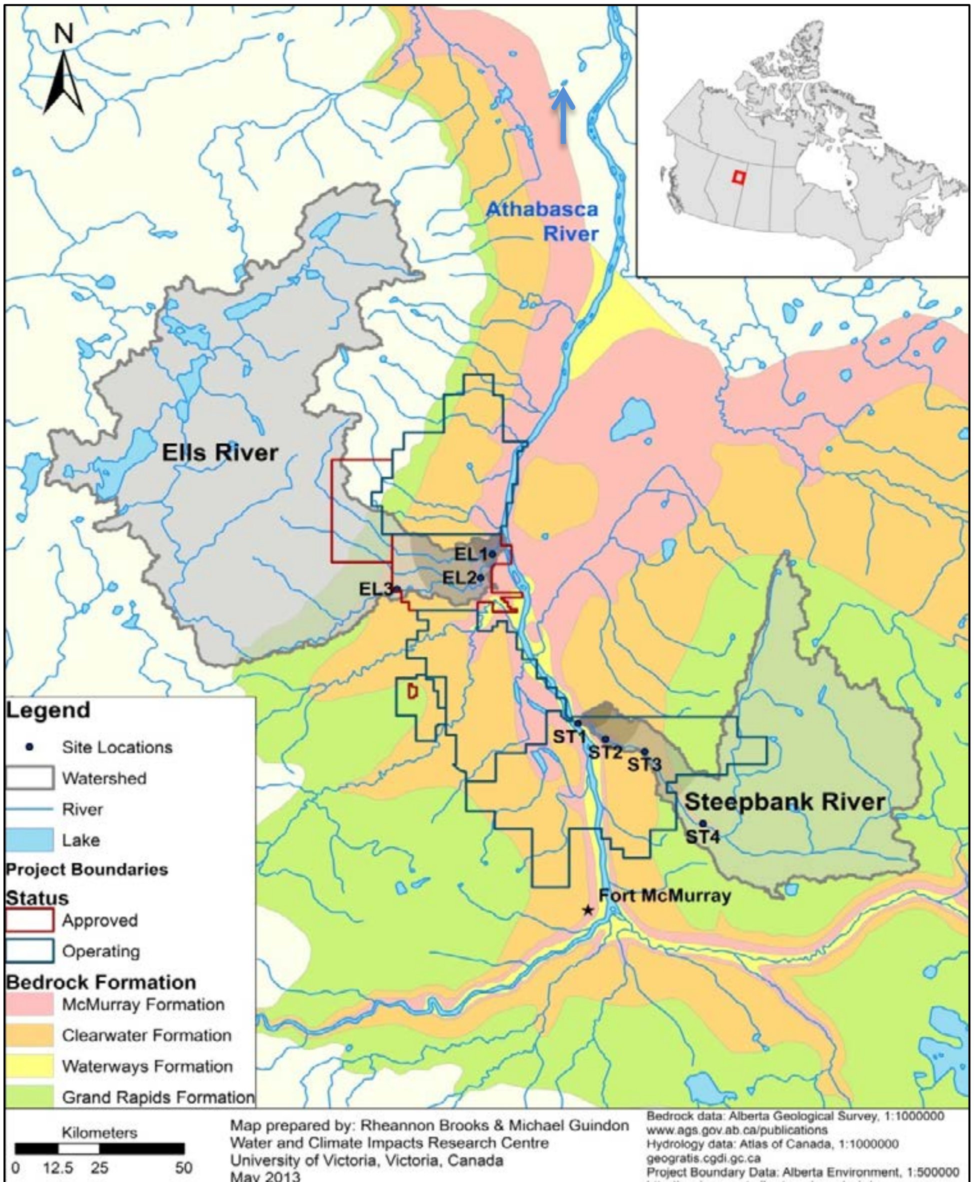

Figure 2.1. The Steepbank and Ells River watersheds and 2012 sampling sites situated in the geomorphology of the Athabasca Oil Sands Region (AOSR). Relevant oil sands (OS) project boundaries for open-pit mining are outlined*. Grey shading in catchments depicts the environmental disturbance gradient from low to high (Steepbank River sites: ST4 to ST1) and low to medium (Ells River sites: EL3 to EL1) from upstream to downstream.

*****Area of open-pit mining project boundaries was adjusted by source after map was produced.

2.1.1 Steepbank River

The Steepbank River originates on the south slopes of Muskeg Mountain at an elevation of approximately 580 m. It drains an area of 1,355 km^2 before discharging into the Athabasca River approximately 40 km downstream of Fort McMurray (RAMP 2012; Figure 2.2). The Steepbank River has a historical mean annual discharge of 6.17 m^3/s (1972-2008), with the spring freshet being the major discharge event, and additional discharge attributed to rainfall events (nival-pluvial regime; WSC 2012). The only major tributary to the Steepbank River is the North Steepbank River, which flows south and drains an area of 525 km^2 (Sekerak and Walder 1980). Periodically, the discharge of the Athabasca River exceeds 1,130 m^3/s, resulting in the water level of the lower Steepbank River to rise, altering the flow regime in the lower reaches (Barton and Wallace 1979).

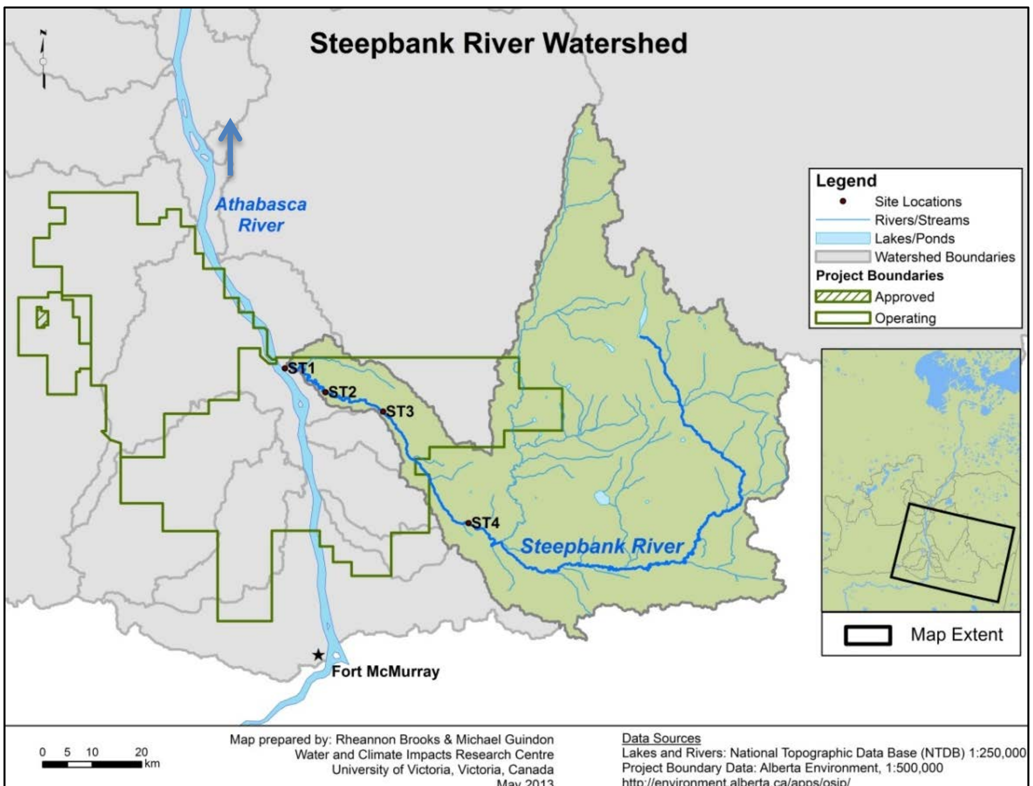

Figure 2.2. The Steepbank River watershed with 2012 sampling sites and relevant OS project leases for open-pit mining.

The Steepbank River is a fifth-order stream (1:50,000) draining mostly muskeg in the uplands. The upper reaches have a strong meandering pattern which becomes more confined with weaker and more irregular meanders as it flows though the McMF in the lower reaches (Headley et al. 2001). The river originates in muskeg but has a gradient of 2.4 m km^{-1} for most of its length and consists of riffles and pools, increasing to 5.7 m km^{-1} in the lower 20 km which consists of long riffles and runs with a few true pools (Barton 1980a). 45% of the watershed is in the uplands, 55% is in the lowlands, and the lower reaches of the river are characterized by steep valley walls (RAMP 2012).

The Steepbank River has a predominately erosional habitat throughout its length (RAMP 2012). River substrates in the upper reaches are composed of fine sediments (Griffiths 1973), whereas the lower reaches have underlying limestone and resemble fine-grained asphalt pavement with patches of limestone rubble lying on top or embedded in the surface (Barton and Wallace 1979). Twenty-four fish species have been documented in the Steepbank River (Golder Associates Ltd. 2004). The area of the single operating project for open-pit mining (as of 2012) was approximately 50.14 km^2, or 3.70% of the total watershed (RAMP 2012; Figure 2.2), with expanded OS development beginning in 2001 on the catchment (Alexander and Chambers in press). Therefore, results from the Steepbank River provide potential information about the effects of OS open-pit mining activities on tributary ecosystems in the AOSR.

2.1.2 Ells River

The Ells River originates in the southeast slopes of the Birch Mountains at an elevation of approximately 730 m, and drains an area of 2,450 km^2 about 64 km northwest of Fort McMurray (RAMP 2012; Figure 2.3). The historical mean annual discharge of the Ells River is 7.17 m^3/s (1976-1986), with the spring freshet being the major discharge event, and additional discharge attributed to rainfall events (nival-pluvial regime; WSC 2012). Numerous small tributaries enter the main stem of the Ells River, including the two largest tributaries, the Joslyn and Chelsea Creek. The Ells River has substantial amounts of the watershed hydrology controlled by lake storage, with 44 lakes present in the watershed; however, most are located in the Gardiner Lakes area in the headwaters of the drainage (i.e., Namur-Gardiner lakes; Sekerak and Walder 1980; Headley et al. 2005).

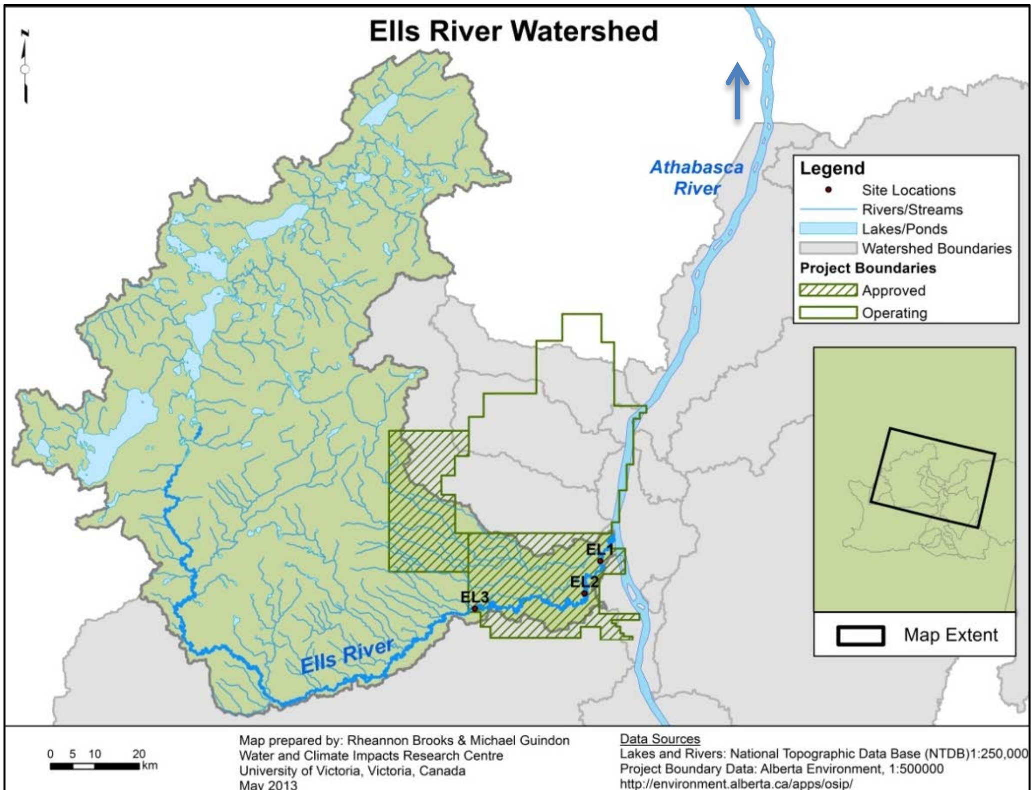

Figure 2.3. The Ells River watershed with 2012 sampling sites and relevant OS project leases for open-pit mining.

The Ells River is a fourth-order stream (1:50,000), with the upper reaches having a strong meandering pattern. It flows through a high gradient reach as it leaves the Birch Mountains, and the river eventually descending to an elevation of approximately 230 m (Sekerak and Walder 1980; Headley et al. 2001). The Ells River then meanders through the boggy landscape of the lower reaches where it cuts down into the McMF, with OS deposits forming part of the bed material (Griffiths 1973). Towards the mouth, the channel becomes more irregular displaying steep cut banks along various lower-gradient reaches (Headley et al. 2001). In general, the Ells River watershed has three distinct physiographic regions - a headwater area (Birch Mountains upland, which is well-drained with numerous streams and lakes, a gradually sloping midstream region (Algar Plain), and a downstream region (Clearwater lowland) of variable gradients (Sekerak and Walder 1980).

The Ells River basin is dominated by a surficial deposit of glacial till overlain by muskeg, and dominant river substrates in the river includes boulders, cobble, and gravel, with some fines (Griffiths 1973). Nineteen fish species have been documented in the Ells River (Golder Associates Ltd. 2004). The total area of the two approved projects (as of 2012) was approximately 260.12 km^2, or 9.6% of the total watershed (Alberta Energy 2012); nevertheless, the area of the single operating project was only 29.05 km^2, or 1.07% of the total watershed (RAMP 2012; Figure 2.3), with initial land-clearing beginning in 2006 on the catchment (Alexander and Chambers in press). Therefore, results from the Ells River will provide baseline data for future assessments of the influence of OS open-pit mining activities on tributary ecosystems in the AOSR.

2.2 Gradient Sampling Design

An environmental disturbance gradient sampling design was implemented to discriminate natural versus anthropogenic non-point source disturbances on the Steepbank and Ells Rivers. In a gradient design, there is no defined reference area, but the response variables are evaluated along the environmental disturbance gradient. This design was utilized to assess any changes in physico-chemical, basal production, and benthic macroinvertebrate variables from upstream to downstream within the Steepbank and Ells Rivers (Table 2.1). This gradient sampling design follows similar protocols as outlined in the Environmental Effects Monitoring (EEM) Technical documents for Pulp and Paper and Metal Mining (Environment Canada 2010a, 2012), as well as Joint Oil Sands Monitoring Program (JOSMP) reports (Environment Canada and AEMERA 2014).

Table 2.1. Environmental disturbance gradient imposed for the site sampling design on the Steepbank and Ells Rivers over the 2012 sampling seasons.

Site	Environmental Gradient
Steepbank River	
ST1	At the mouth of the river nearest to atmospheric deposition sources of upgraders, in the OS geological deposit, and receiving cumulative loadings from upstream disturbances
ST2	Inside the OS deposit with evident upstream land use disturbance from mining operations
ST3	Inside the OS deposit and at the edge of land use disturbance from OS development
ST4	Outside the OS deposit in an undisturbed region of the catchment
Ells River	
EL1	At the mouth of the river receiving cumulative loadings from upstream disturbances and in the OS deposit
EL2	Inside the OS deposit and at the edge of land use disturbance from land-clearing
EL3	Outside the OS deposit in an undisturbed region of the catchment

Sampling sites were also located inside and outside of aerial deposition areas, or "hot spots", with previous studies demonstrating deposition patterns resembled a bulls-eye on the landscape (Kelly et al. 2009; Kirk et al. 2014; Figure 2.4). Under this design, sites located furthest downstream were anticipated to be disturbed the greatest, receiving cumulative loadings from upstream deposition inputs. A further assumption was that deposition rates for atmospheric pollutants should decrease exponentially with increasing distance away from "hot spot" areas, or the geographic center, located close to major OS operations (Garty 2001; Kelly et al. 2009; Cho et al. 2014).

All sampling sites were selected based on previous sampling locations from EC Snow Survey studies (modified from Kelly et al. 2009), RAMP, AOSERP, Phase 1 and Phase 2 of JOSMP, as well as site accessibility and riffle environments (standard habitat type where invertebrate density and diversity are often high; Brown and Brussock 1991). Selected sites varied in cumulative loadings, but were otherwise similar in physical habitat characteristics. A thorough discussion of specific sampling methods for physico-chemical, basal production, and benthic macroinvertebrate community and mercury concentration variables are found in Chapters 3, 4, and 5, respectively.

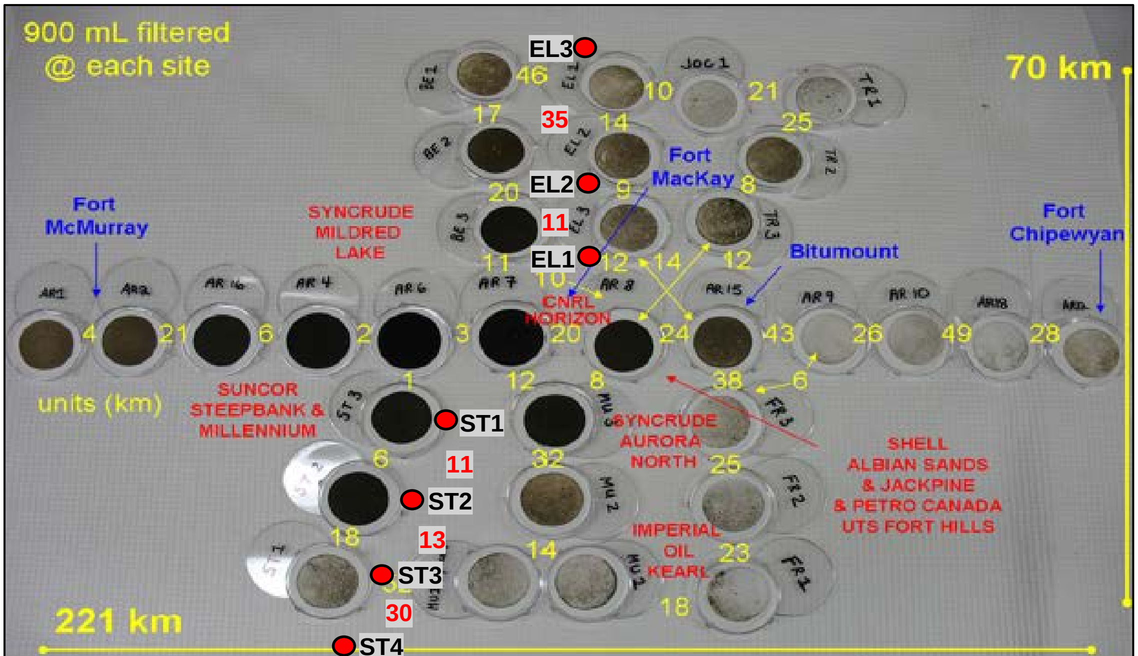

Figure 2.4. "Hot spot" sites sampled in study conducted by Kelly et al. (2009) investigating aerial deposition of OS contaminants. Darker residue on white 0.45-µm Whatman GF/F filters indicates sites with higher levels of airborne particulates, filtered from 900 mL melted snowpack samples. Relevant site locations from this present study on the Steepbank and Ells Rivers are indicated with a red dot. Yellow numbers represent distance (km) between sites in Kelly et al. (2009) study and red numbers represent distance (km) between sites in this study in 2012.

2.3 Sampling Timeline

Sampling for environmental variables, including benthic macroinvertebrate community composition, occurred over four seasons in 2012. Definition of seasons sampled in this study were consistent with other studies conducted in the AOSR (Environment Canada 2011c; RAMP 2012). Each sampling season highlighted temporal differences of environmental variables and community responses from both natural and anthropogenic non-point source perturbations. Specific sampling months and an explanation of what was collected and why is presented in Table 2.2. See Appendix A for a detailed summary of site locations, 2012 sampling seasons, and specific data collected at each site during each sampling period.

Table 2.2. Sampling seasons and data collection which occurred in 2012 for physico-chemical, basal production, and benthic macroinvertebrate community and mercury concentration variables on the Steepbank and Ells Rivers.

Season (2012)	Data Collection
Winter (March)	Under-ice samples were collected during one sampling period. Physico-chemical samples were only collected, as anchor-ice prevented the collection of basal productivity and benthic macroinvertebrate samples. Under-ice sampling was conducted to observe any under-ice chemical and biological activity occurring (Prowse 1994).
Spring (May - June)	Freshet samples were collected during two sampling periods. Physico-chemical, basal productivity, and benthic macroinvertebrate samples were collected during both sampling months. Freshet samples were collected to capture any pulse of terrestrially-derived material or contaminants entering the river ecosystem during spring run-off (Ouyang et al. 2006).
Summer (July - August)	Open-water samples were collected during two sampling periods. Physico-chemical, basal productivity, and benthic macroinvertebrate samples were collected during both sampling months. Open-water samples were collected to investigate the patterns of river ecology during the most productive time of the year (Wetzel 1983).
Fall (September - October)	Before freeze-up samples were collected during two sampling periods. Physico-chemical, basal productivity, and benthic macroinvertebrate samples were collected during both sampling months. Benthic macroinvertebrates are traditionally collected during the fall, due to low flow conditions and most taxa are in their aquatic life stage (Environment Canada 2010b).

2.4 Statistical Design and Analyses

Mixed-Effects Models

Physico-chemical, basal production and benthic macroinvertebrate mercury concentration variables were assessed for differences within- and among-sites along the environmental disturbance gradient using a mixed-effects analysis of variance (ANOVA) over the sampling months ($\alpha = 0.05$; Pinheiro and Bates 2009). Mixed-effects models are comprised of fixed and random effects, with explanatory variables of interest (fixed), and other variables (as nuisance) needed to be accounted for, but not directly interested in (random). The mixed-effects statistical procedure compliments the randomized block design with the block variable set as the random-effect. Moreover, including the random-effects in the analysis increases the power to determine any differences in the fixed-effects (Crawley 2007).

The mixed-effects models used throughout this study were primarily comprised of one fixed and one random-effects parameter. The fixed-effect parameter was sampling site (Steepbank or Ells River), and the random-effect was block which was the sampling month, or the repeated measure over time. The identical two-parameter mixed-effects ANOVA design was used to assess all explanatory variables throughout the study on each river basin. *A posteriori* tests were also performed to determine which factors were significantly different from one another. Mixed-effects models were conducted utilizing R version 2.15.2 (R Development Core Team 2012).

Non-parametric multivariate analysis (PERMANOVA)

Non-parametric methods using permutation tests for multivariate statistical analysis (PERMANOVA) were utilized to determine any significant differences in benthic macroinvertebrate community composition within- and among-sites ($\alpha = 0.05$), while avoiding the strict assumptions of parametric tests. Multivariate analyses are particularly important for intricate ecological systems, such as freshwater ecosystems (Faith et al. 1995; Quinn et al. 1996). Moreover, the assumptions of an ANOVA are not usually met by ecological data, as they rarely fit a normal distribution (Anderson 2001).

PERMANOVA is appropriately conducted for community level data where the responses of multiple non-independent variables are measured in samples from a univariate or multifactorial ANOVA. Pair-wise PERMANOVA *a posteriori* tests were also performed to determine which groups were significant from other groups. Non-parametric multivariate analysis using permutations can be used in experimental designs for ecology that requires multifactorial ANOVA to identify environmental responses from disturbance (Green 1993; Underwood 1993; Glasby 1997). Furthermore, a similarity percentages analyses (SIMPER) was utilized to assess which taxa were most responsible for the spatio-temporal differences within each river basin. The PERMANOVA and SIMPER analyses were conducted utilizing PRIMER version 6.0 (Clarke and Warwick 2013).

Ordination Analyses (RDA)

A canonical redundancy analysis (RDA) was performed to assess which environmental variables directly explained variation in the benthic macroinvertebrate community (Hill and Gauch 1980). Community data and associated environmental measurements typically yield an enormous amount of "noisy" data which are often difficult to interpret. Multivariate methods, such as ordination analyses, provide a means to structure the data by separating relevant variation from noise. Canonical ordination is widely used to identify environmental gradients and their relation to taxonomic composition in ecology, as well as a means of studying seasonal and spatial variation in aquatic communities (ter Braak and Verdonschot 1995). In canonical ordination, biotic data (i.e., taxa) are ordered along environmental gradients, which are constrained linear combinations of environmental variables (Feld and Hering 2007). Ordination analyses were conducted utilizing R version 2.15.2 (R Development Core Team 2012).

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CHAPTER 3: EFFECTS OF NATURAL AND ANTHROPOGENIC NON-POINT SOURCE DISTURBANCES ON PHYSICO-CHEMICAL ENVIRONMENTAL VARIABLES

3.1 Introduction

Human actions at the landscape scale have been shown extensively to disrupt the geomorphic processes that maintain the lotic environment, resulting in habitat that is both degraded and less heterogeneous (Allan 2004). Anthropogenic catchment-scale perturbations in the Athabasca Oil Sands Region (AOSR) can potentially reduce the ecological integrity of river ecosystems through disturbing physical habitat, water and sediment chemistry, and ultimately the biological community via numerous pathways (Allan et al. 1997; Strayer et al. 2003; Townsend et al. 2003). Moreover, matching a specific ecological response to the contributing stressor in the AOSR can be very challenging.

Lotic ecosystems in the AOSR are affected by multiple and interacting disturbances, including co-variation between natural and anthropogenic environmental factors (Allan 2004). Numerous studies have shown that human land uses within a watershed account for the changes in river water quality (Hunsaker and Levine 1995). However, geologic formations within the AOSR as well as shallow groundwater upwelling also contribute inorganic constituents into the surface water of tributaries which flow through reaches with natural oil sands (OS) deposits, specifically the McMurray Formation (McMF; Gorrell 1974; Hackbarth 1981; Maclock et al. 1997). Catchment-scale disturbances can also contribute considerable amounts of fine sediment into aquatic ecosystems (Walling and Fang 2003). Elevated fine sediment levels in the streambed are known to have a wide range of consequences on aquatic biota (Wood and Armitage 1997; Von Bertrab et al. 2013), through changes in habitat and food availability, such as the supply of organic matter and a shift in periphyton quality (Schofield et al. 2004).

Metals and contaminants concentrated in the particulate load are another form of disturbance on the river ecosystem. Fine sediments are the chemically active component of the solid load; thus, many contaminants are transported downstream with the erosion of disturbed landscapes (Axtmann and Luoma 1991). Contaminants of concern may also be transported through natural systems as a result of weathering or leaching from exposed OS seams along the riverbank, in the lower reaches flowing through the McMF (Huang et al. 2014). Nonetheless, organisms living within or near sediments have the potential of being influenced by chronic contaminant exposure from natural and anthropogenic sources (Canfield et al. 1994).

Understanding seasonal variation of physico-chemical variables is also a crucial factor in determining a holistic representation of the fluvial environment in response to catchment-scale disturbance (Ouyang et al. 2006). Seasonality describes the timing of disturbance which is important when characterizing anthropogenic and natural perturbations (Pickell et al. 2014). Spring freshet, summer rainfall and fall freeze-up all influence river discharge and runoff, influencing downstream movement of material, concentration of chemical parameters and transport of terrestrially-derived sediment into the ecosystem (Vega et al. 1998; Woodruff et al. 2001; Ouyang et al. 2006). These events can alter the basic physical and chemical structure of the lotic ecosystem, consequently influencing the river ecology.

Measuring a multitude of physical and chemical environmental variables such as habitat characteristics, water quality parameters and sediment chemistry allows for a thorough evaluation of the rivers abiotic condition to a gradient of land use disturbance. Sampling physico-chemical variables between two basins with varying degrees of land use alteration can assist in determining the consequences of different stages of OS mining activities (i.e., land-clearing vs. open-pit mining) on the integrity of the lotic ecosystem.

Studies to date examining the potential effects of OS development on surrounding river ecosystems in the AOSR have not routinely measured physico-chemical environmental variables utilizing the sampling design implemented in this study (Akena and Christian 1981; Environment Canada 2011b, c; RAMP 2012). Therefore, this chapter evaluates the physico-chemical differences within- and among-sites and between-basins across sampling months, as well as assesses whether observed responses can be attributed to the environmental disturbance gradient. See Chapters 1 and 2 for detailed objectives and predictions for Chapter 3.

3.2 Methods

3.2.1 Field Sampling

Physical Habitat Characteristics were measured within the sampling area at each site and included: 1) flow velocity, 2) water depth 3) substrate composition, and 4) slope. Flow velocity and water depth were measured using a FlowTracker (SonTek) in the riffle at each site during each sampling period. Substrate composition was assessed utilizing the 100 pebble count and slope was measured using surveyor's equipment during simultaneous Canadian Aquatic Biomonitoring Network (CABIN) sampling in low-flow conditions (Environment Canada 2010). Historical average annual discharge and 2012 discharge data were downloaded from the Water Survey of Canada (WSC) and Regional Aquatics Monitoring Program (RAMP) databases for the Steepbank and Ells River hydrometric stations: 07DA006 and 07DA017, respectively.

Water Quality Parameters were collected with a YSI multi-parameter sonde (Yellow Springs Instruments, Idaho, USA) within the sampling riffle at each site and included: water temperature, specific conductivity, dissolved oxygen, and pH. Point samples of field measured water quality parameters were recorded with a calibrated YSI 556 MPS sonde to describe the local sampling conditions during each sampling month, and continuous measurements were recorded every 30 minutes over the 2012 sampling period with a calibrated YSI 6600-V2 sonde. Continuous measurements of standard water quality parameters were collected to highlight any deviations from "normal" conditions, which may not have been documented with a discrete sample (Jarvie et al. 2001).

One bulk water quality sample was collected at each site during each monthly sampling period (May-October 2012). Water samples were taken mid-stream with a 2 L plastic bottle either using a gloved-hand or sampling rod in the water column in areas of constant flow following Standard Operating Procedures (SOPs) outlined by Environment Canada (Environment Canada 2011b). The water was transferred into clean, pre-labeled bottles, and preservative was added as required. Bottles were stored in a chilled cooler and air transported with ice packs within 24 hours of collection for analysis. A comprehensive water quality laboratory analysis was completed on all samples (Nutrients, Cations/Anions, Carbonate Complex, and Physical/Water Quality

Parameters). Water quality total and dissolved metals samples were also collected for future processing and analysis. All water quality parameters and collection methods for samples analyzed in this study are listed in Table 3.1.

Table 3.1. Water quality parameters analyzed in this study and collection methods performed at each site on the Steepbank and Ells Rivers during each monthly sampling period for the 2012 field sampling campaign.

Water Quality Samples	Collection Method	Parameters
Field Measured	YSI 556 MPS sonde, YSI 6600-V2 sonde	Water Temperature (Temp), Specific Conductivity (Cond), Dissolved Oxygen (DO), pH
Nutrients	125 mL NH_3 one time use bottle, filled from 2 L plastic bottle	Total Phosphorus (TP), Total Dissolved Phosphorus (TDP), Total Nitrogen (TN), Total Dissolved Nitrogen (TDN), Dissolved Ammonia (NH_3), Dissolved Potassium (K^+)
Cations/Anions	500 mL plastic bottle, filled from 2 L plastic bottle	Dissolved Calcium (Ca^{2+}), Dissolved Magnesium (Mg^{2+}), Dissolved Sodium (Na^+), Dissolved Chloride (Cl^-), Silicon Dioxide (SiO_2^{2-}), Dissolved Sulfate (SO_4^{2-})
Carbonate Complex	2 L plastic bottle	pH, Total Alkalinity (Alk), Dissolved Organic Carbon (DOC)
Physical/Water Quality Parameters	2 L plastic bottle	Turbidity (Turb), Specific Conductivity (Cond), Total Dissolved Solids (TDS), Total Suspended Solids (TSS)

Sediment Chemistry was analyzed for polycyclic aromatic hydrocarbons (PAHs) and metals chemistry collected from fine sediments contained within rock-basket artificial substrate sediment “traps” (scour pads; Figure 3.1), following a similar design as Simm and Walling (1998). In this study, approximately 50 BBQ ceramic briquettes were placed on top of three scour pads which lined the bottom of three rock-baskets which were deployed at each site within the sampling riffle for a one-month incubation period from March-October 2012. After one month, rock-baskets were retrieved and scour pads were removed and stored in a chilled cooler for the duration of the field sampling day. Scour pads were placed into a -20°C freezer within 8 hours after removal from the river. River-bottom depositional sediment samples were also collected in shallow water using a stainless steel scoop for future sample processing and analysis, following procedures of Headley et al. (2001).

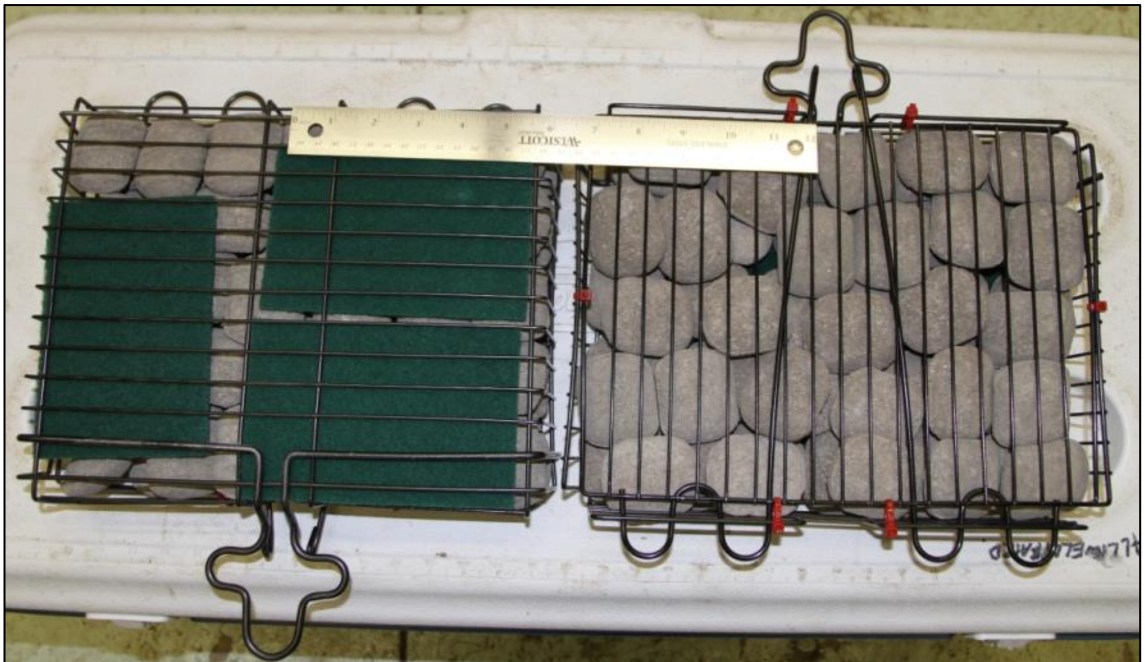


Figure 3.1. Rock-basket artificial substrate top view (right) and bottom view (left) with approximately 50 BBQ ceramic briquettes situated on top of three sediment "traps" (scour pads). Three rock-baskets were deployed at each site on the Steepbank and Ells Rivers during each sampling period and retrieved after one-month from March-October 2012.

3.2.2 Sample Processing

3.2.2.1 Physical Habitat Characteristics

1) Flow velocity (m/s) was measured directly in-front of each of the three rock-baskets during deployment and retrieval at each site and sampling period. Three rock-basket artificial substrates were deployed perpendicular to flow in a row across the river channel within the sampling riffle, where water depth was anticipated to sustain over the one-month. Flow measurements were taken at 0.6 depth for a 30 second duration. Any obstructions in-front of the FlowTracker were removed before recording. Deployment and retrieval success was determined by site-accessibility, wadeable water depth and flow velocity, as well as equipment lost or damaged.

2) Water depth (cm) was measured directly in-front of each of the three rock-baskets during deployment and retrieval using the FlowTracker measurement tool. Rock-baskets were secured to the river bottom with tent pegs and were connected with clothesline to the shore to help prevent equipment loss and damage.

3) Substrate composition (%) was assessed using the 100 pebble count method, which involved the measurement of the intermediate axis of 100 randomly selected pebbles in the sampling area. Substrate sizes were separated into distinct categories to determine substrate percent composition. Assessment of substrate composition was conducted during low-flow conditions (Sep/Oct) to facilitate un-biased selection of all substrata in the sampling area (Environment Canada 2010).

4) Slope (m km^{-1}) was measured at a stable location on the stream bank at each site, which involved one person to walk upstream with a graduated rod that was at least 2 m high. The distance of the person upstream and the height from the water surface was measured using the graduated rod and survey equipment. The person then walked downstream, and the distance of the person downstream and the height from the water surface was measured again. Slope was calculated from the change in height from upstream to downstream over the total distance.

3.2.2.2 Water Quality Parameters

Point measurements of standard water quality parameters were collected during every deployment and retrieval of rock-baskets using the YSI 556 MPS sonde. Data from the continuous logging of the YSI 6600-V2 sonde was downloaded upon retrieval at the end of the 2012 field sampling season. All bulk water quality samples were analyzed using standard protocols at the Environment Canada National Laboratory for Environmental Testing (NLET, Burlington, ON) that has been accredited and certified by CALA to achieve an ISO certification of ISO/IEC 17025.

3.2.2.3 Fine Sediment Chemistry

Scour pad fine sediments were prepared for PAH and metals analysis utilizing the following laboratory protocols:

1) PAHs (ng/g) - Two of the three scour pads from each rock-basket were removed from the -20°C freezer and cut in half using acetone-washed scissors. Acid washed forceps were used to place two halves in an amber glass jar (PAH) and the other two halves in a wide mouth polypropylene container for metals analysis (below). Using Seastar water (AXYS Ltd.), scour pads were rinsed in amber glass jars and shaken 20 times. The two half scour pads were removed from the amber glass jar using acid washed forceps. The water from the glass jar was decanted and the jar was labelled. Samples were sent for analysis of PAHs at AXYS Analytical Services Ltd. (Sidney, BC).

2) Metals (mg/kg) - The other two halves which were placed in a wide mouth polypropylene container (above), received a 16 dram volume amount of Seastar water (AXYS Ltd.) added to the container and shaken 20 times. Two half scour pads were removed from the container using acid washed forceps. The samples were poured into an acid washed 16 dram snap cap container and labelled. A kimwipe and elastic was placed over top of container. Samples were freeze-dried and sent for analysis of recoverable metals and nutrients at NLET (Burlington, ON; Table 3.2).

Twelve elements on the US Environmental Protection Agency (EPA) list of priority pollutants (PPEs) were exclusively included in the data analysis for this study to compliment previous OS studies (Kelly et al. 2010), and are bolded in Table 3.2. Results from fine sediment PAH analysis will be addressed in subsequent papers to the thesis manuscript.

Table 3.2. List of key nutrients, metals and metalloids analyzed from fine sediment samples. Those in Bold print represent the 12 priority pollutant elements (PPEs) which were exclusively included in the data analysis for this study.

Silver (Ag)	Cesium (Cs)	Niobium (Nb)	Tin (Sn)
Aluminum (Al)	**Copper (Cu)**	**Nickel (Ni)**	Strontium (Sr)
Arsenic (As)	Iron (Fe)	Phosphorus (P)	Tellurium (Te)
Boron (B)	Gallium (Ga)	**Lead (Pb)**	Titanium (Ti)
Barium (Ba)	Germanium (Ge)	Palladium (Pd)	**Thallium (Tl)**
Beryllium (Be)	Potassium (K)	Platinum (Pt)	Uranium (U)
Bismuth (Bi)	Lanthanum (La)	Rubidium (Rb)	Vanadium (V)
Calcium (Ca)	Lithium (Li)	Rhodium (Rh)	Tungsten (W)
Cadmium (Cd)	Magnesium (Mg)	Scandium (Sc)	Yttrium (Y)
Cerium (Ce)	Manganese (Mn)	**Antimony (Sb)**	**Zinc (Zn)**
Cobalt (Co)	Molybdenum (Mo)	**Selenium (Se)**	Zirconium (Zr)
Chromium (Cr)	Sodium (Na)		

3.2.3 Statistical Analyses

General linear models (analysis of variance; ANOVA) were used to analyze among-site and monthly differences in physico-chemical environmental variables related to the disturbance gradient hypothesis for the Steepbank and Ells Rivers. ANOVAs tested the following null hypothesis:

- HO1: No change among-sites and months in average physical and chemical variables along the environmental disturbance gradient.

Two-way ANOVAs using mixed-effects models (described in section 2.4), with site as the fixed variable and month (repeated testing over time) as the random, or blocking variable were used to determine site and monthly differences in water depth and flow velocity as well as bulk water and sediment chemistry within the Steepbank and Ells Rivers (a one-way ANOVA was performed for Steepbank River sediment chemistry due to limited site and monthly data). Before running the model, a restricted maximum likelihood (REML) estimation was applied to datasets with smaller sample sizes ($n < 20$) and a maximum likelihood (ML) estimation was applied to datasets with larger sample sizes ($n > 20$), which is a method used for fitting linear mixed models (Kenward and Roger 1997).

Significant interactions between site and month were also examined prior to running the model using a two-factor ANOVA; however, interaction terms were not included in the model to maintain model consistency for all analyses of physico-chemical parameters. After running the model, if a significant difference among-sites and/or months was found ($p < 0.05$), an *a posteriori* test, Tukey HSD, was performed to determine which sites or months were significantly different. All analyses were conducted using R version 2.15.2, utilizing the “nlme” and “multcomp” packages (R Development Core Team 2012).

3.3 Results

3.3.1 Physical Habitat Characteristics

3.3.1.1 Discharge

The historical average daily discharge was determined for the available recorded time period for the Steepbank and Ells River hydrometric stations. Because the historical time period for discharge measurements was different for both rivers, average daily discharge was also determined for consistent years preceding to this study (2009-2011). Discharge data from 2012 was graphed to illustrate the hydrological conditions relevant to when this study was conducted. Historical average daily discharge, average daily discharge from 2009-2011, and the 2012 hydrographs are presented for the Steepbank River in Figure 3.2, and the Ells River in Figure 3.3.

Steepbank River

The historical average daily discharge (1972-2008) of the Steepbank River portrayed a snow-melt driven system with occasional rainfall events throughout the summer months (nival-pluvial regime). Historically, spring freshet began in early April, with freeze-up beginning in early October. In preceding years to this study (2009-2011), the Steepbank River average daily hydrograph had a diminished spring freshet compared to the historical hydrograph, with several precipitation events in the summer and fall months. For 2012, the overall mean annual discharge of the Steepbank River was 6.86 m^3/s. The 2012 hydrograph also displayed a reduced spring freshet compared to the historical hydrograph; however, pronounced discharge events occurred in June/July, as well as in September. The large discharge event in September prevented the monthly collection of bulk water quality samples from all sites (Figure 3.2).

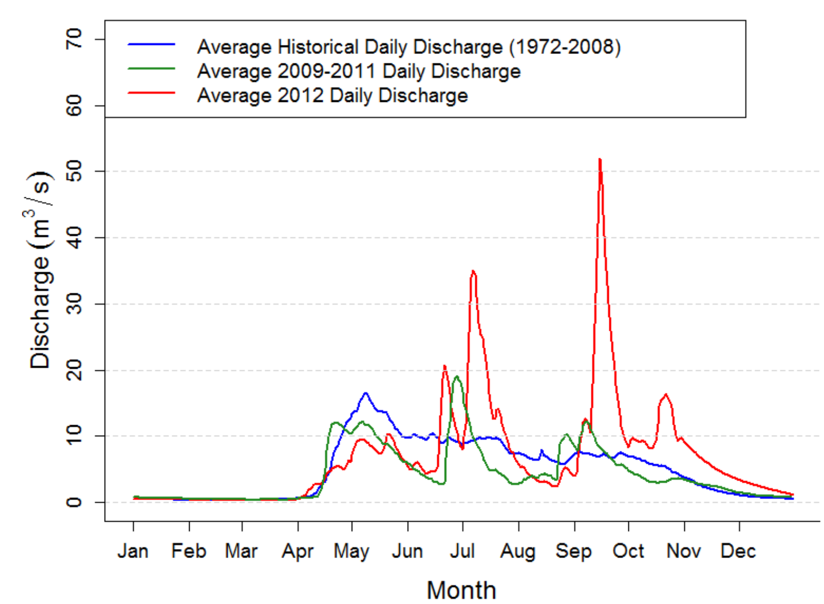


Figure 3.2. Steepbank River historical average daily discharge, average daily discharge (m^3/s) in years preceding this study (2009-2011), and the 2012 sampling year hydrograph.

Ells River

The historical average daily discharge (1975-1986) of the Ells River portrayed a snow-melt driven system with a pronounced freshet and occasional rainfall events throughout the summer months (nival-pluvial regime). Historically, spring freshet began in early April, with freeze-up beginning in late October. In preceding years to this study (2009-2011), the Ells River average daily hydrograph had a diminished spring freshet compared to the historical hydrograph, with greater precipitation-dominated events in the summer. For 2012, the overall mean annual discharge of the Ells River was 8.20 m^3/s. The 2012 hydrograph also displayed a reduced spring freshet compared to the historical hydrograph; however, a spike in discharge occurred at the end of July coinciding with summer precipitation events (Figure 3.3).

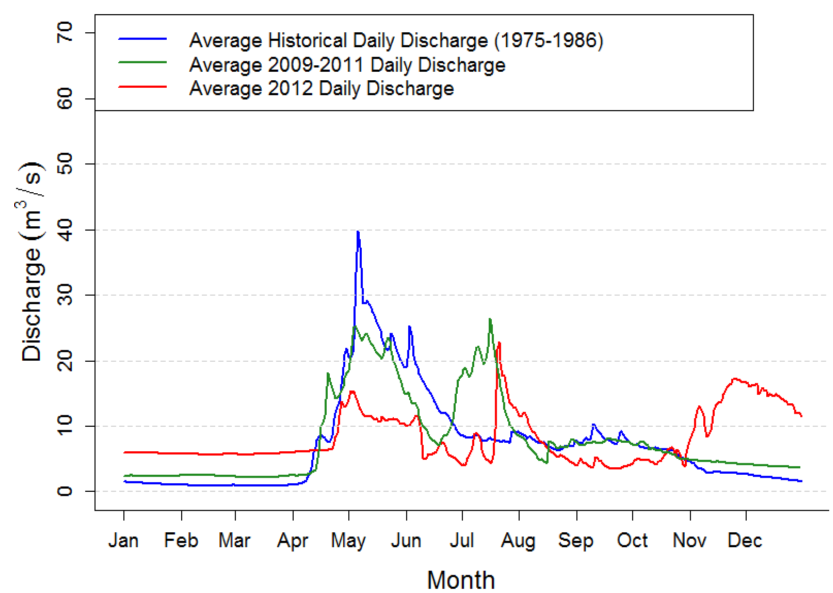


Figure 3.3. Ells River historical average daily discharge, average daily discharge (m^3/s) in years preceding this study (2009-2011), and the 2012 sampling year hydrograph.

3.3.1.2 Flow Velocity and Depth

Steepbank River

Average depth of water where rock-baskets were deployed was significantly greatest at ST2 ($p < 0.05$), and during March deployment ($p < 0.001$). Average flow velocity was also significantly lowest at ST2 ($p < 0.05$), and during March deployment ($p < 0.001$). Due to extreme flooding conditions during the 2012 sampling period on the

Steepbank River, deployment and retrieval success of rock-baskets was limited to the low-flow season (Table 3.3).

Table 3.3. Steepbank River average deployment and retrieval flow velocities (m/s) and water depths (cm; ± standard error of the mean (SE)) for rock-basket artificial substrates for each site and month over the 2012 sampling period. An unsuccessful deployment or retrieval is indicated with "-".

Deployment				Retrieval		
Date (2012)	**Site**	**Average Depth ± SE (cm)**	**Average Flow ± SE (m/s)**	**Date (2012)**	**Average Depth ± SE (cm)**	**Average Flow ± SE (m/s)**
12-Mar	ST1	58.0 ± 0.0	0.32 ± 0.00	-	-	-
13-Mar	ST2	95.0 ± 0.0	0.05 ± 0.00	-	-	-
13-Mar	ST3	103.0 ± 0.0	0.18 ± 0.00	-	-	-
13-Mar	ST4	-	-	-	-	-
11-May	ST1	42.0 ± 2.0	0.62 ± 0.04	-	-	-
09-May	ST2	50.0 ± 5.3	0.83 ± 0.03	-	-	-
09-May	ST3	35.0 ± 3.2	0.68 ± 0.04	-	-	-
09-May	ST4	59.3 ± 3.7	0.29 ± 0.13	-	-	-
20-Jun	ST1	-	-	-	-	-
20-Jun	ST2	-	-	-	-	-
20-Jun	ST3	-	-	-	-	-
20-Jun	ST4	45.0 ± 4.4	0.53 ± 0.10	-	-	-
26-Jul	ST1	-	-	-	-	-
26-Jul	ST2	-	-	-	-	-
26-Jul	ST3	-	-	-	-	-
26-Jul	ST4	41.3 ± 4.7	0.98 ± 0.13	29-Aug	27.7 ± 4.3	0.53 ± 0.18
29-Aug	ST1	37.7 ± 3.2	0.87 ± 0.05	-	-	-
29-Aug	ST2	45.3 ± 2.4	0.67 ± 0.05	-	-	-
29-Aug	ST3	30.3 ± 1.5	0.79 ± 0.07	-	-	-
29-Aug	ST4	48.3 ± 6.9	1.01 ± 0.06	25-Sep	0.0 ± 0.0	0.00 ± 0.00
27-Sep	ST1	48.3 ± 0.3	0.98 ± 0.03	24-Oct	35.0 ± 2.9	0.74 ± 0.04
27-Sep	ST2	-	-	-	-	-
27-Sep	ST3	40.7 ± 0.9	0.87 ± 0.09	24-Oct	47.7 ± 4.3	0.75 ± 0.04
27-Sep	ST4	52.3 ± 6.7	0.47 ± 0.05	24-Oct	54.7 ± 5.9	0.58 ± 0.03

Ells River

Average deployment depth of rock-baskets was significantly lowest at EL2 (p < 0.01), and July had a significantly greater depth than May and June (p < 0.05). There were no significant differences among-sites in average deployment flow velocity, and June and July had significantly greater flows than August and September ($p < 0.001$). Average retrieval depth of rock-baskets was significantly greatest at EL1 ($p < 0.01$), and significantly lowest in August ($p < 0.001$). There were no significant differences among-sites in average retrieval flow velocity, and September had significantly lower flows than June and July ($p < 0.05$). In comparison to the Steepbank River, deployment and retrieval success of rock-baskets was overall greater due to less extreme 2012 hydrological variability on the Ells River (Table 3.4).

Table 3.4. Ells River average deployment and retrieval flow velocities (m/s) and water depths (cm; ± standard error of the mean (SE)) for rock-basket artificial substrates for each site and month over the 2012 sampling period. An unsuccessful deployment or retrieval is indicated with "-".

Deployment				Retrieval		
Date (2012)	**Site**	**Average Depth ± SE (cm)**	**Average Flow ± SE (m/s)**	**Date (2012)**	**Average Depth ± SE (cm)**	**Average Flow ± SE (m/s)**
12-Mar	EL1	-	-	-	-	-
13-Mar	EL2	-	-	-	-	-
13-Mar	EL3	-	-	-	-	-
11-May	EL1	42.7 ± 0.7	0.51 ± 0.00	20-Jun	48.0 ± 0.0	0.75 ± 0.05
11-May	EL2	39.0 ± 2.1	0.90 ± 0.10	20-Jun	45.7 ± 0.9	0.93 ± 0.11
11-May	EL3	-	-	-	-	-
20-Jun	EL1	46.3 ± 3.2	0.75 ± 0.05	26-Jul	62.3 ± 3.9	0.99 ± 0.04
20-Jun	EL2	40.7 ± 3.3	0.98 ± 0.05	-	-	-
21-Jun	EL3	46.0 ± 2.0	0.74 ± 0.02	-	-	-
26-Jul	EL1	62.3 ± 3.9	0.99 ± 0.04	28-Aug	39.7 ± 2.7	0.70 ± 0.09
25-Jul	EL2	39.0 ± 0.0	0.75 ± 0.00	29-Aug	32.0 ± 3.8	0.49 ± 0.23
24-Jul	EL3	59.0 ± 0.0	1.09 ± 0.00	28-Aug	27.0 ± 1.0	0.91 ± 0.06
28-Aug	EL1	50.7 ± 1.8	0.49 ± 0.04	24-Sep	47.7 ± 1.2	0.44 ± 0.04
29-Aug	EL2	53.3 ± 2.9	0.39 ± 0.02	24-Sep	50.0 ± 2.3	0.38 ± 0.05
28-Aug	EL3	45.7 ± 2.0	0.62 ± 0.01	24-Sep	43.0 ± 1.5	0.48 ± 0.00
25-Sep	EL1	50.0 ± 1.2	0.50 ± 0.03	23-Oct	56.0 ± 2.3	0.60 ± 0.03
25-Sep	EL2	41.0 ± 3.5	0.51 ± 0.16	23-Oct	42.7 ± 3.7	0.67 ± 0.14
25-Sep	EL3	46.3 ± 1.9	0.48 ± 0.00	23-Oct	49.7 ± 1.2	0.51 ± 0.01

3.3.1.3 Substrate Composition and Slope

Steepbank River

All four sites on the Steepbank River were composed of cobble, pebble, and gravel, with only ST3 containing small amounts of silt and clay. The presence of cobbles decreased from upstream to downstream, whereas pebbles and gravel increased. Slope decreased from upstream to downstream (Table 3.5).

Table 3.5. Steepbank River % substrate composition quantified using the 100 pebble count method and slope (m km^{-1}) measured once for each site during low-flow conditions in 2012.

Site	Slope (m km^{-1})	% Bedrock	% Boulder	% Cobble	% Pebble	% Gravel	% Sand	% Silt+Clay
ST1	1.25	0	0	16	76	8	0	0
ST2	4.00	0	0	50	45	5	0	0
ST3	7.00	0	0	53.54	37.37	7.07	0	2.02
ST4	6.90	0	0	80	20	0	0	0

Ells River

All three sites on the Ells River were composed of boulder, cobble, pebble, and gravel, with only EL1 containing small amounts of silt and clay, and EL2 containing sand. The presence of cobbles and gravel increased from upstream to downstream, whereas pebbles decreased. Slope was lowest at EL1 and consistent between EL2 and EL3 (Table 3.6).

Table 3.6. Ells River % substrate composition quantified using the 100 pebble count method and slope (m km^{-1}) measured once for each site during low-flow conditions in 2012.

Site	Slope (m km^{-1})	% Bedrock	% Boulder	% Cobble	% Pebble	% Gravel	% Sand	% Silt+Clay
EL1	3.00	0	3	51	33	10	0	3
EL2	4.34	0	2	46	44	2	6	0
EL3	4.36	0	2	27	65	6	0	0

3.3.2 Water Quality Parameters

3.3.2.1 YSI Measurements

The continuous logging of the YSI 6600-V2 sonde produced a comprehensive time series profile of standard water quality parameters over the entire sampling period and the YSI 556 MPS sonde collected point measurements during every sampling month. Each YSI model measured the following water quality parameters: Temperature (Temp; °C), Specific Conductivity (Cond; µS/cm), Dissolved Oxygen (DO; %, mg/L), and pH, for the Steepbank River (Figure 3.4), and the Ells River (Figure 3.5).

Steepbank River

A YSI 6600-V2 sonde was deployed at sites ST1, ST2 and ST4 during the sampling period from March-October 2012. Temp was consistent among-sites, except for extreme variation at ST2 in August. Temp began to increase in early May, coinciding with river ice break-up, and then decreased in October with freeze-up. Cond was also consistent among-sites, with a large decrease during spring freshet. Cond fluctuated around 200 µS/cm for the duration of the sampling period. Temp and Cond measurements from both YSI models were very consistent.

DO at the mouth of the Steepbank River (ST1) was consistent around 100% for the entire sampling period. DO at the most upstream site (ST4) fluctuated around 90% or 10 mg/L, whereas the mid-reach site (ST2) was at approximately 80% or 8 mg/L throughout the sampling period. DO (mg/L) decreased slightly during the summer months for all sites. There were observed differences between DO measurements from the YSI 6600-V2 and YSI 556 MPS sondes, as well as drastic decreases in DO for short time periods at ST1 and ST2. pH measurements also displayed large decreases for short time periods; moreover, probe malfunction is a common occurrence with DO and pH probes on all YSI models (Bienfang 1980; Hartley et al. 2005). Nonetheless, pH maintained slightly basic throughout the sampling period and varied from 7.5-8.5 for all three sites (Figure 3.4).

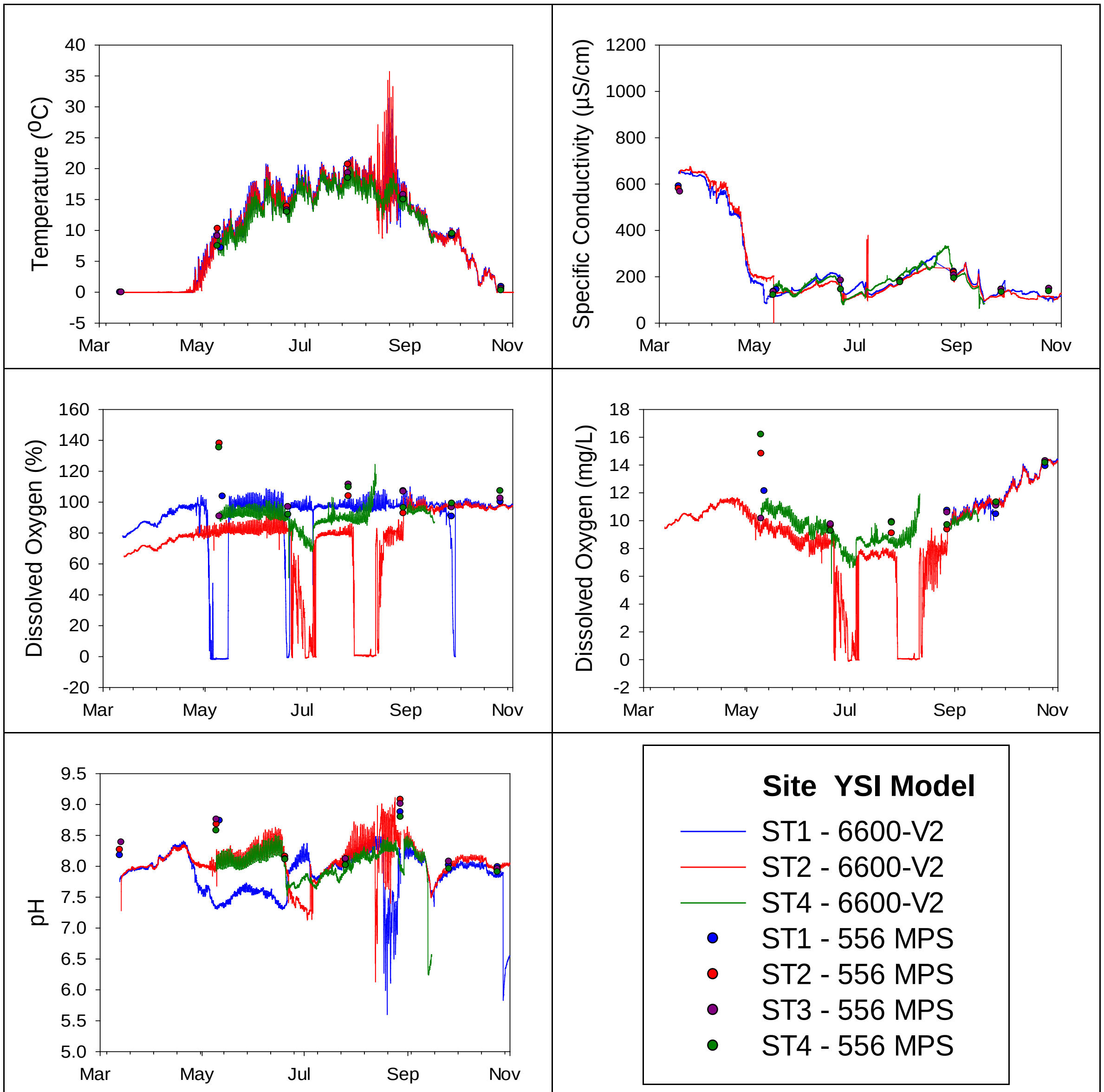


Figure 3.4. Steepbank River water quality parameters (Temperature (°C), Specific Conductivity (µS/cm), Dissolved Oxygen (%, mg/L), and pH) measured using a YSI 6600-V2 sonde for continuous 30 minute measurements and a YSI 556 MPS sonde for point measurements over the 2012 sampling period (March-October).

Ells River

A YSI 6600-V2 sonde was deployed at EL1, EL2 and EL3 during the sampling period from March-October 2012. Temp was consistent among-sites, and began to increase in early May, coinciding with river ice break-up, and decreased in October with freeze-up. Cond was consistent between upstream sites (EL3 and EL2) around 200 μS/cm; however, Cond at the most downstream site (EL1) was extremely variable from May-July. Temp and Cond measurements from both YSI models were very consistent.

DO measurements were comparable among all three Ells River sites which fluctuated around 100% or 10 mg/L for the duration of the sampling period. An increase in DO (mg/L) occurred from summer to fall for all sites. There were a few sudden large decreases in DO which could possibly be attributed to probe malfunction (Bienfang 1980; Hartley et al. 2005). pH maintained slightly basic and fluctuated from 7.5-8.5 for EL1 and EL2; unfortunately, probe failure at EL3 on the YSI 6600-V2 sonde resulted in no pH measurement. There were minor differences in DO and pH measurements between the YSI 6600-V2 and YSI 556 MPS sondes (Figure 3.5).

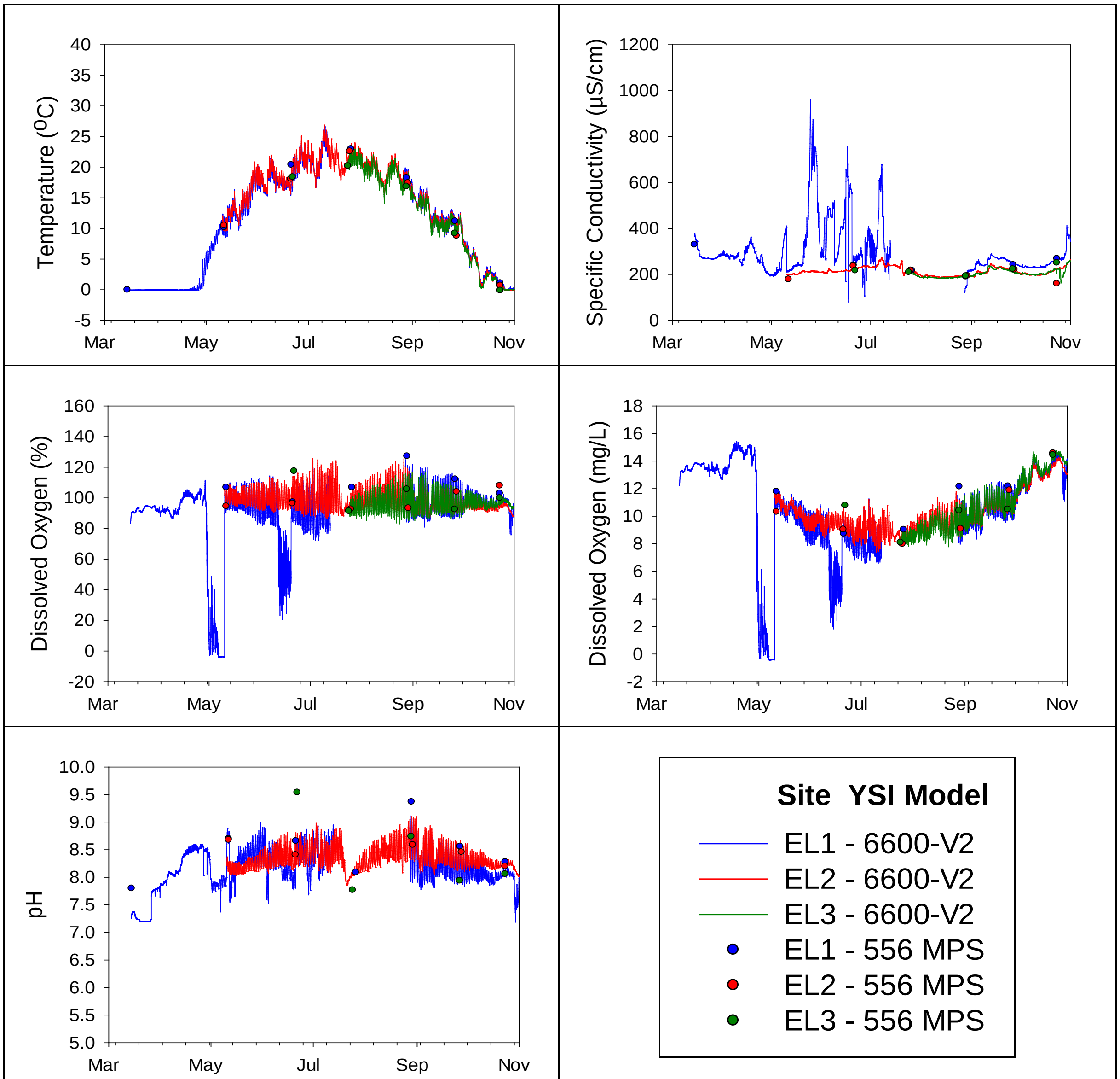


Figure 3.5. Ells River water quality parameters (Temperature (°C), Specific Conductivity (µS/cm), Dissolved Oxygen (%, mg/L), and pH) measured using a YSI 6600-V2 sonde for continuous 30 minute measurements and a YSI 556 MPS sonde for point measurements over the 2012 sampling period (March-October).

3.3.2.2 Bulk Water Quality

Bulk water quality samples were categorized into four functional groups for each river: Nutrients, Cations/Anions, Carbonate Complex, and Physical/Water Quality Parameters, following Moquin (2011). Site and monthly statistical differences are presented for the Steepbank River in Table 3.7, and the Ells River in Table 3.8.

Steepbank River

Nutrients

None of the phosphorus or nitrogen parameters were significantly different among-sites. Potassium (K^+) concentrations were significantly different among-sites, with the most upstream site (ST4) having the significantly lowest concentration. All phosphorus and nitrogen parameters had significantly highest concentrations in June, July and August, whereas K^+ concentration was significantly greatest in May. There was insufficient sample replication for a site x month interaction effect for all nutrient parameters.

Cations/Anions

All cation and anion parameters were significantly different among-sites, with concentrations increasing from upstream to downstream, except for silicon dioxide (SiO_2^{2-}), which had no significant among-site differences. Calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^+) concentrations were significantly highest in August and significantly lowest in October. Chloride (Cl^-) and sulfate (SO_4^{2-}) had significantly greatest concentrations in May, whereas SiO_2^{2-} had significantly highest concentrations in August and significantly lowest concentrations in May and June. There was insufficient sample replication for a site x month interaction effect for all cation/anion parameters.

Carbonate Complex

None of the carbonate complex parameters were significantly different among-sites. pH and total alkalinity (Alk) were significantly highest in August, whereas dissolved organic carbon (DOC) was significantly greatest in July. pH was significantly lowest in July, Alk was significantly lowest in October, and DOC was significantly lowest in May. There was insufficient sample replication for a site x month interaction effect for all carbonate complex parameters.

Physical/Water Quality Parameters

Specific conductivity (Cond) and total dissolved solids (TDS) were the only physical/water quality parameters which were significantly different among-sites with concentrations increasing from upstream to downstream. Turbidity (Turb) and total suspended solids (TSS) concentrations were significantly highest in June, whereas Cond and TDS concentrations were significantly highest in August, and significantly lowest in October. There was insufficient sample replication for a site x month interaction effect for all physical/water quality parameters.

Table 3.7. Summary table of Steepbank River physical/water quality and chemical variables analyzed with a two-way mixed-effects ANOVA, which included a) site and, b) month. Significant *p*-values ($p < 0.05$) for site differences are shaded in grey. Upstream (U/S) to downstream (D/S) changes in variable concentrations are indicated with "+" for increasing and "-" for decreasing, and an interaction column indicates any significant site x month interaction effects. Other than standard chemical abbreviations, the following abbreviations are used: total phosphorus (TP), total dissolved phosphorus (TDP), total nitrogen (TN), total dissolved nitrogen (TDN), total alkalinity (Alk), dissolved organic carbon (DOC), turbidity (Turb), specific conductivity (Cond), total dissolved solids (TDS), and total suspended solids (TSS).

Para-meter	**Fig #**	**Site Differences**	***p*-value**	**U/S-D/S Changes**	**Monthly Differences**	***p*-value**	**Interaction Effect**
Nutrients							
TP	3.6	No	> 0.05	No	> in Jun	< 0.05	No rep.
TDP	3.6	No	> 0.05	No	Oct < Jul, Aug	< 0.01	No rep.
TN	3.7	No	> 0.05	No	> in Jun; Aug > Oct	< 0.01 0.027	No rep.
TDN	3.7	No	> 0.05	No	Jul > May, Jun, Oct; Aug > May, Oct	< 0.01 < 0.01	No rep.
NH_3	3.7	No	> 0.05	No	Jun > Jul, Aug, Oct	< 0.05	No rep.
K^+	3.8	< at ST4	< 0.01	+	> in May; Aug > Jun, Jul, Oct	< 0.001 < 0.05	No rep.
Cations/Anions							
Ca^{2+}	3.9	ST4 < ST1	0.023	+	Aug > Jul > Jun > May = Oct	< 0.01	No rep.
Mg^{2+}	3.9	ST4 < ST2 = ST1	< 0.05	+	Aug > May, Jun, Oct; Jul > May, Oct; Jun > Oct	< 0.001 < 0.01 0.005	No rep.
Na^+	3.10	ST4 < ST3= ST2 < ST1	<0.001	+	Aug > May = Jun = Jul > Oct	< 0.001	No rep.
Cl^-	3.10	ST4 = ST3< ST2; > ST1	< 0.001 < 0.01	+	> in May	< 0.01	No rep.
SiO_2^{2-}	3.11	No	> 0.05	No	Aug > Jul > Oct > Jun = May	< 0.01	No rep.
SO_4^{2-}	3.11	< at ST4	< 0.05	+	> in May; Jun > Jul	< 0.05 0.006	No rep.
Carbonate Complex							
pH	3.12	No	> 0.05	No	< in Jul; Aug > Oct	< 0.05 0.003	No rep.
Alk	3.12	No	> 0.05	No	> in Aug; Oct < Jun, Jul	< 0.05 < 0.05	No rep.
DOC	3.12	No	> 0.05	No	> in Jul; < in May	< 0.01 < 0.001	No rep.

Physical/Water Quality Parameters							
Turb	3.13a	No	> 0.05	No	> in Jun	< 0.05	No rep.
Cond	3.13a	ST4 < ST2 = ST1	< 0.05	+	Aug > May = Jun = Jul > Oct	< 0.01	No rep.
TDS	3.13b	ST4 < ST2 = ST1	< 0.05	+	> in Aug; Oct < Jun, Jul	< 0.01 < 0.05	No rep.
TSS	3.13b	No	> 0.05	No	> in Jun	< 0.05	No rep.

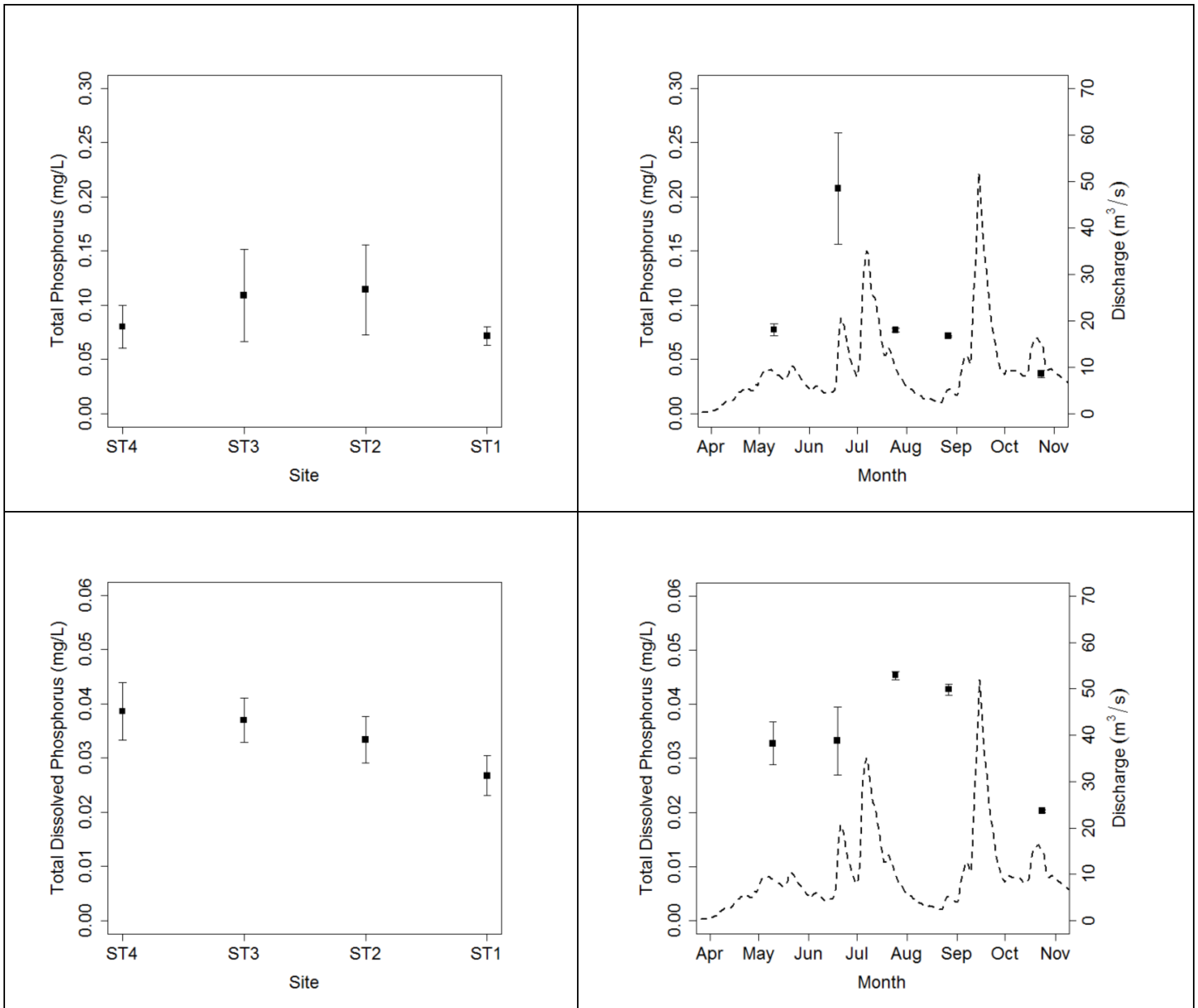


Figure 3.6. *Phosphorus Parameters.* **Top:** Mean concentration of Steepbank River total phosphorus (TP; mg/L) by site (left) and over months (right). There were no significant differences among-sites. TP concentration was significantly greatest in June ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect.
Bottom: Mean concentration of Steepbank River total dissolved phosphorus (TDP; mg/L) by site (left) and over months (right). There were no significant differences among-sites. TDP concentration in October was significantly lower than July ($p = 0.005$), and August ($p = 0.003$). There was insufficient sample replication for a site x month interaction effect.
*Error bars represent the standard error of the mean.

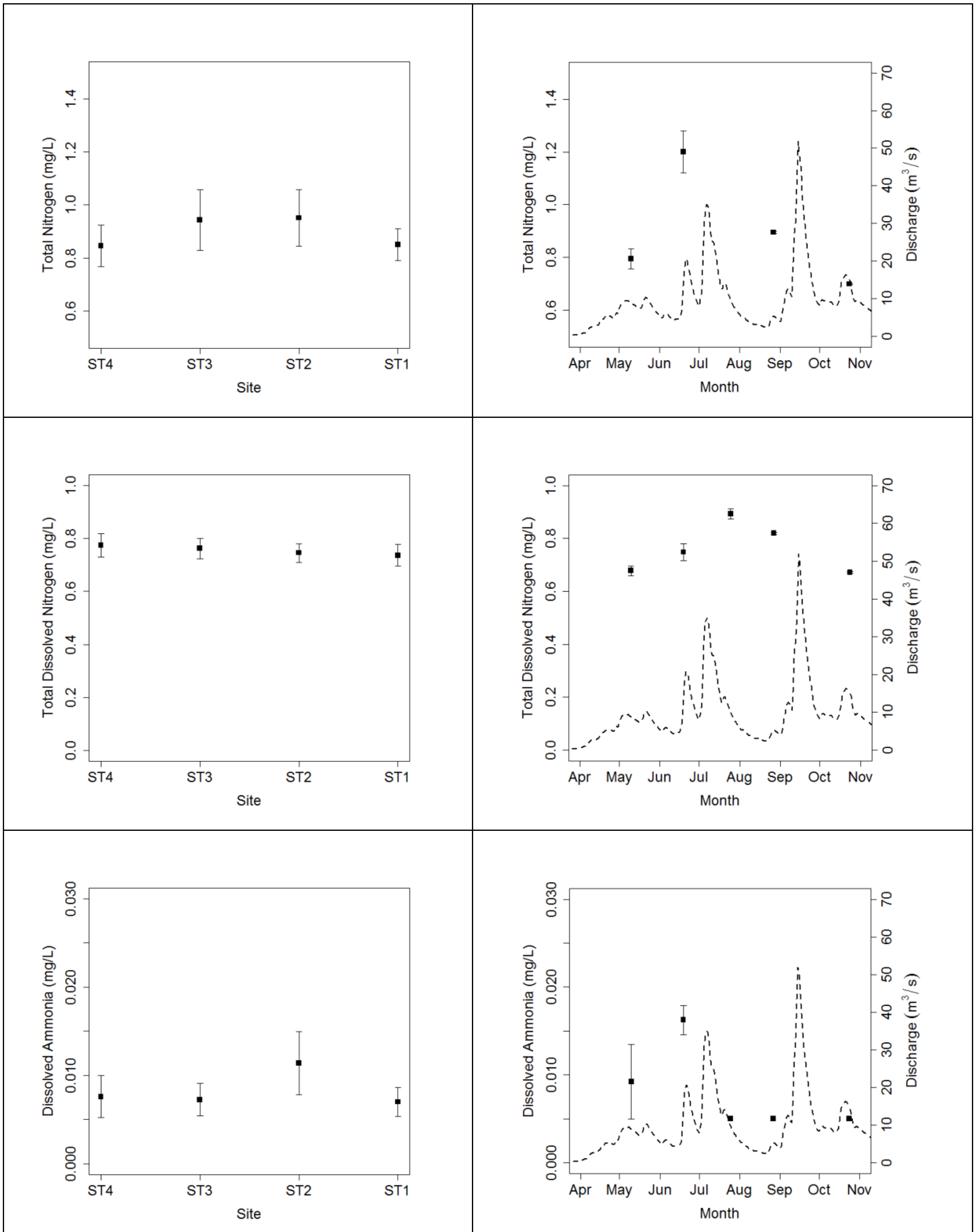


Figure 3.7. *Nitrogen Parameters.* **Top:** Mean concentration of Steepbank River total nitrogen (TN; mg/L) by site (left) and over months (right). There were no significant differences among-sites. TN concentration was significantly greatest in June (p < 0.01); August was significantly greater than October (p = 0.027). There was insufficient sample

replication for a site x month interaction effect.
Middle: Mean concentration of Steepbank River total dissolved nitrogen (TDN; mg/L) by site (left) and over months (right). There were no significant differences among-sites. TDN concentration in July was significantly greater than May, June and October ($p < 0.01$); August was significantly greater than May ($p = 0.003$), and October ($p = 0.002$). There was insufficient sample replication for a site x month interaction effect.
Bottom: Mean concentration of Steepbank River dissolved ammonia (NH_3; mg/L) by site (left) and over months (right). There were no significant differences among-sites. Dissolved NH_3 concentration in June was significantly greater than July, August, and October ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.

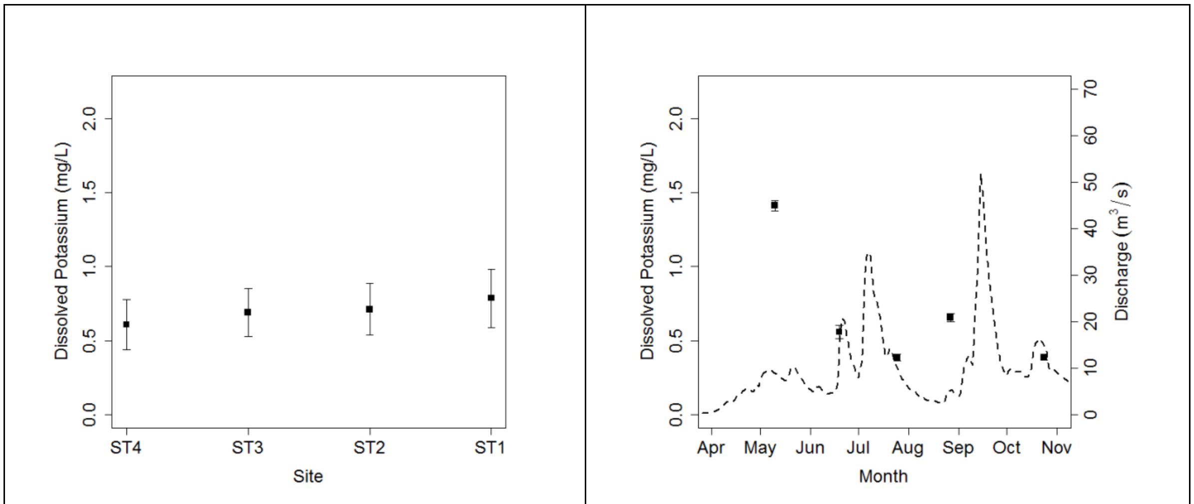


Figure 3.8. Mean concentration of Steepbank River dissolved potassium (K^+; mg/L) by site (left) and over months (right). ST4 was significantly lower than other sites ($p < 0.01$). Dissolved K^+ concentration was significantly greatest in May ($p < 0.001$); August was significantly greater than June, July and October ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect.
*Error bars represent the standard error of the mean.

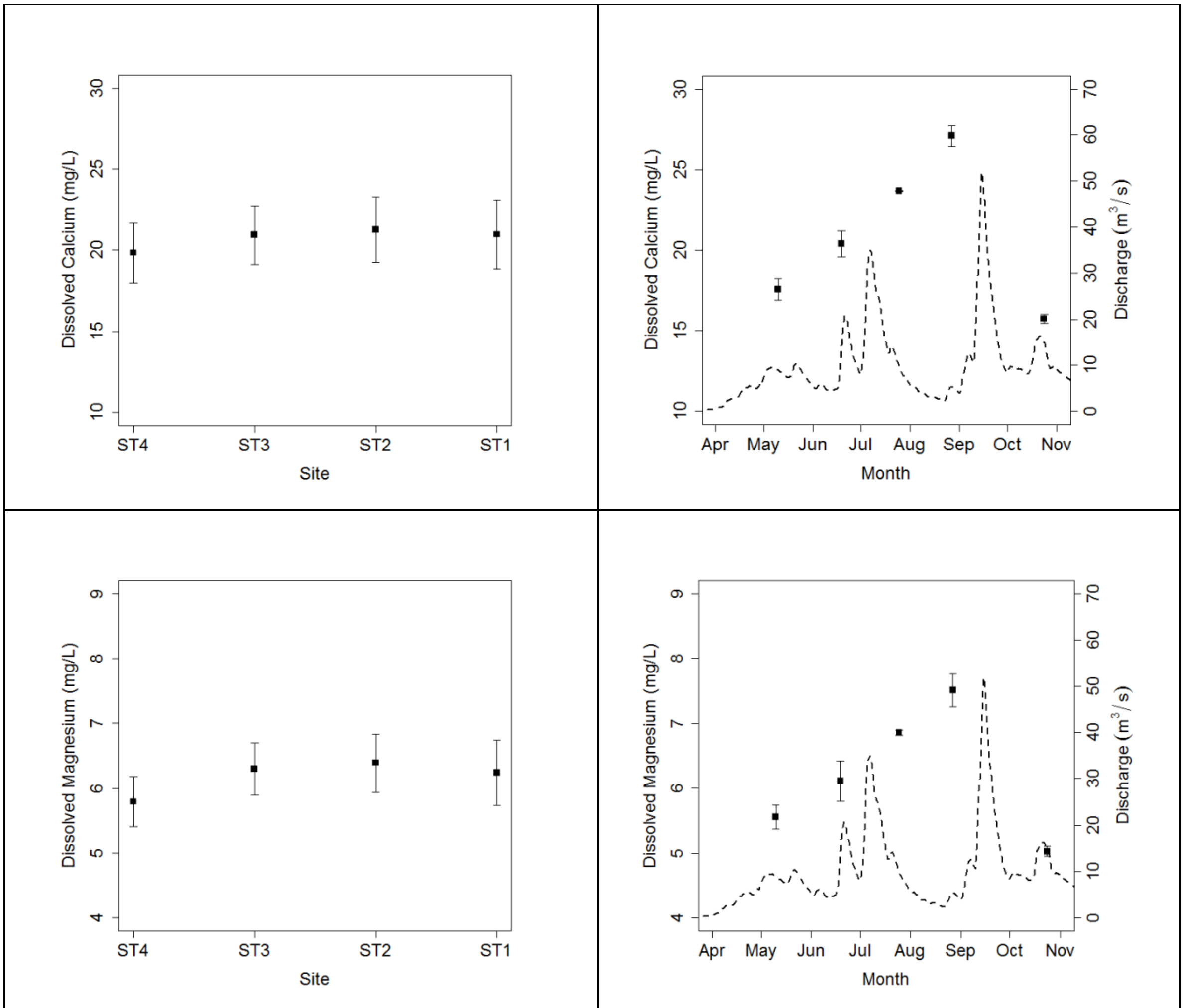


Figure 3.9. *Major Cations.* **Top:** Mean concentration of Steepbank River dissolved calcium (Ca^{+}; mg/L) by site (left) and over months (right). ST4 was significantly lower than ST1 (p = 0.023). Dissolved Ca^{2+} concentration was significantly greatest in August, and lowest in October ($p < 0.01$). There was insufficient sample replication for a site x month interaction effect.
Bottom: Mean concentration of Steepbank River dissolved magnesium (Mg^{2+}; mg/L) by site (left) and over months (right). ST4 was significantly lower than ST2 (p = 0.022), and ST1 (p = 0.033). Dissolved Mg^{2+} concentration in August was significantly greater than May, June and October ($p < 0.001$). Dissolved Mg^{2+} concentration in July was significantly greater than May (p = 0.002), and October (p = 0.000); June was significantly greater than October (p = 0.005). There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.

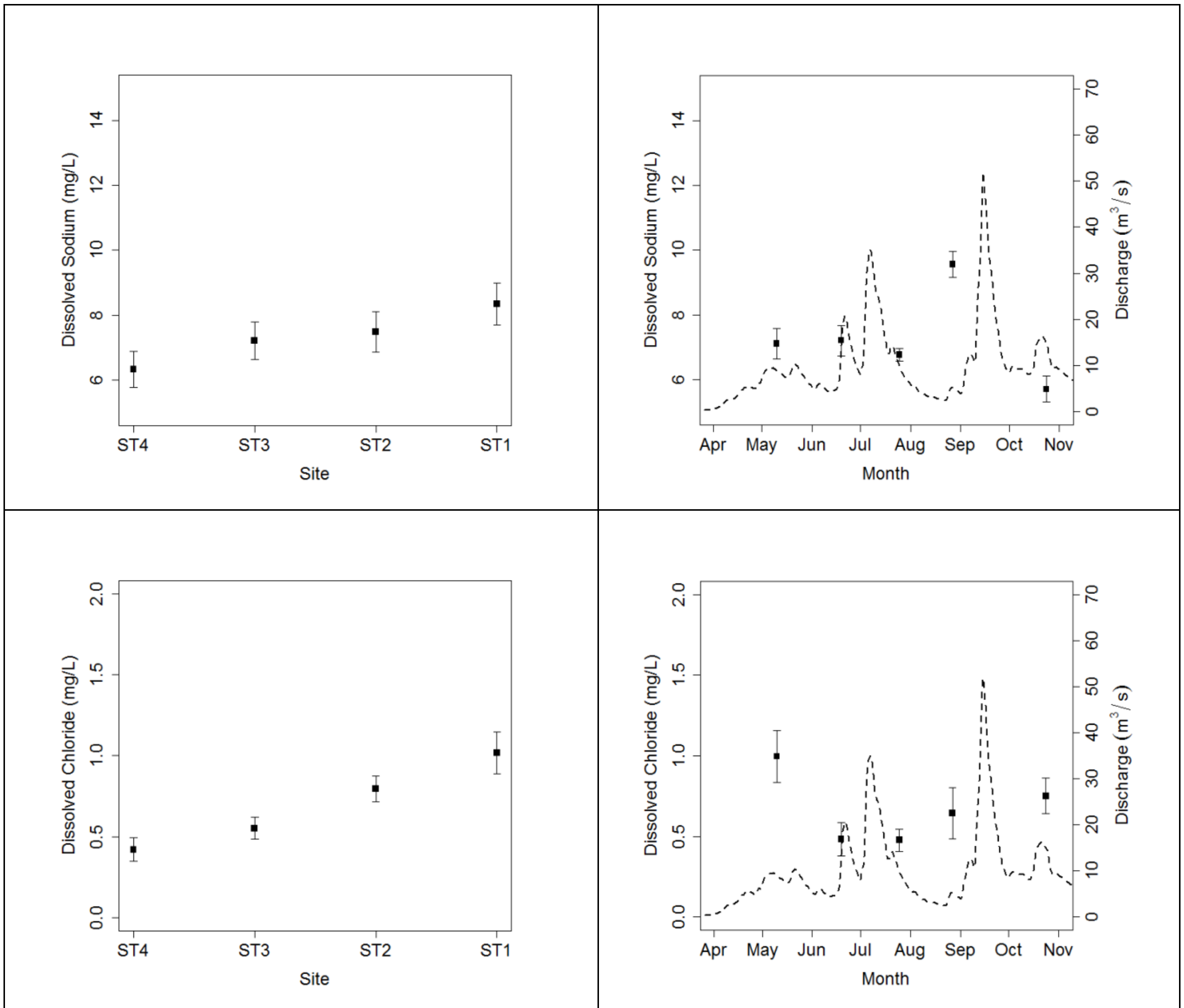


Figure 3.10. *Sodium and chloride ions.* **Top:** Mean concentration of Steepbank River dissolved sodium (Na^+; mg/L) by site (left) and over months (right). ST4 was significantly lower than ST3 and ST2, which were all lower in concentration than ST1 ($p < 0.001$). Dissolved Na^+ concentration was significantly greater in August than May, June and July, which were greater than October ($p < 0.001$). There was insufficient sample replication for a site x month interaction effect.
Bottom: Mean concentration of Steepbank River dissolved chloride (Cl^-; mg/L) by site (left) and over months (right). ST4 and ST3 were significantly lower than ST2 ($p < 0.001$); ST1 was significantly greater than other sites ($p < 0.01$). Dissolved Cl^- concentration was significantly greatest in May ($p < 0.01$). There was insufficient sample replication for a site x month interaction effect.
*Error bars represent the standard error of the mean.

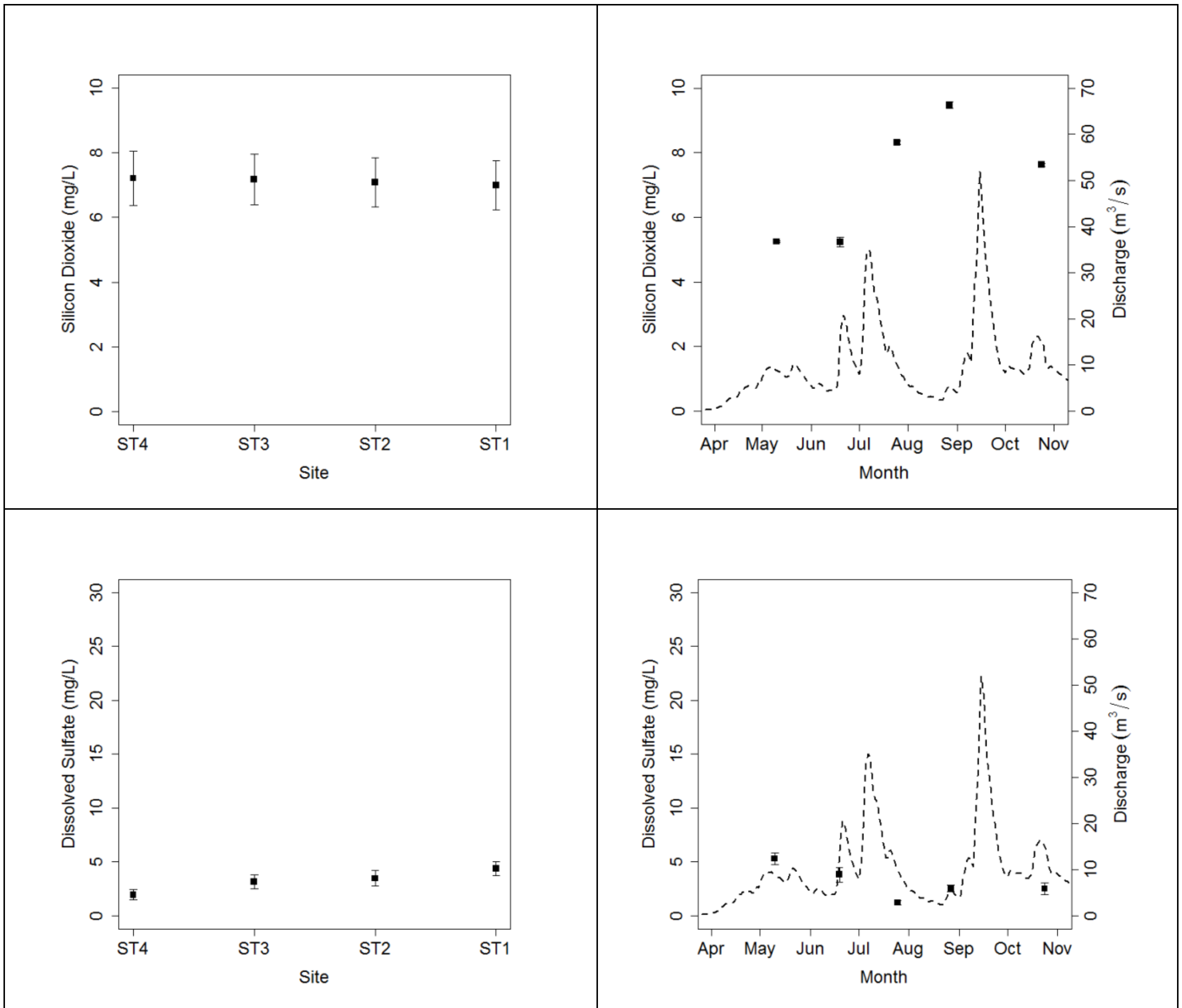


Figure 3.11. *Silicon dioxide and sulfate.* **Top:** Mean concentration of Steepbank River silicon dioxide (SiO_2^{2-}; mg/L) by site (left) and over months (right). There were no significant differences among-sites. SiO_2^{2-} concentration was significantly lowest in May and June, and highest in August ($p < 0.01$). There was insufficient sample replication for a site x month interaction effect.
Bottom: Mean concentration of Steepbank River dissolved sulfate (SO_4^{2-}; mg/L) by site (left) and over months (right). ST4 was significantly lower than other sites ($p < 0.05$). Dissolved SO_4^{2-} concentration was significantly greatest in May ($p < 0.05$); June was significantly greater than July ($p = 0.006$). There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.

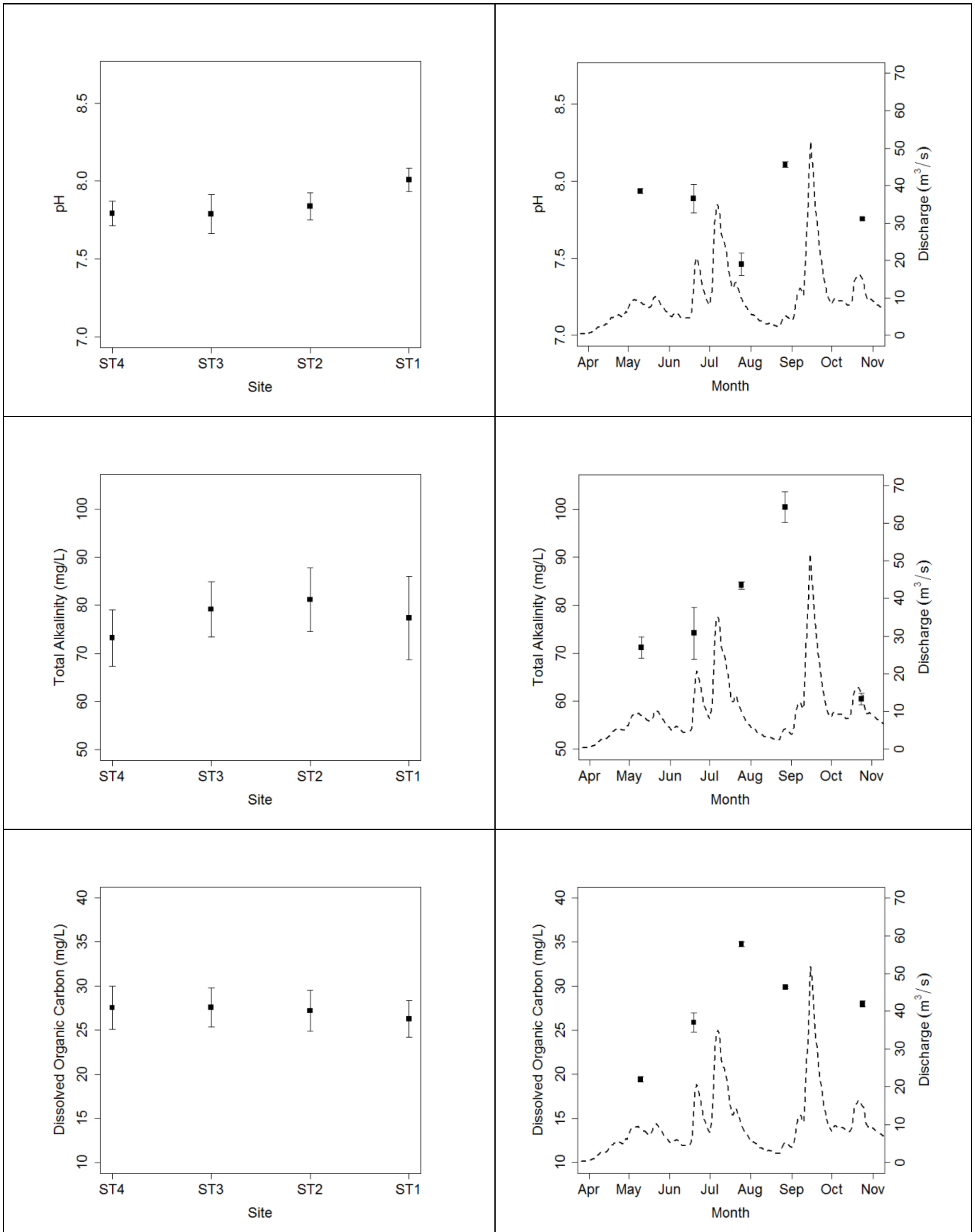


Figure 3.12. *Carbonate Complex.* **Top:** Steepbank River mean pH by site (left) and over months (right). There were no significant differences among-sites. Mean pH was significantly lowest in July (p < 0.05); August was significantly greater than October (p = 0.003). There was insufficient sample replication for a site x month interaction effect. **Middle:** Steepbank River mean total alkalinity (Alk; mg/L) by site (left) and over months

(right). There were no significant differences among-sites. Mean Alk was significantly greatest in August ($p < 0.05$); October was significantly lower than June ($p = 0.044$), and July ($p = 0.002$). There was insufficient sample replication for a site x month interaction effect. **Bottom:** Mean concentration of Steepbank River dissolved organic carbon (DOC; mg/L) by site (left) and over months (right). There were no significant differences among-sites. DOC concentration was significantly greatest in July ($p < 0.01$), and lowest in May ($p < 0.001$). There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.

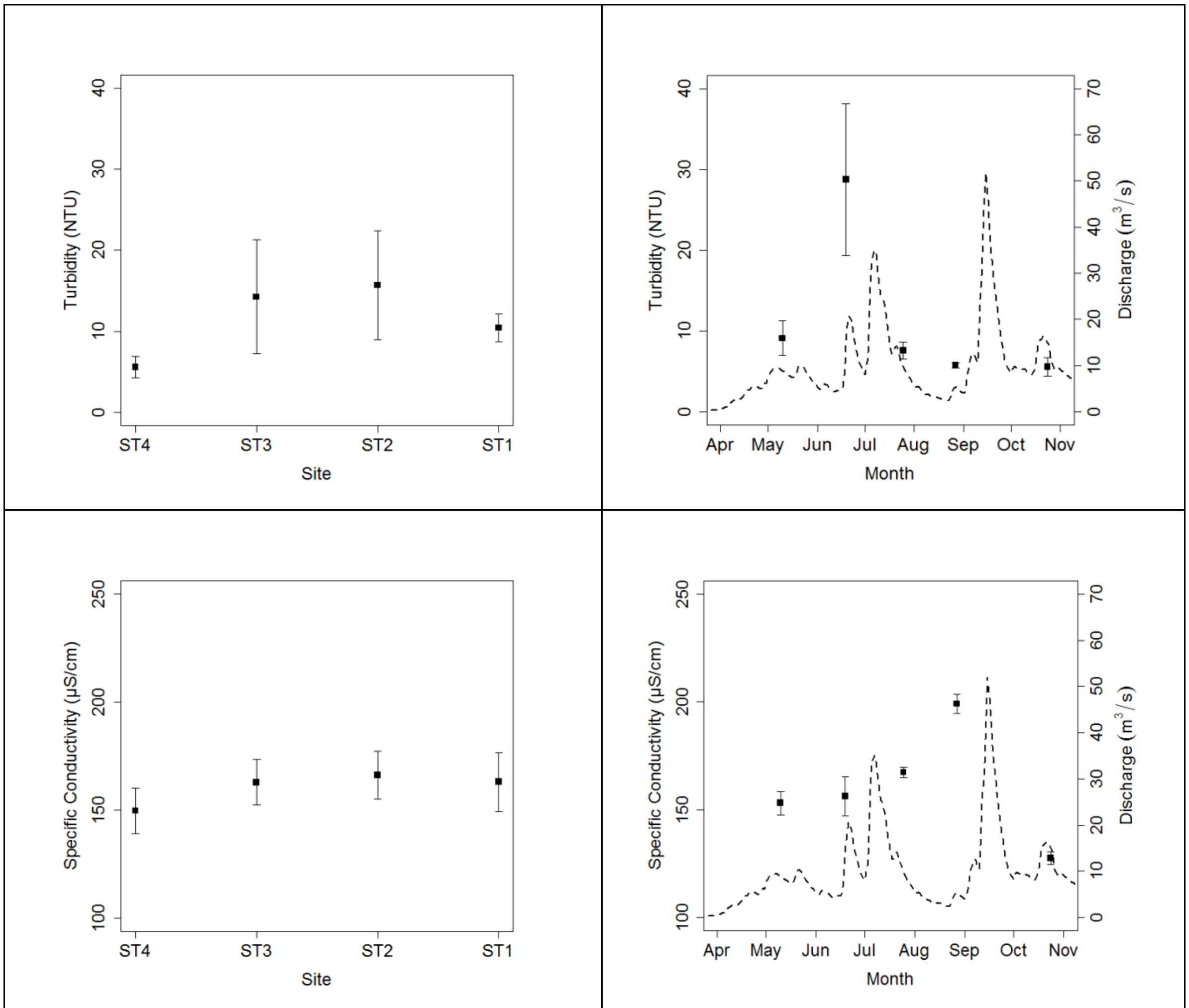


Figure 3.13 a. *Physical/Water Quality Parameters.* **Top:** Steepbank River mean turbidity (Turb; NTU) by site (left) and over months (right). There were no significant differences among-sites. Mean Turb was significantly greatest in June ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect.
Bottom: Steepbank River mean specific conductivity (Cond; µS/cm) by site (left) and over months (right). ST4 was significantly lower than ST2 ($p = 0.015$), and ST1 ($p = 0.050$). Mean Cond was significantly greater in August than May, June and July, which were all greater than October ($p < 0.01$). There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.

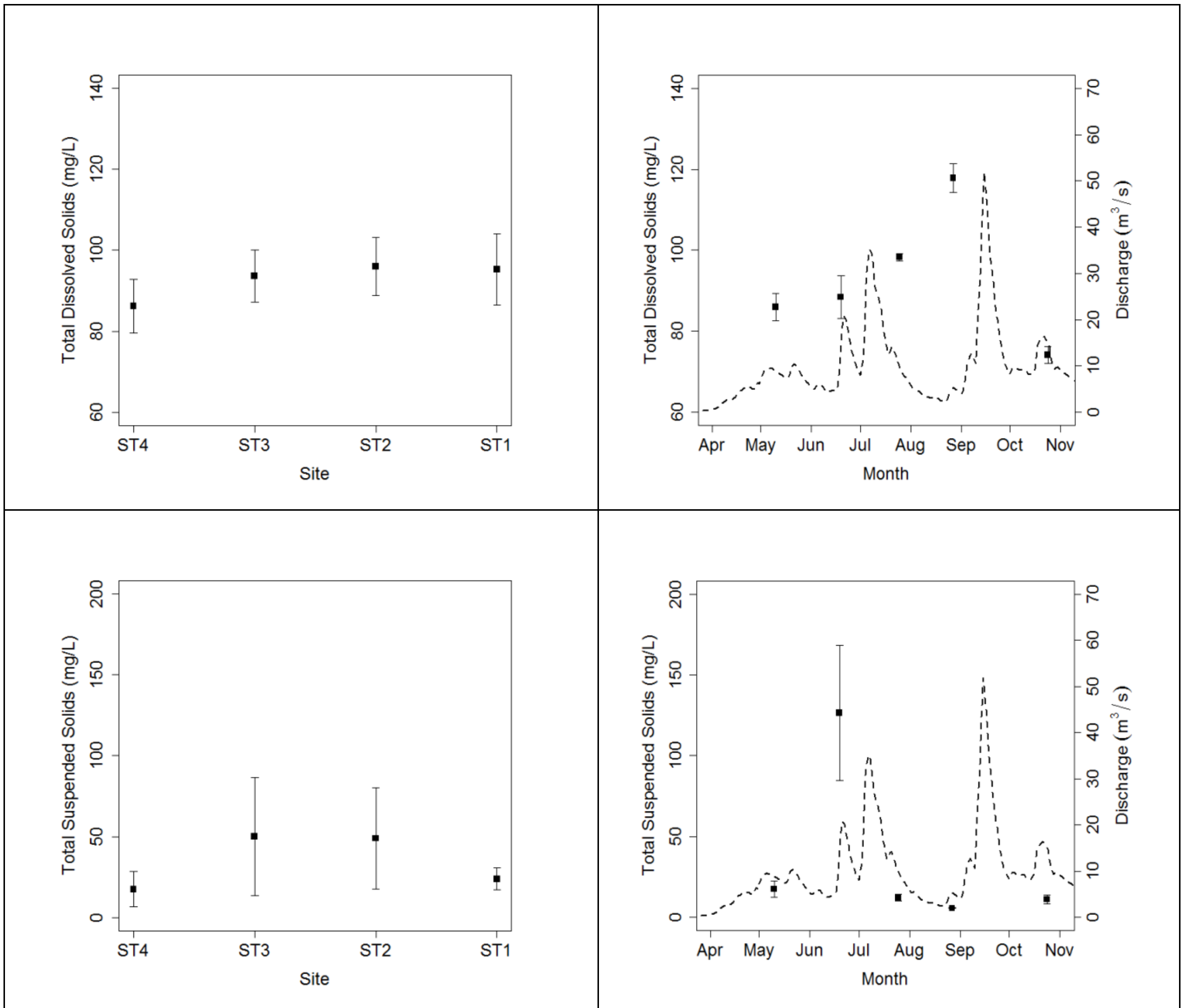


Figure 3.13 b. *Physical/Water Quality Parameters.* **Top:** Steepbank River mean total dissolved solids (TDS; mg/L) by site (left) and over months (right). ST4 was significantly lower than ST2 (p = 0.017), and ST1 (p = 0.017). Mean TDS was significantly greatest in August ($p < 0.01$); October was significantly lower than June (p = 0.026), and July (p = 0.001). There was insufficient sample replication for a site x month interaction effect. **Bottom:** Steepbank River mean total suspended solids (TSS; mg/L) by site (left) and over months (right). There were no significant differences among-sites. Mean TSS was significantly greatest in June ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.

Ells River

Nutrients

None of the nutrient parameters were significantly different among-sites. All nutrient parameters had significantly highest concentrations in May and July, except for

NH_3 which had no significant monthly differences. There was insufficient sample replication for a site x month interaction effect for all nutrient parameters.

Cations/Anions

All cation and anion parameters were significantly different among-sites, with concentrations increasing from upstream to downstream, except for SiO_2^{2-}, which had no significant among-site differences. There were no significant monthly differences for Ca^{2+}, whereas Mg^{2+} concentrations in May and August were significantly lower than June, September and October. Na^+ concentrations were significantly lowest in August and significantly highest in September and October. Cl^- concentrations were significantly lowest in July and August, whereas SiO_2^{2-} concentrations were significantly greatest in July and October. SO_4^{2-} concentrations significantly decreased from May to August, followed by a significant increase in September and October. There was insufficient sample replication for a site x month interaction effect for all cation/anion parameters.

Carbonate Complex

Alk was the only carbonate complex parameter which was significantly different among-sites with concentrations increasing from upstream to downstream. pH values were significantly highest in September, and Alk concentrations were significantly greatest in September and October. DOC concentrations were significantly greatest in July. There was insufficient sample replication for a site x month interaction effect for all carbonate complex parameters.

Physical/Water Quality Parameters

All physical/water quality parameters were significantly different among-sites, with concentrations increasing from upstream to downstream. Turb and TSS concentrations were significantly highest in May and July. Cond and TDS concentrations were significantly highest in September and October, and significantly lowest in August. There was insufficient sample replication for a site x month interaction effect for all physical/water quality parameters.

Table 3.8. Summary table of Ells River physical/water quality and chemical variables analyzed with a two-way mixed-effects ANOVA, which included a) site and, b) month. Significant *p*-values ($p < 0.05$) for site differences are shaded in grey. Upstream (U/S) to downstream (D/S) changes in variable concentrations are indicated with "+" for increasing and "-" for decreasing, and an interaction column indicates any significant site x month interaction effects. Other than standard chemical abbreviations, the following abbreviations are used: total phosphorus (TP), total dissolved phosphorus (TDP), total nitrogen (TN), total dissolved nitrogen (TDN), total alkalinity (Alk), dissolved organic carbon (DOC), turbidity (Turb), specific conductivity (Cond), total dissolved solids (TDS), and total suspended solids (TSS).

Para-meter	**Fig #**	**Site Differences**	***p*-value**	**U/S-D/S Changes**	**Monthly Differences**	***p*-value**	**Interaction Effect**
Nutrients							
TP	3.14	No	> 0.05	No	> in May, Jul	< 0.001	No rep.
TDP	3.14	No	> 0.05	No	> in Jul	< 0.05	No rep.
TN	3.15	No	> 0.05	No	May > Jun, Aug, Oct	< 0.05	No rep.
TDN	3.15	No	> 0.05	No	> in Jul	< 0.001	No rep.
NH_3	3.15	No	> 0.05	No	No	> 0.05	No rep.
K^+	3.16	No	> 0.05	No	> in May; Sep > Jul, Aug	< 0.001 < 0.05	No rep.
Cations/Anions							
Ca^{2+}	3.17	EL2 < EL1	0.011	+	No	> 0.05	No rep.
Mg^{2+}	3.17	EL3 = EL2 < EL1	< 0.01	+	May, Aug < Jun, Sep, Oct	< 0.01	No rep.
Na^+	3.18	EL3 < EL2 < EL1	< 0.001	+	< in Aug ; Sep = Oct > May, Jul, Aug	< 0.001 < 0.05	No rep.
Cl^-	3.18	EL3 < EL2 < EL1	< 0.01	+	Jul < May, Jun, Sep, Oct; Aug < May, Oct	< 0.05 < 0.05	No rep.
SiO_2^{2-}	3.19	No	> 0.05	No	> in Jul, Oct; May > Jun, Aug, Sep	< 0.01 < 0.05	No rep.
SO_4^{2-}	3.19	EL3 = EL2 < EL1	< 0.001	+	May > Jun > Jul = Sep = Oct > Aug	< 0.05	No rep.
Carbonate Complex							
pH	3.20	No	> 0.05	No	> in Sep	< 0.05	No rep.
Alk	3.20	EL3 = EL2 < EL1	< 0.01	+	May < Jun, Sep, Oct; > in Sep, Oct	< 0.01 < 0.01	No rep.
DOC	3.20	No	> 0.05	No	> in Jul; Sep > Jun, Aug	< 0.001 < 0.05	No rep.
Physical/Water Quality Parameters							No rep.
Turb	3.21a	EL3 = EL2 < EL1	< 0.05	+	> in May, Jul	< 0.01	No rep.
Cond	3.21a	EL3 < EL2 = EL1	< 0.01	+	Jul, Aug < Sep, Oct	< 0.05	No rep.
TDS	3.21b	EL3 = EL2 < EL1	< 0.001	+	< in Aug; Sep = Oct > May, Jul, Aug	< 0.01 < 0.05	No rep.
TSS	3.21b	EL2 < EL1	0.029	+	> in May, Jul	< 0.001	No rep.

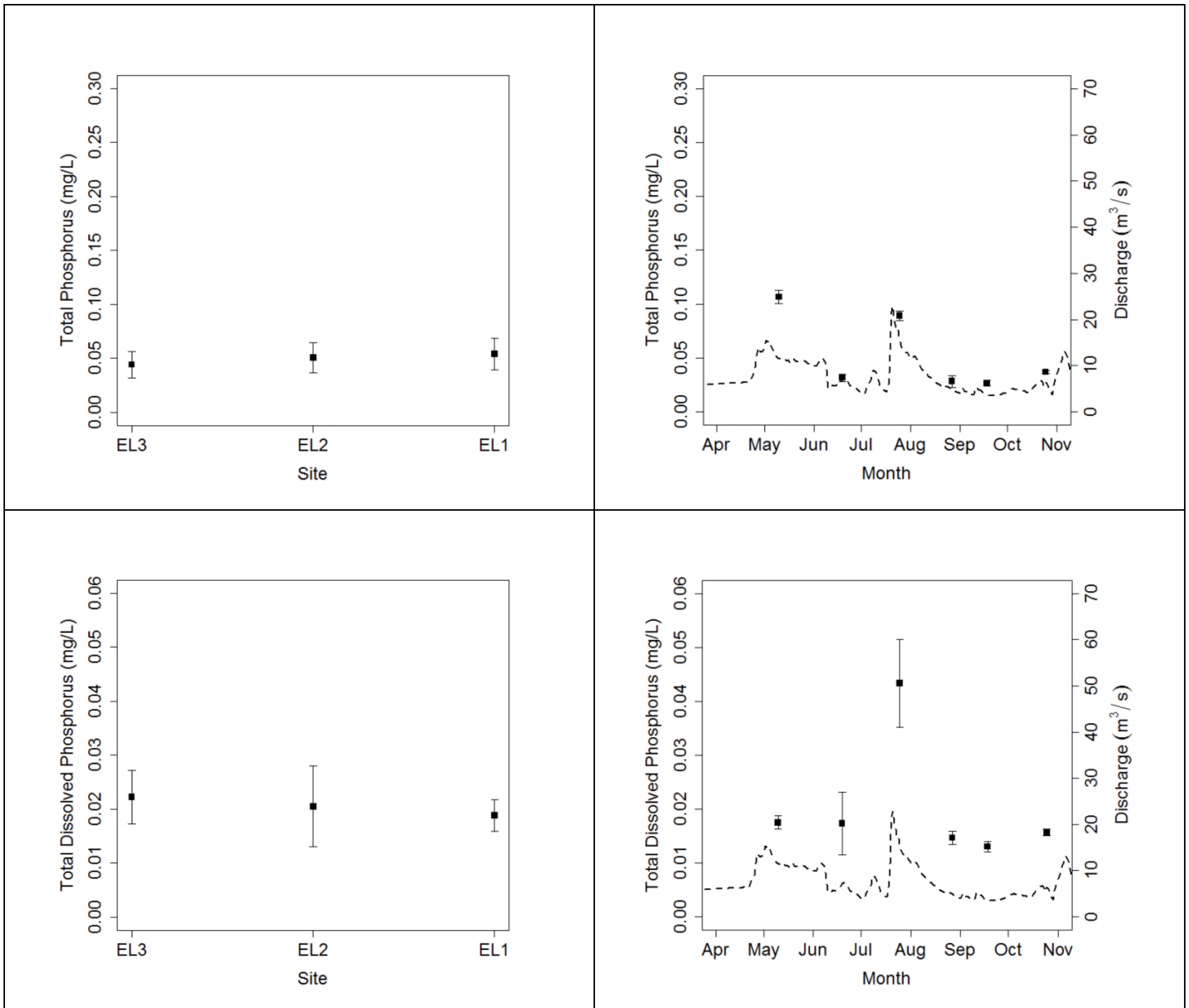


Figure 3.14. *Phosphorus Parameters.* **Top:** Mean concentration of Ells River total phosphorus (TP; mg/L) by site (left) and over months (right). There were no significant differences among-sites. TP concentration was significantly greatest in May and July ($p < 0.001$). There was insufficient sample replication for a site x month interaction effect. **Bottom:** Mean concentration of Ells River total dissolved phosphorus (TDP; mg/L) by site (left) and over months (right). There were no significant differences among-sites. TDP concentration was significantly greatest in July ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect.
*Error bars represent the standard error of the mean.

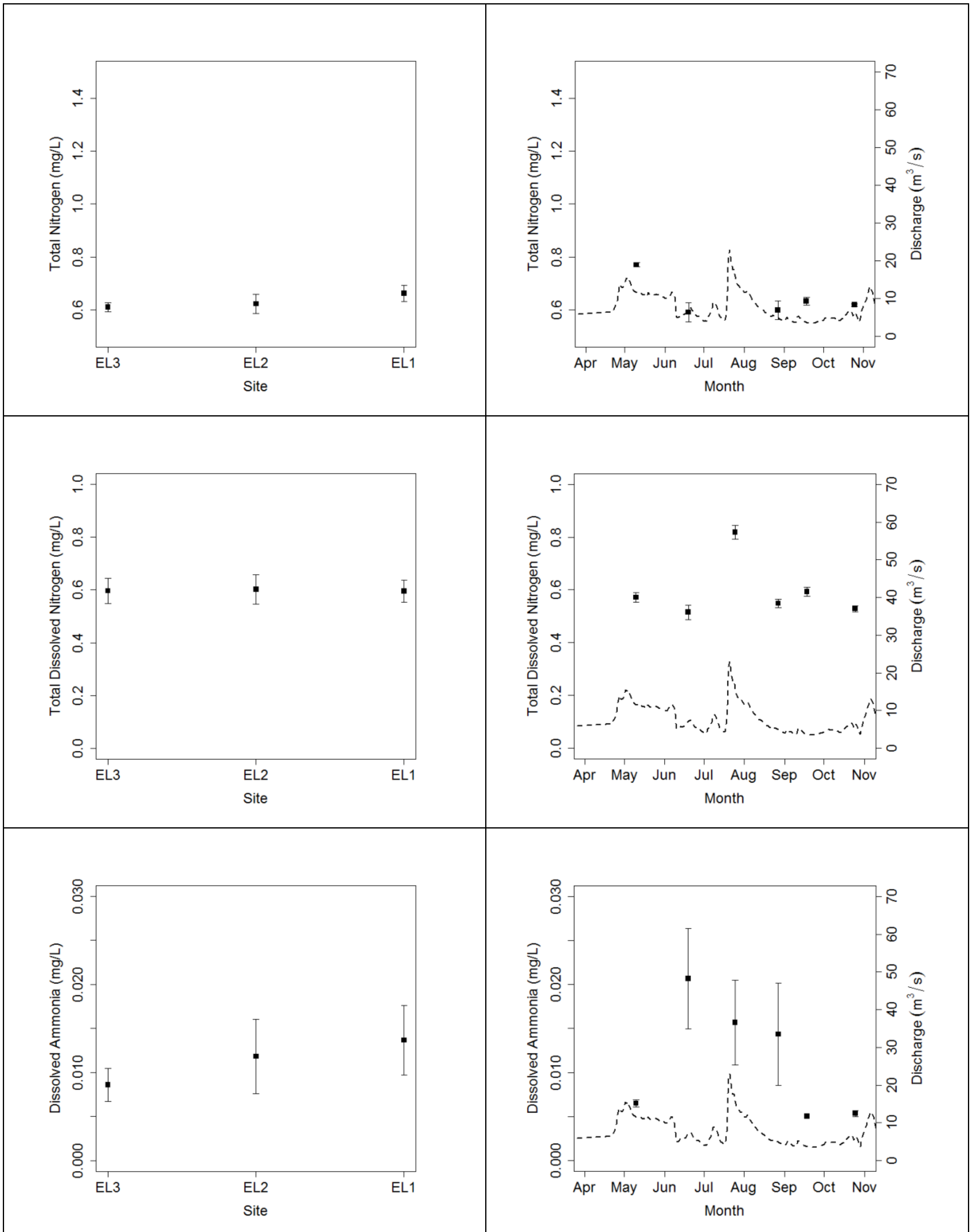


Figure 3.15. *Nitrogen Parameters.* **Top:** Mean concentration of Ells River total nitrogen (TN; mg/L) by site (left) and over months (right). There were no significant differences among-sites. TN concentration in May was significantly greater than June, August and October ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect.

Middle: Mean concentration of Ells River total dissolved nitrogen (TDN; mg/L) by site (left) and over months (right). There were no significant differences among-sites. TDN was significantly greatest in July ($p < 0.001$). There was insufficient sample replication for a site x month interaction effect.
Bottom: Mean concentration of Ells River dissolved ammonia (NH_3; mg/L) by site (left) and over months (right). There were no significant differences among-sites and among-months. There was insufficient sample replication for a site x month interaction effect.
*Error bars represent the standard error of the mean.

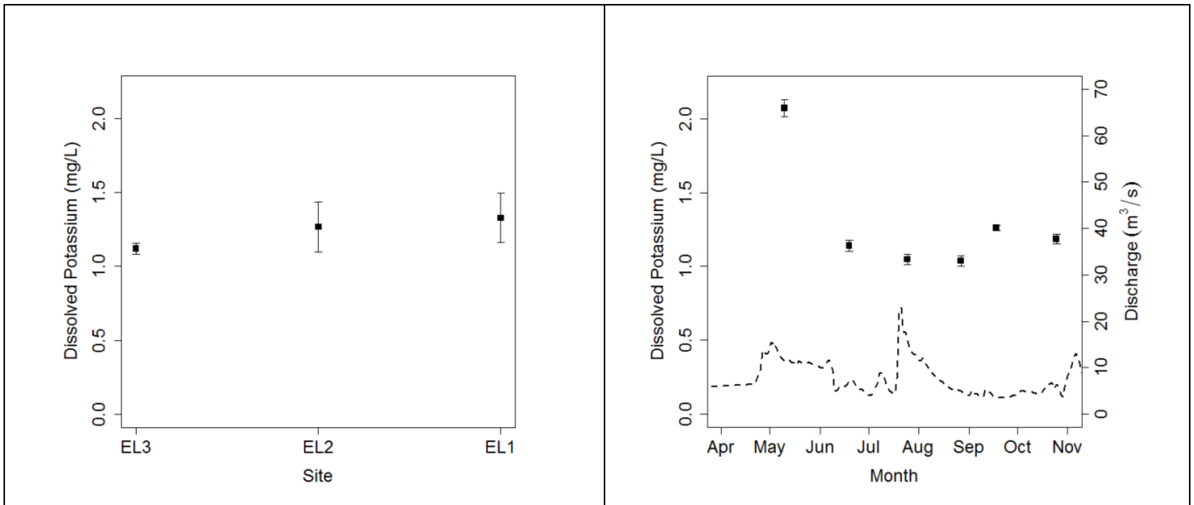


Figure 3.16. Mean concentration of Ells River dissolved potassium (K^+; mg/L) by site (left) and over months (right). There were no significant differences among-sites. Dissolved K^+ concentration was significantly greatest in May ($p < 0.001$); September was significantly greater than July ($p = 0.017$), and August ($p = 0.013$). There was insufficient sample replication for a site x month interaction effect.
*Error bars represent the standard error of the mean.

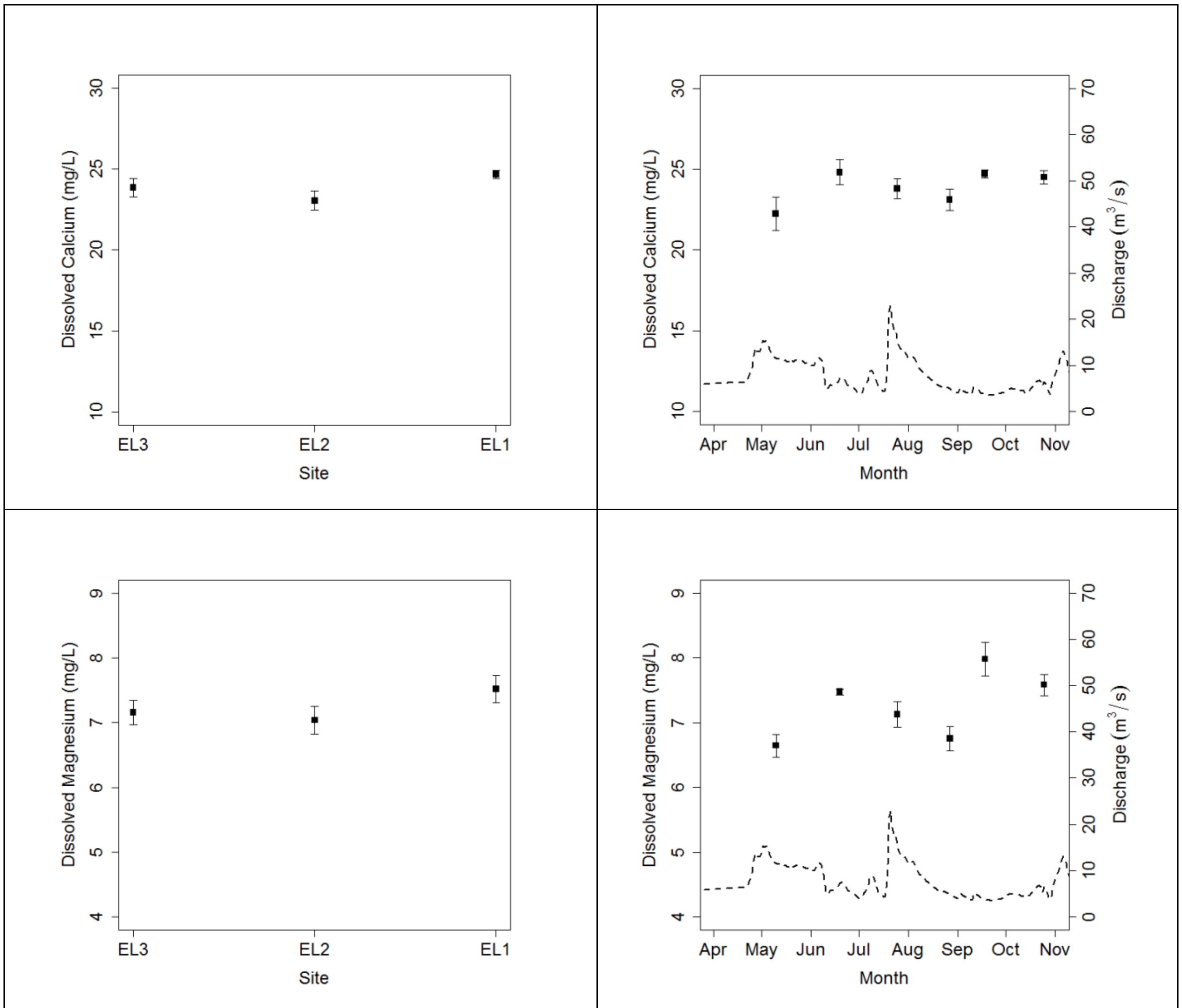


Figure 3.17. *Major Cations.* **Top:** Mean concentration of Ells River dissolved calcium (Ca^{2+}; mg/L) by site (left) and over months (right). EL2 was significantly lower than EL1 (p = 0.011). Dissolved Ca^{2+} concentration was not significantly different among-months. There was insufficient sample replication for a site x month interaction effect.
Bottom: Mean concentration of Ells River dissolved magnesium (Mg^{2+}; mg/L) by site (left) and over months (right). EL3 (p = 0.000) and EL2 (p = 0.002) were significantly lower than EL1. Dissolved Mg^{2+} concentration in May and August was significantly lower than June, September and October ($p < 0.01$). There was insufficient sample replication for a site x month interaction effect.
*Error bars represent the standard error of the mean.

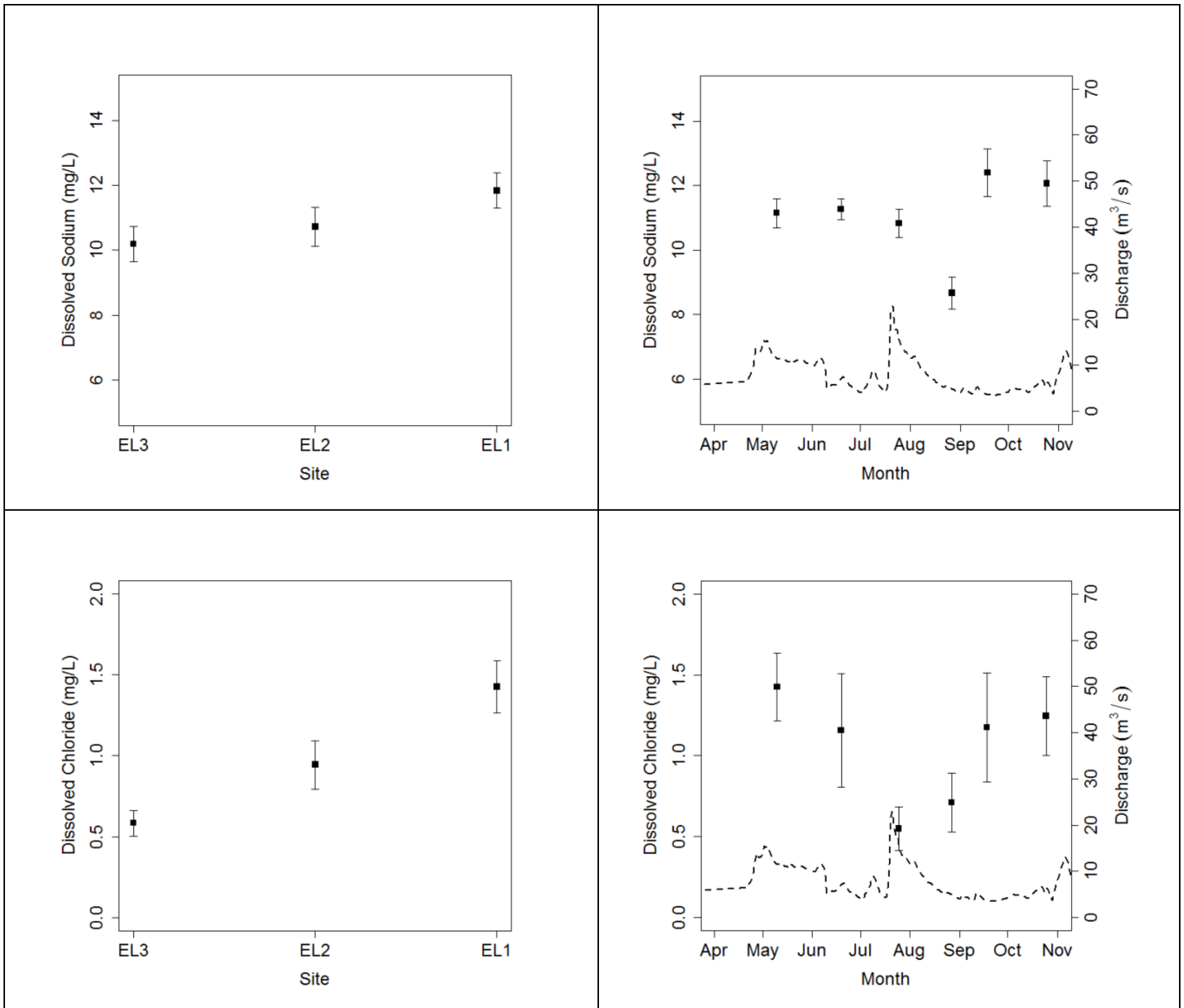


Figure 3.18. *Sodium and chloride ions.* **Top:** Mean concentration of Ells River dissolved sodium (Na^+; mg/L) by site (left) and over months (right). EL3 was significantly lower than EL2 ($p = 0.001$), and both were lower than EL1 ($p < 0.001$). Dissolved Na^+ concentration was significantly lowest in August ($p < 0.001$); September and October were significantly greater than May, July and August ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect.
Bottom: Mean concentration of Ells River dissolved chloride (Cl^-; mg/L) by site (left) and over months (right). EL3 was significantly lower than EL2 ($p = 0.002$), and both were lower than EL1 ($p < 0.01$). Dissolved Cl^- concentration in July was significantly lower than May, June, September and October ($p < 0.05$); August was significantly lower than May ($p = 0.048$), and October ($p = 0.022$). There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.

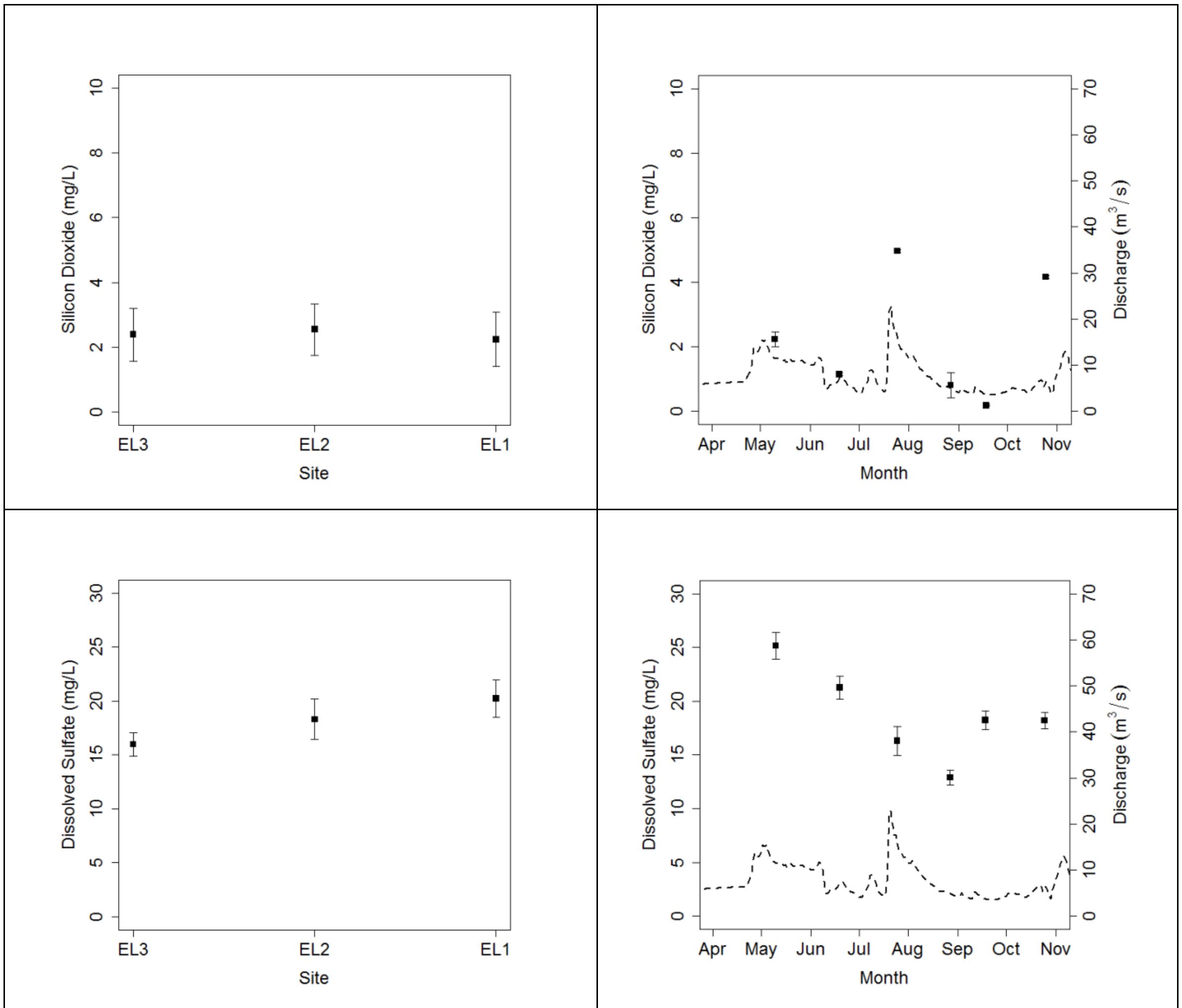


Figure 3.19. *Silicon dioxide and sulfate.* **Top:** Mean concentration of Ells River silicon dioxide (SiO_2^{2-}; mg/L) by site (left) and over months (right). There were no significant differences among-sites. SiO_2^{2-} concentration was significantly greatest in July and October ($p < 0.01$); May was significantly greater than June, August, and September ($p < 0.05$). There was insufficient replication for a site x month interaction effect.
Bottom: Mean concentration of Ells River dissolved sulfate (SO_4^{2-}; mg/L) by site (left) and over months (right). EL3 and EL2 were significantly lower than EL1 ($p < 0.001$). Dissolved SO_4^{2-} concentration was significantly lowest in August, and greatest in May ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect.
*Error bars represent the standard error of the mean.

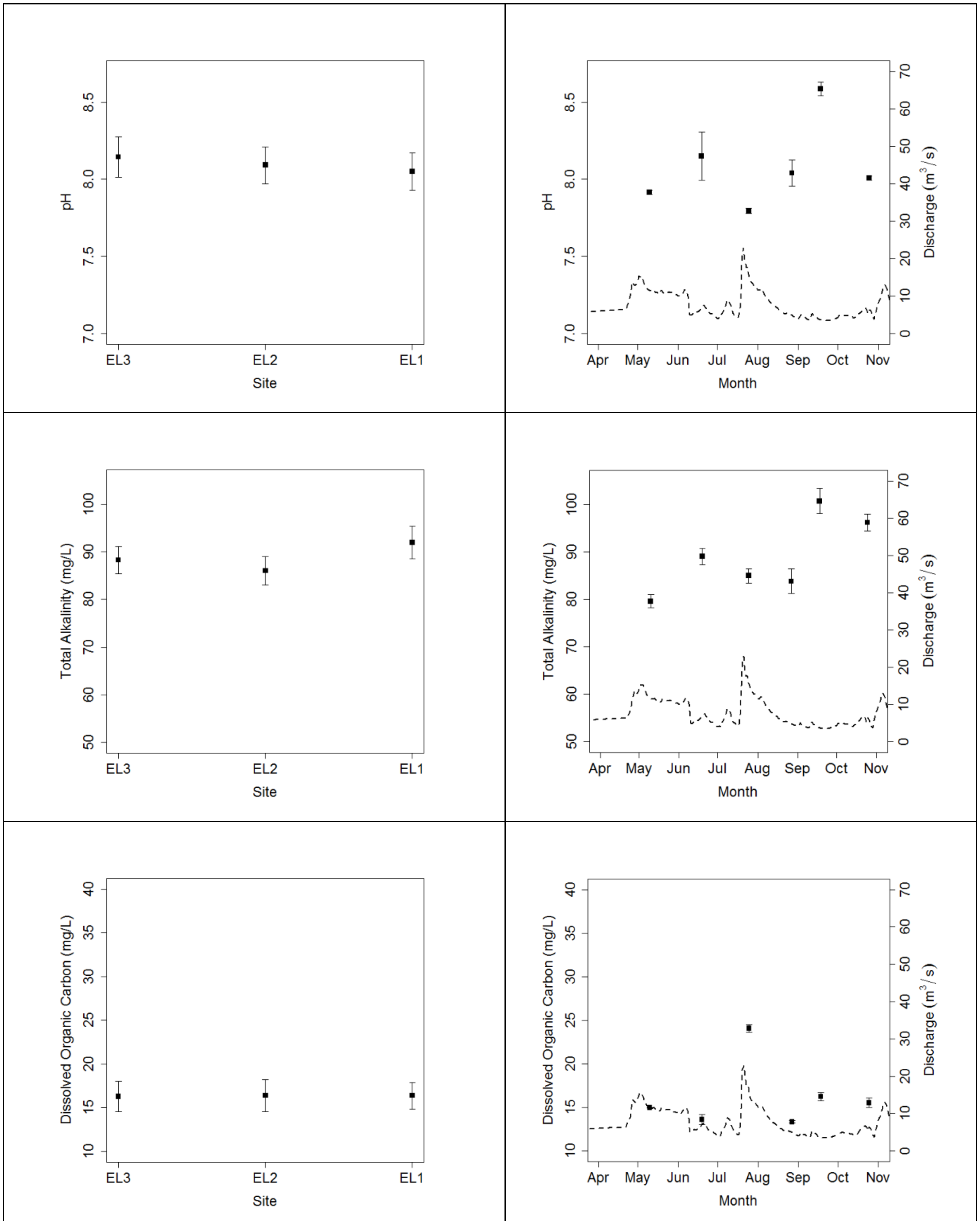


Figure 3.20. *Carbonate Complex.* **Top:** Ells River mean pH by site (left) and over months (right). There were no significant differences among-sites. Mean pH was significantly greatest in September ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect. **Middle:** Ells River mean total alkalinity (Alk; mg/L) by site (left) and over months (right). EL3 ($p = 0.001$) and EL2 ($p = 0.003$) were significantly lower than

EL1. Mean Alk in May was significantly lower than June, September and October ($p < 0.01$); September and October were significantly greater than all other months ($p < 0.01$). There was insufficient sample replication for a site x month interaction effect. **Bottom:** Mean concentration of Ells River dissolved organic carbon (DOC; mg/L) by site (left) and over months (right). There were no significant differences among-sites. DOC concentration was significantly greatest in July ($p < 0.001$); September was significantly greater than June ($p = 0.029$), and August ($p = 0.017$). There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.

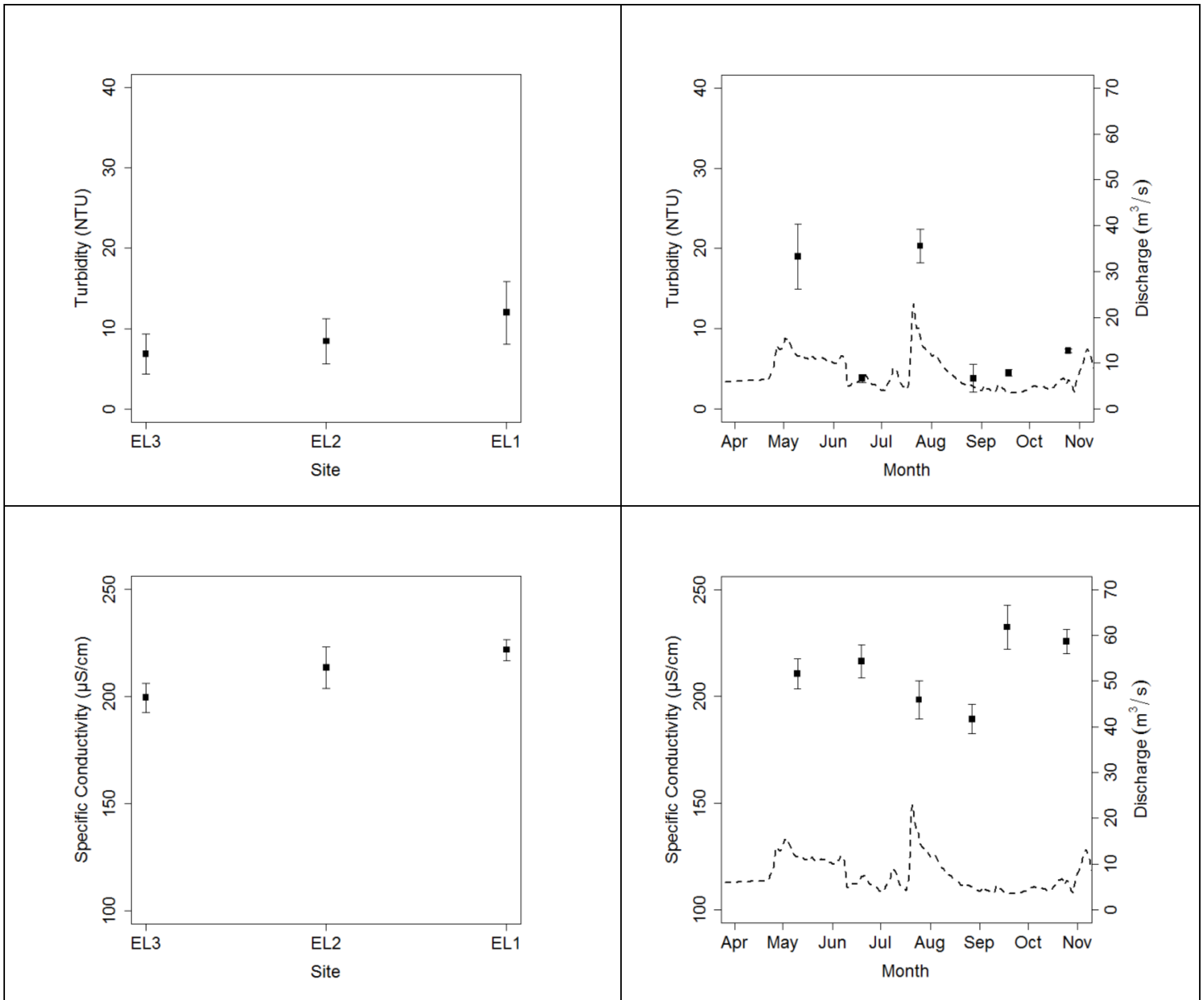


Figure 3.21 a. *Physical/Water Quality Parameters.* **Top:** Ells River mean turbidity (Turb; NTU) by site (left) and over months (right). EL3 ($p = 0.049$) and EL2 ($p = 0.025$) were significantly lower than EL1. Mean Turb was significantly greatest in May and July ($p < 0.01$). There was insufficient sample replication for a site x month interaction effect. **Bottom:** Ells River mean specific conductivity (Cond; µS/cm) by site (left) and over months (right). EL3 was significantly lower than EL2 ($p = 0.010$), and EL1 ($p = 0.001$). Mean Cond in July and August was significantly lower than September and October ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.

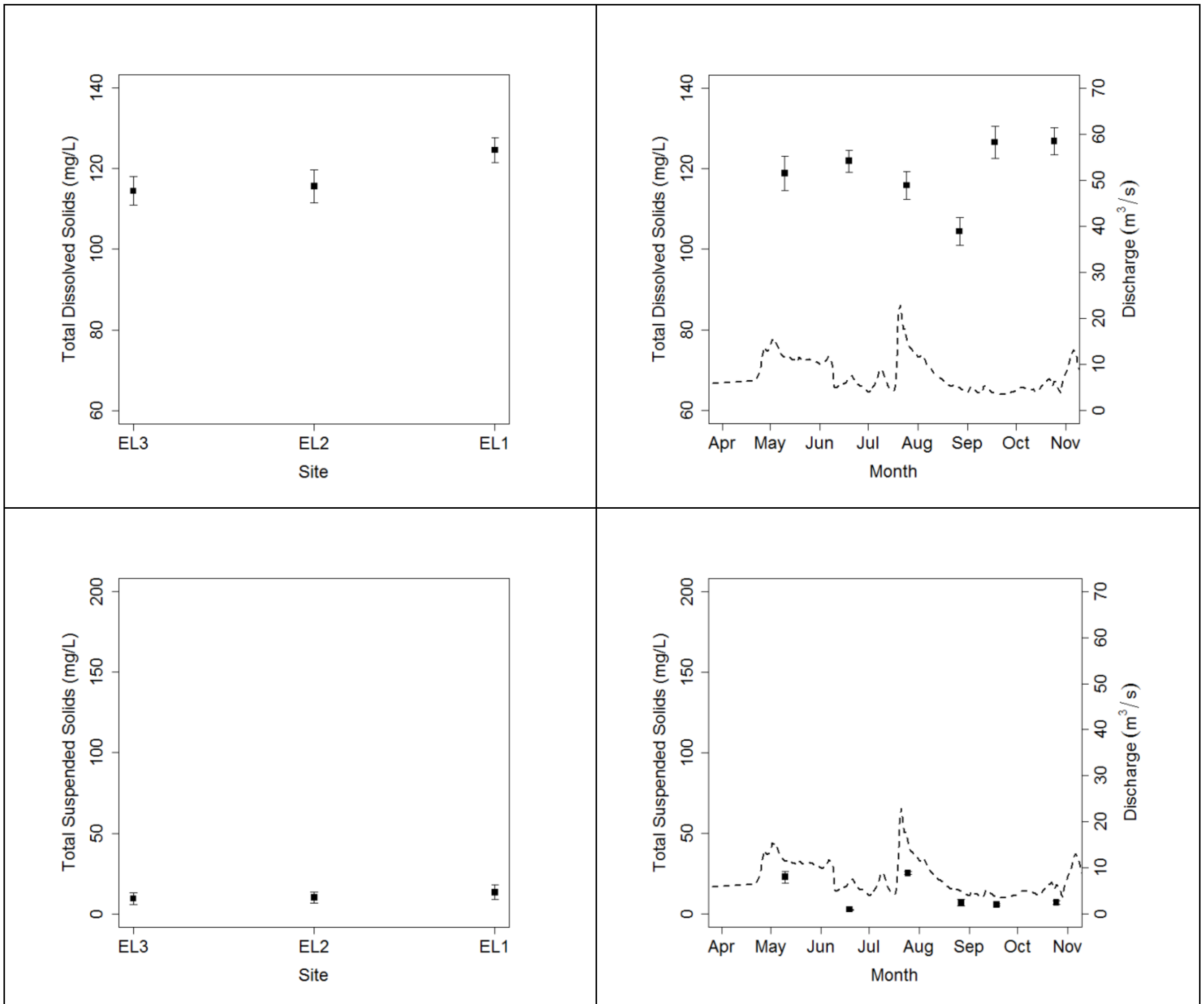


Figure 3.21 b. *Physical/Water Quality Parameters.* **Top:** Ells River mean total dissolved solids (TDS; mg/L) by site (left) and over months (right). EL3 and EL2 were significantly lower than EL1 ($p < 0.001$). Mean TDS was significantly lower in August ($p < 0.01$); September and October were significantly greater than May, July and August ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect.
Bottom: Ells River mean total suspended solids (TSS; mg/L) by site (left) and over months (right). EL2 was significantly lower than EL1 ($p = 0.029$). Mean TSS was significantly greater in May and July ($p < 0.001$). There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.

3.3.3 Fine Sediment Chemistry

Fine sediment metals analysis was categorized into two priority pollutant element (PPE) groups for each river: Low Concentration PPEs and High Concentration PPEs. Site and monthly statistical differences are presented for the Steepbank River in Table 3.9, and the Ells River in Table 3.10.

Steepbank River

Low Concentration Priority Pollutant Elements

All low concentration PPEs in October were significantly different among-sites, with the most upstream site (ST4) having the lowest element concentrations. Silver (Ag), selenium (Se), and thallium (Tl) had significantly highest concentrations in September at ST4. There was insufficient sample replication for a site x month interaction effect for all low concentration PPEs.

High Concentration Priority Pollutant Elements

All high concentration PPEs in October were significantly different among-sites, with ST4 having the lowest element concentrations. Arsenic (As), nickel (Ni), lead (Pb), and zinc (Zn) had significantly highest concentrations in September at ST4. There was insufficient sample replication for a site x month interaction effect for all high concentration PPEs.

Table 3.9. Summary table of Steepbank River fine sediment chemistry for 12 priority pollutant elements (PPEs) analyzed with one-way ANOVAs, which included a) site and, b) month. Upstream (U/S) to downstream (D/S) changes in variable concentrations are indicated with "+" for increasing and "-" for decreasing, and an interaction column indicates any significant site x month interaction effects.

Para-meter	**Fig #**	**Site Differences***	***p*-value**	**U/S-D/S Changes**	**Monthly Differences****	***p*-value**	**Interaction Effect**
Low Concentration PPEs							
Ag	3.22	ST4 < ST1 < ST3	< 0.05	+	Sep > Aug	0.028	No rep.
Be	3.22	ST4 < ST3 = ST1	< 0.001	+	No	> 0.05	No rep.
Cd	3.22	ST4 < ST3 = ST1	< 0.05	+	No	> 0.05	No rep.
Sb	3.22	ST4 < ST3	0.023	+	No	> 0.05	No rep.
Se	3.22	ST4 < ST1 < ST3	< 0.05	+	> in Sep	< 0.05	No rep.
Tl	3.22	ST4 < ST3	0.004	+	> in Sep	< 0.05	No rep.
High Concentration PPEs							
As	3.23	ST4 < ST3 = ST1	< 0.01	+	> in Sep	< 0.001	No rep.
Cr	3.23	ST4 < ST1 < ST3	< 0.05	+	No	> 0.05	No rep.
Cu	3.23	ST4 < ST3 = ST1	< 0.001	+	No	> 0.05	No rep.
Ni	3.23	ST4 < ST3 = ST1	< 0.001	+	> in Sep	< 0.05	No rep.
Pb	3.23	ST4 < ST1 < ST3	< 0.05	+	> in Sep	< 0.01	No rep.
Zn	3.23	ST4 < ST1 < ST3	< 0.05	+	Sep > Oct	0.043	No rep.

*Among-site differences were only analyzed for October because this was the only month where samples were retrieved from more than one-site. **Monthly differences were only analyzed for ST4 because this was the only site where samples were retrieved from more than one-month.

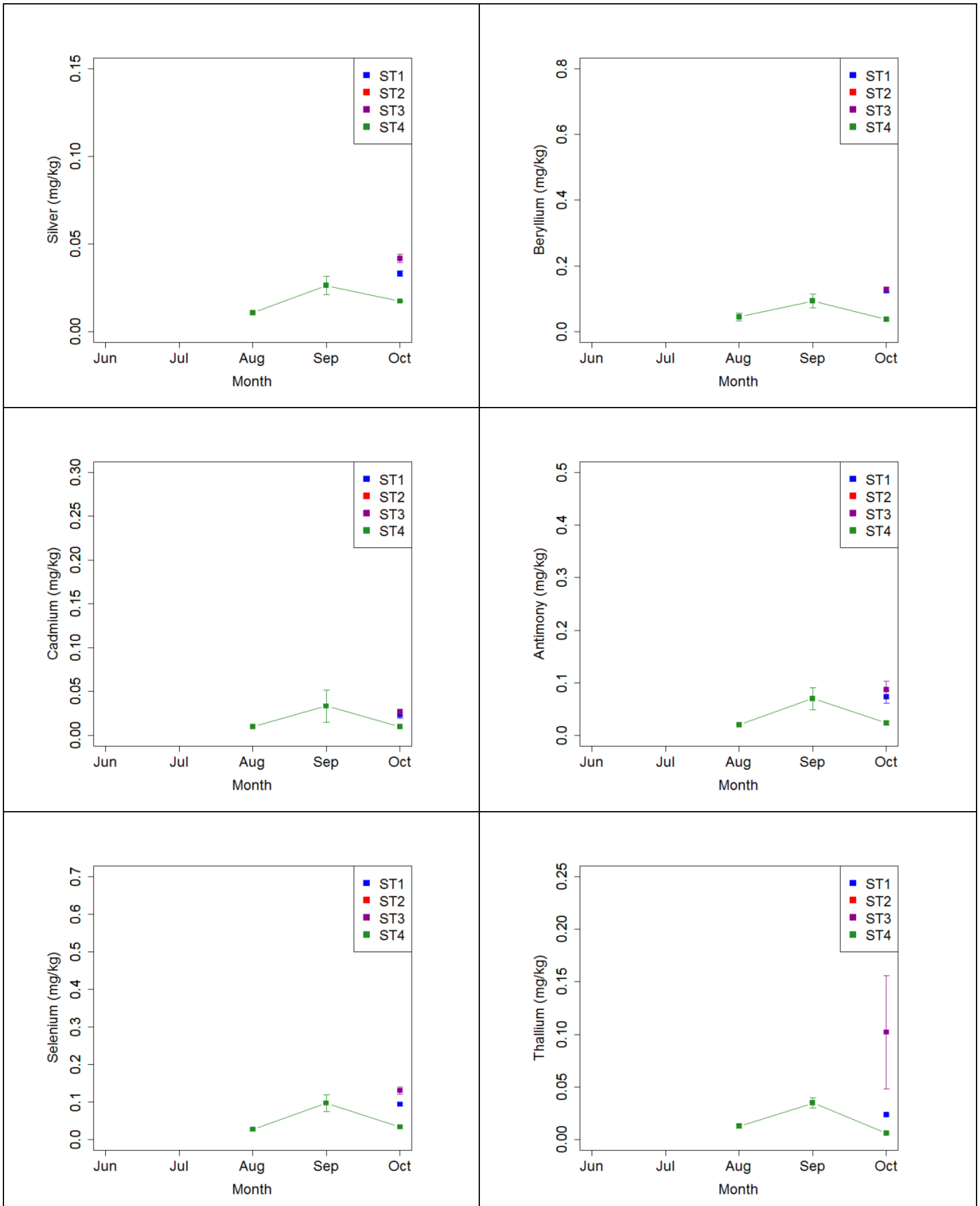
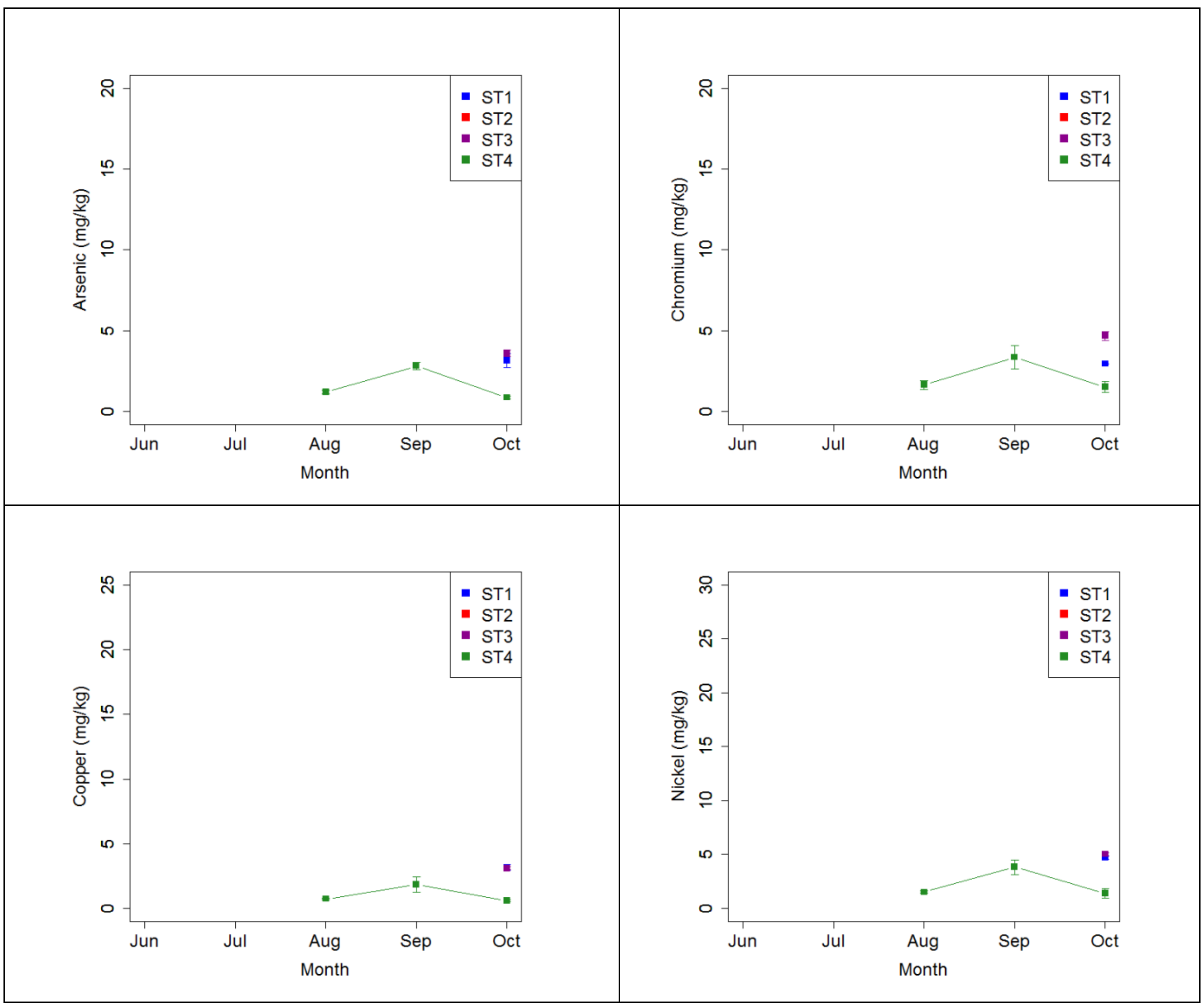


Figure 3.22. *Steepbank River Low Concentration Priority Pollutant Elements.*
Top left: Mean silver (Ag; mg/kg) concentration by site and over months. In October, ST4 was significantly lower than ST1, which was lower than ST3 ($p < 0.05$). Mean Ag concentration at ST4 was significantly greater in September than August ($p = 0.028$). There was insufficient sample replication for a site x month interaction effect.
Top right: Mean beryllium (Be; mg/kg) concentration by site and over months. In October,

ST4 was significantly lower than ST3 and ST1 ($p < 0.001$). Mean Be concentration at ST4 was not significantly different among-months. There was insufficient sample replication for a site x month interaction effect. **Middle left:** Mean cadmium (Cd; mg/kg) concentration by site and over months. In October, ST4 was significantly lower than ST3 and ST1 ($p < 0.05$). Mean Cd concentration at ST4 was not significantly different among-months. There was insufficient sample replication for a site x month interaction effect.
Middle right: Mean antimony (Sb; mg/kg) concentration by site and over months. In October, ST4 was significantly lower than ST3 ($p = 0.023$). Mean Sb concentration at ST4 was not significantly different among-months. There was insufficient sample replication for a site x month interaction effect. **Bottom left:** Mean selenium (Se; mg/kg) concentration by site and over months. In October, ST4 was significantly lower than ST1, which was lower than ST3 ($p < 0.05$). Mean Se concentration at ST4 was significantly greater in September ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect.
Bottom right: Mean thallium (Tl; mg/kg) concentration by site and over months. In October, ST4 was significantly lower than ST3 ($p = 0.004$). Mean Tl concentration at ST4 was significantly greater in September ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.



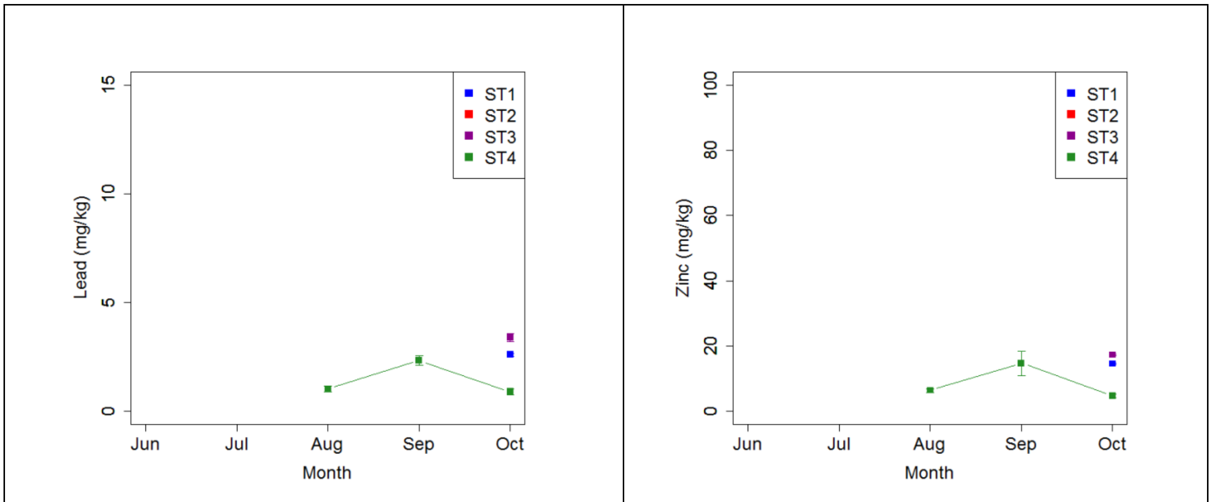


Figure 3.23. *Steepbank River High Concentration Priority Pollutant Elements.*
Top left: Mean arsenic (As; mg/kg) concentration by site and over months. In October, ST4 was significantly lower than ST3 and ST1 ($p < 0.01$). Mean As concentration at ST4 was significantly greater in September ($p < 0.001$). There was insufficient sample replication for a site x month interaction effect.
Top right: Mean chromium (Cr; mg/kg) concentration by site and over months. In October, ST4 was significantly lower than ST1, which was lower than ST3 ($p < 0.05$). Mean Cr concentration at ST4 was not significantly different among-months. There was insufficient sample replication for a site x month interaction effect.
Middle left: Mean copper (Cu; mg/kg) concentration by site and over months. In October, ST4 was significantly lower than ST3 and ST1 ($p < 0.001$). Mean Cu concentration at ST4 was not significantly different among-months. There was insufficient sample replication for a site x month interaction effect.
Middle right: Mean nickel (Ni; mg/kg) concentration by site and over months. In October, ST4 was significantly lower than ST3 and ST1 ($p < 0.001$). Mean Ni concentration at ST4 was significantly greater in September ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect.
Bottom left: Mean lead (Pb; mg/kg) concentration by site and over months. In October, ST4 was significantly lower than ST1, which was lower than ST3 ($p < 0.05$). Mean Pb concentration at ST4 was significantly greater in September ($p < 0.01$). There was insufficient sample replication for a site x month interaction effect.
Bottom right: Mean zinc (Zn; mg/kg) concentration by site and over months. In October, ST4 was significantly lower than ST1, which was lower than ST3 ($p < 0.05$). Mean Zn concentration at ST4 was significantly greater in September than October ($p = 0.043$). There was insufficient sample replication for a site x month interaction effect.
*Error bars represent the standard error of the mean.

Ells River

Low Concentration Priority Pollutant Elements

All low concentration PPEs were significantly different among-sites, with the most downstream site (EL1) having the significantly lowest concentrations for all elements. All low concentration PPEs, except Tl, had significantly lowest concentrations in August. There was a significant site x month interaction for all low concentration PPEs.

High Concentration Priority Pollutant Elements

All high concentration PPEs were significantly different among-sites, with EL1 having the significantly lowest concentrations for all elements. All high concentration PPEs had significantly lowest concentrations in August. There was a significant site x month interaction for all high concentration PPEs.

Table 3.10. Summary table of Ells River fine sediment chemistry for 12 priority pollutant elements (PPEs) analyzed with a two-way mixed-effects ANOVA, which included a) site and, b) month. Upstream (U/S) to downstream (D/S) changes in variable concentrations are indicated with "+" for increasing and "-" for decreasing, and an interaction column indicates any significant site x month interaction effects.

Para-meter	Fig #	Site Differences	*p*-value	U/S-D/S Changes	Monthly Differences	*p*-value	Interaction Effect
Low Concentration PPEs							
Ag	3.24	EL3 = EL2 > EL1	< 0.001	-	Aug < Sep, Oct	< 0.05	0.000
Be	3.24	EL3 = EL2 > EL1	< 0.001	-	Aug < Sep	0.023	0.001
Cd	3.24	EL2 > EL3 > EL1	< 0.01	-	Aug < Sep, Oct	< 0.05	0.011
Sb	3.24	EL3 = EL2 > EL1	< 0.001	-	Aug < Sep, Oct	< 0.05	0.000
Se	3.24	EL2 > EL3 > EL1	< 0.01	-	Aug < Jun, Sep, Oct	< 0.05	0.003
Tl	3.24	EL2 > EL3 > EL1	< 0.05	-	No	> 0.05	0.001
High Concentration PPEs							
As	3.25	EL3 = EL2 > EL1	< 0.001	-	Aug < Sep	0.008	0.000
Cr	3.25	EL3 = EL2 > EL1	< 0.001	-	Aug < Sep, Oct	< 0.05	0.000
Cu	3.25	EL3 = EL2 > EL1	< 0.001	-	Aug < Sep, Oct	< 0.01	0.000
Ni	3.25	EL3 = EL2 > EL1	< 0.01	-	Aug < Sep	0.011	0.000
Pb	3.25	EL3 = EL2 > EL1	< 0.01	-	Aug < Sep, Oct	< 0.05	0.003
Zn	3.25	EL3 = EL2 > EL1	< 0.001	-	Aug < Sep, Oct	< 0.05	0.000

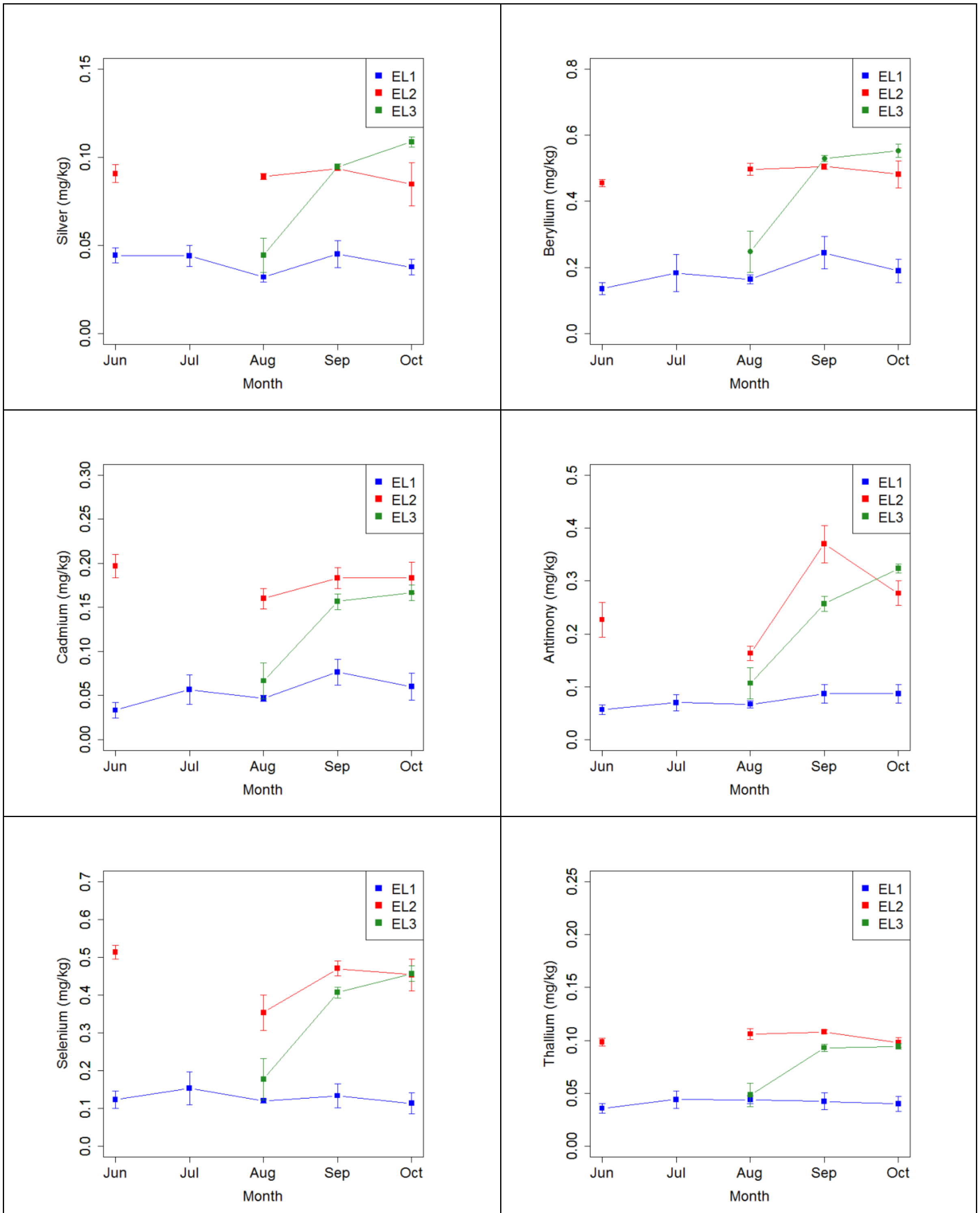
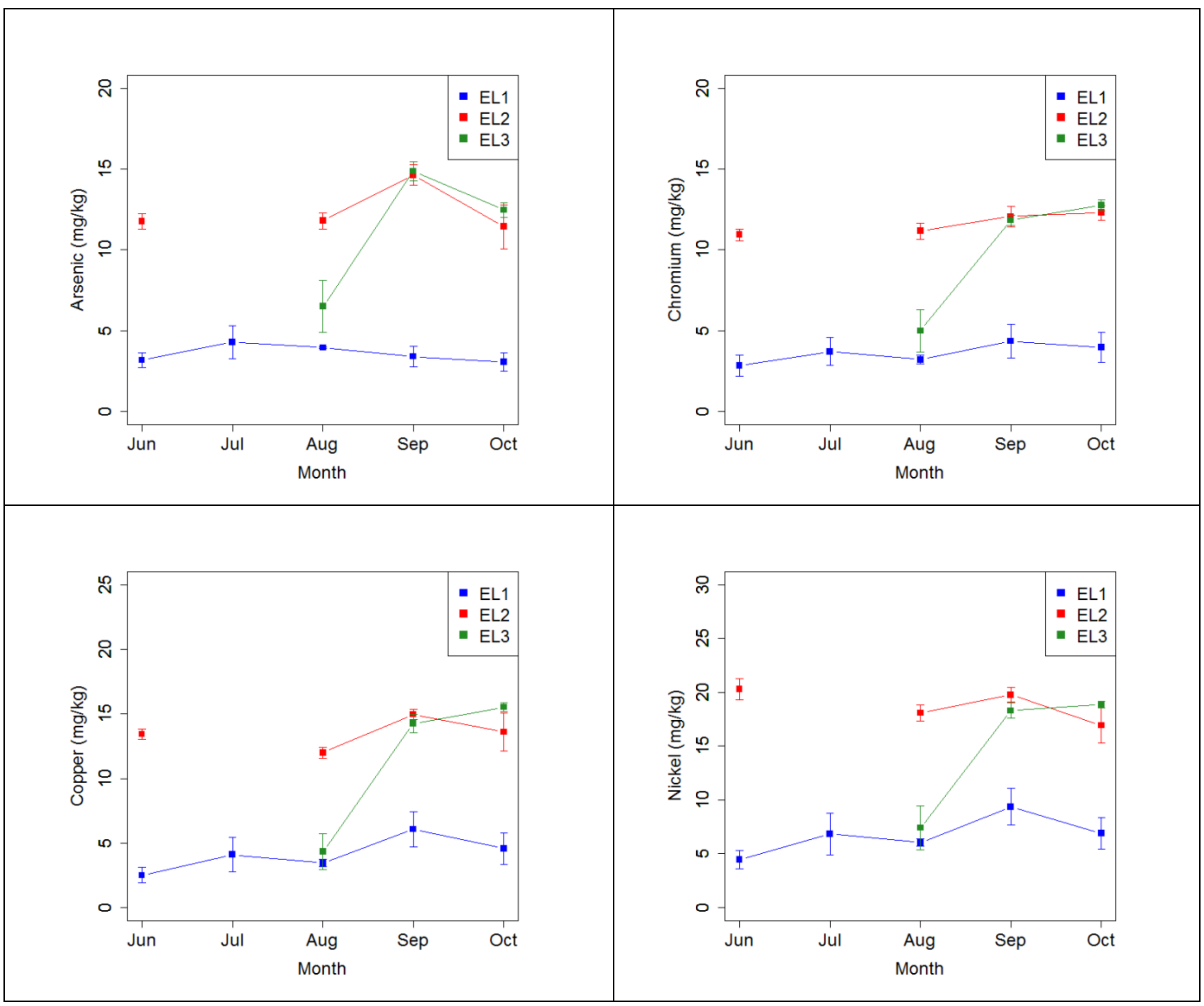
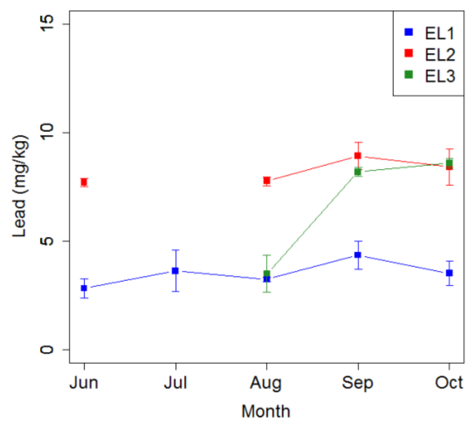


Figure 3.24. *Ells River Low Concentration Priority Pollutant Elements.*
Top left: Mean silver (Ag; mg/kg) concentration by site and over months. EL3 and EL2 were significantly greater than EL1 ($p < 0.001$). Mean Ag concentration in August was significantly lower than September and October ($p < 0.05$). There was a significant interaction for site x month ($p = 0.000$). **Top right:** Mean beryllium (Be; mg/kg) concentration by site and over months. EL3 and EL2 were significantly greater than EL1

($p < 0.001$). Mean Be concentration in August was significantly lower than September (p = 0.023). There was a significant site x month interaction (p = 0.001).
Middle left: Mean cadmium (Cd; mg/kg) concentration by site and over months. EL2 was significantly greater than EL3, which was greater than EL1 ($p < 0.01$). Mean Cd concentration in August was significantly lower than September and October ($p < 0.05$). There was a significant site x month interaction (p = 0.011). **Middle right:** Mean antimony (Sb; mg/kg) concentration by site and over months. EL3 and EL2 were significantly greater than EL1 ($p < 0.001$). Mean Sb concentration in August was significantly lower than September and October ($p < 0.05$). There was a significant site x month interaction (p = 0.000). **Bottom left:** Mean selenium (Se; mg/kg) concentration by site and over months. EL2 was significantly greater than EL3, which was greater than EL1 ($p < 0.01$). Mean Se concentration in August was significantly lower than June, September and October ($p < 0.05$). There was a significant site x month interaction (p = 0.003).
Bottom right: Mean thallium (Tl; mg/kg) concentration by site and over months. EL2 was significantly greater than EL3, which was greater than EL1 ($p < 0.05$). Mean Tl concentration was not significantly different among-months. There was a significant site x month interaction (p = 0.001). *Error bars represent the standard error of the mean.





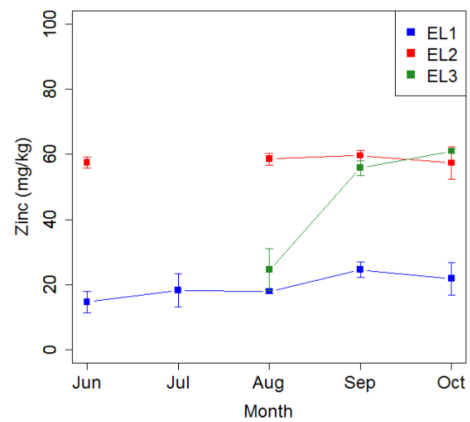


Figure 3.25. *Ells River High Concentration Priority Pollutant Elements.*
Top left: Mean arsenic (As; mg/kg) concentration by site and over months. EL3 and EL2 were significantly greater than EL1 ($p < 0.001$). Mean As concentration in August was significantly lower than September ($p = 0.008$). There was a significant site x month interaction ($p = 0.000$).
Top right: Mean chromium (Cr; mg/kg) concentration by site and over months. EL3 and EL2 were significantly greater than EL1 ($p < 0.001$). Mean Cr concentration in August was significantly lower than September and October ($p < 0.05$). There was a significant site x month interaction ($p = 0.000$).
Middle left: Mean copper (Cu; mg/kg) concentration by site and over months. EL3 and EL2 were significantly greater than EL1 ($p < 0.001$). Mean Cu concentration in August was significantly lower than September and October ($p < 0.01$). There was a significant site x month interaction ($p = 0.000$).
Middle right: Mean nickel (Ni; mg/kg) concentration by site and over months. EL3 and EL2 were significantly greater than EL1 ($p < 0.01$). Mean Ni concentration in August was significantly lower than September ($p = 0.011$). There was a significant site x month interaction ($p = 0.000$).
Bottom left: Mean lead (Pb; mg/kg) concentration by site and over months. EL3 and EL2 were significantly greater than EL1 ($p < 0.01$). Mean Pb concentration in August was significantly lower than September and October ($p < 0.05$). There was a significant site x month interaction ($p = 0.003$).
Bottom right: Mean zinc (Zn; mg/kg) concentration by site and over months. EL3 and EL2 were significantly greater than EL1 ($p < 0.001$). Mean Zn concentration in August was significantly lower than September and October ($p < 0.05$). There was a significant site x month interaction ($p = 0.000$). *Error bars represent the standard error of the mean.

3.4 Discussion

3.4.1 Hydrology and Habitat Characteristics

Discharge measurements on the Steepbank River in 2012 demonstrated a reduced spring-melt event compared to historical hydrographs, with pronounced hydrological extremes occurring in late June/early July. Effects of delayed snow-melt combined with summer rainfall could have possibly contributed to these exacerbated discharge events on the river basin, as explained by Woo and Thorne (2006). Several large pluvial events also occurred in mid-September, which resulted in a large increase in river discharge. The Ells River 2012 hydrograph also displayed a diminished spring-melt event compared to historical hydrographs, with a spike in discharge occurring in late July, coinciding with a sizable summer rainfall event. Backwater at the Ells River hydrometric station created an erroneous increase in discharge from January-April as well as in November due to ice and/or other effects, as described by RAMP (2012). Overall, the Steepbank River experienced major flooding events throughout the sampling period, which could potentially be attributed to an atypical year with extreme flooding occurring on numerous rivers in the lower Athabasca region in 2012.

Flow velocity and water depth where the rock-basket artificial substrates were situated within the Steepbank River exhibited greatest depths and lowest velocities at the downstream site (ST2). Greatest depths and lowest velocities occurred in winter (under-ice), with lowest depths and greatest velocities in summer. Water depth on the Ells River displayed lowest depths at the mid-reach site (EL2), and greatest depths at the most downstream site (EL1). There were no significant differences in flow velocity where rock-baskets were situated from upstream to downstream. Greatest deployment depths were in early summer and lowest retrieval depths in late summer. Highest flow velocities were measured in late spring/early summer coinciding with increased flow during freshet, and lowest flows were observed in the low flow season of late summer/early fall.

Regional precipitation, catchment area, and the presence of lakes can all influence hydrological variation among riverine systems (Chow 1964). Thus, between-basin variations in discharge, water depth and flow velocity in this study were possibly attributed to the differences in catchment size, with the Ells River basin being greater than the Steepbank River basin, lake storage effects from the Namur-Gardiner lakes in the upper Ells River catchment (Headley et al. 2005), as well as geographical separation within the AOSR. Hydrologically connected lakes on a river basin can have buffering effects, ultimately influencing the magnitude and frequency of downstream hydrological events (Emmerton et al. 2007).

Substrate composition within both the Steepbank and Ells Rivers was dominated by cobbles, pebbles, and gravel. Distribution of cobbles decreased, while pebbles increased from upstream to downstream in the Steepbank River with the opposite pattern observed in the Ells River. Natural variation in substratum within the rivers was likely attributed to differences in gradients and water depth at sampling sites. Slope was greatest at upstream sites on the Steepbank River (ST3 and ST4), and decreased in the lower reaches (ST2 and ST1). High-gradient sections of the Steepbank River generally consisted of cobbles and low-gradient sections consisted mainly of pebbles and gravel, as described by Hawkins et al. (1982).

Slope was lowest at the most downstream site on the Ells River (EL1); however, sites were relatively consistent. Differences in water depth can also influence substrate composition, with sufficient tractive force to transport sediment as water depth decreases, or low tractive force so the sediments are deposited as water depth increases (Culp et al. 1986). Upstream to downstream changes in substrate composition within the Steepbank River was also potentially attributed to the steep banks in the lower reaches being susceptible to increased frequency of natural slump events, depositing sediment into the river, as described by Hickin (1974) and Sekerak and Walder (1980).

Moreover, the 100 pebble count has been scrutinized as a substrate sampling method by being biased towards selection of specific-sized substrates when practised incorrectly, and having high sampling error when performed by separate individuals (Marcus et al. 1995). Personal observation of the substratum in the two rivers identified the upstream site on the Steepbank River (ST4) to contain numerous boulders, and the downstream sites on both basins to contain consolidated material, resembling bedrock. This was attributed to large pieces of bitumen eroding from the river bank and being transported downstream to form a bedrock-like substrate in the lower reaches (Barton and Wallace 1979). The substrate composition documented by CABIN sampling did not obtain results in congruence with these personal observations. Based on these findings, application of the 100 pebble count may need to be re-assessed as a method for substrate characterization for tributaries in the AOSR.

Enhanced OS catchment-scale mining activities could have also potentially influenced the hydrological characteristics of the Steepbank River. For example, modification of the runoff-evapotranspiration balance from land use disturbances can cause increases in flood magnitude and frequency, and often lowers base flow if the river is hydrologically connected to the surrounding area (Allan 2004). Anthropogenic catchment-scale disturbances also create impervious landscapes, resulting in “flashy” hydrographs during precipitation events (Dunne and Leopold 1978). Two large discharge events were observed on the more-disturbed Steepbank River basin, compared to the less-disturbed Ells River basin during the 2012 sampling period.

Catchment-scale land disturbance can also increase sedimentation into the river ecosystem (Walling and Fang 2003). According to some studies, human-induced sedimentation exceeds natural rates of sedimentation by a factor of ten (Knox 2006; Leigh and Webb 2006). The large slump event which occurred at the mid-reach site on the Steepbank River (ST3) in spring 2012 created an increase in sediment transported to the downstream environment. Therefore, the Steepbank River possibly contained more pebble and gravel substrate at the downstream sites than the Ells River, from increased erosion from unstable river banks as a consequence of human land use activities, as described by Paul and Meyer (2001). Ultimately, alterations to physical habitat characteristics will influence the river ecology, with more disturbed systems having considerable deviations from the natural flow regime (Allan 2004).

3.4.2 Water and Sediment Chemistry

3.4.2.1 Water Quality Parameters

Nutrients

Among-site differences were not observed for all nitrogen (N) and phosphorus (P) parameters within either the Steepbank or Ells Rivers. Based on a review of previous studies investigating the effects of mining activities on streams and rivers, it was hypothesized that landscape features, such as disturbed lands from OS catchment-scale development, would result in deteriorated environmental conditions with an increase in non-point source nutrient loadings (Carpenter et al. 1998; Bruns 2005). Findings from this study may indicate that leached nutrients within catchment runoff contained insufficient concentrations to produce significant increases in the downstream environment, as described by Likens and Bormann (1974). In a study by Hickman et al. (1980), water quality N and P concentrations were investigated from upstream to downstream within the Steepbank and Ells Rivers, prior to major OS development. Their findings also demonstrated relatively consistent upstream to downstream patterns in N and P concentrations for both rivers. Further investigations of N and P nutrient limitations in these river systems over one-month intervals are evaluated utilizing Nutrient Diffusing Substrates (NDS) in Chapter 4.

The only nutrient parameter which was significantly different among-sites in the Steepbank River was K^+, with the most upstream site (ST4) containing lower concentrations than downstream sites. K^+ has been found to be highly positively correlated with other parameters such as: SO_4^{2-}, Cl^-, Ca^{2+}, Mg^{2+}, Na^+, TDS, and Cond, in other river ecosystems (Vega et al. 1998). Many of these correlated parameters also displayed downstream changes within the Steepbank River. These major ions also account for most of the TDS in surface water. Gorrell (1974) observed elevated TDS concentrations in the lower reaches of the Steepbank River, which flow through the McMF, which in addition to the erosion of saline rocks can also cause a natural flow of salts from the Devonian Formation to the surface water. K^+ has also been found in shallow groundwater chemistry in the lower reaches of tributaries in the AOSR (Hackbarth 1981). Therefore, downstream increases in K^+ concentrations were likely attributed to natural inputs from the OS geological deposit and shallow groundwater upwelling.

Steepbank River N and P parameter concentrations were highest in the late spring/summer for all sites. The Steepbank River hydrograph illustrated increased discharge in the late spring and early summer, likely attributed to a combination of delayed snow-melt and precipitation events, which can result in increased runoff within the river basin, as described by Andersen et al. (2006). The Ells River had highest N and

P parameter concentrations in May and July, which coincided with the onset of spring freshet in May, and elevated discharge in July from summer pluvial events. K^+ concentrations on both river basins were greatest in May followed by a decrease during other months; therefore, K^+ could have been influenced by dilution effects from freshet (Edwards 1973). In general, the Ells River contained greater K^+ concentrations than the Steepbank River, and the Steepbank River portrayed greater overall TP, TDP, TN, and TDN concentrations across sites and months.

Cations/Anions

Both river basins had significant among-site differences for all major cations and anions, except SiO_2^{2-}, with concentrations increasing from upstream to downstream. This finding corroborates with results from other studies investigating the effects of various anthropogenic watershed land use activities on river water chemistry (Dow et al. 2006). During precipitation events, chemical weathering and ions leaching from soils would increase cation/anion concentrations downstream in the river environment during runoff (Raymond et al. 2008). SiO_2^{2-} did not follow this pattern because it is only slowly weathered in most soils (Fisher et al. 1987). Major ions also co-occur with elevated levels of naturally occurring petroleum hydrocarbons in the OS deposit (Maclock et al. 1997). Upwelling of brackish groundwater can also produce high concentrations of major ions in the lower reaches where the Steepbank and Ells Rivers incise through the McMF (Sekerak and Walder 1980; Hackbarth 1981).

Headley et al. (2005) investigated the effects of natural OS bitumen deposits on water chemistry within the Steepbank and Ells Rivers in spring and fall of 1998 and 1999, prior to major OS development. Results from their study demonstrated no consistent upstream to downstream pattern within the Steepbank River, and a slight increase downstream in the Ells River in major ions. In comparison, this study revealed a significant increase in cations and anions from upstream to downstream on both basins, which were not entirely explained by the natural bitumen deposit or shallow groundwater upwelling, indicating increased concentrations downstream were possibly attributed to land use disturbance.

Furthermore, in a study by Alexander and Chambers (in press), historical water quality data (1972-2010) from tributaries in the AOSR was analyzed to evaluate changes in water quality over time in relation to type (open-pit versus *in situ* drilling) and stage (pre-development, early land-clearing and construction, as well as later expanded development) of OS mining activities. They found increased concentrations of five key water quality parameters (dissolved selenium, arsenic and boron, and total uranium and vanadium) downstream of both types of mining operations, particularly during the early stage of development. Their findings indicated that erosion and subsequent runoff associated with land-clearing, construction and early operational activities in the AOSR are altering tributary water quality.

Seasonally, Steepbank River Ca^{2+}, Mg^{2+}, Na^{+} and SiO_2^{2-} concentrations increased from spring to summer, followed by a decrease in fall. With the Steepbank River being characteristic of having increased discharge during pluvial events, ion leaching into the river from the basin may have occurred most frequently during summer (Raymond et al. 2008). Cl^{-} and SO_4^{2-} concentrations were greatest in May; therefore, potentially more influenced by freshet dilution effects. Hackbarth (1981) also found Ca^{2+} and Mg^{2+} concentrations from shallow groundwater chemistry to be lowest in the spring and rise rapidly by early summer, and SO_4^{2-} to be greatest in spring in tributaries of the AOSR. Ells River Mg^{2+}, Na^{+}, Cl^{-} and SO_4^{2-} concentrations decreased from spring to summer with the onset of freshet and increased discharge, followed by an increase into fall. Therefore, Ells River major cations and anions had highest concentrations during low-flow conditions (Edwards 1973), and ion leaching during summer months possibly had a diminished effect on the less-disturbed landscape. Moreover, a buffering effect from upstream Namur-Gardiner lakes could potentially contribute to the observed patterns through moderating runoff.

In general, the Ells River contained greater concentrations of cations/anions parameters than the Steepbank River across sites and months. This was contrary to results from previous studies during early OS mining activities on the Steepbank River basin and prior to land-clearing on the Ells River basin, which demonstrated the Steepbank River to contain greater concentrations than the Ells River (Headley et al. 2005). Findings from this study supported the results from Alexander and Chambers (in press), which suggests early land-clearing for OS development alters tributary water quality in the AOSR.

Carbonate Complex

Among-site differences were only associated with a significant increase in Alk from upstream to downstream within the Ells River. Alk is a parameter that frequently increases in association with catchment-scale land use change (Ometo et al. 2000). Moreover, an increase in Alk from upstream to downstream in the Ells River was likely attributed to the geology in the AOSR, with the porous and permeable Devonian rocks (limestone, dolomite and gypsum) being thicker in the western part than the eastern part of the geologic formation, where there is considerable down cutting into the overlying McMF (Gorrell 1974).

Seasonal differences in Alk and DOC concentrations were observed in the Steepbank River. Spring runoff from snow-melt and summer precipitation likely accounted for increased Alk and DOC concentrations in the river ecosystem in summer. Further, DOC produced from decomposition of organic matter would be faster with higher temperatures, as well as DOC produced *in situ* by algal photosynthesis would also be greater explaining the higher summer DOC concentrations within both rivers (Wetzel 1983). Alk concentrations within the Ells River were greatest during low-flow conditions, attributed to an inverse of the dilution effect, as explained by Ouyang et al. (2006). Overall, the Ells River had greater concentrations of pH and Alk, whereas the Steepbank River had greater concentrations of DOC across sites and months.

Measurements from both of the YSI sondes and bulk water quality demonstrated the Steepbank and Ells Rivers pH values maintained slightly basic over the 2012 sampling period, which was congruent with previous studies on water chemistry within these tributaries (Headley et al. 2005). Stream pH is primarily determined by the surrounding geological composition; moreover, anthropogenic disturbances also influence acidity through changes in catchment geochemistry and aerial contaminant deposition (Monteith et al. 2007). Extreme pH values, either too high (basic) or too low (acidic) can have negative consequences on sensitive benthic macroinvertebrate species (Environment Canada 2010). Extreme pH values were not observed throughout the sampling period, except for a few instances of sudden declines at sites on the Steepbank River; however, this was likely attributed to YSI probe malfunction (Bienfang 1980; Hartley et al. 2005).

Physical/Water Quality Parameters

The Steepbank and Ells Rivers YSI Temp measurements were consistent among-sites and between-basins, with an increase in spring with river ice break-up and a decrease in fall coinciding with freeze-up. High variability occurred in August within the Steepbank River at ST2, which was attributed to the YSI 6600-V2 sonde being situated in very shallow water and exposed to air upon retrieval. YSI DO measurements in the Steepbank River were also relatively similar among-sites and ranged from 80-100% or 8-10 mg/L. DO in the Ells River was very consistent among-sites, fluctuating around 100% saturation or 10 mg/L, being characteristic of a typical fast-flowing river reach. DO concentrations (mg/L) on both basins declined slightly during summer, as equilibrium solubility declined with increasing temperatures, followed by an increase into fall with decreasing temperatures (Wetzel 1983).

Low DO has been identified as a serious water quality problem in freshwater ecosystems. When DO falls below 5 mg/L, sensitive species can be negatively affected (Caraco and Cole 2002). Low DO occurs regularly in the bottom of aquatic systems (Wetzel 1983), systems with heavy organic loads (Clark et al. 1995), and thick macrophyte beds (Suthers and Gee 1986), as well as during sedimentation events (Crisp 1989; Wood and Armitage 1997). Sampling sites on the Steepbank and Ells Rivers did not reach DO levels less than 5 mg/L, except for a few instances of sudden declines; however, this was mostly attributed to YSI probe malfunction (Bienfang 1980; Hartley et al. 2005), but also possibly storm events (Boët et al. 1999).

YSI Cond measurements in the Steepbank River were consistent among-sites, with a large decrease from winter to spring with the onset of freshet and in-flux of freshwater, as described by Mann et al. (2012). YSI Cond measurements in the Ells River were comparable between the two upstream sites, EL3 and EL2; however, the most downstream site, EL1, had extreme variability from spring to summer. This was likely attributed to the YSI 6600-V2 sonde being submerged within a silt-clay environment during spring (Jain et al. 2004); therefore, not adequately representing the water column chemistry. Therefore, this emphasized the importance of comparing various collection techniques of water quality parameters, with equipment failure and inaccurate readings being a common occurrence during sampling (Barbour et al. 1999).

Bulk water quality samples from the Steepbank River portrayed an increase in Cond and TDS from upstream to downstream, whereas the Ells River had a downstream increase in all physical/water quality parameters. These observations were possibly related to anthropogenic land use activities on the basins, resulting in unstable river banks and increased watershed loadings (Walling 2000; Bruns 2005). In a study by Headley et al. (2005), no upstream-downstream patterns in TDS were observed in the Steepbank River in early stages of OS development (1998, 1999). They found TDS slightly increased from upstream to downstream in the Ells River; however, they attributed this to the down cutting of the river through the McMurray and Devonian Formation in the lower reaches. This suggests, enhanced OS mining intensity and land-clearing over time on both catchments may have potentially increased cumulative loadings from upstream sedimentation events, producing increases in TDS and TSS, influencing Cond and Turb, in the downstream river environments. The gradient sampling design used in this study also demonstrated downstream increases which were not entirely explained by natural bitumen deposits for both basins.

Steepbank River Cond and TDS concentrations increased from spring to summer, followed by a decrease in fall. These observations coincided with other nutrient, cation/anion and carbonate complex parameter seasonal patterns within the Steepbank River, explained by delayed snow-melt, increased summer rainfall and discharge within the river basin (Ouyang et al. 2006), as well as seasonal variation in shallow groundwater upwelling (Hackbarth 1981). Turb and TSS concentrations increased in June, which was most likely attributed to a sizeable slump event which occurred at the mid-reach site in spring 2012. Turb and TSS concentrations were greatest in May and July in the Ells River. An increase in discharge coincided with river ice break-up in May, as well as pluvial events in July, which would transport suspended sediments into the lotic ecosystem, as explained by Hooke (1979). Cond and TDS decreased from spring to summer, followed by an increase in fall. These observations coincided with cation/anions and other water quality parameters in the Ells River, explained by the dilution effect during the wet summer season (Ouyang et al. 2006). Both rivers exhibited comparable Turb and TSS concentrations, and the Ells River displayed greater Cond and TDS concentrations across sites and over months, most likely attributed to the geology being thicker in the western part of the formation, causing an increase in the salinity of the surface water (Gorrell 1974).

Furthermore, in a study by Kashyap et al. (2014), major tributaries in the lower Athabasca region were studied to identify where sediment loads were originating from before depositing into the Athabasca River. TSS loads at the inflow and tributary boundaries were determined through discharge rating curves developed from WSC and RAMP data. The results demonstrated that the Ells River was naturally contributing substantial amounts of sediment into the main stem under peak flow conditions, in comparison to the Steepbank, Firebag, Mackay and Muskeg Rivers. These results supported observations from this study demonstrating higher Turb and TSS amounts in the lower Ells River, which will ultimately have implications on suitable habitat availability for algal communities and benthic biota (Schofield et al. 2004), as well as influence fine sediment chemistry.

3.4.2.2 Fine Sediment Chemistry

All low and high concentration PPEs increased in concentration from upstream to downstream within the Steepbank River. These results may potentially be attributed to the transportation of metal-concentrated fine sediments downstream from a basin disturbed by OS mining activities (Axtmann and Luoma 1991), the close proximity of the mouth of the Steepbank River (ST1) to OS upgrading facilities which deposits aerial particulates onto the landscape (Kelly et al. 2009), as well as the lower reaches being situated within the OS geological deposit (Maclock et al. 1997). Previous studies on metals in suspended sediments in the Steepbank River demonstrated no significant increase through reaches that have natural bitumen exposures (Conly et al. 2007), suggesting there could be another possible source of metals within the river ecosystem.

Comparatively, the Ells River most downstream site (EL1) contained depressed levels of low and high concentration PPEs. EL1 is situated within the OS bitumen deposit, and was hypothesized to contain higher levels of naturally sourced metals than upstream sites (Maclock et al. 1997). Therefore, alternative factors may be influencing sediment chemistry within the Ells River. These include: the upper basin comprising a natural origin of high element concentrations (Lechler et al. 2000), or upper basin lake storage effects from the Namur-Gardiner lakes influencing downstream chemistry (Headley et al. 2005). Conly et al. (2007) also documented decreasing fine sediment metal concentrations from upstream to downstream sites within the Ells River. This was explained by longitudinal changes in suspended sediment particle size, which tend to become coarser along the river gradient, with metals not binding as easily to courser particles. Moreover, it was uncertain whether the consistency in particle size from upstream to downstream within the Ells River was due to hydrologic factors or to their sampling design.

All low and high concentration PPEs contained highest levels in the fall in the Steepbank River. Elevated September concentrations were attributed to the large rainfall events which occurred immediately prior to rock-basket retrieval, potentially increasing sedimentation loads into the lotic ecosystem. Moreover, low and high concentration PPEs on the Ells River had depressed concentrations in summer. Decreased element concentrations in August were attributed to the dilution effect during the wet summer season, and reduced sedimentation on a relatively undisturbed catchment. Although there were significant variations in elements from each site in the different sampling months, there was also a significant site x month interaction for all low and high concentration PPEs in the Ells River. Despite this being a regular outcome in freshwater ecological studies investigating variation across space and time (e.g., Butler 1989; Mackey et al. 1984; Thorrold et al. 1998; Kaldy and Dunton 2000), it created difficulties when interpreting the effects of one factor without the other.

For all 12 PPEs, the upstream Ells River sites, EL3 and EL2, exhibited 2-3X greater concentrations compared to the downstream site, EL1, as well as all Steepbank River sites. An alternative explanation of depressed PPE concentrations at EL1 involves the occurrence of naturally high TSS loadings in the lower basin during peak flow conditions (Kashyap et al. 2014). Depressed concentrations at EL1 could have been observed due to greater fine sediment material situated on the sediment trap, creating a "dilution effect" of the PPE concentrations. Therefore, measuring the mass of fine sediment which accumulated on each scour pad would be beneficial to determine if there were actual site differences in PPE concentrations. Overall, this study suggests longitudinal and seasonal changes in fine sediment metal concentrations in both the Steepbank and Ells Rivers were likely attributed to natural variation. Moreover, tracer work would need to be conducted to better determine sources of fine sediment metals in these river ecosystems, as explained by Feng et al. (1999).

3.5 Conclusions

A variety of responses were observed for physical and chemical environmental variables within and between river basins, as well as over seasons in tributaries of the AOSR. Substrate composition at the mouth of the Steepbank River was physically modified from a large slump event which occurred during the 2012 sampling period. Increased erosion from unstable river banks could have been a consequence of catchment-scale disturbances (Paul and Meyer 2001); however, the steep banks characteristic of this river are also susceptible to natural sedimentation events.

In most cases, when a significant among-site difference was observed for a chemical parameter within the surface water or fine sediment, upstream sites contained lower concentrations than downstream sites related to the environmental disturbance gradient. Watershed loadings and transport of materials from upstream land use perturbations may have potentially created increased concentrations in the downstream lotic environment. Moreover, effects from natural bitumen deposits and shallow groundwater upwelling were most likely attributed to changes in water and sediment chemistry from upstream to downstream sites in both the Steepbank and Ells Rivers.

Seasonal differences in the Steepbank River typically portrayed higher concentrations during pronounced discharge events, whereas the Ells River contained greater concentrations during low flow periods. Between-basin differences in monthly concentrations of water quality and fine sediment parameters could possibly be associated with variations in catchment permeability, stage of OS mining development, variability in loadings from runoff during snow-melt and rainfall, major flooding events, natural variation in shallow groundwater chemistry, as well as dilution effects from differences in discharge. Moderating effects of the Namur-Gardiner lakes on the Ells River basin was also clearly reflected in the lack of seasonality in Ells River water chemistry (Headley et al. 2005). Comprehending seasonal variations within and between-basins was imperative at assessing ecosystem responses in relation to non-point source disturbances within tributaries of the AOSR.

Measuring a multitude of parameters allowed a thorough analysis of changes in physical and chemical variables on two river basins with varying levels of anthropogenic disturbance. Notable findings, specifically for the Ells River, which require further investigation included, the potential for naturally elevated sediment chemistry in the upper basin (Lechler et al. 2000), the influence of buffering lake storage effects from the Namur-Gardiner lakes on downstream physico-chemical parameters (Headley et al. 2005), the effects of substantially high suspended sediment loads from the Ells River basin into the Athabasca River (Kashyap et al. 2014), and the relationship of suspended sediment particle size with metal concentrations from upstream to downstream (Conly et al. 2007). Moreover, comparing different stages of OS mining activities on two tributary ecosystems, similar to Alexander and Chambers (in press), provided insight into explaining responses from non-point source disturbances.

Recommendations for future assessments on physico-chemical environmental variables within tributaries of the AOSR would include investigating alternative methods for measuring substrate composition, to facilitate the reduction in bias during sampling as outlined in Sutherland et al. (2010), include information about the physical effects of eroded bitumen material on substrate composition in the lower reaches, as well as measuring substrate compaction to provide critical information on the habitat availability for benthic macroinvertebrates (Gray 2004). Inter-annual sampling of the highly variable Steepbank and Ells Rivers would also assist in determining how differences in hydrology and physical habitat influence water and sediment chemistry, and also highlight whether results from this study would be consistent to other years with less extreme discharge events throughout the sampling period.

Furthermore, collection of multiple bulk water quality samples at a site during a sampling period would produce sample variance, in comparison to a single point measurement. Frequent replacement of the YSI 6600-V2 sondes would reduce the potential for probe fouling and damage, for increased precision of standard water quality measurements (Bienfang 1980; Hartley et al. 2005). Integration of results from water quality total and dissolved metals parameters, PAH concentrations in fine sediments, as well as toxins within depositional sediment samples with the findings from this study would also help determine possible cumulative effects of OS contaminants on the lotic ecosystems (Headley et al. 2005). Moreover, examining wind patterns within the AOSR would identify the locations where OS aerial particulates are being deposited on the surrounding landscapes, to assess which lotic ecosystems along the environmental disturbance gradient have greater potential of increased concentrations of aerial contaminant inputs during catchment runoff (Cho et al. 2014).

Overall, natural variation was the primary driver for changes in physico-chemical variables in both the Steepbank and Ells Rivers. Nevertheless, any deviations from the natural flow regime and "typical" water and sediment chemistry do have consequences on the integrity of freshwater biological communities (Poff et al. 1997). Non-point source disturbances could cause alterations to ecosystem structure and function through changes in basal productivity, nutrient availability and, ultimately benthic macroinvertebrate community composition (Allan 2004).

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CHAPTER 4: EFFECTS OF NATURAL AND ANTHROPOGENIC NON-POINT SOURCE DISTURBANCES ON THE BASAL PRODUCTIVITY OF THE AQUATIC FOOD WEB

4.1 Introduction

Anthropogenic modifications to riverine landscapes can influence the basal productivity of lotic ecosystems (Bunn et al. 1999; Young and Huryn 1999; Houser et al. 2005), affecting the primary food source for many benthic macroinvertebrates (Cummins 1973). Land use perturbations can modify factors controlling basal production through the alteration of flow regimes (e.g., change in intensity or timing of flow; Keppeler and Ziemer 1990; Konrad et al. 2005), and the accelerated transport of nutrients, sediment, organic matter, and pollutants from the watershed into rivers (Johnson et al. 1997; Jordan et al. 1997; Brett et al. 2005). Natural disturbances, such as annual spring freshet, or longitudinal variations in autochthonous and allochthonous contributions along a river channel also control the energy sources in freshwater ecosystems (Vannote et al. 1980; Conners and Naiman 1984; Uehlinger and Naegeli 1998).

Geologic formations within the Athabasca Oil Sands Region (AOSR), specifically the McMurray Formation (McMF), can also have implications on primary productivity and organic matter processing within the lower reaches of tributary ecosystems through potential proximate oil-toxicity (Soto et al. 1975; Miller et al. 1978; Federle et al. 1979; Barsdate et al. 1980), as well as secondary effects resulting from stimulation of bacterial productivity from petroleum hydrocarbons (Lock et al. 1981a; Peterson et al. 1996). In addition, there are a variety of other environmental factors such as nutrient content (Biggs 2000), water temperature (Eulin and Le Cohu 1998), light availability (DeNicola et al. 1992), grazing pressure (Steinman et al. 1990), discharge (Mosisch and Bunn 1997), and substrate stability (Biggs and Gerbeaux 1993) driving temporal and spatial variation of basal production in aquatic food webs.

Epilithic algae, or periphyton, is commonly used as a bioassessment tool to examine the basal production of river ecosystems, by representing the primary producer trophic level (Barbour et al. 1999). Periphyton, primarily composed of photosynthetic algae and associated microbial biofilm, generally have rapid reproduction rates and very short life cycles, making them valuable indicators of short-term perturbations. As primary producers, algae are most directly influenced by physical and chemical environmental variables, with various algal species being sensitive to certain pollutants (Lock et al. 1984). River periphyton have distinct seasonal cycles, with peak abundance and diversity typically occurring in late summer or early fall (DeNicola et al. 1992; Eulin and Le Cohu 1998), while high flows (i.e., during freshet) can scour periphyton away (Stevenson 1990). Because of the high community diversity of algae and the effects of confounding factors on algal growth, various periphyton sampling methods, such as artificial substrates, have been utilized to reduce variability and the number of replicates necessary to collect adequate sample sizes (Morin and Cattaneo 1992).

Artificial substrata are often used to sample epilithon because they simplify the natural complexity by providing uniform colonization time, material, texture and size, and minimize confounding factors (Cairns 1982). They can be easily manipulated in location and incubation time, allow collection in locations that are typically difficult to sample effectively, and facilitate both sampling the periphyton and determination of the sample area (Cattaneo and Amireault 1992). Moreover, several variables considerably influence effectiveness of artificial substrates, such as type of substratum, duration of substrate exposure, placement in the lotic environment with respect to limiting conditions such as shade, temperature or current, as well as location of the sampler relative to surrounding natural substrates (Cairns 1982). Therefore, utilizing both natural and artificial substrates should help determine which method is the most effective bioassessment approach for measuring periphyton in tributaries of the AOSR.

Nutrient availability is often the most critical factor limiting basal production in temperate river ecosystems with minimal riparian shading (Hill and Knight 1988; Tank and Dodds 2003). Research on nutrient limitation on epilithon in streams and rivers has primarily focused on nitrogen (N) and phosphorus (P) concentrations as the limiting nutrients in these systems (Pringle et al. 1986; Tank and Webster 1998; Wold and Hershey 1999). Moreover, it is difficult to relate nutrient concentrations to periphyton growth because of confounding variables (Francoeur et al. 1999). Therefore, a controlled *in situ* bioassay can be used which slowly releases N, P or both, to stimulate the establishment of particular periphyton communities on their outer surfaces, while reducing the influences of extraneous factors (Fairchild and Lowe 1984).

Nutrient diffusing substrata (NDS) have been used widely to study spatial and temporal patterns in nutrient limitation on periphyton growth in numerous lentic and lotic ecosystems (Fairchild and Lowe 1984; Winterbourn and Fegley 1989; Barnese and Schelske 1994), including the main stem Athabasca River (Scrimgeour and Chambers 1997). Changes in nutrient availability along an environmental disturbance gradient could possibly be attributed to nutrient inputs from natural and anthropogenic non-point source land use disturbances (Carpenter et al. 1998). Therefore, nutrient limitation was investigated in this study as a possible mechanism influencing basal production along the environmental disturbance gradient and over various seasons for both the Steepbank and Ells Rivers.

Measuring periphyton biomass utilizing various methods, as well as assessing potential nutrient limitations, at different times of the year, will examine the response of a rivers biotic condition to a gradient of land use disturbance. Comparing two basins with differing anthropogenic land use disturbances can assist in determining any consequences of the different stages of oil sands (OS) mining activities on the basal production of tributary ecosystems in the AOSR. Furthermore, investigating the congruencies and deviations in periphyton abundance between natural and artificial substrates along the environmental disturbance gradient and among-seasons will help determine which sampling method is most effective for the Steepbank and Ells Rivers.

Studies to date examining the potential effects of OS development on surrounding river ecosystems in the AOSR have not routinely measured periphyton biomass, or nutrient limitation, utilizing the sampling design implemented in this study (Hickman et al. 1980, 1983; Environment Canada 2011b, c; RAMP 2012). Therefore, this chapter evaluates the observed differences in periphyton abundance and nutrient limitation within- and among-sites, and between-basins across sampling months, while assessing the disturbance gradient design. See Chapters 1 and 2 for detailed objectives and predictions for Chapter 4.

4.2 Methods

4.2.1 Field Sampling

Periphyton Rock Scrapings were collected from five cobble-sized rocks within the sampling riffle at each site during each sampling period. Two 13.20 cm^2 circles were traced out on each rock using a scalpel and PVC tube with a 4.1 cm internal diameter, following procedures in Hickman et al. (1980). The periphyton samples delineated therein were scraped off from each rock using a scalpel and brush, and rinsed with deionised (DI) water and pooled into two containers. One sample was collected for the analysis of photosynthetic algal biomass through chlorophyll *a* (Chl *a*), and total biofilm mass through ash-free dry mass (AFDM), and the other sample was collected for algal taxonomic identification as outlined by Hauer and Lamberti (2011).

The Chl *a* and AFDM sample was preserved in 95% ethanol (EtOH) and placed in a -20°C freezer for further sample processing and analysis. The algal taxonomy sample was preserved in Lugols and DI water for future identification. A bulk periphyton scraping was also collected from each rock in a sample container for stable isotope analysis, which was stored on ice packs in a cooler during the field day, and transferred to a -20°C freezer for future analysis.

BBQ Briquette Periphyton was collected from each of the three rock-basket artificial substrates at each site (n=3) during monthly retrievals (Figure 4.1). Five BBQ briquettes were removed from the tops of each of the rock-baskets and placed into three separate sample containers pertaining to the tray number. 95% EtOH was immediately added to the containers, enough to cover the BBQ briquettes, and samples were placed in a -20°C freezer upon arrival to the laboratory. BBQ briquette periphyton samples were collected to estimate the monthly accumulation of algal (Chl *a*) and biofilm (AFDM) biomass on a standardized substrate at each site. An additional five BBQ briquettes were collected and placed in separate sample containers with Lugols and DI water for future identification of algal taxonomy.

Nutrient Limitation of periphyton growth was measured utilizing NDS to determine potential N, P, and/or co-limitation (N + P) at each site during each monthly sampling period following methods of Tank and Dodds (2003). Each NDS tray was composed of four treatment sample vials containing: Control (agar only), N addition (0.5 M $NaNO_3$), P addition (0.5 M KH_2PO_4), and N + P combined (0.5 M $NaNO_3$ + 0.5 M KH_2PO_4). Agar surfaces were covered with porous silica discs, and nutrients would diffuse into the river ecosystem during the one-month deployment period. If the lotic environment was N, P and/or co-limited, greater amounts of periphyton would grow on the silica disc pertaining to the treatment sample vial diffusing those nutrients (Figure 4.1).

Three NDS trays were deployed and retrieved while fastened to the rock-baskets at each site (n=3) during every monthly sampling period. During retrieval, four sample vials were removed from the NDS tray and placed into four separately labeled Ziplock bags. Samples were analyzed for algal biomass and total biofilm mass, measured by Chl *a* and AFDM, respectively. NDS samples were stored on ice packs in a cooler during the field day, and transferred to a -20°C freezer for further sample processing and analysis.

Water temperature was also monitored continuously every 30 minutes with Onset® HOBO® data loggers, which were deployed with rock-basket artificial substrates at each site and retrieved after the one-month sampling period (Figure 4.1). Water temperature was measured to identify any thermal microhabitats any of the rock-baskets may be situated in which could influence periphyton growth. Measurements from the temperature data loggers were downloaded upon returning to the laboratory and re-deployed during the subsequent monthly sampling period. See Appendix B for a detailed description of how NDS treatment vials and trays were prepared in the laboratory prior to deployment.

Figure 4.1. A retrieved nutrient diffusing substrata (NDS) fastened to a rock-basket artificial substrate containing 50 ceramic BBQ briquettes and a HOBO® data logger measuring water temperature (°C) every 30 minutes. Greatest periphyton growth was observed on the NDS treatment vial containing the nitrogen and phosphorus (N + P) agar mixture after the one-month deployment period in the example above.

4.2.2 Sample Processing

4.2.2.1 Periphyton Rock Scrapings and BBQ Briquettes

Periphyton rock scrapings and BBQ briquette samples were prepared for Chl *a* and AFDM analysis utilizing the following laboratory protocols performed at NLET (Saskatoon, SK):

GF/C filters were combusted at 500°C for four hours in a muffle furnace prior to sample processing. Periphyton rock scrapings and BBQ briquette samples were removed from the -20°C freezer and brought to room temperature in a dark drawer. Each sample was removed and processed individually, to remain frozen and in the dark to prevent degradation (Hauer and Lamberti 2011). The entire sample was poured into a 100 mL graduated cylinder, with the addition of 90% EtOH to obtain a total volume of 100 mL. The sample was then poured into a blender, and the 100 mL graduated cylinder was rinsed out with EtOH until all visible remnants of the sample were removed. The amount of sample and EtOH that was poured into the blender was recorded. The sample was blended for approximately ten seconds. 10 mL of sample was pipetted into a centrifuge tube for fluorometric analysis of Chl *a* following the methods below. A known portion of the remaining sample was then filtered through the combusted GF/C filter for AFDM with the amount of sample filtered recorded. BBQ briquette periphyton samples did not contain sufficient sample material for AFDM analysis; therefore, only the filtered periphyton rock scraping sample was analyzed following the AFDM methods below.

1) **Chl *a* (g/m^2) -** After completing the sample preparation methods (above), centrifuge tubes containing the samples were placed into a 80°C water bath for seven minutes. Samples were removed from the water bath and placed in a dark drawer for 30 minutes. Samples were then centrifuged to compress all of the material in the bottom of the tube, and the extract volume of the sample was measured with a volumetric centrifuge tube. A portion of the sample was then poured into a culture tube and wiped with a kimwipe to remove external impurities. The culture tube was placed into the fluorometer, the lid was closed and fluorescence was recorded, following the methods of Thompson et al. (1999) without correction for phaeophytin content. If the pigment content of the sample was too high, the sample was diluted by a factor of 10. Dilutions were performed utilizing 1 mL of the extracted sample, which was pipetted into a clean volumetric centrifuge tube and 9 mL of 90% EtOH was added. The centrifuge tube was covered with parafilm, inverted to mix, and placed in the fluorometer, with the dilution factor noted.

2) **AFDM (g/m^2) -** After completing the sample preparation methods (above), the filtered samples were placed in pre-labeled aluminum weighing dishes. The aluminum dishes were placed in a drying oven at 105°C for 24 hours. After 24 hours, filtered samples were cooled and weighed. Each filtered sample was put back into the appropriate aluminum dish and placed in the muffle furnace to combust at 550°C for one hour. Aluminum dishes were removed from the muffle furnace and allowed to cool. Filters were re-wetted with a small volume of DI

water and placed in dishes in the drying oven at 105°C for 24 hours. All filtered samples were re-weighed and recorded.

4.2.2.2 Nutrient Diffusing Substrata

NDS samples were prepared for Chl *a* and AFDM analysis utilizing the following laboratory protocols developed at the University of Calgary (Calgary, AB) by Corbett (2013):

NDS sample vials in Ziplock bags were removed from the -20°C freezer individually for sample processing, to remain frozen and in the dark to prevent degradation (Hauer and Lamberti 2011). Frozen treatment sample vials were placed on laboratory bench and cracked open with a hammer to allow the silica disc top to be removed. Once removed, the silica disc was divided into two equal halves using a hammer and chisel. One half was placed into a centrifuge tube with 5 mL of 100% methanol (MeOH), and sealed with labeled parafilm for spectrofluorometric analysis of Chl *a*. Chl *a* samples were vortexed for a few seconds and centrifuge tubes were placed in dark racks for 8-12 hours in refrigerator. The other silica disc half was placed into a pre-labeled aluminum weighing dish for AFDM analysis and positioned in a 105°C drying oven for 24 hours (see below).

1) Chl *a* (μg/cm^2) - After 8-12 hours of being in the refrigerator (above), eight centrifuge tubes were removed at a time and placed in the centrifuge for seven minutes. Once the first samples were spun down, they were removed and eight more samples were added. Spun down samples were transferred to a fume hood, and 300 μL of extract was pipetted from the centrifuge tube into a cell on a clean 96-well black plate, taking note of what cell contained which sample. The first cells 1-5 in Row A were blanks and filled only with 100% MeOH. Pipetting samples into a 96-well plate was completed when all of the samples were finished, or when the plate was full. Factor 10 dilutions were performed on samples with high pigment concentration. Chl *a* was then analyzed via spectrofluorometry using a SPECTRAmax® GEMINI-XS, following the methods of Thompson et al. (1999) without correction for phaeophytin content.

2) AFDM (mg/cm^2) - After 24 hours of being in the 105°C drying oven (above), samples were removed and allowed to cool. Oven-dried silica discs were then weighed and recorded. Each disc was placed back into the appropriate aluminum dish and positioned in a muffle furnace to combust at 550°C for 3.5 hours. Aluminum dishes were removed from the muffle furnace and allowed to cool. After-combustion, silica discs were weighed again and recorded. The difference between the first and second weight was the AFDM, which was measured as loss on ignition. Samples were wrapped in aluminum foil and labelled for archiving.

4.2.3 Statistical Analyses

General linear models (analysis of variance; ANOVA) were used to analyze among-site and monthly differences in periphyton abundance, as well as treatment differences for nutrient limitation related to the disturbance gradient hypothesis for the Steepbank and Ells Rivers. ANOVAs tested the following null hypotheses:

- HO1: No change among-sites and months in average periphyton abundance along the environmental disturbance gradient.
- HO2: No change among-sites, months and treatments in average nutrient limitation along the environmental disturbance gradient.

Two-way ANOVAs using mixed-effects models (described in section 2.4), with site as the fixed variable and month (repeated testing over time) as the random, or blocking variable were used to determine site and monthly differences for both periphyton rock scraping and BBQ briquette periphyton algal biomass (Chl *a*) and total biofilm mass (AFDM) within the Steepbank and Ells Rivers. Data were first log transformed to fit the assumption of normality for the ANOVA (Berry 1987). Before running the model, a restricted maximum likelihood (REML) estimation was applied to datasets with smaller sample sizes ($n < 20$) and a maximum likelihood (ML) estimation was applied to datasets with larger sample sizes ($n > 20$), which is a method used for fitting linear mixed models (Kenward and Roger 1997). Significant interactions between site and month were also examined prior to running the model using a two-factor ANOVA; however, interaction terms were not included in the model to maintain model consistency for all analyses.

A three-way ANOVA using a mixed-effects model, with site and treatment as the fixed variables, and month as the random variable was used to determine site, month and treatment differences in NDS Chl *a* and AFDM for both rivers. Data were first log transformed to fit the assumption of normality for the ANOVA (Berry 1987). Significant interactions among site, treatment, and month were examined prior to running the model using a three-factor ANOVA; however, interaction terms were not included in the model to maintain model consistency for all analyses of nutrient limitation. REML and ML estimations were applied to datasets before running the model (Kenward and Roger 1997). After running all of the models, if a significant difference among-sites, months, or treatments was found ($p < 0.05$), an *a posteriori* test, Tukey HSD, was performed to determine which sites, months and treatments were significantly different. All statistical analyses were conducted using R version 2.15.2, utilizing the “nlme” and “multcomp” packages (R Development Core Team 2012).

4.3 Results

4.3.1 Rock-Basket Water Temperature

Steepbank River

HOBO® data loggers were successfully retrieved from each of the three rock-baskets at ST4 in August, September and October and at ST3 and ST1 in October. There were no successful retrievals at ST2 throughout the duration of the sampling period due to site inaccessibility from flooding as well as damaged and lost equipment. Water temperature was very consistent within- and among-sites, and decreased from the open-water period in July to freeze-up in October (Figure 4.2).

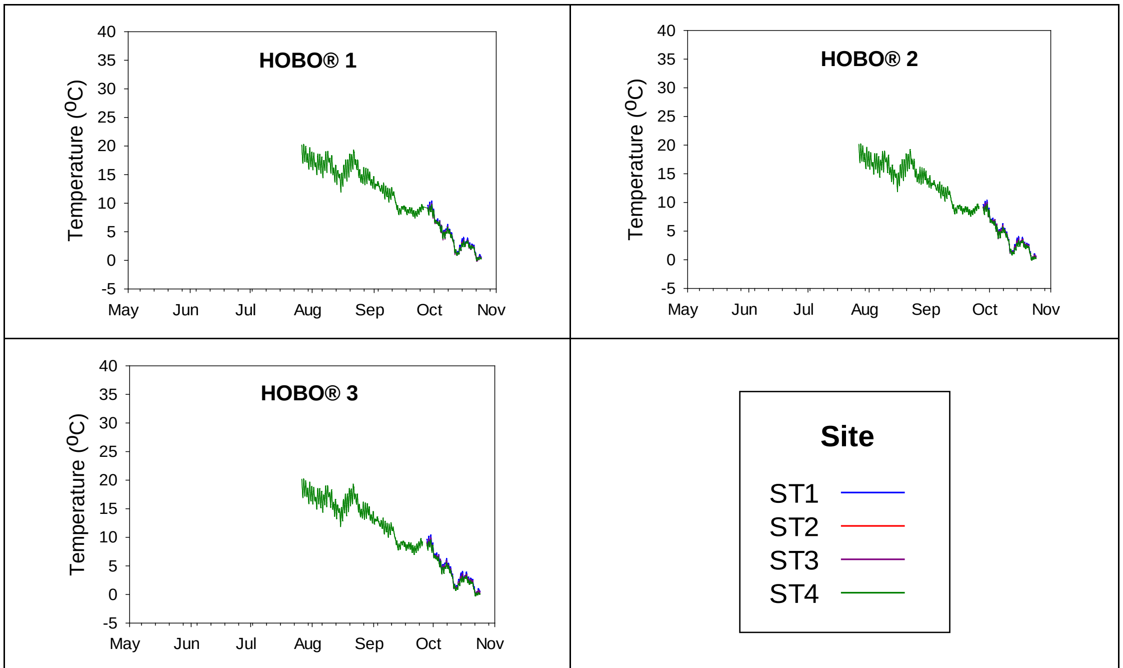


Figure 4.2. Water temperature (°C) recorded every 30 minutes at four sites on the Steepbank River. Data was obtained from three HOBO® data loggers attached to each of the three rock-basket artificial substrates. HOBO® data loggers were deployed and retrieved with each of the rock-baskets over the 2012 sampling period (May-October).

Ells River

HOBO® data loggers were successfully retrieved from each of the three rock-baskets at EL1 each month from June-October. Successful retrievals also occurred at EL2 in June, August, September and October, and at EL3 each month from July-October. Water temperature was very consistent within- and among-sites, and began to increase in May, coinciding with river ice break-up, and decreased in October with freeze-up (Figure 4.3).

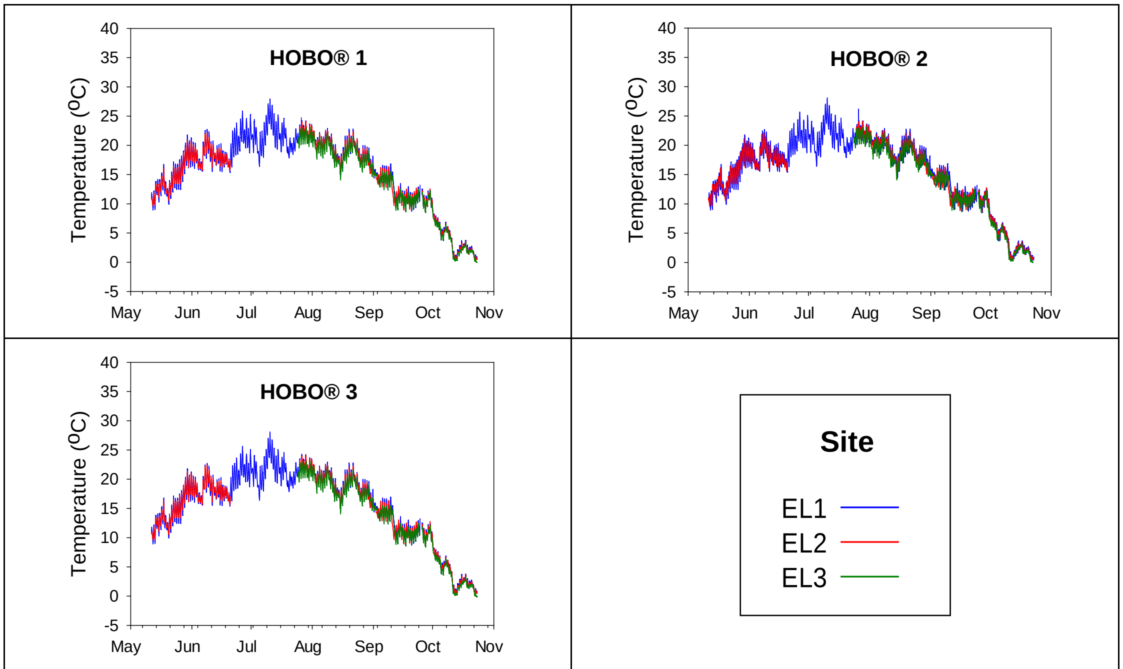


Figure 4.3. Water temperature (°C) recorded every 30 minutes at three sites on the Ells River. Data was obtained from three HOBO® data loggers attached to each of the three rock-basket artificial substrates. HOBO® data loggers were deployed and retrieved with each of the rock-baskets over the 2012 sampling period (May-October).

4.3.2 Algal and Biofilm Biomass

4.3.2.1 Periphyton Rock Scrapings

Steepbank River

Chlorophyll *a*

Log Chl *a* concentrations were significantly different among-sites, with the most upstream site (ST4) having the significantly greatest concentrations, and the most downstream site (ST1) containing the significantly lowest concentrations. Log Chl *a* concentrations were significantly lower in May compared to July, August and October. There was insufficient sample replication for a site x month interaction effect for periphyton rock scraping Chl *a*.

Ash-Free Dry Mass

Log AFDM was significantly different among-sites, with ST1 containing the significantly lowest concentrations. Log AFDM was significantly lower in September than October. There was insufficient sample replication for a site x month interaction effect for periphyton rock scraping AFDM.

Ells River

Chlorophyll *a*

Log Chl *a* concentrations were not significantly different among-sites. Log Chl *a* concentrations were significantly lower in May compared to June and July. There was insufficient sample replication for a site x month interaction effect for periphyton rock scraping Chl *a*.

Ash-Free Dry Mass

Log AFDM was significantly different among-sites, with the most upstream site (EL3) containing significantly lower concentrations than downstream sites (EL2 and EL1). Log AFDM was significantly higher in September compared to May and June. There was insufficient sample replication for a site x month interaction effect for periphyton rock scraping AFDM.

Table 4.1. Summary table of Steepbank and Ells River periphyton rock scraping basal production variables analyzed with a two-way mixed-effects ANOVA, which included a) site and, b) month. Significant *p*-values ($p < 0.05$) for site differences are shaded in grey. Upstream (U/S) to downstream (D/S) changes in variable concentrations are indicated with "+" for increasing and "-" for decreasing, and an interaction column indicates any significant site x month interaction effects. The following abbreviations are used: chlorophyll *a* (Chl *a*), and ash-free dry mass (AFDM).

Para-meter	Fig #	Site Differences	*p*-value	U/S-D/S Changes	Monthly Differences	*p*-value	Interaction Effect
Steepbank River							
Log Chl *a*	4.4	ST4 > ST1	0.013	-	May < Jul, Aug, Oct	< 0.05	No rep.
Log AFDM	4.5	ST4, ST3, ST2 > ST1; ST4 < ST3	< 0.05 < 0.001	-	Sep < Oct	0.024	No rep.
Ells River							
Log Chl *a*	4.6	No	> 0.05	No	May < Jun, Jul	< 0.05	No rep.
Log AFDM	4.7	EL3 < EL2 = EL1	< 0.05	+	Sep > May, Jun	< 0.05	No rep.

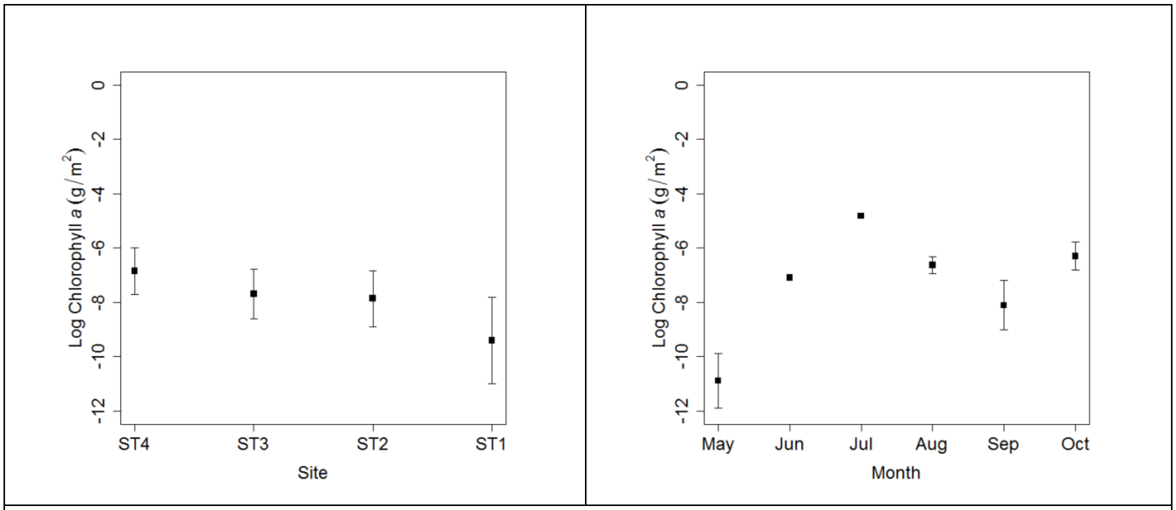


Figure 4.4. Mean concentration of Steepbank River periphyton rock scraping log transformed chlorophyll *a* (log Chl *a*; g/m^2) by site (left) and over months (right). ST4 was significantly greater than ST1 ($p = 0.013$). Log Chl *a* concentration was significantly lower in May than July, August, and October ($p < 0.05$). There was insufficient sample replication for a site x month interaction effect.
*Error bars represent the standard error of the mean.

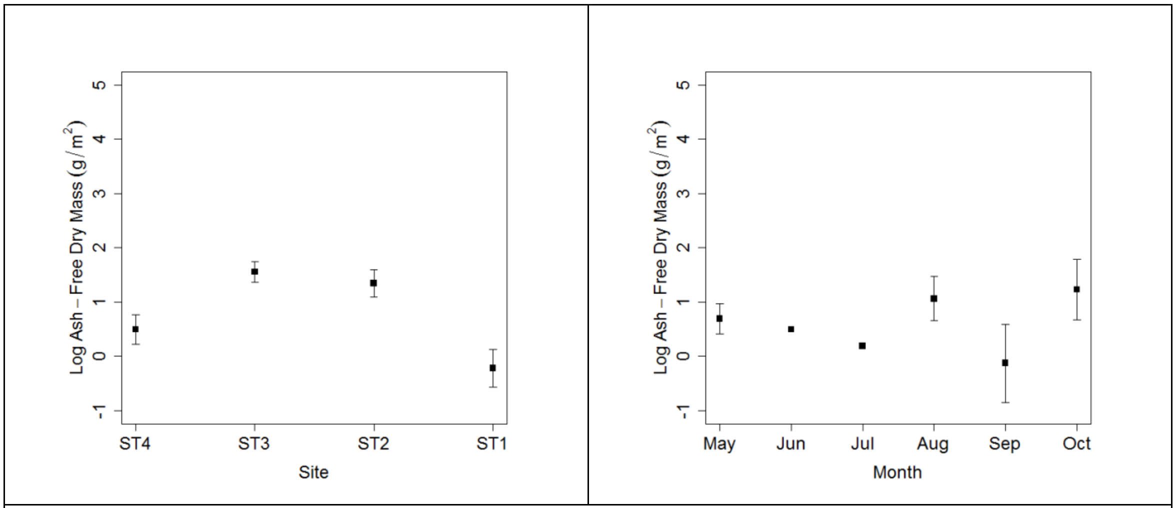


Figure 4.5. Mean concentration of Steepbank River periphyton rock scraping log transformed ash-free dry mass (log AFDM; g/m^2) by site (left) and over months (right). ST4, ST3, and ST2 were all significantly greater than ST1 ($p < 0.05$); ST4 was significantly lower than ST3 ($p < 0.001$). Log AFDM concentration was significantly lower in September than October ($p = 0.024$). There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.

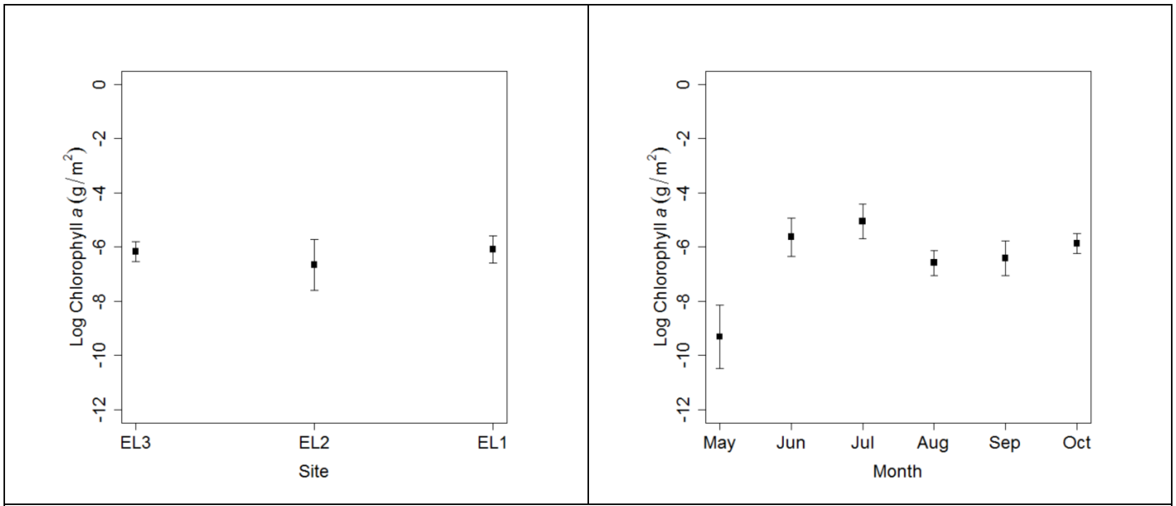


Figure 4.6. Mean concentration of Ells River periphyton rock scraping log transformed chlorophyll *a* (log Chl *a*; g/m^2) by site (left) and over months (right). There were no significant differences among-sites. Log Chl *a* concentration was significantly lower in May than June ($p = 0.050$), and July ($p = 0.023$). There was insufficient sample replication for a site x month interaction effect.
*Error bars represent the standard error of the mean.

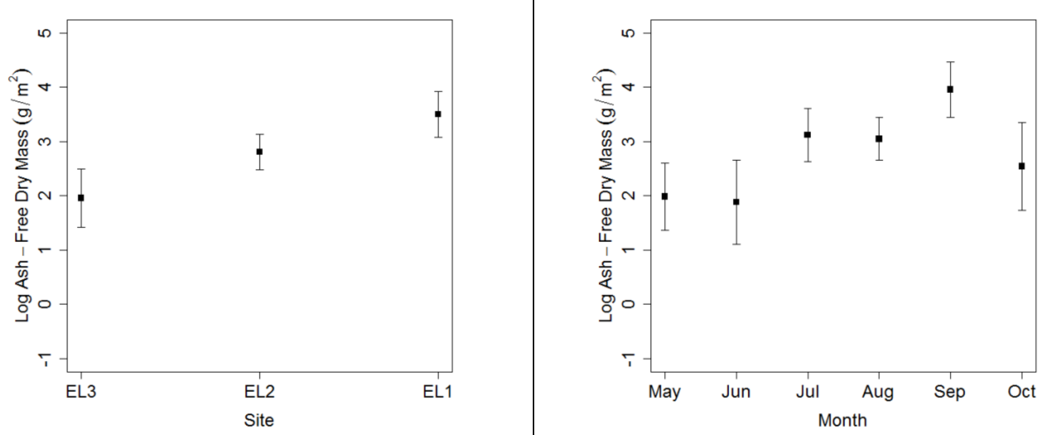


Figure 4.7. Mean concentration of Ells River periphyton rock scraping log transformed ash-free dry mass (log AFDM; g/m^2) by site (left) and over months (right). EL3 was significantly lower than EL2 (p = 0.035), and EL1 (p = 0.000). Log AFDM concentration was significantly greater in September than May (p = 0.031), and June (p = 0.035). There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.

4.3.2.2 BBQ Briquette Periphyton

Steepbank River

Chlorophyll *a*

Log Chl *a* concentrations were not significantly different among-sites. Log Chl *a* concentrations were significantly greatest in August, and significantly lowest in September. There were no successful rock-basket retrievals from the Steepbank River at ST2 during any sampling month, and there were no successful retrievals in June and July at any sites due to site inaccessibility and flooding. There was insufficient sample replication for a site x month interaction effect for BBQ briquette periphyton Chl *a*.

Ells River

Chlorophyll *a*

Log Chl *a* concentrations were significantly different among-sites, with EL3 containing greater concentrations than EL2 and EL1. Log Chl *a* concentrations were significantly greatest in August, and significantly lowest in October. There was no significant site x month interaction for BBQ briquette periphyton Chl *a*.

Table 4.2. Summary table of Steepbank and Ells River BBQ briquette periphyton basal production variable analyzed with a two-way mixed-effects ANOVA, which included a) site and, b) month. Significant *p*-values ($p < 0.05$) for site differences are shaded in grey. Upstream (U/S) to downstream (D/S) changes in variable concentrations are indicated with "+" for increasing and "-" for decreasing, and an interaction column indicates any significant site x month interaction effects. The following abbreviation is used: chlorophyll *a* (Chl *a*).

Para-meter	**Fig #**	**Site Differences**	***p*-value**	**U/S-D/S Changes**	**Monthly Differences**	***p*-value**	**Interaction Effect**
Steepbank River							
Log Chl *a*	4.8	No	> 0.05	No	Aug > Sep, Oct; Sep < Oct	< 0.001 0.044	No rep.
Ells River							
Log Chl *a*	4.9	EL3 > EL2 = EL1	< 0.05	-	Aug > Jun, Sep, Oct; < in Oct	< 0.05 < 0.05	> 0.05

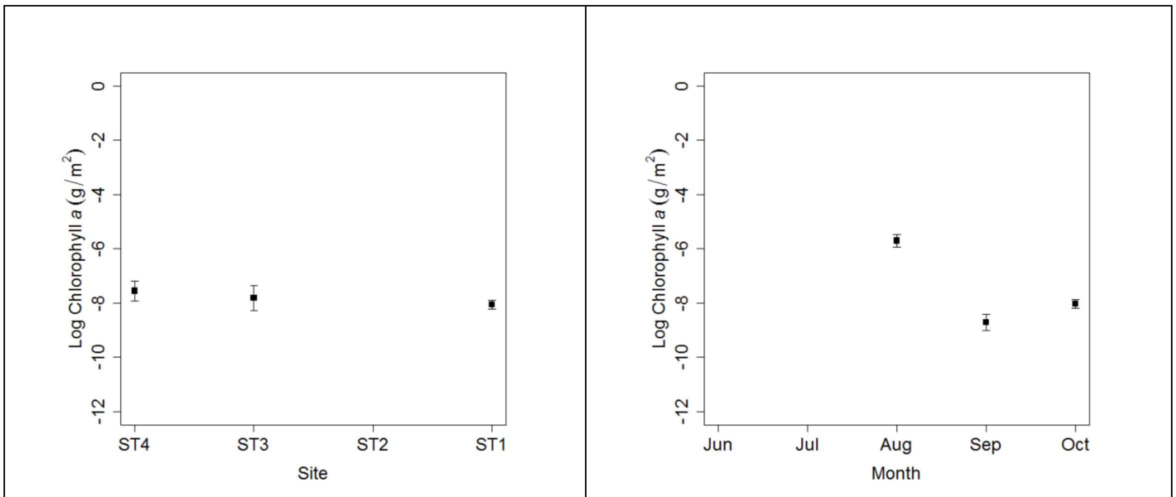


Figure 4.8. Mean concentration of Steepbank River BBQ briquette periphyton log transformed chlorophyll *a* (log Chl *a*; g/m^2) by site (left) and over months (right). There were no significant differences among-sites. Log Chl *a* concentration was significantly greater in August than September and October ($p < 0.001$); September was significantly lower than October ($p = 0.044$). There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.

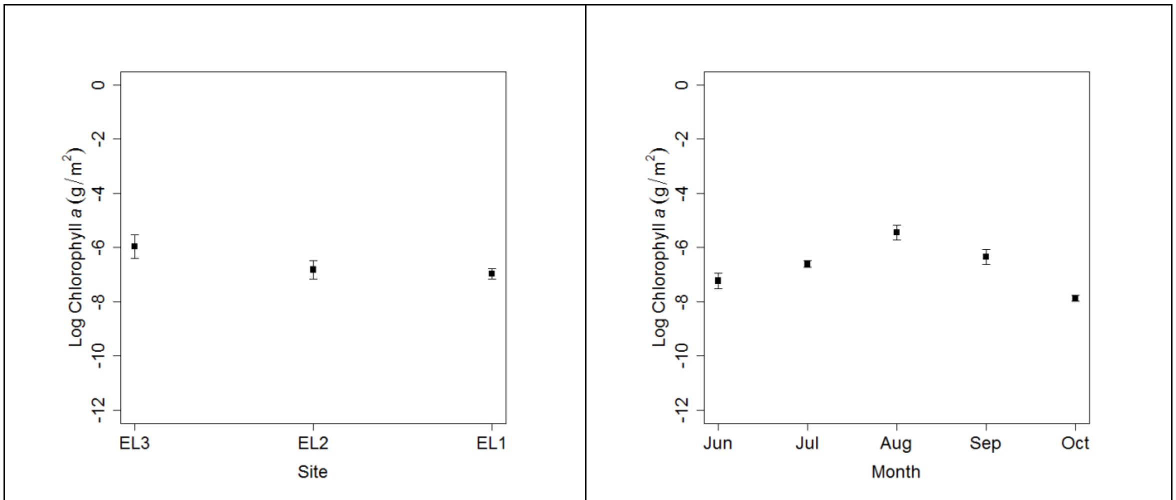


Figure 4.9. Mean concentration of Ells River BBQ briquette periphyton log transformed chlorophyll *a* (log Chl *a*; g/m^2) by site (left) and over months (right). EL3 was significantly greater than EL2 (p = 0.050), and EL1 (p = 0.011). Log Chl *a* concentration was significantly greater in August than June, September and October ($p < 0.05$); October was significantly lower than other months ($p < 0.05$). There was no significant site x month interaction. *Error bars represent the standard error of the mean.

4.3.3 Nutrient Diffusing Substrata

Steepbank River

Chlorophyll *a*

Log Chl *a* concentrations were significantly different among-sites, with ST4 containing significantly greater concentrations than ST1. Log Chl *a* concentrations were significantly greatest in August. There were no NDS treatments significantly different from Control in log Chl *a* concentration. There was a significant site x month interaction for NDS log Chl *a*.

Ash-Free Dry Mass

Log AFDM was not significantly different among-sites. There were no significant differences among-months, and no NDS treatments were significantly different from Control in log AFDM. There were no significant interactions for NDS log AFDM.

Ells River

Chlorophyll *a*

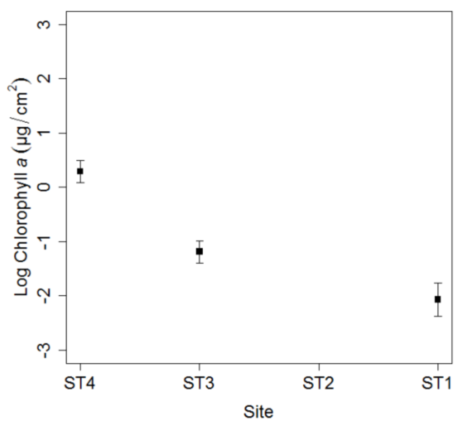
Log Chl *a* concentrations were significantly different among-sites, with EL3 containing significantly greater concentrations than EL1. Log Chl *a* concentrations were significantly lowest in October. N and N + P were significantly greater than Control in log Chl *a* concentration. There was a significant site x month x treatment interaction for NDS log Chl *a*.

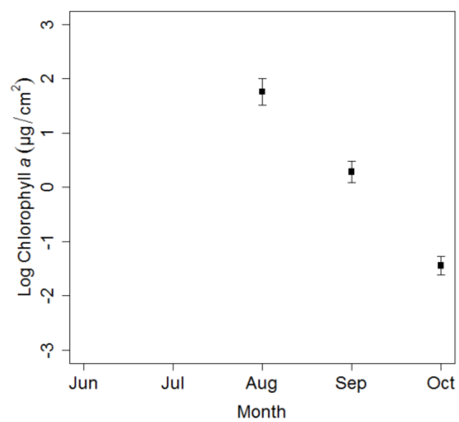
Ash-Free Dry Mass

Log AFDM was not significantly different among-sites. There were no significant differences among-months, and no NDS treatments were significantly different from Control in log AFDM concentration. There were no significant interactions for NDS log AFDM.

Table 4.3. Summary table of Steepbank and Ells River nutrient diffusing substrate (NDS) basal production variables analyzed with three-way mixed-effects ANOVAs, which included a) site, b) month, and c) treatment. Significant *p*-values ($p < 0.05$) for site and treatment differences are shaded in grey. Upstream (U/S) to downstream (D/S) changes in variable concentrations are indicated with “+” for increasing and “-” for decreasing, and an interaction column indicates any significant site x month, site x treatment, month x treatment, or site x month x treatment interaction effects. The following abbreviations are used: chlorophyll *a* (Chl *a*), and ash-free dry mass (AFDM).

Para-meter	Fig #	Site Effect	*p*-value	U/S-D/S Changes	Month Effect	*p*-value	Treatment Effect	*p*-value	Interaction Effect
Steepbank River									
Log Chl *a*	4.10	ST4 > ST1	0.002	-	> in Aug	< 0.001	No	> 0.05	0.000
Log AFDM	4.11	No	> 0.05	No	No	> 0.05	No	> 0.05	> 0.05
Ells River									
Log Chl *a*	4.12	EL3 > EL1	0.040	-	< in Oct	< 0.001	N, N + P > Control	< 0.01	0.007
Log AFDM	4.13	No	> 0.05	No	No	> 0.05	No	> 0.05	> 0.05





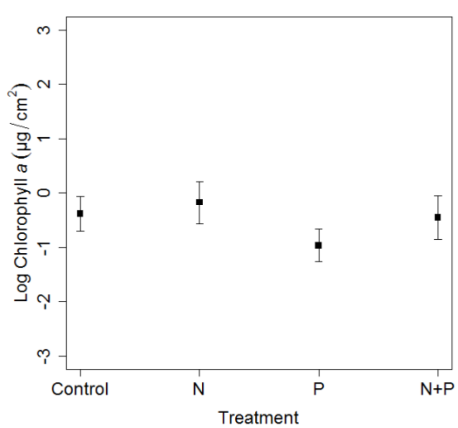
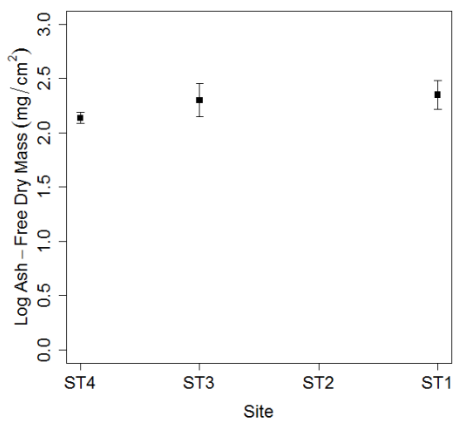
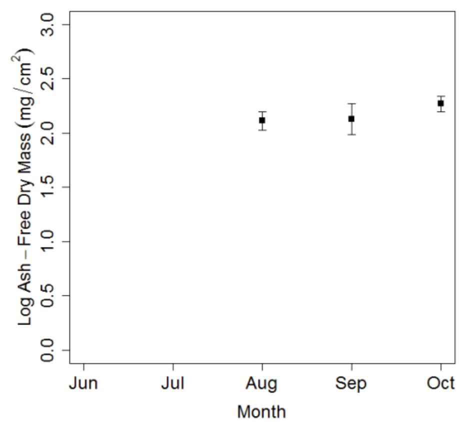


Figure 4.10. Mean concentration of Steepbank River nutrient diffusing substrate (NDS) log transformed chlorophyll *a* (log Chl *a*; µg/cm^2) by site (top left), over months (top right), and by treatment (bottom left). ST4 was significantly greater than ST1 (p = 0.002). Log Chl *a* concentration was significantly greatest in August (p < 0.001). There were no significant differences among-treatments.
There was a significant site x month interaction (p = 0.000).
*Error bars represent the standard error of the mean.





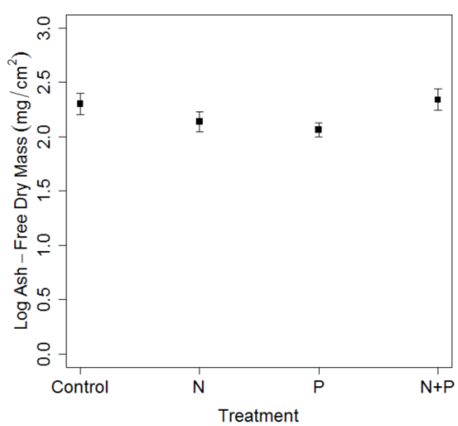
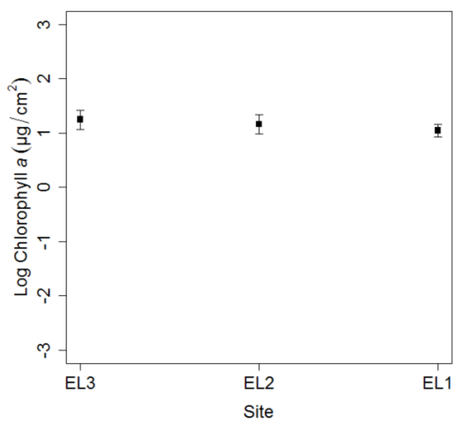
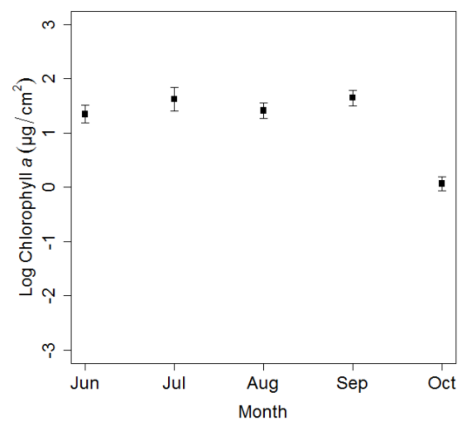


Figure 4.11. Mean concentration of Steepbank River nutrient diffusing substrate (NDS) log transformed ash-free dry mass (log AFDM; mg/cm^2) by site (top left), over months (top right), and by treatment (bottom left).
There were no significant differences among-sites. Log AFDM concentration was not significantly different among-months.
There were no significant differences among-treatments.
There were no significant interactions.
*Error bars represent the standard error of the mean.





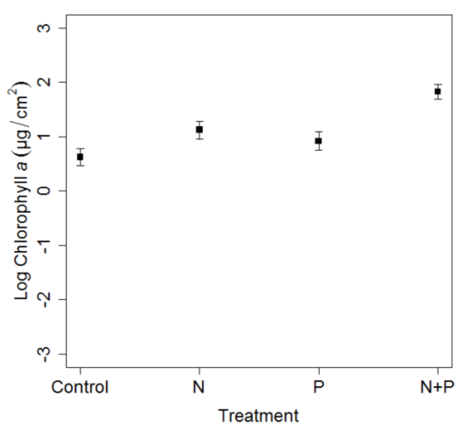
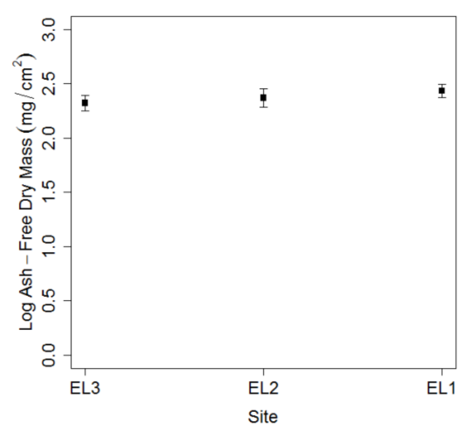
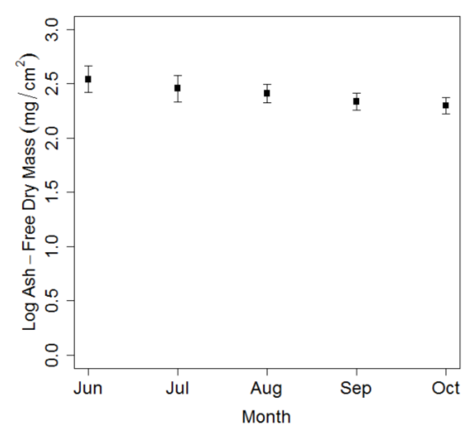


Figure 4.12. Mean concentration of Ells River nutrient diffusing substrate (NDS) log transformed chlorophyll *a* (log Chl *a*; µg/cm^2) by site (top left), over months (top right), and by treatment (bottom left).
EL3 was significantly greater than EL1 (p = 0.040). Log Chl *a* concentration was significantly lower in October than other months ($p < 0.001$). N and N + P treatments were significantly greater than Control ($p < 0.01$). There was a significant site x month x treatment interaction ($p = 0.007$).
*Error bars represent the standard error of the mean.





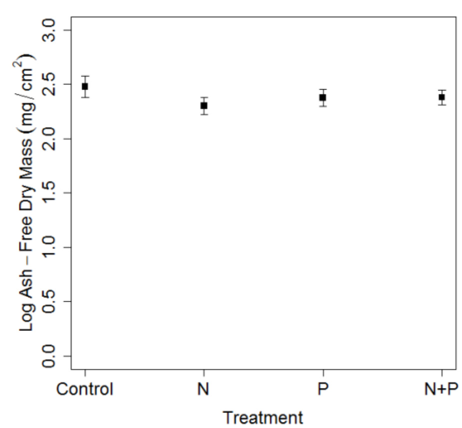


Figure 4.13. Mean concentration of Ells River nutrient diffusing substrate (NDS) log transformed ash-free dry mass (log AFDM; mg/cm^2) by site (top left), over months (top right), and by treatment (bottom left).
There were no significant differences among-sites.
Log AFDM concentration was not significantly different among-months.
There were no significant differences among-treatments. There were no significant interactions.
*Error bars represent the standard error of the mean.

4.4 Discussion

4.4.1 Algal and Biofilm Biomass

Periphyton Rock Scrapings

Among-site differences in algal biomass (Chl *a*), or standing crop, were only observed within the Steepbank River, with a significant decrease from upstream to downstream. Possible mechanisms for downstream decreases in algal standing crop included sedimentation from upstream land use activities which increases algal scouring and abrasion (Hancock 2002), impairs substrate suitability for periphyton growth, and decreases primary production overall (Henley et al. 2000). Accelerated transport of fine particulate organic matter (FPOM; allochthonous inputs) from the terrestrial landscape has also been reported in studies investigating catchment-scale disturbances on river ecosystems, altering autochthonous production in the downstream environment (Webster et al. 1992). Impervious landscapes respond to seasonal storms more drastically, with greater consequences from runoff into the lotic ecosystem compared to natural (usually forested) landscapes. Therefore, catchment runoff on a disturbed basin may have more efficiently transported sediments, contaminants, and nutrients into the river, further degrading in-stream habitat.

A downstream decline in Chl *a* within the Steepbank River was also possibly attributed to natural OS bitumen deposits from the McMF in the lower reaches. Studies on the effects of crude oil on primary production in freshwater ecosystems have shown growth inhibition to occur, with deteriorated algae development and reduced photosynthesis (Bott et al. 1978). Mechanisms for inhibition of algal primary production from natural OS deposits included acute toxicological responses from aromatic compounds (Soto et al. 1975; Miller et al. 1978; Federle et al. 1979; Barsdate et al. 1980), as well as secondary non-toxic responses such as enhancement of metabolism from petroleum hydrocarbons which represent a significant source of organic matter leading to a stimulation of bacterial productivity (Peterson et al. 1996). Furthermore, Lock et al. (1981a) observed bacterial activity to increase over time after being exposed to crude oil substrates in tributaries of the AOSR.

Seasonal effects on Steepbank River Chl *a* concentrations demonstrated a depression in early spring, followed by an increase in summer, and subsequent decrease into fall. The spring algal biomass decline was possibly attributed to scouring during freshet, as described by Stevenson (1990). High turbidity due to large amounts of fine material carried in suspension (Johnston 1922), also could have influenced photosynthetically active radiation (PAR) in the ecosystem, reducing algal primary production in the spring (Wetzel 1983). Moreover, increased water temperature and longer daylight hours during the open-water period in summer likely enabled algal biomass to increase, as explained by DeNicola et al. (1992) and Eulin and Le Cohu (1998). The decrease in fall was attributed to several large pluvial events in September which caused a drastic increase in discharge, possibly resulting in algal scouring. Cooler temperatures and reduced light availability also likely contributed to the suppression in algal growth in the fall.

Seasonal effects on Ells River Chl *a* demonstrated a spring depression coinciding with freshet, with Chl *a* concentrations being consistent during other months. This relative consistency among-seasons was potentially attributed to buffering effects from upper basin Namur-Gardiner lakes influencing downstream physico-chemical and biological variables, as well as moderating runoff from the catchment (Headley et al. 2005). Seasonal storms also have diminished runoff effects on a less disturbed landscape (Golladay et al. 1989), such as the Ells River basin.

Both rivers had significant among-site differences in total biofilm mass (AFDM), with an increase from upstream to downstream. Particulate litter input from the terrestrial landscape controls the allochthonous supply of organic carbon available to freshwater food webs. Thus, downstream sites could be receiving allochthonous inputs of FPOM from upstream loadings and lateral transport from the surrounding landscape, resulting in increased microbial activity within epilithon (Vannote et al. 1980; Conners and Naiman 1984). Algal communities could have also shifted to a heterotrophic state within the lower reaches of both rivers from OS deposit effects (Bott et al. 1978), resulting in increased bacterial activity and biofilm production (Lock et al. 1981a). Moreover, suppression at the mouth of the Steepbank River in both Chl *a* and AFDM was most likely attributed to the large slump event which occurred early within the 2012 sampling season, as well as scouring from the several major discharge events.

Seasonal fluctuations of AFDM in the Steepbank River were minor over spring and summer, but a significant decrease occurred in fall. This depression in AFDM was attributed to an extreme rainfall event in September, resulting in a drastic increase in river discharge and potential substrate abrasion. In the Ells River, AFDM increased from spring to summer and declined in late fall, with a spring depression in AFDM possibly attributed to scouring during freshet. Metabolic rates are also typically greater in summer when warmer temperatures increase biofilm production which are followed by declines in the fall with lower water temperatures (Bott et al. 1985; Boulêtreau et al. 2006). In general, the Ells River had greater levels of Chl *a* and AFDM than the Steepbank River across sites and months. This was likely attributed to the slump event which decreased overall periphyton biomass within the Steepbank River.

Moreover, caution is needed because AFDM does not distinguish algal biomass from other organic material (e.g., fungi, bacteria) in the sample, nor does it account for the physiological state of the organic material (i.e., being senescent). Drying by heat may also volatilize certain organic compounds and carbonates, resulting in an underestimation of AFDM. Thus, AFDM methods may be unsatisfactory for estimating total biofilm mass, especially if there is a large amount of non-algal organic content in the sample (Hauer and Lamberti 2011).

BBQ Briquette Periphyton

Among-site differences in algal biomass (Chl *a*), or standing crop, were only observed within the Ells River, with a significant decrease from upstream to downstream. Possible mechanisms for downstream decreases in algal standing crop size could possibly be attributed to allochthonous inputs of FPOM from the watershed creating a dominantly heterotrophic environment (Vannote et al. 1980; Conners and Naiman 1984), as well as effects from natural OS bitumen deposits from the McMF in the lower reaches (Soto et al. 1975; Bott et al. 1978; Miller et al. 1978; Federle et al. 1979; Barsdate et al. 1980; Lock et al. 1981a). In this study, HOBO® data loggers attached to rock-baskets recorded consistent water temperatures within and among-sites on both the Steepbank and Ells Rivers, indicating temperature differences were not a factor associated with longitudinal variations in periphyton growth.

The Steepbank River Chl *a* concentrations were greatest in summer and lowest in fall. Primary productivity is classically greatest in the summer open-water period with elevated water temperature, and longer daylight hours for photosynthesis. A decline in periphyton abundance in the fall was attributed to the spike in discharge in September, as well as colder temperatures and shorter days. The Ells River had the same seasonal shift from summer to fall, including an increase from spring to summer likely attributed to elevated water temperature and light availability. BBQ briquette periphyton Chl *a* concentration was comparable between river basins, across sites and months. The predictable seasonal pattern illustrated by Ells River BBQ periphyton Chl *a* concentrations illustrated the effectiveness of utilizing an artificial substrate for assessing monthly accumulation rates of periphyton abundance in a lotic ecosystem.

4.4.2 Periphyton Variability on Natural and Artificial Substrata

Epilithon growth variability on natural and artificial substrata among-sites and months was observed in this study. Downstream changes were associated with responses of algal biomass on natural substrates within the Steepbank River, and not the Ells River. In contrast, a downstream change was associated with algal biomass on artificial substrates within the Ells River, and not the Steepbank River. Algal and biofilm biomass on natural substrates for both basins was depressed in spring, whereas artificial substrates had greatest Chl *a* concentrations in summer and lowest in fall.

Deviations between natural and artificial substrata Chl *a* concentrations along the environmental disturbance gradient were possibly attributed to several factors such as differences in retrieval success at sites, slumping events temporarily influencing surrounding natural substrates, large discharge events causing algal scouring, as well as limited within-site variance acquired from periphyton rock scrapings. Furthermore, a broad range of algal responses to natural bitumen deposits were potentially observed, such as stimulation through enhanced bacterial activity as well as inhibition from immediate oil-toxicity. Failure to assess BBQ briquette periphyton would have overlooked the alternative downstream response of algal biomass observed in this study.

Studies by Lock et al. (1981a, b) observed established algal species in tributaries of the AOSR developed a tolerance to the effects of crude oil over time, whereas newly colonized algae on clean substratum were less tolerant to toxic oil compounds. Findings from the Ells River in this study on algal biomass for natural and artificial substrates produced similar results to past studies. Moreover, algal toxicity tests and taxonomic identification would need to be addressed further to better determine mechanistic pathways. Thus, the decline in periphyton rock scraping Chl *a* at the mouth of the Steepbank River was likely attributed to the large slump event altering the downstream environment, whereas the downstream decline in Ells River BBQ briquette Chl *a* was possibly attributed to OS deposit effects on primary production. Alternative mechanisms include allochthonous inputs of particulate organic matter from the terrestrial landscape, as well as potential for shading, light inhibition and increased turbidity from high TSS loadings, which would need further investigations to attribute cause.

Advantages to utilizing artificial substrates to measure periphyton in this study included the increased capability in comparing algal biomass among-sites and seasons through the standardization in substrate shape and size from the ceramic BBQ briquettes which produced more uniform current patterns contributing to greater success of reproducibility (Tuchman and Stevenson 1980). Confounding effects of habitat differences were minimized by providing a standardized microhabitat (Cairns 1982), allowing the potential OS deposit effects to be observed. Time was also saved during field sampling, with the task of scraping off the periphyton and associated potential error being avoided (Meier et al. 1983). Standardized sampling was also practised by eliminating subjectivity in sample collection technique (Cairns 1982). This study also highlighted the advantage of the standardized BBQ briquettes to estimate monthly periphyton growth rates, which would be difficult when sampling natural substrata.

Disadvantages to utilizing artificial substrates included the inability to gather sufficient material to measure AFDM. The only values came from natural substrata. Artificial substrates also required a return trip, and were prone to loss or damage. Furthermore, caution was required when determining the length of deployment time for the artificial substrates, because colonization of a substrate varies greatly with season. For example, periphyton accumulation rates would be greater in summer than in fall; therefore, determining an appropriate incubation time for the entire sampling period was necessary, as indicated by Lowe and Gale (1980).

Overall, the possible responses of epilithon to the natural OS deposits within the lower reaches suggests traditional sampling of periphyton for conducting river bioassessments needs to be re-assessed for producing a comprehensive representation of the responses of periphyton to natural and anthropogenic non-point disturbances within tributaries of the AOSR. Rapid bioassessment approaches, such as artificial substrata, are recommended as a supplementary method to periphyton sampling in these lotic ecosystems; however, the analysis of AFDM should be a requirement of the passive sampler. Moreover, the rock-basket artificial substrate utilized in this study allowed simultaneous sampling of nutrient limitation which was also assessed as a potential mechanism for longitudinal and seasonal alterations in basal production.

4.4.3 Nutrient Limitation

Both the Steepbank and Ells Rivers had significant among-site differences in NDS algal biomass (Chl *a*). The most upstream sites on both rivers contained greater Chl *a* concentrations on all NDS treatments than the most downstream sites. Primary productivity in the lower reaches of the river could have possibly been hindered through larger inputs of allochthonous detritus from the surrounding landscape (Vannote et al. 1980; Conners and Naiman 1984), potential inhibitory effects from natural OS bitumen deposits on newly colonized algae (Soto et al. 1975; Bott et al. 1978; Miller et al. 1978; Federle et al. 1979; Barsdate et al. 1980; Lock et al. 1981a), as well as potential for shading, light inhibition and increased turbidity downstream.

The Steepbank River had no N, P or co-limitations along the environmental disturbance gradient, whereas the Ells River did have nutrient limitations in N and N + P. In a study by Hickman et al. (1983), physical variables were observed to be the controlling factor in algal standing crop size for the Steepbank River, whereas nutrient levels controlled algal biomass within the Ells River over various seasons. Bulk water quality samples from this study also demonstrated greater overall concentrations in N and P parameters within the Steepbank River compared to the Ells River, indicating nutrient availability for algal standing crop size could be an influential factor within the Ells River. Nutrient inputs from non-point source anthropogenic perturbations on the Steepbank River basin could potentially be attributed to this absence of nutrient limitation in the lotic ecosystem, as described by Carpenter et al. (1998); however, this would require further investigation.

Seasonally, Steepbank River NDS Chl *a* had greatest concentrations in summer, with a decline into fall. Primary productivity is generally more abundant in the summer open-water period with elevated water temperature, and longer daylight hours for photosynthesis. Moreover, nutrient loading associated with non-point source pollution often occurs during and after significant precipitation events (Steinman et al. 2011), which occurred more frequently in the summer. A decline in Chl *a* in the fall was attributed to colder temperatures and shorter days. The Ells River had consistent Chl *a* concentrations from spring to summer, with a significant decrease in late fall. This relative consistency among-seasons was likely attributed to buffering effects from upper basin Namur-Gardiner lakes (Headley et al. 2005), as well as diminished nutrient loadings on the ecosystem from a less disturbed catchment, as explained by Howarth and Fisher (1976), Elwood et al. (1981), and Golladay et al. (1989). Moreover, significant interaction effects for site and month were observed for NDS Chl *a* for both the Steepbank and Ells River; therefore, it was difficult to interpret one factor without the other.

Nutrient limitation was only observed within the Ells River, with BBQ briquette periphyton Chl *a* concentrations also significantly declining from upstream to downstream. The Steepbank River did not have significant nutrient limitations, as well as no upstream to downstream changes in BBQ briquette periphyton Chl *a*. Therefore, with nutrient limitation being a major driver in autotrophic production in temperate lotic ecosystems (Hill and Knight 1988; Tank and Dodds 2003), a decrease in periphyton abundance could potentially be associated with nutrient availability in these river ecosystems.

NDS AFDM displayed no significant differences for site, month or treatment on either basin. Moreover, it was hypothesized there would be among-site differences from allochthonous material inputs from the surrounding landscape (Vannote et al. 1980; Conners and Naiman 1984), as well as increased bacterial activity and biofilm growth within the lower reaches of the OS deposit (Lock et al. 1981a; Peterson et al. 1996). No significant differences in NDS AFDM treatments also demonstrated a lack of nutrient limitation for biofilm production within either river. No significant seasonal differences in AFDM were also observed, despite metabolic rates typically being greater in summer with warmer temperatures enabling biofilm to increase, and then decrease in late fall with limited bacterial activity from lower water temperatures (Bott et al. 1985; Boulêtreau et al. 2006). Seasonal inputs of decomposing allochthonous materials could have also potentially influenced monthly AFDM.

Inadequacies of the AFDM method utilized in this study to estimate biofilm biomass could possibly explain the no significant differences among-sites and treatments for both basins. Alternative methods to analyze biofilm organic carbon include pigment analysis and biovolume techniques (Hauer and Lamberti 2011), as well as methods which have been specifically designed for NDS, as outlined by Tank and Webster (1998). Nevertheless, advantages of AFDM analysis used in this study included only requiring basic laboratory instrumentation and being relatively non-labor intensive (Hauer and Lamberti 2011). While alternate methods could be considered for future investigations; the AFDM method employed was sufficient for the purposes of this study.

4.5 Conclusions

Periphyton abundance on natural and artificial substrata demonstrated significant changes along the environmental disturbance gradient, as well as over seasons for both tributaries in the AOSR. In general, algal biomass (Chl *a*) declined, while total biofilm mass (AFDM) increased from upstream to downstream in both the Steepbank and Ells Rivers. Downstream declines in Chl *a* on natural substrata within the Steepbank River was likely attributed to a large slump event as well as several extreme discharge events which occurred during the 2012 sampling season, causing algal scouring at the downstream site.

The Ells River had a depression in Chl *a* on artificial substrata from upstream to downstream. This was possibly attributed to inhibition of algal primary production from acute oil-toxicity from OS deposits on BBQ briquette Chl *a*. Additional possible mechanisms included allochthonous inputs of FPOM from the terrestrial landscape, as well as potential for shading, light inhibition and increased turbidity from high TSS loadings, which would require further investigations. Algal communities shifting to a heterotrophic state over time has also been related to OS deposit effects (Bott et al. 1978), as well as inputs of particulate organic matter from the terrestrial landscape; therefore, an increase in periphyton rock scraping AFDM from upstream to downstream in both rivers was generally observed.

Seasonal effects on periphyton biomass for natural and artificial substrates demonstrated a spring depression likely attributed to algal scouring from freshet, as well as increased turbidity reducing PAR and autotrophic production. This was followed by an increase in summer with warmer temperatures and longer daylight hours, and a decrease in fall with cooler temperatures and shorter days. Seasonal effects within the Ells River were generally less drastic, with potential buffering effects from the upper basin Namur-Gardiner lakes (Headley et al. 2005), as well as diminished nutrient concentrations within runoff from a less disturbed catchment.

Variability in observed results between methods were possibly attributed to rock-basket retrieval success, large slumping and discharge events, as well as limited within-site variance acquired from periphyton rock scrapings. Determining responses of different algal communities to effects from non-point source disturbances was also method dependent, with the potential for algal species on natural substrates to become tolerant to oil-toxicity effects over time, compared to less tolerant species which could have newly colonized on clean substratum, which was observed in studies by Lock et al. (1981a, b). Therefore, rapid bioassessment approaches utilizing artificial substrates were recommended for sampling periphyton abundance to provide greater insight into the diverse behaviour of algal communities to non-point source disturbances within tributaries of the AOSR.

Nutrient limitation on Chl *a* was only observed within the Ells River. Nutrient levels have been shown in previous studies to be a limiting factor in algal biomass in the Ells River, whereas physical forces have been observed to be the controlling factor in algal biomass within the Steepbank River (Hickman et al. 1983). Moreover, an overall decline in NDS Chl *a* was observed from upstream to downstream for both rivers, which was possibly attributed to natural allochthonous inputs from the surrounding landscape, as well as possible inhibitory effects of OS deposits on downstream primary productivity. Seasonal effects on NDS Chl *a* demonstrated similar patterns to natural and artificial substrate periphyton abundance. Site, month and treatment differences for NDS AFDM were not observed, which highlighted potential problems towards replicating the method for AFDM analysis in this study for future research.

Recommendations for future assessments of basal productivity in tributaries of the AOSR include collecting replicate periphyton rock scrapings at a site to provide an estimate of sample variance, as well as reducing sample bias by estimating total periphyton growth on rocks in the sampling riffle instead of limited area scrapings of a single rock (Biggs and Close 1989). Integrating algal taxonomic identification and stable isotope analyses would provide further information on community composition and the relative contributions of resources with distinct isotope signatures to the food web, among-sites and between-basins for both natural and artificial substrata (Cattaneo and Amireault 1992). Algal toxicity tests would also be beneficial at better determining the possible primary and secondary effects of natural bitumen deposits on algal communities within tributaries of the AOSR.

Furthermore, utilizing different material for rock-basket artificial substrates would also determine if substratum-type influenced periphyton growth, as well as potentially permit the accumulation of biofilm for AFDM analysis (Morin and Cattaneo 1992). Experimenting with various NDS devices could potentially increase retrieval success (Scrimgeour and Chambers 1997), and enhanced methods for AFDM analysis would produce more accurate estimations of biofilm production within these river ecosystems (Tank and Webster 1998). N:P ratios could also be determined to assess potential nutrient limitations in the rivers, as outlined by Hecky and Kilham (1988), with deviance from the optimum nutrient ratio possibly leading to limitation by one or the other nutrient.

Light and dark experiments were conducted utilizing *in situ* benthic respirometry chambers to assess longitudinal changes in primary and heterotrophic production, which can be integrated with this study to estimate ecosystem metabolism as a supplementary functional endpoint for determining potential alterations in the river environment (Osborne and Davies 1981). Overall, natural variation was the primary driver for changes in basal productivity variables in both the Steepbank and Ells Rivers. Potential OS deposit effects and influences from nutrient availability on basal production were observed in the Ells River, whereas physical factors were primarily associated with the Steepbank River.

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CHAPTER 5: LONGITUDINAL AND SEASONAL RESPONSES FROM NATURAL AND ANTHROPOGENIC NON-POINT SOURCE DISTURBANCES ON BENTHIC MACROINVERTEBRATE COMMUNITY COMPOSITION

5.1 Introduction

Benthic macroinvertebrate communities play a critical role in the transfer of energy from basal resources (e.g., algae, detritus and associated microbes) to vertebrate consumers in aquatic food webs (Johnson et al. 1999). Invertebrate community composition is controlled by a variety of environmental variables, such as physical habitat (Maddock 1999), water and sediment quality (Hellawell 1986; Reynoldson et al. 1995), autotrophic and heterotrophic production (Vannote et al. 1980), and non-point source contaminants (Clements and Kiffney 1993). Anthropogenic catchment-scale disturbances can alter these parameters through increased sedimentation events (Wood and Armitage 1997), hydrologic alteration (Kaller and Hartman 2004), as well as enhanced nutrient and contaminant inputs from the watershed into the river (Carpenter et al. 1998; Farag et al. 1998). Moreover, natural variation within the watershed is also an essential driver in longitudinal and seasonal changes in community structure (Vannote et al. 1980). For this reason, there is often some degree of uncertainty to whether observed changes in species richness or composition are related to anthropogenic or natural disturbance when performing riverine ecosystem assessments (Kiffney and Clements 1994).

Natural variation of lotic ecosystems controls benthic macroinvertebrate distribution through patterns of watershed loadings, transport, utilization, and storage of physical and biological material (Vannote et al. 1980). Seasonal variability influences timing of catchment runoff, changes in discharge, downstream movement of material, concentrations of chemical parameters and transport of sediment into the river, ultimately affecting the instream biota (Vega et al. 1998; Woodruff et al. 2001; Ouyang et al. 2006). Furthermore, geologic formations within the Athabasca Oil Sands Region (AOSR), specifically the McMurray Formation (McMF), influence the surrounding river ecosystems by altering the natural substrate and introducing chemical constituents into the environment (Barton and Wallace 1979; Maclock et al. 1997), as well as having inhibitory and stimulatory effects on basal production (Soto et al. 1975; Bott et al. 1978; Miller et al. 1978; Federle et al. 1979; Barsdate et al. 1980; Lock et al. 1981a).

Examining community structure or species composition of benthic macroinvertebrates has been widely employed in environmental monitoring and assessments of freshwater ecosystems (Reynoldson and Metcalfe-Smith 1992; Norris and Hawkins 2000; Statzner et al. 2001). Aquatic invertebrates are relatively easy to sample quantitatively utilizing a variety of sampling devices (Carter and Resh 2011), they have recognized community responses to water quality changes (Reynoldson 1984; Hilsenhoff 1987), there is an extensive range of identification keys available (Klemm et al. 2002), the tolerance to pollution of many macroinvertebrate taxa is well documented (Mason 1981; Hellawell 1986; Jeffries and Mills 1990), and the macroinvertebrate community integrates the state of the environment over the previous months (Cairns et al. 1993).

Various sampling devices have been employed to collect benthic macroinvertebrates for river assessments, such as traditional kick-type samplers or artificial substrates (Carter and Resh 2011). Kick-type samplers have been the most commonly used devices in rapid bioassessment approaches for benthic macroinvertebrates (Resh and Jackson 1993). Advantages include low cost, ease of transport, and usefulness in sampling a variety of habitats (including deep-water habitats) more easily than frequently used fixed-quadrat samplers (Carter and Resh 2011). Kick samplers have also been shown to reflect the macroinvertebrate assemblage better than other sampling devices (Buss and Borges 2008). Moreover, discriminating the responses of natural and anthropogenic non-point source perturbations is difficult when there are confounding factors among sampling environments which also contribute to community structure.

Artificial substrata are rapid bioassessment tools which provide a “passive” sampling method and permit standardized sampling by eliminating confounding effects of habitat differences among-sites and seasons (Rosenberg and Resh 1982; Lamberti and Resh 1985; Paller 1996). They also eliminate subjectivity in sample collection, with standardized deployment and retrieval techniques, and can be easily manipulated in location and incubation time (Hellawell 1978). Sampling variability is decreased due to a reduction in microhabitat patchiness allowing the potential for spatial and temporal similarity among-samples. Additionally, replication is simplified with artificial substrates, whereas kick type methods can be arduous when collecting multiple samples per day (Cairns 1982).

Structural endpoints have been traditionally studied utilizing benthic macroinvertebrate communities to identify potential alteration within the freshwater ecosystem (Reynoldson and Metcalfe-Smith 1992; Norris and Hawkins 2000; Statzner et al. 2001). Changes in community structure, specifically indicator species (e.g., loss of sensitive taxa) can be related to potential anthropogenic and naturally induced stresses at multiple spatio-temporal scales (Hynes 1960; Warren 1971). Moreover, breaking apart individual drivers contributing to community changes can facilitate the determination of cumulative effects and sources of disturbance (Dubé et al. 2006).

Although the influences of various parameters have been studied separately, macroinvertebrates in nature are exposed to multiple, simultaneously operating factors. Despite the recognition that all of these variables influence benthic macroinvertebrate communities, the relative contributions of those factors in structuring the benthic macroinvertebrate community are rarely quantified (Peeters et al. 2004). Therefore, multivariate analyses are conducted to relate community composition to multimetric field data, to better understand which natural and anthropogenic environmental variables explain variation in the benthic macroinvertebrate community, as well as to determine whether responses from the community are method dependent (Hill and Gauch 1980).

Furthermore, observed changes in structural characteristics of aquatic insect communities along river gradients have important practical implications for assessing possible contaminant-effects within the lotic ecosystem (Kiffney and Clements 1994). In the AOSR, aerial contaminants from oil sands (OS) mining activities are deposited on the surrounding landscapes, especially on watersheds greatly disturbed by development (Kelly et al. 2009, 2010; Kirk et al. 2014). Natural OS deposits and anthropogenically-sourced contaminants have been previously documented to have significant negative effects on fish species in the AOSR (Colavecchia et al. 2004). Moreover, benthic macroinvertebrates have also been suggested to be reliable indicators of metal bioavailability in contaminated aquatic systems, through the accumulation of contaminants over time (Nehring 1976; Hare and Campbell 1992).

Elemental mercury (Hg) is associated with aerial contaminants from OS mining activities, and higher Hg concentrations have been reported both on the watershed landscapes, as well as in the tributary streams in greatest amounts nearest development sites (Kelly et al. 2010; Kirk et al. 2014). Once deposited, elemental Hg undergoes a number of biogeochemical transformations, which determines its ability to bioaccumulate in the ecosystem. One key process is the microbial methylation of elemental Hg to the toxic bioaccumulative form, methylmercury (MeHg; Driscoll et al. 2013). Thus, bioaccumulation of MeHg in the aquatic food chain was investigated, with benthic macroinvertebrates being a food supply for fish, and fish being an important dietary source forming the major route of MeHg transfer to humans (Langley 1973; Clarkson 2002).

Transportation of Hg in rivers is dependent on many factors, such as physical characteristics of watersheds, biogeochemical controls, and seasonal dynamics, such as storm events, temperature extremes, and snow-melt (Babiarz et al. 1998). Seasonal variations in atmospheric Hg concentrations have been observed globally, but numerous studies fail to identify consistent temporal changes in biota because of the complexity of Hg cycling in the environment (Schroeder and Munthe 1998; Pirrone et al. 2010; Driscoll et al. 2013). For these reasons, Hg was selected as the contaminant to investigate possible modifications to benthic macroinvertebrate community structure along the environment disturbance gradient and over seasons in tributaries of the AOSR.

Measuring benthic macroinvertebrate community composition utilizing various methods, as well as assessing potential alteration from Hg contamination, at different times of the year, will evaluate the response of a rivers biological community to a gradient of land use disturbance. Sampling between two basins with various anthropogenic catchment-scale disturbances can assist in determining possible consequences of the different stages of OS mining activities on the integrity of river ecosystem health in the AOSR. Furthermore, examining the similarities and deviations in community structure between natural and artificial substrates along the environmental disturbance gradient and among-seasons will determine the most effective rapid bioassessment approach for assessing community composition in tributaries of the AOSR.

Studies to date on the potential effects of OS development on surrounding river ecosystems in the AOSR have not routinely assessed benthic macroinvertebrate community composition and Hg concentrations utilizing the sampling design implemented in this study (Barton and Wallace 1979, 1980; Environment Canada 2011b, c; RAMP 2012). The present study also identified physical, chemical, and biological environmental variables which could help explain spatial and temporal shifts in community structure. Therefore, this chapter evaluates the observed differences in benthic macroinvertebrate community composition, and related environmental variables, as well as mercury concentration within- and among-sites, and between-basins, while assessing the disturbance gradient design. See Chapters 1 and 2 for detailed objectives and predictions for Chapter 5.

5.2 Methods

5.2.1 Field Sampling

Three-Minute Kick Nets for benthic macroinvertebrates were performed in the sampling riffle once at each site (n=1) during each sampling period in congruence with the Canadian Aquatic Biomonitoring Network (CABIN) protocol (Environment Canada 2010). A kick net is a triangular metal frame holding a bag with mesh size of 400 µm, with a collection cup connected to the end of the net to facilitate removal of the sample, and a rake handle connected to one end of the metal frame (Figure 5.1). Samples were collected with a kick net over a period of exactly three minutes to standardize the level of effort. A zigzag sampling pattern across the river moving in an upstream direction was practised to integrate benthic macroinvertebrates from various microhabitats in proportion to their occurrence in a sample reach. The substrate was kicked to a depth of approximately 5 to 10 cm, with the disturbed substrate and organisms being swept into the net by the current.

After three minutes, the kick net was washed down to remove organisms attached to the inside of the net. The collection cup was detached, and the sample was emptied into a bucket with water for bucket swirling. Bucket swirling, or elutriation, is a common method used to remove large amounts of inorganic material (e.g., sand and/or gravel) from a sample. Elutriation reduces sorting time, and minimizes the damage that large volumes of sediment can have on the benthic macroinvertebrates (Rosillon 1987; Ciborowski 1991). During elutriation, the sample was agitated or swirled in a bucket with water, dislodging any interstitial benthic macroinvertebrates embedded in the sediment. Lighter organisms and fine particulate matter floated to the surface which was poured into a 250 µm mesh sieve, and the inorganic material was discarded. The sieve was rinsed down and the sample was preserved in a container with 95% ethanol (EtOH) for further sample processing.

Rock-Basket Invertebrates were collected from each of the three rock-basket artificial substrates at each site (n=3) during monthly retrievals. A kick net was positioned downstream of the rock-basket to collect any dislodged invertebrates during the retrieval process. The rock-basket was swiftly removed from its location in the river, and immediately placed into a tray for processing on the river bank. Once the scour pads, HOBO® temperature logger, nutrient diffusing substrate (NDS), five BBQ briquettes for periphyton, and five BBQ briquettes for algal taxonomy were removed from the rock-basket, individual briquettes were rinsed in a bucket to remove any clinging organisms. The empty basket was washed down in the tray and all material from the rock-basket, including briquettes, basket and kick net was collected onto a 250 µm mesh sieve. The sieve was rinsed down and the sample was preserved in a container with 95% EtOH for further sample processing. Benthic macroinvertebrate samples from each of the rock-baskets were placed into three separate sample containers pertaining to the tray number.

Mercury Concentration of benthic macroinvertebrates was analyzed from organisms collected from bulk kick net samples, which were performed in the sampling riffle once at each site (n=1) during each sampling period. Bulk kick nets were identical to three-minute kick nets in procedure, except they were not timed. Kicking continued until sufficient sample material was accumulated. Elutriation was also performed on bulk kick net samples, which were then rinsed into a 250 µm mesh sieve (Figure 5.1). Samples were placed in Ziplock bags, and stored on ice packs in a cooler during the field day, and transferred to a -20°C freezer for further sample processing.

Odonates (Suborder: Anisoptera) were selected as the representative species for determining total Hg (THg) and MeHg concentrations in the benthic macroinvertebrate community due to their large body size, and consistency among-sites. Suitable invertebrates for Hg processing needed to be physically large, as well as have a multiyear lifecycle to determine the body burden of Hg concentration, as described by George and Batzer (2008).

Figure 5.1. Equipment used for three-minute kick net, rock-basket retrieval and bulk kick net sampling for benthic macroinvertebrates at each site on the Steepbank and Ells Rivers during each sampling month (May-October 2012). Picture includes: d-framed 400 μm mesh kick net, 250 μm sieve, squeeze bottle and two buckets for elutriation procedure.

5.2.2 Sample Processing

5.2.2.1 Three-Minute Kick Net Invertebrates

Three-minute kick net benthic macroinvertebrate samples were processed, subsampled and organisms identified to the lowest practical taxonomic level by a certified taxonomist at Cordillera Consulting Inc. (Summerland, BC), in accordance with CABIN benthic macroinvertebrate sample processing protocols (Environment Canada 2010).

5.2.2.2 Rock-Basket Invertebrates

Rock-basket benthic macroinvertebrate samples were processed, subsampled and organisms identified to order level utilizing the following laboratory protocols developed at the University of Victoria (Victoria, BC), which were performed prior to sending to a certified taxonomist (Jack Zloty), who further identified organisms to the lowest practical taxonomic level:

Benthic macroinvertebrate samples were subsampled in accordance with the "Revised Guidance for Subsampling Protocols for Environmental Effects Monitoring (EEM) Benthic Invertebrate Community Surveys" (Glozier et al. 2002). Samples were separated into two size classes of benthic macroinvertebrates (above 850 µm; between 850 µm and 355 µm). Benthic macroinvertebrates greater than 850 µm were subsampled by 25% if sorting time was estimated to be greater than four hours. If the estimated sorting time was less than four hours, all organisms greater than 850 µm were counted. Samples containing benthic macroinvertebrates smaller than 850 µm, but greater than 355 µm, were subsampled by 25% using an Imhoff cone following methods of Wrona et al. (1982). See below for specific subsampling protocols for the 850 µm and 355 µm sieves.

1) Separate sample into two sieves (850 µm and 355 µm) - Inside a large tray, a 850 µm sieve was stacked on top of a 350 µm sieve. The benthic macroinvertebrate rock-basket sample was emptied into the 850 µm sieve, allowing the remnants to fall into the 355 µm sieve below. The top sieve was rinsed down thoroughly and picked apart to allow smaller organisms to fall through the sieve mesh.

2) Subsample top sieve (850 µm) - If the estimated sorting time for the top sieve was greater than four hours, it was subsampled accordingly. To subsample 25% of the benthic macroinvertebrates greater than 850 µm, the top sieve was placed in a large tray filled with water and allowed to float, producing equal distribution of the sample on the sieve. Once the sample was equally dispersed, the water within the tray was poured through the 355 µm sieve to collect any remaining organisms. The 850 µm sieve was then divided into four quadrats, with one quarter randomly selected to sample. All organisms from the one quarter were counted and identified to order level. Samples were checked at random for QA/QC to ensure the 25% subsample was representative of the entire sample. Once all benthic macroinvertebrates were sorted, counted and identified from 25% of the 850 µm sieve, the remaining unsorted sample was returned to the original sample container with 70% EtOH for preservation. The identified organisms were placed into their appropriate order level groups in labelled sample vials with 70% EtOH, in preparation for identification to the lowest practical taxonomic level.

3) **Subsample bottom sieve (355 µm) -** To subsample 25% of the benthic macroinvertebrates between the sizes of 850 µm and 355 µm, an Imhoff cone was utilized to homogenously mix the sample contained within the bottom sieve following methods of Wrona et al. (1982). A 1000 mL Imhoff cone was filled with 500 mL of water, and all of the organisms were rinsed from the bottom sieve into the sampling device. The Imhoff cone was filled to 1000 mL with water, and the air bubbler ran for five minutes to ensure thorough mixing. 25% of the original sample volume was subsampled by removing five subsamples from the mixed solution using a 50 mL test tube. The five subsamples were emptied into a sorting pan and counted, with organisms identified to an order level. Samples were checked at random for QA/QC to ensure the 25% subsample was representative of the entire sample. Once all benthic macroinvertebrates were sorted, counted and identified from 25% of the 355 µm sieve, the remaining unsorted sample was returned to the original sample container with 70% EtOH for preservation. The identified organisms were placed into their appropriate order level groups in labelled sample vials with 70% EtOH, in preparation for identification to the lowest practical taxonomic level.

5.2.2.3 Odonate Mercury Concentration

Bulk kick net Odonate samples were prepared for THg and MeHg analysis by Direct Mercury Analyzer (DMA) utilizing the following laboratory protocols:

Bulk kick net samples in Ziplock bags were removed from the -20°C freezer to thaw the sample slightly before processing. Once thawed, sample contents were emptied into an acid-washed plastic sorting pan, with enough deionized (DI) water added for organisms to float. Samples were processed utilizing acid-washed plastic forceps, removing and counting all Odonate specimens. Removed individuals were placed into a labeled whirlbag pertaining to the specific sample, and returned to the -20°C freezer. Once the bulk kick net sample was processed entirely for Odonates, the remaining sample was returned to the original Ziplock bag and placed in the -20°C freezer.

After all bulk kick net samples were processed, sample whirlbags were removed from the -20°C freezer to thaw slightly before processing. Once thawed, a single Odonate specimen was removed from a whirlbag using acid-washed plastic forceps and blotted on Bibulous paper to remove excess moisture. The individual specimen was weighed in a plastic weigh dish on a micro-scale (pre freeze-dried weight), and placed into an acid-washed labelled scintillation vial. Processing continued until all Odonates from sample whirlbags were weighed. Each specimen was placed into separate vials to facilitate determination of pre and post freeze-dried weights, necessary for calculating % moisture content. Samples were either returned to the -20°C freezer to prevent acquiring moisture, or prepared immediately for freeze-drying.

In preparation for freeze-drying, the caps of the scintillation vials containing pre freeze-dried weighed Odonate specimens were removed, and half of a kimwipe was folded on top, secured with an elastic band. Samples were positioned in the freeze-drier (LABCONCO) for 45 hours at -50°C and 0.016 mBar. After freeze-drying, the Odonate specimens were removed and weighed immediately (post freeze-dried weight). Once all Odonates from a bulk kick net sample were pre and post freeze-dried weighed, samples were separated into three subsamples each containing a minimum of 0.25 g of total dried weight, necessary for THg and MeHg analysis using a DMA. Each subsample was then combined into three separate lysing tubes, and pulverized in a ball-grinder. Samples were sent for THg and MeHg analysis at the Canada Centre for Inland Waters (CCIW; Burlington, ON).

5.2.3 Statistical Analyses

To determine whether benthic macroinvertebrate community structure differed among-sites and months, a nonparametric, permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) based on the Bray-Curtis similarity index was done on % composition of the benthic macroinvertebrate community. Community abundance data was square root transformed prior to calculating the resemblance matrix, to decrease the importance of the very abundant species thereby increasing the importance of the rare species on the outcome of the analyses (Warwick and Clarke 1991). PERMANOVAs tested the following null hypothesis:

- HO1: No change among-sites and months in benthic macroinvertebrate community structure along the environmental disturbance gradient.

Two-way PERMANOVAs using mixed-effects models, with site as the fixed variable and month (repeated testing over time) as the random, or blocking variable were used to determine site and monthly differences for both three-minute kick net and rock-basket invertebrate community composition within the Steepbank and Ells Rivers. The multivariate analysis also simultaneously determined any interaction effects between site and month for multiple taxa. A permutation of residuals under a reduced model was used for 999 permutations, which was the most robust model to avoid Type I and II errors. Pairwise PERMANOVA tests between factor levels were done post hoc to determine which sites and months significantly differed ($p < 0.05$). After the PERMANOVA was run, two-way crossed similarity percentages analyses (SIMPER), were conducted to assess which taxa were most responsible for the differences among-sites and months, for all site and month comparisons (Clarke 1993). The PERMANOVA and SIMPER analyses were conducted in PRIMER version 6.0 (Clarke and Warwick 2013).

If significant site and monthly differences were observed in the PERMANOVA, ordination analyses were subsequently conducted to identify which physico-chemical and biological environmental variables were explaining variations in the three-minute kick net and rock-basket benthic macroinvertebrate communities within the Steepbank and Ells Rivers. Only invertebrate species contributing a cumulative contribution of 70% to the dissimilarity among-sites and months based on the SIMPER analyses was included in the ordination analyses as per Clarke (1993). Invertebrate species were grouped into their respective family groups and the ordination was run at a family taxonomic level. Specific invertebrate species which accounted for a cumulative 70% of the dissimilarity between each site and month included in the ordination analyses are represented in Appendix C.

To determine which constrained ordination technique was to be performed based on the community data, a detrended correspondence analysis (DCA) was conducted which illustrated that variation in the benthic macroinvertebrate data was small (< 2 standard deviations), suggesting that a redundancy analysis (RDA) was the most appropriate ordination method to assess which environmental variables directly explained variation in the benthic macroinvertebrate community (Hill and Gauch 1980; Feld and Hering 2007). Environmental variables were standardized to unit variance prior to analysis to correct for measurements taken with different units (Nerbonne and Vondracek 2001), and benthic macroinvertebrate data were Hellinger-transformed as recommended by Borcard et al. (2011). The Hellinger transformation enables the use of Euclidean-based ordination methods and has the advantage of not disproportionally weighing rare taxa in the analyses (Legendre and Gallagher 2001).

Pre-screening of all physico-chemical and biological parameters for the RDA included removing environmental variables not theoretically related to previous studies explaining changes in benthic macroinvertebrate communities in lotic ecosystems of the AOSR (Barton and Wallace 1979, 1980; Environment Canada 2011b, c; RAMP 2012). Environmental variables included in the three-minute kick net and rock-basket RDA analyses were not identical due to differences in timing and location of sample collection for physico-chemical and biological variables pertaining to the natural or artificial substrates. Remaining variables were run through a Pearson correlation matrix to remove highly correlated parameters to avoid multicollinearity as well as over-fitting, as recommended by Palmer (1993), to produce a full model before performing the RDA. From the full model, the variable with the greatest variance inflation factor (VIF) was entered into a three-step process similar to that used by Hall and Smol (1992), and subsequently by Moquin et al. (2014).

The steps included: 1) examination of the variable with the highest VIF for significant correlations ($p < 0.05$) with the other environmental variables using a Pearson correlation matrix with Bonferroni-adjusted probabilities for the multiple testing of variables (Aickin and Gensler 1996); 2) identification via forward selection of the environmental variable that explained the greatest amount of variance from the correlated subgroup and use of that subgroup in subsequent analyses; 3) use of a series of partial-constrained RDAs to assess whether other members of the correlated variable subgroup exerted an independent influence on the benthic macroinvertebrate community structure. The variable identified in step 2 was used as the sole variable, and each of the other variables in the subgroup was, in turn, loaded as the sole covariable. The significance of the 1st axis was assessed with a Monte Carlo test with 999 random permutations ($p < 0.05$). Covariables that did not explain significant amounts of the benthic macroinvertebrate community structure were removed from subsequent analyses (Hall and Smol 1992; Moquin et al. 2014).

The variable-screening process was repeated with the remaining variables until all had VIF values < 10, indicating low or no multicollinearity (Borcard et al. 2011). Following Borcard et al. (2011), a final RDA with forward selection based on permutational *p*-values (1000 permutations) was used to identify which of the remaining environmental variables explained a statistically significant ($p < 0.05$) amount of variation in community structure. RDA analyses were conducted in R version 2.15.2, utilizing the “psych”, “permute”, and “vegan” packages (R Development Core Team 2012).

Lastly, general linear models (analysis of variance; ANOVA) were used to analyze among-site and monthly differences in Odonate mercury concentration related to the disturbance gradient hypothesis for the Steepbank and Ells Rivers. ANOVAs tested the following null hypothesis:

- HO1: No change among-sites and months in average Odonate mercury concentration along the environmental disturbance gradient.

Two-way ANOVAs using mixed-effects models (described in section 2.4), with site as the fixed variable and month as the random variable were used to determine site and monthly differences in Odonate THg and MeHg concentrations within the Steepbank and Ells Rivers. Data were first log transformed to fit the assumption of normality for the ANOVA (Berry 1987). Before running the model, a restricted maximum likelihood (REML) estimation was applied to the Steepbank River dataset because of the smaller sample size (< 20), and a maximum likelihood (ML) estimation was applied to the Ells River dataset because of a larger sample size (> 20), which is a method used for fitting linear mixed models (Kenward and Roger 1997).

Significant interactions between site and month were also examined prior to running the model using a two-factor ANOVA; however, interaction terms were not included in the model to maintain model consistency for all analyses. In cases where a significant difference among-sites and/or months was found ($p < 0.05$), an *a posteriori* test, Tukey HSD, was performed to determine which sites or months were significantly different. Two-way mixed-effects ANOVAs were conducted using R version 2.15.2, utilizing the “nlme” and “multcomp” packages (R Development Core Team 2012).

5.3 Results

5.3.1 Community Composition and Related Environmental Variables

5.3.1.1 Three-Minute Kick Net Invertebrates

Steepbank River

Benthic macroinvertebrate community composition was significantly different among-sites, with the most downstream site (ST1) being significantly different than upstream sites (ST3, ST4). ST4 and ST3 were also significantly different in benthic macroinvertebrate community structure. Changes in the relative abundance of *Baetis tricaudatus* (Ephemeroptera: Baetidae) had the greatest effect on community structure among-sites, with the upstream sites being more abundant. *B. tricaudatus* abundance accounted for 25% and 40% of the dissimilarity between ST1 vs. ST3, and ST1 vs. ST4, respectively, and 16% of the dissimilarity between ST3 vs. ST4.

Community composition was significantly different in May than July and August, with July being significantly different than August and October. Changes in the relative abundance of *B. tricaudatus* had the greatest effect on community structure among-months, except for May vs. August where changes in the relative abundances of *Ephemerella sp.* (Ephemeroptera: Ephemerellidae) accounted for 13% of the dissimilarity, with August being more abundant. *B. tricaudatus* abundance accounted for 22% and 24% of the dissimilarity between May vs. July, and July vs. August, respectively, with July being more abundant for both comparisons. *B. tricaudatus* abundance explained 37% of the dissimilarity between July vs. October, with October being more abundant. There was insufficient sample replication for a site x month interaction effect for community structure (Table 5.1). Results from the SIMPER analyses are presented in Appendix C (Tables C.1 and C.2).

Table 5.1. Steepbank River results from the permutational multivariate analysis of variance (PERMANOVA) and pairwise comparisons for three-minute kick net benthic macroinvertebrate community composition. Significant *p*-values ($p < 0.05$) for site and monthly differences are shaded in grey. "-" indicates insufficient data for a statistical analysis.

Parameter	Mean square	Pseudo-*F*	*T*	*p*-value
Site	4225	2.57		0.003
Month	2888	1.76		0.003
Site x month	-	-		No rep.
Residuals	1644			
Pairwise comparison				
Site				
ST1 vs ST2			1.06	0.387
ST1 vs ST3			1.65	0.020
ST1 vs ST4			1.91	0.002
ST2 vs ST3			1.13	0.363
ST2 vs ST4			1.47	0.078
ST3 vs ST4			1.46	0.022
Month				
May vs Jun			-	-
May vs Jul			2.31	0.012
May vs Aug			1.40	0.039
May vs Sep			1.31	0.161
May vs Oct			1.13	0.199
Jun vs Jul			-	-
Jun vs Aug			-	-
Jun vs Sep			-	-
Jun vs Oct			-	-
Jul vs Aug			1.87	0.023
Jul vs Sep			1.37	0.068
Jul vs Oct			1.56	0.028
Aug vs Sep			1.11	0.367
Aug vs Oct			1.33	0.103
Sep vs Oct			1.10	0.449

The RDA data screening procedure of environmental variables explaining variation in site differences for the three-minute kick net benthic macroinvertebrate community led to the removal of Cobble, Pebble, silicon dioxide (SiO_2^{2-}), total dissolved solids (TDS), temperature (Temp), dissolved oxygen (DO), total dissolved nitrogen (TDN), sulfate (SO_4^{2-}), and periphyton chlorophyll *a* (Peri-Chl *a*) from further analyses. Forward selection of the remaining variables (total suspended solids (TSS), periphyton ash-free dry mass (Peri-AFDM), total dissolved phosphorus (TDP), calcium (Ca^{2+}), chloride (Cl^-), pH, Depth, and Flow) identified pH and TDP as environmental variables that explained statistically significant levels of total variation in site differences for community composition. The final RDA model (F = 3.06, $p < 0.001$, 999 permutations) explained 38% of the variation in the site differences for benthic macroinvertebrate community structure. The eigenvalues of the first two RDA axes were statistically significant (axis 1: $\lambda_1 = 0.25$, $p = 0.001$, 999 permutations; axis 2: $\lambda_2 = 0.13$, $p = 0.023$, 999 permutations).

Separation from upstream sites (ST4, ST3) and downstream sites (ST2, ST1) was primarily along RDA 1, which was loaded negatively with gradients in pH and TDP. RDA 2 further separated sites along gradients loaded positively with pH and negatively with TDP. In general, downstream sites were associated with higher pH values, whereas upstream sites were associated with higher TDP concentrations (Table 5.2, Figure 5.2).

The RDA data screening procedure of environmental variables explaining variation in monthly differences for the three-minute kick net benthic macroinvertebrate community led to the removal of Cobble, Pebble, Cl^-, SiO_2^{2-}, Ca^{2+}, TDN, DO, Peri-Chl *a*, TDP, SO_4^{2-}, and Temp from further analyses. Forward selection of the remaining variables (TDS, TSS, Peri-AFDM, pH, Depth, and Flow) identified TDS as the only environmental variable that explained statistically significant levels of total variation in monthly differences for community composition. The final RDA model (F = 2.78, $p = 0.002$, 999 permutations) explained 20% of the variation in the monthly differences for benthic macroinvertebrate community structure. The eigenvalue of the first RDA axis was statistically significant (axis 1: $\lambda_1 = 0.20$, $p = 0.002$, 999 permutations).

Separation among-months was only along RDA 1, which was loaded positively with a gradient in TDS. In general, August was associated with higher TDS concentrations (Table 5.3, Figure 5.3).

Table 5.2. Summary results of a redundancy analysis (RDA) on four sites for the Steepbank River three-minute kick net benthic macroinvertebrate community. RDA 1 and 2 are portrayed with loadings of the selected environmental variables for each axis. Significant *p*-values ($p < 0.05$) for eigenvalues of the RDA axes are shaded in grey. Other than standard abbreviations, the following abbreviation is used: total dissolved phosphorus (TDP).

Value	RDA 1	RDA 2
Eigenvalue	3.71	1.99
Proportion explained	0.25	0.13
Cumulative proportion	0.25	0.38
p	0.001	0.023
Environmental variable		
pH	-0.86	0.52
TDP	-0.69	-0.72

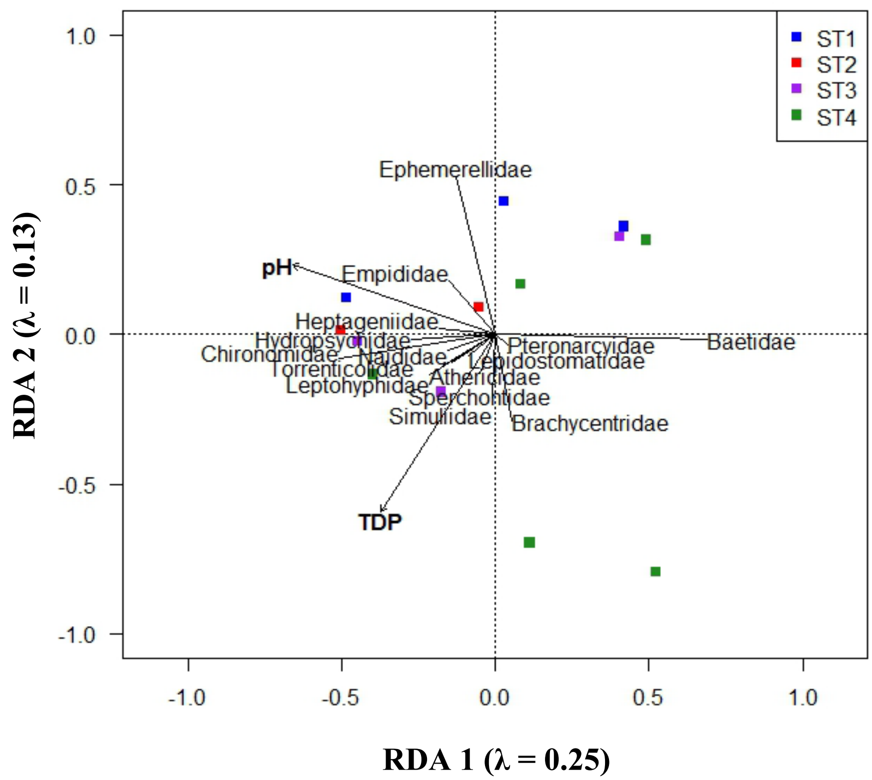
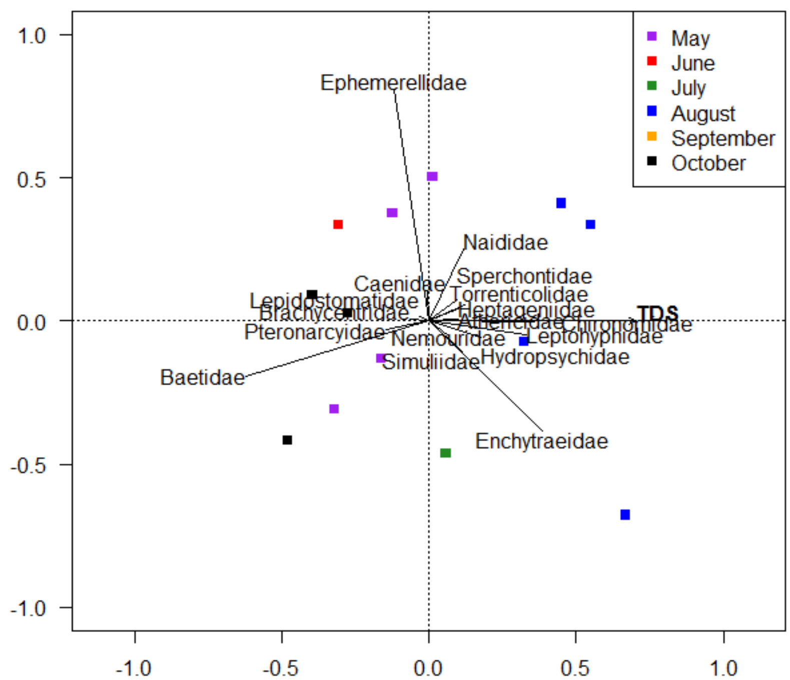


Figure 5.2. Steepbank River redundancy analysis (RDA) triplots showing site scores and associated three-minute kick net benthic macroinvertebrate family groups, and the two environmental variables that independently explain significant amounts of variation in the benthic macroinvertebrate communities at four sites. λ represents the proportion of variation explained by the RDA axis.

Table 5.3. Summary results of a redundancy analysis (RDA) over six months for the Steepbank River three-minute kick net benthic macroinvertebrate community. RDA 1 is portrayed with the loading of the selected environmental variables for the axis. The significant *p*-value ($p < 0.05$) for the eigenvalue of the RDA axis is shaded in grey. The following abbreviation is used: total dissolved solids (TDS).

Value	RDA 1
Eigenvalue	3.43
Proportion explained	0.20
Cumulative proportion	0.20
p	0.002
Environmental variable	
TDS	1.00



RDA 1 (λ = 0.20)

Figure 5.3. Steepbank River redundancy analysis (RDA) triplots showing monthly scores and associated three-minute kick net benthic macroinvertebrate family groups, and the one environmental variable that independently explains significant amounts of variation in the benthic macroinvertebrate communities across six months. λ represents the proportion of variation explained by the RDA axis.

Ells River

Benthic macroinvertebrate community composition was significantly different among-sites, with the most downstream site (EL1) being significantly different than the most upstream site (EL3). Changes in the relative abundance of *Microspectra sp.* (Diptera: Chironomidae) had the greatest effect on community structure between-sites, and accounted for 12% of the dissimilarity, with EL3 being more abundant. Community composition was significantly different in July than September. Changes in the relative abundance of *Microspectra sp.* had the greatest effect on community structure between-months, and accounted for 15% of the dissimilarity, with July being more abundant. There was insufficient sample replication for a site x month interaction effect for community structure (Table 5.4). Results from the SIMPER analyses are presented in Appendix C (Tables C.3 and C.4).

Table 5.4. Ells River results from the permutational multivariate analysis of variance (PERMANOVA) and pairwise comparisons for three-minute kick net benthic macroinvertebrate community composition. Significant *p*-values ($p < 0.05$) for site and monthly differences are shaded in grey. "-" indicates insufficient data for a statistical analysis.

Parameter	Mean square	Pseudo-*F*	*T*	*p*-value
Site	3109	2.59		0.003
Month	1860	1.55		0.012
Site x month	-	-		No rep.
Residuals	1200			
Pairwise comparison				
Site				
EL1 vs EL2			1.52	0.054
EL1 vs EL3			1.74	0.032
EL2 vs EL3			1.56	0.069
Month				
May vs Jun			1.04	0.444
May vs Jul			1.28	0.061
May vs Aug			1.02	0.389
May vs Sep			0.84	0.709
May vs Oct			0.95	0.681
Jun vs Jul			1.21	0.104
Jun vs Aug			1.40	0.056
Jun vs Sep			1.36	0.058
Jun vs Oct			1.44	0.059
Jul vs Aug			1.17	0.260
Jul vs Sep			1.57	0.014
Jul vs Oct			1.35	0.090
Aug vs Sep			1.30	0.111
Aug vs Oct			1.23	0.148
Sep vs Oct			1.02	0.431

The RDA data screening procedure of environmental variables explaining variation in site differences for the three-minute kick net benthic macroinvertebrate community led to the removal of Cobble, SO_4^{2-}, TDS, TDN, SiO_2^{2-}, TSS, Temp, DO, Peri-Chl *a*, and TDP from further analyses. Forward selection with the remaining variables (Pebble, Peri-AFDM, Ca^{2+}, Cl^-, pH, Depth, and Flow) identified Cl^- and Pebble as environmental variables that explained statistically significant levels of total variation in site differences for community composition. The final RDA model (F = 3.40, $p < 0.001$, 999 permutations) explained 34% of the variation in the site differences for benthic macroinvertebrate community structure. The eigenvalues of the first two RDA axes were statistically significant (axis 1: $\lambda_1 = 0.21$, $p < 0.001$, 999 permutations; axis 2: $\lambda_2 = 0.14$, $p = 0.012$, 999 permutations).

Separation from the most upstream site (EL3) and downstream sites (EL2 and EL1) was primarily along RDA 1, which was loaded positively with gradients in Pebble. RDA 2 further separated upstream and downstream sites along gradients of Cl^- and Pebble. In general, greater amounts of Pebble were associated with EL3, and higher Cl^- concentration was associated with EL1 (Table 5.5, Figure 5.4).

The RDA data screening procedure of environmental variables explaining variation in monthly differences for the three-minute kick net benthic macroinvertebrate community led to the removal of Cobble, TDS, SiO_2^{2-}, TSS, TDN, DO, Peri-Chl *a*, TDP, and Temp from further analyses. Forward selection with the remaining variables (Pebble, SO_4^{2-}, Peri-AFDM, Ca^{2+}, Cl^-, pH, Depth, and Flow) identified SO_4^{2-}, Peri-AFDM, and Pebble as environmental variables that explained statistically significant levels of total variation in monthly differences for community composition. The final RDA model (F = 2.81, $p < 0.001$, 999 permutations) explained 41% of the variation in monthly differences for benthic macroinvertebrate community structure. The eigenvalues of the first two RDA axes were statistically significant (axis 1: $\lambda_1 = 0.22$, $p < 0.001$, 999 permutations; axis 2: $\lambda_2 = 0.12$, $p = 0.009$, 999 permutations).

Separation among-months was primarily along RDA 1, which was loaded positively with gradients in Pebble. RDA 2 further separated months along gradients of Peri-AFDM and Pebble. In general, higher concentrations of SO_4^{2-} were associated with spring months, whereas greater amounts of Peri-AFDM and Pebble were associated with summer and fall months (Table 5.6, Figure 5.5).

Table 5.5. Summary results of a redundancy analysis (RDA) on three sites for the Ells River three-minute kick net benthic macroinvertebrate community. RDA 1 and 2 are portrayed with loadings of the selected environmental variables for each axis. Significant *p*-values ($p < 0.05$) for eigenvalues of the RDA axes are shaded in grey.

Value	RDA 1	RDA 2
Eigenvalue	1.67	1.08
Proportion explained	0.21	0.14
Cumulative proportion	0.21	0.34
p	0.001	0.012
Environmental variable		
Cl^-	-0.85	0.53
Pebble	0.98	0.19

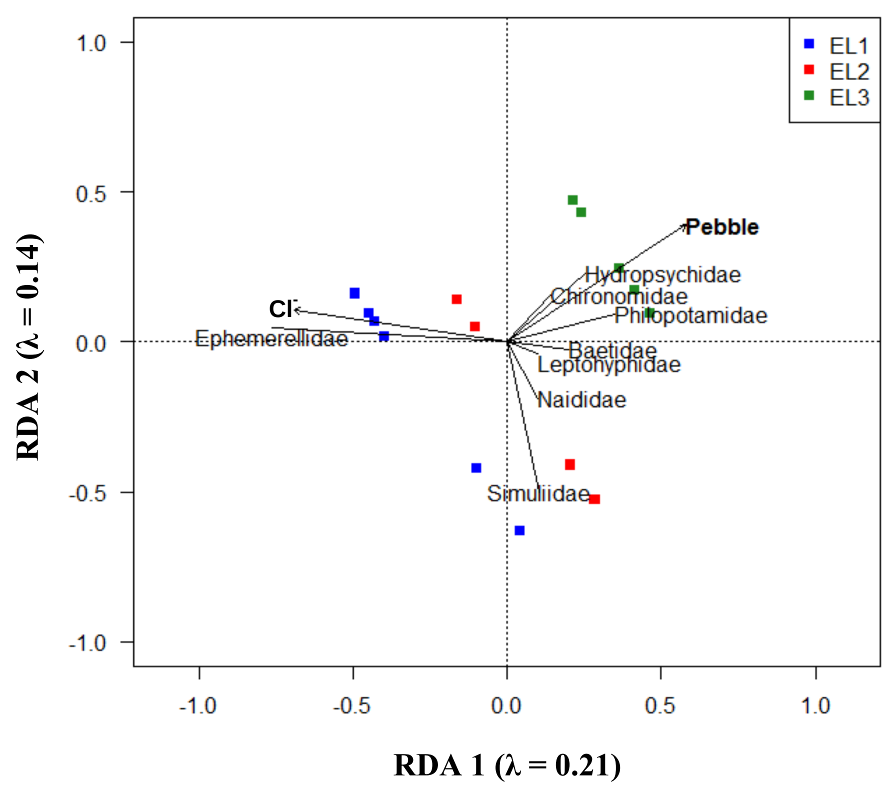


Figure 5.4. Ells River redundancy analysis (RDA) triplots showing site scores and associated three-minute kick net benthic macroinvertebrate family groups, and the two environmental variables that independently explain significant amounts of variation in the benthic macroinvertebrate communities at three sites. λ represents the proportion of variation explained by the RDA axis.

Table 5.6. Summary results of a redundancy analysis (RDA) over six months for the Ells River three-minute kick net benthic macroinvertebrate community. RDA 1 and 2 are portrayed with loadings of the selected environmental variables for each axis. Significant *p*-values ($p < 0.05$) for eigenvalues of the RDA axes are shaded in grey. Other than standard abbreviations, the following abbreviation is used: periphyton ash-free dry mass (Peri-AFDM).

Value	RDA 1	RDA 2
Eigenvalue	2.59	1.48
Proportion explained	0.22	0.12
Cumulative proportion	0.22	0.34
p	0.001	0.009
Environmental variable		
SO_4^{2-}	-0.04	-1.00
Peri-AFDM	-0.90	0.05
Pebble	0.76	0.42

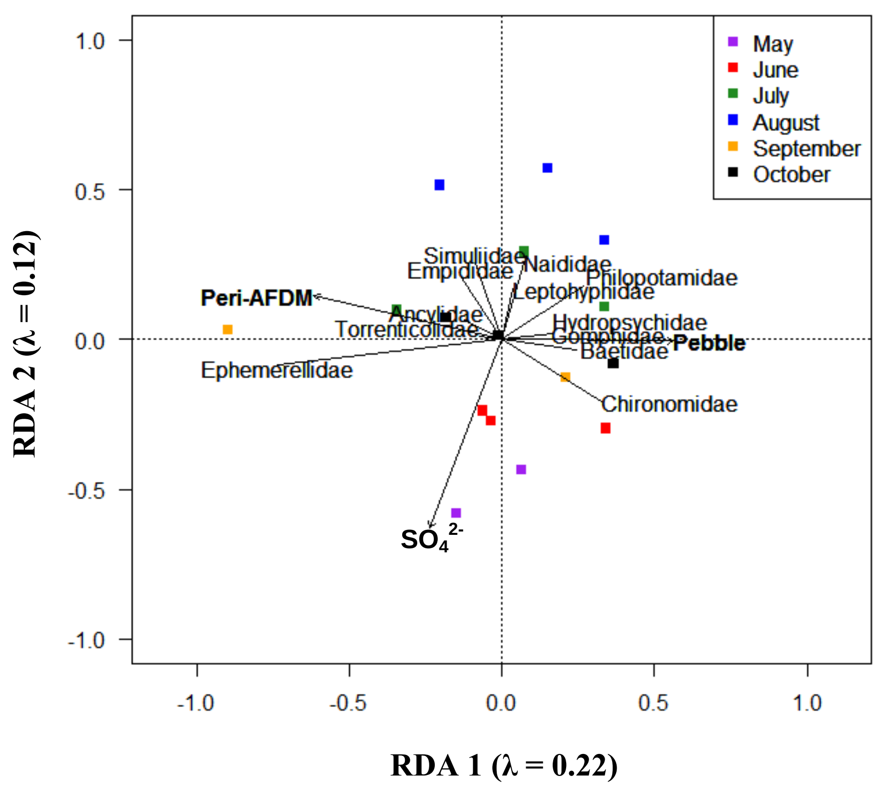


Figure 5.5. Ells River redundancy analysis (RDA) triplots showing monthly scores and associated three-minute kick net benthic macroinvertebrate family groups, and the three environmental variables that independently explain significant amounts of variation in the benthic macroinvertebrate communities across six months. λ represents the proportion of variation explained by the RDA axis.

5.3.1.2 Rock-Basket Invertebrates

Steepbank River

Benthic macroinvertebrate community composition was significantly different among-sites, with the most upstream site (ST4) being significantly different than the most downstream site (ST1). ST4 and ST3 were also significantly different in benthic macroinvertebrate community structure. Changes in the relative abundance of *Baetis spp.* (Ephemeroptera: Baetidae) had the greatest effect on community structure between ST1 vs. ST4, accounting for 27% of the dissimilarity, with ST4 being more abundant. Changes in the relative abundance of *Simulium sp.* (Diptera: Simuliidae) had the greatest effect on community structure between ST3 vs. ST4, accounting for 27% of the dissimilarity, with ST3 being more abundant.

Community composition was significantly different in August than October. Changes in the relative abundance of *Baetis spp.* had the greatest effect on community structure between-months, and accounted for 39% of the dissimilarity, with August being more abundant. There was insufficient sample replication for a site x month interaction effect for community structure (Table 5.7). Results from the SIMPER analyses are presented in Appendix C (Tables C.5 and C.6).

Table 5.7. Steepbank River results from the permutational multivariate analysis of variance (PERMANOVA) and pairwise comparisons for rock-basket benthic macroinvertebrate community composition. Significant *p*-values ($p < 0.05$) for site and monthly differences are shaded in grey. “-” indicates insufficient data for a statistical analysis.

Parameter	Mean square	Pseudo-*F*	*T*	*p*
Site	2999	6.42		0.001
Month	3245	6.94		0.001
Site x month	-	-		No rep.
Residuals	467			
Pairwise comparison				
Site				
ST1 vs ST3			2.45	0.100
ST1 vs ST4			3.04	0.001
ST3 vs ST4			1.82	0.002
Month				
Aug vs Oct			2.64	0.001

The RDA data screening procedure of environmental variables explaining variation in site differences for the rock-basket benthic macroinvertebrate community led to the removal of TDS, Ca^{2+}, Temp, pH, TDP, TDN, SiO_2^{2-}, and SO_4^{2-} from further analyses. Forward selection with the remaining variables (Cl^-, Depth, Flow, and fine sediment arsenic (Sed-As)) identified Depth and Sed-As as environmental variables that explained statistically significant levels of total variation in site differences for community composition. Priority pollutant element concentrations within fine sediments were highly correlated with each other; thus, Sed-As represents general fine sediment metal concentrations. The final RDA model (F = 4.78, $p < 0.001$, 999 permutations) explained 52% of the variation in the site differences for benthic macroinvertebrate community structure. The eigenvalues of the first two RDA axes were statistically significant (axis 1: $\lambda_1 = 0.28$, $p = 0.003$, 999 permutations; axis 2: $\lambda_2 = 0.24$, $p = 0.005$, 999 permutations).

Separation among the most upstream site (ST4) and downstream sites (ST1, ST3) was primarily along RDA 1, which was loaded positively with a gradient in Depth. RDA 2 further separated ST4 from ST1 and ST3, with gradients in Depth and Sed-As. In general, greater Sed-As concentrations and Depth of the rock-basket were associated with downstream sites (Table 5.8, Figure 5.6).

The RDA data screening procedure of environmental variables explaining variation in monthly differences for the rock-basket benthic macroinvertebrate community led to the removal of Ca^{2+}, Temp, TDP, SiO_2^{2-}, Depth, TDN, Cl^-, and pH from further analyses. Forward selection with the remaining variables (TDS, SO_4^{2-}, Flow, and Sed-As) identified TDS as the only environmental variable that explained statistically significant levels of total variation in monthly differences for community composition. The final RDA model (F = 3.67, $p = 0.015$, 999 permutations) explained 27% of the variation in the monthly differences for benthic macroinvertebrate community structure. The eigenvalue of the first RDA axis was statistically significant (axis 1: $\lambda_1 = 0.27$, p = 0.015, 999 permutations).

Separation among-months were primarily along RDA 1, which was loaded negatively with a gradient in TDS. In general, August was associated with higher TDS concentrations (Table 5.9, Figure 5.7).

Table 5.8. Summary results of a redundancy analysis (RDA) on four sites for the Steepbank River rock-basket benthic macroinvertebrate community. RDA 1 and 2 are portrayed with loadings of the selected environmental variables for each axis. Significant *p*-values ($p < 0.05$) for eigenvalues of the RDA axes are shaded in grey. The following abbreviation is used: fine sediment arsenic (Sed-As).

Value	RDA 1	RDA 2
Eigenvalue	1.39	1.18
Proportion explained	0.28	0.24
Cumulative proportion	0.28	0.52
p	0.003	0.005
Environmental variable		
Depth	0.84	-0.54
Sed-As	-0.52	-0.86

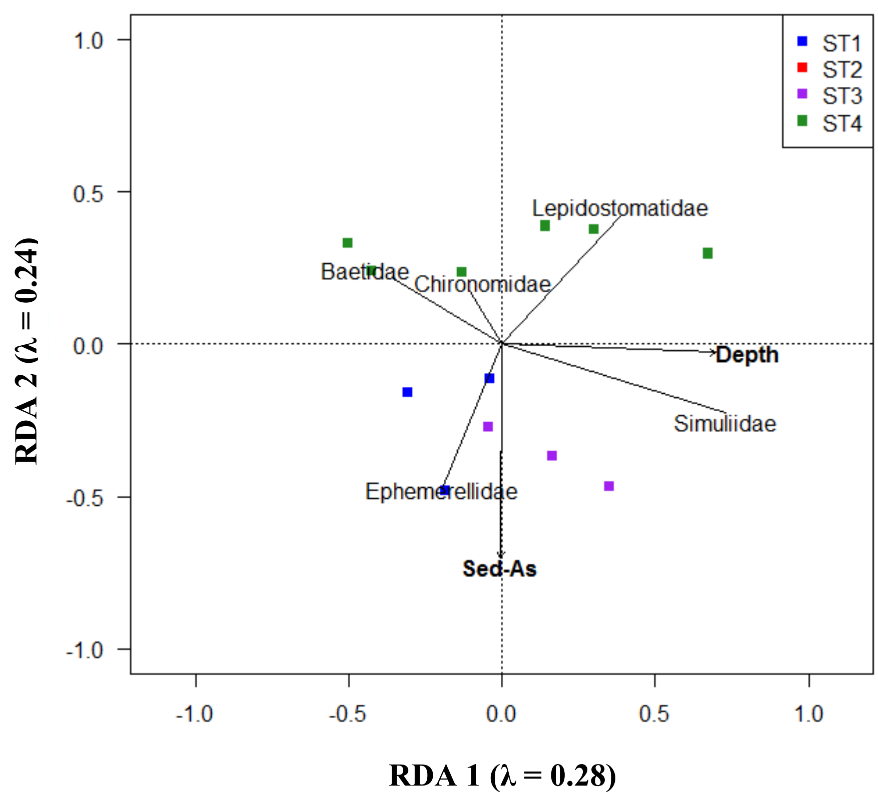


Figure 5.6. Steepbank River redundancy analysis (RDA) triplots showing site scores and associated rock-basket benthic macroinvertebrate family groups, and the two environmental variables that independently explain significant amounts of variation in the benthic macroinvertebrate communities at four sites. λ represents the proportion of variation explained by the RDA axis.

Table 5.9. Summary results of a redundancy analysis (RDA) over five months for the Steepbank River rock-basket benthic macroinvertebrate community. RDA 1 is portrayed with the loading of the selected environmental variable for the axis. The significant *p*-value ($p < 0.05$) for the eigenvalue of the RDA axis is shaded in grey. The following abbreviation is used: total dissolved solids (TDS).

Value	RDA 1
Eigenvalue	1.07
Proportion explained	0.27
Cumulative proportion	0.27
p	0.015
Environmental variable	
TDS	-1.00

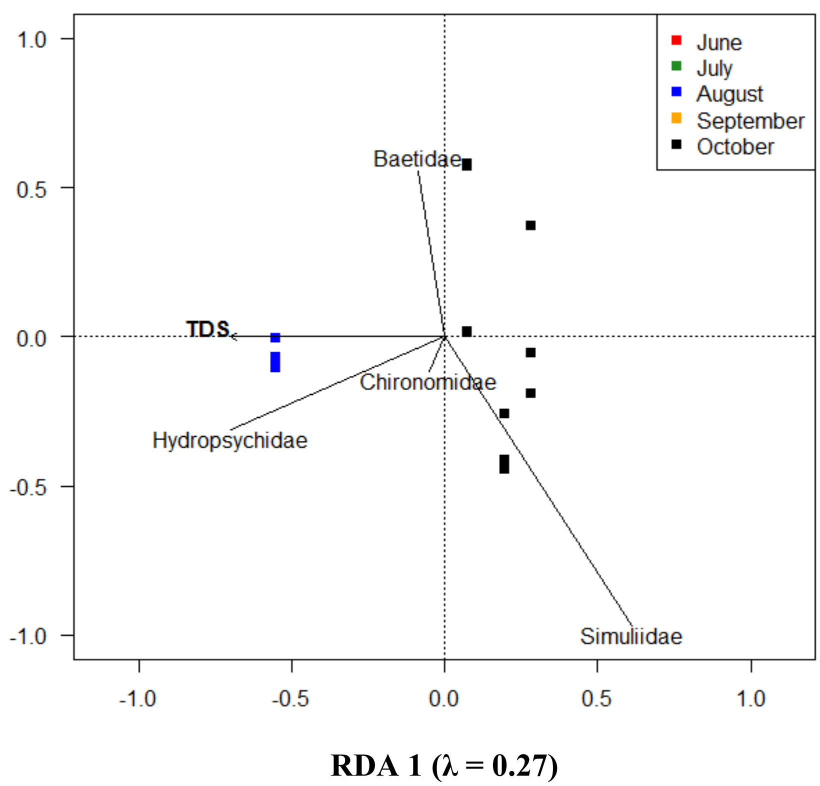


Figure 5.7. Steepbank River redundancy analysis (RDA) triplots showing monthly scores and associated rock-basket benthic macroinvertebrate family groups, and the one environmental variable that independently explains significant amounts of variation in the benthic macroinvertebrate communities across five months. λ represents the proportion of variation explained by the RDA axis.

Ells River

Benthic macroinvertebrate community composition was significantly different among-sites, with the most downstream site (EL1) being significantly different than the most upstream site (EL3). Changes in the relative abundance of *Tvetenia sp.* (Diptera: Chironomidae) had the greatest effect on community structure between-sites, and accounted for 28% of the dissimilarity, with EL3 being more abundant.

Community composition was significantly different among all months. Changes in the relative abundance of *Simulium sp.* had the greatest effect on community structure between June vs. August, September, and October, accounting for 23% of the dissimilarity, for each, with June being most abundant for all comparisons. Changes in the relative abundance of *Tricorythodes minutus* (Ephemeroptera: Leptohyphidae) had the greatest effect on community structure between June vs. July, and accounted for 17% of the dissimilarity, with June being more abundant. Changes in the relative abundance of *Ephemerella sp.* had the greatest effect on community structure between July vs. August, September, and October, accounting for 33%, 41%, and 23% of the dissimilarity, respectively, with July being the least abundant for all comparisons.

Changes in the relative abundance of *Tvetenia* and *Simulium* species had the greatest effect on community structure between August vs. September, and August vs. October, respectively. *Tvetenia sp.* abundance accounted for 15% of the dissimilarity between August vs. September, with September being more abundant, and *Simulium sp.* abundance accounted for 13% of the dissimilarity between August and October, with August being more abundant. Changes in the relative abundance of *Tvetenia sp.* also had the greatest effect on community structure between September vs. October, and accounted for 21% of the dissimilarity, with September being more abundant. There was a significant site x month interaction effect for community structure (Table 5.10). Results from the SIMPER analyses are presented in Appendix C (Tables C.7 and C.8).

Table 5.10. Ells River results from the permutational multivariate analysis of variance (PERMANOVA) and pairwise comparisons for rock-basket benthic macroinvertebrate community composition. Significant *p*-values ($p < 0.05$) for site and monthly differences are shaded in grey.

Parameter	Mean square	Pseudo-*F*	*T*	*p*
Site	5244	3.67		0.003
Month	4940	13.78		0.001
Site x month	1429	3.99		0.001
Residuals	359			
Pairwise comparison				
Site				
EL1 vs EL2			1.46	0.134
EL1 vs EL3			2.83	0.039
EL2 vs EL3			2.02	0.101
Month				
Jun vs Jul			2.86	0.003
Jun vs Aug			3.58	0.001
Jun vs Sep			4.14	0.001
Jun vs Oct			3.48	0.001
Jul vs Aug			4.38	0.003
Jul vs Sep			3.79	0.001
Jul vs Oct			2.80	0.003
Aug vs Sep			3.74	0.001
Aug vs Oct			4.47	0.001
Sep vs Oct			2.50	0.001

The RDA data screening procedure of environmental variables explaining variation in site differences for the rock-basket benthic macroinvertebrate community led to the removal of Ca^{2+}, Depth, BBQ periphyton chlorophyll *a* (BBQ-Chl *a*), TDS, TDN, TSS, fine sediment silver (Sed-Ag), and Cl^- from further analyses. Forward selection with the remaining variables (Temp, DO, TDP, SiO_2^{2-}, SO_4^{2-}, pH, Flow, and Sed-As) identified Temp, Sed-As, pH, SO_4^{2-}, TDP, SiO_2^{2-}, Flow, and DO as environmental variables that explained statistically significant levels of total variation in site differences for community composition. Priority pollutant element concentrations within fine sediments were highly correlated with each other; thus, Sed-As represents general fine sediment metal concentrations. The final RDA model (F = 12.46, $p < 0.001$, 999 permutations) explained 81% of the variation in the site differences for benthic macroinvertebrate community structure. The eigenvalues of the first five RDA axes were statistically significant (axis 1: $\lambda_1 = 0.35$, $p = 0.001$, 999 permutations; axis 2: $\lambda_2 = 0.22$, $p = 0.001$, 999 permutations; axis 3: $\lambda_3 = 0.16$, $p = 0.001$, 999 permutations; axis 4: $\lambda_4 = 0.04$, $p = 0.003$, 999 permutations; axis 5: $\lambda_5 = 0.03$, $p = 0.007$, 999 permutations).

Separation among-sites was primarily along RDA 1, which was loaded positively with gradients in Sed-As, pH, SiO_2^{2-}, and DO. RDA 2 further separated upstream and downstream sites along gradients of SO_4^{2-}, TDP, Flow and associated SiO_2^{2-}, DO and Sed-As concentrations. RDA 3, 4 and 5 also contributed to the separation in site differences; however, considerably less than RDA 1 and 2. Therefore, only RDA 1 and 2 will be examined in this study. In general, higher DO, Sed-As, and SiO_2^{2-} concentrations were associated with the upstream site (EL3), whereas greater pH, Flow, Temp, TDP, and SO_4^{2-} concentrations were associated with mid-reach (EL2) and downstream (EL1) sites (Table 5.11, Figure 5.8).

The RDA data screening procedure of environmental variables explaining variation in monthly differences for the rock-basket benthic macroinvertebrate community led to the removal of Depth, BBQ-Chl *a*, TDS, TSS, TDN, Ca^{2+}, and Sed-As from further analyses. Forward selection with the remaining variables (Temp, DO, TDP, Cl^-, SiO_2^{2-}, SO_4^{2-}, pH, Flow, and Sed-Ag) identified SO_4^{2-}, Temp, Cl^-, pH, TDP, DO, SiO_2^{2-}, and Flow as environmental variables that explained statistically significant levels of total variation in monthly differences for community composition. The final RDA model ($F = 13.13$, $p < 0.001$, 999 permutations) explained 81% of the variation in the monthly differences for benthic macroinvertebrate community structure. The eigenvalues of the first five RDA axes were statistically significant (axis 1: $\lambda_1 = 0.33$, $p = 0.001$, 999 permutations; axis 2: $\lambda_2 = 0.23$, $p = 0.001$, 999 permutations; axis 3: $\lambda_3 = 0.16$, $p = 0.001$, 999 permutations; axis 4: $\lambda_4 = 0.04$, $p = 0.002$, 999 permutations; axis 5: $\lambda_5 = 0.03$, $p = 0.007$, 999 permutations).

Separation among-months was primarily along RDA 1, which was loaded positively with gradients in pH and DO. RDA 2 further separated months along gradients of Temp and Flow. RDA 3, 4 and 5 also contributed to the separation in monthly differences; however, considerably less than RDA 1 and 2. Therefore, only RDA 1 and 2 will be examined in this study. In general, spring months were associated with higher SO_4^{2-}, Flow and Temp, summer/fall months were associated with greater TDP and Cl^-

concentrations, and fall months were associated with larger pH, DO, and SiO_2^{2-} concentrations (Table 5.12, Figure 5.9).

Table 5.11. Summary results of a redundancy analysis (RDA) on three sites for the Ells River rock-basket benthic macroinvertebrate community. RDA 1, 2, 3, 4, and 5 are portrayed with loadings of the selected environmental variables for each axis. Significant *p*-values ($p < 0.05$) for eigenvalues of the RDA axes are shaded in grey. Other than standard abbreviations, the following abbreviations are used: fine sediment arsenic (Sed-As) and total dissolved phosphorus (TDP).

Value	RDA 1	RDA 2	RDA 3	RDA 4	RDA 5
Eigenvalue	2.48	1.55	1.09	0.28	0.20
Proportion explained	0.35	0.22	0.16	0.04	0.03
Cumulative proportion	0.35	0.58	0.73	0.77	0.80
p	0.001	0.001	0.001	0.003	0.007
Environmental variable					
Temp	-0.67	-0.28	-0.09	0.41	-0.14
Sed-As	0.09	0.74	-0.40	-0.14	-0.39
pH	0.56	-0.19	-0.19	-0.27	-0.37
SO_4^{2-}	-0.14	0.13	0.77	-0.52	0.10
TDP	-0.09	0.19	0.57	0.62	0.25
SiO_2^{2-}	0.15	0.46	0.25	0.09	0.76
Flow	-0.68	0.18	-0.11	0.01	0.28
DO	0.43	0.34	-0.41	-0.50	-0.25

Table 5.12. Summary results of a redundancy analysis (RDA) over five months for the Ells River rock-basket benthic macroinvertebrate community. RDA 1, 2, 3, 4, and 5 are portrayed with loadings of the selected environmental variables for each axis. Significant *p*-values ($p < 0.05$) for eigenvalues of the RDA axes are shaded in grey. Other than standard abbreviations, the following abbreviation is used: total dissolved phosphorus (TDP).

Value	RDA 1	RDA 2	RDA 3	RDA 4	RDA 5
Eigenvalue	3.32	2.28	1.64	0.44	0.30
Proportion explained	0.33	0.23	0.16	0.04	0.03
Cumulative proportion	0.33	0.56	0.72	0.77	0.80
p	0.001	0.001	0.001	0.002	0.007
Environmental variable					
SO_4^{2-}	-0.61	-0.69	-0.21	-0.16	-0.17
Temp	-0.45	0.64	-0.07	0.34	0.01
Cl^-	-0.30	-0.54	-0.71	-0.25	-0.13
pH	0.53	-0.09	-0.20	-0.19	-0.51
TDP	-0.25	-0.30	-0.01	0.65	0.59
DO	0.46	-0.18	0.37	-0.47	-0.32
SiO_2^{2-}	-0.04	-0.52	0.25	-0.10	0.72
Flow	-0.55	0.28	0.31	-0.11	0.33

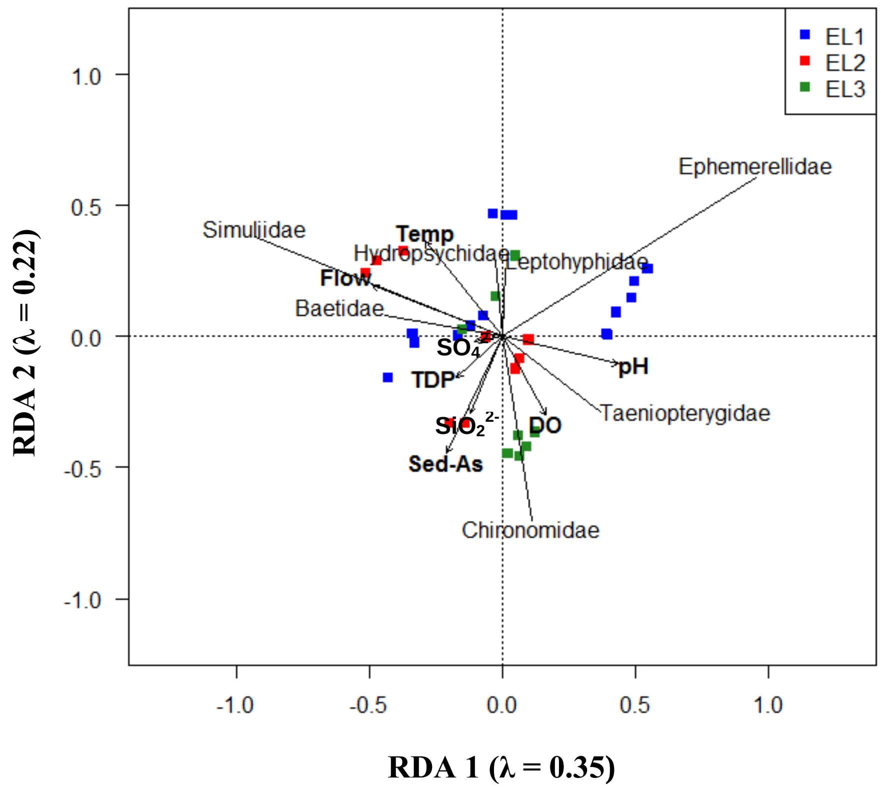


Figure 5.8. Ells River redundancy analysis (RDA) triplots showing site scores and associated rock-basket benthic macroinvertebrate family groups, and the eight environmental variables that independently explain significant amounts of variation in the benthic macroinvertebrate communities at three sites. λ represents the proportion of variation explained by the RDA axis.

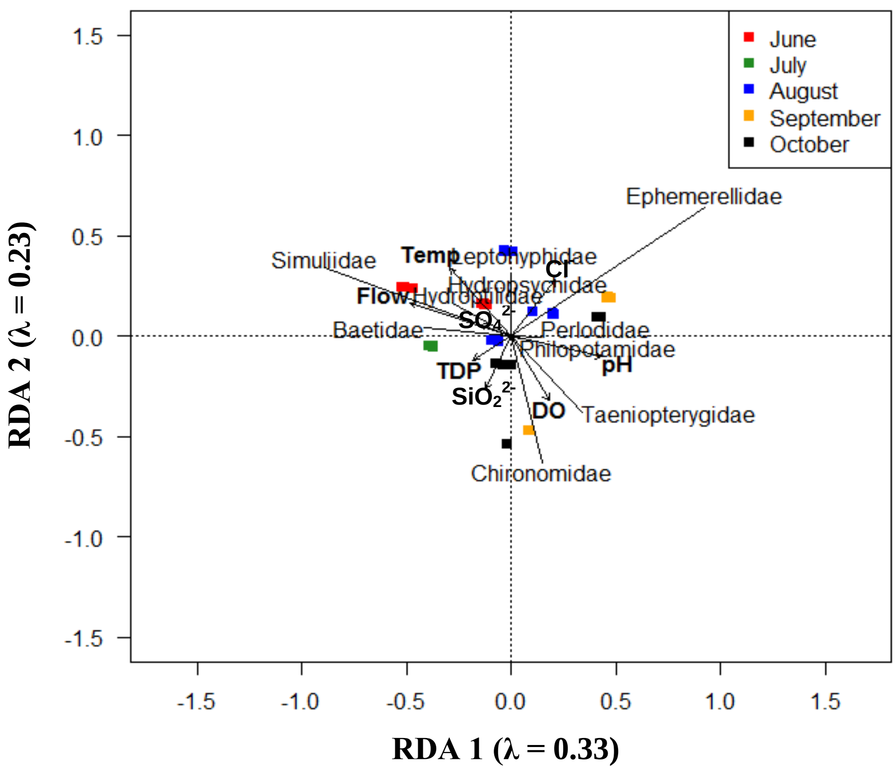


Figure 5.9. Ells River redundancy analysis (RDA) triplots showing monthly scores and associated rock-basket benthic macroinvertebrate family groups, and the eight environmental variables that independently explain significant amounts of variation in the benthic macroinvertebrate communities across five months. λ represents the proportion of variation explained by the RDA axis.

5.3.2 Odonate Mercury Concentration

Steepbank River

Total Mercury

Log THg concentrations were not significantly different among-sites and among-months. There was insufficient sample replication for a site x month interaction effect for Odonate THg.

Methylmercury

Log MeHg concentrations were significantly different among-sites, with the most upstream site (ST4) having significantly lower concentrations than the downstream site

(ST2). Log MeHg concentrations were not significantly different among-months. There was insufficient sample replication for a site x month interaction effect for Odonate MeHg.

Ells River

Total Mercury

Log THg concentrations were significantly different among-sites, with the most upstream site (EL3) having significantly greater concentrations than downstream sites (EL2 and EL1). Log THg concentrations were not significantly different among-months. There was no significant site x month interaction for Odonate THg.

Methylmercury

Log MeHg concentrations were significantly different among-sites, with EL3 having significantly greater concentrations than EL1. Log MeHg concentrations were significantly lowest in June. There was a significant site x month interaction for Odonate MeHg.

Table 5.13. Summary table of Steepbank and Ells River Odonate mercury concentration variables analyzed with a two-way mixed-effects ANOVA, which included a) site and, b) month. Significant *p*-values ($p < 0.05$) for site differences are shaded in grey. Upstream (U/S) to downstream (D/S) changes in variable concentrations are indicated with "+" for increasing and "-" for decreasing, and an interaction column indicates any significant site x month interaction effects. The following abbreviations are used: total mercury (THg), and methylmercury (MeHg).

Para-meter	**Fig #**	**Site Differences**	***p*-value**	**U/S-D/S Changes**	**Monthly Differences**	***p*-value**	**Interaction Effect**
Steepbank River							
Log THg	5.10	No	> 0.05	No	No	> 0.05	No rep.
Log MeHg	5.11	ST4 < ST2	0.004	+	No	> 0.05	No rep.
Ells River							
Log THg	5.12	EL3 > EL2 = EL1	< 0.01	-	No	> 0.05	> 0.05
Log MeHg	5.13	EL3 > EL1	0.003	-	Jun < Jul, Aug, Oct	< 0.01	0.017

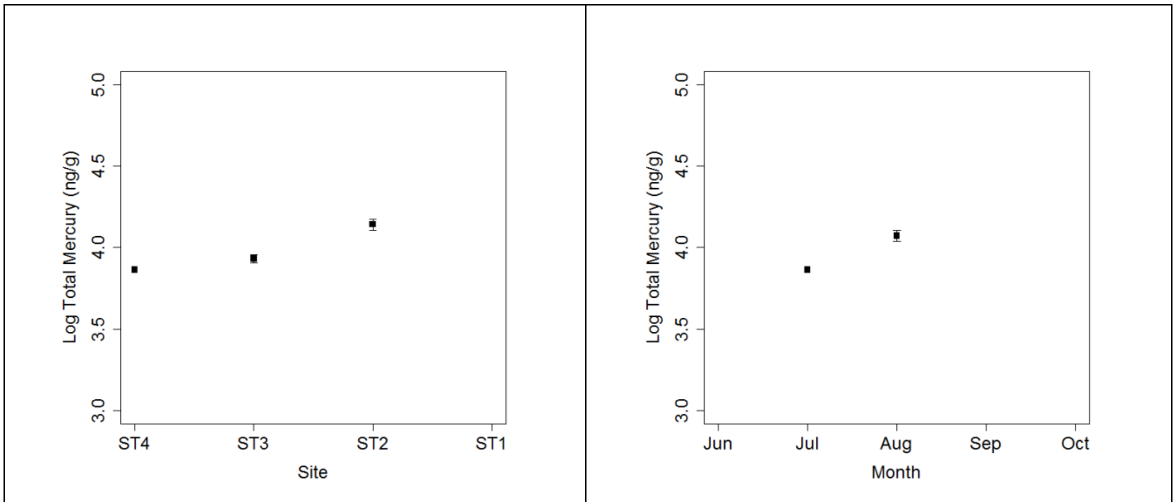


Figure 5.10. Mean concentration of Steepbank River Odonate log transformed total mercury (log THg; ng/g) by site (left) and over months (right). There were no significant differences among-sites and among-months. There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.

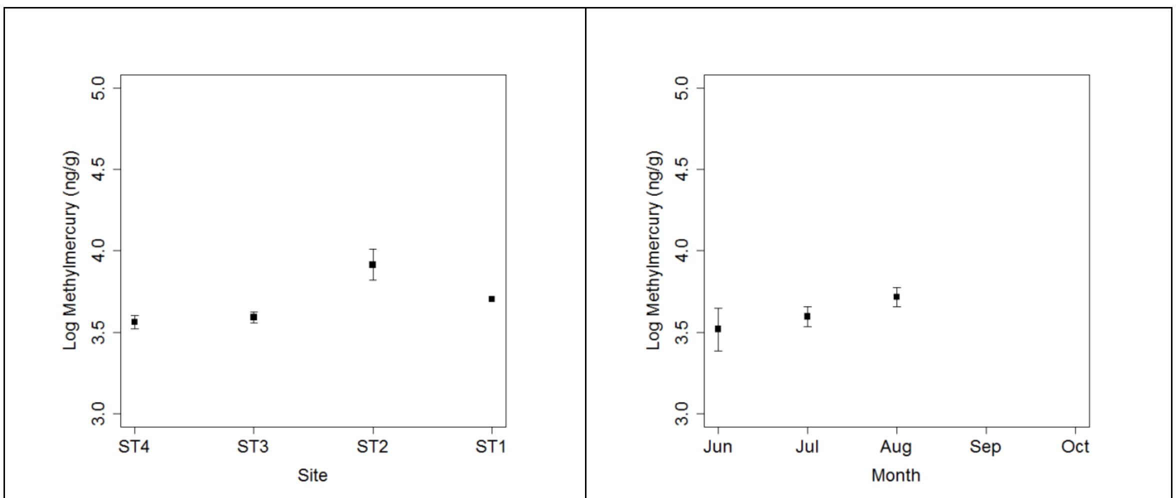


Figure 5.11. Mean concentration of Steepbank River Odonate log transformed methylmercury (log MeHg; ng/g) by site (left) and over months (right). ST4 was significantly lower than ST2 ($p = 0.004$). There were no significant differences among-months. There was insufficient sample replication for a site x month interaction effect. *Error bars represent the standard error of the mean.

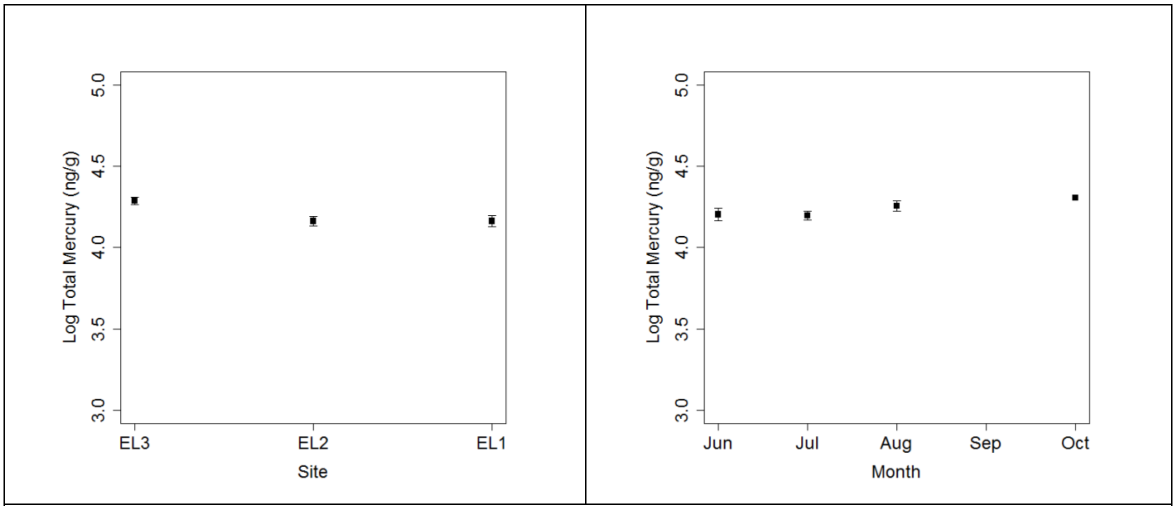


Figure 5.12. Mean concentration of Ells River Odonate log transformed total mercury (log THg; ng/g) by site (left) and over months (right). EL3 was significantly greater than EL2 (*p* = 0.003), and EL1 (*p* = 0.011). There were no significant differences among-months. There was no significant site x month interaction.
*Error bars represent the standard error of the mean.

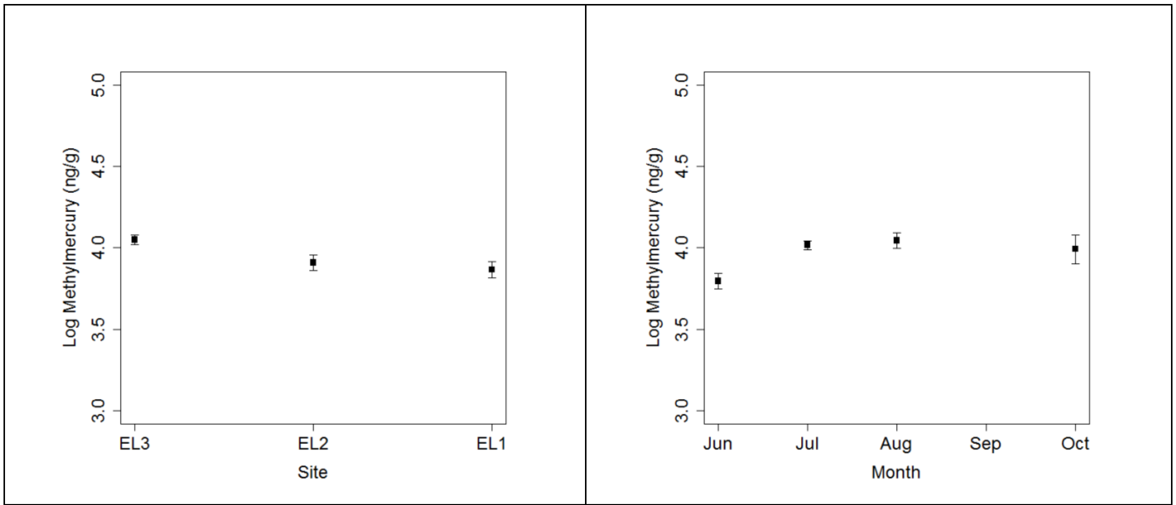


Figure 5.13. Mean concentration of Ells River Odonate log transformed methylmercury (log MeHg; ng/g) by site (left) and over months (right). EL3 was significantly greater than EL1 (*p* = 0.003). Log MeHg concentration was significantly lower in June than July, August and October (*p* < 0.01). There was a significant site x month interaction (*p* = 0.017). *Error bars represent the standard error of the mean.

5.4 Discussion

5.4.1 Longitudinal and Seasonal Variation in Benthic Macroinvertebrate Structure

Three-Minute Kick Net Invertebrates

Human-induced changes in composition were not detected as the observed community shifts were related to natural longitudinal variation for both the Steepbank and Ells Rivers. Changes in the relative abundance of the surface-dwelling Ephemeroptera species, *B. tricaudatus*, had the greatest effect on community structure within the Steepbank River, with upstream sites (ST4 and ST3) having a significantly greater abundance than the most downstream site (ST1). Previous studies on benthic macroinvertebrate community composition within the Steepbank River documented an overall high abundance of *Baetis spp.* from upstream to downstream, with local species expected to be multivoltine (Barton 1980a). Barton and Wallace (1979) observed physical and chemical effects of bitumen deposits did not negatively influence Ephemeroptera (mayflies) within rivers of the AOSR (Barton and Wallace 1979). Therefore, downstream declines in *B. tricaudatus* abundance found in this study were likely attributed to natural perturbations, such as high discharge events and the large sediment slump which occurred on the Steepbank River during the 2012 sampling season.

Changes in the relative abundance of *Microspectra sp*. had the greatest effect on community structure within the Ells River, with the most upstream site (EL3) having a significantly greater abundance than the most downstream site (EL1). Chironomidae are generally considered tolerant to metal-contaminated rivers (Clements 1994). In this study, the upper Ells River basin contained elevated fine sediment metal concentrations; thus, sensitive taxa would likely not be distributed in this location, and tolerant species would dominate. *Microspectra sp.* have also been documented as a relatively metals-sensitive species within the Chironomidae family (Resh and Unzicker 1975; Clements et al. 2000; McHale et al. 2008), which suggested *Microspectra sp*. may be tolerant of the naturally-sourced metals in the upstream Ells River.

Seasonally, spring was significantly different from summer for the Steepbank River, and summer was significantly different from fall for both rivers in benthic macroinvertebrate community composition. Changes in the relative abundance of *B. tricaudatus* contributed the greatest to the dissimilarity in community structure among-seasons for the Steepbank River. This further demonstrated their multivoltine life history and prevalence in this river, despite hydrological extremes during the 2012 sampling season. Changes in the relative abundance of *Microspectra sp.* contributed the greatest to the dissimilarity in community structure among-seasons for the Ells River, by being most abundant in summer. Fine sediment metal concentrations within the Ells River increased in late summer, potentially reflecting *Microspectra sp*. being tolerant of naturally elevated metal concentrations.

Rock-Basket Invertebrates

Anthropogenic catchment-scale disturbances were not detected as the observed community shifts were related to natural longitudinal variation for both the Steepbank and Ells Rivers. Changes in the relative abundance of the rheophilic, surface-dwelling mayflies, *Baetis spp*., had the greatest effect on community structure within the Steepbank River, with the most upstream site (ST4) having a significantly greater abundance than the most downstream site (ST1). Studies by Barton (1980a) and Barton and Wallace (1979) demonstrated overall high abundance of *Baetis spp.* within the Steepbank River, with bitumen deposits not negatively influencing their abundance. Thus, greatest longitudinal differences were likely attributed to natural perturbations, such as the large sediment slump and extreme discharge events which occurred over the 2012 sampling season.

ST4 was also significantly different from the mid-reach site (ST3), which was inside the OS deposit and outside of mining development, with ST3 having a greater abundance of *Simulium sp.*, or black fly larvae. In a study by Barton and Wallace (1979), surface-dwelling *Baetis spp.* and *Simulium sp.* were found to be more abundant at sites within the OS deposit, in comparison to burrowing or negatively phototropic taxa. Observations from their study attributed differences to the physical structure of the substrate rather than toxicity of the OS deposit. Barton and Wallace (1979) also suggested that because *Simulium sp.* have a different feeding mechanism in comparison to other filter feeders within the Steepbank River, they may be less susceptible to fouling by bitumen particles, allowing them to thrive in the OS deposit in the absence of competition from Trichoptera, or caddisfly larvae.

Changes in the relative abundance of *Tvetenia sp.* had the greatest effect on community structure within the Ells River, with the most upstream site (EL3) having a significantly greater abundance than the most downstream site (EL1). *Tvetenia sp.* have also been documented to be a relatively metals-sensitive Chironomidae species (Gower et al. 1994; Clements et al. 2000; Ruse et al. 2000). This suggested a natural gradient in metal concentrations was potentially influencing longitudinal community composition within the Ells River.

Seasonally, summer was significantly different from fall for the Steepbank River, whereas all-seasons were significantly different for the Ells River in benthic macroinvertebrate community composition. Rock-basket retrieval success for the Steepbank River only occurred in August and October, with changes in the relative abundance of *Baetis spp.* contributing the greatest to the dissimilarity in community structure. Community composition for all months was significantly different within the Ells River, which had greater retrieval success than the Steepbank River. Rock-baskets were only deployed for one-month intervals; therefore, distinct colonizations of benthic macroinvertebrates were potentially established depending on monthly environmental conditions as well as life history traits, as explained by Cairns (1982) and Mackay (1992).

Historical studies on tributaries of the Athabasca River have reported *Ephemerella sp.* to be most abundant in mid-July, with changes in the relative abundance contributing greatly to the dissimilarity in community structure from summer to fall. *T. minutus* has been shown to be a fast seasonal/summer species in northeastern Alberta (Clifford 1969); hence, the dissimilarity in abundance from spring to summer. Barton (1980b) also observed *Simulium sp.* to be abundant in tributaries of the Athabasca River during summer months, which potentially explained changes in the relative abundance of *Simulium sp.* contributing to the dissimilarity in community structure from summer to fall. Moreover, *Tvetenia sp.* was abundant in early fall, which could possibly be related to their tolerance of the increase in natural fine sediment metal concentrations in late summer.

Overall, longitudinal and seasonal changes in benthic macroinvertebrate community structure for both the three-minute kick net and rock-baskets were most likely associated with natural variation for both the Steepbank and Ells Rivers. Responses in the benthic macroinvertebrate community from OS development were not directly evident. Moreover, relating environmental variables to community composition can further assist in discriminating possible natural and anthropogenic non-point source disturbances.

5.4.2 Environmental Variables Explaining Patterns in Community Composition

Three-Minute Kick Net Invertebrates

pH and TDP were the most influential environmental variables contributing to benthic macroinvertebrate community structure within the Steepbank River. Higher pH values were mostly associated with downstream sites, which illustrated a positive relationship with Ephemerellidae, Heptageniidae, and Empididae, and a fairly negative relationship with Baetidae. The substrate in the lower reaches of the Steepbank River has been characterized as analogous to bedrock, composed primarily of Devonian limestone (Barton and Wallace 1979), potentially creating an alkaline environment. Seasonal effects may have also contributed to RDA 1 being loaded negatively with gradients in pH and TDP, with most of the sample points to the left of the RDA 1 axis being sites sampled in August.

Furthermore, a historical study on benthic macroinvertebrates in the Athabasca River identified Ephemerellidae, Heptageniidae, and Empididae as dominant taxa residing in bedrock environments, which was attributed to the habitat stability of the substrate (Barton 1980b). Most of the invertebrates collected from bedrock environments in the Athabasca River were also found in stony tributary systems, such as the Steepbank River as documented by Barton and Wallace (1980). The negative association of Baetidae at the downstream sites was likely attributed to other factors aside from the natural OS deposit effects, such as large discharge events as well as the sediment slump event which occurred in the 2012 sampling season.

Upstream sites on the Steepbank River were mostly associated with higher TDP concentrations, which illustrated a positive relationship with the remaining invertebrate families. Nutrients are important for ecosystem-processes, but can be limiting in many temperate lotic ecosystems; thus, they are a crucial determinant in benthic macroinvertebrate distribution (Elwood et al. 1981). Furthermore, in a study by Barton and Wallace (1979), the documented number of taxa collected within the Steepbank River was consistently greater at the upstream sites, compared to downstream sites, even before large-scale OS mining development. They attributed this to the effects of eroding bitumen on natural substrate reducing the habitat availability for many species of benthic macroinvertebrates.

Cl^- concentrations and pebble substrate were the most influential environmental variables on benthic macroinvertebrate community structure in the Ells River. Cl^- was associated with the most downstream site which showed a positive relationship with Ephemerellidae, and a negative relationship with the remaining benthic macroinvertebrate families. Bulk water quality results from this study also demonstrated a significant increase in Cl^- from upstream to downstream within the Ells River. Chemical weathering and ion leaching from soils and rocks on the watershed could have potentially increased Cl^- concentrations in the downstream river environment during runoff (Raymond et al. 2008). Weathering of OS deposits creates increased Cl^- concentrations in the lower reaches of tributaries, specifically in the thicker western part of the formation (Gorrell 1974).

Brackish groundwater upwelling also contributes Cl^- to surface water in the lower reaches of tributaries in the AOSR (Sekerak and Walder 1980; Hackbarth 1981). Moreover, a distinct upstream to downstream pattern in Cl^- has occurred since land-clearing activities began on the Ells River basin, indicating land use disturbance could have possibly attributed to downstream increases over time (Headley et al. 2005). Ephemerellidae have been reported to be abundant in bedrock, non-depositional areas in the Athabasca River (Barton and Wallace 1980), with bedrock-like substrate also observed in the lower reaches of the Ells River. For these reasons, the association of Cl^- and Ephemerellidae at the downstream Ells River site was observed.

The most upstream site on the Ells River (EL3) was associated with a greater percentage of pebble substrate, known to be favourable to many invertebrate families. Traditionally, higher density and diversity in benthic macroinvertebrate community structure have been reported in cobble and pebble lotic environments, while sand and silt-dominated substrates have lower densities and diversities (Erman and Erman 1984; Quinn and Hickey 1990). Overall, environmental variables explaining variation in benthic macroinvertebrate community structure within the Ells River was largely driven by substrate composition. Higher abundance of Ephemerellidae was observed in the downstream environment containing a consolidated and impervious surface related to higher bitumen content resembling a bedrock-like environment, and greater invertebrate diversity in the upstream pebble dominated environment. Furthermore, seasonal effects may have been associated with the distribution of Simuliidae at EL2 and EL1, with sample points to the left of the RDA 2 axis only being sampled in summer months.

Seasonally, TDS was the most influential environmental variable contributing to benthic macroinvertebrate community structure in the Steepbank River. During summer, TDS was positively associated with a majority of the invertebrate families including: Heptageniidae, Leptohyphidae, Nemouridae, Hydropsychidae, Athericidae, Chironomidae, Simuliidae, Sperchontidae, Torrenticolidae, Enchytraeidae, and Naididae, while negatively associated with the remaining families: Ephemerellidae, Caenidae, Lepidostomatidae, Brachycentridae, Pteronarcyidae, and Baetidae. Bulk water chemistry also showed a significant increase in TDS concentrations in the summer for the Steepbank River, which possibly resulted from high summer rainfall on the basin transporting sediments from the surrounding landscape into the river.

Elevated TDS concentrations are typically considered to have negative consequences on sensitive benthic macroinvertebrate taxa, such as EPT (Pond et al. 2008; Timpano et al. 2010). In agreement with this, the invertebrates showing a negative relationship to high TDS concentrations in this study were only EPT taxa, while the majority of the remaining families showing a positive association with TDS were taxa considered relatively insensitive to water quality degradation (Roline 1988; Di Sabatino et al. 2000; Ndaruga et al. 2004). Enhanced TDS concentrations entering rivers during seasonal events have also been linked to human land use activities on disturbed watersheds (Walling 2000; Bruns 2005).

SO_4^{2-}, Peri-AFDM, and pebble substrate were the most influential environmental variables contributing to benthic macroinvertebrate community structure over-seasons for the Ells River. SO_4^{2-} was positively associated with spring, with Chironomidae having a minor positive association. Tolerant taxa such as, Chironomidae, have been shown to be prevalent in lotic ecosystems higher in SO_4^{2-} concentrations (García-Criado et al. 1999; Feio et al. 2006). SO_4^{2-} was negatively associated with summer, which was related to the following benthic macroinvertebrate families: Leptohyphidae, Philopotamidae, Simuliidae, Empididae, and Naididae.

Bulk water chemistry from this study also demonstrated a significant decrease from spring to summer in SO_4^{2-} concentrations in the Ells River, which was attributed to the dilution effect with the onset of spring freshet and in-flux of freshwater into the ecosystem, as described by Mann et al. (2012). Hackbarth (1981) also observed SO_4^{2-} concentrations to be greatest in spring from shallow groundwater upwelling in tributaries of the AOSR. Localized toxicological effects have been observed with moderately high levels of SO_4^{2-} in lotic ecosystems, which destroy the gill surface of some macroinvertebrates, or interferes with their respiratory efficiency and osmoregulation, reducing species diversity (Parsons 1968).

Peri-AFDM and pebble substrate were associated with summer and fall, with Peri-AFDM having a positive relationship with: Ephemerellidae, Torrenticolidae, and Ancylidae, and pebble substrate having a positive relationship with: Baetidae, Hydropsychidae, Gomphidae, and marginally with Chironomidae. Heterotrophic production rates are typically greater in summer with warmer temperatures enabling biofilm to increase, and then decrease in late fall by means of limited bacterial activity from lower water temperatures (Bott et al. 1985; Boulêtreau et al. 2006). Many species of Ephemerellidae and Ancylidae utilize a scraping feeding mechanism, and would distribute themselves in an environment containing higher periphyton biomass (Hauer and Lamberti 2011). Lotic ecosystems with greater periphyton abundance also support richer invertebrate species diversity (Death 2002), creating greater food availability for predacious Torrenticolidae (Di Sabatino et al. 2000).

Substrate composition of the Ells River was only characterized in the low-flow period of summer and early fall; hence, the positive association with these seasons. Numerous studies have shown substrate type to be a major factor in macroinvertebrate distribution (Wright et al. 1984; Richards et al. 1993; Ruse 1996). Many species of Hydropsychidae and Baetidae have been documented to be more abundant within loose pebble and cobble environments because they provide greater areas of refugia from predation and disturbance, compared to cement-embedded substrates (Flecker and Allan 1984; Mishra and Nautiyal 2011).

Furthermore, Gomphidae, or dragonfly larvae, have been associated with a range of substrate types. Substrate size is important in habitat selection by burrowing aquatic insects, such as dragonflies, but little information exists on the burrowing behaviour and factors affecting habitat selection by the species within the Gomphidae family, included in this study (Gibbs et al. 2004). Utilizing rock-baskets to investigate benthic macroinvertebrate community composition eliminated the spatial and temporal variation in substrate composition, by providing a standardized substratum.

Rock-Basket Invertebrates

Depth and Sed-As were the most influential environmental variables contributing to variation in benthic macroinvertebrate community structure within the Steepbank River. Sed-As concentrations were primarily associated with downstream sites, which illustrated a positive relationship with Ephemerellidae. Sed-As concentrations also had a negative association with the most upstream site (ST4), and the respective invertebrate families, Baetidae, Lepidostomatidae, and Chironomidae.

Possible mechanisms for the downstream association of Sed-As included the transportation of metal-concentrated fine sediments downstream from OS mining activities (Axtmann and Luoma 1991), the close proximity of the mouth of the Steepbank River to OS upgrading facilities, which deposits aerial particulates on to the surrounding landscape (Kelly et al. 2009), as well as the lower reaches being situated within the OS geological deposit (Maclock et al. 1997). Previous observations of benthic macroinvertebrate families in the Steepbank River documented Ephemerellidae residing in the lower reaches (Barton and Wallace 1979). High abundance of Ephemerellidae downstream was unanticipated, with this family considered metal-sensitive (Clements 1994), suggesting placement of artificial substrates within the surrounding bedrock-like environment could have potentially influenced rock-basket benthic macroinvertebrate community composition in tributaries of the AOSR.

Depth of the rock-basket in the Steepbank River was mostly associated with Simuliidae distribution. Water depth has been repeatedly documented to be an important factor in artificial substrata placement for larval simuliid colonization (Lewis and Bennett 1974; Ross and Merritt 1978; Gersabeck and Merritt 1979). Therefore, the association between Simuliidae abundance and water depth was a product of the experimental design. Depth may have also been associated with fine sediment material deposited on the scour pads through changes in tractive forces which transports the sediments onto the sediment traps. Seasonal effects could have contributed to RDA 1 being loaded positively with a gradient in depth, with sample points to the right of axis RDA 1 only from the month of October.

Environmental variables explaining variation in benthic macroinvertebrate community structure within the Ells River were associated with natural factors. For example, higher pH values were associated with the downstream distribution of Ephemerellidae, attributed to the limestone bedrock-like habitat in the lower reaches of the Ells River. Greater Sed-As concentrations were also associated with the upstream distribution of Chironomidae. This was possibly attributed to the high metal concentrations observed in the upper Ells River basin, as well as the metal-tolerance of most Chironomidae taxa (Clements et al. 2000).

TDS was the most influential environmental variable on benthic macroinvertebrate community structure over-seasons for the Steepbank River. TDS was associated with summer's frequent rainfall events that elevated sediment runoff, resulting in a positive relationship with Hydropsychidae. Usually, high TDS concentrations are negatively associated with EPT taxa (Pond et al. 2008; Timpano et al. 2010); however, Hydropsychidae have been found in environments with high TDS loadings, indicating their potential ability to endure occasional seasonal TDS increases (Canton and Ward 1981). Site effects likely contributed to Hydropsychidae distribution, with all sample points to the left of RDA 1 axis only from the most upstream site (ST4). Therefore, Hydropsychidae abundance was most likely associated with the upstream site compared to being associated with seasonal TDS increases.

Rock-basket retrieval success was comparably greater for the Ells River, with environmental variables associated with natural variation explaining benthic macroinvertebrate community structure over-seasons. For example, Hydropsychidae abundance was primarily associated with increased water temperature and flow velocity during spring, following river ice break-up and freshet. Multiple studies have identified the importance of flow velocity and temperature on net-spinning hydropsychid larvae. A general pattern of response has been reported, in which the percentage of larvae spinning nets will decrease with a decrease in flow velocity, and increase with an increase in temperature (Philipson 1969; Philipson and Moorhouse 1974; Hauer and Stanford 1982).

When relating water quality parameters from single time grab samples to community structure, it should be acknowledged that they may not be reflective of the invertebrate community structure from past and present conditions (Hauer and Lamberti 2011). Furthermore, confounding factors affect benthic macroinvertebrate communities as a result of intersite habitat and seasonal variation (Hynes 1970; Peeters et al. 2000). In general, environmental variables associated with natural variation in both the Steepbank and Ells Rivers contributed the greatest to benthic macroinvertebrate community composition for both sampling methods. Moreover, the effectiveness of multiple benthic macroinvertebrate sampling methods can be evaluated to determine whether longitudinal and seasonal responses of benthic macroinvertebrates were method dependent when relating macroinvertebrate and environmental data.

5.4.3 Comparison of Benthic Macroinvertebrate Sampling Techniques

To determine whether responses of benthic macroinvertebrates to non-point source disturbances were discriminated based on sampling method, three-minute kick net and rock-basket invertebrate communities were qualitatively compared among-sites and seasons. In general, longitudinal changes within the Steepbank River for both sampling methods were similar in benthic macroinvertebrate structure, with upstream sites being significantly different than downstream sites. Seasonal differences were more apparent within the three-minute kick net benthic macroinvertebrate community; moreover, the reliability of rock-basket retrieval limited sample collection to only two-months for the Steepbank River. *Baetis spp.* contributed the greatest to site and seasonal differences for both sampling methods, illustrating the consistency in results between methods.

Longitudinal changes in community structure within the Ells River for both sampling methods were comparable, with the upstream site (EL3) significantly different than the downstream site (EL1). Seasonal differences were more distinct within the rock-basket benthic macroinvertebrate community, suggesting rock-baskets were potentially more capable of detecting seasonal variation. The two Chironomidae species which contributed the greatest to site and seasonal differences for both sampling methods have similar tolerances to disturbance, illustrating a congruency in results between sampling methods.

Environmental variables explaining longitudinal variation for Steepbank River three-minute kick net benthic macroinvertebrate communities included a positive association with pH downstream, contributing to the distribution of Ephemerellidae, Heptageniidae, and Empididae. Rock-baskets were associated with Sed-As concentrations and water depth downstream, which contributed to the distribution of Ephemerellidae and Simuliidae. Both sampling methods were consistent at identifying the downstream distribution of Ephemerellidae; however, different environmental variables were selected to explain variation in the benthic macroinvertebrate community. Moreover, TDS was the only environmental variable which explained seasonal differences, demonstrating a consistency in seasonal responses for both methods.

Environmental variables explaining longitudinal variation for Ells River three-minute kick net benthic macroinvertebrate communities included a positive association with Cl^- downstream, which contributed to the downstream distribution of Ephemerellidae. Rock-basket samples also identified Ephemerellidae as the only invertebrate family positively associated with the downstream site. Moreover, the rock-basket benthic macroinvertebrate community was associated with a multitude of additional physico-chemical variables (Temp, Sed-As, pH, SO_4^{2-}, TDP, SiO_2^{2-}, Flow, and DO), which were not associated with three-minute kick net invertebrates. Therefore, both methods were similar in illustrating among-site differences in community structure; however, the environmental variables explaining this variation were distinct between methods.

Environmental variables explaining seasonal differences for Ells River benthic macroinvertebrate communities highlighted SO_4^{2-} to be associated with spring for both sampling methods. SO_4^{2-} was attributed to Chironomidae distribution in the natural substrate, and Simuliidae and Baetidae abundance for the rock-basket artificial substrate. Moreover, the rock-basket benthic macroinvertebrate community was associated with several physico-chemical variables (Temp, Cl^-, pH, TDP, DO, SiO_2^{2-}, and Flow), which were not related to three-minute kick net invertebrates.

Advantages to employing artificial substrates to assess benthic macroinvertebrate community composition in this study included the increased capability in comparing community structure among-sites and seasons through the standardization in substrate shape and size. The ceramic BBQ briquettes likely produced more uniform current patterns contributing to greater success of reproducibility in invertebrate colonization, as described by Tuchman and Stevenson (1980). Confounding factors from habitat differences, specifically OS deposit effects on natural substrate, were minimized by providing a standardized microhabitat. Standardized sampling in the field was also accomplished by eliminating subjectivity in sample collection technique, which is difficult to achieve when numerous three-minute kick nets are being performed at multiple sites over various seasons by different field crew. Rock-basket sample collection also required less skill and training than direct sampling of natural substrates.

Disadvantages to utilizing rock-basket artificial substrates included the requirement of two trips to both deploy and retrieve the device. This is in comparison to the three-minute kick net where only one trip is necessary (Cairns 1982). Another disadvantage was their susceptibility to being lost or damaged over the deployment period. Poor rock-basket retrieval success on the Steepbank River created challenges for interpreting the effects of natural and anthropogenic disturbances with large gaps in the dataset. Furthermore, rock-basket benthic macroinvertebrate communities may not be fully representative of the benthic assemblage at a site if the artificial substrate offers different microhabitats than those available in the natural substratum. Artificial substrates often selectively sample certain taxa, misrepresenting relative abundances of these taxa in the natural environment (Cover and Richard 1978).

A critical difference between three-minute kick net and rock-basket sampling techniques was replication. Replication is crucial for producing a robust dataset in riverine ecosystem assessments (Stark 1993). Therefore, three rock-baskets were deployed at each site during each sampling month, compared to a single three-minute kick net. Typically, three or four artificial substrate samplers have been considered sufficient to give a representative benthic macroinvertebrate community sample (Mason et al. 1973; De Pauw et al. 1986).

In general, longitudinal and seasonal responses in benthic macroinvertebrate community composition were comparable between the three-minute kick net and rock-basket methods for both the Steepbank and Ells Rivers. However, the drivers explaining variation in invertebrate communities were method dependent. This variation was most likely attributed to the different variables included in the analysis of each method; moreover, comparing sampling methods provided greater insight into environmental variables associated with benthic macroinvertebrate community structure. For example, substrate composition was only included in the three-minute kick net samples and fine sediment metal concentrations were only used to explain rock-basket communities.

Water quality parameters attributed to community composition for both methods were analyzed from the same sample. Single water quality samples may not be reflective of monthly colonizations of benthic macroinvertebrates; therefore, parameters may have been over-fitted during analysis of relating Ells River rock-basket environmental variables to community composition, as explained by Yee (2006). Overall, the influence of eroded OS deposits on the natural substratum in the lower reaches was identified as a potential contributor to changes in community structure; therefore, to exclusively assess any effects of anthropogenic non-point source perturbations on benthic macroinvertebrate communities in tributaries of the AOSR, artificial substrata are recommended as a supplementary method in combination with traditional sampling techniques.

5.4.4 Bioaccumulation of Mercury in Odonates

Odonate MeHg concentrations were significantly different among-sites within the Steepbank River, with an increase in concentration from upstream to downstream. This was potentially attributed to inputs of non-point source contaminants from catchment-scale disturbances by OS mining activities. Possible sources of aerial contaminants on the Steepbank River basin included: nearby bitumen upgrading facilities, vehicle emissions, volatilization from tailings ponds, and blowing dusts from open pit mines, exposed coke piles, and deforested areas (Kelly et al. 2010; Kirk et al. 2014). Moreover, increased Hg concentrations were also possibly associated with naturally sourced metals from bitumen deposits in the McMF within the lower reaches of the Steepbank River (Maclock et al. 1997).

Odonate THg and MeHg concentrations were significantly different among-sites within the Ells River, with a decrease from upstream to downstream. In this study, relatively high metal concentrations were found in fine sediments at the upstream sites of the Ells River. Increased THg and MeHg concentrations attributed to OS deposits in the lower reaches of the Ells River was not apparent, suggesting either the concentrations in the upper basin were relatively greater or the OS deposit was not a natural source of Hg in tributary ecosystems of the AOSR.

Odonate nymphs, specifically Anisoptera, are predatory invertebrates feeding on primary consumers, such as Chironomids, Baetids and Atyids (Bunn and Boon 1993). The upstream sites on the Ells River contained greater invertebrate diversity, allowing greater prey diversity for Anisoptera. THg and MeHg could have potentially accumulated in a variety of primary consumers, creating greater potential for higher concentrations in the secondary consumers. Thus, THg and MeHg in the downstream environment may have been less bioavailable within the Anisoptera's food source for evident bioaccumulation to have occurred, as explained by Zizek et al. (2007).

Seasonal differences in Odonate THg and MeHg concentrations were not observed within the Steepbank River. Increased loadings during seasonal events, such as snow-melt and summer rainfall, were hypothesized to demonstrate seasonal variation in Hg concentrations, as observed in Kelly et al. (2010) and Kirk et al. (2014). Comparatively, Odonates within the Ells River contained significantly lower MeHg concentrations in spring, compared to summer and fall. This was potentially attributed to spring freshet, causing an in-flux of freshwater into the ecosystem from river ice break-up and increased dilution (Mann et al. 2012). Furthermore, the less disturbed Ells River basin should contain fewer Hg particulates deposited on the snowpack during winter, reducing the concentrations entering the stream during snow-melt (Kelly et al. 2010).

In this study, abundance of sensitive invertebrate species declined and tolerant species increased downstream in the Steepbank River, while MeHg concentrations also increased. Chironomidae species which were relatively tolerant of naturally elevated metal concentrations dominated at the upstream Ells River sites, where greater Hg concentrations were observed. Therefore, Hg contamination on the benthic macroinvertebrate community could be another possible mechanism, in conjunction with natural perturbations, influencing longitudinal changes in community composition which requires further investigation.

Additional studies have also assessed ecotoxicological impacts of sediment exposures on fathead-minnow embryo-larval survival, with samples collected from all sites in this study during 2012. The two downstream sites on the Steepbank River, ST2 and ST1, were the only sites observed to be toxic on embryo-larval survival at 1 g/L (Parrott, J. L. personal communication 2014). Therefore, contaminant-effects on aquatic biota in the Steepbank River were potentially related to OS development, and not the OS bitumen deposit.

Overall, THg and MeHg concentration patterns in aquatic biota did not fully support previous studies investigating the aerial deposition patterns in the AOSR. This present study showed elevated MeHg concentrations in the downstream environment of the Steepbank River, which was also observed in both water and snowpack samples. Furthermore, Odonate Hg concentrations were not significantly greater in the spring for either the Steepbank or Ells Rivers, suggesting any *in situ* pulse of contaminants during spring freshet was not detected within the benthic macroinvertebrate community. The Ells River contained greater Odonate THg and MeHg concentrations in the upper basin, a pattern not seen from water and snowpack samples in previous studies by Kelly et al. (2010) and Kirk et al. (2014). Therefore, this study highlighted the complexity in determining sources and pathways of disturbance from natural and anthropogenic non-point source perturbations, as well as the necessary link between the physico-chemical environment and its influence on the biological community.

5.5 Conclusions

Multi-integrative and multivariate approaches utilizing a variety of environmental variables related to benthic macroinvertebrate community composition was fundamental in assessing possible effects from natural and anthropogenic non-point source disturbances within tributaries of the AOSR. Benthic macroinvertebrate community composition significantly changed along the environmental disturbance gradient for the Steepbank and Ells River basins. Sensitive taxa declined from upstream to downstream in the Steepbank River, whereas tolerant taxa were abundant upstream in the Ells River, for both three-minute kick nets and rock-basket artificial substrates. The greatest longitudinal changes in community composition within the Steepbank River were likely attributed to natural perturbations, such as the sediment slump and large discharge events which occurred over the 2012 sampling season.

The Ells River basin contained higher abundances of relatively metal-tolerant taxa at the upstream sites for both sampling methods. Therefore, the occurrence of elevated metal concentrations observed in the upper basin was possibly related to the distribution of tolerant taxa. Overall, natural environmental drivers explained most of the longitudinal variation in benthic macroinvertebrate community composition within both the Steepbank and Ells Rivers, with little indication of anthropogenic disturbance.

Seasonal differences in benthic macroinvertebrate community composition within the Steepbank River were attributed to changes in the relative abundance of *Baetis spp.*, for both natural and artificial substrates. TDS concentrations also contributed the most to differences in community structure for both sampling methods. Seasonal variation in three-minute kick net benthic macroinvertebrate community composition for the Ells River showed that changes in the relative abundance of Chironomidae species had the greatest influence, with natural variables contributing to changes in community composition. Comparatively, seasonal differences in the rock-basket benthic macroinvertebrate community were highly variable, with natural factors likely contributing the most to variation in community structure.

Variability in observed results between methods were possibly attributed to differences in retrieval success at sites, effects of substrate on the natural invertebrate community, as well as limited within-site variance acquired from three-minute kick nets.

Moreover, environmental variables selected to assess three-minute kick net and rock-basket communities were not consistent as a consequence of when community data and environmental variables were sampled. Due to the confounding effects of substratum on benthic macroinvertebrate community composition observed in this study, artificial substrates were recommended as a rapid bioassessment tool to specifically examine anthropogenic disturbances within tributaries of the AOSR.

Odonate MeHg concentration increased from upstream to downstream sites in the Steepbank River. This was potentially attributed to aerial contaminant deposition on the watershed from nearby OS mining activities, entering the river during runoff, as well as natural sources from OS deposits in the lower reaches. Conversely, Odonate THg and MeHg concentrations decreased from upstream to downstream within the Ells River, possibly due to naturally high metal concentrations in the upper basin. Overall, changes in Hg concentrations among-sites and months within both the Steepbank and Ells Rivers corresponded with benthic macroinvertebrate structure, with a reduction in the abundance of sensitive species and an increase in the abundance of relatively tolerant taxa at sites with higher Hg concentrations. Therefore, Hg could be investigated further as a possible mechanism for explaining longitudinal and seasonal changes in community composition in tributaries of the AOSR.

Recommendations for future assessments of benthic macroinvertebrate community composition in tributaries of the AOSR include collecting replicate three-minute kick nets at a site during a sampling period to produce sample variance. This also includes collecting multiple bulk kick net samples to acquire greater Odonate biomass for Hg analysis. Expanding sample sites upstream on the Ells River is also recommended to further investigate the increase in metal concentrations in the upper basin. Furthermore, more frequent water quality sampling would potentially be more representative of colonized rock-basket benthic macroinvertebrate communities, compared to a single monthly grab sample.

Substrate composition was highlighted as an influential factor in benthic macroinvertebrate distribution in tributaries of the AOSR. Therefore, it is recommended that substrate composition methods should be re-assessed to better comprehend natural intersite habitat variation in river ecosystems of the AOSR. Moreover, surrounding lotic systems in the AOSR have demonstrated high temporal variability. The major flooding events which occurred on the Steepbank River in 2012 could have temporally displaced certain macroinvertebrate taxa. Intensive inter-annual sampling would be highly beneficial in comprehending the possible effects of natural and anthropogenic non-point source perturbations on the invertebrate communities within these tributary ecosystems.

Longitudinal and seasonal responses of benthic macroinvertebrate community composition were mostly attributed to natural variation within tributaries of the AOSR. Furthermore, highly sensitive Ephemerellidae species (e.g., *E. dorothea*, *E. excrucians*) were consistently identified at the downstream sites within both the Steepbank and Ells Rivers. Therefore, at this present time, cumulative effects from non-point source disturbances on benthic macroinvertebrate communities in these river ecosystems were negligible or not detected.

5.6 References

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CHAPTER 6: SUMMARY AND CONCLUSIONS

The overall goal of this thesis was to use a multi-integrative approach to identify spatial and temporal relationships of natural and anthropogenic environmental variables affecting riverine ecosystem structure and function in the Athabasca Oil Sands Region (AOSR). This goal was achieved through conducting a series of inter-related field studies assessing three main components of the freshwater food web utilizing an *a priori* environmental disturbance gradient experimental design. The gradient design was formulated to best discriminate the possible effects of natural and anthropogenic environmental variables on two river basins each having different levels of oil sands (OS) land use disturbance (Table 6.1). The results presented here provide an improved understanding of how key components of the riverine food web are affected both spatially and temporally by natural and anthropogenic non-point source perturbations in tributaries of the AOSR.

Table 6.1. Environmental disturbance gradient imposed for the site sampling design on the Steepbank and Ells Rivers over the 2012 sampling seasons.

Site	Environmental Gradient
Steepbank River	
ST1	At the mouth of the river nearest to atmospheric deposition sources of upgraders, in the OS geological deposit, and receiving cumulative loadings from upstream disturbances
ST2	Inside the OS deposit with evident upstream land use disturbance from mining operations
ST3	Inside the OS deposit and at the edge of land use disturbance from OS development
ST4	Outside the OS deposit in an undisturbed region of the catchment
Ells River	
EL1	At the mouth of the river receiving cumulative loadings from upstream disturbances and in the OS deposit
EL2	Inside the OS deposit and at the edge of land use disturbance from land-clearing
EL3	Outside the OS deposit in an undisturbed region of the catchment

***Component 1* - Examine the effects of natural and anthropogenic non-point source disturbances on physical and chemical environmental variables in Athabasca River tributaries. Investigate the within- and among-site and between-river basin physico-chemical spatial and temporal differences.**

Results from this study document that natural variation explained most longitudinal and seasonal responses of physico-chemical environmental variables for both the Steepbank and Ells Rivers. There were minor indications of anthropogenic catchment-scale disturbances contributing to downstream changes in water and sediment chemistry and differences in the quality and quantity of the physical lotic habitat in tributaries of the AOSR. For example, substrate composition at the mouth of the Steepbank River was physically modified from a large slump event which occurred during the 2012 sampling period. Increased erosion from unstable river banks could have been a consequence of catchment-scale disturbances; moreover, the steep banks in the lower reaches are also susceptible to natural sedimentation events.

Major cation and anion concentrations were greatest at downstream sites for both the Steepbank and Ells Rivers. Cation/anion concentrations were also overall greater within the Ells River, which was possibly attributed to early land-clearing activities occurring on the basin, as documented by Alexander and Chambers (in press). Furthermore, fine sediment metal concentrations were greatest at downstream sites within the Steepbank River. Previous studies by Conly et al. (2007) found no significant change in bed sediment metal concentrations at sites within the OS deposit, suggesting another possible metal source within the Steepbank River.

Moreover, the OS geological deposit and shallow groundwater upwelling in the lower reaches likely contributed the greatest to changes in downstream physico-chemical variables within both the Steepbank and Ells Rivers. Seasonal variations in timing and quantities of catchment runoff that were observed between-basins were potentially a consequence of impervious and unstable landscapes from large-scale OS mining operations on the Steepbank River basin, compared to small-scale land-clearing activities on the Ells River basin. These differences could also be attributed to an anomalous flooding year on the Steepbank River, as well as buffering effects from upper basin lakes on the Ells River basin (Headley et al. 2005).

The Ells River displayed distinct downstream changes in fine sediment metal concentrations which were not observed in the Steepbank River. These responses were hypothesized to be the result of several possible mechanisms operating individually or synergistically. Possible causes for the observed longitudinal patterns include: naturally high metal concentrations in the upper basin (Lechler et al. 2000); lake storage effects from the Namur-Gardiner lakes influencing downstream chemistry and moderating runoff (Headley et al. 2005); "dilution" effects from naturally high sediment loads from the Ells River basin into the lower reaches (Kashyap et al. 2014); or changes in bed sediment particle size which tends to become courser downstream, potentially making binding with metals more difficult in lower reaches of the river (Conly et al. 2007).

Overall, physico-chemical environmental variables within the Steepbank and Ells Rivers basin were mostly attributed to natural variation, with disturbance from OS development either minor or not observed

Component 2 **- Examine the effects of natural and anthropogenic non-point source disturbances on the basal productivity of aquatic food webs in Athabasca River tributaries. Investigate the within- and among-site and between-river basin spatial and temporal differences in algal and biofilm biomass.**

Algal and biofilm biomass displayed significant changes along the environmental disturbance gradient for both river basins. Algal biomass on natural substrates decreased from upstream to downstream sites in the Steepbank River. Possible mechanisms for the downstream decline included OS deposit effects causing deteriorated algae development and reduced photosynthesis (Bott et al. 1978), influences from fine particulate organic matter (FPOM) from the terrestrial landscape on the autochthonous production in the downstream environment (Vannote et al. 1980). Moreover, the suppression in algal and biofilm biomass at the mouth of the Steepbank River was most likely attributed to the large upstream slump event which occurred during the 2012 sampling season, causing evident scouring downstream.

Algal biomass on artificial substrates declined downstream within the Ells River, which was potentially attributed to inhibitory effects from natural bitumen deposits on the photosynthesis of newly colonized algae, as described by Soto et al. (1975), Miller et al. (1978), Federle et al. (1979), and Barsdate et al. (1980). Alternative mechanisms include allochthonous inputs of particulate organic matter from the terrestrial landscape, as well as potential for shading, light inhibition and increased turbidity from high TSS loadings within the Ells River. Biofilm biomass increased longitudinally within both rivers (excluding the most downstream site on the Steepbank River) as a possible result of increased inputs of organic matter from the surrounding landscape, described by Vannote et al. (1980), as well as higher metabolic rates observed linked to the occurrence of natural petroleum hydrocarbons within the OS deposit (Lock et al. 1981a; Peterson et al. 1996).

Seasonal differences for both rivers demonstrated a spring depression in basal production as a likely result of algal scouring from high flows during freshet (Stevenson 1990), as well as possible increased turbidity, reducing photosynthetically active radiation (PAR) and autotrophic production (Johnston 1922; Wetzel 1983). Productivity increased in summer with warmer temperatures and longer daylight hours, followed by a decrease into fall with cooler temperatures and shorter days, as described by DeNicola et al. (1992), Eulin and Le Cohu (1998), and Izagirre and Elosegi (2005). Seasonal effects within the Ells River were generally less variable compared to the Steepbank River, with the upper basin Namur-Gardiner lakes moderating runoff (Headley et al. 2005), as well as diminished inputs of organic material into the system from a relatively undisturbed basin.

This study found that associating responses of algal growth to effects from natural and anthropogenic non-point source perturbations is method dependent. Observations from this study were similar to findings from Lock et al. (1981a, b), which found algal species on natural substrates to become tolerant of OS deposit effects, whereas species newly colonized on clean substratum within artificial substrates were less tolerant to oil-toxicity effects. Moreover, algal taxonomic identification and associated toxicity tests would need to be addressed further to determine mechanistic pathways. Therefore, this study recommends the use of rapid bioassessment approaches utilizing artificial substrata for sampling algal biomass to provide further insights into the diverse responses of algal communities to OS deposits and catchment-scale disturbances within tributaries of the AOSR.

Nutrient limitation on algal growth was only observed within the Ells River. These findings corroborated with results from Hickman et al. (1983), which identified factors which controlled algal standing crop size in tributaries of the AOSR. These factors included nutrient levels within the Ells River, and physical forces within the Steepbank River. Bulk water quality samples demonstrated no significant change in nitrogen and phosphorous parameters from upstream to downstream within both rivers; thus, natural variation most likely explained between-basin differences.

Overall, the results show that basal productivity is most likely controlled by natural factors within the Steepbank and Ells Rivers, with disturbance from OS development either negligible or not detected. Furthermore, changes in algal biomass displayed similar patterns as nutrient availability along the longitudinal gradient in both rivers, suggesting nutrient limitation could be an important environmental driver affecting autotrophic production in these river ecosystems.

Component 3 **- Examine the longitudinal and temporal differences in benthic macroinvertebrate community structure influenced by natural and anthropogenic non-point source disturbances on Athabasca River tributaries. This is assessed by:**

- **Identifying which physico-chemical and basal production variables explain variation in benthic macroinvertebrate community composition.**
- **Determining whether elemental mercury (Hg) can be identified as a contaminant at the base of the aquatic food web.**

Results suggest that human-induced changes in benthic macroinvertebrate community composition were either minor or not observed within the Steepbank and Ells Rivers as the community shifts were likely related to natural longitudinal and seasonal variation. Abundance of sensitive benthic taxa declined from upstream to downstream sites in the Steepbank River. Greatest longitudinal changes in community composition were likely attributed to large discharge events and the sediment slump which occurred throughout the 2012 sampling season. Furthermore, natural environmental variables (pH, total dissolved phosphorus (TDP), fine sediment metal concentration, and water depth) were primarily found to explain longitudinal variations in benthic macroinvertebrate community composition within the Steepbank River.

Relatively tolerant benthic macroinvertebrate taxa dominated upstream within the Ells River, with no downstream decline in sensitive species in relation to the environmental disturbance gradient. The occurrence of relatively tolerant taxa in the upper Ells River basin is possibly related to the elevated metal concentrations observed at upstream sites. Because tolerant taxa were less prevalent downstream in the Ells River, benthic macroinvertebrate communities likely responded to the natural variation of the system from upstream to downstream. Furthermore, natural environmental variables (chloride (Cl^-), pebble substrate, water temperature, fine sediment metal concentration, pH, sulfate (SO_4^{2-}), TDP, silicon dioxide (SiO_2^{2-}), flow velocity, and dissolved oxygen (DO)) were found to explain longitudinal variation in benthic macroinvertebrate community composition within the Ells River.

In the Steepbank River, species composition changes within the Baetidae family contributed the greatest to observed seasonal differences in community structure, with TDS being the most influential environmental variable explaining seasonal variability in community composition. In the Ells River, species composition changes within the Chironomidae family contributed the greatest to observed seasonal differences in community structure, with natural environmental variables (periphyton ash-free dry mass (Peri-AFDM), SO_4^{2-}, pebble substrate, water temperature, fine sediment metal concentration, pH, TDP, SiO_2, flow velocity and DO) explaining seasonal variation in benthic macroinvertebrate community composition.

This study found that spatio-temporal patterns of changes in benthic macroinvertebrate community structure to non-point source disturbances were similar whether traditional kick type or artificial substrate sampling methods were used. Numerous advantages were highlighted for using artificial substrates for sampling benthic macroinvertebrate communities, such as standardization for greater success of reproducibility in invertebrate colonization as well as eliminating subjectivity in sample collection technique. Moreover, because of confounding factors from intersite habitat variation, this study recommends using artificial substrates as a possible rapid bioassessment tool to specifically investigate the potential effects of anthropogenic stressors on benthic macroinvertebrate community composition within tributaries of the AOSR.

Benthic macroinvertebrate Hg concentrations increased from upstream to downstream within the Steepbank River, which was possibly attributed to aerial contaminants deposited on the watershed from nearby OS mining activities as shown by Kelly et al. (2010) and Kirk et al. (2014), as well as natural sources from OS deposits in the lower reaches (Maclock et al. 1997). Hg concentrations decreased from upstream to downstream within the Ells River as a potential result of naturally elevated metal concentrations in the upper basin (Lechler et al. 2000; Headley et al. 2005; Conly et al. 2007; Kashyap et al. 2014). Elevated Hg concentrations within the Steepbank and Ells Rivers were associated with greater abundance of tolerant taxa and lower abundance of sensitive taxa, suggesting potential contaminant-effects on biotic communities within these river ecosystems.

In conclusion, little direct evidence of OS disturbance was observed within both the Steepbank and Ells Rivers. The Steepbank River exhibited minor indications of contaminant-effects within the lower reaches with increased Hg levels; moreover, further investigations are required to determine Hg sources in the ecosystem. Overall, natural variation was the primary driver of longitudinal and seasonal changes in benthic macroinvertebrate community structure in both rivers.

6.1 Cumulative Effects Assessment

The catchment-scale multi-integrative approach utilized in this study investigated the possible effects of multiple anthropogenic stressors on the integrity of freshwater ecosystems in the AOSR over space and time. Cumulative environmental effects results from the incremental, accumulating, and interacting impacts of these anthropogenic stressors on the environment (Dubé et al. 2006). Measuring their cumulative effects was necessary for an improved understanding of the potential consequences of non-point source perturbations from different levels of OS development on the Steepbank and Ells River basins. To assess the cumulative effects, a weight of evidence approach was conducted to determine relative influences and causality of various possible anthropogenic stressors, as outlined by Culp et al. (2000a) in the Northern River Basins Study.

Overall cumulative effects for the Steepbank and Ells River are summarized in Figures 6.1 and 6.2, respectively. Each figure identifies issues of concern at each sampling site based on the weight of evidence approach. At each site, a histogram consisting of seven stacked boxes is provided. Each box represents one of the seven classes of environmental issues in the basin which were addressed in this study (i.e., water quality, sediment chemistry, physical habitat, basal production, nutrient enrichment, contaminants, and benthic macroinvertebrate community structure). The fill pattern within the box reflects the level of concern for that issue, as outlined by this study. A completely dark box indicates potential concern, which is defined as: Study found a significant result at this site which was potentially not attributed to natural factors. A hatched box indicates potential caution, which is defined as: Study found a significant result at this site which could potentially be attributed to either natural or anthropogenic factors. A clear box indicates that the issue is of minimal concern, which is defined as: Study either did not find a significant result at this site or response could likely be attributed to natural factors. Pie diagrams of rock-basket benthic macroinvertebrate community composition are also depicted for each site to illustrate longitudinal shifts in the relative abundances of sensitive and tolerant taxa within the Steepbank and Ells Rivers.

6.1.1 Steepbank River

Water quality issues were designated with minimal concern at downstream sites in the Steepbank River (ST1 and ST2). Downstream increases in cation and anion concentrations were likely attributed to major ions co-occurring with the OS geological deposit and shallow groundwater upwelling in the lower reaches, as well as catchment runoff of ions leached from soils transported downstream. Sites ST3 and ST4 were also of minimal concern by being upstream of land use disturbance and having consistently lower concentrations of water quality parameters than ST1 and ST2.

Sediment chemistry was indicated with minimal concern at ST1, ST2 and ST3. Downstream increases in fine sediment metals were likely attributed to natural metals associated with the OS deposit. For example, ST1 did not have higher metal concentrations than ST3, despite being downstream of OS mining activities; however, both sites were located within the OS deposit. ST4 was also designated with minimal concern by being upstream of land use disturbance and having consistently lower concentrations of fine sediment metals than downstream sites.

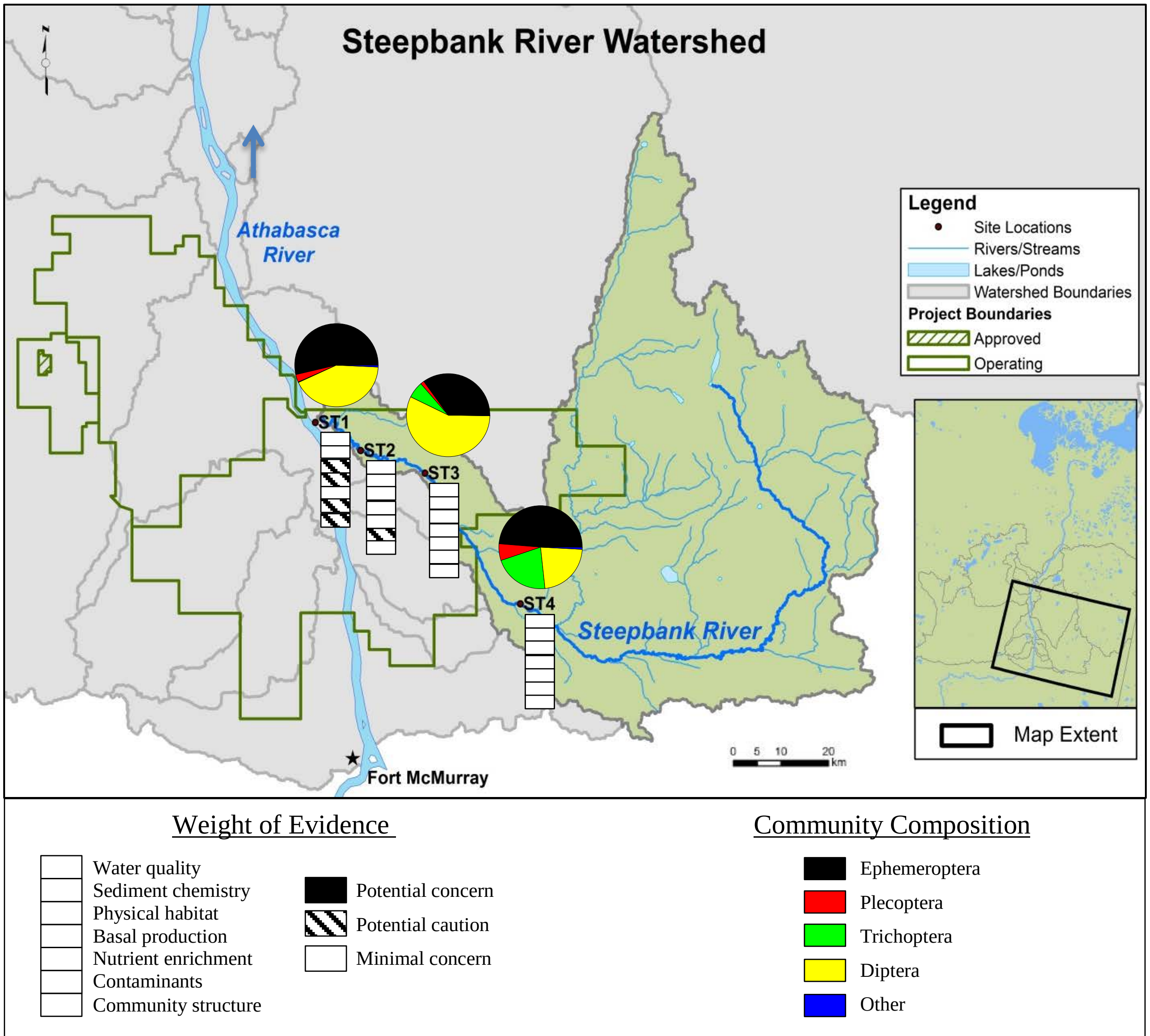

Figure 6.1. Cumulative effects assessment of anthropogenic stressors on the Steepbank River basin based on the weight of evidence from this study. The figure provides a mechanism for identifying issues of concern at sites along the environmental disturbance gradient based on the weight of evidence approach. The histograms consist of seven classes of environmental issues in the basin including water quality, sediment chemistry, physical habitat, basal production, nutrient enrichment, contaminants, and benthic macroinvertebrate community structure. A completely dark box indicates a potential concern and a cross-hatched box indicates potential caution, as outlined by this study. A clear box indicates that, based on information from this study, the issue is of minimal concern. Pie diagrams of rock-basket benthic macroinvertebrate communities illustrate longitudinal changes in relative abundances of sensitive and tolerant taxa, including Ephemeroptera, Plecoptera, Trichoptera, and Diptera from upstream to downstream.

Physical habitat was designated with potential caution at ST1. Results from this study demonstrated enhanced sediment loadings at the mouth of the river from possible upstream land use disturbance resulting in increased erosion from unstable river banks. Moreover, the steep banks in the lower reaches are also susceptible to natural sedimentation events. ST2 was indicated with minimal concern, because no alterations to physical habitat were observed in this study. ST3 and ST4 were also designated with minimal concern by being upstream of OS land use activities. Furthermore, changes to sediment delivery and the hydrologic regime resulted in extensive algal scouring at ST1; thus, basal production was also designated with a level of potential caution. Similarly, ST2, ST3 and ST4 were of minimal concern.

Nutrient enrichment was designated with minimal concern at ST1 and ST2. Results from this study demonstrated no nutrient inputs from upstream watershed loadings in the lower reaches of the Steepbank River. OS mining activities do not directly discharge effluents into the river, such as with pulp mills (Culp et al. 2000b), suggesting nutrient enrichment is an unlikely environmental response in this lotic ecosystem. ST3 and ST4 were indicated with minimal concern by being upstream of OS land use perturbations.

Contaminant effects were of potential caution at ST1 and ST2. Hg concentrations from this study were significantly greater downstream of OS development as well as these sites being located adjacent to one of the largest OS projects in the AOSR (Humphries 2008). Sources of aerial contaminants on the Steepbank River basin included: nearby bitumen upgrading facilities, vehicle emissions, volatilization from tailings ponds, and blowing dusts from open pit mines, exposed coke piles, and deforested areas (Kelly et al. 2010; Kirk et al. 2014). Moreover, natural metals are also associated with the OS geological deposit (Maclock et al. 1997). ST3 and ST4 were designated with minimal concern by being outside the geographical range of aerial contaminant deposition and upstream of OS development.

Benthic macroinvertebrate community structure was indicated with potential caution at ST1. Results from this study demonstrated significant changes in community composition at ST1 compared to upstream sites inside and outside of the OS deposit, which was likely attributed to the alteration of the physical habitat at the mouth of the river. ST2 was designated with minimal concern, with no significant differences in community structure observed from upstream sites in this study. Moreover, ST3 and ST4 were of minimal concern by being upstream of OS development.

Pie diagrams illustrated an overall decline in Plecoptera and Trichoptera species abundance along the environmental disturbance gradient, specifically at ST1. For example, *Zapada cinctipes* (Plecoptera: Nemouridae) and *Lepidostoma sp.* (Trichoptera: Lepidostomatidae) were most abundant at ST4, whereas no Plecoptera or Trichoptera species were most abundant at ST3 or ST1. Diptera taxa increased in relative abundance from upstream to downstream, with *Simulium sp.* (Diptera: Simuliidae) and *Microspectra sp.* (Diptera: Chironomidae) having greater abundance at ST3. Ephemeroptera taxa abundance were relatively consistent from upstream to downstream which was previously observed by Barton and Wallace (1979) and Barton (1980a) prior to enhanced OS development on the Steepbank River basin. However, *Baetis spp.* (Ephemeroptera: Baetidae) abundance declined from upstream to downstream, whereas *Ephemerella sp.* (Ephemeroptera: Ephemerellidae) abundance increased in the lower reaches.

6.1.2 Ells River

Water quality issues were designated with minimal concern at the most downstream site on the Ells River (EL1). Water quality parameters with significant site differences contained the greatest concentrations of major cations/anions and physical/water quality parameters at EL1; however, this was likely attributed to natural variation within the river ecosystem. This included major ions co-occurring with the OS geological deposit, inputs from shallow groundwater upwelling in the lower reaches, as well as catchment runoff of ions leached from soils transported downstream. Sites upstream of OS land use activities (EL2 and EL3) were designated minimal concern with consistently lower concentrations of water quality parameters than EL1.

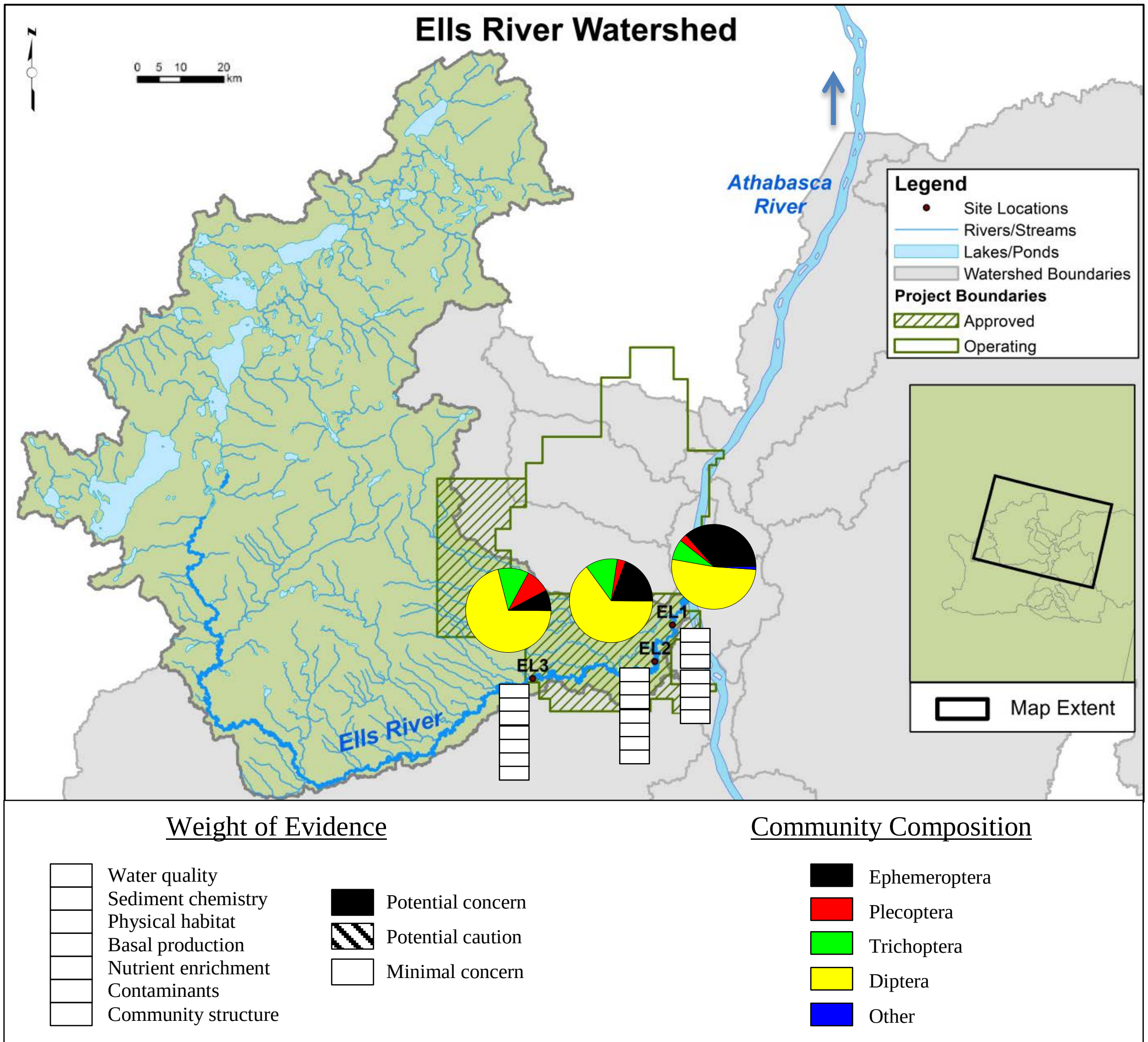

Figure 6.2. Cumulative effects assessment of anthropogenic stressors on the Ells River basin based on the weight of evidence from this study. The figure provides a mechanism for identifying issues of concern at sites along the environmental disturbance gradient based on the weight of evidence approach. The histograms consist of seven classes of environmental issues in the basin including water quality, sediment chemistry, physical habitat, basal production, nutrient enrichment, contaminants, and benthic macroinvertebrate community structure. A completely dark box indicates a potential concern and a cross-hatched box indicates potential caution, as outlined by this study. A clear box indicates that, based on information from this study, the issue is of minimal concern. Pie diagrams of rock-basket benthic macroinvertebrate communities illustrate longitudinal changes in relative abundances of sensitive and tolerant taxa, including Ephemeroptera, Plecoptera, Trichoptera, and Diptera from upstream to downstream.

Sediment chemistry and contaminants were designated with minimal concern at EL1. Increases in concentrations of fine sediment metals or contaminants were not observed at EL1 in this study. EL2 and EL3 were also indicated with minimal concern by being upstream of land-clearing activities and located far from the geographic center of aerial contaminant deposition. Increased metal concentrations at EL2 and EL3 were attributed to natural sources.

Physical habitat was designated with minimal concern at EL1. Results from this study showed greater TDS and TSS concentrations downstream; however, this was likely attributed to naturally high TSS loadings during peak flow conditions within the Ells River (Kashyap et al. 2014). EL2 and EL3 were also indicated with minimal concern by being upstream of land-clearing activities, and having consistently lower concentrations of TDS and TSS than EL1.

Basal production was designated with a level of minimal concern for EL1. Results from this study did not show downstream changes in algal or biofilm biomass in relation to anthropogenic catchment-scale disturbances within the Ells River. EL2 and EL3 were also of minimal concern by being upstream of land-clearing activities. Nutrient enrichment was of minimal concern for all sites on the Ells River, with results from this study suggesting nutrient levels were not altered from catchment-scale perturbations.

Benthic macroinvertebrate community structure was designated with minimal concern for EL1. Results from this study attributed longitudinal changes in community composition to natural variation and not land-clearing activities. Moreover, EL2 and EL3 were also of minimal concern by being upstream of land-clearing disturbances.

Pie diagrams illustrated a slight longitudinal depression in Plecoptera and Trichoptera species abundance along the environmental disturbance gradient. For example, *Taeniopteryx parvula* (Plecoptera: Taeniopterygidae) and *Hydropsyche sp.* (Trichoptera: Hydropsychidae) were more abundant at upstream sites, whereas *Isoperla sp.* (Plecoptera: Perlodidae) had consistent abundance from upstream to downstream. Diptera taxa were most abundant at EL2 and EL3, with large increases in *Tvetenia sp.* and *Microspectra sp.* at EL3, whereas *Simulium sp.* were least abundant at EL3. Ephemeroptera species abundance increased from upstream to downstream, with large increases in abundance of *Ephemerella sp.* and *Tricorythodes minutus* (Ephemeroptera:

Leptohyphidae) at EL1. *Baetis spp.* abundance were relatively consistent from upstream to downstream, whereas *Acerpenna pygmaea* (Ephemeroptera: Baetidae) were more abundant at EL2 and EL3.

6.2 Recommendations and Future Directions

The multi-integrative approach utilized in this study provided an improved investigation of the effects of natural and anthropogenic non-point source disturbances on the key components of tributary ecosystems in the AOSR. Moreover, an inter-annual experimental period is recommended, due to the large temporal variability observed in these river systems. For example, major flooding and discharge events occurred on rivers in the lower Athabasca Region in 2012, specifically the Steepbank River. Furthermore, flow regimes of the lower reaches of the Steepbank River are also altered when the Athabasca River floods, ultimately influencing the downstream lotic environment, as observed by Barton and Wallace (1979). Therefore, multi-year sampling would determine whether extreme hydrological events were responsible for the observed longitudinal and seasonal variability in environmental variables and benthic macroinvertebrate community composition, compared to potential effects from OS mining activities.

Furthermore, the high variability of river ecosystems in the AOSR also suggests increased sample replication of physico-chemical and biological variables is necessary when assessing the effects of natural and anthropogenic non-point source perturbations. For example, bulk water quality parameters, periphyton rock scrapings and three-minute kick nets were only collected as a single sample during each monthly collection period in this study. Therefore, sample variance could not be calculated for these variables for each month, and detection of outliers was limited without sufficient replication (Stark 1993).

Substrate composition was highlighted as a possible factor influencing benthic macroinvertebrate community composition for both the Steepbank and Ells Rivers. River substratum has been repeatedly shown to affect benthic macroinvertebrate distribution in freshwater ecosystems (Wright et al. 1984; Richards et al. 1993; Ruse 1996). The 100 pebble count conducted during routine CABIN sampling did not obtain results in congruence with personal observation of the substratum in the Steepbank and Ells Rivers nor did it account for the bedrock-like substrate in the lower reaches of these rivers.

Based on these findings, application of the 100 pebble count may need to be re-assessed as a method for future studies examining substrate characterization in tributaries of the AOSR, with possible alternative methods outlined in Sutherland et al. (2010).

Rock-basket artificial substrates simplified sample replication of environmental variables and benthic macroinvertebrate community structure as well as provided standardized substratum for easier intersite and seasonal comparisons. Results from this study corroborated with previous findings by Lock et al. (1981a, b), investigating the various responses of algal communities to natural bitumen deposits, which was highlighted exclusively utilizing artificial substrates. Furthermore, effects of eroding bitumen on downstream river substrate also possibly influenced benthic macroinvertebrate habitat availability in this study. Therefore, to investigate the effects of OS development on river ecosystems without confounding factors on algal and invertebrate communities, artificial substrates are recommended as a possible supplementary rapid bioassessment tool for future studies assessing tributary ecosystems in the AOSR.

Future investigations of physico-chemical and biological variables assessed in this study are particularly recommended for the Ells River, specifically examining the sources contributing to elevated metal concentrations in the upper basin. The observed downstream decrease in metal concentrations within the Ells River possibly influenced fine sediment chemistry, benthic macroinvertebrate distribution and mercury concentrations at the base of the aquatic food chain. Several hypotheses were suggested to explain why metal concentrations were greater at the upstream sites. These included: 1) the potential for naturally elevated sediment chemistry in the upper basin (Lechler et al. 2000); 2) the influence of buffering lake storage effects from the Namur-Gardiner lakes on downstream physico-chemical parameters (Headley et al. 2005); 3) the effects of substantially high suspended sediment loads from the Ells River basin into the Athabasca River (Kashyap et al. 2014); and 4) the relationship of suspended sediment particle size with metal concentration along the river gradient (Conly et al. 2007). These hypotheses could be further examined by expanding the number of sample sites in the upper Ells River basin to better understand the mechanistic pathway of this decreasing pattern in metal concentrations from upstream to downstream.

Hg isotope analysis using benthic macroinvertebrates could also investigate Hg cycling in the aquatic food web in the Ells River, as well as potential sources within the environment as described by Paterson et al. (2006). Furthermore, examining changes in particle size from upstream to downstream, as well as mass of fine sediments on scour pad sediment traps could further assess the relationship between sediment particles and metal concentrations within the Ells River, as observed by Conly et al. (2007) and Droppo et al. (2015). These recommendations for future research on the Ells River would gain greater insight into the effects of natural and anthropogenic non-point source disturbances on the integrity of this ecosystem.

This study highlighted the high natural variability of the Ells River. Therefore, classifying the Ells River watershed as a "reference" to compare with more disturbed watersheds in the AOSR, such as the Steepbank River, should be considered with caution due to its natural complexity. This study also demonstrated that developing baseline information on watersheds can be essential at discriminating sources of disturbance, with natural variation potentially confounding with anthropogenic factors. Many studies show that when more than 5 to 10% of a watershed's area is affected by anthropogenic activities, stream biodiversity and water quality suffer (Palmer et al. 2010). Therefore, with only 3.70% of the Steepbank River catchment only currently being disturbed by OS development, the threshold at which change occurs within the river ecosystem may have not yet been reached.

In order to better determine whether anthropogenic non-point disturbances are influencing tributary ecosystems in the AOSR, a causal argument can be constructed based on multiple lines of evidence of OS disturbance on surrounding river ecosystems. Assembly rules for casual arguments have been outlined in Beyers (1998), which supports casual inference based on the results of an unreplicated environmental impact study. Examples of assembly rules include, the association found in the experiment has been observed by other investigators at other times and places, or the causal hypothesis does not conflict with existing knowledge of history and biology. Explicit use of assembly rules for making casual arguments allows investigators to efficiently organize, study, and present available evidence. Moreover, determining mechanisms of action and experimental demonstrations provide the most direct evidence for a causal relationship.

Overall, this study has contributed greatly to understanding the possible effects of both natural and anthropogenic non-point source perturbations on the key components of freshwater food webs, and ultimately the structure and function of tributary ecosystems of the AOSR. This study emphasizes the use of a multi-integrative approach to determine appropriate structural and functional endpoints for detecting changes in freshwater ecosystems, and to discriminate pathways of disturbance in systems experiencing co-variation between natural and anthropogenic gradients. Ultimately, this study has enhanced the mechanistic understanding of natural and anthropogenic environmental variables, which collectively contribute to the cumulative environmental effects of natural and anthropogenic non-point source disturbances within river ecosystems of the AOSR.

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APPENDIX A: SITE SAMPLING SUMMARY TABLES

Table A.1. Summary table of Steepbank River site locations, 2012 sampling seasons, and samples analyzed at each site during each sampling period.

Location	Sampling Station ID	Sampling Station Description	GPS Coordinates		Sample Collection Seasons	Type of Sample Analysis
			Latitude (N)	Longitude (W)		
Steepbank River	ST1	Steepbank River at mouth	57°01.338’	111°28.618’	winter 2012	YSI 6600-V2 sonde & rock-baskets/NDS deployed
					spring 2012	detailed analysis[1,2,3]; rock-baskets/NDS deployed
					summer 2012	detailed analysis[1,2,3,4]; rock-baskets/NDS deployed
					fall 2012	detailed analysis[1,2,3]; rock-baskets/NDS deployed & retrieved[5]
	ST2	Steepbank River d/s of mining, inside OS deposit	56°59.919’	111°24.201’	winter 2012	YSI 6600-V2 sonde & rock-baskets/NDS deployed
					spring 2012	detailed analysis[1,2,3]; rock-baskets/NDS deployed
					summer 2012	detailed analysis[1,2,3,4]; rock-baskets/NDS deployed
					fall 2012	detailed analysis[1]
	ST3	Steepbank River u/s of mining, inside OS deposit	56°58.773’	111°17.914’	winter 2012	rock-baskets/NDS deployed
					spring 2012	detailed analysis[1,2,3]; rock-baskets/NDS deployed
					summer 2012	detailed analysis[1,2,3,4]; rock-baskets/NDS deployed
					fall 2012	detailed analysis[1,2,3]; rock-baskets/NDS deployed & retrieved[5]
	ST4	Steepbank River u/s of mining, outside OS deposit	56°52.144’	111°08.606’	spring 2012	detailed analysis[1,2,3,4]; YSI 6600-V2 sonde & rock-baskets/NDS deployed
					summer 2012	detailed analysis[1,2,3,4]; rock-baskets/NDS deployed & retrieved[5]
					fall 2012	detailed analysis[1,2,3]; rock-baskets/NDS deployed & retrieved[5]

NOTES:
[1] Samples were analyzed for water quality parameters
[2] Samples were analyzed for periphyton rock scrapings
[3] Samples were analyzed for three-minute kick net benthic macroinvertebrates
[4] Samples were analyzed for Odonate mercury concentration
[5] Samples were analyzed for fine sediment chemistry, BBQ periphyton, nutrient limitation, and benthic macroinvertebrates

Table A.2. Summary table of Ells River site locations, 2012 sampling seasons, and samples analyzed at each site during each sampling period.

Location	Sampling Station ID	Sampling Station Description	GPS Coordinates Latitude (N)	GPS Coordinates Longitude (W)	Sample Collection Seasons	Type of Sample Analysis
Ells River	EL1	Ells River at mouth	57°16.826’	111°42.284’	winter 2012	YSI 6600-V2 sonde deployed
					spring 2012	detailed analysis[1,2,3,4]; rock-baskets/NDS deployed & retrieved[5]
					summer 2012	detailed analysis[1,2,3,4]; rock-baskets/NDS deployed and retrieved[5]
					fall 2012	detailed analysis[1,2,3,4]; rock-baskets/NDS deployed & retrieved[5]
	EL2	Ells River u/s of mining, inside OS deposit	57°14.676’	111°44.193’	spring 2012	detailed analysis[1,2,3,4]; YSI 6600-V2 sonde deployed, rock-baskets/NDS deployed & retrieved[5]
					summer 2012	detailed analysis[1,2,3,4]; rock-baskets/NDS deployed & retrieved[5]
					fall 2012	detailed analysis[1,2,3,4]; rock-baskets/NDS deployed & retrieved[5]
	EL3	Ells River u/s of mining, outside OS deposit	57°13.665’	111°57.536’	spring 2012	detailed analysis[1,2,3,4]; rock-baskets/NDS deployed
					summer 2012	detailed analysis[1,2,3,4]; YSI 6600-V2 sonde deployed, rock-baskets/NDS deployed & retrieved[5]
					fall 2012	detailed analysis[1,2,3]; rock-baskets/NDS deployed & retrieved[5]

NOTES:
[1] Samples were analyzed for water quality parameters
[2] Samples were analyzed for periphyton rock scrapings
[3] Samples were analyzed for three-minute kick net benthic macroinvertebrates
[4] Samples were analyzed for Odonate mercury concentration
[5] Samples were analyzed for fine sediment chemistry, BBQ periphyton, nutrient limitation, and benthic macroinvertebrates

APPENDIX B: CONSTRUCTION OF NUTRIENT DIFFUSING SUBSTRATA

B.1 Making Agar Tubes

To make approximately 30 tubes of each treatment (Control, N, P, N + P), agar was dissolved in 250 mL of DDW (double distilled water) in a 500 mL flask and nutrients were dissolved in 250 mL of DDW in a separate 500 mL flask based on the concentrations in Table B.1.

Table B.1. Concentrations of agar and nutrients required to make each treatment within a nutrient diffusing substrate (NDS) device.

Batch	**2% Agar**	**$NaNO_3$**	**KH_2PO_4**
Control	10 g		
0.5 M N	10 g	21.25 g	
0.5 M P	10 g		34.45 g
0.5 M N + 0.5 M P	10 g	21.25 g	34.45 g

Flasks were shaken to completely dissolve the agar and nutrients, covered with a piece of aluminum foil, and autoclaved for 45 minutes (liquid cycle). Once the autoclave cycle was completed, flasks were immediately transferred to a hot plate to prevent agar from gelling. 120 tubes (plastic, 16 dram volume) were pre-labeled and arranged into four treatment groups to facilitate pouring on an aluminum foil lined counter top. Silica discs were also heated on hot plates prior to pouring (300°C).

Nutrients were poured into agar flasks and mixed well before transferring to treatment vial. The tube was filled about half full with the mixture, and a hot silica disc was pressed onto the top of the tube, melting the plastic to create a seal. The tube was inverted onto a clean spot of foil and cooled until solid. Once all tubes were solid, each treatment batch was placed upside down in a black plastic bag in a fridge until NDS trays were to be constructed.

B.2 Making Nutrient Diffusing Substrate (NDS) Trays

Trays were made at least two days prior to deployment to allow the silicone to dry completely.

1. Enough plastic test tube trays were covered with three pieces of duct tape.
2. Tape also covered the bottom of the trays where the silicone attached the tubes to the tray.
3. Four random slits were cut on the top of the trays.
4. Treatments vials were removed from the fridge for the required number of trays.
5. In a fume-hood, silicone was dispensed on the bottom of each of the four treatment vials for a tray.
6. Each tube was inserted, silicone side down, into a slit in the tray. The tube was twisted to secure it to the bottom of the tray.
7. When a tray was full, a damp piece of paper towel was put on top of the tray, and placed into a black plastic bag in an incubator. Ten trays were stacked within each bag.
8. Trays were constructed until there were enough prepared for a particular deployment.

APPENDIX C: SIMPER ANALYSIS TABLES

Table C.1. Similarity percentage (SIMPER) tables for the comparison of three-minute kick net community composition between sites on the Steepbank River. Species accounting for up to 70% cumulative dissimilarity are listed as per Clarke (1993).

	Mean Abundance		Dissimilarity		Contribution	
Species	**ST1**	**ST2**	Mean	SD	%	Cumulative
Ephemerella sp.	42.50	189.50	10.89	1.42	12.87	12.87
Ephemerella dorothea/excrucians	114.00	0.00	8.04	0.71	9.51	22.38
Ephemerella excrucians	12.25	152.50	5.75	0.71	6.79	29.17
Tvetenia sp.	7.25	133.50	5.65	0.77	6.67	35.84
Nais sp.	3.25	108.50	4.47	0.71	5.29	41.13
Tricorythodes sp.	4.50	95.50	4.03	0.74	4.77	45.90
Torrenticola sp.	14.75	120.00	3.84	1.08	4.54	50.44
Baetis tricaudatus	0.00	97.50	3.81	1.63	4.50	54.94
Baetis tricaudatus group	50.50	0.00	3.19	0.72	3.77	58.71
Baetis sp.	54.75	137.00	2.94	0.81	3.48	62.19
Heptagenia sp.	3.00	62.50	2.60	1.11	3.07	65.26
Cricotopus trifascia	0.50	57.00	2.41	0.71	2.85	68.11
Sperchon sp.	0.00	57.50	2.30	10.93	2.72	70.83
ST1 vs. ST2 Average Dissimilarity: 84.59%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**ST1**	**ST3**	Mean	SD	%	Cumulative
Baetis tricaudatus	0.00	725.00	23.92	1.17	25.18	25.18
Ephemerella excrucians	12.25	204.60	7.89	0.93	8.31	33.49
Ephemerella sp.	42.50	132.80	7.23	0.77	7.61	41.10
Ephemerella dorothea/excrucians	114.00	0.00	5.27	0.57	5.54	46.64
Baetis sp.	54.75	45.40	4.64	1.42	4.88	51.52
Heptagenia sp.	3.00	113.20	4.22	1.66	4.44	55.96
Torrenticola sp.	14.75	136.60	3.64	1.36	3.83	59.79
Baetis tricaudatus group	50.50	0.00	2.48	0.71	2.61	62.40
Nais sp.	3.25	58.80	2.12	0.64	2.23	64.63
Atherix spp.	4.50	64.80	1.92	3.06	2.02	66.65
Tvetenia sp.	7.25	37.40	1.63	0.73	1.71	68.36
Brachycentrus occidentalis	0.00	31.40	1.62	0.79	1.70	70.06
ST1 vs. ST3 Average Dissimilarity: 95.02%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**ST2**	**ST3**	Mean	SD	%	Cumulative
Ephemerella excrucians	152.50	204.60	7.30	0.71	16.10	16.10
Baetis sp.	137.00	45.40	4.70	1.15	10.37	26.47
Baetis tricaudatus	97.50	725.00	3.32	0.73	7.32	33.79
Ephemerella sp.	189.50	132.80	2.64	0.71	5.83	39.62
Lopescladius sp.	0.00	32.00	1.74	1.29	3.83	43.45
Sperchon sp.	57.50	54.60	1.64	0.75	3.62	47.07
Torrenticola sp.	120.00	136.60	1.29	1.31	2.85	49.92
Hydropsyche sp.	15.50	27.00	1.27	6.38	2.81	52.72
Stempellinella sp.	23.00	25.80	1.18	0.82	2.61	55.33
Tricorythodes sp.	95.50	65.40	1.18	0.91	2.60	57.93
Tvetenia sp.	133.50	37.40	1.12	1.32	2.48	60.41
Polypedilum sp.	26.00	17.60	1.09	0.73	2.41	62.82
Hemerodromia sp.	55.50	34.60	1.09	0.97	2.40	65.22
Micropsectra sp.	9.50	20.40	1.07	0.93	2.36	67.59
Parametriocnemus sp.	7.50	14.20	0.73	8.75	1.60	69.19
Atherix spp.	31.50	64.80	0.72	3.07	1.59	70.78
ST2 vs. ST3 Average Dissimilarity: 45.34%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**ST1**	**ST4**	Mean	SD	%	Cumulative
Baetis tricaudatus	0.00	638.17	37.91	1.38	40.05	40.05
Ephemerella dorothea/excrucians	114.00	0.00	4.44	0.65	4.69	44.74
Lepidostoma sp.	6.75	59.00	3.79	1.24	4.01	48.75
Acentrella turbida	0.00	73.50	3.54	0.52	3.74	52.49
Baetis sp.	54.72	0.00	3.14	0.85	3.31	55.80
Heptagenia sp.	3.00	43.83	3.12	0.66	3.29	59.10
Ephemerella sp.	42.50	13.50	3.01	1.38	3.18	62.27
Hydropsyche sp.	0.25	30.50	2.63	0.83	2.78	65.05
Baetis tricaudatus group	50.50	0.00	2.38	0.84	2.52	67.57
Baetis bicaudatus	49.25	0.00	2.05	0.50	2.16	69.74
Torrenticola sp.	14.75	53.83	2.04	0.56	2.15	71.89
ST1 vs. ST4 Average Dissimilarity: 94.64%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**ST2**	**ST4**	Mean	SD	%	Cumulative
Baetis tricaudatus	97.50	638.17	10.04	1.00	15.56	15.56
Ephemerella excrucians	152.50	16.17	5.06	0.71	7.83	23.39
Baetis sp.	137.00	0.00	4.35	2.59	6.73	30.12
Ephemerella sp.	189.50	13.50	3.65	0.71	5.65	35.77
Tvetenia sp.	133.50	29.83	3.53	1.25	5.47	41.24
Acentrella turbida	7.00	73.50	3.05	0.71	4.72	45.96
Nais sp.	108.50	8.50	2.65	0.83	4.11	50.06
Pteronarcys dorsata	5.00	29.00	2.27	0.76	3.51	53.58
Lepidostoma sp.	29.50	59.00	2.22	1.24	3.43	57.01
Tricorythodes sp.	95.50	20.17	1.76	0.79	2.73	59.73
Heptagenia sp.	62.50	43.83	1.53	1.41	2.37	62.11
Hydropsyche sp.	15.50	30.50	1.43	0.71	2.22	64.32
Cricotopus trifascia	57.00	0.00	1.40	0.71	2.16	66.49
Sperchon sp.	57.50	19.00	1.39	0.90	2.15	68.63
Stempellinella sp.	23.00	10.17	1.30	2.20	2.01	70.65
ST2 vs. ST4 Average Dissimilarity: 64.57%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**ST3**	**ST4**	Mean	SD	%	Cumulative
Baetis tricaudatus	725.00	638.17	7.22	1.24	16.09	16.09
Ephemerella excrucians	204.60	16.17	4.51	0.80	10.04	26.13
Heptagenia sp.	113.20	43.83	2.49	1.26	5.53	31.66
Ephemerella sp.	132.80	13.50	2.34	0.45	5.22	36.88
Torrenticola sp.	136.60	53.83	2.09	1.15	4.65	41.53
Pteronarcys dorsata	14.00	29.00	1.33	0.57	2.97	44.50
Tvetenia sp.	37.40	29.83	1.29	1.10	2.87	47.37
Tricorythodes sp.	65.40	20.17	1.20	0.58	2.68	50.05
Nais sp.	58.80	8.50	1.19	0.65	2.65	52.69
Brachycentrus occidentalis	31.40	25.00	1.14	0.95	2.53	55.23
Atherix spp.	64.80	24.33	1.00	1.17	2.23	57.46
Sperchon sp.	54.60	19.00	0.99	1.03	2.21	59.67
Simulium sp.	21.40	45.33	0.95	1.56	2.12	61.79
Lepidostoma sp.	25.80	59.00	0.95	1.25	2.11	63.89
Acentrella turbida	34.00	73.50	0.92	0.62	2.04	65.94
Baetis sp.	45.40	0.00	0.91	0.45	2.03	67.97
Hydropsyche sp.	27.00	30.50	0.83	1.80	1.85	69.82
Diphetor hageni	9.40	19.17	0.83	0.69	1.85	71.67
ST3 vs. ST4 Average Dissimilarity: 44.91%						

Table C.2. Similarity percentage (SIMPER) tables for the comparison of three-minute kick net community composition between months on the Steepbank River. Species accounting for up to 70% cumulative dissimilarity are listed as per Clarke (1993).

	Mean Abundance		Dissimilarity		Contribution	
Species	**May**	**June**	Mean	SD	%	Cumulative
Attenella margarita	0.00	531.00	15.69	-	18.97	18.97
Baetis tricaudatus	252.50	119.00	14.06	-	17.01	35.98
Acentrella sp.	0.00	244.00	7.21	-	8.72	44.69
Dannella simplex	0.00	106.00	3.13	-	3.79	48.48
Pteronarcys dorsata	34.75	19.00	3.13	-	3.79	52.27
Simulium sp.	0.00	88.00	2.60	-	3.14	55.41
Acentrella insignificans	0.00	81.00	2.39	-	2.89	58.31
Baetis flavistriga	0.00	75.00	2.22	-	2.68	60.99
Diphetor hageni	18.50	0.00	1.92	-	2.32	63.31
Acentrella turbida	0.00	63.00	1.86	-	2.25	65.56
Ecdyonurus simplicoides	0.00	63.00	1.86	-	2.25	67.81
Brachycentrus occidentalis	2.25	63.00	1.71	-	2.07	69.88
Lepidostoma sp.	45.75	13.00	1.68	-	2.04	71.92
May vs. June Average Dissimilarity: 82.69%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**May**	**July**	Mean	SD	%	Cumulative
Baetis tricaudatus	252.50	747.50	11.52	3.99	21.91	21.91
Tricorythodes sp.	1.25	148.50	5.85	1.08	11.13	33.03
Sperchon sp.	17.25	98.50	3.62	2.03	6.89	39.92
Acentrella turbida	0.00	95.50	3.39	26.25	6.44	46.37
Orthocladius sp.	5.50	82.50	2.54	2.88	4.84	51.21
Pteronarcys dorsata	34.75	0.00	2.02	0.77	3.85	55.06
Torrenticola sp.	65.50	101.00	1.71	0.88	3.26	58.32
Lepidostoma sp.	45.75	8.50	1.65	7.80	3.14	61.46
Stempellinella sp.	29.25	16.00	1.27	2.06	2.42	63.88
Diphetor hageni	18.50	0.00	1.01	0.71	1.92	65.80
Atherix spp.	26.50	54.00	0.97	0.88	1.84	67.64
Eukiefferiella sp.	0.00	22.50	0.81	8.77	1.54	69.19
Micrasema sp.	13.50	0.00	0.78	68.23	1.49	70.68
May vs. July Average Dissimilarity: 52.56%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**June**	**July**	Mean	SD	%	Cumulative
Baetis tricaudatus	119.00	747.50	21.25	-	27.05	27.05
Attenella margarita	531.00	0.00	14.45	-	18.39	45.45
Acentrella sp.	244.00	0.00	6.64	-	8.45	53.90
Dannella simplex	106.00	0.00	2.88	-	3.67	57.57
Orthocladius sp.	25.00	82.50	2.37	-	3.01	60.58
Acentrella insignificans	81.00	13.00	2.20	-	2.81	63.39
Sperchon sp.	0.00	98.50	2.07	-	2.63	66.02
Baetis flavistriga	75.00	2.50	2.04	-	2.60	68.62
Ecdyonurus simplicoides	63.00	0.00	1.71	-	2.18	70.80
June vs. July Average Dissimilarity: 78.56%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**May**	**August**	Mean	SD	%	Cumulative
Ephemerella sp.	36.25	281.00	9.96	1.66	12.68	12.68
Ephemerella dorothea/excrucians	106.75	0.00	6.79	0.50	8.65	21.33
Baetis tricaudatus	252.50	66.00	5.37	0.97	6.84	28.16
Baetis sp.	75.00	89.25	4.02	0.86	5.12	33.28
Tvetenia sp.	20.75	114.50	3.28	1.06	4.17	37.46
Nais sp.	7.50	110.00	3.02	0.99	3.84	41.30
Ephemerella excrucians	92.75	0.00	2.71	0.65	3.45	44.75
Acentrella turbida	0.00	92.00	2.68	0.73	3.41	48.16
Baetis tricaudatus group	42.00	0.25	2.66	0.50	3.38	51.54
Heptagenia sp.	22.25	105.50	2.39	1.30	3.04	54.59
Torrenticola sp.	65.50	103.50	2.31	2.96	2.94	57.52
Tricorythodes sp.	1.25	78.00	2.22	1.02	2.83	60.35
Rheotanytarsus sp.	33.00	13.25	2.14	0.60	2.73	63.08
Lepidostoma sp.	45.75	58.50	1.46	1.61	1.85	64.94
Cricotopus trifascia	0.00	51.75	1.45	0.88	1.85	66.79
Hydropsyche sp.	14.25	60.50	1.36	0.78	1.73	68.51
Simulium sp.	0.00	42.25	1.20	0.91	1.53	70.04
May vs. August Average Dissimilarity: 78.55%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**June**	**August**	Mean	SD	%	Cumulative
Attenella margarita	531.00	3.50	14.15	-	17.60	17.60
Acentrella sp.	244.00	0.25	6.50	-	8.09	25.69
Acentrella turbida	63.00	92.00	5.33	-	6.63	32.32
Heptagenia sp.	0.00	105.50	5.17	-	6.43	38.75
Lepidostoma sp.	13.00	58.50	3.81	-	4.74	43.49
Hydropsyche sp.	0.00	60.50	3.68	-	4.57	48.06
Torrenticola sp.	31.00	103.50	3.01	-	3.75	51.81
Dannella simplex	106.00	0.00	2.82	-	3.51	55.32
Simulium sp.	88.00	42.25	2.34	-	2.92	58.24
Ephemerella sp.	0.00	281.00	2.16	-	2.68	60.92
Acentrella insignificans	81.00	6.00	2.00	-	2.49	63.41
Baetis flavistriga	75.00	5.25	2.00	-	2.49	65.89
Ecdyonurus simplicoides	63.00	0.00	1.68		2.09	67.98
Zapada cinctipes	0.00	23.25	1.68		2.09	70.07
June vs. August Average Dissimilarity: 80.39%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**July**	**August**	Mean	SD	%	Cumulative
Baetis tricaudatus	747.50	66.00	15.64	2.54	24.05	24.05
Ephemerella sp.	0.00	281.00	8.07	0.98	12.18	36.23
Heptagenia sp.	12.00	105.50	3.70	2.51	5.58	41.81
Lepidostoma sp.	8.50	58.50	2.62	1.33	3.96	45.76
Hydropsyche sp.	8.00	60.50	2.61	1.50	3.94	49.71
Nais sp.	2.50	110.00	2.40	0.76	3.63	53.34
Baetis sp.	0.00	89.25	2.37	0.71	3.58	56.92
Acentrella turbida	95.50	92.00	2.23	0.80	3.36	60.28
Tvetenia sp.	26.00	114.50	1.89	0.93	2.84	63.13
Tricorythodes sp.	148.50	78.00	1.87	0.91	2.82	65.95
Orthocladius sp.	82.50	14.25	1.74	0.89	2.62	68.57
Lopescladius sp.	0.00	39.50	1.70	0.90	2.56	71.13
July vs. August Average Dissimilarity: 66.27%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**May**	**September**	Mean	SD	%	Cumulative
Ephemerella dorothea/excrucians	106.75	9.67	7.87	0.58	13.51	13.51
Baetis tricaudatus	252.50	341.67	5.57	0.85	9.55	23.06
Ephemerella excrucians	92.75	80.00	3.64	0.66	6.25	29.31
Baetis tricaudatus group	42.00	11.00	2.67	0.58	4.58	33.89
Rheotanytarsus sp	33.00	1.33	2.41	0.61	4.14	38.03
Ephemerella sp.	36.25	8.33	2.37	0.58	4.07	42.10
Torrenticola sp.	65.50	19.67	2.18	1.35	3.74	45.84
Pteronarcys dorsata	34.75	7.33	2.16	0.70	3.70	49.54
Baetis sp.	75.00	21.00	1.82	0.58	3.12	52.67
Heptagenia sp.	22.25	27.33	1.80	1.30	3.09	55.76
Stempellinella sp.	29.25	0.00	1.76	1.15	3.01	58.77
Lepidostoma sp.	45.75	12.00	1.74	2.58	2.98	61.76
Micropsectra sp.	24.00	0.00	1.51	1.44	2.59	64.35
Tvetenia sp.	20.75	2.33	1.25	1.26	2.15	66.50
Zavrelia sp.	14.75	0.00	1.17	0.58	2.00	68.50
Caenis sp.	15.00	0.00	1.09	0.58	1.87	70.37
May vs. September Average Dissimilarity: 58.26%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**June**	**September**	Mean	SD	%	Cumulative
Attenella margarita	531.00	0.00	20.42	-	23.31	23.31
Baetis tricaudatus	119.00	341.67	15.04	-	17.16	40.47
Acentrella sp.	244.00	0.00	9.38	-	10.71	51.19
Dannella simplex	106.00	0.00	4.08	-	4.65	55.84
Acentrella insignificans	81.00	0.00	3.12	-	3.56	59.39
Baetis flavistriga	75.00	0.00	2.88	-	3.29	62.69
Simulium sp.	88.00	5.00	2.85	-	3.25	65.94
Ecdyonurus simplicoides	63.00	0.00	2.42	-	2.77	68.70
Acentrella turbida	63.00	1.00	2.31	-	2.63	71.33
June vs. September Average Dissimilarity: 87.62%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**July**	**September**	Mean	SD	%	Cumulative
Baetis tricaudatus	747.50	341.67	9.43	1.01	18.71	18.71
Tricorythodes sp.	148.50	6.67	5.32	1.42	10.55	29.26
Ephemerella excrucians	10.50	80.00	3.96	0.90	7.84	37.10
Acentrella turbida	95.50	1.00	3.64	3.10	7.23	44.33
Orthocladius sp.	82.50	0.00	3.24	1.69	6.43	50.76
Sperchon sp.	98.50	15.33	2.90	9.08	5.74	56.50
Torrenticola sp.	101.00	19.67	2.77	5.60	5.50	62.00
Heptagenia sp.	12.00	27.33	1.86	1.50	3.70	65.70
Brachycentrus occidentalis	23.00	21.67	1.56	4.41	3.10	68.80
Atherix spp.	54.00	12.33	1.38	5.19	2.73	71.53
July vs. September Average Dissimilarity: 50.43%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**August**	**September**	Mean	SD	%	Cumulative
Baetis sp.	89.25	21.00	9.70	0.77	11.60	11.60
Ephemerella sp.	281.00	8.33	9.41	1.54	11.25	22.85
Baetis tricaudatus	66.00	341.67	8.16	1.13	9.75	32.59
Acentrella turbida	92.00	1.00	4.15	0.76	4.96	37.56
Baetis tricaudatus group	0.25	11.00	4.10	0.58	4.90	42.46
Ephemerella dorothea/excrucians	0.00	9.67	3.72	0.58	4.44	46.90
Heptagenia sp.	105.50	27.33	3.47	0.94	4.15	51.05
Enchytraeus spp.	8.25	0.00	2.64	0.60	3.16	54.21
Torrenticola sp.	103.50	19.67	2.50	0.88	2.99	57.20
Tvetenia sp.	114.50	2.33	2.38	1.83	2.84	60.04
Tricorythodes sp.	78.00	6.67	2.32	2.28	2.77	62.81
Hydropsyche sp.	60.50	7.00	2.31	1.07	2.77	65.58
Lepidostoma sp.	58.50	12.00	2.19	0.75	2.62	68.20
Ephemerella excrucians	0.00	80.00	1.97	0.72	2.36	70.55
August vs. September Average Dissimilarity: 83.69%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**May**	**October**	Mean	SD	%	Cumulative
Baetis tricaudatus	252.50	1245.33	20.55	1.12	26.97	26.97
Ephemerella dorothea/excrucians	106.75	0.00	8.01	0.58	10.51	37.48
Ephemerella excrucians	92.75	280.67	5.43	0.77	7.13	44.61
Baetis bicaudatus	0.00	65.67	3.70	0.58	4.85	49.46
Torrenticola sp.	65.50	112.33	3.25	1.86	4.26	53.72
Baetis tricaudatus group	42.00	0.00	3.15	0.58	4.14	57.86
Baetis sp.	75.00	0.00	2.91	0.58	3.82	61.67
Ephemerella sp.	36.25	0.00	2.72	0.58	3.57	65.24
Heptagenia sp.	22.25	116.33	2.51	0.83	3.30	68.54
Rheotanytarsus sp.	33.00	4.67	2.32	0.62	3.05	71.59
May vs. October Average Dissimilarity: 76.21%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**June**	**October**	Mean	SD	%	Cumulative
Baetis tricaudatus	119.00	1245.33	35.01	-	41.86	41.86
Attenella margarita	531.00	0.00	13.12	-	15.69	57.55
Acentrella sp.	244.00	0.00	6.03	-	7.21	64.76
Dannella simplex	106.00	0.00	2.62	-	3.13	67.89
Acentrella insignificans	81.00	0.00	2.00	-	2.39	70.28
June vs. October Average Dissimilarity: 83.64%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**July**	**October**	Mean	SD	%	Cumulative
Baetis tricaudatus	747.50	1245.33	20.88	3.29	36.95	36.95
Ephemerella excrucians	10.50	280.67	6.28	0.83	11.11	48.06
Heptagenia sp.	12.00	116.33	3.02	0.89	5.34	53.40
Tricorythodes sp.	148.50	5.67	2.56	2.04	4.53	57.94
Torrenticola sp.	101.00	112.33	2.49	2.46	4.41	62.35
Acentrella turbida	95.50	0.00	2.07	1.79	3.66	66.01
Orthocladius sp.	82.50	9.67	1.63	0.92	2.89	68.89
Sperchon sp.	98.50	19.00	1.48	2.22	2.63	71.52
July vs. October Average Dissimilarity: 56.50%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**August**	**October**	Mean	SD	%	Cumulative
Baetis tricaudatus	66.00	1245.33	20.68	1.13	24.82	24.82
Baetis bicaudatus	0.00	65.67	18.71	0.58	22.45	47.27
Ephemerella excrucians	0.00	280.67	8.18	1.23	9.82	57.09
Ephemerella sp.	281.00	0.00	3.51	0.79	4.21	61.30
Heptagenia sp.	105.50	116.33	3.03	1.73	3.63	64.93
Acentrella turbida	92.00	0.00	2.60	0.73	3.12	68.05
Torrenticola sp.	103.50	112.33	2.15	1.30	2.58	70.63
August vs. October Average Dissimilarity: 83.33%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**September**	**October**	Mean	SD	%	Cumulative
Baetis tricaudatus	341.67	1245.33	21.78	1.13	31.51	31.51
Baetis bicaudatus	0.00	65.67	14.12	0.58	20.43	51.94
Ephemerella excrucians	80.00	280.67	6.75	1.24	9.77	61.71
Baetis sp.	21.00	0.00	4.52	0.58	6.53	68.24
Baetis tricaudatus group	11.00	0.00	2.37	0.58	3.42	71.67
September vs. October Average Dissimilarity: 69.12%						

Table C.3. Similarity percentage (SIMPER) tables for the comparison of three-minute kick net community composition between sites on the Ells River. Species accounting for up to 70% cumulative dissimilarity are listed as per Clarke (1993).

	Mean Abundance		Dissimilarity		Contribution	
Species	**EL1**	**EL3**	Mean	SD	%	Cumulative
Micropsectra sp.	75.00	754.20	8.82	0.64	11.98	11.98
Rheotanytarsus sp.	2048.17	141.80	5.54	1.82	7.53	19.51
Tvetenia sp.	136.83	725.40	4.97	1.04	6.75	26.26
Ephemerella dorothea/excrucians	591.50	4.00	4.88	0.74	6.63	32.89
Tricorythodes sp.	508.00	287.00	4.46	1.27	6.06	38.95
Ephemerella sp.	479.00	32.60	4.44	0.98	6.03	44.99
Baetis sp.	532.83	280.80	3.80	0.87	5.17	50.16
Baetis tricaudatus group	304.33	281.20	3.62	1.22	4.91	55.07
Simulium sp.	416.83	56.20	3.35	0.79	4.56	59.63
Orthocladius sp.	313.50	61.60	2.93	0.89	3.98	63.61
Lopescladius sp.	0.00	190.80	2.08	0.55	2.83	66.44
Chimarra sp.	0.00	177.00	1.78	0.70	2.41	68.86
Acerpenna pygmaea	13.50	166.80	1.55	1.11	2.11	70.97
EL1 vs. EL3 Average Dissimilarity: 73.58%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**EL1**	**EL2**	Mean	SD	%	Cumulative
Rheotanytarsus sp.	2048.17	289.50	14.78	0.71	21.45	21.45
Micropsectra sp.	75.00	957.17	6.08	0.50	8.82	30.27
Ephemerella dorothea/excrucians	591.50	163.83	3.85	0.75	5.58	35.85
Ephemerella sp.	479.00	63.67	3.22	1.09	4.67	40.52
Tanytarsus sp.	60.00	273.00	3.22	0.70	4.67	45.19
Acerpenna pygmaea	13.50	488.50	3.12	0.65	4.53	49.72
Tricorythodes sp.	508.00	118.50	3.11	0.73	4.52	54.24
Baetis sp.	532.83	200.33	3.03	0.84	4.40	58.64
Baetis tricaudatus group	304.33	258.67	2.96	1.05	4.29	62.93
Tvetenia sp.	136.83	370.67	2.17	1.27	3.15	66.08
Simulium sp.	416.83	135.00	1.97	0.61	2.85	68.93
Nais sp.	6.67	224.17	1.83	0.66	2.66	71.59
EL1 vs. EL2 Average Dissimilarity: 68.92%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**EL2**	**EL3**	Mean	SD	%	Cumulative
Micropsectra sp.	957.17	754.20	11.37	0.77	17.18	17.18
Tvetenia sp.	370.67	725.40	5.22	0.76	7.88	25.06
Rheotanytarsus sp.	289.50	141.80	4.57	1.53	6.90	31.96
Nais sp.	224.17	3.40	3.07	0.74	4.64	36.60
Orthocladius sp.	209.83	61.60	2.91	0.88	4.40	41.00
Acerpenna pygmaea	488.50	166.80	2.29	0.53	3.47	44.47
Tanytarsus sp.	273.00	88.00	2.11	0.68	3.19	47.66
Thienemannimyia group	239.83	134.20	1.98	1.63	2.99	50.64
Lopescladius sp.	7.17	190.80	1.96	0.57	2.97	53.61
Baetis tricaudatus group	258.67	281.20	1.70	1.57	2.56	56.17
Cricotopus trifascia	0.00	135.80	1.63	0.56	2.47	58.64
Chimarra sp.	34.67	177.00	1.63	0.74	2.46	61.10
Tricorythodes sp.	118.50	287.00	1.59	0.80	2.40	63.50
Zavrelia sp.	166.67	0.00	1.39	1.05	2.09	65.59
Ephemerella dorothea/excrucians	163.83	4.00	1.28	0.75	1.93	67.52
Cheumatopsyche sp.	0.00	126.60	1.27	0.91	1.92	69.44
Polypedilum sp.	35.00	202.00	1.23	1.14	1.86	71.30
EL2 vs. EL3 Average Dissimilarity: 66.17%						

Table C.4. Similarity percentage (SIMPER) tables for the comparison of three-minute kick net community composition between months on the Ells River. Species accounting for up to 70% cumulative dissimilarity are listed as per Clarke (1993).

	Mean Abundance		Dissimilarity		Contribution	
Species	**May**	**June**	Mean	SD	%	Cumulative
Rheotanytarsus sp.	4050.00	940.00	15.73	0.74	22.60	22.60
Baetis tricaudatus group	465.50	266.67	8.53	0.93	12.25	34.85
Tanytarsus sp.	433.50	113.33	7.83	0.71	11.25	46.10
Baetis sp.	83.50	650.00	5.72	1.53	8.21	54.31
Ephemerella dorothea/excrucians	658.00	83.33	3.08	0.97	4.42	58.73
Tricorythodes sp.	56.50	413.33	2.68	1.04	3.85	62.58
Polypedilum sp.	10.00	356.67	2.63	2.88	3.78	66.36
Acentrella sp.	0.00	180.00	2.37	0.85	3.41	69.77
Orthocladius sp.	11.00	120.00	1.89	1.38	2.71	72.48
May vs. June Average Dissimilarity: 69.62%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**May**	**July**	Mean	SD	%	Cumulative
Rheotanytarsus sp.	4050.00	255.67	29.43	0.85	41.46	41.46
Baetis tricaudatus group	465.50	53.00	6.03	0.72	8.50	49.96
Ephemerella dorothea/excrucians	658.00	0.00	4.81	0.84	6.77	56.73
Simulium sp.	22.00	312.00	4.05	4.73	5.70	62.43
Baetis sp.	83.50	304.67	2.47	3.68	3.49	65.92
Orthocladius sp.	11.00	138.67	2.25	1.19	3.17	69.09
Ephemerella sp.	200.00	10.00	1.30	0.71	1.83	70.92
May vs. July Average Dissimilarity: 70.98%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**June**	**July**	Mean	SD	%	Cumulative
Rheotanytarsus sp.	940.00	255.67	8.78	1.41	13.62	13.62
Micropsectra sp.	113.33	1003.33	8.40	0.66	13.03	26.65
Tvetenia sp.	840.00	167.67	5.63	0.73	8.73	35.38
Tanytarsus sp.	113.33	290.33	4.68	0.72	7.26	42.64
Tricorythodes sp.	413.33	297.67	4.17	1.17	6.47	49.11
Baetis sp.	650.00	304.67	3.65	2.12	5.67	54.78
Polypedilum sp.	356.67	77.67	2.65	1.28	4.12	58.90
Baetis tricaudatus group	266.67	53.00	2.02	1.36	3.14	62.04
Simulium sp.	200.00	312.00	1.99	1.36	3.10	65.13
Torrenticola sp.	230.00	81.33	1.86	1.48	2.89	68.02
Ophiogomphus sp.	146.67	85.67	1.76	1.97	2.74	70.75
June vs. July Average Dissimilarity: 64.43%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**May**	**August**	Mean	SD	%	Cumulative
Rheotanytarsus sp.	4050.00	343.00	15.52	0.79	19.73	19.73
Orthocladius sp.	11.00	782.00	7.74	3.40	9.84	29.58
Baetis tricaudatus group	465.50	499.33	7.37	2.35	9.37	38.95
Tanytarsus sp.	433.50	120.00	6.15	0.93	7.82	46.76
Nais sp.	0.00	251.33	4.54	0.73	5.77	52.53
Simulium sp.	22.00	639.33	4.41	1.27	5.60	58.14
Tricorythodes sp.	56.50	659.00	3.83	1.03	4.87	63.00
Baetis sp.	83.50	656.67	3.72	0.80	4.73	67.74
Ephemerella dorothea/excrucians	658.00	0.00	2.93	0.93	3.72	71.45
May vs. August Average Dissimilarity: 78.66%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**June**	**August**	Mean	SD	%	Cumulative
Tvetenia sp.	840.00	138.00	6.32	0.66	9.70	9.70
Baetis sp.	650.00	656.67	6.00	10.58	9.21	18.90
Orthocladius sp.	120.00	782.00	5.16	1.30	7.92	26.83
Rheotanytarsus sp.	940.00	343.00	4.54	1.59	6.97	33.79
Nais sp.	0.00	251.33	3.46	0.59	5.30	39.10
Simulium sp.	200.00	639.33	2.73	0.81	4.19	43.28
Polypedilum sp.	356.67	0.00	2.55	2.97	3.91	47.19
Baetis tricaudatus group	266.67	499.33	2.40	1.48	3.69	50.88
Thienemannimyia group	170.00	209.33	1.90	0.87	2.91	53.79
Cricotopus trifascia	0.00	183.33	1.78	0.58	2.72	56.52
Ephemerella sp.	46.67	243.67	1.78	1.71	2.72	59.24
Tanytarsus sp.	113.33	120.00	1.67	1.02	2.57	61.81
Hemerodromia sp.	6.67	228.00	1.65	1.48	2.53	64.34
Tricorythodes sp.	413.33	659.00	1.39	0.80	2.14	66.47
Acentrella sp.	180.00	65.33	1.31	1.21	2.01	68.49
Ophiogomphus sp.	146.67	41.33	1.12	0.90	1.72	70.21
June vs. August Average Dissimilarity: 65.18%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**July**	**August**	Mean	SD	%	Cumulative
Micropsectra sp.	1003.33	29.33	10.42	0.60	16.11	16.11
Orthocladius sp.	138.67	782.00	5.04	1.25	7.79	23.91
Tricorythodes sp.	297.67	659.00	5.01	1.02	7.75	31.66
Tanytarsus sp.	290.33	120.00	4.05	0.99	6.25	37.91
Baetis sp.	304.67	656.67	3.69	0.91	5.70	43.62
Simulium sp.	312.00	639.33	3.33	0.86	5.16	48.77
Baetis tricaudatus group	53.00	499.33	3.20	0.76	4.95	53.72
Nais sp.	4.67	251.33	2.73	0.61	4.22	57.94
Cricotopus trifascia	0.00	183.33	1.99	0.58	3.08	61.02
Rheotanytarsus sp.	255.67	343.00	1.91	1.14	2.96	63.98
Ephemerella sp.	10.00	243.67	1.78	1.04	2.76	66.73
Thienemannimyia group	91.00	209.33	1.64	0.79	2.54	69.28
Hemerodromia sp.	108.67	228.00	1.52	2.20	2.35	71.62
July vs. August Average Dissimilarity: 64.68%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**May**	**September**	Mean	SD	%	Cumulative
Rheotanytarsus sp.	4050.00	309.67	30.62	1.05	42.72	42.72
Baetis tricaudatus group	465.50	128.67	5.83	0.73	8.13	50.85
Tanytarsus sp.	433.50	0.00	5.65	0.71	7.89	58.74
Nais sp.	0.00	185.67	3.63	0.71	5.07	63.81
Thienemannimyia group	80.00	66.67	1.68	1.60	2.35	66.15
Ephemerella sp.	200.00	309.33	1.65	1.61	2.31	68.46
Ephemerella dorothea/excrucians	658.00	514.33	1.39	0.80	1.95	70.41
May vs. September Average Dissimilarity: 71.67%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**June**	**September**	Mean	SD	%	Cumulative
Rheotanytarsus sp.	940.00	309.67	11.07	2.06	15.85	15.85
Baetis sp.	650.00	99.67	5.50	27.69	7.87	23.73
Tvetenia sp.	840.00	376.00	5.50	0.94	7.87	31.60
Ephemerella dorothea/excrucians	83.33	514.33	4.27	0.97	6.11	37.71
Tricorythodes sp.	413.33	204.67	2.90	0.89	4.15	41.86
Nais sp.	0.00	185.67	2.78	0.58	3.99	45.85
Ephemerella sp.	46.67	309.33	2.36	0.79	3.38	49.23
Lopescladius sp.	13.33	257.33	2.21	0.64	3.17	52.40
Polypedilum sp.	356.67	142.67	1.92	1.02	2.76	55.15
Simulium sp.	200.00	19.00	1.88	2.12	2.70	57.85
Torrenticola sp.	230.00	185.67	1.85	1.51	2.65	60.50
Acerpenna pygmaea	6.67	152.33	1.75	1.06	2.51	63.01
Thienemannimyia group	170.00	66.67	1.64	4.46	2.34	65.35
Acentrella sp.	180.00	52.33	1.49	1.35	2.13	67.49
Micropsectra sp.	113.33	47.67	1.46	1.21	2.09	69.58
Baetis tricaudatus group	266.67	128.67	1.35	0.84	1.93	71.51
June vs. September Average Dissimilarity: 69.85%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**July**	**September**	Mean	SD	%	Cumulative
Micropsectra sp.	1003.33	47.67	9.86	0.65	14.96	14.96
Ephemerella dorothea/ excrucians	0.00	514.33	7.45	0.72	11.30	26.27
Ephemerella sp.	10.00	309.33	4.27	0.72	6.48	32.75
Simulium sp.	312.00	19.00	3.96	1.12	6.00	38.75
Tanytarsus sp.	290.33	0.00	3.36	0.67	5.09	43.84
Rheotanytarsus sp.	255.67	309.67	3.13	1.26	4.75	48.59
Baetis sp.	304.67	99.67	2.84	0.80	4.31	52.90
Lopescladius sp.	0.00	257.33	2.54	0.62	3.85	56.76
Tvetenia sp.	167.67	376.00	2.20	1.16	3.34	60.10
Nais sp.	4.67	185.67	2.13	0.58	3.24	63.34
Tricorythodes sp.	297.67	204.67	1.72	0.98	2.62	65.95
Chimarra sp.	192.33	38.00	1.51	0.58	2.29	68.25
Ferrissia sp.	24.67	128.67	1.50	0.67	2.28	70.52
July vs. September Average Dissimilarity: 65.90%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**August**	**September**	Mean	SD	%	Cumulative
Orthocladius sp.	782.00	23.67	5.95	1.37	10.10	10.10
Simulium sp.	639.33	19.00	4.04	0.78	6.86	16.97
Rheotanytarsus sp.	343.00	309.67	3.99	1.18	6.77	23.74
Baetis tricaudatus group	499.33	128.67	3.86	1.23	6.56	30.30
Baetis sp.	656.67	99.67	3.83	0.67	6.50	36.80
Ephemerella dorothea/excrucians	0.00	514.33	3.48	0.90	5.91	42.71
Tricorythodes sp.	659.00	204.67	3.44	0.74	5.84	48.55
Lopescladius sp.	34.00	257.33	2.81	0.59	4.77	53.32
Tvetenia sp.	138.00	376.00	2.64	1.13	4.49	57.81
Cricotopus trifascia	183.33	43.00	1.77	0.58	3.01	60.81
Thienemannimyia group	209.33	66.67	1.40	1.67	2.38	63.19
Polypedilum sp.	0.00	142.67	1.39	1.38	2.35	65.54
Hemerodromia sp.	228.00	71.67	1.21	1.27	2.05	67.60
Ephemerella sp.	243.67	309.33	1.14	12.25	1.94	69.54
Chimarra sp.	96.33	38.00	1.07	0.73	1.82	71.36
August vs. September Average Dissimilarity: 58.87%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**May**	**October**	Mean	SD	%	Cumulative
Rheotanytarsus sp.	4050.00	363.33	24.61	0.79	33.08	33.08
Micropsectra sp.	0.00	2127.67	16.51	0.71	22.19	55.27
Acerpenna pygmaea	16.50	908.00	6.73	0.72	9.05	64.32
Ephemerella dorothea/excrucians	658.00	481.00	3.24	8.99	4.36	68.68
Thienemannimyia group	80.00	478.67	2.94	1.69	3.95	72.63
May vs. October Average Dissimilarity: 74.39%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**June**	**October**	Mean	SD	%	Cumulative
Micropsectra sp.	113.33	2127.67	14.43	0.81	20.65	20.65
Rheotanytarsus sp.	940.00	363.33	6.56	1.38	9.39	30.05
Acerpenna pygmaea	6.67	908.00	5.77	0.76	8.26	38.30
Baetis sp.	650.00	167.67	3.64	2.05	5.20	43.51
Ephemerella sp.	46.67	396.67	2.98	0.72	4.26	47.77
Ephemerella dorothea/excrucians	83.33	481.00	2.91	1.21	4.16	51.94
Tvetenia sp.	840.00	526.67	2.80	0.77	4.02	55.95
Tricorythodes sp.	413.33	119.00	2.67	0.67	3.82	59.78
Baetis tricaudatus group	266.67	336.67	2.47	3.46	3.53	63.31
Thienemannimyia group	170.00	478.67	2.09	1.34	2.99	66.30
Polypedilum sp.	356.67	200.00	2.01	1.08	2.87	69.17
Orthocladius sp.	120.00	77.67	1.48	3.86	2.12	71.29
June vs. October Average Dissimilarity: 69.86%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**July**	**October**	Mean	SD	%	Cumulative
Micropsectra sp.	1003.33	2127.67	17.55	1.16	24.61	24.61
Ephemerella sp.	10.00	396.67	5.05	0.63	7.09	31.69
Ephemerella dorothea/excrucians	0.00	481.00	4.92	0.87	6.90	38.59
Acerpenna pygmaea	110.00	908.00	4.70	0.75	6.60	45.19
Thienemannimyia group	91.00	478.67	3.29	1.78	4.61	49.80
Tvetenia sp.	167.67	526.67	3.28	0.73	4.61	54.41
Baetis sp.	304.67	167.67	3.23	0.89	4.53	58.94
Simulium sp.	312.00	12.33	3.09	0.96	4.33	63.27
Baetis tricaudatus group	53.00	336.67	2.15	0.94	3.01	66.28
Orthocladius sp.	138.67	77.67	1.83	7.66	2.56	68.84
Tanytarsus sp.	290.33	0.00	1.72	0.76	2.41	71.25
July vs. October Average Dissimilarity: 71.32%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**August**	**October**	Mean	SD	%	Cumulative
Micropsectra sp.	29.33	2127.67	12.33	0.77	17.30	17.30
Acerpenna pygmaea	94.00	908.00	4.91	0.80	6.89	24.19
Orthocladius sp.	782.00	77.67	4.85	1.37	6.80	31.00
Baetis tricaudatus group	499.33	336.67	4.79	1.49	6.72	37.72
Baetis sp.	656.67	167.67	4.78	0.90	6.70	44.42
Tvetenia sp.	138.00	526.67	3.88	0.69	5.44	49.86
Simulium sp.	639.33	12.33	3.67	0.71	5.15	55.02
Tricorythodes sp.	659.00	119.00	3.24	0.62	4.54	59.56
Ephemerella dorothea/excrucians	0.00	481.00	2.69	1.13	3.77	63.32
Cricotopus trifascia	183.33	0.00	2.03	0.58	2.84	66.17
Polypedilum sp.	0.00	200.00	1.95	0.73	2.73	68.90
Thienemannimyia group	209.33	478.67	1.85	7.08	2.60	71.50
August vs. October Average Dissimilarity: 71.26%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**September**	**October**	Mean	SD	%	Cumulative
Micropsectra sp.	47.67	2127.67	12.18	0.75	22.28	22.28
Acerpenna pygmaea	152.33	908.00	4.34	0.70	7.94	30.22
Thienemannimyia group	66.67	478.67	3.57	2.89	6.53	36.76
Rheotanytarsus sp.	309.67	363.33	2.77	0.90	5.06	41.82
Ephemerella dorothea/excrucians	514.33	481.00	2.73	0.90	4.99	46.81
Lopescladius sp.	257.33	27.67	2.24	0.60	4.10	50.91
Tvetenia sp.	376.00	526.67	2.15	0.82	3.93	54.84
Baetis tricaudatus group	128.67	336.67	1.64	1.13	3.01	57.85
Zavrelia sp.	85.67	233.33	1.63	1.02	2.98	60.83
Ephemerella sp.	309.33	396.67	1.40	0.93	2.55	63.38
Baetis sp.	99.67	167.67	1.35	0.89	2.48	65.86
Ferrissia sp.	128.67	50.00	1.33	0.80	2.42	68.28
Hydropsyche sp.	104.67	58.00	1.29	1.12	2.36	70.64
September vs. October Average Dissimilarity: 54.68%						

Table C.5. Similarity percentage (SIMPER) tables for the comparison of rock-basket community composition between sites on the Steepbank River. Species accounting for up to 70% cumulative dissimilarity are listed as per Clarke (1993).

	Mean Abundance		Dissimilarity		Contribution	
Species	**ST4**	**ST1**	Mean	SD	%	Cumulative
Baetis spp.	592.00	61.67	19.27	4.80	27.34	27.34
Simulium sp.	79.33	2.67	12.07	1.29	17.12	44.46
Lepidostoma spp.	57.00	0.33	11.23	5.27	15.93	60.39
Tvetenia sp.	130.83	8.00	4.66	1.81	6.62	67.01
Ephemerella sp.	40.33	27.67	2.60	1.73	3.69	70.70
ST4 vs. ST1 Average Dissimilarity: 70.49%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**ST4**	**ST3**	Mean	SD	%	Cumulative
Simulium sp.	79.33	214.67	12.09	1.56	27.29	27.29
Lepidostoma spp.	57.00	9.33	6.40	6.21	14.45	41.74
Baetis spp.	592.00	143.67	5.55	1.32	12.53	54.27
Ephemerella sp.	40.33	59.67	4.39	1.87	9.91	64.18
Tvetenia sp.	130.83	54.67	3.32	1.16	7.50	71.67
ST4 vs. ST3 Average Dissimilarity: 44.31%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**ST1**	**ST3**	Mean	SD	%	Cumulative
Simulium sp.	2.67	214.67	27.27	5.48	40.91	40.91
Baetis spp.	61.67	143.67	10.79	2.88	16.19	57.10
Tvetenia sp.	8.00	54.67	6.00	1.09	9.00	66.10
Ephemerella sp.	27.67	59.67	4.39	1.49	6.59	72.69
ST1 vs. ST3 Average Dissimilarity: 66.66%						

Table C.6. Similarity percentage (SIMPER) tables for the comparison of rock-basket community composition between months on the Steepbank River. Species accounting for up to 70% cumulative dissimilarity are listed as per Clarke (1993).

	Mean Abundance		Dissimilarity		Contribution	
Species	**August**	**October**	Mean	SD	%	Cumulative
Baetis spp.	963.33	142.00	27.25	3.28	39.36	39.36
Hydropsyche spp.	349.33	5.00	12.94	5.70	18.68	58.04
Tvetenia sp.	212.67	37.22	6.18	2.11	8.93	66.96
Simulium sp.	42.33	111.22	3.30	1.12	4.77	71.73
August vs. October Average Dissimilarity: 69.24%						

Table C.7. Similarity percentage (SIMPER) tables for the comparison of rock-basket community composition between sites on the Ells River. Species accounting for up to 70% cumulative dissimilarity are listed as per Clarke (1993).

	Mean Abundance		Dissimilarity		Contribution	
Species	**EL1**	**EL2**	Mean	SD	%	Cumulative
Ephemerella sp.	370.73	30.25	15.80	1.30	25.83	25.83
Simulium sp.	212.80	264.75	8.75	1.00	14.31	40.13
Thienemannimyia group	133.67	61.25	5.59	1.42	9.14	49.27
Tvetenia sp.	128.87	235.42	4.90	1.25	8.01	57.28
Baetis spp.	86.07	138.33	4.46	0.97	7.29	64.57
Tricorythodes minutus	85.13	14.75	2.51	1.24	4.10	68.67
Rheotanytarsus spp.	125.87	135.00	2.33	1.32	3.80	72.48
EL1 vs. EL2 Average Dissimilarity: 61.16%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**EL1**	**EL3**	Mean	SD	%	Cumulative
Tvetenia sp.	128.87	1006.00	19.16	1.69	27.73	27.73
Ephemerella sp.	370.73	11.11	13.05	2.15	18.90	46.63
Baetis spp.	86.07	138.44	3.95	1.76	5.72	52.35
Simulium sp.	212.80	102.11	3.45	0.73	5.00	57.35
Thienemannimyia group	133.67	43.44	3.42	1.32	4.95	62.30
Taeniopteryx parvula	22.40	188.56	3.10	1.34	4.48	66.78
Rheotanytarsus spp.	125.87	175.44	2.36	1.32	3.41	70.19
EL1 vs. EL3 Average Dissimilarity: 69.07%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**EL2**	**EL3**	Mean	SD	%	Cumulative
Tvetenia sp.	235.42	1006.00	20.33	1.51	34.70	34.70
Taeniopteryx parvula	11.50	188.56	4.53	1.58	7.74	42.44
Baetis spp.	138.33	138.44	3.53	1.23	6.03	48.47
Rheotanytarsus spp.	135.00	175.44	3.45	1.54	5.89	54.36
Simulium sp.	264.75	102.11	2.56	1.28	4.37	58.73
Hydropsyche spp.	106.17	181.67	2.17	0.90	3.70	62.42
Micropsectra sp.	14.67	95.78	2.10	1.01	3.59	66.01
Polypedilum spp.	14.08	105.44	2.08	1.75	3.55	69.56
Cricotopus/Orthocladius	11.25	71.11	1.79	0.99	3.05	72.61
EL2 vs. EL3 Average Dissimilarity: 58.59%						

Table C.8. Similarity percentage (SIMPER) tables for the comparison of rock-basket community composition between months on the Ells River. Species accounting for up to 70% cumulative dissimilarity are listed as per Clarke (1993).

	Mean Abundance		Dissimilarity		Contribution	
Species	**June**	**July**	Mean	SD	%	Cumulative
Tricorythodes minutus	141.00	12.00	10.40	3.20	17.39	17.39
Tvetenia sp.	188.50	6.67	7.20	6.88	12.05	29.44
Polypedilum sp.	84.17	21.33	5.77	2.37	9.66	39.10
Simulium sp.	544.17	183.33	5.37	1.49	8.98	48.08
Rheotanytarsus spp.	274.00	158.00	4.32	1.49	7.22	55.31
Thienemannimyia group	77.00	37.00	4.11	1.65	6.87	62.17
Mayatrichia spp.	111.83	6.33	3.42	1.38	5.72	67.89
Micropsectra sp.	36.00	0.00	3.40	1.53	5.69	73.58
June vs. July Average Dissimilarity: 59.77%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**June**	**August**	Mean	SD	%	Cumulative
Simulium sp.	544.17	321.11	14.86	1.63	22.63	22.63
Ephemerella sp.	12.00	327.89	10.34	1.13	15.75	38.38
Baetis spp.	246.83	150.78	7.22	1.45	10.99	49.37
Rheotanytarsus spp.	274.00	81.33	4.38	2.40	6.68	56.05
Hydropsyche spp.	90.67	308.22	3.61	1.80	5.49	61.54
Tvetenia sp.	188.50	390.11	3.28	0.95	5.00	66.54
Thienemannimyia group	77.00	84.56	2.63	1.42	4.01	70.56
June vs. August Average Dissimilarity: 65.66%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**July**	**August**	Mean	SD	%	Cumulative
Ephemerella sp.	0.00	327.89	25.51	4.11	32.57	32.57
Simulium sp.	183.33	321.11	13.84	1.61	17.66	50.23
Baetis spp.	28.00	150.78	8.46	4.51	10.80	61.03
Hydropsyche spp.	24.33	308.22	7.57	9.44	9.66	70.69
July vs. August Average Dissimilarity: 78.34%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**June**	**September**	Mean	SD	%	Cumulative
Simulium sp.	544.17	12.78	17.00	1.25	23.26	23.26
Ephemerella sp.	12.00	257.00	10.87	0.97	14.87	38.14
Baetis spp.	246.83	106.67	7.89	0.95	10.79	48.93
Rheotanytarsus spp.	274.00	145.44	6.48	2.33	8.87	57.80
Tricorythodes minutus	141.00	10.67	3.90	1.14	5.33	63.13
Mayatrichia spp.	111.83	0.00	3.52	1.87	4.81	67.94
Thienemannimyia group	77.00	137.11	3.08	1.21	4.21	72.16
June vs. September Average Dissimilarity: 73.08%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**July**	**September**	Mean	SD	%	Cumulative
Ephemerella sp.	0.00	257.00	33.38	6.55	40.77	40.77
Thienemannimyia group	37.00	137.11	11.17	5.19	13.64	54.41
Simulium sp.	183.33	12.78	8.61	3.03	10.51	64.92
Tvetenia sp.	6.67	656.78	6.19	2.45	7.55	72.47
July vs. September Average Dissimilarity: 81.89%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**August**	**September**	Mean	SD	%	Cumulative
Tvetenia sp.	390.11	656.78	8.16	0.95	15.40	15.40
Simulium sp.	321.11	12.78	6.96	1.02	13.14	28.53
Hydropsyche spp.	308.22	82.56	5.52	2.60	10.42	38.95
Baetis spp.	150.78	106.67	4.38	1.60	8.26	47.22
Cricotopus trifasciata	149.22	6.33	3.46	1.50	6.54	53.75
Ephemerella sp.	327.89	257.00	3.38	0.86	6.37	60.12
Thienemannimyia group	84.56	137.11	2.94	1.09	5.55	65.68
Rheotanytarsus spp.	81.33	145.44	2.18	1.38	4.12	69.79
Chimarra spp.	91.56	14.22	1.91	1.24	3.60	73.39
August vs. September Average Dissimilarity: 53.00%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**June**	**October**	Mean	SD	%	Cumulative
Simulium sp.	544.17	52.00	16.18	1.26	23.29	23.29
Baetis spp.	246.83	35.00	7.54	0.96	10.86	34.15
Rheotanytarsus spp.	274.00	103.11	7.19	2.24	10.36	44.51
Tricorythodes minutus	141.00	8.89	4.94	1.11	7.11	51.62
Tvetenia sp.	188.50	359.89	3.95	1.41	5.69	57.31
Mayatrichia spp.	111.83	0.00	3.90	1.95	5.62	62.93
Ephemerella sp.	12.00	76.44	3.79	0.83	5.46	68.39
Polypedilum sp.	84.17	25.67	2.55	0.89	3.67	72.06
June vs. October Average Dissimilarity: 69.44%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**July**	**October**	Mean	SD	%	Cumulative
Ephemerella sp.	0.00	76.44	14.63	2.75	22.67	22.67
Simulium sp.	183.33	52.00	12.62	3.75	19.57	42.23
Thienemannimyia group	37.00	62.56	7.32	4.04	11.35	53.58
Tvetenia sp.	6.67	359.89	6.36	4.41	9.85	63.44
Rheotanytarsus spp.	158.00	103.11	4.73	1.76	7.32	70.76
July vs. October Average Dissimilarity: 64.53%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**August**	**October**	Mean	SD	%	Cumulative
Simulium sp.	321.11	52.00	8.31	1.03	12.95	12.95
Hydropsyche spp.	308.22	23.56	8.25	3.92	12.85	25.80
Ephemerella sp.	327.89	76.44	7.28	0.82	11.34	37.14
Tvetenia sp.	390.11	359.89	7.19	1.23	11.21	48.35
Cricotopus trifasciata	149.22	0.56	4.32	1.57	6.73	55.08
Baetis spp.	150.78	35.00	4.10	1.14	6.38	61.46
Chimarra spp.	91.56	0.67	2.53	1.39	3.94	65.40
Thienemannimyia group	84.56	62.56	2.52	0.80	3.93	69.32
Rheotanytarsus spp.	81.33	103.11	2.38	1.59	3.71	73.03
August vs. October Average Dissimilarity: 64.19%						

	Mean Abundance		Dissimilarity		Contribution	
Species	**September**	**October**	Mean	SD	%	Cumulative
Tvetenia sp.	656.78	359.86	10.02	1.00	20.76	20.76
Ephemerella sp.	257.00	76.44	7.50	0.67	15.55	36.31
Thienemannimyia group	137.11	62.56	3.29	1.19	6.82	43.13
Baetis spp.	106.67	35.00	2.88	1.11	5.96	49.09
Hydropsyche spp.	82.56	23.56	2.55	1.39	5.29	54.38
Simulium sp.	12.78	52.00	2.43	0.59	5.04	59.42
Rheotanytarsus spp.	145.44	103.11	2.33	1.21	4.84	64.26
Taeniopteryx parvula	140.44	51.67	2.30	0.97	4.76	69.02
Isoperla sp.	39.11	8.67	1.50	1.00	3.11	72.13
September vs. October Average Dissimilarity: 48.25%						