

**Aquatic Invertebrates in Wetlands of the  
Oil Sands Region of Northeast Alberta, Canada,  
with Emphasis on Chironomidae (Diptera)**

by

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**A Thesis**

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## Abstract

This thesis describes the effects of oil sands process waters (OSPW) on the benthic macroinvertebrate component of wetland ecosystems through assessment of biological responses at various levels of organization. This was part of an integrated study of multiple biotic components designed to assess a wet landscape reclamation approach being considered by the oil sands mining industry in the Fort McMurray area of northeastern Alberta.

Comparison of the macroinvertebrate communities among OSPW-affected wetlands and environmentally-similar reference wetlands indicated an insignificant trend of reduced taxonomic richness and abundance. Chironomid generic richness was similarly reduced in relation to OSPW input. Also, the general benthic macroinvertebrate community and specifically the chironomid community showed differences in community composition between OSPW and reference wetlands, in terms of the presence and relative abundances of dominant taxa. However, OSPW did not influence rates of oviposition or numbers of adult chironomids caught in shoreline colonization pans.

The incidence of chironomid mouthpart deformities, a biomarker of teratogenicity, was low (3.7 % or less) at three pairs of reference and OSPW wetlands. Similarly, there was no evidence to show that OSPW induces mouthpart deformities, based on a 14 d static sediment bioassay using two traditionally-used laboratory species (*Chironomus tentans* and *C. riparius*) and a population of *C. tentans* which originated from a wetland associated with oil sands mining. However, growth and survival were significantly reduced in both lab and field-derived *C. tentans* larvae exposed to higher concentrations of OSPW.

Development times of *C. riparius* larvae, and to a lesser extent, pupae, were increased in higher OSPW treatments. A moderate degree of tolerance to OSPW was suggested by the responses of the field population.

The toxic effects of OSPW shown in laboratory chironomids may be related to the slightly reduced taxa richness observed at the macroinvertebrate community. The field response may be dependent on other factors including concentration of dissolved organic carbon, wetland trophic status, and biotic interactions, as well as tolerance in field populations of macroinvertebrates. These results support the use of wetlands as a feasible reclamation option.

*For Rusty,  
and the rest of my great family.*

There are those that give with joy,  
and that joy is their reward.  
And there are those who give with pain,  
and that pain is their baptism.  
And there are those who give and know not pain in giving,  
nor do they seek joy, nor give with mindfulness of virtue;  
They give as in yonder valley the myrtle breathes its fragrance into space.  
Through the hands of such as these God speaks,  
and from behind their eyes God smiles upon the earth...  
For in truth it is life that gives unto life -  
while you, who deem yourself a giver, are but a witness.  
- Kahlil Gibran, *The Prophet*

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## **CHAPTER 1 - INTRODUCTION**

### **General Introduction**

Ecotoxicology is the science involved with the study of contaminant effects in ecosystems, thereby combining the disciplines of biochemistry and ecology (Moriarty 1990). It involves the assessment of the direct toxic effects and the indirect physico-chemical effects imposed by contaminants on living beings, but oft forgotten is consideration of the resulting changes in ecological interactions and relationships among these organisms (Swanson 1999). Contaminants are defined here as anthropogenic stresses including chemicals, radioactive and thermal energies, as well as physical stressors, which are imposed on the environment. The use of biological responses (biomarkers) of organisms to assess environmental quality is known as biomonitoring (Rosenberg and Resh 1993, Newman 1998).

Specifically, wetlands in the Athabaskan oil sands mining region of northern Alberta are the focus of this ecotoxicological study. Through a combined effort over several years, including researchers from five universities as well as from the private sector, an integrated assessment of the viability of wetlands receiving oil sands-related waters will be conducted, based on multiple biotic components of the ecosystem. As the significance of change in a community inevitably involves value judgments, be they of economic, scientific, aesthetic, or social nature, criteria delineating an acceptable state of viability must be set. Thus, viability is considered here to be a reflection of the level of biodiversity and production of the ecological community at suitable reference sites (Moriarty 1990).

The objective of this thesis is to assess the possible effects of oil sands mining and development / reclamation on the benthic macroinvertebrate community of wetlands, with particular emphasis on Chironomidae (Diptera).

This thesis evaluates the biological significance of stresses at multiple scales of biological organization. To achieve this, a suite of mining-affected wetlands and suitable (environmentally similar) reference wetlands was selected. The general benthic invertebrate as well as the chironomid communities were then examined, followed by examination of a biomarker, chironomid mouthpart deformities, in field-collected specimens. Life cycle attributes of chironomids were also assessed, in terms of adult behaviour and egg development. Finally, a laboratory bioassay was conducted, exposing field and laboratory chironomid populations to waters from the oil sands extraction process. The various measures described above will together provide an estimate of wetland viability.

This thesis contains seven chapters. This current chapter introduces the principal concepts involved with this thesis, including general biomonitoring, the use of chironomids as bioindicators and their biomarkers (particularly deformities in chironomid mouthparts). It will also provide essential background information pertaining to the habitats under study.

Chapter two examines the process of wetland site selection and characterization of environmental attributes, leading to the grouping of wetlands of similar environment. The results of the analysis of the benthic community, and the chironomid community in particular, is also shown here. The use of multivariate statistics allows for the determination of contaminant effects taking into account the natural heterogeneity in community composition related to differences in environment (e.g., biotic and abiotic factors related to

habitat, microclimate, food, distributions).

To assess sublethal teratogenic effects, chironomid mouthpart deformities were used as a biomarker of development, which is the topic of the third chapter. Following the examination of community level effects (presence and numbers of biota) in chapter 2, this chapter further examines the possible effects of exposure to oil sands-related waters, this time in relation to sublethal effects on chironomids in the field.

Following the measurement of the incidence of deformities in the natural chironomid populations of oil sands and reference wetlands, the question of local adaptation vs. bioavailability was examined in chapter 4. To answer this question, a field (*in situ*) bioassay using a single chironomid species was conducted, where levels of deformities as well as rates of growth and survival could be directly compared between reference and mining-related wetlands without confounding based on differences in interspecific sensitivity.

To further examine the effects of oil sands contaminants on chironomid development and survival and possible adaptation of chironomids, a laboratory bioassay was conducted. Chapter 5 assesses possible differences in sensitivity between two laboratory-reared and a field population to oil sands process water (OSPW).

The current study is important in anticipating future reclamation success of wetlands constructed to receive and detoxify OSPW and how colonizing chironomids will respond to these newly available habitats of wetlands and lakes. Chapter 6 examines the response of different life stages (adults, eggs) of midges to OSPW, in order to provide some insight into the potential colonization of future OSPW wetlands by gravid adults. Behavioural responses of adult midges (flight activity) to OSPW will be discussed, in conjunction with

reproductive effects including oviposition and egg hatching.

The last chapter relates all of the above studies to provide an overall summary, discusses the significance of findings, and also points to areas of research requiring continued effort.

In the case that the constructed wetlands receiving mining inputs are not biologically viable, reduced benthic community richness and altered community composition is expected, as well as possibly increased induction of mouthpart deformities related to metals and polycyclic aromatic hydrocarbons (PAHs) in mine waste, compared to reference sites. Also, some degree of tolerance in chironomids of oil sands wetlands which would not be observed in reference site chironomids may be present. Likewise, laboratory-bred populations are expected to show greater response to OSPW than field-collected chironomids.

### **Biomonitoring**

There are numerous ways of assessing the effects of contaminants in ecosystems. These include chemical analyses of biological and environmental samples, surveys of field populations, and the use of biomonitoring in both the field and the laboratory. It is useful to determine the distributions and concentrations of contaminants found both in biota and their environment, and this may be used to predict the conditions, locations, and organisms in which effects may occur. However, this does not directly provide information regarding adverse biological effects which is a main objective in environmental assessments. The study

of biological responses is validated by the fact that bioavailability factors, exposure regime, interaction effects of chemical mixtures, and differences in sensitivity (interspecific, but also intraspecific) are all integrated in the organism (Moriarty 1990).

Biomonitoring is the scientific process used to evaluate exposure and effects of environmental stress on specified organisms (bioindicators) through observation of their biological responses under specified conditions (Rosenberg and Resh 1993). Biomarkers are the observed physiological, histological, biochemical, or morphological responses within organisms, which imply the presence of biologically significant pollution or an early warning sign of imminent effects (Newman 1998).

Of course, induction of effect depends on a sufficient amount of the chemical reaching the targets within the organisms. This amount is related to the bioavailability of the chemical(s). Bioavailability refers to the degree to which a chemical in the environment is available for uptake by the organism, and is dependent on environmental conditions (Chapman 1997). Factors that determine distribution of contaminants among the various media (food, sediment particles, organic matter, various water phases) as well as chemical fate (biotic and abiotic degradation processes, and biological uptake/ metabolism/ storage/ excretion) are relevant (Wang and Fisher 1999). Bioavailability is affected mainly by pH, dissolved oxygen, redox potential, organic carbon, oxides of iron and manganese, and particularly chemical speciation and acid volatile sulfides for metals (DiToro et al. 1990, Chapman 1997). Levels of other chemicals present, which may bind to or compete for uptake with the specific chemical are also important. Sedimentary and dissolved organic matter (including humic acids), strongly affect uptake of organic toxicants, but pH,

dissolved oxygen, and the specific lipophilicity (manifested as the bioconcentration factor) also play a role (Twiss et al. 1999, Weinstein and Oris 1999). The complexity of these factors supports the use of biological organisms to assess aquatic systems.

Initiating from biomolecular interaction (unless damage is of a general narcotic or physical nature), contamination can result in a wide range of effects, from altered individual physiology to altered population genetic makeup or size structure, to changes in community composition and function. These changes, when adequately understood, can be used to monitor environmental conditions (Newman 1998).

Fish, amphibians, macrophytes, algae, birds, whales, and aquatic invertebrates have all been studied to assess aquatic systems (Niimi 1990, Rosenberg and Resh 1993, Russell et al. 1999). Specifically, benthic invertebrates are used in a myriad of ways in evaluating the state of the environment, including their use in biotic indices, as bioindicator species, in biomonitoring programs, toxicity bioassays, and studies of trophic transfer and biochemical mechanisms of metabolism/fate/behaviour. Zoobenthos, which include various aquatic insects (stoneflies, caddisflies, chironomids, mayflies), oligochaetes, amphipods, and snails, are associated with both aquatic and terrestrial foodwebs (Hudson and Ciborowski 1995).

The use of a single indicator species (or life stage) alone is not recommended as it is unrealistic to assume that any one species can detect and be most sensitive to all stressor types (Moriarty 1990). Community indices can be useful in assessing habitats of comparable species, but can be insensitive where richness is low. Some indices consider identity (and therefore the specific ecological requirements) of species indicative of particular environmental conditions. The use of several bioassessment tools that can measure both

acute and chronic effects can be more effective than any single test in monitoring ecosystems, especially those of which we have very limited understanding (Rosenberg and Resh 1993).

Assessment of effects can be conducted directly in the field or in lab, with often quite different results (Canfield et al. 1994). While field studies maintain realism but lack control of numerous variables, laboratory studies allow for control but lack realism. The two practices have thus become complementary allies used to discern meaningful from meaningless, which is again a value judgment. Field studies are useful in documenting large-scale processes and validating lab results, whereas the latter is essential in testing hypotheses developed from these field studies.

*In situ* toxicity testing permits the semi-controlled assessment of contaminant effects and allows for the natural fluctuations in exposure seen in nature, thereby linking laboratory and field scenarios (Chappie and Burton 1997).

Laboratory studies are conducted under conditions of relatively constant environment, thereby simplifying the nature of the test as well as interpretation of results. Endpoints in bioassays can include rates of survival, growth, development, and reproduction of the test organisms (ASTM 1990).

### *1) Community Level Response*

The community response has been used to assess the biological significance of changes in the environment. However, because community level changes can be quite general, their significance may be unclear. Also, sublethal effects may occur that are not

evident through this observational 'lens', indicating the need to examine effects in closer detail. Changes in community composition appeared to be better indicators of early toxic stress relative to changes in function (Howarth 1990). But what does replacement of one species by another signify to the system? This underlines the need to understand the ecology of the system in order to understand the implications of changes in community composition. Variation rather than absolute measurements have been suggested as a more valid measure of effect (Howarth 1990).

We assume that a more diverse system is more robust and therefore more desirable as an ecosystem objective (Moriarty 1990). High species diversity is generally associated with a more stable, resilient and productive communities (e.g. tropical rainforests)(Elton 1958). However, a high level of diversity is not always necessary for the development of a highly productive or stable ecosystem, as witnessed by the low-diversity but highly productive redwood forests of California (Moriarty 1990). Also, several diverse systems have responded in similar fashion to systems of low diversity, through the loss of sensitive species (Schindler 1987). This implies that, although we may observe changes in levels of diversity and in predominant taxa present, it is difficult to conclude that serious harm has been manifested without further information (Howarth 1990, Moriarty 1990). Spatial and temporal variation in population distribution are important factors to consider in relation to an observed snapshot change in community structure.

Nonetheless, by considering changes at multiple levels of biological organization, we may associate changes at the community level to those of populations and biomarkers, in a comprehensive biomonitoring approach (Rosenberg and Resh 1993).

## ***2) Chironomids as Assessment Tools***

Several taxa have been identified as bioindicators, species which are present under defined environmental conditions (Clements et al. 1990). Knowledge of species tolerances can thus lead to rapid assessment of sites (Armitage and Blackburn 1985).

Midges (Diptera: Chironomidae) are holometabolous insects and thus pass through four life stages, three of which are aquatic (egg, larva, and pupa) and one terrestrial (winged adult). Duration of stages are species-specific. In general, the adult stage is very short (several days), during which mating (but not feeding usually) occurs (Oliver 1971). Females then oviposit egg masses containing hundreds of eggs on or under the water surface. Within a few days, pelagic first instar larvae hatch, and shortly thereafter molt to second instar. The majority of the chironomid life cycle is lived as benthic second to fourth instars, during which time the larvae are in intimate contact with the sediment (Oliver 1971). Many species are detritivorous, though some are carnivorous, omnivorous, or herbivorous. Tube houses are commonly constructed within this family (Pinder 1986).

Chironomid larvae are one of the most widely used benthic invertebrate groups studied, as they fulfill all requirements of a good bioindicator of sediment contamination. These include a ubiquitous distribution, significant links to both aquatic and terrestrial energy and nutrient flow, broad range of sensitivities to various environments, sensitivity to various contaminants, sessile benthic nature (for most of their life), well studied ecology and biology, and ease in field collection and rearing of cultures in laboratory (Wiederholm 1984, Ciborowski et al. 1995). As many persistent organic chemicals and metals become bound to organic constituents in sediments, exposure (and uptake) of these compounds is often quite

high in larvae, especially in detritivores (Harkey et al. 1994, Russell et al. 1999).

Chironomids are thus often used as indicator species, aiding in water quality assessment and characterization of certain conditions, in cytological studies and sediment bioassays, as well as in biomarker studies (Woods et al. 1989, ASTM 1990, Warwick 1990, Bedard et al. 1992).

### *3) Biomarkers*

Adverse effects can occur at different levels of organization than those observable at that of the community. Populations can persist, maintaining species diversity, but stress effects on individuals can alter their normal development and life history. This can be manifested in numerous ways including smaller bodies (affecting predator-prey relationships), altered behaviour, and less energy-efficient metabolism/physiology, ultimately resulting in a slower developing, less fit, or less productive population (Newman 1998).

Biomarkers can be used to enhance toxicity assessment through demonstration of sublethal effects which may complement population level responses. The most effective biomarkers are simple, inexpensive, predictive of adverse effects at higher contamination levels, show a concentration-effect relationship, and are understood in terms of factors that influence its response (Newman 1998). They can also be specific to specific toxicant(s) and related to reduced individual fitness

In some cases, biomarkers have been shown to be more sensitive as signals of toxic effects than more coarse-scale evaluations (van Urk et al. 1992), although the opposite has also been reported (Pardalis 1997). This is expected since effects at the suborganismal level

may not be seen as effects in individuals or populations until more severe contaminant stress is present (Petersen and Petersen 1983, Warwick 1985, Newman 1998). This may be due to differences in tolerance (acclimated and adapted) among individuals and among populations, as only the most sensitive would respond initially. Furthermore, in cases where contaminants may biomagnify, the measurement of sublethal effects in invertebrates may aid in anticipating more serious ecosystem-level effects at higher trophic levels (Hudson and Ciborowski 1995).

In hopes of predicting and avoiding serious damage to the environment, biomarkers can be used along with other measures of population and community effects to afford a deeper understanding of contaminant exposure and biological effects.

#### *4) Chironomid Biomarkers*

Chironomids have been well studied among aquatic invertebrates, and both biochemical and morphological biomarkers are some of the technological fruit of these effort.

Biochemical biomarkers in chironomids include enzyme activity, mixed function oxidases, acetylcholinesterase and general esterases, as well as polytene chromosome puff activity and the production of stress heat shock proteins (Hoffman and Fisher 1994, Hudson and Ciborowski 1995).

Morphological deformities of various headparts (antennae, pecten epipharyngis, mandibles, menta, ligulae) as well as thickened body cuticle and darkened head capsules in chironomids have been used as biomarkers of contaminant toxicity, and varying degrees of

sensitivity have been attributed to each structure (Hamilton and Saether 1971, Warwick 1988, 1990).

### **Chironomid Deformities**

Chironomid deformities may be becoming increasingly common relative to the past, based on subfossil analysis of *Chironomus* and *Procladius* head capsules (Warwick 1980a). Chironomid populations from present-day reference sites, considered relatively uncontaminated, can vary widely in incidence of deformities but generally exhibit between 0 and 4 % deformities (Cushman 1984, Wiederholm 1984, Dermott 1991, Dickman et al. 1992, Groenendijk et al. 1998). Burt (1998) reported a mean mentum deformity level of <3.25% for 5 genera examined from 335 reference sites within the Great Lakes of North America. However, appropriate reference sites must always be used to assess contamination, due to natural differences in background incidence from region to region (Burt 1998). For example, four of five relatively clean sites in the Experimental Lakes Area ranged from 10-16% deformed larvae, with another having no deformed individuals, indicating the variability in natural background levels (Bird et al. 1995).

Studies of spatial variation in incidence of chironomid deformities have been applied in the assessment of lotic and lentic systems. Values of 6% and often much higher indicate probable contamination with toxic metals or organic compounds, but not environmental stress, although environmental conditions that introduce stress can play a strong role in the end result of exposure to teratogens (Wiederholm 1984, Dermott 1991, Dickman et al.

1992, Lenat 1993, Canfield et al.1994, Lester et al. 1994, Burt 1998, Groenendijk et al. 1998).

### *1) Types of Deformities*

There are several structures typically used as teratogenicity biomarkers. Some effort has been made to link particular deformities with exposure to specific contaminant classes, but this has not been clearly shown (Warwick 1990). Antennae show the greatest susceptibility to deformities, followed by menta/ligulae (depending on subfamily), and least sensitive were the mandibles (Warwick 1988). This is consistent with a quantal dose-response hypothesis where various headparts show varying sensitivities based on particular ranges of chemical contamination (Warwick 1989). This pattern was found by Janssens de Bisthoven et al. (1995), who also documented that deformities in antennae and menta were independent of one another. Although mouthparts are probably less sensitive than antennae to contaminant stress, the former do not break as easily and are easier to clearly observe (Warwick 1985, Dermott 1991).

### *2) Mouthpart Deformities*

Chironomid mouthpart (mentum or ligula, depending on the subfamily) deformities have been related to a variety of anthropogenic stresses (pesticides, metals, PAHs, PCBs, and other organic chemicals) in both field and lab settings (Hamilton and Saether 1971, Warwick 1980a, 1987, 1989, 1990, Cushman 1984, Dermott 1991, Dickman et al. 1992, Janssens de Bisthoven et al. 1992, 1998, Lenat 1993, Muir 1993, Cervi 1996, Hudson and

Ciborowski 1996a, 1996b, Burt 1998).

A log-linear relationship between concentration of contaminated sediment containing persistent organics, trace metals, pesticides, organic solvents from the Detroit River and incidence of mentum deformities was observed in *Chironomus salinarius* in the laboratory (Hudson and Ciborowski 1996b). In a bioassay, Cervi (1996) related cadmium exposure to increased frequency of mentum deformities in individually-reared *Chironomus riparius*. She found that deformed larvae experienced delayed development and reduced emergence success relative to normal larvae, and as a result of this, the deformed individuals were larger as pupae. The presence of a deformity was observed to change between instars of individual larvae (Cervi 1996). This suggests that exposure to contaminants is required at each instar for a deformity to appear.

More seriously deformed individuals (multiple cases, or grossly malformed teeth) tend to be found at sites with the highest proportion of deformed individuals (van Urk et al. 1992), although often the highest incidence of mouthpart deformities occurred at intermediate levels of contamination (Muir 1993, Cervi 1996, Hudson and Ciborowski 1996a). This suggests that deformed individuals were less fit and did not survive in more contaminated areas.

It may thus be possible in future to assess the degree of contamination by observing the degree of deformity as well as the proportion of instars deformed, as earlier life stages are known to be generally more sensitive to stress than older ones (Pascoe et al. 1989). However, to simplify assessment, the research conducted for this thesis only considers cases of deformities involving extra or missing teeth on the mentum or ligula.

## **Oil Sands Mining in Northeastern Alberta**

Over the past 30 years, the Athabasca oil sands deposits near Fort McMurray, Alberta, have been mined by Suncor Inc., Oil Sands Group and Syncrude Canada Ltd., which together produce over 17% of Canada's total crude oil production (Bendell-Young et al. 1997). These oil sand deposits contain one third of the world's recoverable oil - more oil than all of Saudi Arabia and the surrounding countries combined (van den Heuvel et al. 1999). However, oil sand must be mined and processed in order to extract the valuable fuel resource. The mining involves an area roughly the size of Lake St. Clair, to be mined tens of metres deep. Production has accelerated in recent years as a result of recent advances in extraction technology. The volume of tailings produced by the year 2025 is estimated to be over one billion cubic metres (Herman et al. 1994).

Natural seepage of hydrocarbons into the Athabasca River and its tributaries is commonly observed. However, there exists a possibility that mining may concentrate toxicants and affect the regional biota (Herman et al. 1994).

### *1) The Mining Process*

Oil sands mining involves the stripping of surface vegetation and topsoil from hundreds of square km of boreal forest, as well as landscaping to channel natural drainage, resulting in serious alteration to wildlife habitat (van den Heuvel et al. 1999).

The hot water flotation process used to extract bitumen from oil sand produces waste water (oil sands process water (OSPW)), fine tails (water and fine particulates), and

residual bitumen (50:50:1) (Herman et al. 1994).

The fine tailings are stored in enormous pits for settling and detoxification. The slurry pumped into the pits separates into a surface layer of toxic pore water and a much deeper subsurface layer of mature fine tailings, an aqueous suspension of 65% water and 35% tailings particles (Fine Tailings Fundamentals Consortium 1995). The volume of tailings produced by the year 2025 is estimated to be over one billion cubic metres (Herman et al. 1994).

OSPW is enriched with several potentially toxic classes of compounds (sulphate and other ions, ammonia, metals, PAHs, naphthenic acids and other hydrocarbons). Thus, another concern is the vast amounts of oil sands process water (OSPW) being produced annually. Acute toxicity relating to the waste water was demonstrated in rainbow trout (96h LC50 <10% (v/v) = concentration lethal to 50% of test organisms over 96h exposure) by MacKinnon and Boerger (1986), who showed that this toxicity was linked to the presence of naphthenic-type organic acids. Naturally occurring in crude oil, naphthenic acids are a complex mixture of mono- and polycyclic alkanes (e.g. cyclohexanes and cyclopentanes) with carboxylated aliphatic side chains of various lengths (Herman et al. 1994, Lai et al. 1996). These compounds are solubilized and concentrated during the bitumen extraction process into salt esters. Being natural surfactants, they can impose osmotic stress on aquatic biota (Gulley 1994). Other evidence for OSPW toxicity is presented below. However, other compounds may also interfere with normal life cycle processes. Trace metals and PAHs can be cytotoxic at high concentrations, and can exert mutagenic and teratogenic effects at low concentrations (Madill et al. 1999). Ammonia can be cytotoxic, particularly to fish (Nix and

Martin 1992, Lai et al. 1996). Elevated salinity can reduce oxygen solubility, and also produces problems of osmotic regulation. Together, these compounds can impair growth, survival and developmental rates.

## *2) Reclamation*

Reclamation of mined lands to produce habitats bearing levels of production and biodiversity similar to pre-mining and reference conditions is required by the Alberta government. This also involves the storage and detoxification of enormous amounts of (OSPW) prior to recycling or release. As part of the strategy to detoxify the OSPW, it is stored in open tailings ponds to allow for photodegradation and biodegradation. Acute toxicity has been shown to decrease within two years of storage (Lai et al. 1996). Phytoplankton, zooplankton and fish are expected colonizers of these reclaimed wetlands, whereas benthic organisms may be challenged by the hypoxic conditions created by microbial degradation of organic chemicals in tailings water (Nix and Martin 1992).

Suncor Ltd. Oil Sands Group and Syncrude Canada Ltd. are two of several major companies engaged in oil sands mining near Fort McMurray, Alberta. They must obey a "no discharge" policy for OSPW production. Due to the enormous volumes to be managed, conventional wastewater treatment systems are not economically feasible.

The development of a viable wastewater management alternative is currently being pursued through various research projects. A wet landscape option involving the incorporation of wetland and lake networks within boreal forests representative of the region is being considered (Lai et al. 1996). To this end, pilot scale wetlands are being

constructed and studied in terms of biotic response to exposure to oil sands effluents. As a pilot experiment, some OSPW is supplemented with gypsum ( $\text{CaSO}_4$ ), a byproduct of bitumen extraction, which acts to initiate coagulation of fine clay particles and precipitate them out of solution to form consolidated tailings (CT) water, thereby accelerating the rate of settling (Lai et al. 1996).

### *3) Effects of Oil Sands Contaminants on Biota*

Several of the components of tailings water, including PAHS, metals, ammonia, and naphthenic acids, have been associated with toxic effects on biota. The effects of such chemicals have been manifested as altered structure and function at the community level (Howarth 1990), altered behaviour, growth and development through life cycles at the individual level (MacKinnon and Boerger 1986, Canfield et al. 1994, Kravitz et al. 1999), as well as suborganismal effects including altered hormone and enzyme activity (corticosteroids and MFOs, metallothioneins, heat shock proteins), immune function, cellular development, and genetic effects including mutation and genotoxicity, increased incidences of mouthpart deformities indicative of developmental stress (Niimi 1990, Warwick 1990, Morcillo and Diez 1996, Sanchez-Dardon et al. 1999).

Polycyclic aromatic hydrocarbons (PAHs) can absorb UV radiation and be modified into more polar and toxic forms. Photoactivation of PAHs (in suspended particulates) in both laboratory and field experiments resulted in increased toxicity to amphipods and oligochaetes. This was confirmed with *in situ Ceriodaphnia dubia* bioassays (Ankley et al. 1994, Ireland and Burton 1994).

The toxicological assessment of chemical mixtures to identify causal agents is made difficult due to our lack of understanding of chemical interactions that take place under varying environmental conditions. However, the first step in assessment is determination of effects of the mixture, which in this case, is OSPW.

Despite the acute toxicity of OSPW, newly constructed wetlands receiving oil sands input support populations of benthic invertebrates including midges, as well as macrophytes, algae, ducks, and swallows (Bendell-Young et al. 1998). Although amphibians and fish appear to be absent, it is possible that, up to the present time, these two groups have not been introduced to the systems. This is possible, as stocked populations of yellow perch, fathead minnow, brook stickleback, and lake chub are still present in ponds at Syncrude sites after six years (van den Heuvel et al. 1999). Rainbow trout were not affected by 3 month exposure to overlying capping water based on body condition and blood parameters (Balch et al. 1995).

Research conducted in 1997/1998 by other scientists indicates that oil sands contaminants do pose a hazard to aquatic life in some cases, mainly in terms of sublethal effects (Bennett and Bendell-Young 1997, Bendell-Young et al. 1998). Considering that present concentrations of chemical constituents are lower than that predicted to occur in future wetlands, these studies should be considered quite relevant in their predictive value if environmental conditions remain similar.

Wetlands receiving oil sands effluent were dominated by chironomids, and had low benthic invertebrate community diversity. The wetlands were surrounded by cattails which grew at normal pace but showed increased photosynthetic rates (Bennett and Bendell-

Young 1997, Bendell-Young et al. 1998). Radiotracer studies pointed to cattail roots as prime repositories of naphthenics, which may translate to strong effects in ducks and benthos that consume this material. Tadpoles reared *in situ* in OSPW-affected wetlands exhibited reduced growth, development, and survival. Fish exposed to water from OSPW-affected wetlands had altered blood chemistry and did not survive longer than 14 d. Ducks also showed possibly reduced growth when exposed to oil sands waters (Bendell-Young et al. 1998).

A 96-h LC50 of <10 mg naphthenic acids/L was observed for rainbow trout in bioassays, and this supported LC50 values of 3-10 % tailings water, which contains 65-75 mg/kg naphthenates (Schram et al. 1984, Nix and Martin 1992). Naphthenic acids are natural surfactants, and can alter ion and gas exchange across permeable membranes, thus imposing physical and chemical stress on the young trout (Gulley 1994). Both growth (stimulated rate initially) and reproduction (delayed spawning and fewer nests produced) were affected in fathead minnows (Siwik and Paszkowski 1998).

Nesting tree swallows did not show any differences in growth or reproduction (clutch size or mass) at reference or tailings wetlands. Immune response of ducks and swallows was also not altered (Smits et al. 1998).

Phytoplankton communities in mature fine tailings ponds up to eight years old differed in composition from reference ponds. However, older ponds were quite similar to reference ponds (Leung and Smith 1998). Zooplankters appear more sensitive than phytoplankton, showing continued effects at the community level that correlated to naphthenic acid concentrations even after 8 years (McCormick and Smith 1998).

Exposure to OSPW has been associated with elevated liver MFO activity and bile PAH equivalent concentration in adult yellow perch, although levels of steroid hormones were not affected. None of these biomarkers could be linked to physiological indices of reproductive development, gonad size, or fecundity (van den Heuvel et al. 1999)

Phenolic compounds are also suspected toxicants in OSPW. These chemical occur at levels between 0.003 and 2.5 mg/L, which are related to toxicity in aquatic biota (Buikema et al. 1979). Microtox (R) toxicity was linked to increased concentrations of phenol in OSPW (Gulley 1994).

Ammonia-ammonium exists at levels (10-15 mg/L) considered highly toxic to fish, based on an LC50 of 0.4 mg/L for trout (Hrudey et al. 1976). Microtox (R) assay results were related to the concentrations of these contaminants in tailings water, suggesting that ammonia also contributes to observed toxicity (Gulley 1994).

The high concentrations of both anions and cations in the OSPW, related to elevated sulphate/chloride salinity, undoubtedly also stresses the biota by overloading the homeostatic mechanisms involved with osmotic and ionic regulation. Yellow perch embryos tolerate only 2-4 g/L total dissolved solids (sulphate saline), and other biotic groups may be more sensitive than perch (van den Heuvel et al. 1999a).

Detoxification of tailings pond water, mainly aerobic, took 8 years to reduce *D. magna* subacute toxicity (Nix and Martin 1992). Phytoplankton growth was stimulated in tailings pond water during field scale trials. Thus, plankton, along with invertebrates and stocked fish, are expected colonizers of the wetlands. However, the anaerobic sludge layer is not expected to support benthos, and had an inhibitory effect on hatch rates of trout eggs

(Nix and Martin 1992). Addition of phosphate and aeration with oxygen greatly increased microbial degradation of naphthenic compounds in laboratory (Nix and Martin 1992, Lai et al. 1996).

*In situ* toxicity to both fathead minnows and sticklebacks was high (LT50<1 d), and this was related to ammonia enrichment (nitrogen and ammonium types), as well as hypoxia (32-50 % saturation) (EVS 1995). Both lethal and sublethal (production of young) effects were strongly reduced in *Ceriodaphnia dubia* in dyke drainage trenches, and these trenches were also related to low survival (6%) of fathead minnows compare to 40% in the Natural Wetlands (EVS 1995).

High acute toxicity (100% mortality) was observed *in situ* with *C. tentans* exposed to tailings sediment in constructed trenches, although natural populations of chironomids were present (EVS 1995). However, species richness was reduced in trenches. Invertebrate species richness was lower in both control (5 chironomid taxa of 16) and dyke drainage (7 chironomid taxa of 17) trenches compared to Natural Wetlands (21 taxa, 7 of which were chironomid taxa) and a reference site (31 taxa, 11 of which were chironomid taxa) (EVS 1995). There were very few chironomid larvae found in trenches containing tailings water, and reduced egg viability was suspected as oviposition was witnessed in all trenches.

In contrast, phytoplankton numbers were highest in tanks containing waters that were most toxic to other biota (in other tests), indicating that algae are stimulated by tailings contaminants (Nix and Martin 1992). However, long-term exposure to oil sands contaminants resulted in a trend of decreased growth rates for total plant biomass. Although Whitehead (1992) observed cattails alone in reclaimed zones, these macrophytes were the

most impacted by exposure to oil sands wastewater of species evaluated. Bulrush seemed to thrive in wastewater relative to control trenches (Nix and Martin 1992).

The accumulation of hydrocarbons and metals by macrophytes is also of concern, particularly because macrophytes are eaten by muskrat, beaver, deer and moose, as well as various waterfowl species which migrate through this region yearly (Gulley 1994). Larvae of *Chironomus* and *Tanypodinae* collected from dyke drainage trenches contained 20-65 mg/kg of naphthenic acids, suggesting that trophic transfer of these contaminants to both aquatic and terrestrial foodwebs is possible. Levels were higher in larvae from sediment containing muskeg compared to those from coke-based sediment. In terms of metals, concentrations of Al and Pb were higher in insects emerging from tailings trenches relative to control water, and Pb concentrations were much higher in larvae from the former sites as well, but concentrations of Hg, Cd and Fe tended to be lower in these individuals (Gulley 1994).

#### *4) Tolerance in Field Populations*

In both field and laboratory studies of various organisms, exposure to chemicals can result in the development of tolerance to the stress (Rosenberg et al. 1977, Warwick 1989). In invertebrates, differences in sensitivity have been related to physiological acclimation, as seen with daphnids and isopods exposed to metals (Fraser 1980, LeBlanc 1982, and Bodar et al. 1990). Tolerance is also developed through genetic adaptation (Brown 1976, Klerks and Levinton 1989), and can be contaminant specific (Dermott 1991). Changes in physiology related to acclimation occur over a small enough time frame to prevent the

individual from being harmed. Adaptation, occurring over a much longer time scale than acclimation, involves selection against the more sensitive individuals in the population, leading to a population of generally more fit individuals, resulting in the eventual 'absence' of effect. The individuals remaining had a physiology that was superior to those organisms affected, conferring on them a better ability to regulate or safely store chemicals (Krantzberg and Stokes 1989). In the case of metals, this is likely related to the production of metallothioneins and metal-storing granules (Groenendijk et al. 1999).

Unlike terrestrial invertebrates that tend to show shorter life-cycles and higher reproductive effort in relation to metal-adaptation, aquatic invertebrates that have adapted tend to show reduced overall fitness under clean conditions. This has been termed the 'costs of tolerance', the energy costs associated with maintaining a tolerance mechanism (Postma et al. 1995). Bervoets et al. (1996) reported optimal survival of tolerant field populations of *Chironomus riparius* at intermediate levels of salinity. Similarly, field populations of *C. riparius* showed tolerance to cadmium, in terms of egg hatchability and larval development, with reduced fitness in controls (Postma et al. 1995). Hoffman and Fisher (1994) showed similar results of tolerance with this species exposed to 4 different pesticides in the field.

Conversely, interspecific differences in sensitivity may not always be manifested. In a multi-species bioassay, the lab species *Chironomus tentans* responded similarly to three wild species. *C. tentans* was relatively sensitive to oil (water-soluble fraction) but was not as sensitive to phenol as three natural populations of chironomids, *Clinotanypus pinguis*, *Einfeldia natchitochae*, and *Tanypus neopunctipennis* (Franco et al. 1984).

### ***5) Integration of Studies***

**An ecosystem functions only through the interaction and transfer of energy among various trophic levels. Overall viability will be limited by the weakest (most sensitive) link in the web. Through the assessment of the various ecosystem components including macrophytes, phytoplankton, zooplankton, macroinvertebrates, fish, birds, and amphibians, a comprehensive measure of the viability of the wetlands related to oil sands production is possible (Bendell-Young et al. 1998). This approach will allow identification of the biotic classes predicted to be most affected by oil sands-related chemical input in the future reclaimed habitats. It will also give indications of the degree of bioavailability and possible trophic transfer of oil sands contaminants in the sediment, water, and biota.**

**My contribution will be to evaluate the susceptibility of the benthic macroinvertebrate component of the ecosystem. Based on research to date, these organisms appear to fit somewhere between fish (highly affected) and more tolerant groups (algae) in terms of sensitivity to contaminants related to oil sands mining. I expect that if contaminants are bioavailable to wetland biota, that the benthic community will be one of the first components to show signs of this, based on probable chemical partitioning to the organic wetland sediments in which they live, and the wide diversity of taxa included in this group.**

## **CHAPTER 2 - Assessment of the Benthic Macroinvertebrate Community in Wetlands as Related to Oil Sands Development**

### **INTRODUCTION**

Water quality monitoring presently emphasizes expensive physico-chemical analyses, with acute and chronic toxicity bioassays using single species exposed to single chemicals (Butterworth 1995). Although this provides a measure of chemical distributions and general toxicity, these studies lack realism, are not comprehensive of all chemicals, do not consider potential chemical interaction, and can only detect effects that are specifically monitored (van der Schalie 1986). The general use of a single species to protect a biological community is most likely not sensible or practical, due to differences in sensitivities among biota that vary between different groups of chemicals, unless it is shown to be a sensitive keystone species.

Benthic macroinvertebrate communities have been used effectively to assess aquatic contamination in a wide variety of ecosystems (Howarth 1990, Rosenberg and Resh 1993, Clements 1994, Bailey et al. 1995). Many contaminants in aquatic systems including metals and organic contaminants such as PAHs are hydrophobic and thus become associated with the organic fractions of sediments (Pesch et al. 1981, Harkey et al. 1994, Russell et al. 1999). Benthos are in intimate contact with the sediments, and thus have the greatest potential for exposure to contaminants. Therefore, they are relevant measures of environmental condition or health of aquatic systems. Benthos are generally sessile, and differences in magnitude of exposure over time are conveniently integrated in responses observable in these biota (Moriarty 1990).

Chironomids are used in a variety of ways to assess viability of aquatic ecosystems. These include their use as indicator species aiding in water quality assessment and characterization of certain conditions (Warwick 1980), in cytological and genetics studies (Woods et al. 1989) and sediment bioassays (ASTM 1990, Bedard et al. 1992), as well as in biomarker studies (Warwick 1990, Hudson and Ciborowski 1995). Their wide use is justified by several points, including their ubiquitous distribution, significant links to both aquatic and terrestrial energy and nutrient flow, broad range of tolerances to various environments (species specific), sensitivity to various contaminants, sessile benthic nature (for most of their life), well studied ecology and biology, and relative ease in field collection and rearing of cultures in laboratory (Warwick 1990, Ciborowski et al. 1995). Their use in assessing sediment contamination is further supported by the fact that the majority of chironomid species are detritivorous (Pinder 1986).

The designation of study sites as reference sites, to be used to compare against sites where reduced environmental quality is suspected, should be scientifically defensible. The selection of inappropriate reference sites will always invalidate conclusions drawn from the best data collection and analyses. Isolation of causal factors requires that reference sites have environmental and ecological properties similar to 'polluted' sites. A knowledge of natural variation/covariation related to populations of macroinvertebrate among reference sites is necessary in order to make decisions of the magnitude of differences that will be considered as indicative of an effect of pollution (Norris et al. 1992). The use of multivariate analysis (e.g. principal components analysis (PCA)) is helpful in unraveling the complexities of considering numerous factors simultaneously by reducing redundancy to

reveal general patterns. Cluster analysis complements this tool on a finer scale.

The goal of this chapter is to determine if there are effects on the benthic macroinvertebrate community related to oil sands mining in pilot-scale wetlands receiving related inputs. (Refer to chapter 1 for a description of the mining process.) In order to accomplish this, suitable reference sites had to be found to be compared to potentially impacted wetlands. Wetlands were deemed suitable as reference sites if they resembled study sites in terms of measured environmental attributes. Environmental characterization of a suite of candidate wetlands was therefore performed, including physical, chemical, and sediment characteristics. The use of multivariate statistics allows for the determination of any effects of oil sands inputs, apart from natural heterogeneity in community structure related to differences in environment (water chemistry, sediment characteristics, physical factors related to habitat, microclimate). Following the pairing of environmentally similar reference and study sites, sampling of the benthos could be carried out.

This study describes the process of wetland site selection, sampling, analysis, and characterization of environmental attributes through the use of PCA and cluster analysis, leading to the grouping of OSPW and reference wetlands of similar environment. The benthic community was sampled and then characterized in terms of taxa richness and standardized abundance, as well as composition of taxonomic groups. The chironomid community in particular was also assessed in terms of richness, composition (subfamily/tribe) and dominant taxa. Together, these measures of community response provided an indication of species sensitivities as well as co-occurrence of benthic taxa compared between reference and OSPW wetlands.

## **METHODS**

### **I Field Methodology**

#### **Environmental Characterization of Study Sites**

##### ***Description of Study Sites:***

In summer 1997, 15 wetlands (sites) were sampled to provide baseline information on the range of environmental variability that characterizes aquatic habitats in the Athabaskan oil sands region near Fort McMurray, Alberta (Table 2.1, Fig. 2.1 and 2.2). Such information is fundamental to the selection of reference sites most appropriate for comparison with wetlands influenced by oil sands mining activity.

This region of Canada is made up of boreal forest composed of poplar, aspen, black spruce, and Jack pine, with numerous wetlands, winding streams, and rivers that run into the Athabasca River flowing northwards to Great Slave Lake. Two large companies involved with oil sands mining (Suncor Inc., Oil Sands Group and Syncrude Canada Ltd.) are located just north of Fort McMurray.

Two of the study sites (Suncor Natural Wetlands and Hummock Wetlands) were 'water affected' (WA), in that they receive some input of mine tailings water. Hummock Wetlands receives consolidated tailings (CT) water piped directly from a tailings pond, and is also supplemented surface water runoff, and by dyke seepage water leaching through the enormous dyke adjacent to the wetland that makes up the south wall of a large tailings pond. CT water is process water that has gypsum added to accelerate precipitation of clay particles out of solution. Natural Wetlands receives water originating from Hummock Wetlands that has passed through 200 m of cattail zone between the sites. This site also

Table 2.1: Reference (REF) and Affected (A) Wetland Sites and Sampling performed. Samples were also a part of concurrent studies (Ciborowski and Whelley 1997, Whelley et al. 1998).

Site	Code	Status	Synoptic		Sediment	Light Trap	Intensive Sampling	<i>In situ</i> tests
			1997	1998				
Natural Wetlands	NW	A	x	x	x	x	x	#2
Natural Wetlands East	NWE	A		x	x			
Hummock Wetlands	HW	A	x	x	x	x	x	#1
High Sulphate Pond	HS	REF	x	x	x		x	#1
Crane Lake	CL	REF	x	x	x		x	
Highway 63 Wetland	HW63	REF		x	x		x	#2
Syncrude Oil Marsh	OM	A	x					
Syncr. Shallow Wetlands	SW	REF	x	x	x	x	x	#2
Demo Pond	DP	A		x		x		
Syncr. North Wetland	SYN	REF	x	x				
South Bison Pond	SB	A	x	x	x	x	x	#2
Syncr. Test Pond 2	TP2	A		x				
Syncr. Test Pond 5	TP5	A		x				
Syncr. Ref. Test Pond	RTP	REF		x				
Syncr. South Ditch	SD	REF		x				
Syncr. West Interceptor Ditch	WID	REF		x				
Syncr. WID Rd. Pond	WIDP	REF		x				
Ruth Lake	RL	REF	x					
Poplar Creek Reservoir	PCR	REF	x	x	x			
Poplar Creek Outflow	PCO	REF		x	x			
Suncor MFT Pond South	MFTS	A		x	x			
Suncor MFT Pond North	MFTN	A		x	x			
Salt Marsh	SM	REF		x	x			
Tower Rd. #1	T1	REF	x	x				
Tower Rd. #2 (Spruce Pond)	T2	REF	x	x	x			
Tower Rd. #3 (channel)	T3C	REF	x	x				
Tower Rd. #3 (marsh)	T3M	REF		x				
Tower Rd. #4	T4	REF	x	x				
Tower Rd. #5	T5	REF	x	x				
Saline Lake	SL	REF		x				
Crane Rd. Marsh	CRM	REF		x				
Crane Rd. West Wetland	CRWW	REF		x				
Horseshoe Lake	HL	REF		x				
Muskeg River Wetland	MR	REF	x	x				
Bridge Wetland	BRW	REF		x				
Barge Marsh	BAM	REF		x				
Intersection HW63 Wetland	I63W	REF		x				
Ft. McKay Wetland	FMW	REF		x				

*Note:* Synoptic sampling refers to single-visit measurement of environmental parameters and sweep samples, whereas intensive sampling refers to collections of chironomids, colonization potential study, and Ekman benthic grabs.

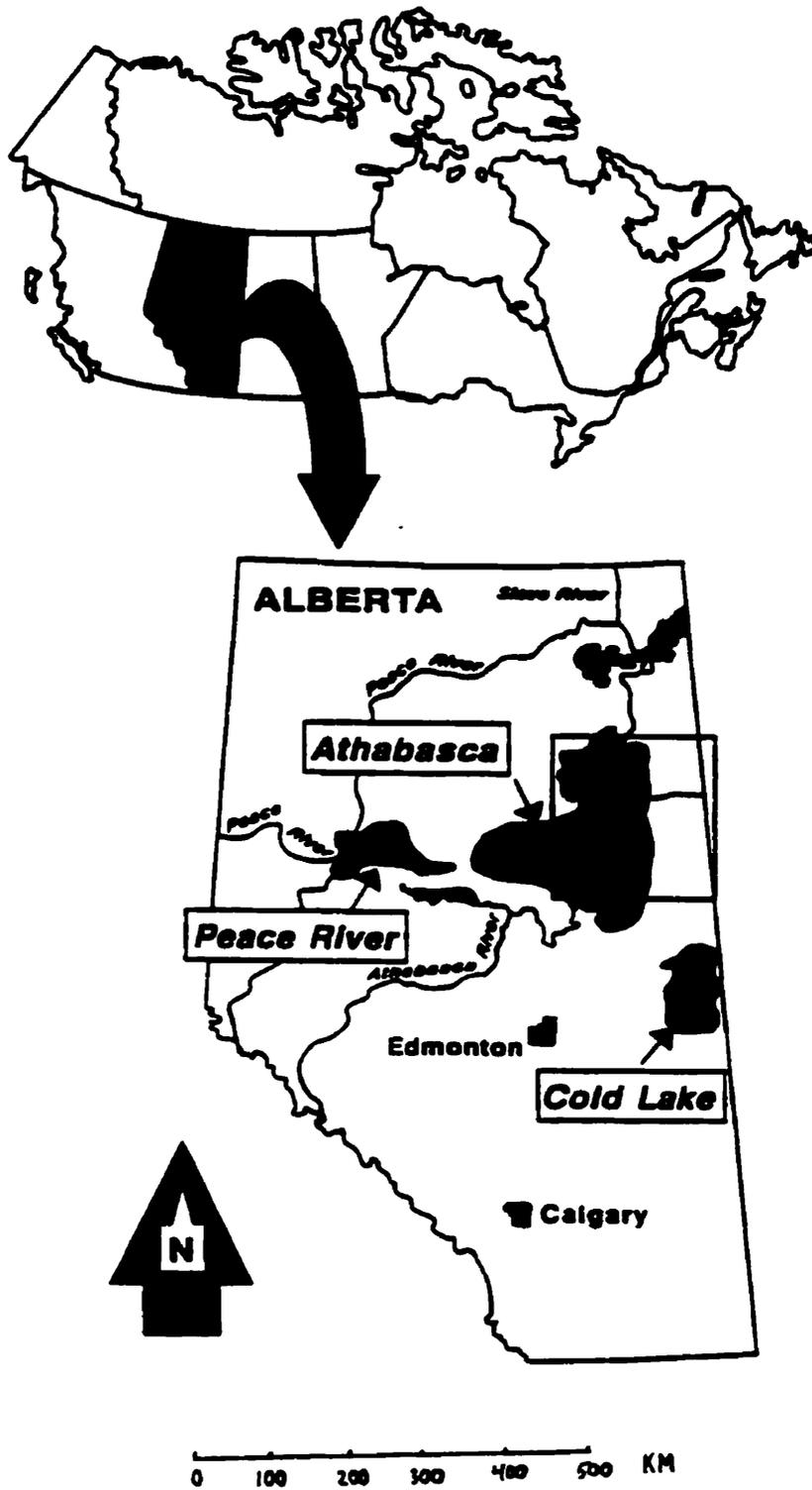


Fig. 2.1: Map of Alberta, with the Fort McMurray study region indicated.



receives surface runoff and dyke seepage water. A third site, Syncrude South Bison Pond, was formed from surface water runoff in a reclaimed mined area, and is highly saline. These three wetlands and their subsequently identified corresponding reference sites (Crane Lake, Suncor High Sulphate Pond, and Syncrude Shallow Wetlands) were designated focal sites and were intensively sampled. Additionally, a borrow pit on the east side the highway entrance to the Suncor property (designated Hwy 63 wetland) was studied to complement reference measurements of amphibians and avifauna made by other university researchers.

In June of 1998, a total of 35 wetland habitats were surveyed, including those 15 sampled the previous summer (Table 2.1). The same focal wetlands from 1997 were intensively sampled in 1998 to provide detailed information on chironomid larval biomass/ density/ developmental condition (Whelley et al. 1998). The exception was the replacement in 1998 of Crane Lake by Highway 63 reference wetland to be compared to Natural Wetlands. Adult aquatic insects were also collected at these sites for concurrent studies.

Water chemistry, sediment characteristics, and benthic community composition (quantitative and qualitative sampling) were evaluated at each site (Table 2.2). Environmental similarity among sites was summarized by multivariate statistical analyses (see below).

***Physico-chemical Data:***

***General Environmental Features:***

Qualitative field notes were made of the general characteristics of each site sampled. Features noted included subjective estimates of composition, relative abundance, and extent

**Table 2.2: Summary of environmental and biological attributes measured at wetlands during synoptic surveys.**

<b>Phase</b>	<b>Attribute</b>	<b>Method Used</b>
<b><u>General</u></b>	<b>Size</b>	<b>qualitative</b>
	<b>Canopy</b>	<b>qualitative</b>
	<b>Macrophytes</b>	<b>qualitative</b>
	<b>Chlorophyll a</b>	<b>filter/acetone extraction</b>
<b><u>Water</u></b>	<b>Dissolved oxygen</b>	<b>YSI meter</b>
	<b>Conductivity</b>	<b>YSI meter</b>
	<b>pH &amp; ORP</b>	<b>Accumet meters</b>
<b><u>Sediment</u></b>	<b>Ekman grab</b>	<b>500 mL composite; frozen</b>
	<b>Texture/odour</b>	<b>qualitative</b>
	<b>Loss on Ignition</b>	<b>550°C 3 h</b>
	<b>Median Particle size</b>	<b>sieving/hydrometry</b>
<b><u>Benthos</u></b>	<b>Community</b>	<b>Ekman grab; n=5 (focal sites)</b>
		<b>Composite dip net sweep</b>

extent of the surrounding terrestrial community, riparian vegetation, and aquatic macrophytes, shoreline characteristics (including evidence of physical disturbance). Notes pertained to the general vicinity sampled rather than the entire water body (Ciborowski and Whelley 1997).

*Water chemistry:*

At each location, measurements of pH, conductivity, salinity, temperature, and dissolved oxygen concentration were taken using YSI meters. Probes were submersed directly in the water of the wetland. Thus, readings pertained to subsurface characteristics, at least 20 cm above the substrate, where there was sufficient depth. Two dissolved oxygen readings were taken - one just above the substrate (epibenthic) and one at subsurface.

*Sediment characteristics:*

Sediment samples were collected with a small trowel from the substrate adjacent to where Ekman grabs had been collected and placed into a clean, plastic tray or bucket. Eight to ten 5-10 cm deep scoops of sediment were gathered and placed into the container. A reading of the sediment's oxidization-reduction potential was taken with an Accumet ORP meter. The composite sample was then mixed, and a precleaned 500-mL amber glass jar was filled with sediment which was sealed with a teflon-lined lid and kept cool for transport back to the freezer at the laboratory. Samples were stored frozen at -40 °C to be later analyzed for contaminants (trace metals, naphthenic acids, PAHs). A second aliquot of sediment was collected into a clear glass jar and frozen, to be analyzed for particle size

distribution and organic content (loss on ignition).

### **Benthic Invertebrate Community Analysis**

#### ***Quantitative Benthic Samples:***

Five replicate benthic samples were collected at approximately 1 m depth from each of the 7 focal sites using a pole-mounted Ekman grab sampler (15 cm x 15 cm). For each replicate, sediment texture, colour, odour, and inclusions of aquatic and terrestrial vegetation were assessed qualitatively and recorded. These samples have been archived for future analysis (Ciborowski and Whelley 1997).

#### ***Qualitative Benthic Samples:***

A heavy duty D-frame aquatic dip net (opening 30 cm across; mesh size 0.5 x 1 mm; BioQuip, Inc., Sacramento, CA) was used to collect benthos from various habitats at depths of 1 m or less. The net was swept through submergent and emergent vegetation, through surficial sediment, and along bank edges. Following a sweep, the net was repeatedly rinsed in the pond to clear it of excess fine sediment. Net contents were then emptied into an enamelled pan, and very large debris (cattail leaves and stems) was rinsed in the pan and discarded. Three or four sweeps (3-min. effort) were emptied into the pan.

#### ***Sample Field Processing and Preservation:***

Each sample (Ekman or dip net) was field-rinsed in a 250- $\mu$ m mesh sieve bucket to remove fine particles. The remaining material and a locality label were placed in a 4-L

polyethylene soil bag to which was added a sufficient amount of formal-ethanol solution (a 5:2.5:1 mixture of water, 95% ethanol, and 100% formalin) to just saturate and cover the entire sample. Bags were sealed with a twist tie. Samples were stored and transported upright in polyethylene buckets, to be later analysed at the University of Windsor. Because samples contained large amounts of organic material, which absorbs preservative, additional preservative (enough to cover bag contents with 1 cm of formal-ethanol mixture) was added to samples 14 d after collection.

## **II Laboratory Methodology**

### **Physico-Chemical Data**

Data were compiled from field notes and supplemented with information (wetland area, surrounding dominant vegetation) from 1:50,000 topographical maps and aerial photographs. Because all water chemistry data were collected in the field using meters, no laboratory processing was necessary.

#### ***Sediment analysis:***

Organic content determination (LOI) and particle size frequency distribution were determined for each sample.

Sediment samples were removed from the freezer and allowed to thaw at room temperature for 24 h. Excess water was poured out of the jars. Approximately 100 g of freshly thawed sediment was removed from its sample jar, spread on a sheet of aluminum foil, and allowed to air-dry at room temperature for 48 h. The sediment was then ground

with a mortar and pestle. Loss on ignition was determined by weighing an oven-dried sample (105°C for 24 h), heating in a muffle furnace (550°C for at least 2 h until colour was uniform), cooling in a desiccator and reweighing. Loss on ignition was the difference between ash and initial oven-dried weight, expressed as a percentage of initial weight. The ashed material was then used to determine particle size frequency distribution.

Particle size distribution of materials >90 µm was determined by hand-sieving each ashed sample through a Standard sieve series (8.00, 4.00, 2.00, 1.00, 0.500, 0.250, 0.125, 0.090 mm). Material retained on each sieve was reground in a mortar and pestle and resieved. Median particle size was determined by interpolation after plotting cumulative sample mass against Wentworth Scale value ( $\text{Log}_2$  (particle diameter)).

### **Benthic Invertebrate Community Sample Processing**

The 15 qualitative benthic samples collected in 1997 were sorted following methodology of Ciborowski (1991). The 1997 Ekman samples and the 1998 dip net and Ekman samples remain to be sorted.

A sample and its preservative were drained on a fine (0.250 mm) sieve and briefly rinsed. The waste preservative was treated with Formalex® to break down residual formalin and discarded.

The sample (or the subsampled proportion to be sorted) was placed in an enamelled tray and repeatedly rinsed. Twigs, whole leaves and tufts of macrophytes and roots were set aside. Organic material was poured off with the water through nested sieves (8.00, 4.00,

1.00, 0.500, 0.250 mm). Remaining inorganic material was examined for cased or shelled organisms and then discarded. Filamentous debris was teased apart beneath water to release finer material, which was poured through the sieves. Material retained in each sieve was returned to a tray and rinsed through sieves again.

Organisms retained in the 8.00 mm and 4.00 mm sieves were separated from debris in trays without the aid of magnification. Filamentous material in these sieves and all finer materials were sorted beneath a dissecting microscope. Filamentous material and the 1.00 mm size fraction were examined at 6.4X magnification in water containing a few drops of diluted dishwashing detergent (a surfactant). Aliquots of the 0.500 mm fraction were placed in a gridded petri dish and scanned once at 6.4X and a second time at 12X magnification. The 0.250 mm fraction was scanned twice at 12X magnification. Invertebrates were transferred to stoppered shell vials and stored in 10% formalin (oligochaetes) or 70% ethanol (other invertebrates). Organisms collected from each size fraction were stored within their own labelled, stoppered shell vial. Vials from the various fractions were kept together in a large vial for later identification, as necessary.

Almost all samples contained large amounts of organic material, requiring subsampling. Randomly selected subsamples of each fraction were examined until a total of at least 100 organisms had been removed or, if this required over 2 h, until one-half of the material had been examined. Up to 4 h or more was occasionally required to achieve this criterion. The proportion of each size fraction sorted was estimated by wet weighing the sorted and unsorted fractions of material (the benthic animals removed represent a negligible proportion of total sample biomass). Unsorted portions of organic material were

placed in jars (by size fraction) and preserved in 95% ethanol or Kahle's fluid for future reference, as necessary.

Organic material that had been sorted was placed on filter paper, dried overnight at 60 °C and weighed to the nearest mg to provide a general estimate of overall sorting effort per sample. This also allowed for numbers of organisms to be standardized against amount of material sorted, to provide a rough estimate of relative densities.

Benthic macroinvertebrates were identified to the generic level where possible using the keys of Clifford (1991; Table 2.3). Arthropod taxa not identified to genus were either damaged or too immature to permit assignment of a generic designation. Chironomids and oligochaetes were stored for later microscope slide-mounting and identification to the genus or species level. A subsample of 20 chironomids from each size fraction sorted was mounted on microscope slides with CMC-9AF aqueous mounting medium for identification with the aid of a compound microscope.

Samples from 15 sites were sorted and identified. Numbers recovered in each size fraction were divided by the proportion of organic material sorted, and the values summed to give an estimate of the total number of animals of each taxon per sample. Estimates based on subsamples that contain at least 100 animals are within 20% of the true total 95% of the time (Ciborowski 1991).

For each sample site, the following data were tabulated:

- 1) estimated total number of animals in each taxon in a sample;
- 2) estimated total number of animals in a sample;

Table 2.3: Taxonomic keys used for identification of wetland benthos and emergent insects.

Taxon		Taxonomic Reference
<b>Aquatic Stages</b>		
Annelida	Oligochaeta	Brinkhurst (1986)
	Tubificidae	
	Hirudinea	Pennak (1978), Clifford (1991)
Crustacea	Amphipoda	Pennak (1978), Clifford (1991)
	other taxa	Clifford (1991)
Hexapoda	Ephemeroptera	Edmunds et al. (1976)
	Trichoptera	Wiggins (1977)
	Diptera-Chironomidae	Oliver and Roussell (1983), Wiederholm (1985)
other orders		Clifford (1991), Merritt & Cummins (1996)
<b>Terrestrial Stages</b>		
Hexapoda	Ephemeroptera	Edmunds et al. (1976)
	Hemiptera	Clifford (1991)
	Trichoptera	Ross (1944), Schmidt (1981)
	Diptera	McAlpine et al. (1981, 1987, 1989)
	Chironomidae	Wiederholm (1985)

- 3) proportion of animals in each taxon in a sample;
- 4) total number of taxa in a sample (excluding Oligochaeta, Chironomidae and microcrustacea).

### **Chironomid Generic Richness**

Chironomids (20 per size fraction of 1.0 mm, 0.50 mm, 0.25 mm where available) collected in dip net samples were examined from each of the 15 sites, based on the procedures of Hudson and Ciborowski (1996a, 1996b) to determine generic identity, and incidence of mouthpart deformities. This provides information on the dominant taxa present, as well as chironomid subfamily and generic richness.

### ***Larval Identification:***

Chironomid larvae were individually mounted on slides for taxonomic identification and morphological examination.

The head capsule of each larva was removed and placed ventral side up on a microscope slide in a drop of CMC-9AF<sup>®</sup> aqueous mounting medium (Master's Chemical Company, Des Plaines, Illinois). A cover slip was placed on the slide and gentle pressure applied to separate the mouthparts and properly orient the head capsule. The cover slip was then ringed with clear nail polish and allowed to dry and clear for at least a week. Chironomids were identified to genus using keys of Oliver and Roussell (1983), Wiederholm (1983) and Coffman and Ferrington (1996) (Table 2.3).

### **III STATISTICAL ANALYSES**

#### **Physico-chemical Data**

The physical, chemical and biological features measured in environmental studies are typically intercorrelated. We used principal component analysis (PCA) to reduce the physicochemical data set to a smaller number of statistically independent compound variables against which to ordinate environmental characteristics of the wetlands sampled. Cluster analysis was performed on the PCA scores to provide information of similarity of sites within the generalized PCA groupings. Data were analyzed using the STATISTICA® software package (StatSoft Inc. 1998). The analysis was performed on the correlation matrix generated from the data set and using Varimax rotation of the derived factors.

We analyzed the 1997 and 1998 data separately. The results of the 1997 analysis allowed us to match the three tailings-affected sites to environmentally-similar reference sites. We could then compare biological attributes measured at the community, population, and developmental levels between paired sites. The 1998 environmental data allowed for comparison of variation in wetlands characteristics on a temporal vs. spatial scale.

Physical (wetland size), chemical (specific conductance [Log transformed], dissolved oxygen, pH), and sediment (LOI, median particle size) characteristics were used in the PCA. Where dissolved oxygen values were suspiciously high (markedly supersaturated), they were replaced with values representing 100% saturation at the given water temperature.

For 1998, environmental data from 33 sites were analyzed in similar fashion to the 1997 data. This permitted inter-year comparison of environmental conditions.

### **Benthic Invertebrate and Chironomid Community Data - 1997 Dip Net Samples**

Data from single dip net samples were summarized in tables in raw form (numbers recovered by sieve size fraction in each sample), and in estimated total numbers per sample (numbers recovered/proportion of sample sorted summed over size fractions) for each taxon identified. We also determined the relative abundance of taxa at each location by dividing the number of representatives of a taxon by the total number of organisms found in each sample.

Taxa were grouped into categories including 'Other' (excluding ubiquitous Chironomidae, Oligochaeta, Ostracoda, and microcrustacean Cladocera and Copepoda), 'Chironomid', or 'Total' ('Other' + 'Chironomid') to calculate taxa richness for each of the fifteen samples. Vertebrates were excluded from these tallies.

Similarity of benthic invertebrate community composition among sites was determined by PCA and by cluster analysis. Multivariate analyses are unduly affected by the influence of rare taxa. Therefore, only 'common' groups of organisms were used for these analyses. To be included in the multivariate analysis, a taxon had to meet the both of the following criteria:

- 1) present at 20 percent of the sites or more (3 or more sites);
- 2) abundance of the taxon was 0.1 percent of the total number of all animals recovered across all sites sampled.

A total of 30 taxa met the criteria. The relative abundances of members of these taxa within each of the sample sites was calculated (percent) and converted to octaves (Gauch 1982) according to the transformation  $Y' = 3.5 + \text{Log}_2(Y + 0.08839)$

The constant 0.08839 (random number) was added to all raw values so that true values of zero would be defined following Log-transformation for normalization. The constant 3.5 was added so that all transformed values would be non-negative.

Principal Components Analysis (PCA) was used to identify suites of taxa whose relative abundance was highly correlated among sites following the same procedures as were used for the environmental data set.

Cluster analysis dendrograms graphically illustrate the degree of similarity among pairs of groups of similar sites, but distort the relationships among members of larger (i.e., more dissimilar) clusters. In contrast, PCA plotting is characterized by faithful representation of similarity between major groups or clusters, but is notorious for distorting apparent similarity between (compositionally) close neighbours (Sneath and Sokal 1973).

Cluster analysis (STATISTICA® software package) was used to group wetland sampling sites that were similar in macroinvertebrate relative composition. Data used for the analysis consisted of the octave-transformed subset derived from the dip net sample. The Euclidean distances between sites was calculated. Sites were then grouped using Ward's method of hierarchical clustering (Wishart 1978) to identify sites that had the most similar relative abundances of the same types of taxa. The number of distinct groups was ascertained by looking for discontinuities in the pattern of linkage distances as a function

of the cumulative number of cluster fusion steps. Group membership was determined by using the k-means clustering option of the Statistica® Cluster Analysis module. The grouping pattern was illustrated by a dendrogram. To determine whether relative abundances of taxa contributing to composition of each group were significantly different, one-way analyses of variance were performed on each taxon (Green and Vascotto 1978).

Finally, taxonomically similar wetlands were related to the patterns of environmental variation seen in the Fort McMurray oil sands area. One-way planned comparison ANOVA was used to test for differences in richness ('other', 'chironomid', and 'total') and standardized abundance as related to OSPW, by comparing the high conductivity reference sites (n=3) to the OSPW sites (n=3). Linear regression was used to test for a relationship between conductivity and the four biotic measures of community response.

## **RESULTS**

### **1997 Field Season**

#### **Physico-Chemical Data**

##### ***Summary of General Environmental Features:***

Data on dominant terrestrial and riparian vegetation, macrophytes and substrate characteristics of the 15 wetland sites sampled in 1997 were summarized in Table 2.4.

Surrounding vegetation tended to fall into one of three classes; spruce forest, poplar/willow overstory, or sedge meadow. Riparian vegetation was dominated by either willow/alder shrubbery or sedges. The dominant emergent macrophyte at most wetlands was cattail (*Typha*), although *Scirpus* and *Equisetum* dominated at sample locations on the larger wetlands (Poplar Creek Reservoir, Ruth Lake, Muskeg River wetland).

General sediment texture varied widely among sites, depending in part upon disturbance history. All wetlands had a surface layer of heavily organic black muck. This layer was underlain by sand or fine gravel at locations that had a history of landscaping in the vicinity.

Table 2.4: Environmental characteristics of 15 sites in Fort McMurray Area (1997)

Site	Terrestrial Area	Riparian Vegetation	Macrophytes	Sediment Texture
TR1	willow, sedge, beaver dam	willow	<i>Typha, Lemna</i>	sandy with peaty top
TR2	willow, poplar, beaver dam	willow, poplar	not sampled	not sampled
SP	spruce forest	sedge, dead growth, alder and spruce	mosses, <i>Valisneria, Potamogeton natans</i>	very sandy, moss cover
TR3(SFU)	roadside, grass, poplar	sedge, bushes	not sampled	not sampled
TR3	stream area,	willow	<i>Typha, Scirpus, Myriophyllum, Potamogeton natans, Equisetum</i>	sand bottom, peat
TR4	spruce/poplar mixed stand	alder, spruce, poplar and sedge	<i>Typha, Equisetum, Myriophyllum</i>	gravel and sand, organic top
TR5	sedge meadow	willow, alder	<i>Typha</i>	organic fine muck
PCR	poplar stands	willows, poplars	<i>Scirpus, Equisetum, Carex, Nuphar, Lemna, Sagittaria, Typha, Myriophyllum, Ceratophyllum</i>	heavy organic matter, black water
RL	willows, grasses	willows	<i>Scirpus, Equisetum, Carex, Nuphar, Lemna, Sagittaria, Typha, Myriophyllum, Ceratophyllum</i>	thin lt brown muck, black thick organic underneath, plus grey sand
SYN	barren/stripped, sandy	sparse; new site	<i>Typha, Equisetum</i>	top yellowy brown muskegy, organic, sandy/gravel substrate
OM	sedge/ bush	sedge, bushes, grass	not sampled	not sampled, perimeter very mucky, dark
SW	sedge, grass bush	shrubs, sedge	<i>Utricularia, Equisetum, Typha, Lemna, Carex, Myriophyllum, Ceratophyllum</i>	mucky yellow brown
SB	grass/ shrub	shrubs, grasses fairly thick	<i>Typha, Equisetum</i>	very heavy muck
CL	willow, poplar, spruce stands	strawberries, spruce, poplar	<i>Typha, Carex, Lemna, Ceratophyllum, Cyanobacteria</i>	mucky, sandy base,
HS	sedges, bushes, willows, poplar	cattail pond	<i>Typha, Equisetum</i>	dk grey sandy substrate, muck top
NW	grass, poplar stands	poplar	<i>Typha, Carex</i>	sandy, muck top, black organic and grey sand
HW	grass, poplar stands	sedge, poplar dead trees	<i>Typha, Carex</i>	heavy organic and algae
MR	spruce stands	spruce	<i>Chara, Equisetum, Nuphar, Carex, Typha, Pontaderia, Utricularia</i>	sand and gravel, little organic matter bridge construction debris

***Water Chemistry:***

Replicate readings of water chemistry features taken with a Hydrolab multimeter are listed in Appendix 2.1 and summarized in Appendix 2.2. Measurements taken with YSI meters on the day of benthic sampling are listed in Appendix 2.3. Measurements of dissolved oxygen recorded were suspiciously high (up to twice the level of oxygen saturation) on some sampling days. In multivariate analyses of environmental data (see below), values that exceeded saturation were replaced with readings taken with a YSI oxygen meter where available. Otherwise, the value for 100% saturation at prevailing water temperature was used (summarized in Table 2.5).

Water temperatures were fairly uniform during the time of sampling and reflected prevailing air temperatures rather than local environmental differences.

Specific conductances ranged from moderate (minimum 150 mhos at Tower Rd. 2) to high (2712 at the High Sulphate wetland; Appendix 2.2). There was a sharp dichotomy between wetlands that had relatively low conductivity (150–400 mhos) and those with high conductivity (>1600 mhos). Crane Lake was exceptional in having intermediate conductivity (955 mhos; Table 2.5).

Dissolved oxygen level tended to correspond with wetland size and depth. Smaller, shallow wetlands had low to subsaturated concentrations. Reservoirs and deeper wetlands were at saturation. The pH ranged from circumneutral to moderately basic (Table 2.5).

Table 2.5: Data used in Principal Components Analysis of environmental characteristics of 13 wetland sites, 1997. OSPW wetland codes are bold-faced. Tower Rd 4 and 5 wetlands did not have complete data sets and were excluded.

Site	Conduct.	D.O. (mg/L)	pH	Size <sup>1</sup>	LOI	Median Particle Size (phi) <sup>2</sup>
TR1	257	3.6	7.2	3	6.7	4
SP	232	5.9	7.4	3	4.4	3.5
TR3	193	2.4	6.9	1	4.4 <sup>3</sup>	3.5 <sup>3</sup>
PCR	252	11.0	8.9	6	11.7	4
RL	248	9.1	9.0	6	8.9	3
SYN	1603	8.5 [14.3] <sup>4</sup>	9.3	4	1.5	2
SW	399	8.5 [17.9] <sup>4</sup>	8.8	2	4.8	3
SB	2266	8.5 [20] <sup>4</sup>	9.3	3	4.8 <sup>3</sup>	4 <sup>3</sup>
CL	955	4.2	8.3	5	7.1	3.5
HS	2712	7.5	8.0	2	13.6	3
NW	1596	3.2	8.1	4	5.3	3.5
HW	2247	6.9	7.8	1	13.6 <sup>3</sup>	3
MR	380	4.7	7.5	3	2.4	2

<sup>1</sup> Size: Subjective ordination ranging from 1 (small) to 6 (very large (= lake))

<sup>2</sup> Phi =  $-\text{Log}_2(\text{diameter (mm)})$ ; see Appendix 2.4.

<sup>3</sup> Data unavailable; estimate based on similar wetland.

<sup>4</sup> Saturation at temperature observed. Measured value [in brackets] was questionable

### ***Sediment Characteristics:***

Sediment particle size frequency distribution and organic content (LOI) are summarized in Appendix 2.4. Organic content ranged from low (1-2%) in areas adjacent to construction zones or receiving tailings, to highly organic (up to 13.6%). Sediments were characterized by having silt or fine sand substrate (median particle size 125  $\mu\text{m}$  or less) unless the site was subject to fill by tailings or adjacent to construction sites.

### ***Comparison of Reference and Water-affected Wetlands:***

To summarize the relative range of environmental variables at reference and water-affected sites, minimum, median and maximum values were determined for quantitative parameters (Table 2.6).

Owing to the larger sample size ( $n=12$ ) variability among reference sites was greater than variability among water-affected sites ( $n=3$ ). Ranges of dissolved oxygen, pH and LOI overlapped broadly. Although the ranges of conductivity also overlapped, most reference sites had low conductivity (median=248 mhos) whereas all water-affected sites had high conductivity ( $>1596$  mhos).

### ***Multivariate Analysis of Environmental Features:***

#### ***Principle Components Analysis:***

Principle components analysis accounted for 81% of the variation in the original environmental 1997 data set (Table 2.7). Three principal components removed significant amounts of variation. Two sites (Tower 4 and 5) were not included in the analysis due to

**Table 2.6: Relative ranges of values at reference and water-affected sites in 1997.**

Parameter	Reference				Water-affected			
	Min.	Median	Max.	n	Min.	Median	Max.	n
Oxygen (mg/L)	2.4	7.2	17.9*	12	3.2	6.9	20.0*	3
Cond (uS/cm)	176	248	2712	12	1596	2247	2266	3
pH	6.91	7.58	9.3	12	7.81	8.08	9.30	3
LOI (% wt.)	1.5	7.1	13.6	9	-	5.3	-	1
Median particle (phi)	4	3.5	2	9	3	-	3.5	2

\* Values exceed 100% saturation; possible meter malfunction

Table 2.7: Factor loadings of environmental variables on 3 principal components derived for 13 wetland sites, 1997. Loadings represent the correlation between the value of the variable and the score of each principal component. Bold faced values are significant at  $p < 0.01$ . 'Prop. explained' is the proportion of the original variance among the 6 environmental variables accounted for by each principal component.

Variable	Factor		
	PC-I	PC-II	PC-III
pH	<b>0.940</b>	0.158	<b>-0.886</b>
Dissolved Oxygen	<b>0.868</b>	0.153	0.166
Size	0.712	<b>-0.515</b>	<b>0.045</b>
Conductivity	0.164	<b>0.875</b>	0.059
LOI	0.126	0.325	<b>0.839</b>
Sed. Particle Size	-0.100	<b>-0.404</b>	<b>0.765</b>
-----			
Prop. Explained	0.366	0.224	0.221
Cumulative Prop.	0.366	0.591	0.812

data gaps.

Values of pH, dissolved oxygen and wetland size were strongly positively associated with the first principal component (PC-I). Thus, wetland sites having high scores for PC-I were large, alkaline and had high levels of dissolved oxygen. This component accounted for 37% of environmental variation among wetland variable measurements.

Specific conductance was strongly positively correlated with the second PC. There was a marginally significant negative correlation between wetland size and PC-II ( $p < 0.05$ ). Thus, wetland sites having high scores for PC-II had high conductivity and tended to be relatively small. The second PC accounted for 22% of environmental variation.

Principal component III was associated with sediment characteristics and pH. Sites with high scores for PC-III had organic sediments (high in LOI) and fine median particle size, and were circumneutral in pH relative to other wetlands. PC-III accounted for 22% of variation among measurements.

The pattern of environmental similarity among the 13 wetland sites included in the multivariate analysis was summarized in a bivariate scatterplot of the principal component scores for PC-I and PC-II (Fig. 2.3).

#### *Cluster Analysis:*

A cluster analysis was performed using the three PCA-derived variables to determine the Euclidean distance relationships among the 13 wetland sites. Ward's method was then used to cluster sites that were most similar to one another. The pattern of clustering was summarized in a dendrogram (Fig. 2.4). The analysis identified 4 general

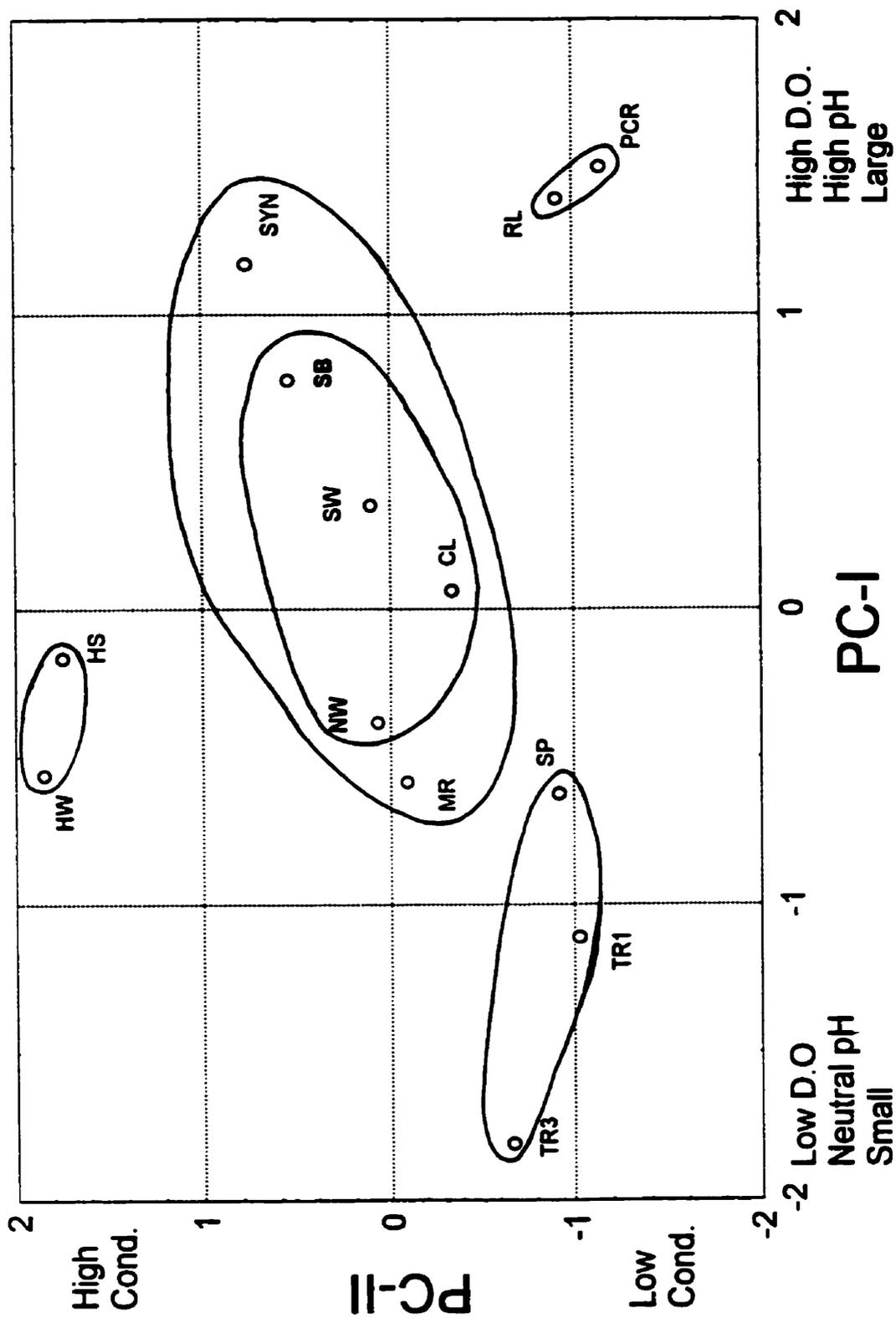


Fig. 2.3: Principal Components Analysis of environmental parameters for 13 wetlands sampled in 1997.

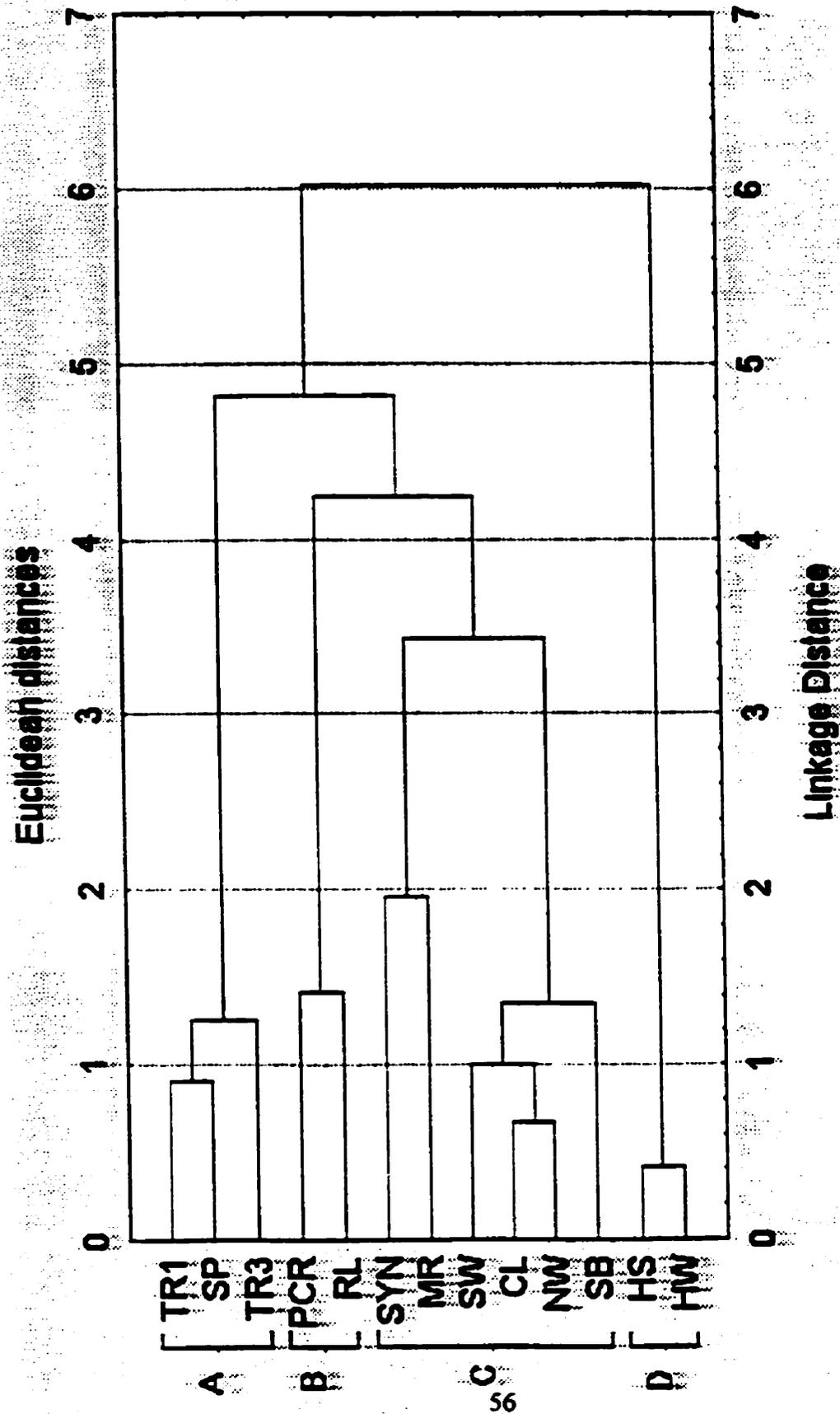


Fig. 2.4: Grouping of 13 wetlands sampled in 1997 based on 3 main Principal Components following PCA of environmental data.

clusters of sites (A,B,C,D) separated from one another by Euclidean distances of 4 units or more (dotted vertical line in Fig. 2.4). The clusters were delineated in the PCA scatterplot by enclosing the sites of each group within an ellipse (Fig. 2.3).

The scatterplot of the positions of the sites based upon the PCA of environmental data was overlaid with hand-drawn ellipses representing cluster analysis groupings by similarity. This served to summarize the relationships among sites in terms of similarities and differences among physico-chemical and associated traits. Sites within an ellipse could be considered to support similar relative abundances of benthic invertebrates.

Cluster A (TR1, TR3, SP) consisted of the 3 reference sites sampled along Tower Road, west of Fort McMurray (Fig. 2.3). These sites were all relatively small, circumneutral and had low conductivity and reduced levels of dissolved oxygen.

Cluster B (PCR, RL) was comprised of the two reservoir sites - large reservoirs with high pH, saturated levels of dissolved oxygen and low conductivity.

Cluster C (MR, NW, CL, SW, SB and SYN) was a large cluster made up of 2 subclusters (SYN-MR and SB-SW-CL-NW; Fig. 2.4)). This group was characterized by intermediate size, pH and dissolved oxygen, but somewhat elevated conductivity. Two of the process-water wetlands fell into this cluster. Of these, the natural wetlands clustered most closely with Crane Lake (Fig. 2.4) . South Bison Wetland was the most dissimilar member of this group.

Cluster D contained the High Sulphate and Hummock wetlands, both of which had extremely high conductivity, and low to intermediate values of variables associated with PC-I (Fig. 2.3). Only the Hummock wetlands contains process-affected water.

## **Benthic Invertebrate Community Analysis**

Benthos from sweep samples were sorted for all 15 sites. Abundances are listed in appendices 2.5a, b, c, and relative abundances are shown in appendices 2.6a, b, c. The amount of organic debris collected in sweeps varied greatly among samples. To provide a general standard measure of approximate absolute abundance, the total number of animals recovered per site was expressed as a proportion of the dry mass of material sorted (Table 2.8). The benthic sample data are listed in Table 2.8, with the 15 wetlands (including Tower Rd. 4 and 5 reference sites) shown in groups based on environmental similarity as determined by PCA and cluster analysis.

### ***Taxonomic Richness and Standardized Abundance:***

The 'total', 'chironomid' and 'other taxa' richness ranged from 9 to 41, 5 to 15 and 4 to 31, respectively (Table 2.8). Reference wetland TR1 had the highest total taxonomic richness (Group A). The Muskeg River wetland also supported an uncommonly large number of taxa (Group C). The process water-affected Hummock Wetland had the most depauperate fauna, with only 9 taxa found, compared to the 20 taxa identified from its most similar reference site, the High Sulphate wetland (Group D).

The abundance of invertebrates ranged from 74 to 1120/g dry mass, with most values between 300 and 850 (Table 2.8). Lowest abundances were found in the reference sites Tower Rd. 5, Tower Rd. 1 and Poplar Creek Reservoir (74, 86 and 139 animals/g dry mass, respectively). The Natural Wetlands and Ruth Lake sites supported the greatest standardized abundances (1,120 and 1,013 animals/g dry mass).

Table 2.8: 'Other' taxonomic richness (all taxa excluding vertebrates, ubiquitous groups and microcrustacea), chironomid richness, total richness ('other' + 'chironomid' richnesses), and standardized abundance of benthic invertebrates in qualitative samples from 15 wetland sites, 1997. Sites most similar in environmental variables are organized by group, reflecting cluster analysis patterns. Wetland receiving process-affected water are bold-faced. Conductivity of water is shown (from Table 2.5).

Cond (uS/cm)	Site	TAXA RICHNESS			Abundance (No./g dry biomass)	Environmental Group
		Other	Chironomid	Total		
257	TR1	31	10	41	86	A
232	SP	22	9	31	469	
193	TR3	15	15	30	306	
252	PCR	10	9	19	139	B
248	RL	17	9	26	1013	
1603	SYN	16	12	28	352	C
380	MR	27	13	40	404	
955	CL	13	6	19	839	
<b>1596</b>	<b>NW</b>	<b>10</b>	<b>8</b>	<b>18</b>	<b>1120</b>	
399	SW	21	8	29	740	
<b>2266</b>	<b>SB</b>	<b>19</b>	<b>8</b>	<b>27</b>	<b>507</b>	
2712	HS	12	8	20	828	D
<b>2247</b>	<b>HW</b>	<b>4</b>	<b>5</b>	<b>9</b>	<b>334</b>	
240	TR4	12	14	26	285	not included in cluster analysis
176	TR5	5	12	17	74	

Wetlands were pooled into 3 groups of sites (Table 2.9). These include the low conductivity reference wetlands (n=9; TR1, SP, TR3, PCR, RL, MR, SW, TR4, TR5), high conductivity reference wetlands (n=3; SYN, CL, HS), and high conductivity OSPW wetlands (n=3; NW, SB, HW). Summary information on standardized abundance (no. animals/g detritus dry mass), 'other taxa' taxonomic richness (excluding vertebrates and ubiquitous taxa - chironomids, oligochaetes, microcrustacea), chironomid generic richness, and total richness ('other taxa' + 'chironomid taxa richness') was then compiled for each group (Table 2.9).

One-way planned comparison ANOVAs for the four biotic measures of community response were nonsignificant ( $p > 0.40$ ) when comparing the high conductivity reference sites to the OSPW sites. Linear regression showed water conductivity to be negatively related to Chironomid generic richness ( $R^2 = 0.29$ ,  $p = 0.04$ ), whereas this parameter was not related to 'Other' ( $R^2 = 0.10$ ,  $p = 0.24$ ) or 'Total' ( $R^2 = 0.11$ ,  $p = 0.08$ ) taxa richness, nor to standardized abundance ( $R^2 = 0.06$ ,  $p = 0.13$ ).

When groups of sites were compared, 'other', chironomid, and total richness all showed a decreasing trend from low-conductivity reference sites > high conductivity reference sites > OSPW sites (Table 2.9). The water-affected high conductivity sites had richness of only half that of the low-conductivity reference sites. However, standardized abundance was lowest in low conductivity reference sites, followed by OSPW sites and highest in high conductivity reference sites (Table 2.9).

Table 2.9: Effects of high conductivity and process-affected water on 'Other', Chironomid, and 'Total' taxa richness (mean +/- SE), and standardized abundance (geometric mean (times/divided by SE factor)).

Attribute	High Conductivity		Low
	WA	Ref.	Conductivity
No. Sites	3	3	9
Abundance	575(se238.46)	801(se31.33)	259(se95.24)
'Other' Richness	11.0+/-4.4	15.3+/-2.8	17.2+/-2.7
Chiron. Richness	6.7+/-0.9	7.3+/-0.7	11.4+/-0.8
Total Richness	17.7+/-5.2	22.7+/-3.2	28.7+/-2.7

***Community Composition:***

The qualitative samples contained a broad diversity of taxonomic groups. Numerically dominant major groups were Oligochaeta, various genera of Cladocera, Copepoda, Ostracoda, and Chironomidae (Appendices 2.5a,b,c to 2.6a,b,c).

A total of 30 widespread and abundant taxa were used for multivariate analysis. Their relative abundances and frequencies of occurrence among sites are listed in Table 2.10. Taxa with the highest incidence of occurrence also tended to be the numerically dominant organisms within each wetland. Chironomidae and Ostracoda were found in all wetlands examined. Oligochaeta (14 sites), Cyclopoida (13 sites), and Ceratopogonidae (12 sites) were also very widely distributed. The remaining taxa occurred at 10 sites or less (Table 2.10).

Predominance of three taxa (Chironomidae, Oligochaeta and Ostracoda) was a characteristic of all 15 wetlands sampled. Of these, Oligochaeta were disproportionately under-represented in the 4 Suncor wetlands (Hummock Wetlands, Natural Wetlands, Crane Lake and High Sulphate Pond) but not at Syncrude sites Shallow Wetlands or South Bison Pond (Table 2.10). This taxon was also rare at the newly formed Syncrude North Reference Wetland, suggesting that the limited abundance of this group depends on wetland age, as South Bison Pond and Shallow Wetlands are older than the Suncor sites.

Chironomid taxa represented between 30-56% of the total richness in the OSPW wetlands, which was well within the range observed for the 12 reference sites (24-71%) (Table 2.8). However, the relative abundance of chironomids was always higher in the OSPW wetland (NW, SB, HW; 29, 31, 95%) compared to its respective reference site (CL,

Table 2.10: Relative abundance of 30 major taxa at 15 wetland sites. Sites have been ordered to reflect groups as determined by cluster analysis of environmental data. Bottom row indicates total number of animals collected per sample. 'Total' column represents total number of animals collected of each taxon. Frequency column represents number of sites at which a taxon was recorded.

Taxon	TR1	SP	TR3	TR4	TR5	PCR	RL	SYN	MR	SW	CL	NW	SB	HS	HW	Total	Freq.
Chironomidae	22.19	54.92	29.34	36.91	17.68	61.90	23.61	47.84	38.40	24.60	29.94	33.92	47.16	5.21	94.96	34971	15
Oligochaeta	25.69	38.44	33.38	30.50	54.71	16.07	46.63	4.19	44.45	59.91	17.35	2.83	29.34	25.31	0.00	33575	14
Ostracoda	8.23	0.52	10.54	29.64	21.20	17.27	26.02	26.17	2.51	6.10	32.36	59.56	9.05	58.98	3.85	23645	15
Ceratopogonidae	0.25	0.54	19.00	0.71	1.85	0.19	0.00	0.00	7.99	0.60	0.47	0.51	0.00	0.49	0.00	1510	12
Hyalella	2.24	0.53	0.00	0.00	0.00	1.36	0.00	4.95	2.96	0.82	7.81	0.00	3.72	0.00	0.00	1111	8
Sphaeriidae	4.49	0.00	3.86	0.05	0.42	0.00	0.93	0.00	0.78	0.00	0.00	0.00	0.00	0.00	0.00	608	6
Gammarus	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	2.36	6.43	0.00	0.00	0.00	0.00	594	3
Hydracarina	1.50	0.41	1.09	0.37	0.00	0.91	0.55	1.40	0.00	0.00	2.56	0.00	1.04	0.00	0.00	592	9
Corixidae	0.25	0.00	0.00	0.37	0.00	0.00	0.23	1.25	0.96	1.56	0.01	2.56	2.80	0.00	0.15	517	10
Promenatus	0.75	0.06	0.04	0.00	3.57	0.00	0.83	0.00	0.22	0.73	0.00	0.00	0.00	0.00	0.00	459	7
Caenis	7.73	0.00	0.48	0.27	0.00	0.00	0.00	1.97	0.33	1.60	0.95	0.00	0.00	0.00	0.00	287	7
Centropilum	0.00	0.24	0.00	0.00	0.00	0.00	0.00	6.40	0.00	0.00	0.00	0.00	0.42	0.00	0.00	236	4
Dysticidae	1.75	0.00	0.02	0.00	0.00	0.00	0.24	0.51	0.10	0.28	0.01	0.12	1.33	0.48	0.00	210	10
Amnicola	0.25	0.95	0.24	0.00	0.00	1.17	0.00	0.00	0.00	0.49	0.47	0.00	0.21	0.00	0.00	182	7
Bactidae	0.25	1.88	0.24	0.43	0.00	0.19	0.00	0.00	0.13	0.00	0.00	0.00	1.33	0.00	0.00	219	8
Trichoptera	0.75	0.60	0.24	0.11	0.59	0.00	0.00	0.86	0.00	0.00	0.00	0.00	0.00	2.94	0.00	176	7
Lestes	3.99	0.00	0.00	0.00	0.00	0.19	0.00	1.31	0.04	0.22	0.00	0.33	0.00	1.14	0.71	155	8
Haliphus	0.00	0.06	0.00	0.00	0.00	0.32	0.23	0.00	0.00	0.27	0.00	0.00	0.64	0.00	0.00	135	5
Hirudinea	4.24	0.01	0.00	0.05	0.00	0.00	0.00	0.00	0.11	0.00	1.50	0.00	0.00	0.00	0.00	134	6
Gerridae	0.00	0.06	0.00	0.00	0.00	0.00	0.23	0.00	0.30	0.00	0.00	0.00	0.00	0.00	0.15	121	4
Gastropoda	4.24	0.52	1.09	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	114	4
Coenoagrionidae	1.50	0.00	0.00	0.00	0.00	0.26	0.24	1.28	0.10	0.06	0.00	0.00	0.21	2.52	0.00	204	8
Armiger	2.99	0.06	0.00	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	108	3
Libel/Coruliidae	3.49	0.09	0.02	0.00	0.00	0.18	0.01	1.53	0.00	0.28	0.00	0.00	0.19	0.00	0.00	96	8
Aeshna	0.25	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.09	0.45	2.34	0.20	95	8
Siphonuridae	2.24	0.00	0.24	0.22	0.00	0.00	0.00	0.00	0.19	0.11	0.00	0.00	1.33	0.00	0.00	81	6
Notonectidae	0.00	0.08	0.02	0.38	0.00	0.00	0.00	0.28	0.00	0.02	0.04	0.09	0.22	0.00	0.00	34	10
Physidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	27	4
Planorbula	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.42	0.49	0.00	25	4
Helisoma	0.75	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.04	0.00	0.00	0.05	0.00	11	5
<b>Total</b>	<b>409</b>	<b>8929</b>	<b>4587</b>	<b>1829</b>	<b>2195</b>	<b>1651</b>	<b>38874</b>	<b>3131</b>	<b>5023</b>	<b>5040</b>	<b>7298</b>	<b>10992</b>	<b>3150</b>	<b>1860</b>	<b>3374</b>	<b>100232</b>	

SW, HS; 17, 23, 35%) (Appendix 2.6a,b,c).

The remaining taxa occasionally achieved dominance in selected wetlands but were rare or absent in others. Consequently, these less abundant taxa defined the unique biological community features of the wetlands. Amphipoda, sphaeriid clams, gastropods and water mites (*Hydracarina*) were locally abundant but less widespread.

Among the three pairs of focal sites, Shallow Wetlands was dominated by oligochaetes (57%) and chironomids (23%), whereas South Bison Pond also had oligochaetes (19%) and chironomids (31%) as well as daphnids (27%). With chironomids (35%), ostracods (18%), chydorids (17%), and daphnids (12%), High Sulphate Pond was much more diverse than Hummock Wetlands (95% chironomids). Finally, Natural Wetlands was mainly dominated by ostracods (51%), as well as chironomids (29%) and daphnids (11%), which was less diverse than Crane Lake which had ostracods (18%), chironomids (17%), daphnids (25%), and cyclopoids (12%).

### ***Multivariate Analysis of Benthic Community Composition:***

#### ***Principal Components Analysis:***

Principal components analysis was used to detect groups of taxa that tended to co-occur among wetland sites in 1997. The analysis identified 9 principal components that accounted for 89 percent of the variation in relative abundance (octaves) of taxa among sites (Table 2.11).

A group of 7 taxa was associated with values of the first Principal Component, accounting for 18% of the total variation in relative abundance of animals among wetlands.

Table 2.11: Factor loadings relating relative abundance (octaves) of 30 major taxa in wetlands to 9 Principal Components. Bold faced entries represent significant correlations ( $p < 0.05$ ) between relative abundance of a taxon and factor score of a wetland on a principal component.

Taxon	FACTOR								
	PC -1	PC-2	PC-3	PC-4	PC-5	PC-6	PC-7	PC-8	PC-9
<i>Armiger</i>	<b>0.889</b>	0.025	0.062	-0.170	-0.126	-0.069	0.234	0.135	-0.006
<i>Helisoma</i>	<b>0.874</b>	-0.011	-0.218	-0.154	0.134	-0.051	0.206	-0.147	0.017
Hirudinea	<b>0.783</b>	-0.093	0.016	-0.011	0.449	-0.058	-0.010	-0.119	-0.094
Gastropoda	<b>0.780</b>	0.155	0.050	0.141	-0.063	-0.282	0.092	-0.198	0.307
Siphonuridae	<b>0.719</b>	0.193	-0.281	0.321	0.029	0.343	-0.048	0.159	0.172
Sphaeriidae	<b>0.705</b>	0.398	0.157	-0.158	-0.268	0.020	-0.146	-0.184	0.053
<i>Caenis</i>	<b>0.567</b>	0.112	0.226	0.071	0.508	0.240	0.352	-0.292	-0.051
Oligochaeta	0.120	<b>0.901</b>	-0.047	0.077	0.147	-0.083	-0.026	0.147	0.020
<i>Promenatus</i>	0.264	<b>0.589</b>	0.275	-0.553	-0.145	0.101	0.053	0.090	-0.041
Chironomidae	-0.089	<b>-0.534</b>	0.468	0.256	0.003	0.180	-0.105	0.211	0.523
<i>Aeshna</i>	0.128	-0.130	<b>-0.937</b>	-0.142	-0.127	0.065	0.057	-0.103	0.002
<i>Planorbula</i>	<b>-0.150</b>	0.106	<b>-0.904</b>	0.171	-0.064	0.004	0.095	0.227	-0.070
Notonectidae	-0.214	-0.070	0.093	<b>0.812</b>	-0.050	0.363	0.184	-0.009	-0.072
Hydracarina	0.407	-0.024	0.258	<b>0.571</b>	0.254	-0.225	0.175	0.298	-0.042
Gerridae	-0.034	-0.153	0.077	<b>-0.539</b>	-0.295	0.242	-0.205	0.035	0.434
<i>Gammarus</i>	-0.068	0.107	0.166	-0.087	<b>0.869</b>	-0.021	-0.196	-0.002	-0.173
<i>Hyaella</i>	0.188	-0.043	-0.019	0.177	<b>0.675</b>	0.206	0.352	0.209	0.290
<i>Amnicola</i>	0.130	0.094	0.122	0.177	<b>0.548</b>	-0.487	-0.009	0.382	0.379
Corixidae	-0.037	-0.082	-0.067	0.125	0.043	<b>0.898</b>	0.139	0.164	-0.005
<i>Centroptilum</i>	-0.230	-0.080	0.094	0.443	-0.017	0.236	<b>0.740</b>	0.046	0.154
Lib/Cord'dae	<b>0.534</b>	-0.024	0.101	0.143	0.168	0.117	<b>0.731</b>	0.136	0.085
Coenagrionidae	0.293	0.006	-0.447	-0.094	-0.051	-0.046	<b>0.718</b>	0.084	-0.229
<i>Lestes</i>	0.379	-0.422	-0.237	-0.225	0.002	0.014	<b>0.616</b>	-0.204	-0.184
Trichoptera	0.119	0.266	-0.289	0.099	-0.238	-0.406	<b>0.584</b>	-0.432	-0.089
Dytiscidae	0.449	0.107	-0.458	0.037	0.025	0.385	<b>0.511</b>	0.281	-0.144
<i>Haliplus</i>	-0.148	0.165	-0.073	0.033	0.087	0.085	0.011	<b>0.912</b>	0.142
Ceratopogonidae	0.041	0.527	0.007	0.009	0.092	-0.081	-0.429	<b>-0.600</b>	0.200
Physidae	-0.014	0.056	-0.449	0.447	-0.016	0.338	-0.067	<b>0.576</b>	0.134
Ostracoda	-0.087	-0.015	-0.069	0.107	-0.027	0.028	0.008	-0.020	<b>-0.950</b>
Baetidae	0.195	0.156	-0.149	0.602	-0.131	-0.133	-0.101	0.244	<b>0.610</b>
Prop. Var. Explained	0.179	0.079	0.104	0.095	0.081	0.073	0.112	0.087	0.079
Prop. of Total Var.	0.179	0.258	0.362	0.457	0.538	0.611	0.723	0.810	0.889

Unidentified, immature and other snails, collectively assigned as ('other') Gastropoda, the snails *Armiger*, *Helisoma*, sphaeriid clams, leeches, and the mayflies *Caenis*, and Siphonuridae (primarily *Siphonurus*) all tended to co-occur.

Relative abundance of oligochaete worms and the snail *Promenetus* were positively associated with factor PC-2. The relative abundance of Chironomidae was negatively associated with these taxa. Thus, samples tended to be dominated by either Chironomidae, or both oligochaetes and *Promenetus*.

Relative abundances of *Aeshna* dragonflies and *Planorbula* snails were strongly negatively associated with values of PC-3. These taxa co-occurred in wetlands that had low values for PC-3.

Aquatic hemipterans, Notonectidae and Gerridae, together with aquatic mites (many species of which parasitize hemipterans) were associated with values of PC-4. But whereas the association of back swimmers (notonectids) and mites with PC-4 was positive, the association with pond-skaters (gerrids) was negative, indicating that the two types of hemipterans seldom occurred together in the same wetland. *Promenetus* was also negatively associated with PC-4.

Amphipods (*Gammarus* and *Hyaella*), as well as the snail *Amnicola*, were all positively associated with PC-5. Relative abundance of water boatmen (Corixidae) was associated with PC-6.

A broad variety of taxa including Odonata (Libellulidae+Corduliidae dragonflies, Coenagrionidae and *Lestes* damselflies), caddisflies (Trichoptera) and predacious water beetles (Dytiscidae) tended to co-occur. Their relative abundance was positively associated

with scores of PC-7.

Relative abundances of the snails Physidae and beetle larvae (*Haliphus*) were positively associated with values of PC-8, whereas relative abundances of biting midges (Ceratopogonidae) were negatively associated with this principal component factor.

The relative abundance of Baetidae mayflies was positively associated with values of PC-9, whereas relative abundances of seed shrimps (Ostracoda) were strongly negatively associated with this factor.

#### *Cluster Analysis:*

A cluster analysis was performed using the full suite of 9 PCA-derived taxon-specific variables to determine the Euclidean distance relationships among the 13 wetland sites. Ward's method was then used to cluster sites that were most similar to one another.

The pattern of clustering is summarized in Fig. 2.5. There was little evidence of consistent similarity in community composition among wetlands - pairs of sites were linked only at relatively large Euclidean distances. There was also little or no correspondence between the grouping pattern observed in this analysis and the pattern found for the wetlands based on similarity in environmental variables.

A second analysis was performed to evaluate similarity of the sites based on only the factor scores from first 3 principal components. Together, these factors described 12 major taxa, but included two of the 3 most numerically dominant organisms (Oligochaeta and Chironomidae). At a Euclidean distance between 2 and 3 (moderate), this analysis partitioned the sites into 5 groups (designated A'-E', Fig. 2.6). Two of these groups

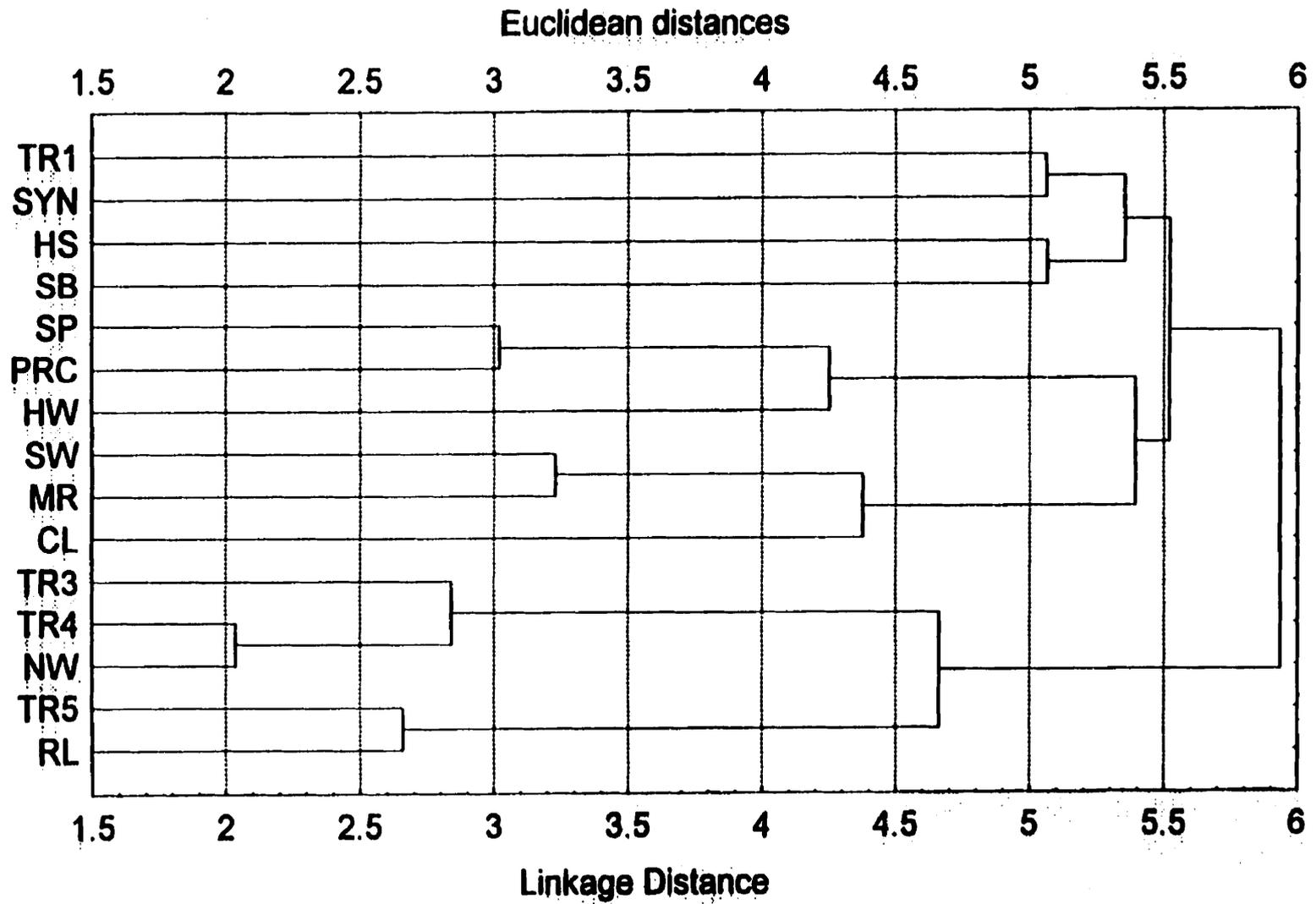


Fig. 2.5: Grouping of 15 wetland sites based on relative abundance of major benthic taxa.

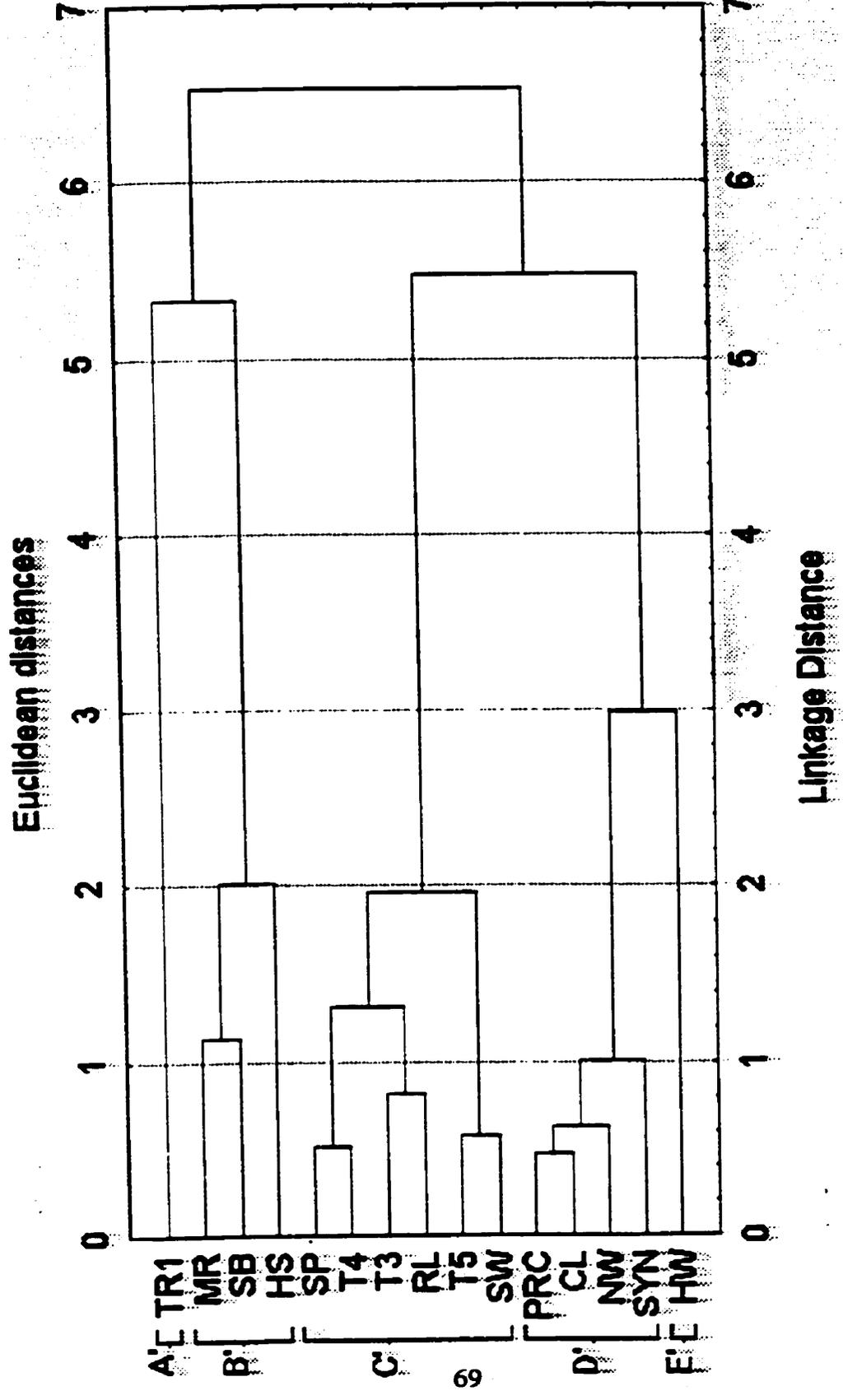


Fig. 2.6: Grouping of 15 wetland sites based on 3 dominant benthic groups from 1997 dip net samples.

consisted of single sites. When group boundaries were drawn around the wetland sites on the environmental Principal Components scatterplot, they overlapped considerably, but appeared to be weakly ordinated with respect to conductivity (Fig 2.7). One single, distinctive site was the reference Tower Road 1 (Group A', Fig. 2.6), which had low conductivity (Fig.2.7) and the greatest richness of all wetlands in the study. Group C' was made up of 6 sites (Fig. 2.6), of which environmental data were available for 4. Three of these 4 sites had low conductivity and one (Shallow Wetland) had intermediate conductivity. Groups B' and D' each was made up of sites with a wide range of conductivity values. The process water-affected Natural Wetland clustered within Group D', whereas South Bison Wetland fell within group B'. The water-affected Hummock Wetland, which had the highest overall conductivity formed the single-site group E', which was distinctive in being almost completely dominated by chironomids.

#### ***Determination of Dominant Chironomid Taxa***

Appendices 2.7a,b,c,d,e list the dominant chironomid taxa present at each of the 15 wetlands sampled in 1997. Twenty chironomids were identified for each size fraction available (of 1.0 mm, 0.50 mm, 0.25 mm) from the qualitative dip net sample. The summary of these data is shown as the number of chironomid taxa per subfamily or tribe (Table 2.12). Chironomid generic richness was lowest at Hummock wetlands, and is lower in the six focal sites of 1997 as compared to all the 9 other reference sites (Table 2.12). Among the 6 focal sites, there was no significant difference in number of chironomid taxa present between reference and tailings sites ( $p=0.84$ ).

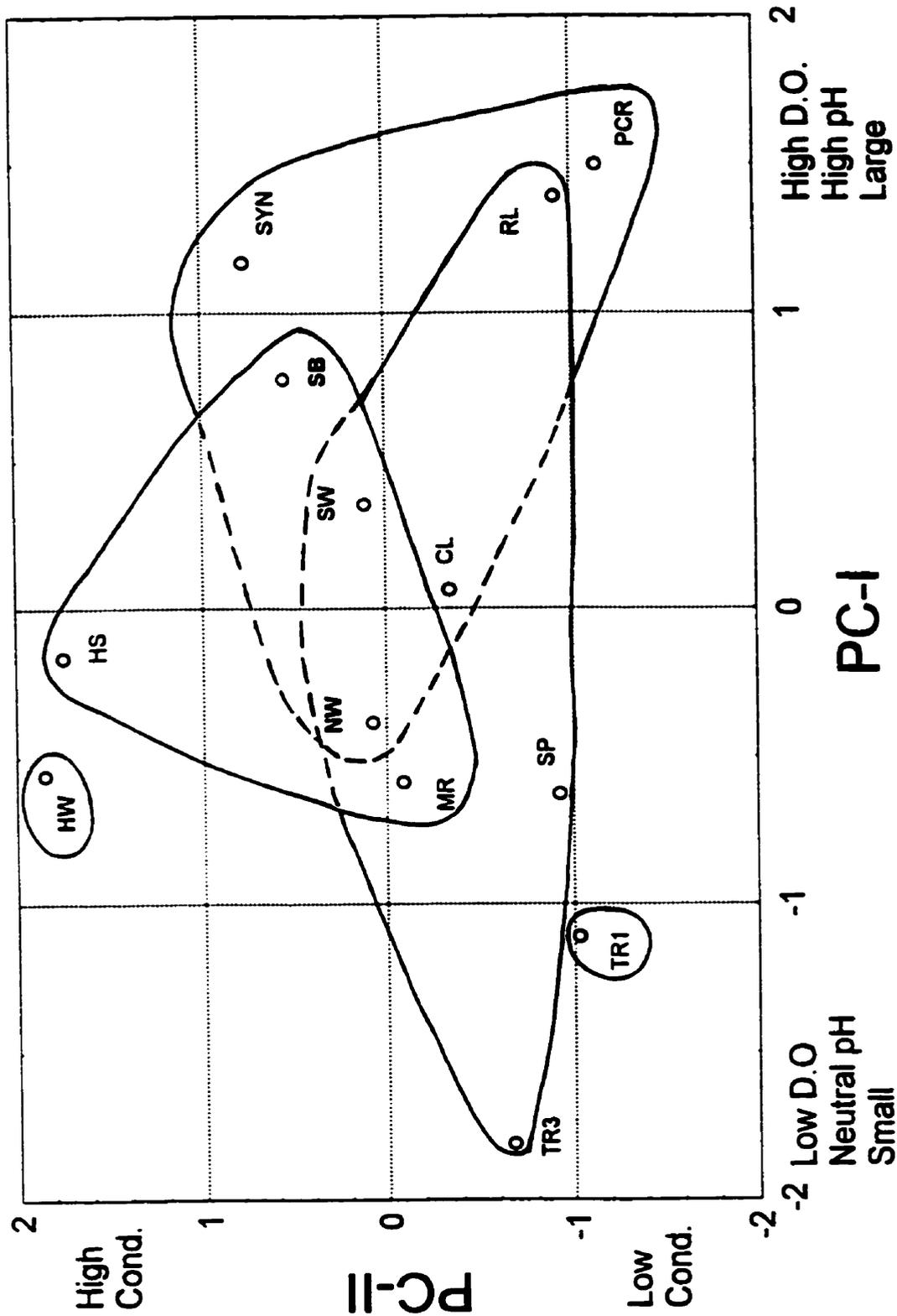


Fig. 2.7: Principal Components Analysis of environmental factors for 13 wetlands sampled in 1997. Ellipses indicate similar benthic assemblages based on cluster analysis of 3 major principal components of benthic data.

Table 2.12: Summary of Chironomid Richness by Subfamily/Tribe for 15 wetlands.

Site	Orthocladinae	Chironominae		Pseudochironominae	Tanypodinae	Total
		Tanytarsini	Chironomini	Pseudochironomini		
TR1		1	6		3	10
SP	3	2	4			9
TR3	3	4	5		3	15
PCR		2	5		2	9
RL	1	2	4		2	9
SYN	4	2	5		1	12
MR	1	1	4	1	6	13
CL	3	1	2			6
NW	2	4	1		1	8
SW	1	2	3	1	1	8
SB	1	2	4		1	8
HS	1	2	2		3	8
HW	4				1	5
TR4	2	3	5		4	14
TR5	4	3	4		1	12
Total	30	31	54	2	27	146
Count	13	14	14	2	13	15

In terms of subfamily, Crane Lake and Spruce Pond were the only sites without Tanypodinae, whereas Tower Rd. #1 and Poplar Creek Reservoir were dominated by Chironomini (Chironominae) but lacked Orthocladinae (Table 2.12). The highest chironomid generic richness was found at Tower Rd #3, Muskeg River, Tower Rd. #5, and Syncrude North Wetland. Chironomini were the most common group observed among sites.

The predominant taxa (in decreasing order) at each of the 6 focal sites are shown in Table 2.13. Seven dominant taxa were observed in the 6 focal wetlands. *Tanytarsus* was dominant at four focal sites, and *Paratanytarsus* was subdominant at all three focal reference sites.

In comparison, the other 9 reference sites showed less dominance of taxa at each site, with more variation in the more common taxa observed (Appendices 2.7a,b,c,d,e). The 9 most common taxa at low-conductivity reference sites included *Chironomus* (Tower Rd. 1, Muskeg R.), *Endochironomus* (Poplar Cr. Res.), *Cladopelma* (Tower Rd. 3), *Tanytarsus* (Tower Rd. 3 and 4, Ruth L., Muskeg R., Syncrude North wetland), *Paratanytarsus* (Poplar Cr. Res.), *Microtendipes* (Tower Rd. 3 and 4, Poplar Cr. Res.), *Cryptotendipes* (Tower Rd. 5, Syncrude. North wetland), *Procladius* (Tower Rd. 1 and 4, Muskeg R.), and *Cricotopus (trifascia)* (Spruce Pond).

Table 2.13: Predominant chironomid genera in dip net samples at 6 paired focal sites of 1997. OSPW wetlands are bold-faced.

Site	Predominant Taxa		
	Dominant	Subdominant	Subdominant
<b>Natural Wetland</b> Crane Lake	<i>Tanytarsus</i> <i>Chironomus</i>	<i>Cricotopus</i> <i>Cricotopus</i>	<i>Derotanypus</i> <i>Paratanytarsus</i>
<b>Hummock Wetland</b> High Sulphate	<i>Psectrocladius</i> <i>Tanytarsus</i>	<i>Derotanypus</i> <i>Chironomus</i>	<i>Cricotopus</i> <i>Paratanytarsus</i>
<b>South Bison Pond</b> Shallow Wetland	<i>Tanytarsus</i> <i>Tanytarsus</i>	<i>Chironomus</i> <i>Paratanytarsus</i>	- <i>Psectrocladius</i>

## **1998 Field Season**

### **Physico-chemical Data**

#### ***General Environmental Features***

General environmental features of the wetlands sampled in 1998 were similar to those studied in 1997.

#### ***Water Chemistry***

Measurements of water chemistry were taken with YSI meters on the day benthic sampling was performed at each site (Appendix 2.8). Measurements of dissolved oxygen were occasionally suspiciously high (up to 50% greater than the level of oxygen saturation). In multivariate analyses of environmental data (see below), water surface oxygen values that exceeded saturation were replaced with the value for 100% saturation at prevailing water temperature. Near-bottom concentrations were expressed as a proportional fraction of the adjusted near-surface value.

#### ***Sediment Characteristics***

Sediment particle size frequency distribution and organic content (LOI) are summarized in Appendix 2.9. Organic content ranged widely in both affected (3.8 - 30.6 % dry mass.) and reference sites (1.7 - 50 % dry mass.).

#### ***Comparison of Reference and Water-Affected Wetlands***

To summarize the relative range of environmental variables at reference and water-affected sites, minimum, median and maximum values were determined for quantitative parameters (Table 2.14).

Table 2.14: Relative ranges of water and sediment (where indicated) parameters at reference and water-affected sites for 1998.

Parameter	Reference				Water-Affected			
	Min.	Median	Max.	n	Min.	Median	Max.	n
Dis. Oxygen (mg/L)	1.2	6.6	10.8	27	2.2	7.2	10.8	9
Conductivity ( $\mu$ S/cm)	170	550	4700	27	500	1680	2000	9
Sediment ORP (mV)	-200	-92	-30	26	-300	-133	-91	8
pH	7.57	8.11	8.80	9	7.87	8.97	9.31	8
Salinity (‰)	0	0.3	3.0	25	0.1	1.0	1.0	9
LOI (% wt.)	1.7	9	50	27	3.8	8.1	30.6	9
Median particle (phi)	5	3	1	27	4	3	3	9

Conductivity of water ranged more widely among the group of reference wetlands (170 to 4,700  $\mu\text{S}/\text{cm}$ ) compared to the tailings-affected group (500 to 2,000  $\mu\text{S}/\text{cm}$ ). The broader range in conductivity of reference wetlands was attributable primarily to two sites - Saline Lake (conductivity 4,700) and High Sulphate Wetland (conductivity 2,700). Median conductivity was lower among the reference group. Reference values of salinity were also broader (0 to 3 ‰) compared to water-affected sites (0.1 to 1 ‰), and again this reflected only a few rare reference sites with high salinity as the group median was lower than for the water-affected group. The pH was slightly higher at tailings-affected sites (median 8.97) relative to reference sites (median 8.11).

### ***Multivariate Analysis of Environmental Features***

#### ***Principle Components Analysis:***

Principle components analysis accounted for 91% of the variation in the original environmental data set among sites, based on five principal components (Table 2.15).

Values of salinity and conductivity were strongly positively associated with the first principal component (PC-I). There were also weaker positive correlations of PC-I with LOI and pH, and a weaker negative correlation with epibenthic concentration of dissolved oxygen. Thus, wetland sites having high scores for PC-I were saline, and tended to be alkaline, have organic sediments, and lower levels of dissolved oxygen. This principal component accounted for 26% of environmental variation among wetland variable measurements (Table 2.15).

Table 2.15: Factor loadings of environmental variables on 5 principal components derived for 33 wetland sites, 1998. Loadings represent the correlation between the value of the variable and the score of each principal component. Bold-faced values are significant at  $p < 0.01$ ; values marked with an asterisk are significant  $p < 0.05$ . "Prop. explained" is the proportion of the original variance (among the 9 environmental variables) accounted for by each principal component. Cumulative proportion is calculated.

Variable	Factor				
	PC-I	PC-II	PC-III	PC-IV	PC-V
Conductivity	<b>0.915</b>	0.089	0.119	-0.220	0.212
Salinity	<b>0.904</b>	0.071	0.189	-0.228	0.139
Area	0.122	<b>-0.947</b>	0.007	0.086	-0.029
Depth	-0.326	<b>-0.880</b>	-0.052	-0.020	0.079
Med. Particle Size	0.060	0.125	<b>0.905</b>	-0.169	0.249
LOI	<b>0.444</b>	-0.156	<b>0.747</b>	0.052	-0.291
Redox	-0.242	-0.146	<b>-0.531</b>	<b>0.907</b>	-0.126
Oxygen	-0.396 •	0.296	-0.272	<b>0.615</b>	0.410*
pH	0.347 •	-0.080	0.116	-0.055	<b>0.874</b>
Prop. Explained	0.257	0.204	0.169	0.149	0.130
Cumulative Prop.	0.257	0.461	0.630	0.779	0.909

Water body depth and surface area were strongly negatively correlated with the second PC. Thus, wetland sites having high scores for PC-II were generally small and shallow. The second PC accounted for 20% of environmental variation.

Principal component III was associated with sediment characteristics. Sites with high scores for PC-III tended to have more organic sediments (high in LOI) and finer median particle size (greater phi-value), as well as lower sediment redox potential. PC-III accounted for 17% of variation among measurements.

Principal components IV and V accounted for 15 and 13 percent of variation, respectively. Dissolved oxygen concentrations and sediment redox potential values were strongly positively correlated with component IV. Thus, sites with high scores for this component had higher epibenthic oxygen concentrations and less reduced (but still typically anoxic) sediments than sites with lower scores for PC-IV. The only factor correlated with values of PC-V was pH. Sites with high values of PC-V were more alkaline than sites with lower PC-V scores.

The pattern of environmental similarity among the 33 wetland sites included in the multivariate analysis was summarized in a bivariate scatterplot of the principal component scores for PC-I and PC-II (Fig. 2.8).

#### *Cluster Analysis:*

A cluster analysis was performed using the five PCA-derived variables to determine the Euclidean distance relationships among the 35 wetland sites. Ward's method was then used to cluster sites that were most similar to one another. The pattern of clustering was

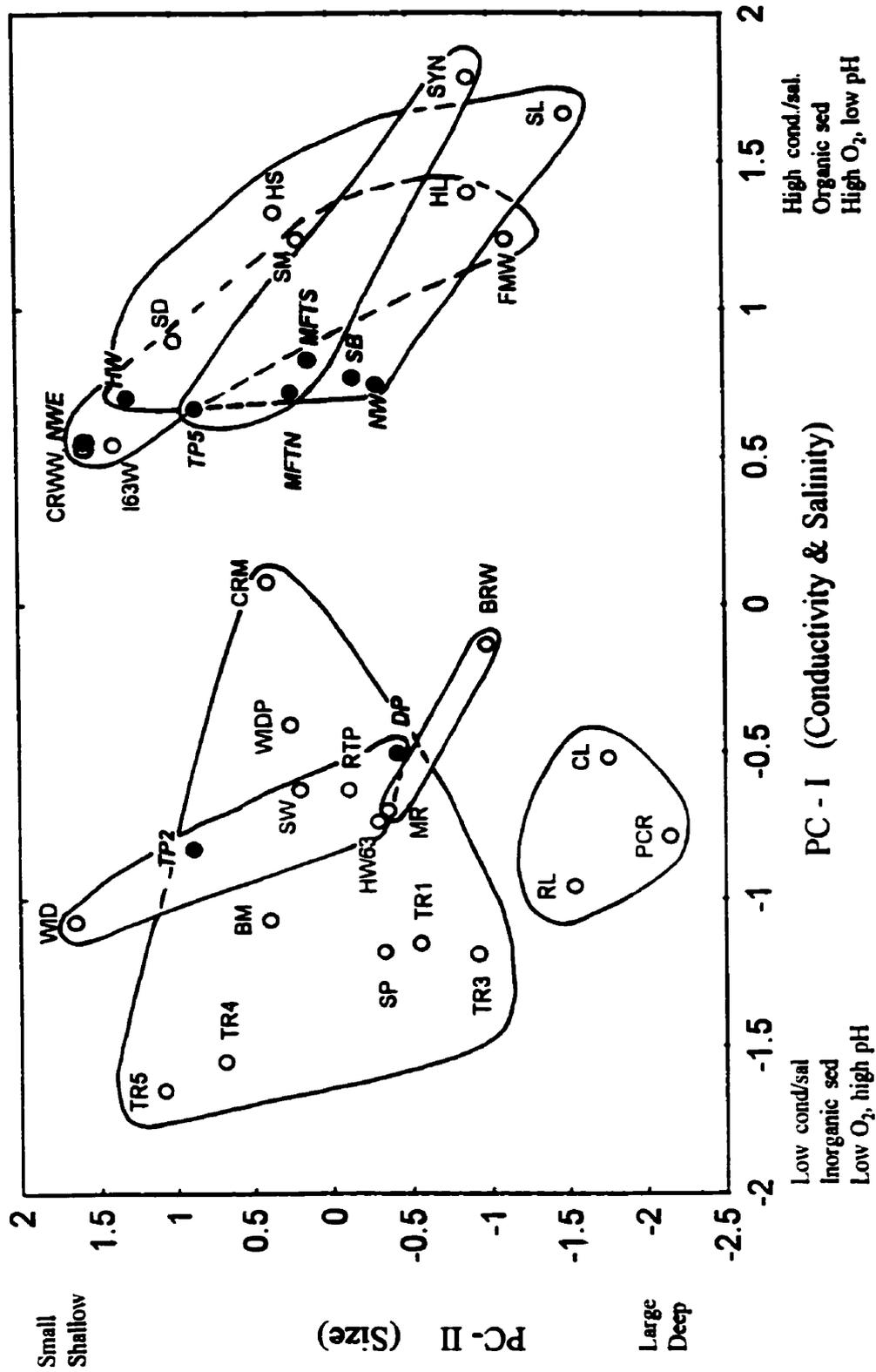


Fig. 2.8: Principal Components Analysis of environmental parameters for 35 wetlands sampled in 1998. Oil sands-affected sites are highlighted.

summarized in a dendrogram (Fig. 2.9).

The analysis identified 6 general clusters of sites (A,B,C,D,E,F) separated from one another by Euclidean distances of 6 units or more (dotted vertical line in Fig. 2.9). The clusters were delineated in the PCA scatterplot by enclosing the sites of each group within an ellipse (Fig. 2.8). The 6 clusters fell within 2 groupings that were clearly distinctive with respect to PC-I. Two clusters of sites (A,B; Fig. 2.9) were high conductivity wetlands and tended to be richer in organic sediments, more alkaline and more hypoxic than other sites (groups to the right in Fig. 2.8). The remaining 4 clusters (C,D,E,F; Fig. 2.9) had complementary characteristics (lower conductivity, more oxygen, less organic sediment, lower pH).

Cluster A contained all three water-affected focal wetlands (Natural Wetland, Hummock Wetland, and South Bison Pond), in addition to the reference sites High Sulphate Wetland and Saline Lake.

Cluster B contained Crane Road West Wetland, Natural Wetland East, Syncrude South Ditch, I63W, Fort Mackay Wetlands, Syncrude Salt Marsh, and Horseshoe Lake, all of which tended to have high conductivity.

Clusters C, D, E, and F were less well spatially separated along the first two Principal Component axes because they were rather similar in terms of conductivity and associated features.

Cluster C contained the majority of tailings-affected wetlands that did not fall into Cluster A, including Suncor Mature Fine Tailings Ponds North and South, Syncrude Test Ponds 2 and 5, Syncrude Demo Pond, Syncrude "S" Pit, and two wetlands by the highway -

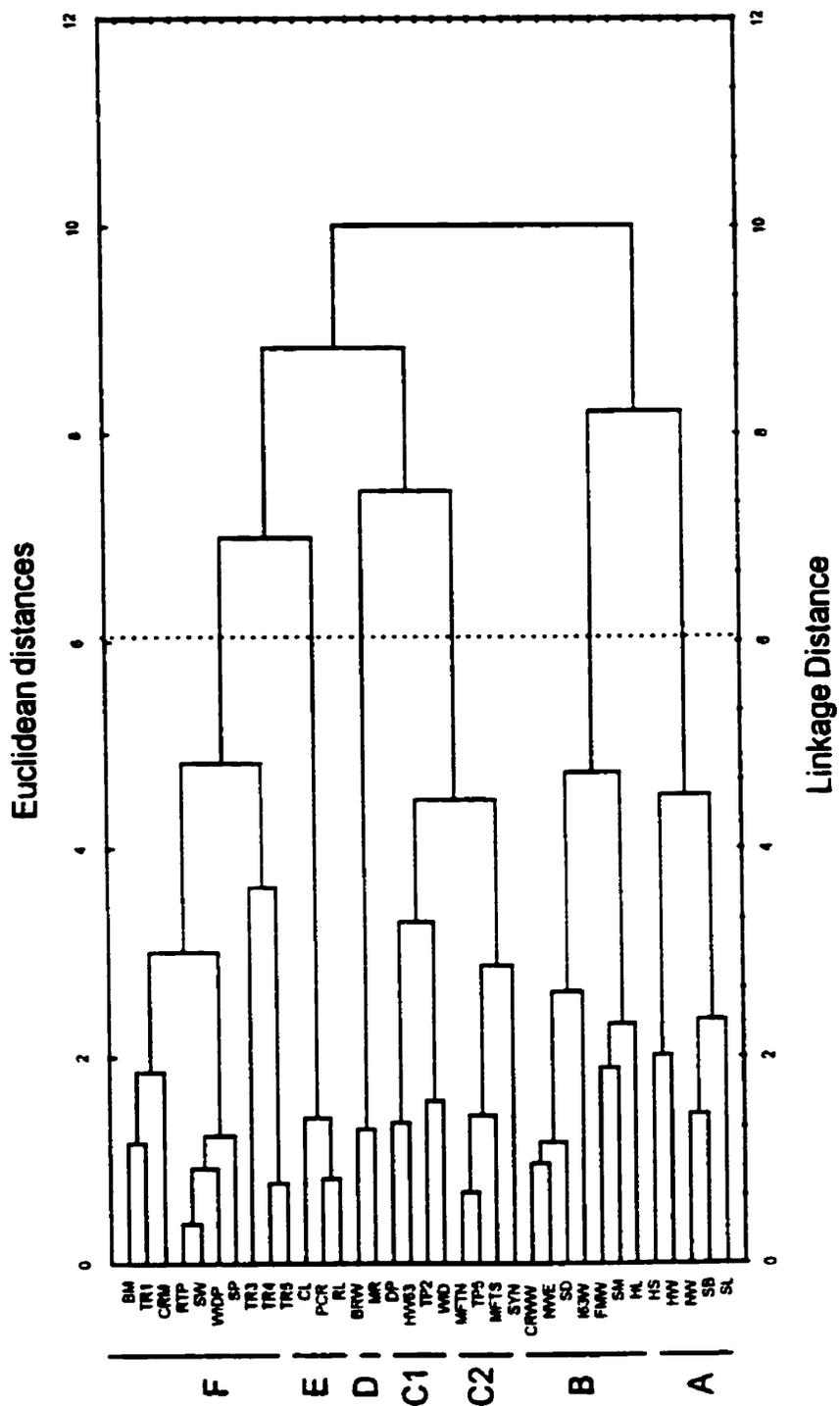


Fig. 2.9: Grouping of 35 wetlands sampled in 1998 based on environmental data.

## **Hwy 63 Wetland and Syncrude West Interceptor Ditch.**

Cluster D was comprised of the Muskeg River wetlands and Barge Marsh Wetlands. Cluster E was contained the 3 largest water bodies - Ruth Lake, Poplar Creek Reservoir and focal wetland Crane Lake. Cluster F was made up largely of the Tower Road reference sites, which tended to be small, shallow and low in conductivity.

## **DISCUSSION**

### **Site Grouping**

The fifteen wetland sites sampled in 1997 contained 3 pairs of reference and OSPW sites of relatively high similarity based on environmental traits. The level of regional variation in environmental attributes was demonstrated among the nine other reference sites. Bailey et al. (1995) advised careful selection of unimpacted sites to be considered as references, and described a range of responses to measure, regarding both community structure and sediment toxicity among reference sites, as opposed to the use of one set response.

Ordination of the 33 wetland locations sampled in 1998 showed that the major gradients that delineated wetland variability in 1997 sampling (conductivity, dissolved oxygen, pH, size and associated factors) were confirmed as predominant in this larger data set. Cluster analysis identified 6 groups of environmentally similar wetlands in 1998. Both tailings-affected and reference wetlands occurred within some of the same clusters (Fig. 2.8, 2.9), indicating good reference candidate sites for ongoing comparisons with tailings-affected wetlands. In particular, High Sulphate Pond and Saline Lake appear to be physico-

chemically similar to tailings-water affected wetlands. High Sulphate Pond is clearly well suited as a reference for comparison with other OSPW wetlands, especially Hummock Wetland. Saline Lake may be an especially suitable candidate for comparison with Natural Wetland and South Bison Pond in future years of study. Wetlands influenced primarily by mature fine tailings fell within another distinctive cluster. Suitable reference wetlands for these water bodies can also be identified from the cluster analysis (Fig. 2.9).

However, there were changes observed in grouping of sites between years. The relative importance of water pH, oxygen, and organic content in sediment were all increased in the 1998 analysis relative to the previous year, and this altered the axes slightly. Between 1997 and 1998 (Fig. 2.3 and 2.8), Crane Lake, Shallow Wetland, and Natural Wetland all shifted moderately in different directions, although the position of South Bison appeared stable. The NW-CL and SB-SW pairings of 1997 were not apparent in 1998 based on the PCA and cluster analyses. However, the pair High Sulphate Pond and Hummock Wetland did not show much change in their relationship over the two years. The increased number of sites sampled in 1998 indicated clear groupings of low and high conductivity wetlands. Faulty meters, especially for D.O., are likely responsible for some of the inter-year variation within sites.

### **Benthic Macroinvertebrate Community**

All three measures of richness (total, chironomid, and other) were reduced in relation to increasing conductivity and the presence of OSPW, although the former appeared to be the dominant determinant. High conductivity lakes in British Columbia had

only one species of *Chironomus*, whereas surrounding lakes of low conductivity supported several species of the genus (Cannings 1975). Although total taxa richness was low to medium in OSPW sites, other factors including wetland age and other habitat attributes not measured may be at least partially responsible. This particularly applied to Hummock Wetland, which receives very erratic input of OSPW and thus experiences huge changes in water levels throughout the growing season. Hummock Wetland is also particularly homogeneous in terms of habitat, which would support the low richness observed.

Although dip nets are of limited quantitative value, and are more useful in assessing simple community composition/ richness, the standardized abundances appeared to be higher at sites with elevated conductivity, although OSPW seemed to partially cancel this effect. Other studies have reported similar results of reduced richness but elevated abundances in degraded systems. Assessment of the Ekman samples collected could be used to more accurately assess the effect of conductivity and OSPW on species abundances. PCA was used to detect reductions in total biomass and number of invertebrate species in metal-contaminated streams, although abundances were not affected (Beltman et al. 1999). This supports the use of taxa richness in assessing disturbance in aquatic systems.

Chironomid taxa richness was clearly reduced in sites of higher conductivity. As chironomids did not totally dominate benthic communities related to OSPW when compared to reference sites (similar relative abundances of this family), diversity in OSPW wetlands does not seem overtly affected.

Benthic macroinvertebrate community composition was generally similar among paired focal sites, with the exception of Hummock Wetlands, which may be due to factors

discussed above. Chironomids, ostracods, and oligochaetes dominate the wetlands of this boreal forest region of Canada, and amphipods, clams, gastropods and water mites showed local abundance at some sites. Differences were noticed at the scale of populations of chironomid genera which showed varied dominance among sites (see below).

In terms of sensitive taxa, oligochaetes were more rare at sites with high conductivity, with relative abundances ranging from 0-19%, compared to reference sites with values of 20% and much higher. However, other patterns of distribution for different taxa were not obvious. Clements (1994) emphasized the importance of considering natural variation in assessing community structure in field studies, in order to accurately characterize the effects of anthropogenic stressors.

Species sensitivities can be effectively used to monitor water quality, although differences among taxa of the same family have been noted (Clements 1994). Ephemeropterans, often sensitive to metal contamination (Clements et al. 1992, Beltman et al. 1999), were absent at both HW and NW. However, mayflies were found at SB, and were not found at other reference sites, indicating that this index is not sufficient for assessing the effects of oil sands waters. Often related to the loss of sensitive mayflies and Tanytarsini is an increased abundance of tolerant organisms including Orthocladinae and Trichoptera (Clements et al. 1992, Clements 1994). A mixture of chlorides, ammonia, organics, and metals was related to reduced mayflies, no effect on caddisflies, and an increase in Orthocladini (Pontasch and Cairns 1991). Orthoclads were quite abundant at HW and included 4 of the 5 chironomid genera observed. Tanytarsini were absent from this site, but present at NW and SB. Thus it appears that HW is most severely affected by oil

sands inputs compared to other sites. Coleoptera larvae were most common in samples from SB and TR1, although this group has also been shown to be sensitive to metals in streams (Beltman et al. 1999). South Bison does not generally appear to be affected by oil sands contamination, based on benthic community richness, and the presence of Tanytarsini, mayflies, and beetles. The history of this site is not clearly known, but there are no active inputs into this pond, as it is being reclaimed to become a bison pasture.

The application of multivariate statistics to problems in ecotoxicology has only recently become popular, mainly due to advances in computing power and software packages available. These tools have advantages over univariate statistics in that they allow study of all observed variables simultaneously, as related to entire communities, and can lead to isolation of important factors in community changes over time (Kedwards et al. 1999). Recently, redundancy analysis was used to account for community response to pesticide concentrations over time (Van den Brink and Ter Braak 1999). This technique could be applied in future to monitor the effect of oil sands mining processes to wetland biota over time.

Erman (1973) used community ordination to compare benthic macroinvertebrate assemblages to environmental characteristics in Bear Lake, Utah, and found silt and clay contents to be the prime factors involved in distributions of benthos. Conductivity, wetland area, and macrophyte abundance were all significant in the ordination of chironomid assemblages in coastal wetlands of Lake Erie (Botts 1997). Ordination of the 1998 sites of this study were also significantly affected by conductivity and water body size. Wetland age and degree of disturbance were also relevant to the wetlands I examined, although ages of

sites are not accurately known.

The results derived from PCA of invertebrate data revealed 9 independent factors relating to benthic groups. From this, the initial cluster analysis indicated very little similarity in benthic community composition among sites. Also, the groupings observed did not correspond to those based on environmental similarity. Thus, overall community composition at this level (*i.e.*, at the level of 30 taxa summarized by 9 independent associations) was largely unrelated to the environmental variables that were measured. Other abiotic or biotic (trophic status, predators, food availability) or natural heterogeneity may be responsible for the observed patterns. Marchant et al. (1997) classified macroinvertebrate assemblages in streams and were able to predict taxa based on models using stepwise discriminate analysis of 22 environmental variables. The inclusion of more variables in the PCA thus may have resulted in a better ability to related environment to community structure in this study. However, the number of wetlands required to attain a sufficient sample size would then be unrealistic based on the resources we had at our disposal.

Cluster analysis of dominant benthic groups (Fig. 2.6, 2.7) derived from PCA showed more distinctive grouping of sites. However, there were still large differences between biological and environmental groupings. Although a weak trend relating to conductivity was suggested among benthic assemblages of the 15 dip nets collected in 1997, there was considerable variability among the five groups described.

## **Chironomid Community**

Analysis of the chironomid community suggests that the environmental characteristics of the 6 high-conductivity focal sites result in different community composition as well as richness and dominant species relative to low conductivity habitat. Two of the OSPW sites were dominated by *Tanytarsus*, as were 6 of the reference sites including 2 of the 3 focal reference sites. However, there was variety among the remaining reference sites in terms of dominant taxa (*Cricotopus*, *Cladopelma*, *Endochironomus*, *Microtendipes*, *Cryptotendipes*, *Procladius*).

These results are somewhat similar to those reported in a study of saline lakes. A *Tanytarsus* sp. and *Cryptotendipes* sp. association was observed in highly saline lakes, whereas *Cricotopus* sp. and *Procladius* sp. were related (at low density) to relatively diverse low conductivity sites (Cannings and Scudder 1978). The saline lakes interestingly also contained *Derotanypus* as the main predatory chironomid, as we observed at both Hummock and Natural Wetlands. The predominance of *Chironomus* at South Bison Pond is not unexpected, based on similar findings in lakes of moderate to heavy salinity (Cannings and Scudder 1978).

The proportion of Tanypodinae, as well as other predators, increased in acid mine-polluted sites, suggesting that these acidophiles were poor competitors (Cranston et al. 1997). The lack of *Psectrotanypus* (*Derotanypus*) at most reference sites (except HS which is a saline, reclaimed pond that may contain residuals oil sands elements) indicates the specialization of this taxa in particular. However, several low conductivity reference sites including MR were home to a few Tanypod taxa.

However, there is little evidence that OSPW water per se has an effect on chironomid generic richness.

### **Future Research**

Data used in our initial multivariate ordination of characteristics of 33 wetlands surveyed in 1998 indicate considerable variability among sites. More refined measures of environmental characteristics (multiple readings on different days, more reliable meters – especially oxygen meter) could clarify the source of variation. In particular, we plan to acquire more accurate estimates of wetland surface area by consulting maps and aerial photographs. Sediment characteristics (median particle size, LOI) require verification through hydrometric analysis of the finest particles. Characteristics of riparian and overstory vegetation, and chlorophyll *a* (a measure of primary production) will provide additional characteristics useful in classifying the wetlands.

Analysis of benthos in subsequent years, especially quantitative characterization, would enable a better understanding of the variability in community structure and composition observed in 1997 samples. A snapshot from dipnet samples provides only general information on community composition, being dependent on the time of year sampled and the specific area sampled at each site. Certainty of observed trends would be possible with proper sample replication, using a quantitative sampler (e.g. Ponar, core sampler, or Ekman).

## **Conclusions**

Data from 1997 collections suggest that high-conductivity wetlands support greater abundance but reduced richness of benthic taxa and of chironomid genera. Comparison of tailings-water affected wetlands suggest a possible trend (though nonsignificant) of reduced abundance and benthic taxonomic richness relative to high conductivity reference wetlands. Chironomid generic richness was relatively similar among the 3 paired high conductivity wetlands. However, the predominance of *Derotanypus* at all three OSPW wetlands indicates that differences between reference and OSPW wetlands do occur in terms of the composition of chironomid communities present.

Hummock Wetland appears most severely affected by oil sands inputs compared to other sites, both in terms of richness (total, and chironomid) as well as composition of the chironomid community and the general benthic community.

Conductivity is related to significant differences in benthic macroinvertebrate (including chironomid) richness. OSPW appears to have a smaller effect on the benthic community, but is related to distinct chironomid community composition.

## **CHAPTER 3 - Field Assessment of Chironomid Mouthpart Deformities Associated with Exposure to Oil Sands Process Water**

### **INTRODUCTION**

Midges are widely used in biomonitoring aquatic ecosystems (Williams et al. 1986, Pascoe et al. 1989, Hudson and Ciborowski 1995, Sibley et al. 1997, Burt 1998). This is related to their world-wide distribution in most types of environments, broad taxonomic diversity, ease of collection in the field (where they exist usually in high densities) and rearing in the laboratory (convenient for bioassays), well studied biology and ecology relating to both aquatic and terrestrial foodwebs, and mainly detritivorous and benthic life history (Pinder 1986, Warwick 1990, Ciborowski et al. 1995). As many contaminants (metals, organic chemicals) tend to distribute into organic components of the sediment layers, these organisms provide excellent indication of aquatic contamination, and have thus been widely applied in field and laboratory toxicology studies (Pesch et al. 1981, ASTM 1990, Bedard et al. 1992, Harkey et al. 1994 ).

Biomarkers that indicate adverse effects in organisms exposed to toxicants are being increasingly used in assessing aquatic systems (Warwick 1980a, Niimi 1990, Hoffman and Fisher 1994, Morcillo and Diez 1996, Olsson 1996, Kravitz et al. 1999, Sanchez-Dardon et al. 1999). Their application has been increasingly proposed as a potential means to link suborganismal responses to contaminant exposure in hopes of efficiently detecting and avoiding ecological catastrophes (Warwick 1985).

Chironomid mouthpart (mentum or ligula, depending on the subfamily) deformities have been used to assess anthropogenic stresses (pesticides, metals, PAHs, PCBs, and

other organic chemicals) in both field and lab settings (Hamilton and Saether 1971, Warwick 1980a, 1987, 1989, 1990, Cushman 1984, Dermott 1991, Dickman et al. 1992, Janssens de Bisthoven et al. 1992, 1998, Lenat 1993, Muir 1993, Cervi 1996, Hudson and Ciborowski 1996a, 1996b, Burt 1998). For a complete discussion on the use of this biomarker, refer to Chapter 1.

In 1996, wetlands in the Fort McMurray region receiving oil sands effluent were dominated by chironomids, with low benthic community diversity, and cattails which grew at normal pace but showed increased photosynthetic rates (Bennett and Bendell-Young 1997, Bendell-Young et al. 1998). Tadpoles and fish were also adversely affected in bioassays, and were absent from these sites. Caged ducks appeared to experience altered growth upon exposure to OSPW wetlands (Bendell-Young et al. 1998). However, natural populations of swallows, ducks, macrophytes, and numerous insects and other invertebrates occur in OSPW-affected wetlands, suggesting that OSPW is not necessarily acutely toxic, but effects observed may be related to sublethal toxicity.

This study assesses the sublethal effects (teratogenicity) of oil sands-related waters through the collection and examination of chironomid larvae from 3 pairs of OSPW and reference wetlands. Sites were paired based on environmental similarity as indicated by PCA and cluster analysis (Chapter 2). In the case that larvae are more frequently deformed at OSPW wetlands than at reference wetlands, it would suggest that contaminants (metals, PAHs) in the OSPW are both bioavailable and teratogenic. This would indicate that this biomarker may be successfully applied, together with community measures, in biomonitoring the water quality of the reclaimed wetlands in the future. On the other hand,

a lack of deformed larvae would suggest that the OSPW does not pose a teratogenic threat to the aquatic biota, at least under the physico-chemical conditions found during sampling.

## **METHODS**

### **I - Field Methodology**

#### **Sample Collection:**

In the summer of 1997, chironomid larvae were hand-picked from the 6 focal sites (NW, CL, SB, SW, HW, and HS) described in Chapter 2. They were to be identified to genus and analyzed for incidence of mouthpart deformities, a biomarker of teratogenicity. Between 225 and 300 chironomid larvae (including at least 100 large red larvae where they were present) were collected at each site.

#### **Sample Field Processing and Preservation:**

A pole-mounted Ekman grab or a dip net was used to collect sediment, which was then sieved in a wooden 60x60 cm sieve box with 2-mm mesh to remove sediment and fine detritus. Chironomids were gently picked from the remaining debris using fine forceps and placed in a pan of water for cleaning and holding. After a substantial number had been collected, they were blotted on paper towelling and transferred to a labelled, 20-mL scintillation vial containing cold Carnoy's solution (3:1 v/v 95% ethanol:glacial acetic acid). The Carnoy's solution was poured off and replaced with fresh solution immediately and after 5 min, 1 h and, 24 h as well as any time afterwards if the solution had darkened

or become cloudy. A minimum of 125 chironomids is the suggested sample size when assessing mouthpart deformities in order to detect a doubling of incidence of deformities from background incidence of teratogenicity (Hudson and Ciborowski 1996a). Where more than one dominant taxon of chironomid was obviously present (as judged by colour and morphology) they were stored in separately marked vials. Vials were stored in a cooler with ice and then kept refrigerated until analysis by taxon.

## **II - Laboratory Methodology**

### **Larval Identification and Determination of Deformities:**

Chironomids collected in dip net samples from each of the 15 wetlands of 1997 (used in assessing chironomid community structure - see Chapter 2), were identified (20 per size fraction per sample) to determine the dominant chironomid taxa present. Following this, the hand-collected chironomids (100-150 per site) stored in Carnoy's were individually mounted on slides for identification and assessment of mouthpart deformities as outlined below.

The following procedures were used to determine generic identity and incidence of deformities, following procedures of Hudson and Ciborowski (1996a, 1996b).

The head capsule of each larva was removed and placed ventral side up on a microscope slide in a drop of CMC-9AF® aqueous mounting medium (Master's Chemical Company, Des Plaines, Illinois). The bodies were stored in a labelled shell vial containing Carnoy's fluid. A cover slip was placed on the slide and gentle pressure applied to separate the mouthparts and properly orient the head capsule. The cover slip was then ringed with

clear nail polish and allowed to dry and clear for at least 7 d. Chironomids were identified to genus using keys of Oliver and Roussel (1983), Wiederholm (1983) and Coffman and Ferrington (1996) (Table 2.3 in Chapter 2).

Each head capsule was examined for the presence of deformities of the ligula (Tanypodinae) or mentum (other subfamilies). The mentum is known to show deformities in larvae of contaminated sites, is heavily sclerotized and clearly visible following mounting, making it advantageous for deformity assessment (Cushman 1984).

A mentum or ligula was classified as either "normal" or "deformed" based on the criteria of Dickman et al. (1992) as modified by Hudson & Ciborowski (1996a). A mentum or ligula that exhibits blunt or chipped teeth is judged to be "worn" (normal) but not deformed. Similarly, a mentum or ligula tooth with jagged edges is considered to be "broken" and also is classified as normal. Only misshapen teeth that have smooth edges are considered by Dickman et al. (1992) to be "deformed". Characteristics of the mentum/ligula that resulted in Dickman et al. (1992) assigning a classification of "deformed" included fused teeth, crossed teeth, extra teeth, missing teeth or teeth of aberrant shape or size. Asymmetry of the mentum/ligula is also an indication of deformity. However, to minimize the necessity for any qualitative assessment of condition, we used a conservative criterion - only an animal that had either missing (including fused), or extra teeth was classified as deformed. Other possible deformities were noted but not considered in analyses. Our designation of "deformed" corresponds with deformity classes II and III of Lenat (1993).

### **Statistical Analysis of the Incidence of Deformities:**

The incidence of deformed larvae from the different study sites was expressed as the proportion  $\pm$  SE of larvae at each site. Standard errors were calculated according to the binomial theorem i.e.,  $SE = \sqrt{pq/k}$  where  $p$  = proportion of deformed specimens,  $q$  = proportion of undeformed specimens, and  $k$  = sample size (Sokal and Rohlf 1981). The G-statistic goodness-of-fit test (Sokal and Rohlf 1981) was used to test for heterogeneity in the incidence of deformities among all sites ( $H_0$ : Incidence of deformities is equal at all sites) and among paired reference and OSPW sites ( $H_0$ : Incidence of deformities is equal among pairs of reference and OSPW sites).

## **RESULTS**

### **Assessment of Mouthparts for Deformities in Field Specimens**

Four of the six focal wetlands, including all three reference sites, appeared to be dominated by *Chironomus* larvae, whereas *Derotanypus* was the most commonly collected genus in two of the three OSPW sites, and was also collected at High Sulphate Pond (Table 3.1). *Tanytarsus* was fairly common at Natural Wetlands.

In contrast to the predominance of *Chironomus* as the taxon collected for deformity analysis, *Tanytarsus* was the predominant taxon observed in dip net sweep samples of four of the six focal sites (Chapter 2). Only one site (Crane Lake) showed the same genus (*Chironomus*) for dominant chironomid taxa present and for the larvae hand-collected for assessment of deformities. The three OSPW wetlands showed fair similarity between the chironomids collected (Chapter 3) and those found in dip nets (Chapter 2).

Table 3.1: Incidence of mouthpart deformities (%  $\pm$  S.E. (n)) of chironomid larvae hand-collected at 6 paired focal wetlands in 1997. OSPW wetlands are bold-faced, and the number of head capsules examined is listed in parentheses.

Site	<i>Derotanypus</i>	<i>Chironomus</i>	<i>Tanytarsus</i>
<b>Natural Wet.</b>	0.7 $\pm$ 0.7 (148)	-	0.0 $\pm$ 0.0 (94)
Crane L.	-	2.0 $\pm$ 1.2 (148)	-
<b>Hummock W.</b>	1.3 $\pm$ 0.9 (149)	-	-
High Sulph.	3.7 $\pm$ 3.6 (27)	1.4 $\pm$ 1.0 (142)	-
<b>South Bison P.</b>	-	2.8 $\pm$ 1.4 (142)	-
Shallow W.	-	2.1 $\pm$ 1.2 (144)	-

*Chironomus* larvae were hand-picked at the other two reference sites, High Sulphate Pond and Shallow Wetlands. However, other taxa predominated over *Chironomus* in dip net samples from both of these sites.

Incidences of deformity were uniformly low at all 6 focal wetlands, and suggested that contaminants at these sites were not causing teratogenicity. The maximum incidence observed (3.7 percent) occurred in a small sample (n=27) from High Sulphate wetland, which was a reference site (Table 3.1). Goodness-of-fit tests indicated that there was no significant heterogeneity among sites (replicated G-test for goodness-of-fit test;  $p < 0.7$ ) or between paired reference and OSPW wetlands (replicated G-test for goodness-of-fit test;  $p < 0.7$  or greater for all three pairs).

The incidence of deformities in the chironomids identified from dip net samples was quite low with the exception of Shallow Wetland (Table 3.2). Furthermore, chironomids from the nine other reference sites also contained no deformed larvae, with the exception of Muskeg River Marsh (1 deformed larvae of 32 head capsules) and Tower Rd #1 (1 deformed larvae of 40, with another having two fused lateral teeth). However, all samples were quite small and included multiple genera, therefore these results must be viewed with caution.

Nevertheless, the results indicate that there was no association between the presence of OSPW in wetlands and elevated incidences of developmental abnormalities. Also, background incidence of deformities is generally very low (3.7 % or less) in this region of Canada.

Table 3.2: Incidence of mouthpart deformities (%  $\pm$  S.E. (n)) of chironomid larvae from dip net sweep samples (all genera, all size fractions pooled together) at 6 paired focal wetlands, and 9 reference sites, in 1997. OSPW wetlands are bold-faced.

Site	No. Deformed	Total	% Deformed $\pm$ SE
<b>Natural Wetland</b>	0* <sup>1</sup>	39	0.0 $\pm$ 0.0
Crane Lake	0	29	0.0 $\pm$ 0.0
<b>Hummock Wetland</b>	0	34	0.0 $\pm$ 0.0
<b>High Sulphate Pond</b>	0* <sup>2</sup>	37	0.0 $\pm$ 0.0
<b>South Bison Pond</b>	0	38	0.0 $\pm$ 0.0
Shallow Wetland	3	39	7.7 $\pm$ 4.3
Syncrude Wetland	0	23	0.0 $\pm$ 0.0
Tower Rd. #4	0	38	0.0 $\pm$ 0.0
Poplar Creek Res.	0	39	0.0 $\pm$ 0.0
Muskeg River Marsh	1	32	3.1 $\pm$ 3.1
Spruce Pond	0	39	0.0 $\pm$ 0.0
Ruth Lake	0	29	0.0 $\pm$ 0.0
Tower Rd. #1	1* <sup>3</sup>	40	2.5 $\pm$ 2.5
Tower Rd. #3	0	39	0.0 $\pm$ 0.0
Tower Rd. #5	0	19	0.0 $\pm$ 0.0
High Sulphate	0	37	0.0 $\pm$ 0.0

\*<sup>1</sup> - deformed dorsomenta plate (*Derotanypus*)

\*<sup>2</sup> - deformed antenna (*Paratanytarsus*)

\*<sup>3</sup> - 2 fused lateral teeth (*Chironomus*)

## DISCUSSION

The incidence of mouthpart deformities in chironomids was relatively low at all six sites studied, regardless of genus or OSPW input.

### *Taxa collected:*

At the time of collection of larvae, the two wetlands (Hummock and Natural Wetlands) that receive OSPW inputs appeared to be dominated by a predatory tanypod species, *Psectrotanypus (Derotanypus) alaskensa*. The third wetland considered to receive oil sands-related inputs, South Bison Pond, is a reclaimed area presently commissioned to be used as a bison pasture, and receives leached berm water as well as surface water runoff. South Bison Pond was dominated by *Chironomus*, as were the three focal reference sites.

The differences in predominant chironomid taxa observed in hand collections vs. dip net samples is likely related to the fact that the hand collections were carried out 2-4 weeks after the dip net sampling was done. This is significant in that taxa collected in mid-June by dip net may have undergone emergence before the later collections for deformity assessment in July. When a genus was less dominant in hand collections compared to the earlier dip net samples, it may be at the latter period of its emergence and thus only the stragglers would have remained.

Sampling bias based on different colour and size of various genera could also be related to the differences in chironomids collected. Larger (3rd/4th instar), more active, and bright red coloured *Chironomus*, and large *Derotanypus* both stood out in sample

trays.

The different sampling devices used are also probably responsible for the differences in chironomids collected. Dip nets were used to collect benthos in the upper sediment layers as well as any organisms living in nearby macrophyte beds, and by design of the sampler, would also collect pelagic biota. The Ekman was used to collect sediment samples to about 8-10 cm depth, from which chironomids were hand picked. Thus it would be expected that some chironomid taxa collected would be specific to either dip net or Ekman samples.

Furthermore, the chiromids collected for analysis of headparts for deformities were generally large red specimens (in hopes of getting a similar genus among all sites), and certain taxa may have been biased against if they were less mobile than active, larger taxa. Regardless of taxa collected by either sampler, there were few deformities observed.

*Field Incidence of Mouthpart Deformities:*

Mouthpart deformities (extra or missing teeth) were rare among chironomids from both reference and OSPW wetlands. Levels of incidence were similar to those reported in studies of unimpacted sites, which generally range from 0 to 4% (Hamilton and Saether 1971, Wiederholm 1984, van de Guchte and van Urk 1989, Dermott 1991, Dickman et al. 1992, Lenat 1993, Hudson and Ciborowski 1996a, Burt 1998, Groenendijk et al. 1998).

Exposure to oil-related contaminants has been linked to increased occurrence of morphological deformities in other studies. Dickman et al. (1992) sampled chironomids at a site contaminated by coal tar, and found 14% of *Chironomus anthracinus* larvae to be

deformed, but only 7.7% following clean-up of the location, and 3-4% at reference sites. Chironomids collected from the heavy metal-contaminated Buffalo River, New York, experienced a high frequency of mouthpart deformities (Stewart and Diggins 1994). A lab study found a log-linear relationship between the concentration of contaminated sediment and the incidence of mentum deformities in chironomids (Hudson and Ciborowski 1996b). Furthermore, contaminant body burdens (PAHs, metals) have been shown to be higher in deformed larvae relative to normal larvae (Dickman et al. 1992, Janssens de Bisthoven et al. 1992).

While some studies have successfully linked contaminant exposure to the incidence of deformities, the use of deformities as a biomarker of OSPW exposure is not recommended based on the results of this study. However, in terms of assessing OSPW effects, this biomarker can still be used in future to assess teratogenicity of wetlands receiving oil sands input. No risk of teratogenicity was shown at the sites sampled in 1997.

Similarly, the incidence of deformities in ligulae (*Procladius*) was a less sensitive indicator of creosote exposure in artificial ponds than was the total invertebrate density (Pardalis 1997). In 1996, chironomids showed slightly higher frequency of mouthpart deformities in Natural Wetlands compared to a control wetland, based on small sample sizes, but these results were not replicated in laboratory trials (Bendell-Young et al. 1997). Metals were related to heavy mortality but not induction of deformities in chironomids (Vermeulen 1995). Also, the incidence of deformed menta, premandibles and antennae was not observed to be linearly related to the concentrations of heavy metals Cd, Pb, and Zn found in streams of an abandoned mine area (Ferringington 1994). Another study

showed overall deformity incidence to be independent of dose of coal liquid applied to experimental ponds, and this biomarker was less sensitive than traditional community measures including taxa richness and biomass (Cushman 1984).

Although this chapter indicated that all chironomid genera were equally unaffected by exposure to oil sands contaminants in terms of teratogenic stress, sensitivity does vary among taxa. Interspecific differences in susceptibility to deformities were apparent in samples downstream from petrochemical industries. *Chironomus* was most frequently deformed, which is consistent with other studies that show this genus, along with *Procladius*, to be tolerant of polluted conditions (Dermott 1991). Burt (1998) suggested a hypothetical gradient of increasing contamination where various taxa would show a response, with *Chironomus* responding in heavier contamination. The oil sands related chemicals may not present a sufficiently teratogenic environment to elicit a response in this genus at South Bison Pond.

The lack of elevated incidences of deformities in oil sands-related wetlands relative to reference sites may be related to one or more of the following possibilities.

Firstly, the concentrations of teratogenic compounds (PAHs, trace metals) in water and sediment may be below the levels that induce deformities. Unfortunately, sediment chemistry analysis was not yet performed on collected sediment samples. However, chemistry analysis of OSPW samples indicated that a majority of total metal concentrations were < 50 µg/L (including Cd, Cu, Pb, and Zn), and only three (Al, B, and Fe) were above 1 mg/L. Concentrations of naphthenic acids were 77-81 mg/kg, but these surfactant-like compounds are not thought to be teratogenic. Considering PAHs in tailings

porewater, none were genotoxic without activation based on the Ames *Salmonella* assay (Madill et al. 1999).

Secondly, the contaminants may have been present at potentially teratogenic concentrations, but not bioavailable. The high levels of dissolved organic carbon (naphthenic acids and others) and highly organic sediments may bind the metals and PAHs (Chapman 1997). Levels of acid volatile sulfides which are important in metal bioavailability are not known (DiToro et al. 1990).

Thirdly, the local chironomid populations may be evolutionarily adapted to these conditions and thus tolerant to these contaminant-induced stresses. Experiments involving the exposure of populations from both OSPW and reference wetlands to the same conditions of chemical stress, either in the field (Chapter 4 discusses *in situ* experiments), or in the laboratory (Chapter 5 discusses an OSPW laboratory bioassay), were designed to answer the question of evolved tolerance.

Finally, individuals that develop deformities in OSPW wetlands may experience greater mortality than non-deformed chironomids, and are thus not alive to be sampled and assessed. Morphological deformities are individual specific, due to differences in exposure related to variation in microhabitat, as well as genetic differences. Deformed individuals are less fit than normal ones, at least in terms of metabolic control of morphological development. Cervi (1996) related deformed mouthparts to delayed and reduced emergence of pupae, thus demonstrating reduced fitness in relation to deformity. Thus it would be expected that, in cases of stress such as those related to colonizing newly formed habitats where chemical stress is not insignificant, mortality may be higher in

larvae that are susceptible to deformities, thus lessening the chance of collecting them in samples.

The low incidence of deformities among larvae of OSPW suggests that native populations are not being exposed to significant concentrations of teratogens. Outside colonizing species would thus not necessarily be inhibited from establishing populations in these reclaimed habitats. This would provide support for the wet landscape option currently being considered. However, the question of field tolerance needs to be addressed.

## **CHAPTER 4 - *In situ* Exposure of Chironomid Larvae to OSPW**

### **INTRODUCTION**

The development of tolerance to stress in field populations of aquatic invertebrates has been reported in relation to both acclimation, where an organism gradually adjusts physiological and biochemical processes to survive current stress (Bodar et al. 1990), and evolutionary adaptation, which occurs over generations (Klerks and Levinton 1989). This phenomenon is of considerable importance when evaluating potentially degraded ecosystems. Tolerance directly relates to the determination of which tools to use in the assessment. Obviously, in the case where local populations have adapted to the surrounding conditions, it would be inappropriate and inaccurate to use laboratory-reared organisms in assessing toxicity, as these organisms would probably be more sensitive to the stressor than pre-exposed populations. This would result in overly-conservative toxicity assessments of environments where the native organisms in fact were not affected. The evaluation of an environment in terms of effects on its resident biota is a logical alternative.

Field populations of chironomids could be used to assess both the degree of tolerance developed in native biota, relative to laboratory populations not pre-exposed to the contaminants of interest. They could then be used to provide a more realistic indication of environmental contamination. Chironomids are used in various facets of water quality assessment, as bioindicators of specific environmental conditions (Warwick 1988), biomarkers of environmental stress and degradation (Janssens de Bisthoven et al. 1998), in benthic community indices (Franco et al. 1984), and in both field and laboratory

toxicology studies (ASTM 1990, Bedard et al. 1992, Harkey et al. 1994). They are one of the most ecologically and taxonomically diverse families of organisms studied, which is reflected in both their occurrence world wide in almost any aquatic environment and to their broad range of sensitivity to various stressors (Wiederholm 1984, Pinder 1986). Living in sediment, which tends to attract contaminants in aquatic systems (metals, organic chemicals), for most of their mainly sessile lives, chironomids are potentially excellent biomonitors of sediment contamination (Pesch et al. 1981, Warwick 1990, Ciborowski et al. 1995).

*In situ* (on site) bioassays are toxicity bioassays conducted in the field using caged organisms to monitor biological responses to stressors (Pereira et al. 1999). The goal of *in situ* bioassays is to combine the advantages of the realism of field trials with the control of laboratory experiments. Comparison of macroinvertebrate responses to copper in field and laboratory streams showed significant differences that underlined the simplicity of lab systems (Clements et al. 1990). Laboratories can not accurately reproduce all the interacting physical, chemical, and biological processes that occur in nature (Pereira et al. 1999). To complement laboratory studies, *in situ* tests can be conducted. These semi-controlled field experiments thus allow for the testing of hypotheses in a more realistic and comprehensive setting than the laboratory, and eliminate artifacts related to sample collection and storage (Chappie and Burton 1997). *In situ* tests have been developed using numerous organisms including phytoplankton, periphyton, various macroinvertebrates, and fish (Pereira et al. 1999).

Pereira et al. (1999) assessed in situ toxicity of acid mine drainage to zooplankton (*Ceriodaphnia dubia* and *Daphnia magna*), and found these tests to be more sensitive to low-moderate contamination than laboratory assays. Conversely, Day et al. (1995) found good correlation between benthic community response and growth in 4 laboratory bioassays (including *C. riparius*, *Hexagenia* sp., *Hyalella azteca*, and *Tubifex tubifex*) and two microbial screening assays. However, results may have been biased as the samples were stored for a year prior to testing, therefore community assessment should not be abandoned for simple single-species laboratory bioassays (Day et al. 1995).

Assessment of the benthic macroinvertebrate community indicated that high conductivity and OSPW were related to reduced taxa richness and also to changes in community composition of chironomids as well as the general benthic assemblages (Chapter 2). Further assessment of possible effects of OSPW in terms of teratogenicity to biota were not apparent, based on the low incidence of mouthpart deformities of chironomids in the field (Chapter 3).

It is important to develop a picture of the degree to which natural populations are already establishing in these pilot-scale OSPW wetlands in order to predict the future success of this wet landscape reclamation option.

In order to more fully understand not only the present effects but also anticipate the future effects of OSPW on the aquatic biota of reclaimed wetlands, in relation to wetland colonization, field experiments were conducted. By exposing one or more populations of chironomids to conditions found at both reference and OSPW wetlands, the question of field adaptation could be addressed. We were able to develop a few

chironomid cultures of field populations, and used two of these, one in each of two experiments.

Ideally, both laboratory populations and field populations from reference sites would be used. Laboratory chironomids quickly died when caged in Natural wetlands which receives OSPW. However, there is an abundant chironomid community at this site. This illustrates that sensitivity to environmental stresses varies considerably. Therefore, there is a need to use native populations to assess field conditions (EVS Consultants 1995). This study was designed to measure mortality, growth, and teratogenicity of chironomid larvae in both OSPW and reference wetlands, in order to assess the tolerance of field populations to water inputs related to oil sands development. Testing interspecies sensitivities would indicate the relevance of using laboratory organisms in assessing field conditions.

In the case that adverse effects (increased mortality and incidence of deformities, and reduced body length) were seen in reference site populations caged at OSPW wetlands and not at reference wetlands, this would indicate not only toxicity of the OSPW, but more importantly, that the natural fauna observed at OSPW sites had evolved mechanisms to cope with this anthropogenic stress not observed in the reference population. However, in the case where no effects are related to reference site chironomids exposed to the OSPW wetlands, it would indicate that the OSPW was not toxic to the population from the reference site, and suggest that adaptation of OSPW species had not occurred or was unnecessary.

In the case where no laboratory or reference site population could be obtained for culturing and testing, a population from an OSPW site would be used. In this case, the population would be expected to show similar responses at OSPW and reference wetlands. However, this population could be affected in reference sites devoid of the influence of oil sands mining, due to costs of tolerance associated with metabolic adaptation (Postma et al. 1995). This would only provide indirect evidence of field adaptation.

## **METHODS**

### **Study Sites**

In situ testing of the toxicity of oil sands tailings water was performed at the 6 focal wetlands including the OSPW sites Natural Wetlands (NW), South Bison Pond (SB), and Hummock Wetlands (HW), as well as their paired reference sites Highway 63 Wetland (HW63), Shallow Wetland (SW), and High Sulphate Pond (HS), respectively. Pairing was based on degree of environmental similarity analyzed using PCA and cluster analysis. Site descriptions and environmental characteristics are described in Chapter 2 (Table 2.4, Appendix 2.8 and 2.9).

### **Bioassay Chamber**

The test containers, based on the design of Sibley (1998), consisted of a 50 cm long x 7 cm diameter cylindrical Plexiglas tubes (transparent) to which was added locally-occurring chironomid larvae. The tubes had two nylon netting-covered (80  $\mu$ m mesh)

windows (20 cm x 5 cm) opposite each other to permit water to exchange between the tube and the surrounding environment (Fig. 4.1). The vertically oriented chamber was closed on the top using an empty yogurt container (pushed into the tube forming a tight seal) and driven 15-20 cm into the sediment.

### **Test Animals**

Test organisms all originated from the same egg masses, collected either from UV light traps at dusk (Whelly et al. 1998) or from colonization water trays used in a field experiment (see Chapter 6). The larvae were carefully counted and placed in 20-mL scintillation vials containing filtered tap water which was acclimated to the aquarium temperature.

### **Inoculation and Recovery Procedures**

In shallow-water trials (< 50 cm deep - the depth of the tubes), chironomid larvae were poured from the vials into each of the tubes while the tops of the cores were above water. The bottoms of the tubes, which were resting on the substrate, were then pushed 15-20 cm deep into the sediment. In deeper water trials, the organisms were pipetted underwater into the submerged tubes through a gap left open between the yogurt container and the top of the core already secured in the sediment bed.

Tubes were removed by slipping a hand under the core to prevent the contents from sliding out, and then raising the core out of the water. The contents were then either assessed on site or preserved for laboratory analysis. Those observed in the field were

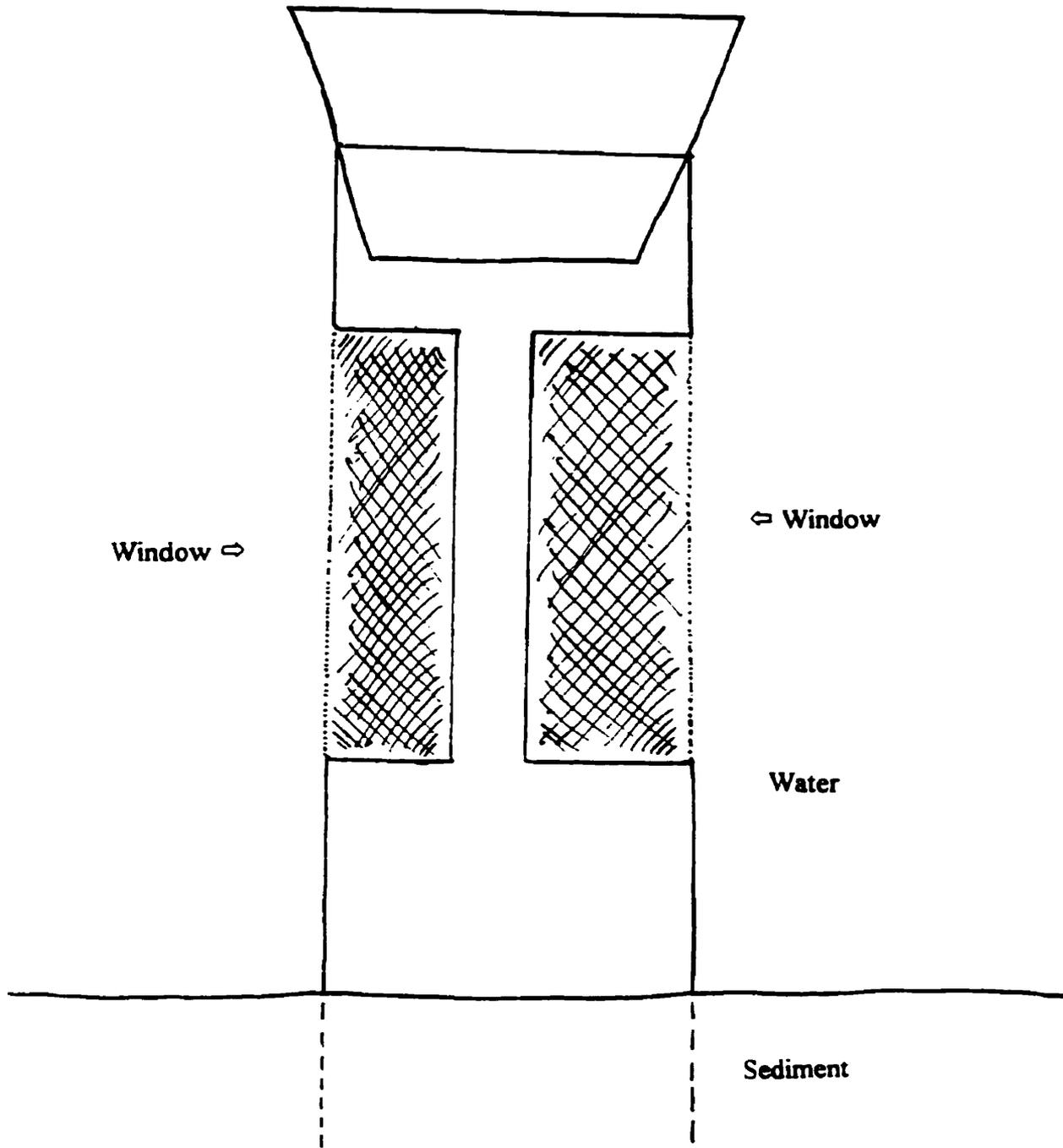


Fig. 4.1: Chamber design for *in situ* testing of chironomids in sediments of wetlands.

sieved using a 0.25 mm sieve bucket to remove fine sediments on site, followed by sorting of all materials in a tray and removing all visible chironomid larvae with fine forceps and preserving them.

In the summer of 1998, two pilot studies were conducted, as well as two main studies.

*Pilot Study 1 (Ojibway Park, Windsor, ON):*

A pilot study was conducted in Ojibway Park, in Windsor (May 15-19, 1998). This pilot study was used to observe the rate of retrieval of test organisms using these new test units. Ojibway Pond was considered to be a relatively similar physical environment to the wetlands of northern Alberta.

Three tubes, each containing 20 third instar *Chironomus riparius* larvae were placed in the pond for one week.

*Pilot Study 2 (HW and HS):*

A 4-day pilot study was conducted (June 23-27, 1998) at Hummock Wetlands (HW) and High Sulphate Pond (HS). The study was designed to test retrieval success of test organisms from tubes. The screening on the chambers was examined for evidence of coverage by algae over this time. Third and fourth instar chironomids were hand picked from sediment at each site (*Derotanypus* at Hummock wetlands; taxonomic identify unconfirmed at High Sulphate Pond but thought to be *Chironomus*), and then placed in tubes at their respective site.

*Main Study 1 (HW and HS):*

The next study (4 d) was conducted from June 27-July 1, 1998, at HW and HS, using a culture of *Chironomus (Chironomus) anthracinus* gp. (identified from larvae) originating from eggs collected in colonization trays from Hummock wetlands and reared in an aquarium at the Suncor Wetlands trailer.

Seven chambers were set up at each of the two sites, with 20 third instar larvae placed in each tube. The experimental duration was kept short to ensure retrieval of test organisms before surviving larvae began to pupate or emerge. In the field, samples were sieved using a 250- $\mu$ m sieve, sorted using forceps and trays, and chironomid larvae and other organisms were stored in vials containing Carnoy's solution. These were transported on ice back to the refrigerator at the Suncor Wetlands Dry Lab. Fresh Carnoy's solution replaced old solutions in all samples.

*Main Study 2 (NW, HW63, SB, and SW):*

The second in situ study (8 d) was conducted using first instar larvae developing from egg masses laid by three identical females (females 'B', 'C', and 'D') collected using light traps at Shallow Wetlands, a reference site at Syncrude. This would permit the investigation of possible adaptation of midges at tailings wetlands to the contaminants found therein. The females were Chironomini, but identification beyond that is not possible in female adult midges. First instar larvae appeared to have a mentum resembling that of *Chironomus*, although at this stage of development, identification of larvae to

genus is usually quite difficult.

The test was conducted for 8 d (July 6-14, 1998). Six tubes, each containing 20 larvae were installed at each of four sites in the following temporal order: Natural Wetlands (NW), Highway 63 (HW63), Shallow Wetlands (SW), and South Bison Pond (SB). The latter sites (SW and SB) were done later in the day, and the larvae may have been heat stressed by that point in the day. Filtered tap water was again used after temperature acclimation as a transfer medium for test organisms being transported to their sites (as in Study 1).

The tubes were checked after 5 d, and no significant algal growth had accumulated on the mesh screens of the tube windows. Only the first few tubes (NW #1-3) were sorted in the field, as cold rainy conditions made sorting in the field impractical. The sediment samples from the remaining tubes were stored in labeled soil bags and preserved in Kahle's solution.

### **Lab Procedures**

Core samples were sieved in the laboratory using 4.00 mm, 1.00 mm, 500 um, and 250 um brass sieves, and sorted using fine forceps beneath a dissecting microscope. Missing larvae were assumed to be dead. Other organisms were identified to genus, including possible competitors (other chironomids) and predators.

Chironomids were mounted on slides, with head capsules removed from the bodies and positioned ventral side up, using CMC-9 mounting medium. They were identified to genus using under a compound light microscope, and taxonomic keys as described in

detail in Chapter 2.

For the main studies, test endpoints include size (body length of larvae both live and dead), mortality, and the incidence of mouthpart deformities of recovered larvae.

## **RESULTS**

### ***In Situ Chironomid Toxicity Testing***

#### ***Pilot Study 1 (Ojibway Park, Windsor, ON):***

After 4 d, we recovered none of the larvae we had introduced to the 3 *in situ* tubes. We assumed that this was because we had started with relatively mature 3rd/4th instars and that these may have died or tried to emerge and then decomposed during the test.

#### ***Pilot Study 2 (HW and HS):***

In Hummock Wetland, we introduced 20 and 22 large green chironomids (*Psectrotanypus (Derotanypus) alaskensis*) into two tubes. After 4 d, we retrieved 13 (65%) and 21 (95%) larvae from the 2 tubes, respectively, although we were not sure if these were the original ones or other native ones. At High Sulphate wetland, we put 20 red chironomids into one tube. We retrieved only 14 chironomids (70%) at the end of the test. Thus, retrieval of test organisms was suboptimal, again possibly related to death of organisms following pupation, or to environmental factors (i.e. low D.O.). However, pupal cases were not observed.

*Main Study 1 (HW and HS):*

At High Sulphate Wetland, we recovered 46 larvae (2/3 of which were alive) out of a total of 140 third and fourth instar larvae added to the 7 cores (Table 4.1). One core (#5) had a broken window (probably the work of a curious duck) and was not included in analysis, as indigenous organisms could have entered the test chamber and killed the *C. anthracinus* test organisms, or these chironomids could have escaped out the window. Core #5 contained 7 dead adult midges (most likely *Tanytarsus*). In the six remaining cores, only 31 chironomids were found alive, 14 were dead, and 1 pupa was observed. Maximum survival in any one core was only 40%. Mean survival was 26%, and mean recovery of test organisms was only 37.5%.

We had similar recovery success at Hummock Wetland and found 38 of 140 larvae put into 7 tubes. In contrast to HS, only one larvae was found alive, and no pupae were observed. Again, one core had broken windows (#5) and was therefore eliminated from the data set. It was the only core to contain a considerable number of live larvae, which were most likely immigrants to the chamber. Mean % survival and % recovery were 1% and 32%, respectively.

Although overall recovery was poor, the difference in mean number of live larvae recovered between wetlands was statistically significant (two-sample t-test of log-transformed data,  $p < 0.05$ ). Geometric mean numbers of living larvae per core tube (times/divided by SE) were  $3.68(x/1.35)$  larvae in the reference High Sulphate Pond vs. only  $0.54(x/1.38)$  larvae in Hummock Wetland.

**Table 4.1: Recovery of red chironomid larvae and pupae from cores at High Sulphate Pond (HS) and Hummock Wetland (HW) during the 4-d *in situ* test #1.**

Site	Rep	live larvae	dead larvae	pupae	Total
HS	1	7	0	0	7
	2	8	0	0	8
	3	5	2	0	7
	4	4	7	0	11
	5(Br)	2	2	0	4
	6	0	1	0	1
	7	7	4	1	12
	total	31	14	1	46
HW	1	0	0	0	0
	2	0	16	0	16
	3	0	2	0	2
	4	0	13	0	13
	5(Br)	10	5	0	15
	6	1	4	0	5
	7	0	2	0	2
	total	1	37	0	38

NOTE:

Br - Broken windows on core (not included in totals)

High Sulphate Pond did not have predatory chironomids (Tanypodinae) in any intact cores, but dragonfly and damselfly larvae were found in five of the seven cores (Table 4.2). There were numerous small red chironomids present in all cores but one, indicating that the test environment was not lethal to all life. Almost all chironomids mounted were *Chironomus*, along with one *Glyptotendipes* larva and one pupa, but many of these could not have been test organisms as they were smaller than the initial size of test animals (Table 4.2).

There were no predatory dragonfly or damselfly larvae in any of the Hummock Wetland cores (Table 4.2). However, there were Tanypodinae present in all cores, thus consumption of test organisms can not be ruled out.

*Main Study 2* (NW, HW63, SB, and SW):

Retrieval of test organisms (*Chironomus*) was extremely poor in this *in situ* test. Only 4 *Chironomus* larvae were found overall, in only 3 of the 24 cores deployed. One of the larvae, at SB, had a deformed mentum (Table 4.3). Survival was not measured, as all samples were immediately placed in sample bags with preservative; only the presence of organisms was noted, and it was assumed that these were alive at takedown.

However, 17 genera of chironomids were observed in the samples, dominated by predatory *Procladius* which occurred in half the cores, and was present at all four sites (Table 4.3). Most other genera occurred in low number at only one or two of the sites. The mean number of genera per core was quite variable, and was highest at NW (4.5) and lowest in HW63 (0.8). The mean number of chironomids per core was highly variable at

Table 4.2: *In situ* test 1 core contents for High Sulphate and Hummock Wetlands (field observations)

Site	Core	damselfly larvae	dragonfly larvae	Tanypodinae	small red chir.	Other
High Sulphate	1	1			12	6 snails
	2	1	1		2	
	3	1	2		1	1 snail, 1 caddisfly larva
	4				5	
	5(Br)			1	6	7 emerged midges
	6	1	1			
	7		1		7	
Hummock W.	1			3	1	
	2			11	1	
	3			11	1	
	4			1	1	
	5(Br)			1	3	
	6			1		
	7			1		

Br - Broken windows on core

Table 4.3: Chironomid genera found in the *in situ* test 2 core samples for 2 reference (SW and HW63) and 2 OSPW-related (SB and NW) wetlands

Taxa	Shallow Wetlands						Highway 63 Wetland						South Bison Pond						Natural Wetlands						
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	
													M	Br					Br			M	M	M	
Chironomus					2	1									1*										
Polypedilum			1																						
Cladopelma				1																					
Endochironomus					1									5											
Dicrotendipes														1											
Glyptotendipes														1					2		1				
Cryptochironomus							1			1					1										
Tanytarsini					2																				
Tanytarsus								1						7			1					1			
Lenziella			1																			5			
Paratanytarsus					2									5					3		7				
Orthocladinae					2			1						1											
Psectrocladius					1									1					2		3				
Tanypodinae					3									2											
Derotanypus					1														2	1	4				
Ablabesmyia					1																				
Clinotanypus					1		2																		
Monopelopia																			1						
Tanypus																	1	5							
Procladius		2	4	1	8	2				2				6				5	3	9	1	4			
<b>TOTAL</b>	<b>0</b>	<b>2</b>	<b>6</b>	<b>2</b>	<b>24</b>	<b>3</b>	<b>3</b>	<b>2</b>	<b>0</b>	<b>3</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>29</b>	<b>2</b>	<b>0</b>	<b>7</b>	<b>8</b>	<b>19</b>	<b>2</b>	<b>25</b>	<b>0</b>	<b>0</b>	<b>0</b>	

NOTE:

M = Missing core

Br = Broken windows of core

Sp = Spilled sample and lost most organisms

\* = Deformed Mentum

all sites except Highway 63 reference site, where few larvae were found. The proportion of chironomids that were tanypods was very high at NW (83%), intermediate at SB and SW (both 46%), and lowest at HW63 (27%) (Table 4.4).

Larvae of beetles, damselflies, and dragonflies were observed at HS, HW63, and SB, but none were detected in the NW cores (Table 4.4). The NW cores were sorted by hand in the field using a 250 um brass sieve, in order to observe survival of organisms. The remaining samples were preserved in the field and sorted in the lab. This may account for some of the differences observed between NW and the other 3 sites.

## **DISCUSSION**

The first main test conducted suggested that *Chironomus* originating from Hummock Wetlands had better survival in High Sulphate Pond than in Hummock Wetlands. This would suggest that HW *Chironomus* are affected by OSPW contaminants and that tolerance is weak to nonexistent in this population. However, it is quite possible that indigenous *Chironomus* were already in the sediment enclosed by the in situ test cores at HS, thereby artificially elevating survival at this site. Densities of chironomids at most sites sampled with dip nets were quite high in 1997 (Chapter 2). This, coupled with the poor rates of retrieval of test organisms due to possibly due to anoxic conditions, suggests that future use of fully enclosed test chambers may be more suitable in these highly organic wetlands, as opposed to the open-bottom design. This would simultaneously eliminate the problem of identifying test organisms among indigenous fauna, and hopefully increase test organism survival. Paper towel substrate and sand are suitable test substrates

Table 4.4: *In situ* test 2 core contents for 2 reference (SW and HW63) and 2 OSPW-related (SB and NW) wetlands

Site	Core	damselfly larvae	dragonfly larvae	Beetles	Tanypodinae	other chir.	Other
Shallow W. (SW)	1						
	2		1			2	
	3		1			6	1 chir. pupa
	4		1			2	1 chir. pupa
	5		1	1 larva, 1 adult	16	12	5 adults, 1 chir. pupa
	6	3			3		
Highway 63 W. (HW63)	1			2 larvae	2	4	
	2(S)		1		7	23	
	3						
	4				2	3	
	5	1					
	6		1	1 larva			
South Bison P. (SB)	1(M)						
	2(Br)				8	21	
	3		1	7 larvae		2	
	4					1	
	5				6	1	
	6				8	1	
Natural W. (NW)	1(Br)				16	3	
	2				1	1	
	3				22	4	
	4(M)						
	5(M)						
	6(M)						

(Br) - Broken windows to core  
(S) - spilled sample  
(M) - Missing core

for these tests (Pereira et al. 1999).

Poor retrieval of test organisms was probably due to anoxic conditions developing in the test chambers over the test periods, although test 1 duration was only 4 days. Unfortunately, water D.O. within test chambers was not measured. Low concentrations of dissolved oxygen (0.8 to 2.1 mg/L) were noted in constructed test trenches containing dyke drainage water from the walls of the tailings pond (EVS Consultants 1995). Reduced D.O. in the presence of ammonia may have caused the heavy mortality (100%) observed in situ in *C. tentans* (EVS Consultants 1995). Food availability to the detritivorous *Chironomus* test organisms is not suspected to have been inadequate in these organic habitats, due to the presence of other taxa, particularly predators that indicate that prey were probably present previously. The presence of natural predators including chironomids as well as damselfly and dragonfly larvae could also have affected survival, as either Tanypodinae or Odonata were commonly seen in cores from all sites, in both tests.

In any case, both main tests 1 and 2 failed to clearly determine if native chironomid populations in OSPW wetlands had developed tolerance. Regarding the testing of this hypothesis, test 2 showed the most promise, in that a reference population was used, instead of a population from an OSPW wetland as in test 1. As mentioned, exposure of a reference population to both its native wetland and an OSPW would allow comparison of incidence of deformities in this population with those observed in chironomids collected from OSPW wetlands. Originally, both laboratory and field populations were to be tested in situ simultaneously, however, egg availability from both sources proved to be the limiting factor. Related to this, it would be preferable to have used more chironomids at

each site to be better able to detect a difference in the incidence of mouthpart deformity among sites. A minimum of 3 replicates of 125 chironomids or more from each site is desirable in order to attain sufficient power to detect differences (Hudson and Ciborowski 1996a, Burt 1998).

*In situ* test development requires patience and time, as Pereira et al. (1999) required 4 seasons and others found little success (42% survival) after as many as 17 tests (Chappie and Burton 1997). So, not surprisingly, this new *in situ* test requires further refinement for use in wetlands of this region. In order to improve the experimental system design, several modifications are suggested.

First, although windows were quite large relative to the surface area of the test cores (maximized window size that maintained structural integrity), a larger mesh size (100 or 120  $\mu\text{m}$ ) may increase water passage through the windows. However, this could compromise the security of the test chamber, as Pereira et al. (1999) found that even a 70  $\mu\text{m}$  mesh size allowed various indigenous invaders (copepods, nematodes, and insect larvae) into test units, and recommended a 50  $\mu\text{m}$  mesh size. Indigenous organisms slightly increased mortality of test organisms *in situ*, and reduced growth of chironomids, amphipods, and mayflies by 90%, 70%, and 50%, respectively (Reynoldson et al. 1994).

Related to this, the use of 2nd or 3rd instars would permit the use of a larger mesh size. Also, compared to first instars used in test 2, these instars are easier to handle and see, and they are probably less prone to handling stress than the first instars. First instars, generally considered the most sensitive (Williams et al. 1986), may be overly affected by slight changes from culture to test conditions, even if proper acclimation procedures are

followed.

The use of blanks at each test site would aid in determination of baseline levels of indigenous *Chironomus*, other chironomids, and other organisms

Fourth, due to the high organic content of the wetland sediments of this region, the use of fully-enclosed test systems is suggested. Similar tests were previously conducted in water-only exposure of chironomids in constructed trenches at Natural Wetlands (EVS Consultants 1995), and in water and sediment exposure of chironomids to metal-contaminated streams, by allowing chironomids to contact the sediment through mesh (Pereira et al. 1999). Substrates are recommended for these types of test systems, and *C. tentans* survival was higher with paper towel than with sand (Chappie and Burton 1997).

Handling stress (shaking) was shown to significantly reduce *Chironomus* survival, thus transport in test tubes is recommended (Chappie and Burton 1997). It is probable that chironomids were exposed to significant shaking and possible heat stress during transport to the various sites.

Finally, acclimation of test organisms to test conditions (temperature, dissolved oxygen, water salinity, pH etc.) is important, but difficult time-wise when using first instars that quickly moult to second instars (Chappie and Burton 1997). Water temperatures at the focal sites were fairly close to room temperature in the summer, so acclimation was likely not a factor in the poor retrieval of test organisms observed.

In conclusion, I was unable to determine if native populations of chironomids at OSPW wetlands were evolutionarily adapted to anthropogenic stresses in their environment due to limitations of the equipment used. The future use of chironomid *in situ*

bioassays requires further development of the test design in terms of increasing the poor retrieval rates observed in 1998. This approach may then have the potential to link laboratory bioassay effects to community response. Interspecies comparisons of sensitivity in the field, using both lab and field populations, would go far in assessing both the relevance of using traditional bioassay organisms and possible tolerance of populations exposed to OSPW.

## **CHAPTER 5 - Laboratory assessment of the comparative sensitivity of three chironomid populations to oil sands process water**

### **INTRODUCTION**

Field studies often lack sufficient control to determine direct causal relationships of toxicants, particularly in the case of chemical mixtures (Moriarty 1990). Also, it is difficult to prove causal linkage between absence of species in the field and chemical contamination, as numerous factors such as sources of colonizing species and habitat characteristics are often not known (Giesy et al. 1988). Laboratory studies utilize controlled environments to isolate causal factors with more certainty. As contaminant stress may result in sublethal as well as lethal effects, survival and growth are popular measures of toxicity (Giesy et al. 1988).

Despite the possibly hazardous input, OSPW-affected wetlands support populations of benthic invertebrates including midges that are apparently unaffected by these compounds. Benthic invertebrate community taxa richness may be slightly lower at OSPW wetlands than in naturally saline wetlands (Chapter 2). However, field collections of chironomids indicated no teratogenic effect being exerted, based on the low incidence of deformities in chironomids (Chapter 3). This may be either because the wetlands receiving the OSPW did not present a developmentally stressful environment to the chironomids (i.e. low bioavailability of potential stressors), or that these populations had become insensitive to the toxic components through selection (i.e. adaptation over generations). Unfortunately, the results of the *in situ* study conducted to examine this question were confounded by complications with test system design (Chapter 4).

In order to determine if low bioavailability of contaminants or adaptation by field populations of chironomids was associated with the observed scarcity of mouthpart deformities, a laboratory bioassay was conducted. This bioassay was conducted using first-instar larvae of two laboratory (*Chironomus riparius* and *C. tentans*) and one field population (*C. tentans*) exposed to OSPW. Chemical sensitivity is known to decrease with increasing age of chironomid larvae (McCahon and Pascoe 1991). Larval survival, growth (estimated by total body length), development to pupal and adult stages (emergence), and incidence of mouthpart deformities were assessed.

This chapter assesses possible differences in sensitivity among two laboratory-reared and one field-collected populations of chironomids to oil sands-related contaminants using a standard 14-d static laboratory bioassay. Variability in response between laboratory species relative to variability from lab to field was analyzed using ANOVA and multiple linear regression to provide data on the toxic equivalence of standard lab-cultured and field-occurring taxa. This information is crucial in assessing the validity and meaning of tests using laboratory-reared organisms to test field conditions.

In the case where tolerance to oil sands contaminants has developed in field populations, I expect that the field population will experience superior survival, growth, emergence and a lower incidence of deformities than laboratory populations. It may be that under field conditions (particularly high humic acid content of the wetlands) that the OSPW components are unavailable to the chironomids. Low bioavailability would result in no adverse effects in any of the species tested, regardless of origin, although adaptation could not be tested. Strong adverse effects observed with all species tested would suggest

that adaptation had not occurred and that contaminants were bioavailable under test conditions.

## **METHODS**

### ***D) Experimental Design and Set-up***

#### ***Design***

Toxicity of oil sands process water (OSPW) to the two laboratory populations (*Chironomus riparius*, and *C. tentans*) and the one field population (*C. tentans*) of chironomids was assessed in terms of larval survival, growth (final body length), and the incidence of mentum deformities.

The experiment was conducted in April-May 1999. It was based on a 3 (populations) x 6 (treatments) randomized design blocked over 7 set-up days, with replicate test jars (n = 4 or 5), each containing 50 test organisms. Lab *C. tentans* had only four replicates due to limited numbers of larvae hatched in the test period. This gave a total of 84 test jars randomly distributed in the environmental test chamber.

#### ***Test Organisms***

The lab populations of *C. riparius* (population R) and *C. tentans* (population T) were reared at the University of Windsor in 20-L aquaria containing silica sand (depth of 3 cm) and continually-aerated dechlorinated tapwater to a depth of 12 cm (Krantzburg 1990). The laboratory cultures of *C. riparius* originated from egg masses obtained from the National Water Research Institute, Environment Canada, Burlington, ON. *C. riparius*

egg masses were received on December 18, 1998. This population had an observed 2 week life cycle, and had undergone 9 generations. The laboratory culture of *C. tentans* originated from egg masses sent from Ministry of Environment in Toronto, Ont., in January 1999. They had gone through 1 to 2 generations.

The field population of *C. tentans* (population L) originated from egg masses collected in early July 1998 at South Bison Pond wetland (56°59.769 N, 111°36.162 W) 30 km north of Fort McMurray, Alberta. Taxonomic identification was carried out in laboratory by mounting specimens on microscope slides. Individuals were identified to the species level using a compound light microscope following the methodology of Wiederholm (1989). Midges were dissected to clearly reveal fine structure of the antennae, ventral view of thorax, three legs from one side, head parts, and abdomen/genitalia. CMC-10 aqueous mounting medium was used to clear and fix tissues. Vinyl spacers were used to aid insect positioning. The field population (known as South Bison "Large") was reared in the same manner as the lab population had been reared, and had undergone 6 to 7 generations since the laboratory culture was started on July 15, 1998.

#### *Test Water*

The OSPW arrived in air-tight 20-L polyethylene pails filled to the top, on April 21, 1999, from Suncor, an oil company of the Fort McMurray area. The OSPW was gently aerated for 2 days (covered) prior to test set-up. The water was a cloudy brown colour and had a strong hydrocarbon odour.

A serial dilution was used (0:1, 15:1, 7:1, 3:1, 1:1, 1:0 v/v OSPW: dechlorinated City of Windsor tap water) to make up the 6 treatments. Water parameters including conductivity, salinity, temperature, pH, dissolved oxygen were measured for each jar on days 0, 7, and 14.

### *Set Up*

Test jars (12 x 12 x 15 cm, 2-L glass) were precleaned with detergent and double rinsed with distilled water, followed by treatment for 24 hours in a 10% nitric acid bath to remove contaminating metals. Upon removal from the acid, jars were immediately triple rinsed with distilled water and left to air dry. Hexane (non UV, residue analytical grade) was then rinsed into all jars to remove any residual organic contaminants from the glass. Jars were given a final double rinse in distilled water. Silica sand (70 mesh) was washed to remove dust/fines/extraneous matter, and 500 g (wet) was added to each jar (approx. 2 cm depth). Following this, 1 L of the appropriate amount of OSPW and/or dechlorinated water was added to each jar.

Newly-laid egg masses were collected from culture aquaria, placed in glass petri plates containing dechlorinated water, and observed daily for hatching. Newly-hatched (<24-h old) first instar larvae were pipetted in groups of 50 into 20-mL scintillation vials containing aerated, dechlorinated water. Test organisms were then transported to the test chamber where the vials were gently immersed into test jars and inverted. Due to limited availability of eggs hatching each day and to time constraints, the larvae were inoculated into test jars on 7 separate days (over an 8-d period) following a randomized block

schedule. New larvae of each day were distributed evenly among treatments to control for differences in fitness/sensitivity and handling among offspring of different mothers/egg masses.

### *Test Conditions/Feeding*

The bioassay was conducted in an environmental chamber set at 21 °C with a 16:8 light:dark photoperiod. Test jars were constantly aerated using a network of capillary tubing attached to an air pump (Corkum and Hanes 1989). Jars were loosely covered with plastic lids to slow evaporation, and distilled water was added to replace any water lost throughout the test.

Larvae were fed a mixture of ground alfalfa pellets and Tetramin in water. Each jar received 1 mg Tetramin /individual/day (Burt 1998), which maximizes growth and survival of *C. riparius*. To elevate total organic carbon levels in the test system, 0.012 mg alfalfa /individual/day was added to each jar. One half of the Tetramin ration was fed daily to larvae in the first week. The full ration was provided daily in the second half of the two-week test so as to minimize buildup of uneaten food which could add physical and chemical stress to the system (reduced oxygen, physical alteration of sediment surface).

### *Chemistry Water Samples*

Separate test jars were set up for water chemistry analysis, and were treated identically to the other replicates in terms of food additions and aeration. However, chironomids were not added to these jars. Water samples were collected from the separate

test jars on days 0 and 14 for analysis of naphthenic acids, nutrients [ammonia, nitrate-nitrite, Total Kjeldahl Nitrogen (TKN), Total Phosphorus (TP)], routine water chemistry, and dissolved/total metals. Due to a higher volume of water required for the tests on d-14, water was also taken from jars containing chironomids. For dissolved metal analysis, all filtration apparatus were carefully washed in detergent, rinsed in distilled water, acid washed (10% nitric), rinsed in distilled water, and rinsed in hexane followed by a triple rinse in distilled water. Water temperature, concentration (mg/L) of dissolved oxygen (D.O.), pH, salinity, and conductivity were recorded prior to sample collection. Samples were then collected and transported in the appropriate precleaned containers to Enviro-Test Laboratories in Winnipeg.

### ***II) Assessing Endpoints of Survival, Growth, and Deformities***

Trials were terminated after 14 d. Survival was assessed during takedown, and verified later during growth assessment. Body lengths and incidences of mouthpart deformities were measured within 3 weeks after completion of the test.

#### ***Survival***

Each jar was examined for emergent adults, which were counted and captured when the lid was removed. The contents of each jar were then emptied into sorting trays (25cm x 30cm x 10 cm deep) using tap water to rinse out residual sand. Larvae, pupae, and pupal exuviae were counted and assessed for survival. An organism was considered alive if it showed signs of mobility or at least appeared viable in form (firm body, usually

curled into a coil). Partially decomposed remains were counted as dead individuals. Chironomids were then hand picked from the trays, blotted dry on paper towel, and preserved in labeled 20-mL scintillation vials containing chilled Carnoy's solution (3:1 mixture of 95% ethanol: acetic acid). Solutions were changed frequently and stored refrigerated to assure sample integrity.

### *Growth*

Larval body length was used as a measure of growth. Vial contents were poured into a deep petri dish, using 70% ethanol to completely rinse out the vial. Adults, pupae and exuviae were recounted and replaced in the vial containing fresh Carnoy's solution. Larvae from a vial were then separated from each other on the dish. A video image was captured using a Hitachi VK-C370 video camera (Olympus 28mm wide angle lens) attached to a Targa 64 computer video digitizing card. Lengths of images were digitally measured on a computer monitor using MOCHA 1.2 (Jandel Scientific), a digital image analysis software package, by manually tracing a line from the apex of the head capsule to the tip of the anal prolegs. The length (mm) was input directly into a spreadsheet, which was then transferred to an Excel 5.0 file. Lengths of all intact larvae bodies were measured regardless of whether they had been alive or dead at takedown, in order to assess growth independently of survival. Bodies were then replaced in fresh Carnoy's solution and refrigerated until required for assessment of deformities.

### *Deformities*

Mouthpart deformities were assessed following the method of Hudson and Ciborowski (1996). Following measurement, the head capsule of each larva was removed beneath a dissecting scope and mounted venter-upward on a microscope slide using CMC-9AF aqueous mounting medium (Masters Company Inc., Bensenville, IL). The bodies were retained in fresh Carnoy's solution for future assessment of chromosomes. Additional head capsules were sometimes found attached to adults and pupae/exuviae, or were found in the sand at takedown, and these were also examined. The mentum of each head capsule was examined under a compound light microscope (100-400x magnification power). Chironomids were considered deformed only if the mentum had one or more extra or missing teeth.

### *III) Statistical Analyses*

A significant number of chironomids emerged before the end of the study in one population (R). Therefore, the proportion of survivors that were still larvae was used to measure developmental effects associated with concentration of OSPW. A total of four endpoints were assessed, including survival, development, growth, and incidence of deformities.

Differences in vigour of first instar larvae were visible on different days, from different egg masses. This was accounted for in the statistical analyses by considering not only "replicate" but also set-up "day" as covariate factors in the data analysis.

Analysis of variance (ANOVA) was conducted to test for any differences among groups. Multiple regression analyses was used to determine if there was any relationship between the dependent variable (i.e. endpoint) and the independent variable (log percentage OSPW). The reference treatment was given a value of log (0+1% OSPW) but it was not used in the regression analyses. Regression analysis was performed for each population separately, as well as for each separate set-up day within each population's data set. This was also done for replicates of each population. All multiple regression analyses were performed using the Statistica© 5.1 software package. All analyses were assessed for significance using an  $\alpha = 0.05$  (one-tailed for OSPW concentration effect, two-tailed for all other tests).

To test for interaction effects of population on survival, growth, development, or incidence of deformities, "population" was coded as a series of dummy variables in the multiple regression. The same procedure was done to test for day x treatment interaction effects on each of the four endpoints, within the data of each population separately.

Following this, values including LC50 (survival), EC50 (lengths), and concentrations required to double the proportion of larvae of all survivors (development) were calculated. This was done directly on graphs using linear regression lines, or in case of apparent threshold effects, using lines of best fit through the appropriate points. Values were calculated for each population using all pooled data, and also for each set-up day, from which a mean set-up day value was determined. An alternative method of analysis called linear-plateau regression could be applied using the SAS(R) software package. This method, also called the threshold or hockey-stick model, has been successfully applied in

assessing threshold responses in fish exposed to pesticides, as well as in aquatic invertebrates exposed to aromatics (Beyers et al. 1994, Penttinen and Kukkonen 1998).

To determine if a consistent pattern of response was present and of what type (e.g. threshold) had occurred, the slopes of the regression lines from different days were compared. This was performed for each population using a student's t-test with unequal variance.

### *Survival*

In counting out the larvae during set up, a possible source of error in introducing the larvae to the test chambers was the inclusion of extra larvae that appeared dead but that were in fact quiescent.

Survival was calculated as the number of survivors divided by 50 (number of 1st instar larvae added to each jar). Survivors included all live larvae, as well as pupae, and the higher value of either the number of pupal exuviae or the number of adults, as each emerging adult would have produced a pupal exuvium. In some cases, more than 50 survivors were counted, but a maximum value of 100% survival was attributed to these test jars. Replicate and day trials in which mean survival in the reference jars was less than 60 % were excluded from analyses.

Survival was expressed as a proportion, and was arcsine square root transformed prior to multiple regression analysis. Treatment concentrations (% OSPW) were  $\log_2$  transformed. The final regression model for populations T and R consisted of 5 replicates, but each had a day excluded. Four replicates were used in calculations for population L.

### *Development*

For the two lab populations T and R, which exhibited emergence during the test, the proportion of survivors that were larvae was compared among treatments (log<sub>2</sub> transformed) within each population. Prior to multiple regression, proportions were arcsine square root transformed and weighted by the square root of the number of total survivors for each replicate jar. The sum of weights was scaled to be equal to the number of samples, for each analysis. Analysis was also done for separate days and replicates within each population.

### *Growth*

Body length was used as a measure of organismal growth. Both body length and biomass are suitable endpoints to assess growth, the former being related to the latter in log proportion. Body length was easier and more appropriate to measure in this experiment. Measurement of individual biomass would have been difficult (continual evaporation of ethanol from bodies) and destructive to samples (as drying them would render them useless for possible future chromosomal assessment).

The effect of OSPW on mean body length was analyzed using variance-weighted multiple regression. The mean body length of larvae in each test jar was weighted by the inverse of the variance of the jar means of all replicates for each treatment. The sum of weights was scaled to be equal to the number of samples, for each separate analysis. A separate regression was performed for each population, as well as for each set-up day and replicate within each population. I also tested for population x treatment interaction

effects, as well as day x treatment effects within each population.

### *Deformities*

The proportion of deformed individuals was compared among treatments (log<sub>2</sub> transformed). Analysis was similar to that described for the development data. The dependent variable was arcsine square root transformed and weighted by the square root of the number of heads examined from that jar. The sum of weights was scaled to equal the number of samples for each analysis.

## **RESULTS**

### **Water Temperature during the bioassay**

The temperature control of the environmental chamber was set at 21°C. However, due to technical difficulties with the cooling unit the temperature in the test jars was actually higher than 21°C until the end of the experiment, thus speeding the development of the larvae and resulting in emergence of many of the *C. riparius* larvae. Water temperatures during the bioassay ranged from 25 to 21 °C (Appendix 5.1). After repair to the cooling system, the temperature of the environmental chamber dropped in the last few test days to 21 °C. The mean was 24.1±0.6 °C before repair and 23.8±1.0°C including the days after repair.

## **Water Parameters**

Water pH, conductivity, salinity and temperature were measured in all test jars on days 0, 7, and 14 (Appendix 5.1).

Conductivity, salinity, and pH all increased proportionately with increasing concentrations of OSPW water for all 3 populations (Fig. 5.1a,b,c, 5.2a,b,c, 5.3a,b,c). Initial oxygen levels were all between 90 and 100% saturation for all treatments of all three populations, but decreased markedly during the 14-d test (Fig. 5.4a,b,c).

Conductivity was relatively invariable within treatments, except at 100% OSPW where slight differences were observed between d-0, d-7 and d-14. These differences were probably related to water evaporation from jars (Fig. 5.1a,b,c).

Salinity followed a similar increasing trend with [OSPW] as was seen with conductivity. Variability was again present only in 100% OSPW treatment, at a minimal level (Fig. 5.2a,b,c).

Water pH significantly decreased with time in most treatments. The greatest decline was seen in the 100% OSPW treatment, and the smallest decline in 0%, suggesting achievement of an equilibrium pH value, possibly affected by the additions of food (Fig. 5.3 a,b,c).

Dissolved oxygen (% saturation) decreased over time from between 90 and 100% on day-0, to between 55 and 65 % on day-14 (Fig. 5.4 a,b,c). Oxygen saturation was fairly invariant among treatments, except for d-14 population R samples. Dissolved oxygen concentrations in test jars containing 12.5% or more OSPW were lower than all other jars.

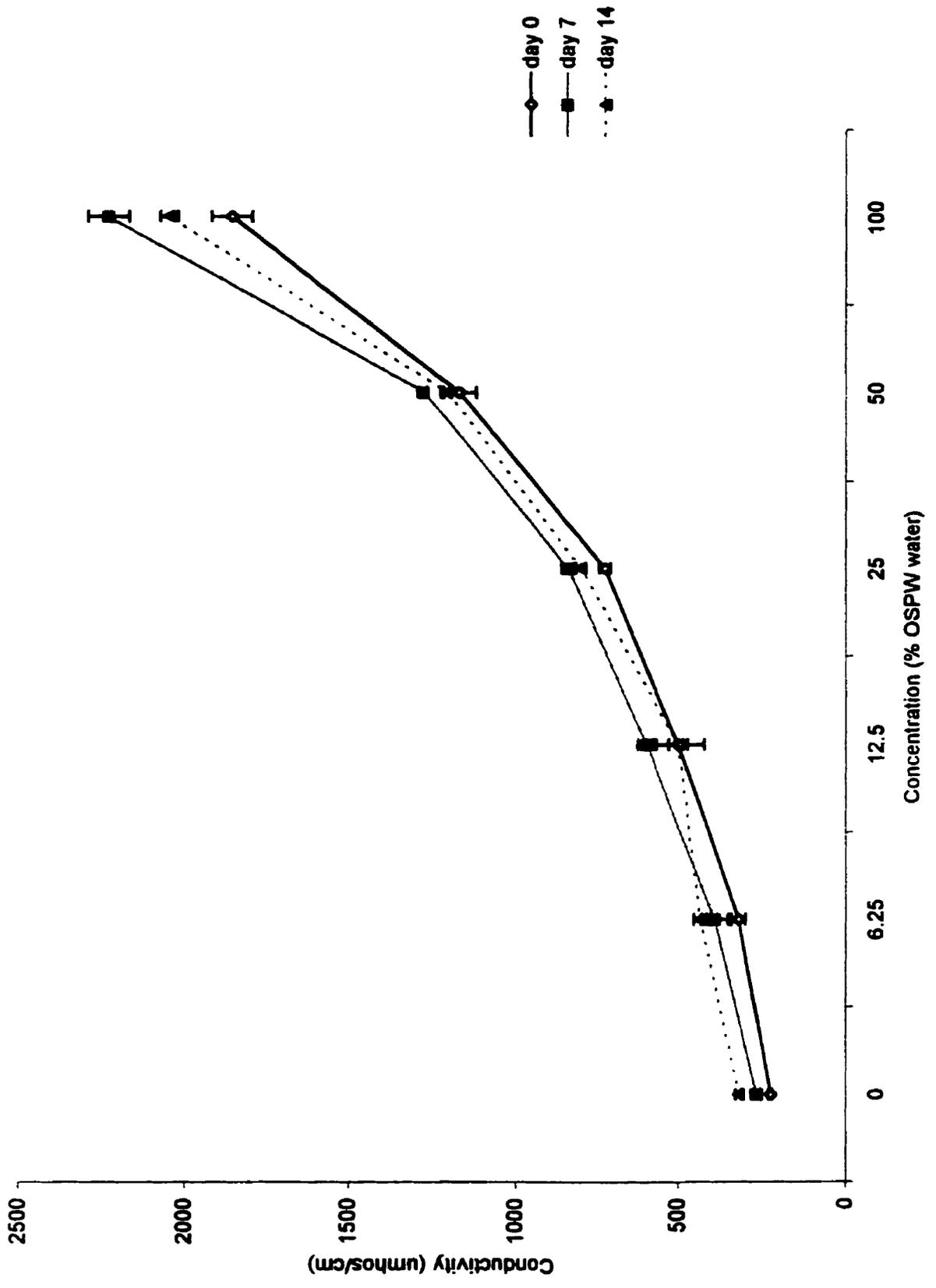


Fig 5.1a: Conductivity of test water in 14d OSPW bioassay on days 0, 7, and 14 for field *C. tentans* (Population L).

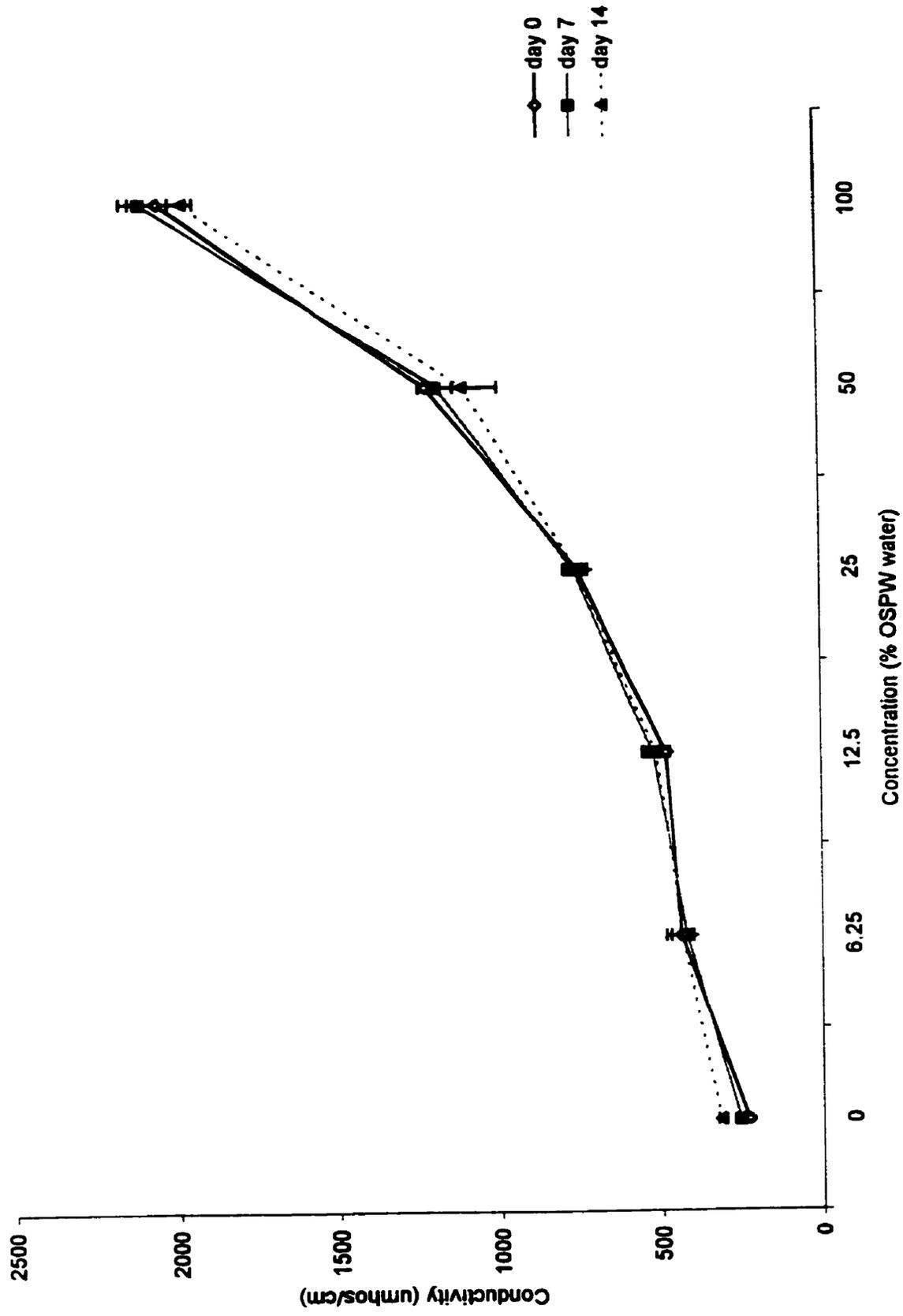


Fig. 5.1b: Conductivity of test water in 14d OSPW bioassay on days 0, 7, and 14 for lab *C. tentans* (population T).

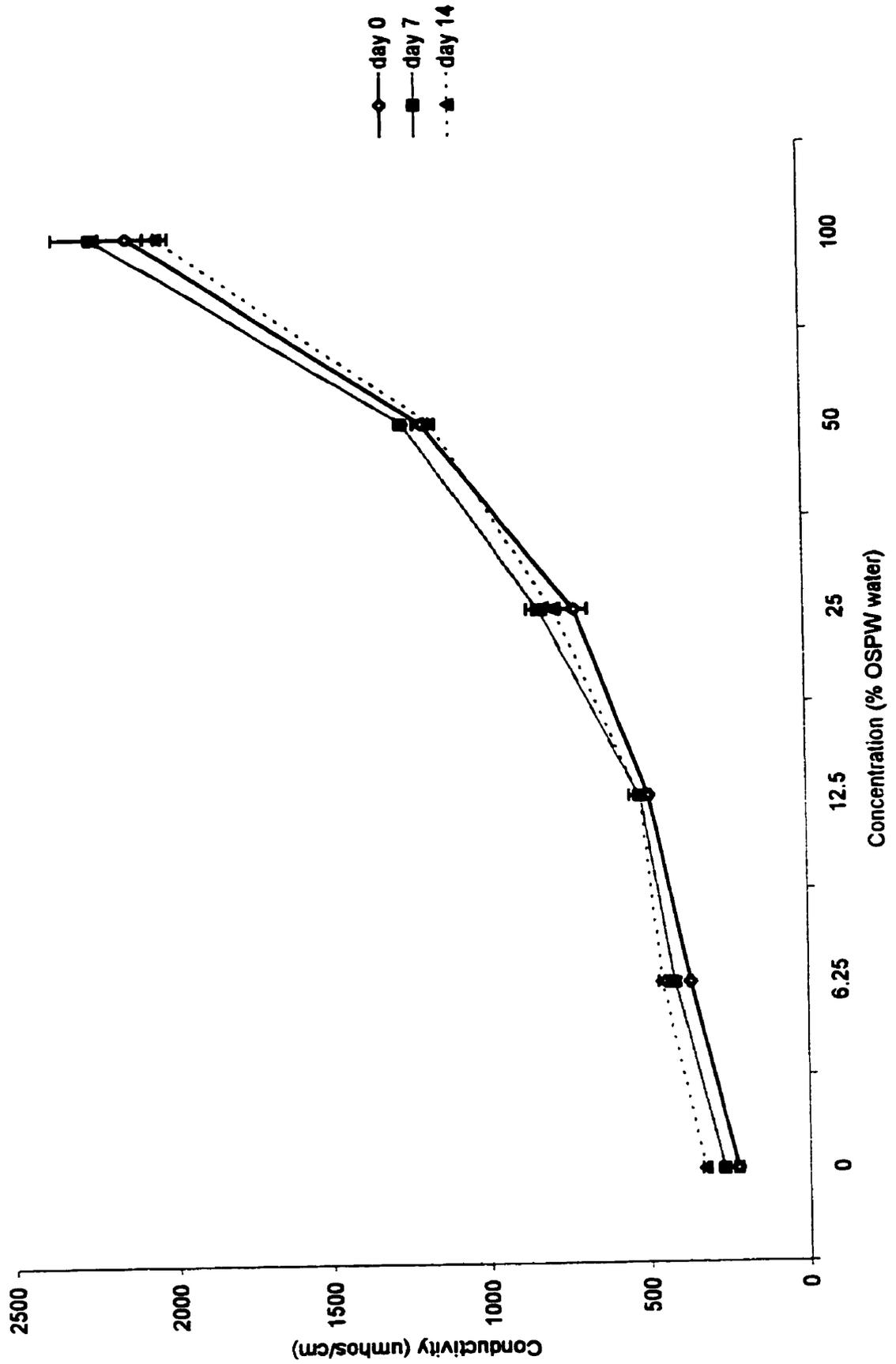


Fig. 5.1c: Conductivity of test water in 14d OSPW bioassay on days 0, 7, and 14 for lab *C. riparius* (population R).

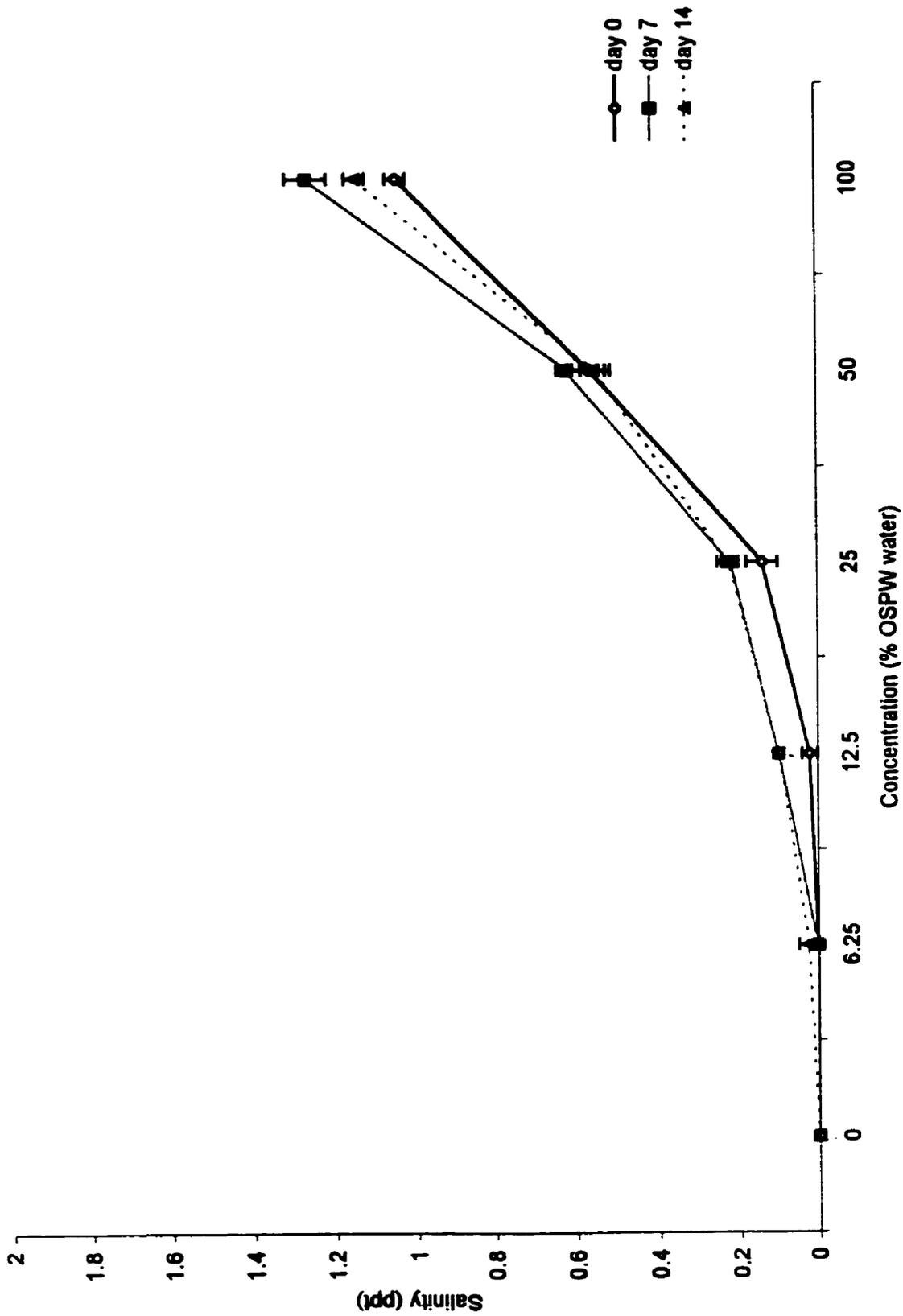


Fig. 5.2a: Salinity (ppt) in 14d OSPW bioassay on days 0, 7, and 14 for field *C. tentans* (population L).

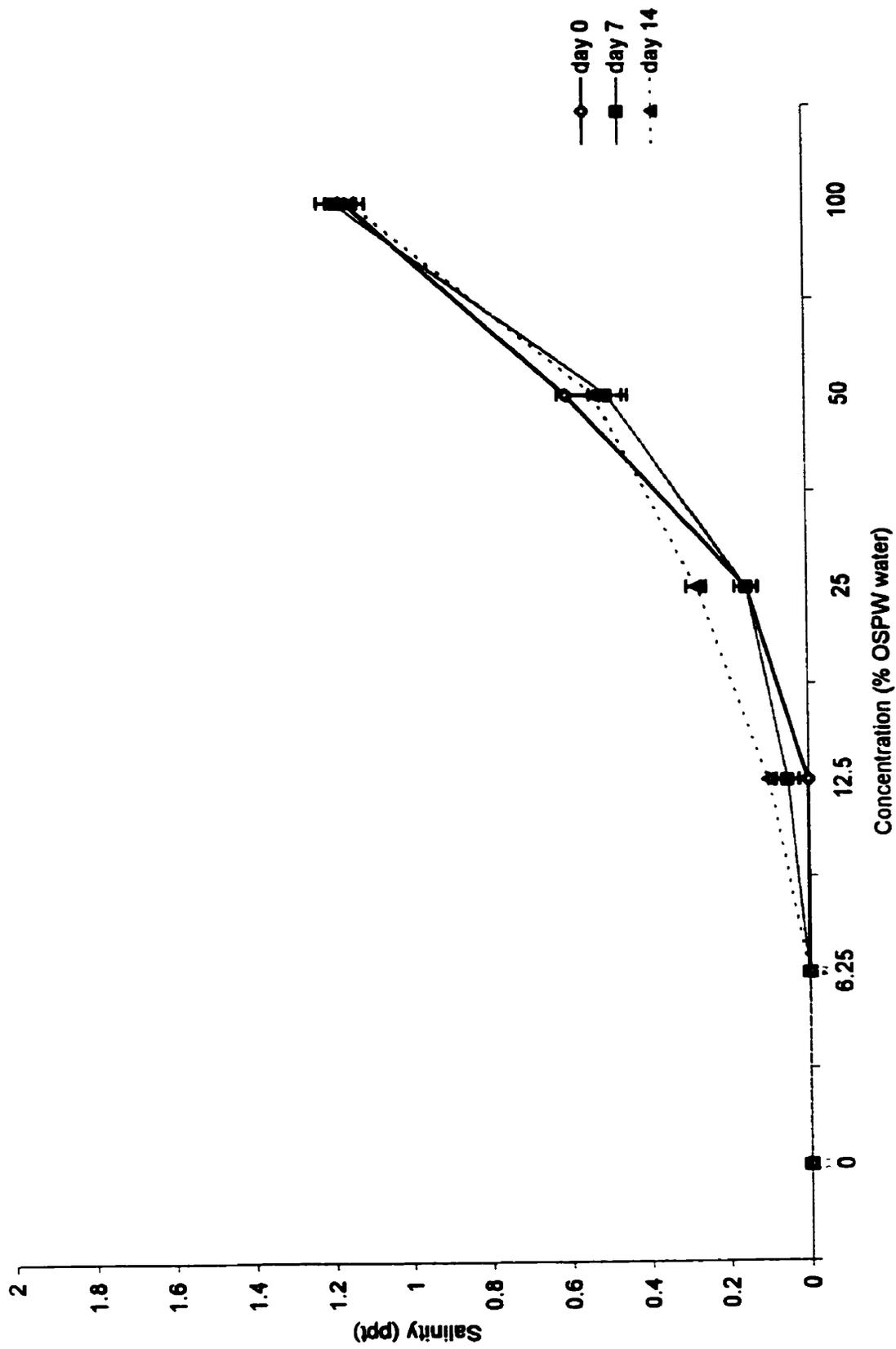


Fig. 5.2b: Salinity (ppt) of test water in 14d OSPW bioassay on days 0, 7, and 14 for lab *C. tentans* (population T).

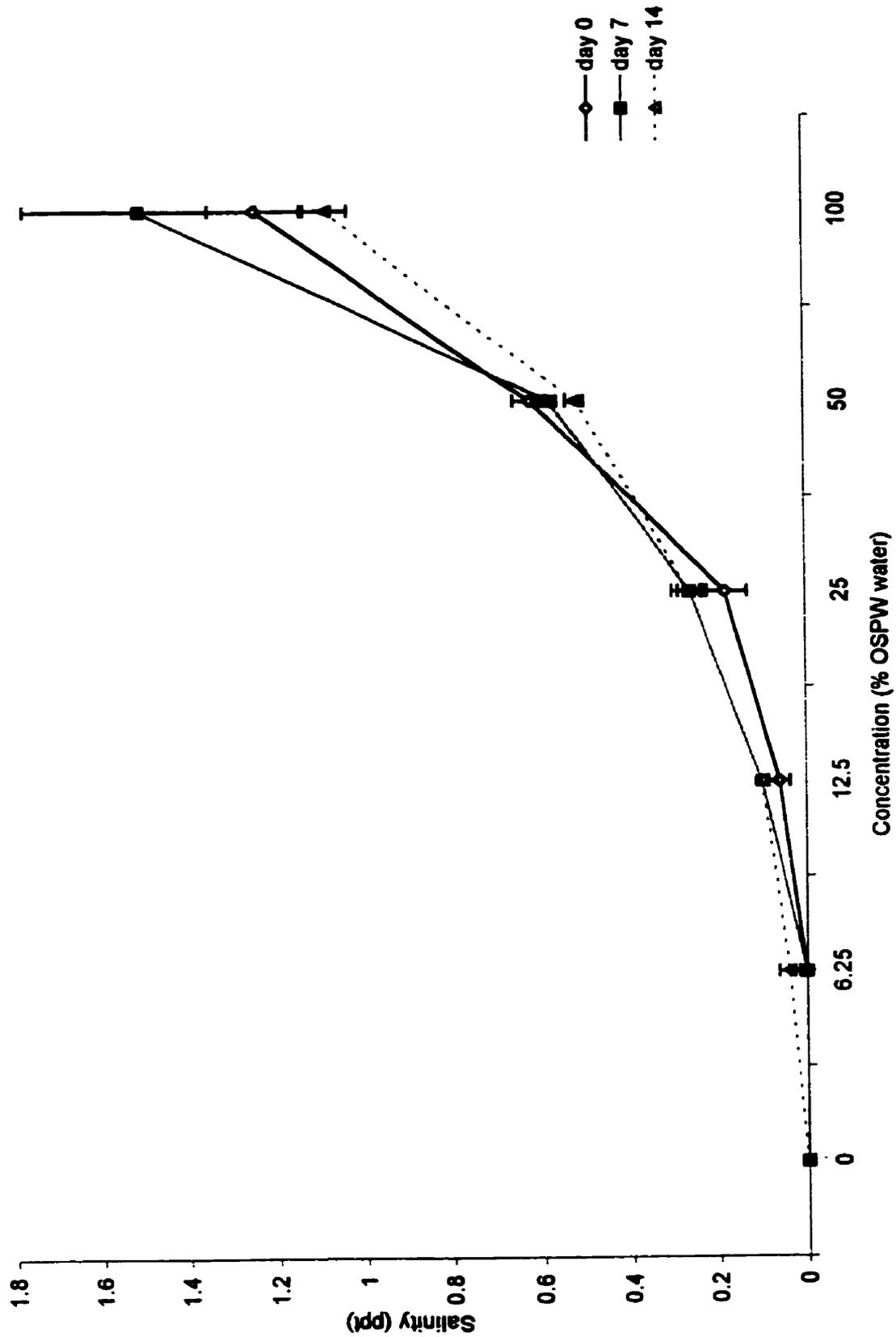


Fig. 5.2c: Salinity (ppt) in 14d OSPW bioassay on days 0, 7, and 14 for lab *C. riparius* (population R).

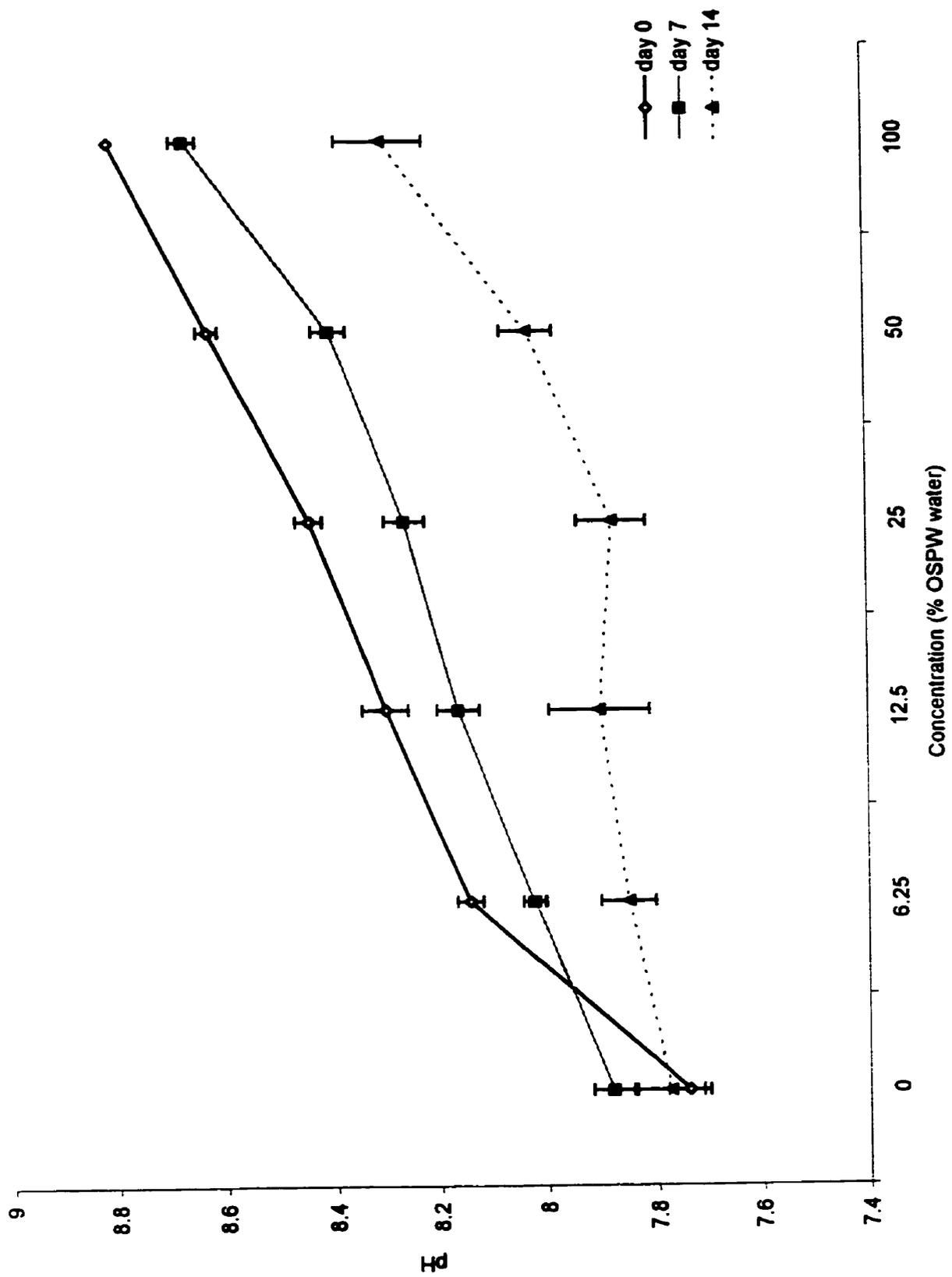


Fig. 5.3a: pH of test water in 14d OSPW bioassay on days 0, 7, and 14 for field *C. tentans* (population L).

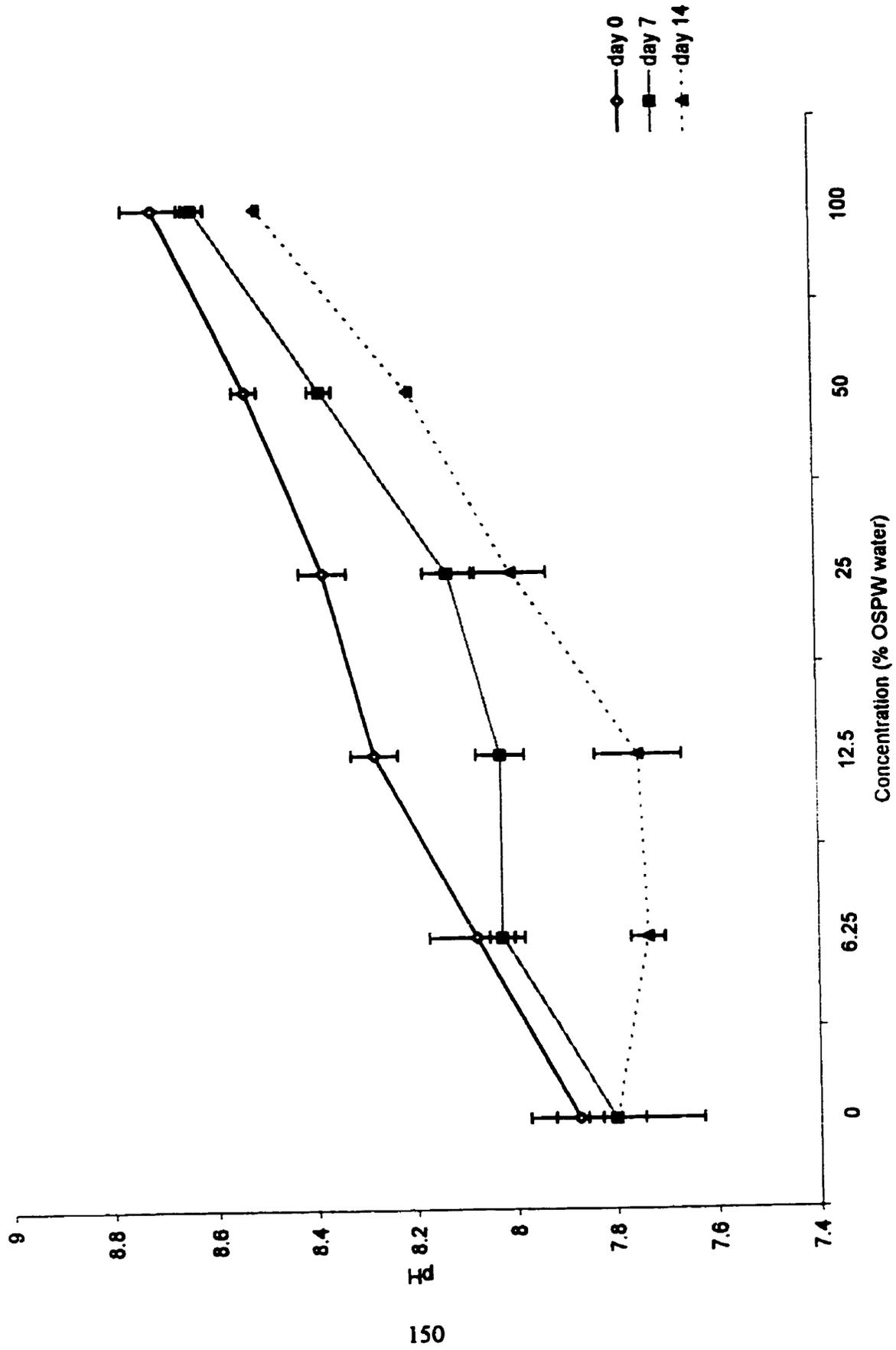


Fig. 5.3b: pH of test water in 14d OSPW bioassay on days 0, 7, and 14 for lab *C. tentans* (population T).

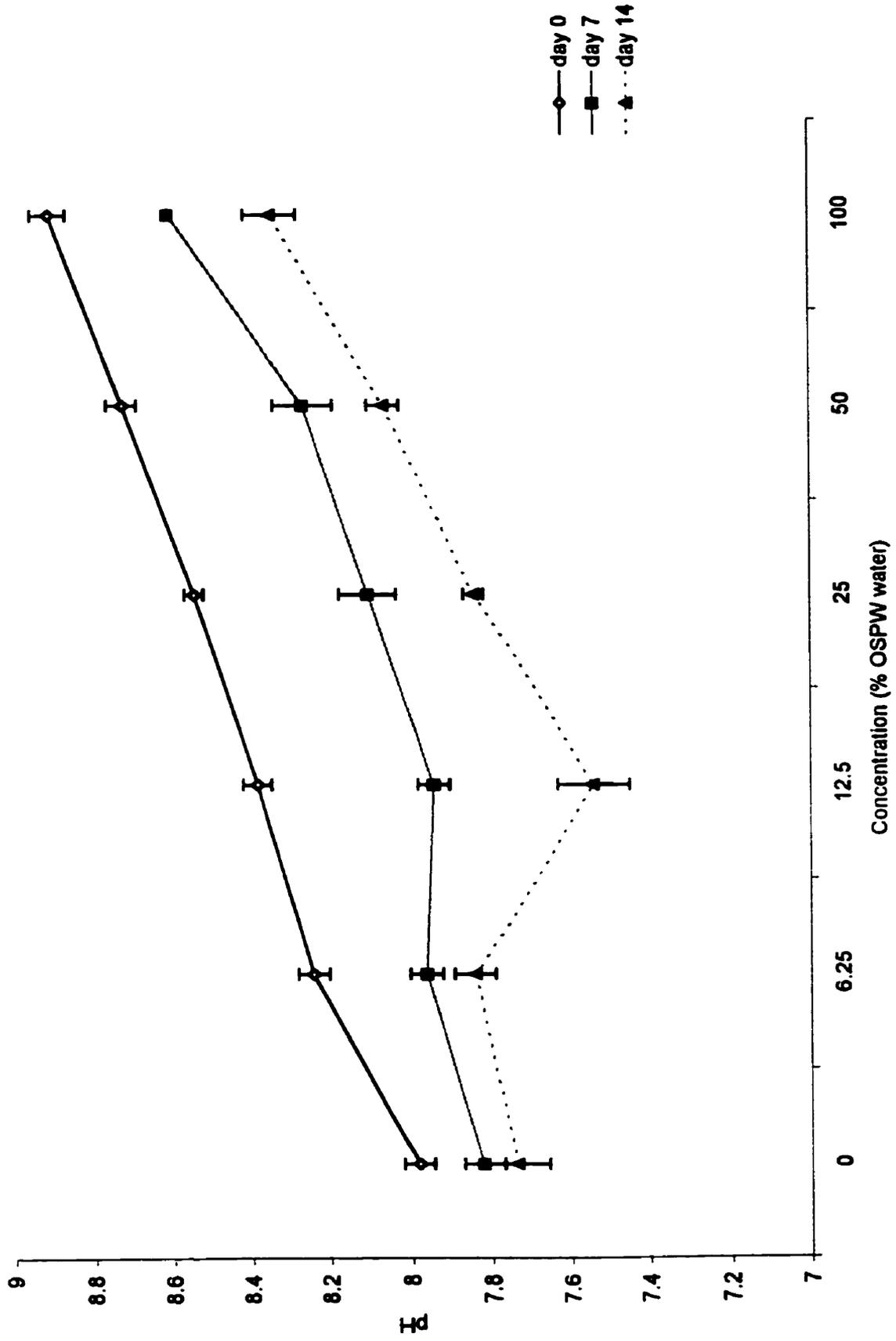


Fig. 5.3c: pH of test water in 14d OSPW bioassay on days 0, 7, and 14 for lab *C. riparius* (population R).

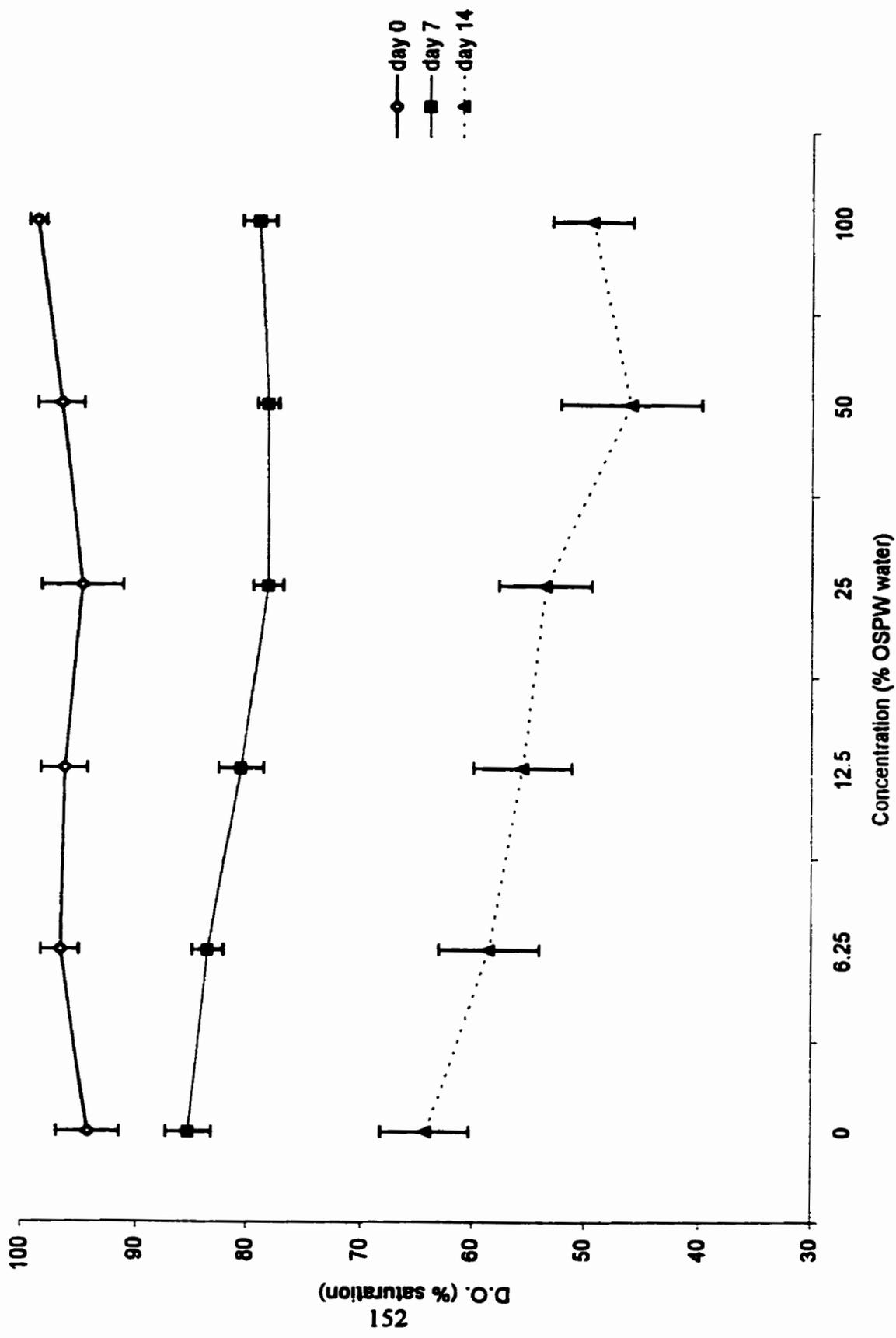


Fig. 5.4a: Dissolved oxygen (% saturation) of test water in OSPW bioassay on days 0, 7, and 14 for field *C. tentans* (population L). (D.O. = 8.5 mg/L at 100% saturation).

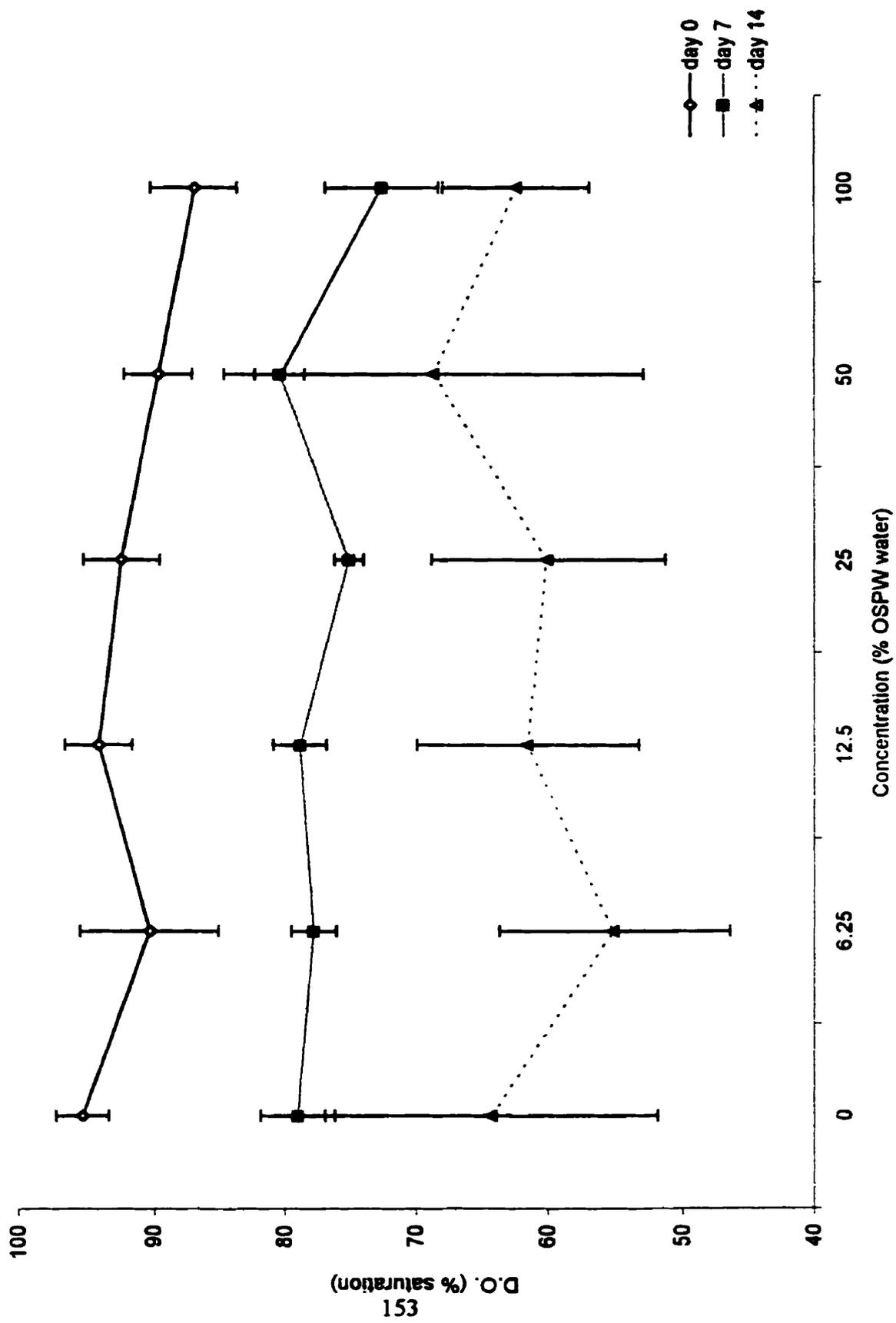


Fig. 5.4b: Dissolved oxygen (% saturation) of test water in 14d OSPW bioassay on days 0, 7, and 14 for lab *C. tentans* (population T). (D.O. = 8.5 mg/L at 100% saturation).

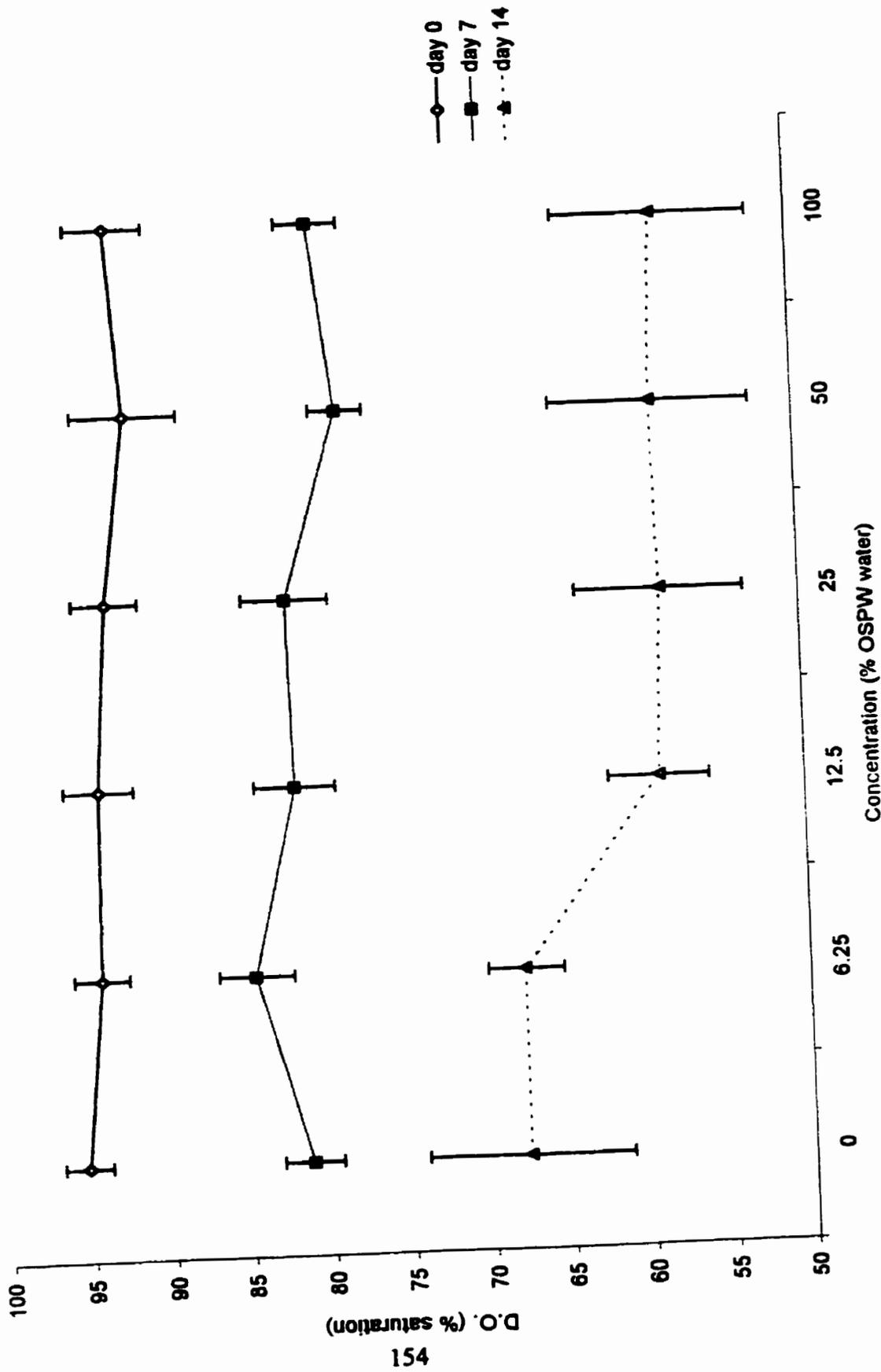


Fig. 5.4c: Dissolved oxygen (% saturation) of test water in 14d OSPW bioassay on days 0, 7, and 14 for lab *C. riparius* (population R). (D.O. = 8.5 mg/L at 100% saturation).

## **Water Chemistry Sample Analysis**

The results of the Enviro-Test Laboratories water chemistry analyses for days 0 and 14 are listed in Appendix 5.2. Samples collected on d 0 shipped for routine nutrient analysis were compromised by delayed delivery by the expeditors, and their values are thus questionable. The concentrations of the dissolved form of some metals (barium, copper, rubidium, and zinc) were higher than the total concentrations. This indicates probable environmental contamination during sample preparation (preparing the filtered dissolved metal samples), as the analyses were repeated twice by the lab and were thus found to be precise and probably accurate. Therefore, although both results are shown, only total metal concentrations will be discussed.

The following section discusses the chemical constituents of OSPW (by comparing control water to 100% OSPW) as well as changes in concentrations that occurred over the test period in relation to [OSPW].

### *Control vs. 100% OSPW*

Overall, the dilution series was reflected in measurements of water conductivity, pH, and naphthenic acid concentrations, which increased proportionately with increasing [OSPW].

#### **Ions and Nutrients:**

The 100% OSPW had much higher concentrations of dissolved solids, dissolved sulphate, total anions, and total cations, as well as higher carbonate, bicarbonate and

calcium carbonate alkalinities than the 0% OSPW treatment. This was true for both d-0 and d-14 samples. The controls had twice as much calcium (32.85 mg/L) as the 100% treatment (13.85 mg/L). Day 14 analyses indicated that dissolved nitrate-nitrite was higher in 100% OSPW (0.72 vs. 0.06 mg/L in controls), but that ammonia (10-16 mg N/L) and total Kjeldahl nitrogen concentrations (19-20 mg N/L) were about equal in 100% OSPW and control water. Food and chironomid waste products almost definitely accounted for most of the nitrogen in the water.

#### Metals:

Pure OSPW contained much higher concentrations of several metals (19 detected here) than control water. On day 0, total metal concentrations of aluminum (12.05 mg/L), barium (0.16 mg/L), boron (2.91 mg/L), cadmium (0.002 mg/L), cesium (0.002 mg/L), chromium (0.015 mg/L), cobalt (0.003 mg/L), iron (2.46 mg/L), lithium (0.25 mg/L), molybdenum (0.86 mg/L), nickel (0.018 mg/L), potassium (19 mg/L), rubidium (0.05 mg/L), sodium (503.5 mg/L), strontium (0.73 mg/L), titanium (0.22 mg/L), uranium (0.009 mg/L), vanadium (0.06 mg/L), and zirconium (0.026 mg/L) were all greater than 5 times higher in OSPW than in control water.

#### *Chemistry changes over time:*

Concentrations of total naphthenic acids dropped slightly from d-0 (79+/-2 m/kg) to d-14 (70.5+/-3.5mg/kg) in the highest treatment, but remained relatively constant in the control treatment (2 mg/kg or less), and a pattern of dilution by a factor of 2 (as designed)

was seen in the d-14 samples through the six treatments (Appendix 5.2).

#### **Ions and Nutrients:**

Total phosphorus and total Kjeldahl nitrogen increased over the 14 days in all treatments, as expected in a bioassay involving feeding. Unfortunately the d-0 sample integrity was compromised, so that no strong conclusions can be drawn regarding nutrients.

#### **Metals:**

By day 14, the 100% to 0% OSPW treatments, Only 11 of the 19 metals remained greater than 5 times higher (total concentration) in 100% OSPW relative to control water. These include aluminum, boron, cadmium, cesium, lithium, molybdenum, rubidium, sodium, titanium, uranium, and vanadium. In particular, molybdenum was 700x higher, lithium was 135x higher, and both boron and sodium were 50x higher.

Total concentrations of barium, iron, tin, vanadium and zirconium all increased over time in the control treatments, but decreased in 100% OSPW treatments, indicating a possible source in the tapwater used.

No metals increased in concentration through time in the 100% OSPW, although several showed strongly decreased concentrations. Metals that showed significant decreases in concentration from the water column include aluminum, chromium, iron, lead, titanium, and zirconium.

***Canadian Water Quality Guidelines (CWQG) for Aquatic Life:***

Dissolved ammonia concentrations were higher than the CWQG in both 0% and 100% OSPW treatments on d-14 (Appendix 5.2; CCREM 1987). Several total metal concentrations from the 100% treatment (both d-0 and d-14) exceeded the CWQG, including arsenic, cadmium, molybdenum, selenium, and particularly aluminum. Both iron and chromium concentrations surpassed the guidelines on d-0 in the pure OSPW treatment, and copper was higher in both 0% and 100% for d-0 and d-14.

***Survival***

Mean survival was excellent for the control treatments of lab populations T ( $91 \pm 5.3 \%$ ) and R ( $89 \pm 5.2 \%$ ), but only moderate for field *C. tentans* population L ( $71 \pm 4.6 \%$ ) (Fig. 5.5). This means that the results of the last population may be suspect due to confounding by ambient conditions.

Survival of *C. riparius* was uniformly high across all OSPW treatments. However, survival of the *C. tentans* populations L and T was poor at the highest concentrations of OSPW. As survival differed significantly among populations (multiple linear regression,  $p < 0.002$ ), analysis was done for each population separately (Fig. 5.5, 5.6, 5.7, 5.8, Table 5.1). Set-up day did not affect survival for any of the populations overall (Table 5.1). However, there was considerable variation in survival within different days (Table 5.2). There were no significant population x [OSPW] interactions, nor were any day x [OSPW] interactions significant for any of the populations, indicating responses among all days and all populations followed a consistent pattern.

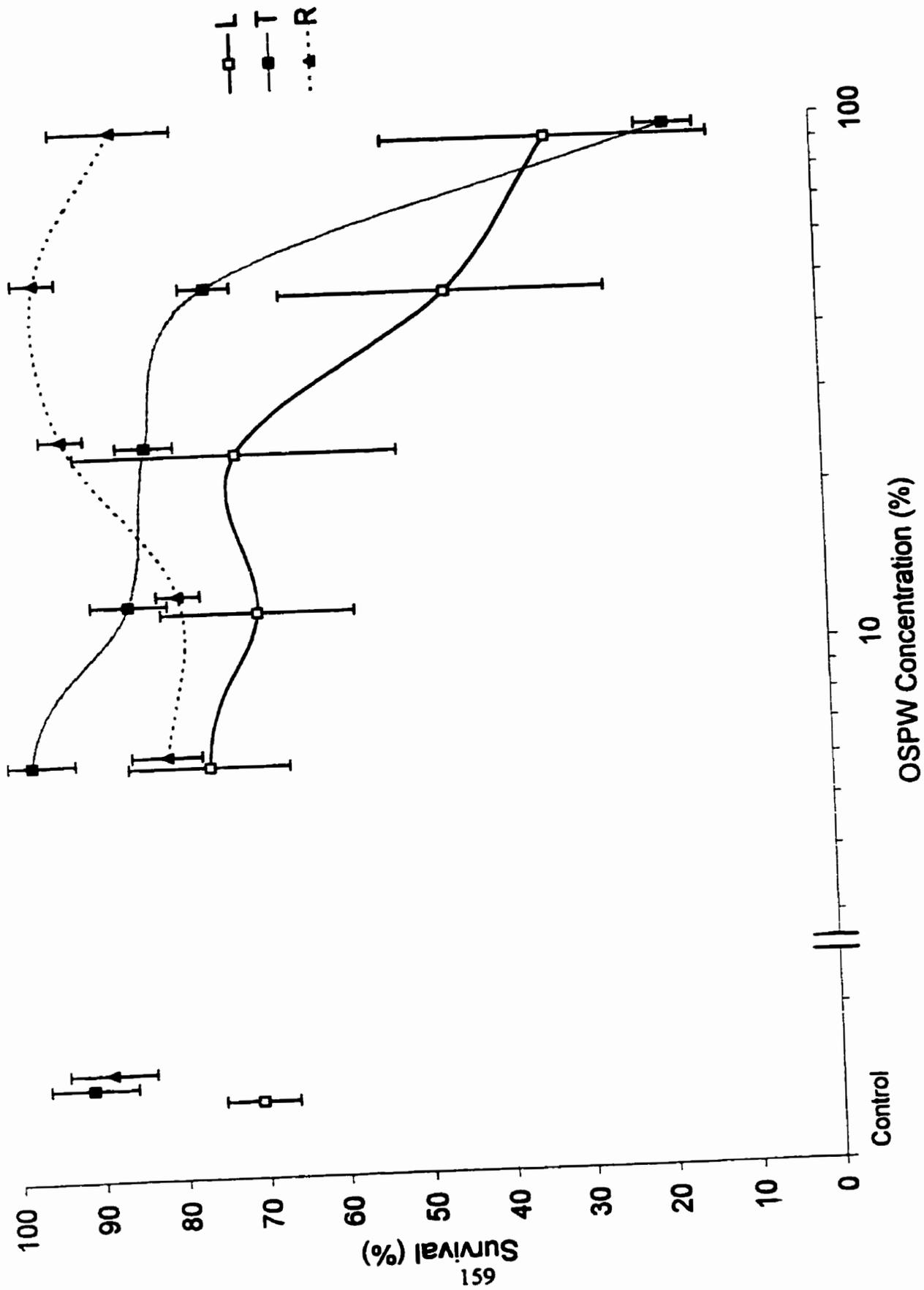


Fig. 5.5: Survival (mean % $\pm$ SE) of chironomid larvae from 3 populations L, T, and R. Number of test jars is 4 (pop L) or 3 (pop R, and pop T except 0% and 6.25% where n=4).

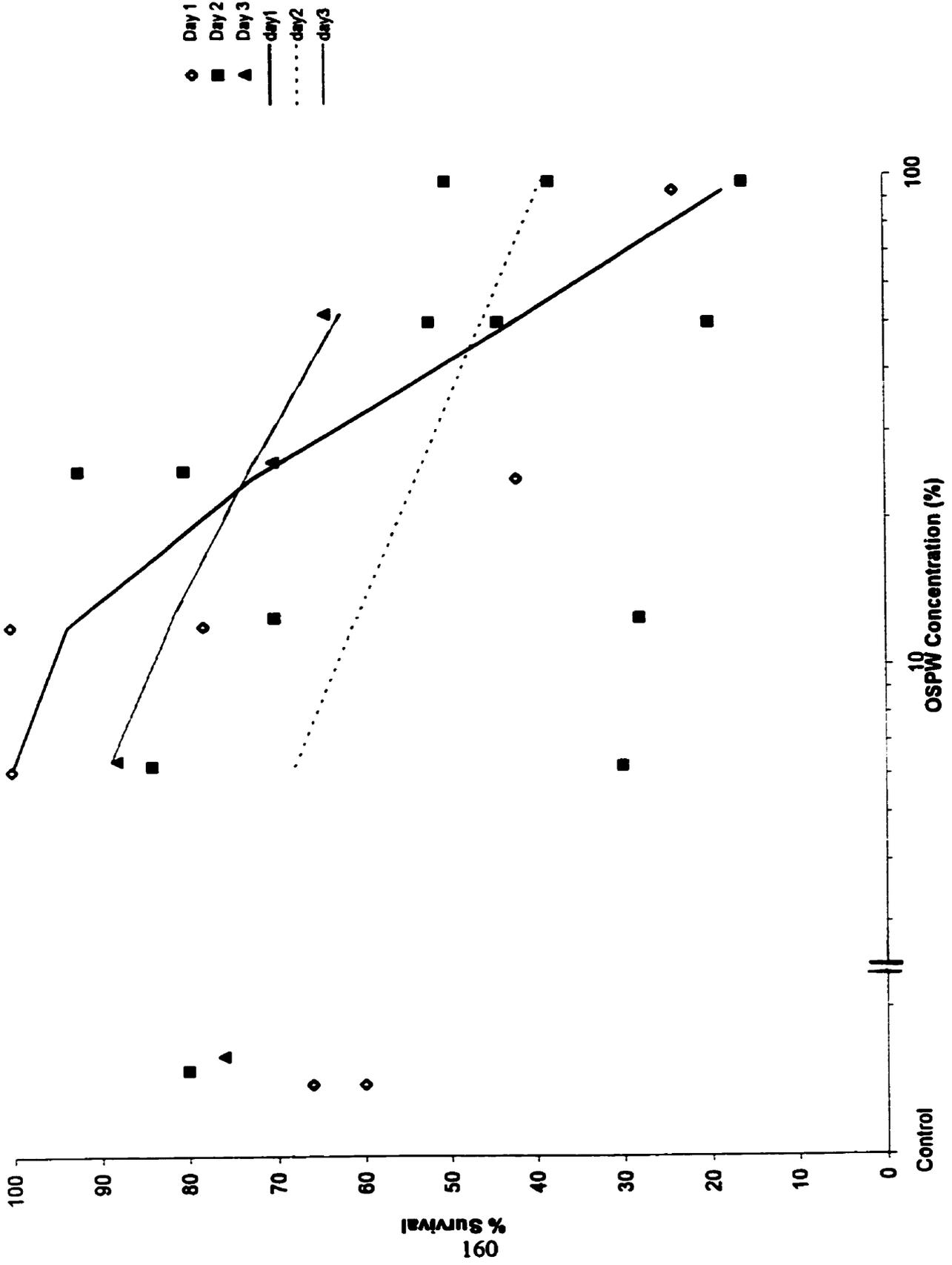


Fig. 5.6: Survival (mean %) of field population L on Days 1, 2, and 3 following exposure to OSPW in a 14-d sediment bioassay. Linear regression lines shown (Day 1  $y=135.64-16.87[\text{OSPW}]$ , day 2  $y=66.68-4.26[\text{OSPW}]$ , day 3  $y=88.55-8.05[\text{OSPW}]$ )

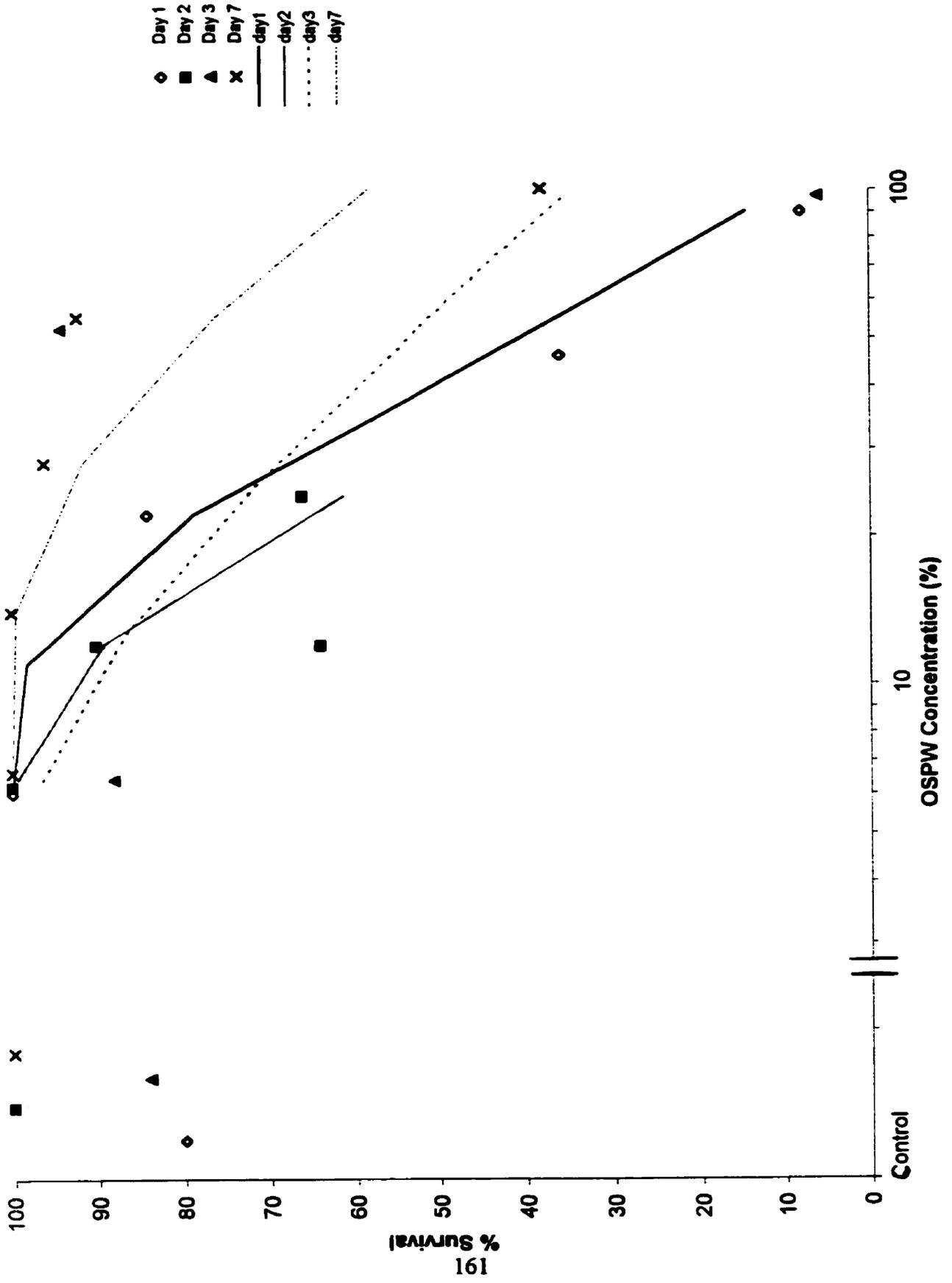


Fig. 5.7: Survival (mean %) of lab population T on days 1, 2, 3, and 7 following 14-d exposure to OSPW. Linear regression lines shown (Day 1  $y=150.2-19.7[\text{OSPW}]$ , day 2  $y=139.6-19.2[\text{OSPW}]$ , day 3  $y=108.5-10.9[\text{OSPW}]$ , day 7  $y=134.2-12.7[\text{OSPW}]$ ).

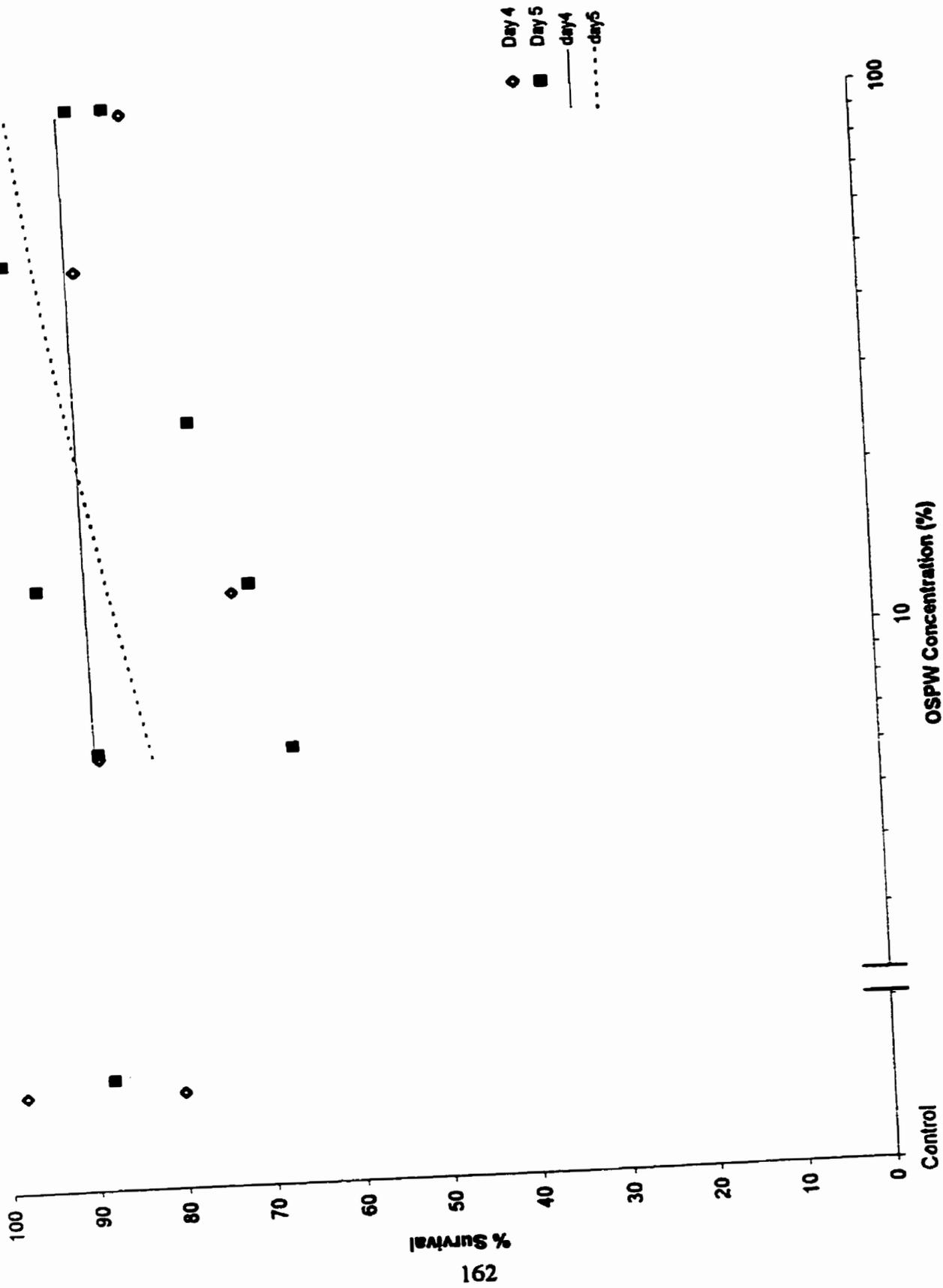


Fig. 5.8: Survival (mean %) of larvae from lab population R on Days 4 and 5 following exposure to OSPW in a 14-d sediment bioassay. Linear regression lines shown (Day 4  $y=69.7+0.19[\text{OSPW}]$ , day 5  $y=58.6+3.1[\text{OSPW}]$ ).

Table 5.1: Multiple regression analyses of survival data for field *C. tentans* (L), lab *C. tentans* (T), and lab *C. riparius* (R). Partial  $R^2$  are summed by day where all components were nonsignificant.

Species	Factor	n	Regr. coef	SE	t	$R^2$
L	intercept	20	93.11	15.37	6.06***	-
	[OSPW]	20	-7.53	2.76	-2.73**	0.27
	sum-days	20	-	-	-	0.03
T	intercept	16	143.43	15.38	9.32***	-
	[OSPW]	16	-14.68	2.93	-5.02***	0.59
	sum-days	16	-	-	-	0.22
R	intercept	15	62.64	13.96	4.49**	-
	[OSPW]	15	1.96	2.54	0.77	0.05
	sum-days	15	-	-	-	0.01

\* :  $p < 0.05$   
 \*\* :  $p < 0.01$   
 \*\*\* :  $p < 0.001$

Table 5.2: Ranges of survival of field *C. tentans* (L), lab *C. tentans* (T), and lab *C. riparius* (R) by day. Number in parentheses were not used in calculations, because max/min. survival proportion was too low. Number of test jars is shown.

DAY	Field <i>C. tentans</i> (L)			Lab <i>C. tentans</i> (T)			Lab <i>C. riparius</i> (R)		
	Low	High	n	Low	High	n	Low	High	n
1	24	100	7	8	100	5			
2	35	86	13	66	100	5	(18)	(45)	(12)
3	64	88	4	6	94	4			
4							72	100	7
5							77	98	11
6				(0)	(14)	(4)			
7				38	100	6			

The within-treatment variability was least for lab population T (Fig. 5.5 and 5.7), which exhibited somewhat of a threshold response: survival declined markedly between 50 and 100 % OSPW.

Survival of field population L was also reduced at 50 % OSPW but the response appeared more linear than the response of the lab population T (Fig. 5.5 and 5.6). Although population T responded more sharply than population L, there was no difference between the slopes of the regression lines (t-test for unequal variance;  $p > 0.10$ ).

Overall, both L and T populations had an LC50 of 71% OSPW (Table 5.3). Mean LC50 values of significant relationships (calculated by day) were 70.3 and 78.5 % OSPW for populations T and L, respectively. Thus the lab population T was slightly more sensitive to OSPW than the field population L, which showed much greater variability among set-up days. LC50 values were not calculable for population R, because survival was uniformly high.

Generally, NOEC and LOEC values were lower for population L than population T. However, variability in the former population was higher than in the latter.

Cases that were excluded due to abnormally low survival include replicate 5 of population L, as well as day 2 and 6 of populations R and T, respectively. The raw data are provided in appendix 5.3 a,b,c.

#### *Survival Analyzed by Day:*

Generally, the results of population T more clearly demonstrated a lethal response than those of the other two populations. Population T clearly exhibited a negative

Table 5.3: Survival LC50, No Observable Effect Concentration (NOEC), and Lowest Observable Effect Concentration (LOEC) values (% OSPW) of two *Chironomus* populations L and T. Population R did not show significant mortality.

Pop - Group	LC50	NOEC	LOEC
L - overall	71	25	50
L - day 1	66	12.5	25
L - day 2	91	25	50
L - day 3	300*	50	-
L - day mean	78.5		
T - overall	71	25	50
T - day 1	49	25	50
T - day 2	30*	6.25	12.5
T - day 3	74	50	100
T - day 7	88	50	100
T - day mean	70.3		

\* extrapolated from weighted regression line on graph

survival-[OSPW] relationship for all 4 set-up days (Fig. 5.7), whereas the relation between survival and [OSPW] of population L was more ambiguous (Fig. 5.6); a strong negative linear trend was observed only on day 1. Survival of population R was always independent of [OSPW].

For the population L, 2 of the 3 days showed direct negative survival-[OSPW] relationships which were both statistically significant but not different from each other (t-test with unequal variances;  $p > 0.20$ ) (Table 5.4). This signified that set-up day did not interact with [OSPW], but that a lethal effect was present. Therefore, although variability between egg masses was significant, the effects of OSPW on survival were consistent among viable cohorts.

A negative survival-[OSPW] relationship was also seen with the days of population T. A negative log-linear relationship (with very similar regression line slope) was observed for the set-up days 1 and 2. On set-up days 3 and 7, a threshold type response ( $> 50\%$  OSPW) was observed, although this was difficult to assess based on the small number of data points for each day.

Survival of population R was independent of [OSPW] for both set-up days (Fig. 5.8). There was high variability among test jars, but no indication of a toxic effect of OSPW at any concentration.

### ***Growth and Development***

Exposure to OSPW water was linked to effects on both growth and development in chironomids. Larval development was slowed but not arrested, as reflected by reduced

Table 5.4: Regression analyses of survival data by day for field *C. tentans* (L), lab *C. tentans* (T), and lab *C. riparius* (R).

Pop	Day	n	B	SE	Intercept	SE (Int)	t	R <sup>2</sup>
L	1	5	-16.87	5.34	135.64	24.19	-3.16*	0.77
L	2	12	-4.26	3.38	66.68	17.41	-1.26	0.14
L	3	3	-6.05	0.82	86.55	3.76	-7.33*	0.98
T	1	4	-19.66	2.55	150.17	13.15	-7.72**	0.97
T	2	4	-19.21	9.01	139.63	34.45	-2.13	0.69
T	3	3	-10.92	13.32	108.52	70.78	0.82	0.40
T	7	5	-12.73	3.61	134.23	17.76	-3.53*	0.81
R	4	5	0.16	4.57	69.72	22.46	0.04	<0.01
R	5	10	3.10	2.92	56.55	14.36	1.06	0.12

\* : p < 0.05

\*\* : p < 0.01

\*\*\* : p < 0.001

body lengths for the field and particularly the lab *C. tentans* populations, and by slowed developmental rates (extended time to emergence) for *C. riparius* at 100% OSPW (Fig. 5.9 and 5.13).

### **I – Body Lengths (Growth)**

The lab *C. tentans* population T was largest (control mean = 18.0+/- mm), followed by the field *C. tentans* population L (control = 15.9+/- mm), and *C. riparius* had the smallest mean body lengths (control = 12.9+/- mm).

Mean body length of chironomid larvae varied significantly among populations, treatments, and their interaction terms (multiple linear regression;  $p=0.015$ ,  $p<0.001$ ,  $p<0.001$ ). Therefore, analysis was performed for each population separately. The set-up day did not significantly affect mean length for any of the populations (Table 5.5, 5.6). The raw data are provided in appendix 5.4 a,b,c.

When data were analyzed by population, body length was significantly affected by [OSPW] for both *C. tentans* populations but not that of *C. riparius* (Table 5.5). Body length was reduced above a threshold [OSPW], decreasing above 25 and 50% OSPW water for *C. tentans* populations T and L, respectively (Fig. 5.9). Body lengths of population T reared in 100% OSPW were 40% as long as control larvae. Larvae of population L reared in 100% OSPW were 75% as long as larvae reared in control water. Lengths of larvae from population R varied little with respect to exposure to OSPW water. However, the larvae reared in water containing  $\geq 25\%$  OSPW were approximately 10% longer than larvae in control or more dilute water (Fig. 5.9).

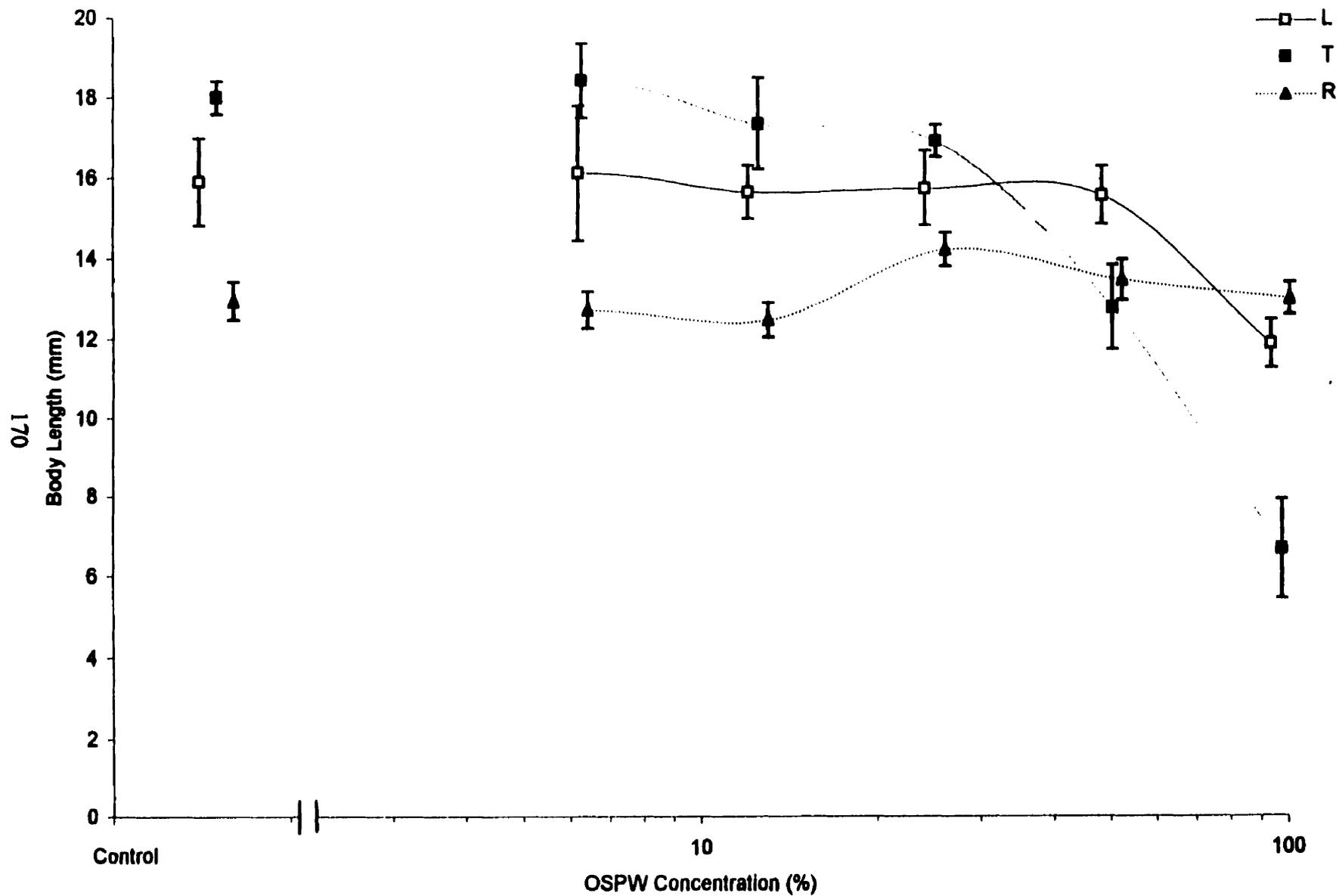


Fig. 5.9: Body Length (mean  $\pm$  SE%) of larvae from 3 populations L, T, and R. Number of test jars is 5 (pop L and R), 4 (pop T 0%, 6.25%, 12.5% OSPW), or 3 (pop T 25%, 50%, 100% OSPW).

Table 5.5: Multiple regression analyses of body length data for field *C. tentans* (L (n=25)), lab *C. tentans* (T (n=17)), and lab *C. riparius* (R (n=25)). Partial R<sup>2</sup> are summed by day where all components were nonsignificant.

Species	Factor	Regr. coef	SE	t	R <sup>2</sup>
L	intercept	21.33	1.65	12.91***	-
	[OSPW]	-1.06	0.30	-3.49**	0.30
	sum-days	-	-	-	0.13
T	intercept	27.93	2.78	10.03***	-
	[OSPW]	-2.65	0.55	-4.79***	0.57
	sum-days	-	-	-	0.04
R	intercept	12.94	0.80	16.16***	-
	[OSPW]	0.08	0.54	0.50	0.01
	sum-days	-	-	-	0.07

\* : p < 0.05  
 \*\* : p < 0.01  
 \*\*\* : p < 0.001

Table 5.6: Range of mean larval body lengths of field *C. tentans* (L), lab *C. tentans* (T), and lab *C. riparius* (R) by Day. Each value represents the arithmetic mean length of all larvae in a single container. Number of test jars is shown.

DAY	Field <i>C. tentans</i> (L)			Lab <i>C. tentans</i> (T)			Lab <i>C. riparius</i> (R)		
	Low	High	n	Low	High	n	Low	High	n
1	10.46	17.32	11	5.05	17.72	5			
2	10.69	21.17	14	17.65	19.61	5	11.11	15.40	12
3	15.72	17.93	5	5.82	20.12	4			
4							11.89	13.86	7
5							12.27	14.53	11
6				16.79		1			
7				9.08	18.99	6			

Overall EC50s for populations T and L were 75 and 200% OSPW, respectively (Table 5.7). Mean EC50 values of significant relationships (by set-up day) were lower and less variable for population T (66% OSPW) than for population L (787% OSPW). As population R showed no significant decrease in body length in any treatments, no EC50 values were calculated.

*Growth Analysed by Day:*

There was considerable variation in body length among different set-up days for populations T and L (Table 5.6). There were no significant day x treatment interactions for any of the populations, indicating that the effect of OSPW was consistent among days within populations. Generally, only larvae from population T (days) showed a consistent reduction in body length with increased OSPW concentration (Fig. 5.10, 5.11, 5.12). The reduction in length of larvae in population L was much less marked, and seemed to occur at concentrations  $\geq 50\%$  OSPW (Fig. 5.10). Mean length of population R larvae covered from treatments with high [OSPW] tended to be larger than controls (Fig. 5.9, 5.12).

For the population L, on 2 of the 3 days there was no relationship between exposure and growth. On the remaining day, statistically significant linearly decreasing response was observed (Table 5.8, Fig. 5.10).

A negative linear dose response was observed on 3 of 4 days for population T, and the other day exhibited a threshold response after 25 % OSPW water (Fig. 5.11).

Body length of Population R was not reduced by exposure to the OSPW water (Table 5.8). Instead, on two set-up days, larvae grown in 25% OSPW were larger than all

**Table 5.7: Growth Effect Concentration (EC50), No Observable Effect Concentration (NOEC), and Lowest Observable Effect Concentration (LOEC) values (% OSPW) of two *Chironomus* populations L and T. Population R did not show reduced growth.**

<b>Pop - Group</b>	<b>EC50</b>	<b>NOEC</b>	<b>LOEC</b>
L – overall	200	50	100
L – day 1	8000*	-	-
L – day 2	787	100	-
L – day 3	-	50	-
L - day mean	787		
T – overall	75	25	50
T – day 1	57	25	50
T – day 2	7300*	-	-
T – day 3	75	50	100
T – day 7	160*	25	50
T - day mean	66		

\* extrapolated from regression line on graph and not used in calculating day mean.

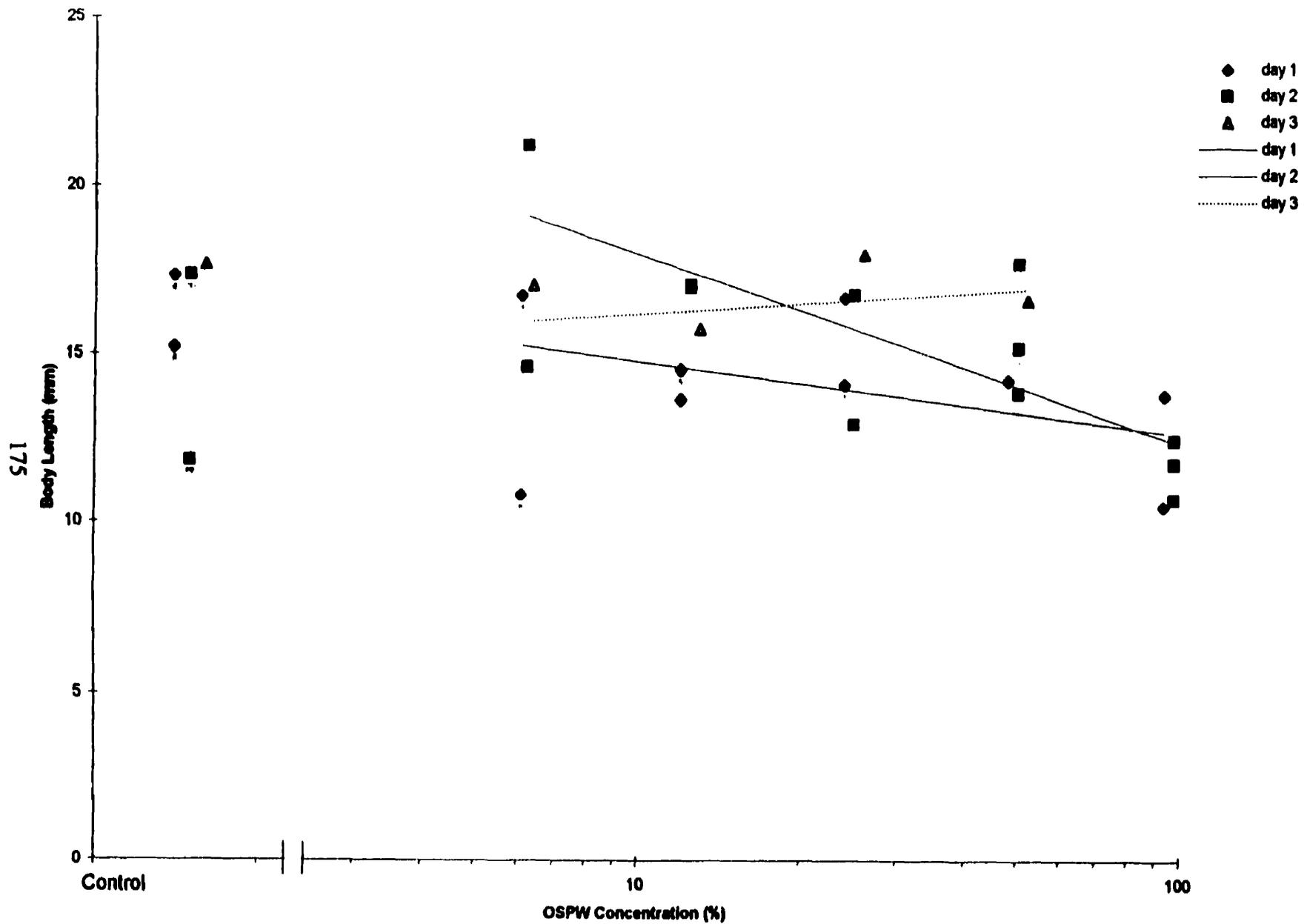


Fig. 5.10: Body Length (mean %) of field population L on Days 1, 2, and 3 following a 14-d exposure to OSPW. Linear regression lines shown (Day 1  $y=16.9-0.7[OSPW]$ , day 2  $y=23.6-1.7[OSPW]$ , day 3  $y=15.2+0.3[OSPW]$ )Page 1

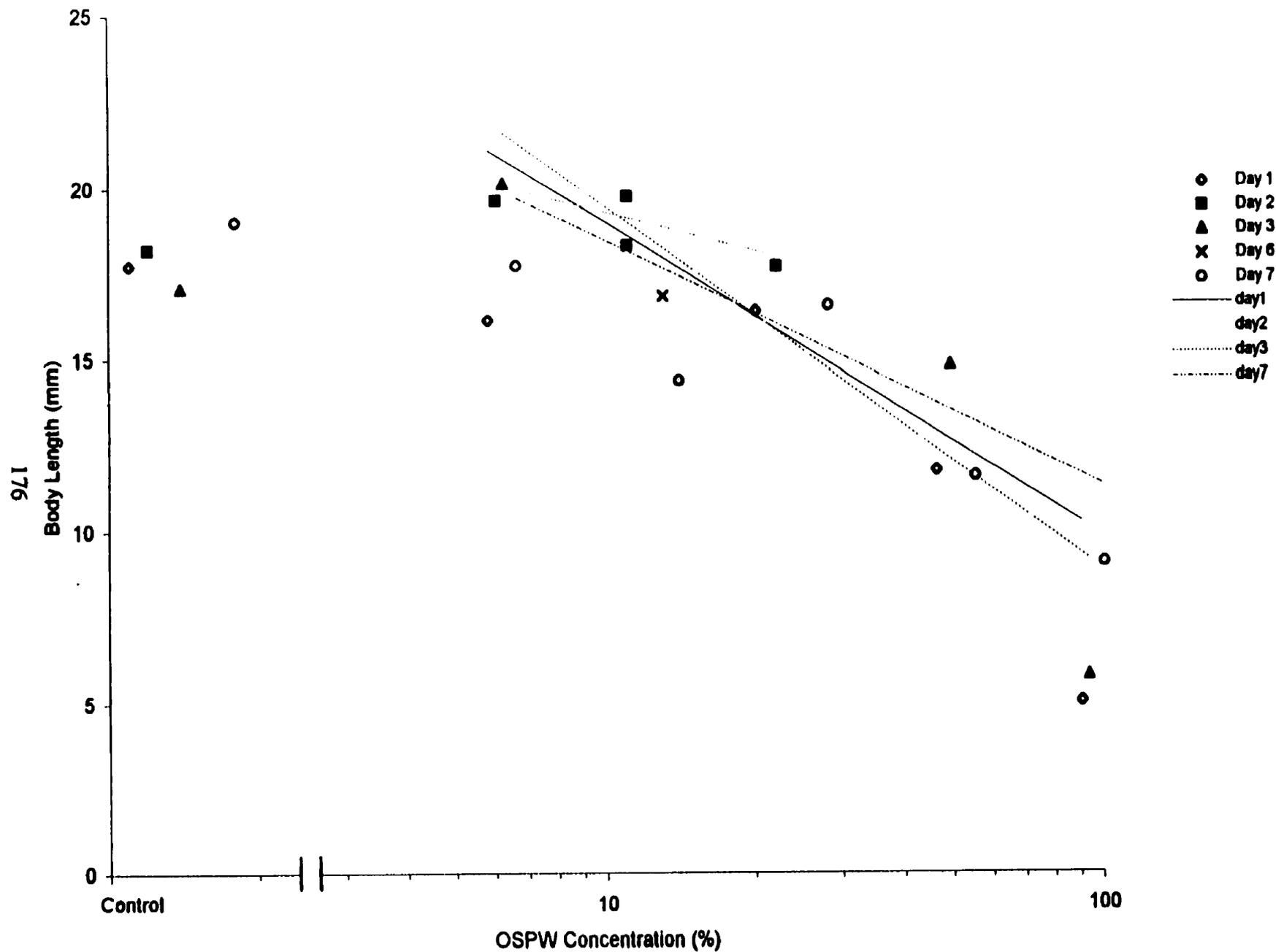


Fig. 5.11: Body Length (mean %) of lab population T on Days 1, 2, 3 and 7 following a 14-d exposure to OSPW. Linear regression lines shown (Day 1  $y=28.1-2.7[\text{OSPW}]$ , day 2  $y=23.0-1.1[\text{OSPW}]$ , day 3  $y=30.0-3.2[\text{OSPW}]$ , day 7  $y=25.5-2.1[\text{OSPW}]$ ).

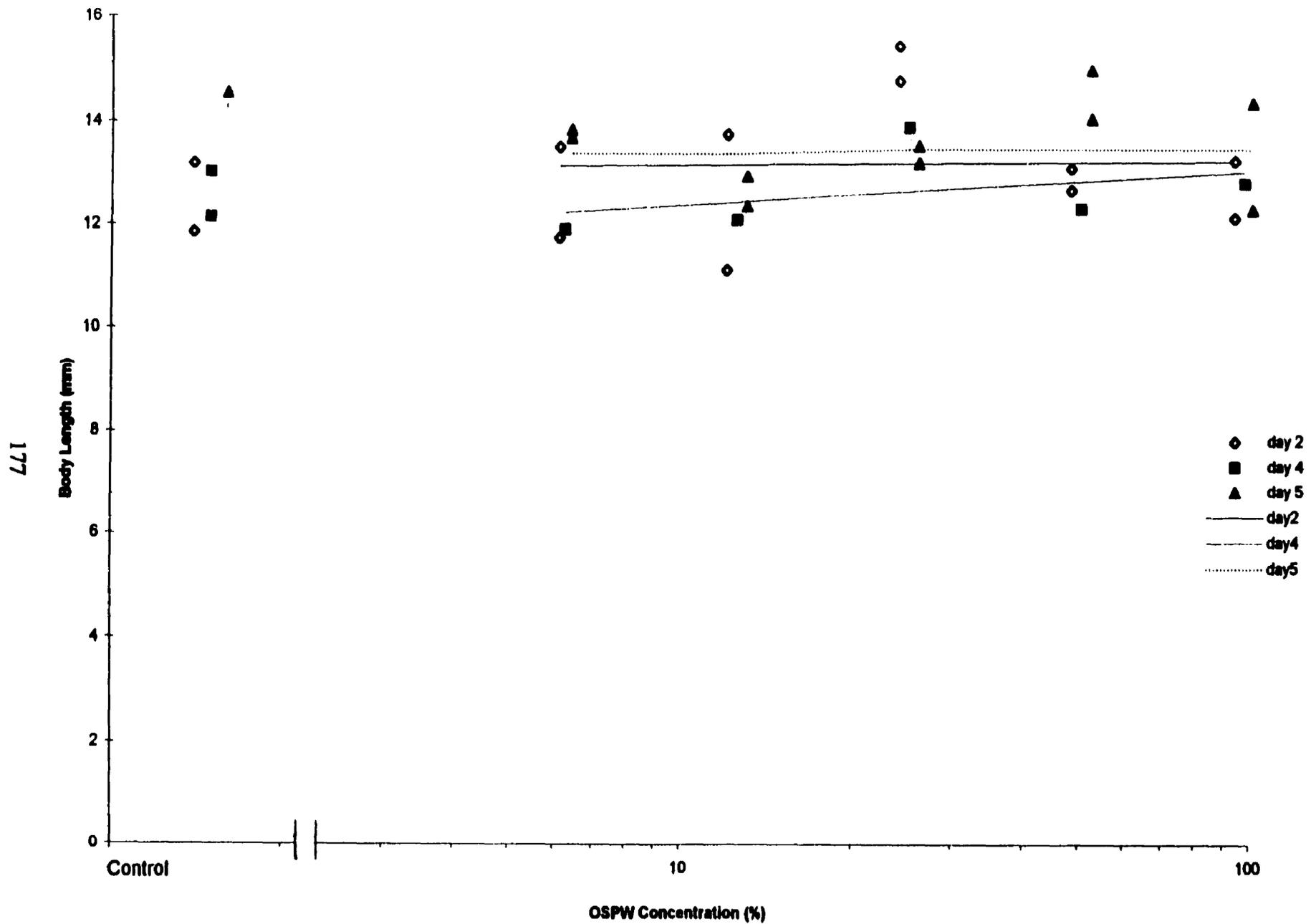


Fig. 5.12: Body length (mean %) of lab population R on days 2, 4, and 5 following a 14-d exposure to OSPW. Linear regression lines shown (Day 2  $y=13.1+0.02[OSPW]$ , day 4  $y=11.7+0.2[OSPW]$ , day 5  $y=13.1+0.08[OSPW]$ ).

Table 5.8: Multiple regression analyses of body length data by Day for field *C. tentans* (L), lab *C. tentans* (T), and lab *C. riparius* (R).

Pop	Day	n	B	SE	Intercept	SE	t	R <sup>2</sup>
L	1	9	-0.65	0.44	16.94	2.36	-1.47	0.24
L	2	12	-1.69	0.42	23.57	2.33	-4.03**	0.62
L	3	4	0.30	0.59	15.17	2.75	0.50	0.11
T	1	4	-2.74	1.75	28.05	8.46	-1.57	0.55
T	2	4	-1.14	0.34	23.04	1.49	-3.38*	0.85
T	3	3	-3.18	1.49	29.97	7.73	-2.13	0.82
T	7	5	-2.13	1.01	25.49	4.81	-2.12	0.60
R	2	10	0.02	0.33	13.09	1.67	0.06	0.00
R	4	5	0.20	0.29	11.70	1.44	0.69	0.14
R	5	10	0.08	0.21	13.08	1.03	0.38	0.02

\* : p < 0.05  
 \*\* : p < 0.01  
 \*\*\* : p < 0.001

others (Fig. 5.12). In trials begun on the third set-up day, larvae from the 50% treatment were the largest, suggesting hormesis or delayed emergence of susceptible individuals (Fig. 5.11), although this was difficult to assess based on the limited number of data points for each day.

## **II – Emergence (Development)**

As emergence was observed in some test jars, the proportion of larvae/survivors was used as a measure of developmental inhibition of larvae in relation to OSPW exposure. Overall, the proportion of survivors that were larvae depended on population and [OSPW] (multiple linear regression;  $p < 0.001$  and  $p < 0.01$ , respectively). Therefore, analysis was done for each population separately. No emergence was observed in population L, and only 4 of 17 jars exhibited emergence for population T (Fig. 5.13). However, significant emergence was observed in population R, tapering off at the higher treatments (Table 5.9). This population demonstrated a threshold response in that a significantly increasing proportion of survivors were larvae in treatments with  $> 25\%$  OSPW water (Fig. 5.13). The EC50 values related to a doubling of % larvae of all survivors (indicating a 50% reduction in living pupae and adults) calculated indicated that the effect of OSPW on the emergence rate of *C. riparius* would be seen mainly at higher concentrations of OSPW (Table 5.10). Overall, the EC50 was 64% OSPW, close to the mean EC50 based on set-up days of 72% OSPW.

In support of the observed relationship of increased proportion of *C. riparius* survivors that are larvae with increased [OSPW], the % pupae of all pupae and adults in

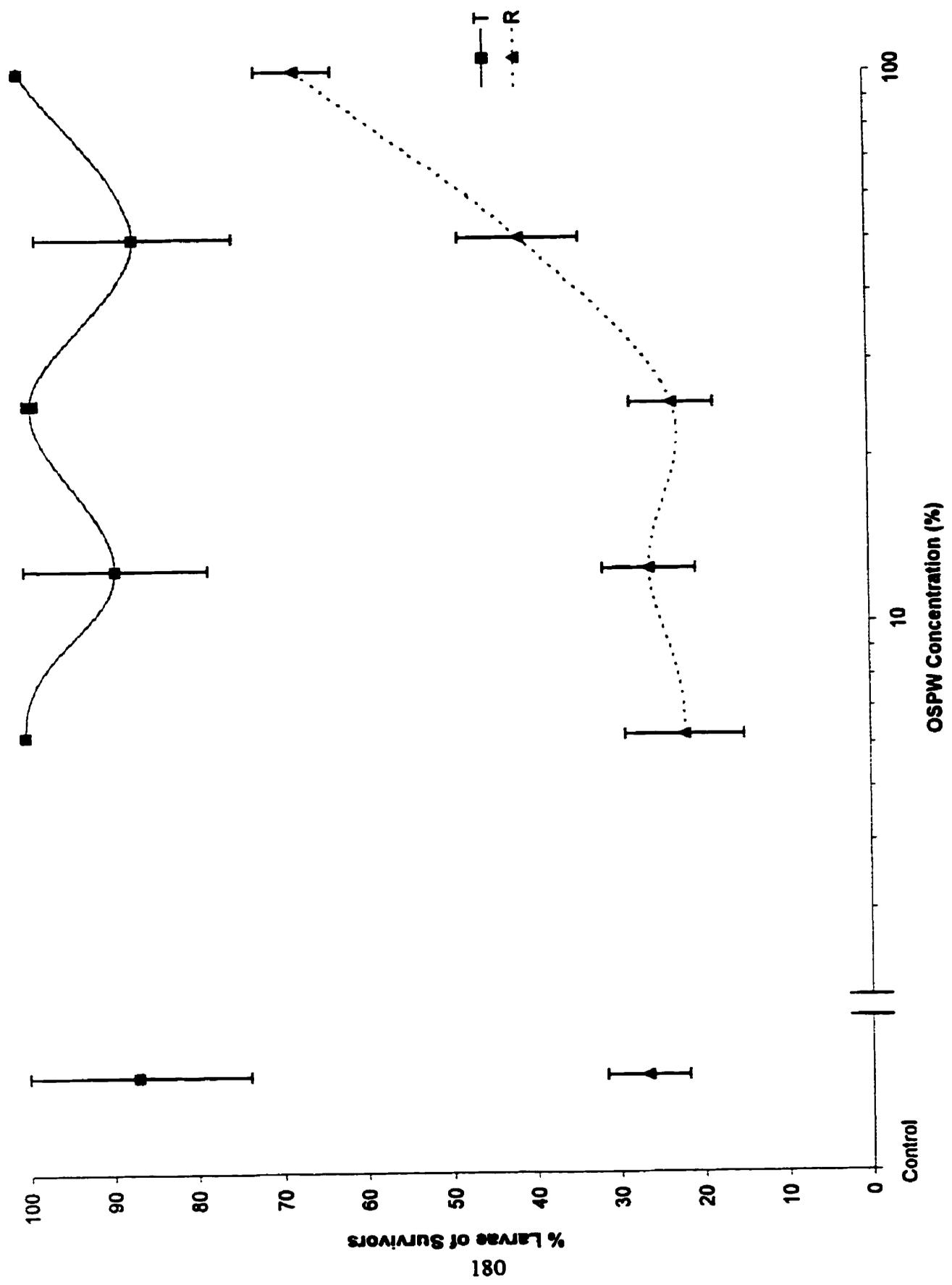


Fig. 5.13: Mean % larvae (+/-SE) of all survivors (larvae, pupae, adults) from lab populations T and R. Number of test jars is 5 (pop R), 4 (pop T 0%, 6.25%, 12.5% OSPW), or 3 (pop T 25%, 50%, 100% OSPW).

Table 5.9: Multiple regression analyses of % larvae of survivors data for lab *C. tentans* (T (n=17)) and lab *C. riparius* (R (n=25)). Partial R<sup>2</sup> are summed by day where all components were nonsignificant.

Pop	Factor	Regr. coef	SE	t	R <sup>2</sup>
T	intercept	102.87	12.62	8.15***	-
	[OSPW]	-2.89	2.66	-1.09	0.06
	sum-days	-	-	-	0.37
R	intercept	5.31	7.43	0.71	-
	[OSPW]	7.40	1.42	5.21***	0.53
	sum-days	-	-	-	0.09

\* : p < 0.05  
 \*\* : p < 0.01  
 \*\*\* : p < 0.001

**Table 5.10: Effects concentrations to double % larvae of survivors and % pupae of pupae and adults. Developmental delay Effect Concentration (EC50), No Observable Effect Concentration (NOEC), and Lowest Observable Effect Concentration (LOEC) values (% OSPW) of *Chironomus riparius*. Population T did not show significant emergence in any treatments.**

Group	EC50	NOEC	LOEC
larval-overall	64	25	50
larval-day 2	49	25	50
larval-day 4	81	50	100
larval-day 5	86	50	100
larval-day mean	72		
pupal-overall	120	50	100

each jar also increased with increasing [OSPW] (Table 5.11, Fig. 5.14). The proportion of pupae and adults that remained in the pupal stage was significantly higher in the 100% OSPW treatment compared to controls (one-way ANOVA;  $p < 0.05$ ). The overall EC50 for pupal development was 120% OSPW, higher than that calculated for larval development. Therefore, the highest treatment induced a higher rate of developmental inhibition in both larvae and pupae, but the larval life stage appeared more sensitive to OSPW than the pupae.

#### *Emergence Analysis by Day:*

No days of population T showed a significant relationship to exposure (Fig 5.15, Table 5.12).

For population R, analysis of the proportion of larvae/survivors by day revealed a strong positive trend with increasing concentration of OSPW (Fig. 5.16). The most highly significant relationships were related to a threshold response (days 2 and 5), whereas a weak linear response was also observed on day 4 (Table 5.11).

Although all three set-up days showed a positive linear relationship between [OSPW] and pupal development, none were statistically significant (Table 5.13).

#### *Deformities*

Incidence of chironomid mouthpart deformities (extra or missing teeth) was used as a teratogenicity biomarker. The incidences observed in this bioassay were generally at or below those observed at relatively pristine sites (Cushman 1984, Dermott 1991, Burt

Table 5.11: Multiple regression analysis of % pupae of (pupae and adults) data for lab *C. riparius* (R). Partial R<sup>2</sup> are summed by day where all components were nonsignificant.

Factor	n	Regr. coef	SE	t	R <sup>2</sup>
intercept	25	26.19	10.56	2.48*	-
[OSPW]	25	4.64	2.02	2.30*	0.19
sum-days	25	-	-	-	0.07

\* : p < 0.05  
 \*\* : p < 0.01  
 \*\*\* : p < 0.001

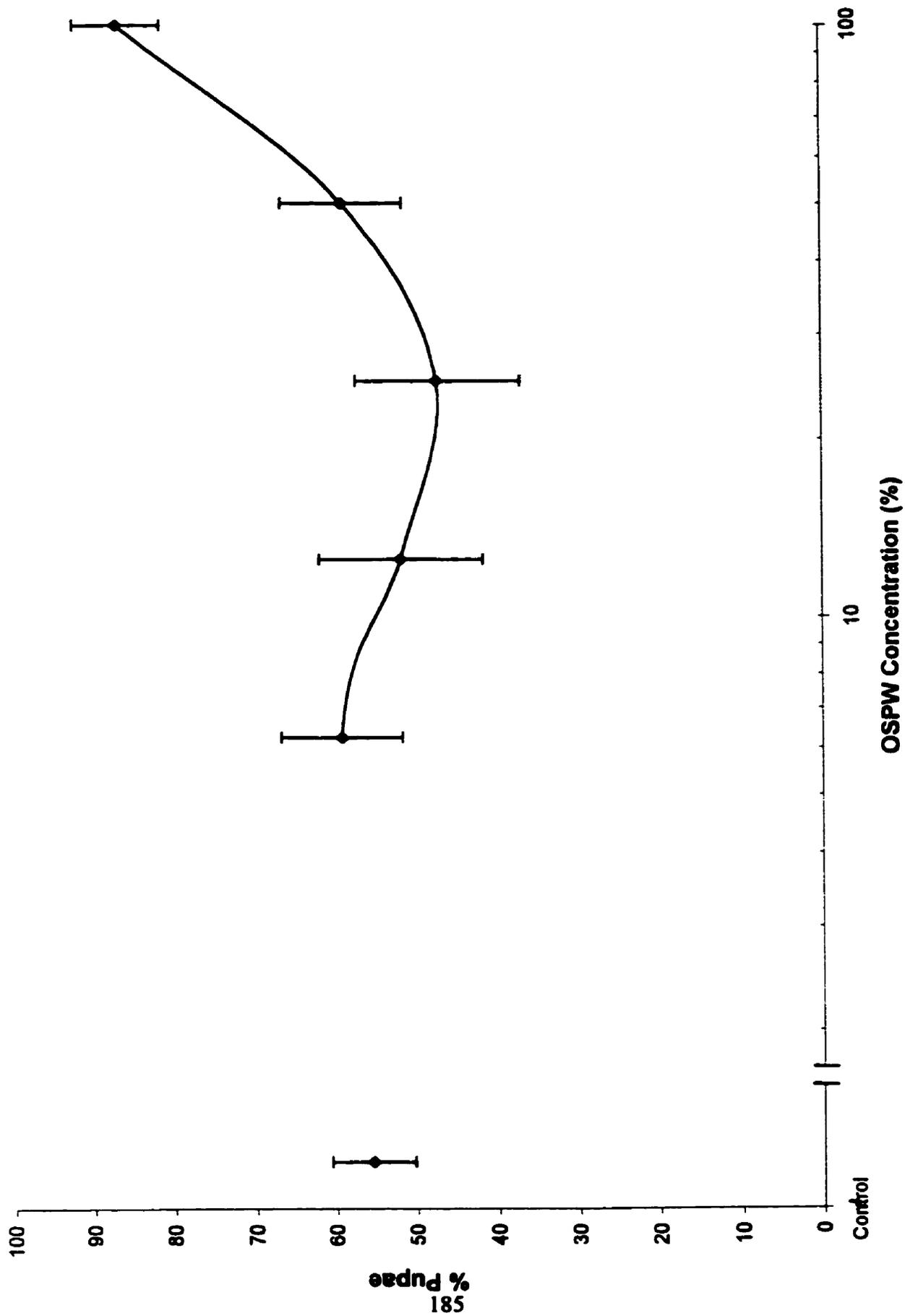


Fig. 5.14: Mean proportion (%  $\pm$  SE;  $n=5$ ) of pupae/(pupae+adults) for *C. riparius* population R following a 14-d sediment bioassay with exposure to OSPW.

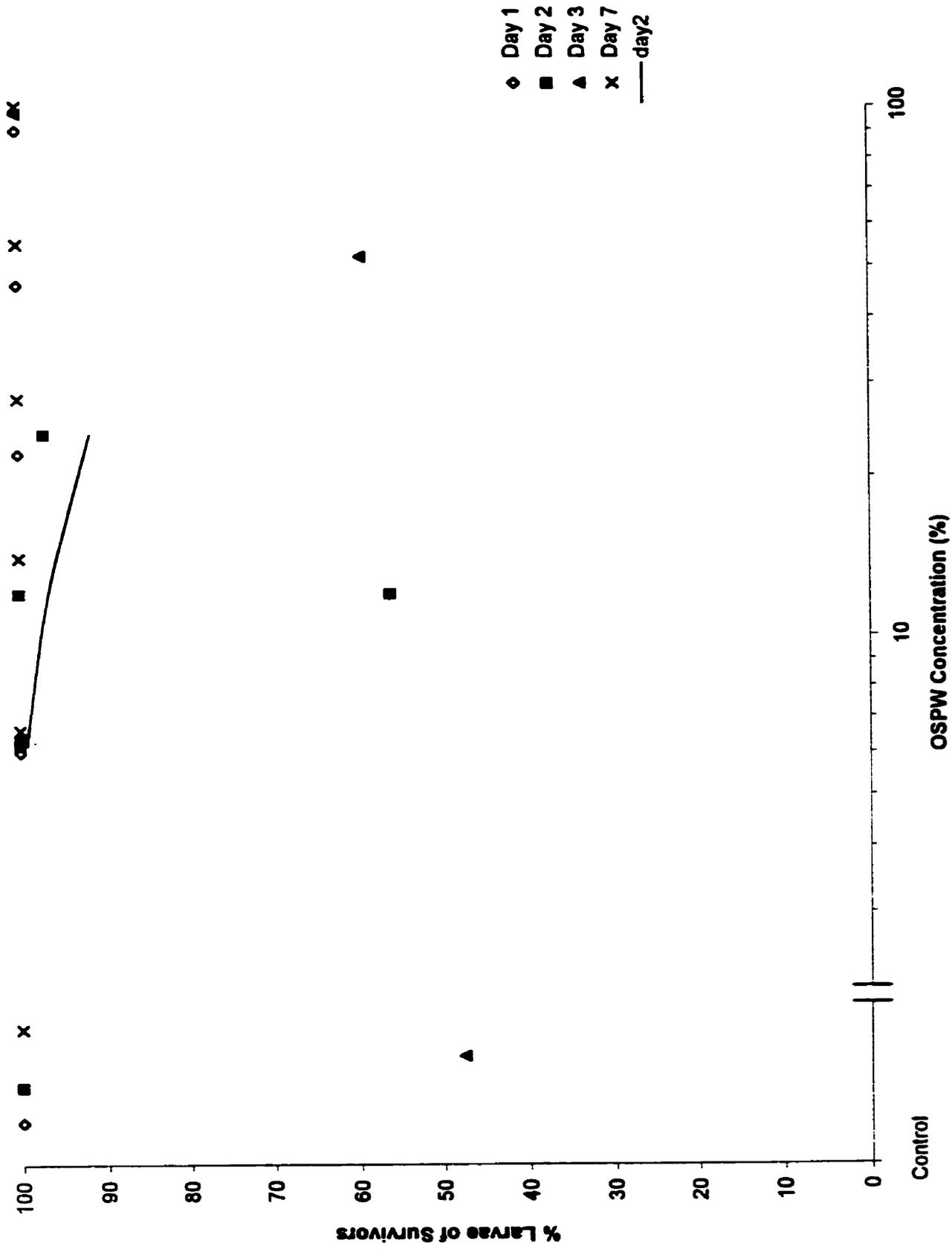


Fig. 5.15: Mean % larvae of all survivors from population T following exposure to OSPW in a 14-d sediment bioassay. Linear regression line shown for day 2:  $y=100.8-6.0[OSPW]$ .

Table 5.12: Regression analyses of % larvae of survivors data by day for lab *C. tentans* (T) and lab *C. riparius* (R). Proportions are arcsine transformed and weighted by the square-root of the number of larvae per jar. Numbers of jars per set-up day are given.

Pop	Day	n	B	SE of B	Intercept	SE (Int)	t	R <sup>2</sup>
T	2	4	-6.02	17.36	100.81	65.55	-0.35	0.06
R	2	10	10.33	2.09	-15.02	10.54	4.94***	0.75
R	4	5	4.58	3.85	10.33	18.93	1.19	0.32
R	5	10	5.65	2.03	13.64	10.05	2.79*	0.49

\* : p < 0.05

\*\* : p < 0.01

\*\*\* : p < 0.001

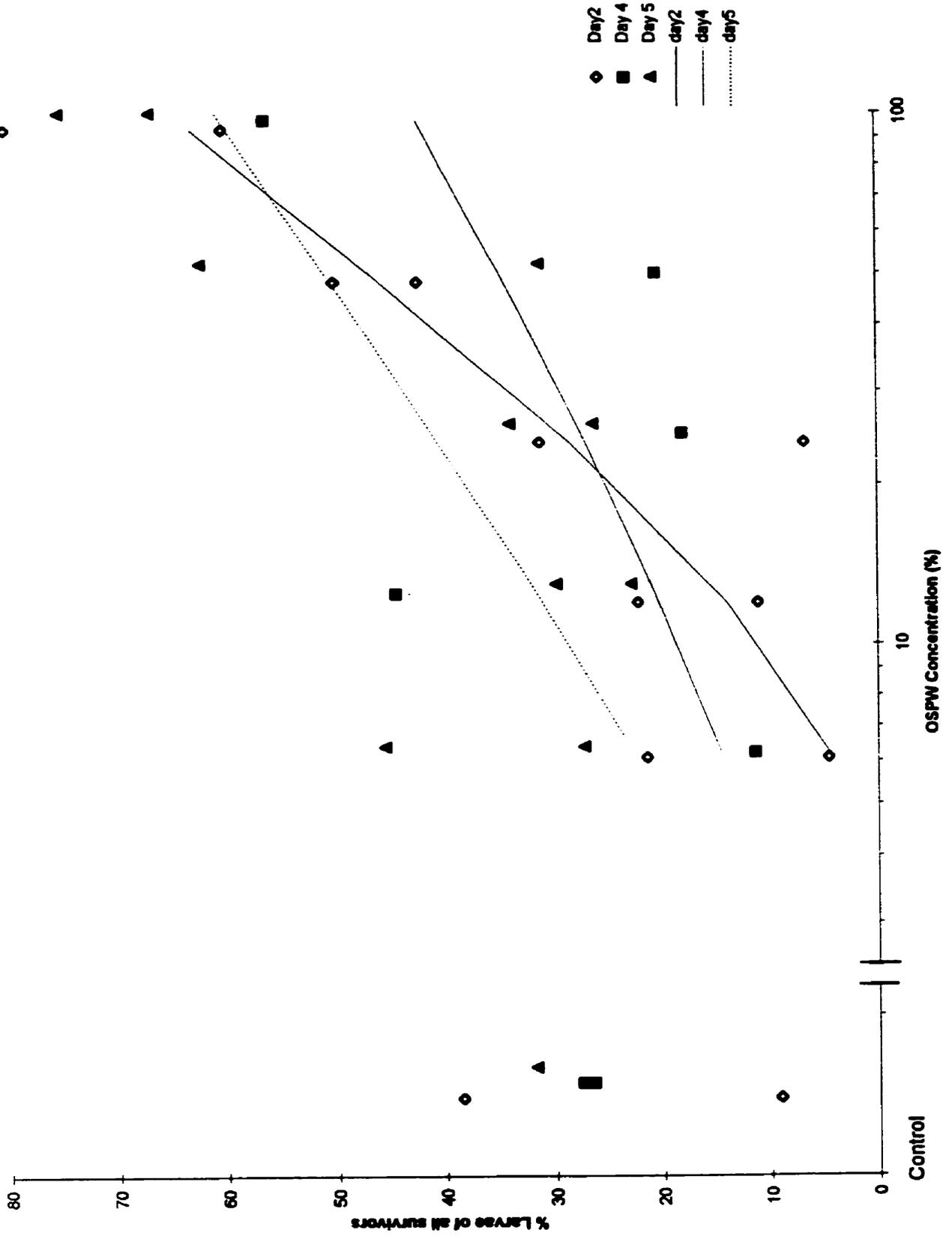


Fig. 5.16: Mean % larvae of all survivors from population R following exposure to OSPW in a 14-d sediment bioassay. Linear regression lines shown (Day 2  $y = -15.0 + 10.3[\text{OSPW}]$ , day 4  $y = 10.3 + 4.6[\text{OSPW}]$ , day 5  $y = 13.6 + 5.7[\text{OSPW}]$ ).

Table 5.13: Regression analyses of % pupae of pupae and adults data by day for lab *C. riparius* (R). Proportions are arcsine transformed and weighted by the square-root of the number of larvae per jar. Numbers of jars per set-up day are given.

Day	n	B	SE(B)	Intercept	SE (Int)	t	R <sup>2</sup>
2	10	3.75	3.22	38.89	16.21	1.16	0.15
4	5	4.97	5.43	27.65	26.72	0.92	0.22
5	10	5.43	3.27	22.41	16.19	1.66	0.26

\* : p < 0.05  
 \*\* : p < 0.01  
 \*\*\* : p < 0.001

1998). Control values were low for lab populations T ( $3.7 \pm 0.8\%$ ) and R ( $3.3 \pm 2.4\%$ ), but were higher and more variable in population L ( $7.5 \pm 4.7\%$ ). Incidences ranged from 1.1 to 7.5% (1.1 to 3.6% if the control group is excluded), 1.8 to 5.2%, and 1.8 to 12.7% (1.8 to 4.6% if the 12.5% treatment is excluded) for field *C. tentans*, lab *C. tentans*, and (lab) *C. riparius*, respectively (Fig. 5.17). These ranges all extend higher than incidences observed in field-collected *Chironomus* ( $< \text{ or } = 2.8\%$ ) or *Derotanypus* ( $< \text{ or } = 2.8\%$ ) (Chapter 3), as seen in previous bioassays at the University of Windsor (Cervi 1996, Burt 1998).

No significantly increased incidences were observed in relation to OSPW exposure either among or within populations (multiple linear regression;  $p=0.06$ ). Population, replicate, and set-up day did not affect the response (multiple linear regression;  $p=0.31$ ) (Table 5.14). Overall, there was no effect of OSPW on the incidence of mentum deformities ( $R^2= 0.05$ ).

The single jar from Day 6 of population R was excluded from analyses as an outlier (one larvae deformed of 7 head capsules; 14.3% deformed).

There was no relationship between [OSPW] and incidence of deformities in larvae from populations L and T. In population R, there was an elevated incidence at 12.5% OSPW compared to all other treatments (Fig. 5.17). This peak was based on a small sample size (4 deformed out of 41 head capsules) from a total of 5 jars, and had a high degree of within-treatment variability (Fig. 5.17). Generally, estimates for population R were based on much smaller sample sizes (head capsules per jar) than either the lab or field *C. tentans*, except at the 100% OSPW treatment, where survival was reduced had

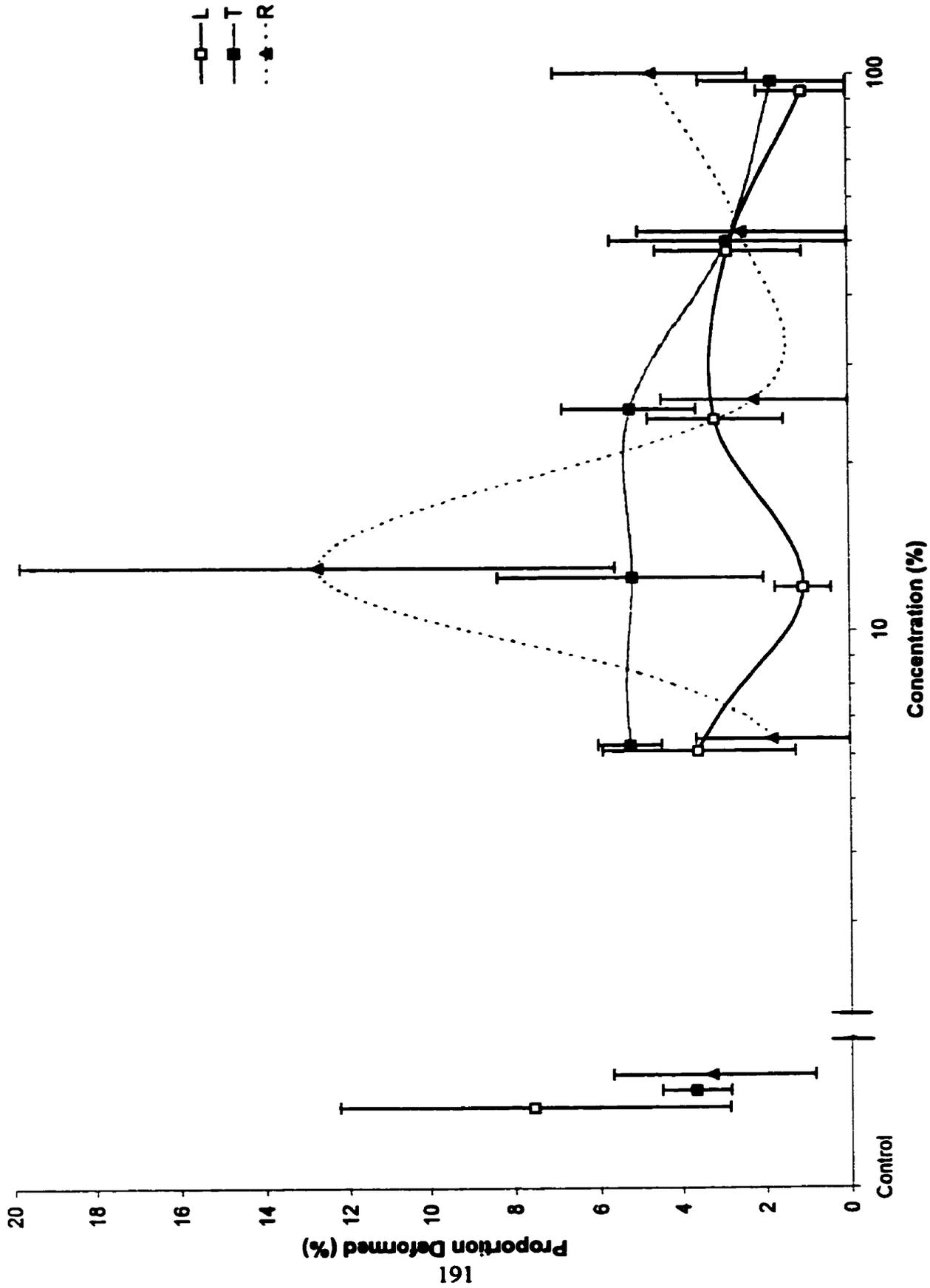


Fig. 5.17: Mean Incidence of Mouthpart Deformities (% +/- SE) in 3 *Chironomus* populations exposed to OSPW water.

Table 5.14: Multiple regression analyses of deformities data for field *C. tentans* (L (n=25)), lab *C. tentans* (T (n=17)), and lab *C. riparius* (R (n=25)). Partial R<sup>2</sup> are summed by day where all components were nonsignificant.

Species	Factor	Regr. coef	SE	t	R <sup>2</sup>
L	intercept	10.17	5.95	1.71	-
	[OSPW]	-0.57	1.14	-0.50	0.01
	sum-days	-	-	-	0.02
T	intercept	20.79	6.36	3.27**	-
	[OSPW]	-1.99	1.26	-1.57	0.13
	sum-days	-	-	-	0.23
R	intercept	9.10	3.46	2.63	-
	[OSPW]	-0.09	1.64	-0.06	<0.001
	sum-days	-	-	-	0.02

\* : p < 0.05

\*\* : p < 0.01

\*\*\* : p < 0.001

occurred in the latter two populations (Fig. 5.17).

#### *Deformities Analysis by Day:*

Populations L and R showed higher variability in the mean proportion of deformed larvae among set-up days, compared to population T (Table 5.15). However, higher incidences were often based on small sample sizes, particularly with population R (Fig. 5.18, 5.19, and 5.20).

Population L showed different responses on different days. The proportion of deformed larvae decreased with [OSPW] on day 1 but increasing on day 3. There was no pattern among day 2 larvae (Fig. 5.18).

The results of the population T, analyzed by set-up day, indicated no relationship between the incidence of mouthpart deformities and the concentration of OSPW (Table 5.15). In fact, for day 1 larvae, there were progressively fewer deformities in treatments with increasing [OSPW] (Fig. 5.19).

The only significant relationship observed between incidence of deformities and [OSPW] occurred with population R larvae on day 2 (Table 5.16). But in contrast, data for days 4 and 5 showed no consistent pattern, with elevated values at 12.5% OSPW, but no significant relationship between deformities and OSPW exposure (Fig. 5.20).

#### **Summary of Toxicological Results**

There were several differences in observed responses between the two lab populations and the field population, and also between the lab populations (Table 5.17).

Table 5.15: Ranges of deformity incidences of field *C. tentans* (L), lab *C. tentans* (T), and lab *C. riparius* (R) by Day. Number of test jars is shown.

DAY	Field <i>C. tentans</i> (L)			Lab <i>C. tentans</i> (T)			Lab <i>C. riparius</i> (R)		
	Low	High	n	Low	High	n	Low	High	n
1	0	12.50	11	0	7.50	5			
2	0	25.00	14	0	6.25	5	0	12.50	12
3	0	8.57	5	0	5.00	4			
4							0	18.75	7
5							0	11.11	11
6					(14.29)	1			
7				1.96	8.51	6			

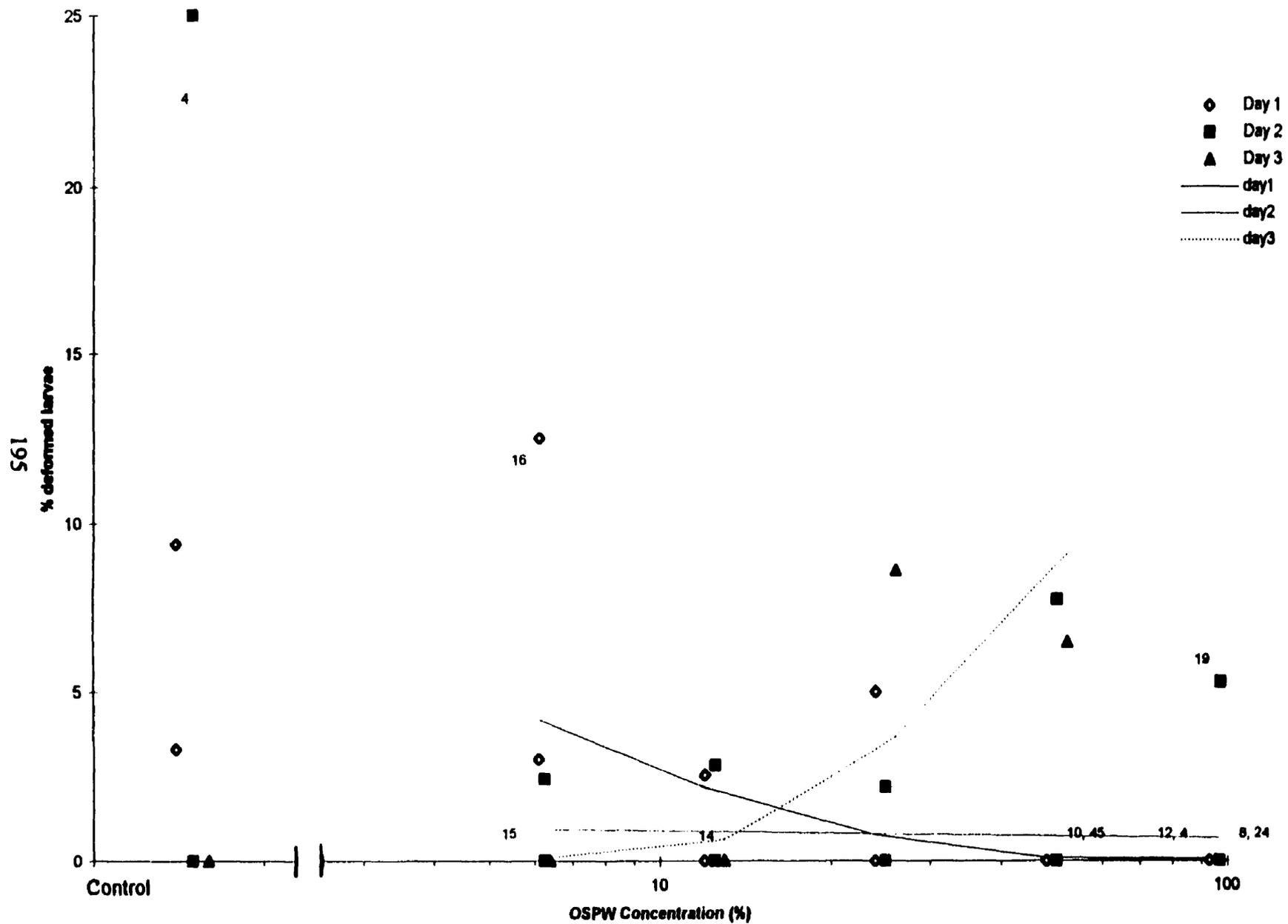


Fig. 5.18: Mentum deformities (%; # heads shown) of chironomids from population L following exposure to OSPW in a 14-d sediment bioassay. Linear regression lines shown (day 1  $y=20.6-3.4[OSPW]$ , day 2  $y=6.2-0.2[OSPW]$ , day 3  $y=-19.3+6.5[OSPW]$ )

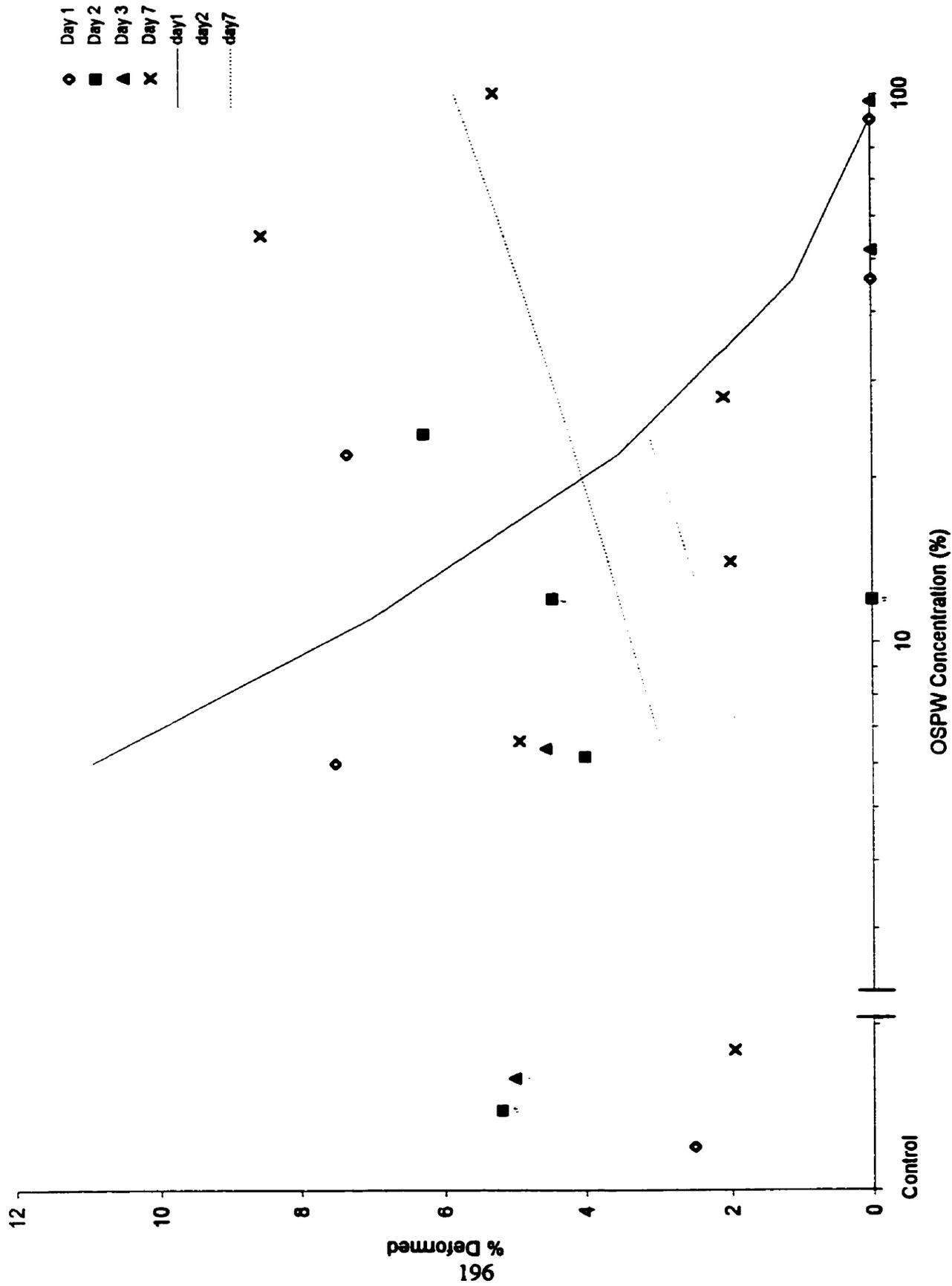


Fig. 5.19: Mentum deformities (%) of chironomids from population T following exposure to OSPW in a 14-d sediment bioassay. Linear regression lines shown (day 1  $y=31.0-4.5[\text{OSPW}]$ , day 2  $y=4.5+1.2[\text{OSPW}]$ , day 7  $y=7.1+1.0[\text{OSPW}]$ ).

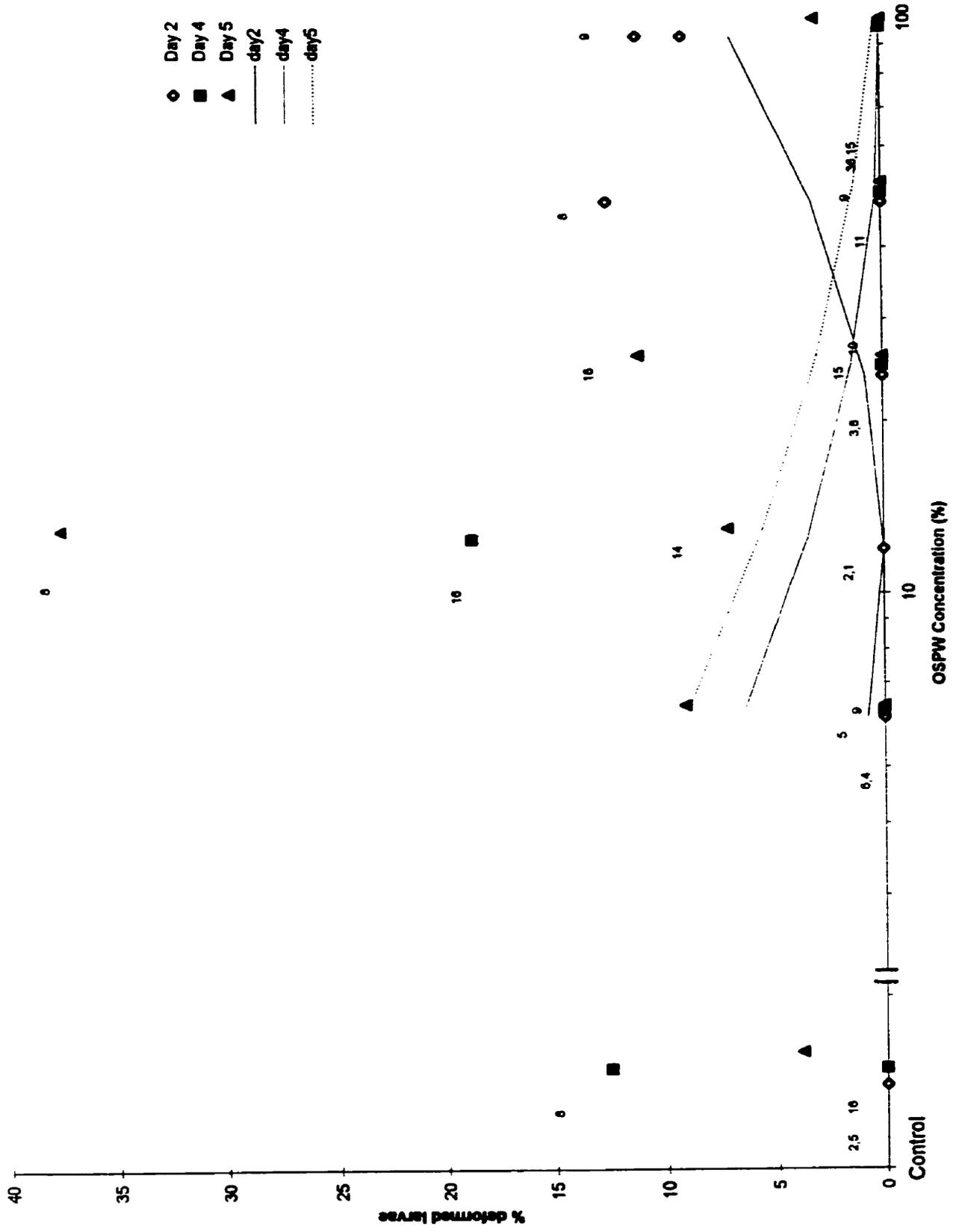


Fig. 5.20: Mentum deformities (%; # heads shown) of chironomids from population R following exposure to OSPW in a 14-d sediment bioassay. Linear regression lines shown (day 2  $y = -18.6 + 5.2[\text{OSPW}]$ , day 4  $y = 25.1 - 3.9[\text{OSPW}]$ , day 5  $y = 27.1 - 3.6[\text{OSPW}]$ ).

Table 5.16: Multiple regression analyses of deformities data by Day for field *C. tentans* (L), lab *C. tentans* (T), and lab *C. riparius* (R).

Pop	Day	n	B	SE	Intercept	SE	t	R <sup>2</sup>
L	1	9	-3.38	1.60	20.64	7.13	-2.11	0.39
L	2	12	-0.20	1.41	6.17	7.10	-0.14	0.00
L	3	4	6.47	2.68	-19.33	11.59	2.41	0.74
T	1	4	-4.53	2.42	31.01	11.36	-1.87	0.64
T	2	4	1.24	6.05	4.47	22.84	-0.21	0.02
T	7	5	1.03	1.40	7.12	6.57	-0.74	0.15
R	2	10	5.18	1.56	-18.60	8.25	3.33**	0.580
R	4	5	-3.91	4.09	25.06	20.92	-0.96	0.23
R	5	10	-3.63	2.48	27.06	12.75	-1.46	0.21

\* : p < 0.05

\*\* : p < 0.01

\*\*\* : p < 0.001

Table 5.17: Summary of observed relationships between survival, growth, emergence, and morphological deformities in 3 chironomid populations in relation to OSPW toxicity.

Pop- Group	Survival	Growth	Emergence (larvae)	Emergence (pupae)	Deformities
L - overall	**	**			
L - day 1	•	MNS			
L - day 2		**			
L - day 3	**				MNS
T - overall	***	***			
T - day 1	**				
T - day 2	MNS	*			
T - day 3					
T - day 6					
T - day 7	•	MNS			
R - overall			***	*	
R - day 2			***		**
R - day 4					
R - day 5			•	MNS	

MNS:  $p < 0.10$  (marginally nonsignificant)

\* :  $p < 0.05$

\*\* :  $p < 0.01$

\*\*\* :  $p < 0.001$

Where one population showed one type of response, another population exhibited effects in a very different manner. Generally, survival, growth, and emergence were all influenced by exposure to OSPW. The biomarker of developmental abnormality, mentum deformities, did not provide any clear indication that OSPW was teratogenic to any of the *Chironomus* populations.

Exposure to OSPW resulted in reduced survival for both the field (L) and lab (T) *C. tentans* populations, but did not affect survival of *C. riparius*, the other lab population tested. This suggests inter-species differences in sensitivity to the contaminants found in OSPW.

Growth was also inhibited by exposure to OSPW in the two *C. tentans* populations but not in *C. riparius*. However, the latter species has a much shorter life cycle, and thus may have been able to grow to maturity during the course of the experiment, regardless of the [OSPW] to which larvae were exposed, as suggested by the significant amount of emergence observed. The two other populations require at least twice the time to develop to adulthood as *C. riparius*, based on observations during rearing. Growth was thus more useful an endpoint in their toxicological assessment.

As mentioned above, emergence was observed in many of the test jars of population R, but was scarce and non-existent in populations T and L, respectively. As well, there was a significant increase in the number of larvae that had failed to metamorphose to pupae (and to adults). There was evidence that exposure to OSPW delayed the development of both chironomid larvae and pupae in population R.

## DISCUSSION

Survival and growth of chironomid larvae in higher OSPW treatments was reduced in both lab and field *C. tentans*. In the other lab species tested (*C. riparius*), both larval and to a lesser extent pupal development was delayed. In no population was evidence of teratogenicity related to OSPW exposure shown.

Due to numerous complications and some technical problems, statistical analyses of the results were quite complicated. This was partly due to the physical limitations imposed on set-up relating to the number of freshly hatched larvae that were available. To account for possible differences in sensitivity or fitness between progeny from different females (egg masses), set-up day became a factor in the analyses. Also, as significant emergence occurred only with the *C. riparius* population, comparison of growth effects of *C. riparius* with the two *C. tentans* populations is not valid; given sufficient time, it is possible that all larvae from all populations would reach a size typical of healthy prepupae. Therefore, there is no way to evaluate growth in *C. riparius* in terms of body length, although it is certain that OSPW exposure does not halt growth in this population.

### *Water Chemistry and Related Oil Sands Toxicology*

Constituting a complex mixture of various compounds, tailings water raises numerous concerns regarding adverse effects in many different groups of living beings. These biota include those that live in or are in contact with organisms that live in habitat receiving input from the oil sands mining industry.

Water chemistry parameters did not generally differ among populations, with the exception of D.O. concentrations which will be discussed below. The mixture of chemical constituents that comprises OSPW complicates the determination of causal links between exposure and effects, in terms of isolating specific stressors. However, that is beyond the scope of this thesis. Regardless, interesting trends in chemical fates were observed. It should be noted that expected future concentrations of chemical constituents in OSPW are higher than present concentrations (Bishay, 1998, pers. com.).

Concentrations of eleven heavy metals were elevated in OSPW treatments throughout the test period, whereas levels of 8 metals found in OSPW had decreased concentrations at take-down. This suggests that these 8 metals became bound to sediment, food, and waste, or were taken up by the chironomids. Further chemical analysis would be necessary to determine the specific fates of these metals. Sediment and pore water analysis would be of interest in terms of bioavailability issues. Tissue analysis would provide valuable information on chemical uptake and storage potential in benthos, in relation to observed effects. But more importantly, the chemistry reveals that many metals were still present in the water column on d-14 of the test. In particular, the five metals (Al, As, Cd, Mo, and Se) that exceed the CWQG for aquatic life are of concern in terms of future toxicity to wetlands flora and fauna of the reclaimed sites (CCREM 1987).

Total extractable hydrocarbons (TEH) at concentrations ranging from 15-75 mg/L were implicated as principal toxicants in both Microtox(TM) tests as well as acute and subacute toxicity observed with *Daphnia magna* and fish (Verbeek et al. 1993, Gulley 1994). A threshold of <14 mg/L for *D. magna* was calculated. But the presence of

naturally-occurring surfactants (naphthenic acids and related compounds) was also suggested to have affected control trenches in field trials (Nix et al. 1993). The major source of acute toxicity was traced to a complex mixture of organic acids believed to be naphthenic acids (Franco et al. 1984, MacKinnon and Boerger 1991, Herman et al. 1994, Bennett and Bendell-Young 1997). A 96-h LC50 of <10 mg naphthenic acids/L was observed for rainbow trout, and this supported LC50 values of 3-10 % in trout following exposure to tailings water (Schram et al. 1984, Nix and Martin 1992). Acting as natural surfactants, these organic acids alter ion and gas exchange across permeable membranes, thus posing physical and chemical stress on the larvae (Gulley 1994). Concentration of TEH was not analyzed in the present study, but concentrations of naphthenic acids in OSPW used (77-81 mg/L) in this bioassay may be toxic to some organisms.

Exposure to OSPW has been associated with elevated liver MFO activity and bile PAH equivalent concentration in adult yellow perch, although levels of steroid hormones were not affected, and none of these biomarkers were linked to physiological indices of reproductive development, gonad size, or fecundity (van den Heuvel et al. 1999)

Phenolic compounds are also suspected toxicants in OSPW that exist at levels between 0.003 and 2.5 mg/L which are related to toxicity in aquatic biota (Buikema et al. 1979). Microtox (TM) toxicity was linked to increased concentrations of phenol (Gulley 1994).

Ammonia-ammonium exists at levels (10-15 mg/L) considered highly toxic, based on an LC50 of 0.4 mg/L for trout (Hrudey et al. 1976). Microtox (TM) results were related to these contaminants in tailings water, suggesting that ammonia also contributes

to observed toxicity (Gulley 1994). However, concentrations of ammonia-nitrogen decreased in trenches still experiencing 100% mortality to *in situ* chironomids, indicating that other stresses were responsible for these results (Gulley 1994).

The high concentrations of both anions and cations in the OSPW, related to elevated sulphate/chloride salinity, undoubtedly also poses stress to the biota in terms of overloading the homeostatic mechanisms involved with osmotic and ionic stress. Yellow perch embryos tolerate only 2-4 g total dissolved solids (sulphate saline)/L, and other biotic groups may be more sensitive than perch (van den Heuvel et al. 1999a). However, OSPW showed a concentration of TDS of only 1.5 ‰.

Detoxification, mainly aerobic, took 8 years to reduce *D. magna* subacute toxicity (Nix and Martin 1992). Phytoplankton growth was stimulated in tailings pond water, thus these organisms, along with invertebrates and stocked fish, are expected colonizers of the wetlands. However, the anaerobic sludge layer is not expected to support benthos, and had an inhibitory effect on hatch rates of trout eggs (Nix and Martin 1992). In the laboratory, phosphate and oxygen greatly increased microbial degradation of naphthenic compounds in tailings water (Nix and Martin 1992, Lai et al. 1996).

*In situ* toxicity of OSPW was high in both fathead minnows and sticklebacks (LT50 < 1 d) exposed in constructed trenches, and this was related to ammonia (nitrogen and ammonium types), as well as low levels of D.O. (32-50 % saturation) (Gulley 1994). Acute toxicity was observed *in situ* with *Chironomus tentans* exposed to tailings sediment in constructed trenches. However, toxicity may have declined from inflow to outflow, indicating either sediment uptake thereby reducing bioavailability of contaminants, or that

these compounds were degraded to non-toxic forms (Gulley 1994). They observed very few chironomid larvae in trenches containing tailings water, and reduced egg viability was suspected as oviposition was witnessed in all trenches. In contrast, phytoplankton numbers were highest in waters that were most toxic to other biota, indicating that algal growth is stimulated by tailings contaminants. However, long-term exposure to oil sands contaminants resulted in a trend of decreased growth rates for total plant biomass. Although Whitehead (1992) observed cattails alone in reclaimed zones, these macrophytes were the most impacted by exposure to oil sands wastewater. However, bulrush seemed to thrive in wastewater relative to control trenches (Nix and Martin 1992).

The accumulation of hydrocarbons and metals by macrophytes is also of concern, particularly for muskrat, beaver, deer and moose, as well as various waterfowl species which migrate through this region yearly (Gulley 1994). Larvae of *Chironomus* and Tanyptodinae collected from wetlands receiving OSPW contained 20-65 mg naphthenic acids/kg water, suggesting that trophic transfer of these contaminants to both aquatic and terrestrial foodwebs is possible (Gulley 1994). Levels were higher in larvae collected from sediment containing muskeg compared to those wetlands constructed with coke-based sediment. In terms of metals, Al and Pb concentrations were higher in emerged insects from tailings trenches relative to control trenches, and Pb levels were much higher in larvae from the former sites as well, but Hg, Cd and Fe tended to be lower in these individuals (Gulley 1994).

In summary, the presence of metals, phenolics, the mixture of TEH (especially naphthenic acids), and ammonia in OSPW has shown to elicit toxic effects in both

laboratory and field studies, to a variety of organisms including fish, invertebrates, and macrophytes, although algae tend to be stimulated in wetlands receiving these mine-related inputs.

*Effect of Oxygen:*

Previously, low oxygen levels of 2-6 mg/L were implicated in reduced microbial degradation observed in tailings water (Gulley 1994). Although some organisms are well suited for hypoxic conditions (e.g. *Chironomus*), other organisms less tolerant to these conditions may be excluded from these stressful environments. The low dissolved oxygen concentrations may present a problem in face of attaining sufficient community diversity and production in wetlands receiving tailings water.

All OSPW treatments of population T experienced similar D.O. concentrations. The D.O. concentrations were lower in the higher treatments of populations L and R. However, only the population L showed a drop in survival and growth at the highest treatment. Population R actually showed increased survival and growth with the lower oxygen levels. Although delays in larval and pupal development in population R were observed, these occurred at higher OSPW treatments than the point at which oxygen concentrations dipped. The responses of population T were not associated with any effect of oxygen concentration. The results incorporated in the three populations therefore indicate that oxygen does not seem to have been a confounding factor in this bioassay.

*Survival:*

Both *C. tentans* populations showed decreased survival at 100% OSPW, whereas survival of *C. riparius* appeared to increase in the higher concentrations. Therefore, comparing only the *C. tentans* populations, field adaptation may be present. However, LC50s were similar, and the NOEC and LOEC values suggest that the field species may be slightly more sensitive. But this is mainly due to the large variability in survival of the field population. The response of *C. riparius*, however, suggests otherwise, unless *C. riparius* is inherently more tolerant to the toxic components of the OSPW compared to *C. tentans*.

Based on analyses done by set-up day, there were some differences in response between larvae from different egg masses. Variation was most obvious between eggs of the lab *C. tentans*. Particularly for population L, there was also a large amount of variation in % survival among replicate jars of the same set-up day and treatment.

The field population of *C. tentans* showed different trends on different set-up days, whereas a trend of decreasing survival with increasing [OSPW] was much more apparent with the lab *C. tentans* population. While two of three days of population L indicated a negative linear relationship between [OSPW] and survival, population T exhibited more of a threshold relationship somewhere between 25 and 50% OSPW.

*Growth:*

Body length, like survival, was related to OSPW exposure in both *C. tentans* populations but not in *C. riparius*. *C. riparius* recorded from the higher treatments were

slightly larger (9% increase at 25% OSPW, dropping to only 2% higher at 100% OSPW, relative to controls) than those in lower treatments. This result was similar to the increased survival observed with this population at higher OSPW concentrations. There are two possible explanations for this increase in survival and growth at high concentrations.

First, the higher OSPW treatments contain proportionately more naphthenic acids and other forms of dissolved organic carbon (D.O.C.), and D.O.C. is known to bind metals, PAHS and other chemicals that could produce toxic effects. Thus the bioavailability of toxicants in OSPW may be low. However, the significant reductions in growth in both *C. tentans* populations, combined with the lack of effect on survival of *C. riparius* suggests that toxicants are able to exert effects. This indicates a difference between the two *Chironomus* species. However, it is very important to restate here that body length was no longer a suitable endpoint for evaluating the effect of OSPW on population R. This was due to the elevated temperatures causing rapid development, and hence emergence, which precluded measuring a majority of the larvae. Only larvae that had not pupated were measured, but by this time, under these conditions, most had had the opportunity to attain full size, even in the highest treatment. Thus the remaining *C. riparius* in the high treatments at takedown were those that experienced delayed larval and pupal development. Cervi (1996) observed similar delays (and reductions also) of emergence, and enlarged pupae, following exposure of this species to cadmium in laboratory trials.

An alternate possibility, though not directly supported, suggests that *C. riparius* is benefiting from the OSPW. It may be that the control water was not as metabolically

beneficial as the OSPW, because the former was dechlorinated water that would lack the myriad of naturally occurring organic and inorganic compounds typically found in outdoor sourced water. The OSPW, being collected in the field, would be expected to contain some of these types of natural ingredients. Burt (1998) observed signs of highly stressed conditions in a bioassay and suggested an unknown stressor was present in the tapwater used, possibly related to nutrient limitation. However, the responses observed in the two *C. tentans* populations are contradictory to this hypothesis, leading me to suspect low bioavailability to be related to the observations with *C. riparius*.

It was clear that neither of the *C. tentans* populations benefited from exposure to 100% OSPW. Evidence was again provided for field adaptation, as the field population did not show nearly as strong a reduction in body length with increasing OSPW concentration as did the lab population. The field population showed a 24 % decrease in body length of chironomids in 100% OSPW relative to controls, but this difference was much larger (60% decrease) in the lab *C. tentans*. This time the regression and ANOVA analyses are in agreement that the field population is less affected than the lab population. There was a strong trend shown among set-up days for population T, in contrast to those of the field population, which were opposing and more variable.

#### *Larval and Pupal Development:*

Neither *C. tentans* population showed significant emergence (population L had none, and population T had a few adults in each of four jars). Population R, however, experienced strong emergence in all but the highest treatment concentration. This was

directly related to both the shorter life cycle of *C. riparius* relative to the two *C. tentans* populations, as well as the elevated temperature of the environmental chamber. All three set-up days of population R related slowed larval development to exposure to high concentrations of OSPW. As mentioned earlier, these results are supported by those found with *C. riparius* exposed to cadmium in laboratory (Cervi 1996). She showed a decreased and reduced rate of emergence of larvae at higher Cd concentrations, and reported that these developmentally-impaired larvae were larger than those in controls. Other studies also showed these responses in chironomids following exposure to trace metals in sediment (Wentzel et al. 1977, Hatakeyama 1988, Pascoe et al. 1989).

Not only did exposure to OSPW slow the developmental of *C. riparius* larvae into pupae, but it also slowed pupal development into emerging adult midges. Therefore, although OSPW was not lethal to *C. riparius*, it was capable of exerting sublethal effects on this population in terms of development. As the design of this bioassay was not focused on assessing pupal development, there was limited data to infer from, yet despite this a trend of decreasing emergence of pupae with increasing [OSPW] was observed. It is possible that this response could be as sensitive as the larval development endpoint.

#### *Deformities:*

Field studies have revealed that chironomid deformities are induced by heavy metals, organochlorine pesticides, and other organic toxicants including PAHs, solvents and oil-related compounds (Dickman et al. 1992, Vermeulen 1995). In laboratory, the proportion of larvae with mentum deformities, was log-linearly related to increasing

concentration of contaminated sediment (Hudson and Ciborowski 1996b). This supports the use of chironomid deformities as a biomarker of degree of contamination.

Chironomid larvae collected from reference (those considered relatively uncontaminated) can vary widely but generally exhibit between 0 and 4 % deformities (Cushman 1984, Wiederholm 1984, Dermott 1991, Dickman et al. 1992, Burt 1998, Groenendijk et al. 1998), depending on the genera and the structure examined. The field survey conducted on wetlands exposed to OSPW in the Athabaskan oil sands region of Alberta supported this value (Chapter 3).

The incidence of mouthpart deformities was not related to exposure to OSPW in any of the populations tested, indicating general non-teratogenicity of the tailings water to chironomid larvae under the test conditions. Polycyclic aromatics in porewater samples from mature fine tailings showed very low mutagenic potential using the Ames assay (Madill et al. 1999). Laboratory incidences of deformities in controls were higher than incidences in field-collected larvae from the Fort McMurray region (Chapter 3), although both results failed to detect any significant levels of teratogenicity related to oil sands contaminants. The higher incidence in lab is common and is probably related to inbreeding stresses (Hudson and Ciborowski 1996b, Burt 1998). In any case, most values were indicative of uncontaminated conditions.

Larvae from field *C. tentans* (population L) controls actually displayed the highest proportion of deformed menta, and this proportion decreased steadily towards the highest treatment concentrations. However, when different set-up days were analyzed, opposing trends were seen for set-up days 1 and 3, the latter showing a marginally nonsignificant

positive relationship between [OSPW] and deformity incidence. Set-up day 2 indicated a flat line trend through all treatments, therefore no strong trend was observed with this field population.

Population T larvae showed low incidence in all treatments, and as seen with population L, a decrease in incidence was observed towards the highest treatments. Also, the set-up days again showed opposing trends; incidence of deformities for day 1 was decreasing as a function of [OSPW], whereas that of days 2 and 7 were positive, although these two days had a large amount of variation, as seen by the low  $R^2$  values.

Higher allelic zygosity was observed in a wild *C. tentans* population (from a pristine site) relative to seven different lab populations, although they were all genetically similar (Woods et al. 1989). Bird et al. (1995) noted a large number of cleft median teeth in *Chironomus* larvae from reference sites, indicating that this type of mentum deformity was probably linked to genetic defects. In this study, population T was the only one to show a significant number of cleft median teeth, suggesting inbreeding stress may have been affecting this population.

Incidence of deformities in population R was highly variable and numbers of head capsules examined per jar was much lower than the 125 heads/treatment recommended by Hudson and Ciborowski (1996a), unlike the other two populations. This sheds uncertainty on the results, although weighting was performed to correct for unequal sample sizes. Although set-up day 2 did show a highly significant positive relationship, results from set-up days 4 and 5 showed no relationship. The results of set-up day 2 were affected mainly by the highest treatments, and very few menta were available from these treatments.

Therefore, although high incidence of deformities was observed in some treatments of population R, the mass emergence of the larvae pre-empted a more powerful analysis for this population.

### *Inter-population Differences*

Differences in response within and among field and laboratory populations of chironomids exposed to contaminants have been documented. Differences in sensitivity have been related to both physiological acclimation (Fraser 1980, LeBlanc 1982, and Bodar et al. 1990) and genetic adaptation (Brown 1976, Klerks and Levinton 1989) in invertebrates. Elevated levels of inert granules (for metal storage), metallothioneins (natural metal-binding proteins) and mixed function oxidases (detoxify organic compounds) have been associated in several organisms including adapted chironomids (Krantzberg and Stokes 1989).

Hoffman and Fisher (1994) noted a 13-250 fold difference in tolerance to four pesticides between lab and field *C. riparius*, and linked this to the field population showing both increased biochemical resistance (acetylcholine esterase and mixed function oxidases) as well as increased fitness related to larger, more fecund individuals, although development was slowed. With sufficient time, *C. riparius* was shown to acclimate to increased salinity and reached maximum emergence at 3 ppt (Bervoets et al. 1996). Groenendijk et al. (1999) observed adapted chironomids with increased metal storage capacity in midguts at contaminated sites. Also, Miller and Hendricks (1996) observed both acclimation and adaptation in *C. riparius* exposed to zinc.

In terms of deformities, interspecies variation in susceptibility has been noted in several studies. *Chironomus* often shows the highest incidence of deformities among suites of genera collected from contaminated habitats (Dermott 1991, Dickman et al. 1992, Hudson and Ciborowski 1996a). Both *Procladius* and particularly *Chironomus* were tolerant to polluted conditions, the latter having an advantage in oxygen-reduced waters (Dermott 1991, Hudson and Ciborowski 1995). Soft organic sediments attain the highest loads of contaminants in aquatic ecosystems (Pesch et al. 1981). Related to this, *Chironomus* appeared to be the most susceptible to deformities, most likely due to its detritivorous feeding habits, although other Chironomini taxa (*Dicrotendipes*, *Phaenopsectra*, *Cryptochironomus*, *Harnischia*) were less susceptible than the tanypod *Procladius* (Warwick 1989, Dermott 1991).

This chapter describes differences in response between a field population (after 8 generations in lab) from a contaminated area in comparison to lab populations. Growth, as measured by body length, was much more affected in the lab population of *C. tentans* compared to the population from South Bison Pond. Survival was also more significantly reduced in the lab population. However, Rosenberg et al. (1977) noted no difference in larval size between two species of *Cricotopus*, although there were significant interspecies differences in numbers found on oiled vs. unoled artificial substrates, and generation time was shortened. This is the opposite of the delayed rates of larval and pupal development observed in this study using *C. riparius*. Interspecific differences can therefore be related to different biological responses, depending on the mode of action of the contaminant and the abiotic and biotic processes (temperature, food, predation) that act to alter

bioavailability of the contaminant as well as behaviour and body condition of the organisms exposed.

Aquatic invertebrates that have adapted tend to show reduced overall fitness under clean conditions. This has been related to 'costs of tolerance' related to alterations in metabolism linked to reproduction and growth (Postma et al.1995). They observed that metal-adapted chironomids experienced superior egg hatching and larval development rates, but showed that in control sediment the mortality was not reduced and even increased to 50% in control (unspiked) sediment. This may in part explain the lower control survival of the field population (Fig. 5.5). Therefore, the history of the site and the chironomid population should be known in order to correctly interpret responses from biomarkers. Also, other environmental stressors (e.g. food) that can affect overall fitness may contribute to the susceptibility of individuals towards stress-related effects including deformities (Burt 1998).

Conversely, interspecific differences in sensitivity may not always be manifested, depending on the class of contaminant. In a multi-species bioassay, the lab species *Chironomus tentans* responded similarly to three field species. *C. tentans* was relatively sensitive to oil (water-soluble fraction) but was not as sensitive to phenol as three natural populations of chironomids, *Clinotanypus pinguis*, *Einfeldia natchitochaeae*, and *Tanypus neopunctipennis* (Franco et al. 1984).

## **Future Research**

It would be highly desirable to be able to more fully compare the 3 populations in terms of all endpoints used in this bioassay. This would require simple changes in exposure duration, depending on the species examined.

First, it would be interesting to assess the rates of development of larvae (time to pupation) and pupae (time to emergence) in the two *C. tentans* populations in order to support the findings from *C. riparius* that indicate delayed metamorphosis and emergence. This would require a longer bioassay (4-6 weeks) to ensure sufficient time for development based on normal conditions.

Additionally, the effect of OSPW on the development of *C. riparius* could be further examined by running a shorter bioassay of only 8 or 10 days at a cooler temperature of 20°C to avoid emergence. I expect that body length of larvae would decrease with increasing concentration of OSPW, as was seen with the two *C. tentans* populations. But there may be differences in sensitivity between the two species. Results from this second bioassay could be compared to the present study and this would provide a truer picture of the effect of OSPW on chironomid development, by combining knowledge of rates of larval growth, pupation, and emergence in both laboratory and field populations.

Also, the inclusion of a positive control in the bioassay would provide better evidence that the OSPW is not teratogenic, and that the bioassay was conducted under appropriate testing conditions. This could be accomplished by spiking the water or sediment of positive control jars with sufficient quantity of a known teratogen (e.g. Cd,

quinone), and then verifying that chironomids in these jars indeed showed elevated incidence of mouthpart deformities.

### **Conclusions:**

OSPW was associated with adverse effects on chironomid survival and growth, as well as on both larval and pupal development, but did not induce mentum deformities. Effects on survival and growth were closely correlated within the three *Chironomus* populations, but these endpoints were not observed in the population showing delayed development. The inter-population variation in response further supports the use of several species in assessing the viability of wetlands receiving OSPW inputs.

There were both inter- and intraspecific differences in responses observed among the three populations exposed to OSPW. Survival and growth were related to OSPW in both lab and field *C. tentans*, but not in the *C. riparius* population. The difference in survival between the two laboratory populations demonstrates that significant differences in sensitivity to OSPW exist between species. Differences in population sensitivity of *C. tentans* based on larval survival, and particularly growth suggest that field adaptation to OSPW compounds may have occurred in population L. Both the LC50 and the observed NOEC/LOECs were lower for the lab population (LC50=75 % OSPW, NOEC = 25 % OSPW) compared to the field one (LC50=200 % OSPW (extrapolated), NOEC = 50% OSPW). However, with only one population of each type to compare, results must be viewed with caution. The *C. riparius* population showed adverse responses to OSPW exposure in terms of decreased development rates both at the larval and the pupal stages

of chironomid development, but survival was not affected. Ecologically, this could result in reduced mating success of the population, depending on the length of delay and if both sexes were effected equally, as emerged chironomids do not live long. Generally, deformities were not related to OSPW exposure in any of the populations. This well-known biomarker of teratogenicity, used in the past to track contaminant exposure in other toxicological studies, indicates that OSPW is probably not exerting teratogenic stress on wetland biota.

The relationships between OSPW exposure and adverse effects on laboratory and field populations, particularly in terms of growth and development, require further study in order to assure that a viable aquatic community can be attained. Particularly, testing several field populations along with traditional lab species is recommended, in order to better determine if field adaptation is present in chironomids of OSPW wetlands. In any case, possible anthropogenic stress related to the input of OSPW (at 50% and 100% concentration) into reclaimed wetlands is suggested by this laboratory study, although field adaptation is apparent to some degree.

#### **Implications for Assessing Viability of Wetlands Associated with Oil Sands**

This bioassay indicates that OSPW caused significant effects in both lab and field populations of chironomids, mainly at 50% and 100% concentration, and that field adaptation may have occurred to some degree, based on larval growth. However, the study was limited as only one field population could be compared to the two lab populations.

Teratogenicity, as measured by rates of mouthpart deformity in chironomid larvae, was not influenced by inputs from the oil sands industry. The determination of field tolerance in chironomids could not be ascertained, thus the evidence provided by this bioassay indicates that OSPW is not teratogenic in either lab or field settings. This biomarker is applicable in the future monitoring of teratogenicity relating to OSPW, although it may not be a good indicator of general exposure, and thus should be combined with the use of other endpoints.

Future biomonitoring of wetlands using cultures originating from the field will allow more relevant determination of remedial success in the reclaimed wetland areas. Based on the toxicity of OSPW observed, the monitoring of wetland sediment and overlying waters in chironomid bioassays in conjunction with assessment of the benthic macroinvertebrate community is recommended, to accommodate possible effects in other, possibly more sensitive taxa.

## **CHAPTER 6 - Colonization Potential of Chironomids in Reclaimed Wetlands Related to Oil Sands Mining**

### **INTRODUCTION**

A wet landscape reclamation option involving the incorporation of wetland and lake networks within boreal forests representative of the region is being considered (Lai et al. 1996). In order for reclaimed mining areas to attain sufficient levels of biological production and diversity, colonization by organisms from surrounding habitats must occur. Colonization is the sequence of events leading to establishment of a population of organisms previously absent, and can occur over broad temporal and spatial scales (Sheldon 1984). Colonization success depends on adult reproductive behaviour (relating to dispersal, and site selection for oviposition) as well as larval requirements (food, sediment substrate). Chironomids are one of the earliest and most rapid colonizers in new aquatic habitats (Davies 1976). Thus, assessing the potential for these 'expert colonizers' to colonize reclaimed wetlands is probably conservative in terms of overall colonization potential, because if these insects can not establish, it is likely that many other groups will not either.

To evaluate the potential for sublethal toxicity of OSPW to aquatic macroinvertebrates, chironomid adults and eggs were studied. Naphthenic acids, acting as natural surfactants that can alter the physico-chemistry of water resulting in reduced ion or gas exchange across biological membranes, may thus affect chironomids (and other emergent insects) in terms of reducing rates of adult oviposition, pupal emergence rates, and normal development. This could inhibit various organisms from establishing self-

sustaining populations in reclaimed oil sands mine leases.

Specifically, to determine the colonization potential of reclaimed mining-influenced wetlands of the future, relative to their reference counterparts, adult chironomid flight activity, egg-laying behaviour, and egg mass development were observed. Estimates of the flight activity related to ovipositioning, as well as rates of egg-laying were measured with shoreline colonization trays filled with either reference or affected wetland water. Eggs collected in these trays were then observed for hatching success in laboratory.

It is expected that adult midges will be more commonly found in their native water trays than in the trays from the other site at each wetland, and that rates of oviposition and egg survival will also follow this pattern, particularly for the chironomids at the reference sites. Reference site adults may avoid OSPW wetland trays, and eggs from these insects may be adversely affected by exposure to water from the OSPW wetlands, in terms of reduced hatching success.

## **METHODS**

### **Field Methods**

#### *Design*

Chironomid abundance and egg-laying activity of midges was evaluated by monitoring eggs masses and insects in pairs of triplicate, water-filled trays placed on the shoreline of the 6 focal monitoring wetlands (Natural Wetlands (NW)-Crane Lake (CL), South Bison Pond (SB)-Shallow Wetland (SW), Hummock Wetland (HW)-High Sulphate Pond (HS)) over a 5 week interval (5 June - 12 July 1998).

### ***Tray Set-up and Maintenance***

At three pairs of reference and tailings-receiving wetlands (grouped together based on similarity of ordinated environmental characteristics), black plastic trays (30 cm x 50 cm) filled with water were placed on the shore. Three pairs of trays were placed around the periphery at each of the 6 focal wetlands (36 trays total). One tray of each pair was filled with water from the wetland at which it was located. The other tray of each pair was filled with water from the complementary wetland (i.e., reference site water if at an oil sands wetland; oil sands wetland water if at a reference wetland). This design was used to observe differences in attraction/avoidance and egg survival of chironomids to both water types, at both reference and oil sands wetlands. All water was always filtered through a plankton net (mesh size 50 $\mu$ m) to prevent the transfer of eggs/larvae from the original wetland into the colonization tray.

Larval and adult insects found in or on the water, as well as egg masses, were collected from the trays every 3-4 d. At this time, water conductivity, salinity, and temperature were measured, and old water was replaced with fresh water from the appropriate wetland. Parameters were then remeasured.

After two weeks, the third replicate of each water was not replaced but was topped off with fresh water to replace water lost to evaporation/ consumption by ducks. This was done to permit development of stray eggs/larvae to occur in the trays over several weeks (compared to only 3-4 days in tray replicates 1 and 2).

By June 22, smaller, shallower white trays were added at each site to see if colonization might be affected by habitat colour, as well as possible differences related to

water temperature/conductivity in these white trays.

### ***Adult Activity***

Adult insects trapped on the water surface of the trays were collected every 3-4 d using a small fish net. Organisms were placed in 20-mL scintillation vials containing 70% ethanol. These were then transported back to the lab for later sorting at the University of Windsor.

### ***Oviposition***

Chironomid egg masses were collected from the water surface every 3-4 days. They were carefully removed and counted using forceps and placed in vials of fresh water of the type they were laid in, and transported to the laboratory at Natural Wetlands for observation of egg survival and development.

## **Laboratory Methods**

### ***Adult Activity***

Vials of 70% ethanol containing adult insects collected from the water surface of colonization trays were sorted using a dissecting microscope and fine forceps. All organisms were identified to at least family level, and adult midges were sexed and identified to subfamily using the key of Oliver (1981).

### ***Egg Hatching***

Waters containing the egg masses were aerated using capillary tubing and needles attached to tygon tubing leading to an air pump. Egg masses were monitored daily beneath a dissection microscope for evidence of development, time to hatching and proportion of individuals in an egg mass that hatched. Larvae were counted, unless they were too numerous (over 25), in which case an estimate was made of the number present. Newly-hatched larvae were transferred to an aquarium containing silica sand and dechlorinated tapwater. These larvae were used to establish ongoing cultures for in situ toxicity studies, or for transport to permanent laboratory facilities (University of Windsor).

### **Statistical Analyses**

#### ***Adult Activity***

The number of adult midges collected in each tray (n=3) was summed over the entire summer sampling period. The mean number of midges per tray for each of the reference and tailings-affected waters was calculated, along with corresponding standard errors ( $SE = SD/\sqrt{n}$ ). This was done for each of the six focal sites.

To determine if there was an effect of site (water-affected vs. reference) or water type (affected or reference wetland source) on the number of adult midges caught in colonization trays, analysis of variance (ANOVA) was carried out by hand based on a randomized block-split plot design with multiple observations, as described in Damon and Harvey (1987).

### ***Oviposition***

The mean number of egg masses collected per tray (n=3) over the entire 1998 summer season was calculated, along with standard error, as in the section above.

Similar ANOVA's were calculated for numbers of egg masses collected (as for numbers of adults) testing for any effects related to water type or site type.

## **RESULTS**

### **Water Parameters**

The water temperature, conductivity, and salinity were measured on every sampling day (every 3-4 days) throughout this 5-week study. Water parameters varied significantly and were related to rates of evaporation. The white water trays showed temperatures similar to the larger black trays. The data are summarized in Appendix 6.1.

### **Adult Activity**

Diptera dominated collections, but damselflies (Zygoptera: Odonata), beetles (Coleoptera) and various types of terrestrial bugs (Heteroptera and Homoptera) were also commonly found in the trays.

Chironomids dominated the Diptera collections. Tanytarsini, Chironomini and Orthoclaadiini were the most commonly caught groups (Appendix 6.2). There was pronounced weather-dependent variation in catches of adults. Most individuals were caught on a few very warm, humid days.

### *Comparison among Wetlands:*

There were marked among-wetland differences in adult activity (Fig. 6.1). Most chironomids were recovered from Natural Wetlands, in Crane Lake water trays. Crane Lake, South Bison Pond, and Hummock Wetlands yielded the fewest adults. However, ANOVAs indicated no significant effect of either water or wetland type on numbers caught, nor were any interactions significant (Table 6.1). There was strong temporal variation in numbers collected in trays, with most adults being caught between June 9-24, and almost none found in the last 3 weeks of testing. Also, females were up to 4 times as common as males, and were predominant at all sites (Appendix 6.2).

Abundances and the taxonomic groups of adults captured at a wetland were independent of the presence of oil sands water. There was also no consistent evidence that chironomids avoided colonization trays containing oil sands-related water at any of the wetlands (Natural Wetland was a possible exception, although there was no significant difference). In contrast, there was a trend for more adults to be captured in trays containing oil sands waters than in trays containing reference water at 2 of the 3 (SW and HS) reference wetlands (Fig. 6.1).

### **Oviposition Activity**

Adult chironomids produced at least some egg masses at all 6 of the focal study wetlands (Fig. 6.2). However, there was substantial variation both in the timing and in the numbers of egg masses produced among wetlands and water types (Appendix 6.3). ANOVA indicated a significant effect ( $p > 0.005$ ) of pairs of wetlands (site effect) on the

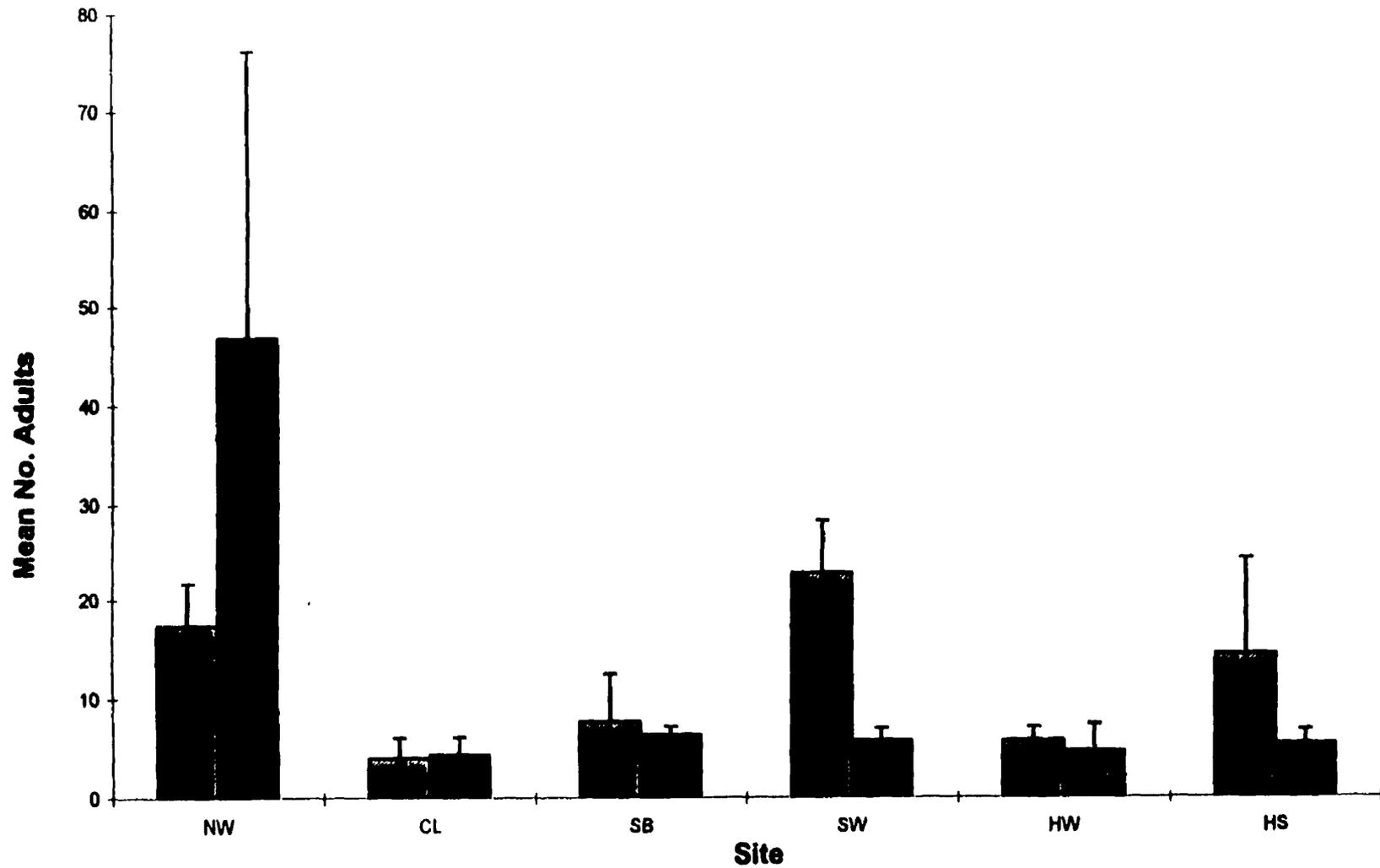


Figure 6.1: Mean ( $\pm$ SE) numbers of adult chironomids per colonization tray captured in oil sands-related water (grey bars) or reference water (black bars) at 6 focal wetlands during 5-week sampling period. Paired sites are grouped adjacently to each other (NW-CL, SB-SW, HW-HS).

Table 6.1: Summary of analysis of variance (ANOVA) of numbers of adult midges and egg masses collected in colonization trays in a field experiment at 6 wetlands in June-July of 1998. Analysis was based on a randomized block-split plot design with multiple observations.

Source	df	Egg Numbers			Adult Numbers		
		SS	MS	F	SS	MS	F
Whole	17	95			6629.4		
site	2	55.17	27.59	16.02***	711.1	355.5	1.26
site type	1	9	9	1.77	256	256	0.22
site x site type	2	10.16	5.08	2.95	2287.5	1143.8	4.07
rep: site x site type	12	20.67	1.72		3374.8	281.2	
Split	18	36			4834.5		
water type	1	0.11	0.11	0.31	0.44	0.44	<0.01
site x water type	2	0.72	0.36	0.18	986.7	493.4	1.98
site type x water type	1	2.78	2.78	0.72	693.4	693.4	8.19
site x site type x water type	2	7.73	3.87	1.88	169.4	84.7	0.34
rep x water type: site x site type	12	24.66	2.06		2984.5	248.7	

\*\*\*p<0.005

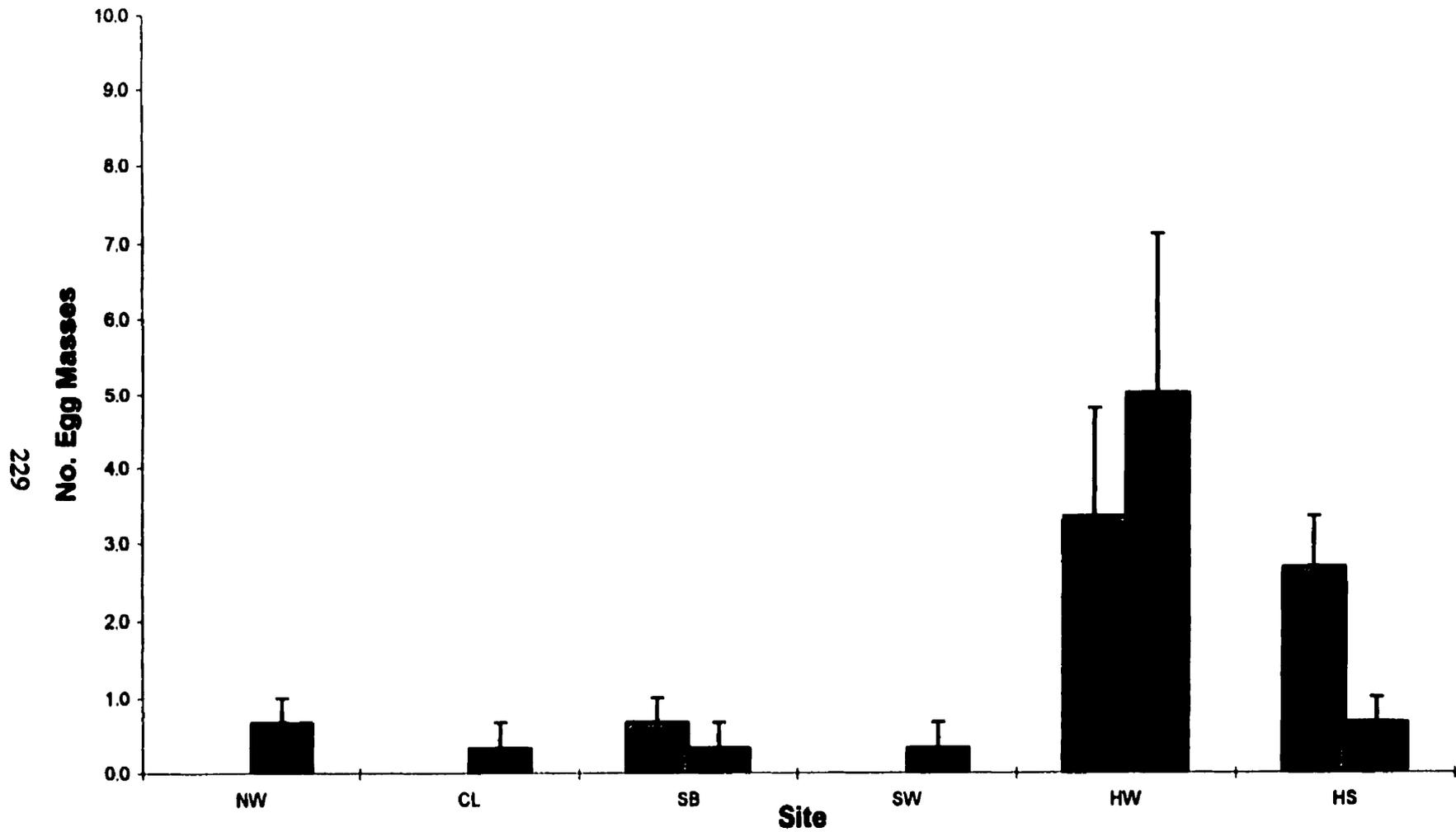


Figure 6.2: Mean ( $\pm$ SE) number of egg masses per pan oviposited into trays containing oil sands-related water (grey bars) or reference water (black bars) at 6 focal wetlands during 5-week sampling period. Paired sites are grouped adjacently to each other (NW-CL, SB-SW, HW-HS).

number of egg masses collected, but neither the type of site nor the type of water affected results, and interactions were also not significant (Table 6.1).

There was marked variation in numbers of egg masses produced among wetlands. Greatest oviposition activity occurred at the paired Hummock and High Sulphate wetlands, in the complementary water type to each site (Fig. 6.2). The fact that mean numbers of egg masses produced varied little between pairs of water-affected and reference wetlands but greatly among pairs suggests that oviposition tendencies may be related to different periods of peak emergence at the different wetlands, but that the tailings process water itself does not appear to inhibit oviposition.

Although eggs were found in reference waters at all six sites, no egg masses were laid in process-affected water trays at either Natural Wetlands, Crane Lake, or Shallow Wetland. However, the overall oviposition activity at these six wetlands was very low (Fig. 6.2).

### **Egg Hatching Study**

Viability of egg masses was variable (0-100 or more larvae per egg mass), but apparently unrelated to the type of water in which eggs had been oviposited (Table 6.2). Oil sands-related waters were not shown to affect rates of egg hatching. In particular, high numbers of hatching larvae were recovered from at least one egg mass laid in water from all three of the OSPW wetlands, implying that there is no effect of oil sands mining-related waters on chironomid egg development (Table 6.2).

Table 6.2: Summary of laboratory egg mass hatching data. "Site" represents wetland at which eggs were oviposited. "Water" indicates the type of water in tray in which eggs were initially oviposited and then maintained. "Hatch success" is a qualitative estimate of the relative degree of egg hatching: none, L = low (1-10 larvae), M = medium (11-35 larvae), H = high (36 + larvae). A typical egg mass contained from 100 to several hundred eggs, depending on the species.

Site	Water	Hatch success per egg mass
NW	CL	H, L
NW	tap	H
CL	CL	L
HS	HS	none, H
HS	HW	L, none, H, none, none
HW	HW	H, L, M, M
HW	HS	M, L, none, M, M, M
SB	SB	L, H
	SW	H
SW	SW	none

## **DISCUSSION**

Numbers of adults and egg masses, as well as hatch rates of egg masses, were not related to exposure to process-affected waters. There appeared weak trends towards a higher number of adults in process-affected water trays at reference sites, suggesting an attraction behaviour. Conversely, a very weak trend suggesting that ovipositioning was reduced in process-affected waters was seen in half the focal sites, but limited numbers of egg masses collected prevented any strong inferences from being drawn.

The pattern of oviposition among wetlands differed from the adult chironomid abundance data (Fig. 6.1 and 6.2). Most adults were captured in Natural Wetlands trays, whereas most egg masses were recovered from trays in the Hummock and High Sulphate wetlands. The extremely high number of midges collected at Natural Wetlands in Crane Lake water was mainly due to an abnormally high number caught on one day. The lack of correspondence between numbers of adults caught and eggs collected at the various sites suggests that adult collections in the trays do not accurately reflect the abundance of midges in the area, but only give a rough measure of diversity of genera. The general lack of adults observed after June 26, 1998, suggests that peak emergence may have occurred prior to June 9, based on the low numbers of adults collected at most sites. In North Dakota, chironomid emergence tended to peak in late spring, and with a gradual-to-abrupt decrease in emergence in summer (King and Wrubleski 1998). Emergence cycles probably occur later in Alberta due to the later onset of spring. Also, egg masses were still being collected into July, providing further evidence that numbers of adults caught were not related to egg collections (Appendix 6.3).

Adult chironomids generally have poor powers of flight and oviposition site selection, and are generally dispersed by wind currents (Davies 1976). Cool temperatures, wind, cloud cover, and relative humidity can affect take-off, flight duration, and swarming (Kovats et al. 1996). Chironomids that fly between ponds and that oviposit in the central part of ponds (*Chironomus* and *Procladius*), as opposed to the periphery which was contaminated with crude oil, have a colonization advantage (Mozley 1978). Dispersal within aquatic systems is mainly left to the first instars which are planktonic and positively phototactic, and can easily disperse along water currents (Davies 1976). Later instars are mainly benthic, but often leave substrate under conditions of environmental degradation (Flanagan 1973). Substrate and vegetation play important roles in determining distributions of chironomid larvae, as seen with *Chironomus* which prefer soft sediment to burrow and build tubes for houses (Davies 1976, Francis and Kane 1995).

In terms of the reclaimed mining areas, chironomids and other important colonizers would be expected to colonize new wetland habitats both by aerial dispersal and by larval drift. Chironomids are one of the earliest and most rapid colonizers in new aquatic habitats (Davies 1976). Factors limiting their dispersal include the distances between wetlands, the degree that wetlands are connected by waterways, as well as habitat characteristics including frequency and severity of disturbance, and permanency (Kovats et al. 1996). Adults chironomids only live for a short period of days (Pinder 1986), and probably do not travel more than 2 km, based on mean inland dispersal distances of 650-1845 of caddisflies and mayflies, which are better fliers than midges (Kovats et al. 1996).

*In situ* test 1 (Chapter 4) suggested that survival of *Chironomus anthracinus* (3rd instar) larvae may have been reduced by exposure to process-affected waters relative to reference waters. Also, *C. tentans* originating from both laboratory and field (South Bison Pond) exhibited reduced survival and growth in relation to OSPW exposure (Chapter 5). The current chapter was designed to assess effects in other life stages (adult, egg, 1st instar) to pinpoint any toxicity manifested in chironomids. A lack of oviposition in process-affected waters at reference sites would strongly suggest that future reclaimed areas would not be rapidly colonized by neighbouring wetlands not receiving oil sands-related inputs.

Newly-hatched first instar chironomids are extremely sensitive to chemicals relative to older life stages (Williams et al. 1986, Pascoe et al. 1989). Process-affected wetland water could thus affect egg masses and first instar larvae from chironomids of reference wetlands much more strongly than those of mining-influenced wetlands. This would also reduce the probability of new habitats containing oil sands contaminants being colonized by reference biota.

Further study of oviposition and related egg development to larvae should be conducted, over a longer sampling season starting in May. This would allow for differences in peak emergence among both species and wetlands, the latter possibly varying depending on mining inputs. McCahon and Pascoe (1991), Pascoe et al. (1989), and Cervi (1996) observed delayed emergence of *Chironomus riparius* exposed to cadmium in bioassays, and similar delays were observed in *C. tentans* exposed to copper (Kosalwat and Knight 1987) and Cd, Zn, and Cr (Wentzel et al. 1978). Laboratory

exposure of *C. riparius* to OSPW resulted in delayed pupation and emergence (Chapter 5). Different generation times were observed between two species of *Cricotopus* exposed to crude oil-contaminated river. Thus, OSPW in reclaimed wetlands may delay development of colonizing larvae to such a degree as to make establishment of self-sustaining populations difficult or even impossible. Should emergence periods of the sexes not temporally overlap, it is possible that mating would not be possible.

In conclusion, there is no evidence that OSPW results in altered oviposition or egg development to larvae. Very few egg masses were obtained overall, although fair numbers were collected at HW and HS. Hatch rates of eggs were quite variable. Collection of adults was highly variable and did not correlate with numbers or dates that eggs were collected in colonization trays.

## **CHAPTER 7 – General Discussion**

In this study, effects of OSPW related to wetland viability were seen in both field and laboratory investigation of chironomids and other macroinvertebrates.

In the field, effects were observed in relation to the presence of OSPW in wetlands. The benthic macroinvertebrate communities at OSPW wetlands exhibited reduced taxa richness and abundances compared to environmentally similar reference wetlands. The chironomid communities also showed reduced taxa richness in relation to OSPW exposure. Most differences were attributable to elevated conductivity, although OSPW added to this effect in the case of richness, but partially negated the effect of conductivity on abundances. Both the general benthic macroinvertebrate community and specifically the chironomid community showed differences in community composition between OSPW and reference wetlands, in terms of the presence and relative abundances of dominant taxa. Among the three OSPW wetlands, these community level responses were strongest at Hummock Wetlands, a small site that experiences erratic water levels through the growing season due to varied CT water input. However, there was no indication of a teratogenic effect of OSPW on native chironomids in the wetlands, based on chironomid mouthpart deformities. Also, no effect of OSPW exposure was detected in wetlands in terms of adult chironomids caught, as well as rates of oviposition.

In the laboratory, no effect of OSPW on hatchability of field-collected eggs was detected. The lack of response with eggs is consistent with a laboratory study which indicated that eggs were the least sensitive life stage of *Chironomus decorus*, and that

larvae were the most sensitive (Kosalwat and Knight 1987). Similarly, a chronic toxicity bioassay revealed significant effects of OSPW on a field population and two laboratory populations of chironomid larvae. Survival of larvae was reduced in both laboratory and field-derived *Chironomus tentans*, and growth was also reduced in these populations, but more so in the laboratory one, suggesting greater tolerance of the field-derived population. No effects of OSPW on survival or growth were seen in *C. riparius*. However, this population showed delayed larval, and to a lesser extent, pupal development in relation to OSPW. These two endpoints could not be evaluated in the *C. tentans* populations which have a longer life cycle and thus did not emerge during the test. Overall, larval survival (lab and field *C. tentans*), growth (lab *C. tentans*), and larval development (*C. riparius*) were all sensitive to OSPW.

The benthic assemblages observed in sampled wetlands were not clearly related to the environmental parameters measured at study sites, indicating that other environmental parameters may regulate the species assemblages which occur, or that natural variation was fairly high. Replicate samples would be useful in assessing this variation. Trophic status of the wetlands could play a role in determining species assemblages. Chironomid communities have been characterized in relation to this parameter (Armitage 1980, Armitage and Blackburn 1985). Furthermore, the numerous interactions among the various organisms present at each site could also be examined in future, in terms of predator and competitor relations. Also, the level of dissolved organic carbon (D.O.C.) related to the humic and naphthenic acids, although not measured, appeared to be high at many sites, judging by the brownish tint to the water. High D.O.C. concentrations could

be acting to regulate effects of the oil sands inputs by binding metals and organic chemicals, thus reducing their bioavailability to the macroinvertebrates. Metal bioavailability would be dependent on water redox potential, pH and dissolved oxygen concentrations which may fluctuate through the year. This effect was suspected by Clements et al. (1990), who considered laboratory results to underestimate toxicity, as effects were stronger in tolerant species in the lab than effects observed in more sensitive taxa in field trials. Total dissolved solids and dissolved organic carbon in field conditions were suspected to be related to the reduced metal toxicity observed (Clements et al. 1990). Laboratory results therefore do not fully represent those expected in the natural environment, but are invaluable in showing causal relationships suggested by field studies. Accordingly, the OSPW bioassay provided support for the observed effects in the field.

Marchant et al. (1997) developed predictive models of macroinvertebrate assemblages in rivers, based on 22 environmental characteristics measured at 49 sites. Conductivity and substrate heterogeneity were two of the five main discriminatory environmental parameters used, which is in accordance with the current study. However, only 15 sites were available for my study of species assemblage patterns. A larger data set may have helped clarify the source of the variability observed. Shelton (1992) noted that the density of invertebrates in samples at mine-associated wetlands varied widely and attributed this to differences in production among microhabitats and sites. King and Wrubleski (1998) reported significant temporal and spatial variability in emerged insect availability in prairie wetlands related to variation in community structure.

With the numerous measures of biological response available to assess ecosystems, it is important to decide which are most useful under particular conditions of stress. Trace metals including cadmium, copper, lead, zinc have been associated with high incidence of chironomid mentum deformities, although larval body length was not affected (Janssens de Bisthoven et al. 1992). This suggests greater sensitivity of the biomarker over individual and population indices. However, and found that exposure of *Chironomus decorus* (Cushman 1984) and *Procladius* sp. (Pardalis 1997) to coal liquid elicited mentum deformities, but Pardalis (1997) concluded that this biomarker was less sensitive than traditional measures including abundance, biomass, number of taxa, and benthic species diversity. However, these chironomids dominated in the crude oil ponds and may have adapted to their environment. Emergence was found to be a more sensitive endpoint than survival in *C. riparius* exposed in lab to azaarenes (Bleeker et al. 1999), and this study showed similar findings with this species in the laboratory. The relative sensitivity of this biomarker in relation to other measures is thus case-specific. These results indicate that use of both this teratogenicity biomarker along with community measures would be most prudent, preferably with knowledge of the contaminant history of the site. Also, the inclusion of a positive control in the bioassay would allow for evaluation of the bioavailability and teratogenicity of OSPW contaminants, and that the bioassay was conducted under appropriate testing conditions. This could be accomplished by spiking the water or sediment of positive control jars (in lab bioassays) or enclosed mesocosms (in the field) with sufficient quantity of a known teratogen (e.g. Cd, quinones). Assessment of larvae in positive controls would then provide quality control for the experiments. If the

teratogenic metals or organic chemicals were present and bioavailable under the present field conditions, the larvae in the positive controls and the OSPW wetlands should show increased incidences of mouthpart deformities relative to relatively uncontaminated reference sites. However, a lack of toxic response in these positive controls would indicate conditions of low bioavailability were present in the wetlands.

Janssens de Bisthoven et al. (1998) suggested the use of multiple biomarkers along with consideration of environmental factors, as in the use of body burdens, sediment characterization, induction of deformities and metallothioneins to assess the impacts of metal contamination. My project utilized measures that encompass suborganismal, organismal, population, and community levels of organization. The main effects of OSPW in wetlands were seen at the level of both the macroinvertebrate and chironomid communities, as well as chronic effects in individual chironomids. This indicates that chironomids are well suited as surrogates of general benthic macroinvertebrate communities in OSPW wetlands.

This study also assessed responses in multiple species, including chironomids as well as the various macroinvertebrates that made up the wetland communities. Toxicity tests including species from all major biotic groups indicate that *C. tentans* is one of the taxa most sensitive to pesticides, but intermediate in sensitivity ranking for phenols, azaarenes and PAHs (Millemann 1984). This study suggested that *C. tentans* is more sensitive to OSPW than *C. riparius*. However, comparison with available literature indicates that no one species is always most or least sensitive to all contaminants, and that

this ranking shifts with environmental conditions and history of the population used (Phipps et al. 1995).

Besides macroinvertebrates, various macrophytes, algae, ducks, and swallows were observed at OSPW wetlands, although no fish were observed. Despite the fact that OSPW contains several potentially toxic classes of compounds (metals, PAHs, other hydrocarbons), wetlands that receive OSPW support populations of midges that are apparently unaffected by these compounds in terms of teratogenicity. *In situ* tests were unable to determine if field populations were adapted to OSPW, due to limitations of the equipment. However, although the lack of teratogenicity seen in field specimens was repeated among both lab and field populations in a laboratory bioassay using sensitive first instar larvae, differences in survival and particularly growth pointed towards adaptation in a field-derived *Chironomus tentans* population.

Wetlands are important not only in terms of energy and nutrient cycling, but also in their ability to improve water quality through settling and removal of contaminants (Hook 1993, Horstman et al. 1998). Aquatic invertebrates play a vital role in nutrient pathways in wetland ecosystems, transferring energy and nutrients fixed by autotrophic organisms into food sources consumed by a variety of wildlife (Horstman et al. 1998).

Wetlands also provide unique habitat for a variety of organisms (Hook 1993, Horstman et al. 1998). Over half of the original area of wetlands in the United States has been lost due to drainage or filling for agriculture or urbanization (Tiner 1984). Similar losses have occurred in various regions of Canada, including the development of 97% of coastal wetlands of the US mainland of the Detroit River (Tulen 1998). This loss of

nesting and foraging habitat is the primary factor in reduced populations of several species of birds in North America, as over 50% of U.S. federally threatened and endangered migratory nongame bird species require wetlands (US Fish and Wildlife Service 1987). However, mine-associated wetlands with suitable hydrology and emergent macrophytes provide replacement habitat and even allow for range expansion of waterfowl (Horstman et al. 1998). Thus the wet landscape option being considered could provide new habitat not only for migratory birds, but also for the other biotic components that are found in wetlands and that may be endangered due to lack of suitable habitat.

The separate analysis of each biotic component of the wetlands, leading to the integrated assessment of viability in the wetland ecosystem may then be realized by examining the conclusions drawn from this study alongside those taken from studies on the other biotic groups. Effects of OSPW on benthos could easily be reflected in other biotic groups to which the macroinvertebrates are linked trophically and ecologically. This could involve ducks and swallows, which feed on benthos (especially chironomids), as well as fish, should any colonize the constructed wetlands (Murkin and Batt 1987). Together, related responses between components can be characterized and compared. This will allow focus to be applied towards safeguarding the more sensitive biota (pending results from other researchers studying the other biota in the wetlands), and ensuring that wetlands receiving OSPW can maintain levels of biological diversity and production seen in pre-mining and reference habitats. Viability of these wetlands will depend on the ability of colonizing organisms to tolerate the OSPW contaminants and establish themselves in these new habitats.

## **Conclusion**

**This study has indicated that OSPW does exert some effects on the benthic macroinvertebrate community in terms of taxa richness. Effects on chironomids were also detected, in terms of individual and community response. The chironomid biomarker used indicated no teratogenicity in relation to OSPW, either in field or laboratory. Thus, depending on the degree to which OSPW is diluted in wetlands, it could directly reduce wetland viability in terms of its benthos.**

**Tolerance of a field population was suggested in a laboratory bioassay. The resident communities present at two of the pilot OSPW wetlands studied indicate that at least a moderate degree of diversity and productivity may be achieved.**

**Thus, the wetland reclamation option appears feasible at the scale currently under use, and should allow for the establishment of viable macroinvertebrate communities. An overall assessment of wetland viability will require evaluation integrated across all trophic levels of the ecosystem.**

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## APPENDICES

Appendix 2.1: Replicate reading of water chemistry values at 15 Fort McMurray Wetlands using Hydrolab multimeter on July 12, 1997.

Site Code	sample no.	temperature (deg C)	depth (cm)	spec. cond (uS/cm)	dis. oxygen (mg/L)	pH
TR1	1	19.96	55	257	4.11	7.19
	2	21.01	29	256	3.87	7.19
	3	20.8	51	257	3.08	7.15
TR2	1	18.91	90	155	3.13	7.08
	2	20.35	54	145	5.99	7.12
SP	1	18.4	48	225	6.26	7.42
	2	17.95	59	236	5.77	7.37
	3	18.53	58	236	5.54	7.39
TR3 (SFU)	1	17.78	100	215	3.59	7.16
TR3 (U of W)	1	16.96	71	193	2.4	6.91
TR4	1	20.93	40	239	7.41	7.6
	2	21.04	47	242	6.91	7.56
TR5	1	18.31	41	176	7.25	7.37
PCR	1	24.64	32	257	11.27*+	8.92
	2	22.93	52	248	11.34*+	9.01
	3	22.31	63	250	10.25*+	8.76
RL	1	24.96	28	248	9.6+	9.06
	2	24	45	247	8.64+	8.99
SYN	1	24.87	46	1605	14*+	9.3
	2	24.45	62	1600	14.6*+	9.33
OM	1	24.6	38	2083	9.63+	8.33
SW	1	23.95	40	407	16.58*+	8.73
	2	24.48	70	390	19.15*+	8.91
SB	1	26.51	29	2266	20*+	9.3
CL	1	24.73	66	962	2.38+	8.14
	2	24.49	100	948	5.98+	8.41
HS	1	24.44	55	2712	8.2+	8.04
	2	24.28	28	2711	6.88+	7.89
NW	1	24.75	38	1596	3.17+	8.08
HW	1	23.22	27	2236	7.01+	7.83
	2	23.54	43	2257	6.7+	7.79

\* values suspect; saturation level greatly exceeded.

+ point at which oxygen values are suspect - meter malfunction?

Appendix 2.2: Mean values of water/ site parameters of 15 Fort McMurray Wetlands measured using Hydrolab multimeter on July 12, 1997

CODE	temperature (deg C)	depth (cm)	spec. cond (uS/cm)	dis. oxygen mg/L	pH	Size of water body	Depth (m)
TR1	21	45	257	3.69	7.18	M-L	moderate
TR2	20	72	150	4.56	7.10		
SP	18	55	232	5.86	7.39	M-L	shallow
TR3(SFU)	18	100	215	3.59	7.16		
TR3	17	71	193	2.40	6.91	S	shallow
TR4	21	44	241	7.16	7.58	M-L	shallow
TR5	18	41	176	7.25	7.37	S	shallow
PCR	23	49	252	10.95*+	8.90	VL	deep
RL	24	37	248	9.12+	9.03	VL	deep
SYN	25	54	1603	14.30*+	9.32	L-VL	shallow
OM	25	38	2083	9.63+	8.33	S	NR
SW	24	55	399	17.87*+	8.82	M	moderate
SB	27	29	2266	20.00*+	9.30	M-L	moderate
CL	25	83	955	4.18+	8.28	VL	deep
HS	24	42	2712	7.54+	7.97	M	shallow
NW	25	38	1596	3.17+	8.08	L	shallow
HW	23	35	2247	6.855+	7.81	S	shallow
MR	NR	NR	NR	NR	NR	M-L	NR

\* values suspect; saturation level greatly exceeded.

+ point at which oxygen values are suspect - meter malfunction?

Appendix 2.3: Water/ site parameters of 15 sites measured on benthos sampling dates (1997) using YSI meters

CODE	pH		Conductivity (uS/cm)		Water Temp (deg C)	D.O. (mg/L)
	water	sediment	water	sediment		
TR1			190		19	
TR2						
SP	8	8	130		15	3.2
TR3(SFU)						
TR3					22	
TR4					17	
TR5					18	
PC			420		19	
RL					18	
SYN	8.9	8	1215		17	
OM						
SW			400		19	
SB	8.2	7.1	1925		20	
CL	8	7.4	800			
HS	7.9	7.1	2350	2150	21	
NW	8	7.6	1360		18	
HW	8	7.4	1600		21	
MR	7.5	6.9	325	390	16	4.7

Appendix 2.4: Substrate Particle Size frequency (%) and Organic Content (LOI) at 15 sites in Fort McMurray Area, collected in summer 1997 (median size represented by bold face entry)

Site Code	LOI (%)	Particle Size (% of total ash weight) retained by sieves (aperture size in mm)								
		8	4	2	1	0.5	0.25	0.125	0.09	<0.09
TR1	6.7	0	1	0	0	1	9	28	21	40
TR2										
SP	4.4	1	0	0	0	0	1	12	48	37
TR3(SFU)										
TR3										
TR4										
TR5										
PC	11.7	0	0	0	0	4	9	23	14	50
RL	8.9	0	0	0	1	3	12	42	20	21
SYN	1.5	7	3	2	2	24	45	12	2	3
OM										
SW	4.8	6	0	0	2	10	14	27	12	28
SB										
CL	7.1	0	1	0	0	1	3	44	24	26
HS	13.6	4	0	1	2	10	24	22	13	24
NW	5.3	2	1	0	1	4	9	31	21	29
HW		1	1	0	0	2	12	48	13	23
MR	2.4	13	5	6	6	13	28	16	4	10
Wentworth $\phi$		-3	-2	-1	0	1	2	3	3.5	$\geq 4$

Appendix 2.5a: Abundance of aquatic taxa at five Fort McMurray Wetlands in 1997

Taxon	Code	TR1	SP	T3	T4	T5
Oligochaeta	AnAnOl	103	3432	1531	558	1201
Hirudinea	AnAnHi	0	1	0	0	0
Glossiphonia complanata	AnAnHiRhGlGl	0	0	0	0	0
Placobdella parasitica	AnAnHiRhGlPl	17	0	0	0	0
Helobdella stagnalis	AnAnHiRhGlHe	0	0	0	0	0
Batrachobdella picta	AnAnHiRhGlBa	0	0	0	1	0
Gastropoda	AnMoGa	17	46	50	0	0
Physidae	AnMoGaPuPh	0	0	0	0	0
Physa	AnMoGaPuPhPh	0	0	0	2	0
Planorbidae	AnMoGaPuPl	0	0	0	0	0
Armiger crista	AnMoGaPuPlAr	12	5	0	0	0
Planorbula campestris	AnMoGaPuPlPu	0	1	0	0	0
Helisoma	AnMoGaPuPlHe	3	1	0	0	0
Promenetus umbilicatellus	AnMoGaPuPlPr	3	5	2	0	78
Stagnicola	AnMoGaPuLySt	2	0	0	0	0
Amnicola limosa	AnMoGaPrHyAm	1	84	11	0	0
Sphaeriidae	AnMoBiLaSp	18	0	177	1	9
Hydrachnidia	AnArArAcHy	6	37	50	7	0
Daphniidae	AnArCrClDa	58	0	0	0	0
Simocephalus	AnArCrClDaSi	0	0	22	0	0
Daphnia	AnArCrClDaDa	0	0	0	0	0
Ceriodaphnia	AnArCrClDaCe	0	0	305	6	0
Polyphemus pediculus	AnArCrClPoPo	0	0	0	0	0
Chydoridae	AnArCrClCh	4	0	0	0	0
Calanoida	AnArCrCa	0	0	0	0	0
Cyclopoida	AnArCrCy	13	92	173	2	0
Harpacticoida	AnArCrHa	0	0	0	0	0
Ostracoda	AnArCrOs	33	46	484	542	465
Amphipoda	AnArCrAm	1	0	0	0	0
Hyalella azteca	AnArCrAmTaHy	9	47	0	0	0
Gammarus lacustris	AnArCrAmGaGa	0	0	6	0	0
immature Ephemeroptera	AnArInEp	2	0	0	18	0
Baetidae	AnArInEpBa	1	147	11	8	0
Baetis	AnArInEpBaBa	0	0	0	0	0
Centroptilum	AnArInEpBaCe	0	21	0	0	0
Caenis	AnArInEpCaCa	31	0	22	5	0
Siphonurus	AnArInEpSiSi	9	0	11	4	0
Parameletus	AnArInEpSiPa	0	0	0	0	0
Anisoptera immature	AnArInOd-1	1	0	0	0	0
Aeshna	AnArInOdAeAe	1	1	1	0	0
Anax	AnArInOdAeAn	0	0	0	0	0
Somatochlora	AnArInOdCoSo	0	0	0	0	0
Cordulia shurtleffi	AnArInOdCoCo	2	1	0	0	0
Sympetrum	AnArInOdLiSy	7	0	1	0	0

Taxon	Code	TR1	SP	T3	T4	T5
Pachydiplax longipennis	AnArInOdLiPx	5	0	0	0	0
Libellula	AnArInOdLiLi	0	0	0	0	0
Leucorrhinia	AnArInOdLiLe	0	7	0	0	0
Zygoptera immature	AnArInOd-2	0	0	0	0	0
Lestes	AnArInOdLeLe	16	0	0	0	0
Enallagma	AnArInOdCeEn	2	0	0	0	0
Ishnura	AnArInOdCels	0	0	0	0	0
Coenagrion	AnArInOdCeCe	4	0	0	0	0
Notonectidae immature	AnArInHeNo	0	0	0	0	0
Buenoa	AnArInHeNoBu	0	0	0	0	0
Notonecta	AnArInHeNoNo	0	0	1	0	0
Corixidae immature	AnArInHeCo-1	1	0	0	7	0
Corixidae male	AnArInHeCo-2	0	0	0	0	0
Corixidae female	AnArInHeCo-3	0	0	0	0	0
Corisella	AnArInHeCoCo	0	0	0	0	0
Gerris	AnArInHeGeGe	0	5	0	0	0
Limnoporus	AnArInHeGeLp	0	5	0	0	0
Mesovelia	AnArInHeMeMe	0	0	0	0	0
Lepidoptera larvae	AnArInLe	0	0	0	0	0
Pyrilidae	AnArInLePy	0	0	0	0	0
Acentria	AnArInLePyAc	0	0	0	0	0
Trichoptera	AnArInTr	3	0	11	0	0
Amiocentrus	AnArInTrBrAm	0	0	0	0	0
Ceraclea	AnArInTrLeCe	0	0	0	0	0
Lepidostoma	AnArInTrLmLm	0	37	0	0	0
Limnephilidae	AnArInTrLi	0	27	0	2	9
Phryganeidae	AnArInTrPg	0	0	0	0	4
Dysticidae larvae	AnArInCoDy	1	0	0	0	0
Graphoderus	AnArInCoDyGr	2	0	0	0	0
Rhantus	AnArInCoDyRh	0	0	0	0	0
Colymbetes	AnArInCoDyCo	0	0	0	0	0
Ilybius	AnArInCoDyll	2	0	0	1	0
Agabus	AnArInCoDyAu	1	0	0	0	0
Dytiscus	AnArInCoDyDy	0	0	1	0	0
Hydaticus	AnArInCoDyHt	1	0	0	0	0
Deronectes	AnArInCoDyDe	0	0	0	0	0
Liodessus	AnArInCoDyLi	0	0	0	0	0
Laccornis	AnArInCoDyLr	0	0	0	0	0
Laccophilus	AnArInCoDyLl	0	0	0	0	0
Gyrinus	AnArInCoGyGy	0	0	0	0	0
Haliphus	AnArInCoHaHa	0	5	0	0	0
Diptera larvae	AnArInDi	0	0	0	0	0
Ceratopogonidae	AnArInDiCe	0	48	871	13	41
Bezzia	AnArInDiCeBe	1	0	0	0	0

Taxon	Code	TR1	SP	T3	T4	T5
Chironomidae larvae	AnArInDiCh-1	88	4899	1324	669	361
Chironomidae pupae	AnArInDiCh-2	1	5	22	6	27
Dixella	AnArInDiDiDi	0	0	0	0	0
Psychodidae	AnArInDiPs	0	0	0	0	0
Tipulidae larvae	AnArInDiTi	0	0	0	0	0
Prionocera	AnArInDiTiPr	0	6	0	0	0
Phalacrocer	AnArInDiTiPh	0	27	0	0	0
Hemerodromia	AnArInDiEmHe	0	0	0	0	0
Sciomyzidae	AnArInDiSz	0	0	0	0	0
tadpole	?	1	0	0	0	0
brook stickleback	?	1	0	0	0	0
fish larva	?	0	0	0	2	19
<b>Total</b>		<b>484</b>	<b>9043</b>	<b>5087</b>	<b>1853</b>	<b>2214</b>
<b>No. 'Other' taxa</b>		<b>31</b>	<b>22</b>	<b>15</b>	<b>12</b>	<b>5</b>
fresh sorted biomass (g)		0.0	64.3	53.0	35.0	123.5
dry sorted biomass (g)		5.6	10.7	7.5	4.8	17.0
				0.0		
fresh unsorted biomass (g)		0.0	61.7	59.7	7.8	76.5
dry unsorted biomass (g)		0.0	8.6	9.1	1.7	12.8
				0.0		
total fresh biomass (g)		0.0	125.9	112.7	42.7	200.0
total dry biomass (g)		5.6	19.3	16.6	6.5	29.8

Appendix 2.5b: Abundance of aquatic taxa at five Fort McMurray Wetlands in 1997

Taxon	Code	RL	PCR	SW	MR	NW
Oligochaeta	AnAnOl	18128	265	3019	2233	312
Hirudinea	AnAnHi	0	0	0	5	0
Glossiphonia complanata	AnAnHiRhGIGI	0	0	0	0	0
Placobdella parasitica	AnAnHiRhGIPI	1	0	0	0	0
Helobdella stagnalis	AnAnHiRhGIHe	0	0	0	0	0
Batrachobdella picta	AnAnHiRhGIBa	0	0	0	0	0
Gastropoda	AnMoGa	0	0	0	1	0
Physidae	AnMoGaPuPh	0	0	0	0	0
Physa	AnMoGaPuPhPh	0	0	22	1	0
Planorbidae	AnMoGaPuPI	0	0	0	0	0
Armiger crista	AnMoGaPuPIAr	91	0	0	0	0
Planorbula campestris	AnMoGaPuPIPu	0	0	0	1	0
Helisoma	AnMoGaPuPIHe	0	0	0	3	0
Promenetus umbilicatellus	AnMoGaPuPIPr	322	0	37	11	0
Stagnicola	AnMoGaPuLySt	0	0	0	2	0
Amnicola limosa	AnMoGaPrHyAm	0	19	25	0	0
Sphaeriidae	AnMoBiLaSp	363	0	0	39	0
Hydrachnidia	AnArArAchHy	214	15	0	0	0
Daphniidae	AnArCrCIDA	0	0	0	0	0
Simocephalus	AnArCrCIDA Si	141	0	147	10	1361
Daphnia	AnArCrCIDA Da	1013	95	30	0	0
Ceriodaphnia	AnArCrCIDA Ce	731	0	28	0	0
Polyphemus pediculus	AnArCrCIPoPo	0	0	0	0	0
Chydoridae	AnArCrCICH	0	784	6	0	0
Calanoida	AnArCrCa	0	0	0	0	51
Cyclopoida	AnArCrCy	3288	663	50	97	470
Harpacticoida	AnArCrHa	0	119	0	0	0
Ostracoda	AnArCrOs	10115	285	308	126	6547
Amphipoda	AnArCrAm	0	0	0	0	0
Hyalella azteca	AnArCrAmTaHy	0	23	41	149	0
Gammarus lacustris	AnArCrAmGaGa	0	0	119	0	0
immature Ephemeroptera	AnArInEp	0	0	0	0	0
Baetidae	AnArInEpBa	0	3	0	6	0
Baetis	AnArInEpBaBa	0	0	0	0	0
Centroptilum	AnArInEpBaCe	1	0	0	0	0
Caenis	AnArInEpCaCa	0	0	80	16	0
Siphonurus	AnArInEpSiSi	0	0	6	9	0
Parameletus	AnArInEpSiPa	0	0	0	0	0
Anisoptera immature	AnArInOd-1	0	3	0	0	0
Aeshna	AnArInOdAcAc	0	0	0	16	10
Anax	AnArInOdAcAn	0	0	0	0	0
Somatochlora	AnArInOdCoSo	0	3	0	0	0
Cordulia shurtleffi	AnArInOdCoCo	0	0	1	0	0
Sympetrum	AnArInOdLiSy	1	0	11	0	0

Taxon	Code	RL	PCR	SW	MR	NW
Pachydiplax longipennis	AnArInOdLiPx	0	0	0	0	0
Libellula	AnArInOdLiLi	1	0	1	0	0
Leucorrhinia	AnArInOdLiLe	0	0	1	0	0
Zygoptera immature	AnArInOd-2	91	0	0	0	0
Lestes	AnArInOdLeLe	0	3	11	2	36
Enallagma	AnArInOdCeEn	1	4	3	0	0
Ishnura	AnArInOdCels	0	0	0	5	0
Coenagrion	AnArInOdCeCe	0	0	0	0	0
Notonectidae immature	AnArInHeNo	0	0	0	0	10
Buenoa	AnArInHeNoBu	0	0	0	0	0
Notonecta	AnArInHeNoNo	0	0	1	0	0
Corixidae immature	AnArInHeCo-1	0	0	78	43	260
Corixidae male	AnArInHeCo-2	0	0	0	0	21
Corixidae female	AnArInHeCo-3	0	0	0	0	0
Corisella	AnArInHeCoCo	0	0	0	5	0
Gerris	AnArInHeGeGe	91	0	0	0	0
Limnoporus	AnArInHeGeLp	0	0	0	15	0
Mesovelia	AnArInHeMcMe	0	0	0	0	0
Lepidoptera larvae	AnArInLe	0	0	0	5	0
Pyralidae	AnArInLePy	0	0	0	1	0
Acentria	AnArInLePyAc	1	0	0	0	0
Trichoptera	AnArInTr	0	0	0	0	0
Amiocentrus	AnArInTrBrAm	0	0	0	0	0
Ceraclea	AnArInTrLeCe	0	0	0	0	0
Lepidostoma	AnArInTrLmLm	0	0	0	0	0
Limnephilidae	AnArInTrLi	0	0	0	0	0
Phryganeidae	AnArInTrPg	0	0	0	0	0
Dysticidae larvae	AnArInCoDy	0	0	0	0	0
Graphoderus	AnArInCoDyGr	1	0	0	0	1
Rhantus	AnArInCoDyRh	1	0	0	0	1
Colymbetes	AnArInCoDyCo	0	0	0	0	1
Ilybius	AnArInCoDyIl	91	0	0	5	10
Agabus	AnArInCoDyAu	0	0	2	0	0
Dytiscus	AnArInCoDyDy	0	0	0	0	0
Hydaticus	AnArInCoDyHt	0	0	0	0	0
Deronectes	AnArInCoDyDe	0	0	6	0	0
Liodessus	AnArInCoDyLi	0	0	6	0	0
Laccornis	AnArInCoDyLr	0	0	0	0	0
Laccophilus	AnArInCoDyLl	0	0	0	0	0
Gyrinus	AnArInCoGyGy	0	0	0	0	0
Haliphus	AnArInCoHaHa	91	5	14	0	0
Diptera larvae	AnArInDi	0	0	0	29	0
Ceratopogonidae	AnArInDiCe	1	3	30	401	56
Bezzia	AnArInDiCeBe	0	0	0	0	0

<b>Taxon</b>	<b>Code</b>	<b>RL</b>	<b>PCR</b>	<b>SW</b>	<b>MR</b>	<b>NW</b>
Chironomidae larvae	AnArInDiCh-1	9036	1009	1210	1924	3631
Chironomidae pupae	AnArInDiCh-2	143	13	30	5	97
Dixella	AnArInDiDiDI	0	0	0	11	0
Psychodidae	AnArInDiPs	0	0	0	5	0
Tipulidae larvae	AnArInDiTi	0	0	6	0	0
Prionocera	AnArInDiTiPr	0	0	0	0	0
Phalacrocer	AnArInDiTiPh	0	0	0	0	0
Hemerodromia	AnArInDiEmHe	0	0	0	11	0
Sciomyzidae	AnArInDiSz	0	0	0	11	0
tadpole	?	0	0	0	0	0
brook stickleback	?	0	0	0	0	0
fish larva	?	0	0	0	0	0
<b>Total</b>		<b>43957</b>	<b>3316</b>	<b>5328</b>	<b>5209</b>	<b>12875</b>
<b>No. taxa</b>		<b>17</b>	<b>10</b>	<b>21</b>	<b>27</b>	<b>10</b>
fresh sorted biomass (g)		158.5	77.9	29.1	130.9	0.0
dry sorted biomass (g)		14.0	10.1	3.8	10.4	4.2
fresh unsorted biomass (g)		247.3	83.3	21.4	23.0	0.0
dry unsorted biomass (g)		29.4	13.8	3.4	2.5	7.3
total fresh biomass (g)		405.8	645.0	50.4	153.9	0.0
total dry biomass (g)		43.4	23.9	7.2	12.9	11.5

Appendix 2.5c: Abundance of aquatic taxa at five Fort McMurray Wetlands in 1997

Taxon	Code	HW	CL	HS	SYN	SB
Oligochaeta	AnAnOl	0	1267	471	131	924
Hirudinea	AnAnHi	0	0	0	0	0
Glossiphonia complanata	AnAnHiRhGIGl	0	36	0	0	0
Placobdella parasitica	AnAnHiRhGIPl	0	0	0	0	0
Helobdella stagnalis	AnAnHiRhGIHe	0	74	0	0	0
Batrachobdella picta	AnAnHiRhGIBa	0	0	0	0	0
Gastropoda	AnMoGa	0	0	0	0	0
Physidae	AnMoGaPuPh	0	0	0	0	2
Physa	AnMoGaPuPhPh	0	0	0	0	0
Planorbidae	AnMoGaPuPl	0	0	3	0	0
Armiger crista	AnMoGaPuPIAr	0	0	0	0	0
Planorbula campestris	AnMoGaPuPIPu	0	0	9	0	13
Helisoma	AnMoGaPuPIHe	0	3	1	0	0
Promenetus umbilicatus	AnMoGaPuPIPr	0	0	0	0	0
Stagnicola	AnMoGaPuLySt	0	0	0	0	0
Ammicola limosa	AnMoGaPrHyAm	0	35	0	0	7
Sphaeriidae	AnMoBiLaSp	0	0	0	0	0
Hydrachnidia	AnArArAchHy	0	187	0	44	33
Daphniidae	AnArCrClDa	0	0	0	0	0
Simocephalus	AnArCrClDaSi	0	906	711	455	1288
Daphnia	AnArCrClDaDa	0	3258	0	0	0
Ceriodaphnia	AnArCrClDaCe	0	0	0	44	0
Polyphemus pediculus	AnArCrClPoPo	0	0	35	0	0
Chydoridae	AnArCrClCh	0	0	1023	0	0
Calanoida	AnArCrCa	0	0	0	0	7
Cyclopoida	AnArCrCy	0	1510	401	89	300
Harpacticoida	AnArCrHa	0	34	0	0	0
Ostracoda	AnArCrOs	130	2362	1097	819	285
Amphipoda	AnArCrAm	0	0	0	0	0
Hyalella azteca	AnArCrAmTaHy	0	570	0	155	117
Gammarus lacustris	AnArCrAmGaGa	0	469	0	0	0
immature Ephemeroptera	AnArInEp	0	0	0	0	0
Baetidae	AnArInEpBa	0	0	0	0	0
Baetis	AnArInEpBaBa	0	0	0	0	42
Centroptilum	AnArInEpBaCe	0	0	0	201	13
Caenis	AnArInEpCaCa	0	70	0	62	0
Siphonurus	AnArInEpSiSi	0	0	0	0	0
Parameletus	AnArInEpSiPa	0	0	0	0	42
Anisoptera immature	AnArInOd-1	0	0	0	0	0
Aeshna	AnArInOdAeAc	7	0	44	0	14
Anax	AnArInOdAeAn	0	0	0	0	0
Somatochlora	AnArInOdCoSo	0	0	9	31	0
Cordulia shurtleffi	AnArInOdCoCo	0	0	0	8	0
Sympetrum	AnArInOdLiSy	0	0	0	0	0

Taxon	Code	HW	CL	HS	SYN	SB
<i>Pachydiplax longipennis</i>	AnArInOdLiPx	0	0	0	0	0
<i>Libellula</i>	AnArInOdLiLi	0	0	1	9	5
<i>Leucorrhinia</i>	AnArInOdLiLe	0	0	0	0	1
Zygoptera immature	AnArInOd-2	0	0	0	0	0
<i>Lestes</i>	AnArInOdiLeLe	24	0	21	41	0
<i>Enallagma</i>	AnArInOdCeEn	0	0	46	40	7
<i>Ishnura</i>	AnArInOdCeIs	0	0	1	0	0
<i>Coenagrion</i>	AnArInOdCeCe	0	0	0	0	0
Notonectidae immature	AnArInHeNo	0	0	0	0	0
<i>Buenoa</i>	AnArInHeNoBu	0	0	0	0	7
<i>Notonecta</i>	AnArInHeNoNo	0	3	0	9	0
Corixidae immature	AnArInHeCo-1	0	1	0	39	88
Corixidae male	AnArInHeCo-2	0	0	0	0	0
Corixidae female	AnArInHeCo-3	0	0	0	0	0
<i>Corisella</i>	AnArInHeCoCo	5	0	0	0	0
<i>Gerris</i>	AnArInHeGeGe	0	0	0	0	0
<i>Limnopus</i>	AnArInHeGeLp	5	0	0	0	0
<i>Mesovelia</i>	AnArInHeMeMe	0	0	0	0	0
Lepidoptera larvae	AnArInLe	0	0	0	0	0
Pyralidae	AnArInLePy	0	0	0	0	0
Acentria	AnArInLePyAc	0	0	0	0	0
Trichoptera	AnArInTr	0	0	55	0	0
<i>Amiocentrus</i>	AnArInTrBrAm	0	0	0	8	0
<i>Ceraclea</i>	AnArInTrLeCe	0	0	0	19	0
<i>Lepidostoma</i>	AnArInTrLmLm	0	1	0	0	0
Limnephilidae	AnArInTrLi	0	0	0	0	0
Phryganeidae	AnArInTrPg	0	0	0	0	0
Dysticidae larvae	AnArInCoDy	0	0	0	8	0
<i>Graphoderus</i>	AnArInCoDyGr	0	0	0	0	0
<i>Rhantus</i>	AnArInCoDyRh	0	1	0	0	0
<i>Colymbetes</i>	AnArInCoDyCo	0	0	0	0	0
<i>Ilybius</i>	AnArInCoDyIl	0	0	9	8	42
<i>Agabus</i>	AnArInCoDyAu	0	0	0	0	0
<i>Dytiscus</i>	AnArInCoDyDy	0	0	0	0	0
<i>Hydaticus</i>	AnArInCoDyHt	0	0	0	0	0
<i>Deronectes</i>	AnArInCoDyDe	0	0	0	0	0
<i>Liodessus</i>	AnArInCoDyLi	0	0	0	0	0
<i>Laccornis</i>	AnArInCoDyLr	0	0	0	0	7
<i>Laccophilus</i>	AnArInCoDyLl	0	0	0	0	7
<i>Gyrinus</i>	AnArInCoGyGy	0	0	0	0	7
<i>Halipus</i>	AnArInCoHaHa	0	0	0	0	20
Diptera larvae	AnArInDi	0	0	0	0	0
Ceratopogonidae	AnArInDiCe	0	35	9	0	0
<i>Bezzia</i>	AnArInDiCeBe	0	0	0	0	0

<b>Taxon</b>	<b>Code</b>	<b>HW</b>	<b>CL</b>	<b>HS</b>	<b>SYN</b>	<b>SB</b>
Chironomidae larvae	AnArInDiCh-1	3167	2184	2088	1475	1486
Chironomidae pupae	AnArInDiCh-2	37	1	9	23	0
Dixella	AnArInDiDiDI	0	0	0	0	0
Psychodidae	AnArInDiPs	0	0	0	0	0
Tipulidae larvae	AnArInDiTi	0	0	0	0	0
Prionocera	AnArInDiTiPr	0	0	0	0	0
Phalacrocer	AnArInDiTiPh	0	0	0	0	0
Hemerodromia	AnArInDiEmHe	0	0	0	0	0
Sciomyzidae	AnArInDiSz	0	0	0	12	0
tadpole	?	0	0	0	0	0
brook stickleback	?	0	0	0	0	0
fish larva	?	0	0	0	0	0
<b>Total</b>		<b>3374</b>	<b>13008</b>	<b>6043</b>	<b>3730</b>	<b>4765</b>
<b>No. taxa</b>		<b>4</b>	<b>13</b>	<b>12</b>	<b>16</b>	<b>19</b>
fresh sorted biomass (g)		100.8	70.8	21.5	27.7	18.7
dry sorted biomass (g)		8.0	7.2	3.3	4.2	2.9
fresh unsorted biomass (g)		26.3	68.1	25.6	47.5	42.0
dry unsorted biomass (g)		2.1	8.4	4.0	6.4	6.5
total fresh biomass (g)		127.1	138.8	47.1	75.1	60.8
total dry biomass (g)		10.1	15.5	7.3	10.6	9.4

Appendix 2.6a: Relative Abundance (% of total per sample) of Aquatic Taxa at five Fort McMurray Wetlands in 1997

Name	Code	TR1	SP	T3	T4	T5
Oligochaeta	AnAnOl	21.3	38.0	30.1	30.1	54.2
Hirudinea	AnAnHi	0.0	0.0	0.0	0.0	0.0
Glossiphonia complanata	AnAnHiRhGI	0.0	0.0	0.0	0.0	0.0
Placobdella parasitica	AnAnHiRhGIPI	3.5	0.0	0.0	0.0	0.0
Helobdella stagnalis	AnAnHiRhGIHe	0.0	0.0	0.0	0.0	0.0
Batrachobdella picta	AnAnHiRhGIBa	0.0	0.0	0.0	0.1	0.0
Gastropoda	AnMoGa	3.5	0.5	1.0	0.0	0.0
Physidae	AnMoGaPuPh	0.0	0.0	0.0	0.0	0.0
Physa	AnMoGaPuPhPh	0.0	0.0	0.0	0.1	0.0
Planorbidae	AnMoGaPuPI	0.0	0.0	0.0	0.0	0.0
Armiger crista	AnMoGaPuPIAr	2.5	0.1	0.0	0.0	0.0
Planorbula campestris	AnMoGaPuPIPu	0.0	0.0	0.0	0.0	0.0
Helisoma	AnMoGaPuPIHe	0.6	0.0	0.0	0.0	0.0
Promenetus umbilicatellus	AnMoGaPuPIPr	0.6	0.1	0.0	0.0	3.5
Stagnicola	AnMoGaPuLySt	0.4	0.0	0.0	0.0	0.0
Amnicola limosa	AnMoGaPrHyAm	0.2	0.9	0.2	0.0	0.0
Sphaeriidae	AnMoBiLaSp	3.7	0.0	3.5	0.1	0.4
Hydrachnidia	AnArArAchHy	1.2	0.4	1.0	0.4	0.0
Daphniidae	AnArCrClDa	12.0	0.0	0.0	0.0	0.0
Simocephalus	AnArCrClDaSi	0.0	0.0	0.4	0.0	0.0
Daphnia	AnArCrClDaDa	0.0	0.0	0.0	0.0	0.0
Ceriodaphnia	AnArCrClDaCe	0.0	0.0	6.0	0.3	0.0
Polyphemus pediculus	AnArCrClPoPo	0.0	0.0	0.0	0.0	0.0
Chydoridae	AnArCrClCh	0.8	0.0	0.0	0.0	0.0
Calanoida	AnArCrCa	0.0	0.0	0.0	0.0	0.0
Cyclopoida	AnArCrCy	2.7	1.0	3.4	0.1	0.0
Harpacticoida	AnArCrHa	0.0	0.0	0.0	0.0	0.0
Ostracoda	AnArCrOs	6.8	0.5	9.5	29.3	21.0
Amphipoda	AnArCrAm	0.2	0.0	0.0	0.0	0.0
Hyalella azteca	AnArCrAmTaHy	1.9	0.5	0.0	0.0	0.0
Gammarus lacustris	AnArCrAmGaGa	0.0	0.0	0.1	0.0	0.0
immature Ephemeroptera	AnArInEp	0.4	0.0	0.0	1.0	0.0
Baetidae	AnArInEpBa	0.2	1.6	0.2	0.4	0.0
Baetis	AnArInEpBaBa	0.0	0.0	0.0	0.0	0.0
Centroptilum	AnArInEpBaCe	0.0	0.2	0.0	0.0	0.0
Caenis	AnArInEpCaCa	6.4	0.0	0.4	0.3	0.0
Siphonurus	AnArInEpSiSi	1.9	0.0	0.2	0.2	0.0
Parameletus	AnArInEpSiPa	0.0	0.0	0.0	0.0	0.0
Anisoptera immature	AnArInOd-1	0.2	0.0	0.0	0.0	0.0
Aeshna	AnArInOdAeAe	0.2	0.0	0.0	0.0	0.0
Anax	AnArInOdAeAn	0.0	0.0	0.0	0.0	0.0
Somatochlora	AnArInOdCoSo	0.0	0.0	0.0	0.0	0.0
Cordulia shurtleffi	AnArInOdCoCo	0.4	0.0	0.0	0.0	0.0
Sympetrum	AnArInOdLiSy	1.4	0.0	0.0	0.0	0.0

Name	Code	TR1	SP	T3	T4	T5
Pachydiplax longipennis	AnArInOdLiPx	1.0	0.0	0.0	0.0	0.0
Libellula	AnArInOdLiLi	0.0	0.0	0.0	0.0	0.0
Leucorrhinia	AnArInOdLiLe	0.0	0.1	0.0	0.0	0.0
Zygoptera immature	AnArInOd-2	0.0	0.0	0.0	0.0	0.0
Lestes	AnArInOdLeLe	3.3	0.0	0.0	0.0	0.0
Enallagma	AnArInOdCeEn	0.4	0.0	0.0	0.0	0.0
Ishnura	AnArInOdCeIs	0.0	0.0	0.0	0.0	0.0
Coenagrion	AnArInOdCeCe	0.8	0.0	0.0	0.0	0.0
Notonectidae immature	AnArInHeNo	0.0	0.0	0.0	0.0	0.0
Buenoa	AnArInHeNoBu	0.0	0.0	0.0	0.0	0.0
Notonecta	AnArInHeNoNo	0.0	0.0	0.0	0.0	0.0
Corixidae immature	AnArInHeCo-1	0.2	0.0	0.0	0.4	0.0
Corixidae male	AnArInHeCo-2	0.0	0.0	0.0	0.0	0.0
Corixidae female	AnArInHeCo-3	0.0	0.0	0.0	0.0	0.0
Corisella	AnArInHeCoCo	0.0	0.0	0.0	0.0	0.0
Gerris	AnArInHeGeGe	0.0	0.1	0.0	0.0	0.0
Limnoporus	AnArInHeGeLp	0.0	0.1	0.0	0.0	0.0
Mesovelia	AnArInHeMeMe	0.0	0.0	0.0	0.0	0.0
Lepidoptera larvae	AnArInLe	0.0	0.0	0.0	0.0	0.0
Pyralidae	AnArInLePy	0.0	0.0	0.0	0.0	0.0
Acentria	AnArInLePyAc	0.0	0.0	0.0	0.0	0.0
Trichoptera	AnArInTr	0.6	0.0	0.2	0.0	0.0
Amiocentrus	AnArInTrBrAm	0.0	0.0	0.0	0.0	0.0
Ceraclea	AnArInTrLeCe	0.0	0.0	0.0	0.0	0.0
Lepidostoma	AnArInTrLmLm	0.0	0.4	0.0	0.0	0.0
Limnephilidae	AnArInTrLi	0.0	0.3	0.0	0.1	0.4
Phryganeidae	AnArInTrPg	0.0	0.0	0.0	0.0	0.2
Dysticidae larvae	AnArInCoDy	0.2	0.0	0.0	0.0	0.0
Graphoderus	AnArInCoDyGr	0.4	0.0	0.0	0.0	0.0
Rhantus	AnArInCoDyRh	0.0	0.0	0.0	0.0	0.0
Colymbetes	AnArInCoDyCo	0.0	0.0	0.0	0.0	0.0
Ilybius	AnArInCoDyIl	0.4	0.0	0.0	0.1	0.0
Agabus	AnArInCoDyAu	0.2	0.0	0.0	0.0	0.0
Dytiscus	AnArInCoDyDy	0.0	0.0	0.0	0.0	0.0
Hydaticus	AnArInCoDyHt	0.2	0.0	0.0	0.0	0.0
Deronectes	AnArInCoDyDe	0.0	0.0	0.0	0.0	0.0
Liodessus	AnArInCoDyLi	0.0	0.0	0.0	0.0	0.0
Laccornis	AnArInCoDyLr	0.0	0.0	0.0	0.0	0.0
Laccophilus	AnArInCoDyLl	0.0	0.0	0.0	0.0	0.0
Gyrinus	AnArInCoGyGy	0.0	0.0	0.0	0.0	0.0
Haliphus	AnArInCoHaHa	0.0	0.1	0.0	0.0	0.0
Diptera larvae	AnArInDi	0.0	0.0	0.0	0.0	0.0
Ceratopogonidae	AnArInDiCe	0.0	0.5	17.1	0.7	1.8
Bezzia	AnArInDiCeBe	0.2	0.0	0.0	0.0	0.0

<b>Taxon</b>	<b>Code</b>	<b>TR1</b>	<b>SP</b>	<b>T3</b>	<b>T4</b>	<b>T5</b>
Chironomidae larvae	AnArInDiCh-1	18.2	54.2	26.0	36.1	16.3
Chironomidae pupae	AnArInDiCh-2	0.2	0.1	0.4	0.3	1.2
Dixella	AnArInDiDiDI	0.0	0.0	0.0	0.0	0.0
Psychodidae	AnArInDiPs	0.0	0.0	0.0	0.0	0.0
Tipulidae larvae	AnArInDiTi	0.0	0.0	0.0	0.0	0.0
Prionocera	AnArInDiTiPr	0.0	0.1	0.0	0.0	0.0
Phalacrocer	AnArInDiTiPh	0.0	0.3	0.0	0.0	0.0
Hemerodromia	AnArInDiEmHe	0.0	0.0	0.0	0.0	0.0
Sciomyzidae	AnArInDiSz	0.0	0.0	0.0	0.0	0.0
tadpole	?	0.2	0.0	0.0	0.0	0.0
brook stickleback	?	0.2	0.0	0.0	0.0	0.0
fish larva	?	0.0	0.0	0.0	0.1	0.8
<b>Total</b>		<b>484</b>	<b>9043</b>	<b>5087</b>	<b>1853</b>	<b>2214</b>
<b>No. taxa</b>		<b>31</b>	<b>22</b>	<b>15</b>	<b>12</b>	<b>5</b>
total fresh biomass (g)		0	125.9	112.7	42.7	200.0
total dry biomass (g)		5.55257	19.3	16.6	6.5	29.8

Appendix 2.6b: Relative Abundance (percent per sample) of Aquatic Taxa at five Fort McMurray Wetlands in 1997

Name	Code	RL	PCR	SW	MR	NW
Oligochaeta	AnAnOl	41.2	8.0	56.7	42.9	2.4
Hirudinea	AnAnHi	0.0	0.0	0.0	0.1	0.0
Glossiphonia complanata	AnAnHiRhGI	0.0	0.0	0.0	0.0	0.0
Placobdella parasitica	AnAnHiRhGIPI	0.0	0.0	0.0	0.0	0.0
Helobdella stagnalis	AnAnHiRhGIHe	0.0	0.0	0.0	0.0	0.0
Batrachobdella picta	AnAnHiRhGIBa	0.0	0.0	0.0	0.0	0.0
Gastropoda	AnMoGa	0.0	0.0	0.0	0.0	0.0
Physidae	AnMoGaPuPh	0.0	0.0	0.0	0.0	0.0
Physa	AnMoGaPuPhPh	0.0	0.0	0.4	0.0	0.0
Planorbidae	AnMoGaPuPI	0.0	0.0	0.0	0.0	0.0
Armiger crista	AnMoGaPuPIAr	0.2	0.0	0.0	0.0	0.0
Planorbula campestris	AnMoGaPuPIPu	0.0	0.0	0.0	0.0	0.0
Helisoma	AnMoGaPuPIHe	0.0	0.0	0.0	0.1	0.0
Promenetus umbilicatellus	AnMoGaPuPIPr	0.7	0.0	0.7	0.2	0.0
Stagnicola	AnMoGaPuLySt	0.0	0.0	0.0	0.0	0.0
Amnicola limosa	AnMoGaPrHyAm	0.0	0.6	0.5	0.0	0.0
Sphaeriidae	AnMoBiLaSp	0.8	0.0	0.0	0.8	0.0
Hydrachnidia	AnArArAcHy	0.5	0.5	0.0	0.0	0.0
Daphniidae	AnArCrCIDA	0.0	0.0	0.0	0.0	0.0
Simocephalus	AnArCrCIDA	0.3	0.0	2.8	0.2	10.6
Daphnia	AnArCrCIDA	2.3	2.9	0.6	0.0	0.0
Ceriodaphnia	AnArCrCIDA	1.7	0.0	0.5	0.0	0.0
Polyphemus pediculus	AnArCrCIPoPo	0.0	0.0	0.0	0.0	0.0
Chydoridae	AnArCrCICH	0.0	23.6	0.1	0.0	0.0
Calanoida	AnArCrCa	0.0	0.0	0.0	0.0	0.4
Cyclopoida	AnArCrCy	7.5	20.0	0.9	1.9	3.6
Harpacticoida	AnArCrHa	0.0	3.6	0.0	0.0	0.0
Ostracoda	AnArCrOs	23.0	8.6	5.8	2.4	50.8
Amphipoda	AnArCrAm	0.0	0.0	0.0	0.0	0.0
Hyalella azteca	AnArCrAmTaHy	0.0	0.7	0.8	2.9	0.0
Gammarus lacustris	AnArCrAmGaGa	0.0	0.0	2.2	0.0	0.0
immature Ephemeroptera	AnArInEp	0.0	0.0	0.0	0.0	0.0
Baetidae	AnArInEpBa	0.0	0.1	0.0	0.1	0.0
Baetis	AnArInEpBaBa	0.0	0.0	0.0	0.0	0.0
Centroptilum	AnArInEpBaCe	0.0	0.0	0.0	0.0	0.0
Caenis	AnArInEpCaCa	0.0	0.0	1.5	0.3	0.0
Siphonurus	AnArInEpSiSi	0.0	0.0	0.1	0.2	0.0
Parameletus	AnArInEpSiPa	0.0	0.0	0.0	0.0	0.0
Anisoptera immature	AnArInOd-l	0.0	0.1	0.0	0.0	0.0
Aeshna	AnArInOdAeAe	0.0	0.0	0.0	0.3	0.1
Anax	AnArInOdAeAn	0.0	0.0	0.0	0.0	0.0
Somatochlora	AnArInOdCoSo	0.0	0.1	0.0	0.0	0.0
Cordulia shurtleffi	AnArInOdCoCo	0.0	0.0	0.0	0.0	0.0
Sympetrum	AnArInOdLiSy	0.0	0.0	0.2	0.0	0.0

Name	Code	RL	PCR	SW	MR	NW
Pachydiplax longipennis	AnArInOdLiPx	0.0	0.0	0.0	0.0	0.0
Libellula	AnArInOdLiLi	0.0	0.0	0.0	0.0	0.0
Leucorrhinia	AnArInOdLiLe	0.0	0.0	0.0	0.0	0.0
Zygoptera immature	AnArInOd-2	0.2	0.0	0.0	0.0	0.0
Lestes	AnArInOdLeLe	0.0	0.1	0.2	0.0	0.3
Enallagma	AnArInOdCeEn	0.0	0.1	0.1	0.0	0.0
Ishnura	AnArInOdCels	0.0	0.0	0.0	0.1	0.0
Coenagrion	AnArInOdCeCe	0.0	0.0	0.0	0.0	0.0
Notonectidae immature	AnArInHeNo	0.0	0.0	0.0	0.0	0.1
Buenoa	AnArInHeNoBu	0.0	0.0	0.0	0.0	0.0
Notonecta	AnArInHeNoNo	0.0	0.0	0.0	0.0	0.0
Corixidae immature	AnArInHeCo-1	0.0	0.0	1.5	0.8	2.0
Corixidae male	AnArInHeCo-2	0.0	0.0	0.0	0.0	0.2
Corixidae female	AnArInHeCo-3	0.0	0.0	0.0	0.0	0.0
Corisella	AnArInHeCoCo	0.0	0.0	0.0	0.1	0.0
Gerris	AnArInHeGeGe	0.2	0.0	0.0	0.0	0.0
Limnoporus	AnArInHeGeLp	0.0	0.0	0.0	0.3	0.0
Mesovelia	AnArInHeMeMe	0.0	0.0	0.0	0.0	0.0
Lepidoptera larvae	AnArInLe	0.0	0.0	0.0	0.1	0.0
Pyralidae	AnArInLePy	0.0	0.0	0.0	0.0	0.0
Acentria	AnArInLePyAc	0.0	0.0	0.0	0.0	0.0
Trichoptera	AnArInTr	0.0	0.0	0.0	0.0	0.0
Amiocentrus	AnArInTrBrAm	0.0	0.0	0.0	0.0	0.0
Ceraclea	AnArInTrLeCe	0.0	0.0	0.0	0.0	0.0
Lepidostoma	AnArInTrLmLm	0.0	0.0	0.0	0.0	0.0
Limnephilidae	AnArInTrLi	0.0	0.0	0.0	0.0	0.0
Phryganeidae	AnArInTrPg	0.0	0.0	0.0	0.0	0.0
Dysticidae larvae	AnArInCoDy	0.0	0.0	0.0	0.0	0.0
Graphoderus	AnArInCoDyGr	0.0	0.0	0.0	0.0	0.0
Rhantus	AnArInCoDyRh	0.0	0.0	0.0	0.0	0.0
Colymbetes	AnArInCoDyCo	0.0	0.0	0.0	0.0	0.0
Ilybius	AnArInCoDyIl	0.2	0.0	0.0	0.1	0.1
Agabus	AnArInCoDyAu	0.0	0.0	0.0	0.0	0.0
Dytiscus	AnArInCoDyDy	0.0	0.0	0.0	0.0	0.0
Hydaticus	AnArInCoDyHt	0.0	0.0	0.0	0.0	0.0
Deronectes	AnArInCoDyDe	0.0	0.0	0.1	0.0	0.0
Liodessus	AnArInCoDyLi	0.0	0.0	0.1	0.0	0.0
Laccornis	AnArInCoDyLr	0.0	0.0	0.0	0.0	0.0
Laccophilus	AnArInCoDyLl	0.0	0.0	0.0	0.0	0.0
Gyrinus	AnArInCoGyGy	0.0	0.0	0.0	0.0	0.0
Haliplus	AnArInCoHaHa	0.2	0.2	0.3	0.0	0.0
Diptera larvae	AnArInDi	0.0	0.0	0.0	0.6	0.0
Ceratopogonidae	AnArInDiCe	0.0	0.1	0.6	7.7	0.4
Bezzia	AnArInDiCeBe	0.0	0.0	0.0	0.0	0.0

<b>Taxon</b>	<b>Code</b>	<b>RL</b>	<b>PCR</b>	<b>SW</b>	<b>MR</b>	<b>NW</b>
Chironomidae larvae	AnArInDiCh-1	20.6	30.4	22.7	36.9	28.2
Chironomidae pupae	AnArInDiCh-2	0.3	0.4	0.6	0.1	0.8
Dixella	AnArInDiDiDl	0.0	0.0	0.0	0.2	0.0
Psychodidae	AnArInDiPs	0.0	0.0	0.0	0.1	0.0
Tipulidae larvae	AnArInDiTi	0.0	0.0	0.1	0.0	0.0
Prionocera	AnArInDiTiPr	0.0	0.0	0.0	0.0	0.0
Phalacrocera	AnArInDiTiPh	0.0	0.0	0.0	0.0	0.0
Hemerodromia	AnArInDiEmHe	0.0	0.0	0.0	0.2	0.0
Sciomyzidae	AnArInDiSz	0.0	0.0	0.0	0.2	0.0
tadpole	?	0.0	0.0	0.0	0.0	0.0
brook stickleback	?	0.0	0.0	0.0	0.0	0.0
fish larva	?	0.0	0.0	0.0	0.0	0.0
<b>Total</b>		<b>43957</b>	<b>3316</b>	<b>5328</b>	<b>5209</b>	<b>12875</b>
<b>No. taxa</b>		<b>17</b>	<b>10</b>	<b>21</b>	<b>27</b>	<b>10</b>
total fresh biomass (g)		405.8074	645.0	50.4	153.9	0.0
total dry biomass (g)		43.37116	23.9	7.2	12.9	11.5

Appendix 2.6c: Relative Abundance (percent per sample) of Aquatic Taxa at five Fort McMurray Wetlands in 1997

Name	Code	HW	CL	HS	SYN	SB
Oligochaeta	AnAnOl	0.0	9.7	7.8	3.5	19.4
Hirudinea	AnAnHi	0.0	0.0	0.0	0.0	0.0
Glossiphonia complanata	AnAnHiRhGIGI	0.0	0.3	0.0	0.0	0.0
Placobdella parasitica	AnAnHiRhGIPI	0.0	0.0	0.0	0.0	0.0
Helobdella stagnalis	AnAnHiRhGIHe	0.0	0.6	0.0	0.0	0.0
Batrachobdella picta	AnAnHiRhGIba	0.0	0.0	0.0	0.0	0.0
Gastropoda	AnMoGa	0.0	0.0	0.0	0.0	0.0
Physidae	AnMoGaPuPh	0.0	0.0	0.0	0.0	0.0
Physa	AnMoGaPuPhPh	0.0	0.0	0.0	0.0	0.0
Planorbidae	AnMoGaPuPI	0.0	0.0	0.0	0.0	0.0
Armiger crista	AnMoGaPuPIAr	0.0	0.0	0.0	0.0	0.0
Planorbula campestris	AnMoGaPuPIPu	0.0	0.0	0.2	0.0	0.3
Helisoma	AnMoGaPuPIHe	0.0	0.0	0.0	0.0	0.0
Promenetus umbilicatellus	AnMoGaPuPIPr	0.0	0.0	0.0	0.0	0.0
Stagnicola	AnMoGaPuLySt	0.0	0.0	0.0	0.0	0.0
Amnicola limosa	AnMoGaPrHyAm	0.0	0.3	0.0	0.0	0.1
Sphaeriidae	AnMoBiLaSp	0.0	0.0	0.0	0.0	0.0
Hydrachnidia	AnArArAchHy	0.0	1.4	0.0	1.2	0.7
Daphniidae	AnArCrCIDa	0.0	0.0	0.0	0.0	0.0
Simocephalus	AnArCrCIDaSi	0.0	7.0	11.8	12.2	27.0
Daphnia	AnArCrCIDaDa	0.0	25.0	0.0	0.0	0.0
Ceriodaphnia	AnArCrCIDaCe	0.0	0.0	0.0	1.2	0.0
Polyphemus pediculus	AnArCrCIPoPo	0.0	0.0	0.6	0.0	0.0
Chydoridae	AnArCrCICH	0.0	0.0	16.9	0.0	0.0
Calanoida	AnArCrCa	0.0	0.0	0.0	0.0	0.1
Cyclopoida	AnArCrCy	0.0	11.6	6.6	2.4	6.3
Harpacticoida	AnArCrHa	0.0	0.3	0.0	0.0	0.0
Ostracoda	AnArCrOs	3.8	18.2	18.2	22.0	6.0
Amphipoda	AnArCrAm	0.0	0.0	0.0	0.0	0.0
Hyaella azteca	AnArCrAmTaHy	0.0	4.4	0.0	4.2	2.5
Gammarus lacustris	AnArCrAmGaGa	0.0	3.6	0.0	0.0	0.0
immature Ephemeroptera	AnArInEp	0.0	0.0	0.0	0.0	0.0
Baetidae	AnArInEpBa	0.0	0.0	0.0	0.0	0.0
Baetis	AnArInEpBaBa	0.0	0.0	0.0	0.0	0.9
Centroptilum	AnArInEpBaCe	0.0	0.0	0.0	5.4	0.3
Caenis	AnArInEpCaCa	0.0	0.5	0.0	1.7	0.0
Siphonurus	AnArInEpSiSi	0.0	0.0	0.0	0.0	0.0
Parameletus	AnArInEpSiPa	0.0	0.0	0.0	0.0	0.9
Anisoptera immature	AnArInOd-l	0.0	0.0	0.0	0.0	0.0
Aeshna	AnArInOdAeAe	0.2	0.0	0.7	0.0	0.3
Anax	AnArInOdAeAn	0.0	0.0	0.0	0.0	0.0
Somatochlora	AnArInOdCoSo	0.0	0.0	0.2	0.8	0.0
Cordulia shurtleffi	AnArInOdCoCo	0.0	0.0	0.0	0.2	0.0
Sympetrum	AnArInOdLiSy	0.0	0.0	0.0	0.0	0.0

Name	Code	HW	CL	HS	SYN	SB
Pachydiplax longipennis	AnArInOdLiPx	0.0	0.0	0.0	0.0	0.0
Libellula	AnArInOdLiLi	0.0	0.0	0.0	0.2	0.1
Leucorrhinia	AnArInOdLiLe	0.0	0.0	0.0	0.0	0.0
Zygoptera immature	AnArInOd-2	0.0	0.0	0.0	0.0	0.0
Lestes	AnArInOdLeLe	0.7	0.0	0.4	1.1	0.0
Enallagma	AnArInOdCeEn	0.0	0.0	0.8	1.1	0.1
Ishnura	AnArInOdCeIs	0.0	0.0	0.0	0.0	0.0
Coenagrion	AnArInOdCeCe	0.0	0.0	0.0	0.0	0.0
Notonectidae immature	AnArInHeNo	0.0	0.0	0.0	0.0	0.0
Buenoa	AnArInHeNoBu	0.0	0.0	0.0	0.0	0.1
Notonecta	AnArInHeNoNo	0.0	0.0	0.0	0.2	0.0
Corixidae immature	AnArInHeCo-1	0.0	0.0	0.0	1.0	1.9
Corixidae male	AnArInHeCo-2	0.0	0.0	0.0	0.0	0.0
Corixidae female	AnArInHeCo-3	0.0	0.0	0.0	0.0	0.0
Corisella	AnArInHeCoCo	0.1	0.0	0.0	0.0	0.0
Gerris	AnArInHeGeGe	0.0	0.0	0.0	0.0	0.0
Limnoporus	AnArInHeGeLp	0.1	0.0	0.0	0.0	0.0
Mesovelia	AnArInHeMeMe	0.0	0.0	0.0	0.0	0.0
Lepidoptera larvae	AnArInLe	0.0	0.0	0.0	0.0	0.0
Pyralidae	AnArInLePy	0.0	0.0	0.0	0.0	0.0
Acentria	AnArInLePyAc	0.0	0.0	0.0	0.0	0.0
Trichoptera	AnArInTr	0.0	0.0	0.9	0.0	0.0
Amiocentrus	AnArInTrBrAm	0.0	0.0	0.0	0.2	0.0
Ceraclea	AnArInTrLeCe	0.0	0.0	0.0	0.5	0.0
Lepidostoma	AnArInTrLmLm	0.0	0.0	0.0	0.0	0.0
Limnephilidae	AnArInTrLi	0.0	0.0	0.0	0.0	0.0
Phryganeidae	AnArInTrPg	0.0	0.0	0.0	0.0	0.0
Dysticidae larvae	AnArInCoDy	0.0	0.0	0.0	0.2	0.0
Graphoderus	AnArInCoDyGr	0.0	0.0	0.0	0.0	0.0
Rhantus	AnArInCoDyRh	0.0	0.0	0.0	0.0	0.0
Colymbetes	AnArInCoDyCo	0.0	0.0	0.0	0.0	0.0
Ilybius	AnArInCoDyIl	0.0	0.0	0.2	0.2	0.9
Agabus	AnArInCoDyAu	0.0	0.0	0.0	0.0	0.0
Dytiscus	AnArInCoDyDy	0.0	0.0	0.0	0.0	0.0
Hydaticus	AnArInCoDyHt	0.0	0.0	0.0	0.0	0.0
Deronectes	AnArInCoDyDe	0.0	0.0	0.0	0.0	0.0
Liodessus	AnArInCoDyLi	0.0	0.0	0.0	0.0	0.0
Laccornis	AnArInCoDyLr	0.0	0.0	0.0	0.0	0.1
Laccophilus	AnArInCoDyLl	0.0	0.0	0.0	0.0	0.1
Gyrinus	AnArInCoGyGy	0.0	0.0	0.0	0.0	0.1
Haliphus	AnArInCoHaHa	0.0	0.0	0.0	0.0	0.4
Diptera larvae	AnArInDi	0.0	0.0	0.0	0.0	0.0
Ceratopogonidae	AnArInDiCe	0.0	0.3	0.2	0.0	0.0
Bezzia	AnArInDiCeBe	0.0	0.0	0.0	0.0	0.0

<b>Taxon</b>	<b>Code</b>	<b>HW</b>	<b>CL</b>	<b>HS</b>	<b>SYN</b>	<b>SB</b>
Chironomidae larvae	AnArInDiCh-1	93.9	16.8	34.6	39.5	31.2
Chironomidae pupae	AnArInDiCh-2	1.1	0.0	0.2	0.6	0.0
Dixella	AnArInDiDiDI	0.0	0.0	0.0	0.0	0.0
Psychodidae	AnArInDiPs	0.0	0.0	0.0	0.0	0.0
Tipulidae larvae	AnArInDiTi	0.0	0.0	0.0	0.0	0.0
Prionocera	AnArInDiTiPr	0.0	0.0	0.0	0.0	0.0
Phalacrocer	AnArInDiTiPh	0.0	0.0	0.0	0.0	0.0
Hemerodromia	AnArInDiEmHe	0.0	0.0	0.0	0.0	0.0
Sciomyzidae	AnArInDiSz	0.0	0.0	0.0	0.3	0.0
tadpole	?	0.0	0.0	0.0	0.0	0.0
brook stickleback	?	0.0	0.0	0.0	0.0	0.0
fish larva	?	0.0	0.0	0.0	0.0	0.0
<b>Total</b>		<b>3374</b>	<b>13008</b>	<b>6043</b>	<b>3730</b>	<b>4765</b>
<b>No. taxa</b>		<b>4</b>	<b>13</b>	<b>12</b>	<b>16</b>	<b>19</b>
total fresh biomass (g)		127.0544	138.8	47.1	75.1	60.8
total dry biomass (g)		10.0755	15.5	7.3	10.6	9.4

Appendix 2.7a: Chironomids Collected in 1997 from wetlands of Fort McMurray Area. Taxa\* indicates that single individual counts were excluded.

TAXA	Tower Rd.1		Tower Rd.3		Tower Rd. 4	
	500 um	1 mm	500 um	1 mm	500 um	1 mm
Orthocladinae						
Cricotopus				1		
Cricotopus (trifascia)						
Cricotopus (Isocladius)						
Cricotopus (Hirvenoja)						1
Eukiefferellia						
Nanocladius			1		2	1
Psectrocladius			2	2		
Orthocladus						
Paratanytarsus			1		2	
Tanytarsus	1		3	2	4	1
Cladotanytarsus				1		
Lenziella				1		
Rheotanytarsus					1	
Endochironomus					1	
Chironomus	11	10	1	1	3	3
Cladopelma			5	2	2	1
Einfeldia						
Cryptotendipes						
Microtendipes	3		5	3	2	2
Paratendipes		1				
Dicrotendipes	1	1		1		1
Glyptotendipes	1					
Polypedilum		1		2		
Pseudochironomus						
Psectrotanypus						
Procladius	2	4	1	1		6
Djalmabatista						1
Ablabesmyia						
Guttipelopia	1	1				1
Paramerima		2				
Monopelopia						
Trissopelopia						
Labrundinia						
Larsia			1	1	1	1
Thienemannimyia				1		
TOTAL	20	20	20	19	18	19
NO. TAXA	7	7	9	13	9	11
No. Taxa *	3	3	4	5	6	3

Appendix 2.7b: Chironomids Collected in 1997 from wetlands of Fort McMurray area. Taxa\* indicates that single individual counts were excluded.

TAXA	Tower Rd.5		Spruce Pond		Ruth Lake	
	500 um	1 mm	500 um	1 mm	500 um	1 mm
Orthocladinae						
Cricotopus						
Cricotopus (trifascia)			8	10		
Cricotopus (Isocladius)			3	1		3
Cricotopus (Hirvenoja)		1				
Eukiefferellia	1					
Nanocladius		1				
Psectrocladius	2	1	2	3		
Orthocladius						
Paratanytarsus	1		3	1	2	5
Tanytarsus		2	2	1	6	6
Cladotanytarsus						
Lenziella		1				
Rheotanytarsus						
Endochironomus			1		1	1
Chironomus			1		1	2
Cladopelma		1				
Einfeldia						
Cryptotendipes		6				1
Microtendipes				3		
Paratendipes		1				
Dicrotendipes						
Glyptotendipes						
Polypedilum	1			1	1	
Pseudochironomus						
Psectrotanypus						
Procladius		1			2	
Djalmabatista						
Ablabesmyia						
Guttipelopia						
Paramerima						
Monopelopia						
Trissopelopia						
Labrundinia						
Larsia						1
Thienemannimyia						
TOTAL	5	15	20	20	13	19
NO. TAXA	4	9	7	7	6	7
No. Taxa *	1	2	5	3	3	4

Appendix 2.7c: Chironomids collected in 1997 from wetlands of Fort McMurray area. Taxa\* indicates that single individual counts were excluded.

TAXA	Poplar Creek Res.		Muskeg River		Syncrude wetland	
	500 um	1 mm	500 um	1 mm	500 um	1 mm
Orthocladinae					2	
Cricotopus					1	
Cricotopus (trifascia)					1	
Cricotopus (Isocladus)						
Cricotopus (Hirvenoja)						
Eukiefferellia						
Nanocladus						
Psectrocladius			1	3		1
Orthocladus						
Paratanytarsus	4	3			2	
Tanytarsus		2	5	1	6	
Cladotanytarsus						
Lenziella						
Rheotanytarsus						
Endochironomus	7	2				
Chironomus	1	3	5			
Cladopelma					1	2
Einfeldia					1	
Cryptotendipes					4	1
Microtendipes	4	5	2	2		
Paratendipes						
Dicrotendipes	1	1	1		1	1
Glyptotendipes						
Polypedilum	1		1			1
Pseudochironomus				1		
Psectrotanypus						
Procladius			1	4		
Djalmabatista						
Ablabesmyia				1		1
Guttipelopia	1	2				
Paramerina		2				
Monopelopia			1	1		
Trissopelopia				1		
Labrundinia				1		
Larsia			1	5		
Thienemannimyia						
TOTAL	19	20	18	20	17	7
NO. TAXA	7	8	9	10	9	6
No. Taxa *	3	7	3	4	4	1

Appendix 2.7d: Chironomids collected in 1997 from wetlands of Fort McMurray area. Taxa\* indicates that single individual counts were excluded.

TAXA	South Bison Pond		Shallow Wetland		Hummocks		High Sulphate Pond	
	500 um	1 mm	500 um	1 mm	500 um	1 mm	500 um	1 mm
Orthocladinae								
Cricotopus								
Cricotopus (trifascia)								
Cricotopus (Isocladius)					1	2		
Cricotopus (Hirvenoja)					1	2		
Eukiefferellia								
Nanocladius								1
Psectrocladius		1	1	6	10	10		
Orthocladius					1			
Paratanytarsus	1	1	5	3			5	4
Tanytarsus	14	6	10	6			9	6
Cladotanytarsus								
Lenziella								
Rheotanytarsus								
Endochironomus		3	2					
Chironomus	2	7	1	2			3	3
Cladopelma		1						
Einfeldia								
Cryptotendipes								
Microtendipes								
Paratendipes								
Dicrotendipes								
Glyptotendipes	2	1	1				1	1
Polypedilum								
Pseudochironomus				2				
Psectrotanypus	1				2	6		1
Procladius							1	
Djalmabatista								
Ablabesmyia				1				3
Guttipelopia								
Paramerima								
Monopelopia								
Trissopelopia								
Labrundinia								
Larsia								
Thienemannimyia								
TOTAL	20	20	20	20	15	20	19	19
NO. TAXA	5	7	6	6	5	4	5	7
No. Taxa *	3	3	3	5	2	4	3	4

Appendix 2.7e: Chironomids collected in 1997 from wetlands of Fort McMurray area. Taxa\* indicates that single individual counts were excluded.

TAXA	Natural Wetlands			Crane Lake	
	250 um	500 um	1 mm	500 um	1 mm
Orthocladinae					
Cricotopus		1			
Cricotopus (trifascia)					
Cricotopus (Isocladus)	1	1	8		7
Cricotopus (Hirvenoja)					
Eukiefferellia					
Nanocladus					1
Psectrocladius					
Orthocladus				1	
Paratanytarsus		6		1	5
Tanytarsus	3	4	6		
Cladotanytarsus	2		1		
Lenziella	4	6			
Rheotanytarsus					
Endochironomus				1	1
Chironomus				7	6
Cladopelma					
Einfeldia					
Cryptotendipes					
Microtendipes					
Paratendipes					
Dicrotendipes		2			
Glyptotendipes					
Polypedilum					
Pseudochironomus					
Psectrotanypus			5		
Procladius					
Djalmabatista					
Ablabesmyia					
Guttipelopia					
Paramerina					
Monopelopia					
Trissopelopia					
Labrundinia					
Larsia					
Thienemannimyia					
TOTAL	10	20	20	10	20
NO. TAXA	4	6	4	4	5
No. Taxa *	3	4	3	1	3

Appendix 2.8: 1998 sites sampled, sampling dates, and environmental parameters / water and sediment chemistry data. Water-affected sites are bold-faced (n=9).

Site	Sample date	Size	air (deg C)	water (deg C)	Cond (uS/cm)	salinity (ppt)	D.O. (mg/L)		Sed. ORP (mV)	pH
		Size					shallow	deep		
NW	03-Jun	m-l	17	15	1700	1	8.6		-162	8.03
NWE	16-Jun	s	16	17	1200	0.7	9	8.9	-91	
HW	06-Jun	s	20	21	1250	0.7	13.6	2.2	-300	7.87
HS	06-Jun	s-m	18	19	2700	1.5	5.8	3	-200	8.03
CL	03-Jun	l	22	18	850	0.4	10.2	10.2	-100	8.8
CRM	17-Jun	s-m	26	18	800	0.3	5	4.4	-58	7.57
CRWW	17-Jun	s	21	24	1350	0.8	7.9		-132	8.1
MFTN	07-Jul	m	25	25	1680	1	7.2	7.8	-133	8.97
MFTS	07-Jul	m	25	25	1690	1	7.4	6.6	-175	8.85
SM	13-Jul	m		20	1600	1.2	9.2	8	-82	8.42
TP5	09-Jun	s-m	19	19	1900	1	9.4	8.2	-110	9.15
TP2	09-Jun	s-m	19	18	500	0.1	10.8	10.8		9.31
RTP	19-Jun	s-m	23	25	720	0.1	6.8	6	-63	8.11
SB	05-Jun	m-l	20	18	2000	1	10.2		-172	8.56
DP	19-Jun	m	23	21	800	0.3	7.6	7.2	-127	9.12
SW	05-Jun	m	18	19	420	0.1	9.8	7	-92	8.29
WID	08-Jun	s-m	23	23	470		13.4	13.4	-105	
WIDP	08-Jun	s-m	23	21	550		13.2		-113	
SYN	11-Jun	l	28	27	2190	1.2	6.8	3.8	-131	
SD	11-Jun	m	26	21	1200	0.8	13.4	5.8	-117	
SL	04-Jun	l	16	16	4700	3	4.8	3		
HW63	07-Jun	m-l	23	20	700	0.3	12.8	10	-190	8.33
TR1	21-Jun	m-l	23	22	220	0	8.2	8	-55	
SP	21-Jun	m	25	22	350	0	8	7.2	-118	8.02
TR3C	21-Jun	s		22	170	0	9.2	8.4	-92	
TR3M	21-Jun	m-l	23	17	180	0	5.2	2.5	-79	
TR4	20-Jun	s-m	27	27	210	0	8.3		-137	
TR5	20-Jun	s	25	24	280	0	8.2	6.6	-173	
HL	26-Jun	l		25	1180	0.5	3.2	1.2	-75	
PCR	10-Jul	l	31	32	390	0	7	6.6	-104	
PCO	10-Jul	l	32	30	345	0	7.6	7.3	90	
MR	25-Jun	m-l	24	23	270	0	9.3	7.6	-106	
BAM	25-Jun	m	26	25	200	0	8.9	8.8	-30	
BRW	25-Jun	l	26	23	450	0	9.4	8.4	-61	
I63W	25-Jun	s	24	21	1420	0.8	10	6.6	-59	
FMW	25-Jun	l	20	20	1490	0.9			-59	

Appendix 2.9: Sediment Characteristics (Water Content, Loss-On-Ignition, and Particle Size Distribution) of Samples Collected in 1998. Water-affected sites are bold-faced.

Site	Moisture % by wt	LOI % d.w.	Particle Size Distribution (mass (g) sediment per size fraction)							
			8 mm	4 mm	1 mm	500 um	250 um	125 um	90 um	<90 um
NW	77.6	30.6	0	0	0.11	0.3	0.83	2.51	2.05	5.38
NWE	37.4	8.1	0.9	0.84	2.09	4.78	13.32	20.49	6.13	12.09
HW	68.3	19.0	0	0	0.23	0.73	3.31	9.93	4.13	7.19
HS	56.6	18.5	0	0.36	0.97	3.78	10.44	7.84	3.36	7.88
CL	46.8	12.1	0	0	0.23	0.41	2.37	4.19	27.11	6.09
HW63	31.5	6.2	1.18	1.28	3.51	7.4	10.69	11.86	10.65	19.83
SW	31.0	6.8	0	0	1.63	10.08	14.49	12.96	13.35	9.97
DP	24.8	3.8	1.32	0.7	5.12	12.63	12.8	14.88	7.95	17.19
SYN	32.1	4.6	0	0	0.92	11.08	28.99	13.26	3.91	8.6
SB	69.8	28.4	0	0	0.08	0.94	3.24	5.06	3.31	8.14
TP2	28.6	5.7	0	0.28	2.59	11.92	17.58	15.74	7.47	14.76
TP5	31.8	4.1	0	1.2	2.67	8.94	15.16	15.34	7.11	14.98
RTP	26.4	4.7	0	0.41	3.64	11.45	14.96	14.68	5.65	18.4
SD	63.2	16.3	0	0.1	1.32	4.96	4.95	4.05	2.46	10.97
WID	41.0	5.6	0	0	0.46	5.85	10.86	10.28	8.43	22.9
WIDP	59.3	15.9	0	0	0.95	3.74	6.54	8.47	4.26	11.23
PCR	64.6	20.4	0	0	0.29	1.85	4.2	5.06	2.96	1.29
PCO	25.1	4.0	15.93	4.94	10.3	13.36	9.89	9.15	2.97	6.12
MFTS	43.6	13.4	0	0	0.42	4.25	14.38	14.71	6.73	8.67
MFTN	31.1	4.6	0	0.17	0.52	3.46	12.01	28.42	11.2	10.06
SM	58.1	16.7	0	1.7	0.42	1.28	3.35	9.95	6.76	11.19
TR1	48.3	10.4	0	0	0.81	3.71	8.99	10.48	4.36	20.29
SP	35.1	8.2	0	0	0.24	1.35	8.73	25.55	7.55	16
TR3M	56.7	15.7	0	0.71	1.07	4.34	5.65	7.7	4.27	11.7
TR4	23.5	3.8	7.18	0.61	1.64	7.45	12.88	16.47	7.06	19.17
TR5	32.7	3.3	0	0	0.03	0.39	15.17	44.19	3.83	5.42
SL	54.6	12.5	0	0	0.11	1.97	4.28	2.96	3.18	25.7
CRM	46.2	9.0	0	0.18	1.39	3.76	9.97	13.73	6.39	14.91
CRWW	31.8	6.2	0	0.31	1.63	7.51	13.81	16.81	7.2	16.47
HL	71.3	21.2	0	0	2.03	2.9	3.36	3.16	1.42	9.52
MRW	16.0	2.1	21.79	8.29	11.12	11.14	20.12	7.91	1.92	4.83
BRW	17.0	1.7	12.6	5.04	17.45	18.29	25.28	6.3	0.53	0.72
BAM	34.2	6.1	0	0	1.7	5.95	17.68	21.78	5.57	9.01
I63W	67.9	27.3	0	0	0.26	0.86	1.58	4.94	4.07	11.27
FMW	81.9	49.9	0	0.13	0.75	0.88	0.87	1.27	1.01	3.21

Appendix 5.1: Water parameters for OSPW Bioassay

day	species	treatment	rep	conductivity uS/cm	salinity ppt	temperature deg C	pH	D. O. mg/L
0	L	1	1	230	0	25	7.6	100
0	L	1	2	205	0	25	7.7	93
0	L	1	3	220	0	25	7.8	93
0	L	1	4	240	0	23	7.8	85
0	L	1	5	230	0	25	7.8	100
0	L	2	1	320	0	25	8.2	100
0	L	2	2	310	0	25	8.2	100
0	L	2	3	290	0	25	8.1	96
0	L	2	4	400	0	23	8.1	91
0	L	2	5	285	0	25	8.1	96
0	L	3	1	490	0	25	8.2	95
0	L	3	2	475	0	25	8.2	97
0	L	3	3	480	0	25	8.3	100
0	L	3	4	445	0	25	8.4	100
0	L	3	5	600	0.1	23	8.4	89
0	L	4	1	720	0.1	25	8.4	97
0	L	4	2	700	0.1	25	8.5	100
0	L	4	3	780	0.3	23	8.4	81
0	L	4	4	680	0.1	25	8.5	100
0	L	4	5	720	0.1	25	8.4	95
0	L	5	1	1220	0.7	23	8.6	89
0	L	5	2	990	0.4	25	8.6	100
0	L	5	3	1200	0.6	25	8.7	100
0	L	5	4	1300	0.6	25	8.6	96
0	L	5	5	1130	0.5	25	8.6	97
0	L	6	1	1720	1	25	8.8	100
0	L	6	2	2000	1.1	25	8.8	97
0	L	6	3	1960	1.1	25	8.8	100
0	L	6	4	1850	1	25	8.8	98
0	L	6	5	1700	1	25	8.8	97
7	L	1	1	280	0	24	8	88
7	L	1	2	280	0	24	7.9	88
7	L	1	3	260	0	24	7.9	85
7	L	1	4	280	0	24	7.8	77
7	L	1	5	230	0	24	7.8	88
7	L	2	1	240	0	24	8	81
7	L	2	2	480	0	24	8.1	85
7	L	2	3	400	0	24	8	86
7	L	2	4	400	0	24	8	79
7	L	2	5	440	0	24	8	86
7	L	3	1	560	0.1	24	8.2	86
7	L	3	2	570	0.1	24	8.1	81
7	L	3	3	580	0.1	24	8.1	77
7	L	3	4	550	0.1	24	8.1	83
7	L	3	5	700	0.1	24	8.3	75
7	L	4	1	810	0.2	24	8.2	78
7	L	4	2	900	0.2	24	8.2	76
7	L	4	3	800	0.2	24	8.4	77
7	L	4	4	860	0.3	24	8.3	83
7	L	4	5	800	0.2	24	8.2	76
7	L	5	1	1280	0.7	24	8.5	78
7	L	5	2	1280	0.6	24	8.4	78

Appendix 5.1: Water parameters for OSPW Bioassay

day	species	treatment	rep	conductivity uS/cm	salinity ppt	temperature deg C	pH	D. O. mg/L
7	L	5	3	1230	0.6	24	8.4	81
7	L	5	4	1300	0.6	24	8.3	75
7	L	5	5	1280	0.6	24	8.4	78
7	L	6	1	2450	1.4	24	8.7	81
7	L	6	2	2100	1.1	24	8.6	79
7	L	6	3	2180	1.3	24	8.7	83
7	L	6	4	2150	1.2	24	8.6	75
7	L	6	5	2200	1.3	24	8.7	76
14	L	1	1	340	0	23.5	7.8	72
14	L	1	2	310	0	24	7.9	70
14	L	1	3	330	0	24	7.8	56
14	L	1	5	300	0	23.5	7.6	59
14	L	2	1	480	0.1	23.5	7.8	48
14	L	2	2	400	0	23.5	7.8	67
14	L	2	3	450	0	24	8	65
14	L	2	5	410	0	24	7.8	54
14	L	3	1	530	0.1	24	8.1	65
14	L	3	2	580	0.1	24	8	60
14	L	3	3	280	0.1	23.5	7.7	45
14	L	3	4	600	0.1	23.5	7.8	52
14	L	4	1	800	0.2	24	7.9	56
14	L	4	2	810	0.2	23.5	7.9	48
14	L	4	4	750	0.3	23.5	7.7	64
14	L	4	5	820	0.2	24	8	46
14	L	5	2	1220	0.6	23.5	8	31
14	L	5	3	1180	0.5	23.5	7.9	54
14	L	5	4	1200	0.5	24	8.1	41
14	L	5	5	1220	0.6	24	8.1	58
14	L	6	1	1980	1.1	23.5	8.2	39
14	L	6	2	2080	1.1	24	8.5	49
14	L	6	3	1970	1.1	23.5	8.1	59
14	L	6	4	2040	1.2	23.5	8.2	45
14	L	6	5	2100	1.2	24	8.5	55
0	R	1	1	200	0	25	7.9	99
0	R	1	2	240	0	23	8	94
0	R	1	3	250	0	23	8	94
0	R	1	4	190	0	25	8.1	98
0	R	1	5	250	0	23	7.9	91
0	R	2	1	380	0	23	8.3	90
0	R	2	2	350	0	25	8.1	99
0	R	2	3	380	0	23	8.3	92
0	R	2	4	380	0	25	8.2	97
0	R	2	5	370	0	23	8.3	92
0	R	3	1	500	0.1	23	8.4	91
0	R	3	2	470	0.1	25	8.3	99
0	R	3	3	510	0.1	23	8.3	89
0	R	3	4	490	0	23	8.5	91
0	R	3	5	500	0	25	8.4	99
0	R	4	1	660	0.1	25	8.5	98
0	R	4	2	810	0.3	23	8.6	91
0	R	4	3	700	0.1	25	8.5	97
0	R	4	4	800	0.3	23	8.6	92

Appendix 5.1: Water parameters for OSPW Bioassay

day	species	treatment	rep	conductivity uS/cm	salinity ppt	temperature deg C	pH	D. O. mg/L
0	R	4	5	600	0.1	23	8.5	87
0	R	5	1	1150	0.7	23	8.8	82
0	R	5	2	1120	0.5	25	8.7	97
0	R	5	3	1220	0.6	25	8.7	97
0	R	5	4	1190	0.6	23	8.6	85
0	R	5	5	1260	0.7	23	8.8	96
0	R	6	1	1920	1	25	8.8	98
0	R	6	2	2320	1.5	23	9	90
0	R	6	3	2300	1.4	23	8.9	85
0	R	6	4	1930	1	25	8.8	97
0	R	6	5	2110	1.3	23	9	91
7	R	1	1	290	0	24	8	84
7	R	1	2	280	0	25	7.8	83
7	R	1	3	240	0	25	7.7	83
7	R	1	4	280	0	24	7.8	74
7	R	1	5	260	0	25	7.8	82
7	R	2	1	410	0	25	7.9	85
7	R	2	2	470	0	24	8	78
7	R	2	3	390	0	25	7.9	89
7	R	2	4	440	0	24	8.1	80
7	R	2	5	390	0	25	7.9	90
7	R	3	1	500	0.1	25	7.8	88
7	R	3	2	530	0.1	24	8	77
7	R	3	3	520	0.1	24	8	76
7	R	3	3	550	0.1	25	8	80
7	R	3	4	500	0.1	25	7.9	87
7	R	4	1	850	0.3	24	8.3	77
7	R	4	2	850	0.3	25	8	83
7	R	4	3	890	0.3	24	8.2	75
7	R	4	4	850	0.3	25	8.1	90
7	R	4	5	680	0.1	25	7.9	84
7	R	5	1	1180	0.5	25	8.2	80
7	R	5	2	1250	0.6	24	8.4	75
7	R	5	3	1250	0.6	24	8.4	79
7	R	5	4	1260	0.6	25	8	74
7	R	5	5	1310	0.6	25	8.3	83
7	R	6	1	2050	1.1	24	8.6	75
7	R	6	2	2380	2.5	25	8.6	76
7	R	6	3	2620	1.6	25	8.6	83
7	R	6	4	2040	1.1	24	8.6	79
7	R	6	5	2050	1.2	25	8.6	85
14	R	1	1	320	0	23.5	7.7	69
14	R	1	2	350	0	25	8	90
14	R	1	3	350	0	25	7.7	59
14	R	1	4	310	0	23.5	7.5	52
14	R	1	5	330	0	25	7.8	68
14	R	2	1	480	0.1	25	8	76
14	R	2	2	440	0	23.5	7.7	61
14	R	2	3	450	0	25	7.9	67
14	R	2	4	480	0.1	23.5	7.8	67
14	R	2	5	450	0	25	7.8	67
14	R	3	1	520	0.1	25	7.4	58

Appendix 5.1: Water parameters for OSPW Bioassay

day	species	treatment	rep	conductivity uS/cm	salinity ppt	temperature deg C	pH	D. O. mg/L
14	R	3	2	500	0.1	23.5	7.4	53
14	R	3	3	630	0.1	25	7.9	54
14	R	3	4	480	0.1	25	7.5	70
14	R	3	5	480	0.1	23.5	7.5	60
14	R	4	1	800	0.3	23.5	7.8	55
14	R	4	2	810	0.3	25	7.8	58
14	R	4	3	790	0.2	23.5	7.8	42
14	R	4	4	790	0.3	25	7.9	66
14	R	4	5	700	0.2	25	7.9	72
14	R	5	1	1160	0.5	25	8	49
14	R	5	2	1200	0.6	23.5	8	42
14	R	5	3	1190	0.5	23.5	8	59
14	R	5	4	1130	0.5	25	8.2	75
14	R	5	5	1120	0.5	25	8.1	69
14	R	6	1	1930	1	23.5	8.1	42
14	R	6	2	2000	1	25	8.4	56
14	R	6	3	2130	1.2	25	8.5	78
14	R	6	4	1970	1.2	23.5	8.3	53
14	R	6	5	2100	1	25	8.4	63
0	T	1	1	270	0	24	7.8	95
0	T	1	2	220	0	25	7.9	97
0	T	1	3	230	0	23	7.8	90
0	T	1	5	200	0	25	8	99
0	T	2	1	380	0	25	8.2	100
0	T	2	2	450	0	24	7.8	76
0	T	2	3	560	0	23	8.2	90
0	T	2	4	350	0	25	8.1	95
0	T	3	1	460	0	24	8.2	92
0	T	3	3	430	0	25	8.3	98
0	T	3	4	520	0	24	8.2	88
0	T	3	5	500	0	25	8.4	98
0	T	4	1	830	0.2	24	8.3	85
0	T	4	2	670	0.1	25	8.4	95
0	T	4	3	800	0.2	24	8.3	91
0	T	4	4	670	0.1	25	8.5	98
0	T	5	1	1170	0.6	23	8.5	83
0	T	5	2	1230	0.6	24	8.5	91
0	T	5	3	1250	0.6	24	8.5	89
0	T	5	4	1200	0.6	25	8.6	95
0	T	6	1	2280	1.3	23	8.8	81
0	T	6	2	2010	1.1	24	8.6	84
0	T	6	3	1880	1.1	25	8.8	96
0	T	6	4	1970	1.1	24	8.6	86
7	T	1	1	230	0	24	7.9	87
7	T	1	2	280	0	24	7.7	75
7	T	1	3	250	0	24	7.9	79
7	T	1	5	260	0	24	7.7	75
7	T	2	1	410	0	24	8	79
7	T	2	2	370	0	24	8	76
7	T	2	3	470	0	24	8.1	82
7	T	2	4	420	0	24	8	74
7	T	3	1	450	0	24	7.9	81

Appendix 5.1: Water parameters for OSPW Bioassay

day	species	treatment	rep	conductivity uS/cm	salinity ppt	temperature deg C	pH	D. O. mg/L
7	T	3	3	570	0.1	24	8	74
7	T	3	4	520	0	24	8.1	83
7	T	3	5	530	0.1	24	8.1	77
7	T	4	1	700	0.1	24	8	74
7	T	4	2	810	0.2	24	8.2	75
7	T	4	3	700	0.1	24	8.1	78
7	T	4	4	810	0.2	24	8.2	73
7	T	5	1	1240	0.5	24	8.4	79
7	T	5	2	1080	0.4	24	8.3	81
7	T	5	3	1100	0.5	24	8.4	85
7	T	5	4	1300	0.6	24	8.4	76
7	T	6	1	2180	1.2	24	8.6	61
7	T	6	2	1980	1.1	24	8.6	71
7	T	6	3	2200	1.3	24	8.7	79
7	T	6	4	1990	1.1	24	8.6	79
14	T	1	1	300	0	21	8.1	89
14	T	1	2	320	0	24	7.8	56
14	T	1	5	330	0	23.5	7.5	48
14	T	2	1	450	0	23.5	7.7	49
14	T	2	2	370	0	21	7.7	72
14	T	2	4	480	0	24	7.8	44
14	T	3	1	400	0.1	21	7.8	81
14	T	3	3	600	0.1	23.5	7.8	47
14	T	3	4	500	0.1	21	7.9	70
14	T	3	5	530	0.1	23.5	7.5	48
14	T	4	1	700	0.2	21	8	63
14	T	4	2	820	0.3	24	8.1	52
14	T	4	3	680	0.3	21	8.1	83
14	T	4	4	780	0.3	23.5	7.8	42
14	T	5	2	1020	0.5	21	8.2	87
14	T	5	3	960	0.4	21	8.2	82
14	T	5	4	1320	0.7	24	8.2	37
14	T	6	2	1900	1.1	21	8.5	64
14	T	6	3	2030	1.1	24	8.5	52
14	T	6	4	1950	1.2	21	8.5	71

Appendix 5.2: Water Chemistry Analyses (mg/L unless stated) for OSPW Biossary. Canadian Water Quality Guidelines for aquatic life are listed.

Test Item	Day 0 0-1	Day 0 0-2	mean Day 0 0%	Day 0 100-1	Day 0 100-2	mean Day 0 100%	Day 14 0-1	Day 14 0-2	Day 14 0%	Day 14 100-1	Day 14 100-2	mean Day 14 100%	Day 0 100%/0%	Day 14 100%/0%	0.00 day 14/day 0	1.00 day 14/day 0	CWQG mg/L
naphthene acid (mg/kg)	<1	2	<1	77	81	79	2	2	2	74	67	70.5					
naphthene acid (mg/kg)																	
(4, 8, 17 mg naphthene acids/kg for treatments 6.25%, 12.5%, and 25% OSPW, respectively)																	
pH																	
phosphorus-total	8.21	8.25	8.23	8.91	8.91	8.91	7.65	7.44	7.545	8.18	8.17	8.175	1.08	1.08	0.92	0.92	1.08
conductivity (uS/cm)	<0.05	0.007	0.007	0.067	0.067	0.067	0.642	1.09	0.866	1.31	1.07	1.19	9.60	1.37	123.70	17.76	17.76
hardness as CaCO3	257	275	266	2300	2290	2295	387	386	386.5	2200	2140	2170	8.63	5.61	1.45	0.95	0.95
nitrate-nitrogen dissolved	105	109	107	95.1	92.5	93.8	105	101	103	110	107	108.5	0.88	1.05	0.96	1.16	1.16
nitrite-nitrogen dissolved	0.86	0.98	0.92	0.19	0.2	0.195	0.1	0.01	0.055	0.01	1.42	0.715	0.21	13.00	0.06	3.67	0.60
nitrogen-total Kjeldahl	<2	0.4	0.4	6.2	6.5	6.35	19.7	21.1	20.4	19.9	17.6	18.75	15.60	3.76	51.00	2.95	2.95
alkalinity as bicarbonate	88	91	89.5	505	503	504	153	154	153.5	593	560	576.5	5.63	3.76	1.72	1.14	1.14
alkalinity as CaCO3	72	75	73.5	495	493	494	126	127	126.5	486	459	472.5	6.72	3.74	1.72	0.96	0.96
alkalinity as carbonate	<20	<20	<20	48	48	48	<20	<20	<20	<20	<20	<20	24.00			0.00	0.00
alkalinity as hydroxide	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10					
ammonia dissolved	0.13	0.16	0.145	5.2	5.5	5.35	15.9	15.3	15.6	11.4	8.76	10.08	36.90	0.65	107.59	1.88	1.88
solids-dissolved	130	140	135	1500	1500	1500	180	180	180	1400	1400	1400	11.11	7.78	1.33	0.93	0.93
sulphate-dissolved	27	30	28.5	628	615	621.5	35	34	34.5	556	528	542	21.81	15.71	1.21	0.87	0.87
total anions (mEq)	2.46	2.61	2.535	25	24.7	24.85	3.74	3.77	3.755	23.2	22.1	22.65	9.80	6.03	1.48	0.91	0.91
total cations (mEq)	2.44	2.52	2.48	22.5	21.9	22.2	2.48	2.63	2.555	21.8	21.5	21.65	8.95	8.47	1.03	0.98	0.98
aluminum-dissolved																	
aluminum-total	0.14	0.15	0.145	11.1	13	12.05	0.03	0.12	0.075	0.26	0.5	0.38	83.10	5.07	0.52	0.03	0.10
antimony-dissolved																	
antimony-total	<0.01	0.001	0.001	0.002	0.002	0.002	<0.001	<0.001	0.001	0.002	0.002	0.002	2.00	2.00	1.00	1.00	1.00
arsenic-dissolved																	
arsenic-total	0.013	0.014	0.0135	0.064	0.063	0.0635	0.029	0.032	0.0305	0.074	0.073	0.0735	4.70	2.41	2.26	1.16	0.01
barium-dissolved																	
barium-total	0.025	0.037	0.031	0.157	0.17	0.1635	0.32	0.0147	0.16735	0.108	0.101	0.1045	12.48	0.62	12.77	0.64	0.64
beryllium-dissolved																	
beryllium-total	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01					
bismuth-dissolved																	
bismuth-total	<0.001	<0.001	<0.001	<0.001	0.0001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001					
boron-dissolved																	
boron-total	0.03	0.03	0.03	2.95	2.87	2.91	0.09	0.02	0.055	2.78	2.8	2.79	97.00	50.73	1.83	0.96	0.96
cadmium-dissolved																	
cadmium-total	<0.002	<0.002	<0.002	0.0016	0.0015	0.00155	<0.002	<0.002	<0.002	0.0018	0.0017	0.00175	7.80	8.75	0.95	1.13	0.00
calcium-dissolved	28.7	30	29.35	11.2	11	11.1	28.6	27.4	28	18	15	16.5	0.38	0.59	0.95	1.49	1.49
calcium-total	30.9	34.8	32.85	13.4	14.3	13.85	27.5	28.9	28.2	18.2	16	17.1	0.42	0.61	0.86	1.23	1.23
cesium-dissolved																	
cesium-total	<0.001	<0.001	<0.001	0.0018	0.0021	0.00195	<0.001	<0.001	<0.001	0.0006	0.0006	0.0006	19.50	4.50	0.23	1.06	0.23
chloride-dissolved	14	15	14.5	62	61	61.5	18	19	18.5	66	64	65	4.24	3.51	1.28	1.06	1.06
chromium-dissolved																	
chromium-total	<0.02	<0.02	<0.02	0.014	0.015	0.0145	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	7.30			0.00	0.01
cobalt-dissolved																	
cobalt-total	<0.002	<0.002	<0.002	0.0031	0.0032	0.00315	0.006	0.008	0.007	0.0026	0.0027	0.00265	15.80	3.79	3.50	0.84	0.84
copper-dissolved																	
copper-total	0.01	0.004	0.007	0.005	0.005	0.005	0.01	0.01	0.01	0.009	0.01	0.0095	0.71	0.95	1.43	1.90	0.00
iron-dissolved																	
iron-total	<0.1	<0.1	<0.1	2.35	2.56	2.455	0.04	0.1	0.07	0.13	0.14	0.135	245.50	1.93	7.00	0.05	0.30
lead-dissolved																	
lead-total	0.014	0.006	0.01	0.003	0.0031	0.00305	<0.005	0.004	<0.005	<0.005	0.0027	0.0001	3.05	1.40	0.00	0.00	0.01
lithium-dissolved																	
lithium-total	0.002	0.002	0.002	0.245	0.248	0.2465	0.002	0.001	0.0015	0.199	0.207	0.2145	123.25	135.33	0.75	0.82	0.82
magnesium-dissolved	8.08	8.28	8.18	16.3	15.8	16.05	8.21	7.86	8.035	15.9	16.8	16.35	1.96	2.03	0.98	1.02	1.02
magnesium-total	8.23	8.78	8.505	18.4	18.3	18.35	8.26	8.52	8.39	17.4	17.6	17.5	2.16	2.09	0.99	0.95	0.95

Appendix 5.2: Water Chemistry Analyses (mg/L, unless stated) for OSPW Bioassay. Canadian Water Quality Guidelines for aquatic life are listed.

Test Item	Day 0 0-1	Day 0 0-2	mean Day 0 0%	Day 0 100-1	Day 0 100-2	mean Day 0 100%	Day 14 0-1	Day 14 0-2	mean Day 14 0%	Day 14 100-1	Day 14 100-2	mean Day 14 100%	Day 0 100% / 0%	Day 14 100% / 0%	0.00 day14 / day 0	1.00 day 14 / day 0	CWQG mg/L
manganese-dissolved							0.0084	0.0381	0.02325	0.0089	0.0182	0.01355		0.58			
manganese-total	0.015	0.0024	0.0087	0.0246	0.0304	0.0275	0.0021	0.0389	0.0205	0.0085	0.0231	0.0158	3.16	0.77	2.36	0.57	
molybdenum-dissolved							0.001	0.001	0.001	0.75	0.639	0.6945		694.50			
molybdenum-total	0.0011	0.001	0.00105	0.853	0.864	0.8585	0.001	0.0012	0.0011	0.762	0.774	0.768	817.62	698.18	1.05	0.89	0.07
nickel-dissolved							0.0029	0.0102	0.00655	0.0112	0.0105	0.01085		1.66			
nickel-total	<.002	<.002		0.017	0.018	0.0175	0.003	0.003	0.003	0.014	0.013	0.0135	8.80	4.50	1.50	0.77	0.07
potassium-dissolved	1.4	1.3	1.35	16.3	15.8	16.05	3.9	4.21	4.055	18.4	16.4	17.4	11.89	4.29	3.00	1.08	
potassium-total	1.3	1.4	1.35	19.1	18.9	19	3.92	3.93	3.925	18.5	18.5	18.5	14.07	4.71	2.91	0.97	
rubidium-dissolved							0.0028	0.0066	0.0047	0.0316	0.0296	0.0306		6.51			
rubidium-total	0.0011	0.0011	0.0011	0.0466	0.0494	0.048	0.0031	0.0032	0.00315	0.0291	0.0287	0.0289	43.64	9.17	2.86	0.60	
selenium-dissolved							<.002	<.002		0.002	0.003	0.0025		1.25			
selenium-total	<.002	<.002		0.005	0.005	0.005	<.002	<.002		0.003	0.004	0.0035	2.50	1.75		0.70	0.00
silicon-dissolved							1.4	1.1	1.25	3.1	3.6	3.35		2.68			
silver-dissolved							<.0002	<.0002		<.0002	<.0002						
silver-total	<.0004	<.0004		<.0004	<.0004		<.0004	<.0004		<.0004	<.0004						0.00
sodium-dissolved	7	7.2	7.1	463	450	456.5	8.61	9.81	9.21	441	438	439.5	64.30	47.72	1.30	0.96	
sodium-total	6.8	7.4	7.1	515	492	503.5	10.1	8.72	9.41	452	474	463	70.92	49.20	1.33	0.92	
strontium-dissolved							0.131	0.13	0.1305	0.682	0.61	0.646		4.95			
strontium-total	0.126	0.145	0.1355	0.72	0.739	0.7295	0.13	0.136	0.133	0.619	0.599	0.609	5.38	4.58	0.98	0.83	
tellurium-dissolved							<.0005	<.0005		<.0005	<.0005						
tellurium-total	<.001	<.001		<.001	<.001		<.001	<.001		<.001	<.001						
thallium-dissolved							<.0001	<.0001		0.0002	0.0001	0.00015		1.50			
thallium-total	<.0001	<.0001		0.0001	0.0002	0.00015	<.0001	<.0001		<.0001	<.0001		1.50			0.00	0.00
tin-dissolved							<.0002	<.0002		<.0002	<.0002						
tin-total	<.0005	<.0005		0.0013	0.0008	0.00105	0.0006	0.0072	0.0039	0.0005	0.0009	0.0007	2.10	0.18	7.80	0.67	
titanium-dissolved							<.0005	<.0005		0.0042	0.0025	0.00335		6.70			
titanium-total	<.0005	0.001		0.17	0.275	0.2225	0.0009	0.0042	0.00255	0.0092	0.0248	0.017	222.50	6.67	2.60	0.08	
tungsten-dissolved							<.0002	<.0002		0.0006	0.0007	0.00065		3.30			
tungsten-total	<.0002	<.0002		0.0008	0.001	0.0009	<.0002	<.0002		0.0006	0.001	0.0008	4.50	4.00		0.89	
uranium-dissolved							0.0005	0.0003	0.0004	0.0061	0.0059	0.006		15.00			
uranium-total	0.0005	0.0006	0.00055	0.0084	0.0086	0.0085	0.0006	0.0005	0.00055	0.0073	0.007	0.00715	15.45	13.00	1.00	0.84	
vanadium-dissolved							0.004	0.003	0.0035	0.021	0.022	0.0215		6.14			
vanadium-total	<.001	<.001		0.061	0.061	0.061	0.003	0.008	0.0055	0.024	0.04	0.032	61.00	5.82	5.50	0.52	
zinc-dissolved							0.006	0.08	0.043	0.067	0.088	0.0775		1.80			
zinc-total	<.02	<.02		<.02	<.02		0.03	0.03	0.03	0.03	0.02	0.025		0.83	1.50	1.30	0.03
zirconium-dissolved							0.0004	<.0002		0.0019	0.0013	0.0016		8.00			
zirconium-total	0.0002	0.0003	0.00025	0.0247	0.0266	0.02565	0.0013	0.0016	0.00145	0.0025	0.0025	0.0025	102.60	1.72	5.80	0.10	

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**Appendix 5.3a: Survival of chironomid larvae from population L following exposure to OSPW in a 14-d bioassay**

**Numbers of larvae alive plus pupae (live or dead) plus adults or pupal exuviae-S (whichever is highest)**

Treatment	Rep	POPULATION L		corrected larvae
		<i>C. tentans</i> larvae	P+(A or S)	
1	1	40	0	40
	2	30	0	30
	3	33	0	33
	4	38	0	38
	5	4	0	
2	1	15	0	15
	2	42	0	42
	3	63	0	50
	4	44	0	44
	5	18	0	
3	1	52	0	50
	2	39	0	39
	3	35	0	35
	4	14	0	14
	5	28	0	
4	1	21	0	21
	2	46	0	46
	3	35	0	35
	4	40	0	40
	5	20	0	
5	1	32	0	32
	2	10	0	10
	3	26	0	26
	4	22	0	22
	5	29	0	
6	1	8	0	8
	2	12	0	12
	3	25	0	25
	4	19	0	19
	5	4	0	

Appendix 5.3b: Survival and % larvae of chironomid larvae from population T following exposure to OSPW in a 14-d bioassay

Numbers of larvae alive plus pupae (live or dead) plus adults or pupal exuviae-S (whichever is highest)

Treatment	Rep	<i>C. tentans</i> total	P+(A or S)	corrected total	larvae	%L
1	1	51	0	50	51	100
	2	40	0	40	40	100
	3	42	22	42	20	48
	4	not used		not used		
	5	58	0	50	58	100
2	1	50	0	50	50	100
	2	60	0	50	60	100
	3	44	0	44	44	100
	4	67	0	50	67	100
	5	not used		not used		
3	1	51	0	50	51	100
	2	not used		not used		
	3	32	14	32	18	56
	4	7	0		7	100
	5	45	0	45	45	100
4	1	0	0		0	
	2	42	0	42	42	100
	3	48	0	48	48	100
	4	33	1	33	32	97
	5	not used		not used		
5	1	47	19	47	28	60
	2	46	0	46	46	100
	3	0	0		0	
	4	18	0	18	18	100
	5	not used		not used		
6	1	3	0	3	3	100
	2	0	0		0	
	3	4	0	4	4	100
	4	19	0	19	19	100
	5	not used		not used		

Appendix 5.3c: Survival, % larvae, % pupae of chironomid larvae from population R following exposure to OSPW in a 14-d bioassay

Numbers of larvae alive plus pupae (live or dead) plus adults or pupal exuviae-S (whichever is highest)

Treatment	Rep	POPULATION R <i>C. riparius</i>		corrected total	larvae	%L	% P of P+(A or S)	n
		total	P+(A or S)					
1	1	13	8		5	38	63	13
	2	49	36	49	13	27	56	49
	3	44	30	44	14	32	43	44
	4	11	10		1	9	70	11
	5	40	29	40	11	28	45	40
2	1	44	39	44	5	11	36	44
	2	14	11		3	21	82	14
	3	44	24	44	20	45	67	44
	4	22	21		1	5	57	22
	5	33	24	33	9	27	54	33
3	1	47	33	47	14	30	45	47
	2	9	8		1	11	50	9
	3	36	20	36	16	44	85	36
	4	35	27	35	8	23	22	35
	5	9	7		2	22	57	9
4	1	15	14		1	7	86	15
	2	53	35	50	18	34	29	53
	3	16	11		5	31	36	16
	4	38	28	38	10	26	46	38
	5	50	41	50	9	18	39	50
5	1	58	22	50	36	62	73	58
	2	14	7		7	50	71	14
	3	26	15		11	42	67	26
	4	44	35	44	9	20	51	44
	5	48	33	48	15	31	33	48
6	1	15	6		9	60	100	15
	2	42	14	42	28	67	86	42
	3	41	18	41	23	56	89	41
	4	30	6		24	80	67	30
	5	44	11	44	33	75	91	44

Appendix 5.4a: Chironomid larval lengths from OSPW bioassay for population L (code = population treatment-rep).  
 (treatment 1-6 corresponds to 0%-100% OSPW respectively)

L 1-1	L 1-2	L 1-3	L 1-4	L 1-5	L 2-1	L 2-2	L 2-3	L 2-4	L 2-5	L 3-1	L 3-2
13.784	25.011	18.111	23.821	10.134	20.512	13.561	11.123	13.709	16.109	21.023	14.314
10.783	22.954	16.557	18.552	14.988	24.009	13.652	11.818	17.5	19.659	20.427	16.903
20.783	18.072	11.617	13.041	15.389	23.133	21.902	11.628	26.015	20.779	15.394	11.239
18.016	18.684	17.871	12.146	7.043	20.023	13.981	10.372	20.014	18.961	16.901	15.76
22.548	15.876	17.255	14.488		21.155	13.489	12.488	17.921	18.483	18.638	12.055
23.359	15.194	6.864	13.003		21.088	11.336	5.832	17.512	21.703	15.452	16.815
18.275	16.17	10.698	14.135		24.349	11.089	10.727	18.827	20.844	18.621	12.223
19.843	18.335	10.545	13.199		26.187	10.268	7.563	17.245	17.56	17.21	19.435
19.705	16.904	8.884	12.235		20.829	15.911	11.322	16.038	18.519	17.91	11.852
19.238	17.269	12.828	19.846		16.99	9.89	10.983	16.465	15.727	16.273	12.346
18.106	17.702	14.738	17.536		19.261	13.073	9.515	10.928	16.741	12.412	15.2
8.803	19.27	17.278	24.541		20.36	11.439	9.628	17.952	18.471	14.437	10.873
19.827	18.071	15.478	19.515		19.757	23.01	10.133	20.588	10.176	12.029	12.788
19.063	17.986	22.195	16.198		21.236	10.09	11.166	19.075	21.742	14.655	20.175
18.377	16.314	23.427	14.243		18.671	12.136	10.942	13.406	10.064	10.724	17.477
21.222	13.189	10.561	20.252			23.318	3.796	18.017	10.224	12.283	17.584
17.779	19.557	22.552	5.857			17.898	12.545	12.775	13.343	13.326	16.914
21.649	14.473	23.151	11.036			17.033	6.568	11.892	11.63	12.741	15.3
19.302	17.27	18.005	18.103			16.711	11.164	10.381		12.086	18.01
10.188	22.799	12.68	16.756			12.21	11.049	24.8		13.115	13.969
20.629	16.158	18.092	18.559			12.666	10.166	17.005		10.369	10.932
16.011	17.162	15.936	16.214			8.719	10.166	20.84		14.234	14.31
20.555	12.432	14.958	23.465			15.189	9.375	20.924		11.644	14.009
11.162	17.252	19.555	20.41			12.369	11.199	17.45		13.764	13.709
15.894	15.425	17.077	20.779			12.619	8.858	12.24		10.88	11.591
16.44	14.28	20.863	18.199			15.464	9.065	21.726		11.958	16.629
21.577	19.496	5.669	19.815			11.603	8.979	12.278		12.306	14.53
17.622	21.401	18.886	22.329			14.491	10.76	19.996		13.414	11.391
12.094	19.078	6.599	16.736			13.277	16.056	15.2		10.401	13.739
12.074	5.721	10.005	19.187			12.624	9.618	18.478		11.966	14.535
17.661		15.018	19.004			12.049	11.372	14.497		13.268	13.768
12.75		12.719	16.168			14.75	12.52	13.261		13.015	16.611
16.904			23.48			13.686	15.422	13.074		14.039	11.078
16.02			22.728			17.117	13.048	17.155		16.538	12.661
19.176			19.327			13.239	16.447	19.819		13.261	13.132
			24.199			14.621	10.952	17.032		11.811	17.389
			12.325			14.248	14.14	13.926		11.959	17.166
			19.196			23.694	8.603	19.066		11.671	18.708
						18.489	11.386	19.319		16.723	9.92
						22.946	11.18	22.956		12.985	
						13.589	12.724	14.773		10.325	
							12.356	17.289		11.335	
							10.294	16.516		9.827	
							11.457	12.655		12.096	
							6.855			12.295	
							14.518			13.19	
							10.232			12.683	
							11.396			13.011	
							10.471			13.35	
							9.935				
							9.733				
							8.269				
							11.732				
							16.252				
							9.191				
							10.9				
							8.871				
							10.893				
							10.732				
							12.555				
							13.147				
							12.125				
						295	9.185				
							9.904				





Appendix 5.4b: Chironomid larval lengths from OSPW bioassay for population T (code = population treatment-rep).											
(treatment 1-6 corresponds to 0%-100% OSPW respectively)											
T 1-1	T 1-2	T 1-3	T 1-4	T 1-5	T 2-1	T 2-2	T 2-3	T 2-4	T 2-5	T 3-1	T 3-2
13.179	18.722	19.223	not used	18.342	21.911	18.472	25.685	16.774	not used	15.024	not used
21.401	18.595	19.072		18.967	18.028	21.975	21.071	18.618		10.798	
16.424	15.109	19.542		13.592	19.287	18.217	19.981	19.099		14.39	
18.069	14.687	12.591		8.727	19.658	17.054	21.407	17.135		16.723	
20.584	18.105	21.276		17.818	20.13	21.281	17.257	18.069		13.307	
20.353	17.275	18.249		15.31	18.626	18.606	17.13	16.591		12.144	
23.598	20.171	20.328		18.858	20.537	10.021	17.202	18.115		9.635	
19.11	15.484	23.731		16.728	18.102	21.19	19.177	18.032		15.798	
21.885	20.065	12.828		17.591	20.69	20.499	22.327	16.931		14.275	
17.957	16.097	4.896		17.18	23.783	17.778	17.073	12.732		12.997	
19.462	18.66	19.108		18.08	19.374	6.511	24.135	18.168		17	
21.053	22.19	21.242		17.422	19.513	19.491	20.564	16.345		14.387	
20.627	20.193	13.099		17.701	12.694	16.991	19.244	7.373		17.109	
21.45	18.747	13.532		17.642	18.688	18.004	22.207	15.102		12.376	
15.375	13.544	21.32		21.093	20.142	16.943	16.768	18.188		15.943	
18.445	16.763	24.87		16.167	19.656	11.763	23.54	15.831		11.419	
9.708	13.189	14.177		22.606	21.405	18.221	21.872	17.703		12.227	
21.066	17.281	13.859		19.567	18.272	21.955	17.988	13.085		14.034	
19.87	22.605	21.843		20.363	18.445	20.307	20.704	17.524		16.512	
19.861	16.984	6.544		16.521	22.155	18.584	20.309	17.482		13.057	
20.504	20.148			15.527	21.603	15.401	21.826	16.535		13.919	
20.372	18.032			19.501	13.256	15.026	19.923	14.908		14.899	
19.284	16.716			15.15	22.397	20.589	17.039	13.781		8.776	
21.019	16.565			17.776	18.457	12.523	20.693	15.697		14.398	
21.733	17.183			17.004	19.323	19.738	12.993	18.67		18.505	
18.012	13.547			19.101	11.158	18.877	19.099	18.621		16.26	
14.171	19.082			21.385	19.618	21.03	15.418	11.521		18.196	
18.649	11.683			19.989	19.138	22.12	21.594	18.63		15.761	
22.842	16.374			21.463	21.441	14.819	21.076	10.95		13.551	
17.73	20.259			19.335	24.156	20.745	20.587	15.753		13.967	
19.832	14.242			20.142	20.105	16.464	22.609	16.88		19.034	
19.429	19.419			19.859	19.555	17.412	14.85	15.195		15.417	
18.488	18.92			17.871	18.941	15.655	24.595	8.795		12.537	
18.356	22.948			18.301	23.886	20.235	19.433	17.063		8.778	
18.703	19.107			18.873	22.698	20.14	20.961	16.901		17.882	
18.974	19.114			21.109	20.658	15.814	19.097	15.881		18.257	
19.746	18.664			18.297	14.238	16.219	25.607	14.764		12.038	
17.066	18.523			19.198	20.03	18.082	21.651	16.759		10.827	
21.848	13.457			16.638	20.379	21.274	20.887	14.271		13.735	
19.288	20.393			17.292	20.537	13.754	21.049	19.667		9.303	
21.404				19.774	19.09	12.58	16.874	11.646		15.135	
7.68				18.981	19.964	21.852	23.86	11.855		12.744	
20.532				19.407	17.835	19.302	21.333	15.76		15.764	
21.602				22.107	21.515	20.363	16.423	12.65		16.501	
16.192				18.291	23.095	15.929		21.12		13.866	
15.944				19.677	15.497	15.923		15.542		16.193	
20.035				15.574	21.077	21.036		17.655		18.717	
20.143				18.624	19.365	11.703		19.975		16.379	
22.196				16.561	19.592	20.668		19.262		17.47	
17.576				17.577	20.603	22.31		9.535		13.62	
19.902				21.554		18.371		18.452		12.096	
				19.222		21.806		18.247			
				16.986		11.795		18.247			
				16.581		18.563		17.218			
				15.844		19.489		20.723			
				16.602		22.313		16.502			
				19.685		12.047		12.52			
				17.482		9.392		17.028			
						14.933		13.822			
						20.858		18.327			
						18.838		19.958			
								11.274			
								16.513			
								17.513			
								15.775			
								15.841			







R 3-3	R 3-4	R 3-5	R 4-1	R 4-2	R 4-3	R 4-4	R 4-5	R 5-1	R 5-2	R 5-3	R 5-4
13.431	8.347	11.567	15.401	14.114	13.989	13.15	14.632	15.184	14.037	13.644	11.26
13.665	13.472	10.646		14.472	13.746	10.12	14.02	14.807	15.026	14.372	14.023
10.828	13.455			13.762	14.881	11.602	14.447	15.339	11.621	14.953	11.15
9.936	13.266			14.273	16.376	12.631	13.619	15.77	14.95	9.033	10.821
10.552	14.105			13.851	14.717	12.956	13.567	15.468	10.21	12.041	14.77
13.023	13.844			14.794		15.186	14.712	15.088	11.669	12.525	14.242
12.106	12.748			13.805		13.953	14.806	12.33	11.084	15.045	13.082
11.125	9.496			12.92		13.433	11.102	14.261			13.19
12.43				13.337		15.117		13.785			7.959
13.198				14.353		13.689		14.015			
11.101				14.233				14.062			
13.749				14.076				14.971			
13.502				14.388				14.075			
12.364				11.765				13.744			
10.056				14.091				11.821			
				14.157				13.74			
				9.461				12.162			
				11.471				11.928			
								15.077			
								12.864			
								15.935			
								10.785			
								15.634			
								12.837			
								14.049			
								14.012			
								13.488			
								14.531			
								15.153			
								13.955			
								14.559			
								12.928			
								13.987			
								14.4			
								14.101			





Appendix 6.1: Colonization parameters for the water trays used at the 6 wetlands in Fort McMurray 1998																	
(continued)																	
CONDUCTIVITY (uS)			old	new	old	new	old	new	old	new	old	old	new	old	old		
Site	Water	Tray #	13-Jun	13-Jun	16-Jun	16-Jun	20-Jun	20-Jun	23-Jun	23-Jun	27-Jun	30-Jun	04-Jul	04-Jul	07-Jul	11-Jul	
HW	HW	1	3350	2400	1770	1800	2780	2320	2780	2350	750	2150	4100	2900	3890	4680	
		2	6200	2400	1750	1870	2850	2310	2900	2290	1060	2100	3300	2950	3600	3550	
		3	2300	2300	1790	1850	2620	2300	2570	2670	1370	2000		2810	3950	4250	
	white										2260	780	2120		2820	3720	3700
		HS	1	10000	2850	2390	2550	4910	3300	4400	3510	2000	4000	7500	3650	6000	7300
			2	7400	2750	2300	2550	4610	3270	4610	3500	1890	3300	5200	3500	4860	5000
	3		7300	2700	2200	2540	4450	3210	4570	4210	2110	3300		4100	5600	6000	
	white										3450	1480	3900		3550	5000	5300
		HS	1	3400	2680	2110	2500	4210	3250	4500	3410		3700	6000	3650	5300	6000
			2	5900	2700	2000	2520	4480	3230	4790	3420		4090	6500	3600	6100	10400
	3		3250	2700	2000	2510	3950	3160	4210	4000		3200	4630	4100	5500	5900	
	white										3210		3800		3640	5000	
HW		1	2100	2220	1650	1870	3050	2350	3050	2400		2130	3800	3080	3820	4200	
		2	2200	2270	1300	2000	3110	2300	2300	2400		2080	3520	2720	4100	5000	
	3		2300	500			2300	2980	2680		1920	2150	3080	4600			
white																	
	SALINITY (ppt)																
	Site	Water	Tray #	old	new	old	new	old	new	old	new	old	old	new	old	old	
HW	HW	1	>40	30	1	1	1.2	1.1	1.3	1	0.5	1.5	2.5	2	2.4	3.3	
		2	>40	29	1	1	1.2	1	1.2	1	0.7	1.2	2.1	2	2	2.5	
		3	>40	29	1	1	1.1	1	1.1	1.2	0.8	1.1		1.9	2.3	3	
	white										1	0.5	1.4		1.8	2	2.8
		HS	1	>40	36	1.2	1.7	2.2	1.5	2	1.7	1.2	2.8	4.5	2.2	3.6	5.2
			2	>40	35	1.2	1.5	2.1	1.5	2.1	1.6	1.1	2.2	3	2.1	3	3.6
	3		26	35	1.2	1.8	2	1.7	2	1.2	1.2	2		2.8	3.5	4.5	
	white										1.6	1	2.8		2.2	3	4
		HS	1	>40	33	1.1	1.5	2	1.5	2	1.8		2.3	3.6	2.3	3.1	4.2
			2	>40	34	1.1	1.3	2	1.6	2.2	1.8		2.2	5.6	2.3	3.8	7.2
	3		>40	34	1.1	1.3	1.9	1.5	2	1.9		2	2.8	2.6	3.2	4	
	white										1.7		2.5		2.4	3	
HW		1	26	27	1	1	1.3	1	1.3	1		1.2	2.3	2	2.3	3	
		2	22	28	0.8	1.1	1.3	1		1.1		1.3	2.2	1.7	2.5	3.6	
	3		29	0.1				1	1.3	1.1		1	2.2	2	2.7	4.3	
white										1		1.2		2	2.8		
	TEMPERATURE (deg C)																
	Site	Water	Tray #	old	new	old	new	old	new	old	new	old	old	new	old	old	
HW	HW	1	24	20	18	18	28	29	28	32	19	28	35	30	31	23	
		2	20	19	18	17	31	28	32	29	19	28	34	28	33	23	
		3	19	20	17	17	29	28	30	30	18	28		29	32	23	
	white										29	18	27		30	33	23
		HS	1	27	19	18	17	32	29	32	30	19	28	35	30	34	23
			2	20	20	18	17	32	28	33	29	19	27	33	30	33	23
	3		19	20	17	17	31	27	32	29	18	26		30	32	23	
	white										29	18	27		30	33	23
		HS	1	15	18	18	23	34	29	33	27		34	34	33	36	25
			2	18	18	18	24	33	27	32	28		35	32	30	34	26
	3		14	17	17	23	31	27	32	31		33	34	30	36	25	
	white										27		32		31	34	
HW		1	15	17	17	24	33	29	33	30		35	34	32	37	25	
		2	18	17	17	24	33	27	33	31		34	31	31	33	26	
	3		18	17			28	32	30		34	32	31	35	25		
white										28		33		32	35		



Appendix 6.2: Colonization trays used in 1998 field season in Fort McMurray		Sites included NW-CL, HW-HS, and SB-SW		Natural Wetland site		Date	Water	Rep	Tanytarsini	Orthocladinae	Tanypodinae	Chironomini	# Groups	Total No. midges	TOTAL
SITE															
						10-Jun	NW	1				1F	1	1	
						14-Jun	NW	1	9F, 2M		5M	2	2	16	
						14-Jun	NW	2	9F, 1M			1	10	52	
						14-Jun	NW	3	4F, 1M	1F		2	6		
						21-Jun	NW	2	8F, 6M		1M	2	15		
						21-Jun	NW	3	3F		1M	1	4		
						10-Jun	CL	1							
						10-Jun	CL	1							
						10-Jun	CL	3							
						14-Jun	CL	1	41F, 21M	2F		2	71		
						14-Jun	CL	2	5F		2M	2	7		
						14-Jun	CL	3	1F		6F	2	7		
						17-Jun	CL	2	1F, 1M			1	2	140	
						21-Jun	CL	1	12F	13M		2	25		
						21-Jun	CL	2	9F, 2M			1	11		
						24-Jun	CL	1	3F		4M	2	7		
						24-Jun	CL	2	2F		3M	2	5		

<b>Appendix 6.2: Colonization trays used in 1998 field season in Fort McMurray</b>											
<b>(continued)</b>											
<b>Crane Lake site</b>											
<b>Date</b>	<b>Water</b>	<b>Rep</b>	<b>Tanytarsini</b>	<b>Orthocladinae</b>	<b>Tanypodinae</b>	<b>Chironomini</b>	<b>Podonominae</b>	<b>UnIDed</b>	<b>No. of groups</b>	<b>Total No. midges</b>	<b>SITE TOTAL</b>
10-Jun	CL	1	1M						1	1	
10-Jun	CL	2			1F	1F			2	2	
17-Jun	CL	2				1F			1	1	
21-Jun	CL	1				1F			1	1	13
21-Jun	CL	2			1F				1	1	
24-Jun	CL	1	1F, 1M		2M	1M			3	5	
24-Jun	CL	2					1M		1	1	
24-Jun	CL	3					1F		1	1	
10-Jun	NW	1	4F						1	4	
21-Jun	NW	1						1F	1	1	
21-Jun	NW	2		2F					1	2	12
24-Jun	NW	1				1F			1	1	
24-Jun	NW	2			2F, 1M			1F	2	4	

Appendix 6.2: Colonization trays used in 1998 field season in Fort McMurray										
(continued)										
Hummock Wetlands										
Date	Water	Rep	Tanytarsini	Orthocladinae	Tanypodinae	Chironomini	UnIDed	No. of groups	Total No. midges	SITE TOTAL
09-Jun	HW	ALL 3	1F			2F		2	3	
13-Jun	HW	1		1F				1	1	
13-Jun	HW	2	3F	2F, 4M		1F	1F	3	11	
20-Jun	HW	1				1F		1	1	
20-Jun	HW	2	2F, 1M					1	3	26
20-Jun	HW	3		1F				1	1	
23-Jun	HW	1	2F					1	2	
23-Jun	HW	2	1F			2F		2	3	
23-Jun	HW	3				1F		1	1	
09-Jun	HS	ALL 3					1	1	1	
13-Jun	HS	1	1F			1F, 1M		2	3	
13-Jun	HS	2		1M			1F	2	2	
20-Jun	HS	1	1F	1M	1F			3	3	20
20-Jun	HS	2	1F	1F, 1M				2	3	
20-Jun	HS	3	2F	1F		2F		3	5	
23-Jun	HS	1	1F	1F				2	2	
23-Jun	HS	3	1F					1	1	

Appendix 6.2: Colonization trays used in 1998 field season in Fort McMurray									
(continued)									
High Sulphate Pond									
Date	Water	Rep	Tanytarsini	Orthocladinae	Tanypodinae	Chironomini	Total No. genus	Total No. midges	SITE TOTAL
09-Jun	HS	2	1M			1F	2	2	
13-Jun	HS	3	2F	1F		1F	3	4	
20-Jun	HS	1		1F	1F		2	2	
20-Jun	HS	2		1M		1F, 1M	2	3	17
20-Jun	HS	3		2F, 1M			1	3	
23-Jun	HS	1	1F				1	1	
23-Jun	HS	2	2F				1	2	
09-Jun	HW	2	1F	2F, 2M		1F	3	6	
09-Jun	HW	3	2M	1M			2	3	
13-Jun	HW	1	6F, 1M	1F, 2M		2F, 1M	3	13	
13-Jun	HW	2	4F, 8M	4F, 2M	4M	2F	4	24	
13-Jun	HW	3	1F				1	1	68
20-Jun	HW	1		2F			1	2	
20-Jun	HW	2		1F			1	1	
23-Jun	HW	1	4F, 1M	4M		1F	3	10	
23-Jun	HW	3	2F, 6M				1	8	

Appendix 6.2: Colonization trays used in 1998 field season in Fort McMurray										
(continued)										
South Bison Pond										
Date	Water	Rep	Tanytarsini	Orthocladinae	Tanypodinae	Chironomini	Unidentified	# Groups	Total No. midges	SITE TOTAL
12-Jun	SB	2				1F		1	1	
19-Jun	SB	1	2F			1F		2	3	
19-Jun	SB	2	2F, 2M					1	4	
19-Jun	SB	3	2F					1	2	
22-Jun	SB	2	1F			1F, 1M		2	3	17
22-Jun	SB	3	1M			1F		2	2	
06-Jul	SB	3	1F	1F				2	2	
12-Jun	SW	1				1F		1	1	
12-Jun	SW	2	2F		1F	1F		3	4	
19-Jun	SW	2				1F		1	1	
19-Jun	SW	3	1F					1	1	14
22-Jun	SW	1	2F					1	2	
22-Jun	SW	2	1F, 1M		2F, 1M			2	5	

Appendix 6.2: Colonization trays used in 1998 field season in Fort McMurray									
(continued)									
Shallow Wetlands (at Syncrude)									
Date	Water	Rep	Tanytarsini	Orthocladinae	Tanypodinae	Chironomini	# Groups	Total No. midges	SITE TOTAL
12-Jun	SW	1	3M	1F			2	4	
12-Jun	SW	2	3F, 1M				1	4	
19-Jun	SW	2	4F				1	4	16
19-Jun	SW	3	3F				1	3	
26-Jun	SW	1	1F				1	1	
12-Jun	SB	1	1F				1	1	
12-Jun	SB	2	1F	1F			2	2	
12-Jun	SB	3	2F, 2M				1	4	
19-Jun	SB	1	3F, 1M				1	4	
19-Jun	SB	2	17F			12M	2	29	43
22-Jun	SB	2	2F, 1M				1	3	

Appendix 6.3: Number of egg masses collected and hatching success of eggs, for 6 wetlands					
ID	Water	Rep	No. egg masses	Start date	Notes on larval hatching
<b>Site: Natural Wetlands</b>					
NW femaleA (light trap) (in tap water)			1	25-Jun	100 (july 4), 45 (july 6), 35 3rd instars (july 10)
NW/CL 2	CL	2	1	24-Jun	8 (1st instars), 30+ (june 28), 30+ (june 29), 30+ (july 3)
NW/CL 3	CL	3	1	01-Jul	3 (july 2), 2 (july 4)
<b>Site: High Sulphate Pond</b>					
HS/HS 3	HS	3	1	23-Jun	0 (june 27)
HS/HS 1	HS	1	1	27-Jun	50 (june 29), 50 (july 3), 100 (july 5)
HS/HW 1	HW	1	3	23-Jun	20 (june 24), 0 (june 27)
HS/HW 3	HW	3	1	23-Jun	
HS/HW 3	HW	3	1	27-Jun	50 (june 30), 50 (july 3)
HS/HW 2	HW	2	2	27-Jun	
HS/HW 1	HW	1	1	04-Jul	
<b>Site: Hummock Wetlands</b>					
HW/HW 3	HW	3	5	09-Jun	lots as 300 taken out for insitu test 1
HW/HW	HW		1	16-Jun	hatched june 25
HW/HW 1+2	HW	1+2	3	20-Jun	hatched, used for aquarium #4...
HW/HW 3	HW	3	1	23-Jun	20 (june 24), 0 (june 27)
HW/HS	HS		7	16-Jun	used for aquar. #2...
HW/HS 3	HS	3	1	20-Jun	3 (june 25, june 27)
HW/HS 1	HS	1	1	23-Jun	0 (june 27)
HW/HS 2	HS	2	4	23-Jun	30 (june 27, 28), 50 (june 29), 30 (july 3), 20 (july 4), 4th instars (july 10)
HW/HS 2	HS	2	1	27-Jun	2 (june 28), 20 (june 29, july 3), 15 (july 4)
HW/HS 2	HS	2	1	04-Jul	20 (july 8)
<b>Site: South Bison</b>					
SB/SB	SB			22-Jun	5 (june 28, 29, july 3)
SB/SB 3	SB	3	1	06-Jul	100 (july 8)
SB/SW 3	SW	3	1	22-Jun	0 (june 23), 100 (june 28, 29, july 3), 35 (july 4)
<b>Site: Crane Lake</b>					
CL/CL 3	CL	3	1	24-Jun	1 (june 27)
<b>Site: Shallow Wetlands</b>					
SW/SW 2	SW	2	1	12-Jun	

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