TESTING LOCAL ADAPTATION IN FIVE POPULATIONS OF *HYALELLA AZTECA* IN NORTHERN ALBERTA'S OIL SANDS REGION

by

Steven R .Beery

FACULTY OF NATURAL RESOURCES MANAGEMENT

LAKEHEAD UNIVERSITY

THUNDER BAY, ONTARIO

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Steven R. Beery

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ABSTRACT

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Canada's oil sands hold the third largest petroleum reserves worldwide. Rapid economic growth has led to increased exploitation of the surrounding boreal forest despite limited understanding of the environmental effects caused by development. Previous studies have typically focused on laboratory animals exposed to commercially available chemicals or extracts of oil sands process-affected material (OSPM; including process-affected water, tailings, and coke). The oil sands region provides an ideal location for studying local adaptations through reciprocal transplant (RT). Local adaptations require certain ecological factors to prevail, such as low gene flow, spatial variability in exposure to environmental effects, and genetic variation in traits associated with tolerance of these effects. The objectives of this research were: (1) to determine if *H. azteca* from habitats with naturally occurring bitumen exhibited increased tolerance to contaminants associated with industrial bitumen extraction compared to *H. azteca* from habitats with no naturally occurring bitumen and (2) to determine if any observed tolerance was attributable to local adaptation or plasticity. The RT occurred in reference wetlands located off oil sands leases and away from oil sands development and reclaimed sites located on oil sands leases and adjacent to mining and upgrading activities. Five populations of *Hyalella azteca* were tested in the RT, four from local wetlands plus one naïve laboratory population. Survival, sensitivity, and behaviour were measured before and after the RT period. Behaviour was tested in a phototaxis assay while sensitivity was assessed using 48 h acute LC50 tests. Survival varied by population and site. Pre-RT sensitivity increased along a gradient of increasing exposure to contaminants. After the RT, sensitivity decreased in every population. There were no significant differences in pre- or post-RT behaviour results for all populations. hese results show that the differences in responses among populations are likely attributable to developmental differences driven by environmental variables and not local adaptation.

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INTRODUCTION

In northern Alberta, native aquatic populations, such as *Hyalella azteca*, are constantly exposed to naturally-high environmental levels of bitumen and other organic compounds because rivers cut through ore deposits. This exposure, over generations, can lead local populations to exhibit a higher tolerance and lower sensitivity to naturally occurring contaminants relative to naïve animals from outside the region. These differences manifest as local adaptations which can be measured using a suite of endpoints such as survival, growth, and reproduction, among others. If the differences between populations are pronounced enough, they could have consequences for the genetic diversity of a species (Hughes et al. 2008). Genetic diversity of a species, in turn, can affect a host of important ecological processes such as primary productivity, population recovery from disturbance, interspecific competition, community structure, and nutrient fluxes (Hughes et al. 2008). As a result, local adaptations that effect a change in genetic diversity can influence population, community, and ecosystem level dynamics.

LOCAL ADAPTATION

What is Local Adaptation?

Local adaptation is the pattern of increased fitness and the process leading to it for animals within their local habitat compared to foreign habitats (Kawecki and Ebert 2004). Local adaptation is driven by divergent selection. Divergent selection is driven by

environmental differences in habitats such that a local population should evolve traits that provide it with some advantage in its local habitat relative to other, non-local genotypes. In the absence of other constraints, this advantage should manifest as a higher relative fitness for resident populations in their local habitat compared with populations originating from other habitats (Hereford 2009). However, local adaptation is not a necessary outcome of divergent selection, and can be confounded and hindered by other forces such as gene flow, genetic drift, temporal variability, and lack of genetic variation (Klerks 2002). Studying adaptation can be difficult, and even impossible, since it necessarily relies on comparisons between the ancestral (less-adapted) trait and the current (more-adapted) trait in order to determine differences attributed to selection forces. In the absence of directional selection, gene flow is expected to reduce genetic differentiation across habitats. Since gene flow acts opposite local adaptation, the existence of a pattern of local adaptation despite gene flow demonstrates the strength of the selection pressures imposed by environmental variables. These environmental pressures can be so strong that selection is readily apparent (e.g., Gallun 1984).

How do Local Adaptations Occur?

Local adaptations are encouraged by a number of ecological factors, including: low gene flow, strong selection against genotypes optimally adapted to other habitats but moderate selection against intermediate genotypes, low temporal variation in the magnitude of selective forces, small differences between habitats in size and quality, the presence of costs or constraints on adaptive plasticity, and genetic variation in traits

associated with contaminant tolerance (Kawecki and Ebert 2004; Pease et al. 2010). For example, small differences in habitat quality, such as contaminant concentration, can drive directional selection towards more tolerant genotypes while less tolerant genotypes may not be able to survive in the contaminated environment. Larger differences in habitat quality elicit stronger selection pressures on intermediate genotypes, making it difficult for a population initially adapted to one habitat to invade new habitats. However, this benefit conferred in the local habitat usually comes with a trade-off cost that manifests as lower relative fitness in a different habitat (Hereford 2009; Kawecki and Ebert 2004). This pattern of local adaptation has been documented in multiple studies and across multiple organisms, from copper-tolerant crustaceans (Khan et al. 2011), through creosote-tolerant killifish (Ownby et al. 2002), to mammals like the Norway rat and its resistance to the rodenticide warfarin (Bishop and Cook 1981).

How are Local Adaptations Tested?

Local adaptation should manifest as improved fitness for a local population in its local habitat compared with a different habitat. This pattern of local adaptation can be tested by studying more than two populations across at least two habitats, which allows a direct comparison between genotypes under similar environmental conditions. Reciprocal transplant (RT) experiments are ideal because they allow the researcher to investigate the effects of the entire habitat; however, they are not always possible for other reasons (logistical, legal, ethical, etc.). A popular alternative is the common garden experiment, in which properties of different habitats are re-created in the

laboratory and tested using different populations. This approach has drawbacks, though. For example, not all variables important to selection may be replicated in the laboratory setting and some animals may be more difficult to rear in a laboratory setting than others. Many examples of studies detecting local adaptations exist (as reviewed by Reznick and Ghalambor 2001), including for animals exposed along a gradient of industrial contamination (e.g. Khan et al. 2011). In fact, the majority of local adaptation studies that document adaptive evolution do so in response to anthropogenic changes in the environment (Reznick and Ghalambor 2001).

What Does Local Adaptation Look Like?

When a population is naturally adapted to environmental conditions it should show improved fitness in its local habitat relative to other non-local populations. This is known as the 'local' vs. 'immigrant' comparison because the comparison is between the 'local' population and the 'immigrant' populations introduced into the 'local' population's habitat. All other factors and constraints being equal, any genetic differentiation observed between populations must be a result of divergent selection driven by environmental factors. Another comparison exists, called the 'home' vs. 'away' comparison. This comparison is less important to local adaptation, however, because it only compares how a single population performs in its 'home' habitat vs. an 'away' habitat. The reason this is less desirable is that differences between environmental variables can confound results. For example, survival of a population optimally-adapted to a resource-poor habitat may increase after transplant to a resource-rich habitat despite it being optimally-adapted for the resource-poor environment. For this reason,

Kawecki and Ebert (2004) argued that the 'local' vs. 'immigrant' comparison should be considered diagnostic of local adaptation. This adaptation can occur on multiple timescales depending on generation time, with some adaptations occurring in as little as a few years (as reviewed by Reznick and Ghalambor 2001).

How Will This Project Contribute to Our Understanding of Local Adaptation?

This project will study four native populations and one non-native population of *H. azteca* from four study wetlands in the oil sands region of northern Alberta. The oil sands region meets the required criteria previously described for local adaptation to prevail. Limited gene flow is achieved by the poor overland dispersal ability of *Hyalella azteca*. Spatial variability is achieved by selecting reference sites located away from oil sands operations while reclaimed sites are located adjacent to operations. Small differences between habitats in size and quality, such as ion and metal concentrations, are present because some sites are located adjacent to oil sands development while others are kilometres away upstream. Lastly, it is assumed that there is at least some genetic variation related to tolerance among the five different populations tested here. By comparing reclaimed wetlands that are adjacent to mining operations but do not incorporate oil sands process-affected material (OSPM) into their construction, to reference wetlands that receive no industrial effluent or input, an estimate can be made as to the effect of oil sands operations on the *H. azteca* metapopulation endemic to the region. Any effects seen in the reclaimed wetlands tested here, therefore, should be a result of environmental factors associated with those reclaimed wetlands that are

adjacent to oil sands operations. Local adaptations have not yet been studied using *Hyalella azteca* in Canada's oil sands region.

In summation, local adaptation is measured by two criteria: comparison between populations within a habitat ('local vs. foreign') and comparison of a population across habitats ('home vs. away') (as reviewed by Kawecki and Ebert 2004). Of these, the most important comparison for local adaptation is between local and foreign populations within each test habitat (Kawecki and Ebert 2004). This comparison is considered diagnostic of local adaptation because it tests divergent natural selection, the driving force behind local adaptation, while environmental variables remain constant. 'Home vs. away' confounds the effect of divergent selection with habitat quality, because it is unknown which environmental variables would be responsible for observed differences (Kawecki and Ebert 2004). Local adaptation, which is expected to confer some fitness-related benefit to an animal in its local habitat regardless of the consequences of these traits in other habitats, can be investigated by exposing several different populations of a species in a spatially heterogeneous environment and measuring responses based on fitness-related traits, such as survival.

My research objectives were: (1) to determine if *H. azteca* from habitats with naturally occurring bitumen exhibit increased tolerance to contaminants associated with industrial bitumen extraction compared to *H. azteca* from habitats with no naturally occurring bitumen and (2) to determine if any observed tolerance is attributable to local adaptation or plasticity. These objectives were accomplished by subjecting the five *H. azteca* populations to a 14 day RT *in situ* at the four study wetlands. Endpoints measured included: survival after RT period, sensitivity to a reference toxicant before

and after the RT period, and behavioural response to light before and after the RT period. The animals' sensitivity to the reference toxicant before and after RT was compared to determine if the wetlands had an effect on their ability to tolerate the reference toxicant. Similarly, a baseline pre-exposure behavioural assay assessed the normal response to light for each population while the post-exposure assays highlighted any deviations from the normal caused by exposure. Phototaxis was investigated because changes in the normal negative phototactic response of *H. azteca* have been documented in amphipods exposed to contaminants (see Phipps 1915). By comparing survival, sensitivity, and behavioural responses of these different populations in habitats characterised by different environmental factors, we can conclude not only whether or not local adaptation has occurred in any of the populations tested, but can also draw conclusions about what environmental factors may be driving such divergent selection.

LITERATURE REVIEW ON OIL SANDS EFFECTS ON AQUATIC INVERTEBRATES

Oil sand is a mixture of naturally occurring bitumen, sand, and water with bitumen saturation levels ranging from 1% to 18% (Government of Alberta 2014). With the development of *in situ* technologies pioneered in the 1990s such as steam assisted gravity drainage and cyclic steam stimulation, bitumen has become a major source of energy in Canada, surpassing the production of conventional crude oil in Alberta in 2001 and comprising 56% of total production by 2013 (Alberta Geologic Survey 2012; CAPP 2014a). As of July 2013, the number of mining, upgrading, and thermal *in situ*

projects grew to include 114 existing installations (i.e. mining projects, *in situ* projects, upgrading facilities, etc.), of which six are major mining projects with three more proposed (Government of Alberta 2014). Alberta's oil sands deposits cover 142,200 km^2 in the Athabasca region of northern Alberta representing the largest oil sands deposit in the world (Alberta Geologic Survey 2012). As of September 2013, 844 km^2 of boreal forest had been disturbed by these projects (Government of Alberta, 2014). Industry operating in the region is required by law to reclaim all disturbed land to an equivalent, but not necessarily an identical, productive state (Government of Alberta 2014).

The large-scale land disturbance required for extraction and open-pit mining of bitumen has placed great importance on reclamation strategies in the region. Bitumen is extracted using the Clark hot water process, which creates large volumes of OSPM laden with environmental contaminants. The water used during the extraction process is pumped to tailings ponds where it is allowed to settle before being reused. As a result, contaminants within OSPM are concentrated. Chief among these contaminants are naphthenic acids (NAs), polycyclic aromatic hydrocarbons (PAHs), metals, and salts.

Alberta's oil sands represent a novel venture in the energy sector. With production and investment increasing at record levels, both environmental monitoring to evaluate effects on the surrounding habitats and effective reclamation strategies are important. With the large volume of OSPM stored on-site recent research has focused on the impacts of OSPM on aquatic organisms, specifically fish, amphibians, and macroinvertebrates. A search of the current literature surrounding oil sands and macroinvertebrates generated 22 publications. The search was initially performed in May 2013 (and again in June 2015) using the following keywords in the Web of Science

online database and Google Scholar web search: oil sands, macroinvertebrates, aquatic macroinvertebrates, and *Hyalella*. The initial results were further narrowed down to studies that had an aquatic invertebrate component in their experimental design and were directly related to Athabasca's oil sands region. Only peer-reviewed literature was selected for this literature review. Of the 22 studies selected for review, 11 were conducted *in situ* and 13 were conducted *ex situ* (two studies had both an *in situ* and *ex situ* component). A number of different stressors were studied, including natural bitumen (one paper), OSPM (19 papers), commercial NAs (one paper), extracted NAs (two papers), PAHs (one paper), and metals (five papers). No studies thus far have examined the effects of diluted bitumen (dilbit) on aquatic macroinvertebrates. All 22 studies focused on animals that are endemic to the Athabasca oil sands region. Different endpoints were investigated depending on the study, with the most common being survival (10 papers), followed by community assemblages (nine papers), development (eight papers), bioconcentration (five papers), behaviour (two papers), biochemical (one paper), and malformations (one paper).

In one recent study, *Chironomus dilutus* larvae were exposed to either untreated OSPM or ozonated OSPM in a 10 d acute toxicity assay and a chronic emergence assay. After the 10 d acute exposure, populations exposed to untreated OSPM had the lowest survival, while survival of larvae in the ozonated treatments was similar to controls (Anderson et al. 2012a). Chronic exposure to untreated OSPM caused significantly lower rates of pupation (31% in untreated OSPM and 71% in controls) and emergence (8% in untreated OSPM vs. 81% in controls). Other studies found similar results of decreased survival with exposure to a stressor (OSPM organic compounds

extract, coke leachates, or NA extracts) in *Chironomus dilutus* (Anderson et al. 2012b), *Ceriodaphnia dubia* (Puttaswamy and Liber 2012; Puttaswamy and Liber 2011; Puttaswamy et al. 2010), and *Daphnia magna* (Armstrong et al. 2009; Frank et al. 2009).

Some of the earliest studies on invertebrates in the region compared benthic macroinvertebrate communities from upstream (no natural bitumen) and downstream (naturally occurring bitumen) sites along the Steepbank River and found that downstream sites supported fewer individuals per unit area and fewer burrowing taxa as well as fewer sensitive taxa such as stoneflies and mayflies (Barton and Wallace 1979a; 1979b). In a pair of studies from 2010, researchers compared community assemblage metrics from reference lakes in the Athabasca region with lakes in a high sulphur deposition region (Parsons et al. 2010a; 2010b). The test lakes in the high sulphur deposition region had lower abundances of benthic macroinvertebrates sensitive to pollutants such as Ephemeroptera, Plecoptera, and Trichoptera. However, the cause of those differences is more likely related to the differences in lake physico-chemical properties than a result of atmospheric deposition (Parsons et al. 2010a). More recently, wetlands that incorporated OSPM into their construction or received input from industry activities showed lower macroinvertebrate trophic diversity, predator biomass, and species richness than those not incorporating OSPM (Bendell-Young et al. 2000; Kovalenko et al. 2013).

Some of the developmental endpoints studied in the oil sands region included growth, emergence and pupation (chironomids), and reproduction and fecundity (daphniids). Water fleas (*Ceriodaphnia dubia*) exposed to two different coke leachates

(one leached at pH 5.5, the other 9.5) had significantly impaired rates of reproduction in seven day chronic tests (Puttaswamy and Liber 2011). The observed toxicity was attributed to nickel in the pH 5.5 treatment and vanadium in the pH 9.5 treatment (Puttaswamy et al. 2010; Puttaswamy and Liber 2011). In a follow-up study, water fleas were exposed to coke leachates that were leached in the presence of different concentrations of inorganic ions (bicarbonate, sulphate, and chloride). In three-brood daphniid tests, fecundity decreased in a concentration-dependent manner for both Ni and V (24 neonates per adult in controls compared with 11 neonates per adult in 2.25 μg L^{-1} Ni or 11 neonates per adult in 500 μg L^{-1} V) independently as well as in mixture assays (four neonates per adult in 2.25 μg L^{-1} Ni + 500 μg L^{-1} V) (Puttaswamy et al. 2012).

Bioaccumulation has also been studied in the Athabasca oil sands region. For example, *H. azteca* were exposed to increasing concentrations of two V species, V(IV) and V(V), for seven days. The *H. azteca* tissues contained V(IV) even when animals were only exposed to V(V), indicating that V is taken up and metabolised by *H. azteca* (Jensen-Fontaine et al. 2013). Another study in which microcosms were constructed using petroleum coke and embedded in a constructed wetland for three years found that *Aeshnid* spp. dragonflies may be accumulating Ni, V, La, and Y, possibly through their diet. They also found that chironomids had the highest tissue concentrations of every metal measured, probably due to their close association with sediment putting them in direct contact with metals (Baker et al. 2012). Yet another study compared food web area and length among sites with three different groupings: low NAs (0-4 mg L^{-1}), medium NAs (4-15 mg L^{-1}), and high NAs (>15 mg L^{-1}). There were no significant

differences in food web area, food web length, or carbon isotopes between low, medium, and high sites. However, differences existed in nitrogen isotopes between sites which they suggested was a result of ammonia from OSPM (Elshayeb et al. 2009). Farwell et al. (2009) found similar results, with high ^{15}N values found in invertebrates along a gradient of increasing exposure to mature fine tailings and consolidated tailings.

Behaviour is one of the lesser-studied endpoints among oil sands macroinvertebrate literature. The two studies reviewed here compared larval chironomid activity during acute and chronic exposures to untreated OSPM and found that those in experimental groups spent more time outside of their larval cases which could increase their risk of predation (Anderson et al. 2012a; 2012b).

Chironomids were also the subjects of studies that looked at malformations and biochemical processes related to oil sands exposure. In one study, researchers found that one group of chironomids collected from a wetland receiving oil sands effluent had a slightly higher frequency of mentum deformities (8%) when compared to controls (0%) (Bendell-Young et al. 2000). The only study to look at biochemical processes affected by OSPM exposure quantified abundances of transcripts related to oxidative stress, such as glutathione-s-transferase, catalase, apoptosis-inducing factor, and glutathione peroxidase, and abundances of transcripts related to endocrine disruption, such as the estrogen-related receptor, the ecysteroid receptor, and ultraspiricle protein. They found that abundances of some transcripts increased (glutathione peroxidise, apoptosis-inducing factor, estrogen-related receptor, ecysteroid receptor, and ultraspiricle protein) in animals exposed to fresh OSPM after seven days relative to controls but not for aged OSPM (Wiseman et al. 2013). Abundances for other transcripts were lower (glutathione-

s-transferase) or not significantly different (catalase) in animals after seven days of exposure to fresh OSPM compared to animals from control treatments (Wiseman et al. 2013).

The results of the reviewed studies highlight the varied responses observed in animals exposed to different concentrations and types of contaminants associated with oil sands operations. Oil sands process-affected material water chemistry is dependent on a number of factors, including ore quality, source, extraction processes, and age of OSPM (Allen 2008). These differences in water chemistry can lead to spatial variability in areas affected by oil sands operations, with nearer sites being more heavily influenced, as well as within reclaimed wetlands incorporating OSPM of different ages and treatment regimes into their construction. As a result, the Athabasca oil sands provide a unique opportunity to study local adaptations, which have not yet been addressed in oil sands literature, and the possible consequences on the *H. azteca* metapopulation endemic to the region.

HYALELLA AZTECA

Range, Distribution, and Abundance

Hyalella azteca is a freshwater amphipod crustacean found ubiquitously throughout North America. It is not uncommon to find *Hyalella azteca* in any permanent water body that reaches 10°C in the summer throughout North America, including lakes, ponds, wetlands, marshes, estuaries, streams, ditches, and even rivers from Mexico to

the tree line in Canada (Bousfield 1958). Populations can occur in large densities (up to 10,000 per m²) under ideal conditions.

As a member of the talitroidean amphipod family Hyalellidae, *Hyalella azteca* has several characteristics that it shares with other members of the same family. In northern Alberta, it is one of two common amphipods, the other being *Gammarus lacustris*. They are easily distinguished based on the presence (*H. azteca*) or absence (*G. lacustris*) of overlapping dorsal plates that look like teeth on segments eight and nine as well as the lack of an accessory flagellum on the first antenna in *H. azteca* (Clifford 1991). They are also very different in adult size, with *H. azteca* growing to about 8mm (females are slightly smaller) while *G. lacustris* is typically about 20 mm long.

Ecology

Hyalella azteca is an epibenthic detritivore. It prefers somewhat alkaline and hard waters with a typical pH range of 6.0-8.0 and typical hardness of < 200 mg L^{-1}. *Hyalella azteca* is also somewhat tolerant of high salinity (as high as 2-3%) and occasionally occupies estuarine habitats. The species occurs abundantly in lentic environments (more so than lotic), especially those with vegetation it can use as both a food source and as cover. It is typically found associated with the surficial 1-2 cm of sediment in an aquatic habitat. The species is also known for its ability to tolerate low dissolved oxygen (30 d lowest-observed-effect concentration < 0.3 mg L^{-1}) and high carbon dioxide (Nebeker et al. 1992; Environment Canada 2014). *Hyalella azteca* is an important food source for many other animals including fish, waterfowl, wading birds, salamanders, and other larger invertebrates (de March, 1981) thus making it a key component of the

benthic environment. In a study of waterfowl from a lake in Saskatchewan, it was found that *H. azteca* accounted for up to 96% of their seasonal diet (Krapu and Reinecke 1992).

Reproduction

Hyalella azteca reproduces sexually and typically reaches sexual maturity about 30 days after birth in 20°C water (Environment Canada 2014). Their life cycle is typically annual, beginning with the spring warm up. Once water temperatures reach about 10°C, overwintering females produce a large clutch of eggs. The female is grabbed by the male in amplexus, who waits for her to moult so that he can fertilise the eggs. She then carries the developing young in her marsupium, or brood pouch, until her next moult when they are released and the cycle begins again. As temperatures increase throughout summer, females continue to mate and produce broods, albeit typically smaller in number of offspring. The previously overwintering females die before the next winter, while the later summer broods will overwinter and begin the cycle again. The newly hatched animals go through a number of instars (5-8) before reaching sexual maturity. It is not uncommon for a single female *Hyalella azteca* to release 1 to 50 offspring per brood (Environment Canada 2014).

Relevance

Hyalella azteca has been used often in toxicology assessments since the 1980s. The species has a few advantageous qualities that make it ideal for toxicity testing, including ease of culture, short generation time, and common distribution throughout

North America (Strong 1972). *Hyalella azteca* was found at almost all of the wetland sites surveyed in the study region including on-site at Suncor in several wetlands, although not in sufficient numbers for toxicity testing. Also, the methodology for acute and chronic testing using *H. azteca* is well established and documented by both Environment Canada and the USEPA. These factors make it an ideal test organism for this project, which employs both acute and chronic testing. Since it is native to the region, the results are directly applicable and may be of specific interest to parties pursuing reclamation, remediation, and protective water quality guidelines in the region. Additionally, by adding a fifth, non-native laboratory culture population, assumptions can be tested about how well standard toxicological testing using laboratory cultures extrapolates to *in situ* situations where animals are exposed to a suite of contaminants at once instead of one or two at a time.

Why Macroinvertebrates?

The use of aquatic invertebrates as model organisms in toxicological tests has many advantages. Principal among these advantages is their size; being much smaller than higher order organisms, aquatic invertebrates require much less space for rearing and experimentation. Another advantage is their lifespan, which is generally short, allowing for multi-generational studies that could take years to complete in vertebrates (Dahms et al. 2011). Ubiquitous throughout northern Alberta, *H. azteca* primarily lives on the benthic surface, which makes it ideal for testing the effects of OSPM since reclamation strategies often incorporate OSPM into wet reclamation landscapes as a component of sediment (Kovalenko et al. 2013). For my testing purposes, the

Environment Canada Environmental Protection Series Biological Test Method for sediment or water-only assays using *H. azteca* was followed (Environment Canada 2014).

Why Reciprocal Transplant?

Reciprocal transplant designs have been used in the past to determine differences in responses to environmental factors between native and foreign populations (as reviewed by Hereford 2009). A RT experiment permits the researcher to determine whether observed differences in population responses to different habitats are caused by plasticity or genetic differentiation based on whether or not the defining pattern of local adaptation (i.e., higher survival in a native wetland population than in a non-native wetland population) is observed in the response variables. The differences in tolerance between native and foreign populations exposed to elevated levels of toxicants are well documented in aquatic invertebrates. Some examples include freshwater unionid clams (Hinch et al. 1986), which showed that growth rate was affected by population source while shell shape was affected by transplant destination. In a more recent study, *Gammarus pulex* from clean and historically impacted sites were transplanted along a Cu and Zn gradient (Khan et al. 2011). Animals from the historically impacted sites accumulated less Cu and Zn and experienced lower levels of oxidative stress and mortality than animals from reference sites.

METHODS AND MATERIALS

STUDY AREA

The study area was located in northern Alberta near Fort McMurray and Suncor's oil sands base plant. Fort McMurray (56°43'44"N 111°23'5"W) is situated south of oil sands development on the banks of the Athabasca and Clearwater Rivers. First settled in 1870, Fort McMurray has recently experienced a surge of growth thanks to oil sands development. In 1999, the population was 42,871, while current estimates put it near 116,407 (RMWB 2015). Twenty six kilometres to the north of Fort McMurray on Highway 63 is Suncor's main base plant, straddling the banks of the Athabasca River. The plant began construction in 1964 and was opened in 1967 ahead of schedule and with an expected output of 45,000 barrels per day (bpd) (Suncor 2015). Suncor experienced moderate growth and expansion into the 1990s, when the bucketwheels of the past were replaced with cost efficient shovels and trucks. It was during this time that Suncor opened its Steepbank (1994) and Millennium (2001) mines, bringing total production up to 288,000 bpd by 2013 (AER, 2014). At the same time, total oil sands production from all producers reached 1.9 million bpd and is expected to continue to increase at a rate of 170,000 bpd annually through 2030 (CAPP 2014a). The increased production has resulted in record profits and investment in the region with $155 billion in annual investment planned over the next 25 years (CAPP 2014b).

The first open-pit mine at Suncor was on the west bank of the Athabasca River (57° 0'14.99"N 111°28'51.91"W) and has since been exhausted. Now, the west bank is home to a series of tailings ponds and the upgrading and power generating facilities. Oil

sands are extracted from open-pit mines using large hydraulic shovels and hauled to the extraction plant by some of the largest haul trucks in the world (capacity: 400 short tons). Once in the extraction plant, bitumen is separated from the sand through a combination of chemical and mechanical processes. The bitumen becomes frothy and rises to the surface of the extraction vessel where it is skimmed off the top and transported for further upgrading. The extraction and upgrading processes produces large amounts of OSPM as by-products, typically in the form of liquid tailings (water, dissolved salts, organic compounds, minerals, residual bitumen), hereafter referred to as oil sands process-affected water (OSPW) and petroleum coke, a solid, carbonaceous, and heterogeneous solid (Allen 2008; Puttaswamy and Liber 2011). Due to a strict zero-discharge policy (Government of Alberta 2010), oil sands producers are not permitted to release OSPW. This policy has created the need for large tailings ponds that act as settling basins for tailings, allowing the heavier components to settle. In this manner, companies reuse their OSPW to limit their withdrawals from the Athabasca River. Tailings ponds occupy roughly 182 km^2 and are expected to persist for decades at current settling rates while the volume of stockpiled coke was 84 million tons in 2014 (AER 2014).

All experimental study sites were located on Suncor's west bank (Figure 1). Several experimental wetlands were considered for study, but ultimately two reclaimed sites were selected from a possible seven. The chosen two were Contaminated-1 wetland (Co-1) and Contaminated-2 wetland (Co-2). These wetlands were initially chosen for a number of reasons: (1) because of previous work conducted at each site, (2) to overlap this project with an amphibian research project in the same region, (3) because *H. azteca* were found in sufficient numbers for testing, and (4) because both

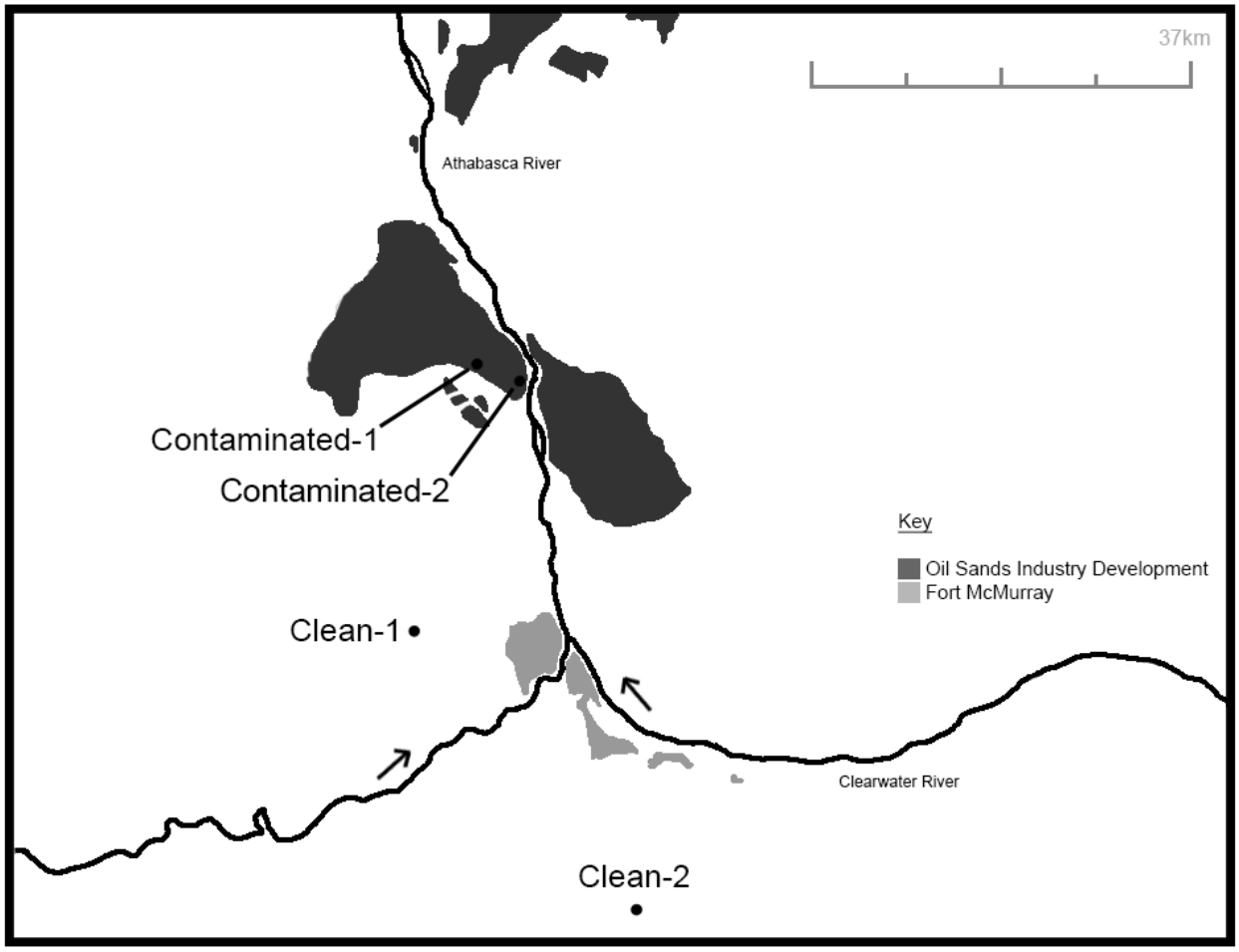

Figure 1. Map of the study area in northern Alberta. Arrows indicate direction of water flow. (H. Beery 2015)

wetlands formed opportunistically in reclaimed areas. Each of these wetlands was designated as reclaimed, meaning that they formed and continue to exist in reclaimed areas on oil sands leases.

The Co-2 wetland formed opportunistically on the site of a reclaimed ex-gravel pit in 2011. Located at 56°58'31.39"N 111°27'34.70"W just outside the east gate of Suncor, Co-2 receives no manmade input. The Co-1 wetland is located at 56°59'15.45"N 111°32'23.24"W. It is a reclamation area where overburden was stored when it was initially stripped for mining. A wetland formed there opportunistically and it is not uncommon to find large numbers of waterfowl there. Located in a reclaimed area to the west of Pond 5, it receives no manmade input.

In contrast, the reference wetlands were located upstream of both Fort McMurray and oil sands industry development. Reference sites were defined as any wetlands not receiving direct or indirect input from oil sands operations. The Cl-1 wetland was located at 56°45'39.73"N 111°37'47.50"W, about 10 km west of Fort McMurray. It is directly adjacent to the road, which is used primarily for recreational access to the boreal forest surrounding Fort McMurray. The Cl-2 wetland is located at 56°31'9.16"N 111°16'45.04"W, approximately 15 km south of the Fort McMurray city limits. It sits adjacent to the highway. These wetlands were chosen for a few reasons: (1) the presence of *H. azteca* in sufficient numbers to support this study, (2) upstream of oil sands and municipal development, (3) minimally influenced by human activity, and (4) ease of accessibility.

EXPERIMENTAL APPROACH

Recall that local adaptations are driven by directional selection imposed by differences in environmental factors between sites. These differences include concentrations of contaminants, such as metals. Alberta`s oil sands region provides an ideal location for testing local adaptations for two reasons: (1) there are differences in habitat quality between reclaimed and reference wetlands (see Table 1, Table 2) and (2) limited gene flow between wetland sites. To test whether or not local adaptations have occurred in the region, I executed a 14 day *in situ* RT experiment using both local and foreign populations of *H. azteca*. Sensitivity was tested because any changes in sensitivity should be driven by the differences in environmental factors associated with each site. Phototaxis was investigated because changes in the normal negative phototactic response of *H. azteca* have been documented in amphipods exposed to contaminants (see Phipps 1915). Sensitivity to a reference toxicant and phototactic response were tested before and after the RT to determine: (1) the effect that caging had on the animals, (2) whether or not differences in environmental factors of sites were pronounced enough to affect population response to a known OSPM-associated toxicant, and (3) whether or not environmental factors differed enough between sites to affect the phototactic response typical of *H. azteca*.

Reciprocal Transplant Design

For the RT, populations were collected from two reference and two reclaimed sites in the Athabasca oil sands region. The two reference sites chosen were Cl-1 and Cl-2 and the two reclaimed sites chosen were Co-1 and Co-2. These sites were chosen

based on the presence of *H. azteca* and because they overlapped with previous and ongoing research. The two reclaimed sites represent the two different age groups in the literature of reclaimed wetlands in the region. The Cl-2 wetland was designated young (< 7 years old) and the Cl-1 wetland was designated old (> 7 years old). These designations were chosen from the literature because reclaimed wetlands that are < 7 years old have higher rates of mortality in tadpoles (Hersikorn et al. 2010) and lower invertebrate richness than > 7 year old wetlands (Kovalenko et al. 2013).

Individuals were collected from among littoral vegetation using small dip nets. In this way, 1500 individuals from each of the four wetlands were collected from July 20th to July 25th, 2014. The remaining 1500 were received via courier from the Canadian Centre for Inland Waters (CCIW, Burlington, ON) and an in-house culture at Lakehead University (which, in turn, was started from the CCIW animal stock). Prior to the RT, the animals were kept in 11 L Rubbermaid replicates in the temperature-controlled onsite laboratory with constant aeration and a 16:8 h light to dark ratio.

For the experiment itself, the five different populations (Cl-1, Cl-2, Co-1, Co-2, and laboratory) were transplanted to the four different wetland sites (Cl-1, Cl-2, Co-1, Co-2). In this way, each population was represented at each exposure site during the test. The exposure period began on July 25th, 2014, with the introduction of the *H. azteca* to the replicates within each wetland. Populations were housed in 11 L Rubbermaid replicates with holes cut in the side and replaced with 500 µm Nitex mesh. The bins each held 100 individuals and were replicated three times per site for a total of 300 individuals from each population housed within each site (1500 individuals per wetland, 6000 individuals total). The replicates were each provided with a standard

meal of 0.27 mg TetraMin commercial fish food flakes per individual every two days (as described by Environment Canada 2014). The exposure period ended on August 8th, 2014, when all animals were counted and collected for post-exposure experiments. At the end of the exposure period, proportional survival was calculated for each population. Survival has been shown to be the most sensitive indicator of chronic toxicity for *H. azteca*, and not reproduction as is the case with *D. magna* (Borgmann et al. 1993; Keithly et al. 2004). By comparing survival of these different populations in habitats characterised by different environmental factors, we can conclude not only whether or not local adaptation has occurred in any of the populations tested, but can also draw conclusions as to which (if any) environmental factors are driving local adaptation.

Sensitivity Study Design

For the sensitivity experiment, a pre-exposure baseline LC50 and a post-exposure LC50 were estimated for each of the five populations. These tests were conducted as 48 h water-only acute assays according to methods described in EPS 1/RM/33 2nd edition (Environment Canada 2014). Cadmium sulphate octahydrate (3 $CdSO_4 \cdot 8\ H_2O$) was chosen as a reference toxicant because Cd is one of the metals of concern associated with oil sands development as well as a USEPA priority pollutant (USEPA 2014) and was present in each wetland study site (unpublished data). The assays were conducted in standard artificial media five-salt (SAM-5S) reconstituted laboratory water at nominal concentrations of 0, 1, 4, 22, and 88 μg L^{-1}. The SAM-5S water was developed for long-term laboratory testing and culturing using *H.* azteca (Borgmann 1996). These concentrations were chosen based on reported literature

values taken from the USEPA's EcoTox Database for *H. azteca* (USEPA 2015). Each test consisted of 10 randomly selected individuals placed into a test vessel at one of the previously mentioned concentrations for 48 h. After 48 h, the survivors were counted and frozen for later use. Each test was replicated three times for a total of 15 test vessels per experiment and 150 individuals. All five populations (Cl-1, Cl-2, Co-1, Co-2, and laboratory) were tested for pre-exposure baseline LC50s.

The post-exposure sensitivity experimental design was the same as the pre-exposure design except using the individuals that were collected after the 14 d reciprocal transplant. The main difference between the pre- and post-exposure assays was the number of treatment groups. While the pre-exposure experiment had five test populations, the post-exposure experiment had 20, one for each of the five populations held in each of the four wetlands.

Behaviour Study Design

The behavioural study experimental design followed the same basic principles as the sensitivity design. A behavioural assay was chosen based on the negative phototactic response in *Hyalella Azteca* (see Phipps 1915). Before and after the RT exposure period, populations were tested for their response to light. The experimental setup used a six-well plate with half of each well occluded with black acrylic paint on the outside. The plate was then placed on top of a custom LED circuit board that had one clear LED situated beneath each well. When the light was turned on, approximately half of each well was illuminated. The entire setup was placed inside a box to prevent interference from outside light sources. A camera placed in the top of the box recorded

the individuals for the duration of the experiment. When it was time to begin the experiment, a pre-determined amount of SAM-5S reconstituted laboratory water was placed into each well of the six-well plate along with one randomly selected individual per well. The individuals were allowed to acclimate to the test chamber for four minutes before the experiment began. Individuals were recorded for the following eight minutes and proportion of time spent in the dark half of the well was determined for each individual.

WATER QUALITY ANALYSES

Water samples were collected at three points during the RT exposure period from each wetland on July 24th, July 31st, and August 9th, 2014. Samples were collected in 1 L Nalgene bottles and immediately placed on ice for storage prior to shipping to the Lakehead University Nutrient Ecology Laboratory in Thunder Bay, ON, Canada. Parameters measured included: specific conductivity, pH, alkalinity (as mg L^{-1} $CaCO_3$), NO_2 + NO_3, total nitrogen, dissolved organic carbon, Ca, K, Mg, Na (Table 1), and trace metals using ICP-MS (Table 2). All trace metals samples were filtered through a 0.45 µm filter and acidified using 3% HNO_3 prior to being analysed.

STATISTICAL TREATMENT

All analyses were conducted using R: A language and Environment for Statistical Computing (R Core Team 2014). The survival results of the RT experiment were tested for normality and homogeneity of variances using Shapiro-Wilks test and Bartlett's test of variance, respectively. The proportional survival data were arcsine square root

transformed to account for any deviations from normality. The RT survival data were analysed using a two-way analysis of variance (ANOVA) with the two factors as source population and exposure site to determine if there were any differences among treatments or statistical interactions. Post-hoc analysis was conducted using Tukey's honest significant difference (HSD) test to determine where the significant differences were. For the sensitivity tests, LC50s were calculated using probit analysis and significance was determined using the ratio test described by Wheeler et al. (2006). Behavioural results, measured as proportion of time spent in the dark half of the well, were tested for normality and homogeneity of variances using Shapiro-Wilks and Bartlett's test, respectively. Once assumptions were verified, results were analysed using a two-way ANOVA with population and site as factors.

Any differences between treatment groups can be attributed to either differences in population characteristics or differences in site-specific variables such as trace metal concentrations. To investigate if water chemistry could explain these differences non-metric multidimensional scaling (NMDS) was performed on all water chemistry parameters. Where values were below the detection limit, half of the detection limit was used. Compared to other forms of ordination, such as principal component analysis, NMDS uses rank orders instead of Euclidean distances, making it a more flexible tool for handling data sets characterised by a large set of analytes, in excess of the number of samples taken (see Clarke 1993). In NMDS, sites and species are grouped together by similarity using a Bray-Curtis dissimilarity matrix and reconstructed in n-dimensional space, where n is chosen by the researcher. As with other ordination techniques, fewer dimensions make for easier interpretation. In NMDS, axes do not represent any specific

variables but rather are oriented arbitrarily in n-dimensional space. However, what is not arbitrary is the position of points relative to one another (Clarke 1993). Here, the relative distance between points represents the relative similarity in water chemistry. In other words, the nearer points are to one another in n-dimensional space, the more similar their water chemistry. In order to further interpret the data, a surf function was used to overlay a contour plot of the distribution of survival within each population on the ordination axes.

RESULTS

HYALELLA AZTECA SURVIVAL

Survival was typically highest in reference sites, with the exception of the Cl-2 population exposed in its native wetland. Mortality was highest (~80%) in the Cl-2 population exposed in both the Cl-1 and Cl-2 reference sites and so the Cl-2 population was removed from further analysis. Survival varied by both site ($F = 9.021$, $df = 3$, $p = 0.0001$) and population ($F = 3.401$, $df = 3$, $p = 0.027$). The remaining populations showed the highest survival in the Cl-1 reference site, with the exception being the Cl-1 population, which saw the highest survival in the Co-1 reclaimed site (Figure 2). This counterintuitive trend was observed in each site, where a foreign population had higher but still not significant survival than the local population. In the Cl-2, Cl-1, and Co-2 sites there were no statistical differences among treatments. In the Co-1 reclaimed site, the Cl-1 reference population showed statistically higher survival than the Co-2 reclaimed

population (t = -3.379, p = 0.039). This was unexpected because the Co-2 population was from a reclaimed site while the Cl-1 population was from a reference site.

For comparisons within populations, survival was highest in the reference sites or Co-1 reclaimed site, which appeared to function more like a reference site based on observed survival. Co-2 reclaimed, Co-1 reclaimed, and laboratory populations all showed the highest survival in the Cl-1 reference site, while Cl-1 showed the highest

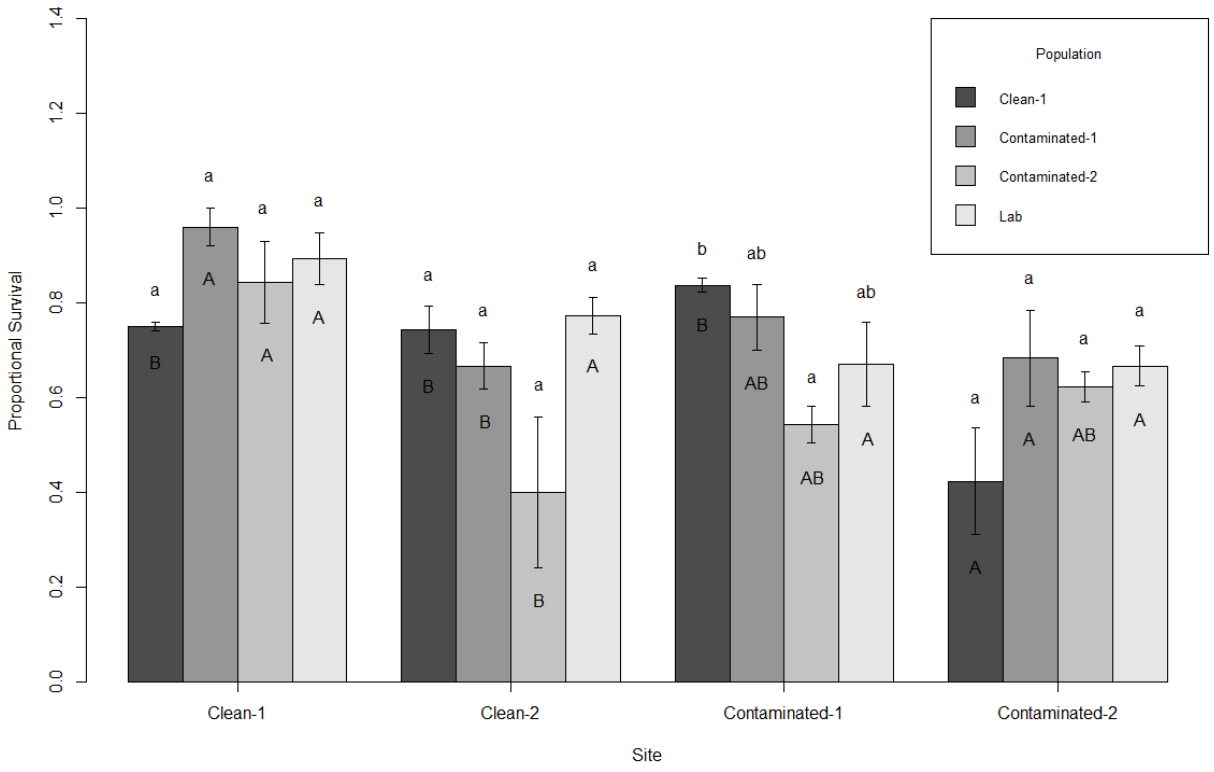

Figure 2. Proportional survival of four populations in each of four study wetlands. Those sharing the same lower-case letter designation represent populations at a single site that are not statistically different from one another while those sharing upper-case letter designation represent one population across all sites.

survival in the Co-1 reclaimed site. The lowest survival for all populations was in the Co-2 reclaimed site with the exception of the Co-2 and Co-1 reclaimed populations, which

saw their lowest survival in the Cl-2 reference site. Survival in the Co-2 reclaimed site was significantly lower for the Cl-1 reference population (Co-2-Cl-1: t = -3.634, p = 0.027; Co-2-Co-1: t = -4.777, p = 0.006; Co-2-Cl-2: t = -3.596, p = 0.029). Within the Co-2 reclaimed population, the only statistical significance was between individuals in the Cl-2 reference site and individuals in the Cl-1 reference site (t = 3.382, p = 0.039). For the Co-1 reclaimed population, survival in the Cl-1 reference site was significantly higher than survival in the Cl-2 reference site or the Co-2 reclaimed site (p = 0.027 and 0.035, respectively). Within the laboratory population, a non-significant trend of higher survival in reference sites than in the reclaimed sites was observed.

WATER CHEMISTRY

The pH of all wetlands was between 7.3 and 8.1 (Table 1), indicating that all sites were slightly alkaline. Specific conductivity was lowest in Cl-1 and increased through Cl-2, Co-1, and Co-2. Alkalinity was highest in Co-1 at 215.1 mg L^{-1} as $CaCO_3$ and lowest in Co-2 at 116.2 mg L^{-1} as $CaCO_3$. The two reference sites were intermediate between them at 209.5 (Cl-1) and 145.8 (Cl-2) mg L^{-1} as $CaCO_3$. The high specific conductivity of the reclaimed sites was also reflected in their high hardness values. Both Co-1 and Co-2 water were almost twice as hard when compared to Cl-1 and Cl-2. Metal

Table 1. Basic water chemistry results for the study wetlands (n = 3).

Wetland		pH	Conductivity ($\mu S\ cm^{-1}$)	Alkalinity (mg L^{-1} as $CaCO_3$)	Hardness (mg L^{-1} as $CaCO_3$)	NO_2+NO_3 ($\mu g\ L^{-1}$)	TN ($\mu g\ L^{-1}$)	DOC (mg L^{-1})	Ca (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Na (mg L^{-1})
Reference												
Clean-1	Mean	7.4	411.00	209.5	170.6	18.72	2.19	38.71	41.68	2.75	16.15	28.06
	SEM	(7.4-7.7)	11.68	3.4	3.1	5.21	0.58	2.71	1.05	0.30	0.25	3.47
Clean-2	Mean	7.7	715.11	145.8	192.2	16.51	1.23	21.77	47.38	2.01	17.95	69.46
	SEM	(7.5-7.7)	4.03	7.0	9.2	5.17	0.69	3.56	3.69	0.16	0.02	9.46
Reclaimed												
Contaminated-1	Mean	7.9	982.44	215.1	374.8	24.97	2.53	40.78	80.30	8.92	42.32	78.86
	SEM	(7.9-8.1)	41.74	22.5	41.4	4.24	0.17	5.94	13.38	0.46	1.94	10.23
Contaminated-2	Mean	7.7	1557.56	116.2	388.9	20.45	2.05	25.84	87.85	4.79	41.17	161.73
	SEM	(7.3-7.7)	64.66	23.3	14.6	6.54	0.77	4.48	5.40	1.31	5.27	25.73

concentrations varied across all four wetlands. Metals that were most associated with reclaimed sites over reference sites included Ni, Cu, and Sr. Nickel values were 10 times higher in reclaimed sites than in reference sites while Cu values were two to eight times higher in reclaimed sites than reference sites (Table 2). Similarly, Sr values in reclaimed sites were almost double those of reference sites. Some metals were higher in reference sites than in reclaimed sites, such as As, Cr, and V. Chromium concentration was almost twice as high in reference sites compared to reclaimed sites. A similar trend was observed in V, which was two times higher in reference sites than reclaimed sites. The remaining metals varied by site with no clear, apparent trends.

Table 2. Mean and standard error of trace metals in the four study wetlands in μg L^{-1} (n = 3). Analytes with no standard error had fewer than three replicates or too many replicates below the detection limit. Bold denotes maximums and minimums.

	Reference				*Reclaimed*			
	Clean-1		Clean-2		Contaminated-1		Contaminated-2	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
As	1.90	0.95	**2.26**	1.78	1.84	1.02	**1.48**	0.84
Ba	44.22	6.95	45.31	22.14	**35.04**	16.24	**75.73**	41.80
Be	0.02	0.00	0.02	0.02	0.02	0.04	**0.04**	0.01
Co	0.22	0.03	**0.14**	0.05	0.21	0.09	**0.38**	0.36
Cr	**22.55**	23.27	18.14	16.61	**8.33**	3.67	12.54	7.96
Cu	**0.55**	0.08	0.83	0.08	1.55	0.67	**3.90**	2.61
Ni	1.17	0.32	**1.09**	0.68	11.03	7.20	**12.92**	8.90
Sr	**202.75**	78.59	207.34	135.21	354.29	190.95	**355.85**	297.81
V	**4.83**	5.02	3.58	3.65	**1.60**	1.17	2.90	1.99

SURVIVAL TRENDS WITH WATER CHEMISTRY MAPPING

Some metals (Al, Li, Mn, Mo, Nb, Pb, Sb, W, Y, Zr) were removed from the ordination because of insufficient measurements to calculate a mean or because too many measurements were below the detection limit (Cd). The Kruskal stress test of the NMDS ordination was 0.03, indicating that the fit of the ordination using two dimensions was good (Clarke 1993). Each site tended to cluster around other points from the same site, indicating that those sites were similar. The Co-2 reclaimed site was characterised by higher Ni, Cu, and specific conductivity, while the Cl-1 reference site was characterised by higher levels of As, DOC, V, and Cr. Intermediate to those two were the Co-1 reclaimed site and Cl-2 reference site. The polygons drawn over each cluster

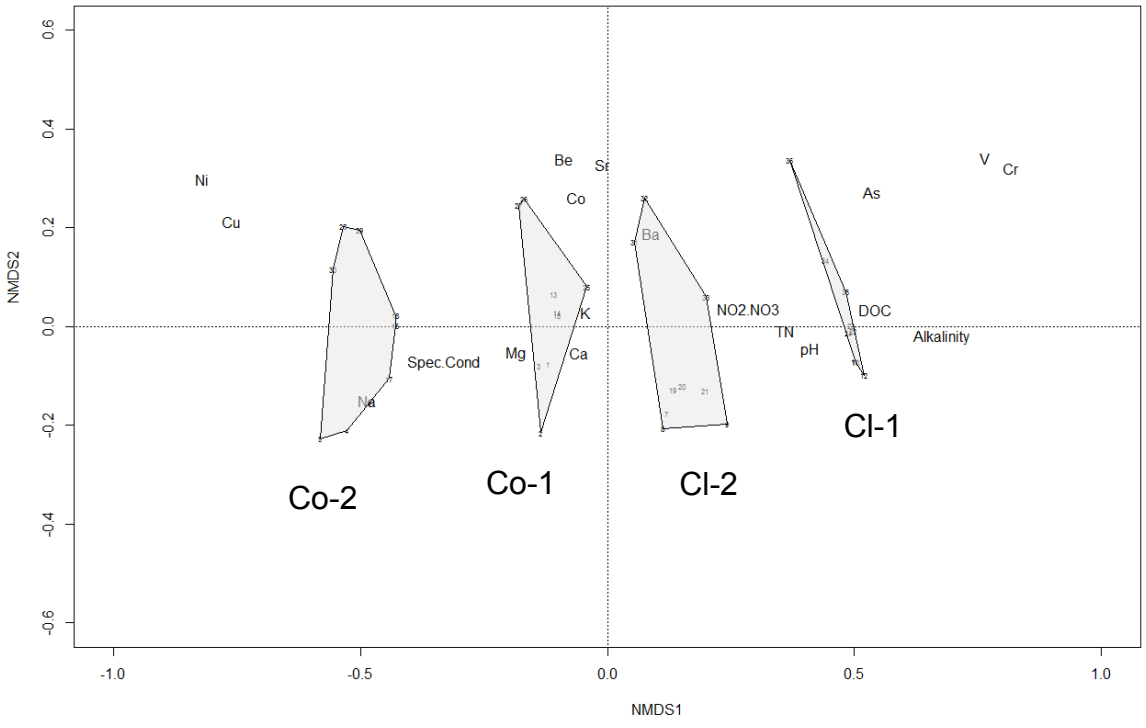

Figure 3. Ordination of wetland sites using NMDS (stress = 0.03) with polygons outlining sites that share similar water chemistry. Large font denotes analytes, small font denotes subsites.

of sites showed that they distinctly separated out from each other (Figure 3).

For the Cl-1 population, survival was lowest in the Co-2 sites (Figure 4) which were characterised by higher Ni, Cu, and specific conductivity than the reference sites. Survival increased near the Co-1 sites reaching a maximum before it decreased slightly in the Cl-1 sites, which were characterised by lower levels of Cu, Ni, and specific conductivity, and higher levels of As, V, Cr, and DOC. Survival was lowest where Ni, Cu, and specific conductivity were highest. Where Ni, Cu, and specific conductivity were lower, survival increased, along with As, V, Cr, and DOC. Similar patterns can be seen in the Co-1 (Figure 5), Co-2 (Figure 6), and laboratory (Figure 7) population survival distribution contour plots.

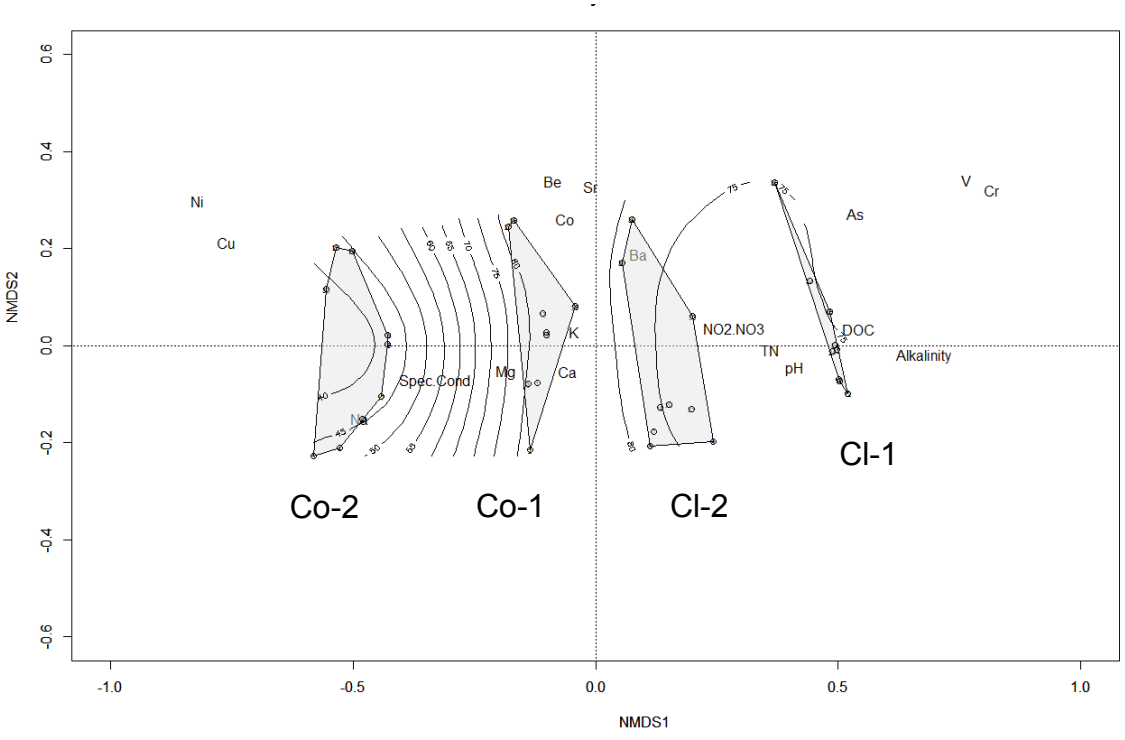

Figure 4. Survival distribution contour plot for the Cl-1 population compared with water chemistry. Numbers on contour lines represent % survival.

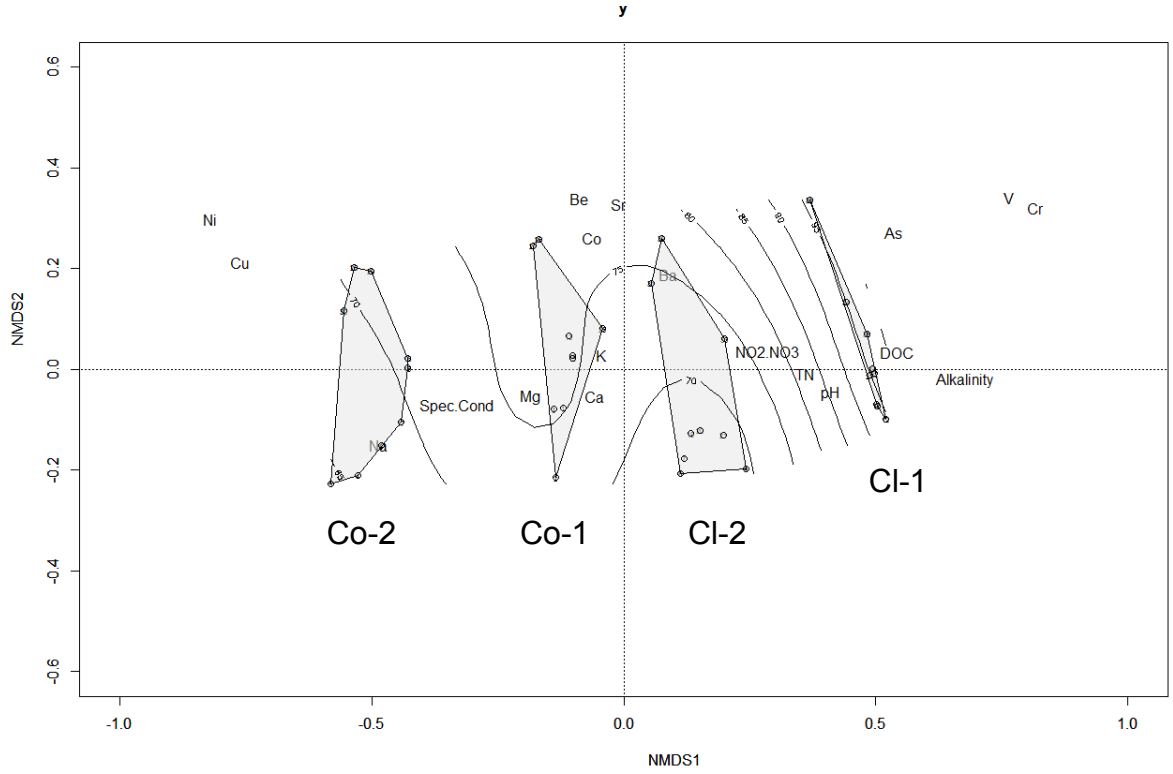

Figure 5. Survival distribution contour plot for the Co-1 population compared with water chemistry. Numbers on contour lines represent % survival.

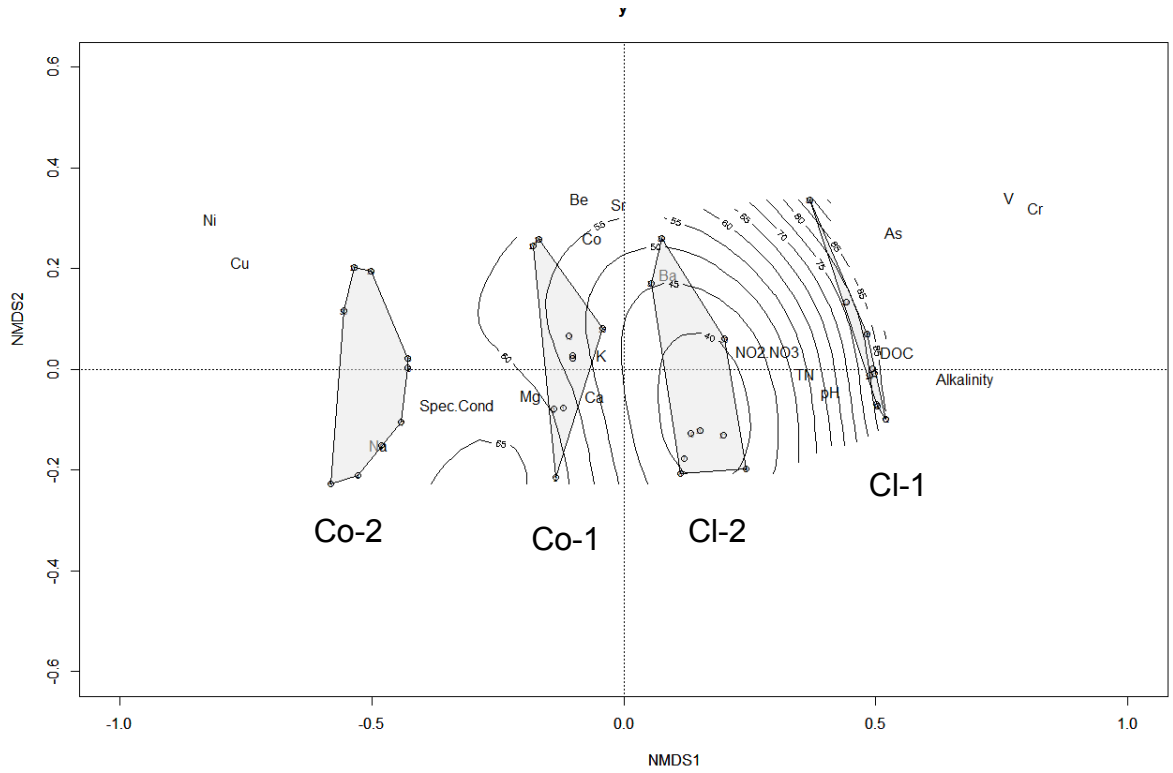

Figure 6. Survival distribution contour plot for the Co-2 population compared with water chemistry. Numbers on contour lines represent % survival.

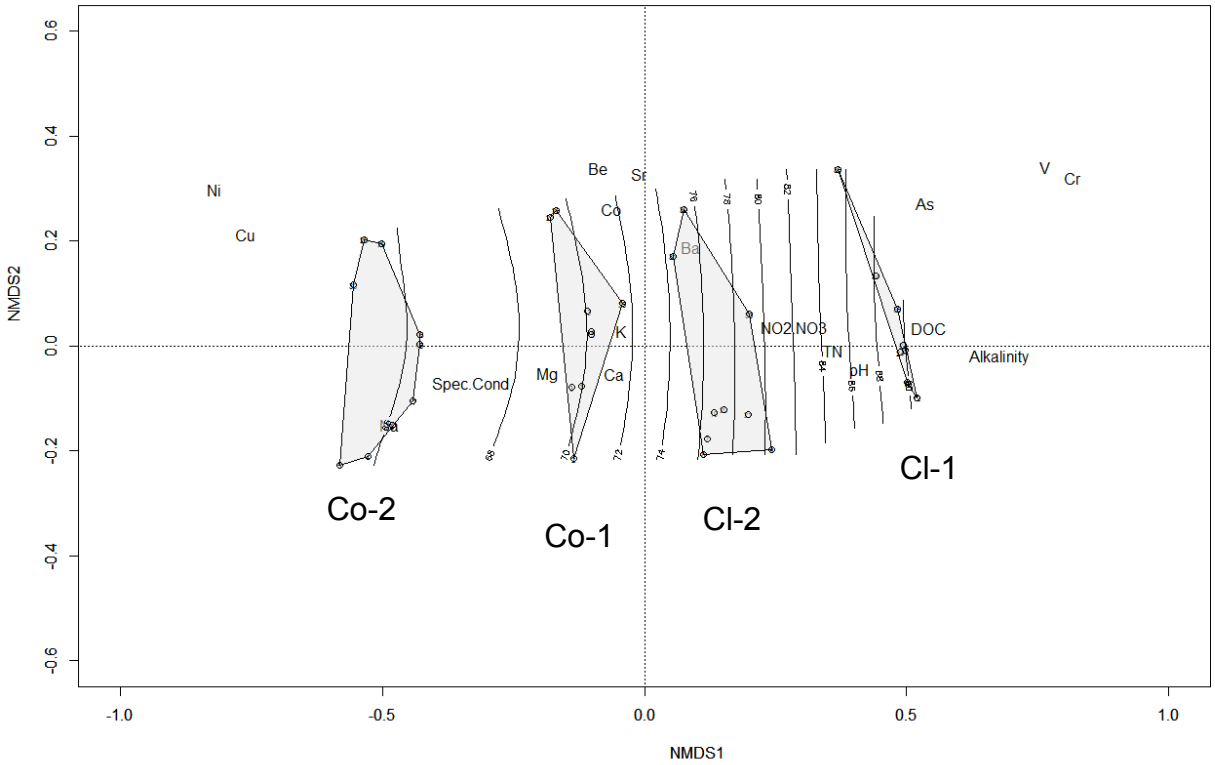

Figure 7. Survival distribution contour plot for the laboratory population compared with water chemistry. Numbers on contour lines represent % survival.

HYALELLA AZTECA SENSITIVITY TESTING

The lowest LC50, corresponding to the highest sensitivity, was observed in the naïve laboratory population and increased through Co-1, Cl-2, and Cl-1 (Table 3). The highest LC50, corresponding to the lowest sensitivity, was observed in the Co-2 reclaimed population. The only two statistically different populations were the laboratory and Co-2 organisms. After the RT exposure period, animals from each treatment group were subjected to a post-exposure sensitivity challenge in the form of a 48 h water-only LC50 assay using the cadmium sulphate octahydrate reference toxicant. The LC50s

calculated for post-exposure populations were unreliable in statistical comparisons because all of the post-exposure LC50s were higher than the highest treatment concentration tested. However, the high LC50s highlighted an observed trend towards decreased sensitivity after the 14 d exposure period in all populations tested relative to pre-exposure values (Figure 8).

Table 3. Estimated pre-exposure LC50 values by population. Those of the same letter are not significantly different.

Population	LC50 (SE) ($\mu g\ L^{-1}$)
Clean-1	76.7 (6.4)^{ab}
Clean-2	76.3 (9.7)^{ab}
Contaminated-1	73.0 (8.4)^{ab}
Contaminated-2	89.0 (8.2)^{b}
Laboratory	61.1 (5.6)^{a}

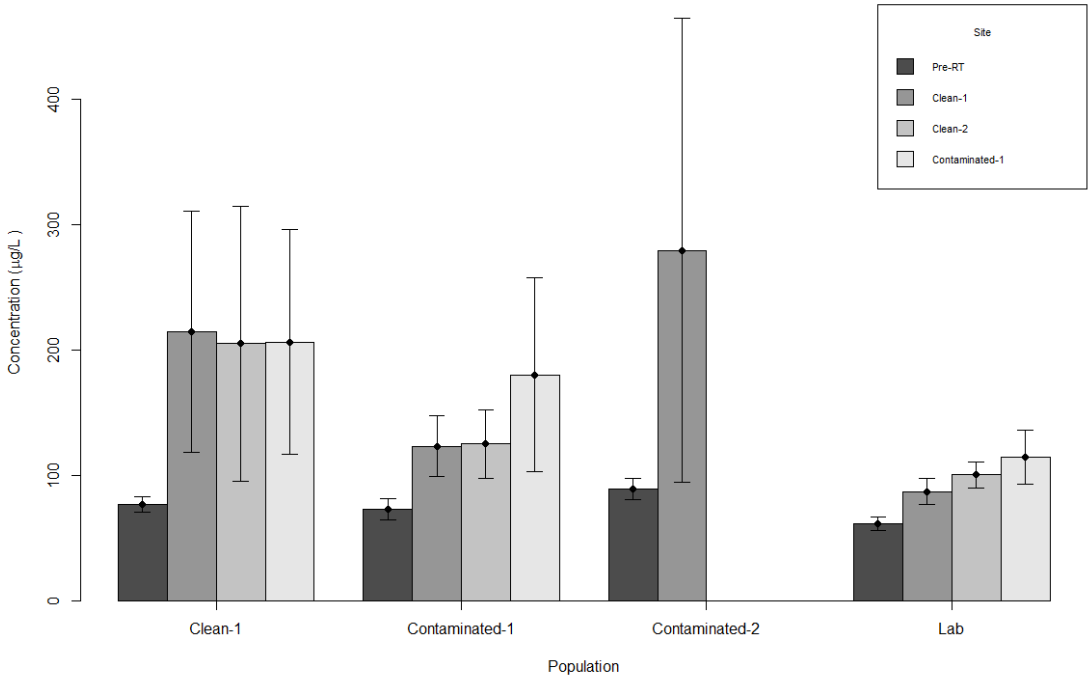

Figure 8. Cadmium LC50s for each population before (pre-RT) and after RT. Error bars represent one SEM. Populations not shown did not survive the RT in sufficient numbers to be tested.

HYALELLA AZTECA BEHAVIOURAL TESTING

For the pre-exposure assays no population was statistically different from any other (Figure 9), but there was an observed trend towards less time spent in the dark for the laboratory population. For the post-exposure behavioural assays (Figure 10), all treatment groups showed similar preference for the dark side (~60-70%) and no treatments were statistically different.

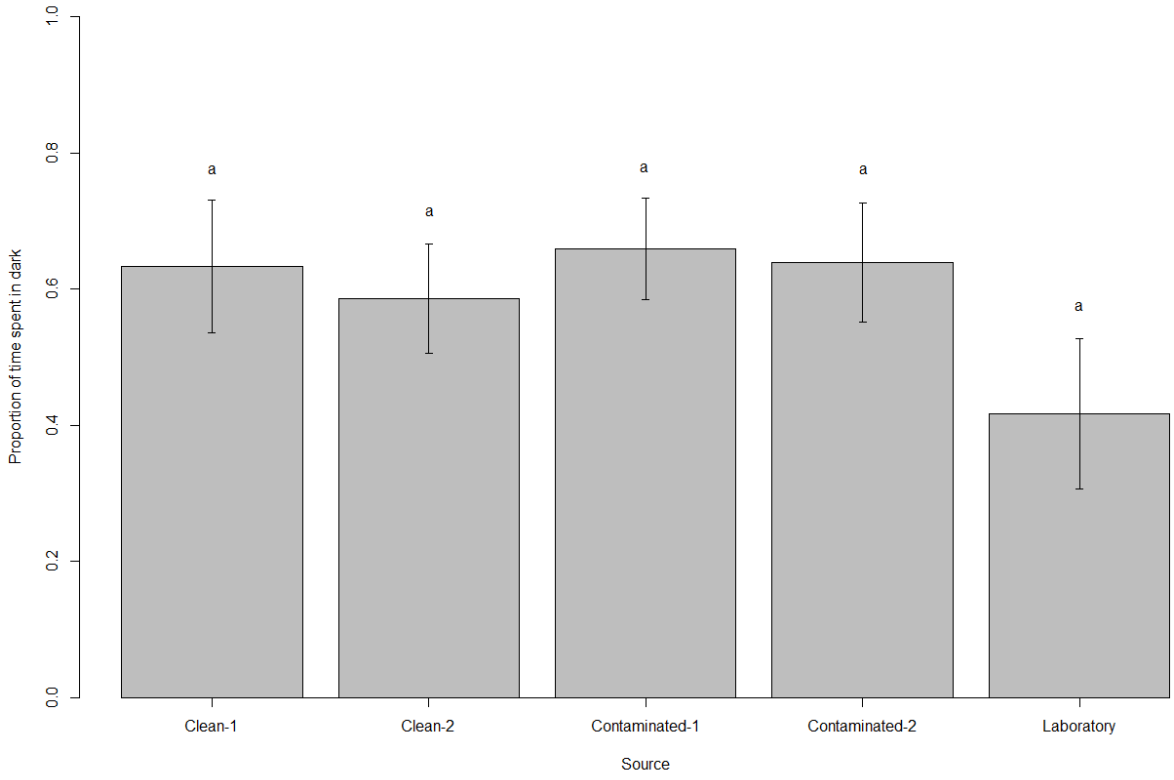

Figure 9. Proportion of time spent in the dark side of the well for the five populations from pre-exposure behaviour assays (n = 12). Those of the same letter are not significantly different.

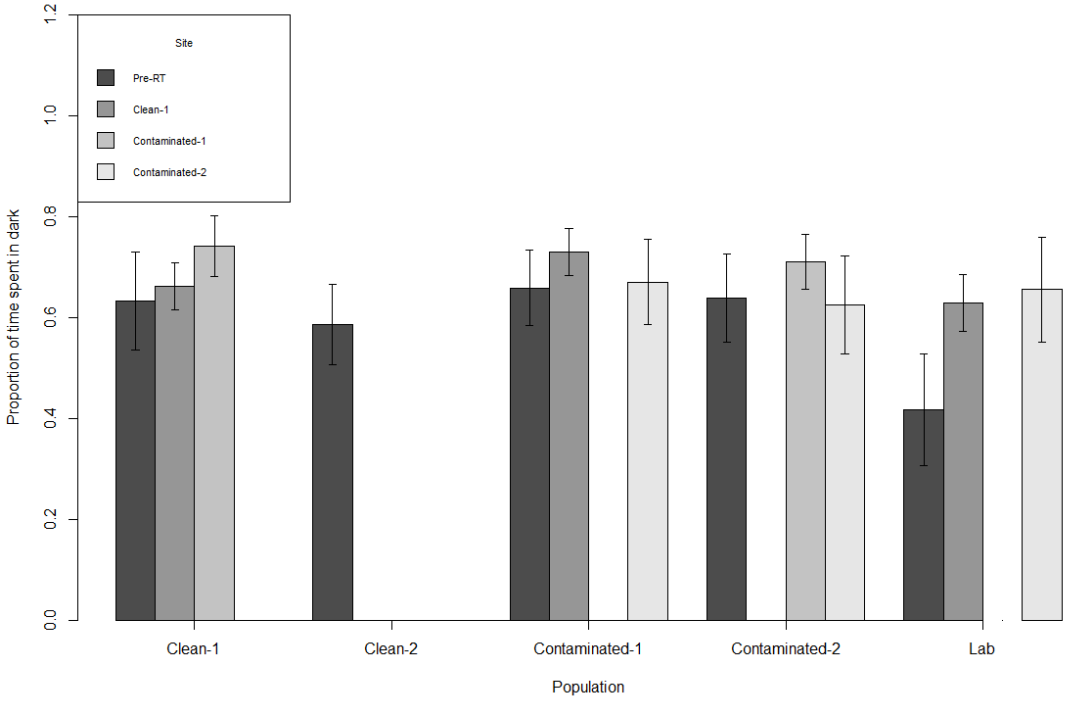

Figure 10. Proportion of time spent in the dark side of the well for the populations tested in behaviour assays (n = 12). Error bars represent 95% confidence intervals. Populations not shown did not survive the RT in sufficient numbers to be tested.

DISCUSSION

Despite clearly demonstrated environmental gradients, we observed no evidence for local adaptation in *Hyalella* populations in northern Alberta. According to Klerks (2002), by definition a population adapted to an environment is less affected by exposure to said environment than a non-adapted population. Based on this description and the data presented previously, local adaptation has likely not occurred in the populations of *H. azteca* tested within the scope of this study. In Figure 2, all populations had lower survival in their local wetland than foreign populations, which is

counter to what is expected in the ‘local vs. foreign’ comparison. For example, within the Cl-1 site, the Cl-1 population had the lowest observed survival. Similarly, the Co-1 population in Co-1 wetland had only the second highest survival and the Co-2 population in the Co-2 wetland had the third highest survival. Furthermore, when comparing results using the ‘home vs. away’ method, all populations had higher survival in an away wetland than their home wetland, which is also counter to what would be expected had local adaptation occurred here. For example, the Co-1 and Co-2 populations had higher survival in the Cl-1 wetland relative to their home wetlands, while Cl-1 had higher survival in both Co-1 and Cl-2 wetlands than in its home wetland.

Other studies have found that the ‘local vs. foreign’ pattern of local adaptation holds true in habitats with contaminant gradients. Two populations, one naïve and one historically impacted, of *Gammarus pulex* held in five sites in the River Hayle (UK) along a polymetal (Cu, Zn) gradient showed that the historically impacted population survived significantly longer than the naïve population at more contaminated sites with higher concentrations of Cu and Zn (Khan et al. 2011). Another study field-collected *Ceriodaphnia pulchella* from reference sites and a site historically impacted by acid mine drainage (AMD) and reared them in the lab for five generations before exposing them to water samples characterised as either reference or AMD contaminated. Both acclimated (lab-reared five generations) and non-acclimated (recently collected) individuals were exposed in order to assess if differences in sensitivity were because of environment-induced physiological alterations or local adaptation. In both experiments, animals from the historically impacted site survived significantly longer than reference animals (Lopes et al. 2005). Another study found that chironomids from a contaminated

site had higher rates of emergence from contaminated sediment than clean sediment while chironomids from a clean site had higher emergence in clean sediment than contaminated, suggesting a trade-off associated with higher fitness in the contaminated environment (Bahrndorff et al. 2006).

Some studies that have investigated local adaptation have found inconsistent or no evidence for local adaptation. One study on *Bromus tectorum* sown at sites with different environmental characteristics found that survivorship and fecundity were affected by site and year of planting but not by seed source population (Rice and Mack 1991). The same pattern was observed in other RT experiments using *Plantago lanceolata* (Antonovics and Primack 1982) and *Chamaecrista fasciculata* (Galloway and Fenster 2000). In each of these studies, local adaptation was observed only in the most extreme habitats or those that were furthest (1000+ kms) apart, suggesting that metapopulation processes and temporal environmental variation hinder local adaptation. These results highlight the importance of plastic responses to varying environmental conditions because genetic bases for variation in fitness can be overwhelmed by environmental determinants of fitness (Hartgerink and Bazzaz 1984).

One possible reason for not seeing the effects of local adaptation in the populations tested here is that oil sands development has only recently accelerated and not enough time has passed for local adaptations to develop. In *G. pulex* from the River Hayle (UK), the environmental pressures driving divergent selection were related to copper and zinc mine drainage that began as early as the seventeenth and eighteenth centuries and continue today (Khan et al. 2011). In contrast, oil sands mining only began in the middle part of the nineteenth century and it was not until the 1990s that it

experienced rapid acceleration. A similar environmental impact timeframe can be seen in a study from Louisiana in which mosquitofish (*Gambusia affinis*) collected from a site historically impacted by petrochemical drainage from 1920 to 1995 initially showed higher tolerance to Pb compared to control fish. However, after being held in clean laboratory water for 34 d, all tolerance to Pb had disappeared from subsequent assays using the historically impacted population, indicating that tolerance was due to acclimation and not adaptation (Klerks, 2002). However, other studies have shown that adaptations can establish relatively quickly in the presence of strong environmental pressures such as contaminant concentration. In one study, least killifish (*Heterandria formosa*) were selected for tolerance to Cd and after six generations, median survival times had increased three-fold in 6 mg L^{-1} Cd exposures (Xie and Klerks 2001). Another study characterised through genetic and physiological evidence the “rapid” invasion of freshwater habitats by a marine copepod (*Eurytemora affinis*) as having occurred within a period of 60 years (Lee 1999). A review of adaptive evolution studies defined “rapid” adaptation as having occurred within the last 200 years (Reznick and Ghalambor 2001).

Significant gene flow can also hinder local adaptation (Klerks 2002; Kawecki and Ebert 2004). Low gene flow can substantially reduce the rate of evolution resistance in house flies (Taylor et al. 1983). In the populations tested here, gene flow is assumed to be low because of the poor overland dispersal ability of *H. azteca* coupled with the somewhat large (in some cases, 50+ km) distances between wetlands, making it unlikely to hinder local adaptation. However, strong selection pressures can overcome the influence of low levels of gene flow (e.g., May and Dobson 1986). In the present study, the environmental factors (i.e., habitat quality) may not be strong enough to drive

adaptive change in the wetlands tested. The largest differences in habitat quality among wetland sites were in specific conductivity, water hardness, and some metals (e.g., Cu, Ni, As, V, Cr), however, the concentrations of metals measured here were relatively low compared to other studies which have shown local adaptation. For example, in the *G. pulex* study in the River Hayle (UK) Cu concentrations ranged from 0.8 to 42.7 μg L^{-1} while the range of Cu concentrations reported here were 0.55 to 3.90 μg L^{-1} (Khan et al. 2011). In the present study, the environmental pressures of habitat quality may not be strong enough to overcome the hindering effects of low gene flow.

Local adaptation typically has a trade-off associated with higher fitness in the local site that manifests as lower relative fitness in foreign sites (Hereford 2009; Kawecki and Ebert 2004). The magnitude of the trade-off is dependent on the magnitude of differences in habitat quality such that larger environmental differences between sites produce larger trade-offs in adapted populations (as reviewed by Hereford 2009). In the present study, the environmental differences between sites were not large enough to elicit an obvious trade-off and this is evidenced by the reclaimed Co-1 population having the highest survival in both a foreign reference wetland and a foreign reclaimed wetland (Figure 2). This does not mean that local adaptation has not occurred, but it does provide a strong rationale for the observed changes being attributable to plasticity rather than adaptation.

Pre-exposure LC50s determined using the reference toxicant showed a trend toward decreasing sensitivity along a gradient of increasing contamination (Table 3). The completely naïve laboratory population showed the highest sensitivity followed by Co-1, Cl-2, Cl-1, and Co-2 in decreasing order. The Co-2 population, which was

collected from the most contaminated and youngest site, showed a significantly higher tolerance for the reference toxicant when compared to the naïve laboratory population. This pattern of increased tolerance along a gradient of increased contamination has been demonstrated in other organisms such as bacteria, plants, and animals (as reviewed by Klerks and Weis 1987). However, this is not necessarily indicative of local adaptation but rather physiological changes related to developmental differences driven by environmental variables such as water chemistry (Klerks and Weis 1987; Lam 1999). For the behavioural experiment, results indicated that phototaxis is not affected by chronic exposure in the wetlands tested here. These results indicate that phototaxis is not a good indicator of sublethal toxicity in the wetlands tested here. A longer exposure period or higher concentration of contaminants may elicit a different response than the results reported here.

In conclusion, this research supports the hypothesis that the four populations of *H. Azteca* from northern Alberta tested here have not undergone local adaptation in response to oil sands development. Additionally, it shows that naïve laboratory populations of *H. azteca* respond similarly to native wild-caught populations from both reference and reclaimed sites. Future research on local adaptation in northern Alberta should look to test populations from reclaimed wetlands incorporating different types of OSPM into their construction.

REFERENCES

Alberta Energy Regulator (AER) (2014). ST98-2014: Alberta's energy reserves 2013 and supply/demand outlook 2013–2023. Alberta Energy Regulator, Calgary, Alberta, Canada. Retrieved from https://www.aer.ca/documents/sts/ST98/ST98-2014.pdf . July 5, 2015.

Alberta Geological Survey (2012). *Alberta oil sands*. Retrieved from http://www.ags.gov.ab.ca/energy/oilsands/. July 5, 2015.

Allen, E.W. (2008). Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. *Journal of Environmental Engineering and Science,* 7, 123–138.

Anderson, J., Wiseman, S.B., Moustafa, A., El-Din, M.G., Liber, K., Giesy, J.P. (2012a). Effects of exposure to oil sands process-affected water from experimental reclamation ponds on *Chironomus dilutus*. *Water Research*, 46, 1662–1672.

Anderson, J.C., Wiseman, S.B., Wang, N., Moustafa, A., Perez-Estrada, L., El-Din, M.C., Martin, J.W., Liber, K., Giesy, J.P. (2012b). Effectiveness of ozonation treatment in eliminating toxicity of oil sands process-affected water to *Chironomus dilutus*. *Environmental Science and Technology,* 46, 486–493.

Antonovics, J., Primack, R. B. (1982). Experimental ecological genetics in *Plantago*: VI. The demography of seedling transplants of *P. lanceolata*. *The Journal of Ecology*, 55-75.

Armstrong, S. A., Headley, J. V., Peru, K. M., Germida, J. J. (2009). Differences in phytotoxicity and dissipation between ionized and nonionized oil sands naphthenic acids in wetland plants. *Environmental Toxicology and Chemistry*, *28*(10), 2167-2174.

Bahrndorff, S., Ward, J., Pettigrove, V., Hoffmann, A. A. (2006). A microcosm test of adaptation and species specific responses to polluted sediments applicable to indigenous chironomids (Diptera). *Environmental pollution*, *139*(3), 550-560.

Baker, L.F., Ciborowski, J.J.H., MacKinnon, M.D. (2012). Petroleum coke and soft tailings sediment in constructed wetlands may contribute to the uptake of trace metals by algae and aquatic invertebrates. *Science of the Total Environment,* 414, 177–186.

Barton, D.R., Wallace, R.R. (1979a). The effects of an experimental spillage of oil sands tailings sludge on benthic invertebrates. *Environmental Pollution,* 18, 305–312.

Barton, D.R., Wallace, R.R. (1979b). Effects of eroding oil sand and periodic flooding on benthic macroinvertebrate communities in a brown-water stream in Northeastern Alberta, Canada. *Canadian Journal of Zoology*, 57, 533–541.

Bendell-Young, L.I., Bennett, K.E., Crowe, A., Kennedy, C.J., Kermode, A.R., Moore, M.M., Plant, A.L., Wood, A. (2000). Ecological characteristics of wetlands receiving an industrial effluent. *Ecological Applications*, 10, 310–322.

Bishop, J. A., Cook, L. M. (1981). *Genetic Consequences of Man Made Change*. Academic Press.

Borgmann, U., Norwood, W. P., Clarke, C. (1993). Accumulation, regulation and toxicity of copper, zinc, lead and mercury in *Hyalella azteca*. *Hydrobiologia*, *259*(2), 79-89.

Borgmann, U. (1996). Systematic analysis of aqueous ion requirements of *Hyalella azteca*: A standard artificial medium including the essential bromide ion. *Archives of Environmental Contamination and Toxicology*, *30*(3), 356-363.

Bousfield, E.L. (1958). Freshwater Amphipod Crustaceans of Glaciated North America. *Can. Field-Nat.,* 72(2): 55-113.

Canadian Association of Petroleum Producers (CAPP) (2014a). The facts on oil sands. Canadian Association of Petroleum Producers, Calgary, Alberta, Canada. Retrieved from http://www.capp.ca/publications-and-statistics/publications/242473. July 5, 2015.

Canadian Association of Petroleum Producers (CAPP) (2014b). Crude oil: Forecast, markets, & transportation. Canadian Association of Petroleum Producers, Calgary, Alberta, Canada. Retrieved from http://www.capp.ca/publications-and-statistics/publications/264419. July 5, 2015.

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, *18*, 117-117.

Clason, B., Duquesne, S., Liess, M., Schulz, R., Zauke, G. P. (2003). Bioaccumulation of trace metals in the Antarctic amphipod *Paramoera walkeri* (Stebbing, 1906):

comparison of two-compartment and hyperbolic toxicokinetic models. *Aquatic Toxicology,* 65, 117-140.

Clifford, H. F. (1991). *Aquatic Invertebrates of Alberta: An Illustrated Guide*. Edmonton, Alberta: University of Alberta.

Dahms, H. U., Hagiwara, A., Lee, J. S. (2011). Ecotoxicology, ecophysiology, and mechanistic studies with rotifers. *Aquatic Toxicology,* 101, 1-12.

de March, B.G.E. (1981). *Hyalella azteca* (Saussure). In *Manual for the Culture of Selected Freshwater Invertebrates* (pp. 61-77). Ottawa, ON: Department of Fisheries and Oceans.

Environment Canada (2014). Biological Test Method: Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca*. Science and Technology Branch, Environment Canada. Retrieved from http://www.ec.gc.ca/faunescience-wildlifescience/default.asp?lang=En&n=0BB80E7B-1. July 5, 2015.

Elshayeb, M., MacKinnon, M.D., Dixon, D.G., Power, M. (2009). The use of carbon and nitrogen stable isotope analysis to characterize food web changes in aquatic systems for reclamation of oil sands process-affected materials. *Water Quality Research Journal of Canada,* 44, 313–322.

Farwell, A.J., Nero, V., Ganshorn, K., Leonhardt, C., Ciborowski, J., MacKinnon, M., Dixon, D.G. (2009). The use of stable isotopes (13C/12C and 15N/14N) to trace exposure to oil sands processed material in the Alberta Oil Sands region. *Journal of Toxicology and Environmental Health*, Part A 72, 385–396.

Frank, R.A., Fischer, K., Kavanagh, R., Burnison, B.K., Arsenault, G., Headley, J.V., Peru, K.M., Van Der Kraak, G., Solomon, K.R. (2009). Effect of carboxylic acid content on the acute toxicity of oil sands naphthenic acids. *Environmental Science and Technology,* 43, 266–271.

Galloway, L. F., Fenster, C. B. (2000). Population differentiation in an annual legume: local adaptation. *Evolution*, *54*(4), 1173-1181.

Government of Alberta (2010). Facts about water in Alberta. Alberta Environment Information Centre, Government of Alberta, Alberta, Canada. Retrieved from http://environment.gov.ab.ca/info/library/6364.pdf. July 5, 2015.

Government of Alberta (2014). *Alberta's Oil Sands*. Alberta Public Affairs Bureau, Government of Canada, Edmonton, Alberta, Canada. Retrieved from http://oilsands.alberta.ca/about.html. July 5, 2015.

Hartgerink, A. P., Bazzaz, F. A. (1984). Seedling-scale environmental heterogeneity influences individual fitness and population structure. *Ecology*, 198-206.

Hereford, J. (2009). A quantitative survey of local adaptation and fitness trade-offs. *The American Naturalist*, *173*(5), 579-588.

Hersikorn, B.D., Ciborowski, J.J.H., Smits, J.E.G., 2010. The effects of oil sands wetlands on wood frogs (*Rana sylvatica*). *Toxicological and Environmental Chemistry,* 92, 1513–1527.

Hinch, S. G., Bailey, R. C., Green, R. H. (1986). Growth of *Lampsilis radiata* (Bivalvia: Unionidae) in sand and mud: a reciprocal transplant experiment. *Canadian Journal of Fisheries and Aquatic Sciences*, *43*(3), 548-552.

Hughes, A. R., Inouye, B. D., Johnson, M. T., Underwood, N., Vellend, M. (2008). Ecological consequences of genetic diversity. *Ecology Letters*, *11*(6), 609-623.

Jensen-Fontaine, M., Norwood, W.P., Brown, M., Dixon, D.G., Le, X.C. (2013). Uptake and speciation of vanadium in the benthic invertebrate *Hyalella azteca*. *Environmental Science and Technology,* 48, 731–738.

Kahle, J., Zauke, G. P. (2002). Bioaccumulation of trace metals in the copepod *Calanoides acutus* from the Weddell Sea (Antarctica): comparison of two-compartment and hyperbolic toxicokinetic models. *Aquatic Toxicology,* 115-135.

Kahle, J., Zauke, G. P. (2003). Bioaccumulation of trace metals in the Antarctic amphipod *Orchomene plebs*: evaluation of toxicokinetic models. *Marine Environmental Research,* 55, 359-384.

Kawecki, T. J., Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, *7*(12), 1225-1241.

Keithly, J., Brooker, J. A., Deforest, D. K., Wu, B. K., Brík, K. V. (2004). Acute and chronic toxicity of nickel to a cladoceran (*Ceriodaphnia dubia*) and an amphipod (*Hyalella azteca*). *Environmental Toxicology and Chemistry*, *23*(3), 691-696.

Khan, F. R., Irving, J. R., Bury, N. R., Hogstrand, C. (2011). Differential tolerance of two *Gammarus pulex* populations transplanted from different metallogenic regions to a polymetal gradient. *Aquatic Toxicology*, 102(1-2), 95-103.

Klerks, P. L., Weis, J. S. (1987). Genetic adaptation to heavy metals in aquatic organisms: a review. *Environmental Pollution*, *45*(3), 173-205.

Klerks, P. L. (2002). Adaptation, ecological impacts, and risk assessment: insights from research at Foundry Cove, Bayou Trepagnier, and Pass Fourchon. *Human and Ecological Risk Assessment*, *8*(5), 971-982.

Kovalenko, K.E., Ciborowski, J.J.H., Daly, C., Dixon, D.G., Farwell, A.J., Foote, A.L., Frederick, K.R., Costa, J.M.G., Kennedy, K., Liber, K. (2013). Food web structure in oil sands reclaimed wetlands. *Ecological Applications*,23, 1048–1060.

Krapu, G.L., Reinecke, K.J. (1992). Foraging ecology and nutrition. In Bruce D. J. Batt, *Ecology and Management of Breeding Waterfowl* (pp. 1-29). Minneapolis, MN: University of Minnesota Press.

Lam, P. K. S. (1999). Methods for distinguishing genetic and environmental variance in stress tolerance. *Ecological Applications*, *9*(2), 449-455.

Lee, C. E. (1999). Rapid and repeated invasions of fresh water by the copepod *Eurytemora affinis*. *Evolution*, 1423-1434.

Lopes, I., Baird, D. J., Ribeiro, R. (2005). Resistance to metal contamination by historically-stressed populations of *Ceriodaphnia pulchella*: Environmental influence versus genetic determination. *Chemosphere*, *61*(8), 1189-1197.

May, R. M., Dobson, A. P. (1986). Population dynamics and the rate of evolution of pesticide resistance. *Pesticide Resistance: Strategies and Tactics for Management*, 170-193.

Nebeker, A. V., Dominguez, S. E., Chapman, G. A., Onjukka, S. T., Stevens, D. G. (1992). Effects of low dissolved oxygen on survival, growth and reproduction of *Daphnia*, *Hyalella* and *Gammarus*. *Environmental Toxicology and Chemistry*, *11*(3), 373-379.

Ownby, D. R., Newman, M. C., Mulvey, M., Vogelbein, W. K., Unger, M. A., & Arzayus, L. F. (2002). Fish (*Fundulus heteroclitus*) populations with different exposure

histories differ in tolerance of creosote-contaminated sediments. *Environmental Toxicology and Chemistry*, *21*(9), 1897-1902.

Parsons, B.G., Watmough, S.A., Dillon, P.J., Somers, K.M. (2010a). A bioassessment of lakes in the Athabasca Oil Sands region, Alberta, using benthic macroinvertebrates. *Journal of Limnology,* 69, 105–117.

Parsons, B.G., Watmough, S.A., Dillon, P.J., Somers, K.M. (2010b). Relationships between lake water chemistry and benthic macroinvertebrates in the Athabasca Oil Sands region, Alberta. *Journal of Limnology*, 69, 118–125.

Pease, C. J., Johnston, E. L., Poore, A. G. (2010). Genetic variability in tolerance to copper contamination in a herbivorous marine invertebrate. *Aquatic Toxicology*, *99*(1), 10-16.

Phipps, C. F. (1915). An experimental study of the behavior of amphipods with respect to light intensity, direction of rays and metabolism. *The Biological Bulletin*, *28*(4), 210-223.

Puttaswamy, N., Turcotte, D., Liber, K. (2010). Variation in toxicity response of *Ceriodaphnia dubia* to Athabasca Oil Sands coke leachates. *Chemosphere*, 80, 489–497.

Puttaswamy, N., Liber, K. (2011). Identifying the causes of oil sands coke leachate toxicity to aquatic invertebrates. *Environmental Toxicology and Chemistry,* 30, 2576–2585.

Puttaswamy, N., Liber, K. (2012). Influence of inorganic anions on metals release from oil sands coke and on toxicity of nickel and vanadium to *Ceriodaphnia dubia*. *Chemosphere*, 86, 521–529.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Regional Municipality of Wood Buffalo (RMWB), 2015. *Archived Census Reports*. Retrieved from http://www.rmwb.ca/Municipal-Government/Census-2015/Municipal-Archived-Census-Reports.htm. July 5, 2015.

Reznick, D. N., Ghalambor, C. K. (2001). The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica*, *112*(1), 183-198.

Rice, K. J., Mack, R. N. (1991) Ecological genetics of *Bromus tectorum*. *Oecologia*, 88(1), 91-101.

Ritterhoff, J., Zauke, G. P. (1997). Bioaccumulation of trace metals in Greenland Sea copepod and amphipod collectives on board ship: verification of toxicokinetic model parameters. *Aquatic Toxicology*, 40, 63-78.

Suncor, 2015. *History*. Retrieved from http://www.suncor.com/en/about/1918.aspx. July 5, 2015.

Strong Jr, D. R. (1972). Life history variation among populations of an amphipod (*Hyalella azteca*). *Ecology*, 1103-1111.

Taylor, C. E., Quaglia, F., Georghiou, G. P. (1983). Evolution of resistance to insecticides: a cage study on the influence of migration and insecticide decay rates. *Journal of Economic Entomology*, *76*(4), 704-707.

United States Environmental Protection Agency (USEPA), 2014. *Priority Pollutants*. Retrieved from http://water.epa.gov/scitech/methods/cwa/pollutants.cfm . July 5, 2015.

United States Environmental Protection Agency (USEPA), 2015. ECOTOX Database. Retrieved from http://cfpub.epa.gov/ecotox/ . July 5, 2015.

Wiseman, S.B., Anderson, J.C., Liber, K., Giesy, J.P. (2013). Endocrine disruption and oxidative stress in larvae of *Chironomus dilutus* following short-term exposure to fresh or aged oil sands process-affected water. *Aquatic Toxicology,* 142, 414–421.

Xie, L., Klerks, P. L. (2001). Selection for contaminant resistance in fish: response and fitness consequences. Presented at: Society of Environmental Toxicology and Chemistry 22nd Annual Meeting, Baltimore, MD, USA. Meeting Abstract Book, 167.