2012

Growth, survival, and community composition of Chironomidae (Diptera) larvae in selected Athabasca oil sands process-affected wetland waters of northeastern Alberta

Kaitlin D. Kennedy

GROWTH, SURVIVAL, AND COMMUNITY COMPOSITION OF CHIRONOMIDAE (DIPTERA) LARVAE IN SELECTED ATHABASCA OIL SANDS PROCESS-AFFECTED WETLAND WATERS OF NORTH-EASTERN ALBERTA

By

Kaitlin D. Kennedy

A Thesis
Submitted to the Faculty of Graduate Studies
through the Department of Biological Sciences
in Partial Fulfillment of the Requirements for
the Degree of Master of Science
at the University of Windsor

Windsor, Ontario, Canada

2012

Growth, survival, and community composition of Chironomidae (Diptera) larvae in selected Athabasca oil sands process-affected wetland waters of north eastern Alberta

by

Kaitlin Kennedy

APPROVED BY:

_____

Carla M. Wytrykush
Syncrude Canada, Ltd.

_____

Dr. Edwin Tam
Department of Civil and Environmental Engineering

_____

Dr. Kenneth G. Drouillard
Great Lakes Institute for Environmental Research

_____

Dr. Jan J.H. Ciborowski, Advisor
Department of Biological Sciences

_____

Dr. Trevor E. Pitcher, Chair of Defense
Department of Biological Sciences

July 23, 2012

DECLARATION OF ORIGINALITY

I hereby certify that I am the sole author of this thesis and that no part of this thesis has been published or submitted for publication.

I certify that, to the best of my knowledge, my thesis does not infringe upon anyone's copyright nor violate any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my thesis, published or otherwise, are fully acknowledged in accordance with the standard referencing practices. Furthermore, to the extent that I have included copyrighted material that surpasses the bounds of fair dealing within the meaning of the Canada Copyright Act, I certify that I have obtained a written permission from the copyright owner(s) to include such material(s) in my thesis and have included copies of such copyright clearances to my appendix.

I declare that this is a true copy of my thesis, including any final revisions, as approved by my thesis committee and the Graduate Studies office, and that this thesis has not been submitted for a higher degree to any other University or Institution.

ABSTRACT

Oil sands process water (OSPW) is toxic to many aquatic organisms. The goal of this study is to determine if or how midge (Diptera: Chironomidae) productivity and community assemblages may differ between OSPW and reference wetlands and the effects of OSPW wetland water, naphthenic acids (NA), and salts on chironomid growth and survival. Although chironomids differed in size, abundance, and community composition among wetlands, the differences were not attributable to the presence or absence of OSPW. Community composition varied with respect to wetland-specific water chemistry attributes (e.g., dissolved oxygen). Ten-d *Chironomus riparius* laboratory bioassays indicated that larvae grew to a smaller size when exposed to OSPW wetland water compared to reference wetland water. When *C. riparius* was reared for 10 d in water mimicking combinations of salts and NA, survival was significantly negatively correlated with salt and NA concentrations, and there was an antagonistic interaction between the two toxicants.

For my parents, Cindy and Dan Kennedy;

for their love and support.

ACKNOWLEDGEMENTS

First and foremost, I would like to acknowledge my supervisor, Dr. Jan Ciborowski. His advice and support guided me through this challenging process like no other supervisor could. I would like to thank my committee members Dr. Edwin Tam and Dr. Ken Drouillard, and my industry mentor Carla Wytrykush (Syndrude Canada).

I would also like to thank my fellow graduate students and field assistants throughout my Masters including Jesse Gardner Costa, Carsten Slama, Josh Martin (now with Suncor), Lyndon Barr, Emily Crisovan, Michael Chen, Katie Williams, Hannah Bagnall, Julie Koloff, Karolina Jurkowski, Jessica Dowson, and Megan Schieck. A special thank you goes out to Sheeva Nakhaie with whom I made many "slap bets"—without her (well-meaning) threat of physical pain hovering over the completion of each chapter, this thesis would not have been possible. Members of the Ciborowski lab that helped me out on the Windsor front included Katya Kovalenko, Mary Sebastian, Li Wang, Michelle Dobrin, Mirna Jajo, Laxmi Pandit, Shubha Pandit, Paige McDonald, Jessica Cuthbert, Nicole Barker, and Kyle Swiston.

In Fort McMurray, AB, many oil sands industry staff members helped tremendously with the logistics of my field research: Chris Beierling (Syncrude), Lori Cyprien (Syncrude), Christine Daly (Suncor), Terry Van Meer (Syncrude), and Richard Kavanaugh (Canadian Natural Resources Limited).

Finally, I'd like to thank my wonderfully supportive family: my parents Cindy and Dan, my brother Mike, and my sister Laura. I'd also like to thank my loving and supportive partner Dave Middleton.

This research was funded by grants provided to Dr. Jan Ciborowski from Syncrude Canada Ltd, Suncor Energy Ltd., PetroCanada, Canadian Natural Resources Ltd., Shell Canada, Total Canada Ltd., Imperial Oil Ltd., the Canadian Water Network, and the Natural Sciences and Engineering Research Council of Canada (NSERC). I gratefully acknowledge receipt of an NSERC Industrial Postgraduate Scholarship and graduate/research assistantships from the University of Windsor.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

OSPW= Oil Sands Process Water

OSPM= Oil Sands Process Material

NA= Naphthenic Acids

TDS= Total Dissolved Solids

PCA= Principal Components Analysis

ANOVA= Analysis of Variance

DO= Dissolved Oxygen

DOC= Dissolved Organic Carbon

CHAPTER I: GENERAL INTRODUCTION

The overarching goal of this research was to determine if oil sands process affected wetlands can support a chironomid community with productivity equivalent to that of natural wetlands. Whether differences in productivity can be attributed to naphthenic acid (NA) concentration, salts, or a combination of the two was also examined.

Chironomidae

Chironomidae are a diverse family of non-biting midges, which are ubiquitous and abundant in North America (Pinder, 1986; Pascoe et al., 1989).It is estimated that there may be up to 15,000 species of Chironomidae world-wide (Cranston, 1995). The life cycle of chironomid species varies greatly in duration and timing; for example, *Chironomus riparius*, a species commonly cultured in the laboratory, has a larval duration of 10 d at 20 degrees C, and can pass through about 7 generations per year in their natural habitat (Learner and Edwards, 1966). At the other extreme, two arctic species of *Chironomus* were found to have a seven year life cycle from egg to adult (Butler, 1982).

Adult female chironomids lay their eggs embedded in a gelatinous matrix on macrophytes, rocks, or leaf litter close to the shoreline (Nolte 1993). A single egg mass can contain between a few dozen eggs for smaller species to several thousand eggs for larger species (Pinder, 1995a). Eggs will typically hatch in 2-6 d. At the time of hatching and for approximately 24 h, the transparent first-instar larvae are planktonic, which causes them to become dispersed via water currents (Pinder, 1995a). Larvae subsequently settle to the substrate, and the larvae are benthic for the remainder of their prepupal

development. Many chironomid species create tubes of mucus, often incorporating other materials. Tubes can secure larvae to substrates, facilitate respiratory efficiency, or provide shelter from predators (Oliver, 1971; Hershey, 1987).

After passing through 4 larval instars, chironomids develop into pupae. During the very brief pupal stage, an individual rises through water column to the surface where it moults and emerges as an adult (Oliver 1971), typically leaving the pupal exuvia floating on the surface of the water. The exuviae, collected as floating windrows in backwaters (Ferrington et al. 1991), or trapped with nets anchored in streams (Boerger 1977) can be identified and enumerated and thus provide a measure of chironomid community composition and abundance.

Chironomids are typically among the most numerous benthic invertebrates in freshwater systems. Larvae can be present in densities of up to 50,000 individuals m^{-2} (Coffman, 1995). The transfer of this aquatic biomass to the terrestrial community through emergence creates an important link between aquatic and terrestrial food webs (Oliver 1971). They are especially important contributors to overall wetland productivity (Pascoe et al. 1989), providing energy to higher trophic levels both within and outside of the wetland communities in which they develop (Oliver, 1971). Chironomid carcass deposition can provide significant nutrient inputs to near-shore terrestrial food webs, and these nutrient inputs can be detected in multiple trophic levels (Hoekman et al., 2012). Because they are so abundant, they can serve as a primary food source for invertebrate predators such as dragonflies (Benke, 1976)) and many vertebrate functional groups,

including dabbling ducks such as mallards (Batzer et al. 1993), and aerial insectivores such as silver-haired bats (Barclay, 1985) and tree swallows (St. Louis et al., 1990). Chironomid larvae have potential to be especially useful bioindicators in that species and genera are differentially sensitive to both disturbance and to specific classes of pollution (Rosenberg 1992, Carew et al. 2007).

Representatives of some multivoltine chironomid genera are amenable to laboratory culturing and are commonly used in biomonitoring freshwater ecosystems. *Chironomus* larvae in particular are standard test organisms used to determine aquatic toxicity (Environment Canada, 1997; EPA, 1996; OECD, 2004).

The Oil Sands Industry and Wet Landscape Reclamation

Unlike traditional oil extraction methods, the shallow nature of the Athabasca oil deposit requires open pit mining. The process of extracting bitumen from the mined oil sand ore (usually the Clark hot water extraction method) requires large volumes of alkaline, hot water and generates large amounts of tailings material. The tailings material is primarily composed of water, sand, and clays, but also contains unrecovered bitumen, salts, naphthenic acids, and trace metals (FTFC 1995). In 2004, the total volume of tailings water on site at Syncrude was approaching 1 billion m^3 (MacKinnon 2004).

The post-mining landscape must be restored to a function equivalent to its pre-mining state, which included 20-40% wetland area (FTFC 1995). Mine tailings material has been used as a component of reclaimed demonstration wetlands (FTFC 1995), but the

consequences to wetland productivity must be determined before this reclamation strategy is implemented on a large scale.

The "wet landscape option" is a proposed reclamation regime comprised of a landscape consisting of series of interconnected wetlands that ultimately drain into end-pit lakes (lakes created by allowing the mined open pit to partially or completely fill with water) (FTFC, 1995). It is currently in the initial stages of being implemented by several oil sands companies because it has the potential to use and permanently store large volumes of process water (FTFC, 1995). However, the practicality of this option at a full scale, however, depends on knowing, among other things, the long-term toxicity of oil sands process water and material on aquatic biota. Initial studies on the use of fresh oil sands process water (OSPW) in reclamation wetlands indicated that OSPW was toxic to a variety of aquatic organisms (MacKinnon and Boerger, 1986). Of the major constituents that comprise OSPW, naphthenic acids (NAs) are commonly cited in the literature as the main contributor to the toxicity (Allen, 2008). However, marine sediments from the late Cretaceous and Devonian eras are close to the surface in the Athabasca oil sands area (Gosselin et al., 2010). Furthermore, sodium hydroxide is used during extraction, and calcium sulphate is often added during the consolidation of tailings, which produces a subsaline environment that adds to the toxicity by imposing osmotic stress on aquatic organisms (MacKinnon et al. 2001). Sodium, calcium, chloride, sulphate, and bicarbonate are the major ions that contribute to the salinity of OSPW (M. MacKinnon, Syncrude Canada Ltd., pers. communication, Gosselin et al., 2010). Concentrations of

naphthenic acids covary with salts in OSPW, making their individual effects difficult to distinguish when conducting field studies (Leung et al. 2003).

Toxicity of Oil Sands Process Water across Taxa

Naphthenic acids are a group of alkyl-substituted carboxylic acids that naturally occur in petroleum (Clemente & Fedorak, 2005; Seifert & Teeter, 1969). They occur in low concentrations (1-2 mg/L) in the Athabasca River and its tributaries, and in natural wetlands, but they can be present in OSPW at concentrations of up to 120 mg/L (M. MacKinnon pers. communication). Recent improvements in technology indicate that the acid extractable portion of OSPW which was previously called NA is not truly NA in its entirety (Rowland et al., 2011). However, since this is a very recent finding, the term "NA" will be used synonymously with "acid extractable portion of OSPW" in this thesis. NAs in freshly produced OSPW are acutely toxic to a variety of microbes, plants, invertebrates, fishes, mammals, and amphibians. Leung et al. (2001) studied phytoplankton community composition and biomass using microcosms in OSPM-affected and reference wetlands. They found significant differences in community composition between reference and OSPM-affected wetlands but no significant differences in biomass.

In yeast, NAs extracted from North Sea oil effluent were found to be weak estrogen receptor agonists and androgen receptor antagonists (Thomas et al., 2009). This means that NAs bind to the estrogen receptor and disrupt binding at the androgen receptor (Thomas et al., 2009). This may explain some of the changes in sex characteristics in

higher trophic organisms (explained further below) such as fathead minnows (e.g. Kavanagh et al. 2011).

Naphthenic acids are acutely toxic to microorganisms (Clemente et al. 2004). Some toxicity assays using *Vibrio fischeri* (Frank et al., 2008a; Frank et al., 2008b) and *Photobacterium phosphorem* (Holowenko et al. 2002) suggested that low molecular weight naphthenic acids are more toxic than higher molecular weight acids. However, these findings must be interpreted with caution because the concentrations of the fractions were reported in mg/L rather than as molar concentrations.

Furthermore, the addition of carboxylic acid, which is expected to reduce hydrophobicity, reduces the toxicity of the acid fraction (Frank et al. 2008a). This relationship between molecular weight, carboxylic acid content, and toxicity was also demonstrated in bioassays using *Daphnia magna* (Frank et al. 2008a).

Water from OSPM-affected ponds reduced the growth rate and slowed the development of northern Canadian toad (*B. boreas*) and wood frog (*R. sylvatica*), tadpoles relative to tadpole growth and development in water from reference ponds (Pollet & Bendell-Young, 2000). When Hersikorn et al. (2010) conducted a similar study, they found that wood frog tadpoles only exhibited high mortality when held in young OSPM-affected wetlands. Tadpoles held in older (>7 years old) OSPM-affected wetlands appeared to have a survival rate similar to tadpoles held in reference wetlands. However, Schock et al. (Keyano College, pers. commun.) found that OSPW slowed the development of wood

frog tadpoles so much that they were unlikely to be able to metamorphose and disperse from ponds prior to winter freeze-up.

The responses of several fish species to OSPW and NA have been studied. Kavanagh et al. (2011) reported disturbances in reproduction and secondary sex characteristics in fathead minnows (*Pimephales promelas*) that were exposed to NA concentrations and conductivities typical of OSPM-affected wetlands; OSPW with NA concentrations of >25 mg/L and conductivities of >2000 µS/cm caused spawning inhibition, lowered sex steroid hormone concentrations, and reduced secondary sex characteristics (nuptials) (Kavanagh et al. 2011). In another assay in which fish were first acclimated in water that was half the conductivity of treatment water prior to the test, spawning and sex steroids in males were significantly reduced but not completely inhibited (Kavanagh et al. 2011), so native or acclimated fathead minnow populations may not be affected to the same extent as those exposed to OSPW quickly. Similarly, fathead minnows in early life stages that were exposed to oil sands process sediments and naturally-occurring oil sands showed delayed hatching, increased mortality, and increased incidence of malformations (Colavecchia et al. 2004). Changes in sex hormone levels detected in goldfish exposed to OSPW may explain the changes in reproductive traits seen in fathead minnows. Both male and female goldfish caged in experimental ponds containing OSPW exhibited significantly lower testosterone and 17β-estradiol concentrations compared to fish held in control ponds (Lister et al. 2008). Furthermore, plasma 17β-estradiol levels were significantly higher in male goldfish that were exposed to a concentration of 6 mg/L naphthenic acids in a 7-d day laboratory bioassay than in fish that were exposed to

reference lake water (Lister et al. 2008). However, these changes in sex steroid levels were not seen in native slimy sculpin (*Cottus cognatus*) and pearl dace (*Margariscus margarita*) collected close to oil sands developments compared to fish collected upstream of developments (Tetreault et al. 2003). But, EROD activity (a biomarker for chemical detoxification of any kind) activity in the liver was significantly higher in slimy sculpin and pearl dace that were collected close to the oil sands developments (Tetreault et al. 2003); this means that although the fish are exposed to potentially toxic chemicals when close to the oil sands developments, this exposure does not necessarily manifest itself in alteration of the sex characteristics studied. In contrast to focusing on sexual differences fish as did the previously described studies, Nero et al. (2006a) examined gill and liver histopathology in goldfish and yellow perch that were held in OSPM-affected and reference ponds. Perch held in a pond with high NA (24 mg/L) concentrations and high conductivity (2657 μS/cm) exhibited significantly higher inflammatory and degenerative liver response indices and significantly higher gill proliferation than fish held in a ponds with lower concentrations of NA and lower conductivities (Nero et al., 2006a). Similarly, goldfish gills showed an inflammatory and degenerative response when held in the high NA and high conductivity pond (Nero et al., 2006a). Nero et al. (2006b) conducted a separate lab bioassay on yellow perch to assess the effect of salinity on NA toxicity. They used a low salt and a high salt treatment on 3 different concentrations of extracted NA and 3 different concentrations of commercial NA. The authors found that all individuals died at 3.6 mg/L NA. However, at lower concentrations of NA the addition of salt reduced mortality by 40-50%. Gill surface area was, however, significantly reduced by the addition of salts to NA (Nero et al. 2006b). This change in gill morphology was

not seen in fathead minnows exposed to OSPW, but the minnows did exhibit very high mortality rates when caged in OSPW ponds compared to control ponds (Farrell et al., 2004). Siwik et al. (2000) conducted two repetitions of a bioassay in which young fathead minnows were held in OSPM-affected wetland water, salty reference wetland water, and lower salt reference wetland water. In the first bioassay, the investigators did not detect any significant differences in growth or survival, but in the second bioassay the minnows exhibited significant differences in survival but not growth (Siwik et al., 2000).

Although many researchers have studied the toxicity of OSPW and NA to fishes, studies on taxa that may be less directly affected by OSPW, such as mammals and birds, are relatively scarce. Rogers et al. (2002) analyzed the short-term toxicity of NA consumption (extracted from OSPW) by rodents at dosages of 3, 30 and 300 mg/kg body weight. High-dose females had significantly higher ovary and spleen weights and high-dose males had significantly higher heart and testes weights than control rats. However, the high dose (300 mg/kg body weight) corresponds to 50 times a "worst-case single-day exposure for wild animals", so results like this would not be seen in nature. In a 90-d test, rats fed 60 mg/kg body weight/d showed increased liver glycogen storage and higher liver weights than rats given lower dosages and controls. The authors suggested that the liver was likely a target organ for NA toxicity in mammals (Rogers et al. 2002). Birds ingest NA in a way similar to mammals-- directly by drinking OSPW, and indirectly by consuming food (e.g., emergent insects) that developed in OSPW. Thyroid hormone concentration (T4) was significantly higher in tree swallow (*Tachycineta bicolour*) nestlings from two OSPM-affected wetlands compared to concentrations in nestlings

from two reference wetlands (Gentes et al. 2007a). However, when tree swallow nestlings were directly fed NA at a rate of 1.5 mg/d for 7 d — an amount corresponding to ten times the worst case scenario exposure—no negative effects were found in growth, organ weight, or blood biochemistry, among other endpoints (Gentes et al. 2007b). Collectively, these studies indicate that ingested NA pose minimal acute toxicity to birds and mammals.

Effect of OSPW on Benthic Invertebrates and Chironomidae

Young (<7 year old) OSPW wetlands support less diverse macroinvertebrate populations than naturally-formed reference or older OSPW wetlands (Leonhardt, 2003). This suggests that toxicity of OSPW may decline over time; naphthenic acids can biodegrade over time (Herman et al. 1994), and this may explain the apparent reduction in toxicity. An alternative explanation may be that more tolerant species may be colonizing OSPW and after seven years the richness may be equivalent, but community composition may still be different.

Bendell-Young et al. (2000) found that Chironomidae larvae were the numerically dominant invertebrates in seven reference wetlands and five OSPW wetlands. OSPW wetlands tended to have lower overall benthic macroinvertebrate diversity, but higher chironomid diversity than reference wetlands. Furthermore, OSPW wetlands had greater chironomid densities and biomass than reference wetlands. Mouthparts of chironomids were also examined for deformities as evidence of teratogenic effects, and no significant difference between wetlands was found (Bendell-Young et al. 2000). Whelly (1999) also found that natural reference wetlands supported greater diversity but lower

macroinvertebrate abundance than constructed wetlands. However, reference constructed wetlands had greater richness than OSPW-affected wetlands of equivalent age. Whelly (1999) also found that the incidence of mouthpart deformities was uniformly low across multiple genera and in all classes of wetlands that he studied.

Anderson et al. (2011) exposed partially grown (2^{nd}-3^{rd} instar) *Chironomus dilutus* (= *C. tentans*) larvae to water from three different OSPW-affected wetlands, two fresh OSPW samples, as well as a freshwater and a saltwater control. No consistent significant differences were found in mean survival among water treatments, but larvae grown in the two fresh OSPW samples did exhibit a lower wet biomass than larvae grown in water from the controls or the reclamation ponds. Larvae grown in two fresh OSPW samples and one OSPW-affected wetland water sample exhibited significantly lower emergence success than controls (Anderson et al. 2011).

Most relevant to this study is a toxicity test done by Whelly (1999) on a laboratory population of *Chironomus riparius* and lab and field derived *C. dilutus* larvae. Mean final body length and survival were not significantly affected by OSPW concentration in the *C. riparius* larvae. But, growth and survival were significantly reduced in both of the *C. dilutus* populations when exposed to high concentrations of OSPW. At 100% OSPW the lab-derived *C. dilutus* population grew to only 40% the size of controls, whereas the field population grew to 75% the size of controls at the same concentration. Whelly (1999) also found that 50% of *C. dilutus* larvae reared in fresh OSPW died at a concentration of 65% OSPW. Although naphthenic acid concentrations of the OSPW used in the

experiment were not known, given the typical range of fresh OSPW (80-100 mg/L; Holowenko et al. 2002), we can estimate that the LC_{50} of naphthenic acids to *C. dilutus* is likely 52- 65 mg/L.

Mode of Action for Naphthenic Acid Toxicity

Although the mode of action of NA toxicity is largely unknown, it has been proposed that it probably acts by causing narcosis—the disruption of cell membrane function (Roberts, 1991; Frank et al. 2008a). Because surfactants can interact with proteins, NAs may affect cell membrane permeability (Abel, 1974). Low molecular weight NAs are more toxic, but are also more readily degraded by microbes (Frank et al. 2008b). The number of carboxylic acid groups in the NA molecular structure also plays a role in toxicity; the greater the carboxylic acid content, the less toxic the mixture is (Frank et al. 2008a). There is also a physical mechanism by which NAs pose stress to aquatic organisms. NAs have surfactant properties, which can cause gill damage and death by asphyxiation (Abel, 1974).

Another alternate mechanism of (sublethal) toxicity was reported recently by Thomas et al (2009); in a yeast bioassay, the authors found NAs to be a weak estrogen receptor agonist and an androgen receptor antagonist. This may explain some of the sex steroid hormone levels and secondary sex characteristic changes in fish that have been detected in other studies (e.g. Kavanagh et al. 2011, Lister et al. 2008). Estrogen is not only used by vertebrates— insects also depend on estrogen to regulate aspects of reproduction (Mechoulam et al. 1984) and may be affected similarly by OSPW. The genomic response

of *E. coli* to NAs was analysed by Zhang et al. (2011) in order to determine how NAs interact with organisms on the cellular level. The main molecular responses were up-regulation of genes involved with NADP or NADPH binding and down-regulation of genes involved with ATP binding.

Recent advances in the analysis of the components of the acidic fraction of OSPW have indicated that different fractionation techniques can yield a different suite of organic acids and not purely NAs (Rowland et al. 2011). Furthermore, the method of analysis used to quantify NAs differs broadly within the scientific community, and determining exactly which acids comprise the suite of extractable organic acids in OSPW has proven to be a very difficult task (Rowland et al. 2011). It is increasingly realized that toxicity tests referring to oil sands extracted "naphthenic acids" actually refer to a broad group of acids, only some of which are toxic.

Toxicity of Salts to Freshwater Biota

High salinities in OSPW and OSPW-affected wetlands pose a great challenge in wetland reclamation. Like NAs, salts can be toxic to freshwater biota, but, unlike NAs, salts do not degrade and therefore decrease in concentration over time. The topography and hydrology of watersheds in the Athabasca oil sands region, is such that annual evaporation often exceeds precipitation (DeVito et al. 2005). Consequently, salinity of wetland waters can increase or remain constant over time. This has been shown to be the case with OSPW-affected wetlands (Macyk et al. 2004).

Salinity can be measured in concentration of ions (i.e., total dissolved solids; parts per thousad (ppt), or mg/L) or by the electrical conductance of the ions in solution, (i.e., conductivity; μS/cm); however, the two measures are very highly positively correlated (Walker et al. 1995).

High salinity can pose a great challenge for freshwater plants and biota. In plants, the sodium, chloride, and sulphate ions can interfere with water transport in a variety of boreal plants (Renault et al. 1998). In a group of OSPW wetlands and natural wetlands surveyed, salinity was found to be negatively correlated with species richness and peak biomass in boreal marsh plants (Trites and Bayley, 2009).

Wojnarowicz (2009) assessed the toxicity of two salts commonly found in OSPW, sodium chloride and sodium sulphate, to *Ceriodaphnia dubia*. She hypothesized that specific ions and the interaction between them may better predict toxicity than the overall concentration of ions or conductivity. However, the effects of sodium chloride and sodium sulphate were additive, and thus there was no interaction between these salts (Wojnarowicz, 2009).

Genera of Chironomidae are differentially sensitive to salinity; as such, chironomid head capsules in sediment cores are often used in paleolimnology in order to infer the water chemistry history of lakes (Walker et al. 1995). For example, highly saline lakes (>10 ppt) are characterized by abundant *Cricotopus* or *Orthocladius* larvae, whereas lakes with lower salinity are characterized by *Chironomus*, *Procladius*, or *Psectrocladius* (Walker et

al. 1995). Because several genera are tolerant of high salinities, chironomids can make up a large component of the benthic fauna in brackish waters (Pinder 1995b). In *Chironomus,* high salinities (conductivity >5000 μS/cm) lower the number of emergent adults, delay the time of emergence, and reduce larval growth rate (Hassell et al. 2006). *Chironomus riparius* larvae reared in water with sodium chloride concentrations of 5, 10 and 20 ppt exhibited significantly reduced survival compared to controls (0 ppt) (Silver et al. 2009). This effect, however, was only seen at temperatures of 22˚C and not at lower temperatures. Furthermore, the survival rate in the controls at 22˚C was low (approximately 30%) (Silver et al. 2009).

Objectives

Although many studies have been done on the effects of OSPW (particularly with fish), the independent and interactive effects of salts and NAs are not well understood. Furthermore, one must understand the impact of these interactions on chironomid productivity in order to assess the likelihood that wetland reclamation strategies in the Athabasca oil sands region can provide enough biomass to support a food web similar to that of natural wetlands. Therefore, the objectives of this thesis were to determine whether OSPW-affected wetlands differ from natural wetlands in emerging chironomid abundance, biomass, and community composition (Chapter II), to assess the effect of water from OSPW-affected wetlands on survival and growth of *C. riparius* relative to the water of reference wetlands under controlled laboratory conditions (Chapter III), and to assess whether the toxicity of OSPW to chironomids primarily due to naphthenic acids, the complement of salts, or an interaction between NAs and salts (Chapter IV).

In general, my expectations were that NAs and salts would each exert stress on chironomid larvae and lower productivity. However, when NAs and salts are present together, I expected there to be an antagonistic effect, thus supporting greater productivity than if the individual effects were added together.

References

Abel, P.D. 1974. Toxicity of synthetic detergents to fish and aquatic invertebrates. Journal of Fish Biology 6: 279-298.

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

Batzer, D. P., M. McGee, V.H. Resh, and R.R. Smith 1993. Characteristics of invertebrates consumed by mallards and prey response to wetland flooding schedules. Wetlands 13: 41-49.

Barclay, R.M.R. 1985. Long- versus short-range foraging strategies of hoary (*Lasiurus cinereus*) and silver-haired (*Lasionycteris noctivagans*) bats and the consequences for prey selection. Canadian Journal of Zoology 63: 2507-2515.

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

Benke, A.C. 1976. Dragonfly production and prey turnover. Ecology 57: 915-927.

Bervoets, L., C. Wils, and R. Verheyen. 1996. Tolerance of *Chironomus riparius* larvae (Diptera: Chironomidae) to salinity. Bulletin of Environmental Contamination and Toxicology 57: 829-835.

Butler, M.G. 1982. A 7-year life cycle for two *Chironomus* species in arctic Alaskan tundra ponds (Diptera: Chironomidae). Canadian Journal of Zoology 60: 58-70.

Carew, M.E., V. Pettigrove, R.L. Cox, and A.A. Hoffmann. 2007. The response of Chironomidae to sediment pollution and other environmental characteristics in urban wetlands. Freshwater Biology 52: 2444-2462.

Clemente, J. S., and P.M. Fedorak. 2005. A review of occurrence, analyses, toxicity, and biodegradation of naphthenic acids. Chemosphere 60: 585-600.

Clemente, J.S., M.D. MacKinnon, and P.M. Fedorak. 2004. Aerobic biodegradation of two commercial naphthenic acids preparations. Environmental Science and Technology 38: 1009–1016.

Coffman, W.P. 1995. Conclusions *In* The Chironomidae: The biology and ecology of non-biting midges. Edited by: Armitage, P.D., Cranston, P.S., and Pinder, L.C.V. Chapman & Hall, London, UK. Pages 436-447.

Colavecchia, M.V., S.M. Backus, P.V. Hodson, and J.L. Parrott. 2004. Toxicity of oil

sands to early life stages of fathead minnows (*Pimephales promelas*). Environmental Toxicology and Chemistry 23: 1709-1718.

Cranston, P.S. 1995. Introduction *In* The Chironomidae: The biology and ecology of non-biting midges. Edited by: Armitage, P.D., Cranston, P.S., and Pinder, L.C.V. Chapman & Hall, London, UK. Pages 1-7.

Dominski, M. 2007. Surface mined oil sand: tailings practices, performance, and projections. Alberta Energy and Utilities Board. *In* Proceedings of the 3rd International Heavy Oil Conference, March 5–7, 2007, Calgary, AB. Alberta Energy and Utilities Board.

Environment Canada. 1997. Biological test method: test for survival and growth in sediment using larvae of freshwater midges (*Chironomus tentans* or *Chironomus riparius*). Report EPS 1/RM/32, Environment Canada, Ottawa, Ontario.

EPA (United States Environmental Protection Agency). 1996. Ecological effects test guidelines OPPTS 850.1790 Chironomid Sediment Toxicity Test. Public Draft. EPA 712-C-96-313. US Environmental Protection Agency, Office of Protection, Pesticides and Toxic Substances, Washington, DC.

Farrell, A.P., C.J. Kennedy, and A. Kolok. 2004. Effects of wastewater from an oil-sand-refining operation on survival, hematology, gill histology, and swimming of fathead minnows. Canadian Journal of Zoology 82: 1519-1527.

Ferrington, L.C., M. Blackwood, and C.A. Wright. 1991. A protocol for using surface-floating pupal exuviae of Chironomidae for rapid bioassessment of changing water quality. Sediment and Stream Water Quality in a Changing Environment: Trends and Explanation 203: 181-190.

Frank, R.A., K. Fischer, R. Kavanagh, B.K. Burnison, G. Arsenault, J.V. Headley, K.M. Peru, G. Van der Kraak, and K.R. Solomon. 2008a. Effect of carboxylic acid content on the acute toxicity of oil sands naphthenic acids. Environmental Science and Technology 43: 266-271.

Frank, R.A., R. Kavanagh, B.K. Burnison, G. Arsenault, J.V. Headley, K.M. Peru, G. Van Der Kraak, and K.R. Solomon. 2008b. Toxicity assessment of collected fractions from an extracted naphthenic acid mixture. Chemosphere 72: 1309-1314.

FTFC (Fine Tailings Fundamentals Consortium). 1995. "Volume II: Fine Tails and Process Water Reclamation" In: Advances in Oil Sands Tailings Research, Alberta Department of Energy, Oil Sands and Research Division, Edmonton, Alberta, Canada.

Gentes, M.L., C. Waldner, Z. Papp, and J.E.G. Smits. 2006a. Effects of oil sands tailings compounds and harsh weather on mortality rates, growth and detoxification efforts in nestling tree swallows (*Tachycineta bicolor*). Environmental Pollution 142: 24–33.

Gentes, M.L., C. Waldner, Z. Papp, and J.E.G. Smits. 2007b. Effects of exposure to naphthenic acids in tree swallows (*Tachycineta bicolor*) on the Athabasca oil sands, Alberta, Canada. Journal of Toxicology and Environmental Health 70A: 1182-1190.

Gosselin, P., S.E. Hrudey, M.A. Naeth, A. Plourde, R. Therrien, G. Van Der Kraak, and Z. Xu. 2010. The Royal Society of Canada Expert Panel: Environmental and Health Impacts of Canada's Oil Sands Industry. Pages 111- 130.

Hassell, K. L., B.J. Kefford, and D. Nugegoda. 2006. Sub-lethal and chronic salinity tolerances of three freshwater insects: *Cloeon* sp. and *Centroptilum* (Ephemeropptera: Baetidae) and *Chironomus* sp. (Diptera: Chironomidae). Journal of Experimental Biology 209: 4024-4032.

Herman, D.C., P.M. Fedorak, M.D. MacKinnon, and J.W. Costerton. 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oil sands tailings. Canadian Journal of Microbiology 40: 467-477.

Hersikorn, B.D., J.J.C. Ciborowski, and J.E.G. Smits. 2010. The effects of oil sands wetlands on wood frogs (*Rana sylvatica*). Toxicology & Environmental Chemistry 92: 1513-1527.

Hershey, A.E. 1987. Tubes and foraging behaviour in larval Chironomidae: implications for predator avoidance. Oecologia (Berlin) 73: 236-241.

Hoekman, D., M. Bartrons, and C. Gratton. 2012. Ecosystem linkages revealed by experimental lake-derived isotope signal in heathland food webs. Oecologia (in press).

Holowenko, F.M., M.D. MacKinnon, and P.M. Fedorak. 2001. Naphthenic acids and surrogate naphthenic acids in methanogenic microcosms. Water Research 35: 2595–606.

Kavanagh, R.J., R.A. Frank, K.D. Oakes, M.R. Servos, R.F. Young, P.M. Fedorak, M.D. MacKinnon, and K. Solomon. 2011. Fathead minnow (*Pimephales promelas*) reproduction is impaired in aged oil sands process-affected waters. Aquatic Toxicology 101: 214-220.

Learner, M.A. and R.W. Edwards. 1966. The distribution of the midge *Chironomus riparius* in a polluted river system and its environs. Air and Water Pollution 10: 757-768.

Leung, S.S., M.D. MacKinnon, and R.E.H. Smith. 2001. Aquatic reclamation in the Athabasca, Canada, oil sands: naphthenate and salt effects on phytoplankton communities. Environmental Toxicology and Chemistry 20: 1532-1543.

Leung, S.S., M.D. MacKinnon, and R.E.H. Smith. 2003. The ecological effects of naphthenic acids and salts on phytoplankton from the Athabasca oil sands region. Aquatic Toxicology 62: 11-26.

Leonhardt, C.L. 2003. Zoobenthic succession in constructed wetlands of the Fort McMurray oil sands region: developing a measure of zoobenthic recovery. M.Sc. Thesis, University of Windsor, Windsor, ON, Canada.

Lister, A., V. Nero, A. Farwell, D.G. Dixon, and G. Van Der Kraak. 2008. Reproductive and stress hormone levels in goldfish (*Carassius auratus*) exposed to oil sands process-affected water. Aquatic Toxicology 87: 170-177.

MacKinnon, M.D. 2004. Oil sands water quality issues: properties, treatment, and discharge options. Canadian Oil Sands Network for Research and Development (CONRAD) Oil Sands Water Usage Workshop, Edmonton, Alberta, February 24–25, 2005.

MacKinnon, M.D. Unpublished data. Summary of water chemistry of selected waterbodies on the Syncrude mine lease area. Prepared for Syncrude Canada, Ltd. Edmonton, AB.

MacKinnon, M.D., J.G. Matthews, W.H. Shaw, and R.G. Cuddy. 2001. Water quality issues associated with composite tailings (CT) technology for managing oil sands tailings. International Journal of Mining, Reclamation and Environment 15: 235-256.

MacKinnon, M.D., and H. Boerger. 1986. Description of two treatment methods for detoxifying oil sands pond water. Water Pollution Research Journal of Canada 21: 496-512.

Macyk, T.M., R.L. Faught, and R.C. Price. 2004. Evaluation of long term changes in reconstituted soils at the operations of Syncrude Canada Ltd. Alberta Research Council. Edmonton, AB.

Mechoulam, R., R.W. Brueggemeier, and D.L. Denlinger. 1984. Estrogens in insects. Experimentia 40: 943-944.

Nero, V., A. Farwell, A. Lister, G. Van Der Kraak, L.E.J. Lee, T. Van Meer, M.D. MacKinnon, and D.G. Dixon. 2006(a). Gill and liver histopathological changes in yellow perch (*Perca flavescens*) and goldfish (*Carassius auratus*). Ecotoxicology and Environmental Safety 63: 365-377.

Nero, V., A. Farwell, L.E.J. Lee, T . Van Meer, M.D. MacKinnon, and D.G. Dixon. 2006. The effects of salinity on naphthenic acid toxicity to yellow perch: Gill and liver histopathology. Ecotoxicology and Environmental Safety 65: 252–64.

Nolte, U. 1993. Egg masses of Chironomidae (Diptera): a review, including new observations and a preliminary key. Entomologica Scandinavica Supplement 43: 1-75.

OECD. 2004. Test No. 218: Sediment-water chironomid toxicity test using spiked sediment. OECD Guidelines for the Testing of Chemicals Section 2: Effects of Biotic Systems. Organisation for Economic Co-operation and Development, Paris.

Oliver, D.R. 1971. Life history of the Chironomidae. Annual Review of Entomology 16: 211-230.

Pascoe, D., K.A. Williams, and D.W.J. Green. 1989. Chronic toxicity of cadmium to *Chironomus riparius* Meigen -- effects upon larval development and adult emergence. Hydrobiologia 175: 109-115.

Pinder, L.C.V. 1986. Biology of freshwater Chironomidae. Annual Review of Entomology 31: 1-23.

Pinder, L.C.V. 1995a. Biology of the eggs and first instar. *In* The Chironomidae: biology and ecology of non-biting midges. Edited by P.D. Armitage, P. S. Cranston, and L.C.V. Pinder. Chapman & Hall, London, UK. Pg. 91-97.

Pinder, L.C.V. 1995b. The habitats of chironomid larvae. *In* The Chironomidae: biology and ecology of non-biting midges. Edited by P.D. Armitage, P. S. Cranston, and L.C.V. Pinder. Chapman & Hall, London, UK. Pg. 131.

Pollet, I., and L.I. Bendell-Young. 2000. Amphibians as indicators of wetland quality in wetlands formed from oil sands effluent. Environmental Toxicology and Chemistry 19: 2589-2597.

Renault, S., C. Lait, J.J. Zwiazek, and M. MacKinnon. 1998. Effect of high salinity tailings waters produced from gypsum treatment of oil sands tailings on plants of the boreal forest. Environmental Pollution 102: 177-184.

Roberts, D.W. 1991. QSAR issues in aquatic toxicity of surfactants. The Science of the Total Environment 109/110: 557-568.

Rogers, V.V., M. Wickstrom, K. Liber, and M.D. MacKinnon. 2002. Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. Toxicological Sciences 66: 347-355.

Rosenberg, D.M. 1992. Freshwater biomonitoring and Chironomidae. Netherlands Journal of Aquatic Ecology 26: 101-122.

Rowland, S.J., A.G. Scarlett, D. Jones, C.E. West, and R.A. Frank. 2011. Diamonds in

the rough: identification of individual naphthenic acids in oil sands process waters. Environmental Science and Technology 45: 3154-3159.

Seifert, W.K., and R.M. Teeter. 1969. Preparative thin-layer chromatography and high resolution mass spectrometry of crude oil carboxylic acids. Analytical Chemistry 41: 786–795.

Silver, P, S.M. Rupprecht, and M.F. Stauffer. 2009. Temperature-dependent effects of road deicing salt on chironomid larvae. Wetlands 29: 942-951.

Siwik, P.L., T. Van Meer, M.D. MacKinnon, and C. Paszkowski. 2000. Growth of fathead minnows in oil sand-processed wastewater in laboratory and field. Environmental Toxicology and Chemistry 19: 1837–45.

St. Louis, V. L., L. Breebaart, and J.C. Barlow. 1990. Foraging behaviour of tree swallows over acidified and nonacidic lakes. Canadian Journal of Zoology 68: 2385-2392.

Tetreault, G.R., M.E. McMaster, D.G. Dixon, and J.L. Parrott. 2003. Using reproductive endpoints in small forage fish species to evaluate the effects of Athabasca oil sands activities. Environmental Toxicology and Chemistry 22: 2775-2782.

Thomas, K.V., K. Langford, K. Petersen, A.J. Smith, and K.E. Tollefsen. 2009. Effect-directed identification of naphthenic acids as important in vitro xeno-estrogens and anti-androgens in North Sea offshore produced discharges. Environmental Science and Technology 43: 8066-8071.

Trites, M., and S.E. Bayley. 2009. Vegetation communities in continental boreal wetlands along a salinity gradient: implications for oil sands mining reclamation. Aquatic Botany 91: 27-39.

Walker, I.R., S.E. Wilson, and J.P. Smol. 1995. Chironomidae (Diptera): quantitative paleosalinity indicators for lakes of western Canada. Canadian Journal of Fisheries and Aquatic Sciences 52: 950-960.

Whelly, M.P. 1999. Aquatic invertebrates in wetlands of the oil sands region of northeast Alberta, Canada, with emphasis on Chironomidae (Diptera). M.Sc. Thesis, University of Windsor, Windsor, ON, Canada.

Wojnarowicz, P. 2009. Effects of salt interactions in oil sands process waters on *Ceriodaphnia dubia* using an isobolographic approach. B.Sc. Thesis. University of Saskatchewan, Saskatoon, SK, Canada.

Zhang, X., S. Wiseman, H. Yu, H. Liu, J.P. Giesy, and M. Hecker. 2011. Assessing the

toxicity of naphthenic acids using a microbial genome wide live cell reporter array system. Environmental Science & Technology 45: 1984-1991.

CHAPTER II: ABUNDANCE, SIZE, AND GENUS RICHNESS OF CHIRONOMIDAE EMERGING FROM REFERENCE AND OIL SANDS PROCESS-AFFECTED WETLANDS

Introduction

As part of the lease agreement with the Alberta government, oil sands companies must restore land to its original level of productivity before mine closure (FTFC, 1995). This means that reclaimed wetlands on site must be as productive as their reference counterparts. As mentioned in the general introduction, one challenge in the reclamation strategy is utilizing the enormous volumes of fine fluid tailings in the wet landscape option. It is therefore of utmost importance that the OSPM-affected reclaimed wetlands sustain a chironomid community.

Biological Productivity and Food Web Structure

Biological productivity is the accumulation of living tissue over a period of time. It is measured in biomass accumulation per unit time per unit area (Huryn and Benke, 2007). In other words, measuring productivity usually involves measuring the growth rate (i.e., change in biomass), and abundance of a group of organisms. High productivity at the bottom of a food web supports greater productivity in the populations at higher trophic levels. Net primary productivity—productivity of autotrophs -- in wetlands can vary greatly and depends on many factors including light, sediment, nutrient availability, and the hydrology of a wetland, i.e., whether it is largely precipitation fed (low primary productivity) or whether it receives inputs of nutrient rich water (higher primary productivity) (Sharitz & Pennings, 2006). Heterotrophic bacteria are likely the greatest

contributors to biomass in wetlands; bacteria play a fundamental role in nutrient cycling, contributing to the decay of organic matter, and providing food for aquatic, detritivorous invertebrates including chironomids (Boon, 2006). Aquatic invertebrates, such as chironomids, are the most important primary consumers in many wetland ecosystems (Jackson & Fisher, 1986), and thus play a major role in food web structure, linking the microbial and macrophyte compartments to upper trophic levels.

The Importance of Chironomidae

Chironomidae dominate the diets of many predators, including fish, waterfowl, and various terrestrial insectivores (spiders, aerial insectivorous birds, bats, etc.) (Hershey, 1985; Batzer et al., 1993; Barclay, 1985). Their abundance and ubiquity make them especially representative of the aquatic emergent component of food webs (Pascoe et al, 1989). Chironomids make up a large portion of the benthic invertebrate biomass of wetlands in northern Alberta, so their productivity influences the overall productivity of the wetland as a whole. In oil sands lease area wetlands, Chironomidae larvae are predominant. The subfamilies Tanypodinae and Orthocladiinae are among the most common invertebrates in young wetlands and Chironominae among the most common invertebrates in older wetlands (Leonhardt, 2003). Chironomids are often the dominant benthic organisms in disturbed ecosystems; for example, they comprised 98% of the total invertebrate biomass in four brackish, constructed ponds located in England (Rehfisch, 1994).

Quantifying Emergence

Chironomids spend most of their life cycle living in the sediment of rivers, lakes and wetlands, passing through four larval instar stages before they pupate. When ready to transform into an adult, a pupa rises to the water surface, sheds its exoskeleton, and emerges as an adult fly. The cast skin (exuvia) typically remains floating on the surface of the water for 24 h or more. The exuviae therefore provide a quantitative measure of the number of chironomids that are emerging per unit area from a wetland (Switerski et al., 2006). Ferrington et al. (1991) recommended the collection of pupal exuviae as an especially effective way of characterizing chironomid community composition.

Because adult chironomids live for only a few days and typically do not feed (Oliver 1971), larval survival and growth are good predictors of the productivity of a population. In a 10-d bioassay, Liber et al. (1996), reared second to third instar *Chironomus tentans* larvae on six different feeding regimes to determine the relationship between larval growth and subsequent emergence and ovipositing success. Survival was >88% in all feeding treatments. Larval growth significantly differed among all treatments, and was highly correlated with emergence success (R^2=0.96). Although fewer females emerged at the lower feeding regimes and their weights were lower, oviposition success (the ability to lay an egg mass) was not affected and second generation larval growth was not affected by maternal growth rate (Liber et al. 1996). However, Butler and Walter (1992) found that female pupal dry biomass was highly correlated (R^2=0.76) with fecundity (number of eggs). The authors studied the correlation by first collecting a total of 79

mature female pupae from three lakes then weighing and dissecting the eggs from these pupae (Butler & Walter, 1992). Together, these studies indicate that assessing the number and size of emergent pupal chironomids permits one to infer larval growth rate and to predict wetland productivity, and ultimately potential adult fecundity.

Toxicity of Oil Sands Process Water

As discussed previously, fresh oil sands process water (OSPW) is toxic to chironomids (Whelly, 1999). In a 10-d bioassay, the LC_{50} of OSPW from a tailings pond to *Chironomus dilutus* occurred at a dilution of approximately 65% OSPW: 35% reference water (Whelly, 1999). The principal toxic constituent in fresh OSPW is thought to be some component of naphthenic acids (Schramm et al., 2000), which is a complex mixture of carboxylic compounds (Grewer et al. 2010). However, this toxicity is expected to decrease over time as naphthenic acids are readily degraded by microorganisms (Del Rio et al., 2006; Frank et al., 2008; Toor 2012). However, naphthenic acids are not the only toxic component in OSPW. Because the Athabasca oil sand deposit is derived from marine sediment, process water becomes enriched in salts during the extraction process. Thus, OSPW contains elevated concentrations of sodium (~500-700 mg/L), bicarbonate (~470-950 mg/L), chloride (~75- 550 mg/L), and sulphate (~200-300 mg/L) (Allen, 2008) typically resulting in conductivity of 1113 to 2400 uS (MacKinnon & Sethi, 1993). These salts can impair growth and survival of aquatic plants and other organisms. When multivariate statistical analyses were used to separate effects of naphthenic acids and elevated conductivity in OSPW, Leung et al. (2001) found that phytoplankton communities were more strongly affected by high conductivity. *Chironomus plumosus* is unable to osmoregulate and is therefore intolerant of salinities greater than the internal

salinity (Lauer, 1969). Salt stress has also been shown to negatively affect emergence. Bervoets et al. (1996) observed significantly lower emergence of *C. riparius* from high salinity water (12 ppt) than in fresh water. Although most *Chironomus* species appear to be salt-intolerant (Cannings & Scudder, 1978), the genera of Chironomidae are differentially sensitive to salt. For instance, Whelly (1999) found that *Tanytarsus*, *Derotanypus,* and *Cricotopus* were dominant in salty, OSPW-affected wetlands. Of these, only *Derotanypus* was completely absent in low-conductivity reference wetlands, making it an indicator of saline conditions (Whelly, 1999).

Objectives

The objectives of this study were to determine 1) whether OSPW-affected wetlands are as productive as reference wetlands by monitoring emergent pupal Chironomidae abundance and size; 2) if different OSPW-affected wetlands support different chironomid community assemblages than reference wetlands; 3) if particular assemblages of emergent chironomid genera can be accounted for by specific physicochemical factors.

Methods

Study Area and Wetlands

The study wetlands were all located in the Athabasca oil sands region north of Fort McMurray, AB. Four OSPM-affected wetlands were studied - “4-m CT”, “Mike’s Pond”, “Natural Wetland”, and “Test Pond 9”. Five reference wetlands were used in this study named “High Sulphate”, “North West Interceptor Ditch” or “NWID”, “V-Notch Weir”, “Shallow Wetland”, and “Suncor Pond 5 Wetland”. The details of these wetlands and dates sampled are described in Table 2.1.

Table 2.1: Description of wetlands used in this study.

Wetland Name	Class	Substrate	Organic carbon class	Water	Age in 2010 (years)	Location (UTM Coordinates)	Property	Sampling Dates (dd/mm/yyyy) – Dates marked (*) used in analyses
4-m CT	OSPW	Four meters of consolidated tailings with small shallow areas containing peat.	Rich	Suncor Oil sands process water.	13	0467670, 6316509	Suncor Energy	06/07/2009*, 17/06/2010, 24/06/2010.
Mike's Pond	OSPW	Clay.	Poor	Syncrude Consolidated tailings oil sands process water.	18	0458752, 6330013	Syncrude Canada Ltd.	23/06/2009*.
Natural Wetland	OSPW	Sand and 15 cm peat mineral mix.	Rich	Suncor Oil sands process water (seepage)	$\geq$24	0468962, 6315305	Suncor Energy	25/07/2009*, 17/06/2010, 24/06/2009.
Test Pond 9	OSPW	Clay.	Poor	Oil sands process water	17	0458007, 6326993	Syncrude Canada Ltd	06/07/2009*, 16/06/2010, 24/06/2010, 29/06/2010.

High Sulphate	Constructed Reference	Sodic overburden and 15 cm peat.	Rich	Fresh (but may be contaminated by seepage from lean oil sands	26	0466395, 6317235	Off-site	25/07/2009*, 17/06/2010, 25/06/2010.
North West Interceptor Ditch (NWID)	Constructed Reference	Sodic overburden	Rich	Fresh	18	0458147, 6330045	Syncrude Canada Ltd.	23/06/2009*.
Shallow Wetland	Constructed Reference	Sodic overburden	Poor	Fresh	18	0458126, 6326649	Syncrude Canada Ltd.	06/07/2009*, 16/06/2010, 23/06/2010, 29/06/2010.
V-Notch Weir	Constructed Reference	Peat	Rich	Fresh	10	0467653, 6316221	Suncor Energy	24/06/2010*.
Pond 5 Wetland	Reference that developed opportunist-ically	Sodic overburden	Poor	Fresh	<7	0466472, 6319087	Suncor Energy	06/07/2009*.

Field Methods

Pupal emergence traps were built from polyvinyl chloride (PVC) piping (26 mm ID x 3 mm) bent into a circle to create floating hoops 30 cm in diameter (Swisterski et al., 2006). Acetate sheets (11 cm wide) were stapled around the circumference of the hoop to provide a barrier against the wind and waves. Each wetland was sectioned radially into five equal areas, and one hoop was placed in an open expanse of water at the boundary between the emergent and submergent vegetation zone of each area at a water depth of approximately 40 cm. Three 25-cm long bamboo stakes driven into the sediment were used to secure each hoop in place (Fig. 2.1, Fig 2.2). Then, a small aquarium net was used to clear the surface of the water of any debris. Material floating on the water surface within the area circumscribed by the hoop was collected after 24 h by gently sweeping the surface of the water with a 0.4-mm mesh aquarium net. The net was inverted into a jar, and 70% ethanol was poured through the net and into the jar. The net was inspected for any remaining exuviae adhering to the mesh, and then more ethanol was poured into the jar. At the time of collection, water pH, conductivity, temperature, and salinity were measured with a YSI model 556MPS meter. Preliminary data indicated that there was very little within-wetland variability in these measures. Consequently, only one set of measurements per wetland per sampling date was taken.

Figure 2.1: Photo of floating hoop emergence traps (Photo by K. Jurkowski, 2009) Hoop diameter is 30 cm.

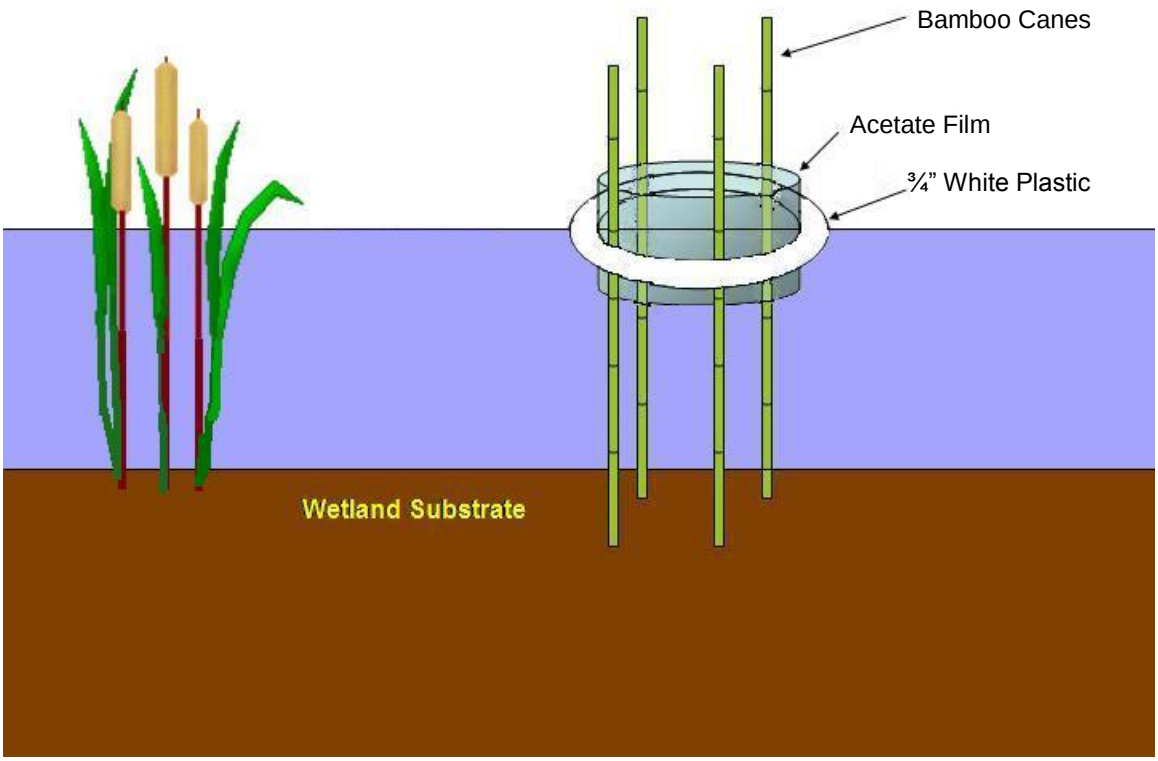

Figure 2.2: Schematic diagram of floating hoop emergence trap (from Swisterski et al., 2006).

Water samples were also taken for later analysis of NA, major ions, dissolved organic carbon, and other water quality characteristics. Analyses were performed by the Syncrude Canada, Ltd. analytical lab. In cases where current data were not available, 2008 data was used instead (see appendix A for all water quality results). If rain occurred during any 24-h sampling period, exuviae were not collected, and hoops were deployed again until dry weather conditions were met.

Laboratory Methods

When samples were brought back to the lab, jar contents were poured through a small 250-µm sieve and emptied into a Petri plate containing water. Exuviae were then counted and sorted beneath a dissecting microscope, placed in a petri dish with glycerin to reduce movement, and photographed using a mounted digital camera. ImageTool 3.0© software (UTHSCSA, San Antonio, TX) was then used to digitally determine the length of each exuvia from the images.

Exuviae were mounted on labeled glass microscope slides with a drop of CMC-9AF mounting medium (Masters Chemical Co., Wood Dale, IL), covered with a cover slip, allowed to dry, and the edges of the cover slip were sealed with nail polish. Pupae were identified to genus or species where possible using the keys of Wiederholm (1986).

Data Analysis

Statistical analyses were performed using STATISTICA 7 software (Statsoft, Inc., Tulsa, OK). Differences in mean abundance and mean pupal length between wetland classes (OSPM vs. reference) were compared by one-way ANOVA. Data were log transformed to meet the assumption of equal variances. A type I error value of 0.05 was chosen to determine whether wetland classes were significantly different. The influence of various water quality parameters (dissolved oxygen, conductivity, salinity, temperature, naphthenic acid concentration, and dissolved organic carbon) on mean abundance and mean length was estimated using forward stepwise multiple regression. A stepwise procedure was used to distinguish among the effects of independent variables that tended to be correlated among themselves (e.g., conductivity and NA concentration).

For diversity data, richness was compared between wetland classes using one-way ANOVA. The relative abundance of each genus within each wetland was expressed as a percentage and transformed to octaves ($\log_2 +1$) to reduce the dominance of the most common taxa (Gauch 1972). Genera that occurred in two or fewer wetlands were discarded from further analysis. The transformed relative abundance data were then analysed by Principal Components Analysis (PCA) in order to reduce the number of variables/genera to a few independent principal components (e.g. Leonhardt, 2003; Barr, 2009). Components were rotated using varimax raw rotation. Multiple regression analyses were then performed to determine relationships between the principal

component scores of the chironomid emergence data (dependent variables) and water quality parameters (temperature, dissolved oxygen, naphthenic acid concentration, salinity, and conductivity – independent variables).

Results

Abundance of Exuviae

On average, about twice as many exuviae per hoop were collected from OSPM wetlands than from reference wetlands (mean ± SE was 10.4 ± 6.1 (n=4) for OSPM wetlands vs. 5.1 ±1.9 for reference wetlands (n=5) per 732 cm^2 hoop). The difference was almost entirely attributable to the large numbers of exuviae collected in Test Pond 9. Because there was marked among-wetland variation in abundance (Fig 2.3) the difference was not statistically significant (p=0.44). There were no significant differences in abundance among all nine wetlands (p=0.13; n=9; one-way ANOVA, Fig. 2.4).

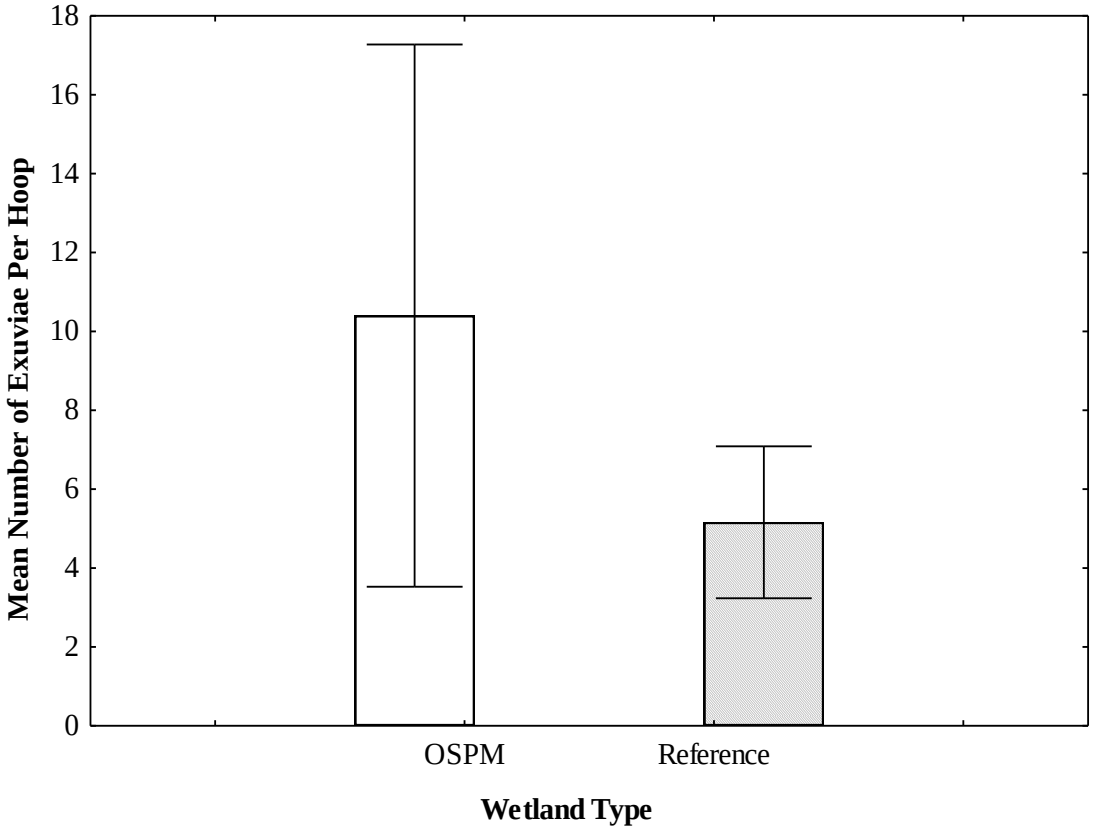

Figure 2.3: Mean (± SE) number of exuviae per 732 cm2 hoop for 4 OSPM-affected wetlands and 5 reference wetlands.

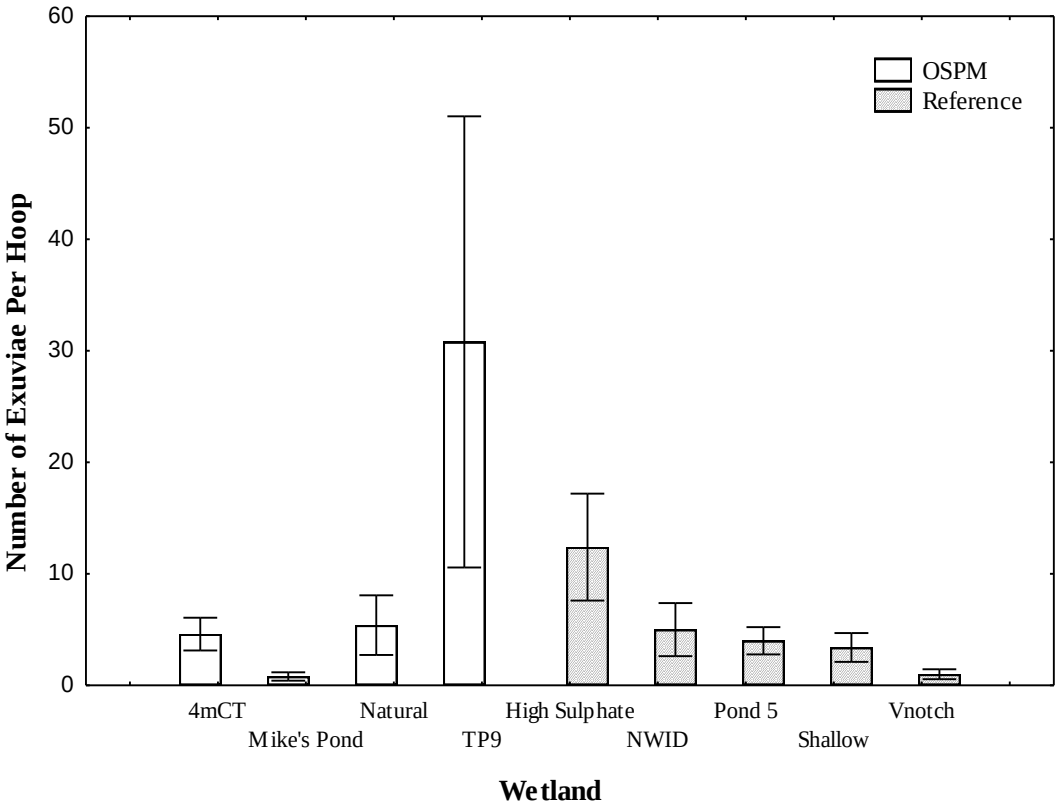

Figure 2.4: Mean (± SE; n=5) number of exuviae per 732 cm^2 hoop in study wetlands.

Size of Exuviae

There was no significant difference in mean exuviae length between OSPM wetlands and reference wetlands ($p=0.13$; n=4 OSPM wetlands and n=5 reference wetlands; one-way ANOVA; Fig.2.5). However, there was significant heterogeneity in mean exuvia length among wetlands ($p<0.001$; n=9; one-way ANOVA; Fig. 2.6).

Richness

There was no significant difference between OSPM and reference wetlands in richness of genera ($p>0.05$; n=4 OSPM wetlands and 5 reference wetlands; one-way ANOVA; Fig. 2.7).

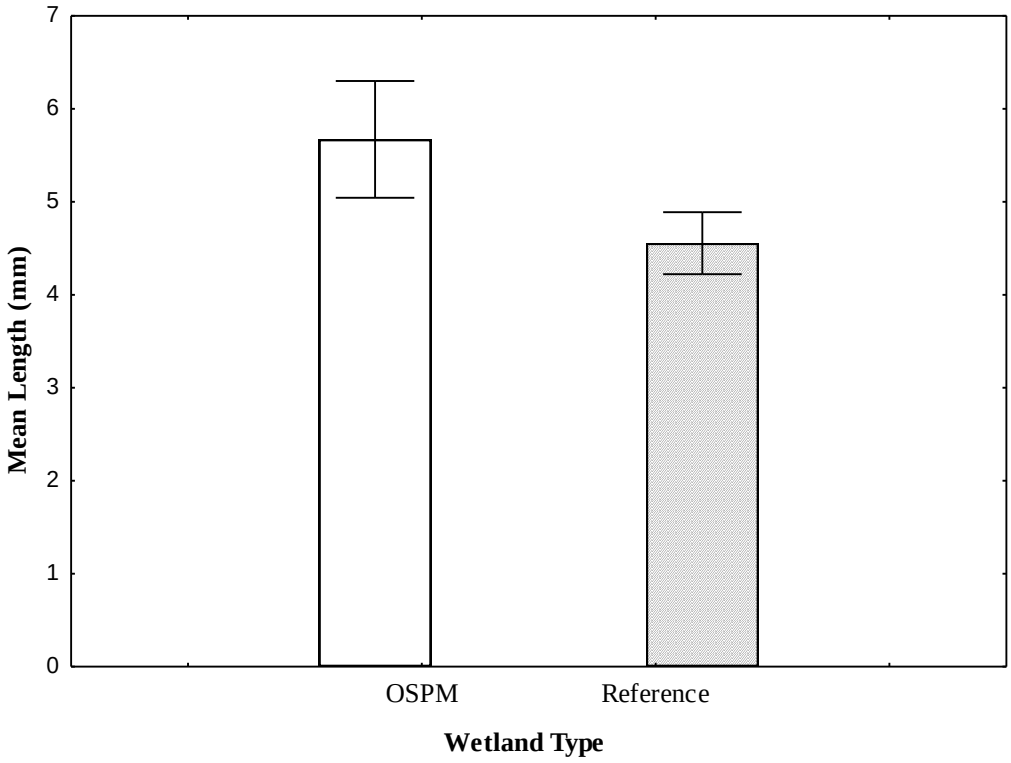

Figure 2.5: Mean exuviae length (± 1 SE) for four OSPM-affected wetlands and five reference wetlands (p=0.13).

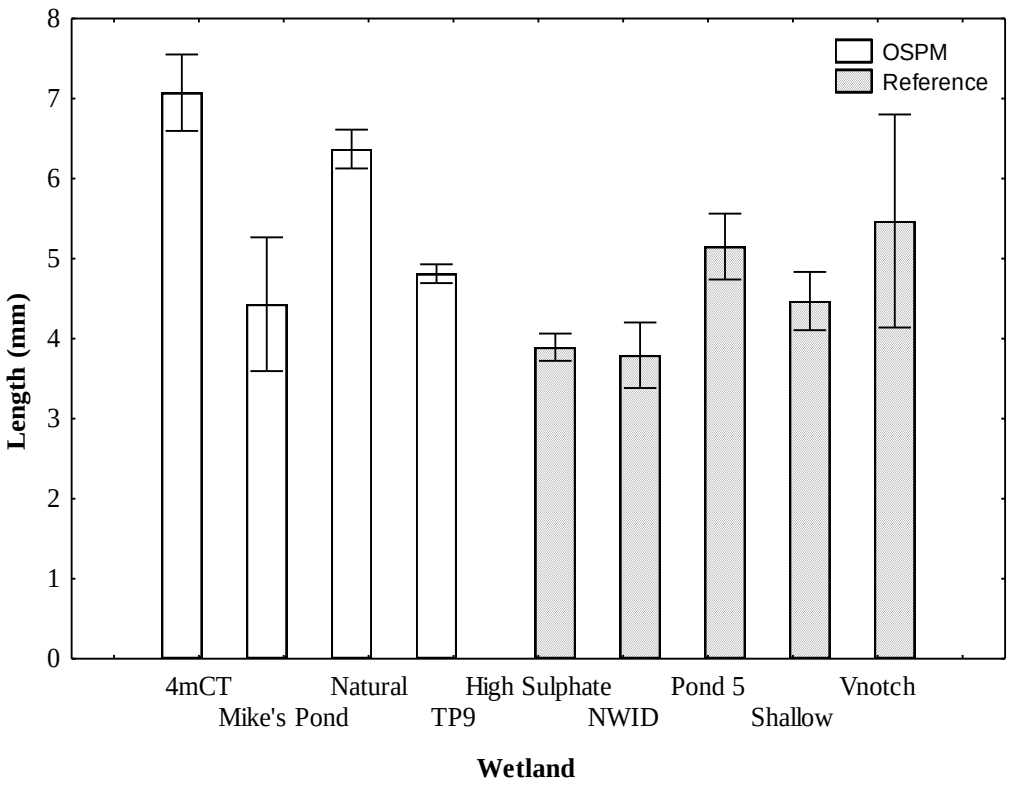

Figure 2.6: Mean exuviae length (± 1 SE) graphed by wetland.

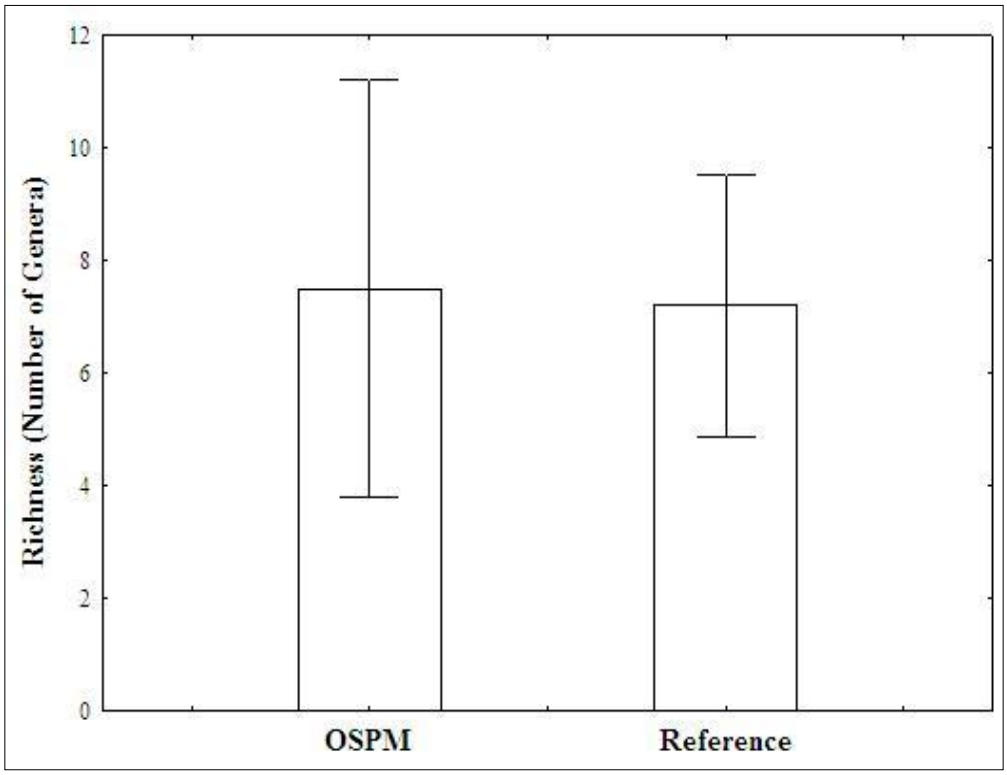

Figure 2.7: Genus richness in OSPM and reference wetlands.

Water Chemistry

Water chemistry measured at the time of sample collection included dissolved oxygen (mg/L), temperature (˚C), conductivity (μS/cm), and salinity (ppt). Naphthenic acid concentrations (mg/L) and dissolved organic carbon (mg/L) values from previous years were used to supplement the data set. Organic level (peat or no peat) was included as a categorical variable in the data set (present (1) or absent (0), depending on whether or not a layer was placed over the inorganic sediments during wetland construction). Water quality data are summarized in Appendix A.

The forward stepwise regression of the water chemistry data on the mean abundance of exuviae revealed no significant relationships (n=9, p=0.23, R^2= 0.20, with dissolved oxygen as the only step). However, the forward stepwise regression of water chemistry data on mean exuviae length revealed a significant relationship with dissolved organic carbon (n=9, p=0.015, R^2=0.67).

Composition of Emergent Chironomidae

Thirty genera were identified from among the 300+ exuviae examined from the nine study wetlands (Appendix B). Twenty-two genera were relatively rare, occurring in only one or two wetlands. Common genera (those that were present in three or more wetlands) belonged to the subfamlies Tanypodinae (*Ablabesmyia*, *Guttipelopia*,

Neozavrelia, *Procladius*, *Tanypus),* Chironominae (*Einfeldia*, and *Tanytarsus)* and Orthocladiinae (*Paracricotopus*,).

Covariation in relative abundances among the 8 common genera in the study wetlands was summarized by principal component analysis (PCA) of the octave-transformed values. Four principal components (PCs) with eigenvalues >1.0 were derived and, together, they explained 84% of the variance in the data set (Table 2.2). The relative abundance of *Einfeldia* was positively correlated with PC-I whereas relative abundances of *Neozavrelia* and *Paracricotopus* were negatively correlated with this principal component (Table 2.3). Relative abundances of *Ablabesmyia* and *Tanytarsus* were positively and negatively correlated with values of PC-II, respectively. The relative abundances of both *Procladius* and *Tanypus* were positively correlated with PC-III, whereas *Guttipelopia* relative abundance was negatively associated with PC-IV. Scatterplots of where each wetland falls with respect to each PC indicate that there was no obvious grouping of wetland type with respect to community composition (Figures 2.8 and 2.9).

Forward stepwise regression was performed on the PC scores for each of the four principlal compoents using water quality data (Table 2.4). Scores of PC-I were significantly negatively correlated with temperature ($p= 0.002$) and significantly positively correlated with dissolved organic carbon ($p= 0.023$). Values of PC-II were

significantly negatively correlated with dissolved oxygen ($p= 0.034$) and conductivity ($p= 0.045$). The scores of PC-III and PC-IV were not significantly correlated with any of the water quality parameters.

Table 2.2: Descriptions of four principal components derived log percent abundance of eight dominant emergent chironomid genera.

	Eigenvalue	Variance Explained (%)	Cumulative Variance Explained (%)
PCI	2.559	32.0	32.0
PCII	1.721	21.5	53.5
PCIII	1.252	15.7	69.1
PCIV	1.162	14.5	83.7

Table 2.3: PC loadings for each of the eight dominant genera. Loadings greater than |0.6| are bold faced.

Genus	PCI	PCII	PCIII	PCIV
Neozavrelia	**-0.87**	0.03	-0.02	0.01
Paracricotopus	**-0.68**	0.36	-0.25	0.01
Einfeldia	**0.66**	0.11	0.17	0.58
Ablabesmyia	-0.28	**0.89**	-0.05	0.15
Tanytarsus	-0.51	**-0.74**	-0.01	0.12
Procladius	0.01	-0.13	**0.96**	-0.17
Tanypus	0.26	0.19	**0.74**	0.49
Guttipelopia	0.04	-0.02	0.08	**-0.94**
Explained Variance	2.07	1.53	1.58	1.52
% of Total Variance Explained	25.8	19.1	19.7	19.0

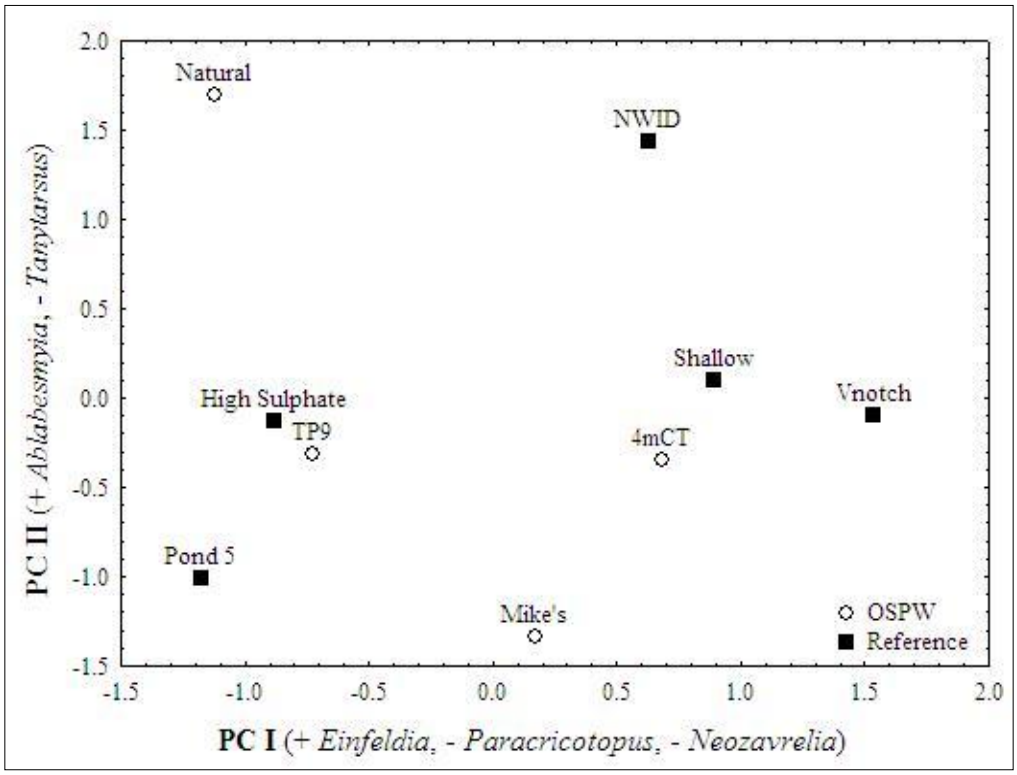

Figure 2.8: Wetlands graphed according to PCI and PCII scores.

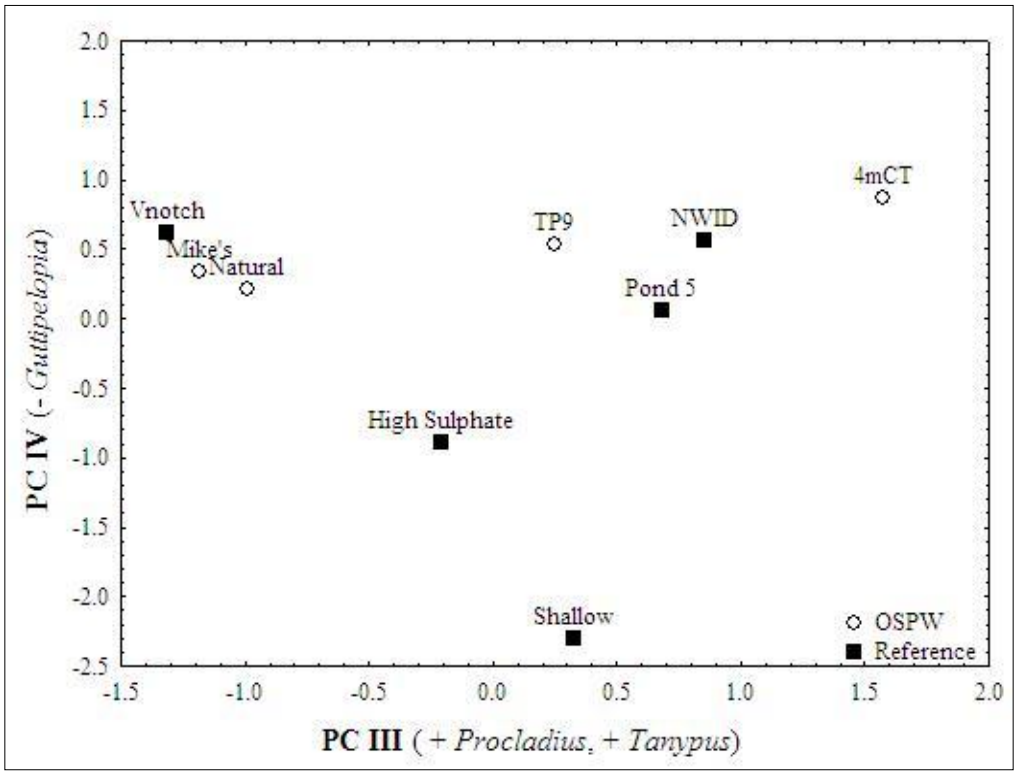

Figure 2.9: Wetlands graphed according to PC III and PCIV scores.

Table 2.4: Results of a forward stepwise multiple regression of conductivity, salinity, naphthenic acid concentration, dissolved oxygen (D.O.), dissolved organic carbon (D.O.C.), and temperature on PCA scores. Statistically significant p-values (<0.05) are indicated by an asterisk (*).

PC-I

R^2= 0.898, N= 9, p= 0.006

	Regression Coefficient	S.E. of Regression Coefficient	Partial R^2	t-value	p-value
Intercept	10.06	1.642		6.127	0.002*
Temperature (C)	-0.497	0.083	0.654	-6.035	0.002*
Organic Level	0.965	0.296	0.137	3.256	0.023*
DOC (mg/L)	-0.015	0.006	0.107	-2.301	0.070

PC-II

R^2= 0.798, N= 9, p= 0.034

	Regression Coefficient	S.E. of Regression Coefficient	Partial R^2	t-value	p-value
Intercept	1.723	0.793		2.172	0.082
DO (mg/L)	-0.218	0.076	0.510	-2.881	0.034*
Conductivity	-0.001	0.001	0.179	-2.650	0.045*
DOC (mg/L)	0.021	0.013	0.109	1.648	0.160

Discussion

Exuvial abundance and size were similar between wetland types, indicating that overall production was equivalent among wetlands, at least within the areas and at the times sampled. The abundance of chironomids emerging from OSPW wetlands was much more variable than abundance of chironomids emerging from reference wetlands but this is largely due to Test Pond 9 data. Swisterski et al. (2006) used the same hoops and found a mean (±S.E.) of 37 (± 5) chironomid pupal exuviae per hoop across 18 samples, which exceeds the mean of this study (7.5 ± 3.0); however, she studied only one wetland (Natural Wetland). The chironomids that emerged from reference wetlands tended to be smaller than chironomids that emerged from OSPW wetlands, but the trend was not statistically significant. The apparent similarity in productivity, as measured by abundance and size at time of emergence, between OSPW wetlands and reference wetlands suggests that overall productivity was similar between wetland classes, which implies similarity at other stages of the life cycle. Adult fecundity is highly correlated with pupal size (Butler and Walker 1992) and larval growth rate correlates with emergence success (Liber et al. 1996). One factor that may explain the lack of relationship between wetland type and emergence is their relatively older age. The OSPW wetlands were 13 to 24 years old. Perhaps toxicity of the OSPW with which the reclaimed wetlands were originally filled has decreased over time, or the chironomids present are tolerant of the water quality in those wetlands. Leonhardt (2003) predicted that community composition of reference and OSPW-affected wetlands would become

indistinguishable after approximately 18 years. However, water quality data suggests that the conductivity has not changed much over time. The conductivities of the OSPW wetlands, all of which were >10 years old remained high. Another explanation for the high variability is that the limited dudration of sampling was not representative of emergence over the season. Different species emerge at different times throughout the year, so a 24-h emergence trap collection provides only a small snapshot of the community emerging and may be subject to bias in size and abundance of chironomids. Assessing emergence over the entire season could indicate how the timing of emergence might vary with respect to wetland characteristics.

In general, emergent chironomid abundance is positively correlated with the volume of submerged aquatic vegetation (e.g. Gerking 1957, Darby 1962, Krul 1970). Pondweed (*Potamogeton pectinatus*), in particular, offers a habitat with greater surface area for colonization than emergent vegetation such as cattail (*Typha latifolia*) (Wrubleski 1987). By observation, wetlands in this study with very little submerged aquatic vegetation, such as Mike's Pond, supported fewer emergent chironomids than wetlands with a greater abundance of submerged aquatic vegetation such as Test Pond 9 and High Sulphate. When analyzing water chemistry data that may have influenced chironomid pupal size and abundance, the only significant relationship detected was between mean length (mm) and dissolved organic carbon (mg/L). This may reflect a difference food quality. Chironomids predominantly feed on detritus, and high dissolved organic carbon can

result in greater bacterial biomass, which is an important, nutritious component of detritus (Boon, 2006; Daly, 2007). Therefore, it is logical that dissolved organic carbon and chironomid size are correlated with one another.

The PCA and forward stepwise multiple regressions on the PCA scores (dependent variables) using the water quality parameters (independent variables) indicated that the relative abundance of *Einfeldia*, as summarized by PC1, was negatively associated with temperature and positively associated with organic level (peat presence or absence); the presence of *Neozavrelia*, also summarized by PC1, was positively associated with temperature and negatively associated with organic level; relative abundance of *Paracricotopus*, the third genus summarized by PC1, was positively associated with temperature and negatively associated with organic level; *Ablabesmyia*, as summarized by PCII, had negative relationships with both dissolved oxygen and conductivity; and finally, the relative abundance of *Tanytarsus*, as summarized by PCII, was positively associated with both dissolved oxygen and conductivity. There were no consistent results regarding community assemblages that were specific to one wetland class (OSPW versus reference) or the other.

Certain assemblages of Chironomidae are usually so closely associated with conductivity that they are used to infer lake history (Walker et al. 1995). Walker et al. (1995) analyzed the chironomid community in 86 British Columbia lakes of varying salinities. *Einfeldia*

are burrowers, so the presence of organic material could make the habitat at the sediment level more favourable—this could explain the positive relationship we see with organic level. Relative abundances of both *Neozavrelia* and *Paracricotopus* were negatively associated with organic level. *Ablabesmyia* is a predacious chironomid and since it does not feed on detritus directly, as the other common genera do, this could explain the lack of relationship with organic level. Only two of the common genera exhibited a significant relationship with conductivity; *Ablabesmyia* relative abundance was negatively related to conductivity, and *Tanytarsus* relative abundance was positively related. This indicates that *Tanytarsus* is a salt-tolerant genus whereas *Ablabesmyia* is salt-intolerant. Similar relationships were found between these two genera and dissolved oxygen; *Ablabesmyia* had a negative relationship with dissolved oxygen levels whereas *Tanytarsus* exhibited a positive relationship. Whelly (1999) found that *Tanytarsus* was a common genus in both OSPW and reference wetlands. However, *Tanytarsus* was the only predominant genus found in common between the study by Whelly (1999) and the present study. It is noteworthy that *Derotanypus*, whose occurrence is strongly associated with elevated salinity (Walker et al., 1995; Whelly, 1999) was not collected on the sampling dates of this study. Clearly, examination of trends throughout the emergence season would strengthen the conclusions of this study.

Conclusion

It appears that reclaimed wetlands affected by OSPW and other process materials do not significantly differ in the abundance of chironomids emerging or the size at time of

emergence over the time period sampled. Reference wetland waters supported chironomids with smaller pupae than did OSPW wetlands. This may be a function of the species present. Dissolved organic carbon concentration was significantly correlated with mean length of chironomid pupae; this is likely related to better food quality in wetlands with high dissolved organic carbon. Differences in chironomid community among wetlands did not correspond to wetland class (OSPW versus reference wetlands), but the relative abundances of certain genera tended to be correlated with some water quality parameters; of particular interest was that *Ablabesmyia* was relatively uncommon in wetlands with high conductivity whereas *Tanytarsus* were most abundant in high conductivity conditions.

References

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

Batzer, D. P., M. McGee, V.H. Resh, and R.R. Smith. 1993. Characteristics of invertebrates consumed by mallards and prey response to wetland flooding schedules. Wetlands 13: 41-49.

Barclay, R.M.R. 1985. Long- versus short-range foraging strategies of hoary (*Lasiurus cinereus*) and silver-haired (*Lasionycteris noctivagans*) bats and the consequences for prey selection. Canadian Journal of Zoology 63: 2507-2515.

Barr, L. 2009. Influence of consolidated mine tailings and oil sands process water on colonization potential of aquatic invertebrates of constructed wetlands in the Athabasca oil sands, Alberta. M.Sc. Thesis, University of Windsor, Windsor Ontario.

Bervoets, L., C. Wils, and R. Verheyen. 1996. Tolerance of *Chironomus riparius* larvae (Diptera: Chironomidae) to salinity. Bulletin of Environmental Contamination and Toxicology 57: 829-835.

Boon, P. I. 2006. Biogeochemistry and bacterial ecology of hydrologically dynamic wetlands. pages 125-126 *In* Ecology of Freshwater and Estuarine Wetlands edited by Batzer, D.P., and Sharitz, R.R. University of California Press.

Butler, M.G., and L. Walker. 1992. Fecundity, reproductive effort, and pupal size in the profundal midge *Chironomus cucini* (Diptera: Chironomidae). Netherlands Journal of Aquatic Ecology 26: 263-267.

Cannings, R.A., and G.G.E. Scudder.1978. The littoral Chironomidae (Diptera) of saline lakes in central British Columbia. Canadian Journal of Zoology 56: 1144-1155.

Daly, C. 2007. Carbon sources, microbial community production, and respiration in constructed wetlands of the Alberta, Canada oil sands mining area. M.Sc. Thesis. University of Windsor, Windsor, ON.

Darby, R.E. 1962. Midges associated with California rice fields, with special reference to their ecology (Diptera: Chironomidae). Hilgardia 32: 1-206.

Del Rio, L. F., A.K.M. Hadwin, L.J. Pinto, M.D. MacKinnon, and M.M. Moore. 2006.

Degradation of naphthenic acids by sediment micro-organisms. Journal of Applied Microbiology 101: 1049-1061.

Ferrington, L.C., M. Blackwood, and C.A. Wright. 1991. A protocol for using surface-floating pupal exuviae of Chironomidae for rapid bioassessment of changing water quality. Sediment and Stream Water Quality in a Changing Environment: Trends and Explanation 203: 181-190.

Frank, R. A., R. Kavanagh, B.K. Burnison, G. Arsenault, J.V. Headley, K.M. Peru, G. Van Der Kraak, and K.R. Solomon. 2008. Toxicity assessment of collected fractions from an extracted naphthenic acid mixture. Chemosphere 72: 1309-1314.

FTFC (Fine Tailings Fundamentals Consortium). 1995. "Volume II: Fine Tails and Process Water Reclamation" In: Advances in Oil Sands Tailings Research, Alberta Department of Energy, Oil Sands and Research Division, Edmonton, Alberta.

Gerking, S.D. 1957. A method of sampling the littoral macrofauna and its application. Ecology 38: 219-226.

Hershey, A. 1985. Effects of predatory sculpin on the chironomid communities in an arctic lake. Ecology 66: 1131-1138.

Jackson, J.K., and S.G. Fisher. 1986. Secondary production, emergence, and export of aquatic insects of a sonoran desert stream. Ecology 67: 629-638.

Lauer, G.J. 1969. Osmotic regulation of *Tanypus nubifer, Chironomus plumosus*, and *Enallagma clausum* in various concentrations of saline lake water. Physiological Zoology 42: 381-387.

Leonhardt, C. L. 2003. Zoobenthic succession in constructed wetlands of the Fort McMurray oil sands region: developing a measure of zoobenthic recovery. M.Sc. Thesis, University of Windsor, Windsor, ON.

Leung, S.S., M.D. MacKinnon, and R.E.H. Smith. 2001. Aquatic reclamation in the Athabasca, Canada, oil sands: naphthenate and salt effects on phytoplankton communities. Environmental Toxicology and Chemistry 20: 1532-1543.

Liber, K., D.J. Call, T.D. Dawson, F.W. Whiteman, and T. Dillon. 1996. Effects of *Chironomus tentans* larval growth retardation on adult emergence and ovipositing success: implications for interpreting freshwater sediment bioassays. Hydrobiologia 323: 155-167.

Krull, J.N. 1970. Aquatic plant- macroinvertebrate associations and waterfowl. Journal of Wildlife Management 34: 707-718.

MacKinnon, M.D., and A. Sethi. 1993. A comparison of the physical and chemical properties of the tailings ponds at the Syncrude and Suncor oil sands plants. *In* Proceedings of Our Petroleum Future Conference, Alberta Oil Sands Technology and Research Authority (AOSTRA), Edmonton, Alta.

Pascoe, D., K.A. Williams, and D.W.J. Green. 1989. Chronic toxicity of cadmium to *Chironomus riparius* Meigen -- effects upon larval development and adult emergence. Hydrobiologia 175: 109-115.

Rehfisch, M. M. 1994. Man-made lagoons and how their attractiveness to waders might be increased by manipulating the biomass of an insect benthos. Journal of Applied Ecology 31: 383-401.

Schramm, L.L., E.N. Stasiuk, and M.D. MacKinnon. 2000. Surfactants in Athabasca oil sands slurry conditioning, flotation recovery, and tailings processes. pp. 365-430 In: Schramm, L.L. (Ed.),Surfactants: Fundamentals and Applications in the Petroleum Industry. Cambridge University Press, Cambridge,

Sharitz, R. R., and S.C. Pennings. 2006. Development of wetland plant communities. Pp. 125-126 *In*: Batzer, D.P. and R.R. Sharitz (eds.) Ecology of Freshwater and Estuarine Wetlands. University of California Press, Berkeley, California, USA.

Slama, C. 2010. Sediment oxygen demand of wetlands in the oil sands region of North-eastern Alberta. M.Sc. Thesis, University of Windsor, Windsor, ON

Swisterski, A., Ciborowski, J.J.H., Wytrykush, C.M., and Beierling, C. 2006. A novel approach to assess chironomid (Diptera: Chironomidae) community composition in the Athabasca oil sands region. Proceedings of the 32nd Annual Toxicity Workshop, Waterloo, ON. October 2005. Canadian Technical Report of Fisheries and Aquatic Science. 2617: 43.

Toor, N.S. 2012. Degradation and aquatic toxicity of oil sands naphthenic acids using simulated wetlands. Ph.D. Dissertation.,University of Saskatchewan, Saskatoon, SK.

Walker, I.R., S.E. Wilson, and J.P. Smol. 1995. Chironomidae (Diptera): quantitative paleosalinity indicators for lakes of western Canada. Canadian Journal of Fisheries and Aquatic Sciences 52: 950-960.

Whelly, M.P. 1999. Aquatic invertebrates in wetlands of the oil sands region of northeast

Alberta, Canada, with emphasis on Chironomidae (Diptera). M.Sc. Thesis, University of Windsor, Windsor, ON.

Wiederholm, T. 1986. Chironomidae of the holarctic region: part II: pupae. Entomologica Scandinavica, Supplement No. 28.

Wrubleski, D.A. 1987. Chironomidae (Diptera) of peatlands and marshes in Canada. Memoirs of the Entomological Society of Canada 140: 141-161.

CHAPTER III

GROWTH RATE AND SURVIVAL OF *CHIRONOMUS RIPARIUS* WHEN EXPOSED TO OIL SANDS PROCESS-AFFECTED WETLAND WATER IN A 10-D BIOASSAY

Introduction

Chironomids play an important role in ecosystems by providing food for higher trophic level organisms (Batzer et al., 1993) and by linking terrestrial and aquatic components of the food web. They are abundant and ubiquitous in water bodies, making them a highly applicable study group (Pascoe et al. 1989). In this study, *Chironomus riparius* larvae were used to assess the interaction of two key toxicants found in oil sands process waters—naphthenic acids and salts—and their individual and combined effects on larval biomass and survival.

The Use of Field Level Bioassays

Laboratory bioassays are useful in assessing toxicity because they are controlled, i.e., responses to the variable of interest are not confounded by the many factors that influence growth and survival under field conditions. However, results from a laboratory bioassay may not be fully transferable to the field precisely because natural variability in factors such as temperature, dissolved organic carbon, environmental heterogeneity, and the effects of other species may alter the organism's response. Whole-effluent toxicity (WET) testing provides a holistic, environmentally-realistic test that can integrate many

interactions of contaminants (Chapman, 2000). This makes whole effluent toxicity testing especially appropriate for determining toxicity in the wetlands of the oil sands region.

Wetland Reclamation in the Athabasca Oil Sands

As part of the lease agreement with the Alberta government, all leased land must be functionally restored to a level of productivity equivalent to what it was before mining took place (FTFC, 1995). Therefore, terrestrial and aquatic reclamation strategies have become an important-- and necessary-- stage of mine closure. Due to the nature of the bitumen extraction process, large volumes of fluid tailings material are generated by the oil sands refining process. Since wetland area comprised 20-40% of the pre-mining landscape (FTFC, 1995), constructing wetlands that incorporate this tailings material and the associated mine process water has become a major goal of landscape reclamation. With the goal of reclamation research in mind, Syncrude Canada Ltd. and Suncor Energy both constructed a series of demonstration OSPM (oil sands process material)-affected and reference wetlands. Natural wetlands that formed opportunistically in the area are also used in this study.

Two components of OSPW can be particularly toxic to aquatic biota—naphthenic acids and salts (Allen 2008, Leung et al. 2001), both of which occur naturally in the oil sands ore but become concentrated by the mining process. Naphthenic acids are a complex mixture of alkyl-substituted carboxylic acids (Holowenko et al., 2002). Due to their surfactant nature, naphthenic acids are expected to cause respiratory stress to chironomids, whereas high ionic concentrations may affect osmoregulation. There is

evidence, however, that there may be an antagonistic relationship between these two compounds (Turcotte et al., 2009). Although studies on the interaction between naphthenic acids and salts are scarce, an antagonistic interaction between other surfactants and salts has been documented in bluegill (Hokanson & Smith, 1971) and goldfish (Gafa Riv, 1974). The reason for this is likely due to precipitation of the surfactant out of solution caused by interactions with bivalent cations (e.g. Ca^{2+} and Mg^{2+}), which causes a reduction in bioavailability (Verge et al. 2001).

Whelly (1999) conducted a 10-d dilution series bioassay of OSPW with several populations of *Chironomus*. He found that fresh OSPW reduced survival and growth. The LC_{50}, that is, the lethal concentration for 50% of organisms, was found to be approximately 65% OSPW (Whelly, 1999), which would correspond to an NA concentration of approximately 32 mg/L given the concentrations of the source process water around the time of the study (Holowenko et al., 2002).

The majority of OSPW or OSPM-affected wetlands in the area contain both NA and salts. As wetland waters age, naphthenic acid concentrations tend to decline over time due to microbial degradation (Del Rio et al. 2006; Frank et al. 2008), but salinity should remain relatively unchanged in the closed systems of constructed study wetlands.

The goal of this study was to determine what toxic effects, if any, are associated with naphthenic acids and salinity. Survivorship and growth rate of *C. riparius* in wetland water that contains a range of naphthenic acids (NAs) and conductivities was studied.

Wetland waters used as replicates in toxicity bioassays as opposed to a dilution series is a novel and highly applicable approach to the question at hand.

It was expected that there would be significant negative relationships between survival and conductivity, biomass and conductivity, survival and NA concentration, and biomass and NA concentration. Both salts and NAs were expected to pose lethal and sublethal toxicity to *C. riparius* larvae, resulting in lower survival and lower final biomass due to reduced growth rate. However, salts and NAs were expected to react antagonistically and yield higher survival and biomass than if their individual effects were added together. This was expected to be reflected in a positive interaction term in the multiple regression on conductivity and NA concentration with both biomass and survival as dependent variables. This antagonism between salts and NAs has been demonstrated in preliminary studies on *Ceriodaphnia dubia* by Turcotte et al. (2009). Other surfactants have also been shown to interact antagonistically with salts (Hokanson & Smith, 1971; Gafa Riv, 1974).

Methods

Organism Origin and Maintenance

Chironomus riparius eggs were provided from a culture maintained at the National Water Research Institute of Environment Canada (Burlington, ON). One set of eggs was used immediately for studies conducted in July 2009. Another set of eggs was used to generate a self-sustaining culture maintained at the University of Windsor following Environment Canada protocols. Studies conducted during July 2010 used first instar larvae hatching from egg masses derived from the University of Windsor daughter culture.

Study Wetlands

Twelve wetlands were selected for this experiment. Wetlands were chosen to represent a broad range of combinations of naphthenic acids (NA) and conductivities. Conductivity was chosen over Total Dissolved Solids (TDS) in this case because insufficient data were available to characterise the concentration of major ions prior to the experiment. Each wetland's class, age, conductivity, concentration of NA, major ions (when data were available), and UTM coordinates are listed in Table 3.1.

Table 3.1: Descriptions of the study wetlands.

Wetland Name	Class	Conductivity (µS/cm)	NA (mg/L)	Lease Property	Geographic Coordinates (UTM)	Major Cation(s) (>100 mg/L)	Major Anion(s) (>100 mg/L)
Recycle Pond	OSPW and OSPM	3430	80[c]	Syncrude	0460010, 6323321	Not Available	Not Available
Trench 4	OSPW	795	22.0 [b]	Suncor	0469261, 6315323	Na^+ [(b)]	HCO_3^-, SO_4^{2-} [(b)]
Dyke 4 Reservoir	OSPW	855	23.0 [b]	Suncor	0467776, 6316302	Na^+, Ca^{2+} [(b)]	SO_4^{2-}, HCO_3^- [(b)]
4-m CT	OSPM and OSPW	4523	41 [b]	Suncor	0467670, 6316509	Na^+, Mg^{2+} [(b)]	HCO_3^-, SO_4^{2-} [(a)]
Test Pond 9	OSPW	2067	20.4[a]	Syncrude	0458007, 6326993	Na^+ [(a)]	HCO_3^- , Cl^- [(a)]
High Sulphate	Reference (but may be contaminated)	3025	12[b]	Off-site	0466395, 6317235	Na^+, Ca^{2+} [(b)]	SO_4^{2-}, HCO_3^- [(b)] (SO_4>>HCO_3)
Shallow Wetland	Reference	370	2.3[a]	Syncrude	0458126, 6326649	None >100mg/L[(a)]	HCO_3^- [(a)]
Natural Wetland	OSPW	1234	64.0[b]	Suncor	0468962, 6315305	Na^+ [(b)]	HCO_3^- [(b)]
V-Notch Weir	Reference	1004	7.0[b]	Suncor	0467653, 6316221	Ca^{2+} [(b)]	Not Available
Peat Pond	Reference	1743	1.0 [a]	Syncrude	0462075, 6316867	Na^+ [(a)]	SO_4^{2-}, HCO_3^- [(a)]
Golden Pond	Reference	1874	3.7[a]	Syncrude	0462066, 6317226	Na^+ [(a)]	SO_4^{2-}, HCO_3^- [(a)]
Salt Marsh	Reference	1238	5.0	Suncor	0467442, 6316830	Na^+, Ca^{2+} [(b)]	Not Available

Mildred Lake	Reference	331	0.9	Syncrude	0463618, 6323259	Not Available	Not Available

[a]Mackinnon, 2008, [b]Martin, 2011, [c]Birks, 2010.

Bioassay Procedures

This study was conducted over a two years. The first 10-d trial occurred in July 2009 and the second 10-d trial occurred in July 2010. Egg masses were placed in Petri dishes filled with dechlorinated tap water and checked daily for evidence of hatching. When first instar chironomids hatched from the eggs, 20 planktonic larvae were collected using a glass Pasteur pipette. In trial one, the chironomids were transferred to a 30-mL holding vial containing dechlorinated tap water before they were gently poured into treatment jars. In trial two, larvae were transferred directly from the Petri dish in which they hatched into the treatment jar. For each egg mass, two sets of 20 larvae were preserved per day to provide a measure of initial size.

New 1-L Mason jars were scrubbed with Fisherbrand Sparkleen detergent and tap water, rinsed with tap water, then triple rinsed with deionized water. Coarse silica sand was used as a substrate with 100 mL of sand in each jar which produced a depth of approximately 2 cm. Jars were then filled with 500 mL of wetland water, which had been filtered through a 280-μm fabric sieve. Five replicate jars per wetland were used. Jars were kept in an evaporation tank 120 cm in diameter filled with 7.5 cm of water to facilitate temperature control. The experiment was done in an unheated trailer on Syncrude Canada Ltd's Mildred Lake site. Jars were aerated for a minimum of 24 h before the addition of larvae and continuously thereafter throughout the study. A branched aeration method as described in Corkum and Hanes (1989) was used. Dissolved oxygen, conductivity, salinity, and pH were measured on days 1 and 0 of the experiments. Larvae were fed a volume of slurry solution equivalent to 10 mg tropical fish flakes per jar, per day. After

10 d, larvae were removed by sieving the contents of each jar through a 250-um sieve and using forceps to remove larvae. Larvae were preserved in chilled Carnoy's solution (3:1, ethanol: glacial acetic acid), which was changed 1 h and 24 h after initial preservation.

Laboratory Procedures

Survival was determined by counting the number of living larvae found after day 10. Final chironomid size was determined by photographing each chironomid using a digital camera mounted on a dissecting microscope. Images of larval length were then measured digitally using ImageTool software. Biomass was then calculated from larval length using the following conversion equation:

$$\text{Biomass} = 0.0018 \times (\text{Length})^{2.617}$$

where biomass is in mg (dry mass) and length is in mm (Benke et al., 1999).

Statistical Analyses

Log-transformed survival ratio (survival divided by mortality plus one) and log-transformed mean biomass across log-transformed NA concentrations and log-transformed conductivities and the interaction term (i.e., the product of NA concentration and conductivity) were analyzed by forward stepwise multiple regression. The class of wetland from which source water was taken (OSPW or reference) and year were included in the regressions as dummy variables, indicated in the model as 0 or 1. Linear regression was also used to determine whether there was a relationship between length and survival—in other words, a density-dependent effect. STATISTICA 7 software was used

for all statistical analyses and a p-value of less than 0.05 was considered statistically significant.

Results

Survival of Chironomid Larvae

Results of the forward stepwise multiple regression of log-transformed survival ratio (survival/mortality) versus log-transformed conductivity, log-transformed naphthenic acid concentration, and their interaction term as well as the class and year dummy variables indicated that chironomid larval survival was affected only by conductivity (Table 3.3). Log survival ratio was positively associated with log conductivity. This indicates that chironomids exhibited higher survival in higher conductivity wetland waters (Fig. 3.1). There was no significant relationship between log NA concentration and survival (Fig. 3.2).

Biomass of Chironomid Larvae

Forward stepwise multiple regression (results in Table 3.4) indicated that mean biomass varied significantly both between years and with respect to wetland class-- larvae attained higher biomass in the 2010 trial than in the 2011 trial-- and reference wetland waters yielded larger chironomids than OSPW wetland waters. There was also a significant, positive relationship between conductivity and chironomid biomass, indicating that chironomid larvae reared in water with higher conductivities grew larger than those reared in lower conductivity wetland water (Fig. 3.3), even after taking into account the differences due to wetland class. There was also a significant, positive effect of log-NA

on log-biomass—this indicates that chironomids grew larger in high NA wetland waters than in lower NA wetland waters (Fig. 3.4). Log conductivity and log NA had a significant, negative interaction on log biomass.

Table 3.2: Results of a forward stepwise multiple regression on log(survival ratio + 1) with class (OSPW or reference), year, log(NA), log(conductivity), and the interaction term of log(NA) and log(conductivity) as independent variables. Significant p-values are indicated by an asterisk (*).

	Regression Coefficient	S.E.	R^2	t-value	p-value
Intercept	-0.6174	0.5535		-1.11	0.269
Log(Conductivity)	0.3571	0.1758	0.246	2.03	0.0463*
Total			0.246		

Table 3.3: Results of a forward stepwise multiple regression with log(biomass) as the dependent variable and class (OSPW or reference), year, log(NA), log(conductivity), and the interaction term of log(NA) and log(conductivity) as independent variable. Significant p-values are indicated by an asterisk (*).

	Regression Coefficient	S.E.	Partial R^2	t-value	p-value
Intercept	0.0589	0.3000		0.1966	0.8450
Year	-0.3603	0.0445	0.4680	-8.0916	<0.0001*
Log(Conductivity)	0.0082	0.1146	-0.4145	0.0718	0.9430
Wetland Class	-0.1923	0.0721	0.9774	-2.6665	0.0104*
Log(NA)	0.8794	0.2246	0.0313	3.9147	0.0003*
Log(NA)*Log (Conductivity)	-0.2812	0.0826	0.0789	-3.4044	0.001*
Total			0.6731		

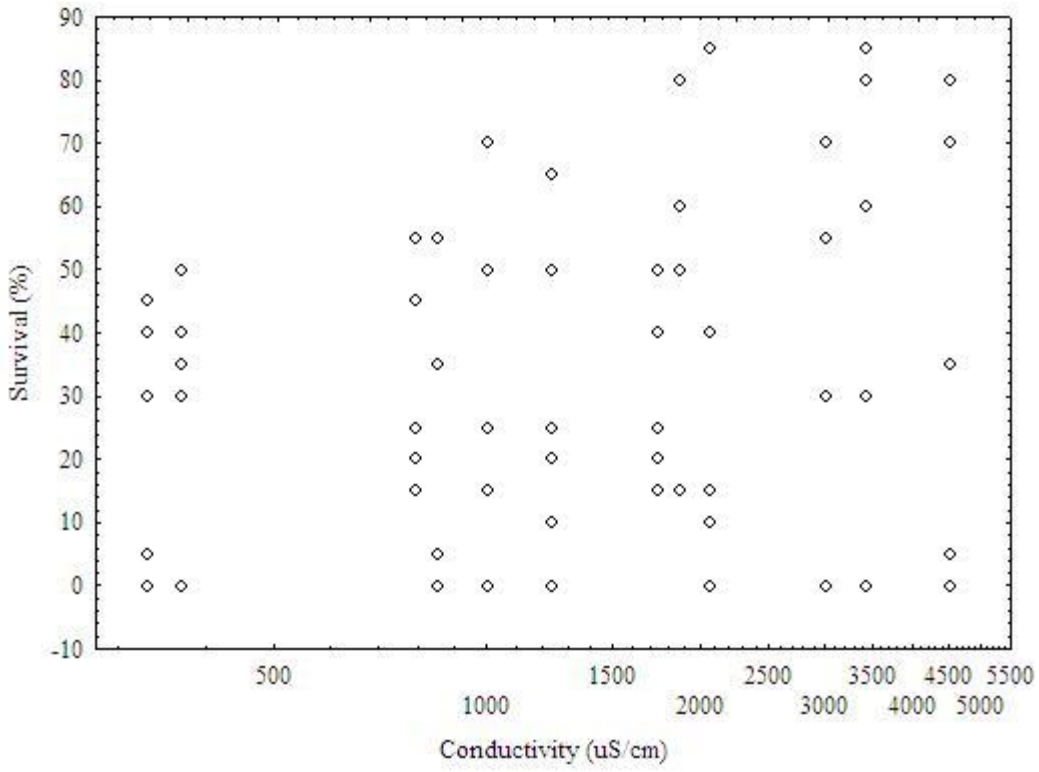

Figure 3.1: Percent larval survival per jar across a range of conductivities (µS/cm) of thirteen wetland waters.

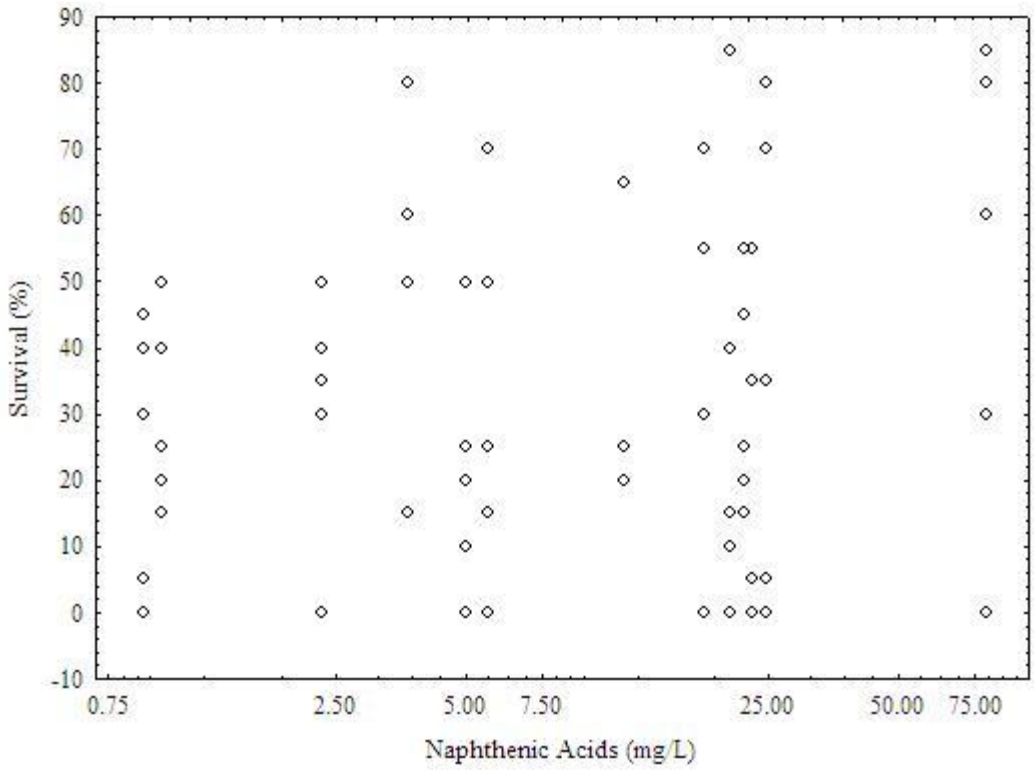

Figure 3.2: Percent larval survival per jar across a range of naphthenic acid concentrations (mg/L) of thirteen wetland waters.

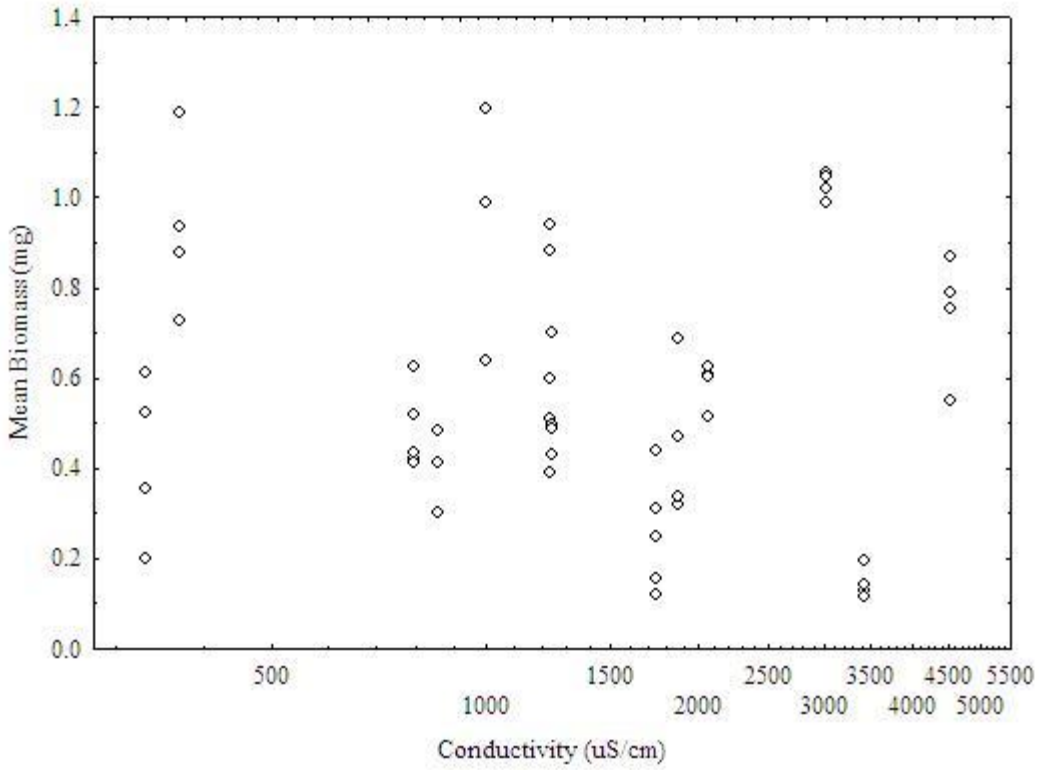

Figure 3.3: Mean larval biomass (mg) per jar across a range of conductivities of thirteen wetland waters.

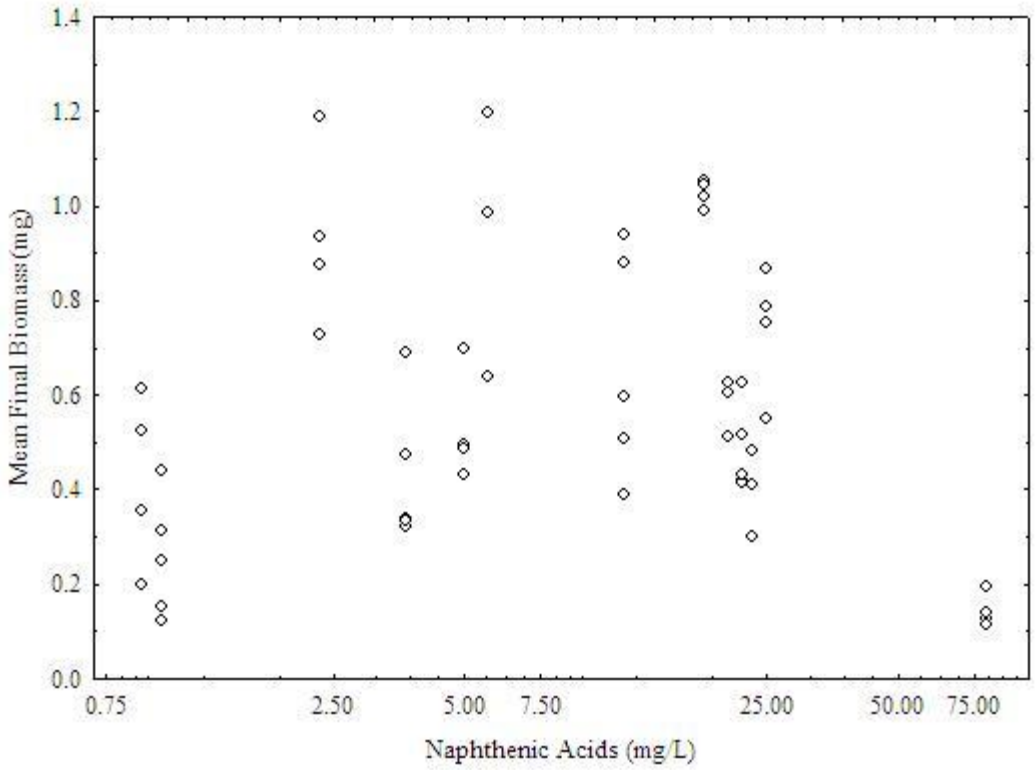

Figure 3.4: Mean larval biomass (mg) per jar across a range of naphthenic acid concentrations (mg/L) of thirteen wetland waters.

Correlation between Length and Survival

In order to determine whether the density of larvae, (which was very variable both among and within treatments) affected their size, a linear regression was performed .Mean length per jar was not significantly related to number of larvae per jar surviving to the end of the bioassay ($p=0.664$; Figure 3.3). This indicates that surviving larvae in each jar did not consume proportionally more food per individual when larvae in the same jar died. The two variables appear to be independent of one another

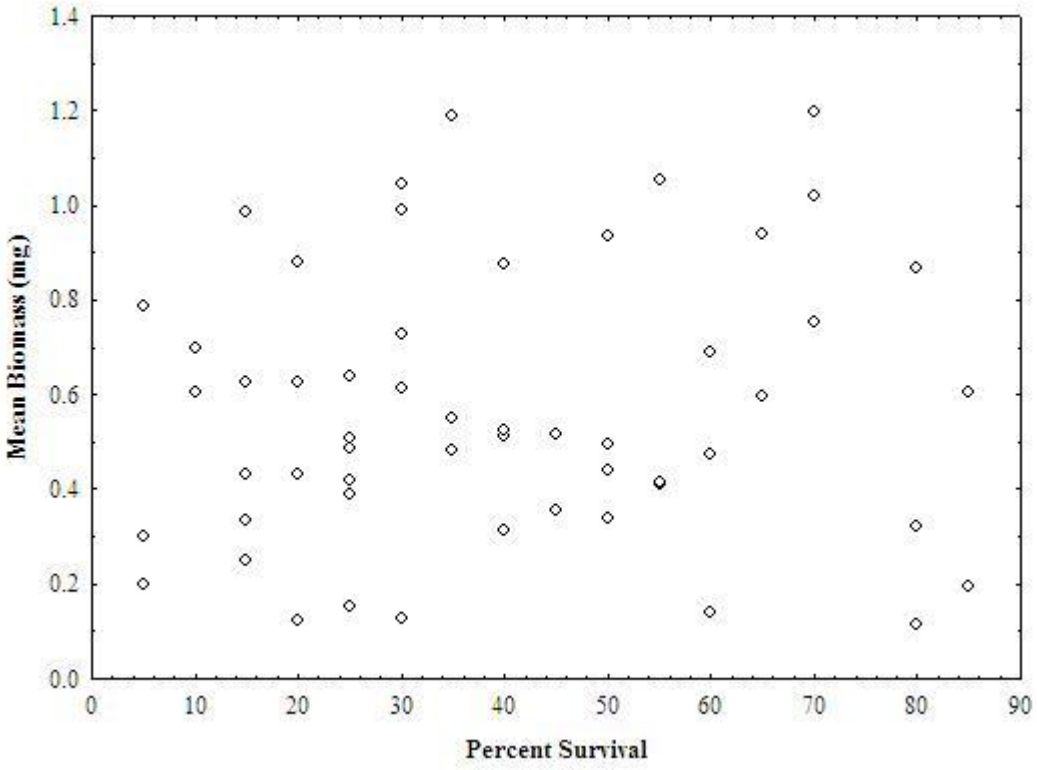

Figure 3.5: Relationship between mean length of chironomids per jar (mm) and the number of individuals surviving to the end of 10-d bioassay.

Discussion

Chironomid larvae survival was low overall, but was significantly positively related to conductivity. In other words, larvae reared in high conductivity wetland waters survived better than those in low conductivity wetland waters. This was unexpected because many species of the genus *Chironomus* are among the least salt tolerant of the family (Cannings and Scudder, 1978). Survival was not significantly affected by year, class, or NA concentration. There was no significant interaction between NA and conductivity on larval survival.

There was a significant positive logarithmic relationship between NA and biomass and between conductivity and biomass. This was contrary to the expectation that these two toxicants would pose sublethal stress leading to lower growth rate. There was also a negative interaction between NA and conductivity—this indicated that there was a synergistic effect and that the two are more likely to limit growth when combined than expected if the effects of each were added together. The 2010 trial yielded larger chironomids than the 2011 trial. This may have been due to temperature differences or the wetlands chosen in each year. Chironomid larvae that were held in reference wetland waters grew to a larger size than those that were held in OSPW wetland waters. This may indicate a sublethal toxicity of OSPW wetland waters from components other than NA and conductivity.

Preliminary findings by Turcotte et al. (2009) indicated that there was an antagonistic interaction between the effects of salts and NA on survival of *Ceriodaphnia dubia* in the laboratory. They hypothesized that the presence of salts caused the acids to precipitate out of solution (Turcotte et al. 2009). If the acids did precipitate out of solution, a planktonic animal such as *C. dubia* would no longer be in contact with the salt-bound acid, but a benthic invertebrate such as *C. riparius* would still be in contact with the surfactant. It is also possible that *C. riparius* are more resilient to these concentrations of salts and NA.

Anderson et al. (2011) studied survival, growth, and emergence of *Chironomus dilutus* exposed to fresh and treated OSPW. There was no consistent, significant trend to indicate that OSPW negatively affected survival, but it did reduce larval size. In the same study, also found that exposure to a saltwater control did not significantly impair growth, survival, or emergence of *C. dilutus* larvae, but concentration of NA did correlate significantly with reductions in these three endpoints (Anderson et al. 2011). The findings of Anderson et al. (2011) are not consistent with the findings of this study.

The effect of naphthenic acids and conductivity on biomass and survival of chironomid larvae did not prove to be significant in this study. Overall, there was high mortality in every treatment, including controls, and this more likely led to non-significant results. This high mortality may have been the result of residual detergent in the jars which may have caused stress to the larvae. Alternatively, the stress caused by handling and

transferring chironomid larvae that were only a few hours hold, may have been responsible for the consistently high mortality.

There was no significant relationship between length and survival of chironomids. This indicates that chironomids did not grow more quickly when other chironomids in the jar died. This is confounded with the overall high mortality, but indicates that adequate space and food was provided for each larva.

Conclusion

Larvae reared in water from wetlands containing OSPW tended to be smaller than larvae reared in water from reference wetlands, all other things being equal. However, low survivorship of the chironomid larvae overall compromised this study and prevented an adequate assessment of the interaction between naphthenic acids and salts and their combined and separate effects on biomass and survival. Limited evidence suggests that *C. riparius* larvae may survive better in water with conductivity higher than the controls used in this study.

References

Allen, E.W. 2008. Process water treatment in Canada's oil 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. 2011.Effects of exposure to oil sands process-affected water from experimental reclamation ponds on *Chironomus dilutus*. Water Research. Accepted December 3, 2011.

Batzer, D. P. , McGee, M, Resh, V.H., and R.R. Smity. 1993. Characteristics of invertebrates consumed by mallards and prey response to wetland flooding schedules. Wetlands 13: 41-49

Benke, A.C., Huryn, A.D., Smock, L.A., and Wallace, J.B. 1999. Length-massrelationships for freshwater macroinvertebrats in North America with particular reference to the southeastern United States. Journal of the North American Benthological Society. 18: 308- 343.

Birks, J. 2011. Personal communication.

Cannings, R.A., and Scudder, G.G.E. 1978. The littoral Chironomidae (Diptera) of saline lakes in central British Columbia. Canadian Journal of Zoology 56: 1144-1155.

Chapman, P. M. 2000. Whole effluent toxicity testing—usefulness, level of protection, and risk assessment. Environmental Toxicity and Chemistry. 19: 3-13.

Corkum, L.D. and E.C. Hanes. 1989. A laboratory aeration system for rearing aquatic invertebrates. Entomological News 100:169-172.

Del Rio, L.F., Hadwin, A.K.M., Pinto, L.J., MacKinnon, M.D., and Moore, M.M. 2006. Degradation of naphthenic acids by sediment micro-organisms. Journal of Applied Microbiology 101: 1049-1061.

Frank, R. A., Kavanagh, R., Burnison, B.K., Arsenault, G., Headley, J. V., Peru, K. M., Van Der Kraak, G., and Solomon, K.R. 2008. Toxicity assessment of collected fractions from an extracted naphthenic acid mixture. Chemosphere 72: 1309-1314.

FTFC (Fine Tailings Fundamentals Consortium). 1995. "Volume II: Fine Tails and Process Water Reclamation" In: Advances in Oil Sands Tailings Research, Alberta Department of Energy, Oil Sands and Research Division, Edmonton, Alberta, Canada.

Gafa Riv, S. 1974. Studies on relationships between acute toxicity to fish and surface activity of anionic surfactants. Sostanze Grasse 51: 183-192.

Hall Jr., L.W., and Anderson, R.D. 1995. The influence of salinity on the toxicity of the various classes of chemicals of aquatic biota. Critical Reviews in Toxicology. 25: 281-346.

Hokanson, K.E.F., and Smith, L.L. 1971. Some factors influencing toxicity of linear alkylate sulphonate (LAS) to the bluegill. Transactions of the American Fisheries Society. 100: 1-12.

Holowenko, F.M., MacKinnon, M.D., and Fedorak, P.M. 2001. Naphthenic acids and surrogate naphthenic acids in methanogenic microcosms. Water Research 35: 2595–606.

Leung, S.S., MacKinnon, M.D., and Smith, R.E.H. 2001. Aquatic reclamation in the Athabasca, Canada, oil sands: naphthenate and salt effects on phytoplankton communities. Environmental Toxicology and Chemistry 20 (7): 1532-1543.

MacKinnon, M.D. 2000. Personal communication.

Martin, J.P. 2011. Personal communication.

Pascoe, D., Williams, K.A., and Green, D.W.J. 1989. Chronic toxicity of cadmium to *Chironomus riparius* Meigen -- effects upon larval development and adult emergence. Hydrobiologia 175: 109-115.

Turcotte, D., Kautzman, M., Peru, K, Headley, J., and Liber, K. 2009. Characterizing salt and naphthenic acids interaction in the toxicity of oil sands process water to freshwater invertebrates. SETAC Poster Presentation.

Verge, C., Morenso, A., Bravo, J., and Berna, J.L. 2001. Influence of water hardness on the bioavailability and toxicity of linear alkylbenzene sulphonate (LAS). Chemosphere 44: 1749-1757.

Whelly, M.P. 1999. Aquatic invertebrates in wetlands of the oil sands region of northeast Alberta, Canada, with emphasis on Chironomidae (Diptera). M.Sc. Thesis, University of Windsor, Windsor, ON.

CHAPTER IV: ASSESSING INDEPENDENT AND INTERACTIVE EFFECTS OF NAPHTHENIC ACIDS AND SALTS ON LARVAE OF *CHIRONOMUS RIPARIUS* (DIPTERA: CHIRONOMIDAE) IN LABORATORY BIOASSAYS

Introduction

Oil sands process water contains a mixture of potential toxicants including, but not limited to, high concentrations of naphthenic acids and salts. The purpose of this study was to determine the independent and combined effects of these two toxicants on the growth and survival of the benthic macroinvertebrate *Chironomus riparius* in the laboratory.

Chironomus riparius as a Study Species

Chironomids often dominate the benthic invertebrate community biomass, so their productivity has potential to influence overall wetland ecosystem productivity and productivity of surrounding terrestrial food webs (Pascoe et al., 1989). For example, Hoekman et al. (2012) found that the deposition and accrual of dying, postmating chironomids can enrich plant and arthropod communities in terrestrial food webs. Their abundance makes large chironomids an important prey species for mallards (Batzer et al., 1993). Adult chironomids are a dominant part of the diets of silver-haired bats (Barclay, 1985) and tree swallows (St. Louis et al., 1990). *Chironomus riparius* is a commonly-used toxicity test organism, so toxicants can be easily compared within this species (Pascoe et al. 1989).

The Toxicity of Oil Sands Process Water

The extraction and refining of oil from mined oil sands generates large volumes of fine fluid tailings material (FTFC, 1995). Oil sands industries then face the challenge of storing the tailings material and process water in an environmentally sound manner. However, oil sands process water/ material (OSPW or OSPM, respectively) contains high concentrations of salts and naphthenic acids and some trace metals, which can cause adverse effects to aquatic biota (reviewed in the General Introduction).

Oil sands process water (OSPW) and water from OSPM-affected wetlands can be toxic to a variety of organisms across several trophic levels. Northern Canadian toad (*Bufo boreas*) and wood frog (*Rana sylvatica*) tadpoles held in OSPM-affected ponds exhibited reduced growth and development (Pollet & Bendell-Young, 2000). Similarly, wood frog tadpoles have exhibited delayed metamorphosis in young OSPM-affected wetlands whereas older OSPM-wetlands had similar metamorphosis timing to reference wetlands (Hersikorn and Smits, 2011). In fathead minnows, OSPW with NA concentrations of >25 mg/L and conductivities of >2000 µS/cm caused a variety reproduction-associated effects including lowered spawning success and reduced sex steroid hormone levels (Kavanaugh et al., 2011). In the laboratory, 96-h exposure to as little as 6.3 % OSPW significantly reduced mean final length and survival of larvae of black flies (*Simulium vittatum*) (Sabo 2003). In one study, first instar *Chironomus dilutus* larvae exhibited a 10-d LC_{50} of 65% OSPW (Whelly, 1999). However, *Chironomus riparius* were more tolerant than *C. dilutus*, exhibiting relatively minor reductions in survival and growth in 100% OSPW (Whelly 1999).

The Toxicity of Salts

There are several ways of measuring the amount of salt in solution (Mackie, 2004). Specific conductance or *conductivity* measures the flow of electrons through the water usually in microsiemens per centimeter (µS/cm). Second, *salinity* refers to the concentration of dissolved salts, usually expressed in parts per thousand (ppt). Third, *total dissolved solids* (TDS) also refers to the concentration of dissolved salts, but it is measured by mass as opposed to number of ions per volume of water and is usually expressed as milligrams per liter (mg/L) (Mackie, 2004).

The marine origin of the oil sands mined in northeastern Alberta causes OSPW to be saline, which results in characteristically elevated conductivity (FTFC, 1995). Chironomids can tolerate varying degrees of salinity, and several species thrive in intertidal or marine conditions (Pinder, 1995). Fourth instar *Chironomus riparius* can tolerate salinity of up to 10 ppt without increases in mortality or reductions in emergence success (Bervoets et al., 1996). The presence of salts, especially particular ions may reduce the bioavailability of other constituents that may themselves be toxic. For example, Hall and Anderson (1995) found that the toxicity of most metals to aquatic biota decreases as salinity increases, likely because metals are more bioavailable in the free ion form (Hall & Anderson, 1995). This suggests that the toxicity of trace metals in OSPW-affected wetlands that contain higher salinities may be lower than metal toxicity in reference wetlands.

The Toxicity of Naphthenic Acids

Naphthenic acids (NAs) are "alkyl-substituted cyclic and aliphatic carboxylic acids that are removed from bitumen during the extraction process" (Allen, 2008). They occur naturally in low levels (<1 mg/L) in waters of the oil sands area but become concentrated in OSPW (reaching concentrations as high as 120 mg/L) during the refining process (M.D. MacKinnon, Syncrude Canada Ltd., unpublished; Headley & McMartin, 2004). In a 96-h toxicity test, NAs had LC_{50} of <10 mg/L in trout (Schramm et al. 1984). The mode of toxicity of NAs has seldom been addressed. One possibility is that the amphipathic nature of NAs causes disruption at the cell membrane (narcosis) which could ultimately lead to cell death (Frank et al., 2008). Zhang et al. (2011) used a genetic approach in order to determine the mode of toxicity for NAs. They found that genes involved in NADP or NADPH binding were up-regulated and genes involved in the ATP-binding cassette transporter complex were down-regulated when *E. coli* was exposed to naphthenic acids (Zhang et al., 2011). Because of the surfactant nature of NA, gill damage and death by asphyxiation is likely the cause of toxicity (Abel, 1974). Surfactants like NA can also interact with proteins and affect the permeability of cell membranes (Abel, 1974).

Objectives

The objectives of this study were to determine the toxicity of naphthenic acids and total dissolved solids to *Chironomus riparius* midge larvae and to determine the relationship of these toxicants when combined. The response variables studied were 10-d survival and larval final length at the end of a standard laboratory bioassay. It was expected that alone, high concentrations of naphthenic acids and added salts would reduce larval survival and

growth due to increased narcosis at the cell membrane and osmoregulation-related stress, respectively.

Methods

Study Organisms

Egg masses from a *Chironomus riparius* culture at the University of Windsor, Windsor, Ontario were used in this study. These insects were originally derived from a lab-reared culture at Environment Canada, Burlington, Ontario. Freshly-laid egg masses were incubated individually in Petri dishes containing dechlorinated tap water at room temperature and were monitored every 12 h until hatching occurred. Planktonic newly hatched larvae that had finished feeding on the gelatinous egg matrix (>12 h old) were removed from their Petri dish by pipette and gently placed in experimental jars in groups of 20. Two groups of 20 newly hatched larvae from each egg mass were preserved in Carnoy's solution (3:1 v/v, acetic acid: ethanol) at this time to be later measured as a proxy for initial size.

Sources of Chemicals and Preparation of Water Treatments

Naphthenic acids were extracted from Mildred Lake Settling Basin fluid tailings and concentrated into a stock solution by Richard Kavanagh (Canadian Natural Resources Limited) in 2008 using methods described by Frank et al. (2006). The salt solution was created by looking at the concentrations of individual ions recorded from Natural Wetland in 2008, which receives OSPW as seepage from an adjacent tailings pond on the Suncor Energy, Inc lease area (MacKinnon, 2008). This yielded a stock salt solution with the same ionic composition and total dissolved solids of a real oil sands process affected

wetland. The concentrations of individual ions in Natural Wetland are listed in Table 4.1 and the salts added to ultrapure water to yield these concentrations are listed in Table 4.2.

Table 4.1: Concentrations of major ions in Natural Wetland.

Ion	Concentration in Natural Wetland (mg/L)
Na	482
K	15
Mg	16
Ca	23
Cl	82
SO_4	240
CO_3	38
HCO_3	908

Table 4.2: Salts added to Millipore filtered water to match major ion concentrations listed in Table 4.1 to yield 10L stock solution.

Salt	Mass (g)
KCl	0.4944
$CaCl_2$	0.6369
NaCl	0.4687
$NaHCO_3$	12.6971
Na_2CO_3	0.6712
Na_2SO_4	2.1805
$MgSO_4$	0.5910

Experimental Design

Several assays were conducted in this study (Table 4.3). The following assays were conducted in a randomized block design in which replicates were blocked through time and space. Jars were randomly arranged under fluorescent light in an environmental chamber under a photoperiod of 16 hours light and 8 hours dark and kept at a constant temperature of 21˚C. The replicated sets of 25 treatments were randomly arranged through space. One-L Jars were washed with Sparkcleen soap, triple-rinsed with dechlorinated tap water, rinsed with 10% HCl, and triple-rinsed with high purity, Millipore-filtered water. A 2-cm depth of autoclaved fine silica sand was placed on the bottom of each jar, after which 1 L of treatment water was added. Jars were aerated individually with capillary tubing using the method of Corkum and Hanes (1989). Twenty newly hatched larvae (<24 h old; previously counted into a holding container) were carefully transferred into each jar. Ten mg of ground Nutrifin7 tropical fish food in a slurry solution was added to each jar daily (0.5mg/larvae/day).

The assays ran for 10 d. On the 11th day of the study (240 h post inoculation) larvae were recovered by pouring the water and sand from a jar through a 250-µm mesh sieve and examining the material retained. Larvae were enumerated, immediately preserved in chilled Carnoy's solution (3:1 v/v acetic acid: ethanol) and stored refrigerated. Larvae were then digitally photographed at 60x magnification beneath a dissection microscope. The body length (distance from posterior end of the anal proleg to the anterior margin of the head) of each larval image was measured to the nearest 100 µm using ImageTool 3.0© (UTHSCSA, San Antonio, TX) image analysis software.

Table 4.3: Description of the concentrations of potentially toxic constituents in each series

Assay	Naphthenic Acids		Total Dissolved Solids		Number of Replicates Completed	Number of Replicates used in Analysis
	Concentration (mg/L)	Percent of Maximum (%)	TDS (mg/L)	Percent of Maximum (%)		
Naphthenic Acid Assay	0	0	335	0	4	3
	10	13	335	0	4	4
	18	22	335	0	4	2
	29	36	335	0	4	4
	48	60	335	0	3	2
	80	100	335	0	4	2
Salt Assay	0	0	335	0	3	3
	0	0	523	13	4	4
	0	0	651	22	3	2
	0	0	853	36	4	4
	0	0	1199	60	3	3
	0	0	1774	100	4	4
Combination Assay	0	0	335	0	4	4
	0	0	1774	100	4	3
	10	13	1199	60	4	3
	18	22	853	36	4	3
	29	36	651	22	4	4
	48	30	523	13	3	2
	80	100	335	0	2	2

Statistical Analyses

In order to analyze independent effects of NA and TDS on length and survival, the single toxicant bioassays were analyzed using linear regression on log transformed variables. A survival ratio (number of larvae that survived divided by number of larvae that died) was used in analysis and an arithmetic mean was taken as average length. Log transforming both the independent (NA and TDS) and dependent (length and survival) variables should produce a dose-response relationship that follows a linear model (De Lean et al., 1978). Two multiple linear regression analyses were performed, to relate each of the two dependent variables (larval survival and mean length-- both log-transformed) to concentrations of NA and TDS and their interaction (NA x TDS).

Results

In all three assays, larval survival was quite low, even in controls, ranging from 0 to 35 percent in controls and 0 to 80 percent overall, with an overall mean of 19 percent. Furthermore, survival was highly variable. It is possible that first instar larvae were so fragile that handling stress occurred early on when larvae were transferred into test jars. Consequently, results should be interpreted with caution. Appendix G provides detailed survival and growth data. Temperature stayed constant over the course of the study beginning at 22.8 ± 1.1˚C and ending at 22.8 ± 1.8˚C. Because water was not changed throughout the 10 d, some evaporation occurred, and conductivity was slightly higher at the end of the experiment than at the beginning. On day 1 the control jars had a mean (±SD) of 470 ± 7 µS/cm, and this increased to 518 ± 43 µS/cm by day 11 Appendix F provides detailed water quality data.

Single Toxicant Bioassays

There was no significant relationship between the concentration of NA alone and larval survival [(Log (survival)/Log(mortality)] (Fig. 4.1; table 4.4). There was also no significant relationship between the concentration of NA and mean larval length (Fig. 4.2; table 4.5).

There was also no significant relationship between TDS alone and larval survival (Fig. 4.3; table 4.6). However, there was a significant, negative relationship between mean larval length (mm) and TDS ($p=0.01$; Fig. 4.4; table 4.7).

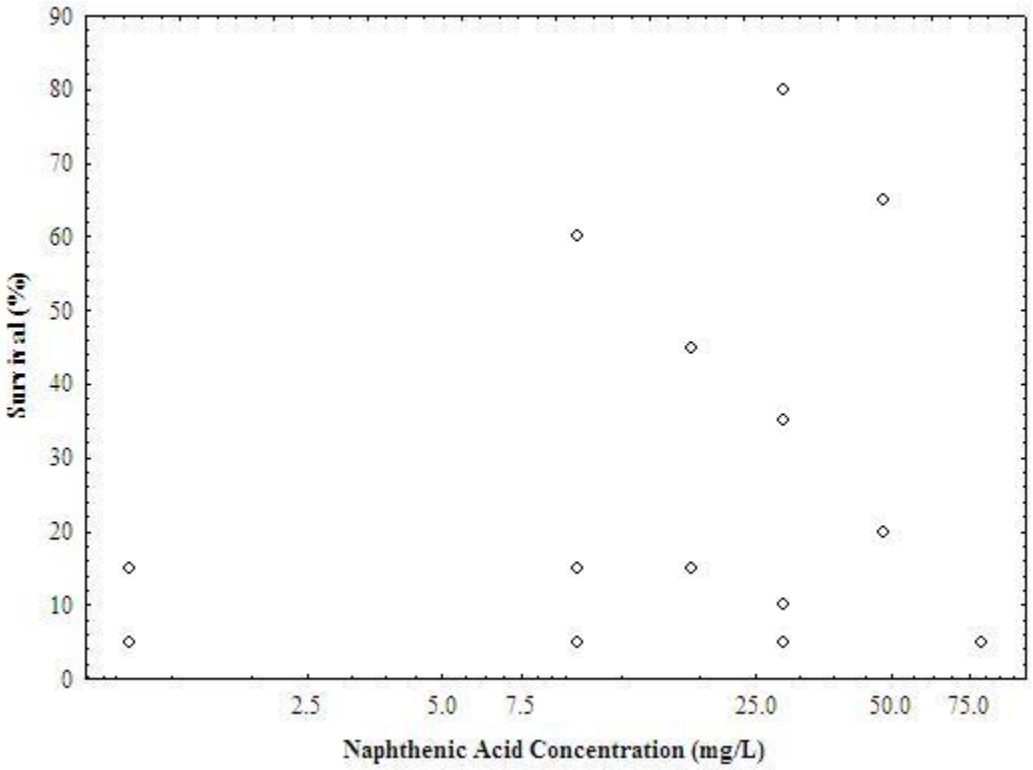

Figure 4.1: Larval survival (percent) in each jar in which at least one larva survived to the end of the study the naphthenic assay series.

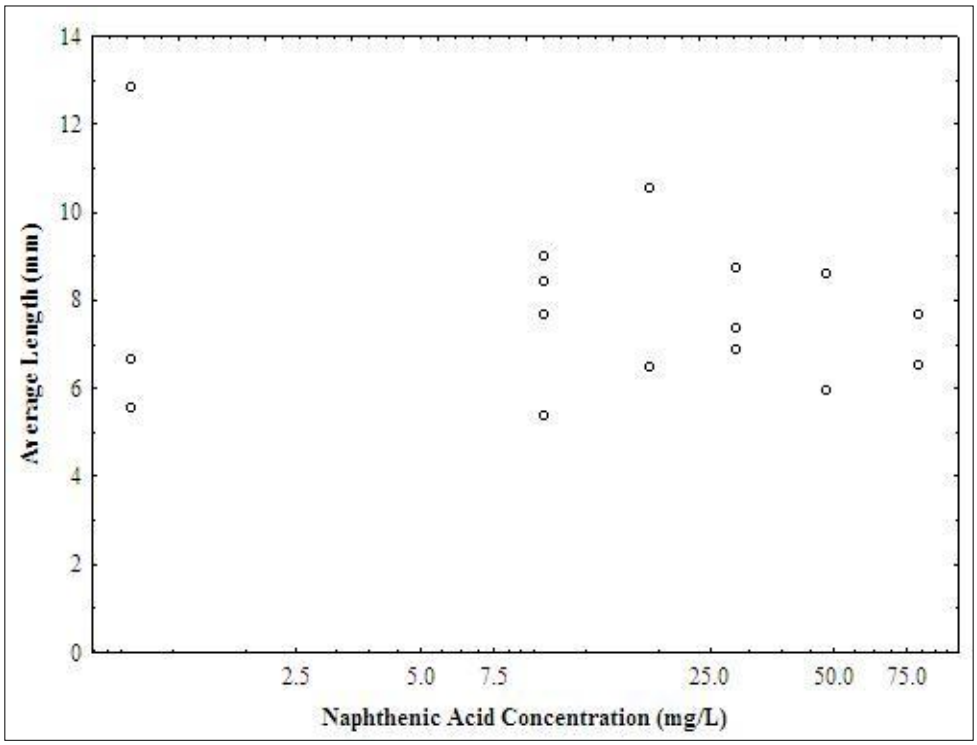

Figure 4.2: Geometric mean body length (mm) of larvae for each jar in which at least one larva survived in the naphthenic acid assay.

Table 4.4: Linear regression results of log transformed NA on log-transformed survival ratio (survival/mortality). Statistically significant p-values (<0.05) are indicated by an asterisk (*). N= 17, df=15.

	Regression Coefficient	S.E.	R^2	t-value	p-value
Intercept	-0.94	0.32		-2.9	0.009*
Log(NA+1)	0.23	0.24	0.057	0.95	0.36

Table 4.5: Linear regression results of log transformed NA on log-transformed mean length (mm). Statistically significant p-values (<0.05) are indicated by an asterisk (*). N=17, df=15.

	Regression Coefficient	S.E.	R^2	t-value	p-value
Intercept	0.90	0.05		16.9	<0.0001*
Log(NA+1)	-0.01	0.04	0.012	-0.43	0.67

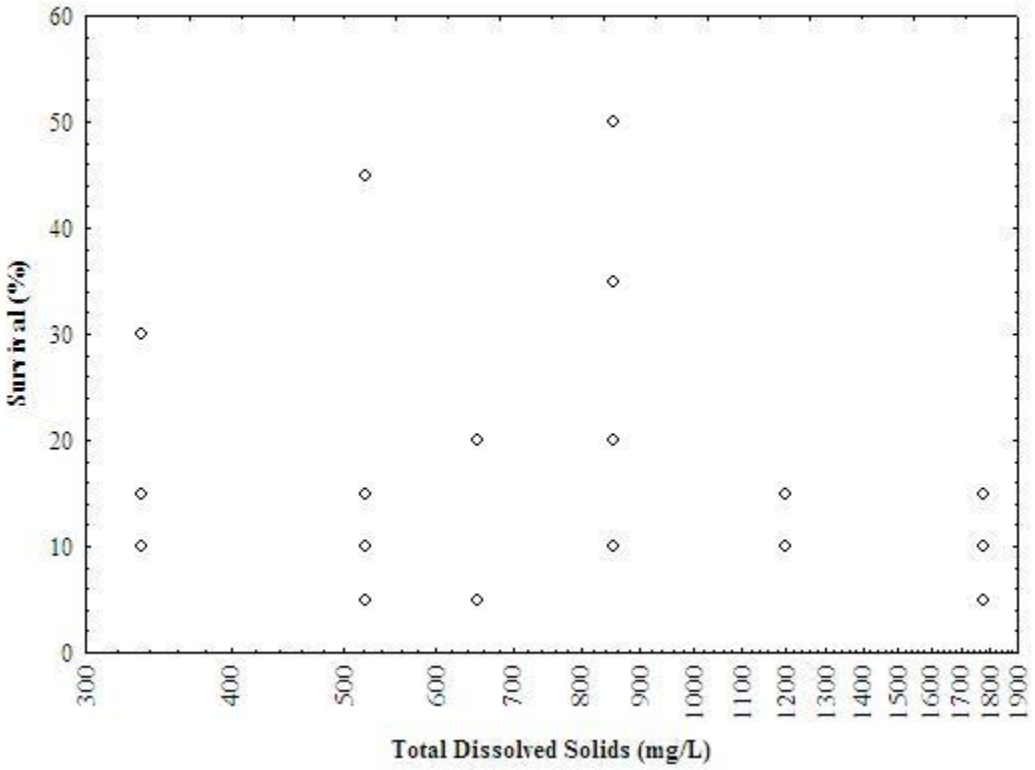

Figure 4.3: Larval survival (percent) in each jar in which at least one larva survived to the end of the study the salt assay series.

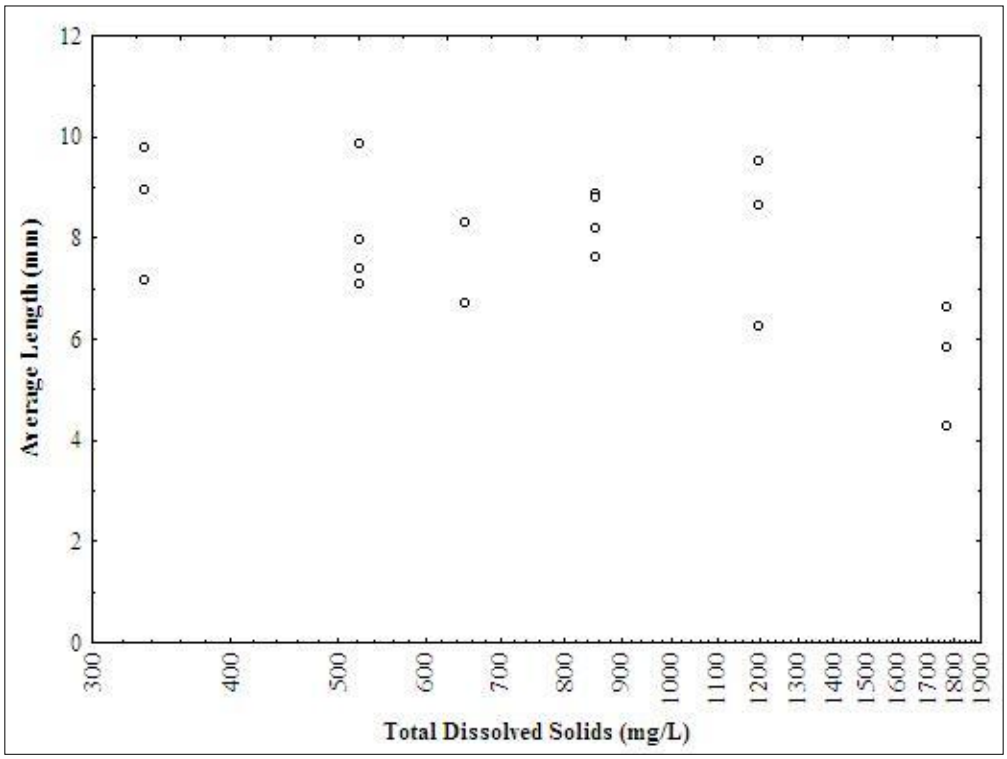

Figure 4.4: Geometric mean body length (mm) of larvae for each jar in which at least one larva survived in the salt acid assay. Note the log scale on the X axis.

Table 4.6: Linear regression results of log transformed TDS on log-transformed survival ratio (survival/mortality). Statistically significant p-values (<0.05) are indicated by an asterisk (*). N=20, df=18.

	Regression Coefficient	S.E.	R^2	t-value	p-value
Intercept	0.17	0.94		0.18	0.86
Log(TDS)	-0.32	0.32	0.05	-0.98	0.34

Table 4.7: Linear regression results of log transformed TDS on log-transformed mean length (mm). Statistically significant p-values (<0.05) are indicated by an asterisk (*). N=20, df=18.

	Regression Coefficient	S.E.	R^2	t-value	p-value
Intercept	1.44	0.21		6.98	<0.0001*
Log(TDS)	-0.19	0.07	0.29	-2.73	0.01*

Combination Bioassay

In the salt and NA combination bioassay, there was a significant negative relationship between NA concentration and survival ($p<0.001$; Fig. 4.5) and TDS and survival ($p=0.02$) (Fig. 4.6; Table 4.8). There was also a significant, positive interaction effect between NA and TDS with respect to survival ($p<0.001$). This indicates that there was an antagonistic relationship between NA and TDS resulting in greater larval survival in treatments in which the two toxicants were combined. In other words, there was a "rescue effect" of the salts on the toxicity of NA. Mean larval length was not significantly influenced by NA concentration (Fig. 4.7), TDS (Fig. 4.8), or the interaction between the two (Table 4.9).

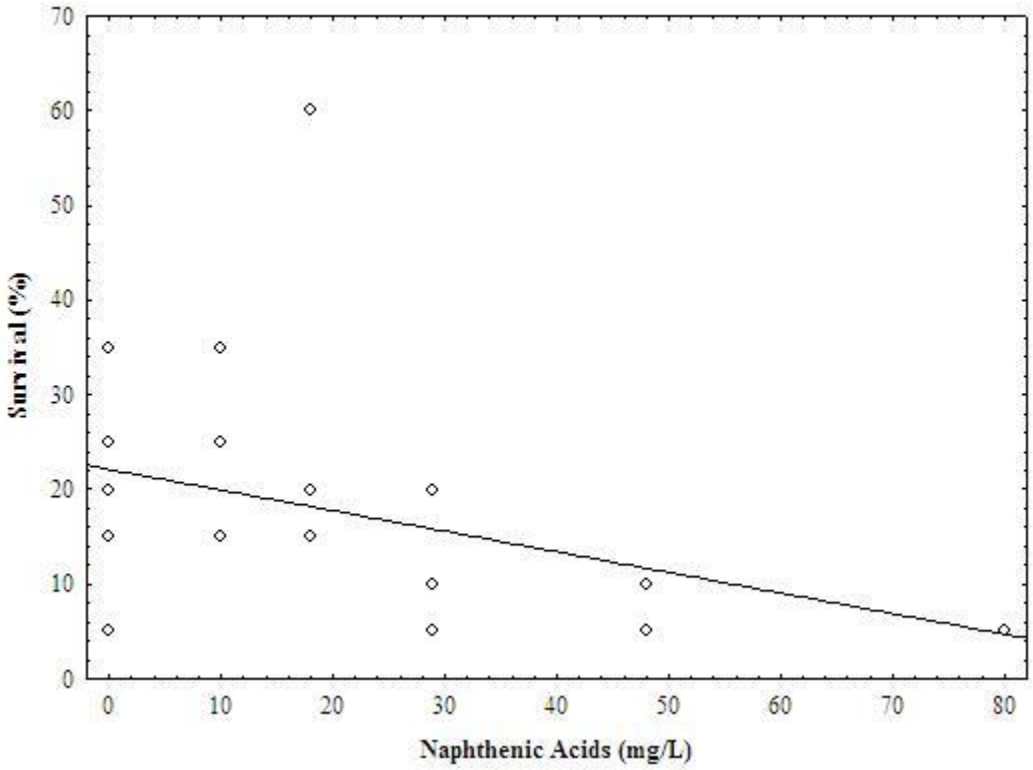

Figure 4.5: Larval survival (%) versus NA in the combination bioassay in which salts (TDS) and NAs were combined.

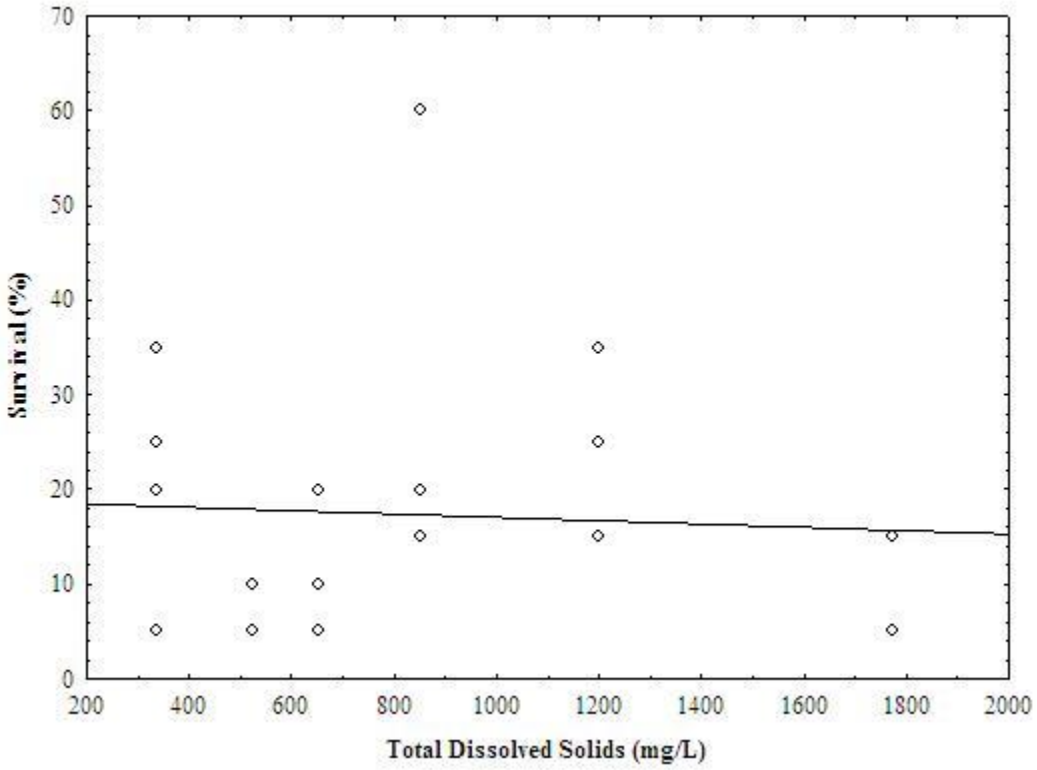

Figure 4.6: Larval survival (%) versus TDS in the combination bioassay in which salts (TDS) and NAs were combined.

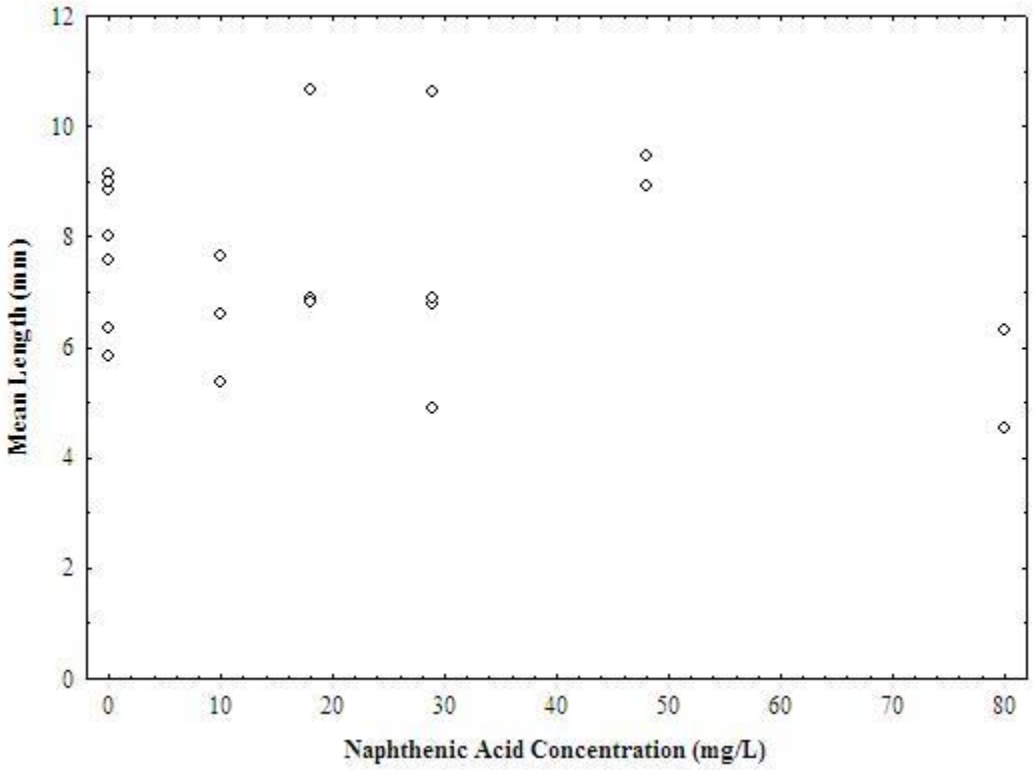

Figure 4.7: Geometric mean larval length (mm) versus NA in the combination assay in which salts (TDS) and NAs were combined.

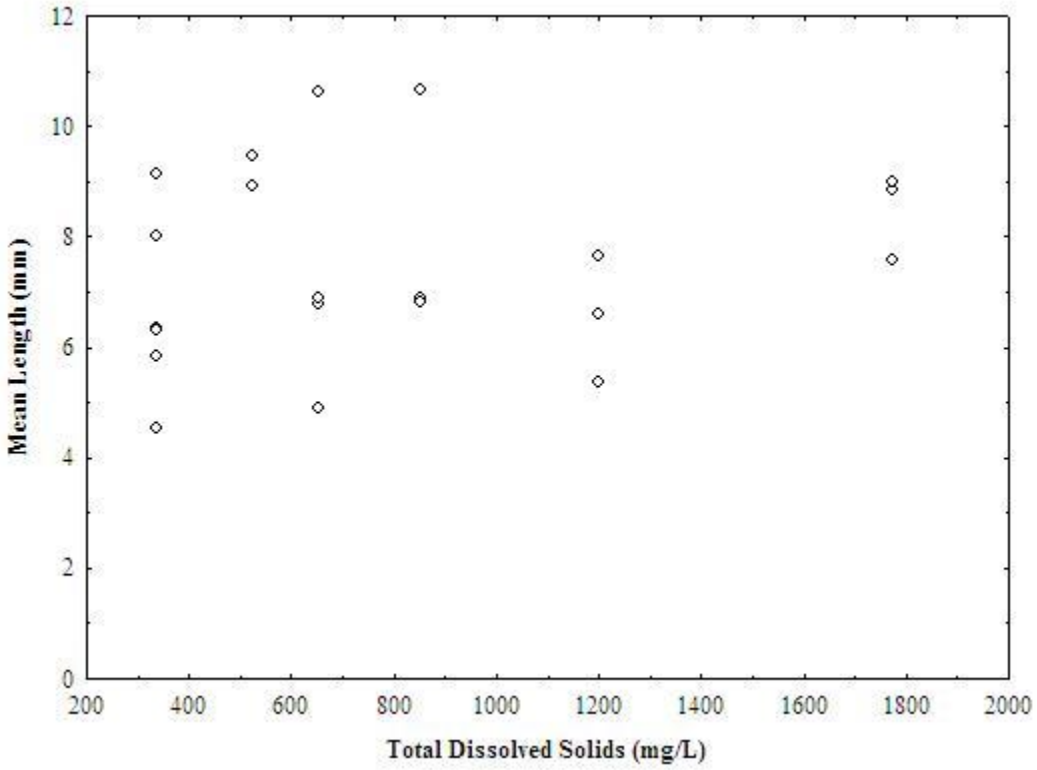

Figure 4.8: Geometric mean larval length (mm) versus TDS in the combination assay in which salts (TDS) and NAs were combined.

Table 4.8: Multiple regression results of log transformed NA, TDS, and interaction effect on log-transformed survival ratio (survival/mortality). Statistically significant p-values (<0.05) are indicated by an asterisk (*).

	Regression Coefficient	S.E.	R^2	t-value	p-value
Intercept	1.41	0.85		1.66	0.12
Log(NA+1)	-4.22	1.01	0.07	-4.18	<0.001*
Log(TDS)	-0.75	0.30	0.29	-2.51	0.02*
NA*TDS	1.49	0.37	0.18	4.06	<0.001*
Total			0.53		

Table 4.9: Multiple regression results of log transformed NA, TDS, and interaction effect on log-transformed mean length (mm). Statistically significant p-values (<0.05) are indicated by an asterisk (*).

	Regression Coefficient	S.E.	R^2	t-value	p-value
Intercept	0.69	0.32		2.14	0.047*
Log(NA+1)	-0.10	0.38	--	-0.25	0.81
Log(TDS)	0.07	0.11	0.05	0.60	0.55
NA*TDS	0.03	0.14	--	0.19	0.85
Total			0.08		

Discussion

In all three bioassays, survival was low. First instar larvae are more sensitive to stress than later instars; handling stress or contamination posed to all larvae possibly caused the low survival rate. Nebeker et al. (1984) found that first instar *C. tentans* larvae were the most sensitive to acute copper exposure followed by second, third, and fourth instars. It is unlikely that dilution water was contaminated by trace metals because salts were added to ultrapure water in order to create reconstituted wetland water. Though unlikely, it is possible that contamination by residual HCl or detergent in the jars from the cleaning process may have been the cause for the low survival rate. The most plausible explanation for the low survival rate is handling stress. Second to third instar *C. tentans* that were transported from the lab to the field for a series of 7-d in situ bioassays exhibited low survival (31.7 ± 21.5%) even when test tubes were used to hold the larvae in an effort to reduce physical stress of sediment contact (Chappie & Burton, Jr., 1997). Given that first instar larvae are more sensitive to chemical stress than later instars (McCahon & Pascoe, 1991; Nebeker et al. 1984), it is expected that they would be more sensitive to physical stress as well.

Single Toxicant Bioassays

In the NA assay, no significant relationship was found between NA concentration and survival or mean larval length. This may mean that the naphthenic acid concentrations used were not high enough to induce a strong response from the larvae. Alternatively, background sources of mortality exceeded the toxic potential of NAs. Another explanation is that the naphthenic acids used contained a low proportion of the lower

molecular weight acids that have been shown to be more toxic and biodegradable by bacteria (Frank et al, 2008). Because these acids were extracted approximately three years prior to this bioassay, it is also possible, though unlikely, that degradation by bacteria may have reduced the stock solution toxicity. The stock solutions were held refrigerated for the entire period since extracted.

In the salt assay, no significant relationship was found between TDS and survival. There was, however, a significant negative relationship between TDS and mean larval length ($p= 0.01$). This may be evidence that salt stress was high enough to reduce growth but not high enough to meaningfully reduce survival. This is not surprising as *C. riparius* is somewhat tolerant of increased salinities (Bervoets et al., 1996). Furthermore, these results are consistent with those of Hassell et al. (2006) who conducted a bioassay on *Chironomus* sp. with 8 conductivity treatments ranging from 150-25,000 µS/cm. As a frame of reference, conductivities in the present salt assay ranged from 503- 2322 µS/cm. Hassell et al. (2006) found that *Chironomus* larvae growth rate (mm/d) steadily declined with increased salinity. Time to emerge was also negatively affected by increased salinity, but size at time of emergence did not depend on salinity (Hassell et al., 2006). The reduction in growth could cause longer emergence times as well as reduced emergence success; third and fourth instar *C. tentans* larvae that were manipulated to grow more slowly than controls exhibited a longer emergence time and drastic reductions in 10-d emergence success (Liber et al. 1996). Oviposition success— the ability to produce an egg mass-- was not found to be correlated with growth rate (Liber et al.

1996). However, the number of eggs produced, or fecundity, of *C. cucini* was negatively affected by reduced size at time of emergence (Butler & Walter, 1992).

Naphthenic Acid and Salt Combination Bioassay

In the combination assay, both NA and TDS had significant negative effects on survival ($p<0.001$ and $p=0.02$, respectively). There was also a significant positive interaction effect of NA with TDS on larval survival. This indicates that there was an antagonistic interaction between NA and salts. The salts may have induced a rescue effect that ameliorated the toxicity of the NA. This effect has also been observedin *Ceriodaphnia dubia* (Turcotte et al., 2009) exposed to NA and salts in OSPW. This indicates that the results of toxicity tests performed using NAs alone may not be applicable to OSPW-affected wetlands and to do so may exaggerate toxicity.

Conclusion

Overall survival in controls was fairly low, due either to the fragility of first instar larvae or to handling stress. Future studies should use later instar larvae with the understanding that these will not be as sensitive to toxicity as first instar larvae.

Salts were found to reduce chironomid larval growth. This is consistent with other findings in the literature (Hassell et al. 2006). This finding also has implies that emergence (Liber et al. 1996) and adult fecundity (Butler & Walter, 1992); may be reduced by the effects of salts.

In the combination assay, salts and NAs were each found to reduce survival, but this effect was reduced when the two classes of compounds were present together (antagonistic interaction). This interaction has seldom been studied, but those that have

investigated this interaction in zooplankton have found similar results (Turcotte et al., 2009).

References

Batzer, D. P., M. McGee, V.H. Resh, and R.R. Smity. 1993. Characteristics of invertebrates consumed by mallards and prey response to wetland flooding schedules. Wetlands 13: 41-49.

Barclay, R.M.R. 1985. Long- versus short-range foraging strategies of hoary (*Lasiurus cinereus*) and silver-haired (*Lasionycteris noctivagans*) bats and the consequences for prey selection. Canadian Journal of Zoology 63: 2507-2515.

Bervoets, L., C. Wils, and R. Verheyen. 1996. Tolerance of *Chironomus riparius* larvae (Diptera: Chironomidae) to salinity. Bulletin of Environmental Contamination and Toxicology 57: 829-835.

Butler, M.G., and L. Walker. 1992. Fecundity, reproductive effort, and pupal size in the profundal midge *Chironomus cuccini* (Diptera: Chironomidae). Netherlands Journal of Aquatic Ecology 26: 263-267.

Chappie, D.J, and G.A. Burton, Jr. 1997. Optimization of in situ bioassays with *Hyalella azteca* and *Chironomus tetans*. Environmental Toxicology and Chemistry 16: 559-564.

Corkum, L.D., and E.C. Hanes. 1989. A laboratory aeration system for rearing aquatic invertebrates. Entomological News 100: 169-172.

De Lean, A., Munson, P.J., and Rodbard, D. 1978. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. American Journal of Physiology. 235: E97-E102.

Frank, R. A., R. Kavanagh, B.K. Burnison, G. J.V. Headley, K.M. Peru, G. Van Der Kraak, and K.R. Solomon. 2006. Diethlaminoethyl-cellulose clean-up of a large volume of naphthenic acid extract. Chemosphere 64: 1346- 1352.

Frank, R. A., R. Kavanagh, B.K. Burnison, G. Arsenault, J.V. Headley, K.M. Peru, G. Van Der Kraak, and K.R. Solomon. 2008. Toxicity assessment of collected fractions from an extracted naphthenic acid mixture. Chemosphere 72: 1309-1314.

FTFC (Fine Tailings Fundamentals Consortium). 1995. "Volume II: Fine Tails and Process Water Reclamation" In: Advances in Oil Sands Tailings Research, Alberta Department of Energy, Oil Sands and Research Division, Edmonton, Alberta, Canada.

Hall Jr., L.W., and R.D. Anderson. 1995. The influence of salinity on the toxicity of the various classes of chemicals of aquatic biota. Critical Reviews in Toxicology 25: 281-346.

Hassell, K. L., B.J. Kefford, and D. Nugegoda. 2006. Sub-lethal and chronic salinity tolerances of three freshwater insects: *Cloeon* sp. and *Centroptilum* (Ephemeroptera:

Baetidae) and *Chironomus* sp. (Diptera: Chironomidae). Journal of Experimental Biology 209: 4024-4032.

Headley, J.V., and D.W. McMartin. 2004. A review of the occurence and fate of naphthenic acids in aquatic environments. Journal of Environmental Science and Health A39: 1989-2010.

Herman, D.C., P.M. Fedorak, M.D. MacKinnon, and J.W. Costerton. 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oil sands tailings. Canadian Journal of Microbiology 40: 467-477.

Hoekman, D., M. Bartrons, and C. Gratton. 2012. Ecosystem linkages revealed by experimental lake-derived isotope signal in heathland food webs. Oecologia (in press).

Kavanagh, R.J., R.A. Frank, K.D. Oakes, M.R. Servos, R.F. Young, P.M. Fedorak, M.D. MacKinnon, and K. Solomon. 2011. Fathead minnow (*Pimephales promelas*) reproduction is impaired in aged oil sands process-affected waters. Aquatic Toxicology 101: 214-220.

Liber, K., D.J. Call, T.D. Dawson, F.W. Whiteman, and T. Dillon. 1996. Effects of *Chironomus tentans* larval growth retardation on adult emergence and ovipositing success: implications for interpreting freshwater sediment bioassays. Hydrobiologia 323: 155-167.

Mackie, G. 2004. Knowing a lake's healthy signs. Pages 103-142. *In* Applied Aquatic Ecosystem Concepts, Second Edition. Mackie, G. (Ed.) Kendall/Hunt Publishing. Dubuque, Iowa.

MacKinnon, M.D. 2008. Unpublished data. Summary of water chemistry of selected waterbodies on the Syncrude mine lease area. Prepared for Syncrude Canada, Ltd. Edmonton, AB.

McCahon, C.P. and D. Pascoe. 1991. Brief-exposure of first and fourth instar Chironomus riparius larvae to equivalent assumed doses of cadmium: effects on adult emergence. Water, Air, and Soil Pollution. 60: 395-403.

Nebeker, A.V, M.A. Cairns, and C.M. Wise. 1984. Relative sensitivity of Chironomus tentans life stages to copper. Environmental Toxicology and Chemistry. 3: 151-158.

Pascoe, D., K.A. Williams, and D.W.J. Green. 1989. Chronic toxicity of cadmium to *Chironomus riparius* Meigen -- effects upon larval development and adult emergence. Hydrobiologia. 175: 109-115.

Pinder, L.C.V. 1995. The habitats of chironomid larvae. *In* The Chironomidae: biology and ecology of non-biting midges. Edited by P.D. Armitage, P. S. Cranston, and L.C.V. Pinder. Chapman & Hall, London, UK. Pg. 131.

Pollet, I. and L.I. Bendell-Young. 2000. Amphibians as indicators of wetland quality in wetlands formed from oil sands effluent. Environmental Toxicology and Chemistry. 19: 2589-2597.

Sabo, E. 2003. *Simulium vittatum* growth, survival, mentum fluctuating asymmetry and incidence of deformity as indicators of contaminant stress. Undergraduate Thesis, University of Windsor, Windsor, ON.

Schramm, L.L., R.G. Smith, and J.A. Stone. 1984. The influence of natural surfactant concentration on the hot water process for recovering bitumen from the Athabasca Oil Sands. AOSTRA Journal of Researcj 1: 5-15.

St. Louis, V. L., L. Breebaart, and J.C. Barlow. 1990. Foraging behaviour of tree swallows over acidified and nonacidic lakes. Canadian Journal of Zoology 68: 2385-2392.

Tammes, P.M.L. 1964. Isoboles, a graphic representation of synergism in pesticides. Netherlands Journal of Plant Pathology. 70:73-80.

Turcotte, D., Kautzman, M., Peru, K, Headley, J., and Liber, K. 2009. Characterizing salt and naphthenic acids interaction in the toxicity of oil sands process water to freshwater invertebrates. SETAC Poster Presentation.

Whelly, M.P. 1999. Aquatic invertebrates in wetlands of the oil sands region of northeast Alberta, Canada, with emphasis on Chironomidae (Diptera). College of Graduate Studies and Research, University of Windsor, Windsor, Ontario, Canada.

Zhang, X., S. Wiseman, H.Yu, H. Liu, J.P. Giesy, and M. Hecker. 2011. Assessing the toxicity of naphthenic acids using a microbial genome wide live cell reporter array system. Environmental Science & Technology. 45: 1984-1991.

CHAPTER V: GENERAL DISCUSSION

Goals

The goals of this study were to determine a) how emerging chironomid abundance, biomass, and community composition differ between young reference and OSPW wetlands, b) how levels of conductivity and naphthenic acids in OSPW and reference wetland waters affect survival and growth of chironomids, and c) whether an interaction between naphthenic acids and salts affects chironomid sensitivity to the two potential toxicants.

Discussion of Key Findings

Chapter II

Neither the abundance nor the length of chironomids that emerged from OSPW wetlands differed from those emerging from reference wetlands. However, there was a non-significant trend towards smaller (shorter length) chironomids emerging from reference wetlands compared to OSPW wetlands. This was not expected given the water quality-related stress posed to chironomids in OSPW wetlands. Because the community composition between OSPW and reference wetlands did not significantly differ, the difference in size is unlikely to be attributable to different species inhabiting the wetland. However, the trend may be the result of increased predation by ducks or other predators. Some ducks, such as mallards, selectively forage on large chironomids (Batzer et al. 1993) so this foraging activity would make the average biomass of emerging chironomids smaller.

Mean length was significantly positively correlated with dissolved organic carbon concentration. This may be related to food quality. Bacterial biofilm is considered the most nutritious component of detritus and high dissolved organic carbon can result in greater bacterial biomass (Boon, 2006).

Although community composition did not significantly differ between reference and OSPW wetlands, the abundance of certain genera was correlated with certain water quality parameters. The abundance of *Einfeldia* was found to be positively associated with the addition of peat to the wetland whereas *Neozavrelia* and *Paracricotopus* were negatively associated with peat. *Einfeldia* also seemed to be more abundant in cool waters whereas *Neozavrelia* and *Paracricotopus* were more abundant in warm waters. *Tanytarsus* was found to be intolerant of low dissolved oxygen whereas *Ablabesmyia* seems to be abundant in low dissolved oxygen conditions. In contrast, *Tanytarsus* was found to be salt-tolerant and *Ablabesmyia* is salt-intolerant. This finding is supported by Cannings and Scudder (1978), who found that *Ablabesmyia* larvae were present only in waters with very low conductivities whereas *Tanytarsus* larvae were either present in all waters or only those with higher conductivities, depending on the species.

Chapter III

In the wetland water bioassay, survival was low and this may have confounded results. There were no significant effects of conductivity, naphthenic acids, or any significant interaction between the two in survival or biomass endpoints. However, there was a slight non-significant trend that chironomids survived better in high conductivity wetland

water. Among all chironomids, *Chironomus* is among the least salt-tolerant genera but it can be found in lakes with conductivities between about 400 and 4100 μS/cm (Cannings and Scudder, 1976), which fall within the approximate range of the conductivities of the wetland waters in this study. The trend towards higher survival in high conductivity wetland waters may be due to the decreased availability of metals when salts are present. The free metal ion is a metals most toxic form and the bioavailability, and thereby toxicity, is greatly reduced when metallic salts are formed (Hall & Anderson, 1995). Although pore waters of constructed wetlands may contain metal concentrations in excess of Canadian drinking water guidelines, there is no evidence that trace metals reach toxic concentrations in OSPW wetlands (Baker et al. 2012).

Chapter IV

In the salt assay, TDS had a negative effect on larval growth/final size. This finding is consistent with those of Hassell et al. (2006) who found that *Chironomus riparius* growth rates were reduced with increased conductivity. This result differs from my findings from Chapter II, which showed a non-significant trend towards larger size in wetland waters that had higher conductivity. In the combination bioassay, both TDS and NA had a significant, negative influence on survival, and there was a significant positive interaction between TDS and NA. This means that alone, salts and NAs may each negatively affect survival, but that there was an antagonistic interaction between these two toxicants. When salts and NAs occur together, chironomids survived better than would be expected if the individual effects were added together. This indicates that OSPW may be less toxic than individual laboratory toxicity tests may imply. No significant negative effect of either toxicant was found in the bioassay tests of whole wetland waters (Chapter III), and also

suggests that the two classes of compounds have mitigative effects on one another. This antagonistic interaction between salts and NAs has also been reported in *Ceriodaphnia dubia* (D. Turcotte, University of Saskatchewan, unpublished data and 2009). Turcotte (2009) proposed that salts caused NAs to precipitate out of solution.

Implications

There were very few differences in the size and length of chironomids emerging from OSPW wetlands compared to reference wetlands. Water chemistry factors that were not specific to OSPW apparently had a greater impact on emerging chironomid numbers than wetland class (OSPW or reference). Dissolved organic carbon had a positive correlation with mean emergent chironomid length. Future research might investigate the value of adding a source of dissolved organic carbon in the reclamation process to facilitate chironomid productivity.

Lower growth in high TDS conditions could be manifested as reduced population size of future generations. Larval size at pupation is highly correlated with eventual emergence success (Liber et al. 1996) and fecundity (Butler and Walker, 1992) so even slight changes could potentially influence future generations of chironomids. This lowered growth rate in high TDS conditions and lowered survival rate in both high TDS and high NA conditions also means that OSPW food webs may be less productive. Ganshorn (2002) analyzed chironomid productivity and food web structure using carbon isotope signatures in many of the same wetlands used in this study. He found no consistent differences between OSPW and reference wetlands in their productivity, but did find that

they had different food web structures. Not only do chironomids contribute to aquatic food web productivity as a food source for higher trophic organisms, but nearby terrestrial ecosystems will gain nutrient inputs from dead chironomid deposition in both plant and insect trophic levels (Hoekman et al., 2012).

Future Studies

There was a low survival rate in the pilot study related to Chapter IV, Chapter IV itself, and Chapter III. This may be due to use of newly hatched first instar chironomid larvae across all of these studies. Although it is common to use first instar larvae in aquatic toxicity tests, later instars tend to be less sensitive to handling stress and therefore may have a higher survival rate. Future studies using *Chironomus riparius* larvae should therefore use older larvae in order to protect against mortality caused by handling. *Chironomus riparius* may possibly be more tolerant than other benthic invertebrate species in the wetlands studied. This may explain the lack of significant differences in growth and survival observed in the naphthenic acid bioassay contrasting OSPW and reference wetland waters. Perhaps more a sensitive indicator of toxicity should be used in future in order to ensure that all invertebrates, no matter what their tolerance to pollution is, will be protected. Whelly (1999) found that *Chironomus dilutus* was slightly less tolerant of OSPW than *C. riparius*.

The mode of action for NA toxicity should be researched further in order to elucidate the mechanism behind the antagonistic relationship with salts if it is confirmed in other bioassays (those supporting higher survival overall). Turcotte (2009) speculated that NAs may precipitate out of solution in the presence of salt but showed no supporting evidence.

Alternatively, results from studies of stoichiometrically similar synthetic detergents (Abel, 1974) may help explain how NA causes toxicity through surfactant action and may shed light on the antagonistic interaction with salt. Surfactants have the potential to interact with proteins and alter the permeability of the cell membrane (Abe,l 1974). Surfactants also cause gill damage and death by asphyxiation (Abel, 1974). However, it is unknown if or how these mechanisms would be alleviated by the presence of salts.

Fresh naphthenic acids may have a different toxicity than the naphthenic acid stock solution used in this study as microbial decomposition can degrade and detoxify these acids. Fresh tailings are not often added to wetlands, but in order to investigate "worst-case scenario" situations, fresh naphthenic acids should be tested for toxicity. Recent studies also indicate that what has previously been thought to be naphthenic acids is actually a broad suite of acids and the composition of these acids differ greatly across the industry depending on refinement techniques used (Rowland et al., 2011). The identification of exactly what constitutes the acid extractable portion of OSPW is essential before toxicity tests can be done on these acids and reclamation procedures reducing the most toxic of these acids can take effect.

Of the 300+ exuviae counted, 257 were identifiable and thirty genera were found. This is on the lower end of typical community assembly studies. Researchers often have to choose between sampling a constant area sampled and collecting a constant number of insects. Because wetlands can be quite variable in chironomid abundances, sampling methods that use the constant number collected approach (such as 300 insects per

wetland) can provide more accurate assessments of community composition. In future studies, an equal number of chironomids per wetland (as opposed to an "equal area" sample) should be sampled and these should be counted in the field in order to ensure all genera have an equal opportunity to be accounted for.

Conclusion

Available data indicated that oil sands process water in itself did not negatively affect chironomid species composition, abundance, size at emergence, larval biomass, or survival. Water chemistry variables such as conductivity affected larval biomass and better accounted for the distribution of common genera among wetlands. Wetlands with higher dissolved organic carbon concentration supported larger chironomid pupae. Organic material, dissolved oxygen, and temperature were also correlated with which genera were collected. These limited results indicate that the OSPW-affected wetlands can support chironomid assemblages that are not consistently different from those in reference wetlands. However, there was limited laboratory evidence that NA and salt concentration individually reduced survival at high concentrations, and there was an antagonistic effect when the two were combined.

References

Abel, P.D. 1974. Toxicity of synthetic detergents to fish and aquatic invertebrates. Journal of Fish Biology 6: 279-298.

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

Batzer, D. P., M. McGee, V.H. Resh, and R.R. Smity. 1993. Characteristics of invertebrates consumed by mallards and prey response to wetland flooding schedules. Wetlands 13: 41-49.

Boon, P. I. 2006. Biogeochemistry and bacterial ecology of hydrologically dynamic wetlands. pages 125-126 *In* Ecology of Freshwater and Estuarine Wetlands edited by Batzer, D.P., and Sharitz, R.R. University of California Press.

Butler, M.G., and L. Walker. 1992. Fecundity, reproductive effort, and pupal size in the profundal midge *Chironomus cucini* (Diptera: Chironomidae). Netherlands Journal of Aquatic Ecology 26: 263-267.

Cannings, R.A., and G.G.E. Scudder.1978. The littoral Chironomidae (Diptera) of saline lakes in central British Columbia. Canadian Journal of Zoology 56: 1144-1155.

Ganshorn, K. Secondary production, trophic position and potential for accumulation of polycyclic aromatic hydrocarbons in predatory diptera in four wetlands of the Athabasca oil sands, Alberta, Canada. M.Sc. Thesis. College of Graduate Studies and Research. University of Windsor, Windsor, ON, Canada.

Hall Jr., L.W., and Anderson, R.D. 1995. The influence of salinity on the toxicity of the various classes of chemicals of aquatic biota. Critical Reviews in Toxicology. 25: 281-346.

Hassell, K. L., B.J. Kefford, and D. Nugegoda. 2006. Sub-lethal and chronic salinity tolerances of three freshwater insects: *Cloeon* sp. and *Centroptilum* (Ephemeropptera: Baetidae) and *Chironomus* sp. (Diptera: Chironomidae). Journal of Experimental Biology 209: 4024-4032.

Hoekman, D., M. Bartrons, and C. Gratton. 2012. Ecosystem linkages revealed by experimental lake-derived isotope signal in heathland food webs. Oecologia (in press).

Liber, K., D.J. Call, T.D. Dawson, F.W. Whiteman, and T. Dillon. 1996. Effects of *Chironomus tentans* larval growth retardation on adult emergence and ovipositing success: implications for interpreting freshwater sediment bioassays. Hydrobiologia 323: 155-167.

Rowland, S.J., A.G. Scarlett, D. Jones, C.E. West, and R.A. Frank. 2011. Diamonds in the rough: identification of individual naphthenic acids in oil sands process waters. Environmental Science and Technology 45: 3154-3159.

Turcotte, D., Kautzman, M., Peru, K, Headley, J., and Liber, K. 2009. Characterizing salt and naphthenic acids interaction in the toxicity of oil sands process water to freshwater invertebrates. SETAC Poster Presentation.

APPENDIX A: WATER QUALITY OF WETLANDS STUDIED IN CHAPTER II

Wetland Type	Wetland	Conductivity (µS/cm)	Salinity (ppt)	N.A. (mg/L)	D.O. (mg/L)	D.O.C. mg/L	Temp (C)	Organic Level (Peat=1, No Peat=0)
OSPM	4mCT	4523	1	41	4.6	86	18.7	1
OSPM	Test Pond 9	2067	1.1	20.4	11.6	48	19.4	0
OSPM	Mike's Pond	1936	2.4	26.2	9.5	43	19	0
OSPM	Natural	1234	0.6	64	4.4	74	22	1
Reference	High Sulphate	3025	1.6	12	6.4	39	22.2	1
Reference	NWID	701	0.3	0.2	3.3	29	19.3	1
Reference	Pond 5	1265	0.6	4	10.1	24	22.5	0
Reference	Shallow	371	0.2	2.3	8.7	18	17.6	0
Reference	V-notch	1004	0.5	7	6.3	22	19.3	1

APPENDIX B: ABUNDANCE OF EMERGENT CHIRONOMID GENERA OF NINE WETLANDS

	4mCT	Test Pond 9	Mike's Pond	Natural	High Sulphate	NWID	Pond 5	Shallow	V-Notch
Procladius	9	7	0	0	1	1	3	2	0
Tanytarsus	1	64	1	0	15	0	2	0	0
Ablabesmyia	0	1	0	4	1	4	0	0	0
Neozavrelia	0	3	0	1	1	0	3	0	0
Einfeldia	6	8	0	0	0	1	0	0	1
Guttipelopia	0	2	0	0	5	0	0	8	0
Paracricotpus	0	27	0	2	3	0	0	0	0
Tanypus	1	5	0	0	0	1	0	0	0
Diplocladius	0	3	0	0	0	1	0	0	0
Doithrix	0	1	0	0	0	1	0	0	0
Endochironomus	1	0	0	0	0	0	0	1	0
Labrundinia	0	0	0	0	2	0	0	0	1
Nanocladius	1	4	0	0	0	0	0	0	0
Neostempellina	0	2	0	0	1	0	0	0	0
Orthocladius	0	0	0	0	2	1	0	0	0
Paratanytarsus	2	0	0	0	1	0	0	0	0
Psectrocladius	0	11	0	0	0	0	0	0	1
Psectrotanypus	0	0	0	1	1	0	0	0	0
Xenopelopia	0	1	0	0	1	0	0	0	0
Zavrelia	0	0	0	0	8	0	2	0	0
Brillia	0	1	0	0	0	0	0	0	0
Chironomus	0	0	0	0	0	1	0	0	0

Cryptochironomus	0	0	0	0	1	0	0	0	0
Glyptotendipes	0	0	0	0	0	0	1	0	0
Lipinella	0	1	0	0	0	0	0	0	0
Monopelopia	0	0	0	0	0	1	0	0	0
Nilothauma	0	0	0	0	2	0	0	0	0
Omisus	0	0	0	0	0	1	0	0	0
Rheotanytarsus	0	1	0	0	0	0	0	0	0
Telmatopelopia	0	2	0	0	0	0	0	0	0

APPENDIX C: MAJOR IONS IN WETLAND WATERS USED IN CHAPTER III

	Major Cations (mg/L)					Major Anions (mg/L)				
Wetland	Na	K	Mg	Ca	NH_4	F	CL	SO_4	CO_3	HCO_3
Recycle Pond	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Trench 4*	291.0	9.7	20.7	36.6	0.3	0	100.0	118.0	36.30	538.0
Dyke 4 Reservoir*	217.0	7.3	45.5	127.0	0.2	0	25.0	542.0	0	359.0
4-m CT*	647	21.3	121	44.8	0.1	0	88	737	N/A	N/A
Test Pond 9	492	1.0	8.7	5.4	0.3	0	230	115	133	525
High Sulphate*	401	15.4	114	146	0.7	0	7.9	1410	0	144
Shallow Wetland	44.9	0.5	11.4	31.9	0.0	0	8.4	18.9	0	232
Natural Wetland*	383	17.8	15	22.1	2.2	0.82	28	64.5	27	834
V-Notch Weir*	76.8	5.9	35.8	114	0.2	0	1.3	312	N/A	N/A
Peat Pond	155	5.0	38.1	90.5	0.1	0	37.0	368	0.0	316
Golden Pond	208	5.3	57.9	142	0.3	0	45.0	783	0	221

Salt Marsh*	206.0	13.7	53.0	144.0	0.630	0.0	2.8	703.0	N/A	N/A
Mildred Lake	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Note: Wetlands marked with an asterisk (*) are from Martin (2010) Suncor Energy Ltd., unpublished data. All other wetland data are from MacKinnon, (2008), Syncrude Canada Ltd. N/A= Not Available

APPENDIX D: MEASURED WATER QUALITY PER JAR IN WETLAND WATER BIOASSAY 2011

Starting Water Chemistry

Wetland Water	Jar	Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Temperature (˚C)	Salinity (ppt)	Start Date	End Date
Mildred Lake	1	335	7.03	18.1	0.1	17-Jun-11	27/06/2011
Mildred Lake	2	325	7.05	18	0.2	18-Jun-11	28/06/2011
Mildred Lake	3	327	7.05	18.3	0.1	18-Jun-11	28/06/2011
Mildred Lake	4	343	6.45	18.3	0.1	17-Jun-11	27/06/2011
Mildred Lake	5	327	6.12	18.3	0.2	18-Jun-11	28/06/2011
Dyke 4 Resevoir	1	863	6.24	18.5	0.4	17-Jun-11	27/06/2011
Dyke 4 Resevoir	2	878	6.04	18.5	0.4	17-Jun-11	27/06/2011
Dyke 4 Resevoir	3	839	6.06	18.4	0.4	17-Jun-11	27/06/2011
Dyke 4 Resevoir	4	852	6.29	18.4	0.4	17-Jun-11	27/06/2011
Dyke 4 Resevoir	5	843	6.3	18.4	0.4	18-Jun-11	28/06/2011
Recycle Pond	1	3091	6.8	18.2	1.9	17-Jun-11	27-Jun-11

Recycle Pond	2	3647	3.52	18.5	1.9	17-Jun-11	28/06/2011
Recycle Pond	3	3632	6.23	18.5	1.9	17-Jun-11	27/06/2011
Recycle Pond	4	3122	5.09	18.5	1.6	17-Jun-11	27-Jun-11
Recycle Pond	5	3659	6.48	18.5	1.9	17-Jun-11	27/06/2011
Trench 4	1	797	6.24	18.4	0.4	17-Jun-11	27/06/2011
Trench 4	2	794	6.26	18.7	0.4	18-Jun-11	28/06/2011
Trench 4	3	792	6.22	18.4	0.4	17-Jun-11	27/06/2011
Trench 4	4	793	6.39	18.7	0.4	18-Jun-11	28/06/2011
Trench 4	5	797	6.06	18.4	0.2	17-Jun-11	27/06/2011
Peat Pond	1	1753	6.57	18.5	0.8	18-Jun-11	28/06/2011
Peat Pond	2	1737	6.69	18.6	0.4	18-Jun-11	28/06/2011
Peat Pond	3	1723	6.15	18.4	0.4	17-Jun-11	27/06/2011
Peat Pond	4	1756	6.57	18.5	0.9	18-Jun-11	28/06/2011
Peat Pond	5	1747	6.25	18.5	0.9	17-Jun-11	27/06/2011

Golden Pond	1	1869	6.5	18.5	1	18-Jun-11	28/06/2011
Golden Pond	2	1886	6.35	18.5	1	18-Jun-11	28/06/2011
Golden Pond	3	1877	6.32	18.4	1	18-Jun-11	28/06/2011
Golden Pond	4	1852	6.26	18.5	0.9	18-Jun-11	28/06/2011
Golden Pond	5	1890	6.36	18.5	1	18-Jun-11	28/06/2011
Salt Marsh	1	1234	6.06	18.5	0.6	17-Jun-11	27/06/2011
Salt Marsh	2	1252	5.8	18.4	0.6	17-Jun-11	27/06/2011
Salt Marsh	3	1243	6.11	18.5	0.6	18-Jun-11	28/06/2011
Salt Marsh	4	1230	6.29	18.5	0.6	18-Jun-11	28/06/2011
Salt Marsh	5	1229	6.32	18.5	0.6	18-Jun-11	28/06/2011

Ending Water Chemistry

Wetland Water	**Jar**	**Conductivity (µS/cm)**	**Dissolved Oxygen (mg/L)**	**Temperature (˚C)**	**Salinity (ppt)**	**End Date**
Mildred Lake	1	466	6.62	17.7	0.2	27/06/2011

Mildred Lake	2	460	6.51	16	0.2	28/06/2011
Mildred Lake	3	456	2.67	16.2	0.2	28/06/2011
Mildred Lake	4	425	6.82	17.5	0.2	27/06/2011
Mildred Lake	5	445	6.52	16.1	0.2	28/06/2011
Dyke 4 Resevoir	1	916	2.24	17.9	0.5	27/06/2011
Dyke 4 Resevoir	2	931	4.88	18	0.4	27/06/2011
Dyke 4 Resevoir	3	909	3.23	18	0.4	27/06/2011
Dyke 4 Resevoir	4	958	0.36	18	0.3	27/06/2011
Dyke 4 Resevoir	5	905	5.95	16.3	0.4	28/06/2011
Recycle Pond	1	3634	6.12	17.9	1.9	27-Jun-11
Recycle Pond	2	3605	6.35	16	2	28/06/2011
Recycle Pond	3	3678	6.23	17.8	1.9	27/06/2011
Recycle Pond	4	3744	6.58	17.9	2	27-Jun-11
Recycle Pond	5	3715	5.79	17.7	1.9	27/06/2011
Trench 4	1	857	6.66	17.7	0.4	27/06/2011
Trench 4	2	878	3.11	16.2	0.4	28/06/2011
Trench 4	3	852	6.41	17.5	0.4	27/06/2011
Trench 4	4	849	6.26	16.1	0.4	28/06/2011
Trench 4	5	857	6.27	17.8	0.4	27/06/2011
Peat Pond	1	1820	6.55	16	0.9	28/06/2011
Peat Pond	2	1806	4.74	16.3	0.9	28/06/2011
Peat Pond	3	1782	0.37	18	0.3	27/06/2011
Peat Pond	4	1817	5.64	16.1	0.9	28/06/2011

Peat Pond	5	1808	5.32	17.9	0.6	27/06/2011
Golden Pond	1	1935	6.46	16.1	1	28/06/2011
Golden Pond	2	1941	6.69	16.1	1	28/06/2011
Golden Pond	3	1933	7.05	16.2	1	28/06/2011
Golden Pond	4	1908	0.37	16.4	1	28/06/2011
Golden Pond	5	1944	6.54	16	1	28/06/2011
Salt Marsh	1	1275	2.41	17.9	0.6	27/06/2011
Salt Marsh	2	1291	6.72	17.7	0.6	27/06/2011
Salt Marsh	3	1305	1.09	16.3	0.7	28/06/2011
Salt Marsh	4	1304	6.77	16	0.7	28/06/2011
Salt Marsh	5	1285	6.6	16.3	0.3	28/06/2011

APPENDIX E: SURVIVAL AND MEAN FINAL SIZE IN WETLAND WATER BIOASSAY

Class	Wetland	Survival (# of larvae)	Percent Survival (%)	Length (mm)	Mean Biomass (mg)	Year	Naphthenic Acids (mg/L)	Conductivity (µS/cm)
OSPM	Dyke 4 Resevoir	0	0	N/A	N/A	2011	23.0	855
OSPM	Dyke 4 Resevoir	1	5	7.06	0.300	2011	23	855
OSPM	Dyke 4 Resevoir	7	35	8.47	0.483	2011	23	855
OSPM	Dyke 4 Resevoir	0	0	N/A	N/A	2011	23	855
OSPM	Dyke 4 Resevoir	11	55	7.97	0.412	2011	23	855
OSPM	Recycle Pond	17	85	6.01	0.197	2011	80	3430.2
OSPM	Recycle Pond	6	30	5.10	0.128	2011	80	3430.2
OSPM	Recycle Pond	12	60	5.29	0.141	2011	80	3430.2
OSPM	Recycle Pond	16	80	4.91	0.116	2011	80	3430.2
OSPM	Recycle Pond	0	0	N/A	N/A	2011	80	3430.2
OSPM	Trench 4	9	45	8.70	0.517	2011	22	794.6
OSPM	Trench 4	4	20	9.36	0.627	2011	22	794.6
OSPM	Trench 4	5	25	8.03	0.420	2011	22	794.6
OSPM	Trench 4	11	55	7.99	0.415	2011	22	794.6

OSPM	Trench 4	3	15	8.13	0.434	2011	22	794.6
OSPM	4mCT	0	0	N/A	N/A	2010	24.9	4523
OSPM	4mCT	0	0	N/A	N/A	2010	24.9	4523
OSPM	4mCT	16	80	10.61	0.869	2010	24.9	4523
OSPM	4mCT	14	70	10.05	0.755	2010	24.9	4523
OSPM	4mCT	7	35	8.92	0.552	2010	24.9	4523
OSPM	4mCT	1	5	10.22	0.788	2010	24.9	4523
OSPM	Natural	13	65	9.19	0.597	2010	11.7	1233.8
OSPM	Natural	4	20	10.66	0.880	2010	11.7	1233.8
OSPM	Natural	5	25	8.64	0.508	2010	11.7	1233.8
OSPM	Natural	5	25	7.80	0.389	2010	11.7	1233.8
OSPM	Natural	13	65	10.93	0.940	2010	11.7	1233.8
OSPM	TP9	8	40	8.67	0.514	2010	20.4	2067.4
OSPM	TP9	2	10	9.25	0.607	2010	20.4	2067.4
OSPM	TP9	17	85	9.22	0.603	2010	20.4	2067.4
OSPM	TP9	3	15	9.35	0.624	2010	20.4	2067.4
OSPM	TP9	0	0	N/A	N/A	2010	20.4	2067.4
Reference	Mildred Lake	1	5	6.07	0.201	2011	0.9	331.4
Reference	Mildred Lake	8	40	8.74	0.524	2011	0.9	331.4
Reference	Mildred Lake	9	45	7.54	0.356	2011	0.9	331.4
Reference	Mildred Lake	0	0	N/A	N/A	2011	0.9	331.4
Reference	Mildred Lake	6	30	9.29	0.614	2011	0.9	331.4

Reference	Peat Pond	10	50	8.17	0.439	2011	1	1743.2
Reference	Peat Pond	8	40	7.17	0.312	2011	1	1743.2
Reference	Peat Pond	3	15	6.58	0.249	2011	1	1743.2
Reference	Peat Pond	4	20	5.00	0.121	2011	1	1743.2
Reference	Peat Pond	5	25	5.48	0.155	2011	1	1743.2
Reference	Golden Pond	10	50	7.40	0.338	2011	3.7	1874.8
Reference	Golden Pond	16	80	7.24	0.320	2011	3.7	1874.8
Reference	Golden Pond	12	60	8.40	0.472	2011	3.7	1874.8
Reference	Golden Pond	3	15	7.38	0.336	2011	3.7	1874.8
Reference	Golden Pond	12	60	9.71	0.689	2011	3.7	1874.8
Reference	Salt Marsh	4	20	8.11	0.431	2011	5.0	1237.6
Reference	Salt Marsh	2	10	9.76	0.700	2011	5.0	1237.6
Reference	Salt Marsh	0	0	N/A	N/A	2011	5.0	1237.6
Reference	Salt Marsh	10	50	8.57	0.497	2011	5.0	1237.6
Reference	Salt Marsh	5	25	8.50	0.486	2011	5.0	1237.6
Reference	Vnotch	3	15	11.14	0.987	2010	5.7	1003.6
Reference	Vnotch	5	25	9.42	0.638	2010	5.7	1003.6
Reference	Vnotch	14	70	11.99	1.197	2010	5.7	1003.6
Reference	Vnotch	0	0	N/A	N/A	2010	5.7	1003.6
Reference	Vnotch	10	50	N/A	N/A	2010	5.7	1003.6
Reference	High Sulphate	14	70	11.27	1.018	2010	17.8	3025.2

Reference	High Sulphate	6	30	11.14	0.988	2010	17.8	3025.2
Reference	High Sulphate	11	55	11.41	1.052	2010	17.8	3025.2
Reference	High Sulphate	6	30	11.38	1.044	2010	17.8	3025.2
Reference	High Sulphate	0	0	N/A	N/A	2010	17.8	3025.2
Reference	Shallow	8	40	10.65	0.877	2010	2.3	370.54
Reference	Shallow	7	35	11.95	1.187	2010	2.3	370.54
Reference	Shallow	10	50	10.91	0.936	2010	2.3	370.54
Reference	Shallow	6	30	9.91	0.727	2010	2.3	370.54
Reference	Shallow	0	0	N/A	N/A	2010	2.3	370.54

APPENDIX F: MEASURED WATER QUALITY FOR CHAPTER IV TEST JARS

Rep	Treatment	Initial Temp. (˚C)	Conductivity (µS/cm)	DO (mg/L)	pH	Final Temp. (˚C)	Conductivity (µS/cm)	DO (mg/L)	pH
1	0NA (Control)	22.84	464	7.35	8.42	22.77	532	7.86	7.62
2	0NA (Control)	23.21	462	6.56	8.38	23.44	485	7.1	7.81
3	0NA (Control)	22.44	482	8.23	8.01	22.8	572	7.86	7.8
1	13NA					23.62	465	7.32	7.62
2	13NA	22.7	459	7.1	8.45	23.11	550	7.52	7.85
3	13NA	22.77	484	10.79	7.47	22.3	555	8.84	7.51
4	13NA					22.33	542	11.06	7.43
1	22NA	22.78	472	7.07	8.32	22.71	566	7.15	7.55
2	22NA	22.77	470	6.47	8.47	23.33	562	7.6	7.82
3	22NA	22.88	480	8.2	7.95	22.42	550	8.23	7.76
4	22NA	22.91	497	8.3	7.71	21.48	558	8.1	7.63
1	36NA	23.03	477	6.24	8.45	22.9	554	7.17	7.78
2	36NA	23.36	473	6.01	8.49	23.67	556	7.62	7.76
3	36NA	22.42	429	8.61	7.78	22.11	570	8.17	7.74
1	60NA	22.6	485	7.16	8.37	22.41	562	8.07	7.71
2	60NA	23.48	490	7.01	8.44	23.52	413	7.37	7.72
3	60NA	22.05	500	8.52	7.53	22.07	566	8.56	7.66
1	100NA	22.87	512	7.23	8.55	22.53	590	7.68	7.81
2	100NA	23.24	496	7.07	8.36	24.55	588	7.76	7.49
3	100NA	21.85	510	8.53	7.77	22.04	583	8.5	7.62
4	100NA	22.9	532	7.21	7.9	22.04	599	8.66	7.73
1	0S (Control)	23.2	480	7.16	8.3	23	568	7.74	7.72

2	0S (Control)	23.16	462	6.74	8.23	24.25	450	8.01	7.39
3	0S (Control)	21.97	478	7.96	7.84	21.93	537	8.65	7.69
1	13S	22.44	706	7.3	8.45	22.77	733	7.79	7.91
2	13S	23.22	715	6.87	8.31	23.71	784	7.61	7.79
3	13S	22.79	713	8.16	7.91	21.67	755	8.4	7.81
4	13S	22.06	711	7.41	7.89	21.75	748	8.07	7.73
1	22S	22.77	891	7.04	8.24	22.57	937	7.87	7.86
2	22S	22.89	984	8.17	7.88	22.69	1034	8.67	7.97
3	22S	22.61	889	7.38	7.92	22.74	919	8.07	7.91
1	36S	22.66	1133	6.92	8.15	23.78	1194	8.19	7.89
2	36S	23.19	1106	6.77	8.33	23.98	1172	7.4	7.95
3	36S	22.67	1145	8.57	7.95	22.83	1266	7.93	8.05
1	60S	22.7	1534	6.72	8.29	22.59	1617	7.63	8.1
2	60S	23.97	1528	7.07	8.18	23.21	1573	7.15	8.08
3	60S	22.89	1555	8.24	8.01	22.02	1583	8.05	8.1
1	100S	22.99	2208	6.6	8.24	23.37	2136	7.64	8.25
2	100S	22.79	2246	7.7	7.78	22.74	2275	8.33	8.02
3	100S	23.04	2217	8.85	8.03	22.08	2297	8.23	8.14
4	100S	23.12	2269	8.73	8.08	21.41	2281	8.18	8.2
1	0NA + 0 S (Control)	23.25	476	7.14	8.33	23.05	447	7.89	7.3
2	0NA + 0 S (Control)	23.2	466	6.86	8.41	23.58	525	7.17	7.7
3	0NA + 0 S (Control)	23.11	465	7.94	7.85	22.57	536	7.98	7.71
4	0NA + 0 S (Control)	22.08	470	7.58	7.91	22.31	530	7.66	7.64
1	0NA + 100S	22.53	2211	7.08	8.36	23.18	2294	7.35	8.19

2	0NA + 100S	23.44	2282	7.53	7.77	23.55	2406	9.45	8.19
3	0NA + 100S	22.51	2228	7.8	7.9	21.95	2269	8.04	8.13
1	100NA+ 0 S	22.8	492	6.81	8.44	23.94	447	7.61	7.56
2	100NA+ 0 S	23.52	541	7.29	8.41	25.04	622	7.56	7.79
3	100NA+ 0 S	22.81	538	7.97	7.77	22.07	573	8.56	7.59
4	100NA+ 0 S	22.94	536	8.01	7.74	21.63	595	8.16	7.64
1	13NA+ 60S	22.72	1539	6.55	8.3	22.93	1602	7.53	8.07
2	13NA+ 60S	22.88	1575	6.95	8.2	24.54	1641	7.86	8.14
3	13NA+ 60S	22.44	1504	8.64	7.71	22.15	1606	8.87	7.89
4	13NA+ 60S	22.97	1575	7.55	8.04	21.79	1604	8.24	8.05
1	22NA + 36S	22.55	1119	7.05	8.24	22.85	1188	7.16	7.92
2	22NA + 36S	23.33	1149	6.85	8.39	23.79	1051	7.42	7.87
3	22NA + 36S	22.59	1076	7.73	7.96	21.71	1122	8.74	7.94
4	22NA + 36S	23.13	1143	7.51	7.94	21.76	1191	8.48	7.9
1	36NA+ 22S	22.87	903	6.33	8.33	22.72	1019	8.66	7.71
2	36NA+ 22S	22.97	902	6.52	8.32	23.13	860	7.66	8
3	36NA+ 22S	22.75	888	8.43	7.78	22.61	939	8.33	7.82
4	36NA+ 22S	21.91	900	8.41	7.86	22.91	955	8.29	7.88
1	60NA + 13 S	22.7	736	7.03	8.36	22.67	678	7.35	7.83
2	60NA + 13 S	22.61	709	6.63	8.44	22	793	7.58	7.88
3	60NA + 13 S	22.63	731	7.35	8.01	21.64	787	8.66	7.97
4	60NA + 13 S	23	744	8.33	8.01	21.84	800	8.44	7.88

APPENDIX G: SURVIVAL AND MEAN LENGTH

FOR CHAPTER IV BIOASSAYS

Salt Assay

TDS (mg/L)	Rep	Mean Length (mm)	% Survival
335	1	8.94	15
335	2	9.77	10
335	3	7.16	30
523	1	9.88	5
523	2	7.98	10
523	3	7.08	45
523	4	7.40	15
651	2	8.32	5
651	3	6.71	20

853	1	8.86	10
853	2	8.79	20
853	3	7.61	35
853	4	8.20	50
1199	1	6.27	10
1199	2	8.64	15
1199	3	9.53	10
1774	1	6.63	5
1774	2	4.27	15
1774	3	6.63	10
1774	4	5.85	10

NA Assay

NA (mg/L)	Rep	Mean Length (mm)	% Survival

0	1	8.04	5
0	2	10.99	5
0	4	6.71	15
10	1	6.14	5
10	2	7.52	5
10	3	6.04	15
10	4	7.00	60
18	2	7.03	15
18	4	9.05	45
29	1	9.73	5
29	2	7.84	35
29	3	9.65	10
29	4	8.28	80
48	3	7.08	20

48	4	7.54	65
80	2	7.12	5
80	3	8.92	5

Combination Bioassay

NA Concentration	TDS	Rep	Average Length (mm)	% Survival
0	335	1	9.13	20
0	335	2	6.36	35
0	335	3	8.00	20
0	335	4	5.84	25
0	1774	1	8.87	5
0	1774	2	9.01	15
0	1774	3	7.57	5
10	1199	2	5.38	25
10	1199	3	7.65	15
10	1199	4	6.62	35
18	853	2	6.91	60
18	853	3	10.68	20
18	853	4	6.82	15
29	651	1	10.61	5
29	651	2	6.79	10
29	651	3	4.88	10
29	651	4	6.89	20

48	523	3	9.47	5
48	523	4	8.91	10
80	335	3	6.33	5
80	335	4	4.55	5

VITA AUCTORIS

Name:	Kaitlin D. Kennedy
Place of Birth:	Windsor, Ontario
Year of Birth:	1987
Education:	F.J. Brennan Catholic High School Windsor, Ontario 2001-2005
	University of Guelph B.Sc. Biological Sciences, Honours Guelph, Ontario 2005-2009
	University of Windsor M.Sc. Biological Sciences, Windsor, Ontario 2009- 2012