INVESTIGATING THE CAUSE(S) OF BENTHIC MACROINVERTEBRATE COMMUNITY IMPAIRMENT DOWNSTREAM OF TWO SASKATCHEWAN URANIUM OPERATIONS

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Saskatoon, Saskatchewan, Canada

Erin Lee Robertson

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ABSTRACT

Past monitoring has noted benthic macroinvertebrate community impairment downstream of both the Key Lake and Rabbit Lake uranium operations in northern Saskatchewan, Canada. The objective of this research was to try to identify the cause(s) of these impacts using a weight-of-evidence approach. Given that sediments generally accumulate contaminants that are related to metal mining activities (such as metals and radionuclides), the initial hypothesis for this research was that contaminated sediments were the primary cause of benthic community impairment at both operations.

In 2003 and 2004 a Sediment Quality Triad (SQT) approach confirmed the presence of an effect on benthic community structure, in addition to significant differences in surface-water, pore-water and whole-sediment chemistry at the immediate down-stream exposure sites at both uranium operations. However, no significant adverse effects were noted in 10-d whole-sediment bioassays with *Hyalella azteca*, although this lack of response could be partially due to sediment pore-water dilution resulting from the automated clean overlying water renewal process employed. Potential causes of benthic community impairment identified through the 2003 and 2004 SQTs for Key Lake include physical sediment composition, surface water pH and total ammonia, in addition to pore-water total ammonia and arsenic. Potential stressors identified at Rabbit Lake included high surface water manganese and uranium concentrations, and increases in pore-water total ammonia, manganese, iron, arsenic, and uranium levels.

In the summer of 2004, 4-d *in-situ* bioassays using *H. azteca* were conducted along with the SQTs to investigate the role both contaminated surface water and sediment played in benthic community impairment *in-situ*. Results from the Key Lake *in-situ* bioassay demonstrated that surface-water was the primary cause of acute toxicity to *H. azteca*. Results from the Rabbit Lake *in-situ* study also demonstrated that surface water as the primary cause of acute toxicity to *H. azteca*, although the relationship was not as strong. The cause of *in-situ* toxicity at Key Lake could not be correlated with any of the variables measured within the *in-situ* study, including trace metals, total ammonia, and pH. Of the measured constituents at Rabbit Lake, only concentrations of uranium in both surface water and pore-water were suspected of causing the observed *in-situ* mortality. Two data sets from two methods of surface water and pore-water collection supported these conclusions.

Due to time constraints and stronger cause-effect relationships, efforts were focused on the *in-situ* toxicity observed at Key Lake. Surface water collected in 2004 at the time of the related *in-situ* study was also found to be acutely toxic to *H. azteca* in separate laboratory surface water bioassays, thus verifying that contaminated surface water, not sediment, was the primary cause of the observed *in-situ H. azteca* mortality. Further information revealed that organic mill-process chemicals, which have been previously linked with sporadic effluent toxicity, were released at the Key Lake operation during the time of the *in-situ* experiment and associated surface water collection. Additional surface water samples collected in June and August, 2005, were not acutely toxic to *H. azteca*. Furthermore, a second bioassay with archived surface waters from the initial 2004 collection demonstrated that the water was no longer acutely toxic (i.e., acute toxicity disappeared after one-year storage). Chemistry comparisons of the toxic and non-toxic surface water samples, verified that trace metals, ammonia, pH, and major ions, including sulphate, were not the cause of toxicity, leaving only organic mill-process chemicals as a possible cause. Subsequent 4-d laboratory toxicity tests demonstrated that these process chemicals (kerosene, amine, and isodecanol) are toxic to *H. azteca* at the levels released in 2004, and are therefore believed to be the cause of the *H. azteca* mortality seen in the earlier *in-situ* experiment.

In short, this weight-of-evidence research provided new information on the possible causes of benthic macroinvertebrate community impairment downstream of both the Key Lake and Rabbit Lake uranium operations.

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
CCME	Canadian Council of Ministers of the Environment
CWQG	Canadian Water Quality Guidelines
DO	dissolved oxygen
DOC	dissolved organic carbon
ICP-MS	inductively coupled plasma mass spectrometry
LC50	median lethal concentration
LIX	aliphatic hydroxylamine
LOEC	lowest observable effect concentration
MSDS	material safety data sheet
NOEC	no observable effect concentration
SD	standard deviation
SE	standard error
SQT	sediment quality triad
TIE	toxicity identification evaluation
TOC	total organic carbon
TU	toxic unit

PREFACE

This thesis has been organized as a series of manuscripts for publication in scientific journals. Thus, there is some repetition of introductions and materials and methods throughout. As such, abstracts for each research chapter are included.

Chapter 3 was submitted to *Environmental Toxicology and Chemistry* on September 26th, 2006, and was accepted (pending revisions) on November 29th, 2006. Chapter 4 will be submitted to *Environmental Monitoring and Assessment* in early 2007. Chapter 2 and Chapter 5 will be submitted to other scientific journals in 2007.

CHAPTER 1
INTRODUCTION

1.1 The Key Lake and Rabbit Lake uranium operations

Saskatchewan is the world's leader in uranium production with some of the largest and richest uranium ore deposits in the world (www.cameco.com). Saskatchewan currently has three active uranium mines (McArthur River, McClean Lake, and Rabbit Lake) and three active uranium mills (Key Lake, McClean Lake, and Rabbit Lake) (Figure 1.1). Uranium mining is the process by which uranium rich ore is extracted from the ground and uranium milling is the process by which uranium rich ore is processed to form a uranium concentrate known as yellowcake (U_3O_8). Uranium mills are built on the premise that uranium-rich ore bodies (uranium mines) are in close proximity. The study sites for this research project are the Key Lake and Rabbit Lake uranium operations.

The Key Lake operation began production in 1983 as a mine and mill site and is currently the largest high-grade uranium milling operation in the world, with a production capacity of 18 million pounds of U_3O_8 annually (www.cameco.com). Although on-site ore has been depleted, the Key Lake mill currently processes ore from the McArthur River mine. The McArthur River ore body is the largest, highest-grade uranium deposit in the world. In addition to U_3O_8 production, Key Lake produces ammonium sulphate fertilizer as a by-product from spent reagents (www.cameco.com).

Currently, the Rabbit Lake operation is the longest-operating uranium production facility in Saskatchewan, having begun production in 1975. Rabbit Lake is the second largest uranium milling operation in the world with a capacity of 12 million pounds of U_3O_8 annually (www.cameco.com). Rabbit Lake is still mining and milling on-site ore.

1.2 Uranium mining and milling processes

In general, the Key Lake and Rabbit Lake operations have similar process

Figure 1.1. Geographic distribution of the current uranium operations in Saskatchewan, Canada, within the Athabasca Basin (enlarged, above right). Adapted from www.cogema.ca.

methods for both mining and milling uranium. As previously mentioned, the final product at both operations is U_3O_8, a uranium concentrate known as yellowcake.

1.2.1 Mining processes

Both uranium operations originally mined ore through open pit mining techniques. At Key Lake, the Gaertner and Deilmann open pit mines were mined out in 1987 and 1997, respectfully (www.cameco.com). At Rabbit Lake, the Rabbit Lake open pit mine and Collins Bay ore bodies were mined out by 1984 and 1997, respectfully (www.cameco.com). Both uranium operations currently use underground mining methods. However, one current difference between the mining methods is that the Key Lake uranium operation uses stockpiled low-grade ore to dilute the underground-derived high-grade ore from McArthur River (www.cameco.com).

1.2.2 Milling processes

Once uranium rich ore has been extracted, it can be transported to a uranium mill for processing. The milling processes at Key Lake and Rabbit Lake are also very similar, with comparable mill process circuits and similar process chemicals used within each circuit/stage of the mill process (Table 1.1 and 1.2) (Cameco Corporation, 2003, 2004). Generally, the milling process at both Key Lake and Rabbit Lake can be divided into two main sections: yellowcake production and waste management/treatment.

1.2.2.1 Yellowcake production

Yellowcake production includes ore grinding, sulphuric acid leaching, counter-current decantation, solution pre-treatment/clarification, solvent extraction, and yellowcake precipitation.

The purpose of grinding the ore is to decrease the particle size in order to increase the efficiency of uranium recovery. Grinding is achieved through impact and attrition using grinding media such as steel balls (Cameco Corporation, 2003, 2004). This process is slightly different at the Key Lake mill as water is added to low-grade ore, mineralized waste, and special waste to form a slurry suitable for blending with the high-grade McArthur River ore (which can be up to approximately 30% U_3O_8) (Cameco

Table 1.1. List of products and chemicals used within the Key Lake uranium mill. Adapted from Cameco Corporation (2004).

Product/Chemical	**Circuit**
Amine	Uranium Stripping
Ammonia	Uranium Stripping and Uranium Precipitation
Barium Chloride	Bulk Neutralization/Effluent Treatment
Ferric Sulphate	Leaching
Flocculent	Bulk Neutralization/Effluent Treatment
Flocculent	Counter-Current Decantation
Flocculent	Grinding
Flocculent	Leaching
Flocculent	Solution Pre-treatment
Flocculent	Tailings Production
Flocculent	Uranium Precipitation
Isodecanol	Uranium Stripping, Molybdenum Removal
Kerosene	Uranium Stripping, Molybdenum Removal
Lime	Tailings Production and Bulk Neutralization/Effluent Treatment
LIX[a]	Molybdenum Removal
Nitrogen Gas	Leaching
Oxygen Gas	Leaching
Silica Reagent	Solution Pre-treatment
Sodium Carbonate	Uranium Stripping
Sodium Hydroxide	Molybdenum Removal
Steel Balls	Grinding
Sulphuric Acid	Leaching, Solution Pre-treatment, Solvent Extraction, Molybdenum Removal, Bulk Neutralization, and Effluent Treatment

[a] LIX = aliphatic hydroxylamine.

Table 1.2. List of products and chemicals used within the Rabbit Lake uranium mill. Adapted from Cameco Corporation (2003).

Product/Chemical	**Circuit**
Amine	Uranium Stripping
Barium Chloride	Effluent Treatment
Ferric Sulphate	Impurity Precipitation and Effluent Treatment
Flocculent	Bulk Neutralization
Flocculent	Counter-Current Decantation
Flocculent	Clarification
Flocculent	Uranium Precipitation
Hydrogen Peroxide	Uranium Precipitation
Isodecanol	Uranium Stripping
Kerosene	Uranium Stripping
Lime	Impurity Precipitation, Bulk Neutralization, and Effluent Treatment
Magnesium Oxide	Uranium Precipitation
Sodium Carbonate	Solvent Extraction
Sodium Chlorate	Leaching
Steel Balls	Grinding
Sulphuric Acid	Leaching, Counter Current Decantation, Solvent Extraction, and Effluent Treatment

Corporation, 2004). High-grade ore is pumped into the blend tank where it is combined with the low-grade product (which includes the addition of a flocculent) to a target of 4% U_3O_8 (Cameco Corporation, 2004). Once ore is grinded to a favourable particle size, it can be pumped to next process step of leaching.

Leaching dissolves the uranium from the ore into solution using sulphuric acid at elevated temperatures under oxidizing conditions (Cameco Corporation, 2003, 2004). At the Key Lake mill, iron is used as a catalyst in the leaching reaction, cycling from the ferric to ferrous oxidation state and back again (Cameco Corporation, 2004). Depending on the iron content of the blended ore, additional iron can be provided through the addition of ferric sulphate. At the Rabbit Lake mill, sodium chlorate is added to maintain oxidizing conditions in the leach slurry. The product from the leaching process is a slurry that contains barren rock particles and an acid solution that contains dissolved metals (including uranium) (Cameco Corporation, 2003, 2004). This product is transferred to the counter-current decantation circuit.

The purpose of the counter-current decantation circuit is to remove the barren rock particles from the uranium bearing solution (also known as the "pregnant solution"). The barren rock and the "pregnant solution" run counter-current to one another, which results in the pregnant solution becoming progressively enriched with uranium while the barren rock becomes progressively depleted in uranium (Cameco Corporation, 2003, 2004). Flocculent is added during this process to maximize underflow densities and overflow clarity (Cameco Corporation, 2003, 2004). The pregnant solution is pumped to solution pre-treatment/clarification and the solids slurry is pumped to the tailings tanks prior to being pumped to the tailings facilities (Cameco Corporation, 2003, 2004).

The solution pre-treatment/clarification stage minimizes gypsum scaling, emulsion formation, and operating difficulties in the solvent extraction circuit through treating the pregnant solution with clarifiers, sand filters and flocculent (Cameco Corporation, 2003, 2004). Once the pregnant solution has been treated, it can move on to the solvent extraction circuit.

The goal of the solvent extraction circuit is to purify and concentrate the pregnant solution through the removal of other dissolved impurities. There are three main stages of the solvent extraction process: extraction, stripping, and impurity removal. During the extraction process, uranium is recovered by running the pregnant solution counter-current to an organic solvent comprised of kerosene, isodecanol, and a tertiary amine (Tri-C_8-C_{10}-Alkylamines) (Cameco Corporation, 2003, 2004). The tertiary amine has a high affinity for uranium, the isodecanol expedites separation of the organic and aqueous phases, and the kerosene adds bulk and is a carrier solvent (Cameco Corporation, 2003). The extraction product is a uranium rich organic solvent called the "pregnant organic". Before the pregnant organic is sent to the uranium stripping section it is "scrubbed" counter-current to a dilute acid to remove arsenic which has entered the pregnant organic (Cameco Corporation, 2003, 2004). Once the pregnant organic has been purified, uranium is stripped from the solution by passing the pregnant organic counter-current to ammonium sulphate and ammonia gas at Key Lake (Cameco Corporation, 2004) and sulphuric acid at Rabbit Lake (Cameco Corporation, 2003). The product of the uranium stripping process is a uranium rich ammonium sulphate based (Key Lake) or sulphuric acid based (Rabbit Lake) solution called the "pregnant strip".

The pregnant strip can contain impurities that must be removed before the uranium is precipitated to form yellowcake (U_3O_8). The Key Lake mill has a molybdenum removal circuit which is only utilized when there are high concentrations of molybdenum present in the ore (Cameco Corporation, 2004). In this process, the pregnant strip is run counter-current to an organic solution comprised of kerosene and a molybdenum extractant consisting of an aliphatic hydroxylamine (also known as LIX) (Cameco Corporation, 2004). The molybdenum in the organic solution is stripped using a dilute sodium hydroxide solution and the resulting molybdenum rich solution is pumped to the tailing tanks (Cameco Corporation, 2004). Conversely, the Rabbit Lake mill has an impurity precipitation stage in which ferric sulphate is added to precipitate arsenic as ferric arsenate and lime is added to remove sulphate and raise the pH of the pregnant strip solution (Cameco Corporation, 2003).

The purified pregnant strip is pumped to the yellowcake precipitation circuit. At the Key Lake mill, the purified pregnant strip is fed into a precipitation tank where ammonia gas is added to the solution to raise the pH to about 7.2 which causes the uranium to precipitate as ammonium diuranate particles (Cameco Corporation, 2004).

Ammonium diuranate particles are sent to the calcining circuit where ammonium diuranate is converted to uranium oxide (U_3O_8) through the addition of spent ammonia scrub from the solvent extraction, addition of a flocculent, and dewatering the formed solution via centrifugation (Cameco Corporation, 2004). Solids are then dried and converted to U_3O_8. In addition to yellowcake production, the Key Lake mill produces ammonium sulphate fertilizer from the removal of ammonia from excess barren strip solution (Cameco Corporation, 2004).

At Rabbit Lake the purified pregnant strip is diluted and cooled prior to the precipitation of the uranium using hydrogen peroxide (Cameco Corporation, 2003). Magnesium oxide is used to maintain the pH in the required range (Cameco Corporation, 2003). When precipitation is complete, the solution and precipitated solids are discharged to the yellowcake thickener where a flocculent is added, the solution is removed (pumped to tailings neutralization), and the precipitated solids (yellowcake) are dried (Cameco Corporation, 2003). Once the yellowcake is dried, the final step for U_3O_8 production at both Key Lake and Rabbit Lake uranium mills is to pack the yellowcake into drums for shipment to uranium refineries.

1.2.2.2 Waste management/treatment

With the production of yellow cake comes the production of both solid and liquid wastes. Solid wastes from the mill circuits are collected and disposed as tailings. Liquid wastes from the mill circuits are treated and released into the receiving environment as effluent. Therefore, the two main functions of waste management/treatment are tailings disposal and effluent treatment. Key Lake and Rabbit Lake have similar waste management/treatment procedures, but have several site-specific differences that depend on the waste management facilities present and the chemistry of the waste.

Prior to 1996, Key Lake tailings were stored in an above ground tailings management facility (Cameco Corporation, 2004). This facility was engineered and constructed with a bentonite liner and drainage collection system (Cameco Corporation, 2004). In 1996, the above ground tailings deposition was successfully changed over to the Deilmann tailings management facility which was initially operated as a sub-aerial deposition, in-pit pervious surround tailings management facility, but was converted to a sub-aqueous tailings deposition in 1999 (Cameco Corporation, 2004).

At Rabbit Lake, solid wastes from the mill circuits form a tailings slurry which is disposed within a tailings pond, lined with a pervious envelope (Cameco Corporation, 2003). Water in the tailings percolates to the drift located at the bottom of the pit which is then pumped to the ground surface and returned to the mill (Cameco Corporation, 2003). Subaerial spigoting or below water discharge methods may also be utilized as required (Cameco Corporation, 2003).

At Key Lake and Rabbit Lake, the general wastewater treatment process includes bulk neutralization (increase pH through the addition of lime) and effluent treatment. Effluent treatment is similar between the operations, but has specific differences at each site. At Key Lake, once the wastewater passes through bulk neutralization, the effluent is treated by adding flocculent, barium chloride (to precipitate radium as barium-radium sulphate), and acid (to adjust/increase pH). (Cameco Corporation, 2004). Treated effluent flows through the discharge launder to one of four monitoring ponds (Cameco Corporation, 2004). Before the effluent is released into the receiving environment from the monitoring ponds, the quality of water is confirmed through sampling and analysis (Cameco Corporation, 2004). If the effluent does not meet the established effluent criteria, the effluent is recycled for re-treatment until all variables in Table 1.3 are within acceptable ranges (Cameco Corporation, 2004).

At Rabbit Lake, once the wastewater passes through bulk neutralization, the effluent is treated by adding flocculent, sulphuric acid (pH adjustment), ferric sulphate (to precipitate soluble arsenic as ferric arsenate), barium chloride (to precipitate soluble radium as barium-radium sulphate) and lime (pH adjustment) (Cameco Corporation, 2003). Treated effluent is pumped to the first settling pond where the supernatant is pumped to the effluent treatment building where additional radium precipitation occurs through the addition of barium chloride (Cameco Corporation, 2003). The effluent then flows to the second settling pond where it is pH adjusted (if required) and polished with pressurized sand filters (Cameco Corporation, 2003). After this stage, the effluent is discharged pass the final point of control into the receiving environment (Cameco

Table 1.3. Maximum allowable monthly mean values and/or ranges of variables measured within the Key Lake uranium operation effluent[a]. Adapted from Key Lake Laboratories (2004).

Variable Measured	Maximum Allowable Monthly Mean Value and/or Range (units)
Temperature	No value
pH	6.0 – 7.0
Conductivity	No value
Ammonia	30 (mg/L)
Arsenic	0.50 (mg/L)
Nickel	0.50 (mg/L)
Lead	0.20 (mg/L)
Molybdenum	No value
Uranium	2.5 (mg/L)
Copper	0.50 (mg/L)
Zinc	0.50 (mg/L)
Radium-226	0.37 (Bq/L)
Total Suspended Solids	15.0 (mg/L)

[a] Limits are part of the conditions of the Key Lake operation operating licence.

Corporation, 2003). Acceptable ranges for the variables measured are presented in Table 1.4.

Considering the mill process is dynamically interconnected, with the recycling of solvents and contaminated water from other sources, effluent constituents discharged into the receiving environments can be of both mining and milling origin (Cameco Corporation, 2003, 2004).

1.3 Discharge basins and study sites

1.3.1 Key Lake

The Key Lake discharge basin is a series of small lakes connected through creeks. The site of effluent discharge is Wolf Lake (the outflow of Wolf Lake is estimated to contain 72% effluent; Golder Associates Ltd., 2003a). From Wolf Lake the effluent flows through Fox Lake entering Yak Creek (estimated to contain 69% effluent) and then David Creek (estimated to contain 37% effluent (Golder Associates Ltd., 2003a)). The diluted effluent then flows through Unknown Lake to Delta Lake (estimated to contain 28% effluent) via David Creek and eventually drains into the Wheeler River (estimated to contain 3% effluent (Golder Associates Ltd., 2003a) (Figure 1.2).

1.3.2 Rabbit Lake

The Rabbit Lake discharge basin is a series of two ponds and a bay. The site of discharge is at the head waters of Horseshoe Creek (estimated to contain 50 to 100% effluent, depending on seasonal variability (Golder Associates Ltd., 2003b)). From Horseshoe Creek the effluent flows into Unknown Pond and then Horseshoe Pond via Horseshoe Creek. From Horseshoe Pond the effluent re-enters Horseshoe Creek which then flows into Hidden Bay, a large bay on Wollaston Lake (Figure 1.3). The concentration of effluent at the inlet of Hidden Bay is estimated to be 2.5 to 5 times less than the head waters of Horseshoe Creek (Golder Associates Ltd., 2003b).

1.4 Environmental concerns with uranium industry effluents

Effluents have long been associated with contamination and adverse biological

Table 1.4. Maximum allowable monthly mean values and/or ranges of variables measured within the Rabbit Lake uranium operation effluent[a]. Adapted from material provided by Kevin Himbeault (2005).

Variable Measured	**Maximum Allowable Monthly Mean Value and/or Range (units)**
Temperature	No value
pH	6.0 – 9.5
Arsenic	0.5 (mg/L)
Copper	0.3 (mg/L)
Lead	0.2 (mg/L)
Nickel	0.5 (mg/L)
Uranium	2.5 (mg/L)
Zinc	0.5 (mg/L)
Radium-226	0.37 (Bq/L)
Thorium-230	1.85 (Bq/L)
Lead-210	0.92 (Bq/L)
Cyanide	1.0 (mg/L)
Selenium	0.6 (mg/L)
Vanadium	0.5 (mg/L)
Un-ionized Ammonia	0.5 (mg/L)
Total Suspended Solids	15.0 (mg/L)

[a] Limits are part of the conditions of the Rabbit Lake operation operating licence.

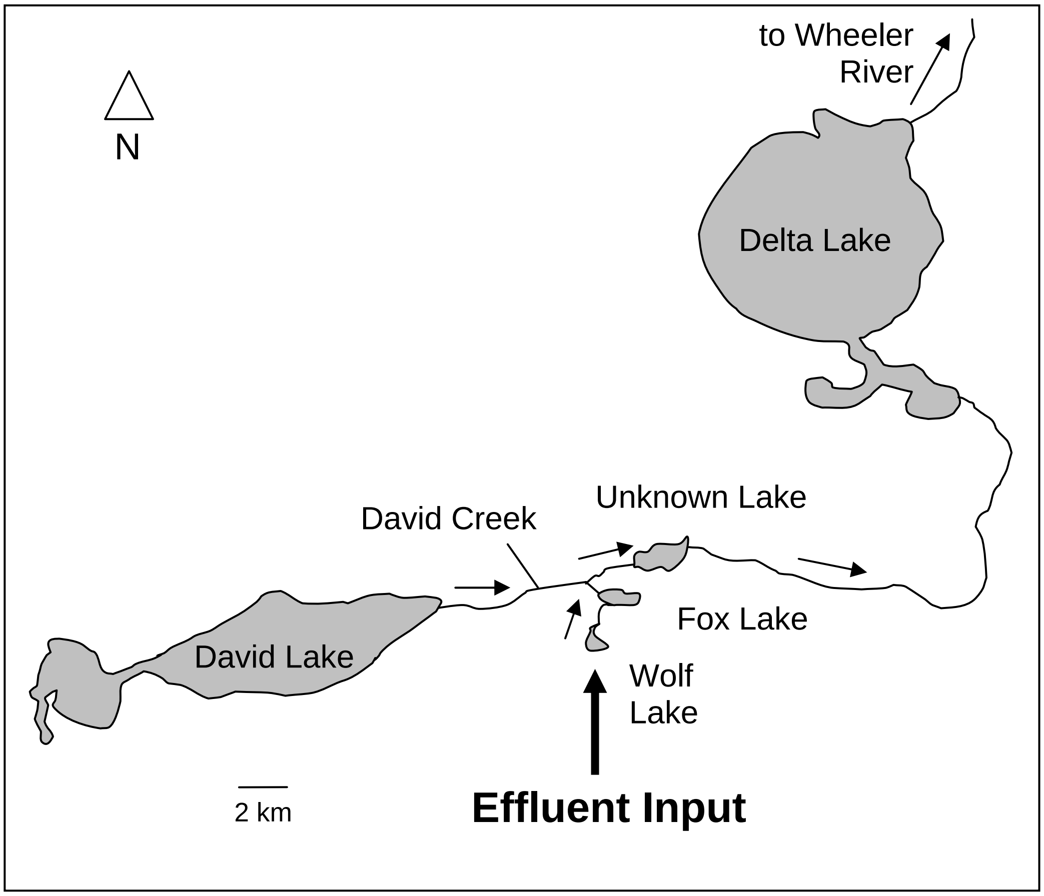

Figure 1.2. The Key Lake uranium operation effluent discharge basin.

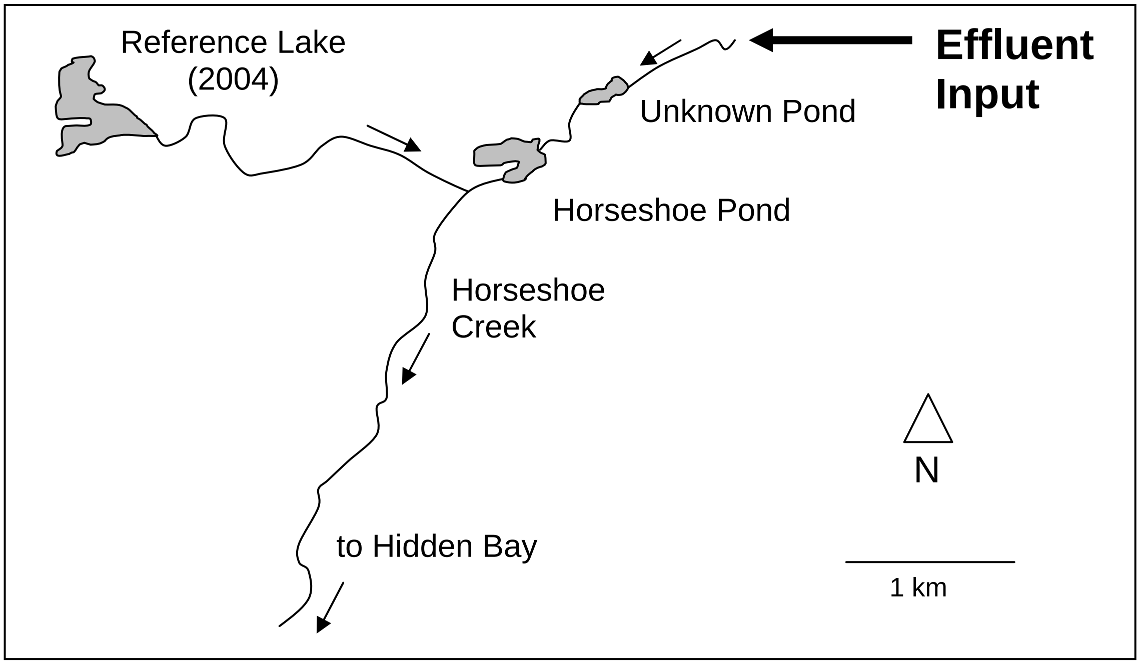

Figure 1.3. The Rabbit Lake uranium operation effluent discharge basin.

effects within their aquatic receiving environments. Because effluents are typically a complex mixture of constituents, constantly changing with time, it is difficult to fully characterize these effluents and quantify exposure to aquatic organisms. Furthermore, determining the cause of adverse biological effects associated with effluents is often very complicated. Two of the major routes of exposure, and hence possible causes of toxicity, to contaminants within effluent impacted areas are surface water and sediment. Surface waters within receiving environments are generally more representative of recent effluent releases than sediments. Surface waters typically act as the main source of exposure to pelagic organisms, but can also affect organisms that live at the surface water/sediment interface. When an "end-of-pipe" effluent toxicity test indicates toxicity, it provides a highly probable explanation for adverse field effects related to surface water exposure. However, it is possible for effluents that pass "end-of-pipe" toxicity tests to produce effects caused by surface water exposure.

Because effluents can comprise significant volumes of surface waters within receiving environments (as is the case with both Key Lake and Rabbit Lake receiving environments), constituents that cause adverse effects in 100% effluent can consequently cause adverse effects in surface waters. Effluent constituents that have the potential to cause adverse effects related to surface water exposure include process chemicals, such as coagulants and flocculants. Coagulants and flocculants were recently identified as the cause of effluent toxicity at the Ekati™ Diamond Mine in Canada's Northwest Territories (deRosemond and Liber, 2004). Therefore, it is important to characterize the specific effluents in question in order to identify potential causes of toxicity.

As suggested earlier, toxicity can occur due to surface water exposure within receiving environments even when "end-of-pipe" effluent is deemed non-toxic. Possible explanations include highly variable effluent, chronic exposure, and/or interactions between the effluent and its receiving environment. In addition, "end-of-pipe" toxicity tests may not be sensitive enough to represent the most sensitive species within the receiving environment or long enough to predict chronic effects. Overall, regardless of whether or not "end-of-pipe" toxicity test exhibit toxic effects, surface waters can be a source of adverse effects in receiving environments.

Sediments can also act as an exposure route and source of toxicity to aquatic organisms. One of the environmental concerns of the uranium industry (including the Key Lake and Rabbit Lake operations), is the release and accumulation of metals within the environment. Metals are a major component of ore and are therefore a major constituent within metal mining/milling effluents. Although the concentration of metals in the effluent may not be toxicologically significant, the accumulation of these metals can represent a higher toxicological risk. The main site of accumulation of these contaminants is generally within the sediments of receiving waters. Because metals do not degrade, their presence within receiving environments poses a long-term environmental risk. Conversely, radionuclides do decay, but because some radionuclides have very long radiological half-lives (the uranium-238 half-life is approximately 4.5 billion years) their accumulation is also of concern. Sediment contaminants could also be a source of redistribution after effluent discharges stop and surface water contamination drops. Since metals and radionuclides partition to and accumulate within the sediment, organisms that live within and/or derive their food from sediments have the greatest risk of long-term exposure to these contaminants.

In short, uranium industry effluent could act as a cause of toxicity to aquatic organisms through surface water and/or sediment environments. Surface waters are usually more representative of current or recent effluent releases, where as sediments are more representative of the accumulation of effluent-derived constituents over time.

1.5 Importance of benthic macroinvertebrates

One group of organisms that are potentially exposed to effluent constituents from both surface water and sediment exposure routes are benthic invertebrates. As suggested by their name, benthic invertebrates inhabit the benthic environment, including sediment. While it is intuitive that sediment and/or sediment pore-water are important routes of exposure for benthic invertebrates, overlying water is also an important (and sometimes overlooked) exposure route for these organisms - it can actually be the primary cause of benthic community impairment (Schmidt et al., 2002). The degree of importance depends on the behaviour and/or niche of the organisms present within the benthic community. For example, benthic invertebrates that burrow within the sediment will have less surface water exposure than epibenthic organisms that inhabit the surface water/sediment interface. Because benthic invertebrates are a diverse group of organisms, benthic communities will likely represent both types of organisms. In addition, diversity also results in a range of sensitivities to different contaminants (Slooff, 1983). Therefore, the composition of benthic communities can often reflect the bioavailability and/or type of stressors present, and/or which environmental phase the stressors predominate from, although mixtures can make this relationship less distinct (Slooff, 1983). Overall, because benthic invertebrates are exposed through both surface water and sediment exposure routes, and because their community structure can change depending on the stressors present, they are ideal organisms to monitor in aquatic receiving environments.

1.6 Past research on benthic macroinvertebrate community health

1.6.1 Key Lake

1.6.1.1 Benthic macroinvertebrate monitoring

Through previous monitoring programs, benthic community impairment has been noted downstream of the effluent discharge site at the Key Lake uranium operation (Terrestrial and Aquatic Environmental Managers Ltd., 1994; Conor Pacific Environmental Technologies Inc., 2000; Golder Associates Ltd., 2002; Golder Associates Ltd., 2005). More specifically, the Key Lake operation has experienced benthic community impairment in Wolf Lake, Fox Lake, Unknown Lake, and Delta Lake (Table 1.5). Although attempts have been made to identify the cause of benthic impairment, no study has clearly determined whether or not the cause is attributed to surface water and/or sediment exposure, or identified the specific constituent or constituents causing these effects.

1.6.1.2 Related effluent and/or surface water studies

The Key Lake operation has had isolated cases of effluent toxicity and Wolf Lake outflow water toxicity (HydroQual Laboratories, 1995a; 2002; 2004). In response to these toxicity events, toxicity identification evaluations (TIEs) were conducted. Toxicity Identification Evaluations are a series of bioassays aimed to characterize

Table 1.5. Summary of benthic community impairment downstream of the Key Lake uranium effluent discharge point. All locations were not sampled during each study.

Lake Sampled	**Year Sampled**	**Findings (Compared to Reference Site)**
Wolf	1993	Decreased total abundance and richness, lower richness and diversity indices, and higher Simpson Dominance index[a]
Fox	1993	Decreased total abundance and richness, lower richness and diversity indices, and higher Simpson Dominance index[a]
Fox	1998	Decreased total abundance and richness[b]
Fox	2001	Decreased total abundance and richness[c]
Fox	2004	Decrease in richness, lower Simpson's Diversity index, and statistically different (higher) Bray-Curtis index[d]
Unknown	1998	Decreased richness[b]
Unknown	2001	Decreased total abundance[c]
Unknown	2004	Statistically different (higher) Bray-Curtis index[d]
Delta	1998	Decreased richness[b]
Delta	2004	Higher Simpson's Diversity index[d]

[a] Terrestrial and Aquatic Environmental Managers Ltd. (1994).
[b] Conor Pacific Environmental Technologies Inc. (2000).
[c] Golder Associates Ltd. (2002).
[d] Golder Associates Ltd. (2005).

(Phase I)(United States, Environmental Protection Agency, 1991), identify (Phase II) (United States, Environmental Protection Agency, 1993a) and confirm (Phase III) (United States, Environmental Protection Agency, 1993b) the cause of acute or chronic toxicity within a sample. The first TIE was initiated after an effluent sample was deemed toxic to rainbow trout (*Oncorhynchus mykiss*) in a standard 4-d test in December 1993 (HydroQual Laboratories, 1995a). The TIE was successful in identifying the toxic constituent of the effluent as isodecanol (HydroQual Laboratories, 1995a). This study also established that Microtox® analysis is effective for identifying effluent toxicity due to isodecanol (HydroQual Laboratories, 1995a). It was determined that effluent Microtox® results that were greater than 30% of the control response were not likely to produce a toxic response due to isodecanol to rainbow trout or fathead minnow (*Pimephales promelas*) (HydroQual Laboratories, 1995a). The Microtox® test measures toxicity by comparing the response of a luminescent bacterium (*Vibrio fischeri*) under treatment and control conditions (SENES Consulting Ltd., 2002). The Key Lake operation currently performs daily Microtox® analysis on both the effluent (from the holding ponds) and Wolf Creek water. If the effluent Microtox® reading is below 30% of the control response, the effluent is recycled for treatment until the Microtox® reading is above 30% of the control response (Kevin Himbeault, personal communication, June 30, 2005).

A follow up study was conducted to evaluate the potential toxicity, fate, and transport of isodecanol, and concluded that isodecanol would have little impact on the aquatic ecosystem (HydroQual Laboratories, 1995b). Another follow up study was initiated to evaluate if the previous releases of isodecanol had an adverse effect on the fish community of Fox Lake (Terrestrial and Aquatic Environmental Managers Ltd, 1995). More specifically, there was a concern that white sucker (*Catostomus commersoni*) could be avoiding Fox Lake. The study confirmed the presence of white sucker in Fox Lake, but also identified a high incidence of individuals with haemorrhaging within the anal fin and skin lesions and/or haemorrhaging in the epidermis (Terrestrial and Aquatic Environmental Managers Ltd., 1995). This discovery expanded the objective of the study to determine the extent and magnitude of these effects downstream. The study examined Fox Lake, Unknown Lake, and Delta Lake.

No reference site was used (Terrestrial and Aquatic Environmental Managers Ltd., 1995). The study found lesions/haemorrhaging within the fish populations at each site, with decreasing incidence with increasing distance from upper Fox Lake (Terrestrial and Aquatic Environmental Managers Ltd., 1995). The lesions were determined to be symptoms of a bacterial infection (Terrestrial and Aquatic Environmental Managers Ltd., 1995). The cause(s) of these lesions was hypothesised to be an especially virulent strain of bacterium introduced to the drainage basin or an immuno-suppression from an anthropogenic and/or natural source (Terrestrial and Aquatic Environmental Managers Ltd., 1995). No conclusions were drawn on the specific cause of the effects found.

Years later, a review of the properties of isodecanol and its degradation products suggested that these compounds are readily biodegradable and are not likely to bioaccumulate (Canada North Environmental Services, 2001). Isodecanol is still used in the Key Lake mill process, but the concentration of this chemical in the final effluent have been reduced through processes that minimize the carry over of isodecanol from the solvent extraction circuit to the final effluent (Kevin Himbeault, personal communication, June 30, 2005).

As mentioned above, water from the Wolf Creek outflow has periodically been deemed toxic. More specifically, 4-d bioassays with water from Wolf Creek outflow showed lethality to rainbow trout in the summer of 2000 and 2001 (SENES Consultants Ltd., 2002). To investigate the cause of this toxicity, phase I and II TIEs were initiated (HydroQual Laboratories, 2004). The first TIE study concluded that the cause of effluent and Wolf Lake outflow water toxicity to fathead minnow was an organic compound removed by a C-18 solid phase extraction, but the compound could not be further identified due to current limitations in LC/MS libraries (HydroQual Laboratories Ltd., 2002). Based on the LC/MS analysis and corresponding Microtox® data, the cause of toxicity was not believed to be isodecanol (HydroQual Laboratories Ltd., 2002). The second TIE study determined that fathead minnow were not sensitive to the samples collected, although toxic responses were found with both the *D. magna* and Microtox® (HydroQual Laboratories, 2004). Toxic effects on bacterial luminescence correlated well with toxicity to *D. magna*, although *D. magna* proved to be the most sensitive test species (HydroQual Laboratories, 2004). The study concluded that the cause of toxicity could be linked to effluent biodegradation and/or interactions between Wolf Lake water and the effluent (HydroQual Laboratories, 2004). Overall, the contribution of these effluent and/or Wolf Lake outflow water toxicity events to *in-situ* benthic community impairment downstream of Key Lake uranium operation is unknown.

Additional studies have been conducted to investigate the toxicity of Fox Lake and Unknown Lake surface waters to fathead minnow larvae (Pyle et al., 2001; 2002). A 7-d *in-situ* study revealed significant mortality (>80%) of caged fathead minnow larvae exposed to Fox Lake and Unknown Lake surface waters compared to caged fathead minnow larvae exposed to David Lake surface water (<20%) (Pyle et al., 2001). Using Principal Component Analysis and pre-existing toxicity data, this study concluded that the cause of acute toxicity was due to dietary selenium from food sources within the exposure environment (Pyle et al., 2001). A companion study found somewhat contradictory results with significant (compared to the lab control) mortality (>80%) in fathead minnow larvae exposed to surface waters collected from Unknown Lake and David Lake, but not in larvae exposed to surface water collected from Fox Lake (<10% mortality) (Pyle et al., 2002).

In 2004, sub-lethal toxicity tests were performed on two samples of final effluent using *D. magna* (48-h), *O. mykiss* (4-d), *P. promelas* (7-d), *Selenastrum capricornutum* (7-d), *Ceriodaphnia dubia* (7-d), and *Lemna minor* (7-d) (Golder Associates Ltd., 2005). No significant effects were seen with either *D. magna* or *O. mykiss* (Golder Associates Ltd., 2005). The *P. promelas* study showed a significant decrease in growth in one of the samples. The *S. capricornutum* study showed significant growth inhibition in both mill effluent samples while *C. dubia* demonstrated significant mortality and reproductive impairment. Mill effluent samples significantly reduced biomass and the number of fronds produced by *L. minor* (Golder Associates Ltd., 2005). The results of these sub-lethal toxicity tests indicate that 100% effluent can cause adverse effects in aquatic species. However, the cause of these results was not addressed, nor was how these results relate to benthic community impairment *in-situ*.

Another issue that has been examined at the Key Lake operation is the low pH of Wolf Lake. Wolf Lake has seasonal variations in pH, with slightly lower levels observed in the early spring and summer (4.5 to 4.7) compared to winter (5.0 to 5.5) months (SENES Consultants Ltd., 2002, 2003). The pH of the final effluent is much higher than the pH of Wolf Lake (regulated to be released between 6.0 and 7.0) (Key Lake Laboratories, 2004), and concern has been raised that constituents of the effluent may be playing a role in this pH depression. To address this concern, a study was conducted to investigate the cause of the seasonal pH variation. It concluded that the drop in pH in the summer months was most likely driven by the naturally acid bog adjacent to Wolf Lake and not by the Key Lake uranium operation effluent (SENES Consultants Ltd., 2002, 2003). It was also noted that low pH levels could be contributing to the observed toxicity in Wolf Lake outflow water samples, especially since low pH conditions are known to increase the toxicity of some metals (due primarily to the higher solubility of metals at lower pH) (SENES Consultants Ltd., 2002). In addition, while low pH can influence the toxicity of other toxicants, it can in itself cause adverse biological effects. For example, studies have shown that low pH levels can cause benthic community impairment independently (Fjellheim et al., 1992; Ledger et al., 2005), as well as in combination with, elevated metal levels (Keller et al., 1992; Soucek et al., 2000). The significance of low pH and/or pH fluctuation to benthic community impairment at the Key Lake operation has yet to be investigated.

One additional issue with the Key Lake operation effluent is the high concentration of ammonia (20-25 mg/L as N (Key Lake Laboratories, 2004). As previously mentioned, ammonia is a process chemical used within the Key Lake mill. Although most of the ammonia is isolated to produce ammonium sulphate fertilizer, some ammonia remains within the final effluent. Ammonia is a common toxicant in aquatic environments and is frequently a cause of aquatic toxicity. Ammonia toxicity is pH-dependent, with the un-ionized, more toxic form predominating at high pH. Although Key Lake operation effluent has a high concentration of total ammonia, due to low pH levels the fraction of un-ionized ammonia is small. However, the role of ammonia in benthic community impairment downstream of Key Lake uranium operation is still under researched.

1.6.1.3 Related sediment studies

Sediment monitoring has been a major component of the historical monitoring programs at Key Lake. The main issue with Key Lake operation sediments is the accumulation of elements, including arsenic, and molybdenum, and radionuclides. In 1998, a Sediment Quality Triad (Long and Chapman, 1985; Chapman, 1996; Chapman et al., 1997; Chapman 2000) study was conducted on the David Creek drainage basin (Conor Pacific Environmental Technologies Inc., 2000). Using this weight-of-evidence approach, it was concluded that the benthic macroinvertebrate community in Fox Lake had been impacted by accumulation of effluent constituents within the sediment. The results of this study found high levels of sediment contamination, an extremely simple and depauperate benthic community, and 100% mortality in the sediment bioassays (using the amphipod *Hyalella azteca* as a test species) (Conor Pacific Environmental Technologies Inc., 2000). No conclusions or speculations were drawn regarding which specific constituents were causing the effects observed. However, closer evaluation of the sediment bioassay methods revealed that overlying water was not changed during the 14-day bioassay and the pH of the overlying water at test termination was < 4 (which exceeds the 4-d LC50 of 4.7 (France and Stokes, 1987)). Needless to say, the conclusions of this sediment bioassay are not valid and prove that further research is required to truly determine if sediment downstream of Key Lake uranium operation is toxic. According to Golder Associates Ltd. (2005), benthic community impairment effects downstream of the Key Lake operation are likely the result of historically contaminated sediments and not current effluent release. However, these studies did not address the possibility that surface water could be contributing to benthic community impairment. Therefore, the role of metal and radionuclide accumulation within sediments in benthic community impairment has yet to be thoroughly examined for the Key Lake uranium operation.

1.6.2 Rabbit Lake

1.6.2.1 Benthic macroinvertebrate studies

Through previous monitoring programs, benthic community impairment has been noted downstream of the effluent discharge site at the Rabbit Lake uranium operation (Terrestrial and Aquatic Environmental Managers Ltd. and SENES Consultants Ltd. 1996; Golder Associates Ltd., 2003c). More specifically, impairment has been observed in Horseshoe Pond, Horseshoe Creek, and Hidden Bay (Table 1.6). As with Key Lake, attempts have been made to identify the cause of benthic community impairment, but no study has clearly determined whether or not the cause is attributed to surface water and/or sediment exposure, or identified the specific constituent or constituents causing these effects.

1.6.2.2 Related sediment studies

In comparison to the Key Lake operation, the Rabbit Lake operation has conducted less research related to finding the cause of benthic community impairment. All of the studies that have been conducted deal directly with sediments. Based on the available literature, no issues have been raised with regards to surface water and/or effluent toxicity. As with the Key Lake operation, sediment monitoring has been a major component of the environmental monitoring at Rabbit Lake, and the main toxicological issue identified is the accumulation of metals and radionuclides. Past research as demonstrated that uranium is of specific concern (Liber et al., unpublished data), but the relative importance of radiotoxicity and chemotoxicity remains unclear (Thomas and Liber, 2001).

1.7 Research goal and objectives

1.7.1 Goal

The overall goal of the research described here was to identify the cause(s) of benthic community structure impairment downstream of the Key Lake and Rabbit Lake uranium operations' discharge sites. The initial hypothesis of this research was that contaminated sediment was the cause of benthic community impairment. In order to address the goal of this research, a weight-of-evidence approach was used which included the use of field studies, *in-situ* bioassays, laboratory bioassays, and toxicity tests.

Table 1.6. Summary of previously observed benthic community impairment downstream of the Rabbit Lake uranium effluent discharge site. All site locations were not sampled during each study.

Lake Sampled	Year Sampled	Findings (Compared to Reference Site)
Horseshoe Pond	1994	Decreased total abundance[a]
Horseshoe Pond	2002	Decreased richness, lower Simpson's Diversity Index, and statistically different (higher) Bray-Curtis index[b]
Horseshoe Creek	1994	Increased total abundance with increase distance from the effluent source[a]
Hidden Bay (shallow)	1994	Decreased total abundance[a]
Hidden Bay (shallow)	2002	Increased richness and statistically different (higher) Bray-Curtis index[b]

[a] Terrestrial and Aquatic Environmental Managers Ltd. and SENES consultants Ltd., (1996).

[b] Golder Associates Ltd, (2003c).

1.7.2 Objectives

To reach its goal, this project was divided into the following specific research objectives:

I. Define the relationships between contaminant concentrations, benthic macroinvertebrate community structure, and the response of *Hyalella azteca* to field-collected sediments for field sites located downstream of the Key Lake and Rabbit Lake uranium operations (Chapter 2).

II. Describe the *in-situ* toxicity to caged *Hyalella azteca* for field sites downstream of the Key Lake and Rabbit Lake uranium operations (Chapter 3).

III. Determine if differences in sampling method can influence estimates of contaminant exposure for pore-water and surface water and how that can affect conclusions drawn with respect to the cause(s) of *in-situ* toxicity to *Hyalella azteca* (Chapter 4).

IV. Identify the cause(s) of *in-situ* toxicity to *Hyalella azteca* at field sites downstream of the Key Lake uranium operation (Chapter 5).

CHAPTER 2

USE OF THE SEDIMENT QUALITY TRIAD TO INVESTIGATE CAUSE(S) OF BENTHIC MACROINVERTEBRATE IMPAIRMENT DOWNSTREAM OF TWO SASKATCHEWAN URANIUM OPERATIONS

2.1 Abstract

Previous monitoring has noted benthic macroinvertebrate community impairment downstream of the Key Lake and Rabbit Lake uranium operations in northern Saskatchewan, Canada. This research focuses on using the sediment quality triad (SQT) as part of a larger weight-of-evidence approach to determine the cause(s) of these effects. The specific objectives of this study were to determine the extent and magnitude of contamination and benthic community impairment downstream of both uranium operations, to develop hypotheses on possible causes of benthic community impairment, and thirdly, to determine the toxicity of field-collected sediments to the amphipod, *Hyalella azteca*. Components incorporated into the SQT included surface water, pore water and sediment chemistry analysis, benthic community assessment, and 10-d whole-sediment bioassays using juvenile, laboratory-reared, *H. azteca*. Results verified an effect on benthic community impairment downstream of the two uranium operations in both 2003 and 2004, along with significant differences in surface water, pore water and sediment chemistry. However, no significant adverse effects were noted from the 10-d whole-sediment bioassays with *H. azteca*. This lack of response could be partially due to pore-water dilution resulting from the automated overlying water renewal process employed. Potential causes of benthic community impairment identified through the 2003 and 2004 SQTs for Key Lake include physical sediment composition, surface water pH and total ammonia, in addition to pore-water total ammonia and arsenic. Potential stressors identified at Rabbit Lake included surface water manganese and uranium, and increases in pore-water total ammonia, manganese, iron, arsenic, and uranium. Because it is still unclear what role contaminated surface water has on benthic

communities downstream of the Key Lake and Rabbit Lake operations, further studies will evaluate the role that both contaminated surface water and contaminated sediment play in benthic community impairment *in-situ*.

2.2 Introduction

The sediment quality triad (SQT) is an integrated assessment approach used to evaluate sediment quality (Long and Chapman, 1985; Chapman, 1996; Chapman et al., 1997; Chapman 2000). The three components of the SQT include measurements of chemistry, toxicity, and infauna (usually in the form of a benthic community assessment) (Long and Chapman, 1985; Chapman, 1996; Chapman et al., 1997; Chapman 2000). The recommended use/uses for the SQT is/are to determine the presence and extent of benthic community impairment and/or the cause(s) of impairment, specifically pollution-induced impairment (Chapman, 1990; Chapman et al., 1997). The SQT has been widely used in both marine and fresh water environments in North America, and has also been utilized in Europe and even Antarctica (Chapman, 2000), proving its wide range use and acceptance.

Through previous monitoring programs, benthic community impairment has been noted downstream of the effluent discharge site at both the Key Lake (Terrestrial and Aquatic Environmental Managers Ltd., 1994; Conor Pacific Environmental Technologies Inc., 2000; Golder Associates Ltd., 2002) and the Rabbit Lake (Terrestrial and Aquatic Environmental Managers Ltd. and SENES Consultants Ltd. 1996; Liber et al., unpublished data; Golder Associates Ltd., 2003c) uranium mining and milling operations in northern Saskatchewan, Canada. However, no study has clearly identified the actual cause or causes of these effects. Because benthic invertebrates live within and/or on sediments, it is hypothesized that contaminated sediments are the cause of benthic community impairment at both the Key Lake (Conor Pacific Environmental Technologies Inc., 2000) and Rabbit Lake uranium operations (Liber et al., unpublished data). Key Lake studies have not identified specific constituents of concern, but studies at Rabbit Lake have demonstrated that uranium within sediments maybe of specific concern to benthic communities (Liber et al., unpublished data), but the relative importance of radiotoxicity and chemotoxicity remains unclear (Thomas and Liber, 2001). Overall, contaminated sediments are still believed to be the cause of benthic community impairment; however, the specific cause(s) still remains to be clearly defined.

The main purpose of this study was to use the SQT to investigate the cause(s) of benthic macroinvertebrate community impairment downstream of the Key Lake and Rabbit Lake uranium operations. More specifically, this study was aimed to determine and/or verify both the extent and magnitude of contamination (in overlying surface water, pore-water, and whole-sediment) and benthic community impairment downstream of the uranium operations, to develop hypotheses on which contaminants could be causing benthic community impairment, and thirdly, to determine the toxicity of field collected sediments to *Hyalella azteca*. This study was done over two years, with a more focused approach in the second year.

2.3 Materials and methods

2.3.1 Study sites

The Key Lake and Rabbit Lake uranium operations are located in northern Saskatchewan, Canada (Figure 1.1). Downstream sampling sites were selected on the basis of previously documented benthic invertebrate community impairment (Terrestrial and Aquatic Environmental Managers Ltd., 1994; Conor Pacific Environmental Technologies Inc., 2000; Golder Associates Ltd., 2002; Terrestrial and Aquatic Environmental Managers Ltd. and SENES Consultants Ltd., 1996; Liber et al., unpublished data; Golder Associates Ltd., 2003c). Because this study was part of a larger weight-of-evidence investigation (including benthic community assessment), selecting sites with similar sediment/habitat was critical. In general, “depositional” sediment types (fine textured, organically enriched) were chosen at water depths ranging from 60 to 100 cm.

2.3.1.1 Key Lake

The Key Lake operation began production in 1983 as a mine and mill site and is currently the largest high-grade uranium milling operation in the world, with a production capacity of 18 million pounds of U_3O_8 annually (www.cameco.com). Although on-site ore has been depleted, the Key Lake mill currently processes ore from another near by mine, McArthur River. Wastewater can be of both mining and milling origin considering many processes recycle/use contaminated water from other sources (Cameco Corporation, 2004).

The Key Lake discharge basin is a series of small lakes connected through creeks. The site of effluent discharge is a pond called Wolf Lake (Wolf Creak outflow is estimated to contain 72% effluent (Golder Associates Ltd., 2003a)). From Wolf Lake the effluent flows through Fox Lake entering Yak Creek (estimated to contain 69% effluent (Golder Associates Ltd., 2003a)) and then David Creek (estimated to contain 37% effluent (Golder Associates Ltd., 2003a)). The effluent then flows through Unknown Lake to Delta Lake (estimated to contain 28% effluent (Golder Associates Ltd., 2003a)) via David Creek and eventually drains into the Wheeler River (estimated to contain 3% effluent (Golder Associates Ltd., 2003a)) (Figure 1.2).

For the Key Lake SQT study, four exposure sites and one reference site were used in 2003 and two exposure sites and one reference site were used in 2004. The exposure sites in 2003 were located near the outflow of Wolf Lake and the inlet of Fox Lake, the inlet of Unknown Lake and the inlet of the large bay in Delta Lake. The reference site was David Lake. In 2004, only Wolf Lake and Fox Lake exposure sites were sampled. David Lake has also been historically used as an environmental reference site for the site operators and their contractors (Terrestrial and Aquatic Environmental Managers Ltd., 1994; Conor Pacific Environmental Technologies Inc., 2000; Golder Associates Ltd., 2002). The sampling dates for the Key Lake SQT studies were September 6-8, 2003 and July 7-12, 2004.

2.3.1.2 Rabbit Lake

Rabbit Lake is the second largest uranium milling operation in the world with a capacity of 12 million pounds of U_3O_8 (www.cameco.com). Rabbit Lake continues to mine and mill on-site ore. Wastewater here can also be of both mining and milling origin (Cameco Corporation, 2003).

The Rabbit Lake discharge basin is a series of two ponds and a bay. The site of discharge is at the head waters of Horseshoe Creek (estimated to contain 50 to 100% effluent, depending on seasonal variability (Golder Associates Ltd., 2003b)). From

Horseshoe Creek the effluent flows into Unknown Pond and then Horseshoe Pond via Horseshoe Creek. From Horseshoe Pond the effluent re-enters Horseshoe Creek which then flows into Hidden Bay, a large bay on Wollaston Lake (Figure 1.3). The concentration of effluent at the inlet of Hidden Bay, is estimated to be 2.5 to 5 times less than at the head waters of Horseshoe Creek (Golder Associates Ltd., 2003b).

The Rabbit Lake SQT study had two exposure sites and one reference site in both 2003 and 2004. In 2003, the first exposure site was near the middle of Horseshoe Pond and the second exposure site was at the junction between Horseshoe Creek and Hidden Bay. The reference site for 2003 was a shallow lake adjacent to Hidden Bay upstream from where the effluent enters Hidden Bay from Horseshoe Creek. In 2004, the first exposure site was at the inlet of Unknown Pond and the second exposure site was again near the middle of Horseshoe Pond. The reference site for 2004 was a different shallow lake located upstream to, and part of, the same drainage basin as, Unknown Pond and Horseshoe Pond. The sampling dates for the Rabbit Lake SQT studies were September 13-14, 2003 and August 6-11, 2004.

2.3.2 Physicochemical characterization

2.3.2.1 Overlying-water

Overlying surface water samples were collected (n = 3 per site in 2003 and n = 4 per site in 2004) using a Wildco® 3.2-L Van Dorn horizontal, acrylic beta water sampler (Wildlife Supply Company, Buffalo, NY, USA) from ~15 cm above the sediment surface. Samples were sieved through a 53-µm sieve to remove planktonic organisms and debris. Samples were analyzed for pH (ORION® PerpHect LogR meter model 370, ORION Research, Beverly, MA, USA, or Beckman® 250 pH/Temp/mV meter and Beckman® pH electrode 511050, Beckman Instruments Inc., Fullerton, CA, USA) and total ammonia (Beckman® 250 pH/Temp/mV meter and Thermo Orion® 95-12 ammonia electrode or ORION® aquafastII photometer) within one week of collection in 2003 and on the day of collection in 2004. Prior to analysis, samples were stored in the dark at 4°C. Samples were also analyzed for conductivity (ORION® ATI conductivity cell 017010 and ORION® ATI meter 170), hardness (Hach Digital Titrator model 16900, Hach Company, Loveland, CO, USA) and alkalinity (Hach Digital Titrator model

16900) within a week of collection. Samples for analysis of total metals were acidified within one week of collection in 2003, and on the day of collection in 2004, and stored in the dark at 4°C until analyzed using ICP-MS (Department of Geological Sciences, University of Saskatchewan). Dissolved oxygen (DO) and temperature were measured *in-situ* ~15 cm above the sediment surface using an ORION® dissolved oxygen meter model 835.

2.3.2.2 Whole-sediment and sediment pore-water

Sediment cores (*n* = 3 per site in 2003 and *n* = 4 per site in 2004) were collected in 5-cm (outside diameter) acrylic core-tubes (Wildlife Supply Company, Buffalo, NY, USA) using a hand-held corer (sealed with plastic core caps). Once sediment cores were collected and the bottom cap in place, the core was carefully topped up with 53-µm sieved site water and the tops capped with no head space. Sediment cores were stored in the dark at 4°C until they were processed (within 1 month of collection in 2003 and within 2 weeks in 2004). Processing included isolating the top 2.5-cm horizon of the sediment cores, homogenizing the sample, and removing sub-samples for determination of total metals (ICP-MS), total organic carbon (TOC, Leco Carbon determinator CR-12, Leco, St. Joseph, MI, USA), particle size (performed by Enviro-Test Laboratories, Saskatoon, SK, Canada), and water content. Pore-water was isolated from sub-samples of sediment cores via centrifugation at 12,000 rpm (17,200 rcf) at room temperature for 20 min. Once isolated, pore-water was filtered through a 0.45-µm membrane (Nalgene® acetate syringe filter, Nalge Nunc International, Rochester, NY, USA) and immediately analyzed for pH, ammonia, hardness, and alkalinity as described above. Sub-samples of pore-water were removed and later analyzed for dissolved metals and dissolved organic carbon (DOC). Dissolved metal samples were acidified at the time of collection and stored in the dark at 4°C until they were analyzed using ICP-MS. Samples for analysis of DOC were stored in the dark at 4°C until analyzed using a Shimadzu total organic carbon analyzer 5050 A (Shimadzu, Tokyo, Japan).

Additional sediment samples were collected (*n* = 2 per site in 2004) for radionuclide chemistry using a standard (15-cm x 15-cm x 15-cm) Ekman grab sampler (Wildlife Supply Company, Buffalo, NY, USA). Sediment samples were placed in a 20-

L pail (one at a time), homogenized, and overlying water (collected with the sample) carefully poured off. The sediment was then transferred to a 1-L polyethylene container and stored in the dark at 4°C until they were analyzed for lead-210, radium-226, and uranium (performed by Saskatchewan Research Council, Saskatoon, SK, Canada). Based on these results, concentrations and/or activities were time corrected and polonium-210 values were extrapolated from lead-210 values (calculation performed by Dr. Patricia Thomas, Saskatoon, SK, Canada).

2.3.3 Benthic community assessment

Samples were collected (n = 3 in 2003 and n = 4 in 2004) with a standard Ekman grab sampler and sieved through a sieve bucket (500-µm in 2003 and 425-µm in 2004) (Wildlife Supply Company, Buffalo, NY, USA). Each sieved sample was immediately preserved with 10% neutralized buffered formalin in a separate 1-L polyethylene container.

Samples collected in 2003 were processed in-house following the Canadian metal mining Environmental Effects Monitoring (EEM) guidance document for benthic community samples (Environment Canada, 2002). First samples were re-sieved in the lab (500-µm in 2003 and 425-µm in 2004) and Rose Bengal dye added (0.25g/L sample) to aid in sorting the benthic invertebrates from the sediment. If a sample contained a large quantity of inorganic matter (rocks and sand) the sample was separated into organic and inorganic fractions by “floating” the sample (achieved by pouring off the less dense organic matter from the inorganic matter). The inorganic matter was then carefully checked for organisms, including clams and snails. If no organisms were found, the inorganic layer was archived and later stored. However, if organisms were found within the inorganic fraction, it was sorted until all organisms were collected. Samples of large volume were separated into coarse (>1mm) and fine fractions (<1mm). All samples were sorted in their entirety (no sub-sampling).

Quality control checks were preformed on at least 10% of the samples. A pass was granted when the number of organisms found was $\leq$10% of the total organisms found within that sample. Samples that failed quality control checks were resorted until the number of organisms found was $\leq$10% of the total number of organisms found in that sample. Sorted organisms were stored in a 70% ethanol and 5% glycerine water mixture, and sent out to an outside taxonomist (Dr. Jan Ciborowski, University of Windsor, ON, Canada) for identification to the lowest practical level. In 2004, samples were sent to the same outside taxonomist for both sorting and identification.

2.3.4 Whole-sediment bioassay

2.3.4.1 Test organisms

Hyalella azteca were obtained from an in-house culture maintained in an environmental chamber with a set photoperiod of 16:8 hour light:dark and a temperature of 23 ± 1°C. Municipal carbon-filtered water with a hardness of ~130 mg/L as $CaCO_3$, an alkalinity of ~80 mg/L as $CaCO_3$, and a pH of ~ 8 was used for culturing. Animals were fed *ad-libidum* with a Tetramin® (Tetra Werke, Melle, Germany) fish food slurry, *Scenedesmus sp.*, and occasionally laboratory-cultured biofilm (unknown composition). Silica sand (particle size = 425 to 850 µm) and cheesecloth gauze were used as substrates. To ensure test species were a known age, adult *H. azteca* were isolated to breed and juveniles collected after 7 days. In 2003, juveniles were aged for seven days (7-14 day old test animals (as recommended in U.S. EPA., 2000)) prior to testing, where as in 2004, juveniles were aged for only two days (2-9 day old test animals (as recommended in Environment Canada, 1997)) prior to testing.

2.3.4.2 Test system

Sediment cores for use in bioassays were collected (n = 3 per site in 2003 and n = 4 per site in 2004) from the same locations as the sediment chemistry cores and benthic community samples, using the same collection methods as for the sediment chemistry cores. Bioassay sediment cores were stored in the dark at 4°C until test set-up (two to three weeks later). Approximately 12 hours before test initiation, sediment cores (~75 mL) were carefully transferred to 300-mL tall-form glass beakers, while maintaining the sediment profile as well as possible, and the beakers placed in a modified sediment testing intermittent renewal (STIR) system (modified from Benoit et al., 1993). The modified STIR system went through 4 automated water changes per day (once every 6 hours) in 2003 and 2 water changes per day (once every twelve hours) in 2004. The overlying water was changed 3 times before test initiation in 2003 and once in 2004. Overlying water (municipal carbon-filtered, described earlier) was aerated and warmed to 23 ± 1°C before use. Each water change renewed approximately one third of the total overlying water (~125 mL) within each beaker. Overlying water within the beakers was aerated from day 4 to day 10 (due to low levels of dissolved oxygen) in 2003 and throughout the entire test in 2004. Diluted Tetramin® fishfood slurry was added to all beakers once per day (500 µL per beaker in 2003 and 100 µL per beaker in 2004).

Test initiation (day 0) commenced with the addition of ten laboratory-reared *H. azteca* per beaker. Dissolved oxygen and temperature were measured daily in all test beakers. Sediment chemistry cores (described above) were assumed to be representative of day 0 pore-water and whole-sediment conditions of the bioassay cores. Hardness, alkalinity, conductivity, ammonia and pH were evaluated at the start (day 0) and finish (day 10) of each bioassay. Overlying water samples were collected in 2004 on day 0 for total trace metals analysis. In 2004, mini-peepers (Doig and Liber, 2000) were inserted into the test sediment at the time the beakers were transferred to the modified STIR system. Mini-peepers were constructed of acrylic with two sample compartments covered with 0.2-µm Supor® membranes (Gelman Sciences, Ann Arbor, MI, USA). Before peepers were added to test vessels, they were stored in a 2-L beaker with ultra-pure (Barnstead NANOpure®) water that was bubbled with nitrogen gas for 24 h. Mini-peepers were placed vertically within the sediment (one mini-peeper per beaker) to ensure that both overlying-surface water samples and pore-water samples could be collected from each peeper. Mini-peeper samples were collected on test take down (day 10) and rinsed with ultra-pure water before they were sampled. Samples from the mini-peeper chambers were collected by piercing the membrane with a 1-mL Eppendorf pipette and carefully withdrawing samples. Samples were acidified immediately after collection and stored in the dark at 4°C until they were analyzed for trace metals using ICP-MS.

Amphipods were removed and enumerated with the use of a light table. Amphipods recovered from the test chambers were rinsed with water and transferred to Petri dishes where excess water was carefully removed. Test organisms were then dried at 60ºC for $\geq$ 24 h, and weighed to obtain their final dry weight.

2.3.5 Statistics and data analysis

All statistics were performed using SigmaStat®, version 3.1 (SPSS Inc., Chicago, IL, USA). If normality or homogeneity of variance failed, all data were transformed using a $\log_{10}$ or, in the case of ratio or percent data, an arcsine square-root transformation (pH data were not transformed). Densities of benthic invertebrate taxa utilized the $\log_{10}(x + 1)$ transformation (due to the presence of zeros in the raw data). For consistency, transformed data sets that still failed tests for normality or homogeneity of variance still underwent parametric analysis. Physicochemical data describing general surface water, pore-water, and sediment characteristics, as well as benthic invertebrate data and survival and final dry weight of *H. azteca*, were analyzed using one-way ANOVA (with lake as the independent factor). If a statistical difference was detected ($p \leq 0.05$), a Dunnett's post-hoc test was run (with the respective reference site as the control). Sites that were sampled both years were compared (with year as the independent factor) using *t*-tests. Comparisons between day 0 and day 10 surface water chemistry measurements and mini-peeper surface water and pore-water measurements (from the *H. azteca* sediment bioassays) were analyzed using two-way ANOVA (with lake and sample day, or lake and water type as the independent factors, respectfully). If a statistical difference was detected ($p \leq 0.05$), a Tukey's all pair-wise multiple comparison post-hoc test was run.

Benthic community structure endpoints included total abundance (organisms/m^2), taxon richness, Simpson's Diversity Index, Bray-Curtis Index, Evenness and the density (organisms/m^2) and proportion of selected taxa. Taxon Richness, Simpson's Diversity Index, Bray-Curtis Index and Evenness were calculated at family or above using the formulas stated in the Metal Mining Guidance document for Aquatic Environmental Effects Monitoring (Environment Canada, 2002).

For ease of interpretation of surface water and pore-water data, only the variables meeting the following criteria were presented/assessed: the mean value exceeded the Canadian water quality guideline (CWQG) for protection of aquatic life (Canadian Council of Ministers of the Environment, 1999); the mean value exceeded published *H. azteca* toxicity values (Borgmann et al., 2005; France and Stokes,1987; Ankley et al., 1995); analytical detection limits exceeded CWQG guidelines or reported toxicity values; or elements that did not have published water quality guidelines and/or toxicity data (Table 2.1). Borgmann et al. (2005) presents 1 week *H. azteca* median lethal concentrations (LC50s) for 63 metals in both soft water (total hardness = 18 mg/L) and hard tap water (total hardness = 124 mg/L). The reference toxicity data for pH, a 96-h LC50 for *H. azteca*, was established using test animals that were cultured at a pH of 6.4 (control animals) and a relatively low hardness (Ca^{2+} = 4.2 mg/L and Mg^{2+} = 1.5 mg/L) (France and Stokes, 1987). Finally, the reference toxicity data used for ammonia, 96-h LC50s for *H. azteca*, were established at a pH of 6.5 for both soft water (total hardness = 42 mg/L) and hard water (total hardness = 240 mg/L) (Ankley et al., 1995).

Other studies investigating the toxicity or bioavailability of metal-contaminated sediments have found that surface water or pore-water concentrations of metals are more predictive of benthic macroinvertebrate community structure than are whole-sediment (total) metals concentrations (Kemble et al., 1994; Chapman et al., 1998; Beltman et al., 1999; Soucek et al., 2000; Schmidt et al., 2002). Therefore, because whole-sediment metal concentrations are generally poor indicators of sediment toxicity, sediment quality guidelines were not used to determine which variables (i.e., trace elements) were of toxicological significance.

2.4 Results

2.4.1 Key Lake

2.4.1.1 Field chemistry

The physicochemical characteristics of surface water, pore-water, and sediment for the Key Lake study sites in 2003 and 2004 are presented in Table 2.2 and Table 2.3, respectively. In 2003 and 2004, all of the exposure lakes sampled had significantly higher surface water total ammonia, hardness and conductivity, and significantly lower pH, than David Lake. In 2003, surface water pH decreased with increasing distance from the effluent source. Pore-waters from exposure lakes (in 2003 and 2004) had significantly higher total ammonia and hardness than the reference lake. In 2004, Wolf Lake and Fox Lake pore-waters had significantly higher pH than David Lake. Overall, David Lake, Fox Lake and Delta Lake sediment compositions in 2003 were similar

Table 2.1. Canadian Water Quality Guidelines (CWQG) and water-only *Hyalella azteca* toxicity data for variables that met one or more of the chosen criteria (see text for details) at the Key Lake or Rabbit Lake study sites. Data are in µg/L, except for pH and total ammonia (mg N/L).

Variable	CWQG[a]	SW LC50[b]	HW LC50[c]
pH	6.5 - 9.0	4.7[d]	n/a[e]
Total Ammonia	3.86 - 55.5[f]	22.8[g]	>204[g]
Li	n/a[e]	650[h]	3130[h]
Mg	n/a[e]	n/a[e]	n/a[e]
Al	5 - 100	89[hi]	>3150[hl]
Si	n/a[e]	n/a[e]	n/a[e]
P	n/a[e]	n/a[e]	n/a[e]
Ca	n/a[e]	n/a[e]	n/a[e]
Cr	8.9[j], 1.0[k]	>1000[hi], 3.1[hl]	>3150[hi], 137[hl]
Mn	n/a[e]	>1000[hi,] 92[hl]	2729[h], 169[hl]
Fe	300	>1000[hi]	>3150[hi]
Ni	25 - 150	75[h]	133[h]
Cu	2 - 4	36[h]	90[h]
As	5	494[h], 581[hl]	426[h], 483[hl]
Se	1	41[h], 43[hk]	118[hi], 371[hl]
Mo	73	>1000[hi], >1000[hil]	>3150[hi], >1000[hil]
Ag	0.1	0.25[h]	1.05[h]
Cd	0.017	0.15[h]	1.6[h]
Tm	n/a[e]	0.01[h]	739[h]
Ta	n/a[e]	2[h]	1977[h]
Hg	0.026	8.4[hi]	2.1[h]
U	n/a[e]	21[h]	1651[h]

[a] CWQG = Canadian Water Quality Guidelines for the Protection of Aquatic Life (Canadian Council of Ministers of the Environment, 1999.).

[b] SW LC50 = soft water median lethal concentration.

[c] HW LC50 = hard water median lethal concentration.

[d] France and Stokes (1987).

[e] n/a = not available.

[f] Based on a pH range of 6 – 7 and a temperature range of 10 – 15ºC.

[g] Ankley et al., Schubauer-Berigan, and Monson (1995).

[h] Borgmann, Couillard, Doyle, and Dixon (2005).

[i] Nominal concentration.

[j] Chromium II.

[k] Chromium IV.

[l] Anion salt.

(Table 2.2). Sediment composition in 2004 was different among all lakes, although Fox Lake and David Lake were generally much more similar than Wolf Lake and David Lake (Table 2.3). Wolf Lake had the highest proportions of silt and clay in both 2003 and 2004.

In 2003 and 2004, whole-sediment, pore-water and surface water from the exposure lakes generally had higher concentrations of trace metals than David Lake and followed a trend of decreasing concentrations with increasing distance from Wolf Lake (Tables 2.4 and 2.5). With respect to sediment concentrations in 2003, exposure sites had significantly higher lithium (Wolf Lake), nickel (all sites but Delta), copper (Wolf and Unknown Lakes), arsenic (all sites but Delta), selenium (Wolf and Unknown Lakes), molybdenum, thulium (all sites but Delta), lead (Wolf and Unknown Lakes), and uranium (all sites but Delta) than David Lake. In 2003, pore-water from exposure sites had significantly higher lithium, phosphorous (all sites but Delta), nickel, arsenic, molybdenum, and uranium (Wolf and Fox Lakes) concentrations than David Lake. Surface water contained significantly higher concentrations of lithium, phosphorous, manganese, nickel, copper (Wolf and Fox Lakes), arsenic (Wolf and Fox Lakes), selenium (Wolf Lake), molybdenum, and uranium.

In 2004, sediment from exposure sites had significantly higher magnesium, aluminum, calcium, arsenic, selenium, and molybdenum than David Lake. In addition, Fox Lake sediment had significantly higher levels of chromium, manganese, iron, copper, and silver than David Lake. Pore-water contained significantly higher concentrations of magnesium, silicon, phosphorous, calcium, arsenic, and molybdenum in exposure lakes than in David Lake. Fox Lake pore-water also had significantly higher manganese and iron concentrations than David Lake. Surface water from exposure lakes had significantly higher concentrations of magnesium, phosphorous, arsenic, and molybdenum. Fox Lake surface water also had significantly higher concentrations of silicon and chromium than David Lake.

Table 2.6 presents selected radionuclide data for David, Wolf, and Fox Lakes in 2004. Uranium activity was higher at the exposure sites than the at reference site. No noteworthy trends were observed with respect to radium-226, lead-210, and polonium-210.

Table 2.2. Mean (± standard deviation, *n* = 3) physicochemical surface water, pore-water, and sediment characteristics for the 2003 study sites at the Key Lake uranium operation.

	Characteristic	David Lake	Wolf Lake	Fox Lake	Unknown Lake	Delta Lake
Surface-water	Depth (cm)	110[a]	100[a]	100[a]	85[a]	75[a]
	Temperature (°C)	16.5[a]	15.1[a]	14.9[a]	15.7[a]	15.8[a]
	DO[b] (mg/L)	9.8[a]	9.1[a]	8.6[a]	9.3[a]	9.9[a]
	pH	6.79 ± 0.02	6.27 ± 0.00[c*]	6.27 ± 0.07[c*]	5.42 ± 0.04[c*]	4.82 ± 0.04[c*]
	Total Ammonia (mg N/L)	<0.1	>20[cd*]	9.6 ± 0.1[c*]	3.8 ± 0.1[*]	1.4 ± 0.0[*]
	Alkalinity (mg/L as $CaCO_3$)	9 ± 3	7 ± 1	7 ± 1	5 ± 1[*]	4 ± 1[*]
	Total Hardness (mg/L as $CaCO_3$)	4 ± 0	860 ± 50[*]	910 ± 20[*]	400 ± 20[*]	290 ± 20[*]
	Conductivity (µS/cm)	15 ± 0	1922 ± 11[*]	1856 ± 10[*]	892 ± 14[*]	702 ± 8[*]
Pore Water	pH	5.87 ± 0.22[c]	6.81 ± 0.17[*]	6.09 ± 0.35[c]	6.78 ± 0.10[*]	4.76 ± 0.64[c*]
	Total Ammonia (mg N/L)	1.20 ± 0.2[e]	19.26 ± 0.56[c*]	16.15 ± 1.49[c*]	>10[cd*]	5.4[ac*]
	Alkalinity (mg/L as $CaCO_3$)	32 ± 20	72 ± 28	37 ± 26	51 ± 10	n/a[f]
	Total Hardness (mg/L as $CaCO_3$)	14 ± 2	1340 ± 100[*]	940 ± 60[*]	480 ± 40[*]	360 ± 80[e*]
Sediment	Sand (%)	93 ± 4	43 ± 17[*]	92 ± 3	68 ± 15[*]	94 ± 4
	Silt (%)	4 ± 3	27 ± 16[*]	7 ± 3	25 ± 8[*]	6 ± 3
	Clay (%)	3 ± 3	30 ± 16[*]	1 ± 0	7 ± 7	1 ± 0
	TOC[g] (%)	7.6 ± 4.6	8.2 ± 1.6	2.5 ± 0.1	16.4 ± 5.38[*]	2.1 ± 0.8
	Water Content (%)	76.7 ± 14.3	86.1 ± 2.0	66.5 ± 0.7	77.2 ± 13.0	40.9 ± 10.60[*]

[a] $n = 1$.

[b] DO = Dissolved oxygen.

[c] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999).

[d] Exceeded the standard curve.

[e] $n = 2$.

[f] n/a = not available

[g] TOC = Total organic carbon.

* Significantly different (one-way ANOVA, Dunnett's post hoc test, $p \leq 0.05$) from David Lake measurement.

Table 2.3. Mean (± standard deviation, $n = 4$) physicochemical surface water, pore-water, and sediment characteristics of the 2004 study sites for the Key Lake uranium operation.

Characteristic	David Lake	Wolf Lake	Fox Lake
Surface-water			
Depth (cm)	98 ± 0	108 ± 4[*]	78 ± 2[*]
Temperature (°C)	18.8 ± 0	18.1 ± 0[*]	19.9 ± 0[*]
DO[a] (mg/L)	8.8 ± 0.6	8.0 ± 0.1[*]	8.1 ± 0.3
pH	6.80 ± 0.02	5.95 ± 0.03[b*]	5.62 ± 0.39[b*]
Total Ammonia (mg N/L)	<0.05	5.0 ± 0.6[b*]	4.0 ± 0.3[b*]
Alkalinity (mg/L as $CaCO_3$)	8 ± 1	7 ± 1	7 ± 1
Total Hardness (mg/L as $CaCO_3$)	6 ± 1	610 ± 20[*]	580 ± 10[*]
Conductivity (µS/cm)	14 ± 0	1190 ± 10[*]	1152 ± 16[*]
Pore Water			
pH	5.28 ± 0.16[b]	6.62 ± 0.41[c*]	6.56 ± 0.13[*]
Total Ammonia (mg N/L)	<0.5	7.7 ± 0.4[bc*]	6.8 ± 1.0[b*]
Alkalinity (mg/L as $CaCO_3$)	16 ± 4[c]	29 ± 20[c]	28 ± 16
Total Hardness (mg/L as $CaCO_3$)	<2	830 ± 60[c*]	600 ± 80[*]
DOC[d] (mg/L)	31.6 ± 8.6[e]	22.0 ± 2.3[c]	27.8 ± 3.5[c]
Sediment			
Sand (%)	98 ± 0[c]	28 ± 3[c*]	84 ± 7[c*]
Silt (%)	1 ± 1[c]	57 ± 3[c*]	12 ± 4[c*]
Clay (%)	1 ± 0[c]	14 ± 2[c*]	5 ± 3[c*]
TOC[f] (%)	1.20 ± 0.37	10.19 ± 0.97[c*]	4.88 ± 1.51[*]
Water Content(%)	40.10 ± 3.35	85.30 ± 3.09[c*]	71.13 ± 5.96[*]

[a] DO = Dissolved oxygen.

[b] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999).

[c] $n = 3$.

[d] DOC = Dissolved organic carbon.

[e] $n = 2$.

[f] TOC = Total organic carbon.

* Significantly different (one-way ANOVA, Dunnett's post hoc test, $p \leq 0.05$) from David Lake measurement.

Table 2.4. Mean (± standard deviation, *n* = 3) trace element concentrations in whole-sediment (dry weight)(mg/kg), pore-water(µg/L), and surface water (µg/L) for the 2003 Key Lake study sites.

Variable	David Lake	Wolf Lake	Fox Lake	Unknown Lake	Delta Lake
Whole Sediment					
Li	4.70 ± 5.55	13.96 ± 4.62*	5.06 ± 1.01	5.92 ± 0.50	2.49 ± 1.10
P	341.4 ± 174.4	632.5 ± 28.2	144.7 ± 18.5	722.9 ± 223.4	89.8 ± 36.3*
Cr	3.11 ± 1.38	21.86 ± 3.29*	3.54 ± 0.64	10.66 ± 4.31	4.60 ± 5.05
Mn	57.13 ± 26.01	25.07 ± 5.61	20.87 ± 2.86	70.51 ± 16.75	52.54 ± 52.12
Ni	1.49 ± 0.58	81.23 ± 46.70*	11.94 ± 0.38*	58.12 ± 9.19*	2.24 ± 0.42
Cu	1.29 ± 0.41	8.27 ± 0.79*	2.96 ± 2.25	4.68 ± 1.98*	0.42 ± 0.22
As	2.2 ± 1.3	479.2 ± 101.69*	106.6 ± 16.9*	350.7 ± 162.0*	3.9 ± 3.0
Se	<1.60	51.60 ± 17.21*	14.01 ± 1.00*	25.01 ± 13.18*	<1.60
Mo	1 ± 0	1100 ± 368*	401 ± 100*	1729 ± 599*	328 ± 156*
Cd	<1.66	<1.66	<1.66	<1.66	<1.66
Tm	0.03 ± 0.00	0.07 ± 0.02*	0.07 ± 0.01*	0.08 ± 0.01*	0.05 ± 0.02
Ta	<10.25	<10.25	<10.25	<10.25	<10.25
Hg	<0.31	<0.31	<0.31	<0.31	<0.31
Pb	3.16 ± 0.65	5.66 ± 1.20*	2.96 ± 0.24	5.31 ± 0.84*	1.77 ± 0.26
U	0.67 ± 0.08	87.89 ± 23.56*	19.01 ± 3.01*	30.28 ± 6.64*	0.94 ± 0.40
Pore Water					
Li	1.9 ± 1.6	984.5 ± 61.4^{b*}	723.0 ± 48.8^{b*}	249.5 ± 21.1*	102.2 ± 13.4*
P	15.8 ± 0.3	569.4 ± 19.4*	390.1 ± 39.0*	223.9 ± 63.5*	86.7 ± 17.1
Cr	<8.29^{ab}	<8.29^{ab}	<8.29^{ab}	<8.29^{ab}	<8.29^{ab}
Mn	191.1 ± 88.8^{ab}	85.0 ± 9.2	447.6 ± 61.0^{ab}	134.5 ± 16.6^{a}	393.4 ± 234.4^{ab}

Ni	<0.26	33.17 ± 13.95^{a*}	31.65 ± 14.21^{a*}	6.99 ± 0.93*	9.46 ± 2.08*
Cu	17.70 ± 4.05^{a}	13.57 ± 4.74^{a}	14.07 ± 3.86^{a}	10.74 ± 0.65^{a}	25.43 ± 15.49^{a}
As	0 ± 0	1820 ± 417^{abc*}	1492 ± 474^{abc*}	332 ± 32^{a*}	8 ± 7^{a*}
Se	<11.81^{a}	<11.81^{a}	<11.81^{a}	<11.81^{a}	<11.81^{a}
Mo	<2	3141 ± 915^{abc*}	6142 ± 3739^{abc*}	2422 ± 590^{abc*}	852 ± 730^{a*}
Cd	<2.70^{abc}	<2.70^{abc}	<2.70^{abc}	<2.70^{abc}	<2.70^{abc}
Tm	<0.01	<0.01	0.01 ± 0.01^{b}	0.01 ± 0.01^{b}	0.01 ± 0.00^{b}
Ta	<14.43^{b}	<14.43^{b}	<14.43^{b}	<14.43^{b}	<14.43^{b}
Hg	<0.13^{a}	<0.13^{a}	<0.13^{a}	<0.13^{a}	<0.13^{a}
Pb	1.82 ± 1.87^{a}	0.79 ± 0.52	0.67 ± 0.32	1.19 ± 1.26^{a}	1.48 ± 1.47^{a}
U	1.1 ± 1.2	127.9 ± 130.1^{b*}	30.8 ± 37.8^{b*}	3.6 ± 1.1	1.2 ± 0.7
<u>Surface Water</u>					
Li	<0	1139 ± 24^{b*}	787 ± 18^{b*}	233 ± 7*	132 ± 6*
P	<32.04	613.63 ± 16.36*	462.78 ± 38.56*	166.06 ± 16.80*	80.76 ± 28.49*
Cr	<8.29^{ab}	<8.29^{ab}	<8.29^{ab}	<8.29^{ab}	<8.29^{ab}
Mn	0.61 ± 0.49	77.85 ± 2.24*	71.74 ± 1.41*	50.84 ± 0.93*	82.65 ± 3.07*
Ni	0.57 ± 0.76	22.65 ± 0.83*	22.31 ± 0.68*	11.28 ± 0.27*	9.17 ± 0.39*
Cu	2.03 ± 0.62^{a}	5.79 ± 1.09^{a*}	3.95 ± 0.18^{a*}	4.00 ± 1.10^{a}	3.83 ± 0.77^{a}
As	<3.21	4.60 ± 0.65*	3.97 ± 0.22*	<3.21	<3.21
Se	<11.81^{a}	14.10 ± 0.68*	<11.81^{a}	<11.81^{a}	<11.81^{a}
Mo	<1.9	208.3 ± 3.0^{a*}	320.2 ± 1.4^{a*}	126.8 ± 1.4^{a*}	34.6 ± 0.8*
Cd	<2.70^{abc}	<2.70^{abc}	<2.70^{abc}	<2.70^{abc}	<2.70^{abc}
Tm	<0.01	<0.01	<0.01	<0.01	<0.01

Ta	<14.43[b]	<14.43[b]	<14.43[b]	<14.43[b]	<14.43[b]
Hg	<0.13[a]	<0.13[a]	<0.13[a]	<0.13[a]	<0.13[a]
Pb	0.05 ± 0.03	0.08 ± 0.04	0.05 ± 0.01	0.09 ± 0.01	0.06 ± 0.01
U	<0.02	0.89 ± 0.06*	0.65 ± 0.07*	0.28 ± 0.03*	0.15 ± 0.01*

[a] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999).

[b] Exceeds soft water median lethal concentration for *Hyalella azteca* (Borgmann, et al., 2005).

[c] Exceeds hard water median lethal concentration for *Hyalella azteca* (Borgmann, et al., 2005).

* Significantly different (one-way ANOVA, Dunnett's post hoc test, $p \leq 0.05$) from David Lake measurement.

Table 2.5. Mean (± standard deviation, *n* = 3) trace element concentrations in whole-sediment (dry weight)(mg/kg), pore-water(µg/L), and surface water (µg/L) for the 2004 Key Lake study sites.

Variable	David Lake	Wolf Lake	Fox Lake
Whole Sediment			
Mg	63.5 ± 10.3	776.3 ± 84.4[*]	380.7 ± 91.7[*]
Al	1501 ± 324	11355 ± 605[*]	7410 ± 788[*]
Si	n/a[a]	n/a[a]	n/a[a]
P	89.3 ± 22.4	367.3 ± 125.1[*]	219.8 ± 73.0
Ca	318 ± 105	6897 ± 1797[*]	5134 ± 1414[*]
Cr	<0.50	12.10 ± 8.50	14.77 ± 4.72[*]
Mn	12.27 ± 2.69	20.89 ± 4.57	26.60 ± 6.38[*]
Fe	1857 ± 307	6558 ± 3267	12479 ± 3840[*]
Cu	0.59 ± 0.15	3.84 ± 2.12	5.21 ± 1.66[*]
As	1.3 ± 0.5	435.4 ± 347.3[*]	602.2 ± 198.0[*]
Se	<4.08	27.15 ± 20.43[*]	31.08 ± 11.19[*]
Mo	<0.06	929.6 ± 548.2[*]	911.9 ± 499.2[*]
Ag	0.09 ± 0.03	0.18 ± 0.04	0.38 ± 0.13[*]
Cd	0.08 ± 0.06	<0.02	<0.02
Hg	<0.22	<0.22	<0.22
Pore Water			
Mg	236 ± 104	21690 ± 1898[*]	18665 ± 420[*]
Al	154.4 ± 104.4[bc]	79.5 ± 132.2[b]	133.6 ± 123.4[bc]
Si	4703 ± 154	17677 ± 4518[*]	7659 ± 565[*]
P	131.0 ± 98.2	384.1 ± 45.1[*]	287.0 ± 14.9[*]

Ca	1069 ± 287	290880 ± 16942*	219292 ± 7588*
Cr	4.86 ± 1.23	14.00 ± 13.48^{bc}	13.55 ± 3.93^{bc}
Mn	82.1 ± 39.3	55.8 ± 10.2	252.5 ± 84.3^{cd*}
Fe	1562 ± 711^{bc}	2827 ± 1897^{bc}	27175 ± 10588^{bcd*}
Cu	1.47 ± 0.41	1.09 ± 0.59	1.90 ± 1.27
As	3 ± 1	4095 ± 2859^{bcd*}	3041 ± 1549^{bcd*}
Se	5.64 ± 7.25^{b}	<2.91^{b}	5.86 ± 7.63^{b}
Mo	6 ± 7	5919 ± 3280^{bcd*}	3536 ± 140^{bcd*}
Ag	4.79 ± 4.26^{bcd}	1.18 ± 0.64^{bcd}	<0.04
Cd	0.12 ± 0.15^{b}	<0.06^{b}	<0.06^{b}
Hg	<0.16^{b}	<0.16^{b}	<0.16^{b}
<u>Surface Water</u>			
Mg	464 ± 16	17973 ± 127*	14518 ± 889*
Al	63.82 ± 16.91^{b}	59.01 ± 33.96^{b}	67.41 ± 38.16^{b}
Si	1438 ± 403	1726 ± 202	509 ± 324*
P	55.3 ± 19.6	314.4 ± 26.5*	316.3 ± 11.1*
Ca	1191 ± 65	190788 ± 4414*	162801 ± 8669*
Cr	<1.11^{b}	2.26 ± 1.39^{b}	4.49 ± 4.50^{bc*}
Mn	16.2 ± 0.5	112.6 ± 2.1*	78.6 ± 8.2*
Fe	550 ± 53^{b}	324 ± 50^{b}	1410 ± 1370^{bc}
Cu	0.51 ± 0.17	1.87 ± 0.45	2.19 ± 1.32^{b}
As	<0.30	12.66 ± 1.20^{b*}	20.26 ± 11.37^{b*}
Se	<2.91^{b}	<2.91^{b}	4.05 ± 3.17^{b}

Mo	1.0 ± 1.0	327.5 ± 1.7[b*]	379.8 ± 5.9[b*]
Ag	0.04 ± 0.03	0.13 ± 0.08[b]	<0.04
Cd	<0.06[b]	0.28 ± 0.44[bc]	<0.06[b]
Hg	<0.16[b]	<0.16[b]	<0.16[b]

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[a] n/a = not available, not measured.

[b] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999).

[c] Exceeds soft water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005)

[d] Exceeds hard water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005)

* Significantly different (one-way ANOVA, Dunnett's post hoc test, $p \leq 0.05$) from David Lake measurement

Table 2.6. Mean (± standard deviation, n = 2) radionuclide activities in whole-sediment (dry weight) from the 2004 Key Lake study sites.

Variable (Bq/kg)	David Lake	Wolf Lake	Fox Lake
U	20.2 ± 7.1	806.4 ± 142.6	1373.4 ± 1479.0
Ra-226	<20	27.5 ± 31.8	<20
Pb-210	85.3 ± 60.3	69.3 ± 37.7	192.0 ± 90.5
Po-210[a]	83.3 ± 58.9	67.7 ± 36.8	187.6 ± 88.4

[a] Extrapolated from lead-210 values (calculation performed by Dr. Patricia Thomas, Saskatoon, SK, Canada).

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2.4.1.2 Benthic community structure

Total abundance, taxon richness, Simpson's diversity index, Bray Curtis index, and evenness for the 2003 and 2004 Key Lake study sites are presented in Table 2.7. Only Unknown Lake (in 2003) had significantly higher total abundance than David Lake. Significantly lower taxon richness was noted in Wolf (2003 and 2004), Fox (2004) and Delta (2003) Lakes. Simpson's diversity index was significantly lower in Wolf (2003 and 2004) and Unknown (2003) Lakes. All exposure sites' Bray Curtis index values (2003 and 2004) for all exposure lakes were significantly different from the respective reference site value. No significant differences were noted with respect to evenness. Temporal comparisons (2003 vs. 2004) of Wolf, Fox, and David Lakes revealed no significant differences.

Density (organisms/m^2) and percent composition of 14 of the most common taxa (plus one pooled group of "other taxa") found at the Key Lake study sites are presented in Tables 2.8 and 2.9, respectively. Generally, significant differences between exposure sites and the reference site (within the year sampled) decreased with increasing distance from the effluent source. Few significant differences were noted between sample years for Wolf Lake and David Lake. Fox Lake had more temporal variation. Comparisons (to the reference site) within each taxon revealed many significant differences with respect to density and percent composition. All exposure sites (within the sample year indicated) had significantly lower densities of Nematoda (2004), Sphaeriidae (2003 and 2004), Hyalellidae (2003), Baetidae (2004), and Corixidae (2004) compared to David Lake. In 2004, Wolf Lake and Fox Lake had significantly higher densities of Ostracods. With respect to percent composition (Table 2.9), all exposure sites (within the sample year indicated) had significantly lower percentages of Nematoda (2004), Sphaeriidae (2003), Hyalellidae (2003), Copepoda (2003), Cladocera (2004), Baetidae (2004), and Corixidae (2004).

2.4.1.3 Whole-sediment bioassay

General water quality data for the 2003 and 2004 sediment bioassays are presented in Table 2.10 and 2.11, respectively. In 2003, average DO levels were significantly lower from day 0 to day 4 when compared to average DO levels from day 5

Table 2.7. Selected benthic community metrics for the 2003 and 2004 Key Lake study sites.

Metric	David Lake		Wolf Lake		Fox Lake	
	2003	2004	2003	2004	2003	2004
Total Abundance (organisms/m^2)	707 ± 243	597 ± 146	424 ± 328	2452 ± 1661	1820 ± 382	4245 ± 3054
Taxon Richness[b]	13 ± 1	12 ± 2	4 ± 2[*]	4 ± 2[*]	10 ± 2	8 ± 2[*]
Simpson's Diversity Index[b]	0.70 ± 0.04	0.61 ± 0.19	0.50 ± 0.07[*]	0.30 ± 0.13[*]	0.61 ± 0.03	0.52 ± 0.07
Bray-Curtis Index[b]	0.27 ± 0.19	0.22 ± 0.11	0.83 ± 0.06[*]	0.78 ± 0.07[*]	0.74 ± 0.05[*]	0.77 ± 0.18[*]
Evenness[b]	0.26 ± 0.04	0.25 ± 0.07	0.55 ± 0.28	0.46 ± 0.33	0.25 ± 0.02	0.30 ± 0.14

Metric	Unknown Lake	Delta Lake
	2003[a]	2003[a]
Total Abundance (organisms/m^2)	4767 ± 1820[*]	889 ± 439
Taxon Richness[b]	9 ± 2	8 ± 2[*]
Simpson's Diversity Index[b]	0.42 ± 0.04[*]	0.63 ± 0.09
Bray-Curtis Index[b]	0.86 ± 0.06[*]	0.57 ± 0.05[*]
Evenness[b]	0.19 ± 0.03	0.37 ± 0.04

[a] Data only available for 2003.

[b] Family or higher.

[*] Significantly different (one-way ANOVA, Dunnett's post hoc test, $p \leq 0.05$) from David Lake measurement.

Table 2.8. Densities (organisms/m2) of selected taxa from the 2003 and 2004 Key Lake study sites.

	David Lake		Wolf Lake		Fox Lake	
Taxon	2003	2004	2003	2004	2003	2004
Nematoda	47 ± 35	20 ± 20	0 ± 0*	0 ± 0*	2 ± 4*	3 ± 4
Sphaeriidae	76 ± 30	30 ± 25	0 ± 0*	2 ± 3*	0 ± 0*	0 ± 0*
Oligochaeta	24 ± 31	8 ± 6	0 ± 0	2 ± 3	467 ± 223	20 ± 27*†
Hyalellidae	4 ± 4	17 ± 22	0 ± 0*	0 ± 0	0 ± 0*	0 ± 0
Copepoda	36 ± 4	23 ± 16	7 ± 7*	30 ± 43	7 ± 7*	100 ± 63†
Cladocera	156 ± 132	45 ± 18	36 ± 50	0 ± 0*	251 ± 129	27 ± 26†
Ostracoda	7 ± 7	30 ± 23	284 ± 200*	327 ± 269*	22 ± 8	2042 ± 1678*†
Baetidae	0 ± 0	23 ± 12†	18 ± 20	0 ± 0*	0 ± 0	2 ± 3*
Corixidae	0 ± 0	23 ± 14†	0 ± 0	3 ± 4*	0 ± 0	5 ± 6*
Chironomini	227 ± 174	110 ± 59	0 ± 0*	2 ± 3*	700 ± 218*	1145 ± 1043*
Tanytarsini	22 ± 23	157 ± 221	0 ± 0	1932 ± 1310†	2 ± 4	487 ± 383†
Orthocladinae	7 ± 12	3 ± 4	2 ± 4	5 ± 6	58 ± 34	22 ± 10*
Tanypodinae	67 ± 27	95 ± 39	76 ± 53	57 ± 21	247 ± 81	370 ± 209*
Chironomidae pupa	0 ± 0	3 ± 7	0 ± 0	88 ± 61	0 ± 0	13 ± 19
Other taxa	36 ± 21	8 ± 13	2 ± 4*	5 ± 10	64 ± 23	10 ± 9†

Taxon	Unknown Lake 2003[a]			Delta Lake 2003[a]		
Nematoda	7	±	7	40	±	27
Sphaeriidae	0	±	0[*]	0	±	0[*]
Oligochaeta	122	±	69[*]	362	±	310[*]
Hyalellidae	0	±	0[*]	0	±	0[*]
Copepoda	31	±	14	0	±	0[*]
Cladocera	53	±	18	49	±	57
Ostracoda	3413	±	1164[*]	7	±	7
Baetidae	2	±	4	0	±	0
Corixidae	0	±	0	0	±	0
Chironomini	884	±	465[*]	171	±	37
Tanytarsini	0	±	0	40	±	64
Orthocladinae	42	±	47	84	±	94
Tanypodinae	187	±	110	93	±	61
Chironomidae pupa	0	±	0	0	±	0
Other taxa	24	±	20	42	±	30

[a] Data only available for 2003.

[*] Significantly different (one-way ANOVA, Dunnett's post hoc test, $p \leq 0.05$) from David Lake measurement.

[†] Significantly different (*t*-test, $p \leq 0.05$) from the respective 2003 measurement.

Table 2.9. Proportion (%) of selected taxa from the 2003 and 2004 Key Lake study sites.

	David Lake		Wolf Lake		Fox Lake		Unknown Lake	Delta Lake
Taxon	2003	2004	2003	2004	2003	2004	2003[a]	2003[a]
Nematoda	7 ± 6	4 ± 3	0 ± 0[*]	0 ± 0[*]	0 ± 0[*]	0 ± 0[*]	0 ± 0[*]	4 ± 1
Sphaeriidae	11 ± 2	6 ± 6	0 ± 0[*]	0 ± 0	0 ± 0[*]	0 ± 0	0 ± 0[*]	0 ± 0[*]
Oligochaeta	3 ± 3	2 ± 1	0 ± 0	0 ± 0	25 ± 11[*]	1 ± 1[†]	3 ± 1	36 ± 15[*]
Hyalellidae	1 ± 1	3 ± 3	0 ± 0[*]	0 ± 0	0 ± 0[*]	0 ± 0	0 ± 0[*]	0 ± 0[*]
Copepoda	5 ± 2	4 ± 2	1 ± 1[*]	1 ± 1	0 ± 0[*]	3 ± 2	1 ± 0[*]	0 ± 0[*]
Cladocera	25 ± 25	8 ± 4	5 ± 6	0 ± 0[*]	14 ± 9	2 ± 3[*†]	1 ± 1	9 ± 13
Ostracoda	1 ± 1	5 ± 5	70 ± 7[*]	19 ± 13[†]	1 ± 1	46 ± 16[*†]	73 ± 4[*]	1 ± 1
Baetidae	0 ± 0	4 ± 3[†]	3 ± 3[*]	0 ± 0[*]	0 ± 0	0 ± 0[*]	0 ± 0	0 ± 0
Corixidae	0 ± 0	4 ± 2[†]	0 ± 0	0 ± 0[*]	0 ± 0	0 ± 0[*]	0 ± 0	0 ± 0
Chironomini	30 ± 17	20 ± 12	0 ± 0[*]	0 ± 0[*]	38 ± 4	26 ± 9	18 ± 4	22 ± 9
Tanytarsini	3 ± 2	22 ± 27	0 ± 0	69 ± 21[*†]	0 ± 0	11 ± 6[†]	0 ± 0	3 ± 5
Orthocladinae	1 ± 1	0 ± 1	2 ± 3	0 ± 0	3 ± 2	1 ± 1[†]	1 ± 1	10 ± 11
Tanypodinae	9 ± 2	16 ± 6	18 ± 2[*]	8 ± 11	14 ± 3	10 ± 3	4 ± 1	10 ± 6
Chironomidae pupa	0 ± 0	0 ± 1	0 ± 0	3 ± 2[*]	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Other taxa	5 ± 1	1 ± 2[†]	0 ± 1[*]	0 ± 0	4 ± 2	0 ± 0[†]	0 ± 0[*]	5 ± 3

[a] Data only available for 2003.

[*] Significantly different (one-way ANOVA, Dunnett's post hoc test, $p \leq 0.05$) from David Lake measurement.

[†] Significantly different (*t*-test, $p \leq 0.05$) from the respective 2003 measurement.

Table 2.10. Mean (± standard deviation) day 0 (d-0) and day 10 (d-10) water quality measurements for the 2003 Key Lake *Hyalella azteca* sediment bioassay.

	David Lake		Wolf Lake		Fox Lake	
Variable	d-0	d-10	d-0	d-10	d-0	d-10
Temperature (°C)	21.3 ± 0.2	21.5 ± 0.4	21.7 ± 0.3	21.9 ± 0.3	21.6 ± 0.4	21.7 ± 0.3
DO[a] (mg/L)	5.5 ± 0.3	7.4 ± 0.4[‡]	5.1 ± 0.4	7.3 ± 0.4[‡]	5.0 ± 0.7	7.0 ± 0.3[‡]
pH	7.91[b]	7.60[b]	7.89[b]	7.96[b]	7.89[b]	7.90[b]
Total Ammonia (mg N/L)	<0.1[b]	0.2[b]	2.3[b]	0.7[b]	0.5[b]	0.5[b]
Alkalinity (mg/L as $CaCO_3$)	61[b]	75[b]	70[b]	73[b]	62[b]	81[b]
Total Hardness (mg/L as $CaCO_3$)	117[b]	122[b]	231[b]	133[b]	165[b]	131[b]
Conductivity (µS/cm)	330[b]	357[b]	568[b]	375[b]	454[b]	373[b]

	Unknown Lake		Delta Lake	
Variable	d-0	d-10	d-0	d-10
Temperature (°C)	21.9 ± 0.4	21.7 ± 0.3	21.4 ± 0.3	21.4 ± 0.4
DO[a] (mg/L)	3.9 ± 0.5[*]	6.9 ± 0.3[‡]	5.7 ± 0.6	7.0 ± 0.3[‡]
pH	7.73[b]	7.69[b]	7.81[b]	7.67[b]
Total Ammonia (mg N/L)	0.9[b]	<0.1[b]	<0.1[b]	0.4[b]
Alkalinity (mg/L as $CaCO_3$)	61[b]	72[b]	59[b]	74[b]
Total Hardness (mg/L as $CaCO_3$)	143[b]	128[b]	126[b]	125[b]
Conductivity (µS/cm)	393[b]	364[b]	359[b]	364[b]

[a] DO = dissolved oxygen.
[b] $n = 1$
[*] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from David Lake measurement.
[‡] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from the respective d-0 measurement.

Table 2.11. Mean (± standard deviation) Day 0 (d-0) and day 10 (d-10) water quality measurements for the 2004 Key Lake *Hyalella azteca* sediment bioassay.

	David Lake		Wolf Lake		Fox Lake	
Variable	d-0	d-10	d-0	d-10	d-0	d-10
Temperature (°C)	23.0 ± 0.5	22.4 ± 0.3	22.9 ± 0.4	22.8 ± 0.3	22.7 ± 0.1	22.7 ± 0.4
DO[a] (mg/L)	7.4 ± 0.1	7.6 ± 0.2[‡]	7.2 ± 0.2	7.3 ± 0.1[*]	7.3 ± 0.1	7.5 ± 0.1[‡]
pH	7.57 ± 0.07	7.99 ± 0.01[‡]	7.78 ± 0.11[*]	7.97 ± 0.01[‡]	7.42 ± 0.02[*]	7.93 ± 0.02[‡]
Total Ammonia (mg N/L)	<0.05	<0.05	0.24 ± 0.03[*]	<0.05[‡]	0.72 ± 0.08[*]	<0.05[‡]
Alkalinity (mg/L as $CaCO_3$)	62 ± 1	72 ± 2[‡]	76 ± 3[*]	67 ± 4[‡]	60 ± 2	65 ± 4[*‡]
Total Hardness (mg/L as $CaCO_3$)	113 ± 1	125 ± 2[‡]	146 ± 6[*]	147 ± 4[*]	169 ± 7[*]	140 ± 2[*‡]
Conductivity (µS/cm)	334 ± 5	357 ± 5[‡]	393 ± 16[*]	403 ± 8[*]	451 ± 10[*]	391 ± 5[*‡]

[a] DO = dissolved oxygen.

[*] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from David Lake measurement.

[‡] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from the respective d-0 measurement.

to day 10. In 2003 and 2004, there were no notable differences between reference and exposure samples with respect to DO, temperature, pH, and alkalinity. Total ammonia levels were elevated on day 0 in 2003 (in Wolf Lake and Unknown Lake test vessels) and 2004 (in Wolf Lake and Fox Lake test vessels) when compared to David Lake samples. In 2003, total ammonia concentrations were higher on day 10 than day 0 in David Lake and Delta Lake test samples. In 2003 and 2004, exposure site test vessels had higher levels of total hardness and conductivity than the reference site test vessels, although these differences were less pronounced on day 10.

Selected trace metal concentrations from 2004 day 0 overlying water and day 10 mini-peeper data are presented in Tables 2.12 and 2.13, respectively. Wolf Lake and Fox Lake test vessels had significantly higher day 0 overlying water levels of arsenic and molybdenum, and significantly lower levels of chromium than David Lake vessels. With respect to mini-peeper data (sampled on day 10), Wolf Lake and Fox Lake test vessels had significantly higher levels of pore-water aluminum, silicon, calcium, manganese, iron, arsenic, and molybdenum when compared to reference conditions. In addition, overlying water levels of arsenic, molybdenum, and silver were higher in exposure lake test vessels when compared to reference conditions.

Mean survival and final dry weight of *H. azteca* for the 2003 and 2004 sediment bioassays are shown in Figure 2.1. No significant differences were noted between the reference and exposure treatments with respect to either endpoint. Comparisons between the 2003 and 2004 bioassays with David Lake sediment, revealed a significantly lower final dry weight for *H. azteca* in 2003.

2.4.2 Rabbit Lake

2.4.2.1 Field chemistry

The physicochemical characteristics of surface water, pore-water, and sediment for the Rabbit Lake study sites in 2003 and 2004 are presented in Table 2.14 and Table 2.15, respectively. In 2003 and 2004, both exposure sites (Unknown Pond and Horseshoe Pond) had significantly higher surface water hardness and conductivity than the reference site. In 2004, exposure sites also had significantly lower surface water pH than the reference site. Exposure sites (in 2003 and 2004) had significantly higher pore-

Table 2.12. Mean (± standard deviation) trace element concentrations ($n = 3$) in overlying water on day 0 of the 2004 Key Lake *Hyalella azteca* sediment bioassay.

Variable (µg/L)	David Lake	Wolf Lake	Fox Lake
Mg	16922 ± 671	19014 ± 596[*]	16603 ± 1149
Al	64.23 ± 17.26[a]	96.11 ± 16.90[ab]	63.82 ± 1.62[a]
Si	<807	1042 ± 108	1132 ± 222
P	213.7 ± 30.6	139.2 ± 29.0	144.5 ± 36.4
Ca	21640 ± 11648	10852 ± 337	15278 ± 409
Cr	3.10 ± 1.32[ab]	<1.37[a*]	<1.37[a*]
Mn	10.72 ± 5.41	1.54 ± 0.15[*]	16.70 ± 2.45
Fe	164.5 ± 9.4	<109.9	669.5 ± 63.5[a*]
Cu	1.94 ± 0.71	3.41 ± 0.58[a]	4.35 ± 1.72[a]
As	1.3 ± 0.9	37.3 ± 4.8[a*]	210.9 ± 51.3[a*]
Se	<7.86	<7.86	<7.86
Mo	1.9 ± 0.6	202.1 ± 22.5[a*]	996.7 ± 86.9[a*]
Ag	<0.09	<0.09	<0.09
Cd	0.64 ± 0.54[ab]	<0.25[ab]	1.47 ± 0.52[ab]
Tm	<0.02[b]	<0.02[b]	<0.02[b]
Hg	<0.70[a]	<0.70[a]	<0.70[a]
Pb	1.23 ± 0.66[a]	0.37 ± 0.16	1.84 ± 0.19[a]

[a] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999).

[b] Exceeds soft water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005).

[c] Exceeds hard water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005).

[*] Significantly different (one-way ANOVA, Dunnett's post hoc test, $p \leq 0.05$) from David Lake measurement.

Table 2.13. Day 10 mean (± standard deviation) trace element concentrations ($n = 3$) in surface water and pore-water collected with mini-peepers from the 2004 Key Lake *Hyalella azteca* sediment bioassay.

Variable	David Lake		Wolf Lake		Fox Lake	
(µg/L)	Surface Water	Pore Water	Surface Water	Pore Water	Surface Water	Pore Water
Mg	15093 ± 537	14025 ± 1486	13932 ± 719	13479 ± 659	15242 ± 853	12904 ± $1641^{\ddagger}$
Al	33.54 ± 3.46^{a}	13.99 ± $4.94^{a\ddagger}$	34.25 ± 11.36^{a}	32.59 ± 7.40^{a*}	35.26 ± 8.64^{a}	52.44 ± $2.13^{a*\ddagger}$
Si	<807	<807	<807	10010 ± $2451^{*\ddagger}$	<807	3963 ± $2050^{*\ddagger}$
P	156.2 ± 100.4	112.3 ± 104.0	<104.5	256.3 ± $114.5^{\ddagger}$	<104.5	294.8 ± $158.4^{\ddagger}$
Ca	21990 ± 1860	24967 ± 2844	27835 ± 559	63617 ± $16531^{*\ddagger}$	24606 ± 1103	45314 ± $8142^{*\ddagger}$
Cr	1.92 ± 2.15^{a}	2.56 ± 1.63^{a}	1.64 ± 0.88^{a}	12.79 ± $10.24^{ab*\ddagger}$	2.81 ± 1.91^{a}	9.77 ± 3.00^{ab}
Mn	<0.42	0.56 ± $0.36^{\ddagger}$	0.46 ± 0.23	12.68 ± $1.03^{*\ddagger}$	2.13 ± 0.92^{*}	31.06 ± $7.03^{*\ddagger}$
Fe	150 ± 53	<109.9	<109.9	4095 ± $4585^{abc*\ddagger}$	<109.9	4604 ± $4117^{abc*\ddagger}$
Cu	1.64 ± 0.90	$<2.25^{a}$	$<2.25^{a}$	$<2.25^{a}$	1.77 ± 1.12	4.62 ± 6.06^{a}
As	0.7 ± 0.4	2.6 ± $0.95^{\ddagger}$	118.1 ± 18.02^{a*}	436.2 ± 488.3^{ac*}	93.2 ± 14.2^{a*}	393.7 ± $299.2^{a*\ddagger}$
Se	$<7.86^{a}$	$<7.86^{a}$	$<7.86^{a}$	$<7.86^{a}$	$<7.86^{a}$	$<7.86^{a}$
Mo	2.1 ± 0.2	1.7 ± 1.1	380.6 ± 39.0^{a*}	376.6 ± 213.7^{a*}	569.6 ± 141.5^{a*}	667.0 ± 44.3^{a*}
Ag	<0.09	0.09 ± 0.08	0.20 ± 0.08^{a*}	$<0.09^{\ddagger}$	0.26 ± 0.03^{ab*}	$<0.09^{\ddagger}$
Cd	$<0.25^{ab}$	0.76 ± 0.59^{ab}	$<0.25^{ab}$	0.27 ± 0.25^{ab}	$<0.25^{ab}$	$<0.25^{ab}$
Tm	$<0.02^{b}$	0.04 ± 0.05^{b}	$<0.02^{b}$	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}	0.03 ± 0.01^{b}
Hg	$<0.70^{a}$	$<0.70^{a}$	$<0.70^{a}$	$<0.70^{a}$	$<0.70^{a}$	$<0.70^{a}$
Pb	0.18 ± 0.14	0.07 ± 0.05	<0.07	0.09 ± 0.06	<0.07	0.09 ± 0.09

[a] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999); [b] Exceeds soft water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005); [c] Exceeds hard water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005); [*] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from David Lake measurement; [‡] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from the respective surface water measurement

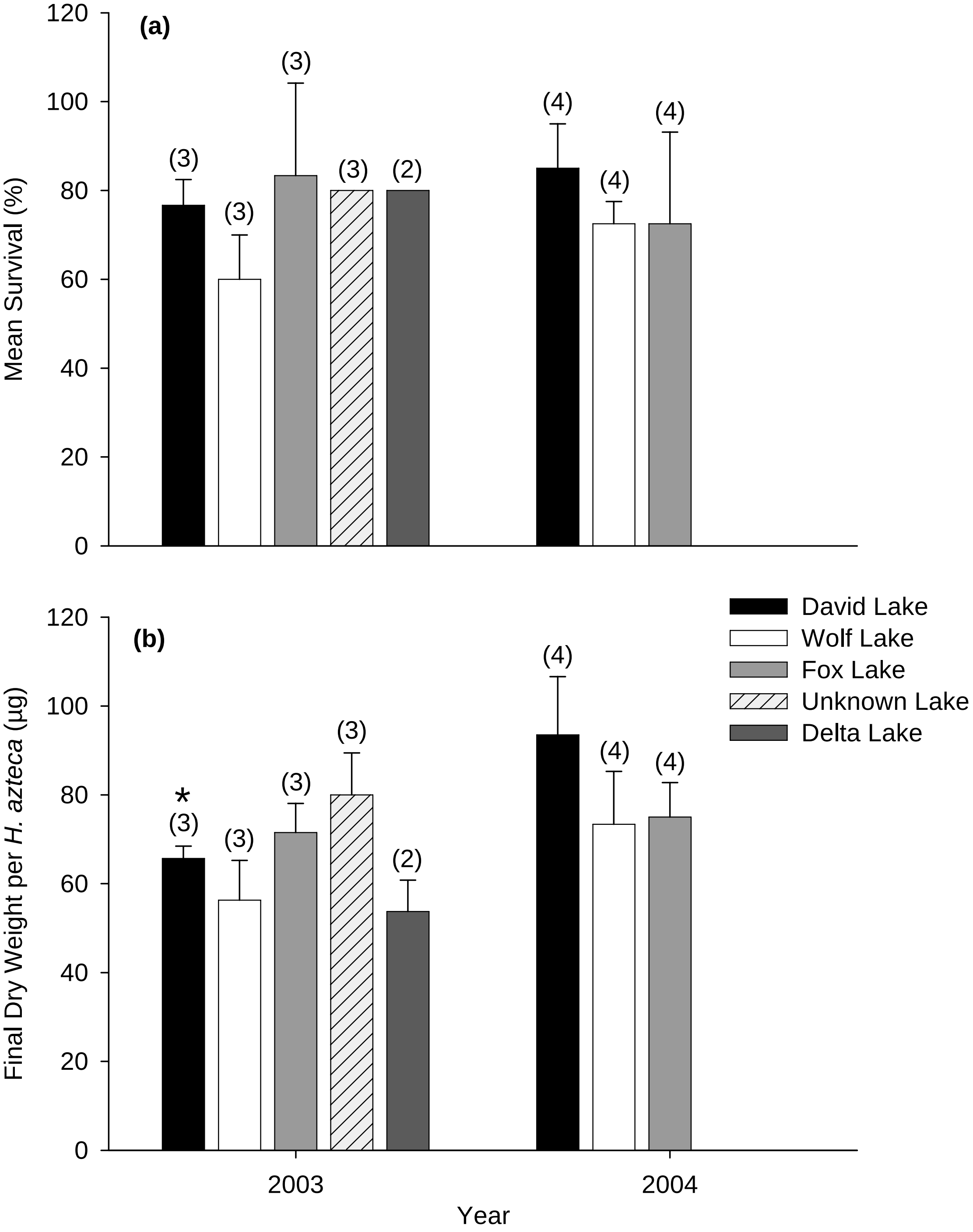

Figure 2.1. Mean survival (± standard deviation) (a) and final dry weight (± standard deviation) (b) of *Hyalella azteca* after a 10-day bioassay with sediments collected from sites at the Key Lake uranium operation in 2003 and 2004. Sample size is noted within parentheses. Unknown Lake and Delta Lake were not sampled in 2004. Asterisk denotes a significant difference between a 2003 measurement and its respective 2004 measurement.

Table 2.14. Mean (± standard deviation, n = 3) physicochemical surface water, pore-water, and sediment characteristics for the 2003 study sites at the Rabbit Lake uranium operation.

Characteristic	Reference Lake	Horseshoe Pond	Hidden Bay
Surface-water			
Depth (cm)	90[a]	85[a]	100[a]
Temperature (°C)	15.0[a]	11.9[a]	12.1[a]
DO[b] (mg/L)	8.2[a]	8.2[a]	8.4[a]
pH	6.79 ± 0.04	6.64 ± 0.10	6.79 ± 0.06
Total Ammonia (mg N/L)	<0.1	<0.1	<0.1
Alkalinity (mg/L as $CaCO_3$)	18 ± 1	11 ± 1[*]	18 ± 1
Total Hardness (mg/L as $CaCO_3$)	11 ± 3	540 ± 40[*]	327 ± 30[*]
Conductivity (µS/cm)	35 ± 2	1306 ± 10[*]	886 ± 6[*]
Pore Water			
pH	6.26 ± 0.07[c]	6.62 ± 0.04[*]	7.24 ± 0.05[*]
Total Ammonia (mg N/L)	0.2 ± 0.3	>10[cd*]	7.2 ± 1.8[c*]
Alkalinity (mg/L as $CaCO_3$)	22 ± 3	63 ± 27	162 ± 28[*]
Total Hardness (mg/L as $CaCO_3$)	7 ± 6	670 ± 20[*]	360 ± 20[*]
Sediment			
Sand (%)	84 ± 6	52 ± 20[*]	29 ± 1[*]
Silt (%)	16 ± 6	44 ± 17[*]	71 ± 1[*]
Clay (%)	1 ± 0	4 ± 4	1 ± 0
TOC[e] (%)	9.1 ± 2.8	13.4 ± 1.2	15.1 ± 1.8[*]
Water Content (%)	75.5 ± 4.3	85.3 ± 2.3[*]	83.0 ± 3.5

[a] n = 1.

[b] DO = dissolved oxygen.

[c] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999).

[d] Exceeded the standard curve.

[e] TOC = Total organic carbon.

[*] Significantly different (one-way ANOVA, Dunnett's post hoc test, $p \leq 0.05$) from Reference Lake measurement.

Table 2.15. Mean (± standard deviation, *n* = 4) physicochemical surface water, pore-water, and sediment characteristics for the 2004 study sites at the Rabbit Lake uranium operation.

Characteristic	Reference Lake	Unknown Pond	Horseshoe Pond
Surface-water			
Depth (cm)	78 ± 2	83 ± 5	64 ± 1*
Temperature (°C)	15.4 ± 0.2	15.7 ± 0.0*	16.9 ± 0.1*
DO^{a} (mg/L)	9.2 ± 0.0	7.7 ± 0.1*	8.0 ± 0.1*
pH	7.3 ± 0.1	6.1 ± 0.0^{b*}	6.6 ± 0.0*
Total Ammonia (mg N/L)	<0.1	<0.1	<0.1
Alkalinity (mg/L as $CaCO_3$)	20 ± 2	7 ± 0*	9 ± 1*
Total Hardness (mg/L as $CaCO_3$)	16 ± 1	800 ± 10*	820 ± 60*
Conductivity (µS/cm)	43 ± 1	1632 ± 5*	1677 ± 79*
Pore Water			
pH	6.7 ± 0.1	7.0 ± 0.1*	6.8 ± 0.1
Total Ammonia (mg N/L)	<0.1	11.6 ± 4.9^{b*}	4.1 ± 1.0^{b*}
Alkalinity (mg/L as $CaCO_3$)	44 ± 6	140 ± 58*	48 ± 10^{c}
Total Hardness (mg/L as $CaCO_3$)	12 ± 2	940 ± 20*	780 ± 60*
DOC^{d} (mg/L)	21.9 ± 7.8^{c}	39.2 ± 1.4^{c*}	27.4 ± 7.7^{c}
Sediment			
Sand (%)	39 ± 5^{c}	35 ± 9^{c}	22 ± 5^{c*}
Silt (%)	44 ± 3^{c}	46 ± 6^{c}	54 ± 4^{c*}
Clay (%)	17 ± 2^{c}	20 ± 4^{c}	24 ± 2^{c}
TOC^{e} (%)	10.0 ± 0.6	24.8 ± 1.4*	14.4 ± 1.0*
Water Content (%)	84.3 ± 1.9	88.2 ± 1.5*	87.1 ± 1.5

[a] DO = dissolved oxygen.

[b] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999).

[c] *n* = 3.

[d] DOC = Dissolved organic carbon.

[e] TOC = Total organic carbon.

[*] Significantly different (one-way ANOVA, Dunnett's post hoc test, $p \leq 0.05$) from Reference Lake measurement.

water total ammonia, and hardness than the respective reference site. In 2003 the exposure sites had significantly higher pore-water pH than the reference site. With respect to sediment composition, the exposure lakes had statistically higher silt and lower sand content in 2003. In 2004, sediment composition was comparable among all lakes with few statistical differences.

Trace metals concentrations in whole-sediment, pore-water, and surface water for the Rabbit Lake study sites are presented in Table 2.16 (2003) and 2.17 (2004). In both years, sediment, pore-water and surface water from the exposure lakes generally had higher concentrations of trace metals than the respective reference lake, and followed a trend of decreasing concentrations with increasing distance from the point of effluent discharge. In 2003, exposure sites had significantly higher phosphorous, chromium, nickel, copper (Horseshoe Pond), arsenic, selenium, molybdenum, and uranium in sediment than the reference lake. Pore-waters from exposure lakes also had significantly higher concentrations of molybdenum and uranium than the reference lake. In addition, only Horseshoe Pond pore-water had significantly higher phosphorous, nickel, and arsenic than the reference lake. Surface water from the exposure sites had significantly higher concentrations of phosphorous, manganese, nickel, copper (Horseshoe Pond), molybdenum, and uranium in 2003.

In 2004, sediment collected from the exposure sites had significantly higher phosphorous, calcium, chromium, manganese, iron, copper, arsenic, selenium, molybdenum, cadmium, and uranium. Associated pore-waters had significantly higher concentrations of magnesium, silicon (Unknown Pond), phosphorous, calcium, chromium, manganese, iron, arsenic, molybdenum, and uranium. Unknown Pond and Horseshoe Pond surface waters contained significantly higher concentrations of magnesium, aluminum, phosphorous, calcium, manganese, iron, copper (Unknown Pond), arsenic, selenium (Horseshoe Pond), molybdenum, and uranium than the reference lake.

Selected radionuclide data for the 2004 Rabbit Lake study sites are shown in Table 2.18. Activity levels for uranium, lead-210, and polonium-210 were higher at the exposure sites than the reference site. No strong trends were observed for radium-226.

Table 2.16. Mean (± standard deviation, *n* = 3) trace element concentrations in whole-sediment (dry weight)(mg/kg), pore-water (µg/L), and surface water (µg/L) for the 2003 Rabbit Lake study sites.

Variable	Reference Lake	Horseshoe Pond	Hidden Bay
Whole Sediment			
P	373.6 ± 144.3	594.1 ± 40.8*	594.0 ± 24.4*
Cr	3.59 ± 1.09	6.33 ± 1.22*	8.29 ± 0.12*
Mn	172.8 ± 70.5	86.8 ± 24.8	173.4 ± 9.8
Ni	4.61 ± 1.57	81.58 ± 43.22*	69.19 ± 4.48*
Cu	6.89 ± 1.00	20.10 ± 8.02*	11.21 ± 0.61
As	1.64 ± 0.53	31.92 ± 19.20*	8.49 ± 0.83*
Se	<1.60	12.01 ± 6.33*	8.16 ± 0.45*
Mo	10 ± 4	1707 ± 1063*	1391 ± 313*
Cd	<1.66	<1.66	<1.66
Tm	0.27 ± 0.13	0.22 ± 0.03	0.22 ± 0.02
Ta	<10.25	<10.25	<10.25
Hg	<0.31	<0.31	<0.31
U	15 ± 4	1009 ± 507*	761 ± 34*
Pore Water			
P	140.2 ± 39.0	224.0 ± 10.7*	101.1 ± 16.2
Cr	<8.29^{ab}	<8.29^{ab}	<8.29^{ab}
Mn	223.9 ± 122.4^{bc}	748.3 ± 414.8^{bc}	297.3 ± 69.5^{bc}
Ni	0.36 ± 0.41	22.31 ± 8.29*	10.64 ± 2.49
Cu	11.29 ± 0.35^{a}	13.73 ± 1.46^{a}	13.41 ± 10.41^{a}
As	0.83 ± 0.30	36.56 ± 4.86^{a*}	6.02 ± 1.15^{a}
Se	<11.81^{a}	<11.81^{a}	<11.81^{a}
Mo	6 ± 2	5452 ± 2170^{abc*}	1068 ± 286^{abc*}
Cd	<2.70^{abc}	<2.70^{abc}	<2.70^{abc}
Tm	0.01 ± 0.01^{b}	0.02 ± 0.02^{b}	<0.01
Ta	<14.43^{b}	<14.43^{b}	<14.43^{b}
Hg	<0.13^{a}	<0.13^{a}	<0.13^{a}
U	2.1 ± 0.4	619.8 ± 643.1^{b*}	426.7 ± 212.8^{b*}
Surface Water			
P	<32.0	313.3 ± 12.5*	141.6 ± 7.1*
Cr	<8.29^{ab}	<8.29^{ab}	<8.29^{ab}
Mn	0.5 ± 0.0	179.0 ± 1.9^{bc*}	79.2 ± 0.8*
Ni	<0.26	32.86 ± 1.59^{a*}	15.69 ± 0.08*

Cu	2.15 ± 0.37[a]	6.41 ± 4.65[a*]	2.68 ± 0.42[a]
As	<3.21	<3.21	<3.21
Se	<11.81[a]	<11.81[a]	<11.81[a]
Mo	3 ± 2	2002 ± 35[abc*]	988 ± 9[a*]
Cd	<2.70[abc]	<2.70[abc]	<2.70[abc]
Tm	<0.005	0.006 ± 0.003	0.005 ± 0.002
Ta	<14.43[b]	<14.43[b]	<14.43[b]
Hg	<0.13[a]	<0.13[a]	<0.13[a]
U	0.1 ± 0.1	129.1 ± 3.1[b*]	32.9 ± 0.6[b*]

[a] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999).

[b] Exceeds soft water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005).

[c] Exceeds hard water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005).

[*] Significantly different (one-way ANOVA, Dunnett's post hoc test, $p \leq 0.05$) from Reference Lake measurement.

Table 2.17. Mean (± standard deviation, *n* = 3) trace element concentrations in whole-sediment (dry weight)(mg/kg), pore-water (µg/L), and surface water (µg/L) for the 2004 Rabbit Lake study sites.

Variable	Reference Lake	Unknown Pond	Horseshoe Pond
<u>Whole Sediment</u>			
Mg	2001 ± 16	2915 ± 726	2349 ± 106
Al	20077 ± 1299	18015 ± 3623	15314 ± 834
Si	n/a[a]	n/a[a]	n/a[a]
P	394 ± 27	1012 ± 71[*]	722 ± 12[*]
Ca	3730 ± 271	21724 ± 1797[*]	12089 ± 197[*]
Cr	4.45 ± 0.16	23.40 ± 3.20[*]	10.83 ± 0.87[*]
Mn	48.3 ± 1.9	127.9 ± 7.9[*]	71.8 ± 3.4[*]
Fe	2762 ± 264	67587 ± 6164[*]	24750 ± 3564[*]
Cu	13.0 ± 0.9	110.4 ± 3.7[*]	45.6 ± 3.0[*]
As	1.8 ± 0.2	622.0 ± 103.1[*]	146.4 ± 29.6[*]
Se	<4.08	98.95 ± 34.80[*]	51.96 ± 3.61[*]
Mo	5 ± 3	3520 ± 1278[*]	5672 ± 1187[*]
Cd	0.06 ± 0.01	1.09 ± 0.21[*]	0.92 ± 0.15[*]
Hg	<0.22	<0.22	<0.22
U	130 ± 7	3681 ± 867[*]	3323 ± 42[*]
<u>Pore Water</u>			
Mg	1430 ± 301	51498 ± 12920[*]	37811 ± 996[*]
Al	445.1 ± 266.0[bc]	<6.5[b*]	43.5 ± 69.9[b*]
Si	10165 ± 1061	14084 ± 2423[*]	8717 ± 582
P	80.2 ± 48.2	451.0 ± 72.3[*]	310.5 ± 44.1[*]
Ca	3134 ± 593	305695 ± 3100[*]	257158 ± 5425[*]
Cr	<1.11[b]	8.15 ± 3.26[bc*]	7.63 ± 2.89[bc*]
Mn	19.0 ± 5.4	324.4 ± 67.6[cd*]	326.0 ± 22.5[cd*]
Fe	108 ± 44	33146 ± 7129[bcd*]	18418 ± 6842[bcd*]
Cu	4.94 ± 2.35[b]	2.16 ± 0.43[b]	2.09 ± 0.18[b]
As	<0.3	359.2 ± 83.7[b*]	55.2 ± 7.3[b*]
Se	5.45 ± 6.91[b]	<2.91[b]	6.00 ± 1.56[b]
Mo	<0	8250 ± 431[bcd*]	7413 ± 1303[bcd*]
Cd	0.17 ± 0.01[bc]	<0.06[b*]	<0.06[b*]
Hg	<0.16[b]	<0.16[b]	<0.16[b]
U	11 ± 4	4587 ± 2605[cd*]	1473 ± 137[c*]

Surface Water			
Mg	2622 ± 113	29417 ± 1467[*]	32772 ± 1079[*]
Al	<6.3[b]	133.9 ± 19.2[bc*]	110.2 ± 66.1[bc*]
Si	1993 ± 506	2465 ± 647	2604 ± 514
P	99.1 ± 20.8	290.1 ± 40.6[*]	376.6 ± 54.1[*]
Ca	3952 ± 398	282824 ± 8188[*]	294673 ± 4124[*]
Cr	<1.11[b]	<1.11[b]	<1.11[b]
Mn	5.2 ± 0.1	188.3 ± 1.6[cd*]	197.2 ± 3.3[cd*]
Fe	18.1 ± 18.6	270.2 ± 31.2[*]	272.2 ± 172.9[*]
Cu	0.87 ± 1.02	3.00 ± 0.35[b*]	2.33 ± 0.56[b]
As	0.22 ± 0.12	9.14 ± 0.75[b*]	6.50 ± 0.55[b*]
Se	4.29 ± 2.51[b]	<2.91[b]	15.50 ± 7.38[b*]
Mo	8 ± 8	5154 ± 68.71[bcd*]	5461 ± 30[bcd*]
Cd	0.06 ± 0.05[b]	<0.06[b]	<0.06[b]
Hg	<0.16[b]	<0.16[b]	<0.16[b]
U	3.0 ± 0.1	123.2 ± 2.3[c*]	102.9 ± 0.7[c*]

[a] n/a = Not available, not measured.

[b] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999).

[c] Exceeds soft water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005).

[d] Exceeds hard water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005).

[*] Significantly different (one-way ANOVA, Dunnett's post hoc test, $p \leq 0.05$) from Reference Lake measurement.

Table 2.18. Mean (± standard deviation, n = 2) radionuclide activities in whole-sediment (dry weight) from the 2004 Rabbit Lake study sites.

Variable (Bq/kg)	Reference Lake			Unknown Pond			Horseshoe Pond		
U	3024.0	±	356.4	86940.0	±	19601.0	25200.0	±	0.0
Ra-226	<20			155.0	±	162.6	<30		
Pb-210	90.4	±	97.8	186.2	±	127.9	223.5	±	301.0
Po-210[a]	88.0	±	95.1	181.2	±	124.4	217.5	±	292.9

[a] Extrapolated from lead-210 values (calculation performed by Dr. Patricia Thomas, Saskatoon, SK, Canada).

2.4.2.2 Benthic community structure

Table 2.19 presents total abundance, taxon richness, Simpson's diversity index, Bray Curtis index, and evenness for the 2003 and 2004 Rabbit Lake study sites. In 2003 and 2004, Horseshoe Pond and Unknown Pond, respectively, had significantly lower total benthic macroinvertebrate abundance than the reference site. Significantly lower taxon richness and Simpson's Diversity index was noted in Unknown Pond (2004), Horseshoe Pond (2003) and Hidden Bay (2004). All exposure sites had Bray Curtis values (in both 2003 and 2004) that were significantly different from the respective reference site value. No significant differences were noted in evenness. Temporal comparisons (2003 vs. 2004) for each of the two reference lakes revealed significant in total abundance and evenness. Horseshoe Pond has significantly different Simpson's diversity indices and evenness in 2003 and 2004.

Tables 2.20 and 2.21 present the density (organisms/m^2) and percent composition of 16 of the most common taxa (plus one pooled group of "other taxa") found at theRabbit Lake study sites. Generally, significant differences between exposure sites and the reference site (within the respective year) decreased with increasing distance from the effluent source. Few significant differences were noted between sampling years for Horseshoe Pond, however, many differences were noted between the two different reference sites (one sampled in 2003 and the other in 2004). Density comparisons (Table 20) within each taxon revealed significantly lower densities of Nematoda (2004), Valvatidae (2004), Sphaeriidae (2003 and 2004), Oligochaeta (2003), Hirudinea (2003), Hyalellidae (2004), Emphemeroptera (2003 and 2004), and Ceratopogonidae (2004) within all exposure sites compared to the respective reference site. All exposure sites had significantly lower percentages of Nematoda (2004), Valvatidae (2004), Sphaeriidae (2003 and 2004), Hirudinea (2003), Hyalellidae (2004), Emphemeroptera (2003 and 2004), Chironomini (2004), and Ceratopogonidae (2004) than the respective reference site.

2.4.2.3 Whole-sediment bioassay

Water quality data for the 2003 Rabbit Lake sediment bioassays are presented in Table 2.22. Average DO levels were significantly lower from day 0 to day 4 than from

Table 2.19. Selected benthic community metrics for the 2003 and 2004 Rabbit Lake study sites.

	Reference Lake		Unknown Pond	Horseshoe Pond		Hidden Bay
Metric	2003	2004	2004[a]	2003	2004	2003[a]
Total Abundance (organisms/m^2)	3816 ± 713	613 ± 138[†]	2735 ± 1183[*]	1120 ± 199[*]	390 ± 223	2547 ± 902
Taxon Richness[b]	18 ± 4	12 ± 2	6 ± 2[*]	7 ± 1[*]	7 ± 5	9 ± 2[*]
Simpson's Diversity Index[b]	0.68 ± 0.09	0.77 ± 0.10	0.52 ± 0.02[*]	0.31 ± 0.15[*]	0.64 ± 0.10[†]	0.34 ± 0.08[*]
Bray Curtis Index[b]	0.12 ± 0.05	0.17 ± 0.03	0.84 ± 0.07[*]	0.52 ± 0.06[*]	0.66 ± 0.12[*]	0.36 ± 0.05[*]
Evenness[b]	0.19 ± 0.03	0.41 ± 0.10[†]	0.41 ± 0.11	0.20 ± 0.03	0.48 ± 0.17[†]	0.18 ± 0.02

[a] Data only available for 2003.

[b] Family or higher.

[*] Significantly different (one-way ANOVA, Dunnett's post hoc test, $p \leq 0.05$) from Reference Lake measurement.

[†] Significantly different (*t*-test, $p \leq 0.05$) than the respective 2003 measurement.

Table 2.20. Densities (organisms/m^2) of selected taxa from the 2003 and 2004 Rabbit Lake study sites.

	Reference Lake		Unknown Pond	Horseshoe Pond		Hidden Bay
Taxon	2003	2004	2004[a]	2003	2004	2003[a]
Nematoda	16 ± 15	47 ± 36	0 ± 0[*]	0 ± 0	2 ± 3[*]	2 ± 4
Valvatidae	0 ± 0	25 ± 10[†]	0 ± 0[*]	0 ± 0	0 ± 0[*]	0 ± 0
Sphaeriidae	147 ± 103	68 ± 36	0 ± 0[*]	0 ± 0[*]	0 ± 0[*]	0 ± 0[*]
Oligochaeta	256 ± 80	8 ± 10[†]	0 ± 0	44 ± 42[*]	15 ± 21	0 ± 0[*]
Hirudinea	44 ± 20	2 ± 3[†]	0 ± 0	0 ± 0[*]	0 ± 0	0 ± 0[*]
Hyalellidae	2 ± 4	55 ± 33[†]	0 ± 0[*]	0 ± 0	2 ± 3[*]	11 ± 19
Copepoda	180 ± 87	3 ± 7[†]	107 ± 70[*]	40 ± 35[*]	12 ± 6	84 ± 23
Cladocera	773 ± 711	42 ± 30[†]	1482 ± 717[*]	51 ± 32[*]	55 ± 80	153 ± 31
Ostracoda	42 ± 38	15 ± 22	2 ± 3	29 ± 30	118 ± 65[*]	133 ± 58
Ephemeroptera	373 ± 76	37 ± 4[†]	0 ± 0[*]	2 ± 4[*]	10 ± 12[*]	80 ± 110[*]
Chironomini	602 ± 104	128 ± 26[†]	278 ± 212	162 ± 53[*]	27 ± 9[*†]	280 ± 177
Tanytarsini	909 ± 110	30 ± 12[†]	70 ± 9	24 ± 4[*]	43 ± 33	1467 ± 626
Orthocladinae	156 ± 125	8 ± 13[†]	527 ± 277[*]	222 ± 132	20 ± 24[†]	13 ± 13[*]
Tanypodinae	289 ± 128	63 ± 25[†]	227 ± 72[*]	502 ± 84	75 ± 46[†]	311 ± 89
Chironomidae pupa	0 ± 0	5 ± 6	20 ± 9[*]	0 ± 0	3 ± 7	0 ± 0
Ceratopogonidae	11 ± 10	63 ± 9[†]	0 ± 0[*]	0 ± 0	0 ± 0[*]	2 ± 4
Other taxa	31 ± 20	13 ± 5	23 ± 16	78 ± 71	8 ± 17[†]	18 ± 31

[a] Data only available for 2003.

[*] Significantly different (one-way ANOVA, Dunnett's post hoc test, $p \leq 0.05$) from Reference Lake measurement.

[†] Significantly different (t-test, $p \leq 0.05$) from the respective 2003 measurement.

Table 2.21. Proportion (%) of selected taxa from the 2003 and 2004 Rabbit Lake study sites.

	Reference Lake		Unknown Pond	Horseshoe Pond		Hidden Bay
Taxon	2003	2004	2004[a]	2003	2004	2003[a]
Nematoda	0 ± 1	7 ± 5	0 ± 0[*]	0 ± 0	0 ± 1[*]	0 ± 0
Valvatidae	0 ± 0	4 ± 1[†]	0 ± 0[*]	0 ± 0	0 ± 0[*]	0 ± 0
Sphaeriidae	4 ± 3	11 ± 4	0 ± 0[*]	0 ± 0[*]	0 ± 0[*]	0 ± 0[*]
Oligochaeta	7 ± 2	1 ± 1[†]	0 ± 0	4 ± 3	3 ± 3	0 ± 0[*]
Hirudinea	1 ± 1	0 ± 0	0 ± 0	0 ± 0[*]	0 ± 0	0 ± 0[*]
Hyalellidae	0 ± 0	8 ± 4[†]	0 ± 0[*]	0 ± 0	0 ± 1[*]	0 ± 1
Copepoda	5 ± 2	0 ± 1[†]	3 ± 2	3 ± 2	4 ± 4	4 ± 2
Cladocera	18 ± 14	6 ± 4	52 ± 9[*]	4 ± 3	11 ± 11	7 ± 4
Ostracoda	1 ± 1	2 ± 3	0 ± 0	2 ± 2	35 ± 15[*†]	5 ± 1
Ephemeroptera	10 ± 3	6 ± 1[†]	0 ± 0[*]	0 ± 0[*]	2 ± 2[*]	3 ± 3[*]
Chironomini	16 ± 3	22 ± 8	9 ± 5[*]	15 ± 6	8 ± 4[*]	10 ± 3
Tanytarsini	25 ± 6	5 ± 2[†]	3 ± 2	2 ± 1[*]	10 ± 9	56 ± 7[*]
Orthocladinae	4 ± 2	2 ± 2	21 ± 9	18 ± 8[*]	4 ± 3[†]	1 ± 0
Tanypodinae	8 ± 4	10 ± 4	10 ± 5	45 ± 12[*]	20 ± 10[†]	13 ± 5
Chironomidae pupa	0 ± 0	1 ± 1	1 ± 0	0 ± 0	1 ± 1	0 ± 0
Ceratopogonidae	0 ± 0	11 ± 4[†]	0 ± 0[*]	0 ± 0	0 ± 0[*]	0 ± 0
Other taxa	1 ± 0	2 ± 1	1 ± 0	6 ± 5	1 ± 3	1 ± 2

[a] Data only available for 2003.

[*] Significantly different (one-way ANOVA, Dunnett's post hoc test, $p \leq 0.05$) from Reference Lake measurement.

[†] Significantly different (*t*-test, $p \leq 0.05$) from the respective 2003 measurement.

Table 2.22. Mean (± standard deviation) Day 0 (d-0) and day 10 (d-10) water quality measurements for the 2003 Rabbit Lake *Hyalella azteca* sediment bioassay.

	Reference Lake		Horseshoe Pond		Hidden Bay	
Variable	d-0	d-10	d-0	d-10	d-0	d-10
Temperature (°C)	21.4 ± 0.2	21.4 ± 0.3	22.0 ± 0.3*	21.9 ± 0.2*	21.4 ± 0.3	21.4 ± 0.3
DO[a] (mg/L)	5.2 ± 0.5	7.3 ± 0.2‡	5.1 ± 0.8	7.2 ± 0.4‡	5.1 ± 0.6‡	7.3 ± 0.4‡
pH	7.84^b	7.75^b	7.93^b	7.96^b	7.93^b	7.91^b
Total Ammonia (mg N/L)	<0.1^b	0.2^b	0.5^b	0.3^b	<0.1^b	0.3^b
Alkalinity (mg/L as $CaCO_3$)	65^b	72^b	65^b	74^b	71^b	75^b
Total Hardness (mg/L as $CaCO_3$)	117^b	119^b	160^b	133^b	146^b	127^b
Conductivity (µS/cm)	336^b	352^b	437^b	368^b	399^b	372^b

[a] DO = dissolved oxygen.

[b] n = 1

[*] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from Reference Lake measurement.

[‡] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from the respective d-0 measurement.

day-5 to day 10. However, there were no notable differences between reference and exposure conditions for DO, temperature, pH, and alkalinity. Total ammonia levels were elevated on day 0 in Horseshoe Pond samples and in all treatment groups on day 10. Exposure site test vessels had higher levels of total hardness and conductivity than the reference site test vessels, although these differences were less pronounced on day 10.

Mean survival and final dry weight of *H. azteca* for the 2003 Rabbit Lake bioassay are presented in Figure 2.2. No significant differences were noted. Unfortunately, the 2004 Rabbit Lake sediment bioassay was un-successful (mean reference site survival = 33%), so results of this bioassay are not presented.

2.5 Discussion

2.5.1 Key Lake

2.5.1.1 Site characterization and field chemistry

Surface water and pore-water variables measured in 2003 and 2004 that appear to have been altered as a result of Key Lake operation effluent include water hardness, conductivity (in surface water), total ammonia (in surface water and pore-water), some metals (surface water and pore-water), and pH (in surface water). Although the Key Lake effluent could be released at lower pH levels than David Lake (~ 6.8), it is unclear to what degree low pH at the exposure sites is natural or anthropogenic, as the pH of the exposure sites generally decreased with increasing distance from the effluent source. Furthermore, previous studies have noted that Wolf Lake has seasonal variations in pH, with slightly lower levels observed in early spring and summer (4.5 to 4.7) compared to winter (5.0 to 5.5) months (SENES Consultants Ltd., 2002, 2003). The same studies determined that the drop in pH in the summer months was most likely driven by the naturally acid bog adjacent to Wolf Lake and not by the Key Lake uranium operation effluent (SENES Consultants Ltd., 2002, 2003), which is regulated to be released at a pH between 6.0 and 7.0 (Key Lake Laboratories, 2004). Surface water and pore-water variables that were significantly different from reference conditions and exceeded their respective Canadian Water Quality Guideline for protection of aquatic life and/or published *H. azteca* toxicity thresholds, and were not exceeded in David Lake in 2003

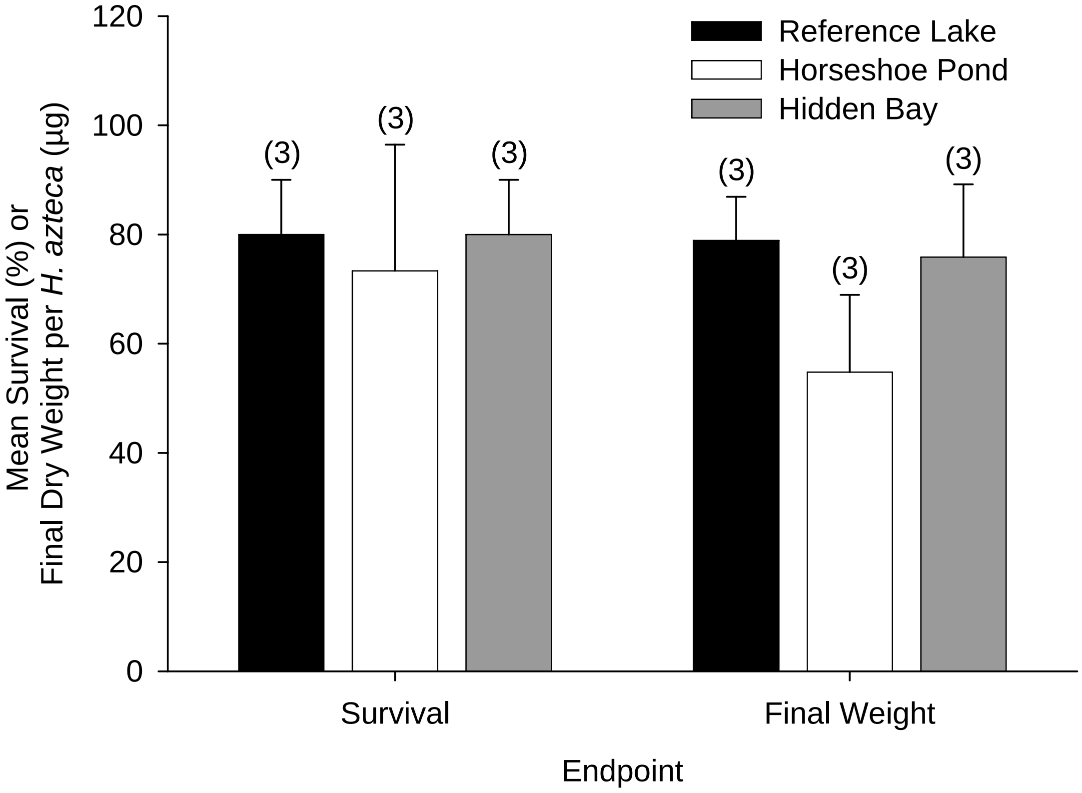

Figure 2.2. Mean survival (± standard deviation) and final dry weight (± standard deviation) per *Hyalella azteca* after a 10-day bioassay with sediments collected from sites at the Rabbit Lake uranium operation in 2003. Sample size is noted within parentheses.

and 2004, include surface water pH, total ammonia, and molybdenum, in addition to pore-water total ammonia, arsenic and molybdenum.

Sediment characteristics also appear to have been influenced by the mine/mill effluent. The elevated levels of fine particles, especially clays, in Wolf Lake are hypothesized to originate from sedimentation of effluent derived constituents. Because this effect is not pronounced at the inlet of Fox Lake, it is further hypothesized that fine particles settle out or are "filtered" out by the time the effluent reaches this location. However, differences in sediment particle size could also be attributed to natural differences between the study sites. The contribution of each of these factors has not been determined. Although whole-sediment contaminant concentrations were not used to determine which variables (i.e., trace elements) were of toxicological significance (as they are generally poor indicators of sediment toxicity), whole-sediment concentrations of selected trace metals were influenced by the mine/mill effluent. Furthermore, radionuclide measurements in 2004 suggest that uranium, radium-226, lead-210, and polonium 210 are not of ecotoxicological concern based on calculation by Thomas and Liber (2001).

2.5.1.2 Benthic community structure

As previously mentioned, other monitoring programs have noted benthic community impairment downstream of the effluent discharge site at the Key Lake uranium operation (Terrestrial and Aquatic Environmental Managers Ltd., 1994; Conor Pacific Environmental Technologies Inc., 2000; Golder Associates Ltd., 2002, 2005). Environment Canada's Metal Mining Environmental Effects Monitoring Guidance Document (Environment Canada, 2002) defines an effect on benthic invertebrate communities as a statistical difference between a reference and an exposure area with respect to total invertebrate density, taxon richness, Simpson's Diversity index, and/or Bray-Curtis index. According to that guidance, the present study identified effects on benthic community structure at all exposure sites in both years. Overall, the degree of benthic impairment appeared to be more severe in Wolf and Fox Lakes than in Unknown and Delta Lakes (hence, the more focused study in 2004 at these near-field sites), with more significant differences (relative to David Lake) in density and/or proportion of the presented taxa and overall lower taxon richness (in Wolf Lake). In addition, sensitive taxa (Sphaeriidae and Hyalellidae) were either present at lower densities and/or proportions at, and/or absent from, all exposure sites.

With respect to the metrics used, all exposure lakes had Bray-Curtis values that were statistically higher than that for the reference lake. The Bray-Curtis index is a distance co-efficient that measures the difference between two sites, reaching a maximum value of 1 for two sites that are entirely different and a minimum value of 0 for two sites that possess identical descriptors (Environment Canada, 2002). The Bray-Curtis index appears to be the most sensitive of the metrics used, a conclusion similarly reached by Faith et al. (1991) who showed that the Bray-Curtis index was superior, when compared to seven other indices, at detecting effects of uranium and gold mines on benthic communities. Previous studies at the Key Lake uranium operation also found that exposure lakes (Fox Lake and Unknown Lake) had Bray-Curtis values that were statistically higher than the reference lake (Wolf Lake was not sampled) (Golder Associates Ltd., 2005). With respect to specific taxa, Sphaeriidae and Hyalellidae were absent from all exposure lakes while present in the reference lake suggesting that they may be among the most sensitive groups to Key Lake effluent exposure. Borgmann et al. (2004) have also noted the absence of Sphaeriidae and Amphipoda (to which Hyalellidae is a part of) in an area influenced by extensive mining and smelting operations. Borgmann et al. (2004) further identified the absence of Ephemeroptera and Tanytarsini at exposure sites. Beltman et al. (1999) and Schmidt et al. (2002) found Ephemeroptera, Plecoptera, and Trichoptera, to be sensitive taxa to effects from mining activities. The Key Lake study did not find any trends with respect to Tanytarsini, Ephemeroptera, Plecoptera, and Trichoptera. The absence and/or low representation of Oligochaeta and Chironomini in Wolf Lake is hypothesized to be related to the flocculent nature of the sediment (possibly a poor habitat for these taxa), as many species of these taxa are generally tolerant to metal contamination (Canfield et al., 1994).

2.5.1.3 Whole-sediment bioassay

The 2003 10-d sediment bioassay revealed no significant differences between David Lake and the exposure sites with respect to *H. azteca* survival or final dry weight. This was not anticipated since exposure lake pore-waters exceeded high hardness LC50 values (Borgmann et al., 2005) for arsenic (Wolf Lake and Fox Lake) and molybdenum (in all exposure lakes except Delta Lake). However, it should be noted that the high hardness LC50 value for molybdenum reported by Borgmann et al. (2005), exceeded the highest experimental concentration, so exceedance of this threshold should be interpreted with caution. Therefore, of the variables measured, pore-water arsenic levels in exposure site sediments were most likely to have been able to adversely affect survival and final weight of test organisms.

As a result of the lack of observed toxicity, it was hypothesized that the four daily water changes (with clean overlying water) used in this bioassay could have diluted sediment pore-water contaminant concentrations over time. This hypothesis was largely based on observed change in day 0 and day 10 overlying water chemistry data (e.g., decreases in levels of total hardness and conductivity). No day 10 pore-water metals data were collected to evaluate this hypothesis.

In 2004, sediment bioassay methods were changed slightly to evaluate the pore-water "dilution hypothesis", while keeping methods similar enough to allow for comparison between years. Overlying water changes were decreased to twice daily in an attempt to decrease pore-water dilution, and additional samples for trace metal analysis were collected to more accurately describe exposure of test organisms (including day 0 overlying water and day 10 overlying water and pore-water using mini-peepers). The separately collected sediment chemistry cores were still assumed to be representative of day 0 pore-water exposure conditions in the bioassay. In addition, younger test animals (2-9 days old) were used and aeration was in place for the entire duration of the test to avoid low DO as a potential confounding factor. Despite these changes in methodology, the 2004 sediment bioassay still showed no significant effect on *H. azteca* survival or final dry weight, although observed reductions in final dry weight (Wolf Lake and Fox Lake) were nearly significant ($p = 0.057$). Interestingly, day 0 pore-water concentrations of iron (Fox Lake), arsenic (Wolf Lake and Fox Lake), and molybdenum (Wolf Lake and Fox Lake) exceeded published *H. azteca* LC50 data (Borgmann et al., 2005). Furthermore, mean day 0 arsenic levels were higher in 2004 than in 2003 (4.1 vs. 1.8 mg/L in Wolf Lake and 3.0 vs. 1.5 mg/L in Fox Lake), perhaps contributing to the lower final dry weight of test organisms from exposure site sediments. Day 10 pore-water variables that exceeded published *H. azteca* high hardness LC50 data (Borgmann et al, 2005) were iron (Wolf Lake and Fox Lake) and arsenic (Wolf Lake), although the LC50 values for iron reported by Borgmann et al. (2005) exceeded their highest experimental concentration, so exceedance of these thresholds should be interpreted with caution. Therefore, of the variables measured in 2004, only arsenic concentrations were expected to have had an effect on the survival and final weight of the test organisms. Furthermore, comparison between day 0 and day 10 pore-water conditions reveal that arsenic concentrations decreased by almost 10-fold in Wolf Lake and Fox Lake sediments over the course of the bioassay. This decrease is believed to be largely caused by pore-water dilution and may have resulted in the lack of significant effects on *H. azteca*. No day 0 or day 10 overlying water chemistry variable exceeded published *H. azteca* LC50 data (Borgmann et al., 2005; France and Stokes, 1987; Ankley et al., 1995).

2.5.1.4 Linking cause and effect

Variables that were considered for the cause of effluent-derived benthic community impairment were significantly different from reference conditions and exceeded their respective Canadian Water Quality Guideline for protection of aquatic life and/or published *H. azteca* toxicity thresholds, and were not exceeded in the reference site for both 2003 and 2004. This excludes all variables that exceed CWQG values and/or published toxicity data in the reference site. With that said, potential cause(s) of benthic community impairment downstream of the Key Lake uranium operation identified through the SQT include physical sediment composition, surface water pH, total ammonia, and molybdenum, and pore-water total ammonia, arsenic, and molybdenum.

Differences in sediment composition (due to either natural and/or anthropogenic origins) can translate to differences in benthic community structure (Hartwell and

Claflin, 2005; Lee et al., 2006). As previously mentioned, significant differences were noted between some exposure sites and the reference site in sediment characteristics. Although the possible effect of physical sediment composition on benthic community structure is acknowledged, it is still hypothesized that surface water and/or sediment contamination played a significant role in the benthic community impairment observed.

One of the possible stressors identified was low surface water pH. While low pH can influence the toxicity of some constituents, it can in itself cause adverse biological effects. For example, studies have shown that low pH levels (generally <6.0) can cause benthic community impairment independently (Fjellheim et al., 1992; Ledger et al., 2005), as well as in combination with elevated metal concentrations (Keller et al., 1992; Soucek et al., 2000). Key Lake effluent is regulated to be released at a pH between 6.0 and 7.0 (Key Lake Laboratories, 2004). However, previous studies have noted that Wolf Lake has historically had lower pH levels than those observed in the present study, ranging from 4.5 to 4.7 (in the early spring and summer) to 5.0 to 5.5 (in the winter months) (SENES Consultants Ltd., 2002, 2003). The same studies determined that the drop in pH in the summer months was most likely driven by the naturally acid bog adjacent to Wolf Lake and not by the Key Lake uranium operation effluent (SENES Consultants Ltd., 2002, 2003). Regardless of the cause of pH depression, surface water pH levels within the downstream environment at the Key Lake operation were at levels low enough to possibly cause changes in benthic community structure. Haines (1981) conducted a review of the consequences of pH depression on aquatic ecosystems. Generally, this review revealed that at pH <6.0 effects on benthic invertebrates include reductions in abundance, production, and growth, and the loss of sensitive species. Of particular interest is that molluscs are highly sensitive to low pH, due to their high $CaCO_3$ requirements and that benthic crustaceans, including *Gammarus lacustris*, *Lepidurus arcticus*, and *Gammarus pulex*, are all sensitive to low pH (Haines, 1981). These findings concur with the observation that Sphaeriidae (fingernail clams) and Hyalellidae (benthic crustaceans) were absent from all exposure lakes, but were present in David Lake. Therefore, low pH could be playing a significant role in benthic community impairment at the Key Lake operation and should be further investigated.

Ammonia is a common toxicant of concern in surface water (Constable et al., 2003) and pore-water (Ankley et al., 1989; Schubauer-Berigan and Ankley, 1991; Van Sprang and Janssen, 1997). In aqueous solutions, ammonia is in a chemical equilibrium between un-ionized ammonia (NH_3), ionized ammonia (NH_4^+) and hydroxide ions (OH^-), an equilibrium that is both pH and temperature dependent. Concentrations of ammonia that are toxic to aquatic organism are often expressed as un-ionized ammonia (NH_3), because NH_3 has been demonstrated to be the principal toxic form of ammonia in the environment (Constable et al., 2003), with few exceptions (Borgmann, 1994). Direct impacts of ammonia include impacts on individual organisms, typically death, reduced growth rate, and reduced reproductive success, while indirect effects generally involve alteration of aquatic habitats, including eutrophication (organic enrichment) or acidification (Constable et al., 2003). Constable et al. (2003) conducted an aquatic ecological risk assessment of ammonia which presented LC50s for un-ionized ammonia on invertebrates that ranged between 0.530 mg/L (a daphnid, *Daphnia magna*) and 10.200 mg/L (a caddisfly, *Philarctus quaeris*). Using published formulas to account for the influences of pH and temperature (Emerson et al., 1975), mean un-ionized ammonia surface water values for exposure lakes in the present study ranged from 0.003 mg/L to 1.013 mg/L in 2003 (0.009 mg/L in reference) and from 0.064 mg/L to 0.152 mg/L in 2004 (0.006 mg/L in reference). Mean pore water values ranged from 0.009 mg/L to 3.353 mg/L in 2003 (0.027 mg/L in reference) and 0.942 mg/L and 1.089 mg/L in 2004. The high end of these ranges (which were noted at the near-field sites) are within the range of published LC50 values for aquatic invertebrates (noted above), demonstrating that ammonia could be having direct impacts on survival, growth, and/or reproduction of some aquatic invertebrates within the receiving environment at Key Lake.

Arsenic has been identified as another contaminant of concern downstream of metal mining activities (Beltman et al., 1999; Canfield et al., 1994), including uranium mining (Mkandawire and Dudel, 2005), and uranium milling (Peterson et al., 2001; Donahue and Hendry, 2003). Water only 4-d LC50 values for the common benthic invertebrates, *H. azteca* and *Chrionomus tentans*, have been described as 1.6 and 7.1 mg/L respectively (Liber and White-Sobey, unpublished data). The value of 1.6 mg/L for *H. azteca* is ~ 3 times higher than the water only 7-d LC50 values published by

Borgmann et al. (2005) for the same species (~0.5 mg/L). Arsenic pore-water concentrations in Wolf Lake and Fox Lake were 1.8 and 1.5 mg/L in 2003 and 4.1 and 3.0 mg/L in 2004. These concentrations suggest that pore-water arsenic could be contributing to benthic community impairment at these sites and could be a contributing factor to the absence of Hyalellidae (to which *H. azteca* is part of) in the Key Lake exposure lakes.

Molybdenum has also been noted as a contaminant of concern downstream of uranium milling activities (Peterson et al., 2001), including the Key Lake uranium operation (Pyle et al., 2000). Water only 4-d LC50 values for molybdenum were found to be >741 mg/L and >2892 mg/L for *H. azteca* and *C. tentans*, respectively (Liber and White-Sobey, unpublished data). The maximum molybdenum concentration measured within the exposure lakes was 6.1 mg/L in Fox Lake surface waters. Although there is relatively little information on the chronic toxicity of molybdenum to aquatic invertebrates, these data suggest that molybdenum is probably not a significant contributor to benthic community impairment *in-situ*, especially as other contaminants of concern (described above) are at or near acute effect concentrations.

2.5.2 Rabbit Lake

2.5.2.1 Site characterization and field chemistry

Water hardness, conductivity (of surface water), pH (of surface water in 2004), total ammonia (in pore-water), and concentrations of some metals (in surface water, pore-water and whole-sediment), in the downstream receiving environment appear to have been altered as a result of Rabbit Lake operation effluent. Surface water and pore-water variables that were significantly different from reference conditions and exceeded the Canadian Water Quality Guideline for protection of aquatic life and/or published *H. azteca* toxicity thresholds, and did not exceed reference conditions include surface water manganese, molybdenum, and uranium, as well as pore-water total ammonia, iron (only measured in 2004), arsenic, molybdenum, and uranium.

Sediment characteristics also appear to have been influenced by the mine/mill effluent. Significant differences were observed in sediment particle size, TOC level, and water content among the study sites. As with the Key Lake study, these differences could be the result of natural differences and/or effluent influences. The elevated levels of fine particles (including silt and clay) and TOC at the 2003 exposure sites compared to the reference site were hypothesized to have been caused by sedimentation of effluent derived constituents. However, comparisons among the 2004 exposure and reference sites do not show the same degree of differences in sediment composition (except for TOC), suggesting that the 2004 reference site is more representative of the exposure site sediment types and possibly that physical sediment composition at these sites has not been heavily influenced by effluent discharge. Although whole-sediment concentrations were not used to determine which variables (i.e., trace elements) were of toxicological significance (as they are generally poor indicators of sediment toxicity), whole-sediment concentrations for selected trace metals were related to mine/mill effluent. Radionuclide data for downstream sediments identified uranium as a potential hazard to benthic communities, although the relative importance of radiotoxicity and chemotoxicity remains unclear (Thomas and Liber, 2001).

2.5.2.2 Benthic community structure

Through previous monitoring programs, benthic community impairment has been noted downstream of the effluent discharge site at the Rabbit Lake uranium operation (Terrestrial and Aquatic Environmental Managers Ltd. and SENES Consultants Ltd. 1996; Golder Associates Ltd., 2003c). According to Environment Canada's EEM criteria for benthic community impairment (Environment Canada, 2002), the Rabbit Lake SQTs described here identified effects on benthic community structure at all exposure sites in both years evaluated. Overall, the degree of benthic impairment appeared to be more severe in Unknown Pond and Horseshoe Pond than in Hidden Bay (hence, the more focused study in 2004 at these near-field sites), with generally greater Bray Curtis values, lower taxon richness, and more significant differences (relative to the reference site) in density and/or proportion of the presented taxa. Sensitive taxa such as Sphaeriidae, Hyalellidae, and Ephemeroptera were also recorded at either lower densities and/or proportions (when compared to reference conditions), and/or were absent from Unknown and Horseshoe Ponds. This trend was not as strong in Hidden Bay, which had higher representation of both Hyalellidae and Ephemeroptera.

As observed in the Key Lake study, all exposure lakes had significantly different Bray-Curtis index values compared to the respective reference site. This further suggests that the Bray-Curtis index might be the most sensitive of the metrics used when evaluating benthic community changes at uranium operations. Previous studies at the Rabbit Lake uranium operation also found that exposure sites (Horseshoe Pond and Hidden Bay) had Bray-Curtis values that were significantly higher than the reference site (Unknown Pond was not sampled) (Golder Associates Ltd., 2003c). With respect to specific benthic invertebrate taxa, Sphaeriidae, Hyalellidae, and Ephemeroptera appear to be the most sensitive groups to the Rabbit Lake uranium operation effluent. Similarly, Borgmann et al. (2004) have noted the absence of Sphaeriidae, Amphipoda, and Ephemeroptera (in addition to Tanytarsini) in an area influenced by extensive mining and smelting operations. Beltman et al. (1999) and Schmidt et al. (2002) have also found Ephemeroptera (in addition to Plecoptera, and Trichoptera) to be sensitive taxa to effects from mining activities.

2.5.2.3 Whole-sediment bioassay

The 2003 Rabbit Lake sediment bioassay revealed no significant differences between exposure sites and the reference site with respect to *H. azteca* survival or final dry weight. Of the variables measured, only day 0 pore-water uranium levels for the exposure sites were high enough to potentially adversely affected survival or final weight of *H. azteca* (Borgmann et al., 2005). However, because overlying water was renewed four times daily, it is believed that sediment pore-water contaminant concentrations were reduced during the 10-d exposure period, thus preventing adverse effects to test organisms.

2.5.2.4 Linking cause and effect

Variables that were considered for the cause of effluent-derived benthic community impairment were significantly different from reference conditions and exceeded their respective Canadian Water Quality Guideline for protection of aquatic life and/or published *H. azteca* toxicity thresholds, and were not exceeded in the reference site for both 2003 and 2004. This excludes all variables that exceed CWQG values and/or published toxicity data in the reference site. With that said, potential cause(s) of benthic community impairment downstream of the Rabbit Lake uranium operation identified through the SQT include physical sediment characteristics, surface water manganese, molybdenum, and uranium levels, and pore-water total ammonia, manganese, iron, arsenic, molybdenum, and uranium concentrations.

Although differences in sediment characteristics were noted between exposure and reference sites in 2003, similar differences were not observed in 2004 suggesting that the effect of sediment composition on benthic community structure (especially in 2004) is largely attributed to something other than sediment structure (e.g., surface water and/or sediment contamination). Differences in benthic community structure in 2003 may have been somewhat influenced by natural differences in sediment type, but because toxicologically relevant levels of some constituents were found in both surface water and sediment (see below), it is believed that contamination played a significant role in benthic community impairment in both 2003 and 2004.

Ammonia was initially identified as a potential stressor of concern within some exposure site pore-waters. Mean un-ionized pore-water values (calculated from total ammonia; Emerson et al., 1975) for exposure lakes ranged from 0.884 mg/L to 2.695 mg/L in 2003 (0.012 mg/L in reference) and from 0.847 mg/L to 3.218 mg/L in 2004 (0.007 mg/L in reference). All of these pore-water values are within the range of published LC50 values for invertebrates (Constable et al., 2003), demonstrating that ammonia could be influencing survival, growth, and/or reproduction of sensitive aquatic invertebrate taxa within the receiving environment at Rabbit Lake. Unlike at Key Lake, ammonia is not a major constituent of the Rabbit Lake effluent. The cause of high ammonia levels at the Rabbit Lake exposure sites is unknown, but could be related to decomposition of organic matter resulting from increase productivity linked to elevated levels of phosphorus in surface water, pore-water, and sediment within the exposure lakes. Significantly higher levels of TOC in the Rabbit Lake exposure sites further support this hypothesis.

As previously discussed, arsenic and molybdenum have both been identified as contaminants of concern downstream from metal mining activities. The average pore-water concentration of arsenic in Unknown Pond is noteworthy (~360 µg/L) when compared to the water only 7-d LC50 values published by Borgmann et al. (2005) (~500 µg/L), suggesting pore-water arsenic might have had an effect on benthic communities in Unknown Pond. Although there is relatively little information published on the chronic toxicity of molybdenum to aquatic invertebrates, molybdenum toxicity data suggest that molybdenum is probably not the primary cause of benthic community impairment at Rabbit Lake.

Schmidt et al. (2002) concluded that benthic community impairment in a stream influenced by mining activity was probably associated with surface water aluminum and iron from acid mine drainage beyond the zone of pH depression. Surface water concentrations of iron from the above study ranged from 1.09 mg/L to 6.80 mg/L. Borgmann et al. (2005) reported a tap water LC50 of >3.150 mg/L. The average iron pore-water concentrations for the 2004 exposure sites (iron was not measured in 2003) ranged from 18.4 to 33.1 mg/L (0.108 mg/L at the reference site). These data suggest that pore-water iron concentrations in Unknown Pond and Horseshoe Pond could possibly be contributing to benthic community impairment within these water bodies and should be further investigated. Schmidt et al. (2002) also noted a similar (although not as pronounced) correlation between surface water manganese, which ranged from 0.35 to 1.11 mg/L, and benthic community impairment. Acute toxicity data of manganese to *H. azteca* can range from 0.169 mg/L for the anion salt in tap water (Borgmann et al., 2005) to 13.7 mg Mn/L for $MnCl_2$ in hard water (Lasier, 2000). *Chironomus plumosus* appears to be even more sensitive with a 96-h LC50 of 0.055 mg/L (Fargašová, 1997). Mean surface water manganese concentrations of the exposure lakes studied here ranged from 0.079 to 0.179 mg/L in 2003 and from 0.188 to 0.197 mg/L in 2004. Mean pore-water manganese concentrations for the exposure lakes ranged from 0.297 to 0.748 mg/L in 2003 and from 0.324 to 0.326 mg/L in 2004. These data suggest that manganese could potentially play a role in benthic community impairment downstream of the Rabbit Lake operation.

Not surprisingly, uranium has been identified as a constituent of concern from uranium mining and milling activities, including the Rabbit Lake uranium operation (Thomas and Liber, 2001; Liber et al., unpublished data). The 14-d LC50 for *H. azteca* for depleted uranium was found to be 1.52 mg/L (Kuhne et al., 2002) which is very comparable to the 7-d LC50 value of 1.651 mg/L reported by Borgmann et al. (2005) (using an atomic absorption uranium standard). Muscatello (2004) found the 10-d LOEC (growth) for *C. tentans* for uranium (as UO^{2+}) to be 157 µg U/L. This exposure concentration also resulted in reductions in adult emergence. Furthermore, Sheppard et al. (2005) derived a freshwater invertebrate ecotoxicity threshold for uranium of 5 µg/L; the reported toxicity endpoints utilized to produce this threshold ranged from 3 µg/L to 5.9 mg/L. Mean uranium concentrations in the surface waters and pore-waters of the Rabbit Lake exposure sites studied here ranged from 32 to 129 µg/L and from 426 to 4587 µg/L, respectively. These data suggest that pore-water uranium concentrations, and to a lesser extent surface water uranium concentrations, are likely contributing to benthic community impairment downstream of the Rabbit Lake operation (especially in Unknown Pond and Horseshoe Pond). Similarly, uranium concentrations could also be a contributing factor to the absence of Hyalellidae from the near-field exposure lakes (Unknown Pond and Horseshoe Pond).

2.6 Conclusions

2.6.1 Key Lake

The toxicologically relevant constituents identified during the Key Lake SQT investigations include physical sediment composition, surface water pH and total ammonia, and pore-water total ammonia and arsenic. Benthic community impairment was noted at all exposure sites. The degree of benthic impairment was more severe in Wolf and Fox Lakes than in Unknown and Delta Lakes, which correlates with higher concentrations of the constituents of concern (except for pH in 2003 which decreased with increasing distance from the effluent source). No toxic response was observed in the *H. azteca* whole-sediment bioassays. This lack of toxicity was hypothesized to be the result of pore-water dilution resulting from the automated overlying water renewal process employed. However, because there were toxicologically relevant levels of some variables in the *in-situ* surface water (such as low pH and total ammonia), the role surface water quality plays in benthic community impairment at the downstream sites is still unknown. Future studies will examine the role both surface water and sediment contamination play in benthic community impairment *in-situ*.

2.6.2 Rabbit Lake

Toxicologically relevant variables that were identified through the Rabbit Lake SQTs include surface water manganese and uranium, and pore-water total ammonia, manganese, iron, arsenic (in Unknown Pond), and uranium. Benthic community impairment was observed at all exposure sites, but was more pronounced in Unknown Pond and Horseshoe Pond than in Hidden Bay. This correlates with lower concentrations of toxicologically relevant constituents in Hidden Bay. As reported for the Key Lake study, there were no significant adverse effects noted in the sediment bioassays, although the 2004 bioassay was invalid due to inadequate reference survival. Again, it is hypothesized that contaminated sediment pore-water was diluted by the automated water renewals and hence did not cause any adverse effects on test organisms. Although uranium in sediment is assumed to be the primary cause of benthic community impairment *in-situ*, this hypothesis will be further evaluated by examining the role both contaminated surface water and sediment play in benthic community impairment at the Rabbit Lake operation.

CHAPTER 3

IN-SITU TOXICITY TO CAGED *HYALELLA AZTECA* DOWNSTREAM OF TWO SASKATCHEWAN URANIUM OPERATIONS

3.1 Abstract

Past monitoring had noted benthic macroinvertebrate community structure impairment downstream of two uranium mine/mill discharge sites in northern Saskatchewan, Canada. This research focused on the use of *in-situ* bioassays, as part of a broader weight-of-evidence approach, in determining the cause(s) of these impacts. The main objectives of this *in-situ* study were to determine if downstream water bodies at the Key Lake and Rabbit Lake uranium operations were toxic to *Hyalella azteca* and, if toxicity was observed, to differentiate between the contribution of surface water and sediment contamination to *in-situ* toxicity. These objectives were achieved by performing 4-d *in-situ* bioassays with laboratory-reared *H. azteca* confined in both surface water and sediment exposure chambers. Results from the *in-situ* bioassays revealed significant mortality at the exposure sites at both Key Lake ($p < 0.001$) and Rabbit Lake ($p = 0.001$). There were no statistical differences between survival in surface water and sediment exposure chambers at either Key Lake ($p = 0.232$) or Rabbit Lake ($p = 0.072$). This suggests that surface water (the common feature of both types of exposure chambers) was the primary cause of *in-situ* mortality of *H. azteca* at both operations, although this relationship was stronger at Key Lake. At Key Lake, the primary cause of aquatic toxicity to *H. azteca* did not appear to be correlated with the variables measured in this study, but most likely with a pulse of organic mill-process chemicals released during the time of the *in-situ* study. The suspected cause of *in-situ* toxicity to *H. azteca* at Rabbit Lake was high levels of uranium in surface water, sediment, and pore-water. The *in-situ* experiments performed provided valuable information for the larger investigation-of-cause studies at both uranium operations.

3.2 Introduction

Through previous monitoring programs, benthic macroinvertebrate community impairment had been noted downstream of the final effluent discharge site at both the Key Lake (Terrestrial and Aquatic Environmental Managers Ltd., 1994; Conor Pacific Environmental Technologies Inc., 2000; Golder Associates Ltd., 2002; Chapter 2) and the Rabbit Lake (Terrestrial and Aquatic Environmental Managers Ltd. and SENES Consultants Ltd., 2006; Liber et al., unpublished data; Golder Associates Ltd., 2003c; Chapter 2) uranium mining and milling operations in northern Saskatchewan, Canada. The present study describes the use of *in-situ* bioassays as part of a broader weight-of-evidence approach to determine the cause(s) of these impacts. Preliminary research using a Sediment Quality Triad (SQT) approach confirmed the presence of an effect on benthic community structure, in addition to significant differences in surface water, pore-water and whole-sediment chemistry at the downstream exposure sites of both uranium operations (Chapter 2). However, results from the 10-d whole-sediment bioassays using laboratory-reared *Hyalella azteca*, and the identification of toxicologically relevant stressors in exposure site surface waters, indicated that contaminated sediments may not be the primary cause of benthic macroinvertebrate community impairment at either uranium operation (Chapter 2). These results triggered a more expanded weight-of-evidence approach which included the use of *in-situ* bioassays.

In-situ bioassays can achieve more realistic exposures than laboratory experiments by integrating complex, site-specific conditions (Chappie and Burton, 2000). Because of this, *in-situ* bioassays have been used to assess the toxicity of both surface water (Pereira et al.,1999; Schmidt et al., 2002; de Bisthoven et al., 2004; Tucker and Burton, 1999; Smolders et al., 2004; Schulz, 2003) and sediment environments (Sibley et al., 1999; Crane et al., 2000; Kater et al., 2001). Some studies have assessed both exposure routes, but did not use the same test species (Shaw and Manning, 1996). This study focused on assessing the toxicity of both environmental matrices to *H. azteca* in order to make stronger comparisons between the two potential exposure pathways. *Hyalella azteca* was chosen for this study because it has been successfully used in caged *in-situ* studies before (Tucker and Burton, 1999; Shaw and Manning, 1996; Chappie and

Burton, 1997) is sensitive to a broad range of toxicants, is native to the study area reference sites (Chapter 2), has standardized experimental protocols (Environment Canada, 1997), and, due to its epi-benthic nature, is suitable for use in both water and surface water/sediment interface environments.

In-situ bioassays can also provide unique and valuable information in both weight-of-evidence and investigation-of-cause research. *In-situ* studies have previously been linked to field bio-monitoring (Maltby et al., 2002) and to laboratory tests (Pereira et al., 1999), as well as used to identify temporal and spatial issues in complex and variable environments (Tucker and Burton, 1999; Schulz, 2003). These qualities made the present *in-situ* study a valuable component of the broader investigation-of-cause research. The specific experimental aim of this study was to determine if waterbodies immediately downstream of the Key Lake and Rabbit Lake uranium operations affected *H. azteca* survival and, if effects were observed, to differentiate between the contribution of surface water and sediment contamination to *in-situ* toxicity.

3.3 Materials and methods

3.3.1 Test organisms

Hyalella azteca were obtained from an in-house culture maintained in an environmental chamber with a set photoperiod of 16:8 hour light:dark and a temperature of 23 ± 1°C. Municipal carbon-filtered water with a hardness of ~130 mg/L as $CaCO_3$, an alkalinity of ~80 mg/L as $CaCO_3$, and a pH of ~ 8 was used for culturing. Animals were fed *ad-libidum* with a Tetramin® (Tetra Werke, Melle, Germany) fish food slurry, *Scenedesmus sp*. and occasionally laboratory-cultured biofilm (unknown composition). Silica sand (particle size = 425 to 850 µm) and cheesecloth gauze were used as substrates.

Hyalella azteca from the stock culture were acclimated for three weeks (10% increase per day) to approximate site-specific water hardness and alkalinity prior to use *in-situ* (based on surface water and pore-water data collected during the previous field season (Chapter 2)). The animals that were designated for use at reference sites were acclimated to a total hardness of ~20 as $CaCO_3$mg/L (low hardness *H. azteca*), whereas the animals that were designated for use at the exposure sites were acclimated to a total hardness of ~900 mg/L as $CaCO_3$ (high hardness *H. azteca*). Both the low hardness and the high hardness animals were acclimated to ~10 mg/L of alkalinity as $CaCO_3$.

Once acclimated, organisms were cultured at the target hardness and alkalinity levels, and adult *H. azteca* isolated into breeding jars. Juveniles were subsequently collected at an age of 0-7 days old and immediately transported to the Key Lake and Rabbit Lake uranium operations (on separate trips) in 250-mL bottles filled with the appropriate reconstituted water and pieces of cheesecloth. Test organisms were fed adequately and kept at room temperature during transportation. Dissolved oxygen (DO) and temperature were monitored using an ORION® dissolved oxygen meter model 835 (ORION Research, Beverly, MA, USA). When DO dropped below 7.0 mg/L, the 250-mL transport bottles were aerated. The organisms used at the reference sites and the nearest downstream sites (Wolf Lake for Key Lake study and Unknown Pond for Rabbit Lake study) were 2-9 days old. However, due to time constraints, the second exposure sites (Fox Lake for Key Lake study and Horseshoe Pond for Rabbit Lake study) used animals that were 3-10 days old.

3.3.3 Study sites

The Key Lake and Rabbit Lake uranium operations are located in northern Saskatchewan, Canada (Figure 1.1). Downstream field sites were selected on the basis of previously documented benthic impairment (Terrestrial and Aquatic Environmental Managers Ltd., 1994; Terrestrial and Aquatic Environmental Managers Ltd. and SENES Consultants Ltd., 1996; Conor Pacific Environmental Technologies Inc., 2000; Golder Associates Ltd., 2002; Golder Associates Ltd., 2003c; Liber et al., unpublished data; Chapter 2). The placement of *in-situ* chambers was dictated by sediment type and water depth. Because this study was part of a larger weight-of-evidence investigation (including benthic community assessment), selecting sites with similar sediment/habitats was critical. In general, “organic” and/or “depositional” sediment types were chosen at water depths ranging from 60 to 100 cm.

3.3.3.1 Key Lake

The Key Lake operation began production in 1983 as a mine and mill site and is currently the largest high-grade uranium milling operation in the world (www.cameco.com). Although on-site ore has been depleted, the Key Lake mill currently processes ore from another near-by mine, McArthur River. Constituents within the wastewater effluent can be of both mining and milling origin considering many processes recycle contaminated water (Cameco Corporation, 2004).

The Key Lake discharge basin is a series of small lakes connected through creeks. The site of effluent discharge is Wolf Lake (Wolf Lake outflow is estimated to contain 72% effluent (Golder Associates Ltd., 2003a)). From Wolf Lake the effluent flows through Fox Lake entering Yak Creek (estimated to contain 69% effluent (Golder Associates Ltd., 2003a)) and then David Creek (estimated to contain 37% effluent (Golder Associates Ltd., 2003a)). The diluted effluent then flows through Unknown Lake to Delta Lake via David Creek and eventually drains into the Wheeler River (Figure 1.2). For the Key Lake *in-situ* study (July 7-12, 2004), two exposure sites and one reference site were used. The exposure sites were located at the outflow of Wolf Lake (July 7-11, 2004) and the inlet of Fox Lake (July 8-12, 2004). The reference site was located in David Lake (July 7-11, 2004).

3.3.3.2 Rabbit Lake

Rabbit Lake is the second largest uranium milling operation in the world (www.cameco.com). Rabbit Lake continues to mine and mill on-site ore. Effluent wastewater constituents can be of both mining and milling origin (Cameco Corporation, 2003).

The Rabbit Lake discharge basin is a series of two ponds and a bay of a large lake. The site of discharge is at the head waters of Horseshoe Creek (estimated to contain 50 to 100% effluent, depending on seasonal variability (Golder Associates Ltd., 2003b)). From Horseshoe Creek the effluent flows into Unknown Pond and then Horseshoe Pond via Horseshoe Creek. From Horseshoe Pond the effluent re-enters Horseshoe Creek which then flows into Hidden Bay, a large bay on Wollaston Lake (Figure 1.3). The exposure sites for the Rabbit Lake *in-situ* study (August 6-11, 2004) were the inlet of

Unknown Pond (August 6-10, 2004) and near the middle of Horseshoe Pond (August 7-11, 2004). The reference site was a nearby shallow lake (Reference Lake) (August 6-10, 2004).

3.3.4 *In-situ* chamber design

Two types of chambers were designed and used. The chambers were constructed from 5-cm diameter acrylic sediment core-tubes with plastic core-caps and were similar to the chambers used by Jacher, (1994) and Chappie and Burton (1997). However, in the present study chambers were placed vertically rather than horizontally. One chamber design represented the surface water exposure route (surface water chamber) and the other represented the combined surface water/sediment interface exposure route (sediment chamber) (Figure 3.1). Both exposure chambers were designed to contain the same volume of water. Surface water chambers were 11-cm tall and sediment chambers were 16-cm tall (to account for a 5-cm deep sediment core). Four 2.5-cm x 4-cm openings were cut into the sides of the chambers and covered with 300-µm Nitex® screen (Wildlife Supply Company, Buffalo, NY, USA) using aquarium silicone adhesive. These Nitex®-covered openings allowed water movement through the chamber during the exposure period, but retained test organisms. Plastic core-caps were used on both the top and bottom of the exposure chambers. The top core-cap had a 1 x 1-cm opening covered with 300-µm Nitex® screen which allowed for the displacement of air bubbles as the chambers were deployed. Chambers were attached to wooden stakes by plastic zip-ties.

3.3.5 *In-situ* chamber deployment and retrieval

Surface water chambers (with the bottom core-cap in place) were filled to the bottom of the Nitex®-covered openings with 53-µm filtered site water. A piece of pre-soaked cheese-cloth (2.5 x 2.5-cm) with laboratory-cultured biofilm (unknown composition) was used as a substrate with associated food source.

For sediment chambers, sediment cores were collected using a hand-held corer (5-cm outside diameter) and extruded into the bottom of the sediment chamber to a depth of approximately 5 cm. Once the sediment core was in place, a bottom core-cap

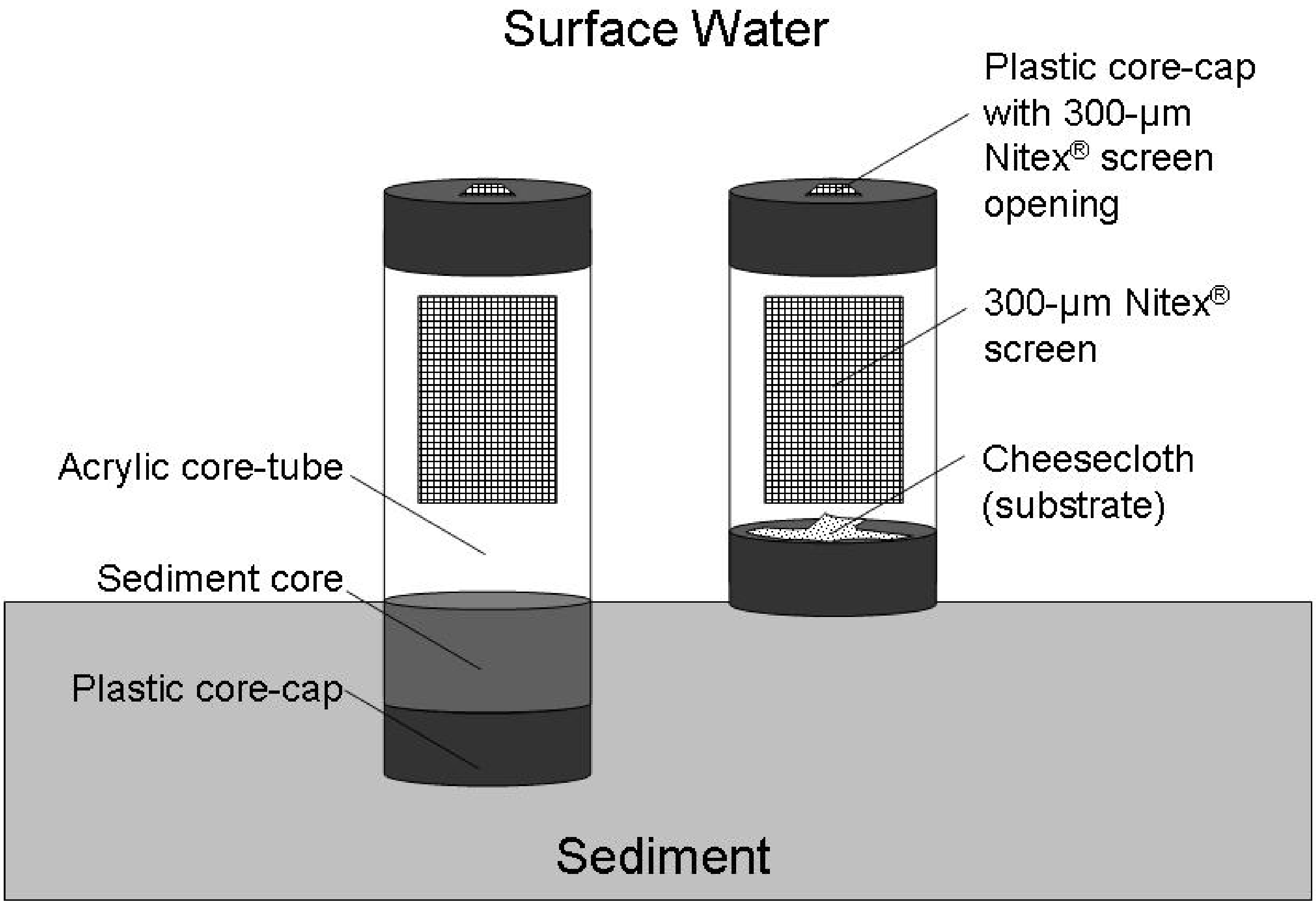

Figure 3.1. Design and relative placement of *in-situ* sediment exposure chambers (left) and surface water exposure chambers (right).

was added. This maintained the natural sediment profile while ensuring that the chamber could be retrieved without losing the sediment and test animals. Once the bottom core cap was secured, 53-µm filtered site water was carefully added to the depth of the bottom of the Nitex®-covered openings while trying to avoid disrupting the sediment profile.

Once chambers were partially filled with filtered site water, ten juvenile *H. azteca* were gently added to each chamber using a plastic pipette. A top core-cap was then added and each chamber attached to a wooden stake by two plastic zip-ties. Surface water chambers were placed so the bottom of the chamber was directly above the sediment surface, while sediment chambers were placed so that the sediment-water interface inside the sediment chamber was in-line with the sediment-water interface of the study site (Figure 3.1). Chambers were placed approximately 30-cm from one another and proper placement was ensured through the use of a Wildco®Aquascope II (Wildlife Supply Company, Buffalo, NY, USA). Four surface water chambers and four sediment chambers were deployed at each site.

Chambers were retrieved four days later by gently pulling the stakes from the sediment, cutting the zip ties, and placing the chambers in a cooler filled with site water. Once retrieved from a site, chambers were immediately brought back to an indoor facility where test animals were removed and enumerated with the use of a light table.

3.3.6 Physicochemical characterization

Surface water samples were collected using a Wildco® 3.2-L Van Dorn horizontal acrylic beta water sampler (Wildlife Supply Company, Buffalo, NY, USA) from ~15 cm above the sediment surface on both day 0 and day 4 of each study. Samples were sieved through a 53-µm sieve to remove zooplankton. Water samples were analyzed for pH (Beckman® 250 pH/Temp/mV meter with a Beckman® pH electrode 511050, Beckman Instruments Inc., Fullerton, CA, USA) and total ammonia (Beckman® 250 pH/Temp/mV meter with a Thermo Orion® 95-12 ammonia electrode or Orion aquafastII photometer, ORION Research, Beverly, MA, USA) on the day of collection. Sub-samples were removed for the analysis of total metals, acidified with double distilled (ultra pure) nitric acid, and stored in the dark at 4°C until later analyzed using ICP-MS (Department of Geological Sciences, University of Saskatchewan). Additional water samples were collected during the length of the *in-situ* bioassay and analyzed within one week of collection for conductivity (Orion ATI conductivity cell 017010 and Orion ATI meter 170), hardness and alkalinity (Hach Digital Titrator model 16900, Hach Company, Loveland, CO, USA). Dissolved oxygen and temperature were measured on day 0 and day 4 outside of the *in-situ* chambers ~5-cm above the sediment surface.

Sediment cores were also collected during the *in-situ* bioassay and processed upon return to the laboratory (within 1 week for Key Lake samples and within 2 weeks for Rabbit Lake samples). Processing included isolating the top 2.5-cm horizon of each sediment core, homogenizing the sample, and taking sub-samples to determine sediment total organic carbon (TOC, Leco Carbon determinator CR-12, Leco, St.Joseph, MI, USA), particle size (performed by Enviro-Test Laboratories, Saskatoon, SK, Canada), and water content. A separate sub-sample was removed to isolate pore-water via centrifugation at 12,000 rpm (17,200 rcf) at room temperature for 20 min. Once collected, pore-water was filtered through a 0.45-µm membrane (Nalgene® acetate syringe filter, Nalge Nunc International, Rochester, NY, USA) and analyzed for pH (ORION® PerpHect LogR meter model 370) ammonia, hardness, and alkalinity on the day of collection. Sub-samples were analyzed for dissolved metals via ICP-MS and dissolved organic carbon (DOC) using a Shimadzu organic carbon analyzer 5050A (Shimadzu, Tokyo, Japan).

Amphipods recovered from the test chambers were rinsed with site-specific reconstituted water, transferred to Petri dishes where excess water was removed, and stored on ice until being transported to the University of Saskatchewan. Test organisms were then dried at 60ºC for $\geq$ 24 h, weighed, and analyzed for metals using ICP-MS.

3.3.7 Statistics and data analysis

All statistics were performed using SigmaStat®, version 3.1 (SPSS Inc., Chicago, IL, USA). If normality or homogeneity of variance failed, data were transformed using a $\log_{10}$ transformation or, in the case of ratio or percent data, an arcsine square-root transformation. pH data were not transformed. For consistency, transformed physicochemical data sets that still failed tests for normality or homogeneity of variance still underwent parametric analysis. Physicochemical data describing general surface water, pore-water, and sediment characteristics were analyzed using one-way ANOVA (with lake as the independent factor). Comparisons between day 0 and day 4 surface water measurements, and between corresponding surface water and pore water measurements, were both analyzed using two-way ANOVA (with lake and sample day, or lake and water type as the independent factors, respectfully). If a statistical difference was detected ($p \leq 0.05$), a Tukey's all pair-wise multiple comparison post-hoc test was run.

For ease of interpretation of surface water and pore-water data, only the variables meeting the following criteria were presented/assessed: the mean value exceeded the Canadian water quality guideline (CWQG) for protection of aquatic life (Canadian Council of Ministers of the Environment, 1999); the mean value exceeded published *H. azteca* toxicity values (Borgmann et al., 2005; France and Stokes,1987; Ankley et al., 1995); analytical detection limits exceeded CWQG guidelines or reported toxicity values; or elements that did not have published water quality guidelines and/or toxicity data (Table 2.1).

The number of amphipods recovered from the *in-situ* chambers was considered the survival data. Damaged chambers (with damaged or loose Nitex® screen), or chambers that had a loss of sediment during enumeration, were not used in the analysis. A two-way ANOVA was conducted with lake and chamber type as the independent factors. If a statistically significant difference was found ($p \leq 0.05$), a Tukey's all pair-wise multiple comparison procedure post hoc test was run.

For ease of interpretation, presentation of trace element data in amphipod tissue has been limited to those elements where surface water and/or pore water concentrations exceeded published high hardness *H. azteca* toxicity thresholds (Borgmann et al., 2005) at exposure sites, but not at reference sites. Due to low amphipod survival at exposure sites and low sample weights, replicates were pooled before analysis (all four replicates for Key Lake samples, and replicates 1 + 4 and 2 + 3 for Rabbit Lake samples). Because of the low samples sizes, statistical analysis was not performed on these data.

3.4 Results

3.4.1 Key Lake

3.4.1.1 Surface water, pore-water and sediment characterization

The physicochemical characteristics of surface water, pore-water, and sediment for the Key Lake study sites are presented in Table 3.1. Wolf and Fox Lakes had significantly higher surface water total ammonia, hardness and conductivity, and significantly lower pH, levels than David Lake. In addition, pore-water from exposure lakes had significantly higher pH, total ammonia, and hardness than the reference lake. Generally, surface water and pore-water conditions at the two exposure sites were very similar. Sediment composition was different among all lakes. Comparisons between exposure sites and the reference site reveal significantly higher silt, TOC, and water content at the exposure sites, and higher sand at the reference site. Water quality variables, including trace elements, measured on days 0 and 4 were generally very similar with few significant differences. These differences were judged to be biologically insignificant, so for simplicity, days 0 and 4 measurements were averaged.

3.4.1.2 Surface water and pore-water comparisons

Surface water and pore-water conditions were generally very different within each site. Generally, pore-water had higher concentrations of total ammonia and alkalinity. Pore-water hardness levels were similar or higher than surface water hardness levels. David Lake surface water pH was lower than the pore-water pH. Conversely, Wolf Lake and Fox Lake surface waters pH was higher than in their respective pore-waters.

Trace metals concentrations in pore-water were generally higher than their respective surface water concentrations (Table 3.2). Among study sites, Wolf Lake and Fox Lake had significantly higher surface water and pore-water magnesium, phosphorus, calcium, arsenic, molybdenum, and uranium than the reference site. Wolf Lake and Fox Lake also had significantly higher surface water manganese and copper than David Lake. Overall, Wolf Lake and Fox Lake trace element concentrations were similar.

Table 3.1. Mean (± standard deviation, *n* = 4) physicochemical surface water, pore-water, and sediment characteristics of the three study sites at the Key Lake uranium operation.

Characteristic	David Lake	Wolf Lake	Fox Lake
Surface-water			
Depth (cm)	103 ± 1	99 ± 2[*]	78 ± 2[*,†]
Temperature (°C)	20.0 ± 0.7[a]	22.2 ± 0.1[a,*]	20.1 ± 0.2[a,†]
DO[b] (mg/L)	7.9 ± 0.4[a]	8.0 ± 0.1[a]	7.3 ± 0.8[a,†]
pH	6.87 ± 0.08[a]	6.12 ± 0.16[a,c,*]	5.74 ± 0.35[a,c,*,†]
Total Ammonia (mg N/L)	<0.05[a]	5.39 ± 0.20[a,c,*]	4.25 ± 0.39[a,c,*,†]
Alkalinity (mg/L as $CaCO_3$)	8 ± 1	7 ± 1	7 ± 1
Total Hardness (mg/L as $CaCO_3$)	6 ± 1	610 ± 20[*]	580 ± 10[*]
Conductivity (µS/cm)	14 ± 0	1190 ± 10[*]	1152 ± 16[*,†]
Pore Water			
pH	5.28 ± 0.16[c]	6.62 ± 0.41[d,*]	6.56 ± 0.13[*]
Total Ammonia (mg/L)	<0.5	7.7 ± 0.4[c,d,*]	6.8 ± 1.0[c,*]
Alkalinity (mg/L as $CaCO_3$)	16 ± 4[d]	29 ± 20[d]	28 ± 16
Total Hardness (mg/L as $CaCO_3$)	<2	830 ± 60[d,*]	600 ± 80[*,†]
DOC[e] (mg/L)	31.6 ± 8.6[f]	22.0 ± 2.3[d]	27.8 ± 3.5[d]
Sediment			
Sand (%)	98 ± 0[d]	28 ± 3[d,*]	84 ± 7[d,*,†]
Silt (%)	1 ± 1[d]	57 ± 3[d,*]	12 ± 4[d,*,†]
Clay (%)	1 ± 0[d]	14 ± 2[d,*]	5 ± 3[d,†]
TOC[g] (%)	1.2 ± 0.4	10.2 ± 1.0[d,*]	4.9 ± 1.5[*,†]
Water Content (%)	40.1 ± 3.4	85.3 ± 3.1[d,*]	71.1 ± 6.0[*,†]

[a] *n* = 8 (average of day 0 (*n* = 4) and day 4 (*n* = 4) measurements).

[b] DO = dissolved oxygen.

[c] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999).

[d] *n* = 3.

[e] DOC = dissolved organic carbon.

[f] *n* = 2.

[g] TOC = total organic carbon.

[*] Significantly different (one-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from David Lake measurement.

[†] Significantly different (one-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from Wolf Lake measurement.

Table 3.2. Mean (± standard deviation) concentrations of trace element surface water ($n = 6$) measurements and pore-water ($n = 3$) measurements from Key Lake study sites that met one or more of the chosen criteria (see text for details).

Variable	David Lake		Wolf Lake		Fox Lake	
(µg/L)	Surface Water	Pore Water	Surface Water	Pore Water	Surface Water	Pore Water
Mg	437 ± 77	236 ± $104^{\ddagger}$	17514 ± 573^{*}	21690 ± 1898^{*}	15168 ± 955^{*}	18665 ± 420^{*}
Al	35.1 ± 33.4^{a}	154.4 ± 104.4^{ab}	62.8 ± 24.0^{a}	79.5 ± 132.2^{a}	67.4 ± 35.5^{a}	133.6 ± 123.4^{ab}
Si	1462 ± 724	4703 ± $154^{\ddagger}$	1381 ± 422	17677 ± $4518^{*\ddagger}$	871 ± 473	7659 ± $565^{\ddagger}$
P	76.6 ± 36.6	131.0 ± 98.2	300.6 ± 41.5^{*}	384.1 ± $45.1^{*\ddagger}$	265.0 ± 68.5^{*}	287.0 ± 14.9^{*}
Ca	1102 ± 105	1069 ± 287	185751 ± 7199^{*}	290880 ± $16942^{*\ddagger}$	173920 ± 15413^{*}	219292 ± $7588^{*\dagger\ddagger}$
Cr	$<1.11^{a}$	4.86 ± $1.23^{ab\ddagger}$	2.24 ± 1.10^{a}	14.00 ± $13.48^{ab\ddagger}$	3.06 ± 3.36^{ab}	13.55 ± $3.93^{ab\ddagger}$
Mn	15.0 ± 1.3	82.1 ± $39.3^{\ddagger}$	117.9 ± 6.0^{b*}	55.8 ± $10.2^{\ddagger}$	79.6 ± $6.1^{*\dagger}$	252.5 ± $84.3^{bc*\dagger\ddagger}$
Fe	532 ± 82^{a}	1562 ± $711^{ab\ddagger}$	390.62 ± 85.73^{a}	2827 ± $1897^{abc\ddagger}$	922 ± 1022^{ab}	27175 ± $10588^{abc*\dagger\ddagger}$
Cu	0.45 ± 0.31	1.47 ± $0.41^{\ddagger}$	2.15 ± 0.46^{a*}	1.09 ± $0.59^{\ddagger}$	1.99 ± 0.87^{*}	1.90 ± 1.27
As	<0	3 ± $1^{\ddagger}$	13 ± 1^{a*}	4095 ± $2859^{abc*\ddagger}$	17 ± 8^{a*}	3041 ± $1549^{abc*\ddagger}$
Se	$<2.91^{a}$	5.64 ± 7.25^{a}	$<2.91^{a}$	2.12 ± 1.15^{a}	2.75 ± 2.46^{a}	5.86 ± 7.63^{a}
Mo	1 ± 1	6 ± 7	338 ± 12^{a*}	5919 ± $3278^{abc*\ddagger}$	389 ± 12^{a*}	3536 ± $140^{abc*\ddagger}$
Ag	<0.04	4.79 ± $4.26^{abc\ddagger}$	0.08 ± 0.08	1.18 ± $0.64^{abc\ddagger}$	<0.04	$<0.04^{*\dagger}$
Cd	0.07 ± 0.08^{a}	0.12 ± 0.15^{a}	0.16 ± 0.31^{ab}	$<0.06^{a}$	$<0.06^{a}$	$<0.06^{a}$
Tm	0.01 ± 0.01^{b}	$<0.01^{\ddagger}$	<0.01	<0.01	<0.01	<0.01
Hg	$<0.16^{a}$	$<0.16^{a}$	$<0.16^{a}$	$<0.16^{a}$	$<0.16^{a}$	$<0.16^{a}$
U	0.03 ± 0.02	0.27 ± $0.20^{\ddagger}$	3.79 ± 0.93^{*}	3.50 ± 4.08^{*}	1.52 ± 0.55^{*}	5.49 ± $0.93^{*\ddagger}$

[a] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999); [b] Exceeds soft water median lethal concentration for *Hyalella azteca* (Borgmann et al.,2005); [c] Exceeds hard water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005); * Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from David Lake measurement; [†] Significantly different ($p \leq 0.05$) from the respective Wolf Lake measurement; [‡] Significant difference ($p < 0.05$) from the respective surface water measurement.

3.4.1.3 *In-situ* bioassay

Mean *H. azteca* survival is presented in Figure 3.2. There was a significant difference between lakes ($p < 0.001$), but not between chamber types ($p = 0.232$). There was no significant interaction ($p = 0.192$). More specifically, there were significant differences when comparing amphipod survival in David Lake surface water chambers with survival in both Wolf Lake ($p < 0.001$) and Fox Lake ($p < 0.001$) surface water chambers. In addition, there were significant reductions in both Wolf Lake ($p < 0.001$) and Fox Lake ($p < 0.001$) sediment chamber survival compared to David Lake sediment chamber survival. There were no significant differences between Wolf Lake and Fox Lake survival data for both chamber types. Concentrations of selected elements in bodies of surviving *H. azteca* are presented in Table 3.3.

3.4.2 Rabbit Lake

3.4.2.1 Surface water, pore-water and sediment characterization

The physicochemical characteristics of surface water, pore-water, and sediment for the Rabbit Lake study sites are presented in Table 3.4. Exposure sites (Unknown Pond and Horseshoe Pond) had significantly higher surface water hardness and conductivity, and lower surface water pH, than the reference site. Unknown Pond and Horseshoe Pond had significantly higher pore-water total ammonia and hardness. Overall, surface water and pore-water conditions at the two exposure sites were very similar. Sediment composition was very comparable among all lakes with few statistical differences. Day 0 and day 4 water quality measurements displayed few significant temporal differences. Therefore, averages of day 0 and day 4 measurements are presented in Table 3.4 (depth, temperature, dissolved oxygen, pH, and total ammonia) and Table 3.5 (selected trace metals).

3.4.2.2 Surface water and pore-water comparisons

Surface water and pore-water conditions were generally very different within each site. Generally, total ammonia and alkalinity were higher in pore-water than in surface water. Pore-water hardness levels were similar to surface water hardness levels.

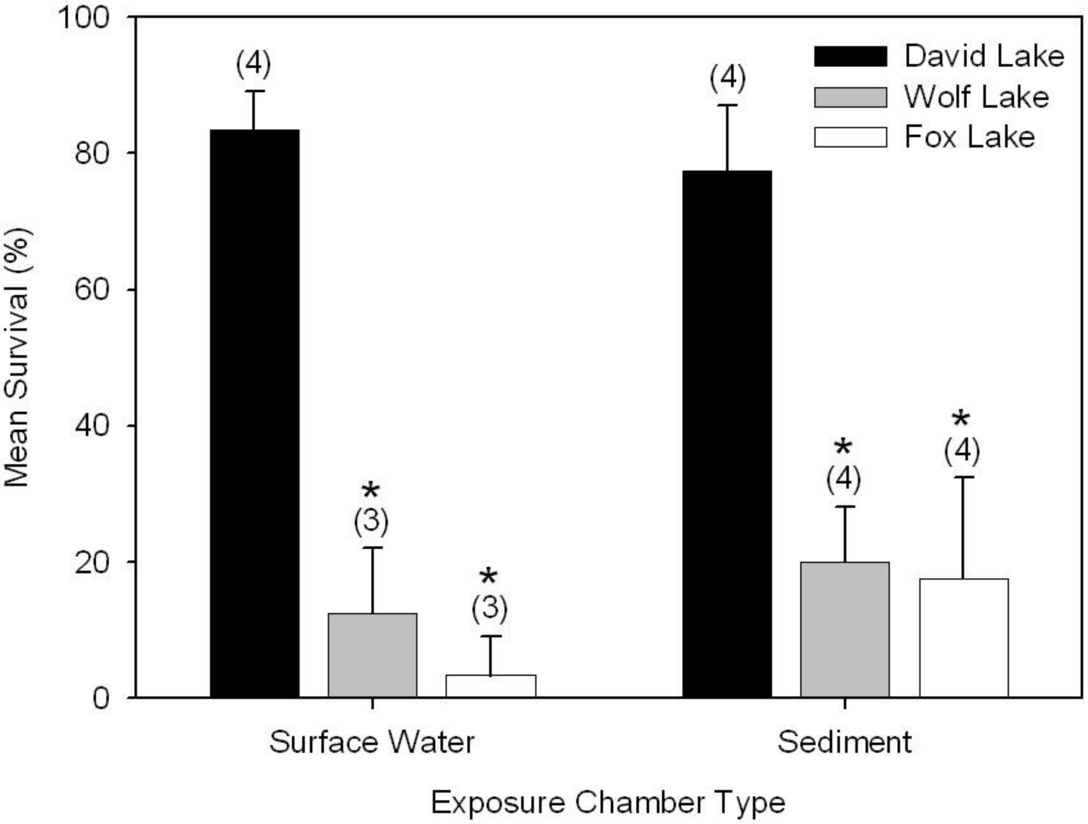

Figure 3.2. Mean percent survival (± standard deviation) of caged *Hyalella azteca* in surface water and sediment exposure chambers at the Key Lake uranium operation. Asterisks denote a significant difference from the respective reference site (David Lake). Sample size is noted within parentheses.

Table 3.3. Tissue concentrations (µg/g) of caged *Hyalella azteca* exposed in both surface water (SW) and sediment (SED) exposure chambers *in-situ* for 4-d at three water bodies near the Key Lake uranium operation.[a]

	David Lake		Wolf Lake		Fox Lake	
Variable	SW	SED	SW	SED	SW	SED
Fe	390.6	463.7	<348.5	<348.5	<348.5	691.9
As	<41.9	<41.9	<41.9	<41.9	<41.9	<41.9
Mo	<3.3	<3.3	4.6	16.2	9.5	26.6

[a] Numbers are single measurements. Due to low amphipod survival at exposure sites and low sample weights, all replicates (n = 4) were pooled before analysis.

Table 3.4. Mean (± standard deviation, $n = 4$) physicochemical surface water, pore-water, and sediment characteristics of the three study sites at the Key Lake uranium operation.

Characteristic	Reference Lake	Unknown Pond	Horseshoe Pond
Surface-water			
Depth (cm)	76 ± 1[a]	85 ± 5[a*]	66 ± 6[a*†]
Temperature (°C)	17.0 ± 1.9[a]	18.0 ± 2.1[a]	15.5 ± 1.2[a†]
DO[b] (mg/L)	9.1 ± 0.3[a]	7.7 ± 0.4[a*]	9.0 ± 0.1[a†]
pH	7.3 ± 0.1[c]	6.3 ± 0.1[ad*]	6.6 ± 0.1[a*†]
Total Ammonia (mg N/L)	<0.1[a]	0.2 ± 0.2[a*]	<0.1[a†]
Alkalinity (mg/L as $CaCO_3$)	20 ± 2	7 ± 0[*]	9 ± 1[*†]
Total Hardness (mg/L as $CaCO_3$)	16 ± 1	800 ± 10[*]	820 ± 60[*]
Conductivity (µS/cm)	43 ± 1	1632 ± 5[*]	1677 ± 79[*]
Pore Water			
pH	6.7 ± 0.1	7.0 ± 0.1[*]	6.8 ± 0.1
Total Ammonia (mg N/L)	<0.1[d]	11.6 ± 4.9[d*]	4.1 ± 1.0[d*†]
Alkalinity (mg/L as $CaCO_3$)	44 ± 6	140 ± 58[*]	48 ± 10[e†]
Total Hardness (mg/L as $CaCO_3$)	12 ± 2	940 ± 20[*]	780 ± 60[*†]
DOC[f] (mg/L)	21.9 ± 7.8[e]	39.2 ± 1.4[e*]	27.4 ± 7.7[e]
Sediment			
Sand (%)	39 ± 5[e]	35 ± 9[e]	22 ± 5[e*]
Silt (%)	44 ± 3[e]	46 ± 6[e]	54 ± 4[e]
Clay (%)	17 ± 2[e]	20 ± 4[e]	24 ± 2[e]
TOC[g] (%)	10.0 ± 0.6	24.8 ± 1.4[*]	14.4 ± 1.0[*†]
Water Content (%)	84.3 ± 1.9	88.2 ± 1.5[*]	87.1 ± 1.5

[a] $n = 8$ (average of day 0 ($n = 4$) and day 4 ($n = 4$) measurements).

[b] DO = dissolved oxygen.

[c] $n = 7$ (average of day 0 ($n = 3$) and day 4 ($n = 4$) measurements).

[d] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999).

[e] $n = 3$.

[f] DOC = dissolved organic carbon.

[g] TOC = total organic carbon.

[*] Significantly different (one-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from Reference Lake measurement.

[†] Significantly different (one-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from Unknown Pond measurement.

Table 3.5. Mean (± standard deviation) concentrations of trace element surface water ($n = 6$) measurements and pore-water ($n = 3$) measurements from Rabbit Lake study sites that met one or more of the chosen criteria (see text for details).

Variable	Reference Lake		Unknown Pond		Horseshoe Pond	
(µg/L)	Surface Water	Pore Water	Surface Water	Pore Water	Surface Water	Pore Water
Mg	2662 ± 139	1430 ± 301‡	29407 ± 1094*	51498 ± 12920*‡	28685 ± 4641*	37811 ± 996*†‡
Al	25.9 ± 41.7^{a}	445.1 ± 266.0^{ab‡}	83.9 ± 58.7^{a}	<6.3^{a*‡}	56.7 ± 72.0^{a}	43.5 ± 69.9^{a*}
Si	2367 ± 536	10165 ± 1061‡	2297 ± 618	14084 ± 2423‡	2471 ± 425	8717 ± 582†‡
P	85.2 ± 24.9	80.2 ± 48.2	349.8 ± 85.5*	451.0 ± 72.3*‡	364.6 ± 52.3*	310.5 ± 44.1*†
Ca	3745 ± 385	3134 ± 593‡	272607 ± 12722*	305695 ± 3100*	258589 ± 39916*	257158 ± 5425*
Cr	<1.11^{a}	<1.11^{a}	1.19 ± 1.20^{a}	8.15 ± 3.26^{ab*‡}	1.75 ± 1.54^{a}	7.63 ± 2.89^{ab*‡}
Mn	5.2 ± 0.4	19.0 ± 5.4‡	185.7 ± 3.1^{bc*}	324.4 ± 67.6^{bc*‡}	171.9 ± 27.9^{bc*}	326.0 ± 22.5^{bc*‡}
Fe	40 ± 39	108 ± 44‡	243 ± 76*	33146 ± 7129^{abc*‡}	249 ± 124*	18418 ± 6842^{abc*‡}
Cu	0.70 ± 0.76	4.94 ± 2.35^{a‡}	2.68 ± 0.72^{a*}	2.16 ± 0.43^{a*}	2.44 ± 0.70^{a*}	2.09 ± 0.18^{a*}
As	<0.3	<0.3	8.4 ± 1.3^{a*}	359.2 ± 83.7^{a*‡}	5.8 ± 1.0^{a*†}	55.2 ± 7.3^{a*†‡}
Se	5.97 ± 4.33^{a}	5.45 ± 6.91^{a}	10.51 ± 9.24^{a}	<2.91^{a‡}	14.53 ± 4.84^{a}	6.00 ± 1.56^{a}
Mo	10 ± 9	<0‡	4945 ± 235^{abc*}	8250 ± 431^{abc*}	4682 ± 858^{abc*}	7413 ± 1303^{abc*}
Ag	0.06 ± 0.06	0.02 ± 0.00	<0.04	0.03 ± 0.01	0.03 ± 0.02	0.02 ± 0.01
Cd	0.04 ± 0.04^{a}	0.17 ± 0.01^{ab‡}	0.06 ± 0.07^{a}	<0.06^{a*}	<0.06^{a}	<0.06^{a*}
Tm	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Hg	<0.16^{a}	<0.16^{a}	<0.16^{a}	<0.16^{a}	<0.16^{a}	<0.16^{a}
U	3 ± 0	11 ± 4‡	117 ± 7^{b*}	4587 ± 2605^{bc*‡}	89 ± 16^{b*}	1473 ± 1376^{b*†‡}

Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999); ^{b} Exceeds soft water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005); ^{c} Exceeds hard water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005); * Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from Reference Lake measurement; † Significantly different ($p \leq 0.05$) from the respective Unknown Pond measurement; ‡ Significant difference ($p < 0.05$) from the respective surface water measurement

Surface water pH was lower in reference site pore-water and higher in the exposure site pore-water when compared to their respective surface waters.

Trace element concentrations in pore-water were, in general, significantly higher than in surface water at each site (Table 3.5). Unknown Pond and Horseshoe Pond had higher surface water and pore-water magnesium, phosphorus, calcium, manganese, iron, arsenic, molybdenum, and uranium when compared to the reference site. The exposure sites also had significantly higher pore-water chromium than the reference lake. Overall, Unknown Pond and Horseshoe Pond had relatively similar trace element concentrations.

3.4.2.3 *In-situ* bioassay

Mean *H. azteca* survival is presented in Figure 3.3. A significant difference was noted among lakes ($p = 0.001$), but not between chamber types ($p = 0.072$). There was no significant interaction ($p = 0.809$). There was significantly lower survival in surface water chambers in Unknown Pond ($p = 0.022$), but not in Horseshoe Pond ($p = 0.141$), and significantly lower survival in sediment chambers in both Unknown Pond ($p = 0.017$) and Horseshoe Pond ($p = 0.036$). Amphipod survival in Unknown Pond and Horseshoe Pond was not significantly different within chamber type (surface water $p = 0.609$; sediment $p = 0.921$). Concentrations of selected elements in bodies of surviving *H. azteca* are presented in Table 3.6.

3.5 Discussion

3.5.1 Site characterization

Surface water and pore-water characteristics that appear to have been altered as a result of uranium operation effluent include water hardness, conductivity (in surface water), total ammonia (in only pore-water for Rabbit Lake), some metals, and pH (in surface water). Statistically significant differences seen among and within lakes that were believed to be independent of uranium operation effluent, and thus not suspected of being the primary cause of *H. azteca* mortality in the exposure lakes, include temperature, dissolved oxygen, and selected metals. While not directly measured, it is not believed that DO dropped to unacceptable levels within the exposure chambers and

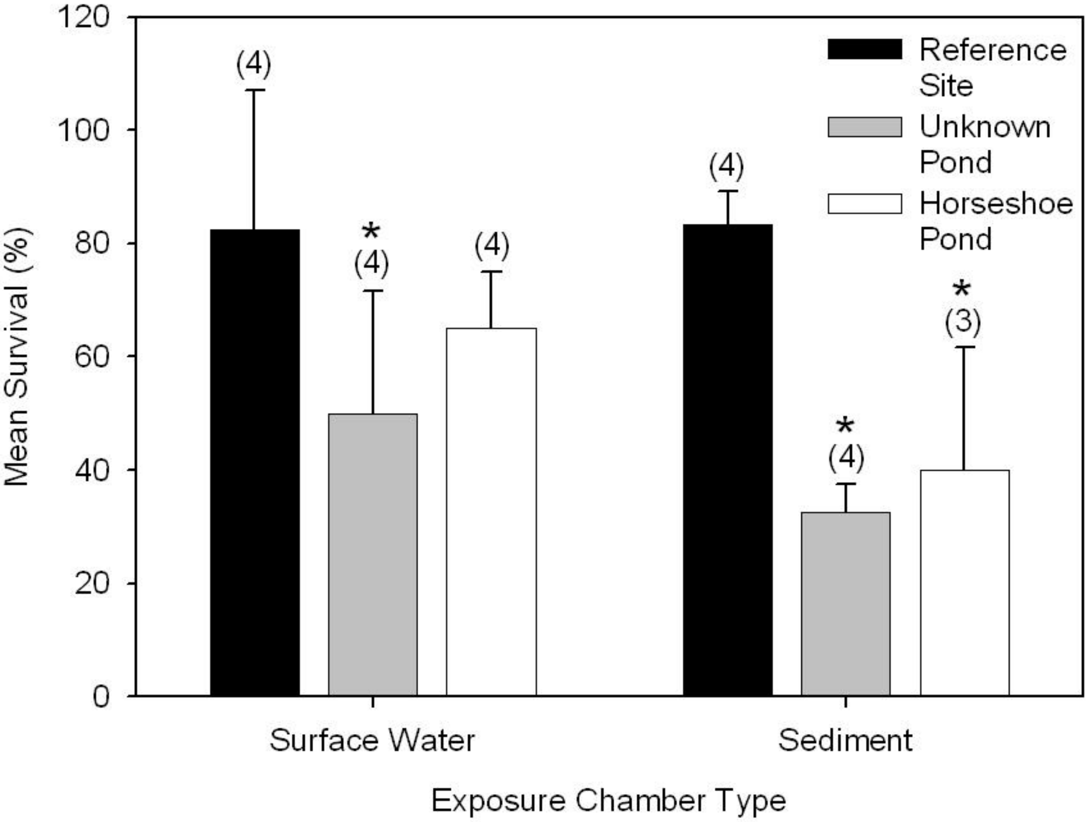

Figure 3.3. Mean percent survival (± standard deviation) of caged *Hyalella azteca* in surface water and sediment exposure chambers at the Rabbit Lake uranium operation. Asterisks denote a significant difference from the respective reference site. Sample size is noted within parentheses.

Table 3.6. Tissue concentrations (µg/g) of caged *Hyalella azteca* exposed in both surface water (SW) and sediment (SED) exposure chambers *in-situ* for 4-d at three water bodies near the Rabbit Lake uranium operation.[a]

	Reference Lake		Unknown Pond		Horseshoe Pond	
Variable	SW	SED	SW	SED	SW	SED
Mn	11.4 ± 12.5	9.6 ± 5.0	9.7 ± 1.1	<5.0	<5.0	9.7 ± 5.4
Fe	408.5 ± 331.3	<348.5	<348.5	<348.5	<348.5	386.4 ± 43.7
Mo	<3.3	<3.3	74.0 ± 14.4	48.9 ± 30.9	16.9 ± 1.8	47.9 ± 13.6
U	2.2 ± 2.2	6.0 ± 7.3	16.9 ± 6.1	13.3 ± 1.1	5.5 ± 0.9	24.7 ± 6.7

[a] Numbers are an average of two measurements. Due to low amphipod survival at exposure sites and low sample weights, replicates 1 + 4 and 2 + 3 were pooled before analysis.

thus could have affected *H. azteca* survival. This is because DO was high (>7 mg/L) directly outside of the *in-situ* chambers, the 300-μm Nitex® screens should have provided adequate water and gas exchange with the chambers, and the *H. azteca* response in reference sediment with high TOC and small particle size (see Rabbit Lake sediment characteristics) was high (83%).

3.5.2 Linking cause and effect

3.5.2.1 Key Lake

The *in-situ* study at the Key Lake operation revealed that the immediate downstream receiving environment (Wolf Lake and Fox Lake) adversely affected *H. azteca* survival. Furthermore, the results suggested that surface water was the primary cause of toxicity since there was no significant difference between amphipod survival in surface water and sediment exposure chambers (the sediment did not appear to influence the *in-situ* toxicity to *H. azteca*). In fact, mean survival in sediment chambers was higher than in surface water chambers (20% vs. 13% in Wolf Lake and 18% vs. 3% in Fox Lake), suggesting that sediment may have acted as a source of protection from the cause of toxicity. Limited protection could result from burrowing (partly avoiding toxic surface water), or from decreased contaminant bioavailability at the surface water/sediment interface. Although it is possible that surface water and sediment had different causes of short-term toxicity to *H. azteca*, the epibenthic, burrowing nature of *H. azteca* (in a non-vegetated environment) suggests this is not likely. Other studies have shown that surface waters from the same drainage basin were also toxic to fathead minnow (*Pimephales promelas*) larvae (Pyle et al., 2001; Pyle et al., 2002); although there were inconsistencies in the results, which make them difficult to interpret.

Due to the very high hardness levels of Wolf Lake and Fox Lake, only variables that exceeded their respective high hardness LC50 value (or the soft water LC50 value for pH, since no high hardness LC50 was available) were considered as candidates for causing the amphipod mortality observed in this study. The only variables that exceeded the high hardness LC50s at Wolf Lake and Fox Lake, but not David Lake, were pore-water iron, arsenic, and molybdenum; no measured surface water variable satisfied this criterion. Therefore, assuming that the cause of the observed toxicity was the same for both surface water and sediment exposure chambers (since the survival of *H. azteca* exposed to both chamber types was not statistically different), the cause should not be iron, arsenic, and molybdenum, as they were only present at "effect" concentrations in pore-water and not surface water. Furthermore, only molybdenum tissue concentrations were higher in amphipods from the exposure compared to those from the reference site, although it should be noted that the detection limit for arsenic was extremely high, with all measurements below detection (Table 3.3). In addition, although these data suggest that molybdenum readily bioaccumulates in amphipods exposed to Wolf Lake and Fox Lake, existing water and sediment molybdenum toxicity data (Borgmann et al., 2005; Liber and White-Sobey, unpublished data) suggests that the environmental concentrations within the present study could not have been the primary cause of the effects observed. Therefore, the primary cause of *in-situ* toxicity to *H. azteca* does not seem to be correlated with the variables measured in this study.

Subsequent discussion with an industry representative revealed that organic mill-process chemicals (kerosene, amine (Tri-C_8-C_{10}-Alkylamines), and isodecanol), which had been historically linked with sporadic effluent toxicity (HydroQual Laboratories Ltd., 1995; 2002; 2004) were released during the time of the *in-situ* experiment. Future research will therefore investigate if the toxicity of these chemicals correlate with the effects observed in the present *in-situ* experiment and could therefore be identified as the likely cause of the aquatic *in-situ* toxicity to *H. azteca* described here.

3.5.2.2 Rabbit Lake

The *in-situ* bioassay at Rabbit Lake revealed that this receiving environment also adversely affected *H. azteca* survival. Furthermore, the study revealed that surface water was the primary cause of toxicity since there was no significant difference between amphipod survival in surface water and sediment exposure chambers. However, comparison of surface water exposure chamber data among lakes only noted significantly lower survival at Unknown Pond, whereas comparisons among sediment exposure chambers revealed significantly lower survival in both Unknown Pond and Horseshoe Pond. Furthermore, mean survival in sediment chambers was lower than survival in surface water chambers (32% vs. 50% in Unknown Pond and 40% vs. 50% in

Horseshoe Pond), suggesting that contaminated sediment may have contributed to the toxicity to *H. azteca*. Contribution from the sediment could have been either through direct exposure to the sediment and its pore-water, or through the sediment-influenced overlying water near the sediment surface.

Because Unknown Pond and Horseshoe Pond have high hardness levels, only variables that exceeded their high hardness LC50 were considered as likely candidates for having caused the amphipod mortality seen in this study. The only variables that exceed these values in both Unknown Pond and Horseshoe Pond, but not in the reference lake, were surface water manganese and molybdenum, and pore-water manganese, iron, and molybdenum. Because Unknown Pond pore-water uranium values exceeded the high hardness LC50 and Horseshoe Pond almost met the same criteria (1473µg/L vs. 1651µg/L (Borgmann et al., 2005)), uranium was also considered as a suspected candidate. Of these elements, only molybdenum and uranium concentrations were higher in amphipods from the exposure sites when compared to those from the reference site (Table 3.6). Based on this, manganese and iron were excluded as causes of the amphipod mortality seen in the present *in-situ* study.

The high hardness LC50 value for molybdenum reported by Borgmann et al. (2005) exceeded the highest experimental concentration, so exceedance of this threshold should be interpreted with caution. Other studies have also shown that molybdenum is relatively non-toxic to *H. azteca*, with a 4-d LC50 that exceeded the highest experimental concentration of 741 mg/L (Liber and White-Sobey, unpublished data). Collectively, these data suggest that although molybdenum was bioavailable and accumulated in *H. azteca*, it was unlikely to have caused the *in-situ* toxicity observed in the present study.

Tissue concentrations of uranium were higher in amphipods from exposure sites than amphipods from the reference site. The one exception was the uranium tissue concentration of amphipods from surface water chambers in Horseshoe Pond. This correlates with the observation that there was no difference in survival in surface water chambers from the reference site and Horseshoe Pond. With respect to uranium concentrations, only Unknown Pond and Horseshoe Pond pore water values exceeded the available high hardness LC50 data (surface water values do not). However, the relatively low surface water uranium concentration measured at Unknown Pond does not correlate with the statistically lower survival of amphipods in Unknown Pond surface water chambers and the high uranium tissue concentration of these amphipods. It is, therefore, hypothesized that the surface water that the test organisms were exposed to in Unknown Pond had a higher concentration of bioavailable uranium than that measured.

Of the variables measured, only uranium appears to have caused, or contributed to, the observed *in-situ H. azteca* mortality. Other unmeasured variables can not be entirely dismissed, although no releases of process chemicals or other potential toxicants were reported. Further work on uranium bioavailability and toxicity to *H. azteca* has been initiated.

3.6 Conclusions

The *in-situ* experimental approach was deemed successful with respect to control survival and minimal loss of test units (two ripped screen tops in the Key Lake study and one chamber that lost sediment in the Rabbit Lake study). Experimental details, such as the provision of a food source (gauze with biofilm) in surface water chambers, the inclusion of screens in the chamber lids, careful acclimation of test animals to site-specific water hardness and alkalinity, and the relative composition and placement of the two chamber types were viewed as positive improvements to *in-situ* testing with *H. azteca*. Furthermore, the study design was successful in accomplishing both objectives of this study: describing the response of *H. azteca* exposed to the receiving environments of both uranium operations, and determining the significance of surface water and sediment to the *in-situ* toxicity to *H. azteca*. Furthermore, both *in-situ* studies produced unique and valuable information not generated through separate Sediment Quality Triad studies (Chapter 2). The toxicity observed in the Key Lake study was hypothesized to be associated with a release of organic mill-process chemicals, whereas the toxicity observed in the Rabbit Lake study may have been associated with high concentrations of uranium. These hypotheses switched the broader investigation-of-cause research towards identifying the exact causes of the observed *in-situ* toxicity to *H. azteca* and the roles these causes could play in benthic community impairment *in-situ*.

CHAPTER 4

EFFECT OF SAMPLING METHOD ON CONTAMINANT MEASUREMENT IN PORE-WATER AND SURFACE WATER AT TWO SASKATCHEWAN URANIUM OPERATIONS: CAN METHOD AFFECT CONCLUSIONS?

4.1 Abstract

This paper describes a comparison of two methods of sediment pore-water sampling and two methods of surface water collection that were used in a broader investigation of causes of adverse effects on benthic invertebrate communities at two Saskatchewan uranium operations. Variables measured and compared included pH, ammonia, DOC, and trace metals. The two types of sediment pore-water samples that were compared are centrifuged and 0.45-µm filtered sediment core samples vs. 0.2-µm dialysis (peeper) samples. The two types of surface water samples that were compared are 53-µm filtered Van Dorn horizontal beta samples vs. 0.2-µm dialysis (peeper) samples. Results showed that 62% of the core sample values were higher than the corresponding pore-water peeper measurements, and that 63% of the Van Dorn surface water measurements were lower than corresponding surface water peeper measurements. Furthermore, only 24% and 14% of the pair-wise surface water and pore-water measurements, respectively, fell within ± 10% range of one another; 73% and 50%, respectively, fell within ± 50%. Although somewhat confounded by differences in filtering method, the observed differences are believed to primarily be related to small, vertical differences in the environment sampled. Despite the observed differences in concentrations of toxicologically relevant variables generated by the different sampling methods, conclusions drawn on the possible cause(s) of acute *in-situ* toxicity to *Hyalella azteca* from a related study were the same at each uranium operation. This study revealed that regardless of sample method, similar conclusions were made.

4.2 Introduction

Selecting a method to sample an environmental matrix depends on the scientific question that is being asked. Reasons for environmental sampling include characterizing toxicity, community structure and physicochemical characteristics of the media sampled. These purposes often require different considerations when selecting a sampling technique; however, it is usually impossible to meet all requirements, as few, if any, sampling methods produce samples that are completely representative of the media assessed. A common problem with method selection is that high spatial resolution comes at the expense of low sample volume and/or the best method is too costly and/or time consuming to implement. As a result of these challenges, the most appropriate sampling technique cannot always be used.

While one sampling technique might be more suitable or representative than another, all sampling methods hopefully characterize the media sampled to an adequate extent. It is therefore likely that there will be measurable differences between samples collected with different methods. Indeed, many studies have discussed the effect of sampling method on sample chemistry (Bufflap and Allen, 1995a, 1995b; Mason et al., 1998; Schults et al., 1992; Frias et al., 1995; Ormaza-González and Statham, 1996; Rausch et al., 2006), usually focusing on which method is more accurate and/or representative of the variables measured. However, few studies, if any, actually compare conclusions derived from the results of different sampling methods and illustrate the relevance of the differences measured. Therefore, the real question is, can measurable differences from different sample methods lead to differences in the conclusions derived from a study?

Through previous monitoring programs, benthic macroinvertebrate community impairment had been noted downstream of the final effluent discharge site at both the Key Lake (Terrestrial and Aquatic Environmental Managers Ltd., 1994; Conor Pacific Environmental Technologies Inc., 2000; Golder Associates Ltd., 2002; Chapter 2) and the Rabbit Lake (Terrestrial and Aquatic Environmental Managers Ltd. and SENES Consultants Ltd. 1996; Liber et al., unpublished data; Golder Associates Ltd., 2003c; Chapter 2) uranium mining and milling operations in northern Saskatchewan, Canada.

Although the presence of benthic community impairment has been well documented, little research as been conducted on identifying the cause(s) of these effects.

The Key Lake and Rabbit Lake operation are the two largest uranium milling operations in the world, with production capacities of 18 and 12 million pounds of U_3O_8 annually, respectively (www.cameco.com). Key Lake began production in 1983 as a mine and mill site, but since on-site ore has been depleted, the Key Lake mill currently processes ore from another near by mine site, McArthur River. Rabbit Lake began production in 1975 and continues to mine and mill on-site ore (www.cameco.com). Wastewater at both operations can be of both mining and milling origin considering many processes recycle contaminated water from other sources (Cameco Corporation, 2003, 2004).

As part of a larger project designed to investigate the cause(s) of benthic community impairment downstream of the Key Lake and Rabbit Lake operations, 4-d *in-situ* bioassays were preformed with caged *Hyalella azteca* using both surface water and sediment exposure chambers (Chapter 3). Results from these bioassays showed that downstream contaminant-influenced environments at both uranium operations were toxic to *H. azteca* and suggested that the primary cause of toxicity was contaminated surface water. Results from the Rabbit Lake study further concluded that sediment may have contributed to the observed toxicity.

As part of the *in-situ* study and larger identification-of-cause investigation at the two uranium operations, pore-water and surface water samples were both collected using two different methods at all exposure and reference sites. Although the initial purpose of collecting these samples was not to compare differences in sampling method, having these data provided an opportunity to evaluate if method-dependent differences in contaminant measurements could lead to differences in conclusions regarding the cause(s) of *in-situ* toxicity to caged *H. azteca* at the two uranium operations. The focus of the comparison was on the use of peepers (*in-situ* dialysis samplers) for both pore-water and near-sediment surface water sampling, compared to the more conventional techniques of grab samples and cores for surface water and sediment, respectively.

4.3 Methods and materials

4.3.1 Study sites

The Key Lake and Rabbit Lake uranium operations are located in northern Saskatchewan, Canada (Figure 1.1). Downstream field sites were selected on the basis of previously documented benthic invertebrate community impairment. Because this study was part of a larger weight-of-evidence investigation (including benthic community assessment), selecting sites with similar sediment/habitats was critical. In general, "depositional" sediment types (fine textured, organically enriched) were chosen at water depths ranging from 60 to 100 cm.

For the Key Lake study, two exposure sites and one reference site were used. The exposure sites were located at the outflow of Wolf Lake and the inlet of Fox Lake. The reference site was located in David Lake (Figure 1.2). The exposure sites for the Rabbit Lake study were at the inlet of Unknown Pond and near the middle of Horseshoe Pond. The reference site was a nearby shallow lake (Reference Lake) (Figure 1.3).

4.3.2 General site conditions

The general overlying water and sediment conditions of both the Key Lake and Rabbit Lake study sites are presented in Table 4.1. Samples were collected as part of the 2004 sediment quality triad assessment for each uranium operation (Chapter 2). Key Lake and Rabbit Lake samples were collected on July 8-10, 2004, and August 7-9, 2004, respectively.

Surface water samples (*n* = 4 per site) were collected using an acrylic 3.2-L Van Dorn horizontal beta water sampler (Wildlife Supply Company, Buffalo, NY, USA) from ~15 cm above the sediment surface and filtered through a 53-µm sieve to remove planktonic organisms and debris. All samples were analyzed for pH (Beckman® 250 pH/Temp/mV meter with a Beckman® pH electrode 511050, Beckman Instruments Inc., Fullerton, CA, USA) and total ammonia (Beckman® 250 pH/Temp/mV meter with a Thermo Orion® 95-12 ammonia electrode or Orion aquafastII photometer, ORION Research, Beverly, MA, USA) on the day of collection. Sub-samples were collected and stored in the dark at 4°C and analyzed for conductivity (Orion ATI conductivity cell 017010 and Orion ATI meter 170), hardness (Hach Digital Titrator model 16900), and

Table 4.1. Mean (± standard deviation, *n* = 4) physico-chemical surface water and sediment characteristics of study sites at the Key Lake and Rabbit Lake uranium operations.

	Key Lake Uranium Operation			Rabbit Lake Uranium Operation		
Variable	David Lake	Wolf Lake	Fox Lake	Reference Lake	Unknown Pond	Horseshoe Pond
Surface water						
Depth (cm)	98 ± 0	108 ± 4	78 ± 2	78 ± 2	83 ± 5	64 ± 1
Temperature (°C)	18.8 ± 0.0	18.1 ± 0.1	19.9 ± 0.1	15.4 ± 0.2	15.7 ± 0.0	16.9 ± 0.1
DO[a] (mg/L)	8.8 ± 0.6	8.0 ± 0.1	8.1 ± 0.3	9.2 ± 0.0	7.7 ± 0.1	8.0 ± 0.1
pH	6.8 ± 0.0	6.0 ± 0.0	5.6 ± 0.4	7.3 ± 0.1	6.1 ± 0.0	6.6 ± 0.0
Alkalinity (mg/L as $CaCO_3$)	8 ± 1	7 ± 1	7 ± 1	20 ± 2	7 ± 0	9 ± 1
Total Hardness (mg/L as $CaCO_3$)	6 ± 1	610 ± 20	580 ± 10	16 ± 1	800 ± 10	820 ± 60
Conductivity (µS/cm)	14 ± 0	1190 ± 10	1152 ± 16	43 ± 1	1632 ± 5	1677 ± 79
Sediment						
Sand (%)	98 ± 0[b]	28 ± 3[b]	84 ± 7[b]	39 ± 5[b]	35 ± 9[b]	22 ± 5[b]
Silt (%)	1 ± 1[b]	57 ± 3[b]	12 ± 4[b]	44 ± 3[b]	46 ± 6[b]	54 ± 4[b]
Clay (%)	1 ± 0[b]	14 ± 2[b]	5 ± 3[b]	17 ± 2[b]	20 ± 4[b]	24 ± 2[b]
TOC[c] (%)	1.2 ± 0.4	10.2 ± 0.97[b]	4.9 ± 1.5	10.0 ± 0.6	24.8 ± 1.4	14.4 ± 1.0
Water Content (%)	40.1 ± 3.4	85.3 ± 3.09[b]	71.1 ± 6.0	84.3 ± 1.9	88.2 ± 1.5	87.1 ± 1.5

[a] DO = dissolved oxygen.

[b] n = 3.

[c] TOC = total organic carbon.

alkalinity (Hach Digital Titrator model 16900) within one week of collection. Major differences in surface water characteristics between reference and exposure sites at both uranium operations included higher hardness and conductivity and lower pH.

Sediment cores were collected (*n* = 4 per site) using a 5-cm diameter hand-corer with 5-cm diameter acrylic core tubes and processed within one week. Processing included isolating the top 2.5-cm horizon of the sediment cores, homogenizing the sample, and removing sub-samples for determination of total organic carbon (TOC, Leco Carbon determinator CR-12, Leco, St. Joseph, MI, USA), particle size (performed by Enviro-Test Laboratories, Saskatoon, SK, Canada), and water content. Sediment characteristics at Key Lake were substantially different among sites, where as sediment characteristics at Rabbit Lake were much more similar with respect to the variables measured.

4.3.3 Surface water sampling – Van Dorn

Van Dorn surface water samples were initially intended to characterize conditions on days 0 and 4 (*n* = 4 per site) of the *in-situ* study. For the purpose of this comparison with the peeper samples, d-0 and d-4 measurements were averaged to provide a single value for each variable measured. Samples were collected as described above, filtered through a 53-µm sieve, and analyzed for pH and ammonia on the day of collection. Sub-samples were taken for the analysis of total metals, acidified on the day of collection, and stored in the dark at 4°C until analyzed using ICP-MS (Department of Geological Sciences, University of Saskatchewan).

4.3.4 Sediment sampling – cores

Sediment cores were collected with a 5-cm diameter hand-corer as described above. Pore-water was isolated from sub-samples of sediment cores via centrifugation at 12,000 rpm (17,200 rcf) at room temperature for 20 min. Once isolated, pore-water was filtered through a 0.45-µm membrane (Nalgene® acetate syringe filter, Nalge Nunc International, Rochester, NY, USA) and immediately analyzed for pH (ORION® PerpHect LogR meter model 370) and ammonia. Sub-samples of pore-water were removed and later analyzed for dissolved metals and dissolved organic carbon (DOC).

Dissolved metal samples were acidified the day of collection and stored in the dark at 4°C until they were analyzed using ICP-MS. Samples for analysis of DOC were stored in the dark at 4°C until analyzed using a Shimadzu total organic carbon analyzer 5050 A (Shimadzu, Tokyo, Japan).

4.3.5 Peeper samples

Peeper samples were originally collected to characterize both the overlying-water and pore-water environment of a 4-d *in-situ* bioassay using the amphipod *H. azteca*. The peepers were deployed on day 0 of the *in-situ* study and retrieved on day 4 (test termination). Peepers were constructed of acrylic and designed to sample the environment vertically, with seven rectangular sample compartments (1-cm x 8-cm) separated vertically by 1 cm and covered with a 0.2-μm Supor® membranes (Gelman Sciences, Ann Arbor, MI, USA). Before deployment, peepers were stored in a plastic pail with ultra-pure (Barnstead NANOpure®) water that was bubbled with nitrogen gas for 24-h. Peepers were placed vertically within the sediment (n = 4 per site) to ensure that both overlying-surface water samples and pore-water samples could be collected from each peeper. Peepers were rinsed with ultra-pure water before they were sampled.

Samples from selected peeper chambers were collected by piercing the membrane with a 1-mL Eppendorf pipette and carefully withdrawing samples. Samples were analyzed for pH immediately after retrieval. Additional sub-samples were analyzed for total ammonia later that day. Other sub-samples were collected for the analysis of DOC and dissolved metals as described previously.

4.3.6 Statistics and data analysis

To simplify the assessment and focus on the objective of evaluating whether sampling method could influence the conclusions drawn regarding the cause(s) of *in-situ* toxicity, only the variables meeting the following criteria were compared: the mean value exceeded the Canadian water quality guideline (CWQG) for protection of aquatic life (Canadian Council of Ministers of the Environment, 1999); the mean value exceeded published *H. azteca* toxicity values (Borgmann et al., 2005; France and Stokes,1987; Ankley et al., 1995); analytical detection limits exceeded CWQG guidelines or reported toxicity values; or elements that did not have published water quality guidelines and/or toxicity data (Table 2.1). Twenty-one variables exceeded these criteria between both uranium operations, nineteen of which exceed the criteria at both uranium operations. For completeness, the twenty-one variables identified over both uranium mines are presented in all data tables.

All statistical analyses were performed using Sigma Stat version 3.1. Physicochemical data were analyzed for normality and homogeneity of variance. If normality or homogeneity of variance failed, data were transformed using a $\log_{10}$ transformation (pH data were not transformed). For consistency among comparisons, transformed data sets that still failed normality or homogeneity of variance still underwent parametric statistical analysis using two-way ANOVA (with sampling method and lake as the independent factors). If a statistical difference was detected ($p \leq 0.05$), Tukey's all pair-wise multiple comparison post-hoc test was run.

To evaluate general differences between results obtained with the different sampling methods, mean percent differences were calculated for both surface water and pore-water, and plotted on a frequency distribution graph. Differences between mean Van Dorn and mean peeper surface water measurements were calculated relative to the peeper measurements. Differences between mean sediment core and mean peeper pore-water measurements were also calculated relative to the peeper measurements. Due to differences in detection limits, differences between measurements were only calculated for means that had >50% of the replicates above the method detection limit. From this criteria, 163 and 182 surface water and pore-water measurement differences were included, respectively (variables included trace metals (Li, Mg, Al, Si, P, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Rb, Sr, Y, Zr, Nb, Mo, Sb, Cs, Ba, La, Pr, Nd, Dy, Ce, Er, Hf, Ta, W, Tl, Pb, Th, and U), ammonia, pH and DOC (pore-water only)). It should be noted that all variables were not equally represented, as concentrations of variables vary among sites. Consequently, some variables were more commonly below detection, than others. All measurements that were below detection, but still utilized for calculation of a mean value, were assigned a value of one-half of the detection limit.

4.4 Results

4.4.1 Differences within selected variables

Key Lake surface water (Table 4.2): Variables in David Lake surface water that showed significant differences between measurement methods included pH and iron (lower in peepers), and arsenic (higher in peepers). Differences in Wolf Lake measurements included lower pH, and higher calcium, iron, nickel, thulium, and thallium peeper measurements compared to Van Dorn measurements. Differences in Fox Lake measurements included lower pH, and higher magnesium, nickel, silver, and thallium peeper measurements compared to Van Dorn measurements.

Key Lake pore-water (Table 4.3): Pore-water variables in David Lake that showed significant differences between measurement methods included zinc, arsenic, and uranium (lower in peepers), and higher calcium, manganese, iron, and thulium (higher in peepers). Wolf Lake measurements that showed statistical differences included lower pH, total ammonia, magnesium, silicon, arsenic, molybdenum, and higher aluminum, manganese, nickel, thulium, and thallium peeper measurements compared to core sample measurements. Differences in Fox Lake measurements included lower manganese, iron, and arsenic, and higher cadmium and thallium in peeper measurements.

Rabbit Lake surface water (Table 4.4): Variables in reference lake surface water that showed significant differences between measurements included pH (lower in peepers) and phosphorus, copper, zinc, arsenic, and thallium (higher in peepers). Significant differences noted in Unknown Pond measurements included lower thallium, and higher pH, total ammonia, magnesium, calcium, chromium, manganese, iron, nickel, arsenic, silver, cadmium, and uranium in peepers compared to Van Dorn samples. Horseshoe Pond measurements that showed statistical differences included lower calcium, and higher pH, total ammonia, magnesium, iron, nickel, zinc, arsenic, and uranium for peeper measurements compared to Van Dorn measurements.

Rabbit Lake pore-water (Table 4.5): Reference lake pore-water variables that showed significant differences between measurements included silicon, copper, and uranium (lower in peepers), and pH, magnesium, arsenic, molybdenum, silver, and cadmium (higher in peepers). Unknown Pond data that showed significant differences

Table 4.2. Mean (± standard deviation) levels of selected variables in Van Dorn surface water samples (n = 6) and peeper surface water samples (n = 3) from the Key Lake uranium operation. Data are in µg/L, except for pH and total ammonia (mg N/L).

Variable	David Lake		Wolf Lake		Fox Lake	
	Van Dorn	Peeper	Van Dorn	Peeper	Van Dorn	Peeper
pH	6.87 ± 0.08^{e}	4.72 ± 0.23^{ad‡}	6.12 ± 0.16^{ae*}	4.94 ± 0.1^{a‡}	5.74 ± 0.35^{ae*†}	4.90 ± 0.11^{a‡}
Total Ammonia	<0.05^{e}	<0.5^{d}	5.39 ± 0.20^{ae*}	5.0 ± 0.3^{a*}	4.25 ± 0.39^{ae*†}	4.1 ± 0.6^{a*†}
Mg	437 ± 77	500 ± 114	17514 ± 573*	18978 ± 10178*	15168 ± 955*	18502 ± 1701*‡
Al	35.1 ± 33.4^{a}	9.2 ± 3.6^{a}	62.8 ± 24.0^{a}	105.6 ± 23.2^{ab*}	67.4 ± 35.5^{a}	99.1 ± 67.9^{ab*}
Si	1462 ± 724	1476 ± 213	1381 ± 422	955 ± 552	871 ± 473	1733 ± 468
P	76.6 ± 36.6	151.2 ± 171.3	300.6 ± 41.5*	332.5 ± 93.1*	265.0 ± 68.5*	229.6 ± 210.8
Ca	1102 ± 105	1380 ± 309	185751 ± 7199*	271964 ± 48660*‡	173920 ± 15413*	238976 ± 100376*
Cr	<1.11^{a}	1.69 ± 1.74^{a}	2.24 ± 1.10^{a}	<1.37^{a}	3.06 ± 3.36^{a}	1.61 ± 1.61^{a}
Mn	15.0 ± 1.3	22.4 ± 17.0	117.9 ± 6.0^{b*}	117.1 ± 1.1^{b*}	79.6 ± 6.1*†	105.9 ± 3.4^{b*}
Fe	532 ± 82.27^{a}	161 ± 46‡	391 ± 86^{a}	937 ± 229^{a*‡}	922 ± 1022^{a}	1000 ± 145^{ab*}
Ni	<4.82	<6.63‡	22.09 ± 2.60*	32.00 ± 5.20^{a*‡}	10.79 ± 3.68*†	27.27 ± 1.64^{a‡}
Cu	0.45 ± 0.31	<2.25^{a}	2.15 ± 0.46^{a*}	1.73 ± 1.06	1.99 ± 0.87*	18.99 ± 29.59^{a}
As	<0.30	0.47 ± 0.26‡	13.35 ± 1.09^{a*}	11.72 ± 0.71^{a*}	16.98 ± 8.16^{a*}	22.16 ± 1.40^{a*†}
Se	<2.91^{a}	<7.86^{a‡}	<2.91^{a}	<7.86^{a‡}	<2.91^{a}	<7.86^{a‡}
Mo	0.9 ± 1.1	0.1 ± 0.0	338.3 ± 12.0^{a*}	384.3 ± 8.3^{a*}	389.5 ± 11.9^{a*}	421.9 ± 2.7^{a*}
Ag	<0.04	0.04 ± 0.00	0.08 ± 0.08	0.04 ± 0.00	<0.04	0.12 ± 0.08^{a‡}
Cd	0.07 ± 0.08^{a}	<0.25^{ab}	0.16 ± 0.31^{ab}	<0.25^{ab}	<0.06^{a}	<0.25^{ab‡}
Tm	0.01 ± 0.01^{b}	<0.02^{b‡}	<0.01	0.02 ± 0.01^{b‡}	<0.01	<0.02^{b‡}
Hg	<0.16^{a}	<0.70^{a‡}	<0.16^{a}	<0.70^{a‡}	<0.16^{a}	<0.70^{a‡}
Tl	<0.02	<0.05	0.53 ± 0.06*	0.79 ± 0.14*‡	0.47 ± 0.07*	0.69 ± 0.10*‡
U	0.03 ± 0.02	<0.03	3.79 ± 0.93*	2.12 ± 0.13*	1.52 ± 0.55*†	1.69 ± 0.36*

[a] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999); [b] Exceeds soft water median lethal concentration for *Hyalella azteca* (Borgmann, et al., 2005; France and Stokes, 1987; Ankley et al., 1995); [c] Exceeds hard water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005; Ankley et al., 1995); [d] $n = 4$; [e] $n = 8$; * Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from David Lake measurement; † Significantly different ($p \leq 0.05$) from the respective Wolf Lake measurement; ‡ Significantly different ($p \leq 0.05$) from the respective Van Dorn measurement.

Table 4.3. Mean (± standard deviation) levels of selected variables in sediment core pore-water samples (n = 3) and peeper pore-water samples (n = 3) from the Key Lake uranium operation. Data are in µg/L, except for pH and total ammonia (mg N/L).

	David Lake		Wolf Lake		Fox Lake	
Variable	Core	Peeper	Core	Peeper	Core	Peeper
pH	5.28 ± 0.16^{ad}	5.17 ± 0.55^{ad}	6.62 ± 0.41^{a*}	4.33 ± 0.25^{abd*‡}	6.56 ± 0.13^{d*}	6.49 ± 0.31^{ad*†}
Total Ammonia	<0.5^{d}	<0.5^{d}	7.7 ± 0.4^{a*}	2.3 ± 1.4^{d*‡}	6.8 ± 1.0^{ad*}	6.2 ± 1.2^{ad*†}
Mg	236 ± 104	1357 ± 278	21690 ± 1898*	18627 ± 1116*‡	18665 ± 420*†	19057 ± 1094*
Al	154.4 ± 104.4^{ab}	11.8 ± 4.6^{a}	79.5 ± 132.2^{a}	257.6 ± 93.4^{ab*‡}	133.6 ± 123.4^{ab}	51.7 ± 18.5^{a†}
Si	4703 ± 154	3761 ± 423	17677 ± 4518*	756.64 ± 320.30*‡	7659 ± 565†	8191 ± 1491*†
P	131.0 ± 98.2	119.7 ± 58.4	384.1 ± 45.1*	411.6 ± 133.6*	287.0 ± 14.9	413.0 ± 61.6*
Ca	1069 ± 287	4980 ± 878‡	290880 ± 16942*	455101 ± 171858*	219292 ± 7588*	271118 ± 231807*
Cr	4.86 ± 1.23^{ab}	3.84 ± 4.05^{ab}	14.00 ± 13.48^{ab}	<1.37^{a}	13.55 ± 3.93^{ab}	10.64 ± 4.78^{ab}
Mn	82.1 ± 39.3	425.8 ± 125.3^{bc‡}	55.8 ± 10.2	100.9 ± 4.1^{b*‡}	252.5 ± 84.3^{bc*†}	117.3 ± 33.4^{b*‡}
Fe	1562 ± 711^{ab}	15530 ± 5502^{abc‡}	2827 ± 1897^{ab}	1691 ± 365^{ab*}	27175 ± 10588^{abc*†}	16976 ± 3546^{abc†‡}
Ni	<4.82	<6.63	19.39 ± 8.31*	49.52 ± 5.16^{a*‡}	11.83 ± 3.09*	11.27 ± 2.05*†
Cu	1.47 ± 0.41	<2.25^{a}	1.09 ± 0.59	3.74 ± 2.44^{a}	1.90 ± 1.27	2.26 ± 1.03^{a}
As	3 ± 1	0 ± 0‡	4095 ± 2859^{abc*}	18 ± 9^{a*‡}	3041 ± 1549^{abc*}	987 ± 278^{abc*†‡}
Se	5.64 ± 7.25^{a}	<7.86^{a}	<2.91^{a}	<7.86^{a}	5.86 ± 7.63^{a}	<7.86^{a}
Mo	6 ± 7	0 ± 0	5919 ± 3278^{abc*}	431 ± 182^{a*‡}	3536 ± 140^{abc*}	1839 ± 406^{abc*}
Ag	4.79 ± 4.26^{abc}	0.07 ± 0.05	1.18 ± 0.64^{abc}	0.09 ± 0.08	<0.04	0.08 ± 0.03
Cd	0.12 ± 0.15^{a}	<0.25^{ab}	<0.06^{a}	<0.25^{ab‡}	<0.06^{a}	0.62 ± 0.85^{ab‡}
Tm	<0.01	0.02 ± 0.01^{b‡}	<0.01	0.02 ± 0.01^{b‡}	<0.01	<0.02^{b‡}
Hg	<0.16^{a}	<0.70^{a‡}	<0.16^{a}	<0.70^{a‡}	<0.16^{a}	<0.70^{a‡}
Tl	<0.02	<0.05	0.35 ± 0.42*	1.55 ± 0.56^{a*‡}	0.09 ± 0.05*	0.12 ± 0.11†
U	0.27 ± 0.20	0.05 ± 0.03‡	3.50 ± 4.08*	2.53 ± 0.49*	5.49 ± 0.93*	12.03 ± 3.95*

[a] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999); [b] Exceeds soft water median lethal concentration for *Hyalella azteca* (Borgmann, et al., 2005; France and Stokes, 1987; Ankley et al., 1995); [c] Exceeds hard water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005; Ankley et al., 1995); [d] n = 4; * Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from David Lake measurement; † Significantly different ($p \leq 0.05$) from the respective Wolf Lake measurement; ‡ Significantly different ($p \leq 0.05$) from the respective core measurement.

Table 4.4. Mean (± standard deviation) levels of selected variables in Van Dorn surface water samples ($n = 6$) and peeper surface water samples ($n = 3$) from the Rabbit Lake uranium operation. Data are in µg/L, except for pH and total ammonia (mg N/L).

Variable	Reference Lake		Unknown Pond		Horseshoe Pond	
	Van Dorn	Peeper	Van Dorn	Peeper	Van Dorn	Peeper
pH	7.3 ± 0.1^{e}	7.2 ± $0.0^{d\ddagger}$	6.3 ± 0.1^{af*}	6.9 ± $0.0^{d*\ddagger}$	6.6 ± $0.1^{f*\dagger}$	6.7 ± $0.2^{*\dagger}$
Total Ammonia	$<0.1^{f}$	$<0.1^{d}$	0.2 ± 0.2^{f*}	0.7 ± $0.8^{d*\ddagger}$	$<0.1^{f\dagger}$	0.1 ± $0.1^{\dagger}$
Mg	2662 ± 139	2786 ± 264	29407 ± 1094^{*}	40787 ± $9015^{*\ddagger}$	28685 ± 4641^{*}	30594 ± $177^{*\dagger}$
Al	25.86 ± 41.65^{a}	75.02 ± 2.31^{a}	83.86 ± 58.69^{a}	27.09 ± 17.82^{a}	56.66 ± 72.00^{a}	58.37 ± 16.92^{a}
Si	2367 ± 536	3120 ± 334	2297 ± 618	4469 ± 3172	2471 ± 425	2077 ± 324
P	85.2 ± 24.9	176.8 ± $105.5^{\ddagger}$	349.8 ± 85.5^{*}	397.0 ± 205.0^{*}	364.6 ± 52.3^{*}	440.2 ± 79.3^{*}
Ca	3745 ± 385	3596 ± 984	272607 ± 12722^{*}	768706 ± $457154^{*\ddagger}$	258589 ± 39916^{*}	202870 ± $3688^{*\dagger}$
Cr	$<1.11^{a}$	0.69 ± 0.00	1.19 ± 1.20^{a}	5.06 ± $4.26^{ab\ddagger}$	1.75 ± 1.54^{a}	1.68 ± 1.06^{a}
Mn	5.2 ± 0.4	4.9 ± 2.8	185.7 ± 3.1^{bc*}	273.1 ± $66.9^{bc*\ddagger}$	171.9 ± 27.9^{bc*}	211.9 ± 35.0^{bc*}
Fe	40 ± 39	55 ± 0	243 ± 76^{*}	10838 ± $14892^{abc*\ddagger}$	249 ± 124^{*}	2582 ± $2203^{ab*\ddagger}$
Ni	<4.82	$<6.63^{\ddagger}$	18.79 ± 2.88^{*}	28.72 ± $5.12^{a*\ddagger}$	20.93 ± 4.53^{*}	37.31 ± $14.32^{a*\ddagger}$
Cu	0.70 ± 0.76	1.12 ± $0.00^{\ddagger}$	2.68 ± 0.72^{a*}	3.33 ± 2.57^{a}	2.44 ± 0.70^{a*}	1.67 ± 0.95
As	<0.30	0.46 ± $0.24^{\ddagger}$	8.36 ± 1.25^{a*}	37.66 ± $34.62^{a*\ddagger}$	5.82 ± 1.03^{a*}	8.31 ± $1.33^{a*\dagger}$
Se	5.97 ± 4.33^{a}	$<7.86^{a}$	10.51 ± 9.24^{a}	$<7.86^{a}$	14.53 ± 4.84^{a}	$<7.86^{a}$
Mo	10 ± 9	2 ± 3	4945 ± 235^{abc*}	8878 ± 4856^{abc*}	4682 ± 858^{abc*}	4667 ± 139^{abc*}
Ag	0.06 ± 0.06	0.06 ± 0.03	<0.04	0.11 ± $0.12^{a\ddagger}$	0.03 ± 0.02	0.08 ± 0.03
Cd	$<0.06^{a}$	$<0.25^{a\ddagger}$	0.06 ± 0.07^{a}	$<0.25^{a\ddagger}$	$<0.06^{a}$	$<0.25^{a\ddagger}$
Tm	<0.01	$<0.02^{b\ddagger}$	<0.01	$<0.02^{b\ddagger}$	<0.01	$<0.02^{b\ddagger}$
Hg	$<0.16^{a}$	$<0.70^{a\ddagger}$	$<0.16^{a}$	$<0.70^{a\ddagger}$	$<0.16^{a}$	$<0.70^{a\ddagger}$
Tl	<0.02	0.03 ± $0.02^{\ddagger}$	0.72 ± 0.17^{*}	0.45 ± $0.23^{*\ddagger}$	0.67 ± 0.13^{*}	0.73 ± 0.11^{*}
U	3 ± 0	2 ± 0	117 ± 7^{b*}	9418 ± $11402^{bc*\ddagger}$	89 ± 16^{b*}	366 ± $333^{b*\dagger\ddagger}$

[a] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999); [b] Exceeds soft water median lethal concentration for *Hyalella azteca* (Borgmann, et al., 2005; France and Stokes, 1987; Ankley et al., 1995); [c] Exceeds hard water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005; Ankley et al., 1995); [d] $n = 4$; [e] $n = 7$; [f] $n = 8$; * Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from Reference Lake measurement; [†] Significantly different ($p \leq 0.05$) from the respective Unknown Pond measurement; [‡] Significantly different ($p \leq 0.05$) from the respective Van Dorn measurement.

Table 4.5. Mean (± standard deviation) levels of selected variables in sediment core pore-water samples (n = 3) and peeper pore-water samples (n = 3) from the Rabbit Lake uranium operation. Data are in µg/L, except for pH and NH3 (mg/L).

	Reference Lake		Unknown Pond		Horseshoe Pond	
Variable	Core	Peeper	Core	Peeper	Core	Peeper
pH	6.7 ± 0.1^{d}	$7.2 \pm 0.1^{d\ddagger}$	$7.0 \pm 0.1^{d*}$	7.0 ± 0.3	6.8 ± 0.1^{d}	$6.8 \pm 0.1^{d*}$
Total Ammonia	$<0.1^{d}$	$<0.1^{d}$	$11.6 \pm 4.9^{ad*}$	$1.3 \pm 1.1^{d*\ddagger}$	$4.1 \pm 1.0^{ad*\dagger}$	$1.5 \pm 0.4^{d*\ddagger}$
Mg	1430 ± 301	$3534 \pm 287^{\ddagger}$	$51498 \pm 12920^{*}$	$47582 \pm 8224^{*}$	$37811 \pm 996^{*}$	$36941 \pm 4020^{*}$
Al	445.1 ± 266.0^{ab}	88.1 ± 55.4^{a}	$<6.29^{a*}$	$31.9 \pm 9.6^{a\ddagger}$	$43.5 \pm 69.9^{a*}$	35.6 ± 11.6^{a}
Si	10165 ± 1061	$2644 \pm 250^{\ddagger}$	$14084 \pm 2423^{*}$	$6883 \pm 2720^{*\ddagger}$	$8717 \pm 582^{\dagger}$	$5140 \pm 1161^{\ddagger}$
P	80.2 ± 48.2	150.9 ± 30.3	$451.0 \pm 72.3^{*}$	358.4 ± 272.5	310.5 ± 44.1	$476.4 \pm 75.8^{*}$
Ca	3134 ± 593	5346 ± 1223	$305695 \pm 3100^{*}$	$809877 \pm 413923^{*\ddagger}$	$257158 \pm 5425^{*}$	$217560 \pm 18285^{*\dagger}$
Cr	$<1.11^{a}$	1.02 ± 0.59^{a}	$8.15 \pm 3.26^{ab*}$	6.83 ± 5.84^{ab}	7.63 ± 2.89^{ab}	$10.42 \pm 3.16^{ab*}$
Mn	19.0 ± 5.4	39.7 ± 31.2	$324.4 \pm 67.6^{bc*}$	$349.6 \pm 55.0^{bc*}$	$326.0 \pm 22.50^{bc*}$	$344.1 \pm 40.1^{bc*}$
Fe	108.30 ± 44.04	174.54 ± 31.48	$33146 \pm 7129^{abc*}$	$35146 \pm 32060^{abc*}$	$18418 \pm 6842^{abc*}$	$28296 \pm 7593^{abc*}$
Ni	<4.82	<6.63	$12.73 \pm 2.05^{*}$	$20.89 \pm 2.97^{*}$	$17.19 \pm 1.62^{*}$	$22.30 \pm 23.10^{*}$
Cu	4.94 ± 2.35^{a}	$1.62 \pm 0.86^{\ddagger}$	2.16 ± 0.43^{a}	1.82 ± 1.21	2.09 ± 0.18^{a}	1.12 ± 0.00
As	<0.3	$1.5 \pm 1.5^{\ddagger}$	$359.2 \pm 83.7^{a*}$	$107.9 \pm 61.8^{a*\ddagger}$	$55.2 \pm 7.3^{a*\dagger}$	$44.2 \pm 5.4^{a*}$
Se	5.45 ± 6.91^{a}	$<7.86^{a}$	$<2.91^{a}$	$<7.86^{a}$	6.00 ± 1.56^{a}	$<7.86^{a}$
Mo	<0	$1 \pm 1^{\ddagger}$	$8250 \pm 431^{abc*}$	$14635 \pm 3733^{abc*}$	$7413 \pm 1303^{abc*}$	$8338 \pm 1793^{abc*}$
Ag	0.02 ± 0.00	$0.08 \pm 0.06^{\ddagger}$	0.03 ± 0.01	$0.09 \pm 0.05^{\ddagger}$	0.02 ± 0.01	0.04 ± 0.00
Cd	0.17 ± 0.01^{ab}	$<0.25^{a\ddagger}$	$<0.06^{a*}$	$<0.25^{a\ddagger}$	$<0.06^{a*}$	$<0.25^{a\ddagger}$
Tm	<0.01	$<0.02^{b\ddagger}$	<0.01	$<0.02^{b\ddagger}$	<0.01	$<0.02^{b\ddagger}$
Hg	$<0.16^{a}$	$<0.70^{a\ddagger}$	$<0.16^{a}$	$<0.70^{a\ddagger}$	$<0.16^{a}$	$<0.70^{a\ddagger}$
Tl	<0.02	0.04 ± 0.02	0.05 ± 0.06	0.16 ± 0.12	0.01 ± 0.00	$0.15 \pm 0.02^{\ddagger}$
U	11 ± 4	$3 \pm 0^{\ddagger}$	$4587 \pm 2605^{bc*}$	$18954 \pm 4563^{bc*\ddagger}$	$1473 \pm 1376^{b*\dagger}$	$6103 \pm 3005^{bc*\dagger\ddagger}$

[a] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999); [b] Exceeds soft water median lethal concentration for *Hyalella azteca* (Borgmann, et al., 2005; France and Stokes, 1987; Ankley et al., 1995); [c] Exceeds hard water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005; Ankley et al., 1995); [d] n = 4; * Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from Reference Lake measurement; † Significantly different ($p \leq 0.05$) from the respective Unknown Pond measurement; ‡ Significantly different ($p \leq 0.05$) from the respective core measurement.

included lower total ammonia, silicon, and arsenic, and higher calcium, silver, and uranium for peeper measurements compared to core sample measurements. Differences in Horseshoe Pond data included lower ammonia and silicon, and higher thallium and uranium for peeper measurements compared to core sample measurements.

4.4.2 Surface water vs. pore-water measurements

Figure 4.1 illustrates the percent difference between mean surface water and mean pore-water measurements made with conventional sampling techniques relative to the respective mean peeper measurements for both study sites combined. For surface water measurements, 63% of the Van Dorn values were less than their respective peeper measurements (represented by a negative number), whereas 62% of the sediment core pore-water measurements yielded greater values than their respective peeper samples (represented by a positive number). Only 24% and 14% of surface water and pore-water measurements, respectively, fell within ± 10% range of one another; 73% and 50%, respectively, fell within ± 50%. Furthermore, the more conventional sampling method values that exceed peeper measurements generally had a greater mean percent difference (up to >+1000%) than values that were less than the respective peeper measurements (up to -100%).

4.5 Discussion

4.5.1 Differences between sampling methods

Sixty-three percent of the mean Van Dorn surface water measurements were lower than the corresponding peeper surface water measurements. Because the Van Dorn samples were only filtered through a 53-µm mesh, and the peeper samples were filtered through a 0.2-µm membrane, one would intuitively expect the opposite trend. However, because the Van Dorn samples were collected ~15 cm above the sediment surface (as close as was possible without inadvertently getting fine surface sediment in the sample) and the peepers sampled the 0-2-cm surface water layer nearest to the sediment, it is believed that the closer association with the sediment surface led to higher concentrations of the variables measured in the peeper samples (the sediments typically contained higher concentrations of most variables). Hence, it is proposed that the peeper

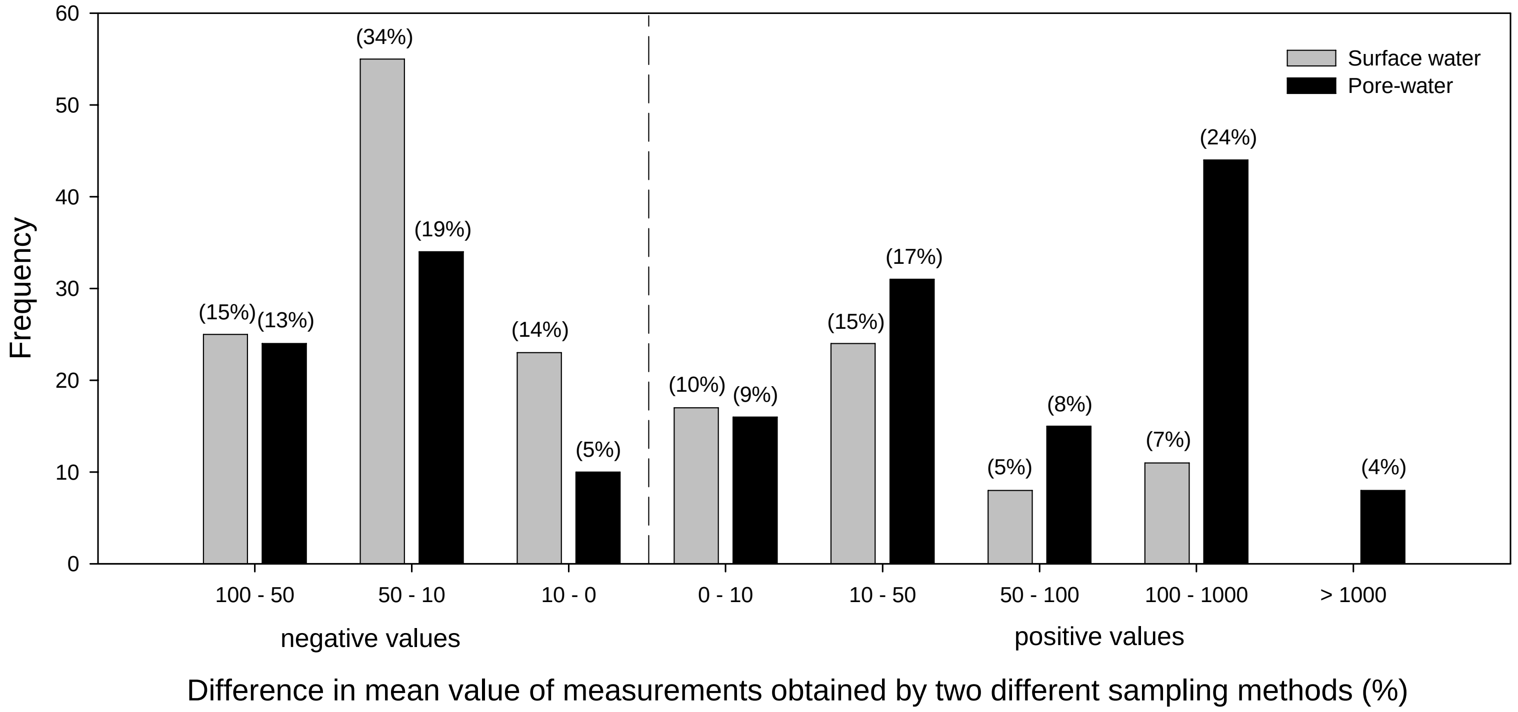

Figure 4.1. Percent difference between Van Dorn surface water and sediment core pore-water measurements (*n* = 163 and *n* = 182 respectively) relative to the corresponding peeper measurements at both the Key Lake and Rabbit Lake uranium operation study sites. Values in parentheses are the percentage of total surface water or pore-water values within that range.

surface water measurements are a more accurate representation of the contaminant concentrations experienced by surface-dwelling and/or epibenthic invertebrates, and likely by *H. azteca* in the associated *in-situ* surface water chambers.

Comparing the differences in pore-water measurements when collected using two different sampling techniques, revealed that 62% of the core samples had higher concentrations of measured variables than the respective peeper samples. Because the pore-water isolated from the sediment core samples were filtered through a 0.45-µm membrane and the peeper samples were equipped with a 0.2-µm membrane, this could be, at least partially, attributed to pore-size differences. However, although the two methods appear to sample similar sediment horizons (pore-water from the core samples represented the top 0-2.5-cm sediment horizon and the top peeper samples represented 1-cm within the top 0-2-cm horizon), it is hypothesized that the methods do not actually sample similar horizons. Because most sediments sampled had a flocculent or unconsolidated surface layer, it is believed this layer was better sampled with the peeper compared to the corer. Visual observations during sampling suggested that some of the flocculent surface layer may have been displaced as the core samples were collected. Therefore, mean core sample measurements were typically higher in metal concentration than the mean peeper measurements because the core sample actually measured a slightly lower sediment horizon that generally contained higher concentrations of the variables measured. The flocculent surface layer would also be more influence by the less contaminated surface water. Consequently, peeper measurements are proposed to be a more accurate representation of the top horizon of sediments *in-situ,* especially for sediments with a flocculent or unconsolidated surface layer. That said, because the associated *in-situ* study utilized sediment cores within the sediment exposure chambers (which were collected in the same matter as the sediment cores utilized for pore-water chemistry), the sediment core pore-water measurements might actually be more representative of the contaminant concentrations experienced by *H. azteca* in the associated *in-situ* study.

In short, it is believed that differences between both methods of surface water and pore-water measurements are more due to capturing different surface water and sediment horizons, respectfully, than due to differences in filtration or actual sampling method used.

4.5.2 Toxicological relevance of measurement differences

While there were differences between measurements from both methods of pore-water and surface water collection, did these dissimilarities lead to differences in the conclusions drawn for the *in-situ* toxicity studies performed at the Key Lake and Rabbit Lake uranium operations? Due to the high water hardness levels at all exposure sites, only contaminants where concentrations exceeded high hardness LC50s were considered likely to have contributed to the toxicity observed in the two *in-situ* studies. To simplify this assessment, conclusions were drawn from the more conventional sampling method measurements (Van Dorn and sediment core measurements) and compared to the conclusions drawn from the peeper measurements.

Key Lake uranium operation: The only variables from the sediment core samples that exceeded the high hardness LC50s at both exposure sites and not the reference site were pore-water iron, arsenic, and molybdenum; no variables measured in the Van Dorn surface water samples exceeded any of the high hardness LC50s. Since there was no difference in the response of the *H. azteca* exposed to surface water and sediment exposure chambers in the associated *in-situ* study, and assuming that the analyses are representative of the *in-situ* environment, the primary cause of the aquatic toxicity to *H. azteca* does not appear to be directly correlated with the variables measured here. Measurements from peeper samples (pore-water and surface water) that exceeded the high hardness LC50 values at an exposure site and not the reference site were pore-water arsenic and molybdenum at Fox Lake; no peeper surface water measurements exceeded any of the high hardness LC50s. Again, because there was no difference in the response of the *H. azteca* exposed in surface water and sediment exposure chambers, the primary cause of the aquatic toxicity to *H. azteca* does not appear to be directly correlated with the variables measured. Overall, while there were differences between values recorded with different sampling methods, there was no difference in the overall conclusion drawn using the two data sets.

Rabbit Lake uranium operation: Variables from Van Dorn and sediment core samples that exceed high hardness LC50 values at both exposure sites and not the reference site were surface water manganese and molybdenum, as well as pore-water manganese, iron, and molybdenum. Because Unknown Pond sediment core pore-water uranium values exceeded the high hardness LC50 and Horseshoe Pond almost met the same criteria (1473µg/L vs. 1651µg/L (Borgmann et al., 2005)), uranium was also considered as a suspected candidate. Variables from peeper samples that exceed high hardness LC50 values at both exposure sites and not the reference site were surface water manganese and molybdenum, as well as pore-water manganese, iron, molybdenum, and uranium. Surface water peeper values exceeded the high hardness LC50 for iron and uranium only in Unknown Pond. Of the above four variables, only molybdenum and uranium concentrations were higher in the surviving amphipods from the exposure sites when compared to those from the reference site (Chapter 3). Based on this, manganese and iron were excluded as likely causes of the amphipod mortality observed in the accompanying *in-situ* study (Chapter 3). Furthermore, molybdenum was secondarily excluded as a potential cause, since molybdenum has been shown to be relatively non-toxic (acutely) to *H. azteca* (the reported LC50 value by Borgmann et al. (2005) and Liber and White-Sobey (unpublished data) exceeded the highest experimental concentrations of 3150 µg/L (1-week) and 741 mg/L (4-d) respectfully).

While tissue concentrations of uranium were usually higher in amphipods from exposure sites than from the reference site, one exception was for amphipods from surface water chambers in Horseshoe Pond (Chapter 3). This correlates well with the observation of no difference in *H. azteca* survival in surface water chambers from the reference site and Horseshoe Pond (Chapter 3). Furthermore, only Unknown Pond and Horseshoe Pond pore water uranium concentrations (from both sampling techniques) exceeded the available high hardness LC50 data. Peeper surface water values exceed the high hardness LC50 for uranium in Unknown Pond; Van Dorn surface water samples did not. Due to the statistically lower survival of amphipods in Unknown Pond surface water chambers and the high uranium tissue concentration of these amphipods, it is believed that the peeper surface water samples were more representative of the exposure conditions experienced by the *H. azteca* within the surface water chambers in Unknown

Pond. Although the Van Dorn method did not produce a toxicologically relevant (acute) concentration of uranium within the surface water, the uranium tissue concentrations of surviving amphipods and the response of the amphipods would still suggest that uranium was the cause of toxicity. Therefore, of the variables measured, it appears that only uranium could have caused, or contributed to, the observed *in-situ H. azteca* mortality. While there were differences in the measurements obtained with the two different sampling methods, conclusions drawn from the more conventional sampling techniques and from the peeper measurements were the same.

Overall, observed differences in concentrations of toxicologically relevant variables related to the use of different sampling methods did not affect the conclusions drawn on the possible cause(s) of *in-situ* toxicity to *H. azteca* at both uranium operations.

CHAPTER 5

INVESTIGATING THE CAUSE OF *IN-SITU* TOXICITY TO *HYALELLA AZTECA* DOWNSTREAM OF THE KEY LAKE URANIUM OPERATION

5.1 Abstract

Past research has found benthic invertebrate community impairment and *in-situ* toxicity to *Hyalella azteca* downstream of the Key Lake uranium operation in northern Saskatchewan, Canada. Using a weight-of-evidence approach, the present study reports findings from subsequent 4-d laboratory surface water bioassays and toxicity tests with *H. azteca* designed to determine the cause of the previously observed *in-situ* toxicity. Surface water collected in 2004 at the time of the related *in-situ* study was toxic to *H. azteca* and therefore verified that contaminated surface water, not sediment, was the primary cause of the observed *in-situ* mortality. Chemical analysis of the surface water showed that trace metals, total ammonia, and pH were not present at levels suspected of causing the observed effects, while high sulphate concentrations could possibly have played a role. Further information revealed that organic mill-process chemicals, which have been previously linked with sporadic effluent toxicity, were released at the Key Lake operation during the time of the *in-situ* experiment and associated surface water collection. Additional surface water samples were collected in June and August, 2005, with the aim of conducting Toxicity Identification Evaluations if a toxic sample was obtained. However, both batches of water were not toxic to *H. azteca*. Furthermore, a second bioassay with archived surface waters from the initial 2004 collection demonstrated that the water was no longer toxic. Chemistry comparisons of the toxic and non-toxic surface water samples, verified that trace metals, ammonia, pH, and major ions, including sulphate, were not likely to be the cause of toxicity, leaving only organic mill-process chemicals as a probable cause. Subsequent 4-d laboratory toxicity tests demonstrated that these process chemicals (kerosene, amine, and isodecanol) are acutely

toxic to *H. azteca* at the levels released in 2004, and are therefore believed to be the cause of the *H. azteca* mortality observed in the earlier *in-situ* experiment.

5.2 Introduction

Benthic community impairment has been observed on several separate occasions downstream of the effluent discharge site at the Key Lake uranium operation in northern Saskatchewan, Canada (Terrestrial and Aquatic Environmental Managers Ltd., 1994; Conor Pacific Environmental Technologies Inc., 2000; Golder Associates Ltd., 2002; Chapter 2). Among these effects that have been observed include significant reductions in taxon richness and total abundance and significantly higher Bray-Curtis index values. Sediment Quality Triad studies conducted in 2003 and 2004 revealed that contaminated sediment might not be the only contributing factor to benthic community impairment (Chapter 2). In response, a 4-d *in-situ* bioassay with laboratory-reared *Hyalella azteca* confined in both surface water and sediment exposure chambers was conducted in the summer of 2004 (Chapter 3) as part of a broader investigation-of-cause research project. Results from the *in-situ* bioassay revealed significant mortality at the exposure sites Wolf Lake and Fox Lake ($p < 0.001$). There was no statistical difference between survival in surface water and sediment exposure chambers ($p = 0.232$), suggesting surface water (the common feature of both types of exposure chambers) was the primary cause of *in-situ* mortality of *H. azteca* at Key Lake. Although the *in-situ* study identified surface water exposure as the primary cause of *in-situ* toxicity, the study concluded that none of the water quality variables measured within that study (including trace metals, total ammonia, and pH) were present levels that could have caused the observed effects (Chapters 3 and 4).

The objective of this study was to investigate the cause of the observed *in-situ* toxicity to *H. azteca*. This objective was achieved through the use of 4-d bioassays with surface water collected from the Key Lake receiving environment and 4-d toxicity tests with suspected constituents. Using a weight-of-evidence approach, constituents are eliminated from the investigation and a probable cause is proposed.

5.3 Methods and materials

5.3.1 Study sites

The Key Lake uranium operation is located in northern Saskatchewan, Canada (Figure 1.1). The operation began production in 1983 as a mine and mill site and is currently the largest high-grade uranium milling operation in the world (www.cameco.com). Although on-site ore has been depleted, the Key Lake mill currently processes ore from another near-by mine, McArthur River. Constituents within the wastewater effluent can be of both mining and milling origin, since many processes recycle contaminated water (Cameco Corporation, 2004). The Key Lake discharge basin consists of a series of small lakes connected through creeks (Figure 1.2). The site of effluent discharge is Wolf Lake. The Wolf Lake outflow is estimated to contain 72% effluent (Golder Associates Ltd., 2003a). Wolf Lake drains through a small creek into Fox Lake. David Lake is an uncontaminated upstream reference lake.

The first batch of water collected for surface water bioassays was collected on day 4 of the *in-situ* bioassays conducted at Wolf, Fox, and David Lakes in July 2004. Samples were collected adjacent to the *in-situ* exposure chambers immediately before the *in-situ* test was terminated. The second and third batches of water for surface water bioassays were collected on June 14, 2005, and August 10, 2005, at the location of the *in-situ* study site in Wolf Lake.

5.3.2 Test organisms

Hyalella azteca were obtained from an in-house culture maintained in an environmental chamber with a set photoperiod of 16:8 hour light:dark and a temperature of 23 ± 1°C. Municipal, carbon-filtered water with a total hardness of ~130 mg/L as $CaCO_3$, an alkalinity of ~80 mg/L $CaCO_3$, and a pH of ~ 8 was used for culturing. Animals were fed *ad-libidum* with a Tetramin® (Tetra Werke, Melle, Germany) fish food slurry, *Scenedesmus sp*., and occasionally laboratory-cultured biofilm (unknown composition). Silica sand (particle size = 425 to 850 µm) and cheesecloth gauze were used as substrates. To ensure test organisms were of known age, breeding adult *H. azteca* were isolated and juveniles collected after 7 days. Juveniles were held for two days, making them 2-9 days old at test initiation (Environment Canada, 1997).

5.3.3 Surface water bioassays

Surface waters were collected using a 3.2-L Van Dorn horizontal acrylic beta water sampler (Wildlife Supply Company, Buffalo, NY, USA) from ~15 cm above the sediment surface. Samples were sieved through a 53-µm sieve to remove planktonic organisms and debris, were transferred into 1-L polyethylene containers, and transported with no head space. Because organic chemicals were identified as a possible cause of previously observed adverse effects prior to the 2005 sampling trips, 10-L polyethylene containers were used to reduce the surface area to volume ratio, thus reducing the potential degree of adsorption of organic material to the polyethylene container walls (collection and transport of water in glass was not practical). Surface water samples were stored in the dark at 4°C until test initiation, which occurred no more than 6 days after collection. Archived batch 1 samples were tested approximately one year after collection. David Lake water was used as a reference for the initial testing of samples from batch 1. For convenience, subsequent bioassays used municipal carbon-filtered water as the control.

Test initiation (day 0) commenced with the addition of ten laboratory-reared juvenile *H. azteca* per test vessel (n = 4 per treatment). Test vessels consisted of 250-mL glass beakers (each with aeration and a 2.5-cm x 2.5-cm piece of cheesecloth gauze as a substrate) for the initial batch 1 and the batch 3 bioassays. The bioassay with archived batch 1 water and batch 2 water (tested concurrently) used 100-mL glass beakers (with aeration and gauze) to accommodate the smaller volume of archived batch 1 water. Regardless of test vessel type, approximately 80% of the water was renewed daily and test organisms within each test vessel fed 100 µL of diluted Tetramin® slurry daily. Dissolved oxygen (DO) and temperature were measured daily in all test vessels using an ORION® dissolved oxygen meter model 835 (ORION Research, Beverly, MA, USA). Hardness and alkalinity (Hach Digital Titrator model 16900, Hach Company, Loveland, CO, USA), conductivity (Orion ATI conductivity cell 017010 and Orion ATI meter 170), ammonia (Beckman® 250 pH/Temp/mV meter, Beckman Instruments Inc., Fullerton, CA, USA and Thermo Orion® 95-12 ammonia electrode or Orion aquafastII photometer), and pH (ORION® PerpHect LogR meter model 370 or Beckman® 250 pH/Temp/mV meter and Beckman® pH electrode 511050) were measured at the start (day 0) and end (day 4) of each bioassay. For both bioassays using samples from batch 1 (initial and archived) and the batch 2 bioassay, overlying surface water samples were collected on day 0 (before the addition of aeration) and day 4 (before test termination) for total trace metals analysis (ICP-MS, Department of Geological Sciences, University of Saskatchewan). To further characterize the different batches of surface water, two 1-L samples of each batch were analyzed for total phosphorus, bicarbonate, hydroxide, carbonate, chloride, nitrate-N, nitrite-N, ion balance, total dissolved solids (TDS) (calculated), calcium, potassium, magnesium, sodium, and sulfate by Enviro-Test Laboratories, Saskatoon, SK. Surviving amphipods were removed and enumerated at test termination.

5.3.4 Toxicity tests

After the previously described *in-situ* study (Chapter 3) and the initial bioassay with batch 1 surface water samples (presented here), discussion with an industry representative revealed that a batch of effluent with 1.2 mg/L of organic mill-process chemicals was released during the time of the *in-situ* study (July 9, 2004). This concentration, measured by the Key Lake operation analytical laboratory through gas chromatography, was for total organic mill-process chemicals, which sums the measurement of all three organic mill-process chemicals used at the facility (kerosene, amine (Tri-C_8-C_{10}-Alkylamines), and isodecanol). The CAS (Chemical Abstracts Service) registry numbers for kerosene, amine, and isodecanol are 8008-20-6, 68814-95-9, and 25339-17-7 respectively (Canadian Centre for Occupational Health and Safety, 2000a, 2000b, 2000c). Test concentrations for the toxicity tests were based around the measured total concentration of 1.2 mg/L (0.12, 0.38, 1.2, 3.8, and 12 mg/L). Because the relative ratio of the three organic mill-process chemicals in this sample was unknown, the chemicals were tested as a mixture at the ratio used within the mill (88% kerosene, 9% amine, and 3% isodecanol), as well as individually. Toxicity tests with the individual chemicals used lower concentrations to account for the possibility that only amine (at 9%) or isodecanol (at 3%) were the toxic component of the mixture.

Test solutions were made with reconstituted water made up to mimic the hardness (~600 mg/L as $CaCO_3$), alkalinity (~5 mg/L as $CaCO_3$), and pH (~6.00) of the samples collected at Wolf Lake and Fox Lake in July 2004. Because the organic mill-process chemicals are volatile and can adsorb to test vessels, 125-mL glass jars (with Teflon-lined lids) were used with no head space, no aeration, and no gauze. For the mixture test, a stock solution was made by combining 88 mL kerosene, 9 mL amine, and 3 mL isodecanol. Chemicals were obtained from the Key Lake uranium operation. The density of kerosene, amine, and isodecanol are 0.8, 0.81, and 0.837 g/mL, respectively (Canadian Centre for Occupational Health and Safety, 2000a, 2000b, 2000c). At the specified ratio, the density of the mixture should be ~0.8 g/mL. Assuming a density of 0.8 g/mL, 15 µL (12 mg) were dissolved in 1 mL of acetone (to ensure solubility at high concentrations) and added to 1 L of reconstituted water to achieve a final concentration of 12 mg/L of the total organic mill-process chemical mixture (the highest test concentration). Similar stock and test solutions were prepared for the individual chemicals. Once the 12 mg/L solution was made for each test, the remaining test solutions were prepared through serial dilution. To account for the addition of acetone (1 mL of acetone in the 12 mg/L treatment), separate solvent controls were run with 1 mL of acetone per litre of reconstituted water. Negative controls (municipal carbon-filtered water with no addition of acetone) were also run.

Test initiation (day 0) commenced with the addition of ten laboratory-reared juvenile *H. azteca* per test vessel (*n* = 4 per treatment). Approximately 80% of the water was renewed daily, and test organisms within each test vessel were fed 100 µL of diluted Tetramin® slurry once a day. Because tests were not aerated and were pH adjusted (through the addition of Omni Trace® HCl, EMD Chemicals Inc., Gibbstown, NJ, USA), DO, temperature and pH were measured before and after all daily water changes. Hardness, alkalinity, conductivity, and ammonia, were measured at the start (day 0) and end (day 4) of each toxicity test. Surviving amphipods were removed and enumerated at test termination.

Analysis for total organic mill-process chemicals was performed on surrogate samples (*n* = 2 per treatment per test). Surrogate samples were used because the method required at least 1 L of sample for a detection limit of 1 mg/L. Due to the 1 mg/L detection limit, only samples from the 1.2, 3.8, and 12 mg/L treatments were extracted and analyzed. For consistency, analysis was performed by the Key Lake operation analytical laboratory through gas chromatography after a hexane extraction step.

5.3.5 Statistics and data analysis

All statistics were performed using SigmaStat®, version 3.1 (SPSS Inc., Chicago, IL, USA). If tests for normality or homogeneity of variance failed, physicochemical data were transformed using a $\log_{10}$ transformation, or in the case of survival data an arcsine square-root transformation. pH data were not transformed. For consistency, transformed data sets that still failed tests for normality or homogeneity of variance still underwent parametric analysis. Physicochemical data describing new/day 0 and old/day 4 water conditions were analyzed using two-way ANOVA. If a statistical difference was detected ($p \leq 0.05$), a Tukey's all pair-wise multiple comparison post-hoc test was run.

For ease of interpretation of surface water and pore-water data, only the variables meeting the following criteria were presented/assessed: the mean value exceeded the Canadian water quality guideline (CWQG) for protection of aquatic life (Canadian Council of Ministers of the Environment, 1999); the mean value exceeded published *H. azteca* toxicity values (Borgmann et al., 2005; France and Stokes,1987; Ankley et al., 1995); analytical detection limits exceeded CWQG guidelines or reported toxicity values; or elements that did not have published water quality guidelines and/or toxicity data (Table 2.1).

For surface water bioassay results, *t*-tests were used to evaluate the difference between the mean survival for the reference/control and the exposure site. For process chemical toxicity tests, a one-way ANOVA was conducted. If a statistical difference was detected ($p \leq 0.05$), a Dunnett's post-hoc test was run (with the solvent control as the designated control). Separate *t*-tests were used to determine if differences existed between the solvent and negative controls. Median lethal concentrations (LC50s) were calculated using the Trimmed Spearman-Karber Method (Program Version 1.5, United States Environmental Protection Agency, Cincinnati, OH, USA).

5.4 Results

5.4.1 Surface water bioassays

The initial bioassay with batch 1 surface water had significantly lower *H. azteca* survival than the respective control (p = 0.022), with a mean survival of 35% (Figure 5.1). There was also a significant reduction in *H. azteca* survival in the batch 2 bioassay of Wolf Lake surface water compared to its control (p = 0.035), but because mean survival was 85%, the sample was not deemed toxic. There was no significant difference in amphipod survival between Wolf Lake and control water in either the bioassay with archived batch 1 water (p = 0.780) or batch 3 water (p = 0.143) (Figure 5.1). With respect to the Fox Lake bioassays, the initial bioassay with batch 1 surface water had significantly lower *H. azteca* survival than its control (p = 0.011), with a mean survival of only 23% (Figure 5.2). The follow-up test with archived batch 1 water revealed that the water was no longer toxic (p = 0.356) after ~1 year storage (Figure 5.2).

General water quality characteristics for the Wolf Lake bioassays are presented in Table 5.1. Although there were differences in temperature, dissolved oxygen, and alkalinity, these variables appear to be reasonably consistent across the different batches of surface waters tested. Trends between day 0 and day 4 measurements (for all bioassays) include higher pH and ammonia on day 4. The bioassay with archived batch 1 water had significantly higher pH and ammonia on day 4 and significantly higher conductivity on both day 0 and day 4 than the initial batch 1 bioassay, although overall the values were very similar. Comparisons between the initial batch 1 water and the batch 2 water revealed significantly higher pH, ammonia, hardness, and conductivity in batch 2 on both day 0 and day 4, with ammonia, hardness and conductivity values approximately two times greater. The third batch of Wolf Lake surface water also had significantly higher pH, ammonia, hardness, and conductivity than the initial batch 1 water, although the levels of ammonia, hardness, and conductivity were not nearly as high as for batch 2 water. Overall, the initial batch 1 bioassay had the lowest pH, ammonia, hardness and conductivity values of all four bioassays.

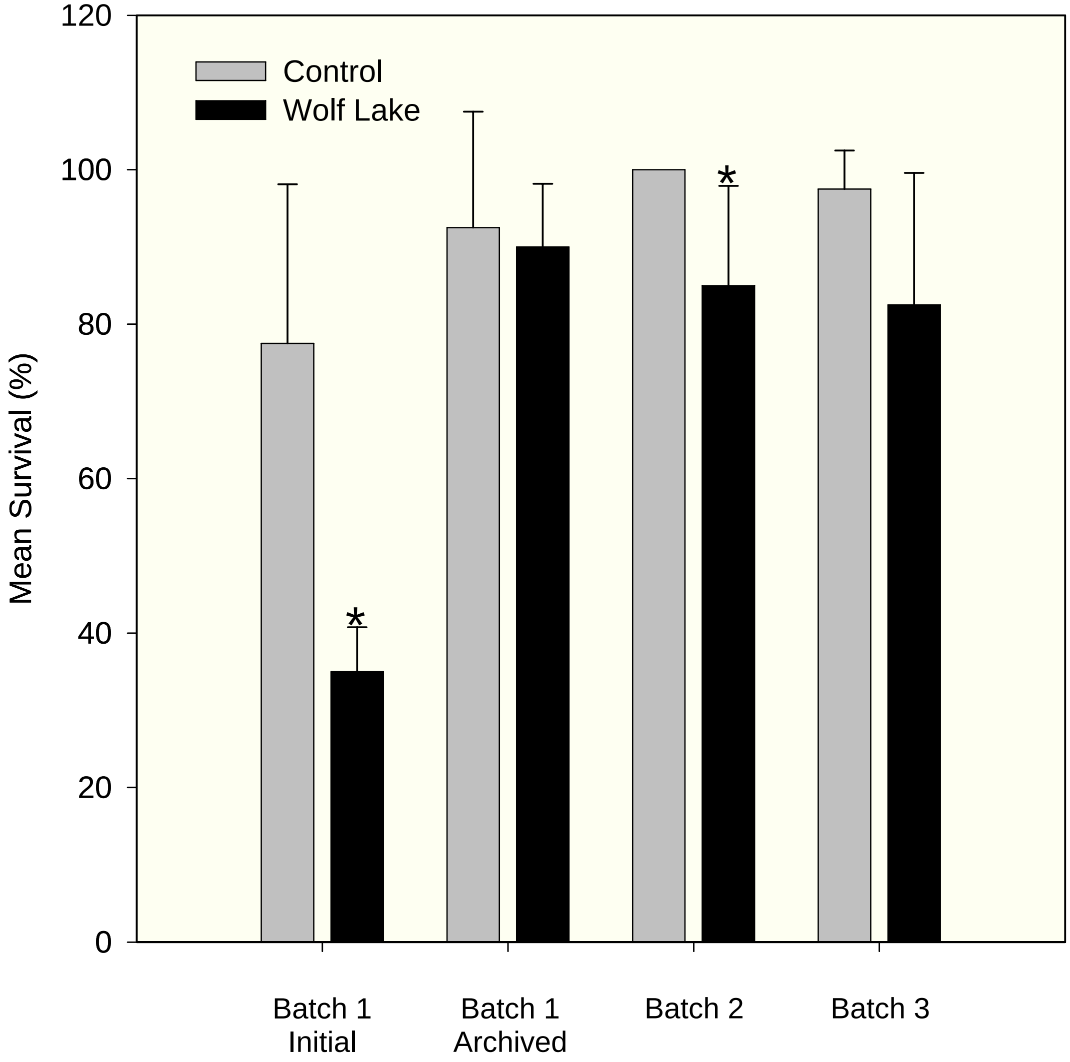

Figure 5.1. Mean *Hyalella azteca* survival (n = 4) from 4-d bioassays with Wolf Lake water and their respective control groups. Asterisk denotes significance from the respective control groups (t-test, $p \leq 0.05$).

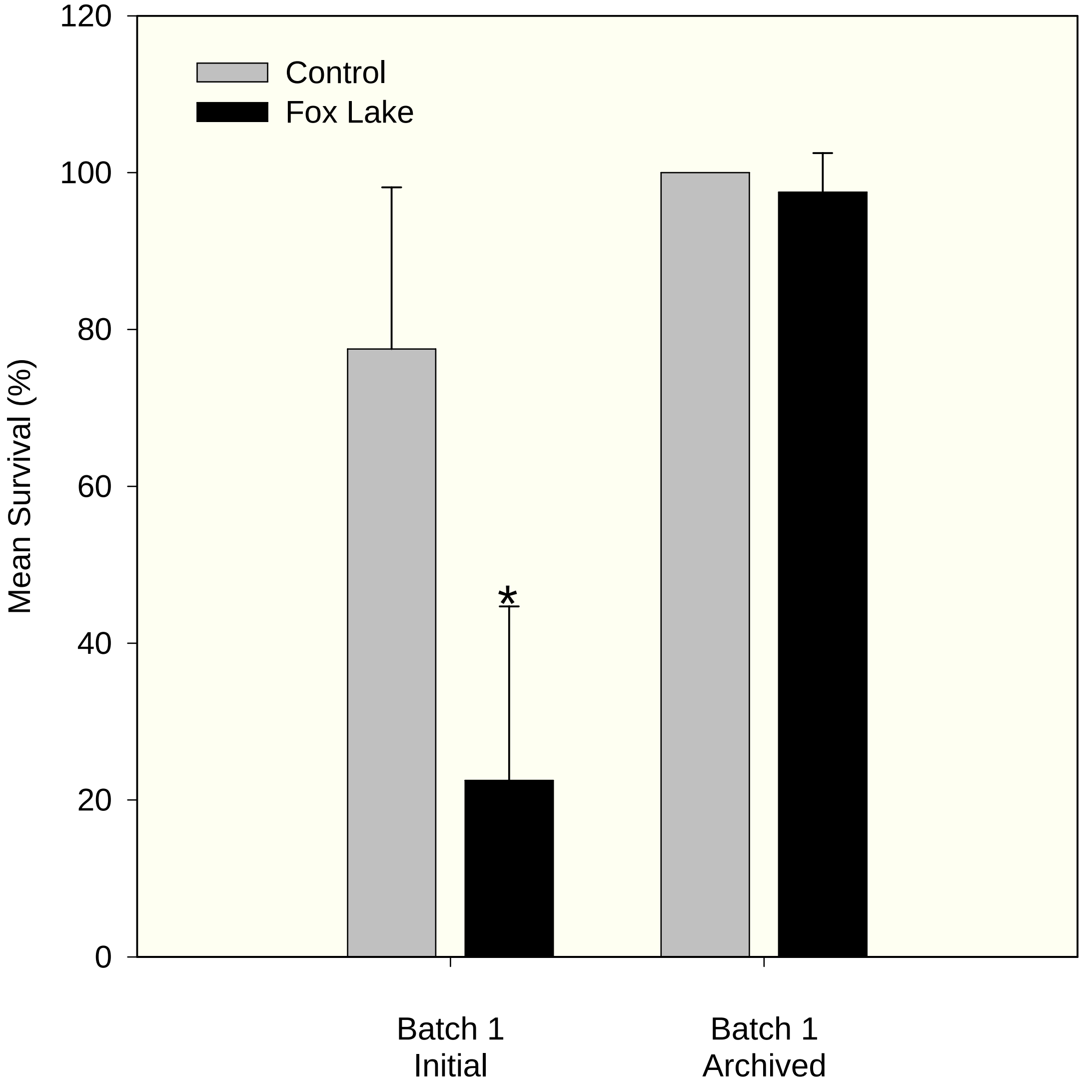

Figure 5.2. Mean *Hyalella azteca* survival (n = 4) from 4-d bioassays with Fox Lake water and their respective control groups. Asterisk denotes significance from the respective control group (t-test, $p \leq 0.05$).

Table 5.1. Mean (± standard deviation, $n = 4$) day 0 (d-0) and day 4 (d-4) water quality measurements for the Wolf Lake *Hyalella azteca* 4-d surface water bioassays.

Variable	Batch 1 - initial (toxic)		Batch 1 - archived (non-toxic)	
	d-0	d-4	d-0	d-4
Temperature (°C)	24.6 ± 0.2	24.8 ± 0.3	23.2 ± 0.4[*]	22.8 ± 0.4[*‡]
DO[a] (mg/L)	7.7 ± 0.4	7.2 ± 0.1[‡]	7.6 ± 0.2	7.6 ± 0.1[*]
pH	6.03 ± 0.02[b]	6.11 ± 0.04[b‡]	6.06 ± 0.05[b]	6.68 ± 0.08[*‡]
Total Ammonia (mg N/L)	5.3 ± 0.4[b]	5.4 ± 0.3[b]	5.6 ± 0.2[b]	6.5 ± 0.3[b*‡]
Alkalinity (mg/L as CaCO$_3$)	5 ± 0	6 ± 1	6 ± 1	7 ± 1[‡]
Total Hardness (mg/L as CaCO$_3$)	610 ± 30	630 ± 20	650 ± 40	680 ± 40
Conductivity (µS/cm)	1154 ± 8	1169 ± 3	1211 ± 7[*]	1292 ± 35[*‡]

Variable	Batch 2 (non-toxic)		Batch 3 (non-toxic)	
	d-0	d-4	d-0	d-4
Temperature (°C)	23.0 ± 0.4[*]	23.3 ± 0.5[*‡]	23.9 ± 0.1[*]	23.6 ± 0.2[*]
DO[a] (mg/L)	7.6 ± 0.2	7.7 ± 0.2[*]	7.9 ± 0.4	7.5 ± 0.1[*‡]
pH	6.19 ± 0.04[b*]	6.24 ± 0.04[b*]	6.45 ± 0.02[b*]	6.74 ± 0.08[*‡]
Total Ammonia (mg N/L)	12.5 ± 0.6[b*]	13.1 ± 0.3[b*‡]	6.8 ± 0.1[b*]	7.3 ± 0.3[b*]
Alkalinity (mg/L as CaCO$_3$)	8 ± 0[*]	7 ± 1[‡]	8 ± 1[*]	7 ± 1[*]
Total Hardness (mg/L as CaCO$_3$)	1140 ± 20[*]	1130 ± 10[*]	700 ± 30[*]	720 ± 20
Conductivity (µS/cm)	2142 ± 21[*]	2130 ± 12[*]	1398 ± 16[*]	1409 ± 18[*]

[a] DO = dissolved oxygen.

[b] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999).

[*] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from the initial Batch 1 measurement.

[‡] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from the respective d-0 measurement.

Table 5.2 presents the general water quality characteristics for the Fox Lake bioassays. Although there were differences between day 0 and day 4 measurements, overall there were no noteworthy trends. The conditions for the initial and second testing of the Fox Lake water were very similar.

Table 5.3 lists major ions data for each batch of Wolf Lake and Fox Lake surface water collected. Due to low sample size (n = 2), statistics were not performed. However, some general trends can be identified. Levels of total phosphorus, bicarbonate, hydroxide, carbonate, and nitrite-N were all slightly above or below the detection limits for all samples. The second batch of Wolf Lake surface water had approximately two times greater levels of chloride, nitrate-N, nitrate+nitrite-N, ion balance, total dissolved solids, calcium, potassium, magnesium, sodium, and sulphate than the other three sets of samples.

Trace metal data for selected Wolf Lake bioassays are presented in Table 5.4. Although significant differences were noted between day 0 and day 4 measurements for each bioassay, these differences did not appear to reflect any trends and were not gauged to be toxicologically significant. Although significant differences were noted between trace metal concentrations for the initial and second (archived) bioassay with batch 1 water, conditions were generally very similar. Furthermore, batch 2 water had higher concentrations of trace metals than the two bioassays with batch 1 water. Table 5.5 presents trace metal data for the Fox Lake bioassays. There were few significant differences noted between tests, or between day 0 and day 4 trace metal measurements for either bioassay. In short, trace element exposure did not appear to differ substantially between comparable tests with toxic and non-toxic water from batch1.

5.4.2 Organic mill-process chemical toxicity tests

General water chemistry for the organic mill-process chemical (mixture, kerosene, amine, and isodecanol) toxicity tests is presented in Table 5.6. Overall, there were significantly lower levels of dissolved oxygen and pH and significantly higher levels of total ammonia on day 4 than day 0 in all tests. Only minor, toxicologically insignificant differences were observed among tests.

Table 5.2. Mean (± standard deviation, $n = 4$) day 0 (d-0) and day 4 (d-4) water quality measurements for the Fox Lake *Hyalella azteca* 4-d surface water bioassays.

Variable	Batch 1 - initial (toxic)		Batch 1 - archived (non-toxic)	
	d-0	d-4	d-0	d-4
Temperature (°C)	24.8 ± 0.4	24.8 ± 0.4	23.2 ± 0.6[*]	23.2 ± 0.5[*]
DO[a] (mg/L)	7.6 ± 0.6	7.3 ± 0.1	7.5 ± 0.2	7.6 ± 0.4
pH	5.79 ± 0.02[b]	5.69 ± 0.09[b]	5.74 ± 0.13[b]	5.92 ± 0.11[b*‡]
Total Ammonia (mg N/L)	4.7 ± 0.5[b]	5.1 ± 0.7[b]	5.4 ± 0.1[b]	5.1 ± 0.1[b]
Alkalinity (mg/L as $CaCO_3$)	5 ± 1	3 ± 1[‡]	6 ± 1[*]	5 ± 1[*]
Total Hardness (mg/L as $CaCO_3$)	610 ± 20	600 ± 20	580 ± 30	580 ± 20
Conductivity (µS/cm)	1150 ± 5	1159 ± 4	1211 ± 7[*]	1200 ± 8[*‡]

[a] DO = dissolved oxygen.

[b] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999).

[*] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from the initial Batch 1 measurement.

[‡] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from the respective d-0 measurement.

Table 5.3. Mean (± standard deviation, *n* = 2) major ion measurements from selected *Hyalella azteca* 4-d surface water bioassays.

	Batch 1 (Wolf)	Batch 1 (Fox)	Batch 2 (Wolf)	Batch 3 (Wolf)
Variable	Toxic	Toxic	Non-toxic	Non-toxic
Total Phosphorus (mg/L)	0.3 ± 0.1	0.3 ± 0.1	<0.2	<0.2
Bicarbonate (HCO_3) (mg/L)	<5	<5	6 ± 0	6 ± 0
Hydroxide (OH) (mg/L)	<5	<5	<5	<5
Carbonate (CO_3) (mg/L)	<5	<5	<5	<5
Chloride (mg/L)	18 ± 0	19 ± 0	33 ± 1	20 ± 0
Nitrate-N (mg/L)	0.6 ± 0.0	0.6 ± 0.0	0.9 ± 0	0.5 ± 0
Nitrite-N (mg/L)	<0.05	<0.05	<0.05	<0.05
Nitrate+Nitrite-N (mg/L)	0.6 ± 0.0	0.6 ± 0.0	0.9 ± 0	0.5 ± 0
Ion Balance (%)	94.3 ± 0.6	94.0 ± 0.6	97.5 ± 4.9	91.7 ± 0.5
TDS (calculated) (mg/L)	998 ± 4	979 ± 8	1920 ± 141	1095 ± 21
Calcium (mg/L)	228 ± 3	221 ± 0	439 ± 66	243 ± 4
Potassium (mg/L)	13 ± 0	15 ± 0	23 ± 1	19 ± 0
Magnesium (mg/L)	19 ± 0	18 ± 1	36 ± 0	15 ± 0
Sodium (mg/L)	21 ± 0	23 ± 0	57 ± 8	31 ± 1
Sulfate (mg/L)	697 ± 2	681 ± 8	1323 ± 81	761 ± 15

Table 5.4. Mean (± standard deviation, $n = 3$) day 0 (d-0) and day 4 (d-4) trace elements measurements from selected Wolf Lake *Hyalella azteca* 4-d surface water bioassays.

Variable	Batch 1 - initial (toxic)		Batch 1 - archived (non-toxic)		Batch 2 (non-toxic)	
(µg/L)	d-0	d-4	d-0	d-4	d-0	d-4
Mg	18956 ± 769	20849 ± 384	18008 ± 1044	19815 ± 232	31851 ± 622[*]	34180 ± 2216[*‡]
Al	69.4 ± 4.6[a]	51.6 ± 2.0[a]	77.9 ± 7.5[a]	82.5 ± 55.3[a]	139.3 ± 27.2[ab*]	170.1 ± 70.5[ab*]
Si	1290 ± 189	1759 ± 27[‡]	<15943[*]	<15943[*]	<15943[*]	<15943[*]
P	268 ± 130	396 ± 84[‡]	<370	<370[*]	<370	<370[*]
Ca	121920 ± 55681	72415 ± 4156	348350 ± 25473[*]	211043 ± 26173[*‡]	411772 ± 6439[*]	427247 ± 14776[*]
Cr	<1.4[a]	<1.4[a]	<340.0[abc*]	<340.0[abc*]	<340.0[abc*]	<340.0[abc*]
Mn	129.0 ± 3.3[b]	130.0 ± 1.5[b]	127.1 ± 3.4[b]	137.3 ± 4.0[b*‡]	91.9 ± 1.3[*]	94.1 ± 1.6[b*]
Fe	1031 ± 44[ab]	858 ± 91[a‡]	546 ± 69[a*]	724 ± 42[a‡]	1175 ± 44[ab*]	1197 ± 84[ab*]
Ni	33.49 ± 1.77[a]	31.53 ± 1.34[a]	31.87 ± 2.99[a]	34.87 ± 2.96[a]	37.95 ± 2.28[a]	58.15 ± 27.20[a*‡]
Cu	16.31 ± 4.13[a]	<2.25[a‡]	<4.57[a*]	<4.57[a*]	6.35 ± 0.38[a*]	4.92 ± 0.59[a*‡]
As	13.22 ± 0.48[a]	11.48 ± 0.83[a‡]	<21.01[a*]	<21.01[a*]	<21.01[a*]	<21.01[a*]
Se	<7.9[a]	<7.9[a]	<194.7[abc*]	<194.7[abc*]	<194.7[abc*]	<194.7[abc*]
Mo	374 ± 2[a]	357 ± 1[a]	381 ± 14[a]	403 ± 9[a*‡]	959 ± 18[a*]	1011 ± 8[abc*]
Cd	<0.25[ab]	<0.25[ab]	<3.58[abc*]	<3.58[abc*]	<3.58[abc*]	<3.58[abc*]
Tm	<0.02[b]	<0.02[b]	<0.20[b*]	<0.20[b*]	<0.20[b*]	<0.20[b*]
Hg	<0.70[a]	<0.70[a]	<12.44[abc*]	<12.44[abc*]	<12.44[abc*]	<12.44[abc*]

[a] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999).

[b] Exceeds soft water median lethal concentration for *Hyalella azteca* (Borgmann, et al., 2005).

[c] Exceeds hard water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005).

[*] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from the initial Batch 1 measurement.

[‡] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from the respective d-0 measurement.

Table 5.5. Mean (± standard deviation, $n = 3$) day 0 (d-0) and day 4 (d-4) trace elements measurements from Fox Lake *Hyalella azteca* 4-d surface water bioassays.

Variable	Batch 1 - initial (toxic)		Batch 1 - archived (non-toxic)	
(μg/L)	d-0	d-4	d-0	d-4
Mg	17015 ± 1407	17587 ± 1413	16369 ± 232	17072 ± 556
Al	80.1 ± 5.5[a]	74.2 ± 5.5[a]	71.9 ± 8.3[a]	118.5 ± 41.3[ab*‡]
Si	782 ± 328	1128 ± 639	<15943[*]	<15943[*]
P	155 ± 45	277 ± 107	<370	<370
Ca	311838 ± 32397	156796 ± 73709[‡]	186231 ± 4676[*]	210614 ± 14762
Cr	0.9 ± 0.4	<1.4[a]	<340.0[abc*]	<340.0[abc*]
Mn	79.30 ± 3.49	79.29 ± 2.42	84.15 ± 1.58[*]	85.00 ± 2.32[*]
Fe	1000 ± 49[ab]	755 ± 57[a‡]	710 ± 65[a*]	689 ± 104[a]
Ni	21.89 ± 1.33	21.05 ± 1.24	20.99 ± 1.72	21.09 ± 2.01
Cu	6.81 ± 3.47[a]	<2.25[a‡]	<4.57[a*]	<4.57[a*]
As	13.56 ± 1.30[a]	9.76 ± 1.94[a‡]	<21.01[a*]	<21.01[a]
Se	<7.9[a]	<7.9[a]	<194.7[abc*]	<194.7[abc*]
Mo	427 ± 15[a]	395 ± 3[a‡]	420 ± 8[a]	411 ± 15[a]
Cd	<0.25[ab]	<0.25[ab]	<3.58[abc*]	<3.58[abc*]
Tm	<0.02[b]	<0.02[b]	<0.20[b*]	<0.20[b*]
Hg	<0.70[a]	<0.70[a]	<12.44[abc*]	<12.44[abc*]

[a] Exceeds Canadian Water Quality Guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 1999).

[b] Exceeds soft water median lethal concentration for *Hyalella azteca* (Borgmann, et al., 2005).

[c] Exceeds hard water median lethal concentration for *Hyalella azteca* (Borgmann et al., 2005).

[*] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from the initial Batch 1 measurement.

[‡] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from the respective d-0 measurement.

Table 5.6. Mean (± standard deviation) new and old water quality measurements for the 4-d *Hyalella azteca* toxicity tests with organic-mill process chemicals.

Variable	Mixture		Kerosene	
	new water	old water	new water	old water
Temperature (°C)	24.0 ± 0.2	24.1 ± 0.2[‡]	24.1 ± 0.1	24.1 ± 0.3
DO[a] (mg/L)	7.5 ± 0.3	6.1 ± 1.2[‡]	6.8 ± 0.2	5.7 ± 0.5[‡]
pH	6.52 ± 0.08	6.33 ± 0.20[‡]	6.51 ± 0.17	6.40 ± 0.21[‡]
Total Ammonia (mg N/L)	>0.1	0.3 ± 0.1[‡]	>0.1	0.2 ± 0.1[‡]
Alkalinity (mg/L as $CaCO_3$)	11 ± 4	8 ± 2[‡]	12 ± 1	9 ± 7[‡]
Total Hardness (mg/L as $CaCO_3$)	660 ± 50	680 ± 30[‡]	680 ± 20	680 ± 20
Conductivity (µS/cm)	1184 ± 65	1230 ± 28[‡]	1271 ± 3	1285 ± 13

Variable	Amine		Isodecanol	
	new water	old water	new water	old water
Temperature (°C)	24.1 ± 0.1	24.1 ± 0.3	23.8 ± 0.2	24.0 ± 0.3[‡]
DO[a] (mg/L)	6.9 ± 0.3	5.6 ± 0.8[‡]	7.0 ± 0.3	5.4 ± 0.6[‡]
pH	6.42 ± 0.11	6.35 ± 0.17[‡]	6.53 ± 0.16	6.34 ± 0.22[‡]
Total Ammonia (mg N/L)	>0.1	0.2 ± 0.1[‡]	>0.1	0.1 ± 0.1[‡]
Alkalinity (mg/L as $CaCO_3$)	11 ± 1	5 ± 3[‡]	7 ± 3	10 ± 1[‡]
Total Hardness (mg/L as $CaCO_3$)	710 ± 20	700 ± 20	700 ± 20	680 ± 20
Conductivity (µS/cm)	1222 ± 16	1164 ± 110[‡]	1295 ± 9	1235 ± 16[‡]

[a] DO = dissolved oxygen.

[‡] Significantly different (two-way ANOVA, Tukey's post hoc test, $p \leq 0.05$) from the respective new water measurement.

Results from chemical analysis of the organic mill-process chemical were on average 2.75 ± 1.15 times lower than nominal concentrations (with the mixture, kerosene, amine, and isodecanol values 2.25 ± 0.54, 4.06 ± 0.74, 3.33 ± 0.35, and 1.38 ± 0.15 times lower, respectively). As a result of this discrepancy, measured values were considered compromised and LC50s, NOECs and LOECs were estimated from nominal concentrations.

Mean survival of *H. azteca* for all four toxicity tests are presented in Figure 5.3. For clarity, only the top five exposure concentrations are shown. Calculation of LC50s, NOECs and LOECs utilized all tested concentrations (Table 5.7). It should be noted that mean survival in the solvent control was significantly lower than in the negative control for both the amine and isodecanol test, but the solvent control was designated as the control for the one-way ANOVAs (Dunnett's post-hoc test). The potency of the chemicals from most toxic to least toxic was amine, kerosene, the industrial mixture, and isodecanol. Overall, LC50s ranged from 0.25 to 1.33 mg/L.

5.5 Discussion

5.5.1 Surface water bioassays

Of the surface water samples tested, only the initial testing of the July 2004 Wolf Lake and Fox Lake surface water samples were toxic to *H. azteca*. This observation supports the conclusion drawn in Chapter 3, that the cause of *in-situ* toxicity to caged *H. azteca* at Wolf Lake and Fox Lake was primarily due to surface water exposure (the water tested was collected on day 4 of the *in-situ* study). Chemical analysis of the toxic Wolf Lake and Fox Lake samples reveal that none of the variables measured (including trace metals, total ammonia, and pH) were at levels believed to be high enough to have caused the observed response (i.e., no variable was in exceedance of their high hardness LC50). These findings concur with the findings presented in Chapters 3 and 4, which concluded that the primary cause of *in-situ* toxicity to *H. azteca* was not correlated with levels of trace metals, total ammonia, and pH. Furthermore, non-toxic surface water samples (including the test with archived batch 1 water that was initially toxic) had similar or higher levels of these variables than the toxic surface water sample, which supports the elimination of trace metals, total ammonia, and pH as potential causes of

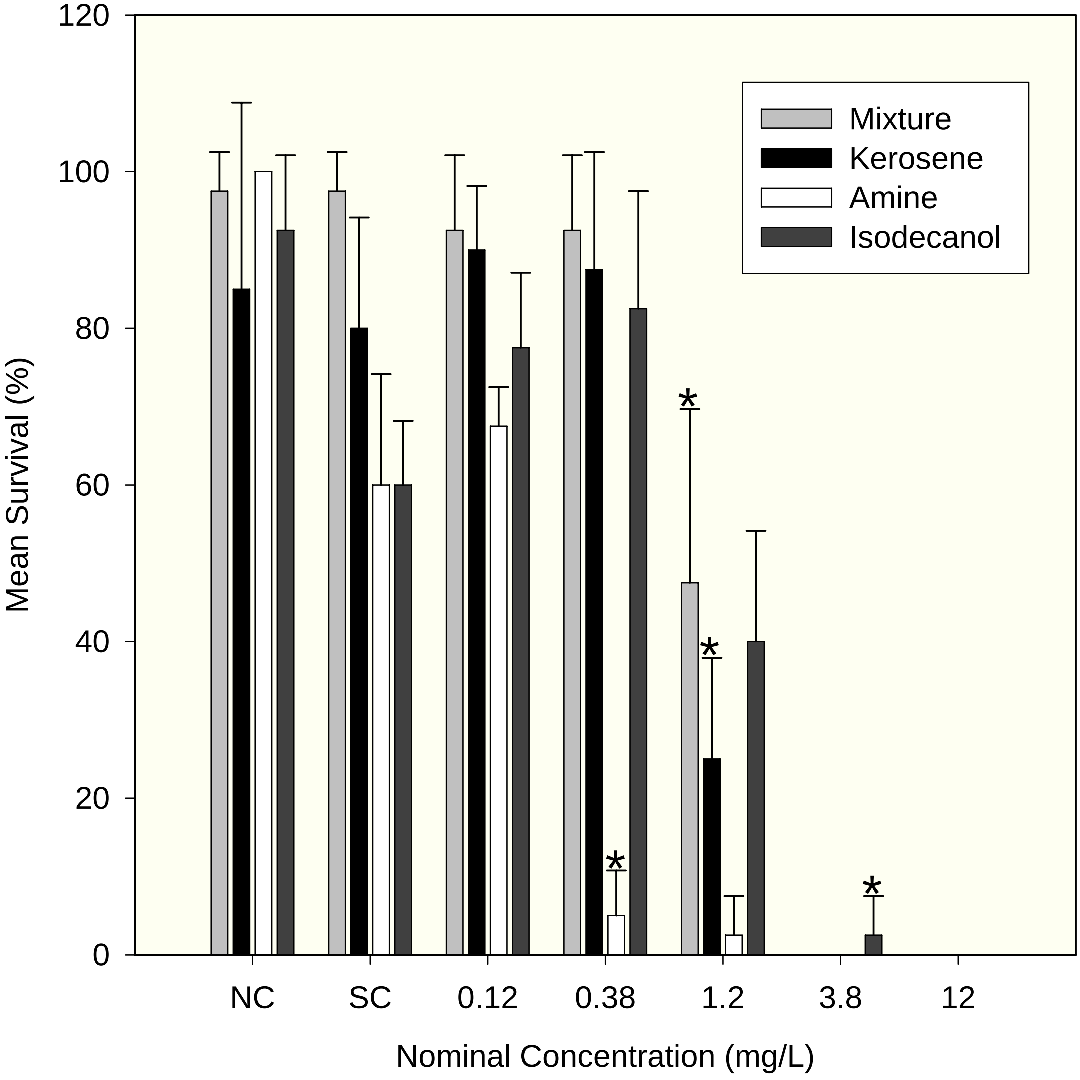

Figure 5.3. Mean *Hyalella azteca* survival (*n* = 4) from 4-d toxicity tests of organic mill-process chemicals in mixture and individually. Asterisk denotes a statistical difference from the respective solvent control group (SC). NC = negative control group.

Table 5.7. Acute toxicity of organic mill-process chemicals in mixture and individually to *Hyalella azteca* in 4-d water-only tests. All data are in mg/L (nominal exposure).

	LC50[a] (95% CL[b])	SK trim[c]	NOEC[d]	LOEC[e]
Mixture	1.15 (0.93-1.41)	5.13%	0.38	1.2
Kerosene	0.94 (0.80-1.11)	0.00%	0.38	1.2
Amine	0.25 (0.22-0.28)	0.00%	0.12	0.38
Isodecanol	1.33 (1.09-1.61)	0.00%	1.2	3.8

[a] Median lethal concentration calculated using the Trimmed Spearman-Karber Method.

[b] Confidence limit.

[c] SK trim = Spearman-Karber trim.

[d] No observable effect concentration.

[e] Lowest observable effect concentration.

toxicity. Although appropriate toxicity data were not available for the major ions presented in Table 5.3, comparisons between toxic and non-toxic surface water samples again revealed that the non-toxic samples had similar or higher concentrations than the toxic samples, thus eliminating these major ions as potential causes of the toxicity observed in the initial bioassays of the July 2004 Wolf Lake and Fox Lake samples. Consequently, it is concluded that the cause of *H. azteca* mortality in the July 2004 surface water samples was not related to trace metals, total ammonia, pH, or major ions.

As previously mentioned, after the completion of the *in-situ* study (Chapter 3) and initial laboratory testing of the first batch of surface water (presented here), discussion with an industry representative revealed that organic mill-process chemicals were released during the time of the *in-situ* study. It was also disclosed that these chemicals had been historically linked with sporadic effluent and/or Wolf Lake outflow toxicity (HydroQual Laboratories Ltd., 1995; 2002; 2004), which further supported the possibility that these chemicals were responsible for the toxicity observed here. Furthermore, because the toxicity of the July 2004 samples disappeared after one year of storage, and the measured inorganic conditions of the toxic and non-toxic water samples from batch 1 were still similar, it was hypothesized that the cause of toxicity to *H. azteca* was a degradable organic compound or mixture of compounds. Comparisons between the toxic responses observed *in-situ* and in the initial batch 1 bioassay reveal that average survival was lower *in-situ* (Wolf Lake = 13% and Fox Lake = 5%) than in the laboratory bioassays (Wolf Lake = 35% and Fox Lake = 23%) suggesting that some of the organic mill-process chemicals could have degraded between collection and test initiation or that exposure *in-situ* peaked prior to day 4 when the water samples for the bioassays were collected.

5.5.2 Organic mill-process chemical toxicity tests

Results from the toxicity tests with organic mill-process chemicals showed that the chemical mixture and the individual chemicals were all toxic to *H. azteca* at concentrations around the total mixture concentration released to Wolf Lake in July 2004 (1.2 mg/L). The observation that all three chemicals are individually toxic at levels around 1.2 mg/L illustrates that toxicity to *H. azteca* would probably have been observed regardless of the relative ratio of the three organic mill-process chemicals released. It should also be noted that if measured exposure concentrations had been utilized (as opposed to nominal), conclusions would have been even stronger since measured chemical concentrations were on average 2.75 ± 1.15 times lower than nominal concentrations.

There is relatively little aquatic invertebrate toxicity data available for the three organic mill-process chemicals used at the Key Lake operation. The MSDS for isodecanol noted a 24-h LC50 for *Daphnia magna* of 11 mg/L (Canadian Centre for Occupational Health and Safety, 2000c). No aquatic invertebrate toxicity data could be found for kerosene and amine.

As previously mentioned, the Wolf Lake outflow (the site of surface water collection at Wolf Lake) is estimated to contain approximately 72% effluent (Golder Associates Ltd., 2003a). Based on this estimate, the concentration of total organic mill-process chemical at the collection site, assuming no degradation, would be ~0.864 mg/L (i.e., 72% of 1.2 mg/L). Kerosene, amine, and isodecanol are all noted to be stable with low water solubilities (insoluble, not available, and <1 g/L, respectively) and low vapour pressures (0.4, 0.1, and 0.01 Torr at 20ºC, respectively) (Canadian Centre for Occupational Health and Safety, 2000a, 2000b, 2000c). Therefore, assuming there are no major differences in the persistence and dissipation of kerosene, amine, and isodecanol within the short time it takes effluent to reach the Wolf Lake outflow, the relative ratio of the three organic mill-process chemicals in the environment is assumed to be similar to the ratio in the chemical mixture. Based on this exposure regime, the toxic units (TU = ratio of exposure concentration to toxicity endpoint (LC50)) for the organic mill-process chemical mixture, kerosene, amine, and isodecanol are 0.751, 0.809, 0.311, and 0.020, respectively. These TUs suggest that kerosene is the most likely cause of the toxic response to *H. azteca*. It is interesting to note that the sum of the TUs for kerosene, amine, and isodecanol is greater than the TU for the mixture, indicating the mixture is less toxic than the sum of its components, although these differences could be due to experimental variability. The TU of the mixture (0.751) further suggests that less than 50% mortality of *H. azteca* should have been observed at the estimated exposure concentrations. The differences between this prediction (calculated through TUs) and the observed toxicity (in the laboratory and *in-situ*) could suggest that *in-situ* exposure was actually higher than estimated, or that organic mill-process chemicals in combination with some other variable caused the observed response.

In conclusion, the evidence provided by the 4-d surface water bioassays and the toxicity tests with organic mill-process chemicals supports the hypothesis that organic mill-process chemicals were the primary, if not sole, cause of *H. azteca* mortality observed in the *in-situ* study (Chapter 3) and the surface water bioassay of July 2004.

CHAPTER 6

GENERAL DISCUSSION

6.1 Project rationale and goal

Past research has noted benthic macroinvertebrate community impairment downstream of both the Key Lake and Rabbit Lake uranium operations in northern Saskatchewan, Canada. Although the presence of these impacts has been well documented, there has been relatively little research directed at identifying the cause(s) of these impairments. Therefore, the overall objective of this research was to identify the cause(s) of benthic macroinvertebrate community impairment at the Key Lake and Rabbit Lake uranium operations using a weight-of-evidence approach, including use of the Sediment Quality Triad (SQT) (field chemistry, benthic community assessment and sediment bioassays), *in-situ* bioassays, surface-water bioassays, and toxicity tests.

6.2 Project summary

6.2.1 Key Lake

Benthic community impairment downstream of the Key Lake uranium operation is well documented. However, relatively little research has been conducted on determining the cause(s) of these impacts. Given sediments accumulate contaminants that are common to metal mining (such as metals and radionuclides), the initial hypothesis for this research was that contaminated sediments were the primary cause of benthic community impairment downstream of the Key Lake uranium operation. Based on this hypothesis, a Sediment Quality Triad (SQT) approach was used in 2003 and 2004. Results confirmed the presence of an effect on benthic community structure, in addition to significant differences in surface-water, pore-water and whole-sediment chemistry. These effects were much more pronounced at the near-field sites, Wolf Lake and Fox Lake. No significant adverse effects were noted from the associated 10-d whole-sediment bioassays with *Hyalella azteca*, although the lack of response was believed to be partially attributed to pore-water dilution resulting from the automated clean overlying water renewal process employed. Potential causes of benthic community impairment identified through the 2003 and 2004 SQTs for Key Lake include physical sediment composition, surface water pH and total ammonia, in addition to increases in pore-water total ammonia, and arsenic.

In the summer of 2004, 4-d *in-situ* bioassays using *H. azteca* were conducted in addition to the SQTs to investigate the role both contaminated surface water and sediment played in benthic community impairment *in-situ*. Results from the *in-situ* bioassays revealed significant mortality at Wolf Lake and Fox Lake ($p < 0.001$), with no statistical differences between survival in surface water and sediment exposure chambers ($p = 0.232$). These results suggest that surface water (the common feature of both types of exposure chambers) was the primary cause of *in-situ* mortality at the Key Lake uranium operation. The cause of *in-situ* toxicity could not be correlated with any of the variables measured within the *in-situ* study (including trace metals, total ammonia, and pH). Two data sets from two methods of surface water and pore-water collection supported these conclusions.

Surface water collected from Wolf Lake and Fox Lake in 2004 at the time of the related *in-situ* study was also toxic to *H. azteca* in separate laboratory surface water bioassays ($p = 0.022$) which verified that contaminated surface water, not sediment, was the primary cause of the observed *in-situ* mortality at Key Lake. Further information from an industry representative revealed that organic mill-process chemicals, which have been previously linked with sporadic effluent toxicity, were released at the Key Lake operation during the time of the *in-situ* experiment and associated surface water collection. Additional Wolf Lake surface water samples were collected in June and August, 2005, with the aim of conducting Toxicity Identification Evaluations if a toxic sample was obtained. However, both batches of water were not toxic to *H. azteca*. Furthermore, a second bioassay with archived surface waters from the initial 2004 collection demonstrated that the water was no longer toxic. Chemistry comparisons of the toxic and non-toxic surface water samples, verified that trace metals, ammonia, pH, and major ions, including sulphate, were not the cause of toxicity, leaving only organic mill-process chemicals as a possible cause. Subsequent 4-d laboratory toxicity tests demonstrated that these process chemicals (kerosene, amine, and isodecanol) are toxic to *H. azteca* at the levels released in 2004, and are therefore believed to be the cause of the *H. azteca* mortality seen in the earlier *in-situ* experiment.

In conclusion, the Key Lake study identified several variables that could cause benthic macroinvertebrate impairment *in-situ*. These stressors were the result of current releases, historical accumulation, and/or natural causes. Potential causes of impairment that are mainly associated with current releases (i.e., surface water) include organic mill-process chemicals, total ammonia, and low pH. Stressors of concern due to historical accumulation (i.e., sediments) include fine particles and arsenic. Natural causes of differences in benthic community structure include sediment composition and low pH. The relative importance of each of these stressors to benthic community impairment *in-situ* was not identified in this thesis, however, because only organic-mill process chemicals were identified as causing acute toxicity, these chemicals are assumed to have a major influence on the health of natural benthic communities, at least at the near-field sites.

6.2.2 Rabbit Lake

Unfortunately the investigation of benthic community impairment for the Rabbit Lake uranium operation was not as successful and involved as that for the Key Lake operation. As with the Key Lake investigation, the cause of benthic community impairment was initially hypothesized to be due to sediment contamination, mainly related to the accumulation of metals and/or radionuclides. In 2003 and 2004, the SQT approach confirmed the presence of an effect on benthic community structure, in addition to significant differences in surface-water, pore-water and whole-sediment chemistry at the near-field sites, Unknown Pond and Horseshoe Pond. No significant adverse effects were noted from the 2003 10-d whole-sediment bioassays with *H. azteca*, although this lack of response could be partially attributed to pore-water dilution resulting from the automated clean overlying water renewal process employed. The 2004 whole-sediment bioassay was not successful due to low control survival. Surface water manganese and uranium, and pore-water total ammonia, manganese, iron, arsenic, and uranium were identified through the 2003 and 2004 SQTs as potential causes of benthic impairment.

In the summer of 2004, a 4-d *in-situ* bioassay using *H. azteca* was conducted in addition to the SQTs to investigate the role both surface water and sediment play in benthic community impairment *in-situ*. Results from the *in-situ* bioassays revealed significant mortality at Unknown Pond and Horseshoe Pond ($p = 0.001$). There were no significant differences between survival in surface water and sediment exposure chambers at Rabbit Lake ($p = 0.072$), although this relationship was much stronger at Key Lake ($p = 0.232$). Of the measured constituents at Rabbit Lake, only concentrations of uranium in both surface water and pore-water were suspected of causing the observed *in-situ* mortality. Two data sets from two methods of surface water and pore-water collection supported these conclusions.

In summary, the Rabbit Lake study identified several variables that could affect benthic macroinvertebrate community structure. Potential causes of impairment that are mainly associated with current effluent releases (i.e., surface water) include uranium and manganese. Stressors of concern due to historical accumulation (i.e., sediments) include uranium, manganese, iron, arsenic, and total ammonia. Natural differences noted in sediment composition could also have affected benthic community structure. Of the stressors identified, uranium (due to historical accumulation) is hypothesized to be the primary cause of benthic community impairment in-situ, especially since it was identified as the most likely cause of acute *in-situ* toxicity to *H. azteca*.

6.3 Comparisons between Key Lake and Rabbit Lake

Although the Key Lake and Rabbit Lake uranium operations have many similarities, the primary constituents of concern to benthic communities are actually very different. For instance, the hypothesized cause of benthic community impairment at Key Lake appears to be related to both surface water and sediment exposure (i.e., both current and historical contamination), while the cause of benthic community impairment at Rabbit Lake is most likely due to the historical accumulation of uranium within sediments and sediment pore-waters. When comparing the specific constituents that were identified as potential causes of benthic community impairment, only pore water total ammonia and pore-water arsenic were identified at both operations.

The suspected cause of acute *in-situ* toxicity to *H. azteca* was also different at both operations. Even though the same organic mill-process chemicals are utilized in both the Key Lake and Rabbit Lake milling process, only Key Lake appears to have had an issue with occasional release of these chemicals to the environment. The cause of organic mill-process chemicals entering the final effluent has been determined to be via waste rock that contains concrete, as concrete does not readily adhere to the solvent extraction process. This problem is currently being addressed by Cameco Corporation in order to avoid organic mill-process chemical from entering the final effluent (Kevin Himbeault, personal communication, June 30, 2005).

Although uranium appears to be the primary cause of benthic community structure impairment at Rabbit Lake, this element is not of concern at Key Lake. This difference cannot be correlated with the grades of ore that are milled, as the McArthur River ore milled at Key Lake is of much higher grade than Rabbit Lake ore, even when it is diluted with Key Lake waste rock. Because Rabbit Lake is an older operation than Key Lake, one could speculate that the accumulation of uranium could be the result of differences in the mill process (i.e., less efficient extraction techniques).

These comparisons demonstrate that operations with similar processes and activities can ultimately have completely different constituents of concern.

6.4 Scientific contributions

This research delivered many significant contributions to the scientific community. Although there are many studies that utilize the SQT, Chapter 2 provides unique information on the potential causes of benthic community impairment at both the Key Lake and Rabbit Lake operations. With the recent increased interest in nuclear power, the use of this information extends beyond the Key Lake and Rabbit Lake uranium operations, to future uranium operations and uranium operation regulators that are interested in cause-effect relationships associated with the uranium mining and milling industry.

In-situ studies are relatively rare in scientific literature and have not been used extensively in Canada. The *in-situ* study described in Chapter 3 is one of the first *in-situ* studies preformed with invertebrates in Canada and provides positive improvements to *in-situ* testing with *H. azteca*. Improvements include provision of a food source (gauze with biofilm) in surface water chambers, the inclusion of screens in the chamber lids, careful acclimation of test animals to site-specific water hardness and alkalinity, and the relative composition and placement of the two chamber types. Furthermore, the study design was successful in accomplishing both objectives of the study: describing the response of *H. azteca* exposed to the receiving environments of both uranium operations, and determining the relative significance of surface water and sediment to the *in-situ* toxicity to *H. azteca*. This method is recommended for environments that have constituents of concern in both surface water and sediment.

Many studies have discussed the effect of sampling method on sample chemistry. However, the research in Chapter 4 is, to my knowledge, the first paper to actually compare conclusions derived from the results of different sampling methods and illustrate the relevance of the differences measured. In addition, Chapter 4 proposes that pore-water peepers measurements provide a more accurate representation of the top horizon of sediments *in-situ*, especially for sediments with a flocculent or unconsolidated surface layer. This recommendation is extremely important for anyone sampling pore-water from sediments (including the derivation of sediment quality guidelines).

The toxicity tests with organic mill-process chemicals presented in Chapter 5 add to the relatively small or non-existent aquatic toxicity data-base for these chemicals. The toxicity data produced can also help Key Lake uranium operation manage their effluent treatment and release.

6.5 Recommendations

6.5.1 Key Lake

With respect to management of the Key Lake uranium operation, it is recommended that there should be an extensive investigation with regards to the organic mill-process chemicals that have been previously linked with effluent and receiving surface water toxicity. Although milling processes have recently been adjusted to decrease the probability of organic mill-process chemicals carrying over to the final effluent, management controls to detect the presence of these chemicals (i.e., measurement through gas chromatography and Microtox$^{®}$) do not appear to be entirely reliable and/or accurate. The current detection limit for "total organic mill-process chemicals" at the Key Lake uranium operation chemistry lab is 1 mg/L. Chapter 5 demonstrates that concentrations at, or around, 1 mg/L are at, or near, acute effect concentrations for *H. azteca*. Screening effluent with this detection limit is therefore ineffective at protecting the receiving environment. In addition, the link between Microtox$^{®}$ readings and organic mill-process chemical concentration should be re-evaluated to gauge the effectiveness of the Microtox$^{®}$ assay in determining the presence of these chemicals in the final effluent. It would also be beneficial for the Key Lake analytical laboratory to analyze each individual organic mill-process chemical, instead of providing a "total" value, to better determine which specific chemical(s) are of concern.

Although previous studies have concluded the cause of pH depression within the Key Lake drainage basin was not caused by the Key Lake uranium operation effluent (including the oxidation of ammonia (nitrification) (SENES Consultants Ltd., 2002, 2003), it is recommended that the relationship between high ammonia concentrations and low pH be re-examined.

Another area which needs further research at Key Lake is to identify the contribution of historical and current contamination to benthic community impairment. In order to accomplish this, more monitoring and research is required on effluent releases and contaminated sediments. With respect to current releases, the stressors of concern should be measured more frequently, to determine the duration of toxicologically relevant levels within the receiving environment. Toxicity tests should be performed more often to characterize the chemical difference between a toxic and non-toxic effluent and the frequency of toxic releases. This information could lead to the development of cause-effect relationships and could ultimately lead to changes in effluent management and treatment. Because there is relatively little knowledge on the toxicity of complex mixtures, characterizing the effluent in tandem with toxicity testing might be the best mechanism to determine the cause(s) of acute effluent toxicity, including the use of Toxicity Identification Evaluations. It would also be beneficial to evaluate the chronic toxicity of effluent releases and contaminated sediments to aquatic invertebrates to determine the chronic effects of both environmental phases and hence determine the primary cause(s) of benthic community impairment. This would include sediment bioassays that minimize sediment and/or pore-water dilution, but also eliminate surface water exposure as a confounding factor. This could be determined by running concurrent surface water and sediment bioassays. This would include two surface water bioassays (one with site water and one with reconstituted water) and two sediment bioassays (one with site sediment and site water and one with site sediment and reconstituted water). Overall, determining whether or not current effluent releases or historical accumulation is the primary factor of benthic community impairment *in-situ* is vital information to effluent treatment and management of the Key Lake uranium operation.

6.5.2 Rabbit Lake

The major constituent of concern downstream of the Rabbit Lake uranium operation appears to be uranium. It is therefore recommended that more research on the effects of uranium on aquatic invertebrates is performed. A lot of research in this area has already commenced, but has yet to enter the scientific community.

As recommended with the Key Lake study, it would also be beneficial to evaluate the chronic toxicity of Rabbit Lake effluent and field collected sediments. In order to evaluate the chronic toxicity of both exposure routes and account for pore-water dilution and surface water exposure, two surface water bioassays and two sediment bioassays could be run concurrently as described for Key Lake.

6.5.3 Uranium industry

With the recent, renewed interest in nuclear energy, it has and will be increasingly important to determine the effects the uranium fuel cycle (including uranium mining and milling) have on receiving environments.

More research is required on constituents present in uranium mining and/or milling effluent including metals and major ions. Many metals and major ions are largely under-studied and desperately require more aquatic toxicity data. The lack of toxicity data might be accredited to the relatively low toxicity of some associated elements, but because these elements are discharged at relatively high concentrations, toxicity thresholds should be identified. Therefore, it is recommended that more chronic studies are conducted on metals and major ions that are released within the part per million and part per thousand ranges such as molybdenum, iron and sulphate. It is also recommended that metals and major ions be tested at water quality conditions that are relevant to effluent releases (such as high hardness and low pH). Although these water quality conditions may be largely irrelevant in other environments, there is a great need of toxicity data that test constituents at water quality conditions that are in accordance with industrial effluents.

It is also recommended that regulators look into regulating more than just well researched constituents such as pH, arsenic, nickel, and radium. Of specific concern at Key Lake is organic mill-process chemicals which are not, to our knowledge, regulated, but have proven to be a problem in the final effluent and receiving environment.

Uranium mines and mills are highly regulated through both federal and provincial governments. As part of their licence agreements and best practices, uranium mines and mills are required to monitor both their releases to the environment and the chemistry and biology of the receiving environment. The data produced from these monitoring programs are largely under utilized. If the data collected through previous monitoring was more effectively utilized and processed into integrated information, emerging toxicological issues and cause-effect relationships could be identified in a timely fashion. To avoid reactive management dictated through regulators, industry needs to better utilize their exhaustive data bases to catch emerging issues before they become a major environmental and/or regulatory problem.

Overall, the extent and severity of benthic community impairment at both the Key Lake and Rabbit Lake uranium operations are a relatively small “foot-print” in comparison to other mining industries, such as the Alberta oil sands. It should also be noted that there are huge economic and social benefits that have been acquired through uranium mining and milling within the province of Saskatchewan. However, more research is required to minimize or negate these effects when new developments are put forth. Although impacts are often expected and observed within the immediate range of effluent release, industries should learn from the impacts within the near-field to prevent these problems from continuing downstream and/or with other uranium mine and/or mill developments.

REFERENCES

Ankley GT, Katko A, Authur JW. 1989. Identification of ammonia as an important sediment-associated toxicant in the lower Fox River and Green Bay, Wisconsin. *Environmental Toxicology and Chemistry* 9:313-322.

Ankley GT, Schubauer-Berigan MK, Monson PD. 1995. Influence of pH and hardness on toxicity of ammonia to the amphipod *Hyalella azteca*. *Canadian Journal of Fisheries and Aquatic Sciences* 52:2078-2083.

Beltman DJ, Clements WH, Lipton J, Cacela D. 1999. Benthic invertebrate metal exposure, accumulation, and community-level effects downstream from a hard-rock mine site. *Environmental Toxicology and Chemistry* 21:1091-1097.

Benoit DA, Phipps GL, Ankley GT. 1993. A sediment testing intermittent renewal system for the automated renewal of overlying water in toxicity tests with contaminated sediments. *Water Research* 27:1403-1412.

Borgmann U. 1994. Chronic toxicity of ammonia to the amphipod *Hyalella azteca*; importance of ammonium ion and water hardness. *Environmental Pollution* 86:329-335.

Borgmann U, Couillard Y, Doyle P, Dixon G. 2005. Toxicity of sixty-three metals and metalloids to *Hyalella azteca* at two levels of water hardness. *Environmental Toxicology and Chemistry* 24:641-652.

Borgmann U, Nowierski M, Grapentine LC, Dixon DG. 2004. Assessing the cause of impacts on benthic organisms near Rouyn-Noranda, Quebec. *Environmental Pollution* 129:39-48.

Bufflap SE, Allen HE. 1995a. Comparison of pore water sampling techniques for trace metals. *Water Research* 29:2051-2054.

Bufflap SE, Allen HE. 1995b. Sediment pore water collection methods for trace metal analysis: A review. *Water Research* 29:165-177.

Burton GA. 1991. Assessing the toxicity of freshwater sediments. *Environmental Toxicology and Chemistry* 10:1585-1627.

Cameco Corporation. 2003. Rabbit Lake Operation: Mining Facility Licensing Manual, Revision 7. Cameco Corporation, Saskatoon, SK, Canada.

Cameco Corporation. 2004. Cumulative Impact Assessment of Proposed Process Changes at Key Lake: Site Information and State of Current Operation. Cameco Corporation, Saskatoon, SK, Canada.

Canada North Environmental Services. 2001. Environmental Fate and Toxic Effects of

Isodecanol and Isodecanoic Acid in Aquatic Systems (Draft report). Prepared for Cameco Corporation, Saskatoon SK, Canada as cited in SENES Consultants Ltd. 2002. Wolf Creek pH Depression and Toxicity Investigations: Key Lake Mine Site. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

Canadian Centre for Occupational Health and Safety. 2000a. Material Safety Data Sheet for Armeen 380 (Tri-C_8-C_{10}-Alkylamines).

Canadian Centre for Occupational Health and Safety. 2000b. Material Safety Data Sheet for Isodecanol.

Canadian Centre for Occupational Health and Safety. 2000c. Material Safety Data Sheet for Kerosene.

Canadian Council of Ministers of the Environment. 1999. Canadian Water Quality Guidelines for the Protection of Aquatic Life. Canadian Council of Ministers of the Environment, Winnipeg, MN, Canada.

Canfield TJ, Kemble NE, Brumbauch WG. 1994. Use of benthic invertebrate community structure and the sediment quality triad to evaluate metal-contaminated sediment in the Upper Clark Fork River, Montana. *Environmental Toxicology and Chemistry* 13:1990-2012.

Chapman PM. 1990. The sediment quality triad approach to determining pollution-induced degradation. *The Science of the Total Environment* 97-98:815-825.

Chapman PM. 1996. Presentation and interpretation of Sediment Quality Triad data. *Ecotoxicology* 5:327-339.

Chapman PM, Paine MD, Arthur AD, Taylor LA. 1996. A triad study of sediment quality associated with a major, relatively untreated marine sewage discharge. *Marine Pollution Bulletin* 32:47-64.

Chapman PM, Anderson B, Carr S, Engle V, Green R, Hameed J, Harmon M, Haverland P, Hyland J, Ingersoll C, Long E, Rodgers J, Salazar M, Sibley PK, Smith PJ, Swartz RC, Thompson B, Windom H. 1997. General guidelines for using the sediment quality triad. *Marine Pollution Bulletin* 34:368-372.

Chapman PM, Wang FW, Janssen C, Persoone G, Allen HE. 1998. Ecotoxicology of metals in aquatic sediment: Binding and release, bioavailability, risk assessment, and remediation. *Canadian Journal of Fisheries and Aquatic Sciences* 18:299-307.

Chapman PM. 2000. The Sediment Quality Triad: then, now and tomorrow. *International Journal of Environment and Pollution* 13:351-356.

Chappie DJ, Burton GA Jr. 1997. Optimization of *in-situ* bioassays with *Hyalella azteca* and *Chironomus tentans*. *Environmental Toxicology and Chemistry* 16:559-564.

Chappie DJ, Burton GA Jr. 2000. Applications of aquatic and sediment toxicity testing *in-situ*. *Journal of Soil Contamination* 9:219-246.

Conor Pacific Environmental Technologies Inc. 2000. McArthur River Pre-Milling Baseline for the Key Lake Study Area, 1998/1999. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

Constable M, Charlton M, Jensen F, McDonald K, Craig G, Taylor KW. 2003. An ecological risk assessment of ammonia in the aquatic environment. *Human and Ecological Risk Assessment* 9:527-548.

Crane M, Higman M, Olsen T, Simpson P, Callaghan A, Fisher T, Kheir R. 2000. An *in-situ* system for exposing aquatic invertebrates to contaminated sediments. *Environmental Toxicology and Chemistry* 19:2715-2719.

de Bisthoven LJ, Gerhardt A, Soares AMVM. 2004. Effects of acid mine drainage on larval *Chironomus* (Diptera, Chironomidae) measured with the multispecies freshwater Biomonitor®. *Environmental Toxicology and Chemistry* 23:1123-1128.

de Rosemond SJC, Liber K. 2004. Wastewater treatment polymers identified as the toxic component of a diamond mine effluent. *Environmental Toxicology and Chemistry* 23:2234-2242.

Doig L, Liber K. 2000. Dialysis minipeeper for measuring pore-water metal concentrations in laboratory sediment toxicity and bioavailability tests. *Environmental Toxicology and Chemistry* 19:2882-2889.

Donahue R, Hendry MJ. 2003. Geochemistry of arsenic in uranium mine mill tailings, Saskatchewan, Canada. *Applied Geochemistry* 18:1733-1750.

Emerson K, Russo RC, Lund RE, Thurston RV. 1975. Aqueous ammonia equilibrium calculations: Effect of pH and temperature. *Journal of the Fisheries Research Board of Canada*. 32:2379-2383.

Environment Canada. 1997. Biological test method: Test for survival and growth in sediment using the freshwater amphipod *Hyalella azteca*. Report: EPS 1/RM/33, Method Development and Application Section, Ottawa, ON, Canada.

Environment Canada. 2002. Metal Mining Guidance document for Aquatic Environmental Effects Monitoring. National Environmental Effects Monitoring Office, Gatineau, QC, Canada.

Faith DP, Humphrey CL, Dostine PL. 1991. Statistical power and BACI design in biological monitoring. Comparative evaluation of measures of community dissimilarity based on benthic macroinvertebrates communities in Rockhole Mine Creek, Northern Territory, Australia. *Australian Journal of Marine & Freshwater*

Research 42:589-602 as cited in Environment Canada. 2002. Metal Mining Guidance document for Aquatic Environmental Effects Monitoring. National Environmental Effects Monitoring Office, Gatineau, QC, Canada.

Fargašová A. 1997. Sensitivity of *Chironomus plumosus* larvae to V^{5+}, Mo^{6+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, and Cu^{+} metal ions and their combinations. *Bulletin of Environmental Contamination and Toxicology* 59:956-962.

Fjellheim A, Raddum GG. 1992. Recovery of acid-sensitive species of Ephemeroptera, Plecoptera, and Trichoptera in River Audna after liming. *Environmental Pollution* 78:173-178.

France RL, Stokes PM. Life stage and population variation in resistance and tolerance of *Hyalella azteca* (Amphipoda) to low pH. 1987. *Canadian Journal of Fisheries and Aquatic Sciences* 44:1102-1111.

Frias J, Ribas F, Lucena F. 1995. Comparison of methods for the measurement of biodegradable organic carbon and assimilable organic carbon in water. *Water Research* 29: 2785-2788.

Golder Associates Ltd. 2002. Key Lake Operation 2001 Monitoring Program, Key Lake Mine Site Saskatchewan. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

Golder Associates Ltd. 2003a. Historical Information and Study Design Report: Key Lake Operation. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

Golder Associates Ltd. 2003b. Historical Information Report: Rabbit Lake Operation, Cameco Corporation, Saskatchewan. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

Golder Associates Ltd. 2003c. Rabbit Lake Environmental Effects Monitoring / Environmental Monitoring Program 2002. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

Golder Associates Ltd. 2005. Key Lake Operation Comprehensive Environmental Effects Monitoring Program Interpretive Report. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

Haines TA. 1981. Acidic precipitation and its consequences for aquatic ecosystem: A review. *Transactions of the American Fisheries Society* 110:669-707.

Hartwell SI, Claflin LW. 2005. Cluster analysis of contaminated sediment data: Nodal analysis. *Environmental Toxicology and Chemistry* 24:1816-1834.

HydroQual Laboratories Ltd. 1995a. Isolation and Identification of the Toxic Constituents Present in Effluent from the Key Lake Operation of Cameco Corporation. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

HydroQual Laboratories Ltd. 1995b. Preliminary Assessment on the Ecological Fate and Effects of Isodecanol in Aquatic Systems. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

HydroQual Laboratories Ltd. 2002. Toxicity Identification Evaluation on Wolf Creek Discharge from the Key Lake Operation of Cameco Corporation. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

HydroQual Laboratories Ltd. 2004. Toxicity Assessment on the Outflow from Wolf Lake. Prepared for Cameco Corporation, Saskatoon Saskatchewan.

Jacher KA. 1994. Toxicity assessment of nonpoint source runoff: *In-situ* field validation using *Hyalella azteca*. M.Sc. thesis. Wright State University, Dayton, OH, USA.

Kater BJ, Postma JF, Dubbeldam M, Prins JTHJ. 2001. Comparison of laboratory and *in-situ* sediment bioassays using *Corophium volutator*. *Environmental Toxicology and Chemistry* 20:1291-1295.

Keller W, Gunn JM, Yan ND. 1992. Evidence of biological recovery in acid-stressed lakes near Sudbury, Canada. *Environmental Pollution* 78:79-85.

Kemble NE, Brumbaugh WG, Brunson EL, Dwyer FJ, Ingersoll CG, Monda DP, Woodward DF. 1994. Toxicity of metal-contaminated sediments from the upper Clark Fork River. *Environmental Toxicology and Chemistry* 13:1985-1997.

Key Lake Laboratories. 2004. Monitoring Pond Monthly Discharge Detail Report: May, June, and July 2004. Key Lake Operation, SK, Canada.

Kuhne WW, Caldwell CA, Gould WR, Fresquez PR, Finger S. 2002. Effects of depleted uranium on the health and survival of *Ceriodaphnia dubia* and *Hyalella azteca*. *Environmental Toxicology and Chemistry* 10:2198-2203.

Lasier PJ, Winger PV, Bogenrieder KJ. 2000. Toxicity of Manganese to *Ceriodaphnia dubia* and *Hyalella azteca*. *Environmental Contamination and Toxicology* 38:298-304.

Ledger ME, Hildrew AG. 2005. The ecology of acidification and recovery: changes in herbivore-algal food web linkages across a stream pH gradient. *Environmental Pollution* 137:103-118.

Lee MR, Correa JA, Seed R. 2006. A sediment quality triad assessment of the impact of copper mine tailings disposal on the littoral sedimentary environment in the Atacama region of northern Chile. *Marine Pollution Bulletin* 52:1389-1395.

Liber K, Wang D, White-Sobey S, Kerrich R, McKee M, Gates T. Unpublished data.

Liber K, White-Sobey S. Unpublished data.

Long ER, Chapman PM. 1985. A sediment quality triad: measures of sediment contamination, toxicity and infaunal community composition in Puget Sound. *Marine Pollution Bulletin* 16:405-415.

Maltby L, Clayton SA, Wood RM, McLoughlin N. 2002. Evaluation of the *Gammarus pulex in-situ* feeding assay as a biomonitor of water quality: robustness, responsiveness, and relevance. *Environmental Toxicology and Chemistry* 21:361-368.

Mason R, Bloom N, Cappellino S, Gill G, Benoit J, Dobbs C. (1998). Investigation of porewater sampling methods for mercury and methylmercury. *Environmental Science and Technology* 32:4031-4040.

Mkandawire M, Dudel EG. 2005. Accumulation of arsenic in *Lemna gibba* L. (duckweed) in tailing waters of two abandoned uranium mining sites in Saxony, Germany. *Science of the Total Environment* 336:81-89.

Muscatello JR. 2004. Chronic toxicity and accumulation of uranium in the aquatic invertebrate *Chironomus tentans*. M.Sc. thesis. University of Saskatchewan, SK, Canada.

Ormaza-González FI, Statham PJ. 1996. A comparison of methods for the determination of dissolved and particulate phosphorus in natural waters. *Water Research* 30:2739-2747.

Pearson TH, Rosenberg R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography and Marine Biology: An Annual Review* 16:229-311.

Pereira AMM, Soares AMVM, Fernando G, Ribeiro R. 1999. Test chambers and test procedures for *in-situ* toxicity testing with zooplankton. *Environmental Toxicology and Chemistry* 18:1956-1964.

Peterson MJ, Smith JG, Southworth GR, Ryon MG, Eddlemon GK. Trace element contamination in benthic macroinvertebrates from a small stream near a uranium mill tailings site. *Environmental Monitoring and Assessment* 74:193-208.

Pyle GG, Swanson SM, Lehmkuhl DM. 2001. Toxicity of uranium mine-receiving waters to caged fathead minnows, *Pimephales promelas*. *Ecotoxicology and Environmental Safety* 48:202-214.

Pyle GG, Swanson SM, Lehmkuhl DM. 2002. Toxicity of uranium mine receiving waters to early life stage fathead minnows (*Pimephales promelas*) in the laboratory. *Environmental Pollution* 116:243-255.

Rausch N, Ukonmaanaho L, Nieminen TM, Krachler M, Roux GL, Shotyk W. 2006. Evaluation of samplers and filter materials for the establishment of trace metal concentration profiles in peat bog porewaters using inductively coupled plasma-mass spectrometry. *Analytica Chimica Acta* 558:201-210.

Schmidt TS, Soucek DJ, Cherry DS. 2002. Integrative assessment of benthic macroinvertebrate community impairment form metal-contaminated waters in tributaries of the upper Powell River, Virginia, USA. *Environmental Toxicology and Chemistry* 22:2233-2241.

Schubauer-Berigan MK, Ankley GT. 1991. The contribution of ammonia, metals and non-polar organic compounds to the toxicity of sediment interstitial water from an Illinois river tributary. *Environmental Toxicology and Chemistry* 10:925-939.

Schults DW, Ferraro SP, Smith LM, Roberts FA, Poindexter CK. 1992. A comparison of methods for collecting interstitial water for trace organic compounds and metal analyses. *Water Research* 26:989-995.

Schulz R. 2003. Using a fresh water amphipod *in-situ* bioassay as a sensitive tool to detect pesticide effects in the field. *Environmental Toxicology and Chemistry* 22:1172-1176.

SENES Consultants Ltd. 2002. Wolf Creek pH Depression and Toxicity Investigations: Key Lake Mine Site. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

SENES Consultants Ltd. 2003. Wolf Creek pH Depression 2002 Investigations: Key Lake Mine Site. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

Shaw JL, Manning JP. 1996. Evaluating macroinvertebrate population and community level effects in outdoor microcosms: Use of *in-situ* bioassays and multivariate analysis. *Environmental Toxicology and Chemistry* 15:608-617.

Sheppard SC, Sheppard MI, Gallerand MO, Sanipelli B. 2005. Derivation of ecotoxicity thresholds for uranium. *Journal of Environmental Radioactivity* 79:55-83.

Sibley PK, Benoit DA, Balcer MD, Phipps GL, West CW, Hoke RA, Ankley GT. 1999. *In-situ* bioassay chamber for assessment of sediment toxicity and bioaccumulation using benthic invertebrates. *Environmental Toxicology and Chemistry* 18:2325-2336.

Slooff W. 1983. Benthic macroinvertebrates and water quality assessment: Some toxicological considerations. *Aquatic Toxicology* 4:73-82.

Smolders R, Bervoets L, Blust R. 2004. *In-situ* and laboratory bioassays to evaluate the impact of effluent discharges on receiving aquatic ecosystems. *Environmental Pollution* 132:231-243.

Soucek DJ, Cherry DS, Currie RJ, Latimer HA, Trent GC. 2000. Laboratory to field validation in an integrative assessment of an acid mine drainage-impacted watershed. *Environmental Toxicology and Chemistry* 19:1036-1043.

Terrestrial and Aquatic Environmental Managers Ltd. 1993. David Creek Baseline Analysis. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

Terrestrial and Aquatic Environmental Managers Ltd. 1994. Aquatic Resources of the David Lake Drainage in the Key Lake Project Area, Saskatchewan, 1993. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

Terrestrial and Aquatic Environmental Managers Ltd., SENES Consultants Ltd. 1994. Cameco Corporation, Key Lake Operation, Status of the Environment Report. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

Terrestrial and Aquatic Environmental Managers Ltd. 1995. Key Lake Operation David Creek Drainage Investigation, 4-10 September, 1995, Field Visit Report. Prepared for Key Lake Operation, Cameco Corporation, Saskatoon, SK, Canada.

Terrestrial and Aquatic Environmental Managers Ltd., SENES Consultants Ltd. 1996. Cameco Corporation, Rabbit Lake Operation, Status of the Environment Report. Prepared for Cameco Corporation, Saskatoon, SK, Canada.

Thomas P, Liber K. 2001. An estimation of radiation doses to benthic invertebrates from sediments collected near a Canadian uranium mine. *Environment International* 27:341-353.

Tucker KA, Burton GA Jr. 1999. Assessment of nonpoint-source runoff in a stream using *in-situ* and laboratory approaches. *Environmental Toxicology and Chemistry* 18:2797-2803.

United States Environmental Protection Agency. 1991. Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures. Second Edition. EPA/600/6-91/003. Office of Research and Development, Duluth, MN, USA.

United States Environmental Protection Agency. 1993a. Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA/600/R-92/080. Office of Research and Development, Duluth, MN, USA.

United States Environmental Protection Agency. 1993b. Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA/600/R-92/081. Office of Research and Development, Duluth, MN, USA.

United States Environmental Protection Agency. 2000. Methods for measuring the toxicity and bioaccumulation of sediment associated contaminants with freshwater invertebrates. Second Edition. EPA/600/R-99/064. Office of Research and Development, Duluth, MN, USA.

Van Sprang PA, Janssen CR. Identification and confirmation of ammonia toxicity in contaminated sediments using a modified Toxicity Identification Evaluation approach. *Environmental Toxicology and Chemistry*. 16:2501-2507.

www.cameco.com. 2004. Cameco Corporation Website.

www.cogema.ca. 2006. AREVA Resources Website.