EFFECTS OF SELENIUM AND OTHER SURFACE COAL MINE INFLUENCES ON FISH AND INVERTEBRATES IN CANADIAN ROCKIES STREAMS

KATHRYN A. KUCHAPSKI
Bachelor of Science, University of Calgary, 2008

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University of Lethbridge
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ABSTRACT

Physical and chemical influences downstream of surface coal mines, including selenium (Se) release, water quality shifts, and habitat alterations can affect aquatic organisms. To evaluate these influences at the community level of organization, fish and macroinvertebrates were studied in mine-affected and reference streams. Se can be toxic to aquatic organisms and was measured in lotic food chains (water, biofilm, macroinvertebrates and juvenile salmonids). Invertebrate Se was significantly related to Se in juvenile fish muscle (westslope cutthroat, bull, rainbow and brook trout) and Se concentrations exceeded proposed individual-level reproductive effects thresholds in some rainbow and cutthroat trout. Community-level effects were only detected in rainbow trout where species specific biomass was negatively related to muscle Se concentration in stream reaches. Macroinvertebrate assemblages varied along a mine-influence gradient defined by Se, alkalinity, substrate embeddedness and interstitial material size. Ephemeroptera were the most sensitive to mining effects and potential mechanisms influencing community composition included Se and ion toxicity and habitat degradation. This project highlights the need to study multiple organisms at different levels of ecological organization in order to understand and manage diverse mining impacts.

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ACA = Alberta Conservation Association
ANCOVA = analysis of covariance
Ck. = creek
DCA = detrended correspondence analysis
df = degrees of freedom
Dg = geometric mean
dw = dry weight
EC: = effective concentration
EC_{10} = concentration that produces toxic effect of interest in 10% of test organisms
EF = enrichment factor
EPT = Ephemeroptera, Plecoptera, Trichoptera
HG-AAS = hydride generation atomic absorption spectrometry
ICP-MS = inductively coupled plasma mass spectrometry
NOEC = no observable effect concentration
NS = not significant
PC = principal component
PCA = principal component analysis
R. = river
RDA = redundancy analysis
Se = selenium
SE = standard error of the mean
TTF = trophic transfer factor

CHAPTER 1.   INTRODUCTION

Surface coal mining influences in aquatic ecosystems

Numerous natural resource development projects are focused on meeting global

energy demands by extracting primary energy sources such as oil and coal. Coal is one of

the world's fastest growing energy industries (International Energy Agency 2013) and

surface mining accounts for approximately 40% of the over 8 billion tonnes of annual

global coal production (World Coal Institute 2005). Surface mining is a process by which

shallow coal seams are accessed through the removal and relocation of overburden rock

using explosives and large mechanical equipment. Canada's largest coal producers are

the provinces of Alberta and British Columbia where current production is principally

from surface mines (World Energy Council 2013) and where watersheds in the Canadian

Rocky mountains and foothills have experienced increasing surface mine development

for the past 40 years (Lussier et al. 2003).

There is growing scientific evidence of environmental impacts caused by surface

coal mining (Palmer et al. 2010) and influences of concern in aquatic ecosystems include

the release of potentially toxic compounds, most notably selenium (Se), changes to

downstream water quality, and alteration of physical aquatic habitats (Lindberg et al.

2011, Maher et al. 2011, Bernhardt et al. 2012, Griffith et al. 2012). At surface coal

mines, large quantities of rock and soil are disturbed, exposing them to air, greater

biological activity, and water dynamics (Ryser et al. 2005). These conditions increase

rock weathering and mobilize solutes in backfill ponds, end-pit lakes, ground water, and

precipitation run-off (Naftz and Rice 1989). Rock spoil piles can also bury headwater

streams and increase sediment export, ultimately altering physical stream channel characteristics (Lindberg et al. 2011). Research into the effects of these impacts on aquatic organisms is central to assessing and managing environmental risks associated with an expanding coal mining industry both globally, and in the Canadian Rockies region. This thesis project investigated surface coal mining influences on fish and macroinvertebrate communities in lotic (flowing water) systems in two watersheds in the Canadian Rockies. Se exposure and toxicity were a major research focus in fish and emergent chemical and physical impacts of concern were also measured and considered, particularly with respect to their influence on macroinvertebrate assemblages.

Selenium exposure and toxicity downstream of mines

Selenium is an essential micronutrient that can produce toxic effects in fish and other aquatic organisms at concentrations above the nutritional requirement. Naturally occurring in surface waters at low concentrations ranging from 0.1- 0.4 µg/L (United States Environmental Protection Agency 2004), Se inputs to aquatic systems can be elevated due to coal and other types of mining, coal burning and smelting, and irrigation agriculture (Lemly 1993a). Se contamination from mining is a widespread issue in North American watersheds with elevated Se concentrations recorded downstream of coal, phosphorus, and uranium mines (Kennedy et al. 2000, Hamilton and Buhl 2004, Holm et al. 2005, Muscatello et al. 2006, Rudolph et al. 2008). In Alberta and British Columbia, surface coal mining has drastically increased Se in streams draining mine sites where waterborne concentrations of over 100 µg/L and increased Se concentrations in the tissues of aquatic organisms have been documented (Casey 2005, Orr et al. 2012).

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Se is present in the natural environment in various chemical forms. Elemental Se (Se (0)) and selenide (Se(-II)) are not readily bioavailable and therefore have low toxicities in aquatic systems (Kulp and Pratt 2004). Oxidized forms including selenite (Se(IV)) and selenate (Se(VI)) are common and highly soluble. They can be taken up from water by bacteria and primary producers (Kulp and Pratt 2004) and transformed into organoselenium forms including selenoamino acids such as selenocysteine and selenomethionine (Fan et al. 2002). Though invertebrates and fish can also take up dissolved Se directly from water, this process is generally sufficiently slow as to be negligible compared with uptake from particulate sources (Presser and Luoma 2010). Therefore, Se accumulation in higher trophic levels of aquatic systems results from transfer of dietary organic Se, particularly selenomethionine, through the aquatic food chain (Simmons and Wallschläger 2005). Because of its essentiality, Se uptake is largely concentration dependent where accumulation rates by organisms are greater when source concentrations are low (DeForest et al. 2007). Additionally, Se enrichment is greatest at low trophic levels (Presser and Luoma 2010) and organisms at higher trophic levels such as birds and fish may not have higher Se concentrations than organisms slightly lower in the food web (e.g. invertebrates) (Presser and Luoma 2010, Stewart et al. 2011). Therefore, toxic effects in fish and birds are generally due to greater Se sensitivity rather than greater Se exposure.

In fish, Se is essential as a component of versatile Se proteins including glutathione peroxidase which is involved in oxidation-reduction reactions that protect cells from oxidative damage and iodothyronine deiodinases which perform thyroid hormone activation/inactivation (Simmons and Wallschläger 2005, Janz et al. 2011). Se

became a contaminant of concern in aquatic ecosystems with the recognition of toxicity

in wild populations of egg laying vertebrates including fish and birds at concentrations

above the essential requirement (Ohlendorf et al. 1986, Lemly 1993b). Toxic effects in

fish can include reduced growth (Hodson and Hilton 1983), reproductive impairment, and

lethality (Lemly 1993b) but the most sensitive toxicity endpoints are reproductive effects

that occur by maternal transfer of Se to eggs causing larval deformities and/or death upon

hatching (Lemly 1993b, Janz et al. 2011). A proposed mechanism of this toxicity with

increasing recent support is one of oxidative stress triggered when excess Se compounds

complex with reduced glutathione and generate superoxide radicals which then cause

cellular damage (Palace et al. 2004).

Se has been studied in fish under field conditions to identify if toxic effects are

occurring and to determine toxicity thresholds. Studies have been conducted downstream

of coal mines in Alberta and British Columbia on exposed salmonid species including

wild populations of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) (Kennedy et

al. 2000, Rudolph et al. 2008), rainbow trout (*Oncorhynchus mykiss*) (Holm et al. 2005),

and brook trout (*Salvelinus fontinalis*) (Holm et al. 2005). In these studies, gametes were

collected from fish at high Se exposure sites and at references sites. Eggs were fertilized

in the field, hatched and reared in the laboratory, then reproductive effects including

larval mortalities and deformities were evaluated. In this way, effective concentrations

(ECs) were determined for reproductive effects in rainbow trout (EC_{10} for larval skeletal

deformities = 21.1 mg Se/kg dw in eggs) (Holm et al. 2005) and cutthroat trout (EC_{10} for

alevin mortalities = 17 − 24.1 mg Se/kg dw in eggs) (Rudolph et al. 2008) and a no

observable effects concentration (NOEC) was determined for brook trout (NOEC > 20.5

mg Se/kg in eggs) (Holm et al. 2005). These studies not only found that reproductive effects were occurring in some fish downstream of mines but that Se sensitivity varies significantly among even closely related cold water salmonid species, and that brook trout, a non-native species in this region, is particularly Se-tolerant.

While field studies have defined toxic ECs, finding practical and effective methods for monitoring Se toxicity and impacts in natural systems with respect to these proposed thresholds is challenging. Waterborne concentrations do not always reflect the potential for Se exposure at higher trophic levels (Orr et al. 2012) and therefore Se monitoring programs in salmonid fishes in the Canadian Rockies have focused on obtaining fish tissues for comparisons with Se toxicity thresholds (Casey 2005, Minnow Environmental Inc et al. 2011). Often adult fish are sampled to obtain eggs or gonads for Se testing because of their direct connection to sensitive toxic endpoints (Presser and Luoma 2010). Two issues that may arise from tissue sampling, especially in lotic systems, include cumulative impacts of repeated fish harvest in exposed populations (Orr et al. 2012) and variable Se exposure due to fish movements.

One proposed method of toxicity monitoring is through food chain transfer modelling (Presser and Luoma 2010, Orr et al. 2012). Se transfer models determine the species and concentration specific transfer of Se at different steps of the food chain in order to use Se concentrations at lower food chain levels (water, biofilm, invertebrates) to predict fish tissue concentrations (Presser and Luoma 2010). These are useful tools as concentrations at lower levels of the food web are often easier to measure and their sampling prevents the need to lethally sample fish from potentially vulnerable populations (Orr et al. 2012).

Tissue Se concentrations in spawning fish caught at exposure locations have been highly variable and this variation has been at least partially attributed to differences in Se exposure resulting from fish movements (Holm et al. 2005, Rudolph et al. 2008). Stream fishes, particularly some life histories of trout, are highly mobile and can move among areas with different Se concentrations. Fish tissue concentrations will begin to reflect dietary concentrations within days of exposure but equilibrium to diet can take weeks or months (Stewart et al. 2011). Therefore, fish movements can confound the relationship of Se tissue to diet concentrations at the point of capture (Janz et al. 2011, Orr et al. 2012). Variable use of Se contaminated habitats by adult fish has been confirmed in studies of rainbow trout, cutthroat trout and mountain whitefish (*Prosopium williamsoni*) residency in Rocky mountain streams (Palace et al. 2007, Friedrich et al. 2011). Se exposure determined by measuring Se accumulated in the annuli of sagittal otoliths from adult rainbow trout, cutthroat trout and mountain whitefish captured in Se-contaminated streams indicated variable lifetime Se exposure and movements between high and low Se habitats (Palace et al. 2007, Friedrich et al. 2011).

Variable Se exposures in fish can create difficulties in predicting Se toxicity effects at a specific site of interest (Holm et al. 2005, Rudolph et al. 2008) and in determining empirical relationships between fish tissue and diet Se concentrations for use in food chain models (Orr et al. 2012). An important research need is the determination of reliable exposure based fish tissue concentrations for comparison to toxicity thresholds and for use in the development of food web models. Long-term laboratory feeding studies such as one carried out with cutthroat trout by Hardy et al. (2010) are an option but are time-intensive and may not reflect Se uptake in natural environments. Exposure-response

relationships can also be determined from field data if the appropriate organisms or life-stages of organisms are sampled. In salmonids, juvenile life stages may be candidates as they are relatively less mobile than adults during spawning. Juvenile salmonids generally spend the first years of their lives rearing in natal tributaries and show restricted movement patterns during the summer feeding months (Rodríguez 2002, Costello 2006, McPhail 2007).

Long-term Se exposure in Canadian Rockies streams also raises concerns over the potential for negative impacts on fish populations (Alberta Selenium Working Group 2010). Fish populations may decline due to reproductive toxicity effects (Janz et al. 2011) and fish community composition may shift as high Se systems favour the survival and range expansion of species that are more Se-tolerant and cause declines and/or extirpations of those that are more sensitive (Alberta Selenium Working Group 2010). In order to manage potential risks to fish populations it is important for proposed toxicity thresholds to be protective of fish at the community level (Van Kirk and Hill 2007). Therefore, Se concentrations that produce community-level impacts in the streams of mined watersheds must be determined.

Macroinvertebrates have generally been considered tolerant to Se exposure and invertebrate Se concentrations have typically only been of interest because invertebrates are dietary sources of Se for fish, amphibians and aquatic birds. However, toxicity effects have been detected in laboratory macroinvertebrate organisms (Ingersoll et al. 1990, Maier and Knight 1993, Malchow et al. 1995, Conley et al. 2011) and invertebrate Se accumulation above reference concentrations has been documented at mine-affected sites in the field (Orr et al. 2006, Wayland and Crosley 2006, Orr et al. 2012). Lab studies

have found that Se toxicity, especially sub-lethal effects, can occur in invertebrates, sometimes at relatively low exposure concentrations and that thresholds protective of fish may not always be protective of their macroinvertebrate prey (deBruyn and Chapman 2007).

Field Se tissue concentrations in invertebrates are variable among sites and taxonomic groups (Wayland and Crosley 2006) but may indicate vulnerability to Se toxicity based on laboratory derived concentrations for acute and chronic effects (deBruyn and Chapman 2007). Few field studies have examined the impacts of Se contamination on macroinvertebrate communities and these investigations have only incorporated a narrow range of relatively low Se exposures (Frenette 2008, Pond et al. 2008).

Other physical and chemical mine related impacts

Recently, influences besides Se have been the focus of scientific studies in surface mining regions. Emerging influences of concern include increases in other solutes weathered from waste rock including Ca^+, Mg^+, SO_4^{2-}, Cl^- and HCO_3^- (United States Environmental Protection Agency 2011) in addition to changes to the physical habitats of receiving environments (Lindberg et al. 2011). These effects have thus far been studied with particular attention to their effects on macroinvertebrate communities.

In the Appalachian Coalfields in the United States, changes in water chemistry variables have been highlighted in recent biomonitoring studies (Hartman et al. 2005, Freund and Petty 2007, Pond et al. 2008, Pond 2010, Cormier et al. 2013). Declines and extirpations of certain macroinvertebrate taxa have been linked to high concentrations of

Ca^+, Mg^+, SO_4^{2-}, Cl^- and HCO_3^- ions in streams draining coal mine sites (Pond et al.
2008, Cormier et al. 2013). The proposed mechanisms of toxicity to macroinvertebrates
are physiological and related to the disruption of cellular ion transport systems on the
gills and integuments of organisms (Cormier et al. 2013). As the mechanisms of toxicity
are assessed based on physiological characteristics shared by many invertebrate taxa
(Cormier and Suter 2013), similar changes in water chemistry may result in
macroinvertebrate community effects in Canadian Rockies streams.

Changes to physical stream habitats also occur in mined watersheds. Direct
impacts occur when headwater streams are buried by valley fills or rock slides (Palmer et
al. 2010) but there are also persistent indirect effects including calcite accumulation and
sediment export (Hartman et al. 2005). The limestone-dominated bedrock and hard water
of Canadian Rockies watersheds produce water chemistry conditions that result in calcite
accumulation in streambeds downstream of mine sites. Calcium carbonate precipitation
as calcite in streams is a natural process caused by the loss of CO_2 from hard water either
to the atmosphere or by photosynthesis of algae, macrophytes or bacteria (House 1990,
Chen et al. 2004). This natural process can be amplified downstream of surface coal
mines as water passing through rock drains becomes supersaturated in CO_2 from
decomposing organic matter which then acts on the carboniferous limestone waste rock,
removing calcium carbonate into solution (Ford and Pedley 1996). CO_2 degassing and
calcite precipitation occurs as the water surfaces (Ford and Pedley 1996) especially in
areas of turbulent flow where the air-water interface is maximized (Chen et al. 2004).
Calcite deposition downstream of mines can be severe, sometimes spanning the entire
channel and developing terraces. Surface mines have also been associated with the export

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of fine sediments although they are often at least partially mitigated by sedimentation ponds and control structures (Pond et al. 2008). Stream habitat alteration resulting from sediment and calcite deposition could affect biota by increasing the embeddedness of stream substrates and decreasing interstitial spaces which can be important habitat for benthic macroinvertebrates (Suttle et al. 2004, Larsen et al. 2011). Calcite accumulation and increases in fine sediments could also have implications for salmonid fish spawning habitat by decreasing accessibility to and quality of spawning gravel which can in turn influence spawning success (Turnpenny and Williams 1980).

Thesis objectives and organization

A diversity of mine impacts in the Canadian Rocky mountains and foothills and the rapidly expanding mine footprint require a greater understanding of effects on ecosystem health in order to assess and manage risks to aquatic resources. This thesis comprises observational field studies of the fish and macroinvertebrate communities in Canadian Rockies streams that experience influences from surface coal mining and those in nearby reference streams. The objective of this thesis is to respond to research needs related to monitoring and managing surface mine effects in the lotic systems of mined watersheds. These include the need to determine appropriate organisms and levels of ecological organization for monitoring Se exposure and managing Se risks, and the need to evaluate relevant mine impacts, in addition to Se contamination, that have the potential to affect aquatic organisms and communities.

The specific objectives of this thesis were:

1) To determine if juvenile salmonid tissues in mountain stream systems reflect food chain Se exposure at the point of capture.

2) To investigate the fish community-level effects of Se exposure and toxicity on salmonid fishes at the stream reach scale.

3) To evaluate the effects on stream macroinvertebrate community assemblages associated with Se exposure and other physical and chemical surface mine influences.

The subsequent chapters of this thesis include rationale, methods, results and discussions of field studies performed in streams of mined watersheds in the Canadian Rocky mountains and foothills. Chapter two presents investigations of Se exposure in juvenile salmonids and of the fish community-level effects of Se contamination. In order to quantify Se exposure and toxicity, Se transfer in stream food chains was determined and compared with toxicity thresholds, and fish biomass was measured at the stream reach scale. Chapter three describes the examination of surface mine influences, including but not limited to Se contamination, on the composition of macroinvertebrate community assemblages. Physical and chemical predictor variables were measured and stream characteristics attributable to mine influences were determined. The response of invertebrate community composition to mine disturbance was then investigated and invertebrate taxa that are sensitive to mine effects were identified. The fourth and final chapter summarizes the key findings of this research and provides recommendations for monitoring and management of Se contamination and other surface mine influences in aquatic ecosystems.

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CHAPTER 2.   FOOD CHAIN TRANSFER AND EXPOSURE EFFECTS OF SELENIUM ON LOTIC FISH COMMUNITIES IN TWO CANADIAN ROCKIES WATERSHEDS

Abstract

Selenium (Se) toxicity impacts on salmonid fishes in surface coal mining regions are challenging to monitor and manage. Se tissue concentrations in stream fishes are difficult to attribute to specific exposure sites and the fish community-level impacts of tissue concentrations above proposed toxicity thresholds have not been defined. To determine if juvenile salmonids reflect local Se exposure concentrations, and to investigate the relationship between Se exposure and toxicity effects at the fish community-level, Se concentrations and fish biomass were examined in the streams of two mined watersheds. Se concentrations were measured in water, biofilm, macroinvertebrates, and juvenile fish muscle tissues from mine-affected and reference streams and significant positive Se transfer relationships were found at each measured level of the lotic food chain. Se accumulation from macroinvertebrates to juvenile fish muscle tissue was not significantly different among fish species including westslope cutthroat, bull, rainbow, and brook trout but indicated a significant relationship between tissue and dietary Se at capture sites. Muscle Se concentrations exceeded proposed individual-level toxic effective concentrations in rainbow and cutthroat trout but fish biomass at the reach scale was only significantly negatively related to average fish muscle tissue Se concentrations for rainbow trout.

Introduction

Selenium (Se) is a contaminant of concern in watersheds of the Canadian Rockies that experience impacts from large-scale surface coal mining projects. Se is released in aquatic ecosystems downstream of mine sites due to high weathering rates of Se-containing disturbed bedrock and soil (Naftz and Rice 1989). Although Se is an essential micronutrient, excess dietary uptake can produce toxic effects in fish as maternal transfer to eggs causes teratogenesis (deformities) and/or death in early life stages (Lemly 1999, Palace et al. 2004). Reproductive effects, mainly increased rates of larval deformities/mortalities, have been identified in rainbow trout (*Oncorhynchus mykiss*) and westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) in Canadian Rockies streams and tissue effective concentrations (EC; the concentration at which a toxic effect is produced) have been proposed (Holm et al. 2005, Rudolph et al. 2008). Se sensitivity and allocation to body tissues varies among fish species (Chapman 2007, Pilgrim 2012) and regional populations of salmonids that may experience toxic Se effects include westslope cutthroat and rainbow trout as well as bull trout (*Salvelinus confluentus*), brook trout (*Salvelinus fontinalis*) and mountain whitefish (*Prosopium williamsoni*).

Monitoring and managing the fish species and region specific risks of Se toxicity in streams of the Canadian Rockies is challenging. Waterborne Se concentrations do not necessarily reflect Se toxicity risks because fish predominantly accumulate Se from their diets (Brix et al. 2005). Se monitoring programs in the region have therefore relied on evaluation of tissue concentrations from lethally sampled fish against toxicity thresholds (Casey 2005, Minnow Environmental Inc et al. 2011). Adult spawning individuals have been targeted (Holm et al. 2005, Rudolph et al. 2008, Orr et al. 2012) in order to collect

eggs and ovaries for monitoring because of their direct connection to reproductive toxicity endpoints (Presser and Luoma 2010). However, Se exposure histories of fish in lotic systems with good hydrological connectivity are uncertain due to the ability of fish, particularly certain life histories of trout, to move in and out of areas of Se exposure (Palace et al. 2007, Friedrich et al. 2011). Therefore tissue concentrations may not reflect exposure potential at a site of interest.

Food chain trophic transfer models have been suggested and developed as methods for improving Se monitoring and reducing lethal fish sampling in vulnerable populations (Presser and Luoma 2010, Orr et al. 2012). By quantifying both enrichment factors (EF; proportional transfer of Se from water into particulate forms at the base of the food chain) and trophic transfer factors (TTF; proportional transfer of Se between organisms at other levels of the food chain) Se sampling in water, biofilm, or invertebrates could be used to model fish tissue concentrations (Presser and Luoma 2010). However, lotic trophic transfer models developed from field data can also suffer from uncertainty introduced by variable Se exposure in mobile fish (Orr et al. 2012).

Field sampling of less mobile life stages of fish during periods of restricted movement might be appropriate for quantifying food chain transfer of Se and monitoring Se concentrations in salmonid species in the Canadian Rockies region. Though some juvenile salmonids can move over long distances (Warnock et al. 2010, Daum and Flannery 2011), generally they spend their first $1 - 3$ years of life rearing in natal tributaries and display restricted movements throughout the summer when stream conditions are stable (Mellina et al. 2005, Costello 2006, McPhail 2007, Muhlfeld et al. 2012). Because fish tissues can take weeks or months to equilibrate to dietary

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concentrations (Stewart et al. 2011), juvenile trout tissues sampled during summer may be more representative of local Se exposure than those of adult fish during spawning, a period of extensive movement. Se concentrations in juvenile salmonids could therefore be useful for comparison to toxicity thresholds or use in food chain models to assess the potential for Se toxicity at a specific site of interest.

Another challenge for the management of Se impacts to fish in Canadian Rockies streams is determining community-level effects. The fish community of a stream reflects the fish species present, their overall population abundance or biomass, and their relative abundances or biomasses. Declines in fish populations may result due to chronic Se exposure that causes individual-level toxicity effects and the composition of fish communities may be altered as a result of differential Se sensitivity among fish species (Janz et al. 2011). The protection of fish populations requires regulatory mechanisms that consider how individual-level Se toxicity translates to impacts at the fish community-level (Van Kirk and Hill 2007).

In this study Se concentrations were sampled in food chains in Canadian Rockies streams with upstream mine impact and in nearby reference streams. Se concentrations were determined in water, biofilm, invertebrates and juvenile salmonids including westslope cutthroat, bull, rainbow and brook trout to determine if juvenile fish muscle Se concentrations reflect reach specific food chain Se exposure, and to evaluate them against proposed Se toxicity effects thresholds. Further, fish biomass and species specific fish biomasses were measured at the reach scale in mine-affected and reference streams to evaluate the hypothesis that Se exposure above toxicity thresholds results in negative impacts at the fish community/population-level.

Materials and Methods

Study areas

Sampling sites were located in two geographically separate watersheds in the eastern Canadian Rocky Mountains and Foothills; the Elk River watershed in south-eastern British Columbia and the McLeod River watershed in west-central Alberta (Figure 2.1). The Elk R. watershed, located in the upper reaches of the Columbia River, contains five operating surface coal mines that drain into tributaries of the Elk R. Surface mine impacts in the basin span over 40 years. Salmonid fish species are the dominant taxa and populations of westslope cutthroat trout, bull trout and mountain whitefish as well as some introduced brook trout are present (McPhail 2007). The McLeod R. watershed is located in the middle reaches of the Athabasca River basin. Surface coal mine impacts in the basin include two active and one reclaimed mine that drain into the McLeod R. and its tributaries. Salmonid fish species in the McLeod R. watershed include mountain whitefish, bull trout, native Athabasca rainbow trout and introduced brook trout (Nelson and Paetz 1992, Rasmussen and Taylor 2009). In total, 13 mine-affected and 11 reference streams were sampled across the two study areas (Table 2.1 and Figure 2.1). Sampled stream reaches spanned stream orders from 2nd to 5th and ranged in elevation between 1250 and 1600 meters above sea level. Streams pass through sub-alpine and montane forests dominated by englemann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*) in the Elk R. basin and lodgepole pine (*Pinus contorta*); white (*Picea glauca*), black (*Picea mariana*) and englemann spruce and aspen (*Populus tremuloides*) and balsam poplar (*Populus balsamifera*) in the McLeod R. region. Streams were

selected to incorporate a range of mine influences and were considered mine-affected if there was any mine impact in the upstream watershed based on mine disturbance boundaries. Three of the mine-affected sampled streams drained mine sites that have been reclaimed for 5 – 10 years (Table 2.1). Reference sites were considered "least disturbed" as some sites experienced impacts from forestry and forestry roads as well as cattle grazing and recreational usage but no influences from surface mines.

Fish community biomass and habitat assessments

Fish biomass was sampled by single pass electrofishing using a Smith-Root LR-24 backpack electrofisher on open populations in 175 – 200 m stream reaches at each site. Fish sampling took place between late July and late August 2011. All fish were identified to species, weighed and measured (fork length). In three of the study streams fisheries inventory projects were performed by government agencies in early September 2011 using sampling methods analogous to those in this study. For these streams, fish biomass data were requested through the government fisheries inventory database (Alberta Sustainable Resource Development 2011). Fish biomass and species biomasses were calculated as the total biomass of fish or biomass of fish of each species captured per unit of stream surface area at each site. Stream surface area was determined as the average wetted stream width of the sampled reach multiplied by reach length.

Fish habitat assessments were done over the same 175 – 200 m stream reaches used to determine fish community biomass. Habitat characteristics were summarized from measurements made according to Johnston and Slaney (1996). Water surface slope was determined as percent slope using a clinometer. Percent riffle, glide and pool were the percent length of the stream reach made up by these habitat unit types. Wetted

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channel width was the average of across channel measurements made in each habitat unit that was assessed (up to n = 24). Channel depth was determined as the mean of all habitat units where the depth was determined as the average of three equidistant measurements taken along a stream transect. Riparian cover and undercut banks were the average percent of the surface area of each habitat unit shaded by first layer vegetation or cutbanks, respectively. Large woody debris with diameter > 10 cm was tallied for the entire reach. Stream substrate was described by performing a one hundred pebble count according to Environment Canada (2010a). Wolman Dg, the geometric mean of intermediate axis length of substrate units measured, was calculated from the pebble count. Substrate embeddedness was summarized as the median percentage depth that a subsample of ten substrate units was buried in the surrounding interstitial material.

Water samples for nutrient analysis (total nitrogen and total phosphorus) were collected between late July and late August 2011. Samples were collected in straight, well-mixed reaches from approximately 10 cm below the surface in acid-washed high-density polyethylene bottles. Samples were held on ice then returned to the laboratory where they were stored at 4^0C until shipped for analysis. Analyses of total nitrogen and total phosphorus were done at the University of Alberta Biogeochemical Analytical Services Laboratory in Edmonton, Alberta, Canada. Some water samples had concentrations of total phosphorus below the method detection limit of 0.001 mg/L and were assigned a value of one half of the method detection limit in statistical analyses. Measurements of dissolved oxygen and pH were made in-situ using a handheld YSI 85 multiparameter instrument (YSI Inc.) and a VWR SP21 Symphony electrode (VWR International, Inc.) respectively.

Food web Se sampling and analysis

Fish were lethally sampled to obtain muscle tissue samples for selenium content analysis. Juvenile fish (< 200 mm fork length) were targeted for lethal sampling and were euthanized with clove oil (160 ppm, emulsified in ethanol). The species and number of lethally sampled fish of each species was determined in accordance with Fish Research Permits issued by provincial governing bodies for each study area. In the Elk River watershed, cutthroat trout (CTTR) and bull trout (BLTR) were sampled and in the McLeod River watershed rainbow trout (RNTR) and brook trout (BKTR) were sampled. While bull trout were captured at McLeod R. sites, Alberta populations are designated as threatened by the Committee on the Status of Endangered Wildlife in Canada (2012) and therefore were not lethally sampled. Euthanized fish were weighed and measured in the field, placed on ice, then frozen until dissections were done in the lab. Fish that were not sacrificed for Se analysis were released to the stream location where they were captured. All fish were handled following animal-welfare protocols approved by the University of Lethbridge Animal Welfare Committee, in accordance with national guidelines. Dorsal white muscle tissue was removed from the left side of fish, above the lateral line and directly anterior to the dorsal fin for Se content analysis. Muscle tissue samples from each fish were dried in a drying oven at 60^0C until a constant weight was reached (approximately 72 hours) and individually homogenated using a nitric acid washed mortar and pestle. Fish ages were determined from sagittal otoliths removed from all lethally sampled fish.

Composite macroinvertebrate samples were collected for Se content analysis between late July and late August 2011, using a 0.09 m^2 surber sampler with 250 µm

mesh size and sampling to approximately 0.1 m depth in the substrate. Three replicate samples were taken per stream each comprising three one minute sampling intervals at three locations along a stream transect. Transects were restricted to shallow riffles, defined as swift water habitats with turbulent flow and broken water surface. Samples were transferred to freezer bags and placed on ice until they could be frozen for further processing. In the laboratory, all macroinvertebrates from each sample were sorted from debris, identified to the family level of taxonomic resolution, and dried in a drying oven at 60^0C until a constant weight was reached (approximately 48 hours). All macroinvertebrates from all three replicate samples from each site were then combined and homogenized in order to obtain a minimum composite tissue sample for Se content analysis (> 0.2 g dry weight).

To represent Se concentrations at the base of the food web, composite biofilm samples (as bacteria, algae, periphyton and/or moss) were collected by scraping material from stream substrates using a stainless steel spatula or forceps. A minimum of 90 mL of sample was collected from at least five substrate units in each sample reach and placed in a polyethylene bag. Due to the patchy distribution of the biofilm community, substrate units that provided sufficient material for analysis were preferentially selected. Excess water was poured off and samples were placed on ice in the field and frozen upon return to the laboratory. Biofilm samples were thawed then dried in a drying oven at 60^0C until constant weight was reached (approximately 48 hours) and homogenated.

Water samples for Se analysis were collected in the same manner as those for nutrient analysis but were acidified to approximately 1% v/v using 16N Omnitrace HNO_3 prior to storage and shipping. Total Se in water was measured by inductively coupled

plasma mass spectrometry (ICP-MS) at ALS Laboratories in Calgary, AB, Canada. Some

water samples had concentrations of total Se below the method detection limit of 0.001

mg/L and were assigned a value of one half of the method detection limit in statistical

analyses. Total Se was measured in dried fish muscle tissue, composite invertebrate

tissues, and composite biofilm by hydride generation atomic absorption spectrometry

(HG-AAS) (detection limit 0.05 mg/kg dry weight) as previously described (Miller et al.

2009). Measured Se tissue concentrations are reported as mg/kg dry weight (dw).

Statistical analysis

Statistical analyses were performed in JMP 10.0 (SAS Institute Inc 2012). Se

concentrations were right skewed and were therefore $\log_{10}$-transformed prior to analysis.

Mean waterborne, biofilm, macroinvertebrate and fish Se concentrations were compared

between mine-affected and reference streams using two-sample t-tests.

Se enrichment and trophic transfer were modelled using Se concentration data for

three steps in the lotic food chain: 1. water to biofilm, 2. biofilm to macroinvertebrates,

and 3. macroinvertebrates to juvenile fish muscle. This model was used as food chain

lengths in Canadian Rockies streams have generally indicated three trophic levels (Orr et

al. 2006). While it is possible for predatory invertebrates to act as secondary consumers

potentially lengthening the food chain, Orr et al. (2006) determined the number of trophic

levels to be ~ 3 in Elk R. streams based on the trophic position of salmonid fishes

estimated by $\Delta\delta^{15}N$ from composite invertebrate samples to fish. At each food chain step

Se concentration in the lower trophic level was a continuous predictor and Se

concentration in the higher trophic level was a response variable. Analysis of covariance

(ANCOVA) was conducted to determine the effect of the continuous predictor Se

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concentration (covariate) and watershed (Elk R. or McLeod R.) on the response Se

concentration in the first two food chain steps. A two factor nested ANCOVA was used

in the third food chain step to investigate the effects of invertebrate Se concentration

(covariate), study watershed location, and fish species nested within watershed location

(bull/cutthroat trout in the Elk R. and rainbow/brook trout in the McLeod R.) on juvenile

fish muscle tissue Se concentration. Initial models contained interaction terms between

factors and covariates to test for homogeneity of within group slopes. As factor by

covariate interactions were not significant, final ANCOVA models were refitted without

interaction terms. In food chain steps where watershed or species factors did not have

significant effects in the refitted model, the enrichment/trophic transfer relationship for

that step is presented as a linear regression of predictor and response Se concentrations

from pooled watershed data.

Fish muscle tissue Se concentrations were the mean concentration of $n = 1 - 10$

fish of the same species captured concurrently at the same site and were weighted by n in

ANCOVA and regression analyses. In an attempt to only represent juvenile fish in food

chain analyses, Se concentrations in any fish with fork length > 200 mm or aged $> 3+$

were not included. Distributions of studentized residuals from linear regressions of Se

relationships at each food chain step were tested as part of initial tests to determine if data

met the assumptions of ANCOVA. One invertebrate (Berry Creek) and two fish (mean

rainbow trout and mean brook trout from Luscar Creek) Se concentrations were found to

be outliers in these distributions and they were therefore removed from the food chain

analyses. Juvenile fish from Luscar Creek were captured in proximity (< 500 m)

downstream of lentic-type habitat formed by a beaver pond on the stream channel. These

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fish had high average muscle Se concentrations of 19.72 mg/kg dw in brook trout and 15.07 mg/kg dw in rainbow trout (compared to 8.76-9.12 mg/kg dw in rainbow trout in Luscar Creek at similar waterborne Se concentrations from 1999 to 2001 (Casey 2005)). These fish may have spent time in the lentic-type environment of the pond and their tissue concentrations may therefore reflect exposure to higher trophic transfer and accumulation of Se typical in lentic food chains compared to the lotic environment where they were captured (Simmons and Wallschläger 2005, Orr et al. 2012).

Mean watershed specific enrichment factors (EF) were determined from EF values calculated as biofilm:waterborne Se concentration at each site. Mean watershed specific trophic transfer factors (TTF) from biofilm to invertebrates were determined from invertebrate:biofilm Se concentration at each site. Finally, mean overall and species specific TTF from invertebrates to juvenile fish muscle were determined as means of invertebrate:fish muscle Se concentration in each individual fish.

Reproductive effective concentrations (ECs) in muscle were calculated from proposed egg Se ECs in order to determine if measured fish muscle Se concentrations indicated the potential for reproductive effects. Muscle Se ECs were calculated from egg Se ECs and species specific egg:muscle relationships determined in field studies on three of the four fish species sampled in the present study (Holm et al. 2005, Rudolph et al. 2008). The calculated muscle Se ECs were 3.01 mg/kg dw EC_{10} (the concentration at which 10% effects were observed) for skeletal deformities in rainbow trout larvae (Holm et al. 2005), >10.25 mg/kg dw NOEC (the highest concentration at which no effects were observed) for craniofacial deformities in brook trout larvae (Holm et al. 2005) and 8.5-12.05 mg/kg dw EC_{10} for alevin mortalities in cutthroat trout (Rudolph et al. 2008). Both

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sub-lethal and lethal thresholds were used as field derived thresholds are rare and equivalent endpoints have not been studied in all species. No studies have determined ECs for reproductive effects in bull trout and therefore bull trout muscle Se concentrations were not compared to an EC threshold.

Linear regression was performed on $\log_{10}$-transformed fish biomass and fish muscle Se concentration to determine the relationship between Se accumulation and the biomass of the fish community in the sampled streams. Fish muscle tissue Se concentrations were the mean concentration of n = 2 – 14 fish of all lethally sampled species captured at each site. Separate linear regressions were performed on $\log_{10}$-transformed species biomass and species specific muscle Se concentrations in Elk R. (cutthroat and/or bull trout) and McLeod R. (rainbow and/or brook trout) streams. Species specific fish muscle tissue Se concentrations were the mean concentration of n = 2 – 10 fish of the same species captured concurrently at the same site. Principal components analysis (PCA) was used to investigate measured fish habitat characteristics other than Se exposure in relation to fish biomass and stepwise multiple linear regressions were used to determine if other measured environmental variables significantly predicted fish biomass. All analyses used $p < 0.05$.

Results

Food chain transfer of Se

Mean waterborne Se, biofilm Se, invertebrate Se and juvenile fish muscle Se concentrations were significantly greater in mine-affected than reference streams (Table 2.2) and significant positive enrichment/trophic transfer relationships were observed at

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each food chain step (Figure 2.2). At the base of the food chain, the overall ANCOVA model predicting biofilm Se concentration from water Se concentration and watershed was significant ($R^2 = 0.5290$, $F_{2,21} = 11.7927$, $p = 0.0004$) with a significant effect of watershed and significant positive effect of water Se (Table 2.3 and Figure 2.2a). An interaction term between water Se and watershed was not significant ($F_{1,20} = 0.0999$, $p = 0.7553$) and was therefore removed from the model. The relationship between biofilm Se and water Se in the Elk R. watershed had a higher regression intercept (0.7240) than the relationship in the McLeod R. watershed (0.4088) (Table 2.4).

In the food chain step from biofilm to macroinvertebrates the model ($R^2 = 0.4724$, $F_{2,20} = 8.9538$, $p = 0.0017$) indicated a significant positive effect of biofilm Se but not study watershed (Table 2.3 and Figure 2.2b) after a non-significant interaction between biofilm Se and study watershed ($F_{1,19} = 1.0900$, $p = 0.3096$) was removed.

Finally, in the third food chain step from invertebrates to fish muscle tissue, interaction terms between species (nested by watershed) and invertebrate Se and between study watershed and invertebrate Se were not significant ($F_{2,15} = 1.0224$, $p = 0.3835$ and $F_{1,15} = 4.2700$, $p = 0.0565$, respectively). The overall model, refitted without interaction terms, was significant ($R^2 = 0.4865$, $F_{4,18} = 4.2648$, $p = 0.0133$) but only the individual effect of invertebrate Se significantly contributed to the fit (Table 2.3 and Figure 2.2c).

Se transfer at each food chain step was described by positive log-linear relationships between predictor and dependent Se concentrations (Figure 2.2). The calculated relationship from water to the base of the food web as biofilm was watershed specific, while the relationships between biofilm and invertebrates and invertebrates and juvenile fish muscle tissue were calculated from pooled watershed and species data

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(Figure 2.2; regression parameters in Table 2.4). Species specific regressions of juvenile fish muscle tissue Se vs. invertebrate Se were also determined (Figure 2.3).

The mean Se EF from water to biofilm was higher in the Elk R. watershed (Table 2.5) but mean trophic transfer of Se from biofilm to invertebrates was greater in the McLeod R. watershed (Table 2.5). Mean fish species specific Se TTF from invertebrates to juvenile salmonids ranged between 1.06 ± 0.14 in bull trout and 1.39 ± 0.08 in rainbow trout with an overall average of 1.24 ± 0.07 and a general transfer factor relationship where TTF rainbow trout > TTF cutthroat trout > TTF brook trout > TTF bull trout (Table 2.5).

Se exposure and fish biomass

Based on calculated muscle Se EC values, average fish muscle Se concentrations in rainbow trout exceeded the EC_{10} for skeletal deformities (3.01 mg/kg dw) in eight of the nine McLeod R. streams in which they were captured. However, where 100% of all individual rainbow trout captured in mine-affected streams had muscle tissue concentrations above the effects threshold (range of site averages $4.71 - 15.07$ mg/kg dw), this proportion was only 56% in reference streams (range of site averages $3.14 - 3.74$ mg/kg dw). Average brook trout muscle Se exceeded the calculated NOEC for craniofacial deformities (10.25 mg/kg dw) at only one mine-affected site, but a single fish at two other sites (one mine-affected, one reference) also exceeded the NOEC. Average cutthroat trout muscle Se was in the EC_{10} range for alevin mortalities ($8.5 - 12.05$ mg/kg dw) at two sites, both of which were mine-affected, but at least one individual fish at each of four mine-affected sites in the Elk R. watershed and one fish at a reference site had muscle Se concentrations within the EC range.

Total fish biomass was not significantly related to average fish muscle Se concentration in the study streams (Figure 2.4). Species biomasses of cutthroat trout and bull trout were also not significantly related to species specific average fish muscle Se concentrations in Elk R. watershed streams (Figure 2.5a). In the McLeod R. basin, rainbow trout biomass was significantly negatively related to rainbow trout fish muscle Se concentrations (Figure 2.5b). Rainbow trout biomass was 2.25 g/m^2 in the reach with the lowest average Se muscle concnetration (3.14 mg/kg dw) and only 0.07 g/m^2 in the reach with the highest average Se muscle tissue concentration (15.07 mg/kg dw). No significant relationship was identified in brook trout (Figure 2.5b) where biomass was between 0.15 and 0.41 g/m^2 over a similar tissue concentration range (3.10 – 18.84 mg/kg dw) in the same streams.

Investigation of measured habitat and water quality characteristics besides Se exposure did not identify any strong predictors of fish biomass. Though PCA indicated that cover variables such as the amount of undercut banks and large woody debris were high and the percent of slow water (pool) habitat was also high in streams with the highest biomass, stepwise multiple linear regression did not identify any significant habitat models that predicted total fish biomass. Therefore, habitat characteristics besides Se exposure are not further discussed in relation to fish biomass but measured values for each stream can be found in Table 2.6 SI.

Discussion

Se transfer in lotic food chains

      Se concentrations in the water and biota of mine-affected streams were greater than in reference streams, confirming that surface mines were significant sources of Se in the studied watersheds. Se accumulation at the base of the food chain, from water to biofilm, differed between the two study watersheds but there was an overall significant positive relationship between waterborne and biofilm Se concentrations. The watershed specific relationships between waterborne and biofilm Se were log-linear with a slope less than one demonstrating higher accumulation of Se by biofilm at low waterborne Se concentrations and a decreasing accumulation rate as waterborne concentrations increased. Concentration dependent accumulation of essential compounds like Se and other metals, where the highest affinity uptake occurs at the lowest source concentration, allows organisms to accumulate enough of the essential compound to meet physiological requirements even when source concentrations are low (DeForest et al. 2007).

      Higher Se accumulation in the biofilm of Elk R. streams at similar waterborne Se concentrations to McLeod R. streams resulted in a significantly greater regression intercept and greater EF in the Elk R. watershed at the first food chain step. Particulate Se enrichment is often site-specific (Presser and Luoma 2010). Interspecific differences in Se enrichment relative to waterborne concentrations exist among algal and bacterial taxa due to differential cellular requirements and abilities for regulating Se uptake (Baines and Fisher 2001, Presser and Luoma 2010). Further, dissolved Se can be present in lotic systems in different forms including selenate (Se VI), selenite (Se IV) or organo-Se (Se II) which are differentially bioavailable to organisms (Maher et al. 2011). Finally,

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selenate is accumulated through carrier-mediated processes in algal and bacterial cells and therefore its uptake can be influenced by the presence of other ions, particularly sulfate, as they compete for transport sites on cell membranes (Fournier et al. 2010, Maher et al. 2011). Therefore, the observed difference in biofilm Se enrichment between the two watersheds could be accounted for by biofilm community variation, Se speciation or the relative concentrations of sulfate and selenate.

In the second food chain step there was a significant positive relationship between biofilm and invertebrate Se concentrations that was not different between watersheds. Invertebrates primarily accumulate Se from their diets and Se accumulation rates can be taxon-specific due to differences in dietary preferences, physiological Se requirements, and capacities for assimilating, retaining and eliminating Se (Andrahennadi et al. 2007, Presser and Luoma 2010, Stewart et al. 2011). Co-incident Se relationships between biofilm and invertebrate Se in the two watersheds are likely a result of overall similarity in the invertebrate communities across watersheds. Se concentrations were measured in composite invertebrate samples containing organisms belonging to a total of 43 families, many of which were rare. Only 13 families (12 of which were aquatic insects) composed an average of >1% of individuals at each site (Chapter 3, this thesis). Of the 13 dominant families, 12 were present in both Elk R. and McLeod R. samples indicating similar Se accumulation capabilities in the organisms that dominate invertebrate assemblages across the two watersheds.

The extent to which the base of the food chain is enriched with Se is important in determining Se contamination throughout the entire food web. Biofilm and therefore invertebrate Se concentrations were lower in McLeod R. streams compared to Elk R.

streams. Average Se TTF from biofilm to invertebrates were within the range for field sampled aquatic insects of 2.1 - 3.2, summarized by Presser and Luoma (2010) however, TTF were higher at McLeod R. sites than Elk R. sites. Higher trophic transfer from biofilm to invertebrates in McLeod R. streams suggests concentration dependent uptake at this food chain step where greater Se accumulation occurs in invertebrates exposed to lower dietary Se concentrations (DeForest et al. 2007). A similar relationship was observed in another lotic trophic transfer study in the Elk R. watershed (Orr et al. 2012) and supports a trophic transfer model where Se uptake rates in stream dwelling invertebrates change in relation to available dietary Se concentrations.

There was a significant relationship in the third step of the food chain between invertebrate and juvenile fish muscle tissue Se. The relationship was also concentration dependent, with higher Se TTFs in fish muscle tissues at lower invertebrate Se concentrations. This supports observations that fish retain more Se from their diets when source concentrations are low (Hardy et al. 2010, Jardine and Kidd 2011) suggesting greater accumulation may be necessary to meet nutritional requirements (Hardy et al. 2010).

While invertebrate Se concentration had a significant effect on fish muscle Se concentration, there was no significant effect of fish species. Generally, Se accumulation in fish is considered species-specific based on variation in dietary preferences, habitat use and physiology (absorption and metabolism) (Stewart et al. 2011) but these sources of variation may not have influenced Se accumulation in fish muscle tissues in this study. The relative magnitudes of the species specific regression slopes of invertebrate vs. fish muscle Se were brook trout > rainbow trout > bull trout > cutthroat trout. However,

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rainbow trout and brook trout in this study were exposed to a lower, narrower range of invertebrate Se concentrations than cutthroat and bull trout. The slightly greater accumulation rates of Se by rainbow trout and brook trout could therefore result from concentration dependent uptake of Se by juvenile fish rather than species specific differences in Se accumulation.

Stomach content analyses on juvenile trout from eastern Canadian Rockies watersheds indicate that they consume similar diets, regardless of species, feeding predominantly on aquatic insects including Ephemeroptera, Plecoptera, Trichoptera and Diptera (Simulidae, Tipulidae, and Chironomidae) (Stantec Consulting Ltd 2004, Casey 2005, Costello 2006, Warnock 2012). In small streams these diets may be supplemented by terrestrial insects and it is therefore important to consider that allochthonous inputs may confound the diet to fish Se accumulation relationship (Jardine and Kidd 2011). However, in the streams of the present study there is evidence that species differences in diet among early life stages of trout in the sampled watersheds are not sufficient to produce differences in Se accumulation.

Species differences in Se tissue concentrations among adult fish collected in previous field studies have also suggested species-specific Se accumulation (Holm et al. 2005, Minnow Environmental Inc et al. 2011). Tissue concentration differences among species may, however, be attributable to the physiological allocation of Se to different tissues rather than differences in absorption and metabolism. In a laboratory study, Pilgrim (2012) measured whole body Se burdens and relative Se allocation to muscle, liver and ovary tissues in adult rainbow, brook and cutthroat trout that were fed Se enriched diets. Though no differences in Se body burdens were reported among species,

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brook trout allocated more Se to muscle than egg tissues compared to rainbow and cutthroat trout (Pilgrim 2012). In field studies, muscle:egg Se ratios also differ among species (1:7 in rainbow trout (Holm et al. 2005), 1:2 in brook trout (Holm et al. 2005, Miller et al. 2013) and 1:2 in cutthroat trout (Rudolph et al. 2008, Orr et al. 2012)) but similarity in overall accumulation is not generally considered due to potential uncertainty of individual Se exposure in field sampled adult fish.

Examinations of Se accumulation and tissue specific allocation in multiple species of juvenile salmonids are rare, but Miller et al. (2013) found that some species of juvenile salmonids may not significantly differ in Se accumulation in confined natural environments. Miller et al. (2013) stocked hatchery reared juvenile rainbow and brook trout into end-pit lakes with elevated Se concentrations in Alberta, Canada and found that after 24 months of exposure muscle Se tissue concentrations and muscle:whole body Se relationships were not significantly different between the species. Therefore, a significant difference in Se accumulation may not have been detected among the muscle tissues of species in the current study because juvenile fish did not differ significantly in their dietary preferences and, as they had not yet developed gonads or had immature gonads, they did not demonstrate differences in Se tissue allocation comparable to those observed in pre-spawning adult fish.

Though there was no effect of species, the relationship between invertebrate Se and juvenile fish muscle Se was significant. Highly variable tissue concentrations in wild trout in lotic systems have been documented (Holm et al. 2005, Rudolph et al. 2008) and in some cases no relationship between fish tissues and diet Se concentrations was found (Orr et al. 2012). Fish movements in lotic systems with good hydrological connectivity

are an important potential factor in producing these Se concentration patterns (Holm et al. 2005, Orr et al. 2012). Variable use of Se-contaminated habitats by adult fish has been confirmed in studies of adult trout residency in Elk and McLeod R. streams (Palace et al. 2007, Friedrich et al. 2011). In these studies, adult fish were captured during the spawning season in order to obtain eggs or gonads for toxicity testing. Spawning in most salmonids, especially those with migratory life histories, is a period of particularly high movement. While Se tissue concentrations can reflect dietary concentrations almost immediately, they can take weeks or months to reach equilibrium (Stewart et al. 2011). Therefore, fish that have recently moved, like those that have recently undertaken spawning migrations may not have tissue concentrations that reflect dietary sources at the location where they are captured. The significant relationship between juvenile fish Se and invertebrate Se supported the prediction that targeting a relatively less mobile life stage in this study would result in a strong relationship between Se concentrations in fish diet and fish tissues.

The significant relationship between juvenile fish muscle Se and invertebrate concentrations at the point of capture also has implications for monitoring and management of Se toxicity risks to fish. It provides field based support for an empirical relationship between dietary Se and accumulation in salmonid tissues in lotic systems. If this relationship can be reliably defined, Se toxicity risks in mine-affected systems could be predicted from sampling at lower levels of the food chain (water, biofilm or invertebrates), reducing impacts on vulnerable fish populations due to lethal sampling (Orr et al. 2012). Further, because juvenile fish of different species do not appear to differ

in Se accumulation it may be possible to generalize food chain models and focus on tissue allocation and species sensitivity differences to determine species specific Se risks. Finally, if fish tissues are required to determine Se toxicity risks in trout, this study indicates that juvenile tissues are representative of Se exposure at the site of interest. Determining differences between juvenile and adult Se tissue allocations will be essential for defining appropriate thresholds for reproductive effects, but juvenile fish may be better candidates for sampling than individuals that have reached maturity and are therefore valuable in maintaining spawning populations.

Individual- and community-level Se exposure effects in fish

Total fish biomass and species specific biomass at the reach scale were not significantly related to average fish muscle Se concentrations except in rainbow trout. In McLeod R. streams, rainbow trout biomass declined as muscle Se concentrations increased, though brook trout biomass in the same streams remained relatively constant at all Se concentrations. Rainbow trout biomass was 2.18 g/m^2 (over 97%) lower at the highest measured Se tissue concentration of 15.07 mg/kg dw than at the lowest Se tissue concentration of 3.14 mg/kg dw. Average rainbow trout muscle Se concentrations were elevated in many of the sampled streams and exceeded the EC$_{10}$ for larval skeletal deformities of 3.01mg/kg dw, which is the lowest field derived EC of any of the sampled species. Therefore, individual-level reproductive effects in rainbow trout likely contributed to declines in biomass.

Though muscle Se concentrations were not related to total fish biomass or species specific biomass in the other sampled species, elevated fish tissue concentrations in the mine-affected study streams suggested individual-level effects may have been occurring.

Two sites had average cutthroat trout muscle Se concentrations above the EC_{10} for alevin mortalities and EC exceedences occurred in individual cutthroat trout at all mine-affected sites in the Elk R. watershed. Potential reproductive effects in bull and brook trout are less certain due to the lack of any EC for bull trout and muscle Se concentrations in brook trout that exceeded a NOEC above which effects are undetermined. In general, Se exposure concentrations in some species may have been great enough to cause individual-level Se toxicity effects but not to produce detectable fish community- or population-level impacts at the reach scale. Small changes in the fish population density and community composition at the reach scale are difficult to detect as they could have been masked by natural population variability, access to uncontaminated refugia or density compensation (Janz et al. 2011).

Stream salmonid populations vary naturally from year to year due to changes in the environmental characteristics of streams. Therefore, in some cases negative impacts due to toxicity must be substantial before they can be detected at the community level (Janz et al. 2011, Environment Canada 2012). Adult fish movements into nearby uncontaminated refugia (Palace et al. 2007, Friedrich et al. 2011) can reduce the community-level impacts of toxicity through access to habitats with low or no Se exposure. The reproductive effects caused by high egg Se concentrations may be mediated if spawning females utilize and feed in uncontaminated habitats during periods of egg formation (Environment Canada 2010b, Janz et al. 2011) and immigration of fish from uncontaminated refugia would suggest that the appropriate spatial unit for measuring/detecting community-level effects is greater than the reach scale (Freund and Petty 2007). Finally, since growth and survival of juvenile trout rearing in streams

depends partially on competition for limited habitat and food resources, when densities are low fish have higher rates of growth and survival (Jenkins et al. 1999, Keeley 2001). This type of density compensation in the sampled streams could prevent the detection of community-level effects if populations compensate for negative individual-level Se impacts by increased growth and survival of remaining individuals (Van Kirk and Hill 2007).

While density compensation may have prevented detection of any reach scale community-level effects that occurred in most of the sampled species, the opposite may have been true for rainbow trout in McLeod R. streams. A demographic model of Se impacts on juvenile cutthroat trout by Van Kirk and Hill (2007) suggests that trout populations can be particularly vulnerable to Se impacts if additional stressors such as non-native species reduce their capacity for density compensation. Brook trout are an introduced species in McLeod R. streams and have been cited as a competitive threat to native rainbow trout populations in the watershed as faster growth, earlier reproduction and relative insensitivity to Se toxicity may provide a competitive advantage (Rasmussen and Taylor 2009). Se exposure that causes individual-level toxicity in rainbow trout but does not affect brook trout reduces overall fish density and could provide an opportunity for density compensation by brook trout, possibly resulting in further rainbow trout declines. The combined effects of Se sensitivity and competition with brook trout may be the reason for apparent impacts on rainbow trout populations at the stream reach scale.

This study contributes valuable information to the continuing development of strategies for monitoring and managing Se impacts in lotic systems. Juvenile fish muscle Se concentrations reflected food chain Se concentrations at capture sites and may

therefore be appropriate candidates for tissue monitoring and use in the creation of food chain models, especially as Se tissue allocation relationships among trout species are further studied and become better understood. Food chain models could eventually allow food web Se concentrations (water, biofilm, invertebrates) to be used to predict and evaluate Se toxicity risks to fish at exposure sites thereby minimizing lethal sampling in at risk populations. Se toxicity effects on fish communities were not detectable in stream reaches at measured muscle concentrations for most fish species. However apparent declines of rainbow trout in McLeod R. streams demonstrated the potential for multiple stressors, including Se toxicity, to influence the ability of fish communities to remain in reference condition.

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Tables and Figures

Table 2.1 Sampling sites listed with study watershed, geographical coordinates and description as reference (Ref), mine-affected (MA), or reclaimed (MA-R).

Stream name	Site type	Watershed	Latitude	Longitude
W. Alexander Ck.	Ref	Elk	49.773	-114.721
Chauncey Ck.	Ref	Elk	50.108	-114.814
Dry Ck.	Ref	Elk	50.035	-114.817
Ewin Ck.	Ref	Elk	50.060	-114.797
Grace Ck.	Ref	Elk	49.984	-114.859
South Line Ck.	Ref	Elk	49.915	-114.767
Deerlick Ck.	Ref	McLeod	53.153	-117.244
Eunice Ck.	Ref	McLeod	53.154	-117.231
Wampus Ck.	Ref	McLeod	53.157	-117.262
Watson Ck.	Ref	McLeod	53.072	-117.259
W. Drinnan Ck.	Ref	McLeod	53.161	-117.544
Cataract Ck.	MA	Elk	50.151	-114.865
E. Crowsnest Ck.	MA	Elk	49.585	-114.693
Erickson Ck.	MA	Elk	49.678	-114.783
Fording R.	MA	Elk	49.894	-114.868
Harmer Ck.	MA	Elk	49.831	-114.822
Line Ck.	MA	Elk	49.892	-114.835
Swift Ck.	MA	Elk	50.158	-114.869
Berry Ck.	MA-R	McLeod	53.095	-117.447
Drinnan Ck.	MA-R	McLeod	53.182	-117.513
Gregg R.	MA	McLeod	53.129	-117.486
Jarvis Ck.	MA	McLeod	53.058	-117.363
Luscar Ck.	MA	McLeod	53.059	-117.311
Sphinx Ck.	MA-R	McLeod	53.125	-117.311

Table 2.2 Measured food chain Se concentrations, descriptive statistics and two-sample t-test (p < 0.05) results for differences in mean Se concentration between mine-affected streams and reference streams.

Variable	Units	Mine-affected Mean ± SE	Range	n	Reference Mean ± SE	Range	n	t	p
Waterborne Se	mg/L	0.100 ± 0.053	0.0005 – 0.543	13	0.0008 ± 0.0001	0.0005 – 0.0017	11	-5.46[*]	<0.001
Biofilm Se	mg/kg	3.57 ± 0.59	0.75 – 8.10	13	1.81 ± 0.24	0.75 – 3.32	11	-2.35	0.028
Invertebrate Se	mg/kg	5.97 ± 0.89	2.65 – 13.41	12	4.01 ± 0.34	2.8 – 6.46	11	-2.49	0.011
Fish muscle Se	mg/kg	7.60 ± 1.01	4.71 – 14.31	9	4.36 ± 0.55	2.6 – 7.36	8	-3.37	0.0023

[*]Indicates use of Welch's t due to unequal between group variances (Levene test; p < 0.05)

Table 2.3 Statistical results of ANCOVA effect tests in food chain Se relationships. In each food chain step, the effects of Se concentration of the lower trophic level and study watershed (Elk R. or McLeod R.) on Se concentration of the higher trophic level were tested. In the food chain step from invertebrates to fish, the effect of fish species (fixed effect nested in watershed location) on fish Se concentration was also tested.

Parameter	Model Effect	df effect	df error	F ratio	p-value
Biofilm Se	Water Se	1	21	4.5603	0.0447
	Watershed	1	21	12.7838	0.0018
Invertebrate Se	Biofilm Se	1	20	7.9742	0.0105
	Watershed	1	20	0.0217	0.8845
Fish Muscle Se	Invertebrate Se	1	18	9.0034	0.0077
	Watershed	1	18	0.1547	0.6987
	Species(Watershed)	2	18	0.7497	0.4867

Table 2.4 Regression parameters for food chain relationships between log$_{10}$-transformed water, biofilm, invertebrate and fish muscle Se concentrations.

	Watershed[*]	R^2	R^2 Adjusted	p-value	Y-intercept	Slope
Water vs. biofilm	Elk R.	0.5290	0.4841	0.0004	0.7240	0.0951
	McLeod R.				0.4088	
Biofilm vs. invertebrate	-	0.4718	0.4467	0.0003	0.5315	0.4290
Invertebrate vs. fish muscle	-	0.4392	0.4126	0.0006	0.2197	0.7645

[*] If watershed is not identified, regression parameters are calculated from linear regressions on pooled data from both study watersheds

Table 2.5 Se enrichment factors (EF) and trophic transfer factors (TTF) in biofilm, invertebrates and fish muscle (overall and species specific). CTTR = westslope cutthroat trout; BLTR = bull trout; RNTR = rainbow trout; BKTR = brook trout.

Transfer step	Watershed	Species	N	Range	Mean ± SE
Water-biofilm (EF)	Elk R.	-	13	7.62 – 9740	2230 ± 798
	McLeod R.	-	11	67.57 – 3140	1400 ± 345
Biofilm-invertebrate (TTF)	Elk R.	-	13	0.69 – 2.84	1.82 ± 0.18
	McLeod R.	-	10	1.50 – 6.01	3.20 ± 0.37
Invertebrate-fish muscle (TTF)		All	80	0.43 – 3.80	1.24 ± 0.07
	Elk R.	CTTR	17	0.52 – 2.80	1.24 ± 0.15
	Elk R.	BLTR	11	0.43 – 2.06	1.06 ± 0.14
	McLeod R.	RNTR	24	0.77 – 2.17	1.39 ± 0.08
	McLeod R.	BKTR	28	0.57 – 3.80	1.19 ± 0.15
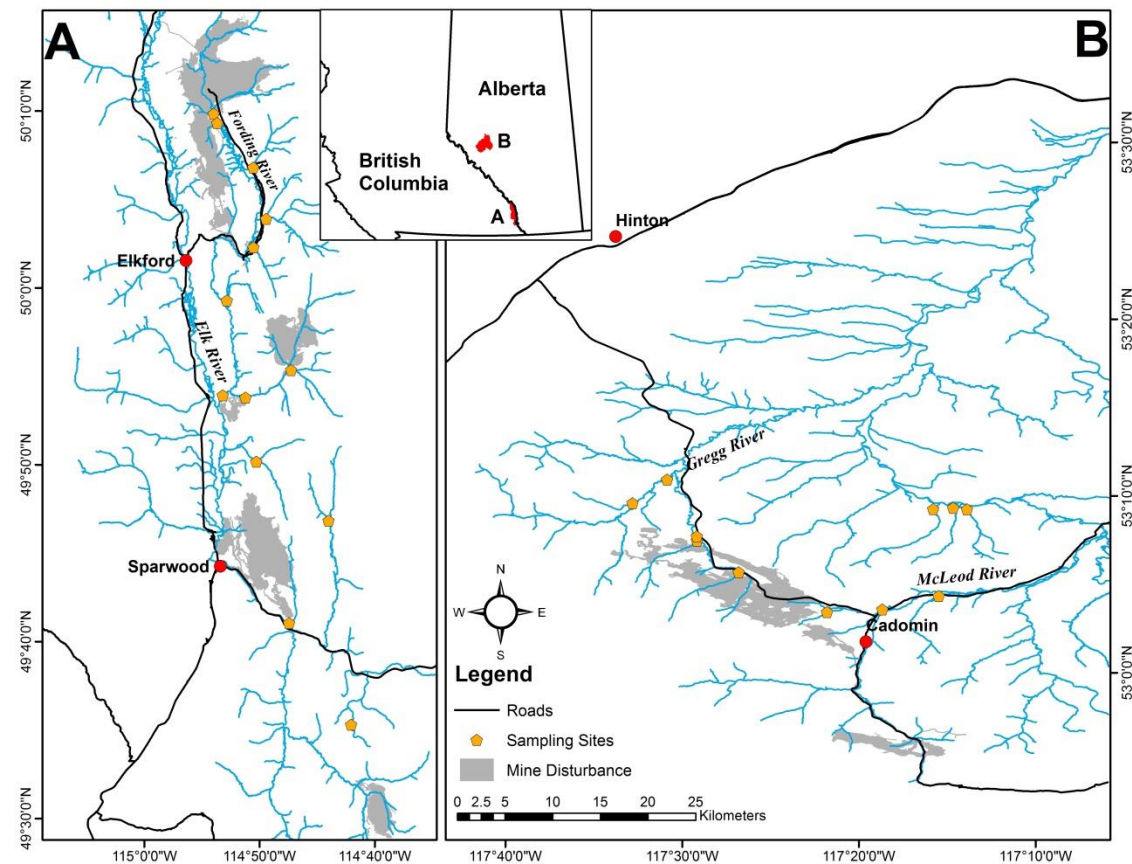
Figure 2.1 Map of study areas in the Elk River watershed, British Columbia, Canada (A) and the McLeod River watershed, Alberta, Canada (B) including sampling sites and disturbance area[1] for active and reclaimed mines.

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[1] Reproduced with the permission of Teck Coal Limited and Sherritt Coal International

(a)

(b)

(c)

Fish muscle Se (mg/kg dw) — y-axis

Invertebrate Se (mg/kg dw) — x-axis
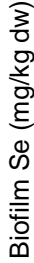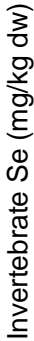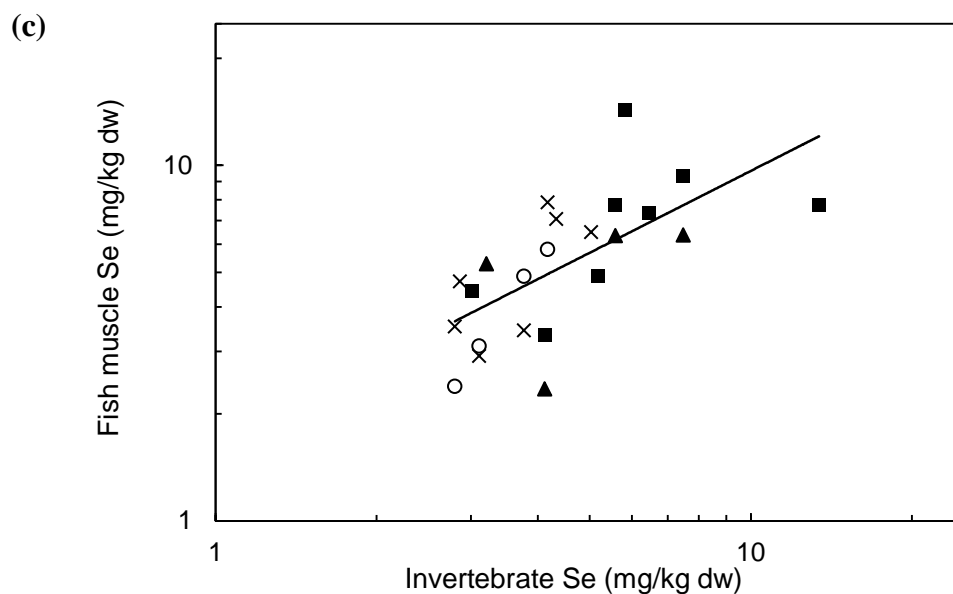
Figure 2.2 Se relationships in three lotic food chain steps in two study watersheds (a) water to biofilm (● and solid line = Elk R.; ○ and dashed line = McLeod R.); (b) biofilm to invertebrates (● = Elk R.; ○ = McLeod R.; line = relationship of pooled watershed data); (c) invertebrates to fish muscle tissue (■ = westslope cutthroat trout; ▲ = bull trout; x = rainbow trout; ○ = brook trout; line = relationship of pooled watershed/species data). Regression parameters are in Table 2.4.
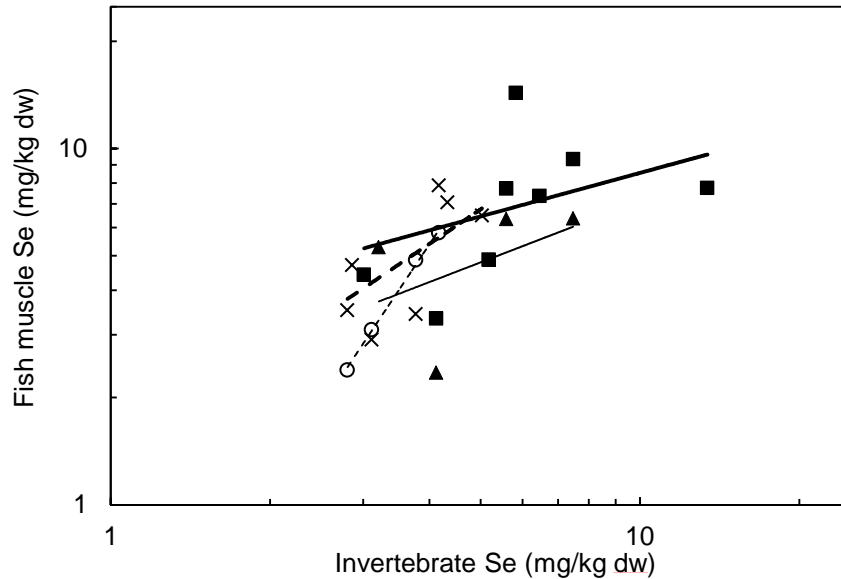
Figure 2.3 Relationship between invertebrate Se concentration and juvenile fish muscle Se concentration in different salmonid fish species. Westslope cutthroat trout = ■ and bold line (R^2 = 0.2141, p = 0.2483); bull trout = ▲ and solid line (R^2 = 0.1709, p = 0.5866); rainbow trout = x and bold-dashed line (R^2 = 0.4641, p = 0.0920); brook trout = ○ and dashed line (R^2 = 0.9963, p = 0.0018).
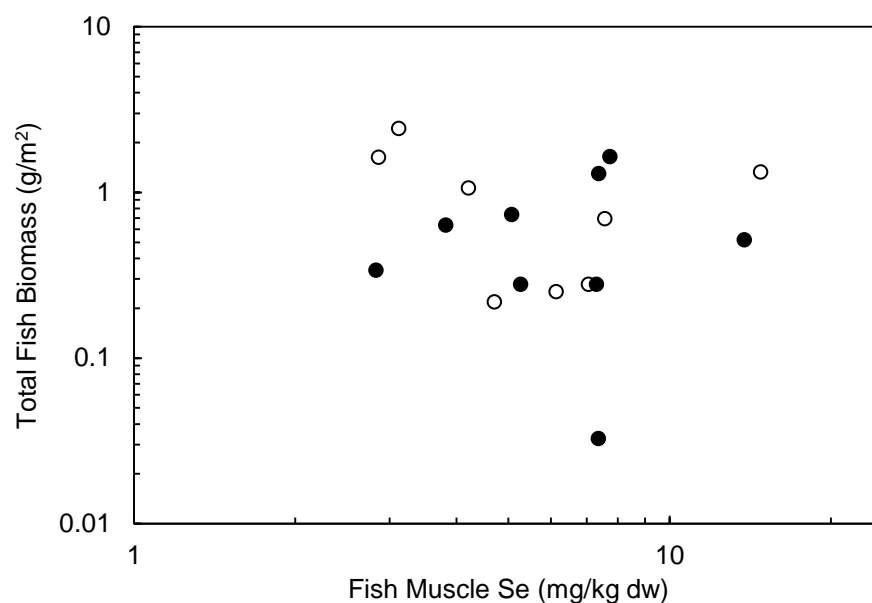
Figure 2.4 Relationship between total fish biomass and average fish muscle tissue Se concentration in sampled stream reaches in the Elk R. watershed (●) and the McLeod R. watershed (○) (pooled watershed data; $R^2 = 0.0165$; p = 0.6236). Fish muscle Se concentrations are the average Se concentration of all fish of all lethally sampled species (n = 2 – 14).

(a)

(b)
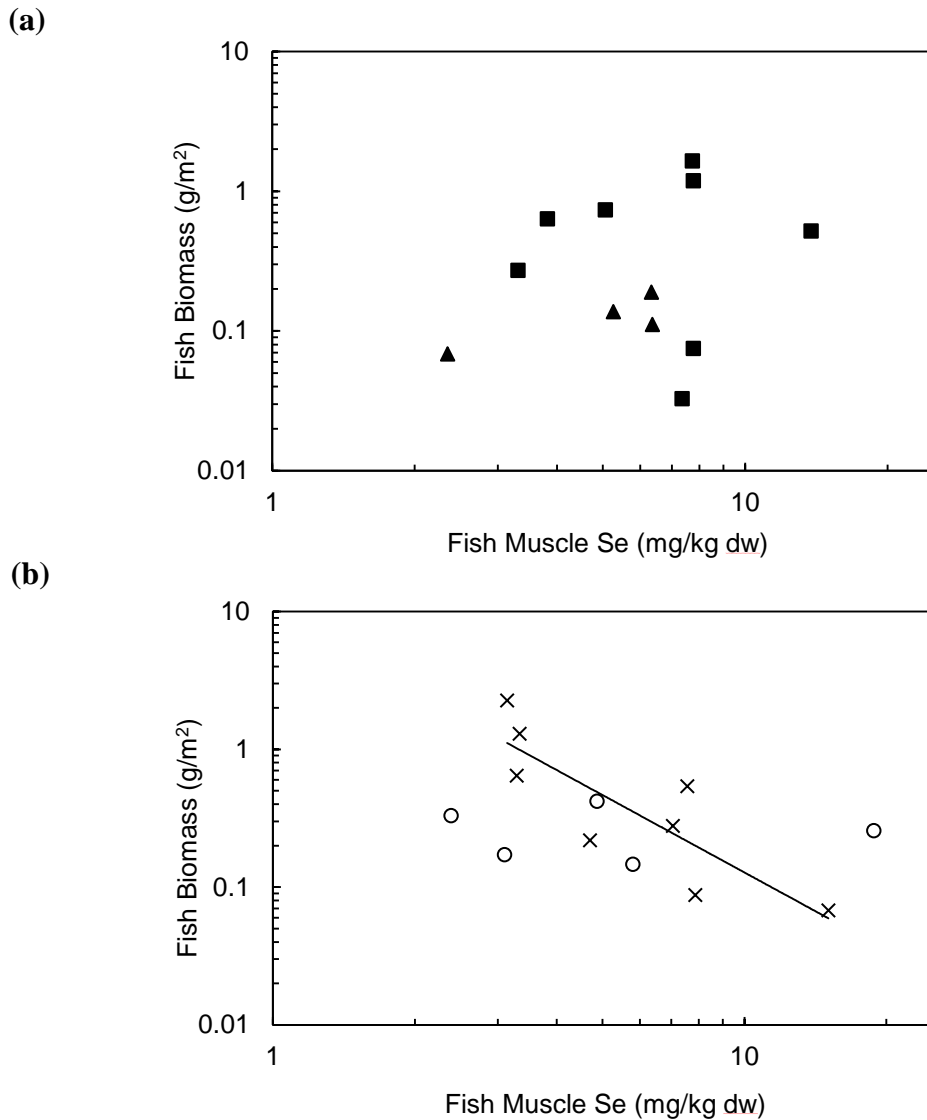
Figure 2.5 Relationship between species biomass and average species specific fish muscle tissue Se concentration in (a) Elk R. streams containing westslope cutthroat trout (■; $R^2 = 0.0007$; p = 0.9516) and/or bull trout (▲; $R^2 = 0.7238$; p = 0.1492) and (b) McLeod R. streams containing rainbow trout (x and solid line; $R^2 = 0.7076$; p = 0.0089) and/or brook trout (○; $R^2 = 0.0034$; p = 0.9255). Fish muscle Se concentrations are the average Se concentrations of fish of the same species from each sampling reach (n = 2 – 10).

Supporting Information

Table 2.6 SI Water quality and habitat characteristics measured in study stream reaches. WS = study watershed (ER = Elk R. and MR = McLeod R.); DO = dissolved oxygen; TN = total nitrogen; TP = total phosphorus; WOLDG = Wolman Dg; EMB = substrate embeddedness; WW = wetted width; LWD = large woody debris; RC = riparian cover; and UCB = undercut banks.

Site	WS	pH	DO (mg/L)	TN (mg/L)	TP (µg/L)	WOLDG (cm)	Slope (%)	Velocity (m/s)	EMB (%)	WW (m)	Depth (m)	Pool (%)	Glide (%)	Riffle (%)	LWD (#)	RC (%)	UCB (%)
Alexander	ER	8.4	10.5	43	19	7.63	2.6	0.44	50	4.8	0.30	41	6	53	21	23	4
Berry	ER	8.3	9.8	667	2	7.35	3.46	0.65	25	4.2	0.20	12	24	64	20	30	4
Cataract	ER	8.1	9.9	30250	0.5	-	11.0	0.59	100	4.0	0.09	24	26	50	10	40	-
Chauncey	ER	8.3	11.8	44	1	8.92	3.1	0.62	25	4.5	0.26	23	26	51	32	40	8
Deerlick	ER	8.2	9.9	103	0.5	7.46	1.6	0.45	25	3.7	0.22	34	35	31	30	11	10
Drinnan	ER	8.2	9.5	57	2	4.77	1.3	0.99	38	9.1	0.47	28	41	31	28	4	5
Dry	ER	8.4	9.6	56	13	4.28	1.2	0.23	25	4.7	0.25	20	20	60	9	17	5
E. Crowsnest	ER	8.3	10.7	33	7	5.32	3.1	0.63	38	3.3	0.18	25	11	64	64	30	5
Erickson	ER	8.3	11.2	6580	11	2.77	7.2	0.80	63	4.5	0.28	13	21	66	22	40	3
Eunice	ER	8.3	10.9	102	4	7.20	3.4	0.61	38	3.2	0.25	21	33	46	33	40	7
Ewin	ER	8.2	11.6	76	1	7.00	3.8	0.80	50	6.6	0.33	42	24	34	10	11	6
Fording	ER	8.4	10.2	7260	2	14.36	0.5	0.80	50	25.0	0.40	24	26	50	10	11	-
Grace	ER	8.4	11.2	62	7	3.47	2.8	0.50	0	3.1	0.23	35	14	51	90	10	10
Gregg	MR	8.4	8.2	1070	2	6.47	4.0	0.84	38	8.3	0.25	10	24	66	15	9	2
Harmer	MR	8.4	10.8	931	10	5.32	2.4	0.71	25	8.0	0.25	22	24	54	165	30	3
Jarvis	MR	8.4	9.1	3670	2	7.13	2.8	0.59	25	3.9	0.18	17	35	48	0	32	2
Line	MR	8.3	11.1	7830	5	15.52	2.0	0.74	50	10.5	0.44	22	8	70	8	13	8
Luscar	MR	8.4	8.6	1960	3	11.8	1.5	0.58	50	5.3	0.23	11	48	41	8	14	5
South Line	MR	8.3	12.5	67	2	11.08	3.8	0.54	50	5.7	0.29	17	17	66	16	5	0
Sphinx	MR	8.4	10.2	337	8	7.14	3.1	0.49	38	5.7	0.24	18	19	63	12	19	5
Swift	MR	8.1	10.3	33650	0.5	1.27	2.1	0.45	100	2.9	0.18	48	24	28	63	7	3
Wampus	MR	8.3	9.7	86	7	7.01	2.6	0.39	50	5.7	0.18	22	32	46	11	26	3
Watson	MR	8.2	8.7	99	5	7.26	2.1	0.38	50	4.0	0.17	26	30	44	1	11	6
W. Drinnan	MR	7.9	9.3	58	2	2.92	1.5	0.52	25	8.6	0.40	21	50	29	23	22	5

CHAPTER 3.   SURFACE COAL MINING INFLUENCES ON MACROINVERTEBRATE ASSEMBLAGES IN STREAMS OF THE CANADIAN ROCKIES

Abstract

Surface coal mine operations can affect downstream aquatic ecosystems through the release of toxicants such as selenium (Se), changes to water chemistry, and physical habitat alterations that affect stream substrate and morphology. Chemical and physical stream characteristics and macroinvertebrate family and community metrics were measured in mine-affected and reference streams in the Canadian Rockies to determine the region specific impacts of surface coal mines on macroinvertebrate community health. Water chemistry was significantly altered in mine-affected streams with elevated conductivity, alkalinity, Se and ion concentrations compared to reference conditions. Trends in physical habitat characteristics, though not significant, were also found along gradients of mine influence where mine-affected streams had higher substrate embeddedness, smaller interstitial materials and steeper slopes. Multivariate redundancy analysis (RDA) showed that macroinvertebrate communities downstream of mine sites demonstrated severe declines in Ephemeroptera family densities and increased densities of Capniidae stoneflies. In RDA ordination of community metrics, family and EPT richness and % Ephemeroptera declined along a gradient of increasing mine influence. Shifts in macroinvertebrate assemblages may have been the result of multiple region specific stressors including Se toxicity, ionic toxicity, or stream substrate modifications.

Introduction

Surface coal mining is an intensive process that involves large-scale removal of overburden rock to access shallow coal seams in coalfield rock formations (Palmer et al. 2010). In addition to major changes in topography and watershed morphology at mine sites (Lindberg et al. 2011), large quantities of disturbed rock and soil are exposed to increased weathering conditions, mobilizing solutes in downstream aquatic systems (Naftz and Rice 1989). Surface coal mining influences lotic systems through the individual and combined effects of stream habitat alteration (Scullion and Edwards 1980), water quality shifts (Pond et al. 2008, Lindberg et al. 2011), and the elevated release of potentially toxic compounds (Wayland and Crosley 2006), most notably selenium (Se) (Palmer et al. 2010). Over the past 40 years, the intensity of surface coal mining has increased dramatically in regions of the Canadian Rocky Mountains in the provinces of Alberta and British Columbia, Canada (Lussier et al. 2003) and the continued development of surface mines emphasizes the need to understand and manage the region specific impacts of mining on stream habitat, water quality and aquatic biota.

Selenium exposure and toxicity have been the focus of many aquatic studies in coal mining regions of the Canadian Rockies (Wayland and Crosley 2006, Wayland et al. 2007, Orr et al. 2012, Miller et al. 2013). Se released from waste rock at mine sites drastically increases background stream concentrations from $0.1 - 0.4$ μg/L (United States Environmental Protection Agency 2004) to concentrations that can reach over 100 μg/L (Orr et al. 2012). The toxic effects of Se bioaccumulation have been extensively studied in egg laying vertebrates including fish (Chapman 2007) and aquatic birds (Wayland et al. 2007). Elevated concentrations of Se have been measured in

macroinvertebrates downstream of surface mines (Wayland and Crosley 2006, Orr et al.

2012) and sub-lethal toxic effects including reduced growth and reproduction have been

observed in certain taxa at relatively low Se tissue concentrations in laboratory studies

(deBruyn and Chapman 2007). Se toxicity threats to valuable aquatic resources, in this

region have led to numerous monitoring plans as well as small and large scale Se

reduction efforts (Abbott et al. 2012).

Alterations in water chemistry besides Se contamination have been the emphasis

of recent biomonitoring studies in the Appalachian Coalfields in the United States

(Cormier et al. 2013a). High stream conductivity and elevated ionic mixtures containing

SO_4^{2-}, Cl^-, HCO_3^-, Ca^+ and Mg^+ released from rock spoil downstream of mountaintop coal

mines have been cited as causes of macroinvertebrate community impairment (Pond et al.

2008, Cormier et al. 2013c). Differences in bedrock geology result in naturally harder

water in Canadian Rockies streams which have background conductivities varying

around 300 μS/cm (Noton 1998, Dessouki and Ryan 2010) compared to the Appalachian

region range of 72 - 153 μS/cm (Cormier et al. 2013a). However, water quality data from

studies in British Columbia and Alberta suggest elevated conductivity and sulfate

concentrations which could affect aquatic biota (Dessouki and Ryan 2010, Miller et al.

2013).

Habitat modifications also occur in the aquatic environments of mined watersheds

and include large-scale land clearance and the complete burial of portions of headwater

streams by rock spoil which increase sediment export and change stream hydrology

(Pond et al. 2008, Fritz et al. 2010). Additionally, limestone and shale dominated bedrock

in the Canadian Rockies creates water chemistry conditions that promote streambed

calcite accumulation. Calcium carbonate precipitation as calcite in streams is a natural process which is intensified downstream of surface coal mines in the region due to the extreme supersaturation of CO_2 and calcite in water passing through rock spoil (Ford and Pedley 1996). Deposition takes place as the water surfaces and successive CO_2 degassing and calcite precipitation occur (Chen et al. 2004). Calcite accumulation in the region can be significant, sometimes spanning the entire width of streams and resulting in concretion or terracing of stream channels.

Macroinvertebrate communities, though not extensively studied in response to mine influences in the Canadian Rockies, are often used to assess the impacts of stressors on the integrity of the biotic community (Clements 2004). Surface mine related studies in other regions have used invertebrate community composition to address questions of mine impact and system recovery (Fritz et al. 2010, Petty et al. 2010). Multiple potential mine impacts in Canadian Rockies streams and a growing coal mine industry highlight the need to understand mining effects on ecosystem health, including macroinvertebrate community integrity in mined watersheds.

The present study examined benthic macroinvertebrate community composition in streams with surface coal mining disturbance in their upstream watersheds and in nearby reference streams. Macroinvertebrate communities as well as water chemistry and physical habitat were investigated. The objectives of this study were to characterize physical and chemical variables in surface mine affected and reference streams, to investigate and quantify the influence of these variables on macroinvertebrate taxa composition and community metrics, and to identify any sensitive taxa that could be used as indicators of mine impact.

Materials and Methods

Study areas

Twenty-four streams were sampled in two separate study areas with long-term influences from surface coal mining (Figure 3.1). The Elk River watershed in south-eastern British Columbia, Canada is an approximately 4450 km^2 portion in the headwaters of the Columbia River basin. Five operating surface coal mines produce metallurgical and thermal coal from the Elk Valley and Crowsnest Coalfields contained in the Mist Mountain Formation of the Jurassic-Cretaceous Kootenay Group (B.C. Ministry of Forests Lands and Natural Resource Operations 2012). These mines drain directly and indirectly into tributaries of the Elk River. The McLeod River watershed is located in the middle reaches of the Athabasca River in west-central Alberta, Canada. Two operating and one reclaimed surface coal mine in the Coalspur and Gates Formations of the Lower Cretaceous Luscar Group (Richardson et al. 1990) drain directly and indirectly into the McLeod River and its tributaries. Across the two study areas, 13 mining affected and 11 reference streams were sampled (Table 3.1 and Figure 3.1). Study sites were located between 1250 and 1600 meters above sea level in elevation in 2nd to 5th order streams. Streams were designated as mine-affected if they received drainage from upstream surface coal mines. Mine-affected streams were selected to represent a range of mine influence based on the area of mine disturbance in the upstream watershed and reference streams were selected in nearby watersheds with no mine influence. Three of the mine-affected streams were influenced by mine areas that have been reclaimed for 5 – 10 years. These sites were grouped with other mine-affected sites in all analyses but are

65

identified as reclaimed in Table 3.1. Despite no mine disturbance in their upstream watersheds, many reference sites experienced other anthropogenic influences from logging, road construction, and recreational usage. Therefore, reference sites do not necessarily represent pristine watersheds but rather the range of anthropogenic effects typical of the region but without the additional influence of mining.

Macroinvertebrate sample collection, processing and identification

Macroinvertebrate samples were collected between late July and late August 2011, using a 0.09 m^2 surber sampler with 250 μm mesh size and sampling to approximately 0.1 m depth into the stream substrate. Three replicate samples were taken per stream each comprising one minute sampling intervals at three locations along a stream transect. Transects were restricted to shallow riffles operationally defined as swift water habitats with turbulent flow less than 0.3 m deep and with broken water surface. All sampled material was transferred to freezer bags, placed on ice in the field, and frozen upon return to the laboratory.

In the laboratory, samples were thawed and washed using Type 1 Milli-Q ultrapure water (EMD Millipore) through a series of 4 sieves (mesh sizes: 2 mm, 1 mm, 500 μm and 250 μm) to remove fine debris. They were then transferred to white plastic trays from which all organisms were sorted from remaining debris and identified to the family level (Clifford 1991), except for organisms of the orders Nematoda and Hydrachnidia which were identified as such. Each family of organisms was enumerated and dried in a drying oven at 60°C until a constant weight was reached (approximately 48 hours). Family density as count per sample and sample dry weight (dw) biomass to the

nearest 0.0001 g were determined. Family densities and biomasses from the three sample replicates collected at each site were averaged for statistical analyses.

Invertebrate community metrics were calculated at the family level and included Shannon-Weiner diversity (Shannon's H), family richness, Ephemeroptera, Plecoptera, Trichoptera (EPT) richness, total sample density, total sample dw biomass, % Ephemeroptera, % Plecoptera, % Trichoptera, and % Diptera. These metrics were chosen to summarize general responses of the invertebrate community to mine disturbance. Diversity and richness metrics are expected to decrease with decreasing water quality (Norris and Georges 1993), while responses of abundance (numbers or biomass) and compositional metrics vary under different types of stresses (Resh and Jackson 1993).

Environmental variables

Environmental variables were measured to describe stream characteristics that influence benthic invertebrate community composition. Measured environmental variables can be classified into two groups; water chemistry (n = 12) and physical habitat (n = 15). All environmental variables measured in this study are listed in Table 3.2.

Water Chemistry

Dissolved oxygen was measured in the field using a handheld YSI 85 multiparameter instrument (YSI Inc.) calibrated for elevation. Water samples for determining total selenium concentration, total nitrogen and total phosphorus were collected at the time of benthic invertebrate sampling. Water samples for other chemistry variables including $NO_2^-+NO_3^-$, Cl^-, SO_4^{2-}, Ca^+, alkalinity (as $CaCO_3$), specific conductance (hereafter referred to as conductivity) and pH were collected in mid-September 2011. Samples were collected at or near the benthic invertebrate sampling

transects from approximately 10 cm below the water surface in well-mixed, swift water

stream units with non-turbulent flow. Samples were collected in acid-washed high-

density polyethylene bottles, placed on ice in the field and stored at 4°C until they were

shipped to the analytical laboratories. Water samples for selenium concentration analysis

were preserved with 16 N Omnitrace HNO_3 to approximately 1% v/v prior to storage.

Analysis for total Se was performed by ALS Environmental, Calgary AB, Canada by

inductively coupled plasma mass spectrometry (ICP-MS). All other water chemistry

analyses were performed at the University of Alberta Biogeochemistry Analytical

Services Laboratory in Edmonton, AB, Canada by standard methods. In some samples,

concentrations of waterborne selenium and total phosphorus were below analytical

method detection limits (0.001 mg/L for both analyses). A value of one half the method

detection limit was assigned to these measurements.

Chronic Se toxicity is often the result of bioaccumulation rather than ambient

exposure (Orr et al. 2006). Therefore Se at the base of the food web was quantified to

reflect dietary exposure of macroinvertebrates in sampled streams (Chapter 2, this thesis).

To determine selenium concentration in the base of the food web, composite biofilm

samples (bacteria, algae, macrophytes and moss) were collected from at least five stream

substrate units near the benthic invertebrate sampling site by scraping material from rocks

using a stainless steel spatula or forceps into polyethylene bags. Samples were placed on

ice in the field and frozen prior to selenium content analysis. In the lab, biofilm samples

were dried in a drying oven at 60°C until constant weight was reached (approximately 48

hours) then homogenized. Biofilm tissues were analyzed for total dw selenium by hydride

generation atomic absorption spectrometry (HG-AAS) as described by Miller et al. (2009).

Physical Habitat

Physical habitat characteristics were measured over 175 – 200 m stream reaches which contained the invertebrate sampling transects. Physical stream habitat characteristics were selected and measured based on protocols adapted from the Canadian Aquatic Biomonitoring Network (Environment Canada 2010) and Johnston and Slaney (1996). Stream substrate size was described using Wolman Dg, the geometric mean of intermediate axis length of substrate units measured in a one hundred pebble count. Substrate units measured in the pebble count were also used to determine the percent composition of the substrate as fines (<0.2 cm), gravel (0.2-6.3 cm), cobble (6.3 - 25.6 cm) and boulder (>25.6 cm).

Substrate embeddedness was measured as the percentage depth that a substrate unit was buried in the surrounding interstitial material and summarized as the median embeddedness of a sub-sample of ten substrate units measured in the one hundred pebble count. Interstitial material was nominally categorized (0 – 9) with higher category numbers corresponding to increasing standard Wentworth substrate size classes (Environment Canada 2010). In some of the study streams substrates were entirely concreted over with calcite. In these locations, interstitial material size was assigned a small value because although concretion conglomerates streambed substrate, if calcite is broken apart macroinvertebrates are often found burrowing in it. Consequently, in terms of invertebrate habitat, calcite is a fine material at least marginally suitable for burrowing organisms. Substrates in these streams were therefore also assigned high embeddedness

scores as larger substrates were buried in finer calcite material and interstitial spaces were drastically reduced or even eliminated.

The water surface slope was determined using a clinometer. Stream velocity was approximated using a velocity head-rod across one of the three invertebrate sampling transects. Percent pool and percent riffle were measured as the percent length of the stream reach comprised of distinct slow and swift water habitat units respectively. Large woody debris was measured as a tally of wood with diameter > 10cm over the entire reach and the percent of each habitat unit shaded by first layer riparian cover was averaged to determine percent riparian cover. Wetted channel width was the average of across channel measurements made in each habitat unit. Channel depth was the average of depths from each habitat unit determined as the mean of three equidistant measurements made along a stream transect.

Statistical analysis

All statistical analyses were performed in JMP 10.0 (SAS Institute Inc 2012) or CANOCO for Windows 4.5 (ter Braak and Šmilauer 2002a) and presented using CanoDraw (ter Braak and Šmilauer 2002a). To reduce the effect of rare taxa in analyses, families that comprised an average of <1% of the organisms at all sampling sites were excluded from analyses. Family abundance was $\log_{10} (y + 1)$ transformed prior to analysis. Invertebrate abundance and biomass metrics were $\log_{10} (y+1)$ transformed, richness metrics were not transformed and percentage metrics were $\arcsin(\sqrt{(y/100)})$ transformed. Transformations were used to reduce skewness in the data and reduce the influence of extreme values in analysis. Where necessary to better approximate normality, water chemistry and direct physical habitat measurements were $\log_{10} (x + 1)$

transformed. Differences in mean value of the environmental variables between mine-affected and reference streams were tested using Welch's t-tests ($p < 0.05$).

Environmental variables were expected to be correlated as some of the measured variables inherently produce redundancy in the dataset and others are influenced by the presence and intensity of mine disturbance in upstream watersheds (Palmer et al. 2010). To both decrease the number of variables and reduce problems caused by multicollinearity in multivariate analyses, a reduced set of environmental variables was selected. Preliminary variable reduction was based on pairwise Pearson-product moment correlations within groups of transformed water chemistry and physical habitat variables. When pairs of water chemistry variables were correlated at $r > 0.80$ and when pairs of physical habitat variables were correlated at $r > 0.70$, one of the pair was dropped from the analysis. Many variables were correlated with more than one other variable. In order to achieve the greatest reduction, variables having the most correlations with coefficients above selected thresholds were chosen to remain in the analysis. Variable reduction resulted in variance inflation factors < 15 in preliminary multivariate analyses (ter Braak and Šmilauer 2002b). The set of environmental variables was further reduced by eliminating environmental variables without significant marginal effects in constrained ordinations. The marginal effect is the variance explained by a single variable and was determined by performing redundancy analysis (RDA) on invertebrate family density or community metrics with each variable individually and determining significance at $p < 0.05$ (Monte-Carlo permutation test) (Lepš and Šmilauer 2003).

In community composition analysis, gradient length is a measure of beta diversity or taxa turnover along a measured or hypothetical environmental gradient where short

gradient lengths indicate low taxa turnover and a linear and/or monotonic response of families or metrics among the samples collected. Preliminary detrended correspondence analyses (DCA) revealed that gradient lengths of both invertebrate family density and invertebrate metrics were short (< 2 and < 1 respectively) indicating that linear response models were appropriate in further ordination analyses (ter Braak and Verdonschot 1995, Lepš and Šmilauer 2003).

Principal components analysis (PCA) and redundancy analysis were used to examine variation in invertebrate family and metric data and to determine variation associated with measured environmental variables. The methods are complimentary as variation missed in one method may be captured by the other (Lepš and Šmilauer 2003). Unconstrained PCA captures the main variation in taxonomic composition while constrained RDA captures the variation in taxonomic composition related to linear combinations of measured environmental predictors (Lepš and Šmilauer 2003).

PCA and RDA were performed on invertebrate family and metric data. Metric data were standardized due to the different unit scales of the metric variables (Lepš and Šmilauer 2003). The statistical significance of the first four RDA axes in each analysis was tested using Monte-Carlo permutation tests with 499 unrestricted permutations ($p <$ 0.05). Intraset correlations were calculated as Pearson's product-moment correlations between the measured values of environmental variables and the site scores on the first two RDA axes (ter Braak 1986). Intraset correlations are used to evaluate the relative importance of each environmental variable in predicting the response of macroinvertebrate assemblages (ter Braak 1986). Correlations were also performed between the measured values of excluded collinear variables and the site scores on the

first RDA axes of family and metric data to determine the relationship of excluded

variables with invertebrate assemblage responses (Griffith et al. 2001). A sequential

Bonferroni correction was applied to account for multiple comparisons with $p < 0.05$ for

the entire test (Rice 1989).

A multiple linear regression was used to model the relationship between the

invertebrate orders with the highest family scores on the first axis of the invertebrate

family RDA and the environmental variables with significant marginal effects that were

most correlated with the axis. This was done to test the significance of relationships

between important environmental predictors and sensitive taxa occurrence.

Results

Environmental characteristics

Mean values and ranges of measured environmental variables grouped by site

type (mine-affected and reference) are listed in Table 3.2. Of the measured

environmental characteristics, some water chemistry variables were significantly different

between mine-affected and reference sites (Welch's t-test, $p < 0.05$, Table 3.2). Streams

with mine influence had significantly higher mean concentrations of waterborne Se, total

nitrogen, $NO_2^-+NO_3^-$, SO_4^{2-}, Cl^-, and Ca^+ than reference streams. Mean biofilm Se

concentration was also significantly higher in mine-affected streams as were mean stream

alkalinity and conductivity. Mean physical habitat characteristics did not differ

significantly between the reference and mine-affected streams except for stream velocity

which was greater at affected sites.

Invertebrate community composition

A total of 47,932 individual invertebrates were identified from 72 samples collected at the 24 sampling locations. Invertebrates comprised 12 orders and 43 families. Many of the identified families were rare and composed, on average, <1% of the individuals at each site. These taxa were excluded from analyses resulting in an invertebrate data set composed of 13 families. Families and abbreviations are listed in Figure 3.2. Variable reduction prior to ordination analysis based on environmental variable correlations initially reduced the set of predictor variables from 27 to 16. Of the remaining 16 predictor variables 7 had significant marginal effects on invertebrate family composition gradients and 6 had significant marginal effects on invertebrate community metric gradients (Monte-Carlo permutation test; $p < 0.05$). Water chemistry variables including waterborne Se, alkalinity and pH and physical habitat variables including slope, Wolman Dg, and substrate embeddedness had significant marginal effects on both family and metric data. Interstitial material size only had a significant marginal effect on invertebrate family composition.

There was a high degree of similarity in the relative locations of the family and metric scores between the PCA and RDA ordinations and similar amounts of variation in the family and metric data were explained by the first two ordination axes in each analysis type. This similarity indicates that the main part of the variability in the family and metric data described by the PCA is explained by the measured environmental variables included in the RDA (ter Braak and Šmilauer 2002b, Lepš and Šmilauer 2003). Because PCA and RDA analyses displayed similar trends in both the family and metric data, only RDA analyses are discussed in further detail.

The RDA analysis of family composition data and the 7 environmental variables with marginal effects as predictor variables produced an ordination in which the first two axes were significant (Monte-Carlo permutation; $p = 0.002$ and $p = 0.018$ respectively). Canonical axes explained a total of 55.2% of the variation in family composition and the first two axes explained 32.4% and 13.9% of the variance in the family data respectively (Figure 3.2). The first two RDA axes had high family-environment correlations of 0.944 and 0.811 and explained a similar percentage of the variation in family composition as the PCA (PC1 = 37.0% and PC2 = 21.5%) signifying a good fit of the family data to the environmental predictor variables (ter Braak 1986).

A gradient of mine influence based on water chemistry and physical habitat attributes was observed in the family RDA plot of Axes 1 and 2 where mine-affected sites were separated from reference sites in ordination space (Figure 3.2). Sites influenced by mining were spread along the positive end of Axis 1 and the negative end of Axis 2 while reference sites had less extreme scores on both axes and were generally grouped in the fourth quadrat of the ordination plot. Alkalinity and waterborne Se concentration, which were both significantly higher at mine-affected sites, as well as substrate embeddedness and slope, were significantly positively correlated to Axis 1 (Intraset correlations; $p < 0.05$, Table 3.3). Interstitial material size was negatively related to Axis 1 (Figure 3.2). The second RDA axis was significantly negatively correlated with pH and waterborne Se concentration (Intraset correlations; $p < 0.05$, Table 3.3). Overall, reference and mine affected sites were arranged along a gradient of increasing mine influence characterized by increasing waterborne Se, alkalinity, substrate embeddedness, and slope and decreasing interstitial material size.

Invertebrate family assemblages in the study streams reflected the gradient of mine influence based on family RDA axis scores (Table 3.4). RDA Axis 1 had large negative scores from three families of the order Ephemeroptera including Baetidae, Ephemerellidae and Heptageniidae, as well as Rhyacophilidae and water mites of the Hydrachnidiae order. Capniidae stoneflies were the only family with large a positive score on Axis 1. Scores on axis 2 were generally of lower magnitude with only Chironomidae having a large negative score.

The RDA on invertebrate community metrics using 6 environmental predictor variables with significant marginal effects explained 47.3% of the overall variation in the metric data (Figure 3.3). The Monte-Carlo permutation test of all canonical axes was significant (p = 0.004). Of the individual axes only the first, which explained 25.1 % of metric variation, was significant (Monte-Carlo permutation; p = 0.010) while the second, which explained 15.2 % of metric variation, was marginally significant (Monte-Carlo permutation; p = 0.082). The metric-environment correlations were high with values of 0.840 and 0.716 on Axis 1 and 2 respectively. As in the analysis of invertebrate family composition, the ordination plot characterized a gradient of mine influence where mine-affected and reference sites were arranged along Axis 1 which was significantly positively correlated to waterborne Se, alkalinity, substrate embeddedness and slope and slightly along Axis 2 which was negatively correlated with Wolman Dg and pH (Intraset correlations; p < 0.05; Table 3.5).

Family richness, EPT richness, % Trichoptera and % Ephemeroptera had large negative scores along the first RDA axis (Table 3.4) indicating a negative relationship to the gradient of mine influence while % Plecoptera had a large positive score and

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therefore positive relationship to the gradient of mine influence (Table 3.4). The second RDA axis had a positive score for % Ephemeroptera and a negative score for % Diptera and the metric scores for total dw biomass and total abundance had negative scores on Axes 1 and 2 respectively (Table 3.4).

Many of the variables that were excluded from the analysis due to multicollinearity were significantly higher at mine-affected vs. reference sites. Significant correlations were observed between these excluded environmental variables and site scores on the first family and metric RDA axes which characterized the gradient of mine influence. The first axis of the family RDA was significantly correlated with Ca^+ ($r = 0.72$), conductivity ($r = 0.77$), Cl^- ($r = 0.65$), SO_4^{2-} ($r = 0.60$), % cobble ($r = -0.64$) and % fine substrates ($r = 0.83$) (Pearson product-moment correlations; $p < 0.05$ with sequential Bonferroni correction). The first axis of the metric RDA was significantly correlated with Cl^- ($r = 0.79$), SO_4^{2-} ($r = 0.75$), Ca^+ ($r = 0.83$), conductivity ($r = 0.88$), total nitrogen ($r = 0.78$), $NO_2^- + NO_3^-$ ($r = 0.70$), % cobble ($r = -0.50$) and % fine substrates ($r = 0.87$) (Pearson product-moment correlations; $p < 0.05$ with sequential Bonferroni correction). These correlations indicate the relationship of collinear variables to the gradient of mine influence and their potential contributions to observed responses in invertebrate community composition.

Regression model

Families of the order Ephemeroptera including Baetidae, Ephemerellidae, and Heptageniidae had the highest combined order score on either of the first two axes in the family composition RDA with family scores of -0.880, -0.715 and -0.782 respectively on Axis 1 (Table 3.4). Ephemeroptera family densities were combined and $\log_{10} (y + 1)$

transformed resulting in an Ephemeroptera density variable. This variable was used in a multiple linear regression model with the environmental predictor variables that were most correlated with the first RDA axis in the family density ordination based on intraset correlations (Table 3.3) including waterborne Se, alkalinity, and substrate embeddedness. Waterborne Se was not significant in the initial regression model and was therefore removed from the final model. The final model predicted Ephemeroptera density from alkalinity and substrate embeddedness ($R^2_{adj} = 0.76$, $F_{2,21} = 39.74$, $p < 0.0001$). In the model alkalinity (t = -4.91, n = 24, $p < 0.0001$) and embeddedness (t = -4.79, n = 24, $p < 0.0001$) were both significant predictors of Ephemeroptera density.

Discussion

The results of this study indicate that impaired macroinvertebrate communities in low- to mid-order streams were related to a gradient of surface mine influence in the Canadian Rockies. Multiple water chemistry variables differed significantly between mine-affected and reference sites and multivariate ordinations separated the site types in ordination space.

Environmental characteristics in mine-affected streams

Waterborne and biofilm Se, alkalinity, conductivity and concentrations of measured ions (Cl^-, Ca^+, SO_4^{2-}, $NO_2^- + NO_3^-$) were highly correlated and significantly greater in mine-affected than reference study streams. Significant increases in waterborne Se concentrations and bioaccumulation in food webs have long been recognized as aquatic impacts of surface mining in the Canadian Rockies and a trend of elevated ionic strength is demonstrated in mine-affected streams of other coal mine regions

(Szczepanska and Twardowska 1999, Fritz et al. 2010, Bernhardt et al. 2012). The high ionic strength and Se concentrations result from exposure of coal minerals and disturbed overburden rock to weathering processes in rock spoil and valley fills (Naftz and Rice 1989). Explosives used to break up the overburden release $NO_2^-+NO_3^-$ and increase total nitrogen downstream. Pyrite coal minerals dissolve and generate sulfuric acid which dissociates, increasing SO_4^{2-} concentrations (Palmer et al. 2010). Highly seleniferous carbonate rock spoil containing Cl^-, Ca^+, Mg^+ and HCO_3^- is weathered by increased water exposure and sulfuric acid (Lindberg et al. 2011). The drainage flowing from surface coal mine and spoil sites thus had high Se, conductivity, alkalinity and dissolved ion concentrations.

Physical habitat variables including substrate embeddedness, interstitial material size, and slope were not significantly different between mine-affected and reference streams but were still identified as significant predictors of macroinvertebrate assemblages along a gradient of mine influence in ordination analyses. Mine-affected streams tended to have higher embeddedness scores and smaller interstitial material sizes. These variables represent both calcite accumulation and fine sedimentation in study streams.

Water surface slopes were not significantly different between reference and mine disturbed sites however there was a significant loading of slope in RDA analyses. In general, lower order streams tend to have steeper slopes but maintain the ability to support taxonomically rich invertebrate communities (Clarke et al. 2008), therefore it is not likely that the individual effect of slope influenced invertebrate community composition. However, most of the measured water chemistry variables were marginally

correlated with slope. This may be because lower order streams have smaller contributing areas and lower flows and therefore chemical inputs from disturbances are less diluted than in higher order streams (Petty et al. 2010). A more concentrated chemical effect in streams with steeper slopes may explain the apparent influence of slope in the RDA analyses.

There were also predictor variables in the RDA that were not related to the gradient of mine influences including pH and substrate size (Wolman Dg). Though all streams had mildly alkaline pH, pH and alkalinity were not significantly correlated. This was probably because most study sites are relatively near to stream headwaters where water enters the stream from subsurface or overland flows and has not yet reached ionic equilibrium. The supersaturation and degassing of CO_2 and the precipitation of calcite may have also influenced this equilibrium (Chen et al. 2004). The RDAs indicated that pH and Wolman Dg influence invertebrate assemblages but their influence is not associated with mining as reference and mine-affected sites did not separate along gradients of these variables in ordination plots.

Macroinvertebrate community impairment downstream of mines

Ephemeroptera families (Baetidae, Heptageniidae and Ephemerellidae) were negatively related to the gradient of mine disturbance in ordination analyses with Baetidae and Ephemerellidae being completely absent from samples taken at 3 of the 13 mine-affected sites. The relationship between the combined densities of these three families was tested using a multiple regression model and found to be significantly related to mine disturbance variables including alkalinity and substrate embeddedness.

Community metrics including family richness, EPT richness and Shannon-Weiner diversity were also negatively related to the mine disturbance gradient.

Ephemeroptera are generally considered sensitive indicators of anthropogenic impact (Resh and Jackson 1993) and declines in Ephemeroptera taxa are often observed in biomonitoring studies evaluating water quality (Pond 2010), sedimentation, and metal toxicity (Scullion and Edwards 1980, Clements 1994). In addition to Ephemeroptera, Plecoptera and Trichoptera are considered relatively more sensitive to stream impairment than other macroinvertebrate taxa (Resh and Jackson 1993, Clements 1994) which is consistent with the decline in EPT richness observed at mine-affected sites. The overall richness and diversity of macroinvertebrate communities are also expected to decline as disturbance increases (Norris and Georges 1993) and families besides Baetidae, Heptageniidae, and Ephemerellidae decreased in abundance relative to the gradient of mine influence, including a strong negative response of the caddisfly Rhyacophilidae and marginal responses of Elmidae and Simulidae dipterans. The decline in overall richness and diversity was therefore not entirely due to losses of Ephemeroptera or EPT taxa as families from other orders were negatively influenced as well.

While the relative abundance of Ephemeroptera and Trichoptera individuals were negatively related to mine disturbance, the relative abundance of Plecoptera had the opposite response. The proportional increase in Plecoptera organisms at mine-affected sites was largely due to higher densities of the family Capniidae. The densities of other Plecoptera including Leuctridae, Peltoperlidae, Perlodidae and Chloroperlidae were not strongly related to the mine disturbance gradient. Varying responses of families of the same order and even genera of the same family are important to consider when

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determining the influence of disturbance on macroinvertebrates. The relative increase of Capniidae in study streams could indicate tolerance to one or more of the mine-influenced water chemistry or habitat variables.

Sample dw biomass and organism density had considerable scores in the metric RDA but their increase was almost perpendicular to the gradient of mine influence. Therefore, while environmental predictors related to mine disturbance influenced community assemblage, they did not appear to be drivers of the overall density or biomass of invertebrates in the study streams. These findings are consistent with those of Hartman et al. (2005) who found differences in taxa composition but no differences in the total density or biomass of organisms present downstream of coal mine valley fills. Other factors including food availability and quality (Clements 1994), land-cover gradients, and stream productivity (Black et al. 2004) which were not quantified in this study can affect invertebrate biomass and density.

Influences of environmental predictors on macroinvertebrate assemblages

Community ordinations and regression analysis emphasized changes in environmental variables that were attributable to surface coal mining and that were significant predictors of macroinvertebrate assemblages in Canadian Rockies streams. Because multiple potential stressors, both chemical and physical, were identified consideration of their mechanisms of influence and their potential as stressors to invertebrate communities in mined systems is required.

Alkalinity, in addition to correlated predictors of ionic strength, was related to the gradient of mine influence along which sites with different macroinvertebrate assemblages were separated and was a significant predictor of Ephemeroptera density.

This is in agreement with other studies where alkalinity, conductivity, and ion concentrations above reference conditions were characteristic of mining influence and where Ephemeroptera density and diversity declined. Pond et al. (2008) found Ephemeroptera family and genus richness was significantly related to the level of upstream mine influence and that Ephemeroptera were nearly absent from mountain-top coal mining affected Appalachian streams. Hartman et al. (2005) found lower densities of Ephemeroptera, scrapers and shredders downstream of mines and Pond (2010) saw a significant decline in the relative abundance and genera richness of mayflies in surface mined streams compared to reference streams or streams with residential influences.

The influences of ionic strength on macroinvertebrates at surface mine sites have recently been the focus of concentrated study in the Central Appalachian coalfields in the eastern United States (Cormier et al. 2013c). Extirpations of certain genera of macroinvertebrates have been attributed to elevated ionic mixtures containing Ca^+, Mg^+, HCO_3^-, SO_4^{2-} and Cl^- in streams that drain valley fills and rock dumps (Cormier et al. 2013a, Cormier et al. 2013b). Ephemeroptera taxa have been identified as some of the most sensitive to increasing ionic strength (Pond 2010, Cormier et al. 2013b).

The proposed effects of elevated ionic strength in freshwater organisms are based on exposure, biochemical and physiological mechanisms described in detail by Cormier et al. (2013b). Generally, exposure to elevated concentrations of dissolved ions passing over the integument and gills of aquatic organisms can influence ion exchange pathways in various external and internal cells (Wichard et al. 1973). Altered or impaired ion exchange can result in deterioration of cell function and physiological stress (Pond et al. 2008). In Ephemeroptera, an example of this proposed effect mechanism is high ambient

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HCO_3^- interfering with ion transport in chloride cells on the gills (Cormier et al. 2013b).

Using conductivity to represent ionic strength and genus extirpation data, Cormier et al.

(2013a) have proposed a field-derived regional benchmark for the protection of

freshwater macroinvertebrates in the Appalachians of 300 µS/cm for ionic mixtures

composed of $HCO_3^- + SO_4^{2-} \geq Cl^-$.

The presence of elevated concentrations of Ca^+, SO_4^{2-}, Cl^- and HCO_3^- at sites with

impaired macroinvertebrate communities in the present study also supports a predicted

toxic effect of ionic strength. If ionic toxicity effects are occurring in the Canadian

Rockies, 300 µS/cm would not be an effective benchmark for the protection of aquatic

life as the average conductivity of reference streams in this study was 315 ± 14 µS/cm

while background conductivities in Appalachian regions were between 72 and 153 µS/cm

(Cormier et al. 2013a). Bedrock geology partially controls regional water chemistry due

to differences in weathering based on rock type and weathering pathways (Spence and

Telmer 2005). Organisms found in streams with shale and limestone geology in the

Canadian Rockies are thus probably adapted to higher background conductivity levels,

potentially indicating higher tolerance to elevated ionic mixtures. Cormier and Suter

(2013) assert however, that despite regional differences in background ionic strength and

invertebrate community assemblages, the causal assessment of toxicity in the

Appalachian region adequately characterizes the main response of representative

organisms to defined ionic mixtures above regional background levels. In support of this

assertion, Black et al. (2004) found that Ephemerellidae, Heptageniidae and Rhyacophila

spp. had low conductivity optima (70.4, 89.3, and 89.3 µS/cm respectively) in streams in

the Pacific North West where average specific conductance was 139 µS/cm and

explained a significant amount of the variance (> 10%) in the occurrence of these taxa (Black et al. 2004). More extensive investigation is required to characterize ion mixtures and concentrations and the macroinvertebrate taxa response in Canadian Rockies streams before an exposure-response relationship can be inferred.

Physical habitat variables including substrate embeddedness and interstitial material size also contributed to the separation of study sites along the mine effects gradient. Data collection methods did not quantitatively distinguish between fine sediment and calcite accumulation with respect to embeddedness and interstitial material size, however, both result in the filling of interstitial spaces and the coating of substrates with fine sediment particles. The loss of interstitial microhabitats can reduce invertebrate density and community diversity (Rabení et al. 2005) and increase invertebrate drift rates (Culp et al. 1986). Large-scale deposition of calcite can also result in terracing which alters channel hydrology and morphology. Finer interstitial material size and slower stream velocities may shift macroinvertebrate assemblages towards organisms capable of burrowing in the substrates and away from organisms that rely on well oxygenated, interstitial spaces and clean substrates to meet their habitat and feeding requirements (Rabení et al. 2005).

Impaired Ephemeroptera communities in mine-affected study streams may have been a result of the general preference of the observed mayfly families for fast flowing, interstitial microhabitats (Clifford 1991). Similarly, a decrease in EPT due to coal particle siltation and ferric hydroxide deposition downstream of coal mining was observed in Wales, UK (Scullion and Edwards 1980). Wood et al. (2005) also experimentally determined that *Baetis rhodani* Ephemeropterans were unable to excavate themselves

from 0.5 cm burial in various sizes of fine particles indicating their unsuitability to habitats dominated by fine sediments.

The larger relative proportion of Capniidae stoneflies observed at mine affected sites compared to reference sites may indicate tolerance of this stonefly family to particular habitat characteristics present (in addition to tolerance to water chemistry stressors). Specifically, the small size of some genera of Capniidae found in the Canadian Rockies (e.g. *Isocapnia*) (Clifford 1991) allows them to penetrate small interstitial spaces and migrate vertically through substrates (Jacobi and Cary 1996). They often inhabit the hyporheic zone (Clifford 1991, Harper et al. 1991) and therefore they may be well adapted to the microhabitat produced by calcite accumulation.

Finally, waterborne Se concentrations in the study streams ranged over several orders of magnitude and were highly correlated with the first two axes of RDA ordinations of macroinvertebrate family assemblages and metrics. The concentration of biofilm Se (representative of dietary exposure for invertebrates) in study streams was significantly correlated with waterborne Se ($r = 0.5$, $n = 24$, $p < 0.05$) but did not have a significant marginal effect on invertebrate families or metrics and was therefore not included as a predictor variable in RDA analyses. While invertebrate Se accumulation above reference concentrations has been documented at sites influenced by mine disturbance (Orr et al. 2006, Wayland and Crosley 2006, Orr et al. 2012), field studies have not directly determined the community level impacts of a wide range of Se contamination. Downstream of surface coal mines, Pond et al. (2008) found that waterborne Se concentrations below 37 µg/L were negatively correlated with multimetric invertebrate indices including taxon richness, EPT richness, Ephemeroptera richness, and

Plecoptera richness; and Frenette (2008) saw proportional decreases in EPT and Ephemeroptera at waterborne Se concentrations at or below 15 µg/L. However, Pond et al. (2008) considered these concentrations to be relatively non-toxic to invertebrates and concluded that while sub-lethal Se effects on macroinvertebrates might be occurring, other stressors present downstream of mines were responsible for a greater portion of the variation in community metrics.

There is evidence that Se toxicity shaped macroinvertebrate assemblages in the present study. The range of waterborne Se concentrations of 0.5 – 543 µg/L at sites in this study was greater than in previous macroinvertebrate assemblage studies (Frenette 2008, Pond et al. 2008) and maximum concentrations of waterborne and dietary exposure were above some laboratory derived chronic effective concentrations (Ingersoll et al. 1990, Maier and Knight 1993, Malchow et al. 1995, Conley et al. 2009). Sub-lethal laboratory effects on the mayfly *Centroptilum triangulifer* to dietary Se exposure of ≥ 4.9 µg/g dw (Conley et al. 2011) could indicate mayfly Se sensitivity, supporting the observed impacts on Ephemeroptera taxa along the gradient of mine disturbance in the study streams. However, Se toxicity has been extensively studied in few invertebrate organisms (deBruyn and Chapman 2007) and the Se sensitivities of taxa and life stages found in the study streams have not been adequately described. Field Se tissue concentrations in invertebrates are also variable among taxonomic groups (Wayland and Crosley 2006, Orr et al. 2012) indicating variable exposure and risk of exceeding laboratory derived concentrations for acute and chronic effects (deBruyn and Chapman 2007, Conley et al. 2009). Therefore, although we found changes in macroinvertebrate community composition along a gradient of mine influence that was strongly

characterized by waterborne Se concentrations, more evidence is required to conclusively link community impairment to Se toxicity at sampling sites.

Macroinvertebrate assemblages and multiple stressors

When multiple potential stressors are present in field macroinvertebrate studies, it can be difficult to attribute changes in the community to any specific impact and different approaches have been used to address this difficulty. For example, Clements (2004) used small-scale experiments to test community response to individual metal contaminants of interest that co-occurred in the field as a method of identifying confounding variables. Cormier et al. (2013b) proposed a method of using field data to assess causation in exposure-response relationships that includes identifying and evaluating the influence of potential confounders (Suter and Cormier 2013).

While ordination analyses in the current study highlighted the potential importance of numerous predictor variables in influencing the responses of invertebrate community assemblages, only substrate embeddedness and alkalinity were significant predictors of Ephemeroptera density in a multiple regression model. Significant correlations between alkalinity and other measured water chemistry variables suggest that a strong relationship between other measures of ionic strength and Ephemeroptera occurrence may also exist. Waterborne Se concentration was included in initial regressions but, despite a strong correlation with mine impact gradients in ordination analyses, did not have a significant relationship with Ephemeroptera density. Therefore, impacts due to Se exposure may be restricted to other taxa or Se co-occurrence may be a confounder of other mine disturbance influences on invertebrates in affected streams.

This study identifies physical and chemical stressors that are altered downstream of surface mines in the Canadian Rockies that have plausible mechanisms for causing negative invertebrate community-level impacts. Previous investigations of coal mining influences on aquatic life in the Canadian Rockies have largely focused on Se contamination and threats to vertebrate organisms. The results of the present study are consistent with studies from other coal-mining regions that demonstrate how multiple stressors shape macroinvertebrate assemblages in mine influenced areas and highlight the need for further investigation into causal links between surface mine influences and benthic community impairment. Future research should focus on the collection and compilation of biomonitoring datasets that reflect local invertebrate assemblages at a range of exposures of the multiple potential stressors. Additionally, lab studies could be used to investigate the effects of individual stressors or combinations of stressors on aquatic organisms. Information resulting from these studies is necessary to develop causal assessments and evaluate confounding variables.

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Tables and Figures

Table 3.1 Sampling sites listed with study watershed, geographical coordinates and description as reference (Ref), mine-affected (MA), or reclaimed (MA-R).

Stream name	Site type	Watershed	Latitude	Longitude
W. Alexander Ck.	Ref	Elk	49.773	-114.721
Chauncey Ck.	Ref	Elk	50.108	-114.814
Dry Ck.	Ref	Elk	50.035	-114.817
Ewin Ck.	Ref	Elk	50.060	-114.797
Grace Ck.	Ref	Elk	49.984	-114.859
South Line Ck.	Ref	Elk	49.915	-114.767
Deerlick Ck.	Ref	McLeod	53.153	-117.244
Eunice Ck.	Ref	McLeod	53.154	-117.231
Wampus Ck.	Ref	McLeod	53.157	-117.262
Watson Ck.	Ref	McLeod	53.072	-117.259
W. Drinnan Ck.	Ref	McLeod	53.161	-117.544
Cataract Ck.	MA	Elk	50.151	-114.865
E. Crowsnest Ck.	MA	Elk	49.585	-114.693
Erickson Ck.	MA	Elk	49.678	-114.783
Fording R.	MA	Elk	49.894	-114.868
Harmer Ck.	MA	Elk	49.831	-114.822
Line Ck.	MA	Elk	49.892	-114.835
Swift Ck.	MA	Elk	50.158	-114.869
Berry Ck.	MA-R	McLeod	53.095	-117.447
Drinnan Ck.	MA-R	McLeod	53.182	-117.513
Gregg R.	MA	McLeod	53.129	-117.486
Jarvis Ck.	MA	McLeod	53.058	-117.363
Luscar Ck.	MA	McLeod	53.059	-117.311
Sphinx Ck.	MA-R	McLeod	53.125	-117.311

Table 3.2 Measured environmental variables, descriptive statistics and Welch's t-test (p < 0.05) results for differences in mean environmental variables between mine-affected streams (n = 13) and reference streams (n = 11).

Variable	Units	Mine-affected Mean ± SE	Mine-affected Range	Reference Mean ± SE	Reference Range	t	p
Water Chemistry							
Waterborne Se	µg/L	100.3 ± 52.8	0.5 – 543.0	0.8 ± 0.1	0.5 – 1.7	-4.67	<0.001
$NO_2^-+NO_3^-$	mg/L	7107 ± 3001	9 – 36750	40 ± 12	2 – 123	-5.64	<0.001
Cl^-	mg/L	2.20 ± 0.63	0.13 – 7.15	0.21 ± 0.05	0.08 – 0.59	-4.37	<0.001
SO_4^{2-}	mg/L	386 ± 144	6 – 1527	21 ± 5	3 – 51	-4.83	<0.001
Ca^+	mg/L	130 ± 35	46 – 407	44 ± 2	38 - 55	-3.81	0.002
Alkalinity (as $CaCO_3$)	mg/L	262 ± 28	171 – 463	154 ± 5	128 – 183	-4.59	<0.001
Conductivity	µs/cm	1099 ± 237	310 – 2890	315 ± 14	272 – 421	-5.28	<0.001
pH	-	8.3 ± 0.02	8.1 – 8.4	8.3 ± 0.04	8.0 – 8.4	-0.95	0.352
DO	mg/L	10.0 ± 0.3	8.2 – 11.2	10.5 ± 0.4	8.7 – 12.5	1.31	0.205
Total nitrogen	mg/L	7253 ± 3142	33 - 33650	72 ± 7	43 – 103	-5.33	<0.001
Total phosphorus	µg/L	4.2 ± 1.0	0.5 – 11.0	5.6 ± 1.8	0.5 – 19.0	0.40	0.692
Biofilm Se	µg/g dw	3.57 ± 0.59	0.75 – 8.10	1.81 ± 0.24	0.75 – 3.32	-2.59	0.018
Physical Habitat							
Wolman Dg	cm	6.8 ± 1.3	0.1 – 15.5	6.7 ± 0.7	2.9 – 11.1	-0.08	0.353
Slope	%	3 ± 1	0.5 – 11	3 ± 1	1 – 4	-0.95	0.750
Velocity	m/s	0.68 ± 0.04	0.45 – 0.99	0.50 ± 0.05	0.23 – 0.80	-2.97	0.007
Wetted width	m	7.28 ± 1.62	2.87 – 25.0	4.88 ± 0.47	3.12 – 8.36	-1.36	0.191
Depth	m	0.26 ± 0.03	0.09 – 0.47	0.26 ± 0.02	0.17 – 0.40	0.08	0.937
Pool	%	21 ± 3	9 – 48	27 ± 3	17 – 42	1.61	0.121
Riffle	%	53 ± 4	28 – 70	47 ± 4	29 – 66	-1.33	0.197
Large woody debris	#	33 ± 12	0 – 165	25 ± 7	1 – 90	0.04	0.968
Riparian cover	%	21 ± 4	4 – 40	22 ± 4	5 – 40	0.18	0.856
Interstitial material	-	2.5 ± 0.4	0 – 5	2.7 ± 0.2	2 – 4	0.40	0.694
Embeddedness	%	50 ± 7	25 – 100	35 ± 5	0 – 50	-1.55	0.135
Fines	%	20 ± 10	0 – 95	4 ± 1	0 – 14	-1.44	0.163
Gravel	%	40 ± 8	0 – 93	44 ± 6	22 – 78	0.64	0.526
Cobble	%	36 ± 6	0 – 69	47 ± 5	2 – 68	1.42	0.168
Boulder	%	5 ± 2	0 – 24	5 ± 1	0 – 11	-0.20	0.842

Table 3.3 Intraset correlation coefficients (Pearson's product-moment correlation) between measured environmental variables and the first two RDA axes for macroinvertebrate family data.

	Axis 1		Axis 2	
Variable	r	p	r	p
Waterborne Se	0.658	0.0005	-0.653	0.0005
Alkalinity	0.773	<0.0001	-0.390	NS
pH	-0.434	0.034	-0.574	0.0034
Wolman Dg	-0.594	0.002	-0.438	NS
Slope	0.602	0.002	-0.126	NS
Interstitial Material	-0.509	0.011	0.197	NS
Embeddedness	0.718	<0.0001	-0.323	NS

NS = not significant

Statistical significance was determined based on p corrected using the sequential Bonferroni method ($p < 0.05$; df = 22)

Table 3.4 Family and metric scores on the first and second axes of family and metric RDA.

	Family RDA			Metric RDA	
	Axis 1	Axis 2		Axis 1	Axis 2
Elmidae	-0.262	0.225	Shannon's H	-0.341	0.321
Chironomidae	-0.239	-0.712	Richness	-0.564	-0.086
Simulidae	-0.297	0.139	EPT Richness	-0.834	-0.059
Baetidae	-0.880	0.019	% Ephemeroptera	-0.534	0.618
Ephemerellidae	-0.715	0.118	% Plecoptera	0.616	0.1622
Heptageniidae	-0.782	0.412	% Trichoptera	-0.467	-0.155
Hydrachnidiae	-0.518	-0.495	% Diptera	-0.131	-0.641
Capniidae	0.777	-0.253	Abundance	-0.235	-0.574
Chloroperlidae	-0.329	-0.103	Biomass	-0.422	-0.280
Leuctridae	-0.147	0.260			
Peltoperlidae	0.017	0.065			
Perlodidae	-0.067	-0.311			
Rhyacophilidae	-0.6932	-0.264			

Table 3.5 Intraset correlation coefficients (Pearson's product-moment correlation) between measured environmental variables and the first two RDA axes for macroinvertebrate metric data.

	Axis 1		Axis 2	
Variable	r	p	r	p
Waterborne Se	0.820	<0.0001	-0.449	NS
Alkalinity	0.795	<0.0001	-0.168	NS
pH	-0.345	NS	-0.799	<0.0001
Wolman Dg	-0.395	NS	-0.531	0.008
Slope	0.623	0.001	-0.029	NS
Embeddedness	0.819	<0.0001	-0.140	NS

NS = not significant

Statistical significance was determined based on p corrected using the sequential Bonferroni method ($p < 0.05$; df = 22)
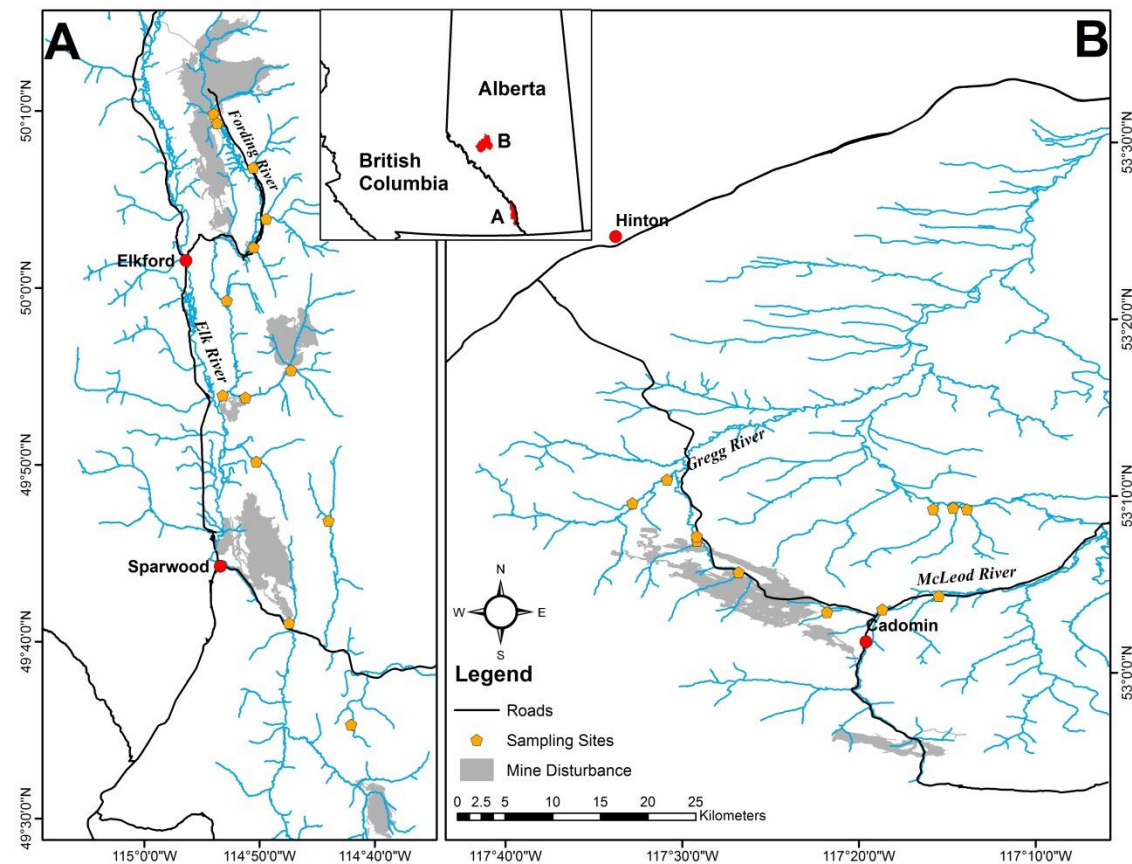
Figure 3.1 Map of study areas in the Elk River, British Columbia, Canada (A) and the McLeod River, Alberta, Canada (B) including sampling sites and disturbance area[1] for active and reclaimed mines.

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[1] Reproduced with the permission of Teck Coal Limited and Sherritt Coal International
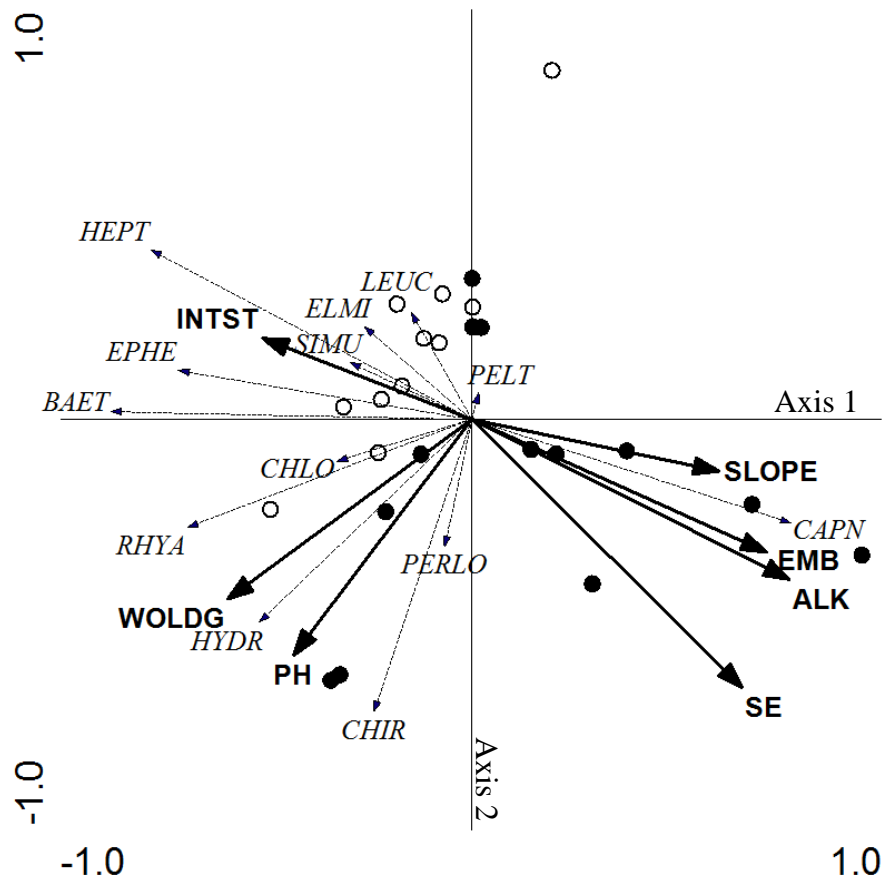
Figure 3.2 First and second axes of RDA triplot of invertebrate family abundance (dashed arrows) and environmental variables (solid arrows). Solid circles represent mine-affected streams and open circles represent reference streams. Environmental variable abbreviations: ALK = alkalinity, EMB = embeddedness, INTST = interstitial material size, PH = pH, SE = waterborne Se, SLOPE = water surface slope, WOLDG = Wolman Dg. Invertebrate family abbreviations: BAET = Baetidae, CAPN = Capniidae, CHIR = Chironomidae, CHLO = Chloroperlidae, ELMI = Elmidae, EPHE = Ephemerellidae, HEPT = Heptageniidae, HYDR = Hydrachnidiae (order), LEUC = Leuctridae, PELT = Peltoperlidae, PERLO = Perlodidae, RHYA = Rhyacophilidae and SIMU = Simulidae.
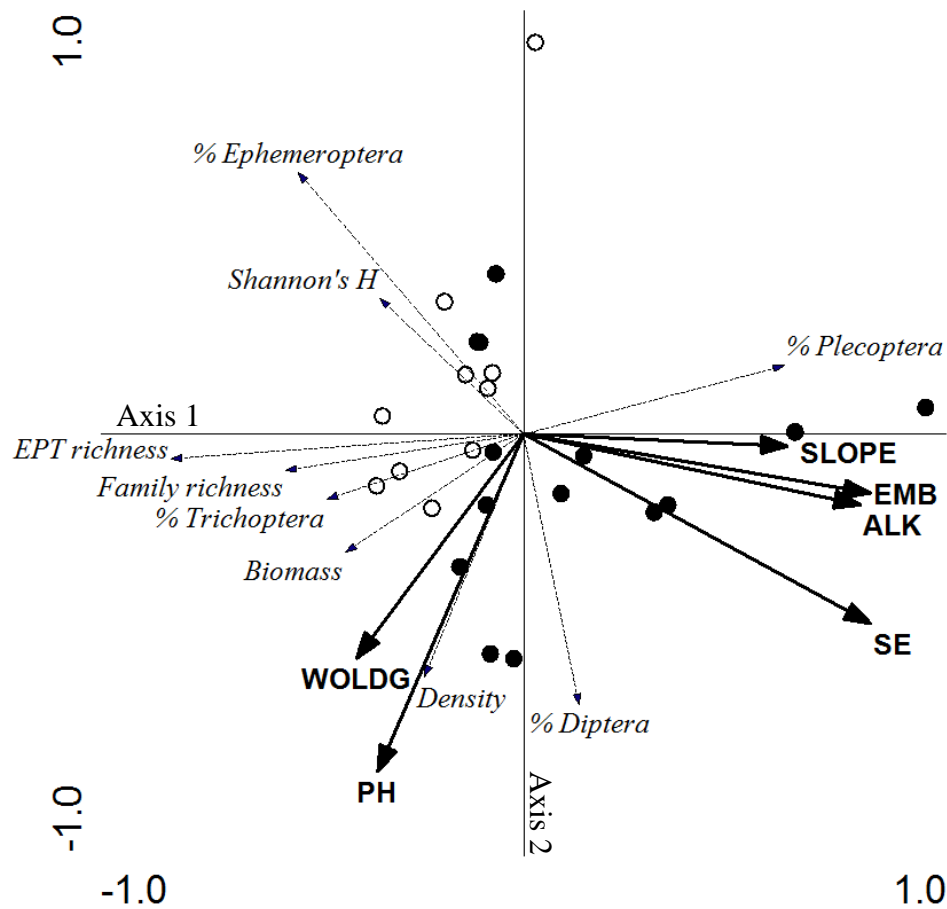
Figure 3.3 First and second axes of RDA triplot of invertebrate metrics (dashed arrows) and environmental variables (solid arrows). Solid circles represent mine-affected streams and open circles represent reference streams. Environmental variable abbreviations: ALK = alkalinity, EMB = embeddedness, PH = pH, SE = waterborne Se, SLOPE = water surface slope, and WOLDG = Wolman Dg. Invertebrate metric abbreviations: EPT = Ephemeroptera, Plecoptera, Trichoptera, Shannon's H = Shannon-Weiner diversity, Density = total density, and Biomass = dw biomass.

CHAPTER 4.   SUMMARY AND CONCLUSIONS

Surface coal mining in the Canadian Rockies is an expanding industry with environmental impacts of concern in aquatic ecosystems. The objectives of this thesis were to investigate surface coal mining influences, particularly selenium (Se) toxicity effects, on fish and macroinvertebrate communities in streams. Conclusions from this research provide important new information for managing and monitoring surface mine influences. Specifically, field studies were carried out in mine-affected and reference streams in two watersheds of the Canadian Rockies in order to:

1) Quantify Se exposure in lotic food chains and juvenile salmonid fishes.

2) Investigate the presence and extent of fish community-level effects resulting from individual-level Se toxicity in salmonid fish species.

3) Evaluate macroinvertebrate community responses downstream of mines and identify invertebrate taxa that are sensitive to mine disturbance.

To determine if juvenile salmonid tissue concentrations better reflected dietary Se concentrations at capture sites than tissues of highly mobile adult life-stages, food web Se concentrations were measured in mine-affected and reference streams (Chapter 2). Se enrichment and trophic transfer were > 1 at each food chain step and there was a significant log-linear relationship between Se in invertebrates and juvenile fish muscle tissues. Additionally, juveniles of salmonid species including westslope cutthroat, bull, rainbow and brook trout did not differ significantly in their muscle Se accumulation.

These results suggest that juvenile salmonid Se tissue concentrations sampled in mid-summer can be used to:

1) Determine Se exposure at specific sites of interest.

2) Improve the predictive capacity of field based food chain trophic transfer models by reducing Se exposure uncertainty.

3) Simplify food chain trophic transfer models due to similarity among species in juvenile muscle Se accumulation rates.

Juvenile salmonids reflected both site specific Se exposure and the potential for toxic Se effects, identifying them as practical organisms/life-stages for Se toxicity monitoring. The significant relationship between Se diet and juvenile tissue concentrations also contributes to refinement of food chain models that can eventually be used as monitoring tools while reducing or eliminating sampling pressure on vulnerable fish populations. Future research comparing juvenile and adult fish tissue Se concentrations and allocations will further improve these food chain models by providing a basis for tissue concentration conversions. Defined tissue concentration and allocation relationships could also improve assessments of toxicity risks to different life stages of fish.

To investigate fish community-level effects resulting from individual-level Se toxicity in salmonids, fish muscle Se concentration and fish biomass were measured at the stream reach scale (Chapter 2). The relationship between total fish biomass and average fish muscle Se concentration was not significant. Species-specific biomass and

muscle Se concentration relationships were not significant except in rainbow trout whose biomass declined with increasing Se tissue concentrations. These results indicate that:

1) The frequency and/or magnitude of Se concentrations that exceeded individual-level toxicity thresholds may not have been great enough to produce community-level toxicity effects in most of the sampled species.

2) Due to compensatory mechanisms such as inter-annual fish community variability, nearby uncontaminated refugia and density compensation community-level effects are difficult to detect at the stream reach scale.

3) The combination of Se sensitivity and the presence of a non-native competitor species (brook trout) may have made rainbow trout particularly vulnerable to community-level effects.

Although fisheries management decisions are often made at the fish community or population level, this research indicates that monitoring programs should not necessarily rely on the detection of community-level effects to indicate risk. Measured Se concentrations above individual-level toxicity thresholds did not always translate to detectable effects at higher levels of ecological organization and Se exposure-response relationships are likely confounded by fish movements, variable Se exposure and density compensation in stream reaches. Therefore monitoring at the stream reach scale cannot be expected to reflect the fish community-level response to Se toxicity and Se impacts may not be apparent until substantial community declines have occurred. Monitoring at larger spatial scales (i.e. watershed/regional scale) may be more appropriate as it would integrate fish movements and variable Se exposure within the monitoring unit. However, collecting detailed community information in addition to defining Se exposure at these

large scales is not practical. Further research into fish movement patterns and exposure histories using otolith microchemistry is recommended as it could strengthen links between Se exposure and toxicity effects. Knowledge of fish movements in mine-affected systems will also help to identify the spatial scale (i.e. reach, stream, watershed, etc.) at which monitoring and management programs should be implemented. However, because of limitations on detection of community-level effects, current Se monitoring focus should remain on individual-level toxicity thresholds.

To investigate how alteration of chemical and physical stream characteristics, including Se exposure, affected macroinvertebrate community assemblages multivariate analyses were used to compare invertebrate family and metric data with environmental predictor variables (Chapter 3). Changes in macroinvertebrate community composition including overall diversity declines and declines of sensitive taxa were found along a gradient of mine disturbance reflected by high waterborne Se, alkalinity, substrate embeddedness and low interstitial material size. These results indicate that:

1) Multiple stressors associated with surface coal mining may influence stream invertebrate community composition.

2) Potential mechanisms of mine impact on macroinvertebrate communities include Se and ion toxicity and physical habitat degradation.

3) Ephemeroptera taxa are particularly sensitive to mine impacts including substrate embeddedness and high alkalinity.

Therefore, environmental management downstream of surface mines must consider impacts besides Se contamination. Identifying priority stressors among the different

potential influences in disturbed systems can be challenging. Methods for the derivation of toxicity thresholds for ionic strength from field data have been proposed and effectively used in other coal mining regions. However, these methods require aquatic community data from sites experiencing a range of each potential stressor to establish causal relationships and identify confounders. Because variables of interest co-occur and are highly correlated downstream of mine sites in the Canadian Rockies region, disentangling influences in the field may not be possible. Cause and effect relationships of different mine effects such as elevated conductivity, alkalinity and ion concentrations, individually and in combination on aquatic organisms could be further examined under controlled laboratory conditions. Additional research in this area will help to shift management of surface coal mine influences in the Canadian Rockies away from an exclusive focus on Se influences which do not fully represent potential risks to aquatic organisms.