DEVELOPING MONITORING STRATEGIES FOR ASSESSING EFFECTS IN PRISTINE NORTHERN RIVERS RECEIVING MINING DISCHARGES

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By

Paula Lynn Spencer

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ABSTRACT

The overall objective of my thesis research was to develop methodologies for assessing effects of mining effluents on pristine and sensitive northern rivers. I used a multi-trophic level approach in field studies to evaluate current monitoring methods and to determine whether metal mining activities had affected two otherwise pristine rivers that flow into the South Nahanni River, NWT; a World Heritage Site. Upstream reference conditions in the rivers were compared to sites downstream and further downstream of mines. The endpoints evaluated included concentrations of metals in river water, sediments and liver and flesh of slimy sculpin (*Cottus cognatus*); benthic algal and macroinvertebrate abundance, richness, diversity, and community composition; and various slimy sculpin measures. Elevated concentrations of copper ($p=0.002$)and iron ($p=0.001$) in liver tissue of sculpin from the Flat River were associated with high concentrations of mine-derived iron in river water and copper in sediments that were above national guidelines. In addition, sites downstream of the mine on the Flat River had increased algal abundances ($p=0.002$) and altered benthic macroinvertebrate communities (($p<0.001$) whereas the sites downstream of the mine on Prairie Creek had increased benthic macroinvertebrate taxa richness ($p=0.050$) and improved sculpin condition (males: $p=0.008$; females: $p=0.001$). Biological differences in both rivers were consistent with mild enrichment of the rivers downstream of current and historical mining activity. Although the effects of mining activities on riverine biota in these northern rivers are currently limited, results of this research show that there is potential for effects to occur with proposed growth in mining activities.

Laboratory exposures were conducted using slimy sculpin, identified as a sentinel fish species in pristine northern rivers, to identify alternative methods for assessing toxicity of contaminants of concern in mining effluents. Ammonia was selected for the exposures based on effluent characteristics of northern mining effluents. Ammonia is known to be an important toxicant in aquatic environments. Although ammonia toxicity has been well studied in many fish species, effects of chronic exposure of slimy sculpin, a critical biomonitoring species for northern aquatic habitats, are not well known. Slimy sculpin were exposed to six concentrations of un-ionized ammonia (NH_3) relevant to concentrations found in northern mining effluents: control (0 ppm), 0.278 ppm, 0.556 ppm, 0.834 ppm, 1.112 ppm, and 1.668 ppm. An LC_{50} of 1.529 ppm was calculated from mortality data. Histopathological examination of gills indicated significant tissue damage, measured as lamellar fusion and epithelial lifting, at 0.834, 1.112, and 1.668 ppm. Using gill endpoints, NOEC and LOEC were calculated as 0.556 ppm and 0.834 ppm respectively. An EC_{50} of 0.775 ppm was determined for lamellar fusion and an EC_{50} of 0.842 ppm for epithelial lifting. Hemorrhage of gills was present in mortalities which occurred at 1.668 ppm of un-ionized ammonia. A significant decrease in liver somatic index (LSI) was seen in both male and female fish at 0.834 and 1.112 ppm, respectively. Gonadosomatic index (GSI) in female fish significantly increased at 1.668 ppm un-ionized ammonia with an associated significant increase in total whole body testosterone concentrations. GSI in male fish also significantly increased at 1.668 ppm but no differences were seen in testosterone concentrations. No significant differences were seen in gonad histopathological assessments or condition factor. Results from this study indicate that ammonia concentrations commonly reported in northern mine effluents hold potential to affect the health of slimy sculpin including acute, chronic, histological and endocrine endpoints.

Results from both the field study and laboratory exposures provide direction for future monitoring programs in pristine northern rivers and emphasize the importance of monitoring tools to detect change in these ecosystems. I recommend that monitoring of northern pristine

rivers focus on a multi-trophic monitoring approach including indicators in algal and benthic macroinvertebrate communities due to their responsiveness. Laboratory exposures using slimy sculpin should be considered to obtain toxicological information for northern contaminants of concern. Gill histopathology endpoints may be a more sensitive indicator for detecting effects in slimy sculpin exposed to ammonia than traditional chronic endpoints. I also recommend monitoring of metal burdens in periphyton and benthic invertebrates for assessment of exposure to mine effluent and causal association in areas of low fish abundance.

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LIST OF ABBREVIATIONS

ANCOVA – Analysis of covariance

ANOVA – Analysis of variance

CCME – Canadian Council for Minister's of the Environment

CEPA – Canadian Environmental Protection Agency

CF – Condition Factor

EEM – Environmental Effects Monitoring

EPA – Environmental Protection Agency (United States)

GSI – Gonadosomatic Index

HCl – Hydrochloric Acid

H_2O_2 – Hydrogen Peroxide

LOEC – Lowest observed effects concentration

LSI – Liversomatic Index

MMER – Metal Mining Effluent Regulations

MVLWB – Mackenzie Valley Land and Water Board

NH_3 – un-ionized ammonia

NOEC – No observed effects concentration

CHAPTER 1.0

GENERAL INTRODUCTION

1.1 Background

Canada's north is currently experiencing large growth in industrial developments and the potential for continued development is high. There is a significant need to assess the sensitivity of aquatic ecosystems in the north and to develop tools to track changes within these northern ecosystems (Lumb et al. 2006, Mallory et al. 2006, Halliwell and Catto 2003, Evans 2000, Lockhart et al. 1992). Northern ecosystems are considered to be especially sensitive to contaminants due to their high-latitude geographical location and extreme climate (Schindler and Smol 2006, Evans et al. 2005). The reduced ice-free period for ecosystems in the north results in low primary production and slow growth cycles (Evans et al. 2005). With invertebrate and fish growth cycles inhibited by climatic factors, energy stores and reproduction are affected (Lumb et al. 2006, Evans 2000). Organisms in northern ecosystems may reproduce every two years compared with annual reproduction in southern watersheds due to reduced energy stores (Evans et al. 2005, Evans 2000). Slower reproduction cycles leave northern species at an increased vulnerability to stressors in the environment (Barrie et al. 1992). Lower energy stores in northern species may also result in a slower growth rate and longer life cycle (Evans et al. 2005, Barrie et al. 1992,), increasing risks to populations from the effects of contaminant toxicity due to increased exposure.

Species diversity in northern ecosystems tends to be reduced due to the harsh climate, limited opportunity for species migration from southern ecosystems, and short period of time since the last glaciation (Evans et al. 2005). Most northern ecosystems are considered to be oligotrophic, with less productivity and species abundance than southern systems (Schindler and Smol 2006, Evans et al. 2005). The increased pressures on northern ecosystems and species by climate, reduced energy stores and lower species diversity, emphasize the importance for effective monitoring programs for detecting changes in northern ecosystems.

The ability of existing monitoring methods to characterize northern aquatic communities and their response to stress requires improvement, as many of these methods (e.g., Environmental Effects Monitoring (EEM) under the federal Fisheries Act, toxicity bioassays) have largely been derived from research in southern ecosystems or with species not found in the north (DDMI 2006, Golder Associates 2006, Jacques Whitford 2006, Azimuth Consulting 2005, DDMI 2005, Golder Associates 2005a, Golder Associates 2005b). Environmental Effects Monitoring (EEM) is designed to provide science-based detection of changes in aquatic ecosystems as a result of development and human activities (Walker et al. 2003, Ribey et al. 2002). Canada's EEM program provides a nationally consistent monitoring approach for assessing effects (Walker et al. 2003), however the methodologies used may not be applicable in sensitive environments with low species abundance (Walker et al. 2003, Munkittrick et al. 2002). Northern ecosystems have naturally low species abundance due to climatic factors and developments are often located in headwaters where species abundance is even lower (Walker et al. 2003). This increases potential impacts of effects monitoring programs on fish populations. Non-lethal sampling methods were developed to avoid impacts to fish populations (Environment Canada 2002, Gray et al. 2002), but toxicological endpoints cannot be obtained (Gray et al. 2002). Also, current non-lethal methodology may be difficult to use in areas of low abundance (Gray et al. 2002). Although non-lethal sampling methods can provide information on age structure, growth, reproductive performance and energy storage (Gray et al. 2002), other EEM endpoints such as liver weight, gonad weight, otolith aging, fecundity and egg size cannot be obtained (Environment Canada 2002). When it became evident that the EEM approach was not suitable for all areas, the use of laboratory exposures was identified as a potential alternative to lethal field sampling and non-lethal sampling (Walker et al. 2003). Laboratory exposures can mimic the natural receiving environment while allowing for experimental manipulation and control of variables in concentration-response experiments (Dubé et al. 2002, Culp et al. 2000). My research used both field studies and laboratory-based experiments to develop monitoring strategies for northern pristine rivers receiving mining discharges.

1.2 Mining in Canada's North

Mining interests in Canada's north began before the 1900's. With the introduction of more advanced exploration methods and mining processes, development in Canada's north has continued to expand. There are currently four operational mines in the Northwest Territories and one in Nunavut. However, there are at least three mines projected to be operational in the Northwest Territories and five in Nunavut by 2010 (Indian and Northern Affairs 2007). In addition to operational and planned mining developments, there were 58 exploration projects conducted in 2007 in the Northwest Territories and over 60 projects in Nunavut (Mining Association of Canada 2007). The long history of mining in Canada's north has resulted in 120 abandoned mines in the Northwest Territories, Nunavut and Yukon Territory (Indian and Northern Affairs 2007). Operational mines, exploration properties and abandoned sites discharge mine effluents to northern waters which have the potential to impact these sensitive ecosystems.

I identified eight properties in the Northwest Territories that discharge effluents directly to surface water, including active mine sites, advanced exploration properties and closed mine sites, through a review of the Mackenzie Valley Land and Water Board (MVLWB) public registry (Table 1.1). The Mackenzie Valley Land and Water Board is the territorial authority responsible for regulating the use of land, water and deposit of waste in unsettled areas in the Northwest Territories. As more land claims become settled, responsibility will transfer to land and water boards established in the settled areas. The MVLWB approves applications and issues Water Licences and Land Use Permits for mining developments and maintains a public registry containing effluent reports for regulated parameters at permitted facilities, required operational reports, and other regulatory information for each development. A summary of common parameters regulated in mining effluent and effluent characterizations of eight mining discharges (average annual effluent concentrations for 2006) are documented in Table 1.2.

Table 1.1. An overview of mining properties in the Northwest Territories

Property	Type	Years of Operation	Discharge Type	Volume of Effluent Discharged (m^3)/year
Giant Mine	Closed Gold Mine	56	Seasonal	343,132
Con Mine	Closed Gold Mine	65	Seasonal	306,916
Prairie Creek	Advanced Exploration	12	Seasonal	79,358
Diavik	Active Diamond Mine	5	Continuous	7,611,334
BHP	Active Diamond Mine	10	Continuous	10,092,221
Tungsten	Active Tungsten Mine	37	Continuous	1,093,396
Snap Lake	Diamond Mine – Construction Phase	1	Continuous	2,819,842
NICO	Advanced Exploration	7	Seasonal	486

Table 1.2 A Summary of regulated parameters and average annual discharge concentrations in mining effluent from eight mining properties in the Northwest Territories. Data was obtained from the MVLWB public registry and average annual concentration was determined from effluent data reported for 2006.

	Giant Mine		**Con Mine**		**Prairie Creek**		**Ekati**		**Diavik**		**Tungsten**		**Snap**		**NICO**	
	RL	EC	RL	EC	RL	EC	RL	EC	RL	EC	RL	EC	RL	EC	RL	EC
pH	6.0-9.5	8.150	6.0-9.5	7.230	6.0-9.5	8.300	6.0-9.0	7.608	NR	7.890	6.0-9.0	8.333	6.0-9.0	7.845	6.0-8.5	8.050
Ammonia (mg/L)	12.000	0.017	NR	12.363	10.000	9.132	4.000	0.019	20.000	1.970	10.000	3.203	20.000	1.426	12.000	46.443
Aluminum (mg/L)	NR	NM	NR	NM	NR	0.090	2.000	0.027	3.000	0.480	NM	115.333	2.000	13.438	NR	1.500
Arsenic (mg/L)	1.000	0.280	1.000	0.089	1.000	NM	1.000	0.001	0.100	0.002	0.400	0.014	0.040	0.000	1.000	0.092
Cadmium (mg/L)	NR	NM	NR	NM	0.010	0.010	NR	0.000	0.003	<0.0002	0.020	0.003	0.002	0.000	0.010	0.001
Copper (mg/L)	0.600	0.015	0.600	0.054	0.200	0.017	0.200	0.003	0.040	0.003	0.400	4.637	0.020	0.009	0.600	0.051
Lead (mg/L)	0.400	0.000	0.400	0.000	0.300	0.080	NR	0.000	0.020	<0.0001	0.400	0.043	0.009	0.001	0.400	0.024
Nickel (mg/L)	1.000	0.048	1.000	0.021	0.400	0.014	0.300	0.004	0.100	0.014	0.800	0.225	0.100	0.002	1.000	0.040
Zinc (mg/L)	0.400	0.008	0.400	0.037	0.600	1.793	NR	0.003	0.020	0.002	0.400	0.510	0.020	0.002	1.000	0.124

RL = Regulated Limit as identified in Water Licences issued to each mining property (MVLWB public registry)
EC = Effluent Concentrations calculated as the Average Annual Concentration
NR = Parameter not regulated in Water Licence
NM = Parameter not measured by mining property

In order to investigate the effects of mining effluents on pristine northern streams and assess current monitoring methodologies in northern environments, I chose to conduct field studies at an active mining property (Tungsten Mine) that has been in operation for a long period and an advanced exploration property that was currently discharging effluent (Prairie Creek exploration property). Both Tungsten Mine and Prairie Creek discharge to the South Nahanni Watershed. Based on an assessment of effluent characterizations and concentrations of contaminants of concern discharged to northern waters by mining properties in the Northwest Territories, ammonia was identifed as an important stressor.

1.3 Pristine Northern Rivers

The South Nahanni River watershed comprises part of Nahanni National Park Reserve, a UNESCO World Heritage Site and Canadian Heritage River, and is considered a sensitive northern environment with pristine aquatic environments (Halliwell and Catto 2003). Two mining developments are located on tributaries (Flat River and Prairie Creek) to the South Nahanni River. Tungsten Mine is located on the banks of the Flat River and began operations in the 1950's continuing through to 1986. At that time the mine went into care and maintenance until it reopened in 2001. Tungsten Mine is currently mining and milling tungsten ore, in the form of shelite. Tailings are deposited into an exfiltration pond system to remove the suspended sediments. The effluent leaches through the soils and into the Flat River. The plume discharge of tailings effluent is approximately 50 km upstream of the confluence with the South Nahanni River (Halliwell and Catto 2003, Environment Canada 1991). Previous biological studies on the Flat River have been limited.

The Prairie Creek advanced exploration program is located approximately 18 km upstream from the confluence with the South Nahanni River (Halliwell and Catto 2003, Environment Canada 1991). The property was initially explored in the 1950's and a mill complex and tailings pond were constructed in the 1980's. Due to financial difficulties, mining and milling did not proceed at the Prairie Creek property and tailings were not generated. Canadian Zinc Corporation took over the property in 1995 and has begun an advanced exploration program for heavy metals (lead/zinc/copper/silver). A portal was constructed at the site for exploration and potential mining purposes. The portal discharges water to Prairie Creek which has potential to be high in several metals (zinc/lead/cadmium). No previous biological studies have been undertaken on Prairie Creek. Tungsten Mine and Prairie Creek exploration property represent ideal systems to conduct EEM programs to assess current monitoring strategies as they are located in a sensitive northern ecosystem (Lumb et al. 2006, Halliwell and Catto 2003) and receive only mining effluent discharges (Beavers 2002, EBA Engineering 2002, Sigma Resources 1978).

1.4 Effects of Mining Effluents

The effects of mining discharges to receiving waters can include chronic exposure to toxic contaminants, bioaccumulation and bioconcentration of contaminants, accumulation of contaminants in sediments and endocrine disruption (Environment Canada 2002). Metal can accumulate in tissues of fish (Farag et al. 1998), invertebrates (Mason et al. 2000) and algae (Meylan et al. 2003) exposed to metal mining effluents. Accumulation of metals has been shown to lead to metal-specific chronic effects such as decreased growth (Levesque et al. 2003), changes in liver size (Dubé et al. 2005), decreased reproductive performance (Rickwood et al. 2006), and decreased survival (Harper et al. 2008).

Ammonia is another important component of mining effluent. Ammonia is known to cause both acute and chronic toxicity in fish as indicated by decreased survival (Fairchild et al. 2005), gill changes (Lease et al. 2003), changes in liver size and function (Milne et al. 2000), reproductive endpoints (Thurston et al. 1986) and decreased swimming performance (Fairchild et al. 2005).

Commonly measured endpoints for assessment of biological impact include condition factor, gonadosomatic index, liversomatic index, behaviour and endurance, reproductive performance, life-cycle indicators (hatching success, deformities), endocrine endpoints such as glycogen storage, triglycerides and sex steroids, as well as histological studies on target organs including liver, gills and gonads (Weber et. al 2007, Rickwood et al. 2006, Dubé et al. 2005, Lease et al. 2003, Environment Canada 2002, Mason et al. 2000, Milne et al. 2000).

1.5 Sentinel Species

Sentinel species are considered representatives or indicators of a larger population or community. They are often measured in field studies to monitor any changes in biological health when community structure is well known or previously characterized. They are also used in systems with low species abundance in order to reduce the potential impact of monitoring programs on resident fish populations. Sentinel species can also be used in conjunction with laboratory exposures to obtain toxicological information on effects endpoints (Lower 1990).

Previous toxicological studies conducted on ecosystems in the north have focused on large-bodied fish species found in southern waters (Evans et al. 2005, Halliwell and Catto 2003, Barrie et al. 1992, Lockhart et al. 1992) for several reasons. Large-bodied fish such as rainbow trout have been extensively studied, provide a greater amount of fish tissue to work with, and are considered more important for commercial reasons (Ankley et al. 2006, Gray et al. 2004). Larger fish are often perceived by the public as more valuable species and provide a comparison to historical monitoring programs (Gray et al. 2004). However, studies conducted with small-bodied fish have indicated several advantages leading to more attention to small-bodied fish in effects monitoring (Gray et al. 2004, Tetreault et al. 2003, Gibbons et al. 1998,). Smaller fish have shorter life cycles which allow for easier assessment of early development stages and full life cycle testing (Ankley and Villeneuve 2006). Small-bodied fish tend to have a smaller home range compared to large-bodied fish which allows for exposure and residency in the zone of influence, as well as representing local conditions (Gray et al. 2004, Munkittrick et al. 2000). These features have increased their use in EEM programs to monitor effects of pulp and paper and metal mining effluents under the federal *Fisheries Act* (Munkittrick et al. 2002). Their size and ease for use in laboratory settings has also increased their utility for toxicity bioassays.

Studies by Sigma and EBA on the Flat River have indicated that slimy sculpin (*Cottus Cognatus*) are the only possible sentinel species that meet selection criteria for the EEM program (EBA Engineering 2002, Sigma Resources 1978). Studies conducted by Halliwell and Catto (2003) confirm they are the most appropriate sentinel species for effects monitoring programs in the South Nahanni watershed. Slimy sculpin are found throughout watersheds in the Northwest Territories (Burr et al. 1998) and are present downstream of most, if not all, major development in the Northwest Territories and Nunavut (DDMI 2006, Golder Associates 2006, Jacques Whitford 2006, Azimuth Consulting 2005, DDMI 2005, Golder Associates 2005a, Golder Associates 2005b). They are a small species of fish with a short life span and limited mobility due to the absence of a swim bladder (Gray et al. 2004), which allows comparison of effects and exposure to contaminants (Munkittrick et al. 2000). Slimy sculpin have been used in the past in mesocosms to measure effects of effluent and contaminants (Dubé et al. 2005). Sculpin have been shown to be a good sentinel species in several field monitoring studies (Galloway et al. 2005, Gray and Munkittrick 2005, Tetreault et al. 2003, Gibbons et al. 1998). Studies conducted by Gray et al. (2005) identified the potential for slimy sculpin as sentinel species based on their work in agricultural areas of the St. John River in New Brunswick, Canada. Limited work has been done on slimy sculpin in northern ecosystems, and their application in northern monitoring programs has not been studied (Lumb et al. 2006, DDMI 2005). Laboratory exposures in my thesis provide an opportunity to examine the response of this northern sentinel species to an important stressor in northern ecosystems.

1.6 Ammonia – An Important Northern Stressor

One of the most common stressors in the north is ammonia (DBCM 2005, DDMI 2005). Ammonia results from blasting residue in mining operations and to a lesser degree the discharge of human waste or sewage from mining facilities (CCME 1999, USEPA 1999). Because federal Metal Mining Effluent Regulations (MMER) do not regulate ammonia, it is important for the provincial and territorial regulatory authorities to ensure discharge limits are protective to the receiving environment. In the Northwest Territories water licences with associated discharge limits are issued by territorial regulatory authorities such as the Mackenzie Valley Land and Water Board (MVLWB) but ammonia is not always a regulated parameter or limits are developed based on toxicological testing using southern species. Because ammonia toxicity varies with pH and temperature, setting generic discharge limits can be difficult (CEPA 2001, CCME 1999, EPA 1985).

Ammonia is a Priority Substance 2 listed in the *Canadian Environmental Protection Act* (CEPA) and although lethal concentrations (LC_{50}) for sensitive species have been reported, these species are not found in northern waters. Acute LC_{50} values range from 0.14-3.44 ppm, with a high variability both within species and between species (Eddy 2005, Lease et al. 2003, CEPA 2001, Wood 2000, Thurston et al. 1986). In rainbow trout (*Oncorhynchus mykiss*), 112 acute toxicity studies have been conducted (CEPA 2001) with a mean LC_{50} of 0.481 ppm (minimum LC50: 0.16 mg/L, maximum LC50: 1.09 mg/L). An acute toxicity study using mottled sculpin (*Cottus bairdi*) yielded a LC_{50} value of 1.390 ppm (Thurston et al. 1981). Data from the Priority Substances List Assessment Report for Ammonia in the Aquatic Environment (CEPA 2001) indicated that white perch (*Morone Americana*) were the most sensitive species, with a mean LC50 of 0.279 ppm based on two studies, and green sunfish (*Lepomis cyanellu*s) were shown to be the most tolerant at 1.860 ppm (mean of six studies).

Previous studies have found chronic toxicity values for un-ionized ammonia to range from as low as 0.068 ppm in rainbow trout to concentrations of 1.1 ppm in other freshwater species such as bluegills (*Lepomis macrochirus*) (Eddy 2005, Milne et al.

2000, Wood 2000). Attempts by Diavik Diamond Mines Inc. (DDMI) and Debeers Canada Mining (DBCM) to conduct toxicity testing on large-bodied sentinel species such as round whitefish (*Genus and species*) have failed due to the low abundance of fish or absence of gametes (DDMI 2006, DBCM 2005). Attempts have been made to capture slimy sculpin for use in toxicity testing as they are present in watersheds receiving discharges but site conditions have limited this option (DDMI 2005). The use of a species of northern relevance would allow for toxicity testing applicable to northern environments and provide the opportunity to use laboratory exposures as an effective way to monitor effects of ammonia and other effluent components.

Concentration-response relationships are necessary for the development of threshold values (Ankley et al. 2006, Rand 1995). In order to use threshold values effectively in the north, it is important that these values are based on concentration-response studies conducted using northern species and under northern conditions such as representative temperature. Threshold values for a northern sentinel species can be used to monitor cumulative effects within watersheds and provide early indication of ecosystem change (Galloway et al. 2005, Gray et al. 2005). These values could also be used in discharge permits for mines which discharge effluent to aquatic receiving environments.

1.7 Research Objectives and Hypotheses

The overall objective for my research was to assess and better understand effects of mining effluents on two pristine and sensitive northern rivers through field and laboratory-based toxicity studies.

My specific research objectives were to:

1) Examine two pristine northern rivers receiving mining discharges (Flat River and Prairie Creek of the South Nahanni watershed) to document current aquatic conditions, assess any changes associated with effluent discharges, and to evaluate suitable indicators and monitoring methods for northern environments;
2) Determine if ammonia, an important stressor discharged by mines in Canada's north, affects the health of slimy sculpin, a northern sentinel species; and
3) Assess how my results will improve future effects monitoring methodologies in northern pristine rivers and how a northern sentinel species can be used to assess effects of an important northern stressor.

I hypothesized that the Flat River and Prairie Creek aquatic systems would show minor changes within the zone of impact in biological communities due to the discharge of mining effluent. Field studies would confirm that slimy sculpin are the only possible sentinel species in the areas of the South Nahanni watershed that were studied. The results of the field program would identify trophic components of importance to focus further monitoring programs. Laboratory studies on ammonia would provide toxicity information for slimy sculpin exposed to concentrations of ammonia found in northern mining effluents and identify responses important for detecting changes as a result of ammonia exposure.

1.8 Research Significance

There is a need to develop monitoring methods specific to the north so changes due to man-made developments can be detected and assessed. Given the increasing development of mining operations in Canada's north, it is crucial that effects monitoring programs are effectively designed to detect changes in sensitive ecosystems, such as the South Nahanni watershed. Recommendations from both field studies and laboratory experiments can be used to customize northern monitoring programs in order to determine effects. Field programs allow for "real-world" assessment of changes and identification of northern sentinel species. Development of concentration-response curves for key northern contaminants using northern sentinel species is important for the development of discharge limits and monitoring programs relevant to northern pristine environments and for providing guidance to regulators. Results of this research will also provide thresholds to assist with cumulative effects assessment in ecosystems receiving multiple discharges containing elevated ammonia.

CHAPTER 2.0

A MULTI-TROPHIC APPROACH TO MONITORING THE EFFECTS OF METAL MINING IN OTHERWISE PRISTINE AND ECOLOGICALLY SENSITIVE RIVERS IN NORTHERN CANADA*

P Spencer, MF Bowman, MG Dubé

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2.1 Introduction

Northern aquatic ecosystems are considered to be sensitive to contaminants due to their high-latitude and extreme climate (Schindler and Smol 2006). The reduced ice-free period results in low primary production, slow growth cycles and reduced energy stores with invertebrates and fish commonly reproducing every two years compared with annual reproduction in southern watersheds (Chambers 2006, Lumb et al. 2006, Evans et al. 2005, Evans 2000). Slower reproduction cycles leave northern species at an increased vulnerability to stressors in the environment (Barrie et al. 1992). Species diversity tends to be reduced due to the harsh climate, limited opportunity for species migration from southern ecosystems, and short period of time since the last glaciation (Evans et al. 2005). Most northern ecosystems are considered to be highly oligotrophic, with less productivity and species abundance than southern systems (Chambers et al. 2006, Schindler and Smol 2006).

Canada's north is currently experiencing large growth in industrial developments and the potential for continued development is extremely high. In 2005, the value of mineral production increased nearly 100% from the mineral production in 2002 (Natural Resources Canada 2005). As an indication of economic significance, diamond mining, which occurs exclusively in the NWT and Nunavut, accounts for 15% of the global market with exports totaling $1.9 billion in 2006 (Mining Association of Canada 2007). Given the significance of industrial development in the north, there is a need to assess the sensitivity of these unique high-latitude ecosystems and to develop tools to track differences over spatial and temporal scales (Mallory et al. 2006, Lockhart et al. 1992). Establishing a baseline of response to anthropogenic stressors is also critical as the advent of climate change and global warming will undoubtedly alter and potentially magnify responses of aquatic systems to stress (Schindler and Smol 2006). The increased pressures on northern ecosystems and their unique characteristics related to climate, reduced productivity, and lower species diversity emphasize the importance of effective monitoring programs which can be used for detecting changes in northern ecosystems and to provide early warning signals for higher trophic levels.

Under the *Fisheries Act*, the 2002 *Metal Mining Effluent Regulations* require metal mines in Canada to conduct environmental effects monitoring (EEM) to assess effects potentially caused by their effluents. The EEM program focuses particular attention on biological monitoring to evaluate whether or not environmental effects are occurring (Walker et al. 2003). An "effect" is a statistically significant response in at least one of the select endpoints (e.g., abundance) in comparisons between biological samples taken from exposure and reference areas. Canada's EEM program provides a nationally consistent monitoring approach for assessing effects and its methods have influenced monitoring practice in other arenas including monitoring under provincial permits and environmental impact assessment (Kilgour et al. 2006). However, methods may not be applicable in sensitive environments with low productivity and species abundance (Walker et al. 2003, Munkittrick et al. 2000). Pristine northern ecosystems, such as the South Nahanni Watershed, have inherently low species abundance and diversity that may impact the utility of traditional monitoring endpoints.

The South Nahanni Watershed includes part of Nahanni National Park Reserve, a UNESCO (*United Nations Educational, Scientific and Cultural Organization*) World Heritage Site and Canadian Heritage River, and is considered a sensitive northern environment with pristine aquatic environments (Halliwell and Catto 2003). Two industrial developments are located on tributaries of the South Nahanni River: Tungsten Mine and Prairie Creek Exploration Property are located on the Flat River and Prairie Creek, respectively. These tributaries are ideal for evaluating the applicability of southern monitoring methods for use in northern systems because they are otherwise pristine and significant impacts were not apparent from previous water quality studies (Lumb et al. 2006, Halliwell and Catto 2003).

Multi-trophic level monitoring allows for determination of biological interactions and assessment of aquatic response patterns in these ecosystems. In the summer of 2006, we conducted a multi-trophic level effects monitoring program on both the Flat River and Prairie Creek in the South Nahanni Watershed. Sentinel fish populations, benthic macroinvertebrates, benthic algal communities, water quality, and sediment quality were sampled at upstream reference areas and compared to near-field and far-field areas exposed to mine effluents. The objectives of this work were to document current aquatic conditions in these rivers and any effects due to mining activities, assess current monitoring methods for northern environments, and make recommendations to improve future effects monitoring in northern waters.

2.2 Materials and Methods

2.2.1 Study Sites

The South Nahanni River is located in the southwest corner of the Northwest Territories (Figure 2.1). The low subarctic climate in the Nahanni Plateau is characterized by cool summers (mean temperature is 9°C) and cold winters (mean temperature is -19.5°C; Environment Canada 1991). Most areas are less than 1372 m above sea level but mountain ranges reach to over 1800 m (Halliwell and Catto 2003).

The terrain is underlain by Palaeozoic carbonates, and is incised by deep and narrow valleys.

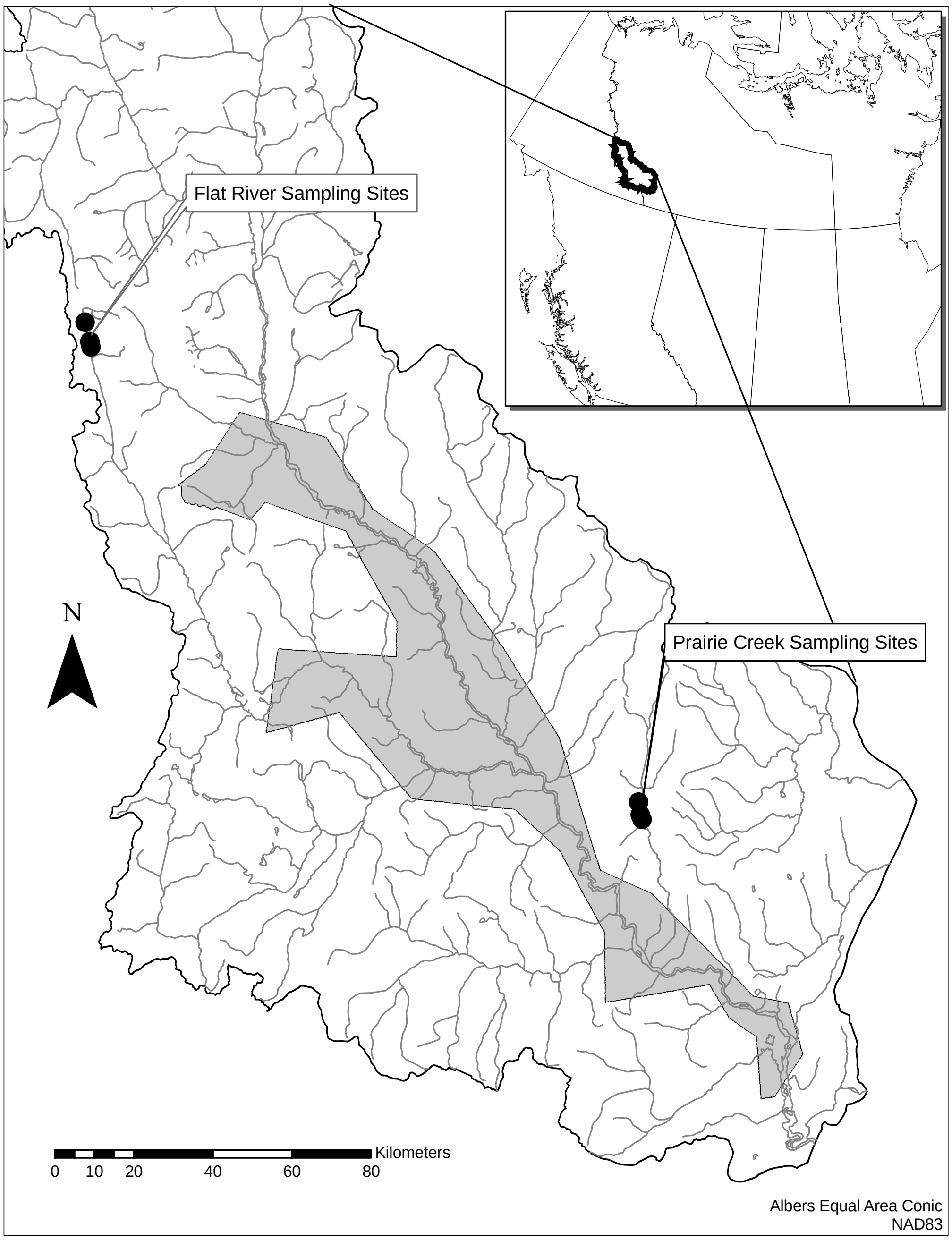

Figure 2.1 Map showing the South Nahanni watershed located in the southwest corner of the Northwest Territories and south-eastern corner of the Yukon Territory. Nahanni National Park is located within the South Nahanni watershed.

Vegetation is sparse at higher elevations but open stands of black spruce with an understory of dwarf birch, Labrador tea, lichen, and moss occur in valleys and at lower elevations (Environment Canada 1991). The South Nahanni River flows in a southerly direction 540 km from ice fields near the Yukon-NWT border through the Mackenzie Mountains into the Liard River which then converges with the Mackenzie River (Environment Canada 1991). The normal range of flows at the flow gauging station upstream of Virginia Falls is 55 to 1500 m^3 per second (Halliwell and Catto 2003).

The South Nahanni River is a UNESCO World Heritage Site and a highly valued national park. Classified as a Canadian Heritage River, the South Nahanni River has water quality considered to be nearly pristine (Halliwell and Catto 2003). Its unique landscapes and ecosystems only emphasize the need to ensure that it is protected from potential stresses of development (Halliwell and Catto 2003, Inland Waters Directorate 1991).

The Flat River and Prairie Creek tributaries of the South Nahanni River have both historical and current industrial activity that could affect this sensitive environment. The Flat River flows into the Nahanni River below Virginia Falls and may exhibit properties more similar to the Mackenzie River. Distinct water quality changes have been detected along the Flat River as a result of high natural variability in sediment loads (Halliwell and Catto 2003). The presence of hot springs and mineral springs also increase the variability within the Flat River watershed. The Flat River valley is filled with glacial and fluvial deposits through which the river meanders in a well defined flood plain (EBA 2002). Limestone, sandstone, and dolostones are the predominant geological forms underlying the Flat River (Environment Canada 1991) and serve the basis for tungsten mining in the area. Flow rates on the Flat River average 247-900 m^3/s (Halliwell and Catto 2003) which is lower than flows in tributaries above Virginia Falls.

Prairie Creek also flows through deposits of dolostones, limestone, and shale (Halliwell and Catto 2003) containing mineralized veins of zinc, lead, copper, and silver. Prairie Creek originates in and flows through upland and steep canyon terrain which leads to reduced suspended sediments compared to other tributaries in the Nahanni watershed (Halliwell and Catto 2003). Due to the underlying geological formations, Prairie Creek precipitates carbonate and other minerals as well as metals from natural mineral deposits (Environment Canada 1991). Flow rates in Prairie Creek are lower than those in both the Flat River and the South Nahanni River, ranging from 0.5 m^3/s in the winter months to 30 m^3/s in the summer months (Inland Waters Directorate 1991).

Tungsten Mine on the Flat River began operations in the 1950's and continued to operate until 1986. Increased silt from direct discharge during this time remains in the river downstream of the mine. In 1986, the mine went into care and maintenance until it reopened in 2001. Tungsten Mine is currently mining and milling tungsten ore, in the form of shelite. Tailings are deposited into an exfiltration pond system to remove the suspended sediments. The exfiltrate leaches through the soils and into the Flat River (Figure 2.2a) approximately 50 km upstream of the confluence with the South Nahanni River (Halliwell and Catto 2003, Environment Canada 1991). Limited information currently exists on the orientation and delineation of the exfiltrate into the Flat River which complicates quantification of biological exposure to current discharges from the mine.

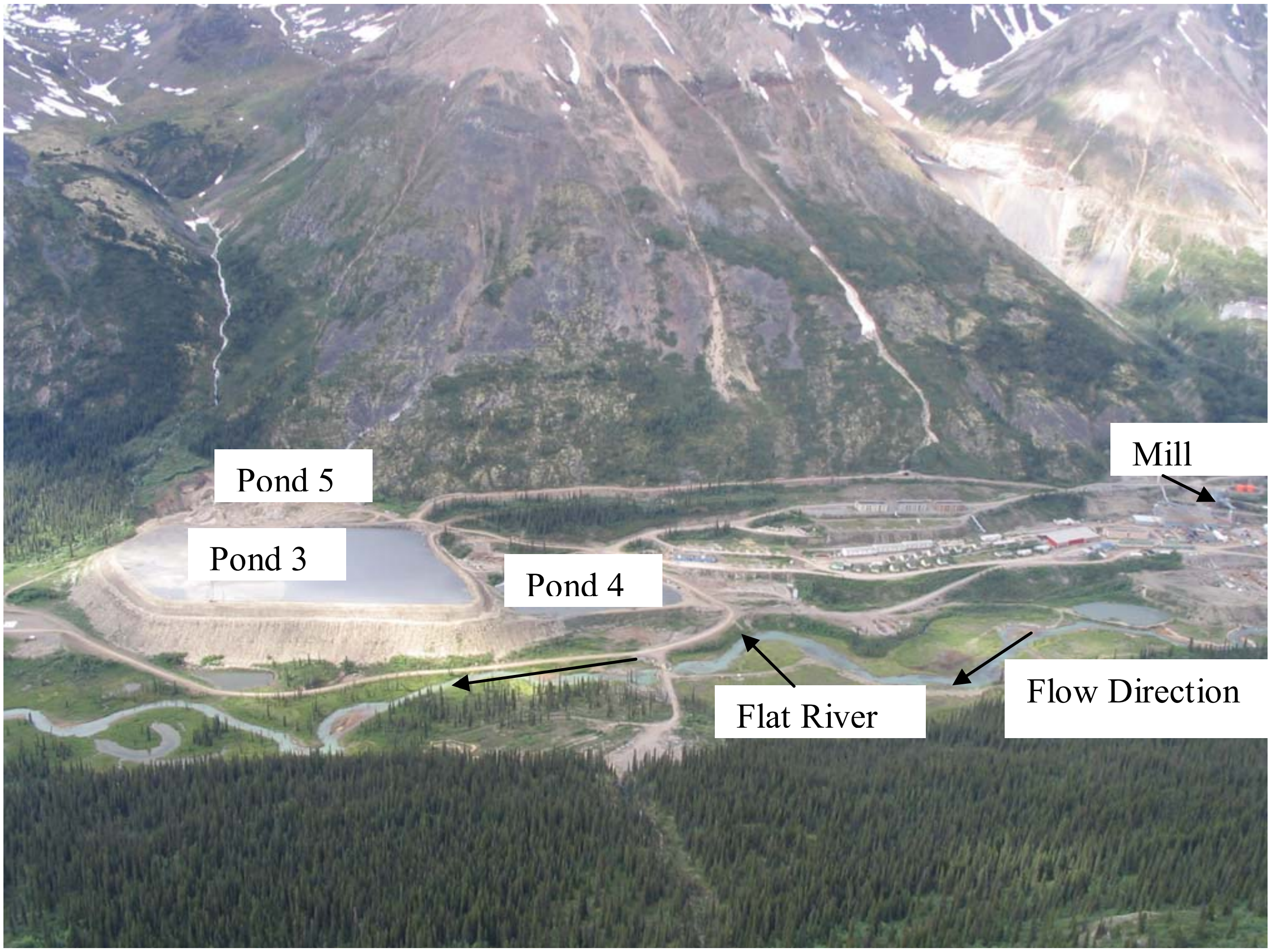

Figure 2.2 (a) Aerial view of Tungsten Mine discharge: Tailings Ponds 3, 4 and 5 comprise an exfiltration system for discharge to the Flat River. Discharges to the tailings ponds include mine water, process water, and sewage treatment plant effluent.

The Prairie Creek Advanced Exploration Program is located approximately 18 km upstream from the confluence with the South Nahanni River (Halliwell and Catto 2003, Environment Canada 1991). The property was initially explored in the 1950's and a mill complex and a tailings pond were constructed in the 1980's (Figure 2.2b). Due to financial difficulties, mining and milling did not proceed at the Prairie Creek property and tailings were not generated. Canadian Zinc Corporation took over the property in 1995 and has begun an advanced exploration program for base metals (i.e., lead, zinc, copper, and silver). A portal was constructed at the site for exploration and potential mining purposes. The portal discharges water to a polishing pond and subsequently to a catchment pond. The water from the catchment pond then discharges to Prairie Creek and has potential to be high in several metals (i.e., zinc, lead, and cadmium). Control-impact monitoring surveys were conducted in the summer of 2006 at reference, near-field (high exposure), and far-field (low-exposure) sites on the Flat River and Prairie Creek using methods outlined in the Metal Mining Effluent Regulations EEM Guidance Document (Environment Canada 2002). In the absence of tracer studies which document the path of flow from the exfiltration basins into the river, the location of the exfiltration basins at Tungsten Mine (Pond 3 and Pond 4, Figure 2.2a) was considered the area of development activity. At Prairie Creek, the portal discharge into Harrison Creek was considered the point source discharge (Figure 2.2b).

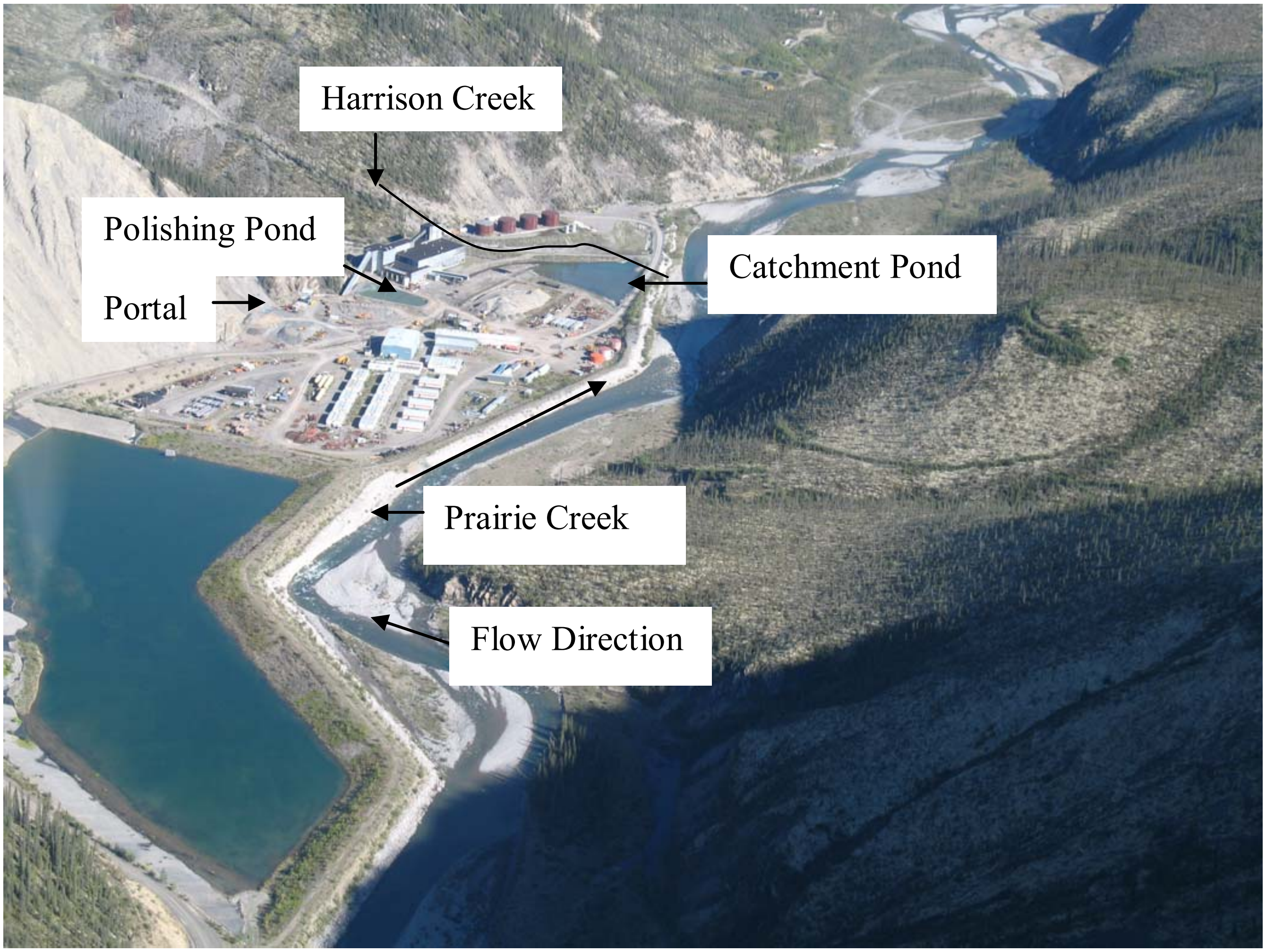

Figure 2.2 (b) Aerial view of the Prairie Creek Exploration Property. Mine water discharge from the portal enters a polishing pond and catchment pond before release into Prairie Creek.

From August 27 - September 2 2006, sentinel fish populations, benthic macroinvertebrates, benthic algal communities, water quality, and sediment quality were sampled at upstream reference areas located 3 km above the zone of influence and compared to near-field and far-field areas exposed to mine effluents. Far-field areas were located approximately 2 km downstream from the near-field sites.

2.2.2 Water chemistry and effluent characterization

Dissolved oxygen, ammonia, conductivity and pH were measured in situ whereas samples for total metals, general water chemistry (conductivity, pH, total suspended solids), and nutrients (nitrate, total phosphorus) were analyzed in a laboratory. Dissolved oxygen was measured with a WTW Dissolved Oxygen Pocket Meter (Model Oxi 330i, WTW Inc., West Wareham, Massachusetts). Ammonia was measured with a Hanna HI 93733 ammonia meter (Hanna Instruments Canada Inc., Laval, Quebec). Conductivity and pH were measured with an Oakton 300 series meter (Oakton Instruments, Vernon Hills, Illinois). Single grab water samples were collected from a location in-stream at each reference, near-field, and far-field site. Effluent samples were collected from points of discharge at both Tungsten Mine (Ponds 3 and 4) and the Prairie Creek Exploration Property (Catchment Pond) to characterize effluents. Water samples were kept at $4^{o}C$ until they were shipped on ice to Taiga Environmental Laboratory in Yellowknife, NWT. Samples for total metals were unfiltered and acidified and analyzed using the inductively coupled plasma-mass spectrometric method (ICP-MS). Conductivity was analysed using the radiometric method, pH using electrometric methodology, and total suspended solids using a gravimetric method. Samples for nitrate and total phosphorus were unfiltered and analyzed by ion chromatography and colorimetric determination, respectively.

2.2.3 Sediment samples

A sediment sample was collected at each sampling area (reference, near-field, and far-field). Grab samples were collected in 500 mL glass jars and analyzed for total metals, particle size (PSA) and total organic carbon (TOC) at Taiga Environmental Laboratory, Yellowknife, NWT. Total metals and minerals were prepared according to EPA 3050B methodology for acid digestion and analysed by ICP-MS or ICP-AES with the exception of mercury which was analysed by cold vapour atomic absorption (EPA 245.5 method). Particle size analysis was conducted using Carter's method 47.3. Total organic carbon was analysed using a dry combustion method.

2.2.4 Benthic algal sampling

Nine 15-25 cm diameter cobbles were randomly selected per reference, near-field and far-field site at each mine. For chlorophyll *a* analysis, one scraping of algae from within a circular template (13.2 cm^2) was taken from each cobble. Three templates were then pooled (representing a total sampled area of 39.6 cm^2 pooled from three rocks) resulting in three replicate chlorophyll *a* samples per site. For algal taxonomy, one circular template was taken from three rocks and pooled resulting in a single taxonomy sample per site. Samples were placed in scintillation vials and preserved in 5% buffered formalin with a final volume of 21 mL (taxonomy samples) or frozen in stream water (chlorophyll *a* samples). For taxonomic analysis, 2 mL sub-samples of epilithon suspension were sonicated for 10-20 s using a Sonifer Cell Disruptor (model w140; Findlay et al. 1999) and gravity settled for 24 h in an Ütermohl chamber (Phycotech, Michigan, USA). Cells were identified, counted and measured from random fields until 100 cells of the dominant species were found. Estimates of cell volume for each species were obtained by measurements of up to 50 cells of an individual species and applying the geometric formula best fitted to the shape of the cell (Rott 1981). Algae were identified to the species level and were then combined to the family level to calculate taxa richness and Simpson's Diversity Index. Methods for fluorometric analysis of chlorophyll *a* are outlined in Bowman et al. (2005).

2.2.5 Benthic invertebrate sampling

Five replicate benthic invertebrate samples were collected from each of the reference, near-field, and far-field sites, consistent with EEM methods. A U-net (0.101 m^3) was used to collect 3 sub-samples which were pooled for each replicate for a total area of 0.303 m^3 at each replicate station. Five randomly selected replicate stations were sampled within each area to provide an area delineated estimate of benthic invertebrate communities. Material was preserved in 95% ethanol, sent to Cordillera Consulting in British Columbia, Canada, where they were sorted, identified to the lowest practical taxonomic level, and counted. Following EEM guidelines, family taxonomic identification was used. Studies indicate that changes in community composition and community responses to disturbances are maintained when species-level data are aggregated to family (Environment Canada 2002). In addition, the predictability of relationships between benthic communities and physical variables can be stronger when family data are used relative to when species level data are used (e.g., Reynoldson et al. 2001). The benthic invertebrate endpoints assessed included total invertebrate density (no. per m^2), taxa richness, Simpson's Diversity Index, and the Bray-Curtis Index. These benthic invertebrate endpoints were selected as they provide quantitative data and important information on community structure and composition (Environment Canada 2002). Total invertebrate density indicated the number of individuals in all families at the selected sites. Taxon richness represented the number of taxonomic families collected for each replicate. Simpson's Diversity Index takes into account abundance patterns as well as taxonomic richness by calculating the proportion of individuals in each taxonomic family and their relative abundance. The Bray-Curtis Index is a dissimilarity index which is a measure of the percentage of difference between sites within a sample area.

2.2.6 Fish collection and sampling

Previous work in the Nahanni National Park has indicated that slimy sculpin (*Cottus cognatus*) are the most abundant fish species and are likely the only possible sentinel fish species for effects monitoring due to low abundance of other species (Halliwell and Catto 2003, EBA Engineering 2002, Sigma Resources 1978). Watershed studies conducted by our lab (Dubé et al., University of Saskatchewan, unpublished) in 2006 concurrent with the sampling described herein also confirmed that slimy sculpin were the only species that could be used as a sentinel based on monitoring guidance under the EEM Program (Environment Canada 2002). Although arctic grayling (*Thymallus arcticus*), bull trout (*Salvelinus confluentus*), and round whitefish (*Prosopium cylindraceum*) were captured in each of the rivers, their presence was sporadic and neither lethal nor non-lethal sampling protocols could be implemented as specified in the EEM guidance document (20 males and 20 females for lethal sampling; approximately 100 fish for non lethal sampling).

Slimy sculpin are a sedentary species and studies have shown limited movement between sites located 200 m apart across the Saint John River in NB, Canada (Galloway et al. 2005, Gray et al. 2004). The distances between all sites on both rivers were chosen 2000-3000 m apart to minimize sculpin movement between sites. Sculpin were collected from upstream, near-field, and far-field sites with backpack electrofishers. Total effort to collect 40 fish was measured in electrofishing seconds. Electrofishing seconds for reference, near-field, and far-field were 12,480, 874, and 6510 seconds, respectively. Twenty males and 20 females collected at each sampling area were sacrificed according to lethal sampling protocols. Non-lethal sampling for this species was also conducted for comparative purposes, although results are not presented here.

Fish were collected and stored alive temporarily in buckets at each site. Upon completion of electrofishing, captured fish were immediately measured for total length and total weight. Sculpin were transported back to the lab at the mine site and euthanized in clove oil prior to dissection. Liver and gonad tissues were removed and weighed (to 0.001 g). Visual observations or presence of parasites were noted during dissections. Fish were analyzed for the standard EEM fish survey endpoints including liver weight, gonad weight, length, weight, condition estimates, age, fecundity, and egg size.

Total metal concentrations in slimy sculpin tissues were investigated in equal numbers of adult males and females from each site. Flesh muscle and liver tissue were analysed from fish collected in the Flat River. Metals were examined only in muscle in fish from Prairie Creek due to lower body weights of the fish captured at the reference, near-field, and far-field sites. Lower body weights resulted in insufficient amounts of liver tissue. Mean sample weights of muscle and liver were 3.5 and 0.3 g respectively.

Samples were analyzed at a commercial laboratory (SRC Laboratories, Saskatoon, SK, Canada) for a suite of 27 metals which were determined by nitric and hydrochloric acid digestion and inductively coupled plasma–mass spectrometer or inductively coupled plasma–optical emission spectrometer following U.S. Environmental Protection Agency method 200.3, except that HCl was used instead of H_2O_2.

Ovaries from each female were placed in 10% buffered formalin. For fecundity estimates, 0.02-0.10 g of follicles (42-217 follicles) were removed from the middle of ovaries, separated, and photographed. Image analysis (Image-Pro Plus; Media Cybernetics, Washington, MD, USA) was used to count and measure the mean diameter of vitellogenic follicles. Counts of vitellogenic follicles were made and fecundity extrapolated as the number of follicles per gram of carcass weight.

Slimy sculpin were aged by counting annuli on sagittal otoliths according to the methods of Gibbons et al. (1998) modified so that otoliths from fish larger than 6 cm or those where annuli were unclear were ground to the nucleus charred over an alcohol flame and read. Briefly, sagittal otoliths were removed surgically from each fish and stored in paper envelopes before being read under water with incident light on a dissecting microscope. Whole otoliths were read three times, charred otoliths twice and Flat River otoliths were read independently by two experienced otolith readers as further verification of assignment of ages.

2.2.7 Statistical analysis

One-way Analysis of Variance (ANOVA) with site (i.e., reference, near-field, far-field) as the factor was conducted for algal biomass, total benthic invertebrate density, invertebrate taxon richness, Simpson's Diversity Index, Bray-Curtis Index, and each of the fish tissue metals. Assumptions of ANOVA were checked for each variable prior to conducting ANOVA. Normality was confirmed using the Shapiro-Wilkes test and equality of variances was compared using Levene's test. All tests were performed using Systat 11. For all analyses, $p<0.05$ was considered significant. Tukey's post hoc test was used to determine site differences when $p<0.05$. Tukey's test using Systat version 11 corrects the significance level automatically for multiple pair-wise post hoc comparisons. Following EEM guidelines, condition, liver weight, gonad weight, and size-at-age were analyzed using ANCOVA for all fish. For condition, length was used as a covariate against body weight. Liver and gonad weights were analysed using body weight as a covariate. Length-at-age ANCOVA was used for growth comparisons. In female fish, ANCOVA was performed for egg size and fecundity using body weight as a covariate. Assumptions were confirmed by checking for normality and equality of variances as described above. Assessment of linearity and interactions were also performed prior to ANCOVA. Mean age for all fish was analysed using ANOVA after checking assumptions as described above.

2.3 Results

2.3.1 Flat River

Concentrations of several metals were higher in the settling ponds compared to river concentrations and increased in the water column downstream of the Tungsten Mine (Table 2.1). Concentrations of iron in Flat River water were 143% higher in near-field compared to reference, remained elevated at the far-field site, and at both exposure sites were above the CCME Water Quality Guideline for the Protection of Aquatic Life (300 µg/L; Table 2.1). Iron was present in extremely high concentrations in both Pond 3 and

Pond 4. Copper showed an increasing trend from reference site to the far-field site but remained below CCME water quality guidelines of 2-4 µg/L. Copper was elevated in water collected from both Pond 3 and Pond 4. Aluminum and tungsten also demonstrated an increasing trend from the reference site to the far-field site, with aluminum above CCME guidelines at all sites. Aluminum and tungsten in Ponds 3 and 4 were orders of magnitude higher in concentration compared to sampling sites in the Flat River. There were little differences in cadmium, mercury, and selenium concentrations among sites, although both mercury and cadmium were elevated above river concentrations in Pond 3 and 4. Selenium was elevated above river concentrations in Pond 3 but not Pond 4. Zinc, chromium, and lead decreased at the near-field site and increased above reference concentrations at the far-field site. Concentrations in Pond 3 and 4 were not elevated above concentrations in the far-field site for chromium and lead but zinc concentrations in the ponds were well above river concentrations. Manganese and uranium concentrations peaked at the near-field site and were elevated above reference site concentrations at the far-field site. Nickel demonstrated a decreasing trend from the reference site to the far-field site.

Consistent with the increase in total iron in water from reference relative to both near-field and far-field sites, iron in sediment showed an increase of 155% from reference to near-field and remained elevated above reference concentrations at the far-field site (Table 2.1). Copper in sediment increased 2900% from reference to both near-field and far-field sites and was above CCME Interim Sediment Quality guidelines at both exposure sites. Tungsten in sediment increased 250% from reference to near-field and remained high at the far-field site. Tungsten was also present in high concentrations in sediments of Pond 3 and Pond 4. Selenium in sediment increased at the near-field and far-field sites and concentrations were higher than those observed in Ponds 3 and 4. Uranium in sediment peaked at the near-field site and decreased again at the far-field site but concentrations were still above those observed at the reference site. All other sediment metals decreased from the reference site to the exposure sites.

General chemistry results for water column samples showed that nitrate and conductivity were elevated in Pond 3 and Pond 4 as well as slightly elevated at the near-field and far-field sites (Table 2.2). Sediment composition samples collected at the three sampling sites along the Flat River showed that the amount of silt at exposure sites was higher than at reference sites but these differences were not statistically significant due to high variability in sediment composition within sites (mean (sd) percent silt at reference, near-field, and far-field sites was 6.7 (8.1), 19.2 (10.0), and 17.7 (18.6), respectively; one-way ANOVA $p = 0.30$).

Algal abundance in the Flat River, measured as chlorophyll *a*, increased significantly ($p=0.002$) from the reference site to the far-field site (Figure 2.3). Mean chlorophyll *a* at the reference, near-field, and far-field sites were 0.46 $\mu g/cm^2$, 1.72 $\mu g/cm^2$, and 4.58 $\mu g/cm^2$, respectively. Algal richness showed a decrease at the near-field site and showed recovery at the far-field site but did not return to reference. Diversity followed the trend of algal richness with a decrease at the near-field site and subsequent increase at the far-field site. There was a substantial decrease of *Achnanthes minutissima* at the near-field site (data not shown).

Algal communities in the Flat River showed distinct differences in taxonomic composition at the near-field site compared to the reference and far-field site (Figure 2.4).

At the near-field site, chlorophytes were not detected, diatom abundance decreased, and cyanophyte abundance increased relative to the reference site. At the far-field site, chlorophytes were detected in the algal community and diatoms were present in a higher abundance than at the reference site. In contrast, cyanophytes decreased below that observed at the reference and near-field sites.

The near-field site on the Flat River had a significantly higher benthic invertebrate density compared to the reference and far-field sites ($p=0.017$, Figure 2.5). There was a statistically significant difference in the Bray Curtis index between the reference and near-field sites as well as between the reference and far-field sites ($p<0.001$). There was no significant difference in richness, and diversity of benthic macroinvertebrates between the reference and two exposure sites. The proportion of Diptera substantially increased at the near-field site (Figure 2.6) with significant Chironomidae proliferation. Diptera remained elevated above reference at the far-field site. Mayflies decreased in density at far-field site. Caddisflies demonstrated an increasing trend from the reference site to the far-field site whereas stoneflies showed a significant decrease at the exposure sites compared to the reference site, with the lowest density of stoneflies at the far-field site.

Table 2.1. Total metal concentrations in Flat River water (µg/L) and sediment (µg/g) for reference, near-field, and far-field sites, effluent in Pond 3 and 4 (n=1 sample per site), and associated Canadian Council of Minister of the Environment (CCME) guidelines[1,2]

Parameter	Detection limit		Upstream		Near-field		Far-field		Pond 3		Pond 4		CCME guidelines	
	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment
Aluminium	0.60	20	35	15000	42	10900	53	11700	4500	19200	6580	11000	5-10	NA
Arsenic	0.20	0.2	0.4	37.3	0.4	23.6	1.0	35.3	14.90	8.4	2.60	21.6	5	5.9
Cadmium	0.10	0.01	0.1	0.65	0.1	0.57	0.1	0.78	5.30	1.84	0.30	0.39	0.017	0.6
Chromium	0.30	0.5	0.6	27.3	0.3	16.4	15.6	17.5	19.60	21	11.00	16.2	9.9	37
Copper	0.30	1	1.0	25	1.1	750	2.20	710	13400.00	1280	183.00	339	2-4	35.7
Iron	50	100	143	42300	347	108000	326	88900	86100	69800	26200	73400	300	NA
Lead	0.10	0.1	2.3	21.7	0.1	11.8	67.60	12	39.00	13	6.90	14.4	1-7	35
Manganese	0.10	10	8.5	556	24.9	463	23.40	441	362.00	2180	629.00	326	NA	NA
Mercury	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.01	3.46	0.02	4.07	0.01	0.026	0.17
Nickel	0.10	0.5	3.6	52.3	2.9	29.5	2.50	32.7	22.10	20.4	8.50	28.5	25-150	NA
Selenium	1.00	0.3	1.0	0.5	1.0	3.1	1.0	2.7	2.00	1.5	0.80	1.7	NA	NA
Tungsten	0.50	0.5	0.8	1.3	2.80	323	3.60	243	7210.00	326	3760.00	238	1	NA
Uranium	0.10	0.5	0.76	1.7	0.98	1.7	0.90	2.4	3.10	1.3	3.28	2.0	NA	NA
Zinc	0.10	1.0	4.51	151	3.56	111	5.00	134	676.00	251	31.60	106	30	123

[1] Water quality guidelines are provided from the CCME for the protection of freshwater aquatic life (CCME 2003).
[2] Sediment quality guidelines are provided from the CCME Interim Sediment Quality Guidelines (CCME 2003).

Table 2.2. General water chemistry results for the Flat River and Prairie Creek collected at reference, near-field, and far-field sites.

Parameter	Detection limit	Flat River upstream	Flat River near-field	Flat River far-field	Pond 3	Pond 4	Prairie Creek upstream	Prairie Creek near-field	Prairie Creek far-field	Catchment pond
Nitrate (mg/L)	0.01	0.02	0.03	0.03	2.27	0.32	0.09	0.10	0.08	-
Total phosphorous (µg/L)	0.01	<0.01	<0.01	<0.01	0.25	0.15	<0.01	<0.01	<0.01	0.2
Conductivity (µS/cm)	0.4	224	253	235	1090	983	452	459	460	826
pH		7.99	7.98	8	8.02	8.21	8.44	8.46	8.42	8.3
Total suspended solids (mg/L)	3	4	6	<3	911	973	<3	<3	<3	<3

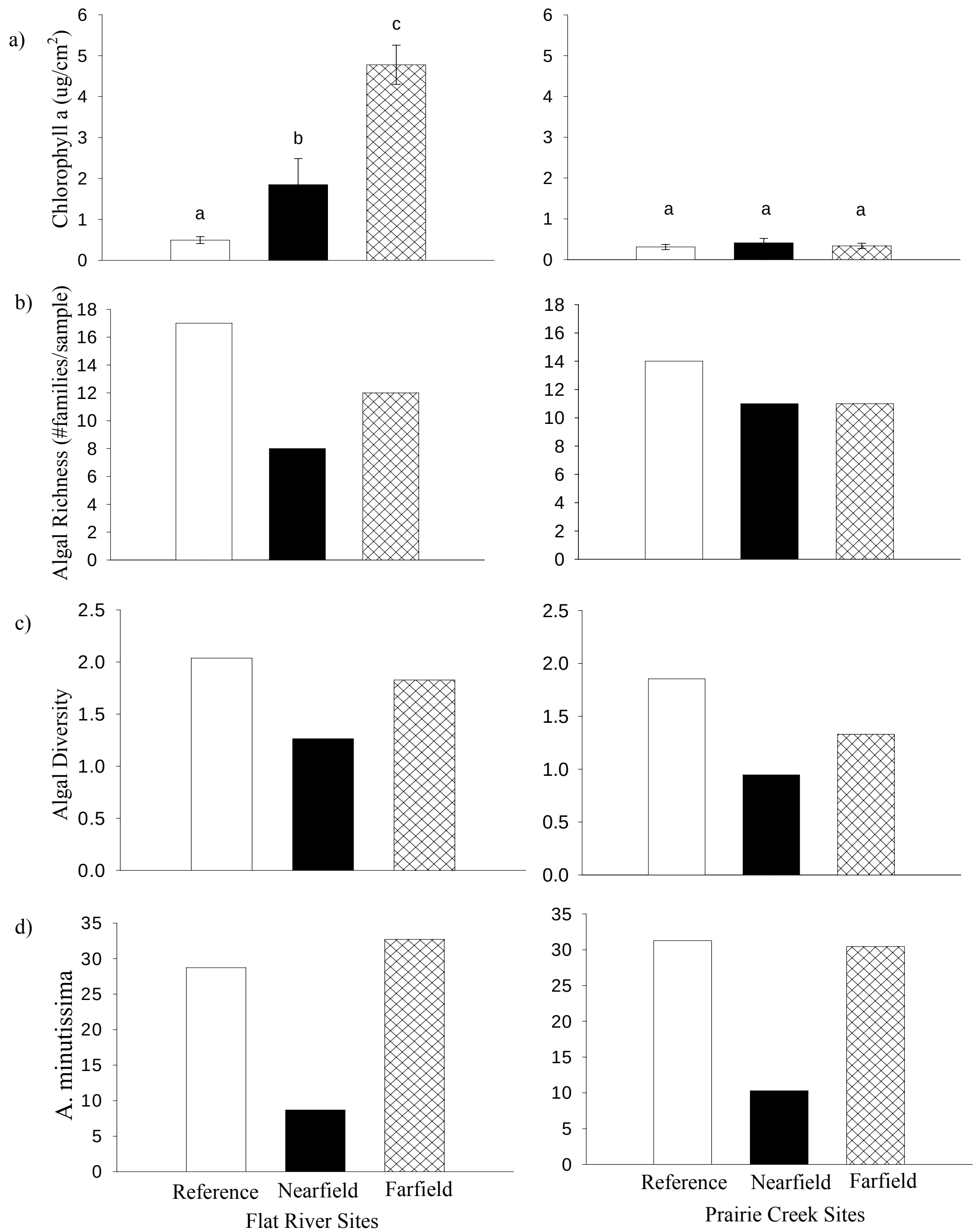

Figure 2.3. Algal endpoints collected in Flat River and Prairie Creek at reference, near-field and far-field sites: (a) mean (±SE) algal biomass reported as chlorophyll a ($\mu g/cm^2$; ANOVA results for Flat River: n=3, F=22.355, p= 0.002 and for Prairie Creek: n=3, F=0.400. p=0.687), (b) algal richness reported as number of families per cm^2 (n=1), (c) algal diversity reported as a diversity index (n=1), and d) % *A. minutissima*. Bars with like letters were not significantly different.

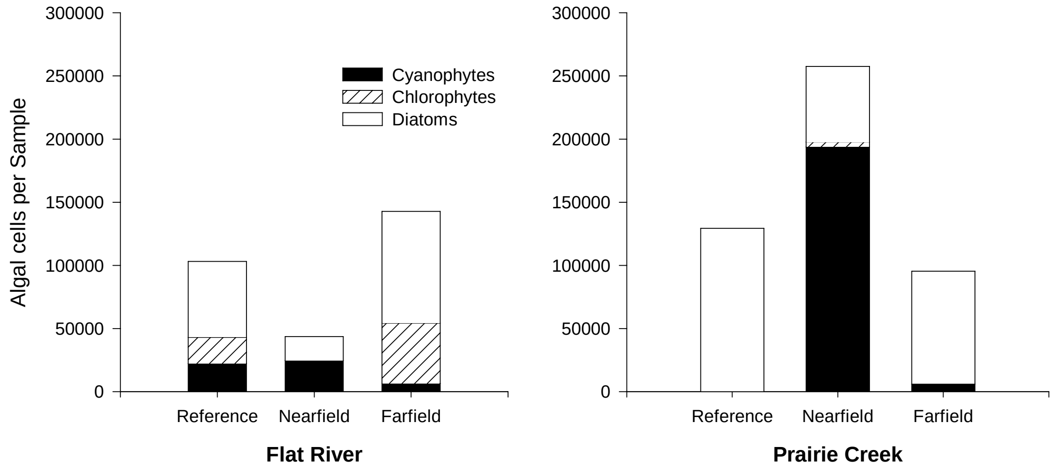

Figure 2.4. Algal taxonomy for the Flat River and Prairie Creek at reference, near-field and far-field sites. Algal community was compared by analyzing algal cells per sample.

The condition of slimy sculpin in the Flat River, as defined as length against weight, showed a significant increase ($p=0.002$) at the far-field site in male fish compared to the reference and near-field site (Table 2.3). Condition was also significantly higher ($p=0.006$) at the far-field site for females when compared to the near-field site but was not significantly different from the reference site. Size-at-age analyses indicated an increase in growth at the far-field site compared to the near-field and reference sites in female fish ($p<0.001$). Male fish, however, showed a decrease in size-at-age at the near-field site and an increase at the far-field site ($p<0.001$). Female fish were older at the far-field site compared to the reference and near-field sites. Age of male fish did not vary significantly among sites. Gonad weights and liver weights for male and female fish were not significantly different between the three sites. Egg size significantly increased from the reference site to the near-field site ($p=0.001$) but no significant difference was observed in fecundity between the sites.

Table 2.3. Mean (± standard deviation) for slimy sculpin metrics and the results of ANOVA and ANCOVA analysis for each river (sites with the same letters were statistically equivalent). Mean age was analyzed using ANOVA. Weight (represented by Condition Factor) was analyzed using ANCOVA with length as a covariate. Gonad size (represented by Gonadosomatic Index) and liver size (represented by Liver Somatic Index) were analyzed with ANCOVA using body weight as a covariate. Slopes among sites were not significantly different (i.e., $p > 0.05$) in any of the ANCOVA analyses (Flat River reference: males n=19, females n=26, Flat River near-field: males n= 30, females n= 22, Flat River far-field: males n=23, females n= 22, Prairie Creek reference: males n=8, females n=17, Prairie Creek near-field: males n= 18, females n=14, Prairie Creek far-field: males n=21, females n=10).

Sex	Parameter	Flat River upstream	Flat River near-field	Flat River far-field	p-value	Prairie Creek upstream	Prairie Creek near-field	Prairie Creek far-field	p-value
Male									
	Mean age	3.34±0.138 A	3.86±0.151 A	3.85±0.223 A	0.089	3.62±0.350 A	4.32±0.261 A	3.91±0.322 A	0.343
	Condition Factor	0.99±0.021 A	0.89±0.020 A	1.02±0.024 B	0.002	0.80±0.026 A	0.91±0.020 B	0.90±0.021 B	0.008
	Liver Somatic Index	1.35±0.093 A	1.35±0.097 A	1.54±0.183 A	0.897	1.35±0.111 A	1.21±0.093 A	1.76±0.168 A	0.062
	Gonadosomatic Index	1.66±0.152 A	1.63±0.167 A	1.69±0.194 A	0.283	1.14±0.271 A	0.88±0.162 A	1.35±0.179 A	0.327
Female									
	Mean age	3.19±0.206 A	3.68±0.224 A	4.73±0.315 B	<0.001	3.50±0.174 A	3.73±0.231 A	4.80±0.539 B	0.023
	Condition Factor	0.91±0.023 A	0.86±0.027 A	0.92±0.013 B	0.006	0.77±0.024 A	0.90±0.045 B	0.90±0.043 B	0.001
	Liver Somatic Index	1.80±0.143 A	1.73±0.129 A	2.29±0.110 A	0.296	1.41±0.108 A	1.73±0.240 A	1.66±0.188 A	0.160
	Gonadosomatic Index	1.73±0.242 A	2.38±0.290 A	3.42±0.160 A	0.172	1.25±0.183 A	0.94±0.266 A	1.23±0.285 A	0.158

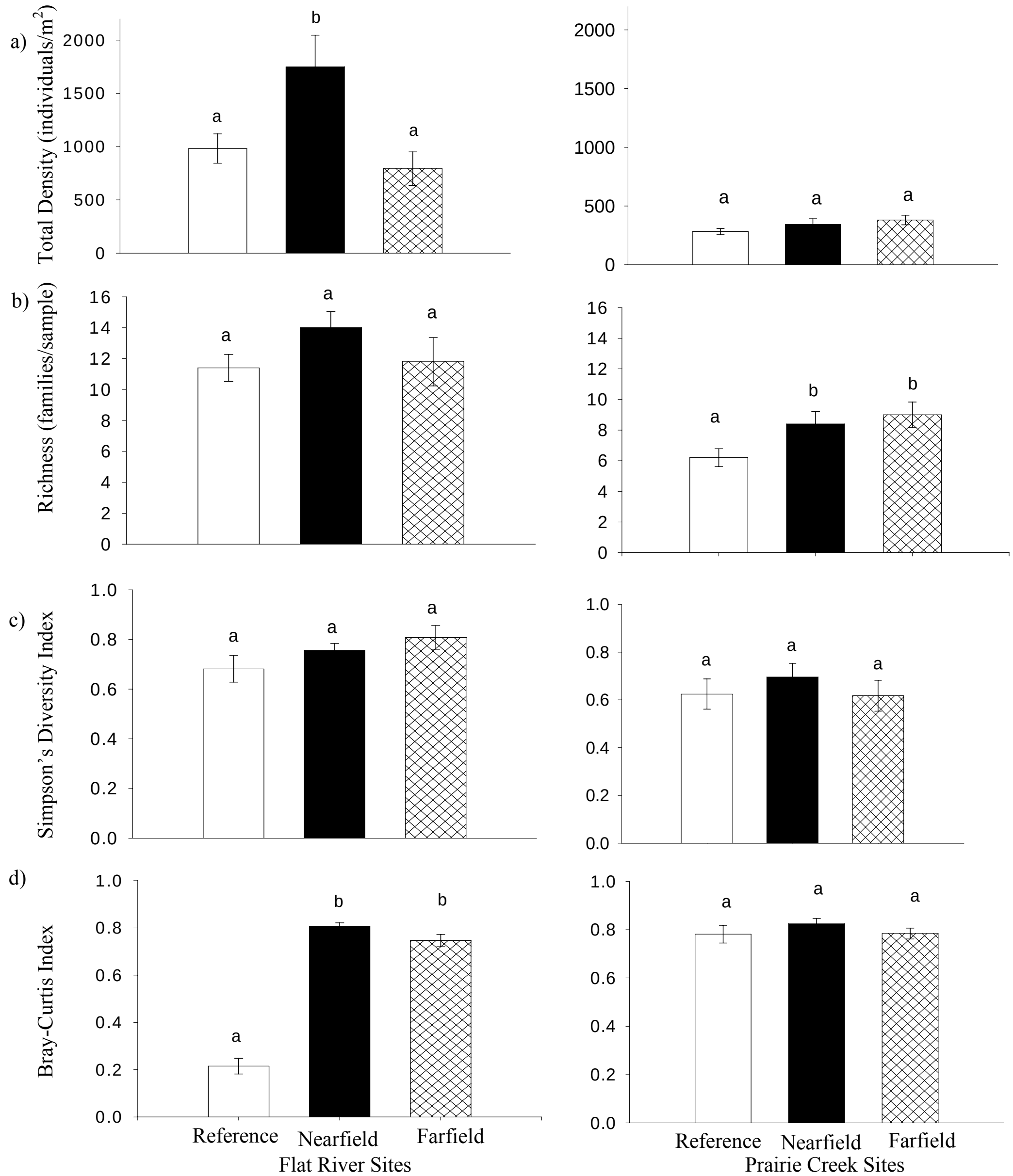

Figure 2.5. Benthic invertebrate endpoints collected in the Flat River and Prairie Creek at reference, near-field and far-field sites: a) mean (±SE) invertebrate density reported as individuals/m^2 (ANOVA results for Flat River: n=5, F=5.851, p=0.017 and for Prairie Creek: n=5, F=1.572, p=0.247), b) mean (±SE) invertebrate richness reported as number of families per sample (ANOVA results for Flat River: n=5, F=1.367, p=0.292 and for Prairie Creek: n=5, F=3.835, p=0.052), c) mean (±SE) invertebrate diversity reported as Simpson's Diversity Index (ANOVA results for Flat River: n=5, F=2.065, p=0.170 and for Prairie Creek: n=5, F=2.065, p=0.170, and d) mean (±SE) community dissimilarity reported as Bray-Curtis Index (ANOVA results for Flat River: n=5, F=163.34, p=0.000 and for Prairie Creek: n=5, F=2.351, p=0.138). Bars with like letters were not significantly different.

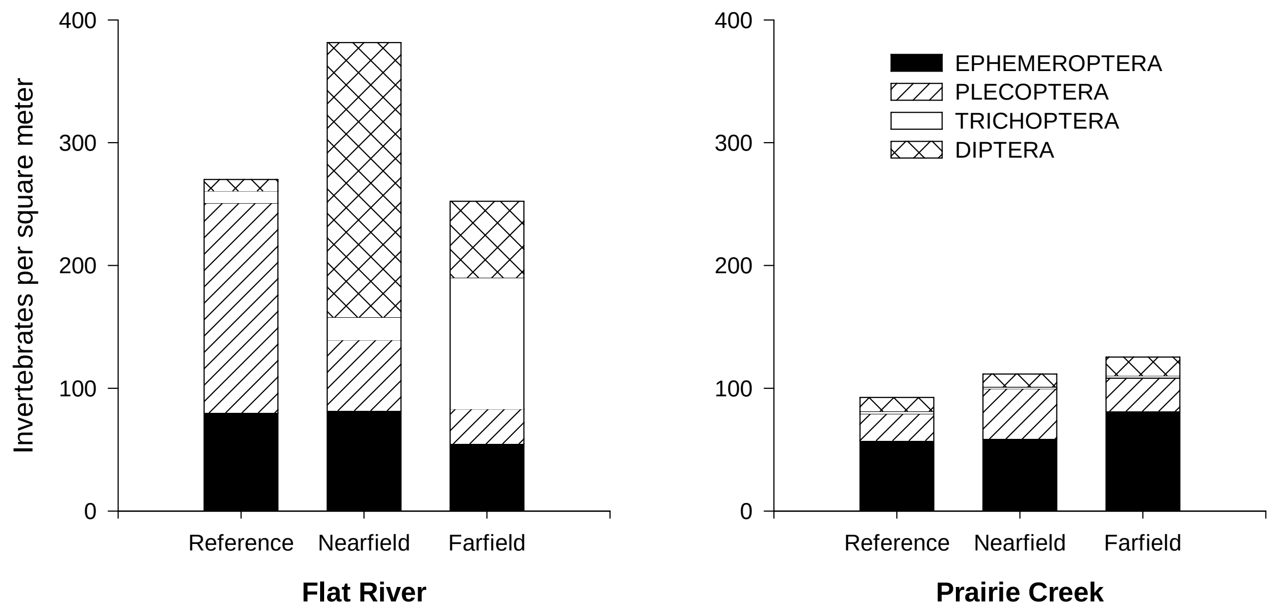

Figure 2.6. Benthic taxonomy for Flat River and Prairie Creek at reference, near-field and far-field sites. Ephemeroptera, Plecoptera, Trichoptera, and Diptera were compared using number of invertebrates per square meter.

Concentrations of metals in liver tissue of Flat River fish indicated a significant accumulation of copper and iron in fish at the near-field site (Table 2.4) when compared to fish from the reference and far-field sites. Copper concentrations in the fish livers at the reference site were 61% lower than those collected at the near-field site ($p=0.002$). Iron in liver tissue was 275% higher in fish from the near-field site compared to the reference site ($p=0.001$). Both copper and iron in liver tissue decreased at the far-field site but remained above reference site concentrations. Selenium in liver tissue was lower at the near-field and far-field sites ($p=0.006$). Cadmium concentrations in liver tissue showed a decreasing trend from the reference site to the far-field site ($p=0.001$). Arsenic concentrations in liver tissue decreased at the near-field site compared to the reference site ($p=0.022$) but increased again at the far-field site above reference concentrations.

Zinc ($p=0.004$) and mercury ($p=0.035$) showed a strong decreasing trend in concentration from the reference site to the far-field site. Arsenic ($p=0.010$) and nickel ($p=0.031$) concentrations in fish muscle decreased at the near-field site compared to the reference site but increased again at the far-field site. Neither copper ($p=0.210$) or iron ($p=0.147$) increased significantly in muscle tissue collected at the near-field site when compared to the reference site despite accumulation of these two metals in liver tissue.

Table 2.4. Tissue metal concentrations for the Flat River and Prairie Creek and ANOVA results (Means with the same letters were not significantly different). An ANOVA was performed for each river and each tissue type (n=5 for Flat River flesh metals, n=5 for Prairie Creek flesh metals, and n=6 for Flat River liver metal concentrations). NM=Not Measured

Parameter	**Flat River upstream**		**Flat River near-field**		**Flat River far-field**		**p-value**		**Prairie Creek upstream**	**Prairie Creek near-field**	**Prairie Creek far-field**	**p-value**
	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Muscle	Muscle	Muscle
Aluminium	4.40±2.40 A	7.54±0.759 A	2.38±0.67 A	11.83±2.60 A	3.82±0.487 A	12.84±3.679 A	0.572	0.369	1.790±0.530 A	2.040±0.326 A	0.650±0.272 B	0.024
Arsenic	0.20±0.032 A	0.13±0.007 A	0.17±0.015 A	0.103±0.008 A	0.280±0.018 B	0.166±0.022 B	0.010	0.022	0.172±0.013 A	0.142±0.017 A	0.066±0.009 B	0.002
Cadmium	0.008±0.002 A	1.042±0.101 A	0.005±0.000 A	0.850±0.102 A	0.004±0.001 A	0.398±0.065 B	0.325	0.001	0.021±0.002 A	0.028±0.003 A	0.016±0.002 B	0.044
Chromium	NM	0.050±0.000 A	NM	0.175±0.072 A	NM	0.710±0.576 A	NM	0.226	NM	NM	NM	NM
Copper	0.578±0.081 A	3.880±0.174 A	0.553±0.210 A	6.233±0.508 B	0.488±0.094 A	4.560±0.277 C	0.210	0.002	0.335±0.014 A	0.340±0.020 A	0.254±0.023 B	0.032
Iron	11.58±3.873 A	60.2±9.162 A	16.33±2.667 A	225.5±22.381 B	19.80±1.960 A	161.8±31.181 C	0.147	0.001	9.525±0.881 A	11.56±0.717 A	6.82±0.635 B	0.018
Lead	0.017±0.004 A	0.021±0.007 A	0.012±0.003 A	0.043±0.019 A	0.015±0.007 A	0.022±0.014 A	0.495	0.488	0.130±0.025 A	0.172±0.047 A	0.077±0.034 A	0.231
Manganese	0.728±0.095 A	1.960±0.129 A	0.610±0.031 A	1.983±0.140 A	0.604±0.064 A	2.180±0.206 A	0.437	0.591	0.335±0.018 A	0.384±0.032 A	0.312±0.024 A	0.160
Mercury	0.112±0.033 A	0.001±0.001 A	0.048±0.032 A	0.003±0.002 A	0.004±0.001 B	0.001±0.001 A	0.035	0.181	0.028±0.018 A	0.066±0.024 A	0.078±0.021 A	0.070
Nickel	0.166±0.044 A	0.288±0.032 A	0.063±0.003 B	0.242±0.048 A	0.128±0.073 A	0.710±0.500 A	0.031	0.436	0.095±0.003 A	0.102±0.030 A	0.024±0.007 B	0.011
Selenium	0.820±0.058 A	6.80±0.636 A	0.850±0.034 A	4.12±0.566 B	0.840±0.068 A	4.08±0.403 B	0.764	0.006	1.250±0.065 A	1.060±0.068 A	1.160±0.087 A	0.465
Tungsten	6.660±4.836 A	100.0±62.780 A	4.600±1.455 A	61.667±9.358 A	6.560±3.323 A	148.40±73.701 A	0.632	0.514	NM	NM	NM	NM
Uranium	0.001±0.001 A	0.004±0.002 A	0.001±0.001 A	0.003±0.000 A	0.001±0.001 A	0.004±0.002 A	0.327	0.595	NM	NM	NM	NM
Zinc	17.4±1.166 A	38.60±3.847 A	13.0±0.365 B	40.67±1.944 A	11.6±0.678 B	33.80±1.934 A	0.004	0.062	43.5±3.279 A	64.60±4.273 A	49.6±8.244 A	0.078

2.3.2 Prairie Creek

Total metals measured in the water column that occurred in higher concentrations in the catchment pond than in Prairie Creek included cadmium, lead, manganese, mercury, and zinc (Table 2.5). Detection limits for metals measured in the catchment pond were higher than detection limits for water samples collected from reference, near-field, and far-field sites as a result of the presence of salts (dissolved solids) above 0.2% in catchment pond water (Warnken, 1999); samples with high dissolved solids are routinely diluted in the lab due to interference with the plasma spectrometer's ability to determine trace metal concentrations. Thus, several metals were measured as non-detectable in the catchment pond yet detection limits were generally higher than concentrations measured at any riverine sites. The same detection limits were used for samples collected at reference, near-field and far-field sites, facilitating comparisons across sites.

Water quality results indicated that total zinc increased from 2.4 µg/L to 12.3 µg/L (400% increase) from the reference site to the near-field site but remained below CCME aquatic quality guidelines for the protection of aquatic life (30 µg/L). Zinc concentrations decreased to 6.8 µg/L at the far-field site. Total aluminum concentrations also increased at the near-field site (12.6 µg/L) compared to the reference site (7.9 µg/L) but decreased at the far-field site (7.3 µg/L). All total aluminum values fell within the range identified in the CCME guidelines. A slight increase in total copper was seen at the near-field site but all copper concentrations were well below aquatic life guidelines (2-4 µg/L). There was little difference in cyanide, nutrient concentrations, or general water chemistry among the sites on Prairie Creek (Table 2.2).

Table 2.5. Metal concentrations (n=1) in Prairie Creek water (µg/L) and sediment (µg/g) for reference, near-field, and far-field sites, effluent in Pond 3 and Pond 4, and associated Canadian Council of Minster of the Environment (CCME) guidelines[1]

Parameter	**Detection limit**		**Upstream**		**Near-field**		**Far-field**		**Catchment Pond**	**CCME guidelines**	
	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment	Water	Water	Sediment
Aluminum	0.60	20	7.9	4820	12.6	3220	7.3	2620	<20	5-10	NA
Arsenic	0.20	0.2	0.2	6.4	0.3	7.6	0.3	6.4	<50	5	5.9
Cadmium	0.05	0.01	0.05	1.24	0.06	1.42	0.05	0.88	3	0.017	0.6
Chromium	0.1	0.5	0.1	9.9	0.1	7.7	0.1	7.8	<5	9.9	37
Copper	0.3	1	0.3	12	0.8	9	0.5	8	<5	2-4	35.7
Iron	50	100	89	12300	89	8900	91	7970	15	300	NA
Lead	0.10	0.1	0.1	25.1	0.2	22.8	0.1	15.2	30	1-7	35
Manganese	0.10	10	0.2	229	0.4	205	0.3	179	234	NA	NA
Mercury	0.02	0.01	0.02	0.06	0.02	0.03	0.02	0.04	0.07	0.026	0.17
Nickel	0.10	0.5	1.2	24.7	1.3	20.3	1.2	20.0	<8	25-150	NA
Selenium	1.00	0.3	1.3	0.6	1.2	0.6	1.2	0.5	<30	1	NA
Uranium	0.10	0.5	3.98	1.7	4.11	1.7	4.30	2.4	NM	NA	NA
Zinc	0.10	1.0	2.4	182	12.3	179	6.8	102	534	30	123

[1] Water quality guidelines are provided from the CCME for the protection of freshwater aquatic life (CCME 2003).
[2] Sediment quality guidelines are provided from the CCME Interim Sediment Quality Guidelines (CCME 2003).

Arsenic in sediment was the only metal that increased in concentration at the near-field site relative to the reference and far-field sites although values were slightly above the CCME interim sediment quality guideline at all locations (Table 2.5). There were no differences in concentrations of cadmium and zinc in sediment collected at the three sites. All other metals demonstrated a decreasing trend from the reference site to the far-field site.

Algal richness decreased at the exposures sites compared to the reference site and diversity also showed a decrease at both exposure sites (Figure 2.3). Abundance of *A. mimutissima* decreased at the near-field site. A significant difference in chlorophyll *a* was not observed between the reference, near-field, and far-field sites. Only diatom species were detected at the reference site (Figure2.4). Diatom densities decreased at the near-field site and increased again in the far-field site with densities slightly below those measured at the reference site. Cyanophytes became dominant at the near-field site and subsequently decreased again at the far-field site. Limited densities of chlorophytes were present at the near-field site and were not detected at the far-field site.

Benthic macroinvertebrate richness increased significantly ($p=0.050$) at both exposure sites compared to the reference site in Prairie Creek (Figure 2.5). Benthic invertebrate density and diversity were not significantly different between reference, near-field, and far-field sites although there seemed to be a slight increasing trend from the reference site to the far-field site. The Bray-Curtis index did not indicate significant dissimilarity between sites. There was an increasing trend in the abundance of mayflies and true flies from the reference to the far-field site (Figure 2.6).

Slimy sculpin condition, as defined as length against weight, showed a significant increase at both the near-field and far-field sites in male ($p=0.008$) and female ($p=0.001$) fish compared to the reference site (Table 2.3). Size-at-age analyses on male fish indicated that fish had higher growth rates at the far-field site compared with the near-field and reference site ($p=0.043$) but significant differences in growth were not seen in female fish. However, mean age analyses in females indicated fish at the far-field site were older ($p=0.023$) than fish at the reference and near-field but no differences were detected in mean age of male fish between the three sites. Gonad weights and liver weights for male and female fish were not significantly different between the three sites. Fecundity did not differ between the three sites but eggs were significantly larger at the near-field site ($p=0.041$).

Metal concentrations in slimy sculpin muscle from Prairie Creek were not significantly different between the reference site and the near-field site. At the far-field site however, aluminum ($p=0.024$), arsenic ($p=0.002$), cadmium ($p=0.044$), copper ($p=0.032$), iron ($p=0.018$), and nickel ($p=0.011$) were significantly lower when compared to the reference and near-field exposure sites. Concentrations of lead, manganese mercury, selenium and zinc did not differ between sites.

A high number of parasites were detected in Prairie Creek (Table 2.6). Parasites were identified as *Ligula intestinalis* by Dr. Terry Dick of the University of Manitoba. Parasite body burdens and percent occurrence did not differ between the three sites on Prairie Creek (Table 2.6).

Table 2.6. Parasite loads for fish in Prairie Creek (Percent occurrence is calculated by comparing the number of parasite-infected fish with the total number of fish captured at the reference, near-field, and far-field sites).

Site	% Occurrence	% Body burden
Reference	25.5	10.4±1.7
Near-field	26.4	14.7±1.6
Far-field	18.8	12.7±3.5

2.4 Discussion

Differences in the water and sediment chemistry, benthos, and sculpin endpoints in both the Flat River and Prairie Creek downstream of mining activity were generally indicative of mild eutrophication, although there was also evidence of contaminant effects. Differences indicative of eutrophication that were observed downstream of the mine on the Flat River were increased algal abundance and increased sculpin egg size. Elevated concentrations of iron, copper, and tungsten in river water and sediments that were above national guidelines, differences in algal and benthic macroinvertebrate community composition, decreased size-at-age in male sculpin, and elevated concentrations of copper and iron in liver tissue of sculpin were indicative of effects of mining activity on the Flat River. Similarly, evidence of mild eutrophication downstream of mining activity in Prairie Creek included increased benthic macroinvertebrate taxa richness and improved condition of sculpin of both sexes along with increased egg size at the near-field site. In contrast, there were increases in water column zinc and aluminum and in sediment arsenic, decreased algal diversity, and differences in taxonomic composition between the reference and near-field sites on Prairie Creek.

Water and sediment chemistry are inherently variable and the interactions between chemical constituents with one another and biological processes are not well understood. Lack of conclusive patterns in water and sediment quality results for these rivers was expected due to the high degree of mineralization, landscape features such as hot springs, and highly variable flow rates (Halliwell and Catto 2003). In addition, higher sampling frequency is typically needed to get an accurate estimate of water and sediment chemistry (Markert et al. 2003). Although water and sediment quality serve as useful indicators of direct effluent exposure, the ability to predict biological effects may be limited in these unique areas. Thus, monitoring studies which focus on biological responses supported by water and sediment quality for exposure assessment are recommended.

The oligotrophic state of the rivers makes them sensitive to nutrient enrichment and other stressors (Vis et al. 1998, Kiffney and Clements 1996). Proposed guidelines for the protection of oligotrophic rivers in Northern Alberta range from 1.9-4.5 $\mu g/cm^2$ chlorophyll *a* (Chambers et al. 2006). Concentrations of chlorophyll *a* in Prairie Creek were consistently below this range at all sites whereas mean concentrations of chlorophyll *a* in the Flat River were below this range at the reference site, within this range at the near-field site, and above this range at the far-field site. The trend of increasing algal abundance from the reference to the far-field site in the Flat River could represent a mine-related enrichment or natural nutrient inputs along the gradient. Typically, enrichment due to a natural river continuum would occur over a far greater spatial distance among sites than what was sampled here. The natural range of algal chlorophyll *a* abundance in these rivers (i.e., 0.3-0.5 μg chl a/cm^2) is lower than the range reported for rivers in northern Alberta (Chambers et al. 2006).

Assessment of algal community composition also suggests mine-related enrichment in the Flat River and Prairie Creek but does not preclude possible effects of metal exposure. Decreased abundance of diatoms in general and of *A. minutissima* in particular as well as reduced overall richness and diversity occurred at the near-field site in both rivers. Diatoms can be sensitive to metal pollution (Gold et al. 2003, Genter et al. 2000, Dixit et al. 1992) but also respond to changes in nutrient status in water bodies

(Canter-Lund and Lund 2005, Markert et al. 2003). *A. minutissima* has been shown to act as an indicator of metal exposure (Gold et al. 2003, Ivorra et al. 2002). In addition, increased abundance of cyanophytes at the near-field site of Prairie Creek can occur naturally but may be associated with enrichment (Canter-Lund and Lund 1995). The results of algal biomass and taxonomy from both rivers suggest that algae are a useful monitoring tool for detecting differences in these sensitive environments. In addition, previous research has indicated that algae accumulate metals and can therefore be used to determine metal bioavailability and accumulation in aquatic ecosystems (Meylan et al. 2003, Ivorra et al. 2002, Gurrieri 1998).

Differences in the community composition of benthic macroinvertebrates at exposure sites in the Flat River are likely a result of tailings that were historically deposited directly into the river whereas increased taxa richness in Prairie Creek is consistent with nutrient enrichment. Although the practice of direct tailings discharge to the Flat River was halted by 1970, tailings are still visible in the Flat River and alter substrate composition in the area (EBA 2002). A higher proportion of silt is present at both exposure sites which would provide a more desirable benthic habitat for dipterans such as chironomids (Courtney and Clements 2002). The substantial decrease in stoneflies at the near-field and far-field sites in the Flat River may represent a sensitivity to mine effluent or differences in sediment composition. Plecoptera, or stoneflies, have been shown to be pollution intolerant in many previous studies (Hodkinson and Jackson 2005).

Unlike in the Flat River, benthic macroinvertebrate richness in Prairie Creek increased at exposure sites but community composition was comparable to the composition at reference sites. The substrate composition among sites in Prairie Creek does not differ as seen in the Flat River; no deposition of tailings has occurred during the 30 years of exploration or care and maintenance phases at the mine. In highly oligotrophic ecosystems, very little nutrient input is required to produce a response (Bowman et al. 2005, Hill et al. 2000, Vis et al. 1998). It is likely that small increases in nutrient or mineral inputs have resulted in increased taxa richness of benthic macroinvertebrates at the exposure sites in Prairie Creek. Benthic macroinvertebrate densities are lower in Prairie Creek than in the Flat River and both rivers have lower densities than systems further south (Benke and Cushing 2005).

Fish community assessments confirmed that slimy sculpin were the only potential sentinel species that could be used for monitoring in the South Nahanni watershed. Slimy sculpin are found throughout watersheds in the Northwest Territories (Burr and Page 1998) and are present downstream of most, if not all, major development in the Northwest Territories and Nunavut (DDMI 2006, Golder Associates 2006, Jacques Whitford 2006, Azimuth Consulting 2005, DDMI 2005, Golder Associates 2005a, Golder Associates 2005b). They are a small species of fish with a short life span and limited mobility due to the absence of a swim bladder (Gray et al. 2004), which allows comparison of effects and exposure to contaminants (Munkittrick et al. 2000). Sculpin have been shown to be a good sentinel species in several monitoring studies (Galloway et al. 2005, Gray and Munkittrick 2005, Tetreault et al. 2003, Gibbons et al. 1998). Limited work has been done on slimy sculpin in northern ecosystems, and their applicability to effects monitoring (Lumb et al. 2006, DDMI 2005).

Analysis of liver tissue but not the flesh of sculpin tracked exposure to metals in the environment. An increase in both copper and iron in the liver tissue of slimy sculpin corresponded to high copper and iron in the effluent of the exfiltration ponds as well as elevated copper in Flat River sediments and iron in river water. Tungsten, which increased in both water and sediment at the exposure sites in the Flat River, significantly decreased in liver tissue suggesting that tungsten is not highly accumulated in slimy sculpin. Because of the considerably smaller fish size at Prairie Creek, only results for flesh metals could be obtained. An exposure pattern was much more difficult to detect in fish collected from Prairie Creek, presumably a consequence of using concentrations of metals in flesh rather than liver. However, the mine on Prairie Creek does not currently discharge a mine effluent per se, but a mine-water portal discharge which currently is treated in their polishing pond and catchment pond. Also, there has been no historical discharge of tailings to the Prairie Creek system.

Although there was some evidence of metal accumulation in fish tissue, increased fish condition at both exposure sites on Prairie Creek and at the far-field site on the Flat River provides further evidence of an enrichment effect. In both rivers, the absence of differences in liver and gonad weights and increased condition suggests that slimy sculpin are not currently adversely affected by mine discharges. The consistent presence of parasites at the reference and exposure sites on Prairie Creek indicated that parasitic infection was not a mine-influenced effect but rather due to other ecological processes.

2.5 Conclusions

Biological differences downstream of mines on the Flat River and Prairie Creek were consistent with mild enrichment of these highly oligotrophic systems. The most responsive endpoints were different in each river reinforcing the need for considering site-specific factors in the design of monitoring programs. Responses to mining activity occurred in each trophic level but were most pronounced at lower trophic levels. Variable water quality and sediment quality results were best analyzed in conjunction with biological differences. Analysis of metal concentrations in benthic algal and/ or macroinvertebrate tissue is recommended for exposure assessment and causal association. The focus of further monitoring programs in sensitive pristine riverine areas exposed to mining activity should include a multi-trophic approach, including adequately replicated benthic algae and macroinvertebrate community samples combined with water and sediment quality and tissue metal burdens. Given the low abundance of fish species in these ecosystems, the effects of lethal monitoring programs on fish populations should be seriously considered. Although the biological effects of mining activities appear to be limited at present, evidence of environmental metal concentrations above national guidelines and associated metal accumulation in the liver tissue of sculpin suggest that there is potential for increased mining activity to affect biological communities in northern rivers. Further, evidence of mild eutrophication which could be exacerbated with the advent of climate change and associated factors (e.g., flow alterations, increased ice-free periods) suggests the need for on-going assessment. This research should serve as a baseline assessment for evaluation of future changes in sensitive tributaries of the South Nahanni River.

CHAPTER 3.0

EFFECTS OF UN-IONIZED AMMONIA ON HISTOLOGICAL, ENDOCRINE, AND WHOLE ORGANISM ENDPOINTS IN SLIMY SCULPIN (*COTTUS COGNATUS*)*

P Spencer, R Pollock, and M Dubé

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3.1 Introduction

Ammonia is a well-known toxicant in aquatic systems. Ammonia in aqueous environments exists in equilibrium between the un-ionized ammonia (NH_3) fraction and ammonium (NH_4^+) fraction (Eddy 2005). This equilibrium is governed by parameters of aqueous solutions such as ionic composition, temperature and pH (USEPA 1999). It is recognized that NH_3 is considered to be the more toxic fraction within aquatic environments as NH_3 is able to permeate tissues (Fairchild 2005). As pH and temperature rise, a higher percentage of NH_3 is present, therefore increasing toxicity. Although increasing temperature increases NH_3 and therefore toxicity, a change of 1 pH unit causes a 10-fold increase in amount of NH_3 (Eddy 2005). It is therefore important to assess NH_3 toxicity under environmentally relevant conditions.

Ammonia is one of the most common contaminants in Canada's north due to its associated uses in mining. High concentrations of ammonia in northern Canada result from the use of ANFO (Ammonium Nitrate Fuel Oil) blasting materials in underground and open pit mining operations, cyanide breakdown in metal mining effluent, and to a lesser degree the discharge of sewage from mining facilities (CCME 1999, USEPA 1999). Ammonia accumulates in effluents from high latitude mining operations due to lower natural breakdown rates associated with shorter ice-free periods and reduced ambient temperatures (CEPA 2001). Current federal Metal Mining Effluent Regulations in Canada do not regulate the discharge of ammonia, increasing the importance for developing threshold limits which are protective of northern species. Water licences with associated discharge limits are issued by territorial regulatory authorities such as the Mackenzie Valley Land and Water Board (MVLWB) but ammonia is not always a regulated parameter or limits are developed based on toxicological testing using southern species. Although ammonia concentrations in effluents discharged from mining operations in northern Canada vary significantly depending on mining and milling processes and effluent retention times, discharge concentrations range from 0.060 mg/L - 2.9 mg/L un-ionized ammonia (Mackenzie Valley Land and Water Board public registry, accessed May 23, 2008)

Many studies conducted with ammonia focus on southern species in more moderate climates. Northern species are considered to be especially sensitive to contaminants due to their high-latitude geographical location and extreme climate (Schindler and Smol 2006, Evans et al. 2005). Because ammonia toxicity varies with pH and temperature, setting discharge limits can be difficult (CEPA 2001, CCME 1999, USEPA 1999). Ammonia is a Priority Substance listed in the Canadian Environmental Protection Act and toxicological thresholds for sensitive species have been reported, however these species are not found in northern waters. Concentration-response experiments are necessary for the development of threshold values (Ankley and Villeneuve 2006, Rand 1995). In order to use threshold values effectively in the north, it is important that these values are based on concentration-response studies conducted using northern species and under test conditions (e.g., temperature and pH) relevant to northern environments.

Sentinel species are considered representatives or indicators of a larger population or community. They are often measured in field studies to monitor any changes in biological health when community structure is well known or previously characterized. They are also used in systems with low species abundance in order to reduce to the potential impact of monitoring programs on resident fish populations. Sentinel species can also be used in conjunction with laboratory exposures to obtain toxicological information on effects endpoints.

Slimy sculpin were identified in previous studies (Spencer et al. 2008) as a northern sentinel species, are found throughout watersheds in the Northwest Territories (Burr et al. 1998) and are present downstream of most, if not all, mining developments in the Northwest Territories and Nunavut (DDMI 2006, Golder Associates 2006, Jacques Whitford 2006, Azimuth Consulting 2005, DDMI 2005, Golder Associates 2005a, Golder Associates 2005b). They are a small fish species with limited mobility and small home ranges due to the absence of a swim bladder (Gray et al. 2004), which facilitates assessment of mine effluent effects in field monitoring programs. Effects assessments are typically more difficult with more mobile, migratory species (Munkittrick et al. 2000). While slimy sculpin have been used in mesocosm studies to measure effects of a mine effluent (Dubé et al. 2005), and in field studies with pulp and paper and sewage effluents (Galloway et al. 2005), agricultural discharges (Gray and Munkittrick 2005) and oil sands contaminants (Tetreault et al. 2003), they have not been used in laboratory studies to assess the toxicity of ammonia.

The main objective of this study was to determine if ammonia, an important contaminant discharged by mines in northern Canada, affects the health of slimy sculpin by assessing histological, endocrine, and whole organism endpoints. Canada's north is currently experiencing increased growth in mining developments and the potential for continued development is high. Threshold values for a northern species are needed to recommend discharge limits, monitor cumulative effects within watersheds and to provide early indication of potential ecosystem change (Galloway et al. 2005, Gray and Munkittrick 2005).

3.2 Methods

Slimy sculpin were collected from the Churchill River in Northern Saskatchewan using electrofishing techniques, transferred back to labs at the University of Saskatchewan, and held in chilled, oxygenated Living Stream tanks (Frigid Units Inc©, Toledo, OH) at 6^{o}C. One week prior to the experiment, fish were transferred to a temperature controlled chamber in which the temperature was increased one degree per day to acclimatize fish to the experimental temperature of 10^{o}C.

3.2.1 Experimental Design

Six concentrations of NH_3 (control (0 ppm), 0.278, 0.556, 0.834, 1.112 and 1.668 ppm) were selected for a 21-day exposure period based on assessment of ammonia concentrations documented in monthly monitoring reports for northern mining operations (MVLWB Public Registry, accessed May 23, 2008). Ammonia concentrations and pH data from 8 northern mining operations (gold, diamond, base metals) were averaged January to December 2006 and used to identify the relevant exposure concentrations for our laboratory experiments (Table 3.1).

In the laboratory, two large head tanks were used to bring water temperature to the desired experimental temperature of 10^{o}C. Water was then transferred to six individual 80 L mixing tanks (one tank for each of the six experimental concentrations). Reagent-grade ammonium chloride was added to each 80 L mixing tank to reach selected experimental concentrations. The pH was adjusted in the 80 L tanks to 8.5±0.1 using NaOH to represent the upper limit of pH values found in northern effluents (Table 3.2).

Table 3.1. Mean (± SE) un-ionized ammonia concentrations, temperatures and pH results from eight mining operations in northern Canada.

	Unionized Ammonia (NH_3) (ppm)	**pH**	**Temperature (°C)**	**Samples (n)**
Giant Mine	0.20±0.02	8.01±0.22	11.2±8.5	6
Con Mine	1.10±0.21	7.55±0.19	9.8±5.2	6
Prairie Creek	1.96±0.26	8.20±0.10	9.6±3.8	5
Diavik	0.031±0.01	7.77±0.41	11.4±6.1	12
BHP	0.08±0.01	7.89±0.30	10.8±4.8	12
Tungsten	0.58±0.56	8.31±0.23	10.2±5.3	12
Snap Lake	0.32±0.09	7.95±0.60	11.0±4.3	12
NICO	2.90±0.56	8.11±0.25	13.1±4.2	5

Table 3.2. Water quality data collected daily during 21-day experiment (n=4). Treatments with the same letter were not significantly different at p=0.05.

	Total Ammonia (ppm)						
Parameter	**Control**	**5**	**10**	**15**	**20**	**30**	**p-value**
Total Ammonia (ppm)	0.0±0.0	4.98±0.17	9.95±0.31	14.98±0.22	19.85±0.45	30.1±1.18	0.001
pH (units)	8.5±.0.1 A	8.5±.0.1 A	8.5±.0.1 A	8.5±.0.1 A	8.5±.0.1 A	8.5±.0.1 A	>0.05
Temperature (°C)	10.1±0.2 A	10.0±0.1 A	10.0±0.2 A	10.2±0.2 A	10.0±0.1 A	9.9±0.1 A	0.784
Dissolved Oxygen (ppm)	8.94±0.19 A	8.92±0.15 A	8.95±0.17 A	8.87±0.22 A	8.96±0.12 A	8.88±0.11 A	0.548

Two peristaltic pumps were set up to transfer water from each 80 L tank to four replicate 10 L tanks per treatment at 13.8 ml/minute through Tygon tubing which allowed for two turnovers per day in each of the 10 L tanks. The four 10 L tanks for each NH_3 concentration represented the experimental units in this design.

Three slimy sculpin greater than 4.5 cm in length were placed into each of the 10 L tanks. Previous work by Brasfield (2007) and Gray et al. (2004) suggested that fish greater than 4.5 cm were more likely to be reproductive adults. Attempts were made to randomly select two females and one male for each 10 L tank to encourage breeding.

However, as sculpin do not display obvious sexual dimorphism, it was not possible to confirm a 2:1 female to male ratio until fish were dissected at the end of the study. An ANOVA was conducted on condition factors for each sex in each of the six treatments to confirm no significant differences were present among the treatments before exposures commenced. Fish were fed frozen bloodworms (San Francisco Bay Brand, Newark, CA) at 5 % of their body weight per day, with feedings in the morning and late afternoon.

Daily water quality measurements were taken to ensure the accuracy and precision of ammonia concentrations, temperature, pH and dissolved oxygen between the four experimental units in each treatment concentration. Total ammonia was measured using the Hanna HI 93733 Photometer (Hanna Instruments, Woonsocket, RI) and un-ionized ammonia values were calculated from total ammonia values for endpoint determinations using pH and temperature measurements. Accuracy of the Hanna ammonia meter was periodically checked using a VMR SympHony pH/ISE Meter Model SB301 (VWR International, PA, USA) using an Orion Ammonia Electrode Probe Model 193-18. Measurements for pH were conducted using an Extech EX800 handheld meter (Extech Instruments, Waltham, MA). Dissolved oxygen concentrations were measured using the YSI85 (YSI Incorporated, Yellow Springs, OH). Temperature measurements were also taken daily using thermometers in each tank and confirmed from temperatures displayed on the YSI probe. Daily checks were made of each tank to assess mortality, egg laying, behavior, and visible fish health.

3.2.2 Chronic Effects Endpoints

After 21 days of exposure, fish were euthanized using clove oil (30uL/1L water) and severed at the spinal cord. The total length and weight of each fish was measured before dissection. Liver and gonads were extracted and weighed and carcass weights were collected after organ extractions were complete. Condition factor (CF) was calculated as comparison of length to body weight using CF=100(body weight/length^3). Gonadosomatic Index (GSI) and Liversomatic Index (LSI) were calculated by comparing respective weights to body weights (organ weight/body weight *100) (Environment Canada 2002). During the second week of the experiment several female fish began dropping eggs. Eggs were weighed and added to the total body weight of the fish. These fish were eliminated from GSI calculations. For the purposes of LSI calculations, the weight of dropped eggs was added to the body weight. The second stage gill arches were removed and placed into histopathology cassettes and preserved in 10% formalin for 24 hours, then transferred to 75% ethanol prior to transfer to the laboratory. Gonads were equally divided and one half of each gonad was immediately frozen at -80^{o}C for hormone analysis and the other half placed in histopathology cassettes and preserved as above for gonads. Histocassettes were submitted to Prairie Diagnostic Services, University of Saskatchewan, for slide preparation.

3.2.3 Gonad Histopathology

Slides were blinded, numbered for identification purposes and separated according to sex. Histological analysis was used to confirm sex in fish that had gonads of undetermined sex during dissections. Gonad histopathology was quantitatively assessed using methods described in Weber et al. (2003). Briefly, female gonads were analyzed at 1000x magnification for stage 1 oogonia and at 400x magnification for pre-vitellogenic, vitellogenic and mature follicles using an Olympus model BH-2 light microscope mounted with a Zeiss 7.2 megapixels 62 Cyber-shot camera (Carl Zeiss Inc., Thornwood, NY, USA). Fibrosis and atretic follicles were also examined. Male gonads were examined for number of spermatocysts per view and fibrosis.

3.2.4 Gill Histopathology

Slides were blinded, numbered for identification and analyzed using an Olympus model BH-2 light microscope mounted with a Zeiss 7.2 megapixels 62 Cyber-shot camera (Carl Zeiss Inc., Thornwood, NY, USA). Digital photographs were taken at 40x and 100x magnification to allow for identification of histopathology features. Lamellar fusion and epithelial lifting were selected as endpoints based on a literature review and their association with reduced respiratory performance (Bryan and Nowak 1998, Takashima and Hibiya 1995). Hemorrhage was also assessed as a measure of severe physical damage. Quantification of gill changes is important for statistical analysis. Selected gill histopathology endpoints were quantified by determining percentage of endpoint occurrence (prevalence) in slimy sculpin gills.

3.2.5 Hormone analysis

Female gonads were analyzed for concentrations of total testosterone and 17-β estradiol while male gonads were analyzed for total testosterone concentrations. A hormone extraction (EIA) buffer was prepared with the following composition: 0.575 g Na_2HPO_4, 0.128 g NaH_2PO_4, and 0.100 g of gelatin (Rickwood et al. 2006). Ingredients were mixed into 95 mL of ultra pure water and heated to 50^{o}C for 15 minutes to dissolve gelatin. Solution was allowed to cool and pH was adjusted to 7.6 with hydrochloric acid and filled to 100 mL with ultra pure water. Previously frozen tissues were allowed to thaw immediately prior to extraction by placing tissue samples in a glass test tube over ice. Thawed tissues were homogenized and ether-extracted for analysis of 17β-estradiol and total testosterone. Samples were pipetted into microcentrifuge tubes and stored at -80°C until hormone concentration analyses were conducted.

ELISA (Enzyme-Linked Immunosorbent Assays) provided a quantitative analysis of hormone concentration based on enzyme conjugate and steroid hormone competition for binding sites on an antibody coated plate. Methods for testosterone and 17-β estradiol were the same but different antibody coated plates and associated standards and conjugates were supplied. ELISA kits were supplied by Oxford Biomedical Sciences. Standards were prepared following procedures outlined in the ELISA kits. Readings were reported directly to SoftmaxPro software. SoftmaxPro provided mean hormone concentrations for the duplicate samples on the plate and gonad hormone concentrations were then calculated (ng/mg tissue) taking into account amount of original tissue sampled and reconstituted volumes.

Extraction efficiency was tested by spiking samples with a known standard (1 ng/mL), extracting hormones as per the procedure described above, and analyzed using ELISA method. Hormone recovery should exceed 80%. Residual extracts were combined and mixed thoroughly to provide a homogenous extract for intra-assay and inter-assay variability. Intra-assay coefficient of variation should be less than 10% and inter-assay coefficient of variation should be less than 15% (Tetreault et al. 2003). This sample was added to 8 wells on the same plate to test for coefficient of variation within the assay ((Standard Deviation/mean) x 100%, n=8) and added to 8 wells on a separate plate on a different day to test for coefficient of variation between assays ((Standard Deviation/mean) x 100%, n=16).

Extraction efficiency (percent hormone extracted) of 4 samples spiked with a 1 ng/ml standard was 85.05% ±0.0747. This was above the required 80% extraction efficiency. The coefficient of variation for intra-assay validation was 11.1 % (mean 0.477, SD 0.053) and inter-assay validation was 14.3% (mean 0.495, SD 0.072). The intra-assay variability was slightly higher than the recommended <10% but the variability between assays was less than 15%.

3.2.6 Statistical Analysis

Previous studies have shown the Analysis of Variance (ANOVA) is useful for determining both No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) in concentration-response experiments (Fairchild 2005, Lease 2003). The NOEC is the lowest non-statistically significant response point and LOEC is the lowest statically significant point, identified by an ANOVA in this experiment (Fairchild 2005, Lease 2003). The NOEC and LOEC for gill histopathology were calculated by using concentrations in which survival was not significantly reduced when compared with control concentrations (Rand 1995). The highest treatment group, 1.668 ppm, was removed from the NOEC and LOEC calculations due to significantly higher mortality than the control (0 ppm) treatment group.

Because concentrations of NH_3 were controlled in this experiment, an ANOVA was conducted to determine if statistically significant differences ($p<0.05$) were present between treatments ($n=4$). Assumptions of ANOVA were checked for each variable prior to conducting ANOVA. Normality was confirmed using the Shapiro-Wilkes test and equality of variances was compared using Levene's test. All tests were performed using Systat 11. Tukey's post hoc tests were conducted to determine which treatments were statistically different. Tukey's test using Systat version 11 corrects the significance level automatically for multiple pair-wise post hoc comparisons.

Linear regression was used to analyze relationships between concentration and response. Where significant relationships were identified during regression analysis, Effect Concentration 50 (EC_{50}), the concentration at which 50% of the population is affected, was determined. Lethal concentration 50 (LC_{50}), the concentration at which 50% of fish have died, was determined using the Trimmed Spearman Karber method. For condition factor, GSI, LSI, hormone concentrations and gonad histopathology, female and male fish were analyzed separately.

3.3 Results

3.3.1 Water Chemistry

A summary of water quality measurements including total ammonia is presented in Table 3.2. Ammonia concentrations were not significantly different among replicate tanks within treatment groups (1.668 ppm: n=4, p=0.464; 1.112 ppm: n=4, p=0.356; 0.834 ppm: n=4, p=0.435; 0.556 ppm: n=4, p=0.422; 0.278 ppm: n=4, p=0.612; 0 ppm: n=4, p=0.712) and there were no significant differences in temperature, dissolved oxygen or pH between treatments. The ANOVA results and Tukey's post hoc tests for ammonia concentrations among six treatment groups (n=4) indicated that concentrations were significantly different (p=0.0001). A summary of average annual ammonia concentrations, temperatures and pH data in effluents from eight northern mine operations in 2006 is summarized in Table 3.1. The two highest ammonia concentrations in Table 3.1 (2.90 ppm and 1.96 ppm) are above the highest experimental concentration used in this study. All other concentrations fall within the treatment concentrations used in this study.

3.3.2 Acute Lethality

In the highest treatment concentration, 1.668 ppm NH_3, 66.7% (±14.14) of exposed fish died in the first 96 hours of this study. A 96-hour LC_{50}, determined using the Trimmed Spearman Karber method, was calculated at 1.529 ppm NH_3 for slimy sculpin. Mortality was not seen in any other treatment concentrations. Of the 12 fish exposed to the highest treatment concentration (three fish for each of four replicate tanks), five were females and seven were males. All five female fish (100%) died in the first 96 hours, while four of the seven male fish (43%) survived the duration of the experiment (21 days).

3.3.3 Chronic Effects Endpoints and Hormone Analysis

A significant increase in GSI at 1.668 ppm NH_3 in female fish ($p<0.001$) corresponded to a significant increase in testosterone in females at 1.668 ppm ($p=0.001$) (Figure 3.1). However, regression analysis did not indicate a relationship between increased GSI and treatment concentrations ($p=0.086$, $R^2=0.467$) or testosterone and treatment concentrations ($p=0.095$, $R^2=0.687$). Estradiol concentrations did not differ between ammonia treatments in female fish ($p=0.449$) nor was a relationship demonstrated ($p=0.449$, $R^2=0.243$). A significant increase in GSI was also detected in male fish at 1.668 ppm ($p=0.035$) but no significant differences were detected in testosterone concentrations among treatments ($p=0.221$) (Figure 3.2). A relationship between GSI and treatment concentrations in males was not present ($p=0.131$, R^2

=0.433), nor was a relationship between testosterone and treatments ($p=0.222$, $R^2 =0.302$).

Liversomatic index decreased significantly in male ($p=0.016$) and female ($p=0.003$) fish at both the 0.834 ppm and 1.112 ppm concentrations (Figure 3.3). Regression analysis did not demonstrate a relationship between LSI and treatment concentrations in either male ($p=0.20$, $R^2 =0.298$) or female fish ($p=0.102$, $R^2 = 0.346$). Condition factor (data not shown) did not differ significantly among treatments for both female ($p=0.77$) and male fish ($p=0.447$) and there was not a significant relationship between condition and treatment concentrations in female ($p=0.747$, $R^2 =0.424$) and male fish ($p=0.445$, $R^2 =0.217$).

3.3.4 Gonad Histopathology

Gonad histopathology was used to confirm sex in each of the fish in this study. No significant differences were found in the number of oogonia, previtellogenic follicles, vitellogenic follicles, or mature follicles in female fish among the treatment concentrations. Assessment of atretic follicles and fibrosis in females did not yield statistically significant results. In male fish, the number of spermatocysts did not differ among treatment groups and evidence of fibrosis was limited and not significant in this study. During the second week of the experiment, female fish began dropping eggs. One fish in the control group dropped eggs, and two fish in each of the 0.556 ppm group, 0.834 and 1.112 ppm treatment group dropped eggs. No fish in the 0.278 ppm or the highest (1.668 ppm) treatment group dropped eggs.

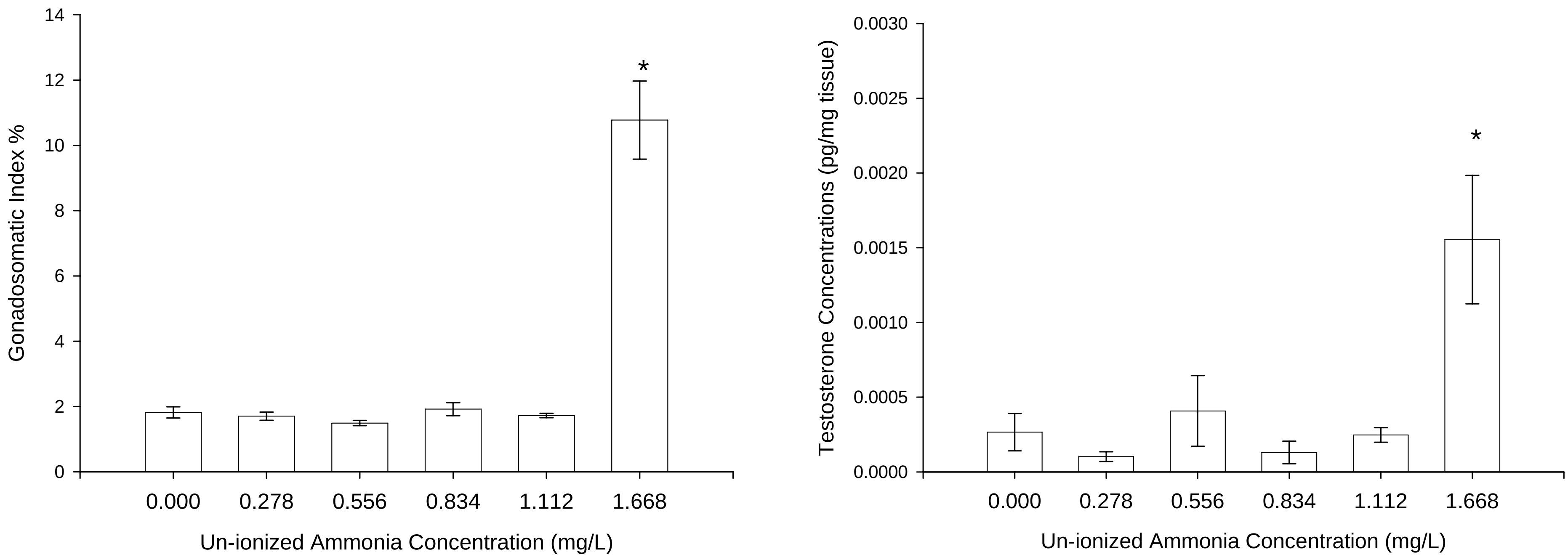


Figure 3.1. Gonadosomatic Index (GSI) values (± 1 SE) and total testosterone concentrations in female fish at 0, 0.278, 0.556, 0.834, 1.112, and 1.668 ppm. The ANOVA results for GSI: $p=0.001$,$n=4$; ANOVA results for total testosterone: $p=0.001$, $n=4$. $p<0.05$ is significant. * represents statistical significance between treatment groups

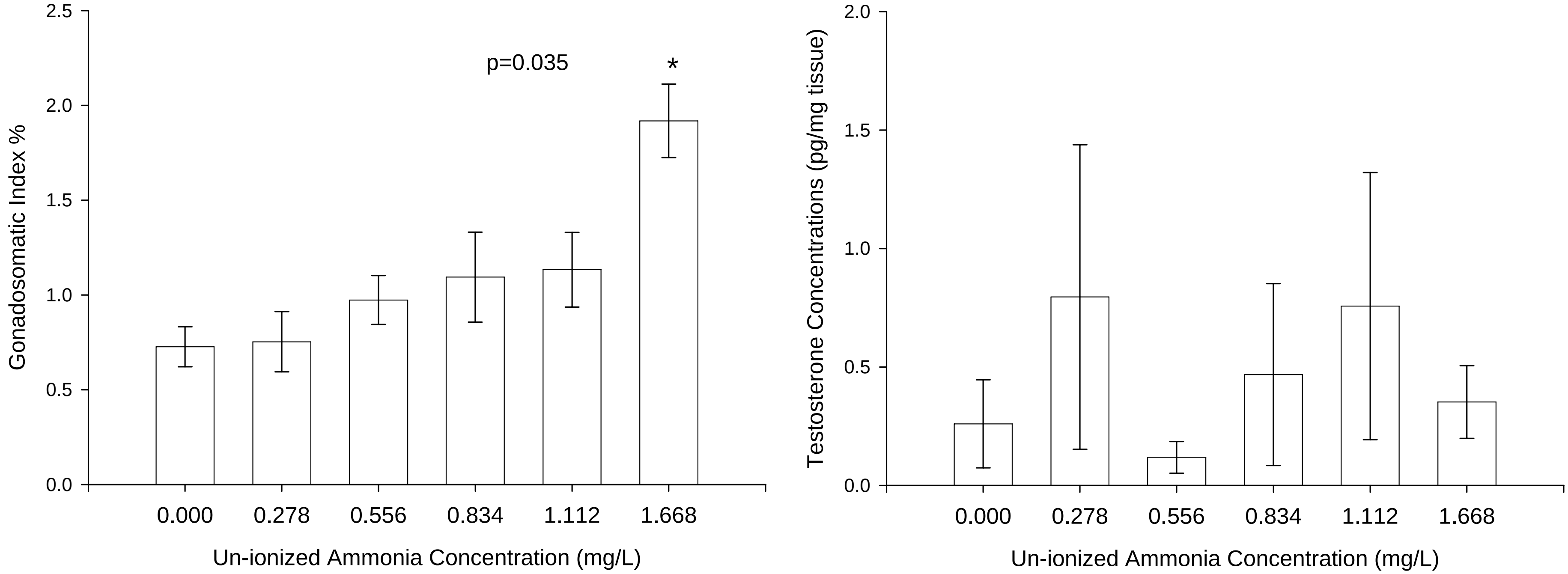


Figure 3.2. Gonadosomatic Index (GSI) values (± 1 SE) and total testosterone concentrations in male fish at 0, 0.278, 0.556, 0.834, 1.112, and 1.668 ppm. The ANOVA results for GSI: $p=0.035, n=4$; ANOVA results for total testosterone: $p=0.221$, $n=4$. $p<0.05$ is significant. * represents statistical significance between treatment groups

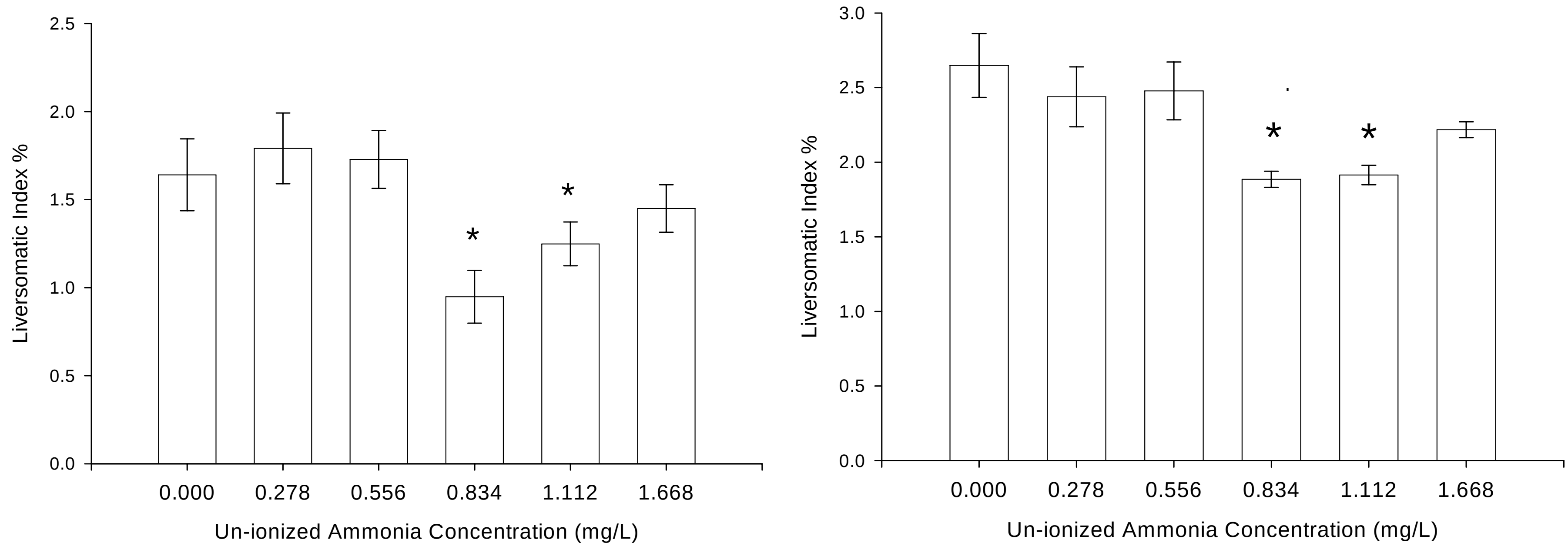


Figure 3.3. Liver Somatic Index (LSI) values (± 1 SE) in male and female fish at 0, 0.278, 0.556, 0.834, 1.112, and 1.668 ppm. ANOVA results for LSI in male fish: p=0.016, n=4; The ANOVA results for LSI in female fish: p=0.003, n=4. p<0.05 is significant. * represents statistical significance between treatment groups

3.3.5 Gill Histopathology

Severe changes in gill structure (lamellar fusion, epithelial lifting, and hemorrhage) were apparent in the three highest NH_3 concentrations (0.834 ppm, 1.112 ppm, 1.668 ppm) used in this study (Figure 3.4). A significant difference was seen in the prevalence of lamellar fusion in slimy sculpin gills at 0.834, 1.112 and 1.668 ppm NH_3 ($p<0.001$). Prevalence of epithelial lifting was also significantly higher at 0.834, 1.112 and 1.668 ppm treatments ($p<0.001$). Linear regression of lamellar fusion ($p=0.001$, $R^2=0.783$, $y=72.765x-5.4429$) and epithelial lifting ($p=0.001$, $R^2=0.843$, $y=70.992x-9.7536$) showed a relationship with prevalence increasing with increasing ammonia concentration. These results allowed for the calculation of NOEC and LOEC using results from an ANOVA. For the purposes of NOEC and LOEC calculations, the 1.668 treatment group was removed as the mortality was significantly different from the control(0 ppm) treatment group (Figure 3.5). The NOEC, defined as the lowest non-statistically significant response point, was 0.556 ppm for both lamellar fusion and epithelial lifting. The LOEC, defined as the lowest statistically significant response point, was 0.834 ppm for both endpoints. The EC_{50} was also calculated for gill histopathology endpoints from regression relationships. The EC_{50} concentrations were calculated at 0.775 ppm for lamellar fusion and 0.842 ppm for epithelial lifting (Figure 3.6). Hemorrhage was assessed in all fish gills but was only present in mortalities from the highest treatment group. Of the eight mortalities in 1.668 ppm NH_3, seven fish gills showed evidence of hemorrhage.

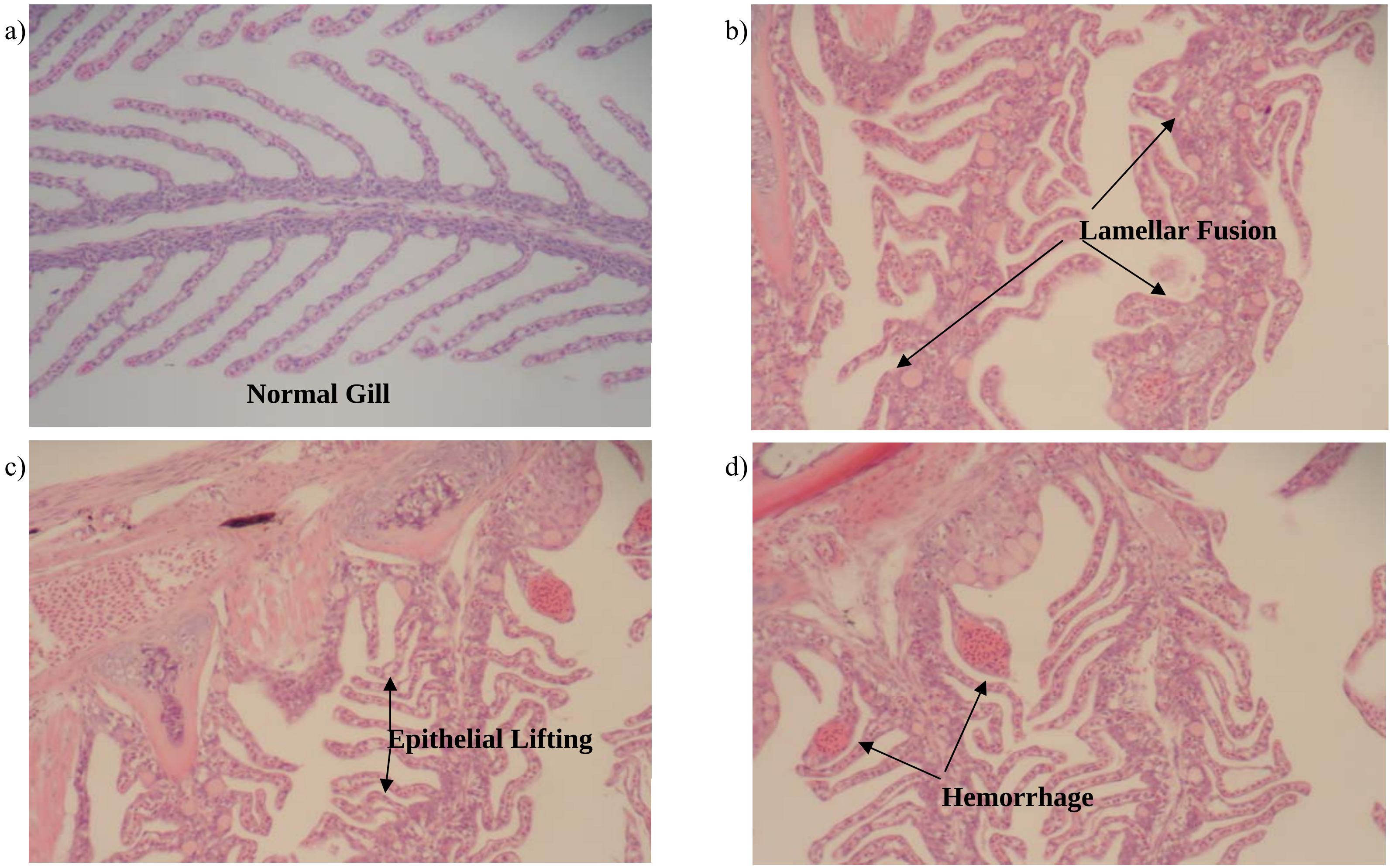
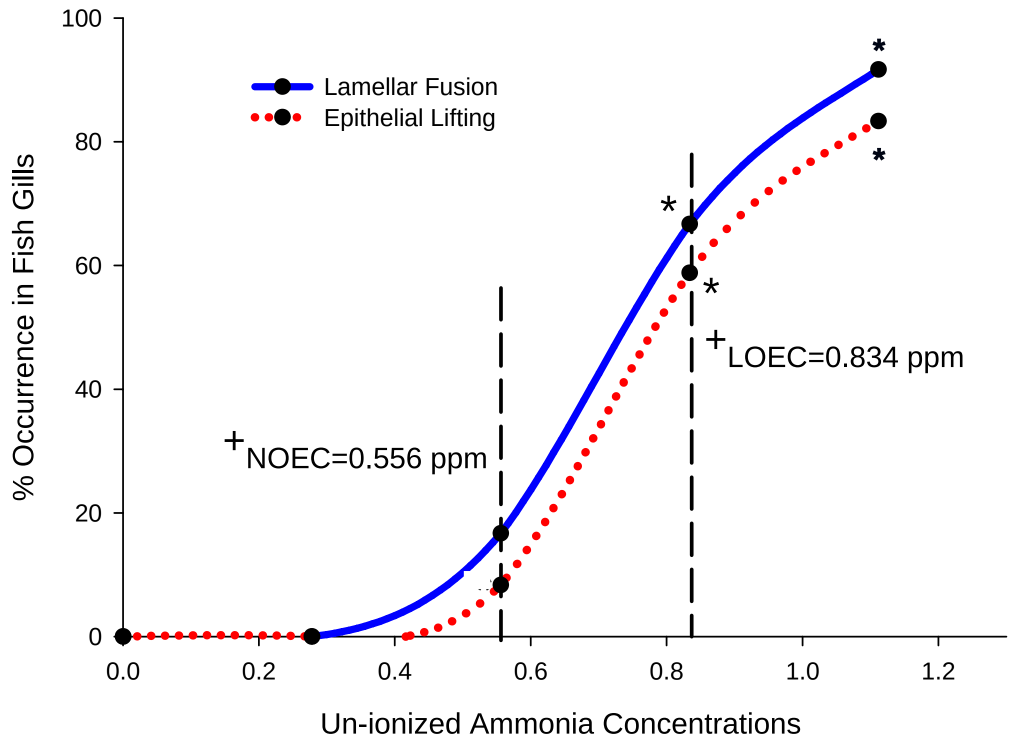


Figure 3.4. Digital photographs of second gill arches a) gill arch collected from fish in control treatment representing normal gill structure in slimy sculpin b) gill arch collected from fish in 0.112 ppm NH_3 showing lamellar fusion in proximal and central regions of lamellae c) gill arch collected from fish in 0.112 ppm NH_3 showing epithelial lifting in lamellae d) gill arch from fish in 1.668 ppm NH_3 showing hemorrhage in lamellae.



+ NOEC is represented by the highest non-statistically significant response point

+ LOEC is represented by the lowest statistically significant response point

* Represents statistically significant responses

Figure 3.5. Concentration-response curve for prevalence of lamellar fusion and epithelial lifting against NH_3 concentration. Toxicological endpoints were calculated: NOEC of 0.556 ppm and LOEC of 0.834 ppm for both lamellar fusion and epithelial lifting. The ANOVA results for lamellar fusion: $p<0.001$ (significant difference between treatment groups), n=4. The ANOVA results for epithelial lifting: $p=0.001$ (significant difference between treatment groups), n=4.

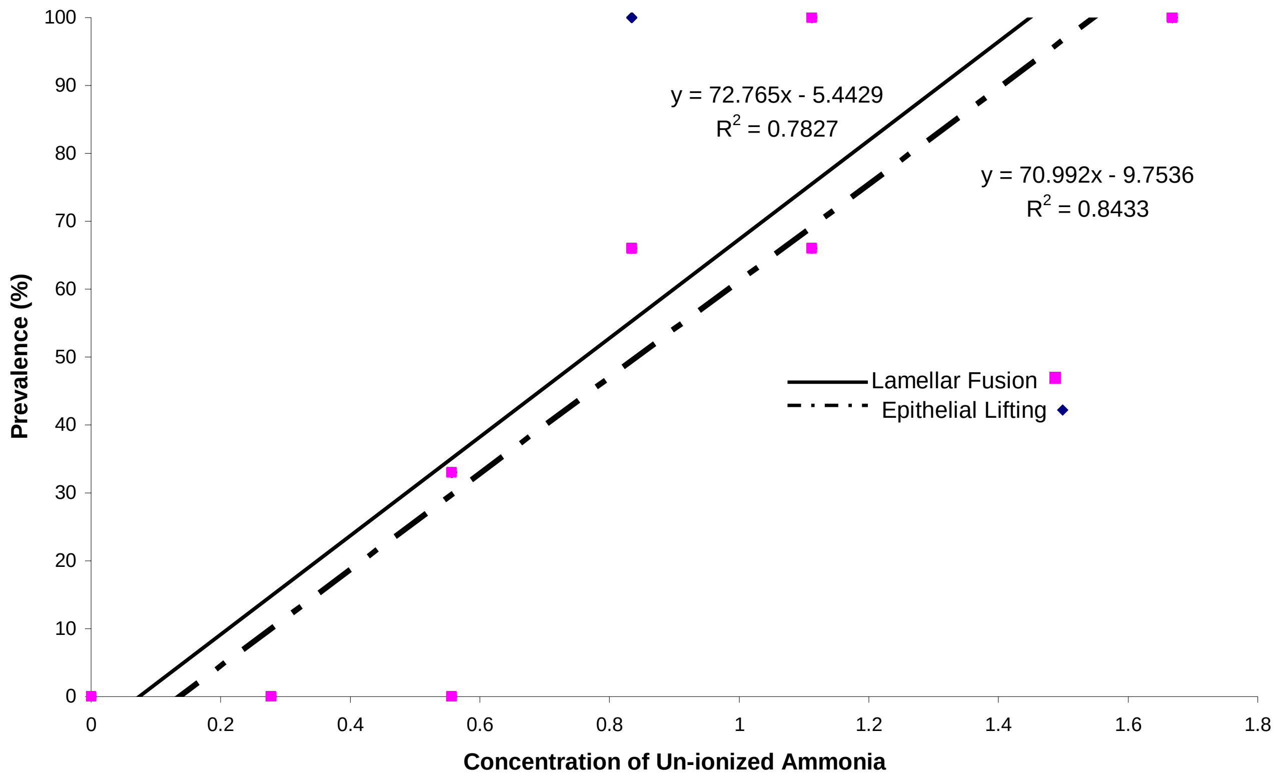


Figure 3.6. The EC_{50} calculation based on regression of lamellar fusion and epithelial lifting against NH_3 concentration. The EC_{50} for lamellar fusion was 0.775 ppm and EC_{50} for epithelial lifting was 0.842 ppm. Regression analysis: Lamellar Fusion ($p<0.000$, $R^2=0.783$) and Epithelial Lifting ($p=0.001$, $R^2=0.843$).

3.4 Discussion

Results of this study have provided toxicological thresholds for gill histological response endpoints in slimy sculpin exposed to concentrations of NH_3 relevant to northern mining effluents. Severe gill damage, measured as lamellar fusion and epithelial lifting, followed a concentration-response pattern, allowing for NOEC and LOEC determinations, as well as EC_{50} calculations. A LC_{50} was obtained from mortalities in the highest treatment group. Gill hemorrhage was apparent in fish mortalities in the high treatment group. Liversomatic indices demonstrated decreased liver size at the 0.834 ppm and 1.112 ppm NH_3 treatment groups in male and female fish. Gonadosomatic index increased in both female and male sculpin at the highest treatment concentration, while testosterone also increased in female fish in the high treatment group.

3.4.1 Ammonia Toxicity

Many factors affect toxicity of NH_3 in aquatic environments. Although pH is documented to be the most important factor influencing toxicity, temperature is also an important element. Tissues, especially branchial tissues, are permeable to NH_3 but relatively impermeable to NH_4^+ (Randall and Tsui 2002). Although temperature increases the amount of NH_3 present, it is also known to increase permeability of tissues to NH_3. The effect of this increased permeability is often overlooked when assessing toxicity (Fairchild 2005). Un-ionized ammonia targets gills due to their one cell-layer structure (Le Francois et. al. 2008), function in respiration and involvement in detoxification and excretion of NH_3 (Randall and Ip 2006, Ip et al. 2001). Un-ionized ammonia exposure leads to non-specific structural and functional damage to gill tissue, such as epithelial lifting and lamellar fusion (Wood 2000) associated with mortality

(Lease et al. 2003), when detoxification and excretion mechanisms are exceeded (Le Francois et al. 2008). Because the gills are crucial for ionoregulation, decreased function can lead to systemic effects (Lease et al. 2003).

A 96 hour acute lethality test resulted in a LC_{50} of 1.529 NH_3. There is a large range of LC_{50} values (0.16-2.0 ppm) reported for acute lethality with a high variability both within species and between species (Eddy 2005, Lease et al. 2003, Wood 2000, Thurston et al. 1986). The LC_{50} for slimy sculpin in this experiment would indicate that they are not as sensitive as other species such as rainbow trout (*Oncorhynchus mykiss*) but less tolerant than species such as bluegills (*Lepomis macrochirus*) (Eddy 2005). Other factors which affect how individual fish respond to NH_3 toxicity include condition, age and presence of disease.

The NOEC and LOEC were calculated at 0.556 ppm and 0.834 ppm respectively in this study. Previous studies have found NOEC and LOEC for un-ionized ammonia to range from as low as 0.068 ppm in rainbow trout to concentrations of 1.1 ppm in other freshwater species such as bluegills (Eddy 2005, Milne et al. 2000, Wood 2000). The effects of NH_3 on slimy sculpin have not been previously tested in a controlled laboratory concentration-response experiment. The results of this study indicate that effect concentrations are within the range of those obtained from other species, however it is well documented that NH_3 toxicity is highly variable within species (Fairchild et al. 2005, Lease 2003). It is important to note that this experiment was conducted at 10^{o}C. As previously discussed, a lower temperature may result in lower gill permeability when exposed to elevated NH_3 concentrations. Temperatures in northern environments may reduce toxicity due to decreased permeability; however many sensitive ecosystems have naturally higher pH values due to underlying mineralogy (Environment Canada 1991) which would increase NH_3 toxicity. In addition to naturally alkaline environments, mine discharges tend to have an alkaline pH (Table 3.1).

3.4.2 Gill Histopathology

Gill structure is an important endpoint when assessing NH_3 exposure given that the gills are the first site of exposure and are involved in NH_3 detoxification (Randall and Tsui 2002). The gill epithelium represents the primary site for oxygen exchange and ionoregulation (Lease et al. 2003) and disruption of these processes leads to decreased ability to function (Smart 1976). Lamellar fusion, which is the fusing of adjacent lamellae, affects gas exchange and can be a direct result of lifting pavement cells (Frances et al. 2008). Lamellar fusion can result from hyperplasia of epithelial cells, indicating advanced structural damage (Smart 1976). Epithelial lifting involves epithelium separation from the basement membrane and usually indicates edema or fluid increases. Cell functioning is disrupted as a result of epithelial lifting, as well disrupted ionoregulation (Pane et al. 2004). Hemorrhage is a result of blood channel disruption and is indicative of severe physical damage (Smart 1976).

Gill damage in fish can result in loss of equilibrium, decreased respiratory effectiveness, decreased swimming performance, and mortality (Fairchild et al. 2005), and is one of the most commonly reported effects of NH_3 exposure (Milne et. al. 2000). In previous studies with fathead minnows and sucker (*Deltistes luxatus*), gill structure was severely compromised yet no changes in growth were observed in fish (Lease et al. 2003, Thurston et al. 1986). This is consistent with results from our study where condition factor remained unchanged after exposure but gill damage was significant.

The absence of mortalities and growth differences in all treatments with the exception of 1.668 ppm NH_3 suggests that changes in gill structure may be a more sensitive indicator of waters high in ammonia than other chronic effect endpoints commonly used in ecological monitoring (Lease et al. 2003, Thurston et al. 1986). It is possible that analyzing slimy sculpin gills from aquatic systems receiving discharges could provide early indications of physiological effects from stressors. Assessment of gill tissue damage in previous studies has lead to more sensitive thresholds than traditional chronic endpoints in rainbow and brown trout (*Salmo trutta*) (Milne et al. 2000). One benefit, as pointed out in Thurston et al. (1986), is the minimal expense of gill histopathology, as a value-added monitoring tool for detecting changes. Using lamellar fusion and epithelial lifting to assess slimy sculpin gills provided important toxicological endpoints in this study and could be used in future studies to identify gill damage in fish exposed to ammonia.

3.4.3 Liversomatic Index

Liversomatic index results for male and female slimy sculpin used in this study were within the range of LSI values found in previous studies with this species (Gray and Munkittrick 2005). Female sculpin had higher LSI than males, which corresponds to results reported by Gray and Munkittrick (2005). Reduced LSI in male and female fish at higher treatment concentrations (0.834 and 1.112 ppm) indicate decreased liver weight relative to body weight in elevated ammonia concentrations. The absence of significant decreases in liver weight in the highest treatment group (1.668 ppm) may be a result of fish mortality within the first 96 hours of the experiment.

Studies by Milne et al. (2000) found significantly lower liver weight ratios with increased ammonia exposure in laboratory studies using both rainbow and brown trout (*Salmo trutta*). One hypothesis for decreased liver size in the above study was the use of energy stored in liver glycogen during periods of stress (Milne et al. 2000). It is possible in our study that stress placed on fish in the 0.834 and 1.112 ppm groups required them to rely on additional energy stores to adapt to the increased pressures of maintaining ionoregulation, equilibrium and respiration in the presence of elevated levels of ammonia. Other studies support this hypothesis of reduced glycogen storage in the liver during ammonia exposures (Smith and Piper 1975) and degeneration due to severe liver tissue damage (Flis 1968). Fish in the highest treatment group likely died before significant liver changes were seen. Histopathological studies of liver tissue were not conducted in this study; however, future histopathology investigations in slimy sculpin liver may provide further insight into ammonia effects.

3.4.4 Gonad Histopathology, Hormone Analysis, and Gonadosomatic Index

Gonad histopathology was important for the confirmation of sex in sculpin used this study. Because female fish began to drop eggs in the second week of the experiment in four of the six treatment groups, several of the fish dissected had visually unidentifiable gonad tissue. Also, differences in age and sexual maturity were not visually apparent at the start of the experiment. Slimy sculpin generally breed once per year, however, evidence suggests that sculpin from northern rivers may only breed once every two years (Van Vilet 1964). This may explain why some fish dropped their eggs while others either reabsorbed eggs throughout the experiment or did not show any evidence of ovary maturation and development. Fish greater than 4.5 cm were selected for this study based on previous research conducted on slimy sculpin indicating that fish below this threshold were unlikely to be reproductive (Brasfield 2007, Gray and Munkittrick 2005). Sculpin were kept at 6^{o}C prior to the experiment which was consistent with temperatures in the Churchill River during that time period. Although it was expected that the increase in water temperature from 6^{o}C to 10^{o}C would trigger gonad development in slimy sculpin in this experiment, limited information available about breeding sculpin in laboratory conditions and evidence for bi-annual breeding in the field may have complicated expected reproductive behaviour (Van Vilet 1964).

Gonadosomatic indices were lower in male (0.75 – 1.20%) and female fish (1.67-1.96) than those reported in Gray and Munkittrick (2005) (male GSI=1.41-2.19%, female GSI=3-12%) with the exception of the highest treatment group (male GSI=1.89 %, female GSI=10.88%). In the highest treatment concentration, GSI increased in both male and female fish, with an associated increase in testosterone in females but not males. Sixty-seven percent of sculpin died with 96 hours of the ammonia exposure in the highest treatment group: 100% of female fish and 43% of male fish. In male fish, an increase in food availability with less fish per tank may account for the increased GSI in surviving males compared to other treatment groups. A possible explanation for increased GSI in female fish may be elevated concentrations of testosterone which could have triggered gonad development.

Increased hormone concentrations can suggest a severe stress response that may have reduced the adaptive capacity of female fish (Tetreault et al. 2003). Only female fish showed a significantly higher testosterone concentration at 1.668 ppm which may help to explain why all female fish in the treatment died. Due to the high variation in testosterone and 17β-estradiol concentrations in sculpin from this study, comparison to biochemical endpoints in previous studies is difficult.

Many previous ammonia studies have focused on non-reproductive endpoints rather than reproductive endpoints. Thurston et al. (1986) however reported significant differences in reproduction (egg production, viability, and egg hatching) in fathead minnows exposed to NH_3 concentrations (0.07-0.96 ppm) in chronic 1-year experiments, with a calculated chronic effects threshold of 0.27 ppm. Although the range of NH_3 concentrations used in experiments by Thurston et al. (1986) are within the range of concentrations used in this experiment (0-1.668 ppm), these endpoints were not measured in this experiment because slimy sculpin have a much longer life-cycle and a hatching period of approximately 28 days (Gray 2005).

3.4.5 Behaviour Changes

Although behaviour was not an endpoint selected in this concentration-response study, it was apparent throughout the experiment that fish in the three highest treatment concentrations (0.834 ppm, 1.112 ppm, 1.668 ppm) were exhibiting behaviour not seen in the control and lower treatment groups (0 ppm, 0.278 ppm, 0.556 ppm). Swimming performance and equilibrium are two physiological processes affected by NH_3 (Lease et al. 2003). Fish in the 1.668 ppm treatment group began to lose equilibrium and were observed swimming in a sideways position prior to death. Other fish in both the 1.112 ppm and 1.668 ppm groups crowded in the corner of the tanks and swam continuously into the corner of the tank during the first week. During the second week, swimming decreased and food consumption decreased in 0.834, 1.112 and 1.668 ppm. Interestingly, this did not correspond with a decreased condition. Those fish that did not die within the first 96 hours remained inactive for the duration of the experiment. This concurs with past studies concluding that NH_3 affects behaviour and functioning at high concentrations (Eddy 2005, Lease et al. 2003), and supports the results obtained from gill histopathology. Quantitative behaviour studies in the future may help to better understand these changes.

3.4.6 Application to Northern Environments

An assessment of effluent characterizations from mining operations in northern Canada provided the range of ammonia concentrations used in this experiment. Ammonia concentrations at two mining operations (1.96 ppm and 2.90 ppm) exceeded the highest concentration used in this study but all other NH_3 concentrations in northern effluents were between 0-1.668 ppm NH_3 . Concentrations were variable depending on the mining and milling processes used, as well as the retention time prior to effluent discharge. Because ammonia toxicity is highly dependent on pH, and to a lesser degree temperature, other effluent characteristics become increasingly important. Un-ionized ammonia fractions are calculated using both temperature and pH, and therefore may provide a more protective discharge limit when compared to using total ammonia, where toxicity can be influenced by both effluent pH and temperature. Un-ionized ammonia thresholds determined in this study, 0.556 ppm NOEC, 0.834 LOEC and 0.842 EC_{50}, are within the range previously reported for chronic effects thresholds in southern species and provide regulators with increased knowledge of the effects of ammonia on slimy sculpin as a northern species. Because there is increased mining development in northern Canada, the need for development of protective limits for ammonia becomes increasingly important. The identification of endpoints sensitive to ammonia exposure in northern species will allow regulators to focus monitoring programs and to detect potential effects of mining effluents with elevated ammonia concentrations.

3.5 Conclusions

Slimy sculpin health is affected by elevated NH_3 concentrations relevant to treated mining effluents discharged to northern Canadian waters. Calculated LC_{50}, NOEC, LOEC, and EC_{50} provide thresholds for a northern species (slimy sculpin) which can be used to provide guidance to regulators when assessing ammonia in effluents and developing discharge criteria protective of northern environments. Gill damage in treatment concentrations indicated that gill histopathology might be an important indicator for detecting changes in fish health in addition to traditional chronic toxicity endpoints. Histopathological work on liver tissue at elevated NH_3 concentrations may provide supportive evidence for the value of tissue histopathology in threshold development. Incorporating behavior studies into NH_3 exposures using slimy sculpin may also be beneficial in future studies.

CHAPTER 4.0

OVERALL DISCUSSION AND RESULTS

The overall objective for my research was to assess and better understand effects of mining effluents on two pristine and sensitive northern rivers through field and laboratory-based toxicity studies with specific objectives to examine two pristine northern rivers receiving mining discharges (Flat River and Prairie Creek of the South Nahanni watershed). My specific objectives were to document current aquatic conditions, assess any changes associated with effluent discharges, and to evaluate suitable indicators and monitoring methods for northern environments. Indicator assessment focused on ammonia as it is an important stressor discharged by mines in Canada's north and affects the health of slimy sculpin, a northern sentinel species. Ultimately my interest was to assess how my results would improve future effects monitoring methodologies in northern pristine rivers and how a northern sentinel species could be used to assess effects of an important northern stressor.

I hypothesized that the Flat River and Prairie Creek aquatic systems would show minor changes in biological communities due to the discharge of mining effluent. Field studies confirmed that minor changes were evident in biological communities in both Flat River and Prairie Creek downstream of effluent discharges. My hypothesis that field studies would confirm that slimy sculpin are the only possible sentinel species in the areas of the South Nahanni watershed that were studied was supported by my research. My research identified trophic components of importance (periphyton and benthic invertebrate communities) to focus further monitoring programs. My objective to use laboratory studies to provide information for slimy sculpin exposed to ammonia and identify responses important for detecting changes was achieved.

4.1 Field studies

Previous studies on pristine rivers within the South Nahanni watershed were limited prior to this research. The South Nahanni watershed has ecological significance and monitoring programs specific to northern rivers are important to detect effects from mining discharges on tributaries. The high-latitude and extreme climate in the South Nahanni watershed combined with the oligotrophic nature of the Flat River and Prairie Creek increases susceptibility to stressors and contaminants. Low productivity is associated with limited nutrient availability, low species density and low biological diversity, which reduces the ability of these systems to tolerate stressors and environmental changes.

Multi-trophic level monitoring programs conducted on the Flat River and Prairie Creek characterized these pristine northern rivers receiving mining discharges and assessed current monitoring methods in northern environments. In addition, northern sentinel species were identified and their associated abundance and distribution were documented. Stressors and contaminants of concern were also identified during multi-trophic monitoring programs on the two rivers and reviews of effluent characterization data.

Environmental Effects Monitoring (EEM) methodologies, as described in the Metal Mining Effluent Regulations, were followed in order to assess their applicability in northern pristine systems. The EEM program provides a nationally consistent monitoring methodology for detecting effects from mining effluents. Although differences were detected between upstream and near-field samples in both rivers in several EEM endpoints, non-EEM endpoints such as algal biomass and algal community composition proved to be good indicators of change in these pristine environments.

Results of the monitoring programs indicated that biological differences downstream of mines on the Flat River and Prairie Creek were consistent with mild enrichment of these highly oligotrophic systems. Because productivity in a system can be an important indicator of change, as well as affecting the sensitivity of systems to some stressors, algal samples should be used to focus future monitoring programs in both these oligotrophic areas and others of similar productivity in northern ecosystems. In addition to algal samples providing information on productivity as an indicator of environmental change, algal community composition also showed differences between reference and exposure sites. Differences in algal community composition support the use of algae as an important monitoring tool in the South Nahanni watershed although it is not currently a monitoring requirement under the EEM program. Benthic invertebrate analysis, which includes effects endpoints assessed in EEM, showed differences in both rivers and was also a useful monitoring tool in the Flat River and Prairie Creek.

Given the low abundance of fish species in these ecosystems, the effects of lethal monitoring programs on fish populations must be considered. Analysis of fish tissue provided information on the accumulation of copper and iron in liver tissue of fish collected from the Flat River. However, due to the low abundance of fish in these rivers, the use of benthic tissue or algal samples to assess metal accumulation may provide an important alternative for assessing effects of mining effluent. Although the biological effects of mining activities appear to be limited at present, evidence of differences between upstream reference and near-field exposure sites suggest that there is potential for increased mining activity to affect biological communities in northern rivers. Further, evidence of mild eutrophication which could be exacerbated with the advent of climate change and associated factors (e.g., flow alterations, increased ice-free periods) suggests the need for focused monitoring programs to detect changes. This research will act as baseline assessment for evaluation of future changes in sensitive tributaries of the South Nahanni River.

4.2 Laboratory Exposures

Slimy sculpin were identified as a key sentinel species for biomonitoring in these rivers based on their abundance and limited mobility. Laboratory exposures were used to generate key toxicological information under controlled conditions for a contaminant of concern (ammonia), a species of northern relevance, and under conditions representative of the north (e.g. temperature of exposure). Because pristine northern rivers are associated with low fish abundance, developing a method for assessing effects in fish without removing them from sensitive systems is critical. Laboratory exposures indicated that slimy sculpin health is affected by elevated NH_3 concentrations relevant to mining effluents from northern Canada.

Calculated LC_{50}, NOEC, LOEC, and EC_{50} provide thresholds for a northern species (slimy sculpin) which can be used in the development of discharge criteria protective of northern environments. Gill damage in treatment concentrations indicated that gill histopathology might be an important indicator for detecting changes in fish health in addition to traditional chronic toxicity endpoints. Histopathological work on liver tissue at elevated NH_3 concentrations may provide supportive evidence for the value of tissue histopathology in threshold development. Incorporating behaviour studies into NH_3 exposures using slimy sculpin may also be beneficial in future studies.

This laboratory exposure examined responses to a single contaminant of concern. Laboratory exposures with complex effluents such as those discharged by mining developments could be used to both identify responsive endpoints in order to further focus field monitoring and act as an alternative to fish monitoring components, such as those required by EEM. The methods used in this research serve as a basis for future laboratory studies with slimy sculpin. Slimy sculpin were shown to be a valuable species for effects monitoring in a laboratory environment and should be considered in future laboratory exposures assessing effects of mining effluents under environmentally relevant conditions. Consideration should be given to try and develop a reproductive partial lifecycle bioassay with this species.

4.3 Future Monitoring Recommendations

The EEM methods were employed in two northern pristine rivers (Flat River and Prairie Creek) receiving mining discharges in order to develop monitoring strategies to detect effects in these unique systems. Because EEM is a nationally legislated monitoring program, using these methods provided comparison to a well-defined and accepted protocol. As required in the EEM program, monitoring of fish, benthos, sediment and water was undertaken, as well as effluent characterization. In addition to traditional EEM endpoints, algal samples were also collected as a measure of productivity and community composition.

Although responses to mining activity occurred in each trophic level, the most responsive endpoints were different in each river reinforcing the need for considering site-specific factors in the design of monitoring programs in pristine northern rivers. Variable water quality and sediment quality results were best analyzed in conjunction with biological differences given the dynamic nature of these systems. Algal endpoints, which are not currently a requirement of EEM, proved to be an important component of the monitoring programs in the Flat River and Prairie Creek.

My research indicated that sentinel fish abundance in these systems was low compared to more southern rivers. Given the low abundance of fish species in these ecosystems, the effects of lethal monitoring programs on fish populations needs to be seriously considered in the design of field studies. Although EEM allows for non-lethal sampling protocols, important information on fish health may not be obtained. Analysis of metal concentrations in benthic algal and/ or macroinvertebrate tissue is recommended for exposure assessment and causal association in rivers where fish populations are of low abundance and the effects of monitoring programs on abundance is of concern.

The focus of further monitoring programs in sensitive pristine riverine areas exposed to mining activity should include a multi-trophic approach, including adequately replicated benthic algae and macroinvertebrate community samples combined with water and sediment quality and benthic tissue/algae metal burdens.

Laboratory exposures with northern sentinel species such as slimy sculpin can provide important toxicological information, as well as providing an alternative to field-based effects assessments. Because monitoring programs in the Flat River and Prairie Creek indicated low fish abundance, it is important to develop alternative monitoring strategies to determine if effects are occurring due to the discharge of mining effluent.

Controlled laboratory exposures allow for identification of responsive endpoints in northern sentinel species for specific contaminants of concern.

In my laboratory exposure, gill histopathology was identified as an effect of ammonia exposure in slimy sculpin. Gill damage in treatment concentrations detected through gill histopathology served as an important indicator for detecting changes in fish health in addition to traditional chronic toxicity endpoints. Calculated LC_{50}, NOEC, LOEC, and EC_{50} provided thresholds for slimy sculpin, which can then be used for developing discharge criteria protective of northern environments and assessment of effluent toxicity to northern species. Gill histopathology methods and results from this research also provide a monitoring tool to detect effects in slimy sculpin exposed to northern mining effluents with elevated ammonia concentrations. Histopathological work on liver tissue at elevated ammonia concentrations may provide supportive evidence for the value of tissue histopathology in threshold development and future monitoring programs.

Parks Canada and Indian and Northern Affairs Canada have used results from the field component of my research to develop a long-term monitoring program for the South Nahanni watershed based on algal and benthic samples combined with water and sediment quality monitoring. An important consideration for future monitoring programs is the use of slimy sculpin in laboratory-based exposures to assess toxicological fish endpoints for specific contaminants of concern or northern mining effluents.

4.4 Conclusions

Results from the multi-trophic monitoring programs have allowed for assessment of current aquatic conditions in the Flat River and Prairie Creek and can be used as a baseline for future monitoring programs in these important ecosystems, as well as other pristine northern rivers receiving mining discharges. Different trophic components responded to mining discharges in each river which confirms the need for considering site-specific factors in the design of monitoring programs. Benthic and algal components were found to be the most responsive and can be used as monitoring tools in pristine environments with low fish abundance. Algal endpoints are not currently used in EEM monitoring programs yet provided important data in these sensitive ecosystems. Monitoring programs in pristine northern rivers receiving mining discharges should be conducted with focus on replicated benthic invertebrate and algal samples, supported by water and sediment quality analyses.

Monitoring programs in the Flat River and Prairie Creek identified slimy sculpin as the only possible sentinel fish species for effects monitoring programs although overall fish abundance was low. In systems with low fish abundance, the effect of removing large numbers of fish for use in monitoring programs needs to be considered. Controlled laboratory exposures can provide key toxicological information when field studies are not practical.

Slimy sculpin were successfully collected from the wild, maintained in a laboratory setting and exposed to ammonia, an important stressor in northern environments. Results provided information on acute toxicity of ammonia in slimy sculpin and indicated that ammonia affected the health of slimy sculpin as indicated by gill damage and changes in liver size. A calculated LC_{50} and NOEC for ammonia provides valuable information when determining effluent discharge limits and conducting cumulative effects assessments in pristine northern environments. Gill histopathology methods and results also provide a monitoring tool to detect effects in slimy sculpin exposed to northern mining effluents with elevated ammonia concentrations.

CHAPTER 5.0

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