The Toxicity and Bioavailability of Nickel and Molybdenum to Standard Toxicity-Test Fish Species and Fish Species Found in Northern Canadian Lakes

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Abstract

Nickel (Ni) and molybdenum (Mo) are two metals that are commonly associated with northern Saskatchewan uranium deposits. Consequently, concentrations of Ni and Mo are elevated above background concentrations in uranium-mine effluent receiving waters. The objectives of this research were: (1) to determine if standard toxicity-test fish species fathead minnows and rainbow trout, were predictive of toxicity to fish species such as northern pike and white suckers that inhabit lakes near northern Canadian uranium mining operations; (2) to determine if toxicity results derived in the laboratory related to toxicity observed in the field; (3) to determine the relative toxicity of Ni and Mo; (4) to determine how water quality parameters, such as hardness, pH, and total suspended solids (TSS) affects Ni toxicity; and, (5) to determine if exposure to Ni or Mo induced metallothionein in fish. Field studies indicated that although Mo concentrations in receiving waters were strongly correlated with larval fathead minnow mortality, dietary Se, which covaried with Mo, was the probable cause of toxicity. Laboratory toxicity tests on field-collected receiving waters gave different results than field tests. Laboratory results were interpreted by considering confounding variables, such as hardness and pH. Waters generally high in Ni, circumneutral or slightly acidic, and with low hardness, caused fathead minnow eggs to hatch earlier than controls. This early hatching is a significant result because time-to-hatch is an ecologically important endpoint often not considered in more conventional toxicity-characterization programs. In laboratory tests involving Ni- and Mo-spiked laboratory dilution water, Ni was much more toxic than Mo. The most sensitive endpoint for Ni toxicity was time required for fathead minnow eggs to hatch. Sensitivity to Ni varied by species in the following order: fathead minnows>northern pike>white suckers>alevin rainbow trout>juvenile rainbow trout. Therefore, water quality criteria based on toxicity data derived from rainbow trout tests are not sufficient to protect sensitive fish species in Ni-contaminated water. However, the Saskatchewan Surface Water Quality Objective (SSWQO) for Ni (0.025 mg/L) was determined to be sufficient to protect larval fish and developing embryos against Ni toxicity. The Maximum Acceptable Toxicant Concentration (MATC) required to protect fathead minnow eggs against Ni toxicity in soft water was 0.14 mg/L, which is 4 times higher than the current SSWQO. However, this MATC is approximately equal to Ni concentrations measured in Key Lake dewatering-effluent receiving waters, and approximately half the concentrations measured in B-Zone pit water, which is proposed to be released into Collins Bay, Wollaston Lake. Consequently, sensitive fish species inhabiting Ni-contaminated waters associated with uranium mining in northern Saskatchewan may be exposed to concentrations that cause sub-lethal effects. Waterborne Mo was not toxic to any life stage of any fish species tested. Water hardness, pH, and TSS reduced Ni toxicity to larval fathead minnows. Metallothionein was induced in juvenile rainbow trout gills, but not livers, in Ni-exposed fish. Molybdenum did not induce metallothionein in juvenile rainbow trout.

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The late English biologist, Sir Peter Medawar, in describing his own writing experience, said, "... it is a proceeding that makes one rather inhuman, selfishly guarding every second of one's time and becoming inattentive about personal relationships..." Consequently, family members require special thanks for their sacrifices during this research effort. My two sons, Tyler and David, helped immeasurably: Tyler's inquisitiveness prompted me to discuss my work, in often excruciating detail, using language comprehensible to a 10-year-old; David frequently dropped everything to work with me in the laboratory as a data recorder and fish feeder. Both have shown a keen interest in the progress of the research. Finally, this work would not have been possible without the love and support from my wife, Laurie. Without her limitless patience and steadfast support, this project would have undoubtedly taken twice as long to complete. Thank you.

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1. Introduction

1.1 Literature Review of Nickel and Molybdenum Toxicity to Fish

1.1.1. Nickel

Nickel (Ni) marks the end of the first transition series on the periodic table (Group VIII). It is a common metal, making up 0.008% of the earth's crust, and is found mostly in ultrabasic igneous rocks ranging in content from 0.016% in basalt, to 0.20% in periodotite (Birge and Black 1980). Nickel is the seventh most abundant transition element, and the twenty-second most abundant element in the earth's crust (Greenwood and Earnshaw 1984). Canada is the world's largest producer of Ni owing mostly to the large deposits of the Sudbury basin (Greenwood and Earnshaw 1984). The recently discovered Ni deposit in Voisey's Bay is reported as the largest in history and promises to maintain Canada's status as the world's largest Ni producer for years to come, pending successful resolution of current environmental and political concerns (Doan 1997).

Like other members of the first transition series (e.g., Cr, Mn, Fe, and Co), Ni is octahedrally coordinated in aqueous systems as $Ni(H_2O)_6^{2+}$ (Greenwood and Earnshaw 1984). Under oxic conditions, the free aquo species is dominant and hydrous oxides of Fe and Mn control solubility. However, hydrous Mn oxides (i.e., Mn-oxyhydroxides) are far more important with respect to Ni solubility than Fe-oxyhydroxides (Richter and Theis 1980). Under anoxic conditions sulphide (if present) controls Ni solubility. Ankley et al. (1991) demonstrated the role of acid-volatile sulphides (AVS) on Ni and Cd bioavailability to estuarine amphipods and oligochaetes. They found that molar

ratios of simultaneously-extracted metals (SEM) to AVS greater than unity consistently produced acute toxicity to *Hyalella azteca* and *Lumbriculus variegatus*.

In natural waters ranging in pH from 5 to 9, the free Ni^{2+} ion is the dominant species (Richter and Theis 1980). Under oxic conditions, Ni forms complexes with naturally occurring ligands to a small degree in the following order: $OH^- > SO_4^{2-} > Cl^- > NH_3$. Under higher pH conditions, the following hydrolysis reactions occur:

at pH 9: $Ni^{2+} + H_20 \rightarrow NiOH^+ + H^+$

at pH 10: $Ni^{2+} + 2H_20 \rightarrow Ni(OH)_2^0 + 2H^+$

at pH 12: $Ni^{2+} + 3H_20 \rightarrow Ni(OH)_3^- + 3H^+$

at pH 14: $Ni^{2+} + 4H_20 \rightarrow Ni(OH)_4^{2-} + 4H^+$

Nickel complexes with the carbonate ion (i.e., $NiCO_3$), although important for other metals, are thought to play a minor role in Ni speciation. However, complexes formed by the carbonate ion with other metals, such as Cu or Pb, tend to free up more binding sites on Mn-oxyhydroxides to accommodate Ni adsorption (Richter and Theis 1980). Therefore, the presence of carbonate plays an indirect role in Ni speciation (Richter and Theis 1980).

Nickel speciation is significantly affected by pH. At high pH, Ni is more likely to adsorb onto Fe or Mn-oxyhydroxides because of increased electrostatic attraction between negatively charged oxide surfaces and positively charged Ni cations. At low pH, competition between Ni and hydrogen ions causes Ni^{2+} to dissociate from electrostatic associations with oxyhydroxides and particulate or colloidal matter, thus causing it to dissolve.

Toxicity of Ni to fish varies with fish species, metal species, and the chemistry of exposure conditions (i.e., hardness, alkalinity, pH, etc.). Fathead minnows, *Pimephales promelas*, exposed to $NiCl_2 \cdot 6H_2O$ in soft and hard water gave LC50 values of 4.0 and 24 mg/L, respectively. The goldfish, *Carassius auratus*, demonstrated 100% mortality when exposed to 10 mg $NiCl_2$/L of soft water, and 8.1 mg $NiCl_2$/L of hard water. Goldfish exposed to $NiCl_2$ in salt water could survive concentrations up to 259 mg/L (Birge and Black 1980).

Rainbow trout (*Oncorhynchus mykiss*, age 1 year old) exposed to $NiSO_4$ under experimental conditions where hardness as $CaCO_3$ was 240 mg/L and pH ranged from 7.3 to 7.5, gave an LC50 value of 32 mg/L (Brown and Dalton 1970). Brown (1968) showed that as water hardness (as $CaCO_3$) varied from 10 to 300 mg/L, 48-h LC50s for Ni increased linearly from 18 to 90 mg/L. Hale (1977), using 2-month old rainbow trout in a mobile continuous-flow laboratory (alkalinity as $CaCO_3$ ranged from 82 to 132 mg/L, pH ranged from 6.4 to 8.3), reported a 96-h TL50 value for Ni of 35.5 mg/L. Data from these studies demonstrate that Ni toxicity decreases with increasing alkalinity and hardness.

In a review paper, Hall and Anderson (1995) reported six studies investigating the effects of salinity on Ni toxicity to fish, bivalves, crustaceans, fungi, and bacterioplankton. In each case, Ni toxicity increased with decreasing salinity. These results further demonstrate the dependence of Ni toxicity on environmental chemistry.

Pickering and Henderson (1966) compared the relative toxicity of Ni to several species of freshwater fish with the toxicity of other metals. When test fish were

maintained under soft-water conditions (20 mg/L as $CaCO_3$) and exposed to $NiCl_2 \cdot 6H_2O$, the 96-h median tolerance limits (TL_M) for the guppy (*Poecilia reticulata*), fathead minnow, bluegill (*Lepomis macrochirus*), and goldfish were 4.45, 4.58-5.18, 5.18-5.36, and 9.82 mg Ni/L, respectively. The same test conducted on fathead minnows and bluegills exposed to $NiCl_2 \cdot 6H_2O$ under hard-water conditions (360 mg Ca/L as $CaCO_3$) gave TL_M's of 42.4-44.5 and 39.6 mg Ni/L, respectively. Furthermore, Cu (as $CuSO_4 \cdot 5H_2O$) and Cd (as $CdCl_2 \cdot 2.5H_2O$) were more toxic than Ni to all fish species, Pb (as $PbCl_2$) was less toxic than Ni, and Zn (as $ZnSO_4 \cdot 7H_2O$) was equally toxic as Ni to bluegills and goldfish, whereas more toxic to fathead minnows and guppies.

Rehwoldt et al. (1971) exposed several species of Hudson River fish (hardness 53 mg/L as $CaCO_3$) to Ni, Cu, and Zn administered as their nitrate salts. The 96-h TL_M's for Ni exposure were 6.2, 8.1, 10.6, 13.0, 13.6, and 46.2 mg/L for striped bass (*Morone saxatilis*), pumpkinseed (*Lepomis gibbosus*), common carp (*Cyprinus carpio*), American eel (*Anguilla rostrata*), white perch (*Morone americana*), and banded killifish (*Fundulus diaphanus*), respectively. Copper was more toxic than Ni in all fish species. Zinc was more toxic than Ni in banded killifish and common carp, whereas Ni and Zn were equally toxic in striped bass, white perch, and American eel. However, in the pumpkinseed, Ni was more toxic than Zn.

Data demonstrating sub-lethal effects of Ni exposure to fish are severely lacking. Pickering (1974) exposed fathead minnows to sub-lethal concentrations of Ni (hardness 207 mg/L as $CaCO_3$, alkalinity 161 mg/L as $CaCO_3$, and pH 7.8) over long periods to

study its chronic effect on reproduction. At Ni concentrations of 730 μg/L, both fecundity and egg hatchability were significantly reduced with respect to controls. Birge and Black (1980) reported on egg hatchability and early-life stage Ni toxicity for several fish species. Under static-renewal test conditions (hardness ranged from 93 to 105 mg/L as $CaCO_3$, alkalinity ranged from 72 to 79 mg/L as $CaCO_3$, and pH ranged from 7.2 to 7.7), 96-h LC50 values for rainbow trout, channel catfish (*Ictalurus punctatus*), largemouth bass (*Micropterus salmoides*), and goldfish were 0.5, 0.71, 2.06, and 2.78 mg/L, respectively. Teratogenesis was most common in channel catfish, goldfish, and rainbow trout, and correlated inversely with egg hatchability. No teratic larvae were observed at exposures below those that significantly affected egg hatchability.

Ellgaard et al. (1995) investigated locomotory responses in goldfish exposed to short-term, sub-lethal concentrations of Ni. Goldfish exposed to 75 mg $NiCl_2 \cdot 6H_2O$/L showed hypoactivity compared to control fish. The authors suggested that the onset of histopathological effects occur very rapidly after initial metal exposure, which causes decreases in ventilation rate and oxygen diffusion across gill lamellae, which, in turn, causes hypoactivity. Further, Ni-induced hypoactivity may reflect the imminent onset of death, which may be observed during chronic-exposure tests, but may not be observed during 96-h toxicity tests. This delayed lethal response suggests that Ni concentrations judged to be "sublethal" from 96-h acute lethality toxicity tests, may actually be lethal when exposure times are increased beyond 96 hours.

Mixtures of Cu, Ni, and Zn have produced additive acute toxic effects to rainbow trout (1970). Similarly, additive toxic effects have been demonstrated for Co-Ni and Zn-Ni mixtures (Birge and Black 1980). Nickel and zinc sulphate mixtures, and Cu-Ni mixtures have been shown to interact synergistically, whereas Ni and Co interact in a less-than-additive manner (Birge and Black 1980). Toxicity demonstrated by rainbow trout and fathead minnows exposed to a complex mixture of metals, including Al, Mn, Fe, Ni, Zn, Cu, and Pb, was shown to be caused only by the presence of Al and Cu (Hickie et al. 1993). Toxic effects that may have been observed when a fish was exposed to a single metal at a time were suppressed by the over-riding influence of Al and Cu toxicity.

1.1.2. Molybdenum

Primary literature on Mo distribution, fate, and effects in aquatic environments is severely lacking, compared to the relatively vast literature concerning Mo in terrestrial systems. Geochemical studies involving the environmental behaviour of Mo have been mostly conducted in oceanic systems (e.g., Legeleux et al. 1994). Very few studies within the past 10 years have been conducted on Mo geochemistry in freshwater-lake systems (Magyar et al. 1993). The following account of Mo geochemistry is largely from Magyar et al. (1993).

Molybdenum can exist in oxidation states II, III, IV, V, and VI, where Mo(VI) is most stable. Consequently, 99% of Mo exists as Mo(VI). The most abundant species of Mo in natural aquatic environments is molybdate (MoO_4^{2-}) and its protonated forms ($HMoO_4^-$ and H_2MoO_4). Other Mo species include the sparingly soluble molybdenum

bromide and chloride (Galvin 1996); and the non-soluble and highly stable thiomolybdates (general formula: $MoO_nS_{4-n}^{2-}$), which exist in the presence of HS^- (Magyar et al. 1993).

Magyar et al. (1993) studied Mo behaviour in a seasonally anoxic lake. During the summer months, as lake water thermally stratified, most Mo was found in the epilimnion with Mo concentration decreasing with increasing depth. Molybdenum minima were observed at the lake bottom during November, just before the onset of autumn turnover. Mo(VI) showed a strong correlation ($r=0.99$) with NO_3^- in water column depth profiles. This high correlation suggests that Mo is being taken up by nitrate-reducing bacteria for synthesis of nitrate reductase (a Mo-containing enzyme) at the sediment-water interface. It was further suggested that Mo could potentially be a limiting factor for primary production in lakes where NO_3^- is the primary source of nitrogen.

Finally, Magyar et al. (1993) calculated distribution coefficients, k_d,[1] and residence times, on which Mo transport is highly dependent. Residence times of Mo were similar to those of water (360-370 d for Mo, and 408 d for water), and k_d values were very low (0.0037-0.0046 L/g). Long residence times coupled with low k_d values suggest that Mo is highly mobile. Scavenging of Mo from the water column takes place by formation of the complex $\{FeS \bullet MoS_3\}$ and by Mo bound to Mn oxides and hydroxides.

Molybdenum is an essential metal and is used as a cofactor for the enzymes xanthine oxidase, sulphite oxidase, and aldehyde oxidase (Goyer 1991; Galvin 1996).

Mammal uptake of Mo is generally facilitated through absorption of Mo^{6-} through the intestinal wall, and is preferentially deposited in the liver, kidney, fat, and blood. Although toxic effects of Mo exposure to ruminants (e.g., "teart" or molybdenosis—a disease caused by an antagonistic association between Mo and Cu causing Cu deficiency, where manifest symptoms include diarrhea, anemia, and poor growth rate; for examples, see several papers in Volume 1 of Chappell and Petersen (1976)) is well known, there are very few data relating Mo toxicity to fish.

Rainbow trout (total length 55 mm) exposed to $Na_2MoO_4 \cdot 2H_2O$ (hardness 25 mg/L as $CaCO_3$, alkalinity 26 mg/L as $CaCO_3$, and pH 6.9) demonstrated a 96-h LC50 of 1320 mg/L (McConnell 1977). Examination of fish that died at the 1500 mg Mo/L exposure concentration revealed fused gill lamellae, gut hemorrhaging, pyloric caeca hemorrhaging, pale livers with hemorrhaging along liver margins, and pale kidney. In the same study, smaller rainbow trout (average size 20 mm) gave lower 96-h LC50 values of 800 mg Mo/L. Rainbow trout exposed for longer periods of time (i.e., one year) at lower concentrations of $Na_2MO_4 \cdot 2H_2O$ (exposure dilutions ranging from 0 to 17 mg/L) showed no significant biological differences in mortality, growth, or hematocrits. Neither eyed eggs, sac-fry, nor fingerling life-stages demonstrated any toxicological differences when compared to controls (McConnell 1977).

Other sub-lethal early-life stage toxicity tests provide contradictory results. Rainbow trout exposed to $Na_2MO_4 \cdot 2H_2O$ from immediately after fertilization to 4 days post-hatch (28 d total exposure, where pH ranged from 6.9 to 7.8, and hardness from 92 to 110 mg/L as $CaCO_3$), demonstrated a chronic threshold effect concentration (TEC) of

0.73 mg/L (Birge 1978). Under roughly the same exposure conditions, Birge et al. (1980) estimated the 28-d LC50 as 0.79 mg/L for rainbow trout exposed to $Na_2MO_4 \cdot 2H_2O$. Eisler (1989) reported a decrease in rainbow trout growth and an increase in mortality at Mo concentrations >50 mg/L. However, 0.79 mg Mo/L was sufficient to reduce egg hatchability to 50% of controls. These results are significant because they demonstrate that early-life stage fish may be significantly more sensitive to Mo than later-life stages, which could lead to significant effects on fish populations (Woltering 1984).

Chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) exposed to $Na_2MO_4 \cdot 2H_2O$ in both soft (hardness 41.7 mg/L as $CaCO_3$, alkalinity 29.6 mg/L as $CaCO_3$, pH 7.57) and brackish (hardness 333 mg/L as $CaCO_3$, alkalinity 36.6 mg/L as $CaCO_3$, pH 7.79, Na^+ 469 mg/L, Cl^- 726 mg/L) waters gave 96-h LC50 values greater than 1000 mg/L (Hamilton and Buhl 1990). Molybdenum did not seem to have any toxic effect on either of these species exposed to any one of several B-Mo-Se mixtures. Dwyer et al. (1992) reported 96-h LC50 values for Mo-exposed (where both salinity and hardness were varied) striped bass as >79.8 mg/L. Their results also showed that complex mixtures of As, B, Cu, Li, Mo, and Sr increased in toxicity to striped bass with decreasing hardness.

Keinholz (1977) showed Mo concentrations in liver and kidney of rainbow trout, exposed in a stream at a sampling station 1.6 km downstream from tailings of a Mo mine, significantly higher than in reference fish. Interestingly, this exposure station downstream of Mo tailings was also high in copper. Kidney residues showed no

significant decrease in Cu between "exposed" and "unexposed" fish, whereas Cu concentrations in liver were significantly higher in exposed fish than in unexposed fish. The observation of elevated liver Cu is contrary to what would be expected from experiments involving Mo-Cu interactions in ruminants, where a Cu reduction would be expected in the presence of elevated Mo concentrations. Saiki et al. (1993) showed that Mo was not biomagnified in aquatic biota, including filamentous algae, plankton, benthic macroinvertebrates, and fish, sampled from the San Joaquin River and its tributaries. Measurements of whole-fish Mo concentrations in striped bass from the same river system showed that Mo was not elevated above reference-site concentrations (Saiki and Palawski 1990).

Fish exposed to complex mixtures of metals (of which Mo is one component) that likely represent metal concentrations in metal-contaminated environments, demonstrate toxic responses that are not associated with Mo toxicity (Hamilton and Wiedmeyer 1990; Buhl and Hamilton 1996). On the other hand, complex mixtures of As-Mo-Se demonstrated interesting interactions with respect to toxicity to *Ceriodaphnia dubia* (Naddy et al. 1995). *Ceriodaphnia dubia* survival data for an As-Mo mixture exposure demonstrated a less-than-additive response (i.e., antagonistic relationship). Only high concentrations of Se added to the As-Mo mixture caused a reduction in *C. dubia* survival, suggesting As-Mo mixtures have an antagonistic effect on Se toxicity. Further, addition of either As or Mo to Se caused a significant reduction in *C. dubia* survival compared to Se exposure alone.

Tong et al. (1974) reported decreasing Mo concentrations in decapitated, definned, and eviscerated whole lake trout (*Salvelinus namaycush*) with increasing age. Ward (1973) measured Mo concentrations in rainbow trout and Kokanee salmon (*Oncorhynchus nerka*) from trace (<6 μg/L), medium (6 μg/L), and high (300 μg/L) concentration environments. Molybdenum concentrations in fish from high-concentration environments showed only slightly higher (13-332 μg/L) concentrations than those from trace (5-118 μg/L) environments. Rainbow trout tended to show higher Mo concentrations in its tissues than Kokanee salmon.

Field investigations into the environmental effects of Mo report conflicting results. For example, Whiting et al. (1994) concluded that Mo concentrations in receiving streams in the vicinity of a molybdenum mine were not sufficient to adversely affect macroinvertebrate communities. In assessing sediment quality in a municipal and industrial harbour, Mo, along with Cd, Cr, Pb, and Ni were identified as factors that reduced sediment quality (Winger and Lasier 1995). Laboratory toxicity tests conducted on field-collected sediment samples, demonstrated that leaf consumption by *Hyalella azteca* was negatively correlated with Mo in sediments (together with unionized ammonia, alkalinity, salinity, pH of pore water, Cd, Ni, and P). Lipsit (pers. comm., 1996) identified Ni, Mo, Na, and U as the four most significant environmental factors affecting benthic macroinvertebrate community structure in water bodies receiving uranium-mining effluent near Collins Bay, northern Saskatchewan.

1.2 Metallothionein

Metallothioneins (MTs) are a family of low molecular weight (6,000-10,000 daltons [Da]) (Zhang and Schlenk 1995), cysteine-rich (~30%) proteins whose purpose has been suggested to regulate divalent cations *in vivo* (Roesijadi 1994). Fish exposed to elevated concentrations of divalent essential and non-essential metals, such as Cu, Zn, Cd, Pb, and Hg, have demonstrated MT induction (Chan 1995). Consequently, MT is now regarded as a potential biomarker for metal-exposed fish (Chan 1995). However, it remains unclear if MT serves well as a biomarker to metal-exposed fish, since most research has been conducted on terrestrial organisms (Arizono et al. 1993; Bauman et al. 1993; Hamer 1986; Kagi and Schaffer 1988; Yamada and Koizumi 1991). There is growing evidence that metal exposure is not the only factor involved in MT induction (Pottinger and Calder 1995).

A metal-binding protein must meet the following criteria to be classified as MT (Hamer 1986; Hamilton and Mehrle 1986; Roesijadi 1992): (1) cytosolic location in the cell; (2) low molecular weight (6,000-7,000 Da from amino acid composition, 10,000-15,000 Da from gel-filtration chromatography); (3) high metal content (4-12 metal atoms per mole of protein); (4) amino acid composition with high cysteine content, and the absence of both aromatic acids and histidine; (5) unique amino-acid sequence, particularly in regard to the locations of cysteine; (6) metal-thiolate clusters; and (7) heat stability (60°C for 5 min). These criteria are based upon horse renal Cd-binding proteins, which were the first MTs discovered (Margoshes and Vallee 1957; Kagi and Vallee 1960). Similar metal-binding proteins that do not meet one or more of these criteria are referred to as "metallothionein-like proteins."

Metallothioneins are further classified as Class I, II, or III, based on the locations of cysteine residues, and the mode of MT synthesis (Roesijadi 1992). Class I MTs are polypeptides with locations of cysteine similar to those of horse kidney. Class II MTs are polypeptides with cysteine locations only distantly related to those of horse kidney. Class III MTs are nontranslationally synthesized metal-thiolate polypeptides referred to as cadystin, phytometallothionein, phytochelatin, or γ-glutamyl-cysteinyl-glycine. Class III MTs have been identified in some plants and fungi.

Each class of MT is further subdivided into several isoproteins, where each isoprotein is designated by a lower case letter (e.g., MT-I_a, MT-I_b, etc.) (Hamer 1986; Kagi and Schaffer 1988). IsoMTs are resolved from a MT-class fraction by high-performance liquid chromatography (HPLC). The organism with the most complex isoMT composition is the human, where up to 10 isoproteins are present, some of which are tissue-specific (Kagi and Schaffer 1988).

A remarkable feature of MT is its similarity, especially with respect to the highly conserved placement of cysteine residues, among species (Andrews 1990). Metallothioneins from higher eukaryotes are straight-chained proteins, 60 to 63 amino acids in length. Cysteine makes up approximately 30% of the total protein (Kagi and Schaffer 1988). Cysteine residues take on three specific arrangements within the protein: Cys-Cys, Cys-X-Cys, and Cys-X-Y-Cys, where X and Y refer to amino acids other than cysteine. Each of these short cysteine sequences are flanked by either argenine or lysine, which implies that argenine and lysine play some part in the metal-binding role of MT (Hamer 1986). Placement of these short sequences in MT is highly

conserved among species (Hamer 1986; Kagi and Schaffer 1988), suggesting MT's slow evolution and probable role in metal regulation (Andrews 1990).

Apo-MT (or thionein, MT without any metals bound to it; i.e., the gene-expression product) is a randomly coiled protein (Andrews 1990). Upon metals binding to apo-MT, rather than forming disulphide bonds, 42 metal-thiolate bonds are produced from cysteine residues, giving stability to the MT secondary structure. Each molecule of MT binds to seven bivalent metal ions, such as Zn(II) or Cd(II), or twelve monovalent metal ions, such as Cu(I). Each bivalent metal ion is held in place with four metal-thiolate bonds in a distorted, tetrahedral-like structure, where two or more metal ions may share thiolate bonds.

Metal binding on MT occurs in two metal-thiolate clusters, named the α- and β-domains, that correspond to the placement of cysteine residues along the protein (Andrews 1990). The α-domain occurs at the carboxyl-terminal region of MT, whereas the β-domain occurs at the amino-terminal region. The α-domain, having 11 cysteines, binds to four bivalent- or five to six monovalent-metal ions, whereas the β-domain, having 9 cysteines, binds to three bivalent or six monovalent ions (Hamer 1986). Metal binding occurs by the α-domain forming a left handed coil around a metal core, whereas the β-domain forms a right-handed coil around its metal core (Andrews 1990). A hinge region separates the two MT domains, and each domain binds to metals independently of the other.

Metal binding to apo-MT is an ordered process (Andrews 1990). Zinc and Cd bind to the α-domain first. Once the α-domain is saturated, Zn or Cd binds to the β-

domain. In contrast, Cu binds to the β-domain first, then to the α-domain. Contradictory to the ordered metal binding to apo-MT, Cd replaces Zn from MT in a random fashion from both domains. Metals are more easily displaced from the β-domain than the α-domain, probably due to a greater number of Cys-metal-Cys cross-links in the α-domain.

Metal binding to apo-MT is a reversible, dynamic-equilibrium process (Andrews 1990). Stability constants for Cu, Cd, and Zn are 10^{19}-10^{17}, 10^{17}-10^{15}, and 10^{14}-10^{11}, respectively (Hamer 1986). In addition to Cu, Cd, and Zn, mammalian MT has also been shown to bind with Hg(II), Co(II), Pb(II), Ni(II), Ag(I), and Au(I).

1.2.1. Metallothionein Function

Many transition metals, such as Cu and Zn, are ubiquitous in the environment and serve important biological functions (Goyer 1991). For example, Zn has been shown to play an important role in over 20 metalloenzymes, such as DNA and RNA polymerases, as well as protein synthesis and degradation enzymes. Copper also occurs in many metalloenzymes, such as cytochrome c oxidase, dopamine β-hydroxylase, superoxide dismutase, and lysyl oxidase. Severe deficiencies of either Zn or Cu lead to impaired biological function, whereas exposure to relatively high concentrations can be toxic. Both Ni and Mo are thought to be essential (Goyer 1991), which suggests that MT may play some role in their regulation in fish.

Other transition metals, such as Cd or Hg, serve no known biological function (Goyer 1991). Toxicity results in organisms exposed to relatively high concentrations

of the essential metals Cu and Zn, whereas Cd and Hg are toxic at very low concentrations.

Metallothioneins have been extensively studied for almost 40 years, yet their *exact* function still remains largely unknown (Hamer 1986). Considering the two aspects of metal metabolism described above, namely metal essentiality and toxicity, MTs may serve two important biological functions: essential-metal regulation (i.e., homeostasis) and metal detoxification (Roesijadi 1992). During essential-metal regulation, MTs donate metals such as Cu and Zn to metalloenzymes and cofactors. Conversely, MTs bind to non-essential metals, or excesses of essential metals, effectively sequestering the metals, thereby protecting the organism from metal toxicity.

Several studies have demonstrated that organisms pre-exposed to low doses of a toxic metal are more tolerant to subsequent higher doses of the same metal (Buckley et al. 1982; Dixon and Sprague 1981; Kito et al. 1982; Pruell and Engelhardt 1980; Roch and McCarter 1984). One hypothesis to explain this phenomenon suggests that when an organism, such as a fish, is exposed to low concentrations of metals, MT production is induced. The increase in MT available to sequester excess metal ions is necessary to reduce any toxicological effects that might arise from the elevated metal concentrations. Because MT production is already at an increased concentration due to the low-concentration metal exposure, any further metal challenge can be more readily accommodated by the excess MT. It is only when MT becomes saturated and excess metal 'spills over' from the MT pool that toxicity results (Hamilton and Mehrle 1986).

Normal physiological processes such as growth, molting, reproduction, development, and cellular differentiation, require increased concentrations of essential metals (Roesijadi 1992). Consequently, MT can be induced during these processes in order to provide necessary essential metals. Although MTs have recently been suggested for use as biomarkers in organisms exposed to elevated concentrations of metals, normal MT metabolism, including increased MT production to accommodate increased metal requirements, may reduce MT's effectiveness as a biomarker.

1.3 Background and Rationale for This Study

1.3.1. Study Area and Historical Background

The Key Lake uranium mine in northern Saskatchewan (Fig. 1.1) holds the long-standing distinction as the most productive uranium mine in the world (Cameco Corp. 1998a). Key Lake produced $5.6\times10^6\pm1.5\times10^6$ kg (mean±SD) of U_3O_8 annually between 1983-1998, for a total output of 90.1×10^6 kg U_3O_8 (Cameco Corp. 1998a; Saskatchewan Energy and Mines 1999a). Average ore grade over the same period was 2.3±0.2% U_3O_8.

The Key Lake uranium operation consists of two open pit mines; the Gaertner and Deilmann pits (Saskatchewan Energy and Mines 1999b). Mining of the Gaertner ore body occurred between 1982-1987, whereas the Deilmann ore body was mined between 1986-1997 (Cameco Corp. 1998a; Cameco Corp. et al. 1995). Although mining ceased in 1997, Key Lake had 5.6×10^6 kg of ore reserve at the end of fiscal year 1998 (Cameco Corp. 1998a). Ore from the new McArthur River mine, approximately

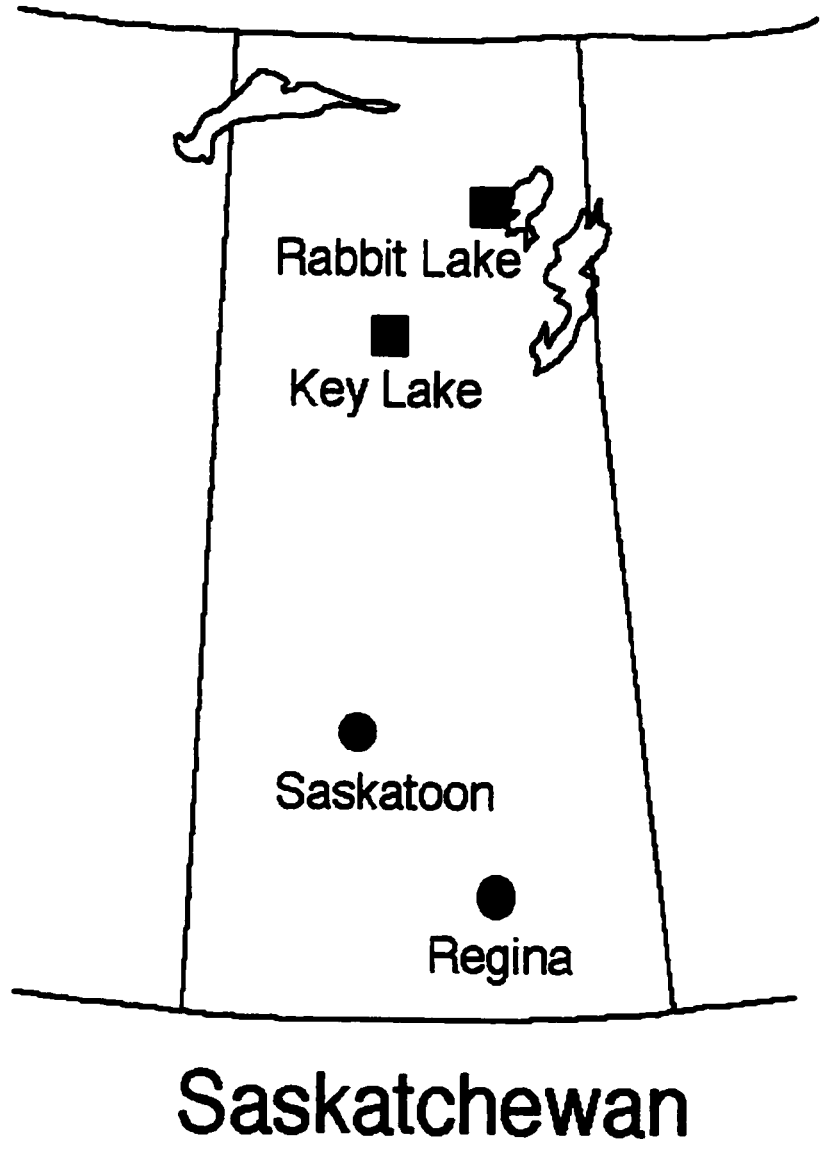

Figure 1.1: Map of Saskatchewan showing the relative locations of the Key Lake and Rabbit Lake uranium operations.

70 km northeast of Key Lake, will be milled at Key Lake for the next twenty years (Cameco Corp. et al. 1995).

Key Lake uranium ore was characterized by a close association between uranium and nickel (Saskatchewan Energy and Mines 1999b). Ore grades varied widely up to a maximum of 35% U_3O_8 and 20% Ni (Saskatchewan Energy and Mines 1999b). Other minerals in the uranium ore included gersdorffite (NiAsS), millerite (NiS), niccolite (NiAs), and bravoite ([Ni,Fe]S_2). Lesser amounts of pyrite (FeS_2), galena (PbS), safflorite ([Co,Fe]As_2), sphalerite (ZnS), chalcopyrite ($CuFeS_2$), hematite (Fe_2O_3), and magnetite (Fe_3O_4) were also present (Saskatchewan Energy and Mines 1999b).

The two effluent types released to the aquatic environment that are of particular concern to this study derive from ore milling and pit dewatering. Crushed uranium ore is fed to the mill as slurry. Uranium is extracted from the ore in an organic solvent (i.e., amine-kerosene mixture). Extracted uranium is recovered using an ammonia sulphate strip solution. Further addition of ammonia causes uranium to precipitate as ammonium diuranate, which is then dried to produce yellowcake (i.e., U_3O_8) (from Thomas 1997). Solid wastes from this process are treated and released to the tailings management facility (TMF). Liquid wastes are treated to remove ^{226}Ra, pH adjusted, and released to the TMF or settling ponds. After a monitoring period liquid from the settling ponds is released to the environment at Wolf Lake (Thomas 1997).

In 1997, 2.54×10^6 m^3 of liquid mill effluent was released to Wolf Lake (Cameco Corp. 1998b). Wolf Lake drains via Wolf Creek into Fox Lake[2]. Fox Lake drains via Yak Creek into David Creek, which in turn empties into Unknown Lake. Historical water quality monitoring of Fox and Unknown lakes has confirmed elevated concentrations of Mo relative to reference waters (i.e., background concentrations)

(Cameco Corp. et al. 1995). At present there are no regulations governing the release of Mo to the aquatic environment (Saskatchewan Environment and Public Safety 1988). However, because Mo release has been identified as an important effluent constituent associated with uranium mining in Canada (Golder Associates Ltd. 1996), mining regulators are currently developing water quality criteria pertaining to Mo (S. Munger[3], pers. comm., 1999).

Nickel is released to the aquatic environment at Key Lake through pit dewatering effluent (Cameco Corp. et al. 1995). Historical monitoring of mass loading of Ni into receiving waters and effluent quality has indicated a rise in Ni concentrations with time (Figs. 1.2 and 1.3). A reverse osmosis (RO) plant was built in 1997 to remove excess Ni from dewatering effluent prior to release into receiving waters; i.e., Horsefly Lake. Horsefly Lake drains immediately into Little McDonald Lake, which in turn drains into McDonald Lake through a short, narrow, shallow channel. In 1997, 8.4×10^6 m^3 of dewatering effluent was discharged into Horsefly Lake at the Key Lake mine site (Cameco Corp. 1998b). Of the total effluent volume, 3.8×10^6 m^3 came from Deilmann pit dewatering, 3.0×10^6 m^3 was from Gaertner pit dewatering, 1.7×10^6 m^3 was released from the RO plant, and 8.6×10^4 m^3 was recycled back into industrial operations (Cameco Corp. 1998b). Historical water quality monitoring of lakes downstream of the dewatering effluent discharge indicate that Ni occurs at concentrations that exceed the Saskatchewan Surface Water Quality Objective for Ni, which is 0.025 mg Ni/L (Saskatchewan Environment and Public Safety 1988).

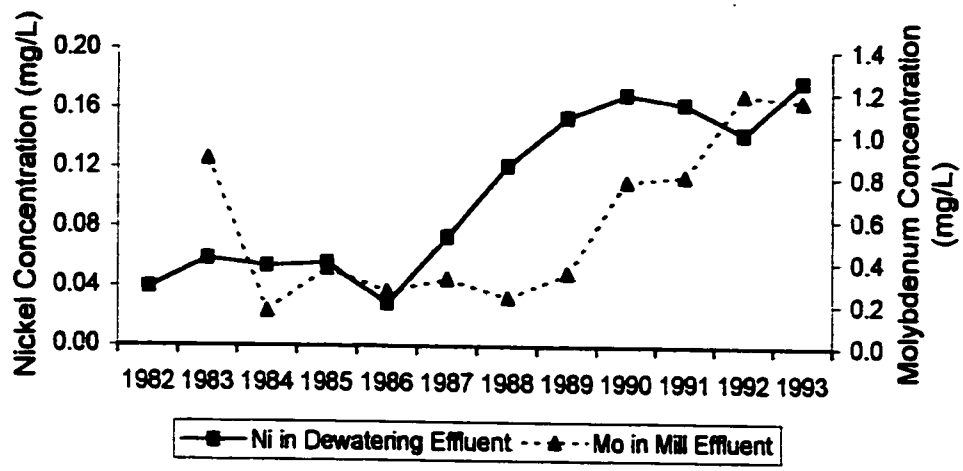

Figure 1.2: Mean annual Ni and Mo concentration in mill and dewatering effluents from the Key Lake mining operation from 1982-1993. Points representing 1993 represent only 9 months of data, whereas other points represent 12 months. Standard deviations are not shown for the sake of clarity. (Data from Cameco Corp. et al. 1995.)

Cameco's Key Lake uranium operation provides an excellent opportunity to study Ni and Mo bioavailability and toxicity to fish. Not only will data be relevant to fish inhabiting receiving waters near the Key Lake mine, but they will also have relevance for fish inhabiting Ni- and Mo-contaminated systems everywhere.

1.3.2. Why Study Nickel and Molybdenum?

The previous section described aquatic systems receiving mine-related effluents from Key Lake uranium operations as being contaminated by Ni and Mo. Temporally increasing Ni and Mo concentrations prompted Cameco to initiate a number of studies, especially with respect to Ni loading to the Horsefly-Little McDonald-McDonald Lake chain (Cameco Corp. et al. 1995). The purpose of these studies was to investigate the effects of increasing Ni and Mo concentrations to indigenous biota (Cameco Corp. et al. 1995).

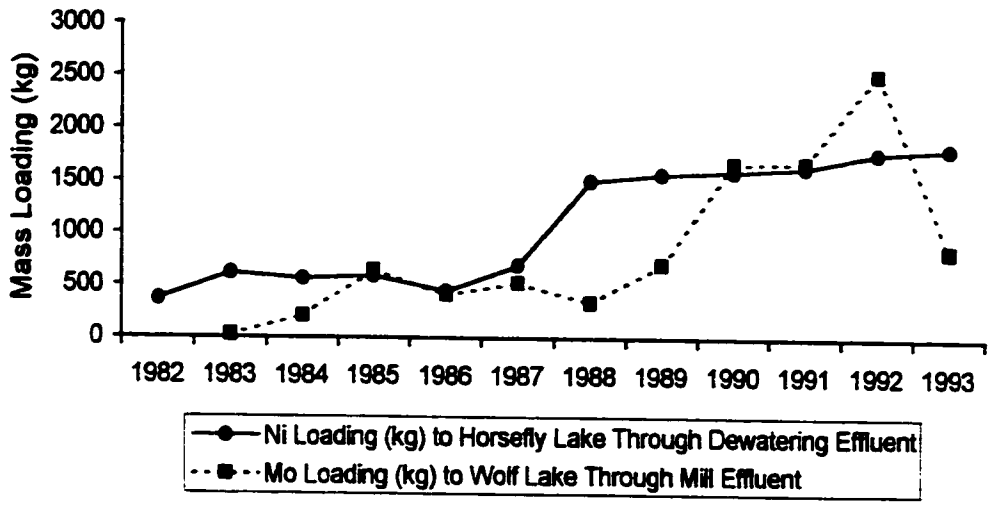

Figure 1.3: Mean mass Ni and Mo loadings (kg) to Horsefly and Wolf Lakes, which receive dewatering and mill effluents, respectively, between 1982-1993. Points representing 1993 represent only 9 months of data, whereas other points represent 12 months. Standard deviations are not shown for the sake of clarity. (Data from Cameco Corp. 1995.)

The problem of elevated Ni and Mo concentrations in aquatic systems, especially in areas around certain industrial operations, is not restricted to Key Lake. In a comprehensive review, the Atomic Energy Control Board (AECB) reported that the most significant contaminants associated with mining and milling of uranium in Canada were As, Ni, and Mo (Golder Associates Ltd. 1996). The same report emphasized the need for more studies addressing the toxicity of Ni and Mo because of the severe lack of data that currently exist. In a review of over 700 documents relating to 95 mine sites, the final Assessment of the Aquatic Effects of Mining in Canada (AQUIMIN), recommended that Ni be included in the revised Metal Mining Liquid Effluent Regulations (MMLER) (AQUAMIN Working Groups 7 and 8 1996).

Nickel contamination has also been reported in aquatic environments affected by uranium operations in the United States, in addition to Ni mining (Brotheridge et al. 1998; Gunn 1995), stainless steel manufacture (Krantzberg and Boyd 1992; Krantzberg

1994; Mayer and Johnson 1994), pesticide formulation, electrical battery manufacture, metal alloy production, and urban runoff (Galvin 1996), among others. Despite the variety of different human activities that introduce Ni into natural waters, surprisingly little research has been done to identify its effects on aquatic biota. More specifically, research that examines factors known to affect metal toxicity to fish (e.g., water hardness, pH, and dissolved organic carbon) has been practically ignored for Ni.

Toxicity of metals to fish has been receiving considerable research attention recently, although the focus has been on Ag (Erickson et al. 1998; Morgan et al. 1997), Cd (Brown et al. 1994), Cu (Welsh et al. 1996), and Zn (Alsop et al. 1999). Metals like Ni and Mo have received very little attention.

Canada's economy is based on its natural resources. Mining represents a significant proportion of those resources, given that Canada is the world's top uranium (The Uranium Institute 1999) and Ni producer (Doan 1997). Because these mining practices are associated with Ni and Mo contamination of aquatic systems, investigations into Ni and Mo toxicity to aquatic biota inhabiting those systems have significant socioeconomic, as well as academic, relevance.

1.3.3. Why Use Toxicity Tests Involving Fish?

The presence of water in a liquid form is thought to be the primary reason why life exists on Earth. Water has unique qualities, such as its high specific heat capacity, its property of occurring in three distinct material phases, its complex temperature-density relationship, its polarity, its ability to act as an acid or base in chemical reactions, and its capacity to dissolve inorganic substances (Wetzel 1983). These

characteristics combine in aquatic systems to form habitats that are qualitatively unique relative to others (Rand et al. 1995). In an aquatic environment, water acts as a chemical solvent that can change the nature of a contaminant through complex chemical reactions (Stumm and Morgan 1981). Relatively non-toxic contaminants can be rendered toxic upon chemical reaction with other dissolved compounds. Pristine aquatic systems are complex and precariously balanced in a state of delicate, yet dynamic, equilibrium among their myriad components. Additions of one or more exogenous chemical components, surpassing the system's assimilative capacity (Cairns 1977), serve only to disrupt this delicate balance. Consequently, essential life-giving qualities of water integrate to form an environment that is sensitive to chemical contamination (Rand et al. 1995).

The primary environmental impact of mining operations that make use of liquid extraction techniques (Ripley et al. 1996) is typically greatest in the aquatic environment (Barbour 1994). Sulphur-bearing ore dug from the ground and concentrated at the surface can generate acid upon contact with water and air. Acid production releases metals, which can then leach into nearby aquatic systems (Ripley et al. 1996). Mining and milling operations make use of large volumes of water in routine processes (e.g., product extraction, pit dewatering, etc.). This water can become contaminated through contact with contaminated surfaces. Contaminated water is often treated to remove potentially harmful constituents prior to release into an aquatic environment. However, many data now exist to show that contaminant concentrations are frequently higher in

receiving waters relative to reference waters upstream of mining operations (Ripley et al. 1996).

Fish represent the resident biota of mine receiving waters and have an intrinsic importance, in terms of both ecology and socioeconomics. Each species in an ecosystem occupies an important niche. All species within a community make up the complex and finely balanced nature of an ecosystem. Disruption of one affects all others in a cascade of events, including shifts in community structure, species abundance, inter- and intra-specific competition, etc. (Carpenter and Kitchell 1992).

Fish are ecologically important in most aquatic ecosystems. They are both predators and prey. Predatory fish, like northern pike (*Esox lucius*), represent the top of the food chain in an aquatic system, which is a trophic position that has been identified as vulnerable to contaminants (Carpenter and Kitchell 1992). Bottom feeding fish, like white suckers (*Catostomus commersoni*), control benthic invertebrate populations. Cyprinids, like fathead minnows, are forage fish; many are omnivorous, and often have diets comprised of mostly plant and algal material. The habitat diversity occupied by fish is suggestive of the many uptake routes potentially available to xenobiotics.

Fish serve as sentinels for environmental perturbations (De Flora et al. 1993; Ewald 1995; Haines and Brumbaugh 1994; LeBlanc and Bain 1997; Parks et al. 1994; Zhang and Schlenk 1995). Water bodies that change from supporting healthy fish populations to ones with few or no fish are immediately suspect of environmental modification. Changes in fish population structure, like age profile, gender distribution, and the absence or presence of young (and their relative abundance) can indicate

environmental flux. Similarly, a contaminated system whose fish population was decimated by contaminant influx, which suddenly shows signs of fish recruitment, is indicative of improving conditions (Keller and Gunn 1995).

Fish are important socioeconomically. When the public considers the impact of mining on aquatic systems, it rarely considers effects on benthic macroinvertebrate communities; instead, it considers effects on fish. Certain fish species that inhabit mine-effluent receiving waters, like lake trout and northern pike, are highly prized sport fish. Upon decommissioning of mining operations, mining companies must restore receiving waters to their original state, or at least one that supports a functioning ecosystem, or to a state that will benefit humans (Ripley et al. 1996). Often, water bodies cannot be returned to their original state after receiving years of mine effluent. However, it is possible to reclaim a water body by returning it to a condition that supports healthy fish populations. Having done so satisfies the condition that the aquatic environment is returned to a state that is useful to humans.

Fish, in addition to benthic invertebrates, have been identified by AQUAMIN as, "key ecosystem components," and are the focus of a large proportion of regulatory compliance efforts (AQUAMIN Working Groups 7 and 8 1996; Cameco Corp. et al. 1995). Owing to their importance in aquatic communities, fish are currently used to characterize and monitor toxicity of mine polluted water (in addition to the water flea, *Daphnia* spp. or *Ceriodaphnia* spp.).

Fish are not necessarily more sensitive to contaminants than other species—in fact, many studies have shown that fish are relatively tolerant to metal intoxication

compared to other non-vertebrate organisms (e.g., Kszos et al. 1992; Masnado et al. 1995). However, aquatic toxicology deals with understanding the effects of contaminants on individuals so that the system as a whole can be more completely understood (Cairns 1986; Moriarty 1983; Newman 1995; Rand et al. 1995). Adequate understanding of a complex system that receives environmental contaminants translates into protection for resident biota via decision-making channels (i.e., from industry, industrial regulators, and society). Because fish have an obvious and intrinsic importance in aquatic systems, it is essential to study effects on fish of contaminants that pollute their environment.

1.3.4. Why is the Laboratory-to-Field Extrapolation So Important?

Relating experimental results derived in the laboratory to a natural situation is the single most important goal of aquatic toxicology. Observation of an effect under laboratory conditions does not necessarily translate to an accurate prediction of the same effect in a natural setting. On the contrary, toxicology laboratories are unrealistic exposure settings because variables such as test organism health, exposure temperature, photoperiod, feeding frequency, food quality, water quality, dissolved form (species) of the contaminant, etc. are controlled. On the other hand, natural systems are dynamic and stochastic.

Fish reared in the laboratory for toxicity testing are held under optimal conditions, compared to fish from natural populations which can be highly stressed or malnourished (Holdway 1992). As a result, the use of well-maintained fish in toxicity tests may lead to a Type II statistical error: that is, determining that a contaminant is safe

when in fact it is hazardous. Cairns (1991) gives five illustrative reasons why laboratory-derived toxicity data may not reflect toxicity in nature: (1) only a small and uniform sample of organisms was tested out of a much larger natural population, (2) a surrogate organism was used rather than one inhabiting the natural system, (3) the time of exposure in the natural system was much longer than that in the laboratory test system, (4) synergistic effects occurring in the natural system were not incorporated into the test procedures in the laboratory system, and (5) the most important or sensitive response was not monitored in the laboratory test.

During environmental evaluations of metal polluted systems, researchers have historically taken measurements of total metal concentrations in the water and attempted to relate those measurements to toxic effects (Stumm and Morgan 1981). Early experimenters realized that weak correlations between total metal concentrations in water and biological effects were probably related to water quality. However, the nature of this relationship did not become apparent until recently (Renner 1997).

The development of the free-ion activity model (FIAM) was an early attempt to resolve this issue (Morel 1983). This model suggests that the free, bivalent metal cation is the most toxic form (i.e., species) to aquatic organisms. As water pH decreases from high (i.e., >7) to low (i.e., <7), metal speciation generally simplifies. At high pH the total metal concentration in the system is spread over a number of different species (e.g., carbonates, hydroxides, sulphides, etc.). At low pH most of the total metal in the system is in the form of the free, bivalent cation (M^{2+})—considered to be the most toxic species to aquatic organisms for most metals. This model proved to be an important and useful

improvement over earlier work, because it could account for why metals were generally more toxic at low pH.

Subsequent work building on the foundation of the FIAM resulted in many significant contributions towards the current understanding of metal bioavailability and toxicity to aquatic biota, including fish. Water hardness generally reduces metal toxicity to fish through competitive exchange reactions between Ca^{2+} and M^{2+} at fish gill surfaces (Alsop et al. 1999; Erickson et al. 1996; Erickson et al. 1998; Meyer et al. 1999; Playle et al. 1992). Dr. Richard Playle and coworkers (Hollis et al. 1996; 1997; Janes and Playle 1995; 1993a; Playle et al. 1993b; Richards and Playle 1998) have demonstrated how dissolved organic carbon strips M^{2+} from the water column, rendering it unavailable for biotic uptake and thereby reducing toxicity. Although improvements upon the FIAM are continuously being published in the primary literature, it has recently been criticized as being too simplistic to account for the tremendous complexity of natural waters (Campbell 1995). Consequently, the state of the science today is not at a level that can extrapolate accurately and consistently laboratory toxicity results to natural waters (Renner 1997).

A thorough understanding of how a particular metal exerts its influence on a biological system requires toxicological knowledge at multiple levels of biological organization. Metallothionein regulates metals and provides protection against metal toxicity at the cellular level in fish. Individual-level responses to metal exposure can be gauged from mortality (i.e., LC50) data, which provide insight into the toxicity of a particular metal relative to other metals. Because fish are not likely to be exposed to

acute concentrations of metal in a natural environment, sublethal toxicity tests on sensitive early-life stage fish provide an indirect means of making predictions about population-level effects, and thereby provide more ecologically relevant toxicological data (Woltering 1984). Laboratory toxicity tests, using reconstituted and artificial water—in addition to field-collected water—performed in conjunction with *in situ* tests, provide information on the relevance of laboratory tests for making predictions about wild, metal-exposed fish.

Currently, guidelines and regulations of water quality criteria are based on single metals. For example, the Saskatchewan Surface Water Quality Objectives (SSWQO) for Ni in soft water is 0.025 mg/L, and for As it is 0.05 mg/L (Saskatchewan Environment and Public Safety 1988). Metal Mine Liquid Effluent Regulations (MMLER) are similarly based. However, in a natural system organisms are exposed to a mixture of contaminants, not single contaminants. That single metals are regulated in complex industrial receiving waters leads to an obvious question about whether legislation regulating metals in the environment is overly protective, or not protective enough, to maintain biological integrity in receiving waters. One recent example makes the point abundantly clear. Meyer et al. (1999) showed that competitive binding of metal cations to binding surfaces (like dissolved organic carbon), in a complex mixture of metals typical of a mine receiving water, increased bioavailability of metals that would otherwise have been predicted unavailable if considered singly. The implication here is that regulations based on laboratory-derived effects of single metals is an overly

simplistic approach to provide adequate protection to aquatic organisms, like fish, in a complex system like a natural water body.

Governmental regulators and industrial environmental managers make important environmental decisions based on results of toxicity data—data that are mostly derived in a laboratory. Good environmental decisions must be based on the most accurate and ecologically relevant data. Consequently, a clear understanding of the environmental behaviour and subsequent biological effects of metal exposure in fish must be attained in order that sound environmental decisions are made (Renner 1997).

1.4 Study Design (Overview)

1.4.1. Five Key Questions and Associated Hypotheses

The purpose of this study was to provide information on Ni and Mo bioavailability and toxicity to freshwater fish. Data derived from this study are relevant not only to the Saskatchewan mining community who must deal with Ni- and Mo-contaminated receiving waters, but also to the wider mining community faced with similar metal contamination in receiving waters. More importantly, this study is meant to provide sound toxicological data to advance understanding of metal behaviour in natural water systems as it relates to fish toxicity. A complete understanding of contaminant effects in contaminated systems will lead to sound environmental decisions that will eventually allow a harmonious relationship between ecology and economy.

The objective of this study was to provide answers to five key questions:

1. Are standard toxicity-test fish species representative of non-standard, native fish species in terms of predicting metal toxicity?

2. How do toxicity results derived in the laboratory relate to toxicity *in situ*?
3. How toxic are nickel and molybdenum to fish?
4. How does nickel speciation affect bioavailability and toxicity to fish?
5. Can metallothionein be induced in fish exposed to metals such as nickel or molybdenum—i.e., metals other than those of Groups IB or IIB of the periodic table?

Each of the questions is discussed below, in terms of its scientific basis and the research approach, under individual headings.

1.4.1.1. Question 1: Are standard toxicity-test fish species representative of non-standard, native fish species in terms of predicting metal toxicity?

The toxicity of mine effluents and receiving waters is routinely characterized via standardized toxicity tests using one or two fish species, like fathead minnows and rainbow trout (Canadian Environmental Protection Service 1990; 1992a; 1992b). Neither of these species inhabits receiving waters of northern Saskatchewan uranium mines. Consequently, it is unclear whether or not toxicity data derived using these species is adequate for protecting indigenous species like northern pike or white suckers.

To answer this question, a series of toxicity tests were conducted using early-life stage fathead minnows, rainbow trout, northern pike and white suckers. Freshly fertilized eggs and newly hatched larvae were placed in serial dilutions of Ni and Mo to determine effects on egg hatchability, time required for hatching, growth, and mortality. Early-life stage fish were used instead of older fish because toxicity results on the former are thought to be more sensitive than the latter. Toxicity results of early-life

stage tests also provide as much information as longer, full-life cycle tests (Woltering 1984). The hypothesis being tested is that there is no difference among the four fish species with respect to egg hatchability, time-to-hatch, growth, or mortality when exposed to acute and sublethal concentrations of Ni and Mo. Put another way, standard toxicity-test fish species serve as acceptable surrogates for predicting Ni and Mo toxicity to fish species indigenous to northern Saskatchewan lakes.

1.4.1.2. Question 2: How do toxicity results derived in the laboratory relate to toxicity in situ?

As discussed above, the primary goal of aquatic toxicology is to link laboratory-based results to the natural world. In order that sound environmental decisions can be made about an aquatic system affected by industrial activities, it is important to ensure that laboratory tests relate to the natural system in question.

To address this question, early-life stage fathead minnows were exposed to natural waters in the laboratory and in the field. Larval fathead minnows were placed in exposure units in five lakes surrounding the Key Lake uranium operation. Two lakes, Fox and Unknown lakes, receive Mo-contaminated mine-mill effluent. Two other lakes, Little McDonald and McDonald lakes, receive Ni-contaminated open pit mine dewatering effluent. David Lake is situated upstream of mining operations and served as a reference. Larval fathead minnows were placed in exposure tubes in each of the lakes for 7 d to monitor growth and mortality. Water samples from each of the four exposure sites per lake were characterized by metal concentration and basic water-quality parameters. Differential responses of larval fathead minnow growth or mortality were

related to exposure-site chemistry using principal components analysis followed by multiple regression.

In the second portion of the study, early-life stage fathead minnows were exposed in the laboratory to water collected from each of the exposure sites of the field study. Freshly fertilized eggs and newly hatched larvae were tested for egg hatchability, time required for hatching, larval growth, and mortality. Results from the laboratory study were compared to results derived in the field in order to determine if laboratory toxicity was related to toxicity observed in the field.

In a separate but related side project, water samples were also collected from an old, mined-out, flooded open-pit uranium mine, called the B-Zone pit, near the Rabbit Lake uranium operation near Wollaston Lake in northern Saskatchewan. Historical water quality monitoring of B-Zone water has demonstrated elevated concentrations of Ni, which makes this study an excellent opportunity to examine effects of Ni contaminated water from a 'natural' system.

Results from the first two tests (i.e., *in situ* toxicity tests and toxicity tests on water samples collected from exposure sites of the *in situ* study) were discussed in terms of how well laboratory results relate to toxicity results *in situ*. The B-Zone study served as an excellent example of how Ni-contaminated water affected by uranium operations affects early-life stage fish. Moreover, the B-Zone study, owing to the relative simplicity of its water chemistry (compared to mill-effluent receiving waters of Key Lake), provided an excellent opportunity to relate laboratory-based toxicity results of a Ni-spiked reconstituted soft water to a naturally soft Ni-contaminated water.

1.4.1.3. Question 3: How toxic are nickel and molybdenum to fish?

An obvious and basic question of this study is; how toxic are Ni and Mo to fish? As previously discussed, several metals have recently been receiving disproportionately more research attention than others have. Nickel and Mo have received little research attention over the years, partially because of their relatively low toxicity to freshwater organisms (as compared to other highly toxic metals like Ag, Cd, and Hg). This paucity of information is particularly true for Mo, which has received almost no research attention.

This question was addressed both directly and indirectly over most of the studies conducted during this investigation. Tests were conducted on early-life stage fathead minnows, rainbow trout, northern pike, and white suckers (see Question 1) in hard laboratory dilution water. Larval fathead minnows were exposed to serial dilutions of Ni in soft, reconstituted waters with varying pH, hardness, and suspended solids to determine relative mortality (LC50; see Question 4 below).

1.4.1.4. Question 4: How does nickel speciation affect bioavailability and toxicity to fish?

A growing consensus in the scientific community suggests that understanding of metal chemistry under varying water quality conditions is fundamental to making meaningful predictions about metal toxicity in nature. Water hardness was the first water quality parameter to emerge from this new school of thought as one of the key parameters affecting metal toxicity to fish. The mechanism for protection occurs via competitive exchange reactions between Ca^{2+} and the free bivalent metal cation (M^{2+}) at the gill surface. Although this effect has been well documented for other metals, it has

been largely neglected for Ni. Increasing pH of exposure water is also thought to decrease metal toxicity by changing metal speciation from M^{2+} to other less available metal complexes or species (e.g., carbonates, hydroxides, and sulphides, etc.). However, tests of some metals under varying levels of pH have resulted in observations that contradict this relatively simplistic idea. Of particular relevance to the mining community is to understand the effects of suspended solids on Ni toxicity. Elevated concentrations of suspended solids are typical in mine-effluent receiving waters. Just as metals bind to dissolved organic carbon and become unavailable for biological uptake, it is possible that metals bind to suspended solids in the same way resulting in reduced toxicity under elevated suspended solids. There currently exists some data to suggest that biological uptake of some metals is reduced in the presence of suspended solids. The effect of suspended solids on Ni toxicity to fish has not been tested.

A series of laboratory toxicity tests were performed with larval fathead minnows in soft, reconstituted water under variable hardness, pH, and total suspended solids (TSS) conditions to determine their effects on Ni toxicity. Reconstituted soft water was used in place of dechlorinated Saskatoon municipal water to closely represent the soft waters of northern Saskatchewan lakes. Toxicity was expressed as 96-h LC50 (i.e., the concentration of Ni required to kill 50% of the test organisms in 96 h) so that relative toxicity could be compared with other values reported in the literature for Ni and other metals.

1.4.1.5. Question 5: Can metallothionein be induced in fish exposed to metals such as nickel or molybdenum—i.e., metals other than those of Groups IB or IIB of the Periodic Table?

Metallothionein (MT) is a cysteine-rich protein that is thought to regulate essential metals, and detoxify metals of Groups IB and IIB of the periodic table (i.e., Ag, Cu, Cd, Hg, and Zn). Fish exposed to metals are known to induce MT, presumably as a means of achieving metal tolerance. There has recently been a strong lobby to utilize fish MT as a biomarker for metal exposure. The new federal Environmental Effects Monitoring (EEM) for Mining has recommended the development of MT as a biomarker for fish inhabiting metal-contaminated water (AQUAMIN Working Groups 7 and 8 1996). Therefore, one purpose of the current study is to determine if exposure to metals like Ni or Mo, neither of which occur in Groups IB or IIB of the periodic table, results in *de novo* MT synthesis.

Juvenile rainbow trout were exposed to high and low concentrations of Ni, Mo, and Cd (a known MT inducer) for 7 d. At the end of the exposure period, fish gills, livers, and kidneys were removed and rapidly frozen in liquid nitrogen. Metallothionein concentration was determined in gills and livers using a mercury-saturation assay (Dutton et al. 1993). Metallothionein could not be detected in kidneys because of the small tissue samples yielded by juvenile fish. Relationships between elevated tissue MT and waterborne metal concentrations were discussed in terms of the usefulness of MT as a viable biomarker.

1.5 Fish Biology

1.5.1. Biology of Fathead Minnows

1.5.1.1. Range, Distribution, and Abundance

Fathead minnows in North America have been used extensively for many decades as one of the most popular baitfish species among anglers. Consequently, it has become difficult to resolve this fish's natural range. Currently, fathead minnows are common in streams, ponds, and lakes east of the Rocky Mountains to Quebec, Canada, and Maine, U.S.A., south to the Susquehanna River and southern Gulf states into Mexico. Fathead minnows west of the Rocky Mountains have been introduced. Fathead minnows have a very high tolerance to low oxygen concentrations in water as well as to extremes in pH, resulting in their ability to survive in many areas where other species cannot (Paetz and Nelson 1970; Eddy and Underhill 1974).

1.5.1.2. Ecology

Fathead minnows tend to inhabit ponds and lakes having muddy bottoms, although Paetz and Nelson (1970) report that they also occur in creeks, streams, and ditches, often with brook sticklebacks. Scott and Crossman (1973) report that fathead minnows prefer the still waters of ponds and lakes to the moving water of streams, and occur in association with five-spine sticklebacks (*Culaea inconstans*), pearl dace (*Margariscus margarita*), and finescale dace (*Phoxinus neogaeus*). Fathead minnows are tolerant to oxygen-poor water, and can occur in waters having salinities in excess of 10,000 mg/L (Scott and Crossman 1973), whereas Paetz and Nelson (1970) report that these minnows can also tolerate extremes in pH.

Fathead minnows are primarily herbivorous. Young fish feed mainly on planktonic algae switching later to filamentous algae. Adults feed on algae, aquatic insect larvae, zooplankton, and entomostracans (Scott and Crossman 1973; Eddy and Underhill 1974). Investigations into stomach contents revealed that large quantities of organic detritus and inorganic material were present, presumably through inadvertent ingestion while feeding on food organisms. Intestinal length of fathead minnows is long, approximately 2.0-2.5 times total body length, which is typical of herbivorous cyprinids.

The fathead minnow's ability to survive under a wide range of water-quality conditions, enabling it to survive in many water bodies, its wide geographic distribution, its ability to spawn all summer making young fish available to predators during summer months, and its high fecundity, make this species an ideal forage fish. All piscivorous fish and birds feed on fathead minnows where they occur.

1.5.1.3. Reproduction

Fathead minnows commence spawning in the spring when water temperatures reach approximately 15.6 °C (Scott and Crossman 1973), and continues throughout the summer. Spawning males turn black with the exception of wide vertical bands which remain light. Three rows of strong tubercles develop on their snout, whereas a spongy pad develops on their back anterior to their dorsal fin. The male herds a female into his spawning territory by nudging her with his snout. Typical spawning sites occur in shallow water and include the bottom of logs, sticks, boards, rocks, or, more rarely, lily pads. After much circling beneath the spawning substrate, spawning commences. The

male moves around usually to the left side of the female where he aligns his urogenital opening with hers. They each begin to vibrate their bodies vigorously against one another, whereas the male lifts the female on her side towards the undersurface of the spawning substrate. Once appropriately positioned, the female deposits a small complement of adhesive eggs. The two spawning fish keep repeating this behaviour until a substantial number of eggs have been fertilized and deposited, after which the male drives off the female. Once the eggs have been deposited, the male aggressively guards them against potential predators, including other male and female fathead minnows. In addition, males constantly stroke and clean the eggs using the dorsal spongy pad and snout tubercles (Eddy and Underhill 1974). Males may seek out a number of females in this fashion such that each nest has eggs from many females and at several different levels of maturation. Egg counts of typical fathead minnow nests had about 600 eggs per nest on average, whereas some nests contained only a few eggs, and others had over 6000 eggs per nest (Eddy and Underhill 1974).

Eggs are approximately 1.15-1.3 mm in diameter (Scott and Crossman 1973). Hatching time is temperature dependent and ranges from 4.5-6 days. At 25 °C eggs will hatch in 5 days. Markus (1934) reported that eggs taken from a natural setting into the laboratory died without an attendant male. However, when eggs were constantly agitated all the eggs hatched. Therefore, the purpose of the male is to clean and aerate the eggs in order to facilitate their successful hatching (Eddy and Underhill 1974). Newly hatched fathead minnows are approximately 5 mm long and white in colour.

Growth is rapid in warm, food-rich water such that sexual maturity can be achieved within one year.

Markus (1934) reported that postspawning mortality is approximately 80%, and seems to be characteristic of the species. However, postspawning mortality has not been identified in Canadian populations (Scott and Crossman 1973). The typical life span of the fathead minnow is approximately two years, although some instances of these fish achieving three years has been reported (Carlson 1967).

1.5.1.4. Relevance to Study

The uranium district of northern Saskatchewan is situated a significant distance north of the northernmost boundary of fathead minnows, which lies somewhere near the Churchill River. Consequently, fathead minnows do not inhabit any of the five study lakes in the Key Lake area. However, the fathead minnow is used extensively as a "standard" toxicity test fish species in aquatic toxicology laboratories across the country. Standard toxicity tests involving fathead minnows have allowed scientists to make inferences about certain contaminants' toxicity to fish inhabiting lakes where fathead minnows do not live. It is the purpose of the current study to determine if these inferences have any meaningful relevance to toxicity in "non-standard" native fish species. Because much is known about the biology and behaviour of fathead minnows, both under laboratory and field conditions, and fathead minnows are easily maintained in the laboratory, they will be used extensively in this study to resolve the bioavailability and subsequent toxicity of Ni and Mo both in the laboratory and in the field.

1.5.2. Biology of Rainbow Trout

1.5.2.1. Range, Distribution, and Abundance

The natural distribution of rainbow trout is in the eastern Pacific Ocean and freshwater west of the Rocky Mountains from Mexico to Alaska. However, due to its popularity as a game-fish species, rainbow trout now occur in many locations worldwide. In Canada, rainbow trout occur from Newfoundland to British Columbia. The northern Canadian range includes southern portions of Newfoundland, Nova Scotia, Quebec, and Ontario, to the central prairies, north to northern British Columbia and southern Yukon Territory. This species is widely abundant in Canada due mainly to its popularity as a sport-fish species, in addition to its economic viability as an aquaculture species. Consequently, fisheries-management organizations maintain rainbow trout populations in water bodies all across Canada.

1.5.2.2. Ecology

Three strains of rainbow trout are recognized by colour: the dark stream-dwelling species is the rainbow trout, a bright silver lake-dwelling species called Kamloops trout, and a large silvery anadromous species called steelhead. These strains, as well as having different colours, also represent three different life habits of rainbow trout; stream dwelling, lake dwelling, and anadromous.

Rainbow trout prefer cool-water habitats having a preferred temperature of 13 °C, and are most successful in water bodies having a temperature of 21 °C. Temperature becomes lethal to trout fingerlings at approximately 24 °C.

Stream-dwelling rainbow trout tend to live in moderate to large ranges within the lower, boulder-laden reaches of resident streams. Lake-dwelling fish tend to inhabit cool, deep lakes with plenty of shallow, productive areas. Paetz and Nelson (1970) report that rainbow trout are tolerant to low-oxygen conditions that occur in lakes during winter. However, extremely low oxygen conditions can result in large winter fish kills.

Young rainbow trout feed mainly on plankton. As size increases, diet shifts to insects and crustaceans, and eventually to other fishes. For the most part, rainbow trout feed along the bottom, although they also take emerging aquatic insects, as well as insects resting on the surface of the water. Large rainbow trout, especially those that are sea-run or from the Great Lakes, require a fish diet to achieve their large size. Rainbow trout restricted to small lakes can exist on a diet exclusively comprised of invertebrates, although they cannot achieve the large size of their sea-run counterparts.

Young rainbow trout residing in western streams are predated upon by other salmonids. In eastern streams, where similar large predatory fish are absent, predation on rainbow trout occurs by aquatic avifauna and various mammals. Young rainbow trout compete with other salmonids for food. In addition, rainbow trout are in direct food competition with other bottom-feeding and predacious fish species.

1.5.2.3. Reproduction

Spawning can take place either in the spring, from mid-April to mid-June, or in the late fall or early winter. Eggs fertilized in spring are likely to hatch in July, whereas eggs fertilized in the fall will not hatch until the following spring (Eddy and Underhill 1974). Although rainbow trout spawn both in the spring and fall, they are mostly spring

spawners (Scott and Crossman 1973). Spawning temperature is between 10.0 and 15.5 °C. Fish migrate up streams to spawn over a gravely substrate. Rainbow trout confined to lakes will spawn on gravely shoals. River-resident rainbow trout will usually ascend secondary tributaries for spawning.

Sexual maturity occurs earlier in males (as early as 1 year old under rare circumstances) than females (as late as 6 years) (Scott and Crossman 1973). In general, sexual maturity occurs between the ages of 3 and 5 years. Rainbow trout spawn annually throughout their adult lives. As spawning season approaches, males develop a kype on their lower jaw. Females move into spawning areas before males and select nesting sites. Once a site has been selected, the female will dig out a redd by turning on her side and fanning her tail rapidly to clear the area of sand and gravel. Typically, redds are longer and deeper than the female's body. Once the redd is complete and the female is ready to spawn, she positions herself in the bottom-centre of the redd and waits for attendant males. Usually, more than one male will attempt to spawn with a single female, although of the males attending a female, one will be dominant over the others. The dominant male positions himself parallel to the female, pressing his body against hers. The two fish arch and vibrate their bodies together, releasing eggs and milt over a few seconds. Fertilized eggs fall into cracks and spaces in the redd. The female then covers the eggs by turning on her side and fanning sand and gravel with her tail from the upstream-edge of the redd.

During a single spawning season, a typical female will spawn either with a single male or with several males in one or more redds. On average, a female will deposit

from 800-1000 eggs per redd. Total egg number per female can be as low as 200 and as high as 13,000, but averages about 4500. Eggs are approximately 3-5 mm in diameter, demersal, and pink to orange in colour (Scott and Crossman 1973).

Eggs hatch in approximately 4-7 weeks, depending on region, habitat, and temperature. After hatching, alevins take approximately 3-7 days to consume the remainder of their yolk sacs before commencing free swimming. Free feeding takes place approximately 15 days post-hatch. Fry of lake-resident spawners either migrate to the lake immediately, or remain in the stream for up to 3 years. Fry of river-resident spawners remain in their birth stream for approximately 2 years.

1.5.2.4. Relevance to Study

Several standard toxicity tests have been designed for early life-stage rainbow trout. Rainbow trout are an excellent toxicity-test fish species because they are relatively easy to maintain under laboratory conditions, they are sensitive to a wide variety of toxicants, and they are easily attainable due to their prolific use in fish-stocking programs, and preferred use in aquaculture. Although the Key Lake uranium mine is within the rainbow trout range in Saskatchewan, these fish do not inhabit lakes in the area. Water samples from Key Lake (and from Canadian mining operations in general) are routinely collected and sent for rainbow trout toxicity tests at laboratories across Canada. Conclusions based on these tests are questionable for several reasons. First, the routine testing only includes 96-h acute lethality tests. These tests are well known for being insensitive, especially under conditions where contaminant concentrations are sub-lethal and natural exposure of resident fish is chronic. Secondly,

by determining the toxicity of receiving water to a non-native fish species tells little about toxicity to native fish species. Thirdly, standardized toxicity testing takes place in the laboratory, and tells little about *in situ* toxicity. One purpose of the current study is to compare standard toxicity testing using rainbow trout with toxicity of other fish species that inhabit northern Canadian lakes.

1.5.3. Biology of Northern Pike

1.5.3.1. Range, Distribution, and Abundance

Northern pike are circumpolar in distribution, and occur only in the Northern Hemisphere. In Canada, it is one of the few species that can be said to occur throughout the country. Other than north of Ungava Bay, Labrador, the Maritime provinces, central and southern British Columbia, northern and eastern portions of the Northwest Territories, Nunavut, and the Arctic archipelago, northern pike occur everywhere else in Canada (Scott and Crossman 1973).

Northern pike have historically been perceived as an "undesirable" species by many people. Northern pike are said to have an extremely "fishy" taste when cooked with the skin still attached, probably because of the thick mucous coating that exists on this species. In addition, northern pike not only compete with other sport-fish species for food; they also prey upon them. This sentiment has led to massive efforts in the past to remove northern pike from certain areas. On the other hand, recent efforts by governments and other fisheries management organizations have turned this negative opinion to a positive one, such that the northern pike is now a considered a highly-prized sport-fish species. This positive attitude towards the northern pike by anglers has led to

massive stocking programs in many Canadian districts. For the most part, northern pike are now considered a desirable sport fish and its distribution has been extended to most parts of North America where appropriate habitat occurs.

1.5.3.2. Ecology

Northern pike inhabit a variety of different habitats, although they prefer warm, shallow, and weedy areas of lakes, and clear, warm, well-vegetated, slow-moving streams and rivers. In the early spring, northern pike can be found in the shallow areas of lakes, where they establish diffuse territories provided appropriate cover and food-availability conditions exist. As the summer progresses and water temperatures increase, northern pike retreat to deeper and cooler sections of the lake.

Eggs and juvenile northern pike are prey to a wide variety of piscivores, including other northern pike, minnows, perches, large aquatic-insect larvae, aquatic avifauna, and aquatic mammals. Adult northern pike are usually the top predator in a lake's food chain, and therefore suffer little predation, aside from the occasional attack from lamprey or angler. During spawning when northern pike move into shallow areas, small adults can be taken by some predatory birds (e.g., eagles and ospreys) and mammals (e.g., bears).

After the yolk sac is consumed, young northern pike feed on large zooplankton and small aquatic insects for about 7-10 days, after which their diet switches mainly to fish. Adult northern pike will consume any appropriately sized moving prey item. Optimum prey size is approximately 0.3-0.5 times the size of the northern pike. Because northern pike are able to achieve massive sizes for freshwater fish, they can

consume just about any moving aquatic vertebrate. Fish make up the main portion of the northern pike's diet, although leeches, crayfish, frogs, mice, ducklings, and muskrats are known prey items. Northern pike are generally opportunistic and will feed on whatever food is available.

It has been determined that 2.3-2.7 kg of food is required to increase a northern pike's weight by 0.5 kg (Scott and Crossman 1973). The huge quantity of food eaten by northern pike each year is testimony to this fish's efficacy as a natural controller of fish populations. Some controversy has flared up in the past that northern pike outcompete or prey upon other more valuable fish species. Although this may be true to a certain extent, northern pike are a critical component to an ecosystem as a voracious predator. Public education by fisheries authorities has curtailed the northern pike's bad reputation for the most part, by shifting focus away from its effects on other fish to its highly desirable characteristics as a sport fish, such as its large size and excellent-tasting flesh. Although some still regard this fish as an undesirable pest, most now recognize and appreciate the northern pike's important place in an aquatic ecosystem.

1.5.3.3. Reproduction

Males achieve sexual maturity earlier than females. In males, sexual maturity is at 2-3 years in southern regions and about 5 years in northern waters. Females reach sexual maturity at 3-4 years in the south and at about 6 years in the north. Spawning takes place for a week or two in the early spring, often before all of the ice has melted from the lake. Suitable spawning grounds include heavily vegetated flood plains of rivers, marshes, and weedy inlets or bays of lakes.

As spawning season approaches, males ripen earlier than females, and move onto spawning beds first. As females ripen, they move onto the spawning beds to initiate spawning. One or two males attend a single female. The male moves along side the female and aligns urogenital pores. With bodies pressed together, both vibrate with the female releasing eggs and the male fertilizing them as they emerge. Each spawning act results in the release of about 50-60 eggs. Total egg number per female is dependent upon the size of the female, where large females produce more eggs than smaller females. One report (cited in Scott and Crossman 1973) tells of a single female producing almost 600,000 eggs. On average, females produce about 9000 eggs per 0.5 kg of fish, with 32,000 eggs per female as the average. Eggs are 2.5-3.0 mm in diameter, clear, yellow or orange in colour, demersal, and very adhesive.

Once eggs are released, both male and female swat their tails causing a thrust of water to randomly disperse the eggs. Eggs attach to stones, sticks, benthic debris, and aquatic vegetation due to their extremely sticky coatings. Once eggs are deposited, they are subject to predation by a variety of piscivores (see *Ecology* section). Some reports (cited in Scott and Crossman 1973) calculate total mortalities of eggs and young northern pike returning from spawning grounds as high as 99.8%. Eggs hatch in 12-14 days at ambient temperatures of about 13-14 °C, but can be hatched in 4-5 days at temperatures of 17.8-20.0 °C.

Young northern pike, 6-8 mm in length, remain attached to vegetation for 6-10 days after hatching due to adhesive glands on their heads. Growth is extremely rapid, such that lengths of 43 mm occur after only one month, and lengths of up to 153 mm is

achieved by the end of the first summer. Growth remains rapid for the first 1-3 years. After sexual maturity, longitudinal growth slows but weight continues to increase with increasing age.

1.5.3.4. Relevance to Study

Northern pike is a common fish species found in the uranium district of northern Saskatchewan. More importantly, northern pike occur in most lakes near mining operations throughout Canada. However, toxicity tests conducted to determine effluent toxicity from mining operations are conducted exclusively on standard toxicity-test species such as fathead minnows and rainbow trout. Because neither of these standard toxicity-test fish species occur in lakes surrounding the Key Lake uranium mine site, it is not clear how toxicity tests conducted in the laboratory provide meaningful information regarding toxicity to native fish species such as northern pike.

Northern pike was chosen as a study species because it is not a standard toxicity-test fish species, it is common in lakes surrounding the Key Lake uranium mine, it is easily captured using angling techniques during spawning season so that eggs and milt can be collected, and it occupies an important trophic position in aquatic ecosystems.

1.5.4. Biology of White Suckers

1.5.4.1. Range, Distribution, and Abundance

The range of the white sucker is restricted to North America. White suckers can be found from central Ungava, Labrador, and Nova Scotia, south to Georgia, along the extreme northern most parts of the Gulf states to northern Oklahoma, north through the eastern boundary of the Rocky Mountains to Alberta and British Columbia, north to the

Mackenzie River delta, where the range turns east, roughly following the tree line to the Hudson Bay Lowlands. This fish species is widely distributed throughout Canada due to its capacity to tolerate a wide range of habitats and conditions (Scott and Crossman 1973).

1.5.4.2. Ecology

White suckers are usually found in warm, shallow lakes, living in 4 to 40 m of water. Coble (1967) found white suckers of South Bay to live in less than 36 m of water. However, white suckers have been found at depths of 50 m in Great Slave Lake (Scott and Crossman 1973). Migrations by white suckers, other than spawning migrations, are usually random (Coble 1967). White suckers are active during the day, but they are most active during dawn and dusk when they move into shallow areas to feed. Older adults tend to move further offshore as they age.

White sucker fry have a terminally located mouth. This adaptation permits them to feed near the surface on plankton and other small invertebrates. As the position of their mouth moves to a more ventral position, there is a transition in their feeding habits. By the time the mouth has become fully positioned ventrally, the white sucker becomes a bottom feeder. The diet includes members from Chironomidae, Trichoptera, Mollusca, Entomostraca, and *Chaoborus*, among others.

At lengths less than 30 cm the white sucker is an important diet item of many predatory fishes, including northern pike, muskellunge, *Esox masquinongy*, smallmouth bass, *Micropterus dolomieu*, largemouth bass, yellow walleye, *Stizostedion vitreum vitreum*, and burbot, *Lota lota*. Some smaller individuals are taken by predaceous birds

and fry are consumed by brook trout, *Salvelinus fontinalis*. During the spawning migration up streams and rivers, adults are caught and eaten by bears and other mammals (Scott and Crossman 1973). Sea lamprey, *Petromyzon marinus*, will prey upon white suckers when lake trout populations are sufficiently low. Predation by sea lamprey has caused a shift in the mean body size of several white sucker populations in the Great Lakes area. However, it is questionable whether this predation is affecting the population size, because sea lamprey tend to attack larger fish of the population (Coble 1967).

There is controversy surrounding the notion that white suckers feed on the eggs of other game fish such as trouts, basses, and sturgeons causing serious damage to these populations. The evidence supporting predation on eggs by white suckers is not conclusive (Scott and Crossman 1973).

Growth of the white sucker is not continuous over its life span. Coble (1967) reported that there was very little growth in the adult white sucker population in South Bay. Geen et al. (1966) reported that adult white and longnose suckers, *Catostomus catostomus*, in Sixteenmile Lake, British Columbia, only grew 10 to 20 mm on average per annum. Circuli were not laid down every year in adults. Those circuli that were laid down during later years formed annuli that were either too close to the margin of the scale to distinguish, or they were resorbed (Geen et al. 1966). Scales can be used with some degree of success as an age index for juvenile fish or those approaching maturity, but not for adults. Consequently, scales used as an aging index of white sucker adults

are not reliable, however, examination of pectoral fin ray annuli provide better age data (Beamish 1973; Chalanchuk 1984; Coble 1967; Geen et al. 1966).

1.5.4.3. Reproduction

White suckers spawn during the spring from early May to early June. Adult fish leave the lakes and migrate up the rivers and streams to seek out a gravel spawning bed in the same stream from which they were hatched. These fish have been known to spawn in rapids and on gravely lake bottoms instead of quiet locations in streams and rivers. Spawning is usually initiated when the temperature of the stream reaches 10°C (Geen et al. 1966). Two to four males surround a single female and press her with their tuberculate anal and caudal fins during irregular spawning acts. Geen et al. (1966) observed spawning to take place between 0600 h and 2130 h in Sixteenmile Lake, British Columbia. No spawning activity was observed after dark. Spawning acts occurred at a rate of approximately 6-40 times per hour. The females produced approximately 20,000 to 50,000 eggs. The fry emerged on average 8-11 days after the spawning period, but migrated to the lake approximately 1 month after the spawning period. This 1-month delay implies that fry remain situated in the gravel for a time before emerging and migrating to the lake. Generally, there is only a 3% survival rate of young.

1.5.4.4. Relevance to Study

White suckers were chosen for this study for similar reasons to those of northern pike; namely, white suckers are not used standard toxicity tests, they are abundant in northern Canadian lakes, and they occupy an important trophic position as a forage fish

in aquatic ecosystems. In addition, white suckers are a very robust fish species, able to inhabit environmental conditions more extreme than other, more economically desirable, and less sensitive fish species. Swanson (1983; 1985) showed that white suckers tended to accumulate radionuclides to a greater extent than other fish species inhabiting the same water systems in northern Saskatchewan. Because white suckers are bottom feeders, and many metal contaminants are found in freshwater sediments, these fish can be expected to receive much greater contaminant exposure due to inadvertent ingestion of benthic debris. Therefore, from an ecological standpoint, understanding the bioavailability, fate, and subsequent toxicity of metals to white suckers is an important consideration.

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Notes

[1] Distribution coefficients are defined by the following equation: $k_d = c_s \times c_w^{-1}$, where k_d is the distribution coefficient (litres/g), c_s is concentration in the solid phase (μg/g), and c_w is concentration in the soluble phase (μg/L). The higher a k_d value, the more strongly associated an element or contaminant is to a solid-phase constituent.

[2] Map of the Key Lake site with respect to study lakes and exposure sites is provided on page 73, in Chapter 2.

[3] Atomic Energy Control Board (AECB), Ottawa, ON

2. Toxicity of Uranium Mine Receiving Waters to Caged Fathead Minnows, *Pimephales promelas*

2.1 Abstract

Larval fathead minnows (*Pimephales promelas*) were placed at four exposure sites for 7 days in each of five lakes surrounding the Key Lake uranium mine in northern Saskatchewan, Canada. Two of the lakes (Fox and Unknown lakes) received uranium mill effluent containing elevated concentrations of molybdenum (Mo). Two other lakes (Little McDonald and McDonald lakes) received open-pit mine dewatering effluent containing elevated concentrations of nickel (Ni). One lake (David Lake) received no mine-related effluent and served as a reference site. Fish placed in lakes receiving Mo-contaminated mill effluent demonstrated higher mortalities than those placed in lakes receiving Ni-contaminated mine-dewatering effluent, which was not significantly different from reference sites. No significant differences were detected in fish growth among the study lakes because of the high (90%) mortality in Fox and Unknown lakes. Principal components analysis characterized exposure sites by total- and dissolved-metal concentration. Stepwise multiple regression of fish mortality on principal components (PCs) generated from total-metal data showed that PC1 could account for 84% of the variance associated with fish mortality. Careful examination of the metals that correlated strongly with PC1 and with fish mortality suggested that dietary Se toxicity probably resulted in the differential fathead minnow mortality observed among the study lakes.

2.2 Introduction

Northern Saskatchewan, Canada, is home to some of the top-producing uranium mines in the world. In particular, Cameco's Key Lake uranium operation is the largest uranium mine in the world, with average annual U_3O_8 production exceeding 5×10^6 kg (Saskatchewan Energy and Mines 1999). During operation, Key Lake uranium ore is extracted from an open-pit mine and is milled on site. Treated pit-dewatering and mill effluents are released to surrounding aquatic systems, causing an elevation of metal concentrations in receiving waters above background concentrations (Cameco Corp. et al. 1995). Mill effluent has previously been characterized as having high concentrations of molybdenum (Mo), whereas dewatering effluent shows high concentrations of nickel (Ni) (Cameco Corp. et al. 1995). Consequently, both Ni and Mo have been identified as 'metals of concern' in aquatic receiving systems around Key Lake.

Traditionally, environmental assessments of mine receiving waters have made use of univariate sample-characterization techniques to identify potential toxicants to indigenous biota (Maund et al. 1999). Organisms occurring in industrially contaminated natural systems are rarely, if ever, exposed to single contaminants. Instead, they are exposed to complex mixtures of contaminants. Attempting to relate biological effects to single contaminants in a complex system through univariate analysis is difficult and often unrealistic (Maund et al. 1999).

Ecotoxicologists have recently recognized this shortcoming and have begun using multivariate techniques to examine several environmental variables simultaneously as a more realistic approach to relating biological effects to complex mixtures (Maund et al. 1999). Multivariate techniques allow variables from a data set

containing many variables to be analyzed simultaneously, rather than individually as in univariate methods. In this way, mutual variance among variables can be investigated allowing for more a realistic characterization of the system under study. Although such analyses are limited by their inability to clearly identify causal relationships, they serve as powerful heuristic tools.

In this study, larval fathead minnows (*Pimephales promelas*) were placed in metal-contaminated and reference lakes for 7 days, after which growth and mortality were observed. Each exposure site per lake was characterized by both total and dissolved metal concentrations, which were ordinated by principal components analysis (PCA). The first six principal components (PCs) generated in each analysis were then used as independent variables in stepwise multiple regression in an attempt to describe potential relationships between metal concentrations and differential fish mortality or growth among study lakes. Multiple regression was also performed using metal-concentrations as independent variables to compare with results from PC regressions.

2.3 Materials and Methods

2.3.1. Study area

The Key Lake uranium mine (57°11' N, 105°34' W; Fig. 2.1) is located in north-central Saskatchewan, approximately 600 km north of Saskatoon, Saskatchewan, Canada. Open-pit mining began at Key Lake in 1983 and ended in 1997 after the ore body was depleted. During that time, the Key Lake mine produced 5.6±1.5 million kg U_3O_8 (mean±SD; n=15) annually, having an average grade of 2.4±0.2% U_3O_8 (n=15) (Saskatchewan Energy and Mines 1999), making it the top-producing uranium mine in

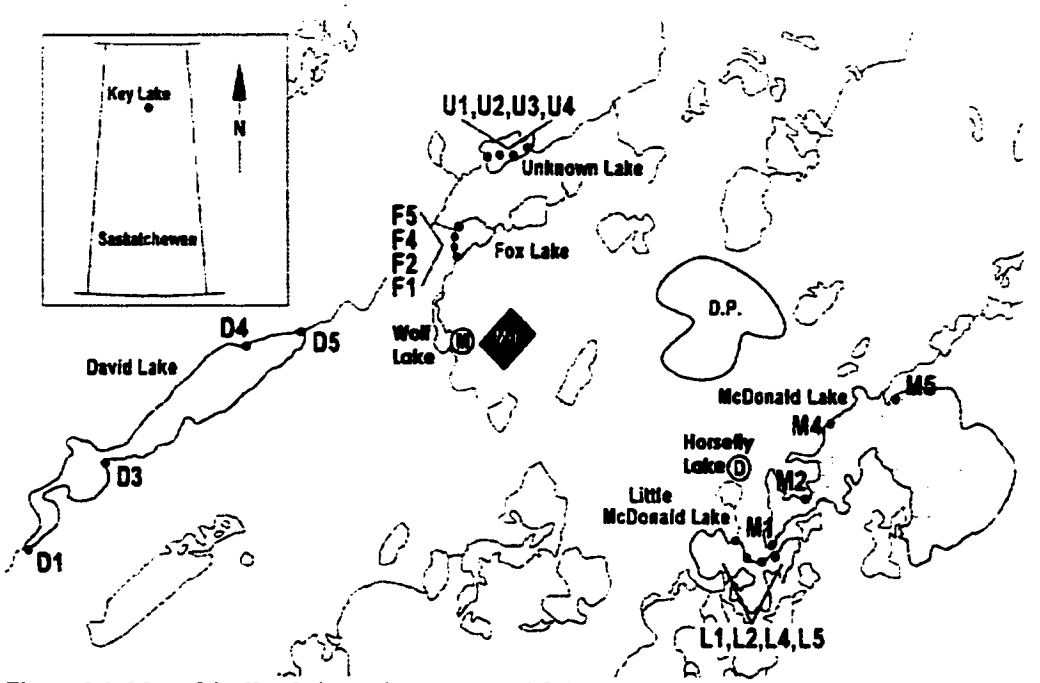

Figure 2.1: Map of the Key Lake study area. Larval fathead minnows were placed in four exposure sites in each of five lakes, David (D), Fox (F), Unknown (U), Little McDonald (L), and McDonald Lakes (M). Mill effluent is discharged directly into Wolf Lake (encircled M), which drains into Fox and ultimately into Unknown Lakes. Deilmann open-pit (D.P. on map) dewatering effluent is discharged into Horsefly Lake (encircled D), which drains into Little McDonald, which in turn drains into McDonald Lake. Exposure sites are represented by dots given alphanumeric labels on each study lake.

the world (Cameco Corp. 1998). Although mining has recently ceased, uranium-ore stockpiles are currently being milled on site. Uranium ore generated at the McArthur River uranium mine, approximately 70 km northeast of Key Lake, will be milled at Key Lake for the next 20 years (Cameco Corp. et al. 1995).

A characteristic of Key Lake uranium deposits was the close association between uranium and nickel. In some places where U_3O_8 concentrations peaked at 35%, nickel concentrations were approximately 20% (Saskatchewan Energy and Mines 1999). Although U_3O_8 and nickel concentrations were correlated within the main ore bodies, nickel concentrations were generally lower than U_3O_8 concentrations. Other minerals in

the uranium ore included gersdorffite (NiAsS), millerite (NiS), niccolite (NiAs), and bravoite ([Ni,Fe]S_2). Lesser amounts of pyrite (FeS_2), galena (PbS), safflorite ([Co,Fe]As_2), sphalerite (ZnS), chalcopyrite ($CuFeS_2$), hematite (Fe_2O_3), and magnetite (Fe_3O_4) were also present (Saskatchewan Energy and Mines 1999).

David Lake is located upstream of mine-related discharges and served as a reference lake. Water drains David Lake via David Creek, which flows directly into Unknown Lake. Wolf Lake (not a study lake), which receives treated mill effluent, drains into Fox Lake via Wolf Creek. Fox Lake is drained by Yak Creek, which flows into David Creek before meeting up with Unknown Lake. Dewatering effluent from the Deilmann open pit is pumped into Horsefly Lake (not a study lake), which then flows into Little McDonald Lake. Little McDonald Lake drains into McDonald Lake through a small, narrow channel. Therefore, Fox and Unknown lakes receive treated mill effluent, whereas Little McDonald and McDonald lakes receive mine-dewatering effluent. Both effluent types are known to have elevated metal concentrations (Cameco Corp. et al. 1995).

2.3.2. Water-quality analyses

The following limnological parameters were measured *in situ* during the study (August 18-25, 1997): temperature, pH, total hardness (as $CaCO_3$), alkalinity (as $CaCO_3$), dissolved oxygen, conductivity, total dissolved solids (TDS), carbon dioxide, and turbidity. Temperature and dissolved oxygen were measured with a Corning electronic dissolved oxygen probe (with built-in temperature sensor) attached to a Corning M90 hand-held meter. Conductivity and TDS were measured with Corning's

electronic conductivity probe and M90 meter. A Lamotte HA-Series pH meter and electrode were used to determine pH. Hardness, alkalinity, and carbon dioxide were determined using titrimetric methods included in the Lamotte water-quality test kit. Turbidity was determined by dissolving farazin in the sample water, and measuring its absorbance using Lamotte's spectrophotometer.

Two water samples and one atmospheric (i.e., sampling) blank were collected from each site in 150 mL, acid-washed, polyethylene bottles. An atmospheric blank was used to account for any atmospheric fallout that may contaminate samples from nearby open pit mining activities. The atmospheric blank was exposed to the open air as water samples were being collected. Both water samples were collected simultaneously by first rinsing each bottle in sample water three times, and then capping the bottle while still submerged. The atmospheric-blank bottle was capped immediately following water-sample collection. All water samples were placed in an ice-filled cooler for transportation back to the laboratory.

After all water samples were collected, one sample from each site was passed through a 0.45-μm filter. Every water sample was acidified with concentrated, ultra-pure nitric acid so that metals would stay in suspension. Samples were kept refrigerated during transportation back to the Department of Geological Sciences at the University of Saskatchewan where inductively coupled plasma-mass spectrophotometry (ICP-MS) analysis was conducted to determine dissolved and total metal concentrations at each study site. Analysis of National Research Council River Water Standard (SLRS-3) showed analytical variation generally <20% between duplicates of a single sample, and

results were within 30% of known values. Metal concentrations were determined after subtracting metal concentrations measured from sampling and analytical blanks.

2.3.3. *In situ* toxicity testing

Four exposure sites were selected in each of five lakes in approximately 1.0-1.5 m of water along the shore between inflow and outflow (total 20 exposure sites). Near-shore sites were selected in this study for several reasons: (i) were fathead minnows to occur in these lakes, larvae would most likely be found in near-shore environments; (ii) near-shore exposure sites are better protected from physical stress (e.g., wind and waves); and (iii) shallow, near-shore exposure sites are easily accessible for daily data collection, even under adverse weather conditions.

Fathead minnow eggs were generated from laboratory breeding stock at the University of Saskatchewan in Saskatoon. Spawning substrates (i.e., 10 cm longitudinal sections of PVC pipe) containing eggs were packed in sealed plastic bags containing laboratory water and oxygen, and shipped to Key Lake by air in a sealed cooler. Egg-hatching chambers were established at Key Lake in a makeshift laboratory. Water in egg-hatching chambers was renewed daily with laboratory water shipped from Saskatoon. Photoperiod and temperature could not be controlled on site and consequently matched ambient conditions. Hatching success and subsequent larvae survival were similar to that observed under controlled laboratory conditions.

Ten larval fathead minnows (<24 h old) were placed in each larval exposure and observation tube (LEOT; described below) at four sites in each of the five study lakes (Fig. 2.1). Each exposure site was constructed by cutting out the bottom of a clean 16 L

food-grade plastic bucket so that it could be fitted over and tied to two wooden stakes which were pounded into near-shore lake sediment (Leis and Fox 1994). The purpose of the plastic bucket was to protect the LEOT from floating debris and other physical damage.

Each LEOT consisted of a 10 cm section of PVC plastic tube (approx. 7 cm inside diameter) capped at each end with 400 μm nitex mesh. At one end, mesh was held in place by an end cap with five 0.5 cm holes cut out of it to allow for water flow. At the other end, mesh was held in place with a screw-cap fitting which allowed for easy access in order to load fish into the LEOT and to count living and dead fish during the test. These exposure units represent a modified version of Kocan's (1996) fish egg exposure unit.

Larval fathead minnows were randomly selected and gently transferred to LEOTs with a glass turkey baster. All LEOTs were loaded at the same time in coolers filled with laboratory water. In order to get LEOTs out to exposure sites in such a manner as to minimize stress to fish, only four LEOTs were transported at once, representing all exposure units for one lake. Once at an exposure site, a single LEOT was carefully lifted out of the cooler by placing a clean plastic cup underneath one end to keep water from falling out and stranding larval minnows on a dry surface. Cup and LEOT were submersed together inside exposure site, the cup was removed, and the LEOT was tied inside the plastic bucket with string.

Fish were exposed in the five study lakes for 7 days. Each day during the test mortality was recorded and dead fish were removed. At the end of the test, surviving

fish were counted and preserved in 10% buffered formalin. Fish were then transported by road to the laboratory in Saskatoon within 24 h of the end of the test, rinsed with distilled water, dried overnight at 110 °C, and weighed to the nearest 10 μg.

2.3.4. Statistical treatment

Mortality (using proportion dead as a metameter of mortality (Newman 1995)) and fish-weight (a surrogate measure for growth) distributions were tested for normality using the Shapiro-Wilks test. Deviations from normality were corrected using a $\log_{10}(x+1)$ transformation. Among-lake mortality and growth comparisons were made using an analysis of variance (ANOVA). Upon detection of significant differences, a Dunnett's test was used to compare mortality or growth of fish from Fox, Unknown, Little McDonald, and McDonald lakes with mortality or growth of fish from David Lake.

To account for significant differences detected in the ANOVA, exposure sites were characterized using a principal components analysis (PCA; variance-covariance matrix) on waterborne, $\log_{10}$-transformed metal concentrations, as a data-reduction technique. A stepwise multiple regression analysis followed PCA by using the first six principal components (PCs) as independent variables and growth or mortality as the dependent variable (Stewart 1996).

Highly intercorrelated variables used in PCA can significantly bias the analysis. Consequently, the data sets were censored to minimize multicollinearity. Metals that could not be detected in at least 10 exposure sites (i.e., half the number of exposure sites) were removed from the analysis. Remaining data points showing "not detected"

were replaced with half the detection limit for that metal. Because dissolved metals were highly correlated with total metals, as expected, each data set was treated separately. Pearson correlation coefficients were calculated for each data set to determine which metals were associated with which. Those metals having correlation coefficients greater than 0.70 for >65% of correlates were removed *a priori* from further analyses. However, certain metals that met these criteria, but are known to be associated with uranium mining in northern Saskatchewan (i.e., As, Cd, Cr, Cu, Fe, Mo, Se, and V), were retained. As a result of *a priori* data censoring, Ag, B, Ba, Cs, Eu, Gd, Hf, Ho, La, Li, Lu, Nb, P, Rb, Sb, Sc, Sn, Sr, Ta, Tb, Th, Ti, Tl, Tm, and Y were removed from both data sets. In addition, Ce, Pr, and Sm were removed from the dissolved-metal data set, and Er and Pb were removed from the total-metal data set.

By definition, the first PC accounts for the most variance associated with metal concentrations at exposure sites. Subsequent PCs account for progressively less variance. Some have argued that later PCs may hold important information that could account for biological effects (Nichols 1977). Therefore, rather than restricting the PCA to the first few PCs (i.e., those having the highest eigenvalues), the analysis was extended to include the first 6 PCs.

Individual-metal concentrations from each data set were then correlated against fish mortality, and PCs identified as being associated with fish mortality. Metals that significantly correlated with both explanatory PCs and fish mortality were compared among the five study lakes. Metals that did not vary among lakes were removed from

subsequent analyses. Remaining metals were retained as independent variables in stepwise multiple regressions to account for variance associated with fish mortality.

Principal components analyses were conducted on SAS/STAT (SAS Institute 1995) statistical software, whereas multiple regressions, Pearson correlations, and descriptive and inferential statistics were computed using SPSS (1988), Shapiro-Wilks tests of normality and Kruskall-Wallis non-parametric ANOVAs were performed on TOXSTAT 3.5 (West and Gulley 1996) computer software.

2.4 Results

2.4.1. Water quality

Water quality of the five study lakes is shown in Table 2.1. Temperature and alkalinity are similar in all study lakes. Median pH values indicate that David, Fox, and Unknown lakes are slightly to moderately acidic (pH < 6.2), with Fox and Unknown lakes (pH < 5.3) more acidic than David. Little McDonald and McDonald lakes were circumneutral. Although soft water occurred in David, Little McDonald, and McDonald lakes (hardness <31 mg/L as $CaCO_3$), very hard water occurred in Fox and Unknown lakes (hardness >550 mg/L as $CaCO_3$) because of receiving mine-mill discharge. This high water hardness was also reflected in high conductivity (>1000 µS/cm), total dissolved solids (TDS; >550 mg/L), and carbon dioxide (CO_2; >8 mg/L) values in Fox and Unknown lakes relative to other lakes. The three smaller, more productive lakes (i.e., David, Fox, and Unknown) tended to have more turbid water (turbidity >16 FTU) compared to the larger, less productive lakes (i.e., Little McDonald and McDonald; turbidity <6 FTU).

Table 2.1: Water qualities of the five study lakes near the Key Lake uranium mine.

	Lake				
	David	Fox	Unknown	Little McDonald	McDonald
Temperature (°C)					
Mean	16.1	18.6	18.7	18.7	17.9
SD	0.9	0.9	1.2	2.1	2.1
n	16	12	9	16	16
pH					
Median	6.1	5.1	5.2	6.7	7.0
Range	5.8-6.3	4.7-5.4	4.4-5.3	6.3-6.8	6.9-7.2
n	16	12	9	16	16
Hardness as $CaCO_3$ (mg/L)					
Mean	9.6	687.0	586.0	29.1	30.1
SD	4.4	190.7	22.7	22.8	41.7
n	8	10	9	8	8
Alkalinity as $CaCO_3$ (mg/L)					
Mean	19.0	22.2	22.6	24.9	24.3
SD	4.4	7.8	6.3	5.9	4.7
n	10	9	9	9	9
Dissolved oxygen (mg/L)					
Mean	7.3	7.4	7.4	8.7	8.8
SD	0.7	0.3	0.7	0.3	0.1
n	16	12	9	16	16
Conductivity (µS/cm)					
Mean	23.0	2111.7	1144.0	76.4	62.5
SD	7.9	92.1	43.4	10.5	2.7
n	16	12	9	16	16
Total dissolved solids (mg/L)					
Mean	11.6	1056.7	577.2	38.5	31.3
SD	4.0	30.6	20.5	5.4	1.3
n	16	12	9	16	16
Carbon dioxide (mg/L)					
Mean	5.3	12.1	8.2	4.6	3.5
SD	1.0	3.9	1.0	1.7	1.0
n	4	4	4	4	4
Turbidity (FTU[a])					
Mean	20.0	33.1	16.1	5.9	5.5
SD	15.6	31.8	9.5	3.9	4.7
n	15	15	15	15	15

a FTU = farazin turbidity units

Total and dissolved metal concentrations (censored metal data used in PCA) of the five study lakes are shown in Tables 2.2 and 2.3, respectively. David Lake had low

Table 2.2: Mean total metal concentrations (μg/L; n=4) and standard deviations (SD) in five lakes near the Key Lake uranium mine. Metal concentrations below detection limit were included in mean calculations as half the detection limit.

	David		Fox		Unknown		Little McDonald		McDonald	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Al	24.08	7.20	25.36	1.40	33.45	4.99	5.82	0.43	4.69	0.47
As	0.38	0.08	43.66	6.49	12.15	0.90	0.25	0.05	0.30	0.15
Cd	0.09	0.17	1.96	0.25	1.11	0.15	0.05	0.04	0.01	0.00
Ce	0.05	0.03	0.08	0.01	0.05	0.01	0.02	0.01	0.01	0.00
Co	0.05	0.02	1.33	0.09	0.75	0.03	2.28	0.71	0.38	0.41
Cr	0.60	0.14	1.06	0.23	0.91	0.27	0.11	0.10	0.15	0.15
Cu	0.41	0.28	1.29	0.11	0.88	0.15	0.59	0.10	0.37	0.19
Dy	0.01	0.01	0.02	0.00	0.02	0.01	0.01	0.01	0.01	0.00
Fe	356.08	216.42	1238.26	56.02	779.61	76.73	61.17	14.28	19.75	10.28
Hg	0.01	0.01	0.05	0.04	0.07	0.03	0.02	0.01	0.01	0.01
Mn	15.50	6.51	67.64	2.59	56.69	3.10	38.19	9.04	12.17	6.96
Mo	0.39	0.44	1397.48	122.46	735.08	45.59	0.72	0.78	0.65	0.89
Nd	0.01	0.02	0.05	0.04	0.04	0.02	0.05	0.03	0.01	0.00
Ni	0.54	0.15	33.25	3.32	17.80	0.84	116.01	9.12	53.56	20.83
Pr	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
Se	0.46	0.75	7.67	1.43	4.51	0.77	0.73	1.36	0.13	0.16
Sm	0.02	0.01	0.01	0.00	0.01	0.02	0.01	0.00	0.01	0.01
U	0.23	0.07	1.24	0.12	0.58	0.05	8.95	1.01	3.13	2.16
V	0.07	0.02	0.87	0.29	0.22	0.05	0.02	0.01	0.03	0.01
W	0.02	0.01	3.13	0.68	0.42	0.04	0.03	0.02	0.02	0.02
Yb	0.03	0.03	0.01	0.01	0.04	0.01	0.02	0.01	0.01	0.01
Zn	2.42	2.61	1.47	0.29	1.49	0.38	12.03	2.07	5.88	3.58
Zr	0.07	0.01	0.05	0.01	0.08	0.03	0.01	0.02	0.01	0.01

concentrations of most metals, but relatively high concentrations of Al, Fe, Mn, and Zn. Fox and Unknown lakes tended to have high metal concentrations, especially with respect to As, Cd, Fe, Mo, Mn, Ni, Se, U and W. McDonald and Little McDonald lakes tended to show less metal contamination than Fox and Unknown, but had elevated concentrations of Co, Ni, U, and Zn, relative to other lakes.

David Lake mean dissolved Cu (0.57 μg/L) and U (0.24 μg/L) were higher than total Cu (0.41 μg/L) and U (0.23 μg/L). The high dissolved U concentration is the result of among-site variability. No single dissolved U datum for each study site exceeded corresponding total U concentrations. Dissolved Cu concentrations exceeding total

Table 2.3: Mean dissolved metal concentrations (µg/L; n=4 unless otherwise noted) and standard deviations (SD) in five lakes near the Key Lake uranium mine. Metal concentrations below detection limit were included in mean calculations as half the detection limit.

	David[a]		Fox		Unknown		Little McDonald		McDonald	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Al	15.21	4.68	17.85	2.08	25.23	5.90	2.93	1.86	3.90	0.59
As	0.30	0.06	41.25	7.84	10.46	1.60	0.17	0.09	0.27	0.12
Cd	0.12	0.19	1.90	0.21	1.07	0.14	0.05	0.04	0.01	0.00
Co	0.05	0.01	1.31	0.08	0.71	0.06	0.97	0.28	0.21	0.19
Cr	0.08	0.06	0.71	0.25	0.21	0.21	0.01	0.00	0.04	0.06
Cu	0.57	0.32	1.18	0.11	0.88	0.15	0.79	0.10	0.57	0.19
Dy	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.00	0.01	0.00
Er	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.00
Fe	162.61	52.90	1114.76	43.44	736.17	72.44	26.13	4.28	12.49	5.63
Hg	0.01	0.01	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.00
Mn	11.44	4.14	68.88	1.90	55.77	3.01	17.48	5.64	3.58	2.94
Mo	0.03	0.01	1391.71	119.73	693.58	28.73	0.01	0.01	0.14	0.16
Nd	0.02	0.02	0.05	0.04	0.02	0.02	0.02	0.01	0.00	0.00
Ni	0.55	0.17	32.13	2.75	15.55	1.96	108.03	5.74	50.96	23.07
Pb	0.19	0.12	0.19	0.13	0.27	0.34	0.01	0.00	0.01	0.00
Se	0.03	0.03	5.88	0.67	2.09	0.87	0.03	0.03	0.02	0.02
U	0.24	0.08	1.24	0.12	0.58	0.05	7.37	1.21	3.04	2.18
V	0.06	0.01	0.72	0.31	0.19	0.05	0.01	0.01	0.01	0.01
W	0.01	0.00	2.82	0.69	0.36	0.05	0.01	0.01	0.01	0.00
Yb	0.02	0.01	0.01	0.00	0.01	0.01	0.01	0.00	0.01	0.00
Zn	2.67	2.82	1.01	0.68	1.01	0.38	11.55	2.07	5.35	3.58
Zr	0.06	0.01	0.04	0.01	0.03	0.01	0.01	0.00	0.01	0.00

a For David Lake filtered water samples, n=3.

concentrations are also reported in Little McDonald (dissolved, 0.79 µg/L; total, 0.59 µg/L) and McDonald lakes (dissolved, 0.57 µg/L; 0.37 µg/L). Dissolved Cu was higher than total Cu at each study site in David, Little McDonald, and McDonald lakes (Table 2.4). Consequently, high mean dissolved Cu concentrations in these three lakes is probably due to sample contamination by Cu.

2.4.2. Fathead minnow mortality and growth

Fathead minnow mortality results are reported in Fig. 2.2. Mortality at two exposure sites in McDonald Lake at sites M4 and M5 was unusually high (i.e., 80% and

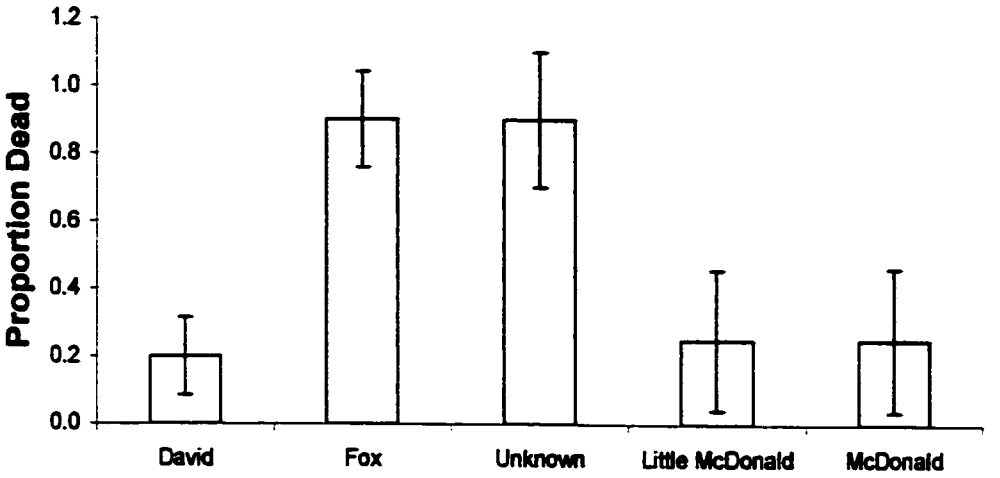

Figure 2.2: Larval fathead minnow mortality after a 7-d exposure in each of the study lakes. Mortality was significantly higher in Fox and Unknown Lakes than other lakes ($p<0.05$), which were not significantly different from one another. Bars represent means±SD ($n=4$; except in McDonald Lake where $n=2$; see text for explanation).

90%, respectively) compared to mortality at other exposure sites in the same lake (i.e., ~40%). Both of these sites were situated on a north-facing shore during a strong weather event originating from the south (i.e., a thunderstorm with high winds causing rough water at M4 and M5). Consequently, LEOTs at these sites were subjected to serious physical disturbances resulting in unusually high mortality owing to physical stress rather than from exposure to contaminants. Mortality and growth data from sites M4 and M5 were therefore removed from further comparative analyses.

Analysis of variance on data collected from remaining exposure sites revealed significant differences ($F_{4,13}=14.11$, $p=0.0001$) in mortality among the five study lakes (Fig. 2.2). Fox and Unknown lakes had significantly (70%) higher mortality than David Lake (Dunnett's t-test, $p<0.05$), whereas mortality in Little McDonald and McDonald lakes was not significantly different from that in David Lake ($p>0.05$). Bartlett's test of

Table 2.4: Total and dissolved copper concentrations (μg/L) at several exposure sites in each of three study lakes. These data report higher dissolved Cu than total suggesting that sample contamination by Cu occurred.

Lake	Exposure Site	Total Cu	Dissolved Cu
David	D1	0.30	—
	D3	0.14	0.26
	D4	0.42	0.54
	D5	0.79	0.91
Little McDonald	L1	0.63	0.83
	L2	0.56	0.76
	L4	0.71	0.91
	L5	0.47	0.67
McDonald	M1	0.60	0.80
	M2	0.43	0.62
	M4	0.18	0.37
	M5	0.28	0.47

homogeneity of variance suggested that there was unequal mortality variance among study sites, probably due to the removal of sites M4 and M5 from the analysis. Therefore, a Kruskall-Wallis non-parametric analysis of variance was performed which confirmed that there were significant mortality differences among study lakes ($p<0.05$).

Fish growth did not vary among the five study lakes ($F_{4,8}=2.00$, $p>0.05$). One hundred percent mortality occurred in five (i.e., sites F1, F2, U1, U2, and U3) of the 18 remaining exposure sites. Because there were no surviving fish from which weight measurements could be made, these sites were excluded from comparison, which may have reduced the effectiveness of analysis of variance for detecting statistically significant growth differences. Therefore, non-parametric analysis of variance was

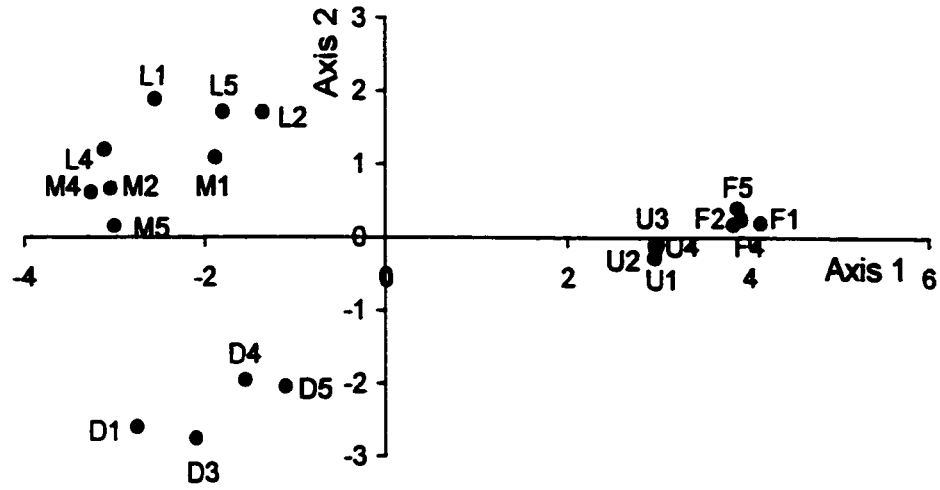

Figure 2.3: Principal components analysis (PCA) ordination of exposure sites characterized by total metal concentration. Points represent exposure sites as shown in Fig. 2.1. Axis 1 is most strongly associated with Mo concentration, and pH, conductivity, TDS, and hardness, while Axis 2 represents Ni concentration, and temperature, dissolved oxygen, and alkalinity.

performed (i.e., Kruskall-Wallis non-parametric analysis of variance), and confirmed parametric results that there were no significant growth differences in fish among study lakes ($p>0.05$).

2.4.3. Principal components and multiple regression analyses

Ordination plots of the first two PCs where exposure sites were characterized by total-metal and dissolved-metal concentrations are reported in Figs. 2.3 and 2.4, respectively. Both ordinations successfully arranged exposure sites according to lake, such that exposure sites from each lake formed tight clusters on each of the plots. Lakes having similar metal concentrations tended to plot near one another. In both cases, sites from Fox and Unknown lakes were most dissimilar from David, Little McDonald, and

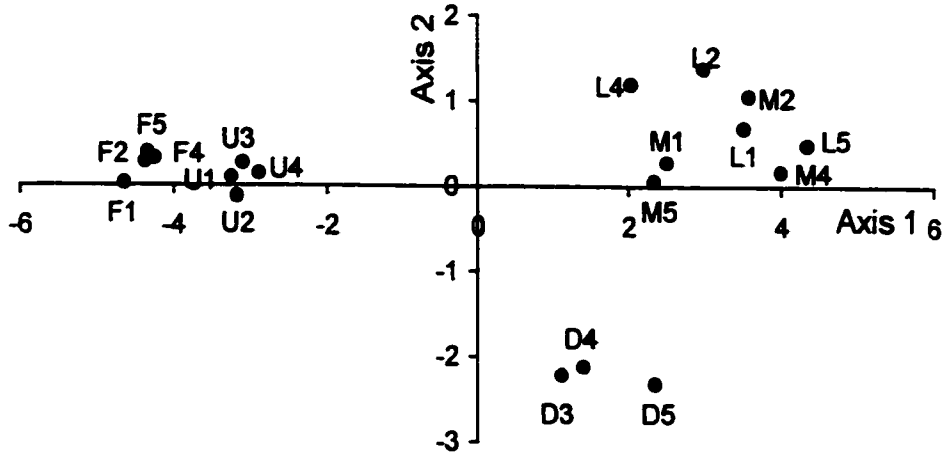

Figure 2.4: Principal components analysis (PCA) ordination of exposure sites characterized by dissolved metal concentration. Points represent exposure sites as shown in Fig. 2.1. Axis 1 is most strongly associated with Mo concentration, and pH, conductivity, TDS, and hardness, while Axis 2 represents Ni concentration, and temperature, dissolved oxygen, and alkalinity.

McDonald lakes, whereas Little McDonald and McDonald lakes were more similar to one another than to David Lake.

Table 2.5 summarizes eigenvalues (Λ), proportion of metal variance explained by each PC, and cumulative metal variance explained by each PC for PCAs computed using total-metal and dissolved-metal data. In both cases, the first PC alone accounted for >70% of the metal variance among exposure sites, whereas the six retained PCs accounted for more than 96% of the metal variance. Each PC after PC3 in both cases accounted for less than 4% of metal variance, but together accounted for as much as 11% of metal variance. The PCA using dissolved-metal data characterized exposure sites slightly better than that computed from total-metal data, as reflected in the higher eigenvalues and percent metal variance explained.

Table 2.5: Eigenvalues, proportion of variance, and cumulative variance explained by the first six principal components of total-metal and dissolved-metal analyses of twenty exposure sites in five lakes near the Key Lake uranium mine.

	Total-Metal Data			Dissolved-Metal Data		
	Eigenvalue	Proportion	Cumulative	Eigenvalue	Proportion	Cumulative
PC 1	166.46	0.70	0.70	209.32	0.79	0.79
PC 2	35.60	0.15	0.85	20.49	0.08	0.87
PC 3	10.39	0.04	0.90	12.58	0.05	0.91
PC 4	6.56	0.03	0.92	5.90	0.02	0.94
PC 5	4.82	0.02	0.94	4.85	0.02	0.95
PC 6	2.90	0.01	0.96	2.81	0.02	0.97

Pearson correlation coefficients ($p<0.05$) for associations between limnological parameters and PCs showed that pH (r_{tot}=–0.91; r_{diss}=0.92), conductivity (r_{tot}=0.94; r_{diss}=–0.94), TDS (r_{tot}=0.94; r_{diss}=–0.94), and water hardness (r_{tot}=0.96; r_{diss}=–0.96) were strongly correlated with PC1 in both PCAs. Water hardness in both analyses had the highest absolute correlation with PC1. The second principal component was correlated with temperature (r_{tot}=0.67; r_{diss}=0.67), dissolved oxygen (r_{tot}=0.55; r_{diss} not significant), and alkalinity (r_{tot}=0.56; r_{diss}=0.57). Only weak correlations between subsequent PCs and limnological parameters were observed.

Eigenvectors (λ) for the two PCAs are reported in Table 2.6. The highest absolute eigenvector loading on the first two axes in both analyses were Mo (λ_{tot}=0.62; λ_{diss}=–0.64) and Ni (λ_{tot}=0.60; λ_{diss}=0.51), respectively. Zinc (λ_{tot}=–0.08; λ_{diss}=0.05) showed the strongest loading in the opposite direction in both analyses. Only U and Zn loaded negatively on PC1 of the total metal analysis, whereas only Ni, U, Yb, and Zn loaded positively on PC1 of the dissolved metal analysis. Because Ni and Mo are the two metals of concern at the Key Lake mine, it is interesting to note on which axes each loads with high magnitude. Nickel loads highest only on PC2 in each analysis.

Table 2.6: Eigenvectors of the first six principal components of PCAs using total-metal and dissolved-metal data. Maximum positive and negative eigenvectors for each PC are highlighted in bold italics.

	PC1	PC2	PC3	PC4	PC5	PC6
Total-Metal Analysis						
Al	0.10	-0.16	0.03	-0.14	-0.05	0.02
As	0.32	-0.01	-0.05	0.14	0.24	-0.18
Cd	0.34	0.07	0.24	-0.20	***-0.53***	***-0.34***
Ce	0.11	-0.10	0.09	-0.35	0.04	-0.14
Co	0.09	0.40	0.03	-0.25	0.24	0.08
Cr	0.16	***-0.27***	***-0.46***	-0.25	0.10	0.20
Cu	0.08	0.06	-0.06	-0.16	-0.03	-0.16
Dy	0.07	0.03	-0.01	-0.03	-0.19	-0.27
Fe	0.21	-0.21	0.06	-0.26	0.19	-0.19
Hg	0.12	0.03	0.09	-0.05	-0.30	0.26
Mn	0.09	0.08	0.04	-0.17	0.16	-0.02
Mo	***0.62***	0.13	-0.34	0.17	-0.22	0.31
Nd	0.08	0.13	0.06	***-0.56***	0.23	0.22
Ni	0.02	***0.60***	-0.04	0.13	0.09	0.06
Pr	0.03	-0.03	0.00	-0.04	0.10	-0.09
Se	0.28	-0.01	***0.65***	0.21	***0.29***	0.09
Sm	0.01	-0.11	-0.02	-0.01	-0.01	0.33
U	-0.05	0.39	-0.07	-0.10	0.12	-0.08
V	0.20	-0.11	-0.10	0.13	0.13	-0.31
W	0.34	0.05	-0.03	***0.22***	0.16	-0.08
Yb	0.04	-0.04	0.37	-0.07	-0.24	***0.41***
Zn	***-0.08***	0.24	-0.03	-0.22	-0.26	-0.18
Zr	0.08	-0.21	0.07	-0.11	0.14	0.04
Dissolved-Metal Analysis						
Al	-0.10	-0.17	-0.06	0.10	0.06	0.08
As	-0.30	0.08	-0.05	-0.08	-0.12	-0.27
Cd	-0.15	-0.09	-0.06	0.11	-0.03	-0.08
Co	-0.07	0.16	-0.13	0.24	-0.33	0.32
Cr	-0.24	-0.19	0.16	***-0.72***	-0.24	***0.40***
Cu	-0.06	-0.04	-0.04	0.15	-0.11	0.00
Dy	-0.06	-0.03	0.09	0.00	-0.23	-0.11
Er	-0.04	-0.01	0.02	0.01	0.01	0.12
Fe	-0.23	-0.17	-0.11	0.19	-0.04	0.27
Hg	-0.06	-0.04	-0.19	0.14	-0.01	-0.26
Mn	-0.14	0.04	-0.08	***0.34***	-0.19	0.39
Mo	***-0.64***	0.10	***0.57***	0.17	0.07	-0.07
Nd	-0.03	-0.08	-0.35	-0.17	***-0.46***	***-0.29***
Ni	0.03	***0.51***	0.04	0.02	-0.33	-0.04
Pb	-0.20	***-0.42***	-0.24	0.20	-0.03	-0.09
Se	-0.33	0.40	***-0.56***	-0.19	***0.47***	0.23
U	0.04	0.26	-0.04	0.13	-0.30	0.15
V	-0.22	-0.16	-0.11	0.01	0.00	-0.15
W	-0.32	0.19	-0.13	-0.10	-0.14	-0.30
Yb	0.02	-0.04	-0.11	-0.20	-0.16	0.01
Zn	***0.05***	-0.20	-0.07	0.06	-0.17	0.18
Zr	-0.11	-0.28	-0.12	0.02	0.00	0.07

Molybdenum has the strongest positive loading on PC1 in both analyses, but also loads

strongly and positively on PC3 (Mo λ_{diss}=0.57) in the dissolved metal analysis. Arsenic, Cd, Fe, Se, V, and W also load highly on PC1 in both analyses. Selenium had the strongest loading on PC3 and PC5 in both analysis, and high loadings on every component in the dissolved metal analysis.

Total and dissolved Al, As, Cd, Cr, Cu, Dy, Hg, Fe, Mn, Mo, Se, V, and W significantly ($p<0.05$) correlated with mortality, in addition to total Ce, and dissolved Co and Er. All of these metals varied by lake ($p<0.05$), except for both total and dissolved Dy, and dissolved Er and Hg, which were removed from subsequent analyses. Remaining metals were significantly associated with PC1 (total and dissolved; $p<0.05$) and retained as independent variables in a multiple regression to account for variance associated with fish mortality.

Results of stepwise multiple regressions of mortality with PCs and metal concentrations as independent variables are shown in Table 2.7. All regressions reported in Table 2.7 were significant ($p<0.05$). The primary axis alone from the total-metals PCA accounted for 84% of the variance associated with fish mortality. A regression using total-metal concentrations as independent variables resulted in Mo alone accounting for 85% of the variability associated with mortality.

Molybdenum concentrations spanned 6 orders of magnitude among study lakes (Tables 2.2 and 2.3), which likely swamped the variability among other independent variables in the analysis. Therefore, the regression using metal concentrations was conducted a second time without Mo. The second total-metals analysis yielded As as the only remaining variable required to account for a significant proportion (82%) of the

Table 2.7: Results of six stepwise multiple regression analyses of fathead minnow mortality on various independent variables.

Data Set	Independent Variables	Intercept (a)	Partial Regression Coefficients: Variable Name	b	S.E. b	β	Sig. T	r^2
Total	PCs 1-6	0.57	PC 1	0.12	0.01	0.92	<0.0001	0.84
	Metals[a]	0.34	Mo	0.18	0.02	0.92	<0.0001	0.85
	Metals-Mo[b]	0.41	As	0.35	0.04	0.91	<0.0001	0.82
Dissolved	PCs 1-6	0.60	PC 1	-0.10	0.02	-0.85	<0.0001	0.72
	Metals[c]	0.87	W	0.29	0.04	0.87	<0.0001	0.75
	Metals-W[d]	0.46	As	0.31	0.05	0.86	<0.0001	0.75

a Metals included: Al, As, Cd, Ce, Cr, Cu, Fe, Hg, Mn, Mo, Se, V, and W.
b Same metals as listed in note (a), but without Mo.
c Metals included: Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Pb, Se, V, and W.
d Same metals as listed in note (c), but without W.

variance associated with fish mortality (Table 2.7). However, Mo and As were strongly correlated with one another ($r=0.96$, $p<0.001$). Dissolved As concentrations in Fox and Unknown lake water were 100 times higher than in David, Little McDonald, and McDonald lakes (Table 2.3).

The same analysis on the dissolved-metal data set also yielded PC1 as the only component required to account for fish mortality ($r^2=0.72$). Substituting dissolved metal concentrations for PCs as independent variables in the regression, W was the only metal the analysis revealed to account for fish mortality ($r^2=0.75$). The same analysis conducted without W gave rise to As being the only variable to account for fish mortality ($r^2=0.75$). Tungsten and As were strongly associated with one another ($r=0.97$, $p<0.001$). Dissolved W was 1000 times higher in Fox Lake than David Lake, although W concentrations in all lakes did not exceed 3.2 mg/L (Table 2.2).

Using metals included in the regression analyses and that were common to both data sets, the proportion of dissolved:total metal concentrations in each lake is reported

Table 2.8: Proportion of dissolved metal in each of the five study lakes.

Metal	David	Fox	Unknown	Little McDonald	McDonald
Al	0.63	0.70	0.75	0.50	0.83
As	0.79	0.94	0.86	0.68	0.90
Cd	1.33	0.97	0.96	1.00	1.00
Cr	0.13	0.67	0.23	0.09	0.27
Cu	1.39	0.91	1.00	1.34	1.54
Fe	0.46	0.90	0.94	0.43	0.63
Mn	0.74	1.02	0.98	0.46	0.29
Mo	0.08	1.00	0.94	0.01	0.22
Se	0.07	0.77	0.46	0.04	0.15
V	0.86	0.83	0.86	0.50	0.33
W	0.50	0.90	0.86	0.33	0.50

in Table 2.8. Chromium, Mo, and Se occurred in their dissolved form at proportionally higher concentrations in Fox and Unknown lakes relative to other lakes. Dissolved As, Cd, Cu, and V was high in each lake, suggesting that these metals occurred predominantly in a dissolved form. Dissolved Fe, Mn, and W was relatively high among all lakes, but was proportionally higher in Fox and Unknown lakes.

2.5 Discussion

Exposure sites were clearly separated into related clusters by PCA using both the total- and dissolved-metal data sets (Figs. 2.3 and 2.4, respectively). Lakes receiving mill effluent (Fox and Unknown lakes) were separated along the first axis from those receiving dewatering effluent (Little McDonald and McDonald lakes). The reference lake (David Lake) was separated from Little McDonald and McDonald lakes along the second axis, and from Fox and Unknown lakes along the first axis. Eigenvector loadings showed that the first axis was most associated with Mo concentrations, whereas the second axis was most associated with Ni concentrations (Table 2.6). Pearson correlations showed that the first axis was also associated with limnological parameters

related to dissolved ions, such as pH, conductivity, TDS, and water hardness, whereas the second axis was related to temperature, alkalinity, and dissolved oxygen (Table 2.1).

The basis for separation of the study sites by PCA into three distinct clusters is because of the nature of the metal-contaminated effluent each lake receives. Nickel is high in dewatering effluent because of the presence of nickel-bearing minerals (such as gersdorffite (NiAsS), millerite (NiS), niccolite (NiAs), and bravoite ($[Ni,Fe]S_2$) in Deilmann-pit geology (Saskatchewan Energy and Mines 1999). Consequently, relatively high concentrations of Ni are present in Little McDonald and McDonald lakes, which receive dewatering effluent. On the other hand, mill effluent is high in Mo owing to the presence of Mo associated with the uranium ore (Cameco Corp. et al. 1995). Therefore, Fox and Unknown lakes, which receive mill effluent, also show relatively high concentrations of Mo. David Lake, the reference site, is low in both Ni and Mo relative to the other four study lakes because it lies upstream of mining activities and receives no contaminated effluent. Given that the first axis is associated most with Mo and the second axis with Ni concentrations in both ordinations generated from total- or dissolved-metal data, a three-cluster separation seems reasonable.

Larval fathead minnow mortality was significantly higher in Fox and Unknown lakes relative to other study lakes (Fig. 2.2). Fish mortality was very strongly associated with the first PC determined from both data sets, as indicated by the first PC's ability to account for large proportions of mortality variability among study sites (Table 2.7). Mortality also correlated strongly with total and dissolved Al, As, Cd, Cr, Cu, Dy, Hg, Fe, Mn, Mo, Se, V, and W, in addition to total Ce, and dissolved Co and Er.

Molybdenum seemed to be most strongly associated with fish mortality. This strong association between Mo concentration and fish mortality was indicated by Mo's strong loading on PC1, its highly significant correlation with fish mortality (r_{tot}=0.92, r_{diss}=0.83; $p<0.001$), in addition to PC1 accounting for 84% of the variance associated with fish mortality in a multiple regression analysis. However, several laboratory studies have shown that Mo is not particularly toxic to fish. For example, McConnell (1977) found that the 96-h LC50 for juvenile rainbow trout (*Oncorhynchus mykiss*) was 1320 mg Mo/L. Hamilton and Buhl (1990) concluded that Mo was relatively non-toxic to chinook salmon (*O. tshawytscha*) and coho salmon (*O. kisutch*) (96-h LC50 >100 mg/L). Molybdenum could not be detected in chinook salmon exposed for 90 d to waterborne Mo at concentrations similar to those of Fox and Unknown lakes (approx. 0.1-0.2 mg Mo/L) (Hamilton and Wiedmeyer 1990). Results from Chapter 4 showed that 100 mg Mo/L did not affect fathead minnow egg hatchability, time-to-hatch, larval growth, or mortality, nor did it affect northern pike (*Esox lucius*) growth or mortality. In the same study, 1000 mg Mo/L was not sufficient to cause significantly more rainbow trout mortalities relative to controls, nor was 2000 mg Mo/L for causing significantly higher white sucker (*Catostomus commersoni*) mortalities relative to controls.

There is an apparent contradiction between results from laboratory studies and those reported here. Laboratory studies suggest that waterborne Mo is relatively non-toxic to fish, whereas *in situ* testing shows that Mo is the metal most strongly associated with fish mortality in lakes around the Key Lake uranium mine. In Fox and Unknown lakes where fish mortality exceeded mortality in other lakes (Fig. 2.2), Mo

concentrations were high (Tables 2.2 and 2.3), and occurred almost exclusively in a dissolved form (Table 2.8).

All of the regression analyses reported in this study were significant ($p<0.05$) and accounted for a large proportion of the variance associated with fish mortality (Table 2.7). Molybdenum concentrations in Fox and Unknown lakes were 3 to 6 orders of magnitude higher than in other study lakes. This high among-lake variability in Mo concentrations obscured the variability associated with the other metals in the analysis. Moreover, metal concentrations used to characterize each exposure site showed a high degree of intercorrelation. Although total Mo was implied as the primary toxicant causing fish mortality, it is more likely that Mo was correlated with the metal (or metals) that actually caused the toxicity.

Analysis of the dissolved-metal data set yielded somewhat similar results. Tungsten was identified as the metal most associated with fish mortality among study lakes. Its concentration in Fox and Unknown lakes was 100 to 1000 times concentrations in other study lakes (Table 2.3), and occurred in a predominantly dissolved form in lakes yielding the highest fish mortality (Table 2.8). However, the toxicity of W to fish is unknown. A concentration of 5000 mg/kg is required to kill 50% of rats intraperitoneally injected with W (i.e., LD50; (Anonymous 1971)). Although its toxicity has not been tested on fish, dissolved W concentrations as low as the highest concentration reported in this study (i.e., 2.8 µg/L in Fox Lake; Table 2.3) are not expected to contribute significantly towards the observed toxicity.

By removing Mo from the total-metal analysis, and W from the dissolved-metal analysis, As concentrations accounted for 82% and 75% of the variance associated with fish mortality in the total and dissolved metal analyses, respectively (Table 2.7). Total Mo and dissolved W were strongly correlated with As ($r_{tot\ Mo}$=0.96, $r_{diss\ W}$=0.97; $p<0.001$). Dissolved As dominated over its insoluble species in all lakes (Table 2.8), and achieved its highest concentrations in lakes demonstrating the highest fish mortality (Fox and Unknown; Tables 2.2 and 2.3). Arsenic concentrations correlated strongly with mortality (r_{tot}=0.91, r_{diss}=0.86; $p<0.001$).

Arsenic is toxic to fish (Sorensen 1991). However, As concentrations required to induce acute toxicity are considerably higher than concentrations reported in Fox and Unknown lakes. Buhl and Hamilton (1991) determined arsenite (the trivalent form of As that is considered as the most toxic As species to fish) 96-h LC50s for arctic grayling (*Thymallus arcticus*), coho salmon, and rainbow trout as 13.7-27.7, 18.5-49.4, 16-91 mg/L, respectively. These concentrations are approximately 3 orders of magnitude higher than As concentrations measured in Fox and Unknown lakes. Sorensen (1991) reported that the acute toxicity (expressed as 24, 48, 72, and 96-h LC50s) of arsenite to bluegills (*Lepomis macrochirus*), rainbow trout, channel catfish (*Ictalurus punctatus*), and goldfish (*Carassius auratus*) ranged in concentration from 15 to 60 mg/L.

Moira Lake, Ontario, is affected by a nearby mining operation and consequently has waterborne As concentrations (56 µg/L) similar to those of Fox and Unknown lakes (Azcue and Dixon 1994). Thirteen species of fish inhabiting the lake had elevated whole body and tissue As concentrations, especially in the intestine. Creek chub

(*Semotilus atromaculatus*) accumulated 10 times more As than the other 12 species. Although the authors concluded that the fish of Moira Lake were not under As stress, they pointed out that: (i) dietary As uptake was possible given high gut As concentrations, and (ii) As accumulation varied considerably among species.

In the present study, Se loaded strongly on almost all PCs generated in both analyses (Table 2.6) and was significantly correlated with fish mortality (r_{tot}=0.76, r_{diss}=0.73; $p<0.001$). Selenium, although an essential nutrient, is extremely toxic to fish (Sorensen 1991). Maier and Knight (1994) reported that toxic thresholds of Se in aquatic systems are exceeded when Se concentrations are elevated by as little as 2 to 5 times background concentrations. Turner and Rudd (1983) reported acute toxicity of waterborne Se to several species of freshwater fish, ranging from about 1 to 80 mg/L, depending on the fish species and oxidation state of dissolved Se. Hamilton and Buhl (1990) determined 96-h LC50s for chinook and coho salmon exposed to Se to range between 13 and 23 mg/L. Sorensen et al. (1984) demonstrated a variety of histopathological and hematological effects in green sunfish (*Lepomis cyanellus*) inhabiting waters with Se concentrations ranging between 8 and 13 mg/L. All of these studies report Se concentrations that were higher than those measured in Fox and Unknown lakes. However, Hodson et al. (1980) demonstrated a variety of subtle, chronic effects on rainbow trout embryos and larvae at waterborne Se concentrations ranging from 4.4 to 53 μg/L. This observation implies that Se concentrations in Fox and Unknown lakes are sufficient to cause chronic toxicity.

However, Se is more toxic to fish through the diet as opposed to waterborne exposure (Hodson and Hilton 1983). Hamilton et al. (1996) demonstrated Se concentrations in food organisms inhabiting water containing 2-3 µg/L that were sufficient (i.e., >3 µg/g) to cause acute toxicity to larval razorback suckers (*Xyrauchen texanus*). Cumbie and Van Horn (1978) reported a complete loss of bluegills from a reservoir having a water Se concentration well below that required for acute toxicity. Dietary Se concentrations above 10 µg/g were attributed as the cause for the loss of bluegills from the reservoir.

Selenium accumulation in fish tissues has been demonstrated under both field (Saiki et al. 1993) and laboratory (Besser et al. 1993) conditions. In a sampling site (i.e., site GT5) from a tributary of the lower San Joaquin River, where water quality characteristics were similar to those in Fox and Unknown lakes, Se in water was 0.009 µg/L, whereas Se in plankton, chironomids, and amphipods was 5.4, 7.2, and 3.3 µg/g, respectively (Saiki et al. 1993). In the same water body, mosquitofish (*Gambusia affinis*), bluegill, and largemouth bass (*Micropterus salmoides*) had whole body Se concentrations of 11, 5, and 7 µg/g, respectively. Therefore, the average bioaccumulation factor (BAF) for Se transfer from water to food is approximately 589, and from food to fish it is approximately 1.4.

Hamilton and Wiedmeyer (1990) reported toxic threshold values for Se accumulation in chinook salmon as low as 3-8 µg/g. Centrarchids exceeding whole body concentrations of 12 µg/g experience severe reproductive problems (Saiki et al. 1993). Fox Lake had the highest waterborne Se concentration of all study lakes (5.9-7.7

µg/L). Assuming BAFs for water:food and for food:fish Se transfer calculated above were similar in Fox Lake, fish inhabiting Fox Lake could expect whole body Se concentrations of 6.4 µg/g, which exceeds the lower limit of Hamilton and Wiedmeyer's (1990) toxic threshold. Therefore, the most likely explanation for the high larval fathead minnow morality observed in Fox and Unknown lakes is dietary Se exposure.

Other metals that were elevated in Fox and Unknown lakes, correlated with mortality, and are known to cause acute toxicity to fish (i.e., Al, Cd, and Cu), were not at sufficient concentrations to cause acute mortality. Aluminum concentrations in Fox and Unknown lakes ranged between 17.9 and 33.5 µg/L. Howells et al. (1983) reported adverse effects on fish exposed to 250 µg/L Al in soft, acidic water. Cadmium and Cu in Fox and Unknown lakes were below 2 µg/L. Acute toxicity of Cd and Cu occurs between 34 and 1000 µg/L in soft water (Pickering and Henderson 1966; Buhl and Hamilton 1991). Water in Fox and Unknown lakes was very hard (hardness 586-687 mg/L as $CaCO_3$), and water hardness is known to ameliorate toxicity (Pickering and Henderson 1966). Therefore, acute toxicity of Cu and Cd is expected to occur at higher concentrations in the hard waters of Fox and Unknown lakes than was observed in the soft water used in the study reported above.

In conclusion, larval fathead minnows placed in lakes around the Key Lake uranium mine demonstrated differential mortality depending on the type of effluent received by the particular lake. Fish placed in lakes receiving Mo-contaminated mill effluent demonstrated higher mortality than those placed in lakes receiving Ni-contaminated mine-dewatering effluent, which was not significantly different from

mortality observed at reference sites. Molybdenum was most strongly associated with PCs that characterized exposure sites by metal concentrations and with mortality. However, previous work has demonstrated that waterborne Mo is not particularly toxic to fish. Although As could account for a considerable proportion of the variance associated with fish mortality, As concentrations in Fox and Unknown lake waters were not high enough to account for the acute toxicity observed in larval fathead minnows. Moreover, As was highly correlated with other metals that also correlated with mortality, notably Se. Selenium concentrations measured in Fox and Unknown lakes were sufficiently elevated relative to reference concentrations (i.e., David Lake) to suggest that dietary Se may explain the elevated fathead minnow mortality observed in these lakes.

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3. Toxicity of Uranium Mine Receiving Waters to Early-Life Stage Fathead Minnows (*Pimephales promelas*) in the Laboratory

3.1 Abstract

Uranium mining activities in northern Saskatchewan, Canada result in elevated metal concentrations in nearby aquatic systems. Of particular concern are arsenic (As), nickel (Ni), and molybdenum (Mo). Arsenic and Ni occur at concentrations that exceed Saskatchewan Surface Water Quality Objectives (SSWQO) in lakes surrounding the Key Lake uranium mine and in the flooded, mined out B-Zone open pit near the Rabbit Lake mine. Early-life stage fathead minnow (*Pimephales promelas*) toxicity was examined in water from five lakes receiving mine-mill and open-pit dewatering effluents near the Key Lake mine, and in water from the B-Zone pit, relative to reference waters and laboratory controls. Results varied in some unexpected ways. In tests of Key Lake water, fathead minnow egg hatchability was reduced by 32-61% in waters receiving mill effluent, whereas time-to-hatch was shortened in dewatering-effluent receiving waters by 33-51% relative to controls. Larval mortality was 16 times higher in one mill receiving water but also in reference water relative to laboratory controls. However, mortality in another mill receiving water was 0%. Larval growth in Key Lake receiving waters did not vary from growth in laboratory controls. Fathead minnow eggs in B-Zone pit waters hatched 26-39% earlier than those in laboratory controls or reference water. Egg hatchability, larval growth and mortality were not significantly affected by B-Zone water. Correlation analysis was insufficient for explaining biological effects. The nature and complexity of water affected by mining

activity determines biological effects. Current regulatory emphasis on single contaminants should be reevaluated in light of the complex interaction among confounding variables such as pH, hardness, conductivity, and multi-metal mixtures.

3.2 Introduction

The toxicity of industrially polluted waters is often characterized through laboratory toxicity testing following standard protocols (Canadian Environmental Protection Service 1990a; 1990b; 1992a; 1992b). One of the primary goals of aquatic ecotoxicology is to relate toxicity results derived in the laboratory with those observed in the field (Chapman 1983). However, this laboratory-field comparison is often difficult due to vastly different exposure conditions in the laboratory relative to the field. In the laboratory, variables are controlled in an unrealistic manner, whereas in the field they fluctuate. Varying experimental conditions lead to widely different results between tests performed in the laboratory and field. Yet, toxicological characterizations of industrially polluted aquatic environments are routinely conducted under laboratory conditions.

Northern Saskatchewan, Canada, is the most productive uranium-mining district in the world. At Key Lake and Rabbit Lake uranium operations in northern Saskatchewan, arsenic (As), molybdenum (Mo), and nickel (Ni) are three environmental contaminants of concern (Cameco Corp. 1990; Golder Associates Ltd. 1996; Cameco Corp. 1997). At Key Lake, open-pit dewatering effluent receiving waters have Ni concentrations that exceed Saskatchewan Surface Water Quality Objectives (SSWQO) of 25 µg/L for Ni in soft water (Saskatchewan Environment and Public Safety 1988) by

a factor of 2-4. Mill effluents at Key Lake result in elevated concentrations of Mo in receiving waters 1-3 orders of magnitude higher than reference waters (Cameco Corp. et al. 1995). However, no SSWQO currently exists in Saskatchewan for Mo (Saskatchewan Environment and Public Safety 1988).

At Rabbit Lake, a flooded, mined-out open-pit, known as the B-Zone pit, shows elevated concentrations of As and Ni that exceed SSWQOs (Saskatchewan Environment and Public Safety 1988; Cameco Corp. 1997). Water in the B-Zone pit is separated from Wollaston Lake by a steel-cell earthen dike (Boojum Research Ltd. 1997; Cameco Corp. 1997). Hydrological exchange between the two water bodies is minimal relative to the volume of the pit (Boojum Research Ltd. 1997; Cameco Corp. 1997). Arsenic occurs in B-Zone pit water at approximately three times the SSWQO for As of 50 μg/L (Saskatchewan Environment and Public Safety 1988), whereas Ni occurs about 10 times the SSWQO (Cameco Corp. 1997). Although As concentrations seem to be falling in association with iron (Fe) in B-Zone pit water, Ni remains a long-term concern (Cameco Corp. 1997). Several proposed decommissioning strategies for the B-Zone pit involve breaching the dike separating B-Zone from Wollaston Lake (Cameco Corp. 1997).

In the uranium district of northern Saskatchewan, toxicity of mine effluents is monitored using rainbow trout (*Oncorhynchus mykiss*) pass/fail tests conducted in the laboratory. Such tests have led to questions about their ecological relevance given that rainbow trout neither inhabit effluent discharge pipes, nor do they inhabit the lakes affected by uranium mining in northern Saskatchewan. In a recent study (Chapter 4), young (alevin and juvenile) rainbow trout were found to be insensitive to Ni relative to

white suckers (*Catostomus commersoni*), a species found in lakes around uranium operations in northern Saskatchewan, and fathead minnows (*Pimephales promelas*), another standard toxicity test fish species. Consequently, sensitive fish species inhabiting uranium-mine receiving waters may not be adequately protected against metal contaminated water when environmental decisions are based on results of rainbow trout toxicity tests (Chapter 4).

Larval fathead minnows were placed in five lakes near the Key Lake uranium mine (Chapter 2). Two lakes received mill effluent, two lakes received open-pit dewatering effluent, whereas a fifth lake upstream of mining activity served as a reference. Mill-effluent receiving waters were more toxic than dewatering-effluent receiving waters as indicated by high larval fathead minnow mortality in the former relative to the latter. A descriptive metal characterization at each exposure site suggested dietary Se in receiving waters were associated with the differential mortality response *in situ*.

The purpose of this study was to: (1) test the toxicity of water from Key Lake and Rabbit Lake uranium operations using early-life stages of a metal-sensitive fish species, the fathead minnow; and, (2) corroborate results of the earlier *in situ* investigation (Chapter 2) in the laboratory using waters collected at the exposure sites used in the field study.

Water samples were collected from Fox and Unknown lakes (mill-effluent receiving waters), Little McDonald and McDonald lakes (open-pit dewatering effluent receiving waters), and David Lake (reference water) from the Key Lake uranium mine.

From the Rabbit Lake operation, water samples were collected from the top and bottom of the B-Zone pit. Samples from the top and bottom of Collins Bay of Wollaston Lake served as reference water for the B-Zone tests. Fathead minnow embryos and larvae were placed in each of the collected water samples plus laboratory controls and were tested for egg hatchability, time-to-hatch, larval survival, and growth. Relationships between observed toxicological effects and aqueous metal concentrations were determined using correlation analysis. Shortcomings of the 'single contaminant' approach were illustrated by the approach.

3.3 Materials and Methods

3.3.1. Key Lake

The Key Lake open-pit uranium mine (57°11' N, 105°34' W) is located in north central Saskatchewan, approximately 600 km north of Saskatoon, SK, Canada (Fig. 3.1). Mean annual production of U_3O_8 at Key Lake between 1983-1998 was 5.6±1.5 million kg (mean±SD; n=16) with an average ore grade of 2.33±0.24% U_3O_8 (n=16), making it the largest uranium operation in the world (Cameco Corp. 1998a; Saskatchewan Energy and Mines 1999a). Ore deposits at the Key Lake site were mined out in 1997, leaving ore stockpile milling as its current primary activity. Ore from a newly developed uranium deposit at McArthur River, approximately 70 km NE of Key Lake, is scheduled for milling at the Key Lake site and is expected to maintain operations at Key Lake for the next 20 years (Cameco Corp. et al. 1995).

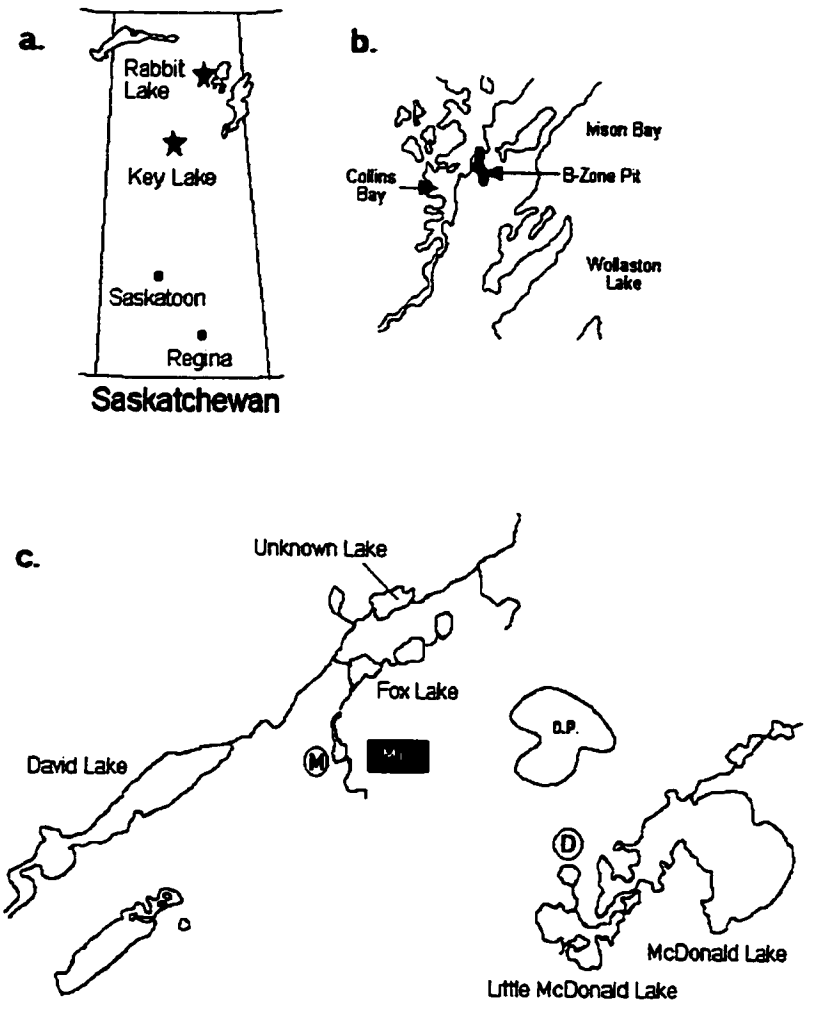

Figure 3.1: Map of study areas. (a) Map of Saskatchewan showing relative locations of the Key Lake and Rabbit Lake uranium operations. (b) The B-Zone pit is located on the Harrison Peninsula of Wollaston Lake on Collins Bay near the Rabbit Lake operation. (c) Five study lakes near the Key Lake operation. D, dewatering effluent discharge into Horsefly Lake; M, mill effluent discharge into Wolf Lake; D.P., Deilmann Pit.

Treated mill effluent is discharged to the environment at Wolf Lake. In 1997,

2.54×10^6 m^3 of effluent was discharged to Wolf Lake. Wolf Lake supplies Fox Lake, which drains via Yak Creek. David Lake, which is situated upstream of uranium operations, drains via David Creek. Yak Creek empties into David Creek before flowing into Unknown Lake. Water samples collected from Fox and Unknown lakes were collected in this study to examine the effects of mill effluent on larval fathead minnows. Water from David Lake served as a reference. Historical water quality monitoring has identified Mo concentrations significantly above background concentrations in this system (Cameco Corp. et al. 1995).

Open-pit dewatering effluent is discharged into Horsefly Lake. In 1997, 6.79×10^6 m^3 of dewatering effluent was discharged into Horsefly Lake from Key Lake's two open pits (Cameco Corp. 1998b). Horsefly Lake drains into Little McDonald Lake, which immediately flows into McDonald Lake. Little McDonald and McDonald lakes are separated by a small, shallow channel. Historical monitoring has identified Ni at concentrations above background concentrations in Little McDonald and McDonald lakes (Cameco Corp. et al. 1995). In Little McDonald Lake, Ni occurs at approximately 2-4 times the SSWQO for Ni, which is 0.025 mg/L (Saskatchewan Environment and Public Safety 1988). Nickel is now recognized as a contaminant of long-term concern at Key Lake (Cameco Corp. et al. 1995).

3.3.2. Flooded B-Zone Pit

The B-Zone ore body was discovered in 1977, and is situated approximately 10 km N of the Rabbit Lake uranium mine (58°11' N, 103°42' W) in northern Saskatchewan. The B-Zone deposit was mined using open-pit techniques between

1984-1991. During that time, the mine produced 18,250 tons of ore with an average grade of approximately 0.69% U_3O_8, and peaks reaching as high as 28.8% U (Saskatchewan Energy and Mines 1999b). Other elements enriched in the ore body include nickel (Ni), arsenic (As), and cobalt (Co).

The B-Zone pit is located on the Harrison Peninsula, which separates Collins Bay from Ivison Bay on Wollaston Lake (Cameco Corp. 1990). The northern portion of the pit extends approximately 150 m into Collins Bay. After the ore body was exhausted in 1991, the pit was force-flooded with water from Collins Bay until it reached stable levels in 1996 (Boojum Research Ltd. 1997). Water from the flooded B-Zone pit is separated from Collins Bay water by a steel-cell earthen dike, and hydrological exchange between the two water bodies is minimal relative to the volume of the flooded pit (Cameco Corp. 1990; Boojum Research Ltd. 1997).

Since the force-flooding of the pit in 1991, limnological analyses of pit water identified: a clear seasonal stratification; spring and fall turnover causing an increase in water-column conductivity over time; and, increasing biological oxygen demand in deep water (Cameco Corp. 1997). These observations, taken together with the fact that little mixing takes place with Collins Bay water, suggest that B-Zone limnology represents an isolated water system. Natural limnological processes are driving water chemistry as in a natural lake. Several proposed decommissioning strategies for the B-Zone pit involve breaching the dike separating B-Zone from Collins Bay (Cameco Corp. 1997). Consequently, it is important to gain an understanding of the toxicological implications of such a breach to fish inhabiting Collins Bay.

Table 3.1: A list of the waters used in toxicity tests of early-life stage fathead minnows.

Water	Label	Description
Laboratory water[a]	LDW	Dechlorinated Saskatoon water; control
Soft-water control[b]		Reconstituted soft water; control
Collins Bay, Top[a]	CT	Collins Bay, 2 m below surface; reference
Collins Bay, Bottom[a]	CB	Collins Bay, 6 m below surface; reference
B-Zone, Top[a]	BT	B-Zone flooded open pit, 2 m below surface
B-Zone, Bottom[a]	BB	B-Zone flooded open pit, 40 m below surface
David Lake	DAV	Upstream of U operations at Key Lake; reference
Fox Lake	FOX	Downstream of mill-effluent discharge at Key Lake
Unknown Lake	UNK	Downstream of Fox and David Lakes
Little McDonald Lake	LMD	Downstream of dewatering-effluent discharge at Key Lake
McDonald Lake	MCD	Downstream of Little McDonald Lake

3.3.3. Toxicity Tests

Two series of static and static-renewal toxicity tests were performed on field-collected water (Table 3.1) using fathead minnow embryos and larvae. The first series of tests examined the toxicity of water collected from lakes surrounding the Key Lake uranium mine. Mine mill receiving waters were collected from Fox and Unknown lakes. Pit-dewatering receiving waters were collected from Little McDonald and McDonald lakes. Water collected from David Lake served as a reference. Dechlorinated Saskatoon municipal water (laboratory water) was used as a laboratory control in Key Lake toxicity tests.

Key Lake water samples were collected at four sites in each lake corresponding to a previous field study (Chapter 2). Samples were collected in clean 20-L collapsible

polyethylene containers that were triple rinsed in sample water. Water samples were immediately placed on ice and kept at 4°C before, during, and after air transportation to the laboratory in Saskatoon. Prior to toxicity test initiation, water samples were warmed to 25°C and water quality data were gathered.

The second series of tests examined the toxicity of water from the flooded B-Zone pit. Water samples were collected from depths of 2 m and 40 m in the B-Zone pit, representing 'top' and 'bottom'. Reference water was collected from Collins Bay near the dyke separating B-Zone from the rest of Wollaston Lake. Samples from Collins Bay were collected at depths of 2 m and 6 m, representing 'top' and 'bottom' samples. In addition to B-Zone and Collins Bay water, additional water samples were collected from Little McDonald Lake.

Water samples were collected by Key Lake and Rabbit Lake environmental personnel in clean and rinsed, 20-L polyethylene containers. Samples were packed on ice and shipped by air to Saskatoon where they were maintained at 4°C until tested. As in Key Lake tests, water samples were warmed to 25°C and water quality data were collected prior to testing. In all experiments, testing began within 72 h of water sample collection.

All tests were conducted in a temperature-controlled room at 25±1°C under a photoperiod of 16 h light and 8 h dark. Tests were conducted in 400-mL plastic cups filled with 300 mL of sample water. Key Lake egg hatchability and all larval growth tests were conducted as static-renewal. Debris was siphoned from each cup daily and

80% of the water was replaced. The B-Zone egg hatchability test was conducted as static (see below).

All fathead minnow growth and survival tests were conducted according to Canadian Environmental Protection Service guidelines (Canadian Environmental Protection Service 1992a). Larval fathead minnows were exposed to each of the study waters, replicated four times, for 168 h. Each replicate contained 10 fish. Controls included dechlorinated Saskatoon municipal water (laboratory water) for Key Lake tests, and laboratory water and a soft, reconstituted water control for tests of B-Zone water. Reconstituted water was prepared by adding reagent-grade $NaHCO_3$, $CaSO_4$, $MgSO_4 \cdot 7H_2O$, and KCl (BDH Chemicals Ltd., Poole, England) to commercially-available reverse osmosis water (Culligan, Saskatoon, SK), according to the specifications of Cooney (1995) for soft water.

Fish were fed brine shrimp (*Artemia salina*; Artemia Canada, Chaplin, SK) nauplii such that live nauplii were available continuously throughout daylight hours. At the end of each growth test, replicate groups of fish were euthanized in hot distilled water, dried at 110°C overnight, and weighed to the nearest 10 µg (Mettler AE240, Mettler-Toledo International, Inc., Greifensee, Switzerland).

Egg hatchability protocol varied between Key Lake and B-Zone tests because of high levels of mortality in control treatments in early tests. During tests of Key Lake water, 10 freshly-fertilized eggs (<12 h) were randomly assigned to each test replicate and water was renewed on a 24-h basis. Tests ended when eggs either hatched or died.

These tests were repeated several times until valid (<20% mortality in controls) results were obtained.

Preliminary testing of B-Zone water samples revealed that static tests begun with eyed eggs yielded equally sensitive results relative to static renewal tests with freshly fertilized eggs, but without high control mortality (unpublished data). Consequently, eggs collected within a 12-h period were placed in an Imhoff settling cone provided with aeration for 48-h. Ten eyed-eggs (48 h) were randomly assigned to test replicates, where they remained until all eggs either hatched or died. Water was not renewed during the test, but mortality data were collected daily.

Water quality was monitored throughout each test for pH, conductivity, total dissolved solids, dissolved oxygen, hardness, and alkalinity. An Orion pH meter (model 290A; Orion Research Inc., Beverly, MA) was used to measure pH. Dissolved oxygen was measured with a Corning (Corning Scientific Instruments, Corning, NY) electronic dissolved oxygen probe (with built-in temperature sensor) attached to a Corning M90 hand-held meter. Conductivity and total dissolved solids (TDS) were measured with Corning's electronic conductivity probe and M90 meter. Hardness and alkalinity were monitored using Hach test kits, models 5-EP MG-L and AL-AP, respectively (Hach Co., Loveland, CO).

Metal concentrations in unfiltered water samples were determined by inductively coupled plasma-mass spectrophotometry (ICP-MS). Key Lake water was analyzed at the Department of Geological Sciences, University of Saskatchewan. Metal concentrations were adjusted by subtracting metal concentrations from sample and

procedural blanks. Concentrations were verified by analysis of National Research Council of Canada River Water Standard (SLRS-3). Coefficients of variation determined for duplicate samples were less than 22%. Measured concentrations were generally within 21% of known values. B-Zone water samples were analyzed at the Saskatchewan Research Council (SRC), Saskatoon, SK. Quality assurance of B-Zone water samples was determined according to SRC's routine in-house QA/QC protocols.

3.3.4. Statistical Treatment

Comparisons among treatments were made using analysis of variance (ANOVA), followed by Dunnett's test to compare treatments against controls, and Tukey's test to compare means among treatments. Normality and homogeneity of variance of endpoint data were tested using Shapiro-Wilks and Bartlett's tests, respectively. Data not meeting ANOVA assumptions were $\log_{10}(x+1)$-transformed. This transformation usually resulted in improvements in data distributions, but not always. In the case where data still did not meet ANOVA assumptions, endpoint means were compared by Kruskall-Wallace tests, and pairwise comparisons were made using Dunn's test. Pearson (parametric) or Spearman (non-parametric) correlation coefficients were computed for associations between test endpoints and aqueous metal concentrations, depending on the distribution of the data. All statistical calculations were conducted on TOXSTAT v3.5 and SPSS statistical software (SPSS 1988; West and Gulley 1996).

Table 3.2: Basic water chemistry of the several waters to which early-life stage fathead minnows were exposed in this study.

Exposure Water	pH[a]	Total Alkalinity[b]	Total Hardness[b]	Conductivity[c]	TDS[d]	Dissolved Oxygen[d]
Key Lake Tests						
LDW	7.5 (7.4-7.6; 5)	60.0 (15.8; 5)	129.6 (45.1; 5)	342.4 (72.3; 5)	170.6 (36.0; 5)	7.7 (0.5; 4)
DAV	7.0 (6.5-7.8; 6)	18.7 (5.1; 6)	13.7 (9.0; 6)	32.8 (11.1; 6)	16.2 (5.5; 6)	8.1 (1.1; 5)
FOX	5.5 (5.2-6.6; 6)	20.7 (3.9; 6)	604.7 (173.3; 6)	2104.2 (207.6; 6)	1011.3 (107.6; 6)	8.3 (1.2; 5)
UNK	5.3 (5.0-5.7; 6)	18.3 (5.2; 6)	401.3 (173.1; 6)	1054.2 (234.5; 6)	510.3 (113.5; 6)	8.3 (1.3; 5)
LMD	6.9 (6.6-7.4; 6)	21.2 (5.5; 6)	25.3 (8.6; 6)	74.6 (14.3; 6)	38.6 (6.9; 6)	8.4 (1.1; 5)
MCD	7.1 (7.0-7.4;6)	22.2 (4.7; 6)	26.0 (17.3; 6)	64.2 (7.2; 6)	32.0 (3.6; 6)	8.2 (1.3; 5)
B-Zone Tests						
LDW	7.8 (7.4-7.9; 7)	68.5 (13.6; 6)	131.3 (25.2; 6)	381.6 (45.8; 7)	189.9 (21.8; 7)	7.5 (0.4; 3)
RW	7.5 (7.3-7.7; 4)	23.8 (11.8; 4)	55.0 (10.0; 4)	186.5 (86.4; 3)	92.6 (42.6; 3)	7.7 (0.5; 3)
CT	7.2 (6.8-7.3;5)	23.3 (4.2; 3)	16.7 (3.1; 3)	30.3 (12.4; 5)	15.7 (4.8; 5)	8.1 (0.9; 4)
CB	7.0 (6.8-7.2; 5)	23.3 (4.2; 3)	14.7 (2.3; 3)	31.4 (6.0; 5)	15.2 (4.3; 5)	8.1 (0.8; 5)
BT	7.3 (6.9-7.5; 5)	45.7 (5.1; 3)	28.0 (2.0; 3)	77.4 (10.4; 5)	37.2 (7.8; 5)	8.1 (1.1; 5)
BB	7.1 (6.9-7.3; 5)	44.7 (4.5; 3)	26.3 (3.2; 3)	75.9 (11.8; 5)	37.4 (6.3; 5)	8.2 (0.9; 5)
LMD	6.9 (6.7-7.3; 4)	22.3 (2.5; 3)	16.0 (0.0; 2)	55.0 (5.2; 4)	27.4 (2.6; 4)	7.8 (0.4; 4)

a pH measured in standard pH units. Values represent medians (range; n).
b Total alkalinity and total hardness are expressed as mean mg $CaCO_3$/L (standard deviation; n).
c Conductivity is expressed as mean µS/cm (standard deviation; n).
d TDS, i.e., Total Dissolved Solids, and dissolved oxygen are expressed as mean mg/L (standard deviation; n).

3.4 Results

3.4.1. Water Quality of Test Waters

Basic water chemistry varied among study waters (Table 3.2). Laboratory

dilution water had higher pH and total alkalinity than waters collected from the Key Lake area. Similarly, laboratory dilution water had higher alkalinity, hardness, pH, conductivity, and total dissolved solids (TDS) than waters collected from Collins Bay or the B-Zone pit. Reconstituted soft water used in B-Zone tests was more similar to study waters than laboratory dilution water; although pH, hardness, conductivity, and TDS were higher than most study waters.

Among Key Lake waters, David, Fox, and Little McDonald Lake waters had circumneutral pH, whereas water from Fox and Unknown lakes were acidic (pH 5.5 and 5.3, respectively). Although laboratory dilution water was hard (hardness 129.6 mg/L as $CaCO_3$), waters from Fox and Unknown lakes were very hard (hardness >400 mg/L as $CaCO_3$). Waters from David, Little McDonald, and McDonald lakes were soft (hardness <25 mg/L as $CaCO_3$). Conductivity of laboratory water was 342.4 µS/cm, and in Fox and Unknown lakes conductivity exceeded 1000 µS/cm. However, conductivity in David, Little McDonald, and McDonald lakes was considerably less (<75 µS/cm). Mean dissolved oxygen (DO) concentrations did not fall below 7.5 mg/L in any treatment of both Key Lake and B-Zone tests.

The pH in B-Zone pit water (pH 7.1-7.3) was similar to that of Collins Bay water (pH 7.2-7.5). However, alkalinity, hardness, conductivity, and TDS concentrations in B-Zone water were approximately twice those in Collins Bay water. Water chemistry from Little McDonald Lake water closely paralleled that from Collins Bay, with the exception of conductivity and TDS, both of which where higher in Little McDonald Lake.

Nickel and Mo are two contaminants of concern at the Key Lake mine, and Ni and As are of concern in B-Zone pit water (Cameco Corp. 1997). Analysis of Key Lake water revealed that Ni concentrations exceeded the SSWQO for Ni (25 μg/L) in water collected from Little McDonald (128 μg/L), McDonald (48 μg/L), and Fox (28 μg/L) lakes (Saskatchewan Environment and Public Safety 1988) (Table 3.3). Arsenic occurred in Fox Lake water above the SSWQO (50 μg/L). Unknown Lake also had elevated As concentrations (28 μg/L), but not exceeding the SSWQO. Molybdenum, however, occurred at very high concentrations in Fox (2552 μg/L) and Unknown (1010 μg/L) Lake water, although no SSWQO currently exists for Mo.

In B-Zone tests, water from the B-Zone pit showed Ni concentrations (218-254 μg/L) exceeding the SSWQO by an order of magnitude. Arsenic concentrations also exceeded the SSWQO of 50 μg/L in B-Zone water (65-76 μg/L) (Saskatchewan Environment and Public Safety 1988). Molybdenum concentrations were highest in water collected from the B-Zone pit regardless of depth (51-53 μg/L), relative to reference water (1 μg/L).

In both Key Lake and B-Zone tests, laboratory dilution water had high concentrations of Al (277 and 236 μg/L, respectively) and Sr (163 μg/L for both tests). Aluminum concentrations in laboratory dilution water were higher than any other water tested. Strontium concentration in laboratory dilution water (163 μg/L) was only exceeded by Sr in Fox (307 μg/L) and Unknown (307 μg/L) lakes. Reconstituted soft water used in B-Zone tests had very low metal concentrations.

Table 3.3: Concentration of selected metals (μg/L), as determined by ICP-MS, in 13 waters used to expose early-life stage fathead minnows. Values represent means (n=4-9). ND, not detected; NA, not analyzed; LDW, lab dilution water; RW, reconstitued water; DAV, David Lake, CT, Collins Bay-Top; CB, Collins Bay-Bottom; BT, B-Zone Pit-Top; BB, B-Zone Pit-Bottom; FOX, Fox Lake; UNK, Unknown Lake; LMD, Little McDonald Lake; MCD, McDonald Lake.

	B-Zone Tests							Key Lake Tests					
Metal	LDW[b]	RW	CT	CB	BT	BB	LMD[b]	LDW[a]	DAV	FOX	UNK	LMD[a]	MCD
Al	236.79	5.00	10.00	9.00	43.17	23.20	5.86	277.33	17.55	46.72	38.04	10.26	4.90
As	0.39	ND	0.33	0.33	65.33	76.33	0.38	0.32	0.36	81.72	28.26	0.32	0.31
B	57.73	23.00	2.80	2.60	20.83	20.60	14.00	58.61	8.07	597.61	284.70	23.71	20.43
Ba	41.73	0.51	3.60	3.20	8.33	9.00	5.86	43.40	3.29	65.19	32.49	10.11	7.86
Cd	0.04	ND	0.44	0.44	0.53	0.44	0.47	0.04	0.11	2.18	0.88	0.11	0.10
Co	0.04	0.03	1.00	1.00	1.00	2.20	1.71	0.04	0.02	1.28	0.77	3.98	0.17
Cu	3.86	0.29	3.20	1.00	1.17	1.00	1.43	3.11	1.52	1.49	0.96	1.09	0.44
Fe	69.13	0.03	102.80	69.40	173.33	322.00	114.43	80.40	NA	NA	NA	NA	NA
Mn	0.38	0.40	19.60	17.80	20.67	66.20	63.57	0.38	NA	NA	NA	NA	NA
Mo	3.18	0.58	1.00	1.00	50.67	52.80	1.00	4.61	0.46	2551.66	1009.88	0.92	0.31
Ni	2.34	1.07	1.00	1.00	218.33	254.00	86.57	3.13	0.53	28.30	16.76	127.93	47.74
Pb	0.04	0.02	2.00	4.20	2.00	2.00	2.29	0.02	0.22	0.24	0.10	0.66	0.11
Sr	163.31	0.62	12.60	12.00	55.50	56.60	36.71	163.31	9.99	603.71	306.78	45.97	37.70
U	0.41	ND	0.90	0.90	4.74	5.16	8.73	0.30	0.05	1.42	0.63	8.65	2.11
Zn	5.68	1.24	5.00	5.00	9.33	5.00	16.29	5.16	6.83	4.23	5.74	19.77	5.47

a Water used in Key Lake tests

b Water used in B-Zone tests

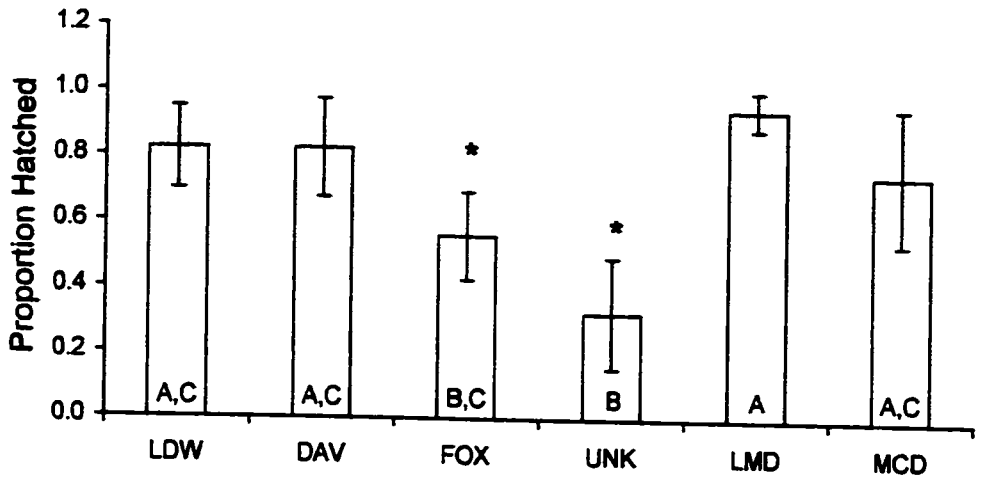

Figure 3.2: Egg hatchability, expressed as proportion hatched, in water collected from lakes in the vicinity of the Key Lake uranium mine. Bars represent means (±SD; n=4). Asterisks indicate significant differences with laboratory water (LDW; $p<0.05$, Dunnett's test). Bars with the same letter designation are not significantly different from one another (Tukey's test; $p>0.05$). LDW, laboratory control; DAV, David Lake water, FOX, Fox Lake water; UNK, Unknown Lake water; LMD, Little McDonald Lake water; MCD, McDonald Lake water.

3.4.2. Key Lake Tests

Egg hatchability (Fig. 3.2) was 82.5% in laboratory and David Lake water, and varied by exposure water ($p=0.0001$). Egg hatchability in Fox and Unknown lakes was 32% and 61% less than in laboratory or David Lake water, respectively. Mean egg hatchability was higher in Little McDonald Lake water (95%) relative to laboratory or David Lake water, although this difference was not significant ($p>0.05$).

In laboratory control water, fathead minnow eggs required 5.7±1.1 d (mean±SD; n=4) to hatch. The time required for fathead minnow eggs to hatch was significantly different among the six exposure waters ($p<0.0001$; Fig. 3.3). Eggs hatched 33-51% earlier in David, Little McDonald, and McDonald lake waters relative to laboratory control water. Time required for fathead minnow eggs to hatch in Fox and Unknown lake waters was not significantly different from laboratory control water ($p>0.05$).

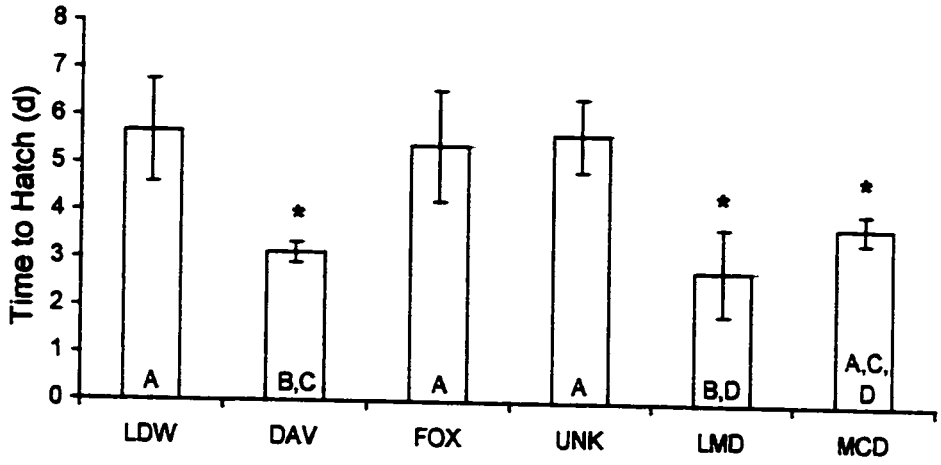

Figure 3.3: Hatching time (d) required for fathead minnow eggs placed in water collected from lakes in the vicinity of the Key Lake uranium mine. Bars represent means (±SD; n=4). Asterisks indicate significant differences with laboratory water (LDW; $p<0.05$, Dunnett's test). Bars with the same letter designation are not significantly different from one another (Tukey's test; $p>0.05$). LDW, laboratory control; DAV, David Lake water, FOX, Fox Lake water; UNK, Unknown Lake water; LMD, Little McDonald Lake water; MCD, McDonald Lake water.

Larval mortality in laboratory control water was 5.3% (Fig. 3.4). However, in David Lake water larval mortality was 82.5%, which was significantly higher than mortality in laboratory water ($p<0.05$). Mortality did not differ from laboratory-water mortality in Fox (0%), Little McDonald (5%), or McDonald (2.5%) lake waters. However, mortality in Unknown Lake water (85%) was significantly higher than laboratory water.

Larval weight was dependent on exposure water ($p=0.02$; Fig. 3.5). In laboratory control water, fathead minnow larvae were 191±81 µg (n=4) after 168 h of exposure. Larvae exposed to David Lake water grew 32% less than those in laboratory control water, although the difference was not significant ($p>0.05$). Larval weight in Fox, Unknown, Little McDonald, and McDonald lake water showed similar gain to that

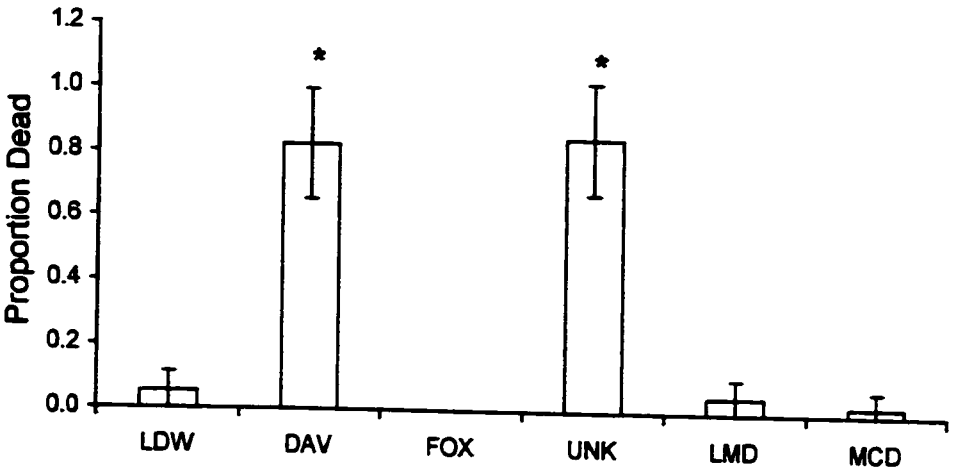

Figure 3.4: Mortality of larval fathead minnows, expressed as proportion dead, in water collected from lakes in the vicinity of the Key Lake uranium mine. Bars represent means (±SD; n=4). Asterisks indicate significant differences with laboratory water (LDW; $p<0.05$, Dunnett's test). LDW, laboratory control; DAV, David Lake water, FOX, Fox Lake water; UNK, Unknown Lake water; LMD, Little McDonald Lake water; MCD, McDonald Lake water.

in laboratory control water. However, fish weight in Unknown Lake water was 63% greater than in David Lake water ($p<0.05$).

Correlations between significant toxicological effects and metal concentrations in exposure waters are reported in Table 3.4. Egg hatchability was negatively correlated ($p<0.05$) with As, B, Ba, Cd, Mo, and Sr. The time required for fathead minnow eggs to hatch was positively correlated with Al, As, B, Ba, Cd, Mo, and Sr, but negatively correlated with U ($p<0.05$). Larval growth as measured by weight was positively correlated with Co and Ni ($p<0.05$). Larval mortality was significantly and negatively related to Ba, Ni, and U concentration ($p<0.05$). Spearman correlation analysis showed no significant ($p>0.05$) association between toxicological endpoints (i.e., egg hatchability, time-to-hatch, larval growth, or mortality) and water-quality parameters (i.e., pH, alkalinity, hardness, conductivity, TDS, or dissolved oxygen).

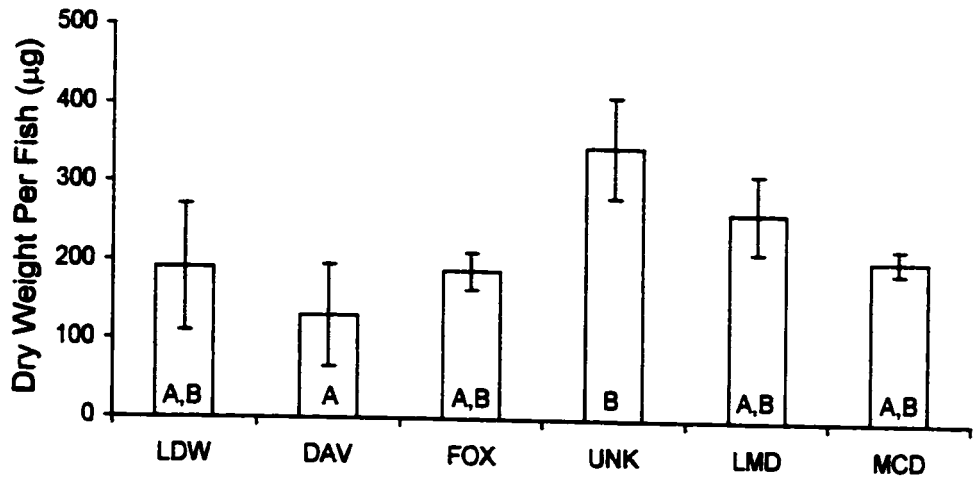

Figure 3.5: Growth of larval fathead minnows placed in water collected from lakes in the vicinity of the Key Lake uranium mine. Bars represent means (±SD; n=4, except in DAV and UNK where n=3 and 2, respectively). Growth was not significantly different in any lake water relative to laboratory control (Dunnett's test; $p>0.05$). Bars with the same letter designation are not significantly different from one another (Tukey's test; $p>0.05$). LDW, laboratory control; DAV, David Lake water, FOX, Fox Lake water; UNK, Unknown Lake water; LMD, Little McDonald Lake water; MCD, McDonald Lake water.

3.4.3. B-Zone Tests

Egg hatchability was 100% in all exposure waters during the B-Zone egg-hatchability test. Eggs exposed to B-Zone pit water hatched earlier than those exposed to other treatments ($p<0.05$; Fig. 3.6). Eggs exposed to water from the top and bottom of the B-Zone pit hatched 30 and 40% earlier, respectively, than those exposed to laboratory control water. Eggs exposed to water from the bottom of the B-Zone pit also hatched 33% earlier than eggs exposed to Little McDonald Lake, and 26-39% earlier than water collected from Collins Bay. Hatching times were strongly and negatively correlated with As ($r=-0.97$), Fe ($r=-0.93$), Mo ($r=-0.95$), and Ni ($r=-0.99$). Spearman correlation analysis showed that hatching time was also negatively related to total alkalinity ($r=-0.77$).

Table 3.4: Relationships between waterborne metal concentrations and toxicological endpoints during tests on early-life stage fathead minnows in Key Lake water. Values represent Pearson correlation coefficients unless otherwise noted. Correlations were based on $\log_{10}(x+1)$-transformed data, and n ranged from 16-23. Significance is indicated by superscript; lack of superscript indicates $p>0.05$.

Metal	Egg Hatchability	Time Required for Hatching	Larval Growth[d]	Larval Mortality[d]
Al	-0.16	0.70[c]	0.00	0.00
As	-0.71[c]	0.56[b]	0.03	-0.10
B	-0.65[a]	0.71[c]	0.20	-0.19
Ba	-0.43[a]	0.77[c]	0.11	-0.44[a]
Cd	-0.63[b]	0.47[a]	0.10	-0.13
Co	0.05	-0.17	0.52[a]	-0.35
Cu	0.05	0.37	0.14	-0.14
Mo	-0.73[c]	0.63[b]	0.04	-0.05
Ni	-0.01	-0.17	0.46[a]	-0.52[a]
Pb	0.18	-0.06	0.00	-0.05
Sr	-0.56[b]	0.74[c]	0.27	-0.23
U	0.29	-0.41[a]	0.19	-0.55[b]
Zn	0.31	0.31	0.44[a]	-0.02

a $p<0.05$
b $p<0.01$
c $p<0.001$
d Spearman correlation coefficients

During the fathead minnow survival and growth test, neither mortality nor growth varied among treatments ($p>0.05$). Larval mortality was 10% or less in all treatments including controls. This low mortality caused mean mortality coefficients of variation (CV) to range between 67-200%. Consequently, a non-parametric analysis of variance (i.e., Kruskall-Wallis Test) was conducted, which corroborated parametric results ($p>0.05$). Overall mean individual fish weight was 183 ± 26 µg (n=28), and did not vary significantly with exposure treatment.

3.5 Discussion

This study showed that the toxicity of waters affected by uranium mining operations to early-life stage fathead minnows varied significantly. Mill-effluent

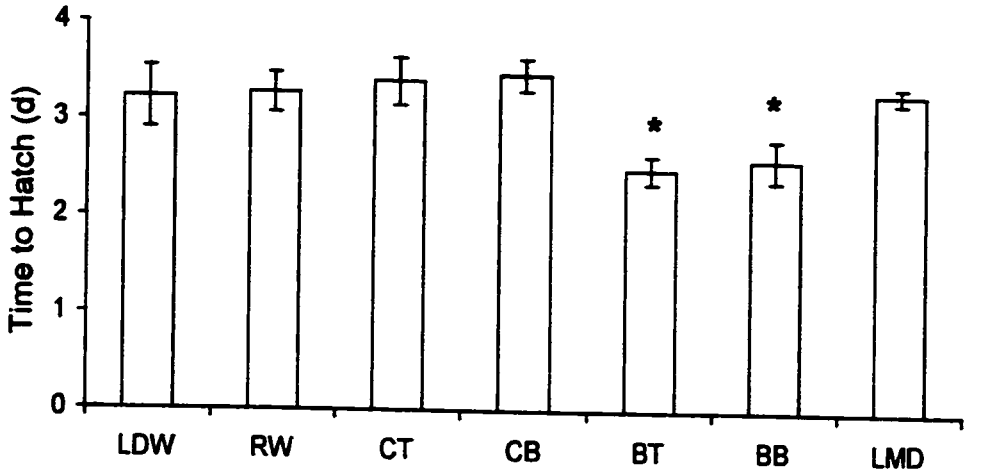

Figure 3.6: Hatching time (d) required for fathead minnow eggs placed in water collected from the flooded B-Zone open pit and Collins Bay of Wollaston Lake in the vicinity of the Rabbit Lake uranium mine, and Little McDonald Lake near the Key Lake mine. Bars represent means (±SD; n=4). Asterisks indicate significant differences with laboratory (LDW) and soft-water (RW) controls ($p<0.05$, Dunnett's test performed separately for each control). BT, B-Zone top (2 m depth); BB, B-Zone bottom (40 m depth); CT, Collins Bay top (2 m depth); CB, Collins Bay bottom (6 m depth); LMD, Little McDonald Lake.

receiving waters caused inhibition of hatching. Dewatering-effluent and B-Zone waters caused shorter hatching times. Effects on mortality were inconsistent, with no mortality in one mill-effluent receiving water (Fox Lake), but 85% mortality in Unknown lake water (Fig. 3.4). Dewatering effluent water had no effect on mortality. Growth in either type of receiving water was not significantly different from laboratory control water (Fig. 3.5).

Results reported here were not completely consistent with a related field study (Chapter 2). The field study showed that mortality of larval fathead minnows was <20% in David, Little McDonald, and McDonald lakes, and >80% in Fox and Unknown lakes. This study showed >80% mortality in David and Unknown lake water, but <10% mortality in Fox, Little McDonald, and McDonald Lake waters. However, growth results were similar; i.e., growth did not vary by lake. Hence, laboratory to field toxicity

extrapolations for larval mortality are problematic, although laboratory results appear to correctly predict lack of growth effects. Relationships between biological effects and exposure metal concentrations were inconsistent between Key Lake and B-Zone tests. Stimulated egg hatching was the only significant result observed in the B-Zone tests, and was found to be strongly and negatively correlated with As ($r=-0.97$), Fe ($r=-0.93$), Mo ($r=-0.95$), and Ni ($r=-0.99$). However, correlations between significant egg hatchability, hatching time, larval growth, and mortality with metals in Key Lake water were weaker and much more complex (Table 3.4). For example, hatching time was not correlated with Ni ($r=-0.17$), but was significantly and positively correlated with As ($r=0.56$) and Mo ($r=0.63$). Moreover, egg hatchability, larval growth and mortality were not affected by B-Zone water, but were by Key Lake receiving waters. Biological effects are difficult to interpret using simple correlation analyses with individual metal concentrations, unless due consideration is given to confounding factors related to water chemistry.

The 11 waters investigated in this study can be divided into four loose categories based on water quality (Table 3.2). Waters having neutral pH, low hardness, and low metals are represented by David Lake and Collins Bay. Little McDonald Lake, McDonald Lake, and B-Zone pit represent waters with neutral pH, low hardness, and high Ni concentrations. Waters with low pH and high hardness, conductivity, Mo, and As are from Fox and Unknown lakes. Finally, laboratory dilution water had neutral pH, high hardness, and low metals (except for Al and Sr).

Biological effects can be interpreted in the light of this water categorization. In low pH, high hardness, high conductivity waters (i.e., Fox and Unknown lakes), effects were variable. In Fox and Unknown lakes there was no effect on time to hatch (Fig. 3.3). However, egg hatchability was reduced in both lakes (Fig. 3.2), and high mortality occurred in Unknown Lake water, but not in Fox Lake water (Fig. 3.4). These waters represent mill-effluent receiving waters, and both lakes have a complex chemical composition (Tables 3.2 and 3.3). The complex chemistry results in opposing mechanisms that influence each water's toxicity to fish. Low pH tends to cause free metal cations (i.e., M^{2+}) to dominate total-metal speciation (Morel 1983). The free cation is thought to be the most toxic form of most metals (Morel 1983). On the other hand, water hardness is known to ameliorate the effects of metals on fish through competitive interactions between hardness (e.g., Ca^{2+}) and metal (M^{2+}) cations at the gill surface (Playle et al. 1992). Given the opposing controlling factors (i.e., pH and hardness), and the complex mixture of toxicants in Fox and Unknown lakes, variable results are not surprising.

Early hatching occurred in neutral, low-hardness, high Ni waters (i.e., B-Zone and Little McDonald lakes) (Figs. 3.3 and 3.6). In waters containing moderate concentrations of Ni (i.e., McDonald, Fox, and Unknown lakes), hatching time was lowest in McDonald Lake water, which also had the softest water of the three lakes. These results suggest a Ni-related reduction in hatching time in soft waters, which was absent in hard waters. Arsenic in B-Zone water exceeded the SSWQO of 50 µg/L, and was negatively correlated with hatching time ($r=-0.97$). Arsenic was also above the

SSWQO in Fox Lake, but hatching times in Fox Lake did not differ from controls. The relatively simple water chemistry of waters showing a significant effect on hatching time yielded consistent effects on hatching time. However, David Lake water (i.e., reference water), also showed a significant reduction in hatching time relative to laboratory dilution water. Conductivity in David Lake water was only 32.8 µS/cm, representing the lowest ionic potential of any water tested. Osmoregulatory dysfunction associated with the low ion content of David Lake water may be responsible for the early hatching times.

Short hatching times demonstrated in dewatering-effluent receiving waters and B-Zone pit waters (neutral, low-hardness, high Ni) seem consistent with laboratory tests. Fathead minnow eggs placed in hard, Ni-spiked laboratory dilution water also showed shorter hatching times relative to controls (Chapter 4). On the other hand, Dave and Xiu (1991) reported a slight (but significant) hatching delay in zebrafish (*Brachydanio rerio*) eggs exposed to Ni-spiked, reconstituted hard water. Because both studies exposed fish under hard water conditions, this discrepancy is likely attributable to species differences.

The effect of shorter hatching times may have significant ecological relevance. Eggs exposed to several metals, like Cu and Pb (Dave and Xiu 1991), commonly demonstrate hatching delays. Delayed hatching response in the presence of metals may be a protective strategy (Dave and Xiu 1991). Developing fish that remain in the protective confines of the chorion for longer periods of time may be more tolerant to elevated environmental metals once they finally hatch. Conversely, those that hatch earlier may be more sensitive to metals. Consequently, failure to recognize a fish

population's vulnerability to an early hatching response could place sensitive fish species at risk.

Larval fathead minnow growth and survival was not significantly affected upon exposure to mill effluent, dewatering, or pit water relative to controls and reference water from Collins Bay. This lack of effects on growth and survival is consistent with the findings of laboratory tests, where larval fathead minnows exposed to Ni in hard (140 mg/L as $CaCO_3$) and soft (20 mg/L as $CaCO_3$) reconstituted water showed 96-h LC50s of 2.28 mg Ni/L (95% confidence interval [CI] 2.05–2.55 mg Ni/L) and 0.49 mg Ni/L (95% CI 0.39–0.62 mg Ni/L), respectively (Chapter 5). Molybdenum-exposed fathead minnow mortality did not vary with controls at concentrations as high as 100 mg Mo/L (Chapter 4). These results show that Ni toxicity is moderated by water hardness, and that Mo is not acutely toxic to fathead minnows. Because the highest concentration of Ni observed in this study was 0.25 ± 0.01 mg Ni/L (bottom of B-Zone pit) in water that was slightly harder than that used in Chapter 5, neither Ni nor Mo were expected to affect fathead minnow survival in B-Zone water.

The lack of acute mortality in this study is consistent with studies of Ni and As toxicity to other fish species. Alevin and juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to Ni and As in soft water (hardness 40 mg/L as $CaCO_3$; pH 7.1-8.0) showed 96-h LC50s of 25.1 and 7.79 mg/L, respectively for Ni, and 91 and 16 mg/L, respectively for As (Buhl and Hamilton 1991). Alam and Maughan (1992) exposed 3.2 cm and 6.0 cm common carp (*Cyprinus carpio*) to Ni (pH 7.1; hardness not reported) and reported 96-h LC50s of 1.3–1.54 mg Ni/L and 1.64–2.3 mg Ni/L, respectively.

These studies show that Ni and As are acutely toxic to fish at concentrations 1-3 orders of magnitude higher than the highest concentrations observed in this study (B-Zone pit).

The lack of effects on growth in this study is also consistent with other studies. Sayer et al. (1991) did not observe any effects on larval brown trout (*Salmo trutta*) growth when exposed to 0.01 mg Ni/L (pH 7.2 in soft water). Results reported in Chapter 4 demonstrated no effect on larval fathead minnow growth after a 168 h exposure to 2 mg Ni/L in hard water (hardness 140 mg/L as $CaCO_3$). Taken together, these results suggest that Ni does not seem to affect fish growth.

Stewart (1999) recently reported that competition among metals in a complex metal mixture (characteristic of mine receiving waters) for particulate binding sites may actually increase bioavailability of certain metals, while simultaneously decreasing bioavailability of others. It is likely that a similar phenomenon occurred in low-pH, high hardness, high-metal waters (i.e., Fox and Unknown lake waters) relative to neutral, low-hardness, high-Ni waters (i.e., Little McDonald, McDonald, and B-Zone waters). Consideration of confounding variables, such as water quality parameters (e.g., pH and hardness), in addition to intercorrelated metal concentrations, is necessary for linking biological effects to environmental conditions. Stewart (1999) emphasized the need to re-evaluate water-quality criteria based on single metals. Results reported here indicated that consideration of single metals to account for biological effects is insufficient.

In conclusion, early-life stage fathead minnow tests demonstrated various responses to water affected by uranium-mining activities, depending on the source and

quality of the water. Effects observed on larval fathead minnows exposed to Key Lake water provided only partial corroboration for earlier results obtained in the field. Correlations between biological effects and metal concentrations were difficult to interpret owing to the complex chemistry of mill-effluent receiving waters. Consideration of elevated metal concentrations, like Ni and As, in the light of confounding factors, such as pH and hardness, allowed for a more meaningful interpretation of biological effects. Nickel seemed to reduce hatching time in soft waters. This result was consistent with laboratory studies of fish eggs in Ni-spiked laboratory dilution water. Hatching stimulation may have significant ecological implications for sensitive species. Mortality results were inconsistent owing to complex interactions with confounding variables, such as pH and hardness. Standard toxicological evaluations of mine receiving waters routinely examine endpoints like growth and mortality. Effects are often attributed to single contaminants exceeding water quality criteria. Results reported here indicate that growth and mortality are less sensitive endpoints than time to hatch, and that analysis of single contaminants is not sufficient for accounting for biological effects.

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4. Nickel and Molybdenum Toxicity to Early-Life Stage Fathead Minnows (*Pimephales promelas*), Rainbow Trout (*Oncorhynchus mykiss*), Northern Pike (*Esox lucius*), and White Suckers (*Catostomus commersoni*)

4.1 Abstract

Nickel (Ni) and molybdenum (Mo) are two metals that are associated with uranium mining in northern Saskatchewan, Canada. Fathead minnows (*Pimephales promelas*) and rainbow trout (*Oncorhynchus mykiss*) are standard toxicity test fish species commonly used to characterize uranium mine effluent and receiving water toxicity. Results from these tests are often used to predict toxicity to indigenous fish species like northern pike (*Esox lucius*) and white suckers (*Catostomus commersoni*). Early-life stage toxicity of Ni and Mo to fathead minnows, rainbow trout, northern pike, and white suckers was tested in hard water. Nickel was generally more toxic than Mo. Fathead minnow eggs exposed to 0.75 mg Ni/L hatched 30% earlier than those in controls, which was also the most sensitive endpoint examined for detecting Ni toxicity. Fathead minnow egg hatchability was reduced by 25% relative to controls at 1.8 mg Ni/L. Nickel 96-h LC50s for larval fathead minnows, sac fry northern pike, sac fry white suckers, alevin rainbow trout, and juvenile rainbow trout were 2.4, >3, 17.9, >20, and 51.2 mg Ni/L, respectively. Nickel did not affect fathead minnow, northern pike, or white sucker growth. During acute Ni exposure, Ni accumulated in rainbow trout gills but not liver, the latter resulting from the short duration of exposure. Molybdenum had no significant effect on fish egg hatchability, growth, or mortality. Sensitive fish species

inhabiting Ni-contaminated receiving waters may not be adequately protected if environmental decisions are based on results of toxicity tests using rainbow trout.

4.2 Introduction

Metals enter aquatic systems through atmospheric deposition from the burning of fossil fuels, surface runoff from urban and industrial activities, or through natural processes such as ground water leaching. The aquatic toxicity of some metals, such as copper, cadmium, and mercury, is well known. However, other metals, like nickel and molybdenum, receive considerably less attention.

Nickel (Ni) and molybdenum (Mo) are naturally occurring elements and are ubiquitous in aquatic environments (Greenwood and Earnshaw 1984). Although both Ni and Mo are considered 'essential' elements (Mertz 1981; Goyer 1991; Galvin 1996), aqueous concentrations exceeding biological thresholds can yield toxic effects in aquatic organisms (Buhl and Hamilton 1991; Dave and Xiu 1991). In natural water ranging in pH between 5 and 9, Ni occurs primarily as Ni^{2+} (Richter and Theis 1980) cation, whereas the dominant Mo species is the highly soluble molybdate (MoO_4^{2-}) anion (Magyar et al. 1993). Nickel can form insoluble complexes with organic and inorganic ligands causing a focus of Ni contamination near its point of discharge. Although Mo can also form insoluble sulphides (e.g., MoS_3 or MoS_2) under anoxic conditions (Viollier et al. 1995), Mo remains primarily dissolved as the molybdate anion in the epilimnion (Magyar et al. 1993). This general abundance of dissolved Mo leads to Mo contamination that can be detected for considerable distances downstream of the discharge point (Magyar et al. 1993).

Uranium mining in northern Saskatchewan is an example of an industrial activity leading to Ni and Mo input to surrounding water bodies (Golder Associates Ltd. 1996). At Cameco Corporation's Key Lake uranium mine, relatively high concentrations of Ni enter receiving waters from open-pit dewatering processes, whereas Mo is introduced to aquatic systems in mill effluent (Cameco Corp. et al. 1995). Dewatering effluent Ni concentrations range between 0.001 and 0.35 mg/L, with a median concentration of 0.11 mg/L (Cameco Corp. et al. 1995). Nearby pit-dewatering receiving waters show Ni concentrations ranging from 54 to 116 µg/L, but reference sites show Ni concentrations of 0.5 µg/L (Chapter 2). Mill effluent Mo concentrations range between 0.01 and 3.71 mg/L, with a median concentration of 0.32 mg/L (Cameco Corp. et al. 1995). Nearby mill-effluent receiving waters show Mo concentrations of 735-1397 µg/L, whereas reference waters have considerably lower concentrations of 0.4 µg/L (Chapter 2). The Saskatchewan Surface Water Quality Objective for Ni in soft, northern Saskatchewan waters is 0.025 mg/L, whereas Mo release is currently unregulated in Saskatchewan (Saskatchewan Environment and Public Safety 1988).

Fathead minnows (*Pimephales promelas*) and rainbow trout (*Oncorhynchus mykiss*) are common toxicity-test fish species used in standard toxicological evaluations. However, neither fathead minnows nor rainbow trout inhabit lakes in the uranium district of northern Saskatchewan. Northern pike (*Esox lucius*) and white suckers (*Catostomus commersoni*) are abundant in northern-Saskatchewan lakes, but very little Ni or Mo toxicity data exist for these species. Therefore, there is a need to study the

effects of Ni and Mo on northern pike and white suckers, especially in areas receiving elevated concentrations of these metals.

The objectives of this research were to determine: (i) the early-life stage toxicity of Ni and Mo to fathead minnows, northern pike, and white suckers, and (ii) if toxicity results from fathead minnows are predictive of Ni or Mo toxicity to northern pike and white suckers. A series of laboratory acute and sublethal, static-renewal experiments were conducted in dechlorinated Saskatoon municipal water to determine the effects of Ni and Mo on fish egg hatchability, time required for hatching, fish growth, and mortality.

4.3 Materials and Methods

4.3.1. General Procedures

A series of static-renewal toxicity tests was carried out to determine the relative toxicity of Ni and Mo to early-life stage fathead minnows, rainbow trout, northern pike, and white suckers. Nickel and Mo were delivered as their chloride ($NiCl_2 \bullet 6H_2O$) and sodium ($Na_2MoO_4 \bullet 2H_2O$) salts, respectively (BDH Chemicals Ltd., Poole, England). Metal exposures included five metal treatments from the geometric dilution series 16, 25, 40, 63, and 100% of maximum nominal concentration (see Table 4.1), plus one control (clean dilution water, 0% maximum concentration). Maximum nominal concentrations were based on Ni or Mo concentrations in receiving waters near the Key Lake uranium mine, concentrations sufficient to cause early-life stage toxicity as reported in the literature, or results of range finding tests. If a particular maximum concentration was insufficient to cause an effect, the test was repeated at a higher

Table 4.1: Summary of toxicity tests performed to determine the effects of Ni and Mo on egg hatchability, time-to-hatch, mortality, and growth in fathead minnows, rainbow trout, northern pike, and white suckers.

Species	Metal	End Point	Exposure Range (mg/L)	Exposure Duration (h)
Fathead minnow	Ni	Egg hatchability	0.48-3.0	72
		Time-to-hatch	0.48-3.0	72
		Mortality	1.2-7.5	96
		Growth	0.32-2.0	168
	Mo	Egg hatchability	16-100	96
		Time-to-hatch	16-100	96
		Mortality	16-100	96
		Growth	16-100	168
Alevin RT[a]	Ni	Mortality	3.2-20	96
Juvenile RT[a]		Mortality	11.9-74.1	96
Alevin RT[a]	Mo	Mortality	160-1000	96
Juvenile RT[a]		Mortality	190.3-1190	96
Northern pike	Ni	Mortality	0.47-3.0	96
		Growth	0.04-0.25	312
	Mo	Mortality	20.4-127.7	96
		Growth	0.27-1.7	312
White sucker	Ni	Egg hatchability	0.04-0.25	336
		Time-to-hatch	0.04-0.25	336
		Mortality	11.8-74.1	96
		Growth	0.04-0.25	528
	Mo	Egg hatchability	0.27-1.7	288
		Time-to-hatch	0.27-1.7	288
		Mortality	320-2000	96
		Growth	0.27-1.7	528

a Rainbow trout

concentration until the solubility limit of the metal salt was achieved (e.g., 2 g/L in the case of Mo-exposed white suckers), a concentration was considered to be unrealistically

high with respect to environmental concentrations (e.g., 100 mg Mo/L in the case of fathead minnow exposure), or the fish supply was exhausted (e.g., northern pike exposures).

Conductivity, total dissolved solids, pH, temperature, and dissolved oxygen were monitored daily throughout the tests. Water hardness and alkalinity were monitored every third day. Nominal metal concentrations were verified by inductively coupled plasma-mass spectrophotometry (ICP-MS; Dept. of Geological Sciences, University of Saskatchewan). Water quality measurements and ICP-MS samples were taken from freshly mixed exposure media prior to test water replacement.

Exposure treatments were prepared by mixing stock solution into dechlorinated Saskatoon municipal water (i.e., laboratory dilution water). Laboratory dilution water quality was as follows (mean±SD; n=40): pH 7.9±0.3, conductivity 371.4±24.1 µS/cm, total dissolved solids (TDS) 185.2±11.8 mg/L, total hardness 111.6±3.0 mg/L as $CaCO_3$, alkalinity 80.4±8.5 mg/L as $CaCO_3$. Dissolved oxygen remained at >80% saturation (n=39). Stock solutions were prepared in distilled, deionized water. With the exception of tests involving juvenile rainbow trout, exposure solutions were freshly mixed every second day. Exposure solutions used in juvenile rainbow trout tests were prepared daily.

Fertilized fathead minnow eggs and larvae were generated from a breeding culture maintained at the Department of Biology, University of Saskatchewan, Saskatoon, SK, Canada, using the methods of the Canadian Environmental Protection Service (1992a). No mortalities occurred among breeding fish or fish from the general

holding population during the 7-d period preceding testing (Canadian Environmental Protection Service 1992a). Breeding fish and fish in general holding tanks showed no signs of disease or injury. Sodium chloride was tested regularly as a reference toxicant to monitor larval fathead minnow sensitivity. Larval fathead minnows generated for this study showed NaCl 96-h LC50s within one standard deviation of our laboratory mean value. Reference toxicants were not evaluated for rainbow trout, northern pike, or white suckers because these species were not routinely maintained in our laboratory.

Fertilized northern pike and rainbow trout eggs (<24 h old and eyed eggs) were donated by the Saskatchewan Fish Culture Station, Ft. Qu'appelle, SK, Canada. Spawning white suckers were netted from Little Bear Lake in north central Saskatchewan. Gametes were stripped from ripe adults and collected in clean, dry plastic containers. After gametes from at least three females and three males were collected, milt was added to unfertilized eggs. Milt and eggs were mixed together with a goose feather. Approximately 125 mL of water was added to the mixture to activate the gametes, while continuously stirring for approximately 60 s. These fertilized eggs were placed in water in a 20-L plastic container and were allowed to harden for 2 h. Once hardened, fertilized eggs were transported via road to the laboratory in Saskatoon.

Toxicity tests followed acceptable protocols (ASTM 1988; 1992; Canadian Environmental Protection Service 1990a; 1992a; 1992b). Procedures involving juvenile rainbow trout varied considerably from other tests owing to the much larger fish involved, and are described later (see *Rainbow Trout Tests*). The remainder of this section describes general procedures for conducting early-life stage tests for fathead

minnows, northern pike, and white suckers. Specific procedures for each species are described below. Test-specific exposure scenarios are summarized in Table 4.1.

Tests were conducted in clean 450-mL polystyrene-derived beverage cups filled with 300-mL exposure medium. Ten fertilized eggs or newly hatched larvae were randomly assigned to each of four test replicates per treatment. Test replicates were randomly assigned positions on shelves to minimize systematic bias of exposure conditions. Debris, including discarded egg cases and dead eggs or larvae, was removed from each replicate daily. Water was renewed by removing and replacing 80% of the test water at 24-h intervals, at which time mortality was recorded.

Experimental endpoints included egg hatchability, time-to-hatch, growth, and mortality. During egg hatchability and time-to-hatch tests, the number of live or dead eggs and larvae was recorded daily. Once hatched, larvae were removed from the test vessel. At the end of growth tests, surviving fish were euthanized in hot distilled water and placed on dry, pre-weighed weighing paper (Fisher Scientific, Nepean, ON). Fish were dried overnight at 110°C and weighed to the nearest 10 μg.

4.3.2. Fathead Minnow Tests

All tests and procedures using fathead minnows were conducted in a temperature-controlled room (25±1°C), under a photoperiod of 16 h light and 8 h dark, and followed the basic principles outlined by the Canadian Environmental Protection Service (1992a). Freshly fertilized fathead minnow eggs were collected twice daily throughout the test. Eggs were gently removed from spawning tiles and transferred to Imhoff settling cones filled with dilution water. Settling cones were supplied with

aeration for 48 h, which is the time required for eggs to reach the eyed stage. Aeration in settling cones was required to minimize fungal infection of eggs. After the 48-h incubation period, eyed eggs were either assigned to egg hatchability or time-to-hatch test replicates, or reared for use in growth or mortality tests. Those reared for use in growth or mortality tests were placed in dilution water in 1-L plastic containers supplied with gentle aeration until hatching. Eighty percent of the water in rearing containers was replaced daily.

Eyed fathead minnow eggs were used in egg-hatchability and time-to-hatch experiments, and larvae <24 h old were used in growth and mortality tests. Egg hatchability refers to the percentage hatched per treatment relative to controls. Time-to-hatch refers to the time required for eyed eggs to hatch once placed in exposure chambers. Egg hatchability tests were complete once the last egg in any treatment either hatched or died. Similarly, time-to-hatch experiments were complete once no live eggs remained in any test replicate. Growth and mortality tests ran for 168 h and 96 h, respectively. Larvae were not fed during 96-h acute exposure tests. During growth tests, larvae were fed live brine shrimp (*Artemia salina*; Artemia Canada, Ltd., Chaplin, SK) nauplii at a rate sufficient to ensure live nauplii were available throughout the daylight hours.

4.3.3. Northern Pike and White Sucker Tests

Tests and procedures involving northern pike or white sucker eggs or larvae followed the general principles outlined by the American Society for Testing and Materials (1988; 1992). Tests were conducted in a temperature-controlled room (12-

14°C) under a photoperiod of 16 h light and 8 h dark. Eggs used in egg hatchability and time-to-hatch tests were assigned to test replicates within 12 h of fertilization. Eggs that were reared for growth and mortality tests were kept suspended in a recirculating-water current throughout their development to minimize fungal infection. Dead and dying eggs, identified by their colour and opacity, were removed at least once per day. Growth and mortality tests were conducted on larvae <24 h old. Growth tests were ended immediately prior to the onset of exogenous feeding, as judged by the degree of yolk sac consumption. Mortality tests lasted 96 h.

4.3.4. Rainbow Trout Tests

All tests and procedures using rainbow trout were conducted in a temperature-controlled room (12-14°C), under a photoperiod of 16 h light and 8 h dark, and followed the general principles outlined by the Canadian Environmental Protection Service (1990a; 1992b). Other egg hatchability and time-to-hatch protocols followed those described above for northern pike and white suckers as much as possible.

Three groups of tests were conducted with rainbow trout, including tests involving freshly fertilized eggs (<24 h old), swim-up fry, and juveniles. Tests using freshly fertilized eggs were conducted in exactly the same manner, and under the same conditions, as those described for white suckers and northern pike. Swim-up fry were reared from eyed eggs obtained from the Saskatchewan Fish Culture Station at 12-14°C under 16 h light and 8 h dark photoperiod. Ten fry were randomly assigned to each of 24 ([5 treatments + 1 control] × 4 replicates) clean 1-L plastic buckets. Debris was removed and water was replaced daily throughout the 96-h exposure duration.

Juvenile rainbow trout (2.1±0.5 g, n=30), donated by the Saskatchewan Fish Culture Station, were also tested under acute Ni and Mo exposure conditions. Fish were held in a 1000 L holding tank for 2 weeks prior to test initiation where they were acclimated to test conditions (i.e., 16 h light and 8 h dark photoperiod, 12-14°C). For each acute lethality test with Ni or Mo, ten juvenile trout were randomly assigned to each of 18 38-L glass aquaria ([5 treatments + 1 control] × 3 replicates). Because tests were conducted as static renewal, exposure tanks were lightly aerated through glass Pasteur pipettes to maintain oxygen concentrations in the water. Debris and dead fish were removed from each tank daily, after which 80% of the water was replaced with freshly mixed solution. Fish were not fed during exposure periods.

Upon test completion, juvenile fish were killed with a sharp blow to the head and weighed to the nearest milligram. Total length was measured from the tip of the snout to the tip of the compressed tail. Livers and entire gill baskets were dissected from each fish and rinsed with distilled, deionized water for subsequent metals analysis. Livers were weighed to the nearest milligram. The hepatosomatic index (fish wet weight (g)/liver wet weight (g)) was calculated for each fish. Gills and livers were dried at 110°C for 24 h. Nickel and Mo concentrations were determined by inductively coupled plasma-mass spectrophotometry (ICP-MS; Department of Geological Sciences, University of Saskatchewan, Saskatoon, SK, Canada).

4.3.5. Statistical Treatment

Tests were considered 'valid' if mortality in the control treatment was <20%. Only results from valid tests were reported here. Mean mortality, egg hatchability, time-

to-hatch, and tissue metal concentrations were compared by one-way analysis of variance (ANOVA). Normality and homogeneity of variance assumptions were tested using Shapiro-Wilks and Levene's tests, respectively. Data not meeting ANOVA-distribution assumptions were $\log_{10}(x+1)$-transformed. Although this transformation was generally successful, those data still not meeting distribution assumptions were compared using a non-parametric Kruskall-Wallis ANOVA. Post-hoc analyses of tests having equal replication included Dunnett's test to compare treatment means with control means, and Tukey's test to compare means among all treatments. Post-hoc comparisons among treatments having different sample sizes were compared using Bonferroni's *t*-test. Results from these analyses were used to determine no observed effect concentrations (NOEC) and lowest observed effect concentrations (LOEC). Inferential statistics were considered significant when $p<0.05$.

Nickel and molybdenum effects on growth and mortality were assessed using point estimate techniques. Mortality effects were estimated using trimmed Spearman-Karber analysis (trim=10%), which also allowed for the calculation of 95% confidence intervals. Effects on fish growth were estimated by determining the metal concentration sufficient to inhibit growth by 25% relative to controls (i.e., IC_{25}). All inferential statistics and point estimates were calculated on SPSS and TOXSTAT statistical software (SPSS 1988; West and Gulley 1996).

4.4 Results

A refrigeration-unit failure during rainbow trout and northern pike egg hatchability and time-to-hatch tests caused a brief temperature spike up to 19°C in test

replicates for approximately 3 h. In the hours and days following the temperature spike, eggs from both control and test replicates in these tests began dying. Because these mortalities can be attributed to temperature fluctuations, data from these tests were omitted from further analysis. No other tests were affected by the temperature spike. However, all rainbow trout eggs that were being reared for growth and mortality tests perished within 3-5 d of the refrigeration-unit malfunction.

4.4.1. Water Quality

Analysis of National Research Council of Canada River Water Standard (SLRS-3) by ICP-MS showed analytical variation generally <20% between duplicates of a single sample, and results were within 30% of known values. Nominal concentrations of Ni and Mo were within 20% of ICP-MS measurements after subtracting Ni and Mo concentrations determined for sample and analytical blanks (i.e., background). Consequently, nominal values are reported below.

4.4.2. Fathead Minnows

Nickel was clearly more toxic to all fathead minnow life stages than Mo (Table 4.2). The IC_{25} for egg hatchability was 1.8 mg Ni/L. Fathead minnow eggs exposed to 1.89 or 3 mg Ni/L showed significantly higher mortality than eggs in controls ($p<0.0001$; Fig. 4.1). An average of 40% egg mortality occurred at 3 mg Ni/L. Egg hatching time from the eyed-egg stage was significantly ($p<0.0001$) reduced from 3 d in controls to 2 d at 3 mg Ni/L (Fig. 4.1). Hatching times were significantly shorter than hatching times in controls at all exposure concentrations greater than 0.48 mg Ni/L. Fathead minnow larvae exposed to Ni showed no mortalities in the control treatment,

Table 4.2: Egg hatchability, time-to-hatch, larval mortality and growth of fathead minnows, rainbow trout, northern pike, and white suckers exposed to Ni and Mo.

Species	Metal	Test	End Point	Conc. (mg/L) (95% C.I.)
Fathead minnow	Ni	Egg hatchability	NOEC	1.2
			LOEC	1.89
			IC_{25}	1.8
		Time-to-hatch	NOEC	0.48
			LOEC	0.75
		Mortality	96-h LC50	2.4 (2.1-2.7)
			NOEC	1.2
			LOEC	1.88
		Growth	NOEC, LOEC, IC_{25}	>2.0
	Mo	Egg hatchability	NOEC, LOEC, IC_{25}	>100
		Time-to-hatch	NOEC, LOEC	>100
		Mortality	96-h LC50	>100
		Growth	IC_{25}	>100
Alevin RT[a]	Ni	Mortality	96-h LC50	>20
			NOEC	8
			LOEC	12.6
	Mo	Mortality	96-h LC50	>1000
Juvenile RT[a]	Ni	Mortality	96-h LC50	51.2 (46.7-56.1)
			NOEC	29.6
			LOEC	46.7
	Mo	Mortality	96-h LC50	>1190
Northern pike	Ni	Mortality	96-h LC50	>3
		Growth	NOEC, LOEC, IC_{25}	>0.25
	Mo	Mortality	96-h LC50	>127.7
		Growth	NOEC, LOEC, IC_{25}	>1.7
White sucker	Ni	Egg hatchability	NOEC, LOEC, IC_{25}	>0.25
		Time-to-hatch	NOEC, LOEC	>0.25
		Mortality	96-h LC50	17.9 (15.3-20.9)
		Growth	NOEC, LOEC, IC_{25}	>0.25
	Mo	Egg hatchability	NOEC, LOEC, IC_{25}	>1.7
		Time-to-hatch	NOEC, LOEC	>1.7
		Mortality	96-h LC50	>2000
		Growth	NOEC, LOEC, IC_{25}	>1.7

a RT, rainbow trout

but significant mortalities at all concentrations higher than 1.2 mg Ni/L ($p<0.0001$; Fig. 4.2). The 96-h LC50 for larval fathead minnows exposed to Ni was 2.4 mg Ni/L (2.1-2.7 mg Ni/L, 95% confidence interval [CI]).

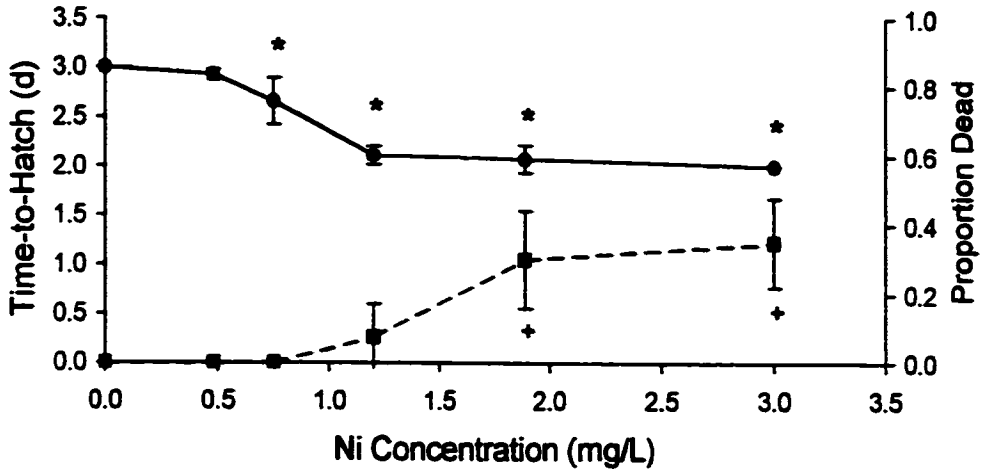

Figure 4.1: Time required for fathead minnow eggs to hatch (•), and egg mortality (■) expressed as proportion dead, upon exposure to Ni. Points represent means±SD (n=4). Both asterisks (*) and plus signs (+) represent significant ($p<0.05$) differences from control (0 mg Ni/L).

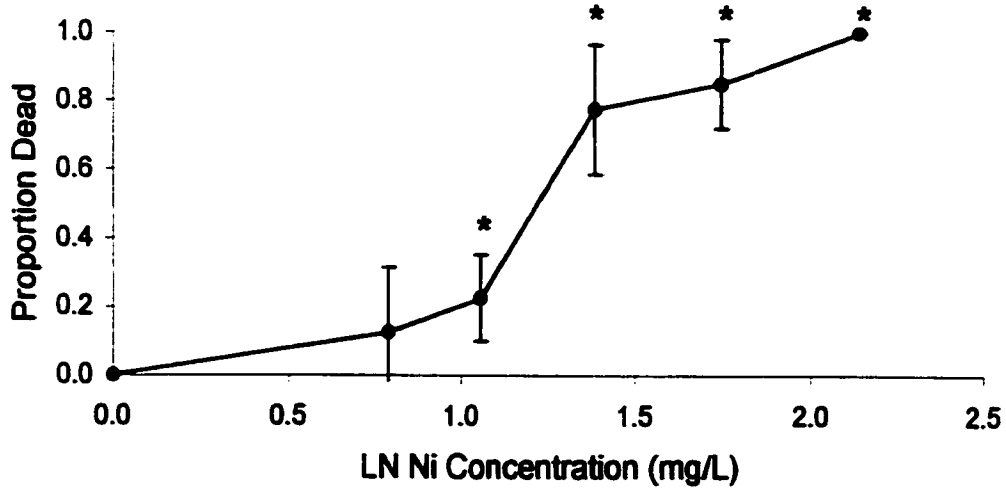

Figure 4.2: Mortality of larval fathead minnows exposed to increasing Ni concentrations. Points represent means±SD (n=4), while asterisks (*) represent significant ($p<0.05$) differences from control (0 mg Ni/L)

In the 168-h fathead minnow larval growth test, some mortality occurred in all treatments including the control. However, larval mortality did not exceed 15%

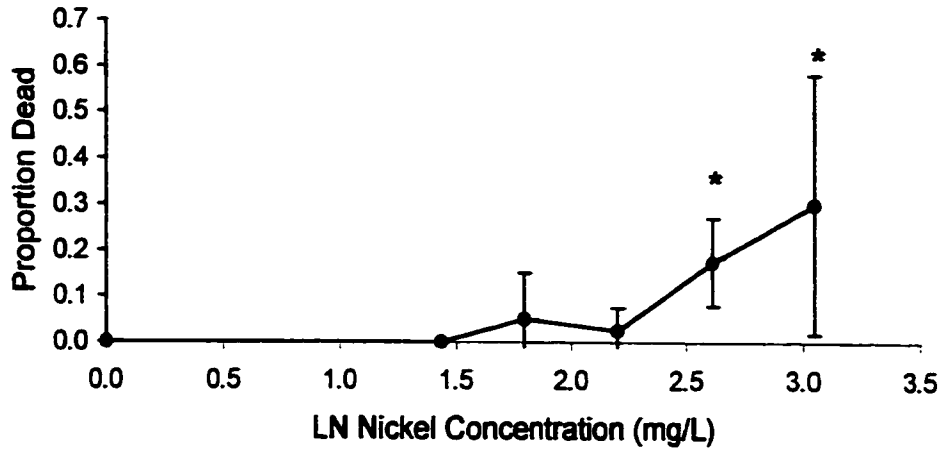

Figure 4.3: Mortality of alevin rainbow trout exposed to increasing Ni concentrations. Points represent means±SD (n=4), while asterisks (*) represent significant ($p<0.05$) differences from control (0 mg Ni/L).

in any treatment, and did not vary significantly ($p>0.05$) with control mortality (5%). Larval weight did not vary ($p>0.05$) with Ni concentrations as high as 2 mg Ni/L after 168-h exposure. Mean larval weight in the control was 415±184 µg (mean±SD; n=4), whereas overall mean weight was 343±160 µg (n=24).

Molybdenum did not significantly affect fathead minnow egg hatchability, time-to-hatch, larval growth, or mortality at any of the concentrations tested relative to controls (Table 4.2). No egg mortalities occurred in any of the Mo-exposure treatments in concentrations as high as 100 mg Mo/L. Egg hatching time ranged from 2.9-3 d, and did not vary by treatment ($p>0.05$). Fathead minnow larval mortality was less than 10% in the control treatment and did not vary with Mo concentration ($p>0.05$). Mean larval weight in the control was 391±44 µg (mean±SD; n=4), whereas overall mean weight was 367±126 µg (n=24).

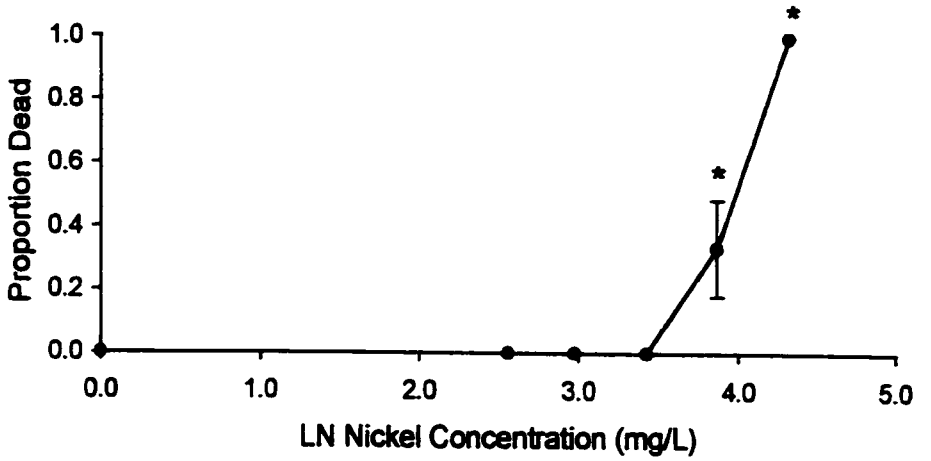

Figure 4.4: Mortality of juvenile rainbow trout exposed to increasing Ni concentrations. Points represent means±SD (n=4), while asterisks (*) represent significant ($p<0.05$) differences from control (0 mg Ni/L).

4.4.3. Rainbow Trout

Alevin rainbow trout mortality increased with Ni concentration ($p=0.006$), although variability was high (Fig. 4.3). No alevin mortalities occurred in the control treatment. The highest concentration in which mortality did not significantly differ from the control (NOEC) was 8 mg Ni/L, whereas the LOEC was 12.6 mg Ni/L. The 96-h LC50 could not be estimated because only 30% mortality occurred at the highest (20 mg Ni/L) concentration tested.

Juvenile rainbow trout mortality significantly increased with Ni concentration ($p=0.005$; Fig. 4.4). No mortalities occurred in the control treatment and 100% mortality occurred at 74.1 mg Ni/L. The NOEC and LOEC were 29.6 and 46.7 mg Ni/L, respectively. The 96-h LC50 was estimated at 51.2 mg Ni/L (46.7-56.1 mg Ni/L, 95% CI). After only 24 h of exposure, 93.3% of all fish in the 74.1 mg Ni/L treatment died. By 48 h, all fish in that treatment were dead. A coughing response was observed in fish exposed to all concentrations of Ni throughout the exposure period. Fish exposed

to 46.7 and 74.1 mg Ni/L experienced a loss of scales not observed in fish from lower-Ni treatments.

Juvenile rainbow trout exposed to Ni for 96 h lost an average 24% of their original body weight of 2.1±0.5 mg (n=30). Final fish weight did not vary by Ni treatment ($p>0.05$) and was 1.6±0.5 mg (n=136). Neither liver weight nor HSI varied with Ni concentration ($p>0.05$). Mean liver weight was 0.021±0.008 g (n=134), whereas mean HSI was 0.013±0.003 (n=134).

Upon examination of Ni concentrations in liver tissue, a suspicious data point was identified. The concentration of liver Ni was measured as 51.6 μg/g in a fish exposed to 18.5 mg Ni/L in the water. Grubb's outlier analysis (Barnett and Lewis 1994) confirmed that the questionable data point was an outlier. The datum was subsequently removed from the analysis, and results reported below were calculated without it.

Fish from the control treatment had 2.2 times more Ni in gills than in liver. At the highest Ni concentration from which gills and livers could be collected, gills contained more than 24 times as much Ni than livers. Gill Ni concentration was 10.9 times higher than Ni concentration in the water. Gill Ni increased with Ni concentration in the water ($p<0.0001$; Fig. 4.5). Mean liver Ni also showed an increasing trend with Ni in water, although the rise in liver Ni was not significant ($p>0.05$; Fig. 4.5). Pearson correlation analysis showed that final fish weight, total length, HSI and liver weight were not significantly correlated with Ni concentration in the water ($p>0.05$).

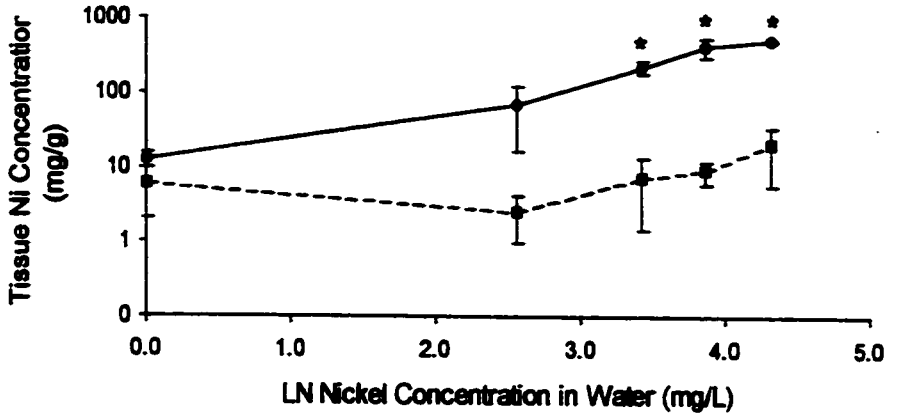

Figure 4.5: Mean (±SD, n=15-30) total Ni concentration in gills (solid line) and livers (dashed line) (μg/g) of fish exposed to increasing waterborne Ni concentrations. Asterisks (*) represent significant differences from control (0 mg Ni/L).

No rainbow trout alevin or juvenile mortalities occurred in any Mo treatment as high as 1000 and 1190 mg Mo/L, respectively, during 96-h exposures. Observations on fish exposed to these high Mo concentrations revealed no obvious external anomalies with respect to control animals.

4.4.4. Northern Pike

Neither northern pike growth nor mortality was affected by any of the Ni or Mo concentrations tested (Table 4.2). Northern pike sac fry mortality in the control of a 96-h mortality test was 11±9% and did not vary by Ni concentration ($p>0.05$), although 25±17% mortality occurred at 0.74 mg Ni/L. Observations on sac fry northern pike during the 96-h test revealed yolk sac hemorrhaging in fish exposed to all Ni treatments. Nickel concentrations as high as 3.0 mg Ni/L during a 312-h exposure had no effect on sac-fry northern pike growth ($p>0.05$). Mean fish weight in the control was 1.85±0.21 mg (n=4), whereas mean weight over all treatments was 1.83±0.18 mg (n=24).

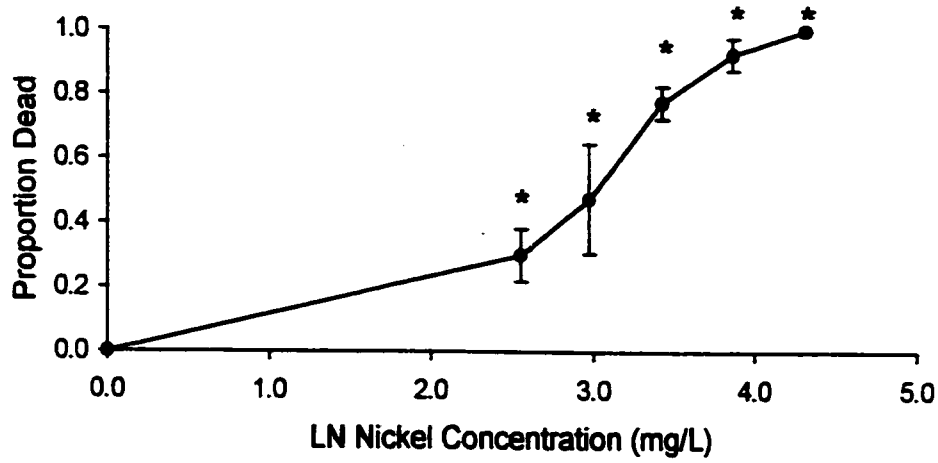

Figure 4.6: Mortality of sac fry white suckers exposed to increasing Ni concentrations. Points represent means±SD (n=4), while asterisks (*) represent significant ($p<0.05$) differences from control (0 mg Ni/L).

Sac fry northern pike exposed for 96 h to 127.7 mg Mo/L showed similar ($p>0.05$) mortality (ranging from 15-30%) to those exposed to control water (Table 4.2). After 312 h in Mo concentrations as high as 1.7 mg Mo/L, northern pike weight was not significantly different from the control ($p>0.05$). Mean northern pike weight in the control was 1.80±0.15 mg (n=4), whereas overall mean northern pike weight was 1.82±0.15 mg (n=24).

4.4.5. White Suckers

Nickel concentrations up to 250 µg/L did not affect white sucker egg hatchability or time required for hatching. Egg mortality was less than 20% in controls and did not vary significantly with Ni concentration ($p>0.05$). The time required for white sucker eggs to hatch was 10.4±0.7 d (n=24), which also did not vary with Ni concentration.

Larval white sucker mortality increased with increasing Ni concentration (Fig. 4.6). White sucker mortalities in all Ni treatments were significantly higher than control

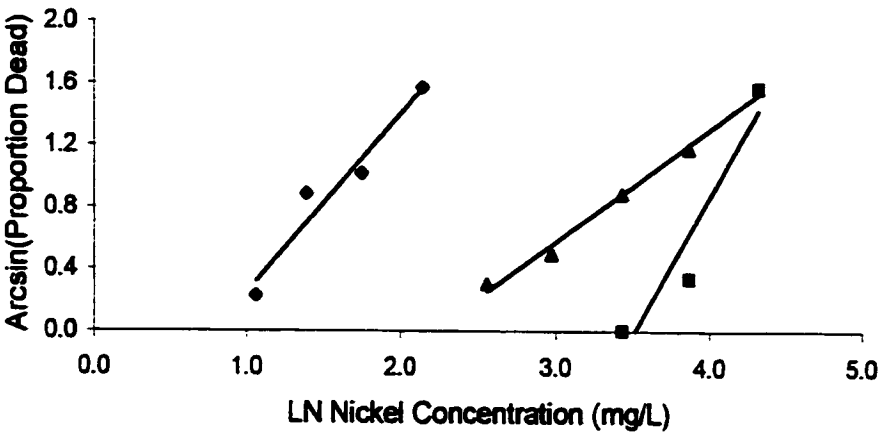

Figure 4.7: Trimmed, arcsine-transformed mortality data for fathead minnows (◆), white suckers (▲), and juvenile rainbow trout (■) exposed to increasing Ni concentrations. Trend lines were fitted with simple linear regression (r^2=0.94, 0.99, and 0.91, respectively). Slopes were compared using the method of Zar (1984), but were not significantly different from one another ($p>0.05$).

mortalities, precluding the calculation of NOEC. The LOEC was 11.9 mg/L, which was the lowest Ni concentration tested. The 96-h LC50 was estimated to be 17.9 mg Ni/L (15.3-20.9 mg/L, 95% CI). White sucker growth was not affected by Ni up to 250 µg/L after 528 h of exposure ($p>0.05$). Sac fry white sucker dry weights in control replicates averaged 1.61±0.23 mg (n=4), whereas overall weight was 1.60±0.34 mg (n=24).

Molybdenum did not significantly affect egg hatchability, time required for egg hatching, larval mortality, or larval growth at any of the concentrations tested. Even at 2000 mg/L, no mortalities were observed among exposed larvae.

4.4.6. Comparing Toxicity Curves

Figure 4.7 represents the significant mortality data reported above for fathead minnows, white suckers, and rainbow trout exposed to Ni. In each case, the concentration response data were censored to remove the flattened leading and trailing edges of the S-shaped dose response curves. Only the central linear portion of each dose

response was analyzed. Mortality data, as proportion dead, were arcsine transformed to straighten each curve. For each fish species, a regression line was generated for the transformed response data, from which the slope was determined. Individual slopes were compared using the method of Zar (1984). Slopes of the regression lines shown in Fig. 4.7 are 1.15 (r^2=0.94), 0.73 (r^2=0.99), and 1.76 % dead/mg Ni/L (r^2=0.91) for fathead minnows, white suckers, and juvenile rainbow trout, respectively. Slopes were not significantly different from one another ($p>0.05$).

4.5 Discussion

Results reported here show that Ni is substantially more toxic to fish than Mo, and early-life stage fathead minnows are more sensitive to Ni than early-life stages of rainbow trout, northern pike, or white suckers. Nickel concentrations as low as 750 µg Ni/L were sufficient to stimulate fathead minnow egg hatching relative to controls (Fig. 4.1). This result is contrary to results reported by Dave and Xiu (1991) that demonstrated hatching inhibition of zebrafish (*Brachydanio rerio*) eggs exposed to 40 µg Ni/L in hard water (100 mg/L as $CaCO_3$). However, the same authors reported hatching stimulation in zebrafish eggs exposed to 16 µg Hg/L. This discrepancy between the two studies might be explained by differences between two fish species.

Although the mechanism for early hatching upon exposure to inorganic toxicants remains largely unknown, it is suspected that contaminants interfere with the hatching process directly. Possible mechanisms include a stimulation of the hatching enzyme chorionase, increasing perivitelline pressure through water uptake by the ovum and/or embryo, or increased muscular contractions causing the egg case to break prematurely

(Denuce 1985; Dave and Xiu 1991). Fish embryos hatching prematurely upon exposure to contaminants are not yet fully developed. Consequently, their survival in the wild may be compromised by their inability to avoid predation or compete for food (as in the case of precocious species, such as the fathead minnow).

In this study, shortened hatching time was matched with an increase in egg mortality when fathead minnow eggs were exposed to at least 1.89 mg Ni/L (Fig. 4.1). Egg hatchability was inhibited by 25% relative to controls (IC_{25}) at 1.8 mg Ni/L (Table 4.2). These results corroborate other studies. For example, fathead minnow eggs exposed to 730 μg Ni/L in hard water (207 mg/L as $CaCO_3$) showed significantly reduced egg hatchability relative to controls (Pickering 1974). Common carp (*Cyprinus carpio*) eggs showed reduced egg hatchability when exposed to 5 mg Ni/L in hard water (128 mg/L as $CaCO_3$) (Blaylock and Frank 1979). On the other hand, zebrafish eggs exposed to Ni concentrations as high as 1.024 mg/L showed no reduction in egg hatchability relative to controls (Dave and Xiu 1991). Again, this discrepancy may be attributed to species differences.

Larval fathead minnows were 21, 8, 7.5, and (at least) 1.3 times more sensitive to Ni than juvenile and alevin rainbow trout, white suckers, and northern pike, respectively, as indicated by their 96-h LC50 values (Table 4.2). Although the 96-h LC50 could not be estimated for northern pike, it is noteworthy that they showed no significant mortalities relative to controls at Ni concentrations greater than those required to kill 50% of the larval fathead minnows. This wide range of acute toxicity values corroborates other work that suggests that Ni toxicity is species dependent. For

example, guppies (*Poecilia reticulata*), fathead minnows, bluegills (*Lepomis macrochirus*), and goldfish (*Carassius auratus*) exposed to Ni in soft water (20 mg/L as $CaCO_3$) gave 96-h LC50s of 4.45, 4.58-5.18, 5.18-5.36, and 9.82 mg Ni/L, respectively (Pickering and Henderson 1966). In hard water (360 mg/L as $CaCO_3$), fathead minnows and bluegills gave 96-h LC50s of 42.4-44.5 and 39.6 mg Ni/L, respectively (Pickering and Henderson 1966). Other species exposed to Ni in relatively soft water (53 mg/L as $CaCO_3$), such as striped bass (*Morone saxatilis*), pumpkinseed (*Lepomis gibbosus*), common carp, American eel (*Anguilla rostrata*), white perch (*Morone americana*), and banded killifish (*Fundulus diaphanus*) gave 96-h LC50s of 6.2, 8.1, 10.6, 13.0, 13.6, 46.2 mg Ni/L, respectively (Rehwoldt et al. 1971). Early-life stage toxicity of Ni to rainbow trout, channel catfish (*Ictalurus punctatus*), largemouth bass (*Micropterus salmoides*), and goldfish in hard water (93-105 mg/L as $CaCO_3$) yielded 96-h LC50 values of 0.5, 0.71, 2.06, and 2.78 mg Ni/L, respectively (Birge and Black 1980). These 96-h LC50 values reported by other studies are similar to those reported here for fathead minnows, white suckers, and rainbow trout. Discrepancies between the current results and those reported in the literature can be attributed to differences among fish species and exposure conditions, like water hardness, which is known to ameliorate metal toxicity to fish (Erickson et al. 1996; Erickson et al. 1998).

Effluent and receiving water toxicity is often evaluated with standard toxicity test fish species such as fathead minnows and rainbow trout. Neither of these species inhabit aquatic systems around uranium mining operations in northern Saskatchewan. This use of non-indigenous species in toxicity tests has led to questions about the

ecological relevance of using toxicity results from these species as a basis for protecting indigenous fish against mine-related metal toxicity. Currently, juvenile rainbow trout are being used in pass/fail tests to monitor effluent and receiving water toxicity at northern Saskatchewan uranium mines. This study showed that juvenile rainbow trout were more Ni-tolerant than fathead minnows, white suckers, or alevin rainbow trout (Table 4.2). In fact, juvenile rainbow trout were 2.9 times more resistant to acute Ni exposure than white suckers, a fish species commonly found in northern Saskatchewan lakes.

Alevin rainbow trout seem to be more sensitive to Ni intoxication than juveniles. Lowest observed effect concentrations for alevins and juveniles were 12.6 and 46.7 mg Ni/L, respectively, and 96-h LC50s were >20 and 51.2 mg Ni/L. These results are similar to those reported elsewhere for juvenile rainbow trout exposed to Ni in hard water (96-h LC50 32-90 mg Ni/L, hardness ranging from 132 to 300 mg/L as $CaCO_3$ (Brown 1968; Brown and Dalton 1970; Hale 1977)). However, another study showed that juvenile rainbow trout (96-h LC50 7.8 mg Ni/L) were more sensitive to Ni than alevins (96-h LC50 25.1 mg Ni/L) in soft (41 mg/L as $CaCO_3$) water (Buhl and Hamilton 1991).

Examination of Ni concentrations in gills and livers from fish exposed under acute Ni concentrations revealed that Ni tended to accumulate in gills but not in livers (Fig. 4.5). Nickel bioaccumulation factors (BAF) for gills ranged between 5.9 and 8.9 with a mean of 7.3±1.3 (SD). Nickel BAFs for liver were considerably lower, ranging between 0.2 and 0.3 with a mean of 0.2±0.03. Rainbow trout exposed to 1.0 mg Ni/L

for 180 d showed little accumulation of Ni in their livers (BAF=0.003) (Calamari et al. 1982). However, common carp tended to accumulate Ni in livers roughly equivalent to that in gills (Sreedevi et al. 1992). Common carp exposed to 8 mg Ni/L for 5 d gave BAFs of 6.0 in gills and 6.4 in livers. After 15 d at the same concentration, BAFs doubled to 12.9 and 12.1 for gills and livers, respectively (Sreedevi et al. 1992). During a 4 d exposure at 40 mg/L, common carp demonstrated BAFs of 5.1 and 2.1 for gills and livers, respectively (Sreedevi et al. 1992). These results suggest that Ni accumulation is both species and time dependent.

Under the pH conditions of this study, Ni mainly occurs as the free bivalent Ni^{2+} cation (Richter and Theis 1980). Fish gill surfaces have a predominantly negative charge owing to the dominant presence of oxygen as a functional binding ligand (Reid and McDonald 1991). Nickel cations, then, bind to gill surfaces where they are taken up to confer a toxic effect. A recent study (Meyer et al. 1999) has shown that Ni concentrations in gills is a better predictor of toxicity than Ni in the water, contrary to the Free-Ion Activity Model (Morel 1983).

Liver did not accumulate Ni to the same extent as gills (Fig. 4.5) probably because of the short exposure time. It is possible that Ni redistribution from gills to liver had not taken place during the 96-h exposure period, as suggested by the lack of significant Ni increase in liver tissue. The trend of increasing Ni in liver with water concentrations, although non-significant, suggests that given enough time Ni accumulation would occur in the liver as well as in the gills. This lack of Ni

accumulation in liver tissue also explains the lack of significance in HSI among fish of various Ni treatments.

Molybdenum caused no significant toxic effect to any life stage or fish species tested, even at concentrations as high as 2 g Mo/L (Table 4.2), relative to controls. Results reported here confirm other studies. Chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) exposed to Mo in both soft (42 mg/L as $CaCO_3$) and brackish (hardness 333 mg/L as $CaCO_3$) water gave 96-h LC50s of >1000 mg Mo/L (Hamilton and Buhl 1990). Striped bass (*Morone saxatilis*) exposed to Mo under variable water hardness and salinity conditions gave 96-h LC50s of >79.8 mg Mo/L (Dwyer et al. 1992). Earlier studies of Mo toxicity to 55-mm rainbow trout in soft water (25 mg/L as $CaCO_3$) showed a 96-h LC50 of 1320 mg Mo/L, whereas 20-mm fish gave a 96-h LC50 of 800 mg Mo/L (McConnell 1977). In the same study, trout exposed to Mo concentrations as high as 17 mg/L for up to 1 year showed no significant toxicological differences relative to controls. Neither eyed eggs, sac fry, nor fingerling life stages demonstrated any toxicological differences relative to controls (McConnell 1977).

Other sub-lethal early-life stage toxicity tests provide contradictory results. Rainbow trout exposed to Mo from immediately after fertilization to 4 days post-hatch (28 d total exposure, hardness 92-110 mg/L as $CaCO_3$), demonstrated a chronic threshold effect concentration of 0.73 mg Mo/L (Birge 1978). Under roughly the same exposure conditions, Birge et al. (1980) estimated the 28-d LC50 as 0.79 mg Mo/L for rainbow trout. Eisler (1989) reported a decrease in rainbow trout growth and an

increase in mortality at Mo concentrations >50 mg/L. However, 0.79 mg Mo/L was sufficient to reduce egg hatchability to 50% of controls.

Nickel concentrations that caused larval mortality in the current study were 100 to 1000 times higher than Ni concentrations observed in lakes surrounding uranium operations in northern Saskatchewan. Because fathead minnows were the most Ni-sensitive species in this study, its Ni-toxicity data can be used to determine a 'safe' concentration to guard against chronic effects among the four species tested in this study. The Maximum Acceptable Toxicant Concentration (MATC; defined as the geometric mean between the NOEC and LOEC) for Ni-exposed fathead minnows was 1.5 mg Ni/L. The fathead minnow MATC can then be used to calculate an application factor (AF_{FHM}=MATC/LC50), 0.626. The fathead minnow AF can then be used to estimate MATCs for northern pike and white suckers, for which NOEC and LOEC data could not be determined (Rand et al. 1995). Therefore, MATCs for alevin rainbow trout, juvenile rainbow trout, northern pike, and white suckers were 10.0, 37.2, 1.9, and 11.2 mg Ni/L, respectively. Even the lowest MATC, which was calculated for fathead minnows, is 10 times higher than that measured in lakes around uranium operations in northern Saskatchewan.

However, an ecologically relevant adverse effect on the time required for fathead minnow egg hatching was observed in hard water at a Ni concentration below the MATC. Other studies (cited above) have shown even more severe effects than stimulated egg hatching, e.g., mortality, at equally low Ni concentrations in hard water (Birge and Black 1980). Blaylock and Frank (1979) showed that common carp eggs

were more sensitive to acute Ni intoxication in hard water than larvae, which corroborates results reported in this study for fathead minnow eggs. In soft northern Saskatchewan lake water, effects are presumed to be more severe.

More than 14 fish species have been identified in water systems around one northern Saskatchewan uranium operation (Cameco Corp. et al. 1995). The range of Ni concentrations reported here that are required to induce toxicity in fish span two orders of magnitude, and those reported in the literature span three or more orders of magnitude for several fish species. More work is required to understand toxicological differences among indigenous fish species to determine if current monitoring practices using standard toxicity test fish species, like fathead minnows and rainbow trout, are ecologically relevant. The current results indicate that by using rainbow trout as a monitor against metal toxicity at northern Saskatchewan uranium mines, early-life stages of sensitive fish species would likely remain unprotected.

In conclusion, species sensitivity to Ni was as follows: fathead minnow>northern pike>white sucker>alevin rainbow trout>juvenile rainbow trout. The most sensitive endpoint for detecting Ni toxicity was the time required for fathead minnow eggs to hatch, which occurred at a concentration (0.75 mg Ni/L) lower than the MATC (1.5 mg Ni/L). Larval fathead minnows that hatch early are not completely developed and may suffer a competitive disadvantage or vulnerability to predation that fully developed larvae do not experience. Rainbow trout, a common species used to monitor the toxicity of uranium mine effluents and receiving water, was the most Ni-tolerant species tested. Molybdenum had no effect on egg hatchability, growth, or mortality in any of the

species tested. Compared to Ni, Mo seems to be relatively non-toxic to the fish species tested. Toxicological monitoring of Ni-contaminated effluents and receiving waters using rainbow trout may not be protective of sensitive fish species. Fourteen fish species have been identified in water bodies surrounding the Key Lake uranium mine in northern Saskatchewan. Nickel sensitivity has only been established in a few of these species. Species as sensitive to Ni as fathead minnows may not be adequately protected using toxicity data generated from rainbow trout tests. Further work is required to establish Ni sensitivity for other indigenous species that are not commonly tested in standard toxicological evaluations.

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5. The Influence of Water Hardness, pH, and Suspended Solids on Nickel Toxicity to Larval Fathead Minnows (*Pimephales promelas*)

5.1 Abstract

Nickel (Ni) is a ubiquitous, naturally occurring metal that is associated with metal mining and other industrial activities. Despite elevated Ni concentrations reported for many industrial receiving waters, Ni receives little research attention addressing factors influencing its toxicity to freshwater fish. This study examined the influence of water hardness, pH, and total suspended solids (TSS) in soft, reconstituted water on Ni toxicity to larval fathead minnows (*Pimephales promelas*). Increasing water hardness from 20 to 140 mg/L (as $CaCO_3$) reduced acute Ni toxicity by 5-fold (96-h LC50s 0.45 and 2.27 mg Ni/L, respectively). Low pH may have had a slight protective effect against Ni toxicity relative to neutral pH conditions. At pH 5.5, the 96-h LC50 was 0.69 mg Ni/L, compared to a 96-h LC50 of 0.54 mg Ni/L at pH 7. However, Ni toxicity was significantly reduced at pH 8.5 where the 96-h LC50 was 2.21 mg Ni/L. These results were explained on the basis of Ni speciation. Total suspended solids also reduced Ni toxicity (expressed as 96-h LC50s) from 0.35 to 1.12 mg Ni/L over a TSS range of 10 to 100 mg/L. This reduction of toxicity due to TSS is significant because mine effluents often have a combination of elevated TSS and metals. The ameliorative effect of TSS was not as significant as high hardness or pH probably because there is a TSS threshold, after which physical irritation to fish gills counteracts any protective effect conferred by TSS. This finding is relevant to choices made in design of mine effluent treatment

systems; i.e., there may be an optimum range of TSS concentrations that protect against effects of metals that remain after treatment.

5.2 Introduction

Several metals, like cadmium (Cd), copper (Cu), zinc (Zn), and more recently silver (Ag), have received considerable research attention to understand their toxic effects on fish. However, nickel (Ni) has received much less attention, and yet it has long been known as an important aquatic contaminant, especially in water bodies surrounding many industrial activities (Nriagu 1980). Elevated Ni concentrations in aquatic systems are associated with steel manufacture (Krantzberg and Boyd 1992), electrical battery manufacture (Greenwood and Earnshaw 1984), pesticide formulations (Galvin 1996), and metal mining (Nriagu et al. 1998; Rutherford and Mellow 1994).

Nickel has been identified as one of the most important aquatic contaminants associated with uranium mining in Canada (Golder Associates Ltd. 1996). The Key Lake open-pit uranium mine in northern Saskatchewan is the largest uranium operation in the world. It produced 6.4×10^6 kg U_3O_8 in 1998 (Cameco Corp. 1998a). At Key Lake, Ni is released to the environment via dewatering effluent from two pit mines. In 1997 alone, 5.6×10^6 m^3 of Ni-contaminated dewatering effluent was released to a nearby lake (Cameco Corp. 1998b). In a recent study (Pyle 1999), Ni in Key Lake receiving waters was 87±20 µg/L (mean±SD; n=7), which exceeds the Saskatchewan Surface Water Quality Objectives (SSWQO) for Ni of 25 µg/L (Saskatchewan Environment and Public Safety 1988). Nickel is currently recognized as a contaminant

having long-term consequences in aquatic systems around Key Lake (Cameco Corp. et al. 1995).

Uranium mine effluent toxicity is often characterized using rainbow trout (*Oncorhynchus mykiss*) in pass/fail-type toxicity tests. Previous work (Chapter 4) showed that rainbow trout were less sensitive to Ni than fathead minnows (*Pimephales promelas*), white suckers (*Catostomus commersoni*), or northern pike (*Esox lucius*). Early-life stage fathead minnows were most sensitive to Ni among the species for which toxicological endpoints could be determined (i.e., fathead minnows, rainbow trout, and white suckers). Therefore, environmental management decisions based on toxicity test results from fathead minnows would provide more protection to Key Lake piscifauna than decisions based on results from rainbow trout tests.

Water hardness and pH are known to influence the metal toxicity to fish (Erickson et al. 1996; Erickson et al. 1998). Water hardness reduces metal toxicity to fish by saturating gill surface binding sites with Ca^{2+} to the exclusion of metal cations (Playle et al. 1992). On the other hand, pH alters metal speciation. Metals are generally considered more toxic at low pH where they occur in their most bioavailable, free ionic form; i.e., M^{2+} (Morel 1983). Therefore, in the soft, acidic uranium-district lakes of northern Canada, toxicity thresholds for Ni released to the aquatic environment are expected to be low.

There are other factors that affect metal toxicity to fish, most notably that of dissolved organic carbon (DOC) which binds waterborne metals rendering them unavailable for uptake in fish (Playle et al. 1993). Lakes surrounding the Key Lake

mine show total organic carbon (TOC) concentrations of 0.2-5.5 mg/L (Cameco Corp. et al. 1995). Although DOC occurs in all lakes to varying degrees, elevated concentrations of total suspended solids (TSS) are usually associated with industrial activity. Dewatering of Key Lake open pits contributes approximately 10^5 kg of suspended solids to nearby receiving waters, or >95% of the total solids loading to the system (Cameco Corp. et al. 1995). Previous studies have suggested a protective role of TSS against metal toxicity to fish (Erickson et al. 1996). Consequently, the high TSS input associated with uranium mining may play a protective role against Ni toxicity to fish inhabiting receiving waters.

The purpose of this study was to investigate the effects of water hardness, pH, and TSS on Ni toxicity using a sensitive fish species such as larval fathead minnows. Three series of three 96-h acute lethality laboratory tests were conducted at low, moderate, and high hardness, pH, and TSS. Nickel concentrations ranged from 0.16 to 3 mg/L. Soft, reconstituted water was used in most tests to represent soft-water conditions of lakes in the uranium district of northern Saskatchewan.

5.3 Materials and Methods

5.3.1. Test Fish and Exposure Conditions

Test organisms were generated from a breeding culture of fathead minnows maintained at the Department of Biology, University of Saskatchewan, Saskatoon, SK, Canada. Both breeding adults and incubating eggs were held in hard, dechlorinated Saskatoon municipal water (i.e., laboratory dilution water), rather than reconstituted water, owing to the large volume of water required. Eggs were collected twice daily and

reared according to Canadian Environmental Protection Service (EPS) Guidelines (1992).

All tests were conducted in a temperature-controlled room (25±1°C) under a 16 h light and 8 h dark photoperiod, and followed general principles and guidelines of Canadian EPS (1992) and ASTM (1992). Each test consisted of five Ni concentrations and at least two controls; reconstituted water or treatment control and laboratory dilution water control. Tests were conducted in 300 mL of exposure solution contained in 450-mL plastic beverage cups. During static renewal tests, dead fish and debris were removed daily prior to 80 % exposure-water replacement. Only dead fish were removed from static tests.

Three series of tests were conducted in this experiment. The first series examined the effect of water hardness on Ni toxicity. Tests were static-renewal and were conducted in reconstituted water having a nominal total hardness of 20, 40, and 140 mg/L as $CaCO_3$ (Table 5.1). Hardness concentrations were chosen to reflect the characteristically soft waters of northern Saskatchewan lakes surrounding the Key Lake uranium mine, in addition to 140 mg/L as $CaCO_3$ which represents Saskatoon municipal water which is routinely used as a laboratory control water. Mill effluent receiving waters show hardness concentrations much higher than the highest hardness concentration tested here (i.e., >300 mg/L as $CaCO_3$).

The second series of tests examined the effect of pH on Ni toxicity. Tests were static-renewal and were conducted in soft reconstituted water pH adjusted to pH 5.5, 7.0, and 8.5. These pH values were chosen to reflect pH in lakes around the Key Lake

Table 5.1: Mineral salt composition (mg/L) of reconstituted waters used as dilution waters in this study. Concentrations were based on those of Cooney (1995). Appropriate proportions of mineral salts were added to reverse-osmosis water to prepare reconstituted dilution waters.

Water	$NaHCO_3$	$CaSO_4$	$MgSO_4 \cdot 7H_2O$	KCl
Tests examining the effects of water hardness on Ni toxicity				
Very soft	12	7.5	11.8	0.5
Soft	12	23	33	0.5
Hard	12	106.6	128	0.5
Tests examining the effects of pH and TSS on Ni toxicity				
Soft	48	30	47	2.0

mine. Mill effluent receiving waters are acidic (pH 5.5), whereas dewatering effluent receiving waters are neutral (pH 7.0). A pH of 8.5 is higher than that observed in Key Lake, but is representative to Saskatoon municipal water. Hydrochloric acid or sodium hydroxide (HCl or NaOH, respectively; PrairieChem, Saskatoon, SK) was added to dilution water to adjust to the appropriate pH. Nominal molar concentrations of reconstituted water constituents were entered into the chemical speciation program MINEQL+ as parameters to estimate Ni speciation in soft reconstituted water over the pH range of 5.5 to 8.5. Components used in the equilibrium calculations included H_2O, H^+, Ca^{2+}, Cl^-, CO_3^{2-}, K^+, Mg^{2+}, Na^+, Ni^{2+}, and SO_4^{2-}.

The third series of tests examined the effect of TSS on Ni toxicity. These tests were conducted as static tests because the opacity of the water under moderate to high TSS conditions was such that surviving fish could not be enumerated properly. In addition, daily additions of TSS solutions in a static-renewal test would provide serial input of suspended solids that would elevate TSS concentrations over time. Clean sculptor's clay (Lewiscraft, Toronto, ON) was used as suspended solids material. An inductively coupled plasma-mass spectrophotometry (ICP-MS; Saskatchewan Research Council (SRC) Laboratory, Saskatoon, SK) analysis was conducted on clay to determine

its metal content. Quality assurance of the clay metals analysis was determined according to SRC's routine in-house QA/QC protocols. Fish were exposed to Ni under 10, 50, and 100 mg TSS/L. Settled solids were resuspended daily by gentle mixing of test replicates. Historical Key Lake dewatering effluent data show TSS concentrations ranging from <1 to >200 mg/L (Cameco Corp. et al. 1995). Although most Key Lake TSS data fall below 15 mg/L, several measurements of TSS over 100 mg/L were reported (Cameco Corp. et al. 1995). Consequently, TSS concentrations used in this study were chosen to reflect the high variability of TSS concentrations measured in Key Lake dewatering effluent.

Ten larval fathead minnows (<24 h old) were randomly assigned to test replicates in each test. Replicates were arranged randomly on the shelf to minimize systematic bias of exposure-condition gradients. All tests were run for 96 h, and LC50 values (with 95% confidence intervals) were estimated using the non-parametric trimmed Spearman-Karber method. All statistics were conducted using the software program TOXSTAT v. 3.5 (West and Gulley 1996).

5.3.2. Reconstituted Dilution Water and Nickel Treatments

Reconstituted water was prepared by adding appropriate amounts of $NaHCO_3$, $CaSO_4$, $MgSO_4 \cdot 7H_2O$, and KCl (BDH Chemicals Ltd., Poole, England) to commercially-available reverse osmosis water (Culligan, Saskatoon, SK) (Table 5.1). During tests examining the influence of water hardness on Ni toxicity, fish were exposed to Ni in very soft, soft, and hard reconstituted water. Mineral salt composition of very soft water was based on Cooney (1995). Soft and hard reconstituted water was

based on mineral salt composition of very soft water, but with higher concentrations of $CaSO_4$ and $MgSO_4 \cdot 7H_2O$ only. This modification of reconstituted water procedure is contrary to Cooney (1995) who recommends corresponding increases of $NaHCO_3$ and KCl in the preparation of soft and hard reconstituted water. However, only $CaSO_4$ and $MgSO_4 \cdot 7H_2O$ were varied to minimize any ameliorative effects of $NaHCO_3$ or KCl on Ni toxicity. All other tests were conducted in soft water, as recommended by Cooney (1995).

Geometric dilution series used in each test were 16, 25, 40, 63, and 100% of maximum Ni concentrations, where maximum concentrations were 1-3 mg Ni/L. During tests of water hardness on Ni toxicity, exposures in very soft and soft water used 1 and 2 mg Ni/L, respectively, as the maximum concentration. All other tests had 3 mg Ni/L as the maximum concentration. Dilution series were prepared by mixing stock solution with the appropriate reconstituted dilution water for the specific test (Table 5.1). Stock solutions were prepared by mixing $NiCl_2 \cdot 6H_2O$ (BDH Chemicals Ltd., Poole, England) into distilled, deionized water. Exposure solutions were prepared daily, whereas stock solutions were freshly mixed weekly.

Nominal concentrations of Ni treatments were confirmed by ICP-MS (Dept. of Geological Sciences, University of Saskatchewan). Nickel concentrations determined for exposure waters and stock solutions were adjusted according to Ni measured in sample and analytical blanks. Nickel concentrations were validated by analysis of an analytical standard (National Research Council of Canada River Water Standard, SLRS-3). Nickel concentrations were always < 18% of known values. Standard deviations

from Ni concentrations in duplicate samples were less than 20% of the mean. Therefore, nominal Ni concentrations are reported below.

Water quality, including temperature, dissolved oxygen, pH, conductivity, total dissolved solids (TDS), hardness, and alkalinity, was monitored three times for every test; upon test initiation, mid-duration, and upon test completion. An Orion pH meter (model 290A; Orion Research Inc., Beverly, MA), calibrated weekly, was used to measure pH. Temperature and dissolved oxygen were measured with a Corning (Corning Scientific Instruments, Corning, NY) electronic dissolved oxygen probe (with built-in temperature sensor) attached to a Corning M90 hand-held meter. Conductivity and TDS were measured with Corning's electronic conductivity probe and M90 meter. Hardness and alkalinity were monitored using Hach test kits, models 5-EP MG-L and AL-AP, respectively (Hach Co., Loveland, CO).

5.4 Results

Dissolved oxygen in test replicates remained >78% saturation in all tests. Dilution water-quality data are shown in Table 5.2. Laboratory dilution water was clearly different from reconstituted dilution waters. Conductivity and TDS was higher in laboratory water relative to reconstituted waters. Total hardness in laboratory water was 160 mg/L as $CaCO_3$, which matched hardness in the hard-reconstituted water. Alkalinity of laboratory water was also higher than any other water, 62.6 mg/L as $CaCO_3$.

Table 5.2: Water quality of laboratory dilution water and reconstituted very soft, soft, and hard dilution waters used in this study.

Water		pH[a]	Conductivity (µS/cm)	TDS[b] (mg/L)	Hardness (mg/L)[c]	Alkalinity (mg/L)[c]
Laboratory	*Mean*	7.7	418.5	206.8	160	62.6
	SD	(7.4-8.1)	38.9	18.6	0	3.0
	N	9	9	9	9	9
Very Soft	*Mean*	6.9	49.0	24.2	20	13.6
	SD	(6.8-7.0)	5.2	2.6	0	0.0
	N	6	6	6	6	6
Soft[d]	*Mean*	7.0	87.4	43.3	55	13.6
	SD	(6.7-7.1)	1.3	0.6	19	0.0
	N	6	6	6	6	6
Soft[e]	*Mean*	7.6	243.7	120.7	50	31.5
	SD	(7.3-7.8)	17.2	8.1	11	7.2
	N	18	18	18	18	18
Hard	*Mean*	7.6	266.0	132	160	20.4
	SD	(7.2-7.8)	18.8	9.2	23	1.4
	N	6	6	6	6	6

a Median pH values are reported instead of means, with pH range represented in parentheses.
b TDS, total dissolved solids.
c as $CaCO_3$.
d Soft reconstituted water used in hardness tests.
e Soft reconstituted water used in pH and TSS tests.

Total hardness of reconstituted waters used in hardness tests were close to nominal concentrations (Table 5.2). Very soft, soft, and hard reconstituted waters gave measured hardness values of 20±0, 55±11, and 160±23 mg/L as $CaCO_3$ (mean±SD, n=6), respectively, compared to 20, 40, and 140 mg/L as $CaCO_3$ nominal concentrations. Soft reconstituted water used in pH and TSS tests was also well within expected hardness parameters (hardness 50±11 mg/L as $CaCO_3$, n=11). Results of ICP-MS analysis of clay samples are reported in Table 5.3. Iron concentration (8.4 mg/g)

Table 5.3: ICP-MS analysis of selected metals in clay used in tests examining the effect of suspended solids on Ni toxicity to larval fathead minnows.

Metal	Concentration (μg/g)
Ag	0.86
Cd	0.21
Cu	18.87
Fe	8443
Ni	18.86
Pb	33.83
Zn	48.25

was at least two orders of magnitude higher than the next highest metal constituent, Zn, which occurred at 48 μg/g.

All tests had <20% mortality in control treatments. Larval fathead minnow mortality did not vary between laboratory water and reconstituted water, or between reconstituted water and treatment controls (*t*-test; $p<0.05$). Consequently, treatment controls were retained and used in subsequent calculations.

Nickel toxicity deceased with increasing hardness (Fig. 5.1). In water having a hardness of 20 mg/L as $CaCO_3$, the 96-h LC50 was 0.45 mg Ni/L for larval fathead minnows. Toxicity was decreased approximately 5-fold at a water hardness of 140 mg/L as $CaCO_3$ (96-h LC50 2.27 mg Ni/L). A doubling of water hardness between 20 and 40 mg/L as $CaCO_3$ had little effect on Ni toxicity (96-h LC50s 0.45 and 0.5 mg Ni/L, respectively).

Nickel toxicity increased slightly between pH 5.5 and 7.0 giving 96-h LC50s of 0.69 and 0.54 mg Ni/L, respectively (Fig. 5.2). However, between pH 7.0 and 8.5 (96-h LC50 2.21 mg Ni/L), Ni toxicity was reduced by 4.1 times. A 3.2-fold decrease in Ni toxicity was observed over the entire pH range from 5.5 to 8.5.

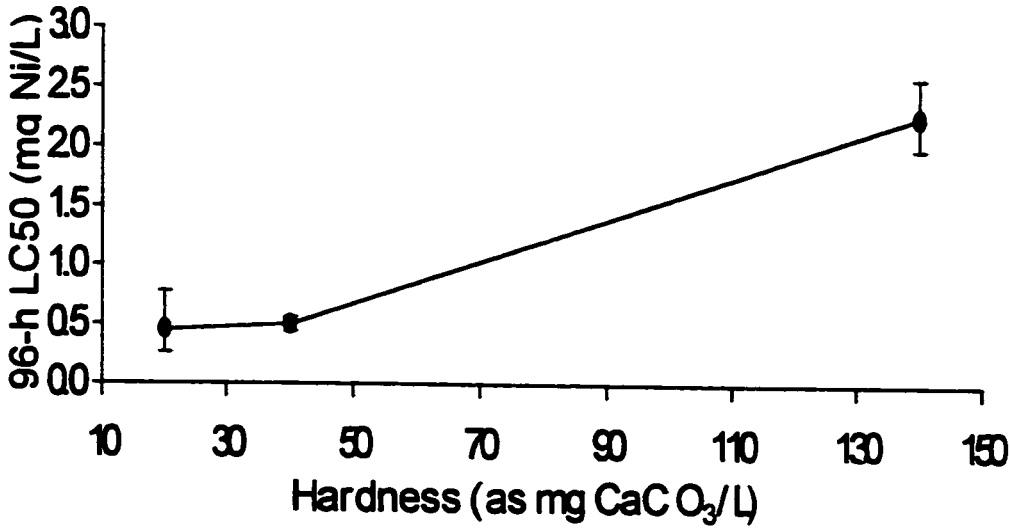

Figure 5.1: The effect of water hardness on Ni toxicity to larval fathead minnows. Points represent 96-h LC50 (mg Ni/L) and error bars represent 95% confidence interval.

Nickel speciation curves estimated by MINEQL+ are shown in Fig. 5.3. In the soft reconstituted water used in pH and TSS tests, free bivalent Ni^{2+} was the dominant species at the lowest pH tested (i.e., pH 5.5), where it accounted for 44.8% of total Ni. The nickel compounds $NiHCO_3^+$, $NiCO_3$ (aqueous), and $NiSO_4$ (aqueous) accounted for 28.9, 23, and 3.3 %, respectively, at pH 5.5. At pH 7, 88.2% of total Ni was in the form of $NiCO_3$ (aqueous), whereas $Ni(CO_3)_2^{2-}$ and $NiHCO_3^+$ accounted for 7.4 and 4.2%, respectively. At pH 8.5, 99.2% of total Ni was in the form of $Ni(CO_3)_2^{2-}$.

Total suspended solids decreased Ni toxicity to larval fathead minnows (Fig. 5.4). The toxicity reduction observed under high-TSS conditions was 51 and 49% less than that observed under high hardness or pH conditions, respectively. Unlike effects of hardness and pH on Ni toxicity, TSS reduced toxicity during the first incremental

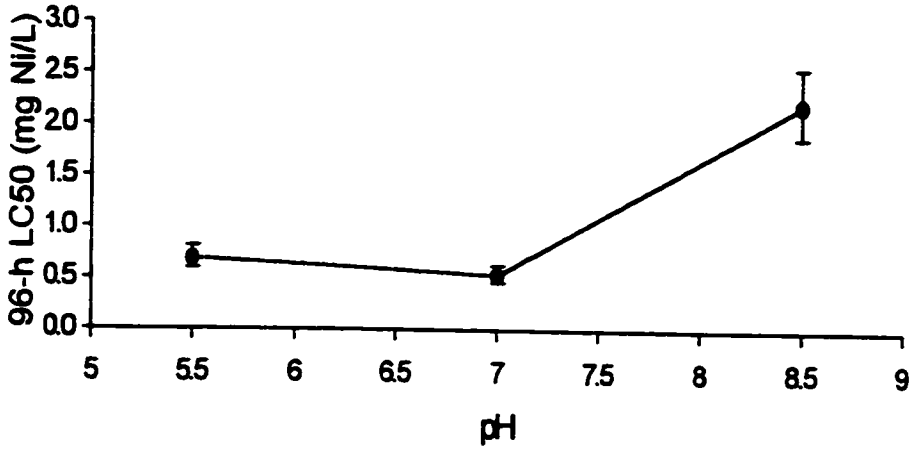

Figure 5.2: The effect of pH on Ni toxicity to larval fathead minnows. Points represent 96-h LC50 (mg Ni/L) and error bars represent 95% confidence interval.

increase. Toxicity was reduced by 61% as TSS increased from 10 to 50 mg/L. Only a 20% additional toxicity reduction was observed as TSS increased from 50 to 100 mg/L. Over a total TSS range of 90 mg/L (i.e., from 10 to 100 mg/L), Ni toxicity was reduced 3.2 times (96-h LC50s 0.35 and 1.12 mg Ni/L, respectively).

5.5 Discussion

Results reported here demonstrate that increasing water hardness (Fig. 5.1) or total suspended solids (Fig. 5.4) reduced Ni toxicity to larval fathead minnows. On the other hand, increasing pH from 5.5 to 7.0 slightly (although not significantly) increased Ni toxicity (Fig. 5.2). However, further increases of pH above 7.0 result in substantial Ni-toxicity reduction. These results suggest three separate mechanisms affecting Ni toxicity to larval fathead minnows: (1) competitive cationic binding at the gill surface (effect of hardness); (2) competitive cationic binding to suspended solids (effect of TSS); and (3) Ni-carbonate formation with increasing pH (effect of pH).

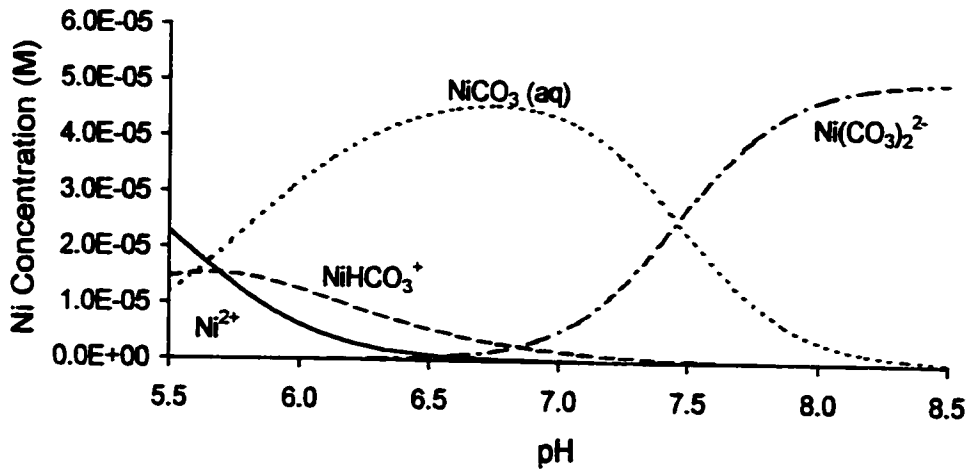

Figure 5.3: The effect of pH on Ni speciation in soft, reconstituted water. The free Ni^{2+} ion dominates the speciation scheme only at the lowest pH. As pH increases to pH 7, aqueous $NiCO_3$ becomes the most dominant Ni species, accounting for 88.2% of the total Ni in the system. A further increase in pH to 8.5 results in $Ni(CO_3)_2^{2-}$ dominating the system, accounting for 99.2% of the total Ni. Toxicity results reflect this speciation scheme (see text for details).

Water hardness is well known to attenuate metal toxicity to fish (Davies et al. 1993; Diamond et al. 1992; Erickson et al. 1996; Erickson et al. 1998; Kallanagoudar and Patil 1997; Moni and S.S.M. 1989; Pascoe et al. 1986). A previous study using adult fathead minnows exposed to Ni under soft (20 mg/L as $CaCO_3$) and hard (360 mg/L as $CaCO_3$) water conditions gave 96-h LC50s of 4.58-5 and 42.4-44.5 mg Ni/L (Pickering and Henderson 1966). Schubauer-Berigan et al. (1993) reported the 96-h LC50 to larval fathead minnows as 3.4 mg Ni/L (1.9-4.0 mg Ni/L 95% CI) in very hard water (300-320 mg/L as $CaCO_3$). This result is similar to the 96-h LC50 reported here for fathead minnows exposed to Ni under hard water conditions (i.e., 2.27 mg/L, 1.99-2.59 mg/L 95% CI), given the difference in hardness between the two studies.

Calcium maintains gill membrane structural integrity by cross binding gill-surface anions (Reid and McDonald 1991; Flik and Verbost 1993). In very soft water, fish are close to their ionoregulatory threshold and gill structural integrity is jeopardized

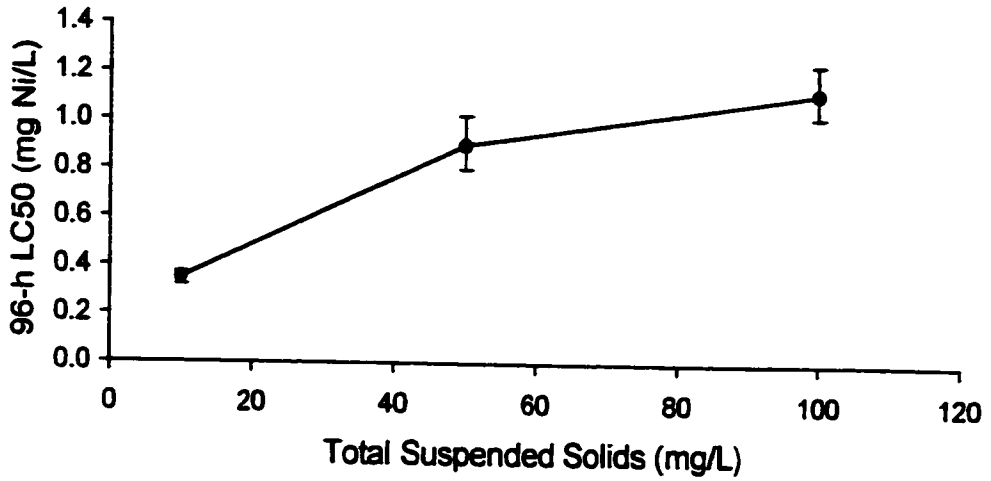

Figure 5.4: The effect of total suspended solids (TSS) on Ni toxicity to larval fathead minnows. Points represent 96-h LC50 (mg Ni/L) and error bars represent 95% confidence interval.

owing to the lack of available Ca^{2+} (McDonald and Rogano 1986). Ambient metals displace Ca^{2+} from the negatively charged gill surface causing structural damage and a reduction in osmotic integrity (Mueller et al. 1991). This alteration of gill tissue is apparent by the observed high toxicity of metals in soft water. On the other hand, high concentrations of Ca^{2+} in the water (i.e., hard water) reduce metal toxicity through competitive inhibition of metal binding to gill surfaces by Ca^{2+} (Hollis et al. 1997).

Nickel toxicity to larval fathead minnows did not vary with increasing pH from 5.5 to 7.0 (Fig. 5.2). Further increases of pH from pH 7.0 to 8.5 resulted in a significant reduction of Ni toxicity. Metals are generally considered more toxic to fish in low-pH water than in high-pH water. At low pH metals generally occur in their most bioavailable form as divalent cations. McDonald et al. (1991) showed that Cu, Cd, and Zn accumulated less in rainbow trout tissues at pH 5 than at pH 7. This reduction of metal accumulation at low pH also resulted in a concurrent reduction of toxicity. This

ameliorating effect of low pH was attributed to H^+ competition with metal ions at gill surfaces.

Further evidence of the ameliorative effect of lower pH on metal toxicity demonstrated that Zn, Cd, and Ni were more toxic to fathead minnows at pH 8.3 than at 6.3 (Schubauer-Berigan et al. 1993). Although the authors did not speculate on the possible mechanism of the protective effect of lower pH against metal toxicity, it is likely due to metal-H^+ competitive-exchange processes. Other studies that also note a reduction of metal toxicity under low pH conditions attribute the effect to either competitive-exchange processes between the metal cation and H^+ at gill surfaces (Cusimano et al. 1985), or a change in metal speciation with changing pH (Erickson et al. 1996).

Nickel speciation calculations (Fig. 5.3) for soft water used here in pH tests indicated that Ni^{2+} was present only at the lowest pH tested (5.5). At pH 5.5, Ni^{2+} competed with H^+ at gill surfaces. At pH 7, aqueous $NiCO_3$ was the dominant species, accounting for >88% of the total Ni in the system. At pH 8.5, 99.2% of all the Ni in the system was in the form of $Ni(CO_3)_2^{2-}$ (Fig. 5.3). The anionic Ni species could not effectively bind to electronegative gill surfaces, which caused a significant reduction in toxicity at pH 8.5 relative to toxicity observed at lower pH.

Total suspended solids reduced toxicity to larval fathead minnows (Fig. 5.4). The protective effect of TSS against Ni toxicity is qualitatively different from protection conferred by water hardness or pH. Rather than out competing Ni for binding sites at gill surfaces or altering aqueous Ni speciation, suspended solids strip Ni from the water

column, limiting the amount of Ni^{2+} available to fish. Playle et al. (1993) calculated equilibrium-based binding constants (log K) for Cu binding to organic carbon in addition to other ligands to model their moderating effects on Cu toxicity to fish. A similar equilibrium-based approach to model the toxicity-modifying effects of TSS would be useful.

Although increasing TSS reduced toxicity, results reported here suggest that most of the ameliorative effect occurred when TSS increased from 10 to 50 mg/L (61% decrease in toxicity) compared to a TSS increase from 50-100 mg/L (20% decrease). It is possible that at very high TSS concentrations (100 mg/L) suspended material settled quickly to the bottom of the test vessel reducing its ability to remove dissolved Ni. Each day during the static TSS tests, settled material—especially in the high-TSS test—had to be resuspended manually. An alternative explanation for the apparent reduction of TSS protection against Ni toxicity is that there is probably a point where protective effects are counteracted by physical irritation to gills. At TSS concentrations observed at the Key Lake mine (i.e., typically <25 mg/L), the presence of TSS in receiving waters may be reducing metal bioavailability to fish. There is a need for future work to study the effects of TSS on Ni toxicity to fish in an exposure system that can provide constant TSS concentrations in test replicates. Results from this work would be important in the design of effluent and mine water treatment systems.

In conclusion, increasing water hardness and TSS reduce Ni toxicity to larval fathead minnows. Water hardness reduces toxicity by out competing Ni for gill-surface binding sites. In very soft water (hardness 20 mg/L as $CaCO_3$), where Ca^{2+}

concentrations are low, Ni is relatively toxic to larval fathead minnows (96-h LC50 0.45 mg Ni/L). Toxicity is reduced by 5-fold when hardness is elevated to 140 mg/L as $CaCO_3$. Total suspended solids (represented by commercially available clay) removes free Ni from the water column reducing Ni bioavailability to fish. However, the protection fish receive from TSS against Ni toxicity might be offset by physical irritation to fish gills caused by high concentrations of TSS. At concentrations commonly observed around the Key Lake operation, TSS is likely rendering Ni unavailable for biological uptake by fish. Consequently, allowing suspended solids to remain in effluent water may reduce total Ni toxicity to fish inhabiting receiving waters. On the other hand, excessive TSS released to the environment may be detrimental to fish by causing physical irritation to their gills. Further study under test conditions that provide constant TSS concentrations is required to confirm this speculation.

At low pH, Ni was equally toxic to larval fathead minnows than at neutral pH. As exposure conditions approached neutrality from low pH, the dominant Ni species changed from Ni^{2+} to $NiCO_3$ (aqueous). The observation that Ni is equally toxic at low and neutral pH suggests that Ni^{2+} and $NiCO_3$ are equally toxic Ni species. Further elevations of pH resulted in another change in Ni speciation such that $Ni(CO_3)_2^{2-}$ became the dominant Ni species resulting in a significant reduction in toxicity.

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6. Metallothionein Induction in Juvenile Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Nickel or Molybdenum

6.1 Abstract

Metallothionein (MT) induction in fish exposed to metals of Groups IB and IIB of the Periodic Table (e.g., Cu, Zn, Ag, Cd, and Hg) is well documented. It is thought that MT regulates essential metals and provides protection to fish against metal toxicity. However, it is not known if MT is induced when fish are exposed to metals other than those mentioned above, like Ni and Mo. Nickel has previously been shown to induce MT in mammals but not in fish. Both nickel and molybdenum are metals of concern in waters surrounding northern Saskatchewan uranium operations. Juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed for 7 days to low and high concentrations of Ni (0.1 and 6.6 mg/L), Mo (1.5 and 1122 mg/L), and Cd (0.1 and 1 μg/L) in an attempt to induce MT in gills and livers. Metallothionein concentration in liver was generally 378-641% higher than in gills, but did not vary ($p>0.05$) with metal treatment or exposure concentration. Lack of MT induction in liver probably reflects short exposure times. Fish exposed to high concentrations of Ni, Mo, or Cd demonstrated 29-115% higher gill MT than control fish ($p<0.05$). High concentrations of Cd contaminated Mo treatments, and probably caused the observed MT induction in those treatments. Metallothionein concentrations did not correlate ($p<0.05$) with hepatosomatic index or metal concentration in the water. Lack of correlation between MT and fish-condition indices or ambient metal concentrations corroborates other studies that suggest MT may have limited utility as an effective biomarker of effects for metal-exposed fish.

6.2 Introduction

Metallothioneins (MTs) are a family of low-molecular weight proteins, rich in cysteine, that are thought to regulate metal metabolism in a wide range of biota (Hamer 1986), including fish (Hamilton and Mehrle 1986). Metallothioneins are thought to sequester essential metals in the intracellular compartment and deliver them to metal-active proteins where needed (Roesijadi 1992). Non-essential metals are sequestered by MT and delivered to excretory organs, like liver and kidney, for subsequent removal thereby conferring protection to fish against metal toxicity (Roesijadi 1992). Fish are known to increase MT production in response to elevated metals in water, presumably as a means of protection against metal toxicity (Hamilton and Mehrle 1986; Roesijadi 1992).

Metallothionein induction has been extensively documented in fish exposed to metals of Groups IB and IIB of the Periodic Table: i.e., Cu (Marr et al. 1995), Zn (Hogstrand et al. 1995a), Ag (Hogstrand et al. 1995b), Cd (Hylland et al. 1996), and Hg (Rema and Philip 1996)). Less is known about MT induction in fish exposed to other metals, like Ni or Mo. Metallothionein induction by Ni has previously been observed in mammals (Arizono et al. 1993; Bauman et al. 1993; Goyer 1991; Hamer 1986), including humans (Yamada and Koizumi 1991), and in cultured cells (Kagi and Schaffer 1988). Ptashynski and Klaverkamp (M.D. Ptashynski, and J.F. Klaverkamp, pers. comm., 1998; manuscript in prep.) have observed MT induction in kidneys and livers of lake whitefish (*Coregonus clupeaformis*) fed 10 mg Ni/g and in the kidneys of lake trout (*Salvelinus namaycush*) fed diets containing 1 and 10 mg Ni/g. However, MT induction by waterborne Ni has not been documented.

Nickel and Mo are two metals of concern in waters surrounding uranium-mining activities in northern Saskatchewan, Canada (Cameco Corp. et al. 1995). For example, the presence of high concentrations of Ni is characteristic of Cameco's Key Lake uranium ore. Dewatering effluent from Key Lake's open pit mine released into nearby receiving water leads to relatively high concentrations of Ni downstream of effluent discharge. Uranium-ore milling operations release high concentrations of Mo to receiving waters. Currently, the Saskatchewan Surface Water Quality Objectives (SSWQO) for Ni in soft water is 0.025 mg/L, yet Ni concentrations in ambient waters near the effluent discharge point are 0.09±0.02 mg/L (n=7;in Little McDonald Lake (Pyle 1999)). There is currently no similar water quality objective for Mo.

There have been several proposals to develop MT measurement as a biomarker for aquatic organisms inhabiting metal-contaminated systems (Chan 1995; Hamilton and Mehrle 1986; Hylland et al. 1996), including Canada's Environmental Effects Monitoring (EEM) for Mining program (AQUAMIN Working Groups 7 and 8 1996). Given the essentiality of both Ni and Mo (Mertz 1981; Goyer 1991; Galvin 1996), and MT's role as an essential-metal regulator, it is possible that fish exposed to either of these metals will show induced MT production. This possible MT induction is especially true for Ni, since Ni shares similar chemical properties with Cu, a known MT inducer in fish, demonstrated by Ni's close proximity to Cu on the Periodic Table.

The purpose of this study was to expose juvenile rainbow trout (*Oncorhynchus mykiss*) to high and low concentrations of Ni and Mo in an attempt to induce MT production. A second objective was to relate elevated MT concentrations with an index

of fish condition (i.e., the hepatosomatic index) or to metal concentration in the exposure water. Because most research into MT induction in fish has focused almost exclusively on metals of Groups IB and IIB of the Periodic Table, the present research was an attempt to demonstrate MT induction by other essential metals.

6.3 Materials and Methods

6.3.1. Exposure Protocol

Juvenile rainbow trout, 8.97±2.70 g (mean±SD; n=48), were donated by the Saskatchewan Fish Culture Station (Ft. Qu'appelle, SK). Fish were acclimated to laboratory conditions (see below) in a 500-L holding tank for 2 weeks prior to test initiation. Feeding occurred daily during acclimation. Fish were allowed to feed for 20 min, after which debris was siphoned from the bottom of the holding tank and 80% of the water was replaced.

Upon exposure initiation, three fish were randomly assigned to each of 16 38-L glass aquaria. Fish were transferred gently and quickly to exposure tanks to minimize stress. Each aquarium was supplied with moderate aeration through a glass Pasteur pipette. Debris was siphoned from each tank daily, and 80% of the water was replaced with freshly mixed exposure solution.

Exposures were conducted for 7 days in a temperature-controlled room maintained at 12-14°C under a photoperiod of 16 h light and 8 h dark. Replicates were arranged on shelves in random order to reduce systematic bias of results. Fish were fed only once (on day 3) during exposure; after which uneaten food was siphoned from each tank and water replaced as per regular daily protocol.

Metal exposures were conducted using a two-level factorial design with replication. At the first level, fish were exposed to four metal treatments: Ni, Mo, Cd (a known MT inducer; positive control), and clean laboratory dilution water (i.e., no metals; negative control). Each treatment was further divided into two concentrations, high and low, at the second experimental level. All eight treatments were replicated twice. Therefore, this experiment was based on a 4×2×2 design.

Nickel, Mo, and Cd stock solutions were prepared by mixing $NiCl_2 \bullet 6H_20$, $Na_2MoO_4 \bullet 2H_20$, and $CdCl_2$ in distilled, deionized water. Exposure solutions were prepared by mixing stock solutions with dechlorinated Saskatoon, SK, municipal water (i.e., laboratory dilution water). High and low exposure concentrations for each metal treatment were based on both preliminary in-house 96-h LC50 determinations for each metal using juvenile rainbow trout, and 96-h LC50s reported in the literature (McConnell 1977; Buhl and Hamilton 1991). Low and high nominal concentrations for each metal treatment was as follows: Cd, 0.1 and 1 µg/L; Ni, 0.1 and 6.6 mg/L; Mo, 1.5 and 1122 mg/L, respectively. The exposure control was laboratory dilution water.

At the end of 7 days of exposure, fish were processed randomly across all treatments to minimize systematic measurement error. Fish were sacrificed quickly by a sharp blow to the head. Total length was measured from the tip of the snout to the tip of the compressed tail. Fish were quickly patted dry with a paper towel and weighed to the nearest 0.001 g. Dissections included removal of the entire gill basket, liver, and kidneys. Tissues were placed in pre-weighed cryovials and weighed to the nearest 0.001

g. Tissues were immediately submersed in liquid nitrogen, and were stored at -80°C until further analysis. This entire process took less than 2 minutes per fish.

6.3.2. Water Quality

The following water-quality parameters were monitored during metal exposures: temperature, pH, total hardness (as $CaCO_3$), alkalinity (as $CaCO_3$), conductivity, total dissolved solids (TDS). Temperature was measured with an alcohol-filled glass thermometer. Conductivity and TDS were measured with Corning's electronic conductivity probe and M90 meter. A Lamotte HA-Series pH meter and electrode were used to determine pH. Hardness and alkalinity were determined using titrimetric methods included in the Lamotte water-quality test kit.

Nominal Cd, Mo, and Ni concentrations were confirmed using inductively-coupled plasma-mass spectrophotometry (ICP-MS; Perkin-Elmer Elan 5000 at the Dept. of Geological Sciences, University of Saskatchewan, Saskatoon, SK). Exposure water was also analyzed by ICP-MS for the presence of other known MT-inducing metals of Groups IB and IIB of the Periodic Table, such as Ag, Cu, Hg, and Zn. Water samples were collected in clean, polyethylene bottles from one replicate in each treatment at the beginning and end of the exposure period. Sample bottles were rinsed in sample water three times prior to collection. Samples were filtered through a 0.45 μm filter, acidified with distilled nitric acid, and refrigerated until further analysis. Analysis of National Research Council River Water Standard (SLRS-3) showed analytical variation generally <20% between duplicates of a single sample, and results were within 30% of known values.

6.3.3. Metallothionein Analysis

A previously described Hg-saturation assay was used to measure MT in fish tissues (Dutton et al. 1993). Briefly, tissues were homogenized in saline solution (0.9% NaCl) at a tissue:saline dilution level of 1:3 w/w for gills and kidneys, or 1:6 w/w for livers. One g of homogenate was heated in a polypropylene centrifuge tube for 5 min at 95°C and cooled on ice for 4 min. The sample was centrifuged at 10,000 rpm for 5 min. The MT-containing supernatant was transferred into a clean 1.5-mL microcentrifuge tube and frozen at –80°C until further analysis.

To 200 µL of thawed supernatant, a solution of $HgCl_2$ and $^{203}HgCl_2$ (Amersham Life Science) mixed in 20% trichloroacetic acid was added to yield a final activity of 10,000 counts per minute (cpm). The sample was vortexed then incubated at room temperature for 10 min. Remaining non-MT bound Hg was removed from the sample through the addition of 400 µL of 50% chicken egg albumin, followed by high-speed vortex and centrifugation at 10,000 rpm for 3 min. Each 500-µL sample of the resulting supernatant was counted on an LKB Wallac 1282 Compugamma gamma counter for 10 min. This counting time generally produced acceptable counting errors within 1%.

As part of a quality-assurance/quality-control program, each group of 20 samples was analyzed concurrently with five total-activity and four blank samples, plus four graduated dilutions of rabbit liver MT-II (M5392, Lot no. 13H95481; Sigma Chemical Co., St. Louis, MO) to produce a standard curve. Total activity samples consisted of only saline solution and ^{203}Hg tracer. Blank samples were also prepared with saline and tracer, and subsequently treated with egg albumin to remove dissolved Hg. The

standard curve dilution series was 8.8, 22, 44, 88, and 176 μg MT-II/mL. Metallothionein concentrations were determined for fish tissues from the standard curve through simple linear regression.

6.3.4. Statistical Treatment

Hepatosomatic indices (HSI) were calculated for individual fish by dividing liver weight (g) by fish weight (g). Fish weight, total length, HSI, and MT concentrations were tested for normality and homogeneity of variance using a Shapiro-Wilks test and Levene's test of equal variance, respectively. Data not meeting normality or variance assumptions were treated with a $\log_{10}(x)$ transformation, which proved successful for treating assumption violations. Paired Student-*t* tests were used to compare MT concentrations between tissues for each permutation of metal treatment and exposure concentration. Student-*t* tests were also used to compare between high and low metal concentration for each permutation of metal treatment and fish tissue. A series of one-way analyses of variance (ANOVA) were conducted to compare mean MT concentration among metal treatments for each permutation of exposure concentration (i.e., high or low) and fish tissue. One-way ANOVAs were performed to detect HSI differences among the four treatments by examining each exposure concentration and fish tissue separately. Post-hoc analysis was by Tukey's test. Pearson correlation coefficients were used to examine the association between tissue MT and HSI, and MT and dissolved-metal concentrations. For all tests, statistical significance was set at α=0.05. All statistical analyses were performed on SPSS statistical software (SPSS 1988).

6.4 Results

Exposure water chemistry remained relatively constant throughout the exposure period (Table 6.1). Variation in conductivity and TDS measurements reflect dissolved ions in the metal treatments. Exposures were carried out at 13.5-13.9°C in circumneutral (pH 7.1-7.3), moderately hard (hardness 109-115 mg/L as $CaCO_3$) water. Nominal metal concentrations were verified in each metal treatment, and were within 15% of expected values (Fig. 6.1), except in the Cd treatment. In the low Cd treatment, actual Cd was 53% higher, and in the high Cd treatment, actual Cd was 23% higher, than nominal concentrations. Other metals showed concentrations similar to those in control water, with the exception of very high concentrations of Cd in Mo treatments. Cadmium concentrations in the lowest-Mo treatment were 8% higher than the highest Cd concentrations measured for the Cd treatments. In the high-Mo treatment, Cd concentrations were 0.9 mg/L, which is >700 times higher than the highest measured value for either Cd treatment. Neither Ag nor Hg were detected in any water samples, and were therefore not included in Fig. 6.1.

No fish mortalities were observed during the exposure period of the experiment. Fish weight (8.97±2.70 g; mean±SD, n=48) and total length (96.27±8.81 mm; n=48) were similar in all treatments. Hepatosomatic indices also did not vary among treatments, and averaged 0.012±0.002 (n=48). However, rejection of the null hypothesis for comparisons of HSI among low-exposure treatments was marginal (i.e., p=0.053). Tukey's test indicated a significant difference between HSI in rainbow trout exposed to low concentrations of Ni (mean HSI 0.0131±0.0020, n=6) and those in the control

Table 6.1: Water quality summary during juvenile rainbow trout exposure to high and low concentrations of nickel, molybdenum, and cadmium.

	Control		Nickel		Molybdenum		Cadmium	
	Low	High	Low	High	Low	High	Low	High
Temperature (°C)								
Mean	13.7	13.5	13.8	13.9	13.9	13.9	13.9	13.8
SD	0.9	0.7	0.8	0.7	0.8	0.8	0.8	0.8
N	4	2	4	4	4	4	4	4
pH								
Median	7.2	7.2	7.2	7.1	7.2	7.3	7.2	7.3
Range	7.1-7.5	7.2	7.1-7.6	7.1-7.6	7.2-7.5	7.3-7.8	7.1-7.6	7.2-7.7
N	3	2	3	3	3	3	3	3
Conductivity (µS/cm)								
Mean	371.8	375	367.3	390.5	365.8	2642.5	368.8	361.5
SD	14.3	14.1	13.6	13.0	9.7	106.9	12.3	4.8
N	4	2	4	4	4	4	4	4
TDS (mg/L)								
Mean	187.8	187.0	184.0	192.8	183.3	1320.0	183.3	183.8
SD	6.1	8.5	6.4	9.5	4.8	49.0	6.7	7.2
N	4	2	4	4	4	4	4	4
Alkalinity (as mg $CaCO_3$/L)								
Mean	81.0		83.5	75.5	85.0	84.6	85.0	76.5
SD	1.4		2.1	20.5	0	24.7	7.1	12.0
N	2		2	2	2	2	2	2
Hardness (as mg $CaCO_3$/L)								
Mean	114.5		112.0	117.0	109.0	115.0	105.0	106.0
SD	2.1		5.7	4.2	1.4	1.4	1.4	2.8
N	2		2	2	2	2	2	2

(mean HSI 0.0099±0.0018, n=6). Pearson correlation analysis showed that HSI and tissue MT were not related, regardless of exposure concentration ($p>0.05$).

Liver MT was always greater than gill MT; however, liver MT was not correlated with metal concentrations in the water. Metallothionein concentrations were 378-641% higher in livers than in gills (Fig. 6.2). Liver MT (mean±SD, 338.6±262.6 µg MT/g wet tissue weight; n=41) remained unchanged between high and low metal

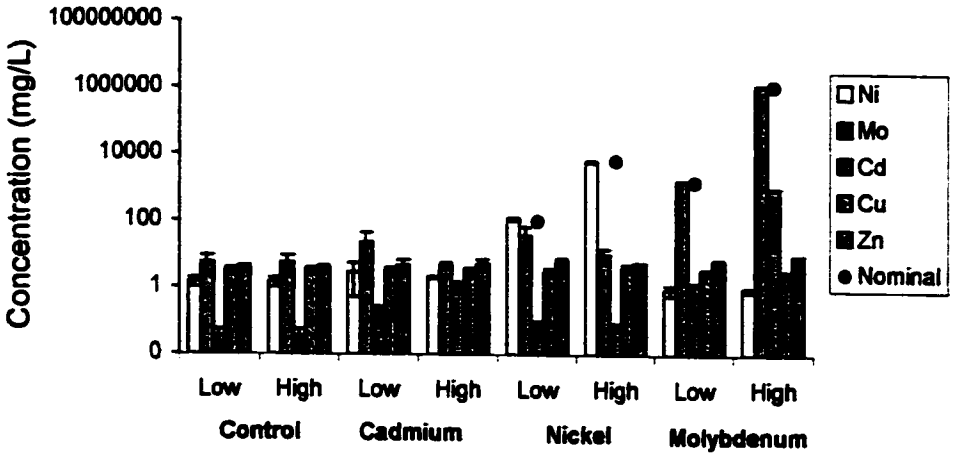

Figure 6.1: Comparison between actual (bars) and nominal (dots) metal concentrations in exposure waters. Bars represent means±SD (n=2). High concentrations of Cd in Mo treatments make subsequent conclusions about Mo-induced MT induction impossible. Neither Ag nor Hg were detected in water samples and were therefore not included in the graph.

concentrations. Liver MT was not correlated with Ni, Mo, Cd, Cu, or Zn in the water ($p>0.05$).

Gill MT, although much lower, showed differences between high and low metal treatments. Gill MT concentrations were higher in the high metal treatments than in the low metal treatments or in the controls (Fig. 6.2). Fish exposed to high-metal treatments had 160-320% more gill MT than control fish ($p<0.05$). Gill MT, however, did not vary among low-metal treatments (mean±SD, 28.7±8.0 μg MT/g wet tissue weight; n=24). Despite the difference between high and low metal treatments, there was no overall correlation with Ni, Mo, Cd, Cu, or Zn concentrations in water. Gill MT was not correlated with liver MT ($p>0.05$).

6.5 Discussion

Results from the current study indicated that juvenile rainbow trout exposed to 6.6 mg Ni/L for 7 days showed elevated concentrations of MT in gills relative to

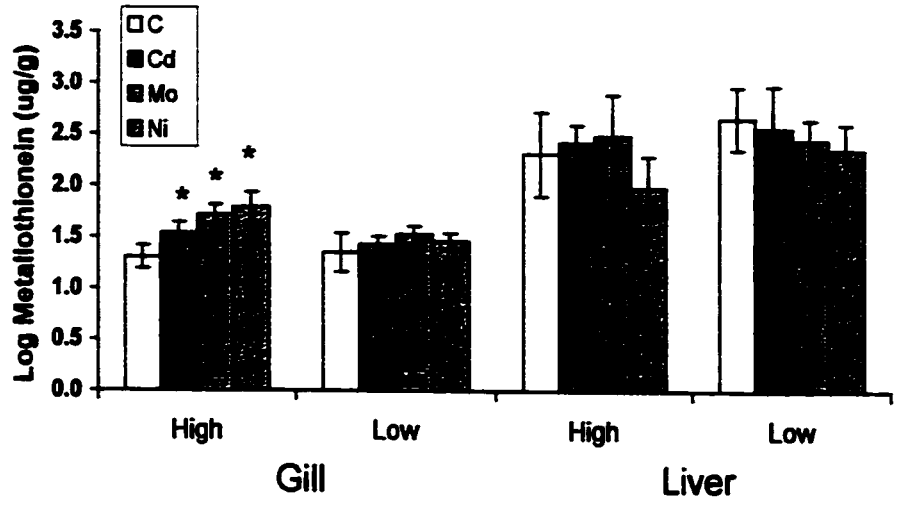

Figure 6.2: Metallothionein induction in juvenile rainbow trout gills and livers exposed to control water (C), nickel (Ni), molybdenum (Mo), and cadmium (Cd). Bars represent means±SD (n=6; n=3 for high-Ni and high-control treatment gills and livers). Asterisks (*) represent significant ($p<0.05$) differences from control.

negative controls, but not in liver (Fig. 6.2). Rainbow trout exposed to Mo treatments also showed elevated MT in gills relative to controls, but this result was suspicious owing to the high concentrations of Cd in Mo treatments (Fig. 6.1). Cadmium is known to induce MT in salmonids (Hamilton et al. 1987), which is likely the reason for the high concentrations of MT observed in Mo-exposed trout gills.

Although MTs have been extensively studied for more than four decades (Margoshes and Vallee 1957; Kagi and Vallee 1960), their exact physiological function has yet to be fully characterized. It is currently thought that MTs regulate essential metals like Cu and Zn, and detoxify non-essential metals like Cd and Hg by sequestering them from reacting with important biomolecules (Roesijadi 1992). In both cases, metal binding to MT is dependent on the binding properties of the metal in question.

All of the metals currently known to bind to MT can be classified as borderline metals with a strong class B (i.e., covalent) character (Nieboer and Richardson 1980; Nieboer and Fletcher 1996). They are 'borderline' in the sense that they can bind to molecules by forming either ionic (class A) bonds with oxygen donor atoms, or covalent (class B) bonds with sulphur or nitrogen donor atoms. However, borderline metals having a strong class B character have a tendency to form covalent bonds with S and N donor atoms preferentially over, but not exclusive of, ionic bonds with O ligands. Metallothioneins are cysteine rich, having a total S content of 30-35% (Hamer 1986; Kagi and Schaffer 1988). As such, it is apparent why borderline metals having a high affinity for S donor ligands (i.e., a high covalent character) bind readily to MT.

Nickel can also be classified as a borderline metal with a strong class B character, like metals of Groups IB and IIB of the Periodic Table. Consequently, Ni can be expected to bind to the sulphur-rich cysteine groups of MT. This high binding potential for Ni suggests that MT induction in Ni-exposed fish may provide protection against Ni toxicity. Consequently, fish inhabiting Ni-contaminated water may develop a metal tolerance, as has been demonstrated for other MT-inducing metals (Duncan and Klaverkamp 1983; Marr et al. 1995).

The MT induction observed in this study occurred only in the gills and not in the liver. Metallothionein is typically associated with gills, liver, kidneys and intestines in fish, all of which are involved with uptake, detoxification, and excretion (Roesijadi 1992). Fish gills represent the interface between external and internal environments, and are the first point of attack by waterborne metals (Laurent and Perry 1991). Nickel

taken up from the water by the gills induced the observed MT response. This *de novo* gill MT probably bound excess Ni where it remained sequestered from further metabolism until it could be redistributed to other organs like liver or kidneys (Olsson and Hogstrand 1987). However, the short exposure time in this study (7 d) was insufficient for translocation of MT-bound Ni to liver. This apparent sequestering of Ni in gills resulted in a lack of MT induction in liver. Future studies implementing longer exposure times to Ni will likely produce MT induction in fish liver.

Metallothionein concentrations measured in gills in the current study did not correlate with HSI or metal concentrations in the exposure medium. Brook trout (*Salvelinus fontinalis*) exposed to Cd for 30 d demonstrated MT induction that was not related to mortality or Cd-exposure concentration (Hamilton et al. 1987). Other studies have also reported MT induction not related to environmental contaminant concentrations or indices of fish health (Cope et al. 1994; Schlenk et al. 1996). Some studies have reported that MT induction was related to other forms of stress than metal contamination (Pottinger and Calder 1995; Weber et al. 1992). These results have led to questions about the usefulness of MT as a biomarker for fish stress. Although MT induction in fish exposed to metals has now been very well established (Hamilton and Mehrle 1986), MT does not appear to be an effective biomarker of effects. Furthermore, it may not be consistently diagnostic of metal exposure.

In conclusion, MT was successfully induced in gills of juvenile rainbow trout gills, but not livers, upon exposure to high concentrations of Ni. Lack of MT induction in livers was likely due to the short exposure time (7 d) in this study. Fish exposed to

Mo also showed MT induction in gills, but that can be attributed to Cd contamination of exposure water. Consequently, no conclusions can be drawn about MT induction upon exposure to Mo. Metallothionein concentrations showed a differential response among metal treatments and could not be related to HSI or metal concentrations in water. This finding corroborates other studies that suggest MT has limited utility as a biomarker.

6.6 References

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7. General Discussion

7.1 Question 1: Are standard toxicity-test fish species representative of non-standard, native fish species in terms of predicting metal toxicity?

The use of surrogate fish species in toxicity tests used to evaluate the toxicity of industrially polluted systems has resulted in concerns about whether toxicity derived from surrogate species represents a similar response in indigenous species. Water quality criteria regulating the release of metals to the environment are based upon laboratory toxicity tests using standard, toxicity-test fish species, like rainbow trout or fathead minnows. If standard species are more tolerant to the contaminant metal than native species inhabiting the contaminated environment, native fish populations will not be adequately protected against metal toxicity.

To assert with 100% confidence that surrogate species provide adequate toxicity information to protect indigenous fish against metal toxicity, all fish species within a system must be tested. In lakes surrounding the Key Lake uranium mine, 14 fish species have been identified (Cameco Corp. et al. 1995). To test each species for acute and chronic metal toxicity would be a huge undertaking in terms of both time and expense. In this study, only two species, northern pike (*Esox lucius*) and white suckers (*Catostomus commersoni*), were tested against results derived from fathead minnows (*Pimephales promelas*) and rainbow trout (*Oncorhynchus mykiss*).

Northern pike and white suckers were chosen as representative species for several reasons: both species are common in lakes of the uranium district of northern Saskatchewan; both species occupy different trophic positions (white suckers are

common prey of northern pike); both species are considered valued ecosystem components; both species have well-characterized biology; gamete-extraction procedures for each species is relatively simple to perform; and early-life stages of both species are easy to maintain in the laboratory. Although it is possible to compare toxicity results of northern pike and white suckers with those of fathead minnows and rainbow trout, it is not known whether toxicological responses by northern pike or white suckers represent those of other native species.

Pickering and Henderson (1966) tested fathead minnows, bluegills (*Lepomis macrochirus*), goldfish (*Carassius auratus*), guppies (*Poecilia reticulata*), and green sunfish (*Lepomis cyanellus*) for their relative sensitivity to Cd, Cu, Ni, Pb, and Zn under various water quality conditions. Fathead minnows were consistently more sensitive to metal intoxication than the other fish species tested.

McKim et al. (1978) tested the relative sensitivities of brook trout (*Salvelinus fontinalis*), rainbow trout, brown trout (*Salmo trutta*), lake trout (*Salvelinus namaycush*), northern pike, white suckers, lake herring (*Coregonus artedii*), and smallmouth bass (*Micropterus dolomieu*) embryos and larvae to Cu. Larvae were consistently more sensitive to Cu than embryos. All species except northern pike showed relatively similar sensitivities to Cu. Northern pike was consistently more tolerant to Cu relative to all other species tested.

McFarlane and Franzin (1978) studied a population of white suckers living in a metals-contaminated lake in Saskatchewan relative to white suckers in a clean lake. They found that the white sucker population inhabiting the metals-contaminated lake

showed reduced spawning success, larval and egg survival, egg size, and adult survival relative to the reference population. The same study also showed that although the white sucker population demonstrated clear signs of metal stress, other fish populations, like lake herring and walleye (*Stizostedion vitreum*) populations, were more sensitive than the white sucker population.

Results reported here suggest that Mo is relatively non-toxic to all fish species tested. Whether tests were conducted on embryos, larvae, or juveniles, Mo caused no effect that was significantly different from control treatments. In this particular case, surrogate species provided identical information to native species. However, conclusions about whether surrogate species represent indigenous species cannot be drawn on negative results.

On the other hand, Ni toxicity was dependent upon fish species. The most sensitive response to Ni was hatching stimulation of fathead minnow eggs at 0.75 mg Ni/L (LOEC). Rainbow trout and northern pike eggs were not tested because they died during a refrigeration unit malfunction. White sucker eggs were exposed to Ni concentrations (0.25 mg Ni/L) twice as high as Ni concentrations reported for dewatering effluent receiving waters near Key Lake (0.1 mg Ni/L). Egg hatchability and time required for hatching were not significantly different from controls. Fathead minnow eggs were repeatedly tested until a significant result was achieved.

Nickel mortality results provided clearer information about species differences. Again, larval fathead minnows were most sensitive to Ni toxicity (96-h LC50 2.4 mg Ni/L), whereas white suckers (96-h LC50 17.9 mg Ni/L) and northern pike (96-h LC50

>3 mg Ni/L) were more tolerant. However, rainbow trout, the species currently being used to characterize effluent and receiving water toxicity, was most Ni-tolerant of any species tested (96-h LC50 >20 and 51.2 mg Ni/L for alevins and juveniles, respectively).

Metal-release regulations are routinely based on toxicity data derived from rainbow trout. Based on the rainbow trout results reported in this study, the Maximum Acceptable Toxicant Concentration (MATC) for Ni was 37.2 mg Ni/L in hard water. The MATC for the most sensitive fish species tested in this study, fathead minnows, was 1.5 mg Ni/L. These values are probably underprotective given that they were derived from data collected from tests conducted in hard water. Northern Saskatchewan lakes around the Key Lake mining district are typically soft. Water hardness has a well-known protective effect against metal toxicity. However, the point of the argument remains uncompromised. Any MATC determined from rainbow trout data for Ni may place native fish species, that have a similar Ni sensitivity to that of the fathead minnow, at risk.

These results raise several important questions. The concentrations of Ni required for toxicity to occur, even in the most sensitive species tested (fathead minnows), are much higher than those observed in Ni-contaminated receiving waters. Also, fathead minnows are a warm-water species that do not occur in the cold, northern Saskatchewan lakes near uranium operations. It is possible that the warmer temperature required for fathead minnow tests (25°C) contributes to a higher metabolic rate than tests in cooler (12-14°C) water for other species. Higher metabolic rates translate into larger

volumes of water passing over gill surfaces relative to water volumes being processed by other species in cooler water. This higher metabolic rate could lead to increased Ni binding at gill surfaces causing toxicity to occur at lower Ni concentrations. Therefore, fathead minnows may not appropriately represent cool water fish like rainbow trout, northern pike, or white suckers.

Rainbow trout used in this study were provided by the Saskatchewan Fish Culture Station (Ft. Qu'appelle, SK). Although eggs were collected and artificially fertilized from fish captured from a natural lake (as were northern pike and white sucker eggs), rainbow trout were originally derived from hatchery stock. White suckers and northern pike used in this study were from wild populations. Rainbow trout from hatchery stock have been artificially selected over many generations for various traits, many of which are beneficial to Saskatchewan's aquaculture industry (e.g., fall spawning, fish size, disease resistance; G. Mitchell[1], pers. comm., 1998). Consequently, it may be that rainbow trout used in this study were more Ni-tolerant than wild fish as an epiphenomenon of their human-selected genetic characteristics. This potential difference in Ni tolerance is illustrative of a point made by Cairns (1991) as to why toxicity results derived in the laboratory, using healthier fish than those in the wild, often do not match toxicity in nature.

Aquatic toxicology laboratories across North America routinely use cultured rainbow trout to characterize metal toxicity in mine effluents and receiving waters. In fact, the Canadian EPS guidelines (1990) provide a list of Environment Canada offices that will assist toxicology laboratories locating disease-free rainbow trout for use in

toxicity tests. Results from this study suggest that cultured rainbow trout used in toxicity evaluations of metals-polluted water may not provide adequate toxicological information to protect indigenous fish.

7.2 Question 2: How do toxicity results derived in the laboratory relate to toxicity in situ?

Extrapolating toxicity results from the laboratory to the field has been a key, long-standing objective of aquatic toxicology (Moriarty 1983). Obviously, toxicity results derived in the laboratory have little meaning if they cannot be related to a natural system. However, results presented in this study suggest that laboratory-derived toxicity of mine-polluted water to early-life stage fathead minnows do not relate to toxicity data derived from a natural system.

Many factors that influence toxicity vary from laboratory to field. The first consideration is with the test organisms themselves. As discussed in the last section, standard toxicity-test fish species are generally healthier than wild fish because of their unnatural rearing conditions. Larval fathead minnows were used to test if toxicity differed between laboratory and field. The fathead minnows that were used came from a United States Environmental Protection Agency (U.S. EPA)-grade genetic line (fish source from Environmental Consulting and Testing, Superior, WI, USA). These fish have been artificially selected over hundreds of generations for optimum reproductive output at a standard laboratory temperature (i.e., 25±1°C). The author's experience with these fish has demonstrated repeatedly that temperature fluctuations as little as 3°C from this optimum range has resulted in significantly-reduced reproductive output and reduced survival of young (pers. obs., 1996-1999). Consequently, to ensure success in

toxicity tests using laboratory-grade fathead minnows a test temperature of 25°C is necessary.

In northern Saskatchewan lakes, like those around the Key Lake mine, water temperature rarely, if ever, achieves 25°C. *In situ* toxicity tests were attempted several times during this study over the course of two summers and 25°C was never measured in any lake. Temperatures were always much cooler than 25°C. In the only successful field toxicity test that was conducted mean temperatures ranged from 16.1-18.7°C, whereas temperature standard deviations ranged from 0.9-2.1°C for the same range. These values represent only instantaneous measurements and they do not reflect diurnal temperature fluctuations. It is suspected that diurnal temperature fluctuations are greater than the instantaneous standard deviations reported. Under natural conditions, fish are free to migrate to areas having optimum temperature conditions. The *in situ* testing protocol used here was such that fish were forced to remain in one place for the duration of the exposure period. Consequently, temperature may have unnaturally affected toxicity results in the field. Under laboratory conditions, temperature was tightly controlled.

Another major difference between laboratory and field tests is that fish used in field tests suffered transportation stress, whereas those used in laboratory tests did not. In fact, field-exposed fish were subjected to two sources of transportation stress that may have influenced their response to metal-contaminated water. Fish eggs were collected in the laboratory in Saskatoon, packaged in an oxygen-filled plastic bag which was placed in a sealed cooler. The cooler was transported to an airport where it was

placed on a plane in an unheated luggage compartment. Transportation to Key Lake required approximately 2 h flight time. The cooler containing the eggs was transported over rough roads to an on-site makeshift laboratory with no temperature control. Although eggs were reared with every effort to minimize temperature fluctuations using a thermal buffer (i.e., a large-volume water bath), temperature fluctuated with ambient temperature. The second source of transportation stress occurred when larval fish were transported from the makeshift laboratory to exposure sites. Fish were transported in small groups to minimize this source of transportation stress. However, some roads leading down to study lakes were unavoidably rough and undoubtedly contributed to fish stress. Fish used in laboratory tests were not subjected to any stress of this kind.

Lakes receiving mine effluents, either from milling or dewatering activities, experience environmental fluctuations (i.e., metal concentrations, organic contaminant inputs, temperature changes, pH changes) in a pulsatile fashion (G. White[2], pers. comm., 1997). Laboratory toxicity tests are conducted in water samples representing conditions at one instant in time. As discussed in the case of temperature fluctuations, wild fish can migrate from a metal plume passing through their habitat (Giattina and Garton 1983; Hartwell et al. 1987). Fish placed in fixed-position cages cannot migrate from metal plumes and are therefore subjected to contaminants that wild fish tend to avoid. Laboratory tests subject fish to relatively constant contaminant concentrations that represent conditions at the time of sampling. Consequently, differences in toxicity responses between a laboratory and field exposure are not surprising.

Field-exposed fish are also subjected to stresses associated with weather conditions. Rough water caused by inclement weather caused significant stress to exposure fish. In the only successful *in situ* exposure, a strong weather event caused rough water sufficient to kill 95% of the test fish at two exposure sites in McDonald Lake. Fish deaths were the result of rough water causing larval exposure and observation tubes to become dislodged from their tether to wooden stakes. However, exposure tubes that remained tethered during rough water still experienced a large amount of movement that was likely sufficient to cause fish stress. This type of stress was not experienced in laboratory-exposed fish.

Another factor that affects metal toxicity differently in a field exposure relative to a laboratory exposure is the feeding status of test animals. During a 168-h laboratory exposure of larval fathead minnows, fish were fed twice daily with live brine shrimp nauplii. During *in situ* exposures, small invertebrates entered exposure tubes through end-cap mesh (pers. obs., 1997) which were available to larval fathead minnows as food. The difference in this regard between laboratory and field exposures is that in the former fish were fed a single food organism at a known rate. In field tests, fish ate whatever organisms passed into exposure tubes, and certainly at rates that varied by exposure site.

Metal toxicity is often the result of ionoregulatory disruption in fish (McDonald and Wood 1993). The mechanism of Ni toxicity is thought to proceed similarly to that of Cu because of similarities between Ni and Cu chemistry (Nieboer and Richardson 1980; Nieboer and Fletcher 1996). Copper toxicity results in a net loss of Na^+, which is

the same effect suspected for Ni (although no studies have tested this hypothesis). Recent evidence suggests that ionoregulatory disruption can be counteracted if a fish replaces Na^+ lost from gills and kidneys with Na^+ taken up from its diet (D'Cruz and Wood 1998). Brine shrimp nauplii are raised in saline water (specific gravity 1.022-1.025) and are consequently high in Na^+. Therefore, Na^+ lost from exposure to Ni is likely regained in laboratory-exposed fish but not in fish exposed *in situ*. These speculations imply that food ration quality and quantity, which is controlled in laboratory but not field exposures, may influence toxicity.

These arguments all indicate that toxicity data derived in the laboratory have little bearing on toxicity under natural conditions. Results reported here show that there is little relationship between laboratory and field toxicity. When a fish responds to a complex suite of environmental contaminants in natural water, the response is integrated through interactions among contaminants, exposure conditions, and feeding status. Even if the response between laboratory and field exposures were the same, it would be difficult to conclude that similar stimuli impinged upon test organisms to yield the same result in the two exposure systems.

7.3 Question 3: How toxic are nickel and molybdenum to fish?

In a series of laboratory toxicity tests Ni was considerably more toxic than Mo. Molybdenum concentrations up to 2 g/L were not sufficient to cause any detectable effects on fathead minnows, rainbow trout, northern pike, or white suckers. In hard water (140 mg/L as $CaCO_3$), Ni caused a significant reduction in the time required for fathead minnow eggs to hatch at 0.75 mg Ni/L, a significant reduction in egg

hatchability at 1.9 mg Ni/L, and was acutely toxic (i.e., 96-h LC50) at 2.4 mg Ni/L. In reconstituted soft (20 mg/L as $CaCO_3$) water, Ni was more toxic to larval fathead minnows (96-h LC50 0.45 mg Ni/L).

Early research on Mo toxicity to fish shows evidence of toxicity at concentrations far below (e.g., <0.1 mg Mo/L) concentrations tested here (literature reviewed in *Introduction*). Results of this study directly contradict those reports.

McConnell (1977) exposed 55 mm and 20 mm rainbow trout to $Na_2MoO_4 \cdot 2H_2O$ in soft water (hardness 25 mg/L as $CaCO_3$) and reported 96-h LC50s of 1320 and 800 mg Mo/L, respectively. Judging by results reported in this study, McConnell's results seem reasonable. Concentrations of 1320 and 800 mg Mo/L would result in Na^+ concentrations of 632.6 and 383.4 mg/L, respectively. It is likely that Na^+ could bind to anionic gill surfaces without having to compete with Ca^{2+} causing ionoregulatory disruption in the fish. Conversely, hard water (140 mg/L as $CaCO_3$) Mo exposures conducted in this study resulted in no detectable toxic effect because Na^+ had to compete for Ca^{2+} at gill surfaces. Calcium can clearly out-compete Na^+ at gill surfaces because gill surfaces have high-affinity Ca^{2+} binding sites (Playle et al. 1992), and because of Ca^{2+} bivalency as opposed to the single charge associated with Na^+. Because Mo occurs as the molybdate anion (MoO_4^{2-}) over a pH range corresponding to pH conditions used in the current study, Mo is not likely to bind to anionic gill surfaces. Therefore, the toxic response reported in McConnell (1977) is probably due to the high Na^+ concentrations, and not Mo. Hamilton and Buhl (1990) reported 96-h LC50s for eyed-eggs, alevins, and fry of chinook salmon (*Oncorhynchus tshawytscha*) and coho

salmon (*O. kisutch*) of >1000 mg Mo/L in water ranging in hardness from 40-333 mg/L as $CaCO_3$, which corroborates results reported here.

Although dietary Se was identified in Key Lake receiving waters as the primary toxicant likely responsible for larval fathead minnow mortality, Mo concentrations were very high (up to 1397 µg/L) in waters showing the highest fish mortalities. Waterborne Mo caused no detectable effects on fish under a controlled laboratory setting. However, at this time there are no data examining the possibility of dietary Mo toxicity. As discussed in the last section, small invertebrates could pass through exposure tube mesh providing test fish with food. If these small invertebrates contained elevated body burdens of Mo, ingestion might be the mechanism by which Mo is taken up by fish from the environment. Molybdenum is an essential element serving as a cofactor for several enzymes, such as xanthine oxidase, sulphite oxidase, and aldehyde oxidase (Goyer 1991; Galvin 1996). Mammalian uptake of Mo takes place via absorption through the intestinal wall (Goyer 1991). However, there is currently no such model in place for fish.

Dietary Mo toxicity was tested several times during this study by hatching brine shrimp in serial Mo dilutions (unpublished data). A regression equation was generated for Mo concentration in brine shrimp hatching water versus brine shrimp nauplii Mo concentration in order to supply fish with various doses of dietary Mo. However, these tests proved to be unsuccessful owing to Cd contamination of Mo salts used as toxicant, and were consequently not reported. There has been a recent recognition of the importance of dietary metal uptake to fish (Dallinger and Kautzky 1985; Dallinger et al.

1987; Farag et al. 1994; Woodward et al. 1995). However, the metals being investigated are ones that cause toxicity while dissolved as well. Dietary Mo toxicity requires further exploration because of its immediate importance in lakes receiving Mo-contaminated mine effluents, and because it might only be toxic to fish if ingested. If this hypothesis turns out to be true, as far as the author is aware, no other metal is toxic to fish *only* in the case of dietary uptake.

The most sensitive indication of Ni toxicity was fathead minnow egg hatching stimulation at 0.75 mg Ni/L. This concentration of Ni is approximately 7.5 times higher than Ni concentrations observed in Ni-contaminated receiving waters at the Key Lake mine. Acute Ni toxicity occurred at 0.45 mg Ni/L in reconstituted soft water, which is approximately 4.5 times higher than concentrations observed at Key Lake. The MATC derived from soft water, Ni-exposed larval fathead minnows tested in this study is 57 µg/L. This MATC is within the SSWQO for Ni of 25 µg/L in soft water. However, the MATC for larval fathead minnow egg hatching time in hard water is 0.6 mg/L. Hatching time was not determined for fathead minnow eggs in Ni-spiked soft water. Consequently, a MATC to protect fathead minnow eggs against Ni toxicity cannot be calculated empirically. However, a MATC can be estimated by using Ni concentrations measured in Little McDonald Lake water (86.6 µg/L; NOEC) and B-Zone pit water (218.3 µg/L; LOEC) (Chapter 3; Table 3.3). The MATC calculated from these data is 0.14 mg/L. This MATC is higher than the SSWQO for Ni, but approximately equal to concentrations measured in dewatering-effluent receiving waters at Key Lake mine, and lower than Ni concentrations measured in the B-Zone pit water near the Rabbit Lake

mine. Therefore, sensitive larval fish and fish embryos are sufficiently protected by the current SSWQO. However, developing fish may be at risk in waters near northern Saskatchewan uranium operations that have Ni concentrations near the MATC of 0.14 mg Ni/L.

Nickel is less toxic than some metals like Cd, but shows similar toxicity to others like Cu and Zn. For example, Playle et al. (1993) reported 96-h LC50s for fathead minnows exposed to Cd as 6.8 μg/L. This value is significantly lower than LC50s reported in this study for Ni. However, fathead minnows exposed to Cu in hard water (hardness 117-121 mg/L as $CaCO_3$, pH 7.3) gave 96-h LC50s of 2.1-2.3 mg/L, which is close to the 96-h LC50 reported in this study for fathead minnows exposed to Ni in hard water (2.4 mg/L) (Lind et al. 1978). Another study reported acute Cu toxicity to fathead minnows as 800 μg/L in hard water (hardness 200 mg/L as $CaCO_3$, pH 8.3) and 200 μg/L in soft water (hardness 45 mg/L as $CaCO_3$, pH 7.9) (Andrew 1976). These values show copper to be somewhat more toxic than Ni toxicity reported here. Fathead minnows exposed to Zn in very hard water (hardness 362-394 mg/L as $CaCO_3$, pH 7.7-7.9) gave 96-h LC50s of 2.2-2.7 mg/L (Carlson and Roush 1985). Because the water hardness was more than twice that of this study, and the 96-h LC50s were similar to those reported here for Ni, Zn is apparently more toxic to fathead minnows than Ni.

Nickel uptake to fish is probably regulated just as Zn or Cu are regulated owing to their essentiality. Once taken up, Ni can accumulate to high concentrations in tissues like gills and livers without significant toxic effect. Only when these regulatory elements (like MT) become saturated and Ni spills over into an "unregulated" pool does

a toxic effect become manifest. This spillover offers a possible explanation as to why Ni is so much less toxic than other metals to fish. Fish that have a propensity to accumulate high quantities of metal in their tissues typically show a tolerance for that metal. Because Ni is essential, its accumulation is regulated. Nickel is expected to accumulate to high concentrations in fish tissues, which is why it is not as toxic as other, non-essential metals.

7.4 Question 4: How does nickel speciation affect bioavailability and toxicity to fish?

The free-ion activity model (FIAM) suggests that the most toxic form of a metal is its free, bivalent cation (i.e., M^{2+}) (Morel 1983). For most metals, M^{2+} dominates over other metal species at low pH. Consequently, metals tend to be more toxic to fish at low pH than at high pH. However, this model has been recently criticized as being too simplistic for predicting toxicity to fish (Campbell 1995). For example, Erickson et al. (1996) exposed juvenile fathead minnows to copper, and found that total Cu 96-h LC50s increased with pH. On first appearances, this result seems in line with the FIAM. However, when based on cupric ion activity, 96-h LC50s increased and then *decreased* with increasing pH. This result suggests that Cu^{2+} is not the only Cu species causing toxicity to fathead minnows, contrary to the FIAM. The authors concluded that some other Cu species, like $CuOH^{+}$ may be as toxic, or more toxic, than Cu^{2+}.

Several examples now exist demonstrating that the free, bivalent metal cation is not necessarily the most bioavailable, or most toxic, species. Rainbow trout accumulated less Cu, Cd, or Zn at pH 5.0 than at pH 7.0, resulting in decreased toxicity at low pH (McDonald et al. 1991). Zinc, Cd, and Ni were more toxic to fathead

minnows at pH 8.3 than at 6.3 (Schubauer-Berigan et al. 1993). Cusimano et al. (1985) demonstrated a reduction of Cu in fish exposed to Cu^{2+} under low pH conditions. Results from these studies indicate that the FIAM is not sufficient for predicting metal toxicity to fish.

Results from this study provide further evidence that the FIAM is not predictive of Ni toxicity to fish. Larval fathead minnows demonstrated a slight protective effect against Ni toxicity at pH 5.5 relative to pH 7.0. Under pH conditions greater than pH 7.0, Ni toxicity was significantly reduced. This pattern of toxicity indicates one of two things: either $NiCO_3$ enters fish gills directly, or $NiCO_3$ dissociates at the gill surface where microenvironmental conditions are acidic, and Ni^{2+} enters gills. These processes are directly analogous to processes previously described for Cu (e.g., Erickson et al. 1996)—a metal whose chemistry is thought to be similar to Ni as a borderline metal with a strong class B character (Nieboer and Richardson 1980; Nieboer and Fletcher 1996).

Water hardness reduced Ni toxicity by 5-fold over a hardness range of 20-140 mg/L as $CaCO_3$. Protection conferred by water hardness against metal toxicity is well documented. Consequently, results reported in this study were not unexpected. Fish used to examine effects of hardness were generated from a breeding culture of fathead minnows maintained in hard water. Eggs were removed from hard water and were placed in soft reconstituted water. The effect of hard to soft water egg transfer with respect to its influence on Ni toxicity is not known. There is currently some evidence to suggest that transferring fish from high to low hardness, or vice versa, may be a source

of stress to fish (L. Taylor[3], pers. comm., 1999). This lack of complete acclimation may have some influence on toxicity.

Water hardness protects fish against metal toxicity by Ca^{2+} competitively out competing M^{2+} for binding sites at the gill surface. Current water quality criteria (WQC) recognize the protective role of water hardness in reducing metal toxicity. However, other factors (like Cl^- providing protection against Ag^+ toxicity) can also affect metal toxicity but are not considered in development of WQC.

Total suspended solids (TSS) reduced Ni toxicity to fish by removing Ni from the water column. Free Ni^{2+} binds to negative binding sites on particle surfaces, rendering Ni^{2+} unavailable for biological uptake. This TSS-reduction in Ni toxicity is the same mechanism as toxicity reduction due to dissolved organic matter (DOM). Very low concentrations of TSS had little effect on toxicity reduction relative to water with 0 mg TSS/L because at the test pH, Ni^{2+} accounts for only a small proportion of total Ni in the system. The effect of TSS on toxicity of some metal that occurs as a free bivalent cation at the test pH is expected to be greater than for Ni.

The TSS study suggested that there was likely a tradeoff between the protection conferred by TSS by binding dissolved Ni, and physical damage caused by TSS itself. This explanation was based on the shape of the 96-h LC50 vs. TSS curve, which appeared to flatten at high TSS concentrations. However, an alternative explanation can account for the same curve shape. At pH 7.6, Ni speciation is dominated by $NiCO_3$. If Ni^{2+} was the dominant species, the expected curve would be linear. Interactions between $NiCO_3$ and a clay particle results in Ni dissociating from $NiCO_3$ and displacing

two H^+ from the clay surface, forming $HCO_3^-+H^+$, H_2CO_3, and Ni-clay complex. This reaction would require more energy to complete than simple binding of Ni^{2+} to clay because of the extra energy required dissociating $NiCO_3$ initially. As TSS increases, so too does $NiCO_3$ dissociation followed by Ni binding on clay particles in a probabilistic manner. Consequently, when TSS reaches very high concentrations (e.g., 100 mg TSS/L) the curve flattens out as most Ni is stripped from the system.

The effects of pH, hardness, and TSS on Ni toxicity to fish demonstrate three distinct mechanisms that reduce toxicity. Changes in pH result in shifts of dominant Ni species, from Ni^{2+} at low pH to $NiCO_3$ at neutral pH, to $Ni(CO_3)_2^{2-}$ at high pH. Hardness represents competitive exchange between Ca^{2+} and Ni^{2+} at gill surfaces. Finally, TSS strips Ni from the water column rendering it unavailable for biological uptake. It might be interesting now to examine how these effects interact with one another to affect toxicity. For example, at low pH, when the dominant species is Ni^{2+}, TSS would be expected as a much more effective toxicity-reducing factor than at pH concentrations where some carbonate Ni species dominates.

7.5 Question 5: Can metallothionein be induced in fish exposed to metals such as nickel or molybdenum—i.e., metals other than those of Groups IB or IIB of the Periodic Table?

Results from this study indicated a clear induction of metallothionein (MT) in gills of juvenile rainbow trout exposed to Ni, but not in livers. Results from Mo exposure were questionable owing to Cd contamination of the Mo salt used as toxicant. That Ni induced MT in rainbow trout gills was an expected result for several reasons. First, Ni is a borderline metal with a strong class B character (Nieboer and Richardson

1980; Nieboer and Fletcher 1996). This strong class B character means that although Ni can form both ionic and covalent bonds with donor ligands, it has a stronger propensity to form covalent bonds with sulphur ligands than ionic bonds with oxygen ligands (Nieboer and Richardson 1980; Nieboer and Fletcher 1996). Metals bind to MT at cysteine residues, and cysteine is a sulphur-containing amino group. As the pre-exposure MT pool becomes saturated with Ni, more MT is produced through a negative feedback system (Roesijadi 1992).

Metallothionein induction was observed in gills and not livers because of the short duration of the exposure (i.e., 7 d). Dissolved Ni taken up by the gills directly from the water probably caused MT induction in gills. If MT were to have been observed in the liver, Ni would need to be mobilized from the gills into the blood, and subsequently transferred to the liver. Once in the liver, MT induction would be expected. Kidneys could not be tested for MT in this study due to the small size of fish used. However, given a sufficient exposure period, MT induction in kidney is also expected.

Gill MT induction demonstrated in this study was not correlated with dissolved Ni. Metallothionein has been demonstrated several times as a useful biomarker for metal-exposed fish. However, there is considerably less evidence that MT induction on its own is a good biomarker for metal effects. Several studies have shown, in fact, that MT induction is not always related to biological effects (Hamilton and Mehrle 1986).

7.6 Future Directions

A major, long-standing question that remains unanswered upon completion of this study is how to relate laboratory-derived toxicity results to a natural system. As discussed above, there is no simple answer to this question, and any potential solution would have to make compromises among laboratory, field, and statistical considerations. In other words, no answer would be *perfect*. However, based on the several—often-unsuccessful—trials conducted in this study, the following *partial* solution is proposed.

The most realistic way to test the toxicity of receiving water would be to set up an exposure system that partially represented field conditions and partially represented laboratory conditions. In this way, a reasonably true measure of toxicity can be established by allowing certain environmental parameters to fluctuate naturally (e.g., pH, metal concentration) while controlling others (e.g., physical perturbation), and simultaneously providing a statistically sound framework from which to test hypotheses. This design would be more difficult, and would require more resources, to conduct than simple laboratory or *in situ* tests. However, the extra effort would be rewarded with a close approximation of receiving water toxicity—something that is not being achieved using current testing protocols.

In order to test the toxicity of remote receiving waters, some sort of laboratory facility should be developed at the remote location. This facility should be able to hold and even culture, test fish. By developing a fish-holding/culturing facility on site, toxicity variance associated with transportation stress would be greatly reduced.

The testing facility should be constructed in such a way as to maximize similarity to field conditions with respect to photoperiod and water quality. Temperature should be allowed to fluctuate on a diurnal cycle, but should be held to within a few degrees of average lake temperature. This temperature fluctuation in the laboratory would allow for a more realistic exposure system because temperature fluctuations occur in nature. By holding the temperature within a few degrees of average lake temperature, the system represents a fish migration to suitable thermal conditions.

Sample water should be collected daily and placed into a head tank where it can be subsequently delivered to test replicates. Water delivered to test replicates should be allowed to flow through in order to minimize metabolic waste build up which could affect fish sensitivity to contaminants. Daily water sample collection and delivery in this way would replicate daily fluctuations in water chemistry. Ideally, water from each test site should be pumped directly into exposure chambers. However, this is probably not a practical or economically feasible approach. Water that is delivered to test chambers should not be filtered to preserve natural metal speciation, or eliminate potential food items. This approach is better than the current approach in that test water chemistry should match ambient water chemistry quite closely, and potentially contaminated food items would still be present exerting their effect on test fish.

By conducting this test in a laboratory setting, fish do not need to be transported to the various exposure sites, further reducing the influence of transportation stress. Tests conducted on a lab bench can be randomized such that experimental replicates can

be interspersed within space and time (Hurlbert 1984), which cannot be done in the field. Consequently, inferential statistics can be applied reliably on test results.

Test fish should be acclimated to reference water and derived from natural stock in order to represent both indigenous species and natural tolerance or sensitivity to contaminants. Fish species should be chosen based on their ecological importance, not their economic importance. Testing should not be restricted to a single species, but should include as many indigenous species as possible. Fish that are tolerant to one contaminant might be exceptionally sensitive to another (Cairns 1986).

This type of study design has several advantages over current designs. Water quality is kept relatively similar to that of study water because it is not treated in any way and it is renewed daily from water coming directly from the source. Current testing protocols require refrigeration of water to be used in toxicity tests, and that tests begin within 72 h of water collection. Refrigerating water samples changes water chemistry (e.g., increases dissolved oxygen, decreases pH) which may lead to changes in contaminant speciation. Speciation changes may lead to a toxicological response in the laboratory that cannot be repeated in the field. Whole water samples also provide test fish with food that they would encounter in nature. Dietary metals are gaining recognition among the scientific community as providing an important contribution towards metal toxicity to fish.

The practice of conducting single-metal toxicity tests in the laboratory and attempting to extrapolate those results to the field should be reevaluated. As discussed, the FIAM was an initial attempt to link laboratory results to field situations. However,

natural systems are highly complex and variable. Simple extrapolation from laboratory results is not sufficient to predict toxicity in the field. Placing fish in contaminated lakes and comparing toxicity results with those derived from laboratory tests of field collected water is the simplest way to determine if laboratory results relate to the field. Once it can be shown that a positive relationship exists, testing protocol can give way to experimentation by spiking water with various contaminants.

It is important to understand how individual metals behave under various water quality conditions, and how this behaviour relates to toxicity. As time passes, more and more studies provide results that the FIAM is not sufficient to predict metal toxicity to fish. Similarly, new water quality parameters are being identified that reduce toxicity (e.g., Cl^- reduces Ag^+ toxicity). Most research efforts until now have focused on metal behaviour in the water, followed by an attempt to relate that behaviour to toxicity. That approach left a wide gap between the chemistry and biology of the system. More research is needed to bridge the gap between chemistry and biology by considering the fish itself as an integral part of the exposure system. The U.S. EPA has recently begun evaluating the biotic ligand model[4] (BLM) which represents a first attempt at resolving this issue. Much more work is required.

Dietary Se was identified as the contaminant most likely to have caused larval fathead minnow mortality in *in situ* tests of Key Lake mill receiving waters. Although laboratory evidence suggests that dietary Mo is relatively non-toxic to fish, there are no data testing its dietary toxicity. Given the high concentrations of Mo measured in Fox and Unknown lakes at the Key Lake uranium-mining operation, it is important to rule

out dietary Mo uptake as a potential source of toxicity to fish. If Mo turns out to be toxic when ingested by fish, this form of metal toxicity, where only the dietary form is toxic, could be the first known to science. Mercury, Pb, and Se are more toxic to fish when taken up by diet relative to aqueous uptake. However, each are still toxic to fish in their dissolved form. Attempts were made during this study to examine the effects of dietary Mo on larval fathead minnow survival and growth. However, these tests were unsuccessful. Therefore, important and interesting research is required to determine if dietary Mo is toxic to fish.

Embryos exposed to metals typically show a hatching delay. Only in the case of a couple of metals, like Hg, is hatching stimulated. Results from this study demonstrated a repeatable hatching stimulation of Ni-exposed fathead minnow eggs. It would be interesting to determine why some metals cause delays whereas others cause stimulation in hatching. It has been postulated that hatching delay may serve as a protective function. The longer that a fish can stay inside the protective egg case the better chances it has of surviving a metal-contaminated environment. Therefore, Ni-induced hatching stimulation may be an ecologically important issue to pursue.

Water quality criteria for metals continues to focus on single metals existing in (or being released to) aquatic systems. This approach has been criticized because fish are rarely exposed to single metals in an aquatic system. Moreover, dietary metals as a source of toxicity are not considered in current WQC. Therefore, studies attempting to extrapolate single-contaminant responses to the field should be abandoned in favour of a more holistic approach as discussed. Furthermore, multivariate statistical techniques

should be developed to accompany the new testing design (Maund et al. 1999; Sparks et al. 1999).

Multivariate statistics seem particularly well placed as a descriptive tool for complex, polluted waters. They can be used as a data-reduction technique that considers all environmental data simultaneously, to identify those responsible for the observed effect. Most multivariate statistics available to biologists were developed by taxonomists and community-structure analysts. Only recently have environmental toxicologists begun using multivariate techniques (Maund et al. 1999; Sparks et al. 1999). These methods should be further developed and implemented by environmental biologists. Sound statistical methods appropriate for complex natural systems are required for the field of aquatic toxicology to fully mature as a scientific discipline (Newman 1995).

Results from this study show that Ni is a potentially toxic metal to fish. However, there is currently very little data concerning the mechanism of Ni toxicity. Nothing is known about the mechanism of Ni uptake from water to gills. Data reported here suggest that $NiCO_3$ may be taken up by gills directly, or it may dissociate in the acidic gill microenvironment causing Ni^{2+} to bind to gills. Direct uptake of $NiCO_3$ would be analogous to direct $CuOH^+$ uptake by gills (Erickson et al. 1996). In addition, results reported here showed a lack of MT induction in fish livers after 168 h of Ni exposure. As an essential element, Ni may be very highly regulated in fish leading to rapid depuration. Alternatively, Ni may have been sequestered in gills by Ni-binding proteins that did not allow it to pass into the serosa for subsequent deposition into liver.

Whatever the case, little work has been done to examine the physiological basis of Ni toxicity to fish. In order to bring current understanding of Ni toxicity in line with other metals, like Cu and Cd, basic physiological research into the mechanism of Ni toxicity is required.

7.7 References

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Notes

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4 The biotic ligand model is currently under public review by the U.S. Environmental Protection Agency (U.S. EPA). Consequently, only a draft document is available and cannot be cited here. The draft document, entitled, "Review of the Biotic Ligand Model of the Acute Toxicity to Metals," can be obtained from the U.S. EPA, or from their Internet web site at http://www.epa.gov/science1/epecblm.pdf.

8. Overall Conclusions

Nickel and Mo toxicity to fish is an important consideration for the mining industry and regulatory bodies. Yet, research concerning Ni and Mo toxicity is lacking compared to other metals like Ag^{+}, Cd^{2+}, and Zn^{2+}. This lack of research is especially true for Mo where toxicity literature is exceptionally scarce for fish. Much of the toxicology literature concerning Mo centres around a Mo-induced Cu deficiency called molybdenosis or teart, which is commonly observed in ruminants grazing on Mo-rich soil. Literature concerning Ni toxicity to fish is largely restricted to older literature conducted during a time before issues like metal speciation, or water chemistry were considered in terms of their potential influence on toxicity (Renner 1997). Given that both Ni and Mo are detected in abiotic and biotic compartments around northern Saskatchewan uranium mines, that Ni is potentially toxic to fish, and that Canada is the world's largest Ni producer, more research on the toxicity of these two metals is required.

Nickel is apparently not as toxic as other metals, like Cd or Cu. The 96-h LC50 for Ni to larval fathead minnows is 2.4 mg/L in hard water, and 0.45 mg/L in soft water. Cadmium has no known biological function and is known to block Ca^{2+} uptake to fish. The 96-h LC50 for Cd is approximately 1-28 µg/L (Playle et al. 1993). Copper, like Ni, is an essential element at trace concentrations, but toxic to fish at higher concentrations. The 96-h LC50 for Cu is 1-25 mg/L (Playle et al. 1993).

The difference between Cd, Cu, and Ni toxicity is that Cd and Cu can cause acute toxicity to fish at concentrations that can potentially occur in metal-polluted water

bodies. Acute Ni toxicity requires Ni concentrations that exceed those that occur around the Key Lake uranium mine in northern Saskatchewan. However, other effects, like egg hatching stimulation, occur at much lower concentrations and may have ecological importance for fish inhabiting mine-receiving waters. Nickel concentrations that occur now in lakes receiving mine dewatering effluent are sufficiently elevated to induce early fathead minnow egg hatching, which can have significant ecological relevance.

Molybdenum did not cause any significant effects to fish under laboratory conditions. Field data suggest that dietary Se may be one of the most important toxicants to account for fish mortality in mill receiving waters. However, dietary Mo as a potential source of toxicity in Mo-contaminated waters cannot be ruled out in the absence of contradictory evidence. Tests examining the effects of dietary Mo failed during this study owing to Cd contamination of Mo toxicant. The hypothesis that Mo is toxic to fish when ingested should be pursued. If it turns out to be correct, as far as the author is aware, it would be the only metal demonstrating this etiology.

Nickel toxicity is generally reduced by increasing hardness, pH, and TSS. The ameliorative effect of hardness is probably due to competitive inhibition of Ni^{2+} binding to gills by Ca^{2+}, as described for other metals. Nickel was not more toxic at low pH relative to neutral pH, contrary to observations made on fish exposed to other metals like Cd and Cu. High pH had a strong protective effect against Ni toxicity. Geochemical equilibrium calculations suggested that at pH 7, the dominant Ni species is $NiCO_3$. Either $NiCO_3$ enters through gills directly and is more toxic to fish than Ni^{2+}, or $NiCO_3$ dissociates at the gill surface causing Ni^{2+} to bind to gills as the first stage of

toxicity. Higher pH (>8) causes a shift in Ni speciation such that the most dominant Ni species is an anion. The predominantly negative gill surface effectively repels the Ni-anion, which gives protection to Ni-exposed fish. Total suspended solids, in the form of clay, provides protection to fish by stripping Ni^{2+} from the water column through competitive exchange with H^+ at the clay surface. Because the dominant Ni species is $NiCO_3$ at circumneutral pH, and not Ni^{2+}, TSS concentrations must be high in order to provide significant protection against Ni toxicity. High TSS concentrations provide more substrate upon which dissociation of $NiCO_3$ and subsequent displacement of H^+ with Ni^{2+} on clay surfaces can occur.

Nickel caused MT induction in rainbow trout gills, but not livers. This lack of MT induction in rainbow trout livers suggests that Ni was sequestered in the gills. Because Ni is an essential nutrient, it may be highly regulated by MT or some other Ni-binding protein in gills in order to maintain homeostasis. Lack of correlation between aqueous Ni and gill MT suggests that MT induction is associated with Ni regulation independently of aqueous Ni concentration.

Protection against metal toxicity of fish in metal-contaminated receiving waters depends on sound toxicological data. Laboratory toxicity results do not relate to toxicity observed in the field. Many factors, such as physical stress, water chemistry, temperature, and feeding status of test organisms, vary between the laboratory and field. Consequently, it is extremely difficult for laboratory and field tests to yield the same results. Furthermore, standard toxicity-test fish species, such as rainbow trout, were more tolerant to Ni than species inhabiting contaminated water bodies near northern

Saskatchewan uranium operations. Consequently, decisions based on laboratory toxicity tests using rainbow trout may put sensitive indigenous fish species at risk.

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