SELENIUM ACCUMULATION AND EFFECTS IN AQUATIC ORGANISMS DOWNSTREAM OF URANIUM MINING AND MILLING OPERATIONS IN NORTHERN SASKATCHEWAN

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By

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ABSTRACT

The overall objective of this thesis was to determine selenium (Se) levels in the major compartments of aquatic ecosystems and correlate these data with potential Se effects on early life stages of two native fish species. This work was conducted at two uranium (U) mines located in northern Saskatchewan, Key Lake mine and McClean Lake mine. In addition, a site fidelity study was conducted at Key Lake mine to evaluate movement patterns of northern pike inhabiting lakes receiving effluent discharges.

At Key Lake mine, Se was accumulated and biomagnified several orders of magnitude higher than its concentration in water (0.7-2.7 µg/L) in lakes receiving discharges, with Se in prey organisms reaching levels above the proposed 3-11 µg/g dry weight (DW) dietary toxicity threshold for fish. Increased concentrations of Se in aquatic biota led to an increase in the Se concentrations in eggs and tissues of northern pike that rely on these organisms as food sources. Furthermore, increases in the incidence of Se-induced deformities above 30% were recorded in fry originating from adults exposed to high levels of dietary Se (> 3µg/g, DW). The increased frequency of deformities found in northern pike fry was associated with a significant increase in the level of Se in northern pike eggs from exposure sites (31.28 - 48.23 µg/g DW) compared to reference (3.19 ± 0.29 µg/g DW).

At McClean Lake mine, Se was accumulated and biomagnified through the aquatic food chain with concentrations in some biota groups (e.g., forage fish) exceeding the lower limit (> 3µg/g DW) of the 3-11µg/g (DW) threshold for dietary Se toxicity in fish. Although both northern pike and white sucker females collected from the exposure site showed greater levels of Se in egg and tissues compared to fish collected from a reference site

(likely caused by exposure to elevated levels of Se in prey organisms), no increases in Se-induced deformities were found in the developing fish larvae. The lack of a toxic response in fish larvae is in agreement with Se thresholds for early life stage deformities, with egg Se concentrations in northern pike and white sucker collected at the exposure site below the proposed 10 μg/g (DW) threshold associated with the presence of developmental abnormalities.

The applicability of the proposed 7.91 μg/g (whole body, DW) Se toxicity threshold to cold water fish is controversial given that most of the research has focused on warm water fish. Therefore, there is an urgent need to conduct studies that allow us to better understand the environmental fate and effects of Se in north temperate (cold water) aquatic systems. The results of my research will contribute valuable information for the establishment of a realistic and environmentally relevant Se threshold for the protection of fish populations in Canadian waters.

During the site fidelity study, fish locations were seasonally and daily recorded using a Lotek SRX_400 receiver with handheld Yagi antenna. The results suggest that tagged pike did not migrate out of the study area throughout the study period, with the mean distance traveled ranging from 50 to 400 m. Differences in movement (distance traveled) and home range were found between reference and exposure sites. Overall data suggest that radio-telemetry is a useful tool in environmental studies. This information on northern pike behavior will be valuable towards developing non-lethal sampling methods that could be applied for assessing the effects of industrial discharges in north temperate aquatic ecosystems.

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In memory of my mother;
forever present, forever missed, forever loved

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LIST OF ABBREVIATIONS

µg/g = micrograms per gram

µS/cm = microsiemens per centimetre

µg/L = micrograms per litre

µm = micrometer

< DL = below detection limit

Ag = silver

Al = aluminum

ANCOVA = analysis of covariance

ANOVA = analysis of variance

As = arsenic

B = boron

Ba = barium

Be = beryllium

Bi = bismuth

$CaCO_3$ = calcium carbonate

CCME = Canadian Council of Ministers of the Environment

Cd = cadmium

CF = condition factor

CH_4Se = methylselenol

$(CH_3)_2Se$ = dimethylselenide

cm = centimetre

Co = cobalt

Cr = chromium

Cu = copper

d = day

DO = dissolved oxygen

DW = dry weight

EC01 = median effect concentration, 1% above background

EC = median effect concentration

Fe = iron

g = gram

Ge = germanium

GPS = geographic positioning system

GPx = glutathione peroxidase

GSH = reduced glutathione

GSI = gonadosomatic index

GSSeH = seleno-persulfide

h = hour

HDPE = high density polyethylene

Hg = mercury

HG-AAS = hydride generation atomic absorption spectrometry

H_2O_2 = hydrogen peroxide

HSI = hepatosomatic index

H_2SeO_4 = selenic acid

H_2SeO_3 = selenious acid

ICP-AES = inductively coupled plasma-atomic emission spectrometry

ICP-MS = inductively coupled plasma-mass spectrometry

ICP-OES = inductively coupled plasma-optical emission spectrometry

K = potassium

kg = kilogram

km = kilometres

km^2 = square kilometres

L = litre

LC50 = median lethal concentration

Log_{10} = logarithm in base 10

m = meter

m^2 = square meters

m^3/d = cubic meters per day

MDPH = minimum displacement per hour

MHz = megahertz

Mg = magnesium

mg = milligram

mg/kg = milligrams per kilogram

mg/L = milligrams per liter

mL = milliliter

mm = millimeter

Mn = manganese

Mo = molybdenum

MS-222 = tricaine methanesulfonate

n = number of samples

N/A = not-available

$NaSeO_3$ = sodium selenite

$NaSeO_4$ = sodium selenate

ng/g =nanogram per gram

ng/L = nanogram per litre

NH_3 = un-ionized ammonia

NH_4^+ = ionized ammonia

Ni = nickel

NOEC = no observed effect concentration

O_2 = oxygen

ºC = degrees centigrade

P = phosphorus

Pb = lead

Po = polonium

Ra = radium

RM-ANOVA = repeated measures ANOVA

S = sulphur

Sb = antimony

Se = selenium

Se-Cys = seleno-cysteine

Se-Met = seleno-methionine

$Se_2O_5^{2-}$ = diselenite

SE = standard error

Sn = tin

Sr = strontium

TDS = total dissolved solids

Tl = thallium

USEPA = United States Environmental Protection Agency

U = uranium

V = vanadium

WW = wet weight

Zn = zinc

CHAPTER 1

General introduction

1.1. Properties and sources of selenium

Selenium (Se) is an essential element with an atomic weight and number of 78.96 and 34, respectively. It occurs in the Periodic Table of Elements in group VIA along with other elements such as sulphur (S). The similar electronic configuration and chemical properties shared between Se and S accounts for many of the interactions between these two elements (Reilly, 2006). Selenium has six stable isotopes with varying degrees of abundance (e.g., ^{74}Se (0.87%) and ^{80}Se (49.82%)) and short-lived isotopes (e.g., ^{75}Se) commonly used in radiology (USEPA, 2004; Reilly, 2006). Selenium is widely distributed throughout the environment and is found in most soils and natural waters at concentrations between 0.01-2 mg/kg and 0.1-0.4 μg/L, respectively (USEPA, 2004; Mayland, 1994). However, higher Se concentrations have been reported to occur in soil (> 38 mg/kg) and waters (> 10 μg/L) of some distinctive areas (e.g., Elk River Valley, BC, Canada; South Dakota, USA) (Kennedy *et al*., 2000; Mayland, 1994).

In North America, significant amounts of Se together with other trace elements such as arsenic (As), boron (B), nickel (Ni), uranium (U) and vanadium (V) can be found in rocks and soils formed during the Carboniferous to Quaternary periods (e.g., Northern Canada) (de Carle, 1986; Wallis *et al*., 1986; Stephens and Waddell, 1998). Natural sources of Se include black shales, phosphate and igneous rocks, limestone and sulphide minerals (e.g., pyrite) (Haygarth, 1994; ATSDR, 2003). In the early 1980s it was recognized that anthropogenic activities had the potential to increase the concentrations of Se found in aquatic environments (Lemly, 1993a). Major anthropogenic sources of Se include metal, phosphate and coal mining operations, sewage treatment plants, agricultural and

horticultural activities, base metal smelting and refining factories, and end-product manufacturers (e.g., semiconductor manufacturers) (ATSDR, 2003; Reilly, 2006).

1.2. Uranium mining and milling operations in northern Saskatchewan

1.2.1. Past, present and future of uranium mines in Saskatchewan

Mining of U in Saskatchewan began in the Beaverlodge district on the north shore of Lake Athabasca in 1962. The larger mines, Eldorado, Gunnar and Lorado, built mills to process their ore and to custom mill ore for the smaller producers. By 1964, falling U prices and the depletion of existing ore bodies left Eldorado as the sole producer in the Beaverlodge area where it continued production until June 1982. Exploration for U continued in Saskatchewan in the intervening years, primarily in the Athabasca sand region, leading to development of open-pit mining at Rabbit Lake in the Wollaston area in 1975 and at Cluff Lake in 1979 (Swanson, 1997).

Today, there are 5 fully operating U mines and mills in northern Saskatchewan: Rabbit Lake, Key Lake, Cigar Lake and McArthur River mine operated by Cameco Corporation and McClean Lake mine operated by AREVA Resources Canada Inc. Other major ore bodies have been discovered at South MacMahon Lake (Midwest project), Collins Bay and Keefe Lake in the Wollaston area and at Maurice Bay on Lake Athabasca. These discoveries are either in the early stages of development, or awaiting development under more favourable economic conditions (e.g., Midwest project, Kiggavik and Sissons exploration in Nunavut). In addition, there are decommissioned (e.g., Cluff Lake mine

owned by AREVA Resources Canada Inc.) or abandoned mines in this region (CEPA, 2001; World Nuclear Association, 2003).

1.2.2. Selenium in aquatic systems receiving mine effluent discharges

Aquatic ecosystems are often the major systems affected by the release of effluents from U mines and mills. Effluents from these mines generally contain elevated levels of trace elements (e.g., As, lead (Pb), cadmium (Cd), molybdenum (Mo), Ni, Se, and U), ions (e.g., sulphate) and radionuclides (e.g., radium-226 (^{226}Ra), lead-210 (^{210}Pb), thallium-228 (^{228}Tl), and polonium-210 (^{210}Po)) (Burns and Finch, 1999). Elevated concentrations of Se have been reported in water, sediments and fish tissues downstream of U mining and milling operations, as a consequence of U ore extractions, dewatering and milling (Klaverkamp *et al*., 2002). In northern Saskatchewan, the background Se concentration in water and sediment ranges from 0.1 to 0.3 μg/L and 0.20 to 0.50 μg Se/g dry weight (DW), respectively. Also, the background Se concentration in fish tissues ranges from 0.5 to 0.75 μg/g DW (converted from 0.2 to 0.3 μg/g wet weight (WW) using 60% moisture) for bone and 1 to 2 μg/g DW (converted from 0.2 to 0.4 μg/g WW using 80% moisture) for muscle (Golder Associates, 2002). However, downstream of U mines and mills the Se concentration in water has been reported to range from 0.1 to 5.88 μg/L (Pyle *et al.*, 2001) and the Se concentration in sediment is often more than 34 μg Se/g DW (Golder Associates, 2002). Not surprisingly, the Se concentration in muscle and bone of fish downstream of U mines and mills is also elevated ranging from 15 to 25 μg/g DW

(converted from 3 to 5 μg/g WW, using 80% moisture) and 15 to 27.5 μg/g DW (converted from 6 to 11 μg/g WW, using 60% moisture), respectively (Golder Associates, 2002).

1.3. Selenium in the aquatic environment

1.3.1. Chemical speciation, transport and partitioning

Selenium is stable in four valence states: selenides (-II), elemental selenium (0), selenites (IV) and selenates (VI). In general, the more soluble and mobile forms of Se (IV and VI) dominate under aerobic and alkaline conditions such as natural waters, while Se (-II) and elemental Se (0) are not soluble in water and precipitate to sediments. In addition, organic forms of Se (organo-selenides) such as seleno-methionine (Se-Met) and seleno-cysteine (Se-Cys), and volatile forms (e.g., dimethylselenide ($(CH_3)_2Se$), are present in natural waters (ATSDR, 2003; USEPA, 2004; Simmons and Wallschläger, 2005). For selenites in solution, equilibria are set up between selenious acid salts (H_2SeO_3, $HSeO_3^-$, SeO_3^{2-}, $HSe_2O_5^-$) and diselenite ($Se_2O_5^{2-}$), whereas dissolved selenate would occur predominantly as selenic acid and its salts (H_2SeO_4, $HSeO_4^-$, SeO_4^{2-}). Selenates can complex with sodium to become extremely mobile in the aquatic environment. Water quality variables such as pH, and other factors (e.g., metals), can affect the partitioning of the various compounds of Se in the aquatic environment (see section 1.4.4; ATSDR, 2003; USEPA, 2004; Simmons and Wallschläger, 2005).

Selenium can be removed from the water-column and deposited in the sediments by chemical (reduction of selenites and selenates to elemental Se), adsorption (e.g., clay, organic carbon, humic substances), complexation (e.g., other metals such as Cd) and co-

precipitation processes (e.g., ferric oxides) (Lemly, 1999; Simmons and Wallschläger, 2005). In addition, most of the Se taken up by aquatic organisms is eventually deposited into the sediments as detritus (Lemly, 1999). Removal of Se from the water column into the sediments is a very efficient process especially in slow-moving water systems such as lakes (Lemly, 1999). Under reducing conditions frequently found in most sediments, Se of different oxidation states is rapidly reduced to selenides and/or elemental Se, with the latter emerging as the dominant fraction (Zawislanski and McGrath, 1998; USEPA, 2004). From the sediments Se can be re-cycled and available for biological uptake by three processes: oxidation of the sediments (e.g., by bioturbation, currents), biotransformation by microorganisms (e.g., oxidation of elemental Se to selenite and/or selenate), and/or uptake by plants or aquatic organisms; the latter being of great importance for the mobilization of selenium into food chains (Lemly, 1999). Some of the Se found in water and sediment can also be converted to volatile forms (e.g., hydrogen selenide (H_2Se), dimethylselenide) by microorganisms (e.g., *Aeromonas*) and released into the atmosphere (Lemly, 1997a; Simmons and Wallschläger, 2005). However, volatilization losses of Se are small and probably of minor significance in the removal of Se from natural waters (Bowie *et al.*, 1996).

1.3.2. Uptake, bioaccumulation and biomagnification

The most important implication of elevated environmental Se is its propensity to accumulate in the aquatic food chain. Thus, even at low water concentrations (< 10 μg/L) Se can be accumulated by aquatic organisms (mainly as Se-Met) 100 to 30,000 times more

than its concentration in water, reaching levels that have the potential to impair fish reproduction (Lemly, 1999). Selenium released into the aquatic environment is effectively taken up and highly assimilated by planktonic organisms (phytoplankton; bacteria and fungi) (Riedel *et al.*, 1991; Besser *et al*., 1993). Selenite appears to be absorbed into these organisms by passive uptake (Ogle *et al*., 1988; Riedel *et al*., 1996) and is the main form of Se available to most algae and bacteria (Simmons and Wallschläger, 2005). Selenate and sulphate are actively transported across cell membranes by a common carrier and/or permease, which may explain the competition for uptake observed between these elements (Ogle *et al*., 1988; Williams *et al*., 1994). Organic forms of Se are taken up by algae 1,000 times more readily than inorganic forms (Amweg *et al.*, 2003) and bacteria have been reported to incorporate Se into amino acids very rapidly (< 10 min.; Ogle *et al*., 1988). In addition, selenates and selenites can be adsorbed to organic compounds on the surface of planktonic organisms (Wang and Dei; 2001). The different forms of dissolved inorganic Se can be accumulated and transformed to organo-selenium by planktonic organisms, significantly contributing to the sedimentation (e.g., by detrital processes) and incorporation of Se into the aquatic food chain (Bowie *et al*., 1996). Furthermore, it has been concluded that phytoplankton and bacteria are primarily responsible for introducing organic forms of Se, mainly Se-Met, into the aquatic food chain (Bowie *et al*., 1996).

Uptake of selenium by aquatic organisms can occur through water or diet. However, dietary uptake (primarily as Se-Met) is usually the dominant pathway of Se accumulation in zooplankton, invertebrates and fish (Lemly, 1993a; Bowie *et al*., 1996; Hamilton, 2004; Stewart *et al*., 2004). Although zooplankton can highly concentrate inorganic and organic Se directly from water, formation of Se-Met in this group requires the ingestion of algae

(i.e., accumulation of Se-Met by ingesting algae) (Saiki and Lowe, 1987). Vertebrates and invertebrates cannot synthesize Se-Met and therefore rely upon lower trophic levels (e.g., fungi, bacteria, phytoplankton) as sources for this amino acid (Alaimo *et al*., 1994). Consequently, any changes in Se uptake by planktonic organisms will have major implications in the Se accumulated by higher trophic levels (e.g., invertebrates, fish) (Bowie *et al.*, 1996; Lemly, 1999).

Sediments are an important repository of Se in aquatic systems. Selenium precipitated into sediments generally by detrital processes (e.g., deposition of phytoplankton, zooplankton and bacteria as main sources of detrital material) (Oremland *et al*., 1990; Bender *et al*., 1991; Graham *et al*., 1992; Bowie *et al*., 1996) can lead to elevated concentrations of Se in sediments and benthic invertebrates (Lemly, 1993a; Saiki *et al*., 1993). Benthic invertebrates can process sediments to obtain food or build their cases and consequently internally sequester (e.g., metal granules) and accumulate elevated levels of Se (2 to 56 µg/g DW) in contaminated environments with no evidence of reductions in abundance or alterations in invertebrate community structure (Sappington, 2002; Lemly, 1993a; Bowie *et al*., 1996; Lemly, 1997a). Therefore, benthic invertebrates can deliver high concentrations of Se (mainly Se-Met) as prey of higher trophic levels (e.g., fish) (Lemly, 1985a; Lemly, 1999; Andrahennadi *et al.*, 2007).

There are limited data regarding the mechanisms of Se uptake in fish. However, Kleinow and Brooks (1986a,b) reported that fathead minnows (*Pimephales promelas*) accumulated more selenite and Se-Met than selenate, even though selenate was more efficiently absorbed in the gastrointestinal tract in a study evaluating the uptake and accumulation of different Se forms. Selenium uptake studies in vertebrates other than fish

(e.g., mice) reported that methionine competes with Se-Met for uptake and selenite was absorbed by passive diffusion in the gastrointestinal tract (Daniels, 1996; ATSDR, 2003). Additionally, selenate appeared to share the same absorption pathway (sodium-mediated carrier) than sulfate (Daniels, 1996; Reilly, 2006). Selenium accumulates in fish tissues primarily in the liver (principal organ of Se metabolism and assimilation) and kidney, followed by gonad and muscle tissue (Ogle *et al*., 1988; Fan *et al*., 2002). The primary excretion route for Se in fish is the urine in addition to Se removal by gills and via the bilary route (Kleinow and Brooks, 1986b). The elimination patterns of selenite and selenate are similar, suggesting a common metabolic pool, and the half-lives of both selenate and selenite are shorter than for Se-Met (Kleinow and Brooks, 1986a).

Biomagnification of Se (defined as increased Se concentrations by successive trophic levels; Lemly, 1997a) has been reported to occur in aquatic environments. Cherry and Guthrie (1977) reported Se biomagnification values ranging from 1.5 to approximately 4 between aquatic plants, invertebrates and fish in a study investigating the toxicity and accumulation of several trace elements in a coal ash disposal drainage in the Savannah River basin, South Carolina, USA. Lemly (1985a) reported biomagnification values ranging from 2 to 6 between plankton, invertebrates and fish in a study evaluating the accumulation of Se in a power plant cooling reservoir (Belews Lake). The general pattern of Se accumulation reported by Lemly (1985a; from smallest to largest) was as follows: periphyton < plankton < invertebrates< forage fish < predatory fish. Conversely, some authors argued that conclusive evidence of Se biomagnification is lacking. Bertram and Brooks (1986) concluded that fathead minnow does not accumulate Se levels greater than their prey after 11 weeks of exposure under laboratory conditions. Saiki *et al.* (1993)

reported no increases in the Se concentrations between invertebrates and fish in a study evaluating the trophic transfer of Se in the San Joaquin River, California, USA. The discrepancies in Se biomagnification between these studies could possibly be due to differences in Se accumulation rates, compartmentalization, and elimination between species, an area that still requires further investigation.

1.3.3. Regulations

In 1980, the U. S Environmental Protection Agency (USEPA) established a Se water quality threshold for the protection of fish populations of 35 µg/L (USEPA, 1980). Lemly (1985a) reported increases in fish mortality and Se-induced deformities (spinal, craniofacial, and fin deformities) and edema in a study evaluating the accumulation and effects of Se in a cooling reservoir (Belews Lake) from a coal-fired power plant in North Carolina. The effects observed by Lemly (1985a) in fish inhabiting Belews Lake occurred even though water concentrations (~ 10 µg/L) did not exceed the 35 µg/L proposed threshold for Se. The decision for later reducing the chronic selenium water threshold to 5 µg/L was largely based on the reported effects of Se on fish population in Belews Lake (USEPA, 1987). In Canada, the threshold for waterborne Se was established at 1 µg/L for the protection of fish (Canadian Council of Ministers of the Environment (CCME), 2003).

Dietary uptake (primarily organo-Se) is usually the dominant pathway of Se exposure in fish (Lemly, 1993a; Hamilton, 2004; Stewart *et al*., 2004) and therefore, the use of a tissue-based criterion for Se is the most logical approach. The USEPA proposed a Se chronic criterion for the protection of fish populations at a whole body fish concentration of 7.91

μg/g DW based on the warm water centrarchid, bluegill sunfish (*Lepomis macrochirus*; USEPA, 2004). The tissue-based approach has the advantage of combining several factors (e.g., exposure duration, chemical forms of Se, and metabolic pathways) into an endpoint (Se tissue level) that can be related to adverse effects occurring in aquatic organisms; particularly fish (USEPA, 2004). However, there are some uncertainties and controversies that require further investigation before the final Se threshold is developed. For example, interactions between Se and other trace elements (e.g., mercury (Hg), As) in aquatic organisms can modify the associations between Se concentration in tissue and its effects (e.g., elevated Se in the tissue, without apparent effect). In addition, few studies have investigated the accumulation and effects of Se in cold water ecosystems (Kennedy *et al*., 2000, deRosemond *et al*., 2005; Holm *et al*., 2005; Rudolph *et al*., 2008) and consequently an important aspect of the debate is focused on whether the proposed Se threshold is applicable to fishes inhabiting north temperate aquatic systems (Lemly, 1993a; Hamilton, 2003).

1.4. Biological role, deficiency and toxicity of selenium

1.4.1. Biological role of selenium

Selenium was first discovered as an essential element in 1957 (Mayland, 1994) and it has been identified as a key component in cellular and tissue anti-oxidant defenses (Reilly, 2006). In vertebrates, Se can be incorporated into functional seleno-proteins, Se-containing proteins and amino acids after absorption (Daniels, 1996). Seleno-proteins specifically incorporate Se-Cys (co-translationally) in their active site and are responsible for all the

biological functions attributed to Se (Patching and Gardiner, 1999; Reilly, 2006). The main seleno-proteins have been identified as glutathione peroxidase (GPx; primary anti-oxidant enzyme, largely responsible for the protection of cells and tissues against oxidative stress), deiodinases (main role in the activation/inactivation of the thyroid hormone), thioredoxin reductases (potential function in mitochondrial defense against oxidative damage), seleno-protein P (metal binding protein in blood, implicated in the transport of Se from the liver to other organs and tissues) and selenophosphate synthetase (synthesis of seleno-phosphate, an intermediate molecule in the synthesis of Se-Cys) (Reilly, 2006). In addition, there are many other seleno-proteins whose functions are not known (e.g., seleno-protein T; Reilly, 2006). Selenium-containing proteins can incorporate exogenous Se-Met non-specifically in the place of methionine. Since vertebrates cannot synthesize Se-Met, any non-metabolized Se-Met is incorporated into places with high rates of protein synthesis (e.g., liver). Although there is no evidence of specific Se storage in vertebrates, these Se-containing proteins that are largely found in skeletal muscle, liver, kidney, stomach and erythrocytes appear to contribute greatly to the Se pool (Schrauzer, 2000; ATSDR, 2003; Reilly, 2006). Selenium can also be found in organisms primarily as part of two amino acids, Se-Met and Se-Cys. In fish, Se is mainly present as seleno-protein forms. Furthermore, the abundance of seleno-proteins (e.g., seleno-protein P) has been reported to highly contribute to the Se accumulation in zebrafish (*Danio rerio*) (Kryukov and Gladyshev, 2000). Fish also appear to utilize seleno-proteins to a larger extent than other vertebrates (Kryukov and Gladyshev, 2000).

1.4.2. Symptoms of selenium deficiency in fish

Selenium deficient fish show decreased GPx activity, decreased levels of Se in liver and plasma, and increased pyruvate kinase and glutathione transferase activity (Bell *et al*., 1986). The increase in glutathione transferase activity appeared to compensate for the loss of GPx activity in Se deficient carp (*Cyprinus carpio*) (Javanovic *et al*., 1997). Hitra disease (muscular and myocardial degeneration, hemorrhages and edema) in farmed salmon is mainly due to Se deficiency in combination with low levels of vitamin E (Watanabe *et al*., 1997). Seleno-protein N deficient zebrafish showed dystrophy of skeletal muscles during early embryogenesis leading to tail malformations and curved body-shape (Deniziak *et al*., 2007). Other symptoms of Se deficiency in fish may include growth reduction, liver degeneration, tissue vacuolation (e.g., in pancreas), lethargy, abnormal swimming patterns and mortality (Hilton *et al*., 1980; Bell *et al*., 1986; Watanabe *et al*., 1997). Environmental concentrations of Se in most natural waters (0.1-0.4 μg/L) and prey organisms (0.1-1 μg/g (DW) are sufficient to prevent deficiencies in wild fish (Hodson and Hilton, 1983).

1.4.3. Mechanisms of selenium toxicity

Uptake of Se by aquatic organisms can occur through water or diet, however, dietary uptake (primarily as Se-Met) is usually the dominant pathway of selenium uptake and accumulation in fish (Lemly, 1993a; Hamilton, 2004; Stewart *et al*., 2004). Selenium presents an interesting paradox, having a very narrow margin between required and toxic levels. Despite being an essential nutrient in fish at dietary concentrations of 0.1 to 0.5 μg/g DW (Lemly, 1997a), toxicity has been reported to occur at dietary concentrations only 7 to

30 times higher than nutritional levels (i.e., > 3 μg/g DW) (Hodson and Hilton, 1983; Lemly, 1993a). Selenium toxicity can occur by three major mechanisms: oxidation of thiol (S-H) groups followed by redox-cycling (Fenton-type reaction) and generation of free radicals (e.g., $O_2^{\bullet-}$), substitution of Se for S in proteins, and inhibition of Se methylation (Spallholz and Hoffman, 2002).

The general metabolism of Se consists of several reductions to produce hydrogen selenide, which can be methylated for excretion or incorporated into seleno-proteins after transformation to Se-Cys (ATSDR, 2003). Two of the intermediate products of Se metabolism, seleno-persulfide (GSSeH) and methylselenol (CH_4Se), are very unstable and remarkable oxidizing catalysts that can enter a Fenton-type reaction, continuously oxidizing thiols such as reduced glutathione (GSH), while reducing oxygen to produce superoxide radicals (Spallholz and Hoffman, 2002; Palace *et al*., 2004). Glutathione is the most important intracellular anti-oxidant, most abundant cellular thiol, and main co-factor of anti-oxidant enzymes, such as GPx. Therefore, GSH depletion will have significant consequences for the prevention of oxidative damage to cells and tissues (Hoffman, 2002). Spallholz and Hoffman (2002) reported free radical production induced by selenite and Se-Cys but not by selenate and Se-Met in a study evaluating the dietary toxicity of Se to aquatic birds. However, Palace *et al.* (2004) showed that rainbow trout (*Oncorhynchus mykiss*) embryos transformed Se-Met to methylselenol, a compound capable of producing superoxide radical in the presence of methioninase.

Another mechanism of Se toxicity is by substitution of Se for S during protein formation (Palace *et al*., 2004). Cells cannot discriminate between Se and S and therefore, when in

excessive amounts, Se is mistakenly substituted for S during protein synthesis (Lemly, 1997b). Selenium and S have similar chemical properties, however at biological pH Se is reduced while S is oxidized, altering the disulfide bonds (S-S) that are necessary for proper protein function (Lemly, 1997b). For example, damage to hair, nail, feathers and hoofs have been attributed to the substitution of Se for S in structural protein (Spallholz and Hoffman 2002). In addition, inhibition of the Se methylation process (a detoxification pathway) may result in more Se available to substitute for S during protein formation and/or formation of superoxide radicals (Spallholz and Hoffman 2002).

1.4.3.1. Selenium toxicity in fish

Developmental malformations in fish are the most important toxicological effect of chronic Se exposure. They are produced as a consequence of parental exposure to Se, maternal deposition of Se (mainly as Se-Met) into eggs and subsequent exposure of the developing larvae during yolk absorption (Lemly,1997b; Palace *et al*., 2004). The poorly developed cytoprotection and repair mechanisms in fish larvae make them highly susceptible to oxidative stress damage (superoxide radical formation from Se-Met by methioninase) and alteration of structural proteins function (substitution of Se by S) (Spallholz and Hoffman, 2002; Palace *et al*., 2004). Selenium-induced deformities appear to occur in fish larvae when egg Se concentrations exceed 10 μg/g DW (Lemly, 1993a). Egg injection studies investigating the teratogenic properties of the different forms of Se in avian embryos reported that the toxicity order for Se compounds was Se-Met>selenite and Se-Cys>selenate (Spallholz and Hoffman, 2002). Furthermore, oviparous vertebrates

emerge as the most sensitive vertebrates to Se, due to the efficient transfer of Se-Met into eggs (Palace *et al*., 2004; Orr *et al*. 2006). Some of the most evident Se-induced terata occurring in fish larvae include: skeletal deformities (lordosis (concave spine), kyphosis (convex spine), scoliosis (lateral curvature of the spine)), craniofacial deformities and missing or deformed fins. Other symptoms of Se exposure that are not true terata include edema, protruding eyes and cataracts (Lemly, 1997b). In wild fish populations adults inhabiting areas with elevated Se appear healthy despite the fact that reproductive impairment is occurring (Lemly, 2002a; Palace *et al*., 2004). A high percentage (> 80%) of deformed larvae do not reach the adult stage, and therefore fish populations can be severely impacted due to the rapid decline in recruitment (Lemly, 1997b).

Chronic exposure to waterborne (40-130 µg/L) and/or dietary (>3 µg/g DW) Se has been reported to cause abnormalities in developing fish. However, as mentioned before, diet and not water is the most important route of Se exposure in fish (Lemly, 1993a; Hamilton, 2004; Stewart *et al*., 2004). Several field and laboratory reproductive studies have investigated the teratogenic effects of Se in fish larvae. Gillespie and Baumann (1986) reported the presence of edema and increased mortality in bluegill sunfish larvae originated from females with egg Se concentration of 39.7 µg/g DW (converted from 7.94 µg/g WW using 80% moisture) in a laboratory study evaluating the impacts of Se in a coal-fired power plant reservoir in North Carolina (Hyco reservoir). Similarly, Woock *et al*. (1987) also reported that exposure of parental bluegill to dietary Se-Met (13-30 µg/g DW) increased the frequency of larval deformities in a laboratory study evaluating the effects of dietary Se exposure. Lemly (1993a) reported increased levels of deformities in fish inhabiting a cooling reservoir (Belews Lake) from a coal-fired power plant in North

Carolina. Although Se-induced deformities were observed in all collected fish species, deformities were more abundant among centrarchids, such as bluegill. The effect of Se in other fish species is less clear. For example, some studies report the presence of Se-induced deformities in fathead minnow exposed to dietary Se in artificial streams (Schultz and Hermanutz, 1990; Hermanutz, 1992), while others report no adverse effect occurring at similar dietary Se concentrations in this fish species (Ogle and Knight, 1989; Pyle *et al*., 2001).

Recent work examining the effects of chronic Se exposure in salmonids inhabiting a coal-mining area has been conducted in British Columbia (Kennedy *et al*., 2000) and Alberta (Holm *et al*., 2005), Canada. Kennedy *et al.* (2000) reported no significant increase in larval deformities in cutthroat trout (*Oncorhynchus clarki*) at egg Se concentrations ranging from 8.7 to 81.3 µg/g DW. Similarly, Holm *et al* (2005) reported no increase in larval deformities in brook trout (*Salvelinus fontinalis*) at egg Se concentrations of 16.9 and 20.0 µg/g DW (converted from 6.6 and 7.8 µg/g WW, using 61% moisture). However, in the same study larval deformities were elevated in rainbow trout at egg Se levels of 22.6 and 29.6 µg/g DW (converted from 8.8 and 10.5 µg/g WW, using 61% moisture) (Holm *et al*., 2005). Although it has been suggested that egg viability (fertilization and hatchability) is not affected by Se levels in the eggs (Gillespie and Baumann, 1986; Ogle and Knight, 1989; Kennedy *et al*., 2000) a recent study by Rudolph *et al.* (2008) reported that fertilization of westslope cutthroat trout (*Oncorhynchus clarki lewisi*) eggs was affected at egg Se concentrations > 80 µg/g DW. It is clear that Se toxicokinetics and its effects in different fish species still require further investigation. Other symptoms of chronic Se exposure in fish occurring when (whole body) Se concentration exceed 4 µg/g DW, include

reduced growth, hemorrhaging of the gill, reduced hematocrit levels with elevated lymphocytes, necrosis of the liver, kidney and ovary, myocarditis, cataracts and juvenile mortality (Sorensen *et al*., 1984; Lemly, 1993a; Kennedy *et al*., 2000).

The concentrations of Se required to kill 50% of the test population (LC50) are shown in Table 1.1. Selenium toxicity depends not only on the fish species but also on the Se form and developmental stage of the organisms. Generally, selenite is more toxic to fish than selenate during water exposures and juvenile fish are the most sensitive, especially after gill development (Buhl and Hamilton, 1991). Selenium concentrations in contaminated aquatic environments are generally less than 10 µg/L, and therefore the required concentration of waterborne Se necessary to elicit acute responses such as mortality in fish are unrealistic (USEPA, 2004). In addition, concentrations of dietary Se (as sodium selenite) ranging from 9 to 13 µg/g DW have been shown to cause mortality in rainbow trout (Hilton *et al*., 1980).

1.4.4. Factors affecting selenium accumulation and toxicity

Selenium bioavailability in aquatic environments depends significantly on water quality parameters, presence of organic matter and microbial activity (Barceloux, 1999; Simmons and Wallschläger, 2005). Water quality variables (e.g., pH, hardness) and presence of certain metal oxides (e.g., Fe and aluminum (Al) oxides) can change the partitioning of the various forms of Se in the aquatic environment and consequently modify its accumulation and toxicity (ATSDR, 2003; USEPA, 2004). Water temperature and day length can also affect the partitioning of the various Se forms in aquatic environments (ATSDR, 2003). In addition, Se can interact with a wide variety of ions, trace elements, and vitamins.

Competition of Se with sulphate has been reported to reduce the availability of selenate to primary producers and its overall toxicity to aquatic organisms (Brix *et al*., 2001; Simmons and Wallschläger, 2005). Competition of phosphate with selenate has also been reported to occur (USEPA, 2004). Interactions of Se with trace elements such as antimony (Sb), As, bismuth (Bi), Cd, copper (Cu), germanium (Ge), Hg, and silver (Ag) have been reported to reduce the tissue accumulation, toxicity and excretion of this element and/or reduce the toxicity of the interacting element (Hill, 1975; Naganuma *et al*., 1983; ATSDR, 2003). Combinations of methionine and vitamin E have also been found to decrease Se toxicity, while vitamin C has been reported to increase the absorption and toxicity of this element (ATSDR, 2003).

1.5. Radiotelemetry as a tool in environmental studies

Anthropogenic activities such as metal mining have the potential to cause significant alterations in the aquatic environment (e.g., invertebrate abundance or diversity, water quality) and consequently affect fish movement and distribution. Behavioural alterations are reliable and sensitive indicators of stress imposed on fish by environmental changes (Baras and Lagardere, 1995; Jakka *et al*., 2007). Radio-telemetry may therefore be used as an early warning system, detecting sub-lethal effects of pollutants at an early stage in aquatic organisms through changes in behaviour. Positional radio-telemetry has been widely used to investigate fish ethology in freshwater systems, including responses to obstruction (e.g., Baxter *et al.*, 2003), habitat selection and biology (e.g., Nettles *et al.*, 1997; Connor and Garcia, 2006), seasonal and daily behaviour (e.g., Kobler *et al*., 2008a),

Table 1.1: Acute selenium (Se) toxicity concentrations in different fish species and life stages. Selenite and selenate are expressed as $NaSeO_3$ and $NaSeO_4$, respectively.

Common name	Scientific name	Life stage	Se form	Endpoint	Se concentration (mg/L)	Reference
Arctic grayling	*Thymallus arcticus*	Alevin	Selenate	LC50 (96hr)	100.0	Buhl and Hamilton (1991)
		Alevin	Selenite	LC50 (96hr)	76.0	Buhl and Hamilton (1991)
		Juvenile	Selenate	LC50 (96hr)	180.0	Buhl and Hamilton (1991)
		Juvenile	Selenite	LC50 (96hr)	34.3	Buhl and Hamilton (1991)
Bluegill	*Lepomis macrochirus*	Adult	Selenite	LC50	28.5	USEPA (2004)
Brook trout	*Salvelinus fontinalis*	Adult	Selenium dioxide	LC50	10.2	USEPA (2004)
Coho salmon	*Oncorhynchus kisutch*	Alevin	Selenate	LC50 (96hr)	379.0	Buhl and Hamilton (1991)
		Alevin	Selenite	LC50 (96hr)	80.0	Buhl and Hamilton (1991)
		Juvenile	Selenate	LC50 (96hr)	74.0	Buhl and Hamilton (1991)
		Juvenile	Selenite	LC50 (96hr)	7.8	Buhl and Hamilton (1991)
Northern pike	*Esox lucius*	Juvenile	Selenite	LC50 (72hr)	11.1	Klaverkamp *et al* (1983)
Rainbow trout	*Oncorhynchus mykiss*	Alevin	Selenate	LC50 (96hr)	47.0	Buhl and Hamilton (1991)
		Alevin	Selenite	LC50 (96hr)	118.0	Buhl and Hamilton (1991)
		Juvenile	Selenate	LC50 (96hr)	32.3	Buhl and Hamilton (1991)
		Juvenile	Selenite	LC50 (96hr)	9.0	Buhl and Hamilton (1991)
White sucker	*Catostomus commersoni*	Juvenile	Selenite	LC50 (96hr)	29.0	Klaverkamp *et al* (1983)
Yellow perch	*Perca flavescens*	Juvenile	Selenite	LC50	4.8	Klaverkamp *et al* (1983)

and conservation programs (e.g., Klefoth *et al*., 2008). However, fewer studies have used radio-telemetry as an environmental monitoring tool (Gagen *et al*., 1994; Cooke and Schreer, 2003; Thorstad *et al.*, 2003; Cooke *et al*., 2004).

Although radio-telemetry has proved to be an important technology to study fish behaviour and migration in real time and in non-constrained organisms (Baras and Largardere, 1995), this technology presents certain limitations. For example, radio signals can be attenuated by deep water (> 5m) and/or water parameters, such as high conductivity (> 500 μS/cm). In addition, electric noise (e.g., high number of radio signals, industrialized areas) can interfere with the valid radio-signal selected for tracking and the cost of the required equipment can be expensive (Lucas and Baras, 2000; Cooke *et al*., 2004). Nevertheless, radio-telemetry is a promising tool for studying exposure of fish to environmental fluctuations, including responses to effluents associated with metal mining.

1.6. Ecological role and importance of northern pike and white sucker

Northern pike (*Esox lucius*) have a circumpolar distribution in the northern hemisphere. Primarily a freshwater fish, they are significant as a commercial and sport fish. Spawning takes place after ice melts in April or May in vegetated areas. Eggs usually hatch between 12-14 days at 18°C. Juvenile pike feed on plankton and invertebrates after absorption of the yolk is complete. Adult pike are classified as omnivorous, however fish represent a high percentage of their diet. Pike abundance and general distribution make them the largest and most important top-level predator in Canadian aquatic ecosystems (Scott and Crossman, 1973; Stewart and Watkinson, 2004).

White sucker (*Catostomus commersoni)* distribution is restricted to freshwater systems in North America. Spawning occurs in the spring between May and June. Adults migrate from lakes into streams or quiet lake margins seeking spawning grounds. Eggs hatch after 10-15 days at 10-15 °C. After yolk absorption, juvenile white sucker feed on plankton and small invertebrates. White sucker are considered schooling fish and classified as omnivorous bottom feeders. They are relevant as a forage species for game and commercial fishing. Their abundance, wide distribution, and wide-ranging habitat use make them one of the most important species at the benthic level in Canada. They also serve as prey for other predatory fishes such as northern pike in most Canadian lakes and streams (Scott and Crossman, 1973; Stewart and Watkinson, 2004).

1.7. Research objectives

The goal of this research project is to evaluate Se levels in the major compartments of the aquatic ecosystem and correlate these data with potential Se effects on early life stages of two native fish species in northern Saskatchewan. Research objectives by chapter are provided in Table 1.2.

The specific research objectives will evaluate:

Objective 1: Determination of Se levels in the major compartments of the aquatic ecosystem (water, sediment, plankton, periphyton, invertebrates, and fish). These data will

be used to calculate biomagnification factors of Se between trophic levels in north temperate lakes receiving U mine effluents.

Ho: *There is no accumulation of Se in abiotic and biotic compartments of aquatic ecosystems downstream of U mine operations.*

Objective 2: Determination of characteristic developmental deformities induced by Se exposure in northern pike and white sucker larvae originated from adult fish exposed to U mining and milling effluents. In addition, Se and other trace element concentrations will be determined in northern pike and white sucker tissues (eggs, muscle, liver, kidney and bone) to evaluate metal accumulation patterns in north temperate fish species.

Ho: *There is no accumulation of Se in eggs and tissues of adult fish inhabiting lakes downstream of U mining and milling operations.*

Ho: *Elevated Se in eggs and tissues of adult fish does not generate developmental malformations in fish larvae.*

Objective 3: Determination of migration patterns, habitat use and home range of adult northern pike within gradients of U mining effluent discharges. These data will be use to evaluate the potential use of behavioural endpoints as an environmental tool.

Ho: *There are no differences between reference and exposure sites in movement pattern, home range and habitat use in northern pike inhabiting lakes downstream of U mines operations.*

Table 1.2: Research objectives by chapter

CHAPTER	OBJECTIVES	CHAPTER DESCRIPTION
1	Introduction	Background information including uranium mining in northern Saskatchewan, selenium in the aquatic environment, ecological uses of radio-telemetry and ecological importance of northern pike and white sucker.
2	Evaluation of selenium concentrations in biotic and abiotic compartments of the aquatic systems at Key Lake uranium mine. *Published Environmental Pollution 156: 387-393, 2008.*	Field collection of samples within a gradient of uranium mining effluent discharge was conducted in summer 2004.
3	Evaluation of selenium-induced deformities in northern pike larvae originated from adults exposed to effluent discharges from Key Lake uranium mine. *Published Environmental Science and Technology 40: 6506-6512, 2006.*	• Egg collection, *in situ* fertilization and laboratory incubations of northern pike embryos conducted in spring 2004 and 2005. • Collection of egg and tissue samples for selenium evaluations in northern pike female from one reference and three exposure sites conducted in spring 2004. • Determination of selenium induced-developmental abnormalities induced by selenium exposure in northern pike larvae.
4	Evaluation of selenium concentrations in biotic and abiotic compartments of the aquatic systems at McClean Lake uranium mine. *Published Science of the Total Environment (In Press)*	Field collection of samples in a lake receiving effluents discharges from McClean uranium mine was conducted in summer 2005.
5	Evaluation of selenium-induced deformities in northern pike and white sucker larvae originated from adults exposed to uranium mine effluent from McClean Lake uranium mine. *Published Environmental Toxicology and Chemistry (In Press).*	• Egg collection, *in situ* fertilization and laboratory incubations of northern pike and white sucker embryos conducted in spring 2006. • Collection of egg and tissue samples for Se evaluations in northern pike and white sucker female from one reference and one exposure site conducted in spring 2006. • Determination of developmental abnormalities induced by selenium exposure in northern pike and white sucker larvae.
6	Determinations of behavioural alterations in northern pike inhabiting aquatic systems receiving effluent discharges from Key Lake uranium mine.	• Northern pike capture and surgical implantation of radio-tags was conducted in summer 2004. • Northern pike locations were recorded seasonally (spring, summer, fall, and winter) from 2004 to 2006.
7	General synthesis and discussion	Summary of results reported in each chapter and key findings. Comparisons between the two study areas and ecological significance of the results. Description of research needs.

CHAPTER 2 [a]

Accumulation of selenium in aquatic systems downstream of Key Lake uranium mine and milling operation

[a] This chapter has been published in Environmental Pollution 56:387-393, under joint authorship with Andrew M Belknap (University of Saskatchewan) and David M Janz (University of Saskatchewan).

2.1. Introduction

Uranium (U) mining and milling operations have the potential to release trace elements such as arsenic (As), nickel (Ni), molybdenum (Mo), selenium (Se), and U and ions (e.g., sulphate, ammonium) into the receiving aquatic ecosystem. Elevated concentrations of Se have been reported in water, sediments and fish tissues downstream of U mining and milling operations in northern Saskatchewan, Canada (Klaverkamp *et al*., 2002; Pyle *et al*., 2002; deRosemond *et al*., 2005, Muscatello *et al*., 2006). Once released into the aquatic environment Se can be accumulated through the food chain reaching levels that can cause deleterious effects (e.g., impaired reproduction) in top predator fish species (Lemly, 1997a).

Selenium can exist in different oxidation states (-II, 0, IV and VI) and as organic compounds (e.g., seleno-methionine) in natural waters. It can be removed from the water-column and deposited in the sediments by adsorption, complexation and co-precipitation processes as well as absorption by aquatic organisms (Lemly, 1999). In sediments, Se can be re-cycled and made available for biological uptake by two processes: oxidation of the sediments (e.g., by bioturbation) and/or uptake by plants or aquatic organisms; the latter being of great importance for the mobilization of selenium into food chains (Lemly, 1999).

Uptake of Se by aquatic organisms can occur through water or diet. However, dietary uptake (primarily organoselenium) is usually the dominant pathway of Se accumulation in upper trophic levels (Lemly, 1993a; Hamilton, 2004; Stewart *et al*., 2004). Although Se is an essential metal for fish at dietary concentrations ranging from 0.1 to 0.5 µg/g dry weight (DW; Lemly, 1997a), toxicity has been reported to occur at dietary concentrations only 7-10 times higher than the dietary Se required for metabolic functions (e.g., > 3 ug/g DW)

(Hodson and Hilton, 1983; Lemly, 1993a). Accordingly, there is a very constricted range between required and toxic dietary levels of Se for fish (Wilber, 1980).

There is currently general agreement that the use of a tissue-based Se exposure criterion for the protection of fish populations is more relevant than a guideline based on water or sediment Se concentrations. The U.S. Environmental Protection Agency (USEPA) proposed a chronic criterion for Se at a whole body fish concentration of 7.91 ug/g (DW) (USEPA, 2004). However, there is still controversy regarding the proposed Se threshold for the protection of fish populations. The information required to establish an appropriate Se threshold in fish that will prevent selenium from reaching levels sufficient to cause toxicity, and consequently reproductive impairment, should include: 1) evaluation of Se accumulation and transfer between trophic levels, 2) association between Se accumulation and adverse biological effects in both field and laboratory, and 3) determination of the most appropriate compartment of the aquatic ecosystem for Se monitoring. Since the majority of Se ecotoxicology research has been conducted in warm water aquatic systems, there is a recognized need for more research focusing on cold water systems.

The purpose of this study was to determine Se accumulation in all major compartments of the aquatic ecosystem including water, sediments, plankton, periphyton, invertebrates, small bodied (forage) fish and predatory fish, downstream of a U mining and milling operation in northern Saskatchewan, Canada. These data were used to evaluate the potential differences in Se accumulation patterns between higher and lower trophic levels and to calculate accumulation factors between trophic levels.

2.2. Materials and Methods

2.2.1. Study area

The Key Lake U milling operation is located in north-central Saskatchewan (57°11'N, 105°34'W), approximately 600 km north of Saskatoon, SK, Canada. Treated mill effluent is discharged at a rate of approximately 6,000 m^3/d to the environment. Treated effluent enters the Yak Creek drainage at Wolf Lake and on average makes up 48% of Yak Creek flow. Yak Creek flows into David Creek before meeting up with Unknown Lake, which is located approximately 2 km from the point of effluent release. Treated effluent is about 23% of the David Creek flow. Water drains from Unknown Lake (via David Creek) into Delta Lake, approximately 10 km downstream of effluent release and continues through into the Wheeler River. Sampling sites in the current study included one reference site, David Lake (1.40 km^2 surface area and 2.44 m average depth) and two exposure sites, Unknown Lake (0.15 km^2 surface area and 1.36 m average depth, referred to as high exposure site) and Delta Lake (2.85 km^2 surface area and 4.42 m average depth, referred to as medium exposure site) (Figure. 2.1). These northern lakes are nitrogen and phosphorus limited and generally oligotrophic (Dillon et al., 2004), however higher nitrogen could occur in exposure lakes as a consequence of mining activities (Bennett and Janz, 2007).

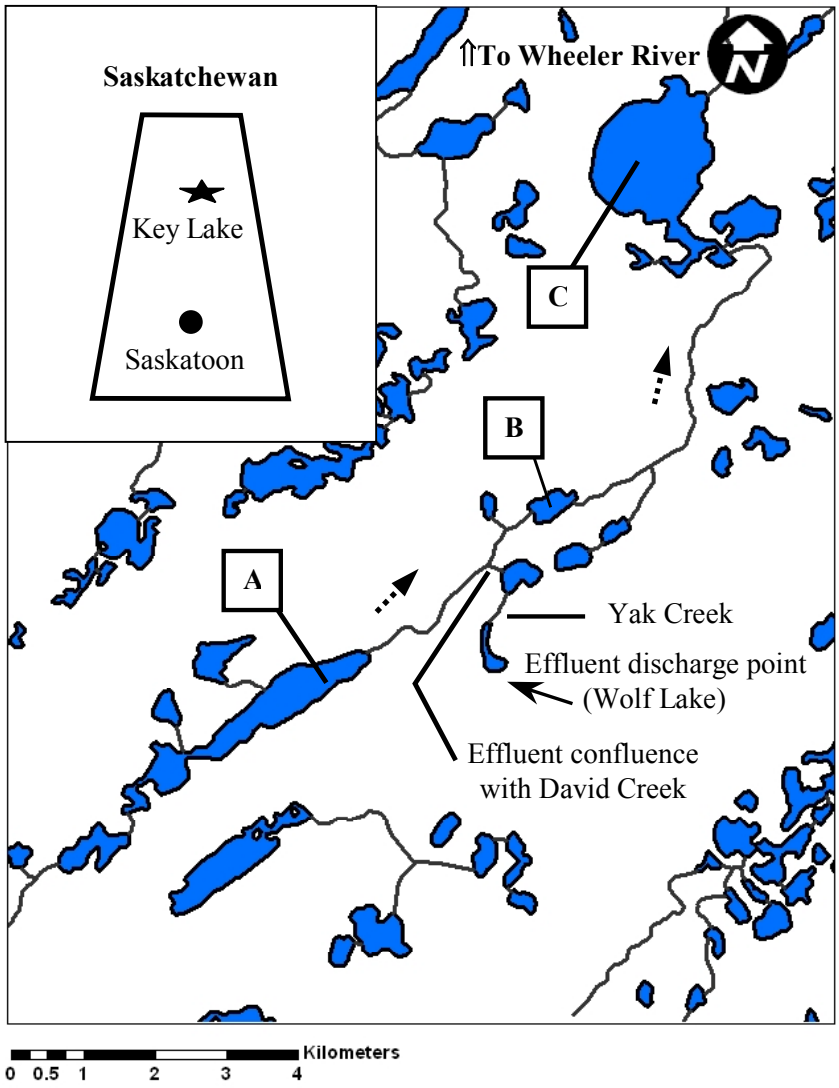

Figure 2.1: Map of Key Lake study area (Saskatchewan, Canada). Insert: map of Saskatchewan showing the relative location of Key Lake uranium mine operations. Sampling sites: A) David Lake (reference site), B) Unknown Lake (high exposure site) and C) Delta lake (medium exposure site). Solid arrow, mill effluent discharge into Wolf Lake. Dashed arrows, flow direction.

2.2.2.Sample collection and processing

Samples were collected in late August 2004 and 2005. Water, periphyton and plankton samples were collected in 2004, while invertebrates, small bodied (forage) fish and predatory fish collection occurred in 2005. Water and biological samples were collected following protocols described elsewhere (ASTM, 2003a; ASTM, 2003b; ASTM, 2003c; ASTM, 2003d; Rosenberg and Resh, 1993; USEPA, 1983). Except for water, all samples were stored frozen at –20 °C until analysis. Within 6 month of collection all the samples were processed and analyzed. Biota samples (plankton, periphyton, invertebrates and fish) were oven dried at 60°C until constant weight was recorded. Sediment Se concentration data from 2004 were obtained from the Cameco Corporation database (Golder, 2005).

2.2.2.1. Water

Surface water samples for total and dissolved Se determinations were collected in triplicate approximately 10 cm below the water surface into pre-cleaned 125-ml high density polyethylene (HDPE) bottles. Samples for dissolved Se determinations were immediately filtered through 0.45 µm disposable filters and acidified to pH < 2 with ultra-pure nitric acid in the field. An additional three replicate samples were collected for sulphate, hardness and alkalinity determinations. Samples were stored at 4°C until analysis. Routine water quality measurements (pH, conductivity, temperature, dissolved oxygen (DO), salinity and dissolved solids) were evaluated on-site using a YSI meter (6 series) with a multi-parameter display system (model 650) attached (YSI Inc., Yellow Springs, OH, USA).

2.2.2.2. Plankton and periphyton

Plankton samples were collected by horizontal towing using a 200-ml collection bucket attached to a 153 μm net mesh (Nitrex®). The contents of several tows were combined resulting in pooled triplicate samples per site. No separation of phytoplankton and zooplankton was made. Periphyton samples (*n*=3) were scraped using plastic tools from rocks or macrophytes close to the shoreline. Samples were collected into plastic whirl-packs and stored frozen until analysis. Percent moisture was 85.0 ± 4.56 and 83.0 ± 2.57 % for plankton and periphyton, respectively.

2.2.2.3. Invertebrates

Invertebrate samples were collected by dip netting in shallow areas or by Eckman grab in deeper locations followed by sieving through a 425 μm mesh. The invertebrates were then sorted by feedings habits into filterers (clams (Bivalvia) *n*= 5, pooled 4-7 bivalves per replicate), detritivores (chironomid larvae (Insecta, Diptera) *n* =5, pooled 5-17 animals per replicate and caddisfly larvae (Insecta, Tricoptera) *n*=5, pooled 3-7 organisms per replicate) and predators (leeches (Hirudinea) *n*=3-4, pooled 1-4 leeches per replicate and dragonfly larvae (Insecta, Odonata) *n*=5, pooled 3-7 animals per replicate) according to Merritt and Cummins (1984). The collected invertebrates were left in the respective site water for 2 to 3 hrs to purge their stomach content. The invertebrate samples were then rinsed with nanopure water to remove any debris, placed into plastic whirl-pack bags and frozen until analysis. Percent moisture were as follows: clams 82.71 ± 3.43 %, chironomids 93.62 ±

1.27 %, caddisflies 87.11 ± 0.88 %, leeches 83.83 ± 0.62 %, and dragonflies 84.70 ± 0.80 %.

2.2.2.4. Fish

Juvenile pike (*Esox lucius*; n=*3*) and spottail shiners (*Notropis hudsonius*; n=5, pooled 6 fish per replicate) were collected using an electrofishing backpack (model LR-24, Smith-Root, Vancouver, WA, USA). Total length and weight were recorded and samples were frozen until analysis. Before analysis, samples were thawed, stomachs were emptied and ageing structures (cleithra and scales) were removed for age determinations. Fish were rinsed with nanopure water before homogenization. Percent moisture was 80.0 ± 2.34 % for juvenile pike and 80.0 ± 5.46 % for shiners.

2.2.3. Analytical procedures

2.2.3.1. Water quality parameters

Water Se and sulphate concentrations were evaluated using inductively coupled plasma-mass spectrometry (ICP-MS) (Saskatchewan Research Council, Saskatoon, SK) and inductively coupled plasma optical emission spectrometry (ICP-OES) (Enviro-Test Laboratories, Saskatoon, SK), respectively. Average selenium recovery > 90 %. Water hardness and alkalinity were measured with a Hach Digital Titrator model 16900 (Hach Company, Loveland, CO, USA).

2.2.3.2. Selenium determination in biological samples

All samples were dried and ground to a powder using an acid-cleaned porcelain mortar and pestle. Then, samples were digested using an Ethos Plus Advanced Microwave (model FAM 50, Milestone Inc., Monroe, CT, USA) with a maximum power of 1000 W and highest operating temperature of 200ºC. One to 2 mg of sample was transferred to teflon digestion vessels. Nitric acid (6 mL) (Omnitrace Grade, EMD Chemicals) and 1 mL of hydrogen peroxide (AnalaR grade, EMD Chemicals) were added in a 86:14 (nitric acid:peroxide) ratio. Samples were digested with the following microwave temperature program: ramp 20ºC to 180ºC over 10 minutes, and held at 180ºC for 20 minutes. Digests were evaporated slowly in teflon jars on a hotplate and were reconstituted in 6 M hydrochloric acid (Omnitrace grade, EMD Chemicals) prior to second microwave heating with the following temperature program: ramp 20ºC to 90ºC over 5 minutes, and held 90ºC for 20 minutes. Samples were syringe filtered using a 0.45 μM disposable filter prior to analysis to remove undigested particulates.

Total Se determinations were made using a Varian (Palo Alto, CA, USA) model SpectrAA 50B atomic absorption spectrometer (wavelength 196 nm) equipped with a hydride generator (model VGA-77, Varian), and electrothermal temperature controller (model ETC-60, Anatech, Sittard, Netherlands). Method detection limit (0.16 μg Se/g) was determined using 7 replicates of DORM-2 certified standard tissue. Average Se recovery (> 90 %) was evaluated using certified tissue (Dogfish muscle, DORM-2) obtained from the National Research Council of Canada.

2.2.4. Statistical analysis

Statistical analyses were performed using SigmaStat version 3.1 (SPSS Inc., Chicago, IL) with a 95% ($\alpha = 0.05$) level of confidence. Significant differences among sites were evaluated using one-way ANOVA followed by Tukey's test when appropriate. Data that failed tests for normality and/or homogeneity of variance were $\log_{(10)}$ transformed prior to the use of the parametric statistical test. If data failed the parametric assumptions, a non-parametric test (Kruskall-Wallis) was used for the evaluation of significant differences among sites. Comparisons between fish from reference and medium exposure sites were evaluated by *t*-test. Analysis of covariance (ANCOVA; SYSTAT® version 10 (SSI Inc., Richmond, CA, USA) was used to compare the body weights fish (forage and predatory) with body length as a covariate. Selenium accumulation factors were calculated on a wet weight basis as defined in USEPA (2000):

Concentration of Se (whole organisms)/ Se in media (water or sediments) or prey organisms (2.1)

2.3. Results

Water collected from the exposure sites was characterized by higher hardness, conductivity and sulphate, and lower pH and alkalinity, compared to the reference site (Table 2.1). The concentration of Se in water was higher in the exposure sites than in the reference site, with approximately 85% of the total recoverable Se in the dissolved fraction (Table 2.2). As with water, sediment Se concentrations were 5 to 10 times higher in the exposure sites than the reference site (Table 2.2).

Table 2.1: Water quality variables measured on-site during sample collection. Data represent the mean (± SE) of three replicate samples. Below detection limit (<).

Variable	Reference	Medium	High
Hardness (mg $CaCO_3$/L)	7.0 ± 1.1	219.3 ± 8.7	289.3 ± 8.3
Alkalinity (mg $CaCO_3$/L)	10.0 ± 2.0	5.3 ± 1.2	6.7 ± 0.7
pH	7.4 ± 0.1	6.5 ± 0.8	6.3 ± 0.2
Conductivity (µS/cm)	12.0 ± 1.0	401.0 ± 14.2	497.6 ± 1.8
Temperature (°C)	10.5 ± 0.2	12.9 ± 0.8	12.3 ± 0.4
DO (mg/L) [a]	10.8 ± 0.3	10.5 ± 0.2	10.4 ± 0.06
TDS (g/L) [b]	0.01 ± 0.001	0.3 ± 0.006	0.4 ± 0.007
Salinity (mg/L)	0.01 ± 0.0	0.2 ± 0.01	0.3 ± 0.004
Sulphates (mg/L)	< 6	257.7 ± 5.0	342.5 ± 6.1

[a] Dissolved oxygen.

[b] Total dissolved solids.

Table 2.2: Water and sediment total selenium concentration in reference and exposure sites. Below detection limit (<).

Variable	Reference	Medium	High
Selenium (µg/L) [a]	< 0.1	0.7 ± 0.0	2.7 ± 0.05
Dissolved selenium (µg/L) [a]	< 0.1	0.6 ± 0.0	2.3 ± 0.2
Bound selenium (µg/L) [b]	N/C [c]	0.1 ± 0.0	0.4 ± 0.1
Sediment selenium (µg/g) [d]	5.7 ± 0.4	25.6 ± 2.9	62.2 ± 4.7

[a] Samples represent the mean (±SE) of three replicate water samples.

[b] Bound selenium in the water column calculated as total selenium – dissolved selenium.

[c] Not calculated.

[d] Selenium concentration reported as sediment dry weight from Golder, 2005.

Although exposure areas have relatively low aqueous Se concentrations, elevated Se concentrations were evident among biota (Figure 2.2). Periphyton and plankton Se levels from the reference site (0.29 ± 0.05 μg/g dry weight (DW) and 1.11 ± 0.11 μg/g DW, respectively) were significantly different ($p < 0.05$) from both medium (1.01± 0.26 μg/g DW and 5.01± 0.70 μg/g DW, respectively) and high (3.75 ± 0.64 μg/g DW and 7.21 ± 1.43 μg/g DW, respectively) exposure sites. Whole body Se concentration in shiners (14.98 ± 0.67 μg/g DW) and juvenile pike (17.02 ± 1.79 μg/g DW) was significantly ($p < 0.05$) higher in the medium exposure site than the reference site (0.87 ± 0.07 and 0.78 ± 0.03 μg/g DW, respectively) (Figure 2.2). Juvenile pike from the high exposure site were not included in any of the statistical analyses ($n = 1$). However, the one juvenile pike from this site presented the highest whole body Se concentration of all sites (28.28 μg/g DW, Figure 2.2). No shiners were collected from the high exposure site. Fish age was 1 year for shiners and 2 to 3 years for juvenile pike. There were no significant differences in the condition factor calculated as:

$$(\text{Body weight}/ (\text{Length})^3) \text{ X } 100 \qquad (2.2)$$

in juvenile pike and shiners between reference (0.66 ± 0.11 and 0.68 ± 0.27, respectively) and medium exposure site (0.70 ± 0.14 and 1.09 ± 0.35, respectively).

When comparing the Se content among the biota within each collection site, plankton selenium levels were significantly higher ($p < 0.05$) than periphyton for all sites, with plankton accumulating approximately 2 to 5 times more Se than periphyton (Figure 2.2). No differences in Se concentrations were found between filterer invertebrates and either plankton or periphyton at each site. However, detritivore and predator invertebrates showed

significant increases in Se concentrations ($p < 0.05$) compared to filterer invertebrates, plankton and periphyton at both medium and high exposure sites (Figure 2.2). Within the medium exposure site there were no significant differences in the Se levels between juvenile pike and shiners; however, both fish species showed significant increases ($p < 0.05$) compared to plankton, periphyton and filterer invertebrates (Figure 2.2). The overall pattern of selenium accumulation (from smallest to largest) was as follows: periphyton < plankton and filterer invertebrates < detritivore and predator invertebrates < small bodied fish (shiners) and predatory fish (juvenile pike; Figure 2.2).

Selenium accumulation factors for the high and medium exposure sites among all the compartments of the aquatic ecosystem are presented in Table 2.3 and Figure 2.3. Selenium in the water was concentrated from 200 to more than 4,000 times in sediments and biota at exposure sites. Biomagnification of Se (defined as increased Se concentrations by successive trophic levels (Lemly, 1997a) also occurred, resulting in approximately 1.5 to 6 fold increase in the Se content between plankton, invertebrates and small bodied fish. However, biomagnification did not appear to occur between the forage (shiners) and predatory (pike) fish species evaluated in this study.

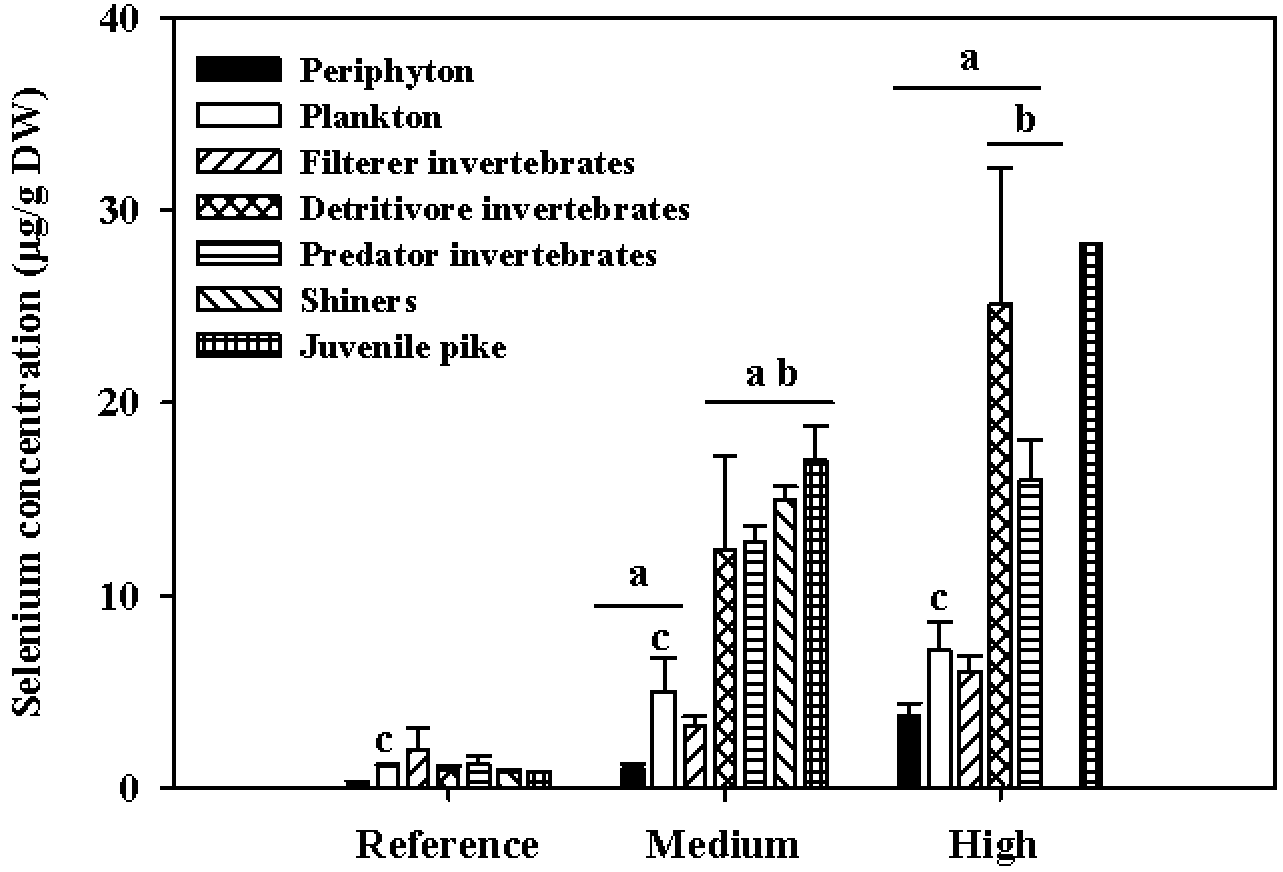

Figure 2.2: Concentration of selenium (µg/g, dry weight) in periphyton, plankton, invertebrates (filterers, detritivores and predators) and fish (juvenile pike and shiners) from reference and exposure sites (see legend in figure). Data represent the mean (± SE). [a] Significantly different from the reference site ($p < 0.05$). [b] Significantly different from periphyton, plankton and filterer invertebrates within each collection site ($p < 0.05$). [c] Significantly different between plankton and periphyton within collection sites ($p < 0.05$).

Table 2.3: Accumulation factors for sediments and biota in both medium and high exposure lakes.

Aquatic compartment	Medium							High						
Sediments	3,157 [a]							3,000 [a]						
Plankton	1,074 [a]							408 [a]						
Periphyton	245 [a]							240 [a]						
Invertebrates														
Filterers	727 [a]	0.23 [b]	0.68 [c]					363 [a]	0.12 [b]	0.90 [c]				
Detritivores	1,480 [a]	0.47 [b]	1.38 [c]					823 [a]	0.27 [b]	2.03 [c]				
Predators	2,868 [a]	0.91 [b]	2.67 [c]	3.95 [d]	1.94 [e]			948 [a]	0.32 [b]	2.33 [c]	2.61 [d]	1.15 [e]		
Fishes														
Shiners	4,280 [a]	1.35 [b]	3.98 [c]	5.89 [d]	2.89 [e]	1.49 [f]		N/A [h]						
Juvenile pike	4,858 [a]	1.54 [b]	4.52 [c]	6.68 [d]	3.28 [e]	1.69 [f]	1.13 [g]	2,126 [a]	0.71 [b]	5.23 [c]	5.86 [d]	2.58 [e]	2.24 [f]	

[a] Accumulation factor calculated as the selenium in sample divided by the total selenium in water (µg/L).

[b] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in sediments. Sediment moisture ranged from 87 to 91%.

[c] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in plankton.

[d] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in filterer invertebrates.

[e] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in detritivore invertebrates.

[f] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in predator invertebrates.

[g] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in shiners.

[h] Not available.

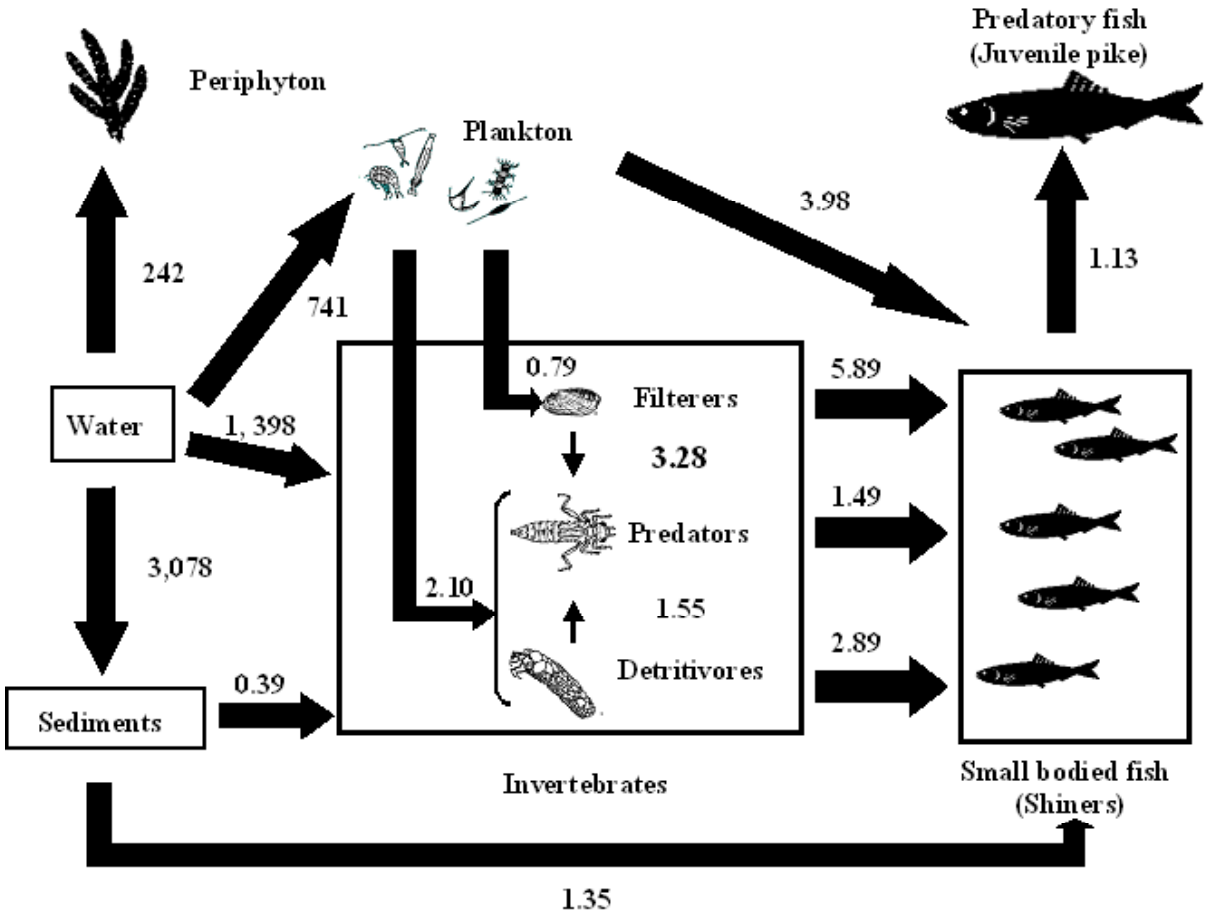

Figure 2.3: Generalized flow diagram showing the accumulation of selenium in aquatic food chains for the Key Lake uranium mine operations watershed. Values represent the mean of calculated accumulation factors for the high and medium exposure sites. Arrows width represents the calculated accumulation factors within (thin arrows) and between (thick arrows) trophic groups

2.4. Discussion

Selenium can be taken up directly from water by aquatic organisms; however diet is the dominant pathway of selenium uptake. The most important implication of elevated environmental Se is its propensity to accumulate in the aquatic food chain, potentially causing adverse effects on fish populations (e.g., impaired reproduction). The major finding of the present study was the accumulation and biomagnification of Se in exposure areas even though water concentrations were low (i.e., medium and high exposure site Se water concentrations were below the current 5 μg/L water criterion established by USEPA and only the medium exposure site water concentration was below the 1 μg/L threshold established by the Canadian Council of Ministers of the Environment (CCME, 2003)), thus having the potential to impair reproduction in top predator fish species such as northern pike. Earlier studies have also suggested that Se concentrations ≤ 1 μg/L in freshwater systems have the potential to accumulate in prey organisms and fish, reaching concentrations high enough to cause deleterious effects in higher trophic levels (Lemly, 1985a; Lemly, 1993a; Hamilton, 2004). Furthermore, several authors have concluded that sediments, as opposed to water, are the major pathway of Se accumulation in aquatic systems (Saiki *et al*., 1993; Hamilton and Lemly, 1999; Orr *et al*., 2006).

Although the Se concentration in plankton at the reference site was significantly higher than periphyton, the measured value for plankton is in agreement with the range reported for other reference sites (0.84 to 6.84 μg/g DW) (Lemly, 1985a; Saiki *et al*., 1993; Orr *et al*., 2006). Selenium concentrations in the evaluated exposure areas were higher in fish, detritivore and predator invertebrates than filterer invertebrates, indicating the importance

of sediments and detrital processes in Se bioaccumulation. Filterer invertebrates feed on the particles suspended in the water column (e.g., plankton), but in contrast, other invertebrates rely on food sources closely related to detrital processes, suggesting a stronger association with sediments.

Biomagnification of Se has been reported by some investigators (Cherry and Guthrie, 1977; Lemly, 1985a; Lemly, 1999) and argued by others (Saiki *et al*., 1993; Barceloux, 1999). In the present study, Se biomagnification was evident between primary producers (e.g., plankton), invertebrates and fish (1.5 to 6 fold increase between successive trophic levels). However, biomagnification between fish species did not seem to occur (no increase in the selenium content between forage and predatory fish). It is clear that a highly pronounced accumulation of Se occurred between lower trophic levels (plankton and invertebrates) and forage fish, but was not observed between higher trophic levels, such as forage and predatory fish (i.e., pike and shiners). Lemly (1985a; Lemly, 1997a) reported biomagnification values for Se ranging from 2 to 6 between primary producers, invertebrates and forage fish in a power plant cooling reservoir. However, the author also reported different accumulation and consequently biomagnification of Se between fishes presenting different feeding modes (e.g., > 2 fold increase between forage and predatory fish). The discrepancies in the Se biomagnification in higher trophic levels could possibly be explained by differences in Se metabolism between species (e.g., differential formation of seleno-proteins leading to differences in selenium accumulation and elimination kinetics), an area that requires further investigation.

Selenium released into the aquatic environment is rapidly taken up and highly assimilated by primary producers (e.g., plankton) (Riedel *et al.*, 1991; Besser *et al*., 1993).

Lower trophic levels (e.g., plankton) can accumulate Se several orders of magnitude relative to the concentration in water (Lemly, 1999). Thus, primary producers play a critical role in accumulating Se present in the water column (e.g., as organo selenium) that can be incorporated into the aquatic food chain (Bowie *et al*., 1996). Selenium precipitated into the sediments generally by depositional processes (e.g., deposition of dead organic material) (Bender *et al*., 1991; Oremland *et a*l., 1990; Graham *et al*., 1992) can lead to elevated concentrations of Se in sediments and benthic invertebrates (Lemly, 1993a; Saiki *et al*., 1993). Benthic invertebrates can process sediments to obtain food or build their cases and consequently can accumulate elevated levels of Se in contaminated environments (Sappington, 2002; Lemly, 1993a; Lemly, 1997a; Bowie *et al*., 1996). Some studies have reported that invertebrates can accumulate levels of Se ranging from 2 to 56 µg/g (DW) with no evidence of reductions in abundance or alterations in invertebrate community structure (Lemly, 1985a; Lemly, 1997a; Crane *et al,* 1992; Hamilton, 2004; Golder, 2005). However, other studies reported toxic effects occurring in benthic invertebrates exposed to water borne (> 100 µg/L) and dietery Se (> 1 µg/g DW) (Debruyn and Chapman, 2007). Nevertheless, benthic invertebrates can deliver high concentrations of Se as prey of higher trophic levels (e.g., fish) (Lemly, 1985a; Lemly, 1999). In this study, plankton, benthic invertebrate and fish (forage and predatory) Se concentrations in exposure areas exceeded the proposed 3 to 11 µg/g (DW) dietary toxicity threshold for fish (Lemly, 1993a; DeForest *et al*., 1999). Furthermore, adult pike inhabiting the same watershed contained high levels of Se in eggs and tissues, which led to an increase in the frequency (> 20% above background levels) of deformities in pike fry (Muscatello *et al*., 2006). Additional data suggest that adult white sucker (*Catostomus commersoni*) inhabiting exposure areas in the

same watershed also accumulated more Se in tissues and eggs (bone 20.14 ±1.6 μg/g DW, muscle 59.54 ± 2.0 μg/g DW, liver 20.36 ± 0.3 μg/g DW, kidney 18.30 ± 4.2 μg/g DW and eggs 42.90 ± 1.0 μg/g DW) than those from the reference site (bone 1.54 ± 0.12, muscle 3.22 ± 0.61 μg/g DW, liver 4.51 ± 0.99 μg/g DW, kidney 3.53 ± 0.34 μg/g DW and eggs 2.41 ± 0.41 μg/g DW).

2.5. Conclusions

Few studies have investigated Se bioaccumulation in north temperate, cold water ecosystems. The goal of the present study was to determine Se accumulation factors in lakes downstream of the Key Lake U mine located in northern Saskatchewan, Canada. The results indicated that Se concentrations in water, sediment and biota were elevated in the exposure sites with the following pattern (from smallest to largest) of Se accumulation in biota: periphyton < plankton and filterer invertebrates < detritivore and predator invertebrates < small bodied and predatory fish. The concentration of Se in detritivore and predator invertebrates was higher than filterer invertebrates, suggesting an important role of the sediment- detrital pathways in Se accumulation. Selenium biomagnification was pronounced between primary producers (e.g., plankton), invertebrates and fish. However, biomagnification between fish species did not appear to occur. Biota Se concentrations at the exposure sites exceeded the proposed 3 to 11 μg/g (DW) dietary toxicity threshold for fish. Based on these findings it is clear that although water concentrations of Se are relatively low, this element is still being incorporated into the food chain via primary producers, gradually building up in sediments and benthic biota, to finally reach levels that

have the potential to cause reproductive impairment in fish (Muscatello *et al*., 2006). There is still controversy regarding the appropriate Se threshold for the protection of aquatic ecosystems, particularly fish populations. The results observed in this study indicate that concentration of Se in water is a poor predictor of Se concentrations in invertebrates and fish.

CHAPTER 3 [a]

Larval deformities associated with selenium accumulation in northern pike (*Esox lucius*) exposed to effluent from Key Lake uranium mine and milling operation

[a] This chapter has been published in Environmental Science and Technology 40, 6506-6512 under joint authorship with Pamela M. Bennett (AREVA Resources Canada Inc.), Kevin T. Himbeault (Cameco Corp.), Andrew M Belknap (University of Saskatchewan) and David M Janz (University of Saskatchewan)

3.1. Introduction

Concerns over the release of selenium (Se) into aquatic systems downstream of industrial activities have led to an increase in investigations on potential effects on fish species. Elevated concentrations of Se have recently been reported in water, sediments and fish tissues downstream of certain uranium (U) mining and milling operations in northern Saskatchewan, as a consequence of U ore extractions, dewatering and milling (Pyle *et al*., 2001; Klaverkamp *et al.*, 2002; Golder, 2005; deRosemond *et al.*, 2005). A major implication of elevated environmental Se levels is associated with its propensity to bioaccumulate in aquatic ecosystems. Although Se is an essential nutrient in fish at dietary concentrations of 0.1 to 0.5 μg/g dry weight (DW) (Lemly, 1997a), contamination of aquatic ecosystems with Se from anthropogenic sources is of concern since toxicity in fish has been reported to occur at dietary concentrations only 7 to 30 times higher than nutritional levels (i.e., > 3 μg/g DW) (Hodson and Hilton, 1983; Lemly, 1993a). The extensive body of Se research conducted since the U. S. Environmental Protection Agency (USEPA) established a chronic water quality criterion of 5 μg/L in 1987 has produced important information, including: (i) dietary exposure to Se (primarily organoselenium) is the most ecologically and toxicologically relevant uptake route in fish, (ii) the major toxicological impacts on fish arise following maternal transfer of Se to eggs during vitellogenesis, (iii) early life stage toxicity of Se in fish arises predominantly when larvae undergo later stages of yolk absorption, and (iv) permanent developmental anomalies (spinal curvatures, missing or deformed fins, and craniofacial deformities) and other

toxicities (e.g., edema) in fish are directly related to elevated Se in eggs (Hodson and Hilton, 1983; Lemly 1993b; Maier and Knight, 1994; Hamilton, 2003)

Due to the bioaccumulative nature of Se through aquatic food webs and potential adverse effects on fish populations due to impaired reproduction, there is currently much focus and debate regarding the ecotoxicology of this element (e.g., Lemly, 1993a, 1993c, Chapman, 1999; Hamilton and Lemly, 1999). From a regulatory perspective, there is currently general agreement that a tissue-based chronic Se exposure criterion for protection of fish populations be developed instead of a guideline based on water-borne or sediment Se concentrations (Hodson and Hilton, 1983; Lemly, 1993a; DeForest *et al.*, 1999; Hamilton, 2002; USEPA, 2004). Recently the U.S. Environmental Protection Agency (USEPA) proposed a chronic criterion for Se at a whole-body fish concentration of 7.91 μg/g (DW) (USEPA, 2004) identifying the warm water centrarchid, bluegill sunfish (*Lepomis macrochirus*), as the most sensitive species (Lemly, 1993b, 1993c). Since the majority of studies documenting Se toxicosis in natural populations of fish have been conducted in warm water fish species, an important aspect of the debate is focused on whether these guidelines are applicable to native cold water fishes inhabiting north temperate aquatic systems (Lemly, 1993a; Lemly, 1997b; DeForest *et al*., 1999). Recent work examining effects of elevated Se levels associated with coal mining on salmonids inhabiting lentic habitats has been conducted in British Columbia and Alberta, Canada (Kennedy *et al.*, 2000; Holm *et al*., 2005). Kennedy *et al*. (2000) reported no significant increase in larval deformities in cutthroat trout (*Oncorhynchus clarki*) at egg Se concentrations ranging from 8.7 to 81.3 μg/g DW (mean 21.2 μg/g). Similarly, Holm *et al*.

(2005) reported no increase in larval deformities in brook trout (*Salvelinus fontinalis*) collected from two sites with mean egg Se concentrations of 6.6 and 7.8 μg/g wet weight (approximately 16.9 and 20.0 μg/g DW based on 61% moisture). However, in the same study (Holm *et al*., 2005) larval deformities were elevated in rainbow trout (*Oncorhynchus mykiss*) at a threshold (i.e., EC_{15}) egg Se concentration of between 8.8 - 10.5 μg/g wet weight (22.6 – 26.9 μg/g DW). Although these studies indicate that cold water salmonids may be less sensitive than centrarchids, such comparisons are confounded by the lack of consistent reporting of threshold values. There is clearly an urgent need for additional studies addressing species differences and potential development of tolerance mechanisms in impacted fish populations, particularly in species inhabiting cold water aquatic ecosystems.

The main objective of the present study was to investigate the presence of characteristic developmental deformities induced by Se exposure in northern pike (*Esox lucius*) fry originating from reproductively mature northern pike collected at several locations downstream of a U milling operation in northern Saskatchewan. Selenium concentrations were determined in multiple pike tissues (eggs, muscle, liver, kidney and bone) to further the understanding of Se bioaccumulation in cold water fish species. Finally, we wished to compare the results of this investigation to effects threshold values currently being proposed by the USEPA and being adopted as effects criteria by other regulatory agencies.

3.2. Materials and Methods

3.2.1. Study area

The Key Lake U milling operation is located in north-central Saskatchewan (57°11'N, 105°34'W), approximately 600 km north of Saskatoon, SK, Canada. Treated mill effluent is discharged at a rate of approximately 6,000 m^3/d to the environment. Sampling sites in the current study included one reference site (Davies Creek) and three exposure sites, David Creek, near-field (high exposure), Delta Lake (medium exposure) and David Creek, far-field (low exposure), located approximately 2, 10 and 15 km downstream of effluent discharge, respectively (Figure 3.1).

3.2.2. Fish collection

Spawning northern pike were collected in May 2004 and 2005. In 2004, pike were collected from the reference site (Davies Creek, n = 5 male, n = 3 female) and two exposure sites, high (David Creek near-field, n = 0 male, n = 1 female) and medium (Delta Lake, n = 5 male, n = 3 female). Eggs from the only female pike collected from the high exposure site were fertilized using milt from males collected at the medium exposure site. In 2005, additional pike were collected from the same reference site (n = 5 male, n = 2 female) and high exposure site (n = 4 male, n = 1 female), as well as a low exposure site (David Creek far-field, n = 5 male, n = 5 female). Ripe males and females were collected using trap nets and held in net-pens for 2 to 5 d prior to gamete collection. Fish were anaesthetized using MS-222, weighed, total lengths taken, and eggs or milt were collected by light pressure on the abdomen. Male fish were released after recovery in fresh water.

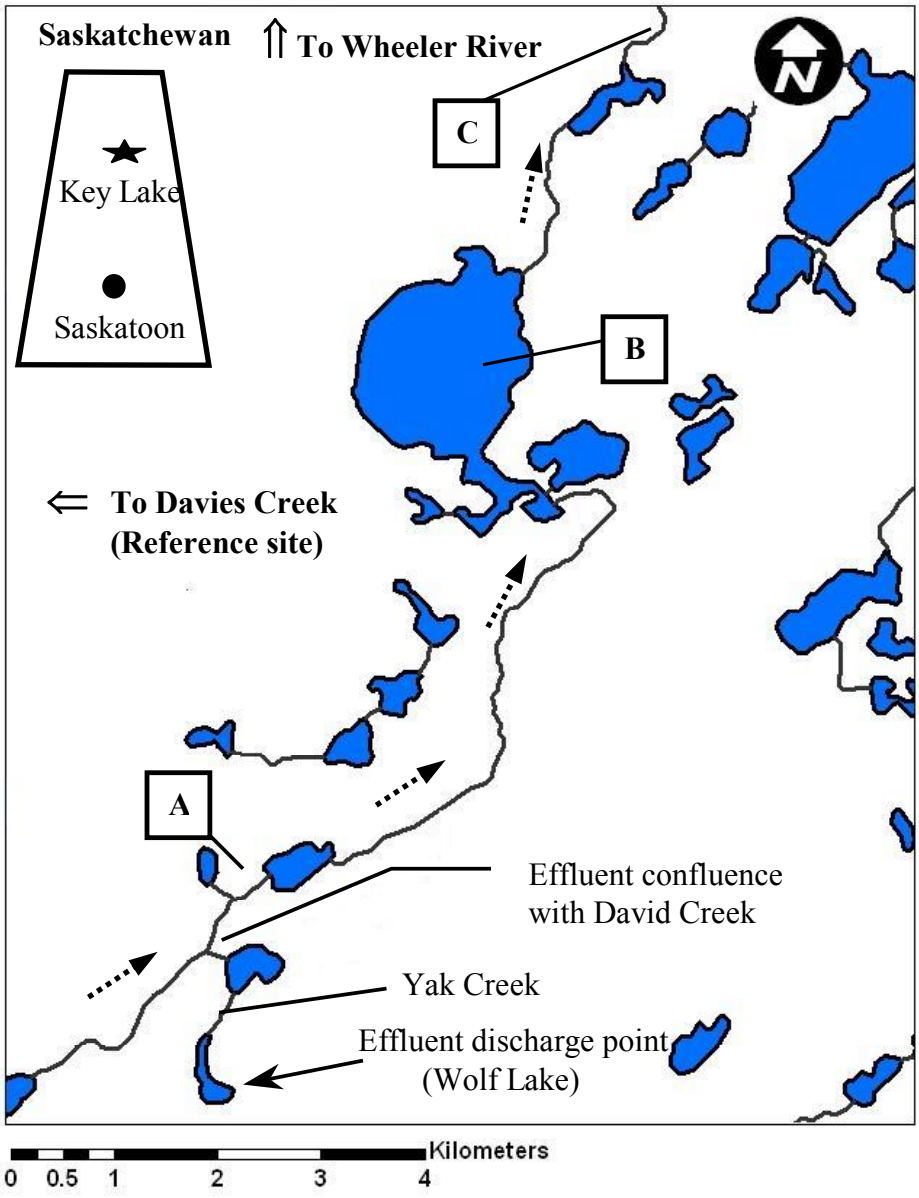

Figure 3.1: Map of Key Lake study area (Saskatchewan, Canada). Insert: map of Saskatchewan showing the relative location of Key Lake uranium mine operations. Sampling sites: Sampling sites: (A) high (David Creek, near-field), (B) medium (Delta Lake) and (C) low (David Creek, far-field) exposure sites. Solid arrow, mill effluent discharge into Wolf Lake. Dashed arrows, flow direction.

Female fish were euthanized for collection of kidney, liver, muscle (caudal region), and bone (spine) for metal analysis. Ageing structures (cleithra and scales) were collected for age determination. Water samples were collected at each site for subsequent basic water chemistry and trace metals analysis.

3.2.3. Egg fertilization

Before fertilization a sub-sample of eggs from each female was collected for trace element analysis. Eggs from each female were fertilized separately with 0.5 mL of pooled milt derived from 4-5 males captured at the same site. Water from each respective site was then added to activate the sperm and gently mixed for 2 min. Eggs were then transferred to 4-L plastic jars filled with site water (for egg hardening), stored on ice and transported to the University of Saskatchewan (U of S) on the day of fertilization.

3.2.4. Laboratory embryo incubations

All quality assurance/quality control procedures for embryo incubations followed as closely as possible the guidelines described by Environment Canada (1998) for early life stage rainbow trout. All materials used during the egg fertilization and embryo incubation procedures were sterilized using a 0.000075% betadyne (povione-iodine) solution. Upon arrival at the U of S, a sub-sample of 100 embryos from each female was stored in 20-ml

scintillation vials containing 70% ethanol for subsequent determination of fertilization success, calculated as:

$$\text{(Number of fertilized eggs / Total number of eggs)} \times 100 \qquad (3.1)$$

Embryo incubations were conducted under static-renewal conditions using 4-L plastic buckets in an environmental chamber with a set photoperiod of 16:8 h light:dark and a temperature of 10 ± 1 °C. Water was collected every 3 days from study sites, shipped to the U of S, and used to replace incubation water every 2 d during embryo development. Water samples for routine water quality analyses and trace elements analysis were collected from three incubation chambers per treatment before and after water renewal. Embryo mortality, temperature and dissolved oxygen were recorded daily in all incubation chambers throughout the study.

Embryos were incubated using a two-way (cross-over) ANOVA experimental design using water obtained either from reference or exposure sites. Thus, embryos originating from reference or exposure site females were incubated in either reference or appropriate exposure site water. In addition, embryos originating from the reference site females were incubated in water from all four study sites. Fifty viable embryos from each individual female fish were randomly transferred to each of four replicate incubation chambers. Cumulative time (degree-days) to the 50% eyed embryo, 50% hatch, and 50% swim up stages were determined for each incubation chamber. The experiment was terminated individually for each incubation chamber when the majority of the fry exhibited swim-up and had absorbed the egg yolk. Fry were euthanized with an overdose of MS-222 (0.8 g/L),

preserved in 10% buffered formalin for 24 h, and then transferred to 70% ethanol for subsequent evaluation of deformities.

3.2.5. Evaluation of morphological deformities

All preserved fry were examined for malformations in a blind fashion using an Olympus model S261 dissecting microscope (Olympus, Melville, NY, USA) with Image-Pro Discovery software version 4.5 (Media Cybernetics Inc., Silversprings, MD, USA). Abnormalities were recorded in four categories: skeletal curvatures, craniofacial, finfold, and edema (Holm et al., 2005). Each category of deformity was calculated as:

(Number of fry presenting one category of deformity/ Total number of fry) x 100 (3.2)

In addition to the frequencies of each category of deformity, the total percentage of deformed fry was calculated as:

(Total number of deformed fry (per female)/Total number of fry per female) x 100 (3.3)

3.2.6. Egg, tissues and water samples for trace element analysis

Total trace element analyses in water, eggs, muscle and bone from female pike were performed by the Saskatchewan Research Council, Saskatoon, SK, Canada using inductively coupled plasma-mass spectrometry (ICP-MS) for water, eggs and muscle, and inductively coupled plasma-atomic emission spectrometry (ICP-AES) for bone. Average trace element recovery > 90 % for all the evaluated elements. Kidney and liver samples

were analyzed only for Se concentrations using hydride generation atomic absorption spectrometry (HG-AAS, recovery > 90% using certified dogfish muscle DORM-2, National Research of Canada). Percent moisture of eggs (74.01 ± 1.54 %), muscle (77.07 ± 0.27 %), bone (58.01 ± 0.99 %), liver (75.10 ± 0.02 %) and kidney (79.70 ± 0.53 %) were determined for each female fish by drying samples for 24 h at 60°C, and all Se residue data are thus expressed on a DW basis.

3.2.7. Statistical analysis

Analysis of covariance (ANCOVA; SYSTAT® version 10 (SSI Inc., Richmond, CA, USA) was used to compare the body weights of adult pike with body length as covariate, and liver weight and egg diameter from female pike with body weight as covariate. The remaining statistical analyses were performed using SigmaStat® version 3.1 (SPSS Inc., Chicago, IL, USA) with a 95% (α= 0.05) level of confidence. Data that failed tests for normality or homogeneity of variance were $\log_{(10)}$ or arcsine square root transformed prior to use of parametric statistical tests. Significant differences among treatments in cumulative time to 50% eyed embryos, 50% hatch and 50% swim-up, mortality and fry deformities were evaluated using two-way ANOVA with egg origin and water source as the two factors. Best fit relationships between the incidence of deformities and Se concentrations in eggs and muscle, and between muscle, bone, kidney, liver and egg Se concentrations were evaluated using regression analysis. Other statistical analyses were performed using one-way ANOVA followed by Dunnett's test as appropriate.

3.3. Results

3.3.1. Water quality

Routine water quality variables were determined in site water at the time of adult fish collection (Table 3.1). Water quality variables and trace elements analysis in embryo incubation chambers were similar when measured before and after 48 h water renewal (Table 3.2; 3.3 and 3.4). In general, water collected from exposure sites was characterized by higher conductivity, hardness and ammonia, and lower pH, compared to the reference site. Water quality variables were consistent among water samples collected from study sites and water collected from embryo incubation chambers (Tables 3.1 and 3.2).

3.3.2. Adult characteristics

Reproductively mature pike were collected from the reference site in 2004 (n=3 female, n=5 male) and 2005 (n=2 female, n=5 male). Statistical comparisons of all endpoints determined in these reference pike revealed no significant differences between years, and thus these data were pooled (Table 3.5). Only the adult female pike collected from the high exposure site in 2004 was in spawning condition, however tissues were collected from two females sampled in 2004 and 2005 at this site. Condition factors (body weight with body length as covariate) of female and male northern pike collected from the high and medium exposure sites were significantly lower than pike collected from the reference site ($p < 0.05$; Table 3.5). There were no differences in hepatosomatic index (liver weight with body weight as covariate) or age among female pike collected from reference and exposure sites (Table 3.5).

3.3.3. Selenium in eggs and larval deformity analysis

Selenium concentration was significantly greater ($p < 0.05$; Table 3.5) in female pike eggs from the medium exposure site (31.28 ± 5.97 μg/g DW) compared to eggs from reference site female pike (3.19 ± 0.29 μg/g DW). Of all nineteen trace elements analyzed, only Se was present at a significantly higher concentration in pike eggs originating from the medium exposure site compared to the reference site (Table 3.6).

The single female pike in spawning condition collected from the high exposure site had the highest egg Se concentration of all sites (48.23 μg/g DW; Table 3.5). No significant difference was found in the mean egg Se concentration in female pike from the low exposure site (3.80 ± 0.16 μg/g DW) when compared to the reference site (Table 3.5). Mean egg diameter and fertilization success did not differ among sites, ranging from 2.6 to 2.8 mm and 70 to 88 %, respectively (Table 3.5). Cumulative embryo mortality throughout incubation was not significantly different among sites, ranging from 45 to 60%. The rates of embryo mortality found in this study are in accordance with the expected mortality for northern pike (Scott and Crossman, 1973). There were no significant differences in condition factor of fry:

$$(\text{Body weight}/ (\text{Length})^3) \times 100 \qquad (3.4)$$

between the reference site (0.41 ± 0.04) and high (0.28), medium (0.34 ± 0.05) or low (0.58 ± 0.13) exposure sites.

Table 3.1: Water quality variables measured on-site at fish collection.

Variable	Reference [a]	High [a]	Medium	Low
Hardness (mg $CaCO_3$/L) [b]	4.3 ± 0.1	142.3 ± 0.6	192.0 ± 1.1	171.0 ± 0.1
Alkalinity (mg $CaCO_3$/L) [b]	12.7 ± 0.3	6.0 ± 0.1	4.7 ± 0.3	10.0 ± 0.1
pH	6.99	5.80	5.78	6.24
Conductivity (µS/cm)	14.1	355.7	479.7	279.3
Temperature (°C)	7.7	8.0	4.5	10.2
DO (mg/L) [c]	12.4	10.0	10.4	10.8

[a] Composite data from years 2004 and 2005.

[b] Data represent the mean (± SE) of three replicate samples.

[c] Dissolved Oxygen

Table 3.2: Water quality variables measured weekly before and after (bold) water renewal in 3 randomly selected incubation chambers during the laboratory embryo incubations**.** Data represent the mean (± SE) of three replicate samples.

Variable	Reference [a]	High [a]	Medium	Low
Hardness (mg $CaCO_3$/L)	5.7 ± 1.4	209.5 ± 72.5	153.5 ± 0.5	149.4 ± 2.7
	4.9 ± 0.6	**182.1 ± 50.0**	**153.5 ± 6.3**	**146 ± 8.3**
Alkalinity (mg $CaCO_3$/L)	11.4 ± 3.0	12.0 ± 1.7	13.3 ± 0.2	11.6 ± 0.4
	14.6 ± 0.2	**9.8 ± 1.1**	**13.1 ± 0.1**	**11.6 ± 0.3**
pH	7.23 ± 0.1	6.51 ± 0.2	6.79 ± 0.01	7.33 ± 0.1
	7.20 ± 0.1	**6.82 ± 0.2**	**6.80 ± 0.1**	**7.43 ± 0.1**
Conductivity (µS/cm)	15.7 ± 4.6	350.0 ± 1.0	367.3 ± 0.1	159.6 ± 1.9
	18.2 ± 0.5	**372.7 ± 3.8**	**367.3 ± 11.2**	**160.0 ± 5.1**
Temperature (°C)	10.8 ± 0.5	10.2 ± 0.6	10.6 ± 0.50	10.2 ± 0.3
	11.5 ± 0.1	**11.4 ± 0.20**	**11.3 ± 0.2**	**11.5 ± 0.3**
DO (mg/L) [b]	10.4 ± 0.23	11.3 ± 0.3	11.2 ± 0.1	9.5 ± 0.4
	11.2 ± 0.5	**10.9 ± 0.9**	**11.5 ± 0.6**	**11.4 ± 0.7**
Ammonia (mg/L)	0.2 ± 0.01	1.8 ± 0.03	0.9 ± 0.01	0.3 ± 0.03
	0.2 ± 0.01	**1.9 ± 0.04**	**0.8 ± 0.02**	**0.4 ± 0.03**

[a] Composite data from years 2004 and 2005.

[b] Dissolved Oxygen.

Table 3.3: Total trace element concentration (µg/L) measured before water renewal in 3 randomly selected incubation chambers during the laboratory embryo incubations**.** Data represent the mean (± SE) of three replicate samples. Reference site trace metal concentrations were not available. Below detection limit (<).

Metals	High [a]	Medium	Low
Ag	< 0.1	< 0.1	< 0.1
Al	44.0 ± 0.0	17.33 ± 1.53	5.27 ± 1.76
As	3.50 ± 0.14	0.46 ± 0.06	0.73 ± 0.06
B	87.0 ± 0.45	70.0 ± 0.0	130.0 ± 10.0
Ba	7.80 ± 0.56	4.07 ± 0.21	8.76 ± 0.25
Be	< 0.1	< 0.1	< 0.1
Cd	< 0.5	< 0.5	< 0.5
Co	0.76 ± 0.15	0.50 ± 0.03	0.30 ± 0.0
Cr	< 5.0	< 5.0	< 5.0
Cu	0.67 ± 0.22	0.40 ± 0.17	< 0.2
Fe	84.0 ± 23.40	71.33 ± 17.95	48.0 ± 44.79
Mn	545.23 ± 19.45	426.66 ± 20.81	75.33 ± 6.11
Mo	125.67 ± 13.67	81.33 ± 4.16	90.0 ± 5.29
Ni	7.34 ± 0.67	2.23 ± 0.30	2.16 ± 0.06
Pb	< 0.1	< 0.1	< 0.1
Sb	3.34 ± 1.23	2.36 ± 2.07	< 0.2
Se	0.80 ± 0.20	0.30 ± 0.0	1.16 ± 0.11
Sn	2.50 ± 0.04	0.23 ± 0.06	0.12 ± 0.07
Sr	94.85 ± 6.70	82.66 ± 3.05	98.33 ± 2.08
Ti	180.0 ± 35.67	0.60 ± 0.0	0.43 ± 0.11
Tl	< 0.2	< 0.2	< 0.2
U	0.45 ± 0.02	0.16 ± 0.06	< 0.1
V	0.35 ± 0.05	< 0.1	< 0.1
Zn	24.46 ± 9.63	12.0 ± 2.64	18.33 ± 8.14

[a] Composite data from years 2004 and 2005.

Table 3.4: Total trace element concentration (µg/L) measured after water renewal in 3 randomly selected incubation chambers during the laboratory embryo incubations**.** Data represent the mean (± SE) of three replicate samples. Below detection limit (<).

Metals	Reference [a]	High [a]	Medium	Low
Ag	< 0.1	< 0.1	< 0.1	< 0.1
Al	17.0 ± 0.0	44.0 ± 0.0	28.66 ± 2.082	6.03 ± 1.76
As	0.10 ± 0.0	3.50 ± 0.14	0.53 ± 0.06	0.48 ± 0.37
B	< 10	50.87 ± 0.34	83.33 ± 20.82	91.66 ± 75.22
Ba	3.75 ± 0.25	9.55 ± 0.35	5.80 ± 0.80	6.50 ± 3.55
Be	< 0.1	< 0.1	< 0.1	< 0.1
Cd	< 0.5	< 0.5	< 0.5	< 0.5
Co	< 0.1	0.30 ± 0.0	0.50 ± 0.0	0.21 ± 0.14
Cr	< 5	< 5	< 5	< 5
Cu	0.30 ± 0.10	0.65 ± 0.35	0.80 ± 0.87	< 0.2
Fe	130.0 ± 0.0	340.0 ± 14.14	91.33 ± 16.92	65.66 ± 32.92
Mn	0.01 ± 0.0	260.34 ± 34.56	270.0 ± 62.45	52.75 ± 45.46
Mo	2.45 ± 1.45	123.50 ± 2.12	64.33 ± 2.51	62.96 ± 53.75
Ni	0.10 ± 0.0	2.30 ± 0.14	2.13 ± 0.35	1.41 ± 1.18
Pb	< 0.1	< 0.1	< 0.1	< 0.1
Sb	< 0.2	< 0.2	1.56 ± 2.36	< 0.2
Se	< 0.1	1.0 ± 0.0	0.53 ± 0.20	1.10 ± 0.10
Sn	0.10 ± 0.0	0.10 ± 0.0	0.30 ± 0.20	0.20 ± 0.10
Sr	11.0 ± 0.0	83.50 ± 0.70	81.0 ± 10.0	66.33 ± 48.80
Ti	5.80 ± 0.10	180.0 ± 0.0	0.70 ± 0.17	0.66 ± 0.06
Tl	< 0.2	< 0.2	< 0.2	< 0.2
U	< 0.1	0.30 ± 0.0	0.15 ± 0.13	< 0.1
V	0.10 ± 0.0	0.25 ± 0.07	0.06 ± 0.03	9.38 ± 16.12
Zn	< 5	25.0 ± 12.45	17.0 ± 2.64	7.0 ± 1.0

[a] Composite data from years 2004 and 2005.

There were no significant differences in the cumulative time to 50% eyed embryos, 50% hatch or 50% swim-up among treatments (Figure 3.2). However, there was a significantly ($p < 0.05$) increased incidence of edema, skeletal deformities, craniofacial deformities and fin deformities in fry originating from pike collected at the medium exposure site (Figure 3.3). There were no differences in the frequencies of deformities observed between embryos originating from the reference and low exposure sites. Fry originating from the single female pike collected at the high exposure site exhibited deformities that were in all cases greater than fry originating from reference, low and medium exposure sites (Figure 3.3).

There were significant linear relationships between Se concentrations in northern pike eggs and the percentage of fry exhibiting edema ($p < 0.001$, $r^2 = 0.78$), skeletal deformities ($p < 0.001$, $r^2 = 0.88$), craniofacial deformities ($p < 0.001$, $r^2 = 0.84$) and fin deformities ($p = 0.021$, $r^2 = 0.63$) (Figure 3.4). There were significant quadratic relationships between Se concentrations in pike muscle and the percentage of fry exhibiting edema ($p = 0.002$, $r^2 = 0.76$), skeletal deformities ($p < 0.001$, $r^2 = 0.91$), craniofacial deformities ($p < 0.001$, $r^2 = 0.80$) and fin deformities ($p = 0.001$, $r^2 = 0.77$) (Figure 3.5). In addition, the percentage of total deformities was significantly different ($p < 0.05$) between reference and medium exposure sites (Figure 3.6 A), showing strong positive relationships with egg ($p < 0.001$, $r^2 = 0.78$) and muscle ($p < 0.001$, $r^2 = 0.80$) Se concentrations (Figures 3.6 B and C, respectively).

Tissue Se concentrations associated with a 1%, 5%, 10% and 20% increase in total deformities above reference values (EC01, EC05, EC10 and EC20, respectively) were

calculated for pike embryos as a function of egg and muscle Se concentrations (Table 3.7). Whole-body EC values for total deformities were derived from the EC values for egg and muscle (Table 3.7), using formulas obtained from the USEPA (2004).

3.3.4. Trace element determinations in adult pike tissues

Muscle, liver, kidney and bone Se concentrations were significantly greater ($p < 0.05$) in female pike collected from the high and medium exposure sites compared to the reference site (Table 3.5). There were positive linear relationships between Se concentrations in northern pike eggs and muscle ($r^2 = 0.83$, $p < 0.001$), liver ($r^2 = 0.87$, $p < 0.001$), kidney ($r^2 = 0.97$, $p < 0.001$) and bone ($r^2 = 0.80$, $p < 0.001$; Figure 3.7). Other trace elements that were significantly different in pike bone between reference and exposure sites (high and medium) were barium (Ba), chromium (Cr), manganese (Mn), molybdenum (Mo), potassium (K) and strontium (Sr) (Table 3.8). However, trace element analyses of northern pike eggs and muscle revealed that Se was the only metal (of nineteen measured) significantly different among sites (Tables 3.5-3.6; 3.8-3.9). No differences in Se concentrations were found between reference and low exposure sites for any of the analyzed tissues (Table 3.5).

Table 3.5: Somatic measurements and concentrations of selenium (Se) in eggs and tissues of (A) female and (B) male northern pike. Data represent the mean (± SE). * Significant difference ($p < 0.05$) between reference and exposure site in pike condition factor (fish weight with length as covariate). ** Significantly different ($p < 0.05$) from reference site using ANOVA followed by Dunnett's test.

A)

Variable	Reference [a] (*n* = 5)	High [a] (*n* = 2)	Medium (*n* = 3)	Low (n = *5)*
Age (yrs)	9.30 ± 0.81	8.50 ± 0.50	7.60 ± 1.20	6.80 ± 0.37
Length (cm)	76.10 ± 6.92	61.40 ± 6.56	65.66 ± 4.09	67.56 ± 1.06
Weight (kg)	4.91 ± 0.85	2.54 ± 0.06	2.34 ± 0.38	3.06 ± 0.14
Condition factor [b]	1.34 ± 0.10	1.11 ± 0.11 *	0.81 ± 0.06 *	1.02 ± 0.25
HSI [c]	0.85 ± 0.09	0.56 ± 0.08	0.92 ± 0.07	0.76 ± 0.08
Egg size (mm)	2.83 ± 0.05	2.66 [d]	2.70 ± 0.06	2.73 ± 0.06
Fertilization (%) [e]	70.01 ± 8.54	78.22 [d]	88.04 ± 2.53	79.11 ± 2.45
Total fry evaluated	727	252	520	274
Egg Se (μg/g) [f]	3.19 ± 0.29	48.23 [d]	31.28 ± 5.97 **	3.80 ± 0.16
Muscle Se (μg/g) [f]	1.64 ± 0.69	38.27 ± 9.53 **	16.58 ± 0.15 **	1.80 ± 0.53
Bone Se (μg/g) [f]	0.55 ± 0.15	13.25 ± 2.94 **	5.61 ± 0.14 **	1.24 ± 0.44
Liver Se (μg/g) [f]	5.60 ± 0.36	51.06 ± 2.48 **	25.76 ± 1.77 **	5.10 ± 0.78
Kidney Se (μg/g) [f]	6.83 ± 0.56	62.21 ± 13.64 **	40.45 ± 5.49 **	7.07 ± 1.07

B)

Variable	Reference [a] (*n* = 10)	High (*n* = 4)	Medium (*n* = 5)	Low (*n* = 5)
Length (cm)	53.23 ± 1.66	54.17 ± 5.65	60.80 ± 3.96	54.02 ± 4.64
Weight (kg)	2.42 ± 0.17	2.12 ± 0.39	1.85 ± 0.34	2.38 ± 0.26
Condition factor [b]	1.63 ± 0.27	1.22 ± 0.12 *	0.78 ± 0.05 *	1.64 ± 0.48

[a] Pooled data from 2004 and 2005 fish collection.

[b] Condition factor calculated as (weight (g)/length^3(cm))

[c] Hepatosomatic index (liver weight/body weight) x 100.

[d] Eggs collected from one female *n* = 1 in 2004.

[e] Fertilization success (%), calculated as (number of fertilized x 100 eggs/total number of eggs) x 100.

[f] Selenium concentration in μg/g dry weight.

Table 3.6: Total trace element concentration (µg/g, dry weight) in northern pike eggs. Data represent the mean (± SEM). Below detection limit (<).

Metals	Reference [a] (*n*= 5)	High (*n*= 2)	Medium (*n*= 3)	Low (*n*= 5)
Ag	< 0.002	< 0.002	< 0.002	< 0.002
Al	1.69 ± 0.77	0.074	1.34 ± 0.35	2.24 ± 0.78
As	0.14 ± 0.09	2.93	0.07 ± 0.03	0.07 ± 0.0
B	< 0.2	0.37	0.37 ± 0.01	0.91 ± 0.60
Ba	0.57 ± 0.20	0.02	0.15 ± 0.06	0.30 ± 0.04
Be	< 0.002	< 0.002	< 0.002	< 0.002
Cd	< 0.002	< 0.002	< 0.002	< 0.002
Co	0.03 ± 0.01	0.12	0.07 ± 0.03	0.04 ± 0.01
Cr	0.28 ± 0.08	0.19	0.18 ± 0.04	0.36 ± 0.01
Cu	2.67 ± 0.21	2.89	3.11 ± 0.19	2.89 ± 0.16
Fe	27.90 ± 6.45	66.79	50.29 ± 16.0	43.81 ± 10.85
Mn	9.75 ± 1.65	2.23	9.37 ± 3.60	6.38 ± 0.42
Mo	< 0.02	0.63	0.26 ± 0.07	< 0.02
Ni	< 0.01	< 0.01	< 0.01	< 0.01
Pb	< 0.005	< 0.005	< 0.005	< 0.005
U	< 0.002	0.01	< 0.002	0.005 ± 0.001
V	< 0.02	< 0.02	< 0.02	< 0.02
Zn	98.22 ± 5.74	85.34	93.14 ± 2.60	112.43 ± 9.82

[a] Pooled data from 2004 and 2005 fish collection .

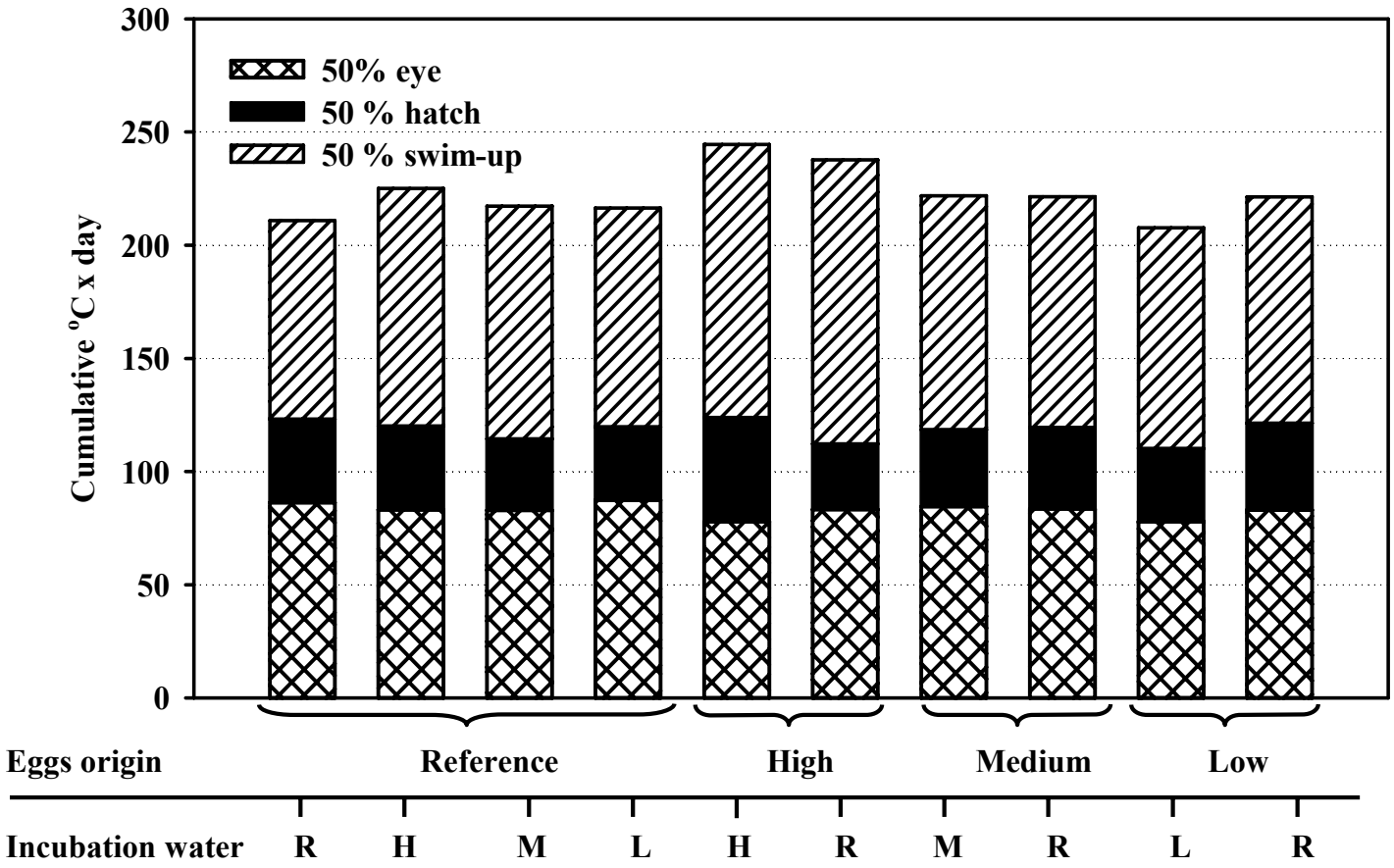

Figure 3.2: Cumulative time (^{o}C x day) to 50% eyed embryo, 50% hatch and 50% swim-up for northern pike embryos incubated in either reference (R), or exposure (high (H), medium (M), low (L)) water. Data represent the mean of four replicate incubation chambers per treatment.

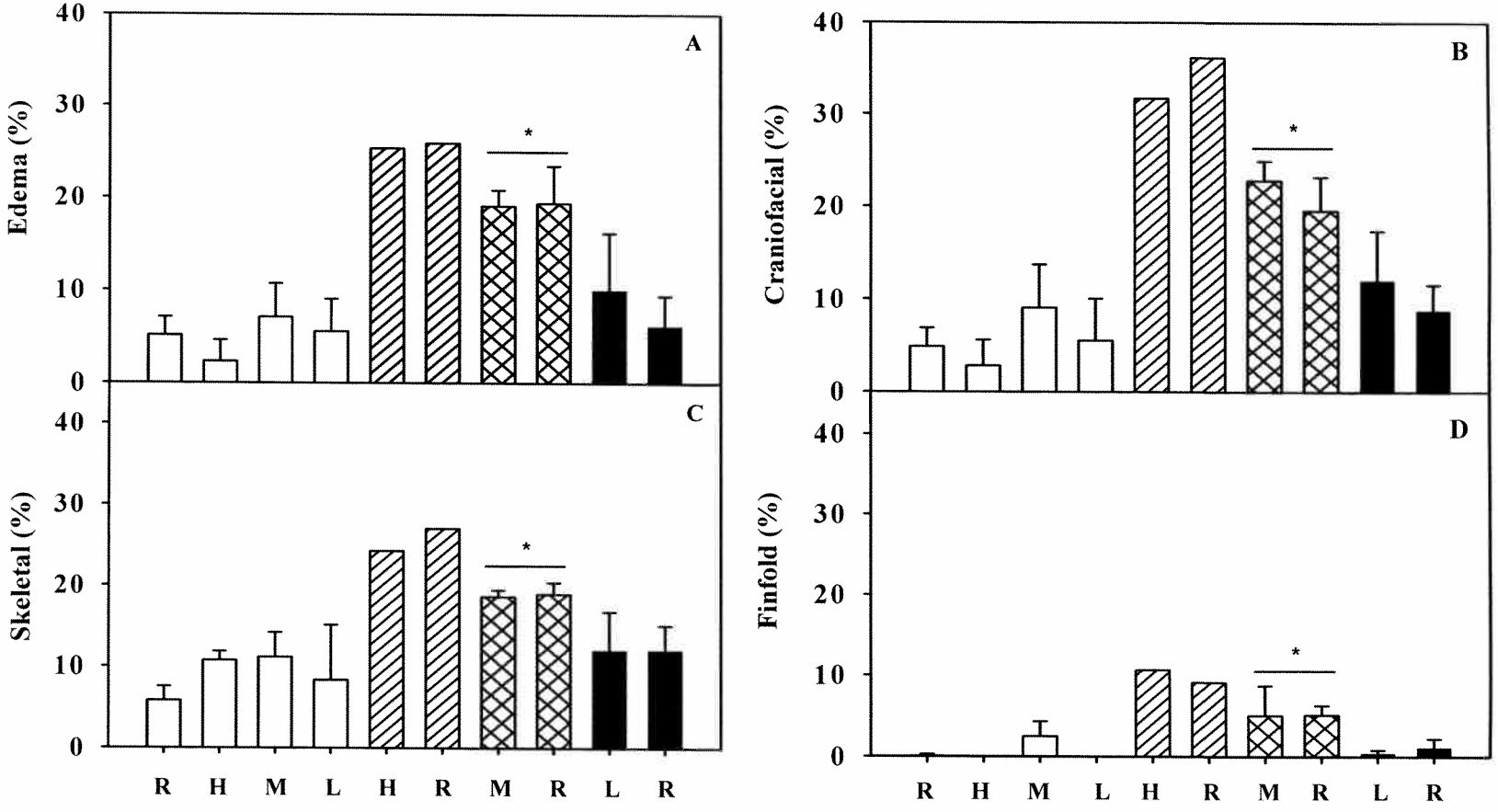

Figure 3.3: Analysis of external malformations (A) edema, (B) craniofacial, (C) skeletal and (D) finfold in northern pike fry originating from reference, high , medium and low exposure sites and incubated in both reference (R) and exposure (high (H), medium (M), low (L)) water. Data represent the mean (± SE) per treatment. * Significant difference ($p < 0.05$) from reference due to egg's origin. Fry from high exposure site were not included in the statistical analysis.

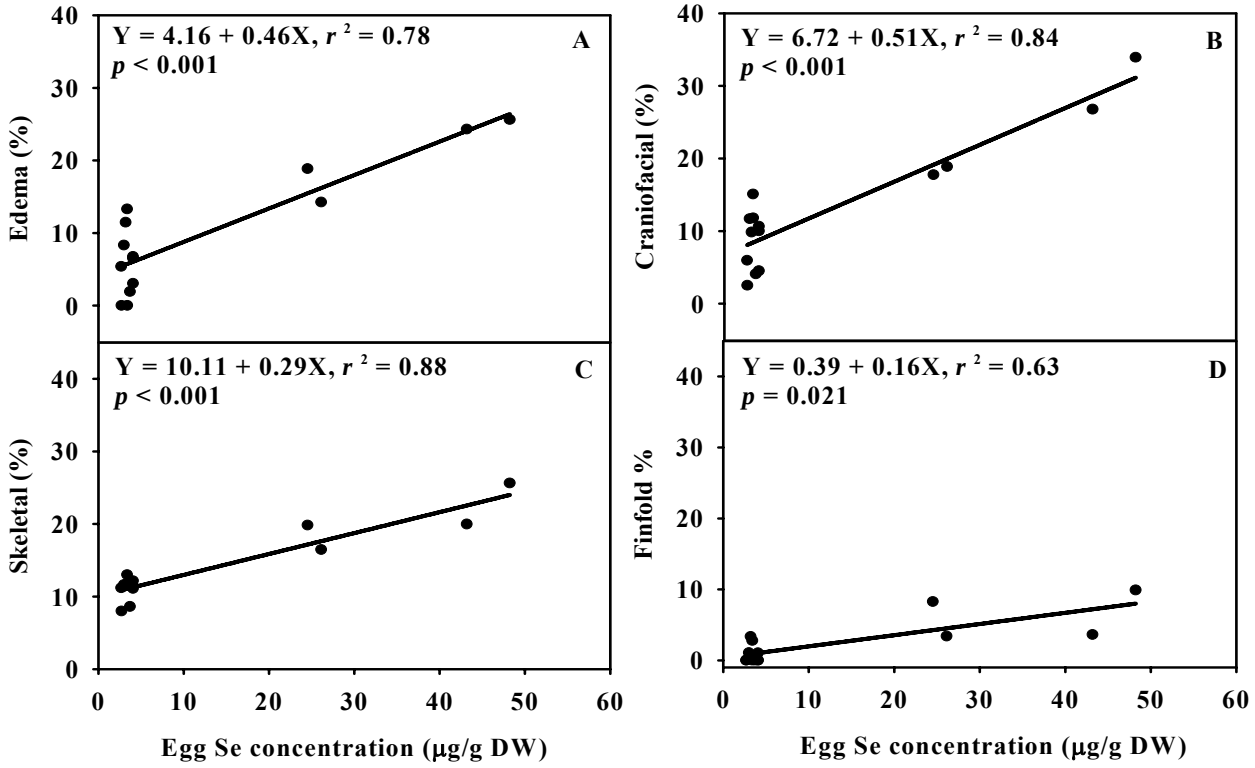

Figure 3.4: Relationships between selenium (Se) concentration (µg/g dry weight) in eggs and (b) muscle obtained from female northern pike collected from reference site (n = 5) and exposure sites (high (n = 1), medium (n = 3), low (n = 5)) and the percentage of deformities: (A) edema, (B) craniofacial, (C) skeletal and (D) finfold in fry originating from each female. Points represent the mean per female.

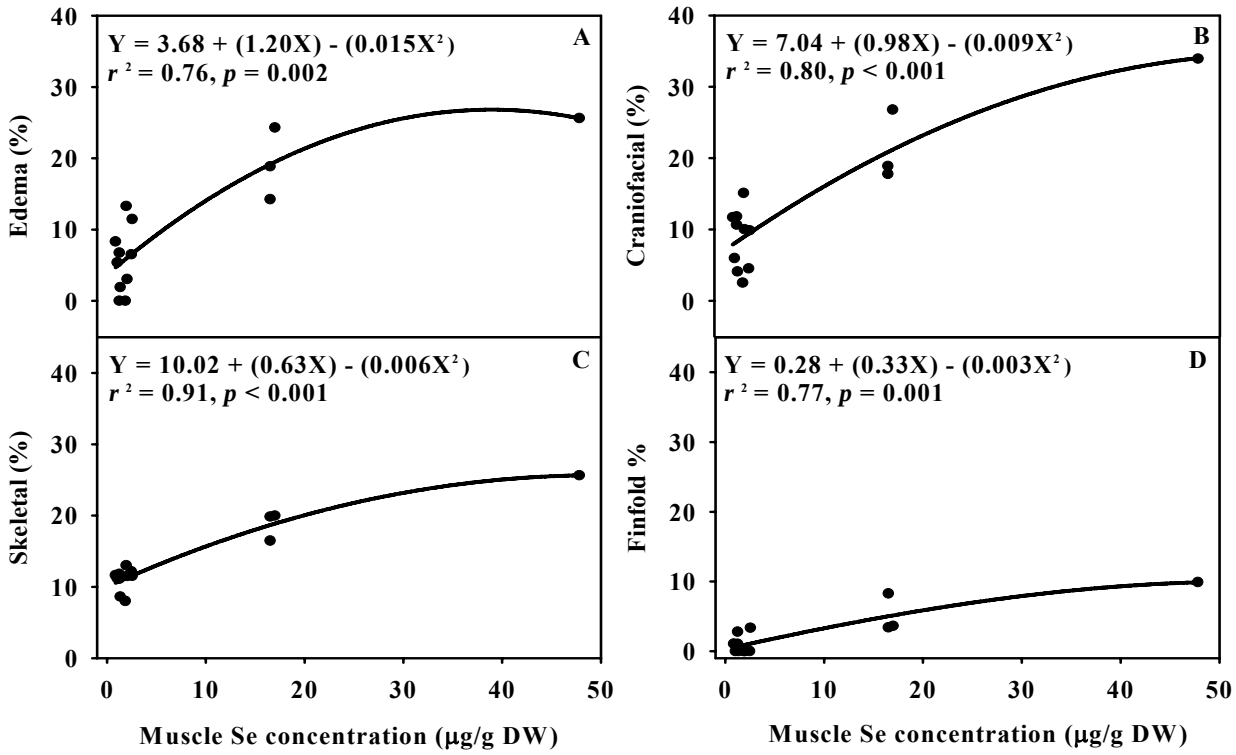

Figure 3.5: Relationships between selenium (Se) concentration (µg/g dry weight) in muscle obtained from female northern pike collected from reference site (n = 5) and exposure sites (high (n = 1), medium (n = 3), low (n = 5)) and the percentage of deformities: (A) edema, (B) craniofacial, (C) skeletal and (D) finfold in fry originating from each female. Points represent the mean per female.

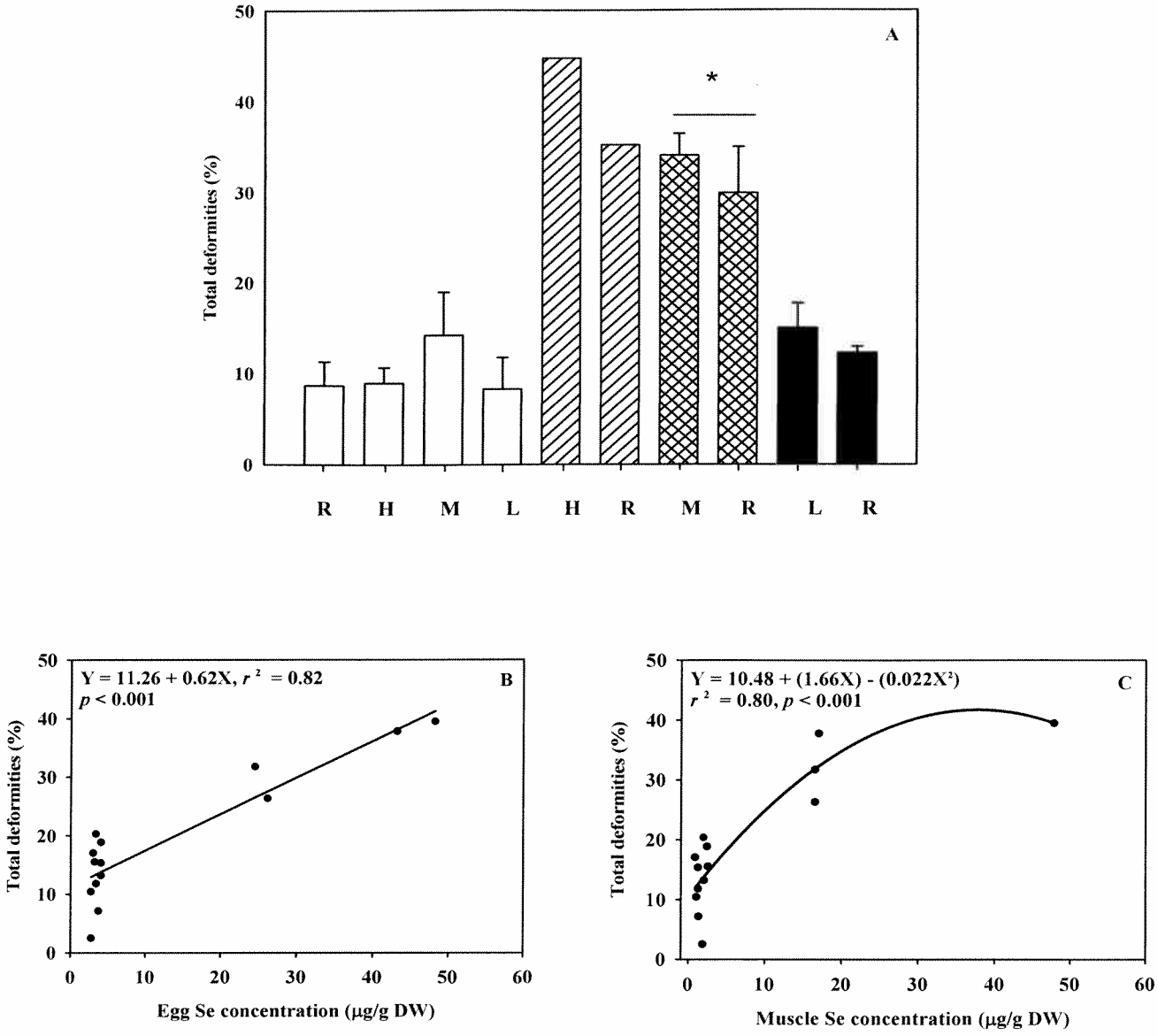

Figure 3.6: Total deformities evaluated in northern pike fry. (A) Analysis of total deformities among embryos originated from ☐ reference, ▨ high, ▩ medium and ■ low exposure sites and incubated in both reference (R) and exposure (high (H), medium (M), low (L)) water. Data represent the mean (± SE) per treatment. * Significant difference ($p < 0.05$) from reference due to egg's origin. Fry from high exposure site were not included in the statistical analysis. Relationship between (B) egg and (C) muscle selenium (Se) concentration (µg/g dry weight) and the total percentage of deformities in embryos originating from reference ($n = 5$) and exposure (high ($n = 1$), medium ($n = 3$), low ($n = 5$)) sites. Points represent the mean of four replicates for each female per treatment.

Table 3.7: Effect Concentration (EC) values (µg selenium/g dry weight) for total deformities in northern pike embryos. Data for egg and muscle represent EC values with 95% confidence interval.

Variable	Egg [a]	Whole-body [b]	Muscle [c]	Whole-body [d]
EC01	8.52 (0.10 - 16.94)	3.96	5.20 (0.0 - 14.53)	4.98
EC05	13.80 (5.61 - 21.96)	6.40	9.05 (0.0 - 18.10)	8.18
EC10	20.38 (11.05 - 29.71)	9.46	13.85 (3.54 - 24.16)	11.96
EC20	33.55 (18.90 - 48.19)	15.56	21.54 (5.35 - 37.73)	17.72

[a] EC values calculated from total deformities (%) in pike embryos as a function of egg selenium concentrations fitted to a linear curve (Figure 3.6 B).

[b] Equation II (USEPA, 2004) was used to convert the egg EC to the whole-body EC.

[c] EC values calculated from total deformities (%) in pike embryos as a function of muscle selenium concentrations fitted to a quadratic curve (Figure 3.6 C).

[d] Equation I (USEPA, 2004) was used to convert the muscle selenium EC to the whole-body EC

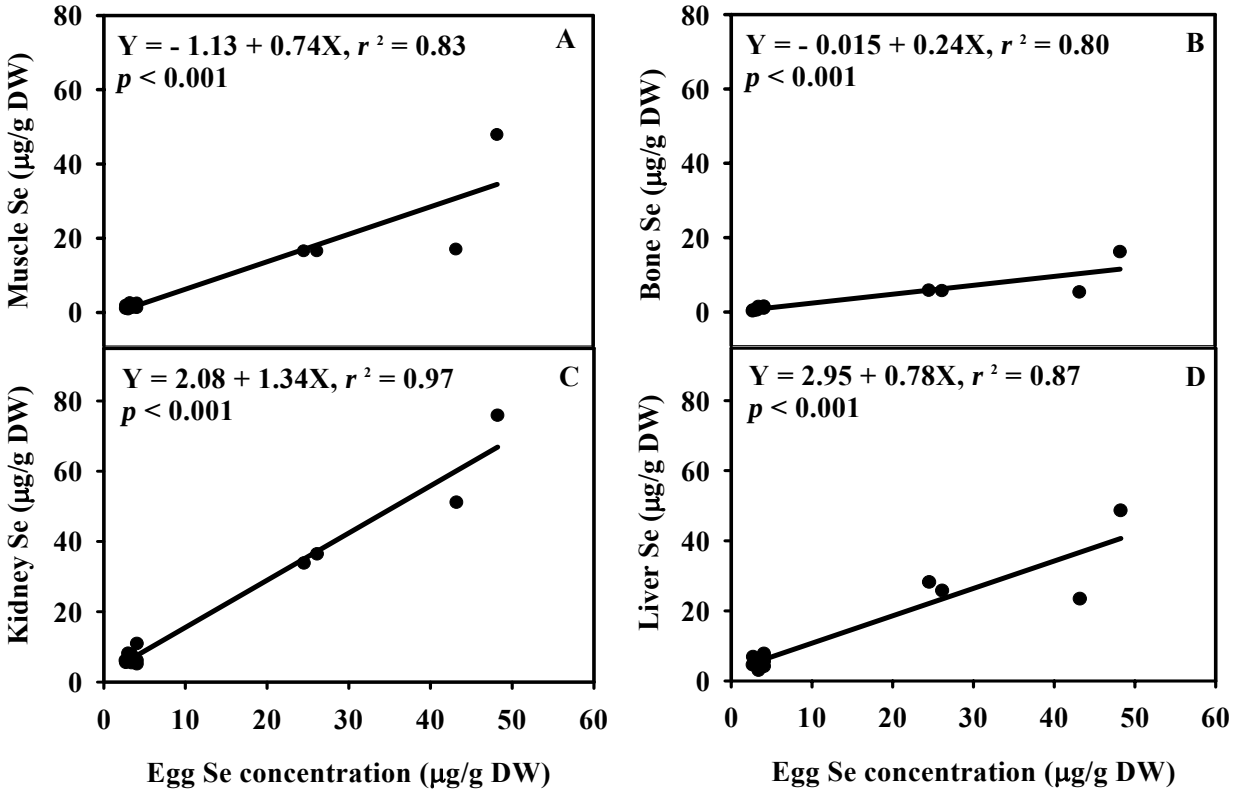

Figure 3.7: Relationships between selenium (Se) concentration in eggs (µg/g dry weight) obtained from female northern pike collected from reference site (n = 5) and exposure sites (high (n = 1), medium (n = 3), low (n = 5)) and Se concentration in adult female pike tissues: (A) muscle, (B) bone, (C) kidney and (D) liver.

Table 3.8: Total trace element concentration (µg/g, dry weight) in northern pike bone. Data represent the mean (± SE). Below detection limit (<).

Metals	Reference [a] (*n* = 5)	High (*n* = 2)	Medium (*n* = 3)	Low (*n* = 5)
Ag	< 0.1	< 0.1	< 0.1	< 0.1
Al	5.83 ± 1.23	4.29	4.23 ± 0.43	7.95 ± 0.90
B	< 0.2	< 0.2	< 0.2	< 0.2
Ba	7.82 ± 0.69	2.38	2.39 ± 1.24*	4.68 ± 0.97
Be	< 0.1	< 0.1	< 0.1	< 0.1
Ca	134,709 ± 30,472	147,857	124,770 ± 8,492	205,777 ±17,168
Cd	< 0.1	< 0.1	< 0.1	< 0.1
Co	< 0.1	< 0.1	< 0.1	< 0.1
Cr	2.31 ± 0.70	1.60	1.52 ± 0.12	4.39 ± 0.46*
Cu	0.40 ± 0.10	0.48	0.46 ± 0.003	0.49 ± 0.05
Fe	24.0 ± 8.92	14.29	7.47 ± 1.03	22.34 ± 5.15
K	3,390 ± 567	4,047	2,319 ± 274	5,665 ± 344*
Mg	1,685 ± 277	1,761	1,431 ± 96	2,271 ± 118
Mn	23.24 ± 1.98	8.10	9.18 ± 2.09*	25.48 ± 2.25
Mo	< 0.1	5.0	2.30 ± 0.34*	< 0.1
Na	6,691 ± 1,051	8,333	5,003 ± 304	8,883 ± 786
Ni	< 0.05	< 0.05	< 0.05	< 0.05
P	67,640 ± 13,837	75,000	62,045 ± 4,322	102,074 ± 8,038
Pb	< 0.1	< 0.1	< 0.1	< 0.1
Sr	144.11 ± 15.82	47.62	30.79 ± 3.21*	100.02 ± 12.35
Ti	0.35 ± 0.10	0.24	0.23 ± 0.002	0.44 ± 0.05
U	0.008 ± 0.0	0.018	0.03 ± 0.004	N/A [b]
V	< 0.1	0.71	< 0.1	< 0.1
Zn	83.32 ± 20.51	95.24	84.69 ± 7.75	141.52 ± 8.94
Zr	0.37 ± 0.13	0.24	0.23 ± 0.002	0.34 ± 0.09

[a] Pooled data from 2004 and 2005 fish collection .

Table 3.9: Total trace element concentration (µg/g, dry weight) in northern pike muscle. Data represent the mean (± SE). Below detection limit (<).

Metals	Reference [a] (*n* = 5)	High (*n* = 2)	Medium (*n* = 3)	Low (*n* = 5)
Ag	< 0.002	< 0.002	< 0.002	< 0.002
Al	2.55 ± 0.87	1.63	3.29 ± 2.30	4.61 ± 0.86
As	< 0.02	15	0.76 ± 0.37	< 0.02
B	< 0.2	< 0.2	< 0.2	< 0.2
Ba	0.24 ± 0.17	0.21	0.17 ± 0.04	0.11 ± 0.04
Be	< 0.002	< 0.002	< 0.002	< 0.002
Cd	< 0.002	< 0.002	< 0.002	< 0.002
Co	0.01 ± 0.005	0.04	0.02 ± 0.006	< 0.005
Cr	< 0.2	< 0.2	< 0.2	< 0.2
Cu	0.70 ± 0.19	1.0	0.78 ± 0.05	0.70 ± 0.05
Fe	10.08 ± 3.61	12.60	11.30 ± 3.91	23.91 ± 10.48
Mn	0.82 ± 0.40	0.78	1.02 ± 0.15	0.77 ± 0.09
Mo	< 0.02	0.13	0.10 ± 0.06	< 0.02
Ni	0.08 ± 0.04	0.02	0.10 ± 0.04	0.47 ± 0.35
Pb	0.05 ± 0.03	0.02	0.02 ± 0.01	0.02 ± 0.008
U	0.04 ± 0.03	0.03	0.01 ± 0.007	0.01 ± 0.003
V	< 0.02	< 0.02	< 0.02	< 0.02
Zn	17.48 ± 4.11	13.04	14.06 ± 3.42	20.34 ± 1.19

[a] Pooled data from 2004 and 2005 fish collection .

3.4. Discussion

Developmental malformations are reliable indicators of chronic Se toxicity in fish (Hodson and Hilton, 1983; Lemly, 1997b). They are produced as a consequence of parental exposure, maternal deposition of Se into eggs during vitellogenesis, and subsequent exposure during yolk resorption in developing larvae (Lemly, 1997b). The major finding of the present study was increased frequencies of edema and deformities (skeletal curvatures, craniofacial deformities and fin deformities) in fry originating from adult pike collected from effluent exposure areas. To our knowledge this is the first study investigating larval deformities associated with Se exposure in northern pike, a dominant top predator with a circumpolar distribution in north temperate and Arctic cold water aquatic ecosystems.

Some of the most evident larval deformities in fish caused by Se are found in the skeleton, fins, head and mouth (Hodson and Hilton, 1983; Lemly, 1993b). In the present study, craniofacial deformities, skeletal deformities, and edema were the most conspicuous developmental deformities observed in northern pike fry. Although the frequency of finfold deformities was also elevated in medium and high exposure site pike, they were less prevalent than the above-mentioned deformities. The increased frequency of deformities found in fry originating from high and medium exposure sites was associated with a 10-15 fold increase in mean Se concentration in eggs compared to reference site eggs. Furthermore, regression analysis revealed significant positive relationships between percentages of each category of deformity as a function of egg and muscle Se concentrations. Given the well documented suite of early life stage deformities associated with similar ranges of Se exposure in other fish species (Gillespie and Baumann, 1986;

Woock *et al,* 1987; Lemly, 1993a, 1993b; Coyle *et al*., 1993; Maier *et al*., 1994; DeForest *et a*l., 1999; Hamilton, 2003; Hamilton, 2004; Holm *et al*., 2005) in addition to Se being the only element significantly different in pike eggs between sites, the larval deformities observed in this study were consistent with Se exposure. Moreover, the increased concentration of Se in pike eggs associated with increased deformity rates in fry originating from the high and medium exposure sites, followed by a decrease in both Se egg concentrations and incidences of deformities in fry originating from the low exposure site (further downstream of effluent discharge), is also consistent with maternal transfer of Se to eggs as the causative factor for the effects observed in fry.

Further evidence supporting Se exposure as the causal factor involved in the increased deformities comes from the other developmental endpoints determined in this study. Earlier studies reported no effect of elevated Se body burden on fertilization success, embryo mortality or hatchability in bluegill sunfish (Gillespie and Baumann, 1986; Woock *et al*, 1987; Coyle *et al*., 1993). More recent studies in salmonids inhabiting cold water systems have also reported no effect of elevated Se body burdens on fertilization success, time to hatch and hatchability (Kennedy *et al*., 2000; Holm *et al*., 2005). The two-way ANOVA experimental design used in the present study allowed discrimination between effects due to maternal transfer compared to effects due to exposure of developing embryos to site water. Importantly, appropriate site water (reference or exposure) was used from the initial activation of sperm and subsequent egg hardening throughout exposures until the swim-up stage of fry. Despite this experimental approach, there were no differences in time to the eyed embryo, hatch, and swim-up stages of larval development among all treatments. These results suggest no toxic effect of high and medium exposure site water on the early

developmental stages of northern pike, and further support the conclusion that maternal transfer of Se to eggs and subsequent exposure of fry during yolk assimilation were responsible for the observed deformities.

The USEPA (2004) recommends a whole-body Se concentration of 7.91 μg/g DW as a chronic criterion for the protection of fish. As indicated by the USEPA (2004), criterion development should be based on a 20 percent increase in response relative to control (*i.e.*, EC_{20}). In this study total deformities increased from 12% overall in fry originating from the reference site to 39% and 31% overall in fry originating from high and medium exposure sites, respectively, indicating a 27% and 19% increase in deformities above naturally occurring levels. Based on these results, a 20 percent increase in total deformities in fish larvae relative to the reference (EC_{20}) was seen at egg and muscle Se concentrations of 33.55 and 21.54 μg/g DW, respectively. Equations provided by the USEPA (2004) were used to convert pike egg and muscle Se concentration to whole-body concentrations, resulting in similar whole body EC_{20}s of 15.56 and 17.72 μg/g, respectively. There is still controversy regarding appropriate Se thresholds for the protection of fish populations. Although there is general agreement that Se regulation requires a tissue-based criterion for fish (DeForest *et al.*, 1999; Hamilton, 2002), there does not appear to be agreement or consistency on the choice of appropriate threshold (i.e., EC) value. Although such inconsistencies make species comparisons difficult, the results observed in the present study indicate that northern pike are within the same range of sensitivity to Se as the majority of warm water (e.g., centrarchids and cyprinids) and cold water (e.g., salmonids) fish species studied to date. Further research is needed to fill existing data gaps before the

final criterion is adopted and to link the extent of Se-induced deformities in individual larval fish with potential negative impacts on fish population dynamics.

3.5. Conclusion

The majority of studies documenting Se toxicosis in fish populations have been conducted in warm water fish species. The objective of this study was to investigate Se toxicosis in north temperate fish larvae originating from reproductively mature northern pike (*Esox lucius*) collected downstream of a U milling operation in northern Saskatchewan, Canada. The major finding of this study was a significant increase in the frequencies of individual deformities (skeletal curvatures, craniofacial deformities and fin deformities) and edema in fry originating from high and medium exposure site females compared to reference site females. The increased deformities rates between reference and exposure site were due to the origin of the eggs and not to the site water use in embryo incubations. These results suggest that maternal transfer of Se as opposed to water exposure was the causative factor of the effects observed in fry. Selenium concentrations resulting in a 20% increase in total deformities above background levels (EC_{20}s) were 33.55 and 21.54 μg/g DW in eggs and muscle, respectively. Mathematical conversion of the egg- and muscle-derived relationships to whole body Se levels resulted in similar EC_{20}s of 15.56 and 17.72 μg/g DW, respectively. These relationships between tissue Se levels and larval deformities suggest that northern pike are within the same range of sensitivity to Se as the majority of warm water (e.g., centrarchids and cyprinids) and cold water (e.g., salmonids) fish species studied to date.

CHAPTER 4 [a]

Selenium accumulation in aquatic biota downstream of McClean Lake uranium mine and milling operation

[a] This chapter has been accepted for publication in Science of the Total Environment 407: 1318-1325, under joint authorship with David M Janz (University of Saskatchewan).

4.1. Introduction

Northern Saskatchewan, Canada, is home to some of the top producing uranium (U) mines in the world. Current U mining and milling practices usually generate elevated concentrations of trace elements (e.g., arsenic (As), molybdenum (Mo), nickel (Ni), selenium (Se) and U) and ions (e.g., sulphate, ammonium) that can be released into the surrounding aquatic systems (Klaverkamp *et al*., 2002; Pyle *et al*., 2001; deRosemond *et al*., 2005, Muscatello *et al*., 2006; Muscatello and Janz, 2008). The presence of elevated Se in the aquatic environment in comparison to reference sites is of concern due to its propensity to accumulate through the food chain and its potential to impair fish reproduction (Lemly, 1999).

Selenium is a non-metal element widely distributed throughout the environment and is found in most ground and surface waters at concentrations between 0.1 and 0.4 μg/L (USEPA, 2004). It presents six stable isotopes (e.g., ^{74}Se) and chemical properties similar to sulphur. Selenium can exist in different oxidation states (-II, 0, IV, VI) and as organic compounds (e.g., seleno-methionine) in natural waters (USEPA, 2004; ATSDR, 2003). Once released into the aquatic environment Se can be removed from the water-column and deposited into the sediments by adsorption, complexation, and co-precipitation processes, as well as, absorption by aquatic organisms (Lemly, 1999). Selenium can be mobilized from the sediments into the food chain by mechanisms present in most aquatic systems (e.g., biological, chemical and physical processes) (Lemly, 1997a, 1999; USEPA, 2004).

Waterborne inorganic Se can be accumulated and transformed into selenoaminoacids by primary producers significantly contributing to the sedimentation (e.g., by detrital

processes) of Se in aquatic systems (Fan *et al*., 2002). Furthermore, several authors have stated that primary producers are important contributors to the Se accumulation and its subsequent transfer to higher trophic levels in aquatic systems (Lemly, 1997a; Fan *et al*., 2002; Baines *et al*., 2004). Although Se can be taken up directly from water, much of the Se accumulated by aquatic consumers is likely the result of dietary intake (Lemly, 1993a; Hamilton, 2004; Stewart *et al*., 2004). Growth inhibition, tissue damage, reproductive impairment, and mortality in fish have occurred at dietary levels > 3 ug/g (dry weight (DW)) (Hodson and Hilton, 1983; Lemly, 1993a; May *et al*., 2008). Furthermore, Se displays an interesting paradox having a very narrow margin between essential (0.1 to 0.5 µg/g DW) and toxic dietary levels for fish (> 3 ug/g DW) (Wilber, 1980).

The U.S. Environmental Protection Agency (USEPA) proposed a chronic criterion for Se at a whole body fish concentration of 7.91 ug/g DW (USEPA, 2004). However, there is an ongoing controversy regarding whether the established threshold is too conservative or too liberal. Factors such as trophic level and habitat preference can significantly modify the accumulation and toxicity of Se in aquatic organisms. Therefore, the information required to establish an appropriate Se threshold for the protection of fish populations should include not only the Se concentrations and associated adverse effects, but also the Se accumulation and biomagnification factors between trophic levels. Since the majority of Se research has focused on the accumulation and effects of Se in warm water aquatic systems, there also is a recognized need for more research focusing on cold water systems.

The objective of this study was to evaluate Se concentrations in water, sediments, plankton, periphyton, invertebrates, and fish downstream of a U mining and milling

operation in northeastern Saskatchewan, Canada. These data were used to calculate the accumulation factors between higher and lower trophic levels.

4.2. Materials and Methods

4.2.1. Study site

The McClean Lake U mining and milling operation is located in north-eastern Saskatchewan (N 58º 23' W 103º 48') approximately 800 km north of Saskatoon, SK, Canada. This mine has been in operation since its opening in 1999. Treated mill effluent is discharged into the Sink Reservoir at a rate of approximately 4,000 m^3/d. Treated effluent enters Vulture Lake before meeting with McClean Lake. The Sink Reservoir and Vulture Lake are together designed as the Sink/Vulture treated effluent management system (Figure 4.1). Samples were collected from a reference site, Indigo Lake located approximately 15 km upstream of the effluent discharge point and one exposure site, Vulture Lake (a closed system), located at the end of the effluent management system (Figure 4.1).

4.2.2. Sample collection and processing

Samples were collected in August 2005. Water, sediment and biological samples were collected following protocols described elsewhere (ASTM, 2003a; ASTM, 2003b; ASTM, 2003c; ASTM, 2003d; ASTM, 2003e; Rosenberg and Resh, 1993; USEPA, 1983). Except for water and sediments, all samples were stored frozen at –20 °C until analysis. Sediments and biota samples (plankton, periphyton, invertebrates and fish) were oven dried at 60°C until constant mass was recorded.

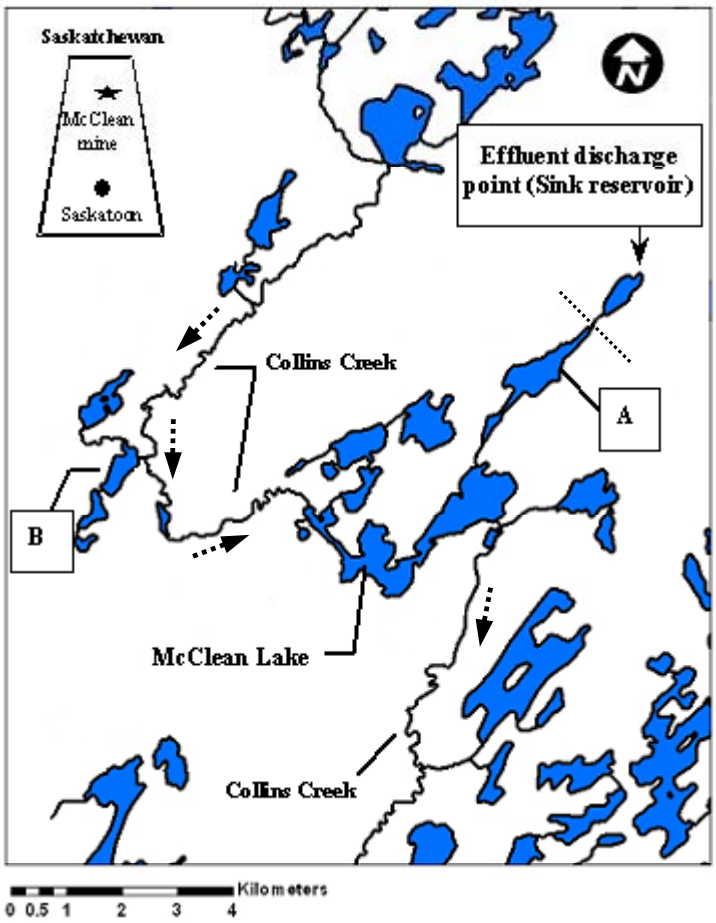

Figure 4.1: Map of McClean Lake mine study area (Saskatchewan, Canada). Insert, map of Saskatchewan showing the relative location of McClean Lake mine operation. Solid arrow, mill effluent discharge into Sink Reservoir. Dashed arrows, flow direction. Dashed lines, dam. Sampling sites: (A) Vulture Lake (exposure site), (B) Indigo Lake (reference site).

4.2.2.1. Water

Water quality variables (pH, conductivity, temperature, dissolved oxygen (DO), salinity, and total dissolved solids (TDS)) were determined on-site using a YSI meter (6 series) with an attached multi-parameter display system (model 650) attached (YSI Inc., Yellow Springs, OH, USA). Surface water samples for total Se determinations were collected in triplicate in the field approximately 10 cm below the water surface in pre-cleaned, 125-ml high density polyethylene (HDPE) bottles and acidified to pH < 2 with ultra-pure nitric acid. An additional three replicate samples were collected for sulphate, hardness, and alkalinity determinations. Samples were stored at 4°C until further analysis.

4.2.2.2. Sediment

Samples were collected in triplicate using 5-cm diameter acrylic sediment core tubes. Sediment cores were kept at 4 °C until transported to the Toxicology Centre, University of Saskatchewan. After arrival to the laboratory, the cores were extruded from the core sampler tubes using a rubber plunge. The first 2 cm of extruded sediment cores were sectioned, transferred to 100-ml pre-cleaned Nalgene® bottles, oven dried, and kept in a dry place until further analysis. Percent moisture for sediments was 80.50 ± 0.10 %.

4.2.2.3. Plankton and periphyton

Plankton samples were collected by horizontal towing using a 200-ml collection bucket attached to a 153-µm mesh (Nitrex®). The contents of several tows were combined

resulting in pooled triplicate samples per site. No separation of phytoplankton and zooplankton was done. Periphyton samples (*n*=3) were scraped using clean plastic tools from rocks or macrophytes close to the shoreline and collected into plastic whirl-packs. Collected samples were stored frozen until further analysis. Percent moisture was 80.47 ± 0.82 and 80.40 ± 1.97 % for plankton and periphyton, respectively.

4.2.2.4. Invertebrates

Samples were collected by dip netting and manual removal from submerged structures (e.g., rocks, vegetation) in shallow locations or by Eckman grab in deeper locations, followed by sieving through a 425-µm mesh. The invertebrates were identified to order (Merritt and Cummins, 1984; Thorp and Covich, 1991) and sorted into Diptera (chironomid larva, *n*=3, pooled 4-10 organisms per replicate), Trichoptera (caddisfly larvae, *n*=3, pooled 3-5 organisms per replicate), Gastropoda (snails, *n*=3, pooled 3-6 organisms per replicate), Odonata (dragonfly larvae, *n*=3, pooled 2-3 organisms per replicate), and Hirudinea (leeches, *n*=3, pooled 2-3 organisms per replicate). The collected invertebrates were left in the respective site water for 2 to 3 hrs to purge their stomach contents and then rinsed with nanopure water to remove any remaining debris. The invertebrate samples were placed into plastic whirl-pack bags and frozen until further analysis. Percent moisture determinations were as follows: Diptera 79.66 ± 1.51%, Trichoptera78.89 ± 2.22 %, Gastropoda 79.59 ± 1.02 %, Odonata 83.22 ± 3.46 %, and Hirudinea 80.78 ± 0.51 %.

4.2.2.5. Juvenile fish

Predatory (juvenile pike (*Esox lucius, n*=4) and forage fish (juvenile white sucker (*Catostomus commersoni, n*=4, stickleback (*Pungitius pungitius, n*= 3-4) and burbot (*Lota lota n*=4)) were collected using an electrofishing backpack (model LR-24, Smith-Root, Vancouver, WA, USA). Total length and weight were recorded and samples were frozen until further analysis. Prior to analysis fish samples were thawed, stomachs were emptied and ageing structures (cleithra (pike), pectoral fins (white sucker), otoliths (burbot) and scales (stickleback)) were removed for age determinations (North/South Consultant Inc., Winnipeg, MB, Canada). Fish were rinsed with nanopure water before homogenization. Moisture content was as follows juvenile pike 78.47 ± 1.27 %, juvenile white sucker 77.52 ± 0.52 %, stickleback 74.20 ± 0.86 % and burbot 73.83 ± 0.64 %.

4.2.3. Analytical determinations

4.2.3.1. Water quality variables

Water total Se and sulphate concentrations were evaluated using inductively coupled plasma-mass spectrometry (ICP-MS) (Prairie Diagnostic Services, Western College of Veterinary Medicine, Saskatoon, SK, Canada) and inductively coupled plasma optical emission spectrometry (ICP-OES) (Enviro-Test Laboratories, Saskatoon, SK, Canada), respectively. Water hardness and alkalinity were measured with a Hach Digital Titrator model 16900 (Hach Company, Loveland, CO, USA). Average Se recovery (> 88 %) was evaluated using a selenium standard solution (2 µg/L; EMD Chemicals, Ltd., Madison, WI, USA).

4.2.3.2. Trace element determinations

Total Se and other trace elements analyses in biota samples was performed by Prairie Diagnostic Services, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada by ICP-MS. Selenium determination in sediment samples was performed by ICP-MS at the Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada. Average selenium recovery (> 86 %) was evaluated using certified fish tissue (Dogfish muscle, DORM-2) and sediments (marine sediments, PACS-2) for biota and sediment samples, respectively. Certified material was obtained from the National Research Council of Canada. Selenium accumulation factors were calculated on a wet weight basis as:

Total concentration of Se (whole organisms)/ total Se in water, sediments or prey organisms (USEPA, 2000; Muscatello *et al*, 2008) (4.1)

4.2.4. Statistical analysis

Statistical analyses were performed using SigmaStat version 3.1 (SPSS Inc., Chicago, IL) with a 95% ($\alpha = 0.05$) level of confidence. Significant differences between sites were evaluated using *t*-test. Data that failed tests for normality and/or homogeneity of variance were $\log_{(10)}$ transformed prior to the use of the parametric statistical test. If data failed the parametric assumptions, a non-parametric test (Mann-Whitney) was used for the evaluation of significant differences between sites. Differences among biota groups within sites were evaluated using a non-parametric test (Kruskall Wallis) due to the lack of normality of the

data. Analysis of covariance (ANCOVA; SYSTAT® version 10 (SSI Inc., Richmond, CA, USA) was used to compare fish body weights with body length as a covariate. Selenium accumulation factors were calculated on a wet weight basis as the total concentration of Se in whole organisms, divided by total Se in either water, sediments or prey organisms (USEPA, 2000; Muscatello et al, 2008).

4.3. Results

4.3.1. Water and sediment

Exposure site water was characterized by higher hardness, conductivity, TDS, salinity and sulphate, and lower pH and alkalinity, compared to the reference site (Table 4.1A). The concentration of Se in water and sediments was higher in the exposure site than the reference site (Table 4.1B).

4.3.2. Selenium and other trace elements in the biota

Selenium concentration in plankton was significantly ($p < 0.05$) higher in the exposure site (2.91 ± 0.56 µg/g DW)) than the reference site (0.73 ± 0.28 µg/g DW; Table 4.2). Other trace elements concentrations also were greater ($p < 0.05$) in plankton samples collected from the exposure site (As, Barium (Ba), Cadmium (Cd), Mo, Ni, lead (Pb), thalium (Tl) and U) while concentrations of other trace elements from the exposure site were significantly ($p < 0.05$) lower than the reference site (chromium (Cr), iron (Fe), manganese (Mn), and vanadium (V); Table 4.3). There was no significant difference in the Se

concentration of periphyton between sites. Concentrations of certain trace elements in periphyton were significantly ($p < 0.05$) higher in the exposure site than the reference site (As, Ba, beryllium (Be), cobalt (Co), Cr, Fe, Mo, Ni, antimony (Sb), Tl, U and V); while concentration of other elements in the exposure site (magnesium (Mg) and strontium (Sr)) were significantly lower ($p < 0.05$; Table 4.3).

Invertebrates. Selenium concentration in Diptera, Trichoptera and Odonata were significantly ($p < 0.05$) higher in the exposure site than the reference site (Table 4.2). There were no significant differences in the Se concentration for Hirudinea and Gastropoda invertebrates between sites (Table 4.2). Concentrations of other trace elements also were significantly different in certain invertebrate groups between reference and exposure sites (Table 4.4). Concentrations of Mo and U were higher in all invertebrate groups collected from the exposure site when compared to reference (Table 4.4). In addition, there were significant ($p < 0.05$) increases in the concentrations of Co and Mn in Gastropoda, Odonata and Hirudinea between sites. Elevated concentration of As were only observed for Odonata collected from exposure site ($p < 0.05$; Table 4.4). In contrast, other concentrations of trace elements in Diptera (Ba, copper (Cu), Mn, Ni and zinc (Zn)), Trichoptera (Ba and V), Gastropoda (Cr, Fe and V) and Odonata (Cd, Cu and Sr) were significantly lower ($p < 0.05$) at the exposure site (Tables 4.4).

Fish. Selenium concentrations in whole body of juvenile white sucker, stickleback and burbot from the exposure site were significantly ($p < 0.05$) higher in the exposure site than the reference site (Table 4.2). There were no significant differences in the Se concentration of juvenile pike between reference and exposure site (Table 4.2).

Table 4.1: A) Water quality variables measured on-site during sample collection. B) Water and sediment total selenium concentration in study sites. Below detection limit (<).

A)

Variable	Reference	Exposure
Hardness (mg $CaCO_3$/L) [a]	5.8 ± 0.13	326.65 ± 4.5
Alkalinity (mg $CaCO_3$/L) [a]	12.70 ± 0.44	9.33 ± 0.44
pH	7.33	7.52
Conductivity (µS/cm)	13.0	625.0
Temperature (°C)	14.42	14.70
DO (mg/L) [b]	11.50	11.92
TDS (g/L) [c]	0.01	0.49
Salinity	0.01	0.38
Sulphates (mg/L) [a]	< 6	331.0 ± 7.33

B)

Variable	Reference	Exposure
Selenium (µg/L)	0.02 ± 0.006	0.43 ± 0.01
Sediment selenium (µg/g, dry weight) [a]	< 0.01	0.54 ± 0.007

[a] Data represent the mean (± SE) of three replicate samples.

[b] Dissolved oxygen.

[c] Total dissolved solids.

Concentrations of trace elements other than Se in whole body fish also were significantly different ($p < 0.05$) between reference and exposure sites (Table 4.5). Thallium and U concentrations in fish were consistently higher ($p < 0.05$) in the exposure site than reference site for all the sampled fish species (Table 4.5). Concentrations of As in juvenile white sucker , stickleback and burbot collected from the exposure site were higher than the reference site ($p < 0.05$; Table 4.5). In addition, stickleback collected from the exposure site had higher concentrations of Ba, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, tin (Sn), Sr, V, and Zn than fish collected from the reference site (Table 4.5). The concentrations of Mo and Sb in burbot and V in juvenile pike also were higher between sites. Concentration of other trace elements in juvenile white sucker (Cd, Co, Cr, Fe, Ni, Pb, Sn, Sr, and V), burbot (Ba, Cr, Mn, Ni, Sr and V) and pike (Sr) collected from the exposure site were lower than the reference site (Tables 4.5).

There were no differences in Se concentrations between the invertebrate groups and either plankton or periphyton from each site. Selenium concentrations in burbot were significantly ($p < 0.05$) higher than periphyton, Gastropoda and Hirudinea within the exposure site (Table 4.2). Selenium concentrations in burbot were also significantly higher ($p < 0.05$) than stickleback for the reference site. No other differences were determined between the invertebrate groups and fish for either site.

Fish age was 1 to 2 years for juvenile white sucker, <1 year for stickleback, 2 to 3 years for burbot, and <1 year for juvenile pike. There were no significant differences in the condition factor of juvenile white sucker and stickleback between reference (0.88 ± 0.05 and 0.67 ± 0.10, respectively) and exposure site (0.85 ± 0.06 and 0.74 ± 0.04, respectively; In contrast, the condition factor of juvenile pike and burbot collected from the reference site

(0.47± 0.12 and 0.64 ± 0.09, respectively) was significantly different from the exposure site (0.54 ± 0.04 and 0.44 ± 0.03, respectively)

The general pattern of Se accumulation (from smallest to largest) in the biota was as follows: periphyton < invertebrates < plankton < predatory fish < forage fish. The concentration of Se in plankton was higher than the Se concentration in periphyton and the majority of the invertebrate groups. The differences in Se accumulation between predatory and forage fish may be due to differences in behaviour and diet preferences that can have important effects in trace elements accumulation. Selenium accumulation factors for all the major compartments of the aquatic ecosystem in the exposure site are presented in Table 4.6 and Figure 4.2. Selenium in the water was concentrated from 150 to more than 6 000 times in sediments and biota at the exposure site. Biomagnification of Se (defined as increased Se concentrations by successive trophic levels (Lemly, 1997)) also was observed, resulting in an approximately 1.5 to 6 fold increase in the Se concentration between plankton, invertebrates and small bodied fish. However, biomagnification did not appear to occur between the forage (juvenile white sucker, stickleback, and burbot) and predatory (pike) fish species.

Table 4.2: Concentration of selenium (μg/g, dry weight) in aquatic biota from reference and exposure sites. * Significantly different from the reference site ($p < 0.05$). # Significantly different from burbot within each collection site ($p < 0.05$).

Variables	Reference	Exposure
Plankton	0.73 ± 0.28	2.91 ± 0.56 *
Periphyton	0.34 ± 0.07	0.35 ± 0.10 #
Invertebrates		
Diptera	0.09 ± 0.06	1.63 ± 0.05 *
Trichoptera	0.27 ± 0.18	3.07 ± 1.36 *
Gastropoda	0.36 ± 0.26	0.52 ± 0.03 #
Odonata	0.43 ± 0.11	1.62 ± 0.14 *
Hirudinea	0.49 ± 0.34	0.86 ± 0.24 #
Juvenile Fishes		
Northern pike	0.75 ± 0.23	1.26 ± 0.06
White sucker	0.99 ± 0.11	3.73 ± 0.15 *
Stickleback	0.01 ± 0.003 #	4.15 ± 0.17 *
Burbot	1.07 ± 0.04	10.96 ± 0.82 *

Table 4.3: Total trace metal concentrations reported on a dry weight basis in plankton and periphyton collected from reference and exposure sites (bold text). Data represent the mean (± SE). * Significantly different ($p < 0.05$) from the reference site. Below instrument detection limit (<).

Metals	Plankton	Periphyton
As (µg/g)	7.88 ± 0.17 **13.09 ± 0.66***	3.26 ± 0.24 **4.35 ± 0.07***
Ba (µg/g)	222.73 ± 3.67 **533.03 ± 44.04***	69.94 ± 4.67 **100.77 ± 7.04 ***
Be (ng/g)	146.0 ± 13.0 **123.0 ± 6.83**	36.60 ± 4.73 **117.0 ± 12.4 ***
Bi (ng/g)	$< 9 \times 10^{-6}$ **$< 9 \times 10^{-6}$**	$< 9 \times 10^{-6}$ **$< 9 \times 10^{-6}$**
Cd (µg/g)	0.28 ± 0.05 **0.69 ± 0.05 ***	0.12 ± 0.03 **0.19 ± 0.01**
Co (µg/g)	4.84 ± 0.03 **4.90 ± 0.67**	1.45 ± 0.14 **2.99 ± 0.19 ***
Cr (µg/g)	4.79 ± 0.16 **3.96 ± 0.09***	1.59 ± 0.17 **4.45 ± 0.17 ***
Cu (µg/g)	5.12 ± 0.70 **6.28 ± 0.15**	4.24 ± 0.76 **2.72 ± 0.22**
Fe (µg/g)	40.343.33 ± 503.0 **16,196.67 ± 616.66 ***	13.753.33 ± 1.838.51 **40,996.67 ± 411.55 ***
Mg (µg/g)	1.599.0 ± 33.33 **1,414.26 ± 206.36 ***	1.063.10 ± 97.70 **593.90 ± 32.21 ***
Mn (µg/g)	3.994.0 ± 73.30 **387.30 ± 77.02 ***	986.0 ± 94.90 **1,335.33 ± 123.91**
Mo (µg/g)	5.41 ± 2.52 **405.17 ± 23.18 ***	0.66 ± 0.41 **87.23 ± 0.89 ***
Ni (µg/g)	7.31 ± 0.97 **40.53 ± 5.05 ***	2.52 ± 0.31 **8.70 ± 0.45 ***
Pb (µg/g)	1.82 ± 0.11 **2.98 ± 0.08***	1.41 ± 1.07 **0.77 ± 0.02**
Sb (ng/g)	446.0 ± 216.0 **302.0 ± 103.0**	6.99 ± 5.79 **57.10 ± 15.70 ***
Sn (µg/g)	0.19 ± 0.05 **0.47 ± 0.21**	0.21 ±0.11 **0.11 ± 0.01**
Sr (µg/g)	66.54 ± 4.16 **28.82 ± 3.22 ***	27.93 ± 1.34 **18.42 ± 2.18 ***
Tl (ng/g)	80.80 ± 5.02 **346.0 ± 38.60 ***	20.70 ± 1.27 **43.90 ± 2.71 ***
U (µg/g)	0.92 ± 0.19 **10.50 ± 0.54 ***	0.26 ± 0.04 **1.16 ± 0.06 ***
V (µg/g)	11.73 ± 0.11 **6.51 ± 0.28 ***	4.91 ± 0.34 **21.09 ± 0.31 ***
Zn (µg/g)	63.71 ± 15.04 **209.62 ± 131.65**	165.09 ± 134.91 **63.33 ± 10.62**

Table 4.4: Total trace metal concentrations reported on a dry weight basis in invertebrates collected from reference and exposure sites (bold text). Data represent the mean (± SE). * Significantly different ($p < 0.05$) from the reference site. Below instrument detection limit (<).

Metals	Diptera	Trichoptera	Gasteropoda	Odonata	Hirudinea
As (µg/g)	2.21 ± 0.62 **1.07 ± 0.05**	0.48 ± 0.16 **0.71 ± 0.06**	1.40 ± 0.20 **1.22 ± 0.02**	0.41 ± 0.01 **1.26 ± 0.06 ***	0.43 ± 0.14 **0.85 ± 0.35**
Ba (µg/g)	117.99 ± 31.93 **2.25 ± 2.24 ***	30.59 ± 6.51 **4.82 ± 4.80 ***	64.09 ± 22.01 **45.83 ± 23.33**	1.98 ± 0.38 **2.08 ± 2.03**	28.68 ± 27.50 **87.53 ± 8.08**
Be (ng/g)	77.30 ± 60.0 $< 6 \times 10^{-5}$	27.60 ± 20.0 **0.70± 0.60**	12.50 ± 9.77 **3.24 ± 0.001**	0.26 ± 0.11 **0.29 ± 0.12**	2.80 ± 1.60 **2.96 ± 1.72**
Bi (ng/g)	$< 9 \times 10^{-6}$ **$< 9 \times 10^{-6}$**	$< 9 \times 10^{-6}$ **$< 9 \times 10^{-6}$**	$< 9 \times 10^{-6}$ **$< 9 \times 10^{-6}$**	$< 9 \times 10^{-6}$ **$< 9 \times 10^{-6}$**	$< 9 \times 10^{-6}$ **$< 9 \times 10^{-6}$**
Cd (µg/g)	2.96 ± 2.63 **0.05 ± 0.006**	0.28 ± 0.05 **0.36 ± 0.21**	0.96 ± 0.65 **0.13 ± 0.005**	0.22 ± 0.02 **0.04 ± 0.01 ***	0.16 ± 0.04 **0.19 ± 0.05**
Co (µg/g)	1.94 ± 0.57 **1.95 ± 0.03**	0.63 ± 0.18 **1.05 ± 0.17**	1.09 ± 0.18 **4.78 ± 0.50 ***	0.34 ± 0.01 **2.19 ± 0.18 ***	0.53 ± 0.19 **4.36 ± 0.28 ***
Cr (µg/g)	6.92 ± 4.16 **0.47 ± 0.05**	2.0 ± 0.60 **0.82 ± 0.25**	2.27 ± 1.18 **0.31 ± 0.02 ***	0.62 ± 0.08 **0.42 ± 0.03**	0.90 ± 0.17 **1.80 ± 0.71**
Cu (µg/g)	27.38 ± 4.93 **7.98 ± 0.15 ***	23.56 ± 6.38 **10.68 ± 1.49**	4.63 ± 1.60 **3.86 ± 0.36**	17.55 ± 0.45 **7.99 ± 0.54 ***	6.07 ± 0.77 **3.82 ± 0.42**
Fe (µg/g)	19,035.3 ± 9,630.9 **331.90 ± 31.60**	5,280.67 ± 691.93 **287.80 ± 58.82**	12,591.67 ± 4,904.76 **683.37 ± 34.63 ***	313.33 ± 4.99 **267.63 ± 50.35**	1,079.77 ± 315.50 **816.80 ± 33.34**
Mg (µg/g)	2,485.66 ± 703.35 **1,869.0 ± 783.01**	2,155.0 ± 503.49 **1,317.33 ± 38.22**	1,181.67 ± 37.47 **1,101.77 ± 75.61**	1,044.13 ± 35.22 **961.57 ± 40.22**	1,063.80 ± 210.28 **1,055.50 ± 178.33**
Mn (µg/g)	1,604.43 ± 559.65 **186.73 ± 3.18 ***	360.69 ± 192.82 **250.28 ± 71.69**	238.28 ± 56.84 **634.60 ± 84.07 ***	43.52 ± 2.63 **196.27 ± 41.26 ***	104.40 ± 83.75 **671.10 ± 54.88 ***
Mo (µg/g)	1.98 ± 0.53 **12.27 ± 0.26 ***	0.65 ± 0.40 **139.46 ± 23.83 ***	0.46 ± 0.08 **21.29 ± 0.80 ***	0.15 ± 0.01 **13.60 ± 1.59 ***	0.67 ± 0.29 **24.44 ± 1.08 ***
Ni (µg/g)	67.04 ± 15.01 **4.69 ± 0.42 ***	19.98 ± 11.54 **4.73 ± 1.99**	3.68 ± 0.64 **12.48 ± 0.83 ***	1.21 ± 0.10 **3.93 ± 0.12 ***	5.66 ± 2.21 **5.62 ± 4.49**
Pb (µg/g)	3.87 ± 2.39 **0.36 ± 0.21**	0.54 ± 0.31 **1.56 ± 1.50**	0.72 ± 0.48 **0.11 ± 0.02**	0.38 ± 0.16 **1.86 ± 1.71**	0.64 ± 0.14 **0.25 ± 0.07**
Sb (ng/g)	28.0 ± 15.20 $<2 \times 10^{-4}$	24.20 ± 10.0 **5.07 ± 3.20**	0.95 ± 0.80 **6.91 ± 0.60 ***	$<2 \times 10^{-4}$ **2.35 ± 0.001**	$<2 \times 10^{-4}$ **5.39 ± 1.29**
Sn (µg/g)	1.60 ± 0.73 **0.08 ± 0.03**	1.18 ± 0.68 **0.10 ± 0.07**	2.21 ± 2.04 **0.01 ± 0.004**	0.18 ± 0.10 **0.06 ± 0.03**	0.03 ± 0.01 **0.02 ± 0.003**
Sr (µg/g)	121.02 ± 60.13 **1.39 ± 0.15**	15.93 ± 4.50 **4.49 ± 0.38**	239.27 ± 117.33 **36.34 ± 35.22**	4.63 ± 0.77 **1.20 ± 0.17 ***	143.31 ± 138.80 **79.93 ± 38.58**
Tl (ng/g)	14.30 ± 5.76 **47.40 ± 2.85 ***	11.30 ± 1.46 **36.80 ± 18.10**	43.90 ± 18.70 **64.30 ± 3.31**	30.0 ± 1.26 **36.80 ± 5.08**	20.0 ± 2.0 **69.80 ± 4.93**
U (µg/g)	0.01 ± 0.009 **0.22 ± 0.02 ***	0.002 ± 0.001 **0.24 ± 0.02 ***	0.38 ± 0.15 **4.75 ± 0.28 ***	0.003 ± 0.002 **0.22 ± 0.05 ***	0.10 ± 0.09 **3.77 ± 0.32 ***
V (µg/g)	4.26 ± 1.61 **0.52 ± 0.04**	2.14 ± 0.76 **0.23 ± 0.03 ***	5.57 ± 3.07 **0.35 ± 0.02 ***	0.30 ± 0.01 **0.36 ± 0.05**	0.54 ± 0.30 **0.37 ± 0.009**
Zn (µg/g)	150.42 ± 36.99 **63.59 ± 0.57 ***	191.07 ± 58.38 **99.96 ± 23.47**	65.33 ± 52.85 **21.70 ± 0.17**	102.72 ± 14.92 **106.34 ± 49.68**	113.73 ± 50.78 **32.99 ± 9.95**

Table 4.5: Total trace metal concentrations reported on a dry weight basis in forage and predatory fish collected from reference and exposure sites (bold text). Data represent the mean (± SE).

* Significantly different ($p < 0.05$) from the reference site. Below instrument detection limit (<).

Metals	Juvenile White sucker	Stickleback	Burbot	Juvenile Pike
As (µg/g)	0.19 ± 0.03 **0.45 ± 0.04 ***	0.003 ± 0.001 **0.58 ± 0.17 ***	0.50 ± 0.06 **1.0 ± 0.05 ***	0.16 ± 0.07 **0.19 ± 0.03**
Ba (µg/g)	5.23 ± 0.47 **4.53 ± 0.29**	0.08 ± 0.01 **8.84 ± 0.45 ***	10.71 ± 2.83 **0.90 ± 0.07***	4.61 ± 0.83 **2.58 ± 0.77**
Be (ng/g)	$< 6 \times 10^{-5}$ **$< 6 \times 10^{-5}$**	$< 6 \times 10^{-5}$ **$< 6 \times 10^{-5}$**	$< 6 \times 10^{-5}$ **$< 6 \times 10^{-5}$**	$< 6 \times 10^{-5}$ **$< 6 \times 10^{-5}$**
Bi (ng/g)	$< 9 \times 10^{-6}$ **$< 9 \times 10^{-6}$**	$< 9 \times 10^{-6}$ **$< 9 \times 10^{-6}$**	$< 9 \times 10^{-6}$ **$< 9 \times 10^{-6}$**	$< 9 \times 10^{-6}$ **$< 9 \times 10^{-6}$**
Cd (ng/g)	54.30 ± 5.94 **18.50 ± 2.57 ***	12.90 ± 12.40 **38.0 ± 11.10**	14.60 ± 1.12 **12.80 ± 2.16**	19.40 ± 7.53 **39.0 ± 13.60**
Co (µg/g)	1.40 ± 0.29 **0.65 ± 0.08 ***	0.12 ± 0.05 **2.86 ± 0.45 ***	0.08 ± 0.01 **0.07 ± 0.01**	0.34 ± 0.14 **0.44 ± 0.09**
Cr (µg/g)	1.53 ± 0.10 **0.65 ± 0.04 ***	0.51 ± 0.16 **58.05 ± 14.59 ***	1.43 ± 0.19 **0.41 ± 0.15 ***	11.73 ± 5.54 **3.30 ± 1.01**
Cu (µg/g)	2.52 ± 0.17 **2.78 ± 0.21**	0.13 ± 0.03 **6.05 ± 0.61***	3.36 ± 0.38 **3.10 ± 0.19**	1.58 ± 0.45 **2.23 ± 0.22**
Fe (µg/g)	238.50 ± 28.62 **110.07 ± 11.76 ***	6.69 ± 1.35 **535.60 ± 94.31***	64.41 ± 10.07 **57.30 ± 8,97**	89.66 ± 35.51 **91.07 ± 29.08**
Mg (µg/g)	1,701.0 ± 109.68 **1,847.50 ± 105.92**	27.04 ± 8.36 **2,779.0 ± 65.13 ***	1,354.25 ± 139.61 **1,295.75 ± 71.55**	1,301.37 ± 288.08 **1,301.85 ± 135.99**
Mn (µg/g)	20.23 ± 3.56 **26.08 ± 2.97**	0.54 ± 0.08 **64.83 ± 0.40 ***	20.89 ± 3.73 **5.66 ± 0.62 ***	16.75 ± 2.87 **17.91 ± 4.37**
Mo (µg/g)	3.49 ± 0.69 **3.85 ± 0.32**	0.11 ± 0.02 **14.26 ± 3.10 ***	0.22 ± 0.04 **3.94 ± 0.25 ***	1.24 ± 0.56 **0.60 ± 0.17**
Ni (µg/g)	1.29 ± 0.09 **0.58 ± 0.03***	0.70 ± 0.12 **76.36 ± 18.82***	1.12 ± 0.19 **0.30 ± 0.12 ***	7.74 ± 3.47 **2.93 ± 0.73**
Pb (ng/g)	61.30 ± 6.13 **24.10 ± 9.64 ***	6.06 ± 3.50 **58.50 ± 26.30**	18.20 ± 7.55 **11.60 ± 6.38**	12.70 ± 6.69 **38.50 ± 37.70**
Sb (ng/g)	4.39 ± 2.70 **0.17 ± 0.09**	3.77 ± 3.41 **39.0 ± 35.0**	$< 2 \times 10^{-4}$ **12.0 ± 8.0 ***	1.41 ± 0.93 **$< 2 \times 10^{-4}$**
Sn (ng/g)	82.20 ± 6.30 **27.60 ± 7.15 ***	3.22 ± 0.68 **119.0 ± 38.60 ***	12.30 ± 0.73 **22.70 ± 10.0**	56.20 ± 10.0 **29.30 ± 11.0**
Sr (µg/g)	76.30 ± 3.91 **20.20 ± 1.89 ***	0.21 ± 0.05 **19.63 ± 1.90 ***	92.47 ± 28.18 **8.69 ± 0.98 ***	63.34 ± 12.47 **9.44 ± 2.62***
Tl (ng/g)	17.90 ± 1.78 **51.40 ± 5.50 ***	0.23 ± 0.09 **29.20 ± 0.93 ***	7.77 ± 0.46 **45.30 ± 2.97 ***	19.80 ± 4.78 **57.70 ± 2.86 ***
U (ng/g)	1.73 ± 0.78 **76.10 ± 8.27 ***	0.025 ± 0.020 **45.50 ± 8.90 ***	$< 1 \times 10^{-5}$ **3.02 ± 0.62 ***	$< 1 \times 10^{-5}$ **16.30 ± 11.40 ***
V (µg/g)	0.24 ± 0.04 **0.14 ± 0.01***	0.006 ± 0.001 **0.45 ± 0.09 ***	0.16 ± 0.01 **0.12 ± 0.01 ***	0.13 ± 0.06 **0.21 ± 0.04**
Zn (µg/g)	72.77 ± 3.91 **73.54 ± 3.57**	0.05 ± 0.04 **1.68 ± 0.49***	46.77 ± 2.37 **43.57 ± 2.93**	92.94 ± 21.29 **107.89 ± 19.42**

Table 4.6: Accumulation factors for abiotic and biotic compartments of the aquatic ecosystem in the exposure site.

Aquatic compartment	Exposure site accumulation factors											
Sediments	231.12 [a]											
Plankton	1,298. 74[a]	5.62 [b]										
Periphyton	154.02 [a]	0.66 [b]										
Invertebrates												
Diptera	758.31 [a]	3.28 [b]	0.58 [c]	4.92 [d]								
Trichoptera	1,513.82 [a]	6.55 [b]	1.16 [c]	9.82 [d]								
Gastropoda	236.88 [a]	1.02 [b]	0.18 [c]	1.54 [d]								
Odonata	707.66 [a]	3.06 [b]	0.54 [c]	4.59 [d]	0.93 [e]	0.48 [f]	2.99 [g]					
Hirudinea	389.38 [a]	0.30 [b]	0.30 [c]	2.54 [d]	0.51 [e]	0.26 [f]	1.64 [g]					
Juvenile Fish												
White sucker	1,965.58 [a]	8.50 [b]	1.51 [c]	12.76 [d]	2.59 [e]	1.30 [f]	8.30 [g]	2.78 [h]	5.05 [i]			
Stickleback	2,477.85 [a]	10.72 [b]	1.91 [c]	16.09 [d]	3.27 [e]	1.64 [f]	10.46 [g]	3.50 [h]	6.36 [i]			
Burbot	6,580.12 [a]	28.47 [b]	5.07 [c]	42.72 [d]	8.68 [e]	4.35 [f]	27.78 [g]	9.30 [h]	16.90 [i]			
Northern pike	645.77 [a]	2.79 [b]	0.50 [c]	4.19 [d]	0.85 [e]	0.43 [f]	2.73 [g]	0.91 [h]	1.66 [i]	0.33 [j]	0.26 [k]	0.10 [l]

[a] Accumulation factor calculated as the selenium in sample divided by the total selenium in water (µg/L).

[b] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in sediments.

[c] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in plankton.

[d] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in periphyton.

[e] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in diptera.

[f] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in trichoptera.

[g] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in gastropoda.

[h] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in odonata.

[i] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in hirudinea.

[j] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in juvenile white sucker.

[k] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in stickleback.

[l] Accumulation factor calculated as the selenium in sample divided by the selenium concentration in burbot.

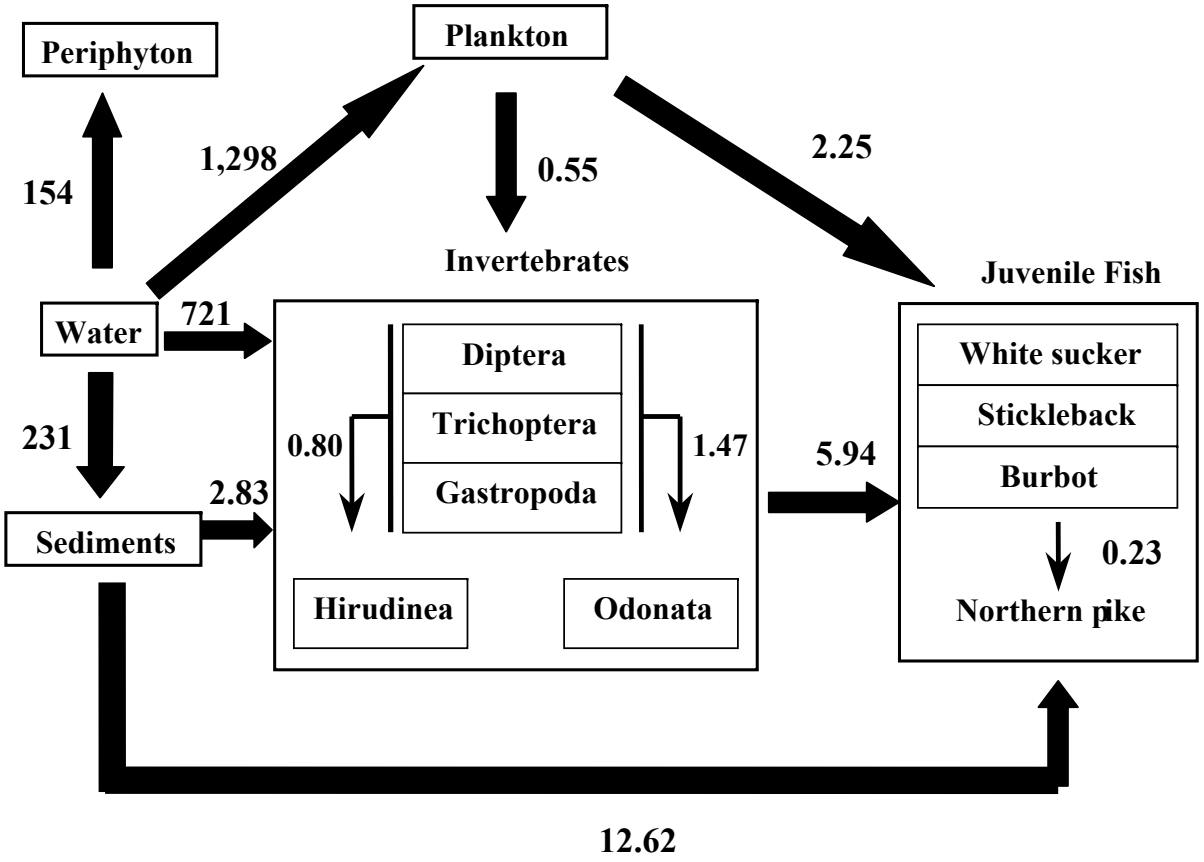

Figure 4.2: Generalized flow diagram showing the accumulation of selenium in aquatic food chain in Vulture Lake (exposure site). For calculation purposes invertebrates and fish were grouped as follow: Diptera, Trichoptera and Gastropoda; Odonata and Hirudinea; forage fish (juvenile white sucker, stickleback and burbot) and predatory fish (juvenile pike). Values represent the mean of calculated accumulation factors for the exposure site. Arrows width represents the calculated accumulation factors between (thick arrows) and within (thin arrows) trophic groups.

4.4. Discussion

A major concern regarding elevated levels of Se in the aquatic environment is its ability to accumulate through the food chain and potentially impact the sustainability of fish populations. In this study, accumulation of Se was greater in plankton, certain invertebrate groups and forage fish from the exposure site compared to reference although water Se concentrations were considered low (i.e., Se concentration in water from the exposure site was below the 1 μg/L and 5 μg/L water criteria established by the Canadian Council of Ministers of the Environment (CCME, 2003) and USEPA (1987), respectively). Other studies also have reported Se accumulation in prey organisms inhabiting aquatic systems with waterborne Se concentrations below 1 μg/L (Lemly, 1993a; Muscatello *et al*., 2008). Furthermore, several authors have concluded that sediments, as opposed to water, play an important role in the accumulation of Se in aquatic systems (Hamilton and Lemly, 1999; Saiki *et al*., 1993; Orr *et al*., 2006). In this study, the Se concentration in sediments was below the 2 μg/g (DW) threshold suggested by Lemly (2002b) to prevent Se concentrations from reaching toxic levels in the aquatic biota. However, the Se concentrations in Trichoptera and forage fish (white sucker, stickleback, and burbot) reported herein exceeded the lower limit of the proposed 3 to 11 μg/g (DW) dietary toxicity threshold for fish (Lemly, 1993a; DeForest *et al*., 1999). The pattern of Se accumulation (from smallest to largest) in the aquatic biota was related as follows: periphyton < invertebrates < plankton < predatory fish < forage fish. Other studies reported similar accumulation trends (periphyton<plankton<invertebrates<fish) for Se in the aquatic biota (Lemly, 1985a; Muscatello *et al*., 2008). The variation in the concentration of Se in plankton reported in

this study could be due to various chemical (e.g., different forms of Se present in the water column) and biological (e.g., preferential uptake for certain Se forms, different composition of plankton community) processes in the environment that have the potential to affect Se uptake and accumulation by primary producers (Riedel *et al*., 1991; Baines and Fisher, 2001; Baines *et al*., 2004).

In addition to Se, varying concentrations of other trace elements were recorded in aquatic biota from the exposure lake when compared to the reference lake. A previous study also reported the presence of other trace elements in exposure lake water (Muscatello and Janz, 2008). Interactions with other trace elements can increase or decrease Se accumulation and have been described elsewhere (Hill, 1975; Naganuma *et al*., 1983; ATSDR, 2003; Hamilton and Palace, 2005). Water quality and environmental variables (e.g., increased hardness, organic matter) can also modify the toxicity and accumulation of metals in aquatic systems. Therefore, the decrease in the concentration of certain trace elements in aquatic organisms from the exposure site reported in this study could be explained by processes such as metal competition (e.g, calcium (Ca^{+2})) and/or complexation (e.g, carbonates) that have the potential to reduce metal bioavailability and accumulation in aquatic systems (Pascoe *et al*., 1986; Winner and Gauss, 1986; Spry and Wiener, 1991). The differences in small bodied fish condition factors between sites reported in this study may be due to differences in food availability (e.g., enrichment of the aquatic ecosystems), fish behaviour and diet preferences which can have important effects on fish condition (Bone and Moore, 2007).

Biomagnification of Se has been reported by some investigators (Lemly, 1985a, 1999; Cherry and Guthrie, 1977) and argued by others (Saiki *et al*., 1993; Barceloux, 1999). In

this study, Se biomagnification ranging from 1.5 to 6 was evident between lower (e.g., plankton) and higher trophic (e.g., fish) levels. However, biomagnification did not occur between forage and predatory fish. This is consistent with previous work examining the accumulation and effects of Se at a different U mine site in northern Saskatchewan that reported similar biomagnification values for Se between trophic levels and no biomagnification occurring between forage and predatory fish (Muscatello *et al.*, 2008). Lemly (1985a, 1997a) reported biomagnification values ranging from 2 to 6 between plankton, invertebrates and fish in a study evaluating the accumulation of Se in a power plant cooling reservoir. However, the author also reported different biomagnification of Se between fishes presenting different feeding modes (e.g., > 2 fold increase between forage and predator fish). The discrepancies in the Se biomagnification in higher trophic levels could possibly be due to differences in Se accumulation, compartmentalization, and elimination between species (e.g., differential formation of seleno-proteins), an area that still requires further investigation.

Selenium released into the aquatic environment can be effectively removed from the water column by primary producers (e.g., plankton) (Riedel *et al*., 1991; Besser *et al*., 1993) and precipitated into the sediments by depositional processes (e.g., deposition of dead organic material) (Bender *et al*., 1991; Oremland *et al*., 1990; Graham *et al*., 1992). Selenium is usually accumulated in the top layer of sediments and can lead to elevated concentrations of Se in benthic invertebrates in contaminated areas compared to reference sites (Lemly, 1993a, 1997a; Saiki *et al.*, 1993). As a result, benthic invertebrates can deliver high concentrations of Se as prey of higher trophic levels (e.g., fish) (Lemly, 1985a, 1999) generally with no apparent reductions in invertebrate abundance or biomass (Lemly,

1997a). In this study, Se concentrations exceeded > 3 µg/g, DW for aquatic biota (certain invertebrate groups and forage fish) from the exposure site, although Se concentrations in water and sediment from the same site were considered low (0.43 µg/L and 0.54 µg/g DW, respectively). A previous study reported that spawning adult white sucker and pike inhabiting the same exposure lake contained elevated levels of Se in eggs (4.89 and 8.02 µg/g, DW, respectively) and tissues (ranging from 1.20 to 10.38 µg/g, DW); however, no increase in the frequencies of Se-induced deformities was recorded in fish fry (Muscatello and Janz, 2008). Although no adverse effect of Se could by identified in this lake, concentrations of this element in some compartments of the aquatic ecosystem (e.g., forage fish) exceeded the lower limit of the proposed 3 to 11 µg/g (DW) dietary toxicity threshold for fish. Consequently, continued environmental monitoring of the aquatic environment should be implemented to prevent potential Se impacts.

4.5. Conclusions

Uranium mining and milling operations have the potential to release trace elements such as U, Ni, As, Se, and Mo and ions (e.g., sulphate, ammonium) into the receiving aquatic ecosystem. The major implication of elevated environmental Se is its propensity to accumulate in the aquatic food chain, potentially impairing fish reproduction. The objective of this study was to investigate the accumulation of Se in the major compartments of aquatic ecosystems (lakes) upstream and downstream of a U mine in northern Saskatchewan, Canada. Selenium concentrations in aquatic biota were elevated in the exposure lake even though water and sediment concentrations were low. Biomagnification

of Se resulted in approximately 1.5 to 6 fold increase in the Se content between plankton, invertebrates and fish. However, no biomagnification was recorded between forage and predatory fish. Concentrations of Se in some compartments of the aquatic ecosystem (e.g., forage fish) exceeded the lower limit of the proposed 3 to 11 μg/g (DW) dietary toxicity threshold for fish. Continued environmental monitoring is recommended to avoid potential Se impacts.

CHAPTER 5 [a]

Assessment of larval deformities and selenium accumulation in northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) in a lake downstream of McClean Lake uranium mining and milling operation

[a] This chapter has been accepted for publication in Environmental Toxicology and Chemistry (In press) under joint authorship with David M Janz (University of Saskatchewan).

5.1. Introduction

Selenium (Se) occurs naturally in aquatic ecosystems with background concentrations typically ranging from 0.1 to 0.4 μg/L (Lemly, 1985b). Anthropogenic activities such as metal mining have the potential to increase Se concentrations in aquatic ecosystems. Elevated concentrations of Se have been reported in water, sediments and fish tissues downstream of certain uranium (U) mining operations in northern Saskatchewan, as a consequence of U ore extractions, dewatering and milling (Pyle *et al*., 2001; Klaverkamp *et al*., 2002; Muscatello *et al*., 2006) Selenium uptake by aquatic biota can occur through water or diet, but dietary uptake is the most relevant route of exposure in fish (Lemly, 1993a; Stewart *et al*., 2004; Hamilton, 2004). Even at low water Se levels (< 5 μg/L) this element can be incorporated into the food chain via primary producers, increasing concentrations in sediments and biota, to finally reach levels that have the potential to cause reproductive impairment in fish (Lemly, 1985a; Lemly, 1997a; Muscatello *et al*., 2006). Thus, a major concern regarding the presence of Se in the aquatic environment is its ability to accumulate through the food chain and have the potential to impact the sustainability of fish populations (Lemly, 1997b; Kennedy *et al.*, 2000).

Selenium is an essential nutrient for fish at dietary concentrations ranging from 0.1 to 0.5 μg/g dry weight (DW) (Hodson and Hilton, 1983). However, there is a very constricted range between required and toxic dietary levels of Se and consequently, dietary concentrations that are seven to thirty times higher than the nutritional levels (i.e., > 3 μg/g DW) can have deleterious effects in fish (Wilber, 1980; Lemly, 1993a; Hamilton, 2003). Selenium incorporated into the eggs during vitellogenesis can cause the appearance of

characteristic developmental malformations when larvae undergo yolk absorption (Lemly, 1997b; Holm *et al*., 2005). Teratogenic effects (spinal curvatures, missing or deformed fins, craniofacial deformities) and other toxicities (e.g., edema) in fish larvae are directly related to elevated (> 10 μg/g DW) Se concentration in eggs (Lemly, 1993b; Maier and Knight, 1994; Holm *et al.*, 2005; Muscatello *et al*., 2006).

The U.S. Environmental Protection Agency (U.S. EPA) proposed a Se chronic criterion for the protection of fish populations at a whole body fish concentration of 7.91 μg/g DW (based on the warm water centrarchid, bluegill sunfish (*Lepomis macrochirus*)) (USEPA, 2004). The use of a tissue-based criterion for Se is the most logical approach for the protection of fish populations. However, there are some uncertainties and controversies that require further investigation before the final Se criterion is developed. For example, only few studies have investigated the accumulation and effects of Se in cold water ecosystems and consequently an important aspect of the debate is focused on whether the proposed selenium threshold is applicable to fishes inhabiting north temperate aquatic systems (Lemly, 1993a; Hamilton, 2003). Recently, work examining the accumulation and effects of Se associated with U mining was conducted in northern Saskatchewan, Canada (Muscatello *et al.*, 2006; deRosemond *et al*., 2005). Muscatello *et al*. (2006) reported high levels of Se in northern pike (*Esox lucius*) eggs (31.28-48.23 μg/g egg DW) and muscle (16.58-38.27 μg/g DW) that were associated with an increase (> 20% above background levels) in the frequencies of total deformities in northern pike fry. deRosemond *et al*. (2005) reported no increase in the percentage of developmental malformations in white sucker (*Catostomus commersoni*) fry with a mean egg Se concentration of 25.6 μg/g DW. Other studies

evaluating Se effects on salmonids have been conducted in coal mining areas in British Columbia (Kennedy *et al*., 2000) and Alberta (Holm *et al*., 2005), Canada. Kennedy *et al*. (2000) reported no increase in the frequencies of deformities in cutthroat trout (*Oncorhynchus clarkii*) larvae with egg Se concentrations ranging from 8.7 to 81.3 μg/g DW. They concluded that the lack of effects might be the result of a developed tolerance to Se in this population. Similarly, Holm *et al.* (2005) reported no increases in larval deformities in brook trout (*Salvelinus fontinalis*) with mean egg Se concentrations ranging from 6.6 to 7.8 μg/g wet weight (16.9 - 20.0 μg/g DW based on 61% moisture). However, in the same study (Holm *et al*., 2005) larval deformities were elevated (approximately 15% above background levels) in rainbow trout (*Oncorhynchus mykiss*) with egg Se concentrations ranging from 8.8 to 10.5 μg/g wet weight (22.6 – 26.9 μg/g DW based on 61% moisture). Since the majority of the Se research on native fish species has been conducted in warm-water aquatic systems, more emphasis should be placed on studies evaluating the effects of Se levels in fish species inhabiting cold-water aquatic ecosystems (Chapman, 2007).

The objective of the present study was to evaluate the presence of characteristic early life stage deformities associated with Se exposure in northern pike and white sucker fry originating from adults inhabiting a lake downstream of a U mining and milling operation in northern Saskatchewan. In addition, Se and other trace element concentrations were determined in northern pike and white sucker tissues (eggs, muscle, liver, kidney and bone) to further the understanding of metal accumulation patterns in north temperate fish species.

5.2. Materials and methods

5.2.1. Study area

The McClean Lake U mining and milling operation has been operating since 1999. The mine is located in northeastern Saskatchewan (58º 23' N, 103º 48' W), approximately 800 km north of Saskatoon, Saskatchewan, Canada. Treated mill effluent is discharged into the Sink Reservoir at a rate of approximately 4,000 m^3/d. Treated effluent enters Vulture Lake before meeting with McClean Lake. The Sink Reservoir and Vulture Lake, are together designed as the Sink/Vulture treated effluent management system (Figure 5.1). Sampling sites in the current study included a reference site, Indigo Lake located approximately 15 km upstream of the effluent discharge point and one exposure site, Vulture Lake (a closed system), located at the end of the effluent management system (Figure 5.1).

5.2.2. Fish collection

Spawning northern pike and white sucker were collected in May and June 2006, respectively. Northern pike were collected from reference (n = 4 male, n = 4 female) and exposure (n = 5 male, n = 5 female) sites. White sucker were collected at the same reference (n = 5 male, n = 5 female) and exposure (n = 4 male, n = 4 female) sites.

Ripe males and females were collected using hoop nets and gill nets and held in net-pens for less than 4 d prior to gamete collection. Fish were anaesthetized using MS-222, weights and total lengths measured, and eggs or milt collected by light pressure on the abdomen.

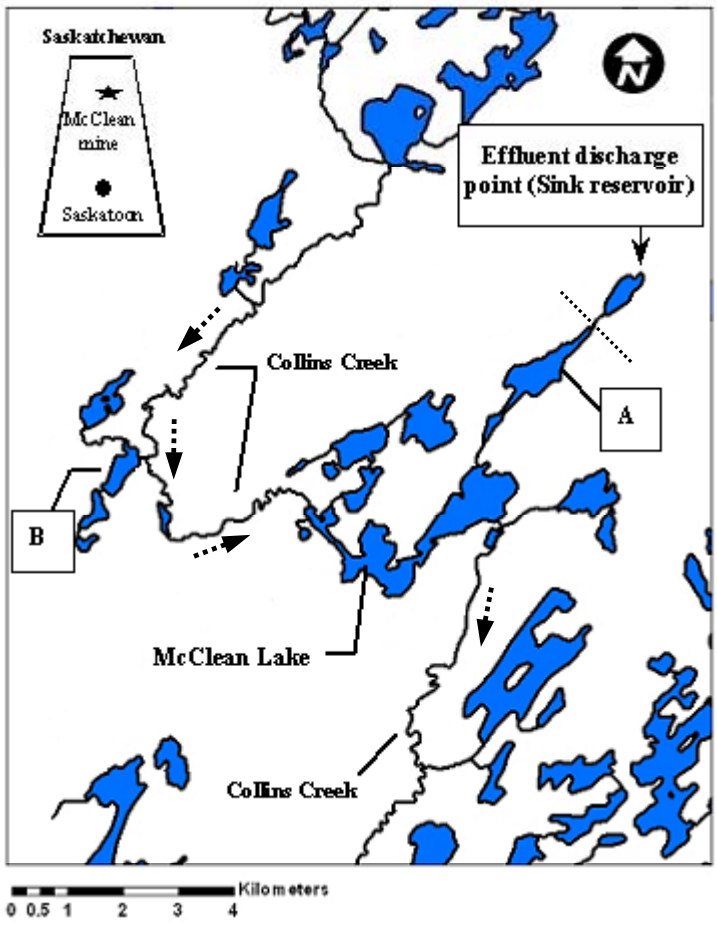

Figure 5.1: Map of McClean Lake mine study area (Saskatchewan, Canada). Insert, map of Saskatchewan showing the relative location of McClean Lake mine operation. Solid arrow, mill effluent discharge into Sink Reservoir. Dashed arrows, flow direction. Dashed lines, dam. Sampling sites: (A) Vulture Lake (exposure site), (B) Indigo Lake (reference site).

Male fish were released after recovery in fresh water and female fish were euthanized for tissue sample collection. Kidney, liver (weight determined on-site), muscle (caudal region), and bone (spine) were collected for trace element analysis and kept frozen until use. Ageing structures were collected from northern pike and white sucker (cleithra and pectoral fin, respectively) for age determination. At each site, pH, conductivity, total dissolved solids (TDS), salinity, temperature and dissolved oxygen (DO) were measured using a YSI model 650 meter (YSI, Yellow Springs, OH, USA), and three replicate samples from each site were collected for subsequent hardness and alkalinity determinations.

5.2.3. Egg fertilization

Sub-samples of eggs (approximately 20 g wet weight) from each female were collected for trace element analysis prior to fertilization. All materials used during the egg fertilization procedure were sterilized using a 0.000075% betadyne (povione-iodine) solution. Eggs from each female were fertilized separately with approximately 0.5 ml of pooled milt derived from four to five males captured at the same site. Sperm activation was achieved by adding water from each respective site and gently mixed for 2 min. Eggs were then transferred to 4-L plastic jars filled with site water (for egg hardening), stored at approximately 4ºC for northern pike and 10ºC for white sucker and transported to the University of Saskatchewan (U of S) on the day of fertilization.

5.2.4. Laboratory embryo incubations

All quality assurance/quality control procedures for embryo incubations were achieved following the guidelines described by Environment Canada (1998) for early life stage rainbow trout. All materials used during the embryo incubation procedure were sterilized using a 0.000075% betadyne (povione-iodine) solution. A sub-sample of approximately 100 embryos from each female was used to evaluate fertilization success (number of fertilized eggs divided by the total number of eggs) as soon as the embryos arrived at the U of S. Embryo incubations were conducted under static-renewal conditions using 4-L plastic buckets in an environmental chamber with a set photoperiod of 16:8 h light:dark and a temperature of 10 ± 1 °C. Water was collected weekly from study sites and shipped to the U of S, where it was used to replace incubation chambers water every 3 d during embryo incubations. Water samples for routine water quality and trace element analyses were collected from three incubation chambers per treatment before and after water renewal throughout the length of the experiments (~ 20-30 d). A total of 36 to 60 samples were collected from northern pike and white sucker incubation chambers, respectively. However, only three randomly selected replicate samples (for each fish specie incubation) were sent for analysis. Mortality, temperature and dissolved oxygen were recorded daily in all incubation chambers throughout the study.

Embryos were incubated using a two-way (cross-over) analysis of the variance (ANOVA) experimental design using water obtained from either reference or exposure sites (Muscatello *et al*., 2006). Fifty viable embryos from each individual female fish were randomly transferred to each of four replicate incubation chambers. Cumulative time

(degree-days) to the 50% eyed embryo, 50% hatch, and 50% swim up stages were determined for each incubation chamber. The experiment was ended individually for each incubation chamber when the majority of the fry exhibited swim-up and had absorbed most of the egg yolk. Fry were euthanized with MS-222 and preserved for the evaluation of deformities as described previously (Muscatello *et al*., 2006). To determine fry condition factor among individual females, three extra replicates per female were incubated in appropriate site water (reference or exposure) and fry length and weight recorded. Condition factor was calculated as

$$(\text{Body weight}/(\text{Length})^3)\text{x } 100 \qquad (5.1)$$

5.2.5. Evaluation of morphological deformities

Evaluation of developmental malformations (skeletal curvatures, craniofacial, finfold, and edema) in northern pike and white sucker fry was performed in a blind fashion using an Olympus model S261 dissecting microscope (Olympus, Melville, NY, USA) with Image-Pro Discovery software version 4.5 (Media Cybernetics, Silversprings, MD, USA) as described previously (Muscatello *et al*., 2006).

5.2.6. Egg, tissue and water sample trace element analysis

Selenium and 21 other trace element analyses in water, eggs and tissues samples were performed by Prairie Diagnostic Services, Western College of Veterinary Medicine, Saskatoon, Saskatchewan, Canada using inductively coupled plasma-mass spectrometry

(ICP-MS). Average selenium recovery for water (> 90 %) and fish egg as well as tissue (> 95 %) samples were evaluated using a Se standard solution (2 µg/L; EMD Chemicals, Ltd., Madison, WI, USA) and certified fish tissue (Dogfish muscle, DORM-2; National Research Council of Canada), respectively. Percent moisture for northern pike and white sucker eggs (73.60 ± 1.50 and 70.22 ± 1.94 %, respectively), muscle (81.56 ± 1.67 and 81.47 ± 1.88 %, respectively), bone (61.71 ± 1.88 and 64.11 ± 3.02 %, respectively), liver (76.03 ± 3.71 and 73.60 ± 1.23 %, respectively) and kidney (80.85 ± 5.29 and 82.46 ± 2.31 %, respectively) were determined for each female fish by drying samples at 60°C until constant weight was recorded. Trace element residue data are thus expressed on a DW basis.

5.2.7. Statistical analysis

Statistical analyses were performed using SigmaStat® version 3.1 (SPSS, Chicago, IL, USA) with a 95% (α= 0.05) level of confidence. Data that failed tests for normality or homogeneity of variance were $\log_{(10)}$ or arcsine square root transformed prior to use of parametric statistical tests. Significant differences among treatments in cumulative time to 50% eyed embryos, 50% hatch and 50% swim-up, mortality and fry deformities were evaluated using two-way ANOVA with egg origin and water source as the two factors. Significant differences between reference and exposure sites in trace element concentrations in female fish eggs, muscle, bone, liver and kidney were evaluated using *t*-tests. Age, egg diameter and fertilization success in female northern pike, and white sucker as well as fry condition factor were also evaluated using *t*-tests. Best fit relationship between the incidence of deformities and selenium concentrations in egg and tissues and

among muscle, bone, kidney, liver and egg were evaluated using regression analysis. Analysis of covariance (ANCOVA; SYSTAT® version 10 (SSI, Richmond, CA, USA) was used to compare the body weights of adult fish with body length as covariate. Analysis of the hepatosomatic index (liver weight with body weight as covariate) using ANCOVA showed a significant interaction ($p < 0.05$) between variable and co-variable (treatment factor) and therefore, was evaluated as liver weight/body weight 100 using a *t-test*.

5.3. Results

5.3.1. Water quality

Water quality variables at the time of adult fish collection and in embryo incubation chambers are shown in Table 5.1 and Table 5.2. Water quality variables were consistent among water samples collected from study sites and embryo incubation chambers. In general, water collected from exposure sites was characterized by higher conductivity, hardness and ammonia, and lower pH, compared to the reference site (Table 5.1 and Table 5.2). Trace elements analyses in embryo incubation chambers were comparable when measured before and after water renewal (Table 5.3 and 5.4). However, variations in the water concentration of certain trace metals (e.g., Antimony (Sb)) before and after water renewals could be the result of only a partial number of water samples analyzed for trace elements. Certain trace elements (arsenic (As), chromium (Cr), iron (Fe), molybdenum (Mo), Se and zinc (Zn)) were above the threshold established by the Canadian Council of Ministers of the Environment (CCME) (2003). Northern pike incubation water showed concentrations of As (> 5 µg/L, exposure site), Cr (> 1µg/L, reference and exposure site)

and Fe (> 300 µg/L, reference site) that were above CCME guidelines. Zinc was higher than 30 µg/L in reference and exposure site water for northern pike before and after water changes. Concentrations of Mo exceeded the 73µg/L threshold established by CCME; however, it should be noticed that this is an interim guideline. Concentrations of waterborne Se were above the established 1µg/L threshold only in northern pike exposure site water before water change.

5.3.2. Adult characteristics

There were no significant differences between study sites in condition factors (body weight with body length as covariate) of adult female northern pike and white sucker (Table 5.5A). However, the condition factor of adult male northern pike and white sucker collected from the reference site were significantly lower than males collected from the exposure site ($p < 0.05$, Table 5.5B). Female northern pike and white sucker collected from the exposure site had significantly lower ($p < 0.05$) hepatosomatic indexes (HSI) than reference site females (Table 5.5A). Statistical comparisons revealed no age differences among female northern pike between sites; however, the average age of female white sucker collected from the reference site was significantly ($p < 0.05$) higher than the exposure site (Table 5.5A).

Table 5.1: Water quality variables measured on-site during (A) northern pike and (B) white sucker collections.

A)

Variable	Hardness [a] (mg $CaCO_3$/L)	Alkalinity [a] (mg $CaCO_3$/L)	pH	Conductivity (µS/cm)	DO [b] (mg/L)	Temperature (°C)	TDS [c] (g/L)	Salinity
Reference	6.20 ± 0.33	12.0 ± 1.33	7.23	10	14.36	7.78	0.01	0.01
Exposure	338.33 ± 23.55	9.66 ± 0.88	6.21	452	13.98	8.20	0.46	0.35

B)

Variable	Hardness [a] (mg $CaCO_3$/L)	Alkalinity [a] (mg $CaCO_3$/L)	pH	Conductivity (µS/cm)	DO [b] (mg/L)	Temperature (°C)	TDS [c] (g/L)	Salinity
Reference	5.76 ± 0.75	13.66 ± 1.11	7.68	11	13.28	13.68	0.01	0.01
Exposure	352.33 ± 3.11	9.33 ± 0.44	6.87	608	13.06	12.30	0.52	0.40

[a] Data represent the mean (± SE) of three replicate samples .

[b] Dissolved oxygen.

[c] Total dissolved solids.

Table 5.2: Water quality variables during (A) northern pike and (B) white sucker embryo incubations measured before and after (in brackets) water renewal. Data represent the mean (± SE) of three replicate samples. Below detection limit (<).

A)

Variable	Hardness (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)	pH	Conductivity (µS/cm)	DO [a] (mg/L)	Temperature (°C)	Ammonia (mg/L)
Reference	4.41 ± 0.59	10.90 ± 0.61	7.48 ± 0.04	21 ± 1.23	9.85 ± 0.07	11.84 ± 0.12	0.06 ± 0.02
	(5.63 ± 0.19)	(11.33 ± 0.48)	(7.46 ± 0.03)	(18.75 ± 0.74)	(9.86 ± 0.07)	(12.21 ± 0.14)	(0.03 ± 0.01)
Exposure	319.17 ± 2.76	9.66 ± 0.65	6.98 ± 0.04	781.50 ± 4.43	9.79 ± 0.12	11.93 ± 0.11	1.57 ± 0.11
	(321.92 ± 2.15)	(11.33 ± 0.81)	(6.91 ± 0.04)	(777.25 ± 6.42)	(9.77 ± 0.08)	(11.74 ± 0.08)	(1.69 ± 0.11)

B)

Variable	Hardness (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)	pH	Conductivity (µS/cm)	DO [a] (mg/L)	Temperature (°C)	Ammonia (mg/L)
Reference	5.36 ± 0.30	9.57 ± 0.80	7.38 ± 0.17	20.43 ± 0.67	9.81 ± 0.07	11.71 ± 0.74	< 0.01
	(5.51 ± 0.13)	(9.71 ± 0.76)	(7.43 ± 0.14)	(20.05 ± 1.48)	(9.93 ± 0.07)	(11.79 ± 0.15)	< 0.01
Exposure	330.05 ± 1.31	8.24 ± 0.60	6.71 ± 0.05	809.38 ± 2.0	9.82 ± 0.07	12.0 ± 0.13	1.69 ± 0.07
	(325.19 ± 1.45)	(9.71 ± 0.35)	(6.73 ± 0.07)	(802.17 ± 1.72)	(9.93 ± 0.08)	(11.80 ± 0.10)	(1.64 ± 0.07)

[a] Dissolved oxygen.

Table 5.3: Total trace element concentration (µg/L) measured in reference water during embryo incubations before and after water change. Data represent the mean (± SE) of three replicate samples. Below instrument detection limit (<).

	Before		After
Metals	**Northern pike**	**White sucker**	
As	0.75 ± 0.39	0.26 ± 0.01	0.30 ± 0.06
Ba	756.53 ± 10.89	139.37 ± 3.95	133.06 ± 5.58
Be	0.25 ± 0.08	$1X10^{-3} \pm 6\ X10^{-4}$	$0.01 \pm 2\ X10^{-3}$
Bi	0.06 ± 0.05	0.20 ± 0.01	$< 9\ X10^{-9}$
Cd	0.95 ± 0.43	0.24 ± 0.06	0.39 ± 0.15
Co	$0.01 \pm 5\ X10^{-3}$	0.05 ± 0.03	0.07 ± 0.06
Cr	1.36 ± 0.66	0.41 ± 0.09	0.26 ± 0.07
Cu	20.54 ± 3.63	5.24 ± 1.13	14.26 ± 5.41
Fe	505.93 ± 42.10	94.41 ± 7.25	88.37 ± 26.86
Mg	251.80 ± 41.58	614.43 ± 14.69	468.20 ± 115.11
Mn	1.87 ± 0.35	1.59 ± 0.49	1.09 ± 0.51
Mo	3.25 ± 0.55	1.34 ± 1.65	0.31 ± 0.02
Ni	< 0.01	0.59 ± 0.16	0.39 ± 0.17
Pb	13.49 ± 3.77	2.14 ± 0.29	3.38 ± 0.21
Sb	1.57 ±0.32	0.29 ± 0.09	$0.02 \pm 4\ X10^{-3}$
Se	0.32 ± 0.03	0.06 ± 0.03	0.1 ± 0.01
Sn	1.43 ± 0.16	0.25 ± 0.13	0.28 ± 0.17
Sr	56.63 ± 1.38	13.45 ± 0.31	10.43 ± 2.49
Tl	$0.04 \pm 8\ X10^{-3}$	$5\ X10^{-3} \pm 2\ X10^{-3}$	$0.01 \pm 3\ X10^{-3}$
U	0.17 ± 0.01	0.05 ± 0.01	0.05 ± 0.04
V	0.52 ± 0.13	0.09 ± 0.01	0.08 ± 0.02
Zn	159.17 ± 57.37	30.75 ± 5.51	96.24 ± 55.31

Table 5.4: Total trace element concentration (µg/L) measured in exposure water during embryo incubations before and after water change. Data represent the mean (± SE) of three replicate samples. Below instrument detection limit (<).

	Before		After
Metals	**Northern pike**	**White sucker**	
As	10.86 ± 0.29	2.55 ± 0.07	2.35 ± 0.12
Ba	914.40 ± 36.22	174.33 ± 4.15	152.36 ± 8.05
Be	0.61 ± 0.03	0.06 ± 0.05	0.07 ± 0.05
Bi	$< 9 \times 10^{-9}$	$<9 \times 10^{-9}$	$0.01 \pm 6 \times 10^{-3}$
Cd	0.81 ± 0.31	0.35 ± 0.05	0.20 ± 0.10
Co	1.50 ± 0.06	0.79 ± 0.61	0.19 ± 0.04
Cr	1.16 ± 0.16	0.35 ± 0.04	0.27 ± 0.06
Cu	21.76 ± 1.04	13.06 ± 3.60	27.06 ± 19.07
Fe	5.08 ± 4.0	19.85 ± 3.14	13.41 ± 2.73
Mg	3,250 ± 782.45	8,655 ± 254.94	7,653 ± 757.19
Mn	54 .17 ± 11.40	32.42 ± 10.60	41.35 ± 16.27
Mo	349.47 ± 7.88	85.66 ± 3.68	77.24 ± 8.32
Ni	50.41 ± 1.24	12.46 ± 0.62	10.81 ± 1.34
Pb	11.46 ± 1.31	2.32 ± 0.61	3.53 ± 0.93
Sb	5.34 ± 0.14	1.1 ± 0.11	0.78 ± 0.05
Se	4.85 ± 0.42	0.90 ± 0.13	0.88 ± 0.02
Sn	0.63 ± 0.08	0.27 ± 0.07	0.15 ± 0.08
Sr	743.90 ± 17.46	200.5 ± 4.86	178.0 ± 16.90
Tl	0.48 ± 0.01	0.13 ± 0.01	0.12 ± 0.01
U	0.77 ± 0.02	0.15 ± 0.02	0.28 ± 0.03
V	1.32 ± 0.23	$0.09 \pm 4 \times 10^{-3}$	0.11 ± 0.01
Zn	44.0 ± 12.13	25.0 ± 6.32	32.0 ± 19.12

5.3.3. Selenium concentration in eggs and larval deformity analysis

Selenium concentrations in northern pike (8.02 ± 0.73 μg/g DW) and white sucker (4.89 ± 0.52 μg/g DW) eggs from the exposure site were significantly higher ($p < 0.05$) than the reference site (2.35 ± 0.20 and 1.94 ± 0.25 μg/g DW, respectively) (Tables 5.6). The measured Se concentration in northern pike eggs from the exposure site was approximately 1.64 times higher than white sucker eggs from the same site. In addition to Se, certain other trace elements in northern pike (Table 5.6) and white sucker (Table 5.6) eggs were significantly ($p < 0.05$) different between sites. Cobalt (Co), Mo, thalium (Tl) and U concentrations were significantly higher ($p < 0.05$) in eggs from exposure site compared to the reference site for both fish species. In contrast, strontium (Sr) was significantly ($p < 0.05$) lower in northern pike and white sucker eggs originating from the reference site. The concentrations of cadmium (Cd) and magnesium (Mg) in eggs originating from the exposure site were significantly ($p < 0.05$) higher than reference only in white sucker eggs (Table 5.6).

Among the four categories of evaluated deformities (spinal curvatures, craniofacial deformities, fin deformities and edema), only edema in white sucker fry was significantly higher (~ 3%, $p < 0.05$) than the reference site (Figure 5.2). In addition, no significant differences in the frequencies of total deformities were observed in either fish species (Figures 5.2 and 5.3). Although exposure northern pike reached the eyed embryo stage earlier (~ 2 d) than reference northern pike ($p < 0.05$) no effect was observed in the overall time (cumulative time to 50% hatch and 50% swim-up) for larval development (Figure. 5.4). White sucker fry did not display significant differences in the cumulative time to 50%

eyed embryo, 50% hatch and 50% swim-up between treatments (Figure 5.5). Cumulative mortality throughout incubations was not significantly different among sites, ranging from 26 to 33% and 74 to 81% for northern pike and white sucker, respectively. The rates of mortality found in the present study are in accordance with the expected mortality for developing northern pike and white sucker embryos Scott and Crossman, 1973). In addition, no differences were found in egg diameter and fertilization success between sites for either fish species (Table 5.5A).

Table 5.5: Somatic measurements in northern pike and white sucker (A) female and (B) male. Data represent the mean (± SE). * Significant difference ($p < 0.05$) between reference and exposure site using *t- test*. ** Significantly different ($p < 0.05$) between reference and exposure site in male condition factor (fish weight with length as covariate) using analysis of covariance.

A)

Variables	Northern Pike		White sucker	
	Reference (*n* = 4)	**Exposure (*n* = 5)**	**Reference (*n* = 5)**	**Exposure (*n* = 4)**
Age (yrs)	5.75 ± 0.85	6.0 ± 0.32	10.6 ± 1.60	5.25 ± 0.25 *
Weight (kg)	1.09 ± 0.27	2.42 ± 0.52	1.26 ± 0.04	0.93 ± 0.08
Length (cm)	56.55 ± 5.23	68.84 ± 1.45	50.30 ± 1.28	40.63 ± 0.82
Cond. Factor [a]	0.56 ± 0.02	0.73 ± 0.15	1.0 ± 0.08	1.38 ± 0.12
HSI [b]	1.42 ± 0.34	0.48 ± 0.07 *	0.62 ± 0.06	0.27 ± 0.08 *
Egg size (mm)	2.54 ± 0.08	2.48 ± 0.09	3.01 ± 0.04	2.96 ± 0.04
Fertilization (%)	88.25 ± 1.03	86.40 ± 0.51	94.80 ± 2.48	98.50 ± 0.65
Total fry evaluated	1,238	1,710	259	470

B)

Variables	Northern Pike		White sucker	
	Reference (*n* = 4)	**Exposure (n = 5)**	**Reference (*n* = 5)**	**Exposure (*n* = 4)**
Weight (kg)	1.18 ± 0.09	2.98 ± 0.09	0.94 ± 0.1	0.68 ± 0.07
Length (cm)	55.0 ± 2.29	66.96 ± 1.38	45.12 ± 1.98	38.26 ± 0.80
Cond. Factor [a]	0.73 ± 0.09	0.99 ± 0.05**	1.02 ± 0.05	1.20 ± 0.07**

[a] Condition factor presented as (weight (g)/length^3(cm)) x 100.

[b] Hepatosomatic index (liver weight/body weight) x 100.

[c] Fertilization success (%), calculated as (number of fertilized eggs/total number of eggs) x 100.

Table 5.6: Female northern pike and white sucker total trace element concentrations in eggs (µg/g dry weight). Data represent the mean (± SE) of 4-5 fish. * Significantly different ($p < 0.05$) from the reference site using *t- test*. Below instrument detection limit (<).

Metals	Northern pike		White sucker	
	Reference	Exposure	Reference	Exposure
As	0.24 ± 0.03	0.38 ± 0.06	0.22 ± 0.03	0.30 ± 0.03
Ba	1.15 ± 0.18	0.66 ± 0.10	0.41 ± 0.09	0.23 ± 0.06
Be	$3x10^{-3}$ ±1 $x10^{-3}$	1 $x10^{-3}$ ± $5x10^{-3}$	8 $x10^{-5}$ ± 5 $x10^{-5}$	7 $x10^{-4}$ ± 4 $x10^{-4}$
Bi	< 9 $x10^{-9}$	< 9 $x10^{-9}$	< 9 $x10^{-9}$	< 9 $x10^{-9}$
Cd	2 $x10^{-3}$ ± 7$x10^{-4}$	7 $x10^{-3}$ ± 2 $x10^{-4}$	9 $x10^{-4}$ ± 2 $x10^{-4}$	0.10 ± 0.05*
Co	0.05 ± 6$x10^{-3}$	0.10 ± 5$x10^{-3}$*	0.04 ± 4 $x10^{-3}$	0.06 ± 5 $x10^{-3}$*
Cr	0.30 ± 0.02	0.36 ± 0.02	0.30 ± 7 $x10^{-3}$	9 $x10^{-3}$ ± 5 $x10^{-3}$
Cu	3.98 ± 0.48	3.42 ± 0.21	2.99 ± 0.12	2.54 ± 0.18
Fe	60.52 ± 7.92	64 .19 ± 5.90	29.88 ± 5.01	35.81 ± 4.02
Mg	1,782 ± 164	2,007 ± 152	793.36 ± 38.0	973.29 ± 45.08
Mn	15.74 ± 3.60	14.15 ± 1.40	11.22 ± 0.65	10.92 ± 2.16
Mo	0.04 ± 0.01	0.11 ± 0.01*	0.05 ± 4 $x10^{-3}$	0.39 ± 0.09*
Ni	0.18 ± 0.03	0.23 ± 0.04	0.25 ± 0.07	0.44 ± 0.18
Pb	0.10 ± 0.03	0.06 ± 0.01	0.09 ± 0.03	0.08 ± 0.02
Sb	4$x10^{-7}$ ± 1$x10^{-8}$	1.3 $x10^{-3}$ ± 8 $x10^{-4}$	< 2 $x10^{-7}$	2 $x10^{-4}$ ± 7 $x10^{-5}$
Se	2.35 ± 0.20	8.02 ± 0.73*	1.94 ± 0.25	4.89 ± 0.52*
Sn	0.10 ± 0.06	0.06 ± 0.02	0.02 ± 5 $x10^{-3}$	0.01 ± 2 $x10^{-3}$
Sr	3.30 ± 0.35	0.81 ± 0.06*	0.58 ± 0.04	0.27 ± 0.08*
Tl	1 $x10^{-3}$ ± 4$x10^{-4}$	3.$x10^{-3}$ ± 3$x10^{-4}$*	7 $x10^{-3}$ ± 5 $x10^{-3}$	0.03 ± 4 $x10^{-3}$*
U	< 1 $x10^{-8}$	45$x10^{-3}$ ± $x10^{-3}$*	6 $x10^{-4}$ ± 2 $x10^{-4}$	0.01 ± 2 $x10^{-3}$*
V	0.16 ± 0.02	0.18 ± 0.01	0.07 ± 8 $x10^{-3}$	0.09 ± 7 $x10^{-3}$
Zn	84.24 ± 7.28	99.99 ±9.35	49.46 ± 2.96	56.13 ± 6.03

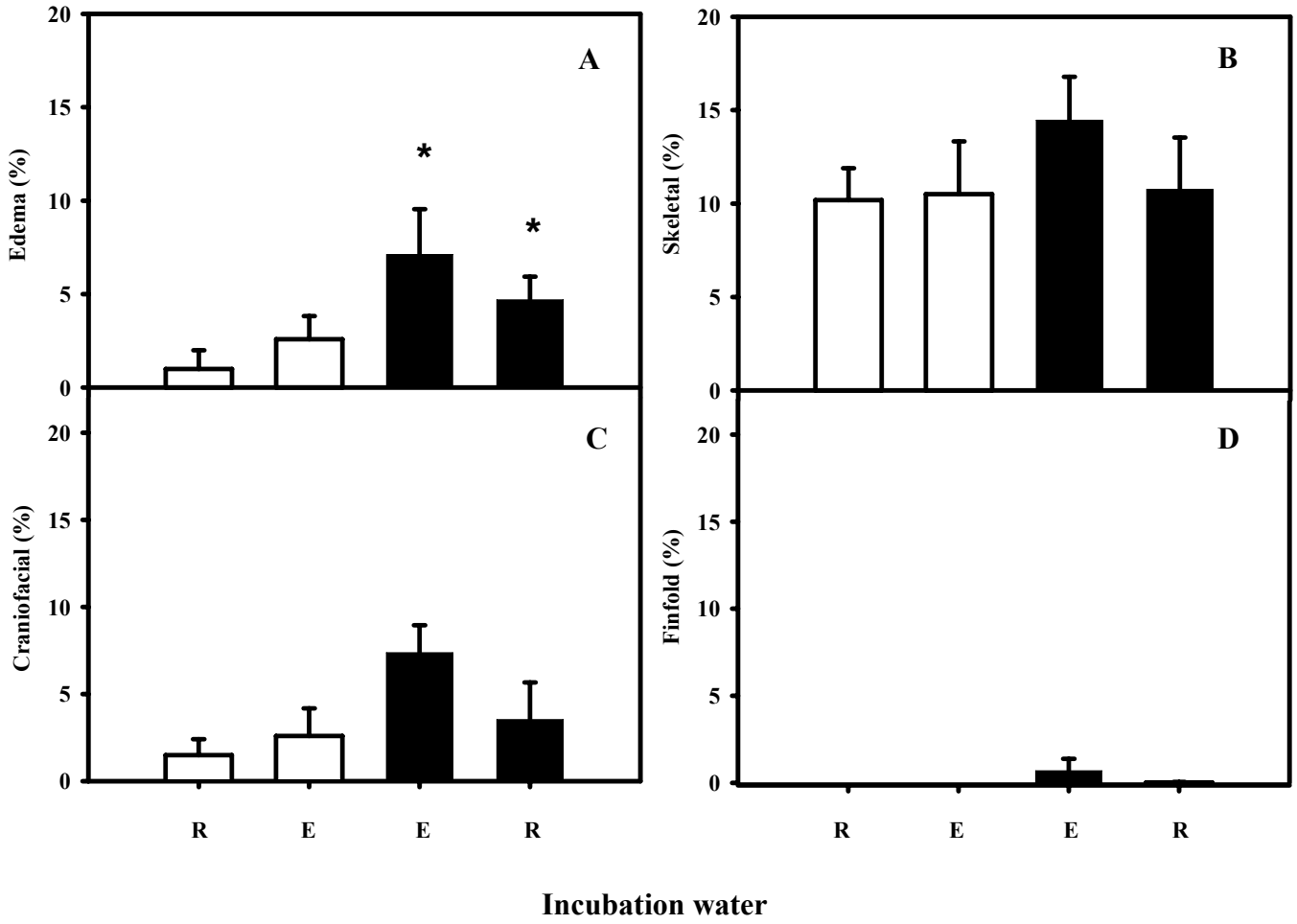

Figure 5.2: Analysis of larval deformities (A) edema, (B) skeletal, (C) craniofacial and (D) finfold in white sucker fry originating from reference (open bars) and exposure (solid bars) sites and incubated in both reference (R) and exposure (E) water. Data represent the mean (± SE) per treatment. * Significant difference ($p < 0.05$) from reference due to egg's origin.

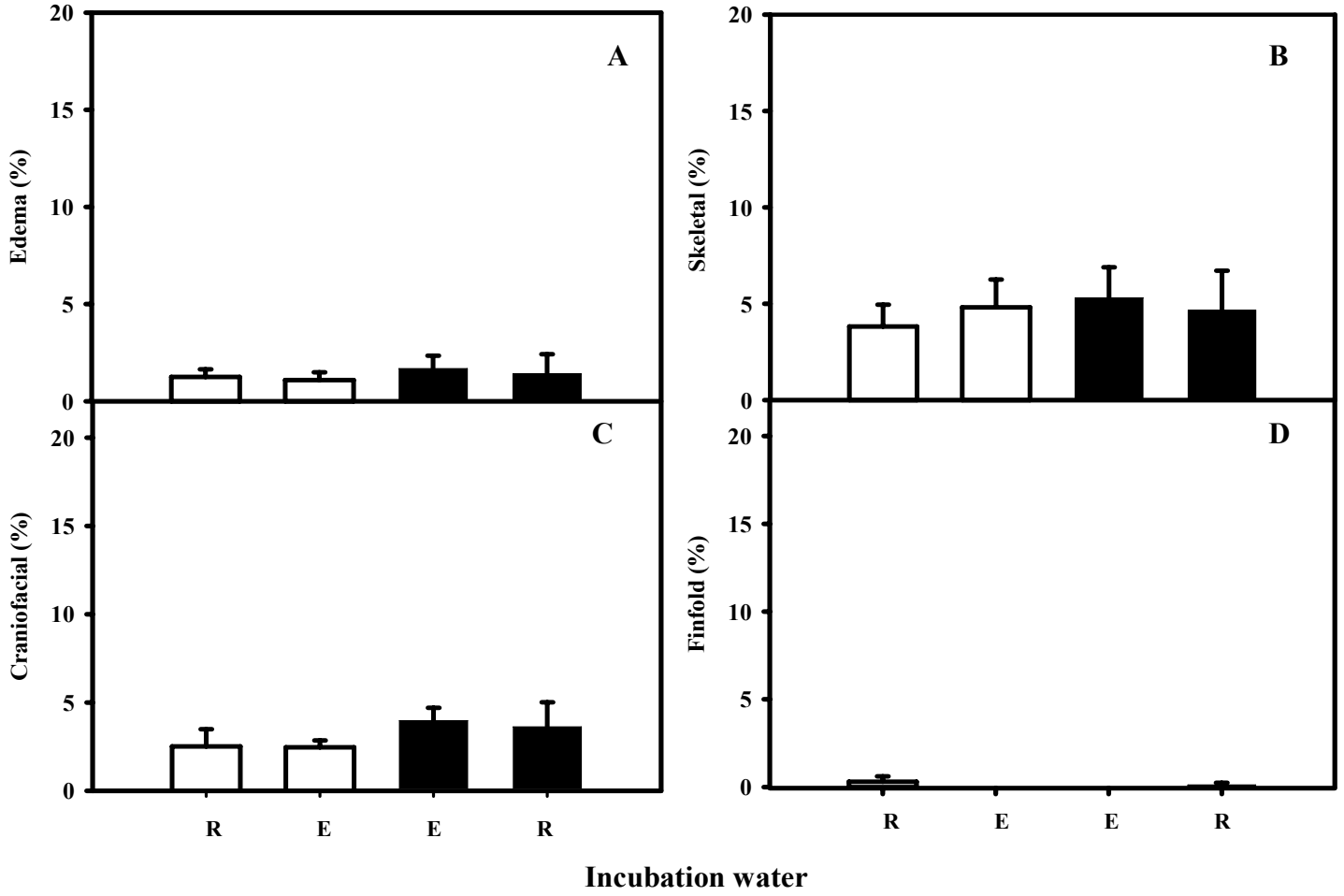

Figure 5.3: Analysis of larval deformities (A) edema, (B) skeletal, (C) craniofacial and (D) finfold in northern pike fry originating from reference (open bars) and exposure (solid bars) sites and incubated in both reference (R) and exposure (E) water. Data represent the mean (± SE) per treatment. * Significant difference ($p < 0.05$) from reference due to egg's origin.

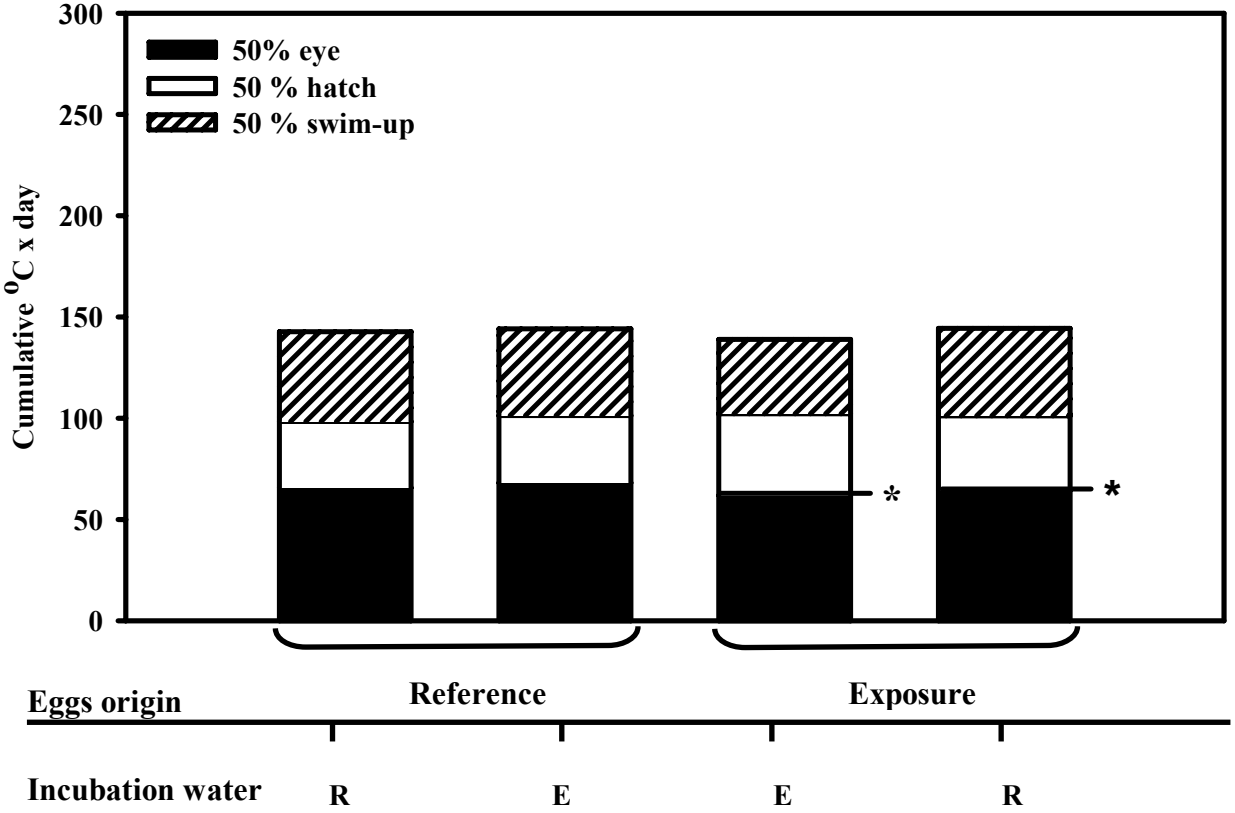

Figure 5.4: Cumulative time (^{o}C x day) to 50% eyed embryo, 50% hatch and 50% swim-up for northern pike embryos incubated in either reference (R) or exposure (E) water. Data represent the mean of four replicate incubation chambers per treatment. * Significantly different ($p < 0.05$) between reference and exposure sites using two-way analysis of variance (ANOVA).

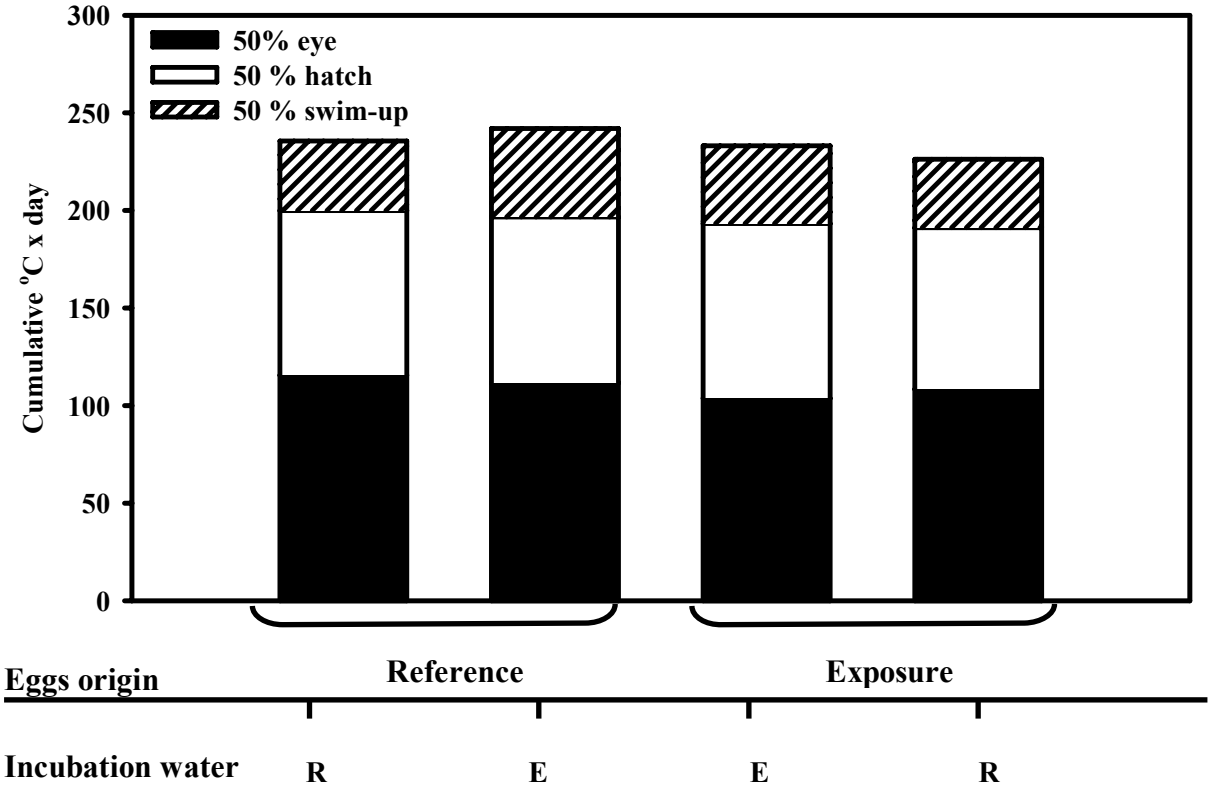

Figure 5.5: Cumulative time (^{o}C x day) to 50% eyed embryo, 50% hatch and 50% swim-up for white sucker embryos incubated in either reference (R) or exposure (E) water. Data represent the mean of four replicate incubation chambers per treatment. * Significantly different ($p < 0.05$) between reference and exposure sites using two-way analysis of variance (ANOVA).

There were no significant differences in the condition factor of northern pike and white sucker fry between reference (1.26 ± 0.06 and 1.40 ± 0.06, respectively) and exposure sites (1.31 ± 0.05 and 1.31 ± 0.04, respectively).

5.3.4. Selenium and other trace elements in adult tissues

Muscle, liver, kidney and bone Se concentrations were significantly greater ($p < 0.05$) in adult female northern pike and white sucker collected from the exposure site compared to the reference site (Tables 5.7 and 5.8, respectively). Muscle and bone Se concentrations were similar between each fish species. In contrast, Se concentrations in liver and kidney of female northern pike were approximately two times higher than female white sucker Tables 5.7 and 5.8. Concentrations of certain trace elements other than Se were also significantly different in northern pike and white sucker tissues between reference and exposure sites (Tables 5.7 and 5.8,). Northern pike females collected from the exposure site showed a significant ($p < 0.05$) increase in muscle concentration of Co, tin (Sn), Tl and Zn when compared to the reference site. White sucker females displayed a significant ($p < 0.05$) increase in the muscle concentration of Tl, Mo and lead (Pb) at the exposure site. Muscle concentrations of other trace elements such as Sr (northern pike and white sucker) and bismuth (Bi; white sucker) showed a decrease in the exposure site compared to the reference site (Tables 5.7 and 5.8). Bone concentrations of Co, Mo and Tl were significantly ($p < 0.05$) higher in the exposure site than the reference site, while Sr concentrations were significantly lower ($p < 0.05$) at the exposure site in both fish species. Liver concentrations of Co, Mo, nickel (Ni), Tl and U were elevated in northern pike and

white sucker females collected from the exposure site, while other metals (Cd (northern pike and white sucker), Cr (northern pike only), Mg and copper (Cu; white sucker only)) were significantly lower ($p < 0.05$) at the exposure site (Tables 5.7 and 5.8). Elevated concentration of As were observed in liver tissue of northern pike females collected from exposure site ($p < 0.05$). There were significant ($p < 0.05$) increases in kidney concentrations of Cu, Mo and Ni in northern pike and white sucker females collected from the exposure site. Kidney concentrations of Co in northern pike and beryllium (Be) in white sucker females also increased ($p < 0.05$) between sites. In contrast, Cd (white sucker and northern pike), Cr and Sr (northern pike only), and Fe (white sucker only) concentrations were lower ($p < 0.05$) in kidney tissues of fish collected from the exposure site (Tables 5.7 and 5.8). There were positive linear relationships between Se concentrations in northern pike eggs and muscle ($r^2 = 0.60$, $p = 0.014$), bone ($r^2 = 0.74$, $p = 0.003$), kidney ($r^2 = 0.72$, $p = 0.004$) and liver ($r^2 = 0.84$, $p < 0.001$; Figure, 5.6). Similarly, strong positive linear relationships were observed in white sucker Se concentrations between eggs and muscle ($r^2 = 0.98$, $p < 0.001$), bone ($r^2 = 0.92$, $p < 0.001$), kidney ($r^2 = 0.88$, $p < 0.001$) and liver ($r^2 = 0.87$, $p < 0.001$; Figure 5.7).

Table 5.7: Female northern pike tissue and bone total trace element concentrations (µg/g dry weight). Data represent the mean (± SE) of 4-5 fish. * Significantly different ($p < 0.05$) from the reference site using *t- test*. Below instrument detection limit (<).

Metal	Muscle		Bone		Liver		Kidney	
	Reference	**Exposure**	**Reference**	**Exposure**	**Reference**	**Exposure**	**Reference**	**Exposure**
As	0.19 ± 0.04	0.32 ± 0.06	0.09 ± 0.03	0.12 ± 0.01	0.36 ± 0.04	0.51 ± 0.04	0.57 ± 0.1	0.75 ± 0.1
Ba	1.15 ± 0.21	0.69 ± 0.33	28.62 ± 1.42	6.14 ± 1.30*	0.08 ± 0.03	0.02 ± 0.01	0.99 ± 0.17	1.10 ± 0.50
Be	$2x10^{-3} \pm 5x10^{-4}$	$2x10^{-3} \pm 6x10^{-4}$	$3x10^{-4} \pm 2x10^{-4}$	$< 6 x10^{-8}$	$< 6 x10^{-8}$	$1x10^{-4} \pm 9.0 x10^{-}$	$2.x10^{-4} \pm 1x10^{-4}$	$5x10^{-4} \pm 4x10^{-4}$
Bi	$5x10^{-3} \pm 9x10^{-4}$	$3x10^{-3} \pm 9x10^{-4}$	$< 9 x10^{-9}$	$< 9 x10^{-9}$	$< 9 x10^{-9}$	$< 9 x10^{-9}$	$< 9 x10^{-9}$	$< 9 x10^{-9}$
Cd	$2 x10^{-3} \pm 7 x10^{-4}$	$4.x10^{-3} \pm 2 x10^{-3}$	$6.x10^{-3} \pm 5 x10^{-4}$	$4 x10^{-3} \pm 1 x10^{-3}$	0.15 ± 0.05	0.04 ± 0.01*	0.37 ± 0.05	0.21 ± 0.03*
Co	$0.01 \pm 8 x10^{-4}$	$0.03 \pm 4.x10^{-3}$*	$0.14 \pm 2x10^{-3}$	$0.17 \pm 7x10^{-3}$*	0.09 ± 0.01	0.23 ± 7.4 x10^{-}	0.41 ± 0.07	1.62 ± 0.01*
Cr	0.77 ± 0.15	0.68 ± 0.11	0.50 ± 0.07	0.24 ± 0.04*	0.44 ± 0.06	0.24 ± 0.01*	1.47 ± 0.38	0.46 ± 0.15*
Cu	1.14 ± 0.13	1.49 ± 0.34	0.98 ± 0.13	0.80 ± 0.07	41.05 ± 17.97	22.08 ± 3.56	6.14 ± 0.44	9.27 ± 0.93*
Fe	14.72 ± 2.64	14.33 ± 2.29	27.64 ± 2.63	35.56 ± 5.46	819.15 ± 443.41	470.56 ± 115.68	245.26 ± 23.13	280.54 ± 28.19
Mg	1,680 ± 52	1,852 ± 150	2,980 ± 104	2,840 ± 181	746.50 ±48.05	828.99 ± 22.28	804.0 ± 26.57	835.22 ± 15.60
Mn	2.55 ± 0.70	2.05 ± 0.76	93.17 ± 18.35	64.98 ± 5.13	6.26 ± 1.20	4.72 ± 0.32	4.24 ± 0.53	0.42 ± 0.19
Mo	$0.05 \pm 8x10^{-3}$	$0.06 \pm 5x10^{-3}$	$0.02 \pm 6x10^{-3}$	0.52 ± 0.06*	0.54 ± 0.03	1.17 ± 0.11*	0.49 ± 0.03	4.32 ± 1.25*
Ni	0.16 ± 0.01	0.23 ± 0.07	2.67 ± 0.28	2.02 ± 0.13	$0.1 \pm 6x10^{-3}$	0.39 ± 0.04*	0.24 ± 0.06	2.13 ± 0.27*
Pb	0.14 ± 0.06	0.10 ± 0.03	$8.8^{-3} \pm 4.5^{-3}$	$1.2^{-3} \pm 1.0^{-3}$	$0.01 \pm 7x10^{-3}$	0.03 ± 0.01	0.04 ± 0.03	0.03 ± 0.01
Sb	$2.x10^{-3} \pm 9 x10^{-4}$	$4x10^{-3} \pm 2x10^{-3}$	$4x10^{-4} \pm 3x10^{-4}$	$2x10^{-3} \pm 1x10^{-3}$	$8 x10^{-4} \pm 3x10^{-4}$	$2x10^{-4} \pm 8x10^{-5}$	0.02 ± 0.01	0.02 ± 0.01
Se	0.70 ± 0.04	4.21± 0.54*	0.38 ± 0.07	1.20 ± 0.11*	4.08 ± 0.51	10.38 ± 0.47*	5.60 ± 0.46	10.27 ± 0.50*
Sn	$0.02 \pm 4 x10^{-3}$	$0.05\pm 5.x10^{-3}$*	0.03 ± 0.01	$0.01 \pm 3.4 x10^{-3}$	$0.015 \pm 3 x10^{-3}$	0.05 ± 0.03	0.03 ± 0.01	0.04 ± 0.01
Sr	6.52 ± 2.08	1.03 ± 0.38*	326.73 ± 16.05	54.91 ± 8.82*	0.44 ± 0.09	0.29 ± 0.07	0.80 ± 0.05	0.41 ± 0.06*
Tl	$0.04 \pm 1.0 x10^{-3}$	$0.08 \pm 8.5 x10^{-}$	$9x10^{-3} \pm 9x10^{-4}$	0.02 ± 9.7 x10^{-}	0.06 ± 0.01	0.13 ± 0.01*	0.09 ± 0.01	0.17 ± 0.01*
U	$6x10^{-4} \pm 2x10^{-4}$	$2x10^{-3} \pm 4x10^{-4}$	0.03 ± 0.01	$0.02 \pm 5 x10^{-3}$	$< 1 x10^{-8}$	$0.01 \pm 4 x10^{-3}$*	0.02 ± 0.01	0.05 ± 0.01
V	0.03 ± 4^{-3}	0.01 ± 3^{-3}	0.16 ± 0.03	0.10 ± 0.02	0.99 ± 0.47	0.33 ± 0.11	0.67 ± 0.25	0.35 ± 0.1
Zn	16.02 ± 2.61	31.93 ± 3.76*	158.93 ± 12.34	170.78 ± 17.88	189.38 ± 34.01	197.14 ± 19.82	351.55 ± 36.85	499.92 ± 58.03

Table 5.8: Female white sucker tissue and bone total trace element concentrations (µg/g dry weight. Data represent the mean (± SE) of 4-5 fish. * Significantly different ($p < 0.05$) from the reference site using *t- test*. Below instrument detection limit (<).

Metal	Muscle		Bone		Liver		Kidney	
	Reference	**Exposure**	**Reference**	**Exposure**	**Reference**	**Exposure**	**Reference**	**Exposure**
As	0.13 ± 0.01	0.94 ± 0.79	0.13 ± 0.01	0.14 ± 0.02	0.26 ± 0.02	0.31 ± 0.01	0.28 ± 0.06	0.30 ± 0.02
Ba	0.99 ± 0.23	0.32 ± 0.12	12.99 ± 1.90	11.63 ± 1.08	0.14 ± 0.04	0.54 ± 0.34	0.39 ± 0.12	0.52 ± 0.12
Be	$8 \times 10^{-4} \pm 4 \times 10^{-}$	$1 \times 10^{-3} \pm 4 \times 10^{-4}$	$< 6 \times 10^{-8}$	$< 6 \times 10^{-8}$	$4 \times 10^{-4} \pm 3 \times 10^{-}$	$3 \times 10^{-3} \pm 3 \times 10^{-3}$	$1 \times 10^{-4} \pm 0.0$	$4 \times 10^{-3} \pm 2 \times 10^{-}$
Bi	$0.01 \pm 3 \times 10^{-3}$	$< 9 \times 10^{-9}$*	$< 9 \times 10^{-9}$	$< 9 \times 10^{-9}$	$< 9 \times 10^{-9}$	$< 9 \times 10^{-9}$	19.20 ± 18.70	$< 9 \times 10^{-9}$
Cd	$5 \times 10^{-3} \pm 2 \times 10^{-3}$	$2 \times 10^{-3} \pm 5 \times 10^{-4}$	$0.01 \pm 5 \times 10^{-3}$	$6 \times 10^{-3} \pm 3 \times 10^{-3}$	0.27 ± 0.03	$0.03 \pm 4 \times 10^{-3}$*	1.27 ± 0.21	0.17 ± 0.05*
Co	$0.02 \pm 3 \times 10^{-3}$	$0.01 \pm 1 \times 10^{-3}$	$0.11 \pm 7 \times 10^{-3}$	$0.16 \pm 7 \times 10^{-3}$*	0.13 ± 0.01	0.30 ± 0.05*	0.45 ± 0.18	0.68 ± 0.22
Cr	1.04 ± 0.61	0.53 ± 0.07	0.32 ± 0.04	0.19 ± 0.02	0.28 ± 0.03	0.35 ± 0.06	0.37 ± 0.07	0.26 ± 0.02
Cu	1.86 ± 0.42	1.39 ± 0.44	1.08 ± 0.08	1.07 ± 0.12	53.91 ± 3.04	22.37 ± 3.73*	7.64 ± 0.18	37.38 ± 9.96*
Fe	2 2.60 ± 5.38	11.96 ± 0.93	33.73 ± 1.95	29.10 ± 2.92	1,156 ± 463.42	1,050 ± 102.34	586.0 ± 83.30	287.50 ± 42.03*
Mg	1,806 ± 104.97	1,703 ± 111.82	2,804 ± 195.97	3,204 ± 124.89	883.70 ± 42.42	1,035 ± 27.07*	898.20 ± 9.62	916.35 ± 49.37
Mn	1.65 ± 0.90	0.64 ± 0.05	97.34 ± 25.99	36.25 ± 6.20	8.21 ± 0.69	7.47 ± 0.81	3.75 ± 0.34	3.07 ± 0.26
Mo	$0.05 \pm 4 \times 10^{-3}$	0.19 ± 0.05*	0.11 ± 0.01	4.29 ± 0.23*	1.02 ± 0.23	2.36 ± 0.12*	0.77 ± 0.06	23.46 ± 6.30*
Ni	0.18 ± 0.03	0.27 ± 0.04	1.80 ± 0.12	1.91 ± 0.09	0.17 ± 0.02	0.53 ± 0.06*	0.31 ± 0.08	1.87 ± 0.53*
Pb	0.02 ± 0.01	0.13 ± 0.04*	$0.01 \pm 7 \times 10^{-3}$	$5 \times 10^{-4} \pm 4 \times 10^{-4}$	0.07 ± 0.03	0.04 ± 0.01	0.05 ± 0.02	0.03 ± 0.01
Sb	$1 \times 10^{-3} \pm 3 \times 10^{-}$	$0.02 \pm 1 \times 10^{-3}$	$< 2 \times 10^{-7}$	$< 2 \times 10^{-7}$	$2 \times 10^{-3} \pm 2 \times 10^{-}$	$3 \times 10^{-3} \pm 2 \times 10^{-}$	$9 \times 10^{-3} \pm 8 \times 10^{-}$	$2 \times 10^{-3} \pm 2 \times 10^{-}$
Se	1.89 ± 0.13	4.37 ± 0.52*	0.73 ± 0.09	1.61 ± 0.12*	3.01 ± 0.30	5.71 ± 0.30*	3.24 ± 0.42	5.89 ± 0.43*
Sn	$0.03 \pm 5 \times 10^{-3}$	$0.05 \pm 9 \times 10^{-3}$	$0.02 \pm 6 \times 10^{-3}$	$0.02 \pm 8 \times 10^{-3}$	0.03 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Sr	11.76 ± 8.94	0.35 ± 0.06*	259.52 ± 19.23	75.34 ± 4.62*	0.79 ± 0.13	1.06 ± 0.55	0.87 ± 0.12	0.57 ± 0.12
Tl	$0.01 \pm 2 \times 10^{-3}$	$0.03 \pm 3 \times 10^{-3}$*	$3 \times 10^{-3} \pm 4 \times 10^{-4}$	0.05 ± 0.03*	$0.01 \pm 2 \times 10^{-3}$	0.08 ± 0.01*	$0.03 \pm 2 \times 10^{-3}$	0.07 ± 0.01
U	$6 \times 10^{-4} \pm 2 \times 10^{-3}$	0.07 ± 0.06	$0.02 \pm 7 \times 10^{-3}$	0.15 ± 0.01*	$5 \times 10^{-3} \pm 5 \times 10^{-3}$	0.05 ± 0.01*	0.02 ± 0.01	0.07 ± 0.02
V	$0.03 \pm 6^{-3} \times 10$	$0.03 \pm 6 \times 10^{-3}$	0.14 ± 0.02	0.19 ± 0.02	0.30 ± 0.15	0.13 ± 0.02	0.62 ± 0.31	0.21 ± 0.04
Zn	15.85 ± 1.27	18.99 ± 3.86	32.72 ± 1.57	38.78 ± 1.88*	91.80 ± 6.07	99.68 ± 9.44	70.94 ± 1.95	89.78 ± 10.39

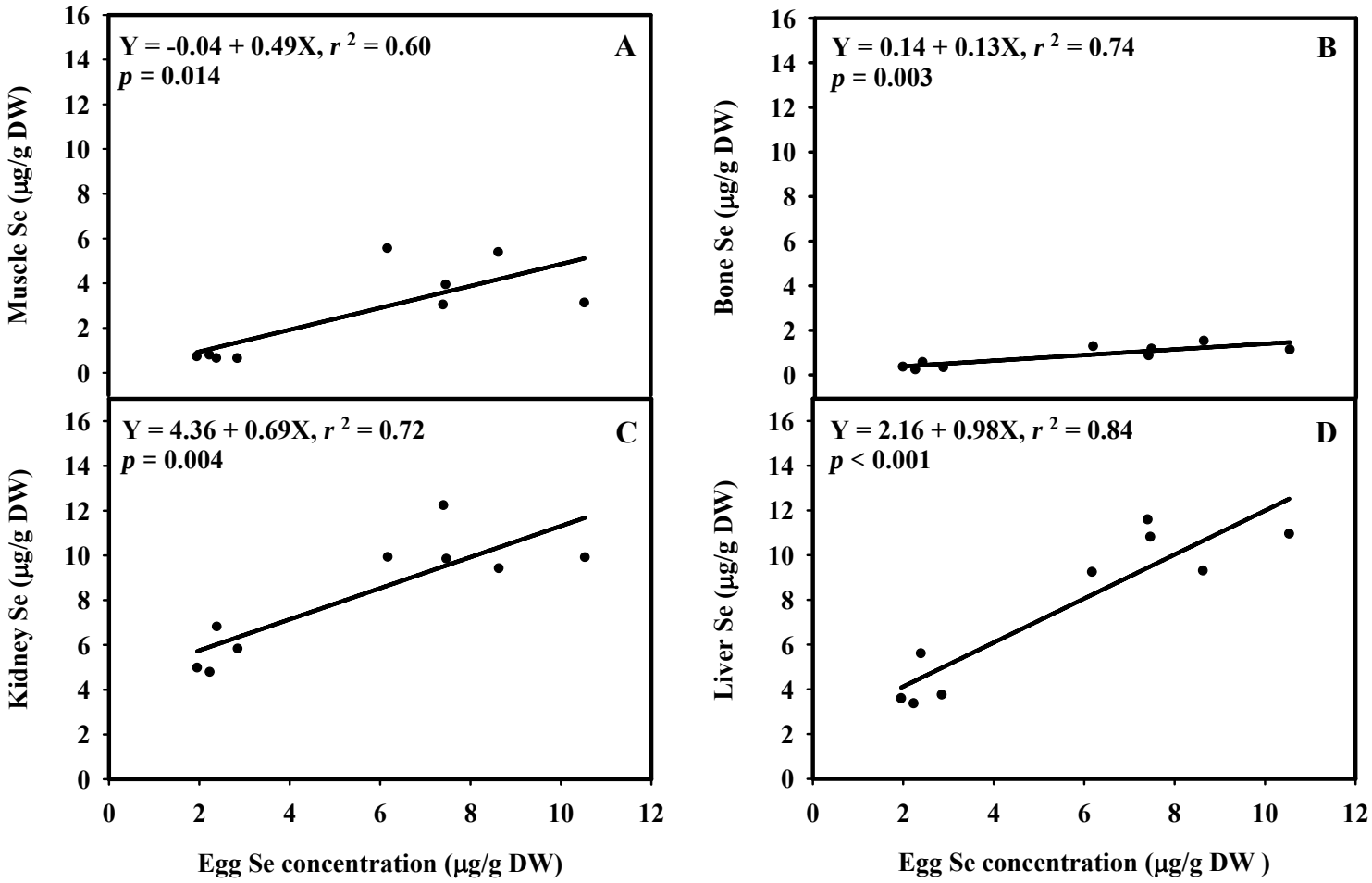

Figure 5.6: Relationships between selenium (Se) concentration in eggs (µg/g dry weight) obtained from female northern pike collected from reference site (n = 4) and exposure site (n = 5) and Se concentration in adult female pike tissues: (A) muscle, (B) bone, (C) kidney and (D) liver.

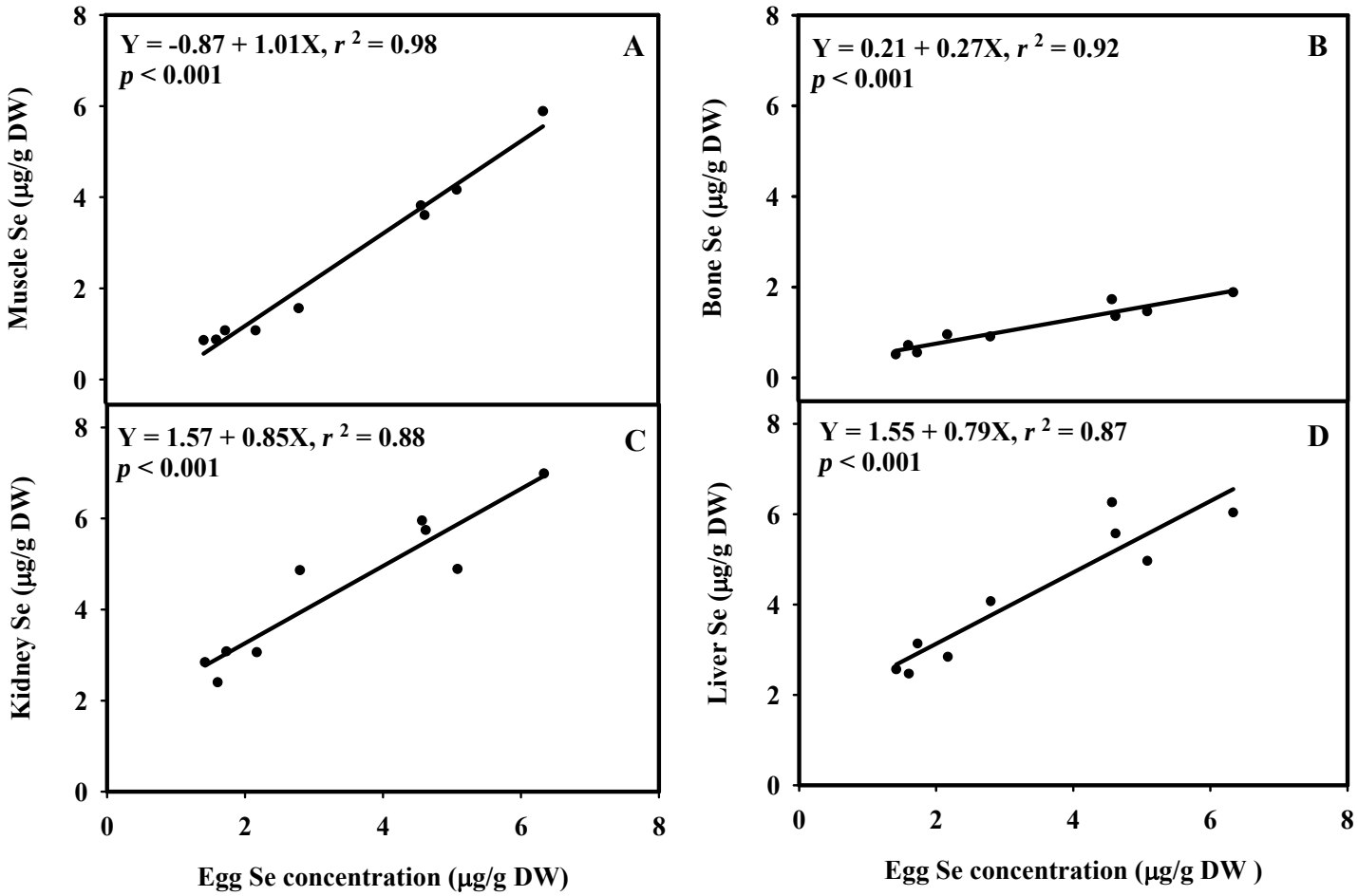

Figure 5.7: Relationships between selenium (Se) concentration in eggs (µg/g dry weight) obtained from female white sucker collected from reference site (n = 5) and exposure site (n = 4) and Se concentration in adult female pike tissues: (A) muscle, (B) bone, (C) kidney and (D) liver.

5.4. Discussion

Elevated concentrations of Se were observed in eggs and tissues of northern pike and white sucker collected downstream of a U mining and milling operation in northern Saskatchewan, Canada. Although the Se levels in northern pike and white sucker eggs from the exposure site were two to three times higher than the reference site, the measured Se concentrations in exposure site eggs were below the reported Se threshold (10 μg/g DW) associated with Se-induced deformities in other fish species (Gillespie and Baumann, 1986; Lemly, 1993a; Coyle *et al*., 1993; Muscatello *et al.*, 2006). Furthermore, whole body Se concentrations for northern pike (3.74 ±0.57 and 4.11 ± 0.88 μg/g DW converted from egg and muscle, respectively) and white sucker (2.40 ± 0.27 and 4.25 ± 0.65 μg/g DW converted from egg and muscle, respectively) calculated using the equations provided by the U.S. EPA (USEPA, 2004) were below the proposed whole body concentration of 7.91 μg/g DW for the protection of fish (USEPA, 2004). There was no evidence of elevated developmental anomalies (spinal curvatures, missing or deformed fins, craniofacial deformities and edema) in northern pike fry originating from the exposure site. However, white sucker fry originating from the exposure site displayed a slight increase in the incidence of edema that could also be associated with several factors (e.g., other metals, organic compounds, ammonia) other than Se (USEPA, 1984; Wilson and Tillitt, 1996; Incardona *et al*., 2004). Overall, there was no apparent effect of exposure lake water on classic early life stage toxicity endpoints (i.e., time to eyed embryo, hatch or swim-up stage).

In the present study, both fish species showed a significant positive relationship between Se concentrations in eggs and tissues, indicating that Se concentration in eggs could be predicted from Se concentrations in adult tissues. Although Se levels in white sucker egg and muscle were similar, the overall pattern (from smallest to largest) of Se accumulation in female northern pike and white sucker tissues was as follows: bone < muscle < eggs < kidney and liver. The pattern of Se accumulation reported in the present study is in accordance with Se accumulation observed in other fish species. (Lemly, 1982; Kennedy *et al.*, 2000; Holm *et al*., 2005; Muscatello *et al*., 2006; Rudolph *et al*., 2008). The differences in Se accumulation between northern pike and white sucker eggs, liver and kidney reported in the present study could be explained by differences in the mobilization of resources for gonad development, Se metabolism (e.g., differential formation of seleno-proteins) and/or different feeding habits (northern pike (piscivorous) and white sucker (benthivorous)), areas that require further investigation.

In addition to Se, elevated concentrations of other trace elements were observed in eggs (Cd, Co, Mo, Tl, and U) and tissues of northern pike (As, Co, Cr, Cu, Mo, Ni, Sn, Tl, U and Zn) and white sucker (Be, Co, Cu, Mg, Mo, Ni, Pb, Sr, Tl, U and Zn) females. In contrast, the concentration of certain trace elements showed a decrease in egg (Sr) and northern pike (Cd, Cr and Sr) and white sucker (Bi, Cd, Cu, Fe and Sr) tissues between sites. Selenium can interact with several trace elements. Whereas some elements (e.g., mercury (Hg), As) have shown protective (antagonistic) effects against the toxicity of selenium (Hill, 1975), others (i.e., boron (B), As-Mo mixtures) can increase its toxicity (Hamilton and Palace, 2005). The complex interactions between Se and trace elements have been described elsewhere (Hill, 1975; Naganuma *et al*., 1983) and still require further investigation. In

addition to metal interactions, other factors such as water quality and environmental variables (e.g., increased hardness, organic matter) can significantly modify metal toxicity and accumulation (Pascoe *et al.*, 1986; Winner and Gauss, 1986; Spry and Wiener, 2001). The decrease in the concentration of certain trace elements in fish eggs and tissues from the exposure site reported in this study could be explained by trace elements competition (e.g., calcium (Ca^{+2})) and/or complexation (e.g., carbonates), processes that can potentially reduce the concentration of bioavailable metals and therefore potentially decrease metal toxicity and accumulation. From a physiological point of view, elevated Ca^{+2} (i.e, high hardness) has been shown to alter gill-metal binding characteristics preventing the accumulation of certain metals (e.g., Cd) (Niyogi and Wood, 2003).

Liver Se concentrations in adult female northern pike and white sucker were below Lemly's (1993a) proposed threshold (12 μg/g DW) for adverse effects in freshwater fish. However, the liver mass (HSI) of female northern pike and white sucker from exposure areas was approximately two times lower than reference. Other studies have reported lower HSI in fish after metal exposure (Rajotte and Couture, 2002; Sindhe and Kulkami, 2004). Nevertheless, there are several factors (e.g., ammonia) other than metal exposure that can also contribute to changes in liver size (Acharva *et al*., 2005). The greater condition factors in adult northern pike and white sucker collected from the exposure site in the present study may be explained by the presence of certain factors (e.g., phosphate) elevated in exposure areas, which can cause enrichment of aquatic ecosystems (e.g., increased productivity and food availability) and consequently have an indirect positive effect on fish condition.

The two-way ANOVA experimental design used in the present study allowed discrimination between maternal transfer and site water effects in two fish species collected downstream of a U mine operation in northern Saskatchewan. The results suggest no overt toxic effects either from egg Se concentration or site water exposure on the early developmental stages of northern pike and white sucker. Overall, the results of the present study are consistent with published Se thresholds for early life stage deformities in other fish species, with egg Se accumulation in northern pike and white sucker collected at this site below the proposed 10 μg/g (DW) biological threshold associated with elevated deformities. Unfortunately, there is still controversy regarding appropriate Se thresholds and particularly the choice of tissue for developing a tissue-based Se guideline. Further research is needed before a Se criterion or guideline for the protection of fish populations is implemented.

5.5. Conclusions

Uranium mining and milling operations in northern Saskatchewan, Canada release effluents elevated in certain trace metals and metalloids, including Se. The goal of the present study was to evaluate the presence of Se-induced deformities in northern pike and white sucker larvae originating from adults collected downstream of a U mine operation. Eggs were incubated in the laboratory following a two-way (cross-over) analysis of variance experimental design to discriminate effects due to maternal transfer *versus* site water exposure in the developing embryos. Selenium concentrations in northern pike and white sucker eggs from the exposure site were approximately two to three times higher than

reference. Among all evaluated deformities (skeletal curvatures, craniofacial deformities, fin deformities and edema), only edema in white sucker fry from the exposure site was slightly elevated (~ 3%) compared to reference. However, the occurrence of edema can be associated with factors other than Se (e.g., other metals, organic compounds). The lack of a clear toxic response in the present study is in agreement with Se thresholds for early life stage deformities reported in other studies, with egg Se concentrations in northern pike and white sucker collected at the exposure site below the 10 μg/g (DW) threshold associated with the presence of deformities. Both fish species displayed strong linear relationships between the Se concentration in eggs and other tissues (muscle, liver, kidney and bone), suggesting that Se concentrations in eggs could be predicted from Se concentrations in adult tissues.

CHAPTER 6

Movement and home range of northern pike (*Esox lucius*) within gradient of mine effluent discharge: Study on the use of radio-telemetry as a monitoring tool

6.1. Introduction

Northern pike (*Esox lucius*) is a widespread specie with a circumpolar distribution and one of the most important predators in north temperate freshwaters (Raat, 1988; Craig, 2008). Although northern pike is an opportunistic predator and will consume a variety of food items, adults are generally piscivores (Scott and Crossman, 1973). Furthermore, it is well known that pike play a major role in structuring the aquatic fauna in cool water habitats (Craig, 2008). In general, pike are described as sedentary (not moving far from their home range) (Vehanen *et al*., 2006; Craig, 2008), displaying higher levels of activity at dusk and the spawning period (Cook and Bergesen, 1988) and having size-specific utilization of vegetated habitats (e.g., small pike restricted to shallow vegetated areas and larger pike likely inhabiting deeper low vegetated areas (pelagic zone)) (Grimm, 1981; Chapman and Mackay, 1984a).

Divergent results have been reported for pike seasonal movement rates and home range. For example, Jepsen *et al*. (2001) and Koed *et al*. (2006) reported increased pike displacement during winter, while Rogers (1998) found less activity in winter than in summer, and Diana *et al*. (1977) found no difference in pike movement between winter and summer. In addition, there are certain discrepancies regarding northern pike home range with some authors reporting restricted home range for pike (Grimm and Klinge, 1996; Eklöv, 1997) and others reporting larger/undefined home ranges with frequent shifts between habitats (Bregazzi and Kennedy, 1980; Chapman and Mackay, 1984b; Cook and Bergersen, 1988). These contradicting results are likely caused by the inherent ability of pike to change its behaviour in response to different environmental conditions (Diana *et al*.,

1977; Chapman and Mackay, 1984b; Headrick and Carline, 1993) in addition to differences in study design (Jepsen *et al*., 2001).

Anthropogenic activities such as metal mining have the potential to significantly modify the structure and integrity of downstream water bodies through physical (e.g., shoreline alterations), chemical (e.g., increased conductivity and metal levels) and biological (eutrophication, changes in invertebrate community or abundance) alterations. These changes in the aquatic environment can modify fish habitat and have an indirect effect in the movement and distribution of fish. Variations in water quality variables (e.g., pH, conductivity, total dissolved solids, trace metal concentrations), invertebrate abundance or diversity and fish reproductive capacity reported to occur downstream of uranium mining and milling operations in northern Saskatchewan (Golder 2005, Muscatello *et al*., 2006) have the potential to affect pike behaviour (e.g., movement). Due to the importance of northern pike as a top predator in aquatic systems and a fisheries resource for commercial and recreational use, understanding the habitat utilization and behaviour of pike plays a major role in the application of effective conservation strategies (Lucas and Baras, 2000). Although positional telemetry (radio and acoustic) have been used to investigate pike ethology in freshwater systems, only a few studies have used geographic positioning system (GPS) to accurately locate fish position (Rogers and Bergesen 1995; Vehanen *et al*., 2006; Kobler *et al.*, 2008 a,b). The goal of this study was to use radiotelemetry in association with GPS techniques to investigate the effect of mining effluent discharges in the seasonal and daily movements and home range of northern pike inhabiting north temperate lakes. This study was conducted to enhance our understanding of the effects of metal mining on northern pike movement and home range. To our knowledge, this is one of

the first studies using radio-telemetry as an environmental monitoring tool in north temperate aquatic ecosystems.

6.2. Materials and Methods

6.2.1. Study area

Key Lake uranium mine is located in north-central Saskatchewan (57°11'N, 105°34'W) approximately 600 km from Saskatoon, SK, Canada. Mining at Key Lake began in 1983 and ceased in 1997. Although, mining activities no longer take place at Key Lake, this mine is currently milling ores transported from McArthur River mine. Treated mill effluent is discharged at a rate of approximately 6,000 m^3/d to the receiving aquatic environment. Treated effluent enters the Yak Creek drainage at Wolf Lake and on average makes up 48% of Yak Creek flow. Yak Creek flows into David Creek before meeting up with Unknown Lake. Treated effluent is about 23% of the David Creek flow. Water drains from Unknown Lake (via David Creek) into Delta Lake, approximately 10 km downstream of effluent release and continues through into the Wheeler River (Figure 6.1). Fish were collected from a reference lake, David site (1.40 km^2 surface area and 2.44 m average depth) and an exposure site, Delta Lake (2.68 km^2 surface area and 4.42 m average depth, Figure 6.1). These northern lakes are nitrogen and phosphorus limited and generally oligotrophic (Dillon *et al*., 2004), however higher nitrogen occurs in exposure lakes as a consequence of effluent release (Bennett and Janz, 2007).

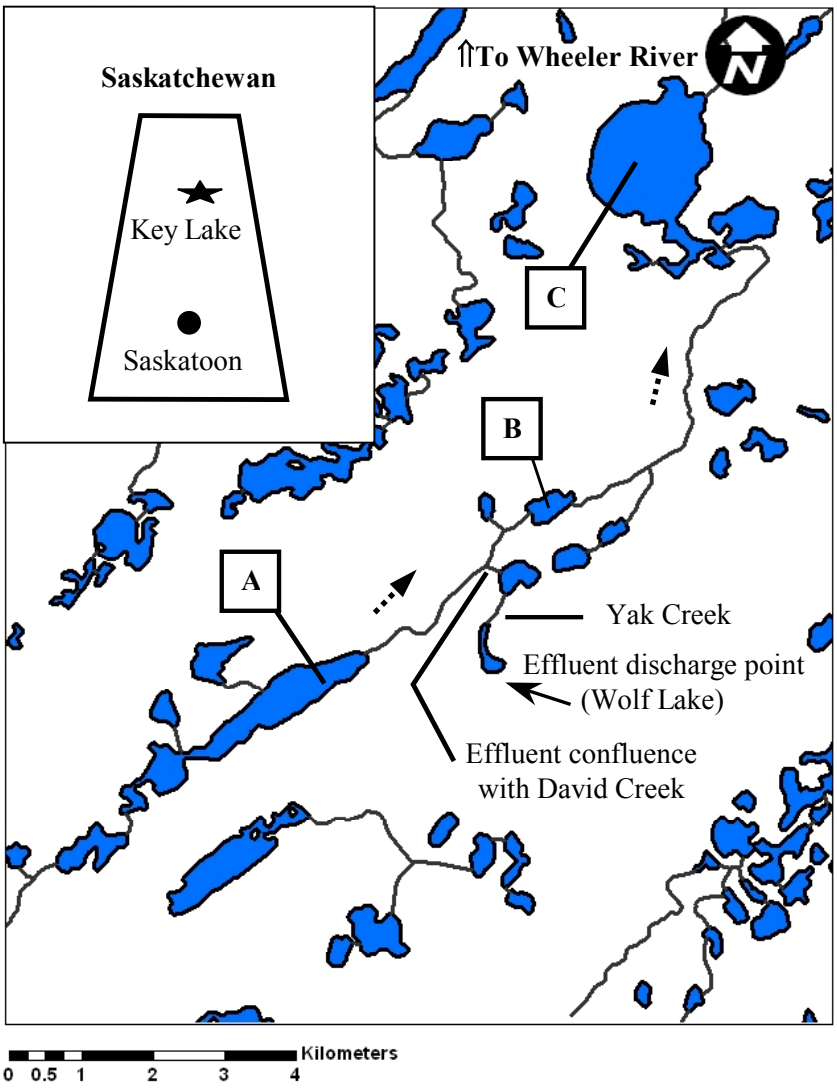

Figure 6.1: Map of Key Lake study area (Saskatchewan, Canada). Insert: map of Saskatchewan showing the relative location of Key Lake uranium mine operations. Sampling sites: A) David Lake (reference site), B) Unknown Lake and C) Delta Lake (exposure site). Solid arrow, mill effluent discharge into David Creek. Dashed arrows, flow direction

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6.2.2. Fish capture and tagging

Northern pike were captured from reference (females *n*=3, males *n*=2) and exposure (females *n*=3, males *n*=2) lakes by angling with barbless hooks and radio-tagged in early September 2004. Prior to tagging, fish were anaesthetized using a 3-aminobenzoic acid (MS 222) solution (~ 0.4 mg/L). Once opercular rate became slow fish were removed from the anaesthetic, and body length and weight determined. Sex was determined as described by Casselman (1974). Northern pike were then placed in a V shaped surgical table and the transmitter was inserted into the body cavity through a 3-4 cm mid-ventral incision posterior to the pelvic girdle. The external antenna was passed through a small hole in the body cavity. The incision was closed using a sterile polypropylene (non-absorbable) filament. The closed wound was rinsed with a broad-spectrum fish antibiotic (MELAFIX®) and covered with a veterinary tissue adhesive (Vetbond™). The duration of the surgical procedure was 3-5 min. Fish were then transferred to a recovery tank containing fresh water and aeration. Fully recovered fish were kept in holding pens and closely monitored for at least 5 hr before release back into the lake. All fish were released no more than 20 m from the site of capture. The transmitters were cylindrical (Model MCFT-3L, Lotek, Newmarket, ON, Canada), 16x75 mm, weighing 26 g (in air). Each transmitter was digitally coded with a continuous burst rate of 5 sec. and operating frequencies within 149.42-149.46 MHz. The tagging method used in the present study has been previously reported to cause no adverse effects on the growth or survival of pike (Jepsen and Aarestrup, 1999).

6.2.3. Tracking and data analysis

Radiotracking was performed at both reference and exposure lakes from a boat using a hand-held receiver (Model SRX400A, Lotek, Newmarket, ON, Canada) and a four-element Yagi antenna. During tracking, fish were approached at low speed to avoid any disturbance and the location was defined by the strongest signal obtained (gain=34, strongest signal 232) or observation of the fish. A GPS device (Garmin ETREX® Legend, Olathe, KS, USA) was used to obtain the location coordinates. Water quality parameters (conductivity, pH, temperature, dissolved oxygen (DO), total dissolved solids (TDS) and salinity) were recorded for each tracking session using a YSI model 650 meter (YSI Inc., Yellow Springs, OH, USA) positioned approximately 1m under the water surface.

Northern pike were tracked from September 29th, 2004 to May 19th, 2006. Fish locations were recorded during fall, spring, summer and winter periods (seasonal tracking). During this tracking session each individual fish position was recorded once per season from 0900 to 1800 h, with one-hour interval between locations (5-6 locations per fish). Due to the low air temperatures experienced in northern Saskatchewan during the winter months (< -35 °C) fish tracking can only be performed from 1200 to approximately 1800 h. In addition, 3-day tracking (daily tracking) was performed in June 2007. During this tracking session, the position of each individual fish was recorded from 0900 to 1800 h (with one-hour interval between locations) during three consecutive days. Each time a fish was located its distance from shore (defined as more than 5 m (> 5 m) or less than 5 m (< 5 m) from shore) and the bottom type were determined by visual observations. Percentages of occurrence were calculated for habitat preference estimations.

Coordinates of seasonal and daily pike locations were incorporated into ArcView GIS version 9.1 (ESRI Inc., Redlands, CA) and linear distances traveled for individual fish were calculated for the morning (0900-1200 h), noon (1200-1300), afternoon (1400-1600 h) and late afternoon (1700-1800 h) periods. Minimum displacement per hour (MDPH) was calculated for the morning, afternoon and per day periods as the ratio between consecutive locations and time elapsed between locations (hours of tracking for each period (4 hrs for morning and afternoon periods, 8 hrs for the day period)). Home ranges were estimated seasonally and daily for individual fish by minimum convex polygons using Hawth's analysis tools for ArcView, version 3.27 (Beyer, 2004).

6.2.4. Statistical analysis

Statistical analyses were performed using SigmaStat version 3.1 (SPSS, Inc., Chicago, IL) with a 95% ($\alpha = 0.05$) level of confidence. Differences between reference and exposure lakes in distance traveled, home range and habitat preference variables (percentages of fish occurrence) over time were evaluated using two-way repeated-measures analysis of variance (RM-ANOVA) with two factors (sex and lake (exposure vs. reference)). One-way RM-ANOVA with one factor (sex) was used to evaluate differences in distances traveled, home range and habitat preference over time within lakes. Data that failed normality or homogeneity of variances was log (x+1) (distances traveled and home range) or arcsine square root (percentages of fish occurrence) transformed prior to use of parametric statistics. Holm-Sidak (post-hoc) test was used when appropriate. Pearson correlation analysis was used to evaluate the possible effects of water parameters and fish body size

(weight and length) on distance traveled. Data that failed normality or homogeneity of variances after transformations were analyzed using a non-parametric correlation test (Spearman rank correlation). Analysis of covariance (ANCOVA, SSI, Inc., Richmond, CA) was used to evaluate the body weight of adult pike with body length as a covariate.

6.3. Results

6.3.1. Adult characteristics

Adult characteristics and radio-tag details are described in Table 6.1. Condition factors (body weight with body length as covariate) of female and male northern pike collected from the exposure lake were significantly lower than pike collected from the reference lake ($p < 0.05$; Table 6.1). All the tagged pike survived and remained in the study area throughout the study period. Therefore, they were classified as resident pike

6.3.2. Seasonal movements

There were no significant differences in the seasonal movement of pike between exposure and reference lakes (Figures 6.2 and 6.3). Mean distances traveled ranged from approximately 50 to 400 m. Although statistically not significant, more active movement was observed during winter for the majority of the fish, with the exception of reference lake females (Figures 6.2 and 6.3). Northern pike from the exposure lake displayed significantly ($p< 0.05$) larger home ranges than pike from the reference lake during summer and winter periods (Figures 6.4 and Figure 6.5). No sex related differences were found within lakes in pike seasonal movement or home ranges within seasons.

Table 6.1: Summary of fish morphometrics and radio-tag information for a total of 10 northern pike at reference and exposure (bold) lakes. Data represent the mean (± SE). * Significantly different from reference ($p < 0.05$) using ANCOVA.

	n	**Weight (kg)**	**Total length (cm)**	**Condition factor**	**Tag frequency (MHz)**	**Tag code**
Females	3	4.7 ± 2.2	76.3 ± 17.4	1.1 ± 0.4	149.42-149.44	11, 14, 15
	3	**3.5 ± 0.4**	**73.0 ± 4.0**	**0.9 ± 0.1***	**149.44-149.46**	**16, 19, 20**
Males	2	3.0 ± 0.50	63.0 ± 1.0	1.2 ± 0.1	149.42	12, 13
	2	**3.1 ± 0.1**	**71.2 ± 1.0**	**0.9 ± 0.0***	**149.46**	**17, 18**

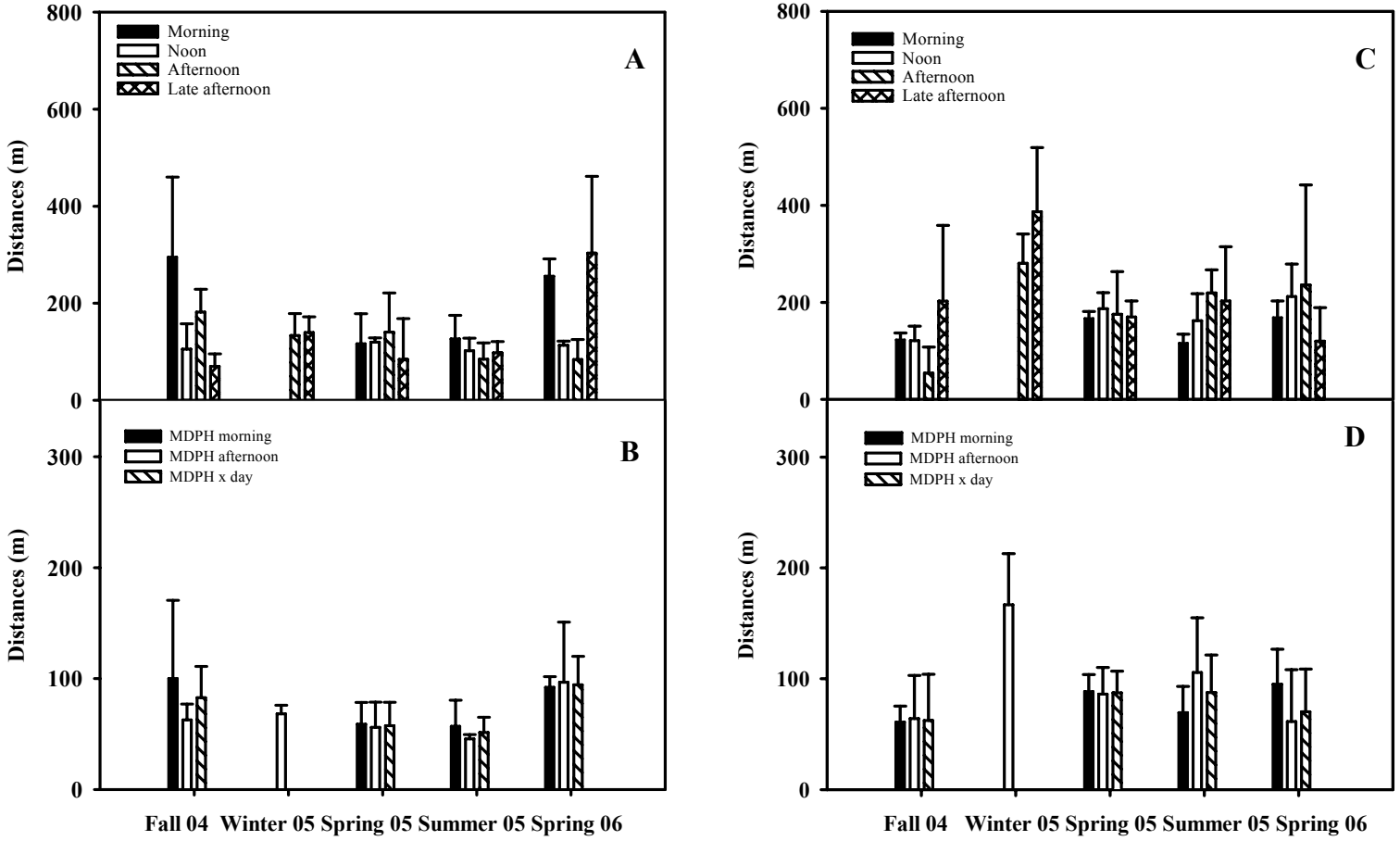

Figure 6.2: Female northern pike seasonal movement ((distance traveled) and minimum displacement per hour (MDPH)) in A) and B) reference lake; C) and D) exposure lake. Data represent the mean (± SE) of three female fish.

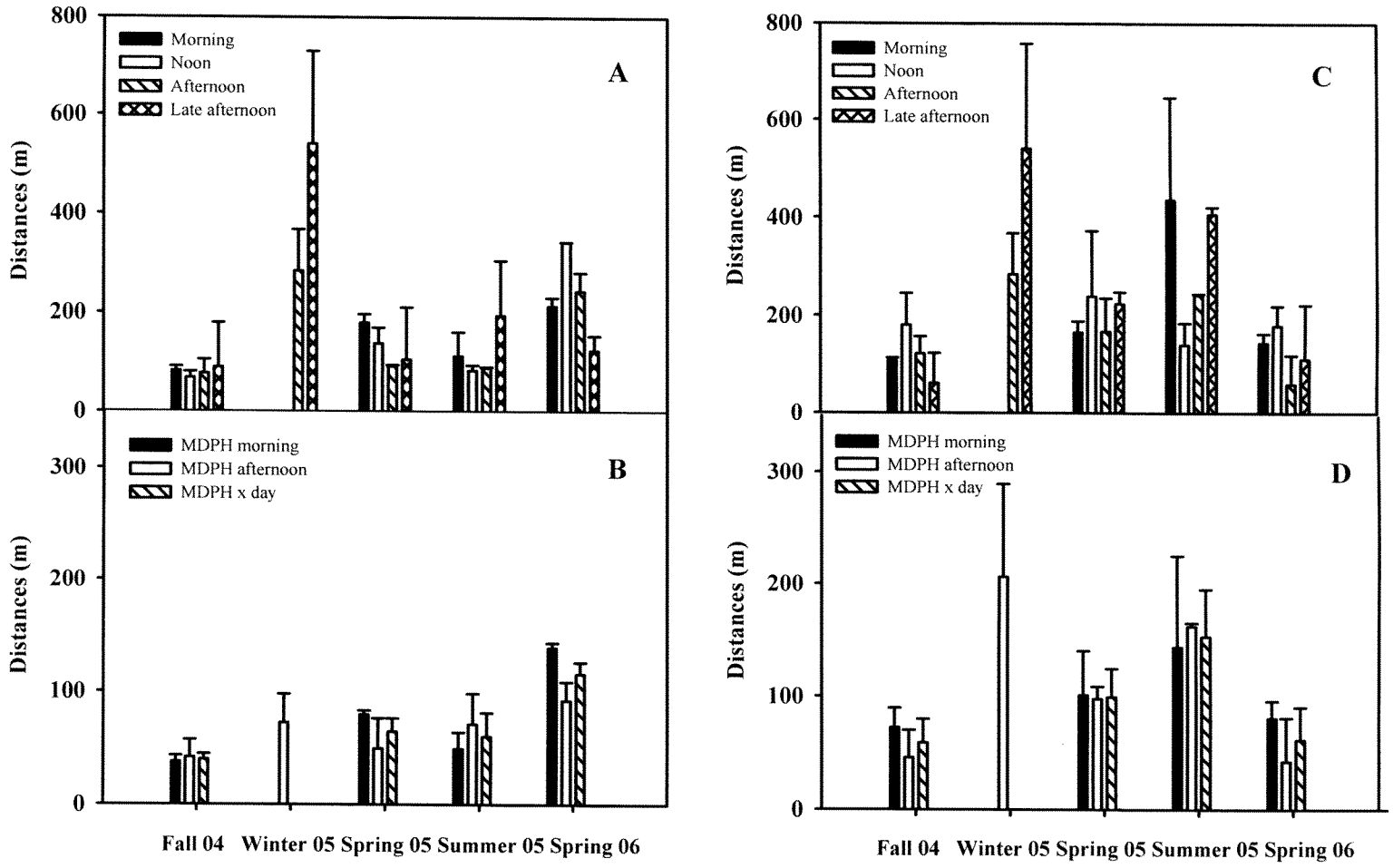

Figure 6.3: Male northern pike seasonal movement ((distance traveled) and minimum displacement per hour (MDPH)) in A) and B) reference lake; C) and D) exposure lake. Data represent the mean (± SE) of two male fish

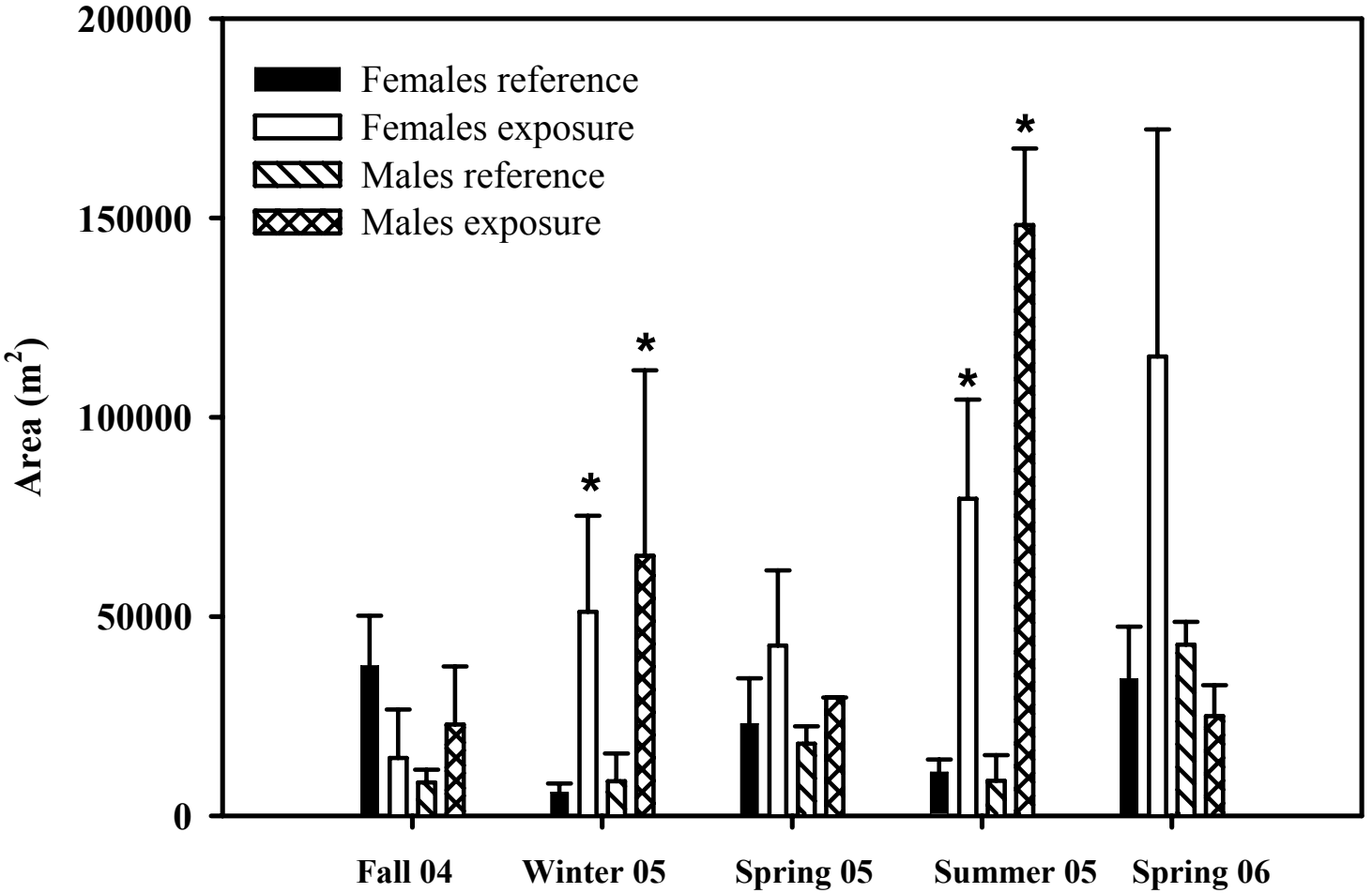

Figure 6.4: Home range for female and male northern pike during different seasons. Area was calculated on 24 hr locations of individual fish per season. Data represent the mean (± SE) of 5 (three females and two males) fish for each study site.

* Significant different ($p < 0.05$) from reference site using two-way repeated measures ANOVA..

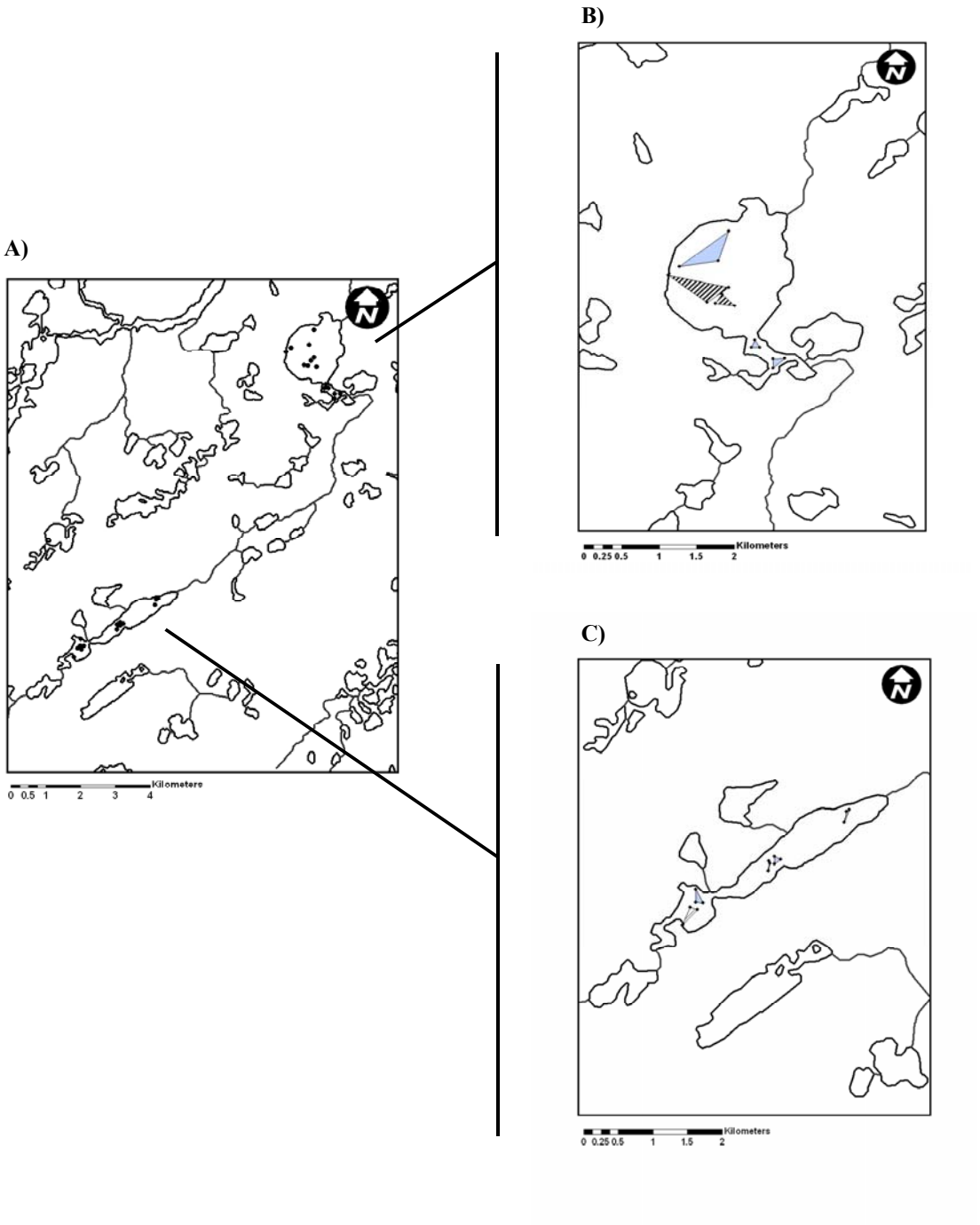

Figure 6.5: Map of the study area showing an example of the winter distribution of northern pike in the reference (*n* =5) and exposure (*n* = 5) lakes (A). A total of three locations are shown for each individual pike. Home range area (m^2) for males (dashed area) and females (full area) in the B) exposure and C) reference lake.

There was no correlation between body size (length and weight) and seasonal home ranges for both reference and exposure lake. Water quality from the exposure lake was characterized by higher conductivity, TDS and salinity and lower pH than the reference lake (Table 6.2). There was no correlation between temperature, TDS and salinity and pike seasonal movement for any of the evaluated lakes.

6.3.3. Daily movements

The daily distances traveled were greater in northern pike males from the exposure lake (137 to 749m) than males from the reference (17 to 136m) lake ($p < 0.05$; Table 6.3). In addition, the morning and afternoon minimum displacement per hour (MDPH) for males collected from the exposure lake was significantly higher than reference lake males (Table 6.3). The daily distance traveled by northern pike females was not different between lakes (Table 6.3). However, the morning MDPH of female pike from the exposure lake was significantly different ($p < 0.05$; Table 6.3) from reference female. Female and male pike from the exposure lake showed differences ($p < 0.05$) in daily movement (distance traveled) and MDPH, with males traveling greater distances than females (Table 6.3). There was no sex related differences in daily pike movement within the reference lake. The 3-day total average daily movement for female and male pike from the reference and exposure lake was 94.4 ± 41.9 and 58.4 ± 33.9 m; and 76.7 ± 13.3 and 418.9 ± 196.7 m, respectively. In the exposure lake, male pike occupied home ranges that were significantly ($p < 0.001$) larger than females (Figure 6.6 and Table 6.4A). In addition, there were significant differences ($p < 0.001$) in male home ranges between lakes (Table 6.4A). There

were no differences in female home ranges between lakes or sex related differences within the reference lake (Table 6.4A). There was no correlation between fish body size and home range in the daily radiotracking. Water quality from the exposure lake was characterized by higher conductivity, TDS and salinity and lower pH than the reference lake (Table 6.4B). There was no correlation between temperature, TDS and salinity and daily pike movement for any of the evaluated lakes. No differences were found in type of habitat used between sexes or lakes (Tables 6.5-6.7). The majority of the pike were found in relatively deep water (> 3m) more than 5m from shore while a small proportion of pike were found close to shore (< 5m) and in vegetated areas (Tables 6.5 –6.7)

Table 6.2: Seasonal water quality variables for reference and exposure (bold) lake. Data represent the mean (± SE) of five measurements per tracking session.

	Fall 2004	Winter 2005 [a]	Spring 2005	Summer 2005	Spring 2006
Conductivity (µS/cm)	9.5 ± 0.5	-	20.0 ± 0.0	11.0 ± 0.0	10.5 ± 0.5
	381.5 ± 6.5	-	**502.0 ± 0.8**	**562.0 ± 18.5**	**494.5 ± 10.5**
pH	8.0 ± 0.03	-	6.61 ± 0.20	7.09 ± 0.0	6.88 ± 0.21
	8.52 ± 0.15	-	**7.12 ± 0.03**	**5.24 ± 0.43**	**6.17 ± 0.28**
Temperature (ºC)	9.9 ± 0.7	-	16.5 ± 0.9	15.2 ± 0.6	12.8 ± 0.6
	10.9 ± 0.6	-	**16.3 ± 0.6**	**16.3 ± 0.6**	**12.5 ± 1.3**
Dissolved oxygen (mg/L)	11.1 ± 0.3	-	7.9 ± 0.6	9.8 ± 0.1	11.6 ± 0.7
	11.2 ± 0.3	-	**12.1 ± 0.2**	**11.0 ± 0.3**	**12.6 ± 0.6**
Total dissolved Solids (g/L)	0.0	-	0.02 ± 0.0	0.01 ± 0.0	0.01 ± 0.0
	0.3 ± 0.0	-	**0.3 ± 0.0**	**0.4 ± 0.01**	**0.4 ± 0.02**
Salinity	0.0	-	0.02 ± 0.0	0.01 ± 0.0	0.01 ± 0.0
	0.3 ± 0.0	-	**0.3 ± 0.03**	**0.3 ± 0.01**	**0.3 ± 0.01**

[a] Not available.

Table 6.3: Northern pike movement ((distance traveled, m) and minimum displacement per hour (MDPH)) during the A) first, B) second and C) third day of consecutive radiotracking in the reference (male n= 2; Female n= 3) and exposure (bold; male = 2 and female n=3) lake. Data represent the mean (± SE) location for each day interval (morning, noon, afternoon and late afternoon). * Significantly different ($p < 0.05$) from reference. # Significant difference ($p < 0.05$) between males and females within lakes.

A	Morning	Noon	MDPH morning [a]	Afternoon	Late afternoon	MDPH afternoon[b]	MDPH x day [c]
Females	137.9 ± 67.7	179.3 ± 75.9	79.3 ± 36.1	46.53 ± 7.55	83.21 ± 31.96	32.4 ± 12.7	55.9 ± 14.1
	70.8 ± 11.4#	**56.6 ± 13.6**	**31.8 ± 2.2***	**75.2 ± 8.0**	**100.4 ±41.0**	**43.9 ± 14.9#**	**37.9 ± 8.5#**
Males	39.4 ± 23.4	31.0 ± 10.5	17.6 ± 8.5	27.5 ± 12.7	75.0 ± 22.9	25.6 ± 8.9	21.6 ± 8.7
	190.3 ± 22.2*	**417.8 ± 5.1***	**152.0 ± 4.3***	**293.6 ± 95.6***	**255.6 ± 69.4***	**137.2 ±6.6***	**144.6 ± 1.2***

B	Morning	Noon	MDPH morning [a]	Afternoon	Late afternoon	MDPH afternoon[b]	MDPH x day [c]
Females	61.9 ± 21.5	55.6 ± 17.6	29.37 ± 9.78	70.72 ± 31.90	135.26 ±48.93	51.50 ± 7.93	40.43 ± 1.07
	97.2 ± 5.5	**91.1 ± 38.7#**	**47.06 ± 12.74 * #**	**67.96 ± 40.15#**	**57.40 ± 8.67#**	**31.34 ± 16.05#**	**39.20 ± 14.39#**
Males	77.2 ± 34.9	33.2 ± 2.5	27.6 ± 9.3	42.2 ± 4.7	23.5 ± 1.2	16.4 ± 1.5	22.0 ± 3.9
	739.2 ± 65.0*	**749.2 ± 186.3***	**372.1 ± 30.3***	**761.2 ± 100.9***	**683.8 ± 2.8***	**361.3 ± 25.9***	**366.7 ± 28.1***

C	Morning	Noon	MDPH morning [a]	Afternoon	Late afternoon	MDPH afternoon[b]	MDPH x day [c]
Females	96.5 ±32.0	114.3 ± 52.9	57.7 ± 24.8	118.2 ± 10.9	32.9 ± 14.5	37.8 ± 3.2	45.2 ± 12.9
	70.7 ± 4.0	**68.4 ± 10.7**	**34.8 ± 2.7***	**96.0 ± 40.6#**	**98.6 ± 44.7#**	**48.7 ± 25.7#**	**41.7 ± 13.8#**
Males	103.1 ± 63.4	76.8 ± 25.3	45.0 ± 22.1	136.2 ± 81.0	37.7 ± 10.2	44.2 ± 17.7	44.6 ± 19.9
	245.8 ± 138.3*	**569.2 ± 158.2***	**203.8 ± 74.1***	**595.0 ± 196.5***	**701.3 ± 49.7***	**324.1 ± 37.7***	**263.9 ± 55.4***

[a] Morning MDPH calculated as the ratio between total distance traveled in the morning and elapsed time of morning radiotracking (4 hrs).

[b] Afternoon MDPH calculated as the ratio between total distance traveled in the afternoon and elapsed time of afternoon radiotracking (4 hrs).

c Daily MDPH calculated as the ratio between total distance traveled during the day and total time of daily radiotracking (8 hrs).

Table 6.4: (A) Home range area (m^2) for the 3-day radiotracking. Data represent the mean (± SE) of daily areas calculated for five fish (males *n*= 2 and females *n* =3) per lake. * Significant different ($p < 0.05$) from the reference lake. # Significantly different ($p < 0.05$) between males and females within lakes. (B) Water quality parameters for the reference and exposure (bold) lake. Data represent the mean (± SE) of five daily measurements.

A)

	Reference	**Exposure**
Females	14, 855 ± 4,085	19,666 ± 4,553
Males	13,324 ± 8,098	556,478 ± 103,804*#

B)

	First day	**Second day**	**Third day**
Conductivity (µS/cm)	11.5 ± 1.5	19.5 ± 1.5	11.5 ± 0.5
	456.5 ± 0.5	**447.0 ± 3.0**	**442.0 ± 4.0**
pH	7.72 ± 0.14	7.73 ± 0.13	7.23 ± 0.12
	7.07 ± 0.06	**7.13 ± 0.02**	**6.84 ± 0.20**
Temperature (ºC)	12.9 ± 0.9	18.6 ± 0.5	19.1 ± 0.1
	15.8 ± 0.9	**16.1 ± 0.2**	**14.0 ± 0.3**
Dissolved oxygen (mg/L)	11.1 ± 0.0	11.4 ± 0.5	9.9 ± 1.0
	10.9 ± 0.01	**10.9 ± 0.0**	**10.8 ± 0.02**
Total dissolved Solids (g/L)	0.01 ± 0.0	0.02 ± 0.0	0.01 ± 0.0
	0.4 ± 0.01	**0.4 ± 0.0**	**0.4 ± 0.0**
Salinity	0.02 ± 0.01	0.01± 0.0	0.01 ± 0.0
	0.3 ± 0.01	**0.3 ± 0.0**	**0.3 ± 0.0**

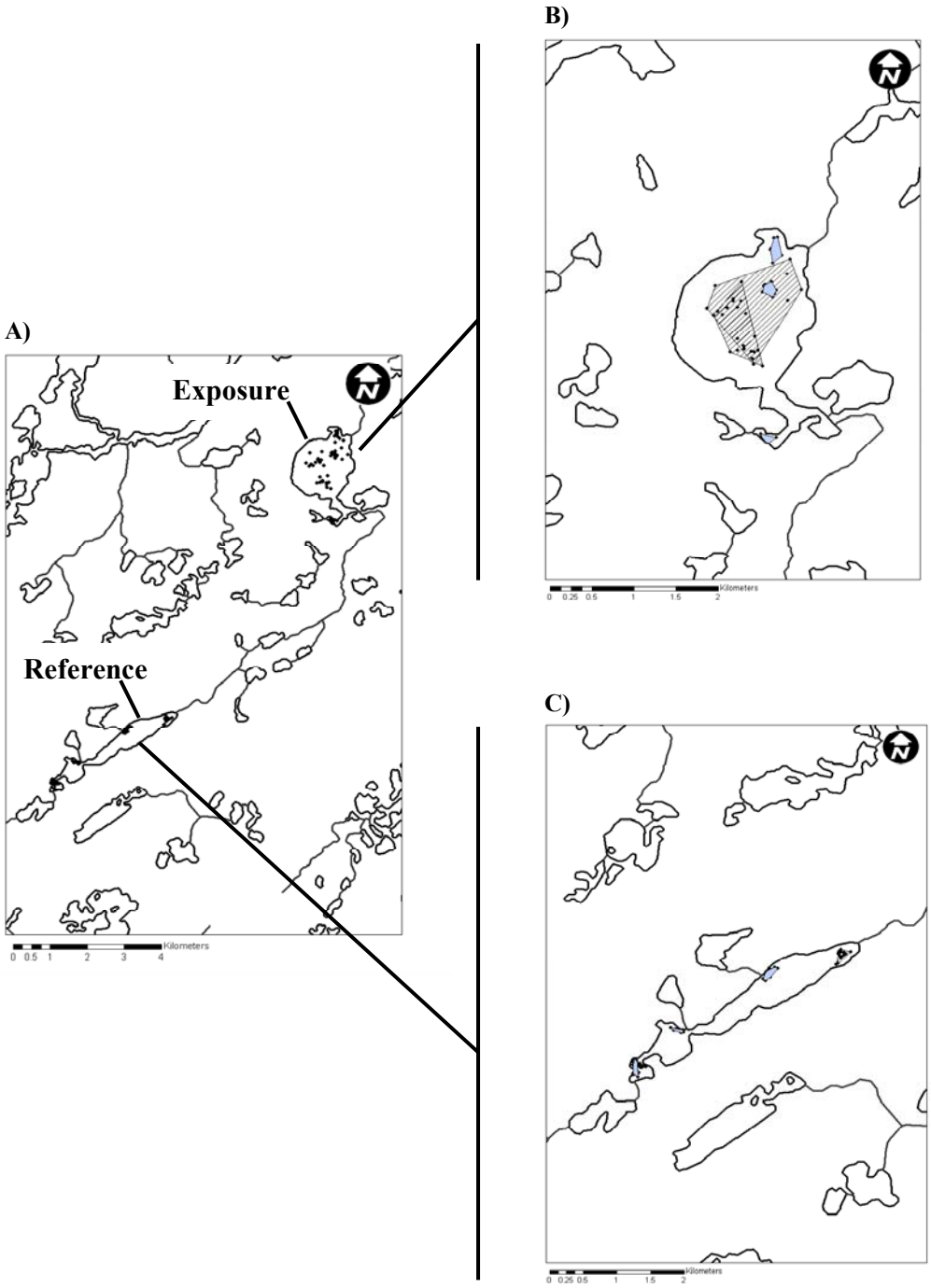

Figure 6.6: Map of study area showing 3-day distribution of northern pike in the reference (*n*= 5) and exposure (*n* = 5) lakes (A). A total of five locations are shown for each individual pike. Home range area (m^2) for males (dashed) and females (full area) in the B) exposure and C) reference lakes was calculated using the total fish locations recorded during the 3-day tracking period

Table 6.5: Daily habitat selection percentages in the reference lake. Data represent the mean (± SE) percentage of fish occurrence in various habitats selected by northern pike.

* Significant differences ($p < 0.05$) between bottom types within lakes. # Significantly different ($p < 0.05$) only from moderate vegetation bottom type within lakes.

Bottom type	Sex	First day		Second day		Third day	
		Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
> 3m	Female	83.3*	55.6*	83.3*	77.8*	66.7*	55.6*
	Male	75.0*	50.0*	25.0*	66.7*	60.0*	33.3*
	Total fish	80.0*	53.3*	50.0*	73.3*	60.0*	46.7*
Rock/organic	Female	0.0	0.0	0.0	0.0	16.7	0.0
	Male	0.0	0.0	0.0	0.0	0.0	0.0
	Total fish	0.0	0.0	0.0	0.0	10.0	0.0
Shallow/organic	Female	16.7	0.0	16.7	0.0	0.0	11.1
	Male	0.0	33.3	25.0	33.3	50.0	0.0
	Total fish	10.0	13.3	20.0	13.3	20.0	6.7
Shallow/rocks	Female	0.0	0.0	0.0	11.1	0.0	0.0
	Male	0.0	0.0	0.0	0.0	0.0	0.0
	Total fish	0.0	0.0	0.0	6.7	0.0	0.0
Sand	Female	0.0	0.0	0.0	0.0	0.0	0.0
	Male	0.0	0.0	0.0	0.0	0.0	0.0
	Total fish	0.0	0.0	0.0	0.0	0.0	0.0
Sand/rock	Female	0.0	0.0	0.0	0.0	0.0	0.0
	Male	0.0	0.0	0.0	0.0	0.0	0.0
	Total fish	0.0	0.0	0.0	0.0	0.0	0.0
Low vegetation	Female	0.0	44.4*	16.7	11.1	16.7	22.2* #
	Male	25.0	16.7*	25.0	0.0	0.0	33.3* #
	Total fish	10.0	33.3*	20.0	6.7	10.0	26.7* #
Moderate vegetation	Female	0.0	0.0	0.0	0.0	0.0	11.1*
	Male	0.0	0.0	25.0	0.0	0.0	33.3*
	Total fish	0.0	0.0	10.0	0.0	0.0	20.0*
High vegetation	Female	0.0	0.0	0.0	0.0	0.0	0.0
	Male	0.0	0.0	0.0	0.0	0.0	0.0
	Total fish	0.0	0.0	0.0	0.0	0.0	0.0

Table 6.6: Daily habitat selection percentages in the exposure lake. Data represent the mean (± SE) percentage of fish occurrence in various habitats selected by northern pike. * Significant differences ($p < 0.05$) between bottom types within lakes.

Bottom Type	Sex	First day		Second day		Third day	
		Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
> 3m	Female	66.7*	33.3	50.0	77.8*	50.0*	33.3*
	Male	100.0*	66.7	0.0	100.0*	100.0*	66.7*
	Total fish	80.0*	0.0	60.0	86.7*	70.0*	46.7*
Rock/organic	Female	0.0	0.0	0.0	0.0	0.0	0.0
	Male	0.0	0.0	0.0	0.0	0.0	0.0
	Total fish	0.0	0.0	0.0	0.0	0.0	0.0
Shallow/organic	Female	33.3	44.4	50.0*	22.2*	0.0	33.3*
	Male	0.0	16.7	75.0*	0.0*	0.0	16.7*
	Total fish	20.0	0.0	20.0*	13.3*	0.0	20.0*
Shallow/rocks	Female	0.0	11.1	0.0	0.0	0.0	0.0
	Male	0.0	0.0	0.0	0.0	0.0	0.0
	Total fish	0.0	0.0	0.0	0.0	0.0	0.0
Sand	Female	0.0	0.0	0.0	0.0	0.0	0.0
	Male	0.0	0.0	0.0	0.0	0.0	0.0
	Total fish	0.0	0.0	0.0	0.0	0.0	0.0
Sand/rock	Female	0.0	22.2	0.0	0.0	16.7	33.3*
	Male	0.0	16.7	0.0	0.0	0.0	16.7*
	Total fish	0.0	0.0	0.0	0.0	10.0	26.7*
Low vegetation	Female	0.0	0.0	0.0	0.0	16.7	0.0
	Male	0.0	0.0	25.0	0.0	0.0	0.0
	Total fish	0.0	0.0	20.0	0.0	10.0	0.0
Moderate vegetation	Female	0.0	0.0	0.0	0.0	0.0	0.0
	Male	0.0	0.0	0.0	0.0	0.0	0.0
	Total fish	0.0	0.0	0.0	0.0	0.0	0.0
High vegetation	Female	0.0	0.0	0.0	0.0	0.0	0.0
	Male	0.0	0.0	0.0	0.0	0.0	0.0
	Total fish	0.0	0.0	0.0	0.0	0.0	0.0

Table 6.7: Northern pike distance from shore for A) reference and B) exposure lakes. Data represent the mean (± SE) percentages of northern pike observations more (< 5m) or less (> 5m) than 5m from shore. *Significantly different ($p < 0.05$) within lakes.

A)

Distance from shore	Sex	First day		Second day		Third day	
		Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
	Female	50.0	66.7*	66.7	88.9*	50.0	50.0
>5	Male	75.0	83.3*	50.0	66.7*	50.0	50.0
	Total fish	50.0	73.3*	60.0	80.0*	50.0	50.0
	Female	50.0	33.3	33.3	11.1	50.0	50.0
<5	Male	25.0	16.7	50.0	33.3	50.0	50.0
	Total fish	50.0	26.7	40.0	20.0	50.0	50.0

B)

Distance from shore	Sex	First day		Second day		Third day	
		Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
	Female	100.0*	100.0*	83.3*	77.8*	66.7*	100.0*
>5	Male	100.0*	100.0*	100.0*	100.0*	100.0*	100.0*
	Total fish	100.0*	100.0*	90.0*	86.7*	60.0*	100.0*
	Female	0.0	0.0	16.7	22.2	33.3	0.0
<5	Male	0.0	0.0	0.0	0.0	0.0	0.0
	Total fish	0.0	0.0	10.0	13.3	40.0	0.0

6.4. Discussion

The use of radio-telemetry in the present study has allowed the collection of valuable data on the habitat use and movement of northern pike inhabiting lakes downstream of a uranium mine in northern Saskatchewan. All northern pike were classified as resident with no migration between the study lakes, which is in agreement with other studies (Craig, 2008; Cook and Bergesen, 1988; Vehanen *et al*., 2006). An important question that often arises in aquatic toxicology investigations using fish in open aquatic ecosystems is whether fish collected from reference and exposure lakes spend time migrating to and from these lakes. Therefore, collecting information regarding fish movement between study areas is important for environmental studies.

6.4.1. Seasonal movement

Northern pike from reference and exposure lakes showed similar movement (distance traveled) over the study period. Diana *et al* (1977) also found no difference in pike seasonal movement between summer and winter time. However, other studies have reported significant movement differences in northern pike between seasons (Cook and Bergesen, 1988; Rogers and Bergesen, 1995). The boost of activity observed during winter may be due to slight increases in water temperature occurring during this period (Cook and Bergesen, 1988; Koed *et al*., 2006) or lower light intensities (pike showed high activity at lower light) under the ice during the day (Kobler *et al*., 2008a). In the present study, female and male pike did not present any differences in their seasonal movement rate. Although

some studies have found no differences in movement between female and male pike (Cook and Bergesen, 1988; Lucas, 1992), other authors have reported that female pike move more extensively than males (Jepsen *et al*., 2001; Koed *et al*., 2006) explained by a more pronounced food consumption in female fish (Koed *et al*., 2006). In general, pike can travel several kilometers/day or stay in the same area all day long (Vehanen *et al*., 2006). The distance traveled by pike in this study was below 400 m and was not influenced by water temperature (Jepsen *et al.*, 2001). However, Vehanen *et al*. (2006) reported such correlation between pike movement and temperature.

In the present study, pike were observed to have large home ranges, moving extensively through certain areas of the lake. Some studies have reported restricted home range with pike utilizing certain areas of rivers and lakes (Lethonen *et al*., 1983; Vehanen *et al.*, 2006) while others reported undefined home ranges, where pike moved over the entire river or lake area (Diana *et al*., 1977; Cook and Bergesen, 1988; Jepsen *et al*., 2001). The differences in home range between these studies can be attributed to the occurrence of two types of strategies or behaviour in pike populations, with one group of individuals occupying restricted areas and a second group moving extensively (Mann, 1980). Clearly, the great variation in home range size found in the scientific literature suggests a high degree of behavioural adaptability that might be expected and advantageous in an opportunist top predator such as northern pike (Bry, 1996). Jepsen *et al.* (2001) suggested that larger home ranges in pike could be associated with low prey availability. Therefore, the larger home ranges observed in this study in exposure pike during winter and summer periods could be attributed to an increased activity searching for food in pike inhabiting the exposure lake. The difference in condition factor between reference and exposure pike

appeared to also support this hypothesis with reference pike presenting higher, and therefore better general health than exposure pike. However, since sample sizes in the present study were small, further research is needed to verify this conclusion. It should be also noted that other factors such as the structure or lack of vegetated areas and distribution of prey between lakes during winter and summer may also cause northern pike to exploit greater areas and therefore influence home range sizes (Kobler *et al*., 2008a). Some studies have reported that home range size is correlated with fish size (length and weight). However, in the present study there was no such correlation within the size of tagged fish and home range (Minns, 1995; Jepsen *et al*., 2001).

6.4.2. Daily movement

Several studies have reported differences in movement rate between sexes with females moving more extensively than males (Jepsen *et al*., 2001; Koed *et al*., 2006). Contrarily, in the present study male pike inhabiting the exposure lake moved more than exposure females and pike inhabiting the reference lake. In addition, males in the exposure lake also presented larger home ranges than the rest of the studied pike. These discrepancies can be caused by several factors (increased foraging, differences in prey distribution, various environmental factors (e.g., oxygen level) and/ or small sample size) that could influence these results. Further research is needed to clarify these findings. In this study, a large percentage of pike found in relatively deep water (> 3m) and non-vegetated habitats further from shore (> 5m). Previous studies have reported that while small pike are usually associated with vegetation, large northern pike (> 25 cm) generally inhabit non-vegetated

and open waters or the less vegetated –open water interface (Grimm and Backx, 1990; Casselman and Lewis, 1996; Vehanen *et al*., 2006). Contrarily, other studies have found a clear association between adult pike and vegetated zones (Headrick and Carline, 1986; Cook and Bergesen, 1988; Jepsen *et al*., 2001). It is clear that this plasticity in habitat use is very advantageous for a top predator such as pike because it allows the predator to exploit food sources from open water and vegetated areas.

Behavioural alterations are reliable indicators of stress imposed on fish by environmental changes (e.g., habitat alterations, water quality) (Israeli-Weinstein and Kimmel, 1998; Jakka *et al*., 2007). Radio-telemetry can therefore be used as an early warning system, detecting sub-lethal effects of pollutants at an early stage in aquatic organisms through changes in behaviour. This study found differences in fish home range and movement (distances traveled) rate between exposure and reference areas even though the sample size was small. We believe that a large data set would have revealed a higher number of behavioural differences in fish between the study lakes. Therefore, additional research increasing the number of tagged fish and locations is required to fully understand the effects of mining effluent discharge on fish behaviour. Nevertheless, we believe that radiotelemetry is a useful monitoring tool that may contribute to the establishment of methods applicable to ensure the sustainability of fish populations inhabiting mining areas.

6.5. Conclusion

Anthropogenic activities such as metal mining can alter fish habitat of downstream areas. Understanding how variation in habitat and water characteristics influences freshwater fish movement, distribution, and home range is crucial to ensure the sustainability of fish populations. The objective of the present study was to determine if radiotelemetry could be used to determine the movement rate, location fidelity and home range of adult northern pike within gradients of uranium mining effluent discharges. Fish were collected from a reference lake and an exposure lake located upstream and downstream of effluent discharge, respectively. Digitally coded radio-tags were surgically implanted into the fish body cavity following sex, weight and length determinations. Fish locations were seasonally and daily recorded using a Lotek SRX_400 receiver with handheld Yagi antenna. Our results suggest that tagged pike did not migrate out of the study area throughout the study period; with the mean distance traveled ranging from 50 to 400 m. Differences in movement (distance traveled) and home ranges were found between pike inhabiting reference and exposure lakes. Our data suggest that the use of radiotelemetry could be a useful addition to environmental studies.

CHAPTER 7

General discussion

7.1. Introduction

The overall goal of the research described in my thesis was to assess the effects of selenium (Se) in aquatic systems receiving effluents from uranium (U) mining operations in northern Saskatchewan. The main objectives of this research project included: Evaluation of Se concentrations in biotic and abiotic compartments of aquatic systems receiving U mine effluent discharges; determination of Se-induced deformities in fish larvae originated from adults inhabiting aquatic systems downstream of U mining and milling operations; potential use of radio-telemetry to detect behavioural alterations in adult fish in aquatic systems downstream of U mining operations.

7.2. Selenium in northern Saskatchewan

7.2.1. Selenium assessment at Key Lake uranium mine

The field-based study described in Chapter 2 was conducted in late summer 2004 to investigate total Se concentrations in water, sediment, plankton, periphyton, invertebrates (filterer, detritivore and predator) and fish (forage and predatory) from aquatic systems upstream and downstream of the Key Lake mining and milling operation. In addition, Se accumulation factors between trophic levels were evaluated for all lakes downstream of Key Lake mine. Although exposure areas had low Se water concentrations (generally < 1µg/L or just above the water criteria established by the Canadian Council of Ministers of the Environment (CCME), 2003), elevated concentrations of Se were evident among aquatic biota. Furthermore, Se in the water was concentrated from 200 to more than 4,000 times in sediments and biota at exposure sites. A significant increase in Se concentrations

was recorded in plankton, periphyton, invertebrates and fish collected from exposure sites compared to reference. Selenium levels in all sampled biota exceeded the 3-11 µg/g dry weight (DW) dietary Se threshold for fish, suggesting that Se released into the aquatic environment can still be accumulated through the food chain reaching levels that have the potential to impair fish reproduction. Biomagnification of Se (defined as increased Se concentrations by successive trophic levels (Lemly, 1997a)) also occurred, resulting in approximately 1.5-6 fold increases in the Se content between plankton, invertebrates and small bodied fish. However, biomagnification did not appear to occur between the forage and predatory fish species evaluated in this study. The observed pattern of Se accumulation (from smallest to largest) was periphyton < plankton and filterer invertebrates < detritivore and predator invertebrates < forage and predatory fishes.

In 2004-2005 a field and laboratory-based study evaluating the effects of Se on reproductive endpoints in northern pike (*Esox lucius*) inhabiting the same watershed was also completed as part of the Se assessment in aquatic ecosystems downstream of Key Lake U mine operation (Chapter 3). The study design focused on answering the following question: were the Se levels in prey organisms high enough to produce reproductive impairment in fish? Fish were collected from three exposure sites (high, medium and low) and one reference site. Both male and female northern pike collected from the exposure site showed significant reductions in condition factor when compared to reference. However, no difference in the female northern pike hepatosomatic index (HSI) was observed between sites. Elevated concentrations of Se were recorded in tissues (muscle, bone, liver, and kidney) and eggs of northern pike females inhabiting exposure lakes compared to reference. Furthermore, of all 22 trace metals analyzed in eggs and muscle in female northern pike

collected from the exposure sites, only Se was present at significantly elevated concentrations compared to reference site females. Significant relationships between egg Se and Se in fish tissues was observed in this study, suggesting that Se levels in eggs could be predicted from fish tissues.

Increases in the incidence of Se-induced deformities (skeletal, craniofacial, and fin deformities) in addition to edema, and total deformities above 30% were recorded in fry originating from female northern pike collected at the high and medium exposure site. The increased frequency of deformities found in northern pike fry was associated with a significant increase in the level of Se in northern pike eggs from exposure sites (31.28 ± 5.97 and 48.23 μg/g DW for medium and high exposure sites, respectively) compared to reference (3.19 ± 0.29 μg/g DW). The use of a two-way cross over ANOVA experimental design with eggs from reference and exposure site incubated in both reference and exposure water allowed the discrimination between maternal transfer of Se and water exposure effects. Furthermore, the observed increased level in Se-induced deformities was due to egg's origin and not to the water used to incubate the embryos, suggesting that maternal transfer of Se, as opposed to water exposure, was responsible for the effects observed in pike fry. In addition, there were no significant differences in the developmental time (e.g., time to 50% eyed embryos) among sites suggesting no effect of the incubation water in early developmental stages of pike larvae. I concluded that maternal transfer of Se into the eggs and subsequent exposure of fry during yolk assimilation was responsible for the observed deformities. Whole body Se concentration resulting in a 20 percent increase in developmental malformations relative to control calculated for northern pike (EC20) was

similar to the EC20 reported for warm water fish species, such as bluegill sunfish (*Lepomis macrochirus*) suggesting similar sensitivity to Se between cold water and warm water fish species (Coyle *et al*., 1993; USEPA, 2004).

7.2.1.1. Integration of results at Key Lake mine

Northern Saskatchewan has been the site of extensive mining activities over the last 50 years. Several investigations have identified and reported increased levels of trace elements such as Se in biotic and abiotic components of aquatic ecosystems downstream of U mine operations (Burns and Finch, 1999; Pyle *et al*., 2001; Klaverkamp *et al*., 2002). In Chapter 2, I reported that Se was accumulated and biomagnified in aquatic systems several orders of magnitude greater than its concentration in water, with aquatic biota reaching levels that exceeded the 3-11 µg/g (DW) dietary Se threshold for fish. Based on these results it is clear that although water Se level in exposure lakes was low (slightly above and/or below < 1µg/L) this element is still being incorporated and accumulated in the aquatic food chain. One plausible explanation for these results is the critical role played by planktonic organisms in the accumulation and assimilation of Se from the water column into organic forms. Furthermore, several authors have reported that planktonic organisms can accumulate Se several orders of magnitude higher than its concentration in water (Lemly, 1993a; Saiki *et al*., 1993; Orr *et al*., 2006), significantly contributing to the sedimentation (e.g., by detrital processes) and incorporation of Se (mainly as Se-Met) into the aquatic food chain (Bowie *et al*., 1996).

Uptake of selenium by aquatic organisms can occur through water or diet. However, dietary uptake (primarily as Se-Met) is usually the dominant pathway of Se accumulation in invertebrates and fish, which cannot synthesize Se-Met and rely upon the lower trophic levels as sources of the amino acid (Alaimo *et al.*, 1994). Thus, Se precipitated into the sediments by detrital processes can lead to elevated concentrations of Se in sediments and benthic invertebrates (Lemly, 1993a; Saiki *et al*., 1993) reaching levels that have the potential to impair fish reproduction. Furthermore, the results reported in Chapter 3 support this latter scenario, with increased level of deformities in northern pike fry originated from adults exposed to high levels of dietary Se. I concluded that the increased frequency of deformities reported in Chapter 3 were produced as a consequence of parental exposure to elevated dietary Se levels (> 3µg/g, DW), maternal deposition of Se into eggs during vitellogenesis, levels in eggs above the toxicity threshold associated with the presence of Se-induced deformities (> 10 µg/g, DW), and subsequent exposure of developing fish larvae during yolk resorption (Lemly, 1997a).

Lemly (1997b) reported a close link between percentages of developmental malformations and population impacts. The author reported that frequencies of developmental malformations above 25% in fish larvae would have a major impact on fish populations. In Chapter 3, the reported percentage of total deformities in fish larvae originating from adults collected from the exposure site was > 30% and therefore a major impact on fish populations could be expected to occur in aquatic systems downstream of the Key Lake U mine. However, the index developed by Lemly (1997b) was based on warm water fishes such as bluegill and therefore its applicability to cold water fish species is controversial.

7.2.2. Selenium exposure and effects at McClean Lake uranium mine

The field study outlined in Chapter 4 was conducted in summer 2005 and included the evaluation of Se levels in aquatic biota, in addition to the evaluation of Se accumulation factors between trophic levels in a lake downstream of the McClean Lake U mining and milling operation. Samples (water, sediment, plankton, invertebrate, and small bodied fish) were collected from one exposure site and one reference site. Although water and sediment Se concentrations were higher in the exposure site than the reference site, water Se concentration was still below the 1 µg/L water criteria established by the CCME (2003).

Selenium concentrations in plankton, invertebrate (Diptera, Trichoptera, and Odonata) and forage fish (white sucker (*Catostomus commersoni*), stickleback (*Pungitius pungitius*), and burbot (*Lota lota*)) were significantly higher in the exposure site than the reference site. Furthermore, Se levels in aquatic biota exceeded the lower limit of the proposed 3 to 11 µg/g (DW) dietary toxicity threshold for fish. Selenium in the water was concentrated from 150 to more than 6,000 times in sediments and biota at the exposure site. Although Se biomagnification ranged from 1.5 to 6 between lower (e.g., plankton) and higher (e.g., fish) trophic levels, no biomagnification was recorded between forage and predatory fish. The overall pattern of Se accumulation (from smallest to largest) in aquatic biota was recorded as follows: periphyton < invertebrates < plankton < predatory fish < forage fish. The differences observed in the accumulation of Se between predatory and forage fish may be due to differences in behaviour (e.g., schooling ((white sucker) *vs* non-schooling (northern pike) fish) and diet preferences (e.g., omnivores (white sucker) *vs* predators (northern pike) fish) that can have important effects in trace elements accumulation.

The field/laboratory study described in Chapter 5 was conducted to evaluate the presence of Se-induced deformities in northern pike and white sucker larvae originating from adults collected at the same reference and exposure lakes as in Chapter 4. In both northern pike and white sucker females, no significant effects were found in condition factor between sites, however a significant reduction was observed in the HSI of females collected from the exposure lake. Greater condition factors were recorded in northern pike and white sucker males collected from the exposure site compared to reference that could be explained by certain factors (e.g., increased productivity and food availability at the exposure site) resulting in an indirect positive effect on fish condition. Significant increases in the Se concentrations in female tissues (muscle, bone, liver, and kidney) were recorded in northern pike and white sucker females collected from exposure site compared to reference. In addition, northern pike accumulated greater Se levels in tissues such as liver and kidney than white sucker females, suggesting a difference in Se toxicokinetics between fish species. Both fish species showed a significant positive relationship between Se concentrations in eggs and tissues, and therefore Se concentration in eggs can be predicted from Se concentrations in adult fish tissues (i.e., muscle). In addition to Se, concentrations of other trace elements (e.g., Mo) were significantly different in female eggs and tissues between sites.

Although Se concentrations in northern pike (8.02 ± 0.73 μg/g DW) and white sucker (4.89 ± 0.52 μg/g DW) eggs from the exposure site were two to three times higher than the reference site (2.35 ± 0.20 and 1.94 ± 0.25 μg/g DW, respectively), Se concentrations in exposure eggs were below the reported threshold (10 μg/g DW) associated with Se-induced

deformities in other fish species (Lemly, 1993a). Embryos were incubated following a two-way cross over ANOVA experimental design, which allowed the discrimination between maternal transfer of Se and potential incubation water effects. Overall, there was no apparent effect either from egg Se concentration or site water exposure on the early developmental stages of northern pike and white sucker larvae.

7.2.2.1. Integration of results at McClean Lake mine

Anthropogenic activities such as U mining have the potential to increase Se concentrations in aquatic ecosystems. Elevated concentrations of Se have been previously reported in some biotic and abiotic aquatic components downstream of U mine operations in northern Saskatchewan, as a consequence of U ore extractions and milling (Pyle *et al*., 2001; Klaverkamp *et al*., 2002). The field investigation described in Chapter 4 reported that Se was highly accumulated and biomagnified through the aquatic food chain in a lake downstream of the McClean Lake U mine, even though water concentrations for this element were low (< 1µg/L). Consequently, concentrations of Se in some biota groups exceeded the lower threshold (> 3µg/g DW) for dietary Se toxicity in fish. Several studies have reported that increased levels of Se in aquatic biota (e.g., invertebrates and forage fish) could lead to an increase in the Se concentrations in egg and tissues of fish that rely on these organisms as food sources (Lemly, 1999; Hamilton, 2004; Orr *et al*., 2006). Furthermore, in this thesis both northern pike and white sucker females collected from the exposure site showed elevated levels of Se in egg and tissues likely caused by exposure to elevated levels of Se in prey organisms (Chapters 4 and 5). However, as described in

Chapter 5 no increases in Se-induced deformities were found in the developing fish larvae. The lack of a clear toxic response in fish larvae is in agreement with Se thresholds for early life stage deformities reported in other studies (Gillespie and Baumann, 1986; Coyle *et al*., 1993; Lemly, 1993b), with egg Se concentrations in northern pike and white sucker collected at the exposure site below the 10 μg/g (DW) threshold associated with the presence of developmental abnormalities. Furthermore, the calculated whole body Se concentration for northern pike and white sucker (< 4 μg/g DW) was below the proposed 7.91 μg/g (DW) whole body Se threshold proposed by USEPA (2004) for the protection of fish populations.

7.2.3. Comparison between mine sites

Elevated Se levels were identified in aquatic biota from both Key Lake and McClean Lake U mines. However, there were differences in the Se exposures and effects between these mine sites. The results are summarized for each mine and Se thresholds are given in Table 7.1. Selenium was accumulated and biomagnified at both mine sites following similar transfer rates between trophic levels. However the aquatic biota at Key Lake mine had Se concentrations that were approximately 4 times higher than the Se concentrations observed in aquatic organisms from McClean Lake mine (Table 7.1). The difference in the years of operation is the most obvious explanation for the variation in the Se concentration in biotic and abiotic compartments of the aquatic ecosystem between mine sites. Key Lake mine has been in operation longer than McClean Lake mine, allowing the aquatic system to accumulate higher levels of Se in sediments, in addition to re-cycling this element from

sediments to the aquatic food chain. Although both Key Lake and McClean Lake mine had low Se concentrations in water (below or slightly above 1μg/L) the Se concentration in sediments in lakes downstream of Key Lake mine was more than 50 times higher than McClean Lake mine. In contaminated environments more than 90% of Se can be found in the top layer of sediments (including overlaying detritus) that can be cycled back into the aquatic food chain by benthic organisms (Simmons and Wallschläger, 2005). Indeed, several authors have highlighted the important role of sediments in the accumulation of Se in aquatic systems (Bowie *et al*, 1996; Lemly, 1999; Simmons and Wallschläger, 2005; Orr *et al*., 2006).

Another plausible explanation for the different Se levels occurring in sediments between mine sites is the potential differences in planktonic community components and resulting deposition rate (by detrital processes) into the sediments. Changes in the Se uptake by planktonic organisms (e.g., presence of different composition of planktonic organisms) will have major implications in the Se deposited into the sediments and its subsequent accumulation by higher trophic levels (e.g., invertebrates, fish) (Bowie *et al.*, 1996; Lemly, 1999). Also, Key Lake mine effluent is released into lakes that are shallow with slow moving water, allowing very efficient Se accumulation, sedimentation and subsequent re-cycling into aquatic food chains. In contrast, the study site at McClean Lake U mine is part of the treated effluent management system, which is a closed lake (with dams in the inflow and outflow) and constant effluent flushing that can decrease Se sedimentation and consequently reduce the Se concentrations in benthic fauna, as well as the Se cycling from sediments into the aquatic food chain.

Despite elevated Se levels in fish eggs collected from the exposure site, no apparent Se-induced deformities were observed in the study site at McClean Lake mine. In contrast, higher levels of Se in fish eggs were associated with elevated frequencies of Se-induced larval deformities at Key Lake mine (Table 7.1). A clear explanation for this difference is that while the Se concentration in fish eggs collected at McClean Lake mine were below the proposed threshold (< 10 µg/g DW) associated with the appearance of Se-induced deformities in developing fish larvae, fish eggs collected from Key Lake mine were above this threshold (Table 7.1). A possible explanation for the discrepancy in the Se concentration in fish eggs between mine sites is associated with the level of Se reported to occur in aquatic biota. While Se concentrations in aquatic organisms at McClean Lake mine site were slightly above the lower limit of the dietary toxicity threshold reported for fish (3 µg/g DW), the Se concentration in invertebrates and fish at Key Lake mine were above the upper limit of the dietary threshold for Se (11 µg/g DW). Therefore, adult fish at Key Lake mine were exposed to dietary Se levels that were high enough to cause elevated Se levels in fish eggs (> 10 µg/g DW) leading to the appearance of Se-induced deformities in fish larvae.

Table 7.1: Summary of selenium (Se) concentrations and effects in aquatic systems downstream of Key Lake and McClean Lake uranium mines.

Variable	Se thresholds	Key Lake mine		McClean Lake mine	
Years of operation		25		10	
Se in abiotic and biotic compartments					
Water Se (µg/L)	< 1 [a]	0.7 - 2.7		0.43	
Sediment Se (µg/g) [e]	2 [b]	26 - 62		0.54	
Se biomagnification factors	N/A [c]	200-4,858		150-6,000	
Se in prey organisms (µg/g) [e]	3-11 [d]	> 11		>3	
Se in adult fish		**Northern pike**	**White sucker**	**Northern pike**	**White sucker**
Egg Se level (µg/g) [e]	10 [f]	31-48	42.90	8.02	4.89
Whole body Se concentrations (µg/g) [e]	7.91 [g]	15-17	19.89	< 4	< 4
Percentage of Se-induced deformities (%)	N/A [c]	31-39	N/A [c]	< 5	< 12

[a] Canadian Council of Ministers of the Environment, 1999; [b] Lemly, 2002b; [d] Lemly, 1993a and DeForest *et al*., 1999; [f] Lemly, 1993b; [g] USEPA, 2004.

[c] Not available.

[e] Se concentration on a dry weight basis.

7.2.4. Conclusion

The association between accumulation of Se and the appearance of adverse biological effects is critical to understand the mechanisms of Se toxicity in aquatic systems. In the laboratory and field research described in Chapters 2 and 3 I addressed this point by 1) identifying the accumulation of Se in aquatic biota (Chapter 2) and 2) linking parental exposure to elevated levels of dietary Se with developmental malformations in fish larvae (reproductive impairment; Chapter 3) in aquatic systems downstream of Key Lake U mine.

The information gathered in Chapter 4 and 5 addressed the gap in knowledge regarding the potential effects of Se concentrations in fish eggs that are slightly below the proposed chronic toxicity threshold (10µg/g DW). I was able to identify elevated Se concentrations in certain biota in a lake downstream of McClean Lake U mine (Chapter 4). Although increased Se concentrations in prey organisms resulted in elevated concentrations of Se in fish eggs, the concentration of this element was below the proposed Se toxicity threshold (< 10µg/g DW) associated with the appearance of Se-induced deformities (Chapter 5). The research described in Chapters 4 and 5 will significantly contribute to the ongoing debate regarding the applicability of Se guidelines to cold water fishes by addressing a critical gap in knowledge regarding the effects of Se concentration in fish tissues that are slightly below the reported chronic toxicity thresholds for this element. In addition, the evaluation of Se accumulation and effects on a variety of fish species is crucial to understand the toxicokinetics of Se in fish presenting different feeding modes and behaviour.

There is an urgent need to conduct studies that allow us to better understand the environmental fate and effects of Se in a variety of aquatic environments, particularly north

temperate (cold water) aquatic systems. In chapter 3 and 5, I was able to identify a strong relationship between the concentration of Se in fish tissues and the presence of developmental malformations in fish larvae that was consistent with previous studies in warm water fish species (Baumann and Gillespie, 1986; Lemly, 1993b; USEPA, 2004). This finding suggests that cold and warm water systems may behave and respond similarly to increased Se inputs in the aquatic environment. However, the applicability of the proposed Se toxicity threshold to cold water fish remains controversial given that most of the research has focused on warm water fish. From a regulatory perspective, there is therefore a clear need for studies documenting Se toxicosis in north temperate aquatic systems. The results of my research have made a significant and unique contribution to further understanding the exposure and effects of Se in north temperate aquatic ecosystems receiving U mine effluent discharges. It will contribute valuable information for the establishment of a realistic and environmentally relevant Se threshold for the protection of fish populations in Canadian waters.

7.3. Use of radio-telemetry as an environmental tool

Anthropogenic activities such as U mining have the potential to alter fish habitat by different processes (e.g., shoreline and water quality alterations, prey abundance). Consequently, these changes in the aquatic habitat can have an indirect effect on fish behaviour (e.g., distribution areas). The goal of the site fidelity study described in Chapter 6 was to determine if radio-telemetry could be used to detect behavioural changes in northern pike inhabiting a lake receiving effluent discharges from Key Lake U mine. Seasonal

(spring, summer, fall, winter) locations were recorded during 2004-2006. In addition, daily (3 consecutive days) locations and records of habitat type were completed in summer 2007. One of the most important questions that aquatic toxicologists face when investigating fish in open aquatic ecosystems is whether fish collected from reference and exposure sites spend time migrating between these sites. Thus, one of the objectives of this thesis included the evaluation of radio-telemetry as a tool for the evaluation of site fidelity as well as movement patterns in fish inhabiting aquatic systems receiving U mine effluent discharges.

As described in Chapter 6, all radiotagged pike were classified as resident pike, remaining in the study lakes with no migration between lakes during the study period. This information is of major significance for studies evaluating the effects of effluent discharges on wild fish, given that fish movement between reference and exposure lakes can significantly modify several monitoring endpoints (e.g., metal concentration in fish tissues, toxic effects).

Seasonal movement of tagged northern pike was not different between reference and exposure sites. However, a significant increase in the home range of northern pike inhabiting the exposure lake was observed for the winter and summer periods. Larger home ranges are often associated with low prey availability in exposure areas, with fish inhabiting these lakes having to move extensively and explore bigger areas in order to find food (Jepsen *et al.*, 2001). Furthermore, the lower condition factor observed in fish collected from exposure site compared to reference could be a consequence of either fish allocating energy resources into moving across larger areas to find food instead of growth and/or low availability of prey organisms in the exposure lake. Future research is needed to support these conclusions.

In the daily tracking, northern pike males in the exposure lake moved more extensively and had larger home ranges compared to the rest of the studied pike. In general, female pike have been reported to move more than males (Jepsen *et al*., 2001; Koed *et al*., 2006), and therefore I concluded that several factors including behavioural adaptability of northern pike as well as small sample size could explain the discrepancies in male movement between my research and other studies (e.g., Jepsen *et al*., 2001; Koed *et al*., 2006). Although juvenile northern pike are reported to favour vegetated shallow areas, adult pike appear to be closely associated with non-vegetated deeper waters. Northern pike inhabiting both reference and exposure lakes follow this latter scenario, with a high occurrence of pike in deep and non-vegetated areas.

In summary, the inclusion of radio-telemetry in my investigation allowed the detection of behavioural differences in northern pike inhabiting a lake receiving U mine effluent discharges. Behavioural alterations are a reliable and sensitive indicator of stress imposed on fish by environmental changes (Israeli-Weinstein and Kimmel, 1998; Jakka *et al*., 2007). Therefore, radio-telemetry can be a useful monitoring tool allowing the detection of sub-lethal and early effects of pollutants in aquatic systems receiving effluent discharges through changes in fish behaviour.

7.4. Research needs and recommendations

7.4.1. Research needs

Recently the U.S. Environmental Protection Agency (USEPA) proposed a chronic threshold for Se at a whole-body fish concentration of 7.91 μg/g DW, identifying the warm

water centrarchid, bluegill sunfish, as the most sensitive species (USEPA, 2004). However, there is still controversy regarding the applicability of this guideline to native cold water fish species inhabiting north temperate aquatic systems. Thus, there is an urgent need to conduct studies that allow us to better understand the environmental fate and effects of Se in a variety of receiving environments, particularly in north temperate aquatic systems where the applicability of the current Se toxicity threshold is controversial given that most of the research has focused on warm water fish (USEPA, 2004). There are some uncertainties and controversies that require further investigation before a final selenium threshold is developed**.** Research needs for Se in aquatic systems include:

- *Understanding the factors that can modify Se toxicity in aquatic environments*. The development of aquatic life criteria has generally been expressed as a function of water quality parameters that can modify the outcome of an adverse effect (Sappington, 2002). Several trace elements (e.g., Hg) and water quality parameters (e.g., pH) can highly modify the toxicity of Se (Lemly, 1999; ATSDR, 2003). Although in this thesis I reported that Se was accumulated and biomagnified through the aquatic food chain (Chapters 2 and 4), investigations of factors influencing Se biogeochemistry are lacking. Therefore, understanding the link between modifying factors (such water quality characteristics and hydrogeology) with Se toxicity in a variety of aquatic environments is critical for the establishment of a relevant Se threshold.
- *Selenium speciation in the aquatic environment*. Se is present in the aquatic environment in different forms. Understanding and predicting the impacts of

elevated Se in aquatic environments should include not only the total Se levels but also the Se form present in different trophic levels. For example, in this thesis I reported elevated concentrations of Se in different aquatic biota groups (Chapters 2 and 4), however no identification of Se forms was made. Studies evaluating the speciation of Se in aquatic biota are required to identify the form of Se that is more toxicologically relevant in aquatic systems.

- *The ecological significance of chronic effects (Se-induced deformities).* In this thesis I reported increases in the percentages of Se-induced deformities in larval fish originating from adults exposed to elevated dietary Se (Chapters 2 and 3). However there is a crucial need for studies evaluating how the severity and prevalence of the different types of deformities can affect fish population dynamics (e.g., recruitment). The relationship between developmental deformities and population-level impacts is central to the determination of an appropriate Se criterion for the protection of fish populations.
- *Species tolerance and sensitivity to Se.* Different sensitivity to Se has been reported to occur between fish species. For example, Holm *et al*. (2005) reported no increase in deformities of brook trout larvae exposed to coal mining effluents, however larval deformities were elevated in rainbow trout. In this thesis, northern pike appeared to be of similar sensitivity to Se than warm water fish species such as bluegill (Chapter 3), however other authors have reported different sensitivity between cold water and warm water fish species (Kennedy *et al*., 2000; Rudolph *et al*., 2008). The different sensitivity to Se observed between fish species could be

due to differences in Se metabolism (e.g., Se-protein levels), type of Se form accumulated in tissues (e.g., seleno-methionine) and/or fish behaviour (e.g., diet preferences; trophic status). Another plausible explanation could be the development of tolerance to Se in certain fish species. Additional research is required to fully understand the physiological and biochemical mechanisms associated with Se toxicity between fish species This is a very critical point because differences in sensitivity between fish species can lead to an inappropriate Se threshold.

7.4.2. Recommendations

During the course of this research project a number of recommendations were highlighted. They are briefly discussed below:

- *Wet weight vs dry weight*. Several studies have reported concentrations of Se in tissues on a wet weight basis (Lemly, 1985a; Kennedy *et al*., 2000; Holm *et al.*, 2005). Wet weight concentrations are generally converted to dry weight using 80% moisture (USEPA, 2004), however certain tissues such as bone have lower moisture content (i.e., 60%). In addition, the moisture content in eggs from different fish species is highly variable (i.e., 61% for salmonids; ~ 75% for northern pike). It should be noted than these conversions can increase the uncertainty of the Se concentration being estimated. Therefore, reporting Se concentrations on a dry weight basis in addition to moisture content for each sampled tissue should be required data for any study reporting Se concentrations in aquatic biota.

- *Two way cross over ANOVA experimental design.* Several studies investigated the effects of Se in developing fish larvae by incubating the embryos in their respective site water (Holm *et al*., 2005) or municipal water (Kennedy *et al*., 2000; Rudolph *et al*.,2008). Although Holm *et al*. (2005) reported the presence of Se-induced deformities in rainbow trout larvae, the link between developmental deformities and maternal transfer of Se was lacking. In this thesis I demonstrated that maternal transfer of Se as opposed to water exposure was responsible for the deformities observed in fry by following a two way cross over ANOVA experimental design (Chapter 3). This design allowed me to identify which of the two factors of variability (egg origin and/or water exposure) was responsible for the effects observed in fry. In addition, it is largely unknown if the presence of other factors in site water (e.g., metals, ammonia, microorganisms, pH) can have effects in developing fish larvae that can lead to the incorrect interpretation of results. Therefore, this general methodology is recommended for use in other research projects evaluating the effects of Se in developing fish larvae.
- *Reported endpoints*. Although it is largely recognized that a Se regulation to protect fish populations should be tissue-based, there is still controversy on the choice of appropriate threshold (e.g., EC value) making comparisons between fish species difficult. In this thesis (Chapter 3) I reported the tissue Se concentrations associated with increases of 1%, 5%, 10% and 20% in Se-induced deformities above background levels (i.e., EC01, EC05, EC10 and EC20 values). Although USEPA (2004) recommended the use of an EC20 in the draft Se criteria guideline, it is

recommended that researchers also report the EC05 and EC10 values for comparison purposes.

- *Deformity evaluation protocols*. Several authors have evaluated the presence of Se-induced deformities in different fish species (Kennedy *et al*., 2000; deRosemond *et al.*, 2005; Holm *et al.*, 2005; Rudolph *et al*., 2008). It is recommended that the evaluation of developmental deformities be completed by experienced laboratory personnel highly familiarized not only with the type of deformities but also with the larval morphology of the studied fish species. There is a critical need for a protocol describing the necessary steps involved in fish larvae deformity analysis. In this thesis (Appendix), I described a protocol for fish collection and fish larvae deformity evaluations that could be followed by other investigators wishing to evaluate Se-induced deformities in developing fish larvae.

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APPENDIX

Fish collection and deformity evaluation protocol

Scope

This guideline describes universal procedures, conditions and recommendations for gamete collection, embryo incubations and evaluation of selenium-induced deformities in freshwater fish. The methods presented in this guideline are based largely on northern pike (*Esox lucius*), white sucker (*Catostomus commersoni*) and lake trout (*Salvelinus namaycush*) embryo incubations and deformity evaluations developed at the Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada. Although this guide is broadly applicable to other freshwater fish species, some modifications from the procedures described here could be justified under special circumstances. In addition, although this guide focuses on embryo incubations carried out under laboratory conditions, the same approach, with modifications, is also applicable to embryo incubations in field settings. Recommendations for experimental design, statistical treatment of data and detailed guidance for performing selenium effects evaluation in fish larvae and fry are provided. This standard guide does not address safety concerns; it is the responsibility of individual investigators to establish appropriate safety practices.

Fish sampling and acquisition of test organisms

All organisms must be obtained from the same source. Gametes may be obtained from brood fish cultured in the laboratory, hatcheries or wild populations. Investigators should be aware that local governments might require permits for wild fish collection and gamete transport (Canadian Council on Animal Care, 2003, 2005).

Field collection and handling of adult fish

Spawning adults are routinely collected using fish traps (e.g., hoop or trap nets) set close to spawning areas. Recording of daily water temperatures is recommended to follow the progression of the spawning process. Ripe males and females are collected and held separated in net-pens (made of durable material that will not harm fish (e.g., synthetic netting)) for no more than 5 d prior to gamete collection. Crowded conditions in net pens should be avoided. Net pens should be covered with dark plastic tarps to avoid direct sun exposure and/or fish jumping out. It is highly recommended to enclose the sides and top of the net pen with wire (e.g., chicken wire) to avoid predation (e.g., raptors, otters). Fish should be carefully observed during holding for signs of physical damage, mortality or other sources of stress (Canadian Council on Animal Care, 2005). It is recommended to record water quality variables (e.g., pH, dissolved oxygen (DO)) at fish collection. Since any handling of the fish will remove the protective body layer of slime, fish should be handled as little as possible using dip nets and soft material gloves.

Anesthetics

Fish must be individually anesthetized (e.g., MS-222) before sample collection to facilitate handling (Canadian Council on Animal Care, 2003, 2005). Anesthetic dosages are usually administered per fish body weight (e.g., for ~ 3-4 Kg pike, 0.4 g/L of MS-222 to obtain sedation). However, the intake of the anesthetic depends on the gill surface area and hence, the same anesthetic solution can be used for several fish presenting moderate variations in size (Gordon, 1967). The anesthetic solution should be prepared using site water. Since the strength of the solution will vary depending on water characteristics (e.g.,

hardness), it is recommended to record water quality parameters. Sedation should be monitored closely. When opercular movement rate is slow or irregular, fish can be removed from the anesthetic and proceed with morphological measurements and gamete extraction (Canadian Council on Animal Care, 2003).

Fish euthanasia and post anesthesia recovery

Euthanasia

Following fish measurements and gamete extraction female fish should be euthanized for tissue sample collection when possible. Use of lethal levels of anesthetics (e.g., MS-222 concentrations > 0.4 g/L) is recommended followed by stunning blow in the head or cervical dislocation (Canadian Council on Animal Care, 2005). Fish carcasses must be disposed of according to appropriate local (e.g., State, Province) regulations.

Recovery

Male fish should be fully recovered from the anesthesia before being released back into the water. The use of a well aerated recovery tank is recommended, containing fresh site water and located in a sheltered area with low lighting. The temperature in the recovery tank should closely match the temperature of the lake or stream to avoid temperature shock. Signs of recovery should be closely monitored. Fish should be gently released back into the water (approximately at the same or close to the collection area) only when normal behavior is observed (e.g., alertness, equilibrium) (Canadian Council on Animal Care, 2003, 2005).

Morphological measurements

External health examination (e.g., ulcers, parasites) of fish before use is desirable. Weight and length measurements are useful for estimating fish condition factor (CF). Balances can ideally be used to measure weight in the field, however, spring balances are a good tool for fieldwork involving larger fish species because they are small and durable. Fish should lie on their side on a hard surface covered with clean paper towels for length measurements. The most common length measurements in fish are total length, fork length and standard length (Anderson and Gutreuter, 1983). Investigators should clearly report the chosen type of length measurement (e.g., fork length). Appropriate length units (e.g., cm) should be used depending of the size of the collected fish.

Tissue collection

Unless whole body selenium concentrations are required, kidney, liver (weight determined on-site), muscle (caudal region), and bone (spine) should be collected for trace metal analysis. Teflon® coated tools are used for the collection of fish tissues. Tools should be cleaned between individual fish samples to avoid cross contamination. Tissue samples should be rinsed with distilled and deionized (nano-pure) water, transfered to plastic bags (e.g., Ziploc® or whirl-pack®) and kept frozen until analysis. In addition to tissue collection, collection of two different ageing structures (e.g., cleithra and scales) is recommended for age determination. Details for ageing structure collection and preparation can be found in Jearld (1983) and Sjolung (1974). It is recommended that the collection of female tissues be completed after gamete fertilization.

Gamete collection and fertilization procedures

Gamete collection

Adult fish for gamete collection should be randomly selected from net pens. Eggs or milt should not be in contact with water before fertilization and thus it is imperative to dry the area surrounding the urogenital opening with paper towels. All the material used for gamete collection should by carefully cleaned and dried. Precautions to avoid fecal, blood or urine contamination should be taken. Gametes must be kept covered to avoid direct sun exposure. It is recommended to collect the gametes from all fish before proceeding with fertilizations.

Milt collection

Milt from collected males should be performed prior to egg collection. Male fish should be anesthetized and gamete collection should proceed after recording weight and length Milt is collected by light pressure on the abdomen into clean eppendorf or falcon tubes. Milt should be collected from a minimum of three males and kept in separate vials on ice until use (Environment Canada, 1998). Care should be taken to prevent the contamination of sperm (e.g., with urine or feces), which can severely reduce sperm viability. However, any urine, feces or blood present in the milt vial after collection should be removed as soon as possible by using a clean plastic transfer pipette. It is recommended to change latex gloves between fish. Male fish can be released back into the water after anesthesia recovery

Egg collection

Female fish should be anesthetized and gamete collection should proceed after recording weight and length. Gently pressure from behind the pectoral fins towards the anus is applied to express the eggs. This process needs to be repeated several times. Check that eggs are released "clean" (e.g., without feces) before starting collection to avoid the contamination of the entire egg batch. Eggs should be obtained from a minimum of 4 females (Environment Canada, 1998), individually collected into pre-cleaned stainless steel bowl and kept covered in a cool place until use. Collected eggs should be closely inspected and eggs with adhered feces, urine or blood discarded by using a clean plastic pipette. After gamete collection female carcasses should be labeled and kept on ice until tissue collection. It is critical to correctly label the females to further match egg and tissue metal concentrations with frequencies of specific deformities.

Fertilization

Before fertilization a sub-sample of eggs (approximately 20g) from each female should be collected using a clean plastic spoon into whirl-pack® bags for trace metals analysis and kept frozen until use. Eggs from each female are fertilized separately with pooled milt derived from the captured males at the same site. Alternatively, a "dry fertilization" procedure can be followed where gametes are transported in isolation to the laboratory prior to fertilization (Holm *et al.*, 2005). Collected milt is pooled in one vial and equal amounts (~ 0.5 ml) poured into each individual egg bowl using a clean plastic pipette. Milt should be evenly distributed on the egg batch. Eggs are combined with the milt by swirling gently by hand for 1 min. Enough site water to cover the eggs is then added to activate the

sperm and gently mixed for 2-3 min. After fertilization, a bentonite clay solution (~ one teaspoon of clay per 200-ml of site water) is added to the eggs to prevent clumping, and thoroughly rinsed 2-3 times after 5 minutes using the same site water to remove any remaining clay (Environment Canada, 1998; ASTM, 2003). Eggs from each individual female are then transferred to 4-L pre-cleaned Eagle picher® (level 1) plastic jars filled with site water (no head space) for egg hardening. Embryos should be carefully oscillated for 1-2 min. every 20-30 min for approximately 2 h to prevent clumping. Egg hardening occurs between 2-3 hours after fertilization.

Tool sterilization and cleaning

Tissue collection

Tools used for tissue collection should be cleaned between samples to avoid cross contamination. It is recommended to rinse all materials with nitric acid (5%) followed by several rinses with deionized water.

Gamete collection

All steps involved in gamete collection must include hygienic (sterile) precautions to avoid cross contamination between different fishes. All materials (e.g., bowls, pipette) used during the egg fertilization procedure should be sterilized using a 0.000075% (~ 0.04 ml in 5 L of water) betadyne (10% povione-iodine, 1% free iodine) solution prepared with deionized water and rinsed with deionized water to remove any remaining iodine. The use of latex examination gloves is strongly recommended during fertilization procedures.

Gloved hands should be washed with betadyne solution (see above), rinsed with deionized water and carefully dried before and after egg handling.

Sample labeling, transport and storage

Location, adult female number, fish species, collection date, and name of researcher should be recorded on the label for each individual sample.

Embryo transport

Embryos (or gametes if conducting dry fertilizations) can then be transported to the laboratory, or to field sites if conducting *in situ* embryo incubations. Embryos should be kept cool (generally at < 10°C) and be water hardened before transportation (ASTM, 2003). All embryo containers should be packed in a cooler(s) with ice or freezer packs to maintain the temperature and avoid severe jolts during transportation. It is recommended that embryos be transported within the first 48 h after fertilization (Environment Canada, 1998; ASTM, 2003). Transported embryos should follow the incubation procedure described above after arrival to laboratory facilities. Unfertilized eggs and milt can be transported for 24 h after collection. Gametes should be kept in plastic bags and on ice for transport (ASTM, 2003). If unfertilized gametes are transported, fertilization procedures as described in the fertilization section should be followed after arrival at research facilities.

Fish eggs and tissues for trace metal analysis

Eggs and tissues should be kept frozen until analysis. After collection samples should be kept in a container with ice or freezer packs until transfer to a freezer (–20°C) for storage.

If the transport period exceeds a few hours, it is recommended to transfer all the whirl-packs containing egg and tissues collected from each individual female into sealed Ziploc® bags to prevent water (from ice melting) entering the sample. Storage time is 6 months to 2 years at –20°C for the majority of trace metals, including selenium (USEPA, 2000)

Ageing structures

Details for ageing structure storage can be found in Jearld (1983) and Sjolung (1974).

Field material disposal

All the disposable material utilized in the field should be collected in garbage bags for disposal. Acid and iodine solutions utilized for tool cleaning should be collected in plastic jars and disposed according to local regulations.

Preparation of egg and tissue samples for metal analysis

Egg and tissue samples should be thawed and wet weight recorded for each individual sample. To prevent cross contamination between samples a plastic foil (e.g., parafilm®) should be placed on the scale and replaced after each weighing. Samples are oven dried at 60°C until constant weight is recorded. It is strongly recommended to record the moisture content for each individual sample. Metal analysis must be performed using hydride generation atomic absorption spectrophotometer (HG-AAS) or inductively coupled plasma-mass spectrometry (ICP-MS) and reported on a dry weight basis.

Egg and muscle

A sub-sample of eggs and muscle is cut from the frozen sample using a scalpel. Samples should be rinsed with nano-pure water and then oven dried in a clean plastic vial. Samples are stored in a dry place until analysis. The remaining sample should be archived at –20°C with collection site location, adult female, fish species, collection date and expiration date recorded on the sample label.

Bone

A sub-sample of bone can be cut from the frozen sample using a scalpel and the remaining sample archived at –20°C with collection site location, adult female, fish species collection date and expiration date recorded on the sample label. Bone sub-samples are dipped into boiling nano-pure water in a clean beaker. Attached flesh should be removed carefully with Teflon® coated tweezers. Clean bone is then rinsed with nano-pure water, carefully dried with a paper towel, weighed (wet weight) and oven dried in a clean plastic vial. The dried sample is ground to a powder in a clean porcelain mortar with a pestle following dry weight determinations. Samples are stored in a dry place until analysis.

Liver and kidney

Liver and kidney samples should be homogenized due to the heterogeneous nature of these organs and to ensure even distribution of contaminants through the samples. Samples are partially thawed, rinsed with nano-pure water and cut in pieces using a scalpel. Samples should be homogenized using a Teflon® pestle with a fitted plastic tube and motor

homogenizer. Homogenates are oven dried in a plastic vial and stored in a dry place until analysis.

Labware cleaning

Labware (e.g., beakers) should be soaked (~ 12h) in detergent and rinsed with deionized water before acid washing. All labware in contact with the samples (except Teflon® coated material) should be soaked for a minimum of 3 h in a nitric acid bath (5%, analytical grade), rinsed with deionized water and finally thoroughly rinsed with nano-pure water. Plastic vials used for sample drying and storage should be rinsed with ultra-pure nitric acid (5%) following acid soak and prior to rinse with deionized and nano-pure water. Teflon® coated materials corrode in acid and therefore a rapid rinse with 10% ultra pure nitric acid, followed by several rinses with nano-pure water is advised. All the washed materials should air dry for at least 24 h before use.

Embryo incubation procedures

Facilities

Embryo incubations should ideally be conducted in an environmental chamber with a set photoperiod and constant temperature. Lighting should be provided by overhead full spectrum fluorescent (or equivalent) tubes with intensity ranging from 500 to 1300 lux. The set photoperiod and temperature will vary depending on the fish species used in the test (e.g., for northern pike and white sucker the set photoperiod and temperature were 16:8 h light:dark and 10 ± 1°C, respectively, while lake trout were incubated in the dark at 10 ±

1°C). During embryo incubations, disturbances of test organisms should be minimized to prevent unnecessary stress.

Materials

All materials or equipment should be cleaned with detergent, rinsed with nitric acid (5 %, analytical grade) and then thoroughly rinsed with deionized water prior to use. Containers (e.g., Nalgene® HDPE bottles) used for the collection of water samples for metal analysis should be soaked in a detergent bath with lids removed for at least 12 h, followed by several rinses with double-distilled (ultra-pure) nitric acid (5%) and nano-pure water. All the washed materials should air dry for at least 24 h before use. Pre-cleaned containers (e.g., Eagle picher®, level 1) do not have to be cleaned prior to use, unless re-used. Materials should not contain substances that can leach into the water, cause sorption of elements from water and/or cause toxic effects in fish embryos. Glass, stainless steel, porcelain, nylon and non-toxic plastics (e.g., polyethylene) may be used (Environment Canada, 1998). Materials that have been in contact with test water (or solution) can be re-used after following the cleaning method mentioned above. No dip nets, pipettes or other tools should be used between treatments or incubation chambers without being cleaned or sterilized. All materials used during embryo incubation setup must be sterilized using a 0.000075% betadyne (povione-iodine) solution.

Incubation chambers

Each incubation chamber is made from a 4-L white plastic (polyethylene) bucket enclosing a smaller bucket (~ 2-L) (Figure A.1). A hole is cut in the lid of the 4-L bucket

allowing the suspension of the small bucket by inserting it through the hole. The sides of the small bucket are replaced by a non-metallic mesh screen (leaving ~ 2 cm from the bottom) to allow water circulation. A non-toxic aquarium approved silicon is used to glue the screen to the bucket. This design allows embryos being immersed in test water at all times during water renewal. A second hole of smaller diameter (~ 1 mm) is cut in the 4-L bucket lid to enable aeration throughout the duration of the test. Air is gently bubbled through a polyethylene capillary tubing which is passed though the 1 mm hole (overhanging close to the bottom of the 2-L bucket) providing continuous aerated water to the embryos.

Water sources and characteristics

Water used in the test should ideally be collected in the field (from reference and exposure areas) and shipped to the laboratory, or be prepared in the laboratory (e.g., reconstituted water). Since all the wastewater should be discarded following local regulations, it is important to consider the volume of the generated wastewater before starting the test.

Field collected water

Containers for transportation must be thoroughly cleaned and rinsed several times with the water to be collected and should be filled to a maximum without leaving air spaces. Each sample container should be filled, sealed and labeled after water collection. When possible, water should be kept at 4 °C during transportation. Upon arrival to the laboratory, a sub-sample for water quality parameters should be collected from each shipped container.

Water is then transferred to clean carboys (~ 50-L, previously rinsed with respective site water) and kept aerated in the environmental chamber to reach test temperature for 24 h prior to its use in the test. The remaining water for subsequent water renewals should be stored in sealed containers in the dark.

Reconstituted water

Information on reconstituted solution preparation can be found in Environment Canada (1998). It is recommended that the prepared solutions follow as close as possible field water characteristics. The use of reconstituted water in early life stages tests is not recommended due to the large volume necessary for these tests (ASTM, 2003).

Test water characteristics

Water characteristics should be acceptable for the survival and growth of the test organisms. Water must be intensively aerated prior to addition to the incubation chambers. Dissolved oxygen in the water should be at 60 to 100% saturation in all the incubation chambers (ASTM, 2003). The pH range should normally be between 6.5 and 8.5 (Environment Canada, 1998). The temperature of test/site water should be adjusted as required for each fish species (e.g., temperature for pike incubations, 10 ± 1 °C).

Procedures

Experimental design

Embryos should ideally be incubated using a two-way (cross-over) ANOVA experimental design using water either from reference/control or exposure sites (Muscatello

et al., 2006; Muscatello and Janz, 2008). Thus, embryos originating from reference or exposure site females are incubated in either reference or exposure site water. Importantly, the two-way ANOVA experimental design allows statistical discrimination between effects due to maternal transfer from effects due to exposure of developing embryos to site water. The minimum desirable number of replicates (incubation chambers) per female is 3. Each chamber should contain at least 40 embryos. The number of organisms per replicate is determined based on the expected size of the larvae at the end of the test and it should not exceed 0.5 g/L (total mass of organisms/liter of water) (ASTM, 2003). It is recommended to add three extra replicates per female for fry weight and length determinations.

Experimental setup

All materials used during embryo incubation procedures are sterilized using a 0.000075% betadyne (povione-iodine) solution. Upon arrival at the laboratory, a random sub-sample of approximately 100 embryos from each female should be collected in 20-ml scintillation vials for the determination of fertilization success and pictures for further evaluation of egg size. Fertilization success should be calculated as soon as the experimental setup is completed. Water in embryo transport containers should be replaced gradually (with respective aerated and at test temperature site water) to achieve test temperature and prevent thermal shock. Before transfer to incubation chambers, embryos should be placed in a 0.000075% betadyne (povione-iodine) solution for approximately 15 min to discourage fungal growth (deRosemond *et al*., 2005). After water hardening egg membranes are impermeable and therefore safe to treat with iodine (Environment Canada, 1998). The temperature of the prepared iodine solution should match the water temperature

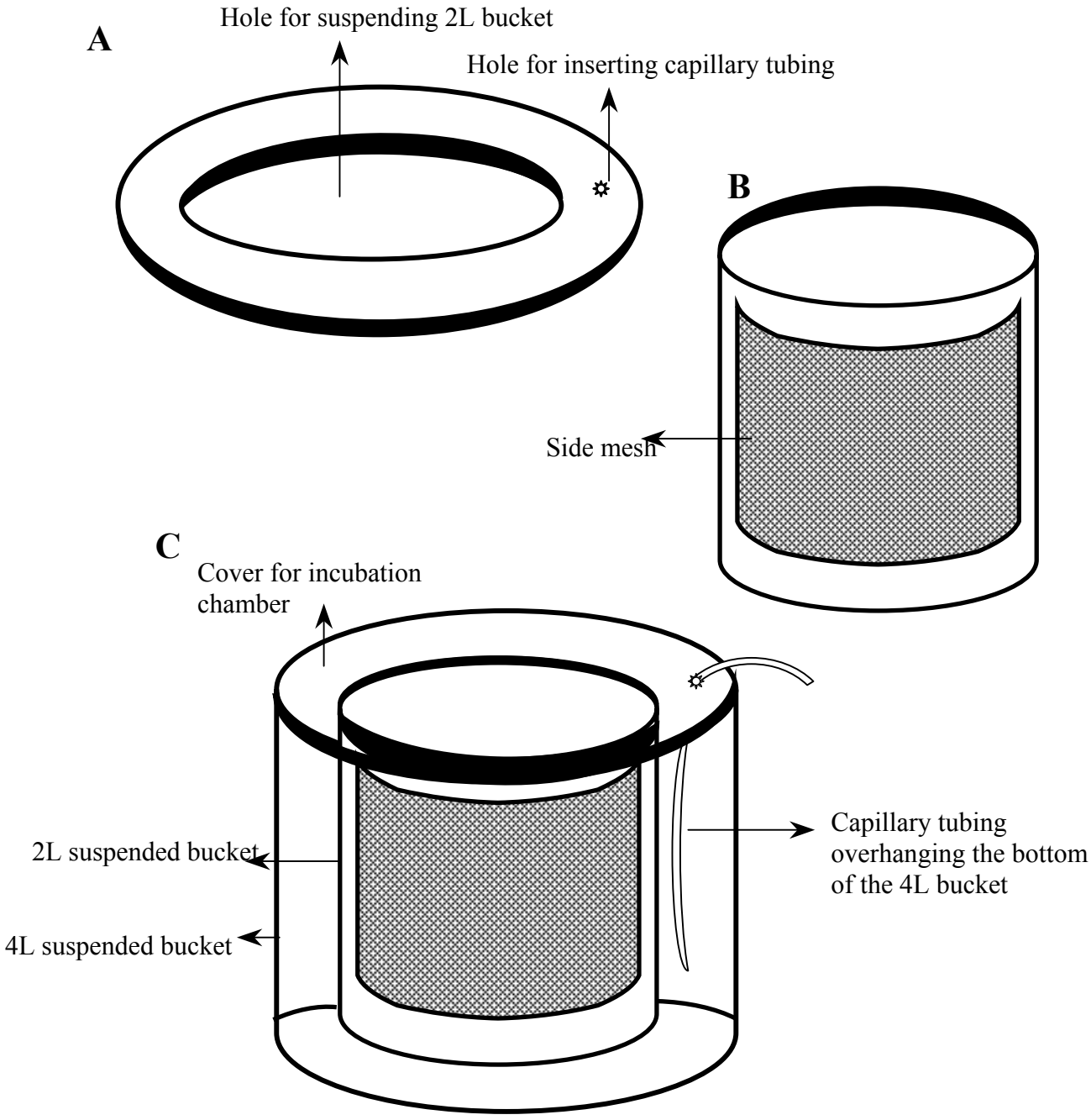
set during the test. Following the iodine treatment the embryos should be rinsed with test/site water to remove any remaining iodine. Fish embryos should be immersed in water at all times. Embryos are gently transferred to a clean glass tray and viable embryos (non-opaque) from each individual female fish randomly transferred (using a plastic pipette with a cut end if necessary) to each replicate incubation chamber. Floating embryos should be gently squirted with water to sink. An identical number of embryos should be added to each incubation chamber. Each replicate should be clearly labeled and randomly allocated in the environmental facility. It should be noted that the pre-eyed stage of embryo development is an extremely sensitive period, and thus it is recommended to not disturb the embryos except for gentle removal of infertile eggs and/or dead embryos (ASTM, 2003; Environment Canada, 1998). The remaining embryos not used in the test should be kept in the environmental chamber provided with aerated and fresh site/test water for at least 7 d as backup.

Test options and water replacement

Static-renewal

Water in incubation chambers is replaced every 2-3 d with new test/site water. The inside bucket (2L) in the incubation chambers containing viable embryos is gently removed and transferred to a new outer bucket (4L) containing fresh site/test water (see Figure A.1). The inside bucket should be gently re-suspended into the new 4L bucket to avoid the embryos floating. Each 4L bucket should be cleaned before use. This procedure allows water renewal without disturbing developing embryos. The incubation chambers should hold a minimum of 2L of water.

Figure A.1: Diagram of proposed incubation chambers for fish embryos. A) Cover lid of the incubation chamber, B) 2L bucket showing side mesh replacement, C) Incubation chamber diagram showing the 2L bucket suspended inside the 4L bucket and capillary tubing.



Flow-through

Flow-through tests continually deliver fresh test/site water to incubation chambers. There are several designs and devices to create successive water renewals. Flow rates should be checked daily throughout the test. Flow rate speed will depend on experimental requirements (Environment Canada, 1998). It is recommended to adjust flow rates to allow complete water renewal every 2 d. Caution to avoid embryo disturbance due to high flow rates should be taken.

Test measurements and endpoints

Water quality variables

Throughout the test, DO and temperature must be recorded daily in all the incubation chambers. Water samples for routine water quality analyses (hardness, alkalinity, conductivity, pH and ammonia) must be collected from three incubation chambers per treatment before and after water renewal.

Water collection for trace metal analysis

Water samples for trace metal analysis should be collected from three incubation chambers per treatment before and after water renewal. It is recommended that the concentration of trace metals be measured at least weekly during the test. Samples for dissolved metal analysis are collected using a 20-ml sterilized plastic syringe and placed in pre-cleaned 8-ml Nalgene® bottles following filtration through a 0.45 mm Nalgene® disposable filter. Samples are then acidified with double distilled (ultra-pure) nitric acid to pH < 2 and stored at 4 °C until analyzed. Samples for total metals should be acidified

without filtration. Metal analysis should be performed using hydride generation atomic absorption spectrophotometer (HG-AAS) or inductively coupled plasma-mass spectrometry (ICP-MS) within 6 months of collection (USEPA, 1983).

Mortality and embryo developmental time

Embryo mortality should be recorded daily in all incubation chambers throughout the study. The criterion for mortality in embryos is usually opaqueness or presenting some fungal growth. For fish larva the mortality criterion is immobility and/or not responding to gently prodding. Dead eggs and embryos should be gently removed using a plastic pipette. Cumulative time (degree-days) to the 50% eyed embryo, 50% hatch, and 50% swim up stages must be recorded when at least one-half of the embryos reach these stages in each incubation chamber. The time required for hatching depends on the incubation temperature and fish species tested (e.g., pike and white sucker hatching time is ~ 10 d at 10 ± 1 °C).

Test duration and fish larva preservation

The experiment is terminated individually for each incubation chamber when the majority (~ 100%) of the fry exhibit swim-up and have absorbed the egg yolk. At this stage fish larvae surface and swim actively. Although the yolk sac is no longer visible, its absorption may not be complete. Therefore, fry exhibiting swimming behavior are a more appropriate indicator of attaining the swim up stage than yolk absorption (Environment Canada, 1998). Fry must be euthanized with an overdose of MS-222 (~ 0.8 g/L), preserved in an appropriate fixative (e.g., 10% buffered formalin) for 24 h, and then transferred to 70% ethanol for subsequent evaluation of deformities. It is recommended to use double-

capped plastic bottles for fry storage to avoid evaporation of ethanol. Fry from extra replicates used for the evaluation of fry condition factor should be euthanized (as previously indicated) prior to weight (wet) and length (either total, fork or standard) evaluations. Individual fish weight is preferred however; small fish can be weighted in groups and individual weight obtained dividing by the total number of animals.

Evaluation of morphological deformities in fish larvae

All the fry should be collected and properly preserved before proceeding with the deformity analysis. The same investigator should perform analyses for a given experiment. Evaluation of developmental malformations must be performed in a blinded fashion by covering identification labels on vials of preserved larvae. It is imperative to be consistent with the decision-making rules for identifying specific deformities. If rules change during the analysis, all the preserved fry should be re-evaluated again taking into consideration the new set of rules. It is highly recommended to perform a preliminary evaluation of all preserved fry (blinded) to develop a plan for the definitive deformity evaluations. The use of a dissecting microscope with an attached camera for recording the different categories of evaluated deformities is ideal. Abnormalities are recorded in four categories: skeletal curvatures, craniofacial, finfold, and edema (Holm *et al*., 2005; Muscatello *et al*., 2006) (Figure A.2).

Procedures

All the pertinent information regarding deformity analysis should be recorded in the appropriate data sheet containing the researcher name, project information, fish species

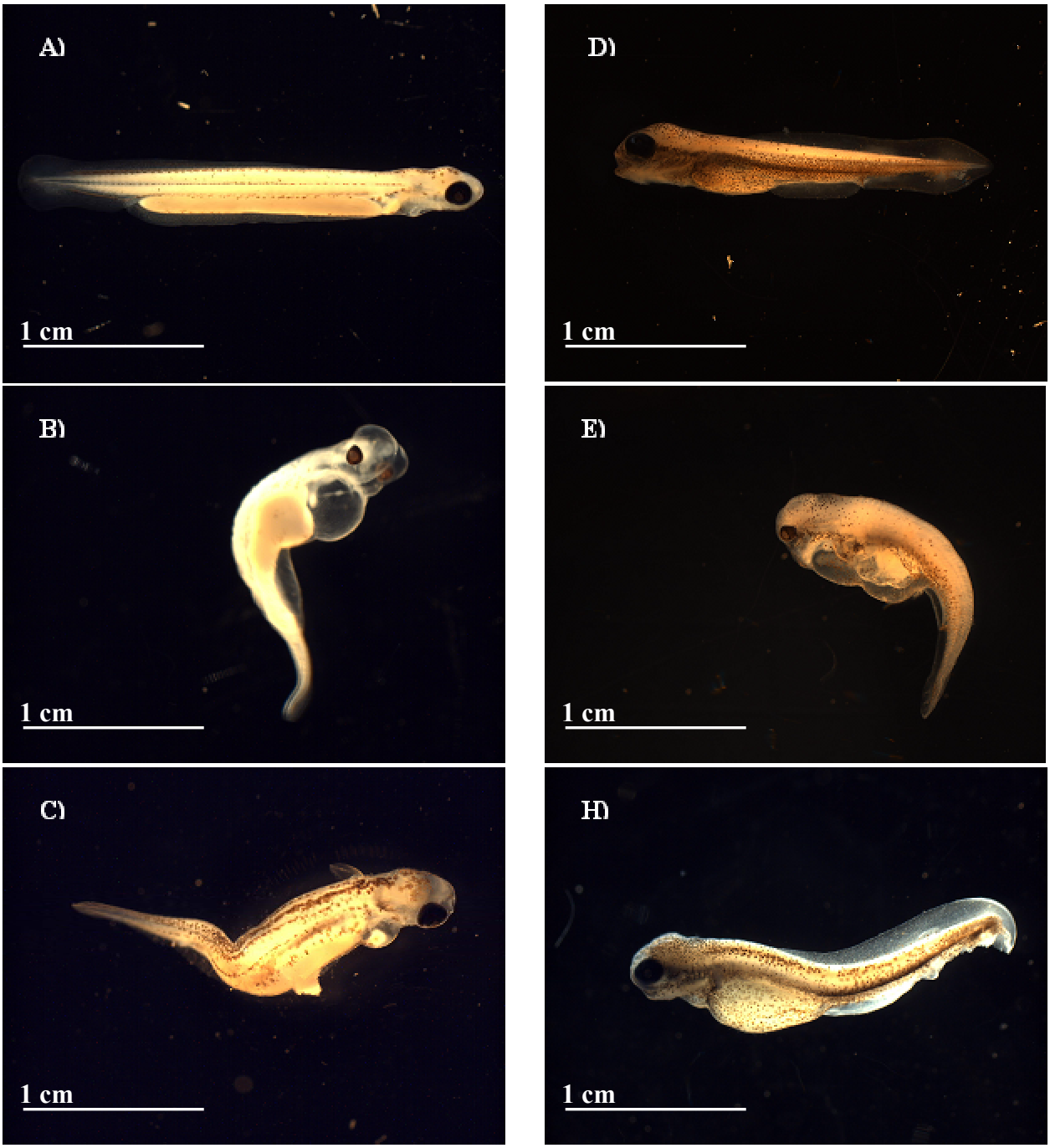


Figure A2: (A) Reference white sucker and (D) northern pike larvae. Developmental malformations in white sucker (B, C) and northern pike (E, H). (B) Spinal curvature (scholiosis), pericardial and craniofacial edema. (C) Spinal curvature (scholiosis) and lacking fin. (E) Craniofacial deformity, microphthalmia (reduction of the eye), pericardial and yolk edema, spinal curvature (kyphosis). (H) Spinal curvature (kyphosis and lordosis) and craniofacial deformity.

evaluated and date on the top of each page. Each work sheet should be numbered and have columns with space to record replicate number and label, categories of deformities and additional notes. The deformity analysis should start with the reference/control group. The investigator should familiarize themselfes with all the morphological characteristics of the reference fish before proceeding with deformity identifications in exposure larva. Deformity evaluations should continue for a minimum of three times before attempting the final analysis. It is recommended that differences between evaluations be less than 10%. It is advised to perform statistical analysis to evaluate (e.g., using ANOVA) differences between deformity evaluation attempts.

Fry should be carefully transferred to a petri dish and the remaining ethanol solution kept in an Erlenmeyer. Fry should be always covered by ethanol during analysis. Fish larvae must be inspected from lateral, dorsal and ventral views. It is recommended to increase the magnification of the dissecting microscope to detect slight deformities present in operculum and jawbones. Tools for larvae handling should be used carefully to prevent damage to the sample and thus it is recommended the use of tweezers with a round end and plastic pipette with a cut end during larva inspections. When larva inspections are finished, fry should be transferred into their respective storage vial and add the rest of the ethanol solution kept in the Erlenmeyer. It is recommended to analyze the presence of deformities one vial at a time.

Graduated severity index

The severity of the evaluated deformities can be recorded using the graduated severity index described in Holm *et al*. (2005). Significant differences among personnel in

evaluating a graduated severity index are likely to occur and therefore the applicability of this index is controversial. It is recommended to conduct frequency analysis (i.e., presence or absence of specific deformities) in order to simplify the deformity evaluations.

Calculations and statistical considerations

Statistical analyses should be performed with a 95% (a= 0.05) level of confidence. Analysis of variance (ANOVA) should be used when more than two treatment groups are being compared. Differences between two treatment groups should be evaluated by t-test. Data that fail tests for normality or homogeneity of variance are transformed (e.g., $\log_{(10)}$ or arcsine square root) prior to use of parametric statistical tests. If data fail the parametric assumptions (normality and/or homogeneity of variance) after transformation, a suitable non-parametric test (e.g., Kruskall-Wallis) should be used on the non-transformed data. Post-hoc tests for parametric (e.g., Tukey's) or non-parametric (e.g., Dunn's) data should then be used when appropriate.

Adult fish age, condition factor (CF) and hepatosomatic index (HSI)

Differences in age between adult fish should be evaluated using ANOVA or t-test. Analysis of covariance (ANCOVA) should be used to compare CF (body weights of adult fish with body length as covariate) and HSI (liver weight with body weight as covariate) between adult fish. If a significant interaction is present between variable and co-variable (treatment factor) differences should be evaluated using ANOVA or t-test (or equivalent non-parametric test) as body weight/(length)3 x 100 and liver weight/body weight x100 for CF and HSI, respectively

Egg and tissue metal concentrations

Differences in egg and tissue metal content among sites or treatments should be evaluated using one-way ANOVA (> 2 treatments) or t-test (2 treatments) followed by post-hoc test when appropriate. Non-parametric statistical tests should be used if data fail assumptions after transformation. Post-hoc evaluations should follow the statistical analysis when appropriate. Best-fit relationships between the incidence of deformities and selenium concentrations in eggs and muscle, and between muscle, bone, kidney, liver and egg selenium concentrations should be evaluated using regression analysis.

Egg size and fertilization success

Fertilization success is calculated as the number of fertilized eggs divided by the total number of eggs. Fertilization success and egg diameter differences between treatment/sites should be evaluated using ANOVA or t-test. If assumptions for parametric test are not fulfilled an equivalent non-parametric test should be used. Post-hoc comparisons of means should be used when appropriate.

Embryo mortality

Total percentage of embryo mortality (calculated as number of dead organisms at the end of the test divided by the total number of organisms per incubation chamber) should be analyzed by two-way ANOVA with egg origin and water source as the two factors of variability. However, percentage of mortality is a nominal variable and thus it is recommended to transform the data using arcsine square root before the statistical analysis. Post-hoc comparisons of means should be used when appropriate.

Embryo developmental time and fry condition factor

Significant differences among treatments in cumulative time to the 50% eyed embryo, 50% hatch and 50% swim-up stages are evaluated using two-way ANOVA with egg origin and water source as the two factors. Fry condition factor is calculated as body weight/length3 x 100 and differences between treatments/groups evaluated using two-way ANOVA (if two factors of variability are significant). If only one of the factors is significant fry can be grouped and differences in condition factor evaluated using ANOVA or t-test. Post-hoc comparisons of means for embryo developmental time and condition factor should be used when appropriate.

Percentages of deformities

The frequencies of each category of deformity should be expressed as percentages and calculated as the total number of fry from each individual female pike that exhibited one category of deformity, divided by the total number of fry preserved for that female. It is also recommended to calculate the total number of deformed fish as the total number of abnormal fish (i.e., exhibiting at least one category of deformity) divided by the total number of fry for each individual female. Percentages of deformed larvae should be recorded for each treatment including control. Fry deformities are evaluated using two-way ANOVA with egg origin and water source as the two factors. Since percentages of deformed fish larvae is a nominal variable, transformation of data using arcsine square root is advised. Post-hoc comparisons of means for embryo developmental time and condition factor should be used when appropriate. It is recommended to calculate effective

concentration (EC) values using appropriate programs (e.g., USEPA-TRAP) and to report EC01, EC05, EC10 and EC20 when possible, with associated 95% confidence intervals.

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